Supplemental Methods

Animals

Male C57BL/6 mice were purchased from Charles River (Sulzfeld, Germany). Male LysM-eGFP mice were generated as described previously [1] and backcrossed on the C57BL/6 background for 6 generations. Male $CX_3CR\text{-}1^{GFP/+}$ mice were generated as described previously [2] and backcrossed to the C57BL/6 background for 10 generations. All experiments were performed using mice at the age of 10 – 12 weeks weighing 20 – 25 g. Animals were housed under conventional conditions with free access to food and water. The experiments were performed according to German legislation for the protection of animals and approved by the local government authorities.

Peritonitis assay

Leukocyte recruitment to the peritoneal cavity was induced by intra-peritoneal (i.p.) injection of PAF (0.5 ml of 10^{-6}M solutions; Sigma Aldrich, Deisenhofen, Germany) or CCL3/MIP-1α (300 µg in 0.5 ml PBS; R&D Systems Europe Ltd.). Three hours after induction of peritoneal inflammation, mice were sacrificed, and their peritoneal cavity was washed with 15.0 ml of ice-cold PBS. The total number of leukocytes recovered from the peritoneal lavage fluid was analyzed using a Coulter ACT counter (Coulter Corp.). To determine the phenotype of leukocytes extravasated to the peritoneal cavity, slide preparations were made in a cytocentrifuge (Cytospin, Frankfurt, Germany) by spinning 200 µl of the peritoneal lavage fluid at 500 rpm for 5 min. Slides were stained (May-Gruenwald-Giemsa) and cell differentiation was made using an oil immersion microscope.

Analysis of leukocyte extravasation using the M. cremaster assay

Surgical preparation

Surgical preparation of the cremaster muscle was performed as originally described by Baez with minor modifications [3]. Mice were anesthetized using a ketamine/xylazine mixture (100 mg/kg ketamine and 10 mg/kg xylazine), administrated by i.p. injection. The left femoral artery was cannulated in a retrograde manner for the administration of microspheres and drugs (see below). The right cremaster muscle was then exposed through a ventral incision of the scrotum. The muscle was opened ventrally in a relatively avascular zone, using careful electrocautery to stop any bleeding, and spread over the transparent pedestal of a custom-made microscopy stage. Epididymis as well as testicle were detached from the cremaster muscle and placed into the abdominal cavity. Throughout the surgical procedure as well as during *in vivo* microscopy the muscle was superfused with warm buffered saline.

In vivo microscopy

The setup for *in vivo* microscopy was centered around an Olympus BX 50 upright microscope (Olympus Microscopy, Hamburg, Germany), equipped for stroboscopic fluorescence epi-illumination microscopy. Light from a 75-W xenon source was narrowed to a near-monochromatic beam of a wavelength of 700 nm or 488 nm by a galvanometric scanner (Polychrome II, TILL Photonics, Graefelfing, Germany) and directed onto the specimen via a FITC filter cube equipped with dichroic and emission filters (DCLP 500, LP515, Olympus). Microscopy images were obtained with Olympus water immersion lenses [20x/numerical aperture (NA) 0.5 and 10x/NA 0.3] and recorded with an analog black-and-white charge-coupled device (CCD) video camera (Cohu 4920, Cohu, San Diego, CA, USA) and an analog video recorder (AG-7350-E, Panasonic, Tokyo, Japan). Oblique illumination was obtained by positioning a mirroring surface (reflector) directly below the specimen and tilting its angle relative to the horizontal plane. The reflector consisted of a round cover glass (thickness, 0.19–0.22 mm; diameter, 11.8 mm), which was coated with aluminum vapor (Freichel,

Kaufbeuren, Germany) and brought into direct contact with the overlying specimen as described previously [4]. For measurement of centerline blood flow velocity, green fluorescent microspheres (0.96 μm diameter, Molecular Probes, Leiden, The Netherlands) were injected via the femoral artery catheter, and their passage through the vessels of interest was recorded using the FITC filter cube under appropriate stroboscopic illumination (exposure, 1 ms; cycle time, 10 ms; λ=488 nm), integrating video images for sufficient time (>80 ms) to allow for the recording of several images of the same bead on one frame. Beads that were flowing freely along the centerline of the vessels were used to determine blood flow velocity (see below).

Quantification of leukocyte kinetics and microhemodynamic parameters

For off-line analysis of parameters describing the sequential steps of leukocyte extravasation, we used the Cap-Image image analysis software (Dr. Zeintl, Heidelberg, Germany). Rolling leukocytes were defined as those moving slower than the associated blood flow and quantified for 30 s per venule. Firmly adherent cells were determined as those resting in the associated blood flow for more than 30 s and related to the luminal surface per 100 μm vessel length. Transmigrated cells were counted in regions of interest (ROI), covering 75 μm on both sides of a vessel over 100 μm vessel length. By measuring the distance between several images of one fluorescent bead under stroboscopic illumination, centerline blood flow velocity was determined. From measured vessel diameters and centerline blood flow velocity, apparent wall shear stress was calculated, assuming a parabolic flow velocity profile over the vessel cross section.

Experimental protocol

Leukocyte recruitment to the cremaster muscle was induced by intra-scrotal (i.s.) injection of PAF (0.4 ml of 10^{-6} M solutions) or CCL3/MIP-1α (300 ng in 0.4 ml PBS). After three hours,

five vessel segments were randomly chosen in a central area of the spread-out cremaster muscle among those that were at least 150 µm away from neighboring postcapillary venules and did not branch over a distance of at least 150 µm. After having obtained recordings of migration parameters, blood flow velocity was determined as described above. After *in vivo* microscopy, blood samples were collected by cardiac puncture for the determination of systemic leukocyte counts using a Coulter ACT Counter (Coulter Corp., Miami, FL, USA). Anesthetized animals were then killed by bleeding to death.

Analysis of leukocyte interstitial migration using the M. cremaster assay

In vivo microscopy

The setup for *in vivo* microscopy was centered around an AxioTech-Vario 100 Microscope (Zeiss MicroImaging GmbH, Goettingen, Germany), equipped with a Colibiri LED light source (Zeiss MicroImaging GmbH) for fluorescence epi-illumination microscopy. Light was directed onto the specimen via filter set 62 HE (Zeiss MicroImaging GmbH) fitted with dichroic and emission filters [TFT 495 + 610 (HE); TBP 527 + LP615 (HE)]. Microscopy images were obtained with an AxioCam Hsm digital camera using a 20x water immersion lens (0.5 NA, Zeiss MicroImaging Gmbh). The images were processed with AxioVision 4.6 software (Zeiss MicroImaging GmbH) [5].

Quantification of leukocyte migration parameters

In vivo microscopy records were analyzed offline using the imaging software ImageJ (National Institutes of Health, Bethesda, MD) as described earlier [5]. Briefly, on the post-injection side of the vessel, 5 transmigrated leukocytes were identified and tracked in the perivascular space within a time period of 6 min. Parameters including migration velocity, directionality, and euclidian as well as accumulated distance were calculated automatically by the software. Accumulated distance is the total distance presented as a line connecting the

positions of the selected leucocyte at each time point. Euclidian distance represents the shortest line connecting the start and end point of the leukocyte migration track. To quantify the directionality of migration, the chemotactic index was calculated by dividing the Euclidian distance by the accumulated distance. Polarization was analyzed offline in digital *in vivo* microscopy images by measuring the eccentricity of the cell that is equal to the ratio of the major axis of the cell (longest straight line that can be drawn across the cell) and minor axis (longest straight line that can be drawn across the cell at 90° to the major axis). Leukocytes with eccentricity of ≥ 1.2 were considered as polarized.

Experimental protocol

Directional interstitial migration of leukocytes was induced in the cremaster muscle after perivenular microinjection (100 µm distance to the vessel under investigation) of 130 pl of PAF (100 nM) or CCL3/MIP-1α (250 nM). Microinjection was performed under visual control using a borosilicate micropipette (tip pressure of 120 hPa for 0.5 s, tip diameter < 1 µm) connected to the injection system involving a semiautomatic micromanipulator (InjectMan NI 2®, Eppendorf, Hamburg, Germany) and a microinjector (FemtoJet®, Eppendorf, Hamburg, Germany). Successful microinjection was verified by the observation of visible swelling of the interstitial tissue during microinjection. The vessel as well as the surrounding tissue were then visualized during a time period of 20 min after microinjection of the inflammatory stimuli. Subsequently, the pharmacological inhibitors or the respective drug vehicle were applied and *in vivo* microscopy was continued for 40 min. Anesthetized animals were then killed by bleeding to death.

Experimental groups

In a first set of experiments, leukocyte migration to the peritoneal cavity was analyzed in control mice with an i.p. injection of PBS as well as in mice receiving either tranexamic acid

(TXA), ε-aminocaproic acid (EACA), the broad-spectrum serine protease inhibitor aprotinin, the broad-spectrum MMP inhibitor GM-6001, or drug vehicle undergoing stimulation with PAF or CCL3/MIP-1α (n=4 each group).

In a second set of experiments, the single steps of the leukocyte extravasation process were analyzed in the cremaster muscle of control mice with an i.s. injection of PBS as well as of mice receiving either TXA, EACA, aprotinin, GM-6001, or drug vehicle undergoing intrascrotal stimulation with PAF or CCL3/MIP-1α (n=4 each group).

In a final set of experiments, directional interstitial migration of leukocytes was induced in the mouse cremaster muscle by perivenular microinjection of PAF or CCL3 and analyzed upon treatment with latrunculin B, blebbistatin, TXA, EACA, aprotinin, GM-6001, or corresponding drug vehicle (n=4 each group).

Reagents

The following inhibitors were used: aprotinin (100.000 KIU kg^{-1} intra-arterially (i.a.); 5 min prior to onset of inflammation as a bolus and then as continuous i.a. infusion/superfusion of the cremaster muscle 100.000 KIU kg^{-1} h^{-1}; Sigma-Aldrich, Deisenhofen, Germany) is a broad-spectrum serine-protease inhibitor. ε-aminocaproic acid (100 mg kg^{-1} i.a. 5 min prior to onset of inflammation as a bolus and then as continuous i.a. infusion/superfusion of the cremaster muscle 100 mg kg^{-1} h^{-1}; Sigma-Aldrich) is a plasmin inhibitor; tranexamic acid (100 mg kg^{-1} i.a. 5 min prior to onset of inflammation as a bolus and then as continuous i.a. infusion/superfusion of the cremaster muscle 100 mg kg^{-1} h^{-1}; Sigma-Aldrich) is a plasmin inhibitor. Latrunculin B (500 nM solution; Sigma Aldrich; as continuous superfusion of the cremaster muscle) is an inhibitor of actin-polymerization. Blebbistatin (500 nM solution; Sigma-Aldrich; as continuous superfusion of the cremaster muscle or together with PAF in 0.4 ml PBS as intrascrotal injection) is a selective inhibitor of non-muscle myosin II. Control animals received equivalent volumes of corresponding drug vehicles. 1A8 (Neutrophil-

depleting anti-Ly-6G mAB; 100 µg i.v.; 24 h prior to induction of inflammation; BD Biosciences, San Jose, CA, USA).

Phenotyping transmigrated leukocytes

To determine the phenotype of transmigrated leukocytes, immunostaining of paraffin-embedded serial tissue sections of the cremaster muscle was performed. Sections were incubated with primary rat anti-mouse anti-Ly-6G, anti-CD45 (BD Biosciences, San Jose, CA, USA), or anti-F4/80 (Serotec, Oxford, UK) IgG antibodies. Then, the paraffin sections were stained with commercially available immunohistochemistry kits (Ly-6G, CD45, Super Sensitive Link-Label IHC detection system, BioGenex, San Ramon, CA, USA; F4/80, Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA), obtaining an easily detectable reddish or brownish end product, respectively. Finally, the sections were counterstained with Mayer's hemalaun. The number of extravascularly localized Ly-6G-, CD45-, or F4/80-positive cells was quantified by light microscopy (objective magnification 40x) on three sections (10 observation fields per section) from each individual animal per experimental group in a blinded manner, respectively. The number of transmigrated Ly-6G-positive cells (neutrophils/monocytes) and F4/80-positive cells (monocytes/macrophages) is expressed as the percentage of total CD45-positive leukocytes.

In addition, the number of transmigrating GFP^- (neutrophils) and GFP^+ (monocytes) leukocytes was evaluated in the cremaster muscle of $CX_3CR\text{-}1^{GFP/+}$ mice (exhibiting GFP^+ monocytes) within the first 120 min upon stimulation with PAF or CCL3 by combining *in vivo* transillumination (total leukocytes) and fluorescence (GFP^+ leukocytes) microscopy.

Multi-photon in vivo microscopy

For *in vivo* two-photon imaging, mice were anesthetized and ears were fixed on a custom built-stage maintaining a physiologic temperature. Images were acquired with a TrimScope

(LaVision Biotech, Goettingen, Germany) connected to an upright microscope with a 20x water immersion objective (Olympus). 4 h after subcutaneous injection of 30 µl PAF (100 nM) or directly upon subcutaneous injection of 30 µl histamine (30 µM; Sigma-Aldrich) into the ventral side of the ear, imaging was performed with an excitation wavelength of 800 nm in a frame of up to 500 µm × 500 µm with 1015 × 1015 pixels, a frame rate of two per minute, and a *z*-step of 1 µm in a range of 10 - 15 µm (20–50 µm below the epidermis). In selected experiments, the macromolecule FITC dextran (5 mg in 0.1 ml saline, *M*r 150,000, Sigma-Aldrich) was infused intra-venously and its leakage into the interstitial tissue was analyzed. Three-dimensional reconstruction was done by using Volocity (Perkin Elmer, Waltham, MA) and intensity measurements by Image J (National Institutes of Health).

Confocal microscopy

For the analysis of the effect of latrunculin B on F-actin and myosin IIA localization in interstitially migrating neutrophils, excised mouse cremaster muscles were fixed in 2 % paraformaldehyde. Tissues were then blocked and permeabilized in PBS, supplemented with 10 % goat serum (Sigma) and 0.5 % Triton X-100 (Sigma). After incubation at 4°C for 12 hours with a polyclonal rabbit anti-mouse antibody to nonmuscle myosin IIA (Abcam, Cambridge, UK), tissues were incubated for 3 hours at room temperature with an Alexa Fluor 488-linked goat anti-rabbit antibody, Alexa Fluor 546 phalloidin, and To-Pro-3 (Invitrogen). Immunostained tissues were mounted in PermaFluor (Beckman Coulter, Fullerton, CA) on glass slides. Confocal z-stacks typically covering 30 µm (z-spacing 0.5 µm) were aquired using a Leica SP5 confocal laser-scanning microscope (Leica Microsystems, Wetzlar, Germany) with an oil-immersion lens (Leica; 63x; NA 1.40). Further image processing and 3D rendering was done using Imaris (Bitplane, Zürich, Switzerland) and Adobe Photoshop 7.0.1 (Adobe Systems, San Jose, CA) software. Images were adjusted for brightness but except for the obvious (cropping, scaling, projections etc.) not otherwise manipulated.

Viability of neutrophils

In order to exclude potential cytotoxic effects of GM-6001, murine bone marrow cells were prepared, exposed to different concentrations of GM-6001 and subjected to an Alamar Blue viability assay (AbD Serotec, Duesseldorf, Germany). Briefly, 2.5 x 10^5 bone marrow cells per well were seeded into 96 well plates in X-Vivo 15 medium (Lonza, Basel, Switzerland) supplemented with 10 % fetal calf serum in a final volume of 150 µl. Indicated concentrations of GM-6001 or DMSO as vehicle control were added and cells were incubated for 20, 60, or 180 min, respectively. The prototypical apoptosis stimulus staurosporine (1 µM, Sigma-Aldrich) served as control. Viability was assessed by addition of 1/10 vol Alamar Blue reagent and incubation for 8 h. Absorption of reduced resorufin was measured at 570 nm (Ref. 590 nm) and measured values were calibrated on 100 % viability of the untreated controls.

Flow cytometry

Anticoagulated whole blood samples or samples from the peritoneal lavage fluid were incubated (20 min; room temperature) with primary antibodies directed against plasmin(ogen) (Santa Cruz Biotechnology, Santa Cruz, CA) as well as against MMP-2, MMP-8, or MMP-9 (Merck Milipore, Schwalbach, Germany) on ice. Isotype-matched controls were also used in all experiments. After lysis of erythrocytes, stained cells were analyzed on a flow cytometer (Gallios, Beckmann Coulter Inc., Brea, CA, USA). Neutrophils were identified by expression of CD45 and CD11b as well as by high expression of Gr-1 and low expression of CD115. Monocytes were identified by expression of CD45 and CD11b as well as by high expression of CD115. Approximately 10,000 – 20,000 gated events were collected in each analysis.

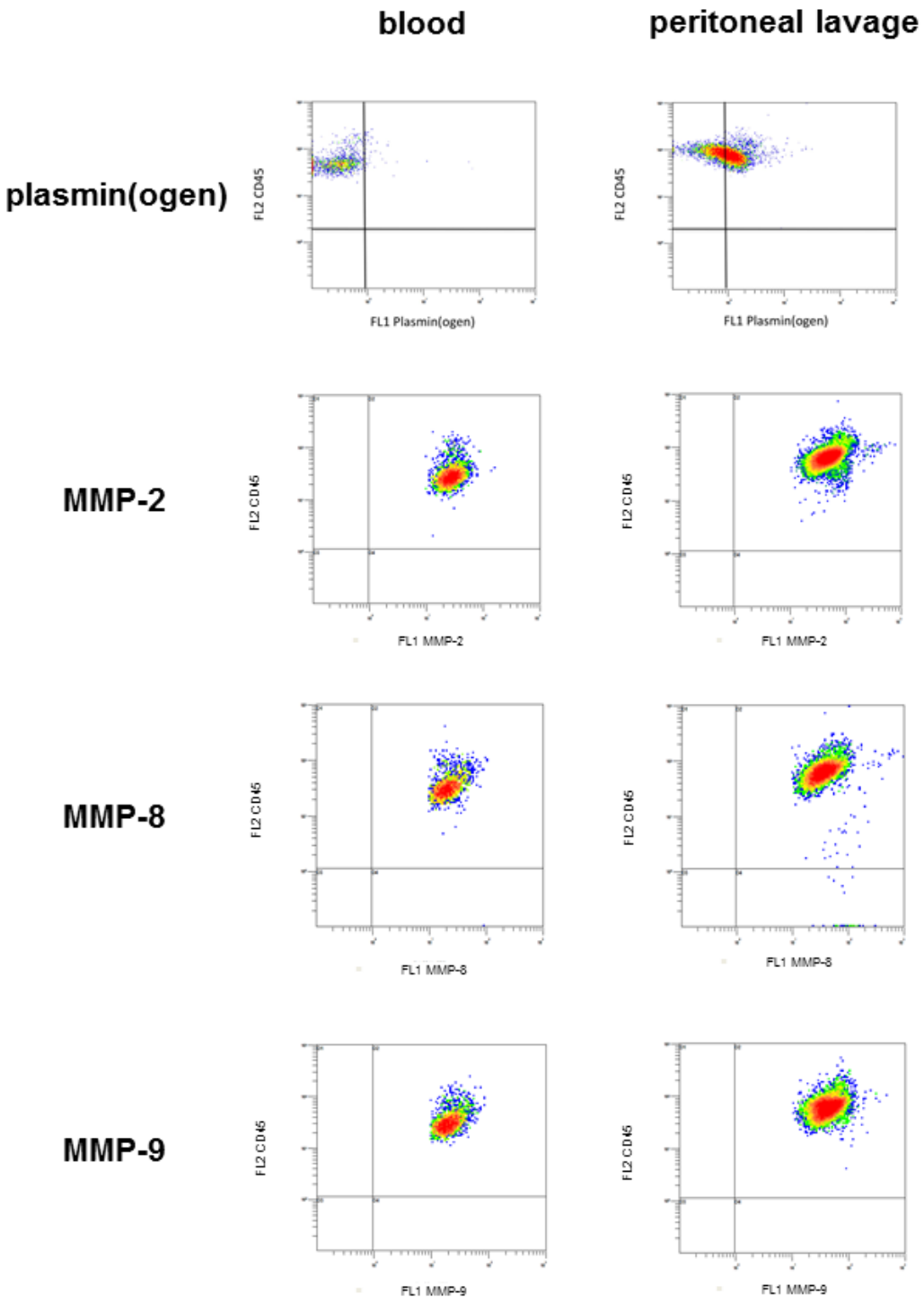
To analyze the effect of GM-6001 on the expression profiles of adhesion/signaling molecules on murine neutrophils and monocytes, anticoagulated whole blood samples were incubated

(20 min; room temperature) with 100 ng ml^{-1} of PAF (with addition of 10 µg ml^{-1} GM-6001 or vehicle) or PBS as negative control. After washing, samples were incubated with primary monoclonal antibodies directed against either CD62L/L-selectin, CD11a/LFA-1, or CD29/β_1 integrin chain (BD Biosciences) on ice. Isotype-matched controls were also used in all experiments. After lysis of erythrocytes, stained cells were analyzed (see above).

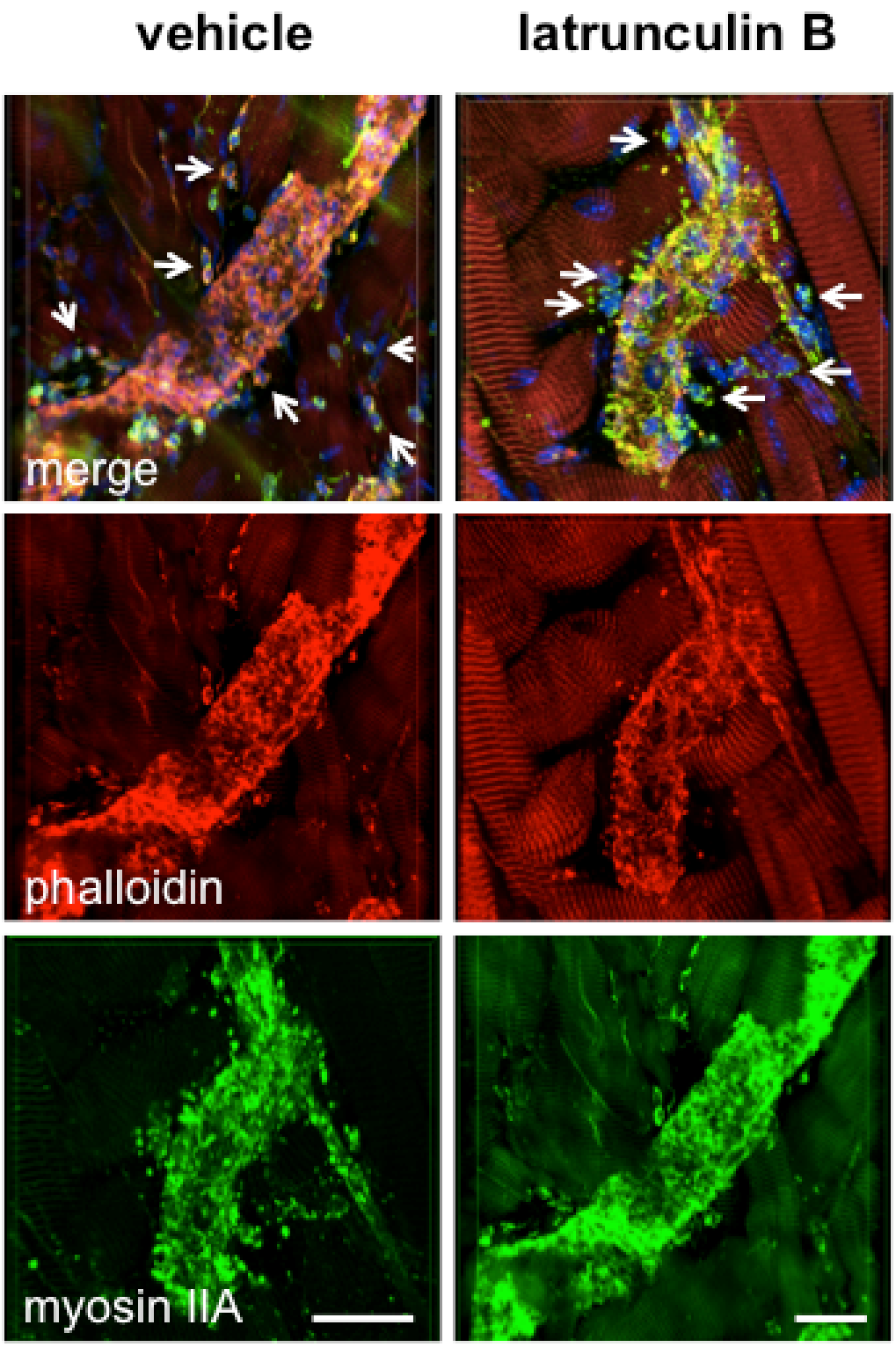
Statistics

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany). After testing normality of data (using the Shapiro-Wilk test), the 1-way ANOVA test followed by the Dunnett (>2 groups) or the *t* test (2 groups) was used for the estimation of stochastic probability in intergroup comparisons. Mean values and SEM are given. *P* values < 0.05 were considered significant.

Supplemental figure I

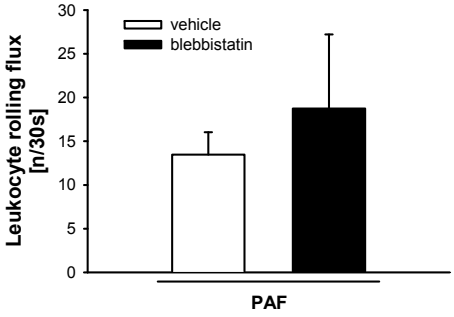


Supplemental figure II



Supplemental figure III

A



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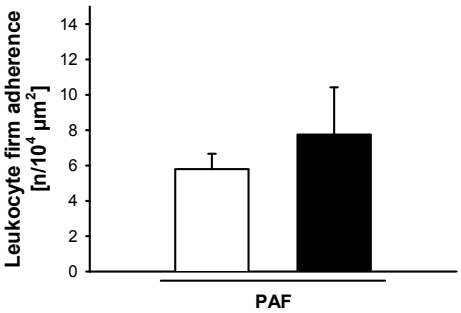

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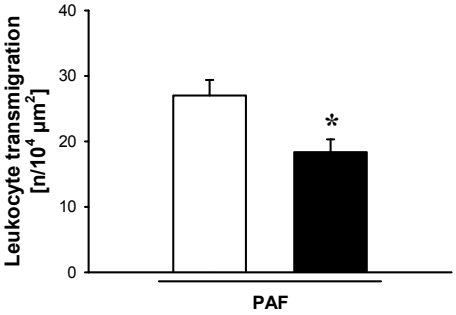
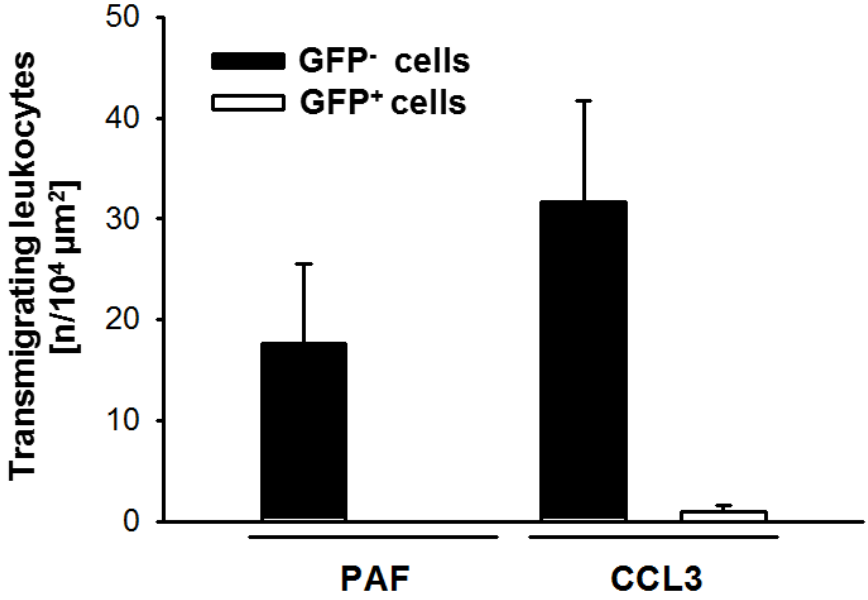


Supplemental figure IV



Supplemental figure V

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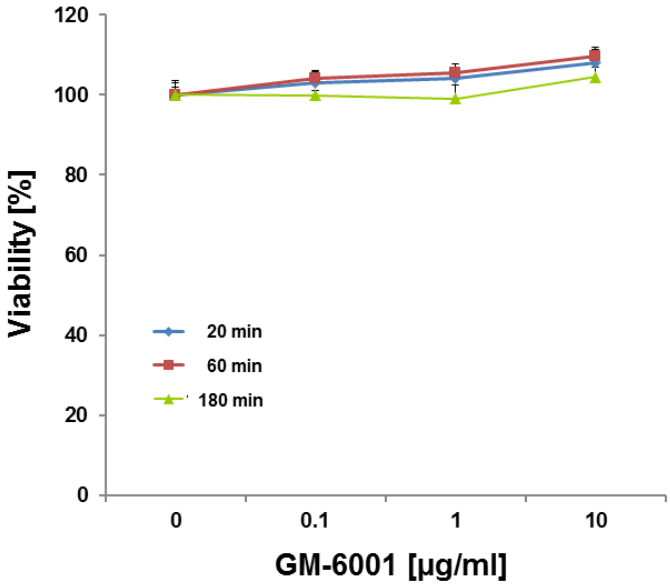

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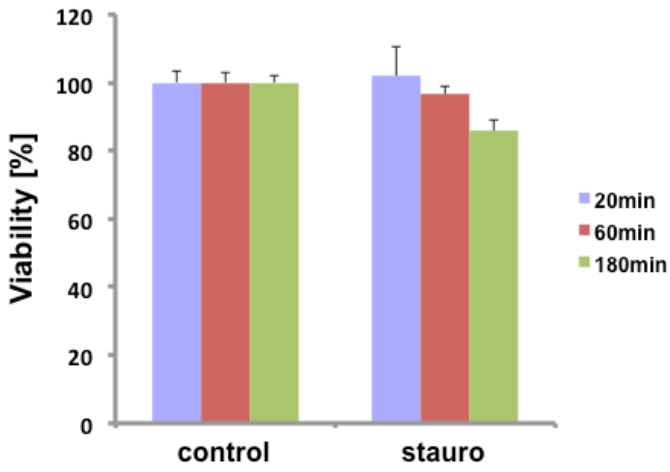

Supplemental table I

Stimulus	Drug	Inner diameter [µm]	Vmean [mm s^{-1}]	Wall shear rate [s^{-1}]	Systemic leukocyte counts [x 10^3 $µl^{-1}$]
PBS	#	26.5 ± 0.3	1.5 ± 0.1	2218.9 ± 42.7	3.5 ± 0.5
PAF	vehicle	26.7 ± 0.2	1.5 ± 0.1	2110.8 ± 13.6	5.3 ± 1.0
PAF	aprotinin	26.5 ± 0.1	1.3 ± 0.1	1908.6 ± 66.0	3.0 ± 0.3
PAF	TXA	25.9 ± 0.1	1.3 ± 0.1	2102.7 ± 70.0	3.3 ± 0.5
PAF	EACA	25.7 ± 0.2	1.1 ± 0.1	1874.1 ± 66.1	3.0 ± 0.7
PAF	GM-6001	24.2 ± 0.4	1.4 ± 0.1	2038.4 ± 72.3	3.0 ± 0.6
CCL3	vehicle	26.0 ± 0.6	1.4 ± 0.1	2110.8 ± 267.0	3.6 ± 0.5
CCL3	aprotinin	26.7 ± 0.2	1.3 ± 0.1	1908.6 ± 66.0	3.6 ± 0.5
CCL3	TXA	26.1 ± 0.1	1.4 ± 0.1	2102.7 ± 72.7	3.3 ± 0.5
CCL3	EACA	25.1 ± 0.1	1.2 ± 0.1	1874.1 ± 66.1	3.9 ± 0.5
CCL3	GM-6001	25.0 ± 0.5	1.3 ± 0.1	2267.8 ± 80.4	3.4 ± 0.8

Supplemental table II

	PBS	PAF + vehicle	PAF + GM-6001
Neutrophils [MFI]			
CD62L	21.1 ± 1.0	13.3 ± 2.6 #	20.9 ± 1.2 *
CD29	83.8 ± 11.0	518.6 ± 124.1 #	173.1 ± 54.5 #*
CD11a	58.1 ± 2.2	84.6 ± 15.4 #	102.0 ± 17.9 #*
CD11b	37.6 ± 7.9	65. ± 2 7.1 #	86.6 ± 8.7 #*
Monocytes [MFI]			
CD62L	38.5 ± 2.7	42.1 ± 0.9	41.2 ± 5.4
CD29	126.8 ± 23.2	128.9 ± 35.4	129.6 ± 29.1
CD11a	86.9 ± 1.4	84.2 ± 2.2	94.4 ± 15.8
CD11b	63.9 ± 5.7	65.0 ± 3.2	75.5 ± 12.1

Supplemental table III

	Neutrophils				Monocytes			
	Blood		Lavage		Blood		Lavage	
	% of total	MFI	% of total	MFI	% of total	MFI	% of total	MFI
MMP-2	99.4 ± 0.1	23.8 ± 2.6	99.7 ± 0.1	42.1 ± 2.5 #	34.9 ± 12.6	144.1 ± 29.4	62.8 ± 8.9 #	287.7 ± 20.8 #
MMP-8	99.1 ± 0.4	23.2 ± 4.5	99.8 ± 0.1	47.0 ± 4.8 #	37.3 ± 9.4	179.0 ± 41.1	68.6 ± 5.8 #	333.3 ± 37.9 #
MMP-9	99.1 ± 0.6	28.1 ± 3.4	99.9 ± 0.1	51.7 ± 2.7 #	34.1 ± 7.2	176.3 ± 43.6	72.6 ± 6.0 #	359.0 ± 40.5 #
plasmin(ogen)	5.4 ± 1.3	1.3 ± 0.1	50.9 ± 8.4 #	1.5 ± 0.1	14.3 ± 1. 7	5.8 ± 0.2	46.5 ± 3.8 #	5.7 ± 0.4

Supplemental Figure I. Surface expression of proteases on naïve and transmigrated neutrophils. Representative dot plots for expression of plasmin(ogen) as well as of MMP-2, -8, and -9 on murine neutrophils isolated from the whole blood or from the inflamed peritoneal cavity.

Supplemental Figure II. Effect of latrunculin B on subcellular localization of actin and myosin II in leukocytes. Representative 3D-rendered confocal microscopy images of the actin cytoskeleton of leukocytes in the interstitial tissue surrounding postcapillary venules of the inflamed mouse cremaster muscle upon topical application of latrunculin B or vehicle. In vehicle-treated tissues, F-actin (red) and myosin IIA (green) are localized at the cell cortex of leukocytes (arrows). Treatment with latrunculin B reduced the leukocyte F-actin content and disrupted F-actin and myosin IIA localization at the leukocyte cell cortex (arrows). Cell nuclei are depicted in blue (scale bars: 40 μm).

Supplemental Figure III. Effect of blebbistatin on leukocyte extravasation. Leukocyte rolling (A), firm adherence (B), and transmigration (C) were quantified three hours after intra-scrotal injection of PAF by *in vivo* transillumination microscopy as detailed in *Material and Methods*. Panels show results for mice receiving blebbistatin or drug vehicle (*$p<0.05$, vs. vehicle for n=3 per group).

Supplemental Figure IV. Effect of PAF or CCL3 on extravasation of monocytes. The number of transmigrating GFP^- (neutrophils) and GFP^+ (monocytes) leukocytes was evaluated in the cremaster muscle of $CX_3CR\text{-}1^{GFP/+}$ mice (exhibiting GFP^+ monocytes) within the first two hours upon stimulation with PAF or CCL3 by *in vivo* microscopy as detailed in *Material and Methods* (mean ± SEM for $n = 3$ per group).

Supplemental Figure V. Effect of GM-6001 on viability of neutrophils. Viability of murine bone marow neutrophils as analyzed upon co-incubation with varying concentrations of GM-6001 (A) or the prototypical apoptosis stimulus staurosporine (B) at different incubations times by using the alamar blue assay (mean ± SEM for $n = 5$ per group).

Supplemental Table I. Systemic leukocyte counts and micro-hemodynamic parameters. Systemic leukocyte counts as well as micro-hemodynamic parameters including inner vessel diameter, blood flow velocity, and wall shear rate were obtained as detailed in *Materials and Methods* (mean ± SEM for $n = 4$ per group).

Supplemental Table II. Effect of GM-6001 on expression of adhesion/signaling molecules on neutrophils and monocytes. The effect of GM-6001 on PAF-elicited changes in the surface expression of L-selectin (CD62L) or integrins (CD11a, CD11b, and CD29) on neutrophils and monocytes was analyzed by flow cytometry as detailed in *Material and Methods* (mean ± SEM for $n = 3 – 5$ per group; #$p<0.05$, vs. PBS; *$p<0.05$, vs. PAF + vehicle).

Supplemental Table III. Surface expression of proteases on naïve and transmigrated neutrophils and monocytes. Surface expression of plasmin(ogen) as well as of MMP-2, -8, and -9 on murine neutrophils and monocytes isolated from the whole blood or from the inflamed peritoneal cavity was analyzed by flow cytometry as detailed in *Material and Methods* (mean ± SEM for $n = 3 – 5$ per group; #$p<0.05$, vs. blood).

Supplemental videos 1A, B. Interstitial migration of neutrophils occurs in areas of low collagen fiber density. Representative multi-photon *in vivo* microscopy videos of neutrophils

(white) migrating within the interstitial tissue. Areas of low (blue), intermediate (yellow), and high (red) collagen density are in indicated in pseudocolors.

Supplemental video 2. Leukocyte being trapped between collagen fibers. Representative multi-photon *in vivo* microscopy video of an interstitially migrating neutrophil (green) encountering collagen fibers (white).

Supplemental video 3. 3-D *in vivo* chemotaxis assay. *In vivo* transillumination microscopy video of interstitially migrating leukocytes in the mouse cremaster muscle upon perivenular microinjection of PAF (injected together with green fluorescence-labeled microspheres for identification of the site of microinjection), the migration track of a single leukocyte is shown in blue (objective magnification 40x).

References

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