**Supplemental figure 1**

**A**, PAGE-Blue, each lane is loaded with a reduced FV-Short variant (20 ul at 133 nM) in sample preparation buffer. To generate FVa, the FV-Short variants (200 nM) were incubated for 30 minutes at 37°C with 50 units/ml thrombin before mixing with the sample preparation buffer. FV756-1458 (1) and the five FV-Short mutants (2-6) were all tested before and after thrombin treatment; FV712-1478 (2), FV712-1481 (3), FV712-1484 (4), FV712-1487 (5), and FV712-1490 (6). Samples were run under reducing conditions on 4-20% gradient gels. PAGE-Blue was used for staining.

Positions of heavy and light chains, thrombin and BSA (bovine serum albumin present in added thrombin) **B**, Samples as in A diluted to 2 nM FV-Short variants and 10 ul loaded. The western blots were developed with monoclonal anti-FVa heavy chain antibody 5146.

**C**, Samples as in A diluted to 2 nM FV-Short variants and 10 ul loaded. The western blots were developed with monoclonal anti-FVa light chain antibody 5112.

**SDS-PAGE and western blots of the new FV-Short variants.** **A**, PAGE-Blue, each lane is loaded with a reduced FV-Short variant (20 ul at 133 nM) in sample preparation buffer. To generate FVa, the FV-Short variants (200 nM) were incubated for 30 minutes at 37°C with 50 units/ml thrombin before mixing with the sample preparation buffer. FV756-1458 (1) and the five FV-Short mutants (2-6) were all tested before and after thrombin treatment; FV712-1478 (2), FV712-1481 (3), FV712-1484 (4), FV712-1487 (5), and FV712-1490 (6). Samples were run under reducing conditions on 4-20% gradient gels. PAGE-Blue was used for staining.

Positions of heavy and light chains, thrombin and BSA (bovine serum albumin present in added thrombin) **B**, Samples as in A diluted to 2 nM FV-Short variants and 10 ul loaded. The western blots were developed with monoclonal anti-FVa heavy chain antibody 5146. **C**, Samples as in A diluted to 2 nM FV-Short variants and 10 ul loaded. The western blots were developed with monoclonal anti-FVa light chain antibody 5112.