SUPPLEMENTAL MATERIAL

Supplemental Methods

Reagents. Recombinant mouse vascular endothelial growth factor A (VEGFA) and interleukin 1β (IL-1β) were purchased from R&D Systems (catalog numbers [#] 493-MV and #401-ML, respectively), the C-X-C motif chemokine receptor 4 (CXCR4) antagonist AMD3100 (#3299) and the combined CXCR4 antagonist and C-X-C motif chemokine receptor 7 (CXCR7) agonist TC14012 (#4300) from Tocris Bioscience.

Beta-arrestin recruitment assay. Beta-arrestin recruitment was measured in CHO-K1 cells expressing human CXCR7 tagged with the ProLink peptide (PathHunter CXCR7 β-arrestin assay, DiscoverX). CHO-K1 cells were incubated with POL5551 or the CXCR7 ligands C-X-C motif chemokine ligand 12 (CXCL12) (R&D Systems, #350-NS) or C-X-C motif chemokine ligand 11 (CXCL11) (R&D Systems, #672-IT) for 90 min at 37 °C before washing, adding the detection reagents, and measuring the chemiluminescent signal according to the manufacturer's instructions.

Mouse strains. DEREG (C57BL/6-Tg(Foxp3-DTR/EGFP)23.2Spar/Mmjax),[1] *Rag1* knockout (B6.129S7-*Rag1*^{tm1Mom}/J),[2] CD11c-Cre (B6.Cg-Tg(Itgax-cre)1-1Reiz/J),[3] iDTR (C57BL/6-Gt(ROSA)26Sortm1(HBEGF)Awai/J),[4] and Tie2-GFP mice (Tg(TIE2GFP)287Sato/J)[5] have previously been described.

Mouse model of reperfused acute MI. All surgical procedures in mice were approved by the

authorities in Hannover, Germany (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit). Mice were housed in individually ventilated cages in a 12 h light/dark cycle in the central animal facility of Hannover Medical School. Food and water were provided *ad libitum*. MI was induced in 8–10-week-old male mice by transient left anterior descending coronary artery (LAD) ligation. Mice were subcutaneously (s.c.) pretreated with 0.02 mg/kg atropine (B. Braun) and 2 mg/kg butorphanol (Pfizer). Anesthesia was induced with 3–4% isoflurane (Baxter) in an induction chamber. After oral intubation, mice were mechanically ventilated (Harvard Apparatus, MiniVent Type 845; respiration rate, 180/minute; tidal volume, 200 μL), and anesthesia was maintained with 1.5–2% isoflurane. Mice were placed on a heating pad connected to a temperature controller (Föhr Medical Instruments) to keep rectal temperature at 37 °C during surgery. A left thoracotomy was performed, and the LAD was ligated by a Prolene 7-0 (Ethicon) suture against a PE-10 tube (ischemia). The tube was removed 1 h later, and reperfusion was visually confirmed by light microscopy. In sham-operated control mice, the ligature around the LAD was not tied.

Echocardiography. High-resolution 2-dimensional transthoracic echocardiography was performed with a linear 30 MHz transducer (Vevo 770, VisualSonics) in mice sedated with 0.5–1% isoflurane via face mask. LV end-diastolic area (LVEDA) and LV end-systolic area (LVESA) were recorded from the long-axis parasternal view. Fractional area change (%) was calculated as [(LVEDA – LVESA) / LVEDA] × 100.

Invasive LV pressure-volume measurements. Mice were s.c. injected with 2 mg/kg butorphanol.

Anesthesia was induced with 4% isoflurane. After oral intubation, mice were intraperitoneally injected with 0.8 mg/kg pancuronium (Actavis), and anesthesia was maintained with 2% isoflurane. LV pressure-volume loops were recorded with a 1.4 F micromanometer-tipped conductance catheter inserted via the right carotid artery (SPR-839, Millar Instruments). Steady-state pressure-volume loops were sampled at a rate of 1 kHz and analyzed with LabChart 7 Pro software (ADInstruments).

Flow cytometry and FACS. After isolation, inflammatory cell suspensions were filtered (40 μm cell strainer, Falcon), washed, and incubated for 5 min at 4 °C in PBS with 4% FCS, 2 mmol/L EDTA, and a CD16/CD32 antibody (clone 2.4G2, mouse BD Fc Block, BD Biosciences) (dilution 1:100). Cells were then stained for 30 min at 4 °C with the following antibodies: CD45R/B220-PE (clone RA3-6B2) (1:500), CD90.2/Thy-1.2-PE (clone 53-2.1) (1:2,500), NK-1.1-PE (clone PK136) (1:500), CD49b/DX5-PE (clone DX5) (1:500), Ly6G-PE (clone 1A8) (1:500), I-Ab-biotin (clone AF6-120.1) (1:500), CD11b-APC (clone M1/70) (1:500), CD11c-biotin (clone HL3) (1:500), Ly6C-FITC (clone AL-21) (1:1,000), and PerCP streptavidin (1:500) from BD Biosciences; F4/80-biotin (clone Cl:A3-1) (1:500) from Bio-Rad; and CD45-Brilliant Violet 570 (clone 30-F11) (1:250) from BioLegend. After washing, cells were added to TruCOUNT tubes (BD Biosciences) and counted on an LSR II flow cytometer (Becton Dickinson) using FACSDiva software (BD Biosciences). Ly6C^{high} monocytes were identified as CD45^{high} CD11b^{high} (CD45R/B220, CD90.2/Thy-1.2, NK-1.1, CD49b/DX5, Ly6G)^{low} (CD11c, F4/80, I-Ab)^{low} Ly6C^{high}, Ly6C^{low} monocytes or macrophages as CD45^{high} CD11b^{high} (CD45R/B220, CD90.2/Thy-1.2, NK-1.1, CD49b/DX5, Ly6G)^{low} (CD11c, F4/80, I-Ab)$^{high\ or\ low}$ Ly6C^{low}, neutrophils as CD45^{high} CD11b^{high} (CD45R/B220, CD90.2/Thy-1.2, NK-1.1,

CD49b/DX5, Ly6G)^{high}, and lymphocytes as CD45^{high} CD11b^{low} (CD45R/B220, CD90.2/Thy-1.2, NK-1.1, CD49b/DX5, Ly6G)^{high}. Conventional and plasmacytoid dendritic cell depletion in CD11c-Cre iDTR mice was confirmed with the following antibodies: CD45R/B220-PE (clone RA3-6B2) (1:200) from BD Biosciences, CD11c-APC-eFluor 780 (clone N418) (1:200) from eBiosciences, and Siglec H-FITC (clone eBio440c) (1:100) from eBiosciences. Data were analyzed with FlowJo (version 10). For Ly6C$^{low/high}$ monocyte and macrophage isolation, inflammatory cells from the infarcted region were incubated with labeled antibodies as described above. After washing, cells were sorted on a FACSAria IIu instrument (Becton Dickinson).[6,7]

Cell surface marker expression on CD4$^+$ Foxp3$^+$/eGFP$^+$ T_{reg} cells. Expression levels of CD62L (official name: lymphocyte selectin), CD69 (CD69 antigen), CD103 (epithelial-associated integrin alpha E), Ctla4 (cytotoxic T-lymphocyte-associated protein 4), Icos (inducible T cell co-stimulator), Klrg1 (killer cell lectin-like receptor subfamily G, member 1), PD-1 (programmed cell death 1), and Tnfrsf18 (tumor necrosis factor receptor superfamily, member 18) were quantified by flow cytometry using the following antibodies: CD62L (eBioscience, conjugate PE, clone MEL-14) (dilution 1:600), CD69 (BioLegend, PE, H1.2F3) (1:200), CD103 (eBioscience, PE, 2E7) (1:200), Ctla4 (eBioscience, PE, UC10-4B9) (1:100), Icos (eBioscience, PE, 7E.17G9) (1:100), Klrg1 (eBioscience, FITC, 2F1) (1:200), PD-1 (eBioscience, APC, RMP1-30) (1:100), and Tnfrsf18 (eBioscience, APC, DTA-1) (1:200).

Suppression assay. T_{reg} cell suppression assays were performed as previously described.[8] To generate antigen-presenting bone marrow-derived dendritic cells (BMDCs), bone marrow cells

were flushed from the femurs and tibias of CD45.2 mice and cultured for 7–8 d in RPMI 1640 GlutaMAX medium (Thermo Fisher Scientific) supplemented with 10% heat-inactivated FCS (Biochrom), penicillin, streptomycin, and 50 μmol/L β-mercaptoethanol in the presence of 5% culture supernatant of a granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing cell line. Naïve $CD4^+$ $CD25^-$ effector T (T_{eff}) cells were enriched from the spleens and lymph nodes of CD45.1 mice by negative magnetic selection using the Dynabeads untouched mouse CD4 cells kit (Thermo Fisher Scientific). $CD25^+$ T cells were depleted by including a functional-grade CD25 antibody (clone PC61.5, Thermo Fisher Scientific) in the antibody cocktail. After enrichment, cells were labeled using the CellTrace violet cell proliferation kit (Thermo Fisher Scientific). Splenic $CD4^+$ $Foxp3^+$/$eGFP^+$ T_{reg} cells were isolated by FACS from DEREG mice. T_{eff} cells (5×10^4 per well) were cultured with T_{reg} cells at different ratios in the presence of BMDCs (3×10^3 per well) and 1 μg/mL CD3ε antibody (clone 145-2C11, Bio X Cell) in 96-well round bottom plates (Greiner Bio-One). After 4 d, $CD4^+$ $CD45.1^+$ T_{eff} cell proliferation was analyzed by flow cytometry. Dead cells were excluded by the LIVE/DEAD fixable aqua dead cell stain kit (Thermo Fisher Scientific).

Quantitative reverse transcription- polymerase chain reaction (RT-qPCR). Total RNA was isolated from $Ly6C^{low/high}$ monocytes and macrophages with the PicoPure RNA isolation kit (Thermo Fisher Scientific) followed by reverse transcription into cDNA (SuperScript III reverse transcriptase, Thermo Fisher Scientific). The mRNA concentrations of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), CXCL12, tumor necrosis factor (TNF), IL-1β, interferon γ (IFN-γ), nitric oxide synthase 2 (NOS2), interleukin 10 (IL-10), and transforming growth factor

β1 (TGF-β1) were measured by qPCR using TaqMan gene expression assays and reagents from Thermo Fisher Scientific.

Tissue collection and histology. Mice were sacrificed at different time points after MI or sham operation, and the hearts were removed. Basal, midventricular, and apical slices of the left ventricle were embedded in OCT compound (Tissue-Tek) and frozen at –20 °C. 8-μm cryosections were prepared. Sections were stained with Masson's trichrome (Sigma-Aldrich), which shows fibrosis in blue on light microscopy. Scar area and total LV area were traced and scar size was calculated as the average ratio of scarred area to total LV area in basal, midventricular, and apical sections. Midventricular cryosections were stained with TRITC-conjugated wheat germ agglutinin (WGA, Vector Laboratories) to visualize cardiac myocyte borders and interstitial matrix and fluorescein-labeled GSL I isolectin B4 (IB4, Vector Laboratories) to visualize capillaries. Ki67 was detected with a rabbit polyclonal antibody (Abcam, #ab15580) (1:100) and a Cy3-labeled polyclonal secondary antibody (Jackson ImmunoResearch, #111-165-144) (1:200). To assess the number of perfused capillaries, Alexa Fluor 488-labeled GS IB4 (Thermo Fisher Scientific) was injected into the tail vein (50 μg per mouse). Mice were sacrificed 1 h later, and midventricular cryosections were stained with a rat CD31 monoclonal antibody (BD Biosciences, clone MEC 13.3) (1:100) and a Cy3-labeled polyclonal secondary antibody (Jackson ImmunoResearch, #712-165-153) (1:200). Images were acquired by fluorescence microscopy (Zeiss Axio Observer.Z1).[9]

Infarct tissue explants. Tie2-GFP mice were euthanized 24 h after MI, and the hearts were

removed. The infarcted region of the left ventricle was dissected into ~1-mm^3 tissue samples and cultured for 3 d in endothelial basal medium (Gibco, #10372019 supplemented with 2% FCS, L-glutamine, penicillin, and streptomycin) on growth factor-reduced Matrigel (Corning) in 48-well plates (1 sample per well). GFP^+ endothelial sprouts were imaged by fluorescence microscopy. Average sprout length was calculated from 8 sprouts per sample.[9]

Endothelial cell proliferation. Human coronary artery endothelial cells were purchased from Provitro and cultured as previously described.[9] Murine cardiac endothelial cells were isolated from the left ventricles of 7–9-day-old wild-type pups and cultured as previously described.[10] Cell proliferation was measured by a colorimetric BrdU incorporation immunoassay (Roche).[9]

Porcine closed-chest model of reperfused MI. All procedures in pigs were approved by the Animal Ethics Committee of the Hungarian National Food Chain Safety Office (approval number: 23.1./02322/009/2008). Domestic (DanBred hybrid) female pigs weighing 28.6 ± 0.4 kg were housed in GLP-accredited facilities at the University of Kaposvar, Hungary. Acute MI was induced by percutaneous balloon occlusion of the mid LAD. The day before the intervention, pigs were orally treated with 300 mg clopidogrel and 250 mg acetylsalicylic acid. Thereafter, pigs received 75 mg clopidogrel and 100 mg acetylsalicylic acid daily. After an overnight fast, pigs were intramuscularly injected with 12 mg/kg body weight ketamine hydrochloride, 1.0 mg/kg xylazine, and 0.04 mg/kg atropine. Anesthesia was induced by inhalation of 2–2.5% isoflurane via face mask. After endotracheal intubation, anesthesia was maintained by isoflurane (2.0–2.5 % in 100% O_2, 3 L/min). The right femoral artery was surgically prepared, a 7-French

arterial sheath was inserted, and unfractionated heparin (200 IU/kg) was injected. The ostium of the left coronary artery was intubated with a 7-French guide catheter under fluoroscopic control. A guide wire was placed into the distal LAD. A 2.75 × 8 mm Maverick PTCA balloon catheter (Boston Scientific) was advanced into the mid LAD and inflated with 4 atm distal to the origin of the second major diagonal branch. Coronary occlusion was confirmed by contrast media injection and ST-segment elevation on the surface ECG. $MgSO_4$ (20.3 mmol, every 60 min) and amiodarone (150 mg diluted in 250 mL saline) were administered during the procedure via an ear vein. After 90 min, the balloon was deflated and removed from the LAD. Coronary reperfusion was documented by angiography. The arterial sheath was then removed, the femoral artery was surgically closed, and the skin was sutured in two layers. Pigs remained connected to the ECG during the intervention and until 60 min after reperfusion to immediately defibrillate ventricular arrhythmias. After extubation, pigs received intramuscular injections of 1 g metamizole, 100 mg benzathine penicillin G, 100 mg procaine penicillin G, and 200 mg dihydrostreptomycin sulphate. 4, 6, 8, and 10 d after MI, pigs were again injected with ketamine hydrochloride, xylazine, and atropine. During isoflurane inhalation via face mask, the pigs then received infusions of POL6326 (3 mg/kg in 60 min) or vehicle via an ear vein.

Magnetic resonance imaging in pigs. 3 d (baseline) and 6 weeks after MI, LV volumes and infarct volume were determined by contrast-enhanced MRI. Anesthesia was induced and maintained as described above. MRI imaging was performed in supine position with a 1.5-T scanner (Siemens) with dedicated cardiac software, using ECG gating and a phased array receiver coil. LV volumes were measured by repeated breath-hold fast gradient echo sequences in a steady state. At first, sagittal and transversal images were obtained to locate the heart according to the

body's coordinate system. Dynamic images were then acquired orthogonal to the longitudinal heart axis from apex to base using ECG-gating, steady-state free precession (true FISP sequence) in short and long-axis views with 1.2 ms echo time (TE), 40 ms repetition time (TR), 25 phases, 50º flip angle, 360 mm field-of-view, 8 mm slice thickness, and a 256 × 256 image matrix. To determine infarct volumes, short and long axis end-diastolic images were obtained 15 min after i.v. application of 0.05 mmoL/kg gadobenate dimeglumine (MultiHance) extracellular contrast agent (Bracco Imaging) by using an inversion recovery-prepared, gradient-echo sequence. All images were evaluated using QMass MR 7.6 software (Medis Medical Imaging Systems) by a researcher blinded to treatment allocation. The endocardial and epicardial borders were traced in all end-diastolic and end-systolic short-axis slices to determine LV end-diastolic and end-systolic volumes (LVEDV and LVESV). LVEF (%) was calculated as [(LVEDV – LVESV) / LVEDV] × 100. LV myocardium showing late contrast enhancement was quantified to assess infarct volume.

Supplemental Table I

Left ventricular pressure-volume measurements in wild-type mice

	Sham	MI, control	MI, POL5551
Heart rate (min^{-1})	492 ± 16	466 ± 8	487 ± 7
LVEDP (mmHg)	9 ± 1	8 ± 1	8 ± 1
LVESP (mmHg)	99 ± 3	90 ± 3*	97 ± 2
LVEDV (μL)	38 ± 3	57 ± 3***	41 ± 3$^{\#\#\#}$
LVESV (μL)	13 ± 2	39 ± 3***	22 ± 3*$^{,\#\#\#}$
LVEF (%)	71 ± 4	37 ± 2***	53 ± 3**$^{,\#\#\#}$
dP/dt_{max} (mmHg/s)	10746 ± 569	6929 ± 378***	9834 ± 401$^{\#\#\#}$
dP/dt_{min} (mmHg/s)	-9079 ± 486	-6072 ± 243***	-7633 ± 148**$^{,\#\#\#}$
τ (ms)	7.2 ± 0.4	9.3 ± 0.3***	7.6 ± 0.3$^{\#\#\#}$
Cardiac output (mL/min)	12.8 ± 1.0	9.0 ± 0.4***	10.3 ± 0.7
Stroke work (mmHg × μL)	2149 ± 103	1312 ± 60***	1671 ± 139*$^{,\#}$

Myocardial infarction (MI) was induced in wild-type mice. Additional mice underwent sham surgery (8 mice). POL5551 (14 mice) or vehicle only (control, 12 mice) were i.p. injected 2, 4, 6, and 8 d after MI. Left ventricular (LV) pressure-volume loops were recorded after 28 d. LVEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; LVEF, LV ejection fraction; dP/dt_{max}, maximum rate of pressure change in the left ventricle; dP/dt_{min}, minimum rate of pressure change in the left ventricle. *P<0.05, **P<0.01, ***P<0.001 vs. sham; $^{\#}$P<0.05, $^{\#\#\#}$P<0.001 vs. MI, control (2-way ANOVA with Tukey post hoc test).

Supplemental Table II

Left ventricular pressure-volume measurements in splenectomized mice

	Sham	MI, SPX, control	MI, SPX, POL5551
Heart rate (min^{-1})	485 ± 15	478 ± 12	473 ± 8
LVEDP (mmHg)	9 ± 0	8 ± 1	10 ± 1
LVESP (mmHg)	101 ± 2	93 ± 4	93 ± 3
LVEDV (μL)	39 ± 3	50 ± 4	51 ± 5
LVESV (μL)	14 ± 2	35 ± 4**	36 ± 5**
LVEF (%)	67 ± 2	39 ± 3***	38 ± 3***
dP/dt_{max} (mmHg/s)	10499 ± 554	7467 ± 290***	7782 ± 396***
dP/dt_{min} (mmHg/s)	-9359 ± 428	-6360 ± 305***	-6272 ± 384***
τ (ms)	7.4 ± 0.3	9.0 ± 0.5*	9.5 ± 0.6*
Cardiac output (mL/min)	12.4 ± 1.0	8.9 ± 0.8*	8.5 ± 0.6**
Stroke work (mmHg × μL)	2172 ± 108	1250 ± 96***	1322 ± 101***

Myocardial infarction (MI) was induced in wild-type mice. Additional mice underwent sham surgery (7 mice). POL5551 (14 mice) or vehicle only (control, 11 mice) were i.p. injected 2, 4, 6, and 8 d after MI. Infarcted mice underwent splenectomy (SPX) immediately before the first injection. Left ventricular (LV) pressure-volume loops were recorded after 28 d. LVEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; LVEF, LV ejection fraction; dP/dt_{max}, maximum rate of pressure change in the left ventricle; dP/dt_{min}, minimum rate of pressure change in the left ventricle. *P<0.05, **P<0.01, ***P<0.001 vs. sham (2-way ANOVA with Tukey post hoc test).

Supplemental Table III

Left ventricular pressure-volume measurements in splenectomized mice treated with splenic mononuclear cells

	Mice that received MNCs from vehicle only-treated, infarcted donors	Mice that received MNCs from POL5551-treated, infarcted donors
Heart rate (min^{-1})	476 ± 10	493 ± 7
LVEDP (mmHg)	9 ± 1	7 ± 1
LVESP (mmHg)	92 ± 3	100 ± 3
LVEDV (μL)	69 ± 4	60 ± 2
LVESV (μL)	51 ± 4	34 ± 2**
LVEF (%)	33 ± 3	52 ± 3***
dP/dt_{max} (mmHg/s)	7623 ± 424	8926 ± 482*
dP/dt_{min} (mmHg/s)	-6414 ± 327	-7500 ± 296*
τ (ms)	9.0 ± 0.3	7.9 ± 0.3*
Cardiac output (mL/min)	10.9 ± 0.9	14.9 ± 0.9**
Stroke work (mmHg × μL)	1433 ± 137	2275 ± 186**

Myocardial infarction (MI) was induced in wild-type donor and recipient mice. 2 d after MI, donor mice were i.p. injected with POL5551 or vehicle only (control) and splenic mononuclear cells (MNCs) were isolated 24 h later. 2 d after MI, recipient mice were splenectomized and then i.v. infused with 17×10^6 donor MNCs. Left ventricular (LV) pressure-volume loops were recorded in recipient mice after 28 d. 10 recipients of MNCs from vehicle only-treated donors, 8 recipients of MNCs from POL5551-treated donors. LVEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; LVEF, LV ejection fraction; dP/dt_{max}, maximum rate of pressure change in the left ventricle; dP/dt_{min}, minimum rate of pressure change in the left ventricle. *P<0.05, **P<0.01, ***P<0.001 (2-independent-sample t tests).

Supplemental Table IV

Left ventricular pressure-volume measurements in DEREG mice

	Saline		DT	
	MI, control	MI, POL5551	MI, control	MI, POL5551
Heart rate (min^{-1})	511 ± 4	509 ± 5	511 ± 2	504 ± 5
LVEDP (mmHg)	8 ± 2	8 ± 1	11 ± 2	10 ± 3
LVESP (mmHg)	87 ± 5	105 ± 4*	91 ± 4	90 ± 5
LVEDV (μL)	55 ± 4	54 ± 3	56 ± 5	59 ± 6
LVESV (μL)	36 ± 4	25 ± 2*	40 ± 4	43 ± 6
LVEF (%)	43 ± 3	63 ± 3***	37 ± 3	37 ± 5
dP/dt_{max} (mmHg/s)	7344 ± 582	11457 ± 508***	7172 ± 965	7715 ± 1076
dP/dt_{min} (mmHg/s)	-5700 ± 593	-8647 ± 379***	-5469 ± 527	-6099 ± 723
τ (ms)	10.0 ± 1.1	8.2 ± 0.5	10.9 ± 0.9	10.5 ± 1.7
Cardiac output (mL/min)	11.3 ± 0.9	16.3 ± 1.2**	10.0 ± 1.0	10.1 ± 1.1
Stroke work (mmHg × μL)	1321 ± 126	2617 ± 238***	1143 ± 203	1330 ± 214

Myocardial infarction (MI) was induced in DEREG mice. Diphtheria toxin (DT, 25 ng/g) or saline were i.p. injected immediately before and 24 h after MI. POL5551 or vehicle only (control) were i.p. injected 2, 4, 6, and 8 d after MI. 8–9 mice per group. Left ventricular (LV) pressure-volume loops were recorded after 28 d. LVEDP, LV end-diastolic pressure; LVESP, LV end-systolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; LVEF, LV ejection fraction; dP/dt_{max}, maximum rate of pressure change in the left ventricle; dP/dt_{min}, minimum rate of pressure change in the left ventricle. *P<0.05, **P<0.01, ***P<0.001 vs. corresponding saline- or DT-injected control mice (2-independent-sample t tests).

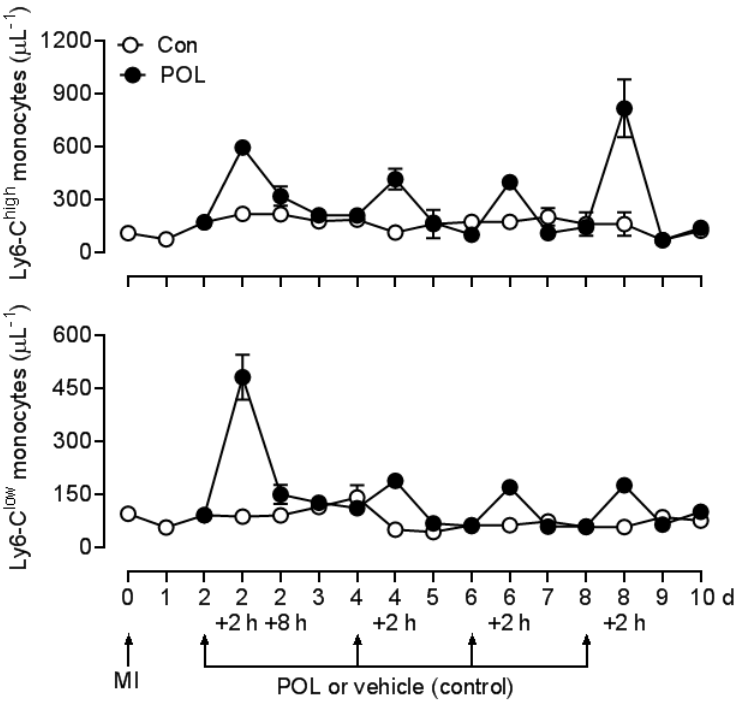
Supplemental Table V

T cell activation marker expression

	Control	POL5551
CD62L	39 ± 2	42 ± 2
CD103	15 ± 1	14 ± 1
Ctla4	37 ± 1	40 ± 2
Icos	8 ± 1	8 ± 1
Klrg1	5 ± 0	5 ± 0
PD-1	24 ± 0	24 ± 1
Tnfrsf18	100 ± 0	100 ± 0

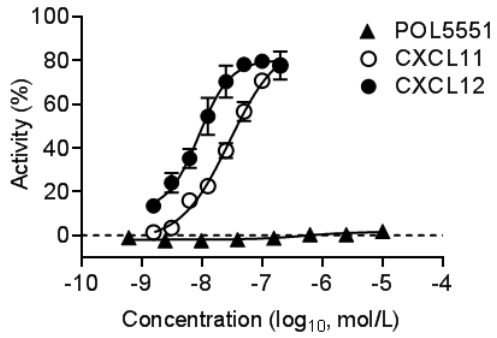
Myocardial infarction (MI) was induced in DEREG mice. 2 d after MI, mice were i.p. injected with POL5551 (6 mice) or vehicle only (control, 5 mice). Splenic $CD4^+$ $Foxp3^+$/$eGFP^+$ T_{reg} cells were isolated 24 h later. Expression (% of all cells) of CD62L (lymphocyte selectin), CD103 (epithelial-associated integrin alpha E), Ctla4 (cytotoxic T-lymphocyte-associated protein 4), Ctla4 (inducible T cell co-stimulator), Klrg1 (killer cell lectin-like receptor subfamily G, member 1), PD-1 (programmed cell death 1), and Tnfrsf18 (tumor necrosis factor receptor superfamily, member 18) was analyzed by flow cytometry.

Supplemental Figure I



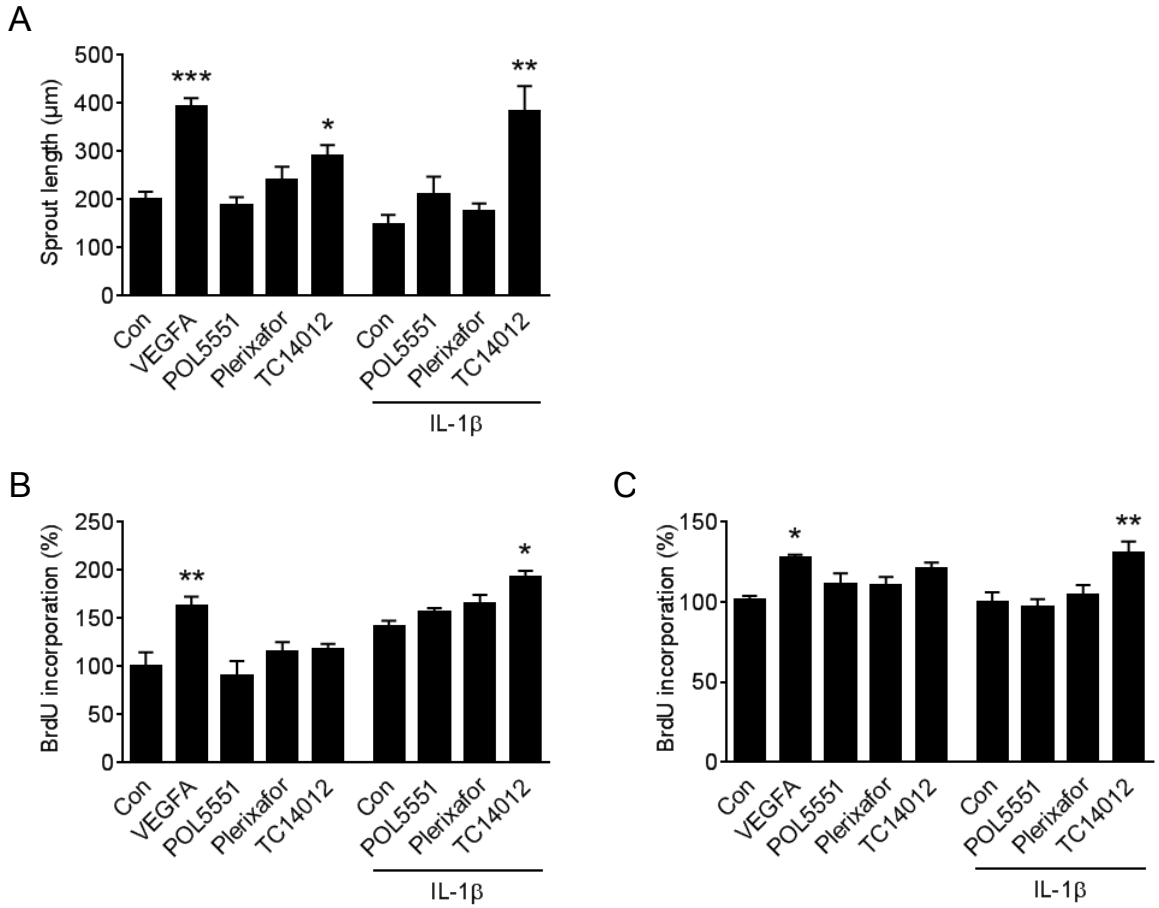
POL5551 mobilizes Ly6C^{high} and Ly6C^{low} monocytes. Myocardial infarction (MI) was induced in wild-type mice. POL5551 (POL) or vehicle only (control, con) were i.p. injected 2, 4, 6, and 8 d after MI. Ly6C^{high} and Ly6C^{low} monocyte numbers in the peripheral blood. 3–5 mice per time point.

Supplemental Figure II



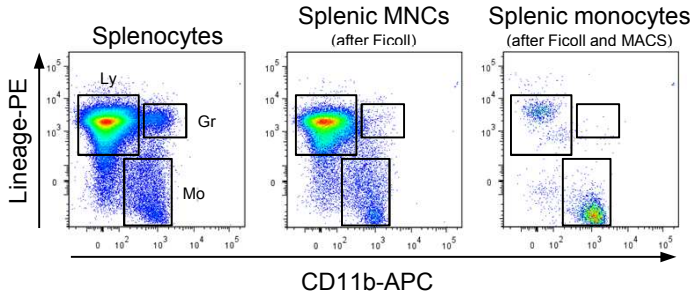
POL5551 does not bind to C-X-C motif chemokine receptor 7 (CXCR7). Beta-arrestin recruitment was measured in CHO-K1 cells expressing CXCR7. Cells were incubated with POL5551, C-X-C motif chemokine ligand 11 (CXCL11), or C-X-C motif chemokine ligand 12 (CXCL12).

Supplemental Figure III



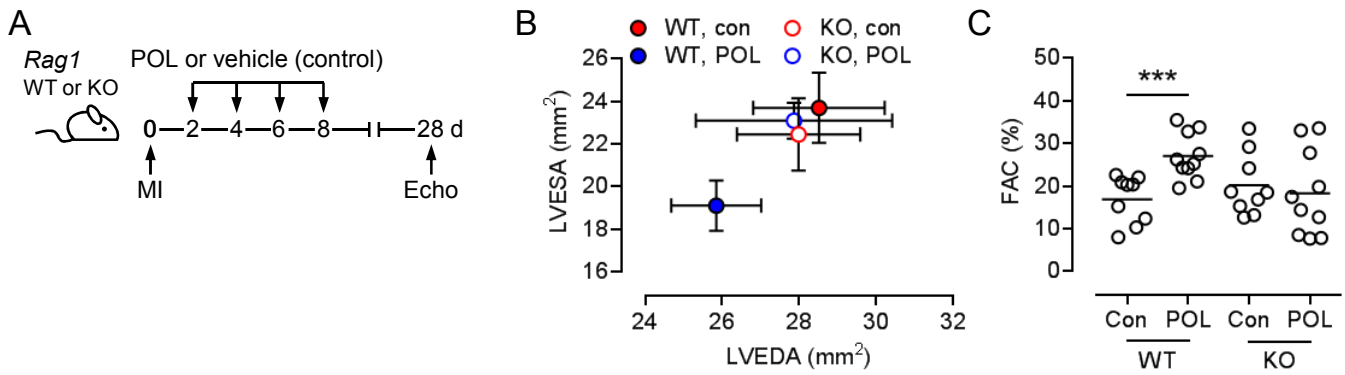
POL5551 does not promote direct angiogenic effects in endothelial cells in vitro. (A) Myocardial infarction was induced in Tie2-GFP mice. After 24 h, tissue samples from the infarcted region were removed and cultured for 3 d in the absence (control, con) or presence of vascular endothelial growth factor A (VEGFA, 50 ng/mL), POL5551 (3 μg/mL), plerixafor (3 μg/mL), TC14012 (100 ng/mL), and/or interleukin 1β (IL-1β, 10 ng/mL). The bar graph depicts average sprout length. Explants from 3–7 mice per group. (B) Mouse cardiac endothelial cells and (C) human coronary artery endothelial cells were stimulated for 16 h and cell proliferation was measured by BrdU incorporation. 3–4 independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. control (1-way ANOVA with Dunnett post hoc test). Consistent with a previous report,[11] the combined C-X-C motif chemokine receptor 4 (CXCR4) antagonist and C-X-C motif chemokine receptor 7 (CXCR7) agonist TC14012 promoted angiogenic effects, especially in the presence of IL-1β which has been shown to enhance CXCR7 expression.[11]

Supplemental Figure IV



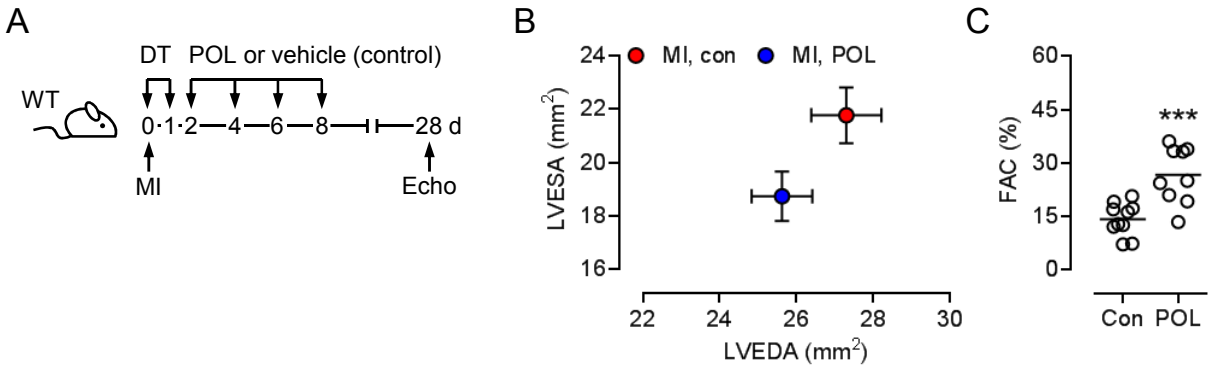
Characterization of donor splenocytes by flow cytometry. Splenocytes were isolated from a wild-type mouse 3 d after myocardial infarction. Mononuclear cells (MNCs) were isolated by Ficoll gradient separation. Monocytes were then enriched by magnetic cell labeling and separation (MACS). Lymphocyte (Ly), granulocyte (Gr), and monocyte (Mo) populations are highlighted.

Supplemental Figure V



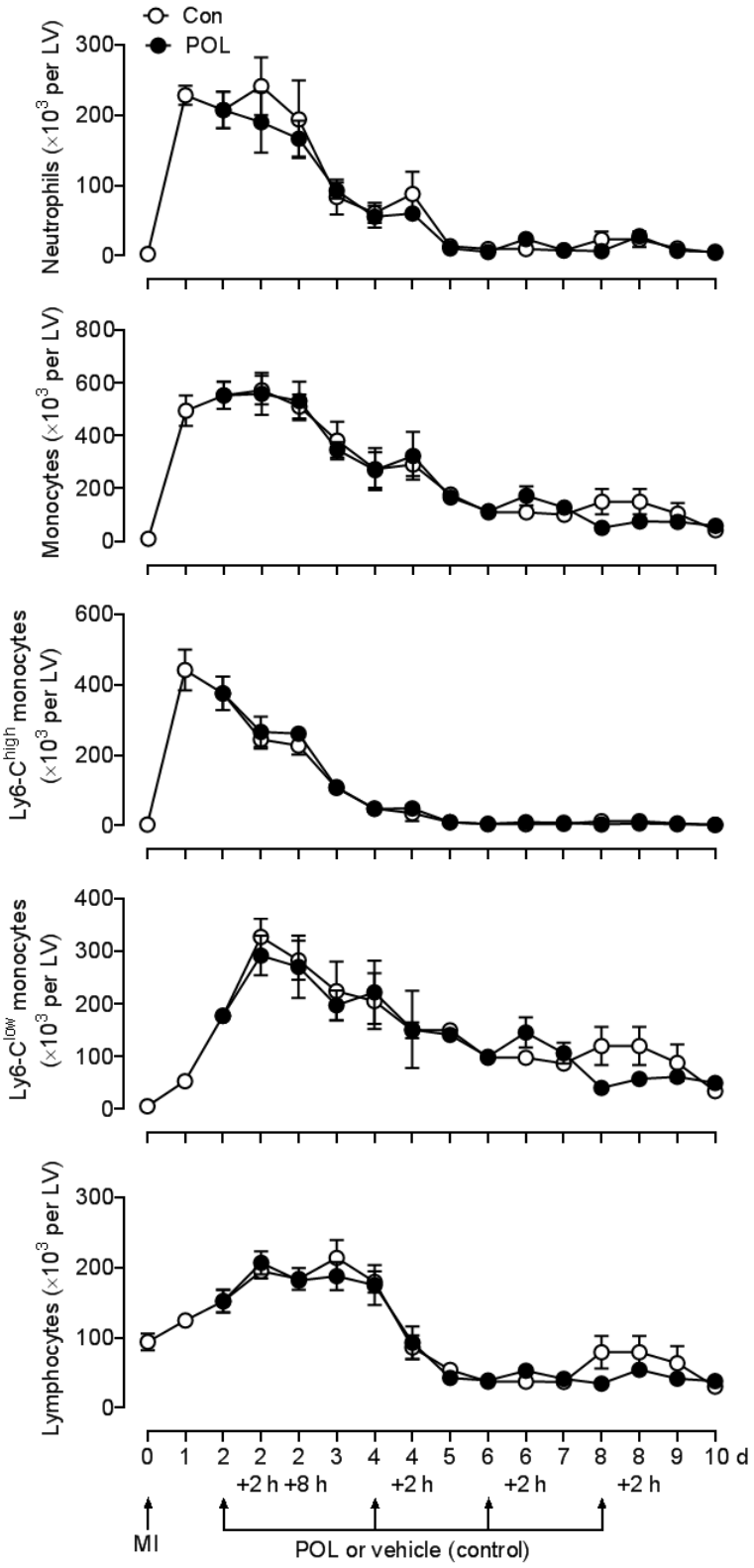
Importance of lymphocytes for the therapeutic effects of POL5551. (A) Experimental setup. Myocardial infarction (MI) was induced in *Rag1* wild-type (WT) or knockout (KO) mice. POL5551 (POL) or vehicle only (control, con) were i.p. injected 2, 4, 6, and 8 d after MI. 9–10 mice per group. (B) Left ventricular (LV) end-diastolic area (LVEDA) and LV end-systolic area (LVESA) as determined by echocardiography 28 d after MI. LVESA, $P<0.05$, vehicle only- vs. POL5551-treated WT mice (2-independent-sample t test). (C) Fractional area change (FAC). ***$P<0.001$ (2-independent-sample t test).

Supplemental Figure VI



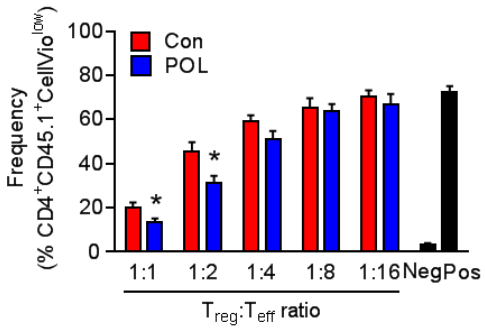
POL5551 treatment effects in diphtheria toxin-injected wild-type mice. (A) Experimental setup. Myocardial infarction (MI) was induced in wild-type (WT) mice. Diphtheria toxin (DT) was i.p. injected immediately before and 24 h after MI. POL5551 (POL) or vehicle only (control, con) were i.p. injected 2, 4, 6, and 8 d after MI. 9–10 mice per group. (B) LV end-diastolic area (LVEDA) and LV end-systolic area (LVESA) as determined by echocardiography 28 d after MI. LVESA: $P<0.05$, POL5551 vs. control (2-independent-sample t test). (C) Fractional area change (FAC). ***$P<0.001$ (2-independent-sample t test).

Supplemental Figure VII



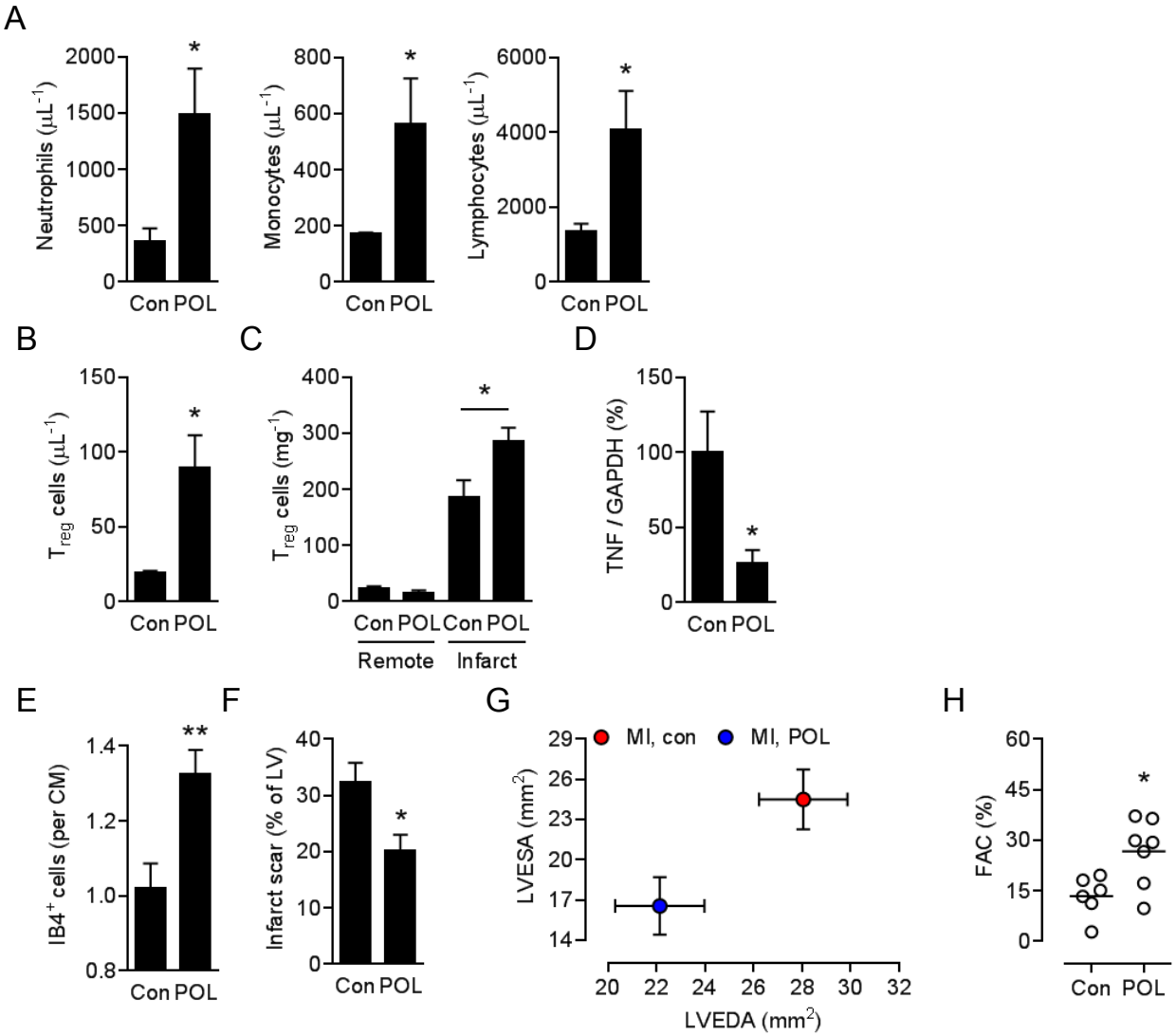
Inflammatory cell accumulation in the infarcted left ventricle. Myocardial infarction (MI) was induced in wild-type mice. POL5551 (POL) or vehicle only (control, con) were i.p. injected 2, 4, 6, and 8 d after MI. Neutrophil, monocyte (all, Ly6C^{high}, Ly6C^{low}), and lymphocyte numbers in the infarcted left ventricle (LV). 3–5 mice per time point.

Supplemental Figure VIII



T_{reg} cell suppression assay. DEREG mice were i.p. injected with POL5551 (POL) or vehicle only (con) 2 d after myocardial infarction. Splenic $CD4^+$ $Foxp3^+$/$eGFP^+$ regulatory T (T_{reg}) cells were isolated 24 h later and used in T_{reg} cell suppression assays. Positive control (Pos) shows effector T (T_{eff}) cell proliferation in the absence of T_{reg} cells; negative control (Neg) shows T_{eff} proliferation in the absence of the CD3ε antibody. Data from 3 independent experiments. *$P<0.05$ vs. same T_{reg}:T_{eff} cell ratio control groups (2-independent-sample t tests).

Supplemental Figure IX



POL6326 treatment effects in mice. (A) Myocardial infarction (MI) was induced in wild-type (WT) mice and POL6326 (POL) or vehicle only (control, con) were i.p. injected after 2 d. Neutrophil, monocyte, and lymphocyte numbers in the peripheral blood at 2 d + 2 h. 4 mice per group. (B) and (C) MI was induced in DEREG mice and POL6326 or vehicle only (con) were i.p. injected after 2 d. $CD4^+$ $Foxp3^+$/$eGFP^+$ regulatory T (T_{reg}) cell numbers (B) in the peripheral blood and (C) in the infarcted and noninfarcted (remote) region of the left ventricle at 2 d + 2 h. 4 mice per group. (D) Infarcted WT mice were i.p. injected with POL6326 or vehicle only (con) 2 d after MI. $Ly6C^{low/high}$ monocytes and macrophages were isolated from the infarcted region 2 d later, and tumor necrosis factor (TNF) expression was determined by RT-qPCR. 4 mice per group. (E) through (H) Infarcted WT mice were i.p. injected with POL6326 or vehicle only (con) 2, 4, 6, and 8 d after MI. (E) Fluorescein-labeled isolectin B4 $(IB4)^+$ capillary density in the infarct border-zone 28 d after MI. CM, cardiomyocyte. 5 mice per group. (F) through (H) 6 vehicle only- and 7 POL6326-treated mice. (F) Scar size 28 d after MI. (G) Left ventricular (LV) end-diastolic area (LVEDA) and LV end-systolic area (LVESA) as determined by echocardiography 28 d after MI. LVEDA: $P<0.05$; LVESA: $P<0.05$, control vs. POL6326 (2-independent-sample t tests). (H) Fractional area change (FAC). *$P<0.05$, **$P<0.01$ (2-independent-sample t tests).

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