Supplemental videos and legends

Supplemental Video 1. HIT antigenicity in vivo with a thrombus. Susceptibility to heparin.
Widefield video in $hPF4^{+}$ mice showing platelet accumulation (red) within a cremaster arteriole laser-induced thrombus and HIT antigenicity detected by KKO (green) prior to and post infusion of 1,000U/kg of unfractionated heparin.

Supplemental Video 2. Exuberant regrowth of a pre-established thrombus in HIT.
Widefield video of HIT mice showing platelet accumulation (red) within a cremaster arteriole laser-induced thrombus prior to and post infusion of KKO. Thrombus growth terminates when the vessel occludes.

Supplemental Video 3. Confocal images of a growing HIT thrombus showing predominant binding of KKO to the perithrombus endothelium.
Confocal image video of HIT mice showing platelet accumulation (red) within a cremaster arteriole laser-induced thrombus and HIT antigenicity (green) post infusion of KKO and initiation of HIT. Note the binding of the HIT antigenicity at the base of the thrombus, the whirlpool recycling of platelets or platelet clumps in the upstream end of the thrombus and the late appearance of HIT antigenicity at the interface of the looser platelet shell and more stable core of the thrombus.

Supplemental figures and figure legends

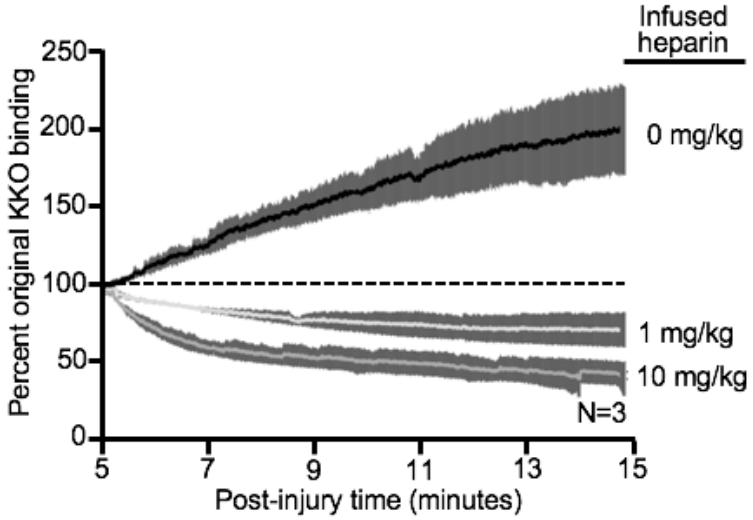

Figure 1 Supplement. Effect of low molecular weight heparin on KKO binding to a thrombus.

Effect of various doses of low molecular weight heparin infused beginning 5 minutes after the cremaster injury. Mean ± 1 SEM for binding of KKO after low molecular weight heparin was infused relative to the 5-minute timepoint is shown. The dashed line indicates the value of KKO binding if there were no change in KKO binding after heparin infusion compared to the 5-minute heparin timepoint.

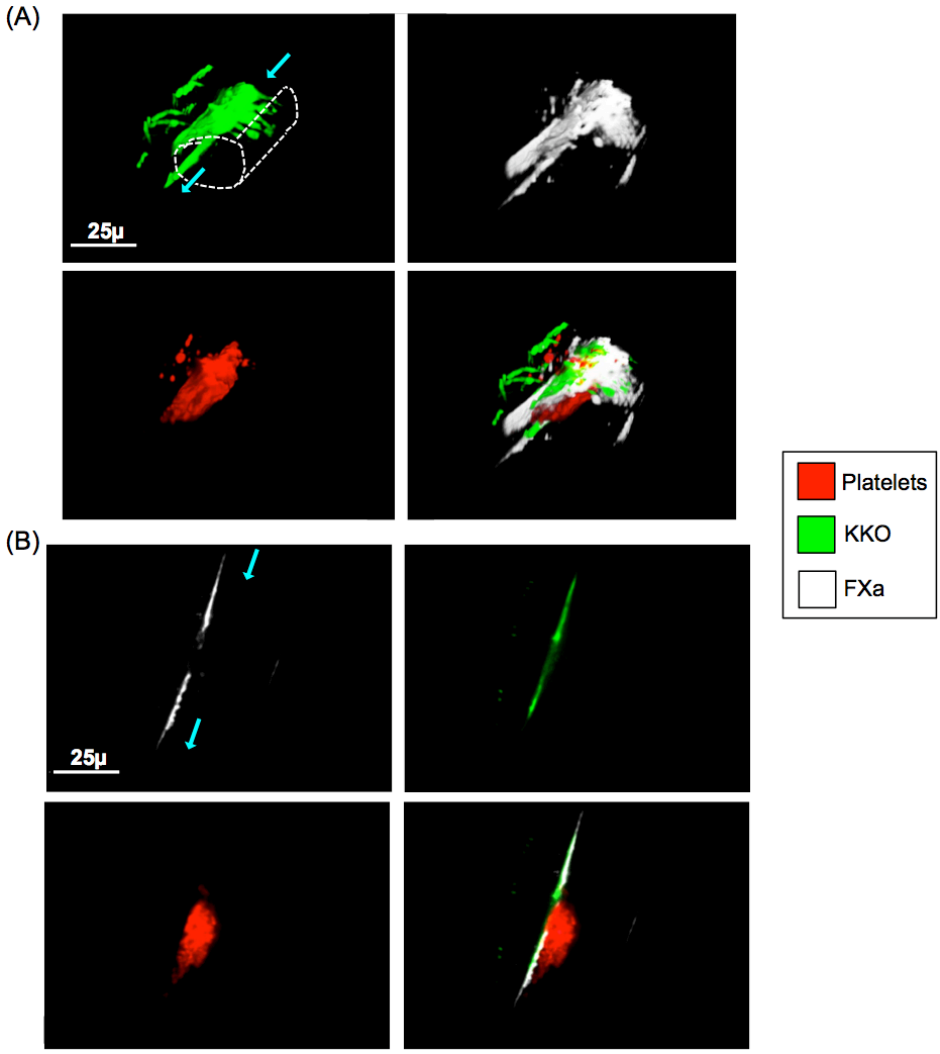

Figure 2 Supplement. Overlap in binding of KKO and FXa as an indicator of activated endothelium at a thrombus.

(**A**) and (**B**) are confocal images of a representative cremaster laser injury thrombus in a HIT mouse done as in Figure 3. (A) is the reconstructed image shown from the top/front with KKO binding in green (top left image), FXa binding in white (top right), accumulated platelets in red (bottom left) and a merged image (bottom right). The direction of blood flow is indicated by the arrows in the KKO alone image as is the magnification. A dotted white outline of the vessel is also included in this image for better visualization. (B) is a more lateral single cut through the same thrombus as in (A) further showing that the KKO binding and FXa binding are at the base of the clot and overlap with directional arrows and magnification in the top left image.

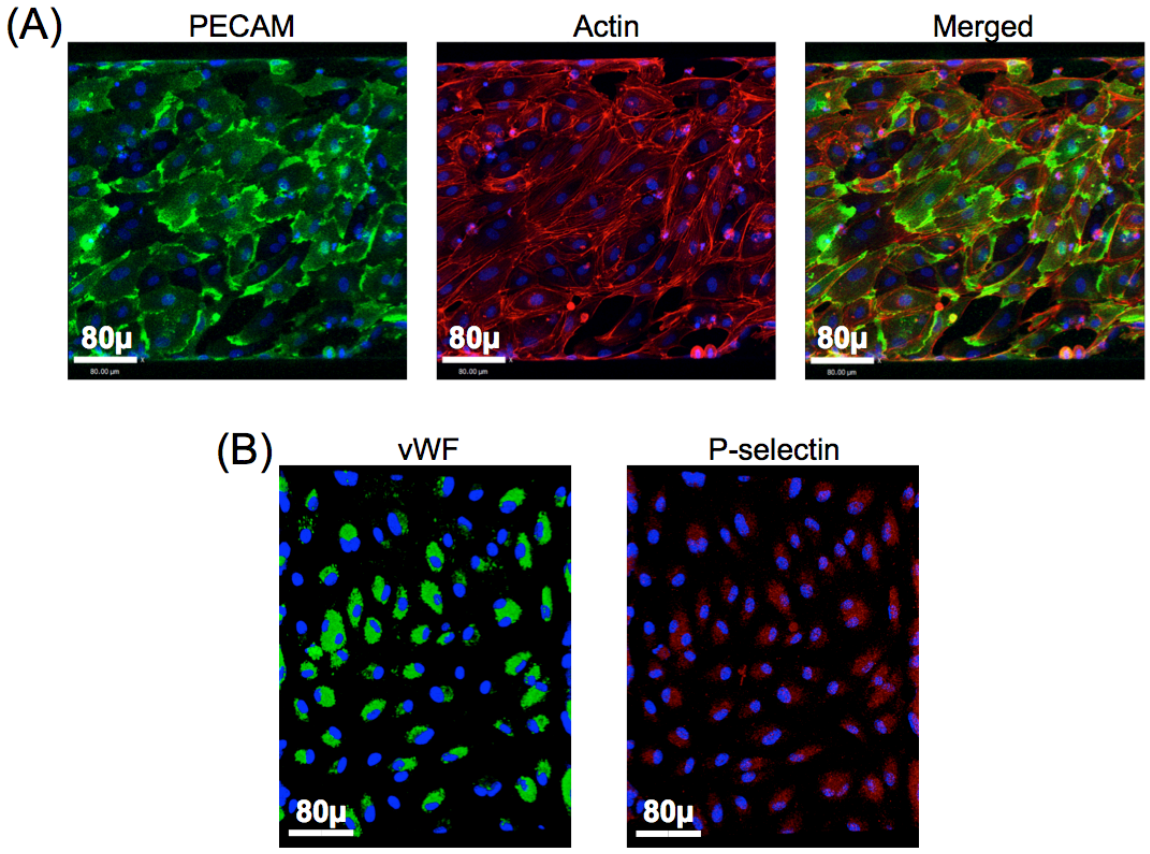

Figure 3 Supplement. Immunostain of HUVECs-coated microfluidic chambers.

(**A**) PECAM staining (green) of confluent HUVECs in the microfluidic chambers as well as cytoskeletal staining for actin (red) with nuclei stained with Hoescht (blue) showing intercellular PECAM staining between adjacent endothelial cells. (**B**) Same as (A), but for vWF and P-selectin showing the quiescent state of these endothelial cells. Scale bar included. In all images, photos were taken at 20X magnification. Size markers are included.

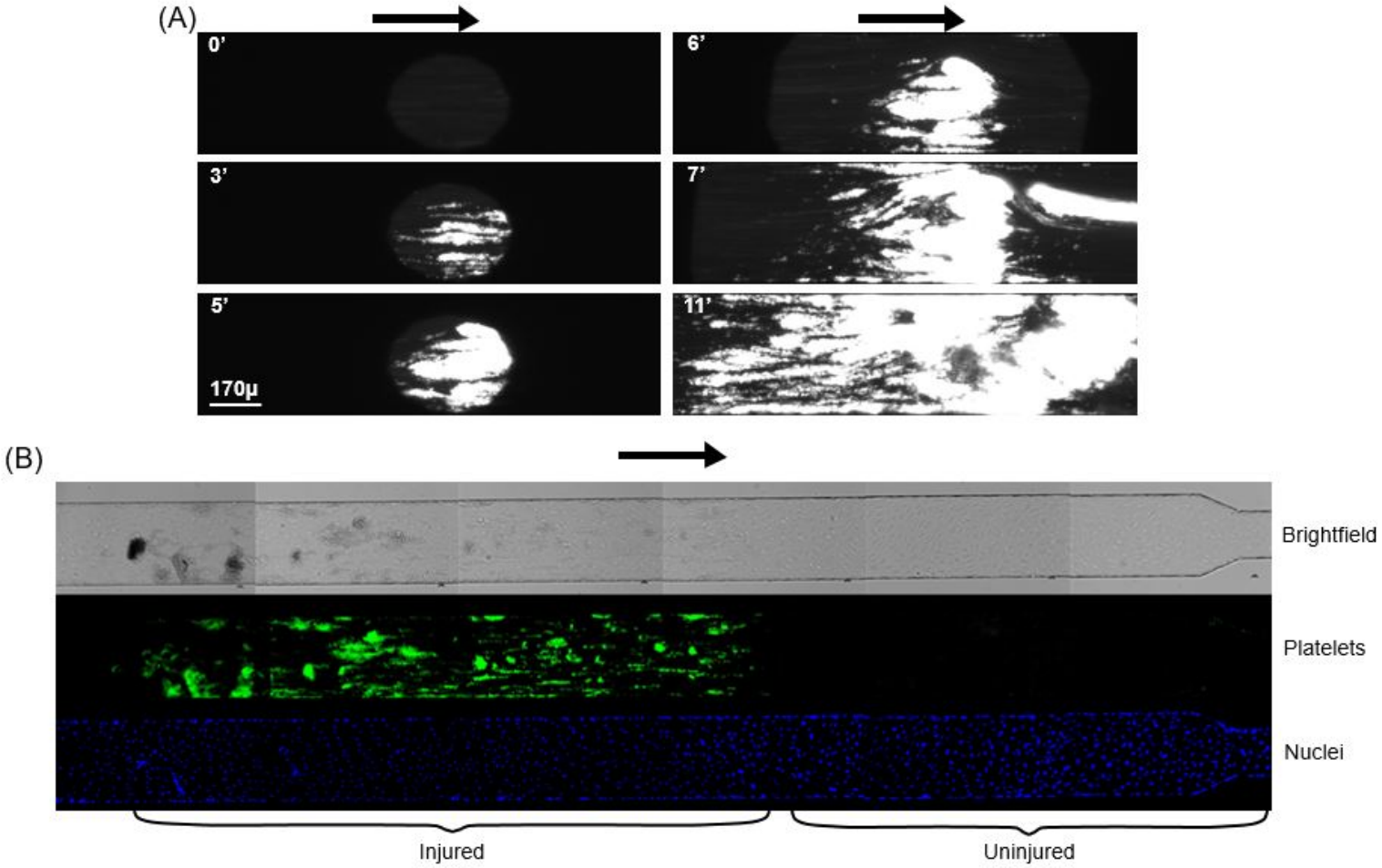

Figure 4 Supplement. Characterization of the microfluidic photochemical injury system. (**A**) Injury made as in Figure 4 but with the diaphragm of the microscope closed then incrementally opened showing injury is localized to the area of the channel exposed to light. The injury is performed as in Figure 4 over the timeframe noted. Accumulating platelets are in white. Scale bar is included. (**B**) Same as Figure 4B, but showing the full channel by brightfield imaging, accumulated platelets (green) and DAPI-stained nuclei (blue). Scale bar is included. In all images, photos were taken at 10X original magnification.

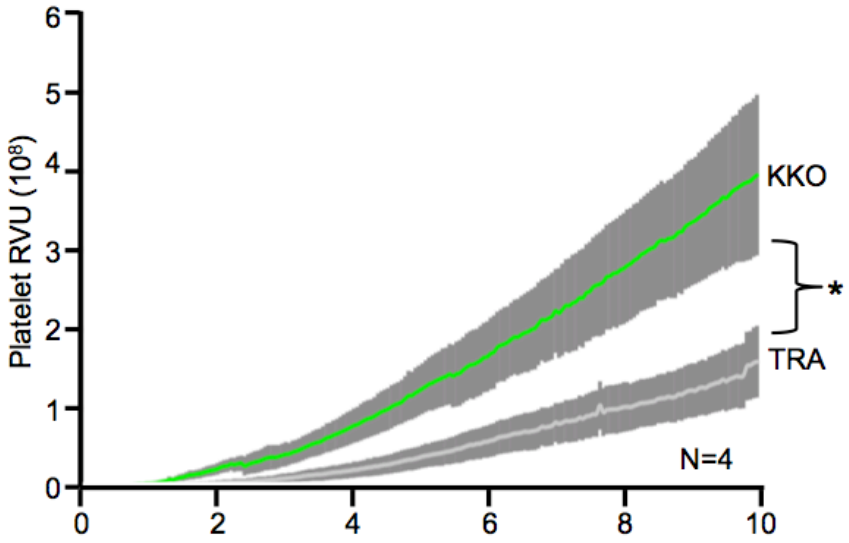

Figure 5 Supplement. Thrombus growth during photochemical injury in the presence of KKO.

Similar studies as in Figure 5A, but microfluidic chamber coated with adult human aortic endothelial cells. Platelet accumulation along endothelium after photochemical injury perfused with whole human blood containing either KKO or TRA for 10 minutes. Mean ± 1 SEM are shown. * = $p<0.01$ for KKO versus TRA exposure.

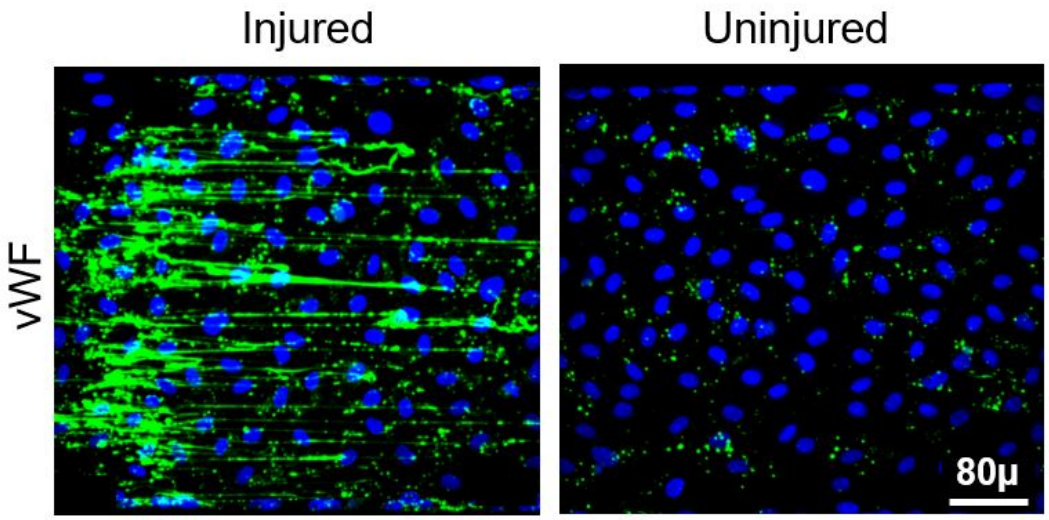

Figure 6 Supplement. Activation of endothelium in area of photochemical injury.
Similar studies as in Figure 4B, but staining for vWF (green) with Hoescht-stained nuclei shown (blue). Scale bar included. Images were taken at 20X original magnification.