Supplemental Material

Detailed Methods

Mice

All animal procedures were performed following the "Guide for the Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio. Adult C57BL/6J wild type (WT, n=76) and MMP-28$^{-/-}$ (n=86) mice of both sexes were used in this study. The MMP-28$^{-/-}$ mice were generated as described previously.[1] All of the MI mice were subjected to coronary artery ligation and were sacrificed at days 1, 3, 5, or 7 post-MI. Day 0 mice served as unoperated naïve controls.

MI Surgery

MI was induced by permanent occlusion of the left coronary artery, as previously described.[2, 3] Briefly, mice were anesthetized with 1-2% isoflurane in a 100% oxygen mix, intubated, and ventilated with a standard rodent ventilator (Harvard Apparatus). The animal was turned onto its right side, and the thoracic cavity was opened through an incision between the 3rd and 4th intercostal space. A rib spreader was introduced to allow for visualization of the heart. The left coronary artery was ligated with an 8-0 suture placed at 1-2 mm distal to left atrium. Infarction was confirmed by observing LV blanching along with ST segment elevation on the electrocardiogram. Immediately after surgery, a one-time injection of buprenorphine (0.1 mg/kg) was intraperitoneally administered.

Echocardiography

The mice were imaged at day 0 for the controls and at days 0, 1, 3, 5 or 7 for the MI mice. Mice were anesthetized with 1-2% isoflurane in a 100% oxygen mix. Transthoracic echocardiography was performed using the Vevo 770 system (VisualSonics). All images were acquired at heart rates >400 bpm to achieve physiologically relevant measurements.[4] Electrocardiogram and heart rate were monitored throughout the imaging procedure. Measurements were taken from the LV parasternal long axis B- and M-mode views. For each parameter, images from 3 cardiac cycles were measured and averaged. The LV remodeling index was calculated as the ratio of end diastolic volume (EDV) to LV mass.[3]

Survival Analysis and Autopsy

The mice were checked daily for the survival analysis. At autopsy, cardiac rupture was confirmed if there were blood clots in the thoracic cavity and the LV rupture site was seen.

Tissue Harvest and Infarct Area Evaluation

At each sacrifice time point, mice were anesthetized with 2% isoflurane in a 100% oxygen mix. The mice were injected with heparin (i.p., 4 USP Units per gram body weight), and 5 min later blood was collected from the common carotid artery and centrifuged for the collection of plasma. The heart was flushed with cardioplegic solution to arrest the heart in diastole and resected as described before.[4] The LV and right ventricle (RV) were separated and weighed individually. The LV was sliced into apex, middle, and base pieces, and stained with 1% 2, 3, 5-triphenyltetrazolum chloride (TTC, Sigma, T8877) for evaluation of infarct area.[3] Infarct area was presented as the percentage of infarct area to total LV area using Photoshop (Adobe). The infarct (LVI) and remote (LVC) regions from the apex and base were isolated, snap frozen in

separate tubes, and stored at – 80°C for biochemical analysis. The middle section was fixed in 10% zinc formalin for histological examination. The lung weight and tibia length were measured and the ratio of lung weight to tibia length (edema index) was calculated.

Protein Extraction and Immunoblotting

Total protein was extracted by homogenizing the samples in protein extraction reagent type 4 (Sigma; 7 M urea, 2 M thiourea, 40 mM Trizma® base, and the detergent 1% C7BzO) and 1x protease inhibitor cocktail (Roche). Protein concentrations were determined by the Quick Start™ Bradford Protein Assay (Bio-Rad) using a 1:40 dilution of protein extract to dilute the urea to a compatible level. Protein expression levels were quantified using the following antibodies: MMP-28 (Sigma, M5066, 1:1000), galectin-3 (R&D, AF1197, 1:1000), collagen type I (Cedarlane, CL50141AP, 1:1000), collagen type III (Cedarlane, CL50341AP, 1:1000), lysyl oxidase (Novus, NB110-41568, 1:2000), and α-smooth muscle actin (SMA, Sigma, A2547, 1:1000).

Total protein (10 μg) for all samples was separated on 4-12% Criterion™ XT Bis-Tris gels (Bio-Rad), transferred to nitrocellulose membrane (Bio-Rad), and stained with MemCode™ Reversible Protein Stain Kit (Thermo Scientific) to verify protein concentration and loading accuracy. After blocking with 5% nonfat milk (Bio-Rad), the membrane was incubated overnight at 4°C with primary antibody, followed by incubation with the secondary antibody (Vector Laboratories) and detection using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientifics).[4] ImageJ was used to measure the densitometry of the entire lane, and the densitometry of the total protein was used as the internal loading control for each lane. The results were quantified as the densitometry of the protein of interest divided by the densitometry of the total protein for that lane.

Immunofluorescence

For immunofluorescence staining, paraffin-embedded 5 μm sections were deparaffinized in citrisolve and rehydrated through graded ethanol. Heat mediated antigen retrieval was performed to expose antigen epitopes (Target Retrieval Solution, Dako, S1699). Sections, blocked with normal horse serum, were incubated with rabbit anti-mouse MMP-28 (Sigma, M5066, 1:100), rat anti-mouse Mac-3 (Cedarlane, CL8493AP, 1:100), or monoclonal anti-mouse α-SMA (Sigma, A2547, 1:100). Secondary antibodies used included Alexa Fluor 488 goat anti-rabbit (Invitrogen, A11008, 1:100), Alexa Fluor 647 chicken anti-rat (Invitrogen, A21472, 1:200), Alexa Fluor 647 donkey anti-rabbit (Invitrogen, A31573, 1:200), and Alexa Fluor 546 donkey anti-mouse antibodies (Invitrogen, A10036, 1:100). Alexa Fluor 546 phalloidin (Invitrogen, A22283, 1:20) was used to stain myocytes. Nuclei were stained with DRAQ5 (CST, #4084, 1:800) or DAPI (Vector Laboratories, H-1200). Co-localization was performed using either an antibody for MMP-28 and phalloidin or antibodies for MMP-28 and Mac-3 to determine the cellular localization of MMP-28.[5] The α-SMA antibody was used to visualize myofibroblasts in the infarct regions at day 7 post-MI or isolated cardiac fibroblasts stimulated with TGF-β1.

Plasma Proteomic Profiling

Plasma samples (80 μL) were analyzed using the Rodent Multi-Analyte Profiling (MAP) version 2.0 (Myriad Rules-Based Medicine, Austin, TX). Concentrations of 58 analytes were measured by CLIA-certified biomarker testing laboratory using reproducible, quantitative, and multiplexed immunoassays.[6] For the plasma TNF-α time course data, values below detection were assigned a 0 value.

Real Time RT^2-PCR

RNA extraction was performed using TRIzol® Reagent (Invitrogen, 15596) according to the manufacturer's instruction. RNA levels were quantified using the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). Reverse transcription of equal RNA content (0.5 μg) was performed using the RT² First Strand Kit (Qiagen, 330401). Real Time RT^2-PCR gene array for Inflammatory Cytokines and Receptors (Qiagen, PAMM-011A) and for Extracellular Matrix and Adhesion Molecules (Qiagen, PAMM-013A) were performed to quantify gene expression levels.[4] The gene levels were normalized to the reference gene hprt1 and the data were reported as $2^{-\Delta Ct}$ values x 100±SEM.

Immunohistochemistry

As described previously, macrophage staining was performed by using a specific antibody against Mac-3 at a dilution of 1:100.[4] Quantification was calculated as the percentage of positively stained area to total area. Ten random scans per section were analyzed and averaged.

LV Macrophage Phenotype Determination

RNA was extracted from day 7 post-MI infarct regions of WT and MMP-28$^{-/-}$ mice. Quantitative real time-PCR (qRT-PCR) was performed using Taqman gene expression assays (Applied Biosystems). IL-6 (Mm00446190_m1), Arg1 (Mm00475988_m1), CD163 (Mm00474091_m1), MRC1 (Mm00485148_m1), and Ym1 (Mm00657889_mH) markers were evaluated. The gene levels were normalized to the reference gene hprt (Mm01545399_m1) and expressed as $2^{-\Delta Ct}$ values x 100±SEM. Ccl3, IFN-γ, IL-1β, TNF-α, and TGF-β1 results were from the day 7 inflammatory array analysis.

Peritoneal Macrophage Isolation and Stimulation

Peritoneal macrophages were isolated from WT and MMP-28$^{-/-}$ mice as previously described.[7] Briefly, the peritoneal cavity was lavaged twice with 10 mL of ice-cold RPMI 1640 media (Gibco) with 10% fetal bovine serum (FBS) and 1% antibiotics. The recovered media were centrifuged at 250 x g for 10 min. The cell pellet was resuspended in 6 mL of RPMI 1640 media. The cells were plated in a 6-well plate ($1x10^6$ cells/ well), incubated at 37°C overnight to allow the cells to adhere, and washed with fresh media to remove unattached cells.

The macrophages were stimulated with interferon (IFN)-γ (20 ng/mL, R&D, 485-MI) and lipopolysaccharide (LPS, 1 μg/mL, Sigma, L 2880) for 4 h to prime the cells to an M1 phenotype, or were stimulated with IL-4 (20 ng/mL, R&D, 404-ML) for 4 h to prime the cells to an M2 phenotype.[8] The cells were incubated with fresh media for the unstimulated negative controls. The cells were harvested for RNA isolation and qRT-PCR. MMP-28 (Mm00712992_m1) in macrophages with different stimuli was measured. For M1 macrophage activation, ccl3 (Mm00441259_g1), IL-1β (Mm01336189_m1), IL-6 (Mm00446190_m1), and TNF-α (Mm00443258_m1) were assessed. For M2 macrophage activation, Arg1, Fizz1 (Mm00445109_m1), MRC1, and Ym1 were assessed.[9] The gene levels were normalized to hprt and expressed as $2^{-\Delta Ct}$ values±SEM for M1 and M2 markers or $2^{-\Delta Ct}$ values x 1000±SEM for MMP-28.

Cardiac Fibroblast Isolation and Stimulation

Cardiac fibroblasts from WT and MMP-28$^{-/-}$ mice were isolated by enzymatic digestion with 600 U/mL collagenase II (Worthington Biochemical, CLS-2) and 60 U/mL DNase I (AppliChem,

A3778). Cells at passage 2 were plated in 6-well plates ($5x10^4$ cells/well), allowed to attach at 37°C overnight, and washed with DMEM/F12 media with 10% FBS and 1% antibiotics to remove unattached cells. Cells were serum starved for 24 h and subsequently stimulated with 10 ng/mL TGF-β1 (Sigma, T7039) for 24 h. One set of cells was incubated with fresh media as the negative control. Cells were collected, total RNA extracted, and the gene expression of α-SMA (Mm00725412_s1), fibronectin 1 (Mm01256744_m1), and connective tissue growth factor (CTGF, Mm01192932_g1) were measured by qRT-PCR. The gene levels were normalized to the hprt and expressed as $2^{-\Delta Ct}$ values±SEM.

Collagen Cross-linking

Collagen cross-linking in day 7 infarct regions of WT and MMP-$28^{-/-}$ mice was evaluated by measuring hydroxylysyl pyridinoline and lysyl pyridinoline. Briefly, infarct tissue was lysed with reagent 4 and the protein concentrations were determined by Quick Start™ Bradford Protein Assay (Bio-Rad). The enzyme-linked immunosorbent assay was performed to determine the concentration of hydroxylysyl pyridinoline (ABIN809022) and lysyl pyridinoline (ABIN773391) following the manufacturer's instruction.

Tensile Strength Measurement

At day 3 post-MI, male mice were sacrificed and the LV was stained with 1% TTC. A ring was sectioned at the LV mid cavity and an incision was made through the remote region to open the ring. The tissue was mounted uniaxially on a Biotester (CellScale, Canada) and stretched in the circumferential direction until failure. Deformation was measured by image tracking analysis. The ultimate cauchy stress was determined from the deformation of the tissue and the measured axial forces as described by Fomovsky and colleagues.[10]

RT-PCR for Foxp3 and PTEN

RNA was extracted from day 7 post-MI infarct regions of WT and MMP-$28^{-/-}$ mice. The expression of Foxp3 (Mm00475162_m1) and PTEN (Mm00477208_m1) were evaluated using Taqman gene expression assays. The gene levels were normalized to the hprt, and expressed as $2^{-\Delta Ct}$ values x 100±SEM for Foxp3 or $2^{-\Delta Ct}$ values±SEM for PTEN.

Statistical Analyses

Performed as described in the manuscript.

Online Tables

Online Table I. Sampling and timing for the individual measurements.

Parameter	Time points (days post-MI)				
	0	1	3	5	7
MMP-28 expression	+	+	+	+	+
Cellular source of MMP-28	+			+	
Echocardiography	+	+	+	+	+
Necropsy	+	+	+	+	+
Plasma analysis, inflammatory and ECM array	+				+
Immunoblotting for galectin-3, collagen I, collagen III, lysyl oxidase, and α-smooth muscle actin			+	+	+
Macrophage immunohistochemistry				+	
In vitro peritoneal macrophage and fibroblast stimulation experiments	+				
Myofibroblast immunofluorescence	+				+
Collagen cross-linking					+
Tensile strength of infarct hearts			+		

Online Table II. Plasma proteomic profiling results for WT and MMP-28$^{-/-}$ mice.
[a]Effects that are MI-dependent; [b]effects that are MMP-28-dependent; [c]effects that are MI+ MMP-28-dependent.

	WT		MMP-28$^{-/-}$	
	Day 0	Day 7	Day 0	Day 7
Apolipoprotein A-I (μg/mL)[a]	28±1	155±14*	25±1	158±10*
CD40 (pg/mL)[a,b]	26±1	38±2*	31±2#	41±1*
CD40 ligand (ng/mL)	2.7±0.3	1.8±0.5	2.5±0.1	1.9±0.1
C-reactive protein mouse (μg/mL)	6.8±1.0	5.5±0.3	6.1±1.0	5.3±0.1
Endothelin-1 (pg/mL)[c]	40±5	30±3	42±2	51±4#
Eotaxin (ng/mL)	1.15±0.13	1.06±0.09	1.26±0.09	0.99±0.04
Epidermal growth factor mouse (pg/mL)	50±12	67±15	50±8	54±5
Factor VII (ng/mL)[a]	33±5	60±5*	38±4	65±5
Fibrinogen (mg/mL)[a]	87±7	146±19*	87±10	126±9
Fibroblast growth factor 9 (ng/mL)	ND	ND	ND	ND
Fibroblast growth factor basic (ng/mL)[a]	115±14	35±4*	130±10	40±2*
Granulocyte chemotactic protein-2 (ng/mL)	4.8±0.8	5.2±0.9	5.6±0.6	5.6±0.8
Granulocyte-macrophage colony-stimulating factor (pg/mL)	ND	ND	ND	ND
Growth-regulated alpha protein (ng/mL)	0.41±0.19	0.13±0.04	0.26±0.13	0.12±0.02
Haptoglobin (μg/mL)	143±14	158±10	117±24	122±13
Immunoglobulin A (μg/mL)	96±29	37±3	75±7	48±4
Interferon-γ (pg/mL)	ND	ND	ND	ND
Interferon-γ induced protein 10 (pg/mL)	109±34	59±6	79±11	60±8
Interleukin-1α (pg/mL)	168±31	138±20	159±23	111±22
Interleukin-1β (ng/mL)	ND	ND	ND	ND
Interleukin-2 (pg/mL)	ND	ND	ND	ND
Interleukin-3 (pg/mL)	ND	ND	ND	ND
Interleukin-4 (pg/mL)	ND	ND	ND	ND
Interleukin-5 (ng/mL)	0.86±0.15	0.63±0.07	0.71±0.06	0.81±0.10
Interleukin-6 (pg/mL)	23±4	24±5	22±6	15±3
Interleukin-7 (ng/mL)	ND	ND	ND	ND
Interleukin-10 (pg/mL)	ND	ND	ND	ND
Interleukin-11 (pg/mL)	ND	ND	ND	ND
Interleukin-12 subunit p70 (ng/mL)	ND	ND	ND	ND
Interleukin-17A (ng/mL)	ND	ND	ND	ND
Interleukin-18 (ng/mL)[a]	7.3±0.3	33.3±1.0*	7.7±0.4	34.6±2.3*
Leukemia inhibitory factor (ng/mL)	0.93±0.07	1.06±0.07	1.06±0.06	1.24±0.07
Lymphotactin (pg/mL)	93±10	96±17	90±17	88±7
Macrophage colony-stimulating factor-1	5.5±0.3	4.8±0.2	4.6±0.2#	4.8±0.2
Macrophage-derived chemokine (ng/mL)[a]	2.0±0.2	1.8±0.2	2.4±0.1	1.9±0.1*
Macrophage inflammatory protein-1α (ng/mL)[a]	3.6±0.4	7.4±0.4*	4.0±0.4	8.0±0.5*
Macrophage inflammatory protein-1β (pg/mL)[c]	92±11	71±6	109±14	143±14#
Macrophage inflammatory protein-1γ (ng/mL)	21±1	22±3	20±2	23±2

Macrophage inflammatory protein-2 (pg/mL)	23±9	10±2	16±5	11±1
Macrophage inflammatory protein-3β (ng/mL)[a]	1.5±0.1	3.4±0.2*	1.9±0.1	3.8±0.2*
Matrix metalloproteinase-9 (ng/mL)[c]	81±6	118±10*	68±7	85±5#
Monocyte chemotactic protein-1 (pg/mL)	154±46	99±22	101±15	70±6
Monocyte chemotactic protein-3 (pg/mL)	331±71	243±53	266±41	170±9
Monocyte chemotactic protein-5 (pg/mL)	28±4	22±4	25±3	16±2
Myeloperoxidase (ng/mL)[c]	84±8	126±10*	109±12	171±15*#
Myoglobin (ng/mL)	71±17	63±16	92±23	58±10
Oncostatin-M (ng/mL)	ND	ND	ND	ND
Serum amyloid P-component (μg/mL)	30±4	35±2	26±4	31±1
Serum glutamic oxaloacetic transaminase (μg/mL)	143±18	116±12	142±16	138±14
Stem cell factor (pg/mL)	ND	ND	ND	ND
RANTES (pg/mL)	ND	ND	ND	ND
Thrombopoietin (ng/mL)[c]	19±3	51±5*	24±2	67±8*#
Tissue factor (ng/mL)[a]	6.7±0.7	13.3±1.1*	8.2±0.8	12.6±0.8*
Tissue inhibitor of metalloproteinases-1	1.7±0.2	3.2±0.6*	1.3±0.2	2.7±0.3*
Vascular cell adhesion molecule-1 (μg/mL)	1.0±0.1	1.1±0.1	1.0±0.1	1.1±0.1
Vascular endothelial growth factor-A (pg/mL)	303±35	310±63	298±58	273±26
Von Willebrand factor (ng/mL)[a]	248±26	429±63*	300±29	459±46*

58 analytes in the plasma were measured. Values are mean±SEM. ND=not detectable. TNF-α data were graphed separately in Online Figure IA. n=8-12/group. *p<0.05 vs. the respective day 0 and #p<0.05 vs. the respective WT.

Online Table III. Inflammatory gene array results for WT and MMP-28$^{-/-}$ mice.
[a]Effects that are MI-dependent; [b]effects that are MMP-28-dependent; [c]effects that are MI+ MMP-28-dependent.

	WT			MMP-28$^{-/-}$		
	Day 0	Day 7		Day 0	Day 7	
	LV	LVC	LVI	LV	LVC	LVI
Abcf1[a]	61±2	52±3	46±3	67±3	40±2*	38±3*
Bcl6	16.3±0.7	11.2±1.7	13.8±1.9	13.0±1.1	8.0±0.9	12.7±1.5
C3[a]	47±5	38±3	76±10*^	42±4	34±5	69±6*^
Casp1[a]	29±3	55±6*	137±10*^	28±2	41±3	134±5*^
Ccl1	0.09±0.02	0.07±0.02	0.12±0.05	0.07±0.01	0.03±0.01	0.06±0.01
Ccl2[c]	4.6±0.5	10.2±0.9	17.3±3.5*^	6.3±1.2	9.2±1.3	10.6±1.9#
Ccl3[a]	0.25±0.04	0.96±0.22	3.70±0.51*	0.34±0.03	0.57±0.12	2.69±0.49*
Ccl4	1.5±0.3	1.9±0.5	5.6±1.2	1.2±0.1	1.4±0.3	4.6±0.6
Ccl5[a]	2.3±1.0	3.5±0.6	7.5±1.4*	2.1±0.6	2.6±0.4	4.9±0.5
Ccl6[c]	24±2	51±14	94±34*	14±2	19±4	35±11#
Ccl7[a]	6.2±0.6	5.4±0.7	16.6±4.1^	7.1±1.1	4.1±1.2	8.1±2.3
Ccl8[a]	4.3±1.4	17.6±3.1	31.2±8.7*	1.8±0.3	11.0±2.5	13.4±3.1
Ccl9[c]	2.1±0.2	8.0±1.1*	18.1±3.2*^	1.7±0.1	3.4±0.2#	9.8±1.7*^#
Ccl11[a]	2.55±0.39	0.56±0.16*	0.36±0.05*	3.21±0.82	0.36±0.11*	0.36±0.08*
Ccl12[a]	4.8±0.7	10.6±2.1	20.1±3.6*	5.3±1.0	6.4±1.3	8.4±1.8
Ccl17[c]	0.16±0.04	1.49±0.23	4.11±0.68*^	0.24±0.06	0.52±0.06	2.46±0.68*^#
Ccl19	7.7±0.6	11.7±3.2	11.4±2.2	7.3±0.7	6.2±0.8	8.4±1.2
Ccl20	0.06±0.01	0.03±0.02	0.03±0.01	0.04±0.02	0.03±0.01	0.03±0.01
Ccl22	0.21±0.04	6.48±3.44	7.48±5.65	0.15±0.03	24.07±13.34	12.83±7.88
Ccl24[a]	0.34±0.04	0.09±0.03	0.06±0.03*	0.37±0.03	0.06±0.03*	0.06±0.02*
Ccl25[a]	0.66±0.04	1.02±0.25	1.18±0.11*	0.91±0.11	0.91±0.16	1.57±0.84
Ccr1[a]	1.3±0.2	5.6±1.5	10.2±2.7*	1.2±0.1	3.2±0.4	5.7±0.9*
Ccr2[c]	1.8±0.4	5.5±1.3*	15.7±0.7*^	1.6±0.3	4.5±1.0*	7.7±0.7*^#
Ccr3[c]	3.5±0.6	11.7±1.9*	33.5±4.4*^	2.1±0.4	8.0±0.9	19.8±2.3*^#
Ccr4	0.29±0.07	0.15±0.07	0.11±0.03	0.26±0.06	0.10±0.01	0.24±0.03
Ccr5[c]	2.5±0.3	10.7±1.5	33.9±5.2*^	1.6±0.3	7.8±0.8	23.3±2.6*^#
Ccr6	0.59±0.13	0.22±0.08	0.43±0.13	0.41±0.06	0.08±0.02	0.35±0.08
Ccr7[a]	0.50±0.08	0.84±0.25	1.68±0.40*^	0.79±0.12	0.48±0.07	1.21±0.14
Ccr8	0.06±0.03	0.03±0.01	0.10±0.03	0.07±0.02	0.09±0.03	0.14±0.08
Ccr9	1.08±0.09	0.58±0.10	0.70±0.18	0.96 ±0.14	0.47±0.11	0.60±0.13
Ccr10	1.53±0.14	0.77±0.14	0.94±0.21	1.46±0.24	0.85±0.15	1.04±0.24
Cd40lg[a]	0.03±0.01	0.04±0.01	0.21±0.09	0.08±0.03	0.03±0.01	0.25±0.07*^
Crp	0.39±0.17	0.13±0.01	0.21±0.01	0.31±0.14	0.38±0.11	0.71±0.32
Cx3cl1[a]	10±1	30±6*	47±7*^	9.0±1.1	21±1	43±5*^
Cxcl1[a,b]	0.50±0.09	0.40±0.07	0.35±0.05	1.40±0.22#	0.26±0.03*	0.35±0.11*
Cxcl5[a]	0.38±0.10	0.66±0.12	3.62±1.22*	0.31±0.05	0.67±0.12	1.70±0.27*

Cxcl9[a]	6.84±1.44	3.78±0.94	2.67±0.93*	5.58±0.91	2.55±0.45	0.82±0.21*
Cxcl10[a]	0.94±0.28	3.87±0.72	7.18±1.41*	1.14±0.40	2.51±0.37	3.29±0.55
Cxcl11	0.11±0.02	0.09±0.02	0.11±0.06	0.12±0.02	0.12±0.03	0.17±0.06
Cxcl12[a]	113±7	91±3	148±13^	127±14	81±9*	110±10
Cxcl13	0.33±0.07	0.33±0.08	0.16±0.05	0.71±0.38	0.26±0.13	0.18±0.05
Cxcl15	ND	ND	ND	ND	ND	ND
Cxcr3[a]	0.16±0.05	0.52±0.13	1.67±0.21*^	0.18±0.04	0.42±0.08	1.63±0.22*^
Cxcr5[a]	2.5±0.5	2.1±0.5	6.6±1.7	2.0±0.3	4.8±1.8	12.0±4.8*
Ifng[a]	0.06±0.02	0.05±0.01	0.09±0.03	0.08±0.02	0.05±0.01	0.15±0.02^
Il1a	0.21±0.03	0.18±0.03	0.35±0.07	0.15±0.03	0.25±0.09	0.44±0.15
Il1b[c]	0.81±0.07	1.57±0.33	2.94±0.28*^	0.98±0.11	0.73±0.12	1.49±0.20#
Il1f6	0.18±0.03	1.04±0.47	2.28±1.90	0.23±0.05	5.58±3.32	0.76±0.46
Il1f8[a]	0.20±0.07	0.16±0.10	0.12±0.05	0.23±0.01	0.04±0.01*	0.18±0.09
Il1r1[a]	6.7±0.4	16.0±1.9*	34.2±2.8*^	6.8±0.3	14.4±1.6*	30.7±2.6*^
Il1r2[a]	0.15±0.04	1.63±0.42	4.47±1.82*	0.19±0.02	2.14±0.79	6.72±4.04*
Il2rb	0.37±0.12	0.27±0.07	0.55±0.12	0.34±0.07	0.23±0.06	0.40±0.07
Il2rg[c]	7.4±0.6	10.7±2.0	15.7±2.4*^	7.8±0.7	7.6±0.5	10.5±1.7#
Il3	0.19±0.01	0.25±0.01	0.33±0.12	0.34±0.01	0.13±0.01	0.27±0.14
Il4[a]	0.17±0.02	0.09±0.03	0.20±0.03^	0.17±0.03	0.07±0.01	0.17±0.02
Il5ra	0.05±0.01	0.03±0.01	0.04±0.01	0.05±0.01	0.12±0.04	0.16±0.08
Il6ra	4.7±0.9	7.7±1.0	20.0±2.7*	5.7±0.5	5.6±0.7	17.0±1.7
Il6st[a]	123±6	98±9	138±16	132±7	81±3*	115±14
Il10[a]	0.12±0.01	0.36±0.07	0.52±0.12*	0.17±0.04	0.23±0.06	0.56±0.11*^
Il10ra[c]	1.24±0.19	3.83±0.48	12.70±1.83*^	0.95±0.13	3.00±0.47	9.43±1.07*^#
Il10rb[a,b]	117±8	62±5*	64±6*	135±11#	56±2*	57±3*
Il11[a]	0.06±0.01	0.19±0.05	0.30±0.03*	0.05±0.01	0.14±0.04	0.26±0.03*
Il13	0.10±0.02	0.55±0.21	0.73±0.44	0.09±0.02	1.23±0.60	3.22±2.48
Il13ra1	21±1	24±3	26±2	20±1	20±1	22±1
Il15[a,b]	34±2	16±2*	14±1*	46±4#	16±1*	12±1*
Il16	2.3±0.1	2.1±0.2	3.5±0.6	2.1±0.2	2.1±0.4	2.8±0.3
Il17b	0.06±0.01	0.08±0.02	0.07±0.01	0.12±0.03	0.05±0.02	0.10±0.04
Il18[c]	0.54±0.02	1.88±0.40*	5.75±0.47*^	0.56±0.07	1.16±0.10	3.97±0.51*^#
Il8rb	0.19±0.05	0.19±0.05	0.78±0.16	0.33±0.13	0.15±0.03	0.38±0.10
Il20	0.25±0.01	0.12±0.01	0.29±0.11	0.29±0.11	0.29±0.15	0.51±0.33
Itgam[a]	4.6±0.7	14.4±1.0	41.0±3.0*	3.8±0.6	11.6±1.5	36.7±1.0*
Itgb2[c]	4.1±0.4	16.6±3.2	58.9±3.6*^	4.2±0.6	10.3±1.7	43.9±8.8*^#
Lta	0.36±0.19	0.30±0.01	0.19±0.01	0.13±0.01	0.27±0.11	0.39±0.14
Ltb	0.65±0.08	0.38±0.03	0.81±0.16	0.47±0.08	0.44±0.11	0.93±0.41
Mif[c]	177±12	239±15*	196±12^	179±9	155±12#	135±15#
Pf4[c]	18±1	26±7	76±18*^	14±1	13±1	34±8#
Scye1[a]	111±6	80±7*	70±5*	120±8	62±3*	51±6*
Spp1[a]	1.4±0.3	168±54	1141±142*^	1.2±0.2	98±40	938±180*^
Tgfb1[c]	19±2	43±3*	70±6*^	18±1	30±2*#	56±4*^#
Tnf	0.42±0.10	0.34±0.05	0.75±0.10	0.55±0.17	0.30±0.04	0.78±0.11

Tnfrsf1a[c]	13±1	25±2*	33±3*^	16±1	15±1#	20±3#
Tnfrsf1b[a]	4.7±0.4	13.9±1.3	33.6±2.9*	4.4±0.4	9.3±1.2	24.5±3.6*
Tollip[a]	25±2	17±1*	15±1*	26±2	15±1*	12±1*
Xcr1	0.14±0.05	0.06±0.02	0.11±0.03	0.10±0.01	0.05±0.01	0.05±0.01

The data are reported as $2^{-\Delta Ct}$ values x 100±SEM. n=6/group. ND=not detectable. LVC=remote region, LVI=infarct region. *p<0.05 vs. the respective day 0, ^p<0.05 vs. the respective LVC, and #p<0.05 vs. the respective WT.

Online Table IV. ECM gene array results for WT and MMP-28$^{-/-}$ mice. [a]Effects that are MI-dependent and [c]effects that are MI+MMP-28-dependent.

	WT			MMP-28$^{-/-}$		
	Day 0	Day 7		Day 0	Day 7	
	LV	LVC	LVI	LV	LVC	LVI
Adamts1[a]	25±3	66±7*	55±8	35±3	54±13	43±12
Adamts2[a]	14±1	111±16*	246±31*^	13±1	97±12*	211±33*^
Adamts5[a]	3.2±0.2	7.1±1.2	13.2±3.4*	3.7±0.3	10.1±2.6	19.8±8.9*
Adamts8[a]	0.62±0.04	4.73±0.68*	2.33±0.31*^	0.60±0.05	3.91±0.58*	2.02±0.22*^
Cd44[a]	8.8±0.7	34.3±4.9*	60.7±5.3*^	8.3±0.8	29.5±1.8*	53.8±9.2*^
Cdh1[a]	0.04±0.01	0.17±0.04	0.47±0.13*^	0.07±0.02	0.11±0.03	0.43±0.14*^
Cdh2[a]	329±21	240±18	170±12*	365±15	250±7	148±16*
Cdh3[c]	0.03±0.01	0.78±0.20	3.51±0.59*^	0.04±0.01	0.31±0.05	2.05±0.31*^#
Cdh4[a]	1.49±0.26	0.46±0.10	0.12±0.03*	0.73±0.11	0.38±0.03	0.11±0.03*
Cntn1	0.06±0.02	0.08±0.03	0.08±0.02	0.09±0.02	0.12±0.05	0.47±0.03
Col1a1[c]	31±3	891±156	3066±483*^	27±3	636±105	2340±284*^#
Col2a1[a]	0.04±0.01	1.31±0.44	9.88±3.32*^	0.08±0.01	0.52±0.10	6.56±1.79*^
Col3a1[c]	195±21	3125±501*	7240±874*^	192±21	2236±399	4516±1090*^#
Col4a1[a]	190±17	541±48*	586±45*	196±20	437±25	484±80
Col4a2[a]	159±10	506±43*	586±48*	161±8	396±28	498±86*
Col4a3	0.77±0.11	1.10±0.13	0.73±0.13	0.99±0.13	1.35±0.24	0.95±0.20
Col5a1[c]	16±1	156±21*	350±44*^	17±2	109±11	244±48*^#
Col6a1[a]	42±3	159±27*	209±24*	45±5	129±16*	168±40*
Ctgf[c]	119±12	1241±168*	2050±246*^	141±22	667±90#	1344±236*^#
Ctnna1[c]	296±13	270±24	174±6*^	309±14	214±16*#	154±16*^
Ctnna2	0.02±0.01	0.05±0.02	0.11±0.03	0.06±0.02	0.03±0.02	0.10±0.05
Ctnnb1	154±10	158±20	202±19	169±12	171±16	175±24
Ecm1[c]	24±2	96±15*	210±13*^	30±2	74±11	160±28*^#
Emilin1[c]	9.4±0.5	59.5±7.2*	119.0±14.5*^	10.4±1.0	43.3±4.3*	91.3±11.1*^#
Entpd1	7.0±0.6	12.9±1.5	13.7±1.5	8.1±0.7	11.2±1.2	11.4±2.8
Fbln1[a]	11.8±1.8	9.5±1.8	18.3±3.9	10.5±1.2	12.3±2.0	23.3±4.8*^
Fn1[c]	13±1	360±75	1225±233*^	13±1	245±41	870±163*^#
Hapln1	ND	ND	ND	ND	ND	ND
Hc	0.07±0.05	0.07±0.02	0.09±0.04	0.04±0.02	0.11±0.03	0.12±0.04
Icam1[a]	8.5±0.6	11.4±0.9	17.2±1.1*^	10.6±1.4	9.8±1.1	14.2±2.0
Itga2[a]	0.66±0.13	0.92±0.15	1.82±0.22*^	0.63±0.08	0.99±0.18	1.75±0.31*^
Itga3	2.9±0.4	3.4±0.4	3.5±0.6	2.9±0.1	3.0±0.4	3.4±0.8
Itga4[c]	0.45±0.05	0.94±0.16	2.58±0.28*^	0.39±0.08	0.86±0.16	1.87±0.40*^#
Itga5[c]	10±1	45±6*	48±5*	11±2	30±1*#	33±3*#
Itgae[a]	0.50±0.12	0.42±0.08	0.72±0.13	0.43±0.12	0.64±0.17	1.28±0.29*^
Itgal[c]	1.10±0.11	1.25±0.22	2.29±0.16*^	1.46±0.29	0.79±0.13	1.72±0.15^#
Itgam[a]	3.8±0.2	14.6±0.8	41.5±4.1*	3.1±0.2	15.6±4.4	42.4±4.4*
Itgav[a]	19±3	32±3	72±9*^	15±1	30±4	78±7*^

Itgax[a]	0.47±0.07	2.22±0.35	8.02±1.73*^	0.67±0.12	2.14±0.59	9.18±1.48*^
Itgb1[c]	223±16	381±17*	502±31*^	231±10	311±12*#	387±23*^#
Itgb2[c]	5.1±0.8	18.2±3.5	64.3±6.0*^	4.3±0.6	11.7±2.1	47.6±8.8*^#
Itgb3[a]	1.6±0.2	7.7±1.1*	10.0±0.7*	1.8±0.2	6.1±0.5*	9.6±1.1*^
Itgb4[a]	0.15±0.02	0.25±0.04	0.37±0.05*	0.17±0.02	0.25±0.06	0.42±0.07*
Lama1	0.06±0.03	0.19±0.05	0.23±0.04	0.09±0.03	0.09±0.02	0.24±0.06
Lama2	28±2	44±4	40±5	31±2	40±5	33±7
Lama3[a]	1.86±0.42	1.43±0.18	0.81±0.12*	1.42±0.07	1.40±0.11	0.97±0.07
Lamb2[a]	112±11	77±5	65±5*	126±21	76±6	72±6*
Lamb3[a]	3.58±0.34	0.85±0.07	0.31±0.02*	3.62±0.46	0.92±0.11	0.31±0.08*
Lamc1	46±4	71±7	68±10	52±6	70±8	52±12
Mmp1a	0.53±0.11	0.29±0.16	0.38±0.12	1.25±0.37	0.08±0.04	0.18±0.06
Mmp2[c]	34±3	99±10	176±30*^	32±4	66±9	91±25#
Mmp3	2.7±0.1	2.4±0.4	2.9±1.3	2.2±0.6	2.2±0.4	1.8±0.4
Mmp7	0.44±0.11	0.22±0.07	0.11±0.07	0.76±0.34	0.15±0.05	0.32±0.10
Mmp8[a]	0.14±0.05	0.37±0.14	2.25±0.41*	0.12±0.05	0.93±0.59	2.47±0.97*
Mmp9[c]	0.46±0.03	0.26±0.11	2.24±0.70*^	0.64±0.27	0.10±0.03	1.15±0.35#
Mmp10	ND	ND	ND	ND	ND	ND
Mmp11[a]	0.19±0.01	0.23±0.03	0.48±0.10*	0.20±0.02	0.25±0.01	0.43±0.08
Mmp12[a]	0.03±0.01	0.16±0.04	0.42±0.14*	0.11±0.03	1.43±1.15	3.08±2.54
Mmp13[a]	0.67±0.14	0.47±0.09	0.65±0.11	0.39±0.05	0.39±0.06	0.86±0.13*^
Mmp14[a]	4.9±0.5	42.3±6.1*	109.5±11.8*^	4.6±0.6	33.4±4.9*	96.4±8.0*^
Mmp15[a]	45±3	19±3*	5.2±0.3*^	47±3	20±2*	4.9±0.4*^
Ncam1[a]	1.3±0.1	8.3±1.3*	15.7±2.0*^	1.6±0.2	6.5±1.0*	13.4±1.1*^
Ncam2	ND	ND	ND	ND	ND	ND
Pecam1[a]	89±8	113±8	72±6^	92±5	97±8	58±6*^
Postn[c]	10±1	495±65*	857±138*^	8±1	363±59*#	522±115*#
Sele[a]	1.16±0.18	2.57±0.43*	1.00±0.16^	1.95±0.30	1.72±0.21	0.84±0.11*
Sell[a]	0.94±0.12	0.97±0.14	1.74±0.22*^	1.36±0.16	0.66±0.10*	1.41±0.17^
Selp[c]	0.58±0.12	2.73±0.56*	4.43±0.42*^	0.83±0.07	1.72±0.15#	3.19±0.43*^#
Sgce[a]	5.3±0.6	15.4±1.3*	21.1±2.5*	8.4±0.8	12.7±1.0	17.0±3.8*
Sparc[a]	114±9	763±73*	1759±215*^	135±15	632±69*	1479±244*^
Spock1	0.08±0.03	0.21±0.05	0.19±0.05	0.23±0.07	0.27±0.05	1.2±0.8
Spp1[a]	1.1±0.3	141±46	1023±146*	1.1±0.2	89±37	824±162*
Syt1	0.06±0.02	0.03±0.02	0.05±0.02	0.04±0.01	0.05±0.02	0.11±0.06
Tgfbi[c]	38±3	96±21	301±27*^	35±3	57±8	172±34*^#
Thbs1[a]	18±4	196±36	717±153*	27±10	133±19	552±91*
Thbs2[c]	17±1	89±17	331±57*^	20±2	60±10	238±51*^#
Thbs3[a]	2.9±0.3	11.5±1.6*	19.5±1.9*^	2.9±0.4	10.7±1.3*	18.6±2.0*^
Timp1[a]	0.19±0.04	1.02±0.18*	1.10±0.14*	0.40±0.09	1.03±0.12	0.87±0.09
Timp2[a]	45±3	124±15*	275±30*^	55±4	109±11	269±32*^
Timp3	41±3	65±8	91±18	66±9	81±14	108±18
Tnc[c]	0.4±0.1	40.0±6.4*	66.9±6.5*^	1.0±0.2	24.0±2.7*#	45.4±9.5*^#
Vcam1[a]	11±2	18±3	40±4*^	12±1	14±2	32±6*^

Vcan[a]	5.4±0.6	28.5±2.0*	33.9±2.6*	7.1±0.8	26.5±2.4*	31.3±4.1*
Vtn[a]	29.0±2.6	18.6±2.5*	9.5±0.8*^	31.0±3.5	13.6±1.3*	5.9±0.7*^

The data are reported as $2^{-\Delta Ct}$ values x 100±SEM. n=6/group. ND=not detectable. *p<0.05 vs. the respective day 0, ^p<0.05 vs. the respective LVC, and #p<0.05 vs. the respective WT.

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Online Figure Legends

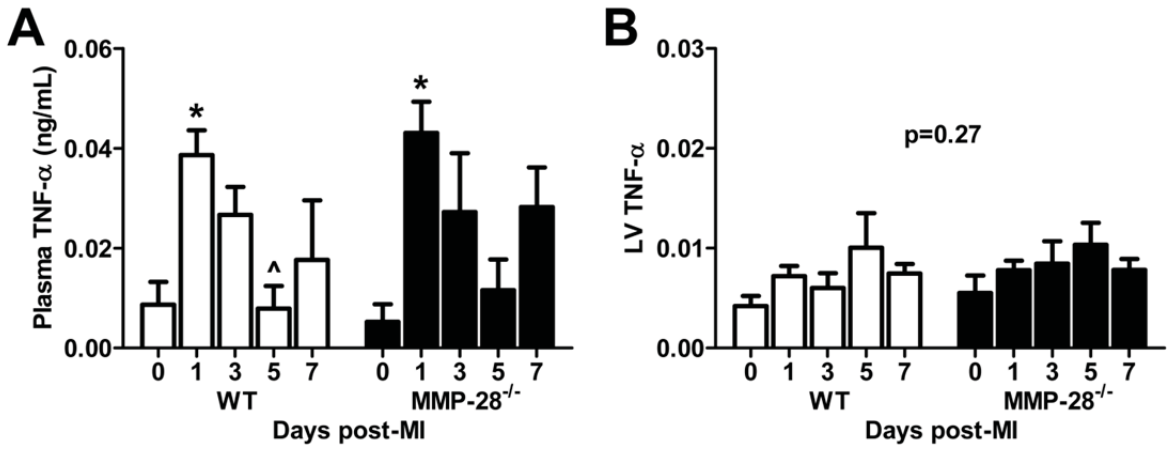
Online Figure I. MMP-28 deletion did not affect TNF-α content. A, Plasma TNF-α levels in WT and MMP-28$^{-/-}$ mice were significantly increased at day 1 and returned to baseline by day 7 post-MI. n=10-16/group. *p<0.05 vs. day 0 and ^p<0.05 vs. day 1. **B**, LV TNF-α mRNA content was not significantly upregulated, compared to respective day 0 levels. n=6/group.

Online Figure II. MMP-28 deletion did not change the LV tensile strength at day 3 post-MI. A, MMP-28 deletion showed no effect on the stretch ratio at failure. **B**, Ultimate tensile strength of infarct hearts was not significantly altered with MMP-28 deletion. n=3-5/group for A and B. **C**, The day 3 post-MI end diastolic volume from mice survived 7 days and mice ruptured at days 4 to 7 post-MI. n=3-8/group. *p<0.05 vs. survived.

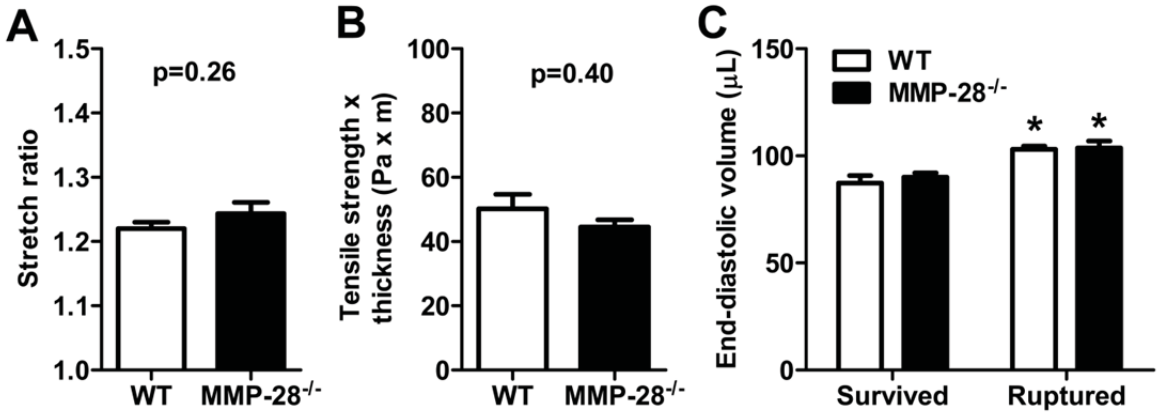
Online Figure III. MMP-28 deletion did not alter regulatory T cell infiltration and PTEN expression. A, Foxp3 mRNA in the day 7 infarct region showed no difference between WT and MMP-28$^{-/-}$ mice. **B**, PTEN mRNA in the day 7 infarct region showed no difference between WT and MMP-28$^{-/-}$ mice. n=6/group for A and B.

Online Figure IV. Schematic illustrating the mechanisms of action of MMP-28 in regulating LV remodeling following MI. MMP-28 deletion aggravated MI-triggered LV remodeling by inhibiting M2 macrophage activation, myofibroblast numbers, ECM synthesis, and collagen cross-linking.

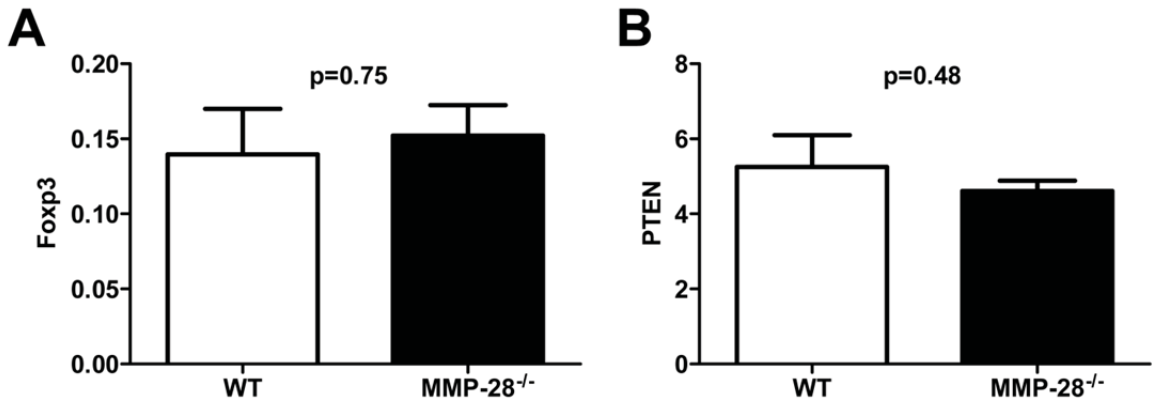
Online Figure I



Online Figure II



Online Figure III



Online Figure IV