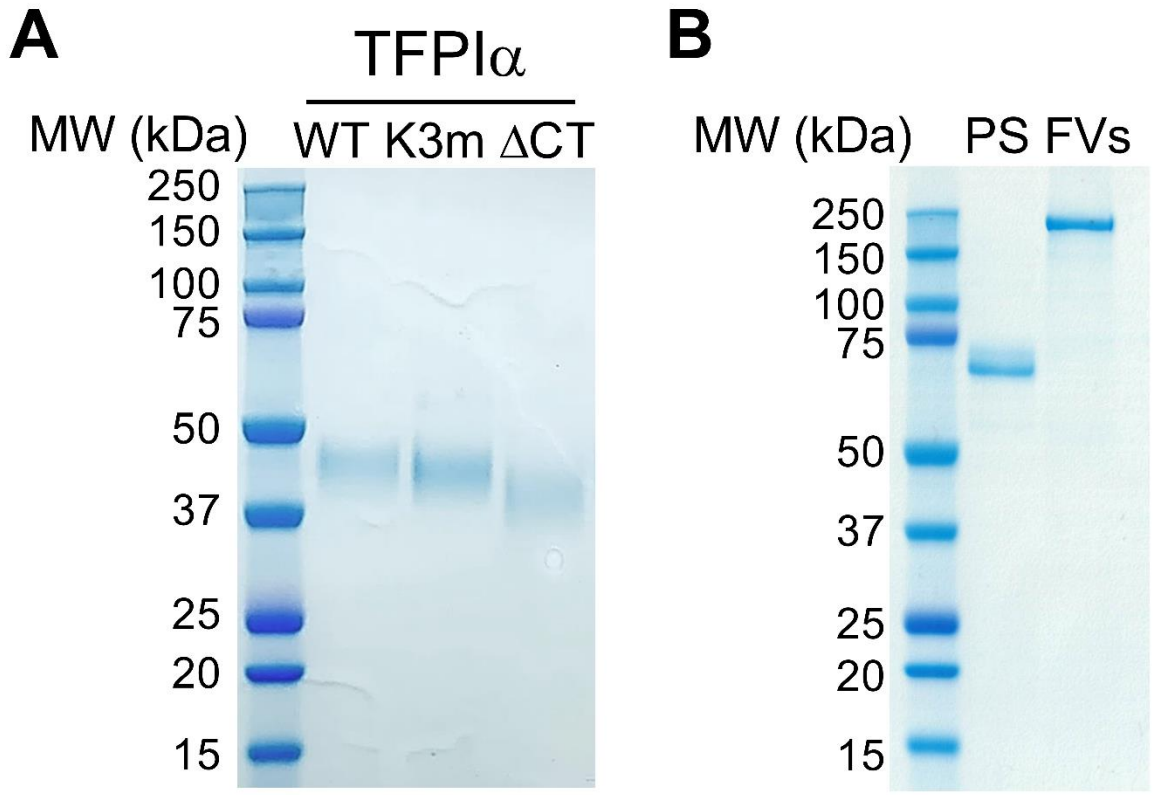
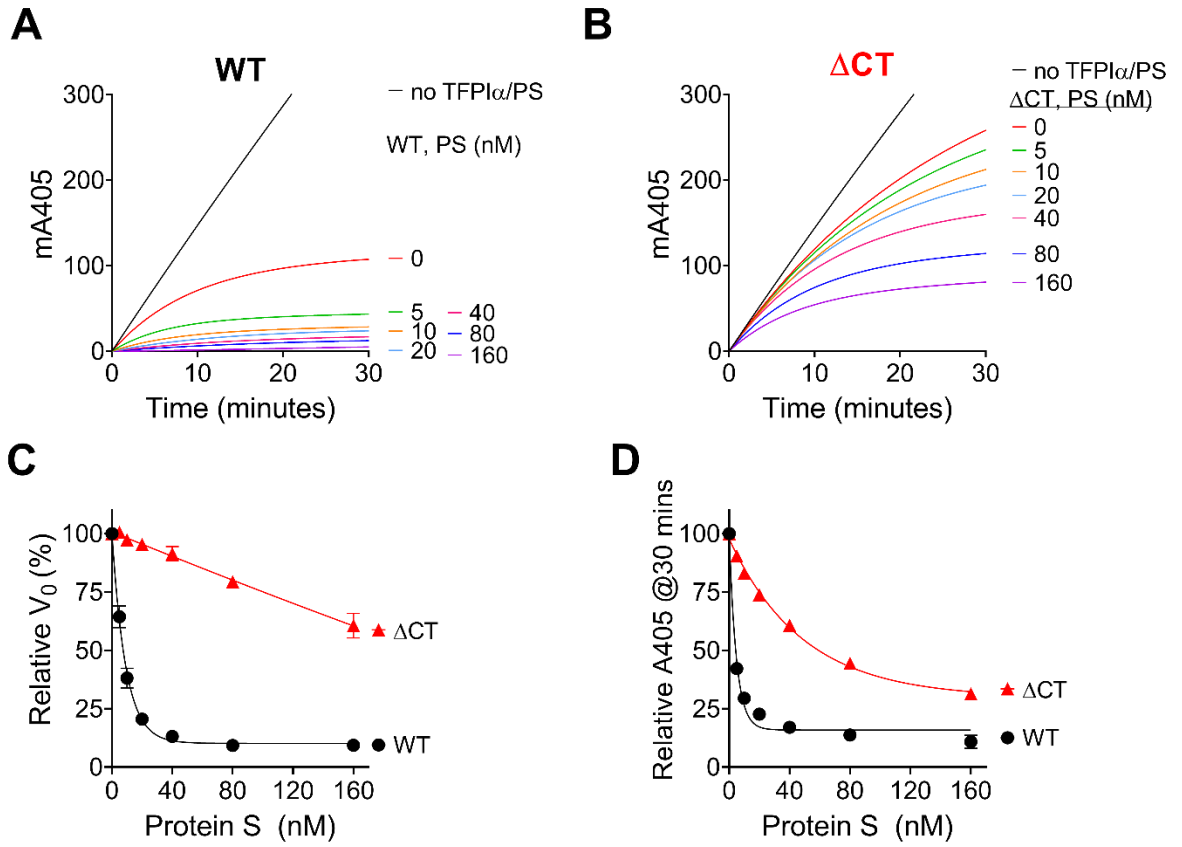
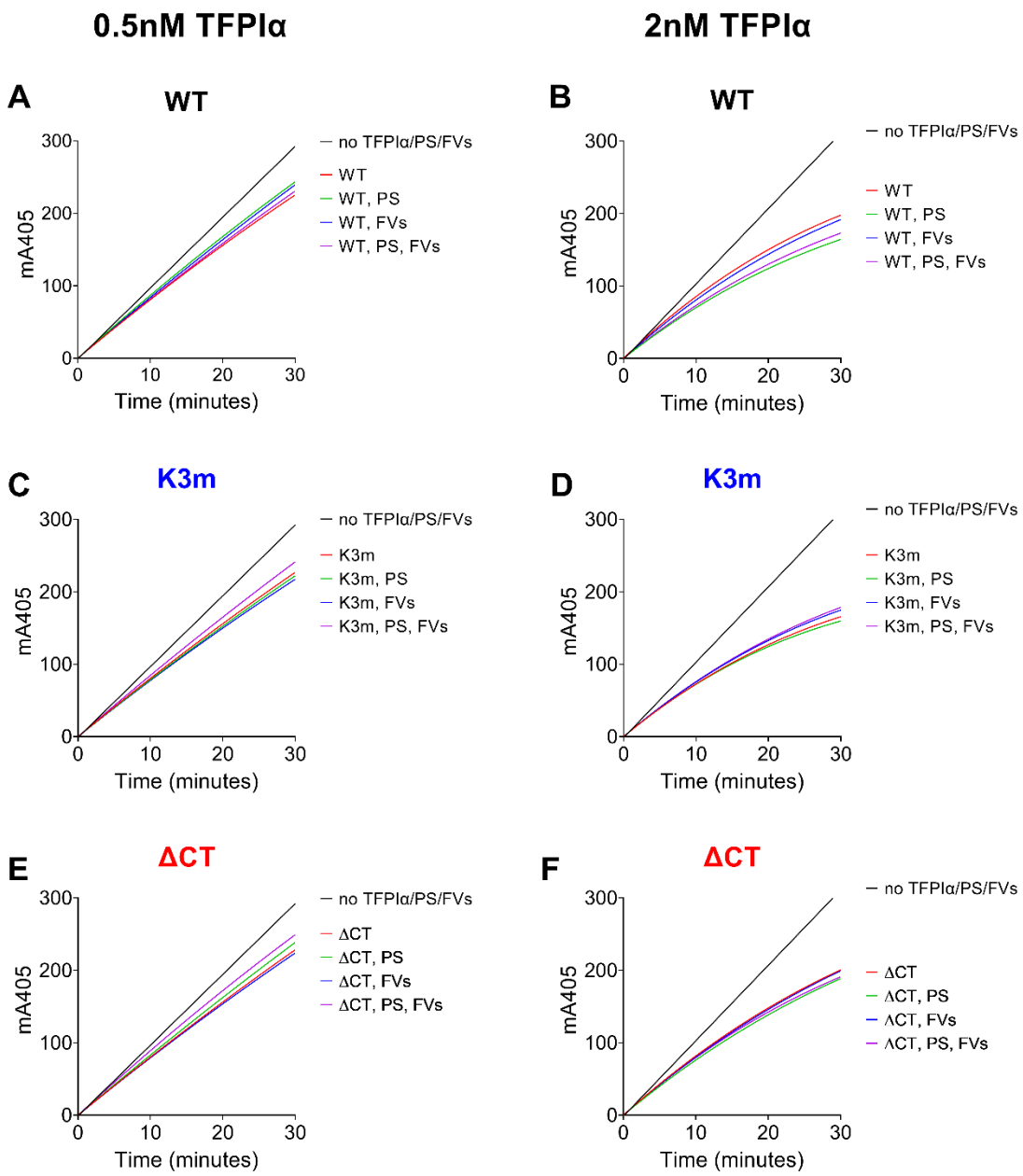
Supplementary results



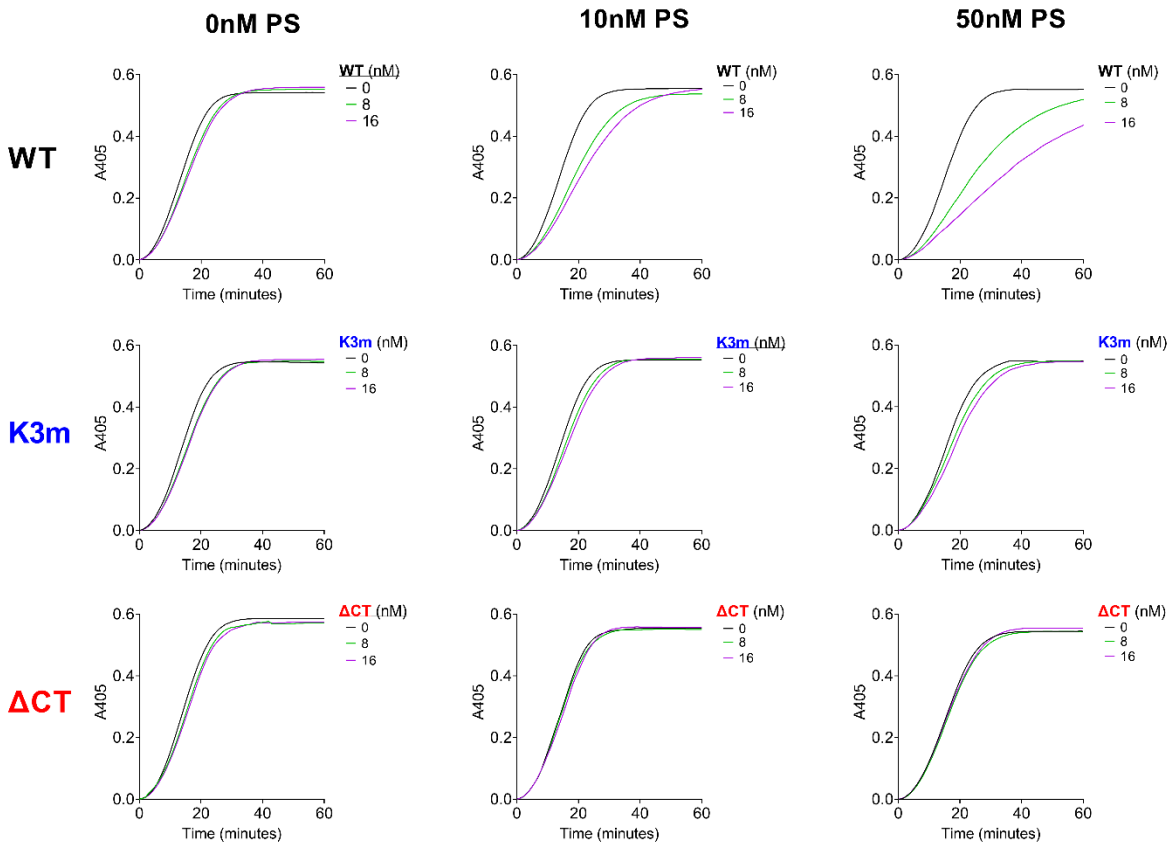
Supplemental Figure 1. WT TFPIα and its variants, protein S and FV-short. Purified WT TFPIα and TFPIα variants (A), protein S and FV-short (B) were analysed by SDS-PAGE, 4-12% gradient gel stained with Instant Blue under unreduced conditions. PS, protein S; FVs, FV-short.



Supplemental Figure 2. Enhancement of 5nM WT TFPIα and TFPIα ΔCT by protein S in the inhibition of FXa. FXa activity (0.5nM) was followed in real-time through cleavage of S-2765 (200µM) at 405 nm in the presence of 25µM phospholipids and absence or presence (5nM) of WT TFPIα (A) or TFPIα ΔCT (B) and increasing concentrations (0-160 nM) of protein S. Results from representative experiments are shown (n = 3). (C) V_0 was calculated for each curve and plotted against protein S concentration. Relative V_0 was calculated as percentage of the V_0 obtained for TFPIα alone. (D) A405 obtained at 30 minutes of FXa inhibition was plotted against concentration of protein S. Relative A405 was calculated as a percentage of the A405 measured for TFPIα alone. Results are given as mean ± SD.



Supplemental Figure 3. Enhancement of TFPIα by protein S and FV-short in the inhibition of FXa in the absence of phospholipids. FXa activity (0.5nM) was followed in real-time through cleavage of S-2765 (200µM) at 405 nm. The experiments were performed in the presence of WT TFPIα (A-B), TFPIα K3m (C-D) or TFPIα ΔCT (E-F) and in the presence and absence of protein S (10nM) and/or FV-short (5nM). Results from representative experiments are shown (n = 2). The experiments were performed at either 0.5nM (A, C, E) or 2nM (B, D, F) TFPIα.



Supplementary Figure 4. The effect of increasing concentrations of TFPIα and protein S on FXa-catalysed prothrombin activation in the presence of FVa. FVa (1nM) was incubated with 0-16nM WT TFPIα, TFPIα K3m or TFPIα ΔCT in the presence of 0-50nM protein S. Prothrombin (1μM), phospholipid membranes and 117 μM substrate S-2238 was added to TFPIα/FVa/protein S, followed by initiation of prothrombin activation by the addition of FXa (0.6pM). The thrombin activity was measured through cleavage of the chromogenic substrate at 405 nm over time. A representative experiment is shown (n=3). PS, protein S.