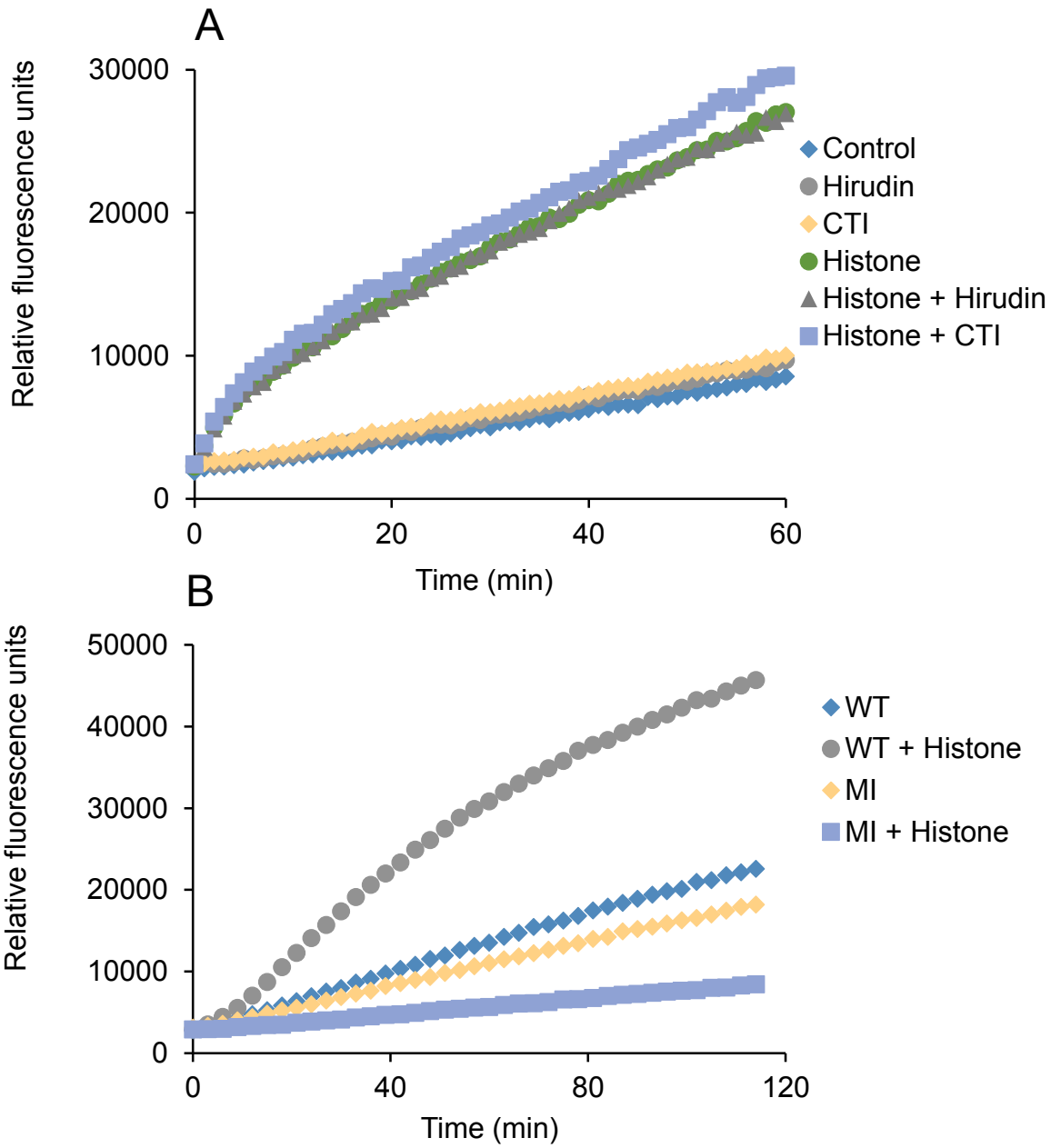
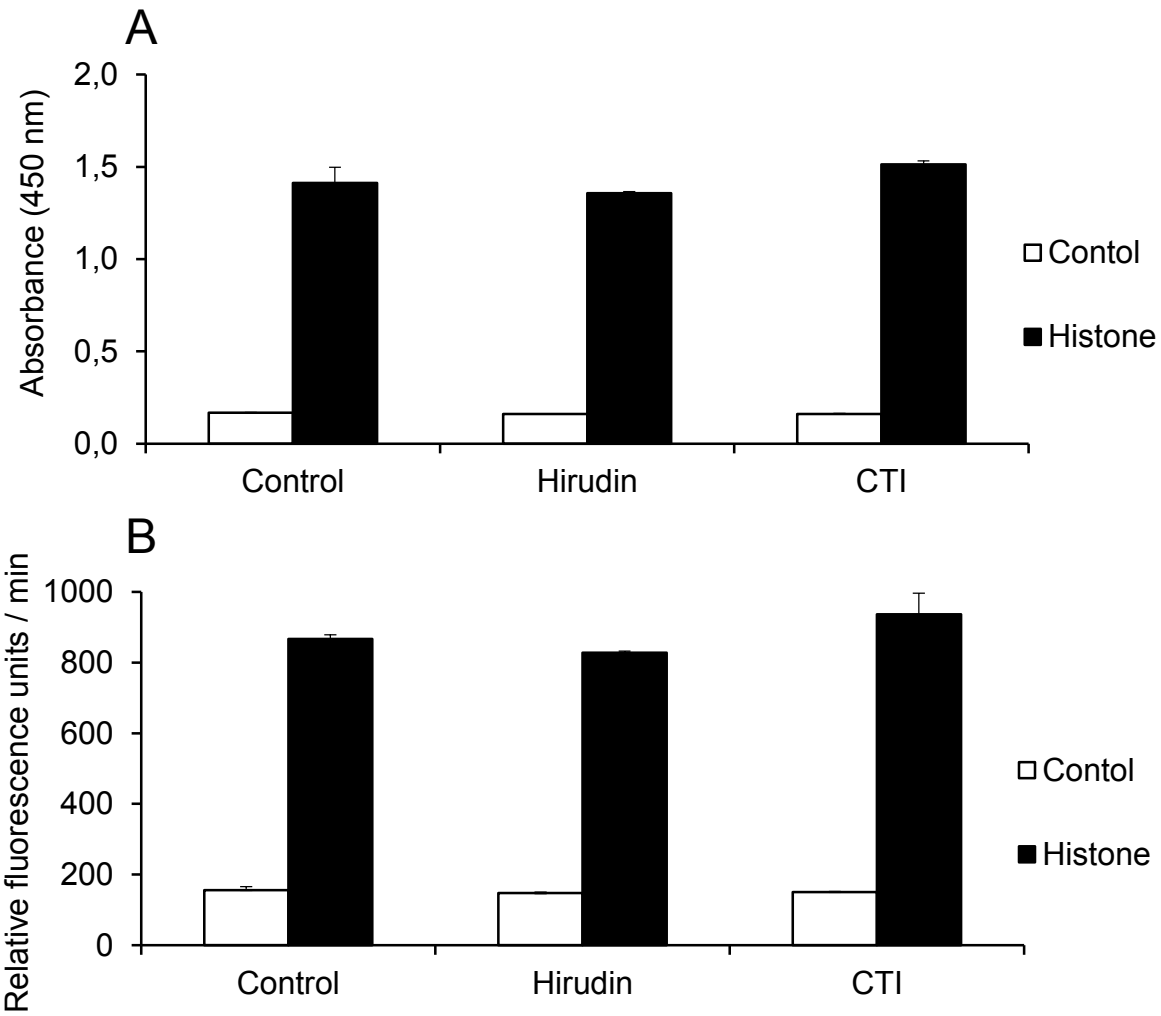
Supplementary Material to Kara et al. “Analysis of the substrate specificity of Factor VII activating protease (FSAP) and design of specific and sensitive peptide substrates”

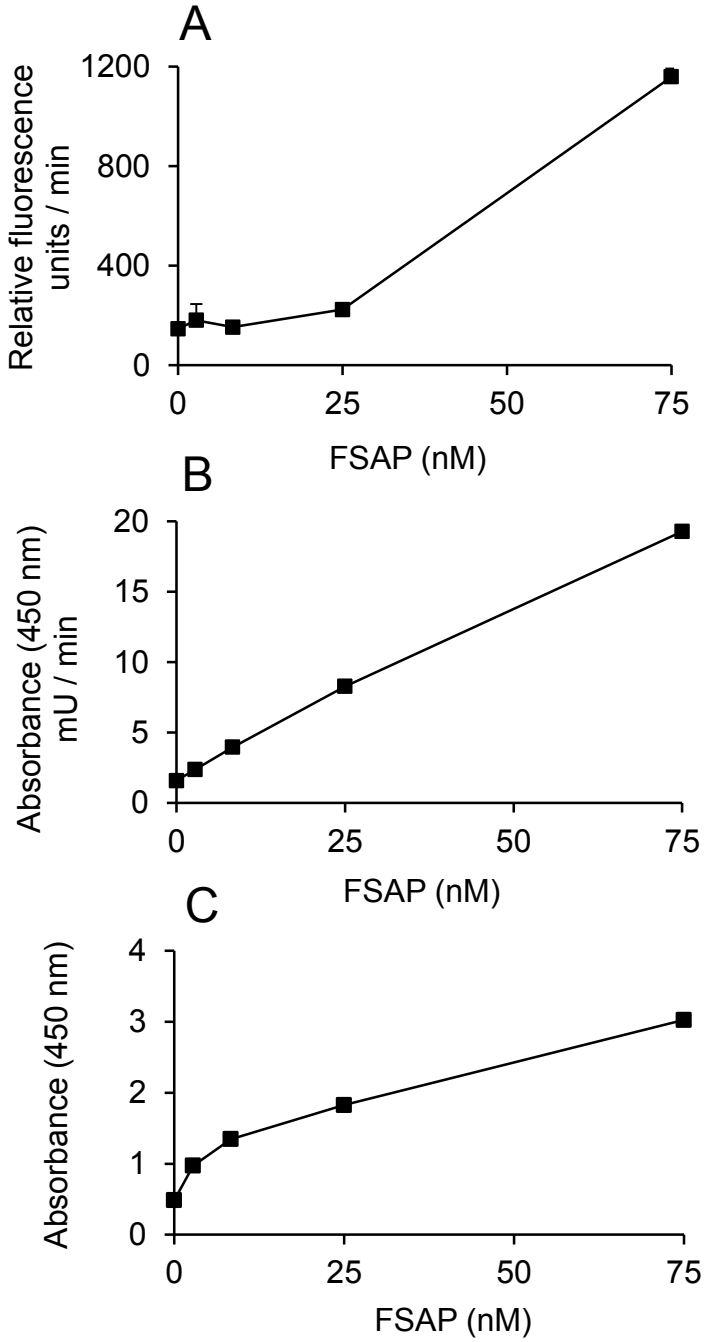
(https://doi.org/10.1160/TH17-02-0087)



Suppl. Figure 1: Determination of pro-FSAP activation in plasma: (A) Normal human citrate plasma (1:5 dilution) was incubated with corn trypsin inhibitor (CTI, 50 µg/ml) or lepirudin (25 µg/ml) in the absence or presence of histones (20 µg/ml). Ala-Lys-Nle-Arg-AMC substrate (40 µM) was added and the proteolytic activity was monitored at 37°C as relative fluorescence units. (B) Same conditions as above except that normal plasma was compared to plasma from a subject homozygous for the MI-SNP. Experiments were performed in duplicate.



Suppl. Figure 2: Determination of FSAP activation in plasma using FSAP: α2 anti-plasmin complex as well as fluorescent peptide turnover: (A) Normal human citrate plasma (1:5 dilution) was incubated with corn trypsin inhibitor (CTI, 40 µg/ml) or lepirudin (12.5 µg/ml) in the absence or presence of histones (20 µg/ml) for 60 min at 37°C. FSAP: α2 anti-plasmin complexes were determined using an ELISA. (B) To plasma samples prepared as above Ala-Lys-Nle-Arg-AMC substrate (40 µM) was added and the proteolytic activity was monitored at 37°C as relative fluorescence units/ min. Experiments were performed in duplicate and are shown as mean $\pm$ SD.



Suppl. Figure 3: Comparison of different assays to measure FSAP activity in plasma: Defined amounts of purified FSAP (0-75 nM) was added to FSAP-deficient plasma for 30 min at 37°C. (A) To these plasma samples Ala-Leu-Lys-Arg-AMC substrate (40 µM) was added and the proteolytic activity was monitored at 37°C as relative fluorescence units/min. (B) Plasma samples were added to FSAP-antibody coated wells to immuncapture FSAP. Activation of pro-uPA turnover was determined by using a chromogenic uPA substrate. (C) FSAP: α2 anti-plasmin complexes were determined using an ELISA. Experiments were performed in duplicate and are shown as mean ± SD.