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(54) Title: CHIMERIC CLOTTING FACTORS

(57) Abstract: Chimeric clotting factors which localize the therapeutic to sites of coagulation (e.g., by being targeted to platelets or being activatable at sites of coagulation), have reduced clearance rates, have improved manufacturability, have reduced thrombogenicity, have enhanced activity, or have more than one of these characteristics are described as are methods for making chimeric clotting factors and methods for improving hemostasis using these clotting factors.

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-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 20 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.

25 [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-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 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 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) 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 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 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

20 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.

25 [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.

30 [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 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.

10 [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: GPIba, 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, and AP3.

35 [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 XTN[®]

[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 structure 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, 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 (Gly₄Ser)_n linker is selected from the group consisting of (Gly₄Ser)6, Ser(Gly₄Ser)6, (Gly₄Ser)4 and Ser(Gly₄Ser)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: GPIba, 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 30 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 XEN[®].

[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 is 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.

[0052] In another embodiment, the invention pertains to a method for producing a

15 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

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 a 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 a 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:CD₂; 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 a 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.

[0061a] Definitions of specific embodiments of the invention as claimed herein follow.

[0061b] According to a first embodiment of the invention, there is provided a chimeric clotting factor comprising:

(i) a clotting factor selected from the group consisting of factor VII (FVII), factor

25 IX (FIX) and factor X (FX);

(ii) a targeting moiety which binds to platelets; and

(iii) a first Fc moiety and a second Fc moiety,

wherein the clotting factor is fused to the first Fc moiety, and

wherein the targeting moiety is fused to the second Fc moiety.

30 [0061c] According to a second embodiment of the invention, there is provided a nucleic acid molecule encoding the chimeric clotting factor of the first embodiment.

[0061d] According to a third embodiment of the invention, there is provided an expression vector comprising the nucleic acid molecule of the second embodiment.

[0061e] According to a fourth embodiment of the invention, there is provided an isolated host

cell comprising the expression vector of the third embodiment.

[0061f] According to a fifth embodiment of the invention, there is provided a method of producing a chimeric clotting factor, said method comprising culturing the host cell of the fourth embodiment in culture medium and recovering the chimeric clotting factor from the medium.

[0061g] According to a sixth embodiment of the invention, there is provided a processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector of the third embodiment in a cell cultured in cell culture medium and isolating the heterodimeric polypeptide from the cell culture medium.

[0061h] According to a seventh embodiment of the invention, there is provided a composition comprising the chimeric clotting factor of the first embodiment and a pharmaceutically acceptable carrier.

[0061i] According to an eighth embodiment of the invention, there is provided a composition comprising the nucleic acid molecule of the second embodiment and a pharmaceutically acceptable carrier.

[0061j] According to a ninth embodiment of the invention, there is provided a method of improving hemostasis in a subject, said method comprising administering to the subject the composition of the seventh or eighth embodiments.

[0061k] The term “comprise” and variants of the term such as “comprises” or “comprising” are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

[0061l] Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

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 which is cleavable in vivo at the site of a clot.

30 [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 GPIIbIIIa 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 illustrating their cleavage.

15 [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 FVIIIC 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 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.

30 [0100] Figure 39 illustrates the results of an assay measuring activation of FX by FVIIa using substrate S2765, which is not cleaved by FVIIa. In this assay, 10 uM 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 FVIIFc." 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, FVIIFc (100 nM) 10 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 FVIIFc (FVII-118) to the high specific activity variant 20 (FVII-127).

[00105] Figure 44A illustrates several targeted constructs. In this instance, an SCE5 scFv which binds to the active conformation of GPIIbIIIa 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 25 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 GPIIbIIIa present on resting and activated platelets. Figure 45B shows the results of thrombin generation assays in platelet-rich FVIII-deficient 30 plasma. Figure 45C shows the results of binding of rFVIIaFc variants to platelets by FACS.

[00107] Figure 46A shows several targeted FVIIa constructs that target GPIb-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 35 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 plasma. In this experiment, the assay was activated with tissue factor (top panel) or by platelet activation (bottom panel).

10 [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.

25 **[00117]** The targeting moiety can be positioned at a number of places in a 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.

30 **[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. 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 SVSQTTSKLTR. Exemplary thrombin cleavage sites include, e.g.,: DFLAEGGGVRL, 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.

15 [00119] The heterologous cleavage site is present in a cleavable linker can be 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.

20 [00120] In one embodiment, a chimeric clotting factor of the invention 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 cleavable scFc regions (comprising a cscFc regions as described herein), less complicated proteins or portions thereof, e.g., XTen polypeptides®, or albumin.

30 [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 occurring (or wild type (WT)) Fc moiety. In another embodiment, the Fc moiety comprises one or more variations in sequence.

35 [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 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.

[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 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, ornithine, 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* 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
15 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
20 hydrophobic interactions and electrostatic interactions.

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

30 [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 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.

10 [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 which may include additional spacer linkers on either the N terminal or 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 (Gly₄ Ser)_n (SEQ ID NO:4). Another exemplary gly-ser polypeptide linker comprises the amino acid sequence S(Gly₄ Ser)_n.

25 [00137] In one embodiment, n=1. In one embodiment, n=2. In another embodiment, n=3, i.e., (Gly₄ Ser)₃. In another embodiment, n=4, i.e., (Gly₄ 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 Ser(Gly₄ 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.

35 [00138] A polypeptide or amino acid sequence "derived from" a designated 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, 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 10 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).

15 **[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 20 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 25 days.

25 **[00149]** As used herein the term "moiety" refers to a component part or constituent of a chimeric polypeptide.

30 **[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 35 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 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.,
15 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
20 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
25 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 35 DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOLV) 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,

10 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).

15 [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,

20 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.

25 Exemplary host cell lines include, but are not limited to, DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HEGLA (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),

30 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-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 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 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 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 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 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) 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.

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

20 [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 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.

30 [00166] A “chimeric protein” or “fusion protein”, as used herein, refers to any 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 V, Factor VII, Factor X or factor XIII, Bernard-Soulier syndrome is a defect or 35 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.

20 [00176] Exemplary chimeric clotting factor constructs of the invention are set forth in the accompanying Figures. Although the Figures generally illustrate the 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.

25 [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 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 CaCl₂ 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 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 (Kcat or Km). 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 (LQQSRKVGDSPN, 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
10 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
15 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
20 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
25 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.,
30 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
35 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 10 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 15 embodiments, a B-domain-deleted Factor VIII has a deletion described in col. 1, lines 25 to col. 2, line 40 of US Patent No. 5,972,885; col. 6, lines 1-22 and example 1 of U.S. Patent no. 6,048,720; col. 2, lines 17-46 of U.S. Patent No. 5,543,502; col. 4, line 22 to col. 5, line 36 of U.S. Patent no. 5,171,844; col. 2, lines 55-68, figure 2, and example 1 of U.S. Patent No. 5,112,950; col. 2, line 2 to col. 19, line 21 and table 2 of U.S. Patent No. 4,868,112; col. 2, line 1 to col. 3, line 19, col. 3, line 40 to col. 4, line 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 25 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 30 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.* 35 *Biochemistry* (1986) 25:8343-8347)), 741 through 1646 (Kaufman (PCT published application No. WO 87/04187)), 747-1560 (Sarver, *et al.*, *DNA* (1987) 6:553-564)), 741 through 1648 (Pasek (PCT application No.88/00831)), or 816 through 1598 or 741 through 1648 (Lagner (Behring Inst. Mitt. (1988) No 82:16-25, EP 295597)), 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 endoplasmatic 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 as prothrombin, coagulation factors VII, IX and X, proteins C, S, and Z. These proteins require vitamin K for the posttranslational synthesis of γ -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 Ca²⁺ 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 L-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

10 [00204] 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 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**

25 [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 than platelets, i.e., the targeting moieties specifically bind to platelets.

30 [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.

35 [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 GpIIb/IIIa as well as CD62P.

30 [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 GPIIbIIIa. 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 GPIIbIIIa. 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 10 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.

15 [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.

20 [00211] In one embodiment, a targeting moiety is fused to a chimeric clotting factor of the invention by a cleavable 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 cleavable linker and, therefore, is not cleaved at the site of a clot.

25 [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.

30 [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 25 an antigen binding portion. The term “antigen-binding portion” refers to a 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 35 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 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 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 VL and/or VH 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 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 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).

[00219] In certain embodiments, a scFv molecule employed in a polypeptide of the invention is a stabilized scFv molecule. In one embodiment, the stabilized scFv 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 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.

[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 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 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 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 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 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

10 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.

15 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-

20 103 (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 30 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

35 sufficiently proximal such that binding of one antibody interferes with binding of the other.

[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 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.
15 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
20 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
25 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.
30 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;
35 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 VH or VL 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 VH 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 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 GPIIbIIIa. 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).

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 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 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 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 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 GPIba 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 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.

20 [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 SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g., DFLAEGGGVR, TTKIKPR, and a sequence comprising or consisting of ALRPR (e.g. ALRPRVVVGGA)).

25 [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 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, XTN, 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, XTN, 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 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 (CDC) and/or to improve manufacturability).

10 [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)

15 [00242] 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 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 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.

20 [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.

25 [00244] In one embodiment, F1 and F2 each comprise a CH₂ and CH₃ moiety.

30 [00245] In one embodiment, after cleavage and substantial excision of the scFc 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 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 15 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.

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 at 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 15 least one Fc moiety comprising a complete CH3 domain (about amino acids 341-438 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 20 one Fc moiety comprising at least a CH3 domain, and at least one of a hinge region (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 30 least one Fc moiety comprising an FcRn binding partner. An FcRn binding partner is 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 KA is higher than 10⁶ M⁻¹, or more preferably higher than 10⁸ 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 direction, and then releases the IgG at relatively higher pH found in the interstitial fluids. It is expressed in adult epithelial tissue (U.S. Pat. Nos. 6,485,726, 6,030,613, 6,086,875; WO 03/077834; US2003-0235536A1) including lung and intestinal epithelium (Israel et al. 1997, Immunology 92:69) renal proximal tubular epithelium (Kobayashi et al. 2002, Am. J. Physiol. Renal Physiol. 282:F358) as well as nasal epithelium, vaginal surfaces, and biliary tree surfaces.

20 [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 been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. The FcRn binding partners include whole IgG, the Fc fragment of IgG, and other fragments of IgG that include the complete binding region of FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of 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, Md.

35 [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 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, 20 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 25 of the biologically active molecule (e.g., EPO, IFN, Factor VII, Factor IX, T20). 30

[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.

10 Mutations believed to impart an increased affinity for FcRn include T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591).

[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 Fc γ RI, Fc γ RII, and Fc γ RIII, 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 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 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.

15 [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 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

20 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

25 Fc domain.

30 [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
15 same, sequence composition (herein termed a “homodimeric Fc region”). In other 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.

[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. Fc γ RI, Fc γ RII, or Fc γ RIII) 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, US2007/0248603, US2007/0286859,
US2008/0057056; 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.

16 [00273] In certain embodiments, a polypeptide of the invention comprises an
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.

17 [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
20 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
25 with decreased FcRn binding affinity are expected to have shorter half-lives, and such
molecules are also useful, for example, for administration to a mammal where a
shortened circulation time may be advantageous, e.g. for in vivo diagnostic imaging
or in situations where the starting polypeptide has toxic side effects when present in
the circulation for prolonged periods. Fc variants with decreased FcRn binding
30 affinity are also less likely to cross the placenta and, thus, are also useful in the
treatment of diseases or disorders in pregnant women. In addition, other applications
in which reduced FcRn binding affinity may be desired include those applications in
which localization the brain, kidney, and/or liver is desired. In one exemplary
embodiment, the 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 region. More preferably, the alteration does not interfere with the ability of the scFc

35 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 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 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 escFc 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 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
15 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.

20

[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
35 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 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
10 upper hinge region (e.g., derived from an IgG1, IgG2, IgG3, or IgG4 molecule), 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 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 integer, 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
15 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
20 50 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,
25 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 SVSQTSLTR. Exemplary thrombin cleavage sites include, e.g., DFLAEGGGVRL, TTKIKPR, LVPRG SEQ ID NO:35) and ALRPRVVVGGA. 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).

15 [00298] The expression vectors can encode for tags that permit for easy purification or identification of the recombinantly produced protein. Examples 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. PreCision Protease (Pharmacia, Peapack, N. J.)) for easy removal of the tag after purification.

25 [00299] For the purposes of this invention, numerous expression vector systems may be employed. These expression vectors are typically replicable in the host 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 30 termination sequences. Preferably, the expression control sequences are eukaryotic 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 MOLV), 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), HEA (human cervical carcinoma), CV1 (monkey kidney line), COS (a derivative of CV1 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

10 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

15 published in 1990. Van den Ouwehand 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/

25 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

30 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.

35 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.

10 [00311] PACE and PACE4 have been reported to have partially overlapping 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; Rehementulla A et al. (1993) *Biochemistry.* 32:11586-90.

15 [00312] U.S. Pat. No. 5,840,529, issued to Seidah et al., discloses nucleotide 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.

20 [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. 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.

25 [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 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 *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.

30 [00315] In addition to prokaryotes, eukaryotic microbes may also be used. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among eukaryotic microorganisms although a number of other strains are commonly available.

35 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. Ycast 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 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
25 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
30 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 30 molecules which comprise a nucleotide sequence encoding a chimeric clotting factor 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, 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

20 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.

25 [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

30 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.

10 [00332] Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, 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 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 20 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 25 subject suffering from, or predisposed to clotting disorders.

30 [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.

35 [00335] Effective doses of the compositions of the present invention, for the 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.

10 [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 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).

25 [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 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 required. Dosage and frequency vary depending on the half-life of the polypeptide in the patient.

35 [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).

15 [00342] As used herein, the administration of polypeptides of the invention in 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.

25 [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 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.

35 [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 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.

15 [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 using techniques known in the art, including via vector, plasmid, liposome, DNA injection, electroporation, gene gun, intravenously injection or hepatic artery infusion. 20 Vectors for use in gene therapy embodiments are known in the art.

25 [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 accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic or prophylactic effect.

30 [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 not limited to, hemostatic disorders.

35 [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.

40 [00350] The chimeric clotting factors of the invention treat or prevent a 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.

45 [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 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.

10 [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 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.

15 [00358] This invention is further illustrated by the following examples which 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); Antibody Engineering Protocols (Methods in Molecular Biology), 510, Paul, S., Humana Pr (1996); *Antibody Engineering: A Practical Approach* (Practical Approach Series, 169), McCafferty, Ed., Irl Pr (1996); *Antibodies: A Laboratory Manual*, Harlow *et al.*, CS.H.L. Press, Pub. (1999); and *Current Protocols in Molecular Biology*, eds. Ausubel *et al.*, John Wiley & Sons (1992).

30 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-BpsEI-Fc-F
AGTCAAGCTTGCGACTCCGGAACTCCACCGCCGATCCACCCCCACCTGATCCACCCCCACCTTAC
BamHI-linker-Fc-R
CATCGGATCCCCGGCACCGGAACCTCCACCGCCGATCCACCCCCACCTGATCCACCCCCACCTTAC
25 CCGGAGACAGGGAGAGG
BcII-Fc-F
CAGTCTTGATCAGACAAACTCACACATGCCACC
scFc-EcoRI-R
ACTGACGAATTCTCATTACCCGGAGACAGGGAG
30 HindIII-Kozak-FVII-F:
CGACAAGCTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
FVII-HC-BpsEI-R:
AGGAGTTCCCCGAGCTCCGCACCCCTCCCAAGCTGTCAGTTTCTCCGATCCCCCCCCACCCGAACTCCA
CCGCCTGATCCACCCCCACCTGATCCGGCCACCCGGAGCCACCTCCGGAGCCACCGCCACCGGGA
35 AAATGGGCTCGCAGGAGG
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

10 the Fc coding region. Next, a 50 μ l 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

15 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 μ l 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

20 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

25 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: Genescrypt-FVII-027-1 and

30 Genescrypt-FVII-026-2. Genescrypt-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

35 overlaps the last 6 bases of Genescrypt-FVII-027-1. In addition, the first 6 bases at the 5' include a SalI site found within the Fc region. Genescrypt-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.

10 [00365] Genescript-FVII-027-1 was cloned into the Sapi and EcoRI sites of 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.

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

Cloning of FVII-037

20 [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 GPIbIIIa is attached to the c-terminus of the second Fc moiety.

25 [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 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 Sall 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, ad 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 the SalI/EcoRI sites of pSYN-FVII-041 to generate pSYN-FVII-044, -045 and -046.

10 **Example 4. Heterodimeric Constructs comprising FVII-Fc and a peptide against GPIb 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 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 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 pSYNFVII-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 GPIb. 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. 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
CTTGGCTTCTCTCCACAGGC

35 [00379] A 50 μ l 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

CGACAAGCTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

BspEI-Fc-FVII-R

15 CGACTCCGGAGCTGGCACGGTGGCATGIGTGAGTTTGTGGAAATGGGCTCGCAGG

[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:

GGGCATGTGAGTTTGTCTGATCCCCCCCCACCGGAACCTCCACCGCTGATCCACCCCCACCTGAT
CCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGAAATGGGGCTCGCAGGAGG

Fc-linker-F:

5 GACAAAACCTCACACATGCCACC

Fc-linker-R:

GCAGAAFFCTCATTTACCCGGAG

[00381] Two 12 μ l 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 μ g 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 FVIIIFc/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 AGTCAAGCTTGTGACTCCCGAACCTCCTGGGGGACCG
BamHI-linker(PACE1)-Fc-R
CATCGGATCCCCCGCCACCGAACCTCCACCCCTGATCCACCCCCACCGATCCGGCCGCCACCGCTCC
GGCGGGCGCCGTTTACCCGGAGACAGGGAGAGG
HindIII-Kozak-FVII-F
10 CGACAAGCTTGCAGGCCACCATGGTCTCCCAGGCCCTCAGG
BspEI-Fc-linker-FVIIIC-R
GAGTTCCGGAGCTGGGCACGGTGGCATG1GTGAGTTTGCTGATCCCCGCCACCGAACCTCCACC
GCCTGATCCACCCCCACCTGATCCGGCCGCCACCCACCTCCGGAGCCACCGCCACCTCGGGC
TTGGGGTTTGCTGG
15 BamHI-2zlink-pace-HC-F
CAGTCTGGATCCGGCGGTGGAGGTTCCGGTGGGGTGGATCAAGGAAGAGGAGAGGATTGTGGGG
GGCAAGGTGTGCC
Fc-EcoRI-R
ATGTCGAATTCTCATTTACCCGGAGACAGGGAGAGG
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-FVIIIC-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-pacc-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-pacc-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 SalI 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 SalI to BsiWI sites of pSYN-FVII-057 was outsourced. This DNA was subcloned into the SalI/BsiWI sites of pSYN-FVII-073 to generate pSYN-FVII-057

Cloning of pSYN-FVII-058, pSYN-FVII-059, pSYN-FVII-060, pSYN-FVII-061

30 **and pSYN-FVII-062**

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

Cloning of pSYN-FVII-066

35 The synthesis of the DNA sequence comprising nucleotides from the SalI to RsrII sites of pSYN-FVII-066 was outsourced. This DNA was subcloned into the SalI/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 SalI to EcoRI sites of pSYN-FVII-067 was outsourced. This DNA was subcloned into the SalI/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 10 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 25 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 30 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 35 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-terminus of the protein followed by a 6x(GGGGS) 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:

AQVQLQESGGGLVQPGGSRLSCAASGFMPFSRYAMSWVRQAPGKGPEWVSGISGSGGSTYYADSVKGRFTVS RDNSKNTLYLQMNSLRAEDTAVYYCARGATYTSRSDVPDQTSFDYWGQGTLTVSSGSASAPKLEE
25 GEFSEARVSELITQDPAVSVALGQTVRITCQGDSLRLNFYASWYQQKPGQAPTLVLYGLSKRPSGIPDRFSASSSCNTASLTITCAQAEDEADYYCLLYCCCCQQCVFGGCTKLTVLRQPKAAPEVTLFPPSSAA

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 Sall to RsrII 35 sites of pSYN-FVII-088 was outsourced. This DNA was subcloned into the Sall/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 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).

10 The coding sequence for human recombinant Fc was obtained by RT-PCR 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 FVIIIFc DNA sequence was cloned into the mammalian dual expression vector pBUDCE4.1 (Invitrogen) under control of the CMV promoter.

15 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.

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

30 **Cloning of pSYN-FVIII-049**

Generated intermediate pSYN-FVIII-048 by cloning NheI/Xhol 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 fragment was subcloned into the RsrII/XbaI sites of pSYN-FVIII-048 to generate

35 pSYN-FVIII-049

Cloning of pSYN-FVIII-108

A SalI/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
AGGAGTCCGGAGCTGGCACGGTGGCATGTGTGAGTTTGTGGATCC
CCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCC
25 ACCGGACCCACCTCCGGAGCCACCGCCACCGGGAAATGGGGCTCGCA
GGAGG

FVII-HC-Hind3-IggKss-f
ACTGACAAGCTGCCGCCACCATGGAGACAGACACACTCCTGCTATGGGT
30 ACTGCTGCTCTGGGTTCCAGGTTCCACTGGTATTGTGGGGGGCAAGGTGTG
C

FVII-LC-NotI-F
ACTGACGCCGCCGCCACCATGGTCTCCCAGG
35 FVII-LC-XhoI-R
ACTGACCTCGAGTTATGCCCTGGGTTGCTGG

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 ad subclone in pSYN-FVII-012 to generate pSYN-FVII-013

10 Primers
 FVII-LC-6xlinker-BamHI-
 RACTGACGGATCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCA
 CCTGATCCGCCACCGGACCCACCTCCGCCGGAGCCACGCCACCTCG
 GCCTTGGGGTTTCTGGC
 HindIII-Kozak-FVII-F
 15 CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
 FVII-HC-NotI-F
 ACTGACGCCGCCGCCACCATGGAGACAGAC
 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 Xla 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 chain with a 1153V 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 H153V 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 H152V mutation was introduced in pSYN-FVII-039, -040 to increase FXIa cleavage efficiency

15 [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 and -040, respectively

Example 9. Transient Transfection of Constructs

20 [00395] For expression of constructs, HEK-293-F cells were grown in suspension in Freestyle media (Invitrogen) supplemented with vitamin K3 (For FVII and FIX transfections only) (Sigma Aldrich, St. Louis, MO) to 2 μ g/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.

25 [00396] Twenty-four hours prior to transfection cells were seeded at a density of 7x10⁵ cells/ml in growth media supplemented with LONGTMR3IGF-1 (Sigma Aldrich, St. Louis, MO) to 20 μ g/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 FVIIIFc Molecules (except FVII-028 and
FVII-053) and FIXFc molecules**

[00397] FVIIIFc 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 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 FVIIIFc proteins

[00400] FVIIIFc 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 FVIIFc were 10 pooled, and total protein was concentrated to 4 mg/ml. The CaCl₂ concentration was raised to 5 mM and the sample was incubated at 4°C for 24 to 48 hours until at least 80% of FVIIFc 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

	FVIIaFc	IU/nM
30	FVII-011	991
	FVII-024	929
	FVII-027	790
35	FVII-037	1131
	FVII-044	1300
	FVII-045	906
40	FVII-046	1145
	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 uM
SFLLRN peptide: in-house synthesis, stock concentration 5 mg/ml (6.7 mM),
working concentration 50 ug/ml (67 uM)

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 ul of ~2-4x10⁸ cells/ml gel-purified platelets to 1 ml of platelet buffer
- Make 100 ul 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 ul of
platelet buffer.
- Add 2.5 ul 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# HCX-0050, lot#X0401, 5.4 mg/ml

40 ATIII: HTI, cat# HCATHI-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 CaCl₂, pH 7.4 was incubated for 5 or 20 minutes, and cleavage of FVIIFc 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 FVIIFc 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-scFc linker-FXIa/thrombin cleavage site-heavy chain-linker-Fc). In some embodiment, the heavy chain will comprise the 1152V 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 10 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. 15 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 20 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 25 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 30 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 35 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 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 FVII-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 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 (LQQSRKVGDSPN, 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 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

[00427] In this example, chromogenic assays were used to measure FVII 30 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. 25 S2765 substrate was added and FXa generation was detected by monitoring substrate 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 FVIIFc 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 GPIIbIIIa
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 GPIIbIIIa. 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 GPIIbIIIa receptor, FVII-066 was tested in thrombin generation assays using mouse FVIII-deficient platelet rich plasma, 10 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 15 GPIIbIIIa

In this example, construct FVII-027 illustrated in Figure 12A was cloned, expressed (with PC5 cotransfection to fully process the escFc 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, 20 a scFv that has been shown to bind to the active conformation of GPIIbIIIa. 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 25 (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 30 for binding to the activated form of GPIIbIIIa, demonstrating the effects are mediated by the interaction of MB9 with the activated form of GPIIbIIIa. 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 35 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 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 10 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 15 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
10 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-
15 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
20 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
25 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.
30 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 FVIIIIFc variant targeted to the active form of GPIbIIIa

[00437] The constructs illustrated in Figure 49A were made as previously described. FVIII-041 is wild type FVIIIIFc, 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 FVIIIIFc. 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 FVIIaFc 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 GPIIbIIIa binding, inhibits thrombin generation
25 activity associated with FVII-053, suggesting that platelet targeting is important for 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 FVIIIFc

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 sample was treated with the irreversible active site inhibitor glutamyl-glycyl-arginyl-
10 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 IU/nmol; FIX068, 5.1 IU/nmol; FIX-088, 3.5 IU/nmol; FIX-090, 13.8 IU/nmol, and
15 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 generation 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

MVSOARL**IC LLLGLCG**CL** AVEVTQ**EE**AL GVLHH**PP**AN AFL**EE**ELRPGS LEPE**EE**KEFQC**
SFEEARE**IFK DA**EP**RT**KL**FIWI SYSD**GD**QC**AS** SPC**Q**NGG**S**C**K**C DQL**Q**SY**IC**FC LFA**FE**GR**NC**E
THKDD**Q**LI**CV N**EN**GC**EQ**YC SDHT**GT**K**RS**C R**CH**E**Y**S**LL**A DG**V**S**C**TPT**VE** Y**PC**G**KI**P**LE**
KRNAS**K**PQ**GR IVGG**KV**CP**KG** E**CP**W**QV**L**LL**L N**GA**O**LC**GG**TL** INT**WV**V**S**A H**CF**D**KI**KN**WR**
NLI**AV**LG**E**RD** L**SE**H**DG**D**EQ**S R**RV**A**QV**I**IP**S TY**VP**G**TT**N**RD** I**AI**L**RL**H**Q**P**V** VL**TD**H**V**P**LC**
LPE**R**TF**S**ER**T L**AF**V**RF**S**LV**S GW**Q**L**LL**D**RG**A T**AE**L**MV**L**LN**V F**R**L**M**T**Q**D**CL**Q Q**SE**K**VG**D**SP**N
ITE**Y**MF**C**AG** Y**DG**S**KD**S**CK**G D**SG**G**PH**A**II**Y R**GT**W**Y**L**TG**I**IV** SW**GQ**G**CA**T**VG** H**FG**V**Y**T**RV**S**Q**
YI**E**W**L**Q**K**LM**R S**E**P**RF**G**V**L**R** A**P**P**FG**GG**GS**G**GS** GGG**SG**GG**GS**G GGG**SD**K**TH**T**C**
PPCPA**E**PL**G G**PS**V**FL**PP**PK** P**KD**T**LM**I**S**R T**PE**V**TC**V**VV**D**D** SH**EP**D**V**E**K**F**N** W**Y**D**GV**E**VH**
AKT**K**PR**EE**QY** N**ST**Y**RV**V**SL** T**VL**H**QD**W**IN** K**YE**K**CK**V**SN**K AL**P**API**EKT**I SK**AK**G**Q**P**RE**
QYV**T**LP**PS**RD** E**LT**P**KN**Q**SL**T C**LV**K**GF**Y**ES**D I**AV**W**E**W**ES**N**Q** PEN**NY**K**TT**PP VL**SD**G**S**F**FL**
YSK**L**TV**D**KS**R W**Q**Q**GN**V**F**S**C**S VM**HE**A**LH**N**HY** T**QK**S**LS**L**SP**G K**RR**R**SG**GG**GG** SGGGG**SG**GGGG
SGGGSG**GGGG SGGGG**SR**K**RR** K**RA**E**VL**Q**V**S G**AE**V**NK**PG**AS**G VK**V**S**CK**A**SG**Y TFT**GY**Y**MH**W**V**
RQ**A**P**G**Q**GLE**W** M**G**W**I**N**P**N**SG**G T**NY**A**QK**F**CG**W V**TM**T**RD**T**S**I S**TAY**M**E**S**RL**R SDD**T**A**VY**Y**CA**
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TFE**V**TC**V**VV**D**D** Q**SH**E**DE**E**EV**K**F** N**WY**V**DG**V**EV**H**I** N**AK**T**K**FREE**Q** Y**ST**Y**RV**V**SL** L**TV**L**HQ**D**LN**
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DNA sequence of FVII-027

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10 cagaqqaqcc tctccctgtc tccggataaa tqaqaattc

15 FVII-037 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 connecting both Fcs sites is shown in bold

20 MVSQLALRLIC LLLGLOGLIA AVEVTOEEAH GVLHRRRRRN AFLLEELRPGS LERECKEEQC
SFEEAREIFK DAERTKLFWI SYSDGDDQAS SFCQNGGSCK DQLQSYICFC LPAGFGRNCE
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25 FPPCAPELLG GPSVFLFPFK FKDTLMISRT PEVTCVVVDV SHEDFEVFKEN WYDVGVEVHN
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FIX-037 DNA sequence

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 gggggatcag gtgggggtgg atcaggccgtt ggagggttccg gtggcggggg atcagcggaa
 gtgcaggtgg tgcagttctgg agtgcagggtg aataaggcttg gggcctcagt gaagggtctcc
 30 tgcacaggctt ctggatcacac ctttacccggc tactatatgc actgggtgcg acaggccccct
 ggacaaggcgc ttgagttggat gggatggatc acccttaaca gttgtggcac aaaactatgca
 cagaagtttcccccagggtggggttccatggc accgtggccatcgccatcgac cccctacatcg
 gagctgagtc ggcgtggatc tgacgacacg cccgtgtatc actgtgcggag aggccgtgt
 ttgtataacccggc gggatcccttccggc gggatcccttccggc gggatcccttccggc
 35 gtcacccgtct ccccaaggag tggatccggc cccaaacccatcga agtgcacccatcga
 tcaagacccatcga cccgtgtatc cccatccatcg cccatccatcg cccatccatcg
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 cccatccatcg cccatccatcg cccatccatcg cccatccatcg cccatccatcg
 40 agggatcccttccggc gggatcccttccggc gggatcccttccggc gggatcccttccggc
 45 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
MVSOALRLIC LLLGLOGCLIA AEVQLVQSGA EVNKPGASVK VSCKASGYTF TGYYMHWVRQ
 APGQGLEWMG WINPNSSGGTN YAQKFQGMV**I** MIVRDTISISTA YMELSLRSD DTAVYYCARG
 RALYNRNDP PNWFDPWGQG TLTVVSSGSA SAPTLKLEEG EFSEARVQAV LTOPPSVSVA
 50 PGQTARITCG GNNIGKSNSQ WYQQKPGQAP VLVYVDDSDR PSGIPERFSG SNSGNM**ATLT**
 ISRVEAGDEA DYYCQVWDSS SDHVVFGGGT KLTVLGQPKA APSVTLFPPS AAARIKLEWI
 SYSDHGTQRC RCHEGYSLLA DGVSCPTIVE YFCGKIFILE KRNASKPQGR RKRKRIVGG
 KVC PKGECPW QVLLLVNGAQ LCGGTLINTPI WVVSAAHCFD KIRKNWRNLIA VLGEHDILSEH
 55 DGDEQSRRVA QVIIPSTYVP GTINHDIAALL RLHQPVVLLTD HVVPLCLPER TFSERTLAFV
 RFSLVSGWGQ LLDRGATALE LMVLNVPRLM TQDCLQQSRK VGDSPNITEY MFCAGYSDGS
 KDSCKGDGGG PHATHYRG**TI** YLTGIVSWGQ GCATVGHFGV YTRVSQYIEW LQKLMRSEPR
 PGVLLRAPFP GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
 FLFPPKPK**ET** LMISRTPEVT CVVVDVSHED PEVKENWYVD GVEVHNAKTK FREEQYNSTY
 60 RVVSVLT**VL**H QDWLNGKEYK CKVSNKALPA PIEKTI**SKAK** GQPREPQVYT LPPSRDEL**TK**
 NQVSLT**CL**VK GFYPSDI**AVE** WESNGQPE**N** YKTTPPV**LD**S DGSFFLYSKL TVD**K**SEWQ**Q**
 NVFSCSVM**II**E ALIHNHY**T**QKS LSLSPGK**GGG** **GS**GGGG**GS**GG**G** DKT H**T**CPPC**P**APE
 LLGGPSV**FL**F PPKPKD**TL**MI SRTPEVTCVV VDVSHE**D**PEV KFNWYVDGVE VHNAKTK**PR**E
 EQYNSTY**RV**V SVLTVLHQ**D**W L**N**KEYK**C**KV SNKALP**AI**E K**T**ISKAK**G**Q**P** REPQVY**T**L**PP**

5 SRDELTKNQV SLTCLVKGFY PSDIAVWES NCQPENNYKT TPPVLDSDGS PFLYSKLTVD
KSRWQGNVF SCSVMHEALH NHYTQKSLSL SPGK

FVII-053 DNA sequence

10	atggcttcggc	aggccctcaq	gtctctctgc	ctttctgttg	ggcttcagggg	ctgcctggct
	gccaagggtgc	agctggtgca	gtctggagct	gagggtaaata	agcctggggc	ctcaagtggaa
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	gccccctggac	aagggttta	gtggatggg	tggatcaacc	ctaacagtgg	tggcacaacaa
	tatgcacaga	agtttcaggg	ctgggttacc	atgaccaggg	acacgttcc	cagcaccggc
	tacatggagc	tgagcaggct	gagatctgac	gacacggccg	tgtattactg	tgccgagaggc
15	cgtgttttgt	ataacccggaa	cgacceggcc	cccaactgg	tegaceccct	ggggccaggaa
	accctggta	ccgttctcc	agggagtgc	tccgccccaa	cccttaaact	tgaagaagg
	gaattttca	aagcaccgt	acagggtgt	ctgactcage	cgccctcggt	gtcaagggtggc
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	tggtaccagg	agaaggccagg	ccaggcccc	gtgtgttgc	totatgtga	tagcaccgg
20	ccctcaggga	tccctgaggc	attctctgg	tecaactctg	gaaacatgg	caccctgacc
	atcaagcagg	togaagccgg	ggatgaggcc	gactattact	gtcaagggtgt	ggatagttagt
	agtgtatcat	tggtattcgg	cgaggggacc	aaagctgaccg	tcctaggtca	gccccaaaggct
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	tcttacagtg	atggggacc	gtgtgttca	agttccatggc	agaatggggg	ctctctgcaag
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	qaogggggatg	agcagagccg	cggggtggcc	caagttcatca	tccccagcac	gtacgtcccg
	ggcaccacca	accacgacat	cgccgtgtcc	cgctgtcacc	agcccgtgt	cctcaactgac
35	catgtgttgc	ccctctgcct	gccccgaa	acgttctgt	agaggacgt	ggcccttcgt
	cgcttctcat	tggtcagccg	ctggggccag	ctgtgttgcacc	gtggggccac	ggcccttcgt
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	ccaggagtc	tcctgcqagc	ccatttcc	gttgggggt	gttccgggg	aggtgggtcc
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	gacaaaactc	acacatgccc	acgtgccc	gttccggaa	tcctggggcc	accgtcagtc
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	cgtgtgtgtc	ggtgttccac	cggttctgc	caaggact	ggagtaca	ggagtaca
	tgcacagg	ccaaacaa	cttcccagcc	ccatcgaga	aaaccatctc	caaaggccaa
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	aaccaggta	gcctgtacc	cttggtccaa	ggttctatc	ccagacat	ccggctggag
	tggggagaga	atgggcaggc	ggagaa	tacaagatca	ccctcccg	gttggactcc
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	aacgttctt	catgttccgt	gatgtatc	gtctgtcaca	accactacac	qcaqaagagc
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	tccctggacc	ctgaggatc	atgtgtgt	gtgtgttgc	gcaacgaa	ccctgtggatc
	aagttaact	ggtacgttgc	cggggggg	gtgtatc	ccaaagacaaa	gggggggggg
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	ctgtatggca	aggagtaca	gtgtatc	cccttccac	cccttccac	cccatctcg
	aaaacatct	ccaaagccaa	aggggccccc	cgagaaccac	agggttacac	cctggccccc
	tccctggat	agctgacca	gaaccagg	agctgtatc	gttgggtca	aggcttctat
	cccaacggaca	tcggccgttgc	gtggggagag	aatggggc	ggagaaacaa	ctacaqacc
65	acgcctcccg	tgttggactc	cgacggctcc	tttttcttct	acagcaagct	caccgtggac

5 aagagcaggt ggcagcaggg gaacgttcttc tcatgtccg tgatgtcatga ggctctgtc
aaccactaca cgcagaagag cctctccatg tctccggta 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 *MVSQLRLILC LILGLQGCIA AVFVTOEEAH* GVLHRRRAN APLEELRPGS LERECKEEQC
SFEAREITFK DAERTKLFWI SYSGDQCAS SPCQNGGSCK DQLQSYICPC I^{PAFEGR}NCE
THKDDQQLICV NENGGCEQYC SDRTGPKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFD1K1KNWR
NLIAVLGEHHD LSEHDGDEQS RRVAVQVIIPS TYVPGTTNHID IALLRLHQPV VLTDHVVPFC
20 LPERTFSERT LAFVFRSLV GWGQOLLDRGA TALELMVNLV PRLMTQDCLQ QSRKVGDSRN
ITEYMFCA^{GY} SDGSKBSCKG DSGCPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMR SEPRPGVLLR APFPGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLPPPK PKUTLMSRT PEVITVVVVD SHEDPEVKFN WYVUDGVVEVHN
AKTKPREEQY NSTYRVVSVL TVLHQDWING KEYKCKVSNK ALPAPIEKT^I SKAKGQ^QPREP
25 QVYILPFSRD ELTKNQVSIT CLVKGFYFSD IAVEWESNGQ PENNYKTTPP VLDSDGSSFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG **KGGGGSGGGG SGGGGSGGGG**
SDKTHTCPPC PAPELLGGPS VFLFPPKFKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNNAKT KPREEQYNST YRVVSVLIVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREFQVY TLPPSRDELT KNQVSLTCLV KGFYFSDIAV EWE^{SNGQ}OPEN NYKTTPPVLD
30 SDGSFFLYSK LTVVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG **GGGGGGGGGG**
GGGGGGGGGG TERWALHNLC GG

FVII-044 DNA sequence

35 atggctctcc aggccttcag gctcccttcgc cttctgttgc ggcttcaggg ctgcctggct
gcacttccq taacccaaqga qqaqcccac ggcgttccttcg accggcccccq qcqcqccaaac
gcgttccctgg aggagctgcg gcccggatcc ctggagaggg agtgcagaagga ggagcgtgc
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40 acgcacaagg atgaccatgt gatctgtgt aacgagaacg gcccgtgtga gcagtactgc
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45 atcaacaccca tctgggttgt ctccggggcc cactgtttcg acaaaatcaa gaactggagg
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55 tacatcgagt ggctgcaaaa gtcacatgcgc tcagagccac gcccaggagt ctccctgcga
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5 ccggagaaca actacaagac cacgcctccc gtgttgact ccgacggctc cttcttctc
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 tcagacaaaaa ctcacacatg cccaccgtgc ccagcacctg aactcttggg aggacgtca
 10 gtcttctctc tccccccaaa acccaaggac accctctatga tctccggac ccctgagggtc
 acatgcgtgg tgggtggacgt gaggccgaa gaccctgggg tcaagtctaa ctggtaacgtg
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 aagtgcataagg tctccaaacaa agccctccca qcctccatcg agaaaaccat ctcacaaagcc
 15 aaaggcgcgc cccgagaacc acagggtgtac accctgcggcc catcccgca tgagctgacc
 aagaaccagg tcagctgac ctgcctggtc aaaggcttct atcccaagcga catcgcgggt
 gagtgggaga gcaatggca gccggagaac aactacaaga ccacgcctcc cgtgttggac
 tccgacggctt cttcttctc ctcacagcaag ctcaccgtgc acaagagcag gtggcagcag
 gggaaacgtct tctcatgtctc cgtgtatgtat gaggctctgc acaaccacta cacgcagaag
 20 agccctctccc tggcttcggg taaagggtggc ggccgatcag gtgggggtgg atcaggcggt
 ggagggttccg gtggcgggggg atcagccgtc accgagcggt gggccctgca caacctgtgc
 ggggggtgtca

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

30 MVSDALRLLC LLLGLOGGLA AVFVTQEEAH GVLHRRKRN AFLEELRPGS IERECKEEQC
 STEEAREI**LPK** DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 THKDDQLICV NEMGGC**EQYC** SDHTGTKRSC RCHEGYISLLA DGVSCTPTVE YPCGKIPILL
 KRNASKPQGR IVGGKVC PKG ECPWQVLLLV NGAQLCGGTI INTIWWVSAA HCFDK1KNWR
 NLIAVLGEHD LSEHDGDEQS RRVAVQVIIFP TYVPGTINHD IALLRLHQPV VLTDHVVPLC
 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVILN PRLMTQDCLQ QSRKVGDSPN
 IITEYMFCAGY SDGSKDSCKG DSGGPHAINY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 35 YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGGGGGGG GGGSGGGGSG GGGSDKTHTC
 PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKEN WYVDGVEVHN
 AKTPKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
 YSKLIVDVKSR WQQGNVFS CS VMHEALHNRY TQKSLISLSPG KGGGGSGGGG SGGGGSGGG
 40 SDKTHTCPPC PAPELIGGPS VFLFPPKPKD FLMTISRTPEV TCVVVVDVSHE DPFVKFNHYV
 DGVEVHNAAKTP KPREEQYNST YRVVSVLTVL HQDWLNGT KCKVSNKALP APIEKTI SKAKGQPREP
 KGQPREFOVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQOPEN NYKTTPPVLD
 SDGSPFLYSK LTVDKSRWQO GNVFSCSVMA EALHNHYTQK SLSLSPGKGG GGGGGGGGGG
 45 GGGGGGGGSAC TERMALHNLC GG

45 FVII-045 DNA sequence

50 atggcttccc aggccctcag gctcctctgc cttctgttgc ggcttcaggg ctgcctggct
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 20 tacagcaagc tcaccgtggc caagacgagg tggcagcgg ggaacgtt ctcatgttcc
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 25 gtcttcctt ccccccaaaa acccaaggac accctcatgtat tctccggac ccctgggt
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 30 aaaggccaggcc cccgagaacc acagggtgtac accctggcccc catcccgca tgagctgacc
 aagaaccagg tcacgttgc ctgcctggc aaaggcttctt atcccaacatgc catcgccgtg
 gagtgggaga gcaatggca gcccggagaac aactacaaga ccacgcctcc cgtgttggac
 tccgacgggtt cttcttcctt ctcacagcgtt ctcacccgtt cacaaggacgtt gttggcagc
 gggaaacgtt ctcatgttccctt cttcttcctt taaagggtggc gggcgttccgtt gttgggggt
 35 ggagggttccgg tggcgggggg atcagcttgc accggacggg tggccctgca caacactgtt
 ggcgggtga

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
 40 underlined, linker connecting both Fcs sites is shown in bold, and OS2 peptide is
 italicized

MVSOAQLRLC LLLGLOGCLA AVFVTQEEAH GVLFHERRRAN AFLEELRPGS LERECKEEQC
 SFEELAREIIFK DAERTKLFWI SYSDGDDQCAS SPCQNGGSCK DQLQSYICFC IPAFEGRNCE
 THKDDQILICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCPTPTVE YPCGKIPFILE
 45 KRNASKPQGR IVGGKVC PKG ECPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
 NLIAVLGEHD LSEHDGDEQS RRVAVQVIIFS TYVPGTTNHID IALLRLHQPV VLTDHVVPLC
 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSRN
 ITEYMPFCAGY SDGSKDSCKG DSGGPHAIHY RGTWYLIGIV SWGQGCATVG HFGVYTRVQ
 50 YLEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
 PPPCPAPELLG GPSVFLFPPK PKDTLMISRT FEVTCVVVVDV SHEDPEVKEN WYVDGVVEVN
 AKTKPREECOY NSTYRVVSVL TVLHQDWING KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 55 QVYTLPPESRD ELTKNQVSLT CLVKGFYFSD IAVEWEWSNGQ PENNYKTTPP VLDSDGSSFL
 YSKLTVDKSR WQQGNVFSOS VMREALHNHY TQKQLSLSPG **KGGGGSGGGG SGGGGSGGGG**
SDKTHTCPPC PAPELGGPS VFLFPPKFD TLMISRTPEV TCVVVDSHE DPEVKENWYV
 DGVEVHNAIKT KPREEQYNST YRVVSVLIVL HQDWLNGKEY KCKVSNKALP APIEKTI SKA
 KGQPREFQVY TLPPSRDELT KNQVSLTCLV KCFYFSDIAV EWESNGOPEN NYKTTPPVLD
 SDGSFFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGGS
GGSGGGGSAC TERDALHNLC GG

60 FVII-046 DNA sequence

atggtctccc agggcctcag gctcttctgc cttctgttttgg ggcttcaggg ctgcctggct
 gcaagtctcg taacccagga qqaqcccac qgcgttctgc accggccccc qccgcqccaaac
 qcggttctgg aggagctgcg gcccggccctc ctggagaggg agtgcaggtt ggagcaggt
 tcccttcgagg agggccggga gatcttcaag qacgcggaga ggacgaagct gttctggatt

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	MVSQALRLIC LLLGLOGLCA AVEVTOEFAAH GVLHRRRRAN AFLEELRPQS LERECKEEQC SEEFAAREIFK DAERTKLFWI SYSDGDDQAS SPCQNGGSCK DQLQSY1CFC LPAFEGRNCE THKDDQLICV NENGGECEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGK1PILE KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTI INTIWWVSAA HCFDK1KNWR NLLIAVLGEHD LSEHDHGDEQS RRAVQVIIIPS TYVPGTTNHID IALLRLHQPV VLTDHVPLC
60	LPERTF'SERT LAFVFRSLSV GWGQQLLDRGKA TALELMVILNV PRLMTQDCLQ QSRKVGDSNP ITEYMFCAKY SDGSKDSCKG DSSGGPHATHY RGTWYLTGIV SWGQGATCIV HFGVYTRVSQ YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC FPCPAPELLG GPSVFLFEPK PKIITLMISRT PEVTCVVVVDV SHEDPEVVKPN WYVGDGVVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP

5 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSSFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGGSGGGGG SGGGGSGGGGG
SGGGGSGGGGG SACTERWALH NLCGGGGGGG GGSGGGGSGG GGSGGGGSGG GGSDKTHTCP
PCPAPELGGG PSVFLITPPKP KDTLM1SRTF EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
10 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSSFLY
SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

FVII-047 DNA sequence

15 atggtcctcc aggccctcaag gtcctctgc ctctctgttg gggttcagggg ctgcctggc
qcatgttccg taaccaggaa ggaagccccac gggttccttc accggggccggc ggcgcgcac
ggttcctgg aggagcttgcg ccggggctcc ctggagaggg agtgcacaggaa ggagcagtgc
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35 tacatcgagt ggtgtggaaa getcatgtgc teagagccac gcccaggagt cttctgtgc
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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

10	MVSQALRLLC	LLLGLOGCIA	AVFVTOEEAH	GVLHRRRRAN	AFLEELRPGS	LERECKEEQC
	SFEEAREIFK	DAERTKLFWI	SYSDGDQCAS	SFCQNGGSCK	DQLOSYICFC	LPAFEGGRNCE
	THKDDQLICV	NENGGCEQYC	SDHTGTKRSC	RCHEGYSLLA	BGVSCPTPTVE	YPCGKIPITLE
	KRNASKFQGR	IVGGKVCPKG	ECPWQVLILV	NGAQLCGGTL	INTIWVVSAA	HCFD1K1KNWR
	NLIAVLGEHD	LSEHDGDEQS	RRVAQVIIIPS	TYVPGTTNHD	IALLRLHQPV	VLTDHVVPCLC
	LPERTFSERT	IAFVRFLSLSV	GWGQLLDRGA	TALEMVNLNV	PRLM1TQDCLQ	QSRKVGDS1PN
15	1TEYMFCA1GY	SDGSK1DSCKG	DSGGPH1AHY	RGTWYLTGIV	SWGQGCATVG	HFGVYTRVSQ
	Y1EWLQKLMR	SEPRPGVLLR	AFFPGGGGSG	GGGSGGGGSG	GGGS1GGGGSG	GGGS1DKTH1TC
	PPCPAPELLG	GPSVFLFFPK	PKD1LM1SRT	PEVTCVVVDV	SHEDPEVKEN	WYDVGVEVHN
	AKTKPREEQY	NSTYRVVSVL	TVLHQDWING	KEYKCKVSNK	ALPAPIEKTI	SKAKGQPREP
	QVYTLPPSRD	ELTKNQVSLT	CLVKGFYFSD	IAVEWESNGQ	FENNYKTTFP	VLDSDGSFFL
	YSKLTVDKSR	WQGNVNFSCS	VMHEALHNHY	TQKSL1SLPG	KGGGGSGGGG	SGGGGSGGGG
20	SGGGGSGGGG	SACTER1MALH	NLCGGGGSGG	GGGGGGGG	GGGGGGGG	GGGS1DKTH1TC
	PCPAPELLGG	PSVFLFFPKP	KDT1LM1SRTP	EVTCVVVDVS	HEDPEVKFNW	YVDGVVEVHNA
	KTKPREEQYN	STYRVVSVLT	VLHQDWLNGK	EYKCKVSNA	LPAPIEKTI	KAKGQPREPQ
	VYTLPPPSRDE	LTKNQVSL1IC	LVKGFYFSD1	AVEWESNGQ	FENNYKTTPPV	LDSDGSFFLY
	SKLTVDKSRW	QOGN1VNFSCSV	MHEALHNHYT	QKSL1SLPGK		

DNA sequence of EVII-048

DNA sequence of pVII-046
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5 ggtggatcaag gggggtggagg ttccgggtggc gggggatccg aaaaaactca acatgecca
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 15 acaagatca ccgtggacaa gaggagggtgg cacggaggggg acgttcttc atgtccgtg
 atgcatgagg ctctgcacaa ccactacacg cagaagagcc tcccctgtc tccggtaaa
 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

MVSQALPLIC LILGLOGCIA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS IERECKEEQC
 SFEEAREI FK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICPC I^{PA}FEGRNCE
 THKDDQLICV NENGGCEQYC SDHTGPKRSC RCHEGYSLLA DGVSTPTVIE YPCGKIPILE
 25 KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTIL INTIWWVSAA HCFDKIKNWR
 NLIAVLGERHD LSEHDGDEQS RRAVQVIIPS TYVPGTINHD IALLRILHQPV VLTDHVVF^LC
 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVNLN PRLMTQDCLQ QSRKVGDS^PN
 I^TEYMF^CAGY SDGSKDSCKG DSGGP^HAHY RGTWYL^TGIV SWGQGCATVG HFGVYTRV^SQ
 YIEWLQKLMR SEPRGVLLR APFP^{GGGG}GSG GGGSGGGGSG GGGSDKTHTC
 30 PCPAP^ELLG GPSVFLFPEK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYDGV^EVHN
 AKTKF^EEEQY NSTYRVVS^VL T^VLHQDW^LNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 QVYTLPPSRD ELTKNQVS^LT CLVKGFYFSD I^AVEWESNGQ PENNYKTT^PP VLDSDGSFFL
 YSKLTV^DKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGCGGGGG
 35 SGGGGSGGGG SACTERDALH NLCGGGGSGG GGGGGGGSGG GGGGGGGSGG GGSIDKTHTC
 PCPAP^ELLG PSVFLFPPKP KDTLMISRT^P PEVTCVVVDV HEDPEVKFNW YVDGV^EVHN
 KTKP^EEEQY NSTYRVVS^VL T^VLHQDW^LNGK EYKCKVSNKA LPAPIEKTI SAKGQPREPQ
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 SKLTVDKSRW QQQN^VFSCSV MHEALHNHY QKSLSLSPGK

40 DNA sequence of FVII-049

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	aacctgtgcg	gtggcgggtgg	cicccggcga	ggttgggtcg	gtggggcggg	atcaggtggg
	ggttggatcaq	gcqgtggagg	tccgggtqgc	gggggatccq	acaaaactca	cacatqccca
	ccgtgcggcag	caccggaaact	cctggggggga	ccgtcagtct	tcttcttccc	cccaaaaaccc
25	aaggacaccc	tcatgtatctc	ccggacccct	gaggtcacat	gctgtgggt	ggacgtgagc
	cacgaagacc	ctgaggtcaa	gtcaactgg	tacgtggacg	gcgtggaggt	gcataatgce
	aagacaaaago	cgccggagga	gcagttacaa	agcacgtacc	gtgtggtcaq	cgttcttacc
	gttctgtcacc	aggactggct	gaatggcag	gagttacaat	gcaagggtctc	caacaaagcc
30	ctccccagecc	ccatcgaaaa	aaccatctcc	aaagccaaag	ggcagccccq	agaaccacacg
	gtgtacaccc	tggcccccatt	ccgggatggq	ctgaccaaga	accagggtcaq	cctgactctgc
	ctggtaaaag	gttttctatcc	cagegcacat	gcccgtgggt	gggagagacaa	tggcagccg
	gagaacaaact	aaacagaccac	gcctccctgt	tttgactccg	acggcttctt	cttctcttac
	agcaagtc	ccgtggacaa	gagcaggtgg	cagcaggggg	acgttcttc	atgtctcggt
	atgtatgagg	ctctgcacaa	ccactacacg	cagaagagcc	tctccctgtc	tccgggtaaa
	tga					

35 FVII-011 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, Glu domain is italicized, linker region connecting FVII to Fc region is underlined, and linker connecting both Fcs sites is shown in bold
MVSGALRLC LLLGLOGGLA AVEVTOEEAH GVLHRRRAN AFLEELRPGS LERECKEEQC
 SPEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 THKDDQQLICV NENGNGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSQCIPTVE YPCGKIPILE
 KRNASKPQGR IVGGKVCPKG ECPWQVLILV NGAQLCGGTL INTIWWVSA A HCFDKIKNWR
 40 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHE IALLRLHQPV VLTDHVVPLC
 LPERTIFSER T LAFVRFSLVS GWGQLLDRGA TALEIMVLNV FRLMTQDCLQ QSRKVGDSQN
 ITEYMFCA GY SDGSKDSCKG DSGGPRAHAIY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 YIIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
 PPCPAPELLG GPSVFLFPK PKDPLMISRT PEVTCVVVDV SHEDPEVKEN WYVDGVEVHN
 45 AKIKPREEQY NSTYRVRVSVL TVLHQDWING KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 QVYTLPPFSRD ELTKNQVSLT CLVKGFYFSD IAEVWESNGQ PENNYKTTTP VLDSDGSFFL
 YSKLTVDKSR WQOQGNVFSCS VMHEALHNRY TQKSLSLSPG KGGGGSGGGGG SGGGGSGGGG
 50 **SDKHTHTCPFC** PAPELLGGPS VFLFPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
 DGVEHVNNAKT PKREEQYNS T YRVSVSLTVL HQWLNGKEY KCKVSNKALP APIEKTI SKAKGQPREP
 KGQPREPVY TLPPSRDELT KRNQVSLTCLV KGFYPSDIAV EWESENQOPEN NYKTTTPVLD
 SUGSEFLYSK LTVLUDKSRWQO GNVEFSCSVMH EALHNRYTOK SLSLSPGK

FVII-011 DNA sequence

55	atggtcctccc aggccctcgat gtcctctgc ctctctgtgg gcttcagggg ctgcctggc gcagtcttcgta accccaggaa ggaaggccac gggttcttcgacc cggcggccggc ggcgcgcac qcggttctggagg agggactcgcc gcccggatcc ctggagaggg agtgcagaaggaa ggagcagtgc tccttcgagg aggcccgggaa gatcttcaag gacggggaga ggacgaagct gttcttgatt tcttacagtg atggggacca gttgtccctca agtccatgcc agaatgggg ctctctgcac gaccagatcc agtccatatact ctgtttctgc ctccctgcct tcgaggccgg qaaactgtgg 60 acgcacaagg atgaccatgt gatctgtgt aacggagaacg cgggtctgtga gcagtactgc agtgaccaca cgggcaccaa ggcgtctgtgt cgggtccacgggggtactc tctgtggca gacgggtgtt aacgttgaa tattccatgtgg gaaaataac tattcttagaa aaaagaaaatg ccagcaaaacc ccaaggccgaa attgtgggggg gcaagggtgtt ccccaaaagggg gagtgtccat ggcaggtctt gttgtttgtgt aatggagctc agtigtgttggg qgggacccctg
----	---

5 atcaacaccca tctgggtggc ctccggggcc cactgtttcg aaaaaatcaa gaactggagg
 aacctgtatcg cgggtgtggg cgagcacgac ctcagcgacg acgacgggga tgagcagagc
 cggcggttgg cgcaggatcat catccccage acgtacgtcc cgggcaccac caaccacgac
 atcgcgtgtgc tccgcgtca ccagccgtg gtcctactg accatgttgt gcccctctgc
 ctggcccgaaac ggacgttctc tgagggacy ctggcccttc tgccgttctc atgggtcagc
 10 ggctggggcc agctgtggc cctgtggggcc aegggccctgg agctcatgtt ctcacacgtg
 cccgggtgtga tgacccagga ctcgtcgag cagtcacggg aggtgggaga ctccccaaat
 atcaggaggat acatgttctg tgccgggtac tggatggca gcaaggactc ctgcaagggg
 gagcgtggag gcccacatgc caccactac cggggcacgt ggtacctgac gggcatcgcc
 agctggggcc agggctgcgc aaccgtgggc cactttgggg tgcacaccag ggttctccag
 15 tacatcgagt ggctgcaaaa gctcatgcgc tcagagccac gcccaggagt ctcctctgcga
 gccccatttc cccgtggcgg tggctccggc ggagggtgggt cccgtggcgg cggatcagg
 ggggggtggat caggcggtgg aggttccgtt ggccggggat cgcacaaaaac tcacacatgc
 ccacccgtgcc cagtcctggc actccctggc ggaccgtca gtcctctt ccccccaaaa
 cccaaaggaca cccatcgat ctccgggacc ctqaaqgtca catqcggtt ggtggacgtg
 20 agccacgcaag accctgggat caagttcaac tggtaacgtgg acggcggtgg ggttcataat
 gccaagacaa agccgggggaa ggacgactt aacagcacgt accgtgtgtt cagcgttctc
 aacgttctgc accaggactg getgaatggc aaggagttaca agtgcacaggat tcacacaaa
 gcccttccaaag ccccatcgaa gaaaaccatc tccaaaggcca aaggcagcc cggagaacca
 cagggttaca ccctgtcccccc atccgggat gagctgacca agaaccaggat cagcgttcc
 25 tgccctgtca aaggcttcta tccacgttgc atccgggtgg agtgggaaqg caatgggaaag
 cccggaaaca actacaagac cacccctccc gtgttggact ccqacggctc ctttttctc
 tacagcaagc tcaccgtggc caagacgagg tggcagcagg ggaacgttctt ctatgttcc
 gtgtatgtcatg aggctctgtca caaccactac acgcagaaga gcttctccct gtctccgggt
 aaagggtggc gggatcagg tgggggtggc tcaaggcggtt gaggttccgg tgggggggg
 30 tcagacaaaaa ctcacacatg cccacccgtgc ccacgttgc aactcttggg aggaccgtca
 qtcttctct tccccccaaa acccaaggac accctcatgtatgatc tctccggac ccctcgaggc
 acatgcgtgg tggtgacgt gggccacgaa gaccctgggg tcaagttcaa ctggtaacgtg
 gacggcggtgg aggtgcataaa tgccaaagaca aaggccggggg aggacgacta caacaccc
 taccgtgtgg tcacgttctt caccgttgc accggactt ggctgaatgg caaggagttac
 35 aagtgcacgg tctccaaacaa aqccctccca gccccccatcg agaaaaccat ctccaaagcc
 aaaggccggc cccgagaacc acagggtgtac accctggccc catcccgca tgagctgacc
 aagaaccagg tcacgttgc acggcttgc ataggcttctt atcccgccga catcccggt
 gagggtggc gcaatggggca gccggagaac aactacaaga ccacgcctcc cgtgttggac
 tccacacggc ctttttctt ctacatcgac ctacccgtgg acaagacgac gtggcagcag
 40 gggaaacgttctc ttcatgttgc ctgtatgtcatg gaggcttgc acaaccaacta caccacaa
 agcccttccccc tggatccggg taaaatga

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.

1 **MQIELSTCFF** LCLLRFCSA TRRYYLGAVE LSWDYMQSDF GELPVNDARFP
 50 S1 PRVPKSFPFN TSVVYKKTLF VEFTIDHLFNI AKPRPPWMGL LGPTIOAEVY
 101 DTVVITLKNM ASHPVSLIHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQTL LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHFLVRNH
 301 RQASLEISPI TFLTIAQTLLM DLQFLFLFCH ISSRQRHDGME AYVKVLDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFTKTREA IGHESGLP LLYGEVGTDL LIIIFKNQASR PYNLYPHGIT
 501 DVRPLYSRRL PKGVKHLKDF LILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSSFVNMER DLASGLIGPL LILCYKESVQDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYLTENI QRFPLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLLTF PFSGETVFM
 701 MENPGLWILG CHNSDFRNRC MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751 SKNNAIEPRS **FQNPPVLKR** HQRERITRTIL QSDQEIEIDYD DTISVEMKKE
 801 DFDIYDDEDEN QSPRSFQKRT RHYFTIAAVER LWDYGMSSSP HVLNRRAQSG
 851 SVPQFKKVVF QETFDGSPFQ PLYRGELNEH LGLLGPyIRA EVEDNIMVTF
 901 RNQASRPYSF YSSLISYEEED QRQGAEPRKIN FVKPNETKTY FWKVKQHHMAP
 951 TKDEFDCKAW AYFSDVDELEK DVRSGLIGPL LVCHTNTLN P AHGRQVITVOE
 1001 FALFFTIFDE TKSWSYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI

5 1051 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1101 YNLYPGVFEI VEMPLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNIKQCTPL
 1151 GMASGHIRDF QITASGQYQG WAPKLAIRLY SGSINAWSTK EPPFSWIKVIDL
 1201 LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1251 FFGNVDSSGI KHNIFNPII ARYIRLHPTH YSIRSTLRLME LMGCDLNSCS
 10 1301 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1351 PKEWLQVDFQ KTMKVGTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
 1401 QNGKVKVFQG NQDSPTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
 1451 CEAQDLY

15 Full length FVIII amino acid sequence: Signal peptide underlined

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVNDARFP
 51 PRVPKSFPFN TSVVYKKTLF VEFTDHFLN AKPRPPWMGL LGPTIQAEVY
 20 101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFFG
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LFPLGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTPFLVRNH
 301 ROASLEISPI TFLITAQIILM DLQFLLFCFCH ISSHQHDGME AVVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPVLIA FDDRSYKSQY LNNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREI AIQHESGILCP LLYGEVGDYL LIIIFKNQASR PYNMILPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRLCTR
 551 YYSSFVNMER DLASGLIGPL LICYKESVDO RGMOIMSDDR NVILLESVFDE
 30 601 NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYLLS IGAQTDPLSV FFSGYTFKKK MVYEDTLLTF PFSGETVFMMS
 701 MENPGLWILG CHNSDFRNRM MTALLKVSST DKNTGDDYYED SYEDISAYLL
 751 SKNNAIPEPRS FSQNSRHPST RQKQFNATTI PENDIEKTDP WFAHRTPMMPK
 801 TQNVSSSDL MLLRQSPPTPH GLSLSLDSLQEA KYETFSDDPS PGAIDSNNSL
 35 851 SEMTHFRPQL HHSGDMVFTP ESGLQLRLNE KLGTTAATEL KKLDFKVSVST
 901 SNNLISTIPS DNLAAGTDTN SSLGPPSPMV HYDSDLQDTTL FGKKSSPLTE
 951 SCGPLSLSEE NNDSKLLESC LMNSQESSSWG KNVSSTESGR LFKGKRAHGP
 1001 ALLTKDNALF KVSISLLKTN KTSNNNGATNR KTHIDGPSLL IENSPSVWQN
 40 1051 ILESDETFKK VTPLTHDRML MDKNATALR NHMSNKTTS KNMEMVQOKK
 1101 EGPIPPDAQN PDMSFFKMLF LPESARWIQR THGKNSLNSG QGPSPKQLVS
 1151 LCPEKSVEGQ NFLSBKNYVV VKGKEPTKDV CLKEMVFPSS RNLPFLTNLDN
 1201 LHENNTHNQE KRIQREIEKK ETLIQENVVL PGIHTVTGTK NPMKRNLLPLS
 1251 TRQNVEGSYD GAYAPVQLQDF RSLNDSTNRT KKHTAHFSKK GEEENLEGLG
 45 1301 NQTKQIVEKY ACTTRISPTN SQQNFVTOQS KRALQFRLP LEEETBLEKRI
 1351 IVDDTSTQWS KNMKHHLTPST LTQIDYNEKE KGAITQSPSLS DCLTRSHSIP
 1401 QANRSPPLIA KVSSFPSIRP IYLTRVLFQD NSSRLPAASY RKKDSGVQES
 1451 SHFLQGAKKN NLSSLALLTLR MTGQDQEVEGS LGTSATNSVT YKKVENTVLP
 1501 KPDLPKTSGK VELLPKVHYI QKDLFPTETNS NGSPGHLDLV EGSSLQGTEG
 50 1551 AIKWNEANRP GKVPLFLRVAT ESSAKTPSKL LDPLAWDNHY GTQIPKREWK
 1601 SQEKSPKTA FKKKDTIILSL NACESNHAIA AINEGQNKPE IEVWAKQGR
 1651 TERLCSQNPP VLKRHQREIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1701 EDENQSPRSF QKTKTRHYFIA AVERLWDYGM SSSPHVLRNR AQEGSVPQFK
 1751 KVVFQEFTDG SFTQPLYRGE LNEHLLGLGP YIRAEVEDNI MVTFRNQASR
 1801 PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEED
 55 1851 CKAWAYFSDV DLEKDVHSGL IGPLLVCNTN TLNPAGHRQV TVQEFALFFT
 1901 IFDETKSWSYF TENMERNCRN PCNIQMEDPT FKENYRPHAI NGYIMDTLPG
 1951 LVMAQDQRIR WYLLSMSGSNE NIHSIHFSGH VFTVRKKEEY KMALVNUVPG
 2001 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLPLVYSNKC QTPLGMASGH
 2051 IRDFQITASC QYQQWAPKLA RLHYSGSINA WSTKEPFWSI KVDLLLAPMII
 60 2101 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WOTYRGNSTG TLMVFFGNVD
 2151 SSGIKNHIFN PPIIARYIIRL HPTHYSIRST LRMELMGCDSL NSCSMPLGME
 2201 SKAISDAQIT ASSYFTRMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 2251 VDFQKTMKVY GTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
 2301 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 65 2351 Y

70 FIX amino acid sequence. Signal sequence is shown in dotted
 underline, propeptide is double underlined
 70 MORVNIMMAE SPGLITICLL GYLSSAECTV FLDHNMANKI LNRPKRYNSG KLEEFVQGNL
 ERECMEEKCC FEEAREVFEN TERTTEFWKQ YVDGDCQESN PCLNGGCKD DINSYEWCP
 FGFEQKNCEL DVTCKNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPPFCGR
 VSVSQTSKLT RAETVFPDVD YVNSTEAEI LDNITQSTQG FNDFTRVVGG EDAKPGQFPW

5 QVVLNLGVDA FCGGSIVNEK WIVTAAHICVE TGVKITVVAQ EHNIEETEHT EQKRNVIRII
 PIHINYNAAIN KYNHDIALLE LDEPLVLKNSY VTFPICTADKE YTNIFLKFGS GYVSGWGRVF
 HKGRSALVQLQ YLVRPLVNDRA TCLRSTKFTI YNNMPMCAGFH EGGRDSCQGD SGGPHVTEVE
 GTSEPLTGIIS WGEECAMKGK YGIYTKVSRV NWIIEKETKIL T

10

FIX DNA sequence

15	atgcagcgcg ggatatctac ctgaatcgcc gagagagaat actgaaagaa ccatgtttaa tttqgatttq	tgaacatgat tcagtgtcga caaagaggtt gtatggaaga caactgaatt atggcggcag aaqqaaaqaa	catggcagaa atgtacagtt taattcaggt aaatttggaaq tttgaagaag tttqtaattt ctqtqaatta	tcaccaggcc tttcttgatc aaatttggaaq tttgaagaag gagatcagtg gacattaatt qatgttaacat	tcatcaccat atgaaaacgc agggaaatcta cagcagaatgt tgagtcaat tttggtgtccc qtaacattaa	ctgcctttta acaaaatt aggaaatcta ttttgaaaac tgagtcaat tttggtgtccc qaatqqcaqa
20	tgcgagcagt tatcgacttg gttctgttt tatgtaaatt tttaatgact	tttggtaaaa cagaaaaacc cataacttc tactgtaaac tcactcggtt	tagtgcgtat gaagtcctgt taagctcacc tgaaaccatt ttgtgataaca	aacaagggtgg gaaccagcag cgtgtctgg tcaactaaag gaagatgcca	tttgcetctg tgcatttc ctgtttttcc tcaactaaag aaccagggtca	tactggggg atgtggaaag ctgtgtggac caccctaaatca attcccttgg
25	caggttgttt tggattgtaa gaacataata cctcaccaca ctggacgaaac	tgaatggtaa ctgtgtgaa ttgaggagac actacaatgc cttttagtgc	agttgtatgca actgggtgtta agaaacataca agctattaaat aaacagctac	ttctgttqqaq aaattacagt gagcaaaagc aagtacaacc gttacacacca	gtctctatgt tgtcgcaagg gaaatgtgtat atgacattgc tttgcattgc	taatgaaaaaa tgcattttcc tcgaattatt ccttctggaa tgacaaggaa
30	tacacgaaca cacaaggaa acatgtctt gaaggaggta gggaccaggtt	tcttcctca gatcagctt gatctacaaa gatcaccatc tcttaacttg	aittggatct agttcttcag gttcaccatc tcaaggagat aatttatttgc	ggctatgtaa taccttagag tataacaaca agttggggac tttgcattgc	gtggctgggg ttccacttgc tggcttccat cccatgttac tgaagtggaa	aagagtcttc tgaccgagcc tggcttccat tgcattttcc gaaaggcaaa
35	tatggaatat ataccaaggat	atcccggtat	gtcaacttgga	ttaaggaaaa	aacaaaagctc	

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

40	MGRPLHLVLL SASLAGLLLL GESLFIRREQ ANNILARVTR ANSFLEEMKK GHLERECMEE TCSYEEAREV FEDSDKTNF WNKYKDGDQC ETSPCQNQGK CKDGLGEYTC TCLEGFEGKN CELFTRKLCS LDNGDCDQFC HEEQNSVVCS CARGYTLADN GKACIPTGPF PCGKQTLERR KRSVAQATSS SGEAPDSITW KPYDAADLDP TENPFDLDF NQTQPERGDN NLTRIVGGQE
45	CKDGECPWQA LLINEENEGF CGGTILSEFY ILTAAHCLYQ AKRFKVRVGD RNTEQEEGGE AVREVEVVIK HNRFTKETYD FDIAVLRLKT PITFRMNVA P ACLFERDWA E STLMQTQTKGI VSGFGRTHEK GRQSTRLKML EVPYVDRNSC KLSSSFIITQ NMFCAGYDTK QEDACQGDSG GPHVTRFKDT YFVTGIVSWG EGCARKGKYG IYTKVTAFLK WIDRSMKTRG LPKAKSHAP VLISSPK

50 FX DNA sequence

50	atggggcgcgc cactgcaccc ctgttcgtctc agtgcctccc tggctggccct cctgtctgtc ggggaaaggat tgttcatccg caggagcag gccaacaaca tccttggcag ggtcacggagg qccaaatccct ttcttgaaga qatgaagaaa qgacacccctcg aaaaqadqatq catggaaqag acctgtctat acgaaagagc ecgcgaggcc tttgaggaca qcgacaaagac gaatgaattc 55
55	tggaaataat acaaagatgg cgaccagtgt gagaccagtc cttggccagaaa ccagggcaaa tgtaaagacg gccttggggaa atacacctgc acctgtttag aaggatcgtg aggcaaaaac tgtqaattat tcacacggaa qctctgcage ctgacacaacg gggactgtga ccagtctgc caacggagaaac agaactctgt ggtgtctcc tgcgccccqcg ggtacacccct ggctgacaaac ggcaaggccct gcattccac agggccctac cccctgtggga aacagacccct ggaacgcagg 60
60	aaggagtctag tggggccaggc caccagcagc agggggggagg cccctgcacag catcacatgg aagccatatgc atgcageccg cttggaaaaacc accgagaacc ccttgcacccct gcttgcattc aaccagacgc acgcgtgagag gggcgacaaac aaccttcacca ggtatgtggg aggccaggaa tgcacaggacg gggagtgtcc ctggcaggcc ctgtctatcc atgaggaaaa cgagggtttc tgttgtggaa ccattcttgcg cggatcttccatccatccaa

5 gccaagagat tcaaggtag ggttagggac cggAACACGG agcaggagga gggccggtag
gagggtcacg aggtggaggt ggtcatcaag cacaaccggc tcacaaggaa gacccatgac
ttcgacatcg cccgtqctccg qctcaagacc cccatcacct tccgcatqaa cgtggccqcc
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10 gaggtgccccct acgtggcccg caacagctgc aagctgtcca gcagcttcat catcacccag
aacatgttct gtgcggctca cgcaccaag caggaggatg cctgcagggg ggacgcggg
gccccggcact caccggctt caaggacacc tacttcgtga caggcatcgt cagctgggg
gaggggtgtg cccgttaaggg gaagtaacggg atctacacca aggtcaccgc ctccctcaag
15 tggatcgaca ggtccatgaa aaccaggggc ttgccccagg ccaagagcca tgccccggag
gtcataaacgt cctctccatt aaagtga

DNA sequence of EVII-066

1 ATGGTCTCCC AGGCCCTCAG GCTCTCTG CTTCTGCTG GGCTTCAGGG
20 CTGGCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCTGC ACCGGCGCCG GCGGGCCAAAC
121 GCGTTCTGG AGGAGCTGCG GCGGGGCTC CTGGAGAGGG AGTGCAGGAA GGAGCAGTGC
181 TCTCTCCAGG AGGCCGGGA GATCTCAAG GACGGGGAGG GGACGAACTT GTTCTGGATT
241 TCTTACAGTGT ATGGGGACCA GTGTCCTCA AGTGCATGCC AGAATGGGGG CTCCCTGCAAG
301 GACCGACATCC AGTCTTATAT CTGCTCTG CTCCTGCTC TGAGGGCCG GAACTGTGAG
361 ACGCACAAGG ATGACCAGT GATCTGTGT AACGAGAACG GCGGCTGTGA GCAGTACTG
421 AGTGACCACA CGGGCACCAA GCGCTCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGTGTG CCTGCACACC CACAGTGAAT TATCAGTGTG GAAAATAC TATCTAGAA
541 AAAAGAAATG CCAGCAAAAC CCAAGGGCGA ATTGTGGGGG GCAAGGTGTG CCCCCAAGGG
601 GAGTGTCCAT GGCAGGTCCT GTTGTGGGT AATGGAGCTC AGTGTGTGG GGGGACCTTG
661 ATCAACACCA TCTGGGTGGT CTGCGGCGC CACTCTTTCG ACAAAATCAA GAACTGGAGG
721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC AGCACGGGA TGAGCAGAGC
781 CGCGGGGTGG CGCAGGTCAAT CATCCCCAGC ACGTACGTCC CGGGCACAC CAACCACGAC
841 ATCGCGTGTG TCCGGCTGCA CCAGGGCGTGT GTCTCTACTG ACCATGTGTG GCCCCCTCTGC
901 CGTCCCCAAC GGACGTTTCG TGAGAGGACG CTGGCTCTCG TGCGCTTCTC ATTGGTCAGC
961 GGCTGGGGCC AGCTCTGGA CGTGGGGCC ACAGGCGCTGG AGCTCATGGT CCTAACCTG
1021 CCCCCGGCTGA TGACCCAGGA CTGCGCTGCA CAGTCACGGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCACGGAGT ACATGTTCTG TCCGGGTCACT CGGGTGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCCACATGC CACCCACTAC CGGGGCACTG GGTACCTGAC GGGCATCGC
1201 AGCTGGGGGAG AGGGCTGCGC AACCGTGGCG CACTTGGGG TGACCCAG GGTCTCCOCAG
1261 TACATCGAGT GGCTGCAAA GCTCATGCGC TCAGCGACAC CGCCAGGAGT CCTCTGCGA
1321 GCCCCATTCG CGCGTGGGG TGGCTCCCGC GGAGCTGGGT CGGTGGGGG CGGATCTAGGT
1381 GGGGGTGGAT CAGGGGGTGG AGGTTCCCGT GGCGGGGGAT CGCACAAAC TCACACATGC
1441 CCACCGTGC CAGCTCCCGA ACTCTGGGA GGACGTCAG TCTCTCTT CCCCCCRAAA
1501 CCCAAGGACA CCCATGAT CTCCCGGACG CCTGAGGTCA CATGGCTGGT GGTGGACGTG
1561 AGCCACAGAAG ACCCTGAGGT CAAGTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
1621 GCGCAAGGAA AGCOSCGGGA GGAGCAGTAC AACACAGGT ACCGTGTGGT CAGCGTCTC
1681 ACCGTCTTGC ACCAGGACCG GCTGAAATGCA AAGGAGTACA AGTGCAGGT CTCAACAAA
1741 GCCCCCTCCAG CCCCCATCGA GAAAACATC TCCAAAGCCA AAGGGCAGCC CGAGAACCA
1801 CAGGTGTACA CCCCTCCCCC ATCCGGGAT GAGCTGACCA AGAACCCAGGT CAGCTGTGCC
1861 TGCGCTGTC AAGGCTTCTA TCCCGAGCAC ATCGCGTGG AGTGGGAGAG CAATGGGCAG
1921 CGCGAGAACACA ACTACAGAC CCTCCCTCCC GTTGTGACT CGCACGGCTC CTTCCTCTC
1981 TACAGCAAGC TCAACCGTGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCACTGCTC
2041 GTGATGCAATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GGCCTCTCCCT GTCTCGGGT
2101 AAACGGGGCC GCGGAGGGGG TGGCGGGGGA TCAGTGGGG GTGGATCAGG CGGTGGAGGT
2161 TCCGGTGGCG GGGGATCGGG CGGTGGAGGT TCCGCTGGGG GTGGATCAGG GAAGAGGAGG
2221 AAGAGGGCGC AGCTCTGAGT CGAGGAGCTT GGGGGAGGT TGTTACAGGC TGGGGGGTCC
2281 CTGAGACTCT CCTGCGTCAGC CTGCGGATT ATGTTGACCA GGTATGCCAT GAGCTGGGTG
2341 CGCCAGGCTC CAGGGAAAGGG CGCAGAGTGG GTCTCAGGTA TTAGTGGTAG TGGTGTGAGT
2401 ACATACTACG CAGACTCCGT GAAGGGGGG TTCACCGTCT CCAGAGACAA TTCAAGAAC
2461 ACGCTGTATC TCCAATGAA CACCTGAGA GCGGAGGACA CGCTGTATA TTACTGCGCC
2521 CGGGGGCCCA CCTACACCG CGGGAGGACG GTGCGGACAG ACACCGAGTT CGACTACTGG
2581 GGCCAGGAA CCTCTGGTCAC CCTCTCTCTCA CGGAGCTGCAT CGGCCCCAAA GCTTGAAGAA
2641 GGTGAATTTC CAGAAGCAGC CGTATCTCAA CTGACTCTAGG ACCTCTGCTG GTCCTGTGGCC
2701 TTGGGACAGA CAGTCAGGAT CACATGCCAA GGAGACAGCC TCAGAAACTT TTATGCAAGC
2761 TGGTACCGC AGAAGCCAGG ACAGGGCCCT ACTCTGTCA TCTATGGTTT AAGTAAGG
2821 CCCTCAGGGA TCCCAGACCG ATCTCTCTGC TCCACCTCTAG GAAACACAGC TTCCCTTGACC
2881 ATCACTCGGG CTCAGGCGGA AGATGAGGCT GACTTACTT GCTGCTGTCA CTACGGGGC
2941 GGCCAGCAGG CGCTGTTCCG CGGGGGGACCC AAGCTGACCGG TCTACGTCA GCCCAAGGGCT
3001 GCCCCCTCGG TCACTCTGTT CGCGCCCTC TCTGCGGCGG GTCAGGCGG GTCAGGCGG
3061 GGTGGGGTCCG GTGGGGCGG ATCAGGTGGG GGTGGATCAG CGGGTGGAGG TTCCGGTGGC

5 3121 GGGGGATCAG ACAAAACTCA CACATGCCCA CCGTCCCCAG CACCGGAACCT CCTGGGCGGA
 3121 CGGTCACTCT TCCCTCTTCCC CCCAAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
 3241 GAGGTCACT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
 3301 TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGGGGGAGGA GCAGTACAAC
 3361 AGCACGTACG GTGTGGTCACT CGTCTCCTACCT GTCTCTGCACC AGGACTGCTT GAATGGCAAG
 10 3421 GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGGCC CCATCGAGAA AACCATCTCC
 3481 AAAGCCAAAG GGCAGCCCCG AGAACCACTAG GTGTACACCC TGCCCCATC CCGGGATGAG
 3541 CTGACCAAGA ACCAGGTCACT CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCCACATC
 3601 CGCGTGGAGT GGGAGAGCAA TGGGAGCGG GAGAACAACT ACAAGACCC GCCTCCCGTG
 3661 TTGGACTCTCG ACGGCTCTCTT CTTCCTCTAC AGCAAGCTCA CGTGGACAA GAGCAGGGGG
 15 3721 CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGATGAGG CTCTGCACAA CCACTACACG
 3781 CAGAAGAGCC TCTCCCTGTC TCCGGTAA TGA

FVII-066 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or SC5 to Fc region is underlined, and linker with proprotein convertase processing sites is shown in bold

20 1 MVSQALRLLC LLLGLOGCIA AVFVTOEEAH GVLRRRRRAN AFLEELRPGS LERECKEEQC
 61 SEEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THRDDQLICV NENGCEQYC SDHTGKTRSC RCHEGYSLLA DGVSCTPIVE YPCGKIPILE
 181 KRNASKEQQR IVGGKVCPCG BCPWQVLLVY NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAVQVIIIPS TYVPCTPNHD IALLRLHQPV VLTDHVVPCLC
 301 LPERTFSERT LAFVRFSLVS GWGQQLLDRGA TALELMVNLV PKLMTQDCLQ QRKRVGDSPN
 361 ITEYMFCAHY SDGSKDSCKG DSGGPHATHY RGTWYLTTGIV SWGQGCATVG HFGVYTRVSQ
 421 YIEWLQLMR SEFPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKHTTC
 481 PPCPAPELLG GFSVFLFFPK PKDTLMISRC PEVIVVVVDY SHEDPEVKFN WYVDCVVEVHN
 541 AKTGPREEQY NSTYRVVSVL TLVHQDWLNG KEYKRCVSNK ALPAPIEKTI SKAKGQFREP
 601 QVYTLPEPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSSFL
 661 YSKLTVDKSKR WQGQNVFSCS VMHEALHNHY TQKSLSLSPG **KRRRRSGGGG** SGGGSGGGGG
 721 **GGGGGGGGGG** **GGGGGGRKRR** KRAQVQLQES CGGIVQPGGS LRLSCAASGF MFSRYAMSIV
 781 RQAPGKGPFW VSGTISGSGGS TYYADSVGR FTVSDRNSKN TLYLQMNSLR AEDTAVYYCA
 841 RGATYKTERSD VFDQTSFDW GQGTLVTVSS GSASAPKLE GEFSEARVSE LTQDPAVSVA
 901 LGQQTVRITCQ GDSLRNFYAS WYQQKPGQAP TLVIYGLSKR PSGIPDRFSA SSSGNTASLT
 961 ITGAQABDEA DYVCLLYYGG QQQGVFGGGP KLTVLQPKA PSVTLFFPS SAAGGGGGGG
 1021 **GGGGGGGGGG** **GGGGGGGGGG** GGSIDKTHTCP PCPAPELLGG PSVFLFPKPK KDTIMISRTP
 1081 EVTCVVVDVS HEDPEVKFNW YVQGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
 1141 EYRKCKVSENKA LPAPIEKTIK KAKGQPREPO VYTLIPPSRDE LTKNOVSLTC LVKGFEYPSDI
 1201 AVEWESNGQP ENNYKTTTPV LDSDGSEFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
 1261 QKSLSTLSPGK *

45 DNA sequence for FVII-057

1 ATGGTCTCCC AGCCCTCTAC GCTCTCTCTCC CTTCCTGCTTG GGCTTCAGGG CTGCTCGGCT
 61 CGAGTCCTCG TAACCCAGGA GGAAGCCAC GGCGTCTCTC ACCGGCGCCG CGCGCGCCAAC
 121 CGCTTCTCTGG AGGAGCTCTG GCGGGCTCC CTGGAGAGGG AGTGCACAGGA GGACCACTGCG
 181 TCCCTCGAGG AGGCGGGGAGA GATCTCAAG GACCGGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCTCTA AGTCCATGCC AGAATGGGGG CTCTGCAAG
 301 GACCACTCTC AGTCTCTATAT CTGCTCTCTC CTCCCTGCT TGAGGGGGGG GAACUTGTGAG
 361 ACAGCACAGG ATGACCAGCT GATCTGTG AACGAGAAC CGGGCTGTGA GCAGTACTGCG
 421 AGTACCCACA CGGGCACCAA CGCGTCCCTG CCGTCCACAG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTGTAA TATCCATGTG CAAAAATAACG TATTCCTAGAA
 541 AAAAGAAATG CCAGCAAAAC CCAAGGGCGA GGTGGCGGTG GTCGGCGGG AGGTGGGTCC
 601 GGTGGCGCG GATCAGGTGG GGGTGGATCA GGCCTGGAG GTTCCGGUCC CGGGGGATCC
 661 GACAAAACCT ACACATGCC ACCGTCCTCCA GCTCGGAAAC TCCCTGGAGG ACCGTCAGTO
 721 TTGCTCTTCC CCCAACAAAC CCAAGCACCC CTCATGTC CCCCCGACCC TGAGGTACAA
 781 TGGCTGTGAG CCACGAAGAC CCTGAGGTCA AGTCTCAACTG GTACGTGGAC
 841 GGCCTGGAGG TGCATAATGC CAAGACAAAG CCGGGGGAGG AGCACTACAA CAGCACGTAC
 901 CGTGTGGTCA GCGTCTCTAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAGGGTCT CCAACAAAGC CCTCCCAAGC CCACTCGAGA AAACCATCTC CAAAGCCAAA
 1021 GGGCAGGCC GAGAACACCA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
 1081 AACCAGGTCA GCTGACCTG CCTGGTCAAAG GGCCTTATTC CCAGCGACAT CGCCGTGGAG
 1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAC TACAGACCA CGCTCTCCCT GTTGAACCTCC
 1201 GACGGCTCT TCTTCTCTA CAGCAAGCTC ACCGTCGACA AGAGCAGGTG CGAGCAGGGGG
 1261 AACGTCTTCT CAGCTCTCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAAGAGC
 1321 CTCTCCCTGT CTCCGGTAA AGGTGGCCGC GGATCAGSTG GGGGTGGATC AGGCCGTGGAG
 1381 GGTTCCGGTG CGGGGGGATC CGGGGGTGGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
 1441 GTTCAAGCG TGACCCAGAC CAGCAAGCTG ACCCGGATTC TGGGGGGCAA GGTGTGCCCC
 1501 AAAGGGGAGT GTCCATGGCA GGTCTGTGTT TTGGTGAATG GAGCTCAGTT GTGTGGGGGG
 1561 ACCCTGATCA ACACCATCTG GGTGGCTCC CGGGCCACT GTTCGACAA AATCAAGAAC

5 1621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA GCGAGCACGA CGGGGATGAG
 1681 CAGAGCCGGC GGGTGGCGCA GGTCATCATC CCCACCCAGT AGTCCCCGGG CACCAACCAAC
 1741 CACGACATCG CGCTGCTCCG CCTGCACCGAG CCCGTGGTCC TCACTGACCA TGTGGTGC
 1801 CTCTGCTGC CGGAACGGAC GTTCTCTGAG AGGACGCTGG CCTCGTGC CTTCTCATTG
 1861 GTCAGCGCT GGGGCCAGCT GCTGGACCGT GGCSCCAAGG CCTGGACCT CATGGTCCTC
 10 1921 AACGTGCCG GGTGATGAC CCAGGACTGC CTGCGCAGT CACGGAAAGT GGGAGACTCC
 1981 CCAAATATCA CGGACTACAT GTTCTGTGCC GGCTACTCGG ATGGCAGCAA GGACTCTGCC
 2041 AAGGGGACCA GTGGAGGCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
 2101 ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTGT CACCAAGGGTC
 2161 TCCCAGTACA TCGACTGGCT CGAAAAGTC ATGCCCTCG ACCCACGCC AGGAGTCTC
 15 2221 CTGCGACCC CAITTCGGG TGGCGGTGGC TCCGGGGAG GTGGGTCGG TGGCGGGGAG
 2281 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAAACCTCAC
 2341 ACATGCCAC CGTCCCCAGC ACCTGCAACT CTGGAGGAGC CCTCAGTCIT CCTCTTCCCC
 2401 CCAAAACCCA AGGACACCTC CATGATCTCC CGGACCCCTG AGGTACATG CGTGGTGGTG
 2461 GACGTGAGCC ACGAAGACCC TCGAGGTCAAG TTCACACTGGT AGCTGGACCG CGTGGAGGTG
 20 2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
 2581 GTCTCTACCG TCTGACCA GGACTGGCTG AATGCCAAGG AGTACAACTG CAAGGTCTCC
 2641 ACACAAAGCC TCCCAAGCCC CATCGAGAAA ACCATCTCA AAGCCAAAGG GCAGCCCCGA
 2701 GAACCAACAGG TGTACACCTC GGGCCATCC CGGGATGAGC TGACCAAGAA CGAGGTCA
 2761 CTGACCTGCC TGGTCAAAGG CTTCATCTCC ACCGACATCC CGTGGACTG GGAGAGCAAT
 2821 GGGCAGCGG AGACAAACTA CAAGACACAGG CCTCCCGTGT TCGACTCCGA CGGCTCTTC
 2881 TTCTCTTACA GCAAGCTCAC CGTGGACAAAG AGCAGCTGGC ACCAGGGAA CGTCTCTCA
 2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
 3001 CGGGGTAAAT 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 Xla cleavage site is shown in bold, and the Factor Xla cleavage site is shown in dashed underline

35 1 MVSQALKLIC LLLGLOGCLA AVEVITQEEAH GVLLHERRKAN AFLEELRPGS LERECKEEQC
 61 SFEEAAREIFK DABRIKLFWI SPYSDGQDQAS SPCCNNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKBDQQLICV NENGCGEQYC SDHTGKRLSC RCHEGYSSLLA DGVSCTPTVY YPCGKIPILE
 181 KRNASKPQGR GGGCGGGGGS GGGGCGGGGS GGGGGGGGGS DKTHTCPCCP APELLGGPSV
 241 FLFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNNAKTK PREEQYNSTY
 301 RVVSVLIVLH QDWLNKEYK CKVSNKALPA PIEKTIKAK QPQREPQVYT LPPSRDELTK
 361 NOVSLPCLVK GTYPSDIAYE WESNGQPENN YKTTFPVLDs DGSSFLYSLI TVDKSRWQOG
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG CGGGGGSGGG CGGGGGSGGG GSGGGGSGGG
 481 GSSVSQTSKL TRTVGGKVCV KGFCPWQVLL IVNGQOLCGG TLTNTIWVVS AAHCFDTKRN
 541 WRNLIAVLGE HDLSEHDGDE QSRRAQVIT PSTYVPGTTN HDIALLRLHQ PVVLTDHVVP
 601 LCLPERTFSE RTLAFMVRFSL VSGWGQLLDR GATALELMVL NVPRLMTQDC LQQSRKVGDS
 661 PNITEYMFCA GYSDGSKDSC KGDGGPHAT HYRGTYWLTG IVSWGQGCAT VCHFGVYTRV
 721 SOYIEWLQKL MRSEPRPCVL RAPFFCCGG SGGGGSGGG SGGGGSGGG SGGGGSDKTH
 781 TCPPCPAPE LGGPSVFLPP PKPKDTMIS RTPEVITVVV DVSHEDPEVK FNWYVUDGVEV
 841 HNAKTKPFREE QYNSTYRVVS VLTVLHQWLN NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 901 EFQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN QGPENNYKTT PPVLDSDGSF
 961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLs PGK*

DNA sequence for FVII-058

5 1 ATGGTCCTCC AGCCCCCTCAG GCTCTCTCTG CTTCTCTCTG CGCTTCAGGG CTGCCTGGCT
 61 GCAGTCCTCG TAACCCAGGA GGAAGCCAC GGCCTCTGC ACCGGCGCCG GCGGCCAAC
 121 CGCTTCCTGG AGGAGCTCGG CGCUGGGCTCC CTGGAGAGGG AGTCCAAGGA GGAGCACTGC
 181 TCCCTCGAGG AGCCCCGGGA GATCTTCAG GACCCGGAGA CGACGAAGCT GTTCTGGATT
 241 CTTTACACTG ATGGGGACCA CTGTCGCTCA AGTCCATGCC AGATGGGGG CTCCCTGCAAG
 301 GACCACTCC AGTCTCTATC CTGCTCTCC CTCCTCTGCT CGAGGGCGG GAACUTGTGAG
 361 ACGCACAGG ATCACCAGC GATCTCTGTG AACGAGAACG CGGGCTGTGA GCACTACTGC
 421 AGTGACCACA CGGGCACCAA CGCCTCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGACACCC CACAGTGA TATUCATGTG GAAAAATACCC TATCTTAGAA
 541 AAAAGAAATG CCAGCAAAAC CCAAGGGCGA CGTGGCGGTG CCTCCGGCGG AGCTGGCTCC
 601 GGTGGCGCG GATCAGGTGG GGGTGGATCA CGCGGGAGG GTTCCGGTGG CGGGGGATCC
 661 GACAAAACTC ACACATGCC ACCGTCGCCA GCTCCGGAAAC TCCCTGGGAGG ACCGTCAGTC
 721 TTCCCTCTCC CCCCCAAACCC CAAGGACACC CTCATGATCT CCCCCACCC TGACGTCA
 781 TGGCTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTCAACTG GTACGTGGAC
 841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CGCUGGGAGG AGCAGTACAA CAGCACTAC
 901 CGTGTGGTCA GCGCTCTCAC CGTCCCTGCAC CAGGACTGGC TGAATGGCAA GGACTACAAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCAAGCC CCCATGAGA AAACCATCTC CAAAGCCAAA
 1021 GGGCAGCCCC GAGAACACCA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
 1081 AACCAAGGTCA GCCTGACCTG CCTGGCAAA GGCTCTATC CGAGCGACAT CGCCGTGGAG

5	1141	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAC	TACAAGACCA	CGCCTCCCGT	GTGGGACTCC
	1201	GACGGCTCT	TCTTCTCTCA	CAGCAAGCTC	ACCGTCGACA	AGAGCAGGTG	GCAGCAGGGG
	1261	AACTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACATACAC	GCAGAAAGAC
	1321	CTCTCCCTGT	CTCGGGTAA	AGGTGGCGGC	GGATCAGGTG	GGGGTGGATC	AGGCGGTGGA
10	1381	GGTCCGGT	GGGGGGGATC	CGGGGGTGTGA	GGTCCGGT	GGGGGGGATC	AGGAGGAGGT
	1441	GGTCAGACT	TCTCTGGCGA	GGGGGGCGGC	GTGCGGATTG	GGGGGGCAGA	GGTGTGCUCC
	1501	AAAGGGGAGT	GTTCATGGCA	GTCCTCTGTG	TTGGTGAATG	GAGCTCAGTT	GTGGGGGGGG
	1561	ACCTGTATCA	ACACCATCTG	GGTGGTCTCC	GCGGCCCACT	TTTTCGACAA	AATCAAGAAC
	1621	TGGAGGAACC	TGATCGGGT	GCTGGGCGAG	CACGACCTCA	GCGAGCACGA	CGGGGATGAG
15	1681	CAGASCGGC	GGGTGGCGCA	GTCATCATC	CCCAGCACGT	ACGTCCCGG	CACCAAC
	1741	CACGACATCG	CGCTCTCTCG	CTGTCACCAAG	CCCGTGTCC	TCACTGACCA	TGTGGTGCCT
	1801	CTCTGCTCG	CGGAACGGAC	GTTCTCTGAG	AGGACGGCTGG	CCTTGTGCG	CTTCATG
	1861	GTCASCGGCT	GGGGGCAAGCT	GCTGGACTG"	GCTGCCACGG	CTTGTGAGGT	ATGGGGCTCC
	1921	AACTGTCCCC	GGCTGTATGAC	CCAGGACTGTC	CTGCAAGACT	CACGGAAAGGT	GGGAGACTCC
20	1981	CCAAATATCA	CGGAGTACAT	GTTCTGTGCC	GGCTACTCGG	ATGGCAGCAA	GGACTCTGC
	2041	AAGGGGGACA	GTGGAGGCC	ACATGCCACC	CACTACCGGG	GCACCTGGTA	CCTGACGGGC
	2101	ATCGTCAGCT	GGGGGCAAGGG	CTEGCACAAC	GTGGGCAACT	TTGGGTTGTA	CACCAAGGTC
	2161	TCCCACTACA	TCAGTGGCT	GCACAAAGCTC	ATGCGCTCA	AGCCACGCC	AGGAGTCTCC
	2221	CTGCGAGGCC	CATTTCCCGG	TCGGGGTGGC	TCCGGGCGAG	GTGGCTCGG	TGGGGCGGAA
25	2281	TCAGGTGGGG	GTGGATCAGC	CGGTGGAGGT	TCCGGCTGGC	GGGGATCAGA	CAAAACTCAC
	2341	ACATGCCAC	CGTGGCCAGC	ACCTGAACCTC	CTGGGAGGAC	CGTCAGTCCT	CCTCTTCCTCC
	2401	CCAAAACCCA	AGGACACCCCT	CATGATCTCC	CGGACCCCTG	AGGTACATG	CGTGGTGTCTG
	2461	GACGTGACCC	ACGAAGAACCC	TCAGGTCAAG	TTCAACTGGT	ACGTGGACGG	CGTGGAGGTG
	2521	CATAATGCCA	AGACRAAGCC	GGGGGAGGAC	GTGACACACA	GCACGTACCC	TGTGGTCAGG
30	2581	GTCTCTACCG	TCTGTCACCA	GGACTGGCTG	AATGGCAAGG	AGTACAAGTG	CAAGGGTCTCC
	2641	AACAAAGCCC	TCCCAGCCCC	CATCGAGAAA	ACCATCTCCA	AGGCCAAAGG	GCAGCCCCGA
	2701	GAACCCACAGG	TGTACACCCCT	GGCCCATATCC	CGGGATGAGC	TGACCAAGAA	CCAGGTCAAG
	2761	CTGACCTGGC	TGGUCAAAGG	CCTCTCTAUC	AGGACACATCG	CGTGGAGGTG	GGAGAACTA
	2821	GGGCAGCGGG	AGAACAACTA	CAAGACCAAC	CCTCCCGTGT	TGGACTCCGA	CGGCTCTTC
	2881	TTCTCTCTACA	GCACAGTCAC	CGTGGACAAAG	AGCAGGTGGC	AGCAGGGGAA	CGTCTTCTCA
	2941	TGCTCTCGTGA	TGCTGAGGGC	TCTGCACAAAC	CACTACACGC	AGAAGAGCCT	CTCCCTGTCT
35	3001	CGGGGTAAAT	GA				

40 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

1	MVSQALKLIC LLLGLQGCLIA AVFVTCQEEAH GVLHRRKKAN AFLFEELRPGS BERECKEEQC
61	CFTEAREPTEFK DAERTKLFWTI SYSDGDQGAS SPCQNGGCK DQIQSYTFCFG TPAFFGRVNP
121	THKKDDQLICV NENGSCCEQYC SDHTGTKRSC RCHEGVSLLA DGVSCTPTEV YPCGKIPFILE
181	KRASKPKQGK GGGGSGGGGS GGGGSGGGGS DKTHTCPCPQ APELLGGPSV
241	FLFPPPKPKDT LMISRPTPEV CIVVVDVSHED PEVKFNWYD EGVHNNAKTRK PREQYNTSY
301	RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYD LPSSRDELTK
361	NQVSLLTCLVK GFYPSDIAVE WESNGQPEENN YKTTTPVPLDS DGSFFLYSKI TVDKERWQOG
421	NVFSCGVMHIE ALHINHYTQKS LSLSPFGKGGG GS GGGGGGGG GS GGGGGS GS GGG GS GGGGGS GGG
481	GSDFLAEGGG YRIVGGKVCP KGECPWQVII LVNGAQLOGG TLINTIYVWS AAHCFDKIKN
541	WRNLIAVILGE HDLSEHGDDE QSRRVAQVII PSTYVPGTT HDIALRLHQ PVVLTHDWV/P
601	LCLPERTFSE RTLAFVFRSL VSGWGQQLIDR GATEALMVLV NVEPLMTQDC LQQRKVKEDS
661	PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWILTG IVSWGQGCAT VGHFGVYTKV
721	SQYJEWLQKL MRSEPRPGVL LRAPFFGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
781	TCPPCPAPFL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVF DVSHEDPEVK FNWYVDGVEV
841	HNAKTKFREY QYINSTTRVVS VLTVLRHQDW NGKFEYKCVS NKALPAPIKE TISKAKCQFPR
901	EEQCVYTLPPS RDLETLKQNVS LTCLIVKGFYD SDAIWRWESNQ GQFENNYKTT PPVLDSDGSE
961	FLYSKLTVDK SRWQOGNVFS CSVMHEALHN HYTQKSLSLS PGK*

60	DNA sequence for FVII-059
	1 ATGGTCTCCC AGGCCCTCAG GCTCCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGGCTGGCT
	61 GCAGTCCTCG CAACCCAGGA GGAAGCCAC GGCCTCTCTGC ACCGGCGCCG GCGCGCCAAC
	121 GCGCTTCTGG AGGAGCTCGC GCGGGCTCTCC CTGGAGAGGG AGTCGCAAGGA GGAGCACTGC
	181 TCCTTCCAGG AGGGCCGGGA GATCTTCAAG GACCGAGGAGA GGACGAAGCT GTTCTGQATT
	241 TCTTACAGTG ATGGGGACCA GTGTCCTCA AGTCCATGCC AAATGGGGGG CTCTCTGCAAG
65	301 GACCAAGCTCG AGTCTCTATAT CTGCTCTCTGC CTCCCTCTGCCT TCGAGGGCCG GAACATGTTGAG
	361 ACGCACAAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG CGGGCTGTGA GCAGTACTGC
	421 AGTGACCACA CGGGCACCAA GCGCTCTCTG CGGTGCCACG AGGGGTACTC TCTGCTGGCA
	481 GACGGGGTGT CCTGCACACC CACAGTTGCA TATCCATCTG GAAAATACAT TATCTACAA
	541 AAAAGAAATG CCAGCAAAAC CCAAGGCCGA GGTGGGGGTG GCTCGGGCGG AGGTGGGTCC
	601 GGTGGCGCGC GATCAGGTGG GGGTGGATCA GGCCTGGAG TCTGGGTGG CGGGGGCATC
	661 GACAAAAACTC ACACATGCC ACCGTGCCA CCTCCGGAAAC TCTCTGGGAGG ACCTGGTAC
70	721 TTCTCTTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGGTCAAC

5 781 TGGCTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTCAACTG GTACGTGGAC
 841 GCGCTGGAGG TGCTATAATGC CAAGACAAAG CCGCCTGGAGG AGCACTACAA CAGCACGTAC
 901 CGCTGTGCTCA CGCTCCCTCAC CGTCTCGAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCGAGC CCCATCGAGA AAACCATCTC CAAAGCCAA
 1021 GGGCAGCCCC GAGAACACCA GGTGTACACC CTGCCCCAT CCCGGGATGA GCTGACCAAG
 10 1081 AACCAGETCA GCCTGACCTG CCTGGICAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
 1141 TGGGAGAGCA ATGGSCAGCC GGAGAACAC TACAAGACCA CGCTCTCCGT GTTGGACTCC
 1201 GACGGGCTCCT TCTTCTCTCA CAGCAAGCTC ACCCGTCGAGA AGAGCAGGTG GCAGCAGGGG
 1261 AACGTCTCTC CATGCTCCGT GATGCTGACA ACCACTACAC GCAGAAGAGC
 1321 CTCTCCCTGT CTCCGGTAA AGGTGGGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
 15 1381 GGTTCCGGTG CGGGGGGATC CGGGGGTGGA GTTTCGGTG GGGGTGGATC AGGAGGAGG
 1441 GGTTCAACCA CCAAGATCAA GCCCCGGATT GTGGGGGCA AGGTGTGCC CAAAGGGGAG
 1501 TGTCTCATGG AGGTCTCTGT GTTGGTGAAT GGAGCTCAGT TGTGTGGGGG GACCCUTGATC
 1561 AACACCATCT GGGTGGTCG CGGGGGCCAC TCTTCTGACA AAATCAAGAA CTGGAGGAAC
 1621 CTGATCCCGG TGCTGGCGA CGACGACCTC ACCGAGCACG AGGGGATGA GCAGACCCGG
 20 1681 CGGGTGCUGC AGGTCTATCAT CCCACAGACG TACGTCCCGG GCACCAACAA CCACGACATC
 1741 GCGCTGCTCC GCCTGCACCA GCGCGGGTC CTCACTGACC ATGTGGTCCC CCTCTGCTG
 1801 CCCGAACGGA CGTCTCTGA GAGGACGCTC GCCTTCGTGC GCTCTCTCATT GTCAAGCGGC
 1861 TGGGGGCCAGG TGCTGGACCG TGCTGGCACG GCCTGGGACG TCACTGGTCTC CAACGTGCC
 1921 CGGCTGTGCA CCCAGGACCTC CCTCCACCGAG TCACCGAAGG TGGAGACTC CCCAATATC
 1981 AGGGAGTACA TGTCTGTGTC CGCTACTCG GATGCCAGCA AGCACTCTC CAAGGGGGAC
 2041 AGTGGAGGCC CACATGCCAC CCAACTACCGG GGCACGTGGT ACCTGACCGG CATCTCAGC
 2101 TGGGGCCAGG GCTGGCAGAC CCGGGGGCAC TTTGGGGTGT ACACCAGGT CTCCAGTAC
 2161 ATCGAGTGGC TGCAAAAGCT CATGCGCTCA GAGCAGCGAG CAGGAGTCTC CTCGCGAGCC
 2221 CGATTTCGGC GTGCGGGTGG CTCCGGCGGA GTGCGGTCCC GTGGCGGCAG ATCAAGTGGG
 30 2281 CGTGGGATCAG GCGCTGGAGG TCCGGGTGGC GGGGGATCAG ACAAAACTCA CACATGCCCA
 2341 CGGTGCCAGG CACCTGAACT CCTGGGAGGA CGCTCAGTCT CCTCTTCCC CCCAAACCC
 2401 AAGGACACCC TCACTGATCTC CGGGACCCCC GAGGTACATC GCGTGGTGGT GGACGTGAGC
 2461 CACCAAGACCC CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCAATAATGCC
 2521 AAGACAAAGC CGGGGGAGGA GCGTGGACAC AGCACTGATC GTGTTGGTCAAG CGTCTCTCACC
 2581 GTCTGCAACC AGGACTGGT GAATGGCAAG GAGTACAAAGT GCAAGGTCTC CAACAAAGGCC
 2641 CTCCCACCC CCATCGAGAA AACATCTCC AAAGCCAAAG GCGAGCCCCG AGAACCCACAG
 2701 GTGTACACCC TGCCCCCATC CGGGGATGAG CTGACCAAGA ACCAGGTACG CCTGACCTGC
 2761 CTGGTCAAAG GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGGCC
 2821 GAGAACAACT ACAAGACCCAC GCCTCCCGTG TTGGACTCGG ACGGCTCCCTT CTTCTCTAC
 40 2881 AGCAAGCTCA CGTGGACAA GAGCAGGTGG CAGCAGGGGA ACCTCTCTC ATGCTCCGTG
 2941 ATGCAATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGTAAA
 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 LLLGLOGCL AVFVQEFH GVLLRRRRA AFLEELRPGS LERECKEEQC
 61 SFEEARIEFK DAERTKLFWI SYSDGDQCA SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 50 121 TIKBDQQLLCV NENGGCCEQYC SDHTGTKRSC RCHEGYSLIA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGGGGGG GGGGGGGGG GGGGGGGGG DKTHTCPCCP APELLGGPSV
 241 FLFPFPKPKDT LMISKTPETV PEVKENWYTV GVEVHNNAKTK PREEQYNSTY
 301 RRVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTIKSK QFQREPQVYT LPPSRDFTLTK
 361 NQVSLSCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDs DGSSFFLYSKL TVDKSRWQQG
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGGGGGG GSGGGGGGGGG GSGGGGGGGGG
 481 **G**STTKTKPEI VGGKVCPRGE CFWQVLLILVN GAQICCGTNTI NTIWWVSAAH CFDKTKNWRN
 541 LIAVGEHDL SEHDGDEQSRV ARAQVIIEST YVPGTTNHDI ALLRLHQPVV LTDHVVPLC
 60 601 PERTFSERTL AFVRFSLVSG WGQLLDRGAT ALEIMVLNPV RLMTQDCLQQ SRKVGDSPNI
 661 TEYMFCAAGYS DGSKDSCKGD SGGPHATHYR GTWVLTGIVS WCGQGCATVGH FGVTVKVSQY
 721 IEWLQKLMRS EPRPGVLLRA PFPGGGGSGG GGSGGGGSGG GGSGGGGSGG GGSDDKTHTCP
 781 PCPAPELLGG PSVFLFPKPK DKTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVBVHNA
 841 KTKPREEQYN STYRVVSVIT VIBCDWINGK FYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
 901 VYTLPPSRLDE LTKNQVSLTC LVKGFPDSI AVEWESNGQP ENNYKTPPPV LDSDGSFFLY
 961 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK *

65 DNA sequence for FVII-060
 1 ATGGTCTCC AGGCCTCTAG GCTCCTCTGC CTTCTGTTG GGCTTCAGGG CTGCTGGCT
 61 CGAGTCTTCG TAACUCAGGA GGAAGCCAC GGCCTCTGC ACCGGCGCCG GCGCCUCAAC
 121 CGCTTCTGG AGGAGCTCG GCGGGGTCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGGCGGGGA GATCTCAGG GACGGGGAGA GGACGAAGCT GTTCTGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG

5 361 ACGCACAAAGG ATGACCACCT GATCTGTCTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCCACA CGGGCACCAA GCGCTCCGT CGGTCACAG AGGGTACTC TCTGCTGGCA
 481 GACGGGTGTG CCTGCACACC CACAGITGAA TATCCATGTG GAAAAATACC TATTCAGAA
 541 AAAAGAAATG CCAGCAAAACCC CCAAGGGCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
 601 GGTGGCGCG GATCAGGTGG GGGTGGATCA GGCCTGGAG GTTCCGGTGG CGGGGGATCC
 10 661 GACAAAACCTC ACACATGCC ACCGTGCCA GCTCCGGAAC CCTCTGGAGG ACCGTCAAGTC
 721 TTCTCTTCC CCCAAAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTCACA
 781 TGGCTGGTGG TGGACGTGAG CCACGAACG CCTGAGGTCA ACTCAACTG GTACGTGGAC
 841 GGCCTGGAGG TGCAATAATG CAAGACAAAG CGCGGGAGG AGCAGTACAA CAGCACGTAC
 901 CGTGTGTCA GCGTCTCAC CGTCTGTAC CAGGACTGGC TGAATGGAA GGAGTACAAG
 15 961 TGCAAGGTCT CCAACAAAGC CCTCCGACCC CCCATGAGA AAACCATCTC CAAACCCAAA
 1021 GGGCAGCCCC GAGAACCCACA GGTGTACACC CTGCCCCAT CCGGGATGA GCTGACCAAG
 1081 ACCAGGTCA GCGCTGACCTG CCTGGTCAAA GGCCTCTATC CGAGCGACAT CGGCGTGGAG
 1141 TGGGAGAGCA ATGGGAGAGCC GGAGAACACR TACAAGACCA CGCTCCCGT GTTGGACTCC
 1201 GACGGCTCTC TCTCTCTCA CAGCACGTC ACCGTGACA AGAGCAGGTG GCAGCAGGGG
 20 1261 AACGTCUTCT CAATCTCGT GATGCAATGAG GCTCTGACA ACCACTACAC GCAGAGAGC
 1321 CTCTCCCTGT CTCCGGTAA AGGTGGCGGC CGATCAGGTG GGGGTGGATC AGGCGGTGGA
 1381 GGTTCGGTGG GCGGGGATC CGGCGGTGGA GTTCCGGTGG GGGGTGGATC AGGAGGAGGT
 1441 GGTTCAGGCC TGCGGCCCCG GGTGGGGCGC GGCCTGGTGG TGGGGGGCAA GTGTCGCCCC
 1501 AAAGGGAGT GTCATGCCA CAGCAGTC ACCGTGACA AGAGCAGGTG GCAGCAGGGG
 1561 ACCCTGATCA ACACATCTG GGTGGTCTC GCGGGGCACT GTTTCGACAA ATAAGAAC
 1621 TGGAGGAACC TGATCGCGT GCTGGGGCAG CACGACCTCA CGAGCACCA CGGGGATGAG
 1681 CAGAGCCGC GGGTGGCCCA GTCATCATC CCCACCACTG AGTCCCCGG CACCACCAAC
 1741 CACGACATCG CGTCTCTCG CCTGCAACAG CCCCTGGTCC TCACTGACCA TGTGGTGGCC
 1801 CTCTGGCTCG CCGAACGAC GTCATCTCGAG AGGAGCTGG CCTTCTGCGG CTTCTCATTC
 1861 GTCAGCGCT GGGGGCAGCT GCGGGGACCT GCGGGCACGG CCGGGGACCT CATGGTCCTC
 1921 AACGTGCCCC GGTGTATGAC CGAGGACTGC CTCAGCAGT CACCGAAGGT GGGAGACTCC
 1981 CAAATAATCA CGGAGTACAT GTTCTGTGCG GGTACTCTGG ATGGCAGCAA GGACTCTGC
 2041 AAGGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACTGGTA CCTGACGGGC
 2101 ATCGTCAGCT GGGGGCAGGG CTGCGCACCC GTGGGGCACT GTGGGGCTTA CACCAAGGGTC
 2161 TCCCTAGTACA TCGAGTGGCT GCAAAAGCTC ATGGCTCTCG AGCCACGCC AGGAGTCCTC
 2221 CTGCGACCC CATTTCGGG TCCGGTGGC TCCGGGGAG GGGATCAGA CAAACTCAC
 2281 TCAGGTTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGATCAGA CAAACTCAC
 2341 ACATGCCAC CGTCCCCAGC ACCTGAACTC CTGGGAGGAC CCTCAGTCTT CCTCTTCCCC
 2401 CCAAAACCC AGGACACCC CATGATCTCC CGGGACCTCG AGTCACATG CGTGGTGGTG
 2461 GACGTCAGGC AGCAAGAGC TGAGGTCAGG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
 2521 CATAATGCCA AGACAAAGCC GGGGGAGGAG CAGTAAACA GCACTGACCG TGTGGTCAGC
 2581 CTCTCTACCG TCCCTGACCA GCACTGGCTG AATGCAAGG ACTACAAGTG CAAGTCTCC
 2641 ACAAAAGCCC TCCCAGGCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAAGCCCGA
 2701 GAACCACAGG TGTACACCCCT GCCCCATCC CGGGATGAGC TGACCAAGAA CGAGGTCAAG
 2761 CTGACCTGCC TGGTCAAAGG CCTCTATCC AGCGACATCG CGTGGAGCTG GGAGAGCAAT
 2821 GGGCAGCCGG AGAACAAACCA CAAGGACAGG CCTCCGGTGG TGGACTCCGA CGGCTCCCTC
 2881 TTCTCTACCA GCAAGCTCAC CGTGGACAGG AGCACTGGC ACCAGGGGAA CCTCTTCTCA
 2941 TGCTCCGTGA TGCATGAGGC TCTGACACAC CACTACACCG AGAAGAGCCT CTCCCTGTCT
 3001 CGGGGTAAAT 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

1 MVSQALRLLC LLLGLQGCLIA AVFVTCEH GVLHRRRRAN AFLEELRPGS LERECKEEQC
 61 SEEEAREIFK DAERTKLEWI SYSDGDQ~~CAS~~ SPCQNGGSCK DQIQS~~Y~~YICFC LPAFEGRNCE
 121 THKDDQ~~L~~LCV NENGSC~~E~~QYC SD~~Y~~TGTRKSC R~~C~~H~~E~~Y~~S~~LLA D~~G~~V~~S~~C~~T~~PT~~V~~E YPCGK~~I~~P~~I~~LE
 181 KR~~N~~ASK~~K~~PFQGR GGGGGGGGG GGGGGGGGG GGGGGGGGG D~~K~~T~~H~~T~~C~~PP~~C~~P APELGGPSV
 241 FLEPP~~P~~KERDT L~~M~~IS~~R~~TP~~E~~V~~T~~ PEV~~K~~N~~W~~Y~~V~~ D~~V~~E~~V~~H~~I~~N~~A~~TK~~P~~ RE~~F~~Q~~Y~~N~~S~~TY
 301 R~~V~~V~~S~~V~~L~~T~~V~~L~~H~~ QD~~W~~LN~~G~~KEY~~K~~ CKVSN~~K~~AL~~P~~A P~~E~~KT~~I~~SK~~A~~T G~~Q~~F~~R~~E~~P~~Q~~V~~YT~~I~~ L~~P~~P~~S~~R~~D~~E~~L~~TK
 361 NQVSL~~T~~CL~~V~~K GFYPS~~D~~IAVE WESNGQ~~P~~ENN Y~~K~~TT~~P~~V~~L~~D~~S~~ DGSFF~~L~~Y~~S~~K~~L~~ TVD~~K~~SR~~W~~Q~~Q~~G
 421 NVFSCS~~V~~M~~H~~E ALU~~N~~HY~~T~~Q~~K~~S L~~S~~LSP~~G~~K~~E~~GG G~~S~~GGGG~~S~~GGGG G~~S~~GGGG~~S~~GGGG G~~S~~GGGG~~S~~GGGG
 481 **G**SA~~L~~R~~P~~R~~V~~VG GAVVGG~~R~~V~~C~~P K~~G~~ECP~~W~~Q~~V~~LL LVNGAQL~~C~~GG TL~~I~~NT~~I~~W~~V~~V~~A~~HCFDK~~I~~KN
 541 WRNLIAV~~L~~G E~~H~~LS~~E~~HD~~G~~DE Q~~S~~R~~R~~V~~A~~V~~I~~ P~~S~~T~~Y~~V~~F~~G~~T~~IN HD~~I~~ALL~~R~~IQ P~~V~~V~~I~~T~~D~~H~~V~~VP
 601 LCLP~~F~~ERT~~F~~SE RT~~L~~A~~F~~V~~R~~ES~~I~~ VSGW~~G~~Q~~L~~DR G~~A~~T~~A~~EL~~M~~VL N~~V~~F~~R~~L~~M~~T~~Q~~DC~~I~~ L~~Q~~Q~~S~~R~~K~~V~~G~~DS
 661 PNITEYMFCA GYS~~D~~G~~S~~K~~D~~SC K~~G~~D~~S~~GG~~G~~HAT HYRG~~T~~W~~Y~~LTG IV~~S~~WG~~Q~~GC~~A~~T VGHFC~~V~~Y~~T~~RV
 721 SQYIEW~~L~~Q~~K~~L MRSE~~P~~RP~~G~~V~~L~~ L~~R~~A~~P~~F~~P~~GGGG S~~G~~GGGG~~S~~GGGG S~~G~~GGGG~~S~~GGGG S~~G~~GGGG~~S~~DK~~T~~H
 781 T~~C~~PP~~C~~PA~~P~~E~~L~~ L~~G~~GP~~S~~V~~E~~LF~~P~~ P~~K~~E~~K~~D~~E~~LM~~I~~S~~R~~ RT~~P~~E~~V~~TC~~V~~V~~V~~ D~~V~~S~~H~~E~~D~~PE~~V~~K~~E~~ F~~N~~W~~V~~D~~G~~V~~E~~V
 841 HNAKTK~~P~~FREE QYNSTY~~R~~V~~V~~S VLT~~V~~L~~H~~Q~~D~~W~~L~~ NG~~K~~Y~~E~~K~~C~~V~~S~~ N~~K~~AL~~P~~AP~~I~~E~~K~~ T~~I~~SK~~A~~K~~G~~Q~~P~~R
 901 E~~P~~Q~~V~~Y~~T~~L~~P~~PS R~~D~~E~~L~~T~~K~~Q~~N~~V~~S~~ L~~T~~CL~~V~~K~~G~~F~~P~~ S~~D~~1~~A~~V~~W~~E~~S~~ S~~Q~~P~~E~~NNY~~K~~TT P~~P~~V~~L~~S~~D~~G~~S~~F
 961 FLYSKL~~T~~V~~D~~K SRWQ~~Q~~GN~~V~~F~~S~~ CSVM~~H~~AL~~I~~H HY~~T~~Q~~R~~S~~L~~S~~L~~S~~F~~ C~~K~~*

5 DNA sequence for FVII-061

1 ATGGCTCTCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
 61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCTCTGC ACCGGCGCCG GCGGCCAAC
 121 CGGTTCTGG AGGAGCTCG GCGGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCAGG AGGCCCGGGA GATCTCAAG GACGGGAGA GGACGAACCT GTTCTGGATT
 10 241 TCTTACAGTG ATGGGGACCA GTGTGCTCTA AGTCATGCC AGAATGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCTTATAT CTGCTCTCTGC CTGCTCTGCCT TGGAGGGCCG GAACTCTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG CGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA CGCTCTCTGT CGGTGCAAGG AGGGTACTC TCTGCTGGCA
 481 GACGGGTGTG CTCACACCC CACAGTCAA TATCCATGTG AAAAATACC TATCTAGAA
 15 541 AAAAGAAATG CCACCAAAAC CCAAGGGCGA GGTGGGGTGTG GCTCCGGCGG AGGTGGGTCC
 601 GGTGGGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCC
 661 GACAAAATC ACACATGCC ACCTGGGCCA GCTCCGGAA TCTGGGAGG ACCGTTAGTC
 721 TTCTCTTCG CCCAAAAACCA CAAGGACACC CTCACTGATCT CCCGGACCC TGAGGTACA
 781 TGGCTGTGG TGGACGTGAG CCTGAAAGAC CCTGAGGTCA ACTTCAACTG GTACTGGAC
 20 841 GGCCTGGAGG TGCTATAATGC CAAGACAAAG CCGGGGGAGG ACCAGTACAA CAGCAOGTAC
 901 CGTGTGCTCA GCGTCTCTAC CGTCTGGCAC CAGGACTGCC TGAATGGCAA GGACTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCGAGC CCCATCGAGA AAACCATCTC CAAAGCCAA
 1021 GGGCAGGCC GAGAACCC GGTGTACACC CTGGCCCCAT CCCGGGAUGA GCTGACCAAG
 1081 AACAGACCTCA CGCTGACCTC CGCTGCTCTA CGCTGACAT CGCGCTGGAG
 1141 TGGGAGAGCA ATGCCACGCC GGAGAACAC TACAGACCA CCCCTCCCGT GTTGGACTCC
 1201 GACGGCTCTC TCTTCTCTA CAGCAAGCTC ACCGTCGACA AGAGCAGCTG GCAGCAGGGG
 1261 AACGTCTCTC CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
 1321 CTCTCCCTGT CTCCGGTAA AGCTGGGGCG GGAATGGGTG GGGGTGGATC AGGCGGTGGA
 1381 GGTTCAGGCC CGCGGGGATG GGTGGGGTGTG GGTTCGGGTG GGGGTGGATC AGGAGGAGG
 1441 GGTTCAGGCC TGGGGGGCCC GGTGGGGCCC GGCCTGGATG TGGGGGGCAA GGTGTGGCCC
 1501 AAAGGGGAGT GTCCATGGCA GGTCTCTGTG TTGGTGAATG SAGCTCAGTT GTGTGGGGGG
 1561 ACCCTGATCA ACACCATCTG GTGTTGCTCC GGGGGCCACT GTTTCGACAA ATCAAGAAC
 1621 TGGAGGAACC TGATCGGGT GCTGGGGCGAG CACGACCTCA CGGAGCACGA CGGGGATGAG
 1681 CAGAGCGGC GGTGAGGCCA GGTGATCATC CCCAACAGT AGTCCCCGG CACCAACCAAC
 1741 CACGACATCG CGCTGCTCCG CCTGACCCAG CGCTGGTGTG TCACTGACCA TGTGGTGGCC
 1801 CTCTGCTCTG CGGAAACGGAC GTTCTCTGAG AGGAGGCTGG CCTCTGGTGG CTTCTCATTG
 1861 GTCAGCGGCT GGGGGCAGCT GGTGGACCGT GGCGCCACGG CCTGGACCT CATGTCCTC
 1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCGACAGT CACGGAAAGGT GGGAGACTCC
 1981 CAAAAATC CCGACTACAT GTTCTGTGCC GGTACTCGG ATGGCAGCAA GGACTCTGCC
 2041 AAGGGGACCA GTGGAGGCC ACATGCCACO CACTACCGG GCACTGGTA CTCGACGGG
 2101 ATCGTCACTG GGGGGCAGGG CTGGCGAACCT GTGGGGCACT TTGGGGTGTG CACCAAGGGTC
 2161 TCCCAGTACA TCGACTGGCT CGAAAAGCTC ATGCCCTCTAG ACCCACGCC AGGAGTCTC
 2221 CTGCGAGGCC CATTCTCCGG TGGGGGTGGC TCCGGGGAG GTGGGTCCGG TGGCGGGGGA
 2281 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAACTCAC
 2341 ACATGCCAC CGTCCCCAGC ACCTGAACTC CTGGAGGAGC CCTCAGTCCTT CCTCTTCCCC
 2401 CCAAAACCCCA AGGACACCCCT CATGATCTCC CGGACCCCTG AGGTCACTATG CTTGGGGTGTG
 2461 GACGTGAGCC AGGAAGACCC TTGAGTCAGG TTCACTGGT AGTGGACGG CGTGGAGGTG
 2521 CATAAACTCA AGACAAAGCC GGGGGAGGAG CAGTACAACA GCACTGACCG TGTGGTCAGC
 2581 GTCTCACCG TCCCTCACCA GGACTGGCTG AATGCGAAGG ACTACAAGTG CAAGGTCTCC
 2541 AACAAACCC TCCAGGCCCATC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAAGCCCCGA
 2701 GAACCCAGGG TGTACACCCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CGAGGTCA
 2761 CTGACCTGCC TGGTCAAAGG CCTCTATCTCC AGCGCACATCG CGTGGGAGTG GGAGAGCAAT
 2821 GGGCAGCCGG AGAACAACTA CAAAGACCCAG CCTCCGGTGT TGGACTCCGA CGGCTCTTC
 2881 TTCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGAA CGTCTCTCA
 2941 TGCTCCGTGA TGCATGAGC TCTGCACAAAC CACTACACCC AGAAGAGCCT CTCCCTGTCT
 3001 CGGGTAAAT GA

60 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 MVSOALRLLC LILGLOGGLA AVFVTCFEEAH GVJHERRRAN AFLEELRPGS LERECKEEQC
 61 SFEAREIIFK DAEFTKLFWI SYSSDGDDQAS SPCQNGGCK DQIQSYICFC LPAFEGRNCE
 121 THKDBQI TCV NFNCGCFQYC SDITGFKRSC RCHFCYSLIA DGVSCPTPTVR YPCGKTPTLE
 181 KRNASKFQGR GGGGGGGGGS GGGGGGGGGS GGGGGGGGGS DKHTHTCP2CP APELGGPSV
 241 FLFPFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNNAKTK PREEQYNSTY
 301 RVSVSLTVLH QDWLNGKEYK CKVSNKALPA EIEKTISSAK QFREPQVYT LPPSRDELTK
 361 RQVSLETCVKE GFYPSDIAVE WESNGCPEENN YKTTPEPVLDs DGSFFPLYSKL TVDKSRWQ2G
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKCGG GSGGGGGGGGG GSGGGGGGGGG GSGGGGGGGGG
 481 GSALRPRVVG GALVGGKVC2 KGECEFWQVLL LVNGAQLOGG TLTNTIWWVG AAIICFDKIKN
 541 WRNLIAVILGE HDLSEHDGDE QSRVVAQVII PSTYVPGTTN HDIALLRLHQ PVVLTDDHVVP
 601 LCLPRTFSE RTLAFVFRSL VSGWGQLLDR GATALELMVL NVPRLMFQDC LQQ3SRKVGDs

5 661 PNITEYMFCA GYSDGSKDSC KGDGGPHAT HYRGTWYLTG IVSWGQGCAT VGIIFGVYTRV
 721 SQYIEWLQLK MRSEFRPGVL LRAFFPGEGG SGGGGGGGGG SGGGGGGGGG SGGGGSDKTH
 781 TCPPCPAPEL LGGPSVFLFP PKPKDTILMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 841 HNAKTKFREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 901 EFQVYLTFLPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT FPVLDSDGSF
 10 961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLN PGK*

DNA sequence for FVII-062

1 ATGGTCTCCC AGGCCCCAG 961 PNITEYMFCA GYSDGSKDSC KGDGGPHAT HYRGTWYLTG IVSWGQGCAT VGIIFGVYTRV
 61 GCAAGTCTCG TAACCCAGGA GGAAGCCAC 921 SQYIEWLQLK MRSEFRPGVL LRAFFPGEGG SGGGGGGGGG SGGGGSDKTH
 121 GCGTTCTGG AGGAGCTGCG GCGGGGCTC 9781 TCPPCPAPEL LGGPSVFLFP PKPKDTILMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 181 TCCTTCGAGG AGGCCCCGGGA GATCTTCAGG GACGGGGAGA 1031 HNAKTKFREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 241 TCTTACAGTG ATGGGGACCA GTGTGCTCTCA AGTCTCATGCC 1081 EFQVYLTFLPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT FPVLDSDGSF
 301 GACCAGCTC AGTCCTATAT CTGCTCTGC 1131 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLN PGK*

15 121 GCGTTCTGG AGGAGCTGCG GCGGGGCTC 9781 TCPPCPAPEL LGGPSVFLFP PKPKDTILMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 181 TCCTTCGAGG AGGCCCCGGGA GATCTTCAGG GACGGGGAGA 1031 HNAKTKFREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 241 TCTTACAGTG ATGGGGACCA GTGTGCTCTCA AGTCTCATGCC 1081 EFQVYLTFLPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT FPVLDSDGSF
 301 GACCAGCTC AGTCCTATAT CTGCTCTGC 1131 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLN PGK*

20 361 ACGGCACAAAGG ATGACAGCCT GATCTGTGTC AACGGAACG 121 GCGTTCTGG AGGAGCTGCG GCGGGGCTC 9781 TCPPCPAPEL LGGPSVFLFP PKPKDTILMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 421 AGTACCCACA CGGGCACCAA GCGCTCTGC 181 TCCTTCGAGG AGGCCCCGGGA GATCTTCAGG GACGGGGAGA 1031 HNAKTKFREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG 1081 EFQVYLTFLPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT FPVLDSDGSF
 541 AAAAGAAATG CCAGCAAAAC 1131 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLN PGK*

25 601 GGTGGCGCG GATCAGGGGG GGGTGGATCA GGCAGGGCG 121 GCGTTCTGG AGGAGCTGCG GCGGGGCTC 9781 TCPPCPAPEL LGGPSVFLFP PKPKDTILMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 661 ACACATGCCA 181 TCCTTCGAGG AGGCCCCGGGA GATCTTCAGG GACGGGGAGA 1031 HNAKTKFREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 721 TTCTCTTCC CCCCAAAACC CAAGGACACC 1081 EFQVYLTFLPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT FPVLDSDGSF
 781 TCGCTGGTGG TGGACGTGAG CCACGAAAGC 1131 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLN PGK*

30 841 GGCCTGCAGG TGCAATAATGC CAAGACAAAG CCGGGGGAGG 121 GCGTTCTGG AGGAGCTGCG GCGGGGCTC 9781 TCPPCPAPEL LGGPSVFLFP PKPKDTILMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 901 CGTGTGTCA GCGTCTCTAC CTCCTCTGCAC CAGGACTGGC 181 TCCTTCGAGG AGGCCCCGGGA GATCTTCAGG GACGGGGAGA 1031 HNAKTKFREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 961 TGCAAGGTCTC CCAACAAAGC CTCCTCCAGC 1081 EFQVYLTFLPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT FPVLDSDGSF
 1021 1081 EFQVYLTFLPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT FPVLDSDGSF
 1141 1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAAC TACAAGACCA CGCTCTCCGT GTTGGACTCC
 1201 1201 GACGGCTCTCT TCTTCTCTCA CAGCAAGCTC ACCGTGACA AGAGCAGGTG GCAGCAGGGG
 1261 1261 AACGCTCTCT CTCCTCCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
 1321 1321 CTCTCCCGT CTGCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGGTGGGA
 1381 1381 GGTTCGGGTG GCGGGGGATC CTCCTCCCGT GGGGTGGATC AGGAGGAGGT
 1441 1441 GGTTCAGGTG GTGGAGGATC CATTGTTGGG GGCAAGGTGT GCGCCAAAGG GGAGTGTCCA
 1501 1501 TGGCAGGTCC TGGTGGTGGT GAATGGAGCT CAGTTGTTGTG GGGGGACCT GATCAACACC
 1561 1561 ATCTGGTGTG CTGCGGGTGC CCACTGTTTC GACAAATCA AGAACTGGAG GAAACCTGATC
 1621 1621 GCGGTGCTGC GCGAGCAGCA CCTCAGGGAG CACCGAGGGG ATGAGCAGAG CGGGGGGGTGC
 1681 1681 GCGCAGGTCA TCATCCCCAG CACGTACAGC CGGGGGACCA CCAACCCACGA CATGGGGCTG
 1741 1741 CTCCGCTTC ACCASCCCGT GGTCTCTAC GACCATGTGG CCCCTCTCTG CCTGCCGAA
 1801 1801 CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGCGCTTCT CATTGGTCAG CGGCTGGGGC
 1861 1861 CAGCTGCTGG ACCGGGGCTG CAGCTCATGG TCTCAACGT GCGGGGGCTG
 1921 1921 ATGACCCAGG ACTGCTGCA GCACTGCAAGG AAGGTGGAG ACTCCCCAA TATCACGGAG
 1981 1981 TACATGTTCT GTGCGGGCTA CTCGGATGGC AGCAAGACT CTCGCAAGGG GGACAGTGGA
 2041 2041 GGGCCACATG CCACCGACTA CGGGGGCAGC TGAGTGTGCA CGGGCATCGT CAGCTGGGGC
 2101 2101 CAGGGCTGCG CAACCGTGGG CCACTTCTGG GTGTACACCA GGGTCTCCCA GTACATCGAG
 2161 2161 TGGCTGAAA AGCTCATGCG CTCAGAGCCA CGCCAGGGAG TCTCCCTCG AGCCCATTT
 2221 2221 CCGGGCTCCGG GTGCGGGCTC CGGAGGTGGG TCCGGTGGCG GCGGATCAGG TGGGGGTGGA
 2281 2281 TCAGGGGGTG GAGGTTCCGG TGGGGGGGGG TCAGACAAA CTACACATG CCCACGGTGC
 2341 2341 CACGGCACCTG AACTCTGGG AGGACGGTCA GTCTCTCTCT TCCCCCCCCA ACCCAAGGAC
 2401 2401 ACCCTCATGA TCTCCGGAC CCCTGAGGTG ACATCTGGTGG TGGTGGAGT GAGGACAGGAA
 2461 2461 GACCCCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCAAA TGCGCAAGACA
 2521 2521 AAGCCGCGGG AGGAGCACTA CAACAGCACG TACCTCTGTG TCAAGTGTCTT CACCGTCTG
 2581 2581 CACCAAGACT GGTGAATGG CAAGGAGTAC AAGTCCAAGG TCTCCAACAA AGCCCTCCCA
 2641 2641 GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CGGGAGAAC ACAGGTGTAC
 2701 2701 ACCCTGGCCC CAACCGGGGA TGAGCTGAGC AAAGAACAGG TCAAGTGTCTT CTCCTGGTC
 2761 2761 AAAGGCTTCT ATCCCAAGGA CATGCCCTG GAGTGGAGA GCAATGGCA CGCGAGAAC
 2821 2821 AACTACAAGA CCACGCCCTC CGTGTGGAC TCCGACGGCT CCTCTCTCT CTACAGCAAG
 2881 2881 CTCACCGTGG ACAAGAGGAG GTGGCAGCAG GGGAACTGTCT TCTCATGCTC CGTGTGTCAT
 2941 2941 GAGGCTCTGC ACAACCACTA CACGAGAAC AGCCTCTCCC TCTCTCCGGG TAAATGA

65 FVII-062 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underline, 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 LLLGLOGLIA AVFVTOEEAH GVLIIRRRAA AFLEELRPGS LERECKEEQC
 61 SFEEAREIIFK DAERTKLFWI SYSDGDQAS SPCCNGGSKC DQLQSYICFC DPATEGRNCE
 121 THKDDQILCV NENGGCEQYC SDITGTRKSC RCHEGYSLLA DVGSCTPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGGGGGGG GGGGGGGGGG GGGGGGGGGG DKTHTCPPCP APELLGGGPSV
 241 FLFPPPKEDT LMISKTPETV CVVVDVSHED PEVKENWYVD GVEVHNNAKTK PREEQYNSTY
 301 RVVSVLTVLH QDWLNNGKEYK CKVSNKALPA PIEKTIKAK QQPREPQVYT LPPSRDELTK

5 361 NQVSLECLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDs DGFFFFYSLI TVDKSRWQG
 421 NNFSCSVMHE ALHNHYTQKS LSLSPRGKEGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
 481 **GSGGGG**IVG GRVCPKGFCP WQVLLIVNGA QLCGTLINT IWVVSAAHCF DKIKNWRNL
 541 AVLGEHDLSE HDGDEQSRRV AQVIIPSTYV PGTTNFDIAL LRLHQPVVLT DVVVPCLPE
 601 RTFSERTLAF VRFSLVSGWG QLLDRGATALL FLMVLNVERL MTQDCLQQR KVGDFNITE
 10 661 YMFCAGYSDG SKDSCKGDG GFATHYRGW WYLIGIVSWG QGCATVGHFG VYTRVQYIE
 721 WLQKLMRSEF RFGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG SDKTHTCPPC
 781 PAPELGGPS VFLFFPKPKD TLMISRTEEV TCVVDVDSHE DPEVKENWVY DGVEVHNNAKT
 841 KFREEQYNST YRVVSVLTVL HQDWLNKEY KCKVSNKALP APIEKTISKA KGQPREFQVY
 901 TLPPSRDELT KNQVSLTCIV GFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK
 15 961 LTVDKSRWQG GNVFSCSVHM EALHNHYTQK SLSISPGK*

DNA sequence for FVII-090

1 ATGGTCTCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCTGGCT
 61 GCAGTCTTCG TAACCCAGGA GGAAGCOCAC GGGCTCTGC ACCGGCGCCG GCGGCCAAC
 121 CGGTTCTGG AGGAGCTCGG GCGGGCTCC CTGGAGAGGG AGTGCAGAGGA GGAGCAAGTG
 181 TCCCTCCAGG AGGCCCGGGGA GATCTTCAGG GACCGGGAGA GGACGAACCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCTCTA AGTCCATGCC AGAATGGGG CTCCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTCTGC CTCCTGCTC AGCAGGGGGG GAACUTGTGAG
 361 ACAGCACAAAG ATGACCAAGC GATCTGCTG AACGAGAACCG CGGGCTGTGA CGACTACTGC
 421 AGTGACCAACA CGGGCACCAA CGCCTCTCT CGGGCCACAG AGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTGTAA TATOCATGTG GAAAAATACC TATCTTGTAGAA
 541 AAAAGAAATG CCAGCAAAACCC CCAAGGGCGA GCTGGCGGTG GCTCCGGCGG AGGTGGTCC
 601 GGTGGCGCG GATCAGGTGG CGGTGGATCA GGCCTGGGAG GTTCCGGTGG CGGGGGATCA
 661 GACAAACACT ACACATGCC ACCGTGCUCA GCTUCGGAAC TCTTGGCGG ACCTCTAGTC
 721 TTGCTCTTCG CCCCCAAACCC CAAGGACACC CTCATGATCT CCGGGACCCC TGAGGTACAA
 781 TGGCTGTGG TGGACGTGAG CGACGAACAC CCTGAGGTCA AGTCAACTG GTACGTGGAC
 841 GGCCTGGAGG TGCATAATGC CAAGACAAAG CCGGGGGAGG AGCAAGTACAA CAGCACGTAC
 901 CGTGTGGTCA GCGTCTCAC CGTCCCTGC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGCTCT CCACAAAGC OCTCCACCC CCCCACGAGA AAACCATCTC CAAACCCAAA
 1021 GGCAGCCCCC GAGAACACCA GGTGTACACC CTGCCCCATC CCGGGGATGA GCTGACCAAG
 1081 ACCAGGTCA GCTTSACCTG OCTGGTCAAA GGCTTCTATC CGACGACAT CGCCCTGGAG
 1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAC TACAAGACCA CGCTCCCGT GTTGGACTCC
 1201 GACGGCTCT TCTTCTCTA CAGCAAGCTC ACCGTCGACA AGAGCAGGTG CGAGCAGGGG
 1261 AACCTCTCT CAGCTCTCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAGAGC
 1321 CTCTCCCTGT CTCGGGTAAG AGGTGGCGG GGTACAGGTG GGGGTGGATC AGGCGGTGGA
 1381 GGTTCCGGTG CGGGGGGATC CGGGGGTGGA GTTCCGGTG GGGGTGGATC AGGAGGAGGT
 1441 GGTTCAGGCC CGCCGGCCCG GATTCTGGGG GGCACAGGTG CCCCCAAAGG GGAGTCTCCA
 1501 TGGCAGGTCC TGTGTGGTGA GAATGGAGCT CAGTTGTGTG GGGGGACCT GATCACACCC
 1561 ATCTGGGTGG TCTCCGGCC CGACTGTTTC GACAAAATCA AGAACTGGAG GAAACCTGATC
 1621 GCGGTGTGG GCGAGCAGCA CCTCAGGGAG CACGGAGGGG ATGAGCAGAG CGGGGGGGTG
 1681 CGCGCAGTCA TCATCCCCAG CACGTACGTC CGGGGACCA CCAACCCACGA CATCCGGCTG
 1741 CTCCGGCTGC ACCAGCCCGT GGTCTCTACT GACCATGTGG TCCCTCTCT CCGCCGGAA
 1801 CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGCCCTCT CATTGGTCAG CGGCTGGGGC
 1861 CAGCTGCTGG ACGTGGCGC CACGGCCTG GAGCTCATGG CCTCAACGT GCGCCGGCTG
 1921 ATGACCCAGG ACTGCTCTCA GCACTGACAGG AAGGTGGAG ACTCCCCAAA TATCACGGAG
 1981 TACATGTTCT GTGCGGGCTA CTGGGATGGC AGCAAGGACT CCTGCAAGGG GGACAGTGGG
 2041 GGCCCCACATG CGACCGCTCA CGGGGGCAGC TGGTACCTGA CGGGCATCGT CAGCTGGGGC
 2101 CAGGGCTCGC CAACCGTGGG CCACTTCTGG GTGTACACCA CGCTCTCCA GTACATCGAG
 2161 TGGCTGCAAA AGCTCATGCG CTACAGAGCCA CGGGCAGGGAG CCTCTCTGG AGCCCCATTG
 2221 CGGGGTGGCG GTGGCTCCGG CGGAGGTGGG TCCGGTGGCG CGGGATCAGG TGGGGGTGGA
 2281 TCAGGGCTGG GAGCTTCCGG TGGCGGGGGA TCAGACAAA CCTCACACATG CCCACCGTGC
 2341 CACAGCACCTG AACTCTGGG AGGACCGTC GTCCTCTCT TCCCCCCTAA ACCCAAGGAC
 2401 ACCCTCATGA TCTCCGGAC CCTCTGAGGTG ACATCCGGTGG CGCTGGACGT GAGCCACGAA
 2461 GACCCCTGAGG TCAAGTTCAA CTGGTACCTG GACGGCGTGG AGTGCATAA TGCAAGACA
 2521 AAGCCGGGG AGGAGCAGTA CAACAGCACG TACCTCTGTG TCAGCGTCCT CACCGTCTG
 2581 CACCAAGGACT GGCCTGAATGG CAAGGAGTAC AAGTCGAAGG CCTCCAACAA AGCCCTCCCA
 2641 GCCCCCATCG AGAAAACCAT CCTCAAAGCC AAAGGGCAGC CGGAGAACCC ACAGGTGTAC
 2701 ACCCTGCCCC CAACCGGGGA TGAGCTGAGC AAGAACCGAG CGACCTGAC CTGCTGGTC
 2761 AAACGCTCT ATCCCTGCGA CATGCCCTC GAGTCGGAGA GCAATGGCA GCGGGAGAAC
 2821 AACTACAAGA CCACGCCCTC CGTGTGGCAG TCCGACGGCT CCTCTCTCT CTACAGCAAG
 2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACTGCT TCTCATGCTC CGTGATGCT
 2941 GAGGCTCTGC ACAACCACTA CACGGCAGAAG AGCCTCTCCG CCTCTCCGGG TAAATGA

70 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

1 MVSQALRLLC LLLGQGCLIA AVFVTQEEAH GVLHERRRAN AFLEELRPGS LERECKEEQC

DNA sequence for FVII-100

DNA sequence for P1/P10		1	ATGGTCTCC	AGGCCUCCTCAG	GCTCCCTCTGC	CTTCTCTCTTG	GGCTTCAGGG	CTGGCTGGCT
25		61	GCAGTCTTCG	TAACCCAGGA	GGAAAGCCAC	GGCGCTCTGC	ACCGGGCGCC	GCGCGCCAAC
		121	CGGTTCTCGG	AGGAGCTGG	GGCGGGCTCC	CTGGAGAGGG	AGTCAAAGGA	GGAGCACTG
		181	TCCTTCGAGG	AGGGCCGGGA	GATCTTCAGA	GAACGCCGAGA	GCACAGGAGT	GTTCCTGATT
		241	TCTTACAGTG	ATGGGACCA	GTGTCCTCA	ACTCTCATGCC	AGATTTGGCC	CTCTCTCAAG
30		301	GACCAAGCTC	AGTCTCTATAT	CTCTCTCTGC	CTCCTCTGCC	TCGAGGGCCG	GAACACTGAG
		361	ACGCACAAGG	ATGACCAAGCT	GATCTGTGTG	AAACGAGAACG	GGGGCTGTGA	GCAGTACTGC
		421	AGTGACCACA	CGGGCACCAC	GGCCTCTCTG	CGGTGCCACG	AGGGGTACTC	TCTGCTGGCA
		481	GACGGGTGT	CCTGCACACC	CACAGETGAA	TATCCATGTG	AAAAAAATAC	TATTCCTAGAA
		541	AAAAGAAATG	CCACCTAAACC	CCRAAGGCCA	GGTTCGGGTG	GTCTCCGGCG	AGGTGGCTCC
		601	CGTGGCGCG	GATCAGGTGG	GGGTGGATCA	GGCGGTGGAG	GTTCGGCTGG	CGGGGGATCC
35		661	GACAAAAC	ACACATGCC	ACCGTGGCCA	GCTCCGGAA	TCTCTGGAGG	ACCGTCACTC
		721	TTCTCTTCC	CCOCAAAC	CAAGGACACC	CTCATGATCT	CCCCGACCC	TGAGGTCAAC
		781	TGCGTGTGG	TGGACGTGAG	CCACGAAGAC	CCTGAGGTCA	AGTCAACTG	GTACGTGGAC
		841	GGGTGEGAGG	TGCTATAATG	CAAGAACAAA	CCGGCGGAGG	AGCAAGTACAA	CAGCACGTAC
		901	CGTGTGTTCA	CGCTTCTCAC	CGTCTGCAAC	CAGGACTGGC	FGAATGGCAA	GGAGTACAAG
40		961	TGCAAGGTCT	CCAAACAAAGC	CTCTCCAGCC	CCCCATGGAA	AAACCATCTC	CAAACCAAA
		1021	GGGCAGCCCC	GAGAACACCA	GGTGTACACC	CTGCCCCAT	CCGGGATGA	GCTGACCAAG
		1081	AACCAGGTCA	GGCTGACCTG	CCTGGTCAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG
		1141	TGGGAGAGCA	ATGCCCAACC	GGAGAACAC	TACAAAGCCA	CCCCCTCCGT	GTGGACTCC
		1201	GACGGCTCTC	TCTCTCTCTA	CAGCAAGCTC	ACCGTCGACA	AGAGCAGCTG	GCAGCAGGGG
45		1261	AACTCTTCT	CATGTCCTGT	GTGTCATGAG	GCTCTGCAAC	ACCACTACAC	CGACAGAACG
		1321	CTCTCCCTGT	CTCGGGGTAA	AGGTGGGCC	GGATCAGGTG	GGGGTGGATC	AGGGGGTGG
		1381	GGTCCCGGTG	CGGGGGGATC	CGGGCGGTGGA	GGTCCGGTG	GGGGTGGATC	AGGAGGAGGT
		1441	GGTCAGCCC	TGCGGCCCCG	GATTGTGCGG	GGCAAGGTGT	GGCCCAAAGG	GGAGTGTCCA
		1501	TGGCAGGTC	TGTGTGTGGT	GAATGGACCT	CAGTTGTGTG	GGGGGGACCT	GATCAACACCC
		1561	ATCTGGGTGG	TCTCGCGGGC	CCACTGTGGT	GACAAAATCA	AGAACCTGGG	GAACCTGTAC
50		1621	GGGGTGTG	GGCAGCAGCA	CTTCAGCGAC	CACGACGGGG	ATGAGCAGAG	CGGGGGTGTG
		1681	GGCAGAGTC	TCATCCCCAG	CACGTACGTC	CCGGGCCACCA	CCAACACAGA	CATCGCGCTG
		1741	CTCCGCTGC	ACCAGCCCCG	GTCCTCACT	GACCATGTGG	TGCCCTCTG	CCTGGCCGAA
		1801	CGGACGTTCT	CTGAGGAGGAC	GCTGGCTCTC	GTGCCCTCT	CATTTGGTCAG	CGGGCTGGGG
		1861	CAGCTGCTGG	ACCGTGGCGC	CACGGCCCCG	GAGCTCATGG	TCTTCACAGG	GGCCCCGGCTG
55		1921	ATGACCCAGG	ACTCGGAGGC	GACGTCACCC	GGCAAGATCA	CGGAGTACAT	GTCTCTGGCTC
		1981	GGCTACTCGG	ATGCCAGCAA	GGACTCTCTG	AAGGGGGACA	GTGGAGGGCCC	ACATGCCCC
		2041	CACTACCGGG	GCACGTGTTA	CCTGACGGGC	ATCGTCAGCT	GGGGCCAGGG	CTGGCCAAACC
		2101	GTGGGGCACT	TTGGGGTGT	CACCGAGGTC	TCCCTAGTACA	TCGAGTGGGT	GCAAAAGCTC
		2161	ATGCGCTCAG	ACCCACGCC	AGGAGTCCTC	CTGCGAGGCC	CATTTCGGCG	TGGGGTGGGC
60		2221	TCCGGCGGG	GTGGGGTCCGG	TGGGGCGGGA	TCAGGTGGG	GTGGATCAGG	CGGGGGAGGT
		2281	TCCGGTGGCG	GGGGATCAGA	CCAAAATCAC	ACATCCCCAC	CTTGGCCAGC	ACCTGAAC
		2341	CTGGGAGGAC	CGTCAGTCCT	CCTCTTCCCC	CCAAAACCCA	AGGACACCC	CATGATCTCC
		2401	CGGACCCCTG	AGGTACACATG	CGTGGGGGTG	GACGTGAGCC	ACGAAGACCC	TGAGGTCAAG
		2461	TTCAACTGGT	ACGTGGACGG	CGTGGGGAGCT	CATAATGCCA	AGACAAAGCC	GGGGGGAGGA
		2521	CACTACAAAC	GCACGTACCG	TGTGGECAGC	GTCCTCACCG	TCTTCACCA	GGACTGGCTG
65		2581	AACTGGCAAG	AGTACAAGTG	CAAGGTCTC	AACAAAGGCC	TCCCGAGCCC	CATCGAGAAA
		2641	ACCATCTCCA	AAAGCCAAAGG	GGAGCCCCCGA	GAACCCACAGG	TCTACACCC	GGCCCCCATCC
		2701	CGGGATGAGC	TGACCAAGAA	CCAGGTCTGC	CTGACCTGCC	TGGTCAAAGG	CTTCTATCCC
		2761	AGGCACATCG	CCGGTGGACTG	GGAGGACAA	GGGAGCCCCG	AGAACACACTA	CAAGACACAG
		2821	CCTCCCCCTGT	TGGACTCCGA	GGGGCTCTTC	CTTCTCTACA	GCACAGTCAC	CGTGGACAAAG
		2881	AGGAGGTGGC	AGCAAGGGAA	GGCTCTCTCA	TGCTCCGGTG	TGGATGAGG	TCTGGCACAAAC
		2941	CACTACACCG	AGAAAGAGCC	CTTCCCTGTC	CCGGCTTAAT	GA	

5

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

1 MVSQALRLLC LLLGLOGGLA AVEVTCEEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
 61 SFEEAREI**FF** DAERTKLF**W**I SYSDGDCQAS SPCQNNGSCK DQLQSYICPC LPAFEGRNC
 121 THKDDQLICV NENG**G**CEQY**C** SDHTGTRKSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQ**G**E GGGSSGGGS GGGSSGGGS GGGSSGGGS DKTHTCPCP APELLGGPSV
 241 FLFPPPKF**D**T IMTSRTPEVT CVVVDVSHED PEVKRNWYVD GVEVHNARTK PREEQYNSTY
 301 RVVSVITV**LH** QDWLN**G**KF**Y**K CKVSNK**AL**EA P**E**KT**T**SK**A**K GQPREPQVYT LPPSKD**E**LT**K**
 361 NQVS**L**TC**L**V**K** GFYPS**D**I**A**VE WESNG**Q**PENN YK**T**TP**P**V**L**DS DGSFFLY**S**KL TVD**K**SR**W**Q**O**Q
 421 NVFSCSV**M**HE ALHNH**Y**TQ**K**S LSLSP**K**GG**G** GS**G**GG**G**GS**G**GG**G** GS**G**GG**G**GS**G**GG**G**
 481 **G**SALRPIVG GK**V**CP**K**GE**C**P Q**V**LL**V**INA**G** QLC**G**GT**L**INT I**W**V**SAAH**C** D**K**I**N**NR**N**
 541 AVL**G**EHD**L**SE RD**G**EQ**S**PRV A**Q**V**I**P**S**EV PG**T**TM**H**DI**A**AL L**R****I****H**Q**P**V**V**LT D**V**V**P**L**C****L****P**
 601 RTF**S**ERT**L**A**F** VR**F**SL**V**SG**W** Q**L**LD**R**GA**T**AL E**L**M**V**LN**V**P**R** L**M**T**D**CE**E**AS**Y****P** G**K**I**T**EY**M****F**
 661 GYSDGSK**D****S**C KGD**S**GG**P**HAT HYRG**T**WY**I**TG IV**S**W**Q**G**C**AT V**G**H**F**G**V**Y**T**RV SQ**I****F**W**L**Q**K**L
 721 M**R****S**E**P**R**F**C**V**L IR**A**PP**F**PG**G**GG**S** SC**G**GG**S**GG**G**GG**S** GG**G**GG**S**DK**P**TH T**C****P****C****A****P****E****L**
 781 LGG**P**SV**F**L**P** PKPKD**T**LM**T** RT**P**EV**T**CV**V** DVS**H**ED**P**EV**K** FN**W**Y**V**D**G**VE**V** H**N**A**K**T**K**P**R**
 841 QYNSTYRV**V**G YLT**V**LI**Q**D**W** L**N**Y**K**C**K**V**S** N**K**A**L**P**A****F**IE**T** K**I**S**K**A**G**Q**F**R EP**Q**Y**V**TL**P**PS
 901 R**D**EL**T**K**N**Q**V**S L**E**CL**V**K**G**F**Y**P SDIA**V**E**W**ES**N** G**Q**P**N**NY**K**TT PP**V**LD**S**D**G**SF FLY**S**KL**T**VD**K**
 961 SRW**Q**Q**G**NV**F**S CS**V**M**U**HE**A**LN**I**N H**Y**T**Q**K**S**LS**L**S PG**K*****

DNA sequence for FVII-115

30 1 ATGGTCTCCC AGGCCTTCAG GCTCCU**C**TGC CTTCTG**C**T**T**G GGCTTCAGGG
 CTGGCCTG**G**CT
 61 G**C**AGTCTTCG TAACCCAGGA GGAAGCCCAC G**G**CG**T**CT**T**GC ACCGGCGCG GCGCGCCAA**C**
 121 GCG**T**TCT**T**GG AGGAG**C**T**G** G**C**CGGG**C**T**C** CTGGAGAGGG AG**T**G**C**AAG**G** G**G**AG**C**AG**T**GC
 181 T**C**CTTC**G**AG**G** AG**G**CC**C**GG**G** G**A**T**C**T**C**TA**G** G**A**CCGG**G**AG**G** G**G**AC**C**GA**G**CT**T**TT**G**ATT
 241 T**C**T**T**CA**G**CT**G** AT**G**GG**G**AC**A** G**T**GT**G**CT**C**TA**G** AG**T**CC**C**AT**G**CC AG**A**AT**T**GG**G**GG C**T**CC**T**GC**A**AG
 301 G**A**CC**A**GT**C**CC AG**T**CC**T**AT**A** G**T**CC**T**CT**C**GC C**T**CC**T**CT**C**GC T**C**GAGGG**CC**GG G**A**ACT**T**GT**G**AG
 361 A**C**GC**A**CA**A**GG AT**G**ACC**A**GG**T** G**A**T**C**T**G**CT**G** AAC**G**AG**A**AC**G** C**G**G**C**T**G**T**G** G**C**AG**T**ACT**G**C
 421 A**G**T**G**AC**A**CA**C** C**G**GC**A**CC**AA** G**G**CG**T**CT**C**GT**G** CG**G**T**G**CC**A**CC AG**GG**GT**T**ACT**C** T**C**T**G**CT**G**GC
 481 G**A**CG**GG**GT**T**GT**G** CCT**G**AC**A**CC C**A**CA**G**TT**G**AA T**A**T**C**AT**G**GT**G** GAAA**A**AT**T**CC T**A**TT**T**CT**G**AG**A**
 541 AAA**A**AA**A**AT**T**G CC**A**CG**A**AA**A**CC C**A**AG**G**GG**G** GG**T**EC**G**GG**G** GT**C**CC**GG**GG**G** AG**G**T**G**GG**G**TC**C**
 601 G**G**T**G**CG**G**CG**G** G**A**T**C**AG**G**GT**G** G**G**GT**G**GG**G**AT**C**CA**G** GT**T**CC**GG**GT**G** G**C**GGGG**G**AT**C**CA
 661 G**A**CA**A**AA**C**T**G** AC**A**CA**G**CC**AA** G**C**T**C**CC**G**AC**A** G**T**CC**GG**GG**G**AC**A** T**C**T**GG**GG**G**GG**G**AC**A**
 721 T**T**CC**T**CT**T**CC C**CCCC**AA**A**AC**A** C**A**AG**G**AC**A**CC C**T**CA**G**AT**T**CT C**CCCC**GG**G**AC**A** TG**A**GG**T**TC**A**CA
 781 T**G**CG**T**GT**G**GG T**G**GA**C**GT**G**AG CC**A**CG**A**AG**A** CCT**G**AG**G**GT**G**CA**G** AG**T**CA**A**CT**G** GT**A**CG**T**GG**G**AC
 841 C**CC**GT**G**CG**G** T**G**CA**T**AA**A**TC C**A**AG**A**CA**A**AG C**G**GC**C**CC**C**AC**G** AG**A**CA**T**AC**A** C**A**GC**A**CT**C**AC
 901 C**G**T**G**GT**G**TC**C** G**C**GT**G**CT**C**AC**G** C**A**GG**G**AT**C**GC**A** AG**A**AT**T**GG**G**AC**A** G**G**ACT**A**CA**G**TC**C**
 961 T**G**CA**A**AA**A**AG**G** C**C**TC**C**CC**G**AC**A** C**A**GG**G**AC**A**CC C**T**GG**G**GG**G**AT**C**AC**A** G**C**GGGG**G**GG**G**AC**A**
 1021 G**G**CC**A**GG**G**GG**G** G**A**GA**C**AC**A**CC C**T**GG**G**GG**G**AT**C**AC**A** C**C**GG**G**AC**A**CC TG**A**GG**T**TC**A**CA
 1081 A**A**CC**A**GG**G**TC**C** G**C**CT**G**AC**A**CT C**C**T**G**GG**G**AA G**G**CT**T**CT**A**TC C**C**AG**C**GA**G**AT C**G**CC**G**GT**G**GG**G**AG
 1141 T**G**GG**G**AG**G**CA A**F**GG**G**CA**G**GG**G** G**A**GA**A**CA**A**AC**A** T**A**CA**A**AG**G**CA C**G**CT**C**CC**C**GT**G** GT**F**GG**G**AC**R**CC
 1201 G**A**CG**G**CC**C**TC**C** T**C**T**C**CT**C**TC**C** C**A**GC**A**CT**G**TC**C** AC**C**CC**G**TC**C**GA**G** AG**A**CC**A**GG**G**TC**C**
 1261 A**A**CG**T**CT**C**TC**C** T**C**AT**C**TC**C**GT**G** G**A**T**C**AT**G**AT**G** G**C**TC**C**GT**G**AC**A** ACC**A**CT**A**AC**A** C**G**CA**A**GG**G**AC
 1321 C**T**CT**C**CT**C**GT**G** CT**C**TC**C**GG**G**IA**A** AG**T**GG**G**GG**G** C**G**AT**C**AG**G**ST**G** G**G**GT**G**GG**G**AT**C** AG**G**GG**G**GT**G**GG**G**
 1381 G**G**TT**C**CG**G**GT**G** G**G**GGGG**G**AT**C** C**G**GG**G**GT**G**GG**G** G**G**GT**G**GG**G**GT**G** G**G**GG**G**AG**G**GG**G**AT**C**
 1441 G**G**TT**C**AG**G**CC**C** TG**G**GG**G**CC**C** G**A**TT**G**GG**G**GG**G** G**G**CA**A**GG**G**ACT**G** G**G**CC**C**AA**A**AG**G** GG**G**AG**G**GT**G**TC**C**
 1501 T**G**GG**G**AG**G**TC**C** T**G**T**G**TT**G**GT**G** GA**A**T**G**GA**G**CT**C** C**A**GT**G**TC**C**GT**G** G**G**GG**G**AC**A**CT**G** G**A**AC**A**AC**A**
 1561 AT**C**T**G**GG**G**GT**G** T**C**T**C**CG**G**CG**G** C**C**ACT**G**CT**C**TC**C** G**A**CA**A**AT**T**CA**G** AG**A**ACT**G**CG**G** G**A**AC**C**T**G**AT**C**
 1621 G**C**GG**G**CT**C**GT**G** G**G**CA**G**AC**A**CA**C** C**C**TC**C**GG**G**AG C**A**GG**G**GG**G**AT**C**AC**A** G**C**GG**G**GG**G**GT**G**
 1681 G**G**CG**C**AG**G**TC**C** T**C**AT**C**CC**C**AG**G** C**A**CG**T**AC**G**TC**C** C**C**GG**G**AC**A**CCA C**C**AA**A**CC**A**AG**G** G**C**AT**C**CG**C**GT**G**
 1741 CT**C**CG**C**CT**C**TC**C** ACC**A**GG**G**CC**C** G**G**GT**G**CT**C**AC**G** T**C**AC**A**AT**G**GT**G** GG**G**CC**C**CT**C**CT**C** G**C**T**C**CC**C**GG**G**AA
 1801 C**G**GA**C**GT**C**TC**C** CT**G**AG**G**AG**G**AC**G** G**C**T**C**GG**G**CT**C**TC**C** GT**G**CG**C**CT**C**TC**C** G**G**GT**G**CT**C**GG**G**AC**A**
 1861 C**A**GG**G**CT**C**GT**G** AC**C**CG**G**GG**G**CG**G** C**A**CG**G**CC**C**GT**G** G**T**AC**T**TC**C**AA**G** T**C**CT**C**AA**C**CT**G** G**G**CC**C**GG**G**GT**G**CT**C**
 1921 AT**G**AC**C**CG**G**AG**G**CT**C**TC**C** G**G**AT**C**AC**G**TC**C**AG**G** A**A**GG**G**GG**G**AG**G** ACT**C**CC**C**AA**A** T**T**AT**C**AC**G**GG**G**
 1981 T**A**CA**T**GT**C**TC**C** GT**G**GG**G**GG**G**CT**C** C**T**CG**G**AT**G**TC**C** G**G**CA**A**GG**G**ACT**G** G**C**T**G**CA**G**AG**G** GG**G**AC**G**GT**G**TC**C**
 2041 G**G**CC**C**AC**A**AT**G** CC**A**CC**C**ACT**C** C**C**GG**G**GG**G**AC**G** T**G**GT**G**AC**G**CT**C** G**G**GG**G**AT**C**CT**C** G**C**AG**T**GG**G**GG**G**GG**G**
 2101 C**A**GG**G**GT**C**TC**C** G**G**AA**C**GT**C**GG**G** CC**A**CT**T**GG**G**GG**G** GT**G**GT**G**AC**A**CCA G**G**GT**G**CT**C**CC**C** G**T**AC**A**TC**C**GG**G**
 2161 T**G**GG**G**CT**C**CAA**A** AG**T**TC**C**AT**G**CG**G** C**T**CG**G**AG**G**CCA CG**G**CC**C**AG**G**AG G**T**CC**C**CT**C**GT**G** G**G**CC**C**CA**A**AT**T**TT
 2221 CCC**G**GT**C**GG**G** G**G**GT**G**CT**C**GG**G** C**G**GG**G**GT**G**GG**G** T**C**CG**G**GT**G**GG**G** G**G**GG**G**AT**C**AG**G** G**T**GG**G**GG**G**GG**G**
 2281 T**C**AG**G**GG**G**GT**G** G**G**GG**G**GG**G**GG**G** T**C**AG**G**GG**G**GG**G** G**G**GG**G**GG**G**GG**G** G**G**GG**G**AT**C**AG**G** G**C**CC**C**GG**G**GT**G**
 2341 CC**A**GC**C**CT**G** A**A**CT**C**CT**G**GG**G** AC**G**AC**C**GT**C** G**T**CT**C**TC**C**CT**C** T**C**CC**C**CC**C**AA**A** ACC**C**AA**G**GG**G**
 2401 ACC**C**CT**C**AT**G**A T**C**TC**C**CC**C**GG**G**AC**G** CC**C**TC**C**GG**G**GT**G** AC**A**CT**G**GT**G**GG**G** T**G**GT**G**GG**G**AC**G**T G**G**AG**C**AC**G**AA
 2461 G**A**CC**C**GT**G**AG**G** T**C**AA**G**TT**C**AA**G** CT**G**GT**G**AC**G**TC**C** G**A**GG**G**GT**G**GG**G** AG**G**GT**G**CA**G**TA**G** A**T**GC**C**CA**G**AC**G**AA

5 2521 AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCCTCCT CACCGTCTG
 2581 CACCAAGACT GGCCTGAATGG CAAGGACTAC AAGTCCAAGG TCTCCAACAA AGCCCTCCCA
 2641 GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC
 2701 ACCCTGCCCC CATCCCAGGA TGAGCTGACC AAGAACAGG TCAGCCTGAC CTGCTGGTC
 2761 AAAGGCTTCT ATCCAGCGA CATGCCCTG GAGTGGGAGA GCAATGGCA GCGGAGAAC
 10 2821 AACTACAAGA CCACGCCCTCC CGTGTGGAC TCCGACGGCT CCTTCCTCCT CTACAGCAAG
 2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAAACGTCT TCTCATGCTC CGTGTGCT
 2941 GAGGCTCTGC ACAACCAGA CACGCACAG AGCCTCTCC TCTCTCCGGG 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 (V158D, E296V and M298Q) are in bold and underlined

1 **MVSQALRLC** MILGLOGGLA AVFVTQEAR GVLHRRRAN AFLEELRPGS LERECKEEQC
 6 61 SFSEAREAR DAPRTTKLFWT SPCCNQGNSCK DQIQSQTFCF IPAFERNCE
 20 121 THRDDQLICV NENGCCQEYC SDHTGTKESC RCHEGYSLLA DGVSCTPVE YPCCKIPILE
 181 KENASKPKQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPCP APPELLGGPSV
 241 FLFPPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFENWYVD GVEVHNNAKTK PREEQYNSTY
 301 KVVSVLVLVH QDWLNGKEYE CKVSNRALK PIERKTIKSA QOPREPQVYT LPPSRSDELTK
 361 NQVSLTCLVK GYYPSPDIAY EWESNGQOPEN YKTTPPVLDs DGSPFLYSLK TVDVKSRWQCG
 421 NVFSCSVNHE ALHNHYTQKS LSLSPGKGGG GSGGGSGG GSGGGSGG
 481 **G**SALRPRIVG GKDCKPKGECP WQVILLIVNGA QLCGCLINT IWWVSAAHCF DKKIKNWRNLI
 541 AVI**E**HEHDLSE RDGDEQSRRV AQVIIIFSTYY PGITNNHDIAL LRLHQPVVLT DRYVPLCLPE
 601 RTFSETRLAF VRFLVSGWG QLLDRGATAL **V**LOVINVPRL MTODCLOQSR KVGDSPNITE
 661 YMFCAGYSDG SKDCKGDSG GPHATHYRGT WLITGVSWG QGATVGHFG VYTRVSYQYIE
 721 WLQKLMRSEFP RGPVLLRAFP PGGGSGG SGGGSGG SDKTHTCPPC
 781 PAPELLGGPS VFLFPPPKPKD TLMISRTPEV TCVVVVDVSHS DPEVKFENWYV DGVEVHNNAKT
 841 KPREEQYNST YRVVSVLVLVH QDWLNGKEYE CKVSNRALK APIEKTIKSA KGQPREPQVY
 901 TLPPSRSDELT KNQVSLTCLV KGFYPSPDIAY EWESNGQOPEN NYKTTPPVLD SDGSFFLYSK
 961 TVDKSRWQ GNVFSCSVNHE BALHNHYTQK SLSLSPGK*

35

DNA sequence for FVII-115

1 ATGGCTCTCC AGGCCCTCAG GCTCCTCTGC CTTCCTGCTG GGCTTCAGGG CTGCTGGCT
 61 GCACTCTTCG TAACCAGGA GGAAGCCAC GGCCTCTGC ACCGGCGCG GCGGCCAAC
 121 GCGTCTCTGG AGGAGCTCG GCGGGGCTCC CTGGAGAGGG AGTGCAGGAA GGAGCAGTGC
 181 TCCCTCGAGG AGGGCGGG GATCTTCAG GACGGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACACTG ATGGGACCA CTGTGCCCA AGTCCATGCC AGATATGGGG CTCTCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTCTGCT CTCCCTGCTC TCGAGGGCG GAACTGTGAG
 361 ACGCACAGG ATGACCAGT GATCTGTGAC AACGAGAACG GCGCTGTGA GCAGTACTGC
 421 AGTGCACACA CGGGCACAA CGCCTCCCTC CGGTCACAGC AGGGTACTC TCTCTCTGCA
 481 GACGGCTGT CCTCCACACC CACAGTGA TATCCATGTC CAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAACCC CAAAGGCCCG CTGCGGCCG CGATTGTGGG GGGCAAGGTG
 601 TGCCCCAAAG GGGAGTGTCC ATGGCAGTC CTGTTGTTGG TGAATGGAGC TCAGTTGTGT
 661 GGGGGACCC TGATCAACAC CATCTGGGTG CTCTCCCGGG CCACACTGTT CGACAAAATC
 721 AAGAACTGGA GGAACCTGAT CGCGGTGCTG GGCAGCAGC ACCTCAGCGA GCACGACGGG
 781 GATGAGCAGA GCGCGCGGT GGCAGCAGTC ATCATCCCCA GCACTGTACG CCCGGGCACC
 841 ACCAACCCAG ACATCGCGCT GCTCCGCTG CACCGCCCG TGGTCTCAC TGACCATGTG
 901 GTGCCCTCTC GCCTGCCCGA CGAGGACGTC TCTGAGGAGA CGCTGGCCTT CGTGCCTTC
 961 TCATTGGTCA GCGGCTGGGG CCAGCTGCTG GACCGTGGCG CCACGGCCCT GGAGCTCATG
 1021 GTCCTCAAG TGCCCCGGCT GATGACCCAG GACTGCTCTG AGCACTACAG GAAGGTGGGA
 1081 GACTCCCTAA ATATCACGGA GTACATGTC TGTGCGGGCT ACTCGGATGG CAGCAAGGAC
 1141 TCCCTGCAAGG GGGACAGTGG AGGCCCCACAT GCCACCCACT ACCGGGGCAC GTGGTACCTG
 1201 ACGGGGCATCG TCAGCTGGGG CCAGGGCTGC GCAACCTGTG CCACATTGGG GGTGTACACC
 1261 AGGGTCTCC AGTACATGGA GTGGCTGCAA AAGCTCATGC CCTCAGAGCC ACGCCCAAGGA
 1321 GTCCTCTGCA GAGCCCCATT TCCCGGTGGC GGTGGCTCG CGGGAGGTGG GTCCGGTGGC
 1381 GGCAGGATCG GTGGGGGTGG ATCAGGGCGT GGAGGTTCCG GTGGCGGGGG ATCCGACAAA
 1441 ACTCACACAT GCCCCACCGTG CCCAGCTCG GAACTCTGG CGGGACCCCTC AGTCTTCCTC
 1501 TTCCCCCAAAC AACCAGGAA CACCCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG
 1561 GTGGTGGACG TGAGGTCAGA AGACCTGTG GTCAGATTCA ACTGGTACGT GGACGCGCTG
 1621 GAGGTGCTACA ATGCCAAGAC AAAGCCCGGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG
 1681 GTCAGCGTCC TCACCGTCCT GCACCAAGGAC TGGCTGAATG GCAAGGAGTA CAAAGTGCAG
 1741 GTCTCCAACA AAGCCCCCTC AGGCCCCATC GAGAAAACCA TCTCCAAACG CAAAGGGCAG
 1801 CCCCGAAACAC CACAGGTCA CACCCCTGCC CCACTCCCGG ATGAGCTGAC CAAGAACCGAG
 1861 GTCAGCGTCA CCTGGCTCTG CAAAGGCTTC TATGCCAGCG ACATCGCCGT GGAGTGGGAG
 1921 AGCAATGGGG AGCCGGAGAA CAACTACAGG ACCAGGCTCC CGTGTGTTGGA CTCCGACGGC
 1981 TCCCTCTTCC TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAAACGTC
 2041 TTCTCATGCT CGTGTGATGCA TGAGGCTCTG CACAACACT ACACGCAGAA GAGCCTCTCC

5 2101 CTGTCTCCGG GTAAAGGTGG CGGCGGGATCA GGTGGGGTG GATCAGGCGG TGGAGGTTCC
 2161 GGTGGCGGGG GATCAGACAA AACTCACACA TGCCCCACCGT GCCCAGCACCC TGAACCTCTG
 2221 GGAGGACCGT CAGTCTTCCT CTTCCCCCCC AAACCCAAGG ACACCCCTCAT GATCTCCCGG
 2281 ACCCCCTGAGG TCACATGGCT GGTGGTGGAC GTGAGCCACG AAGACCCCTGA GGTCAAGTTC
 2341 AACTGGTAGC TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCCCG GGAGGAGCAG
 10 2401 TACAACAGCA CGTACCGTGT GGTCAAGCGTC CTCACCGTCC FGCACCAAGGA CIGGCTGAAT
 2461 GGCAAGGAGT ACAAAGTGCAC GGTCTCCAC AAAGCCTCC CAGCCCCAT CGAGAAAACC
 2521 ATCTCCAAAG CCAAAGGGCA GCCCCGGAGA CCACAGGTGT ACACCCCTGCC CCCATCCCGC
 2581 GATGAGCTGA CCAAAGAACCA GGTCAAGCTG ACCTGAGCTGG TCAAAGGCTT CTATCCCAGC
 15 2641 GACATCCCCG TGGAGTGGGA GAGCAATGG CAGCCCCAGA ACACTACAA GACCAAGCCT
 2701 CCCGTGTTGG ACTCCGACGG CTCCCTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
 2761 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC
 2821 TACACGAGA AGACCCCTCCTC CCTGTCCTCG GGTAATATGA

20 FVII-118 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underline, 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 **MVSQALKLLC** **LLLGLOGGLA** **AVFVTOPEAH** **GVLHPRRAN** AFLEELRPGS LERECKEEQC
 61 SFEAREIPIK DAERTKLFWI **SYSDGDCQAS** SPCQNGGCK DQLQSYICFC LPAFEGRNCE
 25 121 THKDRQLICV NENGCEQYLC SDHTGTRKSC RCHEGYCPCQAS DGVSCTPTVW YPCGKIPILE
 181 KRNASKPQGA LRPKIVGGKV CPKGECPWQV LLLVNGAQLC CGTILINTLW VSAAHCFDKI
 241 KNWRNHLIAVL GEDLSEHNDG DEQSRRVAQV **IIPSTYVPGT** TNHDIALLRL HQ2VVLTDHV
 301 VPLCLPERTF SERTLAFVRF SLVSGWGQLL DRGATALELM VLNVPRIMIQ DCLQGSRKVG
 361 DSNPITEYMF CAGYSDGSKD SCKGDGSCPH ATHYGTWYL TGIVSWGQGC ATVGHFTGVYT
 421 RVSQYIIEWLQ KLMRSEPRVG VLLRAFFFPGG GGGGGGGGG GGGGGGGGG GGGGGGGGGDK
 481 THTCPCCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDEPE VKFRWYVVDGV
 541 EVHNAKTKPR EEQYINSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
 601 PREPVQVYTLPSR PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TPPVVLDSDG
 661 SFFFLYSKLTIV DKSRWQQGNV FSCSVMLHEAL HHNHYTQKSLS LSPGK**GGGGGS** **GGGGSGGGGS**
 721 **GGGGS**DKTHT CFFCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDEPE VKFRWYVVDGV
 781 NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
 841 ISKAKGGPRE PQVYTLPSR DELTKNQVSL TCLVKRGFYP SIAVEWESNG QPENNYKTPP
 901 PVLDSDGSFF LYSKLTVDKS RWQQGNVFC SVMHEALMNH YTQKSLSLSP GK*

40 DNA sequence for FVII-119
 1 ATGGTCCTCCC AGGCCCTCAG GCTCCTCTGC CTTCCTGCTTG GGCTTCAGGG CTGCCTGGCT
 61 CGAGTCCTCG TAACCCAGCA CGAACGCCAC GGCGTCTCTGC ACCGGCGCG GCGGCCAAC
 121 CGCTTCTCTGG AGGAGCTGCG GCGGGGCTCC CTGGAGAGGG AGTGCAGGAA GGAGCAGTGC
 181 TCCCTCGAGG AGGCCCGGAGA GATCTTCAGAG GACGGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACACTG ATGGGACCA **GTGTGCGCTCA** AGTCCATGCC AGATATGGGG CTCCCTGCAAG
 301 GACCACTCTG AGTCCTATAT CTGCTCTCTGC CTCCCTGCCT CGAGGGCCCG GAACTGTCAG
 361 ACGCACAAAGG ATGACAGCTG AACAGGAACG CGGGCTGTGA GCAGTACTGC
 421 AGTACCCACA CGGCCACCAA CGCCTCTCTGC CGGGCTCCACG AGGGTACTTC TCTCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAAATACC TATTCTAGAA
 541 AAAAGRAATG CCAGCAAACC CCAAGGGCGA GGAGGTGGTT CAGCCCTGCG GCCCCGGATT
 601 GTGGGGGGCA AGCTGTGCC CAAAGGGCGAG TGTCCATGCC AGGTCTCTGT GTTGGTGAAT
 661 GGAGGCTCAGT TGTCGTGGGG GACCTGTATC AACACCATCT GGGTGGTCTC CGCGGGCCAC
 721 TGTTCGACA AAATCAAGAA CTGGAGGAGC CTGATCGGG TGCTGGGCGA GCAGACCTC
 781 AGCGAGCACG ACGGGGATGA CGAGAGCCCC CGGGTGGCGC AGGTCACTCAT CCCCAGCACG
 841 TACGGTCCCGG GCACCAACAA CCACGACATC GCGCTGCTCC CCTGCAACCA GCCCCTGGTC
 901 CTCACUACG ATGGGGTGCCTC CCTCTCTCTGC CCCGAACCGA CGTCTCTCTGA GAGGAACGCTG
 961 GCCTTCGTGC GCTTCTCATC GGTCAAGGGC TGGGGGCCAG TGCTGGACCG TGGGCCACG
 1021 GGCCTGGAGC TCATGGTCCT CAACGTGCC CGGCTGATGA CCCAGGACTG CTCACAGCAG
 1081 TCACGGAAAGG TGGGAGACTC CCCAAATATC ACGGAGTACA TGTTCTGTGC CGGCTACTCG
 1141 GATGGCAGCA AGGACTCTG CAAGGGGGAC AGTGGAGGCC CACATGCCAC CCACTACCGG
 1201 GGCACGTGGT ACCTGACGGG CATCGTCAGC TGGGGCCAGG GCTGCGCAAC CGTGGGCCAC
 1261 TTTGGGGTGT ACACCAAGGGT CTCCCACTAC ATCGAGTGGC TGCAAAAGCT CATGCCCTCA
 1321 GAGCCACGCC CAGGAGTCCT CCTGCGAGCC CCAATTCCCG GTGGCGGTGG CCTCCGGCGA
 1381 GGTGGGTCTG GTGGGGCGG ATCAGGTGGG GGTGGATCG CGGGTGGAGG TTCCGGTGGC
 1441 GGGGGATCG ACAAACATCA CACATGCCA CCGTGGCCAG CTCCGGAACT CCTGGGGCGGA
 1501 CCGTCAGTCTC CCTCTCTCC CCAAAACCC AAGGACACCC TCATGATCTC CGGACCCCT
 1561 GAGGTACAT GCGTGGTGGT GGACGTGAGC CACGAGACG CTGAGGTCAA GTTCAACCTGG
 1621 TACGTGGACG GCTGGAGGT GCATAATGCC AAGACAAAGC CGGGGGACCA GCACTACAC
 1681 AGCACGTACG GTGTGGTCAG CGTCCTCACC GTCTGCAAC AGGACTGGCT GAATGGCAAG
 1741 GAGTACAAAGT GCAAGGTCTC CAACAAAGC CTCCCAAGGCC CCATCGAGAA AACCATCTCC
 1801 AAAGCCAAAG GGCAGCCCCG AGAACCAACAG GTGTACACCC TGCCCCCATC CGGGGATGAG
 1861 CTGACCAAGA ACCAGGTCAAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC

5 1821 GCGCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT ACAAGACCAC GCCTCCCGTG
 1881 TTGGACTCCG ACGGCTCCCT CTTCCCTCTAC AGCAAGCTCA CGTGGACAA GAGCAGGTGG
 2041 CAGCAGGGGA ACGTCTTCCTC ATGCTCCGTG ATGCATGAGG CTCAGCACAA CCACTACACG
 2101 CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA GGTGGGGCG GATCAGGTGG GGGTGGATCA
 2161 GCGGGTGGAG GTTCCGGTGG CGGGGGATCA GACAAACTC ACACATGCC ACCGTGCCA
 10 2221 GCACCTGAAAC TCCCTGGAGG ACCGTCACTC TTCCCTTCCC CCCCCAAACC CAAGGACACC
 2281 CTCATGATCT CCCGGACCCC TGAGGTCAAA TGGTGGTGG TGGACGTGAG CCACGAAGAC
 2341 CCTGAGGTCA AGTTCAACTG GTACGTGGAC GGCCTGGAGG TCCATAATGC CAAGACAAAG
 2401 CGCGGGAGG AGCASTACAC CAGCACCTAC CGTGTGGTCA CGCTCTGCAC CGTCTGCAC
 2461 CAGGACTGGC TGAAATGGCAA GAGTACAAAG TGCAAGGTCT CCAACAAAGC CCTCCAGCC
 15 2521 CCCATCGAGA AAACCATCTC CAAAGCCAAA GGGCAGCCCG GAGAACACCA GGTGTACACC
 2581 CTGCCCCCAT CCCCGATGA GCTGACCAAG AACCAAGGTCA GCCTGACCTG CCTGGTCAA
 2641 GGCTTCTATC CCACGCGATCG CGCGGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGACAAAC
 2701 TACAAGACCA CGCCTCCCGT GTTGGACTCC GACGGCTCT TCTTCCCTCA CAGCAAGCTC
 2761 ACCCTGGACCA AGACCGAGGTG CGACGAGGG AACGCTTCT CAGGCTCCGT GATGATGAG
 2821 GCTCTGACCA ACCACTACAC CGAGAAGACG CTCTCCCTGT CTCCGGTAA 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 MVSQALRLLC LELLGQGGLA AVFVLTQEEAH GVLLHERRRAN AFLLEELRPGS LERECKEEQC
 61 SFEAEAREIFK DAERTKLFWI SYSDGDQCAS SPQNNGGSK DQLQSYICFC LPAFEGRNCE
 121 THRDDQLICV NENGCEQYC SDITGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKFILE
 181 KRNASKFQGG GGGSALRPRI VGGKVCPKGE CPWQVLLLVN GAQLCGGTLL NTIWWVSAAH
 241 CFDKIKNWRN LIAVLGEHDL SEADGDEQSK RVAVQVIIIPST YVPGITNHDI ALLRLHQPVV
 30 301 LTDHVVFLCL PERTFSERTL AFVFRFSLVSG WGQILLDRGAT ALELMVILNP RLMTQDCLQQ
 361 SREKVGDSFNPY TEYNFPCAGYS DGSKDSCKGSD SGGPHATHYR CTWYLTGIVS WGQGCATVGH
 421 PGVYTRVSQY IEWLQKLIMRS EPRPGVLLRA PFPFGGGGSGG GGSGGGGSGG GGSGGGGSGG
 481 CGSDDKTHTCP PCPAPELGG PSVFLFPPPKR KDTIMISRTP EVTCVVVDVS HEDPEVKFNW
 541 YVDGVEVHNA KTKPREEQYQ STYRVVSEVLT VLHCDWLNKG EYCKKVSNKA LPAPIEKTS
 601 KAKGQPKREPO VYTLPPSRDE LTKNQVSELTC LVKGYPDSI AVEWESNGQP ENNYKTTTPV
 661 LDSDGSPFLY SKLTVDKSPRQ QQGNVFCSMV HMEALHNHYT QKSLSLSPGK **GGGGSGGGGS**
 721 **GGGGSGGGGS** DKHTHTCPFCP APELLGGESV FLFPFPKPKDT LMISRTPEVII CVVVDVSHED
 781 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 841 PIEKTISKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
 901 YKTTPPVLDs DGSPFLYSLK TVDKSRWQOG NVFSCSVMHE ALHNHYTOKS LSLSPGK*

DNA sequence for FVII-127

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCCTGTTG GGCTTCAGGG CTGCCTGGCT
 61 CGAGTCTTCG TAACCCAGGA GGAAGCCAC CGCGCTCTGC ACCGGCGCCG CGCGGCCAAC
 121 CGCTTCTCTGG AGGAGCTCG CGCGGCCCTC CTGGAGAGGG AGTCAAGGA CGACCACTGC
 181 TCCCTCCAGG AGCCCCCGGA GATCTTCAGG GACCCGGCA CGACCAACCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCTCA AGTCCATGCC AGAATGGGG CTCCCTGCAAG
 301 GACCAAGCTC AGTCTCTATAT CTGCTCTCTGC CTCCCTGCTC CGAGGGGGG GAACGTGCAAG
 361 ACACACAAAG ATGACCAAGCT GATCTGTGTG AACGAGAACG CGGGCTGTGA CGACTACTGC
 421 AGTGACCCACA CGGGCACCAA CGCGCTCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGTGTG CCTGCACACC CACAGTCAA TATCCATGTG CAAAAATAC TATCTAGAA
 541 AAAAGAAATG CGACGGCACCC CCAAGGGGCC CTGCGGCCCG GGATTGTGGG GGGCAAGGTG
 601 TGGCCCAAAG GGGAGTGTCA ATGGCAAGCTC CTGTTCTTGG TGATGGAGC TCAGTCTGT
 661 GGGGGGACCC TGATCANCAC CATCTGGCTG GTCTCCGGGG CCACACTGTIT CGACAAATTC
 721 AAGAACTGGA GGAACCTGAT CGCGGTGCTG GGCGAGCAGC ACCTCAGCGA GCACGACGGG
 781 GATGAATGAG GCGCGCGGGT GGCGCAGCTC ATCATCCCCA GCACGTACCT CCCGGGCAAC
 841 ACCAACACAG ACATCGCGCT GCTCCCTCTG CACCGCCCG TGTCCTCAC TGACCATGTG
 901 GTCGCCCTCTG GCTGCCCTG GCGACGCTC TCTGAGAGGA CGCTGGCCCT CGTCCGCTTC
 961 TCATTGCTCA CGCGCTGGGG CCAGCTCTGT GACCGCTGGCG CCACGGCCCT GGAGCTCATG
 1021 GTCCTCAACG TGCCCCGGCT GATGACCCAG GACTCGAGG CGAGCTACCC CGGCAAGATC
 1081 ACGGAGTACA TGTCTGTGC CGGCTACTCG GATGGCAGCA AGGACTCCCTG CAAGGGGGAC
 1141 AGTGGAGGCC CACATGCCAC CCACATACGGG GGCACGTGGT ACCTGACGGG CATCGTCAGC
 1201 TGGGGCCAGG GCTGGCACAAC CGTGGGGCAC TTGGGGGTGT ACACCAAGGGT CTCCCAGTAC
 1261 ATCGAGTGGC TCAAAAGCT CATGGCTCA GAGCCAGCC GAGGAGTCTT CCTGCGAGCC
 1321 CCATTTCCCG GTGGGGGTGG CTCCGGGGG GGTGGGTCGG GTGGCGGGGG ATCAGGTGGGG
 1381 GGTGGATCA GCGGTGGAGG TTCCGGTGGC GGGGGATCA GCAAAACTCA CACATGCCA
 1441 CGGTGCCAG CTCCGGAACG CCTGGGGGG CCGTCAGTCT TCTCTTCCC CCCAAACCC
 1501 AAGGACACCC TCATGATCTC CGGGACCCCT GAGCTCACAT CGTGGTCTT GGACCTGAGC
 1561 CACGAAGACCG CTGAGGTCAA GTTCAACTGG TACGTGGAGC CGTGGGAGGT GCATAATGCC
 1621 AAGACAAAGC CGCGGGAGGA CGAGTACACG AGCAAGTACCG GTGGGGTCA CGTCCCTCAC
 1681 GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT CCAAGGTCTC CAACAAAGCC
 1741 CTCCCCAGCCC CCATCGAGAA AACCAACTCC AAAGCCAAAG GGCAGCCCCG AGAACACACAG

5 1801 GTGTACACCC TGCCCCCATC CGGGGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC
 1861 CTGGTCAAAG GCTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGAGCCG
 1921 GAGAACAACT ACAAGACCAAC GCCTCCCGTG TTGGACTCG ACGGCTCCCT CTCCTCTAC
 1981 AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTCTC ATGCTCCGTG
 2041 ATGCATGAGG CTCCTCACAA CCACTACACG CAGAAAGGCC TCTCCCTGTC TCCGGTAA
 10 2101 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCA
 2161 GACAAAAC TCACATGCC ACCTGCCCCA GCACCTGAAAC TCCTGGGAGG ACCGTCAGTC
 2221 TCCTCTTCC CCCAAAAAC CAAGGACACC CTACATGATCT CCCGGACCCC TGAGGTACAA
 2281 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTCAACTG GTACGTGGAC
 2341 GCGCTGGAGG TGCTATAATGC AAAGAACAAAG CCGCCGGAGG ACCAGTACAA CAGCACGTAC
 15 2401 CGTGTGTC GCCTCCTCAC CGTCTCTCAC CAGGACTGGC FGAATGGCAA GGAGTACAAG
 2461 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAA
 2521 GGGCAGCCCC GAGAACACCA GGTGTACACC CTGCCCCATC CCCGCGATGA GCTGACCAAG
 2581 AACCAGCTCA GCCTGACCTG CCTGGTCARA GCCTCTATC CCAGCGACAT CGCCGTGGAG
 2641 TGGGAGACCA ATGGGACCC GGAGAACACAA TACAGACCA CCCTCCCGT GTTGGACTCC
 2701 GACGGC'CT TCCTCTCTA CAGCAAGCTC ACCGGTGGACA AGAGCAGGTG GCAGCAGGGG
 2761 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAAGAGC
 2821 CTCTCCCTGT CTCGGGTAA ATGA

25 FVII-127 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underline, the thrombin cleavage site is shown in dashed underline, the trypsin 170 loop region is wavy 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

1 MVSQALLLC LLLGLOGGLA AVFVTOEEAH GYLHEERRAN AFLEELRPGS LERECKEEQC
 61 SFEEAREIFK DAEKIKLFW SYSDGDCAS SPCQNNGSCK DQLQSYICPC LPATEGRNCE
 30 121 THKBDQQLICV NENGCEQYC SDHTGTRSC RCHEGYSLLA DGVSCTPTVE YPCGK1FILE
 181 KRNASKPOGA IPRIPVGKVK CPKGECPWQV LLLNVNGAQLC CGTILINTIIV VSAAHCPDKI
 241 KNWRNLLIAVL GERDLSEHDG DEQSRRVAQV IIPSTYVPGT TNHDIALLRL HQPVVLTQDHV
 301 VPLCLPERTF SERTLAFVRF SLVSGWGQLL DRGATALELM VLNVPRLMTQ DCEASYPGKI
 361 TEYMFAGYS DGSKDSCKGD SGGPHATHYR GTWYLTGIVS WGQGCATVGH FGVTTRVSQY
 421 IEWLQKLMLR EPRPGVLLR EPFPGGGSGG GGSGGGGSGG GGSGGGGGSGG GGSDKTHTCP
 481 PCPAPELLGG PSVFLPPFKP KDTLMISRTP EVTCVVGDVS HEDPEVKFNW YVDGVVBNHA
 541 KTKPREEQYN STYLRVSVLIT VLHQDWLNCK EYRKVYSNRA LPAPIEKUUS RAKGQPREPQ
 601 VYTLPPSRDE LTKNQVSLTC LVAGFYPSDL AVEWESENQQP ENNYKTTPPV LDSDGSSFFLY
 661 SKLTVDKSRM QQGNVFSCSV MHEALHNHYT QKSLSLSPGK GGGCGGGGGG GGGGSSGGGS
 40 721 DKTHTCPPCP APELGGPSV FLFPPPKD LMISRTPEVT CVVVDVSHED PEVKFMWYVD
 781 GVEVHNNAKT PREEQYNSTY RVVSVITVLA QDWLNKEYK CKVSNKALPA PIEKTISKAK
 841 QOPREPCVYT LPPERDELTK NQVSLCILVK GFYPSDIAVE WESNGQPENN YKTTPPVLDs
 901 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*

45 DNA sequence for FVII-125

1 ATGGTCTCCC AGGGCTCTAG GCTCTCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
 61 GCAGTCTCTG TAACCAGGA GGAAGCCCAC GGCGCTCTGC ACCGGCGCCG GCGCGCCAAAC
 121 GCCTTCTCTG AGGAGCTCG CCCGGGCTCC CTGGAGAGGG AGTGCAGGAA GGAGCACTGCG
 181 TCCTTCGAGG AGGGCGGGGA GATCTCAAG GACGGGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCTCTA AGTCCATGCC AGAATGGGG CTCTCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTCTCC CTCCCTGCT TCGAGGGCCG GAACUTGTGAG
 361 ACGCACAAAG ATGACCAGCT GATCTGTTG AAAGGAAGC CGGGCTGTGA GCAGTACTGCG
 421 AGTACCCACA CGGGCACCAA CGCTCCUC CGTGCACACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTGTAA TATCAGTGT AAAAAATAC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAAAC CCAAGGCCGA ATTGTGGGG GCAAGGTGTG CCCCAAAGGG
 601 GAGTGTCTAT GGCAGGCTCT GTTGTGGCTG AATGAGCTC AGTGTGTGUGG GGGGACCCCTG
 661 ATCAACACCA TCIGGGTGGT CTCCGCGGCC CACTGTTCG ACAAAATCAA GAACUTGGAGG
 721 AACCTGATCG CGGTGCTGG CGAGCACGAC CCTACGAGGC AGACGGGGGA TGAGCACAGC
 781 CGGGGGCTGG CGCAGGTCTAT CCTCCCCAGC ACGTACGTCC CGGGCACAC CAACCCACGAC
 841 ATCGCGCTGC TCCCTCTGCA CCAGCCCCCTG GTCTCTACTG ACCATGTGTGT GCCCCCTCTG
 901 CTGGCGGAAC GGACGTTCTC TGAGAGGACG CTGGCTCTCG TGGCTTCTC ATGGTCAAGC
 961 GGCTGGGGCC AGCTGCTGGA CGGTGGCGCC AGGGCCCTGG AGCTCATGTG CCTAACAGTG
 1021 CCCCCGGCTGA TGACCCAGGA CTGGCTCGCAG CAGTCACCGA AGGTGGGAGA CTCCCCAAAT
 1081 ATCACGGAGT ACATGTTCTG TGCCGGGTAC TGCGATGGCA GCAAGGACTC CTGCAAGGGG
 1141 GACAGTGGAG GGCCACATGC CACCCACTAC CGGGGACAGT GGTACCTGAC GGGCATCGTC
 1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACITTTGGGG GTACACCCAG GGTCTCCAG
 1261 TACATOGAGT GGCTGCAAAA GCTCATGCC TCAGAGCCAC GCGCAGGAGT CCTCCCTGC
 1321 GCCCCAATTG CGCGTGGGGG TGGCTCCGGC GGAGGTGGGT CGCGTGGGGG CGGATCAGGT
 1381 GGGGGTGGAT CAGCGGGTGG AGGTTCCCGT GGCGGGGGAT CGAGACATCGT GATGACCCAG
 1441 GCGGCCCGCA GCCTGCCCCG GACCCCCGGC GAGAGCGTGA GCATCAGCTG CGGGAGCAGC
 1501 CGGAGGCTGC TGCAACAGCAA CGGCAACACC TACCTGTCGT GTTCCTCTCA GCGGGCCCGGG
 1561 CAGAGCCCCC AGCTGCTGAT CTACCGGATG AGCAACCTGG CGAGCGGGGT GCGGGACCCGG
 1621 TTCAGCGGCA GCGCGAGGG CACCGCCTTC ACCCTGGGA TCAGCGGGGT GGAGGCCGGAG
 1681 GACGTGGGGC TGTACTACTG CATGCAGCAC CTGGAGTACC CCTTCACCTT CGGCAGCGGG

5 1741 ACCAAGCTGG AGATCAAGCG GGGCGGGCGC GGCACGGCGC GGGCGGGCG CGGCGGGCGC
 1801 GGCAGCCAGG TGCAGCTGCA GCAGACCGGC GCGGAGCTGG TCGGCGCCCG CACCAAGCTG
 1861 AAGATCAGCT GCAAGGCCAG CGGCTACACC TTCAACCAACT ATCGGCTGGG CTGGGTGAAG
 1921 CAGCGGCCCG GCCACGGCCT GGAGTGGATC GGCACATCT ACCCCGGCGG CGGCTACAAC
 1981 AACTACAAAGC AGAACTTCAA GGGCAAGGCC ACCCTGACCG CGACACCCAG CAGCAGCACC
 2041 GCCTACATGCA CGCTGAGGAG CCTGACCCAG GAGGACACCG CGTGTACTT CTGGCGCCCG
 2101 GACTACCGCA ACTACGACTA CGCCATGGAC AGCTGGGCC AGGGCACCAAG CGTGACCGTC
 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
 1 1 MSQALRLLCLLGLQGCL AYFVTCERAN GVLMRRRPRAN AFLEELRPGS LERECKEEQC
 61 SFEEAREIFK DAERTKLFWI SYSDGDCAS SPCONGECK DQLOSYICFC LPAFEGCRNCE
 121 THKDDQLICV NENGCEQYC SDHTGTRKRC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPOGR IVGKVKCPKG BCPWQVLLLV NGACLQCGGL INTIWVVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAVQVIIIPS TYVPGTNTNHD IALLRLHQPV VLTDHVVPIC
 301 LPERTFSEERT LAFVRFSLVS GWGQLLDRGA TALELMLVINR PRIMTQDCLQ QSRKVGDSPV
 361 ITEYMPFCAGY SDGSKDSCKG DSGGPHAIHY RGTWLTGIV SWQOGCATVG HFGVYIRVVSQ
 421 YIEWLQKLMR SEPPPGVLLR APFPGGGSGG GGGSGGGGSGG GGGSDIVMTQ
 481 AAPSVPVTPG ESVSISSCRSS RSLLHSNGNT YLCWELQPRG QSPOLLIYRM SNLASGPVDR
 541 FSGSGSGTAF TLRISRVEAE DVGVVYCMQH LEYPPTFGSG TKLEIKRCGG GSGGGGSGGG
 601 GSQVQLQQSG AELVREGTSV KISCKASGYT FTNYWLGWVK QRPCHGLEWI GDIYPGGGYN
 661 KYNENFRGKA TLTADTSSST AYMQLSSLTG EDSAVYFCAR EYGNYDYAMD SWGQGTSVIV
 721 *SS**

30 DNA sequence for FVII-067
 1 ATGGTCCTCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCTGGCT
 61 GCAAGTCCTGG TAACCCAGGA GGAAGCCAC GGCCTCTGC ACCGGCGCCG GCGGCCAAC
 121 CGCTTCCTGG AGGAGCTGGC CGCGGGCTCC CTGGAGAGGG AGTGCAGGAA GGAGCAGTGC
 181 TCTTCGAGG AGGCCGGGA GATCTTCAAG GACGCCGGAGA GAGCGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGACCA GTGIGCTCA AGTCCATGCC AGAATGGGG CTCCTGCAAG
 301 GACCAAGCTCC AGTCTTATAT CTGCTCTGC CTCCCTGCCTC TCGAGGGGCC GAACTGTGAG
 361 ACGCACAGG ATGACAGCTG GATCTGTGTC AACGAGAACG CGCGCTGTGA CGACTACTGC
 421 AGTGACCCACA CGGGCACCAA GCGCTCTCTG CGGTGCCCCAGC AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CTCGACACCC CACAGTTGAA TATCCATGTG GAAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAAGCAAAAC CCAAGGGCGA ATTGTTGGGG GCAAGGTGIG CCCCAAAGGG
 601 GAGTGTCTAT CGGAGCTCTC GTTGTGTCG AATGAGCTC AGTGTGTG GGGGACCTG
 661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTCTG ACAAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGGACCGACG CTCAGCGAGC AGCACGGGA TGAGCAGAGC
 781 CGGGGGGTGG CGCAGGTCTAT CATCCCCAGC ACCTGGCTCC CGGGCACAC CAACCACGAC
 841 ATCGCGCTGC TCCCTCTGCA CGAGCCCTG GTCTCTACTG ACCATGTGGT GCCCTCTGC
 901 CTGCCCGAAC GGACGTTCTC TGAGACGACG CTGGCCTTCG TGCGCTTCTC ATTGCTCAGC
 961 CGCTGGGCC AGCTGCTGGA CGCTGGGCC ACGGGCTGG AGCTCATGGT CCTAACCGTG
 1021 CCCCGCTGA TGACCCAGGA CTGCTCTGCAG CASTGACGGAA AGTGGGAGA CTCCCCAAAT
 1081 ATCACGGAGT ACATGTTCTG TGCGGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
 1141 GACAGTGGAG GCCACATGC CACCCACTAC CGGGGACCGT GTACCTGAC GGGCATOGTC
 1201 AGCTGGGGGAG AGGGCTGCGC AACCTGGGG CACTTGGGG TGTACACCGAG GGTCTCCCAG
 1261 TACATGGAGT GGCTGCAAA GCTCATGCC TCAGAGGCC ACCCAGGAGT CCTCTGCGA
 1321 GCCCCATTTG CCGGGGGCGG TGGCTCCGGC GGAGGTGGGT CGGTGGCGG CGGATCAGGT
 1381 GGGGGTGGAT CAGGGGGTGG AGGTTCTGGT GGGGGGGGG CCGACAAAC TCACACATGC
 1441 CAACCGCTGCC CAGCTCTGGCA ACTCTGGCC CGAACGTCAG TCTCTCTT CCCCCAAAA
 1501 CCAAGGACA CCTCTCATGAT CTCCGGGAC CCTGAGGTCA CATGGGTGGT GGTGACGCTG
 1561 AGCCACGAAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG AGGGCGTGGA GGTGCTATAAT
 1621 GCGCAAGACAA AGCCGGGGGA GGAGGACATAC AACACGACCT AGCTGTTGGT CAGGGCTCTC
 1681 ACCTGGCTGC ACAGGAGCTG GCTGAATTCG AGGAGTACA ATGTCAGGAT CTCACACATGC
 1741 GCCCCCTCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CGAGAACCA
 1801 CAGGTGTACA CCTCTGGGGAT ATCCCGGGAT GAGCTGACCA AGAACCCAGGT CAGCTGACCC
 1861 TGCGCTGTCA AAGGCTTCTA TCCCAGGAC ATCGCGCTGG AGTGGGAGAG CAATGGGGCAG
 1921 CGGAGAACACA ACTACAAGAC CACGGCTCTC GTGTTGACT CGAGCGCTC CTTCTTCTC
 1981 TACAGCAAGC TCACCGTGGC CAAGAGCAGG TGGCAGCAGG GCAACGCTT CTCATGCTCC
 2041 CTGATGCTGCA AGCTCTGCTCA CAACCACTAC ACCTGGACCA GCTCTCTT GTCTCCGGGT
 2101 AAAGGGTGGCG CGGGATCAGG TGGGGGTGGG TCAAGGGCTG GAGGTTCCGG TGGGGGGGA
 2161 TCAGACACAA CTACACATG CCAACCGCTGC CCAGCACCTG BACTCTGGG AGGACCGTCA
 2221 GTCTTCTCTC TCCCCCCTAA ACCCAAGGAC ACCCTCATGA TCTCCGGAC CCCTGAGGTG
 2281 ACATGGCTGG TGGTGGACGT GAGCCACCAA GACCTGAGG TCAAGTTCAA CTGGTACGTG
 2341 GACGGCTGG AGGTTGATCAA TGCCAAAGCA AAGCCGGGGG AGGAGGAGTCA CAACAGCACG
 2401 TACCGTGTGG TCAGCGTCTC CACCGTCTG CACCGAGACT GGCTGAATGG CAAGGAGTAC
 2461 AAGTGAAGG TCTCCAAACAA AGCCCTCTCA GCCCCCATCG AGAAAACCAT CTCCAAAGCC
 2521 AAAGGGCAGC CCGGAGAAC ACAGGTGTAC ACCCTGCCCC CATCCCGCGA TGAGCTGACCC
 2581 AGAACCCAGG TCACCGTCA CTGCTCTGCA AAAGCTTCT ATCCCAGCGA CATGCCGCTG
 2641 GACTGGGAGA GCAATGGGCA GCGGGAGAAC AACTACAAGA CCACGGCTCC CGTGTGGAC
 2701 TCCGACCGCT CCITCTTCTC CTACAGGACG CTACACGTCG ACAGAGGAG GIGGAGGAG
 2761 GGGAAACGCT TCTCATGCTC CGTGTACAT GAGGCTCTGC ACAACCACTA CACGCAGAAG
 2821 AGCCTCTCCC TGCTCCGGG TAAAGGTGGC GGTCGCTCCG CGGAGGTTGGC GTCCGGTGGC
 2881 GGGGGATCAG GTGGGGGTGG ATCAGGGCTG GGAGGTTCCG GTGGCGGGGG ATCAGCGCAG

5 2941 CTGCACTGAGGAGCTGG GGGAGGCTTG GTACAGCCTG GGGGGTCCCT GAGACTCTCC
 3001 TGTGAGCCCTGCTGATTCTGATTCAT GTTACAGG TATGCCATGA GCTGGGTCGG CCAGGCTCCA
 3061 GGGAAAGGGGC CAGAGTGGGT CTCAGGTATT AGTGGTAGTG GTGGTAGTAC ATACTACGCA
 3121 GACTCGTGA AGGGCGGGT CACCGTCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG
 3181 CAAATGACA GCTTGAGAGC CGAGGACAGC GCTGTATATT ACTGGGCCCC GGGGGCACC
 3241 TACACCCAGCC CGACCGACGT GCCCCGACCAAG ACCACCTTCG ACTACTGGGG CCAGGGAAACC
 3301 CTGGTACCGG TCTCTCAGG GAGTCATTC GCCCCAAAGC TIGAAGAAGG TCAATTTC
 3361 GAAGCAGCG TATCTGAAC GACTCAGGAC CCTGCTGTGT CTGTGGCCTT GGGACAGAC
 3421 GTCAAGGAGC CAGTCAGG AGAGACATT ATGCAAGCTG GTACAGCAG
 3481 AAGCCAGGAC AGGCCCTAC TCTTGTCTC TATGTTTAA GTAAAAGGCC CTCAGGGATC
 3541 CCAGACCGAT TCTCTGCCCTC CAGCTCAGGA AACACAGCTT CCTTGACCAT CACTGGGGCT
 3601 CAGGGCTGAAG ATGAGGCTGA CTATTACTGC CTGCTACT ACGGCGGGCG CCAGCAGGGC
 3661 GTGTGCGCG GCGGACCAA GCTGACGTC CTACGTCAGC CCAAGGCTGC CCCCTCGGTC
 3721 ACTCTGTTC CGCCCTCTTC TCGGGCTCA

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 SCF5 is in bold

1 MVSQALRLLC LLLGLOGCLIA AVFVTEEARH GVILNRRERAN AFLEELRPGS LERECKEEQC
 25 61 SFEEAREIFK DAERTKLFWI SYSDGDCAS SPCONGSCK DQLQSYYICFC LPAFEGRNE
 121 THKDDQLICV NENGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPGR IVGGKVCVKKG BCPWQVLLILV NGACLQCGTTL INTIWVVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RVRQAIVIIP TYVPGTTNRD IALLRLHQPV VLTDHVVPCL
 301 LPERTFSEI LAFVRFLSIV GWGQLLDRGA TALELMVILN PRIMTQDCLQ QSRKVGDSPN
 361 ITEYMFCAKY SDGSKDSCKG DSGGPHATHY RCTWYLTCIV SWQCCATVG HFGVYTRVSQ
 421 YIEWLQKLMR SERRPGVLLR SEPPPGVGGGG GGGEGGGGGG EGEGEGGGGGG GGGEDKTHTC
 481 PPCPAPFLPPK PDKTLMISRT PEVTCVWVVDV SKEDPEVKEN WYVDPGVEVHN
 541 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYRKVSNK ALPAPIEKTI SKAKQOPREP
 601 QVYTLPPSRD ELIJKNQVSLT CLVKGFYPSD IAVEWESNQG PENNYKTTPP VLDSDGSFFL
 661 YSKLTVEKSR WQOGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGGSGGGGG SGGGGEGGGGG
 721 SDKTHTCPPC PAPELGGPS VFLFPKPKFD TLMISRTPEV TCVVVDVSHD DPEVKPNWYV
 781 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
 841 KGQPREPOVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEW NYKTTPPVLD
 901 LTVDKSRWQQ GNVFSCSVMH EALMNHYTQK SLSLSPGKGG GSNSGGGGSG
 961 GGSGGGGGGGG GGSGGGGSAQ VQLQESGGGL VQPGGSSRLS CAAASGMFSR YAMSVVRQAP
 1021 GKGPEWVSGI SGSGGSTYYA DSVKGKFTVS RDNSKNTLYL QMNSLRAEDI AVVYCARQAI
 1081 YTTSRSDVPDQ TSFDWGQQGT LTVVSSDAS APKLEEGEFS EARVSELTQD PAVSVALGQT
 1141 VRITCQDSL RNFYASWYQI YGLSKRPSGI PDRFSASSSG NTASLITITGA
 1201 QAEDEADYYC LLYYGGQQQV VFGGGTAKTV LRQPKAAPS VLFPPSSAA*

45 DNA sequence for FVII-094

1 ATGGTCTCCAGGAGCCTCTAG GCTCCTCTGC CTTCTGCTTG GCGCTCAGGG CTGCCTGGCT
 61 CGAGTCCTCG TAACCCAGGA GGAAGCCAC GGCCTCTGC ACCGGCGCC CGCGCGCAAC
 121 CGGTTCTCTGG AGGAGCTCGG GCGGGCTCC CTGGAGAGGG ACTGCAAGGA CGAGCAAGTC
 181 TCCCTCGAGG AGGGCCCGA GATCTTCAGG CAGCCCGAGA CGACCAAGCT GTTCTGGATT
 241 TCTTACAGTG ATEGGGACCA GTGTGCTCTCA AGTCACATGCC AGAATGGGGG CTCCCTGCAAG
 301 GACCAGCTCC AGTCCCTATAT CTGCTCTCTGC CTCCCTGCGT TCGAGGGCGCG GAACTGTGAG
 361 AOGCACAAAGG ATGACCAAGG GATCTGTGT AACGAGAACG CGCGCTGTGA GCACTACTGC
 421 ATGACCAACA CGGGCACCAA CGCCTCTCTGT CGGTGCGCAGC AGGGGTACTC TCTCTGGCA
 481 GACGGGGTGT CCTGACACACC CACAGTGTAA TATCCATGTG GAAAAAATACC TATTCTAGAA
 541 AAAAGAATATG CCAGCAAAAC CCAAGGGCGA ATTGTGGGGG GCAAGGTGIG CCCAAAGGG
 601 GAGTGTCCCT GGCAGGTCTCT TGAGAGGACG CTGGCCTCTCG TCGGCTCTC ATTSGTCAGC
 661 ATCAACACCA TCTGGGTGGT CTCCGGCGCC CACTGGTCTCG ACAAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGAGCACCGAC CTGCGCGAGC ACAGACGGGGA TGAGCAGAGC
 781 CGGGGGTGT CGCAGGTCTCAT CTCGGCCACCG ACCTGAGTC CCGGACACAC CAACCAAGCAG
 841 ATGGCGCTGC TCCGGCGCTGC CGAGCCCCCTG CTCCCTACTG ACCATGTGGT GCGCCCTCTGC
 901 CTGGCCGAAAC GGACGTTCTC TGAGAGGACG CTGGCCTCTCG TCGGCTCTC ATTSGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CGTGGGGCCC ACGGGCGCTGG AGCTCATGGT CTCACACGTG
 1021 CCCCCGGCTGA TGACCGAGGA CTGCTCTGAG CAGTCACGGG AGGTGGGAGA CTCCCCAAAT
 1081 ATCACGGAGT ACATGTTCTG CTGGCGCTGC TCGGATGGCA CGAAGGACTC CTGCAAGGGG
 1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGCACCGT GCTACCTGAC GGGCATCGTC
 1201 AGCTGGGGCC AGCGCTGGCG AACCGTGGCC CACTTGGGG TGTACACCGAG GGTCTCCAG
 1261 TACATCGAGT GGCTCATGCG TCAGAGCCAC CGCCAGGAGT CTCCTGCGA
 1321 GCCCCCATTC CCGATATCGG TGGCGGTGGC TCCGGGGAGG GTGGGTCCCG TGGCGGGCGGA
 1381 TCAGGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG EGGGATCAGC GCAGGTGGAG
 1441 CTGCACTGAGT CTGGGGAGG CTGGGATCAGG CCTGGGGGGT CCTGAGACT CTCTCTGTGCA
 1501 GCGCTCTGGAT TCAATTTAG CAGGTATGCC ATGAGCTGGG TCGGCCAGGC TCCAGGGAAAG
 1561 GGGCCAGAGT GGCTCTCAGG TATTAGTGGT AGTGGTGGTA GTACATACTA CGCAGACTCC
 1621 GTGAAGGGCC GGTTCACCGT CTCCAGAGAC AATTCACAGA ACACGCTGTA TCTGCAAATG
 1681 AACAGCCTGA GACCGCAGGAGA CACGGCTGTA TATTACTGCG CCGGGGGCGC CACCTACACC
 1741 AGCCGGAGCG ACCTGGCCCGA CCAGACCGAC TTCGACTACT EGGGGCAGGG AACCCGGTC
 1801 ACCGTCCTCTCAGGGAGTGC ATCCGCCCA AAGCTGAAAG AAGGTGAATT TTCAGAAGCA
 1861 CGCGTATCTG AACTGACTCA GGACCCCTCT GTGCTCTGTGG CCTTGGGACA GACAGTCAGG
 1921 ATCACATGCC AAGGAGACAG CCTCAGAAAC TTTATGCAA GCTGGTACCA GCAGAAGCCA
 1981 GGACAGGGCC CTACTCTGTG CATCTATGTT TTAAGTAAA EGCCTCTAGG GATCCAGAC
 2041 CGATTCTCTG CCTCCAGCTC AGGAAACACA GCTCTCTGGA CCATCACTGG GGCTCAGGGCG

5 2101 GAAGAATGAGG CTGACTATTA CTGCCCTGCTG TACTACGGCG GCGGCCAGCA GGGCGTGTTC
 2161 GCGGGCGCGCA CCAAGCTGAC CGCTCTACGT CAGCCCCAAGG CTGCCCCCTTC GGTCACTCTG
 2221 TTCCCCGCCCT CTTCTGCAGC CTGA

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

1 1 MYSQALRLLC LLLGLOGCLL AYEVTCERAN SLYLMLRRRAN AFLEELRPGS LERECKEEQC
 61 SFEEDQIFK DAERTKLFWI SYSDGDDQCS SPCQNGGSCK DQLQSYICFC LPAPFGRNCE
 121 THKDDQLICV NENGCEEQYC SDHTGTKRC RCHEGYVLLA EGVISCTPTVIE YFCGKIPILE
 181 KRNASKPQGR IVSGKVCVPKG BCPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDJKIKNWR
 241 NLIAVLGEHD LSEHDDGDEQS RRAVQVIIIPS TYVFGTTNRD IALILRLHQPV VLTDHVVFLC
 301 LPERTFSERT LAFVVRFSLVS GWGQNLDRGA TALELMVINV PRIMTQDCLQ QSRKVGDSPN
 361 ITEYMFCAKY DSGGGPHATHY RGTWYLTGIV SWQGQCATVG HFGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR APFFPDIGGGG SGGGGGGGGG SGGGGSGGGG SGGGGSAQVQ
 481 LQESGGGLVQ PGGSILRLSCA ASGFMFSRYA MSNVROQAPKGK SFEWVSGISG SGGSTYYADS
 541 VKGRFTVSRD NSKNTLILQNM NSLRAEDTAV YYCARGATPKT SRSVDPDQTS FDYWQGQTLV
 601 TVSSGSAASAP KLEEGEFSEA RVSELTQDPA VSVALQTVR ITCQGDSLKN FYASWYQOKP
 661 QQAPTLIVIYG LSKRPGSIPD RFSASSSSGNT ASLTTITGAQA EDEADYYCLL YYGGQQQGVF
 721 GGGTKLTVLR QPKAAPSVTI FPPSSAA*

25 DNA sequence for FVII-028

1 1 ATGGCTCTCC AGGCCCTCTAG GCTCCCTCTGC CTTCTGCTTG SGCTTCAGGG CTGCCCTGGCT
 61 CGCGGAAGTCG AGCTGGTCA GCTGGGAGCT GAGGTGAATA AGCTGGGGGC CTCAGTGAAG
 121 GTCTCCCTCA AGCTCTCTGG ATACACCTTC ACCGGCTACT ATATGCACTG GGTGGACACAG
 181 GCCCCCTGGAC AAGGGCTTGA GTGGATGGGA TCGATCAACC CTAACAGTGG TGGCACAAAC
 241 TATGCAACAGA AGTTTCAGGG CTGGGTCAAC ATGACAGGG ACACGTCCAT CAGCACCGGC
 301 TACATGGAGC TGAGCAGGCT GAGATCTGAC GACACGGGGC TCTATTACCTG TGGGAGAGGC
 361 CGTGCCTTGTG ATAACCGGAA CGACCGGTCG CCCAACCTGGT TCGACCCCCCTG GGGCACAGGG
 421 ACCCTGGTCA CGGTGCTCTCC AGGGAGTCCA TCCGGCCCCAA CCTTAAACAT TGAAGAAGGT
 481 GAATTCTAGC AAGCACGCGT ACAGCTGTCG CTGACTTCAG CGCCCTCGGT GTCACTGGCC
 541 CCAGGACAGA CGGGCAGGAT TACCTGTGGG GGAACAAACA TTGGAAGTAA AAGTGTGCAG
 601 TGGTACCCAGC AGAAGCCAGG CCAGGGCCCT GTGCTGGTCG TCTATGATGA TAGGCACCGG
 661 CCCTCAGGG A TCCCTGAGCG ATTCTCTGC TCCAACCTGGT GGACACATGGC CACCCCTGACC
 721 ATCAGCAGGG TCGAAGCCCG GGTAGGAGCC GACTTACTT GTCAAGGTGTG GTAGTAGTGT
 781 AGTGTATCATG TGGTATTCTGG CGGAGGGGAC AAGCTGACCG TCTTAGGTCA GCCCAAGGGCT
 841 GCCCCCTCGG TCACTCTGTT CGCGCCGCTC CGGGCCGCTA GGACGAAGCT GTTCTGGATT
 901 TCTTACAGTG ATGGGGACCA GTGTGCTCTCA AGTCCATGCG AGATGGGGG CTCTCTGCAAG
 961 GACCGCTTCG AGTCTTATAT CTGCTCTGGC CTCCCTGGCT TCGAGGGCCG GAACTGTGAG
 1021 AGCCACACAGG ATCACCACTT GATCTCTGTC AACCGACAACC CGCGCTCTGA CCACTACTCC
 1081 AGTGACCCACA CGGGCACCAA CGCCTCTCTG CGGGCCACCG AGGGGTACIC TCTGCTGGCA
 1141 GACGGGGTGT CCTGCACACC CAACAGTGAAT TATCCATGTC GAAAAATACCC TATTCTAGAA
 1201 AAAAGAAATG CCACAGGCGA CCAAGGGCGA ATTGTGGGG CCAAGGTGTG CCCAAAGGG
 1261 GACTGTCCAT GGCAGGTCTC GTTGTGGTG AATGGAGCTC AGTGTCTGG GGGGACCCCTG
 1321 ATCAACACCA TCTGGGTGGT CTGGGGCCCC CACTCTTGGT ACAAAATCAA GAACCTGGAGG
 1381 AACCTGTGCG CGGTGCTGGG CGACCCAGAC CTCAGCAGGC ACACCGGGG TGAGCAGAGC
 1441 CGGGGEGTGG CGCAGGTCTAT CATCCCCAGC ACGTAAGTCC CGGGCACCAAC CAACCCACGAC
 1501 ATCGCGCTGC TCCCTGCTGCA CGACGGCCCTG GTCTCTACTG ACCATGTGGT GCCCCCTCTGC
 1561 CTGGCCGCAAG GGACGTTCTC TGAGAGGACG CTGGCTCTGC TCGGCTTCTC ATTGGTCTCAGC
 1621 GGCTGGGGCC AGCTGCTGGA CGTGGGGCC ACGGGGCTGG AGCTCATGGT CCTCACGTG
 1681 CCCCCGGCTGA TGACCCAGGA CTGCTCGAG CAGTCAGGA AGGTGGGAGA CTCCCCAAAT
 1741 ATCACGGAGT ACATCTCTG TGGCGGTAC TCGGTGGCA GCAAGGACIC TIGCAAGGGGG
 1801 GACAGTGGAG CCCCCACATGC CACCCACTAC CGGGCACCGT GGTACCTGAC GGGCATCGTC
 1861 AGCTGGGGCC AGGGCTGCGC AACCTGGGGC CACTTTGGGG TGTACACCAAG GGTCTCCAG
 1921 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCTGCGA
 1981 GCCCCCAATTG CCGGGCTGGG CGGCTCTGGG CGGAGTGGGT CGGCTGGGGG CGGATCAGGT
 2041 GGGGGGGGAT CAGGGGGGGG AGGTCCTCGG CGGGGGGGAT CGACAAAAC TCAACATGCG
 2101 CCACCGTGCCTC CAGCTCOOGGA ACTCTGCGG GGACCGTCAG TCTTCTCTT CCCCCCAAAA
 2161 CCCAAGGACA CCCCTCATGAT CTCCCGGACCC CCGCTGGTC CAGGGCTGGT GGTGGACGTT
 2221 AGCCACAGGA ACCCTGAGGT CAAGTTAAC TGGTACGTGG ACAGGGCTGGA GGTGCATAAT
 2281 GCCAAGAACAA AGCCCGGGGA GGAGGAGTAC AACACCGAGT ACCTGTGTGGT CAGGGCTCTC
 2341 ACCGCTCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAGGT CTCAACACAA
 2401 GCCCCCTCCUAG CCCCCUATCGA GAAAACCATC TCCAAAGGCA AAGGGCAGCC CGAGAACCCA
 2461 CAGGTGTACAG CCCCCTGGCCC ATCCCGGGGAT GAGCTGACCA AGAACCCAGGT CAGGGTACCC
 2521 TGGCTGTCTA AAGGCTTCTA TCCCGGGAC ATCGGGTGG AGTGGGAGAG CAATGGGGCAG
 2581 CCGGAGAACAA ACTACAAGAC CACGGCTCCC CTGTTGGACT CGACGGGCTC CTTCTTCTC
 2641 TACAGGCAAGC TCAAGGTGGA CAAGGAGCAGG TGGCAGCAGG GGAACGTTCTT CTCACTGCTC
 2701 GTGATGCTATG AGGCTGCTGA CAACCAACTAC ACAGCAGAAGA CGCTCTCCCT GTCTGGGGGT
 2761 AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGGGTG GAGGTTCCGG TGGGGGGGG
 2821 TCCGACAAAAA CTCACACATG CCCACCGTGC CGACGACCTG AACTCTGGG AGGACCGTCA
 2881 GTCTTCTCTC TCCCCCCTAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTG
 2941 ACATGGCTGG TGTTGGACGT GAGCCACCAA GACCTCTGAGG TCAAGTTCAA CGGGTACGTG
 3001 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCCGGGG AGGAGCAGTA CAACAGCACG
 3061 TACCGCTGGG TCACCGCTCT CACCGTCTCTG CACCAAGGACT GCTGAATGG CAAGGAGTAC
 3121 AAGTGAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAGGC
 3181 AAAGGECAGC CCCCAGAACCC ACAGGTGTAC ACCCTGGCCC CATCCCGGGA TGAGCTGACG
 3241 AGAACCGAGG TCAGGCTGAC CTGGCTGGTC AAAGGCTTCT ATCCCAGCGA CATGCCGTG

5 3301 CAGTGGGAGA GCAATGGCCA GCGGGAGAAC AACIACAAGA COACGCCCTCC CGTGTGGAC
 3361 TCGACGGCT CCTCTTCCT CTACACGAG CTCACCGTGG ACAAGAGCAG GTGCCAGCAG
 3421 GGGAACTCT TCTCATGCTC CGTGATGCTA GAGGCTCTGC ACAACCACTA CACGCAGAAG
 3481 AGCCTCTCCC TGTCTCGGG 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 **MVSGAQLLCL** LLIGLQGLA AEVQLVQSGA EVNPKGASVVK VSCKASGYTF TGYYMHNWVRQ
 61 APGQGLEWMG WINPNSGGT VYQKFQGHNVT MTRDT3ISTA YMLSLRRLSD DTAVYYCARG
 15 121 RALYNRNDRS PNWFDPWGQG TLTVTSSGSA SAPTIKLEEG EFSEARVQAV LTQPPSVSA
 181 FGQTARITCG GNNIGSKSVQ WYQQKPGQAF VLVVVYDSDR PSGIFPERFSG SNSGNMATLT
 241 ISRVEAGDEA DYYCQVWDDSS SDHVEWGGT KLTVLGQPKA APSVTLFPPS AAARTKLFW
 301 SYSDGDCAS SPONGGCKQ DQLOSYICPC LPAFEGRNCE THKDDQLICV NENGCEQYC
 361 SDHTGTKRSC RCHRGYSLLA DGVSCTPTVE YPCGKIPILE KRNASPKQGR IVGGKVCPKG
 421 ECPWQVLLV NGAGLCGGTL INTIWIWVSA HCFD1KHNWR MLIAVLGEHD ISEHDGDEQ
 481 RRVQAQVITPS TTVPGTTNHD IALLRLHGPV VLTDHVVPLC LPERTFSERT LAFVRFSLVS
 541 GWGQLLDRGA TALEFLMVLRV PRLMTQDCLQ QSRKVGDSPN ITEYMPGAGY SDGSKDSCKG
 601 DSGGPHATHY RGTWYLTGIV SWGQGCAIVG HFGVYVTRVSG YIENWLQKLMR SEPRPGVLLR
 661 APFPGGGGSG GGGGGGGGG GGGGGGGGG GGGGGGGGG GGGGGGGGG GGGGGGGGG
 25 721 PKDTLMISRT PEVTCVWVVDW SHEDPEVKEN WYVDCVVEVHN AKTPKPREECY NSTYRVVSVL
 781 TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKARGQPREP QVYTLPPSRD ELTKNMQVSLT
 841 CLVKGFYPSD IAEWESENQG PENNYKTTTP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS
 901 VMREALHNHY TQKSLSLSPG KGGGGGGGG SGGGGGGGG SDDKHTCOPC PAPELLGGPS
 961 VFLFPKPKD TLMISRTPEV TLMISRTPEV DPEVRFNWWY DGVEVHNAKT KPREQYNS
 30 1021 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTIKA KGQPREPQVY TLPPSERDELT
 1081 KNQVSLTCLV KGFPYPSDIAV EWESNQFEN NYKTTTPVLD SDGSEFFLYSK LTVDKGRWQ
 1141 GNVFSCSVWHE EALMNHYTQK SLSLSPGK*

35 DNA sequence FVII-039
 1 ATGGTCTCCAG GCTCCTCTGC CTTCCTGTTG GGCTTCAGGG CTGCCCTGGCT
 61 GCAGTCCTCG TAACCCAGGA GGAAGCCAC GGCCTCTGC ACCGGCGCC GCGGCCAAC
 121 GCGTTCTGG AGGAGCTCGC GCGGGCTCC CTGGAGAGGG AGTGCAGGAA GGAGCAGTGC
 181 TCTCTCGAGG AGGCCCGGGA GATCTTCAG GACCCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGCTCA AGTGCCTCTCA AGTCCATGCC AGATGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTCTTCG CTCCCTGCTC TGAGGGCCG GAACTGTGAG
 361 ACGCACAGG ATGACCAGT GATCTGTGTG AACGAGAACG CGGGCTGTGA CGAGTACTGC
 421 AGTGACCCACA CGGCTCCCTGT CGGCTCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGTGTG CTCCTCACCC CACAGTTGAA TATCCTATGTG AAAAATACO TATTCAGAA
 541 AAAAGAAATG CCACCAAAAC CCAAGGGCGA GGGCGAGGAG ACTTCACTCG GTTCTGGGG
 601 GCGCAAGGTG CCGGAAAGG GGAGCTGTCA TGGCGCTTC TGTGTTGGT GAATGGAGCT
 661 CAGTTGTGTG GGGGGACCC GATCAACACC ATCTGGGTTG TCTCCCGCCG CCACTGTTTC
 721 GACAAAATCA AGACTGGAG AACCTGATC GCGGTGCTGG CGAGGACAGA CCTCACCGAG
 781 CACGACGGGG ATCAGCAGAC CGGGGGGTG CGCGCAGGTCA TCATCCCCAG CACCTACGTC
 841 CGGGCACCA CCAACACAGA CATCGCTCG CTCCCTCTGC ACCAGCCCGT GCTCCCTCAI
 901 GACCATCTG TGCCCCCTCG CTCCTCCCGA CGACGCTCTC CTGAGACGGAC GCTGGCCCTTC
 961 GTGCGCTTCT CATTGGTCAG CGGCTGGGG CAGCTGCTGG ACCTGTTGGC CACGGCCCTG
 1021 GAGGCTATGG TCTCTGAAGCT GCCCCGGCTG ATGACCCAGG ACTGCTCTCA GCAGTCACTGG
 1081 AGGTCTGGGAG ACTCCCCAA TATCACGGAG TACATGTTCT GTGCCGGCTTA CTCCGGATGGC
 1141 AGCAAGGACT CTCGAAGGG GGCACTGTG GGCCTCACATG CGGGGGCACG
 1201 TGGTACCTGA CGGGCATCGT CAGCTGGGG CAGGGCTGCG CAACCGTGGG CCACTTGGG
 1261 GTGTACACCA GGGCTCTCCA GTACATCGAG TGGCTGCAA AGCTCATCGC CTACAGGCCA
 1321 CGCCCCAGAG TCCCTCTGGC AGCCCCATT CCCTGCTGG CGGGCTCCGG CGAGGTGGG
 1381 TCCGGTGGCG GCGGATCGG TGGGGGTGGA TCAAGGGTGG GAGGTTCCGG TGGGGGGGG
 1441 TCCGACAAAAT CTCACACATG CCCACCGTGC CGACGCTCGG AACTCTGGG CGGACGGTCA
 1501 GTCTTCTCTC TCCCCCAAGGACCAACCAAGAAC TCCCTCATGA TCTCCCGGAC CCCTGAGGTC
 1561 GACATGGCTGG TGGTGGACGT GAGGGACCAA GACTCTGAGG TCAAGTCAA CTGGTACGGT
 1621 GACGGCGCTGG AGGTGCATAA TGCCAAGACA AAGCCGGGG AGGAGCAGTA CAACAGCACG
 1681 TACCGTGTGG TCAGCGTCTC CACCGTCTG CACCGGACT CGCTGAATGG CAAGGAGTAC
 1741 AAGTGCAGG TCTCCAACAA AGCCCTCTCA GCCCCCATCG AGAAAACCAT CTCCAAAGGCC
 1801 AAAGGGCAGC CCGGAGAAC ACAGGTGATC ACCCTGCCCC CATCCCCGGG TGAGCTGACC
 1861 AAGAACCGAG TCAGCCTGAC CTGCTCTGCTC AAAGGCTTCT ATCCCAGCGA CATGCCGCTG
 1921 GACTGGGAGA GCAATGGGCA GCGGGAGAAC AACTCAAGA CCACGCCCTCC CCGTGTGGC
 1981 TCCGACGGCT CCTCTCTCTC CTACAGCAAG CTACAGCGAG GCAAGAGCAG GTGGCAGCAG
 2041 GGGAACTCTC TCTCATGCTC CGTGATGCTA GAGGCTCTGC ACAACCACTA CACGCAGAAG
 2101 AGCCTCTCCC TGTCTCCGGG TAAAGGTGGC GGCGGATCG GTGGGGGTGG ATCAGGCGGT
 2161 GGAGGTTCCG GTGGGGGGGG ATCAGACAAA ACTCACACATG GCGCACCGTG CCCAGCACCT
 2221 GAACTCTCTG GAGGACCGTC AGTCTCTCTC TTCCCCCCAA AACCCCAAGGA CACCCCTCATG
 2281 ATCTCCCGGA CCCCTGAGGT CACATGCTG GTGGTGGACG TGAGGCCAGA AGACCCCTGAG
 2341 GTCAAGTICA ACTGGTACGT GGACGGCTG GAGGTGCTA ATGCCAAGAC AAAGCCGG
 2401 GAGGAGGAGT ACAACAGCAC GTACCGTGTG GTCAAGCGTC TCACCGTCT GCACCCAGGAC
 2461 TGGCTGAATG GCAAGGAGTA CAAAGTGCAG GTCCTCAACA AAGCCCTCCC AGCCCCCATC
 2521 GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCGAGAAC CACAGGTGTA CACCCCTGCC
 2581 CCATCCUGCG ATGAGCTGAC CAAAGAACAG GTCAACCTGA CCTGCTCTG CAAAGGCTTC
 2641 TATCCCAAGCG ACATGCCGT GGAGTGGGAG AGCAATGGGAG AGCAGGAGAA CAACTACAAG
 2701 ACCACGCCCTC CCGTGTGGA CTCCGACCGC TCCTCTTCC TCTACAGCAA GCTCACCGTG
 2761 GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CGTGTGATGCA TGAGGCTCTG

5 2821 CACAACCAC ACACGCCAGAA GAGCCTCTCC CTGTCCTCCGG 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 MVSQALRLLC LLLGLOGGLA AVFVTQEEAH CVLHRRRERAA AFLLEELRPCS LERECKKEQC
 61 SFEEARIEFK DAERTKLPWI SYEDGEQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 TMKDDQQLICV NENGKCEQYC SDITGTRKSC RCHEGKLLS DGVSCPTFV YPCGKIPILE
 181 KRNASKPQGR GGGDFTRVVG SKVCPKGEC P QVLLILVNGA QLCGGTLINT IWWVSAAHCF
 241 DKKIKNWRNLI AVLGEHDLSE HDGDEQSRRV AQVIIPISTYV PGTTNHDIAL LRLHQFVVLIT
 301 DHVVPCLPFE RTFSERTLAF VRFSLVSSWVG QLLDRGATAL ELMVVLNVPRL MTQDCLQOSR
 361 KVGDSPNITE YMFCAGYSDG SKDSCKGDSG GPHATHYR7T WYLTCGIVSWG QGCATVGHFG
 421 VYTRVSGCIE WLQKLMRSEP RPGVLLRERAPP PGGGGSGGGGG SGGGGSGGGGG SGGGGSGGGGG
 481 **SDKTHTCPPC** PAPELLGGPS VFLFPKKPKD TLMISRTPEV TCVVVDVSHE DPEVKFVNWYV
 541 DGVEVHNNAKT KPRERQYNT YRVVSVLTVL HQDMLNGKEY KCKVSNKALP APIEKTISSKA
 601 KQQPREFQVY TLPPSREDELT KQVQSLTCLV KGFPYPSDIAV EWESNGQOPEN NYKTTTPVLD
 661 SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNEYTQK SLSLSPGKGG GGSGGGGSGG
 721 **GS**GGGGGGSDK THTCPPCPAP ELLGGPSVFL PPPKPKDILM ISRTPEVTCV VVDVSHEDPE
 781 VFKNRYVVDGV EVHNNAKT KPR EEQYNSTYRV VSVLTLVHQD WLNGKEYKCK VSNKALPAPI
 841 EKTISAKQG PREPOVYTLPSRDELT KQVQSLTCLV KQVQSLTCLV YPSDIAVENE SNGQPNNNYK
 901 TPPPVLDSDG SFFLYSKLT VDKSRWQGQNV FSCSVMHEAL HNHYTQKSLS LSPGK*

DNA sequence for FVII-040

30 1 ATGGTCCTCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCTGGCT
 61 GCACTCTTCG TAACCAGGA GGAAGCCRC GCGCTCTGC ACCGGCGCG GCGCGCCAAAC
 121 GCGTTCTCGG AGGAGCTCGG GCGCGCCCTC CTGGAGAGGG AGTCCAAGGA GAGGCAACTG
 181 TCCCTCGAGG AGGCGGGAGA GATCTTCAG GACGGAGAGA SGACGAAGCT GTTCTGGATI
 241 TCTTACAGTG ATGGGGACCA GTGTGCTCA AGTCATGCC AGAATGGGG CTCCCTGCAAG
 301 GACCAGCTCC AGTCTTATAT CTGCTCTGCG CTCCCTGCGT CGAGGGGCCG GAACTGTGAG
 361 AGCACAAGG ATGACCAGT GATCTGTGCG AACGAGAACG CGGGCTGTGA GCAGTACTGC
 421 AGTGACCCACA CGGGCACCAA GCGCTCTGCGT CGGTGCCCCAG AGGGGTACTC TCTCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGGCCG GGAGGAGACT TCACTCGGGT TGTGGGGGGC
 601 AAGGTGTGCC CCAAGGGGA CGGTCCATGG CAGGCTCTGT TGTGGTGAA TGGAGCTCAG
 661 TGTGTGGGG GGACCTGTAT CAACACCATC TGGGGGGTCT CGCGGGCCCA CTGTTTCGAC
 721 AAAATL2AGA ACTGGAGGAA CCTGATCCG CTGCTGGCG AGCACGACCI CAGCAGCAC
 781 GACGGGGGATG AGCAGAGCCG CGGGGGCCG CAGGCTCATCA TCCCAGCAC GTACGGTCCG
 841 GGCACCCACA ACCACGACAT CGCGCTCTC CGCTGCCCCAC AGCCCGTGTG CCTCAGTCAC
 901 CATGTGGTGC CCCCTGCTC GCGCGAACGG ACGTTCTCTG AGAGGACGCT GGCTTCTGGT
 961 CGCTTCATCAI TGGTCAAGCCG CTGGGGCCAG CTGCTGACC CTGGCGCCAC GGCCCTGGAG
 1021 CTCACTGGTCC TCAACGTGCC CGGGCTGTAT ACCCGAGACT GCTGCAGCA GTCACGGAAAG
 1081 GTGGGAGACT CCCCAAATAT CACGGAGTAC ATGTTCTGTG CGGGCTACTC GGATGGCAGC
 1141 AAGGACTCCT GCAAGGGGGG CAGTGCAGGG CCACATGCC CCCACTACCG GGGCACGTGG
 1201 TACCTGACGG GCATCGTCAG CTGGGGCCAG GGCTCGGCA CGTGGGGCTA CTTGGGGGTG
 1261 TACACCAAGG TCTCCCACTA CATCGAGTGG CTGCAAAAGC TCATGCCCTC AGAGCCACCC
 1321 CGAGGACTCC TCCCTGCGAGC CCCATTCTCC GGTCGCGGTG CTCCGGCCG AGGTGGGTCC
 1381 GGTGGGGGG GATCAGGTGG GGGTGGATCA GGCCTGGAG STTCCGGTGG CGGGGGATCC
 1441 GACAAACTC ACACATGCC ACCGTCGCCA GCTCCGGAA TCTGCTGAGC ACCTCTGATC
 1501 TTCCCTCTCC CCCCAAACCA CAAGGACACC CTGATGATCT CGGGGACCCC TGAGGTACA
 1561 TGCCTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTCTCAACTG GTACGTGGAC
 1621 GGCCTGGAGG TGCATAATGC CAAGAACAG CGCGGGGGAG ACCAGTACAA CAGCACGTAC
 1681 CGTGTCTCA CGCTCTGCAC CAGCTGGC TGAATGCCA GGAGTACAAAG
 1741 TGCAAGGGTCT CCACAAAGC CCTCCCAAGG CCCATGGAGA AAACCATCTC CAAAGCCAAA
 1801 GGGCAGCCCC GAGAACCCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
 1861 ACCACGCTCA GCTTGACCTG CCTGGTCAAA GGCTTCTATC CGACGGACAT CGCCGGTGGAG
 1921 TGGGAGAGCA ATGGCGAGCC GGAGAACACAC TACAGACCA CGCTCTCCCGT GTGGAATCTCC
 1981 GACGGCTCT TCTTCCTCTA CGACCAAGCTC ACCGTTGGACA AGAGCAGGTG GCAGCAGGGGG
 2041 AACGTCTTCT CATGCTCGT GATGCATGAG GCTCTGACA ACCACTACAC GCAGAAGAGC
 2101 CTCTCTCTGCT CTCGGGGTAA AGGTGGCCG GGAATCAGGTG GGGGTGGATC AGGGGGTGGGA
 2161 GGGTCCGGTGC CGGGGGGATC AGAACAAAAC CACACATGCC CACCGTGGCC ACCACCTGAA
 2221 CTCCCTGGAG GACCGTCAGT CTTCCCTCTC CCCCCAAAAC CCAAGGACAC CCTCATGATC
 2281 TCCCGGAGCC CTGAGGTCA ATGCGTGGTG CTGGAGCTGA CGCAAGGAGA CGCTGGGTCTC
 2341 AAGTTCRACT GGTACGTGGA CGGGCTGGAG GTGCTTAATG CCAAGACRAA GCGCGGGGAG
 2401 GAGCAGTACA ACACGACGTA CGGTGTGTC AGGCTCTCA CGCTCTCTCA CGAGGACTG
 2461 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCACAAAG CCCCTCCAGC CCCATCGAG
 2521 AAAACCATCT CCAAGGCCAA AGGGCAGGCC CGAGAACAC AGGTGTACAC CTCGCCCCCA
 2581 TCCCGGAGTC AGCTGACCAA GAACCGAGGTC AGCCCTGACCT CGCTGGTCAA AGGCTCTAT
 2641 CCCAGCGACA TCGCGTGGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACRAGACC
 2701 AGGCCTCCCG TGTGGACTC CGACGGCTCC TTCTTCTCT ACAGCAAGCT CACCGTGGAC
 2761 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC
 2821 AACCACTACA CGCGAGAG CCGTCTCCCTG TCTCCGGTA ATAGA

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 MVSQALRILC LLLGLOQGLA AVEVTOERAH GVLLHRRERRAN AFLBEELRPGS LERERECKEEQG
 61 SFEEARERIFK DAERTKLFW¹ SYSDGDDQCAS SPCQNGGSCK DQLQSYICPC LPAPFEGRNC
 121 THKDDQLICV NENGCEEQYC SDHTGTKRSC FCHEGYSLLA DGVSCTPTV² YPCGKIPILE
 181 KRNASKPQGG GGDFTRVVGG KVCPKGECPW QVLLLNVNGA³ LCGGTLINTI WVVSAAHCFD
 241 KIKNWRNLIA VLGRHDLSEH DGDEQSRRVA QVIIIPSTYVP GTTINHDIALL RLHQFVVLTD
 301 HVPVPLCLPER TF3ERTLAFV RPSLVSQWQ⁴ LLDRGATALE LMVLNVPRIM TQDCLQOSRK
 361 VGDSPNITEY MFCA⁵YSDGS KDSCKGDSGG PHATHYRGTW YLTGIVSNQG⁶ GCATVCHFGV
 421 YTRVSQYIEW LQKLMRSEPR PGVLLRAPPF GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS
 481 DKTHTCPKPCP APELLGGPSV FLFPPPKPDT LMISRTPEVT⁷ CVVVDVSHED PEVKPNWYVD
 541 GVEVHN⁸AKTK FREEQYNSTY RVVSVLTVLH QDWLN⁹KEYK CKVENDKALPA PIEKTISKAK
 601 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLD¹⁰
 661 DGSFFFLYSKL TVEKSRWQ¹¹Q NVFSCSVNHE ALHNH¹²YTKQS LSLSFGKGGG GSGGGGGGGG
 721 GSGGGGSDK¹³ HTCPKPCP¹⁴ LLGGPSVFLF PP¹⁵KPDTLM¹⁶ I¹⁷RTPEVTCVV VDVSHEDPEV
 781 KPNWYVEQVGE VHN¹⁸AKTKP¹⁹RE YN²⁰STYRV²¹ SVLTVLHQDW²² LNGKEYKCKV SNKALPAPIE
 841 KTISKANGQP²³ REPQVYTLPP²⁴ SRDELTKNQV²⁵ SLTCLVK²⁶G²⁷Y PSDIAVEWES NGQPENNYKT
 901 TPPVLDSDGS²⁸ FFLYSKL²⁹TVD KSRWQ³⁰QNVF³¹ SCSV³²NHEALH³³ NYTQKSLSL SPGK*

DNA sequence for FIX-042

1 ATTCAGCGCG TGAA³⁴ATGAT³⁵ CATGGCAGAA TCAC³⁶AGGCC³⁷ TCATCACC³⁸CT CTG³⁹CC⁴⁰TTTTA
 61 GGATA⁴¹TAC⁴² TCAGTGC⁴³TA ATG⁴⁴TACAGGT⁴⁵ TTGTT⁴⁶CC⁴⁷TT TTTAA⁴⁸ATA CATTGAG⁴⁹AT⁵⁰
 121 GCTTG⁵¹CC⁵²TT T⁵³AGAT⁵⁴ATAGA⁵⁵ AATATC⁵⁶TGAT⁵⁷ GCTG⁵⁸TCTCT⁵⁹ TCACTAA⁶⁰ATT⁶¹ T⁶²GTATTACAT⁶³
 181 GATTG⁶⁴ACAG⁶⁵ CAATATTGAA GAGTCTAA⁶⁶AC⁶⁷GCCAGCAC⁶⁸GC AGGT⁶⁹GGTAA⁷⁰ GTACTGTGGG
 241 AACAT⁷¹ACACAG⁷² ATTTTGGC⁷³TC⁷⁴CATGCC⁷⁵AA AGAGAATTG⁷⁶ GCTTCAGAT⁷⁷ TATT⁷⁸TGGATT⁷⁹
 301 AAAAACAAAG⁸⁰ AC⁸¹TCTTAA⁸² GAGATCTAA⁸³ AATTTCA⁸⁴TA⁸⁵ TGTTTCTT⁸⁶TT T⁸⁷GTCTAAA
 361 CTAAGA⁸⁸ATT⁸⁹ ATTTCA⁹⁰TTAC⁹¹TCAGT⁹²TT TCCT⁹³GTATCA⁹⁴ IGAAAACGCC⁹⁵ AACAA⁹⁶ATT⁹⁷C
 421 TGAATCGGCC⁹⁸ AAAGAGGT⁹⁹ TAA¹⁰⁰TCAG¹⁰¹TA AAT¹⁰²TTGGAAGA¹⁰³ GTTTGT¹⁰⁴TC¹⁰⁵AA GGGAA¹⁰⁶CTAG¹⁰⁷
 481 AGAGAG¹⁰⁸ATG¹⁰⁹ TATGGA¹¹⁰AA¹¹¹AGTGT¹¹²TTG¹¹³AGA¹¹⁴GG¹¹⁵AC¹¹⁶AGT¹¹⁷TTTGA¹¹⁸AAAC¹¹⁹A
 541 CTGA¹²⁰AAAGAC¹²¹ AACTGA¹²²TTT¹²³GTGAG¹²⁴CT¹²⁵ATGTTG¹²⁶AGATCAG¹²⁷GT¹²⁸G¹²⁹AA¹³⁰TCAT¹³¹
 601 CATG¹³²TTTAA¹³³ TGGCGG¹³⁴CA¹³⁵GT TGCA¹³⁶AGGT¹³⁷AC¹³⁸ATTA¹³⁹TT¹⁴⁰CTAT¹⁴¹CT¹⁴²GT¹⁴³CC¹⁴⁴CT
 661 TTGG¹⁴⁵AA¹⁴⁶TTGA¹⁴⁷ AGGA¹⁴⁸AA¹⁴⁹AC TGTGA¹⁵⁰ATTAG¹⁵¹ ATG¹⁵²ACAT¹⁵³ T¹⁵⁴ACATTAG¹⁵⁵AG¹⁵⁶ATG¹⁵⁷CC¹⁵⁸CT
 721 GCG¹⁵⁹AGCAGT¹⁶⁰ TTGT¹⁶¹AA¹⁶²AGTGT¹⁶³GATA¹⁶⁴ ACAAGG¹⁶⁵GT¹⁶⁶TTG¹⁶⁷CTCT¹⁶⁸TT¹⁶⁹ACTGAGGG¹⁷⁰AT¹⁷¹GG¹⁷²AA¹⁷³GAG
 781 ATCG¹⁷⁴ACTTGC¹⁷⁵ ACAGAAC¹⁷⁶AC¹⁷⁷AGTCTGT¹⁷⁸ G¹⁷⁹CCAC¹⁸⁰AGT¹⁸¹GG¹⁸²AGT¹⁸³CC¹⁸⁴TA¹⁸⁵TG¹⁸⁶GAAGAG
 841 TTCTG¹⁸⁷TTTC¹⁸⁸ ACAAA¹⁸⁹CT¹⁹⁰TCT¹⁹¹ AAG¹⁹²CTCACCC¹⁹³ GTGCTGAGAC¹⁹⁴ TGTTT¹⁹⁵TC¹⁹⁶CT GATGTGG¹⁹⁷AC¹⁹⁸T
 901 ATG¹⁹⁹AAATT²⁰⁰TC TACTGA²⁰¹AGG²⁰²GG²⁰³CT²⁰⁴AC²⁰⁵TT²⁰⁶GG²⁰⁷AG²⁰⁸GT²⁰⁹AC²¹⁰AT²¹¹CA²¹²T
 961 TTA²¹³ATGACT²¹⁴ CACTGGG²¹⁵TT²¹⁶GGTGA²¹⁷AG²¹⁸ATG²¹⁹CCAA²²⁰AC²²¹AGG²²²TC²²³CC²²⁴TTGGC
 1021 AGGTTG²²⁵TTT²²⁶GA²²⁷AA²²⁸TTG²²⁹TG²³⁰AT²³¹CTG²³²TTG²³³AA²³⁴T²³⁵ACAG²³⁶TT²³⁷GT²³⁸CT²³⁹CC²⁴⁰TT
 1081 GGATTG²⁴¹TAAC²⁴² TGCTGCC²⁴³AC²⁴⁴TG²⁴⁵TGAA²⁴⁶ CTGGT²⁴⁷TTA²⁴⁸AAT²⁴⁹ACAG²⁵⁰TT²⁵¹TCGAGGT²⁵²
 1141 ACATA²⁵³AT²⁵⁴AT²⁵⁵AA²⁵⁶AC²⁵⁷AG²⁵⁸AC²⁵⁹AA²⁶⁰GG²⁶¹TT²⁶²AC²⁶³TT²⁶⁴GT²⁶⁵ACT²⁶⁶TT²⁶⁷TT²⁶⁸AT²⁶⁹TT²⁷⁰TT²⁷¹TT²⁷²TT²⁷³TT²⁷⁴TT²⁷⁵TT²⁷⁶TT²⁷⁷TT²⁷⁸TT²⁷⁹TT²⁸⁰TT²⁸¹TT²⁸²TT²⁸³TT²⁸⁴TT²⁸⁵TT²⁸⁶TT²⁸⁷TT²⁸⁸TT²⁸⁹TT²⁹⁰TT²⁹¹TT²⁹²TT²⁹³TT²⁹⁴TT²⁹⁵TT²⁹⁶TT²⁹⁷TT²⁹⁸TT²⁹⁹TT³⁰⁰TT³⁰¹TT³⁰²TT³⁰³TT³⁰⁴TT³⁰⁵TT³⁰⁶TT³⁰⁷TT³⁰⁸TT³⁰⁹TT³¹⁰TT³¹¹TT³¹²TT³¹³TT³¹⁴TT³¹⁵TT³¹⁶TT³¹⁷TT³¹⁸TT³¹⁹TT³²⁰TT³²¹TT³²²TT³²³TT³²⁴TT³²⁵TT³²⁶TT³²⁷TT³²⁸TT³²⁹TT³³⁰TT³³¹TT³³²TT³³³TT³³⁴TT³³⁵TT³³⁶TT³³⁷TT³³⁸TT³³⁹TT³⁴⁰TT³⁴¹TT³⁴²TT³⁴³TT³⁴⁴TT³⁴⁵TT³⁴⁶TT³⁴⁷TT³⁴⁸TT³⁴⁹TT³⁵⁰TT³⁵¹TT³⁵²TT³⁵³TT³⁵⁴TT³⁵⁵TT³⁵⁶TT³⁵⁷TT³⁵⁸TT³⁵⁹TT³⁶⁰TT³⁶¹TT³⁶²TT³⁶³TT³⁶⁴TT³⁶⁵TT³⁶⁶TT³⁶⁷TT³⁶⁸TT³⁶⁹TT³⁷⁰TT³⁷¹TT³⁷²TT³⁷³TT³⁷⁴TT³⁷⁵TT³⁷⁶TT³⁷⁷TT³⁷⁸TT³⁷⁹TT³⁸⁰TT³⁸¹TT³⁸²TT³⁸³TT³⁸⁴TT³⁸⁵TT³⁸⁶TT³⁸⁷TT³⁸⁸TT³⁸⁹TT³⁹⁰TT³⁹¹TT³⁹²TT³⁹³TT³⁹⁴TT³⁹⁵TT³⁹⁶TT³⁹⁷TT³⁹⁸TT³⁹⁹TT⁴⁰⁰TT⁴⁰¹TT⁴⁰²TT⁴⁰³TT⁴⁰⁴TT⁴⁰⁵TT⁴⁰⁶TT⁴⁰⁷TT⁴⁰⁸TT⁴⁰⁹TT⁴¹⁰TT⁴¹¹TT⁴¹²TT⁴¹³TT⁴¹⁴TT⁴¹⁵TT⁴¹⁶TT⁴¹⁷TT⁴¹⁸TT⁴¹⁹TT⁴²⁰TT⁴²¹TT⁴²²TT⁴²³TT⁴²⁴TT⁴²⁵TT⁴²⁶TT⁴²⁷TT⁴²⁸TT⁴²⁹TT⁴³⁰TT⁴³¹TT⁴³²TT⁴³³TT⁴³⁴TT⁴³⁵TT⁴³⁶TT⁴³⁷TT⁴³⁸TT⁴³⁹TT⁴⁴⁰TT⁴⁴¹TT⁴⁴²TT⁴⁴³TT⁴⁴⁴TT⁴⁴⁵TT⁴⁴⁶TT⁴⁴⁷TT⁴⁴⁸TT⁴⁴⁹TT⁴⁵⁰TT⁴⁵¹TT⁴⁵²TT⁴⁵³TT⁴⁵⁴TT⁴⁵⁵TT⁴⁵⁶TT⁴⁵⁷TT⁴⁵⁸TT⁴⁵⁹TT⁴⁶⁰TT⁴⁶¹TT⁴⁶²TT⁴⁶³TT⁴⁶⁴TT⁴⁶⁵TT⁴⁶⁶TT⁴⁶⁷TT⁴⁶⁸TT⁴⁶⁹TT⁴⁷⁰TT⁴⁷¹TT⁴⁷²TT⁴⁷³TT⁴⁷⁴TT⁴⁷⁵TT⁴⁷⁶TT⁴⁷⁷TT⁴⁷⁸TT⁴⁷⁹TT⁴⁸⁰TT⁴⁸¹TT⁴⁸²TT⁴⁸³TT⁴⁸⁴TT⁴⁸⁵TT⁴⁸⁶TT⁴⁸⁷TT⁴⁸⁸TT⁴⁸⁹TT⁴⁹⁰TT⁴⁹¹TT⁴⁹²TT⁴⁹³TT⁴⁹⁴TT⁴⁹⁵TT⁴⁹⁶TT⁴⁹⁷TT⁴⁹⁸TT⁴⁹⁹TT⁵⁰⁰TT⁵⁰¹TT⁵⁰²TT⁵⁰³TT⁵⁰⁴TT⁵⁰⁵TT⁵⁰⁶TT⁵⁰⁷TT⁵⁰⁸TT⁵⁰⁹TT⁵¹⁰TT⁵¹¹TT⁵¹²TT⁵¹³TT⁵¹⁴TT⁵¹⁵TT⁵¹⁶TT⁵¹⁷TT⁵¹⁸TT⁵¹⁹TT⁵²⁰TT⁵²¹TT⁵²²TT⁵²³TT⁵²⁴TT⁵²⁵TT⁵²⁶TT⁵²⁷TT⁵²⁸TT⁵²⁹TT⁵³⁰TT⁵³¹TT⁵³²TT⁵³³TT⁵³⁴TT⁵³⁵TT⁵³⁶TT⁵³⁷TT⁵³⁸TT⁵³⁹TT⁵⁴⁰TT⁵⁴¹TT⁵⁴²TT⁵⁴³TT⁵⁴⁴TT⁵⁴⁵TT⁵⁴⁶TT⁵⁴⁷TT⁵⁴⁸TT⁵⁴⁹TT⁵⁵⁰TT⁵⁵¹TT⁵⁵²TT⁵⁵³TT⁵⁵⁴TT⁵⁵⁵TT⁵⁵⁶TT⁵⁵⁷TT⁵⁵⁸TT⁵⁵⁹TT⁵⁶⁰TT⁵⁶¹TT⁵⁶²TT⁵⁶³TT⁵⁶⁴TT⁵⁶⁵TT⁵⁶⁶TT⁵⁶⁷TT⁵⁶⁸TT⁵⁶⁹TT⁵⁷⁰TT⁵⁷¹TT⁵⁷²TT⁵⁷³TT⁵⁷⁴TT⁵⁷⁵TT⁵⁷⁶TT⁵⁷⁷TT⁵⁷⁸TT⁵⁷⁹TT⁵⁸⁰TT⁵⁸¹TT⁵⁸²TT⁵⁸³TT⁵⁸⁴TT⁵⁸⁵TT⁵⁸⁶TT⁵⁸⁷TT⁵⁸⁸TT⁵⁸⁹TT⁵⁹⁰TT⁵⁹¹TT⁵⁹²TT⁵⁹³TT⁵⁹⁴TT⁵⁹⁵TT⁵⁹⁶TT⁵⁹⁷TT⁵⁹⁸TT⁵⁹⁹TT⁶⁰⁰TT⁶⁰¹TT⁶⁰²TT⁶⁰³TT⁶⁰⁴TT⁶⁰⁵TT⁶⁰⁶TT⁶⁰⁷TT⁶⁰⁸TT⁶⁰⁹TT⁶¹⁰TT⁶¹¹TT⁶¹²TT⁶¹³TT⁶¹⁴TT⁶¹⁵TT⁶¹⁶TT⁶¹⁷TT⁶¹⁸TT⁶¹⁹TT⁶²⁰TT⁶²¹TT⁶²²TT⁶²³TT⁶²⁴TT⁶²⁵TT⁶²⁶TT⁶²⁷TT⁶²⁸TT⁶²⁹TT⁶³⁰TT⁶³¹TT⁶³²TT⁶³³TT⁶³⁴TT⁶³⁵TT⁶³⁶TT⁶³⁷TT⁶³⁸TT⁶³⁹TT⁶⁴⁰TT⁶⁴¹TT⁶⁴²TT⁶⁴³TT⁶⁴⁴TT⁶⁴⁵TT⁶⁴⁶TT⁶⁴⁷TT⁶⁴⁸TT⁶⁴⁹TT⁶⁵⁰TT⁶⁵¹TT⁶⁵²TT⁶⁵³TT⁶⁵⁴TT⁶⁵⁵TT⁶⁵⁶TT⁶⁵⁷TT⁶⁵⁸TT⁶⁵⁹TT⁶⁶⁰TT⁶⁶¹TT⁶⁶²TT⁶⁶³TT⁶⁶⁴TT⁶⁶⁵TT⁶⁶⁶TT⁶⁶⁷TT⁶⁶⁸TT⁶⁶⁹TT⁶⁷⁰TT⁶⁷¹TT⁶⁷²TT⁶⁷³TT⁶⁷⁴TT⁶⁷⁵TT⁶⁷⁶TT⁶⁷⁷TT⁶⁷⁸TT⁶⁷⁹TT⁶⁸⁰TT⁶⁸¹TT⁶⁸²TT⁶⁸³TT⁶⁸⁴TT⁶⁸⁵TT⁶⁸⁶TT⁶⁸⁷TT⁶⁸⁸TT⁶⁸⁹TT⁶⁹⁰TT⁶⁹¹TT⁶⁹²TT⁶⁹³TT⁶⁹⁴TT⁶⁹⁵TT⁶⁹⁶TT⁶⁹⁷TT⁶⁹⁸TT⁶⁹⁹TT⁷⁰⁰TT⁷⁰¹TT⁷⁰²TT⁷⁰³TT⁷⁰⁴TT⁷⁰⁵TT⁷⁰⁶TT⁷⁰⁷TT⁷⁰⁸TT⁷⁰⁹TT⁷¹⁰TT⁷¹¹TT⁷¹²TT⁷¹³TT⁷¹⁴TT⁷¹⁵TT⁷¹⁶TT⁷¹⁷TT⁷¹⁸TT⁷¹⁹TT⁷²⁰TT⁷²¹TT⁷²²TT⁷²³TT⁷²⁴TT⁷²⁵TT⁷²⁶TT⁷²⁷TT⁷²⁸TT⁷²⁹TT⁷³⁰TT⁷³¹TT⁷³²TT⁷³³TT⁷³⁴TT⁷³⁵TT⁷³⁶TT⁷³⁷TT⁷³⁸TT⁷³⁹TT⁷⁴⁰TT⁷⁴¹TT⁷⁴²TT⁷⁴³TT⁷⁴⁴TT⁷⁴⁵TT⁷⁴⁶TT⁷⁴⁷TT⁷⁴⁸TT⁷⁴⁹TT⁷⁵⁰TT⁷⁵¹TT⁷⁵²TT⁷⁵³TT⁷⁵⁴TT⁷⁵⁵TT⁷⁵⁶TT⁷⁵⁷TT⁷⁵⁸TT⁷⁵⁹TT⁷⁶⁰TT⁷⁶¹TT⁷⁶²TT⁷⁶³TT⁷⁶⁴TT⁷⁶⁵TT⁷⁶⁶TT⁷⁶⁷TT⁷⁶⁸TT⁷⁶⁹TT⁷⁷⁰TT⁷⁷¹TT⁷⁷²TT⁷⁷³TT⁷⁷⁴TT⁷⁷⁵TT⁷⁷⁶TT⁷⁷⁷TT⁷⁷⁸TT⁷⁷⁹TT⁷⁸⁰TT⁷⁸¹TT⁷⁸²TT⁷⁸³TT⁷⁸⁴TT⁷⁸⁵TT⁷⁸⁶TT⁷⁸⁷TT⁷⁸⁸TT⁷⁸⁹TT⁷⁹⁰TT⁷⁹¹TT⁷⁹²TT⁷⁹³TT⁷⁹⁴TT⁷⁹⁵TT⁷⁹⁶TT⁷⁹⁷TT⁷⁹⁸TT⁷⁹⁹TT⁸⁰⁰TT⁸⁰¹TT⁸⁰²TT⁸⁰³TT⁸⁰⁴TT⁸⁰⁵TT⁸⁰⁶TT⁸⁰⁷TT⁸⁰⁸TT⁸⁰⁹TT⁸¹⁰TT⁸¹¹TT⁸¹²TT⁸¹³TT⁸¹⁴TT⁸¹⁵TT⁸¹⁶TT⁸¹⁷TT⁸¹⁸TT⁸¹⁹TT⁸²⁰TT⁸²¹TT⁸²²TT⁸²³TT⁸²⁴TT⁸²⁵TT⁸²⁶TT⁸²⁷TT⁸²⁸TT⁸²⁹TT⁸³⁰TT⁸³¹TT⁸³²TT⁸³³TT⁸³⁴TT⁸³⁵TT⁸³⁶TT⁸³⁷TT⁸³⁸TT⁸³⁹TT⁸⁴⁰TT⁸⁴¹TT⁸⁴²TT⁸⁴³TT⁸⁴⁴TT⁸⁴⁵TT⁸⁴⁶TT⁸⁴⁷TT⁸⁴⁸TT⁸⁴⁹TT⁸⁵⁰TT⁸⁵¹TT⁸⁵²TT⁸⁵³TT⁸⁵⁴TT⁸⁵⁵TT⁸⁵⁶TT⁸⁵⁷TT⁸⁵⁸TT⁸⁵⁹TT⁸⁶⁰TT⁸⁶¹TT⁸⁶²TT⁸⁶³TT⁸⁶⁴TT⁸⁶⁵TT⁸⁶⁶TT⁸⁶⁷TT⁸⁶⁸TT⁸⁶⁹TT⁸⁷⁰TT⁸⁷¹TT⁸⁷²TT⁸⁷³TT⁸⁷⁴TT⁸⁷⁵TT⁸⁷⁶TT⁸⁷⁷TT⁸⁷⁸TT⁸⁷⁹TT⁸⁸⁰TT⁸⁸¹TT⁸⁸²TT⁸⁸³TT⁸⁸⁴TT⁸⁸⁵TT⁸⁸⁶TT⁸⁸⁷TT⁸⁸⁸TT⁸⁸⁹TT⁸⁹⁰TT⁸⁹¹TT⁸⁹²TT⁸⁹³TT⁸⁹⁴TT⁸⁹⁵TT⁸⁹⁶TT⁸⁹⁷TT⁸⁹⁸TT⁸⁹⁹TT⁹⁰⁰TT⁹⁰¹TT⁹⁰²TT⁹⁰³TT⁹⁰⁴TT⁹⁰⁵TT⁹⁰⁶TT⁹⁰⁷TT⁹⁰⁸TT⁹⁰⁹TT⁹¹⁰TT⁹¹¹TT⁹¹²TT⁹¹³TT⁹¹⁴TT⁹¹⁵TT⁹¹⁶TT⁹¹⁷TT⁹¹⁸TT⁹¹⁹TT⁹²⁰TT⁹²¹TT⁹²²TT⁹²³TT⁹²⁴TT⁹²⁵TT⁹²⁶TT⁹²⁷TT⁹²⁸TT⁹²⁹TT⁹³⁰TT⁹³¹TT⁹³²TT⁹³³TT⁹³⁴TT⁹³⁵TT⁹³⁶TT⁹³⁷TT⁹³⁸TT⁹³⁹TT⁹⁴⁰TT⁹⁴¹TT⁹⁴²TT⁹⁴³TT⁹⁴⁴TT⁹⁴⁵TT⁹⁴⁶TT⁹⁴⁷TT⁹⁴⁸TT⁹⁴⁹TT⁹⁵⁰TT⁹⁵¹TT⁹⁵²TT⁹⁵³TT⁹⁵⁴TT⁹⁵⁵TT⁹⁵⁶TT⁹⁵⁷TT⁹⁵⁸TT⁹⁵⁹TT⁹⁶⁰TT⁹⁶¹TT⁹⁶²TT⁹⁶³TT⁹⁶⁴TT⁹⁶⁵TT⁹⁶⁶TT⁹⁶⁷TT⁹⁶⁸TT⁹⁶⁹TT⁹⁷⁰TT⁹⁷¹TT⁹⁷²TT⁹⁷³TT⁹⁷⁴TT⁹⁷⁵TT⁹⁷⁶TT⁹⁷⁷TT⁹⁷⁸TT⁹⁷⁹TT⁹⁸⁰TT⁹⁸¹TT⁹⁸²TT⁹⁸³TT⁹⁸⁴TT⁹⁸⁵TT⁹⁸⁶TT⁹⁸⁷TT⁹⁸⁸TT⁹⁸⁹TT⁹⁹⁰TT⁹⁹¹TT⁹⁹²TT⁹⁹³TT⁹⁹⁴TT⁹⁹⁵TT⁹⁹⁶TT⁹⁹⁷TT⁹⁹⁸TT⁹⁹⁹TT¹⁰⁰⁰TT¹⁰⁰¹TT¹⁰⁰²TT¹⁰⁰³TT¹⁰⁰⁴TT¹⁰⁰⁵TT¹⁰⁰⁶TT

5 181 VSVSQISKLT RAESTVFPDVY YVNSTEAETI LDNITQSTQS FNDFTRVVGG EDAKPGQFWR
 241 QVVLNGKVD AFGCGSIVNEK WIVTAAHCV TGVKITVVAG EHNIETEHT EKQRNVIRII
 301 PHRNVNNAIN KYNHDIALLE LDEPILVLSNY VTPICIADKE VTNIFLKFGS GYVSGWGRVFE
 361 HKGRSALVLO YLRVPLVDR A TCLRSTKETI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 421 GTSFLTGIIS WGERCAMKGK YGIYTKVSRV VNWIKEKIKL TDKTHTCPPC PAPELLGGPS
 10 481 VFLFPFPKPKD TIMISRTPEV TCVVVDVSH DPEVFKPNWYV DGVEVHNIAKT KPREEQYINST
 541 YRVVSVLTVL HQDWLNCKEV KCKVSNKALP APIEKTIKSA KQCPREPOVY TLPPSRDELT
 601 KNQVSLTCLV KGFPYPSDIAV EWSNGQPN NYKITTPLVLD SDGSFFFLYSK LIVDKSRWQQ
 661 KNVFSCEVMH EALIENHYTKQ SLSLSPKGKG GGSGGGGGGGK THTCPCPAP
 15 721 ELAGGGSVFL FPPKPKDTLM ISRTPEVTC VVDVSHEDPVE VKFNWYVWDGV EVHNIAKTKPR
 781 BEQYNSTYRV VSVLTVLHQD WLNGKEYRK VSNKALPAPI EKTIKSAKQG PREPQVYTLR
 841 FSRDELTKHQ VSLTCLVKGF YPSDIAVEWE SNGQEPENNYK TTPFVLDSDG SFFLYSKLT
 901 DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGR*

20 DNA sequence for FIX-068
 1 ATGCAGCGCC TGAAACATGAT CATGGCAGAA TCACCAAGGCC TCATCACCAT CTGCCTTTTA
 61 CGATATCTAC TCAGTGCCTGA ATGTACAGGT TTGTTCTCTT TTTTAAAATA CATTGAGTAT
 121 GCTTGCCTIT TAGATATAGA AATATCTGAT GCTGCTTCTC TCACTAAATT TIGATTACAT
 181 GATTTGACAG CAATATTGAA GAGTCATAACG GCCACGCCAGC AGGTGTTGIIA GTACTGTGGG
 241 AACATCACACG ATTTGGCTC CATGCCCTAA AGAGAAATTG GCTTTCAGAT TATTIGGATT
 301 AAAAACAAAG ACTTTCTTAA AGAGATGTTA ATTTCATGATG TGTTCCTCTT TTTGCTAAAA
 361 CTAAGAAATT ATTCTTTTAC ATTTCAGTTT TTCTTGATCA TGAAAACGCC AACAAAATTC
 421 TGAATCGGCC AAAGAGGTAT AATTCAAGTA AATTGGAAGA GTTGTGTTCAA GGGAAATCTAG
 481 AGAGAGAAATG TATGGAAGAA AAGTGTAGT TTGAAGAAGC AGAGAAAGTT TTGAAAACAA
 541 CTGAAAGAAC AACTGAAATT TGGAAGGACT ATGTTGATGG AGATCAGTGT GAGTCACATC
 601 CATCTTTAAA TGGGGCAGT TCCAAGGATG ACATAATTTC CTATGAATGT TGGTGTCCCT
 661 TGGGATTGTA AGGAAAGAAC TGTGAAATTAG ATGTAACATG TAACATTAG AATEGGCAGAT
 721 CGCAGGACTT TTGTTAAAAAT ACTGCTGATCA AACAAGCTGT TTGCTCCCTGT ACTGAGGGAT
 781 ATCGACTTTCG AGAAAACCAAG AGTCTCTG AACAGCAGT GCAATTTCGA TGTGAAAGAG
 841 TTTCCTGTTTC ACACACTCTC AAGCTCACCC GTGCTGAGAC IGTITTTCCF GATGTTGACT
 901 ATGTAATTC TACTGAAGCTT GAAACCAATT TGGTAACAT CACTCAAAGC ACCCAATCAT
 961 TTAATGACTT CACTGGGGTT GTTGGTTGAGA AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
 1021 AGGTTGTTT GAATGGTAAA GTTGATGCT TCTGTGGAGG CTCTATCGTT AATGAAAAT
 1081 GGATTGTAAC TGTGCCCCAC TGTGTTGAACT CTGTTGTTAA AATTACAGTT GTGCAAGGTG
 1141 AACATATAAT TGAGGAGACA GAACATACAG ACAGCAAGGC AATATGTGATT CGAATTATTC
 1201 CTCACCAACAA CTCAATGCA GCTTTAAATA AGTACACCA TGACATTGCCC CTTCTNGAAC
 1261 TGGACGGAACC CTTAGTGCCTA AACAGCTACTG TTACACCTAT TTGCTATTGCT GACAAGGAAT
 1321 ACACGAAACAT CTCCTCTCAA TTGGGATCTG GCTGTTGTAAG TGGCTGGGGAA AGAGTCCTTC
 1381 ACAABGGAG ATCAAGCTTA GTTCTCTACT ACCTTCAAGT TCCACTTGT GACCGAGCCA
 1441 CATCTCTTCG ATCTACAAAC TTCACCAACT ATAACAAACAT CTTCCTGCTC CCCTTCATC
 1501 AAGGAGGTAG AGATTCTACTG CAAGGAGATA GTGGGGGACCC CAATGTTACT GAAGTTCGAAG
 1561 GGACCACTT CTAAACTGGA ATTATTAGCT GGGGTGAAGA CTGTGCAATG AAAGGCAAAAT
 1621 ATGGAATATA TACCAAGGTG TCCCCGTATG TCAACTGGAT FAAGGAAAAA ACAAAAGCTA
 1681 CTGACAAAAC TCACACATGC CCACCGTGC CAGCTCCGGA ACTCTCTGGC GGACCGTCAG
 1741 TCTTCTCTT CCCCCCAAA CCAAGGAGCA CCCTCATGAT CTCCCGGACCC CTCAGGGTCA
 1801 CATGCGCTGGI GTGCGACCTG AGCCACAGAG ACCCTTCAAGT CAAGTTCAAC TGGTACCTGG
 1861 ACGGCCTGGA GGTCATAAT GCCAAGACAA AGCCCGGGGA GGAGCAGTAC AACACGACGT
 1921 ACCGTGTGCTG CAGCGTCTC ACCGTCTGTC ACCAGGACTG GCTGAATGGC AAGGAGTACA
 1981 AGTGCAGGT CTCCAACCAA GCGCCCTCCAG CCCCCCATCGA GAAAACCATC TCCAAGGCCA
 2041 AAGGGCAGCC CGGAGAACCA CAGGTGTACA CCCTCTCCCCC ATCCCGGGAT GAGCTGACCA
 2101 AGAACCAAGGI CAGCTGACCC TGCCCTGGCA AAGGCTCTCA TCCCTGGAC ATCGCCGTGG
 2161 AGTGGGAGAG CAATGGGAG CCGGAGAACCA ACTAACAGAC CACGGCTCTCC GIGTTGGACT
 2221 CGGACGCGTC CTCTCTCTC TACACGAAACG TCACCGTCA CGAGAGCAGG TGGCAGCAGG
 2281 GGAACGCTT CTATGCTCTG GTGATGCTGAG AGGCTCTGCA CAAACACTA ACGCAGAAGA
 2341 GCTCTCTCTT GTCTCCGGT AAACGGCGCC GCGGGAGCGG TGGGGCGGA TCAGGTGGGG
 2401 GTGGGATCAGG CGCTGGGAGGT TCCGGTGGCGG GGGGATCCGG CGGTGGGAGGT TCCGGTGGGG
 2461 GTGGGATCAAG GAAGAGGAGG AAGAGGGCGC AGGIGCAGCTG CGAGGAGTCI GGGGGAGGGCI
 2521 TGGTACAGCC TGGGGGGTCC CTGAGACTCT CCTGTCGAGC CTCTGGATTC ATGTTTACCA
 2581 GGTATGCCAT GAGCTGGCTC CGCCAGGCTC CAGGGAGGGG GCGAGAGTGG GTCTCAGGTA
 2641 TTAGTGGTAGT ACATACATACG CAGACTCCGT GAAGGGCCGG TICACCGTCT
 2701 CCAGAACAAAT TTCAAGAACG ACGCTGTATG TGCAATGAA CAGCCTGAGA GCGCAGGACA
 2761 CGGCTGTATA TTACTGCGCC CCGGGCGCCA CCTACACCAG CGGAGCGAC GTGCCCGACCC
 2821 AGACCACTG CGACTACTGG GCGGAGGAAAC CCCTGCTCAC CGCTCTCTCA GGGAGCTCAT
 2881 CGGCCCCAAA GCTTGAAGATA GTTGAATCTT CAGAGCAGC CGTATCTGAA CTGACTCAGG
 2941 ACCCTGCTGTG GCTGTGGGGT TTGGGACAGA CAGTCAGGAT CACATGCGAA GGAGACAGCC
 3001 TCAGAAACTT TTATGCAAGC TGCTTACCAAC AGAACCCAGG ACAGGCCCCCT ACTCTCTCTA
 3061 TCTATGCTT AACTAAAAGG CCGTCAGGGA TCCCGACCC ATTCTCTGCC TCCAGCTCAG
 3121 GAAACACAGC TTCTCTGACCC ATCACTGGG CTCAGCGGGA AGATGAGGCT GACTTAATCT
 3181 GCTCTGCTGA CTACGGCGGC GGCCAGCAGG GCGTGTCTGG CGCGGGCACC AAGCTGACCG
 3241 TCCCTACGCTA GCCCAAGGCT GCCCCCTCGG TCACTCTGTT CCGGCCCCCT TCTGCGGCCG
 3301 GTGGGCGGTGG CTCCGGCGGA GGTGGGCTCCG GTGGGGCGGG ATCAGGTGGG GGTGGGATCAG
 3361 CGGGTGGAGG TTCCGGTGGC GGGGGATCAG ACAAAACTCA CACATGCCCA CGTGGCCAG
 3421 CACCGGAACCT CCTGGGCGGA CCGTCAGCTC TTCTCTCTCC CCGAAAACCC AAGGACACCC
 3481 TCATGATCTC CGGGACCCCT GAGGTCACT GCGTGTGGGGT CGACGTGAGC CACGAAGACC
 3541 CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC
 3601 CGGGGGAGGA GCAGTACAAAC AGCACGTACG GTGTGGTCAG CGTCCTCACCC GTCCCTGACCC
 3661 AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCGACCC

5	3721	CCATCGAGAA	AACCATCTCC	AAAGCCAAAG	GGCAGCCCCG	AGAACCCACAG	GTGTACACCC
	3791	TGCCCCATC	CCCGATGAG	CTGACCAAGA	ACCAGGTCAG	CCTGACCTGC	CTGGTCAAAG
	3841	GCTTCTATCC	CAGCGACATC	GGCGTGGACT	GGGAGAGCAA	GGGGCAGCCG	GAGAACAACT
	3901	ACAAGACCAC	GGCTCCCGTG	TTGGACTCTCG	ACGGCTCTCTT	CTTCCTCTAC	AGCAAGCTCA
	3961	CGGTGGCAAA	GAGCAGGTGG	CAGCAGGGGA	ACGTCTTCCT	ATGCTCCGTC	ATGCATGAGG
10	4021	CTCTGCCACAA	CCACTACACG	CACAAAGACCC	TCTCCCTGTC	TCCGGTAAAG	TGA

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

1	MQRVNIMIMAE	SPGLLITICLL	GYLLSAECTY	FLDHENANKL	LNRPKREYNSC	KLEEFVQGNL
61	ERECMEEKCS	FEEAREVPEN	TERITTEFWKO	YVDGVSOCESN	PCLNCGSKCD	DINSYECWCP
121	FGPEGKNC	DVICINIKNGR	CEOFCKINSAD	NKVVCSCTEG	YRLAENQKSC	EPAVPFICSC
181	VSVSQTSKLT	RAETTVEFDVD	VYNSTEATTI	LDNTTOSQTS	FNDFTTVVGC	EDAKPGQFPW
241	QVVLNGKVA	FCGGGSIUNEK	WIVTAANCVE	TGVKITVVAG	BNMIEETEHT	EQKRNVRILL
301	PHNHYNAAIN	KYHNDIALLA	LDEFELVLSN	VTPPICIADKE	YTNIFLFKFG	GYVSQGNLFG
361	HKGRSALNVL	YLRLRVLVDRD	TCLRSLSTKFTI	YNNHMPMCAGFH	GGHGRDSCQGD	SGGGHVTEVEV
421	GTSFLTGIIS	WGFEFCAMKGK	YGIYTKVSRV	VNWIKEKTKL	TDKTHTCPPC	PAPELLGGPS
481	VFLFPKPKD	TLMISRTPEV	TCVVVDEVSHE	DPEVKEPNWYV	LGVEVENAKT	KPREEQYNSC
541	YRVVSLLTIVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KQGPREPQVY	TLPPSRELDAT
601	KNQVSLTCLV	KGFPYPSDIAV	WEWSNGQPN	TLPPSRDELT	SDGSFFLYSK	LTVDKSRWQQ
661	GNVFSCSVHM	EALHNHYTQK	SLSLSPGKRR	ERSGGGGGGG	GGGGGGGGGG	GGGGGGGGGG
721	GGSRKRKRRA	VQLQESCGGG	LVQPGGSLRL	SCAASGMFMS	RYAMSVWRQ	PGKGPWEWVG
781	ISGGGGSTYY	ADSVKGRPTV	SDNSKNTLY	LQMNLSRDAE	TAIVYCCARGA	TYTTSRSDVPD
841	QTSFDYWGQG	TLTVTSSGSA	SAFKLEE3EF	SEARVSELTQ	DPAVSCVALQG	TVRITCQGDG
901	LRNRYASWYQ	QKFCQPAFTLV	TYGLLSKRPSC	IDFDRPEACES	GNTASLITIG	AQADEADYV
961	CLLYVGGCQ	GVFGGGTKL	VLRQKKAAPS	VTLLPPSSA	GGGGGGGGGG	GGGGGGGGGG
1021	GGGGGGGGGS	DKHTTCPCP	APELLGGPSV	FLFFFKKFDT	LMISRTPEVY	CVVVDVSHED
1081	PEVKFNWYVD	GVEVHNARTK	PREEQYNSTY	RVVSLLTIVLH	QDWLNGKEYQ	CKVSNKALPA
1141	PIEKTIKAK	GCPREPOVYT	LPPSSRDELT	NQVSLTCLVK	GFYPSDIAVE	WESNGQPNEN
1201	YKTPVVLDS	DGSFFLYSKL	TVDKTSRWWQG	TSVFSCSVME	ALHNHYTOKS	LSSLSPGK*

DNA sequence for FIY-068									
40	1	ATGCAGCGCG	TGACATGAT	CATGGCAGAA	TCACCCAGGCC	TCATCACCCT	CIGCCCTTTA		
	61	GGATACTAC	TCAGTGTGA	ATGTACAGGT	TTGTTTCCTT	TITTTAAAATA	CATTGAGTAT		
	121	GCTGCTCTT	TAGATAATAGA	ATATCTGAT	GCTGCTCTT	TCACIAATTAT	TIGGATACAT		
	181	GATTGACAG	CAATATTGAA	GAGTCTAACCA	GCCAGCACGC	AGGTGGTAA	GTACTGTGGG		
	241	AAACATCACAG	ATTTTGGCTC	CATGCCCTTA	AGAGAAAATG	GCTTCTCAGAT	TATTGGAATT		
	301	AAAAACAAAG	ACTTTCTTAA	GAGATGTTAA	ATTTTCATGA	TGTTTTCTTT	TITGCTAAAAA		
	361	CTAAAGATT	ATTCTTITAC	ATTCAGTTT	TTCTGATCA	TGAAAACGCC	AACAAATTC		
	421	TGAATGGCC	AAAGAGGTAT	AATTTCAGGT	AATTGGAAGA	GTTTGGTCAA	GGGAATCTAG		
	481	AGACAGAATG	TATGGAAGAA	AACTGTTAAT	TTGAAAGAGC	ACGAGAACGTT	TGGAAACACA		
	541	CTGAAAGAC	AACCTGAAATT	TGCAAGGACT	ATGTCATGAT	AGATCAGTGT	TGCTGAACTC		
50	601	CATGTTTAA	TGGGGCGAGT	TGCAAGGATG	ACATTAATTC	CITATGAATGT	TGCTGTCCT		
	661	TGGATTTGA	AGGAAACAAAC	TGTGAAATTAG	ATGTAACATG	AAACATTAAG	AATGGCAGAT		
	721	GGAGACGGTT	TGTGAAATTAAT	ATGTCATGATA	AACTGGGT	TTGCTCTCTG	ACTGAGGGAT		
	781	ATGCGATTCG	AGGAAACCCAG	AACTGCTGTG	AAACCGAGCT	GGCAATTCTCA	TGTTGAGAG		
	841	TTCTGTGTT	ACAAACTTCT	AAGCTCACCC	GTGCTGAGAC	TGTTTTCTCT	GATGTGGACT		
	901	ATGTAATTTC	TACTGAAAGCT	GAACCATTT	TGGATAACATC	CACCTAAACG	ACCCAATCAT		
	961	TTATGACTT	CACTGGGGT	GTGGGTGGAG	AAAGATGCCAA	ACCAAGGTCAA	TTCCCTTGGG		
	1021	AGGGTTTTT	GAATGGTAAA	GTGATGCTAT	TCTGTGGAGG	CITCATCTGTT	AATGAAAAT		
	1081	GGATTGTAAC	TGCTGCCAAC	TCTGTTGAAA	CTGGGCTTAA	AAATTACAGTT	GTGCGCAGCTG		
	1141	AAACATAATAT	TGAGGGAGACA	GAACATACAG	AGCAAAAGCG	AAATGTGATT	CGAATTATT		
	1201	CTCACACABA	CTAACATGCA	GCTTAAATA	AGTACAAACCA	TGACATTGCG	CTCTCTGAAAC		
	1261	TGGACGAACC	CTTAGTGCTA	AAACAGCTACG	TTACACCTAT	TTGCTATTGCT	GACAAGGAAT		
	1321	ACAGAAACAT	CTTCCTCAA	TTTGGATCTG	GCTATGTAAG	TTGGCTGGGG	AGAGTCCTTCC		
	1381	ACAAAGGGAC	ATCAGCTTTA	GTCTCTCTAG	ACCTTGTAGT	TGCACTTGT	GACCAGGCCA		
	1441	CATGTCCTCG	ATCCTACAAAG	TTACACATCT	AAACAAACAT	GTTCCTGTCT	GGCTTCTCATG		
	1501	AAGGAGGTAG	AGATTCTATG	CAAGGAGATA	GTGGGGGACC	CCATGTTACT	GAAGTGGAAAG		
	1561	GGGACCGTTT	CTTAATGTTA	ATTATTAGCT	GGGGTGAAACA	GTGTCATCAAT	AAAGGCAAAAT		
	1621	ATGGAATAAT	TTAACAGGTG	TCCCCGTATG	TCAACTGCTG	TTAGGAAAAAA	ACAAAGCTCT		
	1681	CTGACAAAAC	TCACACATGC	CCACCCCTGCC	CAGCTCCCGA	ACTCTGGGG	GGACCCGTCA		
	1741	TCTTCTCTT	CCCCCCAAAAA	CCCAAGGACCA	CCCTCTAGAT	CTCCCCGGACC	CCTGAGGTCA		
	1801	CATGCGTGGT	GGTGGACGCTG	AGCCACGAAG	ACCTCTGAGGT	CAAGTTCAC	TGGTACCTGGT		
	1861	ACGGCGTGG	GGTCATTAAT	GGCAACAGACA	AGCCCGGGGA	GGAGCAGTAC	AAACAGCACGT		
	1921	ACCGTGTGGT	CAGCGTCTCTC	ACCGTCCTGC	ACCAAGACTG	GCTGAATGGC	AAGGAGTACA		
	1981	AGTGGCAAGGT	CTCCAACAAA	GGCCCTCCCG	CCCCCATCGA	GAACAAACCT	TCCAAAGGCCA		
	2041	AAGGGCAGCC	CGAGAACACA	CAGGTGTACA	CCCTGCCCC	ATCCCCGGAT	GAGCTGACCA		
	2101	AGAACAGGGT	CAGCTGTGACC	TGGCTGGTCA	AAAGGCTCTA	TCCCACTGAC	ATGCCCTGTGG		
	2161	AGTGGGAGAG	CAATGGGCA	CCGGAGAAC	ACTACAAAGAC	CAAGGCTCTCC	GTGTTGGAAT		
	2221	CCGACGGCTC	CTCTCTCTC	TACAGCAAGC	TCACCGTGGCA	CAAGAGCAGG	TGGCAGCAGG		
	2281	GGAAACGCTT	CTCATGTC	GTGATGCTG	AGGCTCTGCA	AAACACTACT	ACGGCAAGAGA		
	2341	GCCTCTCCCT	GTCTCCGGGT	AAAGGTGGG	GGGGATCAGG	TGGGGGTGGA	TCAGGGGGT		
	2401	GAGGTTCCGG	TGGCGGGGGG	TCAGACAACTA	CTCACACATG	CCCCACCGT	CCAGCACCTG		
	2461	AACTCTCGG	AGGACGCC	GTCTCTCT	TCCTCCCCAA	ACCCAAAGGC	ACCCCTCATG		
	2521	TCTCCCGGG	CCCTGGAGGT	ACATGCGTGG	TGGTGGACAGT	GAGCCACGAA	GACCCCTGAGG		

5 2581 TCAAGATCAA CTGCTACGTG GACGGCGTGG AGGTCGATAA IGCAGAGACA AAGCCCGGGG
 2641 AGGAGCAGTA CAACAGCAGC TACCGTGTGG TCACCGCTCT CACCGTCTG CACCAAGGACT
 2701 GGCTGAATGG CAACGGAGTAC AAGTGCAGG TCTCCACAA AGCCCTCCCA GCCCTCATCG
 2761 AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAAC ACAGGTGTAC ACCCTGCC
 2821 CATCCCGGA TGAGCTGACC AAGAACCAGG TCACCGTGCAC CTGCGCTGGTAAAGGCTTCT
 10 2881 ATCCCCAGGA CATCCCGGTG GAGTGCAGA GCANTGGCA ECAGGAGAAC AACTACAAGA
 2941 CCACGGCTCC CGTGTGAC TCCGACCGCT CCTCTCTCCCT CTACAGGACG CTCACCGTCC
 3001 ACAAGAGCAG GTGGCAGCAG GGGAACGCTCT TCTCTGCTC CGTGTGACAT GAGGCTCTGC
 3061 ACAACCACTA CACCGAGAAC AGCCTCTCC TGTGCTCGGG TAAAGGCTGGC GGTGGCTCCG
 3121 GGGGAGSTGG GTCCGGTGGC GGCGGATGAG GTGCGGGTGG ATCAGGGCGGT GGAGGTTCCG
 15 3181 GTGGCGGGGG ATCAGCGCAG GTGCAGCTGC AGGAGTCTGG EGGAGGCTTG GTACAGGCTG
 3241 GGGGGTCCCT GAGACTCTCC TGTGCGACCT CTGGATTCTAT GTTACAGGAG TATGCCATGA
 3301 GCTGGGTGGC CCAGGCTCCA GGGAAAGGGC CAGAGTGGGCTCAGGTTAGTGGTATG
 3361 GTGGTACTAC ATACTACGCA GACTCCCTGCA AGGGCCGGT CACCGTCTCC AGAGACATT
 3421 CCAAGAACAC GCTGTATCTG CAAATGAACA GCCTGAGAGC CGAGGAGAAC GCTGTATATT
 20 3481 ACTCGCGCCG GGGGCCAAC TACACAGACCG GGAGGAGACG GCGCGACCG ACCAGCTTCG
 3541 ACTACTGGG CCACGGGAACT CTGGTCACCG TCTCTCAGG SAGTCATCC GCCCCAAAGC
 3601 TTGAAGAAGGG TGAATTTCA GAAGACCCG TATCTGAACCT GACTCAGGAC CTCCTGTGT
 3661 CIGTGGCTT GGGACAGACA GTCAAGGATCA CATGCCAAGG AGACAGGCCTC AGAAACTTTT
 25 3721 ATGCAAGCTG GTACCGAGCAG AAGCCAGGAC AGGCCCTCTAG TCTCTGCTC TATGGTTAA
 3781 GTAAAAGGCC CTCAGGGATC CGAGACCGAT TCTCTGCTC CAGCTCAGGA AACACAGCTT
 3841 CCTTGACCAT CACTGGGCT CAGGCCGAAG ATGAGGCTGA CTATTACTGC CTGCTGTACT
 3901 ACGGCGCCGG CCAGCAGGGC GTGTTCGCCG GCGGGACCAA GCTGACCGTC CTACGTCAGC
 3961 CCAAGGCTGC CCCCTGGTC ACTCTGTTCC CGCCCTCTTC 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 SC5 is in bold
 1 MQRVNMIMMAESPGLITICLLE GYLLSABCTV FLDHENANKL LNEPKRYNSG KLEEFVQGNL
 61 ERECMEERKCS FEEAREVPEN TERITEFWKQ YVDGDQCESN PCLINGGSCKD DINSYBCWCWP
 121 PGFEGMEKNCLE DVICNIKNNG CEOFICINSAD NKVYCSCTEG YRLAENOKSC EPAPVPPFCGR
 181 VSVSQTSLT RAEFTFEDVD YVNSTRAETI LDNITQS FNDFTRVVGG EDAKPGQFPW
 241 QVVLNGRVDA FCGGSIVNEK WIVTAANCVE TGVKITVVAG EHNIETEHT EQKRNVIRII
 301 PHHNRYNAIN KYNHDIALL LDEFLVILNSY VTPICIAKDE VTNIFLKFGS GYVSGWGRVFE
 361 HKGRSALVHQ YLRVPLVDR A TCLRSTKPTI YNNMPCAGFHK EGGRDSCQGD SGGPHVTEVE
 40 421 GTSFLIGIIS WGEBCAMKGK YGIYTKVSRV VNWIKEKTKL DOKTHTCPPC PAPELGGPS
 481 VFLFPPPKD TLMISRTPEV TCVVVIVSHE DPEVKPNWYV DGVEVENAKT KPREEQYNST
 541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKITSKA KGQPREPVY TLPFPRDELIT
 601 KNQVSLTCLV KGFYPSDIAV EWNSNGQEPN NYKTTPVLD SDGSFFLYSK LTVDKSRWQQ
 661 CNVFSCSVVMH EALHNHYTQK SLSLSPCRGG CGSOGGGSCG CGSOGGGSDK THTCPFCPAP
 721 ELLGGPSVFL FPPKPKDTLM ISRTPEVITV VVDVSHEDPE VKFIVWYVDCV EVHNAKTKP
 781 BEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKANGQ PREPQVYTL
 841 PSRDELTKNQ VSLTCLVKGF YPSDIAVREWE SNGQEPENYY TTPFVLDSDG SFFLVSKLTV
 901 DKSRWQQNV FSCSVMHEAL RNHYTQKSL S LSPCKGGGGS GGGGSGGGGS GGGGSGGGGS
 961 GGGGSAQVQL QESGGGLVQP GGSLRISCAA SGFMSRYAM SWVQAPKGK PEWVEGICSGS
 1021 GGSTYADSV KGRFTVERDN SKNTLYLQMN SLRAEDTAVY YCARGATYTS RSDVPDQTSF
 1081 DYWGQGTIVT VSEGSASAPK LEEGEFSEAR VSELQTDPAV SVALGQTVRI TCQGDLSLRNF
 1141 YASWYQQKPG QAPTLVIYGL SKRPSGIPDR FSASSSGNTA SLITINGAQAB DEADYYCLLY
 1201 YGGGQQGVFG GGTKLTVLRQ FKAAPSVTLP PESSAA*

55 DNA sequence for FIX-069
 1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAAGGCC ICACTACCAT CIGCCTTTTA
 61 CGATATCTAC TCACTGCTGA ATGTACAGT TTGTTTCCTT TTTAAATAA CATTGAGTAT
 121 GCTTGCCCTT TAGATATAGA AATATCTGAT GCTGTCTCTC ICACTAAAT TGTGATTACAT
 181 GATTGACAG CAATATTGAA GAGTCTAACCA GCGACGACAGC AGGGTGGTAA GTACTGTGGG
 241 AACATCACAG ATTTGGCTC CATGCCCTAA AGAGAAATTG CTGTTTCAGAT TATTGGAATT
 301 AAAAACAAAG ACTTCTTAAAGAGATGTTAA ATTTCATGA IGTTTCTTIT TITGCTAAAAA
 361 CTAAGAAATT ATTCTTTAAC ATTTCAGTTT TTCTTGTATCA TGAAAACGCC AACAAAATT
 421 TGAATCGGCC AAAGGAGTAA ATTTCAGGTA ATTTCAGGAA GTTGTGTTAA GGGAACTCTAG
 481 AGAGAGAATG TAIGGAAGAA AAGTGTAGTT TTGAAGAAGC AGGAGAAATT TITGAAAAACA
 541 CTGAAAGAAC AACTGAATTG TGGAAGGACT ATGTTGATGG AGATCAGTGT GAGTCACAT
 601 CATCTTAAA TCGCGCAGT TCCAAGGATG ACAITAATTIC CTATGAATGT TGGTGTCCCC
 661 TGGATTTGA AGGAAAGAAC TGTCAATGAT ATGTAACATG TAACATTAG AATGGCAGAT
 721 CGCGAGCAGT TTGTAAAAAT AGTGTGATGA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT
 781 ATCGACTTGC AGAAAACCAAG AGTCTCTGT AACCAAGCAGT GCACTTTCCA TGTGAAGAG
 841 TTTCTGTTTC ACAAACTCT AAGCTCACCC STGCTGAGAC TGTGTTTCCT GATGTGGACT
 901 ATGTAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
 961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
 1021 AGGTGTTTT GAATGGTAA GTTGATGCT TCTGTGGAGG CTCTATCGTT AATGAAAAT
 1081 GGATTGTAAC TGCTGCCAAC TGTGTTGAA CTGGTGTAA AATTACAGTT GTCGCAGGTG
 1141 AACATAATAT TGAGGAGACAG GAACATACAG AGCAAAAGCG AAATGTGATT CGAATTATTC
 1201 CTCACCAACAA CTACAAATGCA GCTATTAAATA AGTACACCA TGACATTGCC CTTCTGGAAC
 1261 TGGACGAACC CTTAGTGCCTA AACAGCTACG TTACACCTAT TTGCAATTGCT GACAAGGAAT
 1321 ACACGAACAT CTICCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGAGAGTCCTTCC
 1381 ACAAAAGGGAG ATCAGCTTAA GTCTCTTCAAGT ACCTTAGAGT TCCACTTGT GACCGAGCCA
 1441 CATGTCITCG ATCTACAAAG TTCACCATCT ATAACACAT GTTCTGTC GGCCTTCCATG
 1501 AGGGAGGTAG AGATTCAATG CAAGGAGATA GTGGGGACCC ATGTTACT GAAGTGGAG

5 1561 GGACCAGTTT CTTAACTGGA ATTATTAACTT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
 1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA KCAAGCTCA
 1681 CTGACAAAC TCACACATGC CCACCGTGGC CAGCTCCGGA ACTCCCTGGGA GJACCGTCAG
 1741 TCTTCCTCTT CCCCCAAA CCAAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA
 1801 CATGGCTGTT GGTGGACGTG AGCCACCGAG ACCCTGAGGT CAAGTTAAC TGTTACGTGG
 10 1861 ACCGGCTGGA CGTCCATAAT GCCAAGACAA AGCCCGGGA CGACGAGTAC AACAGCACGT
 1921 ACCGGCTGCT CAGCGTCCCTC ACCGTCCCTC ACCAGGACTG CTGAAATGCC AAGGACTACA
 1981 AGTGCAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA
 2041 AAGGGCAGCC CGAGAACCA CAGGTGTACA CCCTCCGGAT GAGCTGACCA
 2101 AGAACCCAGT CAGCCTGACC TGCCCTGGCA AAGGCTTCTA TCCCAAGGAC ATGCCCGTGG
 15 2161 AGTGGGAGAG CAATGGGAG CCGGAGAAC ACTACAAGAC CAGGCTCCC GTGTTGGACI
 2221 CCGACGGCTC CTTCCTCTC TACAGCAAGC TCACCGTCCGA CAAGAGCAGG TGGCAGCAGG
 2281 GGAACGCTCTC CTCATGCTC GTGATGCTAG AGGCTCTGCA CAACCACTAC ACAGCAGAAGA
 2341 GCCTCTCCCT GTCTCCGGGT AAAGGCGCTG CGGTGTCAGG TGGAGGAGGG TCAGGCGGTG
 2401 GTGGATCCGG CGGGGGCGGA TCCGGTGGCG GAGGGTCAGG CGGTGGCGGA TCAGGCTGCA
 20 2461 CCGAGGGGGT GGCCTCTGAC AACCTGTGGC GTGGGGTGGG CTCCGGCGGA GGTGGGTCCG
 2521 GTGGCGCGCG ATCAGGTGGC CGTCCGATCAG CGGTGGAGG TTCCCGTGGC GGGGGATCCG
 2581 ACAAACCTCA CACATGCCA CGCGGCCAG CACCGAACT CCTGGGGCGGA CGGTGAGTCT
 2641 TCCCTCTTCCC CCCCCAACCC AAGGACACCC TCATGATCTC CGGACCCCT GAGGTACACAT
 2701 CGTGTGGTGGT GGACGTGAGC CACGAGAACCC CTGAGGTCAA GTTCAACTGG TACGTGGACG
 25 2761 CGGTGGAGGT GCATAATGCC AAAGAACAAAGC CGCGGAGGAGA CGAGTACAC ACAGCAGTAC
 2821 GTGTGGTCAG CGTCCTCACCC GTCTGCAACC AGGACTGGCT AAATGGCAAG GAGTACAAGT
 2881 GCAAGGTCTC CAACAAAGCC CTCCCAGGCC CCATGAGAA ACCATCTCC AAAGGCAAAG
 2941 GGCAGCCCG AGAACCCACAG GTGTACACCC TGCCCCATC CGGGATGAG CTGACCAAGA
 3001 ACCAGGTCTG CCTGACCTGC CTGGTCAGAAG GCTTCTATCC CAGCGACATC GCGTGGAGT
 3061 GGGAGACAA TGGGAGCCCG GAGAACAACT ACRAACACCAC GCCTCCCGTG TTGGACTCCG
 3121 ACGGCTCTC CTTCCTCTAC AGCAACCTCA CGTGTGGACAA CAGCAGGTGG CAGCAGGGGA
 3181 ACCTCTCTC ATGCTCCGTG ATGCTCATGAGG CTCTGACCAA CCACTACACG CAGAAGAGCC
 3241 TCTCCCTGTC TCCGGTAAA 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 MQRVNIMIMAE¹ SPGLITICL² .GYLLSAETV³ FLDHENANKL⁴ LNRPKRYNSG KLEEFVQGNL
 61 BRECMEEKCS⁵ FEEAREFVN⁶ TERITEFWQK⁷ YVDGQDCESN⁸ PCLINGGSCKD⁹ DINSYECWC¹⁰
 40 121 FGFEKGNC¹¹ DVTCKNIKNG¹² CEQFCKN¹³ NKVVC¹⁴ SCTEG¹⁵ YRLAENQKSC¹⁶ EPAVPPFCGR
 181 VSVSQTSKLT¹⁷ RAETVFPDVD¹⁸ YVNSTEATI¹⁹ LDNITQSTQS²⁰ FNDFTRVVGG²¹ EDAKPGQFPW
 241 QVVLNGKVDA²² FCGGSIV²³ VNEK²⁴ WIVTAANCVE²⁵ TGVKITTVVAG²⁶ EHNIEETHT²⁷ EQKPNVIRII
 301 PHRNNAIN²⁸ KYNHDIALLE²⁹ LDEPLVLSN³⁰ VTPICIAKDE³¹ VTNIFLKFGS³² GYVSGNGRVE
 361 HKGRSALV³³ LRLVPLVDR³⁴ TCLRSTKFTI³⁵ YNNMPCACPH³⁶ EGCQDSCQD³⁷ SSCPHTVTEVE
 421 GTSFLG³⁸ TIS³⁹ WGEBCAMKGK⁴⁰ YGIYTKVSR⁴¹ VNWIKEK⁴² TDKHTHTCP⁴³ PC PAPELLGGPS
 481 VFLFP⁴⁴ PPKPKD⁴⁵ TLMISRTPEV⁴⁶ CVVVVDVSH⁴⁷ DPEVKFNWYV⁴⁸ DGVEVHN⁴⁹ KPREEQYNST⁵⁰
 541 YRVVSVLTVL⁵¹ HQDWLN⁵² GKEYK⁵³ CKVSNKALPA⁵⁴ PIEKTISKAK⁵⁵ QPFRPQVY⁵⁶ LPPSRDEL⁵⁷
 601 KNQVSLTCLV⁵⁸ KGFYPSDI⁵⁹ EWESNGOPEN⁶⁰ NYKTTTPVLD⁶¹ SGSSFFLYSK⁶² LTVDKSRWQQ⁶³
 661 CNVFSCSVMH⁶⁴ EALHNHYTQK⁶⁵ SLSLSPGK⁶⁶ GG⁶⁷ SGGGGGGGG⁶⁸ GG⁶⁹ SGGGGGGGG⁷⁰ GG⁷¹ SGGGGGGGG⁷²
 721 TERMALRN⁷³ L⁷⁴ CGGGSCCGGS⁷⁵ CGGGSCGGGS⁷⁶ CGGGSCGGGS⁷⁷ CGGGSCGGGS⁷⁸ CGGGSCGGGS⁷⁹
 781 FLFP⁸⁰ PPKPKD⁸¹ LMISRTPEV⁸² CVVVVDVSH⁸³ DPEVKFNWYV⁸⁴ DGVEVHN⁸⁵ KPREEQYNST⁸⁶
 841 RVVSVLTVL⁸⁷ HQDWLN⁸⁸ GKEYK⁸⁹ CKVSNKALPA⁹⁰ PIEKTISKAK⁹¹ QPFRPQVY⁹² LPPSRDEL⁹³
 901 NQVSLTCLV⁹⁴ KGFYPSDI⁹⁵ WESNGQ⁹⁶ PENN⁹⁷ YKTTTPVLD⁹⁸ SGSSFFLYSK⁹⁹ LTVDKSRWQQ¹⁰⁰
 961 NVFSCSVMHE¹⁰¹ ALHNHYTQK¹⁰² SLSLSPGK¹⁰³*

55 DNA sequence for FIX-090
 1 ATGCACCGCG TCAACATGAT CATGGCAGAA TCACCAAGGCC TCATCACCAT CTGCCCTTTA
 61 GGATATCTAC TCAGTGTGA ATGTACAGGT TTGTTCTCTT TTAAATTAAT CATTGAGTAT
 121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTTCTCTT TCACATAATT TTGATTACAT
 181 GATTGACAG CAATATTGAA GAGTCACAA GCCCACACCC AGGTTGGTAA GTACITGTGGG
 241 AACATCACAG ATTTTGGCTC CATGCCCTAA AGAGAACATTG GCTTTCAGAT TATTGAGGATI
 301 AAAAACAAAG ACTTTCTTAA GAGATGTAA ATTTCATGA TGTTCCTCTT TTTCTAATAA
 361 CTAAAGATT ATTTCCTTAC ATTTCAGTTT TTCTTCATCA TGAAAACGCC AACAAATTG
 421 TGAATCGGCC AAAGAGGTAT ATTTCAGGTA ATTTCAGGTA ATTTCAGGTA ATTTCAGGTA
 481 AGAGAGATG TATGGAAGAA AAGTGTAGTT TTGAGAACAGC AGAGAACAGT TTTGAAACAA
 541 CTGAAAGAAC AACTGAATTG TGGAACAGT ATGTTGATGG AGATCAGTGT GAGTCACATC
 601 CATGTTTAAAG TGGCUCGAGT TCCAAAGATC ACATTAATTC CTATGAATTT TGGTGTCCCT
 661 TTGGATTGAG AGGAAGAAC TGTGAATTAG ATGTCATGAT TAACATTAAG ATGGCAGAT
 721 GCGAGGAGTT TTGTTAAATAT ATGTCATGATA ACAAGGTGGT TTGCTCTCTGT ACTGAGGGAT
 781 ATCGACATTCG AGAAAACAG AAGTCCCTGT AACCAACACT CCATTTCCA TGTGGAAGAC
 841 TTCTCTGTTTC ACAAAACTTCT AAGCTCACCC GTCTCTGAGAC TCTTTTCTT GTATGTCGACI
 901 AAGTAAATTC TACTGAAGCTT GAAACCAATTG TGGATTAACAT CACTCAAAGC ACCCAATCAT
 961 TTAATGACTT CACTGGGTT GTTGGTGGAG AAGATGCCA ACCAGGTCAA TTCCCTTGGC
 1021 AGGTTGTTTT GAATGGTAA GTTGTGATCAT TCTGTGGAGG CTCTATCGTT AATGAAAAT
 1081 GGATTGTAAC TGCTGCCAC TGTGTTGAAA CTGTTGTTAA AATTACAGTT GTGCGAGGTG
 1141 AACATATAAT TGAGGAGACA GAACATACAG AGCAAAAGCG AATGTTGATT CGAATTATTC
 1201 CTCACCACAA CTACAATGCA GCTATTAATA AGTACACCA TGACATTGCC CTCTGGAAC
 1261 TGGACGACCC CTIAGTGTCA AACAGCTACG TTACACCTAT TIGCATGCGI GACAAGGAAT
 1321 ACACGAACAT CTTCCTCAA TTGAGTGTAGC GCTATGTAAG IGGCTGGGGAA AGAGTCTTCC
 1381 ACACAGGGAG ATCAAGCTTA GTTCTTCAGT ACCTTACAGT TCCACTTGT GACCGAGGCC
 1441 CATGTCTCG ATCTACAAAG TTCACCATCT ATAACAAACAT GTTCTGTGCT GGCTTCCATG

5 1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCACTTACT GAAGTGGAAAG
 1561 GGACCAAGTTT CTTAACCTGGA ATTATTAAGCT GGCGTGAAGA GTGTGCAATG AAAGCCAAAT
 1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAARAGCTCA
 1681 CTGGTGGCGG TGCGCTCCGGC GGAGGTGGGT CGCGTGGCGG CGGATCAGGT GGGGGTGGAT
 1741 CAGCGGTGG AGGTTCGGT GGCGGGGGAT CAGGCCAGGT CGAGCTGCAAG GAGTCCTGGG
 1801 GAGGCCTGGT ACAGCCTGGG CGGTCCCTGA GACTCTCTG TGCGACCTCTG GCAATTCAATG
 1861 TTAGCAGCTA TGCCATGAGC TGGGTCCCCC AGGCCTCAGG GAAGGGGCA GAGTCCTCT
 1921 CAGGTATTAG TGGTAGTGGT GGTAGTACAT ACTACCGAGA CTCCGTGAAG GGCCGGTCA
 1981 CGGTCTCCAG AGACAATTC AAGAACACCC TGATCTGCA ATAGAACAGC CTEAGAGCCG
 2041 AGGACACGGC TGATATTAAC TGCGCCCGG GCGCCTACCA CACCAAGCCG AGCGAAGTGC
 2101 CCGACCAAGAC CAGCTTCGAC TACTGGGGC AGGGAACCTC GGTACCCGTC TCCCTAGGGG
 2161 GTGCATCCGC CCCAAAGCTT GAAGAAGGTG AATTTTCAAGA AGCACCGCTA TCTGAACCTGA
 2221 CTCAGGCCCG TGCTGTCTGG GTGGCCTTGG GACAGACAGT CAGGATCACA TGCCCAAGGAG
 2281 ACAGCCCTAG AAACCTTATG CCAAGCTCTT ACCACCGAA CGCAAGGACAG GCCCTACTC
 2341 TTGTCACTCA TGGTTTAAGT AAAAGGGCCT CAGGGATCCC AGACCGATTC TCTGCTCCA
 2401 GCTCAGGAAAC CACGCTTCC TTGACCATCA CTGGGCTCA GGCGGAAGAT GAGGCTGACT
 2461 ATTACTGCCT GCTGTACTAC GGCGGGGGCC ACCAGGGCCT GTTCCGGGGC GGCACCAAGC
 2521 TGACCGCTCT ACCTCAGGCC AAGGCTGCC CGCTCGTCAC TCTGTTCCCG CCCTCTCTG
 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 SC5 is underlined
 1 *MQRVNMIMAE SPGLITICLL CYLLSACTV PLDHNENAKL LNEPKRYNSG KLBEPVQGNL*
 61 ERECMEEKCS FEEAREVPEN TERTTEFWKQ YVDQDQCESN PCLINGGSCKD DINSYBCWCP
 30 121 FGFEKGKNCLE DVTCKNIKNGR CEFQCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPPFCGR
 181 VSVSQTSKLT RAETVFFDWD VNVSTEAETI LDNITQSTQF FNDFTRVVVG EDAKPQGFPPW
 241 QVVLNGKVDA FCQGSIVNEK WIVTAANICV TGVKITVVAE EINIEETHT EQKRNVIRII
 301 PHENYNNAIN KYNHDIALLE LDEPLVLNSV VTPICIADE YTNIFLRFGS GYVSGWGRVP
 361 HKGRSAALNVLQ YLRVPLVDR A TCRSLRSTFTI YNNMPCAGFH EGGRDSCQGD SGGPHVTEVE
 421 CGFLTIGIIS WGEBCAMIKGK YGIYTKVSRV VNAGKEKTL TGGGGSGGGG SGGGGGGGGG
 481 SGGGGSGGGG SAQVOLQESG GGLVQPGGSL RLSCASGFM PSRYAMSNSR QAPKGPEWV
 541 SGISGSGGST YYADSVKGRF TVSRDNRNLT LYLQMSNLSA EDTAVYYCAR GATYTSRSDV
 601 PDQTSFDYWG QGTLVTVSSG SASAPKLEEG EFSEARVSEL TDPAVSVL GQTVRITCQG
 661 DSLRNFYASW YQQKPGQAPT LVIYGLSKRP SGIPDRFSAS SSGNTASLTI TGAQAEDEAD
 721 YYCLLYYGGG QQGVFGGGT LTVLRQPKAA PSVTLFPPSS AA*

DNA sequence for FVII-088
 45 1 ATGGTCTCCC AGCCCTCAG CCTCCCTCTG CTTCTCTTC CGCTTCACGG CTCCTCGCT
 61 CGAGTCCTTCG TAACCCAGGA CGAAGGCCAC CGGCCTCTCG ACCGGCGGCC GCGGCCCAAC
 121 CGCTTCTCTGG AGGAGCTCTG CGCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCCTCCAGG GATCTTCAGG GACGGGAGA CGACGAAGCT GTTCTGATT
 241 TCTTACACTG ATGGGACCA GTGTGCTCTA AGTCCATGCC AGAATGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCTTATAT CTGCTCTTCG CTCCCTCGCT TCGAGGGCCG GAACCTCTGAG
 361 ACCGCCAACAG ATGACCAAGCT GATCTGTCTG AAGCGAACG CGCGCTCTGA CGACTACTGC
 421 AGTGACCAACA CGGGCACCAA CGCGCTCTGT CGGTCACAGC AGGGGTACTC TCTCTTGGCA
 481 GACGGGGTGT CCTGACACACC CACAGTTGA TATCCATGTG GAAAATACCC TATTCTAGAA
 541 AAAAGGAATAG CGACGAAACCC CAAAGGCCGA ATTGTGGGG CGAAGGTGTG CCCCCAAGGG
 601 GAGTGTCTAT CGCGAGGTCT GTTGTGGTG ATGAGCTC ACTTGTGTG GGGGACCTG
 661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTCTG ACAAAATCRA GAACTGGAGG
 721 ACCCTGATCG CGGTCTCTGG CGAGCAGCAC CTCACCGAGC ACGACGGGG TGAGCAGAGC
 781 CGCGCGCTGG CGCGAGTCAT CACCCCCAGC ACGTACGTCT CGGCACCCAC CAACCACGAC
 841 ATCGCGCTTC TCCCGCTGCA CGAGCCCTGT GTCTCTACTG ACCATGTGGT GCCCCTCTGC
 901 CTGCCCGAAC GGACGTTCTC TGAGAGGAGC CTGGCTCTCG TGGCTCTC ATTGGTCAGC
 961 GGCTGGGGAC AGCTGCTGGA CGCTGGGSCC CGCCGGCTGG AGCTCATGGT CTCACAGTG
 1021 CGCCGGCTGAG TGACCCAGGA CTGGCTCTGAG CAGTCAGGA AGGTGGGAGA CTCCTTAAAT
 1081 ATCACCGAGT ACATGTTCTG TCGCGGTACTC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
 1141 GACAGTGGAG GCCCCACATC CACCCACTAC CGGGGACAGT CGTACCTGAC GGGCATCGTC
 1201 AGCTGGGGAC AGGGCTGCGC AACGCTGGGC CACTTGGGG TGACACCCAG GGTCTCCAG
 1261 TACATCGAGT CGCTGCAAAAC GCTCATGGC TCAGAGGCC CGCCAGGAGT CTCCTGCGA
 1321 GCCCCATTTC CGCGTGGCGG TGGCTCCGGC GGAGGTGGGT CGGTGGCGG CGGATCAGGI
 1381 GGGGGTGGAT CAGGGGGTGG AGGTCTCTGGT CGGGGGGGAT CGACAAACAC TCACACATGC
 1441 CCACCGTGCC CAGCTCCGGG ACTCTGGGA GGACCGTCAG TCTCTCTT CCCCCCAAAA
 1501 CCCAAGGACA CCTCTCATGAT CTCCCGGAC CCTGAGGTCA CATGCGTGTG GGTGACGCTG
 1561 AGCCACGAAG ACCCTGAGGT CAACTTCACAC TGTTACCTGG AGGGCGTGGA GTGCTATAAT
 1621 CGCAAGACAA AGCGCGGGGA GGAGCAAGCT AACACCCACGT ACCTGTGGGT CAGCGCTCTC
 1681 ACCGTCTCTG ACCAGGACTG GCTGAATGGC AAGAGTACA AGTGCAAGGT CTCCAACAAA
 1741 GCCCCCTCAG CCCCCATCGA GAAAACATC TCCAAGCCA AAGGGCAGCC CCGAGAACCA
 1801 CAGGTGTACA CCTCTGGGGC ATCCCCGGGT GAGCTGACCA AGAACCCAGGT CAGCGTGGAC
 1861 TGCGCTGGTCA AAGGCTTCTA TCCCGAGGAC ATCGCGTGG AGTGGGAGAG CAATGGGCGAG
 1921 CGGGAGGACAA ACTACAAGAC CACGCTCTCC GTGTTGGACT CGGACGGCTC CTCTCTCTC
 1981 TACAGCAAGC TCACCGTCGA CAAGACAGG TGCGACGAGG GGAACGTCTT CTCTGCTCC
 2041 GTGATGCTATG AGGCTCTGCA CAAACCAACT ACACGAGAAGA CCTCTCTCC GTCCTCCGGT
 2101 AAACGGCGCC GCGGGAGGGG TGGCGGGCGGA TCAGGTGGGG GTGGATCAGG CGGTGGAGGT
 2161 TCCGGTGGCG GGGGATCCCC CGGTGGAGGT TCCCGTGGGG GTGGATCAGG GAAGAGGAGG
 2221 AAGAGGACAA TCGTGTGAC CCAGGCCGCC CCCACCGTC CGCTGACCCCC CGGCAGAGG

5 2281 CTGAGGCATCA GC1GCCGGAG CAGCCGGAGC CT3CTGCACA GCAACGGCAA CACCTAACCTG
 2341 TGCTGGITOC TGCAGGGGCC CGGOCAGAGC CCCAGCTGC TGATCTACCG GATGAGCAAC
 2401 CTGGCCAGCG GGTGCCCGA CGGGTTCAGC GGCAGGGCA CGGGCACCGG CTTCACCTG
 2461 CGGATCAGCC GGGTGGAGGC CGAGGACGTG GGCCTGTACT ACTGCATCA GCACCTGGAG
 2521 TACCCCTTCA CTTTCGGCAG CGGCACCAAG CTGAGCATCA AGCGGGGGCG CGGGGGCAGC
 10 2581 GCGGGCGGCG CCACGGCCAGC CGGGCCAGC CACCTGCAGC TGCAAGCAGC CGGGGGCGAG
 2641 CTGGTCCGGC CCGGCACCAAG CGTCAAGATC AGCTGCAAGG CCACGGGCTA CACCTTCACC
 2701 AACTACTGGC TGGGCTGGT GAAGCAGCGG CCCGGCCACG GCCTGGASTG GATCGGCAC
 2761 ATCTACCCCG GCGCCACAA CAACAACTAC AACAGGAATC TCAAGGGCAA GGCCACCCCTG
 15 2821 ACCGCCGACA CCACGAGCAG CACCGCTCATC ATGCAGCTGA CGACGCTGAC CAGCGAGGAC
 2881 AGCGCCGTGT ACTTCTGC CCGGGAGTAC GGCAACTACG ACTACGCCAT GGACAGCTGG
 2941 GCCCAAGGCA CCACGGTGA CGTGGAGCAGC GGTGGGGGTG CTCCGGCGG AGGTGGGTCC
 3001 GGTGGCGCG GATCAGGTGG GGGTGGATCA GGCCTGGGG GTCGGGGATCA
 3061 GACAAAATC ACACATGC CGGGCTCCAA GCACGGAAAC TCTCTGGGG ACCGTCAGTC
 3121 TCCCTCTTC CCCCAAACC CAAGGACACC CTCATGATCT CCGGACCCCG TGAGGTACA
 20 3181 TGCCTGGTGG TGCAGGTGAAG CCACGAGACG CTCAGGTCTA AGTCAACTTG GTACGGGAC
 3241 GCGGTGAGG TGCAATATCG ACAGACAAAG CGGGGGAGGC AGCAGTACAA CACCAAGTAC
 3301 CGITGTGGTC GCGTCTCTCAC CGTCTCGAC CAGGAGTGGC TGAATGGCAA GGAGTACAAG
 3361 TCCAAGGTCI CCACAAAGC CCTCCCAAGCC CCCATCGAGA AAACCATCTC CAAAGCCAA
 3421 GGGCAGCOC GAGAACCC CTGAGTACACC CTGCGCCCATC CGGGGGATGA GTCGACCAAG
 25 3481 AACAGGTC GCTGGGACTG CCTGGTCAAGG GGCCTTATC ACAGCGACAT CGGGTGGAG
 3541 TGGGAGAGCA ATGGGCAGCC GGAGAACAC TACAAGACCA CGCTCCCGT GTGGACTCC
 3601 GACGGCTCT TCTTCTCTA CAGCAAGCTC ACCGGTGGACA AGAGCAGGTG GCAGCAGGGG
 3661 AACGTCITCI CAGTCTCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
 3721 CTCTCCCTGT CTCGGGTAA ATGA

30 FVII-088 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underline, 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 MVSQALPILAC LLLHQGCLIA AVFVTQERAH GVLHRRRRAA AFLEELRPGS LERECKEEQC
 61 SFEEAREIFK DAERTKLFWI SYSDGDQ^{AS} SPCQNGGSC^K DQLOSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGCEEQYC SDHTGKTRSC RCHEGSYLLA DGVSCTPTV^E YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPKG ECPWQVLLL^V NGAQLCGGTL INTIWWVSAA HCPDKIKNWR
 40 241 NLIAVLGEHD LSEHDGDEQS RRVQAQVII^{PS} TYVPC^TTNHD IALRLHQFV VLTDHV^VPLC
 301 LPERTFSE^{RT} LAFVRFSLVS GWGQQLLDRGA TALELMV^LNV PRIMTQDCLQ QSRKVGDSPN
 361 I^ETEYMFCA^{GY} SDGSKDSC^{KG} DSGGPHATHY RGTWLTGIV SWQGQCATVG HFGVYTRV^{SQ}
 421 YIEWLQKLMP SEPRPGVLLR AP^FPGGGGGG GGGGGGGGG GGGGGGGGG GGGSDKTHTC
 481 PPCPAPELLG GPSVPLFFPK P^RDTLMISRT P^EVICVVVDV SHEDPVEPN WYVDCVEVHN
 541 AKTKP^REEQY NSTYRVVSVL^L T^VLHQDWLNG KEYR^KVSNK ALPAPIE^XII SKAKQQPREF
 60 601 QVYTLPPSRD ELTKNQVSLT^T CLVKGKFYPSD I^AVEWESNGQ PENNYK^TTP^E VLDSDGSFFL
 661 YSKLTVEKSR WQQCNVFS^CS VMHEALNNH^Y TQKSLSLSPG KRRPRSGGGG SGGGGGGGG
 721 SGGGGSGGGG SGGGGSRKRR KRDIVMTQAA PSVPVTPG^ES VSISCRSSRS LLHSNGNTYL
 781 CWFLQRPQGS PQLLIYRMSN LASGV^PD^RFS GSGSCTAFTL RISRVEAEDV GVYYCMOHLE
 841 YPCTGPGSGT^K LEIKR^RCCGS CGGCGGGGGS QVQLQ^QSGAE LVRPCTSVKI SCKASGYTF^T
 901 NYWLGVWVKQR PGHGLEWIGD I^YPGGGYN^KY NENPFGKATL TADTSS^STA^Y MQLS^LLTSED
 961 SAVYFCA^{RE}Y GNYDYAMDSW CQGTSV^TV^S GGGGGGGGG GGGGGGGGG GGGGGGGGG
 1021 DKTHTCP^CPPV APEL^LGGPSV FLFPPK^PY^D LMI^STP^EFT CVVVDVSHED PEVKPNWYVD
 1081 GVEVHN^ATKT PREQDYN^STY RVVSVL^TY^LH QDWLN^EKEYK CKVSNKALP^A PIEKTISKAK
 1141 QFQFREPQVY^I LPPSRDEL^TK NQVS^LT^CLV^K GFYFSDIAVE WESNGQFENN YK^ITPPV^LDS
 1201 DGSFFLYSKL TVDKSRWQ^QQ N^FV^FCSVM^HE ALHNHYTQ^KS LSLSPGK*

55 DNA sequence for FVIII-041

60 1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTC^GCTT TGCGATTCTG CTTTAGTGCC
 61 ACCAGAAAGT ACTACCTGGG TGCAGTGGAA CTGTC^GATGGG ACTATATGCA AAGTGATCTC
 121 GGTGAGCTG CTGTGGACGC AAGATTC^TCT CCTAGAGTGC CAAATCTT TCAITCAAC
 181 ACCTCAGTCG TGTACAAAAA GACTCTGTT GTAGAATTCA CGGATCACCT TTTCACACATC
 241 GCTAAGCCAA GGCACCCCTG GATGGGTCTG CTAGTCCTA CCATCCAGGC TGAGGTTTAT
 301 GATA^CAGTCG TCTTACACT TAAGAACATG GCTTC^CCTC CTGTCAGTCT TCATGCTGT
 361 GGTGTATCCT ACTGGAAAGC TTCTGAGGG GCTGAATATG ATGATCAGAC CAGTCAAAGG
 421 GAGAAAGAAG ATGATAAAAGT CTTCCCTCGT GGAAGCCATA CATA^TGTCTG CGAGGTCTG
 481 AAGACAGATC GTCCAA^TGTGC CTCTGAGCCA CTGTC^CCTA CCTACTCA^A TCTTTCTCAT
 541 GTGGACCTGG TAAAGACTT GAATTCAGGC CTCAT^TGGAG CCTACTAGT ATGTAGAGAA
 601 GGGAGCTGG C^CAGGAAA GACACAGAC TT^TACAAAT TTATACTACT TTTTGCTGT
 661 TTTGATGAAG GGAAAAGTG GCACTCAGAA ACAAGAACT CCTTGATGCA GGATAGGGAT
 721 GCTGCATCTG CTCGGGCTG GCTAAAGATC CACACAGTCA ATGTTTATGT AAACAGGTCT
 781 CTGCCAGOTC TGAT^TGGATG CCACAGGAA TCAGTCTATT GGCAT^TGTGAT TGGATGGGC
 841 ACCACTCTG AAGTGCACTC AATATT^TCTC GAAGGT^CACA CATTCTTGT GAGGAACCAT
 901 CGCCAGGGT C^CTGGAAAT CTG^CCAATA ACTTTCTCA^T CTGCTCAAAC ACTCTTGATG
 961 GACCTGGAC AGTTTCTACT GTTTCTG^CT ATCTCTTCCC ACCAACATGA TGGCATGGAA
 1021 GCTTATGTC^A AAGTACAGC CTGTC^CAGAG GAACCC^AAC T^ACGAATGAA AAATAATGAA
 1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
 1141 GATGACACTC CTCTT^TCC^TT TATCCAATT CGCCTAGTTG CCAAGAACCA TCCTAAAACI
 1201 TGGGTACATT^A ACAT^TGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCT AGTCC^TCGCC
 1261 CCCGATGACA GAAGTATAA AAGTCAATT TTGACAAATG GCCCTCAGC^G GATTGGTAGG
 1321 AAGTACAAAAA AAGTCCGATT TATGGC^ATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT

5 1381 ATTCAAGGATG AATCAGGAAT CTTGGGACCT TTACTTTATG GCGAAGGTGG AGACACACTG
 1441 TTCATTATAT TTAAGAACATCA AGCAACCGAGA CCATATAAAC A TCTACCCCTCA CGGAATCACT
 1501 GATGTCCTGTC CTTTGTATTG AAGGAGATTA CCAAAGGTG TAAACATTT GAAGGATTTT
 1561 CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAAAGGACAG TGACTGTAGA AGATGGGCCA
 1621 ACTAAATCAG ATCTCTCGGTG CCTGACCCCCG TATTTACTCTA GTTTCGTTAA TATGGAGAGA
 10 1681 GATCTACCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCCT ACAAAGAATC TCTAGATCAA
 1741 AGAGGAACCC AGATAATCTC AGACAACAGG AATCTCATCC TCTTTCTCT ATTTGATCAG
 1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACCTTTC TCCCAATCC AGCTGGAGTG
 1861 CAGCTTGAGT ATCCAGAGT CCAAGCTCC AACATCATCC AGACATCAA TGGCTATGTT
 1921 TTGATAGTT TGCGATTGTC AGTTGGTTG CATGGGTGG CATACTGGTA CATTCTAAGC
 15 1981 ATTGGAGCAC AGACTGACTT CCTTTCTGTC TTCTTCTCTG GATATAACCTT CAAACACAAA
 2041 ATGGTCTATG AAGACACACT CACCCATTC CCAATCTCGAG GAGAAACTCTG CTTCAIGTCG
 2101 ATGGAACACC CAGGTCTATG GATTCTGGGG TGCCCAACTCAGACTTTCG GAACAGAGGC
 2161 ATGACCCCT TACTGAAGGT TTCTGTTGTC GACAGAACCA CTGGTGTATTA TTACAGGGAC
 2221 AGTTATGAAG ATATTCAGC ATACTTGCTG AGTAAAAAAC ATGCCATTGA ACCAAGAAGC
 20 2281 TTCTCTCAAAC CACCCACAGT CTTGACCCAGG CATCACCGGG AAATAACTCG TACTACTCTT
 2341 CACTCAGATC AAGAGGAAT TGAATGATGAT GATACCATAT CAGTTGAAT GAAGAAGGAA
 2401 GATTTGACA TTATGATGA GGATGAAT CAGACCCCCC CGAGCTTICA AAAGAAAACA
 2461 CGACACIATI TTATGCTGAG AGTGGAGAGG CTCCTGGATT ATGGGATGAG TAGCTCCCA
 2521 CATGTTCTAA GAAACAGGGC TCAAGATGGC AGTGTCTCC AGTCAAGAA AGITGTTTTC
 25 2581 CAGGAATTTC CTGATGGCTC CTTTACTCTCAGG CCGCTTATACC GTGGAGAATC AAATGAACAT
 2641 TTGGGACTCC TGGGCCATATA TATAACAGCA GAAGTGAAG ATATATATCAT GTAACTTTG
 2701 AGAAATCAGG CCICCTCGCC CTATTCTTC TATTCTAGG TTATTTCTTA TGAGGAAGAT
 2761 CAGAGGCAAC GAGCAGAAC TAGAAAAAAC TTGTCAGGCT CTAATGAAAC CAAACCTTAC
 2821 TTTGGAAAG TGCAACATCA TATGGCACCC ACTAPAGATG AGTTGACTG CAAAGCCTGG
 30 2881 GCTTAATTCT CTGATGTTGA CCTGGAAAAA GATGTGCACT CAGGCCCTGAT TGGACCCCTT
 2941 CTGGCTCCAC ACATAACAC ACTGACCCCTG GCTGCTGGAG CACAACTGAC ACTACAGGAA
 3001 TTGCTCTGT TTTCACCAT TCTTGTAGAG ACCAAAGCT GCTACTTCAC TGAAAATATG
 3061 GAAAGAAACT CGAGGGCTCC CTGCAATATC CAGATGGAG ATCCCACCTT TAAAGAGAAT
 3121 TATCGCTTCC ATGCAATCAA TGGCTACATTA ATGGTACAC TACCTGGCTT AGTAATGGCT
 3181 CAGGAATCAA GGATTCGATG GTATCTGCTC AGCATGGGC GCAATGAAA CATCCATTCT
 3241 ATTCAATTCA TTGGACATGT GTTCACTCTG CGAAAAAAAG AGGAGTATAA ATGGCACTG
 3301 TACAATCTCT ATCCAGGTGT TTTTGGACAGA GTGGAAATGT TACCATCCAA AGCTGGAATI
 3361 TGGCGGGTGG AATGCCCTAT TGGCGAGCAT CTACATGCTG GGATGAGCAC ACTTTTCTG
 3421 TTGTCAGCAGA ATAAGTGTCA GACTCCCCCTG GGAATGGCTT CTGGACACAT TAGAGATTI
 3481 CAGATTACAG CTTCAGGACA ATATGGACAG TGGGGCCCAA AGCTGGCCAG ACTTCATTAT
 3541 TCCGGATCAA TCAATGCCCTG GAGCACCCAG GAGCCTTTT CTGGATCAA GGTGGATCTG
 3601 TTGGCACCAA TGATTATCA CGGCATCAAG ACCCGGGTG CGCTGAGGAA GTTCTCCAGG
 3661 CCTCTCATCT CTCAGTTTAT CATCATGAT AGTCTTGTAGT GGAAGAAGTC GCGACTTAT
 3721 CCACCAATT CCACCTGAACT CTTAACCTGTC TTCTTGTGCA ATCTGGATTC ATCTGGATA
 3781 AAACACAAATA TTGTTAACCC TCCAATTATT GCTCGATACA TCCGTTTGCA CCCAACTCAT
 3841 TATAGCATTC CGACCACTCT CGCATGGAG TTGATGGCT GTGATTTAAA TAGTTGCAAGC
 3901 ATGCCATTGG GAATGGAGAG TAAAGCAATA TCAGATGGCAG AGATTAATCTG TTCACTCTAC
 3961 TTTACCAATA TGTTCGCCAC CTGGTCTCTC TCAAAAGCTC GACTTCACCT CCAAGGGAGG
 4021 ACTAAATCCTA GGAGACCTCA GGTGAATAT CAAAGAGT GCGTGCAGT GCACTTCCAG
 4081 AAGACAAATG AAGTCACAGG AGTAACTACT CAGGGAGTAA ATCTCTGCT TACCAAGCATG
 4141 TATGTGAGG AGTTCTCTAT CTCCAGGAGT CAAGATGGCC ATCAGTGGAC TCTCTTTTTT
 4201 CAGAAATGGCA AAGTAAGGT TTTCAGGGGAA ATCAAGACT CCTTCACACCC TGTGGTGAAC
 4261 TCTCTAGACCC CACCGTTACT GACTGCTAC CTTCGAATTC ACCCCCCAGAG TTGGGTGCAC
 4321 CAGATTGGCC TGAGGATGGA GGTCTGGGC TGCGGGCAC AGGACCTCTA CGACAAACT
 4381 CACACATGCC CACCGTCCCC AGCTCCAGAA CTCCCTGGCG GACCGTCAGT CCTCTCTTC
 4441 CCCCCCAAAAC CCAAGGACAC CCTCATGATC TCCCCGACCC CTGAGGTGCA AIGCTGGGTG
 4501 TTGGGACCTGA GCCACGAGA CCCTGAGCTC AAGTCATCAACT GGTACGTGGA CGGGCTGGAG
 4561 GTGCATBATTG CCAAGACAAA CGCGCGGGAG GAGGAGTACA ACAGCACGTA CGGTGTGGTC
 4621 AGCGTCTCTA CGTCTCTGCA CCAGGACTGG CTGAATGGCA AGGAGTACAA GTGCAGGGTC
 4681 TCCAAACAAAG CCCCCCTCCAGC CCCCCATCGAG AAAACCATCT CCAAGGCCA AGGGCAGGCC
 4741 CGAGAACCCAC AGGTCTACAC TCCCTGGCTCA TCCCGGGATG AGCTGACCAA GAACCAAGGTC
 4801 AGCCGTACCT GCTCTGGTCAA AGGCTCTAT CCCAGCGACA TCGCCGTGGA GTGGGAGAGC
 4861 ATGGGCAGC CGGAGAACAA CTACAAGACC AGCGCTCCCG TGTGGACTC CGACGGCTCC
 4921 TTCTTCTCTT ACAGCAAGCT CACCGTGACAGG AAGAGCAGGT GGAGCAGGG GAACGTCCTC
 4981 TCATGCTCTG TGATGCTGATCA GGCTCTGCAAC CACCATACA CGCAGAAGAG CCTCTCTCTG
 5041 TCTCCGGGTA AAGGTGGGG CGGATCAGGT GGGGGTGGAT CGGGGGTGG AGGTTCGGG
 5101 GGCGGGGGAT CAGACAAAAC TCACACATCC CCACCGTGC CAGCACCTGA ACTCCCTGGGA
 5161 GGACCGTCAG TCTCTCTCTT CCCCCCCTAA CCAAGGACA CCCTCATGAT CTCCCGGACC
 5221 CCTGAGGTCATCA CTGCGTGGT GGTGGACCTG AGCCACGAAG ACCTCTGGGT CAAGTTAAC
 5281 TTGTACGTGG ACGGCGTGGCA CGTGCATAAT CCAAGACAA AGCCGGGGJA CGAGCACTAC
 5341 AACAGCAAGT ACCGTGTGGT CACCGCTCTC ACCGCTCTGC ACCAGGACTG GCTGAATGGC
 5401 AAGGAGTACA AGTGCAGGT CTCCAAACAA GCCCTCCCGAG CCCCCCATCGA GAAACCATC
 5461 TCCAAAGCCA AAGGGCAGCC CGGAGAACCA CAGGTGTACA CCCTGCCCTC ATCCCGCGAT
 5521 GAGCTGACCA AGAACCGAGT CAGCCTGACCC TGGCTGGTCA AAGGCTTCTA TCCAGCGAC
 5581 ATCGCCGCTGG AGTGGGAGAG CAATGGCAGG CGGGAGAACAA ACTACAAGAC CACGCCCTCCC
 5641 GTGTTGGACT CGCACGGCTC CTTCCTCTC TACACCAAGC TCACCGTGG CAAGAGCAGG
 5701 TGGCAGGAGG GGAACGTCTT CTCATGTCCTC GTGATGCTAC AGGCTCTGCA CAACCAACTAC
 5761 ACGCAGAAGA GCGCTCCCT GTCTCCGGT AAAAGA

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

1 MQIELSTCFE LCLLRFCPFA TPRYVILGAVE LSWDYMQSDL GELPVNDARFP PRVPKSFPFN
 61 TSVVYKKTLE VFTDHLFNI AKPRPFWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
 121 GVSYVWKASEG AEYDDOTSGR EKEDDKVFPG CSHTYVWQVL KENGPMASDP LCLTYSYLSH
 10 131 VDLVKDLNSG LIGALLVCRE GSLAKEKTQT LHKFILLPAV FDEGKSWHSE TKNSLMQDRD
 241 AASARAWPKM HTVNGYVNRS LPGLIGCHRK SVYVHVICMG TPEVHSIPL ECHTFLVRNH
 301 RQASLEISPI TPLTAQTLLM DLGQFLLPCH ISSHMQHDGME AYVKVDSCPE EPQLRMKNNE
 361 EAEDYDDEDDLT DSEMDPVFQI RSVAKKHPKT WVHYIAAEEEE DWDYAPLVLVA
 15 421 PDERSYKSYQV INNGPQRIGR KYKKVRFMAY TDETFKTREA IQHESGLGP LLYGEVCDTL
 481 LIIFKNQASR PYNIYPHGIT DVRPLYSRRL PKGVRLKLDF PIIPGEIFKY KWTVVEDGP
 541 TKSDPRCLTF YYSSFVNMER DLASLGIGL LICYKESVQD RGNQIMSDFR NVILFSVFDE
 601 NRSWYLTENI QRFLPNPAGV QLEDPLFQAS NIMHSGINGV FDSDLQLSVCL HEVAYWYILS
 661 IGAQTDPLSVF FFSGYTFKHK MYVEDILTLF PFSCETVFMN MENFGLWLIG CHNSDPRNRC
 721 MTALLKVSSC DKNTGDIYED SYEDISAYLL SKNNIAEPRS FSONPPVILKR HQREIERTTL
 20 781 QSDQEEIYD DTISSEMVKKE DFDIYDEND QSPRFQKKT RHYFIAAVER LWWDYGMGSSP
 841 HVLRNRAQSG SVPQFKKVFV QEFPTDGSFTQ FLYRGEELNEH LGILGPYIRA EVEDNIMVTF
 901 RNQASRFYSP YSSLISYED QROQAEPRKN FVKVPMETKTY FWKVQPHMAP TKDEPDKAW
 961 AYFSDVLEK DVHSGLIGPL LVCHTNTLNP AHGROVITVQE PALPFTIFD E TKSWYPTENM
 1021 ERNCRAPCNI QMEDPTFKEN RYFHAINGYI MDTLEPLVM QDQKIRWYLL SMGSSENIRS
 25 1081 IRFSGHVFVTV RKKEEYKMAL VNLYPGVET VEMLSKAGI WVECLIGEH LHAGMSTLFL
 1141 VYSNKCQTPL GMASGHIRD QITASCGYQ WAPKLRALHY SGSIINAWSTK EPPFSWIKVDL
 1201 LAPMIHGIK TQGARQKFSS LYISQFLIMY SLDGKWNQTY RGNSTGTLMV FFGNVDSSGI
 1261 KHNIFNPPII ARYIRLHPTM YSIRSLRME LMGCDLNSCS MPLGMESKAI SDAQITASSY
 1321 FTNNMFAITWP SKARLHLQGR SNAWRPQVMN PKEWILQVDFQ KTMKVTGVTT QGVKSLLTSM
 1381 YVKEFLISSS QDGHQWTLFF QNGKVKVFQG NQDSFTPVVV SLDPPLLTRY LRIHPQSWVH
 1441 QIALRMBVLG CEAQDLYDKK HTCPCPAFAE LLGSPSVFLF FFPKPKDFTLMI SRTPEVTCVV
 1501 VDVSHEDPEV KPNWYVGDVE NNAKTYKPRE EQYNTSYRUV SVLTVLHQDW LNLGKBYKCKV
 1561 SNRALKAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCVLVGFY PSDIAVEWES
 1621 NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVVMREALH NHYTQKSLSL
 30 1681 CGPKGGGGSG GGGGGGGSG GGGSDKHTTC PPCPAPELLG GPSVFLFPEK PKDTLMISRI
 1741 PEVTCVVVDV SHEDPEVKFN WYVDGVIEVHN AKTRPEEQQY NSTYRVVSVL TVLHODWLNG
 1801 KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYILPPSRD ELTKNQVSLT CLVKGFYPSD
 1861 IAVEWESNGQ PENNYKTTFP VLSDSGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
 1921 TQKSLSLSPG K*

40 DNA sequence for FVIII-108

1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGIGCCTTT TGGCGATTCTG CTTTAGTGCC
 61 ACCAGAGAT ACTACCTGGG TCCAGTGGAA CTGTCATGGG ACTATATGCA AAGTGTCTC
 121 CCTCAGCTGC CTCCTGGACGC AACATTTCCT CCTAACAGTC CAAATCTTT TCCATTCAAC
 181 ACCTCACTCG TGACAAAAAA GACTCTGTTT GTAGATTCA CGGATCACCT TTTCAACATC
 241 GCTAACGCAA GGCACCCCTG GATGGCTCTG CTAGGCTCTA CCATCCAGGC TGAGGTTTAT
 301 GATAACACTGG TCATTACACT TAAGAACATC GCTTCCCATC CTGTCAGTC TCATGCTGTT
 361 GGTGTATCTT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
 421 GAGAAAGAAC ATGATAAAGT CTTCCTCGT GGAAGCCATA CATAATGTC TGCAGGTCTG
 481 AAAGAGAATG GTCCAATGCC CTCTGACCCA CTGTCCTTA CCTACTCAIA TCITTCCTCAT
 541 GTGGACCTGG TAAAGACTT GAATTCAAGC CTCATCTGGAG CCCTACTAGT ATGAGAGAA
 601 GGGAGCTTGG CCAAGGAAAA GACACAGACC TTGACAAAT TTATACTACT TTTTGTGTA
 661 TTTGATGAAG GGAAAGTGTG CCACTGCAGAA ACAAAAGAAT CCTTGATGCA GGATAGGGAT
 721 GCTGCATCTG CTGGGGCTG GCCTAAATAG CACACAGTC ATGGTTATGT AACAGGTCT
 781 CTGCCAGGTC TGATGGATG CCACAGGAAA TCAGTCATT GGCAAGTGT TGGAAATGGGC
 841 ACCACTCTTG AAGTCACCTC AATATTCTTC GAAGGTCACA CATTTCCTGT GAGGAACCAT
 901 CGCCCAAGCGT CCTTGGAAAT CTGCGCAATA ACTTCTCTTA CTGCTCAAC ACTCTTGATG
 961 GACCTTGACG ATTTTCTACT GTTTTGATC ATCTCTCTCC ACCAACATGA TGGCATGGAA
 1021 GCTTATGTC AAGTAGACAG CTGTCAGAG GAACCCCAAC TAGGAATGAA AAATAATGAA
 1081 GAAGGCGAAG ACTATGATGA TGATCTACT GATTCTTAAA TGGATGTCG CAGGTTTGAT
 1141 GATGACACT CTCTTCCCT TATGCAATTA CGCCTCAGTT CGAACAGACA TCCTAAAACI
 1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCCTCGCC
 1261 CCCGATGACA GAAGTTATAA AAGTCAAAT TTGACAAATG GCCTCTAGCG GATTGGTAGG
 1321 AAGTACAAAAA AAGTCCGATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT
 1381 ATTCACTCATG AATCAGGAAT CTTGGGACCT TTACTTATG EGGAAAGTTGG AGACACACTG
 1441 TTGATIATAT TTARGAATCA AGCAAGCAGA CCAATAACA TCTACCCCTCA CGGAATCCTI
 1501 GATGTCCTGC CTGTTGATTC AAGCAAGTAA CCAAAAGCTG TAAACACATTI GAAGGATTIT
 1561 CCAATTCTGC CAGGAAATAT ATTCAAAAT AAATGGACAG TGACTGTAGA AGATGGGCCA
 1621 ACTAAATCTG ATCCCTGGTG CCTGACCCCC TATTACTCTA GTTTCGTTAA TATGGAGAGA
 1681 GATCTAGCTT CAGGACTCAT TGGCCCTCTC CTCACTCTGCT ACAAAAGAATC TGTAGATCAA
 1741 AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCATCC TGTTCCTGT ATTTGATGAG
 1801 AACCGAAGCT GGTACCTCAC AGAGAAATAA CAACGCTTC TCCCCAAATCC AGCTGGAGTG
 1861 CAGCTTGAGG ATCCAGAGTT CCAAGCTCC AACATCATGC ACAGCATCAA TGGCTATGTT
 1921 TTGATGAGTT TGCAGTTGTC AGTTTGTTG CATGGGTGG CATACTGGTA CATTCTAAGC
 1981 ATGGGAGCAC AGACTGACTT CCTTCTCTG GATATACCTT CAAACACAAA
 2041 ATGGTCTATG AAGACACACT CACCCCTATC CCACTCTCG SAGAAACTGT CTTCTGTC
 2101 ATGGAAAACC CAGGTCTATG GATTCTGGG TGCCACAATC CAGACTTTCG GAACAGAGGC
 2161 ATGACCCCTC TACTGAAGGT TTCTAGTTGT GACAGAAACA CTGGTGATTI TIAAGGAGGAC
 2221 AGTTATGAAAG ATATTTCTGC ATACTCTG AGTAAAAAAC AATGCCATTGA ACCAAGAAGC
 2281 TTCTCTCAA ACCCACCAGT CTTGAAACCC CATCAACGGG AAATAACTCG TAATCTCTT
 2341 CAGTCAGATC AAGAGGAAAT TGACTATGAT GATACCATAT CAGTGAAAT GAAGAAGGAA

5 2401 GATTTTGACCA TTATGATGA GGATGAAAT CAGACCCCCC CGAGCTTCA AAAGAAAAAC
 2461 CGACACTATT TTATGCTGC AGTGGAGG CTCTGGGATT ATGGGATGAG TAGCTCCCCA
 2521 CATGTTCTAA GAAACAGGGC TCAGACTGCG AGTGTCCCTC AGTCAAGAA AGTTTTTTC
 2581 CAGGAATTAA CTGATGGCTC CTTTACTCG CCCITATACC GTGGAGAACT AAATGAACAT
 2641 TTGGGACTCC TGGGCCATA TATAAGACCA GAACCTGAAG ATAATATCAT GTTAACCTTC
 10 2701 AGAAAATCAGG CCTCTCGTCC CTATTCCTTC TATCTTACCC TTATTTCTTA TGAGGAAGAT
 2761 CACAGGCAAG GACACAAAC TAGAAAAAAC TTTCCTCAAGC CTATGAAC CAAAACCTAC
 2821 TTTTGGAAAG TGCAACATCA TATGGCACCC ACTAAGATG AGTTGACTG CAAAGCCTGG
 2881 GCTTATTCTCT CTGATGTTGA CCTGGAAAAA GATGTCAT CAGGCTCTGAT TGGACCCCTT
 2941 CTGGTCCTGC ACACATACAC GATGAAACCT GCTGTGGGA GACAAGTGC AGTACAGGAA
 15 3001 TTTGCTCTGT TTTCACCAT CTTTGATGAG ACCAAAAAGCT GGTACTTCAC TGAAAATATG
 3061 GAAAGAAACT GCAGGGCTCC CTGCAATATC CAGATGGAAG ATCCCACITTT TAAAGAGAAT
 3121 TATCGCTTCC ATGCAATCATCA TGCTCATATA ATGGTACAC TACCTGGCTT AGTAATGGCT
 3181 CAGGATCAAA GGATTCGATG CTATCTGCTC AGGATGGCA GCAATGAAA CATCCATCT
 3241 ATTCAATTCA GTGGACATGT GTTCACTGTA CGAATAAAAAG AGGAGTATAA ATGGCACTG
 3301 TACAATCTCT ATCCAGGTGT TTTTGAGACA GTGGAATGT TACCATCCAA AGCTGGAAATT
 3361 TGGCGGTGCG AATGCTTAT CGTGGAGGAT CTACATCTG CGATGACAC ACTTTTCTG
 3421 GTGTACAGCA ATAAGTGTCA GACTCCCCCTG GGAATGGCTT CTGGACACAT TAGAGATTTT
 3481 CAGATTACAG CTTCAAGGACA ATATGGAGAC TGGGCCCAA AGCTGGCCAG ACITCATTAT
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 25 3601 TTGGCACCAA TGATTTATCA CGGATCACAA ACCCAGGGTG CGGGTCAGAA GTTCTCAGC
 3661 CTCTACATCT CTCAGTTAT CATCATGAT AGTCTGATG GGAAGAAGTG GCAGACTTAT
 3721 CGAGGAATT CCACCTGQAAC CTTAATGGTC TTCTTGGCA ATGTTGGATTC ATCTGGGATA
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 3841 TATAGCATTC GCAGCACTCT CGCAGATGGAG TTGATGGCT GTGATTTAAA TAGTGGCAGC
 3901 ATGCCATTGG GAATGGAGAG TAAACCAATA TCAGATGCC AGATTAATCTG TICATCCTAC
 3961 TTTACCAATA TGTTTGGCAC CTGGTCTCTC TCAAGATCCT CACTTCACCT CCAAGGGAGG
 4021 AGTAATGCTT GGAGACCTCA GGTGAATAT CCAAAAGAGT GGTGCAAGT GGACTTCCAG
 4081 AAGACAAATGA AAGTCACAGG AGTAACACT CAGGGAGTAA ATCTCTCT TACCAAGCATG
 4141 TATGTGAGG AGITTCCTCAT CTCCAGGAGT CAAGTGGCC ATCAGTGGAC TCTCTTTTTT
 4201 CAGAAATGGCA AAGTAAGGTT TTTCAGGGA AATCAAGACT CCTTCACACC TGTGGGAAC
 4261 TCTCTAGACC CACCGTTACT GACTCGCTAC CTTCGAAITTC ACCCCCCAGAG TTGGGTGCAC
 4321 CAGATGGCC TGAGGATGGA GGTCTGGGC TGCGAGGCAC AGGACCTCTA CGACAAAACI
 4381 CACACATGCC CACCGTGCCTG AGCACCTGAA CTCCCTGGAG GACCGTCACT CTTCTCTTC
 4441 CCCCCCAAAAC CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTAC CAGCGTGGT
 4501 GTGGACCTGA GGCACGAAGA CCTCTGAGTC BAGTCAACT GGTACGTGGA CGGGGTGGAG
 4561 GTGCATAATG CCAAGACAAA GCGCGGGAG GAGCAGTACA ACAGCACGTA CGTGTGGTC
 4621 AGCGTCTGCA CGCAGGACTGG CTGATGGCA AGGAGTACAA GTGCAAGGTC
 4681 TCCACAAAG CCCCCTCCAGC CCCCCATCGAG AAAACCATCT CAAAGCCAA AGGGCAAGCCC
 4741 CGACAACCAC ACCTCTACAC CCTCCCCCTA TCCCCCGATC ACCTCACCAA CAACCAAGCTC
 4801 AGCCTGACCT GCTTGGTCAA AGGCTCTCAT CCCACGGAA TGCCCGTGGA GTGGGAGAGC
 4861 AATGGGCAAGC CGGAGAACAA CTACAAAGACC ACGCCCTCCG TGTGGACTC CGACGGCTCC
 4921 TTCTTCTCT ACACCAACT CACCGTCCAC AAGACCGGT GGCAGCAGGG GAACGTCTTC
 4981 TCATGCTCCG TGATGCATGA GGCTCTGCAC AACRACTACA CGCAGAAGAG CCTCTCCTG
 5041 TCTCGGCTA AACCGCGCCG CGGGAGCGT CAGGGCGGAT CAGGTGGGGG TGGATCAGGC
 5101 GGTGGAGGT CCGGTCCCCG GGGATCCGGC CGTGGAGGTT CGGTGGGGG TGGATCAAGG
 5161 AAGAGGAGGA AGAGGGCGCA GGTGAGCTG CAGGAGCTG GGGGAGGTTT GGTACAGGCT
 5221 GGGGGGTCCC TGAGACTCTC CTGTCGACCC TCTGATTCA GTTTAGGAG GTATGCCATG
 5281 AGCTGGGTCC GCGAGGCTCC AGGGAAAGGG CGAGAGTGGG TCTCAGGTTAGTGGTAGT
 5341 GTGGTAGTA CATACTACCG AGACTCCCTG AAGGGCGGT TACCCGTC CAGAGACAAI
 5401 TCCAAGRACA CGCTGTATCT GCAAATGAAAC AGCCCTGAGAG CGAGGAGACAC GGTGTATAT
 5461 TACTGCCCCC GGGGGCGCAC CTACACCGAC CGGAGCGACG ICCCCGACCA GACCACTTC
 5521 GACTACTGGG CCCAGGCAAC GTCTCTCACC GAGTGTGCACT CGGGCCCAAAG
 5581 CTTGAAGAAG GTGAATTTC AGAAGCACCG TGTATCTGAAC TGACTCAGGA CCCTGCTGTG
 5641 TCTGTGGCTT TGGGACAGAC AGTCAGGATC ACATGCCAAG GAGACAGCCT CAGAAACTTT
 60 5701 TATGCAAGCT GGATGCAAGCA GAAGGGCGCA CAGGCCCTA CTCTTGTCT CATATGGTTA
 5761 AGTAAAAGGC CCTCAGGGAT CGCAGACCGA TTCTCTGCTC CGAGCTCAGG AAACACAGC
 5821 TTCTTGACCA TCACTGGGGC TCAGGGCGAA GATGAGGCTG ACTAATACTG CCTCTGTAC
 5881 TACGGCGGGC CGCAGCAGGG CGTGGTCGGC CGGGCGACCA AGCTGACCGT CCTACGTCAG
 5941 CCCAAGGCTG CCCCCCTGGT CACTCTGTC CGGCCCTCTT CTGGGGCGGG TGGCGGTGGC
 65 6001 TCCGGCGGGAG GTGGGTCCCCG TGGCGGGCGA TCAGGTGGGG TTGGATCAGG CGGTGGAGGT
 6061 TCCGGGGCGCG GGGGATCAGA CAAAACCTCA ACAICCCCAC CGTGGCCAGC ACCGGAACCTC
 6121 CTGGGGCGGAC CGTCAGTCCT CCTCTTCCCCC CCAAACCCCA AGGACACCCCT CATGATCTCC
 6181 CGGACCCCTG AGGTCAACATG CGTGGTGGTG GACGTGAGCC AGAAGACCC TGAGGTCAG
 6241 TTCAACTGGT ACCTGGACGG CGTGGAGGTG CATAATGCCA AGACAAAGCC GCGGGAGGAG
 6301 CACTACAACA GCACGTACCG TGTGGTCAGC CTCCCTACCG TCCTGCACCA GGAACGCTC
 6361 AATGGCAAGG AGTACAAGTG CAAGGTCTCC AACAAAGGCC TOCCAGCCCC CATCGAGAAA
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 6481 CGGGATGAGC TGACCAAGAA CGAGGTCACTG CTGACCTGCC TGGTCAAAGG TTCTATCC
 6541 AGCGACATCG CGTGGAGGTG GGAGAGCAAT GGGCAGCCGG AGAACAAACTA CAAGACCAAG
 6601 CCTCCCTGGT TGGACTCCCGA CGGCTCTCTC TTCTCTACCA GCAAGCTCAG CGTGGACAAAG
 6661 AGCAGGTGGC AGCAGGGAA CGTCTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAAC
 6721 CACTACACGC AGAAGAGCCT CTCCCTGTCT CGGGTAAAT 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 **MQIELSTGFF** LCLLRFCCSA **TRRYYLGAVE** LSWDYMQSDFL GELPVNDARFP PRVPKSFPFN
 61 TSVVYKKTLF VEFTDHLFNI AKPRPPWNGL LGPTIQAEVY **DTVITLKNM** ASHPVSLHAV
 10 121 QVSYWKASEG AEYDDQTSQR EKEEDDKVFFG GSHTYVWQVL KENGPMASDP **LCLTYSYLH**
 181 VDLVKDLNSG **LIGALLVCRE** CSLAKEKIQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
 241 AASARAWPKM **HTVNGYVNRS** LPGLIGCHRK SVYWHVIGMG ITPEVEGSIPL EGHFLVNRH
 301 RQASLEISPI **TFLTAQTLIM** DLGQFLLICH ISSHQHDGME AYVKVDSCEPE EPQLRMKNN
 361 EAEDYDDEDLT DSEMDVVRFD DDNSPSFQIQI RSVAKKKPKT WVEYIAAEEF DWDYAPLVLA
 421 PDDRSYKSQY **LNNGFPQRIGR** KYKKVRFMAY TDTETFKTREA IQHESGILGP LLYGEVGDTL
 481 LIIIFKNAQSF PYNIYFNGIT DVRFLYSPRL FKGVKHLKDF PILFGEIJKY KWTYTVEDGE
 541 TKSDPRLTR YYSSFPVNRS DLASGLIGPL LICYKESDQ RGNQIMSDKR NVILFSVFD
 601 NRSWYLTENI QRFLENPAGV QLEDPEFCAS NIMHESINGVY FDSQLQSVCL HEVAYWYILS
 661 IGAQTDFLSV FFSGYTFKHK MYYEDTLJLF PFSGETVFM S MENPGLWILG CHNSDFRNRG
 20 721 MTALLKVSSS DKNITGDDYYE SYEDISAYL SKNNIAIEPRS FSONQPPVLRK HQRELTPTTL
 781 QSDQEEIYDV DTISVEMKKE DFDIYDDEDN QSPRSFQKKT RHYFIAAVER LWDYGMSSSF
 841 HVLRNRAQSG SVPQFKVVF QFFTDGSPIQ PLYRCLENEK LGLLGPYIKA EVEDNTMVTF
 901 RNQASRPFYF YSSLISYBED QRQGAEPPEK FVVKPNETKTY PWKVKQHHMAP TKDEDFCKAW
 961 AFSDVDLKEK DVHSGGLIGPL LVCHTNTLNP AHGROQTVQF EALFFFTIFD E TKSWYFTENM
 1021 ERNCRAPCNI QMEDPTFKEN YRFHAINGYI MDTLPLGLVMA QDQRIRWYLL SMGSNENIHS
 1081 IHFSGHVFVU RKKEEYKMA YNLYPGVFET VEMLESKAGI WRVECLIGEH LAGMSTLFL
 1141 VYSNKQCTPL GMASGHIRD P QITASGQVNG WAPKLAIRLY SGSINAWSIK EPFGSIKVDL
 1201 LAPMIIHMGK TQGARQKFSS LYISQFIIYM SLDGCKWQTY RGNSTGTLMV FFGNRVDSGGI
 1261 KHNIFNPPII ARYIRLHPTH YSIRSTLRLME LMGCCLNSC M P L G M E S K A I SDAQITASSY
 1321 FTNMFMATWSP SKARLHLQGR SNAWRPQVNM PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM
 1381 YVKEPLFISG QDCHQWTLFF QNGKVKVQFQ NGDQFTPVVN SLDPPFLITRY LRIHNPQEWVH
 1441 QIALRMEVLG CEAQDLYDVK MTCPPCPAPE LLGGPSVFLF PPKPKDTIMI SRTPEVTCVV
 1501 DVDSHEDPEV KFNWYVDGVE VHNAKTKPKE EQYNSTYRVV S VLTVLHQDW LNGKEYKCKV
 1561 SNKALPAPIE KTISKAKGQP REPQVYTLPP S R D E L T K N Q V S L T C L V K G F Y PSDIAVEWES
 1621 NGOPENNYKTT TPPVLDSDIGS FFLYSKILTVD KSRWQOGNVF SCVMHEALH NYTOKSLSL
 1681 SPGKRRRRSG GGGSGGGGG GGGSGGGGG KRRKRAQVQL QESGGGLVQP
 1741 GGSLRLSCAA SGFMFSRYAM SWVRQAFGKG PEWVSGISGS GGSTYYADSV
 KGRFTVSRDN
 1801 SKNTLYLQMN SLRAEDTAVY YCARGATYTS RSDVPPDQTSF DYWGQGTLVT
 40 VSSGSASAPK
 1861 LEEGEFSEAR VSELTQDPAV SVALGQTVRI TCQGDSLRNF YASWYQQKPG
 CAPTLVYGL
 1921 SKRPSPGIPDR FSASSSGNTA SLTITGAQAE DEADYYCLLY YGGGQQGVFG
 GGTKLTVLRQ
 45 1981 PKAAPSIVLIF PPSSAAGGGG SGGGGGGGGGG SGGGGGGGGGG SGGGGSDKTH
 TCPPCPAPEL
 2041 LGGPSVFLFP PKPKDTIMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 HNAKTKPREE
 2101 QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQFR
 EPQVYTLPPS
 2161 RDELTKNQVS LTCLVKGFPY SDIAVEWESN QQPENNYKTT PPVLDSDGSF
 FLYSKLTVDK
 2221 SRWQOGNVFS CSVMHEALH NYTOKSLSLS PGK*

55 DNA sequence for pSYN-FVIII-049

1 ATGCCAAATAG AGCTCTCCAC CTGCTTCTT CTGIGGCCCTT TCGCGATTCTG CTTTAGTGCC
 61 ACCAGAAGAT ACTACCTGGG TGCACTGGAA CTGTCATGGG ACTATATGCA BAGTGATCTC
 121 GGTGAGCTGC CTGTGGACGC AAGATTCTCT CCTAGAGTGC CAAATCTTT TCCATTCAAC
 181 ACCTCAGTCG TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTCAACATC
 241 GCTAACCGAA GGCAACCTCG GATGGGTCTG CTAGCTCCATA CCATCCAGGC TGAGGTTTAT
 301 GATAACACTG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCACTCT TCATGCTGTT
 361 GGTGTATCTCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
 421 GAGAAAGAAG ATGATAAAAGT CTTCCTGGT GGAAGCCATA CATATGTCG GCAGGCTCTG
 481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTCCTTA CCTACTCATA TCTTTCTCAT
 541 GTGGACCTGG TAAAGACTT GAATTCAAGC CTCACTGGAG CCCTACTACT ATGTAGAGAA
 601 GGGAGCTGG CCAAGGAAA GACACAGACCC TTGCAAAAT TTATACTACT TTTTCTGTA
 661 TITGATGAAG GGAAAGATGTG GCACTCAGAA ACAAGAAACT CCTTGATGCA GGATAGGGAT
 721 GCTGCATCTG CTGGGGCCTG GCCTAAATG CACACAGTC AATGGTTATGT AAACAGGGTCT
 781 CTGCCAGTC TGATGGATG CCACAGGAA TGACTCTTATT GGCATGTGAT TGGAAATGGGC
 841 ACCACTCCTG AAGTGCACTC AATATTCTC GAAGGTCAAA CATTCTTGT GAGGAACCAT
 901 CGCCGAGCGT CCTTGGAAAT CTCGCCATA ACTTCTCTTA CTGCTCAAC ACTCTTGATG
 961 GACCTTGGAC AGTTCTACT GTTTTGTCTAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
 1021 CCTTATGTC AACTACACAG CTCTCCACAG GAACCCCAAC TACCAATGAA AAATAATGAA
 1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
 1141 GATGACAACCT CTCTTCCCTT TATCCAATT CGCTCAGITG CCAAGAAGCA TCCTAAAAC

5 1201 TCGGTACATT ACATTCGTC TGAAGAGGAG GACIGGGACT ATGCTCCCT AGTCCCTGCC
 1261 CCGGATGACA GAAGTTATAA AAGTCATAT TTGAAACATG CCCTCAGCG GATTTGGTAGG
 1321 AAGTACAAAA AAGTCGGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT
 1381 ATTCAAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGGAAAGTTGG AGACACACTG
 1441 TTGATTATAT TTAAAGATCA AGCAAGGAGA CCATATAACA TCTACCCCTCA CGGAATCACT
 1501 GATGTCGGTC CTTTGTATTC AAGGAGATTA CCANAGGTC TAAACATTG GAAGGATTG
 1561 CCAATTCTGC CAGGAGAAAT ATTCAAAAT AATGGACAG TGACTGTAGA AGATGGGCCA
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 1741 AGAGGAACAC AGATAATGTC AGACAAGGAG AATGTCATCC TGTGTTCTGT ATTGATGAG
 1801 AACCGAAGCT GGTACCTCAC AGAGAAATATA CAACGCTTC TCCCAATCC AGCTGGAGTG
 1861 CAGCTTGAGG ATCCAGAGT CCAAGCTCC AACATCAAGC ACAGCATCAA TGGCTATGTT
 1921 TTTGATAGTT TGCGATGTC AGTTGTTTG CATGGGTG CATACTGGTA CATTCTAAC
 1981 ATTGGACAC AGACTGACTT CCTTTCTCTC TTCTCTCTG GATATAACCTT CAAACACAAA
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 2101 ATGGAAACAC CAGCTCTATG GATTCTGGG TGCCCAACTC CAGACTTTTG GAACAGAGGC
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 2221 AGTTAAGAAG ATATTCAGC ATACTTGTG AGTAAAGAAC ATGCCATTGA ACCAAGAACG
 2281 TCTCTCTAAA ACCCACCAGT CTTGAACACCC CATCAACGGG AAATAACTCG TACTACTCTT
 2341 CAGTCAGATC AAGAGGAAT TTGACTATGAT GATACCATAT CAGTTGAAT GAAGAAGGAA
 2401 GATTTTGACA TTATGATGA GGATGAAAT CAGAGCCCCC SCAGCTTTCA AAAGAAAACA
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 4081 AAGACAATG AAGTCACAGG AGTAACATACT CAGGGAGTAA AATCTCTGCT TACCAAGCATG
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 4201 CAGAAATGGCA AAGTAAAGGT TTTCAGGGAA ATCAAGACT CCTTCACACC TGTTGTGAAAC
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 4321 CAGATTGCCCT TGAGGATGGA GGTCTGGC TGCGAGGCAC AGGACCTCTA CGACAAAACCT
 4381 CACACATGCC CACCGTGCCT AGCACCTGAA CTTCCTGGGAG GACCGTCACT TTCTCTCTT
 4441 CCCCCAAAAC CCAAGGACAC CCTCATGATC TCCCCGACCC CTGAGGTCACT ATGGTGGTGS
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 4561 GTGCTAAATG CCAAGACAA CTACAAGACC ACGCCCTCCCG TGTGGACTC CGACGGCTCC
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 4861 ATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCCTCCCG TGTGGACTC CGACGGCTCC
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 5281 ACCCCCTGAGG TCACATGGCT GGTGGTGGAC GTGAGCCAGG AAGACCCCTGA GGTCAAGTTC
 5341 AACTGGTACG TGGACGGCGT GGAGGTTGAT AATGCCAAGA CAAAGCCGGG GGAGGAGGCAG
 5401 TACAACAGCA CGTACCGTGT GGTCAAGCGTC CTACCGTCC TGACCCAGGA CTGGCTGAAT
 5461 GGCAAGGAGT ACAAGTGCAGA GGTCTCCAC ACAGGCTCC CAGCCCCCAT CGAGAAAACC
 5521 ATCTCCAAAG CCAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCCTGCC CCCATCCGG
 5581 GATGAGCTGA CCAAGAACCA GGTCAAGCTG ACCCCTCTGG TCAAAGGCTT CTATCCCAGC
 5641 GACATCGCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAAATCAA GACCACGGCT
 5701 CCCGTGGTGG ACTCGAGGG CTCTCTCTC CTCTACAGCA AGCTCACCGT GGACAGAGGC
 5761 AGGTGGCAGC AGGGAAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC

5921 TACACGGAGA AGAGCCCTCTC 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

1 MQIELIATCFF LCLLRCGESA TRRYYLGAWE LSNDWYMQSDL GELPFVNDARFP PRVPKSFPPFN
 61 TSVYVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
 121 GVSXWKASEG AEYDDQTSQR EKEDDKVFPG GSHTTYWQVOL KENGPMASDP LCLTYSLSH
 181 VDLVKELNSG LIGALLVCRE GSLLAKETCT LHKFKEILLPAV FDGEKSWHSE TKNSLMDQDR
 241 AASARAWPKM HTVNGVYNRN LPGLIGCHRK CQVWYHIVGMC TITEEVHSIFL ECHTFLVRNH
 301 RQASLEISPI TFLTAQTLIM DLGQFLLFCH ISSHQHDGME AYVKVDSCPF EPQLRMKNNE
 361 EAEDYDDEDTL DSEMDVVRFD DNDSPSFQI Q RSVAKKHPKT WUHYIAAEW DWDYALPVLA
 421 PDDRSYKSQY LINNGPQRIGR KYKVKVRFMAY DTFETKTFREA IQHESGILGP LLYGEVGDTL
 481 LIIIFRNQNSAR PYNIYPHGIT DVRELYSRRL PKGVKHLRDF PILPGEIPFKY KWTWITVEDGP
 541 TKSIDPRCLTR YYSSFVNMRB DLASGLIGPQ LICYKESVDO RGNQIMSDKR NVILPFSVFD
 601 MRSWYLTENI QRFLPNPAGV QLEDPFQAS NIMHSINGVY FDLSQLQSVCL HEVAYWYILS
 661 IGAQTDPLSV FFSGTYFKHHK MYYBDTLLTF PSFGETVFMFS MENPGLWILQ CHNSDRPNRG
 721 MTALKVSSC DKNTGDYED SYEDISAYLL SKNNAAIEPRS FSQNPVVLKR HQREITRTTL
 781 QSDQEEDYD DTISVEMKKE DFDIYDQED QSPRSPFORKT RHYFIAERL WLDYGMGSSSP
 841 HVFLRNRAQSG SVFQPKKKVW QZFTDGSFTQ FLYRGLNHE LGLLGPVYIR A EVDENIMVIT
 901 RNQASRPFYSP YSSLISYEED QROQAEPRKN FVKPNETKTY FWKVQHHMAP TKDEFDCKAW
 961 AYFSVSDLEK DVHSGLICPL LVCHTNTLNP AHGRQVETVQE FALFFTIIFDE TKSWYPTENM
 1021 ERNCRAFCNII QMEDPTFKEN YRFHAINGYI MDTLPGCLVMA QDQRIRWYLL CMGSNEINHS
 1081 IHFSCHVFTV RKEEYKML YNLVFGVFTF VEMPLPSKAGI WRVECLIGERL LHAGMSTLFL
 1141 VYSNKCQTPL GMAEIGHEDF QITASGQYQG WAPKLARLHY SGSIINAWSTK EPPFSWIKVDL
 1201 LAPMIIHGR TQGARQPKSS LY1QSFIIIMY SLDGKQWQTY RGNSTGTLMV FFGNQWVSSGI
 1261 KHNINPPPII AYIRLHLTH YSIRSLTRME LMGCLDNMSC MPLGMESKAI SDAOITASSY
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 1381 YVKELPLISSS QDGHQWTLFF ONGKVKVVFQG NQDSFTPPVWN SLDPPLLTRY LRIHPSWVH
 1441 QIALRMEVLG CEAQDLYDKT HTCEPPCPAPE LLGGPSVFL PPKPKDITMI SRTPEVTCV
 1501 DVDSHEDEPV KFNPWVYDGVCE VHNARTKLPRE BOYNSTYRVRV SVLTVLHQDN LMGKEYKCKV
 1561 SNKAJAPPIE KTISKAKGQP REPVQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVENEWS
 1621 NGQGPNNYKTT TPPVLDSDGS FFLYSLKLTVD KSRWQOGNPFV SC5VMHEALH NYHTQKSLS
 1681 SPGKRRRSGS GGGGGGGGGGG GGGGGGGGGGG GGGGGGGGGGG KRRKDRKTHIT CPCCPAPELL
 1741 GGGSVFLFPP KPKDTLMSIR TPEVTCVVVD VSHEDEPVKF NWYVDGVEVH NAKTKPREEQ
 1801 YNSTYRVVVS LTVLHQDWLN GKEVYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPEPSR
 1861 DELTINQVSE TCLVKGFYPS DIAVEWESNC QPENNYKTTP IVLDSDGSSP LVSKLTVDKS
 1921 RWQCGNWFSSC SVMHEALHN YTOKSLSLSP GK*

45

CLAIMS

1. A chimeric clotting factor comprising:
 - (i) a clotting factor selected from the group consisting of factor VII (FVII), factor IX (FIX) and factor X (FX);
 - (ii) a targeting moiety which binds to platelets; and
 - (iii) a first Fc moiety and a second Fc moiety,
wherein the clotting factor is fused to the first Fc moiety, and
wherein the targeting moiety is fused to the second Fc moiety.
2. 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 a corresponding clotting factor lacking a targeting moiety.
3. The chimeric clotting factor of claim 1 or claim 2, further comprising a cleavable scFc (cscFc) linker interposed between the first Fc moiety and the second Fc moiety, wherein the cscFc linker comprises at least one enzymatic cleavage site which results in cleavage of the cscFc linker.
4. The chimeric clotting factor of claim 3, wherein the at least one enzymatic cleavage site is an intracellular processing site.
5. The chimeric clotting factor of claim 3 or claim 4, wherein the cscFc linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes.
6. The chimeric clotting factor of any one of claims 3 to 5, wherein the cscFc linker has a length of about 10 to about 50 amino acids.
7. The chimeric clotting factor of any one of claims 3 to 6, wherein the cscFc linker has a length of about 20 to about 30 amino acids.
8. The chimeric clotting factor of any one of claims 3 to 7, wherein the cscFc linker comprises a gly/ser peptide.
9. The chimeric clotting factor of claim 8, wherein the gly/ser peptide is comprised of a formula $(\text{Gly}_4\text{Ser})_n$ or $\text{Ser}(\text{Gly}_4\text{Ser})_n$, wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

10. The chimeric clotting factor of claim 9, wherein the gly/ser peptide having the formula (Gly₄ Ser)_n is selected from the group consisting of (Gly₄ Ser)₆, Ser(Gly₄ Ser)₆, (Gly₄ Ser)₄ and Ser(Gly₄ Ser)₄.

5 11. The chimeric clotting factor of claim 1 or claim 2, wherein the clotting factor comprises two polypeptide chains.

12. The chimeric clotting factor of claim 1 or claim 2, wherein the chimeric clotting factor has a structure selected from the group consisting of:

the clotting factor fused to the first Fc moiety and the targeting moiety fused to the second Fc moiety;

10 the clotting factor fused to the first Fc moiety via a spacer moiety and the targeting moiety fused to the second Fc moiety via a spacer moiety;

the clotting factor fused to the first Fc moiety and the targeting moiety fused to the second Fc moiety via a spacer moiety; and

15 the clotting factor fused to the first Fc moiety via a spacer moiety and the targeting moiety fused to the second Fc moiety.

13. The chimeric clotting factor of claim 12, which comprises two polypeptides wherein:

(i) the first polypeptide comprises the clotting factor, a spacer moiety and the first Fc moiety, and the second polypeptide comprises the targeting moiety and the second Fc moiety;

20 (ii) the first polypeptide comprises the clotting factor, a first spacer moiety and the first Fc moiety, and the second polypeptide comprises the targeting moiety, a second spacer moiety and the second Fc moiety;

(iii) the first polypeptide comprises the clotting factor and the first Fc moiety, and the second polypeptide comprises the targeting moiety and the second Fc moiety; or

25 (iv) the first polypeptide comprises the clotting factor and the first Fc moiety and the second polypeptide comprises the targeting moiety, a spacer moiety, and the second Fc moiety, and

wherein the two polypeptides form a covalent bond.

14. The chimeric clotting factor of claim 1 or claim 2, wherein the targeting moiety is fused 30 to the second Fc moiety directly.

15. The chimeric clotting factor of claim 1 or claim 2, wherein the targeting moiety is fused to the second Fc moiety via a spacer moiety.
16. The chimeric clotting factor of claim 1 or claim 2, wherein the targeting moiety is fused to the second Fc moiety via a cleavable linker.
- 5 17. The chimeric clotting factor of any one of claims 1 to 16, 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 and a peptide.
- 10 18. The chimeric clotting factor of any one of claims 1 to 17, wherein the targeting moiety binds to resting platelets.
19. The chimeric clotting factor of any one of claims 1 to 17, wherein the targeting moiety selectively binds to activated platelets.
20. The chimeric clotting factor of claim 18, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIba, GPVI, and the nonactive form of GPIIb/IIIa.
- 15 21. The chimeric clotting factor of claim 19, 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.
22. The chimeric clotting factor of claim 18, wherein the targeting moiety binds to the GPIb complex.
- 20 23. The chimeric clotting factor of claim 17, wherein the targeting moiety is a peptide selected from the group consisting of: PS4, OS1 and OS2.
24. The chimeric clotting factor of claim 17, wherein the targeting moiety comprises an antibody variable region from an antibody selected from the group consisting of: SCES, MB9 and AP3.
- 25 25. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is Factor VII.

26. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is a high specific activity variant of Factor VII.
27. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is Factor IX.
- 5 28. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is a high specific activity variant of Factor IX.
29. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is Factor X.
- 10 30. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is a high specific activity variant of Factor X.
31. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is secreted by a cell in an active form.
32. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is activated *in vivo*.
- 15 33. The chimeric clotting factor of any one of claims 1 to 32, wherein the chimeric clotting factor comprises a heterologous enzymatic cleavage site not naturally present in the clotting factor.
34. The chimeric clotting factor of claim 33, wherein the clotting factor comprises a heavy chain and a light chain, and the heterologous enzymatic cleavage site is genetically fused to the 20 amino terminus of the heavy chain of the clotting factor.
35. A nucleic acid molecule encoding the chimeric clotting factor of any one of claims 1 to 34.
36. An expression vector comprising the nucleic acid molecule of claim 35.
37. The expression vector of claim 36, wherein the vector further comprises a nucleotide sequence encoding an enzyme which cleaves at least one of the enzymatic cleavage sites.
- 25 38. An isolated host cell comprising the expression vector of claim 36 or claim 37..

39. The host cell of claim 38, which expresses an enzyme capable of intracellular processing.
40. The host cell of claim 39, wherein the enzyme is endogenous to the cell.
41. The host cell of claim 39, wherein the enzyme is heterologous to the cell.
- 5 42. A method of producing a chimeric clotting factor, said method comprising culturing the host cell of any one of claims 38 to 41 in culture medium and recovering the chimeric clotting factor from the medium.
- 10 43. A processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector of claim 36 or claim 37 in a cell cultured in cell culture medium and isolating the heterodimeric polypeptide from the cell culture medium.
44. A composition comprising the chimeric clotting factor of any one of claims 1 to 34 and a pharmaceutically acceptable carrier.
- 15 45. A composition comprising the nucleic acid molecule of claim 35 and a pharmaceutically acceptable carrier.
46. A method of improving hemostasis in a subject, said method comprising administering to the subject the composition of claim 44 or claim 45.

Date: 30 November 2015

Generic examples of Enhanced Clotting Factor Fc fusions:
Platelet targeting for enhanced efficacy

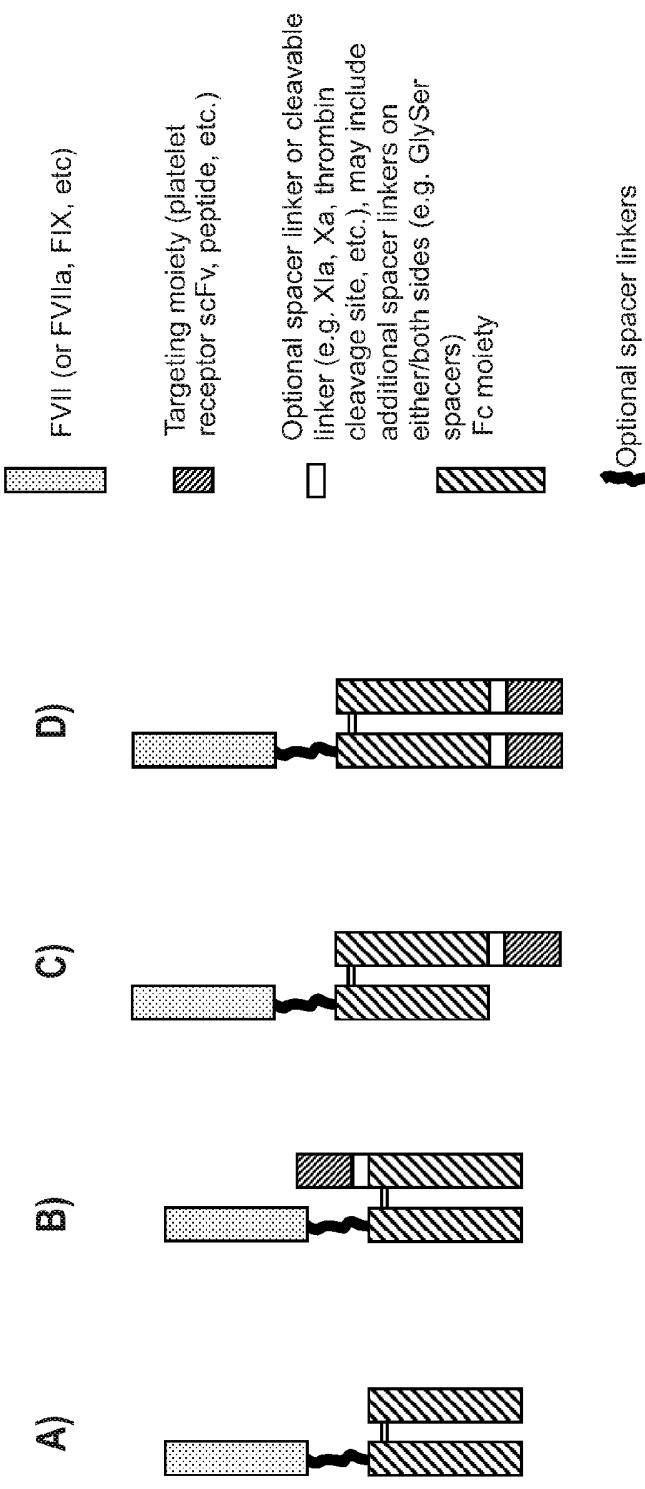


Fig. 1

**Generic examples of Enhanced Clotting Factor Fc fusions
(with protease processing sites): Platelet targeting for enhanced efficacy**

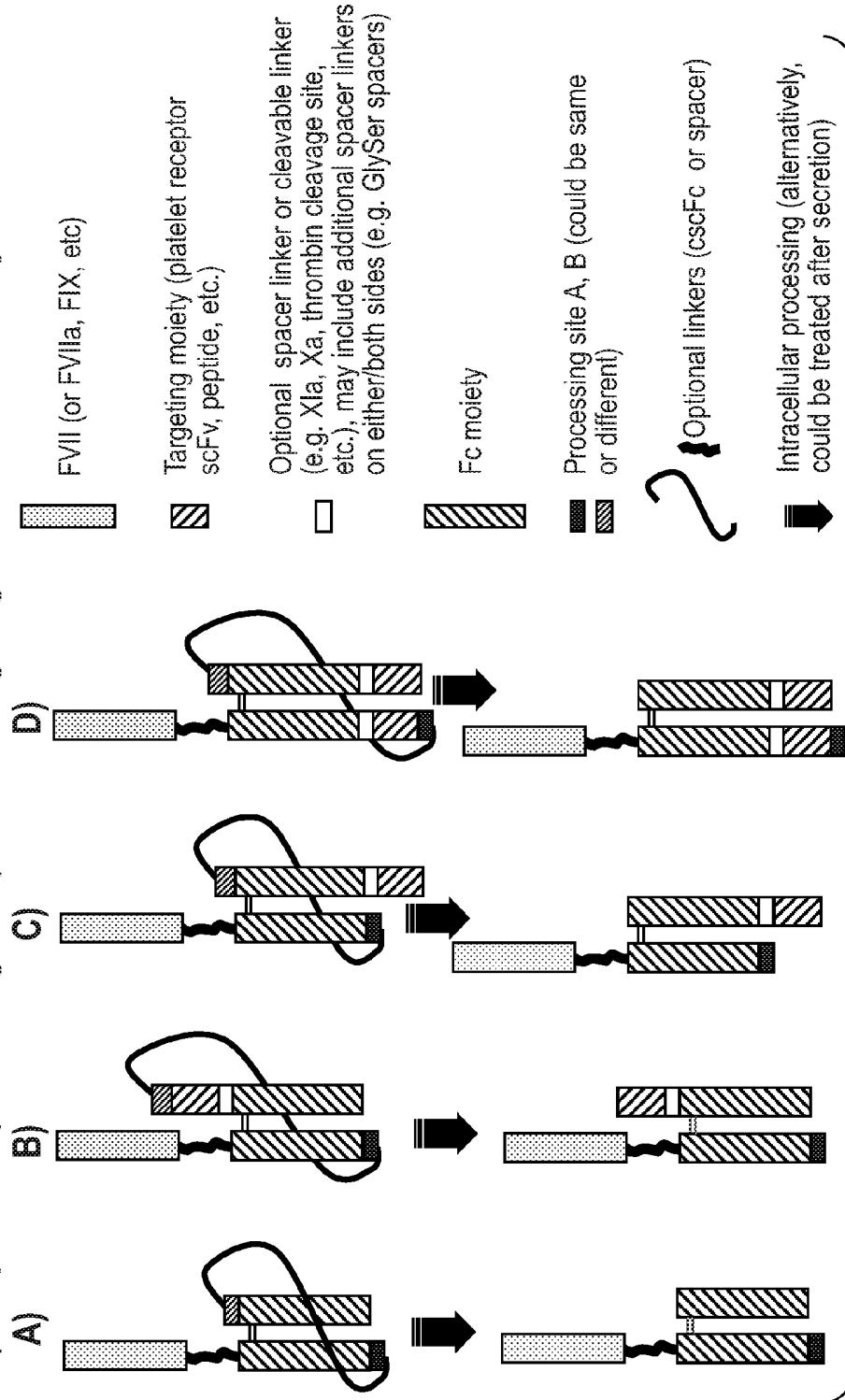


Fig. 2

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance

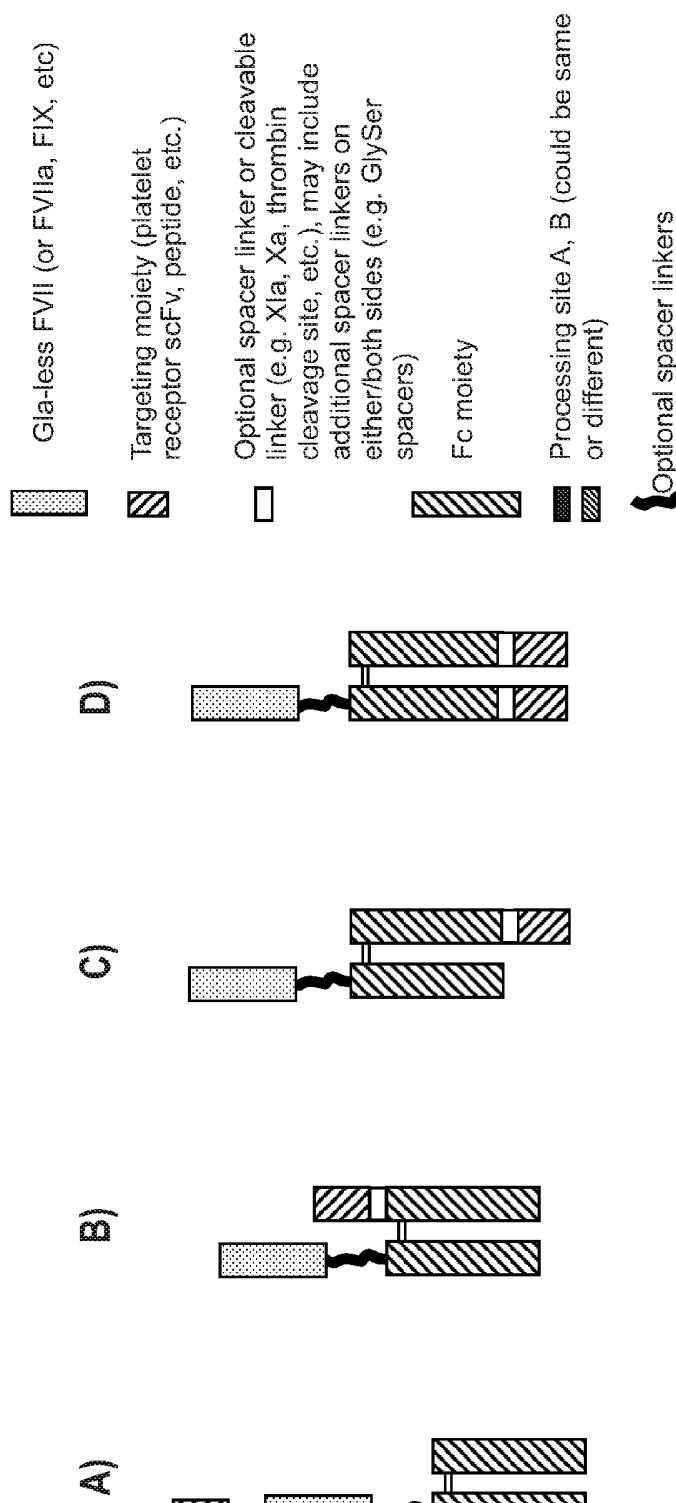


Fig. 3

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance,
with single chain cleavable linkers

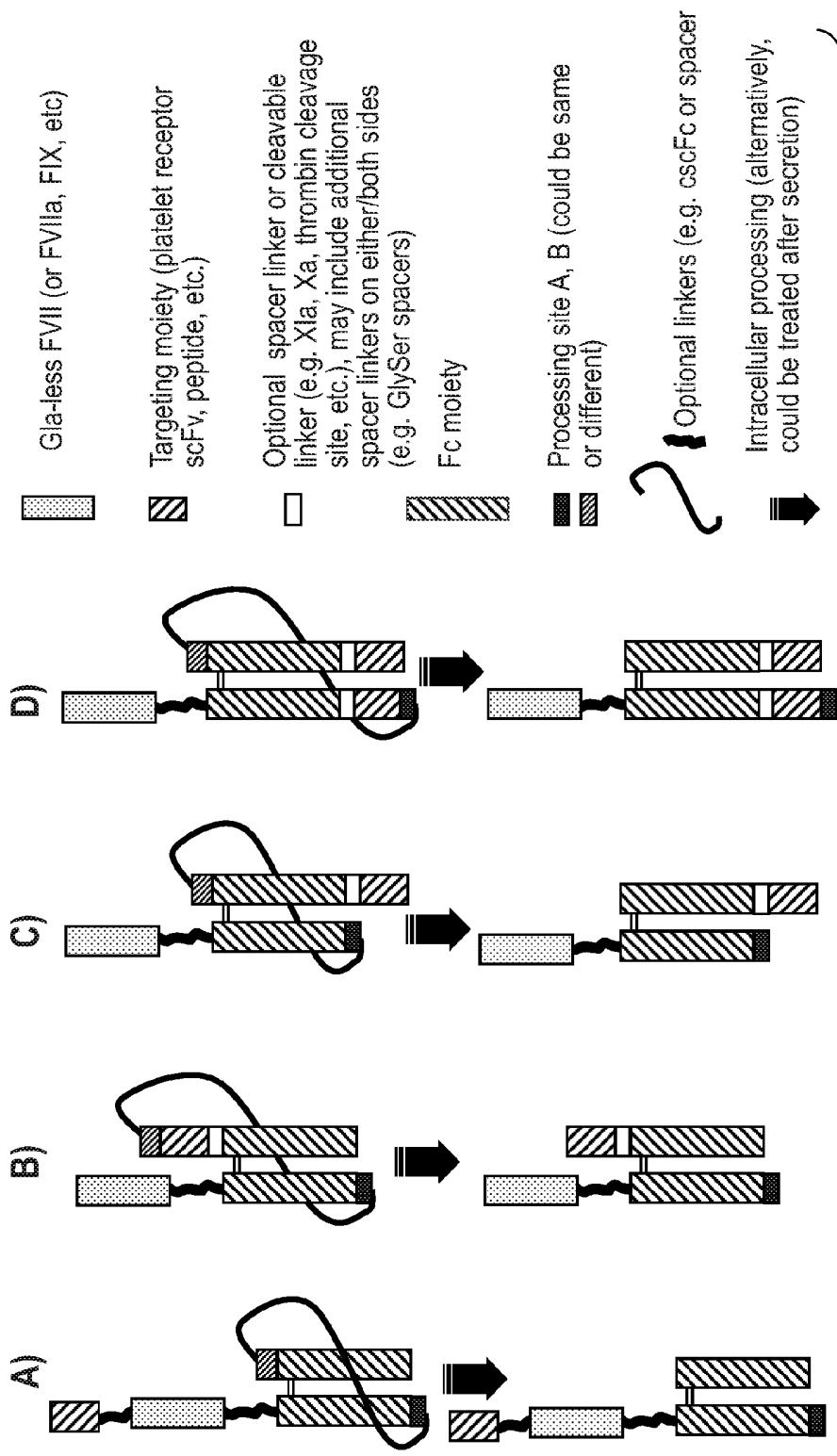
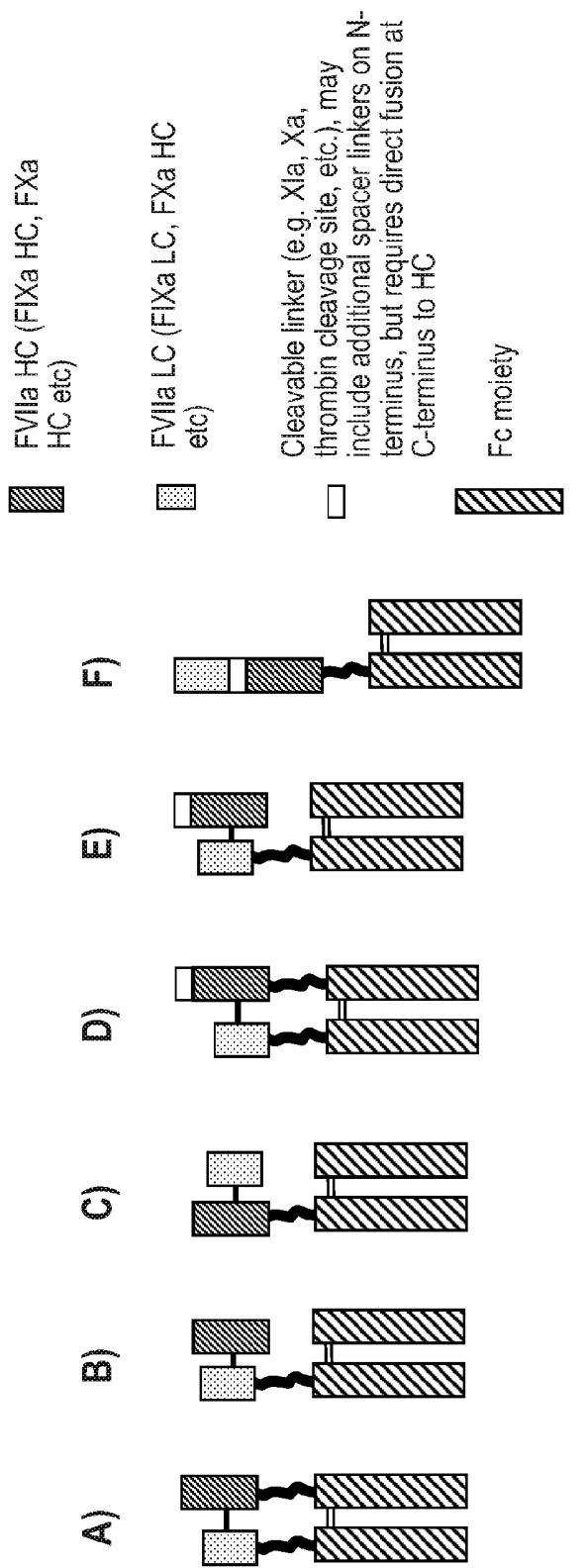


Fig. 4

Generic examples: Activated FVII constructs, activatable FVII constructs



Optional spacer linkers

Fig. 5

Activated FVII constructs, activatable FVII constructs with single chain cleavable linkers

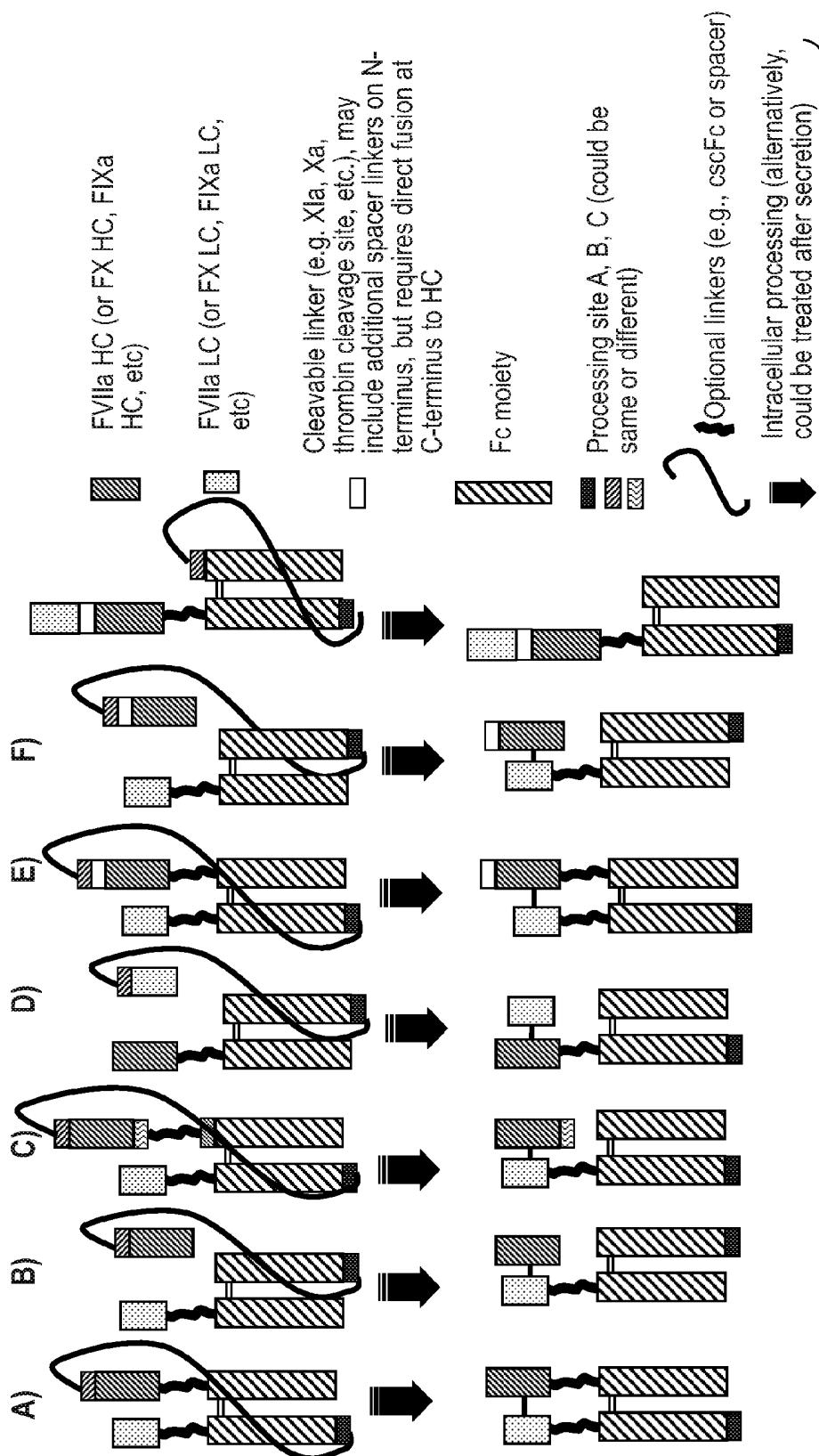


Fig. 6

Generic examples of Enhanced Clotting Factor (nonFc fusions): Platelet targeting for enhanced efficacy

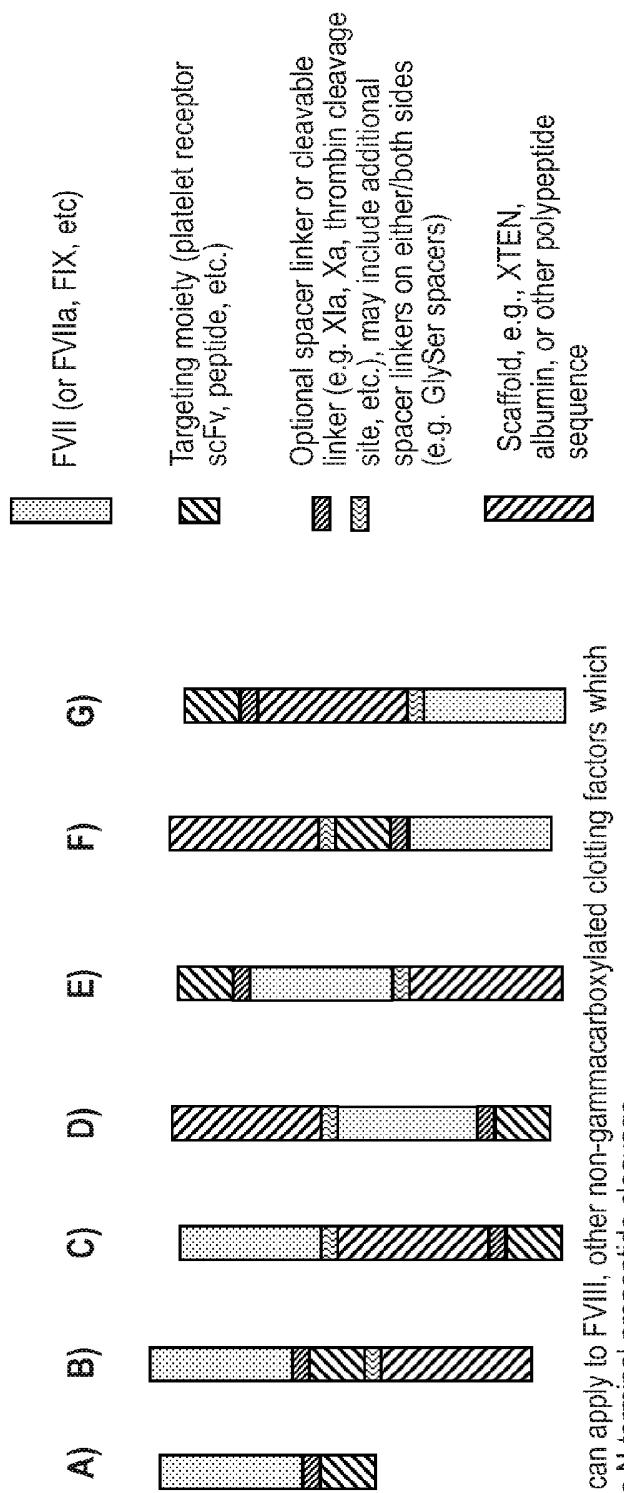
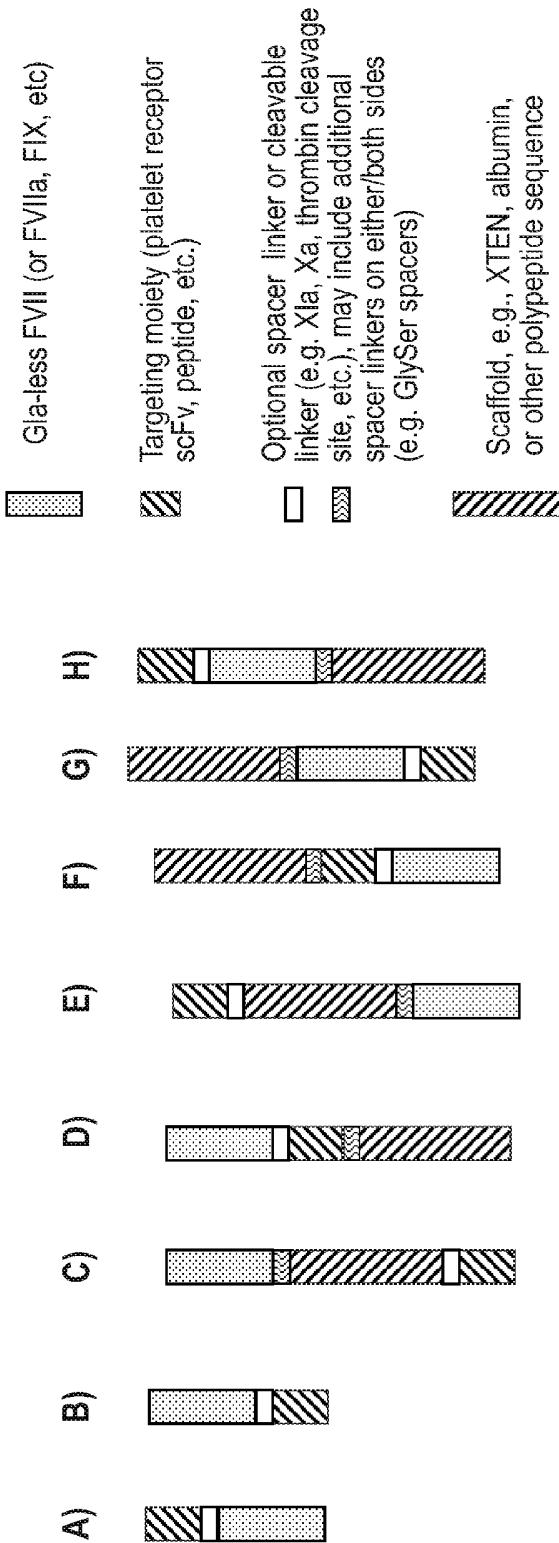


Fig. 7

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Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance, to FVII, FIX, FX (non-Fc fusions)



A, D-F) can apply to FVII, FIX, FX as N-terminal fusion is now possible in Gla domain deleted versions

Fig. 8

Activated FVII constructs, activatable FVII constructs with non-Fc fusions

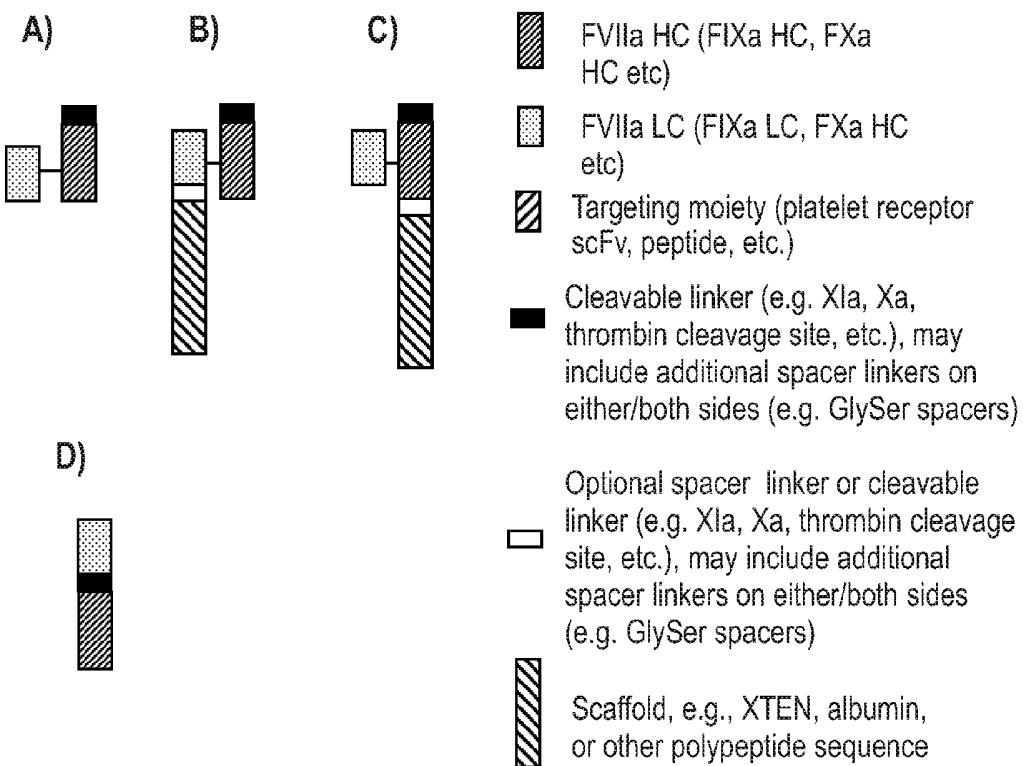


Fig. 9

SDS PAGE for purification and activation of FVII-011

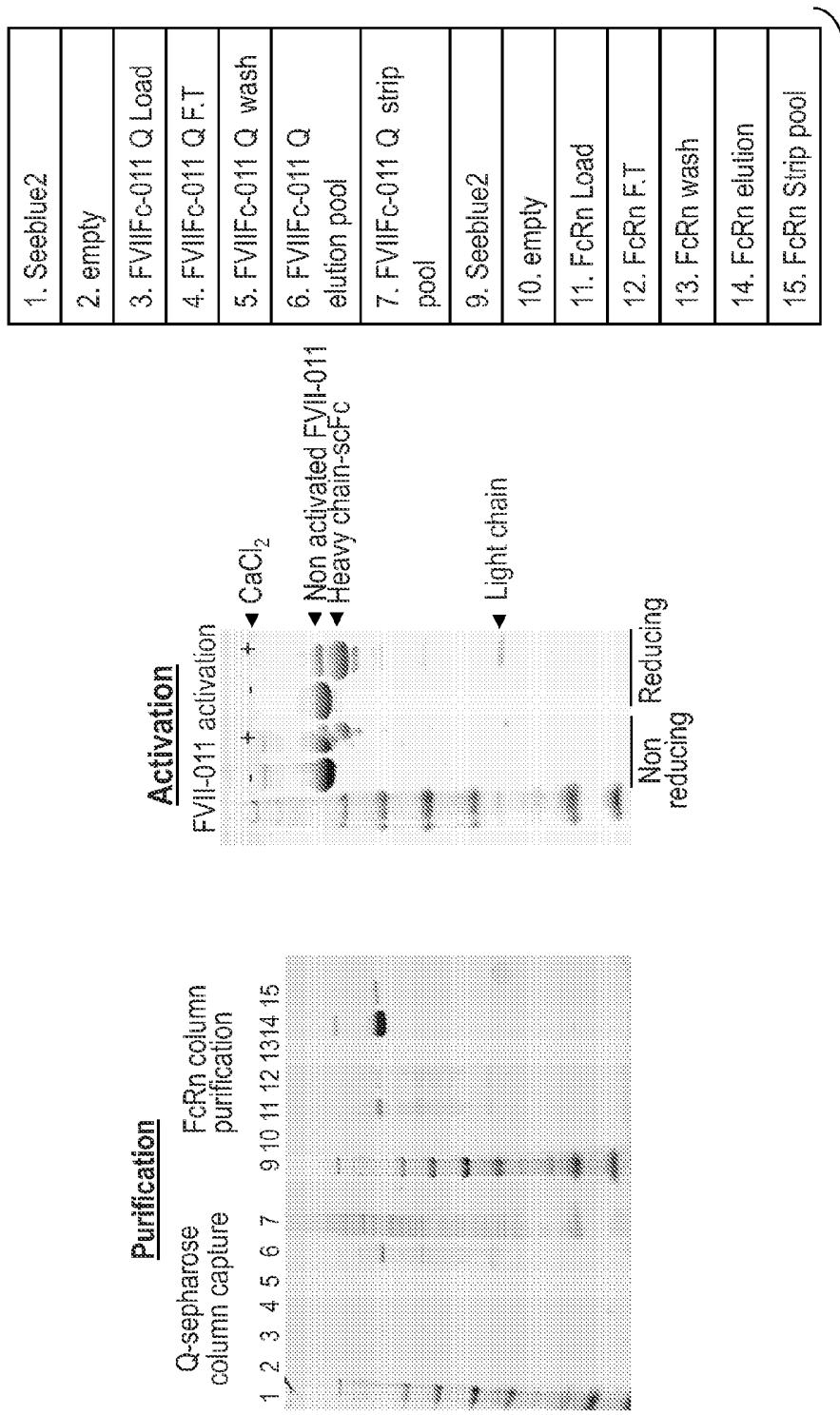
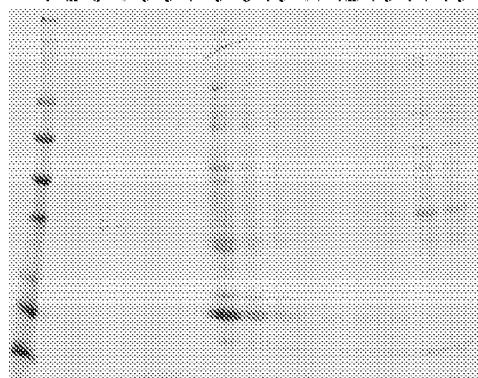


Fig. 10

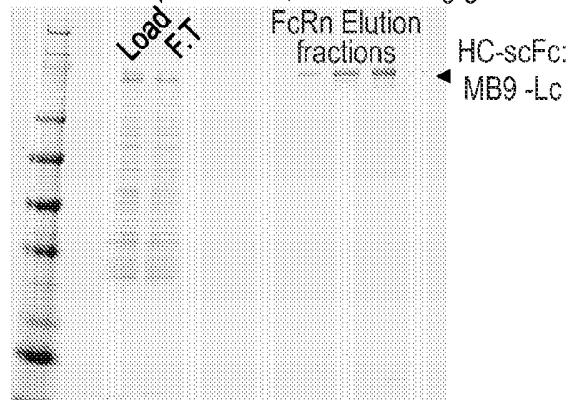
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HIC capture step, non reducing gel

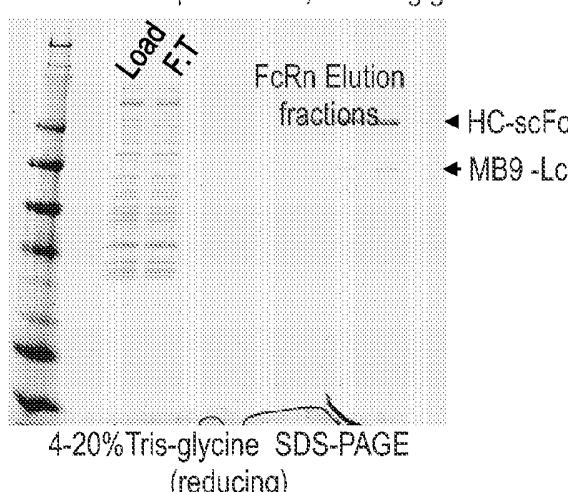
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15



FcRn column purification, non-reducing gel

4-20% Tris-glycine SDS-PAGE
(nonreducing)

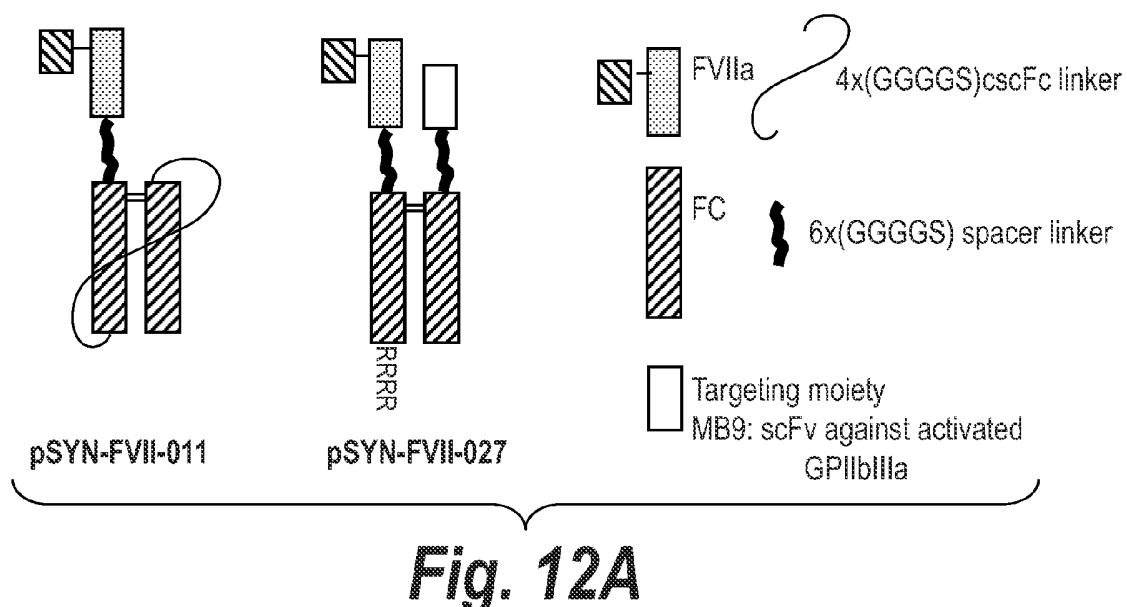
FcRn column purification, reducing gel

4-20% Tris-glycine SDS-PAGE
(reducing)

1. Seeblue2
2. Harvest
3. FVIIIFc-053 HIC Load
4. FVIIIFc-053 HIC F.T.
5. FVIIIFc-053 HIC wash Fraction #A2
6. FVIIIFc-053 HIC wash Fraction #A3
7. FVIIIFc-053 HIC wash Fraction #A4
8. FVIIIFc-053 HIC wash Fraction #A5
9. FVIIIFc-053 HIC wash Fraction #A6
9. FVIIIFc-053 HIC wash Fraction #A6
10. FVIIIFc-053 HIC wash Fraction #B7
11. Empty
12. FVIIIFc-053 HIC Elution Fraction #B6
13. FVIIIFc-053 HIC Elution Fraction #B5
14. FVIIIFc-053 HIC Elution Fraction #B4
15. FVIIIFc-053 HIC Elution Fraction #B3

Fig. 11

Binding of FVII-011 and FVII-027 to activated platelets determined by FACs



Binding of FVII-011 and FVII-027 to activated platelets determined by FACs

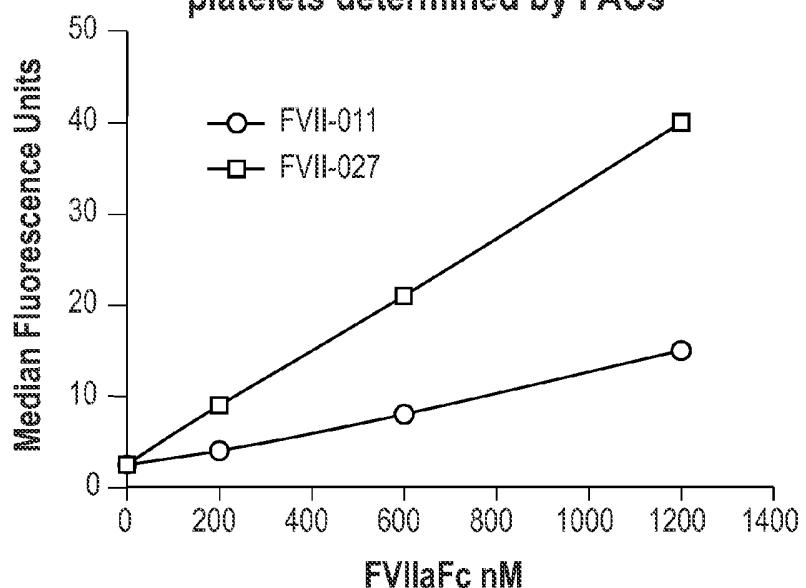


Fig. 12B

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Thrombin generation assay to measure activity of FVII-027, FVII-011 and Novoseven in the presence of activated platelets

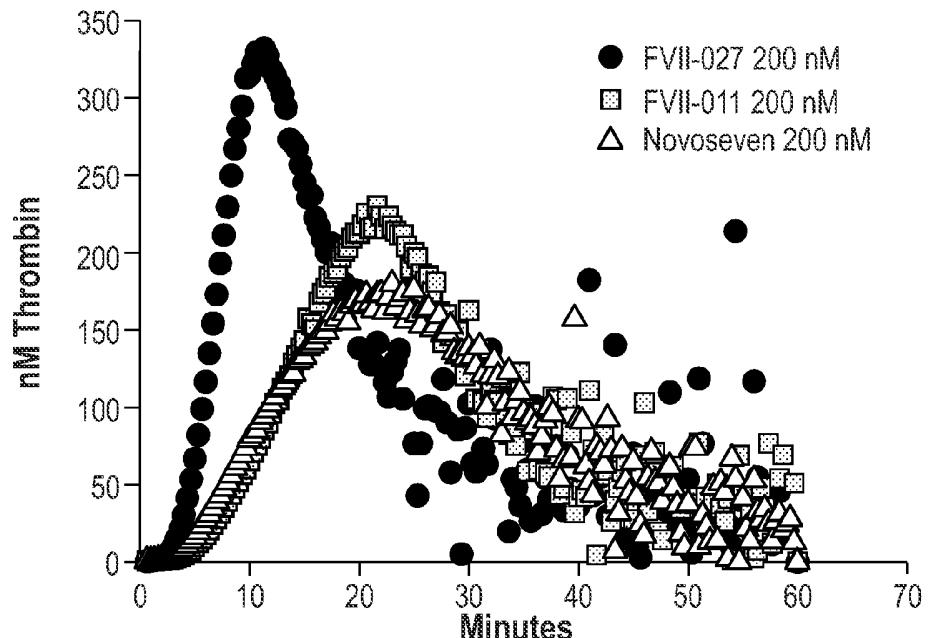


Fig. 13A

Thrombin generation assay to measure activity of FVII-027, FVII-011 and Novoseven in the presence of activated platelets

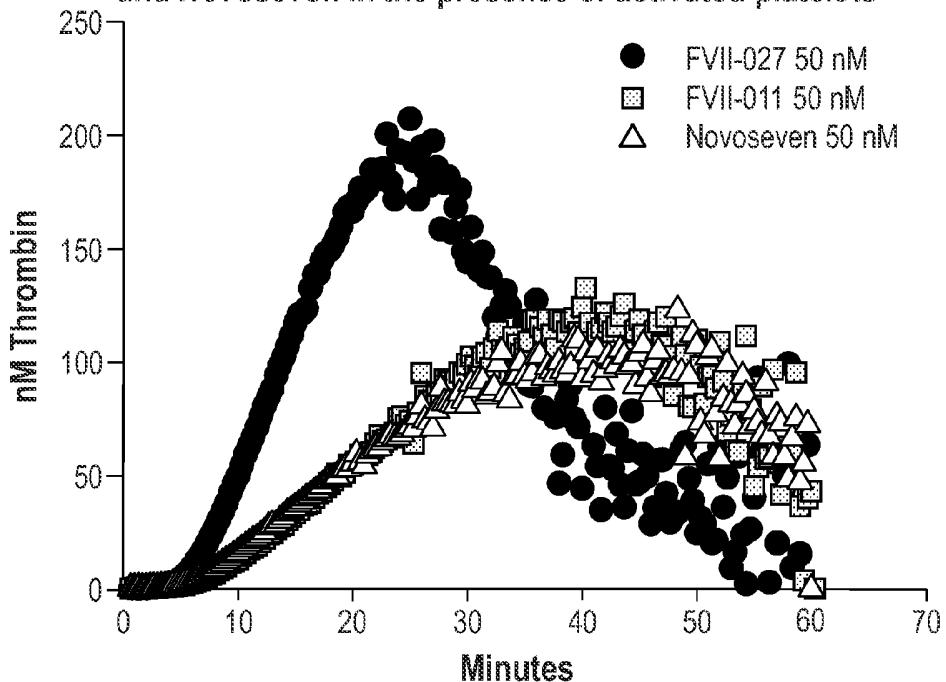


Fig. 13B

14/68

Thrombin generation assay to measure activity of FVII-027, FVII-011 and Novoseven in the presence of activated platelets

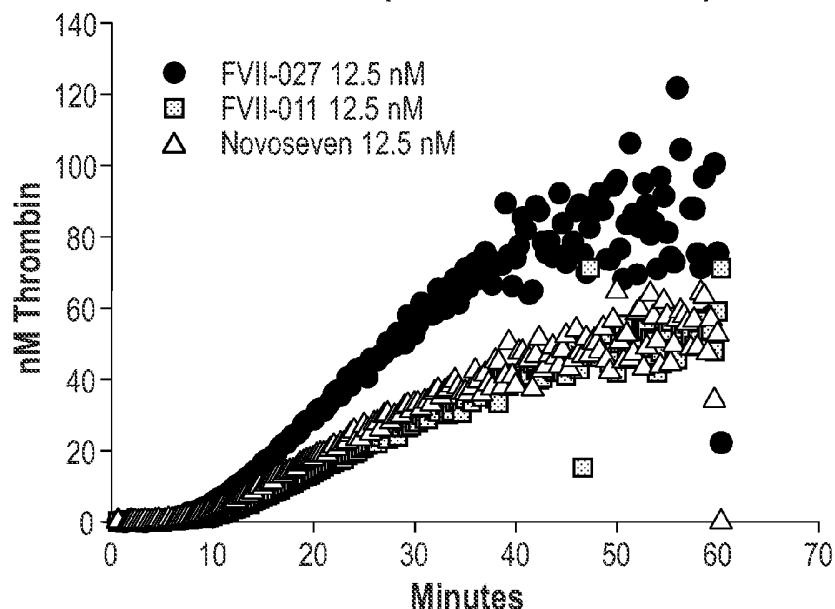


Fig. 13C

Thrombin generation assay to measure activity of FVII-027, FVII-011 and Novoseven in the presence of activated platelets

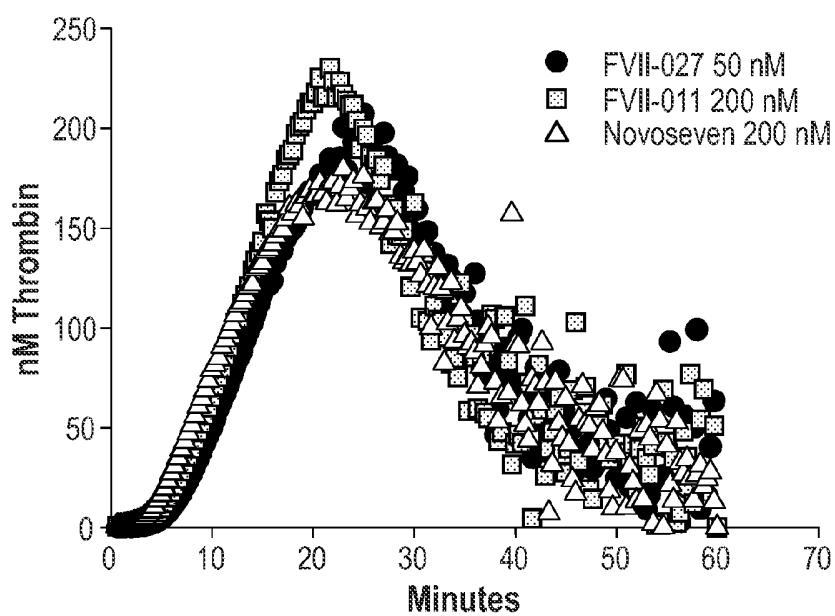


Fig. 13D

15/68

PAC-1 eliminates increased binding to platelets and increased rates of thrombin generation associated with FVII-027

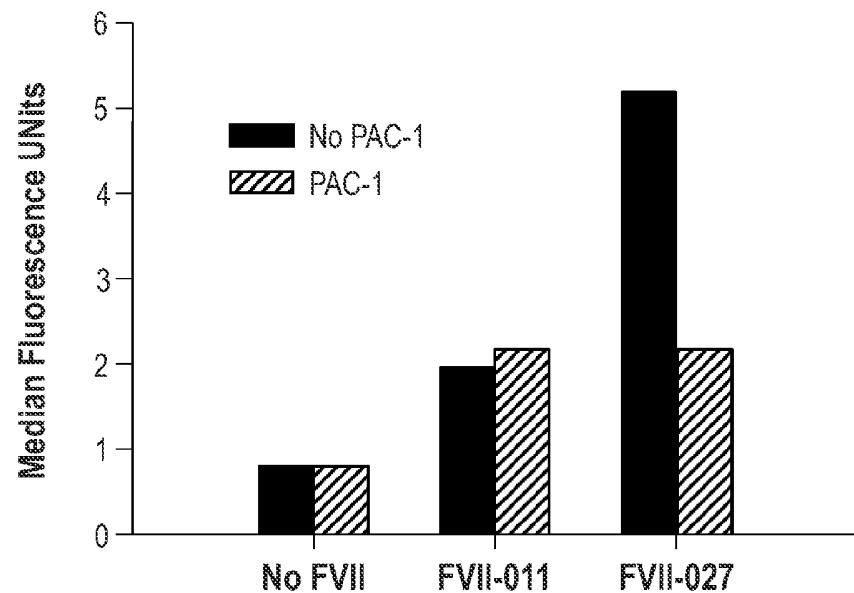


Fig. 14A

PAC-1 eliminates increased binding to platelets and increased rates of thrombin generation associated with FVII-027

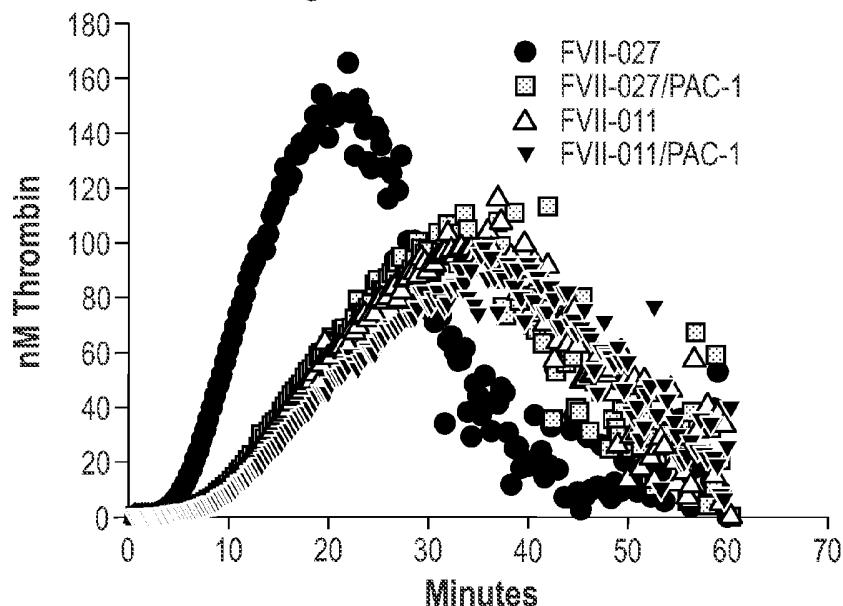


Fig. 14B

16/68

Thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets

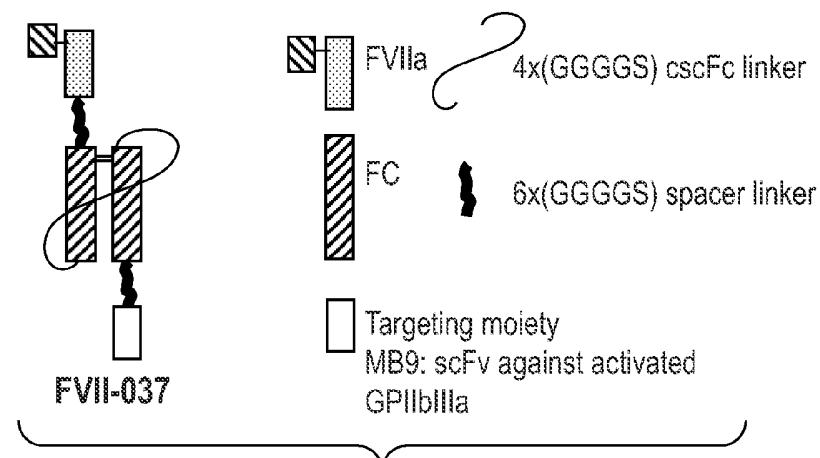


Fig. 15

Thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets

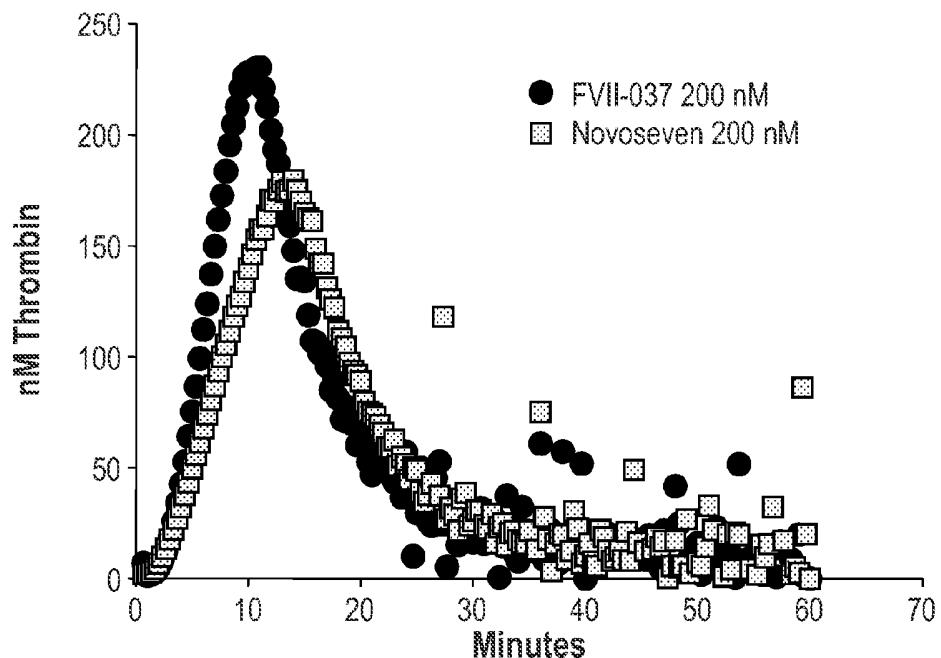


Fig. 16A

17/68

Thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets

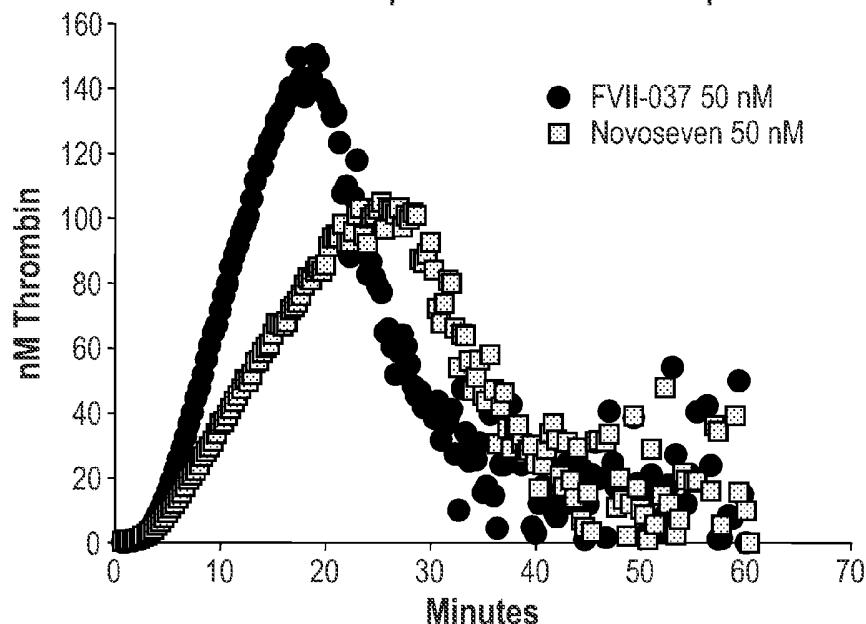


Fig. 16B

Thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets

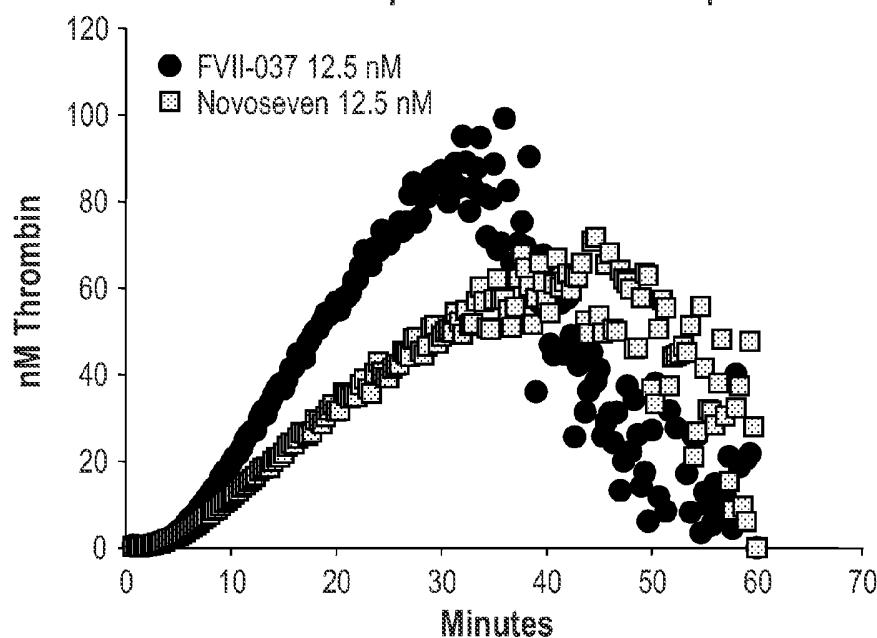
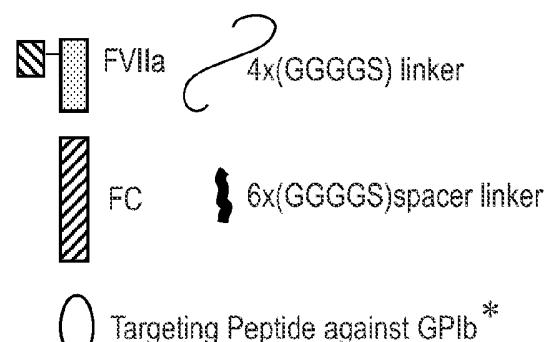
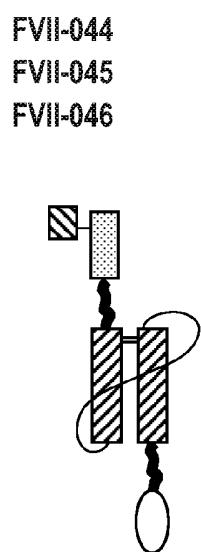


Fig. 16C

Thrombin generation assay to measure activity of FVII-044, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets



Peptide*	Affinity K_D , nM	FVIIFc
PS4	64	-044
OS1	0.74	-045
OS2	31	-046

* Benard et al. Biochemistry 2008, 47: 4674-4682

Fig. 17

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Thrombin generation assay to measure activity of FVII-044, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets

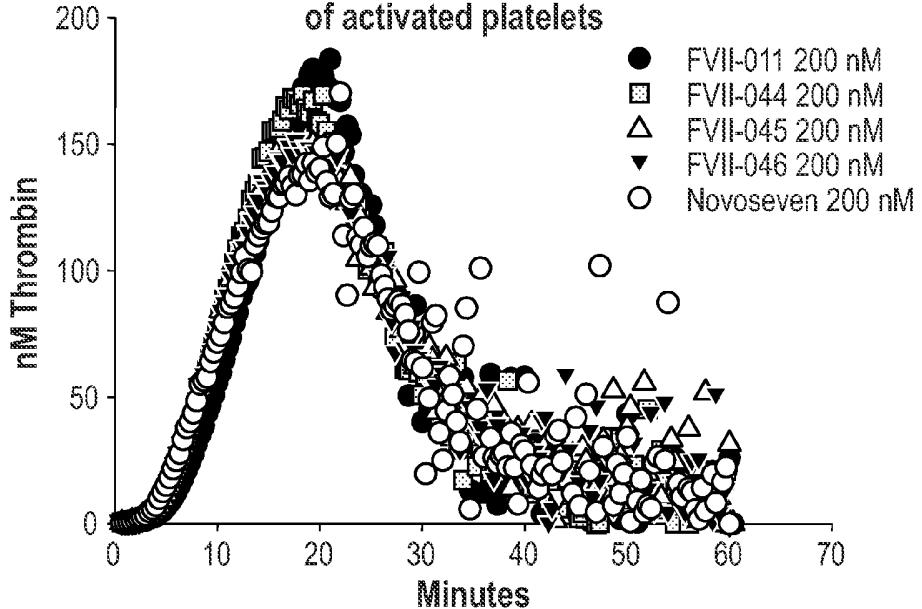


Fig. 18A

Thrombin generation assay to measure activity of FVII-044, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets

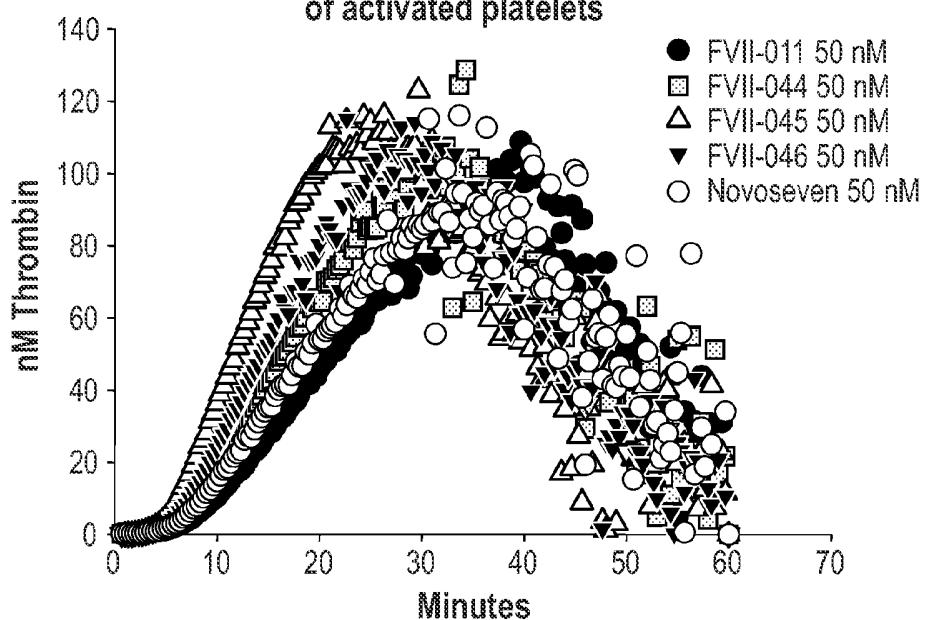
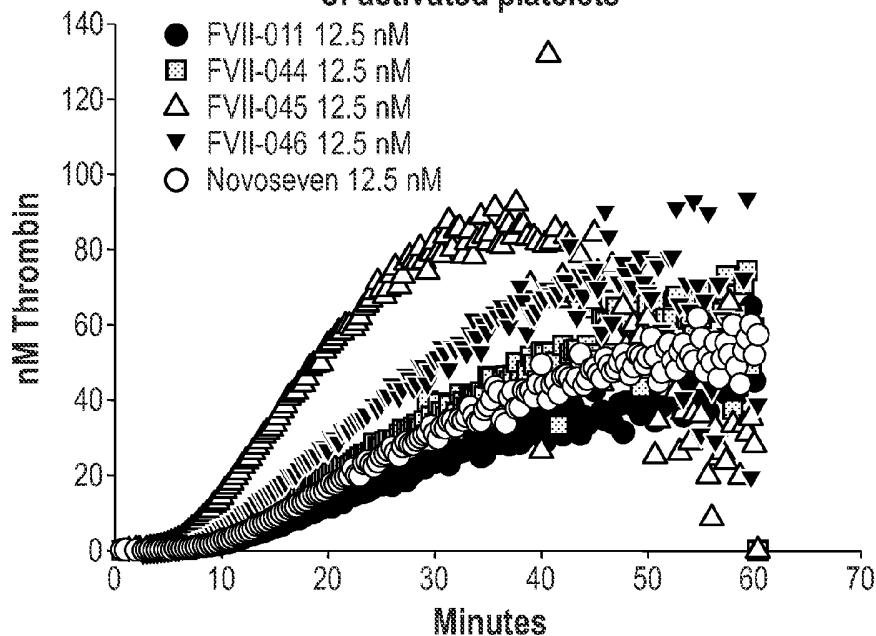


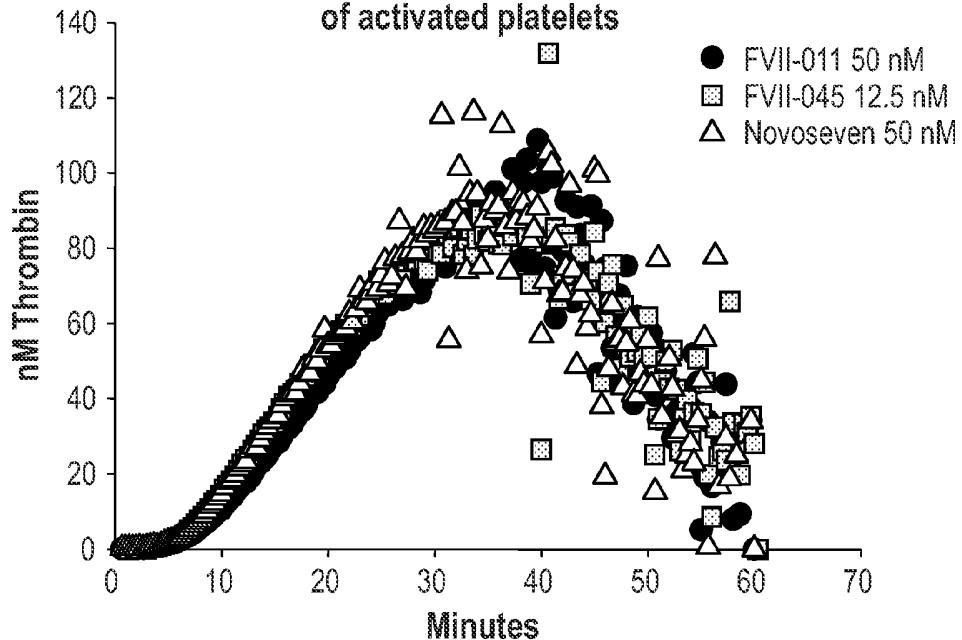
Fig. 18B

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Thrombin generation assay to measure activity of FVII-044, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets

**Fig. 18C**

Thrombin generation assay to measure activity of FVII-045, FVII-011 and Novoseven in the presence of activated platelets

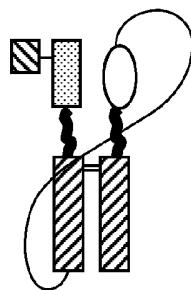
**Fig. 18D**

**Thrombin generation assay to measure activity of FVII-047,
FVII-048, FVII-049, FVII-011 and Novoseven
in the presence of activated platelets**

FVII-047

FVII-048

FVII-049



FVIIa

6x(GGGGS) linker

FC

6x(GGGGS) spacer linker

Targeting Peptide against GPIb *

Peptide*	Affinity K_D , nM	FVIIFc
PS4	64	-047
OS1	0.74	-048
OS2	31	-049

* Benard et al. Biochemistry 2008, 47: 4674-4682

Fig. 19

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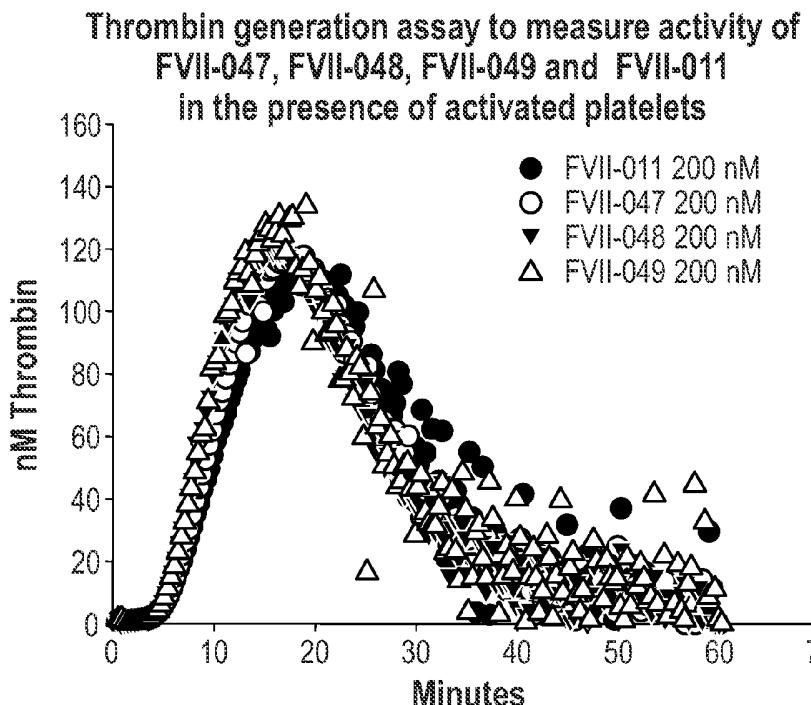


Fig. 20A

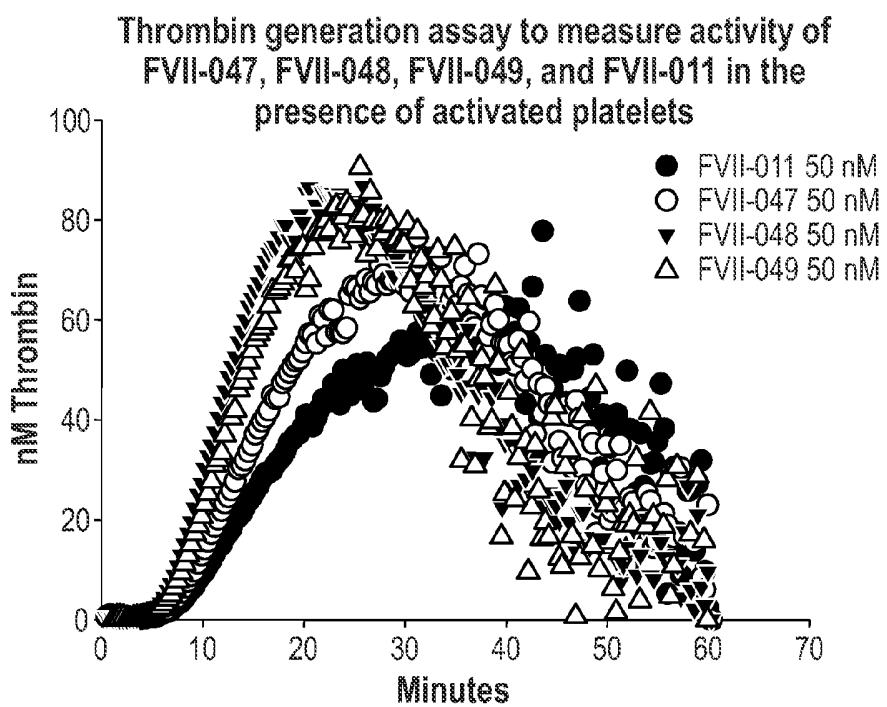


Fig. 20B

23/68

Thrombin generation assay to measure activity of
FVII-047, FVII-048, FVII-049, and FVII-011
in the presence of activated platelets

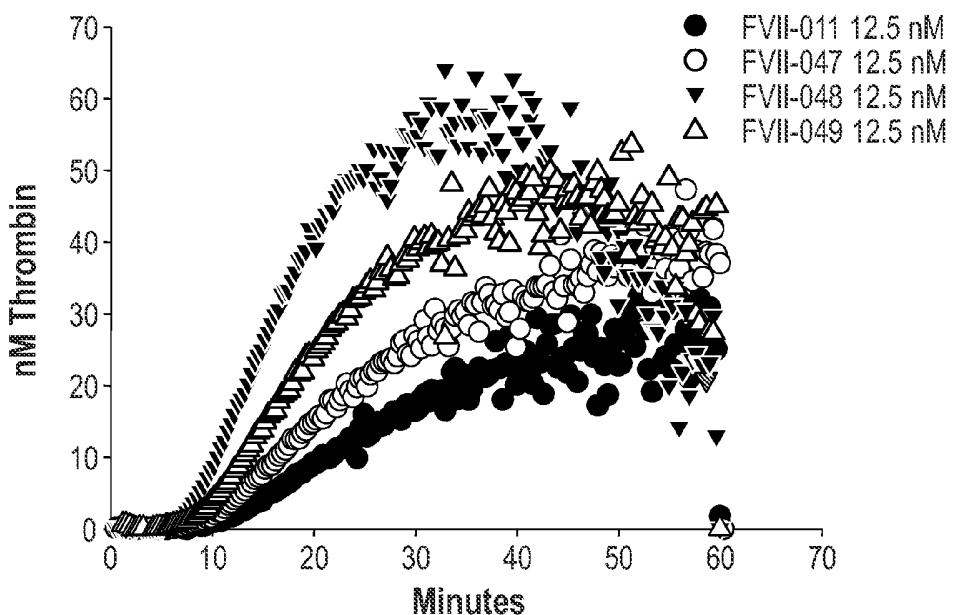


Fig. 20C

Thrombin generation assay to measure activity of
FVII-047, FVII-048, FVII-049, and FVII-011
in the presence of activated platelets

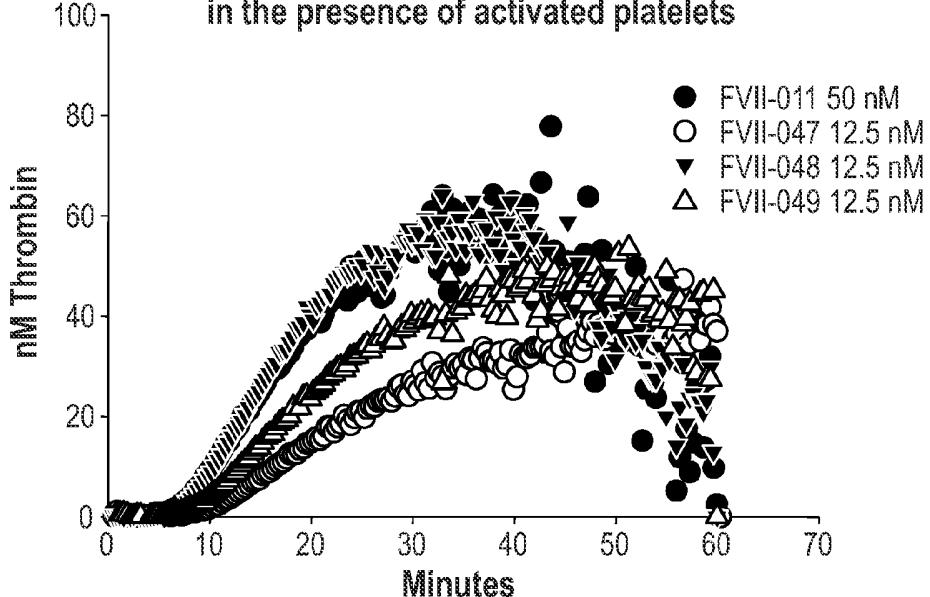


Fig. 20D

**Thrombin generation assay to measure activity of FVII-053
and FVII-011 in the presence of activated platelets**

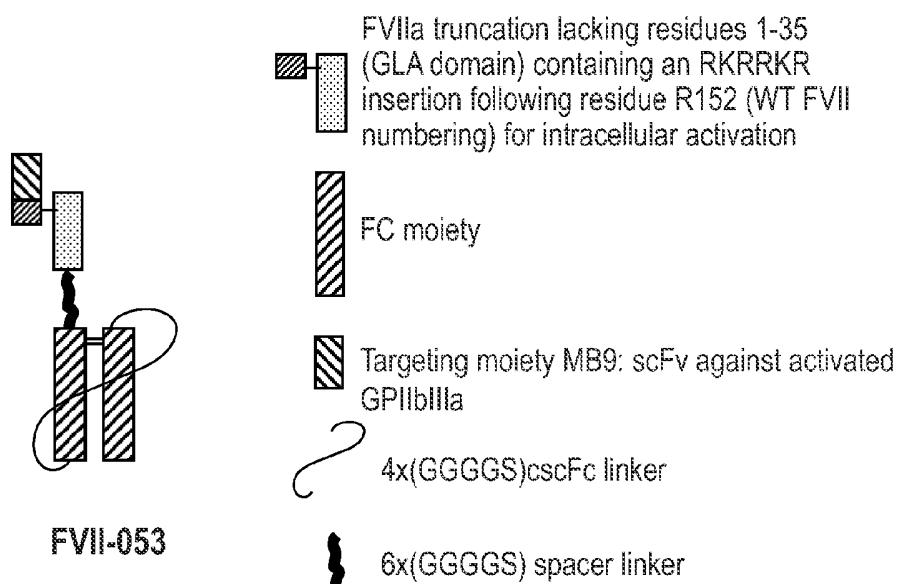


Fig. 21

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Thrombin generation assay to measure activity of FVII-053
and FVII-011 in the presence of activated platelets

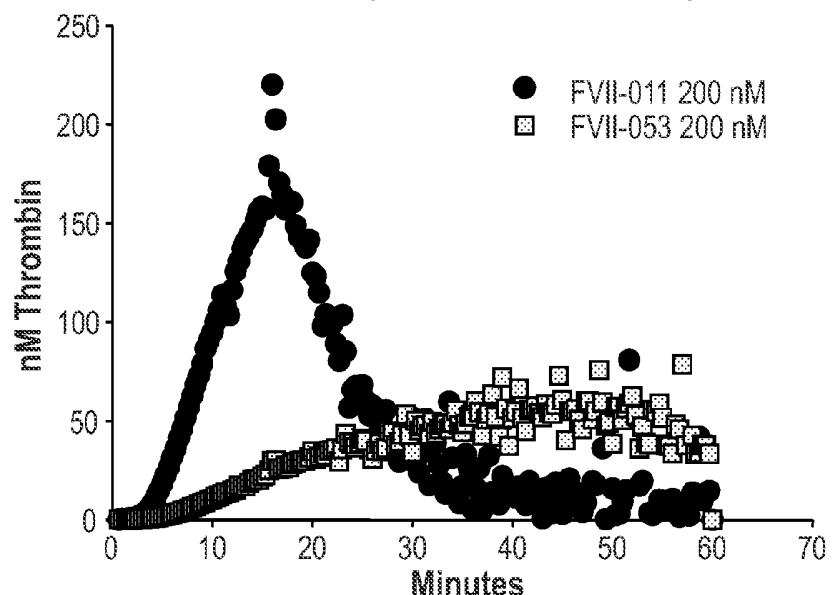


Fig. 22A

Thrombin generation assay to measure activity of FVII-053
and FVII-011 in the presence of activated platelets

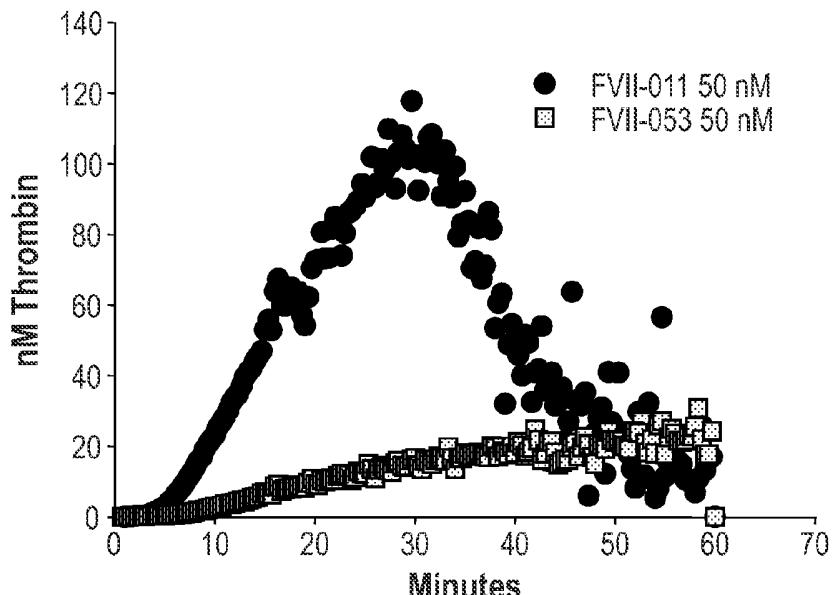


Fig. 22B

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Thrombin generation assay to measure activity of FVII-053
and FVII-011 in the presence of activated platelets

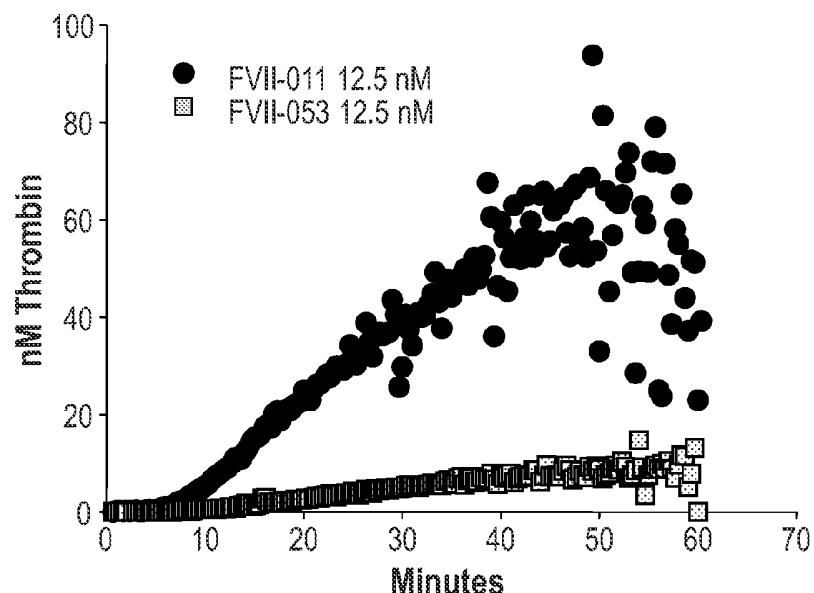


Fig. 22C

Thrombin generation assay to measure activity of FVII-053
and FVII-011 in the presence of activated platelets

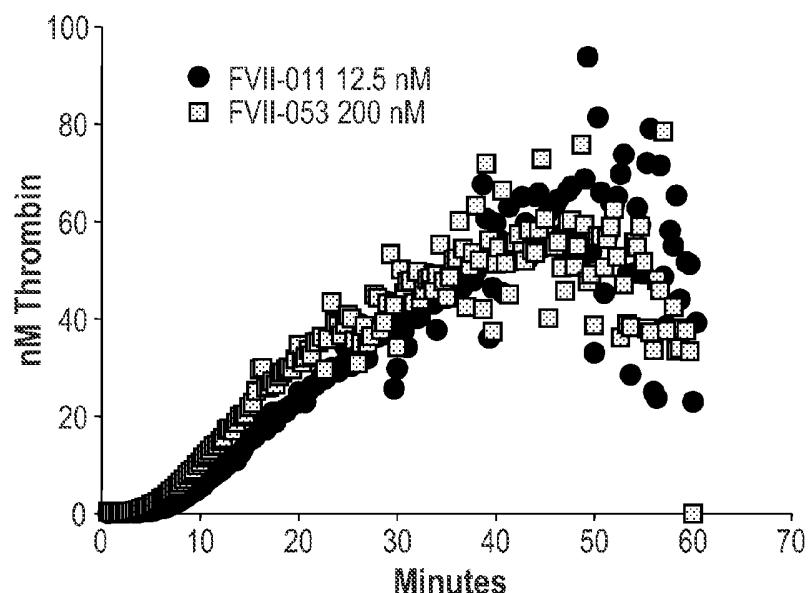


Fig. 22D

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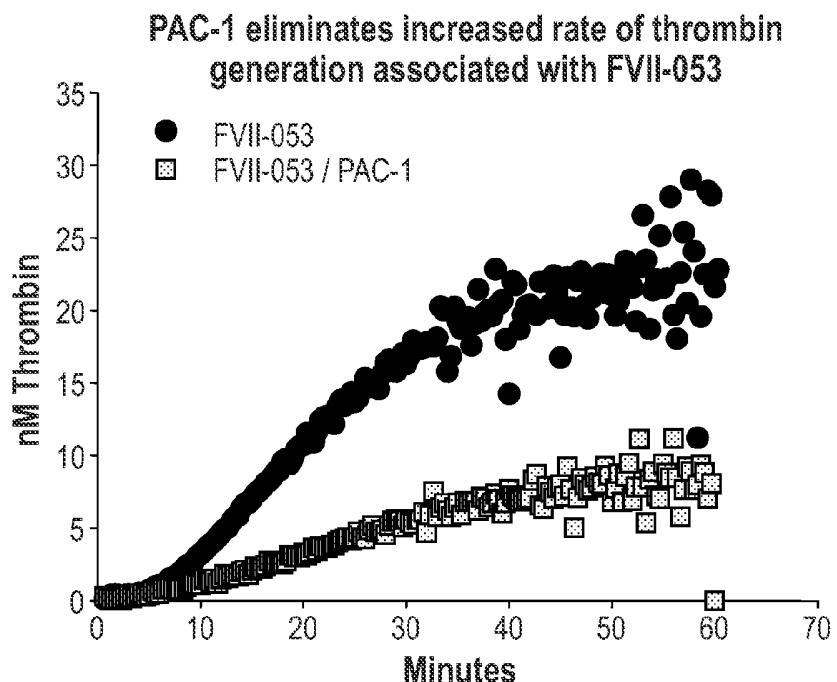


Fig. 23A

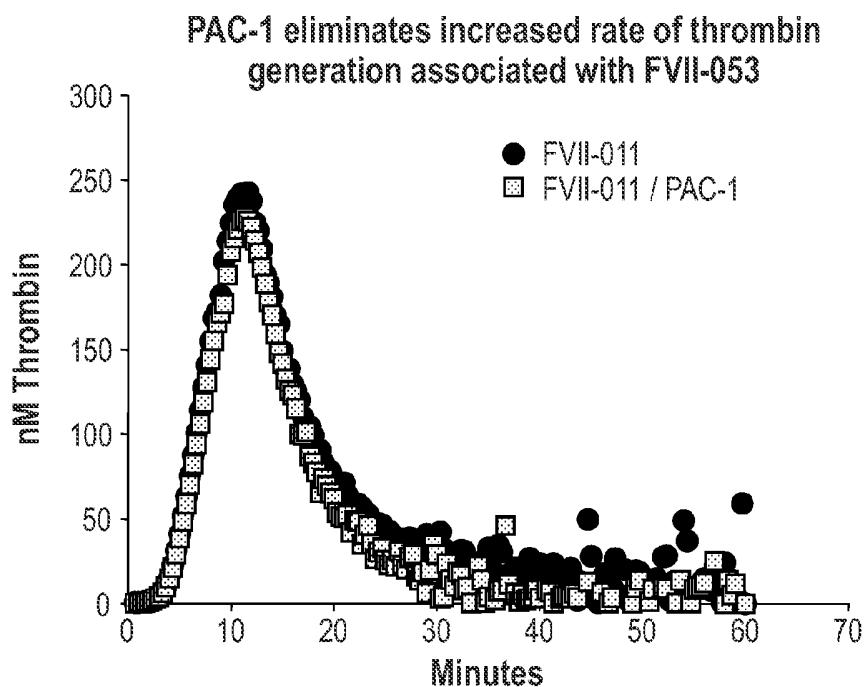


Fig. 23B

Western blot analysis of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown

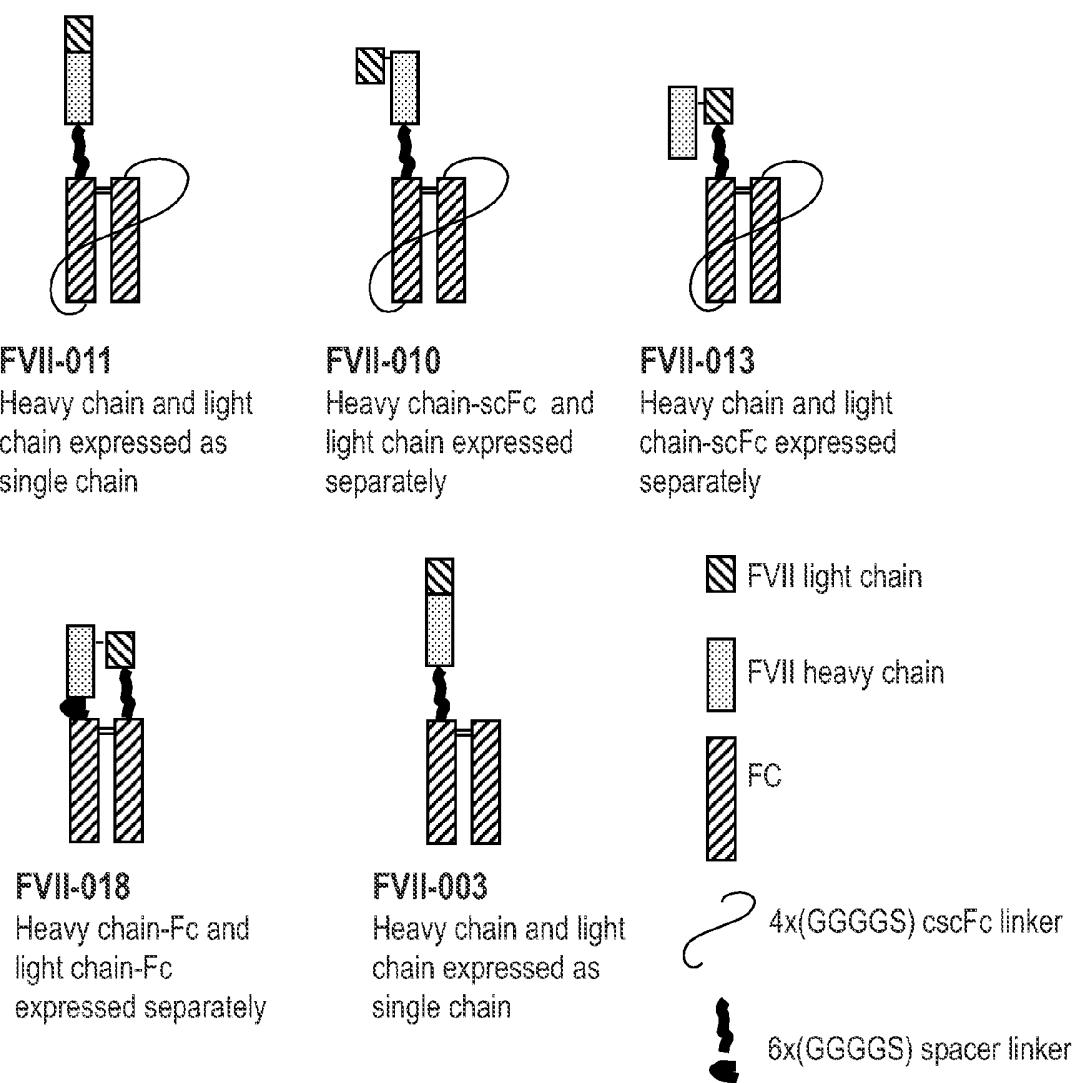


Fig. 24

Western blot analysis of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown

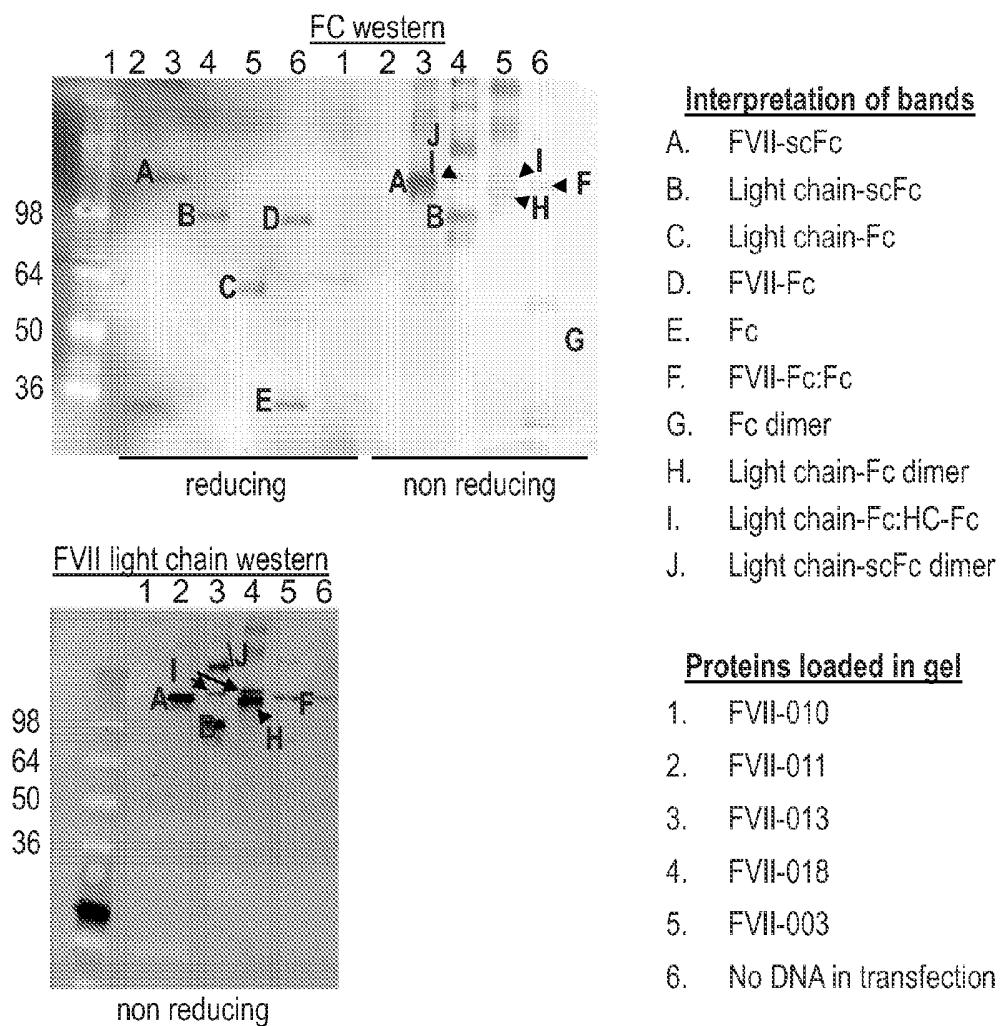


Fig. 25

Western blot of protein A immunoprecipitation following transient transfection of pSYN-FVII-024 with or without pSYN-PC5-003. Lane 1, Seeblue Plus2 marker; lane 2, pSYN-FVII-024, non reducing; lane 3, pSYN-FVII-024+pSYN-PC5-003, non reducing; lane 4, pSYN-FVII-024, reducing; lane 5, pSYN-FVII-024+pSYN-PC5-003, reducing.

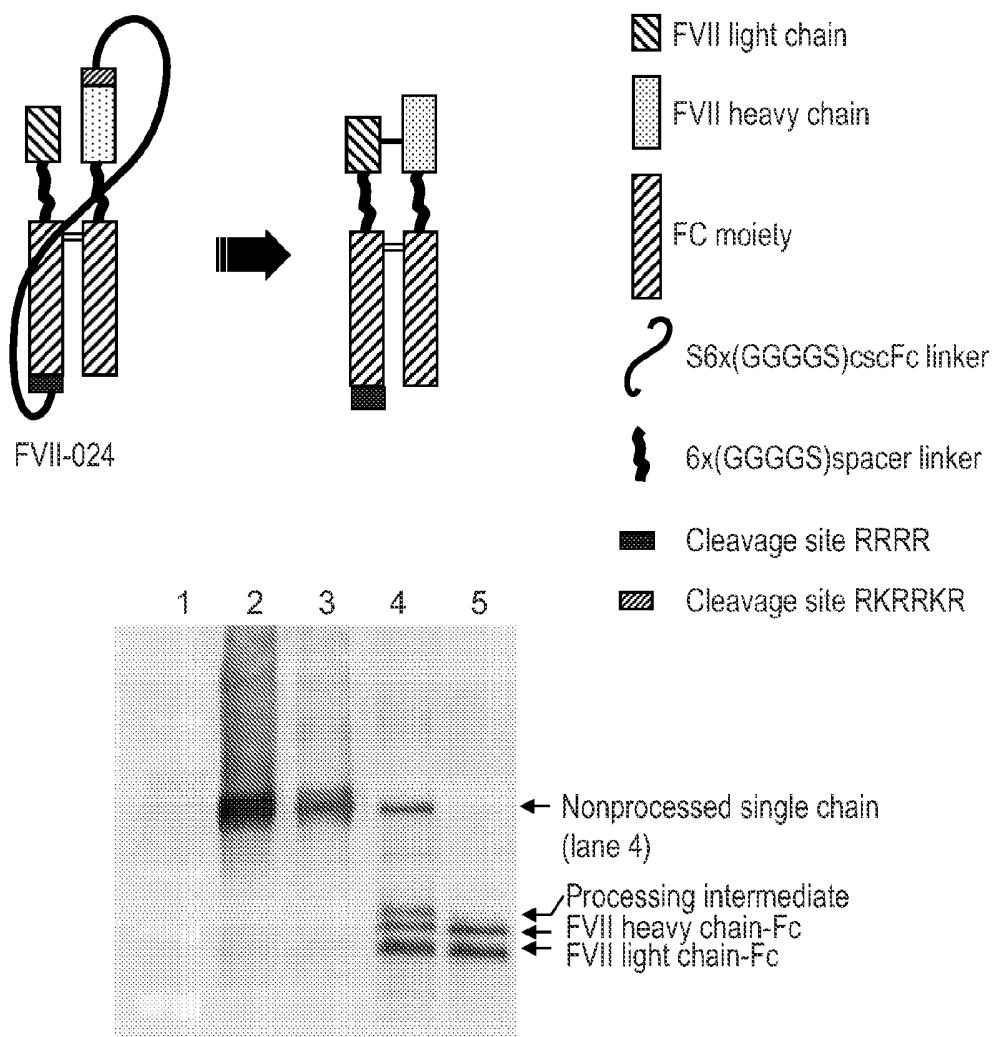


Fig. 26

Western blot analysis (Fc western) of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown

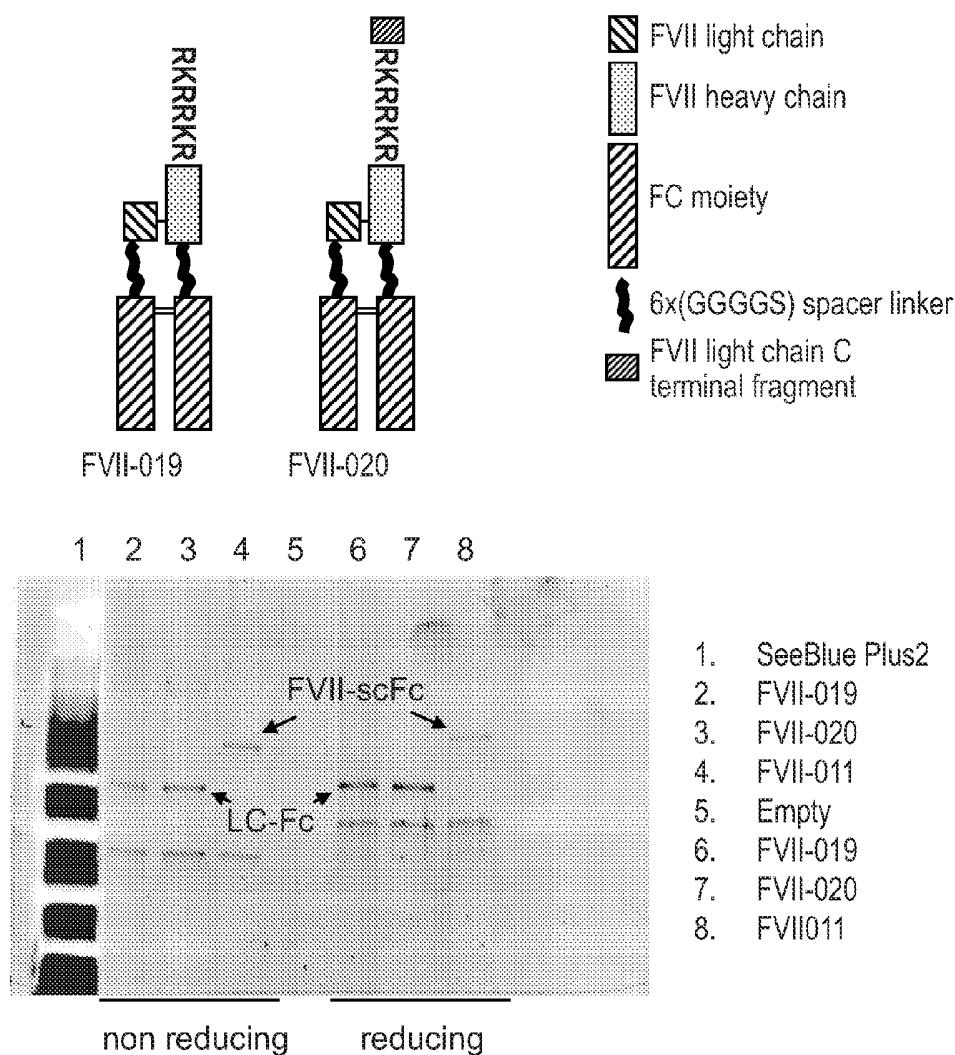
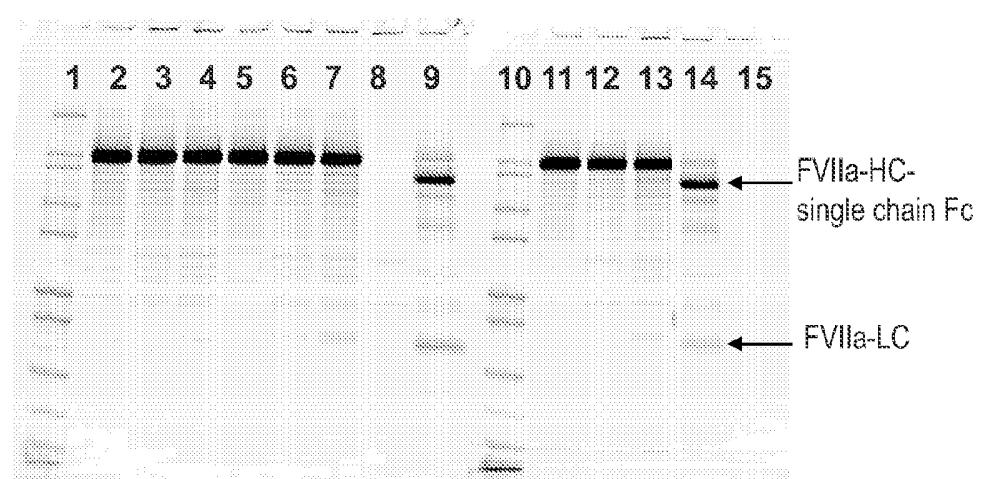
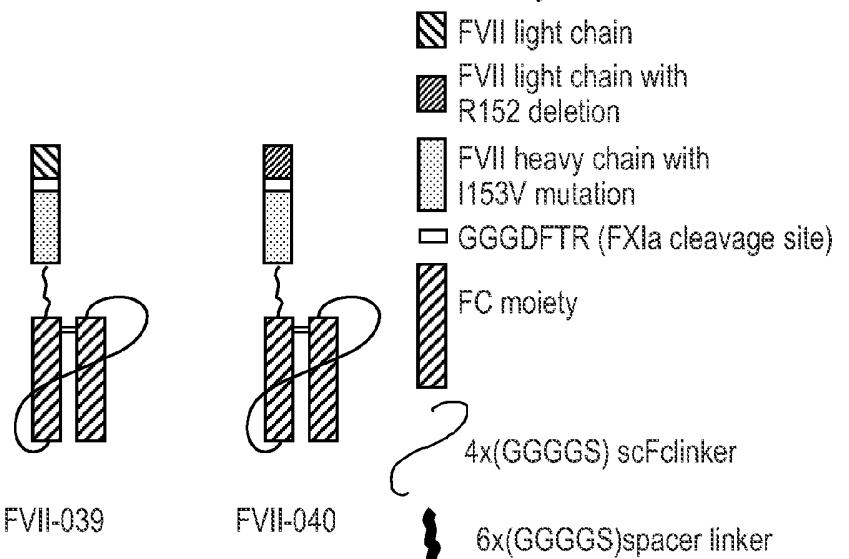


Fig. 27

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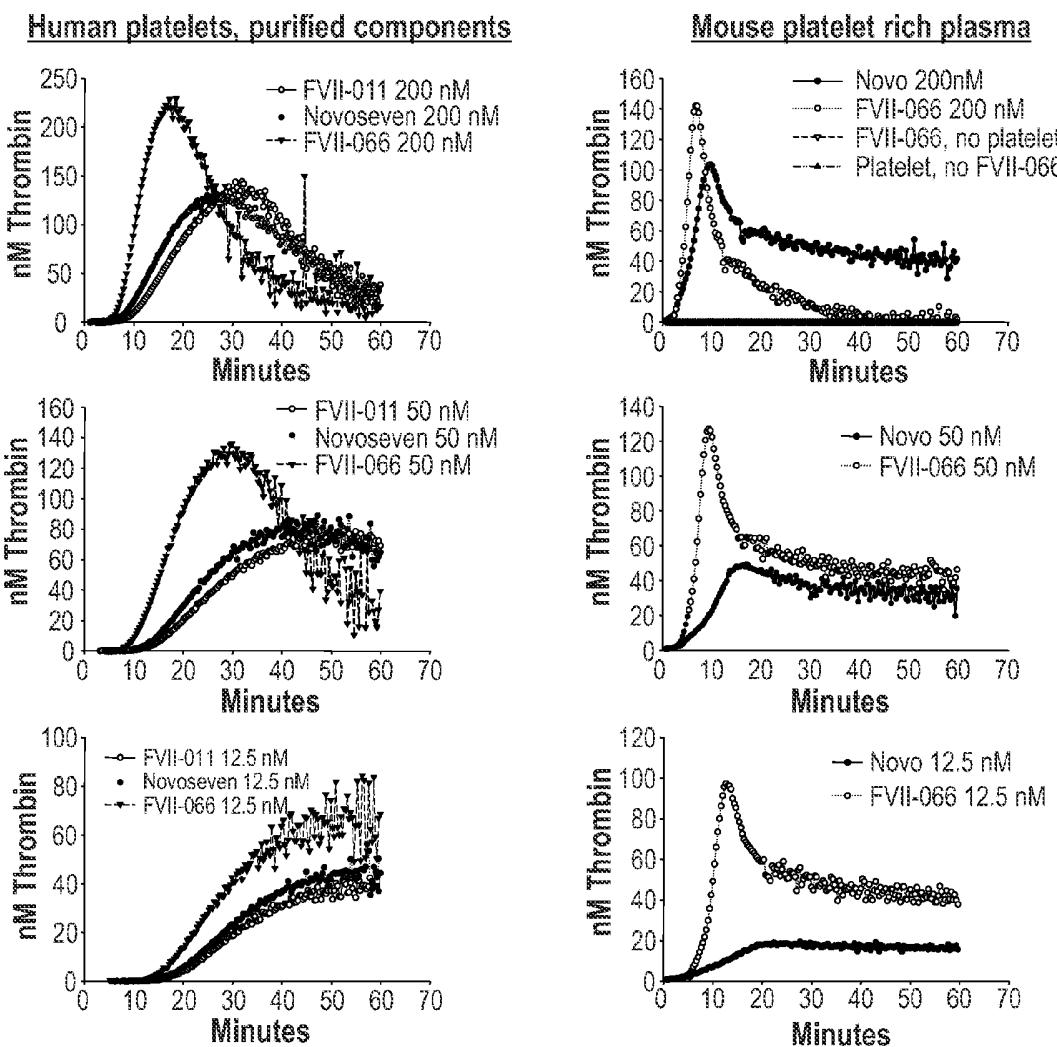
FVII-039 and FVII-040 Treatment by FXIa



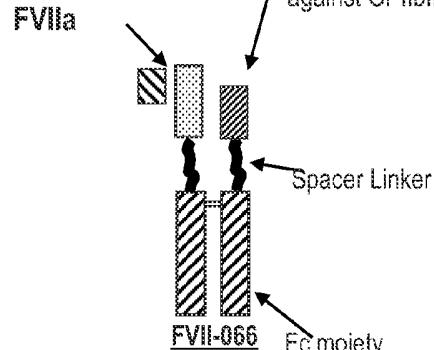
1. Mark-12 (Invitrogen)
2. FVII-011
3. FVII-011+FXIa, 5' incubation
4. FVII-011+FXIa, 20' incubation
5. FVII-039
6. FVII-039+FXIa, 5' incubation
7. FVII-039+FXIa, 20' incubation
8. FXIa
9. FVII-011, activated
10. Mark-12 (Invitrogen)
11. FVII-040
12. FVII-040+FXIa, 5' incubation
13. FVII-040+FXIa, 20' incubation
14. FVII-011, activated
15. FXIa

Fig. 28

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Targeting moiety SCE5: scFv
against GPIIbIIa



SCE5

- scFv against active GPIIbIIa
- Crossreacts with mouse and human receptors

Fig. 29

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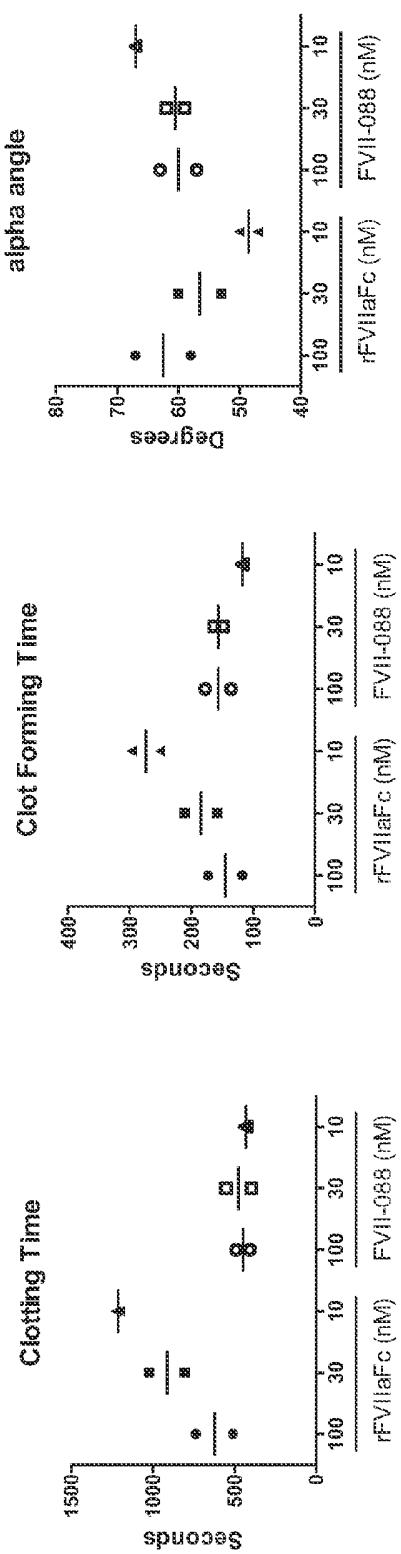


Fig. 30

Activatable ConstructsFXIa cleavage sites

LC-7x(GGGGS)-SVSQTSKLTR-IVGG: FVII-057

Thrombin cleavage sites

LC-7x(GGGGS)-DFLAEAGGGVR-IVGG : FVII-058

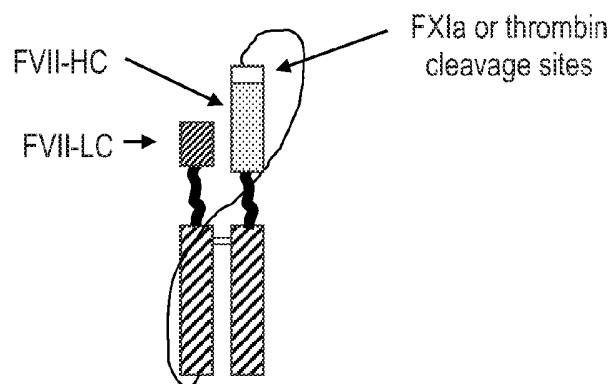
LC-7x(GGGGS)-TTKIKPR-IVGG : FVII-059

LC-7x(GGGGS)-ALRPRVVGGA-VVGG : FVII-060

LC-7x(GGGGS)-ALRPRVVGGA-IVGG : FVII-061

Negative control

LC-8x(GGGGS)-IVGG: FVII-062

***Fig. 31***

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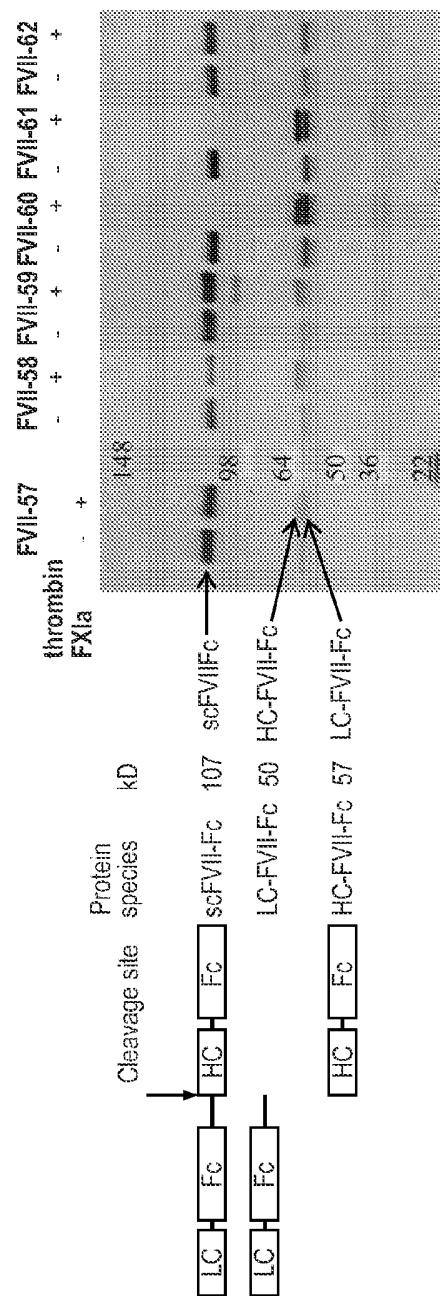


Fig. 32

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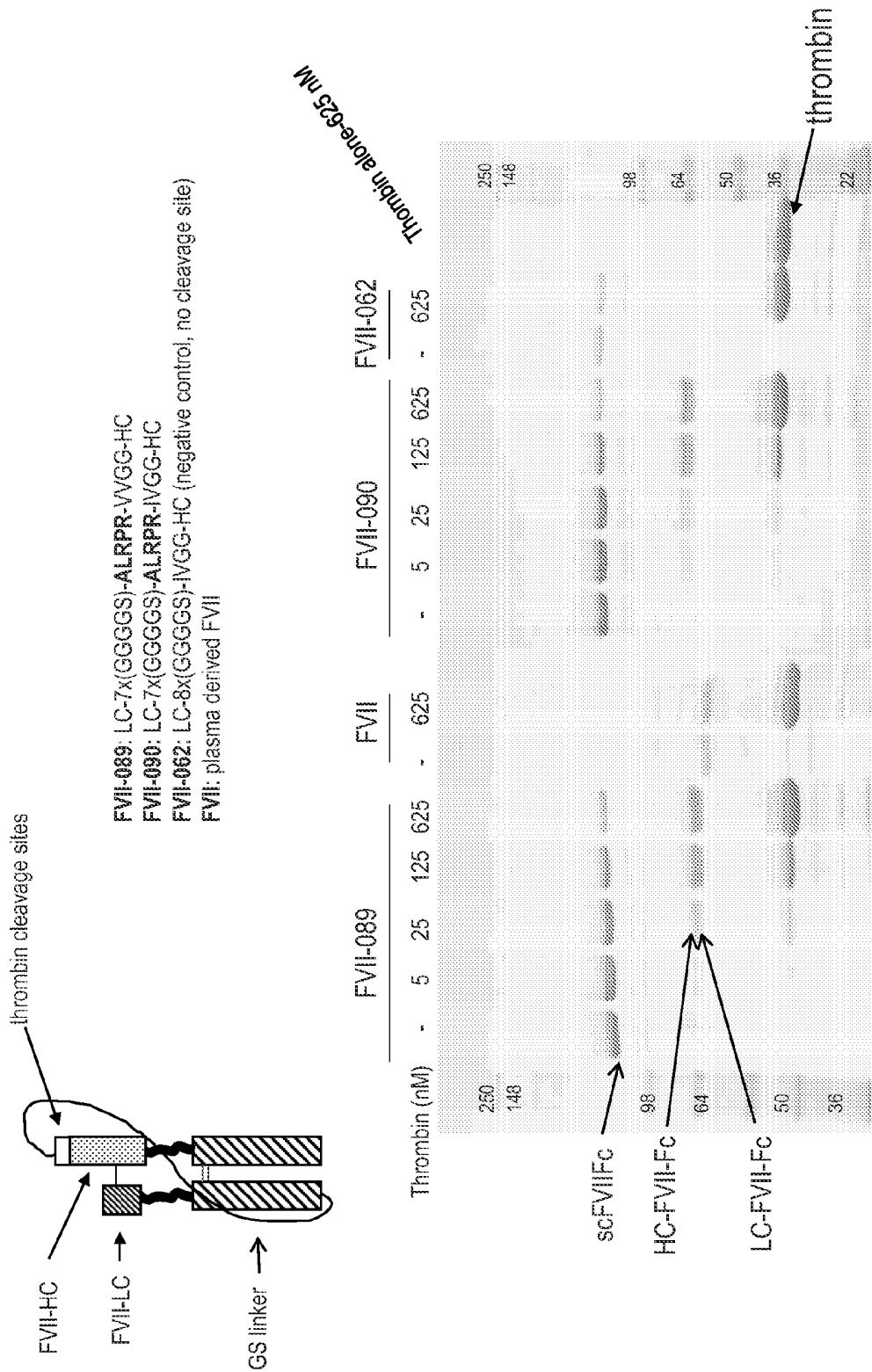


Fig. 33

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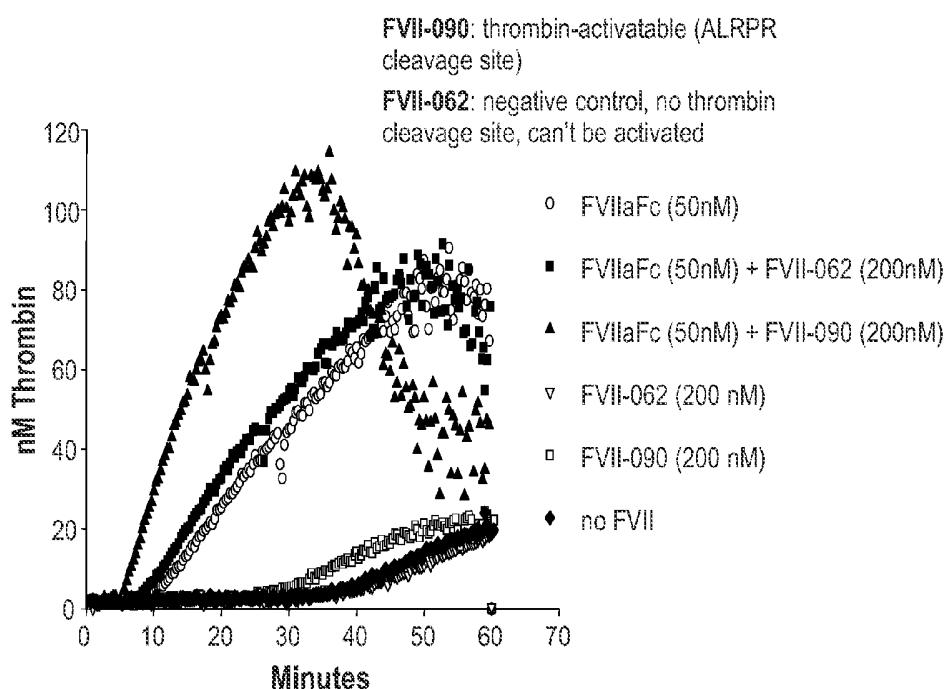


Fig. 34

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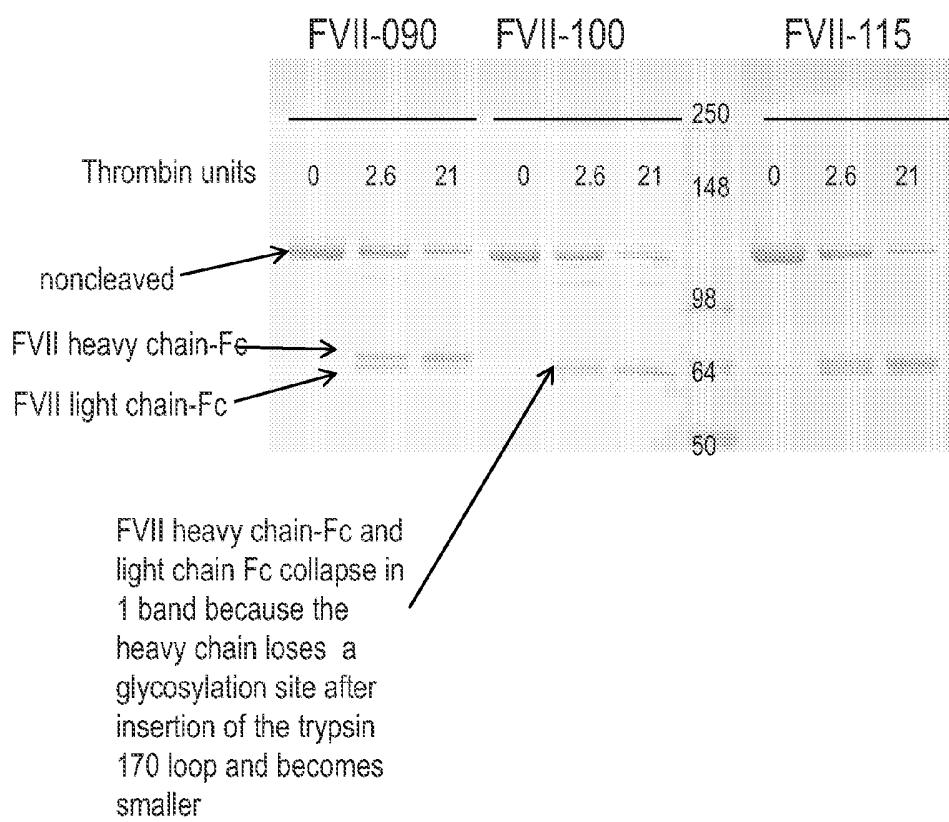


Fig. 35

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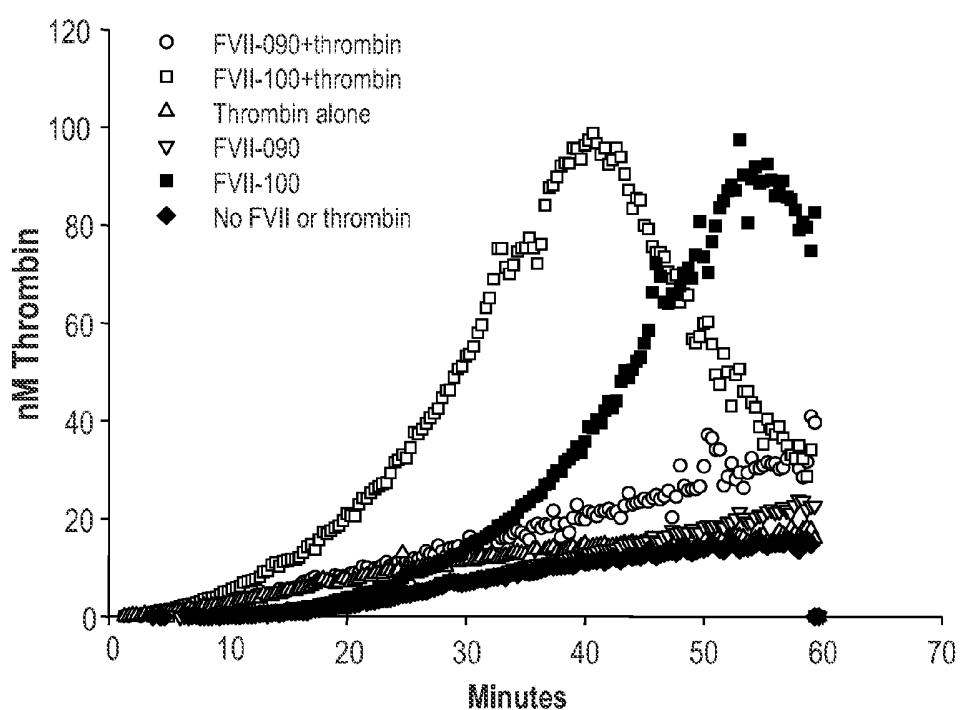


Fig. 36

41/68

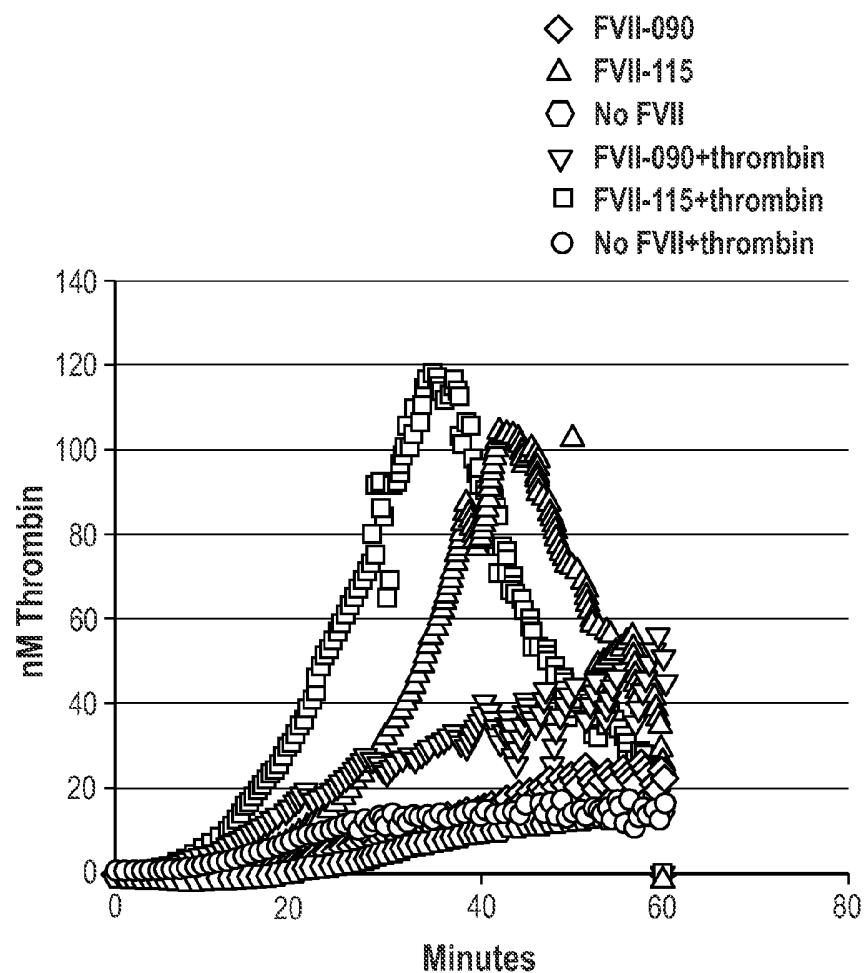


Fig. 37

Amidolytic activity of activatable FVIIFc activated with thrombin

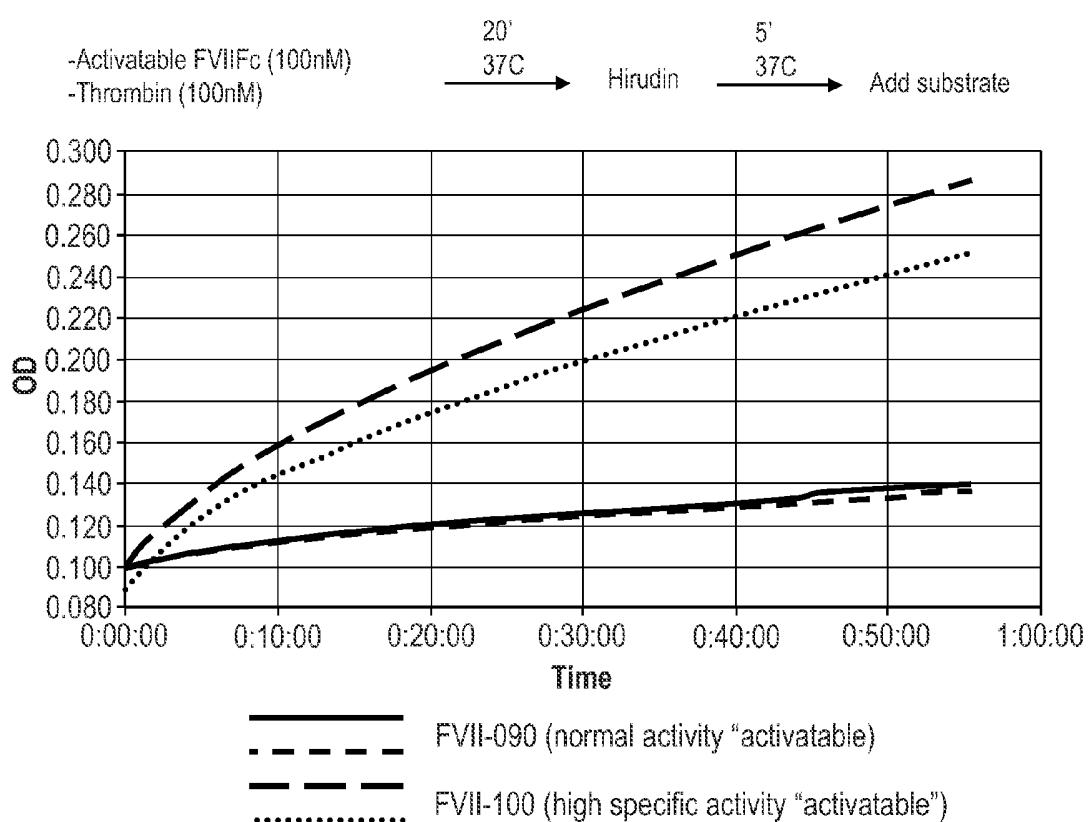


Fig. 38

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Activation of FX by FVIIa

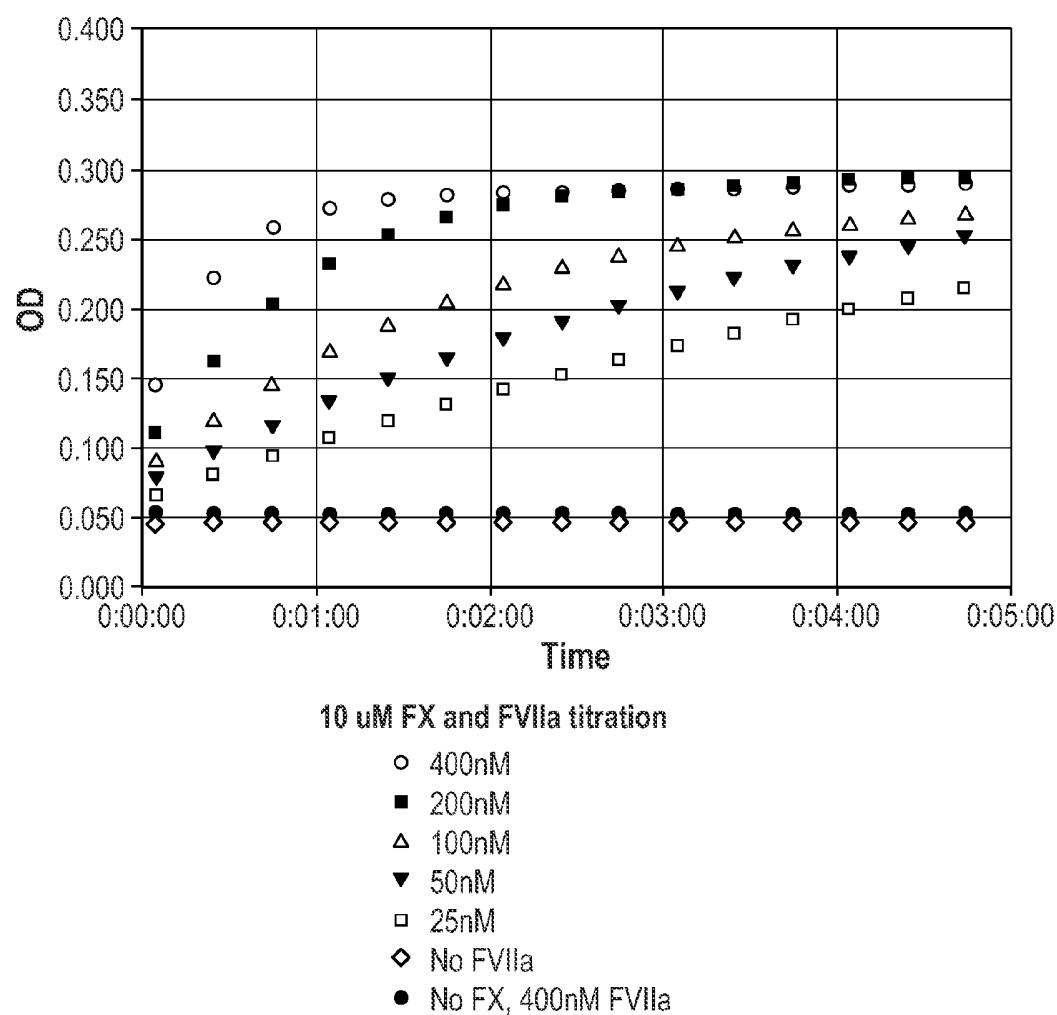


Fig. 39

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FXa generation activity by "activatable" FVIIIFc

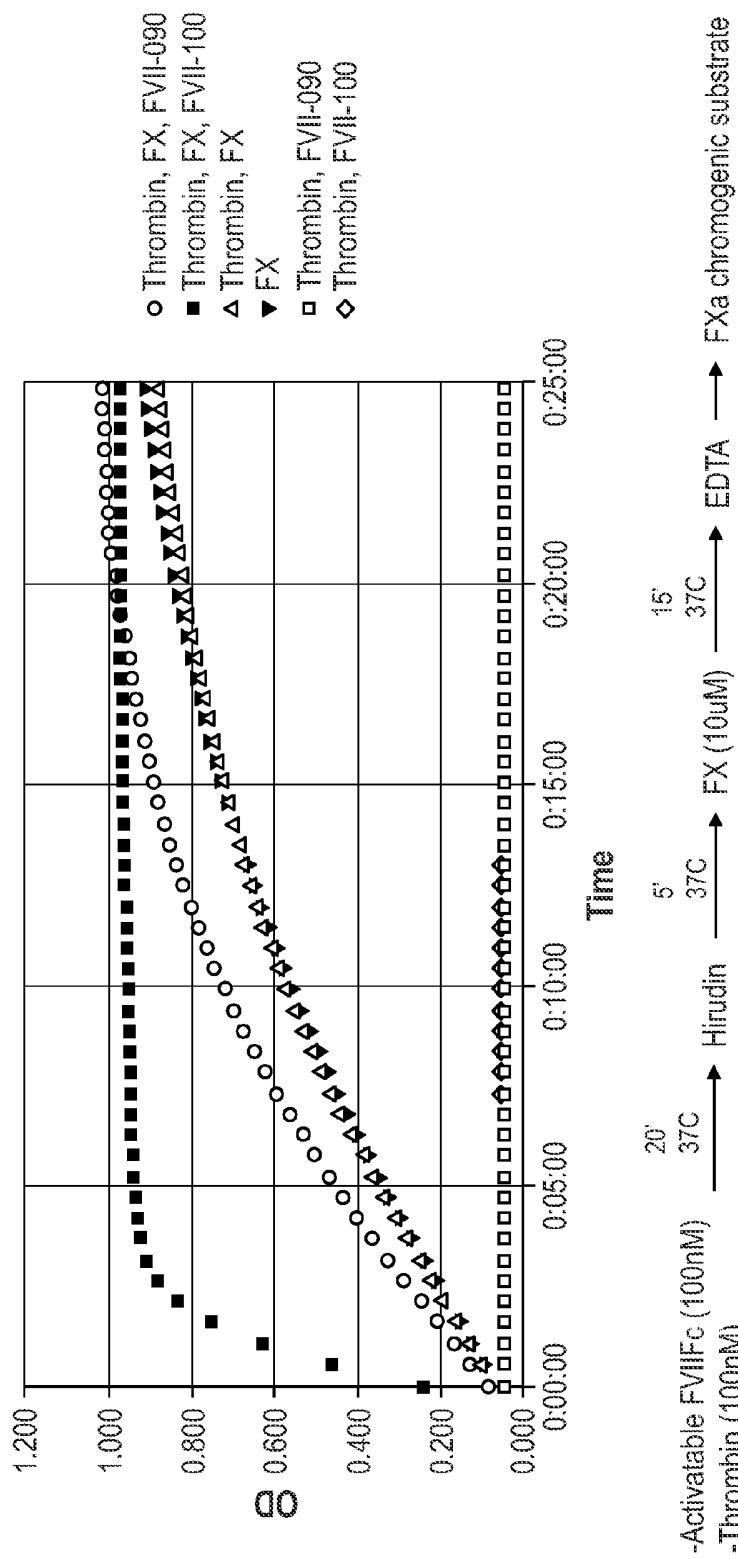
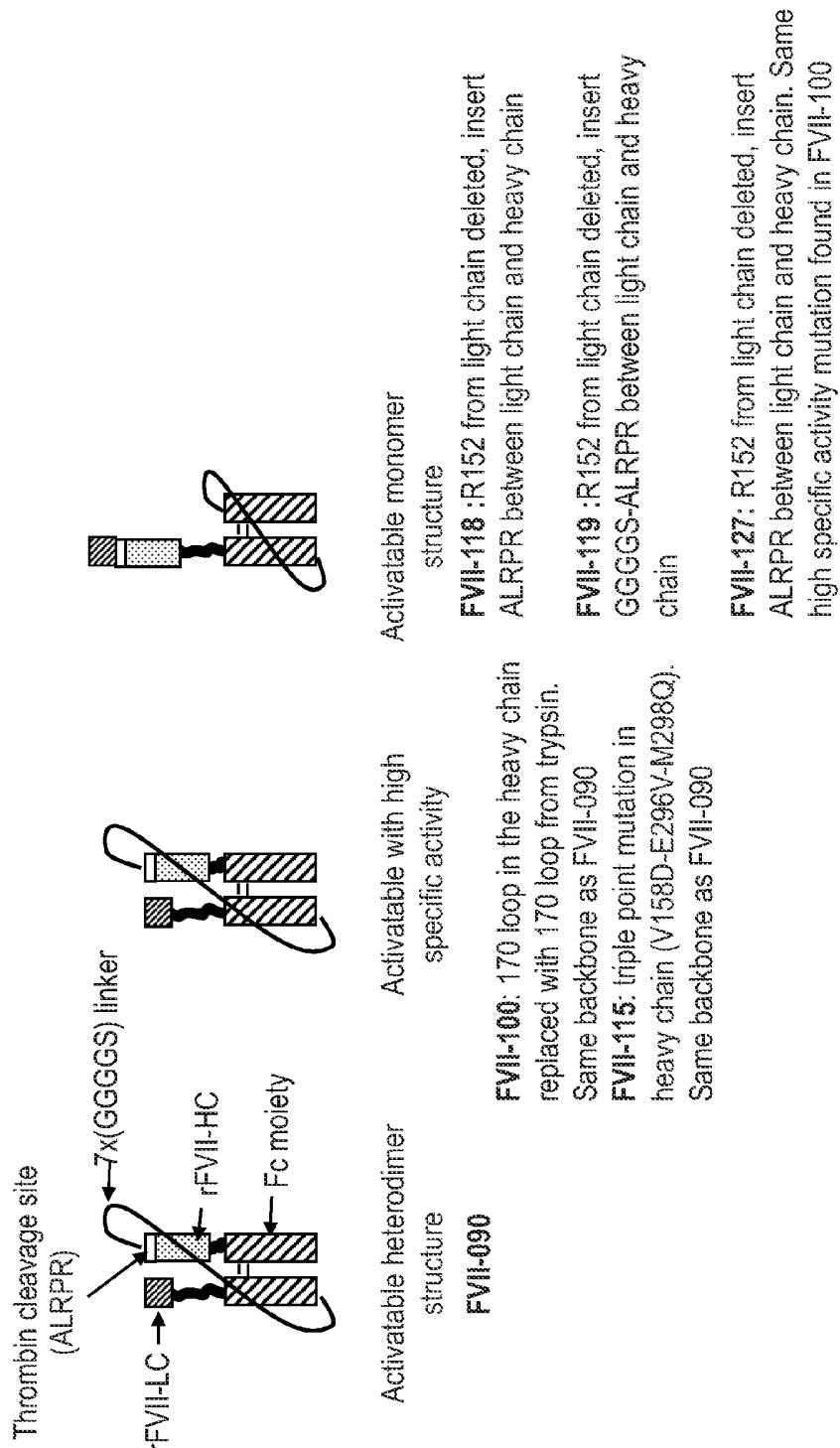


Fig. 40

**Fig. 41**

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Compare efficiency of thrombin cleavage of monomer (FVII-118, -119) vs heterodimer (FVII-090) activatable

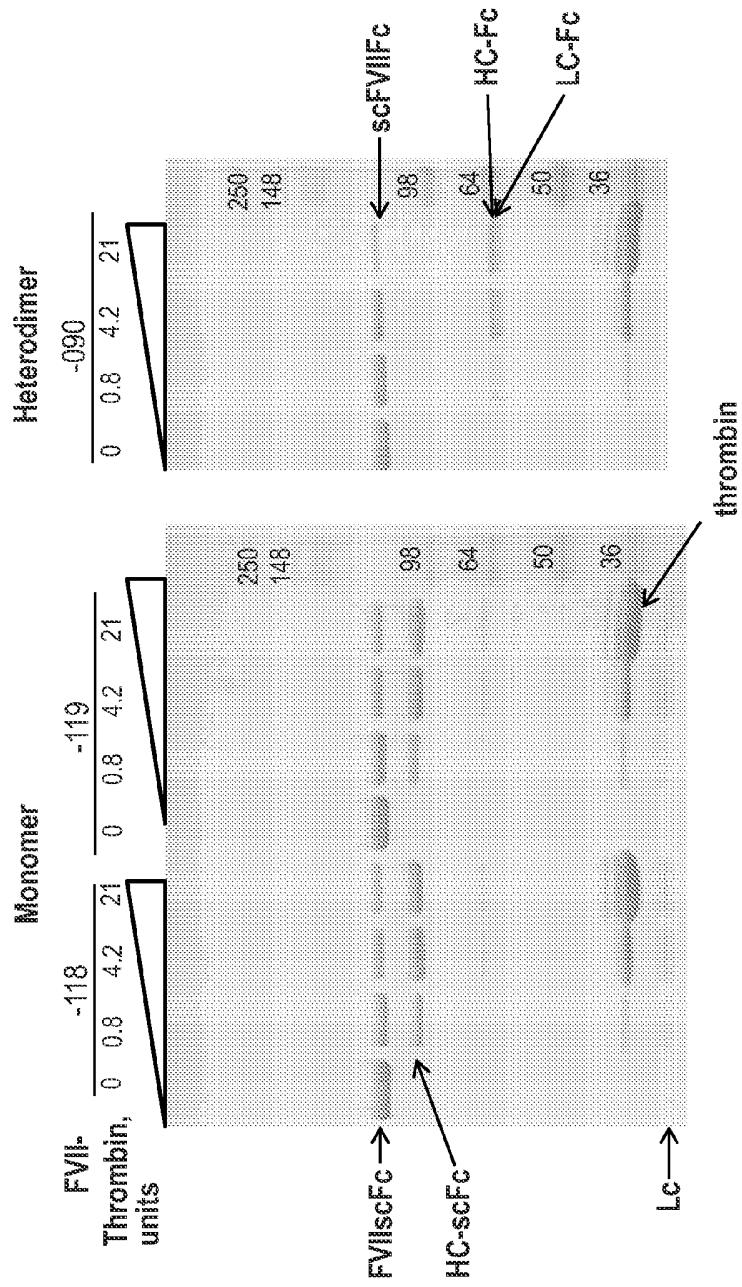


Fig. 42

Thrombin generation assay to compare wild type activatable FVIIFc (FVII-118) to high specific activity variant (FVII-127)

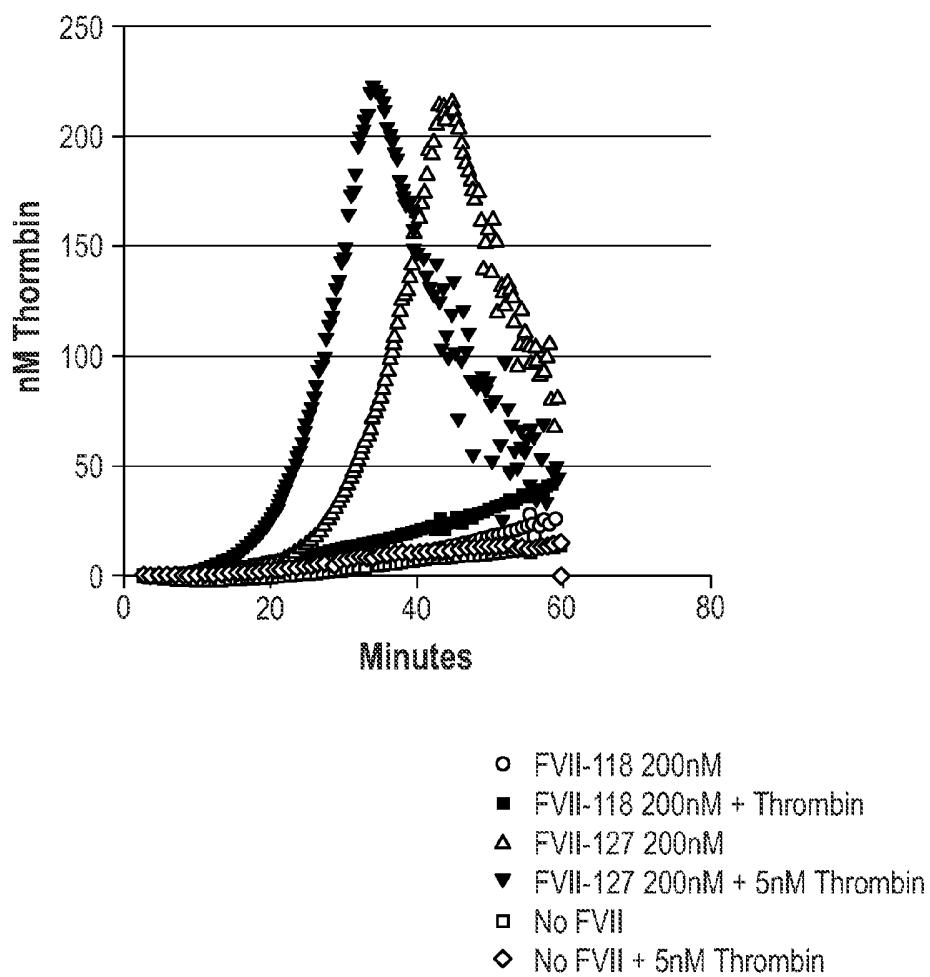


Fig. 43

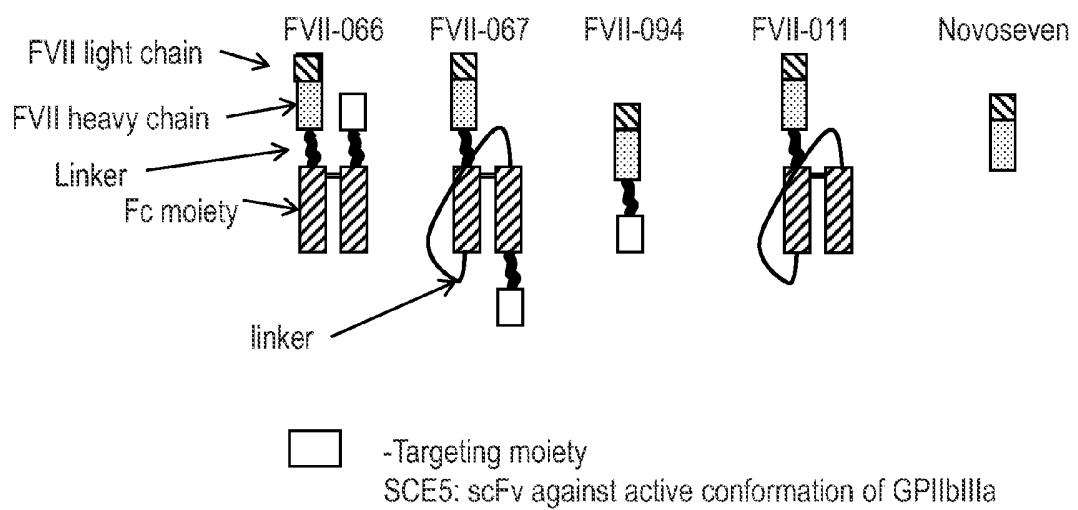
Target FVIIa to active conformation of GPIIbIIIa via scFv (SCE5)

Fig. 44A

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Thrombin generation assays in platelet-rich FVIII-deficient plasma

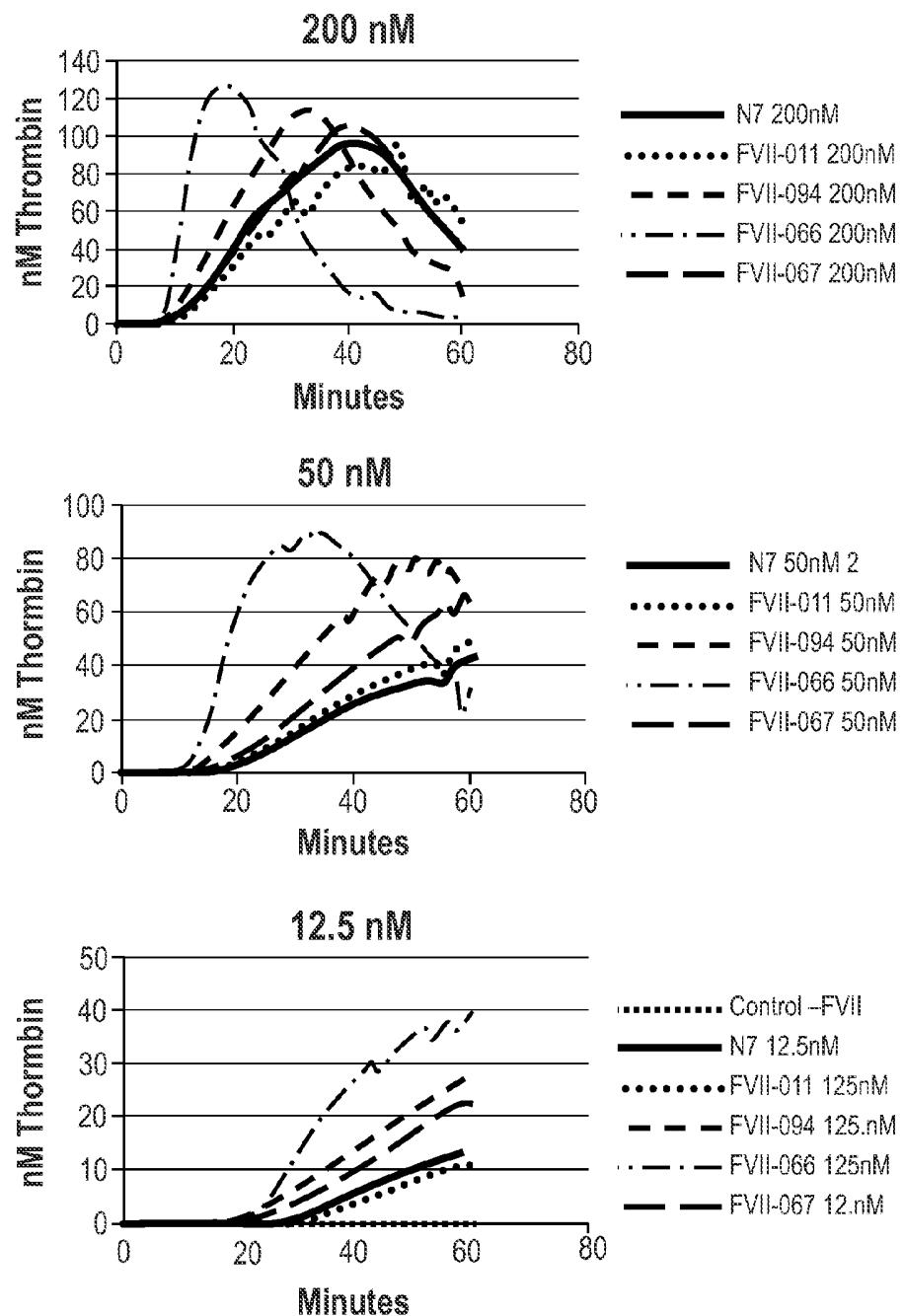


Fig. 44B

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Binding of rFVIIaFc variants to platelets by FACs

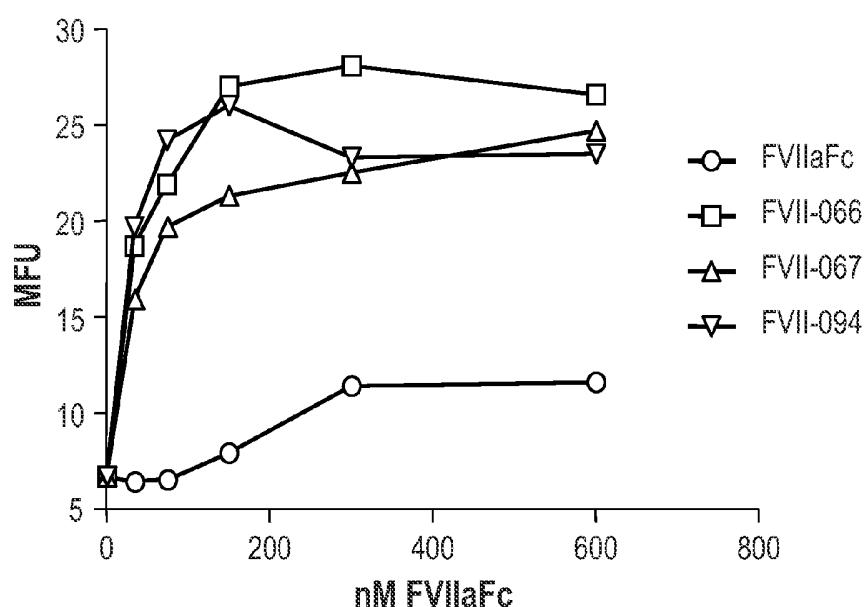
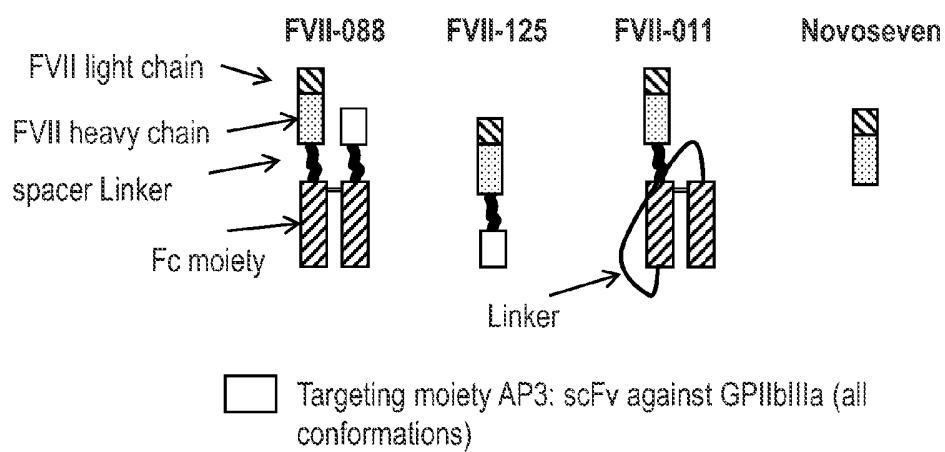


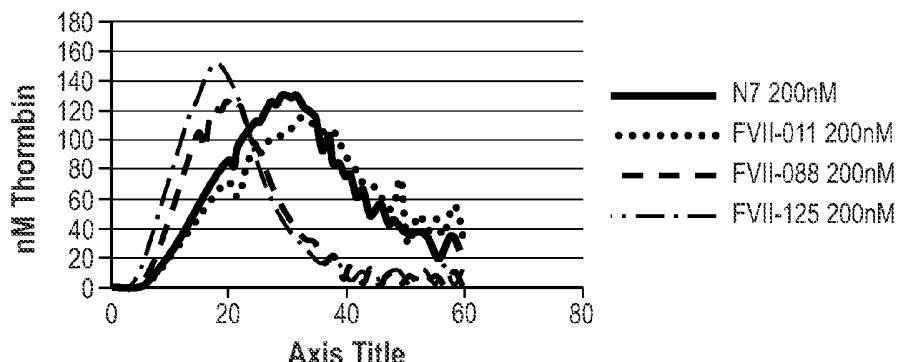
Fig. 44C

Target FVIIa to all conformations of GPIIbIIa via scFv (AP3)*Fig. 45A*

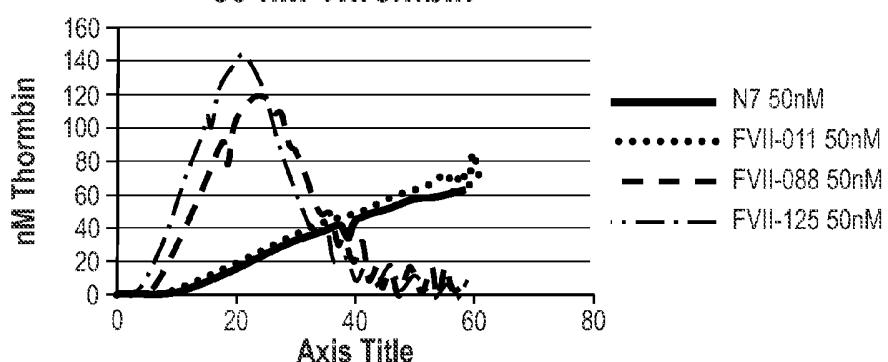
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Thrombin generation assays in platelet-rich FVIII-deficient plasma

200 nM Thrombin



50 nM Thrombin



12.5 nM Thrombin

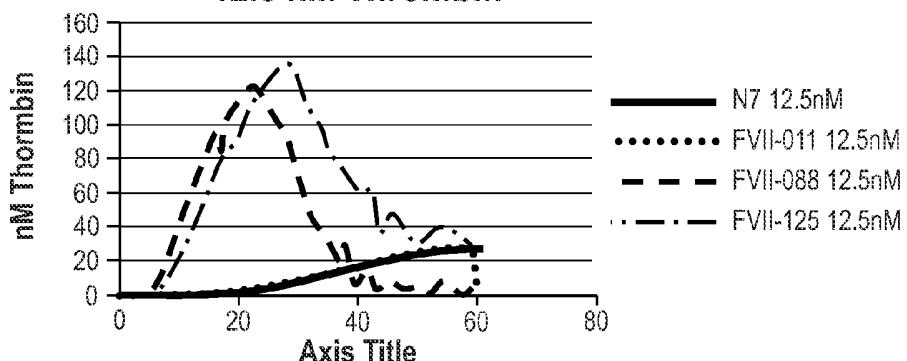


Fig. 45B

Binding of rFVIIaFc variants to platelets by FACs

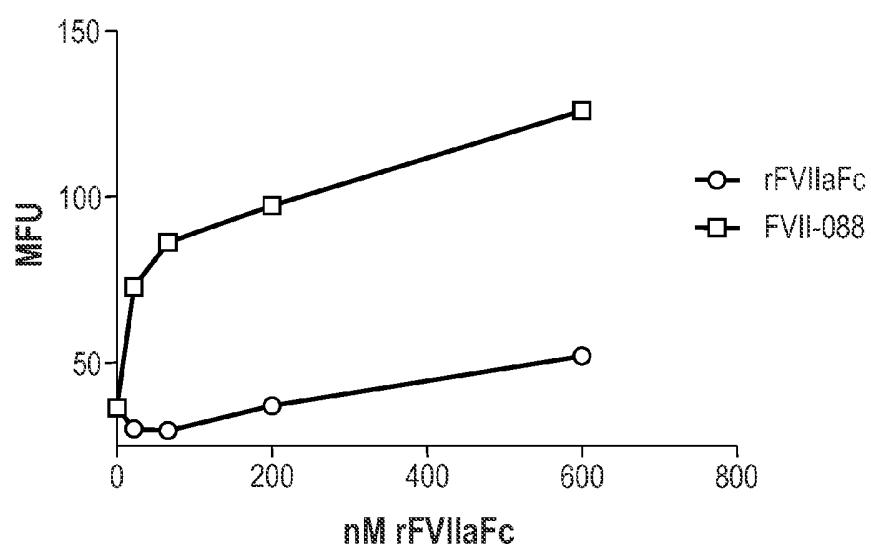
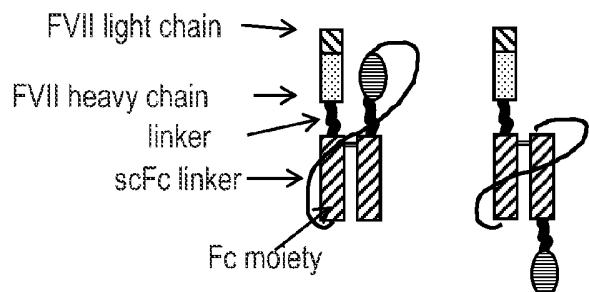


Fig. 45C

Targeting FVIIaFc to GPIb with peptides



○ -Targeting moieties PS4, OS1 and OS2: peptides against GPIb-alpha

<u>N-terminus</u>	<u>C-terminus</u>
-FVII-047 (PS4)	-FVII-044 (PS4)
-FVII-048 (OS1)	-FVII-045 (OS1)
-FVII-049 (OS2)	-FVII-046 (OS2)

Fig. 46A

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Thrombin generation assays in platelet-rich FVIII-deficient plasma

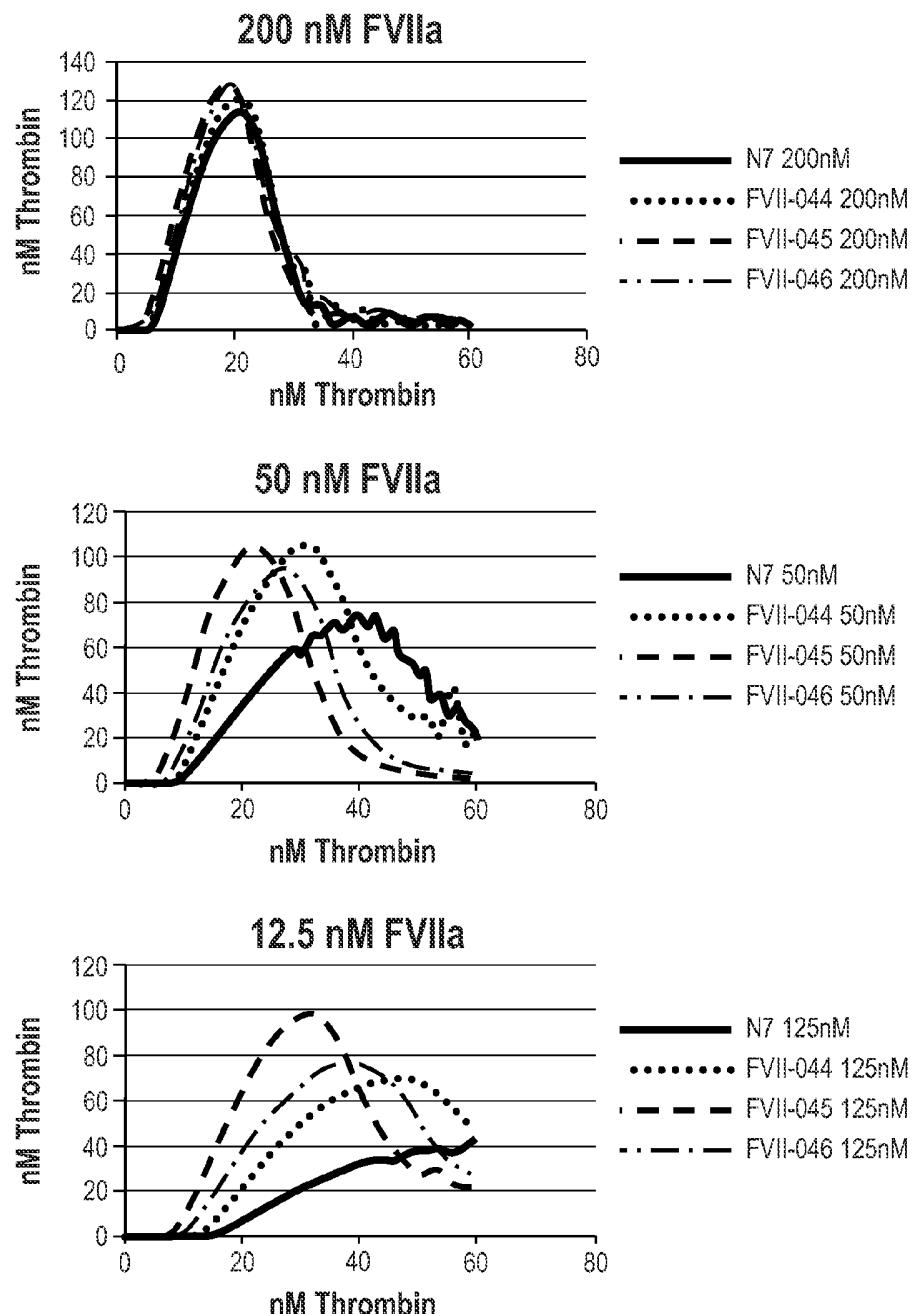


Fig. 46B

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Thrombin generation assays in platelet-rich FVIII-deficient plasma

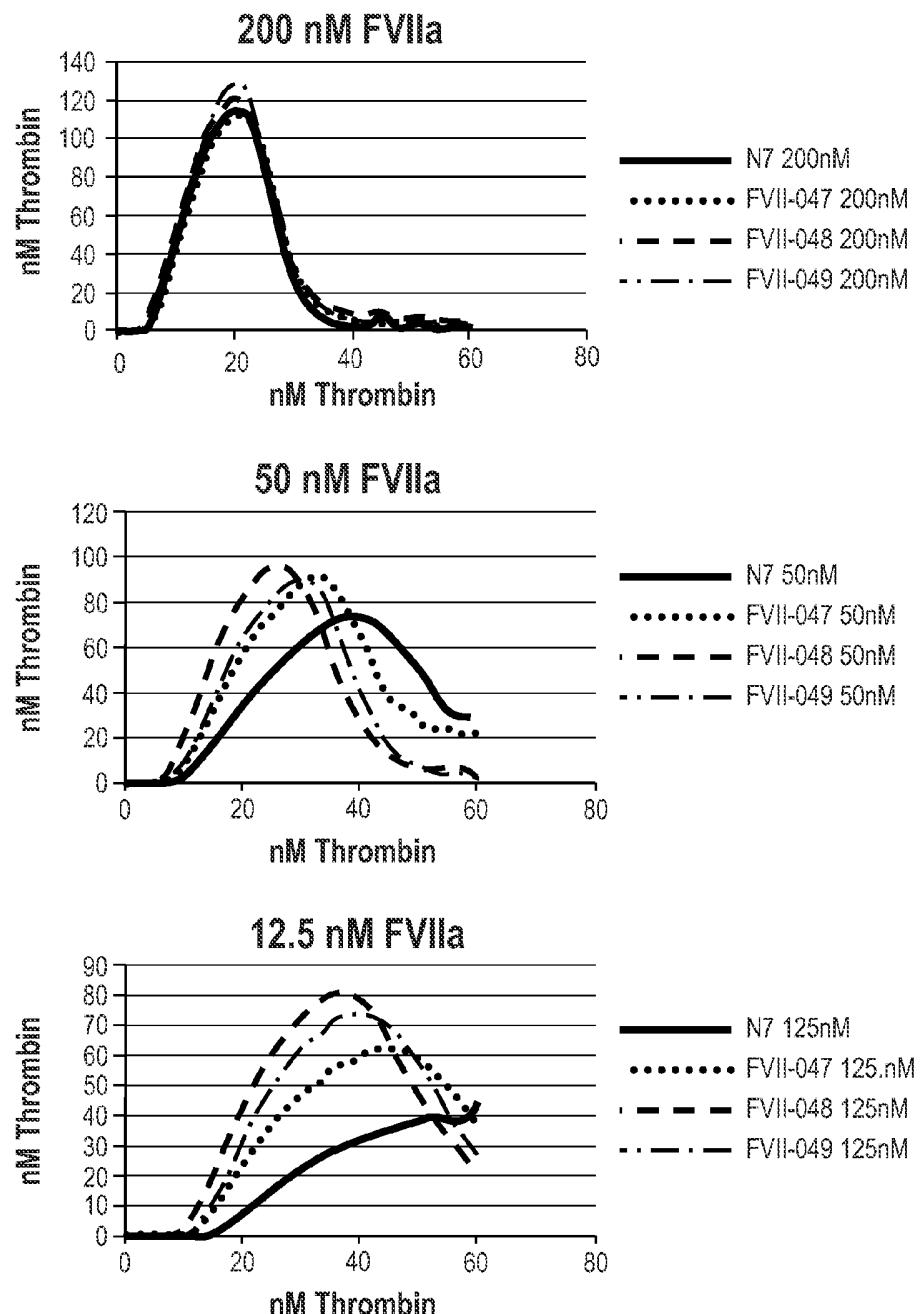


Fig. 47A

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**Thrombin generation assays in platelet-rich FVIII-deficient plasma
Comparison of FVII-045 and FVII-048**

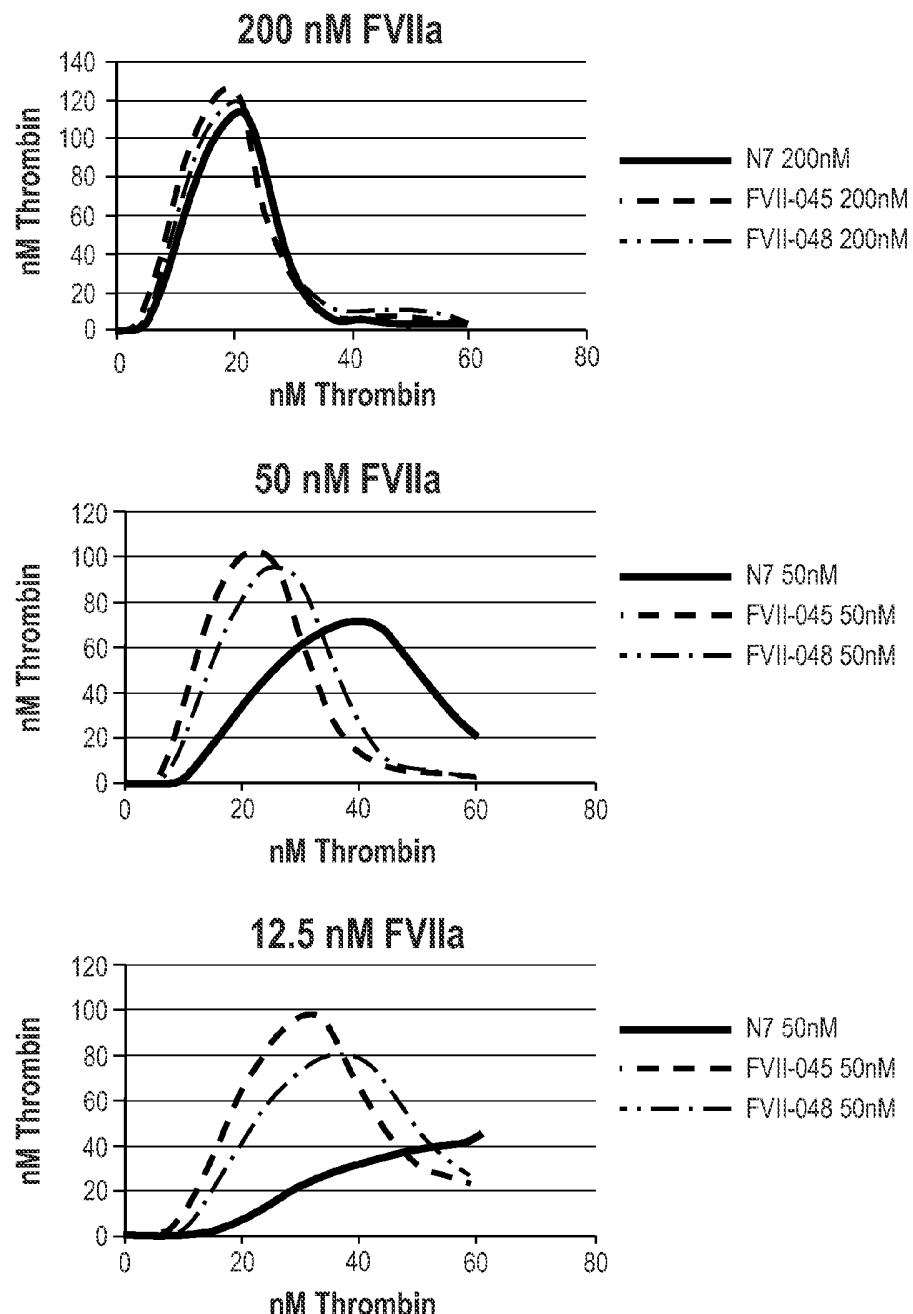
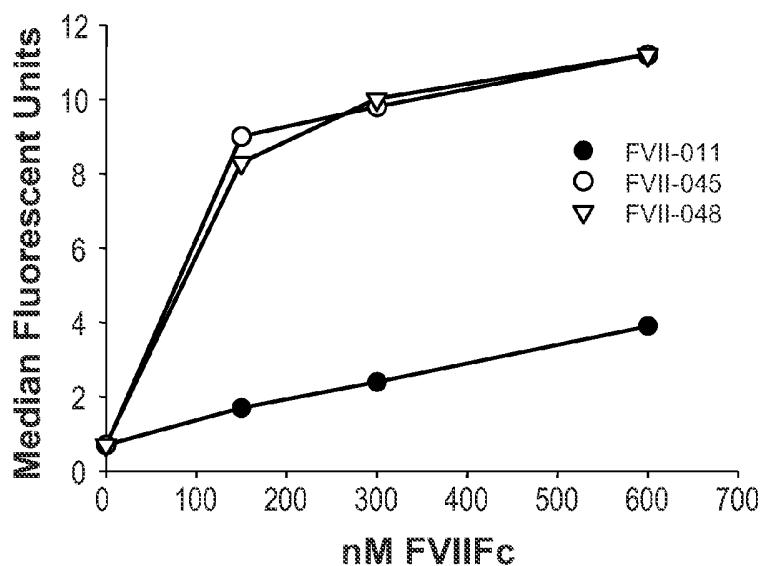


Fig. 47B

Binding of FVII-045, FVII-048 and wild type FVIIaFc to platelets determined by FACS



Affinity for the targeting peptides reported in Benard et al. Biochemistry 2008, 47: 4674-4682

Peptide	Affinity K_D , nM
PS4	64
OS1	0.74
OS2	31

Fig. 48

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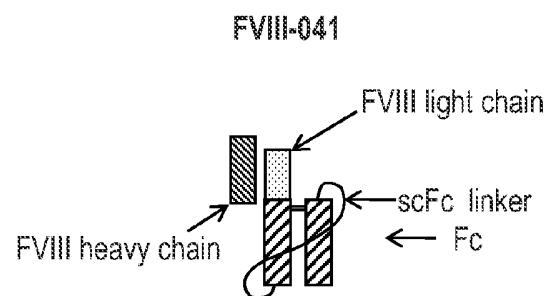
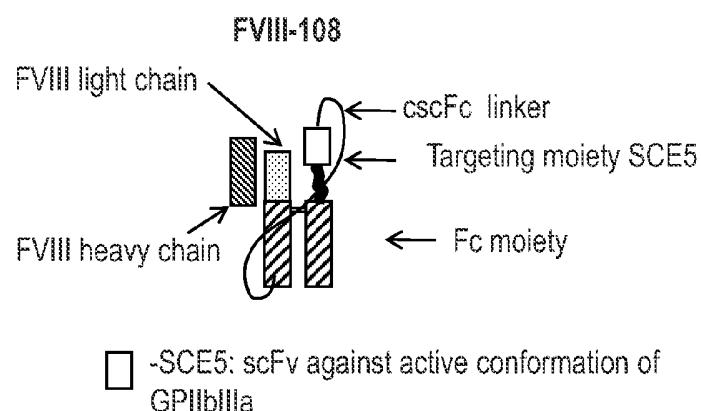
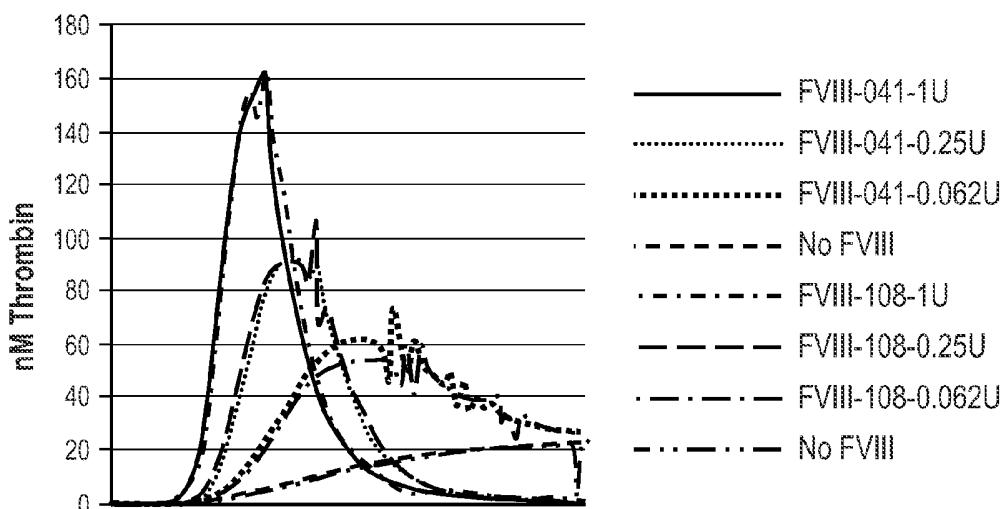


Fig. 49A

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Thrombin generation assays in FVIII deficient platelet-rich plasma

Assay was activated with tissue factor



Assay was activated by platelet activation

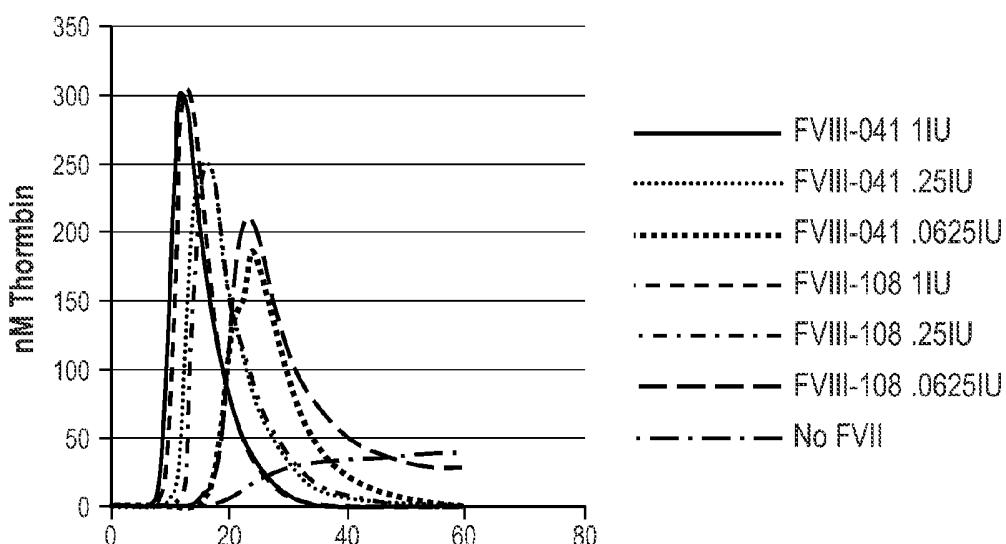


Fig. 49B

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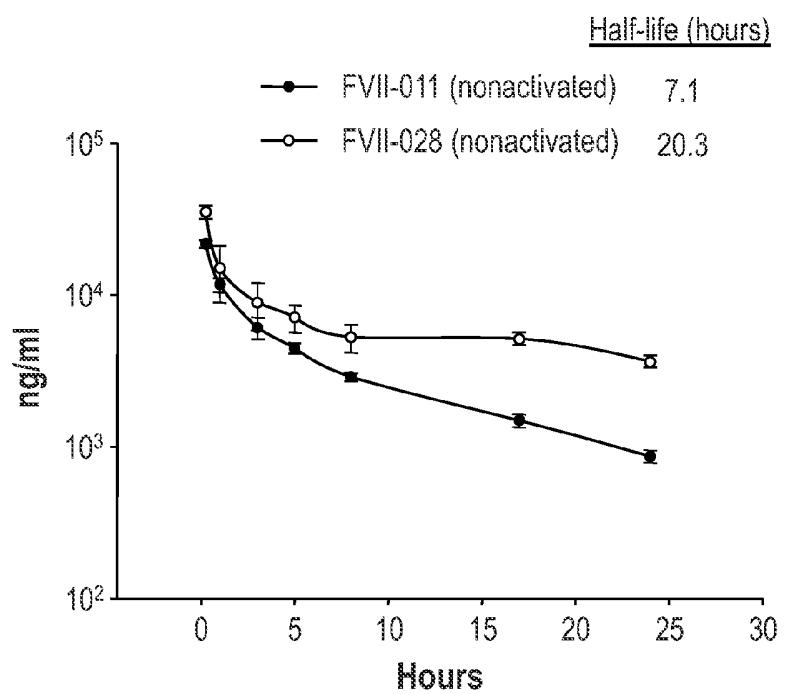
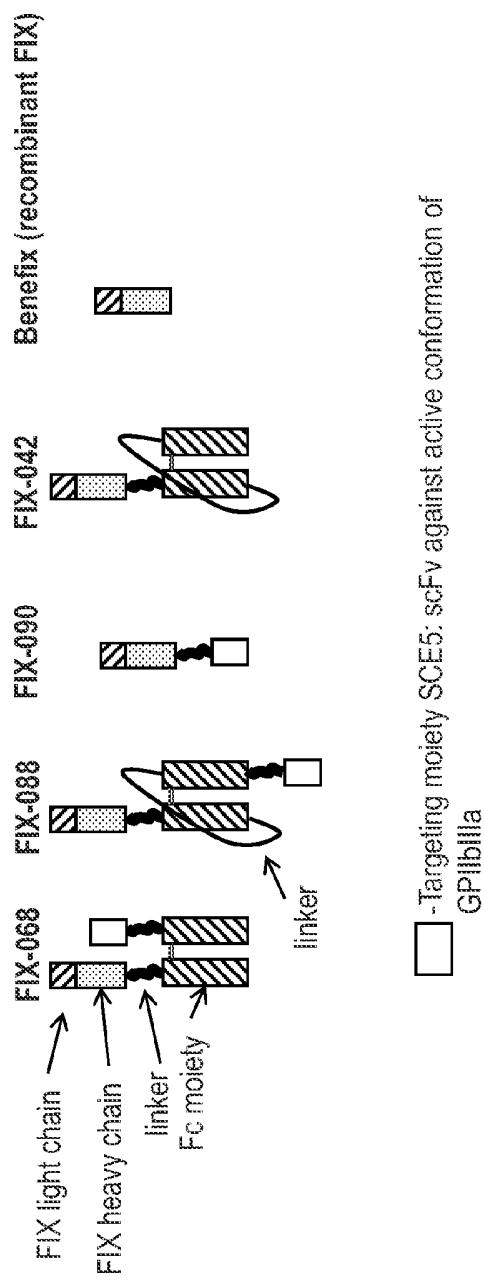


Fig. 50

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Target FIX to active conformation of GPIIbIIa via scFv (SCE5)**Fig. 51A**

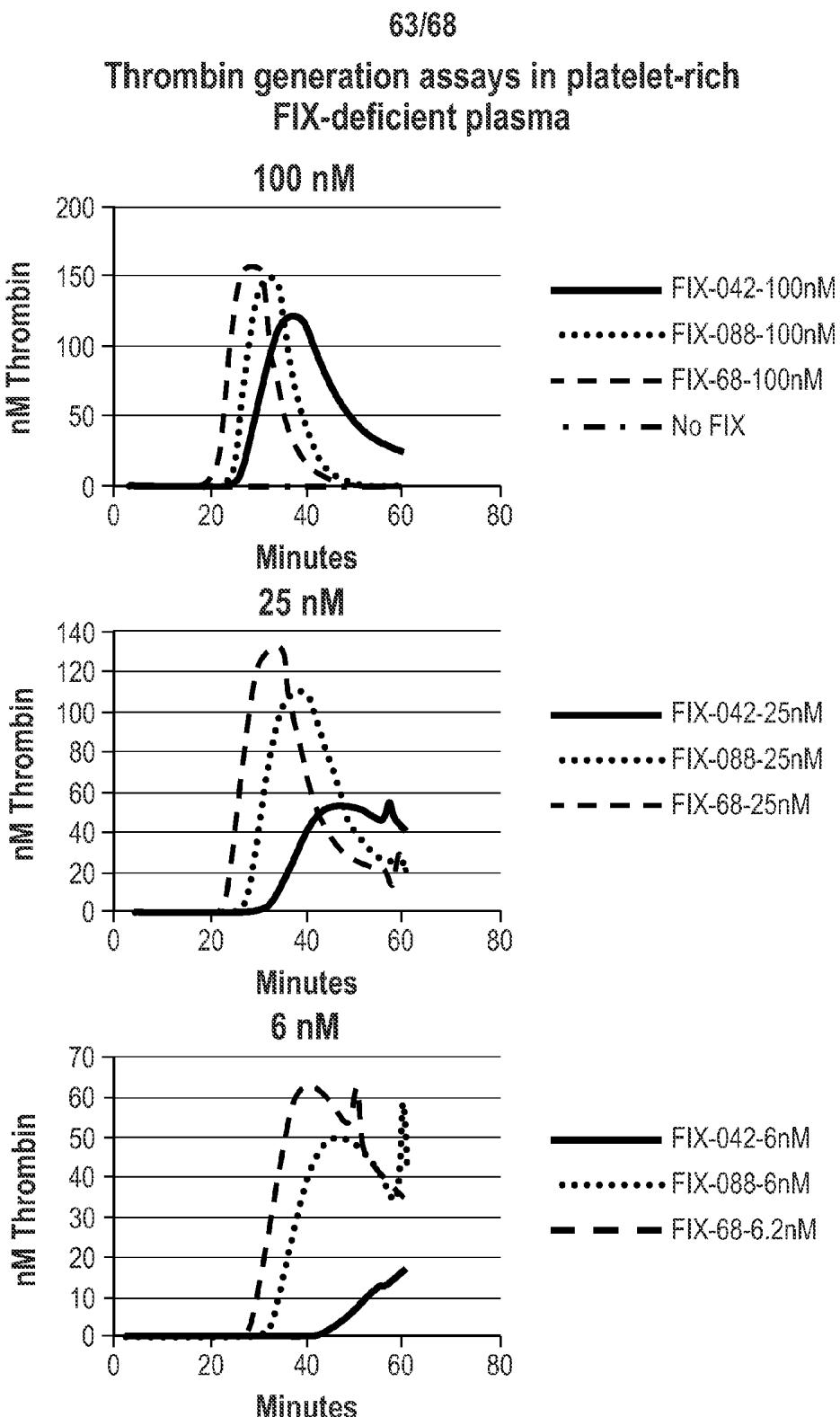


Fig. 51B

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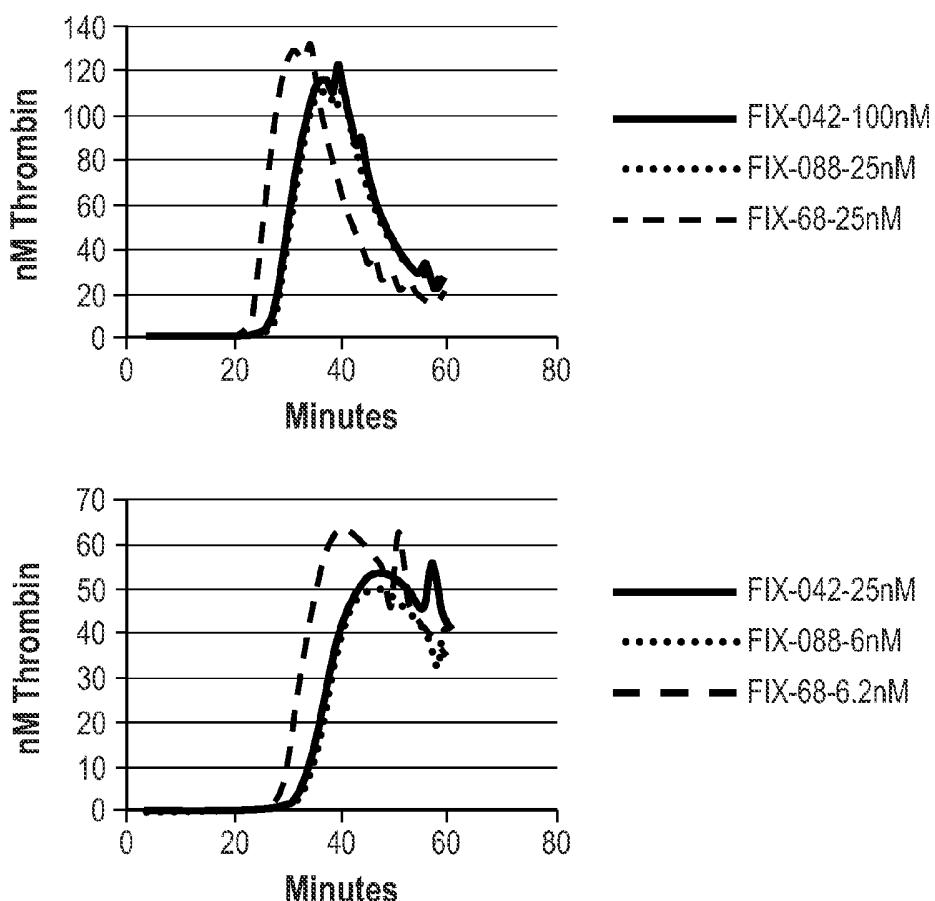


Fig. 51C

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Thrombin generation assays in platelet-rich FIX-deficient plasma

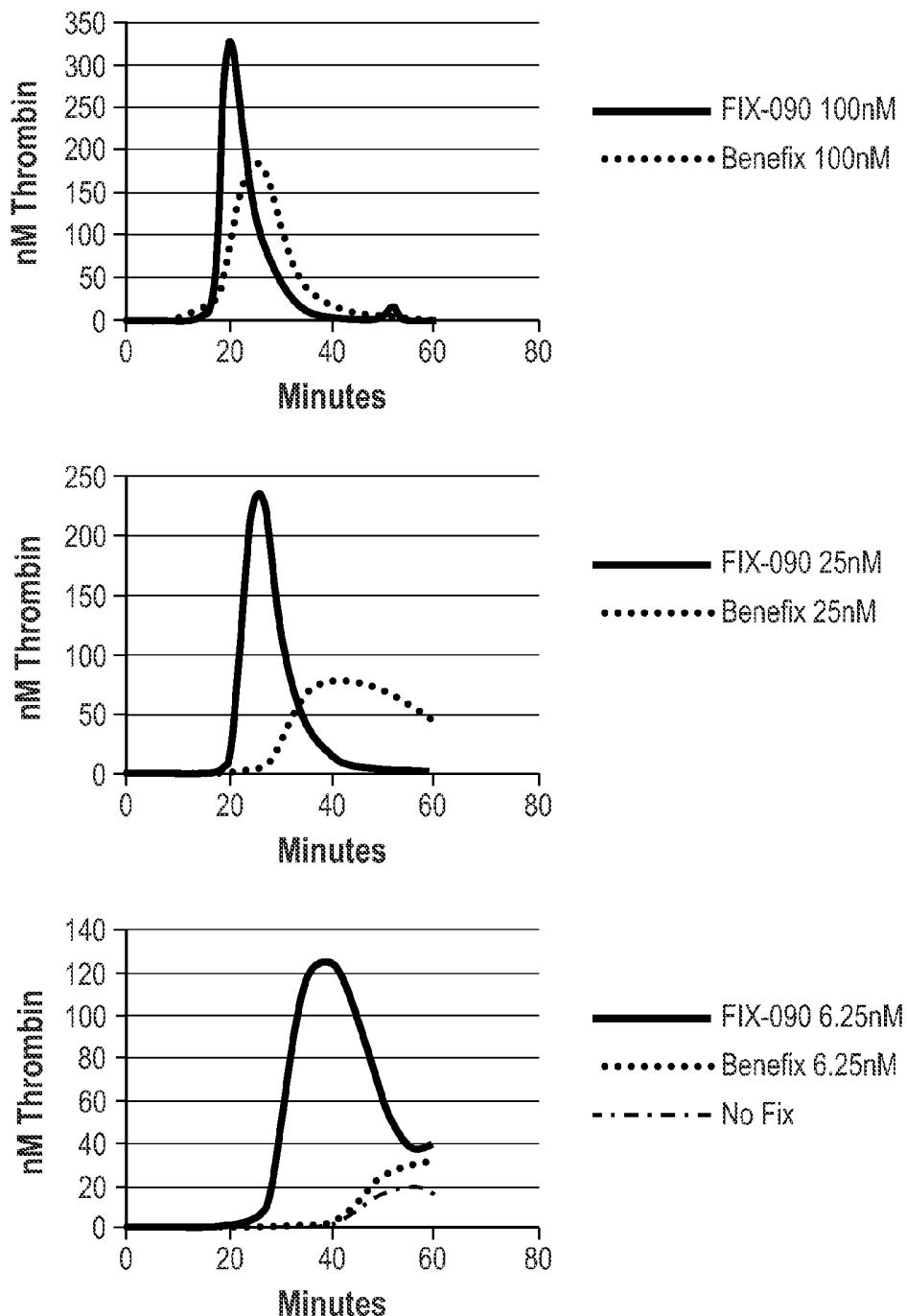
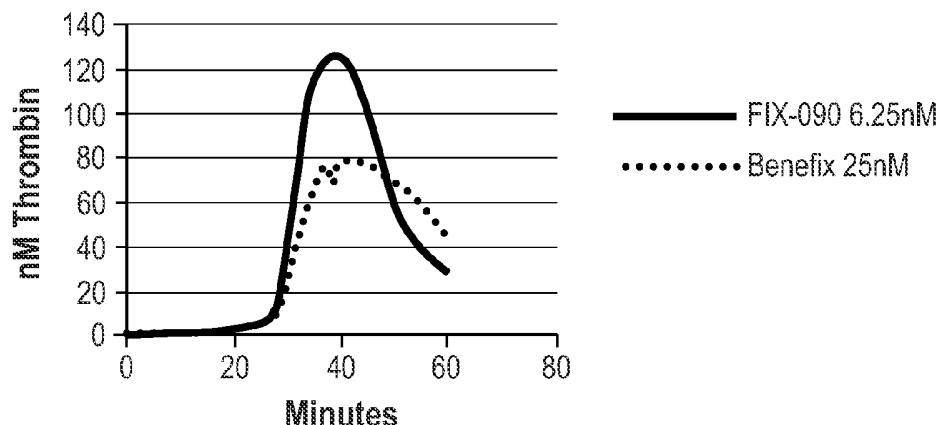
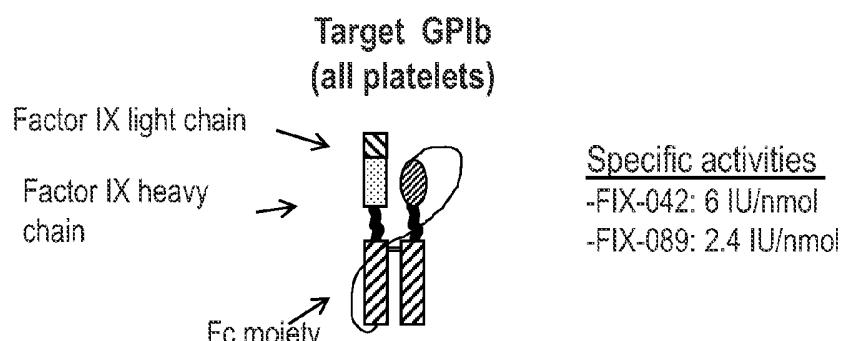


Fig. 52A

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**Fig. 52B**

● -Targetin moiety OS1:peptide
against GPIb-alpha

Fig. 53A

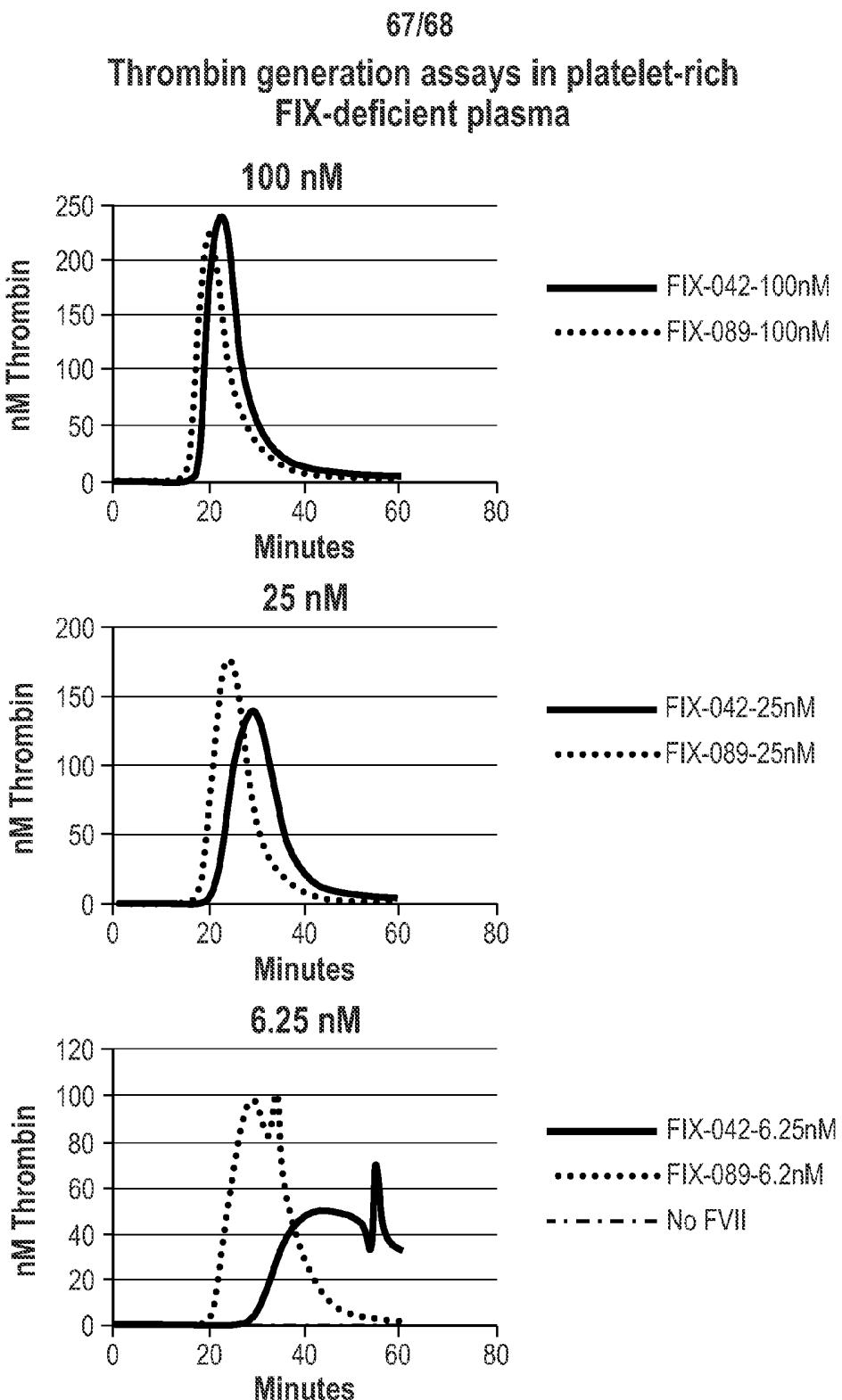


Fig. 53B

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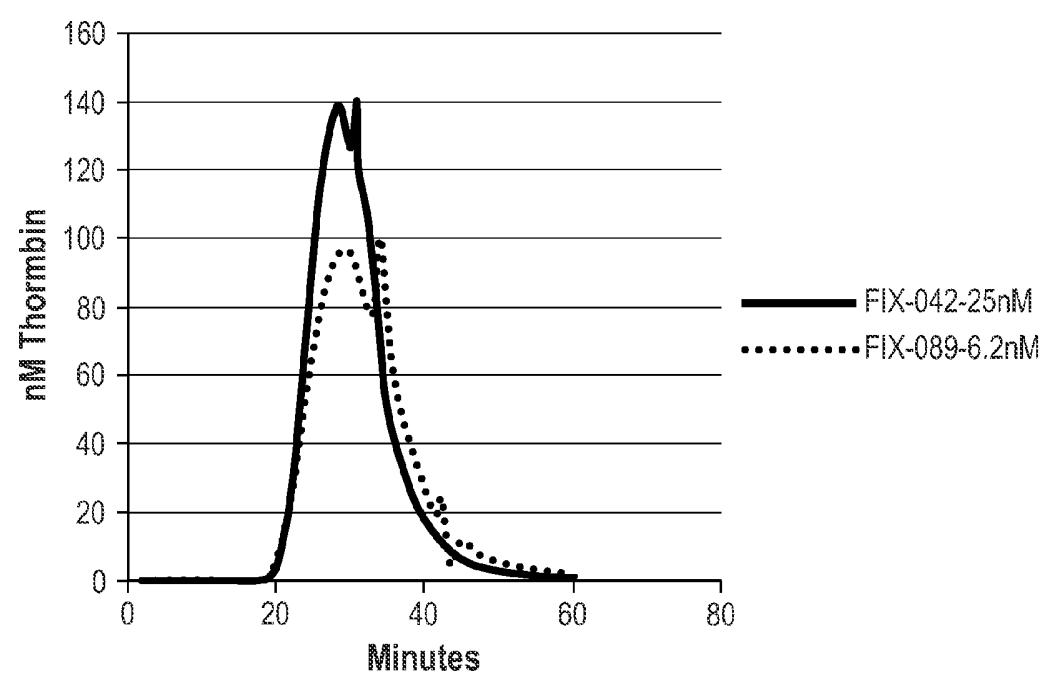


Fig. 53C