Supplemental material
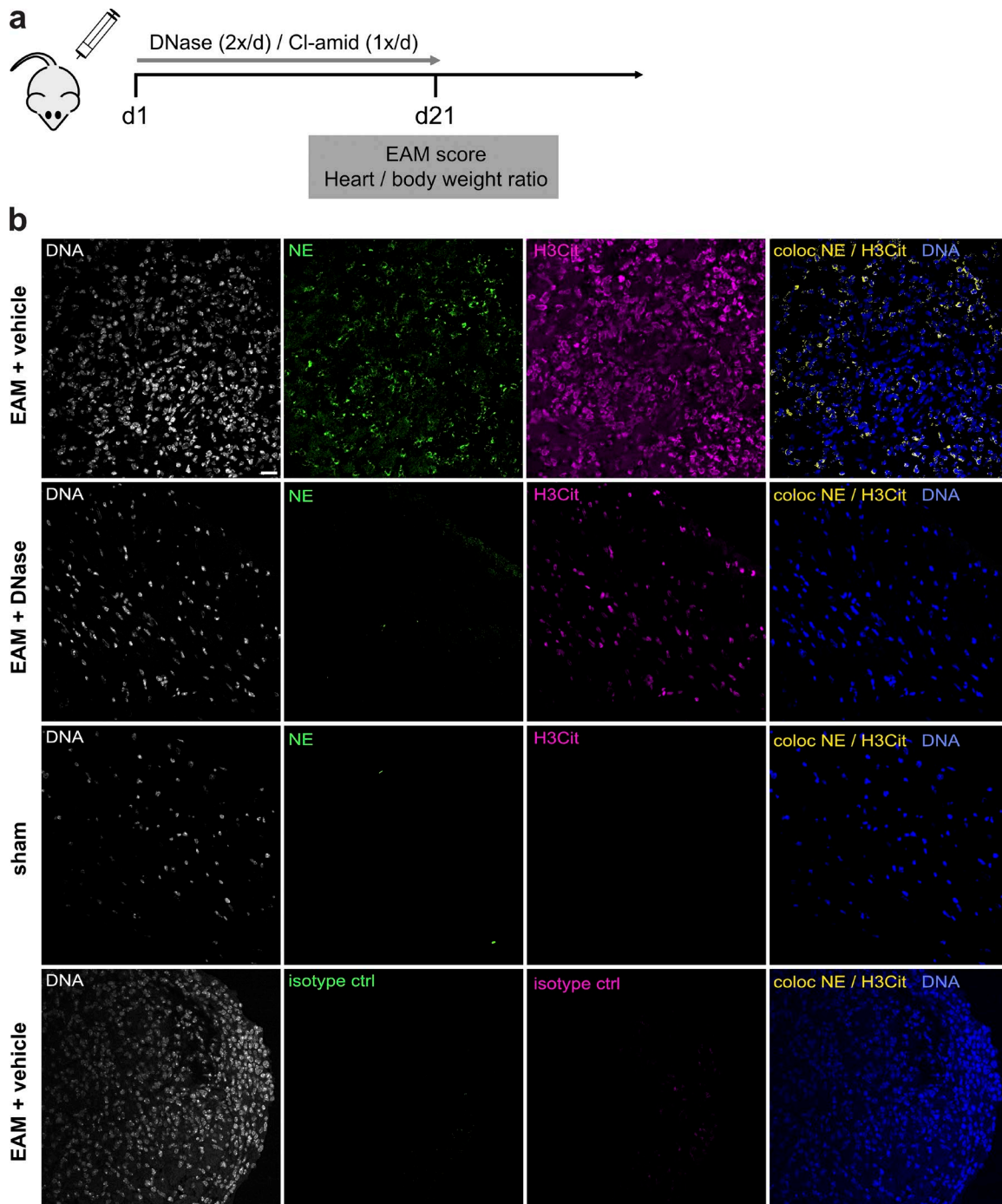
Figure S1. **NETs in myocarditis. (a)** DNase (50 µg i.p.) was administered twice daily, and Cl-amid (250 µg s.c.) was administered once daily for 21 d after induction of EAM. Leukocyte infiltration and the heart/body weight ratio were subsequently analyzed. **(b)** Representative confocal images of cardiac sections of EAM mice after vehicle treatment (EAM + vehicle) or application of DNase (EAM + DNase) as well as sham-treated control mice (sham). Images show staining of DNA, NE, and H3Cit as well as the colocalization of NE and H3Cit (yellow) together with the DNA staining (blue). As control, staining with isotype control Abs for H3Cit and NE of cardiac sections of vehicle-treated EAM mice (EAM + vehicle, isotype control) was conducted. Bar, 20 µm.
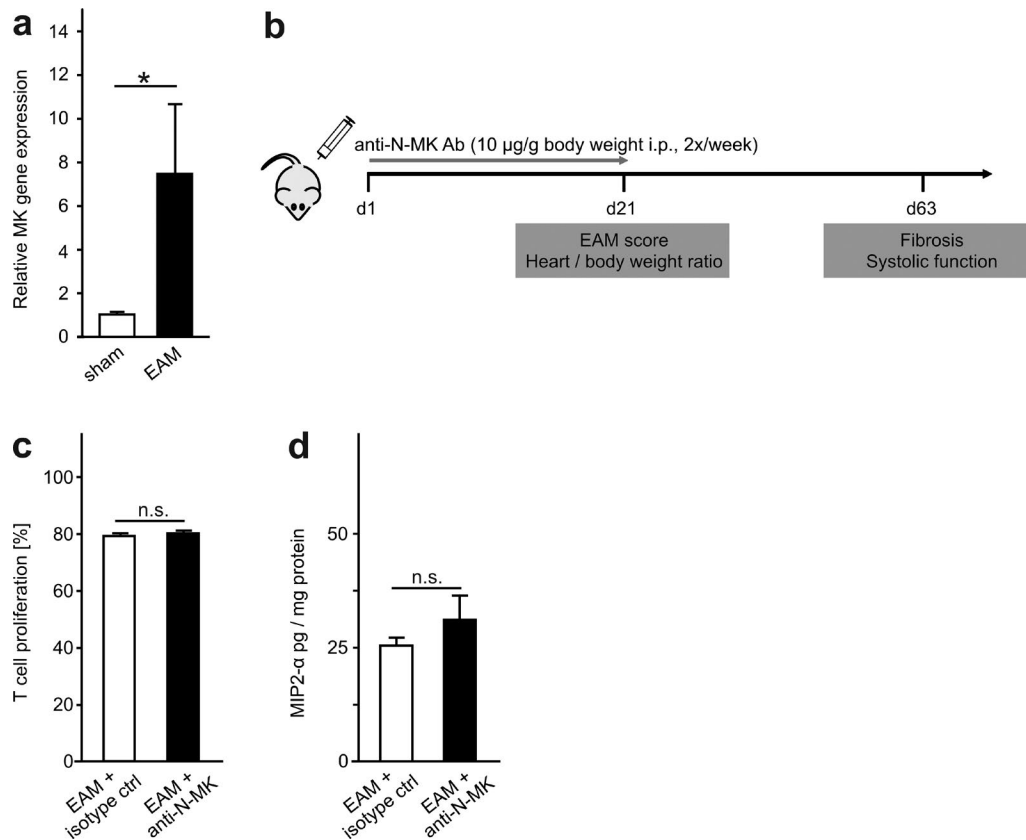
Figure S2. **MK expression and function during EAM. (a)** Relative gene expression of MK in the cardiac tissue of EAM mice at day 21 (7.47 ± 3.17 a.u.) compared with sham-treated control mice at the same time point (1.0 ± 0.10 a.u.) using quantitative real time PCR. $n = 5$ hearts for sham group; $n = 10$ hearts for EAM group. **(b)** Anti-N-MK Ab (10 μg/g body weight i.p.) was administered twice a week for 21 d. Leukocyte infiltration and the heart/body weight ratio were studied at day 21, fibrosis and systolic function at day 63 (see Fig. 3, a–d). **(c and d)** Mice were treated with either isotype control Ab (EAM + isotype ctrl) or with anti-N-MK Ab (EAM + anti-N-MK) from day 1 until day 21 of EAM, as illustrated in b. **(c)** Proliferation of CD4+ T cells in the presence of CD3+/CD28 beads (ratio 1:2 with T cells) was evaluated by flow cytometry after staining for CFSE (EAM + isotype ctrl, 79.4 ± 0.7 [%]; EAM + anti-N-MK, 80.3 ± 1.0 [%]). $n = 10$ for both groups. **(d)** MIP2-α expression was studied in cardiac tissue of EAM mice using ELISA technique. Mice were treated with anti-N-MK Ab (EAM + anti-N-MK, 31.2 ± 5.32 pg/mg) or an isotype control Ab (EAM + isotype ctrl, 25.4 ± 1.87 pg/mg). To determine P values, a Mann–Whitney rank sum test was performed for a, and an unpaired Student's t test was used for c and d. n.s., not significant. *, $P < 0.05$. Data are presented as mean ± SEM.
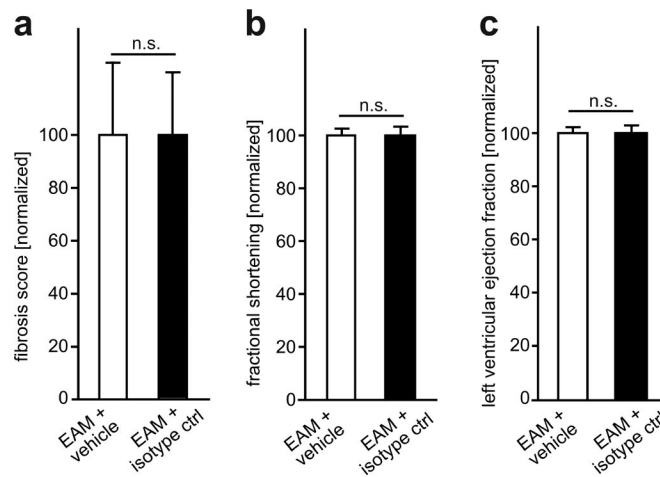
Figure S3. IgG1 control Abs do not affect EAM. (a) Degree of fibrosis in the cardiac tissue as analyzed histologically using a semiquantitative score on day 63 after induction of EAM. Mice were treated with IgG1 isotype Ab from day 1 until day 21 of EAM (EAM + isotype ctrl; 100.0 ± 27.5 [%]) or left untreated for control (EAM; 99.2 ± 23.4 [%]). n = 25 for both groups. **(b and c)** Echocardiographic analysis of left ventricular systolic function on day 63 after induction of EAM. Mice were treated with IgG1 isotype Ab from day 1 until day 21 of EAM (EAM + isotype ctrl) or left untreated for control (EAM). As indicators for systolic function, (b) fractional shortening (EAM + vehicle, 100.0 ± 2.0 [%]; EAM + isotype ctrl, 101.0 ± 2.9 [%]) and (c) left ventricular ejection fraction (EAM + vehicle, 100.0 ± 1.0 [%]; EAM + isotype ctrl, 101.0 ± 2.0 [%]) were determined. n = 25 for both groups. To determine P values, Mann–Whitney rank sum test was performed. n.s., not significant. Data are presented as mean ± SEM.
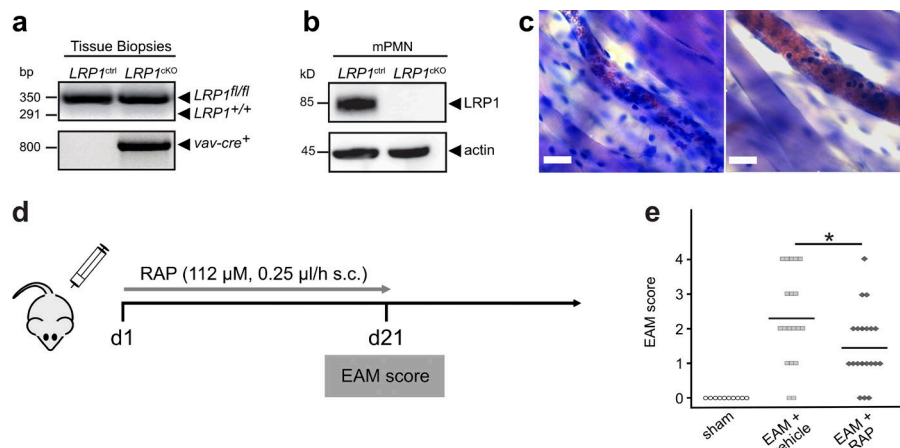

Figure S4. LRP1 is involved in leukocyte infiltration in vivo. (a) Genomic PCR of ear biopsies obtained from *LRP1*ctrl or *LRP1*cKO mice. **(b)** Western blot of BM-derived mPMN of *LRP1*ctrl or *LRP1*cKO mice showing LRP1 and actin expression. n = 3 (a and b). **(c)** Representative images of cremaster muscle whole mounts from *LRP1*ctrl (left) and *LRP1*cKO (right) mice 2 h after intrascrotal administration of TNFα. Whole mounts were stained with Giemsa. Bars, 20 µm. **(d)** RAP (112 µM, 0.25 µl/h) was continuously released from s.c. implanted miniosmotic pumps for 21 d upon induction of EAM. Leukocyte infiltration was subsequently analyzed. **(e)** EAM score at day 21 after induction of EAM or sham immunization (sham: mean EAM score, 0 ± 0). Mice were treated with RAP (EAM + RAP: mean EAM score, 1.45 ± 0.25) or vehicle (EAM + vehicle: mean EAM score, 2.32 ± 0.31). n = 10 for sham group; n = 19 for EAM + vehicle group; n = 20 for EAM + RAP group. To determine P values, a one-way ANOVA with a Holm–Sidak multiple comparisons test was performed. *, P < 0.05. Data represent mean ± individual data points.
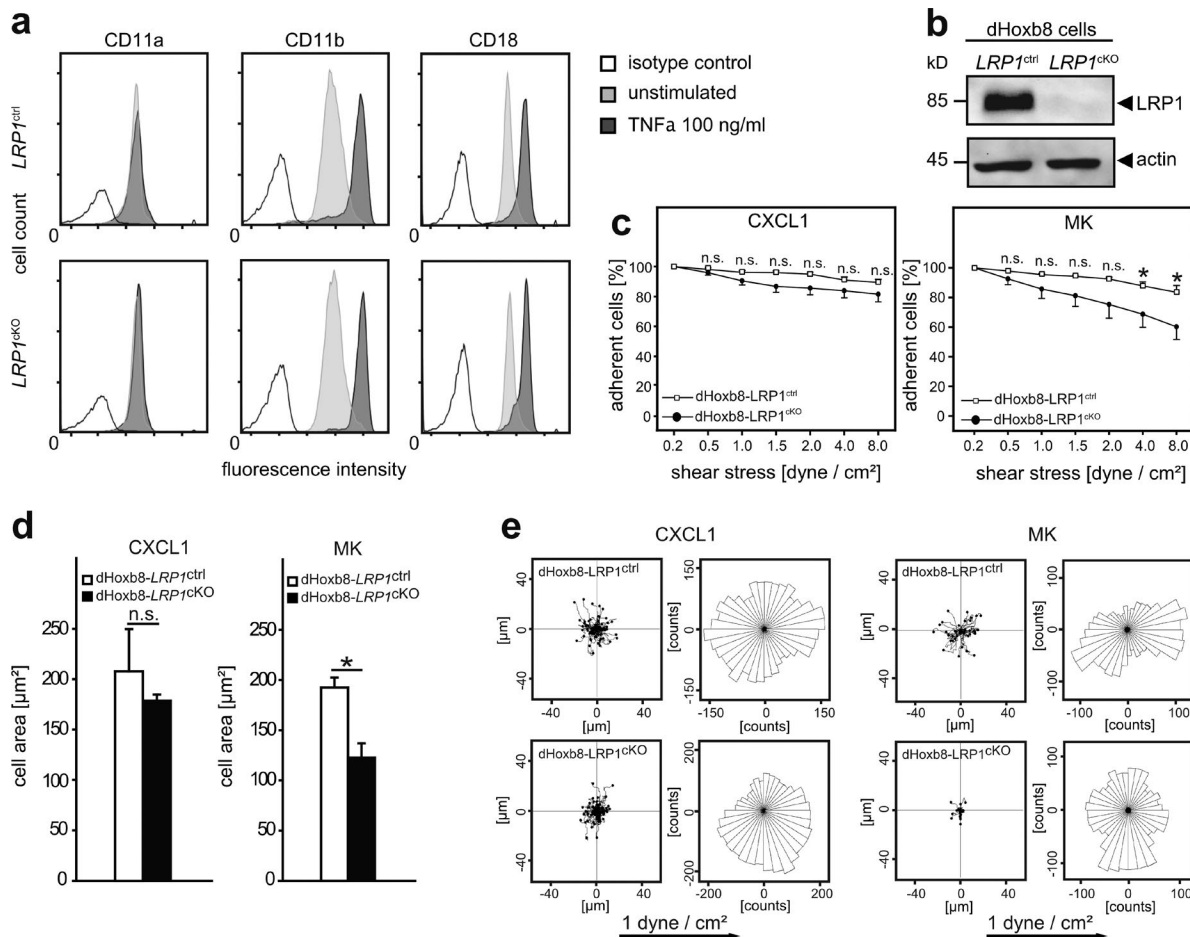
Figure S5. **The MK–LRP1 axis contributes to different PMN recruitment steps. (a)** Cell surface expression of CD11a, CD11b, or CD18 on isolated BM-derived PMNs of *LRP1*ctrl (top) or *LRP1*cKO mice (bottom) as analyzed by flow cytometry. Cells were stimulated for 20 min with TNFα (dark gray) or left untreated for control (light gray). Histograms are representative of three independent experiments. **(b)** Representative Western blot of LRP1 expression in dHoxb-*LRP1*ctrl and dHoxb-*LRP1*cKO cells. *n* = 3. **(c)** Adhesion strengthening of adherent dHoxb8-*LRP1*ctrl (white squares) or dHoxb8-*LRP1*cKO cells (black circles) in the presence of immobilized P-selectin, ICAM-1, and CXCL1 (left, at 8 dyne: dHoxb8-*LRP1*ctrl, 89.4 ± 2.1 [%], dHoxb8-*LRP1*cKO, 81.5 ± 5.1 [%]) or MK (right, at 8 dyne: dHoxb8-*LRP1*ctrl, 84.0 ± 4.1 [%], dHoxb8-*LRP1*cKO, 60.2 ± 8.5 [%]) under increasing shear stress (0.2–8.0 dyne/cm²). Adhesion strengthening was measured as the percentage of adherent cells before the onset of shear stress (100%) at indicated time points. *n* = 4. **(d)** Spreading of dHoxb8-*LRP1*ctrl or dHoxb8-*LRP1*cKO cells on immobilized P-selectin, ICAM-1, and CXCL1 (left, dHoxb8-*LRP1*ctrl, 200.6 ± 48.9 μm², dHoxb8-*LRP1*cKO, 178.5 ± 6.2 μm²) or MK (right, dHoxb8-LRP1ctrl, 192.1 ± 9.9 μm², dHoxb8-LRP1cKO, 122.1 ± 14.4 μm²) after 10 min of flow (1 dyne/cm²). Spreading was analyzed by measuring the cell area. *n* = 3. **(e)** Mechanotactic migration of dHoxb8-*LRP1*ctrl (top) or dHoxb8-*LRP1*cKO cells (bottom) on immobilized P-selectin, ICAM-1, and CXCL1 (left) or MK (right) under flow conditions (1 dyne/cm²). Single-cell migration tracks as well as rose plots are shown. *n* = 3. To determine P values, Mann–Whitney rank-sum test was performed for c, and an unpaired Student's *t* test was used for d. *, P < 0.05. n.s., not significant. Data are presented as mean ± SEM.
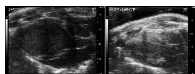

Video 1. **Role of MK for systolic function in EAM.** Short axis parasternal view at day 63 after induction of EAM. Mice were left untreated for control (EAM) or treated with a MK-blocking Ab for 21 d (EAM + anti-N-MK). This video is shown at 25 frames/s.
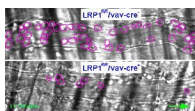

Video 2. **Impact of LRP1 on leukocyte rolling and adhesion in vivo.** Time-lapse transmission light video microscopy of leukocyte rolling and adhesion in postcapillary venules of *LRP1*ctrl (top) and *LRP1*cKO (bottom) mice 2 h after intrascrotal application of TNFα. Arrow indicates direction of flow. This video is shown at 10 frames/s.
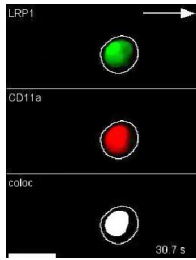
Video 3. **Expression of LRP1 and CD11a during rolling and adhesion.** Time-lapse spinning-disk confocal microscopy showing LRP1 (green), CD11a (red) and the colocalization (white) on murine WT PMN in the presence of P-selectin, ICAM-1, and CXCL1 during rolling and induction of adhesion under flow conditions (1 dyne/cm^2). Bar, 10 µm. This video is shown at 28 frames/s.
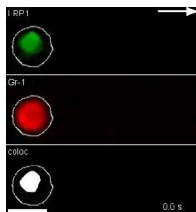
Video 4. **Expression of LRP1 and Gr-1 during rolling and adhesion.** Time-lapse spinning-disk confocal microscopy showing LRP1 (green), Gr-1 (red), and the colocalization (white) on murine WT PMN in the presence of P-selectin, ICAM-1, and CXCL1 during rolling and induction of adhesion under flow conditions (1 dyne/cm^2). Bar, 10 µm. This video is shown at 30 frames/s.
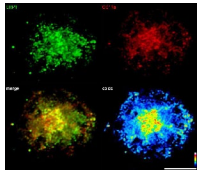
Video 5. **Colocalization of LRP1 and CD11a in adherent dHoxb8 cells.** STED nanoscopy of adherent dHoxb8-_LRP1_ctrl cells showing expression of LRP1 (green), CD11a (red), merge (yellow), and colocalization (pseudocolors) under flow conditions (1 dyne/cm^2) in microflow chambers coated with P-selectin, ICAM-1, and MK. Arrows indicate adhesion site. Bar, 10 µm. This video is shown at 10 frames/s.
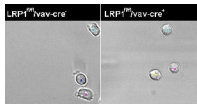
Video 6. **Role of LRP1 for MK-mediated crawling under flow conditions.** Time-lapse transmission light video microscopy of dHoxb8-_LRP1_ctrl and dHoxb8-_LRP1_cKO cells under flow conditions (1 dyne/cm^2) in microflow chambers coated with P-selectin, ICAM-1, and MK. Arrows indicate direction of flow. This video is shown at 5 frames/s.