**Supplementary Material for**

# THE CONTRIBUTION OF AMINO ACID REGION 334-335 FROM FACTOR Va HEAVY CHAIN TO THE CATALYTIC EFFICIENCY OF PROTHROMBINASE

by

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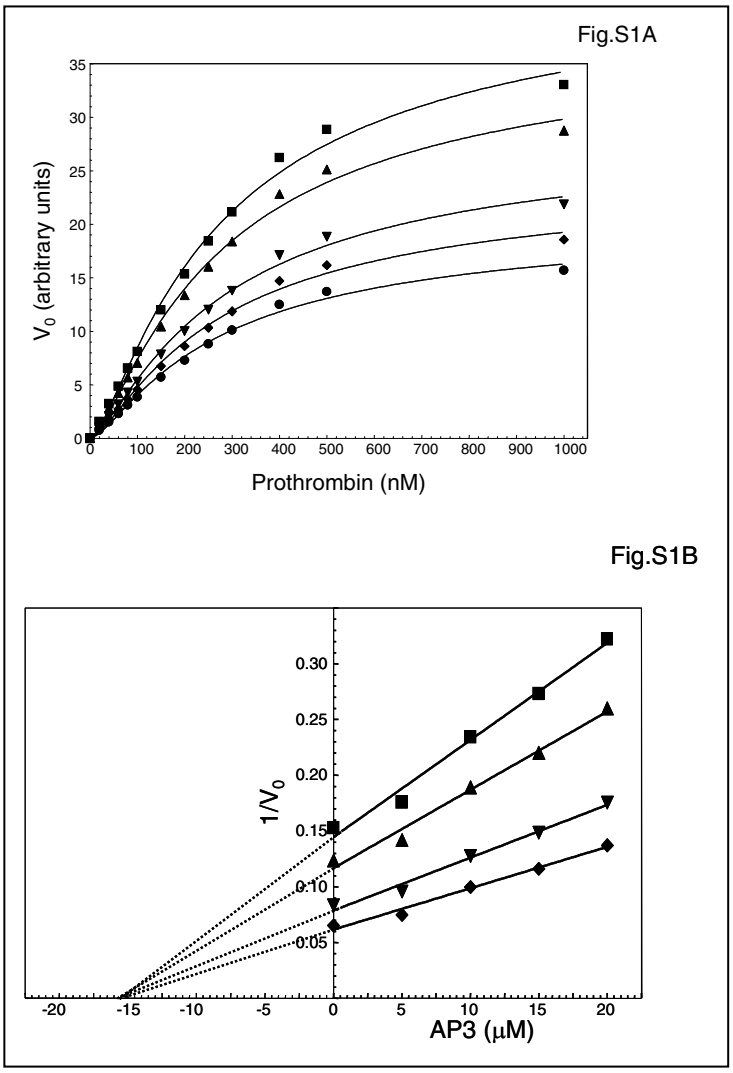


**Figure S1. Kinetic analyses of prothrombin activation by prothrombinase in the presence of AP3.** ***Panel A. Michaelis Menten plots.*** Prothrombin generation experiments were performed as described *(23)* in the absence (*filled squares*) and presence of increasing concentrations of AP3: 5μM *(filled triangles)*; 10μM *(filled inverted triangles)*; 15μM *(filled diamonds)*, and 20μM (*filled circles*) as described in the "*Experimental Procedures*" section using prothrombin concentrations varying from 40nM-1 μM. Initial rates of thrombin formation are plotted as a function of the substrate concentration. ***Panel B. Analysis of the data using the Dixon plots***. The data were analyzed and plotted as $1/V_o$ as a function of inhibitor concentration (AP3, Dixon Plots). For simple linear noncompetitive inhibition a Dixon plot of $1/V_o$ as a function of increasing concentrations of inhibitor is linear at a fixed enzyme and substrate concentration. The apparent inhibition constant ($K_i$) is the value derived from the intercept of each of the four graphs with the x-axis (~15.6 μM). The lines drawn represent the best fit through the points with an $R^2$ varying from

0.9858 (worst) to 0.9875 (best). The concentrations of prothrombin used in the experiments are as follows: 80nM (*filled squares*), 100nM (*filled triangles*), 150nM (*filled inverse triangles*), 200nM (*filled diamonds*).

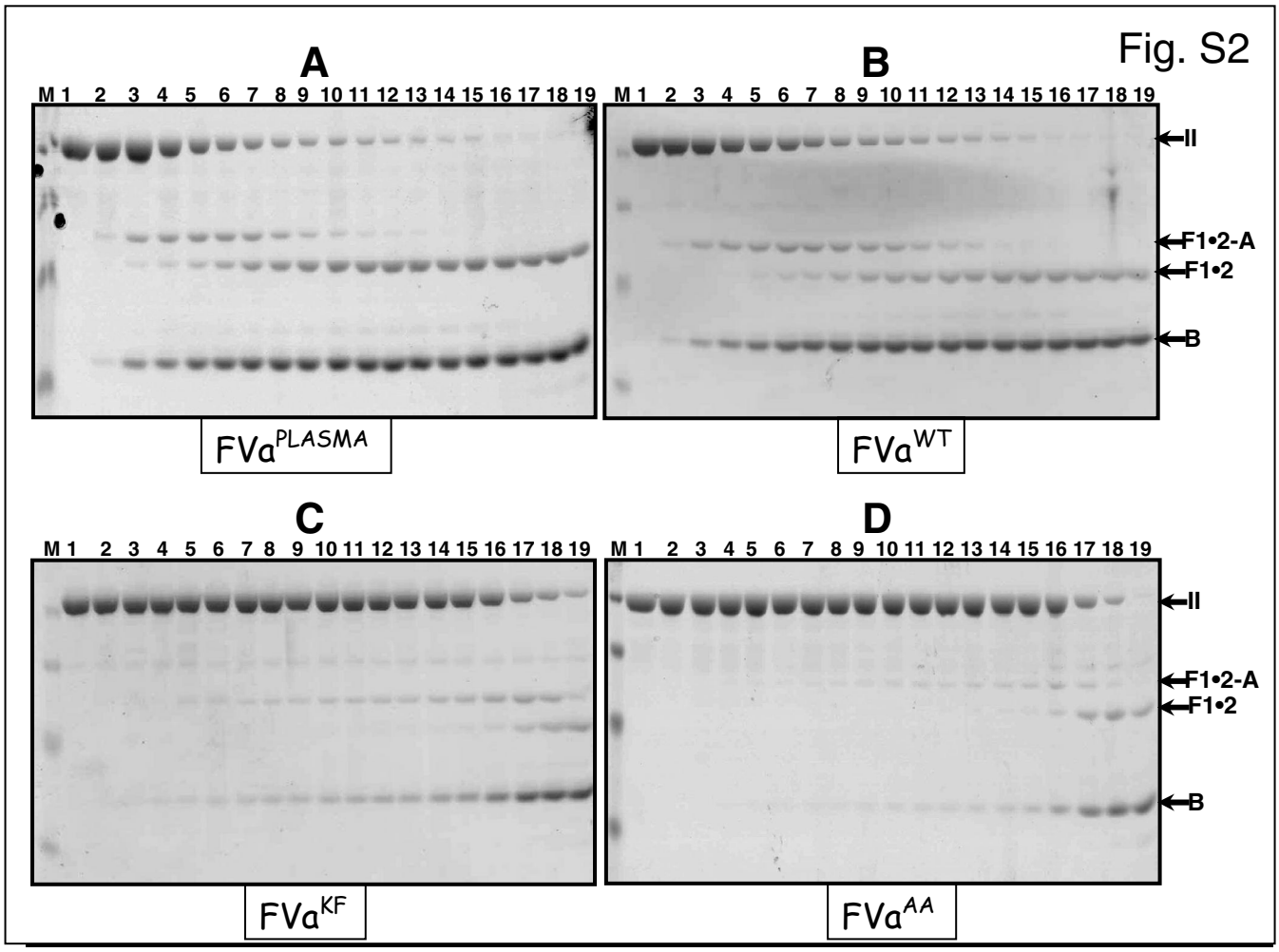


**Figure S2: Prothrombin Activation by Prothrombinase Assembled with Recombinant Factor Va Molecules.** Plasma-derived factor Va, factor $Va^{WT}$, factor $Va^{KF}$, and factor $Va^{AA}$ were incubated with prothrombin, PCPS vesicles, and DAPA as detailed in the "*Experimental Procedures*" section at a final concentration of 10nM. Factor Xa was added to start the reaction to a final concentration of 1nM. Aliquots were withdrawn at selected time intervals and treated as described in the "*Experimental Procedures*". *M* represents the lane with the molecular weight markers (from top to bottom): $M_r$ 98,000, $M_r$ 64,000, $M_r$ 50,000; $M_r$ 36,000. Lanes 1-19, represent samples from the reaction mixture before (0 min) the addition of factor Xa and 20 sec, 40 sec, 60 sec, 80 sec, 100 sec, 120 sec, 140 sec, 160 sec, 180 sec, 200 sec, 220 sec, 240 sec, 5 min, 6 min, 10 min, 20 min, 30 min,

and 60 min respectively following the addition of factor Xa. *Panel A*, factor Xa with plasma-derived factor Va; *panel B* prothrombinase assembled with wild type factor Va; *panel C*, prothrombinase assembled with factor $Va^{KF}$, *panel D* prothrombinase assembled with factor $Va^{AA}$. The legends to the right of the gels indicate the prothrombin activation fragments: II (prothrombin), F1•2-A (Fragment 1•2-A chain), F1•2 (fragment 1•2), and B (B chain of thrombin). Experiments were performed with at least four separate preparations of purified proteins and one representative gel is shown.