Supplementary Table 1: Clinical Description of Studied Missense Variants

Missense Variants (Legacy Position)	HGVS Position	Activity (relative to FIX-WT)	Antigen (relative to FIX-WT)	Genotype-Phenotype	Reference
A148T	194	1	1	Polymorphism	(Bezemer, Arellano et al. 2009)
V182L	228	0.15	1.3	HB causing	(Taylor, Liddell et al. 1990)
N264Y	310	0.25	0.25	HB causing	(Weinmann, Murphy et al. 1998)
H268R	314	0.06	0.02	HB causing	(Costa, Ernault et al. 2000)
R338L	384	6.6	0.78	Thrombophilia	(Simioni, Tormene et al. 2009)
T376N	442	0.15	0.13	HB causing	(Green, Montandon et al. 1991)

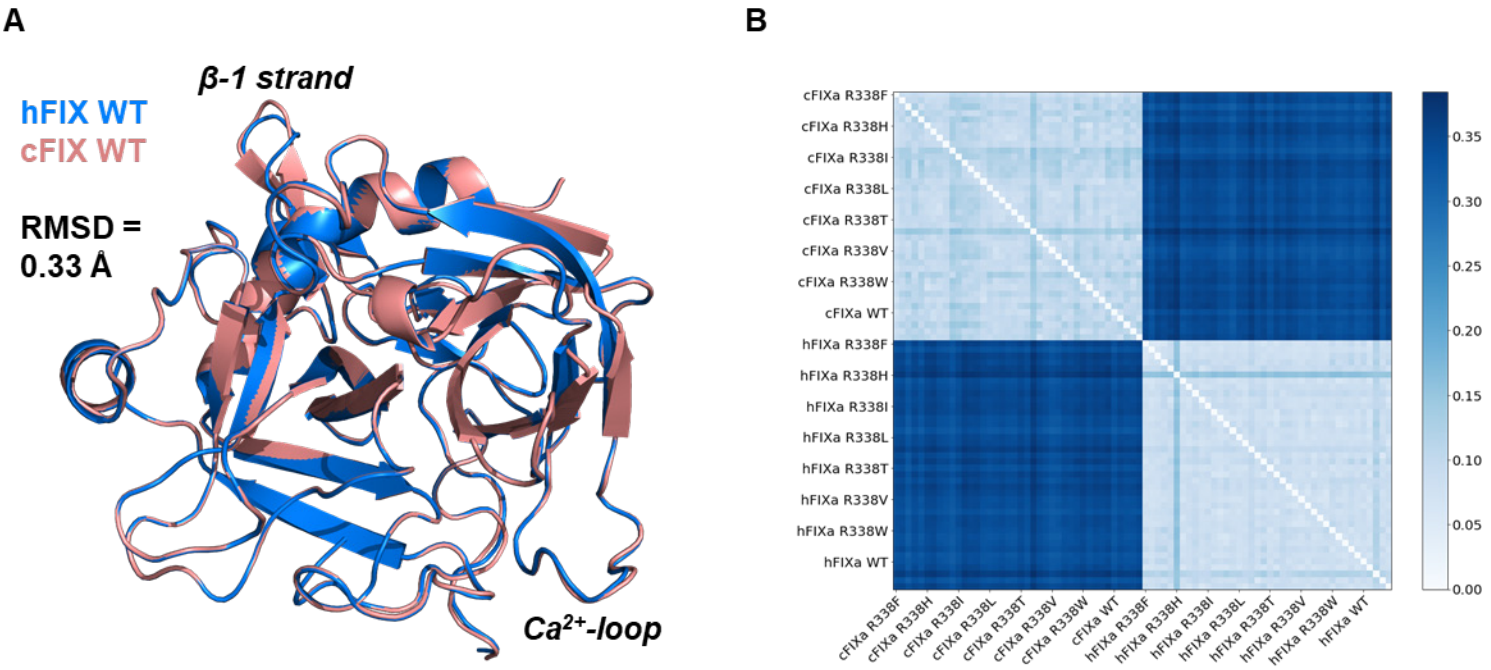
Supplementary Table 2: Characteristics of pediatric thrombosis patients evaluated for *F9* R338 substitutions (n=232)

Characteristic	Percent[†]
Age	
<1	20.7
1-6	10.1
6-13	12.8
13-18	45.7
≥18	10.6
Sex	
Male	55.7
Female	44.3
Catheter-related	28
Inherited thrombophilia	
Antithrombin Deficiency	6
Protein C Deficiency	9
Protein S Deficiency	9
Factor V-Leiden	9
Prothrombin Gene Mutation	4

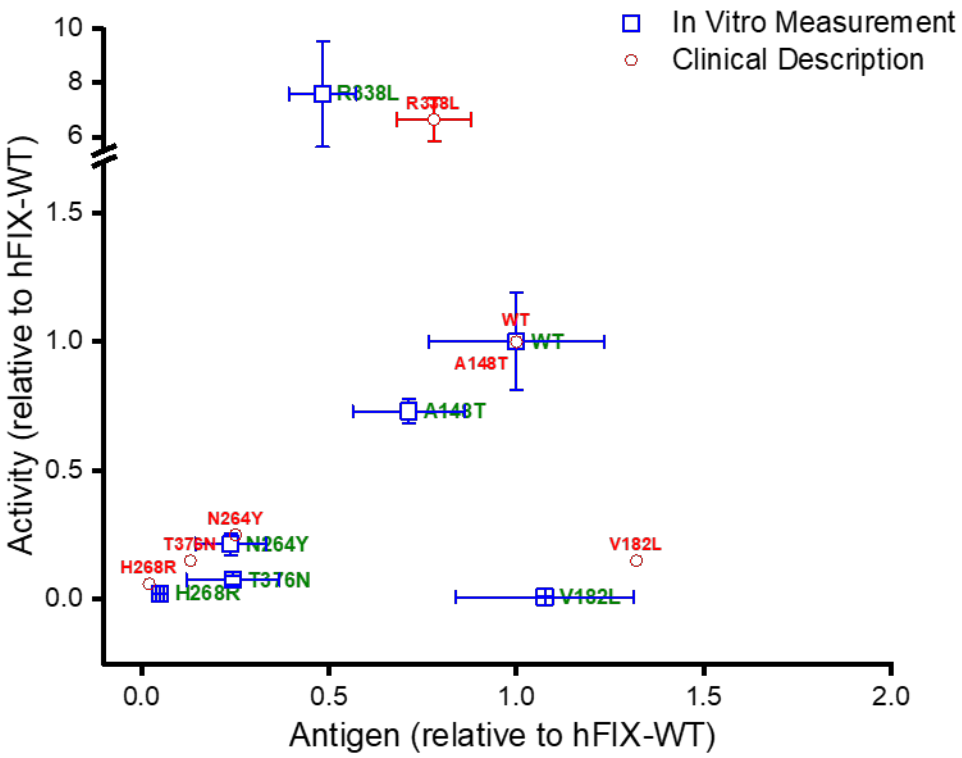
[†]Subjects not tested or missing data removed from denominator

Supplementary Table 3: Summary of negative screenings for FIX R338 thrombophilias

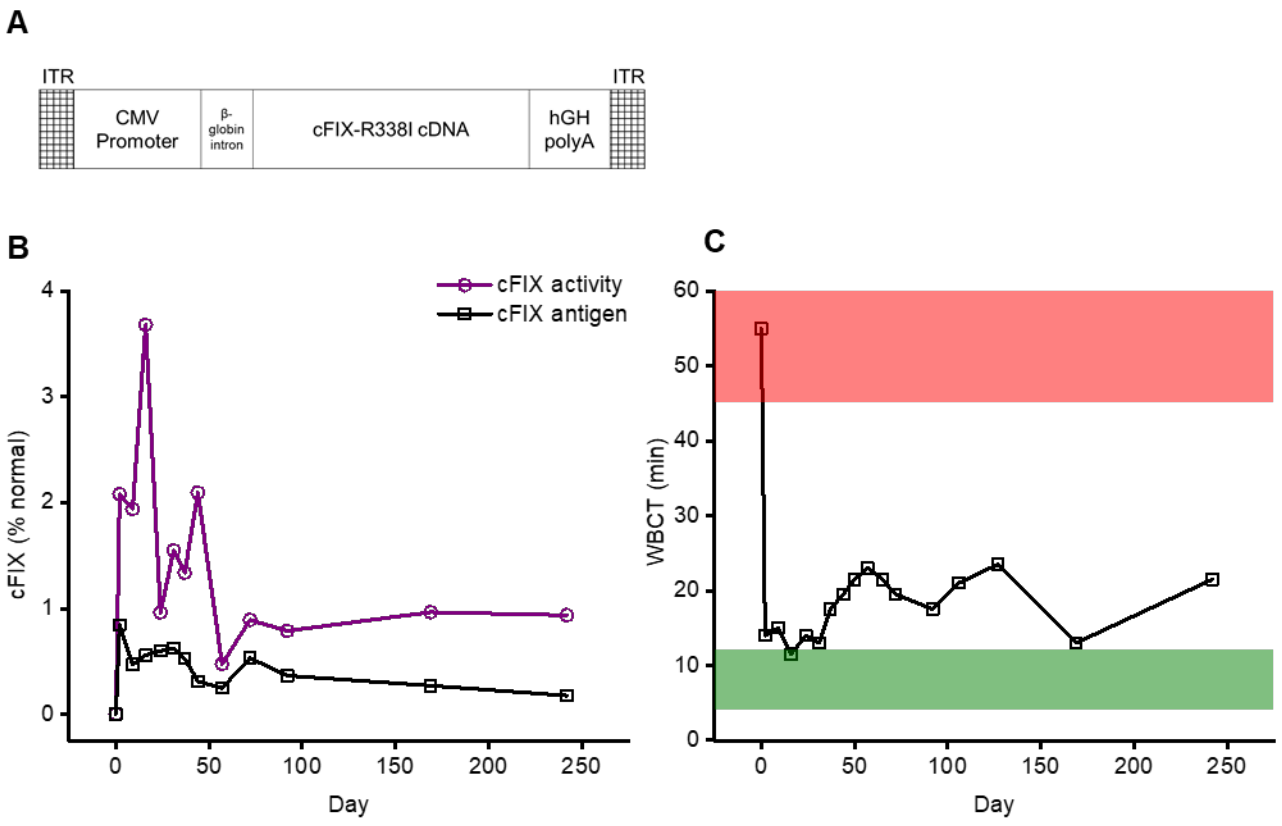
Thrombosis Subjects (N)	Controls (N)	Age (years)	Notes	Reference
200	200	20-80	Italy	(Simioni, Tormene et al. 2009)
19	132	adults	Brazil	(Mazetto, Orsi et al. 2010)
201		adults	Holland	(Koenderman, Bertina et al. 2011)
115	115	20-83	Italy	(Campello, Spiezia et al. 2013)
232		0.01-25	CHOP	*This work*
Total 767	**447**			



Supplementary Figure 1: Homology modeling of hFIX and cFIX variants. (A) Superimposition of the top scoring homology models of hFIX-WT and cFIX-WT. Though predicted structures are overall highly similar, there are minor differences in the β1-strand and the calcium-binding loop. (B) Pairwise RMSD (Å) comparisons between homology models of hFIX-WT and cFIX-WT variants. Deviations are higher between cFIX and hFIX models than within cFIX or hFIX models. 100 homology models were developed for each variant using the RosettaCM (Song, DiMaio et al. 2013) using the crystal structure of hFIXa-WT (PDB# 1RFN) as the template structure. The top five scoring structures for each variant were used for comparison.



Supplementary Figure 2: Comparison of clinically described FIX missense variants with recombinant FIX variants. FIX variants with clinically described activity and antigen levels (red circles) listed in Supplementary Table 1 were expressed in HEK293 cells and the activity and antigen levels were measured from conditioned media (blue squares). HB causing missense variants with a range of expression levels where selected. A148T is the Malmo polymorphism. Blue points represent the activity and antigen of ≥3 independent transient transfections and errors bars represent ± SEM.



Supplementary Figure 3: Gene therapy with AAV6-cFIX-R338I in HB dogs. (A) Schematic of vector used in study. One adult male HB dog (N40) was administered 3 x 10^{12} vg/kg AAV6-cFIX-R338I by peripheral transvenular delivery to the skeletal muscle. (B) cFIX activity (circles) and antigen (squares) over time after vector administration. (C) Whole blood clotting time after vector administration; hemophilia (>45 min) and normal (8 – 12 min) range indicated by red and green box respectively. No FIX inhibitor was detected. There was also no local or systemic toxicity.

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