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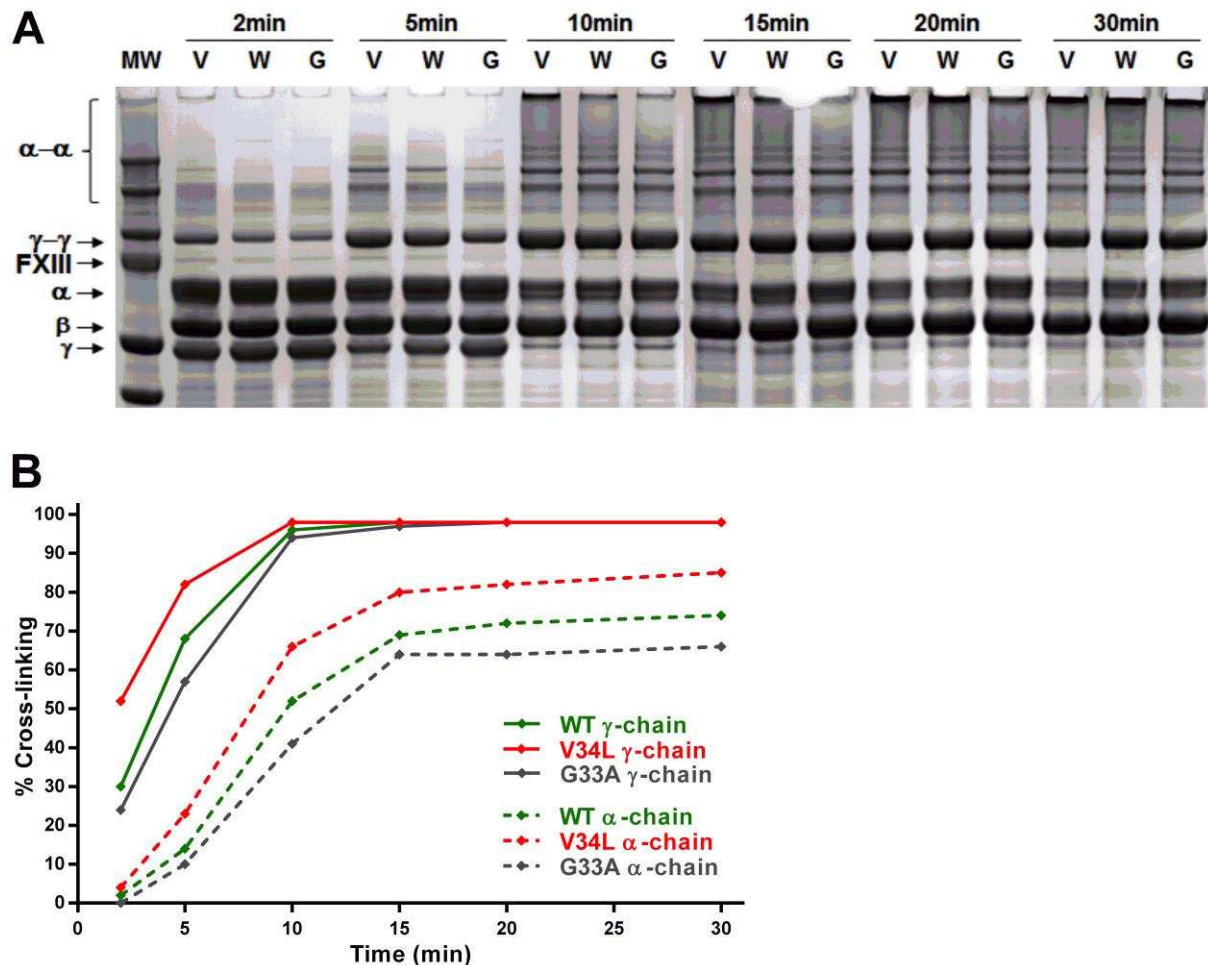


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## SUPPLEMENTAL MATERIAL

### Factor XIII A-subunit V34L variant affects thrombus cross-linking rather than size in a murine model of thrombosis

Cédric Duval, Majid Ali, Waleed W. Chaudhry, Victoria C. Ridger, Robert A.S. Ariëns, Helen Philippou



**Figure 1: Effects of rhFXIII-A<sub>2</sub> variants on formation of fibrin  $\alpha$ - and  $\gamma$ -chain cross-links.** Fibrin (2mg/ml)  $\alpha$ - and  $\gamma$ -chain cross-linking by 15 $\mu$ g/ml rhFXIII-A<sub>2</sub> V34L (V), WT (W) and G33A (G) was observed over time by SDS-PAGE (A), in the presence of 0.5U/ml thrombin and 10mM CaCl<sub>2</sub>. Band densitometry was performed in order to quantify the percentage of  $\alpha$ - and  $\gamma$ -chain cross-linking, both expressed relative to the uncross-linked  $\beta$ -chain band intensities to correct for any loading differences (B). At 2 and 5 minutes,  $\gamma$ -chain cross-linking was increased for V34L and decreased for G33A, compared to WT. From 10 minutes, all the  $\gamma$ -chains were cross-linked, and the  $\alpha$ -chain cross-linking increased over time. Cross-linking of the  $\alpha$ -chain was also increased for V34L and decreased for G33A, compared to WT. Panel A shows a composite of 2 SDS-PAGE gels (lanes 1-10 and 11-19). n=1.

**Table 1: Site-directed mutagenesis primers for rhFXIII-A<sub>2</sub> variants. Mutated bases are highlighted.**

Primer	Forward primer	Reverse primer
<b>T28A</b>	GGAAGATGACCTGCCC <b>G</b> CAGTGGAGCTTCAGGG	CCCTGAAGCTCCACTG <b>C</b> GGGCAGGTCATCTTCC
<b>V29A</b>	GCAGCGGAAGATGACCTGCCACAG <b>C</b> GGAGCTTCAG	GGCACCACGCCCTGAAGCTCC <b>G</b> CTGTGGGCAGGTC
<b>E30A</b>	CCTGCCACAGTGG <b>C</b> GCTTCAGGGCGTGG	CCACGCCCTGAAG <b>C</b> CCACTGTGGGCAGG
<b>L31A</b>	CCTGCCACAGTGGAG <b>GC</b> TCAGGGCGTGGTGC	GCACCACGCCCTGA <b>GC</b> TCCACTGTGGGCAGG
<b>Q32A</b>	CCCACAGTGGAGCTT <b>GC</b> GGGCGTGGTGC	GCACCACGCC <b>GC</b> AAGCTCCACTGTGGG
<b>G33A</b>	GTGGAGCTTCAG <b>G</b> CGTGGTGCCCCGGGGCGTCAAC	GTTGACGCCCCGGGGCACCAC <b>G</b> CCTGAAGCTCCAC
<b>V34A</b>	GGAGCTTCAGGG <b>C</b> CGGTGCCCCGG	CCGGGGCACC <b>G</b> CGCCCTGAACCTCC
<b>V34L</b>	GGAGCTTCAGGG <b>C</b> TGGTGCCCCGG	CCGGGGCACC <b>A</b> GCCCTGAAGCTCC
<b>V34M</b>	GGAGCTTCAGGG <b>A</b> TGGTGCCCCGG	CCGGGGCACC <b>T</b> GCCCTGAAGCTCC
<b>V35A</b>	GAGCTTCAGGGCGT <b>G</b> CGCCCCGGGGC	GCCCCGGGG <b>C</b> GCCACGCCCTGAAGCTC
<b>P36A*</b>	CTTCAGGGCGTGGT <b>G</b> CCCCGGGGCGTCAACC	GGTTGACGCCCCGG <b>C</b> CACCACGCCCTGAAG
<b>R37A</b>	CGTGGTGCCC <b>GC</b> GGGCGTCAACCTGC	GCAGGTTGACGCC <b>GC</b> GGGCACCACG
<b>G38A</b>	CGTGGTGCCCCGG <b>G</b> CGTCAACCTGC	GCAGGTTGAC <b>G</b> CCCCGGGGCACCACG
<b>V39A</b>	CGTGGTGCCCCGGGG <b>C</b> CAACCTGC	GCAGGTT <b>G</b> CGCCCCGGGGCACCACG
<b>N40A</b>	CGGGGCGT <b>GC</b> CCTGCAAGAGTTTCTTAATGTC	GACATTAAGAACTCTTG <b>C</b> AGG <b>GC</b> GACGCCCCG
<b>L41A</b>	GGGCGTCAAC <b>GC</b> GCAAGAGTTTCTTAATGTC	GACATTAAGAACTCTTG <b>C</b> <b>GC</b> GTTGACGCCC

\* P36A variant was successfully mutated but transformation step failed.