Supplemental Methods

Intravital microscopy of the cremaster muscle
An inflammatory response was induced by intrascrotal injection of TNF (50ng). The cremaster muscle was exposed and recordings were made using an Olympus BX51 microscope equipped with a Hamamatsu 9100-02 EMCCD camera (Hamamatsu Photonics) and a 20x water dipping objective. For image acquisition and analysis Olympus cell^{r} software (Olympus) was used. An antibody to Ly6C (ebioscience) was injected i.v. to detect adhesion of Ly6C-positive CM. For luminal detection of CRAMP presented on the endothelium, 50µl of Protein G Fluoresbrite® YG Microspheres (Polysciences) were coupled to 50µg of polyclonal Ab to CRAMP (Innovagen). Beads and antibody were reacted for 30min at room temperature, washed twice and subsequently injected i.v.

Cell culture
Human umbilical vein endothelial cells (HUVEC) (PromoCell) were cultured and propagated on collagen (50 µg/ml, Biochrom) coated tissue culture flask in endothelial growth medium (PromoCell). Peripheral blood mononuclear cells (PBMC) were separated by density gradient centrifugation. Monocyte subsets were then isolated using the CD16^{+} Monocyte Isolation Kit or the Monocyte Isolation Kit II (both Miltenyi Biotech Inc.) for non-classical and classical monocytes, respectively.

Flow adhesion assay
HUVEC cultured in petri dishes were activated with TNF (50ng/ml, 12 h, Peprotech). LL37 (1µg/ml, Anaspec) or mutated LL37 (1µg/ml) were added 15min prior to performance of flow chamber assays. Isolated monocyte subsets were labeled with Calcein AM (Invitrogen) and perfused at 2.5dyne/cm^2 over HUVEC monolayers. In separate experiments, monocytes were pretreated for 30min with inhibitors detailed in the result section, washed twice and then used for flow chamber assays. Adherent cells were quantified in six randomly chosen fields.

Transcytosis assay
HUVECs were cultured on 24-well polycarbonate transwell filter inserts (5µm, Corning Costar) coated with collagen. HUVECs were activated with human TNF (50ng/ml, 12h, Peprotech). FITC-conjugated LL37 (10µg/ml) was added to the bottom chamber. Alternatively, native (unconjugated) LL37 (10µg/ml) was added to the lower chamber of transwells. In order to detect the transcytosed LL37 on the HUVEC cell surface, a FITC conjugated antibody against human LL37 (Innovagen) was used and the fluorescence intensity assessed by a plate reader. For visual analysis, HUVEC were then counter-stained with DAPI (nuclear dye) and CellMask (plasma membrane stain, Invitrogen) and fixed with 4% PFA at 4°C for 30min. After washing, the samples were analyzed using a Leica SP5 MP system with a water dipping 20x; NA 1.00 objective and a Ti:Sa MaiTai DeepSee laser (Spectra Physics) tuned to 790nm. Three Hybrid detectors (HyD) were spectrally tuned for optimal detection efficiency and low bleed through of signal of the used markers (HyD1: 435-485nm, HyD2: 515-550nm, HyD3: 565-630nm). Furthermore, a dye separation protocol (Leica LAS AF software) was performed for removal of any leftover bleed through of signal. Additional image preparation was performed using ImagePro 3D analyzer 7.0 (Media Cybernetics).

Surface plasmon resonance
Interaction between LL37 and chemotactic receptors were studied by Surface Plasmon Resonance on a Biacore X100 system (GE Healthcare). LL37 or mutated LL37 were immobilized on a CM4 sensor chip at a level of 400 response unit (RU) by amine coupling. Receptors FPR1, FPR2, CXCR2 and P2X7 (Abnova) were used as analyte and diluted in a HBS-N running buffer (0.1M HEPES, 1.5M NaCl pH 7.4). Each experiment was performed with a flow of 10µl/min with running buffer. Sensor chip surfaces were regenerated with 50mM NaOH and equilibrated with running buffer prior to the next injection. Results were analyzed with Biaevalution software.

Integrin activation assay
Human PBMCs were incubated 15min with PBS, PMA (50ng/ml, Sigma-Aldrich), MCP1 (50ng/ml, PeproTech), LL37 (1µg/ml, AnaSpec), or mutated LL37 (1µg/ml) and then stained with antibody against CD45, CD14, and CD16 for 30min at 4°C (BD Pharmingen). Antibodies to CD29 (HUTS-21,

BD Pharmingen) and CD11b (CBRM1/5, ebioscience) detecting epitopes of activated integrins only were used to assess integrin activation. Integrin activation was measured by flow using a FACSCanto II (BD Biosciences). The results are analyzed with FlowJo Software (Treestar).

Murine peripheral blood cells were isolated from C57Bl6 mice and suspended in Hanks Balanced Salt Solution containing 1mM $CaCl_2$ and $MgCl_2$ (Invitrogen). Cells were exposed to PMA (50ng/ml, Sigma-Aldrich), MCP1 (50ng/ml, PeproTech), CRAMP (1µg/ml, Innovagen) or an equal volume of PBS, in the presence of ICAM-1/Fc (10µg/ml, R&D Systems) or of VCAM-1/Fc (10µg/ml, R&D Systems) and PE-conjugated anti–human IgG1 (Fc-specific, Southern Biotechnology) for 5 minutes at 37°C. After washing, cells were labeled with antibodies to CD45, CD115, and Gr1 to identify monocyte subsets. Binding of ICAM-1 or VCAM-1 was measured by flow cytometry.

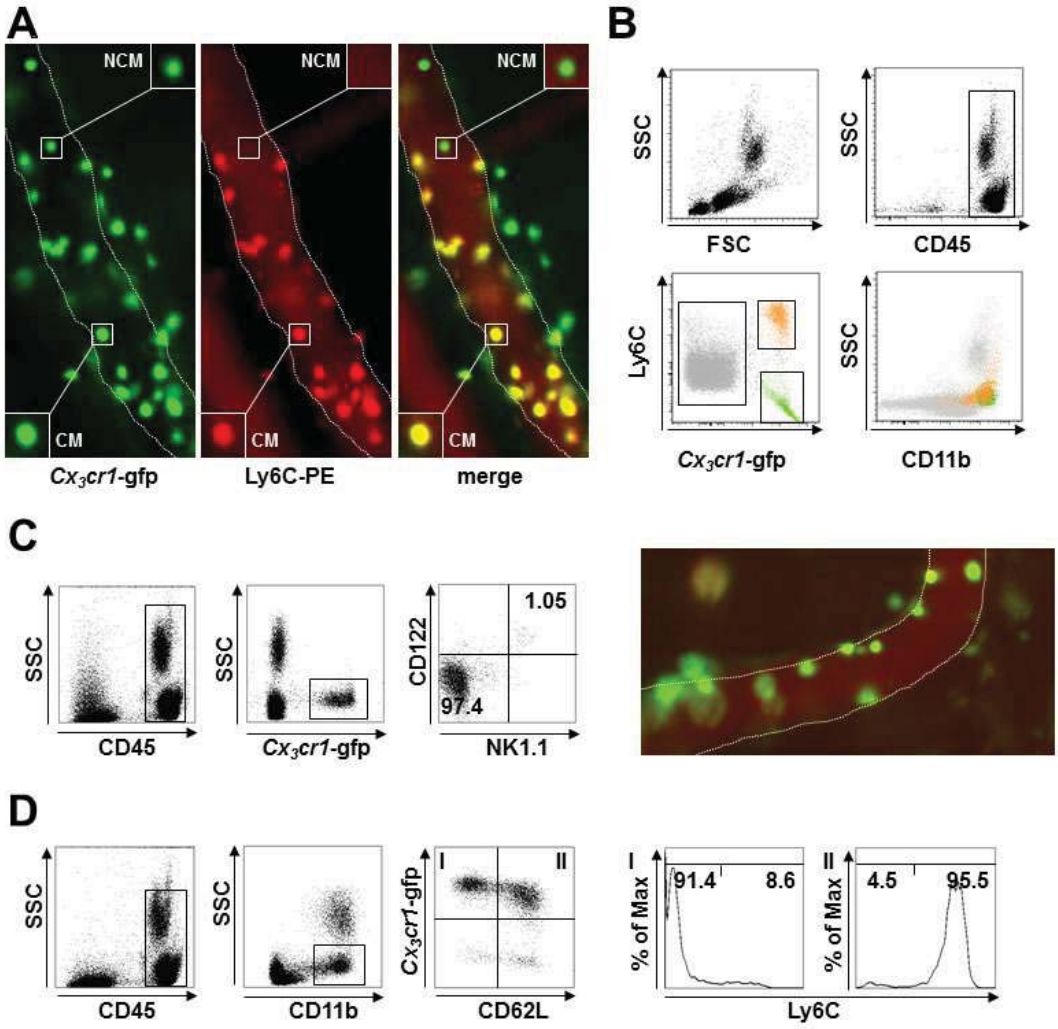
Intracellular Cramp detection

For FACS analysis of cremaster muscle single-cell suspensions, mice were injected intrascrotally with TNF (50ng, 4h). An antibody to Ly6G (1A8, 1µg) was injected 5 minutes prior to sacrifice to label adherent neutrophils. The circulation of mice was flushed with cold PBS and the muscle tissue was excised and digested with 0.25 mg/ml Liberase (Roche) in RPMI1640 + 10% FCS medium at 37 °C for 1h. Surface staining for flow cytometric analysis was conducted using combinations of antibodies against CD11b, Gr1, CD31, and CD45 (all ebioscience). Intracellular labeling of CRAMP (rabbit anti-mouse CRAMP) and an appropriate isotype control (rabbit IgG isotype control, GeneTex) was performed with IC fixation buffer and permeabilization buffer (both from ebiosciene) according to the manufacturer`s protocol for staining of cytosolic proteins. Anti-rabbit IgG (ebioscience) served as fluorescently labeled secondary antibody.

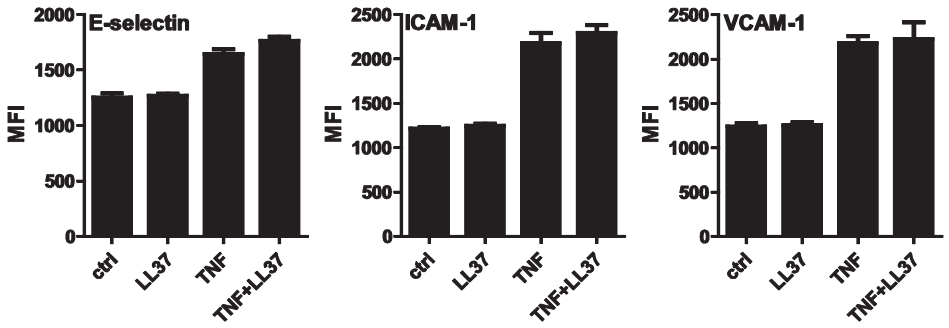
Statistics

All data are expressed as mean±SD. Statistical calculations were performed using GraphPad Prism 5 (GraphPad Software Inc.). Mann-Whitney test, one way ANOVA with Dunnett post-hoc test, or Kruskal-Wallis test with posthoc Dunn tests were used as appropriate. * indicates a p-value < 0.05.

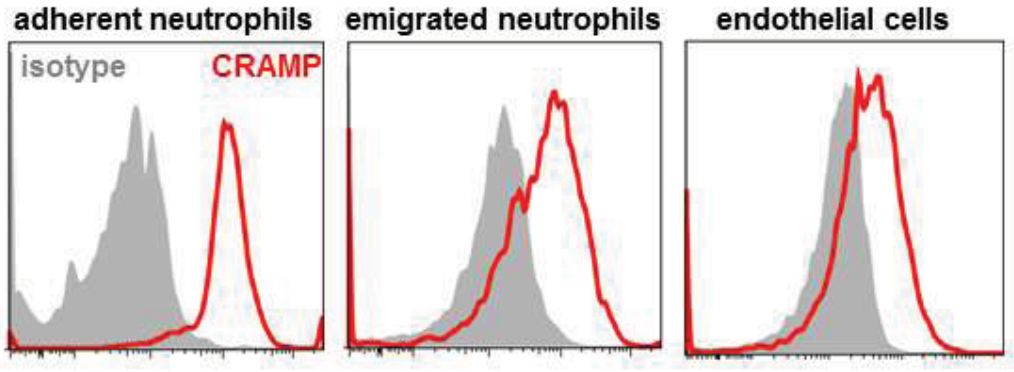
Supplemental Figures



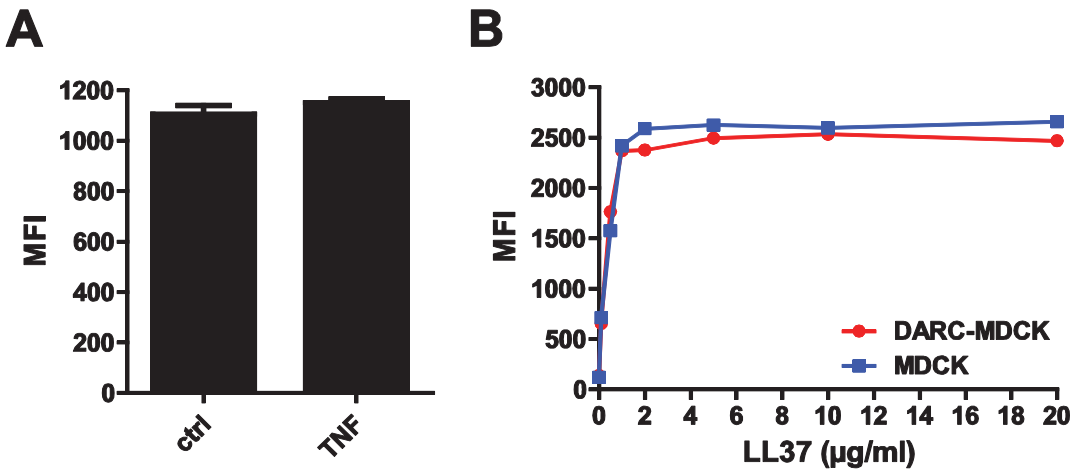
Online Figure I: *In vivo* labeling of classical monocytes. To discriminate between monocyte subsets an antibody to Ly6C (1 µg) was introduced i.v. into *Cx$_3$cr1$^{egfp/wt}$* mice. **A:** Example of intravital microscopy of the cremaster muscle using a beam splitter to allow recording of two emission wavelengths.CM, classical monocyte; NCM, non-classical monocyte. **B:** Blood was drawn from *Cx$_3$cr1$^{egfp/wt}$* mice injected with an antibody to Ly6C and stained with antibodies to CD45 and CD11b. Classical monocytes (Ly6C$^+$gfp$^+$, orange) and non-classical monocytes (Ly6C$^-$gfp$^+$, green) also appear CD11b$^+$SSC^{lo}. In contrast, gfp$^-$ leukocytes (grey) are either CD11b$^+$SSC^{hi} (neutrophils) or CD11b$^-$ SSC^{lo} (lymphocytes). **C:** *Cx$_3$cr1$^{egfp/wt}$* mice were injected i.v. with a PE-conjugated antibody to NK1.1. Labeling of NK cells was confirmed by counterstaining with an antibody to CD122 *ex vivo* (left). *In vivo*, the antibody to NK1.1 did not label adherent gfp$^+$ cells (right). **D:** Intravenous injection of an antibody to Ly6C efficiently labels classical monocytes. Anti-Ly6C-PE (1 µg) was introduced i.v. into *Cx$_3$cr1$^{egfp/wt}$* mice. To assess *in vivo* labeling efficiency, white blood cells were counterstained *ex vivo* with antibodies to CD45, CD11b, and CD62L to allow for identification of monocyte subsets. Anti-Ly6C binding was then quantified on non-classical (I) and classical (II) monocytes.



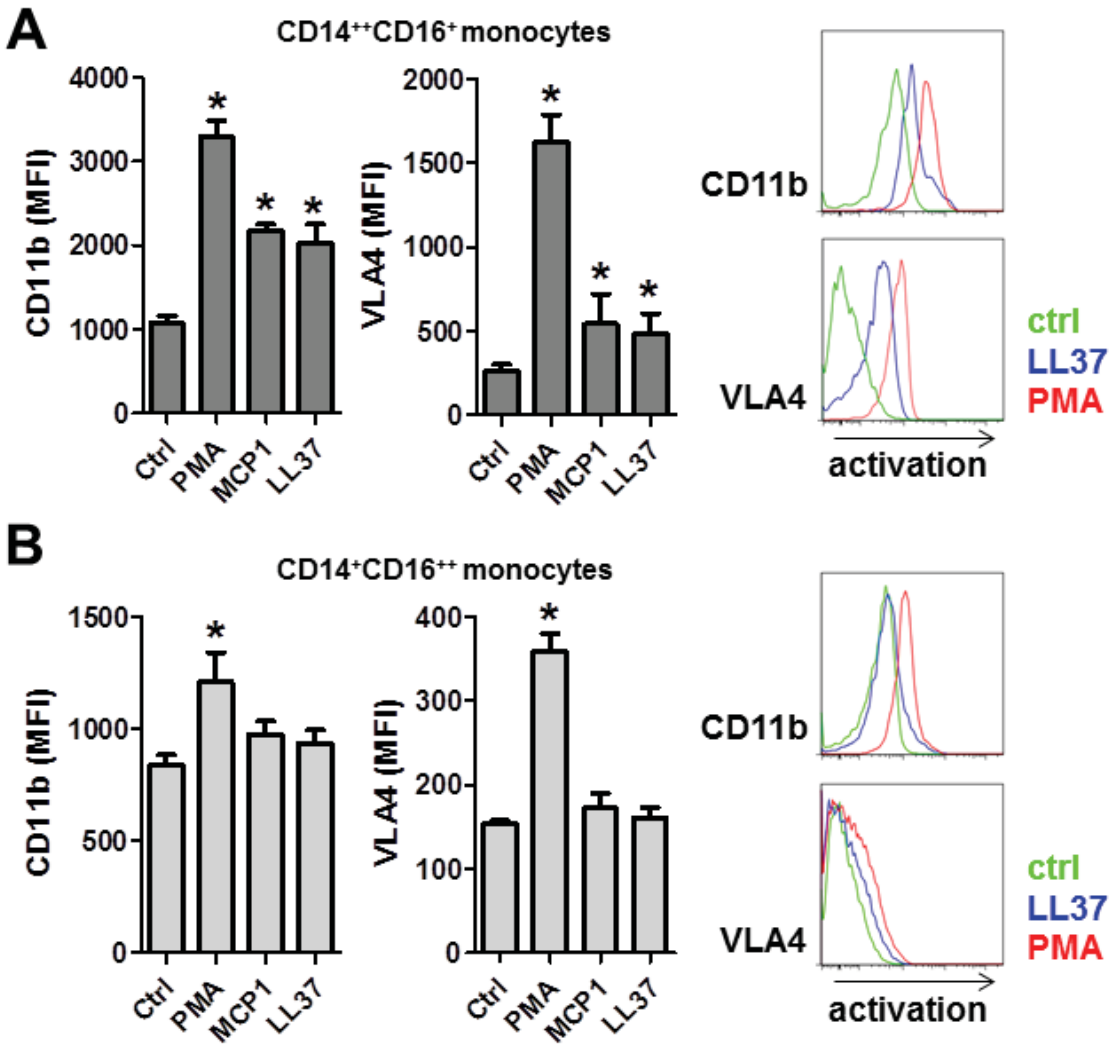
Online Figure II: LL37 does not induce endothelial cell adhesion molecule expression. Human umbilical vein endothelial cells were treated with buffer (ctrl), LL37 (1 µg, 15 min), TNF (20 ng, 12 h), or a combination of both. Expression of E-selectin, ICAM-1, or VCAM-1 was measured by use of a fluorescence plate reader and directly conjugated antibodies towards the respective adhesion molecule. n= 3 for each bar.



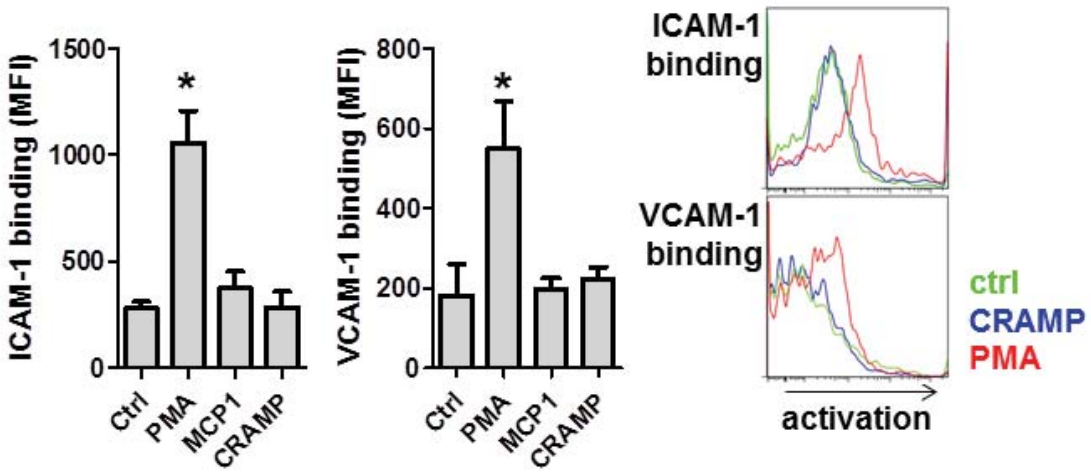
Online Figure III: Neutrophils release CRAMP during extravasation. Mice were injected intrascrotally with TNF (50ng, 4h) and an antibody to Ly6G (1A8, 1µg) was injected 5 minutes prior to sacrifice to label adherent neutrophils. Cremaster muscles were excised and enzymatically digested. Intracellular CRAMP was measured in adherent and emigrated neutrophils as well as in endothelial cells. Representative histograms of three independent experiments display specific CRAMP staining as well as the respective isotype control fluorescence.



Online Figure IV: Cathelicidin does not interact with duffy antigen/receptor for chemokines (DARC). A: TNF does not induce expression of DARC on human umbilical vein endothelial cells (HUVEC). HUVEC were treated with TNF (20 ng, 12 h) and the expression of DARC was analyzed by fluorescence plate reader. n = 3. **B:** LL37 does not bind to DARC. DARC-expressing Madin-Darby canine kidney (DARC-MDCK) or control MDCK cells were incubated with fluorescent LL37 at various concentrations and binding was assessed by flow cytometry. Displayed is one representative experiment.



Online Figure V: LL37 activates integrins on human intermediate but not on non-classical monocytes. Based on the CD16 and CD14 staining properties, $CD14^{++}CD16^{+}$ intermediate monocytes (A) and $CD14^{+}CD16^{++}$ non-classical monocytes (B) were identified within peripheral blood mononuclear cells. Moreover, antibodies to activation epitopes of VLA4 (HUTS-21) and CD11b (CBRM1/5) were added. Cells were treated with PMA (50 ng/ml), MCP-1 (50 ng/ml), or LL37 (1 µg/ml) for 15 minutes and the expression of activated CD11b (left) or VLA4 (middle) was assessed on intermediate and non-classical monocytes. Representative histograms are displayed to the right. * indicates significant difference compared to ctrl.



Online Figure VI: CRAMP does not activate integrins on murine non-classical monocytes. Mouse peripheral leukocytes were treated with PMA (50 ng/ml), MCP-1 (50 ng/ml), or CRAMP (1 µg/ml) in the presence of ICAM-1-Fc (left) or VCAM-1-Fc (right) and an anti-Fc antibody. Monocyte subsets were identified by additional antibody staining (CD45, CD11b, CD115, Gr1). n = 3. * indicates significant difference compared to ctrl.

Online Table I: Differential leukocyte counts in mice with intact WBC and in neutropenic mice. All values are given in count/ml venous blood.

	neutrophils	inflammatory monocytes	resident monocytes	T-lymphocytes	B-lymphocytes
Intact WBC	$9.2x10^5$ +/- $1.4x10^5$	$2.4x10^5$ +/- $0.7x10^5$	$2.0x10^5$ +/- $0.4x10^5$	$2.1x10^6$ +/- $0.8x10^6$	$1.6x10^6$ +/- $0.3x10^6$
neutropenic	$0.6x10^5$ +/- $0.2x10^5$	$3.1x10^5$ +/- $0.5x10^5$	$1.9x10^5$ +/- $0.7x10^5$	$2.2x10^6$ +/- $0.4x10^6$	$1.9x10^6$ +/- $0.4x10^6$
p-value	0.001	0.652	0.905	0.920	0.843