Supplemental Methods, Figures, and Table

Methods

Vector construction of FV mutations (A2086D, W1920R and R506Q)

To generate expression vectors for mutant FV, the FV coding region of pPB-CAG-F5-PGKpuro was mutated as follows using PCR primers shown in **Supplemental Table 1**. First, FV coding regions were PCR-amplified from the pPB-CAG-F5-PGKpuro using primers F1 and R1, and F2 and R2. The R1 and F2 sequences are complementary and contain mutations to be introduced. Second, the PCR products were mixed and amplified by fusion PCR using primers F1 and R2. To generate expression vectors for A2086D and W1920R, PCR products were cloned into the 12.1-kb XhoI fragment of the pPB-CAG-F5-PGKpuro using In-Fusion HD Cloning Kit (TAKARA-Bio), resulting in pPB-CAG-F5-A2086D-PGKpuro and pPB-CAG-F5-W1920W-PGKpuro. To generate the expression vector for R506Q, the PCR product was digested with AflII and ligated to the 11.7-kb AflII fragment of the pPB-CAG-F5-PGKpuro, resulting in pPB-CAG-F5-R506Q-PGKpuro. Nucleotide sequences of PCR-amplified regions were verified by sequencing.

APC sensitivity ratio using activated partial thromboplastin time (aPTT)-based APC-resistance assay

The APC sensitivity ratio (APCsr) were expressed as ratios of aPTT clotting times in the presence of APC divided by clotting times in its absence. This assay was performed with FV-deficient plasma spiked by FV (final concentration; *f.c.* 0.4 U/mL) using CS-2400™ (Sysmex). This assay reflects the effect of APC on the inactivation of both of FVa and FVIIIa; hence, a low level of APCsr indicates a defect in inactivation of FVa and/or FVIIIa and, consequently, reflects APCR.

Diluted PT-based APCR assay

A diluted PT-based APCR assay was performed with minor modification of the aPTT-based APC-resistance assay. One hundred-fold diluted PT reagent (Innovin®) reagent was used instead of aPTT reagent. Results were expressed as the APC sensitivity-ratio (clotting time with APC divided by that without APC). This assay was performed using CS-2400™ (Sysmex) with FV-deficient plasma mixed with rFV (*f.c.* 0.4 U/mL).

Figure

Supplemental Figure 1

(A) ***A schematic diagram of the piggyBac transposon vector expressing full-length human FV***

PB; piggyBac transposon, CAG; CAG promoter, pA; polyadenylation signal, PGK; mouse Pgk1 promoter, puro; puromycin resistance gene, CMV; cytomegalovirus promoter, hyPBase; hyperactive piggyBac transposase.

(B) ***SDS-PAGE analysis of purified recombinant FV protein*** **-** Purified full-length FV-WT and FV mutants (A2086D, W1920R, R506Q) were analyzed on 8% SDS-PAGE. MW; molecular weight

Supplemental Figure 2

Diluted PT using clotting factor-deficient plasma

Diluted PT reagent (50 μL) was mixed with 50 μL TFPI-deficient, FIX-deficient, and FVIII-deficient plasmas and with generated FVa mutant samples *(f.c.* 4.5 nM), followed by the addition of $CaCl_2$. The clotting time was measured in seconds. All Experiments were performed at least 3 separate times, and the average values and standard deviations are shown. -def; deficient plasma. One-way ANOVA was performed on experiments.

Table

Supplemental Table 1. Primers used for the construction of mutant FV expression vectors

Mutation	Sequence*
A2086D	F1: 5'-ACCTCGACAGCACTTTTACCAAACGTGATC-3'
	R1: 5'- AGGGACGTGTGAATGCCTGGCAAGACAAGGCAAACAACAATAAGCAGTG-3'
	F2: 5'- CACTGCTTATTGTTGTTTGCCTTGTCTTGCCAGGCATTCACACGTCCCT-3'
	R2: 5'-GATCAGCGAGCTCTAGATCATCGATGCATC-3'
W1920R	F1: 5'-ACCTCGACAGCACTTTTACCAAACGTGATC-3'
	R1: 5'-CTGCTGCAAGTTTTTCTACACTCCGAGCATTATAAGATCCACCATTGTT- 3'
	F2: 5'-AACAATGGTGGATCTTATAATGCTCGGAGTGTAGAAAAACTTGCAGCAG-3'
	R2: 5'-GATCAGCGAGCTCTAGATCATCGATGCATC-3
R506Q	F1: 5'-GACATTAAAAACTGCCCAAAGAAAACC-3
	R1: 5'-ATGTCTGCTGCCCTCTGTATTCCTTGCCTGTCCAGGGATCTGCTCTTAC-3'
	F2: 5'-GTAAGAGCAGATCCCTGGACAGGCAAGGAATACAGAGGGCAGCAGACAT-3'
	R2: 5'-GTTTCATTGGATTTATGAAGCACCAACG-3'

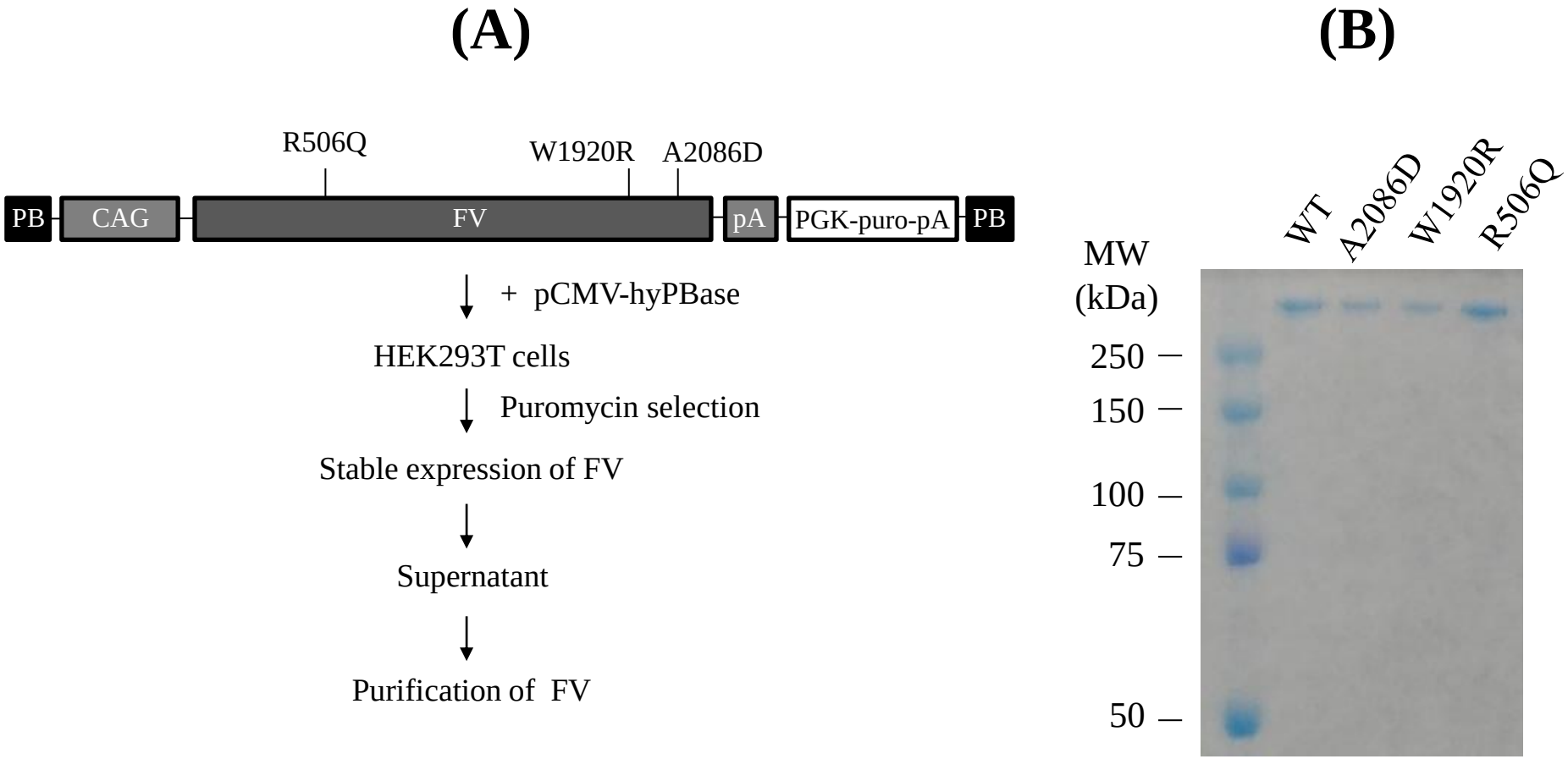
*Underlined letters indicate mutation sites.

Supplemental Table 2. APC sensitivity ratio using diluted PT-based APCR assay

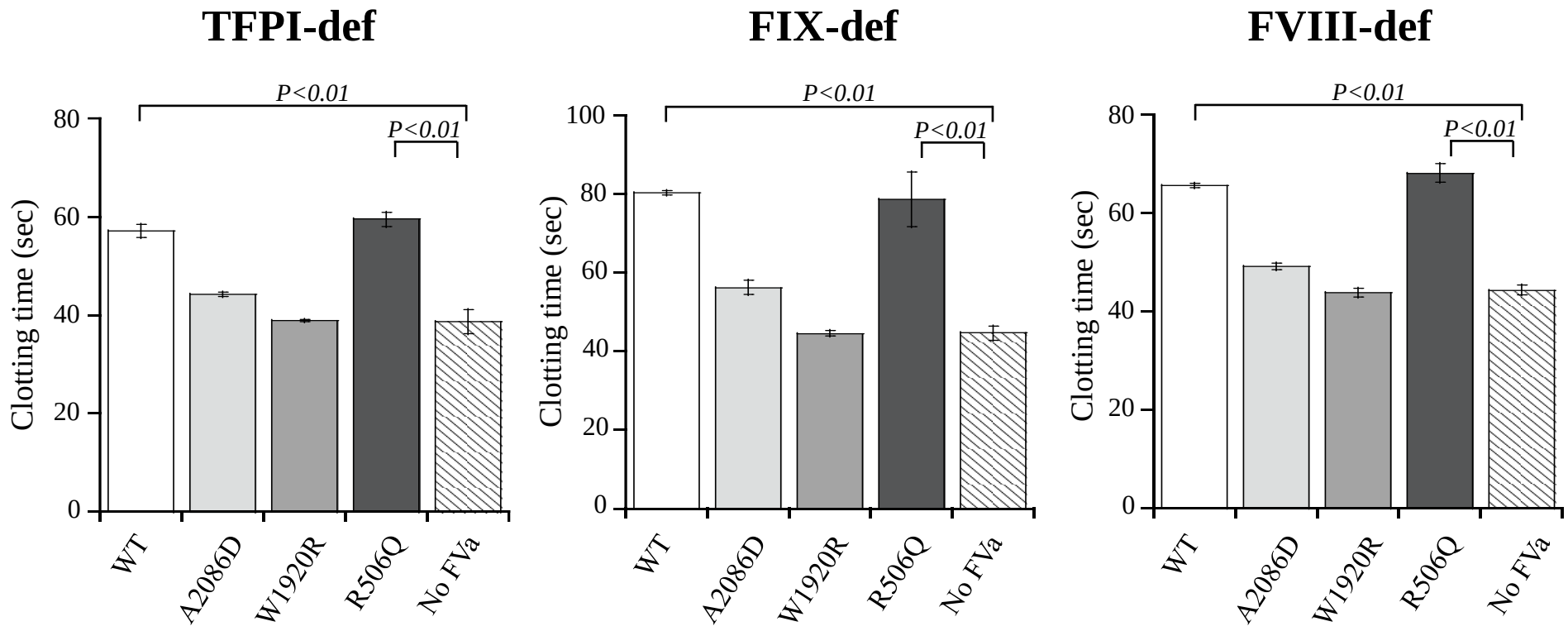
FV mutants	Clotting Time (sec) plus APC	Clotting Time (sec) minus APC	APCsr† (plus/minus APC)
FV-WT	85.9 ± 4.2	58.0 ± 1.7	1.46 ± 0.08
FV-A2086D ($FV_{Besançon}$)	58.0 ± 3.5	54.2 ± 3.5	1.07 ± 0.02
FV-W1920R (FV_{Nara})	63.2 ± 3.0	58.6 ± 2.8	1.08 ± 0.02
FV-R506Q (FV_{Leiden})	63.5 ± 3.8	57.6 ± 3.4	1.10 ± 0.06

Measurements were performed as described in Supplemental Methods. All data were measured 3 times, and the average and standard deviation values are shown.

†; APCsr, APC sensitive ratio was measured by diluted PT-based APCR assay as described in Methods.



Supplemental Figure 1



Supplemental Figure 2