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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

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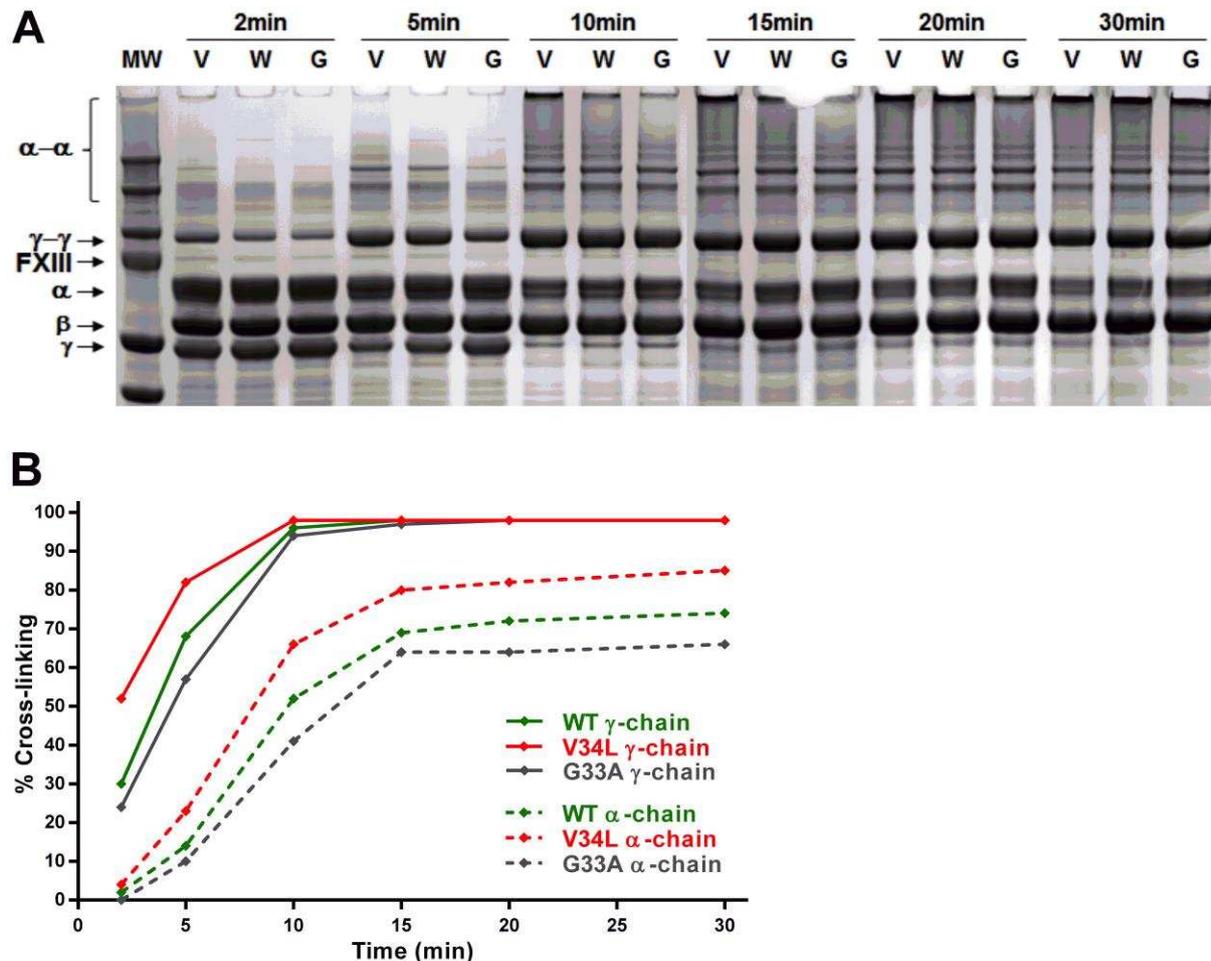


Figure 1: Effects of rhFXIII-A₂ variants on formation of fibrin α - and γ -chain cross-links. Fibrin (2mg/ml) α - and γ -chain cross-linking by 15 μ g/ml rhFXIII-A₂ V34L (V), WT (W) and G33A (G) was observed over time by SDS-PAGE (A), in the presence of 0.5U/ml thrombin and 10mM CaCl₂. Band densitometry was performed in order to quantify the percentage of α - and γ -chain cross-linking, both expressed relative to the uncross-linked β -chain band intensities to correct for any loading differences (B). At 2 and 5 minutes, γ -chain cross-linking was increased for V34L and decreased for G33A, compared to WT. From 10 minutes, all the γ -chains were cross-linked, and the α -chain cross-linking increased over time. Cross-linking of the α -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-A₂ variants. Mutated bases are highlighted.

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

* P36A variant was successfully mutated but transformation step failed.