Supporting Information

Table S1. Effect of Mg^{2+} on Rates of FX Activation by memTF/FVIIa in Solution

TF (WT or mutant)	Initial rates of FX activation (nM/min/nM ×10^3)[a]		
	1.25 mM Ca^{2+} + 0.6 mM Mg^{2+}	1.85 mM Ca^{2+}	1.25 mM Ca^{2+}
WT	122.1 ± 4.0	27.8 ± 3.9	20.4 ± 1.3
Y157A	5.3 ± 0.4	2.2 ± 0.1	1.7 ± 0.1
W158A	30.8 ± 2.8	7.2 ± 0.3	5.4 ± 0.6
K159A	10.3 ± 2.0	2.8 ± 0.2	4.0 ± 1.9
S160A	58.2 ± 4.0	12.1 ± 2.2	7.1 ± 0.2
S161A	189.4 ± 17.2	31.4 ± 2.8	24.3 ± 1.1
S162A	47.3 ± 1.8	9.4 ± 0.6	6.5 ± 0.4
S163A	2.8 ± 0.5	1.0 ± 0.1	0.9 ± 0.1
G164A	1.1 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
K165A	4.8 ± 0.2	1.8 ± 0.1	2.4 ± 1.0
K166A	2.5 ± 0.4	1.5 ± 0.2	1.4 ± 0.3
K169A	133.9 ± 12.9	25.0 ± 1.4	17.4 ± 1.1
E174A	98.3 ± 3.3	18.9 ± 1.0	13.0 ± 1.1
L176A	60.8 ± 4.2	12.5 ± 0.5	7.4 ± 0.4
D178A	129.5 ± 15.0	24.2 ± 1.5	19.1 ± 2.5
D180A	34.4 ± 4.9	13.1 ± 2.3	8.8 ± 0.8
Y185A	2.3 ± 0.2	1.8 ± 0.1	1.4 ± 0.1
R200A	29.0 ± 1.8	5.9 ± 0.5	3.8 ± 0.3

[a]Data are initial rates of FX activation in (nM/min) divided by the memTF/FVIIa concentration (in nM), expressed as mean ± standard error ($n \geq 3$). Reaction conditions were 10 nM FVIIa, 500 nM memTF, 0.1% Triton X-100, 100 nM FX, and 1 mM Spectrozyme Xa.

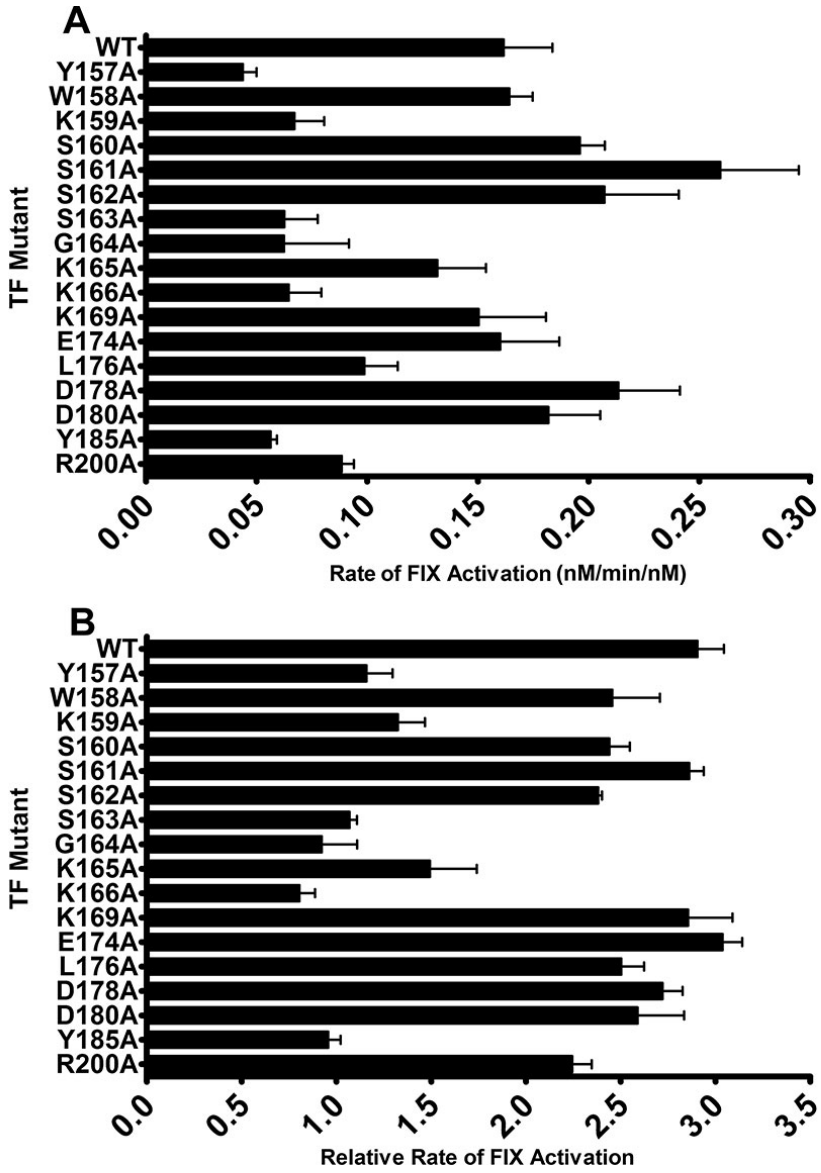

Figure S1. Effect of TF mutations on Mg^{2+}-dependent rate enhancements of FIX activation. (A) Initial rates of FIX activation by WT or mutant memTF/FVIIa in solution (with 0.06% Triton X-100) using 1.25 mM Ca^{2+}. (B) Relative rates of FIX activation by WT or mutant memTF/FVIIa in solution (with 0.06% Triton X-100). In this panel, the rates using 1.25 mM Ca^{2+} + 0.6 mM Mg^{2+} were normalized to those using 1.25 mM Ca^{2+} alone. Data are mean ± standard error (*n* = 3).

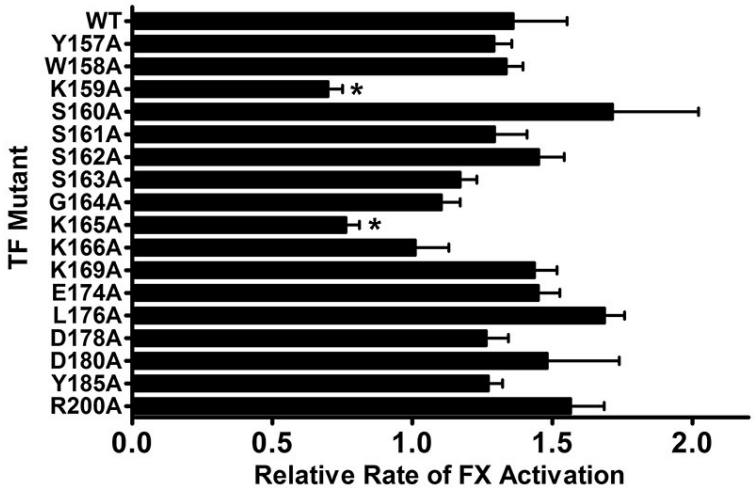

Figure S2. Effect of increased Ca^{2+} concentration on FX activation by memTF/FVIIa in solution. In each case, the initial rates of FX activation by memTF/FVIIa complexes in detergent solution (0.1% Triton X-100) using 1.85 mM Ca^{2+} were normalized to the rates observed with the same version of memTF (mutant or WT) at 1.25 mM Ca^{2+}. The rates of FX activation supported by WT and most memTF mutants were increased approximately 1.3-fold upon increasing the Ca^{2+} concentration from 1.25 to 1.85 mM. However, the rates of FX activation supported by two TF mutants (K159A, p = 0.018; and K165A, p = 0.029) were significantly *decreased* upon increasing the Ca^{2+} concentration (asterisks). Data are mean ± standard error ($n \geq 3$), with statistical significance determined using Student's t-test.

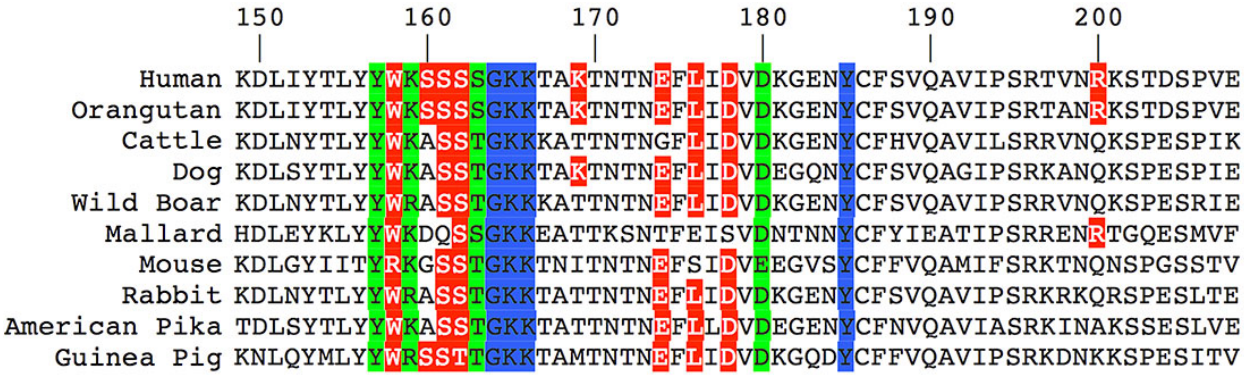

Figure S3. TF sequence alignment of the region of interest across selected mammals and birds. BLAST search was performed using human TF as the standard (with the amino acid numbering also representative of human TF). Highlighted in blue (164-166, 185) were residues most important for Mg^{2+} recognition. Residues in green (157, 159, 163, 180) were intermediate in their importance towards recognizing Mg^{2+}. Mutations of residues in red resulted in no identifiable defect in Mg^{2+} response. We note that the TF residues most important for Mg^{2+} recognition are all highly conserved.

Table S2: K_d Values for TF/FVIIa under Varying Divalent Metal Ion Conditions

TF (WT or mutant)	K_d (nM)[a]		Ratio[b]
	1.85 mM Ca^{2+}	1.25 mM Ca^{2+} + 0.6 mM Mg^{2+}	
WT	0.39 ± 0.1	0.58 ± 0.1	1.48 ± 0.4
Y157A	0.13 ± 0.05	0.23 ± 0.02	1.87 ± 0.7
S163A	0.70 ± 0.3	0.57 ± 0.1	0.81 ± 0.4
G164A	1.03 ± 0.3	1.26 ± 0.06	1.22 ± 0.3
K165A	0.66 ± 0.3	0.94 ± 0.5	1.42 ± 1
K166A	0.44 ± 0.08	0.47 ± 0.04	1.07 ± 0.2
D180A	1.07 ± 0.3	0.86 ± 0.2	0.80 ± 0.3
Y185A	0.43 ± 0.1	0.49 ± 0.1	1.15 ± 0.4

[a]Values for K_d are mean ± standard error ($n \geq 3$). [b]Ratios are mean K_d at 1.25 mM Ca^{2+} + 0.6 mM Mg^{2+} divided by mean K_d at 1.85 mM Ca^{2+} (± standard error).

The K_d values for FVIIa binding to TF in Table S2 were determined using memTF in solution (with 0.1% Triton X-100), under conditions of equal total divalent metal ion concentration with either no Mg^{2+} (1.85 mM Ca^{2+}) or with physiologic concentrations of Mg^{2+} and Ca^{2+} (1.25 mM Ca^{2+} + 0.6 mM Mg^{2+}).