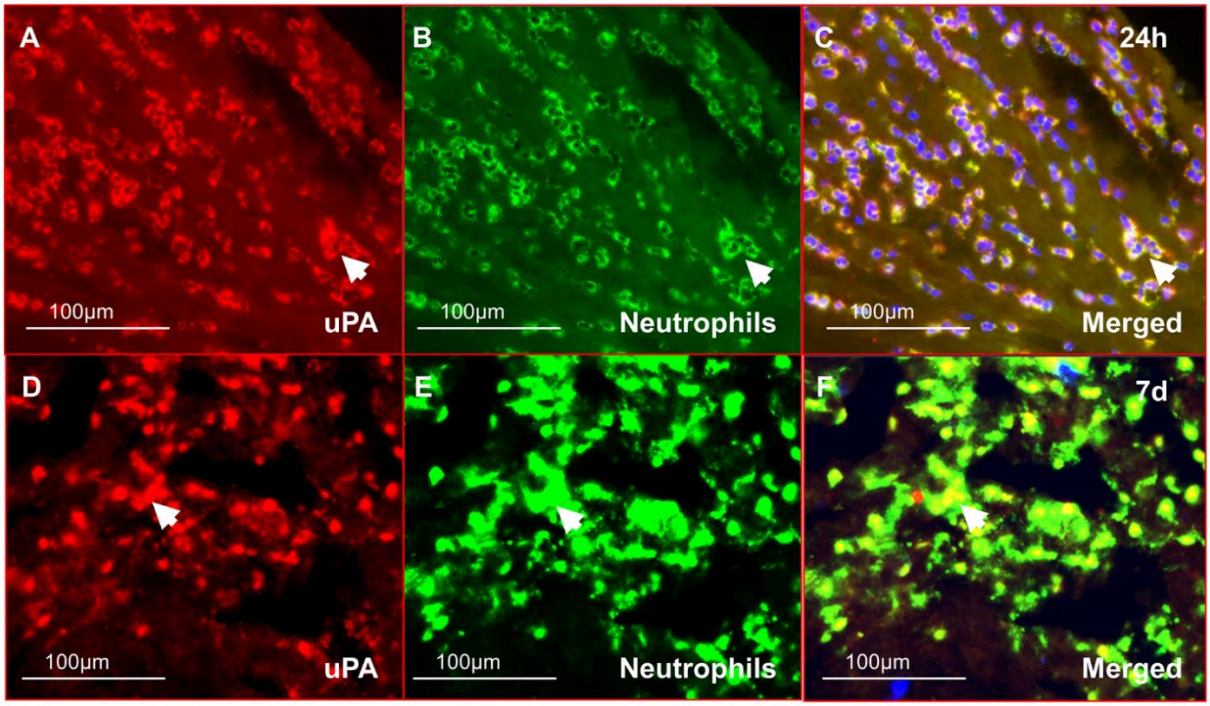
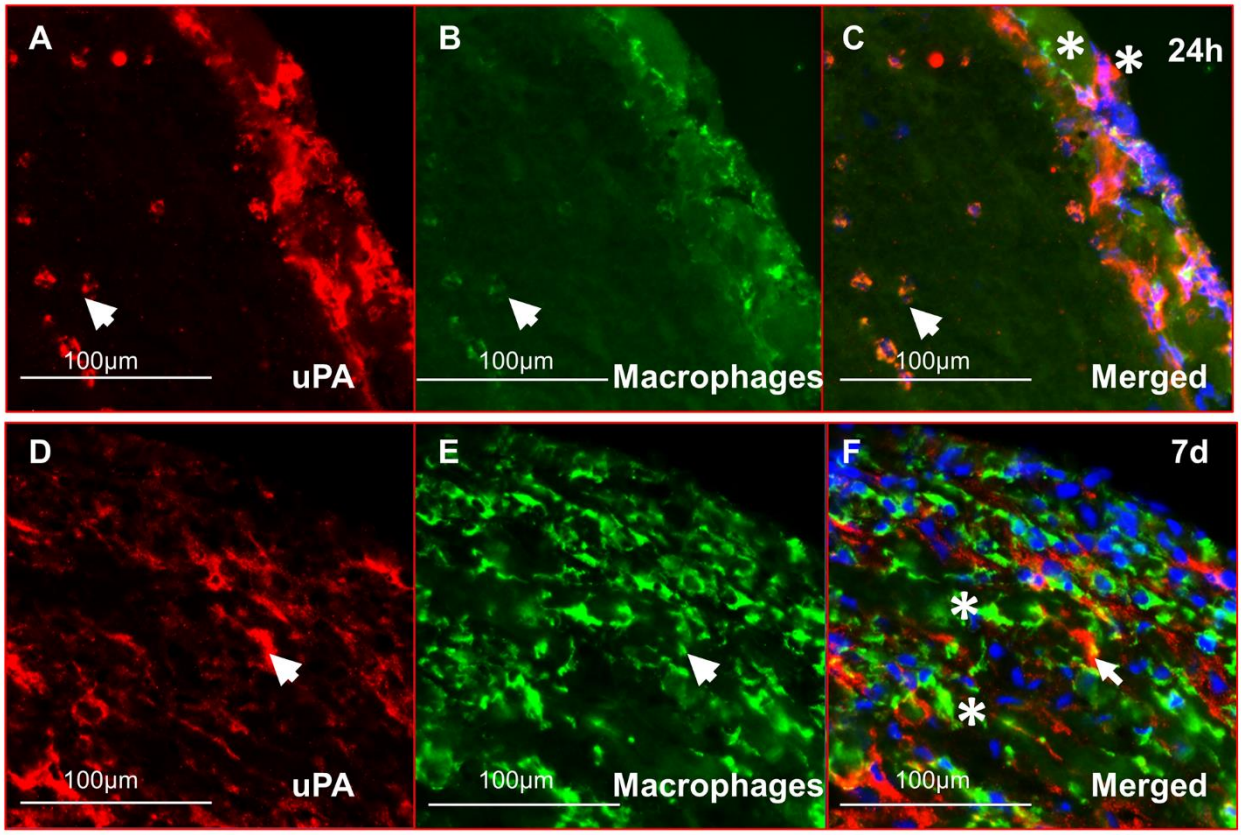
SUPPLEMENTAL MATERIAL

Supplemental Figures and Legends

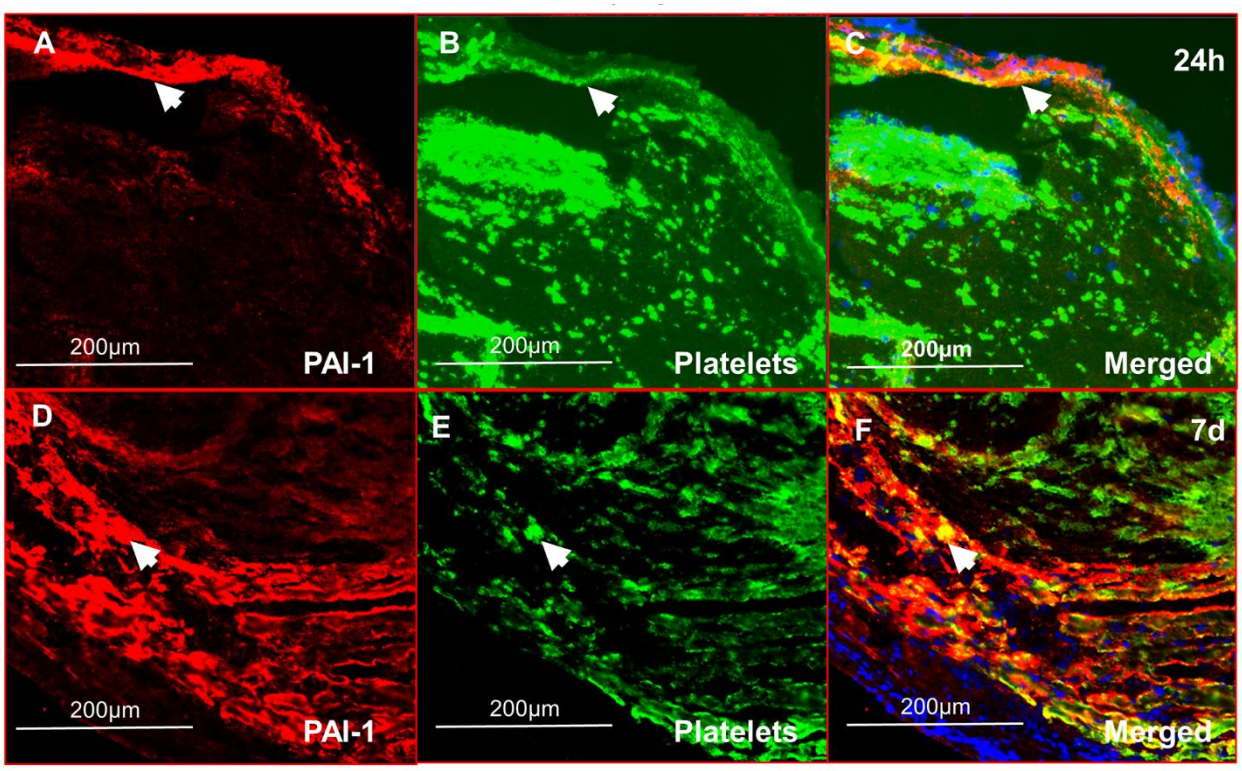


Supplemental figure 1. Neutrophil-associated expression of uPA in 24 h and 7 days IVC thrombi. Cryosections (8 µm) of IVC thrombi (scale bar = 100 µm) showing immunostaining for uPA and neutrophils in representative 24 h (A-C) and 7 day thrombi (D-F). Immunostaining for uPA (A, D) in red (e.g., white arrow) was detected by rabbit-anti mouse uPA and AF555 donkey anti-rabbit antibodies. Immunostaining for neutrophils (B, E) in green (e.g., white arrow) was detected by rat anti-mouse Ly-6G and AF488 donkey anti-rat antibodies. The merged images (C, F) show co-localization of uPA with neutrophils (yellow color, white arrow); nuclei are stained blue with DAPI. Representative images of 24 h and 7 days old IVC thrombi respectively as labelled, N=4 each. Images were captured on Zeiss Axio image M2 upright fluorescence microscope

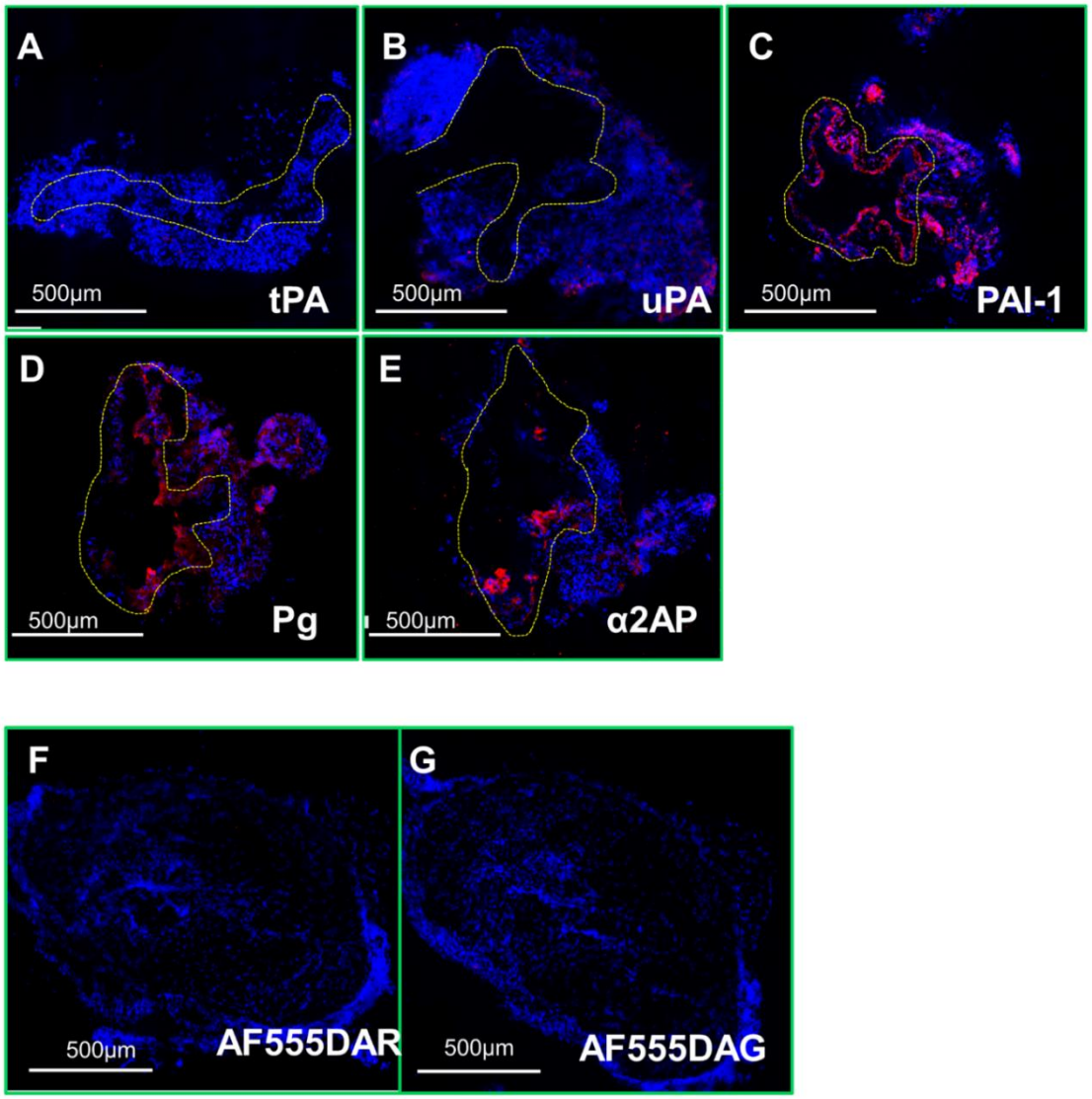
at 40X magnification by an Axiocam 506 digital monochrome camera using Zeiss Zen lite software.



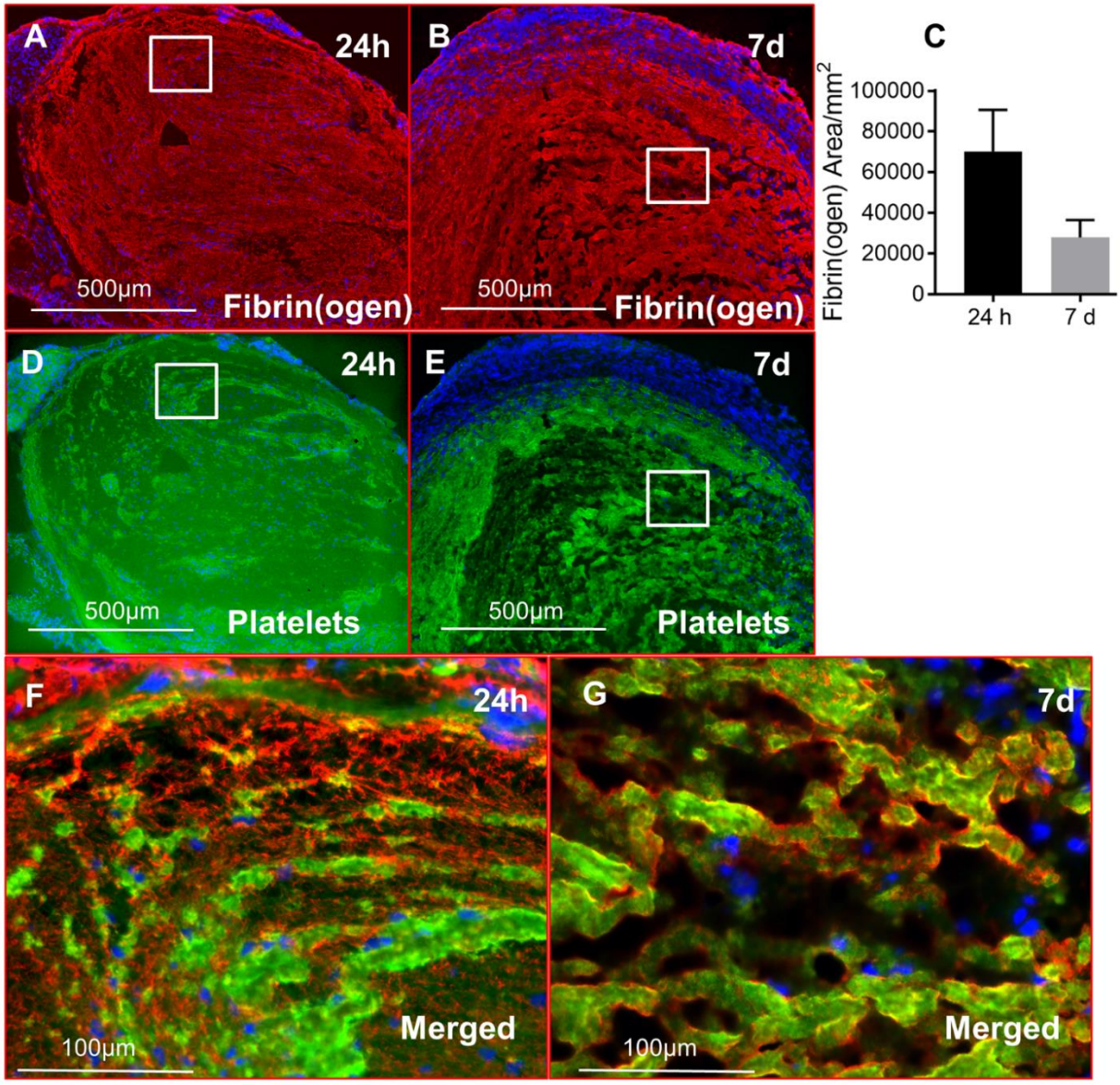
Supplemental figure 2. Expression of uPA by monocytes or macrophages in 24 h and 7 days IVC thrombi. Cryosections (8 µm) of IVC thrombi (scale bar = 100 µm) showing uPA in red (e.g., white arrow A, D) detected by rabbit-anti mouse uPA and AF555 donkey anti-rabbit antibodies. Monocytes or macrophages in green (e.g., white arrow B, E), detected by rat anti-mouse CD68 and AF488 donkey anti-rat antibodies. The merged images (C, F) show partial colocalization of uPA with monocytes/macrophages (yellow/orange color, white arrow); DAPI staining of nuclei is show in blue. Asterisks show areas where macrophages/monocytes did not colocalize with uPA. Panels **A-C** and **D-F** show representative images of 24 h and 7 days old IVC thrombi respectively, N=4 each. Images were captured on Zeiss Axio image M2 upright fluorescence microscope at 40X magnification by an Axiocam 506 digital monochrome camera using Zeiss Zen lite software.



Supplemental figure 3. Detection of platelets and PAI-1 in 24 h and 7 days IVC thrombi. Cryosections (8 µm) of IVC thrombi (scale bar = 100 µm) showing PAI-1 (red, white arrow, A, D) detected by rabbit-anti mouse PAI-1 and AF555 donkey anti-rabbit antibodies. Platelets (green, white arrow, B, E) were detected by rat anti-mouse CD41 and AF488 donkey anti-rat antibodies. The merged images (C, F) show partial co-localization of PAI-1 with platelets (yellow/orange color, white arrow); nuclei stained by DAPI are blue. Panels **A-C** and **D-F** show representative images of 24 h and 7 days old IVC thrombi respectively as labelled, N=4 each. Images were captured on Zeiss Axio image M2 upright fluorescence microscope at 40X magnification by an Axiocam 506 digital monochrome camera using Zeiss Zen lite software.

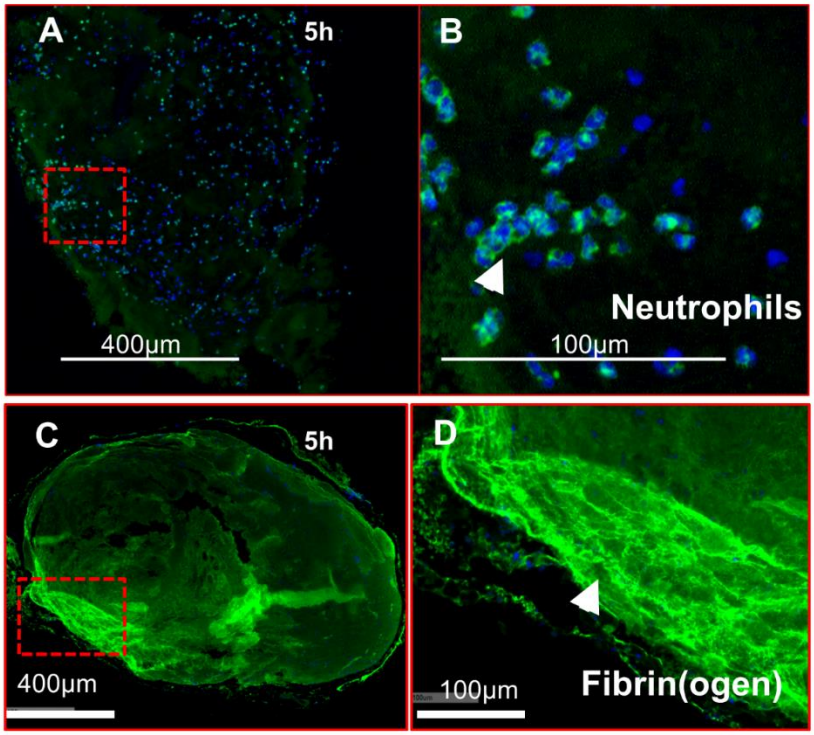


Supplemental figure 4. Detection of fibrinolytic system components in sham controls. Panels A-F show the expression (red color) of **(A)** tPA, **(B)** uPA, **(C)** PAI-1, **(D)** plasminogen (Pg) **(E)** α2AP in sham controls. Panel **(F)** and **(G)** show negative controls for secondary antibodies, Alexa Fluor 555® conjugated donkey anti-rabbit and donkey anti-goat. The primary antibody was not added as a control. One IVC section was always used as a negative control on each slide for comparison. Images (original magnification ×4) of whole IVC sections were captured (500µm). The yellow dotted lines indicate the outline of the vascular lumen.

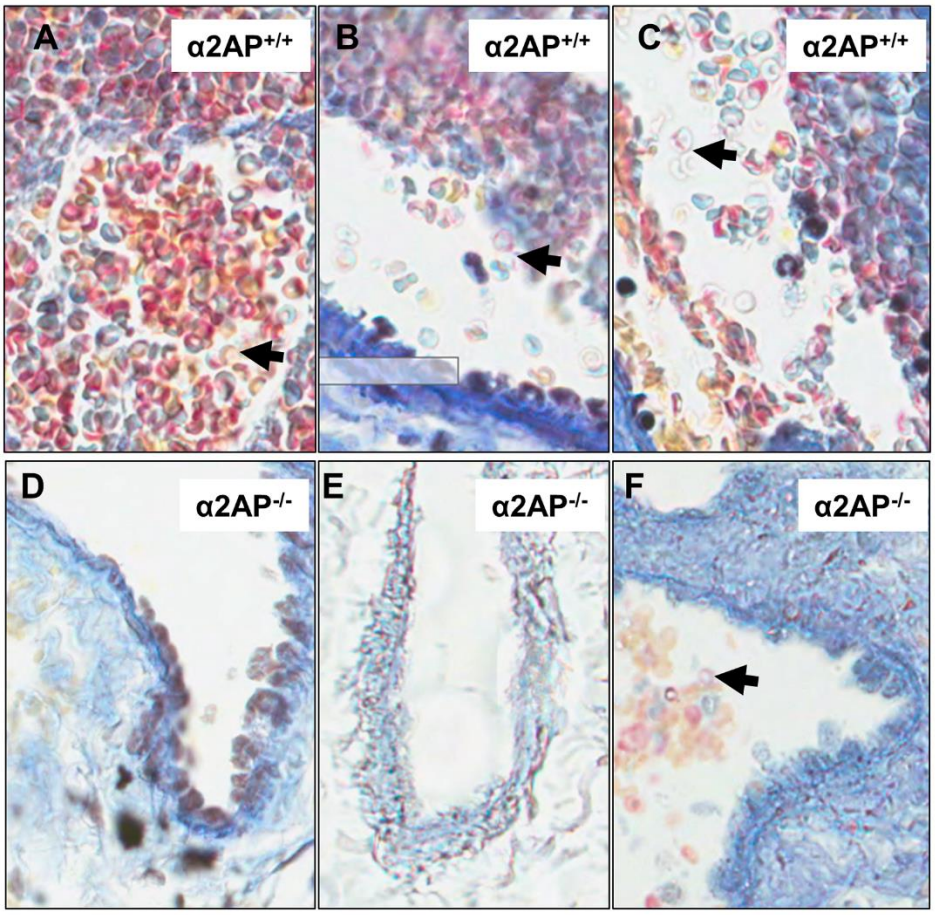


Supplemental figure 5. Fibrin(ogen) and platelet distribution in 24 h and 7 days IVC thrombi. Cryosections (8 µm) of IVC thrombi (scale bar = 100 µm) showing fibrin(ogen) (red, detected by rabbit-anti mouse fibrin(ogen) and AF555 donkey anti-rabbit antibody (A, B). Detection of platelets (green, D, E) by rat anti-mouse CD41 and AF488 donkey anti-rat antibodies. The merged images (F, G) show distribution of fibrin(ogen) and platelets in the thrombi. Panel **A-B** show fibrin(ogen) staining in 24 h and 7 days old IVC thrombi. Panel **C** shows fibrinogen quantification (arbitrary units,

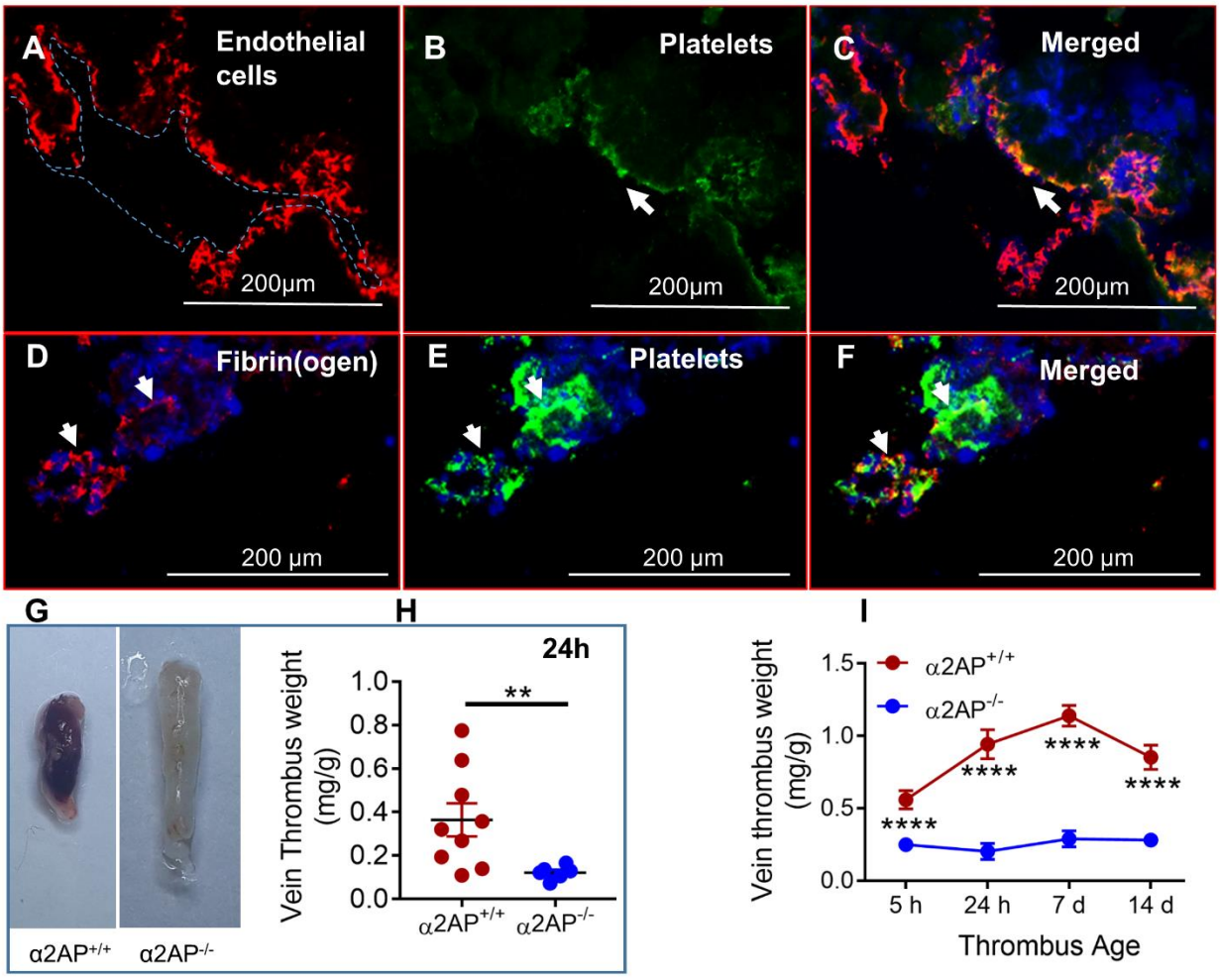
area/mm^2 of the thrombus section), N=4, *P< .105* (non-significant). The quantification was done using Image Pro Plus as described in methods section. Panel **D-E** show representative images of 24 h and 7 days old IVC thrombi respectively for platelets staining as labeled, N=4 each. Panel **F** and **G** show merged magnified images of areas marked by white box in figure A-B and D-E. Images were captured on Zeiss Axio image M2 upright fluorescence microscope at 40X magnification by an Axiocam 506 digital monochrome camera using Zeiss Zen lite software.



Supplemental figure 6. Presence of neutrophils and fibrin(ogen) in 5 h IVC thrombi. Formalin-fixed, paraffin embedded sections (5µm) were immunostained with rat anti-mouse Ly6B.2 antibody followed by AF488 donkey anti-rat antibody (green color). DAPI stained nuclei are blue in color. **(A)** A 4X image of 5h IVC thrombi and **(B)** magnified image (original magnification ×20) of selected area (red box) in (A). The neutrophils are indicated by cell shaped staining (white arrow) in 5 h thrombi. Panel **C-D** show fibrin(ogen) staining, **(C)** 4X image of 5h IVC thrombi and **(D)** Magnified image (original magnification ×20) of selected area (red box) in (C).

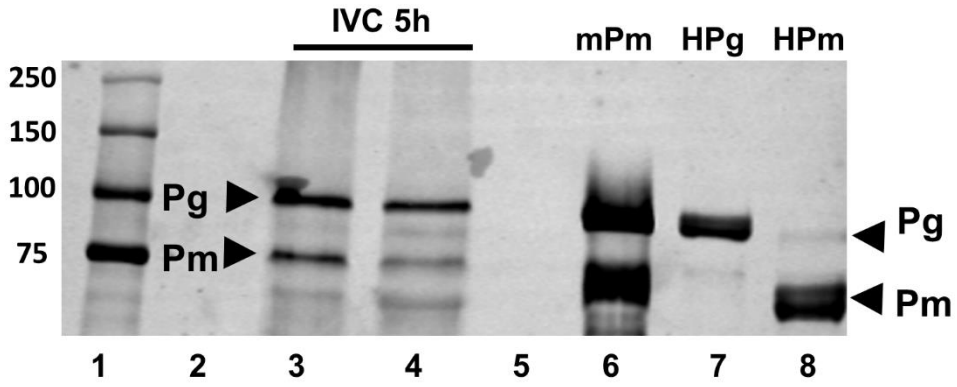


Supplemental figure 7. Presence of red blood cells (RBCs) in IVC of α2AP$^{+/+}$ and α2AP$^{-/-}$ mice after 5 h IVC ligation. Panel **A-C** show representative images of Martius scarlet blue stained sections (at 40X magnification) of IVC thrombi of three α2AP$^{+/+}$ mice after 5 h of IVC ligation as labeled. Panel **D-F** show representative images (at 40X magnification) of IVC of three α2AP$^{-/-}$ mice after 5 h IVC ligation. The black arrows indicate the presence of RBC. There were few (panel F) or no RBCs (panel D-E) were observed.



Supplemental figure 8. Panel (A-C) Platelet deposition on the vascular endothelium in α2AP$^{-/-}$ mice after 24 h IVC ligation. Cryosections (8 µm) of IVC thrombi (Scale bar = 200 µm) showing **(A)** endothelial cells (red, detected by rabbit-anti mouse CD31 and AF555 donkey anti-rabbit) and **(B)** platelets (green, white arrow, detected by rat anti-mouse CD41 and AF488 donkey anti-rat). The blue dotted line in panel A shows the inner vascular boundary of IVC lined by endothelium. The merged image **(C)** showing the presence of platelets on the vascular endothelium (white arrow), N=4 each. **(D-F)** Immunofluorescence images of a microscopic vein thrombus in α2AP$^{-/-}$ mice after 24 h IVC ligation. Panel **D**: fibrin(ogen) (red color, white arrows). Panel **E**:

platelets (green color, white arrows). Panel **F**: merged image showing colocalization of fibrin(ogen) and platelet immunostaining. **Panels G-H** show thrombus formation in $\alpha2AP^{+/+}$ vs $\alpha2AP^{-/-}$ mice in IVC stenosis model at 24 h. Panel **(G)** shows the representative thrombus formation in $\alpha2AP^{+/+}$ mice and empty IVC due to lack of thrombus in $\alpha2AP^{-/-}$ mice as labelled. Panel **(H)** The bar graph s represents vein thrombus weight in mg/g of body weight $^{**}P< .01$ (Mann-Whitney test), N= 15. (**I)** The graph represents vein thrombus weight in mg/g of body weight (mean ± SEM) versus thrombus age at different time points of study post IVC ligation in $\alpha2AP^{+/+}$ vs $\alpha2AP^{-/-}$ mice, Student's t-test at each point. The animal number is as per data from the main figures. $^{****}P< .0001$.



Supplemental figure 9. Plasmin generation in IVC thrombi after 5 h of IVC ligation in α2AP$^{+/+}$ mice. Thrombus lysates (100 µg) were electrophoresed on 10% reducing SDS-PAGE gels along with purified plasminogen and plasmin preparations, and western blotting was done as described in Methods. Each lane is marked: Lane 1- molecular weight marker; lane 2-empty; lane 3,4- thrombus lysates after 5 h of IVC ligation; lane 5-empty; lane 6-mouse plasmin (mPm) (prepared by partial activation of mouse plasminogen with mouse tPA); lane 7- human plasminogen (HPg), lane 8-human plasmin (HPm). The black arrows show plasminogen and the heavy chain of plasmin as detected by rabbit anti-mouse plasminogen antibody followed by goat anti-rabbit 680 IRDye Li-COR secondary antibody (LI-COR biosciences, NE). The blots were scanned by LI-COR Odyssey infrared imaging system.