Supplementary Fig. S1 Anti-factor V (FV) titration of thrombin generation in normal plasma. (A) Thrombin generation in pooled normal plasma was triggered with 2 pM tissue factor (TF) in the presence of increasing concentrations (0–69.5 µg/mL) of an inhibitory antibody against FV (AHV-5101). (B) Peak heights of thrombin generation were plotted against the antibody concentration.

Supplementary Fig. S2 0.5 nM tissue factor pathway inhibitor (TFPI) or HNBSA/Ca²⁺ buffer was incubated at 37°C with 30 µM phospholipid vesicles (20/60/20 DOPS/DOPC/DOPE) and 125 µM CS-11(65) substrate in the absence and presence of 2.5 nM factor V (FV1 or FV2. Reactions were started with 0.1 nM FXa and the absorbance at 405 nm was followed for 50 minutes. Averages of duplicate experiments are shown.
Factor V has Anticoagulant Activity in Plasma in the Presence of TFPIα

van Doorn et al.

FV Purification

All steps were performed at approximately 4°C except for the chromatography steps, which were run at room temperature on an AKTA FPLC (GE Healthcare, Uppsala, Sweden). Plasma was thawed and the following inhibitors were added: phenylalanyl-prolyl-arginine chloromethyl ketone (Calbiochem, La Jolla, California, United States) (2 µM), soybean trypsin inhibitor (Sigma-Aldrich, Zwijndrecht, the Netherlands) (50 mg/L) and benzamidine (Sigma-Aldrich) (10 mM). BaCl2 (Acros organics, Geel, Belgium) was added dropwise (80 mL of 1 M solution per litre of plasma). The formed barium citrate pellet was removed by centrifugation at 4,500 g for 30 minutes. Polyethylene glycol (PEG)-6000 (Merck, Darmstadt, Germany) was added dropwise to a concentration of 7% (w/v) of the remaining supernatant and stirred for 60 minutes. The pellet was removed by centrifugation for 30 minutes at 4,500 × g. PEG-6000 was then added to 13% (w/v) of the supernatant and stirred for 60 minutes. The precipitate was collected by centrifugation at 4,500 × g for 15 minutes to remove any debris. The dissolved pellet was then applied to DEAE-Sepharose (Amersham Pharmacia, Uppsala, Sweden) prepared in a XK-26 Pharmacia column (125 mL resin volume). After application of the sample, fractions were collected by elution with a linear gradient from 100% DEAE buffer A to 100% buffer A plus 1 M NH4Cl (DEAE buffer B). Elution fractions were tested for factor V (FV) activity using a prothrombinase-based assay.1 Samples containing FV were pooled and precipitated using solid (NH4)2SO4 (ammonium sulphate) (Merck) with a 70% saturation. Total FV was purified according to the same protocol, but without separating the FV1 and FV2 peaks on the Resource S and Mono S columns.

References