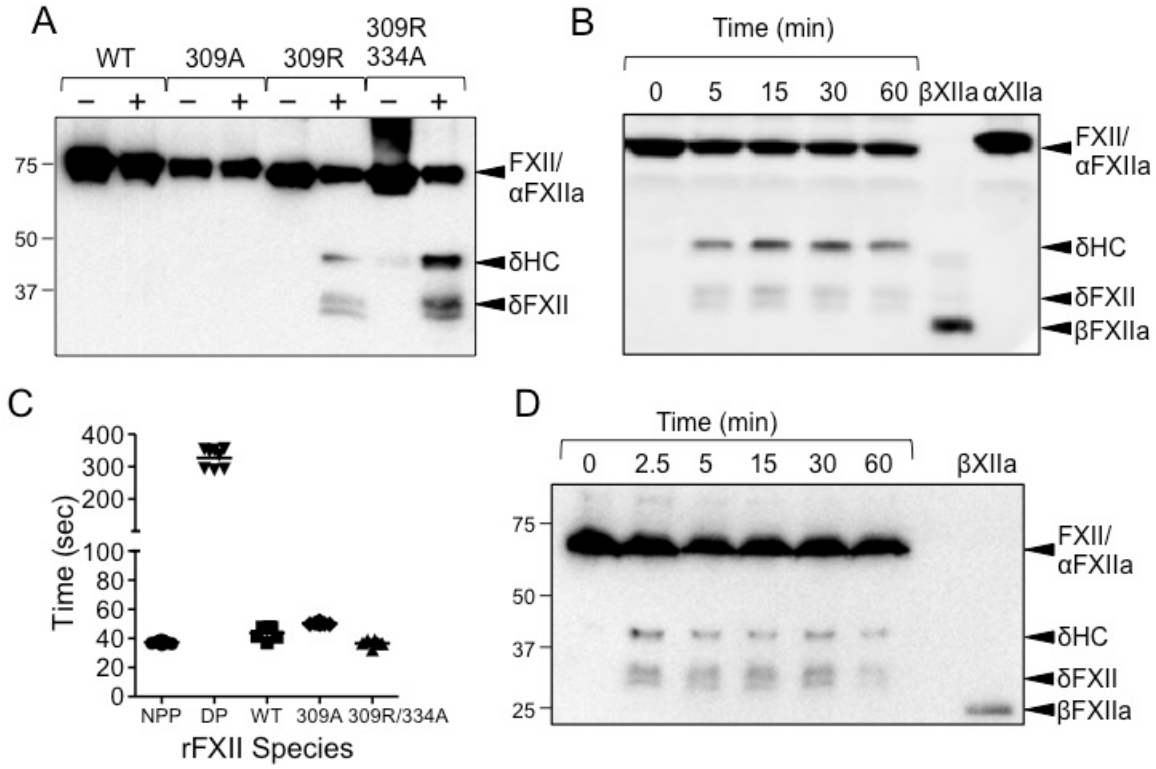
SUPPLEMENTAL FIGURES AND INFORMATION

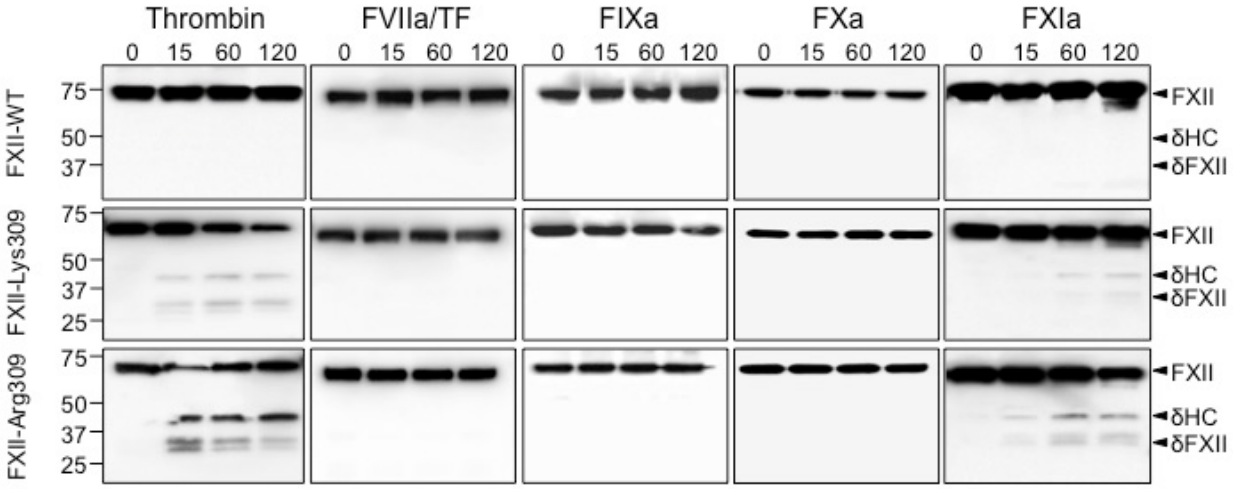
Supplement 1. Cleavage of FXII-Arg^{309} by thrombin. (A) Western blot of 200 nM FXII-WT (WT), FXII-Ala^{309} (309A), FXII-Arg^{309} (309R) or FXII-Arg^{309},Ala^{334} (309R/334A) standard buffer incubated without (–) or with (+) 10 nM thrombin for 2 hrs. **(B)** Western blot of FXII-Arg^{309} (400 nM) in FXII-deficient plasma induced to clot with TF (2.5 pM). At various times after adding TF, samples were removed into non-reducing sample buffer. **(C)** Clotting times in an aPTT assay for normal plasma (NPP), FXII-deficient plasma (DP) or FXII-deficient plasma supplemented with recombinant FXII-WT (WT), FXII-Ala^{309} (309A) or FXII-Arg^{309},Ala^{334} (309R/334A). Each symbol indicates one clotting time. **(D)** Western blot of FXII-Arg^{309},Ala^{334} (400 nM) in FXII-deficient plasma induced to clot with TF (2.5 pM). At various times after adding TF, samples were removed into non-reducing sample buffer. The Western blots in panels **A**, **B** and **D** were probed with polyclonal antibody to human FXII. Also shown in panels **B** and **D** are purified samples of βFXIIa and αFXIIa. To the right of the image are markers for FXII, αFXIIa, βFXIIa, the heavy chain of cleaved FXII-Arg^{309} (δHC) and FXII residues Thr^{310} to Ser^{596} (δFXII). Positions of molecular mass standards in kilodaltons are shown to the left of the images.



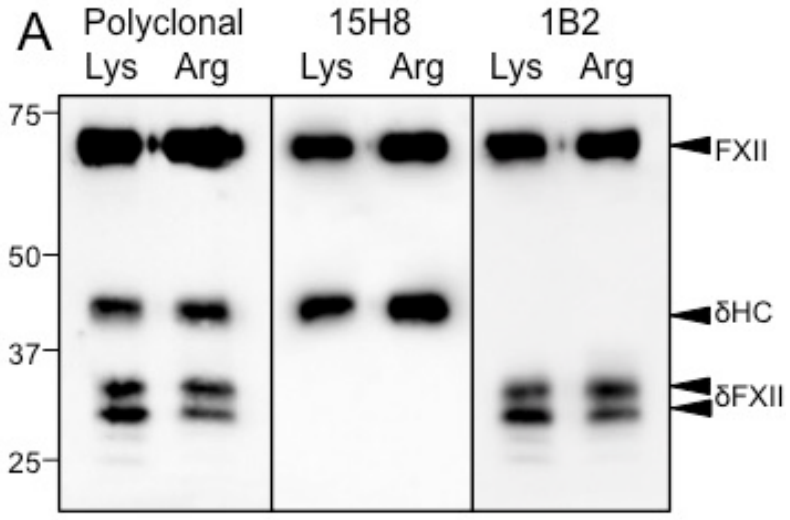
Supplement 2. Mass Spectroscopy analysis of thrombin-cleaved FXII. FXII-WT and FXII-Lys309 were digested with thrombin, fragments were resolved by SDS-PAGE and stained with Coomassie. Bands corresponding to full length and the amino terminal 309 amino acids (as independently confirmed by western blot) were excised, destained, and subjected to in-gel digestion using the arginine specific protease, GingisREX (Genovis, Lund Sweden). Resulting peptides were separated by microcapillary HPLC directly interfaced into a high resolution QExactive plus (ThermoFisher) instrument where both full scan (MS1) and data dependent fragment spectra (MS/MS) were collected as peptides eluted off the column. Using Skyline (Maccoss lab, Seattle WA), full scan MS1 ion chromatograms for the Arg-C fragments for full length FXII (R.LHVPLMPAQPAPPKPQPKTR.T [291 to 310]) and the thrombin-cleaved fragment (R.LHVPLMPAQPAPPKPQPK.T [291 to 308]) were extracted and integrated. The thrombin cleaved band showed essentially zero signal for the full-length Arg-C peptide, while the full length showed very little signal for the shorter Lys309 cleaved peptide. It is possible that this slight residual signal in the full-length band is an artifact of a slight background tryptic activity in the Arg-C enzyme. In summary: Excising both the full length and putative N-terminal 309 fragment produced by thrombin cleavage, we confirmed by mass spectrometry the presence of the Lys309 cleavage specifically in the thrombin-cleaved band.

Supplement 3. FXII cleavage by coagulation proteases.

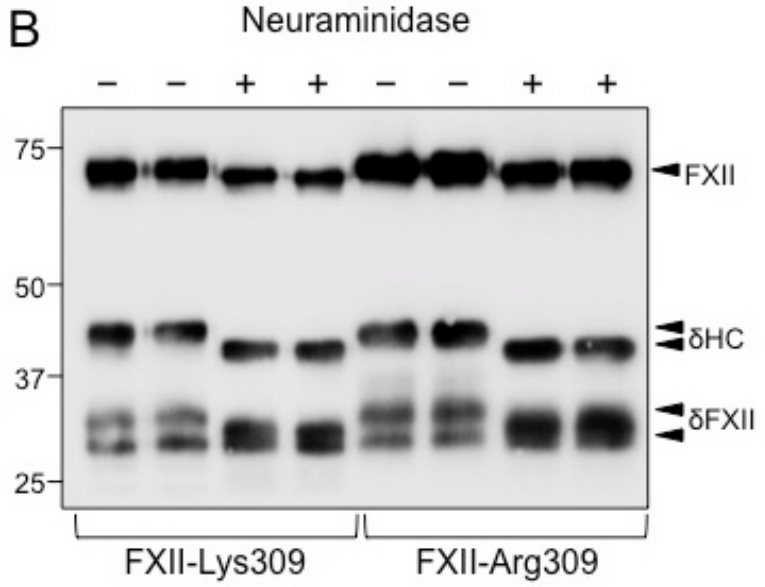
FXII (200 nM) in standard buffer incubated with 10 nM thrombin, factor VIIa/TF, factor IXa, factor Xa, or FXIa. Western blots of time courses were probed with a mixture of monoclonal IgGs to the FXII heavy (15H8) and light (1B2) chains. To the right of images are markers for FXII, the heavy chain of FXII-Lys^{309} or FXII-Arg^{309} cleaved after residue 309 (δHC) and FXII residues Thr^{310} to Ser^{596} (δFXII). Positions of molecular mass standards in kilodaltons are shown to the left of the images.



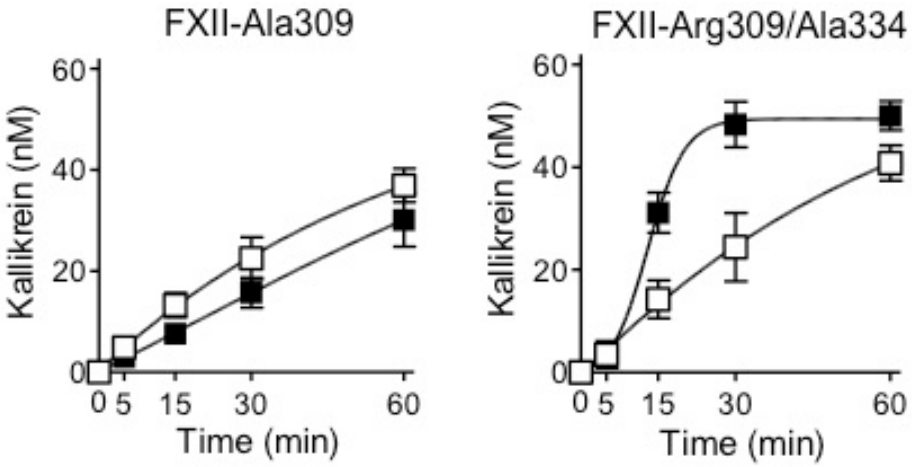
Supplement 4A. Identification of bands produced by cleavage of FXII-Lys309 and FXII-Arg309 by thrombin. FXII-Lys309 (Lys) or FXII-Arg309 (Arg) 200 nM in Standard Buffer were incubated for 2 hours at 37 ºC with thrombin (25 nM). Reactions were stopped by argatroban (125 uM) and products size fractionated by non-reducing SDS-PAGE. Western blots were probed with a polyclonal anti-human FXII IgG (Polyclonal), a monoclonal IgG to the FXII/FXIIa heavy chain (15H8) or a monoclonal IgG to the FXII/FXIIa light chain (1B2). Positions of molecular mass standards in kilodaltons are shown to the left of the images.



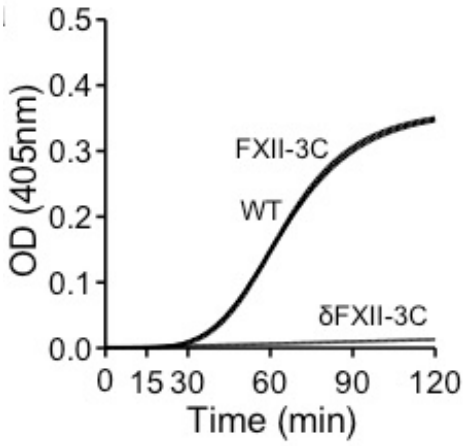
Supplement 4B. Deglycosylation of FXII-Lys309 and FXII-Arg309 fragments after cleavage by thrombin. Shown are western blots of FXII-Lys309 and FXII-Arg309 cleaved by thrombin as in Supplement 4A. After cleavage was stopped with argatroban, mixtures were incubated with vehicle (–) or neuraminidase (+). Western blots of time courses were probed with a mixture of monoclonal IgGs to the FXII heavy (15H8) and light (1B2) chains. To the right are markers for FXII (FXII), the heavy chain of cleaved FXII-Lys/Arg309 (δHC) and the δFXII. Not that bands representing full length FXII, δHC, and δFXII migrate faster, and that the bands of the doublet representing δFXII run closer together after deglycosylation. Positions of molecular mass standards in kilodaltons are shown to the left of the images.



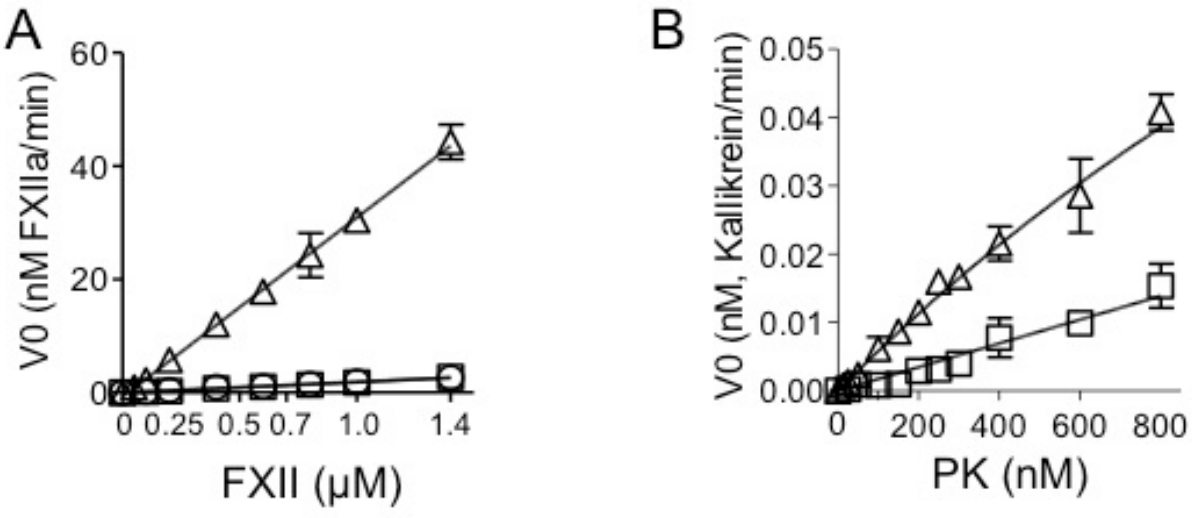
Supplement 5. Effect of thrombin on reciprocal activation of PK and FXII-Ala309 or FXII-Arg309,Ala334. FXII-Ala^{309} or FXII-Arg^{309},Ala^{334}, 100 nM in standard buffer, was incubated with (■) or without (□) thrombin (25 nM) for two hours at 37 ºC. Reactions were stopped with argatroban (125 uM). PK (60 nM) was mixed with 12.5 nM of the preincubated FXII. At various times, aliquots were removed, FXIIa was inhibited with CTI, and kallikrein concentration was determined by chromogenic assay.



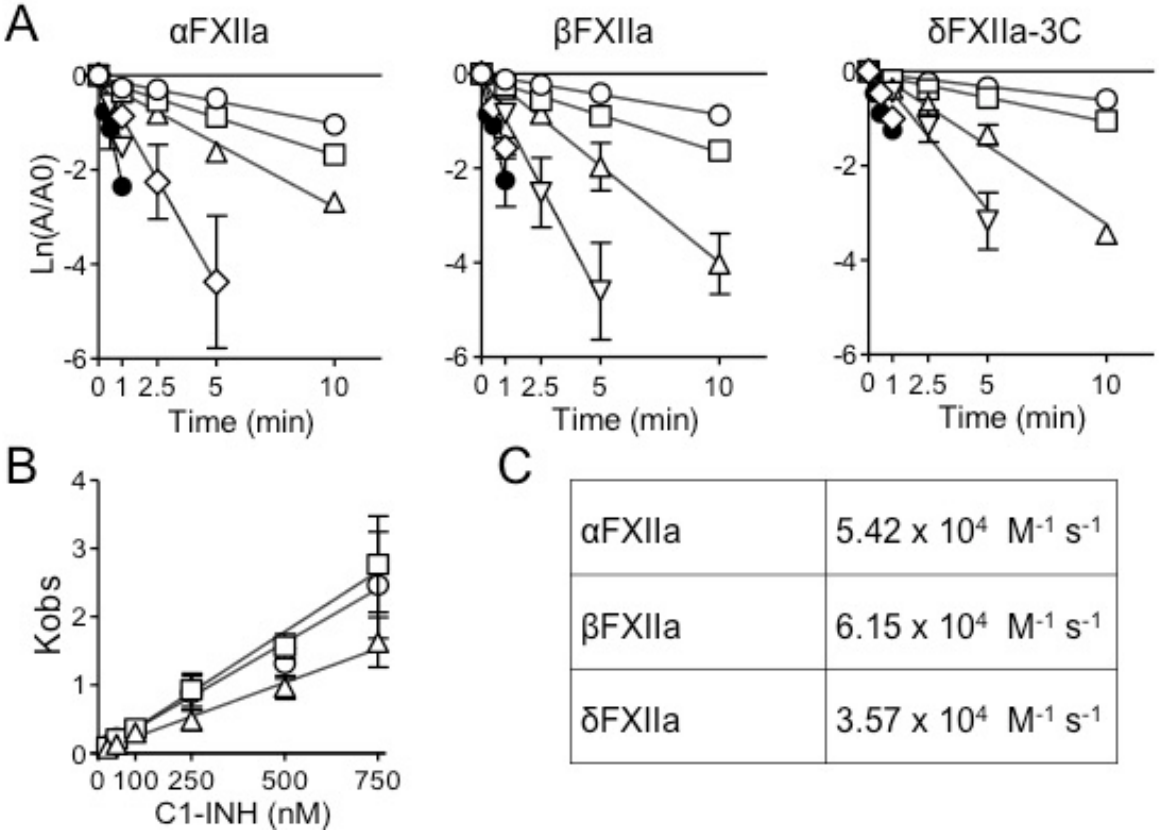
Supplement 6. Cleavage of FXII-3C. Autoactivation of FXII. FXII-WT (WT), FXII-3C, and δFXII-3C (200 nM) were incubated in Standard Buffer at 37 ºC in the presence of 70 μM Poly-P and 200 μM S-2302. Changes in OD 405 nm were continuously monitored on a microplate reader. Data are means of three independent runs.



Supplement 7. Kinetic analyses for FXII and PK activation. (A) FXII-WT (○), FXII-3C (□), or δFXIIa-3C (△) at various concentrations in standard buffer was incubated with 10 nM kallikrein for 15 min. Reactions were stopped with IgG H03, and FXIIa generation was determined by chromogenic substrate assay. **(B)** PK at various concentrations in standard buffer was activated by 15 nM FXII-T (□) or δFXII-T (△) for 30 min. Reactions were mixed with CTI, and kallikrein generation was determined by chromogenic substrate assay. Data are means ± 1 SD. Curves were fit to the Michaelis-Menten equation to derive catalytic efficiency (k_{cat}/K_m).



Supplement 8. FXIIa inhibition by C1-INH. (A) α-FXIIa, β-FXIIa or δXIIa-3C (20 nM) were incubated in Standard Buffer at 37 °C with 0 (straight line), 25 nM (○), 50 nM (□), 100 nM (△), 250 nM (▽), 500 nM (◇) and 750 nM (●) C1-INH. At various times, 20 µl samples were mixed with 20 µl Standard Buffer containing 1 µM S-2302, and the residual FXIIa was determined by measuring ΔOD 405 nm/min (ΔA). Pseudo-first order rate constants were determined using the equation $\ln(\Delta A/\Delta A_o) = -k \times t$, where ΔA_o is the rate of S-2302 cleavage per min by 20 nM FXIIa in the absence of C1-INH, ΔA is the rate of cleavage with C1-INH at time *t*, and *k* is the pseudo-first order rate constant. Data are means ± 1 SD. **(B)** The pseudo-first order rate constants (K_{obs}, the slopes of the individual lines in panel **A** for α-FXIIa (○), β-FXIIa (□) and δFXIIa-3C (△) were plotted against C1-INH concentration using linear regression analysis (Prism 5.0 software). Data are means ± 1 SD **(C)** Second order rate constants for inhibition of αFXIIa, βFXIIa and δFXIIa-3C (δFXIIa) were derived from slopes of the lines in panel **B**.



αFXIIa	5.42 x 10^4 M^{-1} s^{-1}
βFXIIa	6.15 x 10^4 M^{-1} s^{-1}
δFXIIa	3.57 x 10^4 M^{-1} s^{-1}

Supplement 9. Western blot pattern for high molecular kininogen (HK) in mouse plasma. The polyclonal IgG anti-mHKFXI was raised against a peptide sequence representing the binding site for FXI in mouse HK. As such, It is specific for HK and should not recognize the alternatively splice species low molecular weight kininogen. **(A)** Western blot of mouse plasma using anti-mHKFXI showing two major bands in wild type (WT) plasma that are not present in plasma from mice homozygous for a deletion of the *mKng1* gene (*Kng1*$^{-/-}$) that encodes mouse plasma kininogen. **(B)** Western blot of wild type mouse plasma probed with anti-mHKFXI showing the baseline pattern (–), and the change in that pattern after induction of contact activation by adding silica-based PTT-A reagent (+). Note the change in migration of the prominent bands, consistent with cleavage of HK by kallikrein to produce cleaved HK (HKa). Positions of molecular mass standards in kilodaltons are shown to the left of the images.