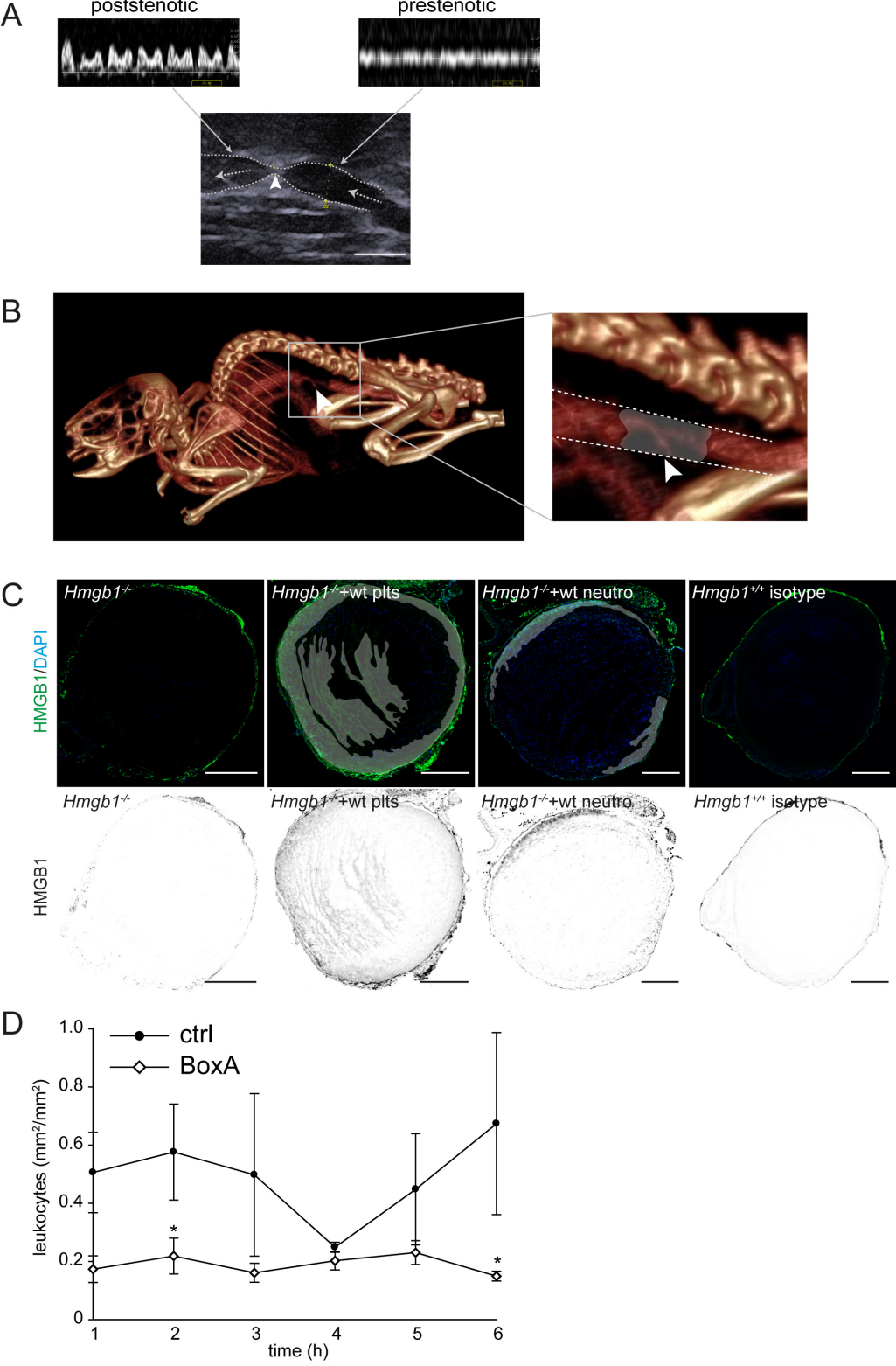


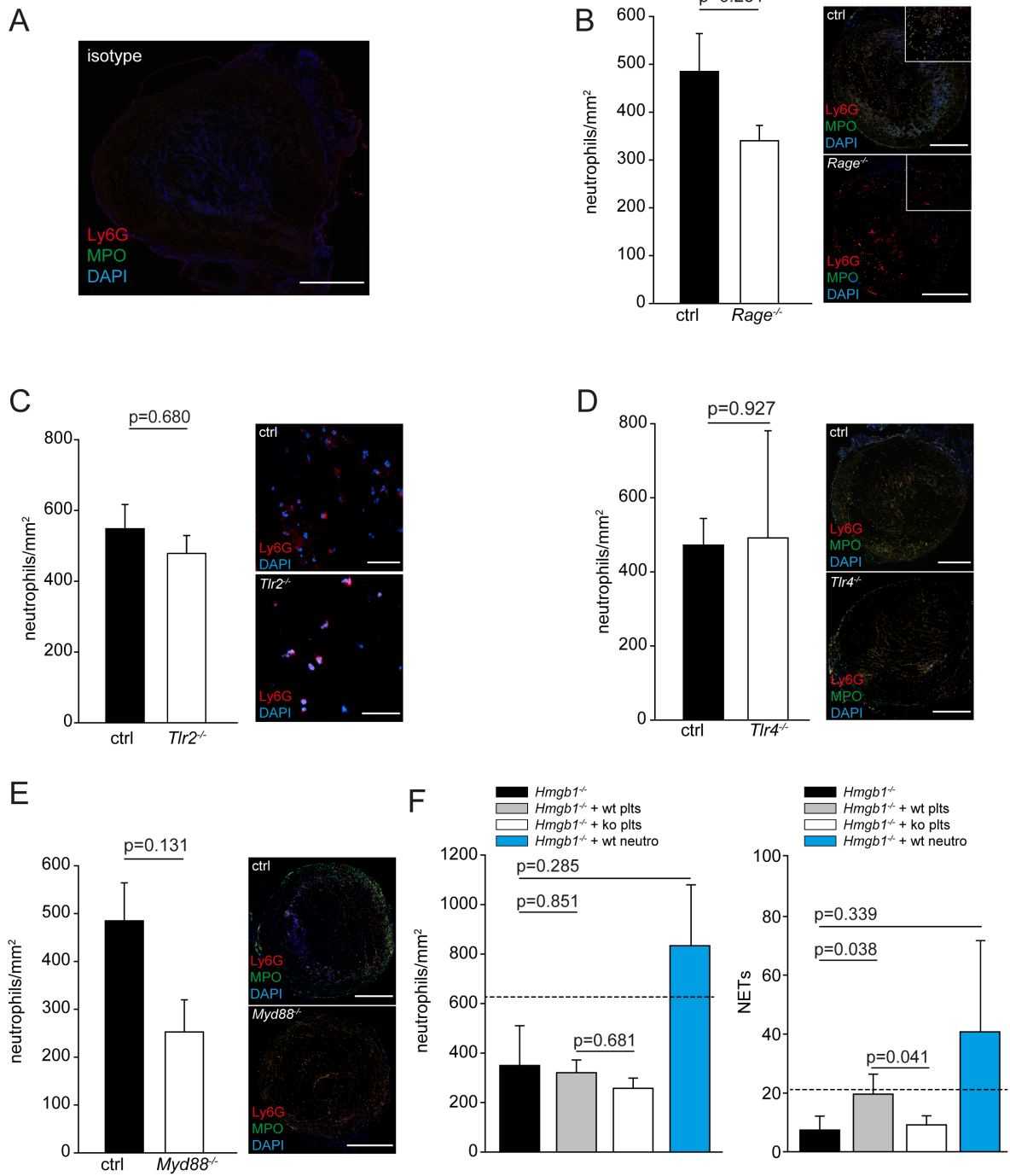
SUPPLEMENTAL DATA

Supplemental figures

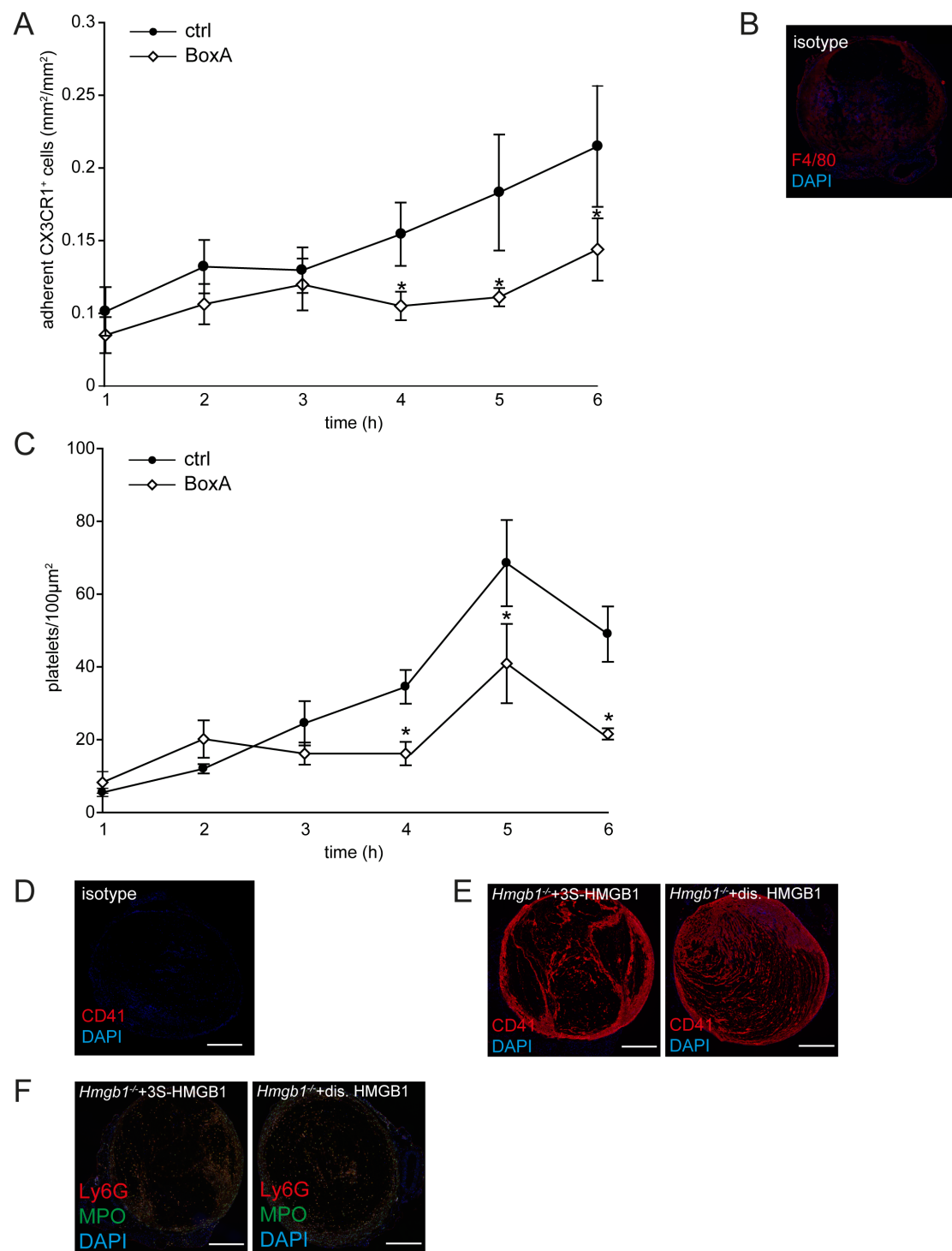
Supplemental Figure 1



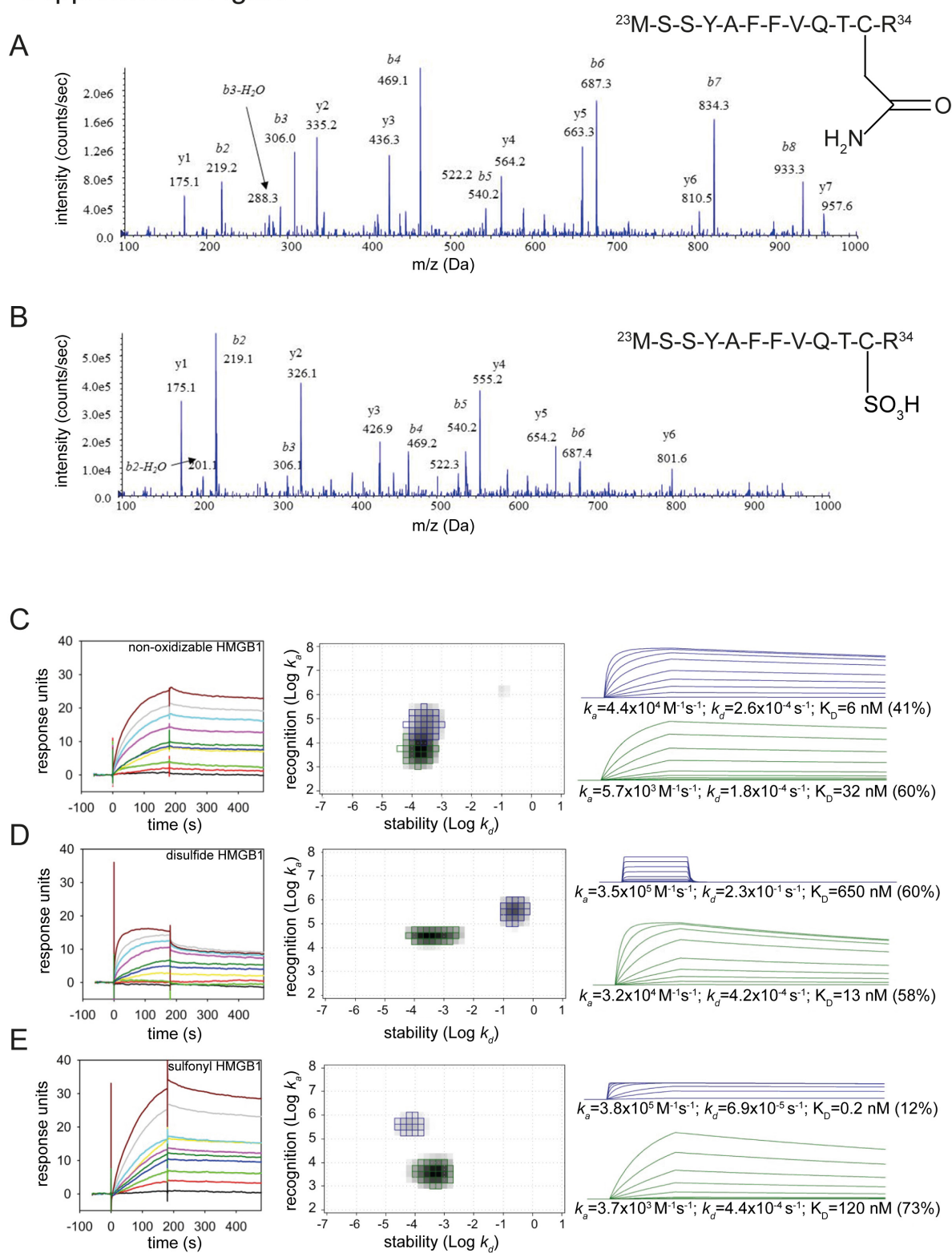
Supplemental Figure 2



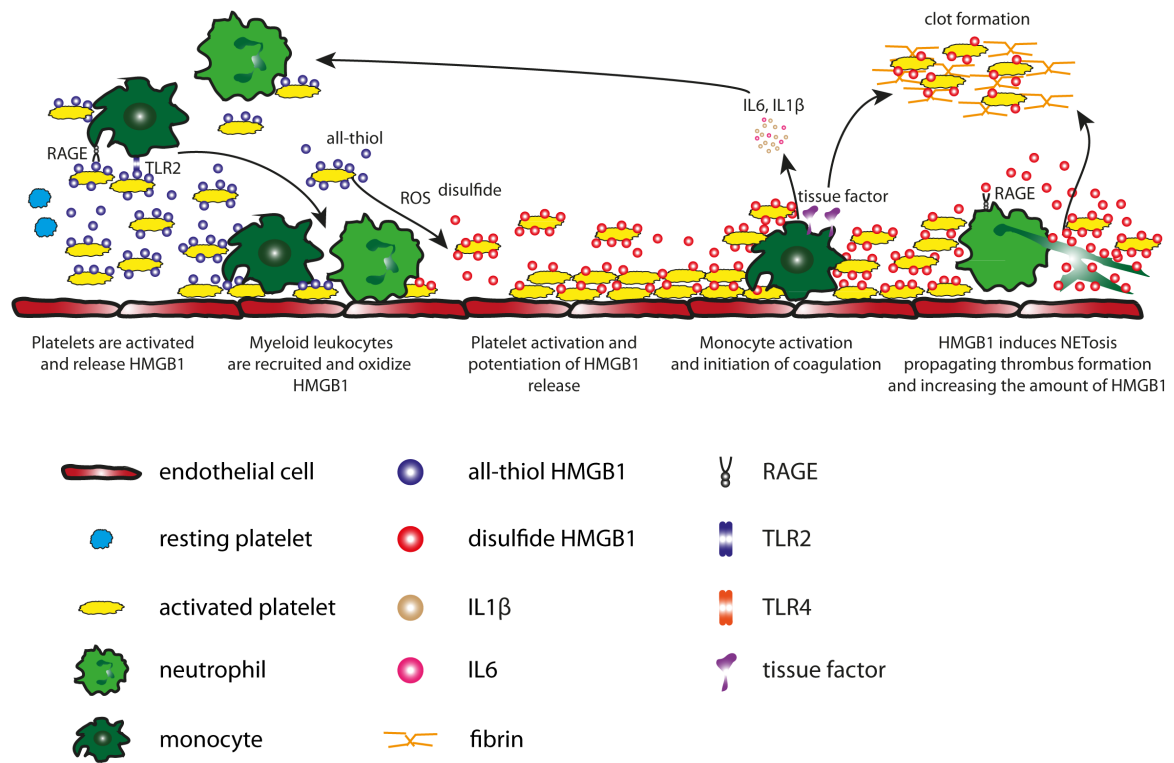
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental figure legends

Supplemental Figure 1. Blood derived HMGB1 contributes to leukocyte recruitment and

NET formation in DVT. A, Ultrasound of the IVC five hours after reduction of blood flow. Top left: pulsed wave Doppler signal of flow in the poststenotic IVC. Top right: pulsed wave Doppler signal of flow in the prestenotic IVC. Dotted arrows indicate direction of flow. Arrowhead points to the stenosis, dotted line indicates outline of the IVC; bar 2 mm. **B,** Left: 3D images from computed tomography *in vivo* showing the thrombus in the IVC upstream to the stenosis indicated by the lack of contrast agent (arrowhead) 48 hours after flow reduction; bar 4 mm. Right: Higher magnification of the IVC (between dotted lines) and thrombus (shaded area) indicated by arrowhead. **C,** Immunofluorescence staining for HMGB1 (green) and isotype control (right) from cross sections of the IVC 48 hours after flow reduction in *Hmgb1*^{-/-} chimeras compared to *Hmgb1*^{-/-} chimeras receiving wildtype platelets or wildtype neutrophils (n=3 each). Top: shaded areas indicate HMGB1 rich regions of the thrombi. Nuclei are counterstained with DAPI (blue); bar 200 μ m. Bottom: signal of HMGB1 in thrombi shown in the top row presented as inverted black and white images. **D,** Quantification of leukocyte over the first six hours after flow reduction in the IVC in mice treated with BoxA (n=4) or control (n=3) by intravital microscopy. Results are mean \pm s.e.m..

Supplemental Figure 2. Impact of pattern recognition receptors and HMGB1 on

neutrophil recruitment. A, Isotype control for MPO and Ly6G staining from cross sections of the IVC 48 hours after flow reduction. Nuclei are counterstained with DAPI (blue); bar 200 μ m. Images representative of n=3 experiments. **B-E,** Left: Quantification of neutrophils within the thrombi. Right: Immunofluorescence staining for Ly6G (red) and MPO (green) from cross sections of the IVC 48 hours after flow reduction. Nuclei are counterstained with DAPI (blue). Images are representative of at least n=3 experiments. **B,** *Rage*^{-/-} mice (bottom) compared to control mice (top); n=5 each, bar 200 μ m. **C,** *Tlr2*^{-/-} mice (bottom) compared to control mice (top); n=3 each, bar 40 μ m. **D,** *Tlr4*^{-/-} mice (bottom) compared to control mice (top); n=3 each, bar 200 μ m. **E,** *Myd88*^{-/-} mice (bottom) compared to control mice (top); n=3 each, bar 200 μ m.

F, Quantification of neutrophils/mm² (left) and neutrophil extracellular traps (right) in *Hmgb1*^{-/-} chimeras compared to *Hmgb1*^{-/-} chimeras receiving wildtype platelets, *Hmgb1*^{-/-} platelets, or wildtype neutrophils. Dotted line indicates mean in *Hmgb1*^{+/+} bone marrow chimeras (n=5 each). Student's t test was used to compare results between two groups, one-way ANOVA followed by LSD-post hoc-test for three groups.

Supplemental Figure 3. HMGB1 fosters platelet accumulation. **A**, Quantification of monocytes in CX3CR1^{GFP/+} mice over the first six hours after flow reduction in the IVC in mice treated with BoxA (n=4) or control (n=4) visualized by intravital microscopy. Results are mean ± s.e.m.. Student's t test was used to compare results, * indicates p<0.05. **B**, Isotype control for F4/80 staining from cross sections of the IVC 48 hours after flow reduction. Nuclei are counterstained with DAPI (blue); bar 200 µm. Images representative of n=3 experiments. **C**, Quantification of platelets over the first six hours after flow reduction in the IVC in mice treated with BoxA (n=5) or control (n=4) visualized by intravital microscopy. Results are mean ± s.e.m.. Student's t test was used to compare results, * indicates p<0.05. **D**, Isotype control (red) for CD41 staining from cross sections of the IVC 48 hours after flow reduction. Nuclei are counterstained with DAPI (blue); bar 200 µm. Images representative of n=3 experiments. **E**, Immunofluorescence staining for CD41 (red) from cross sections of the IVC of *Hmgb1*^{-/-} bone marrow chimeras receiving mutant or disulfide HMGB1. Nuclei are counterstained with DAPI (blue); bar 200 µm. Images representative of n=3 experiments. **F**, Immunofluorescence staining for Ly6G (red) and MPO (green) from cross sections of the IVC of *Hmgb1*^{-/-} bone marrow chimeras receiving mutant or disulfide HMGB1. Nuclei are counterstained with DAPI (blue); bar 200 µm. Images representative of n=3 experiments.

Supplemental Figure 4. Characterization of HMGB1 in platelets and binding properties of different HMBG1 redox variants to RAGE. **A-B**, Mass spectrometry for different HMGB1 redox forms in platelets **A**, Resting platelets contain reduced all-thiol HMGB1. **B**, In thrombin stimulated platelets sulfonyl HMGB1 is present after 30 minutes of stimulation in platelet rich plasma. **C-E**, Surface plasmon resonance and interaction map analysis. RAGE-His was

captured via the His-tag onto a CM5 sensor chip coated with anti-His antibody, and solutions of 10 nM (black), 25 nM (red), 50 nM (light green), 100 nM (blue and yellow), 250 nM (green), 500 nM (turquis), 1000 nM (pink), and 2000 nM (red brown) of non-oxidizable HMBG1 **C**, disulfide HMBG1 **D**, and sulfonyl HMBG1 **E**, were passed over the chip. The experimental data are shown on the left panel. Interaction map® (IM) analyses of the HMBG1-RAGE interactions are shown on the middle and right panel. The green and blue spots of the IMs both represent the two binding events of HMBG1 to RAGE. The sensorgrams were inferred for each specific K_D value, derived from the association (k_a) and dissociation rates (k_d) calculated from the IMs. The blue sensorgram corresponds to the blue spot and the green sensorgram to the green spot from ON/OFF rate map. The calculated affinities, as well as the ON/OFF rates, are indicated below the sensorgrams. The percentage numbers indicate the peak weights, reflecting the stoichiometry of the interactions. Sensorgrams are representative of n=3 experiments.

Supplemental Figure 5. Platelet-derived disulfide HMGB1 as central mediator of DVT.

Platelets adhere to the vessel wall and present reduced HMGB1 on their surface upon activation. This causes the recruitment of monocytes to the developing thrombus, which upon activation release reactive oxygen species (ROS) resulting in the oxidation of HMGB1, thereby rendering it prothrombotic. In an autocrine/paracrine manner, disulfide HMGB1 promotes further platelet activation and aggregation, potentiating the amount of released HMGB1 within the thrombus. This results in the accumulation of monocytes through RAGE and TLR2, inducing the expression of tissue factor and cytokines, setting in motion a vicious circle of coagulation and inflammation eventually leading to obstructive thrombus formation within the vein. Finally, disulfide HMGB1 induces the formation of prothrombotic neutrophil extracellular traps mediated by RAGE, which in turn expose more HMGB1 on extracellular DNA strands and promote DVT propagation.

Supplemental video legends

Video 1: Ultrasound of the poststenotic vena cava inferior five hours after flow reduction.

Video 2: Ultrasound of the prestenotic vena cava inferior five hours after flow reduction.

Video 3: 3D rendering of images from CT-angiography showing the lack of contrast agent in the IVC.