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- (73) Patenthaver: **CSL Behring Lengnau AG, Biotech Innovation Park, 2543 Lengnau BE, Schweiz**
- (72) Opfinder: **Schulte, Stefan, Bauerbacher Strasse 46, 35043 Marburg, Tyskland**  
**Weimer, Thomas, Richard-Wagner-Strasse 8, 35075 Gladenbach, Tyskland**  
**PESTEL, Sabine, Stuempelstal 19, 35041 Marburg, Tyskland**  
**Metzner, Hubert, Im Boden 6, 35041 Marburg, Tyskland**  
**DOWER, Steve, 18 Percy Street, Fitzroy North, Victoria 3068, Australien**
- (74) Fuldmægtig i Danmark: **Patrade A/S, Ceresbyen 75, 8000 Århus C, Danmark**
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**L. L. SWYSTUN ET AL: "FVIII stabilization: VWF D'D3 will do", BLOOD, vol. 124, no. 3, 17 July 2014 (2014-07-17), pages 313-315, XP055330357, & 53RD ANNUAL MEETING AND EXPOSITION OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); SAN DIEGO, CA, USA; DECEMBER 10 -13, 2011 ISSN: 0006-4971, DOI: 10.1182/blood-2014-06-578955**  
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Fortsættes ...



# DESCRIPTION

## FIELD OF THE INVENTION

**[0001]** The present invention relates to products and methods for improving treatment of blood coagulation disorders.

## BACKGROUND OF THE INVENTION

**[0002]** There are various bleeding disorders caused by deficiencies of blood coagulation factors. The most common disorders are hemophilia A and B, resulting from deficiencies of blood coagulation Factor VIII (FVIII) and IX, respectively. Another known bleeding disorder is von Willebrand's disease (VWD).

**[0003]** In plasma FVIII exists mostly as a non-covalent complex with von Willebrand Factor (VWF), and its coagulant function is to accelerate Factor IXa dependent conversion of Factor X to Xa.

**[0004]** Classic hemophilia or hemophilia A is an inherited bleeding disorder. It results from a chromosome X-linked deficiency of blood coagulation FVIII, and affects almost exclusively males with an incidence of between one and two individuals per 10,000. The X-chromosome defect is transmitted by female carriers who are not themselves hemophiliacs. The clinical manifestation of hemophilia A is an increased bleeding tendency.

**[0005]** In severe hemophilia A patients undergoing prophylactic treatment FVIII has to be administered intravenously (i.v.) about 3 times per week due to the short plasma half-life of FVIII of about 12 to 14 hours. Each i.v. administration is cumbersome, associated with pain and entails the risk of an infection especially as this is mostly done at home by the patients themselves or by the parents of children having been diagnosed for hemophilia A. It would thus be highly desirable to increase the half-life of FVIII so that pharmaceutical compositions containing such FVIII would have to be administered less frequently.

**[0006]** Several attempts have been made to prolong the half-life of non-activated FVIII either by reducing its interaction with cellular receptors (WO 03/093313 A2, WO 02/060951 A2), by covalently attaching polymers to FVIII (WO 94/15625, WO 97/11957 and US 4970300), by encapsulation of FVIII (WO 99/55306), by introduction of novel metal binding sites (WO 97/03193), by covalently attaching the A2 domain to the A3 domain either by peptidic (WO 97/40145 and WO 03/087355) or disulfide linkage (WO 02/103024A2) or by covalently attaching the A1 domain to the A2 domain (WO2006/108590).

**[0007]** Another approach to enhance the functional half-life of FVIII or VWF is by PEGylation of

FVIII (WO 2007/126808, WO 2006/053299, WO 2004/075923) or by PEGylation of VWF (WO 2006/071801) The increased half-life of pegylated VWF would indirectly also enhance the half-life of FVIII present in plasma. Also fusion proteins of FVIII have been described (WO 2004/101740, WO2008/077616 and WO 2009/156137).

**[0008]** VWF, which is missing, functionally defect or only available in reduced quantity in different forms of von Willebrand disease (VWD), is a multimeric adhesive glycoprotein present in the plasma of mammals, which has multiple physiological functions. During primary hemostasis VWF acts as a mediator between specific receptors on the platelet surface and components of the extracellular matrix such as collagen. Moreover, VWF serves as a carrier and stabilizing protein for procoagulant FVIII. VWF is synthesized in endothelial cells and megakaryocytes as a 2813 amino acid precursor molecule. The amino acid sequence and the cDNA sequence of wild-type VWF are disclosed in Collins et al. 1987, Proc. Natl. Acad. Sci. USA 84:4393-4397. The precursor polypeptide, pre-pro-VWF, consists of an N-terminal 22-residue signal peptide, followed by a 741-residue pro-peptide and the 2050-residue polypeptide found in mature plasma VWF (Fischer et al., FEBS Lett. 351: 345-348, 1994). After cleavage of the signal peptide in the endoplasmatic reticulum a C-terminal disulfide bridge is formed between two monomers of VWF. During further transport through the secretory pathway 12 N-linked and 10 O-linked carbohydrate side chains are added. More important, VWF dimers are multimerized via N-terminal disulfide bridges and the propeptide of 741 amino acids length is cleaved off by the enzyme PACE/furin in the late Golgi apparatus.

**[0009]** Once secreted into plasma the protease ADAMTS13 can cleave high-molecular weight VWF multimers within the A1 domain of VWF. Plasma VWF therefore consists of a whole range of multimers ranging from single dimers of 500 kDa to multimers consisting of up to more than 20 dimers of a molecular weight of over 10,000 kDa. The VWF-HMWM hereby having the strongest hemostatic activity, which can be measured in ristocetin cofactor activity (VWF:RCo). The higher the ratio of VWF:RCo/VWF antigen, the higher the relative amount of high molecular weight multimers.

**[0010]** In plasma FVIII binds with high affinity to VWF, which protects it from premature elimination and thus, plays in addition to its role in primary hemostasis a crucial role to stabilize FVIII, regulate plasma levels of FVIII and as a consequence is also a central factor to control secondary hemostasis. The half-life of non-activated FVIII bound to VWF is about 12 to 14 hours in plasma. In von Willebrand disease type 3, where no or almost no VWF is present, the half-life of FVIII is only about 2 to 6 hours, leading to symptoms of mild to moderate hemophilia A in such patients due to decreased concentrations of FVIII. The stabilizing effect of VWF on FVIII has also been used to aid recombinant expression of FVIII in CHO cells (Kaufman et al. 1989, Mol Cell Biol 9:1233-1242). Von Willebrand disease type 2N is characterized by low FVIII levels due to mutations in VWF which affect the binding of FVIII to VWF. FVIII levels in VWD type 2N patients are in a range between about 3 IU/dL and 30 IU/dL, typically below 20 IU/dL, depending on the specific mutation in VWF. Von-Willebrand disease type 1 is characterized by a reduced endogenous FVIII activity level compared to the endogenous FVIII activity level in normal human plasma (Sadler J.E. and Blinder M., Von Willebrand Disease: Diagnosis,

Classification, and Treatment; in: Hemostasis and Thrombosis, eds. Colman, Marder, Clowes, George, Aird, and Goldhaber, Lippincott Williams & Wilkins 2006, pp 905-921).

**[0011]** VWF-derived polypeptides, in particular VWF fragments, have been described to stabilize FVIII in vitro and in vivo. WO 2013/106787 A1 is directed at chimeric proteins comprising certain VWF fragments and a FVIII protein. Those chimeric hetero-dimers of FVIII and VWF-fragment do have a fixed molar ratio of VWF to FVIII of 1:1. WO 2014/198699 A2 and WO 2013/083858 A2 describe VWF fragments and their use in the treatment of hemophilia. It was found that bioavailability of FVIII may be significantly improved upon extravascular co-administration with similar molar amounts of VWF fragments. High molar excess of VWF over FVIII was said to be not desirable, and in experiments with VWF fragments co-administered s.c. with FVIII it was found that the VWF dose was not critical for FVIII bioavailability. Thus molar ratios of VWF fragments over FVIII were limited to maximally 50:1 and preferred ranges to maximally 1.5:1. WO 2011/060242 A2 discloses fusion polypeptides comprising certain VWF fragments and an antibody Fc region proposing specific molar ratios of VWF fragment over FVIII of up to 10:1. WO2013/093760 A2 describes a method for preparing a protein, comprising co-expressing FVIII or VWF polypeptides, including truncated forms of VWF, with a recombinant  $\alpha$ -2,3-sialyltransferase. Yee et al. (2014) Blood 124(3):445-452 found that a VWF fragment containing the D'D3 domains is sufficient to stabilize Factor VIII in VWF-deficient mice. However, although a VWF D'D3-Fc fusion protein exhibited markedly prolonged survival when transfused into FVIII-deficient mice, the VWF D'D3-Fc fusion protein did not prolong the survival of co-transfused FVIII.

**[0012]** There is an ongoing need for methods increasing the half-life of FVIII and FVIII products with reduced administration frequency.

## SUMMARY OF THE INVENTION

**[0013]** The invention is as defined in the claims. It has been found by the inventors that the *in vivo* half-life of endogenous Factor VIII can be prolonged by administration of a truncated and half-life extended VWF polypeptide (polypeptide of the invention). It has also been found that the *in vivo* half-life of endogenous Factor VIII can even be prolonged by administration of said polypeptide of the invention without necessary co-administration of exogenous FVIII. The patients have a reduced level of endogenous FVIII before treatment with said polypeptide relative to the level of FVIII in normal human plasma (NHP). The level of endogenous FVIII in said patients is at least 0.5% of the level of endogenous FVIII in normal human plasma (NHP). The polypeptide of the invention is capable of elevating endogenous FVIII levels. This allows for the prophylactic treatment of patients, without necessary co-administration of exogenous FVIII. If exogenous FVIII is co-administered, the follow-up treatment of a bleeding event can be done with the polypeptide of the invention only, i.e. without continued co-administration of exogenous FVIII. It has also been found by the inventors that patients could benefit from a treatment with the polypeptide of the invention by administration of an exogenous FVIII and thereby providing for an endogenous FVIII in said subject, the *in vivo* half-life of such an

endogenous Factor VIII being prolonged.

[0014] The present disclosure therefore provides:

1. [1] A polypeptide comprising a truncated von Willebrand Factor (VWF) and a half-life extending moiety, for use in the treatment of a blood coagulation disorder, said treatment comprising administering the polypeptide to a subject having a blood coagulation disorder and having endogenous Factor VIII (FVIII), wherein the activity level of endogenous FVIII in said subject before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said subject is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP), wherein the polypeptide is capable of binding to endogenous FVIII and wherein the endogenous FVIII level is increased following administration of said polypeptide and wherein
  - (i) said polypeptide is administered for prophylactic prevention of a bleeding event, wherein
    1. a) said treatment either does not comprise co-administration of exogenous FVIII, or
    2. b) said treatment comprising that an exogenous FVIII is administered and thereby providing for the endogenous FVIII in said subject; or
  - (ii) said polypeptide is co-administered together with exogenous FVIII for the treatment of a bleeding event or for initiation of a prophylactic treatment regime, wherein for follow-up treatments said polypeptide is administered without co-administration of exogenous FVIII.

The present invention in particular provides the advantage that a patient may benefit from a remaining low level of endogenous FVIII which can be stabilized by the polypeptide of the invention. The stabilization of endogenous FVIII might allow for higher protective plasma levels of FVIII. According to a certain aspect of the invention the polypeptide optionally allows for an extravascular administration of said polypeptide comprising a truncated VWF and a half-life extending moiety. In addition, the frequency of administration can be reduced by applying the polypeptide of the invention. Administration of the polypeptide allows for an increase of endogenous FVIII activity levels which can be elevated into a physiological range or prolonged in a physiological range. By administration of the polypeptide pathological aPTT values can be reduced to physiological values. Since exogenous FVIII does not necessarily need to be administered according to the therapy of the invention, the potential of formation of inhibitors against FVIII might be reduced. Thus, patients may benefit from a treatment with the polypeptide of the invention even without necessary co-administration of FVIII.

2. [2] The polypeptide for use according to embodiment [1], wherein the truncated von Willebrand Factor (VWF) provides for the capability of the polypeptide's binding to endogenous FVIII.
3. [3] The polypeptide for use according to embodiment [1] or embodiment [2], wherein the endogenous FVIII level is increased following administration of said polypeptide to a

level of at least 1%, or preferably at least 2%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100% of the activity level of endogenous FVIII in normal human plasma (NHP).

4. [4] The polypeptide for use according to any one of the preceding embodiments, wherein the truncated von Willebrand Factor (VWF) is a human truncated von Willebrand Factor (VWF).
5. [5] The polypeptide for use according to any one of the preceding embodiments, wherein the activity level of endogenous FVIII in said subject before treatment with said polypeptide is less than 80%, less than 60%, less than 40%, less than 30%, less than 20% or less than 10% of the activity level of endogenous FVIII in NHP.
6. [6] The polypeptide for use according to any one of the preceding embodiments, wherein the activity level of endogenous FVIII in said subject is at least 1%, at least 2%, at least 3%, at least 4% or at least 5% of the activity level of endogenous FVIII in NHP.
7. [7] The polypeptide for use according to any one of the preceding embodiments, wherein said blood coagulation disorder is hemophilia A or von-Willebrand disease, preferably von-Willebrand disease type 2N, von-Willebrand disease type 3 or von-Willebrand disease type 1.
8. [8] The polypeptide for use according to any one of the preceding embodiments, wherein said blood coagulation disorder is moderate hemophilia A, which is typically characterized by an endogenous FVIII activity level before or without treatment which is in a range between 1% to 5% of the endogenous FVIII activity level in NHP.
9. [9] The polypeptide for use according to embodiment [8], wherein treatment option (i) a) or treatment option (ii) is being applied.
10. [10] The polypeptide for use according to any one of embodiments [1] to [7], wherein said blood coagulation disorder is mild hemophilia A, typically characterized by an endogenous FVIII activity level before or without treatment which is greater than 5% and up to 40% of the endogenous FVIII activity level in NHP.
11. [11] The polypeptide for use according to embodiment [10], wherein treatment option (i) a) or treatment option (ii) is being applied.
12. [12] The polypeptide for use according to any one of embodiments [1] to [7], wherein said blood coagulation disorder is von-Willebrand disease type 3, typically characterized by an endogenous FVIII activity level before or without treatment which is usually in a range between about 1 IU/dL and about 20 IU/dL FVIII activity level corresponding to about 1% to about 20% of the endogenous FVIII activity level in NHP. Most of the patients have an endogenous FVIII activity level below 10 IU/dL, thus a level below 10% of the endogenous FVIII activity level in NHP.
13. [13] The polypeptide for use according to any one of embodiments [1] to [7], wherein said blood coagulation disorder is von-Willebrand disease type 2N, typically characterized by an endogenous FVIII activity level before or without treatment which is in a range between about 3 IU/dL and about 30 IU/dL FVIII activity level, corresponding to about 3% to about 30% of the endogenous FVIII activity level in NHP. Most of the patients have an endogenous FVIII activity level below 20 IU/dL, thus a level below 20% of the endogenous FVIII activity level in NHP. Thus, subjects having von-Willebrand

disease type 2N have an endogenous FVIII activity level from 0.03 IU/mL to 0.3 IU/mL in plasma, typically below 0.2 IU/mL.

14. [14] The polypeptide for use according to any one of embodiments [1] to [7], wherein said blood coagulation disorder is von-Willebrand disease type 1 typically characterized by an endogenous FVIII activity level before or without treatment which is reduced compared to the endogenous FVIII activity level in NHP.
15. [15] The polypeptide for use according to any one of embodiments [12] to [14], wherein treatment option (i) a) or treatment option (ii) is being applied.
16. [16] The polypeptide for use according to any one of embodiments [1] to [7], wherein treatment option (i) b) is being applied.
17. [17] The polypeptide for use according to embodiment [16], wherein said blood coagulation disorder is hemophilia A.
18. [18] The polypeptide for use according to embodiment [17], wherein the coagulation disorder is severe hemophilia A typically characterized by an endogenous FVIII activity level before or without treatment that is below 1% of the endogenous FVIII activity in NHP.
19. [19] The polypeptide for use according to embodiment [17] or [18], wherein said exogenous FVIII has been administered before or after administration of said polypeptide; or wherein said exogenous FVIII is being administered simultaneously with said polypeptide.
20. [20] The polypeptide for use according to embodiment [19], wherein said exogenous FVIII has been administered before said polypeptide.
21. [21] The polypeptide for use according to any one of preceding embodiments, wherein the subject is currently being treated or has been treated by a FVIII gene therapy or FVIII gene transfer approach for provision of endogenous FVIII activity, and whereas the endogenous FVIII activity levels preferably fall below one of the FVIII activity levels as defined in embodiment [5].
22. [22] The polypeptide for use according to embodiment [21], wherein treatment option (i) b) is being applied, whereas the "exogenous" FVIII is provided by the FVIII gene therapy or FVIII gene transfer approach thereby providing for the endogenous FVIII.
23. [23] The polypeptide for use according to any one of embodiments [16] to [20], wherein the activity level of FVIII within the subject's plasma resulting from a exogenous FVIII administered and the activity level of FVIII within the subject's plasma formed by the subject endogenously, if any FVIII is formed by the subject, are being considered together to be at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP). With other words according to this embodiment, the endogenous FVIII being at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP) may be a mixture of FVIII originating from exogenous FVIII, preferably previously administered FVIII, and endogenous FVIII formed by the subject or may be merely a remaining FVIII previously administered in case no FVIII is formed by the subject.
24. [24] The polypeptide for use according to any one of the preceding embodiments, wherein the polypeptide is administered intravenously and/or extravascularly. In case of an extravascular administration, subcutaneous administration is preferred.
25. [25] The polypeptide for use according to any one of embodiments [16] to [23], wherein

- the exogenous FVIII is administered intravenously, i.e. thus providing endogenous FVIII.
26. [26] The polypeptide for use according to embodiment to [25], wherein said polypeptide is administered via a different route of administration than FVIII, preferably said polypeptide is administered subcutaneously.
  27. [27] The polypeptide for use according to any one of embodiments [16] to [25], wherein said polypeptide is administered intravenously.
  28. [28] The polypeptide for use according to any one of the preceding embodiments, wherein the subject is a human being.
  29. [29] The polypeptide is a dimer.
  30. [30] The polypeptide for use according to any one of the preceding embodiments, wherein the truncated VWF comprises (a) amino acids 776 to 805 of SEQ ID NO:4 or (b) an amino acid sequence having a sequence identity of at least 90%, at least 95%; at least 96%, at least 97%, at least 98% or at least 99% to amino acids 776 to 805 of SEQ ID NO:4.
  31. [31] The polypeptide for use according to any one of the preceding embodiments, wherein the truncated VWF comprises (a) amino acids 766 to 864 of SEQ ID NO:4 or (b) an amino acid sequence having a sequence identity of at least 90%, at least 95%; at least 96%, at least 97%, at least 98% or at least 99% to amino acids 766 to 864 of SEQ ID NO:4.
  32. [32] The polypeptide for use according to any one of the preceding embodiments, wherein the truncated VWF comprises amino acids 764 to 1242 of SEQ ID NO:4.
  33. [33] The polypeptide for use according to any one of the preceding embodiments, wherein the truncated VWF consists of (a) amino acids 764 to 1242 of SEQ ID NO:4, (b) an amino acid sequence having a sequence identity of at least 90% to amino acids 764 to 1242 of SEQ ID NO:4.
  34. [34] The polypeptide for use according to any one of the preceding embodiments, wherein the truncated VWF lacks amino acids 1243 to 2813 of SEQ ID NO:4.
  35. [35] The polypeptide for use according to any one of the preceding embodiments, wherein the half-life extending moiety is a heterologous amino acid sequence fused to the truncated VWF.
  36. [36] The polypeptide for use according to embodiment [35], wherein said heterologous amino acid sequence comprises or consists of a polypeptide selected from the group consisting of transferrin and fragments thereof, the C-terminal peptide of human chorionic gonadotropin, an XTEN sequence, homo-amino acid repeats (HAP), proline-alanine-serine repeats (PAS), albumin, afamin, alpha-fetoprotein, Vitamin D binding protein, polypeptides capable of binding under physiological conditions to albumin or immunoglobulin constant regions, polypeptides capable of binding to the neonatal Fc receptor (FcRn), particularly immunoglobulin constant regions and portions thereof, preferably the Fc portion of immunoglobulin, and combinations thereof. The immunoglobulin constant region or portions thereof is preferably an Fc fragment of immunoglobulin G1, an Fc fragment of immunoglobulin G2 or an Fc fragment of immunoglobulin A.
  37. [37] The polypeptide for use according to any one of the preceding embodiments, wherein the half-life extending moiety is conjugated to the polypeptide.

38. [38] The polypeptide for use according to embodiment [37], wherein said half-life-extending moiety is selected from the group consisting of hydroxyethyl starch (HES), polyethylene glycol (PEG), polysialic acids (PSAs), elastin-like polypeptides, heparosan polymers, hyaluronic acid and albumin binding ligands, e.g. fatty acid chains or albumin binding peptides, and combinations thereof.
39. [39] The polypeptide for use according to any one of the preceding embodiments, wherein the pharmacokinetic parameters of the endogenous FVIII are improved by the administration of the polypeptide, in particular wherein the mean residence time (MRT) of the endogenous FVIII is increased and/or the half-life of the endogenous FVIII is prolonged and/or clearance of the endogenous FVIII is reduced.
40. [40] The use of a polypeptide as defined in any one of preceding embodiments [1] to [39] for stabilizing and/or increasing the plasma half-life of endogenous FVIII.
41. [41] The polypeptide for use according to any of embodiments [1] to [40], provided treatment option (ii) is applied and said polypeptide is co-administered together with exogenous FVIII for the treatment of a bleeding event or for initiation of a prophylactic treatment regime, wherein for follow-up treatments said polypeptide is administered without co-administration of exogenous FVIII, wherein the molar ratio of the polypeptide to be co-administered to the exogenous FVIII is greater than 50. The co-administered exogenous FVIII may be plasma derived or any recombinant FVIII. The recombinant exogenous FVIII may be for example a single-chain Factor VIII, preferably consisting of the amino acid sequence SEQ ID NO:5.
42. [42] The polypeptide for use according to any one of embodiments [1] to [41], in case that treatment option (i) b) or treatment option (ii) is applied, wherein the molar ratio of the polypeptide to the exogenous FVIII is at least 50.
43. [43] The polypeptide for use according to embodiment [42], wherein the molar ratio of the polypeptide to the exogenous FVIII is at least 75.
44. [44] The polypeptide for use according to embodiment [42], wherein the molar ratio of the polypeptide to the exogenous FVIII is at least 100.
45. [45] The polypeptide for use according to embodiment [42], wherein the molar ratio of the polypeptide to the exogenous FVIII is at least 200.
46. [46] The polypeptide for use according to embodiment [42], wherein the molar ratio of the polypeptide to the exogenous FVIII is at least 300.
47. [47] The polypeptide for use according to embodiment [42], wherein the molar ratio of the polypeptide to the exogenous FVIII is at least 400 or at least 500.
48. [48] The polypeptide for use according to any one of the preceding embodiments, wherein the polypeptide is a glycoprotein comprising N-glycans, and wherein at least 75 % of said N-glycans comprise, on average, at least one sialic acid moiety.
49. [49] The polypeptide for use according to embodiment [48], wherein at least 85 % of said N-glycans comprise, on average, at least one sialic acid moiety.
50. [50] The polypeptide for use according to embodiment [49] wherein at least 95 % of said N-glycans comprise, on average, at least one sialic acid moiety.
51. [51] The polypeptide for use according to any one of the preceding embodiments, wherein the polypeptide is a dimer, preferably a homodimer comprising two polypeptides (monomers) as defined in one of the herein disclosed embodiments, and the two

monomers forming the dimer are covalently linked to each other via one or more disulfide bridges formed by cysteine residues within the truncated VWF.

52. [52] The polypeptide for use according to embodiment [51], wherein the cysteine residues forming the one or more disulfide bridges is/are selected from the group consisting of Cys-1099, Cys-1142, Cys-1222, Cys-1225, Cys-1227 and combinations thereof, preferably Cys-1099 and Cys-1142, wherein the amino acid numbering refers to SEQ ID NO:4.
53. [53] The polypeptide for use according to any one of embodiments [51] to [52], wherein the affinity of said dimeric polypeptide to FVIII (either exogenous or endogenous FVIII) is greater than the affinity of a monomeric polypeptide to said FVIII, said monomeric polypeptide having the same amino acid sequence as a monomeric subunit of the dimeric polypeptide.
54. [54] The polypeptide for use according to any one of embodiments [51] to [53], wherein the ratio dimer: monomer of the polypeptide of the invention is at least 1.5, preferably at least 2, more preferably at least 2.5 or at least 3. Most preferably all polypeptides of the invention are present as dimers.
55. [55] The polypeptide has a FVIII binding affinity characterized by a dissociation constant  $K_D$  of less than 1 nM, preferably less than 500 pM, less than 200 pM, less than 100 pM, less than 90 pM or less than 80 pM.
56. [56] The polypeptide for use according to embodiment [55], wherein the dissociation constant  $K_D$  ranges from 0.1 pM to 500 pM, from 0.5 pM to 200 pM, from 0.75 pM to 100 pM or most preferred from 1 pM to 80 pM.
57. [57] The polypeptide for use according to any one of embodiments [51] to [56], wherein the dimeric polypeptide has a FVIII binding affinity characterized by a dissociation constant  $K_D$  and said dissociation constant  $K_D$  of the dimeric polypeptide is reduced compared to the dissociation constant  $K_D$  of a monomeric polypeptide, preferably by a factor of at least 10, by a factor of at least 100, by a factor of at least 500 or by a factor of at least 1000.
58. [58] The polypeptide for use according to any one of the preceding embodiments, wherein the pharmacokinetic parameters of the endogenous FVIII are improved by the administration of the polypeptide, preferably wherein the mean residence time (MRT) of the endogenous FVIII is increased and/or the half-life of the endogenous FVIII is prolonged and/or clearance of the endogenous FVIII is reduced, particularly when compared to the corresponding FVIII pharmacokinetic parameters in normal human plasma (NHP) or when compared to the corresponding FVIII pharmacokinetic parameters in a subject not receiving the polypeptide.
59. [59] The polypeptide for use according to embodiment [58], wherein said increase in MRT and/or terminal half-life of the endogenous FVIII is at least 50%.
60. [60] The polypeptide for use according to embodiment [59], wherein said increase in MRT and/or terminal half-life of the endogenous FVIII is at least 100%.
61. [61] The polypeptide for use according to embodiment [58], wherein the clearance of the endogenous FVIII is decreased by the administration of the polypeptide and said decrease is at least 25%.

62. [62] The polypeptide for use according to embodiment [61], wherein said decrease is at least 50%.
63. [63] The polypeptide for use according to embodiment [62], wherein said decrease is at least 100%.
64. [64] The polypeptide for use according to any one of the preceding embodiments, wherein the plasma half-life of said polypeptide is increased when compared to the plasma half-life of endogenous VWF and/or when compared to the plasma half-life of VWF of normal human plasma (NHP).
65. [65] The polypeptide for use according to embodiment [64], wherein the plasma half-life of said polypeptide is at least 25 % higher, in particular, at least 50 % higher, at least 75 % higher or at least 100% higher than the half-life of the endogenous VWF and/or of the half-life of VWF of normal human plasma (NHP).
66. [66] The polypeptide for use according to any one of the preceding embodiments, wherein the MRT of the polypeptide is increased, in particular, at least 25 % higher, at least 50 % higher, at least 75 % higher or at least 100% higher when compared to the MRT of endogenous VWF and/or when compared to the MRT of VWF of normal human plasma (NHP).
67. [67] The polypeptide for use according to any one of the preceding embodiments, wherein the MRT and/or plasma half-life of the polypeptide is increased when compared to that of a reference polypeptide which is identical to said polypeptide except that the reference polypeptide lacks the half-life extending moiety.
68. [68] The polypeptide for use according to any one of the preceding embodiments, wherein the polypeptide increases the maximal concentration ( $C_{\max}$ ) of endogenous Factor VIII as compared to untreated subjects.
69. [69] The polypeptide for use according to embodiment [68], wherein following administration of said polypeptide, the  $C_{\max}$  for FVIII amounts to at least 10 mIU/mL, at least 25 mIU/mL, at least 50 mIU/mL, at least 100 mIU/mL, at least 200 mIU/mL, at least 300 mIU/mL or at least 400 mIU/mL.
70. [70] The polypeptide for use according to any one of the preceding embodiments, wherein the polypeptide increases the peak area under the plasma concentration-time curve from zero to the last measured timepoint (AUC) of endogenous Factor VIII as compared to untreated subjects.
71. [71] The polypeptide for use according to embodiment [70], wherein following administration of the polypeptide, the AUC for the endogenous FVIII is increased to a level of at least 1000 mIU\*h/mL, at least 2000 mIU\*h/mL, at least 3000 mIU\*h/mL, at least 5000 mIU\*h/mL, at least 10000 mIU\*h/mL or at least 20000 mIU\*h/mL chromogenic FVIII activity.
72. [72] The polypeptide for use according to any one of the preceding embodiments, wherein at least one amino acid of the polypeptide is substituted as compared to the amino acid sequence of the wild-type VWF, wherein the binding affinity of such a modified polypeptide to FVIII is being further increased by introduction of said at least one substitution compared to the binding affinity of a reference polypeptide which has the same amino acid sequence except for said modifications.
73. [73] The polypeptide for use according to embodiment [72], wherein said at least one

substitution within the truncated VWF has the capacity to further increase the half-life of endogenous FVIII following administration of the polypeptide and/or may allow for reduction of the to be administered dose of the recombinant polypeptide.

74. [74] The polypeptide for use according to any one of embodiments [72] to [73], wherein the substitutions are selected from the group of combinations consisting of S764G/S766Y, S764P/S766I, S764P/S766M, S764V/S766Y, S764E/S766Y, S764Y/S766Y, S764L/S766Y, S764P/S766W, S766W/S806A, S766Y/P769K, S766Y/P769N, S766Y/P769R, S764P/S766L, and S764E/S766Y/V1083A, referring to the sequence of SEQ ID NO:4 with regard to the amino acid numbering.
75. [75] The polypeptide for use according to embodiment [74], wherein said substitution is either the combination S764E/S766Y or S764E/S766Y/V1083A.
76. [76] The polypeptide for use according to any one of the preceding embodiments, wherein said polypeptide is administered extravascularly, in particular subcutaneously, and following administration the polypeptide exhibits a bioavailability of at least 20%, preferably of at least 30%, of at least 40%, of at least 50%, of at least 60%, of at least 70% or of at least 80%.
77. [77] The polypeptide for use according to any one of the preceding embodiments, wherein following administration of the polypeptide an increase of the subject's endogenous FVIII activity level is achieved, preferably the endogenous FVIII activity level is increased up to the physiological FVIII level (100% = 1 IU/mL) or not substantially increased above the physiological FVIII level following administration of the polypeptide, preferably resulting in an increase of endogenous FVIII activity level not exceeding 300% = 3 IU/mL, more preferably not exceeding 250% = 2.5 IU/mL, not exceeding 200% = 2 IU/mL, not exceeding 150% = 1.5 IU/mL or not exceeding 120% = 1.2 IU/mL of mean FVIII activity level in plasma of normal human plasma, respectively.
78. [78] The polypeptide for use according to any one of the preceding embodiments, wherein following administration of the polypeptide to the subject suffering from a blood coagulation disorder an increased thrombogenic risk is prevented.
79. [79] The polypeptide for use according to embodiment [78], wherein the prevention of a thrombogenic risk is determined or achieved by only a limited increase of endogenous FVIII activity level in the subject following administration of the polypeptide, preferably the endogenous FVIII activity level is increased up to the physiological FVIII level (100% = 1 IU/mL) or not substantially increased above the physiological FVIII level following administration of the polypeptide, preferably resulting in an increase of endogenous FVIII activity level not exceeding 300% = 3 IU/mL, more preferably not exceeding 250% = 2.5 IU/mL, not exceeding 200% = 2 IU/mL, not exceeding 150% = 1.5 IU/mL or not exceeding 120% = 1.2 IU/mL of mean FVIII activity level in plasma of normal human plasma, respectively.
80. [80] The polypeptide for use according to any one of the preceding embodiments, wherein following administration of said polypeptide the maximal concentration ( $C_{\max}$ ) for the polypeptide is at least 20 nmol/kg, at least 40 nmol/kg, at least 60 nmol/kg, at least 80 nmol/kg or at least 160 nmol/kg.
81. [81] The polypeptide for use according to any one of the preceding embodiments, wherein following administration of said polypeptide the area under the concentration

over time curve from  $t=0$  to  $t=\infty$  ( $AUC_{0-\infty}$ ) for the administered polypeptide is at least 2 nmol \* h/mL, at least 3 nmol \* h/mL, at least 4 nmol \* h/mL, at least 20 nmol \* h/mL, at least 40 nmol \* h/mL, or at least 80 nmol \* h/mL.

82. [82] The polypeptide for use according to any one of the preceding embodiments, wherein following administration of said polypeptide the clearance (CL) value for the polypeptide is reduced by a factor of at least 2, at least 5, or at least 10, as compared to a reference treatment, wherein said reference treatment is identical to said treatment, except that the polypeptide to be administered does not comprise a half-life extending moiety.
83. [83] The polypeptide for use according to any one of the preceding embodiments, wherein the polypeptide is administered at an amount of at least 0.01 mg/kg, at least 0.1 mg/kg, at least 0.2 mg/kg, at least 0.5 mg/kg, at least 1 mg/kg or at least 3 mg/kg polypeptide.
84. [84] The polypeptide for use according to any one of the preceding embodiments, wherein the recombinant polypeptide is administered with an amount not exceeding 20 mg/kg, not exceeding 15 mg/kg, not exceeding 10 mg/kg, or not exceeding 5 mg/kg of the polypeptide.
85. [85] The polypeptide for use according to any one of the preceding embodiments, wherein following administration of the polypeptide physiological activated partial thrombin time (aPTT) values are achieved, in particular, pathological aPTT values are reduced to physiological values.
86. [86] The polypeptide for use according to embodiment [85], wherein following administration of the polypeptide the activated partial thrombin time (aPTT) is reduced by a factor of at least 1.5, at least 2, at least 2.5, at least 3, at least 4, at least 5 or at least 10.
87. [87] The polypeptide for use according to any one of the preceding embodiments, wherein the polypeptide is administered repeatedly, preferably with a dosing of once monthly, once every third week, once every second week, once every seven days, twice per week or once every second day.
88. [88] The polypeptide for use according to embodiment [87], wherein following said repeated, i.e. multiple, administration of the polypeptide, a steady state level of endogenous FVIII activity level in the subject is achieved, wherein the steady state FVIII activity level preferably provides a trough level of above 1%, preferably a trough level of above 5%, preferably a trough level of above 10%, more preferably a trough level of above 20%, more preferably a trough level of above 50% and even most preferably the steady state FVIII activity level is essentially within a range of the physiological FVIII activity level.
89. [89] The polypeptide for use according to any one of embodiment [87] to [88], wherein following said repeated, i.e. multiple, administration a sustained reduction of activated partial thrombin time (aPTT) is achieved in the subject, wherein the aPTT preferably is essentially within a physiological range for aPTT.
90. [90] A method of treating a blood coagulation disorder, comprising administering to a patient an effective amount of a polypeptide as defined in any one of embodiments [1] to [89] without co-administering FVIII, said patient having endogenous Factor VIII (FVIII),

wherein the activity level of endogenous FVIII in said patient before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said patient is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP) and whereby the endogenous FVIII level is increased following administration of said polypeptide. Said polypeptide is preferably either administered for prophylactic prevention of a bleeding event, wherein said treatment does not comprise co-administration of exogenous FVIII or said polypeptide is co-administered together with exogenous FVIII for the treatment of a bleeding event or for initiation of a prophylactic treatment regime, wherein for follow-up treatments said polypeptide is administered without co-administration of exogenous FVIII.

91. [91] A method of treating a blood coagulation disorder, comprising administering to a patient an effective amount of a polypeptide as defined in any one of embodiments [1] to [89], said patient having endogenous Factor VIII (FVIII), wherein the activity level of endogenous FVIII in said patient before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said patient is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP) and whereby the endogenous FVIII level is increased following administration of said polypeptide. Said treatment comprising that an exogenous FVIII is administered and thereby providing for the endogenous FVIII in said subject. Said polypeptide is preferably administered for prophylactic prevention of a bleeding event. According to a preferred embodiment, the polypeptide is administered subcutaneously and the FVIII is administered intravenously.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0015]

**Figure 1** shows rD'D3-FP exposure, FVIII activity and aPTT in CD rats after i.v. administration of rD'D3-FP, as described in Example 1.1.

**Figure 1A** shows rD'D3-FP quantified via its albumin component, and data is given as mean  $\pm$  SD for n=2-3 rats per timepoint;

**Figure 1B** shows FVIII quantified as chromogenic FVIII activity in IU/mL, and data is given as mean  $\pm$  SD for n=1-3 rats per timepoint (except for the predose data: n=7). Dotted lines represent the minimum and maximum of predose data. Dotted lines marked as ULN represent the upper limit of normal;

**Figure 1C** shows FVIII quantified as clotting FVIII activity in % of the norm, and data is given as mean  $\pm$  SD for n=1-3 rats per timepoint (except for the predose data: n=7). Dotted lines represent the minimum and maximum of predose data. Dotted lines marked as ULN represent

the upper limit of normal;

**Figure 1D** shows FVIII quantified as chromogenic or clotting FVIII activity as above. AUC was calculated as peak area under the plasma concentration-time curve from time zero to until day 14;

**Figure 1E** shows activated partial thrombin time using Pathromtin® SL, and data is given as mean  $\pm$  SD for n=1-3 rats per timepoint (except for the predose data: n=7). Dotted lines represent the minimum and maximum of predose data;

**Figure 2** shows rD'D3-FP exposure, FVIII activity and aPTT in rabbits after i.v. administration of rD'D3-FP, as described in Example 1.2.

**Figure 2A** shows rD'D3-FP quantified via its albumin component, and data is given as mean  $\pm$  SD for n=1-3 rabbits per timepoint;

**Figure 2B** shows FVIII quantified as chromogenic FVIII activity in IU/mL, and data is given as mean  $\pm$  SD for n=1-3 rabbits per timepoint. Dotted lines represent the minimum and maximum of predose data;

**Figure 2C** shows FVIII quantified as clotting FVIII activity in % of the norm, and data is given as mean  $\pm$  SD for n=1-3 rabbits per timepoint. Dotted lines represent the minimum and maximum of predose data;

**Figure 2D** shows FVIII quantified as chromogenic or clotting FVIII activity as above. AUC was calculated as peak area under the plasma concentration-time curve from time zero to until day 10;

**Figure 2E** shows activated partial thrombin time using Actin® FSL, and data is given as mean  $\pm$  SD for n=1-3 rabbits per timepoint. Dotted lines represent the minimum and maximum of predose data;

**Figure 3** shows rD'D3-FP exposure and FVIII activity in monkeys after i.v. administration of rD'D3-FP, as described in Example 1.3.

**Figure 3A** shows rD'D3-FP quantified against a standard quantified via its albumin component, and data is given as mean  $\pm$  SD for n=3 monkeys per timepoint;

**Figure 3B** shows FVIII quantified as chromogenic FVIII activity in % of the norm, and data is given as mean  $\pm$  SD for n=3 monkeys per timepoint. Dotted lines represent the minimum and maximum of predose data, the dashed line represents the mean of predose data (predose data: n=22).

**Figure 4** shows rD'D3-FP exposure, FVIII activity and aPTT in VWF knockout rats after i.v. administration of rD'D3-FP, as described in Example 1.4.

**Figure 4A** shows rD'D3-FP quantified via its albumin component, and data is given as mean  $\pm$  SD for n=1-4 rats per timepoint;

**Figure 4B** shows FVIII quantified as chromogenic FVIII activity in IU/mL, and data is given as mean  $\pm$  SD for n=1-4 rats per timepoint (except for the predose data: n $\leq$ 8). Predose data in VWF ko rats is below the limit of quantification. The shaded area represents the minimum and maximum of predose data from healthy CD rats; dotted lines marked as ULN represent the upper limit of normal;

**Figure 4C** shows FVIII quantified as clotting FVIII activity in % of the norm, and data is given as mean  $\pm$  SD for n=1-4 rats per timepoint (except for the predose data: n $\leq$ 8). The shaded area represents the minimum and maximum of predose data from healthy CD rats; dotted lines marked as ULN represent the upper limit of normal;

**Figure 4D** shows FVIII quantified as chromogenic or clotting FVIII activity as above. AUC was calculated as peak area under the plasma concentration-time curve from time zero to until day 10;

**Figure 4E** shows activated partial thrombin time using Pathromtin® SL, and data is given as mean  $\pm$  SD for n=1-3 rats per timepoint (except for the predose data: n $\leq$ 7). Dotted lines represent the minimum and maximum of predose data of the VWF ko rats. The shaded area represents the minimum and maximum of predose data from healthy CD rats;

**Figure 5** shows rD'D3-FP exposure and FVIII activity in VWF knockout mice after administration of rD'D3-FP, as described in Example 1.5.

**Figure 5A** shows rD'D3-FP quantified via its albumin component, and data is given as mean  $\pm$  SD for n=3 mice per timepoint;

**Figure 5B** shows FVIII quantified as chromogenic FVIII activity in mIU/mL, and data is given as mean  $\pm$  SD for n=3 mice per timepoint. Vehicle data in VWF ko mice is below the limit of quantification (LLOQ). The shaded area represents the minimum and maximum of predose data from healthy NMRI mice;

**Figure 5C** shows FVIII quantified as clotting FVIII activity in % of the norm, and data is given as mean  $\pm$  SD for n=3-4 mice per timepoint. Vehicle data in VWF ko mice is below the detection limit;

**Figure 5D** shows FVIII quantified as chromogenic or clotting FVIII activity as above. AUC was calculated as peak area under the plasma concentration-time curve from time zero to until day 7;

**Figure 6** shows rD'D3 variant exposure and FVIII activity in VWF knockout rats after i.v. administration of rD'D3 variants, as described in Example 1.6.

**Figure 6A** shows rD'D3-FP WT, rD'D3-FP EY, rD'D3-FP EYA or rD'D3-CTP quantified in VWF ko rats via its albumin component (rD'D3-FP) or via the D'D3 component (rD'D3-CTP), and data is given as mean  $\pm$  SD for n=3-4 rats per timepoint. The dotted lines represent the detection limit for albumin and rD'D3-CTP, respectively;

**Figure 6B** shows FVIII quantified as chromogenic FVIII activity in mIU/mL in VWF ko rats, and

data is given as mean  $\pm$  SD for n=1-4 rats per timepoint;

**Figure 6C** shows FVIII quantified as clotting FVIII activity in % of the norm in VWF ko rats, and data is given as mean  $\pm$  SD for n=1-4 rats per timepoint;

**Figure 6D** shows FVIII quantified as chromogenic or clotting FVIII activity as above. AUC was calculated as peak area under the plasma concentration-time curve from time zero to until day 10;

**Figure 7** shows rD'D3-FP exposure in FVIII knockout, VWF ko and NMRI mice and VWF ko or CD rats or pigs after i.v. or s.c. administration of rD'D3-FP, as described in Example 1.7.

**Figure 7A-1** shows rD'D3-FP quantified in FVIII ko, VWF ko and NMRI mice via its albumin component, and data is given as mean  $\pm$  SD for n=1-3 mice per timepoint;

**Figure 7A-2** shows rD'D3-FP quantified in VWF ko and CD rats via its albumin component, and data is given as mean  $\pm$  SD for n=2-4 rats per timepoint. The dotted line represents the detection limit for rD'D3-FP;

**Figure 7A-3** shows rD'D3-FP quantified in pigs via its albumin component, and data is given as mean  $\pm$  SD for n=1-3 pigs per timepoint. The dashed line represents the detection limit for rD'D3-FP;

**Figure 7B-1** shows FVIII quantified as chromogenic FVIII activity in VWF ko rats, and data is given as mean  $\pm$  SD for n=2-4 rats per timepoint. The dotted lines represent the minimum and maximum of data from untreated healthy CD rats;

**Figure 7B-2** shows FVIII quantified as chromogenic FVIII activity in pigs, and data is given as mean  $\pm$  SD for n=2-3 pigs per timepoint. The dotted lines represent the range coming from predose values from pigs;

**Figure 8** shows rD'D3-FP exposure and FVIII chromogenic activity in FVIII knockout, VWF ko and NMRI mice and VWF ko or CD rats or pigs after i.v. or s.c. administration of rD'D3-FP, as described in Example 1.8.

**Figure 8A** shows rD'D3-FP quantified in VWF ko rats via its albumin component, and data is given as mean  $\pm$  SD for n=3 rats per timepoint. The dashed line represents the detection limit for rD'D3-FP;

**Figure 8B** shows FVIII quantified as chromogenic FVIII activity in VWF ko rats, and data is given as mean  $\pm$  SD for n=2-3 rats per timepoint. The grey shade with dotted lines represent the minimum and maximum of data from untreated healthy CD rats;

**Figure 8C** shows FVIII quantified as clotting FVIII activity in VWF ko rats, and data is given as mean  $\pm$  SD for n=2 rats per timepoint. The grey shade represents the minimum to maximum range of data from untreated healthy CD rats;

**Figure 8D** shows activated partial thrombin time using Pathromtin® SL in VWF ko rats, and

data is given as mean  $\pm$  SD for n=2 rats per timepoint. The grey shade represents the minimum and maximum of predose data of the VWF ko rats. The shaded area represents the minimum and maximum of predose data from healthy CD rats;

**Figure 9** shows rD'D3-FP and FVIII exposure in FVIII ko rats after i.v. or/and or combined with s.c. administration of rD'D3-FP, as described in Example 1.9.

**Figure 9A** shows FVIII quantified as chromogenic FVIII activity in FVIII ko rats, and data is given as mean  $\pm$  SD for n=3 FVIII ko mice per timepoint. The detection limit represent the minimum and maximum of predose data from healthy CD rats;

**Figure 9B** shows FVIII quantified as chromogenic FVIII activity in FVIII ko rats, and data is given as mean  $\pm$  SD for n=2-3 FVIII ko mice per timepoint. The detection limit represents the minimum and maximum of predose data from healthy CD rats;

**Figure 9C** shows FVIII quantified as chromogenic FVIII activity in FVIII ko rats, and data is given as mean  $\pm$  SD for n=2-3 FVIII ko mice per timepoint. The detection limit represent the minimum and maximum of predose data from healthy CD rats;

## DETAILED DESCRIPTION

**[0016]** In a first aspect, the present invention relates to a polypeptide comprising (i) a truncated von Willebrand Factor (VWF) and (ii) a half-life extending moiety, for use in the treatment of a blood coagulation disorder, said treatment comprising administering the polypeptide to a subject suffering from a blood coagulation disorder and having endogenous Factor VIII (FVIII), wherein the activity level of endogenous FVIII in said subject before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said subject is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP), and whereby the endogenous FVIII level is increased following administration of said polypeptide and said polypeptide is administered for prophylactic prevention of a bleeding event, wherein said treatment does not comprise co-administration of exogenous FVIII.

**[0017]** In a second aspect, the present invention pertains to a polypeptide comprising (i) a truncated von Willebrand Factor (VWF) and (ii) a half-life extending moiety, for use in the treatment of a blood coagulation disorder, said treatment comprising administering the polypeptide to a subject suffering from a blood coagulation disorder and having endogenous Factor VIII (FVIII), wherein the activity level of endogenous FVIII in said subject before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said subject is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP) and whereby the endogenous FVIII level is increased following administration of said polypeptide and wherein

said polypeptide is co-administered together with exogenous FVIII for the treatment of a bleeding event or for initiation of a prophylactic treatment regime, wherein for follow-up treatments said polypeptide is administered without co-administration of exogenous FVIII.

**[0018]** A third aspect pertains to a pharmaceutical composition comprising a polypeptide comprising (i) a truncated von Willebrand Factor (VWF) and (ii) a half-life extending moiety, for use in the treatment of a blood coagulation disorder, said treatment comprising administering the polypeptide to a subject suffering from a blood coagulation disorder and having endogenous Factor VIII (FVIII), wherein the activity level of endogenous FVIII in said subject before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said subject is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP), and whereby the endogenous FVIII level is increased following administration of said polypeptide and said polypeptide is administered for prophylactic prevention of a bleeding event, wherein said treatment does not comprise co-administration of exogenous FVIII.

**[0019]** A fourth aspect pertains to a pharmaceutical composition comprising a polypeptide comprising (i) a truncated von Willebrand Factor (VWF) and (ii) a half-life extending moiety, for use in the treatment of a blood coagulation disorder, said treatment comprising administering the polypeptide to a subject suffering from a blood coagulation disorder and having endogenous Factor VIII (FVIII), wherein the activity level of endogenous FVIII in said subject before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said subject is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP) and whereby the endogenous FVIII level is increased following administration of said polypeptide and wherein said polypeptide is co-administered together with exogenous FVIII for the treatment of a bleeding event or for initiation of a prophylactic treatment regime, wherein for follow-up treatments said polypeptide is administered without co-administration of exogenous FVIII.

**[0020]** A fifth aspect pertains to use of a recombinant polypeptide comprising (i) a truncated von Willebrand Factor (VWF) and (ii) a half-life extending moiety for the manufacture of a medicament for the treatment of a blood coagulation disorder, said treatment comprising administering the polypeptide to a subject suffering from a blood coagulation disorder and having endogenous Factor VIII (FVIII), wherein the activity level of endogenous FVIII in said subject before treatment with said polypeptide is reduced relative to the activity level of FVIII in normal human plasma (NHP) provided that the activity level of endogenous FVIII in said subject is at least 0.5% of the activity level of endogenous FVIII in normal human plasma (NHP), and whereby the endogenous FVIII level is increased following administration of said polypeptide and wherein

- said polypeptide is either administered for prophylactic prevention of a bleeding event, wherein said treatment does not comprise co-administration of exogenous FVIII or
- said polypeptide is co-administered together with exogenous FVIII for the treatment of a

bleeding event or for initiation of a prophylactic treatment regime, wherein for follow-up treatments said polypeptide is administered without co-administration of exogenous FVIII.

**[0021]** The polypeptide comprising a truncated von Willebrand Factor (VWF) and a half-life extending moiety will be referred to herein as "polypeptide of the invention".

***The truncated VWF***

**[0022]** The term "von Willebrand Factor" (VWF) as used herein includes naturally occurring (native) VWF, but also variants thereof retaining at least the FVIII binding activity of naturally occurring VWF, e.g. sequence variants where one or more residues have been inserted, deleted or substituted. The FVIII binding activity is determined by a FVIII-VWF binding assay as described in Example 2.

**[0023]** The VWF in accordance with this invention is human VWF represented by the amino acid sequence shown in SEQ ID NO:4, and is a truncated VWF. The cDNA encoding SEQ ID NO:4 is shown in SEQ ID NO:3.

**[0024]** The gene encoding human native VWF is transcribed into a 9 kb mRNA which is translated into a pre-propolypeptide of 2813 amino acids with an estimated molecular weight of 310,000 Da. The pre-propolypeptide contains an N-terminal 22 amino acids signal peptide, followed by a 741 amino acid pro-polypeptide (amino acids 23-763 of SEQ ID NO:4) and the mature subunit (amino acids 764-2813 of SEQ ID NO:4). Cleavage of the 741 amino acids propolypeptide from the N-terminus results in mature VWF consisting of 2050 amino acids. The amino acid sequence of the human native VWF pre-propolypeptide is shown in SEQ ID NO:4. Unless indicated otherwise, the amino acid numbering of VWF residues in this application refers to SEQ ID NO:4, even if the VWF molecule does not comprise all residues of SEQ ID NO:4.

**[0025]** The propolypeptide of native VWF comprises multiple domains. Different domain annotations can be found in the literature (see, e.g. Zhou et al. (2012) Blood 120(2): 449-458). The following domain annotation of native pre-propolypeptide of VWF is applied in this application:

D1-D2-D'-D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK

**[0026]** With reference to SEQ ID NO:4, the D' domain consists of amino acids 764-865; and the D3 domain consists of amino acids 866-1242.

**[0027]** The feature "truncated" means that the polypeptide does not comprise the entire amino acid sequence of mature VWF (amino acids 764-2813 of SEQ ID NO:4). Typically, the

truncated VWF does not comprise all amino acids 764-2813 of SEQ ID NO:4 but only a fragment thereof. A truncated VWF may also be referred to as a VWF fragment, or in the plural as VWF fragments.

**[0028]** The truncated VWF is capable of binding to a Factor VIII. Preferably, the truncated VWF is capable of binding to the mature form of human native Factor VIII. The truncated VWF is capable of binding to endogenous and/or exogenous Factor VIII. In certain embodiments, the truncated VWF is capable of binding to a co-administered recombinant FVIII, preferably to a FVIII as described herein, more preferred to a single-chain Factor VIII consisting of the amino acid sequence SEQ ID NO:5. Binding of the truncated VWF to Factor VIII can be determined by a FVIII-VWF binding assay as described in Example 2.

**[0029]** The truncated VWF of the present invention preferably comprises or consists of an amino acid sequence having a sequence identity of at least 90% to amino acids 776 to 805 of SEQ ID NO:4 and is capable of binding to FVIII. In preferred embodiments the truncated VWF comprises or consists of an amino acid sequence having a sequence identity of at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, to amino acids 776 to 805 of SEQ ID NO:4 and is capable of binding to FVIII. Most preferably, the truncated VWF comprises or consists of amino acids 776 to 805 of SEQ ID NO:4. Unless indicated otherwise herein, sequence identities are determined over the entire length of the reference sequence (e.g. amino acids 776 to 805 of SEQ ID NO:4).

**[0030]** The truncated VWF of the present invention preferably comprises or consists of an amino acid sequence having a sequence identity of at least 90% to amino acids 766 to 864 of SEQ ID NO:4 and is capable of binding to FVIII. In preferred embodiments the truncated VWF comprises or consists of an amino acid sequence having a sequence identity of at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, to amino acids 766 to 864 of SEQ ID NO:4 and is capable of binding to FVIII. Most preferably, the truncated VWF comprises or consists of amino acids 766 to 864 of SEQ ID NO:4.

**[0031]** In another preferred embodiment, the truncated VWF consists of (a) an amino acid sequence having a sequence identity of at least 90% to amino acids 764 to 1242 of SEQ ID NO:4, that the truncated VWF is still capable of binding to FVIII. More preferably, the truncated VWF consists of (a) an amino acid sequence having a sequence identity of at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, to amino acids 764 to 1242 of SEQ ID NO:4, or (b) a fragment thereof, provided that the truncated VWF is still capable of binding to FVIII. Most preferably, the truncated VWF consists of (a) amino acids 764 to 1242 of SEQ ID NO:4 provided that the truncated VWF is still capable of binding to FVIII. As described in more detail below, the polypeptide may be prepared by a method which uses cells comprising a nucleic acid encoding the polypeptide comprising the truncated VWF. The nucleic acid is introduced into suitable host cells by techniques that are known *per se*.

**[0032]** In a preferred embodiment, the nucleic acid in the host cell encodes (a) an amino acid sequence having a sequence identity of at least 90% to amino acids 1 to 1242 of SEQ ID NO:4,

or (b) a fragment thereof, provided that the truncated mature VWF is still capable of binding to FVIII. More preferably, the nucleic acid encodes (a) an amino acid sequence having a sequence identity of at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, to amino acids 1 to 1242 of SEQ ID NO:4, or (b) a fragment thereof, provided that the truncated VWF is still capable of binding to FVIII. Most preferably, the nucleic acid encodes (a) amino acids 1 to 1242 of SEQ ID NO:4, or (b) a fragment thereof, provided that the truncated VWF is still capable of binding to FVIII. Especially if the polypeptide in accordance with this invention is a dimer, the nucleic acid will comprise a sequence encoding amino acids 1 to 763 of VWF (e.g. SEQ ID NO:4), even if the truncated VWF in the polypeptide does not comprise amino acids 1 to 763 of VWF (e.g. SEQ ID NO:4).

**[0033]** In other embodiments the truncated VWF comprises or consists of one of the following amino acid sequences, each referring to SEQ ID NO:4:

776-805; 766-805; 764-805; 776-810; 766-810; 764-810; 776-815; 766-815; 764-815; 776-820; 766-820; 764-820; 776-825; 766-825; 764-825; 776-830; 766-830; 764-830; 776-835; 766-835; 764-835; 776-840; 766-840; 764-840; 776-845; 766-845; 764-845; 776-850; 766-850; 764-850; 776-855; 766-855; 764-855; 776-860; 766-860; 764-860; 776-864; 766-864; 764-864; 776-865; 766-865; 764-865; 776-870; 766-870; 764-870; 776-875; 766-875; 764-875; 776-880; 766-880; 764-880; 776-885; 766-885; 764-885; 776-890; 766-890; 764-890; 776-895; 766-895; 764-895; 776-900; 766-900; 764-900; 776-905; 766-905; 764-905; 776-910; 766-910; 764-910; 776-915; 766-915; 764-915; 776-920; 766-920; 764-920; 776-925; 766-925; 764-925; 776-930; 766-930; 764-930; 776-935; 766-935; 764-935; 776-940; 766-940; 764-940; 776-945; 766-945; 764-945; 776-950; 766-950; 764-950; 776-955; 766-955; 764-955; 776-960; 766-960; 764-960; 776-965; 766-965; 764-965; 776-970; 766-970; 764-970; 776-975; 766-975; 764-975; 776-980; 766-980; 764-980; 776-985; 766-985; 764-985; 776-990; 766-990; 764-990; 776-995; 766-995; 764-995; 776-1000; 766-1000; 764-1000; 776-1005; 766-1005; 764-1005; 776-1010; 766-1010; 764-1010; 776-1015; 766-1015; 764-1015; 776-1020; 766-1020; 764-1020; 776-1025; 766-1025; 764-1025; 776-1030; 766-1030; 764-1030; 776-1035; 766-1035; 764-1035; 776-1040; 766-1040; 764-1040; 776-1045; 766-1045; 764-1045; 776-1050; 766-1050; 764-1050; 776-1055; 766-1055; 764-1055; 776-1060; 766-1060; 764-1060; 776-1065; 766-1065; 764-1065; 776-1070; 766-1070; 764-1070; 776-1075; 766-1075; 764-1075; 776-1080; 766-1080; 764-1080; 776-1085; 766-1085; 764-1085; 776-1090; 766-1090; 764-1090; 776-1095; 766-1095; 764-1095; 776-1100; 766-1100; 764-1100; 776-1105; 766-1105; 764-1105; 776-1110; 766-1110; 764-1110; 776-1115; 766-1115; 764-1115; 776-1120; 766-1120; 764-1120; 776-1125; 766-1125; 764-1125; 776-1130; 766-1130; 764-1130; 776-1135; 766-1135; 764-1135; 776-1140; 766-1140; 764-1140; 776-1145; 766-1145; 764-1145; 776-1150; 766-1150; 764-1150; 776-1155; 766-1155; 764-1155; 776-1160; 766-1160; 764-1160; 776-1165; 766-1165; 764-1165; 776-1170; 766-1170; 764-1170; 776-1175; 766-1175; 764-1175; 776-1180; 766-1180; 764-1180; 776-1185; 766-1185; 764-1185; 776-1190; 766-1190; 764-1190; 776-1195; 766-1195; 764-1195; 776-1200; 766-1200; 764-1200; 776-1205; 766-1205; 764-1205; 776-1210; 766-1210; 764-1210; 776-1215; 766-1215; 764-1215; 776-1220; 766-1220; 764-1220; 776-1225; 766-1225; 764-1225; 776-1230; 766-1230; 764-1230; 776-1235; 766-1235; 764-1235; 776-1240; 766-1240; 764-1240; 776-1242; 766-1242; 764-1242; 764-1464; 764-1250; 764-1041; 764-828; 764-865; 764-1045;

764-1035; 764-1128; 764-1198; 764-1268; 764-1261; 764-1264; 764-1459; 764-1463; 764-1464; 764-1683; 764-1873; 764-1482; 764-1479; 764-1672; and 764-1874.

**[0034]** In certain embodiments the truncated VWF has an internal deletion relative to mature wild type VWF. For example, the A1, A2, A3, D4, C1, C2, C3, C4, C5, C6, CK domains or combinations thereof may be deleted, and the D' domain and/or the D3 domain is retained. In further embodiments the truncated VWF does not comprise the binding sites for platelet glycoprotein Iba (GPIba), collagen and/or integrin  $\alpha$ IIb $\beta$ III (RGDS sequence within the C1 domain). In other embodiments, the truncated VWF does not comprise the cleavage site (Tyr1605-Met1606) for ADAMTS13 which is located at the central A2 domain of VWF. In yet another embodiment, the truncated VWF does not comprise the binding sites for GPIba, and/or does not comprise the binding site for collagen, and/or does not comprise the binding site for integrin  $\alpha$ IIb $\beta$ III, and/or it does not comprise the cleavage site (Tyr1605-Met1606) for ADAMTS13 which is located at the central A2 domain of VWF.

**[0035]** In other embodiments the truncated VWF comprises or consists of an amino acid sequence that has a sequence identity of at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, to one of the amino acid sequences recited in the preceding paragraph, provided that the truncated VWF is capable of binding to FVIII.

**[0036]** A polypeptide of the invention is termed a "dimer" in the present invention if two monomers of polypeptide of the invention are linked covalently. Preferably the two monomeric subunits are covalently linked via at least one disulfide bridge, e.g. by one, two, three or four disulfide bridges. The cysteine residues forming the at least one disulfide bridge are preferably located within the truncated VWF portion of the polypeptide of the invention. In one embodiment, these cysteine residues are Cys-1099, Cys-1142, Cys-1222, Cys-1225, or Cys-1227 or combinations thereof.

**[0037]** The dimer is preferably a homo-dimer, whereby each monomer comprises preferably a half-life extending moiety as disclosed. The truncated VWF preferably comprises or consists of two polypeptides each with an amino acid sequence having a sequence identity of at least 90% to amino acids 764 to 1099, amino acids 764 to 1142, amino acids 764 to 1222, amino acids 764 to 1225, or amino acids 764 to 1227 of SEQ ID NO:4 and is capable of binding to FVIII. In preferred embodiments the truncated VWF comprises or consists of an amino acid sequence having a sequence identity of at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, to amino acids 764 to 1099, amino acids 764 to 1142, amino acids 764 to 1222, amino acids 764 to 1225, or amino acids 764 to 1227 of SEQ ID NO:4 and is capable of binding to FVIII. Most preferably, the truncated VWF comprises or consists of amino acids 764 to 1099, amino acids 764 to 1142, amino acids 764 to 1222, amino acids 764 to 1225, amino acids 764 to 1227 or amino acids 764 to 1242 of SEQ ID NO:4.

**[0038]** The truncated VWF may be any one of the VWF fragments disclosed in WO 2013/106787 A1, WO 2014/198699 A2, WO 2011/060242 A2 or WO 2013/093760 A2, the

disclosure of which is i Z referred to herein. According to further preferred embodiments the truncated VWF as disclosed above may comprise at least one of the amino acid substitutions as disclosed in WO 2016/000039 A1. Those modified versions of the truncated VWF comprise at least one amino acid substitution within its D' domain, as compared to the amino acid sequence of the D' domain of wild-type VWF according to SEQ ID NO: 4. The amino acid sequence of the modified versions of the truncated VWF can have one or more amino acid substitutions relative to the respective wild type sequence. The amino acid sequence of the D' domain of the modified truncated VWF preferably has one or 2 amino acid substitutions relative to the D' domain of SEQ ID NO:4. It is preferred that S at position 764 of SEQ ID NO:4, corresponding to position 1 of SEQ ID NO:2, is substituted with an amino acid selected from the group consisting of G, P, V, E, Y, A and L. It is also preferred that S at position 766 of SEQ ID NO:4, corresponding to position 3 of SEQ ID NO:2 is substituted with an amino acid selected from the group consisting of Y, I, M, V, F, H, R and W. Preferred combinations of substitutions include S764G/S766Y, S764P/S766I, S764P/S766M, S764V/S766Y, S764E/S766Y, S764Y/S766Y, S764L/S766Y, S764P/S766W, S766W/S806A, S766Y/P769K, S766Y/P769N, S766Y/P769R and S764P/S766L, referring to the sequence of SEQ ID NO:4. The binding affinity of the polypeptide of the present invention to FVIII may be further increased by introduction of said substitutions compared to the binding affinity of a reference polypeptide which has the same amino acid sequence except for said modifications. Said substitutions within the truncated VWF may contribute to further increase the half-life of endogenous FVIII or the half-life of co-administered FVIII and/or may allow for reduction of the dose of the recombinant polypeptide of the invention to be administered.

#### ***Half-life extending moiety***

**[0039]** The half-life of endogenous VWF in human plasma is about 16h (Lenting PJ, Christophe OD, Denis CV. von Willebrand factor biosynthesis, secretion, and clearance: connecting the far ends. Blood. 2015.125(13):2019-28).

**[0040]** In addition to the truncated VWF, the polypeptide of the invention further comprises a half-life extending moiety. The half-life-extending moiety may be a heterologous amino acid sequence fused to the truncated VWF. Alternatively, the half-life-extending moiety may be chemically conjugated to the polypeptide comprising the truncated VWF by a covalent bond different from a peptide bond.

**[0041]** In certain embodiments of the invention, the half-life of the polypeptide of the invention is extended by chemical modification, e.g. attachment of a half-life extending moiety such as polyethylene glycol (PEGylation), glycosylated PEG, hydroxyl ethyl starch (HESylation), polysialic acids, elastin-like polypeptides, heparosan polymers or hyaluronic acid. In another embodiment, the polypeptide of the invention is conjugated to a HLEP such as albumin via a chemical linker. The principle of this conjugation technology has been described in an exemplary manner by Conjuchem LLC (see, e.g., US patent No. 7,256,253).

**[0042]** In other embodiments, the half-life-extending moiety is a half-life enhancing protein (HLEP). Preferably, the HLEP is an albumin or a fragment thereof. The N-terminus of the albumin may be fused to the C-terminus of the truncated VWF. Alternatively, the C-terminus of the albumin may be fused to the N-terminus of the truncated VWF. One or more HLEPs may be fused to the N- or C-terminal part of VWF provided that they do not interfere with or abolish the binding capability of the truncated VWF to FVIII.

**[0043]** The recombinant polypeptide further comprises preferably a chemical bond or a linker sequence positioned between the truncated VWF and the HLEM.

**[0044]** Said linker sequence may be a peptidic linker consisting of one or more amino acids, in particular of 1 to 50, 1 to 30, 1 to 20, 1 to 15, 1 to 10, 1 to 5 or 1 to 3 (e.g. 1, 2 or 3) amino acids and which may be equal or different from each other. Preferably, the linker sequence is not present at the corresponding position in the wild-type VWF. Preferred amino acids present in said linker sequence include Gly and Ser. The linker sequence should be non-immunogenic. Preferred linkers may be comprised of alternating glycine and serine residues. Suitable linkers are described for example in WO2007/090584.

**[0045]** In another embodiment of the invention the peptidic linker between the truncated VWF moiety and the HLEM consists of peptide sequences, which serve as natural interdomain linkers in human proteins. Preferably such peptide sequences in their natural environment are located close to the protein surface and are accessible to the immune system so that one can assume a natural tolerance against this sequence. Examples are given in WO 2007/090584. Cleavable linker sequences are described, e.g., in WO 2013/120939 A1.

**[0046]** In a preferred embodiment of the recombinant polypeptide the linker between the truncated VWF and the HLEM is a glycine/serine peptidic linker having or consisting of amino acid sequence 480 - 510 of SEQ ID NO:2.

**[0047]** In one embodiment the polypeptide has the following structure:



**[0048]** Wherein tVWF is the truncated VWF, L1 is a chemical bond or a linker sequence, and H is a HLEP.

**[0049]** L1 may be a chemical bond or a linker sequence consisting of one or more amino acids, e.g. of 1 to 50, 1 to 30, 1 to 20, 1 to 15, 1 to 10, 1 to 5 or 1 to 3 (e.g. 1, 2 or 3) amino acids and which may be equal or different from each other. Usually, the linker sequences are not present at the corresponding position in the wild-type VWF. Examples of suitable amino acids present in L1 include Gly and Ser. The linker should be non-immunogenic and may be a non-cleavable or cleavable linker. Non-cleavable linkers may be comprised of alternating

glycine and serine residues as exemplified in WO2007/090584. In another embodiment of the invention the peptidic linker between the truncated VWF moiety and the albumin moiety consists of peptide sequences, which serve as natural interdomain linkers in human proteins. Preferably such peptide sequences in their natural environment are located close to the protein surface and are accessible to the immune system so that one can assume a natural tolerance against this sequence. Examples are given in WO2007/090584. Cleavable linker sequences are described, e.g., in WO 2013/120939 A1.

**[0050]** Preferred HLEP sequences are described infra. Likewise encompassed by the invention are fusions to the exact "N-terminal amino acid" or to the exact "C-terminal amino acid" of the respective HLEP, or fusions to the "N-terminal part" or "C-terminal part" of the respective HLEP, which includes N-terminal deletions of one or more amino acids of the HLEP. The polypeptide may comprise more than one HLEP sequence, e.g. two or three HLEP sequences. These multiple HLEP sequences may be fused to the C-terminal part of VWF in tandem, e.g. as successive repeats.

#### ***Half-life enhancing polypeptides (HLEPs)***

**[0051]** Preferably, the half-life extending moiety is a half-life extending polypeptide (HLEP), more preferably HLEP is selected from albumin or fragments thereof, immunoglobulin constant region and portions thereof, e.g. the Fc fragment, solvated random chains with large hydrodynamic volume (e.g. XTEN (Schellenberger et al. 2009; Nature Biotechnol. 27:1186-1190), homo-amino acid repeats (HAP) or proline-alanine-serine repeats (PAS), afamin, alpha-fetoprotein, Vitamin D binding protein, transferrin or variants thereof, carboxyl-terminal peptide (CTP) of human chorionic gonadotropin- $\beta$  subunit, polypeptides or lipids capable of binding under physiological conditions to albumin or immunoglobulin constant region.

**[0052]** A "half-life enhancing polypeptide" as used herein is preferably selected from the group consisting of albumin, a member of the albumin-family, the constant region of immunoglobulin G and fragments thereof, region and polypeptides capable of binding under physiological conditions to albumin, to members of the albumin family as well as to portions of an immunoglobulin constant region. It may be a full-length half-life-enhancing protein described herein (e.g. albumin, a member of the albumin-family or the constant region of immunoglobulin G) or one or more fragments thereof that are capable of stabilizing or prolonging the therapeutic activity or the biological activity of the coagulation factor. Such fragments may be of 10 or more amino acids in length or may include at least about 15, at least about 20, at least about 25, at least about 30, at least about 50, at least about 100, or more contiguous amino acids from the HLEP sequence or may include part or all of specific domains of the respective HLEP, as long as the HLEP fragment provides a functional half-life extension of at least 25% compared to the respective polypeptide without the HLEP.

**[0053]** The HLEP portion of the polypeptide of the invention may be a variant of a wild type HLEP. The term "variants" includes insertions, deletions and substitutions, either conservative

or non-conservative, where such changes do not substantially alter the FVIII-binding activity of the truncated VWF.

**[0054]** In particular, the proposed VWF HLEP fusion constructs of the invention may include naturally occurring polymorphic variants of HLEPs and fragments of HLEPs. The HLEP may be derived from any vertebrate, especially any mammal, for example human, monkey, cow, sheep, or pig. Non-mammalian HLEPs include, but are not limited to, hen and salmon.

**[0055]** According to certain embodiments of present disclosure the half-life extending moiety, in particular a HLEP, portion of the polypeptide of the invention may be specified with the alternative term "FP". Preferably, the term "FP" represents a human albumin if not indicated otherwise.

**[0056]** According to certain preferred embodiments, the polypeptide is a fusion protein. A fusion protein in terms of present invention is a protein created by in-frame joining of at least two DNA sequences encoding the truncated VWF as well as the HLEP. The skilled person understands that translation of the fusion protein DNA sequence will result in a single protein sequence. As a result of an in frame insertion of a DNA sequence encoding a peptidic linker according to a further preferred embodiment, a fusion protein comprising the truncated VWF, a suitable linker and the HLEP may be obtained.

#### **Albumin as HLEP**

**[0057]** The terms, "human serum albumin" (HSA) and "human albumin" (HA) and "albumin" (ALB) are used interchangeably in this application. The terms "albumin" and "serum albumin" are broader, and encompass human serum albumin (and fragments and variants thereof) as well as albumin from other species (and fragments and variants thereof).

**[0058]** As used herein, "albumin" refers collectively to albumin polypeptide or amino acid sequence, or an albumin fragment or variant, having one or more functional activities (e.g., biological activities) of albumin. In particular, "albumin" refers to human albumin or fragments thereof, especially the mature form of human albumin as shown in SEQ ID NO:6 herein or albumin from other vertebrates or fragments thereof, or analogs or variants of these molecules or fragments thereof.

**[0059]** According to certain embodiments of present disclosure the alternative term "FP" is used to identify the HLEP, in particular to define albumin as HLEP.

**[0060]** In particular, the proposed polypeptides of the invention may include naturally occurring polymorphic variants of human albumin and fragments of human albumin. Generally speaking, an albumin fragment or variant will be at least 10, preferably at least 40, most preferably more than 70 amino acids long.

**[0061]** Preferred embodiments of the invention include albumin variants used as a HLEP of the polypeptide of the invention with enhanced binding to the FcRn receptor. Such albumin variants may lead to a longer plasma half-life of a truncated VWF albumin variant fusion protein as compared to a truncated VWF fusion with a wild-type albumin.

**[0062]** The albumin portion of the polypeptides of the invention may comprise at least one subdomain or domain of HA or conservative modifications thereof.

### **Immunoglobulins as HLEPs**

**[0063]** Immunoglobulin G (IgG) constant regions (Fc) are known in the art to increase the half-life of therapeutic proteins (Dumont J A et al. 2006. BioDrugs 20:151-160). The IgG constant region of the heavy chain consists of 3 domains (CH1-CH3) and a hinge region. The immunoglobulin sequence may be derived from any mammal, or from subclasses IgG1, IgG2, IgG3 or IgG4, respectively. IgG and IgG fragments without an antigen-binding domain may also be used as HLEPs. The therapeutic polypeptide portion is connected to the IgG or the IgG fragments preferably via the hinge region of the antibody or a peptidic linker, which may even be cleavable. Several patents and patent applications describe the fusion of therapeutic proteins to immunoglobulin constant regions to enhance the therapeutic protein's in vivo half-lives. US 2004/0087778 and WO 2005/001025 describe fusion proteins of Fc domains or at least portions of immunoglobulin constant regions with biologically active peptides that increase the half-life of the peptide, which otherwise would be quickly eliminated in vivo. Fc-IFN- $\beta$  fusion proteins were described that achieved enhanced biological activity, prolonged circulating half-life and greater solubility (WO 2006/000448). Fc-EPO proteins with a prolonged serum half-life and increased in vivo potency were disclosed (WO 2005/063808) as well as Fc fusions with G-CSF (WO 2003/076567), glucagon-like peptide-1 (WO 2005/000892), clotting factors (WO 2004/101740) and interleukin-10 (U.S. Pat. No. 6,403,077), all with half-life enhancing properties.

**[0064]** Various HLEPs which can be used in accordance with this invention are described in detail in WO 2013/120939 A1.

### ***N-Glycans and Sialylation of the polypeptide of the invention***

**[0065]** The polypeptide of the invention preferably comprises N-glycans, and at least 75%, preferably at least 85%, more preferably at least 90% of said N-glycans comprise, on average, at least one sialic acid moiety. In preferred embodiments, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, of said N-glycans comprise, on average, at least one sialic acid moiety. The inventors found that polypeptides comprising highly sialylated VWF fragments not only have a prolonged half-life themselves, but are also capable to extend the half-life of co-administered FVIII further. In

other words, administration of the polypeptide of the invention leads to an extended half-life and/or to a reduced clearance of co-administered FVIII.

**[0066]** The polypeptide of the invention preferably comprises N-glycans, and at least 50% of the sialyl groups of the N-glycans of the glycoproteins are  $\alpha$ -2,6-linked sialyl groups. In general, terminal sialyl groups can be attached to the galactose groups via a  $\alpha$ -2,3- or via a  $\alpha$ -2,6-linkage. Typically, N-glycans of the polypeptide of the invention comprise more  $\alpha$ -2,6-linked sialyl groups than  $\alpha$ -2,3-linked sialyl groups. Preferably, at least 60%, or at least 70%, or at least 80%, or at least 90% of the sialyl groups of the N-glycans are  $\alpha$ -2,6-linked sialyl groups. These embodiments can be obtained by, e.g., co-expressing human  $\alpha$ -2,6-sialyltransferase in mammalian cells.

**[0067]** In one embodiment, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, of the N-glycans of the polypeptide of the invention comprise at least one sialic acid group. In another embodiment, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, of the N-glycans of the polypeptide of the invention comprise at least one sialic acid group.

**[0068]** In another embodiment, less than 15%, less than 12%, less than 10%, or less than 8%, or less than 6%, or less than 5%, or less than 4%, or less than 3%, or less than 2% or even less than 1% of the N-glycans of the polypeptide of the invention are asialo-N-glycans, i.e. they are N-glycans lacking a sialic acid group. In another embodiment, less than 15%, less than 12%, less than 10%, or less than 8%, or less than 6%, or less than 5%, or less than 4%, or less than 3%, or less than 2% or even less than 1% of the N-glycans of the polypeptide of the invention are asialo-N-glycans, i.e. they do not have a sialic acid group.

**[0069]** Suitable methods of producing such glycoproteins are described in pending PCT/EP2016/061440. Accordingly, a method of producing a glycoprotein comprising N-glycans with increased sialylation is described therein, which method comprises (i) providing cells comprising a nucleic acid encoding a polypeptide comprising a truncated von Willebrand Factor (VWF), and (ii) culturing said cells at a temperature of less than 36.0°C. In addition, a method of producing a dimer of a glycoprotein comprising a truncated von Willebrand Factor (VWF), or for increasing the dimerization of said glycoprotein is described, which method comprises (i) providing cells comprising a nucleic acid encoding the amino acid sequence of the glycoprotein, and (ii) culturing said cells at a temperature of less than 36.0°C. Further, a method of producing a glycoprotein comprising N-glycans with increased sialylation is described therein, which comprises (i) providing cells comprising a nucleic acid encoding a polypeptide comprising a truncated von Willebrand Factor (VWF) and a recombinant nucleic acid encoding an  $\alpha$ -2,6-sialyltransferase, and (ii) culturing the cells under conditions that allow expression of the glycoprotein and of the  $\alpha$ -2,6-sialyltransferase.

**[0070]** The above-described embodiments can be combined with each other. Any percentages of N-glycans mentioned above, or any indications of the degree of sialylation, are to be understood as average percentages or degrees, i.e. they refer to a population of molecules,

not to a single molecule. It is clear that the glycosylation or sialylation of the individual glycoprotein molecules within a population of glycoproteins will show some heterogeneity.

### ***Dimers***

**[0071]** The polypeptides of this invention have preferably a high proportion of dimers. The polypeptide of the invention is therefore present as dimer. In one embodiment, at least 50%, or at least 60%, or at least 70% of the polypeptides are present as dimers. In another embodiment, the ratio dimer : monomer of the polypeptide of the invention is at least 1.5, preferably at least 2, more preferably at least 2.5 or at least 3. Most preferably all polypeptides of the invention are present as dimers. The use of dimers is favorable, as the dimer has an improved affinity to Factor VIII as compared to the monomer. The dimer content, and the ratio of dimer to monomer of the polypeptide of the invention can be determined as described in the Examples.

**[0072]** In one embodiment, the affinity of the polypeptide of the invention to Factor VIII is greater than that of human native VWF to the same Factor VIII molecule. The factor VIII affinity may refer to human native Factor VIII, or to the Factor VIII molecule characterized by SEQ ID NO:5.

**[0073]** It has been found that preparations of the polypeptide of this invention with a high proportion of dimers do have an increased affinity to Factor VIII. Such increased affinity to Factor VIII does lead to an enhanced stabilization of Factor VIII by the polypeptides of the present invention. Alternatively to or in combination with an increased dimer proportion also polypeptides in accordance with the invention with mutations within the Factor VIII binding domain which do increase the affinity to Factor VIII are preferred embodiments of the invention. Suitable mutations are disclosed, e.g., in WO 2013/120939 A1.

### ***Preparation of the polypeptide***

**[0074]** The nucleic acid encoding the polypeptide of the invention can be prepared according to methods known in the art. Based on the cDNA sequence of VWF (SEQ ID NO:3), recombinant DNA encoding the above-mentioned truncated VWF constructs or polypeptides of the invention can be designed and generated.

**[0075]** Even if the polypeptide which is secreted by the host cells does not comprise amino acids 1 to 763 of VWF, it is preferred that the nucleic acid (e.g. the DNA) encoding the intracellular precursor of the polypeptide comprises a nucleotide sequence encoding an amino acid sequence having a sequence identity of at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, to amino acids 23 to 763 or preferably to amino acids 1 to 763 of SEQ ID NO:4. Most preferably, the nucleic acid (e.g. the DNA) encoding the intracellular precursor of

the polypeptide comprises a nucleotide sequence encoding amino acids 23 to 763 of SEQ ID NO:4, or amino acids 1 to 763 of SEQ ID NO:4.

**[0076]** Constructs in which the DNA contains the entire open reading frame inserted in the correct orientation into an expression plasmid may be used for protein expression. Typical expression vectors contain promoters that direct the synthesis of large amounts of mRNA corresponding to the inserted nucleic acid in the plasmid-bearing cells. They may also include an origin of replication sequence allowing for their autonomous replication within the host organism, and sequences that increase the efficiency with which the synthesized mRNA is translated. Stable long-term vectors may be maintained as freely replicating entities by using regulatory elements of, for example, viruses (e.g., the OriP sequences from the Epstein Barr Virus genome). Cell lines may also be produced that have integrated the vector into the genomic DNA, and in this manner the gene product is produced on a continuous basis.

**[0077]** Typically, the cells to be provided are obtained by introducing the nucleic acid encoding a polypeptide of the invention into mammalian host cells.

**[0078]** Any host cell susceptible to cell culture, and to expression of glycoproteins, may be utilized in accordance with the present invention. In certain embodiments, a host cell is mammalian. Non-limiting examples of mammalian cells that may be used in accordance with the present invention include BALB/c mouse myeloma line (NSO/ 1, ECACC No: 85110503); human retinoblasts (PER.C6 (CruCell, Leiden, The Netherlands)); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59, 1977); baby hamster kidney cells (BHK, ATCC CCL10); Chinese hamster ovary cells +/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216, 1980); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243 251, 1980); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (HepG2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals NY. Acad. Sci., 383:44-68, 1982); MRC 5 cells; PS4 cells; human amniocyte cells (CAP); and a human hepatoma line (Hep G2). Preferably, the cell line is a rodent cell line, especially a hamster cell line such as CHO or BHK.

**[0079]** Methods suitable for introducing nucleic acids sufficient to achieve expression of a glycoprotein of interest into mammalian host cells are known in the art. See, for example, Gething et al., Nature, 293:620-625, 1981; Mantei et al., Nature, 281:40-46, 1979; Levinson et al. EP 117,060; and EP 117,058. For mammalian cells, common methods of introducing genetic material into mammalian cells include the calcium phosphate precipitation method of Graham and van der Erb (Virology, 52:456-457, 1978) or the lipofectamine™ (Gibco BRL) Method of Hawley-Nelson (Focus 15:73, 1993). General aspects of mammalian cell host system transformations have been described by Axel in US. Pat. No. 4,399,216. For various techniques for introducing genetic material into mammalian cells, see Keown et al., Methods in

Enzymology, 1989, Keown et al., Methods in Enzymology, 185:527-537, 1990, and Mansour et al., Nature, 336:348-352, 1988.

[0080] The cells are cultured under conditions that allow expression of the polypeptide. The polypeptide can be recovered and purified using methods that are known to the skilled artisan.

***Maximal concentration and area under the time-concentration curve of endogenous FVIII***

[0081] According to a preferred aspect of the invention polypeptide as defined hereinabove is used for increasing the  $C_{\max}$  or AUC of endogenous Factor VIII as compared to untreated subjects by administration the polypeptide of the invention alone, i.e. without co-administration of FVIII.

[0082] The maximal concentration ( $C_{\max}$ ) is the highest plasma concentration value measured. Following administration of said recombinant polypeptide, the  $C_{\max}$  for FVIII may be at least 10 mIU/mL, at least 25 mIU/mL, at least 50 mIU/mL, at least 100 mIU/mL, at least 200 mIU/mL, at least 300 mIU/mL or at least 400 mIU/mL.

[0083] The  $AUC_{0-t}$  is the peak area under the plasma concentration-time curve from zero to the last measured timepoint. Following administration of the recombinant polypeptide, the AUC for the endogenous FVIII increase may be at least 1000 mIU\*h/mL, at least 2000 mIU\*h/mL, at least 3000 mIU\*h/mL, at least 5000 mIU\*h/mL, at least 10000 mIU\*h/mL or at least 20000 mIU\*h/mL chromogenic FVIII activity.

***Bioavailability of rD'D3-FP after subcutaneous administration***

[0084] A further aspect of the invention pertains to providing or improving subcutaneous bioavailability of the polypeptide as defined hereinabove.

[0085] The term bioavailability, as used herein, is defined as the percentage of the  $AUC_{0-\infty}$  of polypeptide of the invention after s.c. administration, in relation to the  $AUC_{0-\infty}$  of polypeptide of the invention after i.v. administration. The  $AUC_{0-\infty}$  is the area under the plasma concentration-time curve from zero to infinity. For evaluation of the pharmacokinetic data for calculation of  $AUC_{0-\infty}$ , a two-compartment model (biphasic pharmacokinetic profile) was applied.

[0086] According to certain embodiments, bioavailability of the recombinant polypeptide in the absence of co-administered FVIII is at least 30%, preferably at least 35%, more preferably at least 40%, at least 45% or at least 50%.

**[0087]** According to certain embodiments, bioavailability of the recombinant polypeptide following co-administration with FVIII is at least 30%, preferably at least 35%, more preferably at least 40%, at least 45% or at least 50%.

***Treatment of coagulation disorder***

**[0088]** A further aspect of this invention is a method of treating a blood coagulation disorder, comprising administering to a patient in need thereof an effective amount of a polypeptide as defined hereinabove.

**[0089]** The polypeptides of the invention are useful for treating blood coagulation disorders including hemophilia A and von-Willebrand disease. The term "hemophilia A" refers to a deficiency in functional coagulation FVIII, which is usually inherited. The von-Willebrand disease is selected from the group consisting of von-Willebrand disease type 2N, von-Willebrand disease type 3, and von-Willebrand disease type 1.

**[0090]** In another embodiment, the blood coagulation disorder is von-Willebrand disease type 1 characterized by an endogenous FVIII activity level before treatment which is reduced compared to the endogenous FVIII activity level in NHP.

**[0091]** The patient to be treated may have a reduced activity and/or level of endogenous FVIII as compared to the endogenous FVIII in NHP. The endogenous FVIII activity in the patient may be less than 80%, or less than 70%, or less than 60%, or less than 50%, or less than 40%, or less than 30%, or less than 20%, or less than 20%, or less than 10%, or less than 5% of the endogenous FVIII activity in NHP. The endogenous FVIII activity in the patient to be treated may be less than 0.8 IU/ml, or less than 0.7 IU/ml, or less than 0.6 IU/ml, or less than 0.5 IU/ml, or less than 0.4 IU/ml, or less than 0.3 IU/ml, or less than 0.2 IU/ml, or less than 0.1 IU/ml, or less than 0.05 IU/ml of whole blood.

**[0092]** The polypeptide of the invention is to be administered to a patient having an endogenous FVIII activity of at least 0.005 IU/mL. In certain embodiments, the polypeptide of the invention is to be administered to a patient having an endogenous FVIII activity of 0.01 to 0.4 IU/ml, or 0.02 to 0.3 IU/ml, or 0.03 to 0.2 IU/ml, or 0.04 to 0.1 IU/ml of whole blood.

**[0093]** In one embodiment, the blood coagulation disorder is moderate hemophilia A. Moderate hemophilia A is preferably characterized by an endogenous FVIII activity level which is from about 1% to about 5% of the endogenous FVIII activity level in NHP. Typically, subjects having moderate hemophilia A have an endogenous FVIII activity level from 0.01 to 0.05 IU/mL in plasma.

**[0094]** In another embodiment, the blood coagulation disorder is mild hemophilia A. Mild hemophilia A is preferably characterized by an endogenous FVIII activity level which is from about 5% to about 40% of the endogenous FVIII activity level in NHP. Typically, subjects having

mild hemophilia A have an endogenous FVIII activity level from 0.05 to 0.4 IU/mL in plasma.

**[0095]** In another embodiment, the blood coagulation disorder is severe hemophilia A. Severe hemophilia A is characterized by endogenous FVIII activity level below 1% of the endogenous FVIII activity in NHP.

**[0096]** In another embodiment, the blood coagulation disorder is von-Willebrand disease type 2N. Von-Willebrand disease type 2N is preferably characterized by an endogenous FVIII activity level before treatment which is in a range between about 3 IU/dL and about 30 IU/dL FVIII activity level, corresponding to about 3% to about 30% of the endogenous FVIII activity level in NHP. Most of the patients have an endogenous FVIII activity level below 20 IU/dL, thus a level below 20% of the endogenous FVIII activity level in NHP. Thus, subjects having von-Willebrand disease type 2N have an endogenous FVIII activity level from 0.03 IU/mL to 0.3 IU/mL in plasma, typically below 0.2 IU/mL.

**[0097]** In another embodiment, the blood coagulation disorder is von-Willebrand disease type 3, preferably characterized by an endogenous FVIII activity level before treatment which is usually in a range between about 1 IU/dL and about 20 IU/dL FVIII activity level corresponding to about 1% to about 20% of the endogenous FVIII activity level in NHP. Most of the patients have an endogenous FVIII activity level below 10 IU/dL, thus a level below 10% of the endogenous FVIII activity level in NHP.

**[0098]** The polypeptide of the invention is preferably administered to a subject for the prophylactic prevention of a bleeding event. This treatment does not comprise administration of any exogenous FVIII. The polypeptide of the invention may also be administered to a subject for the treatment of a bleeding event. In that case, the polypeptide of the invention may be co-administered with exogenous FVIII. After such treatment of the bleeding event the follow-up treatment is then carried out without co-administration of exogenous FVIII. That is, the follow-up treatment is done with the polypeptide of the invention only.

**[0099]** Typically, the treatment with the polypeptide of the invention alone, i.e. without co-administration of exogenous FVIII, is continued until a bleeding event occurs. The duration of the treatment with the polypeptide of the invention alone, i.e. without co-administration of exogenous FVIII, is not particularly limited, it is usually continued for at least one week, or at least two weeks, or at least three weeks, or at least four weeks, or at least two months.

**[0100]** Treatment of a disease encompasses the treatment of patients already diagnosed as having any form of the disease at any clinical stage or manifestation; the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of the disease; and/or preventing and/or reducing the severity of the disease.

**[0101]** A "subject" or "patient" to whom a polypeptide of the invention is administered preferably is a human. In certain aspects, the human is a pediatric patient. In other aspects, the human is an adult patient.

**[0102]** Compositions comprising a polypeptide of the invention and, optionally FVIII, are described herein. The compositions typically are supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient).

**[0103]** The term "Factor VIII" and "FVIII" are used interchangeably herein and encompass both plasma derived FVIII and recombinant FVIII. Recombinant FVIII encompasses without limitation full-length FVIII as well as two-chain B-domain deleted or truncated variants as well as single-chain B-domain deleted or truncated variants for example those described in WO 2004/067566 and other FVIII variants with mutations outside the B-domain but having the biological activity of FVIII.

**[0104]** The polypeptide of the invention can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intraperitoneally, intramuscularly, topically or locally. The most suitable route for administration in any given case will depend on the particular polypeptide, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, a polypeptide of the invention will be administered intravenously.

**[0105]** In accordance with this invention, the patient being treated with the polypeptide of the invention may also be treated with blood coagulation Factor VIII, provided that the follow-up treatment is done with the polypeptide of the invention alone, i.e. without co-administration of exogenous FVIII. The polypeptide of the invention and the Factor VIII may be administered simultaneously or in a sequential fashion both modes of administration being encompassed by the term "combination therapy" and "co-administration". The polypeptide of the invention and the Factor VIII may be administered as a mixture, i.e. within the same composition, or separately, i.e. as separate compositions.

**[0106]** The polypeptide and optionally the FVIII are preferably administered intravenously or subcutaneously.

**[0107]** In a first embodiment, both the polypeptide and optionally the FVIII are administered intravenously. In a second embodiment, both the polypeptide and optionally the FVIII are administered subcutaneously.

**[0108]** In another embodiment, the FVIII is administered intravenously, and the polypeptide is administered via a different route. In further embodiments, the polypeptide is administered subcutaneously, and the FVIII is administered via a different route. For example, the polypeptide may be administered subcutaneously, and the FVIII may be administered intravenously.

**[0109]** In further embodiments, the FVIII is administered subcutaneously, and the polypeptide is administered via a different route. In further embodiments, the polypeptide is administered

intravenously, and the FVIII is administered via a different route. For example, the polypeptide may be administered intravenously, and the FVIII may be administered subcutaneously.

**[0110]** Determination of the total number of doses, and length of treatment with a polypeptide of the invention is well within the capabilities of those skilled in the art. The dosage of the polypeptide of the invention to be administered may depend on the concentrations of the endogenous FVIII, the concentration of endogenous VWF in the patient to be treated, or both. An effective dosage based on the ratios described herein can be determined by the skilled person, taking into account the molecular weight of the polypeptide of the invention. Typical dosages for FVIII may range from about 20 U/kg body weight to about 100 U/kg body weight.

**[0111]** The concentration of Factor VIII in the composition used is typically in the range of 10-10,000 IU/mL. In different embodiments, the concentration of FVIII in the compositions of the invention is in the range of 10-8,000 IU/mL, or 10-5,000 IU/mL, or 20-3,000 IU/mL, or 50-1,500 IU/mL, or 3,000 IU/mL, or 2,500 IU/mL, or 2,000 IU/mL, or 1,500 IU/mL, or 1,200 IU/mL, or 1,000 IU/mL, or 800 IU/mL, or 750 IU/mL, or 600 IU/mL, or 500 IU/mL, or 400 IU/mL, or 300 IU/mL, or 250 IU/mL, or 200 IU/mL, or 150 IU/mL, or 125 IU/mL, or 100 IU/mL, or 62.5 IU/mL, or 50 IU/mL, provided the requirements regarding the ratio with respect to the VWF polypeptide of the invention as defined herein are fulfilled.

**[0112]** "International Unit," or "IU," is a unit of measurement of the blood coagulation activity (potency) of FVIII as measured by a FVIII activity assay such as a one stage clotting assay or a chromogenic substrate FVIII activity assay using a standard calibrated against an international standard preparation calibrated in "IU". One stage clotting assays are known to the art, such as that described in N Lee, Martin L, et al., An Effect of Predilution on Potency Assays of FVIII Concentrates, Thrombosis Research (Pergamon Press Ltd.) 30, 511 519 (1983). Principle of the one stage assay: The test is executed as a modified version of the activated Partial Thromboplastin Time (aPTT)-assay: Incubation of plasma with phospholipids and a surface activator leads to the activation of factors of the intrinsic coagulation system. Addition of calcium ions triggers the coagulation cascade. The time to formation of a measurable fibrin clot is determined. The assay is executed in the presence of Factor VIII deficient plasma. The coagulation capability of the deficient plasma is restored by Coagulation Factor VIII included in the sample to be tested. The shortening of coagulation time is proportional to the amount of Factor VIII present in the sample. The activity of Coagulation Factor VIII is quantified by direct comparison to a standard preparation with a known activity of Factor VIII in International Units.

**[0113]** Another standard assay is a chromogenic substrate assay. Chromogenic substrate assays may be purchased commercially, such as the coamatic FVIII test kit (Chromogenix-Instrumentation Laboratory SpA V. le Monza 338 - 20128 Milano, Italy). Principle of the chromogenic assay: In the presence of calcium and phospholipid, Factor X is activated by Factor IXa to Factor Xa. This reaction is stimulated by Factor VIIIa as cofactor. FVIIIa is formed by low amounts of thrombin in the reaction mixture from FVIII in the sample to be measured. When using the optimum concentrations of Ca<sup>2+</sup>, phospholipid and Factor IXa and an excess quantity of Factor X, activation of Factor X is proportional to the potency of Factor VIII.

Activated Factor X releases the chromophore pNA from the chromogenic substrate S-2765. The release of pNA, measured at 405 nm, is therefore proportional to the amount of FXa formed, and, therefore, also to the Factor VIII activity of the sample.

**[0114]** According to some preferred embodiments of the use of the polypeptide, the endogenous FVIII level is increased following administration of said polypeptide to a level of at least 1%, or preferably at least 2%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100% of the activity level of endogenous FVIII in normal human plasma (NHP).

**[0115]** In a further preferred embodiment the endogenous FVIII level is increased following administration of said polypeptide to a level being in a range between 1% to 500%, preferably between 1% to 400%, between 1% to 300%, between 1% to 200%, between 1% to 150%, or between 1% to 100% of the activity level of endogenous FVIII in normal human plasma (NHP).

**[0116]** In a further preferred embodiment the endogenous FVIII level is increased following administration of said polypeptide to a level being in a range between 5% to 400%, preferably between 5% to 300%, between 10% to 200%, between 10% to 150%, between 20% to 150%, or between 40% to 150% of the activity level of endogenous FVIII in normal human plasma (NHP).

**[0117]** In a further preferred embodiment the endogenous FVIII level is increased following administration of said polypeptide to a level as described herein, whereby the dosage of the administered polypeptide and/or the frequency of administration of the polypeptide is adjusted in order to achieve said levels of endogenous FVIII levels in the subject.

### ***Ratios***

**[0118]** In certain embodiments, the polypeptide of the invention is co-administered together with exogenous FVIII for the treatment of a bleeding event or for initiation of a prophylactic treatment regime, wherein for follow-up treatments said polypeptide is administered without co-administration of exogenous FVIII. For those embodiments comprising co-administration of the polypeptide of the invention together with exogenous FVIII, the polypeptide of the invention is preferably administered at a dose such that the molar ratio of the polypeptide to be co-administered to the exogenous FVIII is greater than 50.

**[0119]** The polypeptide of the invention may be a monomer, a dimer, or a mixture thereof. Any molar ratios according to the invention refer to a ratio of the molar concentration of the monomeric subunit of the polypeptide of the invention, whether actually present as monomer or dimer. Ratios are formed over the molar concentration of endogenous FVIII, or, at least for certain embodiments, of the co-administered FVIII or over the molar concentration of the endogenous VWF subunits, if present. Any ratios of polypeptide of the invention over FVIII in this application refer to the amount of monomers comprised in the polypeptide of the invention,

which is preferably present as a dimer, to be administered (in mole) divided by the amount of FVIII to be administered (in mole), unless indicated otherwise. By way of non-limiting example the co-administration of 100  $\mu\text{M}$  of a monomeric polypeptide of the invention with 1  $\mu\text{M}$  of FVIII means a ratio of 100. The same ratio of 100 is obtained if 50  $\mu\text{M}$  of a dimeric polypeptide of the invention are co-administered with 1  $\mu\text{M}$  of FVIII.

**[0120]** The endogenous VWF is, if present, the VWF present in the plasma of the animal or human being to be dosed with the polypeptide of the invention. It usually consists of a range of different oligomers of approximately 2 to 40 monomeric subunits of VWF. Unless indicated otherwise, any ratios of polypeptide of the invention over endogenous VWF in this application refer to the molar plasma concentration of polypeptide of the invention per kg body weight of the treated subject immediately after administration of the polypeptide of the invention, divided by the molar plasma concentration of endogenous VWF per kg body weight of the treated subject. The molar plasma concentration of the polypeptide of the invention per kg body weight of the subject treated immediately after administration of the polypeptide of the invention is calculated assuming a dilution of the polypeptide of the invention administered directly after administration in a plasma volume of 40 ml/kg. The amount of the polypeptide of the invention immediately after administration when administered intravenously is assumed for the purposes of the invention to be identical to the amount administered.

**[0121]** According to one aspect of the invention the molar ratio of the polypeptide of the invention to the endogenous VWF is greater than 0.5. The concentration of endogenous VWF in the plasma of the subject to be treated can be determined by an ELISA or and activity assay, e.g. as described in the Examples. Typically, the concentration measured will be given in U/mL. This value can be converted into a molarity as described in the following.

**[0122]** Normal human plasma (NHP) contains VWF in a concentration of 1 U/mL or 100% by definition. This corresponds to a protein concentration of approximately 10 $\mu\text{g/mL}$  (Haberichter S.L. and Montgomery R.R., Structure and function of von Willebrand factor; in: Hemostasis and Thrombosis, eds. Marder, Aird, Bennett, Schulman and White, Lippincott Williams & Wilkins 2013, pp 197-207). Based on this VWF concentration in NHP and a molecular weight of the mature VWF monomer of approximately 267,500 Da including 18-19% of glycosylation a molar plasma concentration of the VWF monomer unit of approximately  $37 \times 10^{-9} \text{ Mol/L}$  can be calculated for NHP.

**[0123]** For calculation of the molar concentrations of rat or rabbit VWF subunits in normal rat or rabbit plasma, respectively, a molecular weight of the monomeric subunit comparable to human VWF was used (267,500 Da) together with an assumed comparable specific activity (100 U/mg) and the measured endogenous VWF activities in rat or rabbit plasma (refer also to examples).

**[0124]** The concentration of VWF in the human population varies from about 60% to about 200% of VWF concentration in NHP. In certain embodiments of the invention the concentration of endogenous VWF is defined as the concentration in NHP. In other embodiments the

concentration of endogenous VWF is determined in the subject to be treated, and the dose of the polypeptide is based on this individual value.

**[0125]** The molar ratio of the polypeptide of the invention administered to the endogenous VWF is preferably at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8, or at least 9, or at least 10, more preferably at least 15, or at least 20, or at least 25, or at least 30, most preferably at least 40, or at least 50, or at least 75.

**[0126]** The molar ratio of the polypeptide of the invention to be administered to the endogenous VWF may range from 0.5 to 1,000, or from 1 to 500, or from 2 to 400, or from 3 to 300, or from 4 to 250, or from 5 to 200, or from 6 to 150, or from 7 to 140, or from 8 to 130, or from 9 to 120, or from 10 to 110. Preferably, the molar ratio of the polypeptide of the invention administered to endogenous VWF ranges from 3 to 100, or from 4 to 90, or from 5 to 80, or from 6 to 75, or from 10 to 60.

**[0127]** The molar ratio of the polypeptide of the invention to be administered to endogenous FVIII is preferably at least 2, or at least 5, or at least 10, or at least 20, or at least 30, or at least 40, or at least 50, more preferably the ratio is greater than 50, or at least 75, at least 100, or greater than 100, or at least 200, most preferably at least 300, or at least 400, or at least 500.

**[0128]** The molar ratio of the polypeptide of the invention to be administered to endogenous FVIII may range from 2 to 10,000, or from 5 to 5,000, or from 10 to 4,000, or from 20 to 3,000, or from 30 to 2,000, or from 40 to 1,000. Preferably, the molar ratio of the polypeptide of the invention to be administered to endogenous FVIII ranges from 60 to 2,500, or from 110 to 2,000, or from 150 to 1,500, or from 200 to 1,000.

**[0129]** The molar ratio of the polypeptide of the invention to be administered to exogenous FVIII to be co-administered is preferably at least 2, or at least 5, or at least 10, or at least 20, or at least 30, or at least 40, or at least 50, more preferably the ratio is greater than 50, or at least 75, at least 100, or greater than 100, or at least 200, most preferably at least 300, or at least 400, or at least 500.

**[0130]** The molar ratio of the polypeptide of the invention to be administered to FVIII to be co-administered may range from 2 to 10,000, or from 5 to 5,000, or from 10 to 4,000, or from 20 to 3,000, or from 30 to 2,000, or from 40 to 1,000. The molar ratio of the polypeptide of the invention to be administered to FVIII to be co-administered may preferably range from 50 to 10,000, or from 50 to 5,000, or from 50 to 4,000, or from 50 to 3,000, or from 50 to 2,000, or from 50 to 1,000. Preferably, the molar ratio of the polypeptide of the invention to be administered to FVIII to be co-administered ranges from 60 to 2,500, or from 110 to 2,000, or from 150 to 1,500, or from 200 to 1,000.

### ***Pharmaceutical compositions***

**[0131]** Therapeutic formulations of the polypeptide of the invention suitable in the methods described herein can be prepared for storage as lyophilized formulations or aqueous solutions by mixing the polypeptide having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as "carriers"), i.e., buffering agents, stabilizing agents, preservatives, isotonicifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

**[0132]** Buffering agents help to maintain the pH in the range which approximates physiological conditions. They can present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium gluconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

**[0133]** Preservatives can be added to retard microbial growth, and can be added in amounts ranging from 0.2%- 1% (w/v). Suitable preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as "stabilizers" can be added to ensure isotonicity of liquid compositions and include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol,  $\alpha$ -monothioglycerol and sodium thio sulfate; low molecular weight polypeptides

(e.g., peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccharides such as raffinose; and polysaccharides such as dextran. Stabilizers can be present in the range from 0.1 to 10,000 weights per part of weight active protein.

**[0134]** Non-ionic surfactants or detergents (also known as "wetting agents") can be added to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), Pluronic polyols, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.). Non-ionic surfactants can be present in a range of about 0.05 mg/ml to about 1.0 mg/ml, or in a range of about 0.07 mg/ml to about 0.2 mg/ml.

**[0135]** Additional miscellaneous excipients include bulking agents (e.g., starch), chelating agents (e.g., EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), and cosolvents.

**[0136]** The formulation herein can also contain a second therapeutic agent in addition to a polypeptide of the invention. Examples of suitable second therapeutic agents are provided below.

**[0137]** The dosing schedule can vary from once a month to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the patient's sensitivity to the polypeptide of the invention. In specific embodiments, a polypeptide of the invention is administered, twice weekly, every 5 days, once weekly, every 10 days, every two weeks, every three weeks, every four weeks or once a month, or in any range between any two of the foregoing values, for example from every four weeks to every month, from every 10 days to every two weeks, or from two to three times a week, etc.

**[0138]** The dosage of a polypeptide of the invention to be administered will vary according to the particular polypeptide, the subject, and the nature and severity of the disease, the physical condition of the subject, the therapeutic regimen (e.g., whether a second therapeutic agent is used), and the selected route of administration; the appropriate dosage can be readily determined by a person skilled in the art.

**[0139]** It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a polypeptide of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the age and condition of the particular subject being treated, and that a physician will ultimately determine appropriate dosages to be used. This dosage can be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be altered or reduced, in accordance with normal clinical practice.

[0140] The nucleotide and amino acid sequences shown in the sequence listing are summarized in the Table 1.

Table 1

| SEQ ID NO: | Remarks                                                                                                                                            |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| 1          | DNA sequence encoding a polypeptide comprising acids 1 to 1242 of human VWF, a glycine/serine linker and human albumin; nucleotide positions (nt): |
|            | nt 1-6: EcoRI restriction enzyme cleavage site                                                                                                     |
|            | nt 32 - 3757: coding sequence for VWF amino acids 1 to 1242                                                                                        |
|            | nt 3758 - 3850: coding sequence for glycine/serine linker                                                                                          |
|            | nt 3851 - 5608: coding sequence for human albumin                                                                                                  |
|            | nt 5609 - 5616: NotI restriction enzyme cleavage site                                                                                              |
| 2          | Amino acid sequence encoded by SEQ ID NO:1 (mature form): amino acid positions (aa):                                                               |
|            | aa 1 - 479: VWF D'D3 region (VWF amino acids 764 - 1242)                                                                                           |
|            | aa 480 - 510: glycine/serine linker                                                                                                                |
|            | aa 511 - 1095: human albumin                                                                                                                       |
| 3          | DNA sequence encoding the pre-pro form of human native VWF                                                                                         |
| 4          | Amino acid sequence encoded by SEQ ID NO:3                                                                                                         |
| 5          | Amino acid sequence of a single chain Factor VIII molecule                                                                                         |
| 6          | Amino acid sequence of mature human serum albumin                                                                                                  |
| 7          | Amino acid sequence of D'D3-CTP                                                                                                                    |
|            | aa 1 - 479: VWF D'D3 region (VWF amino acids 764 - 1242)                                                                                           |
|            | aa 480 - 511: glycine/serine linker                                                                                                                |
|            | aa 512 - 576: C-terminal peptide of human chorionic gonadotropin-β subunit                                                                         |
|            | aa 577 - 584: polyhistidine tag                                                                                                                    |

[0141] The following Examples illustrate the invention but should not be construed as limiting the present invention to the specific embodiments described herein below.

EXAMPLES

Example 1: FVIII level analysis in plasma after administration of rD'D3-FP

**[0142]** We aimed at characterizing the impact of rD'D3-FP on endogenous FVIII levels, thereby generally supporting a treatment of mild to moderate or severe Hemophilia A patients or certain types of von-Willebrand disease with low level VWF and functional endogenous FVIII. We investigated this effect in different approaches:

- Intravenous administration to models with normal endogenous FVIII and VWF levels, i.e. rats (example 1.1), rabbits (example 1.2) and monkeys (example 1.3), to investigate a potential further increase of endogenous FVIII after intravenous (i.v.) rD'D3-FP administration in a healthy subject.
- Intravenous administration to models with low FVIII levels due to VWF deficiency, i.e. VWF ko rats (example 1.4) and VWF ko mice (example 1.5), to investigate the size of increase of endogenous FVIII after i.v. rD'D3-FP administration in a diseased subject.
- Intravenous administration to models with low FVIII levels due to VWF deficiency, i.e. VWF ko rats, to investigate the size of increase of endogenous FVIII after i.v. administration of rD'D3-FP variants in a diseased subject (example 1.6).
- Subcutaneous administration to models with normal or low FVIII levels, i.e. pig, FVIII ko and VWF ko mouse and FVIII ko and VWF ko rat and (example 1.7) was investigated to compare i.v. and s.c. bioavailability of rD'D3-FP as well as effects on endogenous FVIII.
- A final experiment investigated the effects of multiple rD'D3-FP doses (i.v.) in VWF ko rats (example 1.8).
- Investigation of rD'D3-FP given subcutaneously and rVIII-SingleChain given intravenously in a hemophilia A model, i.e. in FVIII ko rat (example 1.9).

## **Materials and Methods**

**[0143]** For the Examples, a polypeptide comprising a truncated VWF having an amino acid sequence as defined in SEQ ID NO:2 was used. This particular fusion protein consists of an N-terminal amino acid sequence from 1 - 479 representing the VWF D'D3 region (amino acids 764 - 1242 of human native VWF), followed by a 31 amino acid glycine/serine linker peptide and a C-terminal human albumin amino acid sequence from 511 - 1095. This fusion protein having a sequence as defined in SEQ ID NO:2 is referred to as rD'D3-FP or rD'D3-FP WT in the following.

**[0144]** As an alternative for the albumin as half-life extending polypeptide (HLEP), in some Examples another half-life extended rD'D3 variant is used having instead of albumin a CTP (C-terminal peptide of human chorionic gonadotropin- $\beta$  subunit) fused to D'D3 via a glycine/serine linker which is referred to as rD'D3-CTP hereinafter. The fusion protein rD'D3-CTP has a sequence as defined in SEQ ID NO:7.

**[0145]** In certain Examples high affinity variants of rD'D3-FP were used. One particular variant fusion protein consists of an N-terminal amino acid sequence from 1 - 479 representing the

VWF D'D3 region (amino acids 764 - 1242 of human native VWF), followed by a 31 amino acid glycine/serine linker peptide and a C-terminal human albumin amino acid sequence from 511 - 1095, provided that within the D' domain of said polypeptide two amino acid substitutions are present, i.e. S764E and S766Y. This fusion protein consists of a sequence as defined in SEQ ID NO:2 having said two substitutions, namely S764E and S766Y, within the D'D3 region. The VWF amino acid S764 corresponds to the amino acid number 1 within the sequence of SEQ ID NO:2 (see also Table 1). The rD'D3-FP EY variant has been generated as described in WO 2016/000039 A1. Said variant is referred to as rD'D3-FP EY hereinafter.

**[0146]** In certain Examples another high affinity variant of rD'D3-FP was used. This particular variant fusion protein consists of an N-terminal amino acid sequence from 1 - 479 representing the VWF D'D3 region (amino acids 764 - 1242 of human native VWF), followed by a 31 amino acid glycine/serine linker peptide and a C-terminal human albumin amino acid sequence from 511 - 1095, provided that within the D'D3 domain of said polypeptide three amino acid substitutions are present, i.e. S764E, S766Y and V1083A. This fusion protein consists of a sequence as defined in SEQ ID NO:2 having said three substitutions S764E, S766Y, and V1083A within the D'D3 region. Said variant is referred to as rD'D3-FP EYA hereinafter.

### ***Analytics***

**[0147]** In all examples, rD'D3-FP was applied at dose levels quantified by a human albumin ELISA, i.e. measuring the albumin part of the protein. This rD'D3-FP ELISA was used for formulation and plasma samples (except for the monkey plasma samples, who showed relevant human albumin cross-reactivity).

**[0148]** In examples where rD'D3-CTP was used as a rD'D3 variant, the polypeptide was applied at dose levels quantified by OD<sub>280</sub> measurement, and the protein amount was adjusted to a molarity in the same high range as rD'D3-FP.

**[0149]** The ("standard") rD'D3-FP ELISA used a polyclonal goat anti-human albumin capture antibody from Bethyl Laboratories, Inc. (Montgomery, USA). The detection solution consists of a polyclonal peroxidase labelled anti-human albumin detection antibody preparation (Bethyl Laboratories Inc., Montgomery, USA). A chromogenic readout, i.e. TMB from Siemens Healthcare (Eschborn, Germany) was used for quantification in a microplate reader at 450/650 nm (ELx808, BioTek, USA) directly after stopping. As a standard, the drug formulation containing rD'D3-FP was used. rD'D3-FP amounts are given in mg albumin, i.e. no adjustment was done for the D'D3 part of the molecule.

**[0150]** The rD'D3-FP ELISA for monkey plasma samples was set up as a mixed ELISA, where the D'D3 domain was captured and the albumin domain was detected. The assay used an anti-D'D3 monoclonal capture antibody (CSL Behring, in house research grade preparation). The detection solution consists of a polyclonal peroxidase labelled anti-human albumin detection antibody preparation (Bethyl Laboratories Inc., Montgomery, USA). A chromogenic readout, i.e.

TMB from Siemens Healthcare (Eschborn, Germany) was used for quantification in a microplate reader at 450/650 nm (ELx808, BioTek Instruments Inc., Winooski, USA) directly after stopping. As a standard, the drug formulation containing rD'D3-FP was used. rD'D3-FP amounts are given in mg albumin, i.e. no adjustment was done for the D'D3 part of the molecule.

**[0151]** The plasma samples of the PK containing rD'D3-CTP were measured in an anti-D'D3 ELISA. This anti D'D3 ELISA was conducted with two monoclonal anti-human D'D3 antibodies in a sandwich format. Both antibodies for capture and detection were derived from an in house research preparation. The anti-human D'D3 detection antibody was peroxidase labelled. A chromogenic readout, i.e. TMB from Siemens Healthcare (Eschborn, Germany) was used for quantification in a microplate reader at 450/650 nm (ELx808, BioTek Instruments Inc., Winooski, USA) directly after stopping. As a standard, the drug formulation containing rD'D3-CTP was used. rD'D3-CTP amounts are given as rD'D3-CTP concentrations.

**[0152]** Human FVIII:Ag plasma levels were determined with the FVIII Asserachrom ELISA test kit from Stago, S.A.S., France according to the test instruction manual. The Asserachrom testkit contained all reagents with exception of the stop solution, which was obtained from Siemens Healthcare (Eschborn, Germany). As a standard, the drug formulation containing rVIII-SingleChain was used.

**[0153]** FVIII chromogenic activity plasma levels were detected by the COAMATIC® FVIII assay (FVIII:C chromogenic assay, Chromogenix, Instrumentation Laboratory SpA, Milan, Italy) according to the test instruction manual of the manufacturer on a BCS XP analyzer from Siemens Healthcare Diagnostics (Marburg, Germany). For calibration, standard human plasma was used. Alternatively, they were detected by the same COAMATIC® FVIII assay with predilution of the samples in human FVIII deficient plasma from Siemens Healthcare Diagnostics (Marburg, Germany), followed by a readout on the Infinite M200 ELISA-Reader from Tecan (Tecan Trading AG, Switzerland). For calibration, a human plasma-derived von Willebrand factor and FVIII containing product, Haemate® P from CSL Behring, was used. The samples were measured at 405 nm without reference. In general, FVIII chromogenic activity is abbreviated as FVIII:C.

**[0154]** FVIII clotting activity plasma levels were detected using a one-stage clotting assay, which includes Pathromtin® SL, human FVIII deficient plasma and calcium chloride solution, which are all commercially available from Siemens Healthcare Diagnostics (Marburg, Germany). Measures were performed on a BCS XP analyzer from Siemens Healthcare Diagnostics (Marburg, Germany). For calibration, standard human plasma was used. Activated partial thromboplastin time (aPTT) was analysed in plasma samples using Pathromtin® SL reagent (mouse and rat) or Actin® FSL (rabbit) and calcium chloride solution on a BCS XP analyzer from Siemens Healthcare Diagnostics (Marburg, Germany). All reagents are commercially available from Siemens Healthcare Diagnostics (Marburg, Germany).

### ***Experimental animals***

**FVIII ko mouse**

**[0155]** FVIII ko mice (representing a hemophilia A phenotype) were chosen, since they lack exons 16 and 17 of the FVIII gene, and thus have no plasma factor VIII activity (Bi L. et al, Nature genetics, 1995, Vol 10(1), 119-121; Bi L. et al, Blood, 1996, Vol 88(9), 3446-3450). This allows the analysis of FVIII activity levels following treatment with FVIII by quantification of FVIII activity in the plasma of these mice.

**FVIII ko rat**

**[0156]** FVIII ko rats, representing a hemophilia A phenotype, were generated at SAGE Labs (A Horizon Discovery Group Company, Saint Louis, MO 63146, USA) using the CRISPR/Cas9 technology. A 2bp (basepair) deletion and a 1bp insertion at position 23471-23472 within exon 18 was induced, leading to an early stop codon. The generated FVIII ko mutation resulted in a FVIII ko rat with disrupted FVIII function. This allows the analysis of FVIII activity levels following treatment with FVIII by quantification of FVIII activity in the plasma of these rats.

**VWF ko mouse**

**[0157]** VWF knock-out (ko) mice (representing a VWD phenotype) were chosen, since they lack exons 4 and 5 of the VWF gene, and thus have no plasma VWF activity (Denis C. et al, Proc. Natl. Acad. Sci. USA, 1998, Vol 95, 9524-9529). This allows the analysis of D'D3 polypeptides on endogenous FVIII activity levels in the plasma of these mice.

**VWF ko rat**

**[0158]** VWF ko rats, representing a VWD phenotype, were generated at Sigma Advanced Genetic Engineering (SAGE) Labs (Saint Louis, MO 63146, USA) using the CompoZr™ Zinc Finger Nuclease (ZFN) technology and SAGEspeed™ animal Knockout production processes. A 16bp (basepair) deletion at position 33926bp-33941bp in the genomic sequence was induced within exon 7 leading to an early stop codon. The generation of knock-out rats in general using the ZFN technology by Sigma Advanced Genetic Engineering (SAGE) Labs (Sigma-Aldrich Biotechnology) is described in X. Cui et al. (Nature Biotechnology, 2010), in MH. Porteus & D. Carroll (Nature Biotechnology, 2005). The generated VWF ko mutation resulted in a VWF ko rat with disrupted VWF function. This allows the analysis of D'D3 polypeptides on endogenous FVIII activity levels in the plasma of these rats.

**[0159]** Generation of D'D3 albumin fusion protein (D'D3-FP):

**[0160]** The expression cassette for D'D3-FP consisting of cDNA encoding VWF amino acids 1 to 1242, a glycine/serine linker and the cDNA of human albumin was prepared by custom gene synthesis (Eurofins Genomics, Ebersberg, Germany). Through flanking restriction sites (EcoRI, NotI) the expression cassette was excised from the cloning vector supplied and inserted into a pIRESneo3 vector (BD Biosciences, Franklin Lakes, NJ, USA) linearized with EcoRI and NotI. The resulting expression plasmid contained nucleotide sequences encoding the VWF propeptide, D' and D3 (VWF amino acids 1 to 1242 of SEQ ID NO: 4) fused to the albumin coding sequence through a short linker coding sequence under CMV promoter control. The nucleotide sequence of the coding sequence is displayed as SEQ ID NO: 1, the amino acid sequence of the mature D'D3-FP is shown as SEQ ID NO: 2. The presence of the D1D2 VWF propeptide (741 amino acids) during expression is crucial for dimerization of the synthesized polypeptide.

**[0161]** A similar approach was used to generate an expression plasmid for a D'D3 fusion protein to the C-terminal peptide of human chorionic gonadotropin- $\beta$  subunit, linked via a glycine/serine linker and tagged by 8 histidines at the C-terminus of the fusion protein. The amino acid sequence of the mature rD'D3-CTP is shown as SEQ ID NO: 7.

**[0162]** The expression plasmid as described above was grown up in XL10 Gold (Agilent Technologies) and purified using standard protocols (Qiagen, Hilden, Germany).

**[0163]** CHO K1 cells were transfected using the Lipofectamine 2000 reagent (Invitrogen) and grown up in serum-free medium (CD-CHO, Invitrogen) in the presence of 500-1000  $\mu$ g/ml Geneticin. An expression plasmid encoding PACE/furin (pFu-797) as described in WO2007/144173 was cotransfected to maximize propeptide cleavage efficacy. Single cell derived clones were grown up and selected according to their D'D3-FP expression yield as quantified by an albumin specific enzyme immunoassay (see below). The cell line finally selected for D'D3-FP fermentation was called T2050-CL3.

**[0164]** Production of rD'D3-FP was carried out in bioreactors applying a fermentation process in perfusion mode. The fermentation process for the production of rD'D3-FP started with the thaw of cell line T2050-CL3 followed by cell expansion in shake flasks and finally a fermentation process in perfusion mode using the Sartorius BioStat B-DCU 5 L bioreactor and the BioStat STR 50L single-use bioreactors. The BioSeps 10L or 200L (Applikon), respectively, were used as cell retention devices. Cell culture media were either PowerCHO3 (Lonza BESP1204) with 8 mM L-glutamine and 1  $\mu$ M CuSO<sub>4</sub> or ProCHO5 (Lonza BESP1072) with 10 mM L-glutamine and 1  $\mu$ M CuSO<sub>4</sub>.

**[0165]** The seed trains in shake flasks were performed at 37°C, 7.5% CO<sub>2</sub> at a shaker speed of 160 rpm.

**[0166]** The 5L bioreactor was inoculated with a target VCD of  $2.5 \times 10^5$  cells/mL. The cells were cultivated in PowerCHO3 with 8 mM L-glutamine and 1  $\mu$ M CuSO<sub>4</sub> at a temperature of +37.0°C, a pH of 7.00, and at 30 % oxygen saturation. A temperature shift to +34.0 °C (evaluated range +31°C to +35°C) was performed after initial harvests from the bioreactor run at +37°C had been taken. The pH was controlled using CO<sub>2</sub> sparged as acid and NaHCO<sub>3</sub> as base. The overlay air flow rate was set to 0.5 L/min. A ring sparger was used as a sparging unit. The agitation rate was 150 rpm with a 2fold pitch blade impeller in down pull mode.

**[0167]** The 50L bioreactor was inoculated with a target VCD of  $3.0 \times 10^5$  cells/mL. The cells were cultivated in ProCHO5 medium with 10 mM L-glutamine and 1  $\mu$ M CuSO<sub>4</sub> at a temperature of +37.0°C, a pH of 6.90, and at 30 % oxygen saturation. A temperature shift to +34.0 °C was performed after the initial one or two harvests. PH control as above, the overlay air flow rate was set to 2 L/min. A micro sparger was used as a sparging unit. The agitation rate was 90 rpm with a 2fold pitch blade impeller in down pull mode.

**[0168]** The perfusion was initiated when the VCD in the bioreactor was  $\geq 1.0 \times 10^6$  cells/mL. The perfusion rate was set to 1.0 volume/volume/day. The BioSep was operated in back flush mode with 5 (10) minutes runtime and 10 seconds back flush at a power input of 7 (30) W (numbers in brackets refer to the 50L bioreactor). The perfusate and the bleed were filtered inline and collected in bags over 48 hours at +2 to +8°C. The VCD was controlled by active bleeding using a turbidity probe using glucose consumption as parameter with a target of 2 g/L glucose. Harvest and bleed were filtered inline, the harvest system consisting of a disposable filter and disposable bag was changed every second day.

**[0169]** To prepare material for the PK analyses described below harvests were purified by affinity and size exclusion chromatography. Briefly, the cell-free harvest from the bioreactor was concentrated 30-fold using a TFF system (e.g. Pall Centramate 500 S) with a 30 kD membrane (e.g. Pall Centramate OS030T12). That concentrate was spiked with NaCl and EDTA to a final concentration of 0.75 M NaCl and 5 mM EDTA and loaded overnight on a CaptureSelect Human Albumin column (Life Technologies) which was pre-equilibrated with 20 mM Tris buffer pH 7.4. After washing the column with equilibration buffer rD'D3-FP was eluted with elution buffer (20 mM Tris, 2 M MgCl<sub>2</sub>, pH 7.4). The eluate was then 10-fold concentrated and dialyzed against 50 mM Tris, 150 mM NaCl, pH 7.4 using Ultra Centrifugal Filters with a 30 kD cut-off (e.g. Amicon. UFC903024). To separate the rD'D3-FP dimer from the monomer portion that material was loaded on a Superdex 200 pg column (GE Healthcare Code: 17-1069-01) pre-equilibrated with 50 mM Tris, 150 mM NaCl, pH 7.4 and the peak fractions containing the D'D3-FP dimer were pooled. The area under the curve for the dimer and monomer peak fractions were used to calculate dimer to monomer ratio. Dimer preparations of said D'D3 albumin fusion protein were used for the pharmacokinetic experiments in Examples 1.1-1.6. Such dimer preparations are referred to as D'D3-FP or rD'D3-FP in the following, if not indicated otherwise.

**[0170]** The rD'D3-FP EY and EYA variants have been generated by equivalent method steps.

**Example 1.1: Impact of intravenous treatment with rD'D3-FP on physiological endogenous FVIII levels in rats****Animals**

**[0171]** Female Crl:CD (Sprague Dawley) rats in a weight range of 200-294 g were bred at Charles River Laboratories (Sulzfeld, Germany). In house, the animals were kept at standard housing conditions, i.e. at 20-24°C under a 12 h/12 h light-darkness cycle. Animals were fed ad libitum with standard mouse and rat diet (Ssniff-Versuchsdäten, Soest, Germany). Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

**[0172]** For the 1 mg/kg group the group size was n=9, divided in 3 cohorts, except for the control (n=3 animals only). The group size of the 1 mg/kg group was n=6, divided in 2 cohorts. Thus, n=3 animals per time-point were used always.

**Experimental details**

**[0173]** The test articles were administered i.v. by a single injection into the lateral tail vein of the rats (n=3 per group). rD'D3-FP was applied at a dose level of 1 mg/kg or 3 mg/kg based on human albumin values. Blood samples of the 1 mg/kg group were taken retroorbitally (the terminal sampling at day 10 and 14 by Vena cava puncturing) at pre-dose, 6 h and 1, 2, 3, 4, 5, 7, 10 and 14 days post administration (p.a.) after intravenous bolus injection. Blood samples of the 3 mg/kg group were drawn at 0.083, 3, 8h and 1, 2, 3 and 4 days post administration. They were anticoagulated using sodium citrate (1 part sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at approx. -70°C for the determination of rD'D3-FP and/or FVIII activity.

**[0174]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using a human albumin ELISA. FVIII activity plasma levels were detected using a chromogenic assay as well as a one-stage clotting assay.

**[0175]** Calculation of the total peak area under the curve (AUC) was done with GraphPad Prism (GraphPad Software, La Jolla, California, USA) over the period of 14 days using the pretreatment values as baseline and identifying peaks with a  $\leq 30\%$  distance from minimum to maximum values.

**Results**

**[0176]** rD'D3-FP was quantified up to 4 d (3 mg/kg) or 14 d (1 mg/kg) p.a., and measured data were well above the detection limit over the whole observation period (Fig. 1A). A linear dose-dependency was observed for the 2 tested doses.

**[0177]** FVIII activity was measured as chromogenic and one-stage clotting activity for the group treated with 1 mg/kg rD'D3-FP, and both FVIII activity tests showed transient increases in endogenous FVIII levels as compared to the saline control after administration of rD'D3-FP (Fig. 1B and 1C), with a peak between days 2 (chromogenic activity) or 1 (clotting activity) to day 5 p.a. Again for both assays, mean FVIII concentrations hardly exceeded the maximum predose values, i.e. 3.1 IU/mL for chromogenic FVIII activity and 454 % (4.5 IU/mL) for clotting FVIII activity. It shall be mentioned, that the upper limit of normal (ULN) with the dilution of 1:4 used for quantification of the samples was not reached for any of the animals. In line with this, AUC (evaluated over days 0 to 14 p.a.) as shown in Fig. 1D increased with the chromogenic as well as with the clotting assay at a dose of 1 mg/kg rD'D3-FP.

**[0178]** In line with the increase in FVIII activity, the physiological activated partial thrombin time (aPTT) decreased (Fig. 1E), starting immediately after administration with a peak until about day 7 p.a. as compared to vehicle-treated animals. Again, mean values ranged within the predose range, and only on day 1 p.a. mean values were below the lowest predose value of 16.5 s.

**[0179]** With the given exposure to rD'D3-FP for at least 14 days, a slight increase in FVIII activity was observed. A peak value was given between days 1-5 p.a., which was in any case <2-fold above saline-treated animals (FVIII chromogenic activity: maximal increase by ~1 IU/mL or 100% to -3.5 IU/mL = 350%; FVIII clotting activity: maximal increase by -300% to -700%, mostly by -100% to -500% - in line with higher baseline values in these animals as compared to men) and mostly within the physiological variation. This slight increase of FVIII activity led to a shortening of aPTT, with mean values only slightly and shortly below the minimum predose value. Thus, in healthy rats, slight increases in FVIII levels were observed at a dose of 1 mg/kg rD'D3-FP, but barely changing these values out of the physiological range.

### **Example 1.2: Impact of intravenous treatment with rD'D3-FP on physiological endogenous FVIII levels in rabbits**

#### **Animals**

**[0180]** Female CHB rabbits in a weight range of 2.0-3.2 kg (Bauer, Neuental, Germany) were housed one per cage in wire-steel cages at standard housing conditions, i.e. at 20-23°C and 50% relative humidity under a 12 h/12 h light-darkness cycle. The animals were provided tap water ad libitum and fed rabbit pellets (Deukanin®, Deutsche Tiernahrung Cremer GmbH & Co. KG, Düsseldorf, Germany). Animal husbandry and study procedures complied with the

German Animal Welfare law and European Union regulations.

### **Experimental details**

**[0181]** The test articles were administered i.v. by a single injection into the lateral ear vein of the rabbits (n=3 per group). rD'D3-FP was applied at a dose level of 1, 3 or 10 mg/kg based on human albumin values. Blood samples were taken from the ear artery at pre-dose, 1, 3 and 6 h, followed by daily sampling up to 10 days post administration (p.a.) after intravenous bolus injection. They were anticoagulated using sodium citrate (1 part sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at approx. -70°C for the determination of rD'D3-FP and/or FVIII activity.

**[0182]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using a human albumin ELISA. FVIII activity plasma levels were detected using a chromogenic assay as well as a one-stage clotting assay. Further, aPTT was quantified using the Actin® FSL test. Any clotted samples were excluded from evaluation.

**[0183]** Calculation of the total peak area under the curve (AUC) was done with GraphPad Prism (GraphPad Software, La Jolla, California, USA) over the period of 10 days using the pretreatment values as baseline and identifying peaks with a  $\leq 30\%$  distance from minimum to maximum values.

### **Results**

**[0184]** rD'D3-FP was quantified up to 10 d p.a., and measured data were well above the detection limit of 16 or 31 ng/mL over the whole observation period, except for a drop observed in some animals from day 7 on or later (Fig. 2A). This sudden drop in exposure was observed in single animals, beginning on days 7-10, and especially for the higher doses. One potential cause could be the formation of anti-drug-antibodies - in line with the lower homology of the D'D3 region in rabbits and men as compared to the other species tested.

**[0185]** FVIII activity was measured as chromogenic and one-stage clotting activity, both showing increases in endogenous levels as compared to the saline control (Fig. 2B and 2C). The data suggest a slight increase of endogenous FVIII levels after administration of rD'D3-FP, with a peak between days 3-7 p.a. Nevertheless, the mean values only transiently increased the range of the predose values of 2.8 IU/mL in the chromogenic and 611 % of the norm (6.1 IU/mL) in the clotting FVIII activity test. In line with this, AUC (evaluated over days 0 to 10 p.a.) as shown in Fig. 2D increased: with the chromogenic assay, starting at the dose of 3 mg/kg, while the effect with the clotting assay was visible only with 10 mg/kg. This is potentially related to the higher variation of baseline values in the clotting assay, which were already slightly higher in the treated groups.

**[0186]** Even though FVIII activity showed slight increases, no decrease was observed in the aPTT (Fig. 2E).

**[0187]** With the given exposure to rD'D3-FP for at least 6 days, a slight increase in FVIII activity was observed, which slightly exceeded levels observed in saline treated animals. A peak value was given between days 3-7 p.a., which was <2-fold above saline treated animals (FVIII chromogenic activity: maximal increase by ~1 IU/mL or 100% to ~3.5 IU/mL = 350%; FVIII clotting activity: maximal increase by ~400% to ~900% - in line with higher baseline values in these animals as compared to men). These increased FVIII activity levels only slightly exceeded the physiological levels in rabbits on days 3 to 6 at a dose of 10 mg/kg, and did not reduce aPTT. Thus in healthy rabbits, only slight changes in FVIII levels were observed.

### **Example 1.3: Impact of intravenous treatment with rD'D3-FP on physiological endogenous FVIII levels in monkeys**

#### **Animals**

**[0188]** Male Cynomolgus monkeys in a weight range of approximately 4-7 kg, age approximately 5-6 years (obtained from Vietnam - documentation includes health screening and any treatment administered prior to arrival - to Huntingdon Life Sciences, Cambridgeshire, UK) were housed in pairs in cages specifically designed to house non-human primates at standard housing conditions, i.e. at 15-24°C and 40-70% relative humidity under a 12 h/12 h light-darkness cycle. The animals were provided tap water ad libitum and fed Old World Monkey Diet (200 g daily per animal) plus supplemental diet (two biscuit supplements, approximately 25 g each, and fresh fruit produce).

#### **Experimental details**

**[0189]** The test articles were administered i.v. by a single injection into the saphenous vein of the monkeys (n=3 per group). rD'D3-FP was applied at a dose level of 2.5 mg/kg based on human albumin values. Blood samples were taken from the femoral vein at pre-dose, and 5, 30 min, 2, 6, 16, 30, 48, 72, 96, 120, 144, 168 hours p.a. after intravenous bolus injection. They were anticoagulated using sodium citrate (1 part sodium citrate 3.2% + 9 parts blood), processed to plasma and stored at about -70°C for the determination of FVIII activity and/or rD'D3-FP.

**[0190]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using an antibody capturing the D'D3 part of the molecule and using a detection antibody for albumin. FVIII chromogenic activity plasma levels were detected by the

Chromogenix assay.

## **Results**

**[0191]** rD'D3-FP was quantified using an ELISA binding to both of its components, albumin and D'D3, and measurements were performed up to 168 h (7 d) p.a. Measured data were well above the detection limit over the whole observation period (Fig. 3A).

**[0192]** FVIII activity was measured as chromogenic activity as shown in Fig. 3B. Basal endogenous levels varied relevantly, with a range of 83.6-314.8 % of the norm (0.8-3.1 IU/mL = dotted lines in Fig. 3B) and a mean 176.4 % of the norm (1.8 IU/mL, dashed line in Fig. 3B). As in rats and rabbits, only a small increase of endogenous FVIII levels up to a mean maximum of 258.9 % of the norm was observed, and variability was quite high. Nevertheless, an increase in endogenous FVIII levels by up to ~1 IU/mL or -100 % of the norm (to -2.5 IU/mL or -2.5 IU/mL - in line with higher baseline values in these animals as compared to men) as compared to the individual pre-dose values was demonstrated, which (as mean) nevertheless did not exceed the physiological FVIII levels of monkeys.

**Example 1.4: Impact of intravenous treatment with rD'D3-FP on endogenous FVIII levels in VWF ko rats**

## **Animals**

**[0193]** Male and female VWF ko rats in a weight range of 261-598 g were bred at Charles River Laboratories (Sulzfeld, Germany). In house, the animals were kept at standard housing conditions, i.e. at 20-24°C under a 12 h/12 h light-darkness cycle. Animals were fed ad libitum with standard mouse and rat diet (Ssniff-Versuchsdäten, Soest, Germany). Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

**[0194]** The group size of the 1 and 10 mg/kg groups was n=9, divided in 3 cohorts, except for the control (n=3 animals only). Thus, n=3 animals per time-point were used for all vehicle, 1 and 10 mg/kg rD'D3-FP timepoints. For the 3 mg/kg group, the group size was n=4 per timepoint.

## **Experimental details**

**[0195]** The test articles were administered i.v. by a single injection into the lateral tail vein at a total volume of 2 or 3 mL/kg. rD'D3-FP was applied at dose levels at 1, 3 or 10 mg/kg based on

human albumin values. Blood samples were taken retroorbitally under short term anaesthesia at predose, 6, 24, 48, 72, 96, 120, 168, 240, 336 h (1 and 10 mg/kg) p.a. using an alternating sampling scheme, or from each individual animal from the saphenous vein at predose, 1, 24, 48, 72, 120, 192, 240 and 336 h p.a. (3 mg/kg). The PK profile was taken from three cohorts of rats per group (1 and 10 mg/kg) or from individual animals (3 mg/kg). Blood samples were anticoagulated using sodium citrate (1 parts sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at -70°C for the determination of FVIII activity and/or albumin.

**[0196]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using a human albumin ELISA. Further, FVIII chromogenic and clotting activity as well as aPTT was measured (the latter only in groups treated with 1 and 10 mg/kg rD'D3-FP). Calculation of the area under the curve (AUC) was done with MATLAB R2017a (Natick, Massachusetts, USA) using trapezoidal method from zero to day 10.

## **Results**

**[0197]** rD'D3-FP was quantified using an ELISA against human albumin, and measurements were performed up to 14 d p.a. All measured data were well above the detection limit over the whole observation period up to day 14 (Fig. 4A). A linear dose-dependency was shown from 1-10 mg/kg rD'D3-FP.

**[0198]** FVIII activity measured as chromogenic activity as shown in Fig. 4B did not give relevant baseline FVIII levels: 22 of 23 samples were below the limit of detection (0.005 IU/mL or 0.5%) and one animal had a value of 0.007 IU/mL. In the VWF ko rats (in contrast to the CD rats), the upper limit of normal (ULN, 4 IU/mL) was reached with the dilution of 1:4 used for quantification of the samples of both rat strains: After administration of rD'D3-FP, levels increased within 2 days, with the 10 mg/kg dose even exceeding the upper limit of quantification of the assay of 4 IU/mL (or 400%). In VWF ko rats, the FVIII activity levels after dosing of 10 mg/kg - but not 1 or 3 mg/kg - rD'D3-FP transiently exceeded the values measured in healthy CD rats. FVIII chromogenic activity values were after administration of 10 mg/kg rD'D3-FP also higher than in the other healthy species, i.e. rabbits (dosed up to 10 mg/kg rD'D3-FP) or monkeys (2.5 mg/kg rD'D3-FP). The effect in rats after administration of 10 mg/kg rD'D3-FP lasted up to 10 days. Even at a dose of 1 mg/kg rD'D3-FP, a highest plasma concentration of 2.36 IU/mL (or 236%) was reached on day 2, and the effect was still weakly seen on day 7. Similarly with 3 mg/kg rD'D3-FP, a highest FVIII plasma concentration was reached on day 1 (2.09 IU/mL or 209%). With these lower 2 doses, the mean FVIII concentrations were just within the range of normal levels from CD rats.

**[0199]** When FVIII activity was measured with the clotting assay as depicted in Fig. 4C, baseline FVIII levels were measurable (undiluted samples: mean 22.3%, minimum 11.7% and maximum 40.9% of the norm, n=17; and with a detection limit of 40%, 20 samples remained <40%). Thus, as in CD rats and rabbits, baseline values were highly variable. It needs to be mentioned that all samples after administration of rD'D3-FP needed a dilution step of 1:8,

thereby generating an ULN of 1186.4 % of the norm. Again, after administration of rD'D3-FP at a dose of 10 mg/kg, endogenous FVIII levels increased within 2 days even over the ULN of the assay, i.e. higher than in rabbits. This effect lasted up to 14 days at the high dose of 10 mg/kg rD'D3-FP. Even at a dose of 1 mg/kg, a highest plasma concentration of 388.8% of the norm was reached on day 2, and the effect was still seen on day 7. Similarly at a dose of 3 mg/kg, the highest plasma concentration measured on day 2 was 435.2% of the norm. Similar to the chromogenic FVIII activity, the physiological range of FVIII activity was exceeded after administration of 10 mg/kg and just reached after administration of 1 or 3 mg/kg rD'D3-FP.

**[0200]** Thus, chromogenic and clotting activity data are in line, with clotting activity data showing slightly stronger responses, assumingly related to the measurement of baseline rat FVIII against a human FVIII standard. An increase in FVIII activity was observed, which reached levels observed in healthy CD rats (1 and 3 mg/kg) or exceeded them (10 mg/kg). For the 10 mg/kg dose, peak values were above the upper detection limit, thus an x-fold increase above normal ranges could not be determined.

**[0201]** This is in line with the calculated increases in AUC (day 0-10), showing increases after a dose of 1, 3 and 10 mg/kg rD'D3-FP as depicted in Fig. 4D. Increases after doses with 1 and 3 mg/kg rD'D3-FP were -10-fold for chromogenic and -80-fold for clotting FVIII activity as compared to vehicle treatment, while those with 10 mg/kg rD'D3-FP reached -50-fold for chromogenic and 430-fold effects on AUC<sub>0-10d</sub>.

**[0202]** In line with these increases in FVIII levels, activated partial thrombin time (aPTT) decreased relevantly from pathological predose or vehicle data to a lowest mean value of 12.5 s in the 10 mg/kg rD'D3-FP dose group (Fig. 4E). This decrease lasted in both measured dose groups (1 and 10 mg/kg) up to 14 days, and brought the aPTT values in (1 mg/kg rD'D3-FP) or slightly below (10 mg/kg rD'D3-FP) a range observed in healthy CD rats (range 16.5-36.6 s).

**Example 1.5: Impact of intravenous treatment with rD'D3-FP on endogenous FVIII levels in VWF ko mice**

### Animals

**[0203]** Male and female VWF ko mice in a weight range of 30-45 g were breed at Charles River Laboratories (Sulzfeld, Germany). In house, the animals were kept at standard housing conditions, i.e. at 20-24°C under a 12 h/12 h light-darkness cycle. Animals were fed ad libitum with standard mouse and rat diet (Ssniff-Versuchsdäten, Soest, Germany). Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

**[0204]** The group size was n=12, divided in 4 cohorts, except for the control (n=4 animals).

Thus, n=3-4 animals per time-point were used.

### **Experimental details**

**[0205]** The test articles (rD'D3-FP or vehicle (isotonic saline)) were administered i.v. by a single injection into the lateral tail vein at a total volume of 5 mL/kg. rD'D3-FP was applied at a dose level of 10 mg/kg based on human albumin values. Blood samples were taken retroorbitally under short term anaesthesia at 4, 7, 16, 24, 48, 72, 96 and 168 h p.a. using an alternating sampling scheme from the rD'D3-FP-dosed animals, and at 4 and 168 h p.a. from the vehicle-treated animals. The PK profile was taken from four cohorts of mice per group. Blood samples were anticoagulated using sodium citrate (1 parts sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at -70°C for the determination of FVIII activity and/or albumin.

**[0206]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using a human albumin ELISA. Further, FVIII chromogenic and clotting activities were measured.

**[0207]** Calculation of the total peak area under the curve (AUC) was done with GraphPad Prism (GraphPad Software, La Jolla, California, USA) over the period of 7 days identifying peaks with a  $\leq 10\%$  distance from minimum to maximum values.

### **Results**

**[0208]** rD'D3-FP was quantified using an ELISA against human albumin, and measurements were performed up to 7 d p.a. All measured data were well above the detection limit over the whole observation period (Fig. 5A).

**[0209]** FVIII activity measured as chromogenic activity did not give relevant baseline FVIII levels: all 6 measured samples from vehicle-treated animals were below the limit of detection (10 mIU/mL or 1%). For comparison, chromogenic activity of healthy NMRI mice ranged at about 96-300 mIU/mL, median 230 mIU/mL, mean 206 mIU/mL (range 10-30% of the norm, unpublished data), i.e. lower than observed in other animal species or men. According to Fig. 5B, after administration of rD'D3-FP, levels increased quickly within 4 hours to a mean value of 138 mU/mL (14%), and increased further, reaching a maximum at 48 h p.a., with a mean of 421 mU/mL (42%) to 72 h p.a. (429 IU/mL or 43%). At the last time-point at 168 h (7 d) p.a., still 194 mU/mL (20%) were measured. With this, FVIII chromogenic activity after treatment with 10 mg/kg rD'D3-FP slightly exceeded physiological FVIII plasma levels as measured in NMRI mice, and effects were comparable to VWF ko rats.

**[0210]** When FVIII activity was measured with the clotting assay, baseline FVIII levels were

measurable (vehicle-treated animals: mean 31.1, minimum 25.8 and maximum 41.8 % of the norm, n=8). Thus, as in the other species, baseline values were highly variable. Again, after administration of rD'D3-FP, levels increased quickly as depicted in Fig. 5C, and could only be measured in a dilution of 1:60 (while vehicle-treated animals, used for the quantification of baseline values, were measured with a dilution of 1:10). At the first sampling point of 4 h p.a., mean values were already at 137 % of the norm, and increased further up to 16 h p.a., with a mean of 218 % of the norm. The maximum exposure was reached at 72 h p.a. with a mean of 304 % of the norm. At the last time-point at 168 h (7 d) p.a., still 254 % of the norm were measured.

**[0211]** Thus, chromogenic and clotting activity data were generally in line, with clotting activity data showing stronger responses, assumingly related to the measurement of mouse FVIII (in VWF ko animals) against a human FVIII standard. An increase in FVIII activity was observed, which exceeded levels observed in healthy NMRI mice <2-fold (FVIII chromogenic activity: maximal increase by 0.1 IU/mL = -10% to -0.4 IU/mL = 40% - in line with lower baseline values in these animals as compared to men; FVIII clotting activity: maximal increase to -300% (no range from NMRI mice determined)). This is in line with the calculated increases in AUC (day 0-7), showing large increases after a dose of 10 mg/kg rD'D3-FP as compared to vehicle treated animals as depicted in Fig. 5D.

#### **Example 1.6: Impact of intravenous treatment with different rD'D3 polypeptides on endogenous FVIII levels in VWF ko rats**

##### **Animals**

**[0212]** Male and female VWF ko rats in a weight range of 261-559g were breed at Charles River Laboratories (Sulzfeld, Germany). In house, the animals were kept at standard housing conditions, i.e. at 20-24°C under a 12 h/12 h light-darkness cycle. Animals were fed ad libitum with standard mouse and rat diet (Ssniff-Versuchsdäten, Soest, Germany). Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

**[0213]** The group size was n=4 for each group.

##### **Experimental details**

**[0214]** The test articles were administered i.v. by a single injection into the lateral tail vein at a total volume of 3 mL/kg. rD'D3-FP was applied at a dose level of 3 mg/kg based on human albumin values. Blood samples were taken from the saphenous vein at pre-dose, 1, 24, 48, 72, 120, 192, 240 and 336 h p.a. from the rD'D3-FP-dosed animals, and at pre-dose, 1, 24, 48,

72, 96, 168, 240 and 336h from the rD'D3-CTP treated animals. Blood samples were anticoagulated using sodium citrate (1 parts sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at -70°C for the determination of FVIII activity and/or albumin.

**[0215]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using a human albumin ELISA. rD'D3-CTP was measured by an ELISA technique using antibodies against anti-human D'D3. Further, FVIII chromogenic and clotting activities were measured.

**[0216]** Calculation of the area under the curve (AUC) from 0 to day 10 was done with MATLAB R2017a (Mathworks, Natick, Massachusetts, USA) using the trapezoidal method.

## **Results**

**[0217]** rD'D3-FP was quantified using an ELISA against human albumin, and measurements were performed up to 14 d p.a. All measured data of the rD'D3-FP variants were well above the detection limit over the whole observation period, while rD'D3-CTP reached baseline values on day 14 (Fig. 6A). A slight advantage of rD'D3-FP WT was observed in recovery as compared to the variants EY and EYA, but no relevant difference was seen with regard to clearance. rD'D3-CTP was quantified using an ELISA against human rD'D3 and measurements were well above the detection limit until and including d 10 p.a. (Fig. 6A).

**[0218]** FVIII activity measured as chromogenic activity did not give relevant baseline FVIII levels: all 18 measured predose samples were below the limit of detection (20 mIU/mL or 2%). According to Fig. 6B, after administration of rD'D3-FP variants, levels increased already at 1h p.a. (WT  $197 \pm 20$  mIU/mL or 5%, EY  $171 \pm 84$  mIU/mL or 17%, EYA  $203 \pm 117$  mIU/mL or 20% and CTP  $110 \pm 102$  mIU/mL or 11%) and even further at the next measured timepoints. A maximum was reached between day 1 to day 8 with means of 2088 mU/mL (209%) for WT, of 2889 mU/mL (289%) for EY, of 1044 mU/mL (104%) for EYA, and of 2214 mU/mL (221%) for CTP. The last measurable FVIII activity was observed for WT at day 10 ( $237 \pm 225$  mU/mL (24%)), for EY at day 10 ( $512 \pm 603$  mU/mL (51%)), for EYA at day 14 (last measured timepoint,  $179 \pm 107$  mU/mL (18%)), and for CTP at day 14 ( $22 \pm 4$  mU/mL (2%)). This led to highest observed  $AUC_{0-10d}$  after i.v. administration for the rD'D3-CTP variant (Tab. 2, Fig. 6D).

**[0219]** When FVIII activity was measured with the clotting assay, baseline FVIII levels were also not measurable (n=20 predose treated animals, detection limit 40%). As depicted in Fig. 6C, again, after administration of rD'D3-HLP, levels increased already at 1h p.a. (WT  $47 \pm 4$  %, EY  $41 \pm 1$  %, EYA  $45 \pm 5$  % and CTP  $40 \pm 1$  %) and even further at the next measured timepoints. A maximum was reached between day 1 to day 8 with means of 435 % for WT, of 453% for EY, of 779 % for EYA, and of 358% for CTP. The last measurable FVIII activity was observed for WT at day 10 ( $85 \pm 39$  %), for EY at day 10 ( $130 \pm 84$  %), for EYA at day 14 (last measured timepoint,  $46 \pm 7$  %), and for CTP at day 7 ( $57 \pm 29$  %). The highest observed

AUC<sub>0-10d</sub> was achieved after i.v. administration with rD'D3-FP EYA (Tab. 2, Fig. 6D).

[0220] Thus, chromogenic and clotting activity data were generally in line, again with clotting activity data showing stronger responses. An increase in FVIII activity was observed to about 1.5-2 IU/mL (150-200%) for chromogenic FVIII activity and to -300-700 % for clotting activity, and thus slightly below values in CD rats for chromogenic FVIII activity and slightly above values in CD rats for clotting FVIII activity (see Fig. 4B and Fig. 4C in example 1.4) - in line with baseline values in these animals differing from those of men.

Table 2: AUC<sub>0-10d</sub> of rD'D3 and FVIII activity after i.v. administration of rD'D3 variants in VWF ko rat

|                              | FVIII chromogenic activity<br>AUC <sub>0-10d</sub> [h*IU/mL] | FVIII clotting activity AUC <sub>0-10d</sub> [h*%/mL] |
|------------------------------|--------------------------------------------------------------|-------------------------------------------------------|
| rD'D3-FP WT 3<br>mg/kg i.v.  | 156                                                          | 46789                                                 |
| rD'D3-FP EY 3<br>mg/kg i.v.  | 106                                                          | 50101                                                 |
| rD'D3-FP EYA 3<br>mg/kg i.v. | 191                                                          | 90633                                                 |
| rD'D3-CTP 3<br>mg/kg i.v.    | 271                                                          | 33542                                                 |

[0221] Thus, chromogenic and clotting activity data were generally in line, with clotting activity data showing higher absolute FVIII concentrations related to higher absolute AUC<sub>0-10d</sub> values. It shall be mentioned that effects on FVIII activity were stronger after rD'D3-CTP administration in the chromogenic as compared to the clotting assay. While all 4 rD'D3-FP variants showed about comparable exposure, the rD'D3-FP EYA variant with the highest binding affinity to FVIII showed longest and highest effects on endogenous FVIII.

**Example 1.7: Subcutaneous availability of rD'D3-FP in FVIII ko, VWF ko and NMRI mice, VWF ko and CD rats and pigs, and its impact on endogenous FVIII levels in VWF ko rats and pigs**

Animals

*FVIII ko mice*

[0222] Male and female FVIII ko mice in a weight range of 20-30 g were breed at Charles River Laboratories (Sulzfeld, Germany). The group size was n=12, divided in 4 cohorts. Thus,

n=3 animals per time-point were used.

***VWF ko mice***

**[0223]** Male and female VWF ko mice in a weight range of 25-40 g were bred at Charles River Laboratories (Sulzfeld, Germany). The group size was n=12, divided in 4 cohorts. Thus, n=3 animals per time-point were used.

***NMRI mice***

**[0224]** Female NMRI mice in a weight range of 27-34 g were bred at Charles River Laboratories (Sulzfeld, Germany). The group size was n=12, divided in 4 cohorts. Thus, n=3 animals per time-point were used.

***CD rats***

**[0225]** Female rats Crl:CD (Sprague Dawley) in a weight range of 250-302 g were bred at Charles River Laboratories (Sulzfeld, Germany). The group size was n=6, divided in 2 cohorts. Thus, n=3 animals per time-point were used.

***VWF ko rats***

**[0226]** Male and female VWF ko rats in a weight range of 222-559 g were bred at Charles River Laboratories (Sulzfeld, Germany). The group size was n=4.

**[0227]** In house, mice and rats were kept at standard housing conditions, i.e. at 20-24°C under a 12 h/12 h light-darkness cycle. Animals were fed ad libitum with standard mouse and rat diet (Ssniff-Versuchsdäten, Soest, Germany). Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

***Pigs***

**[0228]** Pigs were chosen, since they represent a good model for subcutaneous bioavailability with respect to its predictivity for men. The group size was 2 (intravenous) or 3 (subcutaneous).

**[0229]** Male pigs in a weight range of 23-27 kg were bred at Schlosser (Schwalmtal, Germany). In house, the animals were kept in a stable on straw at 18-21°C. Animals were fed with bruised grain. Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

### **Experimental details**

#### ***FVIII ko, VWF ko and NMRI mice***

**[0230]** The test articles were administered i.v. by a single injection into the lateral tail vein at a total volume of 5 mL/kg or s.c. by a single injection into the neck at a total volume of 5 mL/kg. Blood samples were taken retroorbitally under short term anaesthesia using an alternating sampling scheme at 3, 8, 16, 24, 32, 48, 72 and 96 h p.a., and i.v. additionally at 5 min p.a.

**[0231]** Blood samples were anticoagulated using sodium citrate (1 parts sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at -70°C.

**[0232]** rD'D3-FP exposure was determined by measurement of the albumin part of the protein using a human albumin ELISA.

#### ***VWF ko rats***

**[0233]** The test articles were administered i.v. by a single injection into the lateral tail vein at a total volume of 3 mL/kg or s.c. by a single injection one side in the flank at a total volume of 2 mL/kg.

**[0234]** Blood samples from the s.c. group were taken from the saphenous vein at pre-dose, 4, 24, 48, 72, 96 and 168 h p.a. from each animal, and from the i.v. group at pre-dose, 1, 24, 48, 72, 120, 192, 240 and 336 h p.a.

**[0235]** Blood samples were anticoagulated using sodium citrate (1 parts sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at -70°C.

**[0236]** rD'D3-FP exposure was determined by measurement of the albumin part of the protein using a human albumin ELISA. Further, FVIII chromogenic activities were measured.

#### ***CD rats***

**[0237]** The test articles were administered i.v. by a single injection into the lateral tail vein at a

total volume of 3 mL/kg or s.c. by a single injection one side in the flank at a total volume of 3 mL/kg.

**[0238]** Blood samples were taken retroorbitally under short term anaesthesia using an alternating sampling scheme at 3, 8, 24, 48, 72 and 96 h p.a., and i.v. additionally at 5 min p.a.

**[0239]** Blood samples were anticoagulated using sodium citrate (2 parts sodium citrate 3.13% + 8 parts blood), processed to plasma and stored at -70°C.

**[0240]** rD'D3-FP exposure was determined by measurement of the albumin part of the protein using a human albumin ELISA.

### ***Pigs***

**[0241]** The test articles were administered s.c. in the flanks or i.v. into the ear vein by a single injection, at a total volume ranging from 0.211 to 0.751 mL/kg.

**[0242]** Blood samples were taken from the ear or saphenous vein. Timepoints in the 10 mg/kg rD'D3-FP s.c. groups were pre-dose, 3, 12, 24, 32, 48, 72, 96, 120, 144 and 168 h p.a. , and in the i.v. group pre-dose 5 min, 3, 12, 24, 32, 48, 72, 96, 120, 144 and 168 h p.a. Timepoints in the 3 mg/kg rD'D3-FP s.c. groups were pre-dose, 1, 3, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240 and 264 h p.a.

**[0243]** The PK profile was taken from individual animals. Blood samples were anticoagulated using sodium citrate (1 parts sodium citrate 3.13% + 9 parts blood), processed to plasma and stored at -70°C for the determination of FVIII antigen and albumin.

**[0244]** rD'D3-FP exposure was determined by measurement of the albumin part of the protein using a human albumin ELISA. Further, FVIII chromogenic activities were measured.

### ***General***

**[0245]** rD'D3-FP was applied at a dose level of 3, 3.5 or 10 mg/kg based on human albumin values. In mice, in some groups, rVIII-SingleChain was co-administered at a dose of 100 or 200 IU/kg. The PK profile was taken from four cohorts of mice or 2 cohorts of rats per group, respectively, or from individual pigs.

**[0246]** Calculation of the area under the curve (AUC) from 0 to infinity was done with MATLAB R2017a (Natick, Massachusetts, USA).

### **Results**

[0247] rD'D3-FP was quantified using an ELISA against human albumin, and measurements were performed up to 4 d p.a. in mice and 14 days p.a. in rats and pigs. All measured data were well above the detection limit over the whole observation period (Fig. 7A).

[0248] Fig. 7A-1 shows PK profiles of rD'D3-FP in different mouse strains, with no visual impact of rFVIII co-administration. Curves in all three strains were about comparable, nevertheless in NMRI mice, exposure declined quicker than in FVIII ko or VWF ko animals.

[0249]  $AUC_{0-inf}$  and the resulting bioavailabilities of rD'D3-FP are summarized in Table 3. It was shown in FVIII ko mice that i.v. administration of rD'D3-FP with or without rVIII-SingleChain did no impact on the  $AUC_{0-inf}$  of rD'D3-FP (with FVIII set to 100 % -> without FVIII calculates to 89 %). Thus, there was no relevant effect of rVIII-SingleChain on the rD'D3-FP PK profile in this experiment. Comparison of  $AUC_{0-inf}$  in the different strains suggests no major difference between the three strains, with a ranking from FVIII ko (1590 h\* $\mu$ g/mL) over VWF ko animals (1197 h\* $\mu$ g/mL) to agina slightly lower  $AUC_{0-inf}$  in NMRI animals (940 h\* $\mu$ g/mL).

[0250] PK profiles in rats are shown in Fig. 7A-2, suggesting comparable clearance of rD'D3-FP after s.c. and i.v administration in CD and VWF ko rats. Nevertheless, s.c. availability was lowest in VWF ko rats as compared to CD rats. Fig. 7A-3 shows PK profiles in pigs, suggesting comparable exposure at same doses after at the latest 2 days, and no impact from rVIII-SingleChain on the PK profile of rD'D3-FP.

[0251] Table 4 summarizes the bioavailabilities of rD'D3-FP over species. In rats, evaluation of  $AUC_{0-inf}$  (CD rats) and  $AUC_{0-inf}$  (VWF ko rats) for calculation of bioavailability showed values of 40 % and 11 %, respectively, i.e. a better bioavailability was seen in FVIII-competent rats as compared to VWF ko animals. This is in contrast to the observations in mice  $AUC_{0-inf}$  inf evaluation, suggesting about comparable data from VWF ko and NMRI animals. If at all, VWF ko mice would have better  $AUC_{0-inf}$  as compared to NMRI mice. In pigs,  $AUC_{0-inf}$  of rD'D3-FP ranged between 59 to 187%. Taken together, compared over species, the pig showed the highest rD'D3-FP bioavailability.

Table 3:  $AUC_{0-inf}$  of rD'D3-FP quantified as albumin in different mice strains

|                                             | $AUC_{0-inf}$ [h* $\mu$ g/mL] | Bioavailability [%] |
|---------------------------------------------|-------------------------------|---------------------|
| NMRI                                        | 940                           | n.d.                |
| 200 IU/kg rFVIII + 3 mg/kg rD'D3-FP<br>s.c. |                               |                     |
| FVIII ko                                    | 1590                          | 48                  |
| 3 mg/kg rD'D3-FP s.c.                       |                               |                     |
| 100 IU/kg rFVIII + 3 mg/kg rD'D3-FP<br>i.v. | 3702                          |                     |
| 3 mg/kg rD'D3-FP i.v.                       | 3286                          |                     |

|                                             | AUC <sub>0-inf</sub> [h*µg/mL] | Bioavailability [%] |
|---------------------------------------------|--------------------------------|---------------------|
| VWF ko                                      | 1197                           | n.d.                |
| 200 IU/kg rFVIII + 3 mg/kg rD'D3-FP<br>s.c. |                                |                     |
| n.d. not determined                         |                                |                     |

Table 4: Bioavailabilities of rD'D3-FP quantified as albumin in different species

|                     | Mice | Rat  | Pig     |
|---------------------|------|------|---------|
| FVIII competent     | n.d. | 40%  | 59-187% |
| FVIII ko            | 48%  | n.d. | n.d.    |
| VWF ko              | n.d. | 11%  | n.d.    |
| n.d. not determined |      |      |         |

[0252] Endogenous FVIII chromogenic activity increased not only after intravenous (see before, examples 1.1-1.5) but also after subcutaneous rD'D-FP administration (Fig. 7B).

[0253] Fig. 7B-1 shows that in rats, values increased with a maximum at day 1 (3 mg/kg i.v.) or day 4 (10 mg/kg s.c.). This calculates for a reduction in effect on FVIII AUC<sub>0-inf</sub> after s.c. as compared to i.v. rD'D3-FP administration (independent of the rD'D3-FP dose) to 17% (chromogenic) and 14% (clotting) activity. This slight but nevertheless relevant increase in endogenous FVIII activity was observed for i.v. as well as s.c. treatment (mainly ranging from -0.5-1 IU/mL or 50-100%), and thus ranging slightly below the levels reached in CD rats (compare Fig. 4B).

[0254] In pigs (Fig. 7B-2), an increase in FVIII chromogenic activity was observed at about 1 day after s.c. administration of rD'D3-FP. This effect lasted over the whole period of 11 days. This increase was maximally 2-fold above predose values (maximal increase by ~5 IU/mL or 500% to ~10 IU/mL = 1000% - in line with higher baseline values in these animals as compared to men, i.e. in line with the small effects after i.v. compound administration to FVIII competent animals.

[0255] Thus, these data suggest that treatment with rD'D3-FP can be done not only using i.v. but also using s.c. compound administration.

**Example 1.8: Impact of multiple intravenous doses of rD'D3-FP on endogenous FVIII levels in VWF ko rats**

**Animals**

**[0256]** Male and female VWF ko rats in a weight range of 281-504 g were bred at Charles River Laboratories (Sulzfeld, Germany). The group size was n=11, divided in 4 cohorts. Thus, n=2-3 animals per time-point were used.

**[0257]** In house, the animals were kept at standard housing conditions, i.e. at 20-24°C under a 12 h/12 h light-darkness cycle. Animals were fed ad libitum with standard mouse and rat diet (Ssniff-Versuchsdäten, Soest, Germany). Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

### **Experimental details**

**[0258]** The test articles were administered i.v. by multiple injections into the lateral tail vein at a total volume of 3 mL/kg on day 0 (cohort 1), day 0+7 (cohort 2), 0+7+14+21 (cohort 3) or day 0+7+14 (cohort 4). Two blood samples per animal were taken retroorbitally under short term anaesthesia at the cohort-specific timepoints (cohort 1: predose + 7 days p.a., cohort 2: 3 + 10 days p.a., cohort 3: 17 + 24 days p.a., cohort 4: 14 + 21 days p.a.). rD'D3-FP was applied at a dose level of 3 mg/kg based on human albumin values. The PK profile was taken from four cohorts of VWF ko rats. Blood samples were anticoagulated using sodium citrate (2 parts sodium citrate 3.13% + 8 parts blood), processed to plasma and stored at -70°C for the determination of albumin.

**[0259]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using a human albumin ELISA. Further, FVIII chromogenic and clotting activity and aPTT using Pathromtin® SL was measured.

### **Results**

**[0260]** rD'D3-FP was quantified using an ELISA against human albumin, and measurements were performed up to day 24 d. All measured data were well above the detection limit over the whole observation period (Fig. 8A). With the 4 administrations, a slight accumulation was observed in peak (to 151 %) as well as trough (to 128 %) levels.

**[0261]** FVIII chromogenic (Fig. 8B) activity levels were increased from values below to the detection limit after the 2<sup>nd</sup> administration into the range observed in CD rats (maximal increase to -1.8 IU/mL = 180%). FVIII clotting activity (Fig. 8C) was even slightly above the data measured in CD rats (maximal increase to -800% - in line with higher baseline values in these animals as compared to men). Nevertheless, no accumulation was seen for the two FVIII activity assays over a period of 4 weeks.

**[0262]** In line with this, aPTT decreased from above normal values from CD rats to values at

the lower range of the normal range of CD rats (Fig. 8D).

**[0263]** Taken together, these data suggest that multiple treatments with rD'D3-FP can be done with slight accumulation of rD'D3-FP, but without accumulation of FVIII levels and with normalization of aPTT.

**Example 1.9: Investigation of rD'D3-FP given subcutaneously and rVIII-SingleChain given intravenously in a hemophilia A model, i.e. in FVIII ko rat.**

### **Animals**

**[0264]** Male and female FVIII ko rats in a weight range of 220-487 g were bred at Charles River Laboratories (Sulzfeld, Germany). The group size was n=6, divided in 2 cohorts. Thus, n=3 animals per time-point were used.

**[0265]** In house, the animals were kept at standard housing conditions, i.e. at 20-24°C under a 12 h/12 h light-darkness cycle. Animals were fed ad libitum with standard mouse and rat diet (Ssniff-Versuchsdäten, Soest, Germany). Tap water was supplied ad libitum. Animal husbandry and study procedures complied with the German Animal Welfare law and European Union regulations.

### **Experimental details**

**[0266]** The test articles were administered s.c. in the neck (rD'D3-FP) or i.v. (rVIII-SingleChain) into the lateral tail vein of FVIII ko rats by a single injection, at a total volume of 3 mL/kg. rD'D3-FP was applied s.c. in a dose of 3 mg/kg based on human albumin values 10 minutes prior to rVIII-SingleChain. Animals were treated intravenously with rVIII-SingleChain at a dose of 200 IU/kg chromogenic FVIII activity. rVIII-SingleChain was reconstituted with water for injection, and rD'D3-FP was thawed in a water bath. In every case, a dose volume of 3 mL/kg was administered, with dilution buffer for FVIII (rVIII-SingleChain) or isotonic saline (rD'D3-FP) being used for dissolution of the compounds as necessary.

**[0267]** Blood samples were taken by cannulation of the tail vein. Timepoints in the groups were 0.083, 1, 4, 8, 16, 24, 32, 48 and 72 h p.a. The PK profile was taken from two cohorts of rats per group, and n=3 per timepoint. Blood samples were anticoagulated using sodium citrate (2 parts sodium citrate 3.13% + 8 parts blood), processed to plasma and stored at -70°C for the determination of FVIII activity and FVIII antigen.

**[0268]** rD'D3-FP exposure was determined by measurement of the albumin part of the construct using a human albumin ELISA. Further, FVIII chromogenic activity and human FVIII

antigen was measured.

[0269] Estimation of the maximal concentration ( $C_{\max}$ ), the area under the concentration over time curve from  $t=0$  to  $t=\infty$  ( $AUC_{0-\infty}$ ), mean residence time (MRT), clearance (CL) and terminal half-life ( $t_{1/2}$ ) was done by two-compartmental modelling in the i.v. calculations, and by two-compartmental-resorption modelling in the s.c. calculations. For parameter estimation, a weighted least-squares cost function was applied. Bioavailability was calculated as the percentage of the  $AUC_{0-\infty}$  after s.c. administration as compared to i.v. administration. Time to 1, 5 and 10 % trough levels was calculated by setting the model equation equal to 0.01, 0.05 or 0.1 IU/mL and solving for time.

## Results

### *Evaluation of D'D3 data*

[0270] rD'D3-FP was absorbed after s.c. administration. rD'D3-FP could be quantified over the whole period of observation of 72 h; i.e. it remained above the detection limit of 27 ng/mL (Fig. 9A).

[0271]  $C_{\max}$ ,  $AUC_{0-\infty}$ , clearance, MRT and  $t_{1/2}$  are given in Table 5 and confirm relevant exposure of rD'D3-FP over time after subcutaneous administration.

Table 5: Pharmacokinetic parameters of rD'D3-FP after s.c. administration of rD'D3-FP followed by i.v. administration of rVIII-SingleChain in FVIII ko rats

| Treatment                                                      | $C_{\max}$ ,<br>extrap.<br>[ $\mu\text{g/mL}$ ] | Clearance<br>[mL/kg/h] | MRT<br>[h] | Half-life,<br>terminal<br>[h] | $AUC_{0-\infty}$<br>[ $\mu\text{g}\cdot\text{h/mL}$ ] |
|----------------------------------------------------------------|-------------------------------------------------|------------------------|------------|-------------------------------|-------------------------------------------------------|
| 3 mg/kg rD'D3-FP s.c.&<br>200 IU/kg rVIII-<br>SingleChain i.v. | 32                                              | 1.0                    | 77         | 36                            | 2959                                                  |

### *Evaluation of FVIII data*

[0272] The FVIII PK profile of rVIII-SingleChain after subcutaneous preadministration of rD'D3-FP was prolonged as compared to rVIII-SingleChain given alone for FVIII chromogenic activity (Fig. 9B) as well as FVIII antigen (Fig. 9C).

[0273] FVIII chromogenic activity (Fig. 9B) was well above the detection limit until the last timepoint of 72 h p.a. when rD'D3-FP was preadministered, while it reached the baseline at 72

h when FVIII was given alone. Visually, exposure was improved with subcutaneous preadministration of rD'D3-FP starting at 32 h p.a.

[0274] Similar observations were done for FVIII:Ag (Fig. 9C), but exposure was already improved with subcutaneous preadministration of rD'D3-FP starting at 24 h p.a., and baseline was reached already at 48 h p.a. with FVIII given alone, probably related to lower variability of the measured concentrations.

[0275] Predosing of rD'D3-FP improved clearance, MRT and  $t_{1/2}$  for both FVIII chromogenic activity and FVIII:Ag.

[0276]  $AUC_{0-inf}$  improved by 41 % for FVIII chromogenic activity (Table 6) and by 49 % for FVIII:Ag (Table 8).  $C_{max}$  improved by 30 % for FVIII chromogenic activity and 23 % for FVIII:Ag (Table 6 and 8, respectively).

[0277] Time to trough was calculated for both administration schemes with and without rD'D3-FP for chromogenic activity (Table 7) and for FVIII:Ag (Table 9). As for  $AUC_{0-inf}$ , predosing of rD'D3-FP showed also favourable trough levels for chromogenic activity as well as FVIII antigen. Prolongation was 6% and 18% for 1% trough, 9% and 19% for 5% trough and 8% and 18% for 10% trough for chromogenic FVIII activity and FVIII:Ag, respectively.

Table 6: Pharmacokinetic parameters of FVIII chromogenic activity after administration of rD'D3-FP s.c. followed by i.v. administration of rVIII-SingleChain in FVIII ko rats

| Treatment                                                | $C_{max}$ ,<br>extrap.<br>[mIU/mL] | Clearance<br>[mL/kg/h] | MRT [h] | Half-life,<br>terminal [h] | $AUC_{0-inf}$<br>[mIU*h/mL] |
|----------------------------------------------------------|------------------------------------|------------------------|---------|----------------------------|-----------------------------|
| 3 mg/kg rD'D3-FP s.c. & 200 IU/kg rVIII-SingleChain i.v. | 4036                               | 4.2                    | 12      | 8                          | 47435                       |
| 200 IU/kg rVIII-SingleChain i.v.                         | 3107                               | 6.0                    | 11      | 7                          | 33428                       |

Table 7: Time to trough levels of rVIII-SingleChain (FVIII chromogenic activity) after s.c. administration in FVIII ko rats

| Treatment                                                | 1% trough [h] | Time to 5%<br>trough [h] | 10% trough [h] |
|----------------------------------------------------------|---------------|--------------------------|----------------|
| 3 mg/kg rD'D3-FP s.c. & 200 IU/kg rVIII-SingleChain i.v. | 53            | 35                       | 27             |
| 200 IU/kg rVIII-SingleChain i.v.                         | 50            | 32                       | 25             |

Table 8: Pharmacokinetic parameters of FVIII antigen after administration of rD'D3-FP s.c. followed by i.v. administration of rVIII-SingleChain in FVIII ko rats

| Treatment                                                | C <sub>max</sub> ,<br>extrap.<br>[mIU/mL] | Clearance<br>[mL/kg/h] | MRT<br>[h] | Half-<br>life,<br>terminal<br>[h] | AUC <sub>0-inf</sub><br>[mIU*h/mL] |
|----------------------------------------------------------|-------------------------------------------|------------------------|------------|-----------------------------------|------------------------------------|
| 3 mg/kg rD'D3-FP s.c. & 200 IU/kg rVIII-SingleChain i.v. | 3662                                      | 3.8                    | 14         | 10                                | 53957                              |
| 200 IU/kg rVIII-SingleChain i.v.                         | 2983                                      | 5.8                    | 12         | 8                                 | 36211                              |

Table 9: Time to trough levels of rVIII-SingleChain (FVIII antigen) after s.c. administration in FVIII ko rats

| Treatment                                                | 1% trough [h] | Time to 5%<br>trough [h] | 10% trough<br>[h] |
|----------------------------------------------------------|---------------|--------------------------|-------------------|
| 3 mg/kg rD'D3-FP s.c. & 200 IU/kg rVIII-SingleChain i.v. | 66            | 43                       | 33                |
| 200 IU/kg rVIII-SingleChain i.v.                         | 56            | 36                       | 28                |

#### Conclusion from *in vivo* animal experiments

[0278] These studies demonstrate that i.v. or s.c. administration of rD'D3-FP slightly increases endogenous levels of FVIII in healthy rats, rabbits, pigs and monkeys, even with the already physiological FVIII levels in these animals. Predose values were hardly exceeded, thereby only slight or no shortening of aPTT was observed.

[0279] In animals that show a haemophilia A bleeding type (VWF ko rats or VWF ko mice, that have relevantly reduced FVIII activity levels), this increase of endogenous FVIII activity is stronger than in the healthy animals, with increases of absolute FVIII levels to levels about equal or above those of healthy mice and rats. In these animals, physiological levels were either restored (levels within the upper end of the physiological variability, for example up to 200-300% of the norm chromogenic FVIII activity after administration of 1 and 3 mg/kg rD'D3-FP in VWF ko rats - in line with higher baseline values in these animals as compared to men) or exceeded the physiological range (for example  $\geq 400\%$  of the norm chromogenic FVIII activity after administration of 10 mg/kg rD'D3-FP in VWF ko rats). In the VWF ko mouse as an example with lower baseline values regarding chromogenic FVIII activity as compared to men, -40% of the norm were reached at a dose of 10 mg/kg rD'D3-FP, i.e. an increase  $< 2$ -fold above levels observed in healthy NMRI mice. This suggests that in individuals with initially reduced FVIII levels, i.e. a haemophilia A phenotype, a FVIII elevating effect may potentially be stronger than in healthy subjects. Similar effects were achieved by co-administration of rD'D3-FP EYA or EY variant, or by rD'D3-CTP.

[0280] Multiple doses of rD'D3-FP in VWF ko rats led to a slight accumulation of rD'D3-FP over

time, while FVIII activity reached (chromogenic activity) or slightly exceeded (clotting activity) physiological levels, and aPTT was restored to normal values.

**[0281]** Without wishing to be bound by theory, this may be explained by a physiological down-regulation of FVIII synthesis in healthy animals with physiological FVIII levels as compared to the VWF ko rats and mice. It may thus be speculated that the effect on FVIII in human haemophilia A patients should be comparable to that observed in VWF ko rats and mice, and thus should achieve long lasting physiological levels. These effects have been shown after i.v. and s.c. administration. Further bioavailabilities of rD'D3-FP ranged between 11-187%, dependent on species and genotype, with highest values achieved in pigs, known to be a well predictive model for s.c. bioavailability. Thereby, these data are suggesting that s.c. treatment is feasible as well.

#### **Example 2: Determination of FVIII affinity to VWF fragment dimer and monomer**

**[0282]** A VWF fragment (1-1242) albumin fusion (D'D3-FP) was expressed in a bioreactor; after purification as described above and isolation of monomer and dimer, the affinity of FVIII to these preparations was assessed through surface plasmon resonance via a Biacore instrument (T200, GE Healthcare).

**[0283]** An anti-albumin antibody (MA1-20124, Thermo Scientific) was covalently coupled via its N-terminus to an activated CM 3 chip by NHS (N-Hydroxysuccinimide) and EDC (Ethanolamine hydrochloride), both contained in the amine coupling kit (BR1000-50) from GE Healthcare. For immobilization 3 µg/mL of the antibody were diluted in sodium acetate buffer (10 mM, pH 5.0) and the antibody solution was flown over the chip for 7 min. at a flow rate of 10 µL/min. After the immobilization procedure non-coupled dextran filaments were saturated by flowing ethanolamine solution (1 M, pH 8.3) over the chip for 5 min (at a flow rate of 10 µL/min). The aim of saturating the flow cell was to minimize unspecific binding of the analytes to the chip. A reference flow cell was set up by saturating an empty flow cell with ethanolamine by using the same procedure as above.

**[0284]** Dimeric and monomeric D'D3-FP proteins, respectively, were immobilized to the covalently coupled anti-albumin antibody by a flow of the D'D3-FP proteins (5 µg/mL) over the chip for 3 min (flow rate of 10 µL/min).

**[0285]** To create binding curves for FVIII, each D'D3-FP protein preparation was diluted in running buffer (HBS-P+: 0.1 M HEPES, 1.5 M NaCl and 0.5% v/v Surfactant P20, pH 7.4; product code BR100671, GE Healthcare) to concentrations of 0.25 nM, 0.5 nM, 1 nM, 3nM and 4 nM. By performing a single cycle kinetic, samples with ascending concentrations of each dilution were flown over the chip for 2 min (flow rate 30µL/min.), followed by a dissociation time of 10 min. with running buffer HBS-P+. All measurements were performed twice. The temperature for the measuring procedure was adjusted to +25°C.

[0286] Binding parameters were calculated using BiaEvaluation Software. The curve fitting methods were based on Langmuir equations. The input data for calculations were the molar mass of the analyte FVIII (rVIII-SingleChain) of 170 kDa, other parameters like max. RU and slopes were automatically extracted out of the fitted association and dissociation curves. The outputs of BiaEvaluation Software are the association rate constants and the dissociation rate constants, from which the affinity constants were calculated. The results are shown in Table 10.

Table 10: FVIII affinity data for D'D3-FP dimer and monomer

| D'D3-FP preparation | ka [1/Ms]         | kd [1/s]             | KD [M]                |
|---------------------|-------------------|----------------------|-----------------------|
| D'D3-FP Dimer       | $4.5 \times 10^7$ | $1.5 \times 10^{-3}$ | $3.4 \times 10^{-11}$ |
| D'D3-FP Monomer     | $9.9 \times 10^5$ | $3.0 \times 10^{-2}$ | $3.0 \times 10^{-8}$  |

[0287] The dimeric D'D3-FP shows a significantly ( $K_D = 34 \text{ pM}$ ) increased affinity to FVIII compared to the D'D3-FP monomer ( $K_D = 30 \text{ nM}$ ) which results both from a faster association and a slower dissociation of rVIII-SingleChain.

SEQUENCE LISTING

[0288]

- <110> CSL Behring Recombinant Facility AG
- <120> Truncated Von Willebrand Factor Polypeptides for Treating Hemophilia
- <130> 2016\_L003\_A249
- <150> EP 16198501.5
- <151> 2016-11-11
- <160> 7
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| ctgtgaggat ggcttcatgc actgtaccat gagtggagtc cccggaagct tgctgcctga  | 2280 |
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| cgtgtcctc agcagtcctc tgtctcatcg cagcaaaagg agcctatcct gtcggccccc   | 2340 |
| catggtcaag ctggtgtgtc ccgctgacaa cctgcgggct gaagggtcgc agtgtaccaa  | 2400 |
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| ccccccgggc atggtccggc atgagaacag atgtgtggcc ctggaaagggt gtcctgctt  | 2520 |
| ccatcagggc aaggagtatg cccctggaga aacagtgaag attggctgca acacttgtgt  | 2580 |
| ctgtcgggac cggaagtgga actgcacaga ccatgtgtgt gatgccacgt gctccacgat  | 2640 |
| cggcatggcc cactacctca ccttcgacgg gctcaaatac ctgttccccg gggagtgcc a | 2700 |
| gtacgttctg gtgcaggatt actgcggcag taaccctggg acctttcgga tcctagtggg  | 2760 |
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| gaaagtgtgt ggcctgtgtg ggaattttga tggcatccag aacaatgacc tcaccagcag  | 3060 |
| caacctccaa gtggaggaag acctgtgga ctttgggaac tcctggaaag tgagctcgca   | 3120 |
| gtgtgctgac accagaaaag tgctcttgg ctcacccct gccacctgcc ataacaacat    | 3180 |
| catgaagcag acgatggtgg attcctcctg tagaatcctt accagtgacg tcttccagga  | 3240 |
| ctgcaacaag ctggtggacc ccgagccata tctggatgtc tgcatttacg acacctgctc  | 3300 |
| ctgtgagtc attggggact gcgcctgctt ctgcgacacc attgctgcct atgcccacgt   | 3360 |

|                                                                    |      |
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| acctgcctgt caagtcacgt gtcagcacc ctagccactg gcctgccctg tgcagtgtgt   | 3540 |
| ggagggctgc catgcccact gccctccagg gaaaatcctg gatgagcttt tgcagacctg  | 3600 |
| cgttgaccct gaagactgtc cagtgtgtga ggtggctggc cggcgttttg cctcaggaaa  | 3660 |
| gaaagtcacc ttgaatccca gtgacctga gcactgccag atttgccact gtgatgttgt   | 3720 |
| caacctcacc tgtgaagcct gccaggagcc gggaggctcg agcgggggat ctggcgggtc  | 3780 |
| tggaggctct ggagggctcg gaggctctgg aggctctggg ggcctctggc ggtctggagg  | 3840 |
| gtcgggatcc gatgcacaca agagtgaggt tgctcatcgg tttaaagatt tgggagaaga  | 3900 |
| aaatttcaaa gccttggtgt tgattgcctt tgctcagtat cttcagcagt gtccatttga  | 3960 |
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| gagaaatgaa tgcttcttgc aacacaaaga tgacaaccca aacctcccc gattggtgag   | 4200 |
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| accagaggtt gatgtgatgt gcactgcttt tcatgacaat gaagagacat ttttgaaaaa  | 4260 |
| atacttatat gaaattgcc aagacatcc ttacttttat gccccggaac tccttttctt    | 4320 |
| tgctaaaagg tataaagctg cttttacaga atgttgccaa gctgctgata aagctgcctg  | 4380 |
| cctgttgcca aagctcgatg aacttcggga tgaagggaag gcttcgtctg ccaaacagag  | 4440 |
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| atgctgtgaa aaacctctgt tggaaaaatc ccactgcatt gccgaagtgg aaaatgatga  | 4740 |
| gatgcctgct gacttgcctt cattagctgc tgattttgtt gaaagtaagg atgtttgcaa  | 4800 |
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| gcttgagagag tacaaattcc agaatgcgt attagttcgt tacaccaaga aagtaccca   | 5100 |
| agtgtcaact ccaactcttg tagaggtctc aagaaacctg ggaaaagtgg gcagcaaatg  | 5160 |
| ttgtaaacat cctgaagcaa aaagaatgcc ctgtgcagaa gactatctat ccgtggtcct  | 5220 |
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| cacagaatcc ttggtgaaca ggcgacctg cttttcagct ctggaagtgc atgaaacata   | 5340 |
| cgttcccaaa gagtttaatg ctgaaacatt caccttccat gcagatatat gcacactttc  | 5400 |
| tgagaaggag agacaaatca agaaacaaac tgcacttggt gagctcgtga aacacaagcc  | 5460 |
| caaggcaaca aaagagcaac tgaaagctgt tatggatgat ttgcgagctt ttgtagagaa  | 5520 |
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5616

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| Ser | Leu | Ser | Cys | Arg | Pro | Pro | Met | Val | Lys | Leu | Val | Cys | Pro | Ala | Asp |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Leu | Arg | Ala | Glu | Gly | Leu | Glu | Cys | Thr | Lys | Thr | Cys | Gln | Asn | Tyr |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Leu | Glu | Cys | Met | Ser | Met | Gly | Cys | Val | Ser | Gly | Cys | Leu | Cys | Pro |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gly | Met | Val | Arg | His | Glu | Asn | Arg | Cys | Val | Ala | Leu | Glu | Arg | Cys |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Cys | Phe | His | Gln | Gly | Lys | Glu | Tyr | Ala | Pro | Gly | Glu | Thr | Val | Lys |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Gly | Cys | Asn | Thr | Cys | Val | Cys | Arg | Asp | Arg | Lys | Trp | Asn | Cys | Thr |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | His | Val | Cys | Asp | Ala | Thr | Cys | Ser | Thr | Ile | Gly | Met | Ala | His | Tyr |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Thr | Phe | Asp | Gly | Leu | Lys | Tyr | Leu | Phe | Pro | Gly | Glu | Cys | Gln | Tyr |
|     |     | 115 |     |     |     | 120 |     |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Leu | Val | Gln | Asp | Tyr | Cys | Gly | Ser | Asn | Pro | Gly | Thr | Phe | Arg | Ile |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys  
 145 150 155 160

Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly  
 165 170 175

Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val  
 180 185 190

Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser  
 195 200 205

Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr  
 210 215 220

Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln  
 225 230 235 240

Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val  
 245 250 255

Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg  
 260 265 270

Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met  
 275 280 285

Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val  
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Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val  
 305 310 315 320

Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys  
 325 330 335

Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly  
 340 345 350

Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu  
 355 360 365

Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn  
 370 375 380

Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu  
 385 390 395 400

Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro  
 405 410 415

Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp  
 420 425 430

Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|
| 435 |     | 440 |     | 445 |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
| Val | Thr | Leu | Asn | Pro | Ser | Asp | Pro | Glu | His | Cys | Gln | Ile | Cys | His | Cys |  |  |  |  |
| 450 |     |     |     |     |     | 455 |     |     |     |     |     |     |     |     | 460 |  |  |  |  |
| Asp | Val | Val | Asn | Leu | Thr | Cys | Glu | Ala | Cys | Gln | Glu | Pro | Gly | Gly | Ser |  |  |  |  |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |  |  |  |  |
| Ser | Gly | Gly | Ser | Gly | Gly | Ser | Gly | Gly | Ser | Gly | Gly | Ser | Gly | Gly | Ser |  |  |  |  |
|     |     |     |     | 485 |     |     |     |     |     | 490 |     |     |     |     | 495 |  |  |  |  |
| Gly | Gly | Ser | Gly | Gly | Ser | Gly | Gly | Ser | Gly | Gly | Ser | Gly | Ser | Asp | Ala |  |  |  |  |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |  |  |  |  |
| His | Lys | Ser | Glu | Val | Ala | His | Arg | Phe | Lys | Asp | Leu | Gly | Glu | Glu | Asn |  |  |  |  |
|     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |  |  |  |  |
| Phe | Lys | Ala | Leu | Val | Leu | Ile | Ala | Phe | Ala | Gln | Tyr | Leu | Gln | Gln | Cys |  |  |  |  |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |  |  |  |  |
| Pro | Phe | Glu | Asp | His | Val | Lys | Leu | Val | Asn | Glu | Val | Thr | Glu | Phe | Ala |  |  |  |  |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |  |  |  |  |
| Lys | Thr | Cys | Val | Ala | Asp | Glu | Ser | Ala | Glu | Asn | Cys | Asp | Lys | Ser | Leu |  |  |  |  |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |  |  |  |  |
| His | Thr | Leu | Phe | Gly | Asp | Lys | Leu | Cys | Thr | Val | Ala | Thr | Leu | Arg | Glu |  |  |  |  |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |  |  |  |  |
| Thr | Tyr | Gly | Glu | Met | Ala | Asp | Cys | Cys | Ala | Lys | Gln | Glu | Pro | Glu | Arg |  |  |  |  |
|     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |  |  |  |  |
| Asn | Glu | Cys | Phe | Leu | Gln | His | Lys | Asp | Asp | Asn | Pro | Asn | Leu | Pro | Arg |  |  |  |  |
|     | 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |  |  |  |  |
| Leu | Val | Arg | Pro | Glu | Val | Asp | Val | Met | Cys | Thr | Ala | Phe | His | Asp | Asn |  |  |  |  |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     |     | 640 |  |  |  |  |
| Glu | Glu | Thr | Phe | Leu | Lys | Lys | Tyr | Leu | Tyr | Glu | Ile | Ala | Arg | Arg | His |  |  |  |  |
|     |     |     |     | 645 |     |     |     |     | 650 |     |     |     |     | 655 |     |  |  |  |  |
| Pro | Tyr | Phe | Tyr | Ala | Pro | Glu | Leu | Leu | Phe | Phe | Ala | Lys | Arg | Tyr | Lys |  |  |  |  |
|     |     |     | 660 |     |     |     |     | 665 |     |     |     |     | 670 |     |     |  |  |  |  |
| Ala | Ala | Phe | Thr | Glu | Cys | Cys | Gln | Ala | Ala | Asp | Lys | Ala | Ala | Cys | Leu |  |  |  |  |
|     |     | 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |  |  |  |  |
| Leu | Pro | Lys | Leu | Asp | Glu | Leu | Arg | Asp | Glu | Gly | Lys | Ala | Ser | Ser | Ala |  |  |  |  |
|     | 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |  |  |  |  |
| Lys | Gln | Arg | Leu | Lys | Cys | Ala | Ser | Leu | Gln | Lys | Phe | Gly | Glu | Arg | Ala |  |  |  |  |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |  |  |  |  |
| Phe | Lys | Ala | Trp | Ala | Val | Ala | Arg | Leu | Ser | Gln | Arg | Phe | Pro | Lys | Ala |  |  |  |  |
|     |     |     |     | 725 |     |     |     |     | 730 |     |     |     |     | 735 |     |  |  |  |  |

Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His  
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 Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala  
 755 760 765  
 Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys  
 770 775 780  
 Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile  
 785 790 795 800  
 Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala  
 805 810 815  
 Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala  
 820 825 830  
 Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His  
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 Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu  
 850 855 860  
 Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr  
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 Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn  
 885 890 895  
 Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys  
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 Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly  
 930 935 940  
 Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu  
 945 950 955 960  
 Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys  
 965 970 975  
 Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val  
 980 985 990  
 Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val  
 995 1000 1005  
 Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile  
 1010 1015 1020  
 Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala  
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Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln  
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Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys  
1055 1060 1065

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Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr  
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gcc cga tgc agc ctt ttc gga agt gac ttc gtc aac acc ttt gat ggg 144  
Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly  
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Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu  
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Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys  
115 120 125

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Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly  
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Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly  
145 150 155 160

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| 165 |     |     |     |     |     |     |     | 170 |     |     |     | 175 |     |     |     |      |
| gaa | ggg | acc | ttg | acc | tcg | gac | cct | tat | gac | ttt | gcc | aac | tca | tgg | gct | 576  |
| Glu | Gly | Thr | Leu | Thr | Ser | Asp | Pro | Tyr | Asp | Phe | Ala | Asn | Ser | Trp | Ala |      |
|     |     |     | 180 |     |     |     |     |     |     | 185 |     |     |     | 190 |     |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| ctg | agc | agt | gga | gaa | cag | tgg | tgt | gaa | cgg | gca | tct | cct | ccc | agc | agc | 624  |
| Leu | Ser | Ser | Gly | Glu | Gln | Trp | Cys | Glu | Arg | Ala | Ser | Pro | Pro | Ser | Ser |      |
|     |     |     | 195 |     |     |     |     |     |     | 200 |     |     |     | 205 |     |      |
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| tca | tgc | aac | atc | tcc | tct | ggg | gaa | atg | cag | aag | ggc | ctg | tgg | gag | cag | 672  |
| Ser | Cys | Asn | Ile | Ser | Ser | Gly | Glu | Met | Gln | Lys | Gly | Leu | Trp | Glu | Gln |      |
|     |     |     | 210 |     |     |     |     |     |     | 215 |     |     |     | 220 |     |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| tgc | cag | ctt | ctg | aag | agc | acc | tcg | gtg | ttt | gcc | cgc | tgc | cac | cct | ctg | 720  |
| Cys | Gln | Leu | Leu | Lys | Ser | Thr | Ser | Val | Phe | Ala | Arg | Cys | His | Pro | Leu |      |
|     |     |     | 225 |     |     |     |     |     |     |     |     |     | 235 |     |     | 240  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| gtg | gac | ccc | gag | cct | ttt | gtg | gcc | ctg | tgt | gag | aag | act | ttg | tgt | gag | 768  |
| Val | Asp | Pro | Glu | Pro | Phe | Val | Ala | Leu | Cys | Glu | Lys | Thr | Leu | Cys | Glu |      |
|     |     |     | 245 |     |     |     |     |     |     |     |     |     | 250 |     |     | 255  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| tgt | gct | ggg | ggg | ctg | gag | tgc | gcc | tgc | cct | gcc | ctc | ctg | gag | tac | gcc | 816  |
| Cys | Ala | Gly | Gly | Leu | Glu | Cys | Ala | Cys | Pro | Ala | Leu | Leu | Glu | Tyr | Ala |      |
|     |     |     | 260 |     |     |     |     |     |     |     |     |     |     |     | 270 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| cgg | acc | tgt | gcc | cag | gag | gga | atg | gtg | ctg | tac | ggc | tgg | acc | gac | cac | 864  |
| Arg | Thr | Cys | Ala | Gln | Glu | Gly | Met | Val | Leu | Tyr | Gly | Trp | Thr | Asp | His |      |
|     |     |     | 275 |     |     |     |     |     |     |     |     |     |     |     | 285 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| agc | gcg | tgc | agc | cca | gtg | tgc | cct | gct | ggt | atg | gag | tat | agg | cag | tgt | 912  |
| Ser | Ala | Cys | Ser | Pro | Val | Cys | Pro | Ala | Gly | Met | Glu | Tyr | Arg | Gln | Cys |      |
|     |     |     | 290 |     |     |     |     |     |     |     |     |     |     |     | 300 |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| gtg | tcc | cct | tgc | gcc | agg | acc | tgc | cag | agc | ctg | cac | atc | aat | gaa | atg | 960  |
| Val | Ser | Pro | Cys | Ala | Arg | Thr | Cys | Gln | Ser | Leu | His | Ile | Asn | Glu | Met |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| 305 |     |     |     | 310 |     |     |     | 315 |     |     |     | 320 |     |     |     |      |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| tgt | cag | gag | cga | tgc | gtg | gat | ggc | tgc | agc | tgc | cct | gag | gga | cag | ctc | 1008 |
| Cys | Gln | Glu | Arg | Cys | Val | Asp | Gly | Cys | Ser | Cys | Pro | Glu | Gly | Gln | Leu |      |
|     |     |     | 325 |     |     |     |     |     |     |     |     |     |     |     |     | 335  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| ctg | gat | gaa | ggc | ctc | tgc | gtg | gag | agc | acc | gag | tgt | ccc | tgc | gtg | cat | 1056 |
| Leu | Asp | Glu | Gly | Leu | Cys | Val | Glu | Ser | Thr | Glu | Cys | Pro | Cys | Val | His |      |
|     |     |     | 340 |     |     |     |     |     |     |     |     |     |     |     |     | 350  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| tcc | gga | aag | cgc | tac | cct | ccc | ggc | acc | tcc | ctc | tct | cga | gac | tgc | aac | 1104 |
| Ser | Gly | Lys | Arg | Tyr | Pro | Pro | Gly | Thr | Ser | Leu | Ser | Arg | Asp | Cys | Asn |      |
|     |     |     | 355 |     |     |     |     |     |     |     |     |     |     |     |     | 365  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| acc | tgc | att | tgc | cga | aac | agc | cag | tgg | atc | tgc | agc | aat | gaa | gaa | tgt | 1152 |
| Thr | Cys | Ile | Cys | Arg | Asn | Ser | Gln | Trp | Ile | Cys | Ser | Asn | Glu | Glu | Cys |      |
|     |     |     | 370 |     |     |     |     |     |     |     |     |     |     |     |     | 380  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| cca | ggg | gag | tgc | ctt | gtc | aca | ggt | caa | tca | cac | ttc | aag | agc | ttt | gac | 1200 |
| Pro | Gly | Glu | Cys | Leu | Val | Thr | Gly | Gln | Ser | His | Phe | Lys | Ser | Phe | Asp |      |
|     |     |     | 385 |     |     |     |     |     |     |     |     |     |     |     |     | 400  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| aac | aga | tac | ttc | acc | ttc | agt | ggg | atc | tgc | cag | tac | ctg | ctg | gcc | cgg | 1248 |
| Asn | Arg | Tyr | Phe | Thr | Phe | Ser | Gly | Ile | Cys | Gln | Tyr | Leu | Leu | Ala | Arg |      |
|     |     |     | 405 |     |     |     |     |     |     |     |     |     |     |     |     | 415  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| gat | tgc | cag | gac | cac | tcc | ttc | tcc | att | gtc | att | gag | act | gtc | cag | tgt | 1296 |
| Asp | Cys | Gln | Asp | His | Ser | Phe | Ser | Ile | Val | Ile | Glu | Thr | Val | Gln | Cys |      |
|     |     |     | 420 |     |     |     |     |     |     |     |     |     |     |     |     | 430  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| gct | gat | gac | cgc | gac | gct | gtg | tgc | acc | cgc | tcc | gtc | acc | gtc | cgg | ctg | 1344 |
| Ala | Asp | Asp | Arg | Asp | Ala | Val | Cys | Thr | Arg | Ser | Val | Thr | Val | Arg | Leu |      |
|     |     |     | 435 |     |     |     |     |     |     |     |     |     |     |     |     | 445  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| cct | ggc | ctg | cac | aac | agc | ctt | gtg | aaa | ctg | aag | cat | ggg | gca | gga | gtt | 1392 |
| Pro | Gly | Leu | His | Asn | Ser | Leu | Val | Lys | Leu | Lys | His | Gly | Ala | Gly | Val |      |
|     |     |     | 450 |     |     |     |     |     |     |     |     |     |     |     |     | 460  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| gcc | atg | gat | ggc | cag | gac | gtc | cag | ctc | ccc | ctc | ctg | aaa | ggt | gac | ctc | 1440 |

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Ala Met Asp Gly Gln Asp Val Gln Leu Pro Leu Leu Lys Gly Asp Leu<br>465 470 475 480                                                                    |      |
| cgc atc cag cat aca gtg acg gcc tcc gtg cgc ctc agc tac ggg gag<br>Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu<br>485 490 495     | 1488 |
| gac ctg cag atg gac tgg gat ggc cgc ggg agg ctg ctg gtg aag ctg<br>Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu<br>500 505 510     | 1536 |
| tcc ccc gtc tat gcc ggg aag acc tgc ggc ctg tgt ggg aat tac aat<br>Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn<br>515 520 525     | 1584 |
| ggc aac cag ggc gac gac ttc ctt acc ccc tct ggg ctg gcg gag ccc<br>Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro<br>530 535 540     | 1632 |
| cgg gtg gag gac ttc ggg aac gcc tgg aag ctg cac ggg gac tgc cag<br>Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln<br>545 550 555 560 | 1680 |
| gac ctg cag aag cag cac agc gat ccc tgc gcc ctc aac ccg cgc atg                                                                                       | 1728 |
| Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met<br>565 570 575                                                                        |      |
| acc agg ttc tcc gag gag gcg tgc gcg gtc ctg acg tcc ccc aca ttc<br>Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe<br>580 585 590     | 1776 |
| gag gcc tgc cat cgt gcc gtc agc ccg ctg ccc tac ctg cgg aac tgc<br>Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys<br>595 600 605     | 1824 |
| cgc tac gac gtg tgc tcc tgc tgc gac ggc cgc gag tgc ctg tgc ggc<br>Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly<br>610 615 620     | 1872 |
| gcc ctg gcc agc tat gcc gcg gcc tgc gcg ggg aga ggc gtg cgc gtc<br>Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val<br>625 630 635 640 | 1920 |
| gcg tgg cgc gag cca ggc cgc tgt gag ctg aac tgc ccg aaa ggc cag<br>Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln<br>645 650 655     | 1968 |
| gtg tac ctg cag tgc ggg acc ccc tgc aac ctg acc tgc cgc tct ctc<br>Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu<br>660 665 670     | 2016 |
| tct tac ccg gat gag gaa tgc aat gag gcc tgc ctg gag ggc tgc ttc<br>Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe<br>675 680 685     | 2064 |
| tgc ccc cca ggg ctc tac atg gat gag agg ggg gac tgc gtg ccc aag<br>Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys<br>690 695 700     | 2112 |
| gcc cag tgc ccc tgt tac tat gac ggt gag atc ttc cag cca gaa gac<br>Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp<br>705 710 715 720 | 2160 |
| atc ttc tca gac cat cac acc atg tgc tac tgt gag gat ggc ttc atg<br>Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met<br>725 730 735     | 2208 |
| cac tgt acc atg agt gga gtc ccc gga agc ttg ctg cct gac gct gtc<br>His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val<br>740 745 750     | 2256 |
| ctc agc agt ccc ctg tct cat cgc agc aaa agg agc cta tcc tgt cgg<br>Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg<br>755 760 765     | 2304 |

|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| ccc ccc atg gtc aag ctg gtg tgt ccc gct gac aac ctg cgg gct gaa<br>Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu<br>770 775 780     | 2352 |
| ggg ctc gag tgt acc aaa acg tgc cag aac tat gac ctg gag tgc atg<br>Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met<br>785 790 795 800 | 2400 |
| agc atg ggc tgt gtc tct ggc tgc ctc tgc ccc ccg ggc atg gtc cgg<br>Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg<br>805 810 815     | 2448 |
| cat gag aac aga tgt gtg gcc ctg gaa agg tgt ccc tgc ttc cat cag<br>His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln<br>820 825 830     | 2496 |
| ggc aag gag tat gcc cct gga gaa aca gtg aag att ggc tgc aac act<br>Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr<br>835 840 845     | 2544 |
| tgt gtc tgt cgg gac cgg aag tgg aac tgc aca gac cat gtg tgt gat<br>Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp<br>850 855 860     | 2592 |
| gcc acg tgc tcc acg atc ggc atg gcc cac tac ctc acc ttc gac ggg<br>Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly<br>865 870 875 880 | 2640 |
| ctc aaa tac ctg ttc ccc ggg gag tgc cag tac gtt ctg gtg cag gat<br>Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp<br>885 890 895     | 2688 |
| tac tgc ggc agt aac cct ggg acc ttt cgg atc cta gtg ggg aat aag<br>Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys<br>900 905 910     | 2736 |
| gga tgc agc cac ccc tca gtg aaa tgc aag aaa cgg gtc acc atc ctg<br>Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu<br>915 920 925     | 2784 |
| gtg gag gga gga gag att gag ctg ttt gac ggg gag gtg aat gtg aag<br>Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys<br>930 935 940     | 2832 |
| agg ccc atg aag gat gag act cac ttt gag gtg gtg gag tct ggc cgg<br>Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg<br>945 950 955 960 | 2880 |
| tac atc att ctg ctg ctg ggc aaa gcc ctc tcc gtg gtc tgg gac cgc<br>Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg<br>965 970 975     | 2928 |
| cac ctg agc atc tcc gtg gtc ctg aag cag aca tac cag gag aaa gtg<br>His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val<br>980 985 990     | 2976 |
| tgt ggc ctg tgt ggg aat ttt gat ggc atc cag aac aat gac ctc acc<br>Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr<br>995 1000 1005   | 3024 |
| agc agc aac ctc caa gtg gag gaa gac cct gtg gac ttt ggg aac<br>Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val Asp Phe Gly Asn<br>1010 1015 1020          | 3069 |
| tcc tgg aaa gtg agc tcg cag tgt gct gac acc aga aaa gtg cct<br>Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg Lys Val Pro<br>1025 1030 1035          | 3114 |
| ctg gac tca tcc cct gcc acc tgc cat aac aac atc atg aag cag<br>Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met Lys Gln<br>1040 1045 1050          | 3159 |
| acg atg gtg gat tcc tcc tgt aga atc ctt acc agt gac gtc ttc<br>Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val Phe<br>1055 1060 1065          | 3204 |

|                                                                                                                                              |      |
|----------------------------------------------------------------------------------------------------------------------------------------------|------|
| cag gac tgc aac aag ctg gtg gac ccc gag cca tat ctg gat gtc<br>Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val<br>1070 1075 1080 | 3249 |
| tgc att tac gac acc tgc tcc tgt gag tcc att ggg gac tgc gcc<br>Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala<br>1085 1090 1095 | 3294 |
| tgc ttc tgc gac acc att gct gcc tat gcc cac gtg tgt gcc cag<br>Cys Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln<br>1100 1105 1110 | 3339 |
| cat ggc aag gtg gtg acc tgg agg acg gcc aca ttg tgc ccc cag<br>His Gly Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln<br>1115 1120 1125 | 3384 |
| agc tgc gag gag agg aat ctc cgg gag aac ggg tat gag tgt gag<br>Ser Cys Glu Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu<br>1130 1135 1140 | 3429 |
| tgg cgc tat aac agc tgt gca cct gcc tgt caa gtc acg tgt cag<br>Trp Arg Tyr Asn Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln<br>1145 1150 1155 | 3474 |
| cac cct gag cca ctg gcc tgc cct gtg cag tgt gtg gag ggc tgc<br>His Pro Glu Pro Leu Ala Cys Pro Val Gln Cys Val Glu Gly Cys<br>1160 1165 1170 | 3519 |
| cat gcc cac tgc cct cca ggg aaa atc ctg gat gag ctt ttg cag<br>His Ala His Cys Pro Pro Gly Lys Ile Leu Asp Glu Leu Leu Gln<br>1175 1180 1185 | 3564 |
| acc tgc gtt gac cct gaa gac tgt cca gtg tgt gag gtg gct gcc<br>Thr Cys Val Asp Pro Glu Asp Cys Pro Val Cys Glu Val Ala Gly<br>1190 1195 1200 | 3609 |
| cgg cgt ttt gcc tca gga aag aaa gtc acc ttg aat ccc agt gac<br>Arg Arg Phe Ala Ser Gly Lys Lys Val Thr Leu Asn Pro Ser Asp<br>1205 1210 1215 | 3654 |
| cct gag cac tgc cag att tgc cac tgt gat gtt gtc aac ctc acc<br>Pro Glu His Cys Gln Ile Cys His Cys Asp Val Val Asn Leu Thr<br>1220 1225 1230 | 3699 |
| tgt gaa gcc tgc cag gag ccg gga ggc ctg gtg gtg cct ccc aca<br>Cys Glu Ala Cys Gln Glu Pro Gly Gly Leu Val Val Pro Pro Thr<br>1235 1240 1245 | 3744 |
| gat gcc ccg gtg agc ccc acc act ctg tat gtg gag gac atc tcg<br>Asp Ala Pro Val Ser Pro Thr Thr Leu Tyr Val Glu Asp Ile Ser<br>1250 1255 1260 | 3789 |
| gaa ccg ccg ttg cac gat ttc tac tgc agc agg cta ctg gac ctg<br>Glu Pro Pro Leu His Asp Phe Tyr Cys Ser Arg Leu Leu Asp Leu<br>1265 1270 1275 | 3834 |
| gtc ttc ctg ctg gat ggc tcc tcc agg ctg tcc gag gct gag ttt<br>Val Phe Leu Leu Asp Gly Ser Ser Arg Leu Ser Glu Ala Glu Phe<br>1280 1285 1290 | 3879 |
| gaa gtg ctg aag gcc ttt gtg gtg gac atg atg gag cgg ctg cgc<br>Glu Val Leu Lys Ala Phe Val Val Asp Met Met Glu Arg Leu Arg<br>1295 1300 1305 | 3924 |
| atc tcc cag aag tgg gtc cgc gtg gcc gtg gtg gag tac cac gac<br>Ile Ser Gln Lys Trp Val Arg Val Ala Val Val Glu Tyr His Asp<br>1310 1315 1320 | 3969 |
| ggc tcc cac gcc tac atc ggg ctc aag gac cgg aag cga ccg tca<br>Gly Ser His Ala Tyr Ile Gly Leu Lys Asp Arg Lys Arg Pro Ser<br>1325 1330 1335 | 4014 |
| gag ctg cgg cgc att gcc agc cag gtg aag tat gcg ggc agc cag<br>Glu Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly Ser Gln<br>1340 1345 1350 | 4059 |

|                             |                     |             |      |
|-----------------------------|---------------------|-------------|------|
| 1340                        | 1345                | 1350        |      |
| gtg gcc tcc acc agc gag gtc | ttg aaa tac aca ctg | ttc caa atc | 4104 |
| Val Ala Ser Thr Ser Glu Val | Leu Lys Tyr Thr Leu | Phe Gln Ile |      |
| 1355                        | 1360                | 1365        |      |
| ttc agc aag atc gac cgc cct | gaa gcc tcc cgc atc | gcc ctg ctc | 4149 |
| Phe Ser Lys Ile Asp Arg Pro | Glu Ala Ser Arg Ile | Ala Leu Leu |      |
| 1370                        | 1375                | 1380        |      |
| ctg atg gcc agc cag gag ccc | caa cgg atg tcc cgg | aac ttt gtc | 4194 |
| Leu Met Ala Ser Gln Glu Pro | Gln Arg Met Ser Arg | Asn Phe Val |      |
| 1385                        | 1390                | 1395        |      |
| cgc tac gtc cag ggc ctg aag | aag aag aag gtc att | gtg atc ccg | 4239 |
| Arg Tyr Val Gln Gly Leu Lys | Lys Lys Lys Val Ile | Val Ile Pro |      |
| 1400                        | 1405                | 1410        |      |
| gtg ggc att ggg ccc cat gcc | aac ctc aag cag atc | cgc ctc atc | 4284 |
| Val Gly Ile Gly Pro His Ala | Asn Leu Lys Gln Ile | Arg Leu Ile |      |
| 1415                        | 1420                | 1425        |      |
| gag aag cag gcc cct gag aac | aag gcc ttc gtg ctg | agc agt gtg | 4329 |
| Glu Lys Gln Ala Pro Glu Asn | Lys Ala Phe Val Leu | Ser Ser Val |      |
| 1430                        | 1435                | 1440        |      |
| gat gag ctg gag cag caa agg | gac gag atc gtt agc | tac ctc tgt | 4374 |
| Asp Glu Leu Glu Gln Gln Arg | Asp Glu Ile Val Ser | Tyr Leu Cys |      |
| 1445                        | 1450                | 1455        |      |
| gac ctt gcc cct gaa gcc cct | cct cct act ctg ccc | ccc cac atg | 4419 |
| Asp Leu Ala Pro Glu Ala Pro | Pro Pro Thr Leu Pro | Pro His Met |      |
| 1460                        | 1465                | 1470        |      |
| gca caa gtc act gtg ggc ccg | ggg ctc ttg ggg gtt | tcg acc ctg | 4464 |
| Ala Gln Val Thr Val Gly Pro | Gly Leu Leu Gly Val | Ser Thr Leu |      |
| 1475                        | 1480                | 1485        |      |
| ggg ccc aag agg aac tcc atg | gtt ctg gat gtg gcg | ttc gtc ctg | 4509 |
| Gly Pro Lys Arg Asn Ser Met | Val Leu Asp Val Ala | Phe Val Leu |      |
| 1490                        | 1495                | 1500        |      |
| gaa gga tcg gac aaa att ggt | gaa gcc gac ttc aac | agg agc aag | 4554 |
| Glu Gly Ser Asp Lys Ile Gly | Glu Ala Asp Phe Asn | Arg Ser Lys |      |
| 1505                        | 1510                | 1515        |      |
| gag ttc atg gag gag gtg att | cag cgg atg gat gtg | ggc cag gac | 4599 |
| Glu Phe Met Glu Glu Val Ile | Gln Arg Met Asp Val | Gly Gln Asp |      |
| 1520                        | 1525                | 1530        |      |
| agc atc cac gtc acg gtg ctg | cag tac tcc tac atg | gtg acc gtg | 4644 |
|                             |                     |             |      |
| Ser Ile His Val Thr Val Leu | Gln Tyr Ser Tyr Met | Val Thr Val |      |
| 1535                        | 1540                | 1545        |      |
| gag tac ccc ttc agc gag gca | cag tcc aaa ggg gac | atc ctg cag | 4689 |
| Glu Tyr Pro Phe Ser Glu Ala | Gln Ser Lys Gly Asp | Ile Leu Gln |      |
| 1550                        | 1555                | 1560        |      |
| cgg gtg cga gag atc cgc tac | cag ggc ggc aac agg | acc aac act | 4734 |
| Arg Val Arg Glu Ile Arg Tyr | Gln Gly Gly Asn Arg | Thr Asn Thr |      |
| 1565                        | 1570                | 1575        |      |
| ggg ctg gcc ctg cgg tac ctc | tct gac cac agc ttc | ttg gtc agc | 4779 |
| Gly Leu Ala Leu Arg Tyr Leu | Ser Asp His Ser Phe | Leu Val Ser |      |
| 1580                        | 1585                | 1590        |      |
| cag ggt gac cgg gag cag gcg | ccc aac ctg gtc tac | atg gtc acc | 4824 |
| Gln Gly Asp Arg Glu Gln Ala | Pro Asn Leu Val Tyr | Met Val Thr |      |
| 1595                        | 1600                | 1605        |      |
| gga aat cct gcc tct gat gag | atc aag agg ctg cct | gga gac atc | 4869 |
| Gly Asn Pro Ala Ser Asp Glu | Ile Lys Arg Leu Pro | Gly Asp Ile |      |
| 1610                        | 1615                | 1620        |      |
| cag gtg gtg ccc att gga gtg | ggc cct aat gcc aac | gtg cag gag | 4914 |
| Glu Val Val Pro Ile Glu Val | Glu Pro Asn Ala Asn | Val Gln Glu |      |

|      |     |     |     |     |     |      |     |     |     |     |      |      |     |     |  |      |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|------|-----|-----|--|------|
| Gln  | Val | Val | Pro | Ile | Gly | Val  | Gly | Pro | Asn | Ala | Asn  | Val  | Gln | Glu |  |      |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     |      | 1635 |     |     |  |      |
| ctg  | gag | agg | att | ggc | tgg | ccc  | aat | gcc | cct | atc | ctc  | atc  | cag | gac |  | 4959 |
| Leu  | Glu | Arg | Ile | Gly | Trp | Pro  | Asn | Ala | Pro | Ile | Leu  | Ile  | Gln | Asp |  |      |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |      |     |     |  |      |
| ttt  | gag | acg | ctc | ccc | cga | gag  | gct | cct | gac | ctg | gtg  | ctg  | cag | agg |  | 5004 |
| Phe  | Glu | Thr | Leu | Pro | Arg | Glu  | Ala | Pro | Asp | Leu | Val  | Leu  | Gln | Arg |  |      |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |      |     |     |  |      |
| tgc  | tgc | tcc | gga | gag | ggg | ctg  | cag | atc | ccc | acc | ctc  | tcc  | cct | gca |  | 5049 |
| Cys  | Cys | Ser | Gly | Glu | Gly | Leu  | Gln | Ile | Pro | Thr | Leu  | Ser  | Pro | Ala |  |      |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |      |     |     |  |      |
| cct  | gac | tgc | agc | cag | ccc | ctg  | gac | gtg | atc | ctt | ctc  | ctg  | gat | ggc |  | 5094 |
| Pro  | Asp | Cys | Ser | Gln | Pro | Leu  | Asp | Val | Ile | Leu | Leu  | Leu  | Asp | Gly |  |      |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |      |     |     |  |      |
| tcc  | tcc | agt | ttc | cca | gct | tct  | tat | ttt | gat | gaa | atg  | aag  | agt | ttc |  | 5139 |
| Ser  | Ser | Ser | Phe | Pro | Ala | Ser  | Tyr | Phe | Asp | Glu | Met  | Lys  | Ser | Phe |  |      |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |      |     |     |  |      |
| gcc  | aag | gct | ttc | att | tca | aaa  | gcc | aat | ata | ggg | cct  | cgt  | ctc | act |  | 5184 |
| Ala  | Lys | Ala | Phe | Ile | Ser | Lys  | Ala | Asn | Ile | Gly | Pro  | Arg  | Leu | Thr |  |      |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |      |     |     |  |      |
| cag  | gtg | tca | gtg | ctg | cag | tat  | gga | agc | atc | acc | acc  | att  | gac | gtg |  | 5229 |
| Gln  | Val | Ser | Val | Leu | Gln | Tyr  | Gly | Ser | Ile | Thr | Thr  | Ile  | Asp | Val |  |      |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |      |     |     |  |      |
| cca  | tgg | aac | gtg | gtc | ccg | gag  | aaa | gcc | cat | ttg | ctg  | agc  | ctt | gtg |  | 5274 |
| Pro  | Trp | Asn | Val | Val | Pro | Glu  | Lys | Ala | His | Leu | Leu  | Ser  | Leu | Val |  |      |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |      |     |     |  |      |
| gac  | gtc | atg | cag | cgg | gag | gga  | ggc | ccc | agc | caa | atc  | ggg  | gat | gcc |  | 5319 |
| Asp  | Val | Met | Gln | Arg | Glu | Gly  | Gly | Pro | Ser | Gln | Ile  | Gly  | Asp | Ala |  |      |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |      |     |     |  |      |
| ttg  | ggc | ttt | gct | gtg | cga | tac  | ttg | act | tca | gaa | atg  | cat  | ggg | gcg |  | 5364 |
| Leu  | Gly | Phe | Ala | Val | Arg | Tyr  | Leu | Thr | Ser | Glu | Met  | His  | Gly | Ala |  |      |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |      |     |     |  |      |
| cgc  | ccg | gga | gcc | tca | aag | gcg  | gtg | gtc | atc | ctg | gtc  | acg  | gac | gtc |  | 5409 |
| Arg  | Pro | Gly | Ala | Ser | Lys | Ala  | Val | Val | Ile | Leu | Val  | Thr  | Asp | Val |  |      |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |      |     |     |  |      |
| tct  | gtg | gat | tca | gtg | gat | gca  | gca | gct | gat | gcc | gcc  | agg  | tcc | aac |  | 5454 |
| Ser  | Val | Asp | Ser | Val | Asp | Ala  | Ala | Ala | Asp | Ala | Ala  | Arg  | Ser | Asn |  |      |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |      |     |     |  |      |
| aga  | gtg | aca | gtg | ttc | cct | att  | gga | att | gga | gat | cgc  | tac  | gat | gca |  | 5499 |
| Arg  | Val | Thr | Val | Phe | Pro | Ile  | Gly | Ile | Gly | Asp | Arg  | Tyr  | Asp | Ala |  |      |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |      |     |     |  |      |
| gcc  | cag | cta | cgg | atc | ttg | gca  | ggc | cca | gca | ggc | gac  | tcc  | aac | gtg |  | 5544 |
| Ala  | Gln | Leu | Arg | Ile | Leu | Ala  | Gly | Pro | Ala | Gly | Asp  | Ser  | Asn | Val |  |      |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |      |     |     |  |      |
| gtg  | aag | ctc | cag | cga | atc | gaa  | gac | ctc | cct | acc | atg  | gtc  | acc | ttg |  | 5589 |
| Val  | Lys | Leu | Gln | Arg | Ile | Glu  | Asp | Leu | Pro | Thr | Met  | Val  | Thr | Leu |  |      |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |      |     |     |  |      |
| ggc  | aat | tcc | ttc | ctc | cac | aaa  | ctg | tgc | tct | gga | ttt  | gtt  | agg | att |  | 5634 |
| Gly  | Asn | Ser | Phe | Leu | His | Lys  | Leu | Cys | Ser | Gly | Phe  | Val  | Arg | Ile |  |      |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |      |     |     |  |      |
| tgc  | atg | gat | gag | gat | ggg | aat  | gag | aag | agg | ccc | ggg  | gac  | gtc | tgg |  | 5679 |
| Cys  | Met | Asp | Glu | Asp | Gly | Asn  | Glu | Lys | Arg | Pro | Gly  | Asp  | Val | Trp |  |      |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |      |     |     |  |      |
| acc  | ttg | cca | gac | cag | tgc | cac  | acc | gtg | act | tgc | cag  | cca  | gat | ggc |  | 5724 |
| Thr  | Leu | Pro | Asp | Gln | Cys | His  | Thr | Val | Thr | Cys | Gln  | Pro  | Asp | Gly |  |      |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |      |     |     |  |      |
| cag  | acc | ttg | ctg | aag | agt | cat  | cgg | gtc | aac | tgt | gac  | cgg  | ggg | ctg |  | 5769 |
| Gln  | Thr | Leu | Leu | Leu | Ser | His  | Arg | Val | Asn | Cys | Asn  | Arg  | Gln | Leu |  |      |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |      |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|------|
| Gln  | Thr | Leu | Leu | Lys | Ser | His  | Arg | Val | Asn | Cys | Asp  | Arg | Gly | Leu |      |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |      |
| agg  | cct | tcg | tgc | cct | aac | agc  | cag | tcc | cct | gtt | aaa  | gtg | gaa | gag | 5814 |
| Arg  | Pro | Ser | Cys | Pro | Asn | Ser  | Gln | Ser | Pro | Val | Lys  | Val | Glu | Glu |      |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |      |
| acc  | tgt | ggc | tgc | cgc | tgg | acc  | tgc | ccc | tgc | gtg | tgc  | aca | ggc | agc | 5859 |
| Thr  | Cys | Gly | Cys | Arg | Trp | Thr  | Cys | Pro | Cys | Val | Cys  | Thr | Gly | Ser |      |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     | 1950 |     |     |     |      |
| tcc  | act | cgg | cac | atc | gtg | acc  | ttt | gat | ggg | cag | aat  | ttc | aag | ctg | 5904 |
| Ser  | Thr | Arg | His | Ile | Val | Thr  | Phe | Asp | Gly | Gln | Asn  | Phe | Lys | Leu |      |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     | 1965 |     |     |     |      |
| act  | ggc | agc | tgt | tct | tat | gtc  | cta | ttt | caa | aac | aag  | gag | cag | gac | 5949 |
| Thr  | Gly | Ser | Cys | Ser | Tyr | Val  | Leu | Phe | Gln | Asn | Lys  | Glu | Gln | Asp |      |
| 1970 |     |     |     |     |     | 1975 |     |     |     |     | 1980 |     |     |     |      |
| ctg  | gag | gtg | att | ctc | cat | aat  | ggt | gcc | tgc | agc | cct  | gga | gca | agg | 5994 |
| Leu  | Glu | Val | Ile | Leu | His | Asn  | Gly | Ala | Cys | Ser | Pro  | Gly | Ala | Arg |      |
| 1985 |     |     |     |     |     | 1990 |     |     |     |     | 1995 |     |     |     |      |
| cag  | ggc | tgc | atg | aaa | tcc | atc  | gag | gtg | aag | cac | agt  | gcc | ctc | tcc | 6039 |
| Gln  | Gly | Cys | Met | Lys | Ser | Ile  | Glu | Val | Lys | His | Ser  | Ala | Leu | Ser |      |
| 2000 |     |     |     |     |     | 2005 |     |     |     |     | 2010 |     |     |     |      |
| gtc  | gag | ctg | cac | agt | gac | atg  | gag | gtg | acg | gtg | aat  | ggg | aga | ctg | 6084 |
| Val  | Glu | Leu | His | Ser | Asp | Met  | Glu | Val | Thr | Val | Asn  | Gly | Arg | Leu |      |
| 2015 |     |     |     |     |     | 2020 |     |     |     |     | 2025 |     |     |     |      |
| gtc  | tct | gtt | cct | tac | gtg | ggt  | ggg | aac | atg | gaa | gtc  | aac | gtt | tat | 6129 |
| Val  | Ser | Val | Pro | Tyr | Val | Gly  | Gly | Asn | Met | Glu | Val  | Asn | Val | Tyr |      |
| 2030 |     |     |     |     |     | 2035 |     |     |     |     | 2040 |     |     |     |      |
| ggt  | gcc | atc | atg | cat | gag | gtc  | aga | ttc | aat | cac | ctt  | ggt | cac | atc | 6174 |
| Gly  | Ala | Ile | Met | His | Glu | Val  | Arg | Phe | Asn | His | Leu  | Gly | His | Ile |      |
| 2045 |     |     |     |     |     | 2050 |     |     |     |     | 2055 |     |     |     |      |
| ttc  | aca | ttc | act | cca | caa | aac  | aat | gag | ttc | caa | ctg  | cag | ctc | agc | 6219 |
| Phe  | Thr | Phe | Thr | Pro | Gln | Asn  | Asn | Glu | Phe | Gln | Leu  | Gln | Leu | Ser |      |
| 2060 |     |     |     |     |     | 2065 |     |     |     |     | 2070 |     |     |     |      |
| ccc  | aag | act | ttt | gct | tca | aag  | acg | tat | ggt | ctg | tgt  | ggg | atc | tgt | 6264 |
| Pro  | Lys | Thr | Phe | Ala | Ser | Lys  | Thr | Tyr | Gly | Leu | Cys  | Gly | Ile | Cys |      |
| 2075 |     |     |     |     |     | 2080 |     |     |     |     | 2085 |     |     |     |      |
| gat  | gag | aac | gga | gcc | aat | gac  | ttc | atg | ctg | agg | gat  | ggc | aca | gtc | 6309 |
| Asp  | Glu | Asn | Gly | Ala | Asn | Asp  | Phe | Met | Leu | Arg | Asp  | Gly | Thr | Val |      |
| 2090 |     |     |     |     |     | 2095 |     |     |     |     | 2100 |     |     |     |      |
| acc  | aca | gac | tgg | aaa | aca | ctt  | gtt | cag | gaa | tgg | act  | gtg | cag | cgg | 6354 |
| Thr  | Thr | Asp | Trp | Lys | Thr | Leu  | Val | Gln | Glu | Trp | Thr  | Val | Gln | Arg |      |
| 2105 |     |     |     |     |     | 2110 |     |     |     |     | 2115 |     |     |     |      |
| cca  | ggg | cag | acg | tgc | cag | ccc  | atc | ctg | gag | gag | cag  | tgt | ctt | gtc | 6399 |
| Pro  | Gly | Gln | Thr | Cys | Gln | Pro  | Ile | Leu | Glu | Glu | Gln  | Cys | Leu | Val |      |
| 2120 |     |     |     |     |     | 2125 |     |     |     |     | 2130 |     |     |     |      |
| ccc  | gac | agc | tcc | cac | tgc | cag  | gtc | ctc | ctc | tta | cca  | ctg | ttt | gct | 6444 |
| Pro  | Asp | Ser | Ser | His | Cys | Gln  | Val | Leu | Leu | Leu | Pro  | Leu | Phe | Ala |      |
| 2135 |     |     |     |     |     | 2140 |     |     |     |     | 2145 |     |     |     |      |
| gaa  | tgc | cac | aag | gtc | ctg | gct  | cca | gcc | aca | ttc | tat  | gcc | atc | tgc | 6489 |
| Glu  | Cys | His | Lys | Val | Leu | Ala  | Pro | Ala | Thr | Phe | Tyr  | Ala | Ile | Cys |      |
| 2150 |     |     |     |     |     | 2155 |     |     |     |     | 2160 |     |     |     |      |
| cag  | cag | gac | agt | tgc | cac | cag  | gag | caa | gtg | tgt | gag  | gtg | atc | gcc | 6534 |
| Gln  | Gln | Asp | Ser | Cys | His | Gln  | Glu | Gln | Val | Cys | Glu  | Val | Ile | Ala |      |
| 2165 |     |     |     |     |     | 2170 |     |     |     |     | 2175 |     |     |     |      |
| tct  | tat | gcc | cac | ctc | tgt | cgg  | acc | aac | ggg | gtc | tgc  | gtt | gac | tgg | 6579 |
| Ser  | Tyr | Ala | His | Leu | Cys | Arg  | Thr | Asn | Gly | Val | Cys  | Val | Asp | Trp |      |
| 2180 |     |     |     |     |     | 2185 |     |     |     |     | 2190 |     |     |     |      |
| agg  | aca | cct | gat | ttc | tgt | gct  | atg | tca | tgc | cca | cca  | tct | ctg | gtt | 6624 |
| 2195 |     |     |     |     |     | 2200 |     |     |     |     | 2205 |     |     |     |      |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |      |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|------|
| Arg  | Trp | Pro | Asp | Phe | Cys | Ala  | Met | Ser | Cys | Pro | Pro  | Ser | Leu | Val |      |
| 2195 |     |     |     |     |     | 2200 |     |     |     |     | 2205 |     |     |     |      |
| tat  | aac | cac | tgt | gag | cat | ggc  | tgt | ccc | cgg | cac | tgt  | gat | ggc | aac | 6669 |
| Tyr  | Asn | His | Cys | Glu | His | Gly  | Cys | Pro | Arg | His | Cys  | Asp | Gly | Asn |      |
| 2210 |     |     |     |     |     | 2215 |     |     |     |     | 2220 |     |     |     |      |
| gtg  | agc | tcc | tgt | ggg | gac | cat  | ccc | tcc | gaa | ggc | tgt  | ttc | tgc | cct | 6714 |
| Val  | Ser | Ser | Cys | Gly | Asp | His  | Pro | Ser | Glu | Gly | Cys  | Phe | Cys | Pro |      |
| 2225 |     |     |     |     |     | 2230 |     |     |     |     | 2235 |     |     |     |      |
| cca  | gat | aaa | gtc | atg | ttg | gaa  | ggc | agc | tgt | gtc | cct  | gaa | gag | gcc | 6759 |
| Pro  | Asp | Lys | Val | Met | Leu | Glu  | Gly | Ser | Cys | Val | Pro  | Glu | Glu | Ala |      |
| 2240 |     |     |     |     |     | 2245 |     |     |     |     | 2250 |     |     |     |      |
| tgc  | act | cag | tgc | att | ggt | gag  | gat | gga | gtc | cag | cac  | cag | ttc | ctg | 6804 |
| Cys  | Thr | Gln | Cys | Ile | Gly | Glu  | Asp | Gly | Val | Gln | His  | Gln | Phe | Leu |      |
| 2255 |     |     |     |     |     | 2260 |     |     |     |     | 2265 |     |     |     |      |
| gaa  | gcc | tgg | gtc | ccg | gac | cac  | cag | ccc | tgt | cag | atc  | tgc | aca | tgc | 6849 |
| Glu  | Ala | Trp | Val | Pro | Asp | His  | Gln | Pro | Cys | Gln | Ile  | Cys | Thr | Cys |      |
| 2270 |     |     |     |     |     | 2275 |     |     |     |     | 2280 |     |     |     |      |
| ctc  | agc | ggg | cgg | aag | gtc | aac  | tgc | aca | acg | cag | ccc  | tgc | ccc | acg | 6894 |
| Leu  | Ser | Gly | Arg | Lys | Val | Asn  | Cys | Thr | Thr | Gln | Pro  | Cys | Pro | Thr |      |
| 2285 |     |     |     |     |     | 2290 |     |     |     |     | 2295 |     |     |     |      |
| gcc  | aaa | gct | ccc | acg | tgt | ggc  | ctg | tgt | gaa | gta | gcc  | cgc | ctc | cgc | 6939 |
| Ala  | Lys | Ala | Pro | Thr | Cys | Gly  | Leu | Cys | Glu | Val | Ala  | Arg | Leu | Arg |      |
| 2300 |     |     |     |     |     | 2305 |     |     |     |     | 2310 |     |     |     |      |
| cag  | aat | gca | gac | cag | tgc | tgc  | ccc | gag | tat | gag | tgt  | gtg | tgt | gac | 6984 |
| Gln  | Asn | Ala | Asp | Gln | Cys | Cys  | Pro | Glu | Tyr | Glu | Cys  | Val | Cys | Asp |      |
| 2315 |     |     |     |     |     | 2320 |     |     |     |     | 2325 |     |     |     |      |
| cca  | gtg | agc | tgt | gac | ctg | ccc  | cca | gtg | cct | cac | tgt  | gaa | cgt | ggc | 7029 |
| Pro  | Val | Ser | Cys | Asp | Leu | Pro  | Pro | Val | Pro | His | Cys  | Glu | Arg | Gly |      |
| 2330 |     |     |     |     |     | 2335 |     |     |     |     | 2340 |     |     |     |      |
| ctc  | cag | ccc | aca | ctg | acc | aac  | cct | ggc | gag | tgc | aga  | ccc | aac | ttc | 7074 |
| Leu  | Gln | Pro | Thr | Leu | Thr | Asn  | Pro | Gly | Glu | Cys | Arg  | Pro | Asn | Phe |      |
| 2345 |     |     |     |     |     | 2350 |     |     |     |     | 2355 |     |     |     |      |
| acc  | tgc | gcc | tgc | agg | aag | gag  | gag | tgc | aaa | aga | gtg  | tcc | cca | ccc | 7119 |
| Thr  | Cys | Ala | Cys | Arg | Lys | Glu  | Glu | Cys | Lys | Arg | Val  | Ser | Pro | Pro |      |
| 2360 |     |     |     |     |     | 2365 |     |     |     |     | 2370 |     |     |     |      |
| tcc  | tgc | ccc | ccg | cac | cgt | ttg  | ccc | acc | ctt | cgg | aag  | acc | cag | tgc | 7164 |
| Ser  | Cys | Pro | Pro | His | Arg | Leu  | Pro | Thr | Leu | Arg | Lys  | Thr | Gln | Cys |      |
| 2375 |     |     |     |     |     | 2380 |     |     |     |     | 2385 |     |     |     |      |
| tgt  | gat | gag | tat | gag | tgt | gcc  | tgc | aac | tgt | gtc | aac  | tcc | aca | gtg | 7209 |
| Cys  | Asp | Glu | Tyr | Glu | Cys | Ala  | Cys | Asn | Cys | Val | Asn  | Ser | Thr | Val |      |
| 2390 |     |     |     |     |     | 2395 |     |     |     |     | 2400 |     |     |     |      |
| agc  | tgt | ccc | ctt | ggg | tac | ttg  | gcc | tca | acc | gcc | acc  | aat | gac | tgt | 7254 |
| Ser  | Cys | Pro | Leu | Gly | Tyr | Leu  | Ala | Ser | Thr | Ala | Thr  | Asn | Asp | Cys |      |
| 2405 |     |     |     |     |     | 2410 |     |     |     |     | 2415 |     |     |     |      |
| ggc  | tgt | acc | aca | acc | acc | tgc  | ctt | ccc | gac | aag | gtg  | tgt | gtc | cac | 7299 |
| Gly  | Cys | Thr | Thr | Thr | Thr | Cys  | Leu | Pro | Asp | Lys | Val  | Cys | Val | His |      |
| 2420 |     |     |     |     |     | 2425 |     |     |     |     | 2430 |     |     |     |      |
| cga  | agc | acc | atc | tac | cct | gtg  | ggc | cag | ttc | tgg | gag  | gag | ggc | tgc | 7344 |
| Arg  | Ser | Thr | Ile | Tyr | Pro | Val  | Gly | Gln | Phe | Trp | Glu  | Glu | Gly | Cys |      |
| 2435 |     |     |     |     |     | 2440 |     |     |     |     | 2445 |     |     |     |      |
| gat  | gtg | tgc | acc | tgc | acc | gac  | atg | gag | gat | gcc | gtg  | atg | ggc | ctc | 7389 |
| Asp  | Val | Cys | Thr | Cys | Thr | Asp  | Met | Glu | Asp | Ala | Val  | Met | Gly | Leu |      |
| 2450 |     |     |     |     |     | 2455 |     |     |     |     | 2460 |     |     |     |      |
| cgc  | gtg | gcc | cag | tgc | tcc | cag  | aag | ccc | tgt | gag | gac  | agc | tgt | cgg | 7434 |
| Arg  | Val | Ala | Gln | Cys | Ser | Gln  | Lys | Pro | Cys | Glu | Asp  | Ser | Cys | Arg |      |
| 2465 |     |     |     |     |     | 2470 |     |     |     |     | 2475 |     |     |     |      |
| tca  | ggc | ttc | act | tac | gtt | ctg  | cat | gaa | ggc | gag | tgc  | tgt | gga | agg | 7479 |

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Ser Gly Phe Thr Tyr Val Leu His Glu Gly Glu Cys Cys Gly Arg
2480 2485 2490

tgc ctg cca tct gcc tgt gag gtg gtg act ggc tca ccg cgg ggg 7524
Cys Leu Pro Ser Ala Cys Glu Val Val Thr Gly Ser Pro Arg Gly
2495 2500 2505

gac tcc cag tct tcc tgg aag agt gtc ggc tcc cag tgg gcc tcc 7569
Asp Ser Gln Ser Ser Trp Lys Ser Val Gly Ser Gln Trp Ala Ser
2510 2515 2520

ccg gag aac ccc tgc ctc atc aat gag tgt gtc cga gtg aag gag 7614
Pro Glu Asn Pro Cys Leu Ile Asn Glu Cys Val Arg Val Lys Glu
2525 2530 2535

gag gtc ttt ata caa caa agg aac gtc tcc tgc ccc cag ctg gag 7659
Glu Val Phe Ile Gln Gln Arg Asn Val Ser Cys Pro Gln Leu Glu
2540 2545 2550

gtc cct gtc tgc ccc tgc ggc ttt cag ctg agc tgt aag acc tca 7704
Val Pro Val Cys Pro Ser Gly Phe Gln Leu Ser Cys Lys Thr Ser
2555 2560 2565

gcg tgc tgc cca agc tgt cgc tgt gag cgc atg gag gcc tgc atg 7749
Ala Cys Cys Pro Ser Cys Arg Cys Glu Arg Met Glu Ala Cys Met
2570 2575 2580

ctc aat ggc act gtc att ggg ccc ggg aag act gtg atg atc gat 7794
Leu Asn Gly Thr Val Ile Gly Pro Gly Lys Thr Val Met Ile Asp
2585 2590 2595

gtg tgc acg acc tgc cgc tgc atg gtg cag gtg ggg gtc atc tct 7839
Val Cys Thr Thr Cys Arg Cys Met Val Gln Val Gly Val Ile Ser
2600 2605 2610

gga ttc aag ctg gag tgc agg aag acc acc tgc aac ccc tgc ccc 7884
Gly Phe Lys Leu Glu Cys Arg Lys Thr Thr Cys Asn Pro Cys Pro
2615 2620 2625

ctg ggt tac aag gaa gaa aat aac aca ggt gaa tgt tgt ggg aga 7929
Leu Gly Tyr Lys Glu Glu Asn Asn Thr Gly Glu Cys Cys Gly Arg
2630 2635 2640

tgt ttg cct acg gct tgc acc att cag cta aga gga gga cag atc 7974
Cys Leu Pro Thr Ala Cys Thr Ile Gln Leu Arg Gly Gly Gln Ile
2645 2650 2655

atg aca ctg aag cgt gat gag acg ctc cag gat ggc tgt gat act 8019
Met Thr Leu Lys Arg Asp Glu Thr Leu Gln Asp Gly Cys Asp Thr
2660 2665 2670

cac ttc tgc aag gtc aat gag aga gga gag tac ttc tgg gag aag 8064
His Phe Cys Lys Val Asn Glu Arg Gly Glu Tyr Phe Trp Glu Lys
2675 2680 2685

agg gtc aca ggc tgc cca ccc ttt gat gaa cac aag tgt ctg gct 8109
Arg Val Thr Gly Cys Pro Pro Phe Asp Glu His Lys Cys Leu Ala
2690 2695 2700

gag gga ggt aaa att atg aaa att cca ggc acc tgc tgt gac aca 8154
Glu Gly Gly Lys Ile Met Lys Ile Pro Gly Thr Cys Cys Asp Thr
2705 2710 2715

tgt gag gag cct gag tgc aac gac atc act gcc agg ctg cag tat 8199
Cys Glu Glu Pro Glu Cys Asn Asp Ile Thr Ala Arg Leu Gln Tyr
2720 2725 2730

gtc aag gtg gga agc tgt aag tct gaa gta gag gtg gat atc cac 8244
Val Lys Val Gly Ser Cys Lys Ser Glu Val Glu Val Asp Ile His
2735 2740 2745

tac tgc cag ggc aaa tgt gcc agc aaa gcc atg tac tcc att gac 8289
Tyr Cys Gln Gly Lys Cys Ala Ser Lys Ala Met Tyr Ser Ile Asp

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| 2750                        | 2755                | 2760        |      |
|-----------------------------|---------------------|-------------|------|
| atc aac gat gtg cag gac cag | tgc tcc tgc tgc tct | ccg aca cgg | 8334 |
| Ile Asn Asp Val Gln Asp Gln | Cys Ser Cys Cys Ser | Pro Thr Arg |      |
| 2765                        | 2770                | 2775        |      |
| acg gag ccc atg cag gtg gcc | ctg cac tgc acc aat | ggc tct gtt | 8379 |
| Thr Glu Pro Met Gln Val Ala | Leu His Cys Thr Asn | Gly Ser Val |      |
| 2780                        | 2785                | 2790        |      |
| gtg tac cat gag gtt ctc aat | gcc atg gag tgc aaa | tgc tcc ccc | 8424 |
| Val Tyr His Glu Val Leu Asn | Ala Met Glu Cys Lys | Cys Ser Pro |      |
| 2795                        | 2800                | 2805        |      |
| agg aag tgc agc aag tga     |                     |             | 8442 |
| Arg Lys Cys Ser Lys         |                     |             |      |
| 2810                        |                     |             |      |

&lt;210&gt; 4

&lt;211&gt; 2813

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 4

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile |  |
| 1 5 10 15                                                       |  |
| Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr |  |
| 20 25 30                                                        |  |
| Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly |  |
| 35 40 45                                                        |  |
| Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly |  |
| 50 55 60                                                        |  |
| Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys |  |
| 65 70 75 80                                                     |  |
| Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu |  |
| 85 90 95                                                        |  |
| Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro |  |
| 100 105 110                                                     |  |
| Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys |  |
| 115 120 125                                                     |  |
| Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly |  |
| 130 135 140                                                     |  |
| Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly |  |
| 145 150 155 160                                                 |  |
| Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln |  |
| 165 170 175                                                     |  |
| Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala |  |
| 180 185 190                                                     |  |

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser  
 195 200 205  
 Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln  
 210 215 220  
 Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu  
 225 230 235 240  
 Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu  
 245 250 255  
 Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala  
 260 265 270  
 Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His  
 275 280 285  
 Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys  
 290 295 300  
 Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met  
 305 310 315 320  
 Cys Gln Glu Arg Cys Val Asp Gly Cys Ser Cys Pro Glu Gly Gln Leu  
 325 330 335  
 Leu Asp Glu Gly Leu Cys Val Glu Ser Thr Glu Cys Pro Cys Val His  
 340 345 350  
 Ser Gly Lys Arg Tyr Pro Pro Gly Thr Ser Leu Ser Arg Asp Cys Asn  
 355 360 365  
 Thr Cys Ile Cys Arg Asn Ser Gln Trp Ile Cys Ser Asn Glu Glu Cys  
 370 375 380  
 Pro Gly Glu Cys Leu Val Thr Gly Gln Ser His Phe Lys Ser Phe Asp  
 385 390 395 400  
 Asn Arg Tyr Phe Thr Phe Ser Gly Ile Cys Gln Tyr Leu Leu Ala Arg  
 405 410 415  
 Asp Cys Gln Asp His Ser Phe Ser Ile Val Ile Glu Thr Val Gln Cys  
 420 425 430  
 Ala Asp Asp Arg Asp Ala Val Cys Thr Arg Ser Val Thr Val Arg Leu  
 435 440 445  
 Pro Gly Leu His Asn Ser Leu Val Lys Leu Lys His Gly Ala Gly Val  
 450 455 460  
 Ala Met Asp Gly Gln Asp Val Gln Leu Pro Leu Leu Lys Gly Asp Leu  
 465 470 475 480  
 Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu  
 485 490 495

Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu  
500 505 510

Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn  
515 520 525

Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro  
530 535 540

Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln  
545 550 555 560

Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met  
565 570 575

Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe  
580 585 590

Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys  
595 600 605

Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly  
610 615 620

Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val  
625 630 635 640

Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln  
645 650 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu  
660 665 670

Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe  
675 680 685

Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys  
690 695 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp  
705 710 715 720

Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met  
725 730 735

His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val  
740 745 750

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg  
755 760 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu  
770 775 780

Glu Tyr Glu Asp Thr Tyr Thr Asp Glu Asp Thr Tyr Glu Asp Met

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met  
785 790 795 800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg  
805 810 815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln  
820 825 830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr  
835 840 845

Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp  
850 855 860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly  
865 870 875 880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp  
885 890 895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys  
900 905 910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu  
915 920 925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys  
930 935 940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg  
945 950 955 960

Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg  
965 970 975

His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val  
980 985 990

Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr  
995 1000 1005

Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val Asp Phe Gly Asn  
1010 1015 1020

Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg Lys Val Pro  
1025 1030 1035

Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met Lys Gln  
1040 1045 1050

Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val Phe  
1055 1060 1065

Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val  
1070 1075 1080

Cys Ile Thr Asn Thr Cys Ser Cys Glu Ser Ile Glu Asp Cys Ala

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Cys  | Ile | Tyr | Asp | Ile | Cys | Ser  | Cys | Glu | Ser | Ile | Gly  | Asp | Cys | Ala |
| 1085 |     |     |     |     |     | 1090 |     |     |     |     | 1095 |     |     |     |
| Cys  | Phe | Cys | Asp | Thr | Ile | Ala  | Ala | Tyr | Ala | His | Val  | Cys | Ala | Gln |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |
| His  | Gly | Lys | Val | Val | Thr | Trp  | Arg | Thr | Ala | Thr | Leu  | Cys | Pro | Gln |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
| Ser  | Cys | Glu | Glu | Arg | Asn | Leu  | Arg | Glu | Asn | Gly | Tyr  | Glu | Cys | Glu |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
| Trp  | Arg | Tyr | Asn | Ser | Cys | Ala  | Pro | Ala | Cys | Gln | Val  | Thr | Cys | Gln |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
| His  | Pro | Glu | Pro | Leu | Ala | Cys  | Pro | Val | Gln | Cys | Val  | Glu | Gly | Cys |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
| His  | Ala | His | Cys | Pro | Pro | Gly  | Lys | Ile | Leu | Asp | Glu  | Leu | Leu | Gln |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
| Thr  | Cys | Val | Asp | Pro | Glu | Asp  | Cys | Pro | Val | Cys | Glu  | Val | Ala | Gly |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
| Arg  | Arg | Phe | Ala | Ser | Gly | Lys  | Lys | Val | Thr | Leu | Asn  | Pro | Ser | Asp |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
| Pro  | Glu | His | Cys | Gln | Ile | Cys  | His | Cys | Asp | Val | Val  | Asn | Leu | Thr |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
| Cys  | Glu | Ala | Cys | Gln | Glu | Pro  | Gly | Gly | Leu | Val | Val  | Pro | Pro | Thr |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
| Asp  | Ala | Pro | Val | Ser | Pro | Thr  | Thr | Leu | Tyr | Val | Glu  | Asp | Ile | Ser |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
| Glu  | Pro | Pro | Leu | His | Asp | Phe  | Tyr | Cys | Ser | Arg | Leu  | Leu | Asp | Leu |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
| Val  | Phe | Leu | Leu | Asp | Gly | Ser  | Ser | Arg | Leu | Ser | Glu  | Ala | Glu | Phe |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
| Glu  | Val | Leu | Lys | Ala | Phe | Val  | Val | Asp | Met | Met | Glu  | Arg | Leu | Arg |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
| Ile  | Ser | Gln | Lys | Trp | Val | Arg  | Val | Ala | Val | Val | Glu  | Tyr | His | Asp |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
| Gly  | Ser | His | Ala | Tyr | Ile | Gly  | Leu | Lys | Asp | Arg | Lys  | Arg | Pro | Ser |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |
| Glu  | Leu | Arg | Arg | Ile | Ala | Ser  | Gln | Val | Lys | Tyr | Ala  | Gly | Ser | Gln |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |
| Val  | Ala | Ser | Thr | Ser | Glu | Val  | Leu | Lys | Tyr | Thr | Leu  | Phe | Gln | Ile |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
| Phe  | Ser | Lys | Ile | Asp | Arg | Pro  | Glu | Ala | Ser | Arg | Ile  | Ala | Leu | Leu |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |

|                 |                     |                 |                 |      |
|-----------------|---------------------|-----------------|-----------------|------|
| 1370            |                     | 1375            |                 | 1380 |
| Leu Met<br>1385 | Ala Ser Gln Glu Pro | Gln Arg Met Ser | Arg Asn Phe Val |      |
| Arg Tyr<br>1400 | Val Gln Gly Leu Lys | Lys Lys Lys Val | Ile Val Ile Pro |      |
| Val Gly<br>1415 | Ile Gly Pro His Ala | Asn Leu Lys Gln | Ile Arg Leu Ile |      |
| Glu Lys<br>1430 | Gln Ala Pro Glu Asn | Lys Ala Phe Val | Leu Ser Ser Val |      |
| Asp Glu<br>1445 | Leu Glu Gln Gln Arg | Asp Glu Ile Val | Ser Tyr Leu Cys |      |
| Asp Leu<br>1460 | Ala Pro Glu Ala Pro | Pro Pro Thr Leu | Pro Pro His Met |      |
| Ala Gln<br>1475 | Val Thr Val Gly Pro | Gly Leu Leu Gly | Val Ser Thr Leu |      |
| Gly Pro<br>1490 | Lys Arg Asn Ser Met | Val Leu Asp Val | Ala Phe Val Leu |      |
| Glu Gly<br>1505 | Ser Asp Lys Ile Gly | Glu Ala Asp Phe | Asn Arg Ser Lys |      |
| Glu Phe<br>1520 | Met Glu Glu Val Ile | Gln Arg Met Asp | Val Gly Gln Asp |      |
| Ser Ile<br>1535 | His Val Thr Val Leu | Gln Tyr Ser Tyr | Met Val Thr Val |      |
| Glu Tyr<br>1550 | Pro Phe Ser Glu Ala | Gln Ser Lys Gly | Asp Ile Leu Gln |      |
| Arg Val<br>1565 | Arg Glu Ile Arg Tyr | Gln Gly Gly Asn | Arg Thr Asn Thr |      |
| Gly Leu<br>1580 | Ala Leu Arg Tyr Leu | Ser Asp His Ser | Phe Leu Val Ser |      |
|                 | 1585                |                 | 1590            |      |
| Gln Gly<br>1595 | Asp Arg Glu Gln Ala | Pro Asn Leu Val | Tyr Met Val Thr |      |
| Gly Asn<br>1610 | Pro Ala Ser Asp Glu | Ile Lys Arg Leu | Pro Gly Asp Ile |      |
| Gln Val<br>1625 | Val Pro Ile Gly Val | Gly Pro Asn Ala | Asn Val Gln Glu |      |
| Leu Glu<br>1640 | Arg Ile Gly Trp Pro | Asn Ala Pro Ile | Leu Ile Gln Asp |      |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Phe  | Glu | Thr | Leu | Pro | Arg | Glu  | Ala | Pro | Asp | Leu | Val  | Leu | Gln | Arg |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
| Cys  | Cys | Ser | Gly | Glu | Gly | Leu  | Gln | Ile | Pro | Thr | Leu  | Ser | Pro | Ala |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
| Pro  | Asp | Cys | Ser | Gln | Pro | Leu  | Asp | Val | Ile | Leu | Leu  | Leu | Asp | Gly |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
| Ser  | Ser | Ser | Phe | Pro | Ala | Ser  | Tyr | Phe | Asp | Glu | Met  | Lys | Ser | Phe |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |
| Ala  | Lys | Ala | Phe | Ile | Ser | Lys  | Ala | Asn | Ile | Gly | Pro  | Arg | Leu | Thr |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
| Gln  | Val | Ser | Val | Leu | Gln | Tyr  | Gly | Ser | Ile | Thr | Thr  | Ile | Asp | Val |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |
| Pro  | Trp | Asn | Val | Val | Pro | Glu  | Lys | Ala | His | Leu | Leu  | Ser | Leu | Val |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |
| Asp  | Val | Met | Gln | Arg | Glu | Gly  | Gly | Pro | Ser | Gln | Ile  | Gly | Asp | Ala |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |     |     |     |
| Leu  | Gly | Phe | Ala | Val | Arg | Tyr  | Leu | Thr | Ser | Glu | Met  | His | Gly | Ala |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |     |     |     |
| Arg  | Pro | Gly | Ala | Ser | Lys | Ala  | Val | Val | Ile | Leu | Val  | Thr | Asp | Val |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |     |     |     |
| Ser  | Val | Asp | Ser | Val | Asp | Ala  | Ala | Ala | Asp | Ala | Ala  | Arg | Ser | Asn |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |
| Arg  | Val | Thr | Val | Phe | Pro | Ile  | Gly | Ile | Gly | Asp | Arg  | Tyr | Asp | Ala |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
| Ala  | Gln | Leu | Arg | Ile | Leu | Ala  | Gly | Pro | Ala | Gly | Asp  | Ser | Asn | Val |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
| Val  | Lys | Leu | Gln | Arg | Ile | Glu  | Asp | Leu | Pro | Thr | Met  | Val | Thr | Leu |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |
| Gly  | Asn | Ser | Phe | Leu | His | Lys  | Leu | Cys | Ser | Gly | Phe  | Val | Arg | Ile |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |
| Cys  | Met | Asp | Glu | Asp | Gly | Asn  | Glu | Lys | Arg | Pro | Gly  | Asp | Val | Trp |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |
| Thr  | Leu | Pro | Asp | Gln | Cys | His  | Thr | Val | Thr | Cys | Gln  | Pro | Asp | Gly |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |     |     |     |
| Gln  | Thr | Leu | Leu | Lys | Ser | His  | Arg | Val | Asn | Cys | Asp  | Arg | Gly | Leu |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |
| Arg  | Pro | Ser | Cys | Pro | Asn | Ser  | Gln | Ser | Pro | Val | Lys  | Val | Glu | Glu |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |

|                                                                               |
|-------------------------------------------------------------------------------|
| Thr Cys Gly Cys Arg Trp Thr Cys Pro Cys Val Cys Thr Gly Ser<br>1940 1945 1950 |
| Ser Thr Arg His Ile Val Thr Phe Asp Gly Gln Asn Phe Lys Leu<br>1955 1960 1965 |
| Thr Gly Ser Cys Ser Tyr Val Leu Phe Gln Asn Lys Glu Gln Asp<br>1970 1975 1980 |
| Leu Glu Val Ile Leu His Asn Gly Ala Cys Ser Pro Gly Ala Arg<br>1985 1990 1995 |
| Gln Gly Cys Met Lys Ser Ile Glu Val Lys His Ser Ala Leu Ser<br>2000 2005 2010 |
| Val Glu Leu His Ser Asp Met Glu Val Thr Val Asn Gly Arg Leu<br>2015 2020 2025 |
| Val Ser Val Pro Tyr Val Gly Gly Asn Met Glu Val Asn Val Tyr<br>2030 2035 2040 |
| Gly Ala Ile Met His Glu Val Arg Phe Asn His Leu Gly His Ile<br>2045 2050 2055 |
| Phe Thr Phe Thr Pro Gln Asn Asn Glu Phe Gln Leu Gln Leu Ser<br>2060 2065 2070 |
| Pro Lys Thr Phe Ala Ser Lys Thr Tyr Gly Leu Cys Gly Ile Cys<br>2075 2080 2085 |
| Asp Glu Asn Gly Ala Asn Asp Phe Met Leu Arg Asp Gly Thr Val<br>2090 2095 2100 |
| Thr Thr Asp Trp Lys Thr Leu Val Gln Glu Trp Thr Val Gln Arg<br>2105 2110 2115 |
| Pro Gly Gln Thr Cys Gln Pro Ile Leu Glu Glu Gln Cys Leu Val<br>2120 2125 2130 |
| Pro Asp Ser Ser His Cys Gln Val Leu Leu Leu Pro Leu Phe Ala<br>2135 2140 2145 |
| Glu Cys His Lys Val Leu Ala Pro Ala Thr Phe Tyr Ala Ile Cys<br>2150 2155 2160 |
| Gln Gln Asp Ser Cys His Gln Glu Gln Val Cys Glu Val Ile Ala<br>2165 2170 2175 |
| Ser Tyr Ala His Leu Cys Arg Thr Asn Gly Val Cys Val Asp Trp<br>2180 2185 2190 |
| Arg Thr Pro Asp Phe Cys Ala Met Ser Cys Pro Pro Ser Leu Val<br>2195 2200 2205 |
| Tyr Asn His Cys Glu His Gly Cys Pro Arg His Cys Asp Gly Asn<br>2210 2215 2220 |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Val  | Ser | Ser | Cys | Gly | Asp | His  | Pro | Ser | Glu | Gly | Cys  | Phe | Cys | Pro |
| 2225 |     |     |     |     |     | 2230 |     |     |     |     | 2235 |     |     |     |
| Pro  | Asp | Lys | Val | Met | Leu | Glu  | Gly | Ser | Cys | Val | Pro  | Glu | Glu | Ala |
| 2240 |     |     |     |     |     | 2245 |     |     |     |     | 2250 |     |     |     |
| Cys  | Thr | Gln | Cys | Ile | Gly | Glu  | Asp | Gly | Val | Gln | His  | Gln | Phe | Leu |
| 2255 |     |     |     |     |     | 2260 |     |     |     |     | 2265 |     |     |     |
| Glu  | Ala | Trp | Val | Pro | Asp | His  | Gln | Pro | Cys | Gln | Ile  | Cys | Thr | Cys |
| 2270 |     |     |     |     |     | 2275 |     |     |     |     | 2280 |     |     |     |
| Leu  | Ser | Gly | Arg | Lys | Val | Asn  | Cys | Thr | Thr | Gln | Pro  | Cys | Pro | Thr |
| 2285 |     |     |     |     |     | 2290 |     |     |     |     | 2295 |     |     |     |
| Ala  | Lys | Ala | Pro | Thr | Cys | Gly  | Leu | Cys | Glu | Val | Ala  | Arg | Leu | Arg |
| 2300 |     |     |     |     |     | 2305 |     |     |     |     | 2310 |     |     |     |
| Gln  | Asn | Ala | Asp | Gln | Cys | Cys  | Pro | Glu | Tyr | Glu | Cys  | Val | Cys | Asp |
| 2315 |     |     |     |     |     | 2320 |     |     |     |     | 2325 |     |     |     |
| Pro  | Val | Ser | Cys | Asp | Leu | Pro  | Pro | Val | Pro | His | Cys  | Glu | Arg | Gly |
| 2330 |     |     |     |     |     | 2335 |     |     |     |     | 2340 |     |     |     |
| Leu  | Gln | Pro | Thr | Leu | Thr | Asn  | Pro | Gly | Glu | Cys | Arg  | Pro | Asn | Phe |
| 2345 |     |     |     |     |     | 2350 |     |     |     |     | 2355 |     |     |     |
| Thr  | Cys | Ala | Cys | Arg | Lys | Glu  | Glu | Cys | Lys | Arg | Val  | Ser | Pro | Pro |
| 2360 |     |     |     |     |     | 2365 |     |     |     |     | 2370 |     |     |     |
| Ser  | Cys | Pro | Pro | His | Arg | Leu  | Pro | Thr | Leu | Arg | Lys  | Thr | Gln | Cys |
| 2375 |     |     |     |     |     | 2380 |     |     |     |     | 2385 |     |     |     |
| Cys  | Asp | Glu | Tyr | Glu | Cys | Ala  | Cys | Asn | Cys | Val | Asn  | Ser | Thr | Val |
| 2390 |     |     |     |     |     | 2395 |     |     |     |     | 2400 |     |     |     |
| Ser  | Cys | Pro | Leu | Gly | Tyr | Leu  | Ala | Ser | Thr | Ala | Thr  | Asn | Asp | Cys |
| 2405 |     |     |     |     |     | 2410 |     |     |     |     | 2415 |     |     |     |
| Gly  | Cys | Thr | Thr | Thr | Thr | Cys  | Leu | Pro | Asp | Lys | Val  | Cys | Val | His |
| 2420 |     |     |     |     |     | 2425 |     |     |     |     | 2430 |     |     |     |
| Arg  | Ser | Thr | Ile | Tyr | Pro | Val  | Gly | Gln | Phe | Trp | Glu  | Glu | Gly | Cys |
| 2435 |     |     |     |     |     | 2440 |     |     |     |     | 2445 |     |     |     |
| Asp  | Val | Cys | Thr | Cys | Thr | Asp  | Met | Glu | Asp | Ala | Val  | Met | Gly | Leu |
| 2450 |     |     |     |     |     | 2455 |     |     |     |     | 2460 |     |     |     |
| Arg  | Val | Ala | Gln | Cys | Ser | Gln  | Lys | Pro | Cys | Glu | Asp  | Ser | Cys | Arg |
| 2465 |     |     |     |     |     | 2470 |     |     |     |     | 2475 |     |     |     |
| Ser  | Gly | Phe | Thr | Tyr | Val | Leu  | His | Glu | Gly | Glu | Cys  | Cys | Gly | Arg |
| 2480 |     |     |     |     |     | 2485 |     |     |     |     | 2490 |     |     |     |
| Cys  | Leu | Pro | Ser | Ala | Cys | Glu  | Val | Val | Thr | Gly | Ser  | Pro | Arg | Gly |
| 2495 |     |     |     |     |     | 2500 |     |     |     |     | 2505 |     |     |     |

|      |      |     |     |     |     |      |     |     |     |     |      |     |     |      |
|------|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|------|
| Asp  | Ser  | Gln | Ser | Ser | Trp | Lys  | Ser | Val | Gly | Ser | Gln  | Trp | Ala | Ser  |
| 2510 |      |     |     |     |     | 2515 |     |     |     |     | 2520 |     |     |      |
| Pro  | Glu  | Asn | Pro | Cys | Leu | Ile  | Asn | Glu | Cys | Val | Arg  | Val | Lys | Glu  |
|      | 2525 |     |     |     |     | 2530 |     |     |     |     |      |     |     | 2535 |
| Glu  | Val  | Phe | Ile | Gln | Gln | Arg  | Asn | Val | Ser | Cys | Pro  | Gln | Leu | Glu  |
| 2540 |      |     |     |     |     | 2545 |     |     |     |     | 2550 |     |     |      |
| Val  | Pro  | Val | Cys | Pro | Ser | Gly  | Phe | Gln | Leu | Ser | Cys  | Lys | Thr | Ser  |
| 2555 |      |     |     |     |     | 2560 |     |     |     |     | 2565 |     |     |      |
| Ala  | Cys  | Cys | Pro | Ser | Cys | Arg  | Cys | Glu | Arg | Met | Glu  | Ala | Cys | Met  |
| 2570 |      |     |     |     |     | 2575 |     |     |     |     | 2580 |     |     |      |
| Leu  | Asn  | Gly | Thr | Val | Ile | Gly  | Pro | Gly | Lys | Thr | Val  | Met | Ile | Asp  |
| 2585 |      |     |     |     |     | 2590 |     |     |     |     | 2595 |     |     |      |
| Val  | Cys  | Thr | Thr | Cys | Arg | Cys  | Met | Val | Gln | Val | Gly  | Val | Ile | Ser  |
| 2600 |      |     |     |     |     | 2605 |     |     |     |     | 2610 |     |     |      |
| Gly  | Phe  | Lys | Leu | Glu | Cys | Arg  | Lys | Thr | Thr | Cys | Asn  | Pro | Cys | Pro  |
| 2615 |      |     |     |     |     | 2620 |     |     |     |     | 2625 |     |     |      |
| Leu  | Gly  | Tyr | Lys | Glu | Glu | Asn  | Asn | Thr | Gly | Glu | Cys  | Cys | Gly | Arg  |
| 2630 |      |     |     |     |     | 2635 |     |     |     |     | 2640 |     |     |      |
| Cys  | Leu  | Pro | Thr | Ala | Cys | Thr  | Ile | Gln | Leu | Arg | Gly  | Gly | Gln | Ile  |
| 2645 |      |     |     |     |     | 2650 |     |     |     |     | 2655 |     |     |      |
| Met  | Thr  | Leu | Lys | Arg | Asp | Glu  | Thr | Leu | Gln | Asp | Gly  | Cys | Asp | Thr  |
| 2660 |      |     |     |     |     | 2665 |     |     |     |     | 2670 |     |     |      |
| His  | Phe  | Cys | Lys | Val | Asn | Glu  | Arg | Gly | Glu | Tyr | Phe  | Trp | Glu | Lys  |
| 2675 |      |     |     |     |     | 2680 |     |     |     |     | 2685 |     |     |      |
| Arg  | Val  | Thr | Gly | Cys | Pro | Pro  | Phe | Asp | Glu | His | Lys  | Cys | Leu | Ala  |
| 2690 |      |     |     |     |     | 2695 |     |     |     |     | 2700 |     |     |      |
| Glu  | Gly  | Gly | Lys | Ile | Met | Lys  | Ile | Pro | Gly | Thr | Cys  | Cys | Asp | Thr  |
| 2705 |      |     |     |     |     | 2710 |     |     |     |     | 2715 |     |     |      |
| Cys  | Glu  | Glu | Pro | Glu | Cys | Asn  | Asp | Ile | Thr | Ala | Arg  | Leu | Gln | Tyr  |
| 2720 |      |     |     |     |     | 2725 |     |     |     |     | 2730 |     |     |      |
| Val  | Lys  | Val | Gly | Ser | Cys | Lys  | Ser | Glu | Val | Glu | Val  | Asp | Ile | His  |
| 2735 |      |     |     |     |     | 2740 |     |     |     |     | 2745 |     |     |      |
| Tyr  | Cys  | Gln | Gly | Lys | Cys | Ala  | Ser | Lys | Ala | Met | Tyr  | Ser | Ile | Asp  |
| 2750 |      |     |     |     |     | 2755 |     |     |     |     | 2760 |     |     |      |
| Ile  | Asn  | Asp | Val | Gln | Asp | Gln  | Cys | Ser | Cys | Cys | Ser  | Pro | Thr | Arg  |
| 2765 |      |     |     |     |     | 2770 |     |     |     |     | 2775 |     |     |      |

Thr Glu Pro Met Gln Val Ala Leu His Cys Thr Asn Gly Ser Val  
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Val Tyr His Glu Val Leu Asn Ala Met Glu Cys Lys Cys Ser Pro  
 2795 2800 2805

Arg Lys Cys Ser Lys  
 2810

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<211> 1444

<212> PRT

<213> Artificial Sequence

<220>

<223> amino acid sequence of a single chain factor viii molecule

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Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro  
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Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys  
 35 40 45

Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro  
 50 55 60

Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val  
 65 70 75 80

Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val  
 85 90 95

Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala  
 100 105 110

Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val  
 115 120 125

Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn  
 130 135 140

Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser

145 150 155 160

His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu  
 165 170 175

Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu  
 180 185 190

His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp  
 195 200 205  
 His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser  
 210 215 220  
 Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg  
 225 230 235 240  
 Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His  
 245 250 255  
 Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu  
 260 265 270  
 Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile  
 275 280 285  
 Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly  
 290 295 300  
 Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met  
 305 310 315 320  
 Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg  
 325 330 335  
 Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp  
 340 345 350  
 Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe  
 355 360 365  
 Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His  
 370 375 380  
 Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu  
 385 390 395 400  
 Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro  
 405 410 415  
 Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr  
 420 425 430  
 Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile  
 435 440 445  
 Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile  
 450 455 460  
 Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile  
 465 470 475 480  
 Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys  
 485 490 495

His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys  
 500 505 510

Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys  
 515 520 525

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala  
 530 535 540

Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp  
 545 550 555 560

Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe  
 565 570 575

Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln  
 580 585 590

Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe  
 595 600 605

Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser  
 610 615 620

Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu  
 625 630 635 640

Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr  
 645 650 655

Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro  
 660 665 670

Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp  
 675 680 685

Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala  
 690 695 700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu  
 705 710 715 720

Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala  
 725 730 735

Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro Ser Thr Arg  
 740 745 750

Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Thr Thr Leu Gln  
 755 760 765

Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met  
 770 775 780

Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro  
 785 790 795 800

Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu  
 805 810 815  
 Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn  
 820 825 830  
 Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln  
 835 840 845  
 Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu  
 850 855 860  
 Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu  
 865 870 875 880  
 Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser  
 885 890 895  
 Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala  
 900 905 910  
 Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe  
 915 920 925  
 Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys  
 930 935 940  
 Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His  
 945 950 955 960  
 Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn  
 965 970 975  
 Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe  
 980 985 990  
 Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu  
 995 1000 1005  
 Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr  
 1010 1015 1020  
 Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met  
 1025 1030 1035  
 Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg  
 1040 1045 1050  
 Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile  
 1055 1060 1065  
 His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr  
 1070 1075 1080  
 Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val  
 1085 1090 1095

|         |                 |      |                 |      |             |
|---------|-----------------|------|-----------------|------|-------------|
| Glu Met | Leu Pro Ser Lys | Ala  | Gly Ile Trp Arg | Val  | Glu Cys Leu |
| 1100    |                 | 1105 |                 | 1110 |             |
| Ile Gly | Glu His Leu His | Ala  | Gly Met Ser Thr | Leu  | Phe Leu Val |
| 1115    |                 | 1120 |                 | 1125 |             |
| Tyr Ser | Asn Lys Cys Gln | Thr  | Pro Leu Gly Met | Ala  | Ser Gly His |
| 1130    |                 | 1135 |                 | 1140 |             |
| Ile Arg | Asp Phe Gln Ile | Thr  | Ala Ser Gly Gln | Tyr  | Gly Gln Trp |
| 1145    |                 | 1150 |                 | 1155 |             |
| Ala Pro | Lys Leu Ala Arg | Leu  | His Tyr Ser Gly | Ser  | Ile Asn Ala |
| 1160    |                 | 1165 |                 | 1170 |             |
| Trp Ser | Thr Lys Glu Pro | Phe  | Ser Trp Ile Lys | Val  | Asp Leu Leu |
| 1175    |                 | 1180 |                 | 1185 |             |
| Ala Pro | Met Ile Ile His | Gly  | Ile Lys Thr Gln | Gly  | Ala Arg Gln |
| 1190    |                 | 1195 |                 | 1200 |             |
| Lys Phe | Ser Ser Leu Tyr | Ile  | Ser Gln Phe Ile | Ile  | Met Tyr Ser |
| 1205    |                 | 1210 |                 | 1215 |             |
| Leu Asp | Gly Lys Lys Trp | Gln  | Thr Tyr Arg Gly | Asn  | Ser Thr Gly |
| 1220    |                 | 1225 |                 | 1230 |             |
| Thr Leu | Met Val Phe Phe | Gly  | Asn Val Asp Ser | Ser  | Gly Ile Lys |
| 1235    |                 | 1240 |                 | 1245 |             |
| His Asn | Ile Phe Asn Pro | Pro  | Ile Ile Ala Arg | Tyr  | Ile Arg Leu |
| 1250    |                 | 1255 |                 | 1260 |             |
| His Pro | Thr His Tyr Ser | Ile  | Arg Ser Thr Leu | Arg  | Met Glu Leu |
| 1265    |                 | 1270 |                 | 1275 |             |
| Met Gly | Cys Asp Leu Asn | Ser  | Cys Ser Met Pro | Leu  | Gly Met Glu |
| 1280    |                 | 1285 |                 | 1290 |             |
| Ser Lys | Ala Ile Ser Asp | Ala  | Gln Ile Thr Ala | Ser  | Ser Tyr Phe |
| 1295    |                 | 1300 |                 | 1305 |             |
| Thr Asn | Met Phe Ala Thr | Trp  | Ser Pro Ser Lys | Ala  | Arg Leu His |
| 1310    |                 | 1315 |                 | 1320 |             |
| Leu Gln | Gly Arg Ser Asn | Ala  | Trp Arg Pro Gln | Val  | Asn Asn Pro |
| 1325    |                 | 1330 |                 | 1335 |             |
| Lys Glu | Trp Leu Gln Val | Asp  | Phe Gln Lys Thr | Met  | Lys Val Thr |
| 1340    |                 | 1345 |                 | 1350 |             |
| Gly Val | Thr Thr Gln Gly | Val  | Lys Ser Leu Leu | Thr  | Ser Met Tyr |
| 1355    |                 | 1360 |                 | 1365 |             |

Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp  
 1370 1375 1380

Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn  
 1385 1390 1395

Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu  
 1400 1405 1410

Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln  
 1415 1420 1425

Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu  
 1430 1435 1440

Tyr

<210> 6

<211> 585

<212> PRT

<213> Homo sapiens

<400> 6

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Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln  
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Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu  
 35 40 45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys  
 50 55 60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu  
 65 70 75 80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro  
 85 90 95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu  
 100 105 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His  
 115 120 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg  
 130 135 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg  
 145 150 155 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala  
 165 170 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser  
 180 185 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu  
 195 200 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro  
 210 215 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys  
 225 230 235 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp  
 245 250 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser  
 260 265 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His  
 275 280 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser  
 290 295 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala  
 305 310 315 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg  
 325 330 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr  
 340 345 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu  
 355 360 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro  
 370 375 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu  
 385 390 395 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro  
 405 410 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys  
 420 425 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys  
 435 440 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His  
 450 455 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser  
 465 470 475 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr  
485 490 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp  
500 505 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala  
515 520 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu  
530 535 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys  
545 550 555 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val  
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Ala Ala Ser Gln Ala Ala Leu Gly Leu  
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<210> 7

<211> 584

<212> PRT

<213> Artificial sequence

<220>

<223> His-tagged CTP fusion protein

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<222> (1)..(479)

<223> VWF D'D3 region (VWF amino acids 764 - 1242)

<220>

<221> MISC\_FEATURE

<222> (480)..(511)

<223> glycine / serine linker

<220>

<221> MISC\_FEATURE

<222> (512)..(576)

<223> C-terminal peptide of human chorionic gonadotropin beta subunit

<220>

<221> MISC\_FEATURE

<222> (577)..(584)

<223> Polyhistidine tag

<400> 7

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp

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Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro
35      40      45
Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys
50      55      60
Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys
65      70      75      80
Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr
85      90      95
Asp His Val Cys Asp Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr
100     105     110
Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr
115     120     125
Val Leu Val Gln Asp Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile
130     135     140
Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys
145     150     155     160
Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly
165     170     175
Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val
180     185     190
Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser
195     200     205
Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr
210     215     220
Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln
225     230     235     240
Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val
245     250     255
Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg
260     265     270
Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met
275     280     285
Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val
290     295     300

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Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val  
 305 310 315 320  
 Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys  
 325 330 335  
 Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly  
 340 345 350  
 Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu  
 355 360 365  
 Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn  
 370 375 380  
 Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu  
 385 390 395 400  
 Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro  
 405 410 415  
 Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp  
 420 425 430  
 Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys  
 435 440 445  
 Val Thr Leu Asn Pro Ser Asp Pro Glu His Cys Gln Ile Cys His Cys  
 450 455 460  
 Asp Val Val Asn Leu Thr Cys Glu Ala Cys Gln Glu Pro Gly Gly Ser  
 465 470 475 480  
 Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly  
 485 490 495  
 Gly Ser Gly Gly Ser Gly Gly Gly Ser Gly Gly Ser Gly Ser Ser Ala  
 500 505 510  
 Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg  
 515 520 525  
 Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln Ala Ser Ser Ser  
 530 535 540  
 Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly  
 545 550 555 560  
 Pro Ser Asp Thr Pro Ile Leu Pro Gln Ala Gly Gly Ser Gly Gly Ser  
 565 570 575  
 His His His His His His His His  
 580

# REFERENCES CITED IN THE DESCRIPTION

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**PATENTKRAV**

1. Et polypeptid omfattende en trunkeret von Willebrand-Faktor (VWF) og en halveringstidsforlængende del til anvendelse til behandling af en blodkoagulationsforstyrrelse, nævnte behandling omfatter administration af polypeptidet til et individ med en blodkoagulationsforstyrrelse og med endogen Faktor VIII (FVIII), hvor aktivitetsniveauet for endogent FVIII hos nævnte individ før behandling med nævnte polypeptid er reduceret i forhold til aktivitetsniveauet for FVIII i normalt humant plasma (NHP) forudsat at aktivitetsniveauet for endogent FVIII i nævnte individ er mindst 0,5% af aktivitetsniveauet for endogent FVIII i normalt humant plasma (NHP), hvor den trunkerede VWF omfatter en aminosyresekvens med en sekvensidentitet på mindst 90% til aminosyrer 764 til 1242 i SEQ ID NO:4, og hvor polypeptidet er i stand til binding til endogent FVIII, hvor den trunkerede von Willebrand-Faktor (VWF) tilvejebringer muligheden for polypeptidet til binding til endogent FVIII; hvor polypeptidet er en dimer, og affiniteten af nævnte dimer til FVIII er større end affiniteten af et monomert polypeptid til FVIII, nævnte monomere polypeptid har den samme aminosyresekvens som en monomer underenhed af det dimere polypeptid; hvor polypeptidet har en FVIII-bindingsaffinitet, **karakteriseret ved** en dissociationskonstant  $K_D$  på mindre end 1 nM; hvor den halveringstidsforlængende del er en heterolog aminosyresekvens fusioneret til den trunkerede VWF eller er konjugeret til polypeptidet; og hvor det endogene FVIII-niveau forøges efter administration af nævnte polypeptid, og hvor

- (i) nævnte polypeptid administreres til profylaktisk forebyggelse af en blødningshændelse, hvor

a) nævnte behandling enten ikke omfatter samtidig administration af eksogent FVIII, eller

b) nævnte behandling omfatter, at der administreres et eksogent FVIII og derved tilvejebringer det endogene FVIII i nævnte individ; eller

- (ii) nævnte polypeptid administreres samtidig med eksogent FVIII til behandling af en blødningshændelse eller til initiering af et profylaktisk be-

handlingsregime, hvor til opfølgingsbehandling administreres nævnte polypeptid uden uden samtidig administration af exogent FVIII.

- 5 2. Polypeptid til anvendelse ifølge krav 1, hvor det endogene FVIII-niveau forøges efter administration af nævnte polypeptid til et niveau på mindst 1%.
3. Polypeptid til anvendelse ifølge krav 1 eller 2, hvor den trunkerede von Willebrand-Faktor (VWF) er en human trunkeret von Willebrand-Faktor (VWF).
- 10 4. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor aktivitetsniveauet af endogent FVIII hos nævnte individ før behandling med nævnte polypeptid er mindre end 80%, mindre end 60%, mindre end 40%, mindre end 30% , mindre end 20% eller mindre end 10% af aktivitetsniveauet for endogent FVIII i NHP.
- 15 5. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor aktivitetsniveauet for endogent FVIII i nævnte individ før behandling foretrukket er mindst 1%, mindst 2%, mindst 3%, mindst 4% eller ved mindst 5% af aktivitetsniveauet for endogent FVIII i NHP.
- 20 6. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor blodkoagulationsforstyrrelsen er udvalgt blandt hæmofili A og von-Willebrand sygdom.
- 25 7. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor individet er et menneske.
8. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor polypeptidet administreres enten intravenøst eller ekstravaskulært.
- 30 9. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor polypeptidet har en FVIII-bindingsaffinitet **kendetegnet ved** en dissociationskonstant  $K_D$  på mindre end 500 pM, foretrukket mindre end 200 pM, mindre end 100 pM, mindre end 90 pM eller mindre end 80 pM.

10. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor den trunkeerede VWF består af (a) aminosyrer 764 til 1242 af SEQ ID NO:4, (b) en aminosyresekvens med en sekvensidentitet på mindst 90% til aminosyrer 764 til 1242 i SEQ ID NO:4.

5

11. Polypeptidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor den halveringstidsforlængende del er en heterolog aminosyresekvens fusioneret til den trunkeerede VWF, og hvor nævnte heterologe aminosyresekvens omfatter eller består af et polypeptid udvalgt blandt gruppen bestående af albumin eller fragmenter deraf, transferrin eller fragmenter deraf, det C-terminale peptid af humant choriongonadotropin, en XTEN-sekvens, homo-aminosyre-gentagelser (HAP), prolin-alanin-serin-gentagelser (PAS), afamin, alfa-fetoprotein, Vitamin D-bindende protein, polypeptider, der er i stand til binding under fysiologiske betingelser til albumin eller til immunglobulin-konstante regioner, polypeptider, der er i stand til binding til den neonatale Fc-receptor (FcRn), især immunglobulin-konstante regioner og dele deraf, foretrukket Fc-delen af immunglobulin, og kombinationer deraf.

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12. Polypeptid til anvendelse ifølge et hvilket som helst af kravene 1 til 10, hvor den halveringstidsforlængende del er konjugeret til polypeptidet, og hvor nævnte halveringstidsforlængende del er udvalgt fra gruppen bestående af hydroxyethylstivelse (HES), polyethylenglycol (PEG), polysialinsyrer (PSA'er), elastinlignende polypeptider, heparosanpolymerer, hyaluronsyre og albuminbindende ligander, f.eks. fedtsyre-kæder eller albuminbindende peptider og kombinationer deraf.

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13. Polypeptidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor de farmakokinetiske parametre for det endogene FVIII forbedres ved administration af polypeptidet, især hvor den gennemsnitlige opholdstid (MRT) for det endogene FVIII forøges og/eller halveringstiden for det endogene FVIII er forlænget, og/eller clearance af det endogene FVIII reduceres.

30

14. Polypeptid til anvendelse ifølge et hvilket som helst af de foregående krav, hvor plasma-halveringstiden for polypeptidet er højere end plasma-halveringstiden for endogent VWF og/eller højere end plasma-halveringstiden for VWF for normalt humant plasma (NHP).

15. Polypeptid til anvendelse ifølge krav 14, hvor polypeptidets plasma-halveringstid er mindst 25% højere end halveringstiden for den endogene VWF og/eller mindst 25% højere end halveringstiden for VWF for normalt humant plasma (NHP).

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16. Polypeptidet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor der efter administration af polypeptidet opnås en forøgelse af individets endogene FVIII-aktivitetsniveau, foretrukket forøges det endogene FVIII-aktivitetsniveau op til det fysiologiske FVIII-niveau (100% = 1 IU/ml) eller ikke væsentligt forøget over det fysiologiske FVIII-niveau efter administration af polypeptidet, foretrukket resulterende i en forøgelse af det endogene FVIII-aktivitetsniveau, der ikke overstiger 300% = 3 IU/ml, mere foretrukket ikke overstiger 250% = 2,5 IU/ml, ikke overstiger 200% = 2 IU/ml, ikke overstiger 150% = 1,5 IU/ml eller ikke overstiger 120% = 1,2 IU/ml af gennemsnitlig FVIII-aktivitetsniveau i plasma af henholdsvis normalt humant plasma.

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# DRAWINGS

Figure 1A

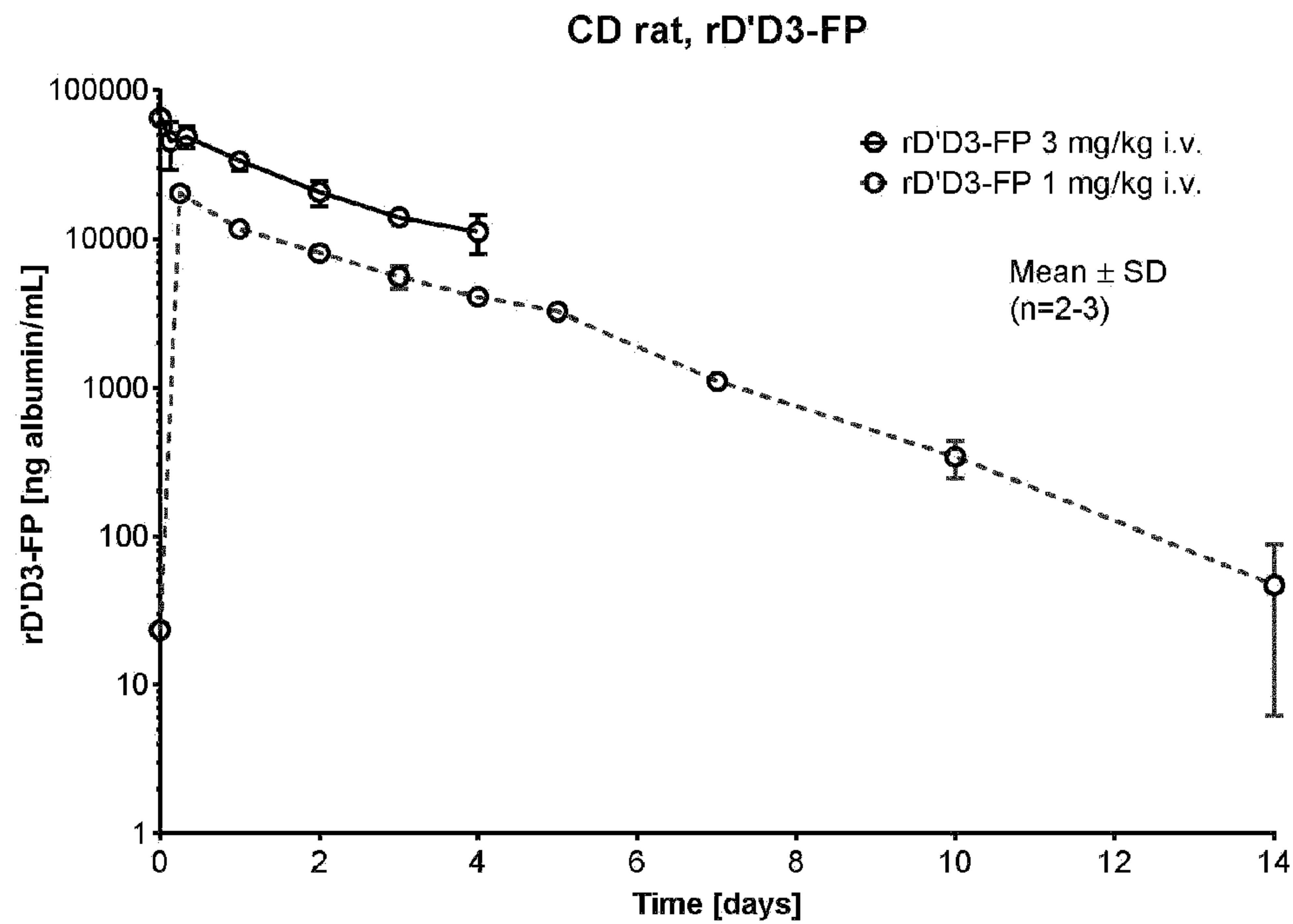


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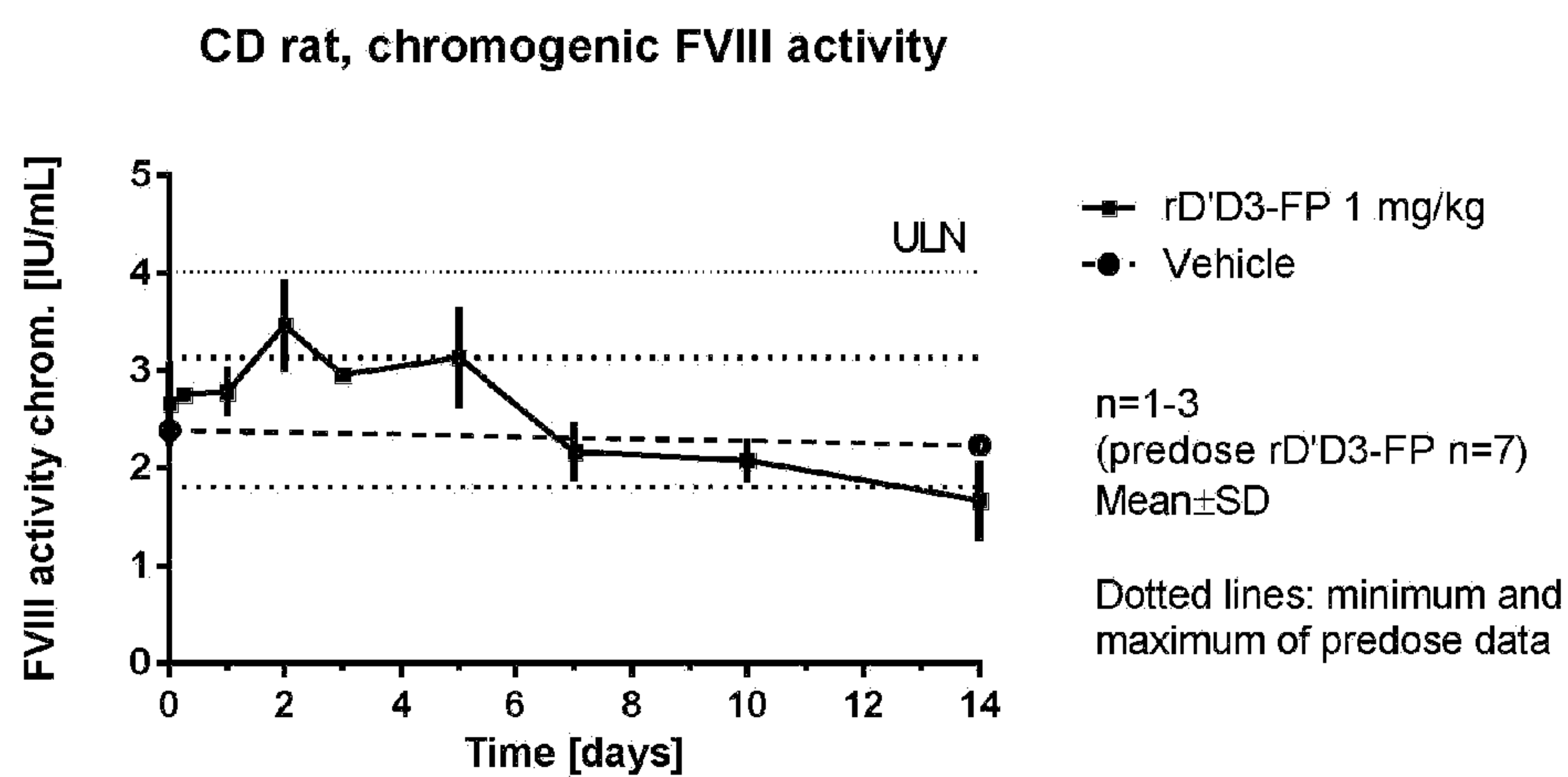


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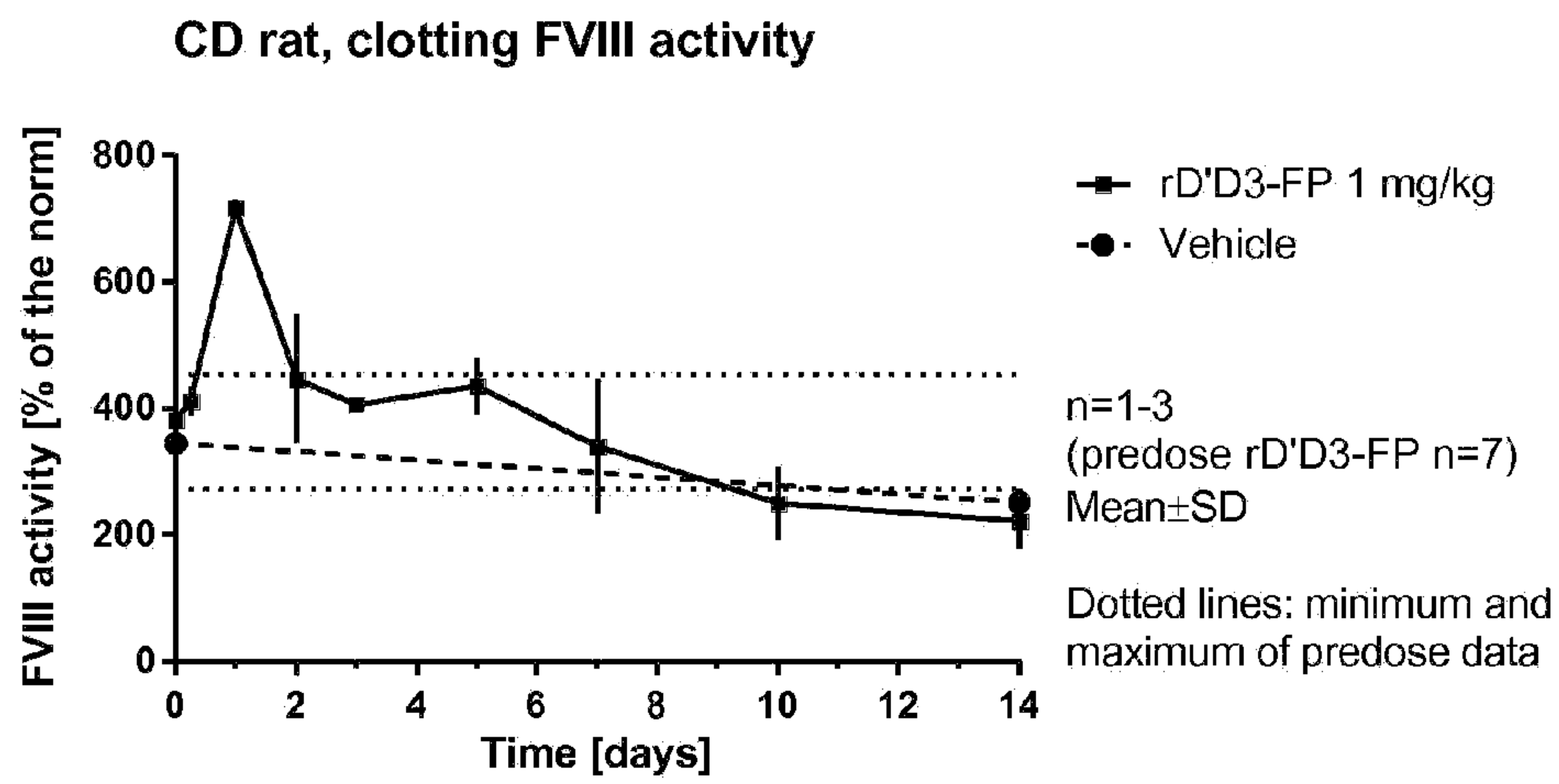


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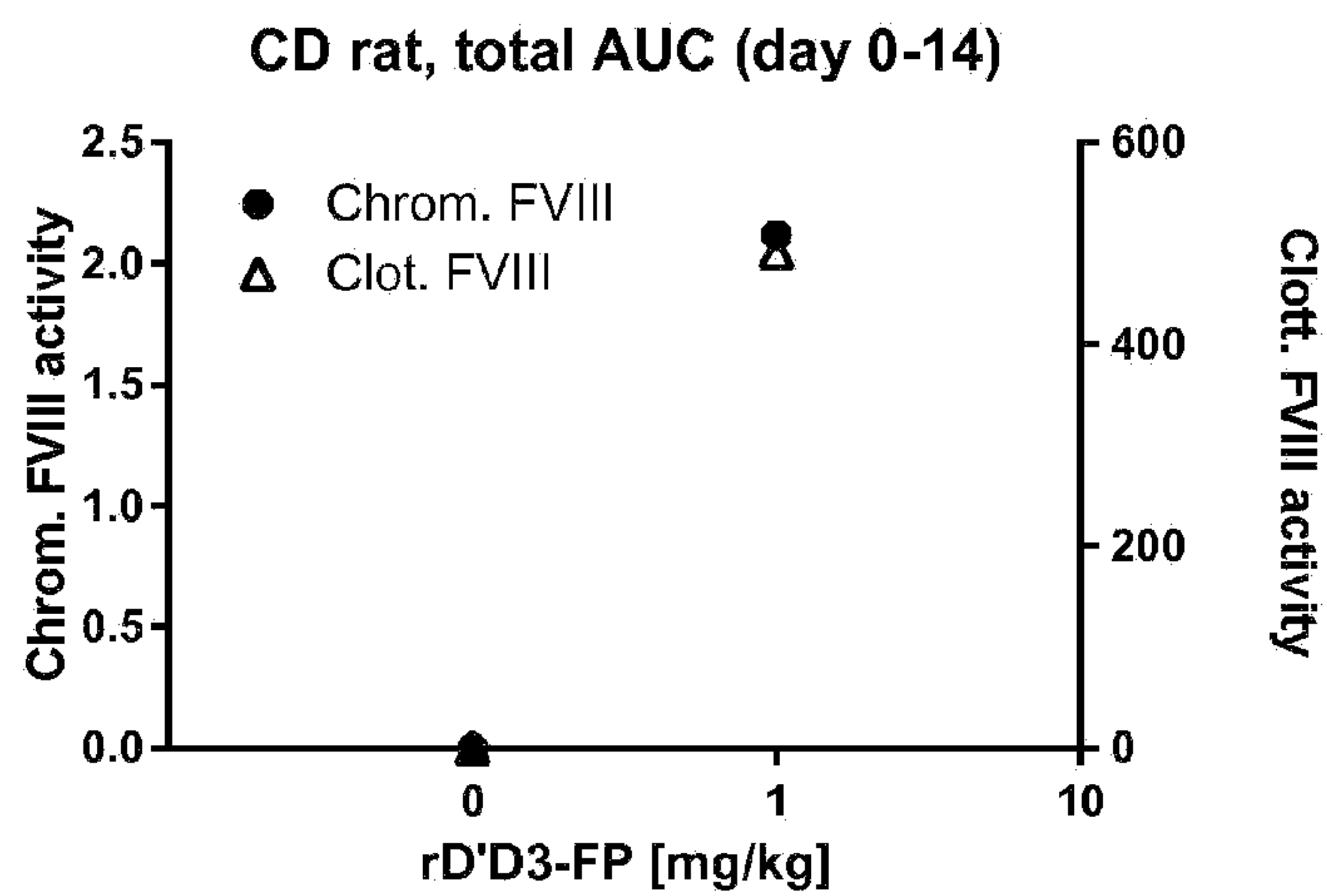


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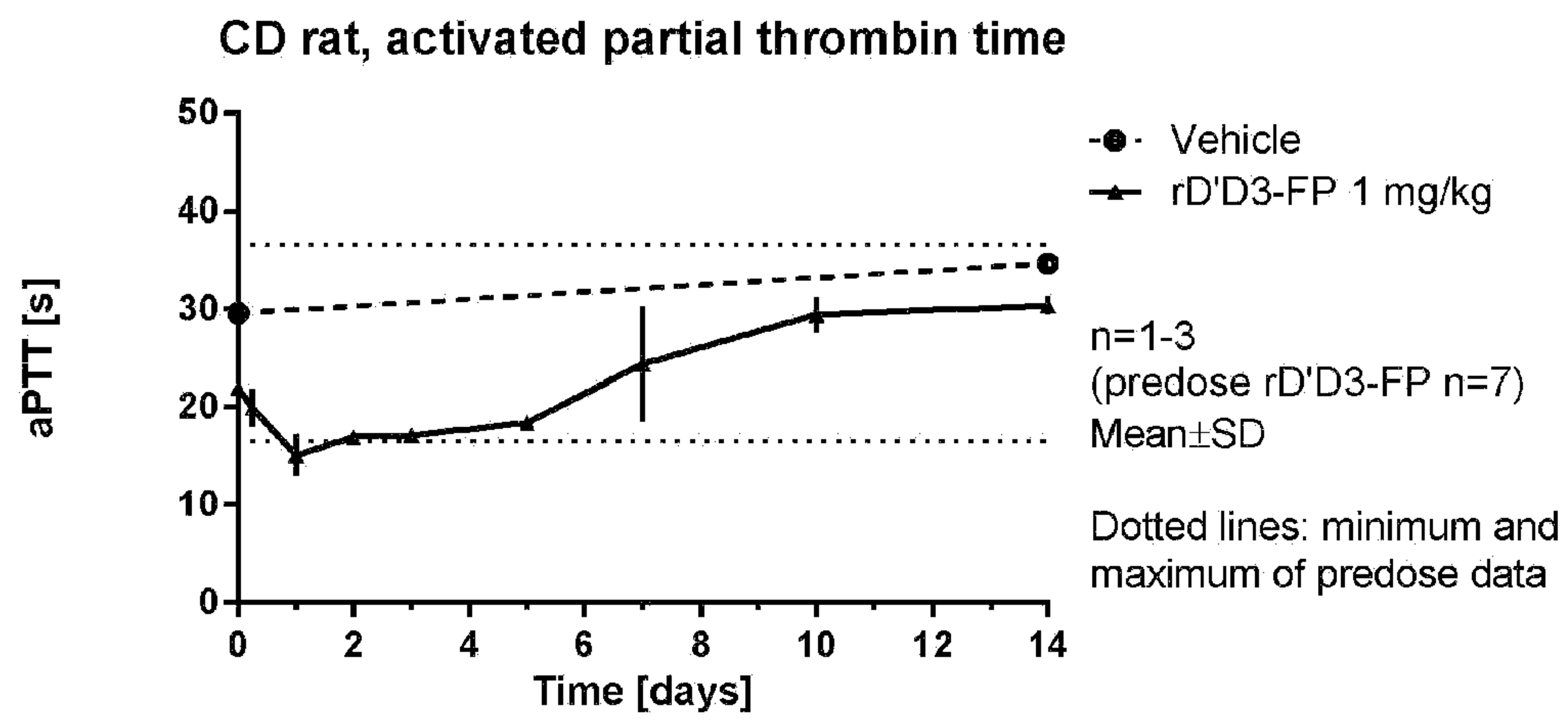


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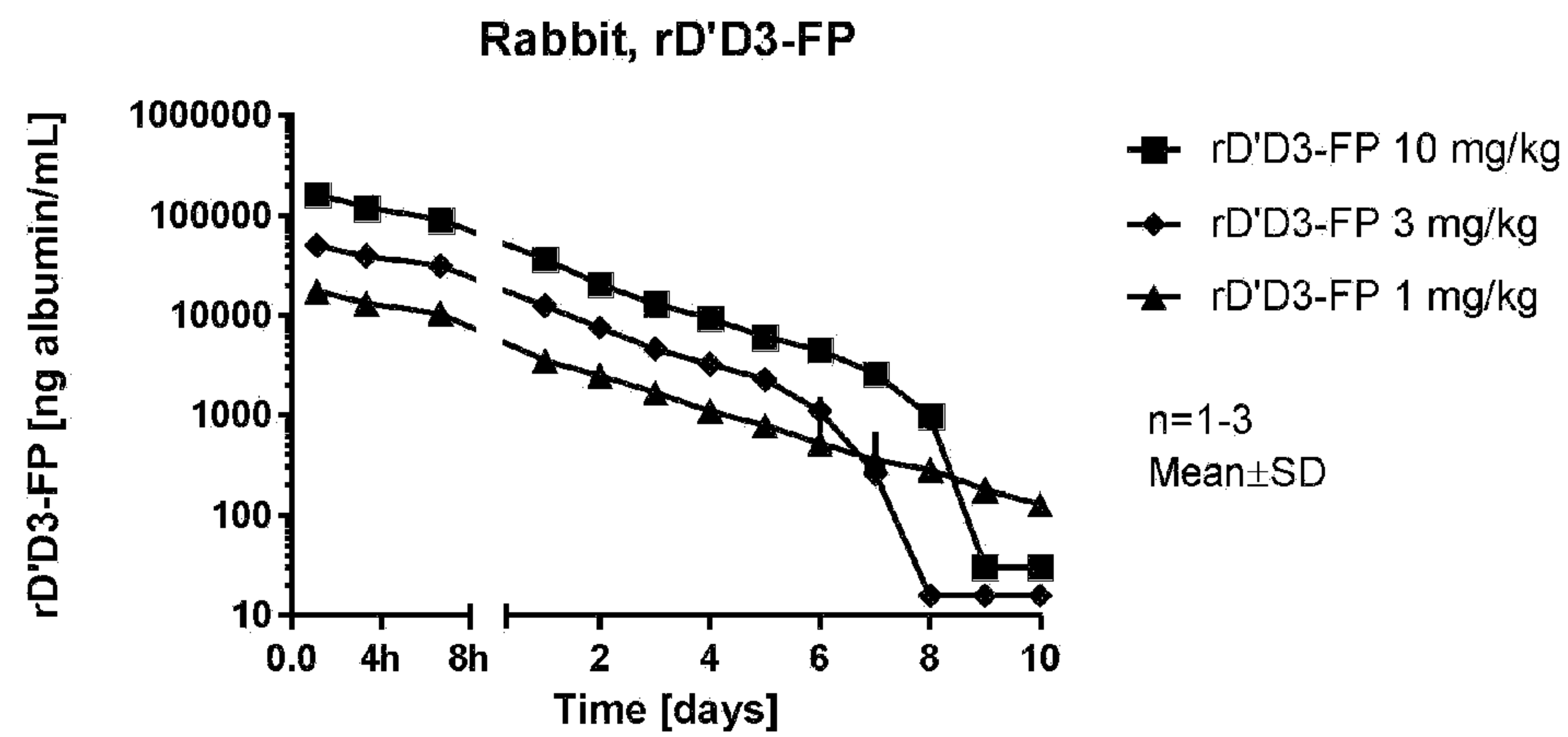


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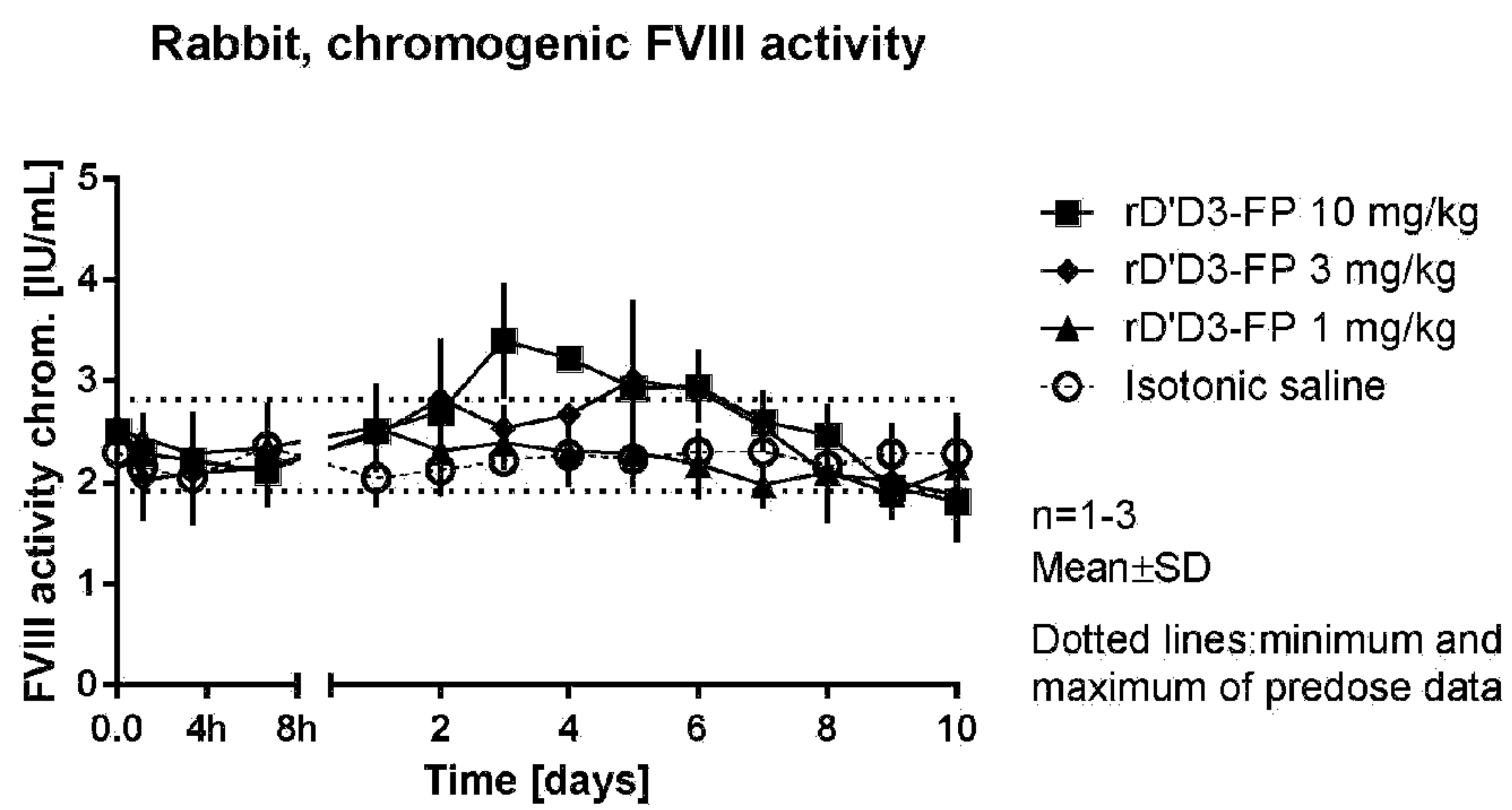


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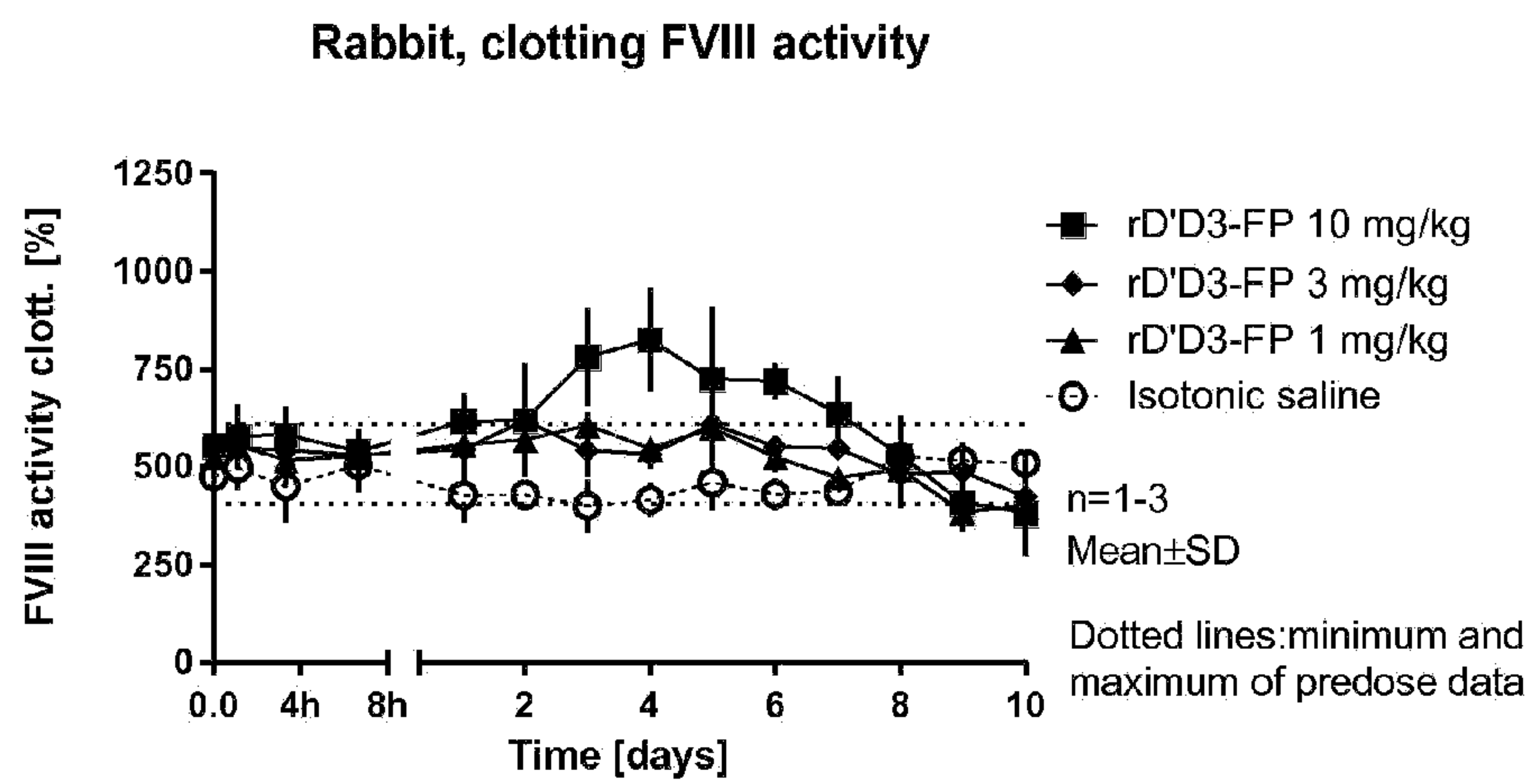


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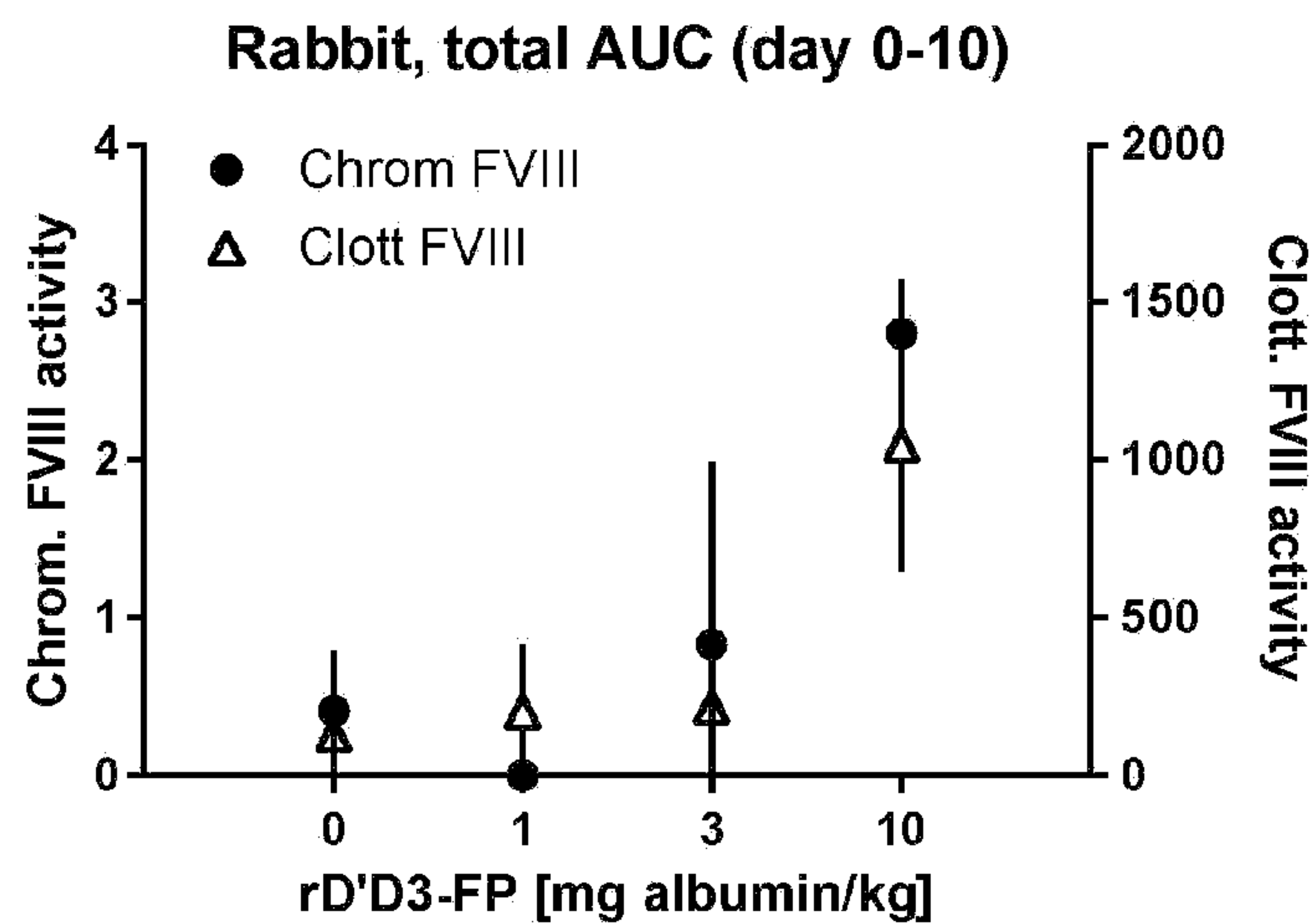


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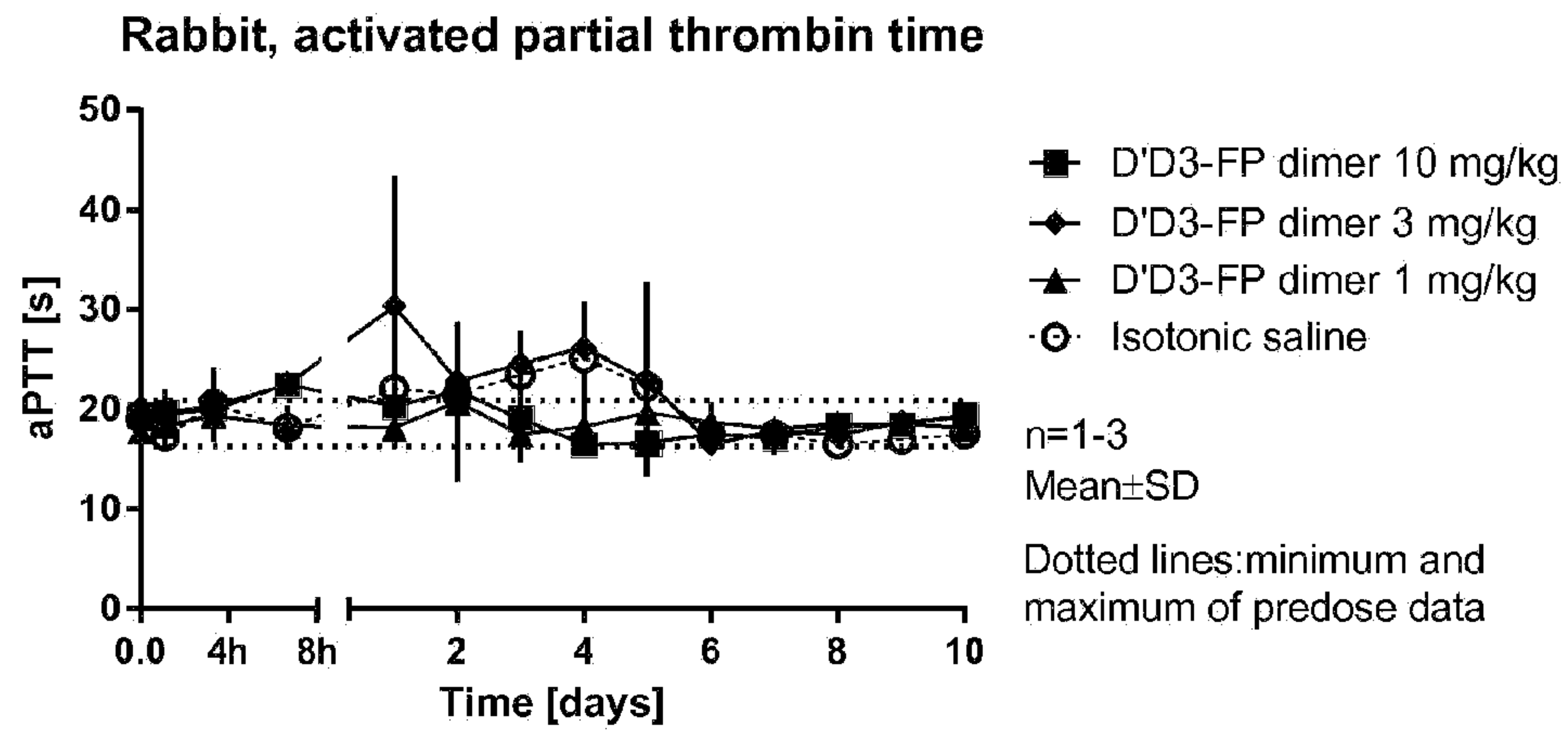


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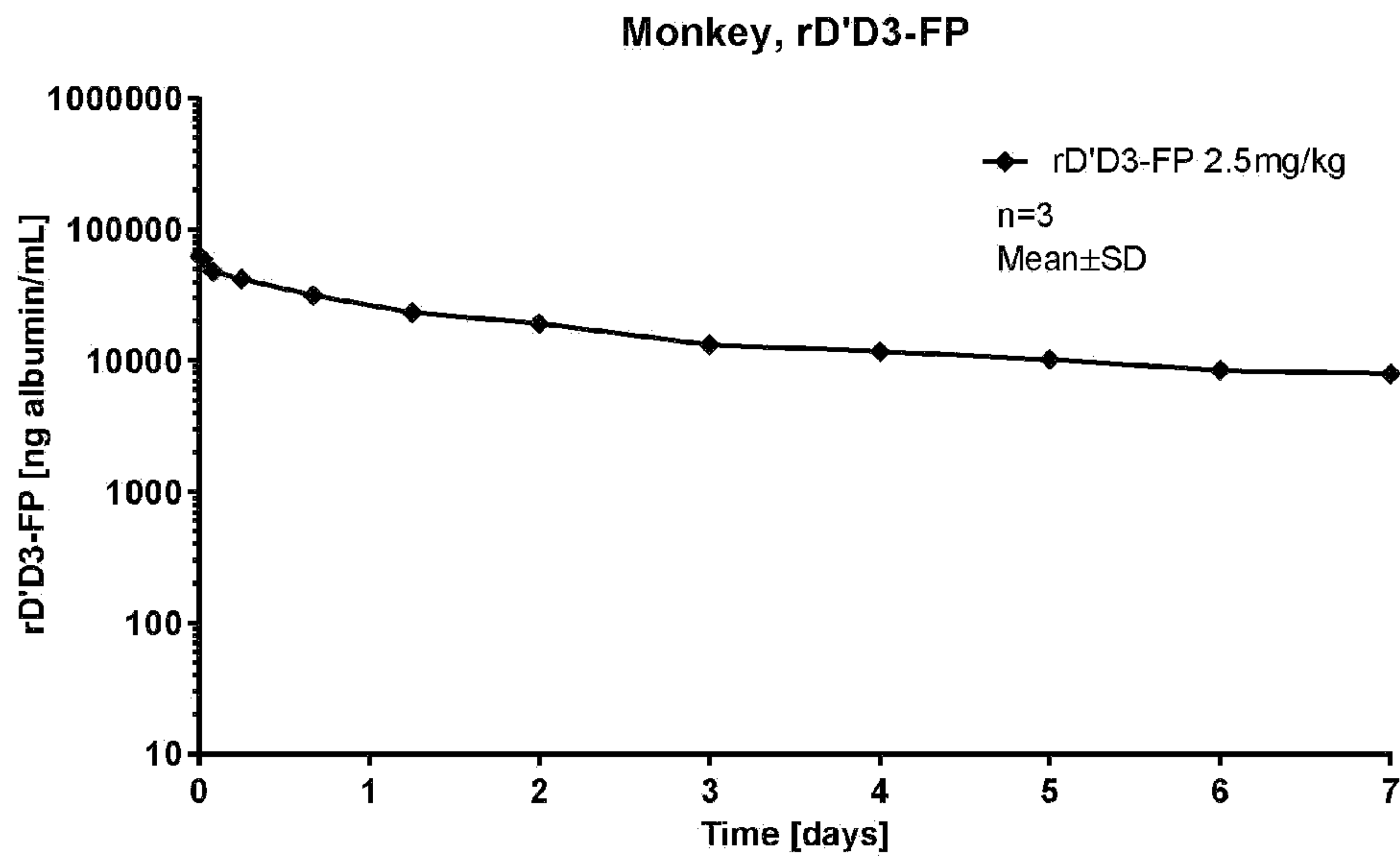


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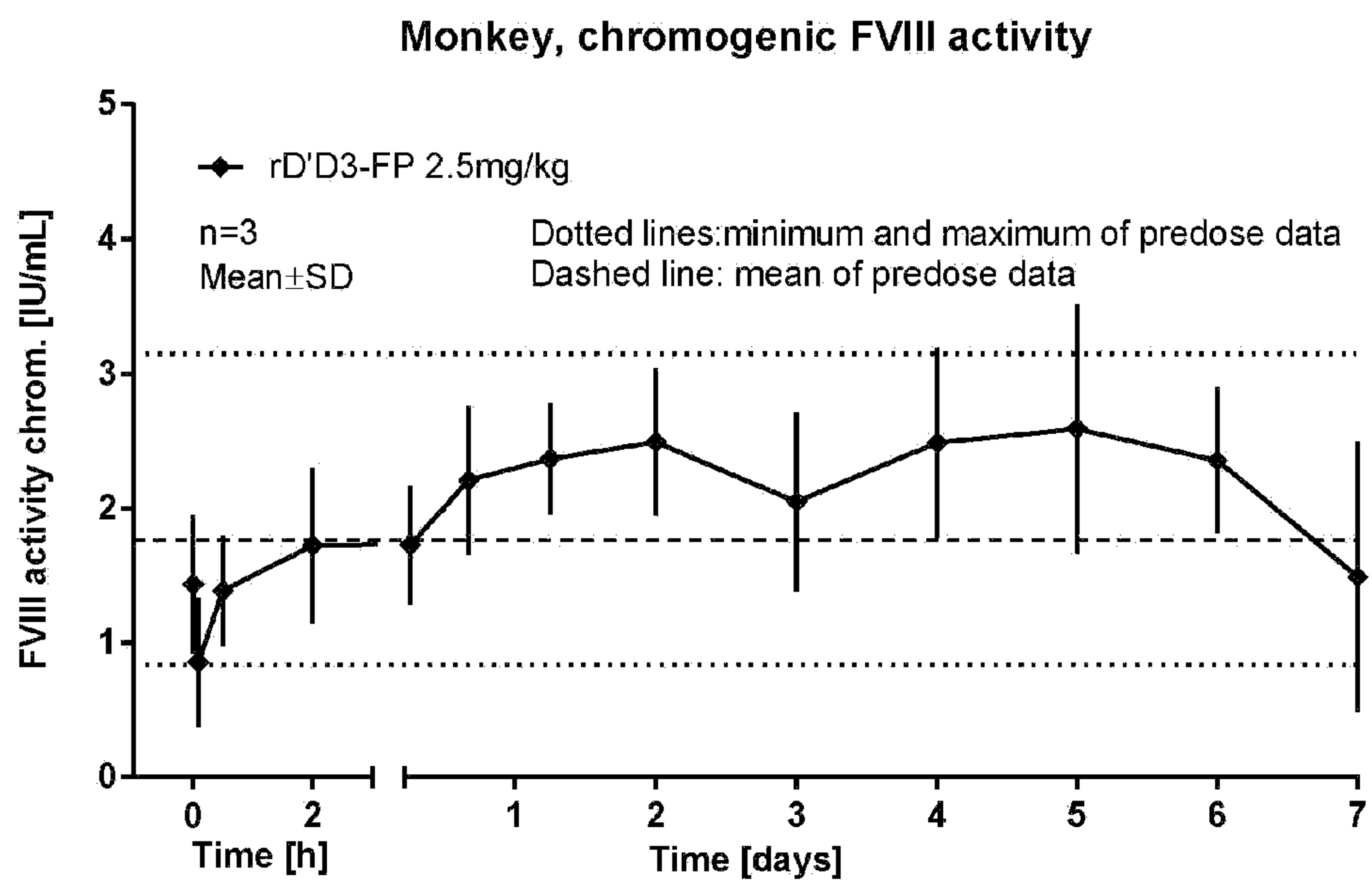


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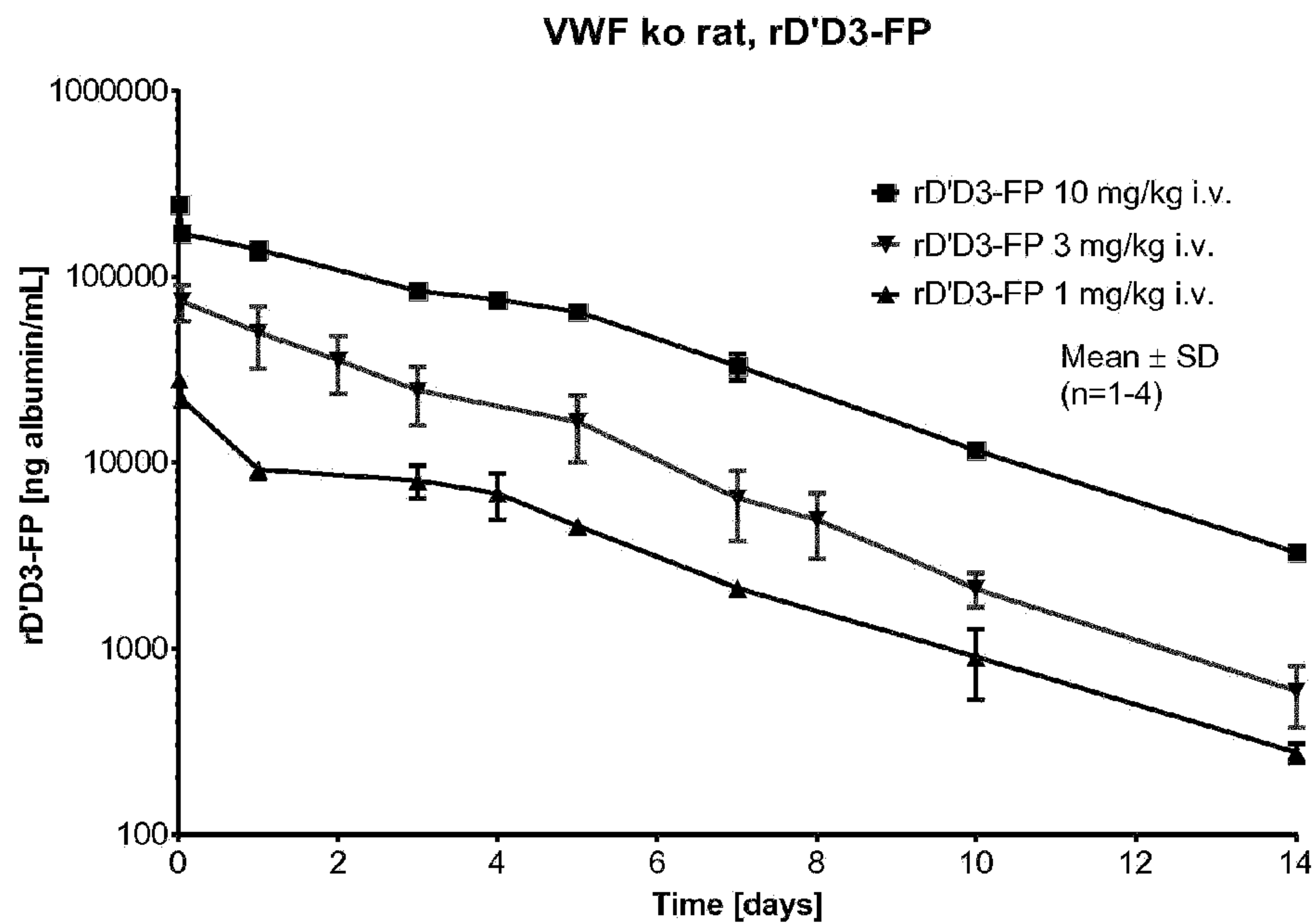


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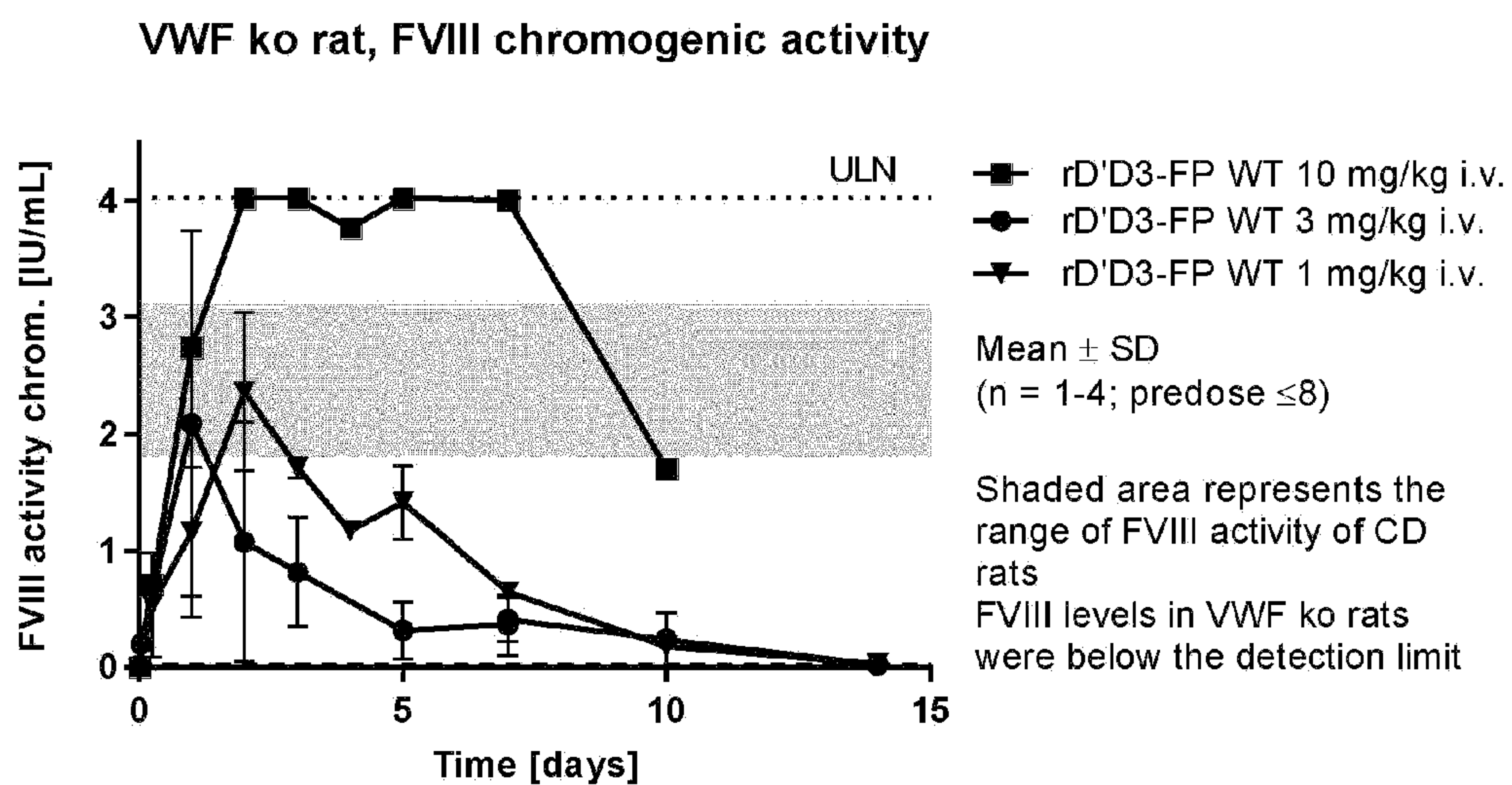


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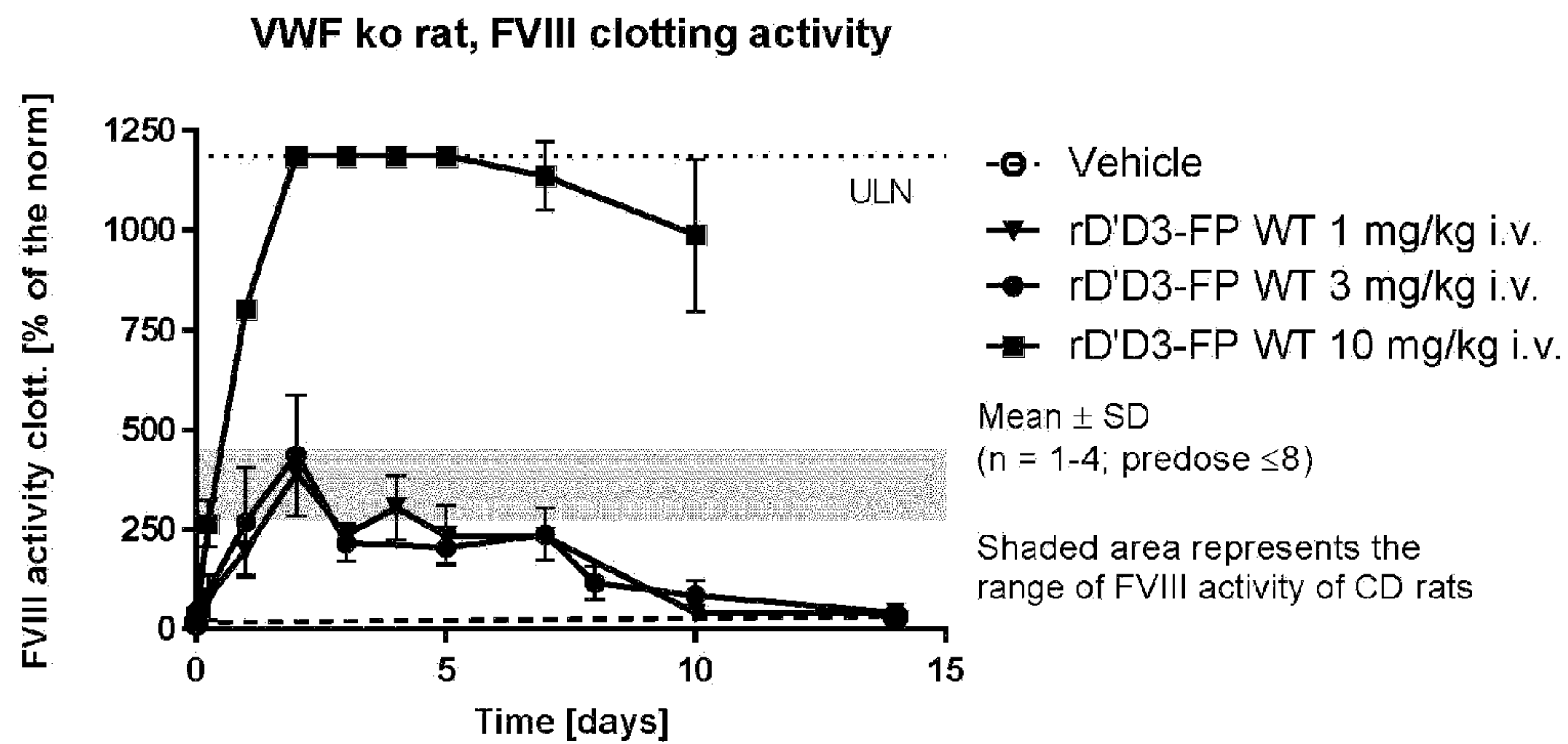


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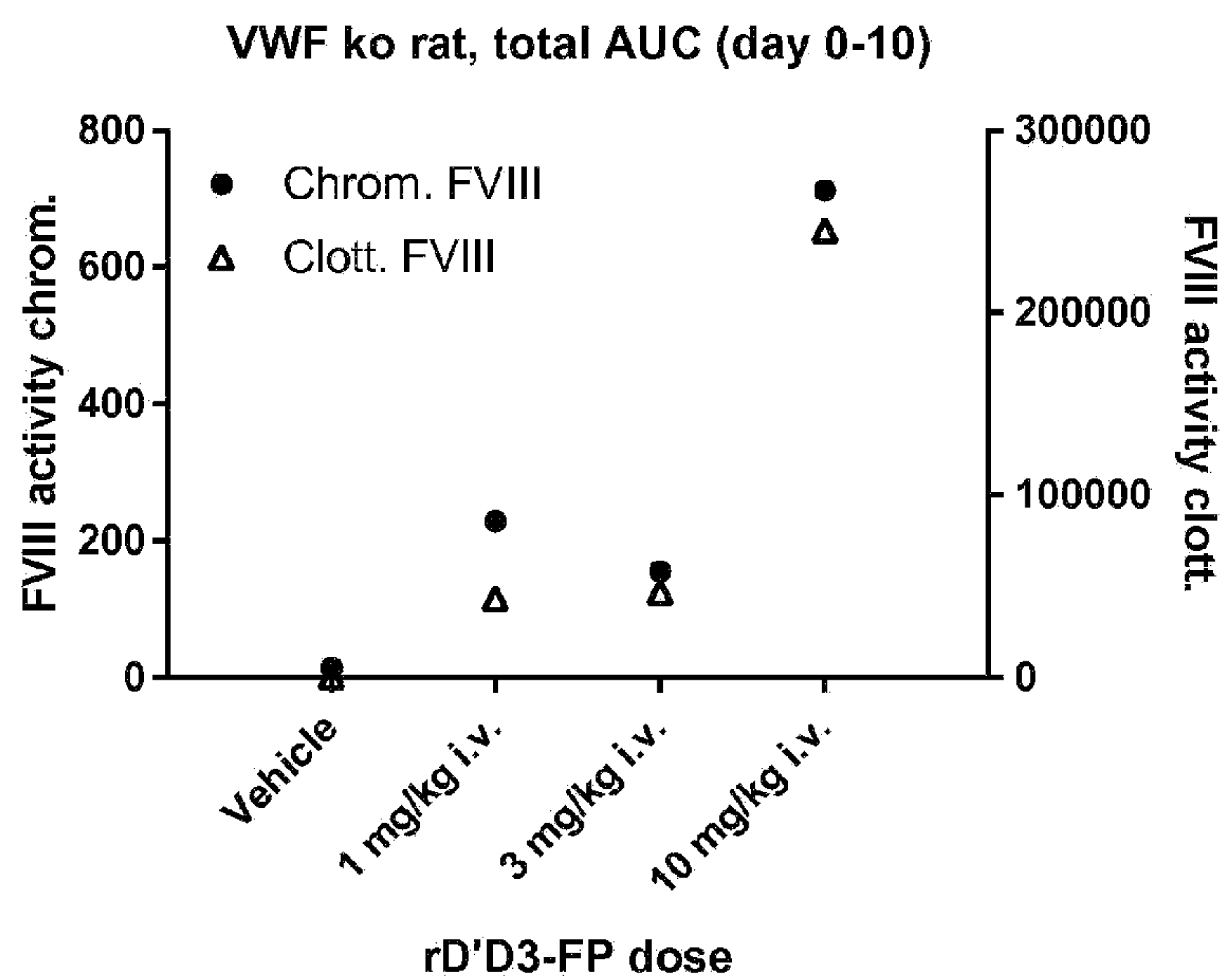


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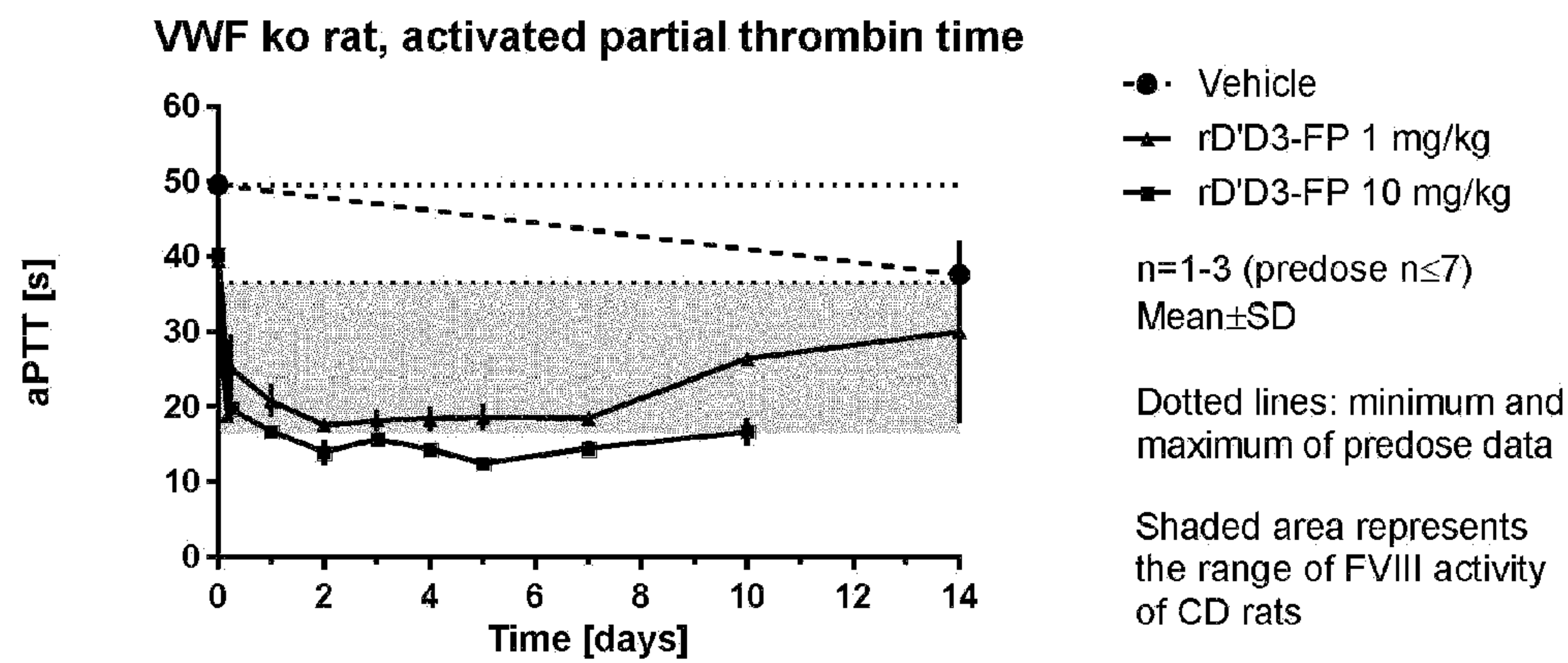


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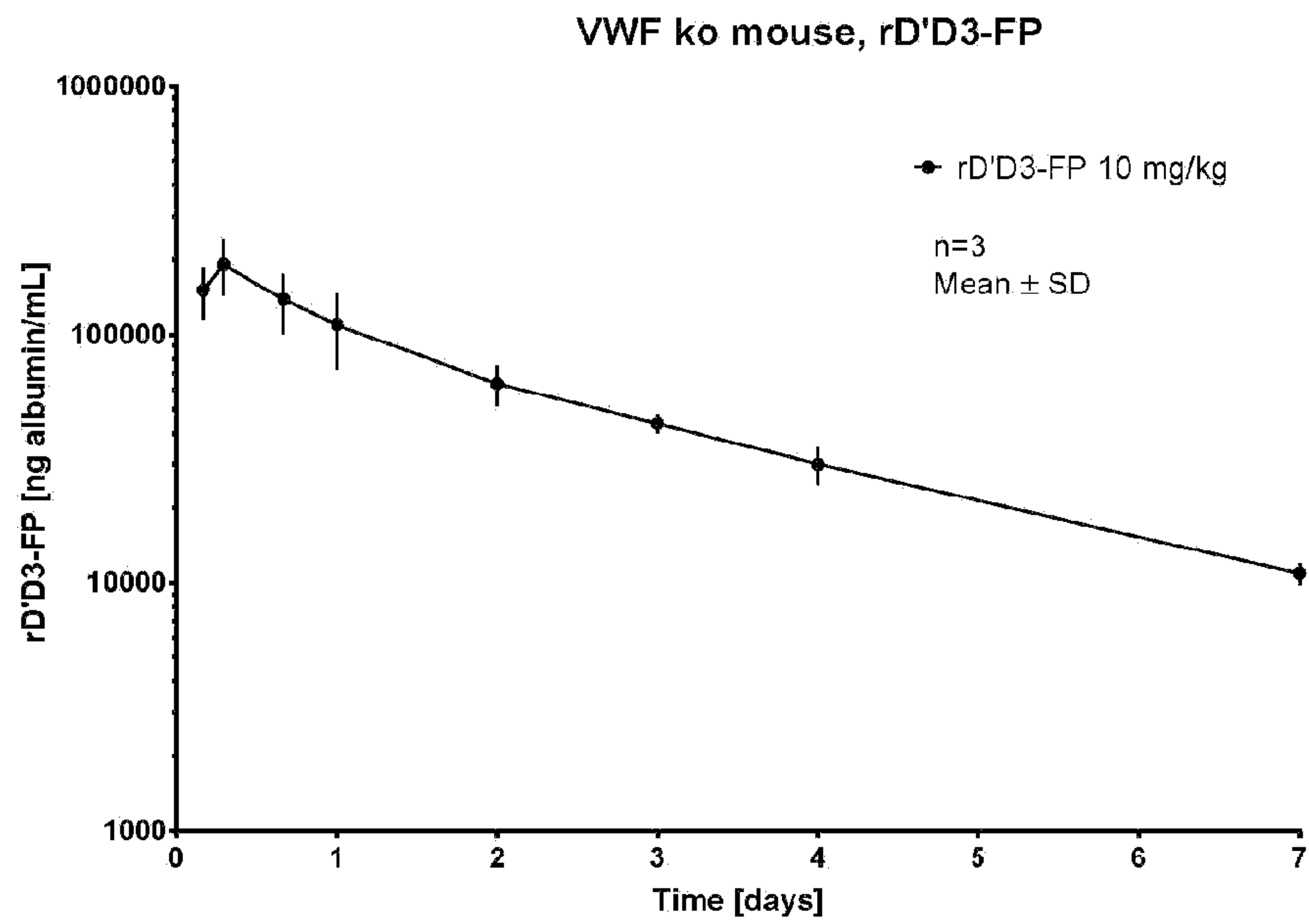


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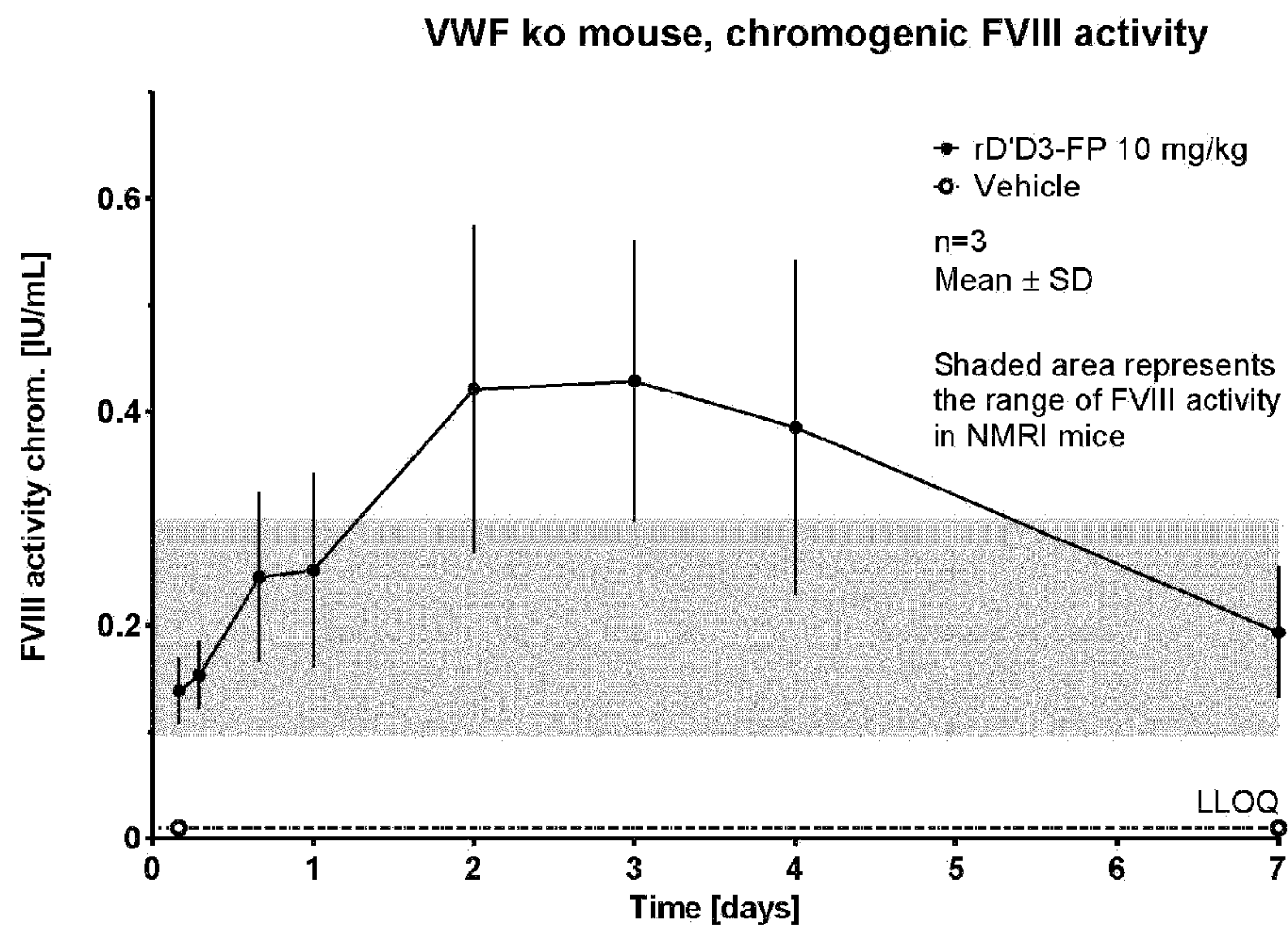


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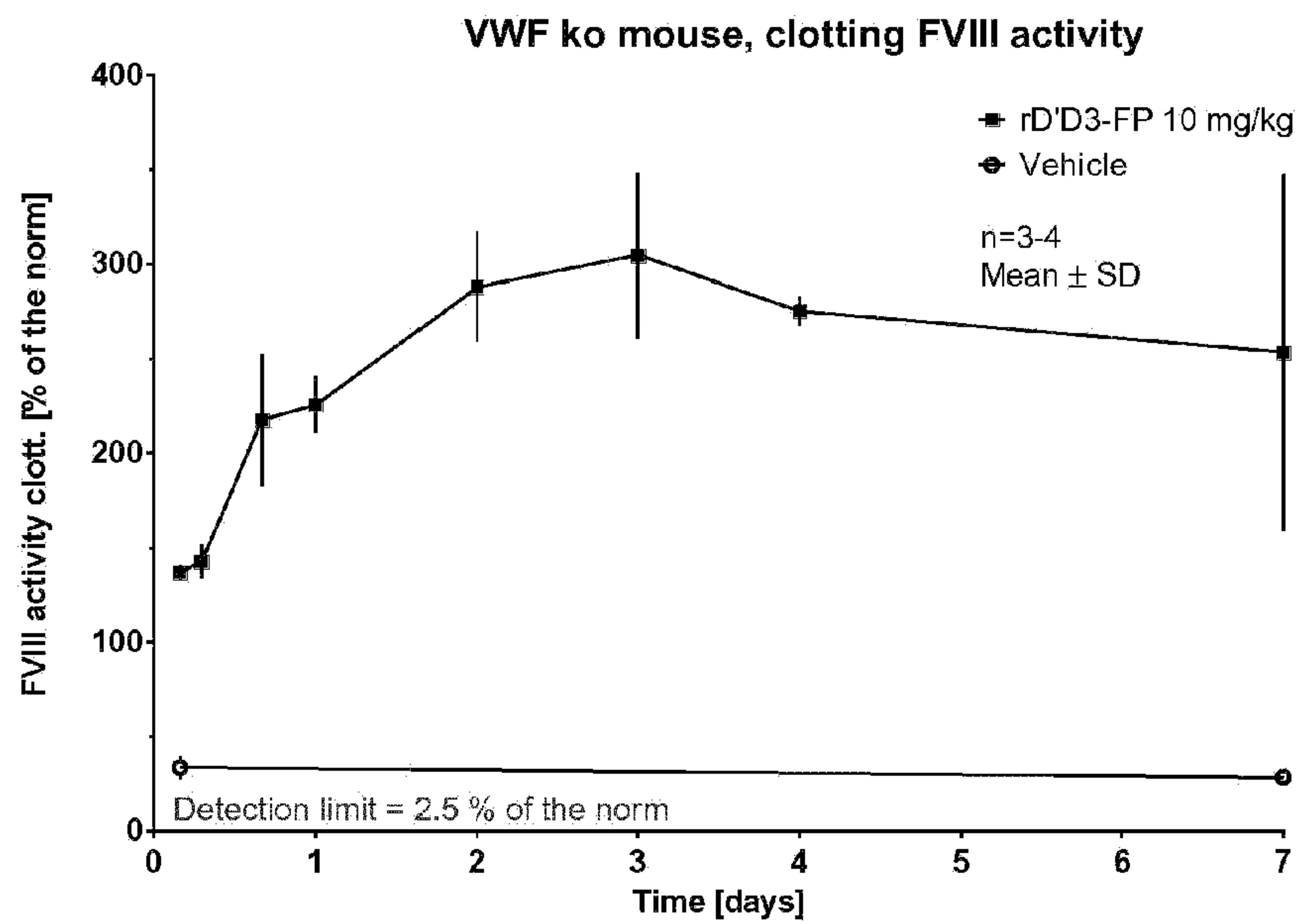


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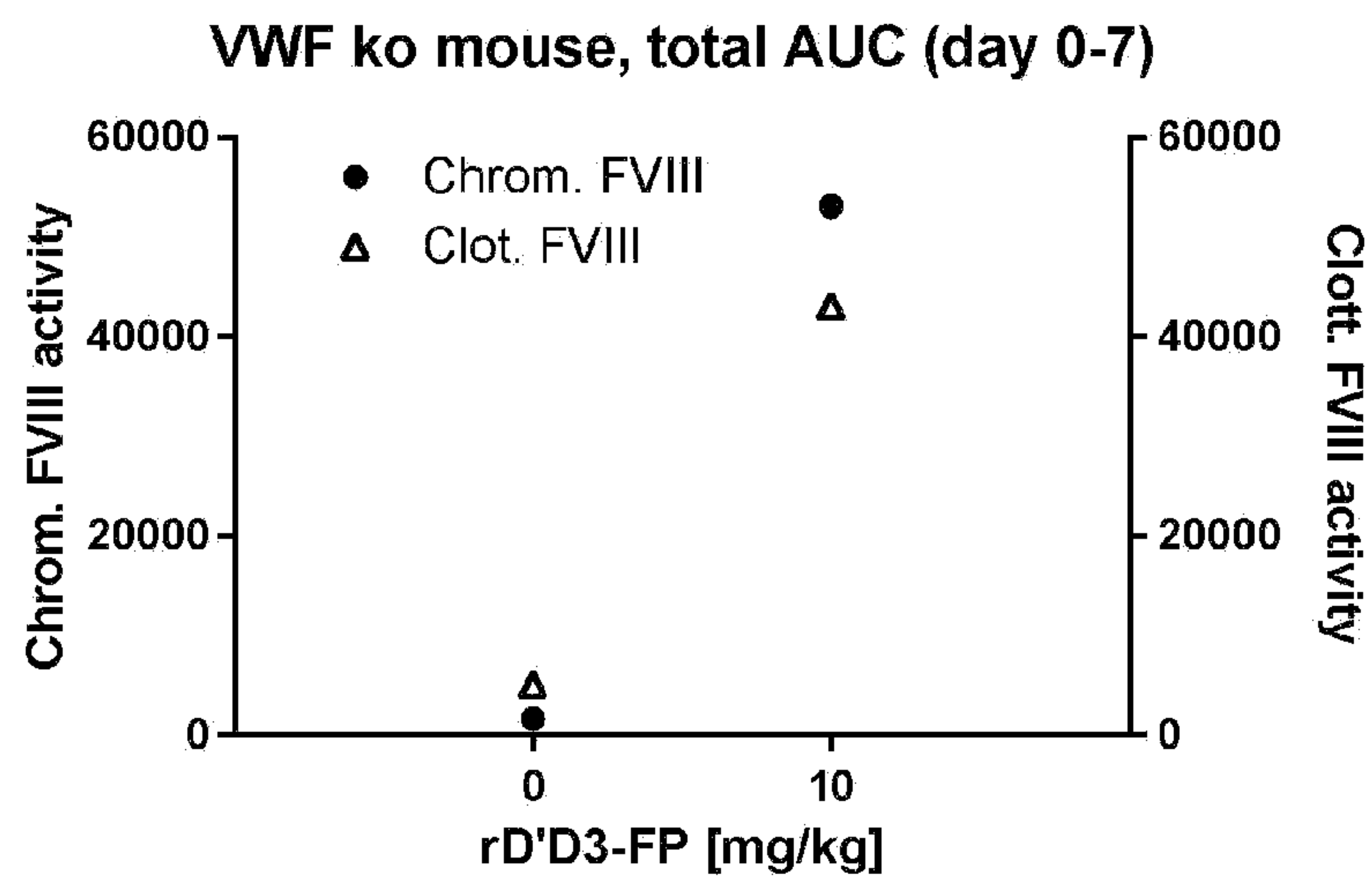


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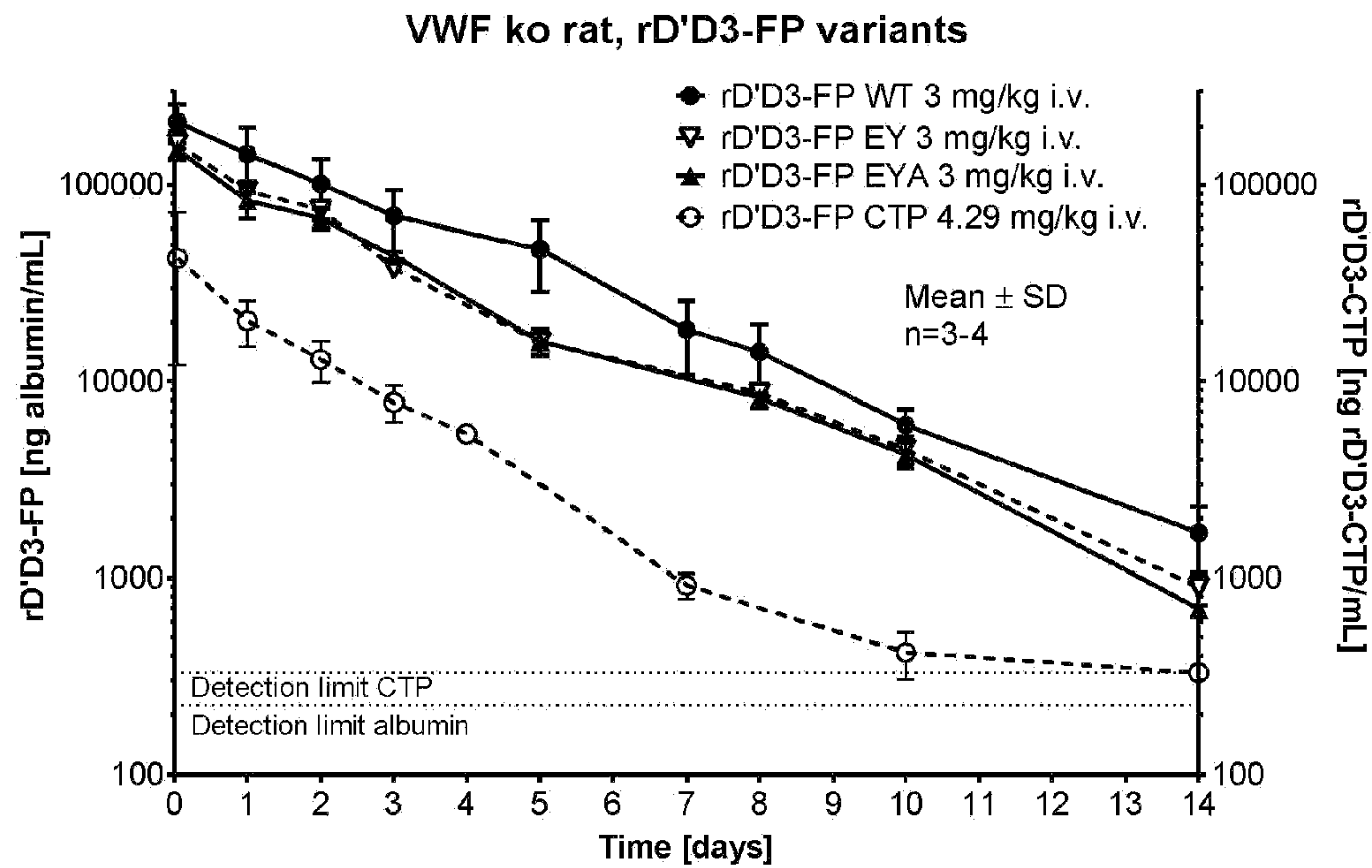


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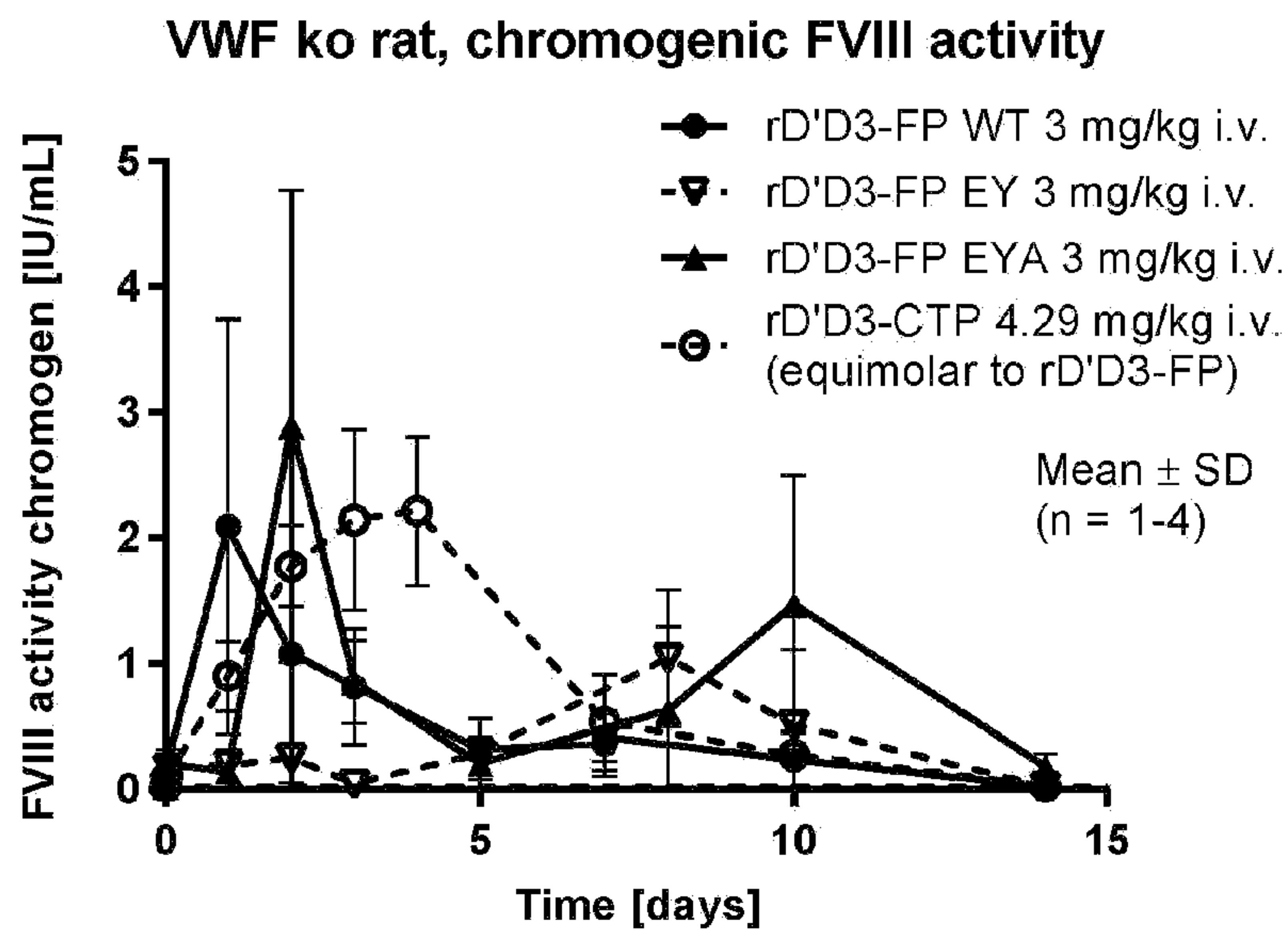


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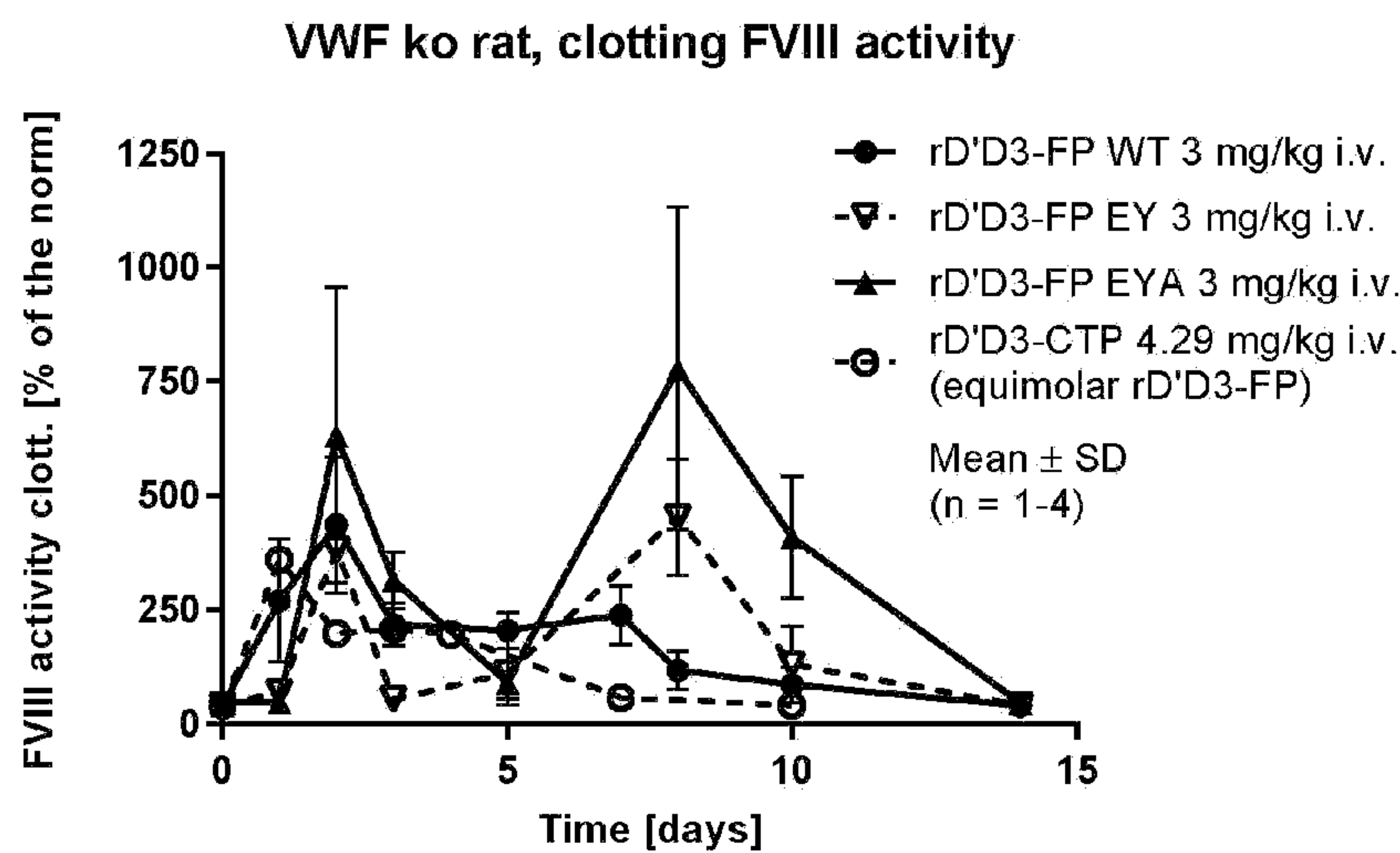


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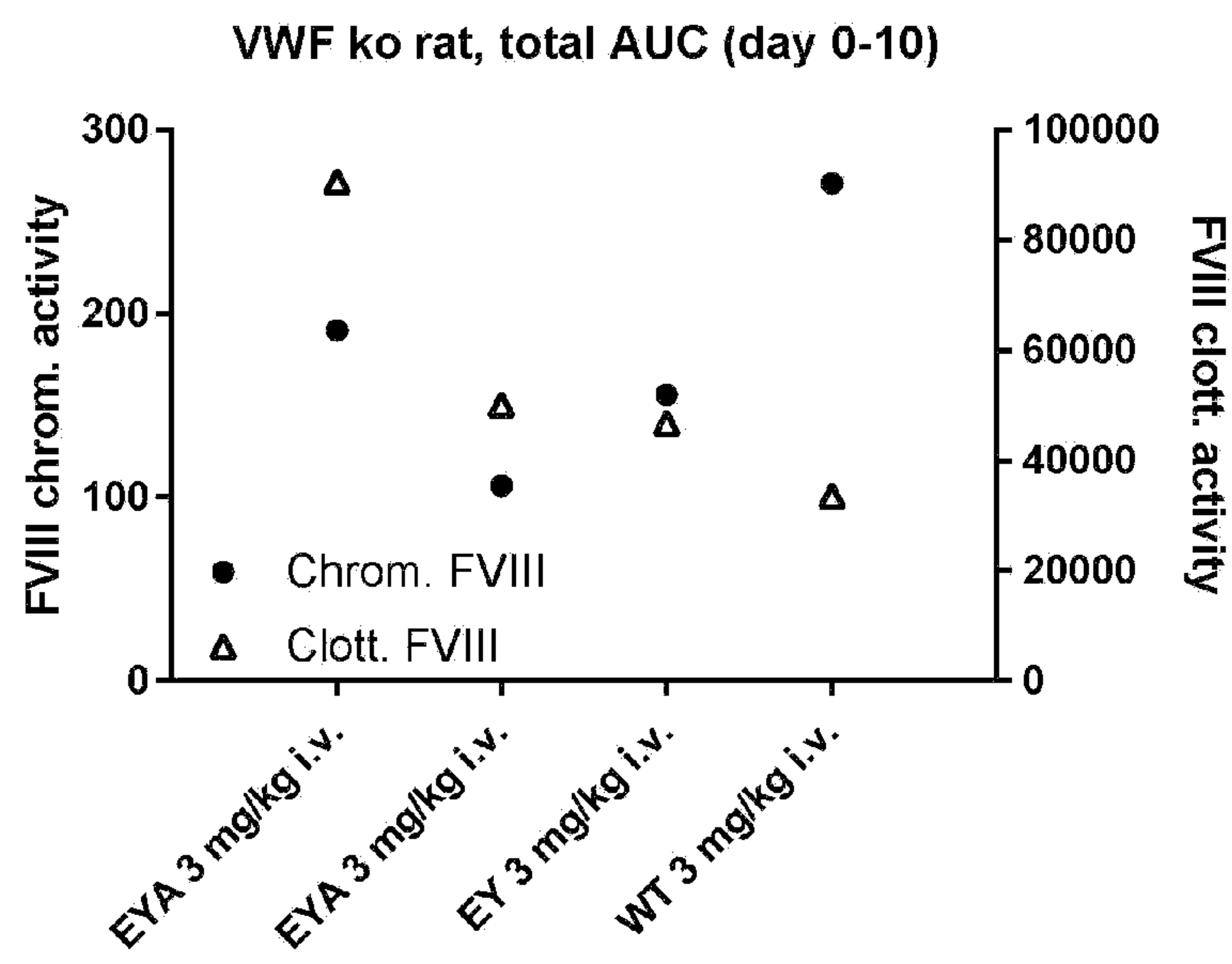


Figure 7A-1

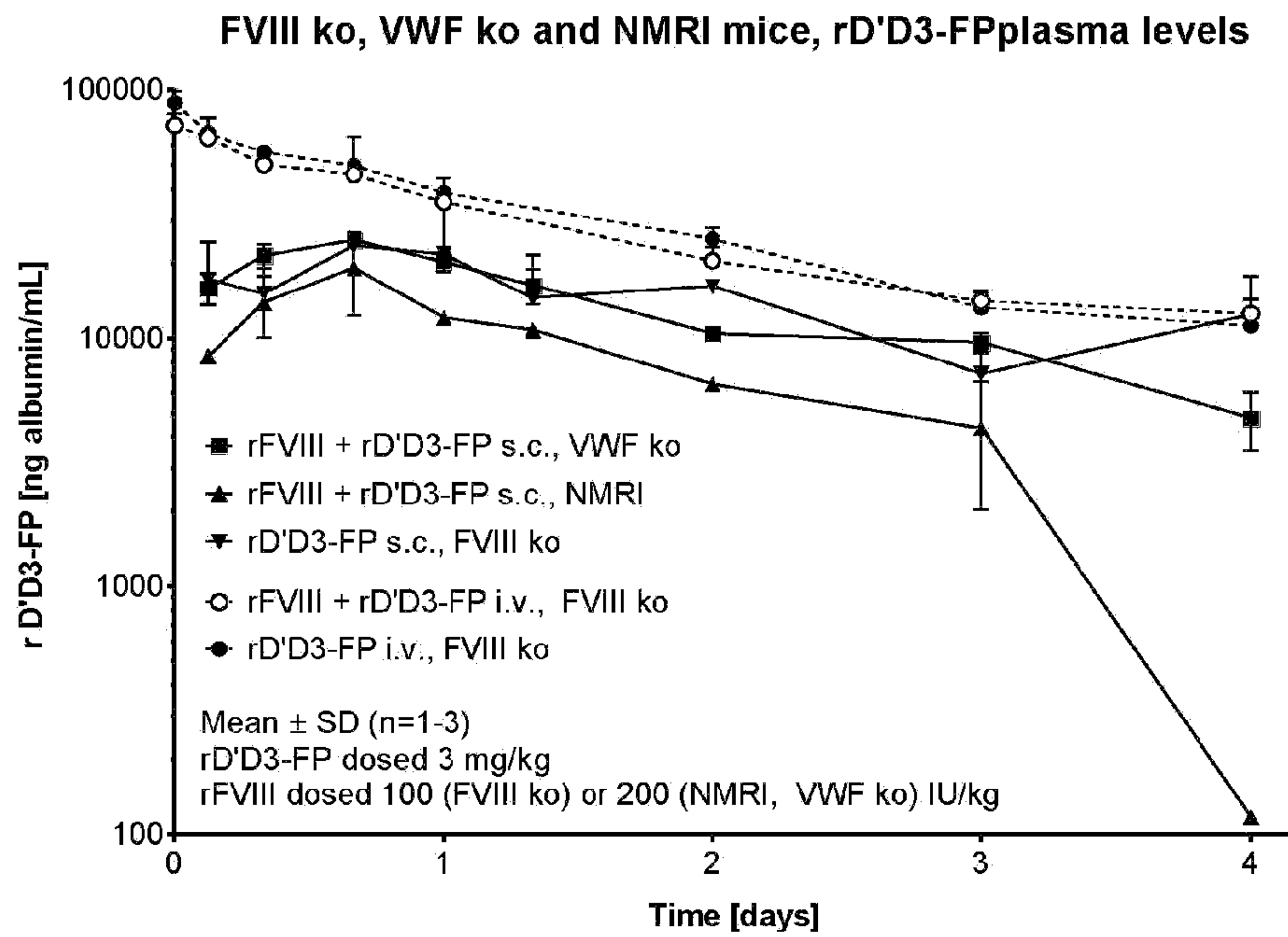


Figure 7A-2

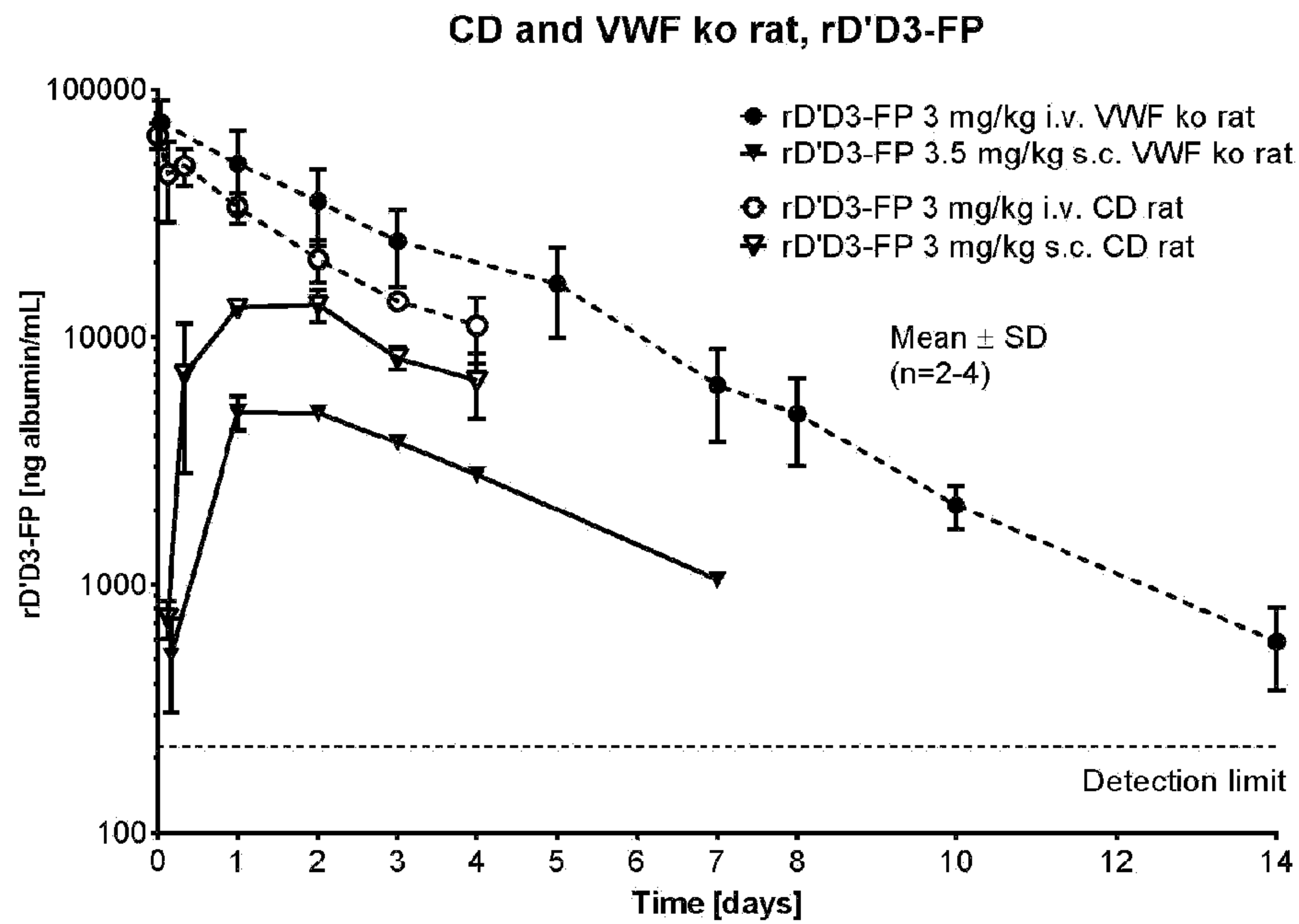


Figure 7A-3

Fig, rD'D3-FP plasma levels

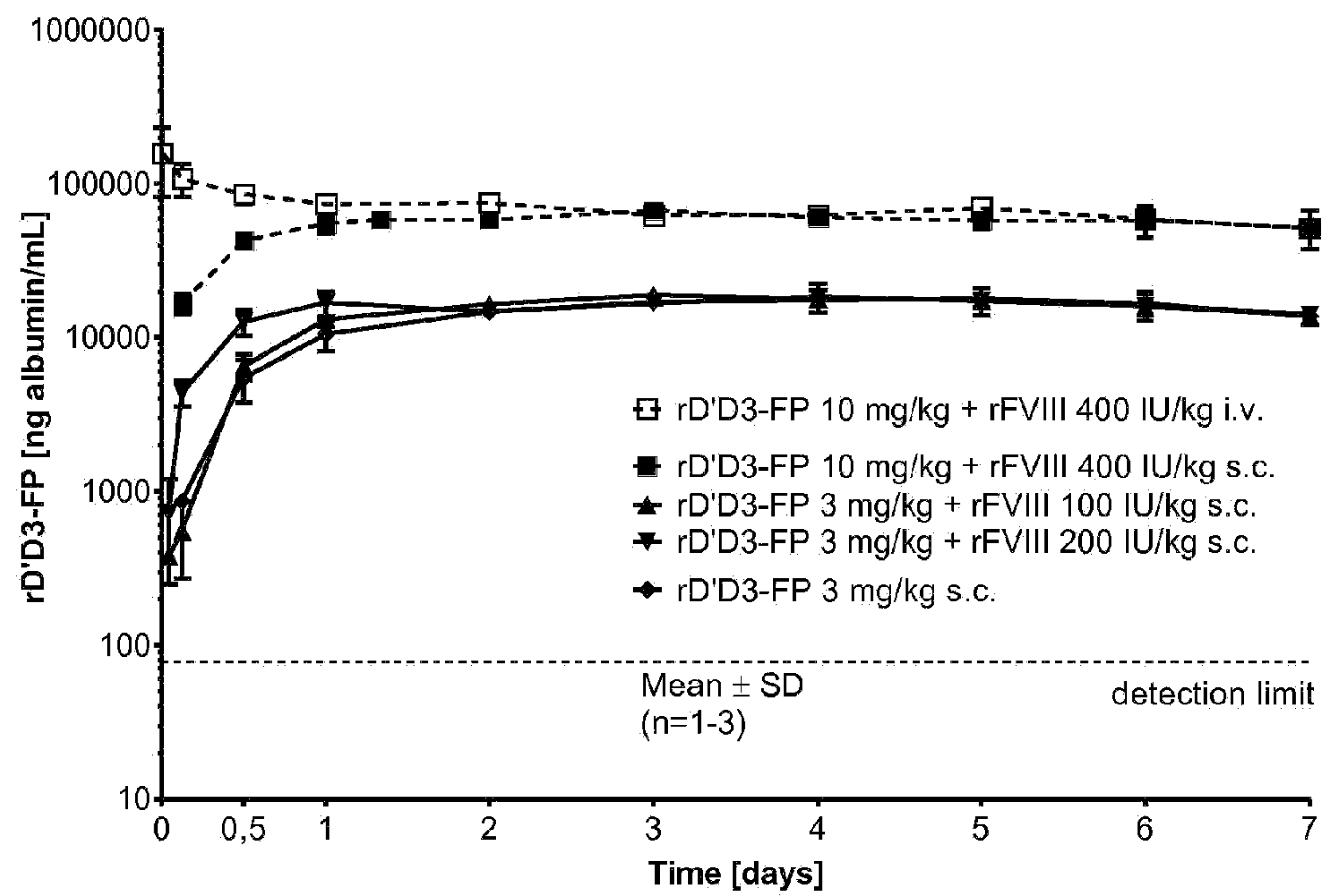


Figure 7B-1

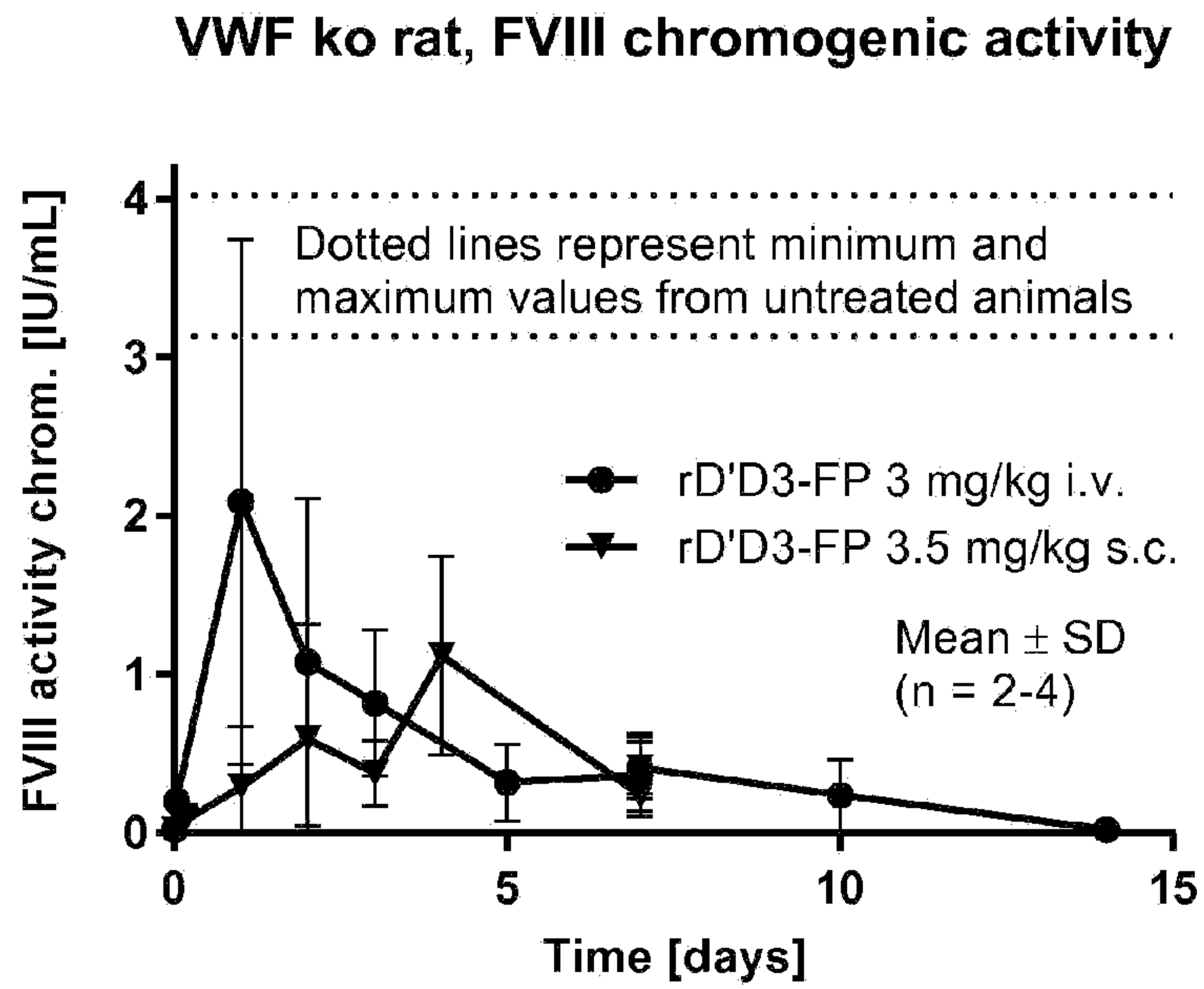


Figure 7B-2

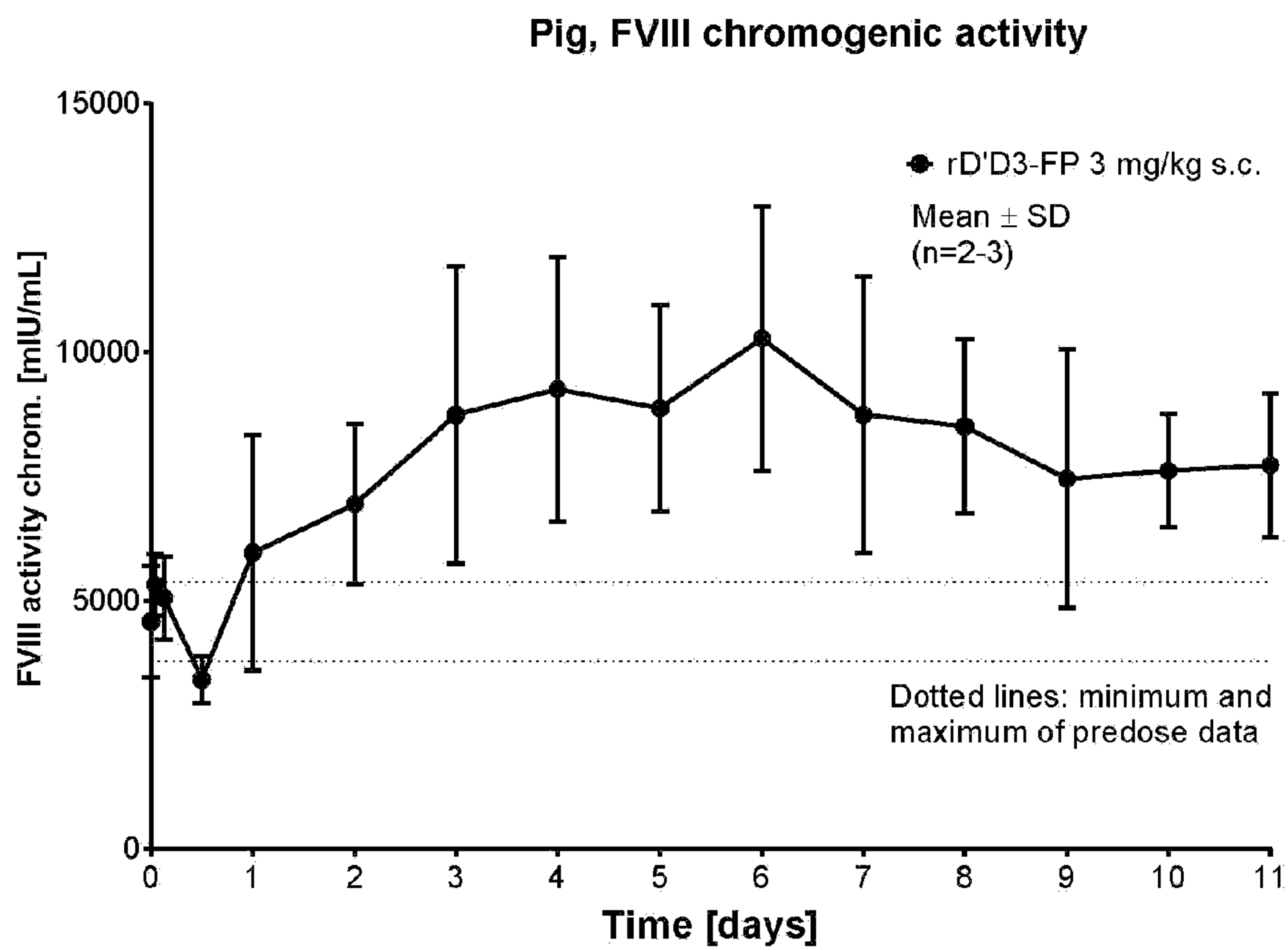


Figure 8A

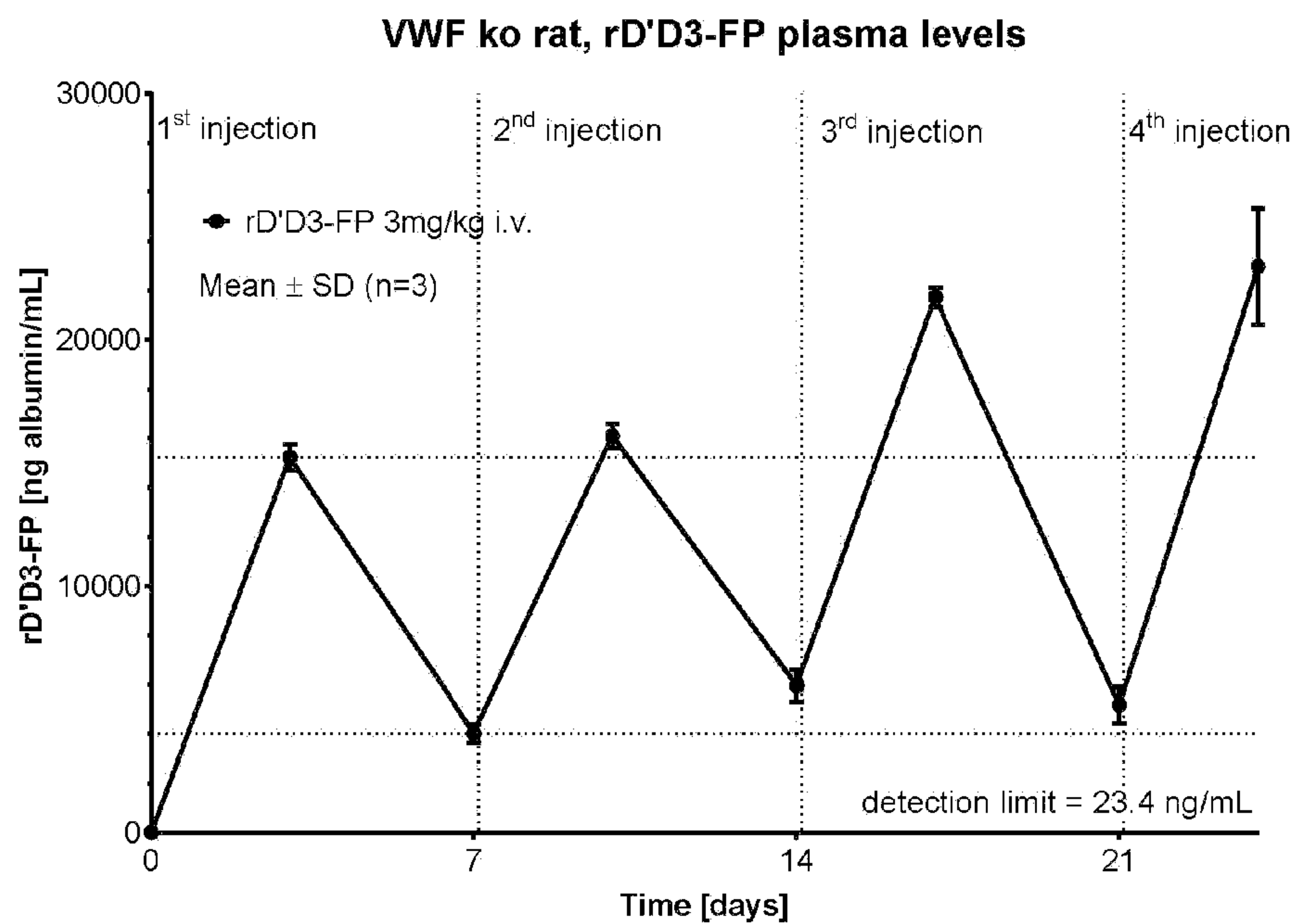


Figure 8B

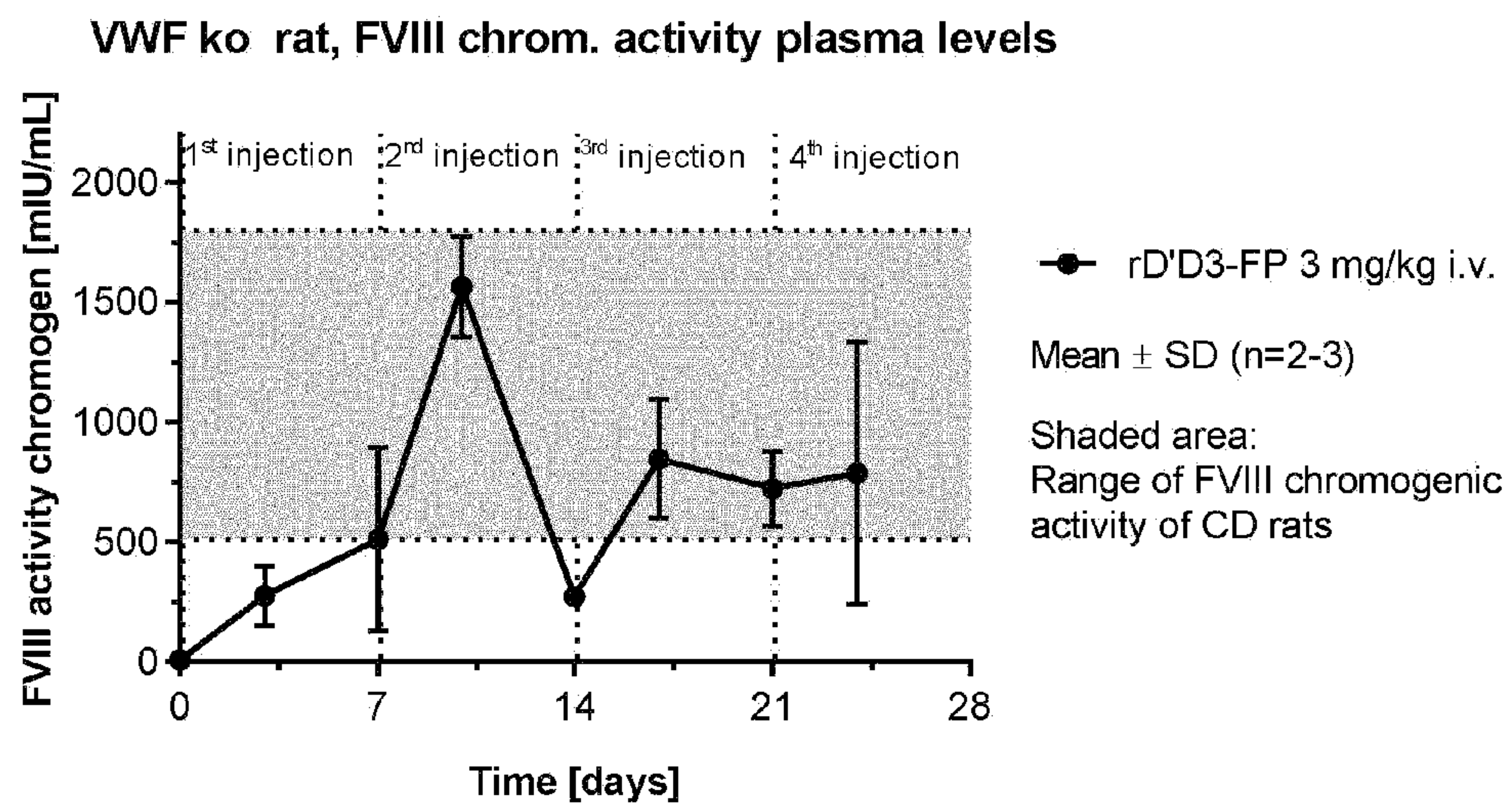


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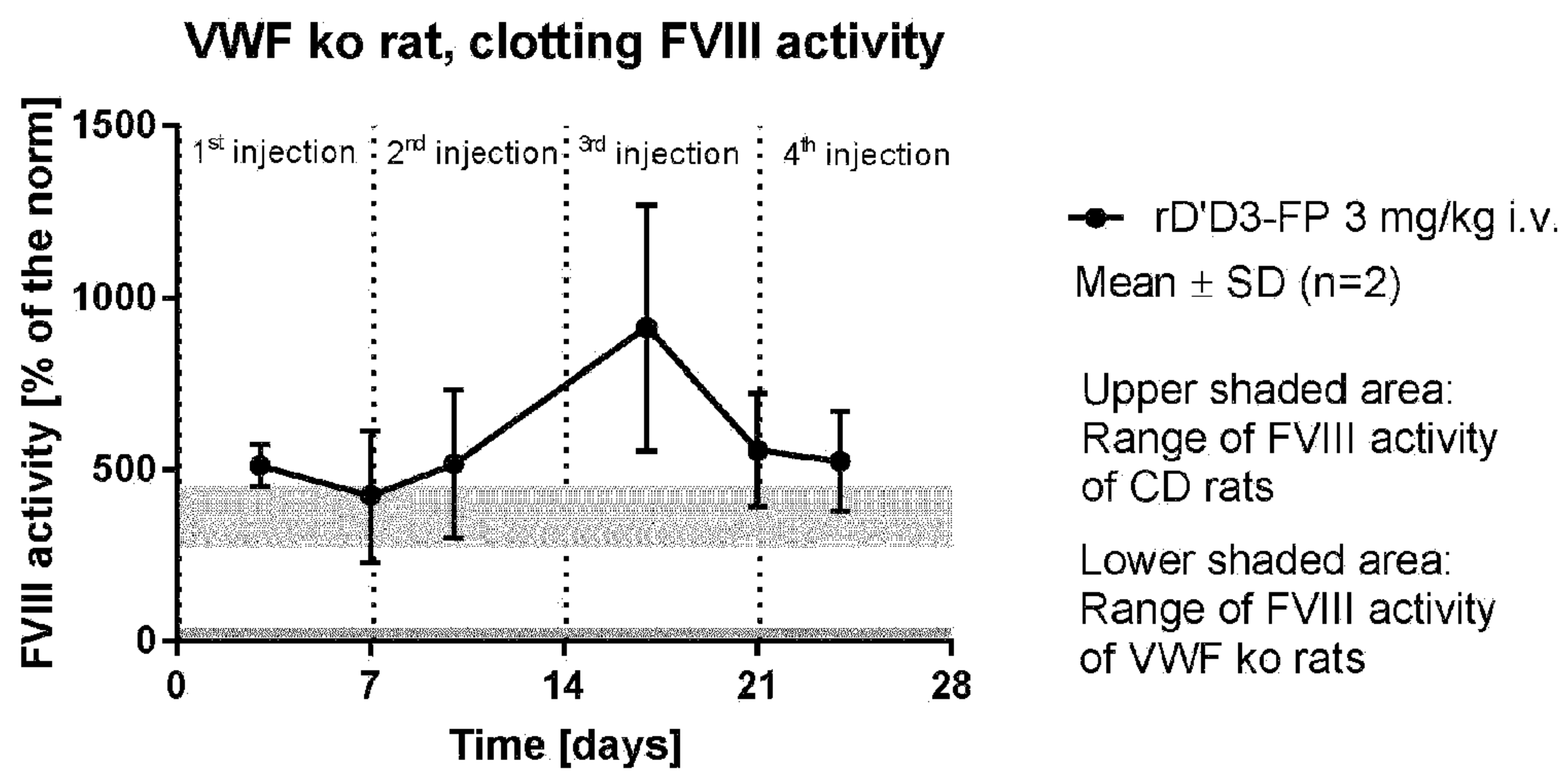


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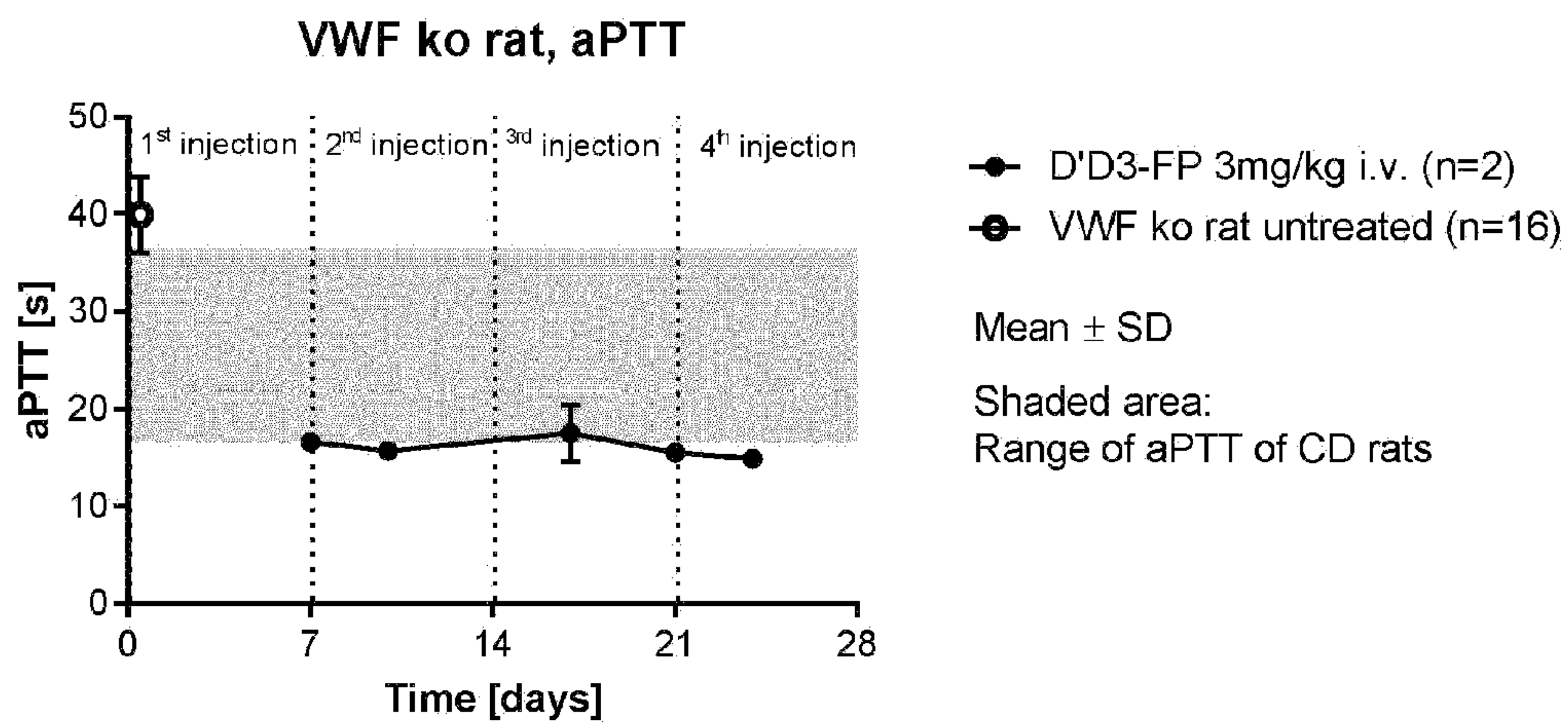


Figure 9A

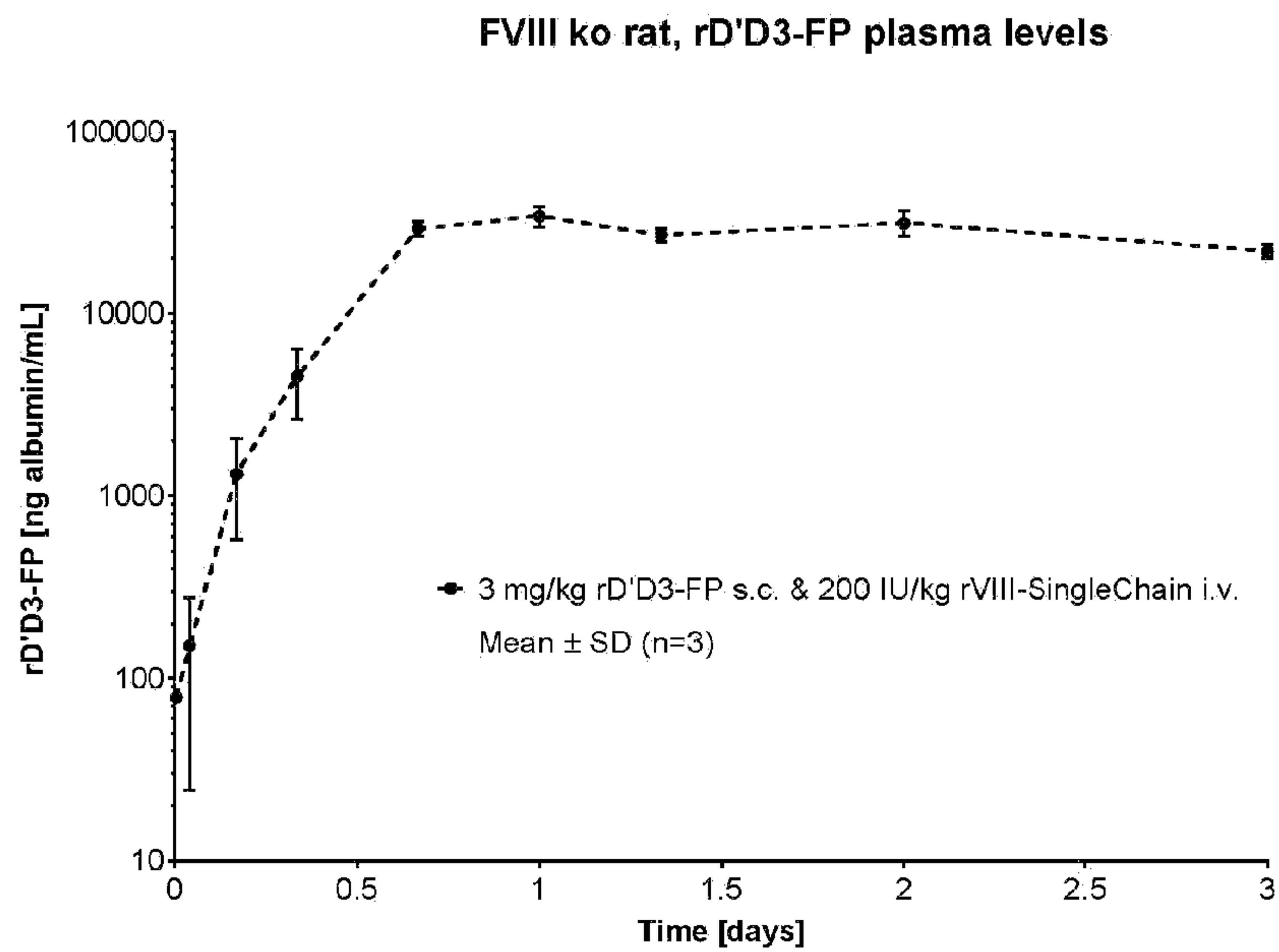


Figure 9B

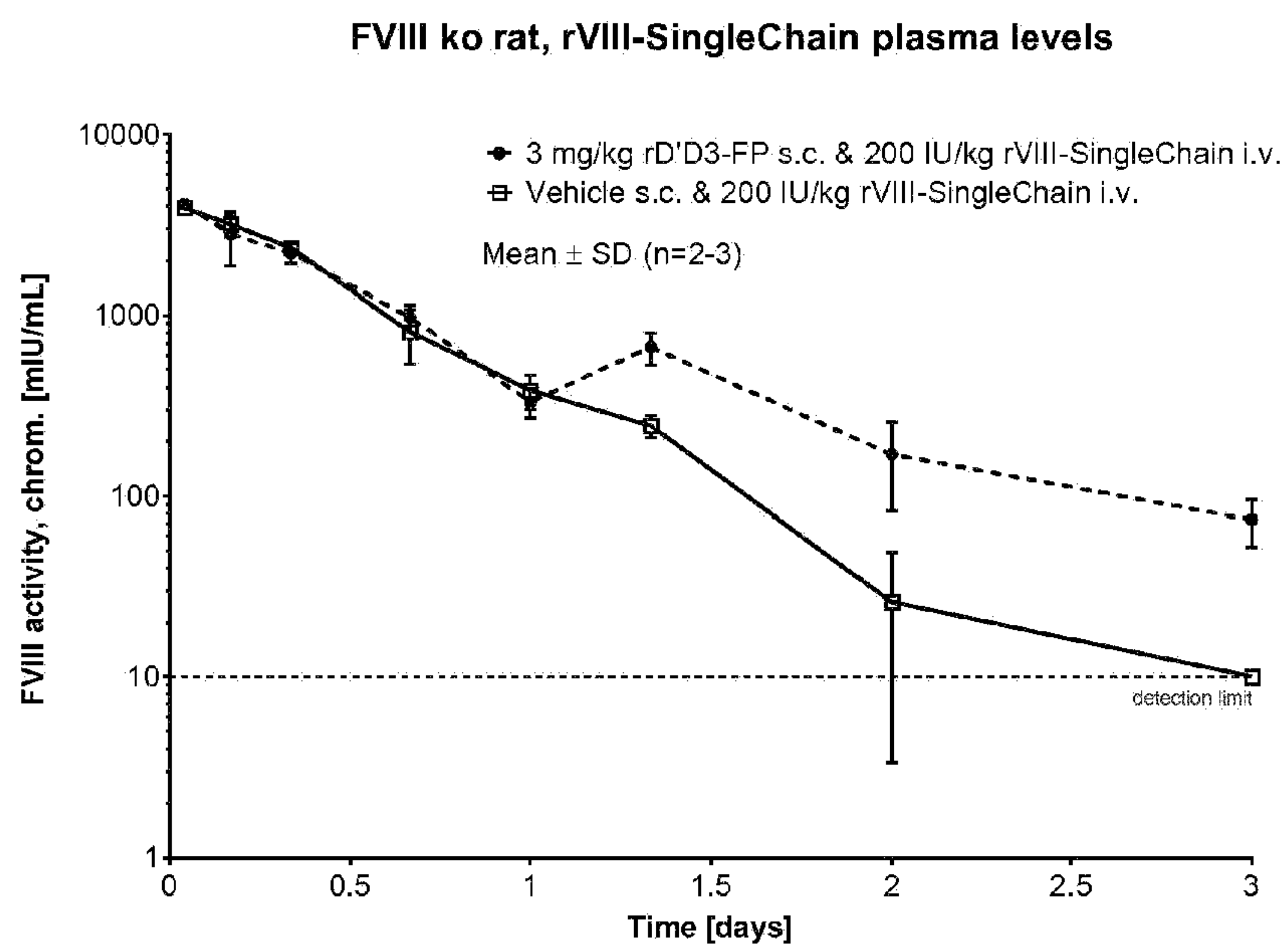


Figure 9C

