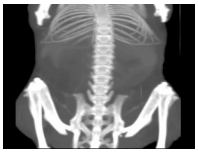
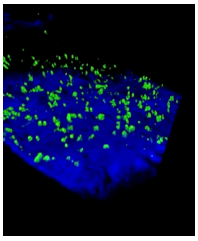
SUPPLEMENTAL MATERIAL

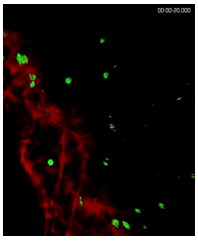
von Brühl et al., http://www.jem.org/cgi/content/full/jem.20112322/DC1



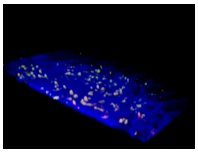
Video 1. Computed tomography 3D movie of the thrombosed IVC in vivo. The dimension of DVT formation was evaluated by contrast-enhanced computed tomography in anaesthetized mice. Animals were imaged with the Inveon small animal PET/CT scanner. Arrows point to the cranial and caudal border of the thrombus in the IVC (frame rate: 10.03 frames/s).



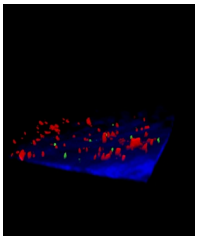
Video 2. Leukocytes covering the vessel wall of the IVC. 3D movie of IVC thrombus 48 h after the flow-restricting procedure. Leukocytes (green) attached to the vessel wall (blue) visualized by two-photon microscopy (frame rate: 6.03 frames/s).



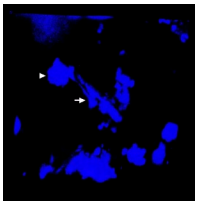
Video 3. Crawling neutrophils shortly after DVT induction. Neutrophils (green, labeled with an anti–Ly6G-FITC antibody) crawling along the venous vessel wall (red) 2 h after the flow restricting procedure (video still in Fig. 3 A). Tracking of a 4D reconstruction of images acquired by intravital time lapse two-photon microscopy (acquisition rate: 3 frames/min; frame rate: 8.11 frames/s).



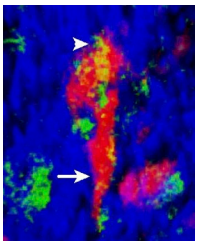
Video 4. Neutrophils recruited to the developing thrombus. GFP^+ cells (green) in LysM-eGFP are also stained by a PE-labeled anti-Ly6G antibody (red), indicating neutrophils (yellow; video still in Fig. 3 F). Thrombus in the IVC 6 h after DVT induction visualized by two-photon microscopy is presented in a 3D rendering (frame rate: 5.03 frames/s).



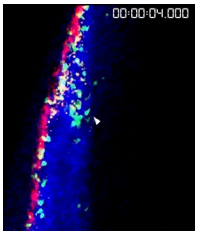
Video 5. Monocytes attached to venous vessel wall. Representative movie of thrombi from CX3CR1-eGFP (green) mice analyzed by two-photon microscopy showing the recruitment of monocytes (green) to the vessel wall (blue) 6 h after DVT induction (video still in Fig. 3 F). Neutrophils were stained by i.v. application of a PE-labeled anti-Ly6G antibody (red; frame rate: 6.02 frames/s).



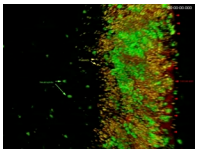
Video 6. NET in the thrombus visualized in 3D. Representative movie of a cross sections of WT thrombi analyzed by two-photon microscopy showing the 3D structure of NETs (blue) visualized by Hoechst staining. Arrowhead, cell nucleus; arrow, NET (frame rate: 25 frames/s).



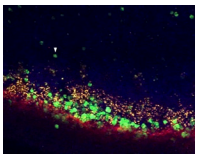
Video 7. NETs in vivo. Time-lapse movie of the IVC 4 h after flow reduction acquired by intravital two-photon microscopy. Extracellular DNA was visualized by Sytox orange (red; arrow) originating from neutrophils (green, labeled with an anti–Ly6G-FITC antibody; arrowhead) attached to the venous vessel wall (blue; frame rate: 10 frames/s).



Video 8. Platelets and neutrophils forming a thrombus in the IVC. Intravital time-lapse movie of a cross section of the IVC vessel wall (red) imaged by two-photon microscopy in LysM-eGFP mice (video still in Fig. 7 C). A developing thrombus (arrowhead) consisting of neutrophils (green) and platelets (yellow) is forming 4 h after DVT induction. Plasma is colored in blue (acquisition rate: 30 frames/min; frame rate: 8.04 frames/s).



Video 9. Surface of the IVC vessel wall covered by neutrophils and platelets. 4D movie of the developing mural thrombus visualized by intravital two-photon microscopy in LysM-eGFP mice. Neutrophils (green) and platelets (yellow) are densely packed on the luminal surface of the IVC wall (red) 6 h after DVT induction. Arrowheads pointing to neutrophils recruited to the thrombus (acquisition rate: 3 frames/min; frame rate: 6.19 frames/s).



Video 10. Platelets support neutrophil recruitment to the thrombus. Cross section of the venous vessel wall (red) in the IVC visualized by intravital two-photon microscopy in LysM-eGFP mice. Neutrophils (green), covered with platelets (yellow), are recruited from the blood stream (blue) to the forming thrombus (arrowheads) 6 h after DVT induction (acquisition rate: 3 frames/min; frame rate: 5.19 frames/s).

Table S1. List of antibodies, providers, and detection methods used for immunohistochemistry and immunofluorescence microscopy

Antigen	Primary antibody	Clone	Provider	Secondary antibody	Provider
CD 41	Rat	MWReg30	BD	Donkey anti–rat Alexa Fluor 594	Invitrogen
CD 45	Rat	30-F11	eBioscience	Donkey anti–rat Alexa Fluor 594	Invitrogen
Ly-6G	Rat	1A8	BD	Donkey anti–rat Alexa Fluor 594	Invitrogen
CD 62P	Rat	RB40.34	BD	Goat anti–rat Alexa Fluor 488	Invitrogen
MPO	Rabbit	A0398	Dako	Donkey anti–rabbit Alexa Fluor 488	Invitrogen
NE	Rabbit	ab68672	Abcam	Goat anti–rabbit Alexa Fluor 594	Invitrogen
F4/80	Rat	BM8	eBioscience	Anti–R-PE	Rockland
Histone H3	Rabbit	ab1791	Abcam	Goat anti–rabbit Alexa Fluor 594	Invitrogen
H2A-H2B-DNA	Mouse	Losman et al., 1992	V. Brinkmann	Goat anti–mouse Alexa Fluor 594	Invitrogen
TF	Rabbit		ADI	Donkey anti–rabbit Alexa Fluor 594	Invitrogen
PDI	Mouse	RL90	Thermo Fisher Scientific	Goat anti–mouse Alexa Fluor 594	Invitrogen
fibrinogen	Rabbit	A0080	DAKO	Donkey anti–rabbit Alexa Fluor 594	Invitrogen
vWF	Rabbit	AB7356	Millipore	Donkey anti–rabbit Alexa Fluor 488	Invitrogen

PDI, protein disulfide isomerase.

REFERENCES

Losman, M.J., T.M. Fasy, K.E. Novick, and M. Monestier. 1992. Monoclonal autoantibodies to subnucleosomes from a MRL/Mp(-)+/+ mouse. Oligoclonality of the antibody response and recognition of a determinant composed of histones H2A, H2B, and DNA. *J. Immunol.* 148:1561–1569.