Figure 1: Different VWF Constructs

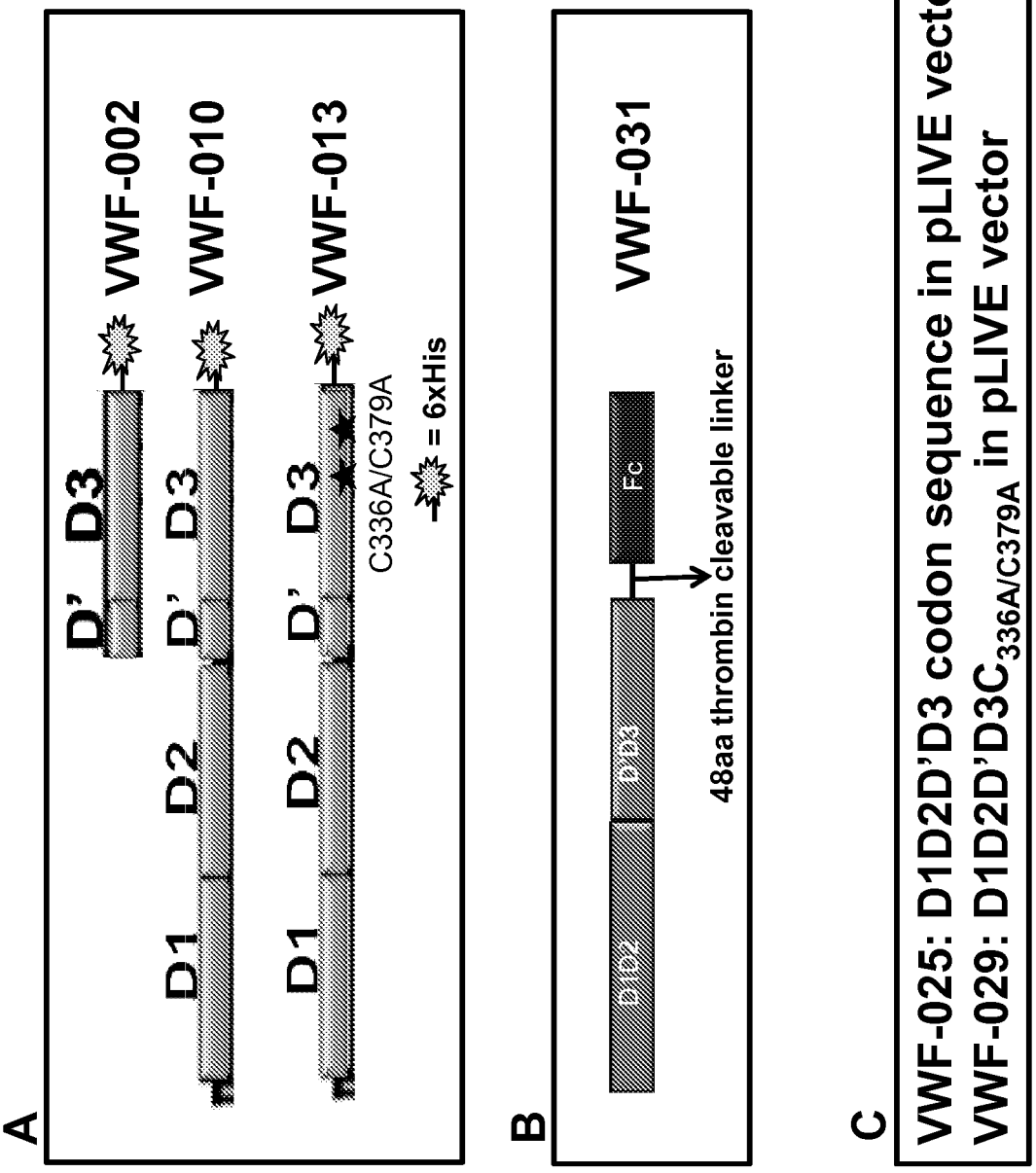
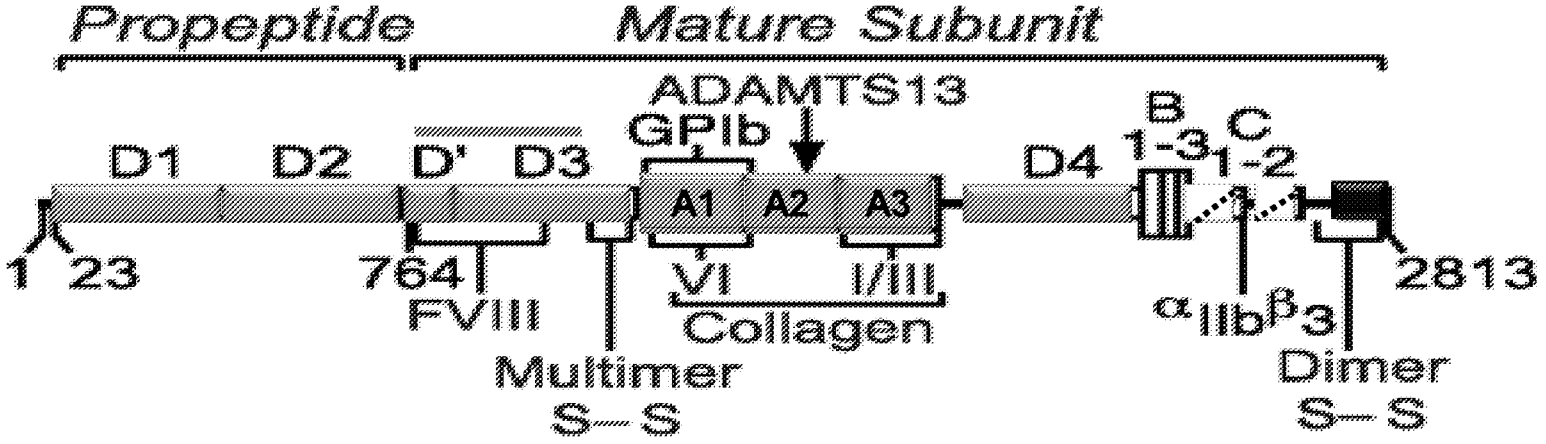


Figure 1D. Von Willebrand Factor



- ~ 250 kDa protein, forms multimers (> 20 MDa) by disulfide bonding

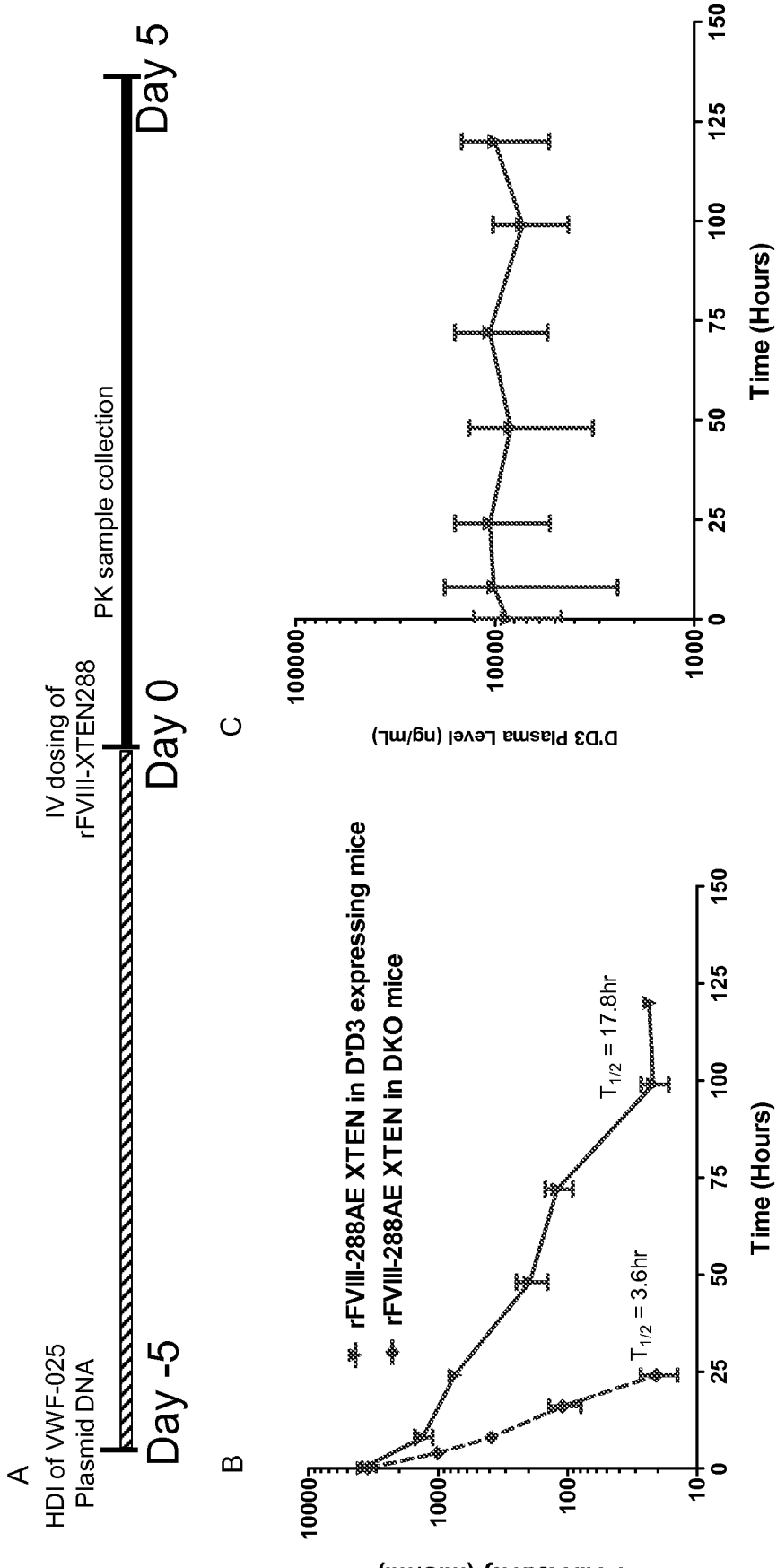
- Associates with FVIII (95-98%) in non-covalent complex
  - Protects FVIII from protease cleavage/activation
  - Stabilizes heavy & light chain
  - Prevents clearance of FVIII by scavenger receptors

  } **Extends half-life**

  - Clearance of FVIII-vWF complex through vWF-receptors
  - Prevents pinocytosis and recycling of rFVIIIFc?

  } **Limits half-life**

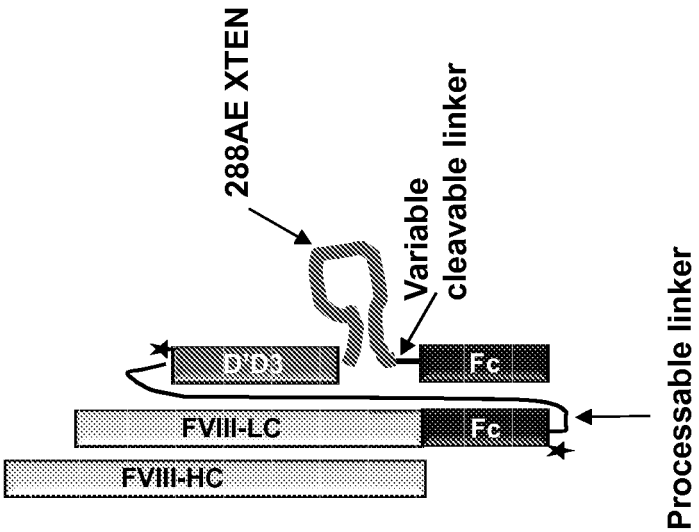
Figure 2: rFVIII-XTEN PK in VWF D'D3 Expressing Mice



5 fold additional half-life extension of FVIII-XTEN from VWF D'D3 fragment

Figure 3: FVIIIFC/VWF heterodimer Constructs (variable linker)

DNA construct	Linker between VWF and Fc
FVIII-161	288AE XTEN + IS{5X(GGGGS)}LVPRGSGG
D'D3= 1-477aa with C336A/C379A)	



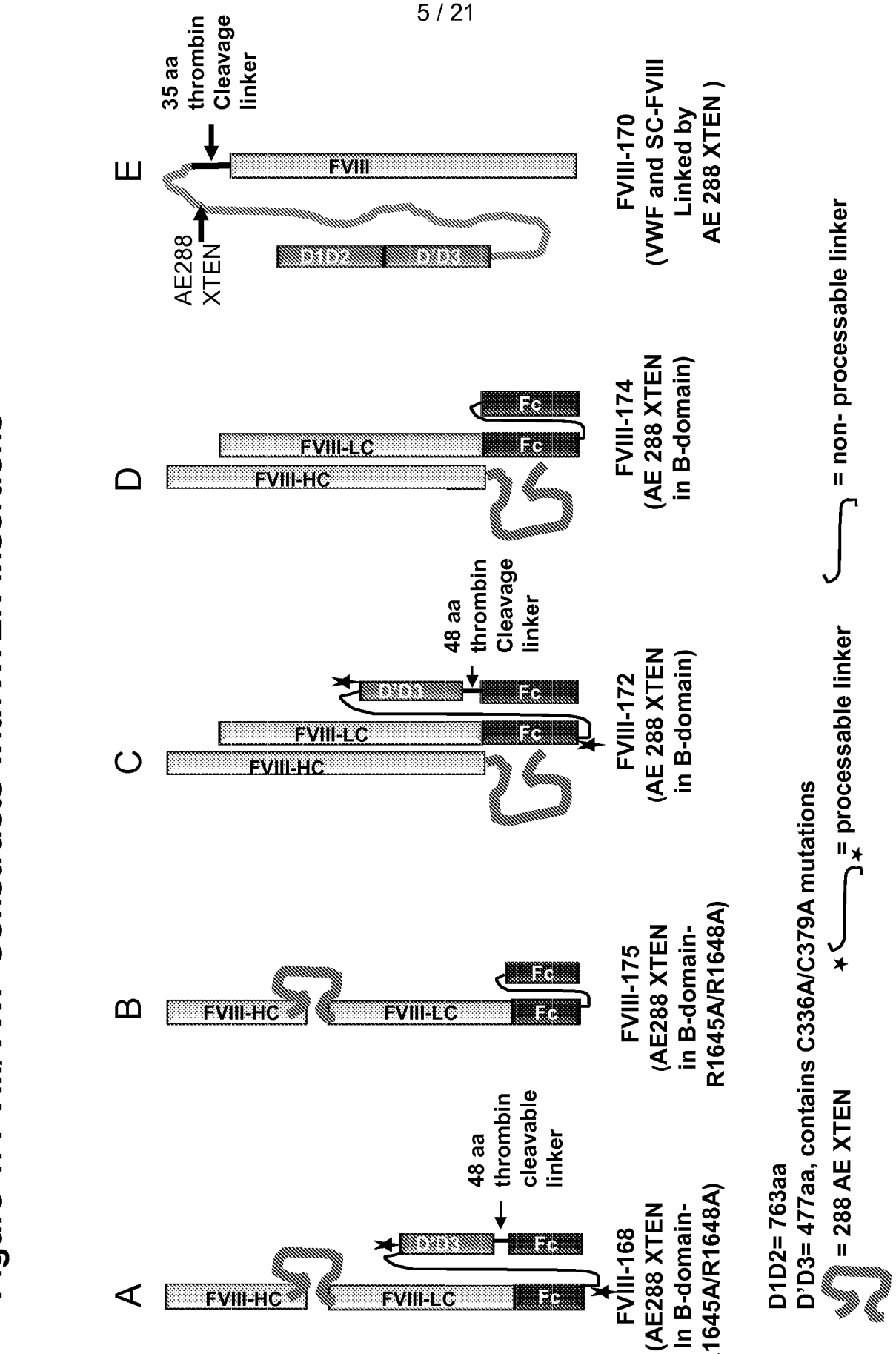

Figure 4: FVIII/VWF Constructs with XTEN insertions
A
FVIII-HC
FVIII-LC
Fc
D'D3
48 aa thrombin cleavable linker
FVIII-168 (AE288 XTEN In B-domain-R1645A/R1648A)
B
FVIII-HC
FVIII-LC
Fc
FVIII-175 (AE288 XTEN in B-domain-R1645A/R1648A)
C
FVIII-HC
FVIII-LC
Fc
D'D3
48 aa thrombin Cleavage linker
FVIII-172 (AE 288 XTEN in B-domain)
D
FVIII-HC
FVIII-LC
Fc
FVIII-174 (AE 288 XTEN in B-domain)
E
AE288 XTEN
D1D2
D'D3
FVIII
35 aa thrombin Cleavage linker
FVIII-170 (VWF and SC-FVIII Linked by AE 288 XTEN)
D1D2= 763aa
D'D3= 477aa, contains C336A/C379A mutations
= 288 AE XTEN
= processable linker
= non- processable linker

Figure 5: XTEN insertion improves the PK of FVIII/VWF heterodimers

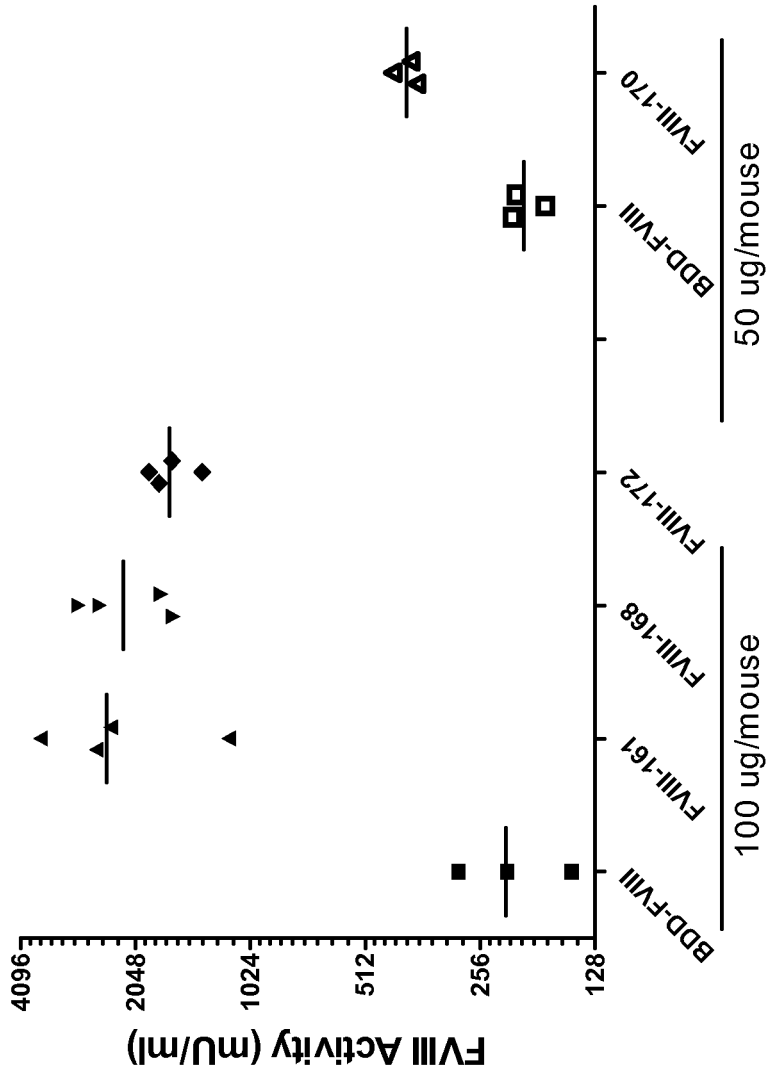
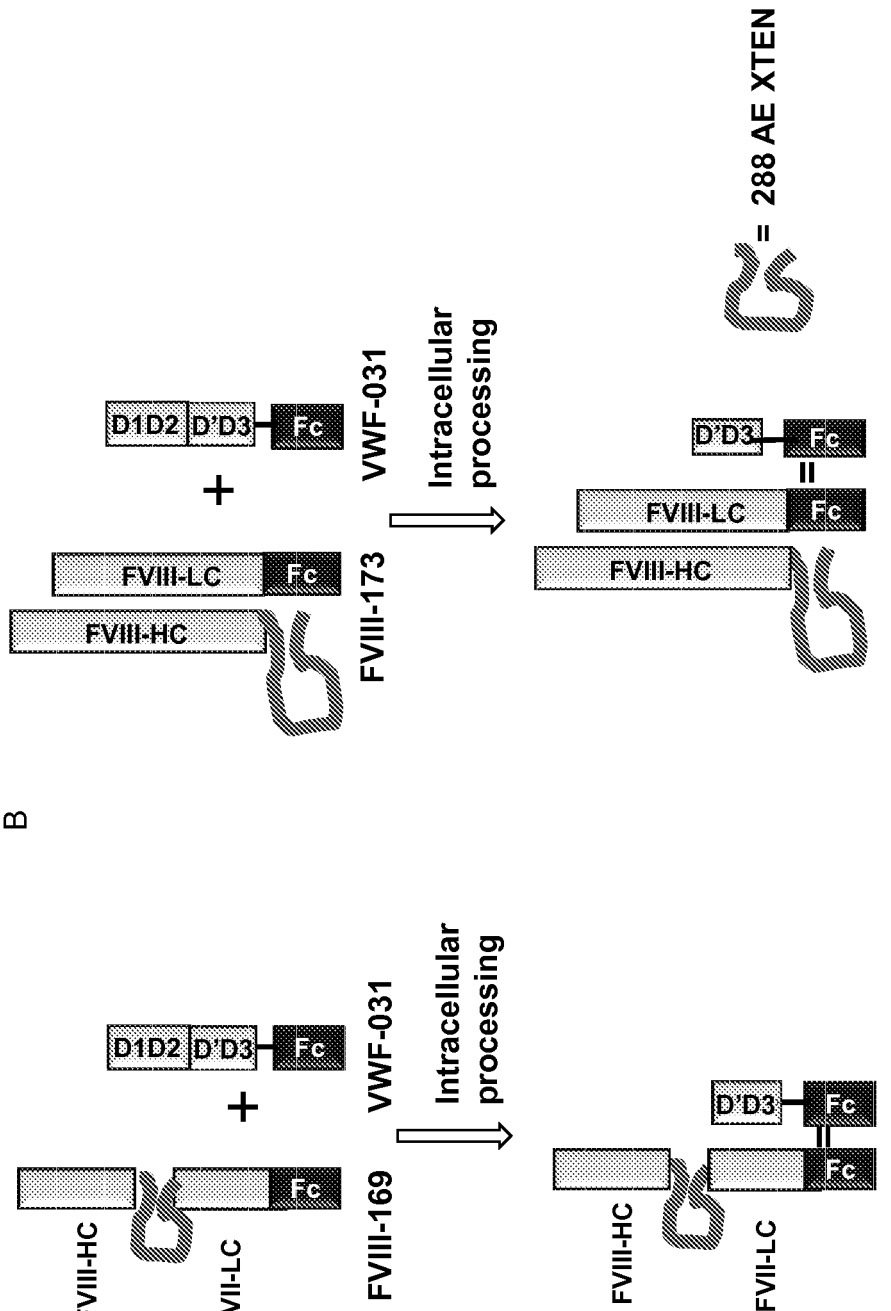


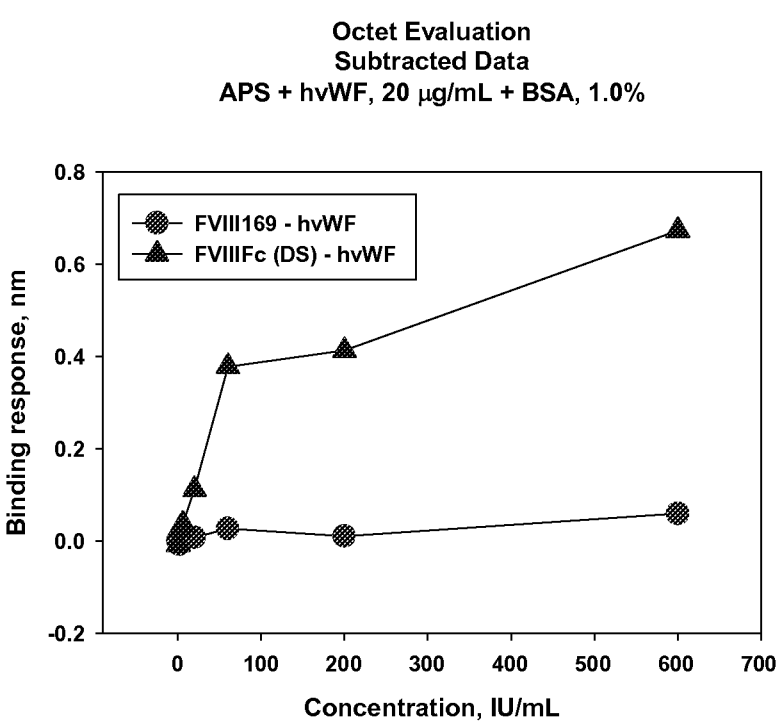
Figure 6: FVIII Constructs (Co-transfection system)



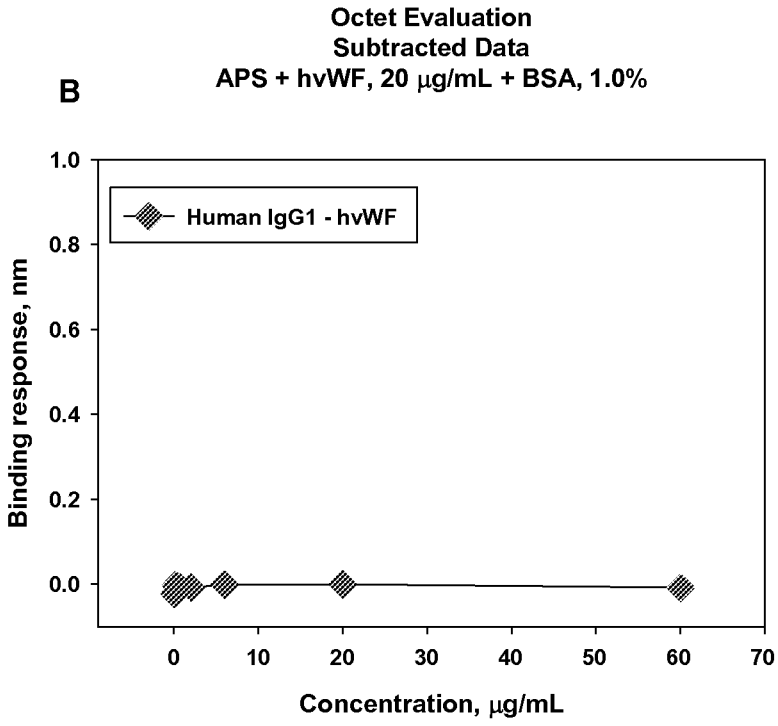
FVIII-169	SC- FVIII R1645A/R1648A with 288 AE XTEN B-domain insertion directly fused to a single Fc
FVIII-173	Dual chain - FVIIIwith288 AE XTEN B-domain insertion directly fused to a single Fc
VWF-031	D1D2D'D3 (763aa-477aa /C336A/C379A) fragment linked to a single Fc with a 48aa thrombin cleavage linker

Figure 7: FVIII-169/VWF-31 has no detectable binding toward immobilized hVWF

A



B



Analyte	APS Probe + hVWF	Protein G Probe
FVIII-169/VWF-031	-	+
rFVIIIFc	+	+
Human IgG1	-	+

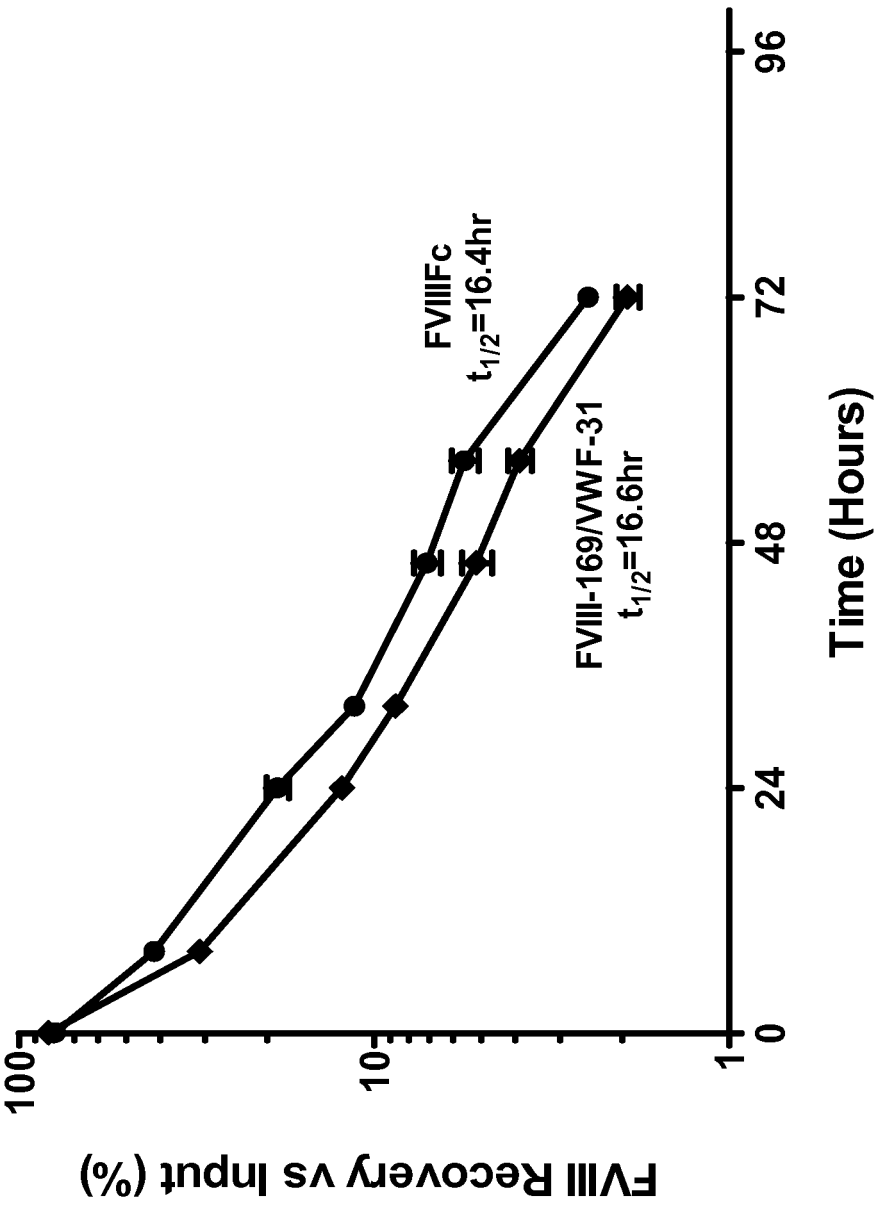


Figure 8A: FVIII-169/VWF-031 PK in HemA

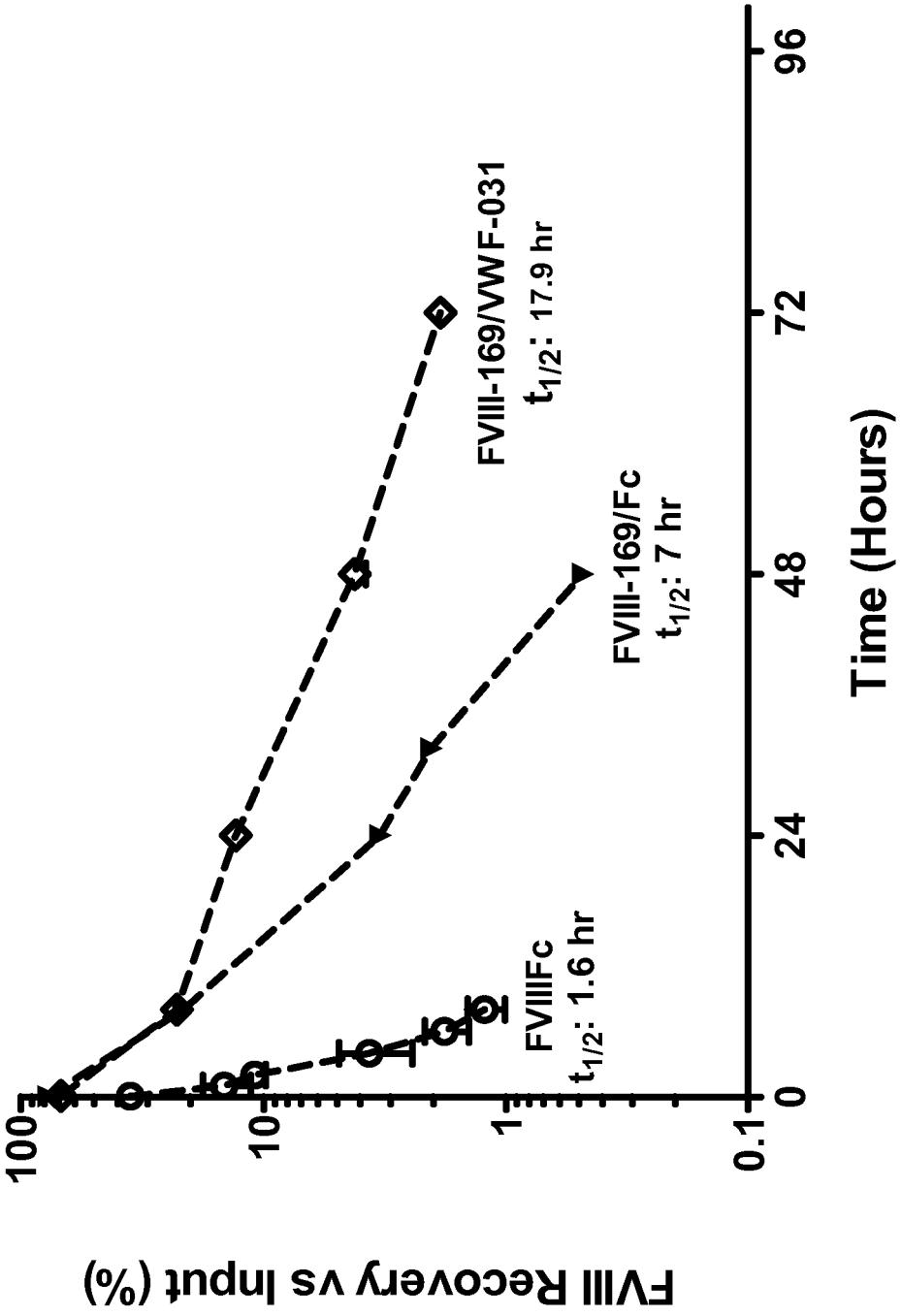


Figure 8B: FVIII-169/VWF-031 PK in FVIII/VWF DKO Mice

Figure 9A: FVIII-XTEN variants half-life in D'D3 expressing FVIII/VWF DKO mice

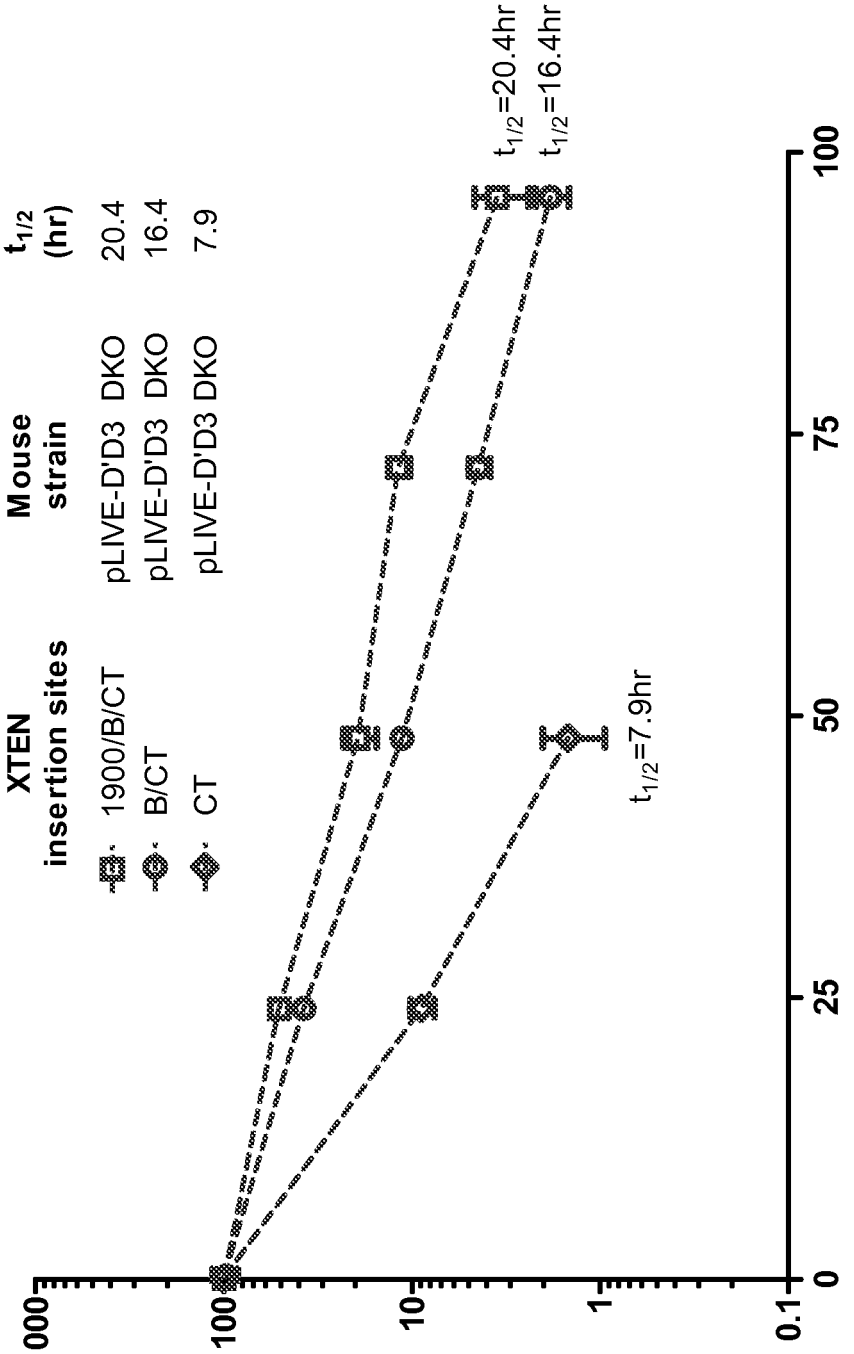


Figure 9B: PK of FVIII-XTEN with three XTEN insertions

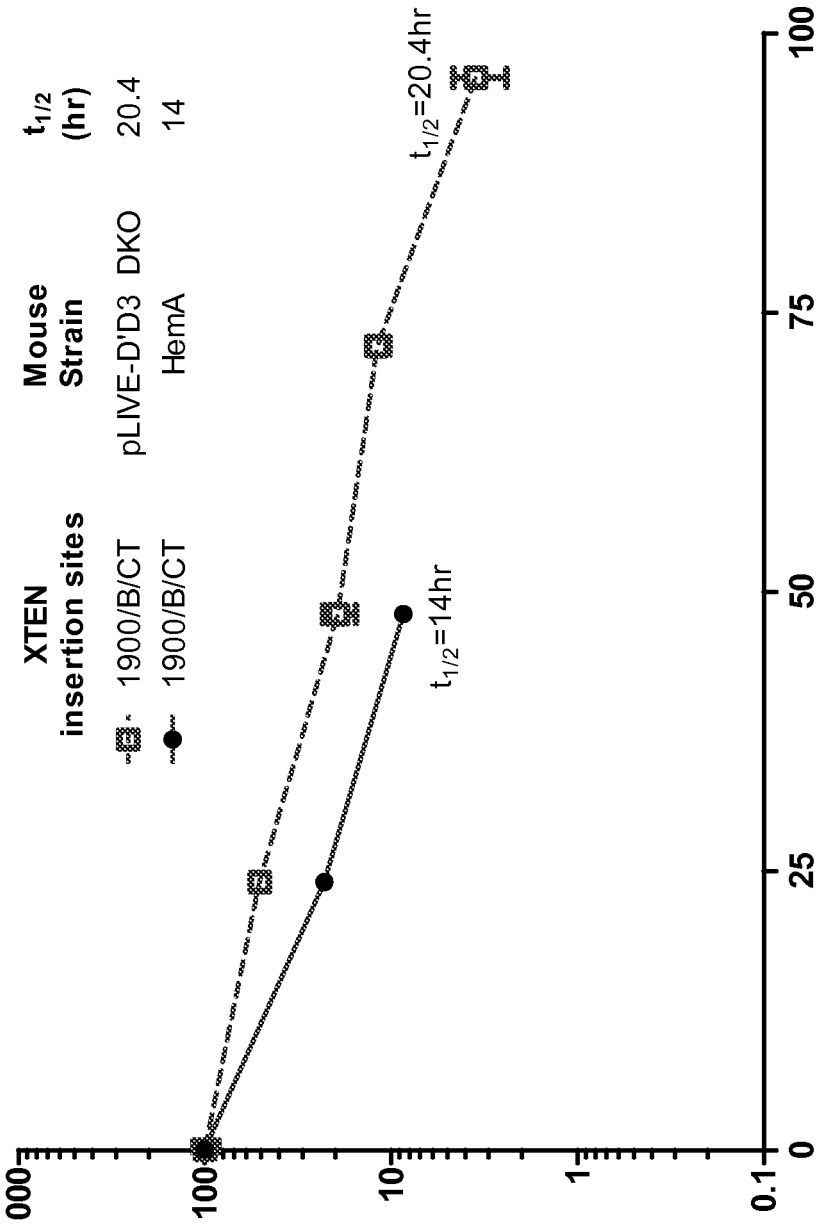
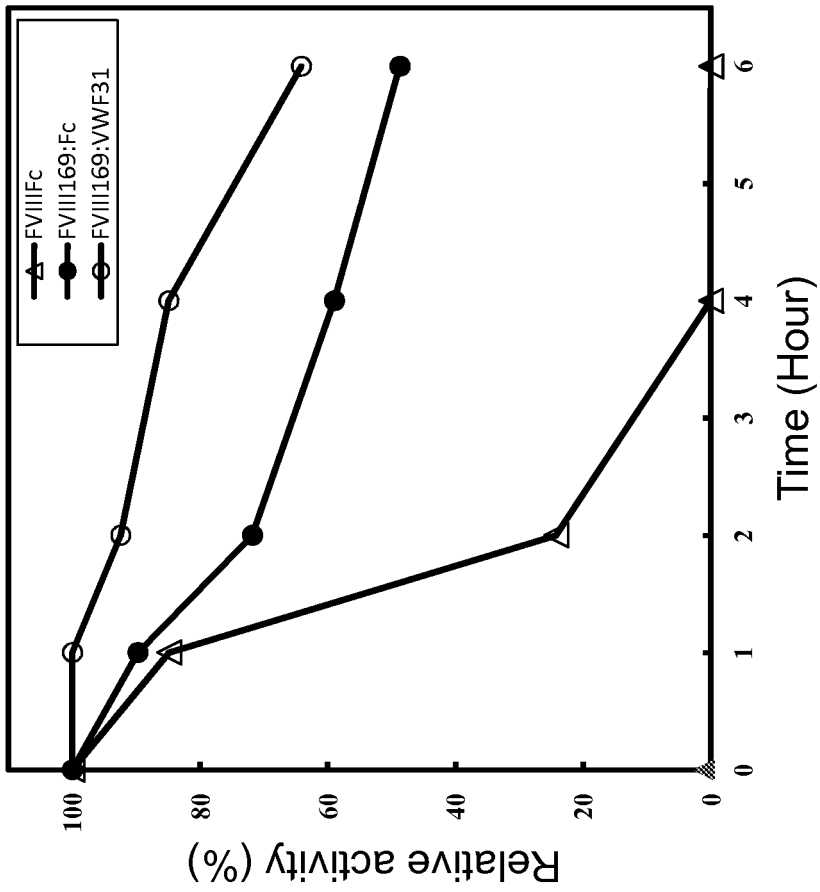


Figure 10: FVIIIFc activity in mouse DKO plasma



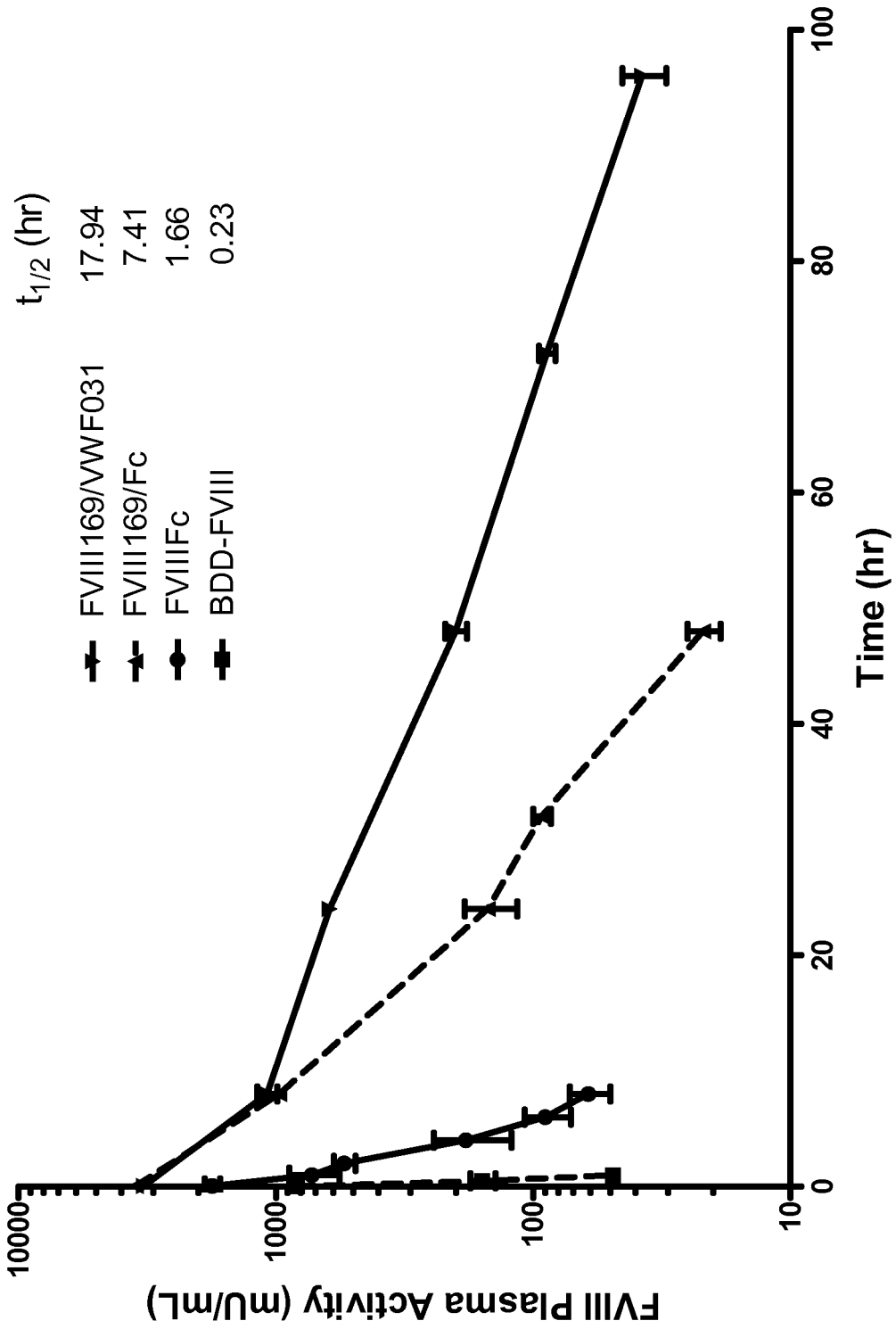


Figure 11: Additive effect of Fc, XTEN and VWF-D'D3 fragment on FVIII half-life extension

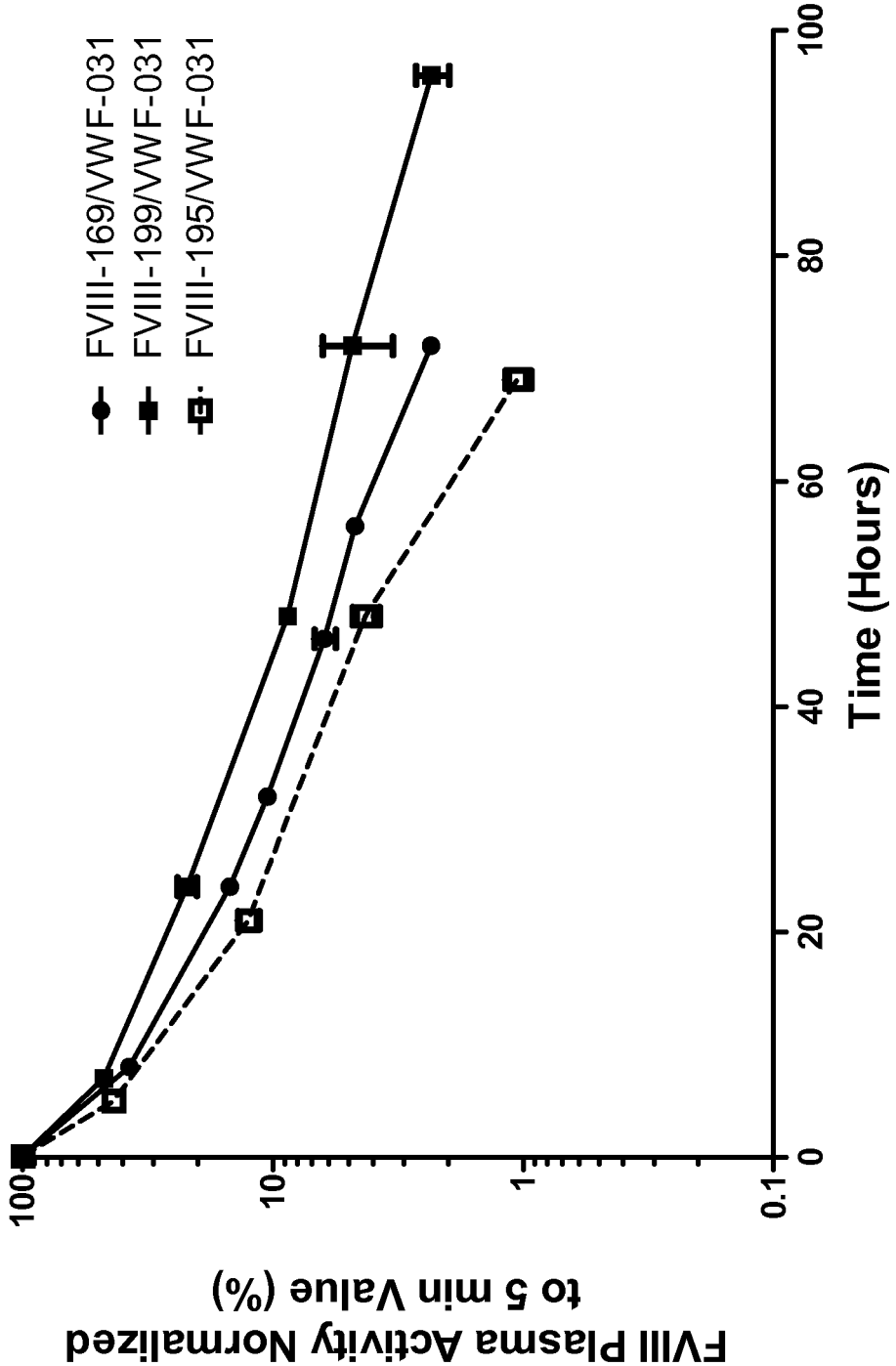


Figure 12A: Effect of different XTEN on rFVIII-XTEN/VWF heterodimer PK in HemA mice

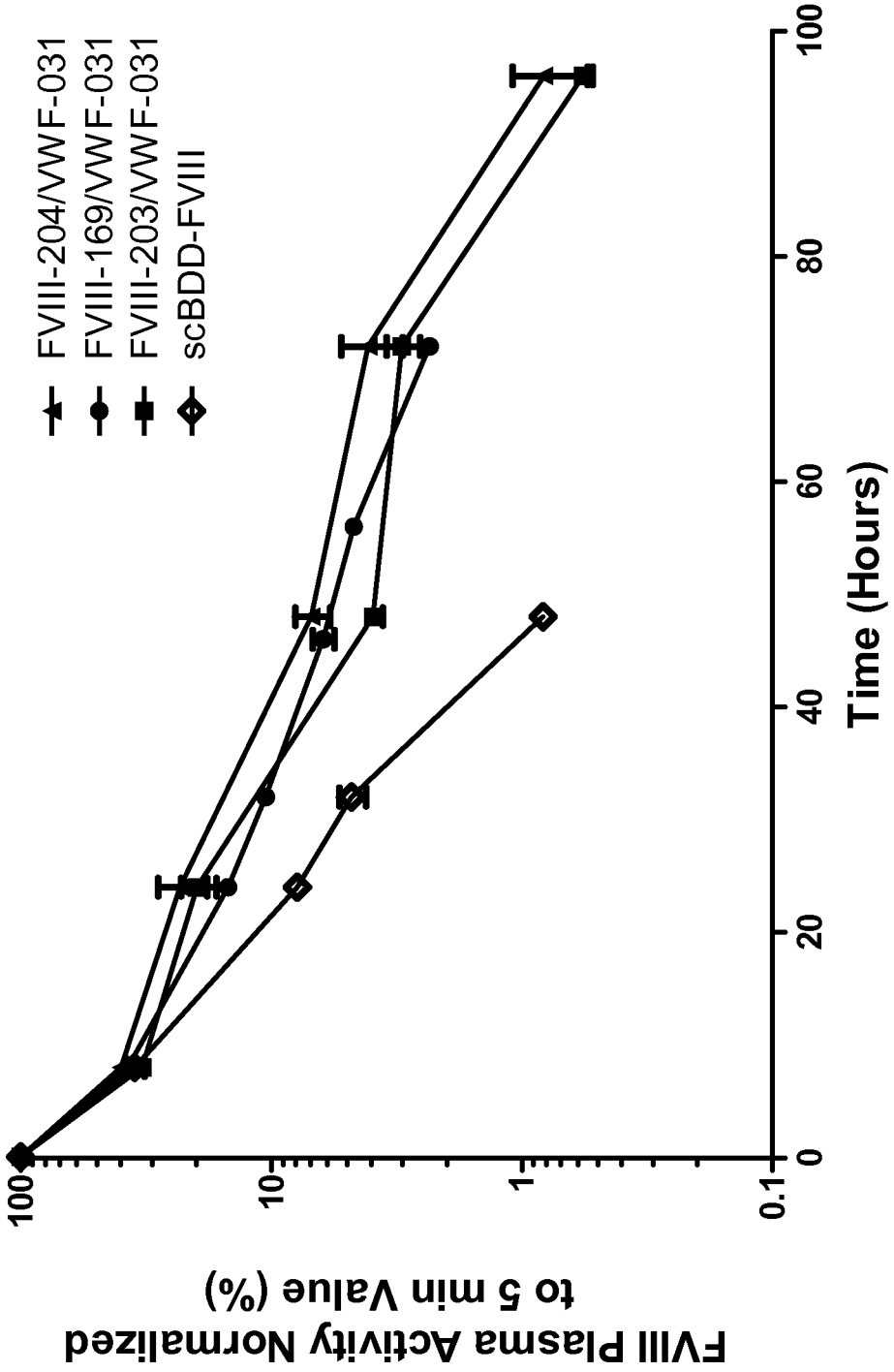


Figure 12B: Effect of different XTEN on rFVIII-XTEN/VWF heterodimer PK in HemA mice

Figure 12C: Effect of different XTEN on rFVIII-XTEN/VWF heterodimer PK in HemA mice

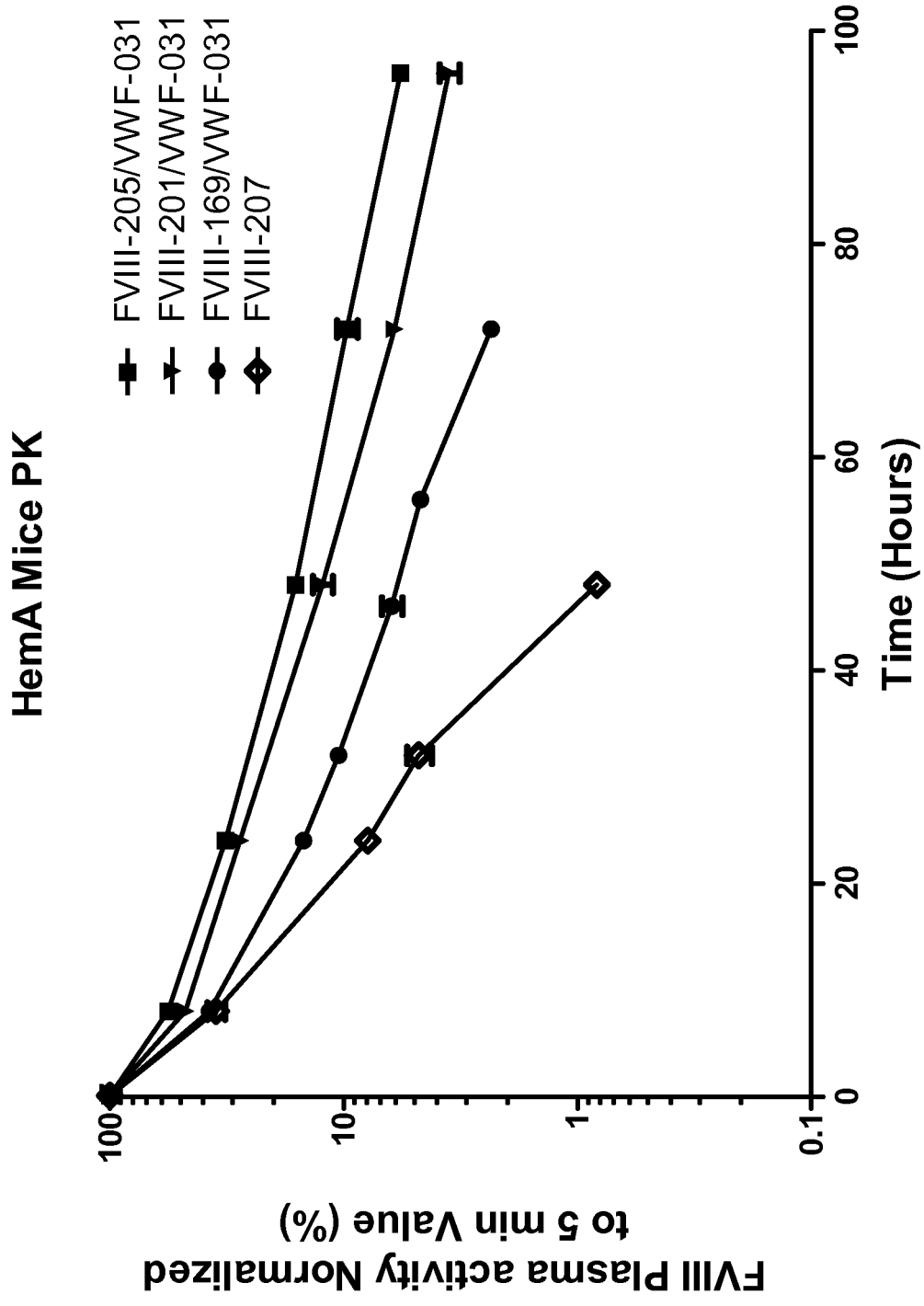


Figure 13: rFVIII-XTEN/VWF-XTEN heterodimer PK in FVIII/VWF DKO mice

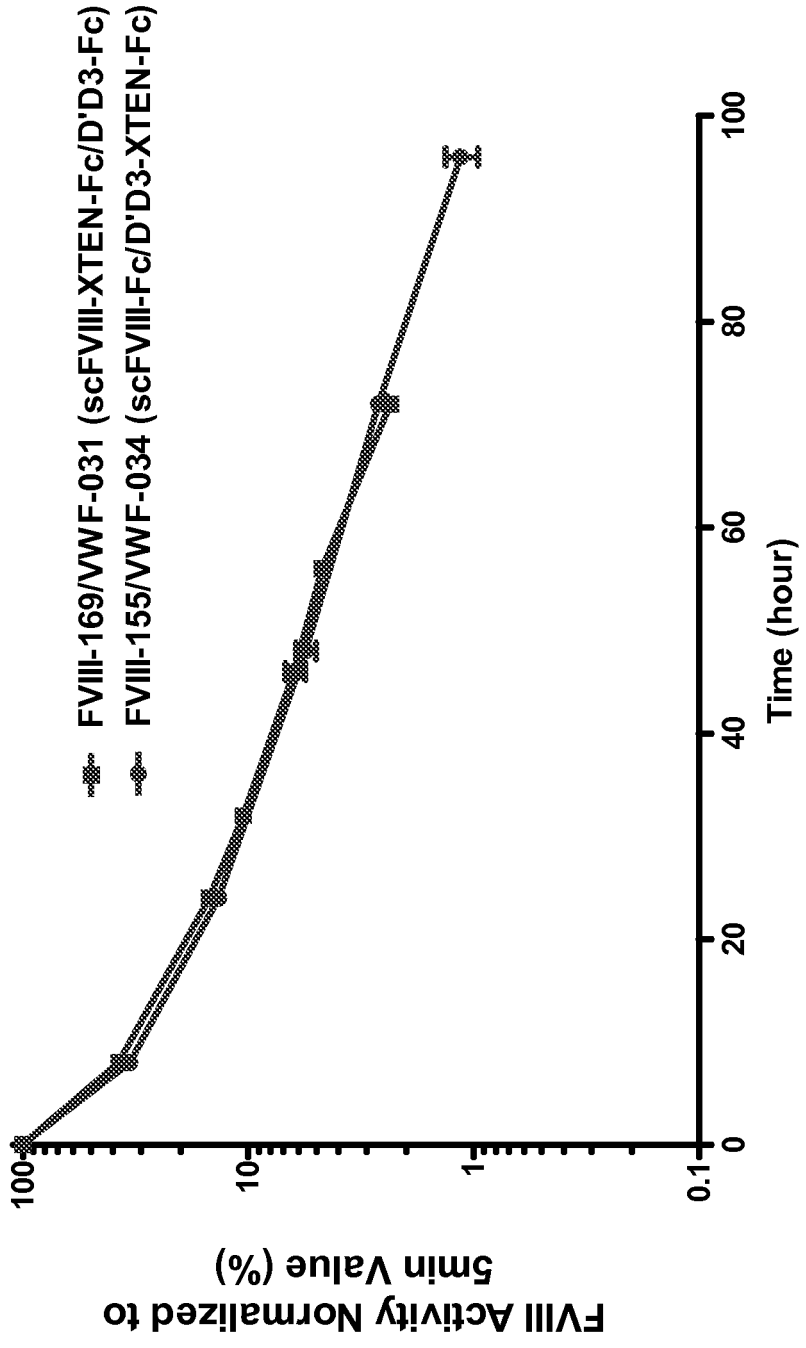


Figure 14. rFVIII-XTEN/VWF Heterodimer PK In HemA Mice

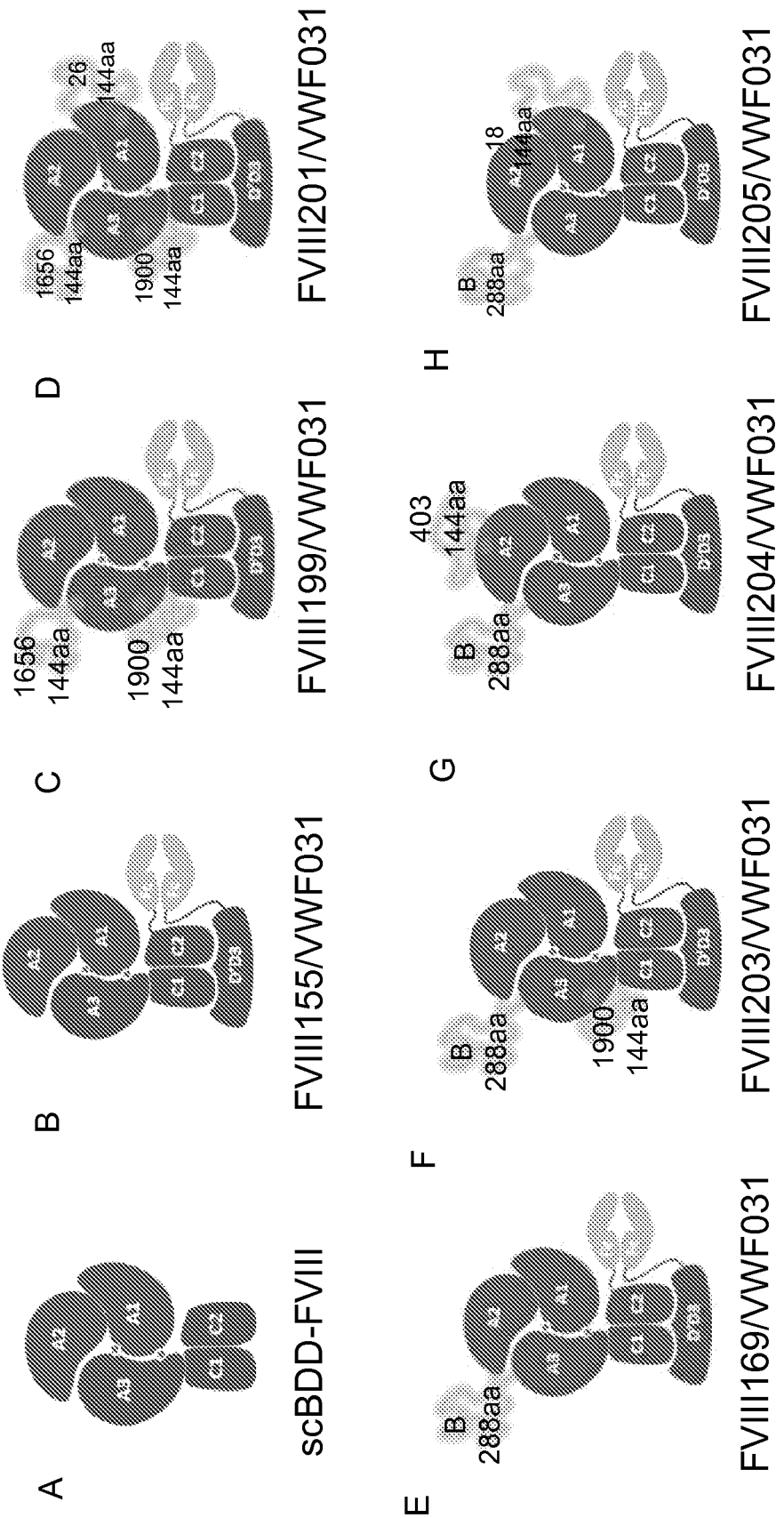
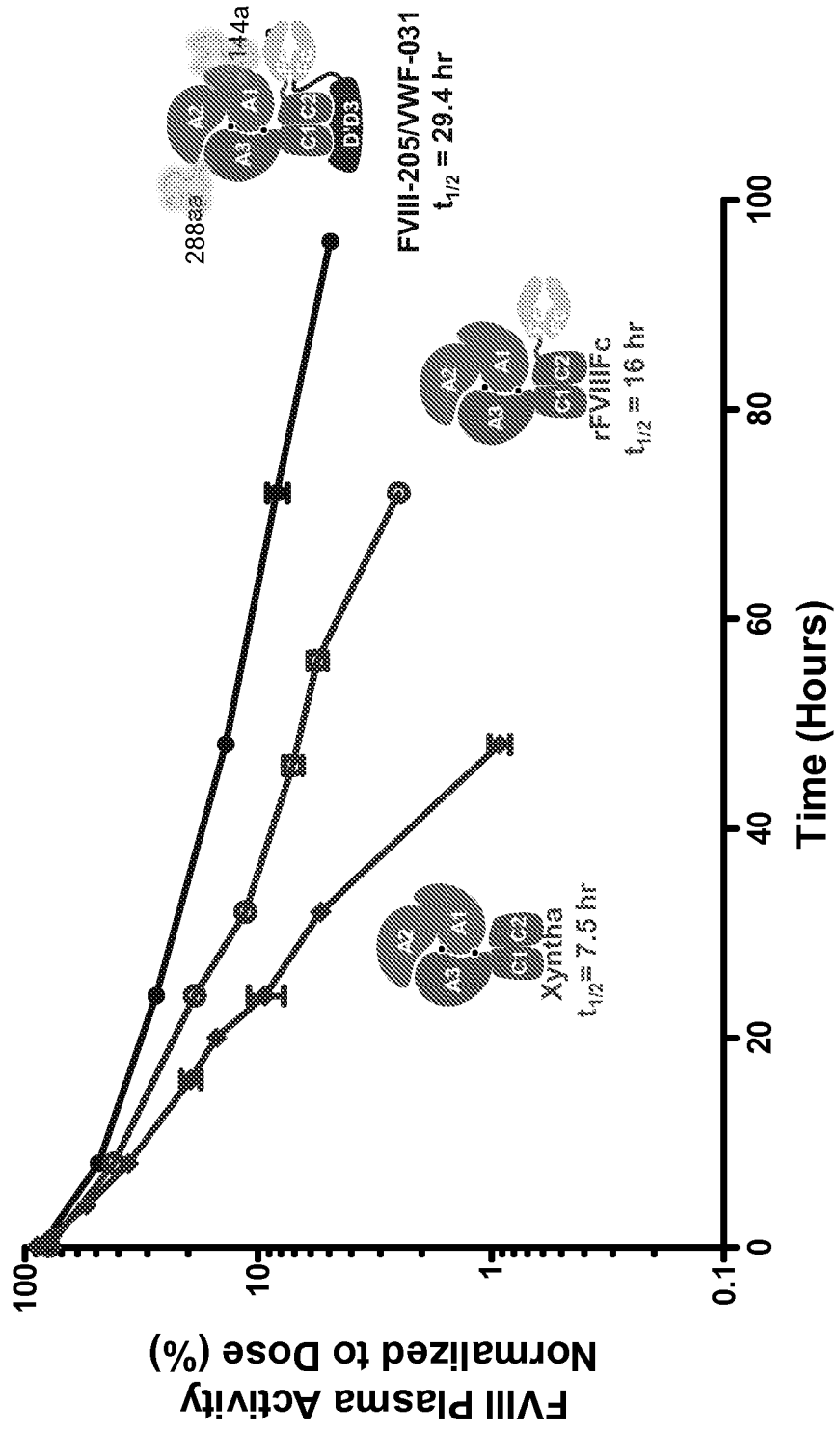


Figure 15:
Half-life extension in HemA mice by rFVIII-XTEN/VWF



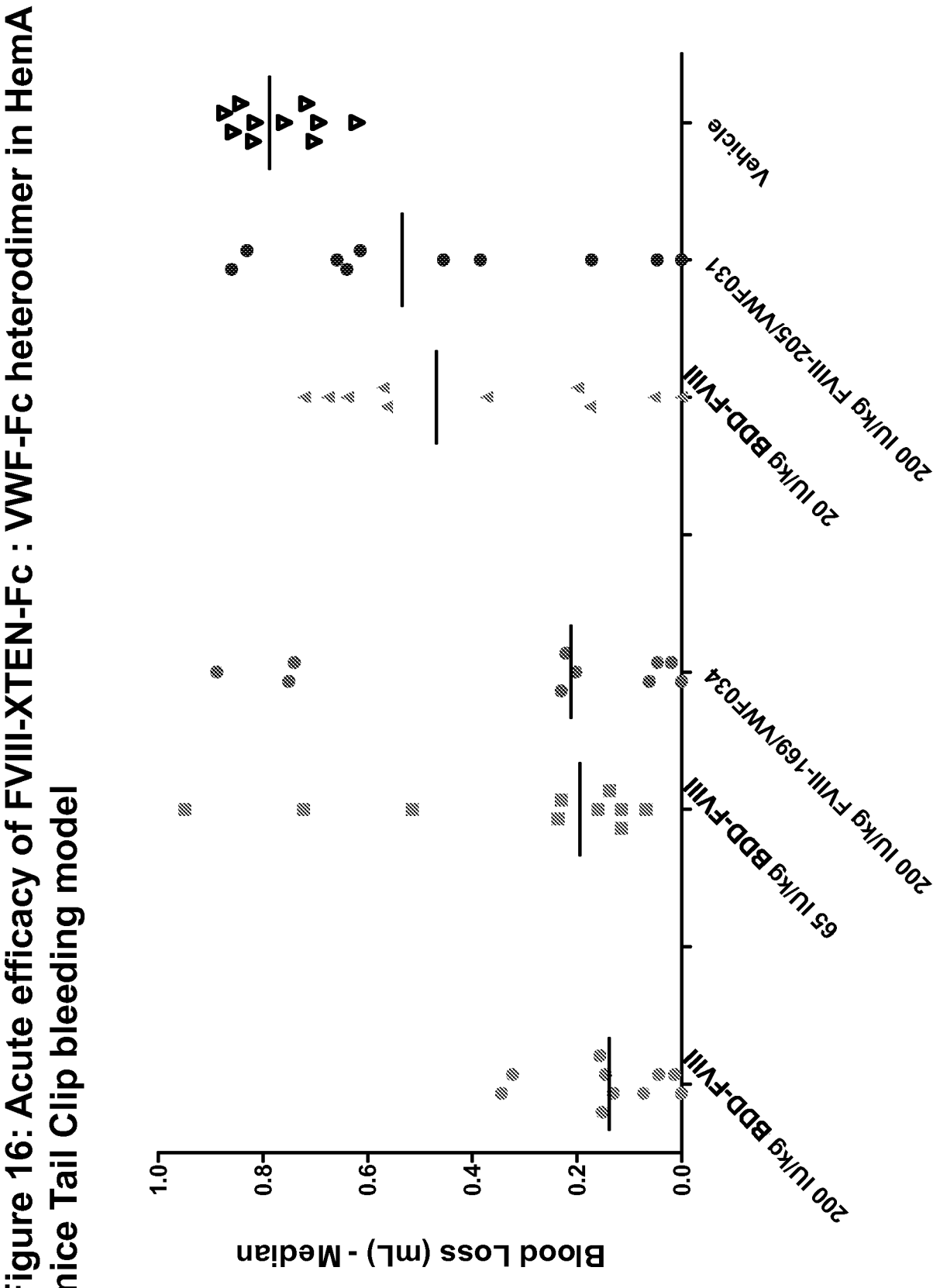


Figure 16: Acute efficacy of FVIII-XTEN-Fc : VWF-Fc heterodimer in HemA mice Tail Clip bleeding model