

CHIMERIC CLOTTING FACTORS

5 RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application Serial No. 61/363,183 filed July 9, 2010; U.S. Provisional Patent Application No. 61/363,186 filed July 9, 2010; U.S. Provisional Patent Application No. 61/442,029 filed February 11, 2011; U.S. Provisional Patent Application No. 10 61/442,150 filed February 11, 2011; U.S. Provisional Patent Application No. 61/442,055 filed February 11, 2011; U.S. Provisional Patent Application No. 61/467,880 filed March 25, 2011; and U.S. Provisional Patent Application No. 61/491,762 filed May 31, 2011. The entire contents of the above-referenced provisional patent applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

15 [0002] Initiation of the extrinsic clotting pathway is mediated by the formation of a complex between tissue factor, which is exposed as a result of injury to a vessel wall, and Factor VIIa. This complex then converts Factors IX and X to their active forms. Factor Xa converts limited amounts of prothrombin to thrombin on the tissue factor-bearing cell. This resulting thrombin is then able to diffuse away from the tissue-
20 factor bearing cell and activate platelets, and Factors V and VIII, making Factors Va and VIIIa. During the propagation phase of coagulation, Factor Xa is generated by Factor IXa (in complex with factor VIIIa) on the surface of activated platelets. Factor Xa, in complex with the cofactor Factor Va, activates prothrombin into thrombin, generating a thrombin burst. The cascade culminates in the conversion of fibrinogen
25 to fibrin by thrombin, which results in the formation of a fibrin clot. Factor VII and tissue factor are key players in the initiation of blood coagulation.

[0003] Factor VII is a plasma glycoprotein that circulates in blood as a single-chain zymogen. The zymogen is catalytically inactive. Although single-chain Factor VII may be converted to two-chain Factor VIIa by a variety of factors in vitro, Factor Xa
30 is an important physiological activator of Factor VII. The conversion of zymogen

5 Factor VII into the activated two-chain molecule occurs by cleavage of the peptide bond linking the Arginine residue at amino acid position 152 and the Ile residue at amino acid position 153. In the presence of tissue factor, phospholipids and calcium ions, the two-chain Factor VIIa activates Factor X or Factor IX. Factor VIIa is thought to be the physiologic initiator of the clotting cascade by acting at the surface of a TF-
10 bearing cell, typically a damaged endothelial cell, and generating the initial amount of thrombin that then diffuses to platelets to activate them and prime them for the propagation phase of thrombin generation. Therapeutically, recombinant FVIIa acts by activating Factor X on the surface of activated platelets, bypassing the need for FIXa or FVIIIa to generate a thrombin burst during the propagation phase of
15 coagulation. Since FVIIa has relatively low affinity for platelets, recombinant FVIIa is dosed at supra-physiological levels. This process is thought to be tissue factor-independent

[0004] Human factor IX circulates as a single-chain glycoprotein (mol wt 57,000). It is present in plasma as a zymogen and is converted to a serine protease, Factor
20 IXa β (more commonly referred to as FIXa), by Factor XIa (activated plasma thromboplastin antecedent) in the presence of calcium ions. In the activation reaction, two internal peptide bonds are hydrolyzed in Factor IX. These cleavages occur at a specific arginyl-alanine peptide bond and a specific arginyl-valine peptide bond. This results in the release of an activation peptide (mol wt approximately equal to 11,000)
25 from the internal region of the precursor molecule and the generation of Factor IXa β (mol wt approximately equal to 46,000). Factor IXa β is composed of a light chain (mol wt approximately equal to 18,000) and a heavy chain (mol wt approximately equal to 28,000), and these chains are held together by a disulfide bond.

[0005] Factor X is also synthesized as a single-chain polypeptide containing the light
30 and heavy chains connected by an Arg-Lys-Arg tripeptide. The single-chain molecule is then converted to the light and heavy chains by cleavage of two (or more) internal peptide bonds. In plasma, these two chains are linked together by a disulfide bond, forming Factor X. Activated Factor X, Factor Xa, participates in the final common pathway whereby prothrombin is converted to thrombin, which in turn converts
35 fibrinogen to fibrin.

[0006] Clotting factors have been administered to patients to improve hemostasis for some time. The advent of recombinant DNA technology has significantly improved

- 5 treatment for patients with clotting disorders, allowing for the development of safe and consistent protein therapeutics. For example, recombinant activated factor VII has become widely used for the treatment of major bleeding, such as that which occurs in patients having haemophilia A or B, deficiency of coagulation Factors XI or VII, defective platelet function, thrombocytopenia, or von Willebrand's disease.
- 10 Recombinant factor IX is therapeutically useful as well.
- [0007] Although such recombinant molecules are effective, there is a need for improved versions which localize the therapeutic to sites of coagulation, have improved pharmacokinetic properties, have reduced clearance rates, have improved manufacturability, have reduced thrombogenicity, or have enhanced activity, or more
- 15 than one of these characteristics.

SUMMARY OF THE INVENTION

- [0008] The instant invention relates to chimeric clotting factors which have enhanced
- 20 activity. The present invention features *inter alia* methods for making chimeric clotting factors, the chimeric clotting factors made using these methods, and methods for improving hemostasis using these clotting factors. The chimeric clotting factors of the invention possess enhanced pharmacokinetic properties, have reduced clearance rates, have improved manufacturability, have reduced thrombogenicity, have
- 25 enhanced activity, or more than one of these characteristics. In one embodiment, improved clotting factors of the invention have increased activity where needed, e.g., by targeting the clotting factor to platelets or by being present in a subject in an activatable form (a non-naturally occurring activatable form) that is activated at the site of clot formation.
- 30 [0009] In one aspect, the invention pertains to a chimeric clotting factor which comprises a clotting factor selected from the group consisting of FVII, FIX and FX and a targeting moiety which binds to platelets and optionally a spacer moiety between the clotting factor and the targeting moiety.
- [0010] In one embodiment, the clotting factor comprises a structure represented by
- 35 the formula A B C, wherein A is the clotting factor; wherein B is a spacer moiety; and wherein C is at least one targeting moiety which binds to platelets.

- 5 [0011] In one embodiment, the clotting factor comprises a structure from amino terminus to carboxy terminus represented by a formula selected from the group consisting of: A B C; C B A
- [0012] In one embodiment, the clotting factor exhibits increased generation of thrombin in the presence of platelets as compared to an appropriate control lacking
- 10 the at least one targeting moiety.
- [0013] In one embodiment, the clotting factor comprises a scaffold moiety and, optionally, a second spacer moiety.
- [0014] In one embodiment, the clotting factor further comprises D and E, wherein D is a spacer moiety; and E is a scaffold moiety and wherein the chimeric clotting factor
- 15 comprises a structure from amino terminus to carboxy terminus represented by a formula selected from the group consisting of: A B C D E; A D E B C; E D A B C; C B A D E; E D C B A; and C B E D A.
- [0015] In one embodiment, E is a dimeric Fc region comprising a first Fc moiety, F1 and a second Fc moiety, F2.
- 20 [0016] In one embodiment, the clotting factor is expressed as a polypeptide comprising a cleavable scFc (cscFc) linker interposed between two Fc moieties, wherein the cscFc linker is adjacent to at least one enzymatic cleavage site which results in cleavage of the cscFc polypeptide linker.
- [0017] In one embodiment, the cscFc linker is adjacent to at least one enzymatic
- 25 cleavage site which results in cleavage of the cscFc linker.
- [0018] In one embodiment, the chimeric clotting factor of claim 9, wherein the at least one enzymatic cleavage site is an intracellular processing site.
- [0019] In one embodiment, wherein the polypeptide linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes.
- 30 [0020] In one embodiment, the polypeptide linker has a length of about 10 to about 50 amino acids.
- [0021] In one embodiment, the polypeptide linker has a length of about 20 to about 30 amino acids.
- [0022] In one embodiment, the polypeptide linker comprises a gly/ser peptide.
- 35 [0023] In one embodiment, the gly/ser peptide is of the formula (Gly₄Ser)_n, or Ser(Gly₄Ser)_n wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In one embodiment, the (Gly₄ Ser)_n linker is selected

5 from the group consisting of (Gly₄ Ser)₆, Ser(Gly₄ Ser)₆, (Gly₄ Ser)₄ and Ser(Gly₄ Ser)₄.

[0024] In one embodiment, the clotting factor comprises two polypeptide chains.

[0025] In one embodiment, the chimeric clotting factor has a structure selected from the group consisting of: A linked to F1 via a spacer moiety and C linked to F2; A
10 linked to F1 via a spacer moiety and C linked to F2 via a spacer moiety; A linked to F1 and C is linked to F2 via a spacer moiety; A linked to F1 via a spacer moiety and C is linked to F2 via a spacer moiety.

[0026] In one embodiment, a chimeric clotting factor comprises two polypeptides wherein the first polypeptide comprises the moieties A B F1; A B F1; A B F1; or A B
15 F1 D C and the second polypeptide comprises the moieties C F2; C D F2; F2 D C; or F2 D C, wherein the two polypeptide chains form an Fc region.

[0027] In one embodiment, the targeting moiety is fused to at least one of the polypeptide chains of the Fc region. In one embodiment, the targeting moiety is fused to at least one of F1 and F2 directly. In one embodiment, the targeting moiety is
20 fused to at least one of F1 and F2 via a spacer moiety. In one embodiment, the targeting moiety is fused to at least one of F1 and F2 via a cleavable linker. In one embodiment, the targeting moiety is selected from the group consisting of: an antibody molecule, an antigen binding fragment of an antibody molecule, an scFv molecule, a receptor binding portion of a receptor, a peptide. In one embodiment,
25 wherein the targeting moiety binds to resting platelets. In one embodiment, the targeting moiety selectively binds to activated platelets. In one embodiment, the targeting moiety selectively binds to a target selected from the group consisting of: GPIIb, GPVI, and the nonactive form of GPIIb/IIIa. In one embodiment, the targeting moiety selectively binds to a target selected from the group consisting of:
30 the active form of GPIIb/IIIa, P selectin, GMP-33, LAMP-1, LAMP-2, CD40L, and LOX-1. In one embodiment, the targeting moiety binds to the GPIb complex. In one embodiment, the targeting moiety is a peptide selected from the group consisting of: PS4, OS1, and OS2. In one embodiment, the targeting moiety comprises an antibody variable regions from an antibody selected from the group consisting of: SCE5, MB9,
35 and AP3.

[0028] In one embodiment, wherein the clotting factor is Factor VII.

[0029] In one embodiment, the clotting factor is a high specific activity variant of Factor VII. In one embodiment, the clotting factor is Factor IX. In one embodiment,

- 5 the clotting factor is a high specific activity variant of Factor IX. In one embodiment, the clotting factor is Factor X. In one embodiment, clotting factor is a high specific activity variant of Factor X.
- [0030] In one embodiment, the clotting factor is secreted by a cell in active form. In one embodiment, the clotting factor is activated in vivo.
- 10 [0031] In one embodiment, the chimeric clotting factor comprises a heterologous enzymatic cleavage site not naturally present in the clotting factor.
- [0032] In one embodiment, the enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.
- [0033] In one embodiment, the clotting factor comprises a scaffold moiety is a protein
- 15 molecule which increases the hydrodynamic radius of the chimeric clotting factor. In one embodiment, the scaffold moiety, if present, is selected from the group consisting of albumin and XTEN[®]
- [0034] In another aspect, the invention pertains to a polypeptide comprising FVII, which FVII comprises a heterologous enzymatic cleavage site activatable by a
- 20 component of the clotting cascade.
- [0035] In one embodiment, the polypeptide comprises a scaffold moiety and, optionally, a spacer moiety.
- [0036] In one embodiment, the scaffold moiety is a dimeric Fc region comprising a first Fc moiety, F1 and a second Fc moiety, F2.
- 25 [0037] In one embodiment, the clotting factor comprises two polypeptide chains.
- [0038] In one embodiment, the chimeric clotting factor has a struture selected from the group consisting of: the clotting factor linked to the first Fc moiety via a spacer moiety; the clotting factor linked to the second Fc moiety via a spacer moiety; the clotting factor is directly linked to F1; and the clotting factor is directly linked to F2.
- 30 [0039] In one embodiment, the chimeric clotting factor further comprises a targeting moiety.
- [0040] In one embodiment, the chimeric clotting factor is synthesized as a single polypeptide chain comprising a cscFc linker. In one embodiment, the cscFc linker is linked to (e.g., directly linked or adjacent to) at least one enzymatic cleavage site
- 35 which results in cleavage of the linker.
- [0041] In one embodiment, the at least one enzymatic cleavage site is an intracellular processing site. In one embodiment, the cscFc linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes. In one

5 embodiment, the cscFc linker has a length of about 10 to about 50 amino acids. In one embodiment, the cscFc linker has a length of about 20 to about 30 amino acids.

[0042] In one embodiment, the cscFc linker comprises a gly/ser peptide.

[0043] In one embodiment, wherein the gly/ser peptide is of the formula (Gly₄Ser)_n, or Ser(Gly₄Ser)_n wherein n is a positive integer selected from the group consisting of
10 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In one embodiment, the (Gly₄ Ser)_n linker is selected from the group consisting of (Gly₄ Ser)₆, Ser(Gly₄ Ser)₆, (Gly₄ Ser)₄ and Ser(Gly₄ Ser)₄.

[0044] In one embodiment, the clotting factor is a high specific activity variant of Factor VII. In one embodiment, the heterologous enzymatic cleavage site present in
15 the chimeric clotting factor is cleaved at the site of clot formation. In one embodiment, the cleavage site is selected from the group consisting of: a factor XIa cleavage site, a factor Xa cleavage site, and a thrombin cleavage site. In one embodiment, the enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.

20 [0045] In one embodiment, the targeting moiety binds to resting platelets. In one embodiment, the targeting moiety selectively binds to activated platelets.

[0046] In one embodiment, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIIb_a, GPVI, and the nonactive form of GPIIb/IIIa. In one embodiment, the targeting moiety selectively binds to a target
25 selected from the group consisting of: the active form of GPIIb/IIIa, P selectin, GMP-33, LAMP-1, LAMP-2, CD40L, and LOX-1.

[0047] In one embodiment, the scaffold moiety is a protein molecule which increases the hydrodynamic radius of the chimeric clotting factor. In one embodiment, the scaffold moiety, if present, is selected from the group consisting of albumin and

30 XTEN[®]

[0048] In one aspect the invention pertains to a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: A B C; C B A; A B C D E; A D E B C, E D A B C, C B A D E, E D C B A, C B E D A, wherein A an activatable clotting factor, B is absent or is a linker, C is a targeting
35 moiety, D is absent or is a linker, and E is a scaffold moiety.

[0049] In one embodiment, the clotting factor comprises a light and heavy chain of a clotting factor and each of the light and heavy chains are expressed as separate polypeptide chains.

- 5 [0050] In one embodiment, the invention pertains to a nucleic acid molecule encoding a chimeric clotting factor of the invention. In one embodiment, the nucleic acid molecule is present in a vector. In one embodiment, the vector further comprises a nucleotide sequence encoding an enzyme which cleaves at least one of the enzymatic cleavage sites.
- 10 [0051] In one embodiment, the invention pertains to a host cell comprising the expression vector of the invention. In one embodiment, the host cell expresses an enzyme capable of intracellular processing. In one embodiment, the enzyme is endogenous to the cell. In one embodiment, the enzyme is heterologous to the cell.
- 15 [0052] In another embodiment, the invention pertains to a method for producing a chimeric clotting factor comprising culturing the host cell in culture and recovering the chimeric clotting factor from the medium.
- [0053] In another embodiment, the invention pertains to a processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector in a cell cultured in cell
- 20 culture medium and isolating the mature, heterodimeric polypeptide from the culture medium.
- [0054] In one embodiment, the invention pertains to a composition comprising a chimeric clotting factor and a pharmaceutically acceptable carrier.
- [0055] In another embodiment, the invention pertains to a composition comprising
- 25 the nucleic acid molecule of the invention and a pharmaceutically acceptable carrier.
- [0056] In another embodiment, the invention pertains to a method for improving hemostasis in a subject, comprising administering the composition of the invention.
- [0057] in one aspect, the invention pertains to an chimeric clotting factor which comprises a light chain moiety and a heavy chain moiety of a clotting
- 30 factor, and at least one targeting moiety, wherein said targeting moiety (i) specifically binds to platelets, (ii) is not interposed between the light and heavy chains of the clotting factor, and wherein said chimeric clotting factor exhibits increased generation of thrombin in the presence of platelets as compared to an appropriate control lacking the at least one targeting moiety.
- 35 [0058] In another aspect, the invention pertains to an chimeric clotting factor, which comprises the moieties A-B-C-D-E in linear sequence wherein A is a clotting factor, an activatable clotting factor, or an activated clotting factor; B

5 is absent or is a linker; C is a targeting moiety; D is absent or is a linker; and E is absent or is a scaffold moiety.

[0059] In still another aspect, the invention pertains to an chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: ABC; ABCDE; ADEBC, EDABC, CBADE, EDCBA, CBEDA, wherein A is a clotting factor, an activatable clotting factor or an activated clotting factor, B is absent or is a linker, C is a targeting moiety, D is absent or is a linker, and E is a scaffold moiety.

[0060] In yet another aspect, the invention pertains to an chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: ABF1:F2; ABF1:CDF2; ABF1:F2DC, ABF1DC:F2DC, wherein A is a clotting factor, an activatable clotting factor or an activated clotting factor, B is absent or is a linker, C is a targeting moiety, D is absent or is a linker, and F1 and F2 are each an Fc moiety, and : represents dimerization mediated by the F1 and F2 chains of two polypeptide chains.

[0061] In still another aspect, the invention pertains to an chimeric clotting factor which comprises a light chain moiety and a heavy chain moiety of a clotting factor, and at least one targeting moiety, wherein said targeting moiety specifically binds to platelets, wherein the chimeric clotting factor comprises a disulfide linked Fc region which comprises two polypeptide chains.

BRIEF DESCRIPTION OF THE DRAWINGS

[0062] Figure 1 illustrates exemplary chimeric clotting factor constructs comprising a targeting domain. These exemplary constructs comprise an Fc region.

[0063] Figure 2 illustrates exemplary chimeric clotting factor constructs comprising a targeting domain. These exemplary constructs comprise a cleavable single chain Fc (cscFc) in which the scFc linker is processed by a cell in which it is expressed to form a two chain Fc region.

[0064] Figure 3 illustrates exemplary chimeric clotting factor constructs which comprise a targeting domain and wherein the clotting factor moiety lacks a Gla domain. These exemplary constructs comprise an Fc region.

- 5 [0065] Figure 4 illustrates exemplary chimeric clotting factor constructs which comprise a targeting domain and wherein the clotting factor moiety lacks a Gla domain. These exemplary constructs comprise a cleavable single chain Fc (cscFc) in which the scFc linker is processed by a cell in which it is expressed to form a two chain Fc region.
- 10 [0066] Figure 5 illustrates exemplary chimeric clotting factor constructs which are activated (e.g., after activation in vitro or by separate expression of the light and heavy chains of the clotting factor) or which are activatable, i.e., comprise a moiety which is cleavable in vivo at the site of a clot (see panels D and E). These exemplary constructs comprise an Fc region. Constructs A, B, and C did not express well in
- 15 early experiments.
- [0067] Figure 6 illustrates exemplary chimeric clotting factor constructs which are activated (e.g., after activation in vitro or by separate expression of the light and heavy chains of the clotting factor) or which are activatable, i.e., comprise a moiety which is cleavable in vivo at the site of a clot. These exemplary constructs comprise a
- 20 cleavable single chain Fc (cscFc) in which the scFc linker is processed by a cell in which it is expressed to form a two chain Fc region.
- [0068] Figure 7 illustrates exemplary chimeric clotting factor constructs comprising a targeting domain.
- [0069] Figure 8 illustrates exemplary chimeric clotting factor constructs which
- 25 comprise a targeting domain and wherein the clotting factor moiety lacks a Gla domain.
- [0070] Figure 9 illustrates exemplary chimeric clotting factor constructs which are activated (e.g., after activation in vitro or by separate expression of the light and heavy chains of the clotting factor) or which are activatable, i.e., comprise a moiety
- 30 which is cleavable in vivo at the site of a clot.
- [0071] Figure 10 shows SDS PAGE for purification and activation of FVII-011.
- [0072] Figure 11 shows SDS PAGE for purification of active FVII-053.
- [0073] Figure 12 shows schematics of FVII-011 and FVII-102 and shows binding of FVIII-011 and FVII-027 to activated platelets determined by FACS.
- 35 [0074] Figure 13 shows thrombin generation assay to measure activity of FVII-027, FVII-011 and Novoseven in the presence of activated platelets.
- [0075] Figure 14 shows that PAC-1 eliminates increased binding to platelets and increased rates of thrombin generation associated with FVII-027.

- 5 [0076] Figure 15 shows constructs used in a thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets.
- [0077] Figure 16 shows a thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets.
- [0078] Figure 17 shows the constructs used in the thrombin generation assay to
- 10 measure activity of FVII-044, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets shown in Figure 18.
- [0079] Figure 18 shows a thrombin generation assay to measure activity of FVII-044, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets.
- [0080] Figure 19 shows the constructs used in the thrombin generation assay to
- 15 measure activity of FVII-047, FVII-048, FVII-049, FVII-011 and Novoseven in the presence of activated platelets shown in Figure 20.
- [0081] Figure 20 shows a thrombin generation assay to measure activity of FVII-047, FVII-048, FVII-049, FVII-011 and Novoseven in the presence of activated platelets.
- [0082] Figure 21 shows the construct used in the thrombin generation assay to
- 20 measure activity of FVII-053 and FVII-011 in the presence of activated platelets shown in Figure 22.
- [0083] Figure 22 shows a thrombin generation assay to measure activity of FVII-053 and FVII-011 in the presence of activated platelets
- [0084] Figure 23 shows that PAC-1 eliminates increased rate of thrombin generation
- 25 associated with FVII-053
- [0085] Figure 24 shows the constructs used in the Western blot analysis of FVIIIFc species following transient transfection of HEK 293 cells and protein A pulldown shown in Figure 25.
- [0086] Figure 25 shows Western blot analysis of FVIIIFc species following transient
- 30 transfection of HEK 293 cells and protein A pulldown.
- [0087] Figure 26 shows Western blot of protein A immunoprecipitation following transient transfection of pSYN-FVII-024 with or without pSYN-PC5-003. Lane 1, pSYN-FVII-024, non reducing; lane 2, pSYN-FVII-024, non reducing; lane 3, pSYN-FVII-024, reducing; lane 4, pSYN-FVII-024, reducing.
- 35 [0088] Figure 27 shows Western blot analysis (Fc western) of FVIIIFc species following transient transfection of HEK 293 cells and protein A pulldown.
- [0089] Figure 28 shows FVII-039 and FVII-040 treatment by FXIa.

- 5 [0090] Figure 29 shows that an FVIIaFc variant targeted to active form of GPIIb/IIIa shows an increased rate of thrombin generation.
- [0091] Figure 30 shows a Rotation Thromboelastometry (ROTEM) assay to measure the activity of FVII-088 and wild type recombinant FVIIaFc in hemophilia A human blood. Clotting Time, Clot Forming Time and Alpha Angle parameters are shown.
- 10 [0092] Figure 31 shows exemplary cleavage sites and illustrative positions of such cleavage sites in activatable clotting factor constructs. In this Figure FVII is used as an example.
- [0093] Figure 32 shows cleavage of the constructs illustrated in Figure 31.
- [0094] Figure 33 shows additional activatable constructs and a Western blot
- 15 illustrating their cleavage.
- [0095] Figure 34 shows the results of a thrombin generation assay using the FVII-062 and -090 constructs. FVII-062 is a negative control which lacks a thrombin cleavage site, so the construct cannot be activated. FVII-090 contains the ALRPR cleavage site and so is activatable by thrombin.
- 20 [0096] Figure 35 illustrates the cleavage of high specific activity FVII variants. FVII heavy chain-Fc and light chain Fc collapse in 1 band because the heavy chain loses a glycosylation site after insertion of the trypsin 170 loop and becomes smaller.
- [0097] Figure 36 illustrates the results of a thrombin generation assay using FVII-090 and FVII-100.
- 25 [0098] Figure 37 illustrates the results of a thrombin generation assay using FVII-090 and FVII-115.
- [0099] Figure 38 illustrates amidolytic activity of activatable FVIIaFc activated with thrombin. Amidolytic activity of the activatable variants can be measured following thrombin activation and there is increased amidolytic activity for the high specific
- 30 activity variants as compared to FVII-090. In these assays, after activation of the activatable molecule by thrombin, hirudin is added to inhibit thrombin cleavage of the chromogenic substrate. In this manner, the thrombin does not interfere with the ability to detect FVIIa activity.
- [0100] Figure 39 illustrates the results of an assay measuring activation of FX
- 35 by FVIIa using substrate S2765, which is not cleaved by FVIIa. In this assay, 10 μ M of FX was incubated with FVIIaFc for 15 minutes at 37°C. The reaction was quenched with EDTA and substrate was added. Figure 39 shows the results of the

5 control experiment which demonstrates that FX activation by FVIIaFc can be detected.

[00101] Figure 40 shows FXa generation activity by “activatable FVIIc.” The experiment shown in Figure 40 shows that there is an increase in FX activation activity for the high specific activity variants. In this experiment, FVIIc (100 nM) was activated with thrombin (100 nM). Hirudin was added to inhibit the thrombin. FX (10uM) was added, followed by EDTA to inhibit the reaction. The activity of FX was measured by detecting the FXa substrate.

[00102] Figure 41 illustrates exemplary activatable construct formats, including an activatable monomer structure used in FVII-118, FVII-119, and FVII-127.

15 [00103] Figure 42 illustrates the efficiency of thrombin cleavage of activatable constructs, specifically monomeric (FVII-118 and -119) as compared to the heterodimeric (FVII-090).

[00104] Figure 43 illustrates the results of a thrombin generation assay to compare wild type activatable FVIIc (FVII-118) to the high specific activity variant (FVII-127).

20 [00105] Figure 44A illustrates several targeted constructs. In this instance, an SCE5 scFv which binds to the active conformation of GPIIb/IIIa was included at various sites in the construct. Figure 44B illustrates the results of thrombin generation assays in platelet-rich FVIII-deficient plasma using these constructs. N7 is the Novoseven control. Figure 44C illustrates the binding of recombinant FVIIaFc variants to platelets by FACS.

[00106] Figure 45A illustrates several targeted FVIIa constructs which include AP3, an scFv against GPIIb/IIIa present on resting and activated platelets. Figure 45B shows the results of thrombin generation assays in platelet-rich FVIII-deficient plasma. Figure 45C shows the results of binding of rFVIIaFc variants to platelets by FACS.

30 [00107] Figure 46A shows several targeted FVIIa constructs that target GPIIb-alpha using peptides that bind to that molecule, specifically, the PS4, OS1, and OS2 peptides. Figure 46B shows the results of thrombin generation assays in platelet-rich FVIII-deficient plasma using the C terminal peptide constructs shown in Figure 46A.

[00108] Figure 47A shows the results of thrombin generation assays in platelet-rich FVIII-deficient plasma using the N terminal peptide constructs shown in Figure 46A. Figure 47B shows a direct comparison of FVII-045 and FVII-048.

5 [00109] Figure 48 shows the binding of FVII-045 and FVII-048 and wild type FVIIaFc to platelets as determined by FACS. The figure also shows the affinity of the targeting peptides as reported in Bernard et al. Biochemistry 2008, 47:4674-4682.

[00110] Figure 49A shows an exemplary targeted FVIII construct. Figure 49B shows the results of a thrombin generation assay in FVIII deficient platelet-rich
10 plasma. In this experiment, the assay was activated with tissue factor (top panel) or by platelet activation (bottom panel).

[00111] Figure 50 shows results of an experiment measuring half life of a targeted FVII construct comprising a gla domain (FVII-011) and lacking a gla domain (FVII-028).

15 [00112] Figure 51A shows several FIX construct comprising targeting moieties, in this case SCE5 scFv. Figure 51B shows the results of thrombin generation assays in platelet-rich FIX-deficient plasma using the constructs of Figure 51A. Figure 51C illustrates that both FIX-068 and FIX-088 have at least 4 times more activity than FIX-042 as measured by Thrombin generation.

20 [00113] Figure 52A shows the results of a thrombin generation assay comparing FIX-090 and Benefix. Figure 52B shows that the activity of FIX-090 is almost 4 times that of Benefix.

[00114] Figure 53A shows a targeted FIX construct comprising a peptide that binds to GPIb, present on resting and activated platelets. Figure 53 B shows the
25 results of thrombin generation assays in platelet-rich FIX deficient plasma. Figure 53C demonstrates that FIX-089 is roughly 4-times stronger than FIX-042 as measured by thrombin generation, while having a lower specific activity.

DETAILED DESCRIPTION OF THE INVENTION

30 [00115] The present invention relates to chimeric clotting factors. The present invention is based, at least in part, on the development of novel ways to enhance the efficacy, pharmacokinetic properties, and/or manufacturability of clotting factors. In one embodiment, improved clotting factors of the invention have increased activity where needed, e.g., by targeting the clotting factor to platelets or by being present in a
35 subject in an activatable form (a non-naturally occurring activatable form) that is activated at the site of clot formation. This can be accomplished, e.g., by targeting the clotting factors or by making them in an activatable form.

5 [00116] In one embodiment, the subject clotting factors are targeted to the site of coagulation. By incorporating a targeting moiety which targets the clotting factor to resting or activated platelets, the activity of a clotting factor can be enhanced. For example, in the case of factor VII, unlike endogenous FVII that is likely activated by tissue factor (TF) at endothelial cell surfaces to generate activated factor X (FXa),
10 exogenous FVIIa likely generates FXa/FIXa in a TF independent manner, most effective at the surface of activated platelets where other clotting factors are localized. However, physiologically FVIIa acts at the surface of a TF-bearing cell, such as an endothelial cell, and has low affinity for platelets. It has been hypothesized that therapeutic recombinant FVIIa acts by converting Factor X into Factor Xa on the
15 surface of activated platelets. To overcome low platelet affinity and be effective at treating bleeds, recombinant FVIIa is dosed at supra-physiological levels. Therefore, in the case of FVIIa, targeting to platelet surfaces could significantly increase the efficacy of this molecule. Although other clotting factors (e.g. FIX, FVIII, FX) have higher affinity to platelets, these too may exhibit enhanced activity by incorporating
20 platelet targeting moieties. In addition, FVIIa has a relatively short half-life (~2.3 hours) in humans. This short half-life likely contributes to the need to dose recombinant FVIIa multiple times to control a bleed. Thus, targeting clotting factors, and in particular FVIIa, to platelets improves efficiency.

[00117] The targeting moiety can be positioned at a number of places in a
25 chimeric clotting factor. Exemplary structures of targeted chimeric clotting factors are set forth, e.g., in Figures 1-4, 7, 8, 17, 19, 21, 44, 46, 49, 51, and 53.

[00118] In another embodiment, a chimeric clotting factor of the invention is made in a form that is activatable at the site of coagulation. For use in bypass therapy exogenous clotting factors are only efficacious when given in the activated form.
30 However, such activated clotting factors are rapidly inactivated by endogenous pathways (e.g. antithrombin III, TFPI), leading to clearance of the active form and a short effective half life. Giving higher doses does not solve this problem as it can result in thrombogenic effects. Thus, in one embodiment, the invention pertains to an "activatable" chimeric clotting factor constructs which comprise a heterologous
35 enzymatic cleavage site not normally present in the clotting factor. These molecules circulate as enhanced zymogens and have a longer half life due to the lack of inactivation upon dosing, but can readily be activated at the site of clotting by cleavage by an enzyme. In one embodiment, such a heterologous enzymatic cleavage

5 site is one for an enzyme produced during the clotting cascade. For example, in one embodiment, the heterologous cleavage site of an activatable construct comprises a Factor XIa, Xa, or thrombin cleavage site. Exemplary FXIa cleavage sites include, e.g.,: TQSFNDFTR and SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g.,: DFLAEGGGVR, TTKIKPR, and ALRPR. In one embodiment, a
10 heterologous cleavage site is interposed between the light and heavy chains of the clotting factor. In another embodiment, a heterologous cleavage site is not interposed between the two chains of the clotting factor. In one embodiment, the heterologous cleavage site is amino terminal to the heavy chain of the clotting factor.

[00119] The heterologous cleavage site is present in a cleavable linker can be
15 positioned at a number of places in a chimeric clotting factor. Exemplary structures of activatable chimeric clotting factors are set forth, e.g., in Figures 5, 6, 9, 29, 27, 31, and 41. Exemplary such constructs are activated in the presence of clot formation and are described in more detail below.

[00120] In one embodiment, a chimeric clotting factor of the invention
20 comprises a scaffold, e.g., to enhance the hydrodynamic radius of the molecule. For example, a chimeric clotting factor of the invention may be a fusion protein. Exemplary scaffolds include, e.g., FcRn binding moieties (e.g., complete Fc regions or portions thereof which bind to FcRn), single chain Fc regions (ScFc regions, e.g., as described in US 2008/0260738, WO 2008/012543, or WO 2008/1439545),
25 cleaveable scFc regions (comprising a cscFc regions as described herein), less complicated proteins or portions thereof, e.g., XTen polypeptides[®], or albumin.

[00121] In one embodiment, a chimeric clotting factor of the invention employs an Fc region or an FcRn binding portion thereof as a scaffold moiety. In one embodiment, the Fc moiety to which the chimeric clotting factor is fused is a naturally
30 occurring (or wild type (WT)) Fc moiety. In another embodiment, the Fc moiety comprises one or more variations in sequence.

[00122] In another embodiment, the Fc moiety is a scFc moiety (e.g., comprising a non-cleavable or a cscFc linker). In a construct comprising a cscFc linker an unprocessed molecule comprises a cleavable single chain Fc region in which
35 the component Fc moieties are genetically-fused in a single polypeptide chain forming a functional, single chain, dimeric Fc region. The cscFc linker can link the Fc moieties that will comprise the dimeric Fc region of the polypeptide in tandem or may link one Fc moiety to a non-Fc moiety of the construct, e.g., a clotting factor or

5 targeting moiety, which is, in turn, linked to a second Fc moiety. The cscFc linker is interposed between the Fc moieties that comprise the scFc region and is flanked by at least one enzymatic cleavage site, e.g., an intracellular enzymatic processing sites. In one embodiment, the scFc linker is flanked by two enzymatic cleavage sites resulting in the excision of the linker (e.g., all or substantially all of the linker) when the protein
10 encoded by the nucleic acid molecule is processed in a cell. In another embodiment, the scFc linker is adjacent to at least one enzymatic cleavage site that allows for excision of the linker in vitro after the polypeptide has been secreted by a cell or comprises at least one enzymatic cleavage site that allows for cleavage of the linker in vivo after the construct is administered to a subject. Thus, in one embodiment,
15 although the such a polypeptide comprises scFc region(s) encoded in a single open reading frame (ORF) as part of one contiguous nucleotide sequence in unprocessed form, the cscFc linker is enzymatically cleaved (e.g., prior to administration or in vivo after administration), resulting in a polypeptide which is a heterodimeric molecule comprising an Fc region which is not fused in a single amino acid chain, i.e., the
20 resulting processed construct has a Fc region which comprises two polypeptide chains. In such embodiments, all or substantially all of the linker is excised, while in some embodiments, a portion of the cleavage site may remain, e.g., four arginines of the RRRR cleavage site.

[00123] In one embodiment, the scFc linker is flanked by two processing sites
25 for cleavage. The two processing sites can be the same or different. In one embodiment, at least one processing site is a cluster of basic amino acid residues as recognized by arginine kex2/furin enzymes. Such enzymes cleave immediately C-terminal to an arginine residue. In another embodiment, at least one cleavage site is one that may be cleaved in vivo, for example a cleavage site recognized by thrombin.

30 [00124] In one embodiment, a chimeric clotting factor of the invention is manufactured in an activated form in the context of an scFc molecule comprising a cscFc linker. For example, Factor VII, is generally produced recombinantly as a zymogen, and requires activation during manufacturing to produce the active form for administration. In one embodiment, a chimeric clotting factor of the invention is
35 secreted from the cell in which it is expressed in active form to improve manufacturability. As is set forth in more detail below, such clotting factors can be produced by incorporating a single chain Fc region into the molecule. Single chain Fc regions are formed by dimerization of Fc moieties which are present in a single

5 polypeptide chain. In one embodiment, such a construct comprises an scFc polypeptide linker linking the two Fc moieties of the scFc which is adjacent to at least one intracellular processing site. Cleavage of such a construct is delayed until late in the secretory pathway, e.g., when the protein colocalizes with active processing enzymes in the trans-Golgi apparatus.

10 [00125] In one embodiment, a cell expressing a construct encoding a polypeptide of the invention endogenously expresses an enzyme which cleaves the scFc linker at one or more processing sites resulting in a dimeric molecule comprising two polypeptide chains. In another embodiment, a cell expressing a construct encoding a polypeptide of the invention exogenously expresses an enzyme which
15 cleaves the scFc linker at one or more processing sites.

[00126] In one embodiment, a chimeric clotting factor of the invention can combine two or more of these features to create an optimized construct e.g. targeting an activatable fusion protein construct to resting platelets, such that it can be activated efficiently as well as at a higher local concentration at the site of active coagulation.

20 Exemplary such combination constructs include chimeric clotting factors that are both targeted and comprise an scFc linker for enhanced processing. In another embodiment, a construct of the invention is targeted and activatable.

[00127] Exemplary constructs of the invention are illustrated in the accompanying Figures and sequence listing. In one embodiment, the invention
25 pertains to a polypeptide having the structure as set forth in the Figures. In another embodiment, the invention pertains to a polypeptide having the sequence set forth in the accompanying sequence listing or the the nucleic acid molecule encoding such polypeptides. In one embodiment, the invention pertains to a mature form of a polypeptide having the sequence set forth in the accompanying sequence listing. It
30 will be understood that these constructs and nucleic acid molecules encoding them can be used to improve hemostasis in a subject.

[00128] In order to provide a clear understanding of the specification and claims, the following definitions are provided below.

35 I. Definitions

[00129] As used herein, the term "protein" or "polypeptide" refers to a polymer of two or more of the natural amino acids or non-natural amino acids.

5 **[00130]** The term "amino acid" includes alanine (Ala or A); arginine (Arg or R); asparagine (Asn or N); aspartic acid (Asp or D); cysteine (Cys or C); glutamine (Gln or Q); glutamic acid (Glu or E); glycine (Gly or G); histidine (His or H); isoleucine (Ile or I); leucine (Leu or L); lysine (Lys or K); methionine (Met or M); phenylalanine (Phe or F); proline (Pro or P); serine (Ser or S); threonine (Thr or T);

10 tryptophan (Trp or W); tyrosine (Tyr or Y); and valine (Val or V). Non-traditional amino acids are also within the scope of the invention and include norleucine, omithine, norvaline, homoserine, and other amino acid residue analogues such as those described in Ellman *et al.* Meth. Enzym. 202:301-336 (1991). To generate such non-naturally occurring amino acid residues, the procedures of Noren *et al.* Science

15 244:182 (1989) and Ellman *et al.*, *supra*, can be used. Briefly, these procedures involve chemically activating a suppressor tRNA with a non-naturally occurring amino acid residue followed by in vitro transcription and translation of the RNA. Introduction of the non-traditional amino acid can also be achieved using peptide chemistries known in the art. As used herein, the term "polar amino acid" includes

20 amino acids that have net zero charge, but have non-zero partial charges in different portions of their side chains (e.g. M, F, W, S, Y, N, Q, C). These amino acids can participate in hydrophobic interactions and electrostatic interactions. As used herein, the term "charged amino acid" include amino acids that can have non-zero net charge on their side chains (e.g. R, K, H, E, D). These amino acids can participate in

25 hydrophobic interactions and electrostatic interactions.

[00131] An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence (an amino acid sequence of a starting polypeptide) with a second, different "replacement" amino acid residue. An "amino acid insertion" refers to the incorporation of at least one

30 additional amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present larger "peptide insertions", can be made, e.g. insertion of about three to about five or even up to about ten, fifteen, or twenty amino acid residues. The inserted residue(s) may be naturally occurring or non-naturally occurring as disclosed above. An "amino

35 acid deletion" refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

[00132] Polypeptides may be either monomers or multimers. For example, in one embodiment, a protein of the invention is a dimer. A dimeric polypeptide of the

5 invention may comprise two polypeptide chains or may consist of one polypeptide chain (e.g., in the case of an scFc molecule). In one embodiment, the dimers of the invention are homodimers, comprising two identical monomeric subunits or polypeptides (e.g., two identical Fc moieties or two identical biologically active moieties). In another embodiment, the dimers of the invention are heterodimers,
10 comprising two non-identical monomeric subunits or polypeptides (e.g., comprising two different clotting factors or portions thereof or one clotting factor only). See, e.g., U.S. patent 7404956, incorporated herein by reference.

[00133] As used herein, the term “polypeptide linkers” refers to a peptide or polypeptide sequence (e.g., a synthetic peptide or polypeptide sequence) which
15 connects two domains in a linear amino acid sequence of a polypeptide chain. In one embodiment, the the polypeptides of invention are encoded by nucleic acid molecules that encode polypeptide linkers which either directly or indirectly connect the two Fc moieties which make up the construct. These linkers are referred to herein as “scFc linkers” and the scFc linker is interposed between the two Fc moieties of a
20 polypeptide which comprises it. If the scFc linker connects two Fc moieties contiguously in the linear polypeptide sequence, it is a “direct” linkage. In contrast, the scFc linkers may link the first Fc moiety to a binding moiety which is, in turn, linked to the second Fc moiety, thereby forming an indirect linkage. These scFc linkers permit the formation of a single chain genetic construct. In one embodiment,
25 the polypeptides also comprise enzymatic cleavage sites which result in the scFc linker being cleavable (a cscFc linker) and, in one embodiment, substantially excised (e.g., during processing by a cell). Thus, the resulting processed polypeptide is a dimeric molecule comprising at least two amino acid chains and substantially lacking extraneous linker amino acid sequences. In some embodiments, all or substantially all
30 of the linker is excised, while in some embodiments, a portion of the cleavage site may remain, e.g., four arginines of the RRRR cleavage site.

[00134] In another embodiment, another type of polypeptide linker, herein referred to as a “spacer” may be used to connect different moieties, e.g., a clotting factor or targeting moiety to an Fc moiety on the polypeptide. This type of linker may
35 provide flexibility to the polypeptide molecule. Spacers are not typically cleaved; however in certain embodiments, such cleavage may be desirable. Exemplary positions of spacers are shown in the accompanying drawings. Spacers can be located between the clotting factors, targeting moieties, and/or scaffolds, e.g., at the N or C

5 terminus of these moieties. In one embodiment, these linkers are not removed during processing.

[00135] A third type of linker which may be present in a chimeric clotting factor of the invention is herein referred to as a "cleavable linker" which comprises a heterologous cleavage site (e.g., a factor XIa, Xa, or thrombin cleavage site) and
10 which may include additional spacer linkers on either the N terminal of C terminal or both sides of the cleavage site. Exemplary locations for such sites are shown in the accompanying drawings and include, e.g., placement adjacent to targeting moieties. In another embodiment, such linkers may be adjacent to a clotting factor or portion thereof. For example, in one embodiment, a cleavable linker may be fused to the N
15 terminus of the heavy chain of a clotting factor to make an activatable form of the clotting factor. In such cases, the cleavable linker may include additional spacer linkers at the N terminus of the cleavage site, but requires direct fusion at the C-terminus of the cleavage site to the amino terminus of the heavy chain of the clotting factor.

20 [00136] As used herein, the term "gly-ser polypeptide linker" refers to a peptide that consists of glycine and serine residues. An exemplary gly/ser polypeptide linker comprises the amino acid sequence $(\text{Gly}_4 \text{Ser})_n$ (SEQ ID NO:4). Another exemplary gly/ser polypeptide linker comprises the amino acid sequence $\text{S}(\text{Gly}_4 \text{Ser})_n$.

25 [00137] In one embodiment, $n=1$. In one embodiment, $n=2$. In another embodiment, $n=3$, i.e., $(\text{Gly}_4 \text{Ser})_3$. In another embodiment, $n=4$, i.e., $(\text{Gly}_4 \text{Ser})_4$ (SEQ ID NO:6). In another embodiment, $n=5$. In yet another embodiment, $n=6$. In another embodiment, $n=7$. In yet another embodiment, $n=8$. In another embodiment, $n=9$. In yet another embodiment, $n=10$. Another exemplary gly/ser polypeptide
30 linker comprises the amino acid sequence $\text{Ser}(\text{Gly}_4 \text{Ser})_n$ (SEQ ID NO:26). In one embodiment, $n=1$. In one embodiment, $n=2$. In a preferred embodiment, $n=3$. In another embodiment, $n=4$. In another embodiment, $n=5$. In yet another embodiment, $n=6$.

[00138] A polypeptide or amino acid sequence "derived from" a designated
35 polypeptide or protein refers to the origin of the polypeptide. Preferably, the polypeptide or amino acid sequence which is derived from a particular sequence has an amino acid sequence that is essentially identical to that sequence or a portion thereof, wherein the portion consists of at least 10-20 amino acids, preferably at least

5 20-30 amino acids, more preferably at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the sequence.

[00139] Polypeptides derived from another peptide may have one or more mutations relative to the starting polypeptide, e.g., one or more amino acid residues which have been substituted with another amino acid residue or which has one or
10 more amino acid residue insertions or deletions. Preferably, the polypeptide comprises an amino acid sequence which is not naturally occurring. Such variants necessarily have less than 100% sequence identity or similarity with the starting antibody. In a preferred embodiment, the variant will have an amino acid sequence from about 75% to less than 100% amino acid sequence identity or similarity with the
15 amino acid sequence of the starting polypeptide, more preferably from about 80% to less than 100%, more preferably from about 85% to less than 100%, more preferably from about 90% to less than 100% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) and most preferably from about 95% to less than 100%, e.g., over the length of the variant molecule. In one embodiment, there is one amino acid difference
20 between a starting polypeptide sequence and the sequence derived therefrom. Identity or similarity with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (*i.e.* same residue) with the starting amino acid residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

25 [00140] Preferred polypeptides of the invention comprise an amino acid sequence (e.g., at least one clotting factor or Fc moiety or domain) derived from a human protein sequence. However, polypeptides may comprise one or more amino acids from another mammalian species. For example, a clotting factor, Fc domain, or targeting moiety may be derived from a non-human species and included in the
30 subject polypeptides. Alternatively, one or more amino acids may be present in a polypeptide which are derived from a non-human species. Preferred polypeptides of the invention are not immunogenic.

[00141] It will also be understood by one of ordinary skill in the art that the polypeptides of the invention may be altered such that they vary in amino acid
35 sequence from the naturally occurring or native polypeptides from which they were derived, while retaining the desirable activity of the native polypeptides. For example, nucleotide or amino acid substitutions leading to conservative substitutions or changes at "non-essential" amino acid residues may be made. An isolated nucleic

- 5 acid molecule encoding a non-natural variant of a polypeptide derived from an immunoglobulin (e.g., an Fc domain, moiety, or antigen binding site) can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the immunoglobulin such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.
- 10 Mutations may be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

[00142] The polypeptides of the invention may comprise conservative amino acid substitutions at one or more amino acid residues, e.g., at essential or non-essential amino acid residues. 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, a nonessential amino acid residue in a polypeptide may be replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members. Alternatively, in another embodiment, mutations may be introduced randomly along all or part of a coding sequence, such as by saturation mutagenesis, and the resultant mutants can be incorporated into polypeptides of the invention and screened for their ability to bind to the desired target.

[00143] In the context of polypeptides, a "linear sequence" or a "sequence" is the order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.

35 [00144] As used herein, the terms "linked," "fused", or "fusion" refer to linkage via a peptide bonds (e.g., genetic fusion), chemical conjugation or other means. For example, one way in which molecules or moieties can be linked employs polypeptide linkers which link the molecules or moieties via peptide bonds. The terms

5 "genetically fused," "genetically linked" or "genetic fusion" are used interchangeably and refer to the co-linear, covalent linkage or attachment of two or more proteins, polypeptides, or fragments thereof via their individual peptide backbones, through genetic expression of a single polynucleotide molecule encoding those proteins, polypeptides, or fragments. Such genetic fusion results in the expression of a single
10 contiguous genetic sequence. Preferred genetic fusions are in frame, i.e., two or more open reading frames (ORFs) are fused to form a continuous longer ORF, in a manner that maintains the correct reading frame of the original ORFs. Thus, the resulting recombinant fusion protein is a single polypeptide containing two or more protein segments that correspond to polypeptides encoded by the original ORFs (which
15 segments are not normally so joined in nature). In this case, the single polypeptide is cleaved during processing to yield dimeric molecules comprising two polypeptide chains.

[00145] As used herein, the term "Fc region" is defined as the portion of a polypeptide which corresponds to the Fc region of native immunoglobulin, i.e., as
20 formed by the dimeric association of the respective Fc domains of its two heavy chains. A native Fc region is homodimeric and comprises two polypeptide chains. 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
25 contiguous genetic sequence).

[00146] As used herein, the term "Fc domain" refers to the portion of a single immunoglobulin 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,
30 a complete Fc domain comprises at least a hinge domain, a CH2 domain, and a CH3 domain.

[00147] As used herein, the term "Fc domain portion" or "Fc moiety" includes an amino acid sequence of an Fc domain or derived from an Fc domain. In certain embodiments, an Fc moiety comprises at least one of: a hinge (e.g., upper, middle,
35 and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, or a variant, portion, or fragment thereof. In other embodiments, an Fc moiety comprises a complete Fc domain (i.e., a hinge domain, a CH2 domain, and a CH3 domain). In one embodiment, a Fc moiety comprises a hinge domain (or portion

5 thereof) fused to a CH3 domain (or portion thereof). In another embodiment, an Fc moiety comprises a CH2 domain (or portion thereof) fused to a CH3 domain (or portion thereof). In another embodiment, an Fc moiety consists of a CH3 domain or portion thereof. In another embodiment, an Fc moiety consists of a hinge domain (or portion thereof) and a CH3 domain (or portion thereof). In another embodiment, a Fc moiety consists of a CH2 domain (or portion thereof) and a CH3 domain. In another embodiment, a Fc moiety consists of a hinge domain (or portion thereof) and a CH2 domain (or portion thereof). In one embodiment, an Fc moiety lacks at least a portion of a CH2 domain (e.g., all or part of a CH2 domain).

[00148] 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. Therefore, in a preferred embodiment, 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.

[00149] As used herein the term “moiety” refers to a component part or constituent of a chimeric polypeptide.

[00150] As used herein, the term “targeting moiety” refers to a molecule, fragment thereof or a component of a polypeptide which localizes or directs the polypeptides of the invention to a desired site or cell. In one embodiment, a construct of the invention comprises a “targeting moiety” which enhances the activity of the polypeptide, e.g., by localizing the molecule to a desired site. Such a moiety may be, e.g., an antibody or variant thereof (e.g., and scFv) or a peptide. In another embodiment, such a targeting moiety may be a polypeptide, a receptor binding portion of a ligand, or a ligand binding portion of a receptor which is linked to a polypeptide of the invention and binds to the desired target, e.g., on a cell or tissue. The targeting moiety may be genetically fused to a construct, chemically conjugated to the construct

5 or linked to the construct via a spacer. For example, targeting moieties may be attached to a construct of the invention by formation of a bond between the targeting moiety and an Fc moiety of a construct, where the targeting moiety comprises a first functional group and the Fc moiety comprises a second functional group, and where the first and second functional groups are capable of reacting with each other to form
10 a chemical bond (see, e.g., U.S. patent 7381408). In one embodiment, a targeting moiety binds to platelets. Exemplary targeting moieties are described in more detail below.

[00151] In one embodiment a targeting moiety for use in a construct of the invention comprises an antibody variant. The term “antibody variant” or “modified
15 antibody” includes an antibody which does not occur in nature and which has an amino acid sequence or amino acid side chain chemistry which differs from that of a naturally-derived antibody by at least one amino acid or amino acid modification as described herein. As used herein, the term “antibody variant” includes synthetic forms of antibodies which are altered such that they are not naturally occurring, e.g.,
20 antibodies that comprise at least two heavy chain portions but not two complete heavy chains (such as, domain deleted antibodies or minibodies); multispecific forms of antibodies (e.g., bispecific, trispecific, etc.) altered to bind to two or more different antigens or to different epitopes on a single antigen); heavy chain molecules joined to scFv molecules; single-chain antibodies; diabodies; triabodies; and antibodies with
25 altered effector function and the like.

[00152] As used herein the term “scFv molecule” includes binding molecules which consist of one light chain variable domain (VL) or portion thereof, and one heavy chain variable domain (VH) or portion thereof, wherein each variable domain (or portion thereof) is derived from the same or different antibodies. scFv molecules
30 preferably comprise an scFv linker interposed between the VH domain and the VL domain. ScFv molecules are known in the art and are described, e.g., in US patent 5,892,019, Ho et al. 1989. *Gene* 77:51; Bird et al. 1988 *Science* 242:423; Pantoliano et al. 1991. *Biochemistry* 30:10117; Milenic et al. 1991. *Cancer Research* 51:6363; Takkinen et al. 1991. *Protein Engineering* 4:837.

35 [00153] A “scFv linker” as used herein refers to a moiety interposed between the VL and VH domains of the scFv. scFv linkers preferably maintain the scFv molecule in a antigen binding conformation. In one embodiment, a scFv linker comprises or consists of an scFv linker peptide. In certain embodiments, a scFv linker

5 peptide comprises or consists of a gly-ser polypeptide linker. In other embodiments, a scFv linker comprises a disulfide bond.

[00154] The term “glycosylation” refers to the covalent linking of one or more carbohydrates to a polypeptide. Typically, glycosylation is a posttranslational event which can occur within the intracellular milieu of a cell or extract therefrom. The
10 term glycosylation includes, for example, N-linked glycosylation (where one or more sugars are linked to an asparagine residue) and/or O-linked glycosylation (where one or more sugars are linked to an amino acid residue having a hydroxyl group (*e.g.*, serine or threonine). In one embodiment, a molecule of the invention is glycosylated. In another embodiment, a molecule of the invention is aglycosylated. In yet another
15 embodiment, a molecule of the invention has reduced glycosylation as compared to that in a wild type Fc region.

[00155] As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally
20 occurring IgG molecules, the CH1 and CL regions are linked by native disulfide bonds and the two heavy chains are linked by two native disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[00156] The term “vector” or “expression vector” is used herein to mean
25 vectors used in accordance with the present invention as a vehicle for introducing into and expressing a desired polynucleotide in a cell. As known to those skilled in the art, such vectors may easily be selected from the group consisting of plasmids, phages, viruses and retroviruses. In general, vectors compatible with the instant invention will comprise a selection marker, appropriate restriction sites to facilitate cloning of the
30 desired gene and the ability to enter and/or replicate in eukaryotic or prokaryotic cells.

[00157] Numerous expression vector systems may be employed to produce the chimeric clotting factors of the invention. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or
35 MOMLV) or SV40 virus. Additionally, cells which have integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics) or resistance to heavy metals

5 such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. In one embodiment, an inducible expression system can be employed. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals. In one embodiment, a secretion signal, e.g., any one of several well characterized bacterial leader peptides (e.g., pelB, phoA, or ompA), can be fused in-frame to the N terminus of a polypeptide of the invention to obtain optimal secretion of the polypeptide. (Lei *et al.* (1988), *Nature*, 331:543; Better *et al.* (1988) *Science*, 240:1041; Mullinax *et al.*, (1990). *PNAS*, 87:8095).

10 [00158] The term "host cell" refers to a cell that has been transformed with a vector constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of proteins from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of protein unless it is clearly specified otherwise. In other words, recovery of protein from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells. The host cell line used for protein expression is most preferably of mammalian origin; those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for the desired gene product to be expressed therein.

20 Exemplary host cell lines include, but are not limited to, DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), PerC6 cells, HAK (hamster kidney line), SP2/O (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte) and 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature. The polypeptides of the invention can also be expressed in non-mammalian cells such as bacteria or yeast or plant cells. In this regard it will be appreciated that various unicellular non-

35 mammalian microorganisms such as bacteria can also be transformed; *i.e.* those capable of being grown in cultures or fermentation. Bacteria, which are susceptible to transformation, include members of the enterobacteriaceae, such as strains of *Escherichia coli* or *Salmonella*; Bacillaceae, such as *Bacillus subtilis*; *Pneumococcus*;

5 Streptococcus, and Haemophilus influenzae. It will further be appreciated that, when expressed in bacteria, the polypeptides typically become part of inclusion bodies. The polypeptides must be isolated, purified and then assembled into functional molecules.

[00159] In addition to prokaryotes, eukaryotic microbes may also be used. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used
10 among eukaryotic microorganisms although a number of other strains are commonly available including *Pichia pastoris*. For expression in *Saccharomyces*, the plasmid YRp7, for example, (Stinchcomb *et al.*, (1979), *Nature*, 282:39; Kingsman *et al.*, (1979), *Gene*, 7:141; Tschemper *et al.*, (1980), *Gene*, 10:157) is commonly used. This plasmid already contains the TRP1 gene which provides a selection marker for a mutant strain of
15 yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, (1977), *Genetics*, 85:12). The presence of the *trp1* lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

[00160] As used herein the term "endogenous" refers to molecules (e.g. nucleic
20 acid and/or protein molecules) that are naturally present in a cell. In contrast, the term "exogenous" or "heterologous" refers to such molecules that are not normally found in a given context, e.g., in a cell or in a polypeptide. For example, an exogenous or heterologous molecule may be introduced into a cell and are only present after manipulation of the cell, e.g., by transfection or other forms of genetic engineering or a
25 heterologous amino acid sequence may be present in a protein in which it is not naturally found.

[00161] 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
30 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 and SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g. DFLAEGGGVR, TTKIKPR, LVPRG SEQ ID NO:35)
35 and ALRPR. Other enzymatic cleavage sites are known in the art.

[00162] As used herein, the term "processing site" or "intracellular processing site" refers to a type of enzymatic cleavage site in a polypeptide which is the target for enzymes that function after translation of the polypeptide. In one embodiment, such

5 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
10 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., furin, PC2, PC1/Pc3, PC4, PACE4, PC5/PC6, and LPC/PC7/PC8/SPC7. Other processing sites are known in the art.

[00163] In constructs that include more than one processing or cleavage site, it
15 will be understood that such sites may be the same or different.

[00164] *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
20 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.

25 [00165] As used herein, the phrase “subject that would benefit from administration of a polypeptide” includes subjects, such as mammalian subjects, that would benefit from administration of polypeptides of the invention, e.g., to improve hemostasis.

[00166] A “chimeric protein” or “fusion protein”, as used herein, refers to any
30 protein comprised of a first amino acid sequence derived from a first source, bonded, covalently or non-covalently, to a second amino acid sequence derived from a second source, wherein the first and second source are not the same. A first source and a second source that are not the same can include two different biological entities, or two different proteins from the same biological entity, or a biological entity and a
35 non-biological entity. A chimeric protein can include for example, a protein derived from at least 2 different biological sources. A biological source can include any non-synthetically produced nucleic acid or amino acid sequence (e.g. a genomic or cDNA sequence, a plasmid or viral vector, a native virion or a mutant or analog, as further

5 described herein, of any of the above). A synthetic source can include a protein or nucleic acid sequence produced chemically and not by a biological system (e.g. solid phase synthesis of amino acid sequences). A chimeric protein can also include a protein derived from at least 2 different synthetic sources or a protein derived from at least one biological source and at least one synthetic source. A chimeric protein may
10 also comprise a first amino acid sequence derived from a first source, covalently or non-covalently linked to a nucleic acid, derived from any source or a small organic or inorganic molecule derived from any source. The chimeric protein may comprise a linker molecule between the first and second amino acid sequence or between the first amino acid sequence and the nucleic acid, or between the first amino acid sequence
15 and the small organic or inorganic molecule.

[00167] As used herein, the term "clotting factor," refers to molecules, or analogs thereof, naturally occurring or recombinantly produced which prevent or decrease the duration of a bleeding episode in a subject. In other words, it means molecules having pro-clotting activity, i.e., are responsible for the conversion of
20 fibrinogen into a mesh of insoluble fibrin causing the blood to coagulate or clot.

[00168] Clotting activity, as used herein, means the ability to participate in a cascade of biochemical reactions that culminates in the formation of a fibrin clot and/or reduces the severity, duration or frequency of hemorrhage or bleeding episode.

[00169] Hemostasis, as used herein, means the stopping or slowing of bleeding
25 or hemorrhage; or the stopping or slowing of blood flow through a blood vessel or body part.

[00170] 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.

30 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), Von Willebrand disease, factor XI deficiency (PTA deficiency), Factor XII deficiency, deficiencies or structural abnormalities in fibrinogen, prothrombin, Factor
35 V, Factor VII, Factor X or factor XIII, Bernard-Soulier syndrome 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

5 failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this may increase bleeding risk.

[00171] 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
10 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, or stem cell transplantation

15 [00172] On-demand treatment 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,
20 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
25 surgery, intrathoracic surgery, or joint replacement surgery.

[00173] 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.

30 [00174] 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, the prophylaxis of one or more symptoms
35 associated with a disease or condition.

II. Clotting Factors

5 [00175] In particular, the invention pertains to improved versions of factors VII, IX, and X. These factors are all structurally related in that in each the amino terminal end of the light chain is not amenable to the incorporation of additional moieties. Similarly, the amino terminal end of the heavy chain of these three clotting factors is not amenable to the incorporation of additional moieties, with the exception
10 of cleaveable moieties, i.e., moieties linked via a cleavage site or moieties which consist of a cleavage site. The chimeric clotting factor constructs of the invention were designed based on these shared properties and it will be understood that although factor VII is often shown to illustrate exemplary embodiments of the invention, the subject constructs may be made using factor VII, IX, or X. For
15 example, one of skill in the art would understand that the FVII portion of a construct of the invention could be substituted with a FVIII, FIX or FX portion to make an enhanced version of one of these clotting factors.

[00176] Exemplary chimeric clotting factor constructs of the invention are set forth in the accompanying Figures. Although the Figures generally illustrate the
20 clotting Factor as a single chain (in its zymogen form) it will be understood that the clotting factor may also be present in its active form in a construct of the invention, e.g. as a two chain, disulfide bonded form.

[00177] In one embodiment, a chimeric clotting factor of the invention is expressed by a cell in active form. In another embodiment, a chimeric clotting factor
25 is expressed in inactive form and is subsequently activated under appropriate conditions in vitro such that the active form of the clotting factor is present in the construct. In another embodiment, a chimeric clotting factor of the invention comprises a clotting factor in inactive form and the clotting factor is activated in vivo after administration.

30 [00178] In one embodiment, an scFc scaffold can be used to produce an active form of a molecule. Certain clotting factors are produced recombinantly as zymogens and, therefore, require activation during manufacturing. Active forms of Factors VII, IX, and X are comprised of dimeric molecules in which the heavy and light chain are linked only by a disulfide bond.

35 [00179] In one embodiment, a chimeric clotting factor is activated prior to administration to a subject to improve hemostasis. Methods for activating clotting factors are known in the art. For example, in one embodiment, a chimeric clotting

5 factor of the invention is contacted with media containing CaCl_2 at a concentration of approximately 5 mM.

[00180] In another embodiment, a chimeric clotting factor of the invention is secreted in active form by a cell in which it is expressed. In one embodiment, an active chimeric clotting factor is made by expressing the heavy and light chain of a
10 clotting factor as separate polypeptides.

[00181] In another embodiment, the N-terminus of the heavy chain of the clotting factor is modified to comprise an intracellular processing site which delays the activation of the clotting factor during synthesis until later in the secretory pathway, (i.e. until protein colocalizes with active processing enzymes in the trans-
15 Golgi network), leading to greater productivity. Exemplary such intracellular processing sites include those recognized by furin. Exemplary cleavage sites for this family of enzymes include an amino acid sequence comprising the motif Arg-Xaa-Lys/Arg-Arg.

[00182] In a preferred embodiment, an active construct of the invention is made
20 in the context of an Fc fusion protein, e.g., using an scFc linker (e.g., a cscfc linker).

[00183] Exemplary constructs are shown in the accompanying figures.

[00184] In one embodiment, the invention pertains to processed (e.g., mature) polypeptides in which the at least one cleavage site adjacent to an scFc polypeptide linker has been cleaved such that the molecule is no longer a single polypeptide chain
25 such that the polypeptide is comprised of at least two polypeptide chains (owing to cleavage at the enzymatic cleavage site(s) P1 and/or P2).

[00185] In one embodiment, such processed polypeptides comprise a clotting factor or portion thereof linked to the second Fc moiety (i.e., the second Fc moiety when counting from the amino terminus to the carboxy terminus prior to cleavage of
30 the polypeptide linker) which has a free amino terminus after cleavage of the polypeptide linker.

[00186] In one embodiment, a clotting factor attached to the N-terminus of the second Fc moiety is catalytically active, e.g., has enzymatic activity. In another embodiment, a clotting factor attached to the N-terminus of the second Fc moiety is
35 secreted by a cell as a zymogen requiring further enzymatic processing of the clotting factor in order to be fully activated.

[00187] In one embodiment, the invention pertains to clotting factors which are secreted from cells in active or activated form without the need for further activation

5 during processing. For example, Factor VII is generally produced recombinantly as a zymogen and requires activation during manufacturing to produce the active form for administration. In one embodiment, a polypeptide of the invention is secreted from the cell in which it is expressed in active form to improve manufacturability. As is set forth in more detail below, such clotting factors can be produced by expressing the
10 light chain of a clotting factor and the heavy chain of a clotting factor separately in the context of an scFc molecule comprising a cscFc linker. Activation of such a construct is delayed until late in the secretory pathway during processing, e.g., when the protein colocalizes with active processing enzymes in the trans-Golgi apparatus.

[00188] In one embodiment, a clotting factor of the invention is a mature form
15 of Factor VII or a variant thereof. Factor VII (FVII, F7; also referred to as Factor 7, coagulation factor VII, serum factor VII, serum prothrombin conversion accelerator, SPCA, proconvertin and eptacog alpha) is a serine protease that is part of the coagulation cascade. FVII includes a Gla domain, two EGF domains (EGF-1 and EGF-2), and a serine protease domain (or peptidase S1 domain) that is highly
20 conserved among all members of the peptidase S1 family of serine proteases, such as for example with chymotrypsin. FVII occurs as a single chain zymogen, an activated zymogen-like two-chain polypeptide and a fully activated two-chain form. As used herein, a "zymogen-like" protein or polypeptide refers to a protein that has been activated by proteolytic cleavage, but still exhibits properties that are associated with
25 a zymogen, such as, for example, low or no activity, or a conformation that resembles the conformation of the zymogen form of the protein. For example, when it is not bound to tissue factor, the two-chain activated form of FVII is a zymogen-like protein; it retains a conformation similar to the uncleaved FVII zymogen, and, thus, exhibits very low activity. Upon binding to tissue factor, the two-chain activated form
30 of FVII undergoes conformational change and acquires its full activity as a coagulation factor.

[00189] Exemplary FVII variants include those with increased specific activity, e.g., mutations that increase the activity of FVII by increasing its enzymatic activity (K_{cat} or K_m). Such variants have been described in the art and include, e.g., mutant
35 forms of the molecule as described for example in Persson et al. 2001. PNAS 98:13583; Petrovan and Ruf. 2001. J. Biol. Chem. 276:6616; Persson et al. 2001 J. Biol. Chem. 276:29195; Soejima et al. 2001. J. Biol. Chem. 276:17229; Soejima et al. 2002. J. Biol. Chem. 247:49027. In one embodiment, a variant form of FVII

5 includes the mutations Exemplary mutations include V158D-E296V-M298Q. In another embodiment, a variant form of FVII includes a replacement of amino acids 608-619 (LQQRKVGDSPN, corresponding to the 170-loop) from the FVII mature sequence with amino acids EASYPGK from the 170-loop of trypsin. High specific activity variants of FIX are also known in the art. For example, Simioni et al. (2009 N.E. Journal of Medicine 361:1671) describe an R338L mutation. Chang et al. (1988 JBC 273:12089) and Pierri et al. (2009 Human Gene Therapy 20:479) describe an R338A mutation. Other mutations are known in the art and include those described, e.g., in Zogg and Brandstetter. 2009 Structure 17:1669; Sichler et al. 2003. J. Biol. Chem. 278:4121; and Sturzebecher et al. 1997. FEBS Lett 412:295. The contents of
10 these references are incorporated herein by reference.

[00190] Full activation, which occurs upon conformational change from a zymogen-like form, occurs upon binding to its co-factor tissue factor. Also, mutations can be introduced that result in the conformation change in the absence of tissue factor. Hence, reference to FVIIa includes both two-chain forms thereof, the
15 zymogen-like form and the fully activated two-chain form.

[00191] In one embodiment, a clotting factor of the invention is a mature form of Factor VIII or a variant thereof. FVIII functions in the intrinsic pathway of blood coagulation as a cofactor to accelerate the activation of factor X by factor IXa, a reaction that occurs on a negatively charged phospholipid surface in the presence of
20 calcium ions. FVIII is synthesized as a 2351 amino acid single-chain polypeptide having the domain structure A1-A2-B-A3-C1-C2. Wehar, G. A. et al., Nature 312:337-342 (1984) and Toole, J. J. et al., Nature 312:342-347 (1984). The domain structure of FVIII is identical to that of the homologous coagulation factor, factor V (FV). Kane, W. H. et al., PNAS (USA) 83:6800-6804 (1986) and Jenny, R. J. et al.,
25 PNAS (USA) 84:4846-4850 (1987). The FVIII A-domains are 330 amino acids and have 40% amino acid identity with each other and to the A-domain of FV and the plasma copper-binding protein ceruloplasmin. Takahashi, N. et al., PNAS (USA) 81:390-394 (1984). Each C-domain is 150 amino acids and exhibits 40% identity to the C-domains of FV, and to proteins that bind glycoconjugates and negatively
30 charged phospholipids. Stubbs, J. D. et al., PNAS (USA) 87:8417-8421 (1990). The FVIII B-domain is encoded by a single exon and exhibits little homology to any known protein including FV B-domain. Gitschier, J. et al., Nature 312:326-330 (1984) and Cripe, L. D. et al., Biochemistry 31:3777-3785 (1992).

5 [00192] FVIII is secreted into plasma as a heterodimer of a heavy chain (domains A1-A2-B) and a light chain (domains A3-C1-C2) associated through a noncovalent divalent metal ion linkage between the A1- and A3-domains. In plasma, FVIII is stabilized by binding to von Willebrand factor. More specifically, the FVIII light chain is bound by noncovalent interactions to a primary binding site in the amino
10 terminus of von Willebrand factor. Upon proteolytic activation by thrombin, FVIII is activated to a heterotrimer of 2 heavy chain fragments (A1, a 50 kDa fragment, and A2, a 43 kDa fragment) and the light chain (A3-C1-C2, a 73 kDa chain). The active form of FVIII (FVIIIa) thus consists of an A1-subunit associated through the divalent metal ion linkage to a thrombin-cleaved A3-C1-C2 light chain and a free A2 subunit
15 associated with the A1 domain through an ion association. Eaton, D. et al., Biochemistry 25: 505 (1986); Lollar, P. et al., J. Biol. Chem. 266: 12481 (1991); and Fay, P. J. et al., J. Biol. Chem. 266: 8957 (1991). This FVIIIa heterotrimer is unstable and subject to rapid inactivation through dissociation of the A2 subunit under physiological conditions.

20 [00193] In one embodiment, a clotting factor comprises a B-domain deleted version of factor VIII. "B-domain" of Factor VIII, 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 Factor VIII. The other human Factor VIII domains are defined by the following
25 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-
30 domains, for porcine, mouse and canine Factor VIII are also known in the art. In one embodiment, the B domain of Factor VIII is deleted ("B-domain-deleted factor VIII" or "BDD FVIII"). An example of a BDD FVIII is REFACTO® (recombinant BDD FVIII with S743/Q1638 fusion), which is known in the art.

[00194] A "B-domain-deleted Factor VIII" may have the full or partial
35 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, each of which is incorporated herein by reference in its entirety. In some embodiments, a B-domain-deleted Factor VIII

5 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 version Factor VIII) (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: 6, i.e., SEQ ID NO: 2). In some embodiments, a B-domain-deleted Factor VIII 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 15 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 20 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 Factor VIII 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, which is incorporated herein by reference in its entirety. In some embodiments, a B-domain-deleted Factor VIII 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), incorporated herein by reference in its entirety. A B-domain-deleted Factor VIII may also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of Factor VIII. Meulien P., *et al. Protein Eng.* 2(4): 301-6 (1988), incorporated herein by reference in its entirety. 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 though 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)), each of

5 which is incorporated herein by reference in its entirety. Each of the foregoing deletions may be made in any Factor VIII sequence. In one embodiment, the invention pertains to a targeted version of FVIII, wherein the targeting (i) specifically binds to platelets, (ii) is not interposed between the light and heavy chains of the clotting factor, and wherein said chimeric clotting factor exhibits increased generation
10 of thrombin in the presence of platelets as compared to an appropriate control lacking the at least one targeting moiety.

[00195] In one embodiment, a clotting factor of the invention is a mature form of Factor IX or a variant thereof. Factor IX circulates as a 415 amino acid, single chain plasma zymogen (A. Vysotchin et al., J. Biol. Chem. 268, 8436 (1993)). The
15 zymogen of FIX is activated by FXIa or by the tissue factor/FVIIa complex. Specific cleavages between arginine-alanine 145-146 and arginine-valine 180-181 result in a light chain and a heavy chain linked by a single disulfide bond between cysteine 132 and cysteine 289 (S. Bajaj et al., Biochemistry 22, 4047 (1983)). The structural organization of FIX is similar to that of the vitamin K-dependent blood clotting
20 proteins FVII, FX and protein C (B. Furie and B. Furie, supra). The approximately 45 amino acids of the amino terminus comprise the gamma-carboxyglutamic acid, or gla, domain. This is followed by two epidermal growth factor homology domains (EGF), an activation peptide and the catalytic "heavy chain" which is a member of the serine protease family (A. Vysotchin et al., J. Biol. Chem. 268, 8436 (1993); S. Spitzer et al.,
25 Biochemical Journal 265, 219 (1990); H. Brandstetter et al., Proc. Natl. Acad. Sci. USA 92, 9796 (1995)).

[00196] In one embodiment, a clotting factor of the invention is a mature form of Factor X. Factor X is a vitamin-K dependent glycoprotein of a molecular weight of 58.5 kDa, which is secreted from liver cells into the plasma as a zymogen. Initially
30 factor X is produced as a prepropeptide with a signal peptide consisting in total of 488 amino acids. The signal peptide is cleaved off by signal peptidase during export into the endoplasmic reticulum, the propeptide sequence is cleaved off after gamma carboxylation took place at the first 11 glutamic acid residues at the N-terminus of the mature N-terminal chain. A further processing step occurs by cleavage between
35 Arg182 and Ser183. This processing step also leads concomitantly to the deletion of the tripeptide Arg180-Lys181-Arg182. The resulting secreted factor X zymogen consists of an N-terminal light chain of 139 amino acids (M, 16,200) and a C-terminal heavy chain of 306 amino acids (M, 42,000) which are covalently linked via a

5 disulfide bridge between Cys172 and Cys342. Further posttranslational processing steps include the .beta.-hydroxylation of Asp103 as well as N- and O-type glycosylation.

[00197] It will be understood that in addition to wild type (WT) versions of these clotting factors or biologically active portions thereof, the present invention may
10 also employ precursor truncated forms thereof that have activity, allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the mature form of the clotting factor and which retain the ability to promote clot formation. For example,
15 modified FVII polypeptides and variants thereof which retain at least one activity of a FVII, such as TF binding, factor X binding, phospholipid binding, and/or coagulant activity of a FVII may be employed. By retaining activity, the activity can be altered, such as reduced or increased, as compared to a wild-type clotting factor so long as the level of activity retained is sufficient to yield a detectable effect. Exemplary
20 sequences of clotting factors that can be used in the constructs of the invention are found in the accompanying sequence listing.

[00198] Exemplary modified polypeptides include, but are not limited to, tissue-specific isoforms and allelic variants thereof, synthetic molecules prepared by translation of nucleic acids, proteins generated by chemical synthesis, such as
25 syntheses that include ligation of shorter polypeptides, through recombinant methods, proteins isolated from human and non-human tissue and cells, chimeric polypeptides and modified forms thereof. The instant clotting factors may also consist of fragments or portions of WT molecules that are of sufficient length or include appropriate regions to retain at least one activity (upon activation if needed) of a full-length
30 mature polypeptide. Exemplary clotting factor variants are known in the art.

[00199] As used herein, the term "Gla domain" refers to the conserved membrane binding motif which is present in vitamin K-dependent proteins, such as prothrombin, coagulation factors VII, IX and X, proteins C, S, and Z. These proteins require vitamin K for the posttranslational synthesis of gamma-carboxyglutamic acid, an
35 amino acid clustered in the N-terminal Gla domain of these proteins. All glutamic residues present in the domain are potential carboxylation sites and many of them are therefore modified by carboxylation. In the presence of calcium ions, the Gla domain interacts with phospholipid membranes that include phosphatidylserine. The Gla

5 domain also plays a role in binding to the FVIIa cofactor, tissue factor (TF). Complexed with TF, the Gla domain of FVIIa is loaded with seven Ca^{2+} ions, projects three hydrophobic side chains in the direction of the cell membrane for interaction with phospholipids on the cell surface, and has significant contact with the C-terminal domain of TF.

10 [00200] The Gla domain of factor VII comprises the uncommon amino acid γ -carboxyglutamic acid (Gla), which plays a vital role in the binding of clotting factors to negatively charged phospholipid surfaces.

[00201] The GLA domain is responsible for the high-affinity binding of calcium ions. It starts at the N-terminal extremity of the mature form of proteins and
15 ends with a conserved aromatic residue. A conserved Gla-x(3)-Gla-x-Cys motif is found in the middle of the domain which seems to be important for substrate recognition by the carboxylase.

[00202] Using stopped-flow fluorescence kinetic measurements in combination with surface plasmon resonance analysis, the Gla domain has been found to be
20 important in the sequence of events whereby the protease domain of FVIIa initiates contact with sTF (Biochemical and Biophysical Research Communications. 2005. 337:1276). In addition, clearance of clotting factors may be significantly mediated through Gla interactions, e.g., on liver cells and clearance receptors, e.g., EPCR.

[00203] In one embodiment, targeted clotting factors are modified to lack a Gla
25 domain. The Gla domain is responsible for mediating clearance of clotting factors via multiple pathways, such as binding to liver cells, clearance receptors such as EPCR, etc. Thus, eliminating the Gla domain has beneficial effects on half life of clotting factors. Though Gla domain is also generally required for activity by localizing clotting factors to sites of coagulation, the inclusion of a platelet targeting domain
30 moiety targets the Gla deleted clotting factor to platelets. In one embodiment, a clotting factor of the invention comprises a targeting moiety and lacks a Gla domain. For example, in the case of Factor VII, the Gla domain is present at the amino terminus of the light chain and consists of amino acids 1-35. The Gla domains of exemplary clotting factors are indicated in the accompanying sequence listing. This
35 domain can be removed using standard molecular biology techniques, replaced with a targeting domain, and the modified light chain incorporated into a construct of the invention. In one embodiment, a cleavage site may be introduced into constructs lacking a Gla domain to facilitate activation of the molecule. For example, in one

5 embodiment, such a cleavage site may be introduced between the amino acids that are cleaved when the clotting factor is activated (e.g., between amino acids 152 and 153 in the case of Factor VII). Exemplary clotting factors lacking a Gla domain are shown in the accompanying figures

[00204] In one embodiment, a cleavage site may be introduced into constructs
10 lacking a Gla domain to facilitate activation of the molecule. For example, in one embodiment, such a cleavage site may be introduced between the amino acids that are cleaved when the clotting factor is activated (e.g., between amino acids 152 and 153 in the case of Factor VII). Exemplary clotting factors lacking a Gla domain are shown in the accompanying figures.

15 [00205] Exemplary clotting factors are those of mammalian, e.g., human, origin. The sequences of exemplary clotting factors are presented in the accompanying sequence listing, e.g., alone or in the context of a chimeric clotting factor construct.

20 III. Targeting Moieties

[00206] In one embodiment, a clotting factor of the invention is targeted to platelets to enhance its efficacy by localizing the clotting factor to the site of coagulation using a "targeting moiety" which binds to a target molecule expressed on platelets. Preferably the targeted molecules are not expressed on cells or tissues other
25 than platelets, i.e., the targeting moieties specifically bind to platelets.

[00207] In one embodiment, receptors/conformations found on resting platelets are targeted. By doing so, sites for coagulation could be primed for enhanced efficacy. Targeting such molecule may also extend half life of the clotting factor and/or prevent clearance. Examples of such targets include GpIb of the GpIb/V/IX complex, and GpVI and nonactive form of GPIIb/IIIa.
30

[00208] In one embodiment, receptors/conformations only found on activated platelets are targeted in order to localize the clotting factor to site of active coagulation. Examples of such targets include, e.g., the active form of GpIb/IIIa as well as CD62P.

35 [00209] In one embodiment, a polypeptide of the invention comprises a "targeting moiety" which has affinity for and binds to platelets. For example, in one embodiment, a targeting moiety binds to the GPIb complex, e.g. GPIb-alpha. Examples of such targeting moieties include the peptides PS4, OS1, and OS2 which

5 bind to both active and nonactive platelets (Benard et al. 2008 Biochemistry 47:4674); In another embodiment, a targeting moiety binds to the active conformation of GPIIb/IIIa. Examples of such targeting moieties include SCE5 and MB9 variable regions which bind active platelets only (Schwarz et al. 2004 FASEB Journal express article 10.1096/fj.04-1513fje; Schwarz et al. 2006 Circulation Research. 99:25-33; U.S. Patent publication 20070218067). In another embodiment, a targeting moiety binds to both the active/nonactive conformation of GPIIb/IIIa. An example of such a targeting moiety is the variable region of the AP3 antibody (Peterson et al. 2003. Hemostasis, Thrombosis, and Vascular Biology 101:937; WO 2010115866). Other targets and targeting moieties are known in the art. Another version of factor IX (the triple mutant V86A/E277A/R338A) with augmented clotting activities has been described by Lin et al. 2010. Journal of Thrombosis and Haemostasis 8: 1773). The contents of these references are incorporated herein by this reference.

[00210] The chimeric clotting factors of the invention can comprise one or more than one targeting moiety. Exemplary configurations are set forth in the accompanying Figures. Additionally, two or more targeting moieties may be linked to each other (*e.g.*, via a spacer) in series, and the tandem array operably linked to a construct of the invention. When two or more targeting moieties are present in a chimeric clotting factor of the invention, the moieties may be the same or different.

25 [00211] In one embodiment, a targeting moiety is fused to a chimeric clotting factor of the invention by a cleaveable linker which may be cleaved to remove the targeting moiety at the site of a clot. In another embodiment, a targeting moiety is not attached via a cleaveable linker and, therefore, is not cleaved at the site of a clot.

[00212] In one embodiment, the targeting moiety is located on the N- or C-terminus of factor VIII. In another embodiment, a targeting moiety is located on the C-terminus of FVII, FIX, FX, or the C-terminus of either or both chains of FVIIa, FIXa, or FXa. In embodiments in which an Fc region or portion thereof is employed, the targeting moiety may be positioned at the N or C terminus of the second Fc chain, or the C-terminus of either or both Fc chains.

35 [00213] In one embodiment, a targeting moiety is not genetically fused directly to a construct, but rather is linked via a spacer or a chemical bond to the construct. For example, targeting moieties may be attached to a construct of the invention by formation of a bond between the targeting moiety and an Fc moiety of a construct,

5 where the targeting moiety comprises a first functional group and the Fc moiety comprises a second functional group, and where the first and second functional groups are capable of reacting with each other to form a chemical bond (see, e.g., U.S. patent 7381408).

[00214] In one embodiment, a polypeptide of the invention comprises at least
10 one of an antigen binding site (*e.g.*, an antigen binding site of an antibody, antibody variant, or antibody fragment), a polypeptide, a receptor binding portion of ligand, or a ligand binding portion of a receptor which specifically binds to platelets, *e.g.*, resting or activated platelets. Exemplary targeting moieties include scFv molecules or peptides which bind to molecules to be targeted. Examples of targeting moieties
15 are found in the instant examples and Figures. Other molecules useful as targeting moieties can readily be selected by one of skill in the art based upon the teaching herein.

A. Antigen Binding Sites Which Bind to Platelets

20 [00215] In certain embodiments, a polypeptide of the invention comprises at least one antigen binding portion (*e.g.*, binding site) of an antibody. In one embodiment, the antigen binding portion targets the polypeptide to platelets

[00216] In other embodiments, a polypeptide of the invention may comprise an antigen binding portion. The term “antigen-binding portion” refers to a
25 polypeptide fragment of an immunoglobulin, antibody, or antibody variant which binds antigen or competes with intact antibody (*i.e.*, with the intact antibody from which they were derived) for antigen binding (*i.e.*, specific binding). For example, said antigen binding portions can be derived from any of the antibodies or antibody variants described *supra*. Antigen binding portions can be produced by recombinant
30 or biochemical methods that are well known in the art. Exemplary antigen-binding portions include Fv, Fab, Fab', and (Fab')₂ as well as scFv molecules.

[00217] In other embodiments, a chimeric clotting factor of the invention may comprise a binding site from single chain binding molecule (*e.g.*, a single chain variable region or scFv). Techniques described for the production of single chain
35 antibodies (U.S. Pat. No. 4,694,778; Bird, *Science* 242:423-442 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); and Ward *et al.*, *Nature* 334:544-554 (1989)) can be adapted to produce single chain binding molecules. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region

5 via an amino acid bridge, resulting in a single chain antibody. Techniques for the assembly of functional Fv fragments in E coli may also be used (Skerra *et al.*, *Science* 242:1038-1041 (1988)).

[00218] In certain embodiments, a polypeptide of the invention comprises one or more binding sites or regions comprising or consisting of a single chain variable
10 region sequence (scFv). Single chain variable region sequences comprise a single polypeptide having one or more antigen binding sites, *e.g.*, a V_L domain linked by a flexible linker to a V_H domain. The V_L and/or V_H domains may be derived from any of the antibodies or antibody variants described *supra*. ScFv molecules can be constructed in a V_H-linker-V_L orientation or V_L-linker-V_H orientation. The flexible
15 linker that links the V_L and V_H domains that make up the antigen binding site preferably comprises from about 10 to about 50 amino acid residues. In one embodiment, the polypeptide linker is a gly-ser polypeptide linker. An exemplary gly/ser polypeptide linker is of the formula (Gly4Ser)_n, wherein n is a positive integer (*e.g.*, 1, 2, 3, 4, 5, or 6). Other polypeptide linkers are known in the art. Antibodies
20 having single chain variable region sequences (*e.g.* single chain Fv antibodies) and methods of making said single chain antibodies are well-known in the art (see *e.g.*, Ho *et al.* 1989. *Gene* 77:51; Bird *et al.* 1988 *Science* 242:423; Pantoliano *et al.* 1991. *Biochemistry* 30:10117; Milenic *et al.* 1991. *Cancer Research* 51:6363; Takkinen *et al.* 1991. *Protein Engineering* 4:837).

25 [00219] In certain embodiments, a scFv molecule employed in a polypeptide of the invention is a stabilized scFv molecule. In one embodiment, the stabilized cFv molecule may comprise a scFv linker interposed between a V_H domain and a V_L domain, wherein the V_H and V_L domains are linked by a disulfide bond between an amino acid in the V_H and an amino acid in the V_L domain. In other embodiments, the
30 stabilized scFv molecule may comprise a scFv linker having an optimized length or composition. In yet other embodiments, the stabilized scFv molecule may comprise a V_H or V_L domain having at least one stabilizing amino acid substitution(s). In yet another embodiment, a stabilized scFv molecule may have at least two of the above listed stabilizing features.

35 [00220] Stabilized scFv molecules have improved protein stability or impart improved protein stability to the polypeptide to which it is operably linked. Preferred scFv linkers of the invention improve the thermal stability of a polypeptide of the invention by at least about 2°C or 3°C as compared to a conventional polypeptide

5 Comparisons can be made, for example, between the scFv molecules of the invention. In certain preferred embodiments, the stabilized scFv molecule comprises a (Gly₄Ser)₄ scFv linker and a disulfide bond which links V_H amino acid 44 and V_L amino acid 100. Other exemplary stabilized scFv molecules which may be employed in the polypeptides of the invention are described in US Provisional Patent
10 Application No. 60/873,996, filed on December 8, 2006 or US Patent Application No. 11/725,970, filed on March 19, 2007, each of which is incorporated herein by reference in its entirety.

[00221] Polypeptides of the invention may comprise a variable region or portion thereof (e.g. a VL and/or VH domain) derived from an antibody using art recognized
15 protocols. For example, the variable domain may be derived from antibody produced in a non-human mammal, e.g., murine, guinea pig, primate, rabbit or rat, by immunizing the mammal with the antigen or a fragment thereof. See Harlow & Lane, *supra*, incorporated by reference for all purposes. The immunoglobulin may be generated by multiple subcutaneous or intraperitoneal injections of the relevant antigen (e.g., purified
20 tumor associated antigens or cells or cellular extracts comprising such antigens) and an adjuvant. This immunization typically elicits an immune response that comprises production of antigen-reactive antibodies from activated splenocytes or lymphocytes.

[00222] While the variable region may be derived from polyclonal antibodies harvested from the serum of an immunized mammal, it is often desirable to isolate
25 individual lymphocytes from the spleen, lymph nodes or peripheral blood to provide homogenous preparations of monoclonal antibodies (MAbs) from which the desired variable region is derived. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Mice are typically used for making monoclonal antibodies. Monoclonal antibodies can be prepared against a fragment by injecting an antigen
30 fragment into a mouse, preparing "hybridomas" and screening the hybridomas for an antibody that specifically binds to the antigen. In this well known process (Kohler *et al.*, (1975), *Nature*, 256:495) the relatively short-lived, or mortal, lymphocytes from the mouse which has been injected with the antigen are fused with an immortal tumor cell line (e.g. a myeloma cell line), thus, producing hybrid cells or "hybridomas" which are
35 both immortal and capable of producing the antibody genetically encoded by the B cell. The resulting hybrids are segregated into single genetic strains by selection, dilution, and regrowth with each individual strain comprising specific genes for the formation of a

5 single antibody. They produce antibodies which are homogeneous against a desired antigen and, in reference to their pure genetic parentage, are termed "monoclonal".

[00223] Hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. Those skilled in the art will appreciate that reagents, cell lines and media for the formation, selection and growth of hybridomas are commercially available from a number of sources and standardized protocols are well established. Generally, culture medium in which the hybridoma cells are growing is assayed for production of monoclonal antibodies against the desired antigen. Preferably, the binding specificity of the monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* assay, such as a radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). After hybridoma cells are identified that produce antibodies of the desired specificity, affinity and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp 59-103 (Academic Press, 1986)). It will further be appreciated that the monoclonal antibodies secreted by the subclones may be separated from culture medium, ascites fluid or serum by conventional purification procedures such as, for example, affinity chromatography (e.g., protein-A, protein-G, or protein-L affinity chromatography), hydroxylapatite chromatography, gel electrophoresis, or dialysis.

25 [00224] Optionally, antibodies may be screened for binding to platelets of a specific activation state or to a specific region or desired fragment of the antigen without binding to other nonoverlapping fragments of the antigen. The latter screening can be accomplished by determining binding of an antibody to a collection of deletion mutants of the antigen and determining which deletion mutants bind to the antibody. Binding can be assessed, for example, by Western blot or ELISA. The smallest fragment to show specific binding to the antibody defines the epitope of the antibody. Alternatively, epitope specificity can be determined by a competition assay in which a test and reference antibody compete for binding to the antigen. If the test and reference antibodies compete, then they bind to the same epitope or epitopes sufficiently proximal such that binding of one antibody interferes with binding of the other.

35 [00225] DNA encoding the desired monoclonal antibody or binding site thereof may be readily isolated and sequenced using any of the conventional procedures

5 described *supra* for the isolation of constant region domain sequences (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The isolated and subcloned hybridoma cells serve as a preferred source of such DNA. More particularly, the isolated DNA (which may be synthetic as described herein) may be used to clone the desired variable
10 region sequences for incorporation in the polypeptides of the invention.

[00226] In other embodiments, the binding site is derived from a fully human antibody. Human or substantially human antibodies may be generated in transgenic animals (e.g., mice) that are incapable of endogenous immunoglobulin production (see e.g., U.S. Pat. Nos. 6,075,181, 5,939,598, 5,591,669 and 5,589,369, each of
15 which is incorporated herein by reference). For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of a human immunoglobulin gene array to such germ line mutant mice will result in the production of human antibodies upon antigen challenge.
20 Another preferred means of generating human antibodies using SCID mice is disclosed in U.S. Pat. No. 5,811,524 which is incorporated herein by reference. It will be appreciated that the genetic material associated with these human antibodies may also be isolated and manipulated as described herein.

[00227] In other aspects, the polypeptides of the invention may comprise
25 antigen binding sites, or portions thereof, derived from modified forms of antibodies. Exemplary such forms include, e.g., minibodies, diabodies, triabodies, nanobodies, camelids, Dabs, tetravalent antibodies, intradiabodies (e.g., Jendreyko et al. 2003. J. Biol. Chem. 278:47813), fusion proteins (e.g., antibody cytokine fusion proteins, proteins fused to at least a portion of an Fc receptor), and bispecific antibodies. Other
30 modified antibodies are described, for example in U.S. Pat. No. 4,745,055; EP 256,654; Faulkner et al., Nature 298:286 (1982); EP 120,694; EP 125,023; Morrison, J. Immun. 123:793 (1979); Kohler et al., Proc. Natl. Acad. Sci. USA 77:2197 (1980); Raso et al., Cancer Res. 41:2073 (1981); Morrison et al., Ann. Rev. Immunol. 2:239 (1984); Morrison, Science 229:1202 (1985); Morrison et al., Proc. Natl. Acad. Sci.
35 USA 81:6851 (1984); EP 255,694; EP 266,663; and WO 88/03559. Reassorted immunoglobulin chains also are known. See, for example, U.S. Pat. No. 4,444,878; WO 88/03565; and EP 68,763 and references cited therein.

5 [00228] In another embodiment, a chimeric clotting factor of the invention comprises an antigen binding site or region which is a diabody or an antigen binding site derived therefrom. Diabodies are dimeric, tetravalent molecules each having a polypeptide similar to scFv molecules, but usually having a short (e.g., less than 10 and preferably 1-5) amino acid residue linker connecting both variable domains, such
10 that the V_L and V_H domains on the same polypeptide chain cannot interact. Instead, the V_L and V_H domain of one polypeptide chain interact with the V_H and V_L domain (respectively) on a second polypeptide chain (see, for example, WO 02/02781). In one embodiment, a polypeptide of the invention comprises a diabody which is operably linked to the N-terminus and/or C-terminus of at least one genetically-fused
15 Fc region (i.e., scFc region).

[00229] In certain embodiments, a polypeptide of the invention comprises a single domain binding molecule (e.g. a single domain antibody) as a targeting moiety. Exemplary single domain molecules include an isolated heavy chain variable domain (V_H) of an antibody, i.e., a heavy chain variable domain, without a light chain variable
20 domain, and an isolated light chain variable domain (V_L) of an antibody, i.e., a light chain variable domain, without a heavy chain variable domain,. Exemplary single-domain antibodies employed in the binding molecules of the invention include, for example, the Camelid heavy chain variable domain (about 118 to 136 amino acid residues) as described in Hamers-Casterman, et al., Nature 363:446-448 (1993), and
25 Dumoulin, et al., Protein Science 11:500-515 (2002). Other exemplary single domain antibodies include single V_H or V_L domains, also known as Dabs® (Domantis Ltd., Cambridge, UK). Yet other single domain antibodies include shark antibodies (e.g., shark Ig-NARs). Shark Ig-NARs comprise a homodimer of one variable domain (V-NAR) and five C-like constant domains (C-NAR), wherein diversity is concentrated
30 in an elongated CDR3 region varying from 5 to 23 residues in length. In camelid species (e.g., llamas), the heavy chain variable region, referred to as VHH, forms the entire antigen-binding domain. The main differences between camelid VHH variable regions and those derived from conventional antibodies (VH) include (a) more hydrophobic amino acids in the light chain contact surface of VH as compared to the
35 corresponding region in VHH, (b) a longer CDR3 in VHH, and (c) the frequent occurrence of a disulfide bond between CDR1 and CDR3 in VHH. Methods for making single domain binding molecules are described in US Patent Nos 6,005,079 and 6,765,087, both of which are incorporated herein by reference. Exemplary single

5 domain antibodies comprising VHH domains include Nanobodies® (Ablynx NV, Ghent, Belgium).

[00230] Exemplary antibodies from which binding sites can be derived for use in the binding molecules of the invention are known in the art. Examples of such targeting moieties include SCE5 and MB9 variable regions which bind active
10 platelets only (Schwarz et al. 2004 FASEB Journal express article 10.1096/fj.04-1513fjc; Schwarz et al. 2006 Circulation Research. 99:25-33; U.S. Patent publication 20070218067). In another embodiment, a targeting moiety binds to both the active/nonactive conformation of GPIIb/IIIa. An example of such a targeting moiety is the variable region of the AP3 antibody (Peterson et al. 2003. Hemostasis,
15 Thrombosis, and Vascular Biology 101:937; WO 2010115866).

B. Non-Immunoglobulin Platelet Binding Molecules

[00231] In certain other embodiments, the polypeptides of the invention comprise one or more platelet binding sites derived from a non-immunoglobulin
20 binding molecule. As used herein, the term “non-immunoglobulin binding molecules” are binding molecules whose binding sites comprise a portion (e.g., a scaffold or framework) which is derived from a polypeptide other than an immunoglobulin, but which may be engineered (e.g., mutagenized) to confer a desired binding specificity to a platelet target. Other examples of binding molecules
25 comprising binding sites not derived from antibody molecules include receptor binding sites and ligand binding sites which bind to platelets.

[00232] Non-immunoglobulin binding molecules may be identified by selection or isolation of a target-binding variant from a library of binding molecules having artificially diversified binding sites. Diversified libraries can be generated
30 using completely random approaches (e.g., error-prone PCR, exon shuffling, or directed evolution) or aided by art-recognized design strategies. For example, amino acid positions that are usually involved when the binding site interacts with its cognate target molecule can be randomized by insertion of degenerate codons, trinucleotides, random peptides, or entire loops at corresponding positions within the
35 nucleic acid which encodes the binding site (see e.g., U.S. Pub. No. 20040132028). The location of the amino acid positions can be identified by investigation of the crystal structure of the binding site in complex with the target molecule. Candidate positions for randomization include loops, flat surfaces, helices, and binding cavities

- 5 of the binding site. In certain embodiments, amino acids within the binding site that are likely candidates for diversification can be identified using techniques known in the art. Following randomization, the diversified library may then be subjected to a selection or screening procedure to obtain binding molecules with the desired binding characteristics, e.g. specific binding platelets using methods known in the art.
- 10 Selection can be achieved by art-recognized methods such as phage display, yeast display, or ribosome display. In one embodiment, molecules known in the art to bind to platelets may be employed in the constructs of the invention. For example, peptides which bind to GPIIb as described in the art (e.g., PS4, 0S1, or 0S2) may be used (Benard et al. 2008. *Biochemistry* 47:4674-4682).

15

IV. Activatable Clotting Factors

- [00233] Clotting factors given for bypass therapy are efficacious when given in the activated form, since exogenous clotting factors are often not activated with sufficient kinetics to be effective. However, they are also rapidly inactivated by
- 20 endogenous pathways (e.g., by antithrombin III or TFPI), leading to clearance of the active form and a short effective half life. In one embodiment, a chimeric clotting factor of the invention is "activatable." Such activatable constructs circulate as an enhanced zymogen with a longer half life, but can be readily cleaved at the site of clotting when necessary.
- 25 [00234] In one embodiment, an activatable construct of the invention comprises a cleavable linker comprising, e.g., a factor XIa, Xa, or thrombin cleavage site (which is cleaved by factor XIa, Xa, or thrombin, respectively) leading to formation of the active form of the clotting factor at the site of a clot. Exemplary factor FXIa cleavage sites include, e.g., TQSFNDFTR and SVSQTSLTR. Exemplary thrombin cleavage
- 30 sites include, e.g., DFLAEGGGVR, TTKIKPR, and a sequence comprising or consisting of ALRPR (e.g. ALRPRVVGGA)).
- [00235] In one embodiment, the cleavable linker may be flanked on one or more sides (upstream, downstream or both) by a spacer moiety.
- [00236] In one embodiment, the cleavable linker is interposed between the light
- 35 chain and heavy chain of the clotting factor. In another embodiment, the cleavable linker is not interposed between the light chain and heavy chain of the clotting factor. In one embodiment, the cleavable linker is located amino terminal to the heavy chain.

- 5 [00237] Exemplary activatable constructs are shown in the accompanying Figures and following Examples.

V. Scaffold Moieties

- Some embodiments of the invention comprise a scaffold moiety, which can be
10 selected from, e.g., a protein moiety, cscFc region, a Fc moiety, albumin, XTEN, etc.

A. Protein Moieties

- In one embodiment, the scaffold is a protein moiety. Such a moiety may
comprise a complete protein or a portion thereof, or a synthetic molecule. Preferred
protein moieties are of a sufficient molecular size that they improve the half life of a
15 chimeric clotting factor of the invention when incorporated into a construct. For
example, in one embodiment, an artificial protein, XTEN, may be included in a
construct as a scaffold (Schellenberger et al. 2009. 27:1186). In another
embodiment, albumin (e.g., human serum albumin) may be included in a construct of
the invention. For example, as known in the art, serum albumin (for example, HSA)
20 can be used as a protein scaffold. In particular various domains and sub-domains of
HSA, have a structure that is quite amenable to mutation or randomization for the
generation of serum albumin scaffold-based protein libraries. Examples of albumin,
e.g., fragments thereof, that may be used in the present invention are known. e.g., U.S.
Patent No. 7,592,010; U.S. Patent No. 6,686,179; and Schulte, Thrombosis Res. 124
25 Suppl. 2:S6-S8 (2009), each of which is incorporated herein by reference in its
entirety.

B. scFc Regions

- [00238] In one embodiment, the invention provides for polypeptides
comprising at least one genetically fused Fc region or portion thereof within a single
30 polypeptide chain (i.e., polypeptides comprising a single-chain Fc (scFc) region) in
one embodiment, comprising a cscFc.

- [00239] In one embodiment, a chimeric clotting factor which comprises a
clotting factor selected from the group consisting of FVII, FIX and FX and a targeting
moiety which binds to platelets and optionally a spacer moiety between the clotting
35 factor and the targeting moiety. In another embodiment, polypeptide comprising FVII,
which FVII comprises a heterologous enzymatic cleavage site activatable by a
component of the clotting cascade.

- 5 [00240] In one embodiment, the invention provides unprocessed polypeptides in which at least two Fc moieties or domains (*e.g.*, 2, 3, 4, 5, 6, or more Fc moieties or domains) within the same linear polypeptide chain that are capable of folding (*e.g.*, intramolecularly or intermolecularly folding) to form one functional scFc region which is linked by an Fc polypeptide linker. For example, in one preferred
- 10 embodiment, a polypeptide of the invention is capable of binding, via its scFc region, to at least one Fc receptor (*e.g.* an FcRn, an FcγR receptor (*e.g.*, FcγRIII), or a complement protein (*e.g.* C1q)) in order to improve half life or trigger an immune effector function (*e.g.*, antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC) and/or to improve manufacturability).
- 15 [00241] A variety of polypeptides of alternative designs are within the scope of the invention. For example, in one embodiment, a polypeptide comprises the moieties:

A-F1-P1- L-P2-B-F2 (I)

- [00242] in linear sequence from the amino to carboxy terminus wherein A, if
- 20 present, is a clotting factor or portion thereof, F1 is a first Fc moiety or domain, P1 is an enzymatic cleavage site, L is an ScFc linker, P2 is an enzymatic cleavage site B, if present, is a clotting factor or portion thereof, F2 is a second Fc moiety or domain and “-” represents a peptide bond. Formula (I) comprises at least an A or B and optionally both. A and B, if both present, can be the corresponding heavy and light chains of a
- 25 clotting factor. Formula (I) comprises at least a P1 or P2 and optionally both. P1 and P2, if both present, can be the same or different. Formula (I) comprises at least a F1 and F2. F1 and F2, if both present, can be the same or different.

- [00243] Exemplary polypeptides according to formula I include: A-F1-P1- L-P2-F2; F1-P1- L-P2-B-F2; A-F1-P1- L- F2; F1-P1- L- B-F2; A-F1- L-P2-F2; and F1-
- 30 L-P2-B-F2.

[00244] In one embodiment, F1 and F2 each comprise a CH2 and CH3 moiety.

- [00245] In one embodiment, after cleavage and substantial excision of the cscFc linker (L), a polypeptide of the invention comprises two polypeptide chains where the first polypeptide chain comprises A linked to a first Fc moiety and where
- 35 the second polypeptide chain comprises B linked to a second Fc moiety, where F1 and F2 dimerize to form an Fc region. In one embodiment, A and B are optionally present and are clotting factors or portions thereof.

5 [00246] In one embodiment, A is the light chain of a clotting factor and B is the heavy chain of a clotting factor. In one embodiment, B is the light chain of a clotting factor and A is the heavy chain of a clotting factor. In one embodiment, when A and B associate in the polypeptide, the polypeptide then forms a functional clotting factor, e.g., FVII, FIX or FX. . In one embodiment, such a polypeptide is enzymatically
10 active upon secretion from a cell.

i) Fc Moieties or Domains

[00247] Fc moieties useful as F1 and F2 for producing the polypeptides of the present invention may be obtained from a number of different sources. In preferred
15 embodiments, an Fc moiety of the polypeptide is derived from a human immunoglobulin. It is understood, however, that the Fc moiety may be derived from an immunoglobulin 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 polypeptide Fc domain or portion thereof may be
20 derived from any immunoglobulin class, including IgM, IgG, IgD, IgA and IgE, and any immunoglobulin isotype, including IgG1, IgG2, IgG3 and IgG4. In a preferred embodiment, the human isotype IgG1 is used.

[00248] A variety of Fc moiety gene sequences (*e.g.* human constant region gene sequences) are available in the form of publicly accessible deposits. Constant
25 region domains comprising an Fc moiety 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 Fc moiety sequences (*e.g.* hinge, CH2, and/or CH3 sequences, or portions thereof) can be derived from these sequences
30 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.

[00249] Fc moiety sequences can be cloned, *e.g.*, using the polymerase chain
35 reaction and primers which are selected to amplify the domain of interest. To clone an Fc moiety sequence 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.

5 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

10 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, which is incorporated by reference herein.

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20 [00250] The polypeptides of the invention may comprise two or more Fc moieties (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more Fc moieties). These two or more Fc moieties can form a Fc region. In one embodiment, the Fc moieties may be of different types. In one embodiment, at least one Fc moiety present in the polypeptide comprises a hinge domain or portion thereof. In another embodiment, the polypeptide

25 of the invention comprises at least one Fc moiety which comprises at least one CH2 domain or portion thereof. In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one CH3 domain or portion thereof. In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one CH4 domain or portion thereof. In

30 another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one hinge domain or portion thereof and at least one CH2 domain or portion thereof (e.g., in the hinge-CH2 orientation). In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one CH2 domain or portion thereof and at least one CH3 domain or

35 portion thereof (e.g., in the CH2-CH3 orientation). In another embodiment, the polypeptide of the invention comprises at least one Fc moiety comprising at least one hinge domain or portion thereof, at least one CH2 domain or portion thereof, and least

5 one CH3 domain or portion thereof, for example in the orientation hinge-CH2-CH3, hinge-CH3-CH2, or CH2-CH3-hinge.

[00251] In certain embodiments, the polypeptide comprises at least one complete Fc region derived from one or more immunoglobulin heavy chains (*e.g.*, an Fc domain including hinge, CH2, and CH3 domains, although these need not be
10 derived from the same antibody). In other embodiments, the polypeptide comprises at least two complete Fc regions derived from one or more immunoglobulin heavy chains. In preferred embodiments, the complete Fc moiety is derived from a human IgG immunoglobulin heavy chain (*e.g.*, human IgG1).

[00252] In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a complete CH3 domain (about amino acids 341-438
15 of an antibody Fc region according to EU numbering). In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a complete CH2 domain (about amino acids 231-340 of an antibody Fc region according to EU numbering). In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising at least a CH3 domain, and at least one of a hinge region
20 (about amino acids 216-230 of an antibody Fc region according to EU numbering), and a CH2 domain. In one embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a hinge and a CH3 domain. In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a hinge, a
25 CH₂, and a CH₃ domain. In preferred embodiments, the Fc moiety is derived from a human IgG immunoglobulin heavy chain (*e.g.*, human IgG1). In one embodiment, an Fc moiety comprises or consists of amino acids corresponding to EU numbers 221 to 447.

[00253] In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising an FcRn binding partner. An FcRn binding partner is
30 a molecule or portion thereof that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the FcRn binding partner. 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
35 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 K_A is higher than 10^6 M^{-1} , or more preferably higher than 10^8 M^{-1} . If necessary, non-specific binding can be reduced without

5 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.

10 [00254] 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 immunoglobulin classes such as IgA, IgM, IgD, and IgE) at relatively low pH, actively transports the IgG transcellularly in a luminal to serosal
15 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
20 epithelium, vaginal surfaces, and biliary tree surfaces.

[00255] FcRn binding partners of 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
25 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
30 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 immunoglobulins or immunoglobulin fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U.S. Department of Public Health, Bethesda,
35 Md.

[00256] The Fc region 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

5 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 γ 1) 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, 10 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, 15 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 wildtype proline substituted by alanine at position number 20 238. As an example, one 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 wildtype amino acids at the positions specified above. Mutations may be introduced singly into Fc giving rise to more than one hundred FcRn binding partners distinct from native Fc. Additionally, combinations of two, 25 three, or more of these individual mutations may be introduced together, giving rise to hundreds more FcRn binding partners. Moreover, one of the FcRn binding partners of a construct of the invention may be mutated and the other FcRn binding partner not mutated at all, or they both may be mutated but with different mutations. Any of the mutations described herein, including N297A, may be used to modify Fc, regardless 30 of the biologically active molecule (e.g., EPO, IFN, Factor VII, Factor IX, T20).

[00257] Certain of the above mutations may confer new functionality upon the 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 FcRn binding partner, 35 and to render the FcRn binding partner incapable of binding to Fc γ RI, Fc γ RIIA, Fc γ RIIB, and Fc γ RIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et

- 5 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. Mutations believed to impart an increased affinity for FcRn include T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591).
- 10 [00258] 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
- 15 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.
- 20 [00259] In one embodiment, the FcRn binding partner is a polypeptide including the sequence PKNSSMISNTP (SEQ ID NO: 12) and optionally further including a sequence selected from HQSLGTQ (SEQ ID NO: 13), HQNLSDGK (SEQ ID NO: 14), HQNISDGK (SEQ ID NO: 24), or VISSHLGQ (SEQ ID NO: 25) (U.S. Pat. No. 5,739,277).
- 25 [00260] 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
- 30 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.
- [00261] The constant region domains or portions thereof making up an Fc moiety of a polypeptide of the invention may be derived from different
- 35 immunoglobulin molecules. For example, a polypeptide of the invention may comprise a CH2 domain or portion thereof derived from an IgG1 molecule and a CH3 region or portion thereof derived from an IgG3 molecule. In another example, a

5 polypeptide can comprise an Fc moiety comprising a hinge domain derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. As set forth herein, it will be understood by one of ordinary skill in the art that an Fc moiety may be altered such that it varies in amino acid sequence from a naturally occurring antibody molecule.

10 [00262] In another embodiment, a polypeptide of the invention comprises an scFc region comprising one or more truncated Fc moieties that are nonetheless sufficient to confer Fc receptor (FcR) binding properties to the Fc region. For example, the portion of an Fc domain that binds to FcRn (i.e., the FcRn binding portion) comprises from about amino acids 282-438 of IgG1, EU numbering (with the
15 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 moiety of a polypeptide 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
20 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.

[00263] In one embodiment, a polypeptide of the invention lacks one or more constant region domains of a complete Fc region, i.e., they are partially or entirely
25 deleted. In a certain embodiments polypeptides of the invention will lack an entire CH2 domain (Δ CH2 constructs). Those skilled in the art will appreciate that such constructs may be preferred due to the regulatory properties of the CH2 domain on the catabolic rate of the antibody. In certain embodiments, polypeptides of the invention comprise CH2 domain-deleted Fc regions derived from a vector (e.g., from IDEC
30 Pharmaceuticals, San Diego) encoding an IgG₁ human constant region domain (see, e.g., WO 02/060955A2 and WO02/096948A2). This exemplary vector is engineered to delete the CH2 domain and provide a synthetic vector expressing a domain-deleted IgG₁ constant region. It will be noted that these exemplary constructs are preferably engineered to fuse a binding CH3 domain directly to a hinge region of the respective
35 Fc domain.

[00264] In other constructs it may be desirable to provide a spacer moiety between one or more constituent Fc moieties. For example, a spacer moiety may be

5 placed between a hinge region and a CH2 domain and/or between a CH2 and a CH3 domains. For example, compatible constructs could be expressed wherein the CH2 domain has been deleted and the remaining CH3 domain (synthetic or unsynthetic) is joined to the hinge region with a 5 – 20 amino acid spacer moiety. Such a spacer moiety may be added, for instance, to ensure that the regulatory elements of the
10 constant region domain remain free and accessible or that the hinge region remains flexible. Preferably, any linker peptide compatible with the instant invention will be relatively non-immunogenic and not prevent proper folding of the scFc region.

[00265] In certain embodiments, the polypeptides of the invention may comprise a dimeric Fc region comprising Fc moieties of the same, or substantially the same, sequence composition (herein termed a “homodimeric Fc region”). In other
15 embodiments, the polypeptides of the invention may comprise a dimeric Fc region comprising at least two Fc moieties which are of different sequence composition (*i.e.*, herein termed a “heterodimeric Fc region”). In one exemplary embodiment, the heterodimeric Fc region comprises an amino acid substitution in a first Fc moiety
20 (*e.g.*, an amino acid substitution of Asparagine at EU position 297), but not in a second Fc moiety.

[00266] In certain embodiments, the Fc region is hemi-glycosylated. For example, the heteromeric scFc region may comprise a first, glycosylated, Fc moiety (*e.g.*, a glycosylated CH2 region) and a second, aglycosylated, Fc moiety (*e.g.*, an
25 aglycosylated CH2 region), wherein a linker is interposed between the glycosylated and aglycosylated Fc moieties. In other embodiments, the Fc region is fully glycosylated, *i.e.*, all of the Fc moieties are glycosylated. In still further embodiments, the Fc region may be aglycosylated, *i.e.*, none of the Fc moieties are glycosylated.

30 [00267] In certain embodiments, an Fc moiety employed in a polypeptide of the invention is altered, *e.g.*, by amino acid mutation (*e.g.*, addition, deletion, or substitution). For example, in one embodiment, an Fc moiety has at least one amino acid substitution as compared to the wild-type Fc from which the Fc moiety is derived. For example, wherein the Fc moiety is derived from a human IgG1 antibody,
35 a variant comprises at least one amino acid mutation (*e.g.*, substitution) as compared to a wild type amino acid at the corresponding position of the human IgG1 Fc region.

[00268] The amino acid substitution(s) of an Fc variant may be located at a position within the Fc moiety referred to as corresponding to the position number that

5 that residue would be given in an Fc region in an antibody (as set forth using the EU numbering convention). One of skill in the art can readily generate alignments to determine what the EU number corresponding to a position in an Fc moiety would be.

[00269] In one embodiment, the Fc variant comprises a substitution at an amino acid position located in a hinge domain or portion thereof. In another embodiment, 10 the Fc variant comprises a substitution at an amino acid position located in a CH2 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH3 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH4 domain or portion thereof.

15 [00270] In certain embodiments, the polypeptides of the invention comprise an Fc variant comprising more than one amino acid substitution. The polypeptides of the invention may comprise, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid substitutions. Preferably, the amino acid substitutions are spatially positioned from each other by an interval of at least 1 amino acid position or more, for example, at 20 least 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid positions or more. More preferably, the engineered amino acids are spatially positioned apart from each other by an interval of at least 5, 10, 15, 20, or 25 amino acid positions or more.

[00271] 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., 25 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

30 [00272] The polypeptides of the invention may employ art-recognized Fc variants which is 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, 35 WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2,

5 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,
 10 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; and 7,317,091, each of which is
 incorporated by reference herein. In one embodiment, the specific change (e.g., the
 15 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.

[00273] In certain embodiments, a polypeptide of the invention comprises an
 20 amino acid substitution to an Fc moiety which alters the antigen-independent effector
 functions of the antibody, in particular the circulating half-life of the antibody.

[00274] Such polypeptides exhibit either increased or decreased binding to
 FcRn when compared to polypeptides lacking these substitutions and, therefore, have
 an increased or decreased half-life in serum, respectively. Fc variants with improved
 25 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
 30 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
 35 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 polypeptides of the invention exhibit reduced transport across the

5 epithelium of kidney glomeruli from the vasculature. In another embodiment, the polypeptides of the invention exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In one embodiment, a polypeptide with altered FcRn binding comprises at least one Fc moiety (e.g, one or two Fc moieties) having one or more amino acid substitutions within the “FcRn binding
10 loop” of an Fc moiety. The FcRn binding loop is comprised of amino acid residues 280-299 (according to EU numbering) of a wild-type, full-length, Fc moiety. In other embodiments, a polypeptide of the invention having altered FcRn binding affinity comprises at least one Fc moiety (e.g, one or two Fc moieties) having one or more amino acid substitutions within the 15 Å FcRn “contact zone.” As used herein, the
15 term 15 Å 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). In preferred embodiments, a polypeptide of the invention having altered FcRn binding affinity comprises at least one Fc moiety (e.g, one or two Fc moieties) having one or more
20 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 which is incorporated by reference herein.

25 [00275] A polypeptide of the invention may also comprise an art recognized amino acid substitution which alters the glycosylation of the polypeptide. For example, the scFc region of the binding polypeptide may comprise an Fc moiety 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.,
30 a low fucose or fucose-free glycan).

[00276] In other embodiments, a polypeptide of the invention comprises at least one Fc moiety having engineered cysteine residue or analog thereof which is located at the solvent-exposed surface. Preferably the engineered cysteine residue or analog thereof does not interfere with an effector function conferred by the scFc
35 region. More preferably, the alteration does not interfere with the ability of the scFc region to bind to Fc receptors (e.g. FcγRI, FcγRII, or FcγRIII) or complement

- 5 proteins (e.g. C1q), or to trigger immune effector function (e.g., antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC)).
- [00277] In one embodiment, an unprocessed polypeptide of the invention may comprise a genetically fused Fc region (i.e., scFc region) having two or more of its constituent Fc moieties independently selected from the Fc moieties described herein.
- 10 In one embodiment, the Fc moieties of a dimeric Fc region are the same. In another embodiment, at least two of the Fc moieties are different. For example, the Fc moieties of the polypeptides 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
- 15 residues, about 15 residues, about 20 residues, about 30 residues, about 40 residues, or about 50 residues). In yet other embodiments, the Fc moieties of the polypeptides of the invention may differ in sequence at one or more amino acid positions. For example, at least two of the Fc moieties 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,
- 20 about 20 positions, about 30 positions, about 40 positions, or about 50 positions).

VI. Polypeptide Linkers

- [00278] As used herein, the term “polypeptide linkers” refers to a peptide or polypeptide sequence (e.g., a synthetic peptide or polypeptide sequence) which
- 25 connects two domains in a linear amino acid sequence of a polypeptide chain. The polypeptides of invention are encoded by nucleic acid molecules that encode polypeptide linkers which either directly or indirectly connect the two Fc moieties which make up the construct,. These linkers are referred to herein as “scFc linkers”. If the scFc linker connects two Fc moieties contiguously in the linear polypeptide
- 30 sequence, it is a “direct” linkage. In contrast, the scFc linkers may link the first Fc moiety to a binding moiety which is, in turn, linked to the second Fc moiety, thereby forming an indirect linkage. These scFc linkers (L) result in the formation of a single chain genetic construct. However, in one embodiment, the scFc polypeptides also comprise enzymatic cleavage sites which result in the scFc linker being cleavable (an
- 35 cscFc linker) and, in one embodiment, substantially excised (e.g., during processing by a cell). Thus, the processed molecule is a dimeric molecule comprising at least two amino acid chains and substantially lacking extraneous linker amino acid sequences. In some embodiments, all or substantially all of the linker is excised,

5 while in some embodiments, a portion of the cleavage site may remain, e.g., four arginines of the RRRR cleavage site.

[00279] In another embodiment, another type of polypeptide linker, herein referred to as a “spacer” may be used to connect different moieties, e.g., a clotting factor or a targeting moiety to an Fc moiety. This type of polypeptide linker may
10 provide flexibility to the polypeptide molecule. Spacers are not typically cleaved, however such cleavage may be desirable. Exemplary positions of spacers are shown in the accompanying drawings. Spacers can be located between the clotting factors, targeting moieties, and/or scaffolds, e.g., at the N or C terminus of these moieties. In one embodiment, these linkers are not removed during processing.

15 [00280] A third type of linker which may be present in a chimeric clotting factor of the invention is a cleavable linker which comprises a cleavage site (e.g., a factor XIa, Xa, or thrombin cleavage site) and which may include additional spacer linkers on either the N terminal or C terminal or both sides of the cleavage site. These cleavable linkers when incorporated into a clotting factor result in a chimeric
20 molecule having a heterologous cleavage site. Exemplary locations for such sites are shown in the accompanying drawings and include, e.g., adjacent to targeting moieties. In another embodiment, such linkers may be adjacent to a clotting factor or portion thereof. For example, in one embodiment, a cleavable linker may be fused to the N terminus of the heavy chain of a clotting factor to make an activatable form of the
25 clotting factor. In such cases, the cleavable linker may include additional spacer linkers at the N terminus of the cleavage site, but require direct fusion at the C-terminus of the cleavage site to the amino terminus of the heavy chain of the clotting factor.

[00281] In one embodiment, an unprocessed polypeptide of the instant
30 invention comprises two or more Fc domains or moieties linked via a cscFc linker to form an Fc region comprised in a single polypeptide chain. The cscFc linker is flanked by at least one enzymatic cleavage site, e.g., a site for processing by an intracellular enzyme. Cleavage of the polypeptide at the at least one enzymatic cleavage site results in a polypeptide which comprises at least two polypeptide chains.
35 In one embodiment, an cscFc linker links F1 or F2 to, e.g., a clotting factor, optionally via a cleavage site.

[00282] As is set forth above, other polypeptide linkers may optionally be used in a construct of the invention, e.g., to connect a clotting factor or targeting moiety to

5 an Fc moiety. One type of polypeptide linker is referred to here as spacers. Some exemplary locations of spacers that can be used in connection with the invention include, e.g., polypeptides comprising GlySer amino acids such as those set forth in the accompanying figures and described in more detail below. In one embodiment, a spacer may be adjacent to one or more moieties each independently selected from
10 clotting factor, scaffold moiety, e.g., Fc, cleavage site, and a targeting moiety.

[00283] In one embodiment, the polypeptide linker is synthetic, i.e., non-naturally occurring. In one embodiment, a polypeptide linker includes peptides (or polypeptides) (which may or may not be naturally occurring) which comprise an amino acid sequence that links or genetically fuses a first linear sequence of amino
15 acids to a second linear sequence of amino acids to which it is not naturally linked or genetically fused in nature. For example, in one embodiment the polypeptide linker may comprise non-naturally occurring polypeptides which are modified forms of naturally occurring polypeptides (e.g., comprising a mutation such as an addition, substitution or deletion). In another embodiment, the polypeptide linker may
20 comprise non-naturally occurring amino acids. In another embodiment, the polypeptide linker may comprise naturally occurring amino acids occurring in a linear sequence that does not occur in nature. In still another embodiment, the polypeptide linker may comprise a naturally occurring polypeptide sequence.

[00284] For example, in certain embodiments, a polypeptide linker can be used
25 to fuse identical Fc moieties, thereby forming a homomeric scFc region. In other embodiments, a polypeptide linker can be used to fuse different Fc moieties (e.g. a wild-type Fc moiety and an Fc moiety variant), thereby forming a heteromeric scFc region.

[00285] In another embodiment, a polypeptide linker comprises or consists of a
30 gly-ser linker. In one embodiment, an scFc or cscFc linker comprises at least a portion of an immunoglobulin hinge and a gly-ser linker. As used herein, the term "gly-ser linker" refers to a peptide that consists of glycine and serine residues. An exemplary gly/ser linker comprises an amino acid sequence of the formula (Gly₄Ser)_n (SEQ ID NO: 4), wherein *n* is a positive integer (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). A preferred gly/ser linker is (Gly₄Ser)₂ (SEQ ID NO:29), (Gly₄Ser)₄ (SEQ ID NO:6), or (Gly₄Ser)₆. (SEQ ID NO: 5) Another exemplary gly-ser linker is GGGSSGGGSG (SEQ ID NO: 30). In certain embodiments, said gly-ser linker may be inserted
35 between two other sequences of the polypeptide linker (e.g., any of the polypeptide

5 linker sequences described herein). In other embodiments, a gly-ser linker is attached at one or both ends of another sequence of the polypeptide linker (e.g., any of the polypeptide linker sequences described herein). In yet other embodiments, two or more gly-ser linker are incorporated in series in a polypeptide linker. In one embodiment, a polypeptide linker of the invention comprises at least a portion of an upper hinge region (e.g., derived from an IgG1, IgG2, IgG3, or IgG4 molecule), at
10 at least a portion of a middle hinge region (e.g., derived from an IgG1, IgG2, IgG3, or IgG4 molecule) and a series of gly/ser amino acid residues (e.g., a gly/ser linker such as (Gly₄Ser)_n) (SEQ ID NO:4).

[00286] Polypeptide linkers of the invention are at least one amino acid in
15 length and can be of varying lengths. In one embodiment, a polypeptide linker of the invention is from about 1 to about 50 amino acids in length. As used in this context, the term "about" indicates +/- two amino acid residues. Since linker length must be a positive interger, the length of from about 1 to about 50 amino acids in length, means a length of from 1-3 to 48-52 amino acids in length. In another embodiment, a
20 polypeptide linker of the invention is from about 10-20 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 15 to about 50 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 20 to about 45 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 15 to about 35 or about 20 to about
25 30 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, or 60 amino acids in length. In one embodiment, a peptide linker of the invention is 20 or 30 amino acids in length.

[00287] Polypeptide linkers can be introduced into polypeptide sequences using
30 techniques known in the art. Modifications can be confirmed by DNA sequence analysis. Plasmid DNA can be used to transform host cells for stable production of the polypeptides produced.

VIII. Enzymatic Cleavage Sites

35 [00288] In one embodiment, one or more enzymatic cleavage site(s) is linked to e.g., flanks or is adjacent to, a cscFc linker (L) of an unprocessed polypeptide of the invention. Such cleavage sites can be upstream or downstream of the cscFc linker or both. For example, in one embodiment of a construct encoding a polypeptide of the

5 invention, a cleavage site is linked (e.g., directly or indirectly) to one or both ends of a cscFc linker (L).

[00289] For example, in one embodiment, a nucleic acid molecule of the invention specifies a polypeptide represented by the formula:

A-F1-P1-L-P2-B-F2 (I)

10 [00290] in linear sequence from the amino to carboxy terminus wherein A, if present, is a clotting factor or portion thereof, F1 is a first Fc moiety or domain, P1 is an enzymatic cleavage site, L is a cscFc linker, P2 is an enzymatic cleavage site B, if present, is a clotting factor or portion thereof, F2 is a second Fc moiety or domain and “-” represents a peptide bond. Formula (I) comprises at least an A or B and optionally
15 both. A and B, if both present, can be the corresponding heavy and light chains of a clotting factor. Formula (I) comprises at least a P1 or P2 and optionally both. P1 and P2, if both present, can be the same or different. Formula (I) comprises at least a F1 and F2. F1 and F2, if both present, can be the same or different.

[00291] In another embodiment, a Factor XIa or Xa cleavage site may be
20 incorporated into a construct of the invention, e.g., in a cleavable linker. Exemplary FXIa cleavage sites include, e.g., TQSFNDFTR and SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g., DFLAEGGGVR, TTKIKPR, LVPRG SEQ ID NO:35) and ALRPRVVGGA Other useful cleavage sites are known in the art.

[00292] In one embodiment, some portion of the linker may remain after cleavage at
25 the at least one enzymatic cleavage site. In order to minimize the presence of extraneous amino acid sequences, two cleavage sites may be included in a polypeptide of the invention. In some embodiments, all or substantially all of the linker is excised, while in some embodiments, a portion of the cleavage site may remain, e.g., four arginines of the RRRR cleavage site.

30

Preparation of Polypeptides

[00293] A variety of methods are available for recombinantly producing a
chimeric clotting factor of the invention. In one embodiment, the invention relates to a nucleic acid construct comprising a nucleic acid sequence encoding the chimeric
35 proteins of the invention. It will be understood that because of the degeneracy of the code, a variety of nucleic acid sequences will encode the amino acid sequence of the polypeptide. The desired polynucleotide can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an earlier prepared polynucleotide.

- 5 **[00294]** Oligonucleotide-mediated mutagenesis is one method for preparing a substitution, in-frame insertion, or alteration (*e.g.*, altered codon) to introduce a codon encoding an amino acid substitution (*e.g.*, into an Fc variant moiety). For example, the starting polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA
- 10 polymerase is used to synthesize an entire second complementary strand of the template that incorporates the oligonucleotide primer. In one embodiment, genetic engineering, *e.g.*, primer-based PCR mutagenesis, is sufficient to incorporate an alteration, as defined herein, for producing a polynucleotide encoding a polypeptide of the invention.
- 15 **[00295]** For recombinant production, a polynucleotide sequence encoding the chimeric protein is inserted into an appropriate expression vehicle, *i. e.* , a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation.
- 20 **[00296]** The nucleic acid encoding the chimeric protein is inserted into the vector in proper reading frame. The expression vector is then transfected into a suitable target cell which will express the polypeptide. Transfection techniques known in the art include, but are not limited to, calcium phosphate precipitation (Wigler et al. 1978, Cell 14 : 725) and electroporation (Neumann et al. 1982, EMBO, J. 1 : 841). A
- 25 variety of host-expression vector systems may be utilized to express the chimeric proteins described herein in eukaryotic cells. In one embodiment, the eukaryotic cell is an animal cell, including mammalian cells (*e. g.* 293 cells, PerC6, CHO, BHK, Cos, HeLa cells). When the chimeric protein is expressed in a eukaryotic cell the DNA encoding the chimeric protein may also code for a signal sequence that will permit the
- 30 chimeric protein to be secreted. One skilled in the art will understand that while the protein is translated the signal sequence is cleaved by the cell to form the mature chimeric protein. Various signal sequences are known in the art *e. g.* , native factor VII signal sequence, native factor IX signal sequence and the mouse IgK light chain signal sequence. Alternatively, where a signal sequence is not included the chimeric
- 35 protein can be recovered by lysing the cells.
- [00297]** The chimeric protein of the invention can be synthesized in a transgenic animal, such as a rodent, goat, sheep, pig, or cow. The term "transgenic animals" refers to non-human animals that have incorporated a foreign gene into their

5 genome. Because this gene is present in germline tissues, it is passed from parent to offspring. Exogenous genes are introduced into single-celled embryos (Brinster et al. 1985, Proc. Natl. Acad.Sci. USA 82 : 4438). Methods of producing transgenic animals are known in the art. including transgenics that produce immunoglobulin molecules (Wagner et al. 1981, Proc. Natl. Acad. Sci. USA 78: 6376; McKnight et al. 10 1983, Cell 34 : 335; Brinster et al. 1983, Nature 306: 332; Ritchie et al. 1984, Nature 312: 517; Baldassarre et al. 2003, Theriogenology 59 : 831 ; Robl et al. 2003, Theriogenology 59: 107; Malassagne et al. 2003, Xenotransplantation 10 (3): 267).

[00298] The expression vectors can encode for tags that permit for easy purification or identification of the recombinantly produced protein. Examples 15 include, but are not limited to, vector pUR278 (Ruther et al. 1983, EMBO J. 2: 1791) in which the chimeric protein described herein coding sequence may be ligated into the vector in frame with the lac z coding region so that a hybrid protein is produced; pGEX vectors may be used to express proteins with a glutathione S-transferase (GST) tag. These proteins are usually soluble and can easily be purified from cells by 20 adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The vectors include cleavage sites (e. g. PreCission Protease (Pharmacia, Peapack, N. J.)) for easy removal of the tag after purification.

[00299] For the purposes of this invention, numerous expression vector systems may be employed. These expression vectors are typically replicable in the host 25 organisms either as episomes or as an integral part of the host chromosomal DNA. Expression vectors may include expression control sequences including, but not limited to, promoters (e.g., naturally-associated or heterologous promoters), enhancers, signal sequences, splice signals, enhancer elements, and transcription termination sequences. Preferably, the expression control sequences are eukaryotic 30 promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Expression vectors may also utilize DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV), cytomegalovirus (CMV), or SV40 virus. Others involve the use of polycistronic systems with internal 35 ribosome binding sites.

[00300] Commonly, expression vectors contain selection markers (e.g., ampicillin-resistance, hygromycin-resistance, tetracycline resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA

5 sequences (see, *e.g.*, Itakura *et al.*, US Patent 4,704,362). Cells which have integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be
10 directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation.

[00301] A preferred expression vector is NEOSPLA (U.S. Patent No. 6,159,730). This vector contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth
15 hormone polyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, the dihydrofolate reductase gene and leader sequence. This vector has been found to result in very high level expression of antibodies upon incorporation of variable and constant region genes, transfection in cells, followed by selection in G418 containing medium and methotrexate amplification. Vector systems are also taught in U.S. Pat.
20 Nos. 5,736,137 and 5,658,570, each of which is incorporated by reference in its entirety herein. This system provides for high expression levels, *e.g.*, > 30 pg/cell/day. Other exemplary vector systems are disclosed *e.g.*, in U.S. Patent No. 6,413,777.

[00302] In other embodiments the polypeptides of the invention of the instant
25 invention may be expressed using polycistronic constructs. In these expression systems, multiple gene products of interest such as multiple polypeptides of multimer binding protein may be produced from a single polycistronic construct. These systems advantageously use an internal ribosome entry site (IRES) to provide relatively high levels of polypeptides of the invention in eukaryotic host cells.
30 Compatible IRES sequences are disclosed in U.S. Pat. No. 6,193,980 which is also incorporated herein. Those skilled in the art will appreciate that such expression systems may be used to effectively produce the full range of polypeptides disclosed in the instant application.

[00303] More generally, once the vector or DNA sequence encoding a
35 polypeptide has been prepared, the expression vector may be introduced into an appropriate host cell. That is, the host cells may be transformed. Introduction of the plasmid into the host cell can be accomplished by various techniques well known to those of skill in the art. These include, but are not limited to, transfection (including

- 5 electrophoresis and electroporation), protoplast fusion, calcium phosphate precipitation, cell fusion with enveloped DNA, microinjection, and infection with intact virus. See, Ridgway, A. A. G. "*Mammalian Expression Vectors*" Chapter 24.2, pp. 470-472 Vectors, Rodriguez and Denhardt, Eds. (Butterworths, Boston, Mass. 1988). Most preferably, plasmid introduction into the host is via electroporation. The
- 10 transformed cells are grown under conditions appropriate to the production of the light chains and heavy chains, and assayed for heavy and/or light chain protein synthesis. Exemplary assay techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or fluorescence-activated cell sorter analysis (FACS), immunohistochemistry and the like.
- 15 **[00304]** As used herein, the term "transformation" shall be used in a broad sense to refer to the introduction of DNA into a recipient host cell that changes the genotype and consequently results in a change in the recipient cell.
- [00305]** Along those same lines, "host cells" refers to cells that have been transformed with vectors constructed using recombinant DNA techniques and
- 20 encoding at least one heterologous gene. In descriptions of processes for isolation of polypeptides from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of polypeptide unless it is clearly specified otherwise. In other words, recovery of polypeptide from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and
- 25 the suspended cells.
- [00306]** The host cell line used for protein expression is most preferably of mammalian origin; those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to,
- 30 DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), P3.times.63-Ag3.653 (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte),
- 35 PerC6, and 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.

5 [00307] In one embodiment, a host cell endogenously expresses an enzyme (or the enzymes) necessary to cleave an scFc linker (e.g., if such a linker is present and contains intracellular processing site(s)) during processing to form the mature polypeptide. During this processing, the scFc linker may be substantially removed to reduce the presence of extraneous amino acids. In another embodiment of the invention, a host cell is transformed to express one or more enzymes which are exogenous to the cell such that processing of an scFc linker occurs or is improved.

[00308] In one embodiment an enzyme which may be endogenously or exogenously expressed by a cell is a member of the furin family of enzymes. Complete cDNA and amino acid sequences of human furin (i.e., PACE) were published in 1990. Van den Ouweland A M et al. (1990) Nucleic Acids Res. 18:664; Erratum in: Nucleic Acids Res. 18:1332 (1990).

[00309] U.S. Pat. No. 5,460,950, issued to Barr et al., describes recombinant PACE and the coexpression of PACE with a substrate precursor polypeptide of a heterologous protein to improve expression of active, mature heterologous protein.

20 [00310] U.S. Pat. No. 5,935,815, issued to van de Ven et al., likewise describes recombinant human furin (i.e., PACE) and the coexpression of furin with a substrate precursor polypeptide of a heterologous protein to improve expression of active, mature heterologous protein. Possible substrate precursors disclosed in this patent include a precursor of Factor IX. Other family members in the mammalian furin/subtilisin/Kex2p-like proprotein convertase (PC) family in addition to PACE are reported to include PC1/PC3, PC2, PC4, PC5/6 (hereinafter referred to simply as PC5), PACE4, and LPC/PC7/PC8/SPC7. While these various members share certain conserved overall structural features, they differ in their tissue distribution, subcellular localization, cleavage specificities, and preferred substrates. For a review, see Nakayama K (1997) Biochem J. 327:625-35. Similar to PACE, these proprotein convertases generally include, beginning from the amino terminus, a signal peptide, a propeptide (that may be autocatalytically cleaved), a subtilisin-like catalytic domain characterized by Asp, His, Ser, and Asn/Asp residues, and a Homo B domain that is also essential for catalytic activity and characterized by an Arg-Gly-Asp (RGD) sequence. PACE, PACE4, and PC5 also include a Cys-rich domain, the function of which is unknown. In addition, PC5 has isoforms with and without a transmembrane domain; these different isoforms are known as PC5B and PC5A, respectively. Comparison between the amino acid sequence of the catalytic domain of PACE and

5 the amino acid sequences of the catalytic domains of other members of this family of proprotein convertases reveals the following degrees of identity: 70 percent for PC4; 65 percent for PACE4 and PC5; 61 percent for PC1/PC3; 54 percent for PC2; and 51 percent for LPC/PC7/PC8/SPC7. Nakayama K (1997) Biochem J. 327:625-35.

[00311] PACE and PACE4 have been reported to have partially overlapping
10 but distinct substrates. In particular, PACE4, in striking contrast to PACE, has been reported to be incapable of processing the precursor polypeptide of FIX. Wasley L C et al. (1993) J Biol Chem. 268:8458-65; Rehemtulla A et al. (1993) Biochemistry. 32:11586-90.

[00312] U.S. Pat. No. 5,840,529, issued to Seidah et al., discloses nucleotide
15 and amino acid sequences for human PC7 and the notable ability of PC7, as compared to other PC family members, to cleave HIV gp160 to gp120 and gp41.

[00313] Nucleotide and amino acid sequences of rodent PC5 were first
described as PC5 by Lusson J et al. (1993) Proc Natl Acad Sci USA 90:6691-5 and as PC6 by Nakagawa T et al. (1993) J Biochem (Tokyo) 113:132-5. U.S. Pat. No.
20 6,380,171, issued to Day et al., discloses nucleotide and amino acid sequences for human PC5A, the isoform without the transmembrane domain. The sequences of these enzymes and method of cloning them are known in the art.

[00314] Genes encoding the polypeptides of the invention can also be
expressed in non-mammalian cells such as bacteria or yeast or plant cells. In this
25 regard it will be appreciated that various unicellular non-mammalian microorganisms such as bacteria can also be transformed; i.e., those capable of being grown in cultures or fermentation. Bacteria, which are susceptible to transformation, include members of the enterobacteriaceae, such as strains of Escherichia coli or Salmonella; Bacillaceae, such as Bacillus subtilis; Pneumococcus; Streptococcus, and
30 Haemophilus influenzae. It will further be appreciated that, when expressed in bacteria, the polypeptides typically become part of inclusion bodies. The polypeptides must be isolated, purified and then assembled into functional molecules.

[00315] In addition to prokaryotes, eukaryotic microbes may also be used.
Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used
35 among eukaryotic microorganisms although a number of other strains are commonly available.

For expression in *Saccharomyces*, the plasmid YRp7, for example, (Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene,

5 10:157 (1980)) is commonly used. This plasmid already contains the TRP1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, Genetics, 85:12 (1977)). The presence of the *trp1* lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence
10 of tryptophan.

Other yeast hosts such *Pichia* may also be employed. Yeast expression vectors having expression control sequences (*e.g.*, promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters
15 include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for methanol, maltose, and galactose utilization.

[00316] Alternatively, polypeptide-coding nucleotide sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (see, *e.g.*, Deboer *et al.*,
20 US 5,741,957, Rosen, US 5,304,489, and Meade *et al.*, US 5,849,992). Suitable transgenes include coding sequences for polypeptides in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.

[00317] *In vitro* production allows scale-up to give large amounts of the desired
25 polypeptides. 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
30 by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, chromatography over DEAE-cellulose or (immuno-)affinity chromatography, *e.g.*, after preferential biosynthesis of a synthetic hinge region polypeptide or prior to or subsequent to the HIC chromatography step described herein. An affinity tag sequence (*e.g.* a His(6) tag) may optionally be attached or
35 included within the polypeptide sequence to facilitate downstream purification.

[00318] In one embodiment, a host cell of the invention comprises a genetic construct encoding a polypeptide comprising an scFc scaffold and one or more

5 enzymes that can process a cscFc linker. The construct and the enzyme(s) can be expressed using a single vector or two vectors.

[00319] In one embodiment, the invention pertains to nucleic acid molecules which encode a polypeptide of the invention. In one embodiment, the nucleic acid molecule encodes a chimeric clotting factor selected from the group consisting of
10 FVII, FIX and FX and which comprises a targeting moiety which binds to platelets and optionally a spacer moiety between the clotting factor and the targeting moiety. In another embodiment, the invention pertains to a nucleic acid molecule encoding a polypeptide comprising FVII, which FVII which comprises a heterologous enzymatic cleavage site activatable by a component of the
15 clotting cascade.

[00320] Once expressed, the chimeric clotting factor can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity column chromatography, HPLC purification, gel electrophoresis and the like (see generally Scopes, Protein Purification (Springer-Verlag, N.Y., (1982)) and see
20 specifically the methods used in the instant Examples. Substantially pure proteins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses.

IX. Methods of Administering Polypeptides of the Invention

25 [00321] In another embodiment, the invention relates to a method of treating a subject with a hemostatic disorder comprising administering a therapeutically effective amount of an enhanced clotting factor of the Invention.

[00322] Compositions for administration to a subject include nucleic acid molecules which comprise a nucleotide sequence encoding a chimeric clotting factor
30 of the invention (for gene therapy applications) as well as polypeptide molecules.

[00323] In one embodiment, an enhanced clotting factor composition of the invention is administered in combination with at least one other agent that promotes hemostasis. Said other agent that promotes hemostasis is a therapeutic with demonstrated clotting activity. As an example, but not as a limitation, hemostatic
35 agent can include Factor V, Factor VII, Factor VIII, Factor IX, Factor X, Factor XI, Factor XII, Factor XIII, prothrombin, or fibrinogen 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.

5 **[00324]** In one embodiment of the invention, the composition (e.g., the polypeptide or nucleic acid molecule encoding the polypeptide) is one in which the clotting factor is present in active form when administered to a subject. Such an activated molecule may be expressed by a cell in active form or may be activated in vitro prior to administration to a subject. In another embodiment, the composition is
10 one in which the clotting factor is present in activatable form and the clotting factor is activated in vivo at the site of clotting after administration to a subject.

[00325] The chimeric clotting factor of the invention can be administered intravenously, subcutaneously, intramuscularly, or via any mucosal surface, e.g., orally, sublingually, buccally, sublingually, nasally, rectally, vaginally or via
15 pulmonary route. The chimeric protein can be implanted within or linked to a biopolymer solid support that allows for the slow release of the chimeric protein to the desired site.

[00326] For oral administration, the pharmaceutical composition can take the form of tablets or capsules prepared by conventional means. The composition can also
20 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
25 include flavoring, coloring and sweetening agents. Alternatively, the composition can be presented as a dry product for constitution with water or another suitable vehicle.

[00327] For buccal and sublingual administration the composition may take the form of tablets, lozenges or fast dissolving films according to conventional protocols.

[00328] For administration by inhalation, the compounds for use according to
30 the present invention are conveniently delivered in the form of an aerosol spray from a pressurized pack or nebulizer (e.g. in PBS), with a suitable 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
35 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.

5 **[00329]** In one embodiment, the route of administration of the polypeptides of the invention is parenteral. The term parenteral as used herein includes intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal or vaginal administration. The intravenous form of parenteral administration is preferred. While all these forms of administration are clearly contemplated as being within the scope of
10 the invention, a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. Usually, a suitable pharmaceutical composition for injection may comprise a buffer (e.g. acetate, phosphate or citrate buffer), a surfactant (e.g. polysorbate), optionally a stabilizer agent (e.g. human albumin), etc. However, in other methods compatible with the teachings herein, the
15 polypeptides can be delivered directly to the site of the adverse cellular population thereby increasing the exposure of the diseased tissue to the therapeutic agent.

[00330] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil,
20 and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In the subject invention, pharmaceutically acceptable carriers include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Other common parenteral vehicles include sodium phosphate solutions, Ringer's
25 dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

30 **[00331]** More particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of
35 manufacture and storage and will preferably be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures

5 thereof. The proper fluidity 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.

[00332] Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,
10 ascorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

15 [00333] In any case, sterile injectable solutions can be prepared by incorporating an active compound (*e.g.*, a polypeptide by itself or in combination with other active agents) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active
20 compound into a sterile vehicle, which 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, which yields a powder of an active ingredient plus any additional desired ingredient from a previously sterile-filtered
25 solution thereof. The preparations for injections are processed, filled into containers such as ampoules, bags, bottles, syringes or vials, and sealed under aseptic conditions according to methods known in the art. Further, the preparations may be packaged and sold in the form of a kit. Such articles of manufacture will preferably have labels or package inserts indicating that the associated compositions are useful for treating a
30 subject suffering from, or predisposed to clotting disorders.

[00334] 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.

[00335] Effective doses of the compositions of the present invention, for the
35 treatment of conditions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals

5 including transgenic mammals can also be treated. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[00336] In one embodiment, the dose of a biologically active moiety (e.g., comprising FIX) can range from about 25 to 100 IU/kg, e.g., 0.417 mg/kg to 1.67
10 mg/kg. In another embodiment, the dose of a biologically active moiety (e.g., comprising FVIII) can range from about 25 to 65 IU/kg, e.g., 0.003125 mg/kg to 0.008125 mg/kg. In another embodiment, the dose of a biologically active moiety (e.g., comprising FVII), can range from about 90 to 270 ug/kg or 0.090 to 0.270 mg/kg.

15 [00337] Dosages can range from 1000 ug/kg to 0.1 ng/kg body weight. In one embodiment, the dosing range is 1ug/kg to 100 ug/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-
20 CLOT VIIa-rTF 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).

[00338] Doses intermediate in the above ranges are also intended to be within the scope of the invention. Subjects can be administered such doses daily, on
25 alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. In some methods, two or more polypeptides may be administered simultaneously, in which case the dosage of each polypeptide administered falls within the ranges indicated.

30 [00339] Polypeptides of the invention can be administered on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of modified polypeptide or antigen in the patient. Alternatively, polypeptides can be administered as a sustained release formulation, in which case less frequent administration is
35 required. Dosage and frequency vary depending on the half-life of the polypeptide in the patient.

[00340] The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications,

5 compositions containing the polypeptides 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 minimize effects of disease. Such an amount is defined to be a "prophylactic effective dose." A relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive
10 treatment for the rest of their lives.

[00341] Polypeptides of the invention can optionally be administered in combination with other agents that are effective in treating the disorder or condition in need of treatment (e.g., prophylactic or therapeutic).

[00342] As used herein, the administration of polypeptides of the invention in
15 conjunction or combination with an adjunct therapy means the sequential, simultaneous, coextensive, concurrent, concomitant or contemporaneous administration or application of the therapy and the disclosed polypeptides. Those skilled in the art will appreciate that the administration or application of the various components of the combined therapeutic regimen may be timed to enhance the overall effectiveness of the treatment.
20 A skilled artisan (e.g. a physician) would be readily be able to discern effective combined therapeutic regimens without undue experimentation based on the selected adjunct therapy and the teachings of the instant specification.

[00343] It will further be appreciated that the polypeptides of the instant invention may be used in conjunction or combination with an agent or agents (e.g. to
25 provide a combined therapeutic regimen). Exemplary agents with which a polypeptide of the invention may be combined include agents that represent the current standard of care for a particular disorder being treated. Such agents may be chemical or biologic in nature. The term "biologic" or "biologic agent" refers to any pharmaceutically active agent made from living organisms and/or their products
30 which is intended for use as a therapeutic.

[00344] The amount of agent to be used in combination with the polypeptides of the instant invention may vary by subject or may be administered according to what is known in the art. See for example, Bruce A Chabner *et al.*, *Antineoplastic Agents*, in GOODMAN & GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS
35 1233-1287 ((Joel G. Hardman *et al.*, eds., 9th ed. 1996). In another embodiment, an amount of such an agent consistent with the standard of care is administered.

[00345] As previously discussed, the polypeptides of the present invention, may be administered in a pharmaceutically effective amount for the *in vivo* treatment

5 of clotting disorders. In this regard, it will be appreciated that the polypeptides of the invention can be formulated to facilitate administration and promote stability of the active agent. Preferably, pharmaceutical compositions in accordance with the present invention comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. Of course, the
10 pharmaceutical compositions of the present invention may be administered in single or multiple doses to provide for a pharmaceutically effective amount of the polypeptide.

[00346] In one embodiment, a chimeric clotting factor of the invention can be administered as a nucleic acid molecule. Nucleic acid molecules can be administered
15 using techniques known in the art, including via vector, plasmid, liposome, DNA injection, electroporation, gene gun, intravenously injection or hepatic artery infusion. Vectors for use in gene therapy embodiments are known in the art.

[00347] In keeping with the scope of the present disclosure, the chimeric clotting factors of the invention may be administered to a human or other animal in
20 accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic or prophylactic effect.

[00348] The chimeric proteins of the invention have many uses as will be recognized by one skilled in the art, including, but not limited to methods of treating a subject with a disease or condition. The disease or condition can include, but is are
25 not limited to, hemostatic disorders.

[00349] In one embodiment, the invention relates to a method of treating a subject having a hemostatic disorder comprising administering a therapeutically effective amount of at least one chimeric clotting factor of the invention.

[00350] The chimeric clotting factors of the invention treat or prevent a
30 hemostatic disorder by promoting the formation of a fibrin clot. The chimeric clotting factor of the invention can activate any member of a coagulation cascade. The clotting factor can be a participant in the extrinsic pathway, the intrinsic pathway or both.

[00351] A chimeric clotting factor of the invention can be used to treat hemostatic disorders, e.g., those known to be treatable with the particular clotting
35 factor present in the chimeric clotting factor. The hemostatic disorders that may be treated by administration of the chimeric protein of the invention include, but are not limited to, hemophilia A, hemophilia B, von Willebrand's disease, Factor XI deficiency (PTA deficiency), Factor XII deficiency, as well as deficiencies or

5 structural abnormalities in fibrinogen, prothrombin, Factor V, Factor VII, Factor X, or Factor XIII.

[00352] In one embodiment, the hemostatic disorder is an inherited disorder. In one embodiment, the subject has hemophilia A, and the chimeric protein comprises Factor VII or Factor VIIIa. In another embodiment, the subject has hemophilia A and
10 the chimeric clotting factor comprises Factor VII or Factor VIIa. In another embodiment, the subject has hemophilia B and the chimeric clotting factor comprises Factor IX or Factor IXa. In another embodiment, the subject has hemophilia B and the chimeric protein comprises Factor VII or Factor VIIa. In another embodiment, the subject has inhibitory antibodies to Factor VII or Factor VIIIa and the chimeric
15 clotting factor comprises Factor VII or Factor VIIa. In yet another embodiment, the subject has inhibitory antibodies against Factor IX or Factor IXa and the chimeric protein comprises Factor VII or Factor VIIa.

[00353] The chimeric clotting factor of the invention can be used to prophylactically treat a subject with a hemostatic disorder. The chimeric clotting
20 factor of the invention can be used to treat an acute bleeding episode in a subject with a hemostatic disorder.

[00354] In one embodiment, the hemostatic disorder is the result of a deficiency in a clotting factor, e.g., Factor IX, Factor VIII. In another embodiment, the hemostatic disorder can be the result of a defective clotting factor.

25 [00355] In another embodiment, the hemostatic disorder can be 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 autoimmune disease, or pregnancy. The acquired disorder can result from old age or from medication to treat an underlying secondary disorder (e.g. cancer
30 chemotherapy).

[00356] The invention also relates to methods of treating a subject that does not have a hemostatic disorder or a secondary disease or condition resulting in acquisition of a hemostatic disorder. The invention thus relates to a method of treating a subject in need of a general hemostatic agent comprising administering a therapeutically
35 effective amount of at least one chimeric clotting factor of the invention. For example, in one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric clotting factor of the invention can be administered prior to or after surgery as a prophylactic. The chimeric

5 clotting factor 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, or stem cell transplantation.

[00357] In another embodiment, the chimeric clotting factor of the invention can be used to treat a subject having an acute bleeding episode who does not have a
10 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.

[00358] This invention is further illustrated by the following examples which
15 should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

20

[00359] Throughout the examples, the following materials and methods were used unless otherwise stated.

General Materials and Methods

25 [00360] 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);
30 *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.*, C.S.H.L. Press, Pub. (1999); and *Current Protocols in Molecular Biology*, eds. Ausubel *et al.*, John Wiley & Sons (1992).

35 **Example 1. Heterodimeric Constructs comprising FVII-Fc and MB9-Fc at the amino terminus of the second Fc chain**

Cloning of pSYN-FVII-027

5 [00361] The FVII-027 construct comprises cscFc for cleavage when processed during manufacture in a cell. The construct comprises a targeting moiety, a scFv moiety that binds to GPIIb/IIIa, MB9.

[00362] Plasmid (pSYN-FVII-027) was generated for the expression FVII-Fc and MB9-Fc heterodimer, where MB9 is a scFv previously shown to bind to receptor
 10 GPIIb/IIIa on activated platelets. Protein from pSYN-FVII-027 is expressed in the cell as a single polypeptide where the C-terminus of the FVII-Fc subunit is linked to the N-terminus of the MB9-Fc subunit by a (GGGGS)_{6x} polypeptide linker. Furthermore, RRRRS and RKRRKR sequences were inserted at the 5' and 3' end of the polypeptide linker, respectively, for intracellular cleavage by proprotein convertases
 15 following the last Arg at each sequence. Consequently, cells will express a 2 chain FVII-Fc/MB9-Fc heterodimer where the FVII-Fc chain has a RRRRS sequence at the C-terminus, but the remainder of the linker and the RKRRKR sequence have otherwise been removed.

[00363] As a first step a series of intermediate plasmid were generated using
 20 the following primers:

HindIII-SalI-BspEI-Fc-F

AGTCAAGCTTGTGCGACTCCGGAACCTCCTGGGCGGACC

BamHI-linker-Fc-R

CATCGGATCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCACCTGATCCGCCGCCACCTTTAC

25 CCGGAGACAGGGAGAGG

BclI-Fc-F

CAGTCTTGATCAGACAAAACCTCACACATGCCCCACC

scFc-EcoRI-R

ACTGACGAATTCTCATTTACCGGAGACAGGGAG

30 HindIII-Kozak-FVII-F:

CGACAAGCTTGTCCGCCACCATGGTCTCCAGGCCCTCAGG

FVII-HC-BspEI-R:

AGGAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTGCGGATCCCCGCCACCGGAACCTCCA

CCGCCTGATCCACCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGA

35 AATGGGGCTCGCAGGAGG

A 50 ul PCR reaction was carried out with 25 pmol of HindIII-SalI-BpEI-Fc-F and BamHI-linker-Fc-R and template pSYN-Fc-001 using the following cycle: 95° C 2 minutes; 30 cycles of (95° C 30 seconds, 54° C 30 seconds, 72° C 1 minute). The expected sized band (~700 bp) was gel purified with a Gel Extraction kit (Qiagen,
 40 Valencia, Calif.) and cloned into the HindIII and BamHI restriction sites of

5 pBUDCE4 (Invitrogen, Carlsbad, Calif.) to generate intermediate pSYN-FVII-007. Primers HindIII-SalI-BpEI-Fc-F and BamHI-linker-Fc-R amplify the Fc region starting at amino acid 221 (EU numbering) and add a HindIII and a SalI restriction enzyme site immediately upstream of site Fc region, as well as a DNA fragment encoding a (GGGGS)_{4x} linker followed by a BamHI site immediately downstream of the Fc coding region. Next, a 50 ul reaction was carried out with 25 pmol of BclI-Fc-F and scFc-EcoRI-R, and template pSYN-Fc-011 using the same cycles as above. The expected sized band (~700 bp) was gel purified as above, cut with restriction enzymes BamHI and EcoRI, and cloned in the BclI/EcoRI restriction sites of pSYN-FVII-007 to generate the intermediate plasmid pSYN-FVII-008. The primer pair BclI-Fc-F and scFc-EcoRI-R amplifies the Fc region while adding a BclI and EcoRI restriction sites immediately upstream and downstream of the Fc coding region, respectively. To generate the last intermediate plasmid, a 50 ul PCR reaction was carried out with 25 pmol of HindIII-Kozak-FVII-F and FVII-HC-BspEI-R and template pSYN-FVII-001 using the following cycle: 95° C 2 minutes; 30 cycles of (95° C 30 seconds, 55° C 30 seconds, 72° C 90 seconds). The primer pair amplifies the FVII coding region while adding a DNA fragment at the 3' end of the molecule encoding a (GGGGS)_{6x} polypeptide linker followed by a fragment of the Fc region ending at amino acid 221 (EU numbering). Primer HindIII-Kozak-FVII-F generates a HindIII restriction site at the 5' of the molecule followed by a Kozak sequence directly upstream of the FVII coding region. The FVII-HC-BspEI-R primer introduces DNA encoding the polypeptide linker as well as the Fc portion. The expected sized band (~1500 bp) was gel purified as above and cloned into the HindIII/BspEI sites of pSYN-FVII-008 to generate pSYN-FVII-011.

[00364] Next, 2 DNA fragments were synthesized: Genescript-FVII-027-1 and Genscript-FVII-026-2. Genescript-FVII-027-1 consists of a DNA fragment encoding a portion of the Fc region (starting at nucleotide 1306, EU numbering) followed by the sequence RRRRS-(GGGGS)_{6x}-RKRRKR followed by a portion of the MB9 scFv (residues 1-142). An EcoRI site was introduced in the coding sequence of MB9 using the degeneracy of the genetic code to preserve the proper amino acid sequence and overlaps the last 6 bases of Genescript-FVII-027-1. In addition, the first 6 bases at the 5' include a SapI site found within the Fc region. Genscript-FVII-026-2 consists of a DNA fragment encoding a portion of the MB9 (residues 143-273) followed by a

5 (GGGGS)_{6x} polypeptide linker followed by the Fc region and an EcoRI site. An EcoRI site was introduced in the coding sequence of MB9 using the degeneracy of the genetic code to preserve the proper amino acid sequence and overlaps the first 6 bases of Genescript-FVII-026-2.

[00365] Genescript-FVII-027-1 was cloned into the SapI and EcoRI sites of
10 pSYN-FVII-011 to generate pSYN-FVII-036. Next, Genscript-FVII-026-2 was cloned into the EcoRI site of pSYN-FVII-036 to generate pSYN-FVII-027. Correct orientation of the last cloning step was confirmed by restriction enzyme analysis and DNA sequencing.

Example 2. Heterodimeric Constructs comprising FVII-Fc and MB9-Fc, MB9 at
15 **the carboxy terminus of the second Fc chain**

Cloning of FVII-037

[00366] The FVII-037 construct is made using an scFc scaffold which is not cleaved during processing. In this construct the targeting moiety, again the MB9 scFv which binds to GPIIb/IIIa is attached to the c-terminus of the second Fc moiety.

20 [00367] Synthesis of DNA fragment Genscript-FVII-037 was outsourced (Genscript) This fragment comprises a portion of the Fc region (residues 434 to 447, EU numbering) followed by a (GGGGS)_{4x} polypeptide linker and the MB9 scFv. A SapI/EcoRI fragment of Genscript-FVII-037 was subcloned into the SapI/EcoRI of pSYN-FVII-011 (refer to P0830) to generate an intermediate construct. A SapI
25 fragment from pSYN-FVII-011 was subcloned into the SapI sites of the intermediate construct to generate pSYNFVII-037.

Example 3. Heterodimeric Constructs comprising FVII-Fc and a peptide against GPIb at the carboxy terminus of the second Fc chain

Cloning of THE pSYN-FVII-041 intermediate construct.

30 [00368] In order to make this construct, the FVII-041 construct was first made as an intermediate. Synthesis of DNA molecule Genscript-FVII-041 was outsourced (Genscript). This fragment was digested with SapI and cloned into SapI sites of pSYN-FVII-011 to generate pSYN-FVII-041. This process introduces a unique SalI site (residues 412-413 EU numbering, GTG GAC to GTC GAC) in the second Fc.

35 Cloning of pSYN-FVII-044-, -045 and -046.

[00369] The FVII-041 construct was used as the starting material to generate several constructs that comprise targeting moieties which are peptides that bind to GPIb. The PS4 peptide is used in the -044 construct, the OS1 peptide in the -045

5 construct, and the OS2 peptide in the -046 construct. In these constructs an scFc scaffold is used and the peptides are attached via a linker to the C-terminus of the second Fc moiety.

[00370] Synthesis of Genscript-FVII-044, -045 and -046 was outsourced (Genscript). These DNA fragments were cleaved with SalI/EcoRI and subcloned into
10 the SalI/EcoRI sites of pSYN-FVII-041 to generate pSYN-FVII-044, -045 and -046.

Example 4. Heterodimeric Constructs comprising FVII-Fc and a peptide against GPIIb at the amino terminus of the second Fc chain

Cloning of the pSYN-FVII-043 intermediate.

[00371] In order to make this construct, the FVII-043 construct was first made
15 as an intermediate. Synthesis of DNA fragment Genscript-FVII-043 was outsourced (Genscript). This fragment comprises a DNA molecule encoding a portion of the Fc region (residues 232 to 447, EU numbering) followed by a (GGGGS)₄x polypeptide linker and another portion of the Fc region (residues 221 to 238, EU numbering). This DNA fragment was digested with BspEI and RsrII and subcloned into the BspEI/RsrII
20 sites of pSYN-FVII-042 to generate pSYN-FVII-050. This process introduces a unique SalI site (residues 412-413 EU numbering, GTG GAC to GTC GAC) in the first Fc. A HindIII/EcoRI fragment of pSYN-FVII-050 was subcloned into the HindIII/EcoRI sites of pSYN-FVII-011 to generate pSYN-FVII-043.

Cloning of pSYN-FVII-047, -048 and -049.

25 [00372] The FVII-043 construct was used as the starting material to generate several constructs that comprise targeting moieties which are peptides that bind to GPIIb. The PS4 peptide is used in the -047 construct, the OS1 peptide in the -048 construct, and the OS2 peptide in the -049 construct. In these constructs an scFc scaffold is used and the peptides are interposed between the scFc linker and a linker
30 which is attached to the N-terminus of the second Fc moiety.

[00373] Synthesis of DNA molecules Genscript-FVII-047, -048 and -049 was outsourced (Genscript). A SalI/RsrII fragment from Genscript-FVII-047, -048 and -049 was subcloned into SalI/RsrII sites of pSYN-FVII-043 to generate pSYN-FVII-047, -048 and -049, respectively.

35 **Example 5. Heterodimeric Constructs comprising a Gla-deleted FVII-Fc and a targeting molecule**

Cloning of the FVII-028 intermediate

- 5 [00374] In order to make this construct, the FVII-028 construct was first made as an intermediate. Synthesis of DNA fragment Genscript-FVII-028 was outsourced (Genscript). This fragment was cut with HindIII/XbaI and subcloned into pSYN-FVII-011 to generate pSYN-FVII-028.

Cloning of FVII-053

- 10 [00375] The FVII-028 construct was used as the starting material to generate a construct that comprises a targeting moiety and employs a clotting factor that lacks a Gla domain. For this construct, amino acids 1-35 were removed from FVII and an RKRRKR insertion was added after residue R152 (WT FVII numbering) to facilitate intracellular activation. The MB9 scFv served as the targeting moiety.
- 15 [00376] DNA molecule Genscript-FVII-025 was outsourced and an XbaI/BsiWI fragment from this molecule was subcloned into XbaI/BsiWI sites of pSYN-FVII-028 to generate pSYN-FVII-053

Example 6. Heterodimeric Constructs comprising a Factor VII heavy and light chains as two separate polypeptides.

20

Cloning of pSYN-FVII-024 intermediate construct

- [00377] The FVII-024 construct is one in which the heavy and light chains of factor FVII are not contiguous in a single chain molecule. The construct employs cscFc such that the cscFc linker is cleaved by proteases in the trans-Golgi network
- 25 This cleavage results in linker removal as well as activation of FVII, resulting in the expression of activated FVIIaFc.

- [00378] The coding sequence of FVII was obtained by reverse transcription coupled to polymerase chain reaction from a human liver mRNA library (Ambion, Austin, Texas) using the following primers:

- 30 FVII-F1
GGGAATGTCAACAGGCAGGG
FVII-R1
CTTGGCTTTCTCTCCACAGGC

- 35 [00379] A 50 µl reaction was carried out with 10 pmol of each primer using the Superscript One-step RT-PCR with Platinum Taq system (Invitrogen, Carlsbad, Calif.) according to the manufacturer's standard protocol in a MJ thermocycler. The cycle used was 50° C for 30 minutes for the reverse transcription followed by

- 5 denaturing at 94° C for 2 minutes and 30 cycles of (94° C 30 seconds, 53° C 30 seconds, 72° C 90 seconds) followed by 10 minutes at 72° C. The expected sized band (~1400 bp) was gel-purified with a Gel Extraction kit (Qiagen, Valencia, Calif.) and cloned in pCR2.1 TOPO using the TOPO TA Cloning kit (Invitrogen, Carlsbad, Calif.) to produce the intermediate plasmid pSYN-FVII-001. To construct a plasmid
10 for the expression of a two-chain FVII-Fc and Fc heterodimer, the FVII coding sequence was PCR-amplified using the following primers:

HindIII-Kozak-FVII-F

CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

BspEI-Fc-FVII-R

- 15 CGACTCCGGAGCTGGGCAACGGTGGGCATGTFGTGAGTTTTGTCTCGGAAATGGGGCTCGCAGG

[00380] The forward primer HindIII-Kozak-FVII-F adds a HindIII restriction site followed by a Kozak sequence immediately upstream of the FVII coding region. The reverse primer BspEI-Fc-FVII-R adds a fragment of the constant region of IgG1 (the Fc region) comprising amino acids 221-233 (EU numbering). This process also
20 incorporates a BspEI restriction site at amino acids 231-233 using the degeneracy of the genetic code to preserve the correct amino acid sequence (EU numbering). A 50 ul reaction was carried out with 15 pmol of each primer and template pSYN-FVII-001 using Platinum Pfx DNA Polymerase system according to manufacturer's protocol in a MJ Thermocycler using the following cycles: 95° C 2 minutes; 30 cycles of (95° C
25 15 seconds, 49° C 30 seconds, 68° C 90 seconds); 68° C 10 minutes. Plasmid pSYN-FIX-027 (pBUD FIXFc/Fc) was digested with HindIII and BspEI and the expected sized band for the vector (approximately 5800 bp) was purified away from the FIX insert (expected size band approximately 1480 bp) with a Gel Extraction kit (Qiagen, Valencia, Calif.). Next, the PCR-amplified FVII sequence was subcloned into HindIII
30 and EcoRI sites of the vector derived from pSYN-FIX-027 after removing the FIX insert. This generated pSYN-FVII-002 (pBUD FVIIFc/Fc). Next, A (GGGGS)_{6x} polypeptide linker was added between FVII and the Fc region coding sequences in pSYN-FVII-002 using the following primers:

FVII-linker-F:

- 35 CATCCCCAGCACGTACGTCC

FVII-Linker-R:

GGGCATGTGTGAGTTTTGTCTGATCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGAT

CCGCCGCCACCGGACCCACCTCCGCCGAGCCACCGCCACCGGAAATGGGGCTCGCAGGAGG

Fc-linker-F:

5 GACAAACTCACACATGCCCACC

Fc-linker-R:

GCAGAATTCTCATTACCCGGAG

[00381] Two 12 µl PCR reactions were carried out with either 12 pmol of FVII-linker-F and FVII-Linker-R (reaction 1) or Fc-linker-F and Fc-linker-R (reaction 10 2) using Expand High Fidelity System (Boehringer Mannheim, Indianapolis, Ind.) according to manufacturer's standard protocol in a MJ Thermocycler. The first and second reactions were carried out with 1 µg of pSYN-FVII-002 as template using the following cycle: 94 °C. 2 minutes; 14 cycles of (94°C. 30 seconds, 55°C. 30 seconds, 72°C. 2 minutes); 72°C. 10 minutes. The expected sized bands (532 bp for reaction 1 15 and 670 bp for reaction 2) were gel purified with a Gel Extraction kit (Qiagen, Valencia, Calif.), then combined in a PCR reaction with 25 pmol of FVII-linker-F and Fc-linker-R as before, but with 30 rounds of amplification. The expected sized band (1200 bp) was gel purified with a Gel Extraction kit (Qiagen, Valencia, Calif.) and digested with restriction enzymes KpnI and EcoRI. The expected sized band (920 bp) 20 was gel purified as before and cloned into the KpnI/EcoRI sites of pSYN-FVII-002 to generate pSYN-FVII-003 (pBUD FVIIFc/6x(GGGGS)/Fc).

Cloning of pSYN-FVII-024 to express a two-chain heterodimer

[00382] Plasmid (pSYN-FVII-024) was generated for the expression of a two-chain heterodimer where one chain consists of the FVII light chain (residues 1-152) 25 followed by a (GGGGS)_{6x} linker followed by the Fc region, while the other chain contains a FVII heavy chain (residues 153 to 406) followed by a (GGGGS)_{6x} linker followed by the Fc region. The plasmid is designed to express the heterodimer as a single polypeptide where the C-terminus of the FVII heavy chain-linker-Fc chain is connected to the N-terminus of the heavy chain-linker-Fc chain by the following 30 polypeptide sequence: RRRRS-(GGGGS)_{6x}-RKRRKR, where the RRRRS and RKRRKR sequences are proprotein convertase cleavage sites. Intracellular cleavage by proprotein convertases following the last Arg at each cleavage site can result in removal of the polypeptide linker. Consequently, cells will express a 2 chain heterodimer where the FVII light chain-linker-Fc chain has a RRRRS sequence at the 35 C-terminus, but the remainder of the linker and the RKRRKR sequence have otherwise been removed. Construction of the pSYN-FVII-024 and several intermediate plasmids required the use of the following primers:

HindIII-SalI-BpEI-Fc-F

- 5 AGTCAAGCTTGTGCGACTCCGGAACCTCCTGGGCGGACC
 BamHI-linker(PACE1)-Fc-R
 CATCGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCACCTGATCCGCCGCCACCGCTCC
 GGCGGCGCCGTTTACCCGGAGACAGGGAGAGG
 HindIII-Kozak-FVII-F
- 10 CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
 BspEI-Fc-linker-FVIIIC-R
 GAGTTCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTGTCTGATCCCCCGCCACCGGAACCTCCACC
 GCCTGATCCACCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCTCGGCC
 TTGGGGTTTGCTGG
- 15 BamHI-2xlink-pace-HC-F
 CAGTCTGGATCCGGCGGTGGAGGTTCCGGTGGGGGTGGATCAAGGAAGAGGAGGAAGAGGATTGTGGGG
 GGCAAGGTGTGCC
 Fc-EcoRI-R
 ATGTCTGAATTCTCATTTACCCGGAGACAGGGAGAGG
- 20 **[00383]** To generate the first intermediate plasmid, a PCR reaction was
 performed with 25 pmol of primers HindIII-Sall-BpEI-Fc-F and BamHI-
 linker(PACE1)-Fc-R and template pSYN-Fc-001 using Expand High Fidelity System
 (Boehringer Mannheim, Indianapolis, Ind.) according to manufacturer's standard
 protocol in a MJ Thermocycler. The following cycles were used: 95° C 2 minutes; 30
- 25 cycles of (95° C 30 seconds, 58° C 30 seconds, and 72° C 1 minute); 72° C 10
 minutes. The correct sized band (approximately 730 bp) was gel purified as above and
 cloned into the HindIII/BamHI sites of pBUDCE4 vector (Invitrogen, Carlsbad,
 Calif.), generating pSYN-FVII-014. PCR amplification with primers HindIII-Sall-
 BpEI-Fc-F and BamHI-linker(PACE1)-Fc-R generated a DNA fragment encoding a
- 30 portion of the Fc region (Amino A X-Y) followed by an RRRRS sequence and
 (GGGGS)_{2x} polypeptide linker. Primer HindIII-Sall-BpEI-Fc-F introduces a HindIII
 and Sall restriction site at the 5' end of the molecule, while primer BamHI-
 linker(PACE1)-Fc-R introduces a BamHI at the 3' end that overlaps the codons
 encoding the last 2 residues of the GGGGS linker (residues GS with codons GGA
- 35 TCC). Next, another PCR reaction was performed as above with primers HindIII-
 Kozak-FVII-F and BspEI-Fc-linker-FVIIIC-R and template pSYN-FVII-002 using
 the same conditions described for cloning of pSYN-FVII-014, but with an annealing
 temperature of 57° C The expected sized band (approximately 700 bp) was gel
 purified and cloned into the HindIII and BspEI sites of pSYN-FVII-014 to generate
- 40 pSYN-FVII-023. Primers HindIII-Kozak-FVII-F and BspEI-Fc-linker-FVIIIC-R

5 amplified a DNA fragment encoding the FVII light chain followed by a (GGGGS)_{6x} polypeptide linker and a portion of the Fc region up to amino acid 232 (EU numbering). Primer HindIII-Kozak-FVII-F introduces a HindIII restriction site at the 5' end of the molecule followed by a Kozak sequence while primer BspEI-Fc-linker-FVII-LC-R adds a BspEI site at the 3' end of the molecule.

10 In the final step a PCR reaction was carried out as above with primers BamHI-2xlink-pace-HC-F and Fc-EcoRI-R and template pSYN-FVII-003 with the following cycles: 95° C 2 minutes; 30 cycles of (95° C 30 seconds, 55° C 30 seconds, and 72° C 2 minute); 72 ° C 7 minutes. This PCR reaction generated a DNA molecule encoding a (GGGGS)_{2x} polypeptide linker followed by a RKRRKR sequence followed by the
 15 FVII heavy chain. Primers BamHI-2xlink-pace-HC-F and Fc-EcoRI-R introduce a BamHI site and an EcoRI site at the 5' and 3' end of the molecule, respectively. The expected sized band (approximately 1600 bp) was cloned into the BamHI and EcoRI sites of pSYN-FVII-023 to generate pSYN-FVII-024.

20 Cloning of intermediate pSYN-FVII-073

A silent mutation was introduced in the first Fc moiety of FVII-024 by PCR-based site-directed mutagenesis methods, resulting in the generation of a Sall site at DNA region encoding amino acids in position 412 and 413 (EU numbering). This generated the intermediate construct FVII-073

25 **Cloning of pSYN-FVII-057**

The synthesis of the DNA sequence comprising nucleotides from the Sall to BsiWI sites of pSYN-FVII-057 was outsourced. This DNA was subcloned into the Sall/BsiWI sites of pSYN-FVII-073 to generate pSYN-FVII-057

30 **Cloning of pSYN-FVII-058, pSYN-FVII-059, pSYN-FVII-060, pSYN-FVII-061 and pSYN-FVII-062**

These constructs were cloned as described for pSYN-FVII-057 (outsourced synthesis of DNA from Sall to BsiWI and subcloned into pSYN-FVII-073)

Cloning of pSYN-FVII-066

35 The synthesis of the DNA sequence comprising nucleotides from the Sall to RsrII sites of pSYN-FVII-066 was outsourced. This DNA was subcloned into the Sall/RsrII sites of pSYN-FVII-043 to generate pSYN-FVII-066

Cloning of pSYN-FVII-067

- 5 The synthesis of the DNA sequence comprising nucleotides from the Sall to EcoRI sites of pSYN-FVII-067 was outsourced. This DNA was subcloned into the Sall/EcoRI sites of pSYN-FVII-041 to generate pSYN-FVII-067

Cloning of pSYN-FVII-090

- The synthesis of the DNA sequence comprising nucleotides from the BamHI to BsiWI sites of pSYN-FVII-090 was outsourced. This DNA was subcloned into pSYN-FVII-061 by 3-way ligation (where the outsourced DNA was cut with BamHI/BsiWI and pSYN-FVII-061 with BamHI/BsiWI/NotI) to generate pSYN-FVII-090

Cloning of pSYN-FVII-100

- 15 A portion (amino acids 311 to 322 of the FVII mature sequence) of the 170 loop of FVII was replaced with the 170 loop of trypsin (amino acids EASYPGK). This mutation was introduced by standard overlapping PCR methods using the pSYN-FVII-090 as template and backbone structure to generate pSYN-FVII-100

Cloning of pSYN-FVII-115

- 20 A triple point mutation (V158D, E296V and M298Q; mature FVII sequence numbering) was introduced into the FVII coding region of pSYN-FVII-090 by PCR-based site-directed mutagenesis to generate pSYN-FVII-115

Cloning of pSYN-FVII-118

- The synthesis of the DNA sequence comprising nucleotides from the XbaI to BsiWI sites of pSYN-FVII-118 was outsourced. This DNA was subcloned into the XbaI/BsiWI sites of pSYN-FVII-011 to generate pSYN-FVII-118

Cloning of pSYN-FVII-119

- The synthesis of the DNA sequence comprising nucleotides from the XbaI to BsiWI sites of pSYN-FVII-119 was outsourced. This DNA was subcloned into the XbaI/BsiWI sites of pSYN-FVII-011 to generate pSYN-FVII-119

Cloning of pSYN-FVII-127

- A DNA fragment comprising the 170 loop of trypsin was generated by PCR using pSYN-FVII-100 as template. This PCR reaction generated BsiWI and BspEI restriction sites at the 5' and 3', respectively. The DNA fragment was subcloned into the BsiWI/BspEI sites of pSYN-FVII-118 to generate pSYN-FVII-127.

Cloning of pSYN-FIX-042

- 5 A HindIII/BspEI fragment from pSYN-FIX-030 (as described in US Patent 7566565) was subcloned into the HindIII/BspEI sites of pSYN-FVII-011 to generate pSYN-FIX-042

Cloning of pSYN-FIX-068

- A HindIII/BspEI fragment from pSYN-FIX-030 (plasmid described in full in
10 **US7566565**) was subcloned into the HindIII/BspEI sites of pSYN-FVII-066 to generate pSYN-FIX-068

Cloning of pSYN-FIX-088

A BspEI-EcoRI fragment from pSYN-FIX-067 was subcloned into BspEI-EcoRI sites of pSYN-FIX-053 to generate pSYN-FIX-088

- 15 **Cloning of pSYN-FIX-089**

A BspEI-EcoRI fragment from pSYN-FIX-048 was subcloned into BspEI-EcoRI sites of pSYN-FIX-053 to generate pSYN-FIX-089

Cloning of pSYN-FIX-090

- A DNA fragment comprising the FIX coding region from the XbaI site to the C-
20 terminus of the protein followed by a 6x(GGGGGS) linker, the SCE5 coding sequence and an EcoRI site was outsourced for synthesis and subcloned into the XbaI/EcoRI sites of pSYN-FIX-053 to generate pSYN-FIX-090. The SCE5 sequence is set forth below:

- 25 A QVQLQESGGGLVQPGGSLRLSCAASGFMFSRYAMSWVRQAPGKGP EWVSGISGSGGSTYYADSVKGRF
TVSRDNSKNTLYLQMNSLRAEDTAVYYCARGATYTSRSDVPDQTSFDYWGQGT LVTVSSGSASAPKLEE
GEFSEARVSELTQDPAVSV ALGQTVRI TCQGD SLRNFYASWYQQKPGQAPT LVIYGLSKRPSGIPDRFS
ASSSGNTASLTITGAQAEDEADYYCLLYGGGQQGVFGGGTKLTVLRQPKAAPSVTLFPPSSAA

Cloning of pSYN-FVII-094

- A DNA fragment comprising a sequence encoding a 6x(GGGGS) linker followed by
30 the SCE5 coding sequence was synthesized (outsourced) and cloned into the EcoRV/EcoRI sites of a pSYN-FVII-011 variant that had been previously modified to generate an EcoRV site at the C-terminus of the FVII coding region

Cloning of pSYN-FVII-088

- The synthesis of the DNA sequence comprising nucleotides from the SalI to RsrII
35 sites of pSYN-FVII-088 was outsourced. This DNA was subcloned into the SalI/RsrII sites of pSYN-FVII-066 to generate pSYN-FVII-088

Cloning of pSYN-FVII-125

A DNA fragment was PCR amplified from pSYN-FVII-088, comprising the AP3 region and part of the linker. This PCR reaction generated BamHI and EcoRI sites at

5 the 5' and 3' of the DNA fragment, respectively. This DNA fragment was subcloned into the BamHI/EcoRI sites of pSYN-FVII-011 to generate pSYN-FVII-125

Cloning of pSYN-FVIII-041

The coding sequence of human recombinant B-domain deleted FVIII was obtained by reverse transcription-polymerase chain reaction (RT-PCR) from human liver poly A
 10 RNA (Clontech) using FVIII-specific primers. The FVIII sequence includes the native signal sequence for FVIII. The B-domain deletion starts after serine 743 (S743; 2287 bp) and ends before glutamine 1638 (Q1638; 4969 bp) for a total deletion of 2682 bp (SQ version).

The coding sequence for human recombinant Fc was obtained by RT-PCR
 15 from a human leukocyte cDNA library (Clontech) using Fc specific primers. Primers were designed such that the B-domain deleted FVIII sequence was fused directly to the N-terminus of the Fc sequence with no intervening linker. The FVIII-Fc DNA sequence was cloned into the mammalian dual expression vector pBUDCE4.1 (Invitrogen) under control of the CMV promoter.

20 A second identical Fc sequence including the mouse Igk signal sequence was obtained by RT-PCR and cloned downstream of the second promoter, EF1 α , in the expression vector pBUDCE4.1. This final construct was designated pSYN-FVIII-013.

A second plasmid was created from similar constructs using PCR and standard
 25 molecular biology techniques, in order to express rFVIII-BDD-Fc-Fc in which the rFVIII-BDD-Fc coding sequence was fused to the second Fc sequence with a (GGGGS)₄ linker, allowing for production of only the rFVIII-BDD-Fc monomer-dimer hybrid in transient transfection. This construct was designated pSYN-FVIII-041.

Cloning of pSYN-FVIII-049

Generated intermediate pSYN-FVIII-048 by cloning NheI/XhoI fragment from pBUD-CE4.1 into pSYN-FVIII-013. The synthesis of a DNA fragment comprising the region from RsrII to XbaI sites of pSYN-FVIII-049 was outsourced. This
 35 fragment was subcloned into the RsrII/XbaI sites of pSYN-FVIII-048 to generate pSYN-FVIII-049

Cloning of pSYN-FVIII-108

A Sall/RsrII fragment from pSYN-FVII-066 was subcloned into pSYN-FVIII-049 to generate pSYN-FVIII-108

5 **Example 7. Additional Attempts at Expression of Activated Constructs**

[00384] Several other constructs were made with the goal of expressing activated FVII. However, these constructs did not successfully express activated molecules. By Western blot it was demonstrated that that the FVII heavy chain cannot be expressed with a free N terminus using a common method of fusing a
10 heterologous signal peptide to the N-terminus of the heavy chain.

Cloning of pSYN-FVII-010

[00385] The FVII-010 construct is one in which the heavy chain of factor VII was expressed in the context of an scFc scaffold and the light chain was expressed separately.

15 [00386] PCR-amplify with primer pairs FVII-HC-Hind3-IggKss-F/FVII-HC-BspEI-R, using pSYN-FVII-001 (see supra.). Clone in BspEI/HindIII sites of pSYN-FVII-008 (see supra), generating pSYN-FVII-009.

[00387] PCR amplify FVII light chain from pSYN-FVII-003 (refer to P0830) with primers FVII-LC-NotI-F/ FVII-LC-XhoI-R and clone in pSYN-FVII-009 to
20 generate pSYN-FVII-010

Primers

FVII-HC-BspEI-R

AGGAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTTCGGATCC
CCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCGGCC
25 ACCGGACCCACCTCCGCCGAGCCACCGCCACCGGGAAATGGGGCTCGCA
GGAGG

FVII-HC-Hind3-IggKss-f

ACTGACAAGCTTGCCGCCACCATGGAGACAGACACACTCCTGCTATGGGT
30 ACTGCTGCTCTGGGTTCCAGGTTCCACTGGTATTGTGGGGGGCAAGGTGTG
C

FVII-LC-NotI-F

ACTGACGCGGCCGCGCCGCCACCATGGTCTCCCAGG

35

FVII-LC-XhoI-R

ACTGACCTCGAGTTATCGGCCTTGGGGTTTGCTGG

40 Cloning of pSYN-FVII-013

[00388] The FVII-013 construct is one in which the light chain was expressed in the context of an scFc scaffold and the heavy chain was expressed separately.

[00389] PCR-amplify with primer pair FVII-LC-linker-BamHI-R/ HindIII-Kozak-FVII-F from pSYN-FVII-001 (refer to P0830) and clone in BamHI/HindIII

- 5 sites of pSYN-FVII-011, generating pSYN-FVII-012. PCR-amplify FVII-HC from pSYN-FVII-009 using primer pair FVII-HC-NotI-F/FVII-HC-XhoI-R and subclone in pSYN-FVII-012 to generate pSYN-FVII-013

Primers

- 10 FVII-LC-6xlinker-BamHI-
 RACTGACGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCCA
 CCTGATCCGCGCCACCGGACCCACCTCCGCGGAGCCACCGCCACCTCG
 GCCTTGGGGTTTGCTGGC
 HindIII-Kozak-FVII-F
 15 CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
 FVII-HC-NotI-F
 ACTGACGCGGCCGCGCCGCCACCATGGAGACAGAC
 FVII-HC-XhoI-R
 ACTGACCTCGAGTTAGGGAAATGGGGCTCGCAGGAG

20

Cloning of pSYN-FVII-018

[00390] For the FVII-018 construct, the heavy chain of FVII was expressed as an Fc fusion protein and the light chain of FVII was separately expressed as a separate Fc fusion protein.

- 25 [00391] Primers FVII-HC-Hind3-IggKss-F/scFc-EcoRI-R were used to PCR amplify HCFVII-linker-Fc, using pSYN-FVII-010 as template. Subclone in HindIII/EcoRI sites of pBUDCE4. This makes pSYN-FVII-017. Next, PCR-amplify from pSYN-FVII-013 with primers FVII-LC-NotI-F/FC-XHOI-R and subclone in XhoI/NotI sites of FVII-017. This makes PSYN-FVII-018

30

Primers

scFc-EcoRI-R
 ACTGACGAATTCTCATTACCCGGAGACAGGGAG

Fc-XhoI-R

- 35 AGCTCTCGAGTCATTACCCGGAGACAGGG

Example 8. Attempts at Expression of Activatable Constructs

Cloning of FVII-039, -040

- 40 [00392] Several constructs were made in an attempt to generate constructs in which Factor VII can be activated in vivo at the site of clotting using an appropriate cleavage site, in this case the DFTR Factor XIa cleavage site.

[00393] The 039 construct was made in the context of an scFc scaffold. The construct included the FVII light chain, the FXIa cleavage site, and the FVII heavy

- 45 chain with a I153V mutation in linear sequence attached to the N-terminus of the first Fc moiety.

5 The 040 construct was also made in the context of an scFc scaffold. The construct included the FVII light chain with an R152 deletion, the FXIa cleavage site, and the FVII heavy chain with an I153V mutation in linear sequence attached to the N-terminus of the first Fc moiety. The DFTR cleavage sequence is a natural FXIa sequence found in FIX. In FIX, the DFTR sequence is followed by a valine, so an
10 I152V mutation was introduced in pSYN-FVII-039, -040 to increase FXIa cleavage efficiency

[00394] Synthesis of DNA molecule Genscript-FVII-039 and -040 was outsourced (Genscript). An XbaI/BsiWI fragment from Genscript-FVII-039 and -040 was subcloned into XbaI/BsiWI sites of pSYN-FVII-011 to generate pSYN-FVII-039
15 and -040, respectively

Example 9. Transient Transfection of Constructs

[00395] For expression of constructs, HEK-293-F cells were grown in
20 suspension in Freestyle media (Invitrogen) supplemented with vitamin K3 (For FVII and FIX transfections only) (Sigma Aldrich, St. Louis, MO) to 2 µg/liter (growth media) as suspension cells at 37° C/10% CO₂. Cells were subcultured every three to four days by seeding at cell density of 5x10⁵ cells/ml.

[00396] Twenty-four hours prior to transfection cells were seeded at a density
25 of 7x10⁵ cells/ml in growth media supplemented with LONGTMR3IGF-1 (Sigma Aldrich, St. Louis, MO) to 20 µg/liter (transfection media). On the day of transfection, a transfection solution was made with a volume equal to 5% of the total volume of the cell culture to be transfected. In the transfection solution DNA was added (final concentration 20 mg/L) to a freshly made solution of PEI (60 mg/L) in
30 transfection media. The solution was swirled for 30 seconds and incubated for five minutes at room temperature before adding directly to the cell culture. Four hours later a volume equal to the cell culture volume of OptiCHO (Invitrogen) supplemented with vitamin K3, LONGTMR3IGF-1 and 200 mM L-glutamine was added to the cells. The cell culture was allowed to grow as shown above and daily
35 media samples were taken to assess protein expression. On the day of harvest, the cells were spun down and the media filtered in preparation for protein purification or protein analysis by protein A pulldown/western blot.

5 **Example 10. Protein Purification of FVIIFc Molecules (except FVII-028 and FVII-053) and FIXFc molecules**

[00397] FVIIFc molecules were purified from conditioned media using the following columns: 1) Anion exchange chromatography with pseudo-affinity elution (e.g. Q sepharose 4FF (GE Healthcare) followed by elution with varying levels of
10 CaCl₂ to selectively elute the most active species), followed by 2) shFcRn (soluble human FcRn) affinity (NHS- coupled shFcRn with sepharose 4FF beads) chromatography, binding Fc-containing proteins at low pH (e.g. pH 6.2) and eluting at neutral pH (e.g. pH 8.0). In some cases, an additional step was included utilizing cation exchange chromatography with NaCl elution. These purification steps utilized
15 standard methods known to those in the art to generate purified proteins of >95% purity by SEC analysis and SDS-PAGE. FIXFc proteins were purified as previously described in US Patent 7,566,565.

Example 11. Protein Purification of FVII-028 and FVII-053

[00398] FVII-028 and -053 were purified from conditioned media using the
20 following columns: 1) Hydrophobic interaction chromatography (e.g. Phenyl FF (high sub) (GE Healthcare)), followed by 2) Anion/cation exchange chromatography with salt elution. These purification steps utilized standard methods known to those in the art to generate purified proteins of >95% purity by SEC analysis and SDS-PAGE.

Example 12. Purification of FIX-090

25 [00399] FIX-090 was purified through a 2-step chromatography process, first using an immunoaffinity chromatography step with an anti-GLA domain antibody, followed by anion exchange chromatography using pseudoaffinity elution similar to FIXFc proteins described above. These purification steps utilized standard methods known to those in the art to generate purified proteins of >95% purity by SEC
30 analysis and SDS-PAGE. .

Example 13: Purification of FVIII-Fc proteins

[00400] FVIII-Fc proteins were purified from clarified and chemically defined harvest media using a two or three column purification process, including a FVIII-specific affinity purification step (McCue 2009) followed by a combination of anion
35 exchange with standard NaCl elution and/or shFcRn (soluble human FcRn) affinity (NHS- coupled shFcRn with sepharose 4FF beads) chromatography, binding Fc-containing proteins at low pH (e.g. pH 6.2) and eluting at neutral pH (e.g. pH 8.0).

- 5 These purification steps utilized standard methods known to those in the art to generate purified proteins of >95% purity by SEC analysis and SDS-PAGE.

Example 14. Activation of FVII constructs

- [00401] Fractions eluted from the FcRn column containing FVIIIFc were
 10 pooled, and total protein was concentrated to 4 mg/ml. The CaCl_2 concentration was raised to 5 mM and the sample was incubated at 4°C for 24 to 48 hours until at least 80% of FVIIIFc was activated. The extent of activation was assessed by SDS PAGE (Figure 10)

15 **Example 15. FVIIa activity assays, soluble tissue factor method**

- [00402] Specific activity of the FVIIaFc variants was determined by the soluble tissue factor method. Unlike lipidated full length tissue factor, soluble tissue factor (extracellular portion of tissue factor) can't activate FVII into FVIIa, but it acts as an activator of the conversion of factor X into factor Xa by FVIIa. To determine the
 20 specific activity of FVIIaFc variants, A STACLOT[®] FVII-rTF kit (Diagnostica Stago, Asnieres, France) was used following manufacturer's recommendations. Table 1 summarizes the data and shows comparable specific activity for all variants.

25 **Table 1.** Specific activity of FVIIaFc variants based on the soluble tissue factor method

30	FVIIaFc	IU/nM
	FVII-011	991
	FVII-024	929
	FVII-027	790
35	FVII-037	1131
	FVII-044	1300
	FVII-045	906
	FVII-046	1145
40	FVII-047	924
	FVII-048	973
	FVII-049	1130
45	FVII-053	929

5

Example 18. FACs assays to study binding of FVIIaFc and platelets

10 [00403] In this example, the following reagents and methods were used:

Reagents

ADP: Sigma Aldrich, cat# A2754, stock 1 mM, working concentration 10 μ M

SFLLRN peptide: in-house synthesis, stock concentration 5 mg/ml (6.7 mM),

working concentration 50 μ g/ml (67 μ M)

15 FVII antibody-FITC-labeled: Affinity Biologicals SAFVII-APFTC

Platelet buffer: 15 mM HEPES, 138 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 5 mM CaCl₂, 5.5 mM dextrose and mg/ml BSA, pH 7.4

Method

- Count platelets
- 20 • Add 20 μ l of $\sim 2\text{--}4 \times 10^8$ cells/ml gel-purified platelets to 1 ml of platelet buffer
- Make 100 μ l aliquot for each sample
- Add agonist and FVIIaFc (to desired concentration) as needed Incubate at 37C for 15 minutes
- Add equal volume of HBS/5 mM CaCl₂/1.5% formaldehyde, incubate 20' at RT
- 25 • Spin 15' at 3000g
- Wash in HBS/5mM CaCl₂/1 mg/ml BSA, spin again and resuspend in 100 μ l of platelet buffer.
- Add 2.5 μ l of FVII antibody-FITC-labeled and incubate for 30 ' at room temperature.
- 30 • Analyze by FACs

Example 17. Thrombin Generation assay

[00404] In this example, the following reagents and methods were used:

35

Reagents

FV: HTI, cat#HCV-0100, lot#Z0413, 5.1 mg/ml

Prothrombin: HTI, cat#HCP-0010, lot# Z0128, 4.8 mg/ml

FX: HTI, cat# HCV-0050, lot#X0401, 5.4 mg/ml

40 ATIII: HTI, cat# HCATIII-0120, lot#Y0401, 8.2 mg/ml

- 5 TFPI: American Diagnostica, cat# 4900PC, lot# 081031, 100 ug/ml
 Reader: Fluorskan, Thermo Electron Fluorometer
 Thrombin Calibrator: Thrombinoscope, cat# TS20.00
 Fluca: Thrombinoscope, cat# TS50.00
 Platelet buffer: 15 mM Hepes pH 7.4, 138 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 5.5
 10 mM Dextrose, supplemented with 1 mg/ml BSA before using
 ADP: Sigma Aldrich, cat# A2754, stock 1 mM, working concentration 10 uM
 SFLLRN peptide: in-house synthesis, stock concentration 5 mg/ml (6.7 mM),
 working concentration 50 ug/ml (67 uM)

15

	Primary stock (mg/ml)	Working solution (ug/ml)	[final] ug/ml
FV	5.1	105.6	4.4
FII	4.8	1200	54
FX	5.4	120	5
ATIII	8.2	1800	75
TFPI	0.1	1.44	0.06
Platelet	2-10E8	1.74E8	0.6E8
FVIIaFc	1 mg/ml (10 uM)	1200 nM	200 nM
FVIIaFc	1 mg/ml (10 uM)	200 nM	62.5 nM
FVIIaFc	1 mg/ml (10 uM)	62.5 nM	12.5 nM

- 20 Method
 [00405] -Set up software according to manufacturer's recommendations
 [00406] -Prewarm water and Fluca buffer
 [00407] -Make clotting factor mix. Dilute stock concentration of FV, FII, FX, ATIII and TFPI to make working solution. Need 5 ul/well, so for a 30 well assay
 25 prepare 180 ul of each Mix all the clotting factor solutions in a single bulk solution
 [00408] -Premake FVIIaFc dilutions. Make 1200 nM solution (12 ul into 88 ul of buffer) in 100 ul and dilute 4-fold twice (25 ul into 75 ul of buffer) to obtain 200 nM and 62.5 nM solutions
 [00409] -Make calibrator solution (1 ml of warm water in calibrator vial)
 30 [00410] -Add 20 ul of buffer or calibrator to the wells

- 5 [00411] -Add 25 ul of clotting factor mix to the wells (or 25 ul of buffer to calibrator wells)
- [00412] -Add 20 ul of FVIIaFc to the wells (or buffer to calibrator wells)
- [00413] -Add 35 ul of platelets (previously add ADP and SFLLRN). Add platelets to calibrator well
- 10 [00414] -Put plate in instrument, prepare Fluca buffer and start reaction (add 20 ul Fluca/well) 5 minutes after putting plate into instrument.

Example 18. Analysis of protein generated from transient transfections

- 15 [00415] For analysis of protein from transient transfections, conditioned media from transfections of pSYN-FVII-010, 011, -013, -018, -003, -019 -020 and -024 were subjected to protein A immunoprecipitation. Briefly, cell culture supernatant was mixed with approximately 50 ul of protein A-Sepharose 50% slurry and
- 20 incubated at 4°C with rocking for 1 hour, then centrifuged to pellet the protein A beads. Beads were washed twice by resuspending in 1 ml of PBS, spinning and aspirating. The beads were resuspended with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or nonreducing conditions, heated for 5 minutes at 100°C, spun down and loaded on SDS-PAGE gels and run according
- 25 to standard protocols. Gels were transferred to nitrocellulose membranes and Western blots were performed to detect the Fc region or the FVII light chain. For Fc detection, the antibody used was a goat anti-human IgG (Fc specific)-horseradish peroxidase conjugate (Pierce ImmunoPure antibody, catalog #31413). For FVII light chain detection an anti light chain monoclonal antibody was used (Green Mountain, clone
- 30 6MA-219). The antibodies were diluted 1:15,000 (for Fc detection) or 1:200 (for light chain detection) in PBST (PBS with 0.1% Tween-20) with 5% nonfat dry milk and incubated with the membrane for 1 hour at room temperature. The membrane was then washed in PBST 3 times for 10 minutes and signal was detected by a chemiluminescent method for Fc detection. For FVII light chain detection, the
- 35 membrane was further incubated for one hour in a solution containing HRP-labeled goat anti-mouse antibody (Southern Biotech, # 1010-05) diluted 1:5000 in PBST. The membrane was also washed in PBST 3 times for 10 minutes and the signal was detected by a chemiluminescent method. Chemiluminescent detection was performed

5 using ECL Plus Western Blotting Detection System (Amersham Biosciences catalog #RPN2132) according to manufacturer's protocol. Signal was visualized in a Storm 840 Phosphorimager (Molecular Devices).

[00416] The effect of PC5 on the processing of the proprotein convertase cleavage sites in the FVII-024 linker was tested as shown in Figure 26. Under
10 nonreducing conditions the effect of PC5 on cleavage site processing can not be detected because the FVII light chain-Fc and FVII heavy chain-Fc subunits remain linked via 2 disulfide bonds in the Fc region (lanes 2 and 3). Under reducing conditions we observed partial processing of FVII-024 generated from cells not cotransfected with PC5 (lane 4), but full processing when the cells were cotransfected
15 with PC5 (lane 5). Full processing of the linker results in secretion of activated FVII (FVIIa), since a free N-terminus of the heavy chain is required and sufficient to activate the protein.

20 **Example 19. Cleavage of FVII-039 and FVII-040 by FXIa**

[00417] The activation FVII-039 and FVII-040 by FXIa, as a result of the FXIa cleavage site inserted immediately upstream of the FVII light chain in these proteins, was characterized in vitro. A 1.5 μ M solution of FVII-039, FVII-040 or FVII-011
25 (non activated) containing 15 nM FXIa in 50 mM Tris-HCl, 100 mM NaCl, 10 mM CaCl_2 , pH 7.4 was incubated for 5 or 20 minutes, and cleavage of FVII-Fc was determined by SDS PAGE under reducing conditions, followed by SYPRO Ruby staining (Invitrogen). FXIa failed to cleave FVII-039, FVII-040 and nonactivated FVII-011, as shown in Figure 28.

30

Example 20. Alternative Activatable FVII-Fc constructs

Failure of FXIa to cleave FVII-039 and FVII-040 may have been caused by inaccessibility of the protease to the cleavage site by steric hindrance. To improve
35 FXIa or thrombin cleavage site accessibility, the sites will be placed upstream of the heavy chain in a structure where the heavy chain is not preceded by the light chain (Light chain-linker-Fc-scFclinker-FXIa/thrombin cleavage site-heavy chain-linker-Fc). In some embodiment, the heavy chain will comprise the I152V mutation. Once the best cleavage site is determined, a cscFc will be introduced so that the cell secretes

5 a heterodimeric protein with the following structure: light chain-linker-Fc which is disulfide bonded to a second chain: FXIa/thrombin cleavage site-heavy chain-linker Fc.

[00418] In order to improve the level of activation observed with the constructs shown in Figure 28, a second generation of activatable variants illustrated in Figure 31 (similar in structure to those shown in Figure 6E) was used to increase accessibility of the cleavage site. In this example, FXIa and thrombin cleavage sites were used for these constructs (See Figure 31). Constructs were transiently transfected as previously described. FVIIFc was captured from media with protein A. FVIIFc bound to the beads was put in buffer and FXIa or thrombin was added and incubated. FVIIFc was eluted from beads with SDS PAGE loading buffer at 100 C for 5 minutes. The gel was loaded and western blot performed to detect Fc as previously described and the results are shown in Figure 32. As shown in Figure 32, both the thrombin and factor XIa cleavage sites could be cleaved to yield FVII heavy and light chain molecules in the presence of the appropriate enzyme. Best cleavage was observed for constructs FVII-060 and FVII-061, while no cleavage was observed for the negative control (FVII-062) in the presence of thrombin.

Example 21. A Factor VII Activatable Construct

[00419] The constructs depicted in Figure 33 were (FVII-090, FVII-089 and FVII-062) were cloned, expressed and purified as previously described (these proteins do not require activation). Due to a cloning error a "VVGGA" sequence was inserted after the ALRPR thrombin cleavage sequence of FVII-060 and FVII-061, but while this insertion would be expected to affect the activity, it would not be predicted to affect the assessment of cleavage by thrombin in SDS-PAGE based assays. This sequence was removed in FVII-089 and FVII-090. To 125 nM of FVII-090, FVII-089, FVII-062, or plasma-derived FVII (FVII) increasing concentrations of thrombin were added and incubated for 10 minutes at 37°C. The mixture was run on SDS-PAGE gel to determine cleavage by thrombin (Figure 33). Generation of FVII light chain-Fc and FVII heavy chain-Fc was observed for FVII-089 and FVII-090 after incubation with thrombin. The fact that there was no cleavage of plasma-derived FVII or the FVII-062 negative control by thrombin shows specificity. No significant difference in cleavage efficiency was observed for FVII-089 and FVII-090.

5 [00420] Thrombin generation assays were used to measure activity of
 activatable variant FVII-090. A thrombin generation assays in FVIII-deficient
 platelet-rich plasma was performed as previously described, but replacing clotting
 factors and platelets with FVIII-deficient platelet-rich plasma. The results depicted in
 Figure 34 are from an assay in which thrombin generation was activated with 50 nM
 10 of FVIIaFc. As shown in Figure 34, thrombin is generated by 50 nM of FVIIaFc.
 The addition of 200 nM FVII-090 (not FVII-062, the negative control) to 50 nM of
 FVIIaFc results in a significant increase in thrombin generation, suggesting that FVI-
 090 becomes activated by thrombin generated by FVIIaFc. FVII-090 in the absence of
 any FVIIaFc activation also shows increased thrombin generation relative to FVII-062
 15 in the absence of activation. This could be caused by activation of FVII-090 from
 small amounts of thrombin generated by residual levels of tissue factor or contact
 pathway activation.

20 **Example 22. A High Specific Activity Factor VII Activatable Construct**

[00421] To make the high specific activity version of Factor VII, FVII-100,
 amino acids 311 to 322 of the FVII mature sequence (LQQRKVGDSPN,
 corresponding to the 170- loop) from FVII-090 , were replaced with amino acids
 EASYPGK from the 170-loop of trypsin. This substitution has been shown to confer
 25 high specific activity.

[00422] An additional high specific activity version of Factor VII, FVII-115,
 was constructed. In this version, the 170 loop is wild type, but there are three point
 mutations in the heavy chain of FVII, V158D, E296V and M298Q. FVII-100 and
 FVII-115 are illustrated in Figure 41

30 [00423] In a soluble tissue factor assay, the specific activity of FVII-011(wild
 type FVIIaFc) is 10,000 IU/mg. FVII-090 has a specific activity of 0.32 IU/mg, FVII-
 100 has a specific activity of 0.25 IU/mg, and FVII-115 has a specific activity of 14
 IU/mg. Thus, each of the activatable forms (prior to activation by the appropriate
 enzyme) is essentially inactive in this assay.

35 [00424] In the context of activated FVII, such high specific activity variants
 have the potential to be more efficacious, but also to be more susceptible to inhibition
 by proteins such as antithrombin. This inhibition depends on FVIIa being active;
 therefore, high specific activity variants which are activatable (dosed as nonactive

5 proteins) should be more resistant to antithrombin inhibition while having the potential to have high specific activity once activated at the site of injury.

[00425] The cleavage of purified FVII-090 and FVII-100 and 115 (high specific activity variants) by thrombin was tested as previously described. The results are shown in Figure 35. SDS PAGE analysis showed how the 3 proteins were cleaved
10 by thrombin with comparable efficiency. For FVII-100, FVII heavy chain-Fc and light chain Fc collapse in 1 band because a glycosylation site is removed from the heavy chain after insertion of the trypsin 170 loop, reducing the mass of the FVII HC-Fc band which therefore migrates faster on the gel and comigrates with the FVII LC-Fc band. Thrombin generation assays were used to measure activity of activatable
15 variant FVII-090 and high specific activity variant FVII-100. As set forth previously herein, thrombin generation was tested in a reconstituted system with human platelets, Factor X, Factor V, prothrombin, antithrombin and tissue-factor pathway inhibitor. Activity was measured with or without 5 nM thrombin activation.

[00426] As shown in Figure 36, activity of FVII-090 is enhanced in the
20 presence of thrombin, suggesting activation of FVII-090 by thrombin. However, activity is significantly increased in the context of the high specific activity variant FVII-100 with thrombin activation. High activity with longer initiation time for FVII-100 in the absence of thrombin, suggests that residual levels of tissue factor, thrombin or contact pathway activation can generate enough thrombin to activate FVII-100
25 without exogenous addition of thrombin. Figure 37 shows that similar results were obtained for the other high specific activity variant, FVII-115.

Example 23. Confirmation of activity of activatable variants using various assays

30 [00427] In this example, chromogenic assays were used to measure FVII activity. One of the assays used measures the amidolytic activity of FVIIaFc by measuring the cleavage of a chromogenic substrate by FVIIa. Another of these measures the FX activation activity by measuring the ability of FVIIa to activate FX, as determined by measuring levels of a chromogenic substrate that is cleaved by
35 activated FX (FXa).

[00428] In amidolytic assays, the chromogenic substrate Chromozyme t-PA was used. FVIIa cleaves this substrate in a dose dependent manner. The substrate is

5 also cleaved by thrombin, but the cleavage by thrombin can be inhibited by hirudin (data not shown).

[00429] As shown in Figure 38, amidolytic activity of the activatable variants can be measured following thrombin activation and there is increased amidolytic activity for the high specific activity variants as compared to FVII-090. In these
10 assays, after activation of the activatable molecule by thrombin, hirudin is added to inhibit thrombin cleavage of the chromogenic substrate. In this manner, the thrombin does not interfere with the ability to detect FVIIa activity.

[00430] The activation of FX by FVIIa is also enhanced in the high specific activity FVII activatable variants. To measure activation of FX by FVIIa, substrate
15 S2765 was used. This chromogenic substrate is also recognized by FX. In the assay, 10 uM of FX was incubated with FVIIaFc for 15 minutes and the reaction was quenched with EDTA. Figure 39 shows the results of the control experiment which demonstrates that FX activation by FVIIaFc can be detected.

[00431] The experiment shown in Figure 40 shows that there is an increase in
20 FX activation activity for the high specific activity activatable variant FVII-100. In this experiment, FVIIIFc (100 nM) was activated with thrombin (100 nM) for 20 minutes at 37C. Hirudin was added to inhibit the thrombin. FX (10uM) was added and incubated for 15 minutes at 37C, followed by EDTA to inhibit the reaction. S2765 substrate was added and FXa generation was detected by monitoring substrate
25 cleavage

Example 24. Monomeric Fc molecules can also be synthesized in activatable form

[00432] Three monomeric constructs were made as shown in Figure 41. In
30 FVII-118, an ALRPR cleavage site was inserted between the light chain and heavy chain. In FVII-119, the sequence GGGGS-ALRPR was inserted between the light chain and heavy chain. For FVII-127, the construct was made like FVII-118, but with the same high specific activity mutation used in FVII-100. The specific activity of the non-activated purified forms of these constructs was tested in a soluble tissue factor
35 assay and compared to FVII-011 (wild type FVIIaFc which had an activity of 10,000 IU/mg). FVII-118 had an activity of 4.5 IU/mg and FVII-127 had an activity of 1.8 IU/mg, demonstrating that these molecules had essentially no activity in their activatable form.

5 [00433] Thrombin cleavage reactions of FVII-118, FVII-119 and FVII-090 followed by SDS PAGE analysis were performed as previously described. As shown in Figure 42, the cleavage site in an activatable construct can be cleaved in the context of both the monomer and heterodimer Fc molecules. In the figure, the decrease in the intensity of the nonactivated FVII-Fc band with increasing thrombin concentration is
10 similar for the FVII-118, FVII-119, and FVII-090 constructs.

[00434] Another activatable monomeric construct, FVII-127, was made and tested. FVII-127 has the backbone of FVII-118, but the same 170 loop substitution used in FVII-100 to confer high specific activity. As shown in Figure 43, the activity of FVII-127 is significantly increased as compared to FVII-118 lacking the high
15 specific activity amino acid substitution. High activity with longer initiation time for FVII-127 in the absence of thrombin, suggests that residual levels of tissue factor, thrombin or contact pathway activation can generate enough thrombin to activate FVII-127 without exogenous addition of thrombin. FVII-127 activity is accelerated by thrombin.

20

Example 25. FVIIaFc variants targeted to the active form of GPIIb/IIIa

In this example the constructs illustrated in Figure 44A were cloned, transiently expressed, purified and activated as previously described. FVII-066 was cotransfected with PC5 to fully process the cscFc linker, described in the protein sequence,
25 connecting the first Fc moiety to the platelet targeting moiety. These constructs employed the targeting moiety SCE5, a scFv against the active conformation of GPIIb/IIIa. SCE5 has been shown to crossreact with mouse and human receptor. The SCE5 targeting moiety was placed at the N-terminus (FVII-066) or C terminus (FVII-067) of the second Fc moiety of FVIIaFc. In addition, the SCE5 was placed at the C-
30 terminus of FVIIa (FVII-094). FVIIaFc (FVII-011) and Novoseven were used as controls. As shown in Figure 44B, these proteins were tested by thrombin generation assays in FVIII-deficient human plasma as previously described. These experiments revealed increased rates of thrombin generation for all the proteins containing the SCE5 targeting moiety relative to the controls. The highest rates of thrombin
35 generation were observed for FVII-066, followed by FVII-094 and FVII-067, suggesting that the placement of the SCE5 targeting moiety can have a significant effect on the activity of the protein. Binding of these proteins to activated human platelets was determined by FACS assays as previously described (Figure 44C). All

5 the FVIIa proteins containing the SCE5 targeting moiety showed increased binding to platelets relative to the FVIIaFc control. This shows that attaching the SCE5 targeting moiety to FVIIa can increase its affinity from platelets. Since the SCE5 targeting moiety has been shown to interact with the mouse GPIIb/IIIa receptor, FVII-066 was tested in thrombin generation assays using mouse FVIII-deficient platelet rich plasma, as well as in a reconstituted system using human purified components and platelets, as previously described (Figure 29). We observed increased rates of thrombin generation for FVII-066 relative to the controls in both systems.

Example 26. Additional FVIIaFc variants targeted to the active form of GPIIb/IIIa

15 In this example, construct FVII-027 illustrated in Figure 12A was cloned, expressed (with PC5 cotransfection to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety), purified and activated as previously described. This construct employs the targeting moiety MB9, a scFv that has been shown to bind to the active conformation of GPIIb/IIIa. FACS assays were performed as previously described to assess binding to activated platelets, and FVII-027 was shown to bind to activated platelets with higher affinity than the FVII-011 control (Figure 12B). Thrombin generation assays were performed with reconstituted purified human proteins and platelets as previously described (Figures 13A, 13B and 13C). FVII-027 showed increased rates of thrombin generation relative to the controls. Figure 13D illustrates that FVII-027 has four times more activity than FVII-011 or Novoseven, based on thrombin generation assays. Figure 14 illustrates that the enhanced platelet binding and thrombin generation activity of FVII-027 were abrogated by PAC1, an antibody that competes with MB9 for binding to the activated form of GPIIb/IIIa, demonstrating the effects are mediated by the interaction of MB9 with the activated form of GPIIb/IIIa. The MB9 targeting moiety was also placed at the C-terminus of the second Fc moiety of FVIIaFc to generate FVII-037 illustrated in Figure 15. Thrombin generation assays in a FVIII-deficient reconstituted system with platelets revealed increased rates of thrombin generation for FVII-037 relative to the Novoseven control (Figure 16)

Example 27. Factor VII constructs targeted to both activated and nonactivated platelets

5 [00435] In this example, the constructs illustrated in Figure 45A were cloned, expressed, purified and activated as previously described. FVII-088 was cotransfected with PC5 to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety. FVII-088 and FVII-125 employed the AP3 targeting moiety, a scFv that binds to both active
10 and nonactive conformations of human GPIIbIIIa. The results in Figure 45B show thrombin generation assays in FVIII-deficient platelet-rich plasma, and both FVII-088 and FVII-125 showed increased rates of thrombin generation relative to the controls, demonstrating that targeting FVIIaFc or FVIIa to the active and nonactive conformation of GPIIbIIIa results in increased activity. Binding of FVII-088 and
15 FVIIaFc to activated human platelets was tested by FACS (Figure 45C). These data reveal that FVII-088 binds to platelets with higher affinity than FVIIaFc (FVII-011), showing that the AP3 targeting moiety can increase the affinity of FVIIaFc for platelets.

 Rotation Thromboelastometry (ROTEM®, Pentapharm GmbH, Munich,
20 Germany) is another method to evaluate platelet-targeted FVIIa constructs, since it allows for the characterization of several coagulation parameters in whole blood (in the presence of platelets). The ability of FVII-088 and wild type recombinant FVIIaFc (rFVIIaFc) to form firm and stable clots was evaluated by ROTEM with Calcium Chloride as activator (NATEM) following manufacturer's recommendations.
25 Hemophilia A blood from a human donor was spiked with FVIIIFc to a final concentration of 100, 30 or 10 nM. The NATEM reaction was initiated by the addition of CaCl₂. Coagulation parameters, including Clotting Time (relates to coagulation initiation time), Clot Formation Time (relates to rates of coagulation) and Alpha Angle (relates to rates of coagulation) were assessed as shown in Figure 30.
30 FVII-088 showed a significant reduction in the Clotting Time and Clot Forming Time and an increase in the alpha angle relative to wild type rFVIIaFc, consistent with enhanced coagulation kinetics for FVII-088. These data demonstrate that FVII-088 has enhanced activity relative to wild type FVIIaFc in agreement with the thrombin generation assay data

35

Example 28. Use of peptides for targeting FVIIa to platelets

[00436] The constructs illustrated in Figure 46A were cloned, expressed, purified and activated as previously described. These proteins were made using

5 peptides that bind to platelet receptor GPIb-alpha (found in both activated and nonactivated platelets), specifically PS4, OS1, and OS2 as platelet targeting moieties. In making these molecules the peptide was attached to either the N or the C terminus of the second Fc moiety of the construct. The FVII-044 construct employed the PS4 peptide attached to the C terminus of the second Fc moiety of the construct; FVII-045 employed the OS1 peptide attached to the C terminus of the second Fc moiety of the construct; and the FVII-046 construct employed the OS2 molecule attached to the C terminus of the second Fc moiety of the construct. In contrast, the FVII-047 construct employed the PS4 peptide attached to the N terminus of the second Fc moiety of the construct; the FVII-048 molecule employed the OS1 peptide attached to the N-terminus of the second Fc moiety of the construct; and the FVII-049 molecule employed the OS2 peptide attached to the N-terminus of the second Fc moiety of the construct. Thrombin generation assays were performed using FVIII-deficient platelet rich plasma as previously described. As shown in Figure 46B, when the assay was performed with limiting concentrations of FVIIa, each of the FVII-044, FVII-045, and FVII-046 C-terminal fusion constructs exhibited enhanced thrombin generation as compared to the Novoseven control. A similar result is shown in Figure 47A for the N-terminal fusion constructs. Figure 47B shows that the FVII-045 construct may be marginally better than the FVII-048 construct in this assay, but that again both the N and C terminal fusions are better than the Novoseven control. In addition, there is a correlation between the published GPIb-alpha affinity for each peptide (Figure 48) and the increase in the activity associated with that peptide when recombinantly fused to FVIIaFc. Figure 48 shows the binding of FVII-049, FVII-048 and wild type FVIIaFc (FVII-011 control) to activated platelets as determined by FACS as well as the affinity for the targeting peptides reported in Bernard et al. Biochemistry 2008. 47:4674-4682. FACS data revealed increased affinity of FVII-045 and FVII-048 for platelets relative to the FVII-011 control

Example 29. An FVIII-Fc variant targeted to the active form of GPIIb/IIIa

[00437]

The constructs illustrated in Figure 49A were made as previously described. FVIII-041 is wild type FVIII-Fc, while FVIII-108 has a SCE5 platelet targeting moiety at the N-terminus of the second Fc moiety. For expression, FVIII-108 was cotransfected with PC5 to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety.

5 These proteins were tested in thrombin generation assays using FVIII deficient platelet rich plasma as previously described, but in addition the thrombin generation assay was also activated with tissue factor As shown in Figure 49B, no significant improvement was seen using the targeted version of FVIII-Fc. It is noteworthy that the thrombin generation assays described herein measure thrombin generation on the
10 surface of platelets and, therefore, are an accurate measure of activity.

Example 30. Making and testing a version of FVII targeted to platelets that lacks a Gla domain

[00438] In this example a version of FVIIa-Fc illustrated in Figure 21 was
15 generated. This protein has the MB9 scFv at the N-terminus and a deletion that removes the Gla domain. FVII-053 contains an RKRRKR sequence inserted between the light and heavy chain for intracellular activation. FVII-053 was transiently expressed (cotransfected with PC5 for processing of the RKRRKR sequence which results in activation of the protein) and purified as previously described. Thrombin
20 generation assays with purified components and platelets reveal that FVII-053 has some activity (Figure 22), even though this activity is compromised relative to the FVII-011 control (Figure 22D). Data in Figure 23 show how the PAC1 antibody, which competes with MB9 for GPIIb/IIIa binding, inhibits thrombin generation activity associated with FVII-053, suggesting that platelet targeting is important for
25 activity. Another construct identical to FVII-053, but without the RKRRKR insertion was generated (FVII-028) and tested in a PK study in the nonactivated together with nonactivated FVII-011. As shown in Figure 50, the terminal half-life of the targeted, Gla-less FVII-028 molecule was nearly three times longer (20.3 hours) than that of the FVII-011 control (7.1 hours), suggesting that removing the Gla domain increases
30 the terminal half-life of FVII-Fc

Example 31. Platelet Targeted FIX Molecules

[00439] In this example, the FIX constructs illustrated in Figure 51A were made and tested. FIX-068 was cotransfected with PC5 to fully process the cscFc
35 linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety. FIX-068 has the SCE5 platelet targeting moiety at the N-terminus of the second Fc moiety, while FIX-088 has SCE5 at the C-terminus of the second Fc moiety. FIX-090 is a FIX construct without an Fc domain and has the SCE5 moiety

5 attached at the C-terminus of the FIX protein. FIX-042 is a FIXFc as a single chain Fc without a targeting moiety and was made as a control. BeneFIX (Pfizer) was also used as a control. To remove trace amounts of activated FIX (FIXa) from the BeneFIX sample that cause misleading results in thrombin generation assays, the BeneFIX
10 sample was treated with the irreversible active site inhibitor glutamyl-glycyl-arginyl-chloromethylketone (Hematologic Technologies). BeneFIX was incubated with an excess amount of the inhibitor for 180 minutes at room temperature. The sample was then dialyzed to remove unbound inhibitor. The treated BeneFIX is hereafter referred to as BeneFIX. The specific activities of the molecules made were FIX-042, 6
15 IU/nmol; FIX068, 5.1 IU/nmol; FIX-088, 3.5 IU/nmol; FIX-090, 13.8 IU/nmol, and BeneFIX, 12 IU/nmol. When these constructs (FIX-068, FIX-088 and FIX-042) were tested in a thrombin generation assay in platelet-rich FIX-deficient plasma as shown in Figure 51B, each of the targeted molecules had a higher activity than the FIX-042 control. Figure 51C illustrates that both FIX-068 and FIX-088 have at least 4 times more activity than FIX-042 as measured by thrombin generation. Since the specific
20 activity of FIX-042 is higher than FIX-068 and FIX-088, the increased activity observed in the thrombin generation assays may be underestimated, and therefore the increased activity by platelet targeting may be greater than 4-fold.

[00440] As shown in Figure 52A, FIX-090 (which lacks an Fc) also shows increased activity relative to BeneFIX, suggesting that targeting FIX to platelets in the
25 absence of Fc also increases activity. Figure 52B shows that the activity of FIX-090 is at least 4 times that of BeneFIX. Since both FIX-090 and BeneFIX have similar specific activities, the 4-fold increase in activity in thrombin generations assays must be caused by the platelet targeting effect

30 **Example 32. Use of peptides for targeting FIX to platelets**

[00441] In this example, the FIX-089 construct illustrated in Figure 53A was cloned, transiently expressed and purified as previously described. The molecule comprises the OS1 peptide, which binds to GPIb-alpha receptor, attached to the N-terminus of the second Fc moiety of the construct. The specific activity of the FIX-
35 089 construct was 2.4 IU/nmol as compared to 6 IU/nmol for the control FIX-042 molecule.

[00442] As shown in Figure 53B, the FIX-089 molecule is more active than the FIX-042 control in thrombin generation assays with FIX-platelet rich plasma; this is

- 5 particularly evident at limiting concentrations of FIX. Figure 53C demonstrates that
FIX-089 is roughly 4-times stronger than FIX-042 as measured by thrombin
generation, while having a lower specific activity. This further suggests that targeting
to GPIb increases the activity of FIXFc.

10

5 DRAFT SEQUENCE LISTING

FVII-027 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or MB9 to Fc region is underlined, and linker with proprotein convertase processing sites is shown in bold

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10 MVSOALRLIC LLGLQGCLA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
   SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
   THKDDQLICV NENGGCEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTTVE YPCGKIPILE
   KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
15 NLI AVLGEHD LSEHDGDEQS RRVAQVIIPS TYVFGTTNHD IALLRLHQPV VLTDHVVPPLC
   LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSFN
   ITEYMFECAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
   YIEWLQKLMR SEPRFGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
   PPCPAPELLG GPSVFLFPPK PKDTLMISR PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
20 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKT SKAKGQPREP
   QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP VLDSGGSFFL
   YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KRRRRSGGGG SGGGSGGGG
   SGGGSGGGG SGGGSGRKR KRAEVQLVQS GAEVNKPGAS VKVSCASGY TFTGYMHWV
   RQAPGQGLEW MGWINPNSGG TNYAQKFQGW VTMRDTSIS TAYMELSLRL SDDTAVYYCA
   RGRALYNRND RSPNWFDPWG QGTLVTVSSG SASAPTLKLE EGEFSEARVQ AVLTQPPSVS
25 VAPGQTARIT CGGNIGSKS VQWYQKPGQ APVLVYDDSD DRPSGIPERF SGSNSGNMAT
   LTISRVEAGD EADYYCQVWD SSSDHVVFEGG GTKLTVLGQP KAAPSVTLFP PSAAAGGGGS
   GGGSGGGGSG GGGSGGGGSG GGGGSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
   TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVSV LTVLHQDWLN
   GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS
30 DIAVEWESNG QPENNYKTPP PVLDSGGSFF LYSKLTVDKS RWQGNVFSC SVMHEALHNH
   YTQKSLSLSP GK

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DNA sequence of FVII-027

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35 atggtctctcc aggcctctcag gctcctctgc cttctgcttg ggttcaggg ctgectgget
   gcagtctctcg taaccacagga ggaagccac gccgtctctg accggcgccg gcgcgccaac
   gcttctctcg aggagctcg gcgggctcc ctggagaggg agtgcaagga ggagcagtc
   tctctcgagg aggcocggga gatcttcaag gacgcggaga ggaogaagct gttctgatt
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   gacggggtgt cctgcacacc cacagttgaa tatccatgtg gaaaaatacc tattctagaa
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   atcaacacca tctgggtggt ctccgcggc cactgtttcg acaaaatcaa gaactggagg
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   gcccatttc ccggtggcgg tggctccggc ggaggtgggt ccggtggcgg cggatcaggt
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5 tgccctgggtca aaggtcttcta tcccagcgac atcgcctgtg agtgggagag caatgggag
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 tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcagtgtcc
 gtgatgcatg aggtctotgca caaccactac acgcagaaga gcctctccct gtctccgggt
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 aagagggcgg aagtgcagct ggtgcagctt ggagctgagg tgaataagcc tggggcctca
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Genscript-FVII-027-1 DNA sequence

40 gaagagcctc tccctgtctc cgggtaaacg gcgcgcgcgg agcgggtggc gcggtatcagg
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 tgggggtgga tcaaggaaga ggaggaagag ggcgggaagt cagctggtgc agtctggagc
 tgaggtgaat aagcctgggg cctcagtgaa ggtctcctgc aaggcttctg gatacacctt
 caccggctac tatatgcact ggggtgcgaca ggcccttgga caagggttg agtggatggg
 45 atggatcaac cctaacagtg gtggcacaaa ctatgcacag aagtttcagg gctgggtcac
 catgaccagg gcacagtcga tcagcacgcg ctacatggag ctgagcaggc tgagatctga
 cgacacggcc gtgtattact gtgcgagagg ccgtgctttg tataaccgga acgaccggtc
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50

Genscript-FVII-026-2 DNA sequence

gaattctcag aagcacgcgt acaggctgtg ctgaactcag cgcctcgggt gtcagtggcc
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 tggtagcagc agaagccagg ccaggccctt gtgctgggtg tctatgatga tagcgaccgg
 55 cctcaggga tccctgagcg attctctggc tccaactctg ggaacatggc caccctgacc
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 ggtgggtccg gtggcggcgg atcaggtggg ggtggatcag gcggtggagg ttccgggtgg
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 65 gagtacaagt gcaagggtct caacaaagcc ctcccagccc ccatcgagaa aaccatctcc

5 aaagccaaaag ggcagcccccg agaaccacag gtgtacaccc tgcccccatc ccgcgatgag
 ctgaccaaga accaggtcag cctgacctgc ctgggtcaaaag gcttctatcc cagcgacatc
 gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gctcccgctg
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 10 cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg
 cagaagagcc tctccctgtc tccgggtaaa tgagaattc

FVII-037 amino acid sequence. Signal sequence is shown in dotted underline,

15 propeptide is double underlined, linker region connecting FVII or MB9 to Fc region is underlined, and linker connecting both Fcs sites is shown in bold

MVSOALRLLC LLLGLOGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 HKDDQLICV NENGGEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTPTVE YPCGKIPILE
 20 KRNASKPQGR IVGGKVCCKG ECPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
 NLIIVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSFN
 ITEYMFACGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 YLEWLQKLNR SEPRPGVLLR AFFFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
 25 PFCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP VLDSGDSFFL
 YSKLTVDKSR WQGNVVFSCS VMHEALHNHY TQKSLSLSPG **KGSGSGSGSG** **SGSGSGSGSG**
 30 **SDKTHTCPPC** PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
 DGVEVHNAKT KPREEQYNST YRVVSVLTFLV HQDWLNGKEY KCKVSNKALP APIEKTISKA
 KGQPREPQVY TLPPSRDEL TKNQVSLTCLV KGFYPSDIAV EWESNGQFEN NYKTTTPVLD
 SDGSFFLYSK LTVDKSRWQQ GNVVFSCVMH EALHNHYTQK SLSPGKGG GSGSGGGSGG
 GSGSGGGSGG GSGSGGSAE VQLVQSGAEV NKPASVSKV CKASGYTFG YMHWVRQAP
 35 GQGLEWMGWI NPNSGGTNYA QKFGQWVTMT RDTISSTAYM ELSRLRSDDT AVYYCARGRA
 LYNRNDRSPN WFDWQGGTL VTVSSGSASA PTLKLEEGEF SEARVQAVLT QPPSVSVAPG
 QTARITCGGN NIGSKSVQWY QQKPGQAFVL VVYDDSDRPS GIFERFSGSN SGNMATLTIS
 RVEAGDEADY YCQVWDSSSD HVVFGGGTKL TVLGQPKAAP SVTLFPPSAA A

FIX-037 DNA sequence

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 tctctcgagg aggcctcgga gatcttcaag gacgcggaga ggacgaagct gttctggatt
 45 tcttacagtg atggggacca gtgtgctca agtccatgcc agaattgggg ctctgcaag
 gaccagctcc agtctatat ctgcttctgc ctccctgctc tcgagggccg gaactgtgag
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 gacgggggtgt cctgcacacc cacagttgaa tatccatgtg gaaaaatacc tattctagaa
 50 aaaagaaatg ccagcaaac ccaaggccga attgtggggg gcaaggtgtg ccccaaaggg
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5 cccaaggaca cccatcatgat ctcccggacc cctgaggtca catgcgtggt ggtggacgtg
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 30 tgcaaggctt ctggatacac cttcaccggc tactatatgc actgggtgog acaggccctc
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 cagcagaagc caggccaggc cctgtgtctg gtgtcttatg atgatagcga ccggccctca
 gggatccctg agcagattct tggctccaac tctgggaaca tggccacctc gaccatcagc
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 catgtggtat tcggcggagg gaccaagctg accgtcctag gtcagcccaa ggtgcccc
 tcggctactc tgttcccgcc gtcgcgggcc gcttga

FVII-053 amino acid sequence. Signal sequence is shown in dotted underline, linker region connecting FVII to Fc region is underlined, linker connecting both Fcs sites is shown in bold, and MB9 is italicized

45 MVSOALRLLC LLLGLOGCIA AEVQLVQSGA EVNKPASVK VSCKASGYTF TGYIMHWVRQ
 APGQGLEWMG WINPNSGGTN YAQKFQGWVT MTRDTSISTA YMELSLRLSD DTAVYYCARG
 RALYNRNDRS PNWFDPWGQG TLVTVSSGSA SAPTLKLEEG EFSEARVQAV LTQPPSVSVA
 50 PGQTARITCG GNNIGSKSVQ WYQQKPGQAP VLVVYDDSDR PSGIPERFSG SNSGNMATLT
 ISRVEAGDEA DYYCQVWDSS SDHVVFSGGT KLTVLGQPKA APSVTLFPSP AAARTKLEWI
 SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE THKDDQLICV NENGGCEQYC
 SDHTGTRKRC RCHGYSLLA DGVSCPTPVE YPCGKIPILE KRNASKPQGR RKRRKRIVGG
 KVCPKGECPW QVLLLVNGAQ LCGGTILINTI WVVSAAHCFD KIKNWRNLIA VLGEHDLSEH
 55 DGDEQSRRVA QVIIPSTYVP GTTNHDIAL RLHQPVVLT HVVPLCLPER TFSERTLAFV
 RFSLVSGWGQ LLDRGATALE LMVLNVPRIM TQDCLQQSRK VGDSPNITEY MFCAGYSDGS
 KDSCKGDSGG PHATHYRGTW YLTGIVSWGQ GCATVGHFGV YTRVSQYIEW LQKLMRSEPR
 PGVLLRAPFP GGGSGGGGGS GGGSGGGGGS GGGSGGGGGS DKHTTCPPCP APELLGGPSV
 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 60 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG
 NVFSCSVME ALHNHYTQKS LSLSPGKGGG **GGGGGGSGGG** **GGGGGGSGGG** **GGGGGGSGGG** DKTK HTCPPCPAPE
 LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
 EQYNSTYRVV SVLTFLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP

5 SRDELTRKNQV SLTCLVKGFY PSDIAVEWES NGQFENNYKT TPPVLDSDBGS FFLYSKLTVD
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK

FVII-053 DNA sequence

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65 acgcctcccg tgttggactc cgacggctcc ttcttctct acagcaagct caccgtggac

5 aagagcaggt ggcagcaggg gaacgtcttc tcatgctcgg tgatgcatga ggetctgcac
aaccactaca cgcagaagag cctctccctg tctccgggta aatga

10 FVII-044 amino acid sequence. Signal sequence is shown in dotted underline,
propeptide is double underlined, linker region connecting FVII or PS4 to Fc region is
underlined, linker connecting both Fcs sites is shown in bold, and PS4 peptide is
italicized

15 MVSOALRLLC LLIGLQGCLA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
THKDDQLICV NENGGCEQYC SDHTGTPKRS RCHEGYSLLA DGVSCPTTVE YPCGKIPILE
KRNASKPQGR IVGKVCVFKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLI AVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLDTHVVPCL
20 LPERTFSERT LAFVRFSLVS GWQQLLDGRG TALELMVLNV PRLMTQDCLQ QSRKVGDSFN
ITEYMFCAQY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HPGVYTRVSQ
YIEWLQKLMR SEPFGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKITPP VLDSGDSFLL
25 YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG **KGGGGSGGGG** **SGGGSGGGG**
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDEL TKNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKITPPVLD
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGSGG
30 GGSGGGGSAC TERWALHNLC GG

FVII-044 DNA sequence

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   ggcgggtga

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25 FVII-045 amino acid sequence. Signal sequence is shown in dotted underline,
 propeptide is double underlined, linker region connecting FVII or OS1 to Fc region is
 underlined, linker connecting both Fcs sites is shown in bold, and OS1 peptide is
 italicized

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MVSOALRLLC LLLGLOGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
SFEEAREIFK DAERTKLFWI SYSDDGQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
30  THKDDQLICV NENGGEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTPVE YPCGKIPILE
   KRNASKPQGR IVGGKVC PKG ECPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
   NLI AVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPFC
   LPERTFSERT LAFVRFSLVS GWQQLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSFN
   ITEYMFACGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
35  YIEWLQKLMR SEPRPGVLLR APFPGGGSG GGGSGGGSG GGGSGGGSG GGGSDKTHTC
   PPCPAPELLG GPSVFLFPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
   AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
   QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP VLDSGDSFFL
   YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSGGGG
40  SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHR DPEVKFNWYV
   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
   KGQPREPQVY TLPFSRDEL TNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLD
   SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GSGGGSGGGG
   GSGGGGSAC TERMALHNLC GG

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45

FVII-045 DNA sequence

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 35 ggaggttccg gtggcggggg atcagcctgc accgagcggg tggcctgca caacctgtgc
 ggcggtgga

FVII-046 amino acid sequence. Signal sequence is shown in dotted underline,
 propeptide is double underlined, linker region connecting FVII or OS2 to Fc region is
 underlined, linker connecting both Fcs sites is shown in bold, and OS2 peptide is
 italicized

40 MVSOALRLLC LLLGLOGCLA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 THKDDQLICV NENGGCEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTTVE YPCGKIPFILE
 45 KRNASKPQGR IVGGKVC PKG ECPWQVLLV NGAQLCGGTL INTIIVVSAA HCFDKIKNWR
 NLIIVLGEHD LSEHDGDEQS RRVAQVIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPPLC
 LPERTFSERT LAFVRFSLVS GWGQLLD RGA TALELMVLNV PRLMTQDCLQ QSRKVGDSFN
 ITEYMFACGY SDGSKDSCCK DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 YIEWLQKLMR SEPRPGVLLR APFPGGGSG GGGSGGGSG GGGSGGGSG GGGSDKTHTC
 50 PPCPAPELLG GPSVFLFPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
 AKTKFREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP VLDSDGSFFL
 YSKLTVDKSR WQQGNVFS SCS VMHEALHNHY TQKSLSLSPG **KGGGSGGGG** **SGGGSGGGG**
 55 **SDKTHTCPPC** PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
 KGQPREPQVY TLPPSRDEL TKNQVSLTCLV KGFYPSDIIV EWESNGQPEN NYKTPPVLD
 SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGGSGGGSGG
GGSGGGGSAC TERDALHNLC GG

60 FVII-046 DNA sequence

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   agcctctccc tgtctccggg taaaggtggc ggcggtacag gtgggggtgg atcaggcggt
50  ggaggttccg gtggcggggg atcagcctgc accgagcggg acgcctgca caacctgtgc
   ggggggtga

```

FVII-047 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or PS4 to Fc regions is underlined, and PS4 peptide is italicized

```

55  MVSOALRLLC..LLLGLOGCLA AVFVTOEEAH GVLRRRRRAN AFLEELRPGS LERECKEEQC
   SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
   THKDDQLICV NENGCEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTTVE YPCGKIPILE
   KRNASKPQGR IVGKVCVFKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
   NLI AVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
60  LPERTFSERT LAFVRFSLVS GWGQLLDLGRG TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
   ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
   YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKHTHC
   PPCPAPELLG GPSVFLFPPK PKDTLMISRT FEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
   AKTKPREEQY NSTYRVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP

```

5	QVYTLFPSRD	ELTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ	PENNYKTTPP	VLDSGGSFFL
	YSKLTVDKSR	WQQGNVFSCS	VMHEALHNHY	TQKSLSLSPG	KGGGGSGGGG	SGGGSGGGG
	SGGGSGGGG	SACTERWALH	NLCGGGGSGG	GGSGGGSGGG	GGSGGGSGGG	GGSDKTHTCP
	PCPAPELLGG	PSVFLFPEKP	KDTLMISRTF	EVTCVVVDVS	HEDPEVKFNW	YVDGVEVHNA
	KTKPREEQYN	STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ
10	VYTLFPSRDE	LTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY
	SKLTVDKSRW	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK		

FVII-047 DNA sequence

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15	gcagtctctg	taaccacagga	ggaagcccac	ggcgctctgc	acggcgcccg	gcgcgccaac
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	tccttcgagg	aggcccgga	gatcttcaag	gacgcggaga	ggacgaagct	gttctggatt
	tcttacagtg	atggggacca	gtgtgcctca	agtccatgcc	agaatggggg	ctcctgcaag
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	ggtggatcag	gcggtggagg	ttccgggtgg	gggggatccg	acaaaactca	cacatgcccc
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	ctcccagccc	ccatcgagaa	aaccatctcc	aaagccaaaag	ggcagccccg	agaaccacag
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	gagaacaact	acaagaccac	gcctcccgtg	ttggactccg	acggctcctt	cttctctctc
	agcaagctca	ccgtggacaa	gagcaggtgg	cagcagggga	acgtcttctc	atgctccgtg
	atgcctgagg	ctctgcacaa	ccactacacg	cagaagagcc	tctccctgtc	tcggggtaaa
65	tga					

- 5 FVII-048 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or OS1 to Fc regions is underlined, and OS1 peptide is italicized

	<u>MVSOALRLLC</u>	<u>LLLGLOGCLA</u>	<u>AVFVTQEEAH</u>	<u>GVLHRRRRAN</u>	AFLEELRPGS	LERECKEEQC
	SFEAREIFK	DAERTKLFWI	SYSDGDQCAS	SFCQNGGSCK	DQLQSYICFC	LPAFEGRNCE
10	THKDDQLICV	NENGGCEQYC	SDHTGTRKSC	RCHEGYSLLA	DGVSCPTPVE	YPCGKIPILE
	KRNASKPQGR	IVGGKVCPKG	ECFWQVLLLV	NGAQLCGGTL	INTIWWVSAA	HCFDKIKNWR
	NLIAVLGEHD	LSEHDGDEQS	RRVAQVIIPS	TYVPGTTNHD	IALRLHQPV	VLTDHVVPLC
	LPERTFSERT	LAFVRFSLVS	GWGQLLDRGA	TALELMVLNV	PRLMTQDCLQ	QSRKVGDSFN
	ITEYMFACGY	SDGSKDSCKG	DSGGPHATHY	RGTWYLTGIV	SWGQGCATVG	HFGVYTRVSQ
15	YIEWLQKLMR	SEPRPGVLLR	APFFGGGGSG	GGGSGGGGSG	GGGSGGGGSG	GGGSDKTHTC
	PFCPAPELLG	GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDFEVKFN	WYVDGVEVHN
	AKTKPREEQY	NSFYRVVSVL	TVLHQDWLNG	KEYCKKVSNN	ALPAPIEKTI	SKAKGQPREP
	QVYTLPPSRD	ELTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ	PENNYKTTPF	VLDSGGSFFL
	YSKLTVDKSR	WQQGNVFSCS	VMHEALHNHY	TQKSLSLSPG	KGGGGSGGGG	SGGGSGGGG
20	<u>SGGGSGGGG</u>	<u>SACTERMALH</u>	<u>NLCGGGGSGG</u>	<u>GGSGGGSGG</u>	<u>GGSGGGSGG</u>	<u>GGSDKTHTCF</u>
	PCPAPELLGG	PSVFLFPPKP	KDTLMISRT	EVTCVVVDVS	HEDFEVKFNW	XVDGVEVHNA
	KTKPREEQYN	STYKRVSVLT	VLHQDWLNGK	EYCKKVSNNK	LPAPIEKTIS	KAKGQPREPQ
	VYTLPPSRDE	LTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGGSFFLY
	SKLTVDKSRW	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK		

25

DNA sequence of FVII-048

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30	gcgttccttg	aggagctgcg	gccgggctcc	ctggagaggg	agtgcacgga	ggagcagtg
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	aaaggcgggtg	gcggttcagg	tggaggagg	tcaggcgggtg	gtggatccgg	cggggcgga
	tcgggtggcg	gagggtcagg	cgggtggcga	tcagcctgca	ccgagcggat	ggccctgcac
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5 ggtggatcag gcggtggagg ttccgggtggc gggggatccg acaaaactca cacatgcccc
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 tga

20 FVII-049 amino acid sequence. Signal sequence is shown in dotted underline,
 propeptide is double underlined, linker region connecting FVII or OS2 to Fc regions
 is underlined, and OS2 peptide is italicized

MVSOALRLLC LLLGLOGCLA AVFVTQEEAH GVLHPRRRAN AFLEELRPGS LERECKEEQC
 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 THKDDQLICV NENGGCEQYC SDHTGTRSC RCHEGYSLLA DGVSCPTTVE YPCGKIPILE
 25 KRNASKPQGR IVGKVCVCPKG ECPWQVLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
 NLIIVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPPLC
 LPERTFSERT LAFVRFSLVS GWGQLLDGRG TALELMVLNV PRLMTQDCLQ QSRKVGDSFN
 ITEYMFACAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 YIEWLQKLMR SEPRPGVLLR APFPGGGSG GGGSGGGSG GGGSGGGSG GGGSDKTHTC
 30 PCPAPPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYCKVSNK ALPAPIEKTI SKAKQPREP
 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPPP VLDSGDSFFL
 YSKLTVDKSR WQGNVVFSCS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSGGGG
 SGGGGSGGGG SACTERDALH NLCGGGGSGG GGSGGGSGG GGSGGGSGG GGSDDKTHTCF
 35 PCPAPPELLG PSVFLFPPKP KDTLMISRT PEVTCVVVDV HEDPEVKFNW YVDGVEVHNA
 RTKPREEQYN STYRVVSVLT VLHQDWLNGK EYCKVSNKA LPAPIEKTIS KAKQPREPQ
 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTPPPV LDSDGDSFFLY
 SKLTVDKSRW QQGNVVFSCSV MHEALHNHYT QKSLSLSPGK

40 DNA sequence of FVII-049

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 gccccatttc ccggtggcgg tggtccggc ggaggtgggt ccggtggcgg cggatcaggt
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5  ccaccgtgcc cagctccgga actcctggga ggaccgtcag tcttcctctt cccccaaaa
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   agccacgaag accctgaggt caagttcaac tggtagctgg acggcgtgga ggtgcataat
   gccaaagcaa agccggggga ggagcagtac aacagcaagt accgtgtggt cagcgtcttc
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10  gccctccag ccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca
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   tacagcaagc tcaccgtcga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc
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   tccggtggcg gaggttcagg cgggtggcga tcagcctgca ccgagcggga cgcctgcac
   aacctgtgcg tggcggtggt ctcggcgga ggtgggtccg gtggcgggcg atcaggtggg
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   cagcaagacc ctgaggtcaa gttcaactgg tacgtggaag gcgtggaggt gcataatgcc
   aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtgggtcag cgtctcacc
   gtctgcacc aggaactggt gaatggcaag gagtacaagt gcaaggtctc caacaaagcc
25  ctcccagccc ccctcgagaa aacctctcc aaagccaaag ggcagccccc agaaccacag
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   gagaacaact acaagaccac gctctccgtg ttggaactcg acggctcctt cttctctac
   agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgtctcgtg
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   tga

```

FVII-011 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, Gla domain is italicized, linker region connecting FVII to Fc region is underlined, and linker connecting both Fcs sites is shown in bold

```

35  MVSQALRLLC LLGLQGCLA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
   SFEEAREIFK DAERTKLFWI SYSDDGQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
   THKDDQLICV NENGGEQYC SDHTGTRKSC RHEGYSLLA DGVSTPTVE YPCGKIPILE
   KRNASKPQGR IVGGKVC PKG ECPWQVLLV NGAQLCGGTL INTIWWVSAA HCFD KIKNWR
40  NLI AVLGEHD LSEHDGDEQS RRVAQVII PS TYVPGTTNHD IALLRLHQPV VLT DHVPLC
   LPERTFSERT LAFVRFSLVS GWQQLLD RGA TALELMVLNV PRLMTQDCLQ QSRKVG DSPN
   ITEYMF CAGY SDGSKDCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
   YIEWLQKL MR SEPRPGVLLR APFPGGGSG GGGSGGGSG GGGSGGGSG GGGSDKTHTC
   PPCPAPELLG GPSVFLFPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
45  AKTKPREEQY NSTYRVSVL TVLHQDWLNG KEYCKVSNK ALPAPIEKTI SKAKGQPREP
   QVYTLFP SRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDS DGSFFL
   YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGSGGGG SGGGSGGGG
   SDKTHTCFPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
50  KGQPREPQVY TLPPSRDEL T KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
   SDGSFFLYSK LTVDKSRWQQ GNVFSCSV MH EALHNHYTQK SLSLSPGK

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FVII-011 DNA sequence

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55  atggtctctc aggcctcag gctcctctgc cttctgcttg ggcttcaggg ctgcctgggt
   gcagtcctcg taaccacagga ggaagccac ggctcctgc accggcgccg gcgcgccaac
   gcgttccttg aggagctgc gccgggctcc ctggagaggg agtgcaagga ggagcagtg
   tctctcgagg aggcgggga gatcttcaag gacgcggaga ggacgaagct gttctggatt
   tcttacagtg atggggacca gtgtgcctca agtccatgcc agaattgggg ctctgcaag
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   gacggggtgt cctgcacacc cacagttgaa tatccatgtg gaaaaatacc tattctagaa
   aaaagaaatg ccagcaaac ccaaggcga attgtggggg gcaaggtgtg ccccaagggg
   gagtgtccat ggcaggctct gttgttggtg aatggagctc agttgtgtgg ggggacctg

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5 atcaaacacca tctgggtggt ctccgcggcc cactgtttcg acaaaatcaa gaactggagg
 aacctgatcg cgggtgctggg cgagcacgac ctccagcgagc acgacgggga tgagcagagc
 cggcggggtgg cgcaggtcat catccccagc acgtacgtcc cgggcaccac caaccacgac
 atcgccgtgc tccgcctgca ccagcccggtg gtccctcaetg accatgtggt gcccctctgc
 ctgcccgaac ggacgtttctc tgagaggacg ctggccctcg tgcgcttctc attggtcagc
 10 ggctggggcc agctgctgga cgtggcgcc acggccctgg agctcatggt cctcaacgtg
 ccccggtgta tgaccagga ctgcctgcag cagtcaaggga aggtgggaga ctccccaaat
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 gacagtggag gccacatgc caccactac cggggcacgt ggtacctgac gggcatcgtc
 agctggggcc agggctgccc aaccgtgggc cactttgggg tgtacaccag ggtctcccag
 15 tacatcgagt ggctgcaaaa gctcatgccc tcagagccac gccaggagt cctcctgcga
 gcccatttc ccggtggcgg tggctccggc ggaggtgggt ccggtggcgg cggatcaggt
 cgggggtggat caggcgggtg aggttccggt ggccggggat ccgacaaaac tcacacatgc
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 cccaaggaca cctcatgat ctcccggaac cctgaggtca catgctggtt ggtggaagtg
 20 agccacgaag accctgaggt caagttcaac tggtagctgg acggcgtgga ggtgcataat
 gccaaagaaa agccggggga ggagcagtac aacagcacgt accgtgtggt cagcgtcttc
 accgtcctgc accaggactg gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa
 gccctcccag ccccatcgga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca
 caggtgtaca ccctgcccc atcccggtat gagctgacca agaaccaggt cagcctgacc
 25 tgcttggtca aaggttctc tccagcgac atcgccgtgg agtgggagag caatgggcag
 ccggagaaca actacaagac acgcctccc gtgttggaet ccgacggctc cttctctctc
 tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc
 gtgatgcatg aggtctgca caaccactac acgcagaaga gcctctccct gtctccgggt
 aaaggtggcg gcggtacagg tgggggtgga tcaggcgggtg gaggttccgg tggcggggga
 30 tcagacaaaa ctacacatg cccaccgtgc ccagcacctg aactcctggg aggaccgtca
 gtcttctctt tcccccaaaa acccaaggac accctcatga tctcccgga cctgaggtc
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 gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcagc
 35 tacgtgtgg tcagcgtct caccgtcctg caaccaggact ggctgaatgg caaggatgac
 aagtgcagg tctccaacaa agccctccca gcccctatcg agaaaacca ctccaagcc
 aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccgoga tgagctgacc
 aagaaccagg tcagcctgac ctgcctgggt aaaggttct atcccagcga catcgccgtg
 gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgttgga
 40 tccgacggct ccttctctct ctacagcaag ctcccggtg acaagagcag gtggcagcag
 gggaacgtct tctcatgctc cgtgatgcat gaggtctctg acaaccacta cagcagaag
 agcctctccc tgtctccggg taaatga

45 **B domain deleted FVIII amino acid sequence:** Signal peptide
 underlined; 14 amino acid linker (containing the remaining B domain)
 between the HC and LC sequence is double underlined, with the
 S743/Q1638 fusion site indicated in bold.

50 1 MOIELSTCEFF LCLLRFCFSA TRYYLGAVE LSWDYMQSDL GELPVDARFP
 51 PRVPKSFPEF TSVVYKKTLE VEFTHLLENI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPF
 151 GSHTYVWQVL KENGPMSADP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 55 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCEP
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIQR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGDIT LIIFKNQASR PYNIPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 60 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYLTEI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
 751 SKNNAIEPRS FSQNPPVLKR HQREITRTTL QSDQEEIDYD DTISVEMKKE
 65 801 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
 851 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 901 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FVKVQHMAP
 951 TKDEFDCKAW AYFSDVDLEK DVHSGILIGPL LVCHTNTLNP AHGRQVTVQE
 1001 FALFFTIFDE TKSIFYTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI

5 1051 MDTLPLGLVMA QDQIRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1101 YNLYPGVFET VEMPLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
 1151 GNASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1201 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1251 PFGNVDSSGI KHNI FNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 10 1301 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1351 PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISS QDGHQWTLFF
 1401 QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
 1451 CEAQDLY

15

Full length FVIII amino acid sequence: Signal peptide underlined

1 MQIELSTCFF LCLLRFCFSA TRRYLGAIVE LSWDYMOSDL GELPVDARFP
 51 PRVPKSFPPN TSVVYKKTFL VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 20 101 DTVVITLKNM ASHPVSLHAV GVS YWKASEG AEYDDQTSQR EKEDDKVFPF
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRSLPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 25 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPTK
 401 WVHYIAAEEE DWDYAPLVLA PDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETPTKREA IQHESGILGP LLYGEVGDITL LIIFKNQASR PYNIPYHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YSSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 30 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYLLS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751 SKNNAIEPRS FSQNSRHPST RQKQFNATTI PENDIEKTDP WFAHRTMPMK
 801 IQNVSSSDLL MLLRQSPTPH GLSLSDLQEA KYETFSDDPS PGAIDSNNSL
 35 851 SEMTHFRPQL HSGDMVFPT ESGLQLRLNE KLGTAAATEL KKLDKVSST
 901 SNLISTIPS DNLAAGTDNT SSLGPPSMPV HYDSQLDITL FGKKS SPLTE
 951 SGGPLSLSEE NNDSKLLESG LMNSQESSWG KNVSTTESGR LFKGKRAHGP
 1001 ALLTKDNALF KVSISLLKTN KTSNNSATNR KTHIDGPSLL IENSPSVWQN
 1051 ILESDETFKK VTPLIHDRML MDKNATALRL NHMSNKTTS KMMEMVQQKK
 40 1101 EGPIPPDAQN PDMSFFKMLF LPESARWIQR THGKNSLNSG QGSPFKQLVS
 1151 LGPEKSVGGQ NFLSEKNKV VKGGEFTKDV GLKEMVFPS RNLFLTLNLDN
 1201 LHENNTHNQE KKIQEEIEKK ETLIQENVVL PQIHTVTGTH NFMKNLFLLS
 1251 TRQNVESYD GAYAPVLQDF RSLNDSTNRT KKHTAHFSKK GEENLEGLG
 1301 NQTKQIVEKY ACTTRISPNT SQQNFVTQRS KRALKQFRLP LEETELEKRI
 45 1351 IVDDTSTQWS KNMKHLTPST LTQIDYNEKE KGAITQSPLS DCLTRSHSIP
 1401 QANRSPLEPIA KVSSFPSIRP IYLTREVLFQD NSSHLPAASY RKKDSGVQES
 1451 SHFLQGAKN NLSLAITLLE MTGDQREVGS LGTSATNSVT YKKVENTVLP
 1501 KPDLPKTS GK VELLPKVHIY QKDLFPTETS NGSPGHLDLV NGSLQLQTEG
 50 1551 AIKWNEANRP GKVPFLRVAT ESSAKTPSKL LDPLAWDNHY GTQIPKEEWK
 1601 SQEKSPEKTA FKKKDTILSL NACESNHAIA AINEGQNKPE IEVTWAKQGR
 1651 TERLCSQNPP VLKRHQREIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1701 EDENQSPRSF QKTRHYFIA AVERLWDYGM SSSPHVLNRN AQSGSVQPFK
 1751 KVVFOEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
 1801 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 55 1851 CKAWAYFSDV DLEKDVHSG LIGPLLCHTN TLNPAHGRQV TVQEFALFFT
 1901 IFDETKSWYF TENMERNCR PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1951 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 2001 VPETVEMLP KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 2051 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
 60 2101 HGKIQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 2151 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 2201 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 2251 VDFQKTMKVT GVTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
 2301 VFQGNQDSFT PVVNSLDPPL LTRYLRHPQ SWVHQIALRM EVLGCEAQDL
 65 2351 Y

FIX amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined

70 MORVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
 ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSKD DINSYECWCP
 FGFEGKNCEL DVTCKIKNGR CEQFCNKNSAD NKVVCSTEG YRLAENQKSC EPAVFPFCGR
 VSVSQTSKLT RAETVFPDND YVNSTEAEI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW

5 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVIIRII
 PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIAADKE YTNIFLKFPS GYVSGWGRVF
 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 GTSFLTGIIS WGEECAMK GK YGIYTKVSRY VNWIKEKTKL T

10

FIX DNA sequence

atgcagcgcg tgaacatgat catggcagaa tcaccaggcc tcatacccat ctgcctttta
 ggatatctac tcagtgcctga atgtacagtt tttcttgatc atgaaaacgc aaaaaatt
 15 ctgaatcggc caaagaggta taattcaggt aaattggaag agtttggtca agggaaatcta
 gagagagaa gtatggaaga aaagtgtagt tttgaagaag cagcagaagt ttttgaaaac
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 gtttctgttt cacaaaacttc taagctcacc cgtgctgaga ctgtttttcc tgatgtggac
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 tttaatgact tcaactcgggt tgttggtgga gaagatgcca aaccagggtca attcccttgg
 25 cagggttgtt tgaatggtaa agttgatgca ttctgtggag gctctatcgt taatgaaaaa
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 30 tacacgaaca tcttcctcaa atttggtatc ggctatgtaa gtggctgggg aagagtcttc
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 gggaccagtt tcttaactgg aattattagc tggggtgaag agtgtgcaat gaaaggcaaa
 35 tatggaatat ataccaaggt atcccgggtat gtcaactgga ttaaggaaaa aacaaagctc
 acttga

FX amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined

40 MGRPLHLVLL SASLAGLLLL GESLFIRREQ ANNILARVTR ANSFLEEMKK GHLERECMEE
 TCSYEEAREV FEDSDKTNEF WNKYKGDGQC ETSPCQNGK CKDGLGEYTC TCLEGFEGKN
 CELFTRKLCs LDNGDCDQFC HEEQNSVCS CARGYTLADN GKACIPTGPFY PCGKQTLERR
 KRSVAQATSS SGEAPDSITW KPYDAADLDP TENPFDLLDF NQTQPERGDN NLTRIVGGQE
 CKDGECFWQA LLINEENEGF CGGTILSEFY ILTAACHLYQ AKRFKVRVGD RNTEQEEGGE
 45 AVHEVEVVIK HNRFTKETVD FDIIVLRLKT PITFRMNVP ACLPERDWAE STLMTQKTGI
 VSGFGRTHEK GRQSTRKLML EVFYVDRNSC KLSSSFIITQ NMFCAGYDTK QEDACQGDSDG
 GPHVTRFKDT YFVTGIVSWG EGCARKGRYG IYTKVTAFLK WIDRSMKTRG LPAKAKSHAPE
 VITSSPLK

50 FX DNA sequence

atggggcgcc cactgcacct cgtcctgctc agtgccctcc tggctggcct cctgctgctc
 ggggaaaagtc tgttcacccg caggagcag gccaaacaaca tcttggcgag ggtcacgagg
 gccaatctct tcttggaaga gatgaagaaa ggacacctcg aaagagagtg catggaagag
 55 acctgctcat acgaagaggg ccgcgaggtc tttgaggaca gcgacaagac gaatgaattc
 tggataaaat acaaagatgg cgaccagtgt gagaccagtc cttgccagaa ccaggggcaaa
 tgtaaaagac gctcggggga atacacctgc acctgttttag aaggattcga agggcaaaaac
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 60 aagaggtcag tggcccaggc caccagcagc agcggggagg cccctgacag catcacatgg
 aagccatctg atgcagccga cctggacccc accgagaacc ccttcgacct gcttgacttc
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5 gccaagagat tcaaggtgag ggtaggggac cggaacacgg agcaggagga gggcggtgag
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 10 gaggtgcccc acgtggaccg caacagctgc aagctgtcca gcagcttcat catcacccag
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 gagggctgtg cccgtaaggg gaagtacggg atctacacca aggtcacccg ctctctcaag
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 15 gtcataacgt cctctccatt aaagtga

DNA sequence of FVII-066

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG
 20 CTGCCCTGGCT
 61 GCAGTCTTTC TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCTTGCAAG
 25 301 GACCAGCTCC AGTCCATATAT CTGCTTCTGC CTCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACCAAG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCACTGTGTC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTAATC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAGAGGG
 30 601 GAGTGTCCAT GGCAGGTCTT GTTGTGGTGT AATGGAGCTC AGTTGTGTGT GGGGACCCCTG
 661 ATCAACACCA TCTGGGTGGT CTCGCGGCC CACTGTTTCG ACAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
 781 CGGGGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACAC CAACCACGAC
 841 ATCGCGCTGC TCCGCTGCA CCAGCCCGTG GTCTCACTG ACCATGTGGT GCCCTCTGTC
 35 901 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TCGGCTTCTC ATTGGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CCGTGCGGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
 1021 CCGCGGCTGA TGACCCAGGA CTGCCCTGCA CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
 1081 ATCACGGAGT ACATGTTCTG TCCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
 1141 GACASTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGCATCGTC
 40 1201 AGCTGGGGCC AGGGCTGCGC AACCCTGGGC CACTTTGGGG TGTACACCAG GGTCTCCAG
 1261 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCTGCGA
 1321 GCCCCATTTT CCGGTGGCGG TGGCTCCGCG GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
 1381 GGGGGTGGAT CAGGGGTGAG AGGTTCGGGT GCGGGGGAT CCGACAAAAC TCACACATGC
 1441 CCACCGTGCC CAGCTCCGGA ACTCCTGGGA GGACCGTCAG TCTCTCTCTT CCCCCAAAA
 45 1501 CCAAGGACA CCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
 1561 AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGG GGTGCATAAT
 1621 GCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCTCT
 1681 ACCGTCTGTC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT TTCCAACAAA
 1741 GCCTCCCGAG CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
 50 1801 CAGGTGTACA CCTGCCCTTC ATCCCGGGAT GAGCTGACCA AGAACAGGT CAGCCTGACC
 1861 TGCTGTGTA AAGGCTTCTA TCCAGCGAC ATCGCGCTGG AGTGGGAGAG CAATGGCAG
 1921 CCGGAGAACA ACTACAAGAC CACGCTCTCC GTGTGGACT CCGACGGCTC CTCTCTCTCT
 1981 TACAGCAAGC TCACCGTGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
 2041 GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
 55 2101 AAACGGCGCC GCGGAGCGG TGGCGCGGGA TCAGGTGGGG GTGGATCAGG CGGTGGAGGT
 2161 TCCGCTGGCG GGGGATCCGG CCGTGGAGGT TCCGCTGGGG GTGGATCAAG GAAGAGGAGG
 2221 AAGAGGGCGC AGGTGCAGCT GCAGGAGTCT GGGGAGGCT TGGTACAGCC TGGGGGTCC
 2281 CTGAGACTCT CCTGTGCAGC CTCTGGATTC ATGTTTAGCA GGTATGCCAT GAGCTGGGTCT
 2341 CGCCAGGCTC CAGGGAAGGG GCCAGAGTGG GTCTCAGGTA TTAGTGGTAG TGGTGGTAGT
 60 2401 ACATACTACG CAGACTCCGT GAAGGGCCGG TTCAACGCTT CCAGAGACAA TTCCAAGAAC
 2461 ACGCTGTATC TGCAAAATGAA CAGCCTGAGA GCCGAGGACA CCGCTGTATA TTAAGTAAAGG
 2521 CCGGGCGCCA CCTACACCAG CCGGAGCGAC GTGCCCCACC AGACCAGCTT CGACTACTGG
 2581 GCGCAGGGAA CCTGTGTCAC CTTCTCTCTA GGGAGTGCAT CCGCCCCAAA GCTTGAAGAA
 2641 GGTGAATTTT CAGAAGCAGC CTTATCTGAA CTGACTCAGG ACCCTGCTGT GTCTGTGGCC
 65 2701 TTGGGACAGA CAGTCAGGAT CACATGCCAA GGAGACAGCC TCAGAAACTT TTATGCAAGC
 2761 TGGTACAGC AGAAGCCAGG ACAGGCCCTT ACTCTGTGTA TCTATGTTT AAGTAAAGG
 2821 CCCTCAGGGA TCCCAGACCG ATTCTCTGCC TCCAGCTCAG GAAACACAGC TTCTTTGACC
 2881 ATCACTGGGG CTCAGGCGGA AGATGAGGCT GACTATTACT GCCTGTGTA CTACGGCGGC
 2941 GCGCAGCAGG GCGTGTTCGG CCGCGGACC AAGCTGACCG TCCTACGTCA GCCAAGGCT
 70 3001 GCCCCCTCGG TCACTCTGTT CCGGCCCTCT TCTGCGGCCG GTGGCGGTGG CTCCGGCGGA
 3061 GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG GGTGATCAG GCGGTGGAGG TTCCGGTGGC

5	3121	GGGGGATCAG	ACAAAACCTCA	CACATGCCCA	CCGTGCCCCAG	CACCGGAACT	CCTGGGCGGA
	3181	CCGTGAGTCT	TCCTCTTCCC	CCCCAAACCC	AAGGACACCC	TCATGATCTC	CCGGACCCCT
	3241	GAGGTACAT	GCGTGGTGGT	GGACGTGAGC	CACGAAAGACC	CTGAGGTCAA	GTTCAACTGG
	3301	TACGTGGACG	GCGTGGAGGT	GCATAATGCC	AAGACAAAGC	CGCGGGAGGA	GCAGTACAAC
	3361	AGCACGTACC	GTGTGGTCAG	CGTCCTCACC	GTCTGTCACC	AGGACTGGCT	GAATGGCAAG
10	3421	GAGTACAAGT	GCAAGGTCTC	CAACAAAGCC	CTCCAGCCCC	CCATCGAGAA	AACCATCTCC
	3481	AAAGCCAAAG	GGCAGCCCCG	AGAACCACAG	GTGTACACCC	TGCCCCATC	CCGGGATGAG
	3541	CTGACCAAGA	ACCAGGTACG	CCTGACCTGC	CTGGTCAAAG	GCTCTATCC	CAGCGACATC
	3601	GCCGTGGAGT	GGGAGAGCAA	TGGGCAGCCG	GAGAACAAC	ACAAGACCAC	GCCTCCCGTG
	3661	TTGGACTCCG	ACGGCTCCTT	CTTCCTCTAC	AGCAAGCTCA	CCGTGGACAA	GAGCAGGTGG
15	3721	CAGCAGGGGA	ACGTCTTCTC	ATGCTCCGTG	ATGCATGAGG	CTCTGCACAA	CCACTACACG
	3781	CAGAAGAGCC	TCTCCCTGTC	TCCGGGTAAA	TGA		

FVII-066 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or SCE5 to Fc region is underlined, and linker with proprotein convertase processing sites is shown in bold

20	1	MVSQALRLLC	LLLGLQGCLA	AVFVTQEEAH	GVLHRRRRAN	AFLEELRPGS	LERECKEEQC
	61	STEEAREIFK	DAERTKLEWI	SYSDDQDCAS	SPCQNGGSCK	DQLQSYICFC	LPAFEGRNCE
	121	THKDDQLICV	NENGGCEQYC	SDHTGTRKSC	RCHEGYSLLA	DGVSCPTVE	YPCGKIPILE
	181	KRNASKPQGR	IVGGKVCPRG	ECFQWVLLLV	NGAQLCGGTL	INTIWWVSAA	HCFDKIKNWR
25	241	NLIAVLGEHD	LSEHDGDEQS	RRVAQVIIPS	TYVPGTTNHD	IALLRLHQPV	VLTDHVVPIC
	301	LPERTFSERT	LAFVRFSLVS	GWGQLLDRC	TALELMVLNV	PRLMTQDCLQ	QSRKVGDSFN
	361	ITYEMFCAGY	SDGSKDSCRG	DSGGPHATHY	RGTWYLTGIV	SWGQGCATVG	HPGVYTRVBSQ
	421	YIEWLQKLMR	SEPRPGVLLR	APFPGGGSSG	GGGSGGGSSG	GGGSGGGSSG	GGGSKDTHTC
	481	PPCPAPELLG	GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN
30	541	AKTKPREEQY	NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKTI	SKARGQPREP
	601	QVYTLPPSRD	ELTKNQVSLT	CLVKGFPYPS	IAVEWESNGQ	PENNYKTTPF	VLDSDGSFFL
	661	YSKLTVDKSR	WQQGNVFSKS	VMHEALHNHY	TQKSLSLSPG	KRRRRSGGGG	SGGGSGGGGG
	721	SGGGSGGGGG	SGGGSSRKRR	KRAQVQLQES	GGGLVQPGGS	LRLSCAASGF	MFSRYAMSWV
	781	RQAPGKGPFW	VSGISGSGGS	TYYADSVKGR	FTVSRDNSKN	TLYLQMNLSL	AEDTAVYYCA
35	841	RGATYTSRSD	VFDQTSFDYW	GGQTLTVVSS	GSASAPKLEE	GEFSEARVSE	LTQDPAVSV
	901	LGQTVRITCQ	GDLSLNFYAS	WYQQKPGQAP	TLVIYGLSKR	PSGIPDRFSA	SSSGNTASLT
	961	ITGAQAEDEA	DYYCLLYYGG	QQQGVFGGGT	KLTIVLRQPKA	APSVTLFFPS	SAAGGGGSGG
	1021	GGSGGGGSGG	GGSGGGGSGG	GGSDKTHTCP	PCPAPELLGG	PSVFLFPPKP	KDTLMISRTF
	1081	EVTCVVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN	STYRVVSVLT	VLHQDWLNGK
40	1141	EYKCKVSNKA	LPAPIEKTI	KAKGQPREPQ	VYTLPPSRDE	LTKNQVSLTC	LVKGFYPSDI
	1201	AVEWESNGQP	ENNYKTTPPV	LDSGGSFFLY	SKLTVDKSRW	QQGNVFSCSV	MHEALHNHYT
	1261	QKSLSLSPGK	*				

45	DNA sequence for FVII-057							
	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCCTGGCT	
	61	GCAGTCTTTC	TAACCCAGGA	GGAAGCCCAC	GGCGTCCCTG	ACCGGCGCCG	GCGCGCCAAC	
	121	GCGTTTCCTG	AGGAGCTGCG	GCCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCACTGC	
	181	TCTTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAAGCT	GTTCCTGCATT	
50	241	TCTTACAGTG	ATGGGGACCA	GTGTGCCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG	
	301	GACCAGCTCC	ATGCTCTATAT	CTGCTTCTGC	CTCCCTGCCT	TCGAGGGCCG	GAAGTGTGAG	
	361	ACGCACAAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	GCAGTACTGC	
	421	AGTGACCACA	CGGGCACCAC	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA	
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATTTCTAGAA	
55	541	AAAAGAAATG	CCAGCAAAGC	CCAAGGCCGA	GGTGGCGGTG	GCTCCGGCGG	AGGTGGGTCC	
	601	GGTGGCGCGG	GATCAGGTGG	GGGTGGATCA	GCGCGTGGAG	GTTCGGGTGG	CGGGGGATCC	
	661	GACAAAACTC	ACACATGCCC	ACCGTGCCCA	GCTCCGGAAC	TCCTGGGAGG	ACCGTCAGTC	
	721	TTCCTCTTCC	CCCCAAAACC	CAAGGACACC	CTCATGATCT	CCCGGACCCC	TGAGGTACAA	
	781	TGCGTGGTGG	TGGACGTGAG	CCACGAAGAC	CCTGAGGTCA	AGTTCAACTG	GTACGTGGAG	
60	841	GGCGTGGAGG	TGCATAATGC	CAAGACAAAG	CCGCGGGAGG	AGCAGTACAA	CAGCAGCTAC	
	901	CCTGTGGTCA	GCGTCTCACC	CGTCTGTGAC	CAGGACTGGC	TGAATGGCAA	GGAGTACAAG	
	961	TGCAAGGTCT	CCAACAAAGC	CCTCCAGGCC	CCCATCGAGA	AAACCATCTC	CAAAGCCAAA	
	1021	GGGCAGCCCC	GAGAACACAA	GGTGTACACC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	
	1081	AACCAAGTCA	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	
65	1141	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GTGGGACTCC	
	1201	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC	ACCGTGCACA	AGAGCAGGTG	GCAGCAGGGG	
	1261	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	
	1321	CTCTCCCTGT	CTCCGGGTAA	AGGTGGCGGC	GGATCAGGTG	GGGGTGGATC	AGGCGGTGGA	
	1381	GGTTCGGGTG	GCGGGGGATC	CGGCGGTGGA	GGTTCGGGTG	GGGGTGGATC	AGGAGGAGGT	
70	1441	GGTTCAGCG	TGAGCCAGAC	CAGCAAGCTG	ACCGGATTG	TGGGGGGCAA	GGTGTGCCCC	
	1501	AAAGGGGAGT	GTCCATGGCA	GGTCTGTGTT	TTGGTGAATG	GAGCTCAGTT	GTGTGGGGGG	
	1561	ACCCTGATCA	ACACCATCTG	GGTGGTCTCC	GCGGCCCACT	GTTTCGACAA	AATCAAGAAA	

5
1621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA GCGAGCACGA CGGGGATGAG
1681 CAGAGCCGGC GGGTGGCGCA GGTTCATCATC CCCAGCACGT ACGTCCCGGG CACCACCAAC
1741 CACGACATCG CGCTGCTCCG CCGTGCACAG CCGGTGGTCC TCACTGACCA TGTGGTGCC
1801 CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTGCG CTCTCATTTG
10 1861 GTCAGCGGCT GGGGCCAGCT GCTGGACCGT GCGGCCACGG CCTGGAGCT CATGGTCCTC
1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
1981 CCAAAATATCA CGGAGTACAT GTTCTGTGCC GGCTACTCGG ATGGCAGCAA GGACTCCTGC
2041 AAGGGGGACA GTGGAGGCC ACATGCCACC CACTACCGGG GCACGTGGTA CTGACGGGC
2101 ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTCTA CACCAGGGTC
2161 TCCAGTACA TCGAGTGGCT GCAAAAGCTC ATGCGCTCAG AGCCACGCC AGGAGTCCTC
15 2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
2281 TCAGGTGGGG GTGGATCAGT CGGTGGAGGT TCCGTGGCG GGGGATCAGA CAAAACCTAC
2341 ATCGTCCCCC CGTGGCCAGC ACCTGAATC CTGGGAGGAC CGTCACTCTT CCTCTTCCCC
2401 CCAAAACCCA AGGACACCCCT CATGATCTCC CGGACCCCTG AGGTACATG CGTGGTGGTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
20 2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581 GTCCTACCGT TCCTGCACCA GCACTGGCTG AATGGCAAGG AGTACAAGT CAAGCTCTCC
2641 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACCACAGG TGTACACCCCT GCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTACAGC
2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
25 2821 GGGCAGCCGG AGAACAACCTA CAAGACCACG CCTCCCCTGT TGGACTCCGA CGGCTCCTTC
2881 TTCTCTACA GCAAGCTCAC CCGTGGACAAG AGCAGGTGGC AGCAGGGCAA CGTCTTCTCA
2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001 CCGGATAAAT GA

30
FVII-057 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the Factor XIa cleavage site is shown in bold, and the Factor XIa cleavage site is shown in dashed underline

35 1 MVSQALRLLC LLLGLQGCLA AVFVTOEFAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLFWI SYSDGDDQAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THRDDQLICV NENGCEQYC SDHTGTRKSC RCHGYSLLA DGVSCPTVE YPGKIPILE
181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTCPCPCP APBLLGGPSV
241 FLFPFKPRDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHMAKTK PREEQYNSTY
40 301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPSSRDELTK
361 NQVSLTCLVK GFYPDSIAVE WESNGQPENN YKTPPVLDSD DGSFFLYSKL TVDKSRWQQG
421 NVFSCSVME ALHNHYTQKS LSLSPGKGGG **SGGGSGGGSG** **SGGGSGGGSG** **SGGGSGGGSG**
481 **GS**SVSQTSLK TRIVGGKVCP KGECEFWQVLL LVNGAQLCGG TLINTIWWVS AAHCDFDKIKN
541 WRNLI AVLGE HDLSEHDGDE QSRRAVQVII PSTYVPGETN HDIALRLRHQ PVVLT DHVVP
601 LCLPERTFSE RTLAIVRFSL VSGWGQLDR GATALELMVL NVPRLMTPQC LQSRKVGDS
45 661 PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFVGYTRV
721 SQYIEWLQKL MRSEPRPGVL LRAPFPGGGG SGGGSGGGSG SGGGSGGGSG SGGGSGDKTH
781 TCPPCPAPEL LGGPVFLFPP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
841 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
901 EPQVYTLPPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN GQPENNYKTT PPVLDSDGSF
50 961 FLYSKLTVDK SRWQGNVFS CSVMHEALHN HYTKSLSLSL PGK*

DNA sequence for FVII-058

55 1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTCTGCTTGG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCGG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGAT
241 TCTTACAGTG ATGGGGACCA GTGTGCTTCA AGTCCATGCC AGAATGGGGG CTCTGCAAG
60 301 GACCAGCTCC AGTCCTATAT CTGCTCTGCG CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
361 ACGCACAAAG ATGACCAGCT GATCTGTGTG AACGAGAACG CCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CCGGCACCAA GCGCTCCTCT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGCCCGA GGTGGCGGTG CCTCCGCGCG AGGTGGGTCC
601 GGTGGCGCGG GATCAGGTGG GGGTGGATCA GCGCGTGGAG GTTCCGCTGG CCGGGGATCC
65 661 GACAAAACCT ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCACTC
721 TTCTCTCTTC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTACAC
781 TCGPGCTGGT TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GCGGTGGAGG TGCAATATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901 CGTGTGGTCA GCGTCTCTAC CGTCTGTCAC CAGGACTGGC TGAATGGCAA GGAGTACAA
70 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CCGCTGGAG

5	1141	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GTTGGACTCC
	1201	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC	ACCGTCGACA	AGAGCAGGTG	GCAGCAGGGG
	1261	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACCTACAC	GCAGAAGAGC
	1321	CTCTCCCTGT	CTCCGGGTAA	AGGTGGCGGC	GGATCAGGTG	GGGGTGGATC	AGGCGGTGGA
	1381	GGTCCCGGTG	GCGGGGGATC	CGGCGGTGGA	GGTCCCGGTG	GGGGTGGATC	AGGAGGAGGT
10	1441	GGTTCAGACT	TCCTGGCCGA	GGGCGGCGGC	GTGCGGATTG	TGGGGGGCAA	GGTGTGCCCC
	1501	AAAGGGGAGT	GTCCATGGCA	GCTCCTGTTG	TTGGTGAATG	GAGCTCAGTT	GTGTGGGGGG
	1561	ACCGTGATCA	ACACCATCTG	GGTGGTCTCC	GCGGCCCACT	GTTTCGACAA	AATCAAGAAC
	1621	TGGAGGAACC	TGATCGCGGT	GCTGGGCGAG	CACGACCTCA	GCGAGCACGA	CGGGGATGAG
	1681	CAGAGCCGGC	GGGTGGCGCA	GGTCATCATC	CCCAGCACGT	ACGTCCCGGG	CACCACCAAC
15	1741	CACGACATCG	CGCTGCTCCG	CCTGCACCAG	CCCGTGGTCC	TCACTGACCA	TGTGGTGCCC
	1801	CTCTGCCTGC	CCGAACGGAC	GTTCTCTGAG	AGGACGCTGG	CCTTCGTGCG	CTTCTCATTT
	1861	GTACGCGGCT	GGGGCCAGCT	GCTGGACCGT	GCGGCCACGG	CCCTGGAGCT	CATGGTCCTC
	1921	AACGTGCCCC	GGCTGATGAC	CCAGGACTGC	CTGCAGCAGT	CACGGAAGGT	GGGAGACTCC
	1981	CCAAATATCA	CGGAGTACAT	GTTCTGTGCC	GGCTACTCGG	ATGGCAGCAA	GGACTCCTGC
20	2041	AAGGGGGACA	GTGGAGGCCC	ACATGCCACC	CACTACCGGG	GCACGTGGTA	CCTGACGGGC
	2101	ATCGTCAGCT	GGGGCCAGGG	CTGCGCAACC	GTGGGCCACT	TTGGGGTGTG	CACCAAGGTC
	2161	TCCAGTAGTA	TCGAGTGGCT	GCAAAAGCTC	ATGCGCTCAG	AGCCACGCCC	AGGAGTCCTC
	2221	CTGCGAGCCC	CATTTCCCGG	TGGCGGTGGC	TCCGGCGGAG	GTGGGTCCGG	TGGCGGCGGA
	2281	TCAGGTGGGG	GTGGATCAGG	CGGTGGAGGT	TCCGGTGGCG	GGGGATCAGA	CAAAACTCAC
25	2341	ACATGCCCCAC	CGTGCCACGC	ACCTGAACTC	CTGGGAGGAC	CGTCAGTCTT	CCTCTTCCCC
	2401	CAAAAACCCA	AGGACACCTT	CATGATCTCC	CGGACCCCTG	AGGTCACATG	CGTGGTGGTG
	2461	GACGTGAGCC	ACGAAGACCC	TCAGGTCAAG	TTCAACTGGT	ACGTGGACGG	CGTGGAGGTG
	2521	CATAATGCCA	AGACAAAGCC	GCGGGAGGAG	CAGTACAACA	GCACGTACCG	TGTGGTCAGC
	2581	GTCTCACCAG	TCCTGCACCA	GGACTGGCTG	AATGCCAAGG	AGTACAAGTG	CAAGGTCTCC
30	2641	AACAAAGCCC	TCCCAGCCCC	CATCGAGAAA	ACCATCTCCA	AAGCCAAAGG	GCAGCCCCGA
	2701	GACCCACAGG	TGTACACCCCT	GCCCCCATCC	CGGGATGAGC	TGACCAAGAA	CCAGTTCAGC
	2761	CTGACCTGCC	TGGTCAAAGG	CTTCTATCCC	ACCGACATCG	CCGTGGAGTG	GGAGAGCAAT
	2821	GGGACGCCGG	AGAACAACCT	CAAGACCACG	CCTCCCGTGT	TGGACTCCGA	CGGCTCCTTC
	2881	TTCCTCTACA	GCAAGCTCAC	CCTGGACAA	AGCAGGTGGC	AGCAGGGGAA	CGTCTTCTCA
35	2941	TGCTCCGTGA	TGCATGAGGC	TCTGCACAAC	CACCTACACG	AGAAGAGCCT	CTCCCTGTCT
	3001	CGGGTAAAT	GA				

FVII-058 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

40	1	MVSGALRLLC	LLLGLQGCLA	AVFVTOEEAH	GVLHRRRRAN	AFLEELRPGS	LERECKEQEC
	61	SFEAREIFK	DAERTKLEWI	SYSDGDQCAS	SPCQNGGSCK	DQLQSYICFC	LPAFEGRNCE
	121	THKDDQLICV	NENGGCEQYC	SDHTGTRKSC	RCHEGYSLLA	DGVSTPTVE	YPCGKIPILE
	181	KRNASKPQGR	GGGGSGGGGS	GGGGSGGGGS	GGGGSGGGGS	DKTHTCPFCP	APELLGGPSV
45	241	FLFFPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY
	301	RVS SVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPQVYT	LPFSRDELTK
	361	NQVSLTCLVK	GFYPSTDAVE	WESNGQPENN	YKTPPVFLDS	DGSFFLYSKL	TVDKSRWQGG
	421	NVFSQSVNHE	ALHNHYTQKS	LSLSFGKGGG	GGGGGGGGGG	GGGGGGGGGG	GGGGGGGGGG
	481	GSDFLAEGGG	VRIVGGKVCP	KGECFWQVLL	LVNGAQLCGG	TLINTIWWVS	AAHCFDKIKN
50	541	WRNLI AVLGE	HDLSEHDGDE	QSRRVAQVII	PSTVYVPGTTN	HDIALRLRHQ	PVVLTDHVVV
	601	LCLPERTFSE	RTLAFVRFSL	VSGWGQLLDR	GATALELMVL	NVPRLMTQDC	LQSRKVGDS
	661	PNITEYMFCA	GYS DGS KDSC	KGDSGGPHAT	HYRGTYLTLG	IVSWGQGCAT	VGHFGVYTRV
	721	SQYIEWLQKL	MRSEPRPGVL	LRAPFPGGGG	SGGGSGGGGG	SGGGSGGGGG	SGGGSGDKTH
	781	TCPPCPAPEL	LGGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV
55	841	HNAKTKPREE	QYNSTYRVVS	VLTVLHQDWL	NGKEYKCKVS	NKALPAPIEK	TISKAKGQPR
	901	EPQVYTLPPS	RDELTKNQVS	LTCLVKGFYP	SDIAVEWESN	GQPENNYKTT	PPVLDS DGSF
	961	FLYSKLTVDK	SRWQQGNVFS	CSVMHEALHN	HYTQKSLSL	PGK*	

DNA sequence for FVII-059

60	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
	61	CGAGTCTTTC	TAACCCAGGA	GGAGGCCAC	GGCGTCTTGC	ACCGGCGCCG	GCGGCCCAAC
	121	GCGTTCCTGG	AGGAGCTGCG	GCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	TCCTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAAGCT	GTTCTGGATT
	241	TCTTACAGTG	ATGGGGACCA	GTGTGCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG
65	301	GACCAGCTCC	AGTCTTATAT	CTGCTTCTGC	CTCCTGCTTC	TCGAGGGCCG	GAAGTGTGAG
	361	ACGCACAAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	CGAGTACTGC
	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATTCTAGAA
	541	AAAAGAAATG	CCAGCAAACC	CCAAGGCCGA	GGTGGCGGTG	GCTCCGCGCG	AGGTGGGTCC
70	601	GGTGGCGGCG	GATCAGGTGG	GGGTGGATCA	GGCGGTGGAG	GTTCCGGTGG	CGGGGGATCC
	661	GACAAAATCT	ACACATGCC	ACCGTGCCCA	GCTCCGGAAC	TCCTGGGAGG	ACCGTCAGTC
	721	TTCCTCTTCC	CCCCAAAACC	CAAGGACACC	CTCATGATCT	CCCGGACCCC	TGAGGTACACA

5
781 TGGCTGGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GGGGTGGAGG TGCATAATGC CAAGACAAAG CCGGGGGAGG AGCAGTACAA CAGCAGGTAC
901 CGTGTGGTCA GCGTCTCTAC CGTCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAAG
961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
10
1021 GGGCAGCCCC GAGAACCCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGACAA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
15
1381 GGTTCGGGTG GCGGGGGATC CGCGGGTGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCGAACCA CCAAGATCAA GCGCCGGATT GTGGGGGGCA AGGTGTGCCC CAAAGGGGAG
1501 TGTCCATGGC AGGTCTGTGT GTTGGTGAAT GGAGCTCAGT TGTGTGGGGG GACCCTGATC
1561 AACACCATCT GGGTGGTCTC CGCGGGCCAC TGTTCGACA AAATCAAGAA CTGGAGGAAC
1621 CTGATCGCGG TGCTGGGCGA GCACGACCTC AGCGAGCAGC ACGGGGATGA GCAGAGCCGG
20
1681 CGGGTGGCGC AGGTCTATCAT CCCCAGCAGC TACGTCCCGG GCACCACCAA CCACGACATC
1741 CGGCTGCTCC GCCTGCACCA GCGCGTGGTC CTCACTGACC ATGTGGTGGC GTTCTGCTG
1801 CCGGAACGGA CGTTCTCTGA GAGGACGGTG GCCTTTCGTG GCTCTCTATT GGTTCAGCGC
1861 TGGGGCCAGC TGCTGGACCG TGGCGCCACG GCGCTGGAGC TCATGGTCTT CAACGTGCCC
1921 CGGCTGATGA CCCAGGACTG CCTGCAGCAG TCACGGAAGG TGGGAGACTC CCCAAATATC
25
1981 ACGGAGTACA TGTTCCTGTG CCGCTACTCG GATGGCAGCA AGGACTCCTG CAAGGGGGAC
2041 AGTGGAGGCC CACATGCCAC CCACTACCGG GGCACGTGGT ACCTGACGGG CATCGTCAGC
2101 TGGGGCCAGG GCTGCGCAAC CGTGGGCCAC TTTGGGGTGT ACACCAGGGT CTCCCAGTAC
2161 ATCGAGTGGC TGCAAAAGCT CATGCGCTCA GAGCCACGCC CAGGAGTCTT CCTGCGAGCC
2221 CCATTTCCCG GTGGCGGTGG CTCCGGCGGA GGTGGGTCCG GTGGCGCGCG ATCAGGTGGG
30
2281 GGTGGATCAG GCGGTGGAGG TTCCGGTGGC GGGGGATCAG ACAAACCTCA CACATGCCCA
2341 CCGTSCCCAG CACCTGAACCT CCGGGGAGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC
2401 AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTACATC GCGTGGTGGT GGACGTGAGC
2461 CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGAGC GCGTGGAGGT GCATAATGCC
2521 AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCAGGTACC GTGTGGTTCAG CGTCTCAGC
35
2581 GTCTGSCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC
2641 CTCCTCAGCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCGG AGAACACAG
2701 GTGTACACCC TGCCCCCACC CCGGGATGAG CTGACCAAGA ACCAGGTACG CCTGACCTGC
2761 CTGCTCAAAG GCTTCTATCC CAGCGACATC GCGTGGAGT GGGAGAGCAA TGGGCAGCCG
2821 GAGAACAAC TACAAGACCAC GCGTCCCGTG TTGGACTCCG ACGGCTCCTT CTCTCTCTAC
40
2881 AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTCTCT ATGCTCCGTG
2941 ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTCTC TCCGGGTAAA
3001 TGA

45 FVII-059 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
50 61 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGEQYQC SDHTGTRKSC RCHEGYSLLA DGVSCPTPVE YPCGKIPILE
181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTCTPPCP APPELLGGPSV
241 FLFFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
361 NQVSLTCLVK GFYPSTDAVE WESNGQPENN YKTTPPVLDG DGSFFLYSKL TVDKSRWQQG
55 421 NVFSCSVMEH ALHNHYTQKS LSLSFGKGGG GGGGGGGGGG GGGGGGGGGG GGGGGGGGGG
481 GSTTKIKPRI VGGKVCCKGE CPWQVLLLVN GAQLCGGTLI NTIWWVSAAH CFDKIKNWRN
541 LIAVLGEHDL SEHDGDEQSR RVAQVIFEST YVPGTTHNDI ALLRLHQFVV LTDHVVPLCL
601 PERTFSERTL AFVRFSLVSG WGQLLDRGAT ALELMVLNVP RLMTQDCLQQ SRKVGDSPTNI
661 TEYMFCAGYS DGSKDSCKGD SGGPHATHYR GTWYLTGIVS WQGCATVGH FGVTTRVSQY
60 721 IEWLQKLMRS EPRPGVLLRA PFPGGGSGGG GSGGGGSGGG GSGGGGSGGG GSGDKHTHTCP
781 PCPAPELLGG PSVFLFPPKP KDTLMISRTPEVTCVVVDVS HEDPEVKFNW YVDGEVFNHNA
841 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYCKVSNKA LPAPIEKTIS KAKGQPREPQ
901 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
961 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK *

DNA sequence for FVII-060

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTCTGCTTGG GGCTTCAGGG CTGCGCTGGCT
50 61 GCAGTCTTCG TAACCAGGGA GGAAGCCAC GCGTCTCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
70 181 TCCTTCAGAG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTCAAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
301 GACCAGCTCC AGTCTATATAT CTGCTTCTGC CTCCTGCCT TCGAGGGCCG GAACTGTGAG

5
361. ACCGACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421. AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481. GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541. AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
10 601. GGTGGCGGCG GATCAGGTGG GGGTGGATCA GCGGTGGAG GTTCCGGTGG CGGGGGATCC
661. GACAAAACCTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCACTC
721. TTCCTCTTCC CCCCCAAACC CAAGACACC CTCATGATCT CCGGAACCCG TGAGCTCACA
781. TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
841. GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901. CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
15 961. TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021. GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCGGGATGA GGTAGCCAA
1081. AACCAAGTCA GCCTGAACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1141. TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201. GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGACAA AGAGCAGGTG GCAGCAGGGG
20 1261. AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321. CTCTCCTTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381. GGTTCGGGTG GCGGGGGATC CCGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441. GGTTCAGCCC TGCGGCCCGG GGTGGTGGGC GCGCGCGTGG TGGGGGGCAA GGTGTGCCCC
1501. AAAGGGGAGT GTCCATGGCA GGTCTCTGTG TTGGTGAATG GAGCTCAGTT GTGTGGGGGG
25 1561. ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGCCCACT GTTTCGACAA AATCAAGAAC
1621. TACATGACAC TGATCGCGGT GCTGGGCGAG CAGCAGCTCA GCGAGCACGA GGTAGACTAG
1681. CAGAGCCGGC GGGTGGCGCA GGTCTCATC CCCAGCACGT ACGTCCCGGG CACCACCAAC
1741. CACGACATCG CGCTGCTCCG CCTGCACCAG CCGGTGGTCC TCACTGACCA TGTGGTGGCC
1801. CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CTTCTGTCGG CTCTCTCATG
30 1861. GTCAGCGGCT GGGGCCAGCT GCTGGACCGT GCGGCCACGG CCTTGGAGCT CATGGTCTCT
1921. AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
1981. CCAAATATCA CGGAGTACAT GTTCTGTGCC GGCTACTCGG ATGGCAGCAA GGACTCCTGC
2041. AAGGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
2101. ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTCTA CACCAGGGTC
35 2161. TCCCAGTACA TCGAGTGGGT GCAAAAGCTC ATGCGCTCAG AGCCACGCCC AGGAGTCTCT
2221. CTGCGAGCCC CATTTCGGGT TCGCGGTGGC TCGCGCGGAG GTGGGTCCGG TGGCGCCGGA
2281. TCAGGTGGGG GTGGATCAGG CCGTGGAGGT TCGGTGGCG GGGGATCAGA CAAAACCTAC
2341. ACATGCCCAC CGTGGCCAGC ACCTGAACTC CTGGGAGGAC CGTCAGTCTT CCTCTTCCCC
2401. CCAAAACCCA AGGACACCCCT CATGATCTCC CCGACCCCTG AGGTACATG CGTGGTGGTG
40 2461. GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
2521. CATTAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581. GTCTCACCG TCCTGCACCA GCACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
2641. AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701. GAACACAGG TGTACACCCCT GCGCCCATCC CCGGATGAGC TGACCAAGAA CCAGGTACAG
45 2761. CTGACCTGCC TGGTCAAAGG CTTCTATCCC ACCGACATCG CCGTGGAGTG GGAGAGCAAT
2821. GGGCAGCCGG AGAACAACCT CAAGACCAGC CCTCCCGTGT TGGACTCCGA CGGCTCTCTC
2881. TTCTCTACA GCAAGCTCAC CCTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941. TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001. CCGGGTAAAT GA

50

FVII-060 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

55 1. MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRFGS LERECKEEQC
61. SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121. THKDDQLICV NENGCEQYC SDHTGTRKSC RCHBGYSLLA DGVSCPTTVE YPCGKIPILE
181. KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTHTCPFCP APELLGGPSV
241. FLFFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
301. RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPSPRDELTK
60 361. NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTFPPVLDG DGSFFFLYSKL TVDKSRWQQG
421. NVFSCSVME ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
481. GSALRPRVVG GAVVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWWVS AAHCFDKIKN
541. WRNLIATLGE HDLSEHDGDE QSRKVAQVII PSTYVPGTTN HDIALRLRHQ PVVLTLDHVP
601. LCLPERTFSE RTLAFAVRFSL VSGWGQLLDR GATALELMVL NVPRLMFQDC LQSRKVGDS
65 661. PNITEYMFCA GYSDGSKDSC KGDSSGPHAT HYRGTWYLTG IVSWGQGCAT VGHFVGYTRV
721. SGIIEWLQKL MRSEPRPGVL LRAPFPGGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
781. TCPPCPAPEL LGGPVFLFPE PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
841. HNARTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAIEK TISKAKGQPR
901. EPQVYTLPPS RDELTKNQVLS LTKLVKGFYP SDIAVEWEEN GQPENNYKTT PPVLDSDGSF
70 961. FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLG PGK*

5 DNA sequence for FVII-061

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1 ATGCTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCTCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
10 241 TCTTACAGTG ATGGGGACCA GTGTGCCCTCA AGTCCATGCC AGAATGGGGG CTCTCTGCAAG
301 GACCAGCTCC AGTCTATAT CTGCTTCTGC CTCCTGCTTC TCGAGGGCCG GAACTGTGAG
361 AGGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
15 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
601 GGTGGCGGGG GATCAGGTGG GGGTGGATCA GCGGTGGAG GTTCCGGTGG GGGGGGATCC
661 GACAAAACTC ACACATGCC ACCGTGCCCA GCTCCGGAAC TCCGAGGAGG ACCGTGAGTC
721 TTCCTCTTCC CCCCCAACCC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTACAA
781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
20 841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901 CGTGTGGTCA GCGTCTCACC CGTCTGACAC CAGGATGGC TGAATGGCAA GGAGTACAA
961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCCAA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
25 1141 TGGGAGAGCA ATGGGCGAGC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGAGCA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCGCGTG GCGGGGGATC CCGCGGTGGA GGTTCGCGTG GGGGTGGATC AGGAGGAGGT
30 1441 GGTTGAGCCC TGCGGCCCGG GGTGGTGGGC GCGGCCATTG TGGGGGGCAA GGTGTGCCCC
1501 AAAGGGGAGT GTCCATGGCA GGTCTCTGTG TTGGTGAATG GAGCTCACTT GTGTGGGGGG
1561 ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGCCACT GTTTCGACAA AATCAAGAAC
1621 TGAGGGAACC TGATCGCGGT GCTGGGCGAG CAGGACCTCA GCGAGCACGA CGGGGATGAG
1681 CAGAGCCGCG GGGTGGCGCA GGTCTATCAT CCCAGCACGT ACCTCCCGGG CACCACCAAC
35 1741 CACGACATCG CGCTGCTCCG CCTGCACCAG CCCGTGGTCC TCACTGACCA TGTGGTGCCT
1801 CTTGCTCCTG CCGAAGCGAC GTTCTCTGAG AGGACGCTGG CTTCTGTCGG CTCTCATATG
1861 GTCAGCGGCT GGGGCCAGCT GCTGGACCGT GCGGCCACGG CCTGGAGCT CATGGTCTCT
1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
1981 CCAAATATCA CCGAGTACAT GTTCTGTGCC GCTTACTCGG ATGGCAGCAA GGACTCCTGC
40 2041 AAGGGGACAA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGCTA CCTGACGGGG
2101 ATGCTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTCTA CACGAGGTCT
2161 TCCCAGTACA TCGAGTGGCT GCAAAAGCTC ATGCCCTCAG AGCCACGCCC AGGAGTCTCT
2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGCGGGAG GTGGGTCCGG TGGCGGCGGA
2281 TCAGGTGGGG GTGGATCAGG CCGTGGAGGT TCCGTGGCG GGGGATCAGA CAAAACTCAC
45 2341 ACATGCCACG CGTGGCCAGC ACCTGAACTC CTGGGAGGAC CGTCAGTCTT CCTCTTCCCC
2401 CCAAAACCCA AGGACACCC CTGATCTCC CCGACCCCTG AGGTACATG CTGGTGGGTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581 GTCCTCACCG TCCTGCACCA GCACTGGCTG AATGCCAAGG AGTACAAGTG CAAGGTCTCC
50 2641 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACACAGG TGTACAACCT CCGCCCATCC CCGGATGAGC TGACCAAGAA CCAGTTCAGC
2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
2821 GGGCAGCCGG AGAACAACCT CAAGACCAG CCTCCCGTGT TGGACTCCGA CGGCTCCTTC
2881 TTCTCTTACA GCAAGCTCAC CGTGGACAA AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
55 2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001 CCGGGTAAAT GA

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FVII-061 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

```

1 MVSQALRLLC LLGLQGOLA AVEVTQEEAH GVLHRRRRAN AFLEELRFGS LERECKEEQC
61 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGEQYIC SDHTGTRKSC RCHGYSLLA DGVSCPTTVE YPCGKIPILE
65 181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTHTCPFCP APELLGGPSV
241 FLFFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPFSRDELTK
361 NQVSLTCLVK GFYPSTIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG
421 NVFSCSVMH EALHNHYTQKS LSLSPGKGGG GGGGGGGGGG GGGGGGGGGG GGGGGGGGGG
70 481 GSALRPRVVG GAIVGGKVC PKECPWQVLL LVNQAQLCGG TLINTIWWVS AAHCFDKIKN
541 WRNLIIVLGE HDLSEHDGDE QSRVAQVII PSTYVPGETN HDIALRLHQ PVVLTDHVVP
601 LCLPRTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL NVPRLMFQDC LQQSRKVGDS

```

5 661 PNITEYMFCA GYSDGSKDSC KGDSSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV
 721 SQYIEWLQKL MRSEPRPGVL LRAPFPGGGG SGGGSGGGG SGGGSGGGG SGGGSGDKTH
 781 TCPPCPAPEL LGGPSVFLFF PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 841 HNAKTKFREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 901 EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
 10 961 FLYSKLTVDK SRWQQGNVFS CSMVHEALHN HYTQKSLSL S PKG*

DNA sequence for FVII-062

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
 61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGCCAAC
 15 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCCTCA AGTCCATGCC AGAATGGGGG CTCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAAG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 20 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GAAGGGGTGT CCGTGCACAC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAAATG CCAGCAAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGCGCG AGGTGGGTCC
 601 GGTGGCGGCG GATCAGGTGG GGTGGATCA GCGGTGGAG GTTCCGGTGG CCGGGGATCA
 661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTCACTC
 25 721 TTCTCTTTC CCCCCAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTACAA
 781 TGGCAGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTCAACTG GTACGTGGAC
 841 GCGGTGGAGG TGCAATAATG CAAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 901 CGGTGTGTCA GCGTCTCAC CGTCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
 30 1021 GGGCAGCCCC GAGAACCAACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
 1081 AACCAGGTCA GCCTGACCTG CCGGTCTAAA GGCTTCTATC CCAGCGACAT CGCGGTGGAG
 1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
 1201 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGACAA AGAGCAGGTG GCAGCAGGGG
 1261 AACGTCTTCT CATGTCCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
 35 1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
 1381 GCGTCCGGTG GCGGGGGATC CCGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGTGGAT
 1441 GGTTCAGGTG GTGGAGGATC CATTGTGGGG GGCAAGGTGT GCCCCAAAGG GGAGTGTCCA
 1501 TGGCAGGTCC TGTGTGTGGT GAATGGAGCT CAGTTGTGTG GGGGGACCTT GATCAACACC
 1561 ATCTGGGTGG TCTCCGCGGC CCACTGTTCG GACAAAATCA AGAATGGAG GAACCTGATC
 40 1621 GCGGTGCTGG GCGAGCACGA CCTCAGCGAG CACGACGGGG ATGAGCAGAG CCGGGGGGTG
 1681 TCATCCCCAG CAGGTACGTC CCGGCGACCA CCAACCACGA CATCGCGCTG
 1741 CTCCGCTTGC ACCAGCCCGT GGTCTCACT GACCATGTGG TGCCCTCTG CCGCCCGAA
 1801 CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGCGCTTCT CATTGGTCAG CGGCTGGGGC
 1861 CAGCTGCTGG ACCGTGGCGC CACGGCCCTG GAGCTCATGG TCCTCAACGT GCCCGCGCTG
 45 1921 ATGACCCAGG ACTGCCTGCA GCAGTCAAGG AAGGTGGGAG ACTCCCCAAA TATCACGGAG
 1981 TACATGTTCT GTGCCGGCTA CTCGGATGGC AGCAAGGACT CCTGCAAGGG GGACAGTGGG
 2041 GGCCACATG CCACCCACTA CCGGGGCACG TGGTACCTGA CCGGCATCGT CAGCTGGGGC
 2101 CAGGGCTGCG CAACCGTGGG CCACTTTGGG GTGTACACCA GGGTCTCCCA GTACATCGAG
 2161 TGGCTGCAAA AGCTCATGCG CTCAGAGCCA CGCCAGGAG TCCTCTGCG AGCCCCATT
 50 2221 CCGGTGGCG GTGGCTCCGG CCGAGGTGGG TCCGGTGGCG GCGGATCAGG TGGGGGTGGA
 2281 TCAGSCGGTG GAGGTTCGGG TGGCGGGGGA TCAGACAAAA CTCACACATG CCCACGTGTC
 2341 CCAGCACCTG AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCAAA ACCCAAGGAC
 2401 ACCCTCATGA TCTCCCGGAC CCCTGAGGTG ACATGCGTGG TGGTGGACGT GAGCCACGAA
 2461 GACCTGAGG TCAAGTTCAA CTGGTACGTG GACGCGCTGG AGGTGCATAA TGCCAAGACA
 55 2521 AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCTT CACCGTCTCT
 2581 CACCCAGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
 2641 GCCCCATCG AGAAAAACAT CTCCAAAGCC AAAGGGCAGC CCGGAGAACC ACAGGTGTAC
 2701 ACCCTGCCCC CATCCCGGGA TCAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
 2761 AAAGGCTTCT ATCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCGGAGAAC
 60 2821 AACTACAAGA CCACGCTCC CGTGTGGAC TCCGACGGCT CCTCTCTCTT CTACAGCAAG
 2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGAACGTCT TCTCATGCTC CGTGATGCAT
 2941 GAGGCTCTGC ACAACCACTA CACGAGAAAG AGCCTCTCCC TGTCTCCGGG TAAATGA

FVII-062 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVIIa light chain or heavy chain to Fc region is underlined, and linker region connecting the Fc and the FVIIa heavy chain is shown in bold

1 MVSQALRLLC LLLGQGGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
 61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCPTPVE YPCGKIPILE
 70 181 KRNASKPQGR GGGSGGGGGS GGGSGGGGGS GGGSGGGGGS DKTHTCPFCP APPELLGGPSV
 241 FLFPKPKDPT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 301 RVVSVLTVLH QDWNLNKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPSPRDELTK

5	361	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTFPVLDS	DGSFFLYSKL	TVDKSRWQQG
	421	NVFSQSVHME	ALHNHYTQKS	LSLSFGKGGG	GSGGGGSGGG	GSGGGGSGGG	GSGGGGSGGG
	481	GSGGGG IVG	GKVC PKGECF	WQVLLLVNGA	QLCGGTLLNT	IWVVSAAHCF	DKTKNWRNLI
	541	AVLGEHDLSE	HDGDEQSRRV	AQVIIPSTYV	PGTGNHDIAL	LRLHQPVVLT	DHVVPCLCLPE
10	601	RTFSERTLAF	VRFSLVSGWG	QLLDRGATAL	ELMVLNVFRL	MTQDCLOQSR	KVGDSFNITE
	661	YMFCAQYSDG	SKDSCKGDSG	GFHATHYRGT	WYLTGIVSWG	QGCATVGHFG	VYTRVSQYIE
	721	WLQKLMRSEP	RPGVLLRAPF	PGGGGSGGGG	SGGGGSGGGG	SGGGGSGGGG	SGGGGSGGGG
	781	PAPELLGGPS	VFLFPKPKD	TLMISRTFEV	TCVVVDVSHE	DPEVKFNWYV	DGVEVHNAKT
	841	KPREEQYNST	YRVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KGPQPREPQVY
15	901	TLPPSRDELT	KNQVSLTCLV	KGFYPSDIAV	EWESNGQFEN	NYKTTFPVLD	SDGSFFLYSK
	961	LTVDKSRWQQ	GNVFSQSVHME	EALHNHYTQK	SLSLSFGK*		

DNA sequence for FVII-090

	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
20	61	GCAGTCTTCG	TAACCCAGGA	GGAAGCCAC	GGCTCCTGC	ACCGGCGCCG	CGCGGCCAAC
	121	GCGTTCCTGG	AGGAGCTGCG	GCCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	TCCTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAAGCT	GTTCTGGATT
	241	TCCTACAGTG	ATGGGGACCA	GTGTGCCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG
	301	GACCAGCTCC	AGTCTTATAT	CTGCTTCTGC	CTCCCTGCCT	TCGAGGGCCG	GAAGTGTGAG
25	361	ACGCACAAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	GCAGTACTGC
	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATTCATGAA
	541	AAAAGAAATG	CCAGCAAACC	CCAAGGCCGA	GGTGGCGGTG	GCTCCGCGCG	AGGTGGGTCC
	601	GGTGGCGGCG	GATCAGGTGG	GGGTGGATCA	GGCGGTGGAG	GTTCCGGTGG	CGGGGGATCA
	661	GACAAAACTC	ACACATGCCC	ACCGTGCCCA	GCTCCGGAAC	TCCTGGGCGG	ACCGTCAAGT
30	721	TTCCTCTTCC	CCCCAAAACC	CAAGGACACC	CTCATGATCT	CCCGGACCCC	TGAGGTACAA
	781	TTGCTGGTGG	TGGACGTGAG	CCACGAAGAC	CCTGAGGTCA	AGTTCACCTG	GTACGTGGAC
	841	GGCGTGGAGG	TGCATAATGC	CAAGACAAG	CCGCGGGAGG	AGCAGTACAA	GCAGCAGTAC
	901	CGTGTGGTCA	GCGTCTCACC	CGTCTGCAC	CAGGACTGGC	TGAATGGCAA	GGAGTACAAG
35	961	TGCAAGGTCT	CCAACAAAGC	CCTCCCAGCC	CCCATCGAGA	AAACCATCTC	CAAAGCCAAA
	1021	GGGCAGCCCC	GAGAACCCACA	GGTGTACACC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG
	1081	AACCCAGTCA	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCGTGGAG
	1141	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GTTGGACTCC
	1201	GACGGCTCCT	TCTTCTCTCA	CAGCAAGCTC	ACCGTCGACA	AGAGCAGGTG	GCAGCAGGGG
40	1261	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC
	1321	CTCTCCCTGT	CTCCGGGTAA	AGGTGGCGGC	GGATCAGGTG	GGGGTGGATC	AGGCGGTGGA
	1381	GGTTCGCGTG	GCGGGGGATC	CGGCGGTGGA	GCTTCCGGTG	GGGGTGGATC	AGGAGGAGGT
	1441	GGTTCAGCCC	TGCGGCCCCG	GATTGTGGGG	GGCAAGGTGT	GCCCCAAAGG	GGAGTGTCCA
	1501	TGGCAGGTCC	TGTTGTTGGT	GAATGGAGCT	CAGTTGTGTG	GGGGGACCC	GATCAACACC
45	1561	ATCTGGGTGG	TCTCCGCGGC	CCACTGTTTC	GACAAAATCA	AGAATGGAG	GAACCTGATC
	1621	GCGGTGCTGG	GCGAGCACGA	CCTCAGCGAG	CACGACGGGG	ATGAGCAGAG	CCGGCGGGTG
	1681	GCGCAGGTCA	TCATCCCCAG	CAGTACGTC	CCGGGCACCA	CCAACACAGA	CATCGCGCTG
	1741	CTCCGCCTGC	ACCAGCCCGT	GGTCTCACT	GACCATGTGG	TGCCCTCTCT	CCTGCCCGAA
	1801	CGGACGTTCT	CTGAGAGGAC	GCTGGCCTTC	GTGCGCTTCT	CATTGGTCAG	CGGCTGGGGC
50	1861	CAGCTGCTGG	ACCGTGGCGC	CACGGCCCTG	GAGCTCATGG	TCCTCAACGT	GCCCCGGCTG
	1921	ATGACCCAGG	ACTGCCTGCA	GCAGTCAAGG	AAGGTGGGAG	ACTCCCCAAA	TATCACGGAG
	1981	TACATGTTCT	GTGCCGGCTA	CTCGGATGGC	AGCAAGGACT	CCTGCAAGGG	GGACAGTGA
	2041	GGCCACATG	CCACCCACTA	CCGGGGCAGC	TGGTACCTGA	CGGGCATCGT	CAGCTGGGGC
	2101	CAGGGCTGCG	CAACCGTGGG	CCACTTTGGG	GTGTACACCA	GGGTCTCCCA	GTACATCGAG
	2161	TGGCTGCAAA	AGCTCATGCG	CTCAGAGCCA	CGCCAGGAG	TCCTCTGCGG	AGCCCCATT
55	2221	CCCGGTGGCG	GTGGCTCCCG	CGGAGGTGGG	TCCGCTGGCG	GCGGATCAGG	TGGGGGTGGA
	2281	TCAGGCGGTG	GAGGTTCGCG	TGGCGGGGGA	TCAGACAAAA	CTCACACATG	CCCACCGTGC
	2341	CCAGCACCTG	AACTCCTGGG	AGGACCGTCA	GTCTTCCTCT	TCCCCCAAAA	ACCCAAGGAC
	2401	ACCCTCATGA	TCTCCCGGAC	CCCTGAGGTC	ACATCCGTGG	TGGTGGACGT	GAGCCACGAA
60	2461	GACCCGTAGG	TCAAGTTCAA	CTGGTACGTG	GACGCGGTGG	AGGTGCATAA	TGCCAAGACA
	2521	AAGCCGCGGG	AGGAGCAGTA	CAACAGCAGC	TACCGTGTGG	TCAGCGTCTT	CACCGTCTCT
	2581	CACCCAGGACT	GGCTGAATGC	CAAGGAGTAC	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA
	2641	GCCCCATCG	AGAAAAACAT	CTCCAAAGCC	AAAGGGCAGC	CCCGAGAACC	ACAGGTGTAC
	2701	ACCCTGCCCC	CATCCCGGGA	TCAGCTGACC	AAGAACCAGG	TCAGCCTGAC	CTGCCTGGTC
	2761	AAAGGCTTCT	ATCCCAGCGA	CATCGCGGTG	GAGTGGGAGA	GCAATGGGCA	GCCGGAGAAC
65	2821	AACTACAAGA	CCACGCTCC	CGTGTGGGAC	TCCGACGGCT	CCTTCTTCTT	CTACAGCAAG
	2881	CTACCGTGG	ACAAGAGCAG	GTGGCAGCAG	GGGAACGTCT	TCTCATGCTC	CGTGATGCAI
	2941	GAGGCTCTGC	ACAACCACTA	CACGCAGAAG	AGCCTCTCCC	TGTCTCCGGG	TAAATGA

FVII-090 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

70	1	<u>MVSQALRLLC</u>	<u>LLGLGQGLA</u>	<u>AVFVTQEEAH</u>	<u>GVLHRRRRAN</u>	<u>AFLEELRPGS</u>	<u>LERECKEEQC</u>
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5	61	SFEEAREIFK	DAERTKLEWI	SYSDGDQCAS	SPCQNGGSCK	DQLQSYICFC	LPAFEGRNCE
	121	THKDDQLICV	NENGGCEQYC	SDHTGTRKSC	RCHEGYSLLA	DGVSCPTTVE	YPCGKIPILE
	181	KRNASKFQGR	GGGSGGGGGS	GGGSGGGGGS	GGGSGGGGGS	DKTHTCPFCP	APELLGGPSV
	241	FLFFPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	FREEQYNSTY
10	301	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPQVYT	LPFSRDELTK
	361	NQVSLTCLVK	GFYPSTDAVE	WESNGQPENN	YKTPFPVLDS	DGSFFLYSKL	TVDKSRWQQG
	421	NVFSQSVMHE	ALHNHYTQKS	LSLSFGKGGG	GGGGGGGGGG	GGGGGGGGGG	GGGGGGGGGG
	481	GSALRPRIVG	GKVCCKGECF	WQVLLLVNGA	QLCGGTLINT	IWVVSAAHCF	DKIKNWRNLI
	541	AVLGEHDLSE	HDGDEQSRRV	AQVIIPSTYV	PGTNNHDIAL	LRLHQPVVLT	DHVVPCLLPE
	601	RTFSERTLAF	VRFSLVSGWG	QLLDRGATAL	ELMVLNVFRL	MTQDCLQSSR	KVGDSFNITE
15	661	YMFCAQYSDG	SKDSCKGDSG	GFHATHYRGT	WYLTGIVSWG	QGCATVGHFG	VYTRVSQYIE
	721	WLQKLMRSEF	RPGVLLRAPF	PGGGSGGGGG	SGGGSGGGGG	SGGGSGGGGG	SDKTHTCPPC
	781	PAPELLGGPS	VFLFPKPKPK	TLMISRTPEV	TCVVDVSHED	DPEVKFNWYV	DGVEVHNAKT
	841	KFREEQYNST	YRVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KGQPREPQVY
	901	TLFPSRDEL	KNQVSLTCLV	KGFYPSDIAV	EWESNGQFEN	NYKTPFPVLD	SDGSFFLYSK
20	961	LTVDKSRWQQ	GNVFSQSVMH	EALHNHYTQK	SLSLSFGK*		

DNA sequence for FVII-100

	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
25	61	GCAGTCTTCG	TAACCCAGGA	GGAAGCCAC	GGCGTCTGCT	ACCGGCGCCG	GCGCGCCAAC
	121	GCGTTCCTGG	AGGAGCTGCG	GCCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	TCCCTCCAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAAGCT	GTCTCTGATT
	241	TCTTACAGTG	ATGGGGACCA	GTGTGCTTCA	AGTCCATGCC	AGAATGGGGG	TCCTGCAAG
	301	GACCAGCTCC	AGTCTTATAT	CTGCTTCTGC	CTCCCTGCCT	TCGAGGGCCG	GAAGTGTGAG
30	361	ACGCACAAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	GCAGTACTGC
	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATTTAGAA
	541	AAAAGAAATG	CCAGCAAACC	CCAAGGCCGA	GGTGGCGGTG	GCTCCGCGCG	AGGTGGGTCC
	601	GGTGGCGGCG	GATCAGGTGG	GGGTGGATCA	GGCGGTGGAG	GTTCCGGTGG	CGGGGGATCC
35	661	GACAAAACTC	ACACATGCCC	ACCGTGCCCA	GCTCCGGAAC	TCCTGGGAGG	ACCGTCAAGT
	721	TTCTCTTCTC	CCCCAAAACC	CAAGGACACC	CTCATGATCT	CCCGGACCCC	TGAGGTCAAC
	781	TGCGTGGTGG	TGGACGTGAG	CCACGAAGAC	CCTGAGGTCA	AGTTCAACTG	GACGTGGAC
	841	GGCGTGGAGG	TGCATAATGC	CAAGACAAG	CCGCGGGAGG	AGCAGTACAA	GCAGCAGTAC
	901	CGTGTGGTCA	GCGTCTCACC	CGTCTGCAC	CAGGACTGGC	TGAATGGCAA	GGAGTACAAG
40	961	TGCAAGGTCT	CCAACAAAGC	CCTCCCAGCC	CCCATCGAGA	AAACCATCTC	CAAAGCCAAA
	1021	GGGCAGCCCC	GAGAACCCACA	GGTGTACACC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG
	1081	AACCCAGTCA	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCGGTGGAG
	1141	TGGGAGAGCA	ATGGGCGAGC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GTTGGACTCC
	1201	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC	ACCGTCGACA	AGAGCAGGTG	GCAGCAGGGG
45	1261	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC
	1321	CTCTCCCTGT	CTCCGGGTAA	AGGTGGCGGC	GGATCAGGTG	GGGGTGGATC	AGGCGGTGGA
	1381	GGTTCGGGTG	GCGGGGGGAT	CGGCGGTGGA	GCTTCCGGTG	GGGGTGGATC	AGGAGGAGGT
	1441	GGTTCAGCCC	TGCGGCCCCG	GATTGTGGGG	GGCAAGGTGT	GCCCCAAGAG	GGAGTGTCCA
	1501	TGGCAGGTCC	TGTTGTTGGT	GAATGGAGCT	CAGTTGTGTG	GGGGGACCC	GATCAACACC
50	1561	ATCTGGGTGG	TCTCCGCGGC	CCACTGTTTC	GACAAAATCA	AGAAGTGGAG	GAACCTGATC
	1621	GCGGTGCTGG	GCGAGCACGA	CCTCAGCGAG	CACGACGGGG	ATGAGCAGAG	CCGGCGGGTG
	1681	GCGCAGGTCA	TCATCCCCAG	CACGTACGTC	CCTGGCACCA	CCAACACAGA	CATCGCGCTG
	1741	CTCCGCTTGC	ACCAGCCCCG	GCTCCTCACT	GACCATGTGG	TGCCCCCTCT	CCTGCCCGAA
	1801	CGGACGTTCT	CTGAGAGGAC	GCTGGCCTTC	GTGGCGTTCT	CATTGGTTCAG	CGGCTGGGGC
55	1861	CAGCTGCTGG	ACCGTGGCGC	CACGGCCCTG	GAGCTCATGG	TCCTCAACGT	GCCCCGGCTG
	1921	ATGACCCAGG	ACTGCGAGGC	CAGCTACCCC	GGCAAGATCA	CGGAGTACAT	GTTCTGTGCC
	1981	GGCTACTCGG	ATGGCAGCAA	GGACTCCTGC	AAGGGGGACA	GTGGAGGCC	ACATGCCACC
	2041	CACTACCGGG	GCACGTGGTA	CCTGACGGGC	ATCGTCAGCT	GGGGCCAGGG	CTGCGCAACC
	2101	GTGGGCCACT	TTGGGGTGTG	CACCAGGGTC	TCCCAGTACA	TCGAGTGGCT	GCAAAAGCTC
60	2161	ATGCGCTCAG	AGCCACGCCC	AGGAGTCCCT	CTGCGAGCCC	CATTTCCCGG	TGGCGGTGGC
	2221	TCCGGCCGAG	GTGGGTCCCG	TGGCGGCGGA	TCAGCTGGGG	GTGGATCAGG	CGGTGGAGGT
	2281	TCCGGTGGCG	GGGGATCAGA	CAAAACTCAC	ACATGCCCCA	CGTGCCGAGC	ACCTGAACCT
	2341	CTGGGAGGAC	CGTCAGTCTT	CCTCTTCCCC	CCAAAACCCA	AGGACACCC	CATGATCTCC
	2401	CGGACCCCTG	AGGTACACATG	CCTGGTGGTG	GACGTGAGCC	ACGAAGACCC	TGAGGTCAAG
	2461	TTCAACTGGT	ACGTGGACGG	CCTGGAGGTG	CATAATGCCA	AGACAAGGCC	CGGGGAGGAG
65	2521	CAGTACAACA	GCACGTACCG	TGTGGTCAGC	GTCTTCACCC	TCCTGCACCA	GGAGTGGCTG
	2581	AATGGCAAGG	AGTACAAGTG	CAAGGTCTCC	AACAAAGCCC	TCCAGGCC	CATCGAGAAA
	2641	ACCATCTCCA	AAGCCAAAGG	GCAGCCCCGA	GAACCACAGG	TGTACACCC	GCCCCATCC
	2701	CGGGATGAGC	TGACCAAGAA	CCAGGTACAG	CTGACCTGCC	TGGTCAAAGG	CTTCTATCCC
	2761	AGCGACATCG	CCGTGGAGTG	GGAGAGCAAT	GGGCGAGCCG	AGAACAATA	CAAGACCACG
70	2821	CCTCCCGTGT	TGGACTCCGA	CGGCTCCTTC	TTCTCTTACA	GCAAGCTCAC	CGTGGACAAG
	2881	AGCAGGTGGC	AGCAGGGGAA	CCTCTCTCTA	TGCTCCGTGA	TGCATGAGGC	TCTGCACAAC
	2941	CACTACACGC	AGAAGAGCCT	CTCCCTGTCT	CCGGGTAAAT	GA	

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FVII-100 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, the thrombin cleavage site is shown in dashed underline, and the trypsin 170 loop region is wave underlined

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1  MVSQAIRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61  SPEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGEQYIC SDHTGTRKSC RCHEGYSLLA DGVSCETPVE YPCGKIFILE
181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTTCPPCP APELLGGPSV
241 FLFFPKPKDT LMISRTPEVT CVVVDVSHED FEVKFNWYVD GVEVHNAKTK PREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPVLDS DGSFFLYSKL TVDKSRWQQG
421 NVFSCSVMEH ALHNHYTQKS LSLSFGKGGG GGGGGGSGGG GGGGGGSGGG GGGGGGSGGG
481 GSALRPRIVG GKVCPRGECF WQVLLLVNGA QLCGGTLINT IWVVSAAHCF DKIKNWRNLI
541 AVLGEHDLSE HDGDEQSRV AQTIIPTSTYV PGTTHNDIAL LRLHQPVVLT DHVVPCLLPE
601 RTEPERTLAF VRFSIVSGWG QLLDRGATAL ELMVLNVPR LMTQDCEASYP GKITEYMFCA
661 GYSDGSKDSC KGDGSGPHAT HYRGTYWLTG IVSWGQGCAT VGHFGVYTRV SQYIEWLQKL
721 MRSEPRPGVL LRAPFFGGGG SGGGSGGGGG SGGGSGGGGG SGGGSGGGGG SGGGSGGGGG
781 LGGSPSVFLP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNATKTPREE
841 QYNSTYRVVS VLTVLHQDWL NGKEYRCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
901 RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSE FLYSKLTVDK
961 SRWQQGNVFS CSVMHEALHN HTQKSLSLG PGK*

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DNA sequence for FVII-115

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1  ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG
CTGCCTGGCT
61  GCAGTCTTCG TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCGGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCCTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGAIT
241 TCTTACAGTG ATGGGGACCA GTGTGCTTCA AGTCCATGCC AGAATGGGGG CTCTCGCAAG
301 GACGAGCTCC AGTCCATAT ATGCTTCTGC CTCCCTGCTC TCGAGGGCCG GAAGTGTGAG
361 ACGCAACAAG ATGACAGAGT GATCTGTGTG AACGAGAACG CGGGCTGTGA CGAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAAAC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
601 GGTGGCGGGG GATCAGGTGG GGTGGATCA GCGGTGGAG CTTCGGGTGG CGGGGATCA
661 TGCAAAAGTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTGATC
721 TTCTCTTTC CCCCCAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTACAA
781 TGGTGGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GCGGTGGAGG TGCAATAATG CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901 CGTGTGGTCA GCGTCTCAC CGTCTGAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
961 TGCAAGGTCT CCAACAAAGC CCTCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCGGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCGGTGCAAA GGCTTCTATC CCAGCGACAT CGCGGTGGAG
1141 TGGGAGAGCA ATGGGCGAGC GGAGAACAAC TACAAGACCA CGCTCCCGT GTTGGACTCC
1201 GACGAGCTCT TCTTCTCTTA CAGCAAGCTC ACCGTGACCA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCTGAG GCTCTGCACA ACCACTACAC CAGAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCGGGTG GCGGGGGATC CGCGGGTGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCCC TGCGGCCCGG GATTGTGGGG GGCAAGGACT GCGCCAAAGG GGAGTGTCCA
1501 TGGCAGGTCC TGTGTGTGGT GAATGGAGCT CAGTGTGTG GGGGGACCCT GATCAACACC
1561 ATCTGGGTGG TCTCCGCGGC CACTGTGTTC GACAAAATCA AGAATGGAG GAACCTGATC
1621 GCGGTGCTGG GCGAGCACGA CCTCAGCGAG CACGACGGGG ATGAGCAGAG CCGCGGGGTG
1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCACGA CATCGCGCTG
1741 CTCCGCTTGC ACCAGCCCGT GGTCTTCACT GACCATGTGG TGCCCCCTCG CCGCCCCGAA
1801 CCGAGCTTCT CTGAGAGGAC GCTGGCTTTC GTGGCTTCT CATTTGGTCA GGGTCTCCAG
1861 CAGCTGCTGG ACCGTGGCGC CACGGCCCTG GTACTCCAAG TCCTCAACGT GCGCCGGCTG
1921 ATGACCCAGG ACTGCCTGCA GCAGTCAAGG AAGGTGGGAG ACTCCCCAAA TATCACGGAG
1981 TACATGTTCT GTGCGGGCTA CTCGGATGCC AGCAAGGACT CCGCAAGGG GGACAGTGGG
2041 GGCCACATG CCACCCACTA CCGGGGCACG TGGTACCTGA CCGGCATCGT CAGCTGGGGC
2101 CAGGGCTGCG CAACCGTGGG CCACTTTGGG GTGTACACCA GGGTCTCCCA GTACATCGAG
2161 TGGCTGCAAA AGCTCATGCG CTCAGAGCCA CGCCAGGAG TCCTCCTGCG AGCCCCATTT
2221 CCGGGTGGCG GTGGCTCCGG CGGAGGTGGG TCCGGTGGCG CCGGATCAGG TGGGGGTGGA
2281 TCAGGCGGTG GAGGTTCGGG TGGCGGGGGA TCAGACAAAA CTCACACATG CCCACCGTGC
2341 CCAGCACCTG AACTCCTGGG AGGACCGTCA GTCTTCTCT TCCCCCAA ACCCAAGGAC
2401 ACCCTCATGA TCTCCGGAC CCGTAGGTG ACATGCGTGG TGGTGGACGT GAGCCACGAA
2461 GACCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA

```

5 2521 AAGCCGCGGG AGGAGCAGTA CAACAGCAGC TACCGTGTGG TCAGCGTCCT CACCGTCCTG
 2581 CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
 2641 GCCCCATCG AGAAAACCAT CTCCAAGGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC
 2701 ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
 10 2761 AAAGGCTTCT ATCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC
 2821 AACTACAAGA CCACGCCTCC CGTGTGGGAC TCCGACGGCT CCTTCTCTCT CTACAGCAAG
 2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACTGCT TCTCATGCTC CGTGATGCAT
 2941 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGA

15 FVII-115 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, the thrombin cleavage site is shown in dashed underline, and the three point mutations in FVIIa (V151D, E296V and M298Q) are in bold and underlined

1 MVSQALRLLC LLLGLOGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
 61 SFEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGCK DQLQSYICFC LPAFEGRNCE
 20 121 THKDDQLICV NENGGEQYC SDHTGTRSC RCHGYSLA DGVSCPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGSGGGG GGGGSGGGG GGGGSGGGG DKHTCTPPCP APELLGGPSV
 241 FLFPKPKRDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNATK PREEQYNSTY
 301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDL DGSFFLYSKL TVDKSRWQQG
 25 421 NVFSCSVME ALHNHYTQKS LSLSFGKGGG SGGGSGGGG SGGGSGGGG SGGGSGGGG
 481 GSALRPRIV GKDCPKGEC WQVLLLVNGA QLCGGTINT IWVSSAAHCF DKIKNWRNLI
 541 AVLGEHDLSE HDGDEQSRV AQVLIPTTYV PGTNHDIAL LRLHQPVVLT DHVPLCLPE
 601 RTFSERTLAF VRFSLVSGWG QLLDRGATAL VLQVLNVPR MTQDCLQSR KVGDSFNITE
 661 YMFCAQYSDG SKDSCKGDGSG GPHATHYRGT WYLTGIVSWG QGCATVGHFG VYTRVSQYIE
 30 721 WLQKLMRSEP RFGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG SDKTHCTPPC
 781 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHD DPEVKFNWYV DGVEVHNATK
 841 KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
 901 TLPPSRDELTK KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLDL SDGSFFLYSK
 961 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSFGK*

35

DNA sequence for FVII-118

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
 61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGCCAAC
 40 121 GCGTTCCTGG AGGAGCTGCG GCCGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGAIT
 241 ACTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGG CTCTGCAAG
 301 GACCAGCTCC AGTCTTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAAG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA CGAGTACTGC
 45 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTAGAA
 541 AAAAGAAATG CCAGCAAAACC CCAAGGCCCC CTGCGGCCCC GGATTGTGGG GGGCAAGGTG
 601 TGCCCCAAAG GGGAGTGTCC ATGGCAGGTC CTGTTGTTGG TGAATGGAGC TCAGTTGTGT
 661 GGGGGGACCC TGATCAACAC CATCTGGGTG GTCTCCGCGG CCCACTGTTT CGACAAAATC
 50 721 AAGAACTGGA GGAACCTGAT CGCGGTGCTG GCGGAGCACG ACCTCAGCGA GCACGACGGG
 781 GATGACGAGA GCCGCGGGGT GCGCGAGGTC ATCATCCCCA GCACGTACGT CCGGGCACCC
 841 ACCAACCAGG ACATCGCGCT GCTCCGCTG CACCAGCCCG TGGTCCCTAC TGACCATGTG
 901 GTGCCCCCTC GCCTGCCCGA ACGGACGTTT TCTGAGAGGA CGCTGGCCTT CGTGCGCTTC
 961 TCATTGGTCA GCGGCTGGGG CCAGCTGCTG GACCGTGGCG CCACGGCCCT GGAGCTCATG
 55 1021 GTCTCAACG TGCCCCGGCT GATGACCCAG GACTGCCTGC AGCAGTCACG GAAGGTGGGA
 1081 GACTCCCCAA ATATCACGGA GTACATGTTT TGTGCCGGCT ACTCGGATGG CAGCAAGGAC
 1141 TCCTGCAAGG GGGACAGTGG AGGCCACAT GCCACCCACT ACCGGGGCAC GTGGTACCTG
 1201 ACGGGCATCG TCAGCTGGGG CCAGGGCTGC GCAACCGTGG GCCACTTTGG GGTGTACACC
 1261 AGGGTCTCCC AGTACATCGA GTGGCTGCAA AAGCTCATGC GCTCAGAGCC ACGCCAGGA
 60 1321 GTCTCTCTGC GAGCCCCATT TCCCGTGGC GGTGGCTCCG GCGGAGGTGG GTCCGCTGGC
 1381 GGCGGATCAG GTGGGGGTGG ATCAGGCGGT GGAGGTTCGG GTGGCGGGGG ATCCGACAAA
 1441 ACTCACACAT GCCCAACGTG CCCAGCTCCG GAACTCCTGG GCGGACCGTC AGTCTTCTC
 1501 TTCCCCCACC AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG
 1561 GTGGTGGACG TGAGCCACGA AGACCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG
 65 1621 GAGGTGCATA ATGCCAAGC AAAGCCCGCG GAGGAGCAGT ACAACAGCAC GTACCGTGTG
 1681 GTACAGCTCC TCACCGTCTC GCACAGGAC TGGCTGAATG GCAAGGAGTA CAAGTCAAG
 1741 GTCTCCAACA AAGCCCTCCC AGCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
 1801 CCCCCAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG ATGAGCTGAC CAAGAACCAG
 1861 GTACAGCTGA CCTGCCTGGT CAAAGGCTTC TATCCAGCG ACATCGCCGT GGAGTGGGAG
 70 1921 AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCCT CCGTGTGGGA CTCCGACGGC
 1981 TCCTTCTTCC TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGACGCTC
 2041 TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGAGAA GAGCCTCTCC

5	2101	CTGTCTCCGG	GTAAAGGTGG	CGGCGGATCA	GGTGGGGGTG	GATCAGGCGG	TGGAGGTTC
	2161	GGTGGCGGGG	GATCAGACAA	AACTCACACA	TGCCACCGT	GCCCAGCACC	TGAATCCTG
	2221	GGAGGACCGT	CAGTCTTCT	CTTCCCCCA	AAACCCAAGG	ACACCCCAT	GATCTCCCGG
	2281	ACCCCTGAGG	TCACATGCGT	GGTGGTGGAC	GTGAGCCACG	AAGACCTGA	GGTCAAGTTC
10	2341	AACTGGTACG	TGGACGGCGT	GGAGGTGCAT	AATGCCAAGA	CAAAGCCGCG	GGAGGAGCAG
	2401	TACAACAGCA	CGTACCGTGT	GGTCAGCGTC	CTCACCGTCC	TGCACCAGGA	CTGGCTGAAT
	2461	GGCAAGGAGT	ACAAGTGCAA	GGTCTCCAA	AAAGCCCTCC	CAGCCCCAT	CGAGAAAACC
	2521	ATCTCCAAAG	CCAAAGGGCA	GCCCCGAGAA	CCACAGGTGT	ACACCCGTGC	CCCATCCCGC
	2581	GATGAGCTGA	CCAAGAACCA	GGTCAGCCTG	ACCTGCCTGG	TCAAAGGCTT	CTATCCCAGC
	2641	GACATCGCCG	TGGAGTGGGA	GAGCAATGGG	CAGCCGGAGA	ACAACACAAA	GACCACGCCCT
15	2701	CCCGTGTGG	ACTCCGACGG	CTCCTTCTTC	CTCTACAGCA	AGCTCACCGT	GGACAAGAGC
	2761	AGGTGGCAGC	AGGGGAACGT	CTTCTCATGC	TCCGTGATGC	ATGAGGCTCT	GCACAACCAC
	2821	TACACGCAGA	AGAGCCTCTC	CCTGTCTCCG	GGTAAATGA		
20	FVII-118 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the thrombin cleavage site is shown in dashed underline, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold						
	1	<u>MVSQALRLLC</u>	<u>LLLGLOGCLA</u>	<u>AVFVTQEEAH</u>	<u>GVLHRRRRAN</u>	AFLEELRPGS	LERECKKEEQC
25	61	SFEAREIFK	DAERTKLEWI	SYSDGDQCAS	SPCNGGGSCK	DQLQSYICFC	LPAFEGRNCE
	121	THKDDQLICV	NENGGEQYQ	SDHTGTRKSC	RHEGYSLLA	DGVSCPTIVE	YPCGKIPILE
	181	KRNASKPQGA	LRPRIVGGKV	CPKGECPWQV	LLLVNGAQLC	GGTLINTIWF	VSAAHCFDKI
	241	KNWRNLIAVL	GEHDLSEHDG	DEQSRRAQV	IIPSTYVPGT	TNHDIALRL	HQPVVLTDHV
	301	VPLCLPERTF	SERTLAFVRF	SLVSGWQQLL	DRGATALELM	VLNVPRIMTQ	DCLQQSRKVG
	361	DSPNITEYMF	CAGYSDGSKD	SCKGDSGGPH	ATHYRGTWYL	TGIVSWGQGC	ATVGHFQVYT
30	421	RVSQYIEWLQ	KLMRSEPRPG	VLLRAFFPGG	GGSGGGGSGG	GGSGGGGSGG	GGSGGGGSDK
	481	THTCPPCPAP	ELLGGPSVFL	PPPKPRDTLM	ISRTPEVTCV	VVDVSHEDPE	VKNWYVDGV
	541	EVHNAKTRPR	EEQYNSTYRV	VSVLEVLHQD	WLNGKEYKCK	VSNKALPAPI	EKTISKAKGQ
	601	PREPQVYTLF	PSRDELTKNF	VSLTCLVKGF	YPSDIAVEWE	SNGQPENNYK	TTPFVLDSDG
	661	SFLYSLKTLV	DKSRWQQGNV	FSCSVMHEAL	HNHYTQKSLS	LSPGKGGGGS	GGGGSGGGGS
35	721	GGGGSDKTHT	CPPCPAPELL	GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF
	781	NWYVDGVEVH	NAKTKPREEQ	YNSTRVSVSV	LTVLHQDWLN	GKEYKCKVSN	KALPAPIET
	841	ISKAKGQPRE	PQVYTLPPSR	DELTKNQVSL	TCLVKGFYPS	DAIVEWESNG	QPENNYKTTT
	901	PVLDSGDSFF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH	YTQKSLSLSP	GK*
40	DNA sequence for FVII-119						
	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
	61	GGAGTCTTCG	TAACCCAGGA	GGAAGCCAC	GGCGTCTGTC	ACCGGCGCGC	GGCGGCCAAC
	121	GCGTTCTCTG	AGGAGCTGCG	GCGGGCTTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
45	181	TCTTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAAGCT	GTCTCTGGAAT
	241	TCTTACAGTG	ATGGGGACCA	GTGTGCCCTCA	AGTCCATGCC	AGAATGGGGG	CTCTGCAAG
	301	GACCAGCTCC	AGTCTATAT	CTGCTTCTGC	CTCCTGCTTC	TGAGGGGCGC	GAAGTGTGAG
	361	ATGACCAAGG	ATGACCAAGG	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	CGAGTACTGC
	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATCTAGAA
50	541	AAAAGAAATG	CCAGCAAACC	CAAAGCGGGA	GGAGGTGTTT	CAGCCCTGCG	GCCCCGGATT
	601	TGGGGGGGCA	AGGTGTGCC	CAAAGGGGAG	TGTCCATGCC	AGGTCTGTTT	GTGTGTGAAT
	661	GGAGCTCAGT	TGTGTGGGGG	GACCTGATC	AACACCATCT	GGGTGGTCTC	CGCGGCCAC
	721	TGTTTTCGACA	AAATCAAGAA	CTGGAGGAAC	CTGATCGCGG	TGCTGGGCGA	GCACGACCTC
	781	AGCGAGCAGC	ACGGGGATGA	GCAGAGCCGG	CGGTGGGCGC	AGGTCAATCAT	CCCCAGCAGC
55	841	TACGTCCCAG	GCACACCAA	CCACGACATC	GCGTGTCTCC	GCCTGCACCA	CCCCGTGGTC
	901	CTCACTGACC	ATGTGGTGCC	CCTCTGCTTG	CCGAAACGGA	CGTCTCTGTA	GAGGACGCTG
	961	GCCTTCGTGC	GCTTCTCATT	GGTCAGCGGC	TGGGGCCAGC	TGCTGGACCG	TGGCGCCACG
	1021	GCCCTGGAGC	TCATGGTCTT	CAACGTGCCC	CGGTGATGTA	CCCAGGACTG	CCTGCAGCAG
	1081	TCACGGAAGG	TGGGAGACTC	CCCAAAATATC	ACGGAGTACA	TGTTCTGTGC	CGGTACTCTG
60	1141	GATGGCAGCA	AGGACTCCTG	CAAGGGGGAC	AGTGGAGGCC	CACATGCCAC	CCACTACCGG
	1201	GGCAGCTGGT	ACCTGACGGG	CATCGTCAGC	TGGGGCCAGG	ECTGCGCAAC	CGTGGGCCAC
	1261	TTTGGGGTGT	ACACCAAGGT	CTCCAGTAC	ATCGAGTGGC	TGCAAAAGCT	CATGCGCTCA
	1321	GAGCCACGCC	CAGGAGTCTT	CCTGCGAGCC	CCATTTCCCG	GTGGCGGTGG	CTCCGGCGGA
	1381	GGTGGGTCCG	GTGGCGGCGG	ATCAGGTGGG	GGTGGATCAG	GCGGTGGAGG	TTCCGGTGGG
65	1441	GGGGGATCCG	ACAAAACCTCA	CACATGCCCA	CCGTGCCACG	CTCCGGAATC	CCTGGGCGGA
	1501	CGTTCAGTCT	TCCTCTTCCC	CCCAAAACCC	AAGGACACCC	TCATGATCTC	CCGGAACCTT
	1561	GAGGTACAT	GCCTGGTGGT	GGACGTGAGC	CACGAAGACC	CTGAGGTCAA	GTTCAACTGG
	1621	TACGTGGACG	GCGTGGAGGT	GCATAATGCC	AAGACAAAGC	CGCGGGAGGA	GCAGTACAAC
	1681	AGCACGTACC	GTGTGGTTCG	CGTCTCACC	GTCTGCACC	AGGACTGGCT	GAATGGCAAG
70	1741	GAGTACAAGT	GCAAGGTCTC	CAACAAAGCC	CTCCAGCCCC	CCATCGAGAA	AACCATCTCC
	1801	AAAGCCAAAG	GGCAGCCCCG	AGAACCACAG	GTGTACACCC	TGCCCCATC	CCGGATGAG
	1861	CTGACCAAGA	ACCAGGTCAG	CCTGACCTGC	CTGGTCAAAG	GCTTCTATCC	CAGCGACATC

5	1921	GCCGTGGAGT	GGGAGAGCAA	TGGGCAGCCG	GAGAACAAC	ACAAGACCAC	GCCTCCCGTG
	1981	TTGGACTCCG	ACGGCTCCTT	CTTCCTCTAC	AGCAAGCTCA	CCGTGGACAA	GAGCAGGTGG
	2041	CAGCAGGGGA	ACGTCTTCTC	ATGCTCCGTG	ATGCATGAGG	CTCTGCACAA	CCACTACACG
	2101	CAGAAGAGCC	TCTCCCTGTC	TCCGGGTAAA	GGTGGCGGCG	GATCAGGTGG	GGGTGGATCA
10	2161	GGCGGTGGAG	GTTCGGGTGG	CGGGGGATCA	GACAAAAC	ACACATGCC	ACCGTGCCCA
	2221	GCACCTGAAC	TCCTGGGAGG	ACCGTCAGTC	TTCTCTTCC	CCCCAAAACC	CAAGGACACC
	2281	CTCATGATCT	CCCGGACCCC	TGAGGTCACA	TGCGTGGTGG	TGGACGTGAG	CCACGAAGAC
	2341	CCTGAGGTCA	AGTTCAACTG	GTACGTGGAC	GGCGTGGAGG	TGCATAATGC	CAAGACAAAG
	2401	CCGCGGGAGG	AGCAGTACAA	CAGCACGTAC	CGTGTGGTCA	GCGTCTTCAC	CGTCTGCAC
	2461	CAGGACTGGC	TGAATGGCAA	GGAGTACAAG	TGCAAGGTCT	CCAACAAAGC	CCTCCAGACC
15	2521	CCCATCGAGA	AAACCATCTC	CAAAGCCAAA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC
	2581	CTGCCCCCAT	CCCGGATGTA	GCTGACCAAG	AACGAGGTCA	GCCTGACCTG	CGTGGTCAAA
	2641	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	ATGGGCGAGC	GGAGAACAAC
	2701	TACAAGACCA	CGCCTCCCGT	GTTGGACTCC	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC
	2761	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG
20	2821	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGA

FVII-119 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the thrombin cleavage site with GGGS linker is shown in dashed underline, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

25	1	<u>MVSQALRLLC</u>	<u>LLLGLOGCLA</u>	<u>AVFVTQEEAH</u>	<u>GVLHRRRRAN</u>	AFLEELRPGS	LERECKEEQC
	61	SFEAREIFK	DAERTKLEWI	SYSDGDQCS	SPCQNGGSK	DQLQSYICFC	LPAPFGRNCE
	121	THKDDQLICV	NENGGCEQYC	SDHTGTKRSC	RCHGYSLLA	DGVSCFTPVE	YPCGKIPILE
	181	KRNASKPQGG	<u>GGGSALRPRI</u>	VGGKVC PKGE	CPWQVLLLVN	GAQLCGGTLI	NTIWWVSAAH
	241	CFDKIKNWRN	<u>LI AVLGEHDL</u>	SEHDGDEQSR	RVAQVIIIPST	YVPGTINHDI	ALLRLHQPVV
30	301	LTDHVVPCL	PERTFSERTL	AFVRFSLVSG	WGQLLDRGAT	ALELMVLNVP	RLMTQDCLQQ
	361	SRKVGDSFNI	TEYMFAGYS	DGSKDSCKGD	SGGPHTHYR	GTWYLTGIVS	WGQCATVGH
	421	FGVYTRVSQY	IEWLQKLMRS	EPRPGVLLRA	PFPGGGGSGG	GGSGGGSGG	GGSGGGSGG
	481	<u>GGSDKTHTCP</u>	<u>PCPAPELLGG</u>	<u>PSVFLFPKPK</u>	<u>KDTLMISRTF</u>	<u>EVTCVVVDVS</u>	<u>HEDPEVRFNW</u>
	541	YVDGVEVHNA	KTKPREEQYN	STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS
35	601	KAKGQPREPQ	VYTLPPSRDE	LTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTTPV
	661	LDSGDSFFLY	SKLTVDKSRW	QQGNVFSQSV	MHEALHNHYT	QKSLSLSPGK	GGSGGGSGG
	721	GGSGGGSGG	DKTHTCPPCF	APELLGGFSV	FLFPKPKPDT	LMISRTPEVT	CVVVDVSHED
	781	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA
	841	PIEKTISKAK	GQPREPQVYT	LPFSRDELTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN
40	901	YKTFPVLDLS	DGSFFLYSKL	TVDKSRWQQG	NVFSQSVNHE	ALHNHYTQKS	LSLSPGK*

DNA sequence for FVII-127

	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
	61	GCAGTCTTCG	TAACCCAGGA	GGAAGCCAC	GGCGTCTG	ACCGGCGCCG	GGCGGCCAAC
45	121	GCGTTCCTGG	AGGAGCTGCG	GCCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	TCCTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAAGCT	GTTCTGGAIT
	241	CTTTACAGTG	ATGGGGACCA	GTGTGCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG
	301	GACCAGCTCC	AGTCTATAT	CTGCTTCTGC	CTCCCTGCCT	TGGAGGGCCG	GAACTGTGAG
	361	ACGCACAAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	GCAGTACTGC
50	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATTCAGAA
	541	AAAAGCAATG	CCAGCAAAAC	CCAAGGCCCC	CTGCGGCCCC	GGATTGTGGG	GGGCAAGGTG
	601	TGCCCCAAAG	GGGAGTGTCC	ATGGCAGGTC	CTGTGTGTGG	TGAATGGAGC	TCAGTTGTGT
	661	GGGGGGACCC	TGATCAACAC	CATCTGGGTG	GTCTCCGCGG	CCCCTGTGTT	CGACAAAATC
55	721	AAGAACTGGA	GGAACCTGAT	CGCGGTGCTG	GGCGAGCACG	ACCTCAGCGA	GCACGACGGG
	781	GATGAGCAGA	GCCGGCGGGT	GGCGCAGGTC	ATCATCCCCA	GCACGTACGT	CCCGGGCACC
	841	ACCAACCACG	ACATCGCGCT	GCTCCGCTTG	CACCAGCCCG	TGGTCCCTAC	TGACCATGTG
	901	GTGCCCCCTC	GCCTGCCCGA	ACGGACGTTT	TCTGAGAGGA	CGCTGGCCTT	CGTGGCCTTC
	961	TCATTGGTCA	GCGGCTGGGG	CCAGCTGCTG	GACCGTGGCG	CCACGGCCCT	GGAGCTCATG
60	1021	GTCTTCAACG	TGCCCCGGCT	GATGACCCAG	GACTGCGAGG	CCAGCTACCC	CGGCAAGATC
	1081	ACGGAGTACA	TGTTCTGTGC	CGGCTACTCG	GATGGCAGCA	AGGACTCTTG	CAAGGGGGAC
	1141	AGTGGAGGCC	CACATGCCAC	CCACTACCGG	GGCACGTGGT	ACCTGACGGG	CATCGTCAGC
	1201	TGGGGCCAGG	GCTGCGCAAC	CGTGGGCCAC	TTTGGGGTGT	ACACCAGGGT	CTCCAGTAC
	1261	ATCGAGTGGC	TGCAAAAGCT	CATGCGCTCA	GAGCCACGCC	CAGGAGTCC	CCTGCGAGCC
65	1321	CCATTTCCTG	GTGGCGGTGG	TCCCGGCGGA	GGTGGGTCCG	GTGGCGGCGG	ATCAGGTGGG
	1381	GGTGGATCAG	GCGGTGGAGG	TTCCGGTGGC	GGGGGATCAG	ACAAAAC	CACATGCCCA
	1441	CCGTGCCCCAG	CTCCGGAAC	CCTGGGCGGA	CCGTCACTCT	TCCTCTTCCC	CCCCAAACCC
	1501	AAGGACACCC	TCATGATCTC	CCGACCCCT	GAGGTCACAT	CGGTGGTGGT	GGACGTGAGC
	1561	CACGAAGACC	CTGAGGTCAA	GTTCAACTGG	TACGTGGACG	GCGTGGAGGT	GCATAATGCC
70	1621	AAGACAAAGC	CGCGGGAGGA	GCAGTACAAC	AGCACGTACC	GTGTGGTTCAG	CGTCTCACC
	1681	GCTCTGCACC	AGGACTGGCT	GAATGGCAAG	GAGTACAAGT	GCAAGGTCTC	CAACAAAGCC
	1741	CTCCAGCCCC	CCATCGAGAA	AACCATCTCC	AAAGCCAAAG	GGCAGCCCCG	AGAACCACAG

5	1801	GTGTACACCC	TGCCCCCATC	CCGGGATGAG	CTGACCAAGA	ACCAGGTCAG	CCTGACCTGC
	1861	CTGGTCAAAG	GCTTCTATCC	CAGCGACATC	GCCGTGGAGT	GGGAGAGCAA	TGGGCAGCCG
	1921	GAGAACAAC	ACAAGACAC	GCTTCCCGTG	TTGGACTCCG	ACGGCTCCTT	CTTCTCTAC
	1981	AGCAAGCTCA	CCGTGGACAA	GAGCAGGTGG	CAGCAGGGGA	ACGTCTTCTC	ATGCTCCGTG
	2041	ATGCATGAGG	CTCTGCACAA	CCACTACACG	CAGAAGAGCC	TCTCCCTGTC	TCCGGGTAAA
10	2101	GGTGGCGGCG	GATCAGGTGG	GGGTGGATCA	GGCGGTGGAG	GTTCCCGTGG	CGGGGGATCA
	2161	GACAAAATC	ACACATGCCC	ACCGTGCCCA	GCACTGAAC	TCCTGGGAGG	ACCGTCACTC
	2221	TTCTCTTCC	CCCCAAAACC	CAAGGACACC	CTCATGATCT	CCCGGACCCC	TGAGTACACA
	2281	TGCGTGGTGG	TGGACGTGAG	CCACGAAGAC	CCTGAGGTCA	AGTTCAACTG	GTACGTGGAC
	2341	GGCGTGGAGG	TGCATAATGC	CAAGACAAAG	CCGCGGGAGG	AGCAGTACAA	CAGCACGTAC
15	2401	CGTGTGGTCA	GGCTCTCAC	CGTCTGCAC	CAGGACTGGC	TGAATGGCAA	GGAGTACAAG
	2461	TGCAAGSTCT	CCAACAAAG	CCTCCCAGCC	CCATCGAGA	AAACCATCTC	CAAGCCCAAA
	2521	GGGCGACCCC	GAGAACCACA	GGTGTACACC	CTGCCCCCAT	CCCGCGATGA	GCTGACCAAG
	2581	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG
	2641	TGGGAGAGCA	ATGGGAGACC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GTGGGACTCC
20	2701	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG
	2761	AAOGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC
	2821	CTCTCCCTGT	CTCCGGGTAA	ATGA			

FVII-127 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the thrombin cleavage site is shown in dashed underline, the trypsin 170 loop region is wave underlined, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

30	1	<u>MVSQALRLLC</u>	<u>LLGLQGCLA</u>	<u>AVFVTQEEAH</u>	<u>GVLHRRRRAN</u>	AFLEELRPGS	LERECKEEQC
	61	<u>SFEAREIFK</u>	<u>DAERTKLEWI</u>	<u>SYSDDGDCAS</u>	<u>SPCQNGGSCK</u>	<u>DQLQSYICFC</u>	<u>LPAFEGRNCE</u>
	121	THKDDQLICV	NENGGEQYC	SDHTGTRKSC	RCEGYSLLA	DGVSCPTIVE	YPCGKIPILE
	181	KRNASKPQGA	LRPRIVGGKV	CPKGECPWQV	LLLVNGAQLC	GGTLINTIWW	VSAAHCFDKI
	241	KNWRNLIAVL	GEHDLSEHDG	DEQSRRAQV	IIPSTYVPGT	TNHDIALRL	HQPVVLTDHV
	301	VPLCLPERTF	SERTLAFVRF	SLVSGWGQLL	DRGATALELM	VLNVPRMTQ	<u>DCEASYPGKI</u>
	361	TEYMFCAQYS	DGSKDSCKGD	SGGPHATHYR	GTWYLTGIVS	WGQGCATVGH	FGVYTRVSQY
35	421	IEWLQKLMRS	EPRPGVLLRA	PFPGGGGSGG	GGSGGGGSGG	GGSGGGGSGG	GGSDKTHTCP
	481	PCPAPELLGG	PSVFLPPPKP	KDTLMISRTF	EVTCTVVVDVS	HEDPEVKFNW	YVDGVEVHMA
	541	KTKPREEQYN	STYRVSVLT	VLHQDWLNGK	EYKCKVSNRA	LPAPIERTIS	KAKGQPREPQ
	601	VYTLPPSRDE	LTRNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKITPPV	LDSGGSFFLY
	661	SKLTVDKSRW	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK	GGGGSGGGGS	GGGGSGGGGS
40	721	DKTHTCPPCP	APELLGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD
	781	GVEVHNARKT	PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTIKAK
	841	GQPREPQVYT	LPPSRDELTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTPPVLDLS
	901	DGSFFLYSKL	TVDKSRWQQG	NVFSQSVME	ALHNHYTQKS	LSLSPGK*	

45 DNA sequence for FVII-125

50	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
	61	GCAGTCTTCG	TAACCCAGGA	GGAAGCCAC	GGCTTCCTGC	ACCGGCGCCG	CGGCGCCAAC
	121	GCGTTCCTGG	AGGAGCTGCG	GCGGGCTTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	TCTTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAAGCT	GTCTCTGGAAT
	241	TCTTACAGTG	ATGGGGACCA	GTGTGCCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG
	301	GACCAGCTCC	AGTCTATAT	CTGCTTCTGC	CTCCCTGCC	TCGAGGGCCG	GAAGTGTGAG
	361	ACGCAACAAG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	CAGTACTGTC
	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATCTAGAA
55	541	AAAAGAAATG	CCAGCAAACC	CCAAGCCCGA	ATTGTGGGGG	GCAAGGTGTG	CCCCAAAGGG
	601	GAGTGTCCAT	GGCAGTCCCT	GTGTGTGGTG	AATGGAGCTC	AGTTGTGTGG	GGGGACCTTG
	661	ATCAACACCA	TCTGGGTGGT	CTCCGCGGCC	CACTGTTTTCG	ACAAAATCAA	GAAGTGGAGG
	721	AACCTGATCG	CGGTGCTGGG	CGAGCACGAC	CTCAGCGAGC	ACGACGGGGA	TGAGCAGAGC
	781	CGGCGGGTGG	CGCAGGTCAT	CATCCCCAGC	ACGTACGTCC	CGGGCACCAC	CAACCACGAC
60	841	ATCGCGCTGC	TCCGCTGCA	CCAGCCCCTG	GTCTTCACTG	ACCATGTGGT	GCCCCCTCTG
	901	CTGCCCGAAC	GGACGTTCTC	TGAGAGGACG	CTGGCCTTTCG	TGCGCTTCTC	ATTGGTCAGC
	961	GGCTGGGGCC	AGCTGCTGGA	CCGTGGGCCC	ACGGCCCTGG	AGCTCATGGT	CCTCAACGTC
	1021	CCCCGGCTGA	TGACCCAGGA	CTGCCTGCAG	CAGTCAACGA	AGGTGGGAGA	CTCCCCAAAT
	1081	ATCACGGAGT	ACATGTTCTG	TGCCGGCTAC	TCGGATGGCA	GCAAGGACTC	CTGCAAGGGG
65	1141	GACAGTGGAG	GCCCCATGTC	CACCCACTAC	CGGGGCACGT	GGTACCTGAC	GGGCATCGTC
	1201	AGCTGGGGCC	AGGGCTGCGC	AACCGTGGGC	CACITTTGGG	TGTACACCAG	GGTCTCCAG
	1261	TACATCGAGT	GGCTGCAAAA	GCTCATGCGC	TCAGAGCCAC	GCCAGGAGT	CTCCTGCGA
	1321	GCCCCATTTC	CCGGTGGCGG	TGGCTCCGGC	GGAGGTGGGT	CCGGTGGCGG	CGGATCAGGT
	1381	GGGGGTGGAT	CAGGCGGTGG	AGGTTCGGGT	GGCGGGGGAT	CCGACATCGT	GATGACCCAG
70	1441	GCCGCCCCCA	GCGTGCCCGT	GACCCCGGCG	GAGAGCGTGA	GCATCAGCTG	CCGGAGCAGC
	1501	CGGAGCCTGC	TGCACAGCAA	CGGCAACACC	TACCTGTGCT	GGTTCCTGCA	CGCGCCCGGC
	1561	CAGAGCCCCC	AGCTGCTGAT	CTACCGGATG	AGCAACCTGG	CCAGCGCGGT	GCCCGACCGG
	1621	TTACAGCGGA	GCGGAGCGGG	CACCGCCTTC	ACCCTGCGGA	TCAGCCGGGT	GGAGGCCGAG
	1681	GACGTGGGCG	TGTACTACTG	CATGCAGCAC	CTGGAGTACC	CCTTCACCTT	CGGCAGCGGC

5 1741 ACCAAGCTGG AGATCAAGCG GGGCGGCGGC GGCAGCGGCG GCGGCGGCGC CGGCGGCGGC
 1801 GGCAGCCAGG TGCAGCTGCA GCAGAGCGGC GCCGAGCTGG TGCGGGCCGG CACCAGCGTG
 1861 AAGATCAGCT GCAAGGCCAG CGGCTACACC TTCACCAACT ACTGGCTGGG CTGGGTGAAG
 1921 CAGCGGCCCG GCCACGGCCT GGAGTGGATC GGCACATCT ACCCGGCGG CGGCTACAAC
 1981 AAGTACAACG AGAACTTCAA GGGCAAGGCC ACCCTGACCG CCGACACCAG CAGCAGCACC
 10 2041 GCCTACATGC AGCTGAGCAG CCTGACCAGC GAGGACAGCG CCGTGTACTT CTGCGCCCGG
 2101 GAGTACGGCA ACTACGACTA CGCCATGGAC AGCTGGGGCC AGGGCACCAG CGTGACCGTG
 2161 AGCAGCTGA

15 FVII-125 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker region connecting FVII to AP3 is bold, and AP3 scFv is italicized

20 1 MVSOAIRLLC LLLGLGCGCLA AVEVTOERAN GVLHRRRRRAN AFLEELRPGS LERECKEEQC
 61 SPEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCQYQC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPKG BCFWQLLLLV NGAQLCGGTL INTIWVVSA HCFDKIKNWR
 241 NLTAVLGEHD LSEHDGDEQS RVVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
 301 LPERTFSERT LAFRFLSLVS GWGQLLDRGA TAELMLVNLV PRLMTQDCLQ QSRKVGDSPN
 361 ITEYMPCAGY SDGSKDSCRG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HPGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGSGGGGGSG GGSGGGGGSG GGSGDIVMTQ
 25 481 AAPSVPVTPG ESVSISCRSS RSLLHSNGNT YLCNFLQRFG QSPQLLIYRM SNLASGVPDR
 541 FSGSGSGTAF TLRISRVEAE DVGVYYCMQH LEYPFTFGSG TKLEIKRGSG GSGGGSGGSG
 601 GSQVQLQSG AELVRPGTSV KISCKASGYT FTNYWLGWVK QRPGHGLEWI GDIYPGGGYN
 661 KYNENFKGKA TLTADTSSST AYMLSLSLTS EDSAVYFCAR EYGNYDYAMD SWQGTSSVTV
 721 SS*

DNA sequence for FVII-067

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
 35 61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCAGAG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCTTCA AGTCCATGCC AGAATGGGGG CTCTGCAAG
 301 GACCAGCTCC AGTCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA CGACTACTGC
 40 421 ACCGACCACA CGGGCAACAA CGTCTCCTGT CGTGCCACAG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTAGAA
 541 AAAAGAAATG CCAGCAAAAC CCAAGGCCGA ATTGTGGGGG GCAAGGGTGT CCCCAGAGGG
 601 GAGTGTCCAT GGCAGTCCCT GTTGTGTGTG AATGGAGCTC AGTTGTGTGG GGGGACCTGT
 661 ATCAACACCA TCTGGGTGGT TCCGCGGCC CACTGTTTCG ACAAATCAA GAACTGGAGG
 45 721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
 781 CGCGGGGTGG CGCAGGTGAT CATCCCCAGC ACGTACGTCC CGGGCACAC CAACCCAGC
 841 ATCGCGCTGC TCCGCTGCA CCAGCCGCTG GTCCTCACTG ACCATGTGGT GCGCTCTGCT
 901 CTGCCCCAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TCGCTTCTC ATTGGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
 50 1021 CCGCGGCTGA TGACCCAGGA CTGCTGTCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
 1081 ATCACGGAGT ACATGTTCTG TGCCGGCTAC TCGSATGGCA GCAAGGACTC CTGCAAGGGG
 1141 GACAGTGGAG GCCCCATGCG CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCTGC
 1201 AGCTGGGGCC AGGGCTGGGC AACCTGGGG CACTTGGGG TGTAACACAG GGTCTCCCG
 1261 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
 55 1321 GCCCCATTTT CCGGTGGCGG TGGCTCCGCG GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
 1381 GGGGGTGGAT CAGGCGGTGG AGGTTCGGGT GCGCGGGGAT CCGACAAAAC TCACACATGC
 1441 CACCGCTGOC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG TCTTCTCTTT CCCCCAAA
 1501 CCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
 1561 AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
 60 1621 CCGAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCTCCTC
 1681 ACCGTCTTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
 1741 GCCTCCCAAG CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
 1801 CAGGTGTACA CCCTGCCCCC ATCCCGGAT GAGCTGACCA AGAACAGGT CAGCCTGACC
 1861 TGCTTGGTCA AAGGCTTCTA TCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
 65 1921 CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTGGACT CCGACGGCTC CTCTCTCCTC
 1981 TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGCTTT CTCATGCTCC
 2041 GTGATGCAAG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGT
 2101 AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG GAGGTTCGGG TGGCGGGGGA
 2161 TCAGACAAAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG AGGACCGTCA
 70 2221 GTCTTCTCTT TCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
 2281 ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 2341 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCAGC
 2401 TACCGTGTGG TCAGGTCTCT CACCGTCTCT CACGAGGACT GGCTGAATGG CAAGGAGTAC
 2461 AAGTGCAAGG TCTCCAACAA GCCCTCTCCA GCCCTCTCCA AGAAAAACCA TCCAAAGGCC
 75 2521 AAAGGGCAGC CCGGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGCGA TGAGCTGACC
 2581 AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCAGCGCA CATCGCCGTG
 2641 GAGTGGGAGA GCAATGGGCA GCGGAGAAC AACTACAAGA CCACGCCTCC CGTGTGGAC
 2701 TCCGACGGCT CCTTCTTCTT CTACAGCAAG CTCACCGTCC ACAAGAGCAG GTGGCAGCAG
 2761 GGAACGCTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCGAAG
 80 2821 AGCCTCTCCC TGTCTCCGGG TAAAGGTGGC GGTGGCTCCG GCGGAGTGG GTCCGCTGGC
 2881 GCGGATCAG GTGGGGGTGG ATCAGGCGGT GGAGTTCCG GTGGCGGGG ATCAGCGCAG

5	2941	GTGCAGCTGC	AGGAGTCTGG	GGGAGGCTTG	GTACAGCCTG	GGGGGTCCCT	GAGACTCTCC
	3001	TGTGCAGCCT	CTGGATTTCAT	GTTTAGCAGG	TATGCCATGA	GCTGGGTCCG	CCAGGCTCCA
	3061	GGGAAGGGGC	CAGAGTGGGT	CTCAGGTATT	AGTGGTAGTG	GTGGTAGTAC	ATACTACGCA
	3121	GACTCCGTGA	AGGGCCGGTT	CACCGTCTCC	AGAGACAATT	CCAAGAACAC	GCTGTATCTG
10	3181	CAATGAACA	GCCTGAGAGC	CGAGGACACG	GCTGTATATT	ACTGCGCCCG	GGGCGCCACC
	3241	TACACCAGCC	GGAGCGACGT	GCCCGACCAG	ACCAGCTTCG	ACTACTGGGG	CCAGGGAACC
	3301	CTGGTCACCG	TCTCCTCAGG	GAGTGCATCC	GCCCCAAAGC	TTGAAGAAGG	TGAATTTTCA
	3361	GAAGCACCGG	TATCTGAAGT	GACTCAGGAC	CCTGCTGTGT	CTGTGGCCTT	GGGACAGACA
	3421	GTACAGATCA	CATGCCAAGG	AGACAGCCTC	AGAAACTTTT	ATGCAAGCTG	GTACCAGCAG
	3481	AAGCCAGGAC	AGGCCCTTAC	TCTTGTCTAT	TATGGTTTAA	GTAAAAGGCC	CTCAGGGATC
15	3541	CCAGACCGAT	TCTCTGCCTC	CAGCTCAGGA	AACACAGCTT	CCTTGACCAT	CACCTGGGGT
	3601	CAGGCGGAAG	ATGAGGCTGA	CTATTACTGC	CTGCTGTACT	ACGGCGGGCG	CCAGCAGGGC
	3661	GTGTTCCGGC	GCGGCACCAA	GCTGACCGTC	CTACGTCAGC	CCAAGGCTGC	CCCCTCGGTC
	3721	ACTCTGTTCC	CGCCCTCTTC	TGCGGCCTGA			
20	FVII-067 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVIIa to Fc region is underlined, linker connecting both Fc regions is dashed underlined and linker connecting the Fc region to SCE5 is in bold						
25	1	<u>MYEQALRLLC</u>	<u>LLILGQGLA</u>	<u>AVEVTQEEAH</u>	<u>GVLHRRRRAN</u>	AFLEELRPGS	LERECKEEQC
	61	SFEAREIFK	DAERTKLEWI	SYSDDGDCAS	SPCQNGGSCK	DQLQSYICFC	LPAFEGRNCE
	121	THKDDQLICV	NENGCEQYVC	SDHTGTRKSC	RCHGYSLLA	DGVSCPTVE	YPCGKIPILE
	181	KRNASKPQGR	IVGGKVCFKG	BCPWQVLLLV	NGAQLCGGTL	INTIWWVSAA	HCPDKIKNWR
	241	NLTAVLGEHD	LSEHDGDEQS	RRVAQVIIIPS	TYVPGTTNHD	IALLRLHQPV	VLTDHVVPIC
	301	LPERTFSERT	LAFVRFSLVS	GWGQLLDRA	TALELMVLNV	PRMTQDCLQ	QSRKVGDSPN
30	361	LTBYMFCAGY	SDGSKDSCKG	DSGGPHATHY	RGTWYLTGIV	SWGQGCATVG	HPGVYTRVSO
	421	YIEWLQKLMR	SEPRPGVLLR	APFPGGGGSG	GGGGGGGGSG	GGGGGGGGSG	GGGSDKHTTC
	481	PPCPAPELLG	GPSVFLFFPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN
	541	AKTKPREEQY	NSTYRVVSVL	TVLHQDWLNG	KEYRCKVSNK	ALPAPIEKT	SKAGQPREP
	601	QVYTLPPSRD	ELTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ	PENNYKITPP	VLDSDGSFFL
35	661	YSKLTVDKSR	WQQGNVFSKS	VMHEALHNHY	TQKSLSLSPG	KGGGGGGGGG	SGGGGGGGGG
	721	SDKHTTCCPC	PAPELGGPS	VFLFPPKPKD	TLMSRTPEV	TCVVVDVSH	DPEVKFNWVY
	781	DGEVHNNAKT	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA
	841	KGQPREPQVY	TLPPSRDEL	KNQVSLTCLV	KGFYPSDIAV	EWESNGGPN	NYKTTTPVLD
40	901	SDGSFFLYSK	LTVDKSRWQQ	GNVFSKSVMH	EALHNHYTQK	SLSLSPGKGG	GGGGGGGGGG
	961	<u>GGSGGGGSAQ</u>	<u>GGSGGGGSAQ</u>	<u>VQLQESGGGL</u>	<u>VQPGGSLRLS</u>	CAASGFMFSR	YAMSWVRQAP
	1021	GKSPFVWSGI	SGSGGSTYYA	DSVKGRFTVS	RDNSKNTLYL	QMNSLRAEDT	AVYYCARGAT
	1081	YTSRSDVPDQ	TSFDYWGQGT	LVTVSSGSAS	APKLEEGEFS	EARVSELTQD	PAVSVLGGT
	1141	VTITCGDGL	RNFYASWYQQ	KPGQAPTLVI	YGLSKRPSGI	PDRFSASSSG	NTASLTITGA
45	1201	QAEDADYYC	LLYYGGGQQC	VFGGCTKLT	LRQPKAAPS	TLFPPSSAA*	
	DNA sequence for FVII-094						
	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
	61	GCAGTCTTCG	TAACCCAGGA	GGAAGCCAC	GGCGTCTG	ACCGGCGCCG	GCAGGCCAAC
50	121	GCGTTCCTGG	AGGAGCTGCG	GCCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	TCTTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAGCT	GTCTTGGATT
	241	TCTTACAGTG	ATGGGACCA	GTGTGCCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG
	301	GACCAGCTCC	AGTCTTATAT	CTGCTTCTGC	CTCCCTGCCT	TCGAGGGCCG	GAAGTGTGAG
	361	ACGCACAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAAGC	GCGGCTGTGA	CGAGTATCTG
55	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATTCTAGAA
	541	AAAAGAAATG	CCAGCAAACC	CAAAGGCCGA	ATTGTGGGGG	GCAAGGTGTG	CCCCAAAGGG
	601	GAGTGTCCAT	GGCAGGTCTC	GTGTGTGGTG	AATGGAGCTC	AGTTGTGTGG	GGGGACCTCG
	661	ATCAACACCA	TCTGGGTGGT	TCCCGCGGCC	CACTGTTTCG	ACAAAATCAA	GAAGTGGAGG
	721	AACCTGATCG	CGGTGCTGGG	CGAGCACGAC	CTCAGCGAGC	ACGACGGGGA	TGAGCAGAGC
60	781	CGGCGGGTGG	CGCAGGTCTC	CATCCCCAGC	ACGTACGTCC	CGGGCACACC	CAACCCAGAC
	841	ATCGCGCTGC	TCCGCTTGCA	CCAGCCCGTG	GTCTCACTG	ACCATGTGGT	GCCCCCTCTG
	901	CTGCCCCAAC	GGACGTTCTC	TGAGAGGACG	CTGGCCTTCG	TGCGCTTCTC	ATTGGTCAGC
	961	GGCTGGGGCC	AGCTGCTGGA	CGGTGGCGCC	ACGGCCCTGG	AGCTCATGGT	CCTCAACGTC
65	1021	CCCCGGCTGA	TGACCCAGGA	CTGCCCTGCAG	CAGTCACGGA	AGGTGGGAGA	CTCCCCAAAT
	1081	ATCACGGAGT	ACATGTTCTG	TGCCGGCTAC	TCGGATGGCA	GCAAGGACTC	CTGCAAGGGG
	1141	GACAGTGGAG	GCCCCATGTC	CACCCACTAC	CGGGGCACGT	GGTACCTGAC	GGGCATCGTC
	1201	AGCTGGGGCC	AGGGCTGCGC	AACCGTGGGC	CACCTTGGGG	TGTACACCCG	GGTCTCCGAG
70	1261	TACATCGAGT	GGCTGCAAAA	GCTCATGCGC	TCAGAGCCAC	GCCCAGGAGT	CCTCCTGCGA
	1321	GCCCCATTTC	CCGATATCGG	TGGCGGTGGC	TCCGGCGGAG	GTGGGTCCGG	TGGCGGCGGA
	1381	TCAGGTGGGG	GTGGATCAGG	CGGTGGAGGT	TCCGGTGGCG	GGGGATCAGC	GCAGGTGCAG
	1441	CTGCAGGAGT	CTGGGGGAGG	CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CTCCTGTGCA
	1501	GCTCTGGGAT	TCATGTTTAG	CAGGTATGCC	ATGAGCTGGG	TCCGCCAGGC	TCCAGGGAAG
	1561	GGGCCAGAGT	GGGTCTCAGG	TATTAGTGGT	AGTGGTGGTA	GTACATACTA	CGCAGACTCC
75	1621	GTGAAGGGCC	GGTTCAACCT	CTCCAGAGAC	AATTCACAGA	ACACGCTGTA	TCTGCAAAAT
	1681	AACAGCCTGA	GAGCCGAGGA	CACGGCTGTA	TATTACTGCG	CCCGGGGCGC	CACCTACACC
	1741	AGCCGGAGCG	ACGTGCCCCG	CCAGACCAGC	TTCCGACTACT	GGGGCCAGGG	AACCTTGGTC
	1801	ACCGTCTCCT	CAGGGAGTGC	ATCCGCCCCA	AAGCTTGAAG	AAGGTGAATT	TTCAGAAGCA
	1861	CGCGTATCTG	AACTGACTCA	GGACCTGCTG	GTGTCTGTGG	CCTTGGGACA	GACAGTCAGG
	1921	ATCACATGCC	AAGGAGACAG	CCTCAGAAAC	TTTATGCAA	GCTGGTACCA	GCAGAGGCCA
80	1981	GGACAGGGCC	CTACTCTTGT	TAAAGTAAAG	GGCCCTCAGG	GATCCAGAC	
	2041	CGATTCTCTG	CCTCCAGCTC	ATGAAACACA	GCTTCTCTGA	CCATCACTGG	GGCTCAGGGC

5 2101 GAAGATGAGG CTGACTATTA CTGCCTGCTG TACTACGGCG GCGGCCAGCA GGGCGTGTTC
 2161 GCGGCGCGCA CCAAGCTGAC CGTCCTACGT CAGCCCCAAG CTGCCCCCTC GGTCACTCTG
 2221 TTCCCGCCCT CTTCTGCGGC CTGA

10 FVII-094 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker region connecting FVII to SCE5 is underlined

1 MYSQAIRLLC LLLGLQGCLA AVEVTQEEAH GVLHRRERAN AFLEELRPGS LERECKEEQC
 61 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTPVE YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPEKG ECFWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAVIIPS TYVPGTTNHD IALLRLHOPV VLTDHVVPLC
 301 LPERTFSERT LAFVRFSLVS GWGQLLDRA TALELMVLNV PRLMTQDCLQ QSRKVGDSNP
 361 ITEYMFCAFY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HPGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR AFFFIDIGGGG SGGGSGGGGG SGGGSGAQVQ
 481 LQESGGGLVQ PGGSLRLSCA ASGFMFERYA MSWVRQAPGK GPEWVSGISG SGGSTYYADS
 541 VKGRFTVSRD NSKNTLYLQM NSLRAEDTAV YYCARGATYT SRSDVPDQTS FDXWQGQTLV
 601 TVSSGSASAP KLEEGEFSEA RVSELTQDPA VSVALGQTVR ITCQGDLSRN FYASWYQKQP
 661 GQAPTLVIYG LSKRPSGIPD RFSASSSGNT ASLTITGAQA EDEADYYCLL YYGGGQQGVF
 721 GGTKLTLVLR QPKAAPSCTL FPPSSAA*

25 DNA sequence for FVII-028

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
 61 GCGGAAGTGC AGCTGGTGCA GTCTGGAGCT GAGGTGAATA AGCCTGGGGC CTCAGTGAAG
 121 GTCTCCTGCA AGGCTTCTGG ATACACCTTC ACCGGCTACT ATATGCACTG GGTGCGACAG
 181 GCGCCTGGAC AAGGGCTTGA GTGGATGGGA TGGATCAACC CTAACAGTGG TGGCACAACC
 241 TATGCACAGA AGTTTCAGGG CTGGGTCAAC ATGACCAGGG ACACGCTCAT CAGCACCGCC
 301 TACATGGAGC TGAGCAGGCT GAGATCTGAC GACACGGCCG TGTATTACTG TGCAGAGGGC
 361 CGTGCTTTGT ATAACCGGAA CGACCGGTCC CCCAACTGGT TCGACCCCTG GGGCCAGGGA
 421 ACCCTGGTCA CCGTCTCCTC AGGGAGTGCA TCCGCCCCAA CCCTTAAACT TGAAGAAGGT
 481 GAATTTTTCAG AAGCACGCGT ACAGGCTGTG CTGACTCAGC CGCCTCGGTG GTCAGTGGCC
 541 CCAGGACAGA CGGCCAGGAT TACCTGTGGG GGAAACAACA TTGGAAGTAA AAGTGTGCAG
 601 TGGTACCAGC AGAAGCCAGG CCAGGCCCTC GTGCTGGTGC TCTATGATGA TAGCGACCGG
 661 CCTCAGGGA TCCCTGAGCG ATTCTCTGGC TCCAACCTCT GGAACATGGC CACCTTGACC
 721 ATCAGCAGGG TCGAAGCCGG GGATGAGGCC GACTATTACT GTCAGGTGTG GGTAGTAGT
 781 AGTGATCATG TGGTATTCCG CGGAGGGACC AAGCTGACCG TCCTAGGTCA GCCCAAGGCT
 841 GCGCCCTCGG TCACTCTGTT CCGCCGCTCC GCGGCCGCTA GGACGAAGCT GTTCTGGATT
 901 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 961 GACAGACTCC AGTCTATAT CTGCTTCTGC CTCCTGCTCC TCGAGGGCCG GAATGTGAG
 1021 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACC GCGGCTGTGA GCAGTACTGC
 1081 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 1141 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTAGAA
 1201 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCGCAAGGG
 1261 GAGTGTCCAT GGCAGTCCCT GTTGTGTGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
 1321 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAATCAA GAATGGAGG
 1381 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGACGAGAGC
 1441 CGGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACCA CCAACCACGAC
 1501 ATCGCGCTGC TCCGCTTGCA CCAGCCCTGG GTCTCCTACT ACCATGTGGT GCCCTCTGCG
 1561 GCGGCAAGAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TCGCTTCTC ATTGCTCAGC
 1621 GGCTGGGGCC AGCTGCTTGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
 1681 CCGCGGCTGA TGACCCAGGA CTGCTGTCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
 1741 ATCAGCGAGT ACATGTTCTG TGCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
 1801 GACAGTGGAG GCGCCATGCG CCGGCACTAC CGGGGCACTG GGTACCTGAC GGGCATCGTC
 1861 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCAG
 1921 TACATCGAGT GGTGCAAAA GCTCATGCGC TCAGAGCCAC GCGCAGGAGT CCTCCTGCGA
 1981 GCGCCATTTC CCGGTGGCGG TGGCTCCGCG GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
 2041 GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GCGGGGGGAT CCGACAAAC TCACACATGC
 2101 CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG TCTTCTCTT CCCCCAAAA
 2161 CCGAAGGAGC CCTCATGAT CTGAGGTGCA CATGCGTGGT GGTGAGCATG GGTGACATAT
 2221 GCGGAGGAGC ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGG GGTGACATAT
 2281 GCGGAGGAGC AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCTCT
 2341 ACCGTCTTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
 2401 GCGCTCCGAG CCGCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
 2461 CAGGTGTACA CCTGCCCCC ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC
 2521 TGCTTGGTCA AAGGCTTCTA TCCAGCGGAC ATCGCGGTGG AGTGGGAGAG CAATGGGCGA
 2581 CCGGAGAAC AACTACAAGC CACGCTCCCG GTGTGGACT CCGACGGCTC CTCTCTCTCT
 2641 TACAGCAAGC TCACCGTGGG CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
 2701 GTGATGCATG AGGCTCTGCA CAACCATAC ACGCAGAGA GCCTCTCCCT GTCTCCGGGT
 2761 AAAGGTGGCG GCGGATCAGG TGGGGTGGG TCAGGCGGTG GAGGTTCCGG TGGCGGGGGA
 2821 TCCGACAAA CTCACACATG CCCACCTGCG CACGACCTG AACTCCTGGG AGGACCGTCA
 2881 GTCTTCTCT TCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCTGTAGGTC
 2941 ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 3001 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCGCGGG AGGAGCAGTA CAACAGCAGC
 3061 TACCGTGTGG TCAGCGTCTT CACCGTCTTG CACCGAGACT GGCTGAATGG CAAGGAGTAC
 3121 AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCGCCCATCG AGAAAACCAT CTCCAAGGCC
 3181 AAAGGGGAGC CCGGAGAAC ACCCTGCTAC ACCCTGCCCC CATCCGCGCA TGAGCTGACC
 3241 AAGAACCAGG TCAGCTGAC CATGCTGGTC AAAGGCTTCT ATCCACGCGA CATGCGCGTG

5	3301	GAGTGGGAGA	GCAATGGGCA	GCCGGAGAAC	AACTACAAGA	CCACGCCTCC	CGTGTGGGAC
	3361	TCCGACGGCT	CCTTCTTCCT	CTACAGCAAG	CTCACCGTGG	ACAAGAGCAG	GTGGCAGCAG
	3421	GGGAACGTCT	TCTCATGCTC	CGTGATGCAT	GAGGCTCTGC	ACAACCACTA	CACGCAGAAG
	3481	AGCCTCTCCC	TGTCTCCGGG	TAAATGA			
10	FVII-028 amino acid sequence. Signal sequence is shown in dotted underline, linker region connecting FVII to Fc region is underlined, linker connecting both Fcs sites is shown in bold, and MB9 is italicized						
	1	<u>MYSQALRLLC</u>	<u>LLLGLQGCLA</u>	<u>AEVOLVQSGA</u>	<u>EVNKPASVK</u>	<u>VSCASGYTF</u>	<u>TGYMHVVRQ</u>
15	61	<u>APQGLEWMG</u>	<u>WINFNSGGTN</u>	<u>YAKFQGWVT</u>	<u>MTRDTSISTA</u>	<u>YMELSRRLSD</u>	<u>DTAVYYCARG</u>
	121	<u>RALYNRNDRS</u>	<u>PNWFDPWQGG</u>	<u>TLVTVSSGSA</u>	<u>SAPTLLKLEBG</u>	<u>EFSEARVQAV</u>	<u>LTQPPSVSVA</u>
	181	<u>PGQTARITCG</u>	<u>GNNIGSKSVQ</u>	<u>WYQQKPGQAP</u>	<u>VLVYVDDSDR</u>	<u>PSGIFERFSG</u>	<u>SNNGNMATLT</u>
	241	<u>ISRVEAGDEA</u>	<u>DYYCQVWDS</u>	<u>SDHVVFQGGT</u>	<u>KLTVLGQPKA</u>	<u>APSVTLPPPS</u>	<u>AAARTKLFWI</u>
	301	<u>SYSDGDQCA</u>	<u>SPCQNGGSC</u>	<u>DQLQSYICFC</u>	<u>LPAPFGRNCE</u>	<u>THKDDQLICV</u>	<u>NENGGCEQYC</u>
20	361	<u>SDHTGTRKSC</u>	<u>RCHEGYSLA</u>	<u>DGVSCPTFVE</u>	<u>YPCGKIPILE</u>	<u>KRNASKPQGR</u>	<u>IVGKVCVPGK</u>
	421	<u>ECPWQVLLLV</u>	<u>NGAQLCGGTL</u>	<u>INTIWWVSA</u>	<u>HCFDKIKNWR</u>	<u>NLIAVLGEHD</u>	<u>LSEHDGDEQS</u>
	481	<u>RRVAQVILPS</u>	<u>TYVPGTTNHD</u>	<u>IALLRLHQP</u>	<u>VLTDHVVPCL</u>	<u>LPERTFSERT</u>	<u>LAFFVRFSLVS</u>
	541	<u>GWGQLLDRGA</u>	<u>TALELMVLNV</u>	<u>PRLMTQDCLQ</u>	<u>QSRKVGDSNP</u>	<u>ITEYMFPCAG</u>	<u>SDGSKDSCKG</u>
	601	<u>DGGGPHATHY</u>	<u>RGTWYLTGIV</u>	<u>SWGQGCATVG</u>	<u>HFGVYTRVSQ</u>	<u>YIEWLQRLMR</u>	<u>SEPRPGVLLR</u>
25	661	<u>APFPQGGGSG</u>	<u>GGGSGGGGSG</u>	<u>GGGSGGGGSG</u>	<u>GGGSGDKTHTC</u>	<u>PPCPAPELLG</u>	<u>GPSVFLFPPK</u>
	721	<u>PKDTLMISRT</u>	<u>PEVTCVVDV</u>	<u>SHEDPEVKFN</u>	<u>WYVDGVEVHN</u>	<u>AKTKPREEQY</u>	<u>NSTYRVVSVL</u>
	781	<u>TVLHQDWLNG</u>	<u>KEYKCKVSNK</u>	<u>ALPAPIEKTI</u>	<u>SKAKGQPREP</u>	<u>QVYTLPPSRD</u>	<u>ELFTKNQVSLT</u>
	841	<u>CLVKGFFPSD</u>	<u>IAVEWESNGQ</u>	<u>PENNYKTPPP</u>	<u>VLDSDGSPFL</u>	<u>YSKLTVDKSR</u>	<u>WQGNVVFSCS</u>
	901	<u>VMHEALHNHY</u>	<u>TQKSLSLSPG</u>	<u>KGGGGSGGGG</u>	<u>SGGGSGGGGG</u>	<u>SDKTHTCPPC</u>	<u>PAPELLGGPS</u>
30	961	<u>VFLFPPKPKD</u>	<u>TLMISRTPEV</u>	<u>TCVVVDVSHE</u>	<u>DPEVKFNWYV</u>	<u>DGVEVHNAKT</u>	<u>KPREEQYNST</u>
	1021	<u>YRVVSVLTVL</u>	<u>HQDWLNGKEY</u>	<u>KCKVSNKALP</u>	<u>APIEKTI</u>	<u>SKAKGQPREP</u>	<u>QVYTLPPSRD</u>
	1081	<u>KNQVSLTCLV</u>	<u>KGFYPSDIAV</u>	<u>BWESNGQPEN</u>	<u>NYKTPPPVLD</u>	<u>SDGSPFLYSK</u>	<u>LTVDKSRWQQ</u>
	1141	<u>GNVVFCSVMH</u>	<u>EALHNHYTQK</u>	<u>SLSLSPGK*</u>			
	DNA sequence FVII-039						
35	1	ATGGTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
	61	GCAGTCTTCG	TAACCCAGGA	GGAAGCCAC	GGCGTCCTGC	ACCGGCGCCG	CGCGCCCAAC
	121	GGCTTCTTGG	AGGAGCTCGC	GCCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	TCCTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACCGGGAGA	GGACCAAGCT	GTCTCTGGATT
40	241	TCCTACAGTG	ATGGGGACCA	GTGTGCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG
	301	GACCACTTCC	AGTCTTATAT	CTGCTTCTGC	CTCCTGCCTC	TCGAGGGCCG	GAAGTGTGAG
	361	ACGCACAAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	GCAGTACTGC
	421	AGTGACCACA	CGGGCAACCA	GCGCTCTCTG	CGGTGCCACG	AGGGGTACTC	TCGTCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATCTAGAA
45	541	AAAAGAAATG	CCAGCAAACC	CCAAGGCCGA	GGCGGAGGAG	ACTTCACTCG	GGTGTGGGGG
	601	GGCAAGGTGT	GCCCCAAAGG	GGAGTGTCCA	TGGCAGGTCC	TGTTGTGTGG	GAATGGAGCT
	661	CAGTTGTGTG	GGGGGACCC	GATCAACACC	ATCTGGGTGG	TCTCCGCGCG	CCACTGTTTC
	721	GACAAATCA	AGAACTGGAG	GAACCTGATC	GCGGTGCTGG	GCGAGCACGA	CCTCAGCAGG
	781	CACGACGGGG	ATGACGAGAG	CGGGCGGGTG	GCGCAGGTCA	TCATCCCCAG	CACGTACGTC
50	841	CCGGGCACCA	CCAACCACGA	CATCGCGCTG	CTCCGCTGCT	ACCAGCCCGT	GGTCTCTACT
	901	GACCATGTGG	TGCCCTCTCT	CTGCCCCGAA	CGGACGTTCT	CTGAGAGGAC	GCTGGCCTTC
	961	GTGCGCTTCT	CATTGGTCAG	CGGCTGGGGC	CAGCTGCTGG	ACCGTGGCGC	CACGGCCCTG
	1021	GAGCTCATGG	TCCTCAACGT	GCCCCGGCTG	ATGACCCAGG	ACTGCTTGCA	GCAGTACACG
	1081	AAGGTGGGAG	ACTCCCCAAA	TATCACGGAG	TACATGTTCT	GTGCGCGCTA	CTCGGATGGC
55	1141	AGCAAGGACT	CCTGCAAGGG	GGACAGTGGG	GGCCACATG	CCACCCACTA	CCGGGGCACC
	1201	TGGTACCTGA	CGGGCATCGT	CAGCTGGGGC	CAGGCTGCGC	CAACCGTGGG	CCACTTTGGG
	1261	GTGTACACCA	GGGTCTCCCA	GTACATCGAG	TGGCTGCAAA	AGCTCATGCG	CTCAGAGCCA
	1321	CGCCAGGAGG	TCCTCCTGCG	AGCCCCATT	CCCGGTGGCG	GTGGCTCCCG	CGAGGTGGGG
	1381	TCCGGTGGCG	GCGGATCAGG	TGGGGGTGGA	TCAGGCGGTG	GAGGTTCCGG	TGGCGGGGGA
	1441	TCCGACAAAA	CTCACACATG	CCCCCGGTGC	CCAGCTCCGG	AACTCCTGGG	CGGACCGTCA
60	1501	GCTTCTCTCT	TCCCCCAAAA	ACCAAGGAC	ACCTCATGTA	TCTCCCGGAC	CCCTGAGGTC
	1561	ACATGCGTGG	TGGTGGACGT	GAGCCACGAA	GACCCTGAGG	TCAAGTTCAA	CTGGTACGTC
	1621	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG	AGGAGCAGTA	CAACAGCACG
	1681	TACCGTGTGG	TCAGGTCCTG	CACCGGACT	GGCTGAATGG	CAAGGAGTAC	CAAGCCGCGG
	1741	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA	GCCCCCATCG	AGAAAAACAT	CTCCAAAGCC
65	1801	AAAGGGCAGC	CCCGAGAACC	ACAGGTGTAC	ACCTGCCCCC	CATCCCGGGA	TGAGCTGACC
	1861	AAGAACCAGG	TCAGCCTGAC	CTGCCTGGTC	AAAGGCTTCT	ATCCCAGCGA	CATCGCCGTG
	1921	GAGTGGGAGA	GCAATGGGCA	GCCGGAGAAC	AACTACAAGA	CCACGCCTCC	CGTGTGGGAC
	1981	TCCGACGGCT	CCTTCTTCTC	CTACAGCAAG	CTCACCGTGG	ACAAGAGCAG	GTGGCAGCAG
	2041	GGGAACGTCT	TCTCATGCTC	CGTGATGCAT	GAGGCTCTGC	ACAACCACTA	CACGCAGAAG
70	2101	AGCCTCTCCC	TGTCTCCGGG	TAAAGGTGGC	GGCGGATCAG	GTGGGGGTGG	ATCAGGCGGT
	2161	GGAGGTTCCG	GTGGCGGGGG	ATCAGACAAA	ACTCACACAT	GCCCCCGGTG	CCCAGCACCT
	2221	GAACTCCTGG	GAGGACCGTC	AGTCTTCTCT	TTCCCCCAAA	AACCCAGGGA	CACCCCTCAT
	2281	ATCTCCCCGA	CCCCTGAGGT	CACATGCGTG	GTGGTGGACG	TGAGCCACGA	AGACCTTGAG
	2341	GTCAGATTCA	ACTGGTACGT	GGACGGCGTG	GAGGTGCATA	ATGCCAGAGC	AAAGCCGCGG
75	2401	GAGGAGCAGT	ACAACAGCAC	GTACCGGTGT	GTACAGGTCC	TCACCGTCTC	GCACCGAGAC
	2461	TGGCTGAATG	GCAAGGAGTA	CAAGTGCAAG	GTCTCCAACA	AAGCCCTCCC	AGCCCCCATC
	2521	GAGAAAACCA	TCTCCAAGAG	CAAAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCTTGCCC
	2581	CCATCCCGCG	ATGAGCTGAC	CAAGAACCAG	GTACGCTGTA	CCTGCCCTGG	CAAAGGCTTC
	2641	TATCCACAGC	ACATCGCCGT	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG
80	2701	ACCAAGCCTC	CCGTGTTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG
	2761	GACAAGAGCA	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG

5

3821 CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAATGA

FVII-039 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the FXIa cleavage site is shown in dashed underline, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

10

1 MYSQALRLLC LLLGLOGCLA AVFVTOEEAH GVLHRRREAN AFLEELRPGS LERECKKEEQC
 61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTRKSC RCHBGYSLLA DGVSCPTPVE YPCGKIPILE
 181 KRNASKPQGR GGGDFTREVVG GKVCCKGCEP WQVLLLVNGA QLCGGTILINT IWVVSAAHCF
 241 DKIKNWRNLI AVLGEHDLSE HDGDEQSRV AQVIIPSTYV PGTTNHDIAL LRLHQFVVLIT
 301 DHVVPCLCLPE RTFSERTLAF VRFSLVSGWG QLLDRGATAL ELMVLNVPR MTQDCLQOSR
 361 KVGDSNPITE YMFCAQYSDG SKDSCKGDSG GPHATHYRGT WYLTGIVSWG QGCATVGHFG
 421 VYTRVSQYIE WLQKLMRSEP RPGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG
 481 SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHS DPEVKFNWYV
 541 DGVEVHNAKT KPREQYNST YRVSVLTVL YRVSVLTVL YRVSVLTVL YRVSVLTVL
 601 KGQPREPQVY TLPPSRDEL KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLD
 661 SDGSFFLYSK LTVDKSRWQ GNVFSVSMH EALHNHYTQK SLSLSPGRKG GGGGGGGGSG
 721 GGSGGGGSDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 781 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHSD WLNGKEYCK VSNKALPAPI
 841 EKTISKAKGQ PREPQVYTL PSRDELTKNQ VSLTCLVKG YPSDIAVEWE SNQGPENNYK
 901 TTPFVLDSG SFFLYSKLTV DKSRWQQGNV FSCSVMH HNHYTQKSL LSPGK*

25

DNA sequence for FVII-040

30

1 ATGGTCTCTCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCTTGGCT
 61 GCAGTCTTCC TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCGGGGCTCC CTGGAGAGGG AGTGCAAGGA GAGGACAGTC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCTGCAAG
 301 GACCAGCTCC AGTCTTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAAG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACACCA CGGGCACCA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCGGC GGAGGAGACT TCACTCGGGT TGTGGGGGGC
 601 AAGGTGTGCC CCAAAGGGGA GTGTCCATGG CAGGTCTGTG TGTGGTGAA TGGAGCTCAG
 661 TGTGTGGGGG GGACCTGAT CAACACCATC TGGGTGGTCT CCGCGGCCG CCGTTTCGAC
 721 AAAATCAAGA ACTGGAGGAA CTTGATCGCG GTGTGGGGCG AGCACGACCT CAGCGAGCAC
 781 GACGGGGGATG AGCAGAGCCG GCGGGTGGCG CAGGTTCATCA TCCCCAGCAC GTACGTCCCG
 841 GGCACACCA ACCACGACAT CGCGCTGCTC CGCTTGCACC AGCCCGTGGT CCTCACTGAC
 901 CATGTGGTGC CCCTCTGCCT GCCCGAACCG ACCTTCTCTG AGAGGACGCT GGCCTTCGTG
 961 CGCTTCTCAT TGGTCAGCGG CTGGGGCCAG CTGTGGGACC GTGGCGCCAC GGCCTTGGAG
 1021 CTCATGGTCC TCAAGTGCC CCGGCTGATG ACCAGGACT GCCTGCAGCA GTCACGGAAG
 1081 TGGGGAGACT CCCCCAATAT CACGGAGTAC ATGTCTGTGT CCGGCTACTC GGATGACAGC
 1141 AAGGACTCCT GCAAGGGGGA CAGTGGAGGC CCACATGCCA CCCACTACCG GGCACAGTGG
 1201 TACCTGACGG GCATCGTCAG CTGGGGCCAG GGCTGCGCAA CCGTGGGGCA CTTTGGGGTG
 1261 TACACACAGG TCTCCAGTA CATCAGTGG CTGCAAAAGC TCATGCGCTC AGAGCCACCG
 1321 CCAGGAGTCC TCTGCGAGC CCCATTTCCT GGTGGCGGTG GCTCCGCGCG AGGTGGGTCC
 1381 GGTGGCGGGG GATCAGGTGG GGGTGGATCA GCGGTGGAG GTTCCGCTGG CCGGGGATCC
 1441 GACAAAATC ACACATGCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTGAGT
 1501 TTCTCTTCC CCCCCAACCC CAAGGACACC CTCTGATCT CCCGGACCCC TGAGGTGACA
 1561 TCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 1621 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 1681 CGTGTGGTCA GCGTCTCAC CGTCTGTGAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 1741 TGCAAGGTCT CCAACAAAGC CCTCCAGGCC CCATCGAGA AAACCATCTC CAAAGCCAAA
 1801 GGCAGGCCCG GAGAACCACA GGTGTACACC CTGCCCCAT CCCGGGATGA GCTGACCAAG
 1861 AACCAAGTCA GCCTGACCTG CCGGTCAAA GGCTTCTATC CCAGCGACAT CGCGGTGGAG
 1921 TGGGAGAGCA ATGGGACGCC GGAGAACAAC TACAAGACCA CGCTTCCGTG GTTGGACTCC
 1981 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
 2041 AACCTCTTCT CATGCTCGT GATGCTGAG GCTCTGCACA ACCACTACAC GCAGAGAGAC
 2101 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGCGGTGGGA
 2161 GGTTCGCGTG GCGGGGGATC AGACAAAAC CACACATGCC CACCGTCCCC AGCACCTGAA
 2221 CTCTGGGAG GACCGTCAGT CTTCTCTTTC CCCCCAAAAC CCAAGGACAC CCTCATGATC
 2281 TCCCGGACCC CTGAGGTGAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA CCTGAGGTG
 2341 AAGTTCAACT GGTACGTGGA CCGGTGGAG GTGCATAATG CCAAGACAAA GCGCGGGGAG
 2401 GAGCAGTACA ACAGCAAGTA CCGTGTGGTC AGCGTCTCA CCGTCTGCA CCAGGACTGG
 2461 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCTCCGAGC GCGCATCGAG
 2521 AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA
 2581 TCCGCGATG AGCTGACCAA GAACAGGTC AGCTGACCT GCCTGGTCAA AGGCTTCTAT
 2641 CCCAGCGACA TCGCGTGGG GTGGGAGAGC AATGGGAGC CGGAGAACA CTACAAGACC
 2701 ACAGCTCCCG TGTGGAGTCC CAGCGGCTCC TTCTTCTCT ACAGCAGCT CACCGTGGAC
 2761 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC
 3821 AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGA

65

80

FVII-040 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the FXIa cleavage site is shown in dashed underline, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

5
1 MVSQAIRLLC LILGLQCCIA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEAREIIPK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGCGEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTIVE YPCGKIPILE
181 KRNASKPQGG GGDFTRVVG KVC PKGECFW QVLLLVNGAQ LCGGTLLINTI WVVSAAHCFD
10 241 KTKNWRNLIA VLGEHDLSEH DGDEQSRRA QVIFSTYVP GTTNHDIALL RLHQFVVLTD
301 HVVPLCLPER TFSERTLAFV RPSLVSGWGQ LLDRGATALE LMVLNVPRLM TQDCLQOSRK
361 VGDSFNITEY MFCAGYSDGS KDSCKGDSGG PHATHYRGTW YLTGIVSWGQ GCATVGHFV
421 YTRVSQYIEW LQKLMRSEPR PGVLLRAPFP GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS
481 GKATTCGPCP APELLGGPSV FLFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
15 541 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTIKAK
601 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPFVLD
661 DGSFFLYSKL TVDKSRWQGG NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGSGGGG
721 GSGGGSGDKT HTCCPPCPAPE LLGGPSVPLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV
781 KFNWYVDGVE VHNNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNKKEYKCKV SNKALPAPIE
20 841 KTSISKAGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
901 TFPVLDSGDS FFLYSKLTVL KSRWQGGNVF SCSVMHEALH NHYTQKSLSL SPGK*

DNA sequence for FIX-042

1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCCTTTTA
61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCCTT TTTTAAATA CATGAGTAT
25 121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGCTTCT TCACTAATTT TGTATTACAT
181 GATTTGCAGC CAATATTGAA GAGTCTAACA GCCAGCACGC AGGTTGTGTA GTACTGTGG
241 AACATCACAG ATTTTGGCTC CATGCCCTAA AGAGAAATTG GCTTTCAGAT TATTTGGATT
301 AAAAACAAAG ACTTTCCTTAA GAGATGTAAA ATTTTCATGA TGTTCCTTTT TTTGCTAAAA
361 CTAAAGAATT ATCTTTTAC ATTTTCAGTT TTCTTGATCA TGAAGACGCC AACAAAATTC
421 TGAATTCGGC AAAGAGGTAT AATTGAGTA AATTGGAAGA GTTTGTCAA GGAATCTAG
30 481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
541 CTGAAAGAAC AACTGAATTT TGAAGCAGT ATGTTGATGG AGATCAGTGT GAGTCCAAATC
601 CATGTTTAAA TGGCGCGCAGT TGCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
661 TTGGAATTTGA AGGAAAGAAC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT
35 721 GCGAGCAGTT TTGTAAGAAAT AGTGCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT
781 ATCGACTTGC AGAAAACGAG AAGTCTGTG AACCAGCAGT GCCATTCCA TGTGGAAGAG
841 TTTCTGTTTC ACAACTTCT AAGCTCAGCC GTGCTGAGAC TGTTCCTCTT GATGTGGACT
901 ATGTAAATTC TACTGAAGCT GAAACCAATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
40 1021 GAATGTGTTT GAATGTGATA GTTGATGCAT TCTGTGGAGG CTCTATGCTT AATGAAAAAT
1081 GGATTGTAAAC TGCTGCCCAC TGTGTTGAAA CTGTTGTTAA AATTACAGTT GTGCGAGGTG
1141 AACATAATAT TGAGGAGACA GAACATAAG AGCAAAAGCG AAATGTGATT CGAATTATTC
1201 CTCACCAACA CTACAATGCA GCTATTAATA AGTACAACCA TGACATTGCC CTCTGTGAAC
45 1261 TGGACGAACC CTTAGTGCTA AACAGCTACG TTACACCTAT TTGCATTGCT GACAAGGAAT
1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC
1381 ACAAGGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
1441 CATGTCTTCG ATCTACAAAG TTCACCATCT ATACCAACAT GTTCTGTGCT GGCTTCCATG
1501 AAGGAGGTAG AGATTGATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAG
1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCCAAAT
50 1621 ATGGATGATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAGCTCA
1681 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG
1741 TCTTCCTCTT CCCCCAAAA CCCCAGGACA CCCCCTATGAT CTCCCGGACC CCTGAGGTCA
1801 CTCACCAACA GGTGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAC TGGTACGTGG
1861 ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCCGGGGA GGAGCAGTAC AACAGCACGT
55 1921 ACCGTGTGGT CAGCGTCCTC ACCGTCTGTC ACCAGGACTG GCTGAATGGC AAGGAGTACA
1981 AGTGCAAGGT CTCCAACAAA GCCCTCCAG CCCCCTATG GAAAACCATC TCCAAAGCCA
2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCGTCCGCCC ATCCCGGATG GAGCTGACCA
2101 AGAACCAGGT CAGCCTGACC TGCCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATGCGCGTGG
60 2161 AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCTCCCT GTGTTGGACT
2221 CCGACGGCTC CTTCCTCCTC TCACGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
2281 GGAACGTCTT CTCATGCTCC GTGATGCAAT AGGCTCTGCA CAACCACTAC ACGCAGAAGA
2341 GCCTCTCCCT GTCTCCGGGT AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG
2401 GAGGTTCCGG TGGCGGGGGA TCAGACAAA CTACACATG CCCACCGTGC CCAGCACCTG
65 2461 AACTCCTGGG AGGACCGTCA GTCTTCTCTT TCCCCCAAA ACCCAAGGAC ACCCTCATGA
2521 TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCTGAGG
2581 TCAAGTTCAA CTGGTACGTG GACGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG
2641 AGGAGCAGTA CAACAGCACG TACCCTGTGG TCAGCGTCTT CACCGTCTG CACCGAGCT
2701 GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG
70 2761 AGAAAACCAT CTCCAAGGCC AAAGGGCAGC CCGGAGAACC ACAGGTGTAC ACCCTGCCCC
2821 CATCCCGGCA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCTGGTGC AAAGGCTTCT
2881 ATCCAGCGCA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
2941 CCAGGCCTCC CGTGTGGAC TCCGACGCT CTTCTCTCT CTACAGCAAG CTCACCGTGG
3001 ACAAGAGCAG GTGGCAGCAG GGAACGCTCT TCTCATGCTC CGTGTATGCAT GAGGCTCTGC
75 3061 ACAACCACTA CACGAGAAG AGCCTCTCCC TGTCTCCGG TAAATGA

FIX-042 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker region connecting the Fc regions is underlined

80 1 MORVNMIMAE SEGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
61 ERECMEEKCS FEEAREVFN TERTTEFWKQ YVDGDQCESN PCLNGGSKCD DINSYECWCP
121 PGFEGKNCEL DVTCTNIKNGR CEQFCKNSAD NKVVCSTEG YRLAENQKSC EPAVFPFCGR

5	181	VSVSQTSKLT	RAETVFPDVD	YVNSTEAEETI	LDNITQSTQS	FNDFTRVVGG	EDAKPGQFPW
	241	QVVLNGKVDA	FCGGSIVNEK	WIVTAAHCVE	TGVKITVVAG	EHNIIEEHT	EQKRNVIIRI
	301	PHHNYNAAIN	KYNHDIALLE	LDEPLVLNSY	VTPICIADE	YTNIFLKFGS	GYVSGWGRVF
	361	HKGRSALVLQ	YLRVPLVDRA	TCLRSTKFTI	YNNMFCAGFH	EGGRDSCQGD	SGGFHVTEVE
10	421	GTSFLTGIIS	WGEECAMKGG	YGIYTKVGRY	VNWIKEKTKL	TDKTHTCFPC	PAPELLGGPS
	481	VFLFPPKPKD	TLMISRTFEV	TCVVVDVSHE	DPEVKFNWYV	DGVEVHNAKT	KPREEQYNST
	541	YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KGQPREPQVY	TLPPSRDEL
	601	KNQVSLTCLV	KGFYPSDIAV	EWESNGQPEN	NYKITPPVLD	SDGSFFLYSK	LTVDKSRWQQ
	661	KNVFSCSVHM	EAHNNHYTQK	SLSLSPGKGG	GGSGGGGGGG	GGSGGGGGEDK	THTCPPCPAP
	721	ELGGGSPVFL	FPPKPKDTLM	ISRTPEVTCV	VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR
15	781	EEQYNSTYRV	VSVLTVLHQD	WLNKKEYKCK	VSNKALPAPI	EKTISKAKGQ	PREPQVYTLF
	841	PRDELTKNQ	VSLTCLVKGK	YPSDIAVEWE	SNGQPEPNYK	TTPPVLDSDG	SFFLYSKLTV
	901	DKSRWQQGNV	FSCSVMHEAL	HNHYTQKSLS	LSPGK*		
DNA sequence for FIX-068							
20	1	ATGCAGCGCG	TGAACATGAT	CATGGCAGAA	TCACCAGGCC	TCATCACCAT	CTGCCCTTTTA
	61	GGATATCTAC	TCAGTGCTGA	ATGTACAGGT	TTGTTTCCTT	TTTTAAATA	CATTGAGTAT
	121	GCTTGCCTTT	TAGATATAGA	AATATCTGAT	GCTGTCTTCT	TCATAAAT	TTGATTACAT
	181	GATTTGACAG	CAATATTGAA	GAGTCTAACA	GCCAGCACGC	AGGTTGGTAA	GTTACTGTGGG
25	241	AACATCACAG	ATTTTGGCTC	CATGCCCTAA	AGAGAAATTG	GCTTTCAGAT	TATTTGGATT
	301	AAAAACAAAG	ACTTTCCTTAA	GAGATGTAAA	ATTTTCATGA	TGTTTTCTTT	TTTGCTAAAA
	361	CTAAAGAAAT	ATTCTTTTAC	ATTTTCAGTTT	TTCTTGATCA	TGAAAACGCC	AACAAAATTC
	421	TGAATCGGCC	AAAGAGGTAT	AATTCAGGTA	AATTGGAAGA	GTTTGTTCAT	GGGAATCTAG
	481	AGAGAGAAAT	TATGGAAGAA	AAGTGTAGTT	TTGAAGAAGC	ACGAGAAGTT	TTTGAAGAAC
30	541	CTGAAAGAAC	AACTGAATTT	TGGAAGCAGT	ATGTTGATGG	AGATCAGTGT	GAGTCCAATC
	601	CATGTTTAAA	TGGCGGCTAG	TGCAAGGATG	ACATTAATTC	CTATGAATGT	TGTTGTCCCT
	661	TTGGATTTGA	AGGAAAGAAC	TGTGAATTAG	ATGTAACATG	TAACATTAAG	AATGGCAGAT
	721	GCGAGCAGTT	TTGTAAAAAT	AGTGTCTGTA	ACAAGGTGGT	TTGCTCCTGT	ACTGAGGGAT
	781	ATCGACTTGC	AGAAAACCCAG	AAGTCCCTGTG	AACCAGCAGT	GCCATTTCCT	TGTGGAAGAG
35	841	TTTCTGTTTC	ACAAACTTCT	AAGCTCACCC	GTGCTGAGAC	TGTTTTTCCT	GATGTGGACT
	901	ATGTAAATTC	TACTGAAGCT	GAAGCCATTT	TGGATAACAT	CACTCAAAGC	ACCCAATCAT
	961	TTAATGACTT	CACTCGGTTT	GTTGGTGGAG	AAGATGCCAA	ACCAGGTCAA	TTCCCTTGGC
	1021	AGGTTGTTTT	GAATGGTAAA	GTTGATGCAT	TCTGTGGAGG	CTCTATCGTT	AATGAAAAAT
40	1081	GGATTGTAA	TGCTGCCAC	TGTGTTGAAA	CTGGTGTAA	AATTACAGTT	GTCCGAGGTG
	1141	AACATAATAT	TGAGGAGACA	GAACATACAG	AGCAAAAGCG	AAATGTGATT	CGAATTATTC
	1201	CTCACCACAA	CTACAATGCA	AGTATTAATA	AGTACAACCA	TGACATTGCC	CTTCTGGAAC
	1261	TGGACGAACC	CTTAGTGCTA	AACAGCTACG	TTACACCTAT	TTGCATTGCT	GACAAGGAAT
	1321	ACACGAACAT	CTTCTCCTAA	TTTGGATCTG	GCTATGTAA	TGGCTGGGGA	AGAGTCTTCC
	1381	ACAAAGGGAG	ATCAGCTTTA	GTTCTTCAGT	ACCTTAGAGT	TCCACTGTGT	GACCGGCCCA
45	1441	CATGTCTTCG	ATCTACAAAG	TTCAACCATCT	ATAACAACAT	GTTCTGTGCT	GGCTTCCATG
	1501	AAGGAGGTAG	AGATTTCATGT	CAAGGAGATA	GTGGGGGACC	CCATGTTACT	GAAGTGGAA
	1561	GGACCAAGTT	CTTAAGTGA	ATTATTAGCT	GGGGTGAAGA	GTGTGCAATG	AAAGGCAAT
	1621	ATGCAATATA	TACCAAGGTG	TCCCGGTATG	TCAACTGGAT	TAAGGAAAAA	ACAAAGCTCA
	1681	CTGACAAAAC	TCACACATGC	CCACCGTGCC	CAGTCCCGGA	ACTCCTGGGC	GGACCGCTCAG
50	1741	TCTTCTCTTT	CCCCCAAAAA	CCCAAGGACA	CCCTCATGAT	CTCCCGGACC	CCTGAGGTCA
	1801	CATGCGTGGT	GGTGGACGTG	AGCCACGAAG	ACCCTGAGGT	CAAGTTCAAC	TGGTACGTGG
	1861	ACGGCGTGGA	GGTGCATAAT	GCCAAGACAA	AGCCGCGGGA	GGAGCAGTAC	AACAGCACGT
	1921	ACCGTGTGGT	CAGCGTCTTC	ACCGTCTCTG	ACCAGGACTG	GCTGAATGGC	AAGGAGTACA
	1981	ATGTCACAGG	CTCCAACAAA	GCCCTCCAG	CCCCATCGA	GAAAACCATC	TCCAAGGCCA
55	2041	AAGGGCAGCC	CCGAGAACCA	CAGGTGTACA	CCCTGCCCCC	ATCCCGGGAT	GAGCTGACCA
	2101	AGAACCAGGT	CAGCCTGACC	TGCCTGGTCA	AAGGCTTCTA	TCCCAGCGAC	ATCCCGGTGG
	2161	AGTGGGAGAG	CAATGGGCGC	CCGGAGAACA	ACTACAAGAC	CACGCCCTCC	GTGTTGGACT
	2221	CCGACGGCTC	CTTCTTCTCT	TACAGCAAGC	TCACCGTCTG	CAAGAGCAGG	TGGCAGCAGG
	2281	GGAACGTCTT	CTCATGCTCC	GTGATGCATG	AGGCTCTGCA	CAACCACTAC	ACGCAGAAGA
60	2341	GCCTCTCCCT	GTCTCCGGGT	AAACGGCGCC	GCCGGAGCGG	TGGCGGCGGA	TCAGGTGGGG
	2401	GTGGATCAGG	CGGTGGAGGT	TCCGGTGGCG	GGGGATCCGG	CGGTGGAGGT	TCCGGTGGGG
	2461	GTGGATCAAG	GAAGAGGAGG	AAGAGGGCGC	AGGTGCAGCT	GCAGGAGTCT	GGGGGAGGCT
	2521	TGGTACAGCC	TGGGGGGTCC	CTGAGACTCT	CCTGTGCAGC	CTCTGGATTC	ATGTTTAGCA
	2581	GGTATGCCAT	GAGCTGGGTC	CGCCAGGCTC	CAGGGAAGGG	GCCAGAGTGG	GTCTCAGGTA
65	2641	TTAGTGGTAG	TGGTGGTAGT	ACATACTACG	CAGACTCCGT	GAAGGGCCGG	TTACCCGTCT
	2701	CCAGAGACAA	TTCCAAGAAC	ACGCTGTATC	TGCAAAATGAA	CAGCCTGAGA	GCCGAGGACA
	2761	CGGCTGTATA	TTACTGCGCC	CGGGGCGCCA	CCTACACCAG	CCGGAGCGAC	GTGCCCCGAC
	2821	AGACCAGCTT	CGACTACTGG	GGCCAGGGAA	CCCTGGTCA	CGTCTCTCTA	GGGAGTGCA
	2881	CCGCCCAAAA	GCTTGAAGAA	GCTGAATTTT	CAGAAGCACG	CGTATCTGAA	CTGACTCAGG
	2941	ACCCTGCTGT	GTCTGTGGCC	TTGGGACAGA	CAGTCAGGAT	CACATGCCAA	GGAGACAGCC
70	3001	TCAGAAACTT	TTATGCAAGC	TGGTACCAGC	AGAAGCCAGG	ACAGGCCCTT	ACTCTTGCTA
	3061	TCTATGGTTT	AAGTAAAAGG	CCCTCAGGGA	TCCCAGACCG	ATTCTCTGCC	TCCAGCTCAG
	3121	GAAACACAGC	TTCTTGAACC	ATCACTGGGG	CTCAGGCGGA	AGATGAGGCT	GACTATTACT
	3181	GCCTGCTGTA	CTACGGCGGC	GGCCAGCAGG	GCGTGTTCGG	CGGCGGCACC	AAGCTGACCG
75	3241	CTCTACGTCA	GCCCAAGGCT	GCCCTCTGG	TCACTCTGTT	CCCGCCCTCT	TCTGCGGCGG
	3301	GTGGCGGTGG	CTCCGGCGGA	GGTGGGTCCG	GTGGCGGCGG	ATCAGGTGGG	GGTGGATCAG
	3361	GCGGTGGAGG	TTCCGGTGGC	GGGGGATCAG	ACAAAACCTCA	CACATGCCCA	CCGTGCCCGC
	3421	CCCGGGAACF	CCTGGGCGGA	CCGTCACTCT	TCCTCTTCCC	CCCAAAACCC	AAGGACACCC
	3481	TCATGATCTC	CCGGACCCCT	GAGGTCAACAT	GCGTGGTGGT	GGACGTGAGC	CACGAAGACC
	3541	CTGAGGTCAA	GTTTCAACTGG	TACGTGGACG	GCGTGGAGGT	GCATAATGCC	AAGACAAAGC
80	3601	CGCGGGAGGA	GCAGTACAAC	AGCACGTACC	GTGTGGTCA	CGTCCCTCAC	GTCCTGCACC
	3661	AGGACTGGCT	GAATGGCAAG	GAGTACAAGT	GCAAGGTCTC	CAACAAAGCC	CTCCAGCCCC

5 3721 CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC
 3781 TGCCCCCATC CCGCGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
 3841 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAAC
 3901 ACAAGACCAC GCCTCCCGTG TTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA
 3961 CCGTGGACAA GAGCAGGTGG CAGCAGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG
 10 4021 CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA TGA

FIX-068 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting SCB5 to Pc region is underlined, and linker with proprotein convertase processing sites is shown in bold

15 1 MQRVNMIMAE SPGLITICLL GYLLSAECTY FLDHENANKI LNRPKRYNSG KLEEFVQGNL
 61 ERECEMEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
 121 PGFEGKNCEL DVTCTNKNR CEQFCNSAD NKVVCSTEG YRLAENQKSC EPAVPFPCGR
 181 VSVSQTSLKT RAETVFEDVD YVNSTEAEIT LDNITQSTQS FNDFTRVVGG EDAPKQGFPPW
 20 241 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVRIRI
 301 PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICADKE YTNIFLKFGS GVVSGWGRVF
 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKPTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 421 GTSFLTGIIS WGEECAMKKG YGIYTKVSRY VNWIKETKRL TDKTHTCPPC PAPELLGGPS
 481 VFLFPPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELTK
 25 601 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLVD SDGSFFLYSK LTVDKSRWQQ
 661 GNVFSCSVMH EALHNHYTQK SLSLSPGKRR **RRSGGGGSGG** **GGSGGGGSGG** **GGSGGGGSGG**
 721 **GGSRKRRKRA** QVQLQESGGG LVQPGGSLRL SCAASGFMFS RYAMSWVRQA PGKGPPEWVSG
 781 ISGSGGSTYY ADSVKGRFTV SRDNSKNTLY LQMNSLRAED TAVYYCARGA TYTSRSDVPD
 841 QTSFDYWGQG TLVTVSSGSA SAPKLEEGER SEARVSELTQ DPAVSVAGLG TVRITCQGLS
 30 901 LRNFYASWYQ QKPGQAPTIV IYGLSKRPSG IEDRFSSASS GNTASLTITG AGAEDEADYV
 961 CLLYYGGGQQ GVFGGGTKLT VLRQPKAAPS VTLPFPSSAA GGGGSGGGGS GGGGSGGGGS
 1021 GGGGSGGGGS DKHTTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
 1081 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 35 1141 PIEKTISKAK GQPREPQVYT LPSPSRDELTK NQVSLTCLVK GFYPSDIAVE WEENGSQPENN
 1201 YKTPPVLDLS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*

DNA sequence for FIX-068

40 1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTTAA
 61 GGATATCTAC TCAGTGCCTGA ATGTACAGGT TTGTTTCCTT TTTTAAATA CATTGAGTAT
 121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT
 181 GATTTGACAG CAATATTGAA GAGTCTAACA GCCAGCACGC AGGTTGGTAA GTACTGTGGG
 241 AATCATACAG ATTTTGGCTC CATGCCCTAA AGAGAAATTG GCTTTTCAGT TAATTTGGATT
 301 AAAAACAAAG ACTTTCTTAA GAGATGTAAA ATTTTCATGA TGTTTTCTTT TTTGCTAAAA
 361 CTAAAGAAAT ATTCTTTTAC ATTTTCAGTT TTCTTGATCA TGAAAACGCC AACAAAATTC
 421 TGAATCGGCC AAAGAGGTAT AATTTCAGTA AATTGGAAGA GTTTGTTCAT GGAATCTAG
 481 AGAGAGAAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
 541 CTGAAAGAAC AACTGAATTT TGGAAGCAGT ATGTTGATGG AGATCAGTGT GAGTCCAATC
 601 CATGTTTAAA TGGCGGCAGT TGCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
 661 TTGGATTTGA AGGAAAGAAC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT
 721 GCGAGCAGTT TTGTAAAAAT AGTGCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT
 781 ATCGACTTGC AGAAAAACCA AAGTCCGTGT AACCAGCAGT GCCATTTCCT TGTGGAAGAG
 841 TTTCTGTTTC ACAACTTCT AAGCTCACCC GTGCTGAGAC TGTTTTCTCT GATGTGGACT
 901 ATGTAAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
 961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGTCAA TTCCCTTGCC
 1021 AGGTTGTTTT GAATGGTAAA GTTGATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
 1081 GGATTGTAAAC TGCTGCCACAC TGTGTTGAAA CTGGTGTAAA AATTACAGTT GTCGAGGTG
 1141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAGCG AAATGTGATT CGAATTATTC
 1201 CTCACCAAAA CTACAATGCA GCTATTAAATA AGTACAAACA TGACATTGCC CTCTGGAAC
 60 1261 TGGACGAACC CTTAGTGCTA AACAGCTACG TTACACCTAT TTGCAATTGT GACAAGAAAT
 1321 ACACGAACAT CTTCCFCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC
 1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
 1441 CATGTCTTCG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
 1501 AAGGAGGTAG AGATTCAATG CAAGAGGATA GTGGGGGACC CCATGTTACT GAAGTGGAAAG
 65 1561 GGACCAAGTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
 1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAGGCTCA
 1681 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG
 1741 TCTTCTCTCT CCCCCAAAAA CCAAGGACA CCCTCATGAT CTCCCGGACC CTTGAGGTCA
 1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
 70 1861 ACGGCGTGGG GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCAGT
 1921 ACCGTGTGGT CAGCGTCTTC ACCGTCTGTC ACCAGGACTG GCTGAATGGC AAGGAGTACA
 1981 AGTGCAAGGT CTCCAACAAA GCCCTCCAG CCCCACATCGA GAAAACCATC TCCAAAGCCA
 2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCTGCCCCC ATCCCGGGAT GAGCTGACCA
 2101 AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCAGCGAC ATCGCCGTGG
 75 2161 AGTGGGAGAG CAATGGGCAG CCGGAGAAC ACTACAAGAC CACGCCTCCC GTGTTGGACT
 2221 CCGACGGCTC CTTCTTCTCT TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
 2281 GGAACGTCTT CTCATGCTCC GTGATGCTAG AGGCTCTGCA CAACCACTAC ACCGAGAAGA
 2341 GCCTCTCCCT GTCTCCGGGT AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG
 2401 GAGGTTCCGG TGGCGGGGGA TCAGACAAA CTCACACATG CCCACCGTGC CCAGCACCTG
 80 2461 AACTCCTGGG AGGACCGTCA CTCTTCTCT TCCCCCAA ACCCAAGGAC ACCCTCATGA
 3521 TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCTGAGG

5	2581	TCAAGTTCAA	CTGGTACGTG	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG
	2641	AGGAGCAGTA	CAACAGCACG	TACCGTGTGG	TCAGCGTCCT	CACCGTCCTG	CACCAAGGACT
	2701	GGCTGAATGG	CAAGGAGTAC	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA	GCCCCCATCG
	2761	AGAAAACCAT	CTCCAAAGCC	AAAGGGCAGC	CCCAGAAACC	ACAGGTGTAC	ACCTTGCCCC
10	2821	CATCCCGCGA	TGAGCTGACC	AAGAACCAGG	TCAGCCTGAC	CTGCCTGGTC	AAAGGCTTCT
	2881	ATCCAGCGGA	CATCGCCGTG	GAGTGGGAGA	GCAATGGGCA	GCCGGAGAAC	AACTACAAGA
	2941	CCACGCCTCC	CGTGTGGGAC	TCCGACGGCT	CCTTCTTCCT	CTACAGCAAG	CTCACCGTCG
	3001	ACAAGAGCAG	GTGGCAGCAG	GGGAACGTCT	TCTCATGTCT	CGTGTATGAT	GAGGCTCTGC
	3061	ACAACCACTA	CACGCAGAAG	AGCCTCTCCC	TGTCTCCGGG	TAAAGGTGGC	GGTGGCTCCG
	3121	GCGGAGGTGG	GTCCGGTGGC	GCGGATCAG	GTGGGGGTGG	ATCAGGCGGT	GGAGGTTCGG
15	3181	GTGGCGGGGG	ATCAGCGCAG	GTGCAGCTGC	AGGAGTCTGG	GGGAGGCTTG	GTACAGCCTG
	3241	GGGGGTCCCT	GAGACTCTCC	TGTGCAGCCT	CTGGATTCTAT	GTTTAGCAGG	TATGCCATGA
	3301	GCTGGGTCCG	CCAGGCTCCA	GGGAAGGGGC	CAGAGTGGGT	CTCAGGTATT	AGTGGTAGTG
	3361	GTGGTAGTAC	ATACTACGCA	GACTCCGTGA	AGGCGCGGTT	CACCGTCTCC	AGAGACAATT
	3421	CCAAGAACAC	GCTGTATCTG	CAAATGAACA	GCCTGAGAGC	CGAGGACACG	GCTGTATATT
20	3481	ACTGGCGCCG	GGGCGCCACC	TACACCAACC	GGAGCGACGT	GCCCGACACG	ACCGAGCTTCG
	3541	ACTACTGGGG	CCAGGGAACC	CTGGTCACCG	TCTCTCAGG	GAGTGCATCC	GCCCCAAAGC
	3601	TTGAAGAAGG	TGAATTTTCA	GAAGCACGCG	TATCTGAAC	GACTCAGGAC	CCTGCTGTGT
	3661	CTGTGGCCTT	GGGACAGACA	GTCAGGATCA	CATGCCAAGG	AGACAGCCTC	AGAAACTTTT
	3721	ATGCAAGCTG	GTACCAGCAG	AAGCCAGGAC	AGGCCCTTAC	TCTTGTCTAT	TATGGTTTAA
25	3781	GTAAAGAGCC	CTCAGGGATC	CCAGACCGAT	TCTCTGCCTC	CAGCTCAGGA	AAACACAGCT
	3841	CCTTGACCAT	CACTGGGGCT	CAGGCGGAAG	ATGAGGCTGA	CTATTACTGC	CTGCTGTACT
	3901	ACGGCGGGCG	CCAGCAGGGC	GTGTTCCGGC	GCGGCACCAA	GCTGACCGTC	CTACGTCAGC
	3961	CCAAGGCTGC	CCCCTCGGTC	ACTCTGTTC	CGCCTCTTTC	TGCGGCCTGA	
30	FIX-088 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker connecting both Fc regions is underlined and linker connecting the Fc region to SCE5 is in bold						
	1	<u>MQRVNMIMAE</u>	<u>SPGLITICLL</u>	<u>GYLLSAECTV</u>	<u>FLDHENANKI</u>	<u>LNRPKRYNSG</u>	<u>KLEEFVQGNL</u>
35	61	ERECMEEEKCS	FEEAREVFEN	TERTFEFWKQ	YVDGDQCESN	PCLNGGSCKD	DINSYECWCP
	121	FGFEGKNCCL	DVTCNINKGR	CEQFCNHSAD	NKVVCSCTEG	YRLAENQKSC	EPAPVPFCGR
	181	VGSFNGSKLT	RAETVFDPDV	YVNSTEAETI	LDNITQSTQS	FNDFTRVVG	EDAKPGQFPW
	241	QVVLNGKVDA	FCGGSIIVNEK	WIVTAHCVE	TGVKITVVAG	EHNIETETHT	EQKRNVIIRI
	301	PHHNYNAAIN	KYNHDIALLE	LDEPLVLNSY	VTPICIADKE	YTNIFLKFGS	GVVSGWGRVP
40	361	HKGRSALVLQ	YLRVPLVDRA	TCLRSTKPTI	YNNMFCAGFH	EGGRDSCQGD	SGGPHVTEVE
	421	PSRFLTGIIIS	WGEBCAMKGG	YGIYTKVSRY	VNWIKEKTKL	TDKHTCPPC	PAPELLGGPS
	481	VFLFPPPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV	DGVEVHNAKT	KPREEQYNST
	541	YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KGQPRPQVY	TLPPSRDEL
	601	KNQVSLTCLV	KGFYPSDIAV	EWESNGQPEN	NYKTTPFVLD	SDGSFELYSK	LTVDKSRWQQ
45	661	GNVPSCSVMH	EALHNHYTQK	<u>SLSLSPGRGG</u>	<u>GGSGGGGGGG</u>	<u>GGSGGGGGSDK</u>	<u>THTCPPCPAP</u>
	721	ELLGGPSVPL	FPPPKKDTLM	ISRTPEVTCV	VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR
	781	EEQYNSTYRV	VSVLTVLHQD	WLNGKEYKCK	VSNKALPAPI	EKTISKAKGQ	PREPQVYTL
	841	PSRDELTKNQ	VSLTCLVKG	YPSDIAVEWE	SNGQPENNYK	TPPPVLDSDG	SFELYSKLTV
	901	DKSRWQQGNV	FSCSVMEHAL	HNHYTQKSL	<u>LSLPGKGGGG</u>	<u>GGGGSGGGGS</u>	<u>GGGGSGGGGS</u>
50	961	<u>GGGGS</u> AQVQL	<u>QESGG</u> LVQP	<u>GGSLRL</u> SCAA	<u>SGFMFS</u> RYAM	<u>SWVRQAP</u> PGK	<u>PEWVSG</u> ISGS
	1021	GGSTYYADSV	KGRFTVSRDN	SKNTLYLQMN	SLRAEDTAVY	YCARGATYTS	RSDVDPQTSF
	1081	DYWGQGTLLV	VSSGSASAPK	LEEGEFSEAR	VSELTQDPAV	SVALGQTVRI	TCQGSSLRNF
	1141	YASWYQQKPK	QAPTLV IYGL	SKRPSGI PDR	FSASSSGNTA	SLTITGAQAE	DEADYYCLLY
	1201	YGGGQQGVFG	GGTKLTVLRQ	PKAAPSVTLF	PPSSAA*		
55	DNA sequence for FIX-088						
	1	ATGCAGCGCG	TGAACATGAT	CATGGCAGAA	TCACCAGGCC	TCATCACCAT	CTGCCTTTTAA
	61	GGATATCTAC	TCAGTGTCTGA	ATGTACAGGT	TTGTTTCCTT	TTTTAAATAA	CATTGAGTAT
	121	GCTTGCCTTT	TAGATATAGA	AATATCTGAT	GCTGTCTTCT	TCATAAATT	TTGATTACAT
60	181	GATTTGACAG	CAATATTGAA	GAGTCTAACA	GCCAGCACGC	AGGTTGGTAA	GTACTGTGGG
	241	AACATCACAG	ATTTTGGCTC	CATGCCCTAA	AGAGAAATTG	GCTTTCAGAT	TATTTGGATT
	301	AAAAACAAAG	ACTTTCTTAA	GAGATGTAAA	ATTTTCATGA	TGTTTTCTTT	TTTGCTAAAA
	361	CTAAAGAATT	ATTCTTTTAC	ATTTTCAGTT	TTCTTGATCA	TGAAAACGCC	AACAAAATTTC
	421	TGAATCGGCC	AAAGAGGTAT	AATTTCAGTA	AATTGGAAGA	GTTTGTTCAT	GGGAATCTAG
65	481	AGAGAGAATG	TATGGAAGAA	AAGTGTAGTT	TTGGAAGAAGC	ACGAGAAGTT	TTTGAAAAACA
	541	CTGAAAGAAC	AACTGAATTT	TGGAAGCAGT	ATGTTGATGG	AGATCAGTGT	GAGTCCAATC
	601	CATGTTTAAA	TGGCGGCAGT	TGCAAGGATG	ACATTAATTC	CTATGAATGT	TGGTGTCCCT
	661	TTGGATTTGA	AGGAAAGAAC	TGTGAATTAG	ATGTAACATG	TAACATTAAG	AATGGCAGAT
	721	GCGAGCAGTT	TTGTAAAAAT	AGTGCTGATA	ACAAGGTGGT	TTGCTCCTGT	ACTGAGGGAT
70	781	ATCGACTTGC	AGAAAACACG	AAGTCCTGTG	AACCAGCAGT	GCCATTTCCT	TGTGGAAGAG
	841	TTTCTGTTTC	ACAAACTTCT	AAGCTCACCC	GTGCTGAGAC	TGTTTTTCTC	GATGTGGACT
	901	ATGTAAATTC	TACTGAAGCT	GAAACCATTT	TGGATAACAT	CACCTCAAAGC	ACCCAATCAT
	961	TTAATGACTT	CACCTCGGTT	GTTGGTGGAG	AAGATGCCAA	ACCAGGTCAA	TTCCCTTGCG
75	1021	AGTTTGTTTT	GAATGGTAAA	GTTGATGCAT	TCTGTGGAGG	CTCTATCGTT	AATGAAAAAT
	1081	GGATTGTAA	TGCTGCCCAT	TGTGTTGAAA	CTGGTGTAAA	AATTACAGTT	GTCCGAGGTG
	1141	AACATAATAT	TGAGGAGACA	GAACATACAG	AGCAAAAGCG	AAATGTGATT	CGAATTATTTC
	1201	CTCACCACAA	CTACAATGCA	GCTATTATAA	AGTACAACCA	TGACATTGCC	CTTCTGGAAC
	1261	TGGACGAACC	CTTAGTGCTA	AACAGCTACG	TTACACCTAT	TTGCATTGCT	GACAAGGAAT
	1321	ACACGAACAT	CTTCTTCAAA	TTTGGATCTG	GCTATGTAA	TGGCTGGGGA	AGAGTCTTCC
	1381	ACAAAGGGAG	ATCAGCTTTA	GTTCTTCAGT	ACCTTAGAGT	TCCACTTGTT	GACCGAGCCA
80	1441	CATGTCTTCG	ATCTACAAAG	TTCAACATCT	ATAACAACAT	GTTCTGTGCT	GGCTTCCATG
	1501	AAGGAGGTAG	AGATTTCATG	CAAGGAGATA	GTGGGGGACC	CCATGTTACT	GAGGTGGAAG

5	1561	GGACCAGTTT	CTTAACCTGGA	ATTATTAGCT	GGGGTGAAGA	GTGTGCAATG	AAAGGCCAAAT
	1621	ATGGAATATA	TACCAAGGTG	TCCCGGTATG	TCAACTGGAT	TAAGGAAAAA	ACAAAGCTCA
	1681	CTGACAAAAC	TCACACATGC	CCACCGTGCC	CAGCTCCGGA	ACTCCTGGGA	GGACCGTCAG
	1741	TCCTCCTCTT	CCCCCAAAA	CCCAAGGACA	CCCTCATGAT	CTCCCGGACC	CCTGAGGTCA
10	1801	CATGCGTGGT	GGTGGACGTG	AGCCACGAAG	ACCCTGAGGT	CAAGTTCAAC	TGGTACGTGG
	1861	ACGGCGTGGA	GGTGCATAAT	GCCAAGACAA	AGCCGCGGGA	GGAGCAGTAC	AACAGCACGT
	1921	ACCGTGTGGT	CAGCGTCCTC	ACCGTCCCTG	ACCAGGACTG	GCTGAATGGC	AAGGAGTACA
	1981	AGTGCAAGGT	CTCCAACAAA	GCCCTCCCAG	CCCCCATCGA	GAAAACCATC	TCCAAAGCCA
	2041	AAGGGCAGOC	CCGAGAACCA	CAGGTGTACA	CCCTGCCCCC	ATCCCGGGAT	GAGCTGACCA
	2101	AGAACCAAGT	CAGCCTGACC	TGCCTGGTCA	AAGGCTTCTA	TCCCAGCGAC	ATCGCCGTGG
15	2161	AGTGGGAGAG	CAATGGGCAG	CCGGAGAACA	ACTACAAGAC	CACGCCCTCC	GTGTTGGACT
	2221	CCGACGGCTC	CTTCTTCTCT	TACAGCAAGC	TCACCGTCGA	CAAGAGCAGG	TGGCAGCAGG
	2281	GGAACGTCTT	CTCATGCTCC	GTGATGCTAT	AGGCTCTGCA	CAACCACTAC	ACGCAGAAGA
	2341	GCCTCTCCCT	GTCTCCGGGT	AAAGGCGGTG	GCGGTTCAAG	TGGAGGAGGG	TACGGCGGTG
	2401	GTGGATCCGG	CGGGGGCGGA	TCCGGTGGCG	GAGGCTCAGG	CGGTGGCGGA	TCAGCCCTGCA
20	2461	CCGAGCGGAT	GGCCCTGCAC	AACCTGTGCG	GTGGCGGTGG	CTCCGGCGGA	GGTGGGTCCG
	2521	GTGGCGGCGG	ATCAGGTGGG	GGTGGATCAG	GCGGTGGAGG	TTCCGGTGGC	GGGGGATCCG
	2581	ACAAAACCTCA	CACATGCCCA	CCGTGCCCAG	CACCGGAAC	CCTGGGCGGA	CCGTCACTCT
	2641	TCCTCTTCCC	CCCAAAACCC	AAGGACACCC	TCATGATCTC	CCGGACCCCT	GAGGTACAT
25	2701	GCGTGGTGGT	GGACGTGAGC	CACGAAGACC	CTGAGGTCAA	GTTCAACTGG	TACGTGGACG
	2761	GCGTGGAGGT	GCATAATGCC	AAGACAAAGC	CGCGGGAGGA	GCAGTACAAC	AGCACGTACC
	2821	GTGTGGTTCAG	CGTCTTCACC	GTCTGTCACC	AGGACTGGCT	GAATGGCAAG	GAGTACAAGT
	2881	GCAAGGTCTC	CAACAAAGCC	CTCCCAGCCC	CCATCGAGAA	AACCATCTCC	AAAGCCAAAG
	2941	GGCAGCCCCG	AGAACCACAG	GTGTACACCC	TGCCCCCATC	CCGGGATGAG	CTGACCAAGA
30	3001	ACCAGGTTCAG	CCTGACCTGC	CTGGTCAAAG	GCTTCTATCC	CAGCGACATC	GCCGTGGAGT
	3061	GGVAGAGCAA	TGGGCGAGCC	GAGAACAAC	ACAAGACCAC	GCTCCCGTGG	TTGGGCTCCG
	3121	ACGGCTCCTT	CTTCTCTTAC	AGCAAGCTCA	CCGTGGACAA	GAGCAGGTGG	CAGCAGGGGA
	3181	ACGTCTTCTC	ATGCTCCGTC	ATGCATGAGG	CTCTGCACAA	CCACTACACG	CAGAAGAGCC
	3241	TCTCCCTGTC	TCCGGGTAAA	TGA			
35	FIX-089 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker regions connecting OS1 to Fc regions are underlined, and OS1 peptide is italicized						
	1	<u><u>MQRVNMIMAE</u></u>	<u><u>SPGLITICLL</u></u>	<u><u>GILLSAECTV</u></u>	<u><u>FLDHENANKI</u></u>	<u><u>LNRPKRYNSG</u></u>	<u><u>KLEEFVQGNL</u></u>
40	61	<u><u>ERECEMEKCS</u></u>	<u><u>FEEAREVFEN</u></u>	<u><u>TERITTEFWKQ</u></u>	<u><u>YVDGQDCESN</u></u>	<u><u>PCLNGGSCKD</u></u>	<u><u>DINSYECWCP</u></u>
	121	<u><u>FGFEGKNCEL</u></u>	<u><u>DVTCTNKNGR</u></u>	<u><u>CEQFCNNSAD</u></u>	<u><u>NKVVCSCTEG</u></u>	<u><u>YRLAENQKSC</u></u>	<u><u>EPAPVFPQCR</u></u>
	181	<u><u>VSVSQTSKLT</u></u>	<u><u>RAETVFPDVD</u></u>	<u><u>YVNSTEAEIT</u></u>	<u><u>LDNITQSTQS</u></u>	<u><u>FNDFTRVVG</u></u>	<u><u>EDAKPGQFPW</u></u>
	241	<u><u>QVVLNGKVDA</u></u>	<u><u>FCGGSIVNEK</u></u>	<u><u>WIVTAACHVE</u></u>	<u><u>TGVKITVVAG</u></u>	<u><u>EHNIEEETHT</u></u>	<u><u>EQKRNVIIRI</u></u>
	301	<u><u>PHHNYNAAIN</u></u>	<u><u>KYNHDIALLE</u></u>	<u><u>LDEPLVLNSY</u></u>	<u><u>VTPICIAADKE</u></u>	<u><u>YTNIFLKFSG</u></u>	<u><u>GVVSGWGRVF</u></u>
45	361	<u><u>HKGRSALVLQ</u></u>	<u><u>YLRVPLVDRA</u></u>	<u><u>TCLRSTKPTI</u></u>	<u><u>YNNMFCAGFH</u></u>	<u><u>EGGRDSCQGD</u></u>	<u><u>SGGPHVTEVE</u></u>
	421	<u><u>GTSFLTGIIS</u></u>	<u><u>WGEBCAMKKG</u></u>	<u><u>YGIYTKVSRY</u></u>	<u><u>VNWIKEKTRL</u></u>	<u><u>TDKHTTCCPP</u></u>	<u><u>PAPELLGGPS</u></u>
	481	<u><u>YFLFPPKPKD</u></u>	<u><u>TLMISRTPEV</u></u>	<u><u>TCVVVDVSHE</u></u>	<u><u>DPEVKFNWYV</u></u>	<u><u>DGVEVHNAKT</u></u>	<u><u>KPREEQYNST</u></u>
	541	<u><u>YRVVSVLTVL</u></u>	<u><u>HQDWLNGKEY</u></u>	<u><u>KCKVSNKALP</u></u>	<u><u>APIEKTISKA</u></u>	<u><u>KQPREPQVY</u></u>	<u><u>TLPPSRDEL</u></u>
	601	<u><u>KNQVSLTCLV</u></u>	<u><u>KGFYPSDIAV</u></u>	<u><u>EWESNGQPEN</u></u>	<u><u>NYKTTPFVLD</u></u>	<u><u>SDGSFFLYSK</u></u>	<u><u>LTVDKSRWQQ</u></u>
50	661	<u><u>GNVFSCSVMH</u></u>	<u><u>EALHNHYTQK</u></u>	<u><u>LSLSLSPGKGG</u></u>	<u><u>GGSGGGGSGG</u></u>	<u><u>GGSGGGGSGG</u></u>	<u><u>GGSGGGGSGG</u></u>
	721	<u><u>TERMALHNL</u></u>	<u><u>GGGGSGGGGS</u></u>	<u><u>GGGGSGGGGS</u></u>	<u><u>GGGGSGGGGS</u></u>	<u><u>DKHTTCCPP</u></u>	<u><u>APELLGGPSV</u></u>
	781	<u><u>FLFPPKPKDT</u></u>	<u><u>LMISRTPEVT</u></u>	<u><u>CVVVDVSHED</u></u>	<u><u>PEVKFNWYVD</u></u>	<u><u>GVEVHNAKTK</u></u>	<u><u>PREEQYNSTY</u></u>
	841	<u><u>RVVSVLTVLH</u></u>	<u><u>QDWLNGKEYK</u></u>	<u><u>CKVSNKALPA</u></u>	<u><u>PIEKTISKAK</u></u>	<u><u>GQPREPQVYT</u></u>	<u><u>LPFSRDELTK</u></u>
	901	<u><u>NQVSLTCLVK</u></u>	<u><u>GFYPGDIAVE</u></u>	<u><u>WESNGQPENN</u></u>	<u><u>YKTTFPVLDS</u></u>	<u><u>DGSFFLYSKL</u></u>	<u><u>TVDKSRWQQG</u></u>
55	961	<u><u>NVFSCSVMHE</u></u>	<u><u>ALHNHYTQKS</u></u>	<u><u>LSLSLSPGK*</u></u>			
	DNA sequence for FIX-090						
	1	ATGCAGCGCG	TGAACATGAT	CATGGCAGAA	TCACCAGGCC	TCATCACCAT	CTGCCCTTTTA
	61	GGATATCTAC	TCAGTGCTGA	ATGTACAGGT	TTGTTTCCTT	TTTTAAATA	CAITGAGTAT
60	121	GCTTGCCTTT	TAGATATAGA	AATATCTGAT	GCTGTCTTCT	TCATAAAT	TTGATTACAT
	181	GATTTGACAG	CAATATTGAA	GAGTCTAACA	GCCAGCACGC	AGGTTGGTAA	GTACTGTGGG
	241	AACATCACAG	ATTTTGGCTC	CATGCCCTAA	AGAGAAATTG	GCTTTCAGAT	TATTTGGATT
	301	AAAAACAAAG	ACTTTCTTAA	GAGATGTAAA	ATTTTCATGA	TGTTTTCTTT	TTTGCTAAAA
	361	CTAAAGAATT	ATTCTTTTAC	ATTTCAAGTT	TTCTTGATCA	TGAAAACGCC	AACAAAATTC
65	421	TGAATCGGCC	AAAGAGGTAT	AATTCAGGTA	AATTGGAAGA	GTTTGTTCAT	GGGAATCTAG
	481	AGAGAGAATG	TATGGAAGAA	AAGTGTAGTT	TTGAAGAAGC	ACGAGAAGTT	TTTGAAAACA
	541	CTGAAAGAAC	AACTGAATTT	TGGAAGCAGT	ATGTTGATGG	AGATCAGTGT	GAGTCCAATC
	601	CATGTTTTAA	TGGCGGCAGT	TGCAAGGATG	ACATTAATTC	CTATGAATGT	TGGTGTCCCT
	661	TTGGATTTGA	AGGAAAGAAC	TGTGAATTAG	ATGTAACATG	TAACATTAAG	AATGGCAGAT
	721	GCGAGCAGTT	TTGTAAAAAT	ACTGCTGATA	ACAAGGTGGT	TTGCTCCTGT	ACTGAGGGAT
70	781	ATCGACTTGC	AGAAAACCAG	AAGTCTGTG	AACCAGCAGT	GCCATTTCCA	TGTGGAAGAG
	841	TTTCTGTTTC	ACAAACTTCT	AAGCTCACCC	GTGCTGAGAC	TGTTTTTCCT	GATGTGGACT
	901	ATGTAAATTC	TACTGAAGCT	GAACCAATTT	TGGATAACAT	CACTCAAGC	ACCCAATCAT
	961	TTAATGACTT	CACTCGGGTT	GTTGGTGGAG	AAGATGCCAA	ACCAGGTCAA	TTCCCTTGGC
75	1021	AGGTTGTTTT	GAATGGTAAA	GTTGATGCAT	TCTGTGGAGG	CTCTATCGTT	AATGAAAAAT
	1081	GGATTGTAA	TGCTGCCAC	TGTGTTGAAA	CTGGTGTAA	AATTACAGTT	GTGCGAGGTG
	1141	AACATAATAT	TGAGGAGACA	GAACATACAG	AGCAAAAGCG	AAATGTGATT	CGAATTATTC
	1201	CTCACCACAA	CTACAAATGCA	GCTATTAATA	AGTACAACCA	TGACATTGCC	CTTCTGGAAC
	1261	TGGACGAACC	CTTAGTGCTA	AACAGCTACG	TTACACCTAT	TTGCATGCT	GACAAGGAAT
	1321	ACACGAACAT	CTTCTCAAAA	TTTGGATCTG	GCTATGTAAG	TGGCTGGGGA	AGAGTCTTCC
80	1381	ACAAAGGGAG	ATCAGCTTTA	GTTCTTCACT	ACCTTAGAGT	TCCACTTGT	GACCGAGCCA
	1441	CATGTCTTCG	ATCTACAAAG	TTCACTTCT	ATAACAACAT	GTTCTGTGCT	GGCTTCCATG

5	1501	AAGGAGGTAG	AGATTTCATGT	CAAGGAGATA	GTGGGGGACC	CCATGTTACT	GAAGTGGGAA
	1561	GGACCAGTTT	CTTAACCTGGA	ATTATTAGCT	GGGGTGAAGA	GTGTGCAATG	AAAGGCCAAAT
	1621	ATGGAATATA	TACCAAGGTG	TCCCGGTATG	TCAACTGGAT	TAAGGAAAAA	ACAAAGCTCA
	1681	CTGGTGGCGG	TGGCTCCGGC	GGAGGTGGGT	CCGGTGGCGG	CGGATCAGGT	GGGGGTGGAT
10	1741	CAGGCGGTGG	AGGTTCCGGT	GGCGGGGGAT	CAGCGCAGGT	GCAGCTGCAG	GAGTCTGGGG
	1801	GAGGCTTGGT	ACAGCCTGGG	GGGTCCCTGA	GACTCTCCTG	TGCAGCCTCT	GGATTTCATGT
	1861	TTAGCAGGTA	TGCCATGAGC	TGGGTCCGCC	AGGCTCCAGG	GAAGGGGCCA	GAGTGGGTCT
	1921	CAGGTATTAG	TGGTAGTGGT	GGTAGTACAT	ACTACGCAGA	CTCCGTGAAG	GGCCGGTTCA
	1981	CGGTCTCCAG	AGACAATTCC	AAGAACACGC	TGTATCTGCA	AATGAACAGC	CTGAGAGCCG
	2041	AGGACACGGC	TGTATATTAC	TGCGCCCGGG	GCGCCACCTA	CACCAGCCGG	AGCGACGTGC
15	2101	CGGACCAGAC	CAGCTTCGAC	TACTGGGGCC	AGGGAACCCCT	GGTCACCGTC	TCCTCAGGGA
	2161	GTGCATCCGC	CCCAAAGCTT	GAAGAAGGTG	AATTTTCAGA	AGCACGCGTA	TCTGAACCTGA
	2221	CTCAGGACCC	TGCTGTGTCT	TGCGCCTTGG	GACAGACAGT	CAGGATCACA	TGCCAAGGAG
	2281	ACAGCCTCAG	AAACTTTTAT	GCAAGCTGGT	ACCAGCAGAA	GCCAGGACAG	GCCCTACTCT
	2341	TTGTCTCTTA	TGTTTAAAGT	AAAAGGCCCT	CAGGATCCCT	AGACCGATTG	TCTGCCTCCA
20	2401	GCTCAGGAAA	CACAGCTTCC	TTGACCATCA	CTGGGGCTCA	GGCGGAAGAT	GAGGCTGACT
	2461	ATTACTGCCT	GCTGTACTAC	GGCGGCGGCC	AGCAGGGCGT	GTTCCGGCGG	GGCACCAGGC
	2521	TGACCGTCTT	ACGTCAGCCC	AAGGCTGCCC	CCTCGGTAC	TCTGTTCCTG	CCCTCTTCTG
	2581	CGGCCTGA					
25	FIX-90 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker regions connecting FIX to SCE5 is underlined						
	1	MORVNMIMAE	SEGLITICLL	GYLLSAECTV	FLDHENANKI	LNRPKRYNSG	KLEEFVQGNL
30	61	ERECMEKCS	FEEAREVFN	TERTEFWKQ	YVDGQCESN	PCLNGGSCDK	DINSYECWCP
	121	FGFEGKNCEL	DVTCKIKNGR	CEQFCNKSAD	NKVVCSTEG	YRLAENQKSC	EPAPVPFCGR
	181	VVSQTSKLT	RAETVFDPD	YVNSTEAETI	LDNITQSTQS	FNDFTRVVG	EDAKFGQFPW
	241	QVVLNGKVDA	FCGGSIVNEK	WIVTAAHCEV	TGVKITVVAG	EHNIETEHT	EQRNVIRII
	301	PHNYNAAIN	KYNHDIALLE	LDEPLVLNSY	VTPICIAADKE	VTNIFLRFGS	GVVSGWGRVP
35	361	HKGRSALVLQ	YLRVPLVDRA	TCLRSTKPTI	YNNMFCAGFH	EGGRDSCQGD	SGGPHVTEVE
	421	GTSFLTGIIS	WGEECAMKKG	YGIYTKVSRV	VNWIKEKTKL	TGGGGSGGGG	SGGGSGGGGG
	481	SGGGSGGGST	SAQVQLQESG	GGLVQPGGSL	RLSCAASGFM	FSRYAMSWVR	QAPGKGPWV
	541	SGISGSGGST	YYADSVKGRF	TVSRDNSKNT	LYLQMNLSRA	EDTAVVYCAR	GATYTSRSDV
	601	PDQTSFDYWG	QGTLLVTSSG	SASAPKLEEG	EFSEARVSEL	TQDPAVSVAL	QGTVRITCQG
40	661	DSLRFYASW	YQKPGQAPT	LVIYGLSKRP	SGIPDRFSAS	SSGNTASLTI	TGAQAEDDAD
	721	YYCLLYYGGG	QQGVFGGGTK	LTVLRQPKAA	PSVTLFPPSS	AA*	

DNA sequence for FVII-088

45	1	ATGCTCTCCC	AGGCCCTCAG	GCTCCTCTGC	CTTCTGCTTG	GGCTTCAGGG	CTGCCTGGCT
	61	GCAGTCTTTC	TAACCCAGGA	GGAGGCCAC	GGCTCCTGC	ACCGGCGCCG	GCGCGCCAAC
	121	GCTTCTCTGG	AGGAGCTGCG	GCGGGCTCC	CTGGAGAGGG	AGTGCAAGGA	GGAGCAGTGC
	181	CGCTTCGAGG	AGGCCCGGGA	GATCTTCAAG	GACGCGGAGA	GGACGAGCT	GTTCTGGATT
	241	TCTTACAGTG	ATGGGGACCA	GTGTGCCTCA	AGTCCATGCC	AGAATGGGGG	CTCCTGCAAG
	301	GACCAGCTCC	AGTCTTATAT	CTGCTTCTGC	CTCCCTGCCT	TCGAGGGCCG	GAAGTGTGAG
50	361	ACGCACAAGG	ATGACCAGCT	GATCTGTGTG	AACGAGAACG	GCGGCTGTGA	GCAGTACTGC
	421	AGTGACCACA	CGGGCACCAA	GCGCTCCTGT	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
	481	GACGGGGTGT	CCTGCACACC	CACAGTTGAA	TATCCATGTG	GAAAAATACC	TATTTAGAA
	541	AAAAGAAATG	CCAGCAAACC	CCAAGGCCGA	ATTGTGGGGG	GCAAGGTGTG	CCCCAAAGGG
	601	GAGTGTCCAT	GGCAGGTCTT	GTTGTTGGTG	AATGGAGCTC	AGTTGTGTGG	GGGGACCCTG
55	661	ATCAACACCA	TCTGGGTGGT	CTCCGCGGCC	CACGTCTTCG	ACAAAATCAA	GAAGTGGAGG
	721	AACCTGATCG	CGGTGCTGGG	CGAGCACGAC	CTCAGCGAGC	ACGACGGGGA	TGAGCAGAGC
	781	CGCGGGGTGG	CGCAGGTCTC	CATCCCCAGC	ACGTACGTCC	CGGGCACACC	CACCCAGCAC
	841	ATCGCGCTGC	TCCGCTTGCA	CCAGCCCGTG	GTCTCACTG	ACCATGTGGT	GCCCTCTGTC
	901	CTGCCCCAAG	GGACGTTCTC	TGAGAGGACG	CTGGCCTTCG	TGCGCTTCTC	ATTGCTCAGC
60	961	GGCTGGGGCC	AGCTGCTGGA	CGGTGGCGCC	ACGGCCTTGG	AGCTCATGGT	CCTCAACGTG
	1021	CCCCGGCTGA	TGACCCAGGA	CTGCCTGCAG	CAGTCACGGA	AGGTGGGAGA	CTCCCCAAAT
	1081	ATCACGGAGT	ACATGTTCTG	TGCCGGCTAC	TCGGATGGCA	GCAAGGACTC	CTGCAAGGGG
	1141	GACAGTGGAG	GCCCCATGCG	CACCCACTAC	CGGGGCACGT	GGTACCTGAG	GGGCATCGTC
	1201	AGCTGGGGCC	AGGGCTGCGC	AACCCGTGGC	CACTTTGGGG	TGTACACCAG	GGTCTCCAG
65	1261	TACATCGAGT	GGCTGCAAAA	GCTCATGCGC	TCAGAGCCAC	GCCCAGGAGT	CCTCTGCGCA
	1321	GCCCCATTTC	CCGGTGGCGG	TGGCTCCGGC	GGAGGTGGGT	CCGGTGGCGG	CGGATCAGGT
	1381	GGGGGTGGAT	CAGGCGGTGG	AGGTTCCGGT	GGCGGGGGAT	CCGACAAAAC	TCACACATGC
	1441	CCACCGTGCC	CAGCTCCGGA	ACTCCTGGGA	GGACCGTCAG	TCTTCTCTTT	CCCCCAAAA
	1501	CCCAAGGACA	CCCTCATGAT	CTCCCGGACC	CCTGAGGTCA	CATGCGTGGT	GGTGGACGTG
70	1561	AGCCACGAAG	ACCCTGAGGT	CAAGTTCAAC	TGGTACGTGG	ACGGCGTGGG	GGTGACATAAT
	1621	GCCAAGACAA	AGCCGCGGGA	GGAGCAGTAC	AACAGCACGT	ACCGTGTGGT	CAGCGTCTCT
	1681	ACCGTCTCTC	ACCAGGACTG	GCTGAATGGC	AAGGAGTACA	AGTGCAAGGT	CTCCAACAAA
	1741	GCCCTCCCAG	CCCCATCGA	GAAGAACATC	TCCAAAGCCA	AAGGGCAGCC	CCGAGAACCA
	1801	CAGGTGTACA	CCCTGCCCCC	ATCCCGGGAT	GAGCTGACCA	AGAACCAGGT	CAGCTGACCC
75	1861	TGCTTGGTCA	AAGGCTTCTA	TCCAGCGAC	ATCGCCGTGG	AGTGGGAGAG	CAATGGGCGC
	1921	CCGGAGAACA	ACTACAAGAC	CACGCCTCCC	GTGTTGGACT	CCGACGGCTC	CTTCTTCTCT
	1981	TACAGCAAGC	TCACCGTCCA	CAAGAGCAGG	TGGCAGCAGG	GGAAACGTCT	CTCATGCTCC
	2041	GTGATGCATG	AGGCTCTGCA	CAACCACTAC	ACGCAGAAGA	GCCTCTCCCT	GTCTCCGGGT
	2101	AAACGGCGCC	GCCGGAGCGG	TGGCGGGCGA	TCAGGTGGGG	GTGGATCAGG	CGGTGGAGGT
80	2161	TCCGGTGGCG	GGGGATCCGG	CGGTGGAGGT	TCCGGTGGGG	GTGGATCAAG	GAAGAGGAGG
	2221	AAGAGGGACA	TCGTGATGAC	CCAGGCCGCC	CCGAGCGTGC	CCGTGACCCC	CGCGAGAGGC

5	2281	GTGAGCATCA	GCTGCCGGAG	CAGCCGGAGC	CTGCTGCACA	GCAACGGCAA	CACCTACCTG
	2341	TGCTGGTTCC	TGCAGCGGCC	CGGCCAGAGC	CCCCAGCTGC	TGATCTACCG	GATGAGCAAC
	2401	CTGGCCAGCG	GCGTGCCCGA	CCGGTTTCAGC	GGCAGCGGCA	GCGGCACCGC	CTTCAACCCTG
	2461	CGGATCAGCC	GGGTGGAGGC	CGAGGACGTG	GGCGTGTACT	ACTGCATGCA	GCACCTGGAG
10	2521	TACCCCTTCA	CCTTCGGCAG	CGGCACCAAG	CTGGAGATCA	AGCGGGGCGG	CGGCGGCAGC
	2581	GGCGGGCGCG	GCAGCGGCGG	CGGCGGCGAG	CAGGTGCAGC	TGCAGCAGAG	CGGCGCCGAG
	2641	CTGGTGCGGC	CCGGCACCCAG	CGTGAAGATC	AGCTGCAAGG	CCAGCGGCTA	CACCTTCACC
	2701	AACTACTGGC	TGGGCTGGGT	GAAGCAGCGG	CCCGGCCACG	GCCTGGAGTG	GATCGGCGAC
	2761	ATCTACCCCG	GCGGCGGGTA	CAACAAGTAC	AACGAGAACT	TCAAGGCGAA	GGCCACCCTG
15	2821	ACCGCCGACA	CCAGCAGCAG	CACCGCCTAC	ATGCAGCTGA	GCAGCCTGAC	CAGCGAGGAC
	2881	AGCGCCGTGT	ACTTCTGCGC	CCGGGAGTAC	GGCAACTACG	ACTACGCCAT	GGACAGCTGG
	2941	GGCCAGGGCA	CCAGCGTGAC	CGTGAGCAGC	GGTGGCGGTG	GCTCCGGCGG	AGGTGGGTCC
	3001	GGTGGCGGCG	GATCAGGTGG	GGGTGGATCA	GGCGGTGGAG	GTTCCGCTGG	CGGGGGATCA
	3061	GACAAAACCTC	ACACATGCCC	ACCGTGCCCA	GCACCGGAAC	TCCTGGGCGG	ACCGTTCAGTC
20	3121	TTCCTCTTCC	CCCCAAAACC	CAGGACACCC	CTCATGATCT	CCCGGACCCC	TGAGGTTCACA
	3181	TGCGTGGTGG	TGGACGTGAG	CCACGAAGAC	CCTGAGGTCA	AGTTCAACTG	GTACGTGGAC
	3241	GGCGTGGAGG	TGCATAATGC	CAAGACAAAG	CCGCGGGAGG	AGCAGTACAA	CAGCACGTAC
	3301	CGTGTGGTCA	GCGTCTCTAC	CGTCTGTCAC	CAGGACTGGC	TGAATGGCAA	GGAGTACAAG
	3361	TGCAAGGTCF	CCAACAAGAG	CCTCCCAGCC	CCCATCGAGA	AAACCATCTC	CAAGGCCAAA
25	3421	GGGACGCCCC	GAGAACCCACA	GGTGTACACC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG
	3481	AACGAGGTCA	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG
	3541	TGGGAGGACA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GTGGAGTCC
	3601	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG
	3661	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACATACAC	GCAGAAGAGC
30	3721	CTCTCCCTGT	CTCCGGGTAA	ATGA			

FVII-088 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or AP3 to Fc region is underlined, the AP3 scFv italicized, and linker with proprotein convertase processing sites is shown in bold

35	1	<u>MVEQALRLLC</u>	<u>LLGLQGCLA</u>	<u>AVEVTQEEAH</u>	<u>GVLHRRRRAN</u>	AFLEELRFGS	LERECKEEQC
	61	SPFEAREIFK	DAERTKLFWI	SYSDDQDCAS	SPCQNGGSCK	DQLQSVICFC	LPAFEGRNCE
	121	THKDDQLICV	NENGGCEQYC	SDHTGTRKSC	RCHEGYSLLA	DGVSCPTPVE	YPCGKIPILE
40	181	KRNASKPQGR	IVGGKVCPEK	ECPWQVLLLV	NGAQLCGGTL	INTIIVVSA	HCFDKIKNWR
	241	NLLAVLGEHD	LSEHDGDEQS	RRVAQVIIPS	TYVPGTTNHD	IALLRLHQPV	VLTDHVVPLC
	301	LPERTFSERT	LAFVRFSLVS	GWGQLLDRC	TAELMVLNV	PRIMTQDCLQ	QSRKVGDSNP
	361	ITBYMFCAFY	SDGSKDSCCK	DSGGPHATHY	RGTWYLTGIV	SWGQGCATVG	HPGVYTRVSG
	421	YIEWLQKLMR	SEPRPGVLLR	APFPFGGGSG	GGGSGGGGSG	GGGSGGGGSG	GGGSDKTHTC
45	481	PPCPAPELLG	GPSVFLFPPK	PRDILMISRT	PEVICVVVDV	SHEDPEVKFN	WYVDGVEVHN
	541	AKTKPREEQY	NSTYRVVSVL	TVLHQDWLNG	KEYRCKVSNK	ALPAPIEKTI	SKAKGQPREP
	601	QVYTLPPSRD	ELTKNQVSLT	CLVKGFPYSD	IAVEWESNGQ	PENNYKITTP	VLDSDGSFPL
	661	YSKLTVDKSR	WQQGNVFSCS	VMHEALHNHY	TQKSLSLSPG	KRRRRSGGGG	SGGGSGGGGG
	721	SGGGSGGGGG	SGGGSGSRKR	KRDIVMTQAA	PSVPVTPGES	VSISCRSSRS	LLHSNGNTYL
	781	CWFLQRPQGS	PQLLIYRMSN	LASGVPDRPS	GGSGGTAFTL	RISRVEAEDV	GVYYCMQHLE
50	841	YPPTFGSGTK	LEIKRGGGGS	GGGSGGGGGS	QVQLQQSGAE	LVRPGTSVKI	SCKASGYTFT
	901	NYWLGWVKQR	PGHGLEWIGD	IYPGGGYNKY	NENPKGKATL	TADTSSSTAY	MLLSSLTSED
	961	SAVYFCAREY	GNVDYAMDSW	GQGTSTVTSS	GGGGSGGGGG	GGGGSGGGGG	GGGGSGGGGG
	1021	DVHTTCPPCP	APELLGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD
55	1081	GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK
	1141	GQPREPQVYT	LPPSRDELTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTTPVLDS
	1201	DGSFFLYSKL	TVDKSRWQQG	NVFSQCSVMHE	ALHNHYTQKS	LSLSPGK*	

DNA sequence for FVIII-041

60	1	ATGCAAATAG	AGCTCTCCAC	CTGCTTCTTT	CTGTGCCTTT	TGCGATTCTG	CTTTAGTGCC
	61	ACCAGAGATG	ACTACCTGGG	TGCAGTGGA	CTGTCATGGG	ACTATATGCA	AAGTGATCTC
	121	GGTGAGCTGC	CTGTGGACGC	AAGATTTCCT	CCTAGAGTGC	CAAAATCTTT	TCCATTCAAC
	181	ACCTCAGTCG	TGTACAAAAA	GACTCTGTTT	GTAGAATTCA	CGGATCACCT	TTTCAACATC
	241	GCTAAGCCAA	GGCCACCCCTG	GATGGGTCTG	CTAGGTCCTA	CCATCCAGGC	TGAGGTTTAT
65	301	GATACAGTGG	TCATTACACT	TAAGAACATG	GCTTCCCATC	CTGTCACTGT	TCATGTTTAT
	361	GGTGTATCCT	ACTGGAAAGC	TCTCTAGGGA	GCTGAATATG	ATGATCAGAC	CAGTCAAAGG
	421	GAGAAAGAAG	ATGATAAAGT	CTTCCCTGGT	GGAGGCCATA	CATATGCTCTG	GCAGGTCTCTG
	481	AAAGAGAATG	GTCCAATGGC	CTCTGACCCA	CTGTGCCTTA	CCTACTCATA	TCCTTCTCAT
	541	GTGGACCTGG	TAAAAGACTT	GAATTCAGGC	CTCATTGGAG	CCCTACTAGT	ATGTAGAGAA
	601	GGGAGTCTGG	CCAAGGAAAA	GACACAGACC	TTGCACAAAT	TTATACTACT	TTTTGCTGTA
70	661	TTTGATGAAG	GGAAAAGTTG	GCACTCAGAA	ACAAAGAACT	CCTTGATGCA	GGATAGGGAT
	721	GCTGCATCTG	CTCGGGCCTG	GCCTAAAAATG	CACACAGTCA	ATGGTTATGT	AAACAGGTCT
	781	CTGCCAGGTC	TGATTGGATG	CCACAGGAAA	TCAGTCTATT	GGCATGTGAT	TGGAATGGGC
	841	ACCACTCCTG	AAGTGCACCT	AATATTCCTC	GAAGGTCACA	CATTTCTTGT	GAGGAACCAT
	901	CGCCAGGCGT	CCTTGGAATC	CTCGCCAATA	ACTTTCCTTA	CTGCTCAAAC	ACTCTTGATG
75	961	GACCTTGGAC	AGTTTCTACT	GTTTGTGTCAT	ATCTCTTCCC	ACCAACATGA	TGGCATGGAA
	1021	GCTTATGTCA	AAGTAGACAG	CTGTCCAGAG	GAACCCCAAC	TACGAATGAA	AAATAATGAA
	1081	GAAGCGGAAG	ACTATGATGA	TGATCTTACT	GATCTTGAAA	TGGATGTGGT	CAGGTTTGAT
	1141	GATGACAACT	CTCCTTCCTT	TATCCAAATT	CGCTCAGTTG	CCAAGAAGCA	TCCTAAAACI
	1201	TGGGTACATT	ACATTGCTGC	TGAAGAGGAG	GACTGGGACT	ATGCTCCCTT	AGTCTCCGCC
80	1261	CCCGATGACA	GAAGTTATAA	AAGTCAATAT	TTGAACAATG	GCCCTCAGCG	GATTGGTAGG
	1321	AAGTACAAAA	AAGTCCGATT	TATGGCATAC	ACAGATGAAA	CCTTTAAGAC	TCGTGAAGCT

5	1381	ATTCAGCATG	AATCAGGAAT	CTTGGGACCT	TTACTTTTATG	GGGAAGTTGG	AGACACACTG
	1441	TTGATTATAT	TTAAGAATCA	AGCAAGCAGA	CCATATAACA	TCTACCCCTCA	CGGAATCACT
	1501	GATGTCCGTC	CTTTGTATTC	AAGGAGATTA	CCAAAAGGTG	TAAAACATTT	GAAGGATTTT
	1561	CCAATTCTGC	CAGGAGAAAT	ATTCAAAAT	AAATGGACAG	TGACTGTAGA	AGATGGGGCA
10	1621	ACTAAATCAG	ATCCTCGGTG	CCTGACCCGC	TATTACTCTA	GTTTCGTAA	TATGGAGAGA
	1681	GATCTAGCTT	CAGGACTCAT	TGGCCCTCTC	CTCATCTGCT	ACAAAAGATC	TGTAGATCAA
	1741	AGAGGAAACC	AGATAATGTC	AGACAAGAGG	AATGTCATCC	TGTTTTCTGT	ATTTGATGAG
	1801	AACCGAAGCT	GGTACCTCAC	AGAGAATATA	CAACGCTTTC	TCCCCAATCC	AGCTGGAGTG
	1861	CAGCTTGAGG	ATCCAGAGTT	CCAAGCCTCC	AACATCATGC	ACAGCATCAA	TGGCTATGTT
	1921	TTTGATAGTT	TGCAGTTGTC	AGTTTGTTTG	CATGAGGTGG	CATACTGGTA	CATTCTAAGC
15	1981	ATTGGAGCAC	AGACTGACTT	CCTTTCTGTC	TTCTTCTCTG	GATATACCTT	CAAACACAAA
	2041	ATGGTCTATG	AAGACACACT	CACCCATTTC	CCATTCTCAG	GAGAAACTGT	CTTCATGTCT
	2101	ATGGAAGAAC	CAGGTCTATG	GATTCTGGGG	TGCCACAAC	CAGACTTTTC	GAACAGAGGC
	2161	ATGACCGCCT	TACTGAAGGT	TTCTAGTTGT	GACAAGAACA	CTGGTGATTA	TTACGAGGAC
20	2221	AGTTATGAAG	ATATTTGAGC	ATATCTGCTG	AGTAAAGACA	ATGCCATTGA	ACCAAGAAGC
	2281	TCTCTCAAA	ACCCACCAGT	CTTGAAACGC	CATCAACGGG	AAATAACTCG	TACTACTCTT
	2341	CAGTCAGATC	AAGAGGAAAT	TGACTATGAT	GATACCATAT	CAGTTGAAAT	GAAGAAGGAA
	2401	GATTTTGACA	TTTATGATGA	GGATGAAAT	CAGAGCCCCC	GCAGCTTTCA	AAAGAAAACA
	2461	CGACACTATF	TTATTTGCTG	AGTGGAGAGG	CTCTGGGATT	ATGGGATGAG	TAGCTCCCCA
25	2521	CATGTTCTAA	GAAACAGGGC	TCAGAGTGGC	AGTGTCCCTC	AGTTCAAGAA	AGTTGTTTTT
	2581	CAGGAATTTA	CTGATGGCTC	CTTTACTCAG	CCCTTATACC	GTGGAGAACT	AAATGAACAT
	2641	TTGGGACTCC	TGGGGCCATA	TATAAGAGCA	GAAGTTGAAG	ATAATATCAT	GGTAACTTTT
	2701	AGAAATCAGG	CCTCTCGTCC	CTATTCCTTC	TATCTAGGCC	TTATTTCTTA	TGAGGAAGAT
	2761	CAGAGGCAAG	GAGCAGAAAC	TAGAAAAAAC	TTTGTCAAGC	CTAATGAAAC	CAAAACTTAC
	2821	TTTTGGAAAG	TGCAACATCA	TATGGCACCC	ACTAAAGATG	AGTTTGACTG	CAAAGCCTTG
30	2881	GCTTATTTCT	CTGATGTTGA	CCTGGAAAAA	GATGTGCACT	CAGGCTGATG	TGAGCCCTTT
	2941	CTGGTCTGCC	ACACTAACAC	ACTGAACCTT	GCTCATGGGA	GACAAGTGAC	AGTACAGGAA
	3001	TTTGCTCTGT	TTTTCAACAT	CTTTGATGAG	ACCAAAAGCT	GGTACTTCAC	TGAAAAATAT
	3061	GAAAGAAACF	GCAGGGCTCC	CTGCAATATC	CAGATGGAAG	ATCCCACCTT	TAAAGAGAAT
35	3121	TATCGCTTCC	ATGCAATCAA	TGGCTACATA	ATGGATACAC	TACCTGGCTT	AGTAATGGCT
	3181	CAGGATCAAA	GGATTTCGATG	GTATCTGCTC	AGCATGGGCA	GCAATGAAAA	CATCCATCTT
	3241	ATTCATTTC	GTGGACATGT	GTTCACCTGT	CGAAAAAAG	AGGAGTATAA	AATGGCACTG
	3301	TACAATCTCT	ATCCAGGTGT	TTTTGAGACA	GTGGAAATGT	TACCATCCAA	AGCTGGAATT
	3361	TGGCGGGTGG	AATGCCTTAT	TGGCGAGCAT	CTACATGCTG	GGATGAGCAC	ACTTTTTCTG
	3421	GTGTACAGCA	ATAAGTGTCA	GACTCCCCTG	GGAAATGGCT	CTGGACACAT	TAGAGATTTT
40	3481	CAGATTACAG	CTTCAGGACA	ATATGGACAG	TGGGCCCCAA	AGCTGGCCAG	ACTTCATTAT
	3541	TCCGGATCAA	TCAATGCCTG	GAGCACCAAG	GAGCCCTTTT	CTTGGATCAA	GGTGGATCTG
	3601	TTGGCACCAA	TGATTATTCA	CGGCATCAAG	ACCCAGGGTG	CCCGTCAGAA	GTCTCTCCAG
	3661	CTGTACATCT	CTCAGTTTAT	CATCATGTAT	AGTCTTGATG	GGAAGAAGTG	GCAGACTTAT
45	3721	CGAGGAAATF	CCACTGGAAC	CTTAATGGTC	TTCTTTGGCA	ATGTGGATTTC	ATCTGGGATA
	3781	AAACACAATA	TTTTTAAACCC	TCCAATTATT	GCTCGATACA	TCCGTTTGCA	CCCAACTCAT
	3841	TATAGCATTC	GCAGCACTCT	TGCGATGGAG	TTGATGGGCT	GTGATTTAA	TAGTTGCAGC
	3901	ATGCCATTGG	GAATGGAGAG	TAAAGCAATA	TCAGATGCAC	AGATTACTGC	TTCATCTTAC
	3961	TTTACCAATA	TGTTTGCCAC	CTGGTCTCCT	TCAAAAGCTC	GACTTCACTT	CCAAGGGAGG
50	4021	AGTAATGCCT	GGAGACCTCA	GGTGAATAAT	CCAAAAGAGT	GGCTGCAAGT	GGACTTCCAG
	4081	AAGACAATGA	AAGTCACAGG	AGTAACCTACT	CAGGGAGTAA	AATCTCTGCT	TACCAGCATG
	4141	TATGTGAAGG	AGTTCTCAT	CTCCAGCAGT	CAAGATGGCC	ATCAGTGGAC	TCTCTTTTTT
	4201	CAGAATTGGCA	AAGTAAAGGT	TTTTTCAGGA	AATCAAGACT	CCTTCACACC	TGTGGTGAAC
	4261	TCTCTAGACC	CACCGTTACT	GACTCGCTAC	CTTCGAATTC	ACCCCCAGAG	TTGGGTGCAC
55	4321	CAGATTGCCC	TGAGGATGGA	GGTTCTGGGC	TGCGAGGCAC	AGGACCTCTA	CGACAAAAC
	4381	CACACATGCC	CACCGTGCCC	AGCTCCAGAA	CTCCTGGGCG	GACCGTCAGT	CTTCTCTTTC
	4441	CCCCCAAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACCC	CTGAGGTCAC	ATGCGTGGTG
	4501	GTCGACGTGA	GCCACGAAGA	CCCTGAGGTC	AAGTTCAACT	GGTACGTGGA	CGCGGTGGAG
	4561	GTGCATTAATG	CCAAGACAAA	GCCGCGGGAG	GAGCAGTACA	ACAGCACGTA	CCGTGTGGTC
60	4621	AGCGTCTCTA	CCGTCTGTCA	CCAGGACTGG	CTGAATGGCA	AGGAGTACAA	GTGCAAGGTC
	4681	TCCAACAAAG	CCCTCCAGC	CCCCATCGAG	AAAACCATCT	CCAAAGCCAA	AGGGCAGCCC
	4741	CGAGAACCAC	AGGTGTACAC	CCTGCCCCCA	TCCCGGGATG	AGCTGACCAA	GAACCAGGTC
	4801	AGCCTGACCT	GCCTGGTCAA	AGGCTTCTAT	CCCAGCGACA	TCGCGGTGGA	GTGGGAGAGC
	4861	AATGGGCAGC	CGGAGAACAA	CTACAGAGAC	ACGCCTCCCG	TGTTGGAGTC	CGACGCTTCC
	4921	TTCTTCTCT	ACAGCAAGCT	CACCGTGAGC	AAGAGCAGGT	GGCAGCAGGC	GAACGTCTTC
65	4981	TCATGCTCCG	TGATGCATGA	GGCTCTGCAC	AACCACTACA	CGCAGAAGAG	CCTCTCCCTG
	5041	TCTCCGGGTA	AAGGTGGCGG	CGGATCAGGT	GGGGGTGGAT	CAGGCGGTGG	AGGTTCCGGT
	5101	GGCGGGGGAT	CAGACAAAAC	TCACACATGC	CCACCGTGCC	CAGCACCTGA	ACTCTGGGA
	5161	GGACCGTCAG	TCTTCTCTTT	CCCCCAAAA	CCCAAGGACA	CCCTCATGAT	CTCCCGGACC
70	5221	CCTGAGGTCA	CATGCGTGGT	GGTGGACGTG	AGCCACGAAG	ACCCTGAGGT	CAAGTTCAAC
	5281	TGGTACGTGG	ACGGCGTGGA	GGTGCAATA	GCCAAGACAA	AGCCGCGGGA	GGAGCAGTAC
	5341	AACAGCACGT	ACCGTGTGGT	CAGCGTCTTC	ACCGTCTCTG	ACCAGGACTG	GCTGAATGGC
	5401	AAGGAGTACA	AGTGCAAGGT	CTCCAACAAA	GCCCTCCCAG	CCCCCATCGA	GAAAACCATC
	5461	TCCAAAGCCA	AAGGGCAGCC	CCGAGAACCA	CAGGTGTACA	CCCTGCCCCC	ATCCCGCGAT
75	5521	GAGCTGACCA	AGAACCAAGT	CAGCTTGACC	TGCCTGGTCA	AAGGCTTCTA	TCCCGCGAC
	5581	ATCGCCGTGG	AGTGGGAGAG	CAATGGGCGG	CCGGAGAACA	ACTACAGAC	CAGCCCTCCC
	5641	GTGTTGGACT	CCGACGGCTC	CTTCTTCTTC	TACAGCAAGC	TCACCGTGGA	CAAGAGCAGG
	5701	TGGCAGCAGG	GGAACGTCTT	CTCATGCTTC	GTGATGCATG	AGGCTCTGCA	CAACCACTAC
80	5761	ACGCAGAAGA	GCCTCTCCCT	GTCTCCGGGT	AAATGA		

5 FVIII-041 amino acid sequence. Signal sequence is shown in dotted underline, and linker region connecting the Fc regions is underlined

1 MQIELSTCEE LCLLRECFSA TRRYVLGAVE LSWDYMOSDL GELPVDARFP PRVPKSFPPFN
 61 TSVVYKKTLE VEFTHLLENI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
 121 GVSYYKASEG AEYDDQTSQR EKEDDKVFPQ GSHTYVWQVL KENGPMASDP LCLTYSYLGH
 181 VDLVKDLNSG LIGALLVCRE GSLAKEKTOT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
 241 AASARAWPKM HTVNGYVNRSLPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHFTFLVRNH
 301 RQASLEISPI TFLTAQTLML DLGQFLLPCH ISSHQHDCME AYVKVDSCPE EPQLRMKNNE
 361 EABDYDDDLT DSEMDVVRFD DNNSPSFIQI RSVAKKHPRK WVHYIAEEEE DRDYAPLVLA
 421 PDDRSYKSQV LNNGPQRIGR KYKKVRFMAY TDETFKTRTA IQHESGILGF LLNGEVGDTL
 481 LIIFKNQASR PYNIPYHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP
 541 TKSDPRCLTR YYSSFVNMR DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL HEVAYWYILS
 661 IGAQTDFLSV FFSGYTFKHK MVEDTLTLF PFSGETVFMS MENPGLWILG CHNSDFRNRG
 721 MTALLKVSSC DKNTGDYED SYEDISAYLL SKNNAIEPRS FSNPNPVLKR HQREITRTTL
 781 QSDQEEIDYD DTISVEMKKE DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
 841 HVLNRNAQSG SVPOFKKVVF QEFITDGSFTQ FLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 901 RNQASRPYSP YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQHMAP TKDEFDCRAW
 961 AYPDSDVLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE PALPFTTFDE TKSIFYTENM
 1021 ERNCRAPCNI QMEDPTFKEN YRFHAINGYI MDTLPGLVMA QDQIRIRWYLL SMSGNENIHS
 1081 LHFSGHVFV RKKEEYKML YNLYPGVFET VEMLPKSKAGI WRVECLIGE HLAGMSTLFL
 1141 VYSNKQCTPL GMASGHIRDF QITASQYVQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1201 LAPMIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGILMV FFGNVDSSGI
 1261 KHNIIFNPPII ARYIRLHPHT YSIRSTLRME LMGCDLNSCS MPLGMESKAI SDAQITASSY
 1321 FYNMFATWSP SKARLHLQGR SNAWRPQVNN PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM
 1381 YKEFLISS QDGHQWTLFF QNGKVKVFQO NQDSFTPVVN SLDPLLLTRY LRIHPQSWVH
 1441 QALRMEVLG CEADLYDKT HTPCPCPAPE LLGGFSVFLF PPKPKDTIMI SRTPEVTCVV
 1501 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNKKEYCKKV
 1561 SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES
 1621 NGQPENNYKT TPPVLDSGDS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
 1681 SPKGGGGGGG GGGGGGGGGG GGGGSKTHTC PPCPAPELLG GPSVFLFPKP PKITLMISIT
 1741 PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPEREQV NSTYRVVSVL TLVHQDWLNG
 1801 KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD
 1861 IAVEWESNGQ PENNYKTPP VLDSDGSFFL YSKLTVDKSR WQQGNVPSCS VMHEALHNHY
 1921 TQKSLSLSPG K*

DNA sequence for FVIII-108

1 ATGCAAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TCGGATTCTG CTTTAGTGCC
 61 ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG ACTATATGCA AAGTGATCTC
 121 GGTGAGCTGC CTGTGGACGC AAGATTTCCT CTTAGAGTGC CAAAATCTTT TCCATTCAAC
 181 ACCTCAGTCG TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
 241 GCTAAGCCAA GGGCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 301 GAATACATGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT TCATGCTGTT
 361 GGTGTATCCT ACTGGAAGAG TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
 421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGCTCT GCAGGTCCTG
 481 AAAGAGAAAT GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
 541 GTGGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
 661 TTTGATGAAG GGAAAGTTG GCACTCAGAA ACAAGAAGT CTTGTATGCA GGATAGGGAT
 721 GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCCT
 781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAAATGGGC
 841 ACCACTCTCT AAGTGCACCT AATATTCTCT GAAGGTCACA CATTTCTTGT GAGGAACCAT
 901 CGCCAGGCGT CTTTGGAAAT CTGCCCATAA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
 961 GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
 1021 GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
 1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
 1141 GATGACAACT CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACI
 1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCTCGGCC
 1261 CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG GCCCTCAGCG GATTTGATAG
 1321 AAGTACAAAA AAGTCCGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT
 1381 ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGAAGTGGG AGACACACTG
 1441 TTGATTATAT TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCTTCA CGGAATCACI
 1501 GATGTCCGTC CTTTGTATTG AAGGAGATTA CCAAAAGGTG TAAAACATTT GAAGGATTTT
 1561 CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG TGACTGTAGA AGATGGGCCA
 1621 ACTAAATCAG ATCCTCGGTG CCGTACCCGC TATTACTCTA GTTTCTGTAA TATGGAGAGA
 1681 GATCTAGCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCT ACAGAGAACT TGTAGATCAA
 1741 AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCATCC TGTCTTCTGT ATTTGATGAG
 1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC AGCTGGAGTG
 1861 CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC ACAGCATCAA TGGCTATGTT
 1921 TTTGATGATT TGCAGTTGTC AGTTTGTGTT CATGAGGTGG CATACTGGTA CATTCTAAGC
 1981 ATTGGAGCAC AGACTGACTT CTTTCTCTG TTCTTCTCTG GATATACCTT CAAACACAAA
 2041 ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTTATGTCT
 2101 ATGGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAAC TCGACTTTCC GAGACAGGCG
 2161 ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
 2221 AGTTATGAAG ATATTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGAAGC
 2281 TTCTCTCAAA ACCCACCAGT CTTGAAACGC CATCAACGGG AAATAACTCG TACTACTCTT
 2341 CAGTCCAGATC AAGAGGAAAT TGACTATGAT GATACCATAT CAGTTGAAAT GAAGAAGGAA

5	2401	GATTTTGACA	TTTATGATGA	GGATGAAAT	CAGAGCCCC	GCAGCTTCA	AAAGAAAACA
	2461	CGACACTAT	TTATGCTGC	AGTGGAGAG	CTCTGGGATT	ATGGGATGAG	TAGCTCCCCA
	2521	CATGTTCTAA	GAAACAGGGC	TCAGAGTGGC	AGTGTCCCTC	AGTTCAAGAA	AGTGTGTTTT
	2581	CAGGAATTTA	CTGATGGCTC	CTTTACTCAG	CCCTTATACC	GTGGAGAACT	AAATGAACAT
10	2641	TTGGGACTCC	TGGGGCCATA	TATAAGAGCA	GAAGTTGAAG	ATAATATCAT	GGTAACCTTC
	2701	AGAAATCAGG	CCTCTCGTCC	CTATTCCCTC	TATTCTAGCC	TTATTTCTTA	TGAGGAAGAT
	2761	CAGAGGCAAG	GAGCAGAACC	TAGAAAAAAC	TTTGTCAGC	CTAATGAAAC	CAAAACTTAC
	2821	TTTGGAAAG	TGCAACATCA	TATGGCACCC	ACTAAAGATG	AGTTTGACTG	CAAAGCCTGG
	2881	GCTTATTTCT	CTGATGTTGA	CCTGGAAAAA	GATGTGCACT	CAGGCTGAT	TGGACCCCTT
	2941	CTGGTCTGCC	ACACTAACAC	ACTGAACCCCT	GCTCATGGGA	GACAAGTGAC	AGTACAGGAA
15	3001	TTTGCTCTGT	TTTTCACCAT	CTTTGATGAG	ACCAAAAGCT	GGTACTTCAC	TGAAAAATATG
	3061	GAAAGAAACT	GCAGGGCTCC	CTGCAATATC	CAGATGGAAG	ATCCCACCTT	TAAAGAGAAAT
	3121	TATCGCTTCC	ATGCAATCAA	TGGCTACATA	ATGGATACAC	TACCTGGCTT	AGTAATGGCT
	3181	CAGGATCAAA	GGATTGATG	GTATCTGCTC	AGCATGGGCA	GCAATGAAAA	CATCCATTCT
	3241	ATTCATTTCA	GTGGACATGT	GTTCACTGTA	CGAAAAAAG	AGGAGTATAA	AATGGCACTG
20	3301	TACAATCTCT	ATCCAGGTGT	FTTTGAGACA	GTGGAAATGT	TACCATCCAA	AGCTGGAATF
	3361	TGGCGGGTGG	AATGCCTTAT	TGGCGAGCAT	CTACATGCTG	GGATGAGCAC	ACTTTTTCTG
	3421	GTGTACAGCA	ATAAGTGTCA	GACTCCCCTG	GGAAATGGCTT	CTGGACACAT	TAGAGATTTT
	3481	CAGATTACAG	CTTCAGGACA	ATATGGACAG	TGGGCCCCAA	AGCTGGCCAG	ACTTCATTAT
	3541	TCCGGATCAA	TCAATGCCTG	GAGCACCAAG	GAGCCCTTTT	CTTGGATCAA	GGTGGATCTG
25	3601	TTGGCACCAA	TGATTATTCA	CGGCATCAAG	ACCCAGGGTG	CCCGTCAGAA	GTCTCCAGC
	3661	CTCTACATCT	CTCAGTTTAT	CATCATGTAT	AGTCTTGATG	GGAAGAGTG	GCAGACTTAT
	3721	CGAGGAAATF	CCACTGGAAC	CTTAATGGTC	TTCTTTGGCA	ATGTGGATTG	ATCTGGGATA
	3781	AAACACAATA	TTTTTAACCC	TCCAATTATT	GCTCGATACA	TCCGTTTGCA	CCCAACTCAT
	3841	TATAGCATTC	GCAGCACTCT	TCCGATGGAG	TTGATGGGCT	GTGATTTAA	TAGTTGCAGC
30	3901	ATGCCATTGG	GAATGGAGAG	TAAAGCAATA	TCAGATGCAC	AGATTACTGC	TTCATCTTAC
	3961	TTTACCAATA	TGTTTGCCAC	CTGGTCTCCT	TCAAAAGCTC	GACTTCACCT	CCAAGGGAGG
	4021	AGTAATGCCT	GGAGACCTCA	GGTGAATAAT	CCAAAAGAGT	GGCTGCAAGT	GGACTTCCAG
	4081	AAGACAATGA	AAGTCACAGG	AGTAACACT	CAGGGAGTAA	AATCTCTGCT	TACCAGCATG
35	4141	TATGTGAAGG	AGTTCTTCAT	CTCCAGCAGT	CAAGATGGCC	ATCAGTGGAC	TCTCTTTTTT
	4201	CAGAATTGGC	AAGTAAAGGT	TTTTTCAGGA	AATCAAGACT	CCTTCACACC	TGTGGTGAAC
	4261	TCTCTAGACC	CACCGTTACT	GACTCGCTAC	CTTCGAATTC	ACCCCCAGAG	TGCGGTGCAC
	4321	CAGATTGCC	TGAGGATGGA	GGTTCTGGGC	TGCGAGGCAC	AGGACCTCTA	CGACAAAAC
	4381	CACACATGCC	CACCGTGCCC	AGCACCTGAA	CTCCTGGGAG	GACCGTCAGT	CTTCTCTTTC
	4441	CCCCCAAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACCC	CTGAGGTCAC	ATGCGTGGTG
40	4501	GTGGACGTGA	GCCACGAAGA	CCCTGAGGTC	AAGTTCAACT	GGTACGTGGA	CGCGTGGAG
	4561	GTGCATAATG	CCAAGACAAA	GCCGCGGGAG	GAGCAGTACA	ACAGCACGTA	CCGTGTGGTC
	4621	AGCGTCCCTA	CCGTCTGTGA	CCAGGACTGG	CTGAATGGCA	AGGAGTACAA	GTGCAAGGTC
	4681	TCCAACAAAG	CCCTCCAGC	CCCAATCGAG	AAAACCATCT	CCAAAGCCAA	AGGGCAGCCC
45	4741	CGAGAACCAC	AGGTGTACAC	CCTGCCCCCA	TCCCGCGATG	AGCTGACCAA	GAACCAGGTC
	4801	AGCCTGACCT	GCCTGGTCAA	AGGCTTCTAT	CCCAGCGACA	TCCCGGTGGA	GTGGGAGAGC
	4861	AATGGGACAG	CGGAGAACAA	CTACAAGACC	ACGCTTCCCG	TGTTGGACTC	CGACGGCTCC
	4921	TTCTTCTCT	ACAGCAAGCT	CACCGTCGAC	AAGAGCAGGT	GGCAGCAGGG	GAACGCTCTC
	4981	TCATGCTCCG	TGATGCATGA	GGCTCTGCAC	AACCACTACA	CGCAGAGAG	CCCTCTCCCTG
50	5041	TCTCCGGGTA	AACGGCGCCG	CCGGAGCGGT	GGCGGCGGAT	CAGGTGGGGG	TGGATCAGGC
	5101	GGTGGAGGTT	CCGGTGGCGG	GGGATCCGCG	GGTGGAGGTT	CCGGTGGGGG	TGGATCAAGG
	5161	AAGAGGAGGA	AGAGGGCGCA	GGTGCAGCTG	CAGGAGTCTG	GGGGAGGCTT	GGTACAGCCT
	5221	GGGGGGTCCC	TGAGACTCTC	CTGTGCAGCC	TCTGGATTCA	TGTTTAGCAG	GTATGCCATG
	5281	AGTCTGGTCC	GCCAGGCTCC	AGGGAAGGGG	CCAGAGTGGG	TCTCAGGTAT	TAGTGGTAGT
55	5341	GGTGGTAGTA	CATACTACGC	AGACTCCGTG	AAGGGCCGGT	TCACCGTCTC	CAGAGACAAT
	5401	TCCAAGAACA	CGCTGTATCT	GCAAAATGAAC	AGCCTGAGAG	CCGAGGACAC	GGCTGTATAT
	5461	TACTGCGCCC	GGGGCGCCAC	CTACACCAGC	CGGAGCGACG	TGCCCCAGCA	GACCAGCTTC
	5521	GACTACTGGG	GCCAGGGAAC	CCTGGTCACC	GTCTCCTCAG	GGAGTGCATC	CGCCCCAAAG
	5581	CTTGAAGAAG	GTGAATTTTC	AGAAGCACGC	GTATCTGAAC	TGACTCAGGA	CCCTGCTGTG
60	5641	TCTGTGGCCT	TGGGACAGAC	AGTCAGGATC	ACATGCCAAG	GAGACAGCCT	CAGAAACTTT
	5701	TATGCAAGCT	GGTACCAGCA	GAAGCCAGGA	CAGGCCCTTA	CTCTTGTCAT	CTATGGTTTA
	5761	AGTAAAAGGC	CCTCAGGGAT	CCCAGACCGA	TTCTCTGCC	CCAGCTCAGG	AAACACAGCT
	5821	TCCTTGACCA	TCACTGGGGC	TCAGGCGGAA	GATGAGGCTG	ACTATTACTG	CCTGCTGTAC
	5881	TACGGCGGCG	GCCAGCAGGG	CGTGTTCGGC	GGCGGCACCA	AGCTGACCCG	CCTACGTCAG
65	5941	CCCAAGGCTG	CCCTCTGGGT	CACTCTGTTC	CCGCCCCCTT	CTGCGGCCGT	TGGCGGTGBC
	6001	TCCGGCGGAG	GTGGGTCCGG	TGGCGGCGGA	TCAGGTGGGG	GTGGATCAGG	CGGTGGAGGT
	6061	TCCGGTGGCG	GGGGATCAGA	CAAAACTCAC	ACATGCCAC	CGTGCCCGAG	ACCGGAACCT
	6121	CTGGGCGGAC	CGTCAGTCTT	CCTCTTCCCC	CCAAAACCCA	AGGACACCC	CATGATCTCC
	6181	CGGACCCCTG	AGGTACATG	CGTGGTGGTG	GACGTGAGCC	ACGAAGACCC	TGAGGTCAAG
70	6241	TTCAACTGGT	ACGTGGACGG	CGTGGAGGTG	CATAATGCCA	AGACAAAGCC	GCGGGAGGAG
	6301	CAGTACAACA	GCACGTACCG	TGTGGTCAGC	GTCTCACC	TCCTGCACCA	GGACTGGCTG
	6361	AATGGCAAGG	AGTACAAGTG	CAAGGTCTCC	AACAAAGCCC	TCCCAGCCCC	CATCGAGAAA
	6421	ACCATCTCCA	AAGCCAAAGG	GCAGCCCCGA	GAACCAACAG	TGTACACCC	GCCCCATCC
	6481	CGGGATGAGC	TGACCAAGAA	CCAGGTCAGC	CTGACCTGCC	TGGTCAAAGG	CTTCTATCCC
75	6541	AGCGACATCG	CCGTGGAGTG	GGAGAGCAAT	GGGACGCCGG	AGAACAAC	CAGACCCAGC
	6601	CCTCCCGTGT	TGGACTCCGA	CGGCTCCTTC	TTCTCTTACA	GCAAGCTCAC	CGTGGACAAG
	6661	AGCAGGTGGC	AGCAGGGGAA	CGTCTTCTCA	TGCTCCGTGA	TGCATGAGGC	TCTGCACAAC
	6721	CATACACGC	AGAAGAGCCT	CTCCCTGTCT	CCGGGTAAAT	GA	

5 FVIII-108 amino acid sequence. Signal sequence is shown in dotted underline, linker region connecting SCE5 to Fc region is underlined, and linker with proprotein convertase processing sites is shown in bold

1 MQIELSTCFE LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP PRVFKSFPPN
 61 TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
 10 121 GVSYWKASEG AEYDDQTSQR EKEDDKVFPFG GSHTYVWQVL KENGPMASDP LCLTYSYLSH
 181 VDLVKDLNSG LIGALLVCRE GSLAKEKTQT LHKPILLFAV FDEGKSWHSE TKNSLMQDRD
 241 AASARAWPKM HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCEP EPQLRMKNNE
 15 361 EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT WVHYIAAEE DWYAPLVLA
 421 PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY TDETFKTREA IQHESGILGP LLYGEVGDTL
 481 LIIFKNQASR PYNIPYHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGF
 541 TKSDPRCLTR YYSSFVNMR DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYLTEINI QRFLPNPAGV QLEDPEPQAS NIMHSINGYV FDSLQLSVCL HEVAYWYILS
 20 661 IGAQTDFLSV FFSGYTFKKH MVYEDTLTLF PFSGETVFMS MENPGLWILG CHNSDFNRNG
 721 MTALLKVSSC DKNTGDYDED SYEDISAYLL SKNNAIEPRS FSNPPEVLKR HQREITRITL
 781 QSDQEEIDVD DTISVEMKKE DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LDWYGMSSSP
 841 HVLNRNRAQSG SVPQPKKVVF QEFIDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 901 RNQASRPYSE YSSLISYEED QROGAEPKRN FVKPNETKTY FWKVQHMAP TKDEFDCRAW
 25 961 AYFSDVDLEK DVHSLIGLPL LVCHTNTLNP AHGRQVTVQE FALFFTIFDE TKSIFYTENM
 1021 ERNCRAPCNI QMEDPTFKEN YRFHAINGYI MDLPLGLVMA QDQIRIRWYL SMGSNENIHS
 1081 IHFSGHVFTV RKKEEYKMAL YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL
 1141 VYSNKCQTPL GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK EPPSWIKVDL
 1201 LAEMIHHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV FPGNVDSSGI
 30 1261 KHNIFNPPII ARYIRLHPHT YSIRSTLRME LMGCIDLNSC MPLGMESKAI SDAQITASSY
 1321 FTNMFATWSP SKARLHLQGR SNAWRPQVMN PKEWLQVDFQ KTMKVTVGVT QVKSLLTSM
 1381 YVKEFLISS QDGHQWTLFF QNGKVKVFGQ NQDSFTFVVN SLDPPLTRY LRIHPQSWVH
 1441 QIALRMEVLG CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTIMI SRTPEVTCVV
 1501 VDVSHEDPEV KFNWYVDGVE VHNATKPRE EQYNSTYRVV SVLTVLHQDW LNKKEYKCKV
 35 1561 SNKALPAPIE KTISKAKQGP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES
 1621 NGQPPENNYKT TPPVLDSGDS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
 1681 SPGRRRRRSG GGGSGGGGSG GGGSGGGGSG GGGSGGGGSG RRRRRAQVQL QESGGGLVQP
 1741 GGSRLRLSCAA SGFMFSRYAM SWVRQAPGKG PEWVSGISGS GGSTYYADSV
 KGRFTVSRDN
 1801 SKNTLYLQMN SLRAEDTAVY YCARGATYTS RSDVPDQTSF DYWGQGTLVLT
 40 VSSGSASAPK
 1861 LEEGEFSEAR VSELTQDPAV SVALGQTVRI TCQGDSLRNF YASWYQQKPG
 QAPTLVIYGL
 1921 SKRPSGIPDR FSASSSGNTA SLTITGAQAE DEADYYCLLY YGGGQQGVFG
 GGTKLTVLRQ
 45 1981 PKAAPSVTLF PPSSAAGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
 TCPPCPAPEL
 2041 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 HNAKTKPREE
 2101 QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKQPR
 50 EPQVYTLPPS
 2161 RDELTKNQVS LTCLVKGFYF SDIAVEWESN GPENNYKTT PPVLDSGDSF
 FLYSKLTVDK
 2221 SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*

55 DNA sequence for pSYN-FVIII-049

1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCATCTCTG CTTTAGTGCC
 61 ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCAATGGG ACTATATGCA AAGTGATCTC
 121 GGTGAGCTGC CTGTGGACGC AAGATTTCTT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
 181 ACCTCAGTCG TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
 60 241 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
 301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCACTCT TCATGCTGTT
 361 GGTGTATCCT ACTGGAAGAG TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
 421 GAGAAAGAAG ATGATAAAGT CTCTCCCTGGT GGAAGCCATA CATATGCTG GCAGGTCCTG
 481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCCTTA CCTACTCATA TCTTTCTCAT
 65 541 GTGGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
 661 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAAGT CCTGTATGCA GGATAGGGAT
 721 GCTGCATCTG CTCGGGCCTG GCCTAAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
 781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAAATGGC
 70 841 ACCACTCTG AAGTGCACCT AATATTCTC GAAGGTACAC CATTCTTGT GAGGAACCAT
 901 CGCCAGGCGT CCTTGGAAAT CTGCCCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
 961 GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
 1021 GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
 1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
 75 1141 GATGACAACT CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT

5	1201	TGGGTACATT	ACATTGCTGTC	TGAAGAGGAG	GACTGGGACT	ATGCTCCCTT	AGTCTCGGCC
	1261	CCCGATGACA	GAAGTTATAA	AAGTCAATAT	TTGAACAATG	GCCCTCAGCG	GATTGGTAGG
	1321	AAGTACAAAA	AAGTCCGATT	TATGGCATACT	ACAGATGAAA	CCTTTAAGAC	TCTGGAAGCT
	1381	ATTTCAGCATG	AATCAGGAAT	CTTGGGACCT	TTACTTTTATG	GGGAAGTTGG	AGACACACTG
10	1441	TTGATTATAT	TTAAGAATCA	AGCAAGCAGA	CCATATAACA	TCTACCTCA	CGGAATCACT
	1501	GATGTCCGTC	CTTTGTATTC	AAGGAGATTA	CCAAAAGGTG	TAAAAACATT	GAAGGATTTT
	1561	CCAAITCTGC	CAGGAGAAAAT	ATTCAAAATAT	AAATGGACAG	TGACTGTAGA	AGATGGGCCA
	1621	ACTAAATCAG	ATCCTCGGTG	CCTGACCCGC	TATFACTCTA	GTTTCGTAA	TATGGAGAGA
	1681	GATCTAGCTT	CAGGACTCAT	TGGCCCTCTC	CTCATCTGCT	ACAAAAGAATC	TGTAGATCAA
	1741	AGAGGAARAC	AGATAATGTC	AGACAAGAGG	AATGTCATCC	TGTTTTCTGT	ATTGTATGAG
15	1801	AACCGAAGCT	GGTACCTCAC	AGAGAATATA	CAACGCTTTC	TCCCCAATCC	AGCTGGAGTG
	1861	CAGCTTGAGG	ATCCAGAGTT	CCAAGCCTCC	AACATCATGC	ACAGCATCAA	TGGCTATGTT
	1921	TTTGATAGTT	TGCAGTTGTC	AGTTTGTTFG	CATGAGGTGG	CATACTGCTA	CATTCTAAGC
	1981	ATTGGAGGCAC	AGACTGACTT	CCTTTCTGTC	TTCTTCTCTG	GATATACCTT	CAAAACACAA
20	2041	ATGGTCTATG	AAGACACACT	CACCTATTC	CCATTCTCAG	GAGAACTGT	CTTCATGTGG
	2101	ATGGAAAAAC	CAGGTCTATG	GATTCCTGGG	TGCCACAAC	CAGACTTTCG	GAACAGAGGC
	2161	ATGACCGCCT	TACTGAAGGT	TTCTAGTTGT	GACAAGAACA	CTGGTGATTA	TTACGAGGAC
	2221	AGTTATGAAG	ATATTTCAGC	ATACTTGCTG	AGTAAAAACA	ATGCCATTGA	ACCAAGAAGC
	2281	TTCTCTCAAA	ACCCACCAGT	CTTGAACGCG	CATCAACGGG	AAATAACTCG	TACTACTCTT
25	2341	CAGTCAGATC	AAGAGGAAAT	TGACTATGAT	GATACCATAT	CAGTTGAAAT	GAAGAAGGAA
	2401	GATTTTGACA	TTTATGATGA	GGATGAAAT	CAGAGCCCC	GCAGCTTTCA	AAAGAAAAACA
	2461	CGACACTATT	TTATTGCTGC	AGTGGAGAGG	CTCTGGGATT	ATGGGATGAG	TAGCTCCCCA
	2521	CATGTTCTAA	GAAACAGGGC	TCAGAGTGGC	AGTGTCCCTC	AGTTCAGAA	AGTTGTTTTT
	2581	CAGGAATTTA	CTGATGGCTC	CTTTACTCAG	CCCTTATACC	GTGGAGAACT	AAATGAACAT
30	2641	TTGGGACTCC	TGGGGCCATA	TATAAGAGCA	GAAGTTGAAG	ATAATATCAT	GGTAACCTTC
	2701	AGAAATCAGG	CCTCTCGTCC	TATTTCTGTC	TATTTCTAGCC	TTATTTCTTA	TGAGGAAGAT
	2761	CAGAGGCAAG	GAGCAGAACC	TAGAAAAAAC	TTTGTCAGGC	CTAATGAAC	CAAAACTTAC
	2821	TTTTGGAAG	TGCAACATCA	TATGGCACCC	ACTAAAGATG	AGTTTGACTG	CAAAGCCTGG
	2881	GCTTATTTCT	CTGATGTTGA	CCTGGAATAA	GATGTGCAC	CAGGCCGTGAT	TGGACCCCTT
35	2941	CTGGTCTGCC	ACACTAACAC	ACTGAACCTT	GCTCATGGGA	GACAAGTGAC	AGTACAGGAA
	3001	TTTGCTCTGT	TTTTCAACAT	CTTTGATGAG	ACCAAAAGCT	GGTACTTCAC	TGAAAAATATG
	3061	GAAAGAACT	GCAGGGCTCC	CTGCAATATC	CAGATGGAAG	ATCCCACCTT	TAAAGAGAA
	3121	TATCGCTTCC	ATGCAATCAA	TGGCTACATA	ATGGATACAC	TACCTGGCTT	AGTAATGGCT
	3181	CAGGATCAAA	GGATTGATG	GTATCTGCTC	AGCATGGGCA	GCAATGAAA	CATCCATTCT
40	3241	ATTCATTTCA	GTGGACATGT	GTTCACTGTA	CGAAAAAAG	AGGAGTATAA	AATGGCACTG
	3301	TATCAATCTT	ATCCAGGTGT	TTTTGAGACA	GTGGAAATGT	TACCATCCAA	AGCTGGAAT
	3361	TGGCGGGTGG	AATGCCTTAT	TGGCGAGCAT	CTACATGCTG	GGATGAGCAC	ACTTTTTCTG
	3421	GTGTACAGCA	ATAAGTGTCA	GACTCCCCTG	GGAAATGGCT	CTGGACACAT	TAGAGATTTT
	3481	CAGATTACAG	CTTCAGGACA	ATATGGACAG	TGGGCCCCAA	AGCTGGCCAG	ACTTCATTAT
45	3541	TCCGGATCAA	TCAATGCCCTG	GAGCACCAAG	GAGCCCTTTT	CTTGGATCAA	GGTGGATCTG
	3601	TTGGCACCAC	TGATTATTCA	CGGCATCAAG	ACCCAGGGTG	CCCGTCAGAA	GTTCCTCCAGC
	3661	CTCTACATCT	CTCAGTTTAT	CATCATGTAT	AGTCTTGATG	GGAGAAGTG	GCAGACTTAT
	3721	CGAAGTAAAT	CCACTGGAAC	CTTAATGGTC	TTCTTTGGCA	ATGTGGATTG	ATCTGGGATA
	3781	AAACACAATA	TTTTTAACCC	TCCAATTATT	GCTCGATACA	TCCGTTTGCA	CCCAACTCAT
50	3841	TATAGCATTC	GCAGCACTCT	TCGCATGGAG	TTGATGGGCT	GTGATTTAA	TAGTTGCAGC
	3901	ATGCCATTGG	GAATGGAGAG	TAAAGCAATA	TCAGATGCAC	AGATTACTGC	TTCATCTCAC
	3961	TTTACCAATA	TGTTTGCCAC	CTGGTCTCCT	TCAAAAGCTC	GACTTCACCT	CCAAGGGAGG
	4021	AGTAATGCCT	GGAGACCTCA	GGTGAATAAT	CCAAAAGAGT	GGCTGCAAGT	GGACTTCCAG
	4081	AAGACAATGA	AAGTCACAGG	AGTAACACT	CAGGGAGTAA	AATCTCTGCT	TACCAGCATG
55	4141	TATGTGAAGG	AGTTCTCAT	CTCCAGCAGT	CAAGATGGCC	ATCAGTGGAC	TCTCTTTTTT
	4201	CAGAATGGCA	AAGTAAAGGT	TTTTTCAGGGA	AATCAAGACT	CCTTCACACC	TGTGGTGAAC
	4261	TCCTAGACC	CACCGTTACT	GACTCGCTAC	CTTCGAATTC	ACCCCCAGAG	TTGGGTGCAC
	4321	CAGATTGCC	TGAGGATGGA	GGTTCTGGGC	TGCGAGGCAC	AGGACCTCTA	CGACAAAAC
	4381	CACACATGCC	CACCGTGCCC	AGCACCTGAA	CTCCTGGGAG	GACCGTCAGT	CTTCTCTTTC
	4441	CCCCCAAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACCC	CTGAGGTAC	ATGCGTGGTG
60	4501	GTGGAGCTGA	GCCACGAAGA	CCCTGAGGTC	AAGTTCAACT	GGTACGTGGA	CGCGGTGGAG
	4561	GTGCATAATG	CCAAGACAAA	GCCGCGGGAG	GAGCAGTACA	ACAGCACGTA	CCGTGTGGTC
	4621	AGCGTCTCTA	CCGTCTGCA	CCAGGACTGG	CTGAATGGCA	AGGAGTACAA	GTGCAAGGTC
	4681	TCCAACAAAG	CCCTCCAGC	CCCCCTCGAG	AAAACCATCT	CCAAAGCCAA	AGGCGAGCCC
	4741	CGAGAACCAC	AGGTGTACAC	CCTGCCCCCA	TCCCGCGATG	AGCTGACCAA	GAACCAAGTC
65	4801	AGCCTGACCT	GCCTGGTCAA	AGGCTTCTAT	CCCAGCGACA	TCCGCGTGGA	GTGGGAGAGC
	4861	AATGGGCGAG	CGGAGAACAA	CTACAAGACC	ACGCCTCCCG	TGTTGGACTC	CGACGGCTCC
	4921	TTCTTCTCTF	ACAGCAAGCT	CACCGTCGAC	AAGAGCAGGT	GGCAGCAGGG	GAACGTCTTC
	4981	TCATGCTCCG	TGATGCATGA	GGCTCTGCAC	AACCACTACA	CGCAGAAGAG	CCTCTCCCTG
	5041	TCTCCGGGTA	AACGGCGCCG	CCGGAGCGGT	GGCGCGGAT	CAGGTGGGGG	TGGATCAGGC
70	5101	GGTGGAGGTT	CCGGTGGCGG	GGGATCCGGC	GGTGGAGGTT	CCGGTGGGGG	TGGATCAAGG
	5161	AAGAGGAGGA	AGAGGGACAA	AACTCACACA	TGCCCAACCG	GCCCAGCTCC	AGAAGCTCTG
	5221	GGCGGACCGT	CAGTCTTCTT	CTTCCCCCCA	AAACCCAAAG	ACACCCCTCAT	GATCTCCCCG
	5281	ACCCCTGAGG	TCACATGCGT	GGTGGTGGAC	GTGAGCCACG	AAGACCTGGA	GGTCAAGTTC
	5341	AACTGGTACG	TGGACGGCGT	GGAGGTGCAT	AATGCCAAGA	CAAAGCCGCG	GGAGGACGAG
75	5401	TACAACAGCA	CGTACCGTGT	GGTCAGCGTC	CTCACCGTCC	TGCACCAAGG	CTGGCTGAAT
	5461	GGCAAGGAGT	ACAAGTGCAA	GGTCTCCAAC	AAAGCCCTCC	CAGCCCCCAT	CGAGAAAAAC
	5521	ATCTCCAAAG	CCAAAGGGCA	GCCCCGAGAA	CCACAGGTGT	ACACCCCTGCC	CCCATCCCCG
	5581	GATGAGCTGA	CCAAGAACCA	GGTCAGCCTG	ACCTGCCTGG	TCAAAGGCTT	CTATCCCAGC
	5641	GACATCGCCG	TGGAGTGGGA	GAGCAATGGG	CAGCCGGAGA	ACAACATCAA	GACCAAGCCT
80	5701	CCCGTGTGG	ACTCCGACGG	CTCTTCTTTC	CTCTACAGCA	AGCTCACCTG	GGACAAGAGC
	5761	AGGTGGCAGC	AGGGGAACGT	CTTCTCATGC	TCCGTGATGC	ATGAGGCTCT	GCACAACCA

5 5821 TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGA

FVIII-049 amino acid sequence. Signal sequence is shown
in dotted underline, and linker with proprotein
convertase processing sites is shown in bold

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1 MQIELSTCEE.LCLLRECFSA TRRYYLGAWE LSWDYMQSDL GELPVDARFP PRVPKSFPPN
61 TSVVYKRTLF VEFTHLHFN AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
121 GVSYWKASEG AEYDDQTSQR EKEDDKVFPG GSHTYVWQVL KENGPMASDP LCLTYSYLSH
181 VDLVKDLNSG LIGALLVCRE GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
241 AASARAWPKM HTVNGYVNR LPLGLGCHRK SVYWHVIGMG TPEVHSIFL EGHFTFLVRNH
301 QASLEISPI TFLTAQTLLM DLGQFLFLFCH ISSHQHDGME AYVKVDSCPE EPQLRMKNNE
361 EABDYDDDLT DSEMDVVRFD DDNSPSPFIQI RSVAKKHPKT WVRHIAAEEE DWDYAPLVLA
421 PDRSYKSQY LNNGPQRIGR KYKKVRFMAY TDETFKTREA IQHESGILGP LLYGEVGDTL
481 LIIFKNQASR PYNIPHGIT DVRPLYSRRL PRGVRHLKDF PILPGEIFKY KWTVTIVEDGP
541 TKSDPRCLTR YYSSFVNMR DLASGLIGPL LICYKESVDQ RGNQIMSDEK NVILFSVFDE
601 NRSWYLTEI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL HEVAYWYILS
661 IGAQTDFLSV FFSGYTFKHK MVEDTLTLF PFSGETVFMS MENPGLWILG CHNSDFRNRG
721 MTALLKVSSC DKNTGDYED SYEDISAYLL SKNNAIEPRS PSQNPPVLKR HQREITRTTL
781 QSDQEEIDYD DTISVEMKKE DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
841 HVLNRNAQSG SVPQFKKVVF QEFTDGSFTQ FLYRGELNEH LGLLGPYIRA EVEDNIMVTF
901 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQHMAP TKDEFDCXAW
961 AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE FALFFTIFDE TKSIFYTENM
1021 ERNCRAPCNI QMEDPTFKEN YRFHAINGYI MDTLPLGLVMA QDQRIRWYLL SMGSNENIHS
1081 IHFSGHVFTV RKKEEYKMAL YNLYPGVPET VEMPLPSKAGI WRVECLIGE HLAGMSTLFL
1141 VYSNKCQTPL GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1201 LAPMIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGILMV FFGNVDSSGI
1261 KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS MPLGMESKAI SDAQITASSY
1321 FTMNFATWSP SKARLHLQGR SNAWRPQVNN PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM
1381 YVKEFLISS QDGHQWTLFF QNGKVKVPQG NQDSFTPVVN SLDPPLTRY LRIHPQSWVH
1441 QIALRMEVLG CEAQDLYDKT HTCPCCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1501 VDVSHEDPEV KFNWYVDGVE VHNARTKPRE EQYNSTYRVV SVLTVLHQDW LNKKEYKCKV
1561 SNKALPAIE KTISKAKGP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES
1621 NGQPENNYKT TPPVLDSGSG FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHTQKSLSL
1681 SPGKRRRRSG GGGSGGGSG GGGSGGGSG GGGSGGGSG KRRKRDKTHT CPFCPAPELL
1741 GGSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
1801 YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
1861 DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTP PVLDSDGSEF LYSKLTVDKS
1921 RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK*

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What is claimed is:

1. A chimeric clotting factor which comprises i) a clotting factor
 10 selected from the group consisting of FVII, FIX and FX and ii) a
 targeting moiety which binds to platelets and, optionally, iii) a spacer
 moiety between the clotting factor and the targeting moiety.
2. The chimeric clotting factor of claim 1, which comprises a structure
 represented by the formula A B C, wherein A is the clotting factor;
 15 wherein B is a spacer moiety; and wherein C is at least one targeting
 moiety which binds to platelets.
3. The chimeric clotting factor of claim 2, which comprises a structure
 from amino terminus to carboxy terminus represented by a formula
 selected from the group consisting of: A B C; C B A.
- 20 4. The chimeric clotting factor of claim 1, wherein said chimeric
 clotting factor exhibits increased generation of thrombin in the presence
 of platelets as compared to an appropriate control lacking the at least
 one targeting moiety.
5. The chimeric clotting factor of claim 1, further comprising a
 25 scaffold moiety and, optionally, a second spacer moiety.
6. The chimeric clotting factor of claim 5, further comprising D and E,
 wherein D is a spacer moiety; and E is a scaffold moiety and wherein
 the chimeric clotting factor comprises a structure from amino terminus
 to carboxy terminus represented by a formula selected from the group
 30 consisting of: A B C D E; A D E B C; E D A B C; C B A D E; E D C B
 A; and C B E D A.
7. The chimeric clotting factor of claim 6, wherein E is a dimeric Fc
 region comprising a first Fc moiety, F1 and a second Fc moiety, F2.
8. The chimeric clotting factor of claim 7, wherein the clotting factor
 35 is expressed as a polypeptide comprising a cleavable scFc (cscFc) linker
 interposed between two Fc moieties, wherein the cscFc linker is
 adjacent to at least one enzymatic cleavage site which results in
 cleavage of the cscFc polypeptide linker.

- 5 9. The chimeric clotting factor of claim 8, wherein the cscFc linker is adjacent to at least one enzymatic cleavage site which results in cleavage of the cscFc linker.
10. The chimeric clotting factor of claim 9, wherein the at least one enzymatic cleavage site is an intracellular processing site.
- 10 11. The chimeric clotting factor of claim 9, wherein the polypeptide linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes.
12. The chimeric clotting factor of claim 9, wherein the polypeptide linker has a length of about 10 to about 50 amino acids.
- 15 13. The chimeric clotting factor of claim 9, wherein the polypeptide linker has a length of about 20 to about 30 amino acids.
14. The chimeric clotting factor of claim 9, wherein the polypeptide linker comprises a gly/ser peptide.
15. The chimeric clotting factor of claim 14, wherein the gly/ser peptide
20 is of the formula (Gly₄Ser)_n, or Ser(Gly₄Ser)_n wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.
16. The chimeric clotting factor of claim 15, wherein the (Gly₄ Ser)_n linker is selected from the group consisting of (Gly₄ Ser)₆, Ser(Gly₄ Ser)₆, (Gly₄ Ser)₄ and Ser(Gly₄ Ser)₄.
- 25 17. The chimeric clotting factor of claim 8, wherein the clotting factor comprises two polypeptide chains.
18. The chimeric clotting factor of claim 7, wherein the chimeric clotting factor has a struture selected from the group consisting of: A linked to F1 via a spacer moiety and C linked to F2; A linked to F1 via a spacer moiety and C linked to F2 via a spacer moiety; A linked to F1 and C is linked to F2 via a spacer moiety; A linked to F1 via a spacer moiety and C is linked to F2 via a spacer moiety.
- 30 19. The chimeric clotting factor of claim 18, which comprises two polypeptides wherein the first polypeptide comprises the moieties A B F1; A B F1; A B F1, A B F1 D C and the second polypeptide comprises the moieties C F2; C D F2; F2 D C, F2 D C, wherein the two polypeptide chains form an Fc region.
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- 5 20. The chimeric clotting factor of claim 7, wherein the targeting moiety is fused to at least one of the polypeptide chains of the Fc region.
21. The chimeric clotting factor of claim 7, wherein the targeting moiety is fused to at least one of F1 and F2 directly.
- 10 22. The chimeric clotting factor of claim 7, wherein the targeting moiety is fused to at least one of F1 and F2 via a spacer moiety.
23. The chimeric clotting factor of claim 7, wherein the targeting moiety is fused to at least one of F1 and F2 via a cleavable linker.
24. The chimeric clotting factor of claim 1, wherein the targeting moiety is selected from the group consisting of: an antibody molecule, an antigen binding fragment of an antibody molecule, an scFv molecule, a receptor binding portion of a receptor, a peptide.
- 15 25. The chimeric clotting factor of claim 1, 2, 5 or 7 wherein the targeting moiety binds to resting platelets.
- 20 26. The chimeric clotting factor of 1, 2, 5 or 7 wherein the targeting moiety selectively binds to activated platelets.
27. The chimeric clotting factor of claim 25, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIIb, GPVI, and the nonactive form of GPIIb/IIIa.
- 25 28. The chimeric clotting factor of claim 26, wherein the targeting moiety selectively binds to a target selected from the group consisting of: the active form of GPIIb/IIIa, P selectin, GMP-33, LAMP-1, LAMP-2, CD40L, and LOX-1.
29. The chimeric clotting factor of claim 25, wherein the targeting moiety binds to the GPIb complex
- 30 30. The chimeric clotting factor of claim 25 wherein the targeting moiety is a peptide selected from the group consisting of: PS4, OS1, and OS2.
31. The chimeric clotting factor of claim 25, wherein the targeting moiety comprises an antibody variable regions from an antibody selected from the group consisting of: SCE5, MB9, and AP3.
- 35 32. The chimeric clotting factor of claim 1, 2, 5, or 7 wherein the clotting factor is Factor VII.

- 5 33. The chimeric clotting factor of claim 1, 2, 5, or 7, wherein the clotting factor is a high specific activity variant of Factor VII.
34. The chimeric clotting factor of claim 1, 2, 5, or 7 wherein the clotting factor is Factor IX.
35. The chimeric clotting factor of claim 1, 2, 5, or 7 wherein the
10 clotting factor is a high specific activity variant of Factor IX.
36. The chimeric clotting factor of claim 1, 2, 5, or 7 wherein the clotting factor is Factor X.
37. The chimeric clotting factor of claim 1, 3, 4, or 5 wherein the clotting factor is a high specific activity variant of Factor X.
- 15 38. The chimeric clotting factor of claim 8, wherein the clotting factor is secreted by a cell in active form.
39. The chimeric clotting factor of claim 1, wherein the clotting factor is activated in vivo.
40. The chimeric clotting factor of claim 39 wherein the chimeric
20 clotting factor comprises a heterologous enzymatic cleavage site not naturally present in the clotting factor.
41. The chimeric clotting factor of claim 40, wherein enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.
- 25 42. The chimeric clotting factor of claim 5, wherein the scaffold moiety is a protein molecule which increases the hydrodynamic radius of the chimeric clotting factor.
43. The chimeric clotting factor of claim 42, wherein the scaffold moiety, if present, is selected from the group consisting of albumin and
30 XTEN[®].
44. A polypeptide comprising FVII, which FVII comprises a heterologous enzymatic cleavage site activatable by a component of the clotting cascade.
45. The polypeptide of claim 44, wherein the polypeptide comprises a
35 scaffold moiety and, optionally, a spacer moiety.
46. The chimeric clotting factor of claim 45, wherein the scaffold moiety is a dimeric Fc region comprising a first Fc moiety, F1 and a second Fc moiety, F2.

- 5 47. The chimeric clotting factor of claim 44, wherein the clotting factor comprises two polypeptide chains.
48. The chimeric clotting factor of claim 44, wherein the chimeric clotting factor has a structure selected from the group consisting of: the clotting factor linked to the first Fc moiety via a spacer moiety; the
10 clotting factor linked to the linked to the second Fc moiety via a spacer moiety; the clotting factor is directly linked to F1; and the clotting factor is directly linked to F2.
49. The chimeric clotting factor of claim 47, further comprising a targeting moiety, C.
- 15 50. The chimeric clotting factor of claim 47, wherein the chimeric clotting factor is synthesized as a single polypeptide chain comprising a cscFc linker.
51. The chimeric clotting factor of claim 49, wherein the cscFc linker is adjacent to at least one enzymatic cleavage site which results in
20 cleavage of the linker.
52. The chimeric clotting factor of claim 50, wherein the at least one enzymatic cleavage site is an intracellular processing site.
53. The chimeric clotting factor of claim 50, wherein the cscFc linker is flanked by two enzymatic cleavage sites which are recognized by the
25 same or by different enzymes.
54. The chimeric clotting factor of claim 49, wherein the cscFc linker has a length of about 10 to about 50 amino acids.
55. The chimeric clotting factor of claim 49, wherein the cscFc linker has a length of about 20 to about 30 amino acids.
- 30 56. The chimeric clotting factor of claim 49, wherein the cscFc linker comprises a gly/ser peptide.
57. The chimeric clotting factor of claim 56, wherein the gly/ser peptide is of the formula (Gly₄Ser)_n, or Ser(Gly₄Ser)_n wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and
35 10.
58. The chimeric clotting factor of claim 56, wherein the (Gly₄ Ser)_n linker is selected from the group consisting of (Gly₄ Ser)₆, Ser(Gly₄ Ser)₆, (Gly₄ Ser)₄ and Ser(Gly₄ Ser)₄.

- 5 59. The chimeric clotting factor of claim 45, wherein the clotting factor is a high specific activity variant of Factor VII.
60. The chimeric clotting factor of claim 44 wherein the heterologous enzymatic cleavage site not naturally present in the clotting factor is cleaved at the site of clot formation.
- 10 61. The chimeric clotting factor of claim 44, wherein the cleavage site is selected from the group consisting of: a factor XIa cleavage site, a factor Xa cleavage site, and a thrombin cleavage site.
62. The chimeric clotting factor of claim 59, wherein enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.
- 15 63. The chimeric clotting factor of claim 48 wherein the targeting moiety binds to resting platelets.
64. The chimeric clotting factor of 48, wherein the targeting moiety selectively binds to activated platelets.
- 20 65. The chimeric clotting factor of claim 63, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIIb, GPVI, and the nonactive form of GPIIb/IIIa.
66. The chimeric clotting factor of claim 64, wherein the targeting moiety selectively binds to a target selected from the group consisting of: the active form of GPIIb/IIIa, P selectin, GMP-33, LAMP-1, LAMP-2, CD40L, and LOX-1.
- 25 67. The chimeric clotting factor of claim 64, wherein the scaffold moiety is a protein molecule which increases the hydrodynamic radius of the chimeric clotting factor.
- 30 68. The chimeric clotting factor of claim 67, wherein the scaffold moiety, if present, is selected from the group consisting of albumin and XTEN[®].
69. The chimeric clotting factor of claim 44, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: A B C; C B A; A B C D E; A D E B C, E D A B C, C B A D E, E D C B A, C B E D A, wherein A an activatable clotting factor, B is absent or is a linker, C is a targeting moiety, D is absent or is a linker, and E is a scaffold moiety.
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- 5 70. The chimeric clotting factor of claim 44, wherein the clotting factor comprises a light and heavy chain of a clotting factor and each of the light and heavy chains are expressed as separate polypeptide chains.
71. A nucleic acid molecule encoding a chimeric clotting factor of any one of claims 1, 2, 5, 7, 44, 45, 45 and 58.
- 10 72. The nucleic acid molecule of claim 71, wherein the nucleic acid molecule is present in a vector.
73. The vector of claim 72 wherein the vector further comprises a nucleotide sequence encoding an enzyme which cleaves at least one of the enzymatic cleavage sites.
- 15 74. A host cell comprising the expression vector of claim 72.
75. A host cell comprising the expression vector of claim 72, wherein the host cell expresses an enzyme capable of intracellular processing.
76. The host cell of claim 75, wherein the enzyme is endogenous to the cell.
- 20 77. The host cell of claim 75, wherein the enzyme is heterologous to the cell.
78. A method for producing a chimeric clotting factor comprising culturing the host cell of claim 76 or 77 in culture and recovering the chimeric clotting factor from the medium.
- 25 79. A processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector of claim 78 in a cell cultured in cell culture medium and isolating the mature, heterodimeric polypeptide from the culture medium.
- 30 80. A composition comprising a chimeric clotting factor of any one of claims 1, 2, 5, 7, 44, 45, 48 and 58 and a pharmaceutically acceptable carrier.
81. A composition comprising the nucleic acid molecule of claim 71 and a pharmaceutically acceptable carrier.
- 35 82. A method for improving hemostasis in a subject, comprising administering the composition of claim 80 or 81.