Supplement Methods

Recombinant proteins and antibodies

Recombinant hPF4 was isolated from S2 cell media using the Drosophila Expression System (Invitrogen), purified, and characterized as described[26]. Final protein concentrations were determined using the bicinchoninic acid protein assay (Pierce) using bovine serum albumin (BSA) as the standard. KKO and TRA[24], a monoclonal IgG isotype control, were purified from hybridoma supernatants. Other antibodies used in these studies include a polyclonal rabbit anti-hPF4 antibody (Abcam), a polyclonal rabbit anti-hVWF antibody (DAKO) and a FITC-prelabeled polyclonal sheep anti-hVWF antibody (Abcam). $F(ab')_2$ fragments of the monoclonal anti-mouse CD41 antibody MWReg30 (BD Biosciences) were used to detect murine platelets in the cremaster laser injury model. Anti–fibrin 59D8 monoclonal antibody was provided by Hartmut Weiler of the BloodCenter of Wisconsin (Milwaukee, Wisconsin, USA). Bound HIT-patient IgG was detected using a rabbit polyclonal anti-human IgG Fc-specific antibody (Jackson Research Laboratories). All antibodies were either labeled using Alexa Fluor antibody-labeling kits (Fisher) or were detected using species-appropriate Alexa Fluor-conjugated secondary antibodies (Fisher). The FcγRIIA-blocking monoclonal antibody IV.3[21] was produced in hybridoma cells and purified on Protein G (Invitrogen). The mouse anti-CD42b monoclonal antibody AK2[22] was from Biorad.

Analysis of microfluidic studies

All imaging was performed on a Zeiss Axio Observer Z1 inverted microscope or a Zeiss LSM 710 laser scanning confocal microscope[10]. Data were analyzed using ImageJ or Volocity 6.3 for confocal images. To perform fluorescence colocalization studies, labeled, elongated strings of VWF versus labeled, punctate, unextended VWF were identified by an investigator blinded to the PF4 or KKO staining in the experimental images. Colocalization of fluorescent signals for VWF and KKO, taken via confocal imaging, was analyzed by Imaris software (9.5.1) using co-localization technique and the final Pearson's coefficient in the region of interest volume (ROI vol) calculated for every VWF string in the field for co-localization with KKO[29]. Statistical significance was performed further using GraphPad Prism 6.0 (GraphPad Software) followed by multiple t test.

Biophysical interaction between VWF and hPF4

The interaction between soluble plasma hVWF and hPF4 was examined using DLS as described[7]. Sample solutions (500 µl) containing hPF4 (10 µg/ml), VWF (0-10 µg/ml), or hPF4 (10 µg/ml)

together with different concentrations of VWF (0-10 µg/ml) were prepared at room temperature for 15-20 minutes, transferred to zeta disposable cuvettes (Fisher), and the size distribution of the resultant soluble particles was measured in a Zetasizer Nano ZS (Malvern)[7].

Supplement videos

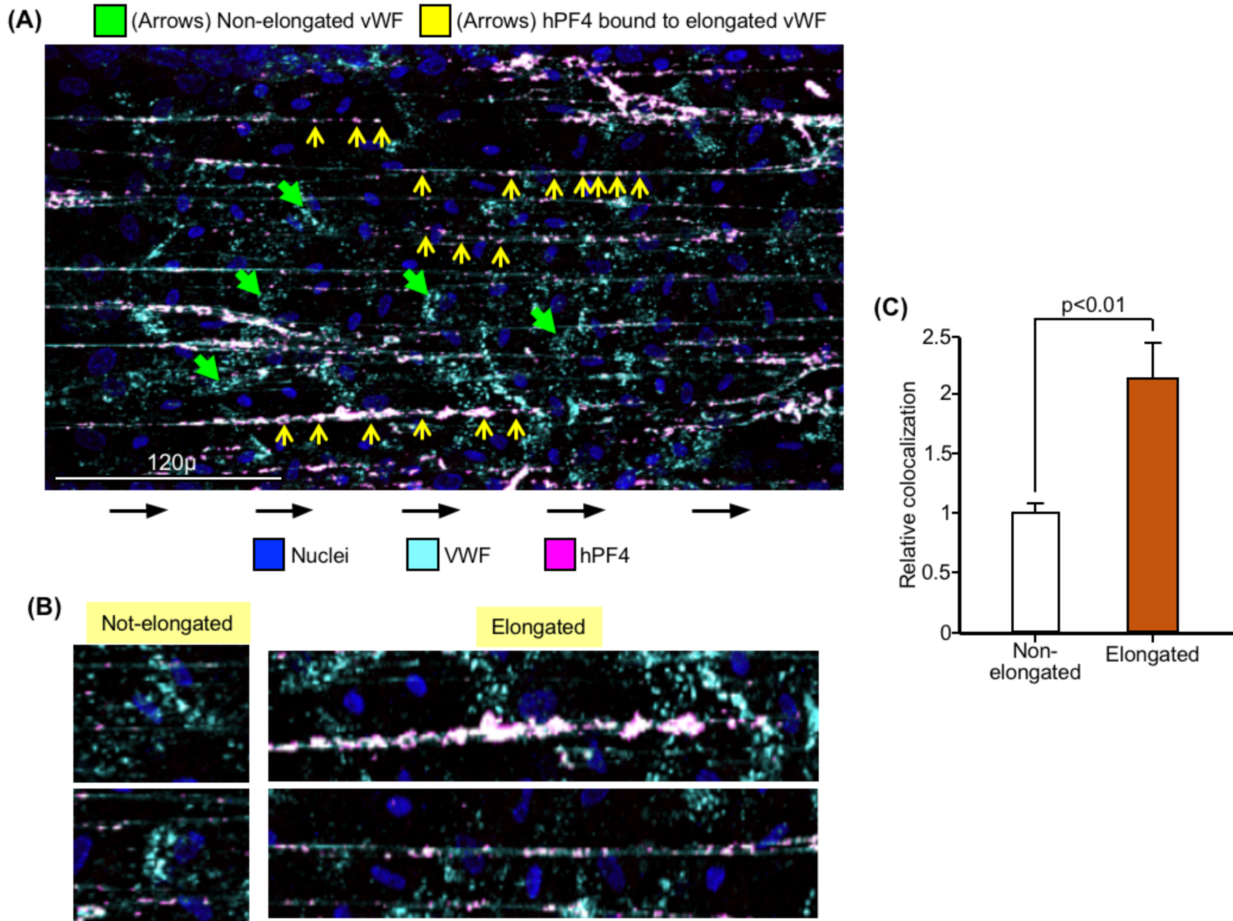
Supplement Video 1. hPF4 binds elongated strands of VWF released from injured HUVECs.

A 3D rendering composed of confocal image stacks taken of the injured endothelialized channel after flowing heparin-free BCM containing hPF4. The image displays binding of hPF4 (magenta) along with VWF (cyan) released within the injured area of the channel with nuclei shown in blue. The video is taken in the direction of flow, within the lumen, along the HUVEC monolayer lining the bottom of the channel.

Supplement Video 2. KKO binds hPF4-VWF complexes in vitro.

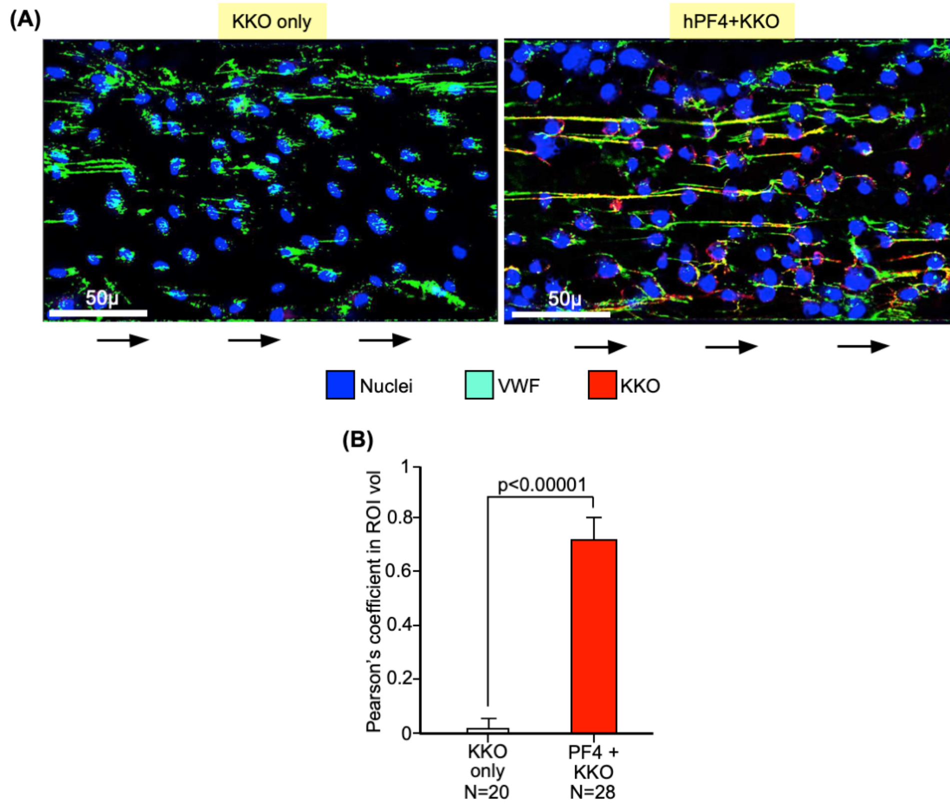
A 3D rendering composed of confocal image stacks taken of the injured endothelialized microfluidic channel after flowing heparin-free BCM containing hPF4 and then KKO. The image displays binding of KKO (red) along with VWF (cyan) released within the injured area of the channel with nuclei shown in blue. The video is taken in the direction of flow, within the lumen, along the HUVEC monolayer lining the bottom of the channel.

Supplement Figures and legends



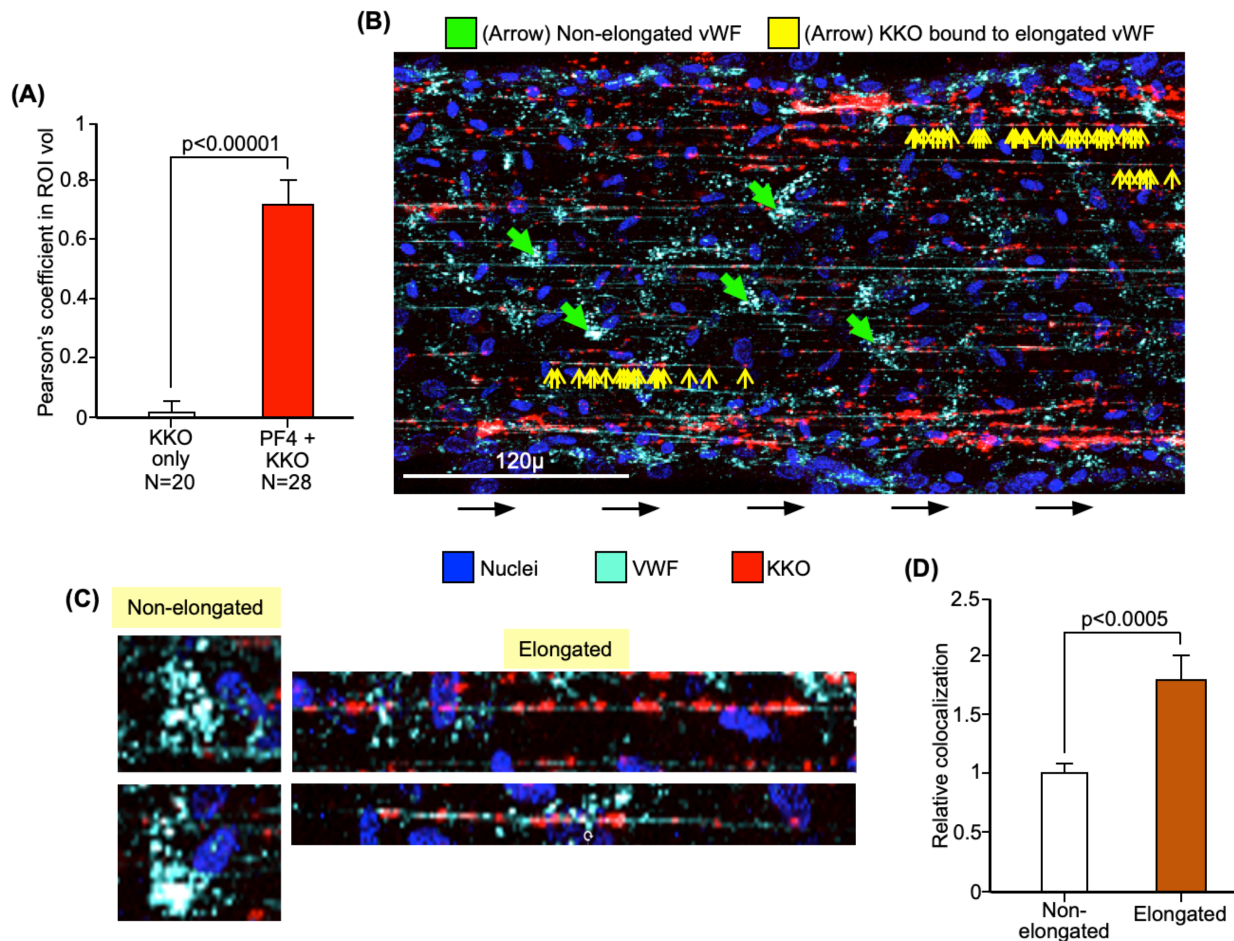
Supplement Figure 1. hPF4 binds selectively to elongated strands of VWF released from injured HUVECs in a microfluidic system.

(A) shows a representative microfluidic channel injured area after infusion of hPF4 (25 µg/ml) with nuclei, VWF and bound hPF4 shown. Yellow arrows indicate VWF strands. Green arrows indicate non-elongated VWF. Direction of flow is indicated by black arrows. **(B)** shows enlargement from (A). **(C)** relative colocalization of hPF4 with VWF strings versus non-elongated VWF. Means ± 1 SEM are shown with N ≥3 separate studies. P value was determined using Student t-test comparing hPF4 binding to the two-types of VWF.



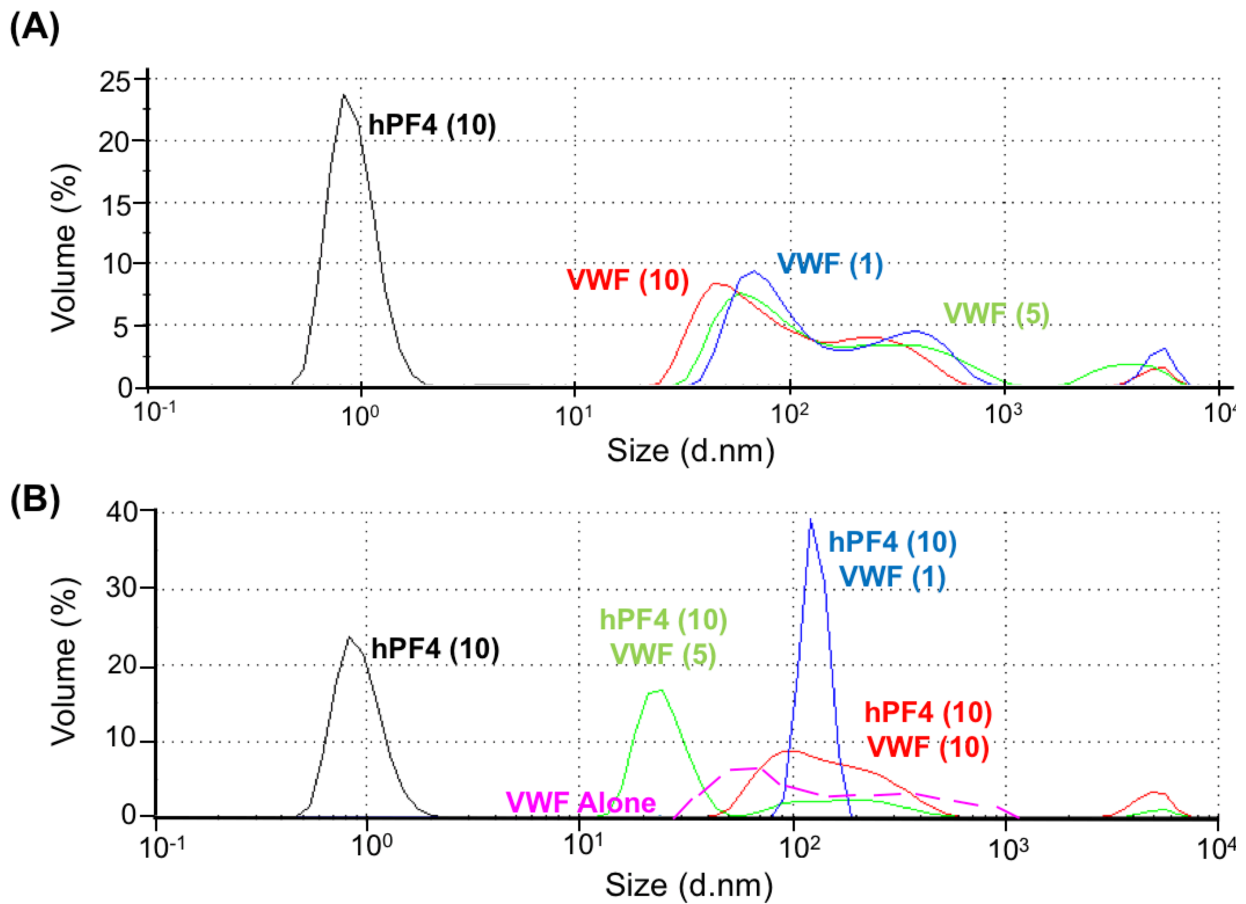
Supplement Figure 2. hPF4-KKO binds selectively to VWF.

Same as in Supplement Figure 1, but both either with KKO (10 µg/ml) alone or along with hPF4 (25 µg/ml) infused. **(A)** shows representative examples of injury with or without hPF4 infusion. **(B)** show selective binding of KKO to VWF. Mean ± 1 SEM for N ≥3 fields studied.



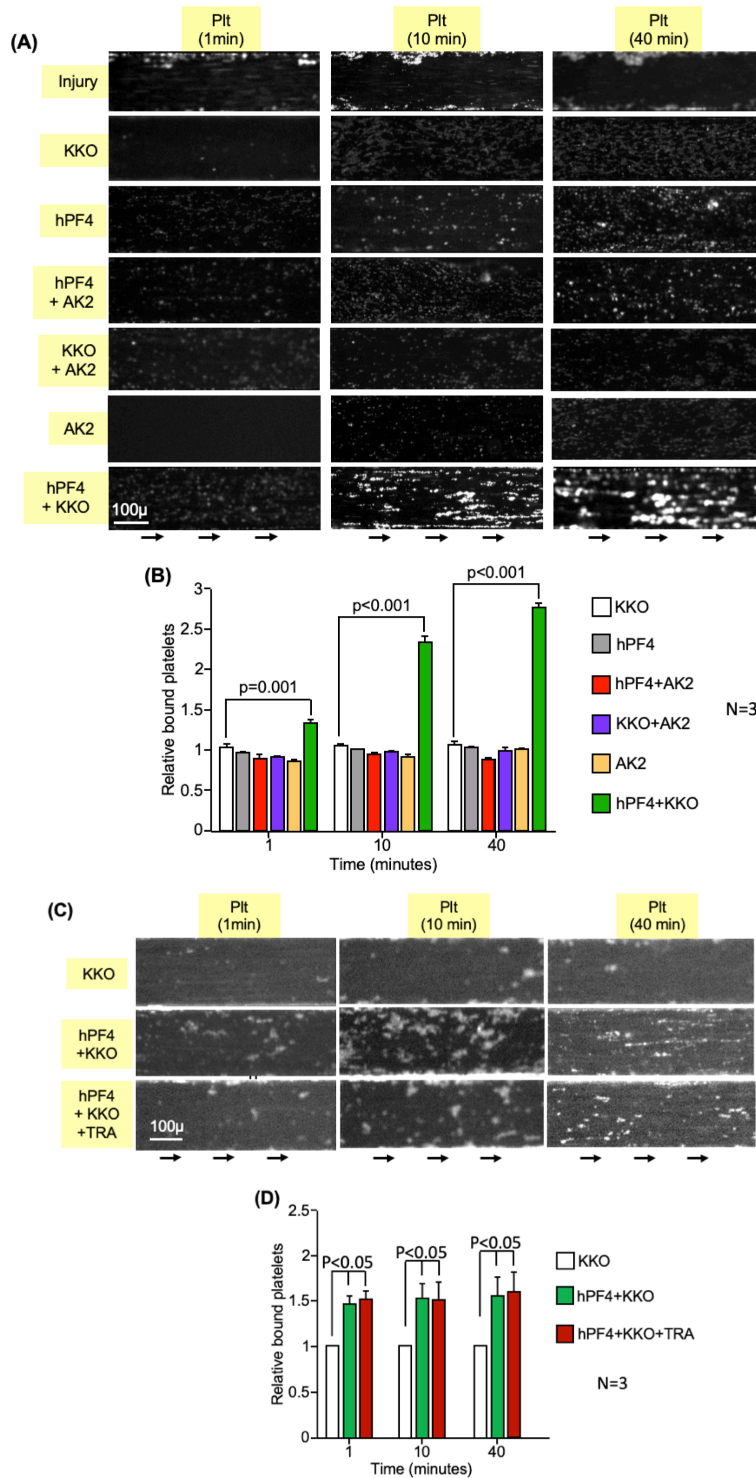
Supplement Figure 2. hPF4-KKO binds selectively to elongated strings of VWF released from injured endothelial cells in a microfluidic system.

Same as in Supplement Figure 1, but both hPF4 (25 µg/ml) and KKO (10 µg/ml) were infused. **(A)** shows analysis of colocalization of the observed binding of PF4+KKO to VWF compared to KKO in the absence of added PF4. N = number of VWF fibers counted. **(B)-(D)** show selective binding of PF4+KKO to elongated VWF strings. (B) and (C) are selective examples and (D) shows mean ± 1 SEM for N ≥3 fields studied.



Supplement Figure 3. Physical interactions of hPF4 and VWF using DLS.

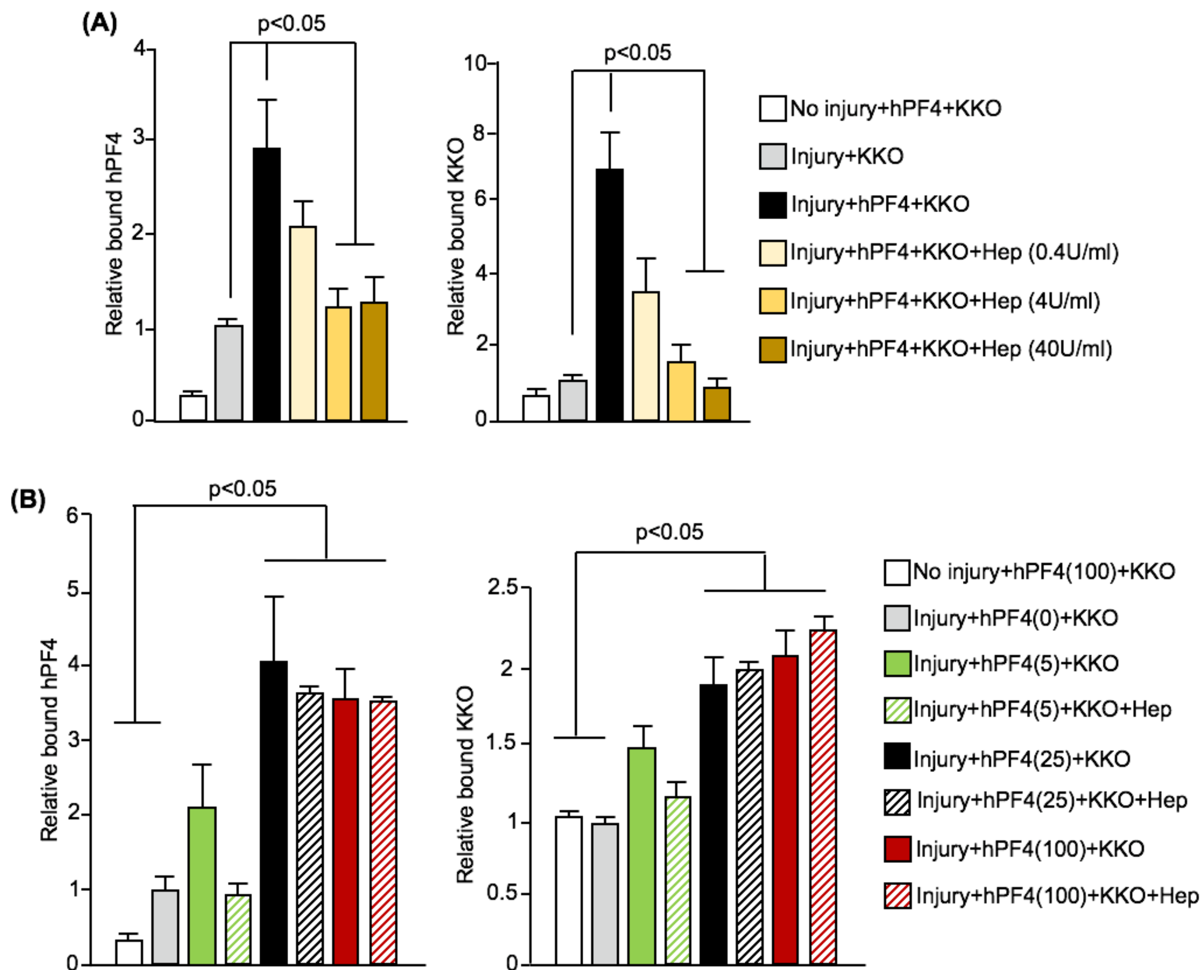
Volume readouts from DLS looking at hPF4 (10 µg/ml = black) in comparison to varied concentrations of VWF (1 µg/ml = blue, 5 µg/ml = green, 10 µg/ml = red) in both isolated and mixed solutions. Curves are a single representative result of 4-5 independent experiments. **(A)** Volume readouts of samples containing the individual proteins at the concentrations indicated in parenthesis. **(B)** Volume readouts from samples containing a mixed solution of hPF4 and VWF at the concentrations indicated in parenthesis in µg/ml.



Supplement Figure 4. Additional controls for the binding of platelets to hPF4-VWF-HIT antibody complexes.

Studies as in Figure 5, but showing additional controls. **(A)** shows representative images and **(B)** shows mean ± 1 SEM for analysis of three independent channels for each condition. P are studies

by Student T test versus KKO only. **(C)** and **(D)** are similar to (A) and (B), but for an additional control where TRA is added as a control antibody.



Supplement Figure 5. The ability of heparin to remove hPF4 from hPF4-VWF-KKO complexes.

(A) Complexes with VWF were formed by flowing hPF4 (25 µg/ml) and KKO (10 µg/ml) along the damaged vasculature followed by exposing the channel to heparin (0-40 U/ml) only for ten minutes. Means ± SEM are shown with N ≥4. P values were determined by one-way ANOVA analysis compared to injury plus PF4 and KKO alone. **(B)** Studies showing how binding of hPF4 (25 µg/ml) and KKO (10 µg/ml) within hPF4-VWF-KKO complexes changed in the presence of a solution containing heparin (0 or 0.4 U/ml) in addition to hPF4 (5-100 µg/ml) and KKO (10 µg/ml) for ten minutes. Means ± SEM are shown with N ≥4. P values were determined by one-way ANOVA analysis.