Supplemental Material

Methods

Generation of genetically engineered Grem2 mice

In order to inactivate the *Grem2* gene and generate *Grem2*$^{-/-}$ mice, we first constructed an insertion vector containing two fragments from the *Grem2* gene locus (fragments B and C, each 0.5 kb in length) flanking a *kanamycin selection* gene cassette under the synthetic *EM7* prokaryotic promoter (*EM7neo*) and a fusion puromycin/truncated herpes simplex virus *thymidine kinase* gene [*pu(Δ)TK*; Figure 2A)]. Fragment B is located within the *Grem2* single intron just upstream of exon 2, which contains the entire *Grem2* coding sequence and 3' untranslated area (UTR). Fragment C resides within the 3' UTR. The truncated thymidine kinase was incorporated to facilitate clone selection in future recombination strategies. Homologous recombination between a BAC containing the *WT Grem2* locus and the insertion vector replaced the entire coding sequence and part of the 3' UTR of the *Grem2* gene with the *pu(Δ)TK/EM7neo* cassette. A vector containing two additional small fragments of the *Grem2* gene locus (Fragments A and D), also 0.5 kb in length, were used to retrieve the resultant targeting vector from the modified BAC (Figure 2A).

200 μg of the targeting vector were linearized and double-electroporated into 3.5 x 10^7 129/Sv mouse embryonic stem cells at the Vanderbilt Transgenic Mouse/Embryonic Stem Cell Shared Resource (TMESCSR). After puromycin selection at 1.5 μg/ml, 483 colonies were picked and 25 colonies were identified as having properly recombined by Southern blotting using 5' and 3' probes outside the targeting vector (the location of the probes are marked in Figure 2A). The targeting efficiency was 5.2%. Six positive clones were subsequently expanded and confirmed by secondary screening. Two selected clones were then injected into C57BL/6 blastocysts and blastocysts were transplanted into pseudopregnant females. Both clones gave germline transmission generating two independent *Grem2*$^{-/-}$ mouse lines that displayed identical physiological phenotypes and response to myocardial ischemic injury. The *Grem2*$^{-/-}$ mice and littermate *WT* controls (*WT*^{mix}) were kept on a mixed C57BL/6 and 129/Sv background.

The *αMHC-Grem2* plasmid was generated by inserting the full-length *Grem2* cDNA into the *αMHC* (*Myh6*) gene promoter-polyA hGH cloning vector 1 (kindly provided by Dr. J. Robbins).[1] The *αMHC-Grem2* transgenic (*TG*^{Grem2}) mice were generated by pronuclear microinjection of the construct into fertilized oocytes at the TMESCSR. *TG*^{Grem2} mice and *WT*

littermate controls were raised in C57BL/6 background.

Histological, molecular and flow cytometric analyses were conducted using male mice at 12-16 weeks of age fed with a normal chow diet.

Experimental myocardial infarction (MI) and administration of Grem2 protein and DMH1

Mice underwent open chest surgery, a 10-0 nylon suture was placed through the myocardium into the anterolateral left ventricular wall around the left anterior descending (LAD) artery and the vessel was permanently ligated.[2] Mice were euthanized at defined time points following surgery to obtain cardiac tissue for molecular, histological and flow cytometric analyses.

For injection of Grem2 protein, we synthesized, purified and measured activity as previously described.[3,4] *WT* mice were injected with 1 μg Grem2 protein per gram of body weight or vehicle (sterile 1X PBS) via intraperitoneal injection (IP) once per day at day 2, 3, and 4 following MI. For injection of DMH1 (Sigma), *Grem2*$^{-/-}$ mice were injected IP with 13 μg DMH1[5] per gram of body weight or vehicle (DMSO) once per day at 2, 3 and 4 days following MI. [5,6]

Echocardiography

Mice underwent echocardiography measurements in order to assess cardiac function post-surgery. Mice were rested and calmed before echocardiography was performed. All mice were conscious and unsedated during imaging using the VEVO 2100 machine and transducer MS-400 (VisualSonics) to measure and calculate cardiac parameters. The left ventricle was located in B-Mode and was traced over five consecutive beats in M-Mode. Left ventricular internal dimension and volume in diastole and systole ($LVID_d$, $LVID_s$, $LVvol_d$, $LVvol_s$) were measured from M-Mode using the short axis and used to calculate fractional shortening and ejection fraction.[7]

RNA analysis by Reverse Transcription and quantitative Polymerase Chain Reaction (RT-qPCR)

Whole hearts were dissected at the indicated time points after MI, perfused to remove blood cells and RNA was obtained using TriZol Reagent according to the manufacturer's instruction (Life Technologies). RNA was obtained from cells in culture using the RNeasy Mini Kit (Qiagen). Reverse transcription was conducted by incubating 100 ng of $oligo(dt)_{15}$

(Promega) with 3 μg RNA for 5 min at 70°C. 20 mM of dNTPs (GE Healthcare), 200 U/μl of Mo-MLV reverse transcriptase with 5x associated buffer (Promega), 40 U/μl RNasin (Promega) and water were added to the RNA solution and incubated at 40°C for 1 hour, followed by a 5 minute incubation at 95°C in order to inactivate enzyme activity. 1:100 of the final cDNA solution or ~20 ng served as template for quantitative Real Time PCR with GoTaq qPCR Master Mix (Promega) using a C1000 Thermal Cycler (BioRad) as previously described.[2,7] 0.5 μM of *Gapdh* primers were included as an internal control and relative gene induction levels were determined using the $2^{(-DDCt)}$ formula.[8,9] Experiments were done in triplicates. The sequences of gene-specific primers have been included in the Online Table III.

Immunofluorescence and immunohistochemistry analyses

For IF on cardiac tissue sections, freshly isolated hearts were perfused with 1X Phosphate Buffered Saline (PBS), bisected transversely, embedded in Optimal Cutting Temperature (OCT) compound, frozen on dry ice, cut into 10 μm thick sections and stored at -70°C until use. Before antibody staining, slides were thawed at room temperature, immersed in cold 1:1 acetone:methanol and fixed for 5 minutes on ice. Slides with cardiac tissue sections were washed three times in 1X PBS for 5 minutes each wash, and incubated with blocking buffer containing 1% bovine serum albumin (BSA) and 0.05% saponin in 1X PBS for 1 hour at room temperature. Next, sections were stained with primary antibodies overnight at 4°C in blocking buffer. Afterwards, slides were washed five times in 1X PBS for 5 minutes each, incubated with secondary antibodies and DAPI for 1 hour at room temperature in blocking buffer, washed in 1X PBS three times for 5 minutes each, and mounted with VECTASHIELD fluorescent mounting medium (Vector Laboratories). The Vanderbilt Histology Core performed histological services, including tissue sectioning and hematoxylin and eosin staining. Bright field images were taken on the Zeiss AxioImager Z1.

Primary antibodies used for IF analysis were as follows: rabbit polyclonal anti-human Tie1 (Santa Cruz Biotechnology, 1:100, Cat. No. sc-342 (C-18), rat monoclonal anti-mouse CD31/PECAM1 (BD Pharmingen; 1:100, Cat. No. 553370), rabbit monoclonal anti-mouse p-Smad1/5/8 (Cell Signaling; 1:50, Cat. No. 9511), rat monoclonal anti-mouse CD45 (BD Pharmingen; 1:100, Cat. No. 550539), mouse monoclonal anti-mouse MF20 (Developmental Studies Hybridoma Bank; 1:5, Cat. No. MF 20, RRID:AB_2147781), rabbit polyclonal anti-human Grem2 (GeneTex; 1:100, Cat. No. GTX108414), rat monoclonal anti-mouse CD62E/E-selectin (BD Pharmingen; 1:100, Cat. No. 550290), and mouse monoclonal α-Actinin (Sigma;

1:800, Cat. No. A7811). Secondary antibodies used for IF were: goat anti-mouse Cy3-conjugated (Cat. No. 115-165-146), goat anti-rat Cy3 (Jackson ImmunoResearch, Cat. No. 712-165-150), and goat anti-rabbit Alexa-Fluor-488-conjugated (Life Technologies, Cat. No. A21206). Cy3 antibodies were used at a 1:200 dilution and Alexa-Fluor-488 was used at a 1:400 dilution. Cardiac tissue sections were stained with the fluorescent dye 4',6-diamidino-2-phenylindole (DAPI, 1:5000 dilution; Invitrogen) to mark cellular nuclei. Control images using isotype controls and minus primary antibody controls are shown in Online Figure XII. Images were taken on the Olympus FV-1000 inverted confocal microscope and processed using the FV10-ASW 1.6 Viewer software (Olympus).

p-Smad1/5/8^{+} cardiomyocytes were quantified using ImageJ 1.46r (NIH) color thresholding, as a percentage of cells double positive for MF20 and p-Smad1/5/8 amongst all DAPI positive cells in the viewing field; at least 4 viewing fields were used for calculations. N=3 mice for each group.

Flow Cytometry

We prepared single cell suspensions of cardiac cells depleted of cardiomyocytes from freshly isolated whole hearts perfused with 1X PBS to remove blood cells. Next, hearts were digested with Collagenase D (2 mg/ml; Roche) and DNase I (100 μg/ml) in a solution of RPMI 1640 (Gibco) containing 10% FBS using an AUTOMacs Dissociator (Miltenyi Biotech), and then incubated at 37°C for 30 minutes in an orbital shaker to prepare single cell suspensions. The digested tissue was then passed through a 70-micron cell strainer and centrifuged at 500 g for 10 minutes. The cell pellet was suspended in 2 ml 1X PBS and centrifuged at 300 g for 5 minutes. Two more centrifugation/wash steps followed, and the pellet was suspended in 100 μl of FACS buffer (1% BSA, 0.5% NaN_3 in 1X PBS). We then added 2 μl of Fc blocker (eBioscience) and cells were incubated for 10 minutes at 4°C to prevent non-specific antibody binding, then washed with 1 ml of FACS buffer and centrifuged at 300 g for 5 minutes. The cells were resuspended in 100 μl of FACS buffer and antibodies were added at 1 μl or 0.25 μg per 1 million cells and incubated for 30 minutes at 4°C. The antibodies used were Brilliant Violet 510–conjugated (BV510) anti-CD45 antibody (Biolegend, Cat. No. 103107), Alexa Fluor 488–conjugated anti-F4/80 antibody (Biolegend, Cat. No. 123119), PE-Cy conjugated anti-Ly6C (eBioscience, Cat. No. 25-5932-80), Brilliant Violet 421-conjugated anti-Ccr2 (Biolegend, Cat. No. 150605), PE-conjugated anti-Ly6G (Biolegend, Cat. No. 127607), and APC-Cy7-conjugated anti-CD3e (BD Pharmingen, Cat. No. 557596).

After incubation, cells were centrifuged at 300 g for 5 minutes and washed twice with 1 ml FACS buffer. 5 µl of 7-AAD (eBioscience) was added to 100 µl of the solution and incubated for 10 minutes at room temperature for live/dead staining. After 300 µl of FACS staining buffer was added, cell samples were analyzed by flow cytometry using the BD FACSCanto II cytometer. Total cell number was determined by adding 50 µl of counting beads (~49500-52000 beads per µl; Life Technologies). Flow-minus-one was used for gating. Low voltage gating was conducted in order to capture the counting bead population. All leukocyte populations were quantified within the $CD45^+$ gate, and pro-inflammatory monocyte cell numbers were quantified within the $F4/80^+$ gate. Data acquisition was completed using FloView.

Regarding flow cytometry of blood leukocytes, 100 µl of fresh heparinized blood was directly stained with 1.5 µl of each of the previously mentioned antibodies for 30 minutes at 4°C. The samples were then washed with FACS buffer. 2 ml of red blood cell lysis buffer was applied per 100 µl of blood for 4 minutes at room temperature. Two additional rounds of washing and centrifugation at 1500g for 3 minutes followed. 7-AAD staining and incubation with primary and secondary antibodies was conducted as described above.

TTC Staining

Whole mouse hearts were isolated 24 hours post-MI, flash frozen and cut into 1 mm sections. The sections were incubated in 1.5%TTC at 37°C for 30 minutes and then fixed in 10% formalin overnight, and finally imaged. ImageJ 1.46r (NIH) was used to outline infarct (white) tissue. Infarct size is reported as a percentage of the total left ventricular (LV) area.

Cell culture

Human Microvascular Endothelial cells (HMECs)[10] were kindly provided by Dr. Sergey Ryzhov. Cells used for experiments were between the third and fourth passages and cultured in 199 media (Gibco 11150) containing 15% FBS, 10 U/ml Heparin (Sigma), and 30 µg/ml endothelial cell growth supplement (Biomedical Technologies). Cells were grown in full growth serum and then seeded in 12-well plates. Prior to growth factor addition, cells were incubated with serum starvation media (same as normal media with 1% FBS) over night. Cells were subsequently treated with rhTNFα (R&D; 10 ng/ml), rhBMP2 (R&D; 100 ng/ml-200 ng/ml), Grem2 (100 ng/ml), and DMH1 (10µM)[11] or the equivalent volume of vehicle solution (PBS or

DMSO). After 4 hours or 24 hours of treatment, cells were lysed for RNA extraction. Data are representative of at least two independent experiments.

For binding assays, HMEC cells were grown for three days until they reached monolayer confluency (3 x 10^4 cells/well within a 96-well plate). Calcein AM labeled (1 μM, 30 min) human monocytes (THP-1 cells) were added to the HMEC monolayer at a concentration of 10 x 10^4 cells per well (HMEC:THP-1 ratio 1:3) and incubated together for 30 minutes. After incubation, non-adherent THP-1 cells were aspirated off and the remaining cells were washed with PBS 5 times. The fluorescence intensity was measured using the Modulus microplate multimode reader. The number of adherent THP-1 cells was calculated from a calibration curve prepared using increasing concentrations (ranging from 0.1 to 100 x 10^3 cells) of THP-1 cells. 300 ng/ml of BMP2, 100 ng/ml of Grem2 and 100ng/ml of TNFα were used alone or combination for 24 hours to treat either HMEC or THP-1 cells.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software. Data are represented as the mean ± SEM. Student's two-tailed unpaired *t*-test was used for comparison between two groups, *one-way ANOVA* was used to compare multiple groups, and *two-way ANOVA* was used to compare gene induction in each mouse model over time. Dunnett's and Bonferroni's multiple comparisons test was used post-hoc. *$*P<0.05$*, *$**P<0.01$*, *$***P< 0.001$*, *$****P<0.0001$* were considered significant.

Online Methods References

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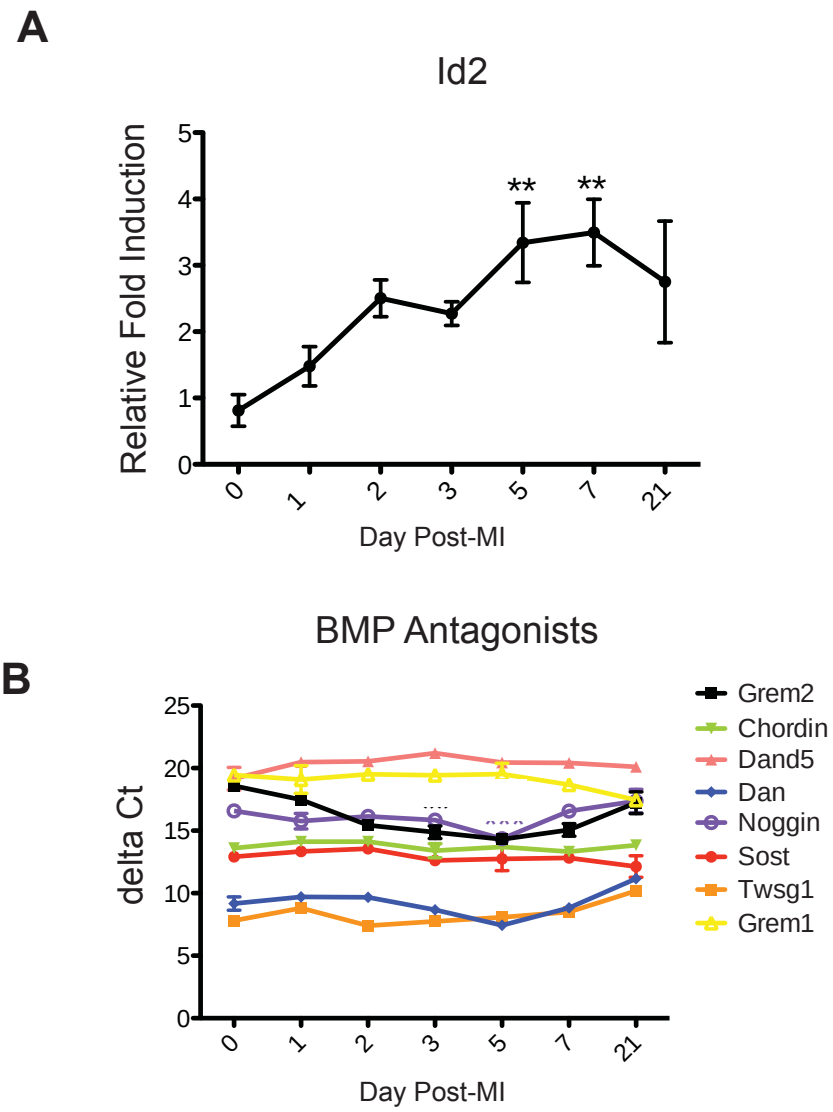
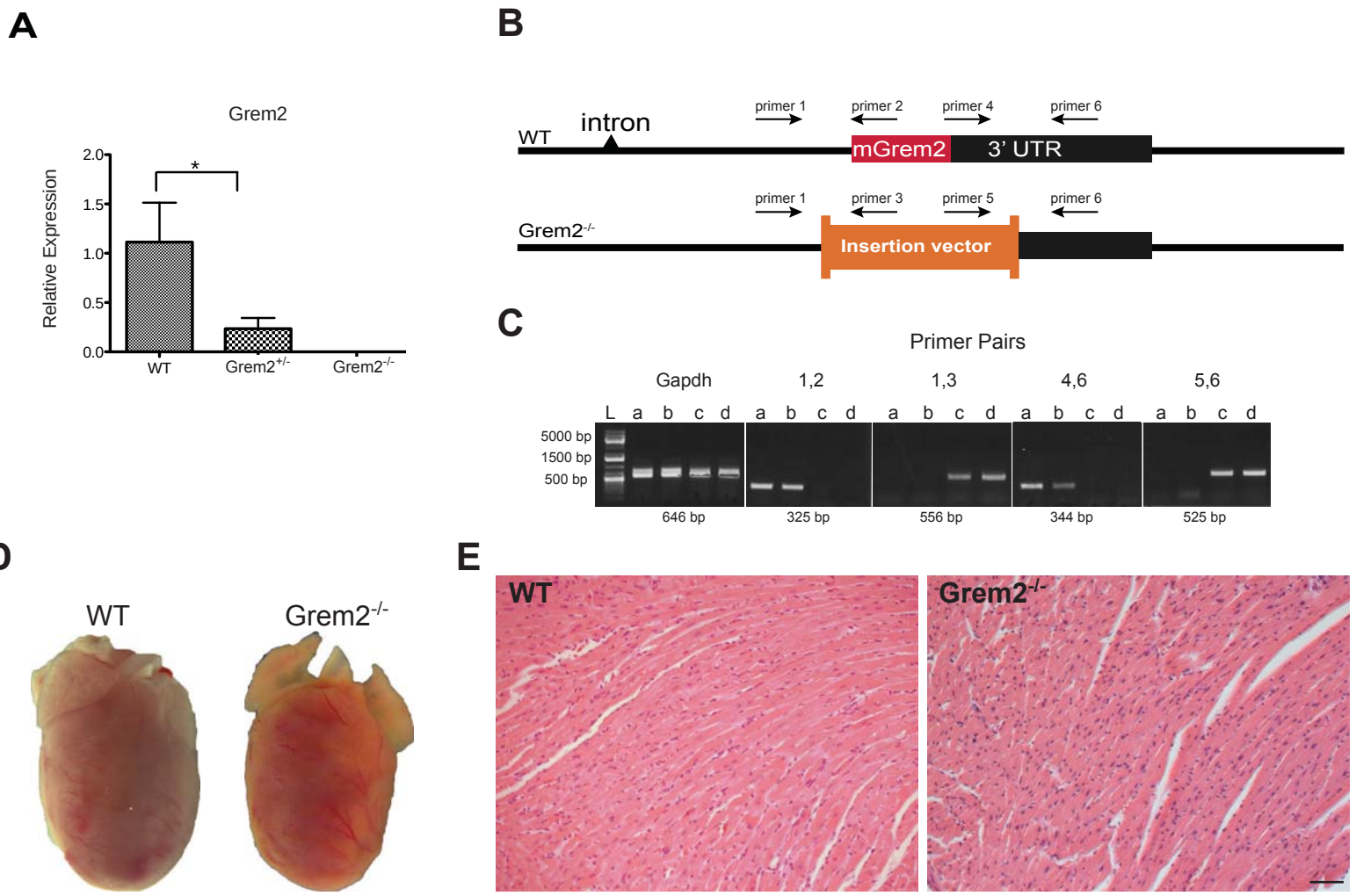
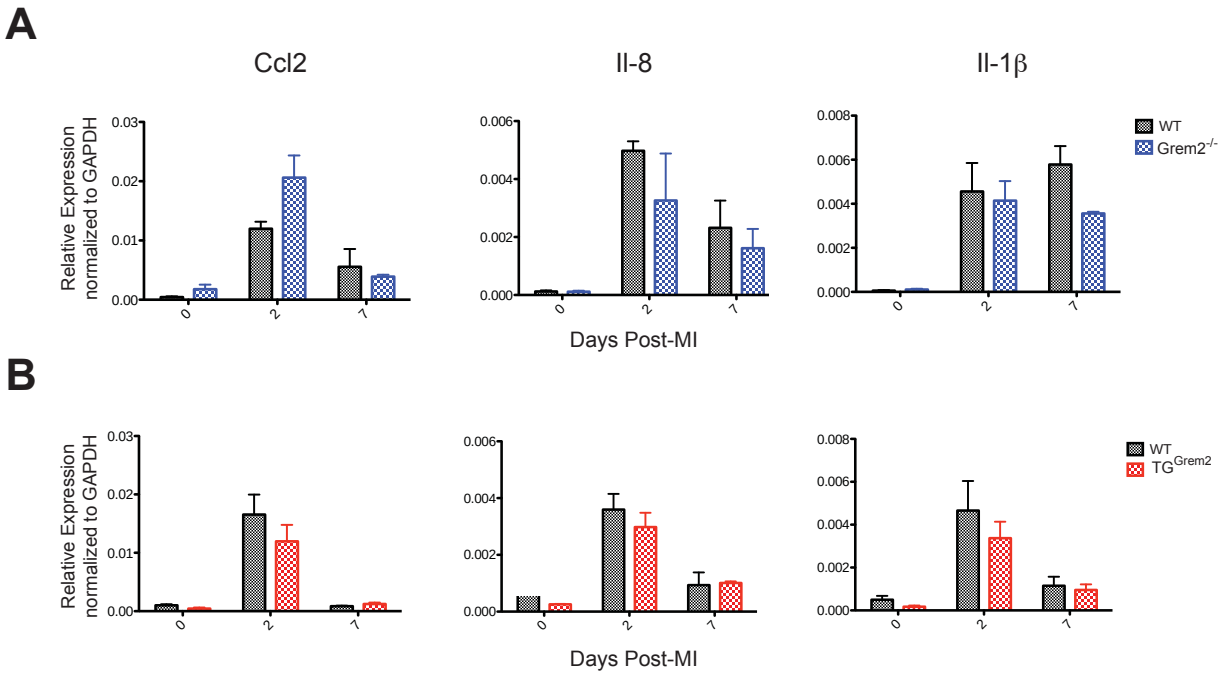


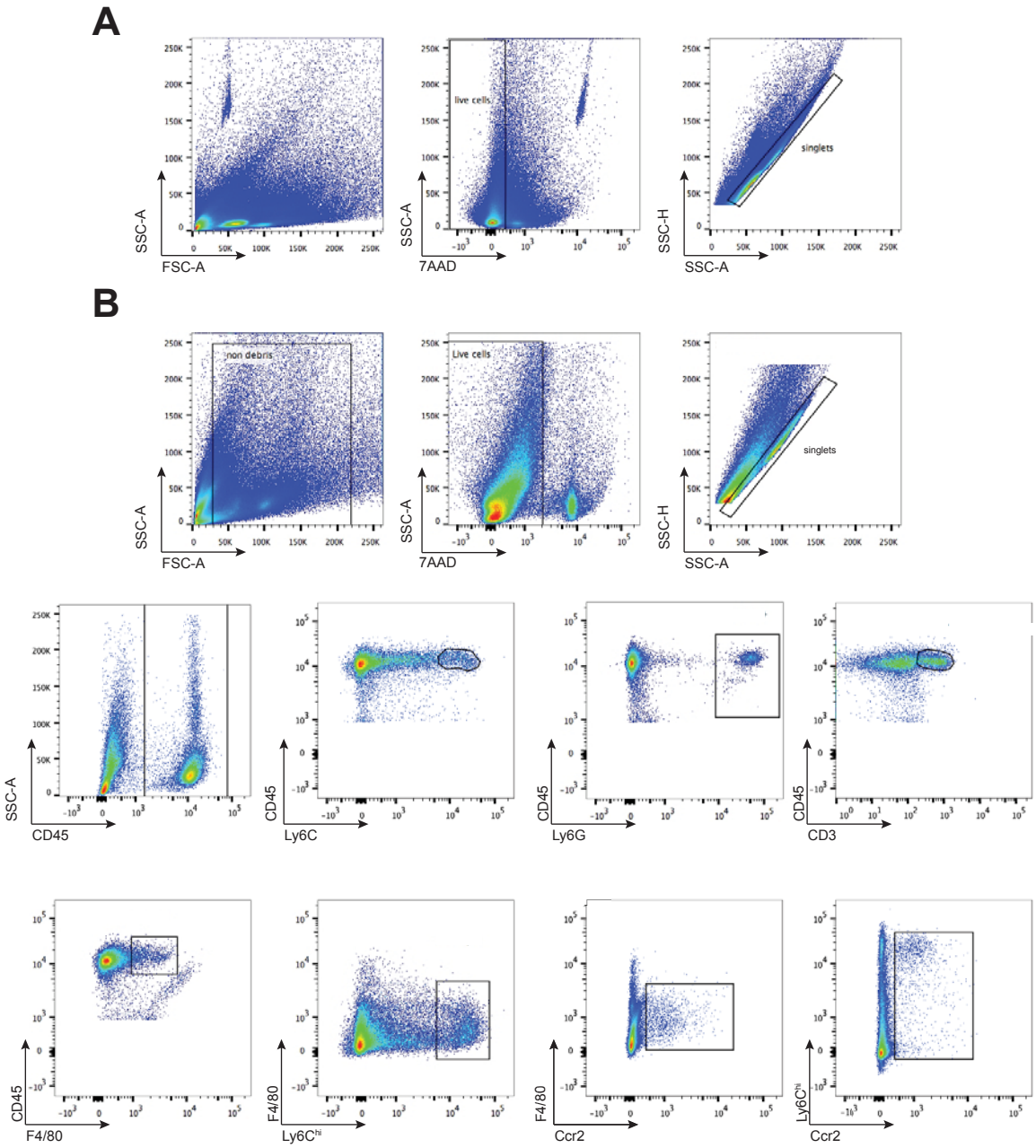
Online Figure I. Induction of canonical BMP signaling target gene *Id2,* as well as relative expression of BMP antagonists after MI. (A) qPCR analysis of whole heart RNA samples isolated from *WT* mice at days 0, 1, 2, 3, 5, 7 and 21 post-MI shows *Id2* gene induction throughout the cardiac tissue repair process. ** *P < 0.01* compared to day 0. One-way ANOVA with Dunnett's multiple comparisons test. N=3 for all time points. All data are means ± SEM. *Id2: Inhibitor of DNA binding 2*. **(B)** Absolute expression of BMP antagonist genes following normalization to *Gapdh*. delta Ct= Ct_{gene}- Ct_{Gapdh}. All data points (N=3) are means ± SEM.



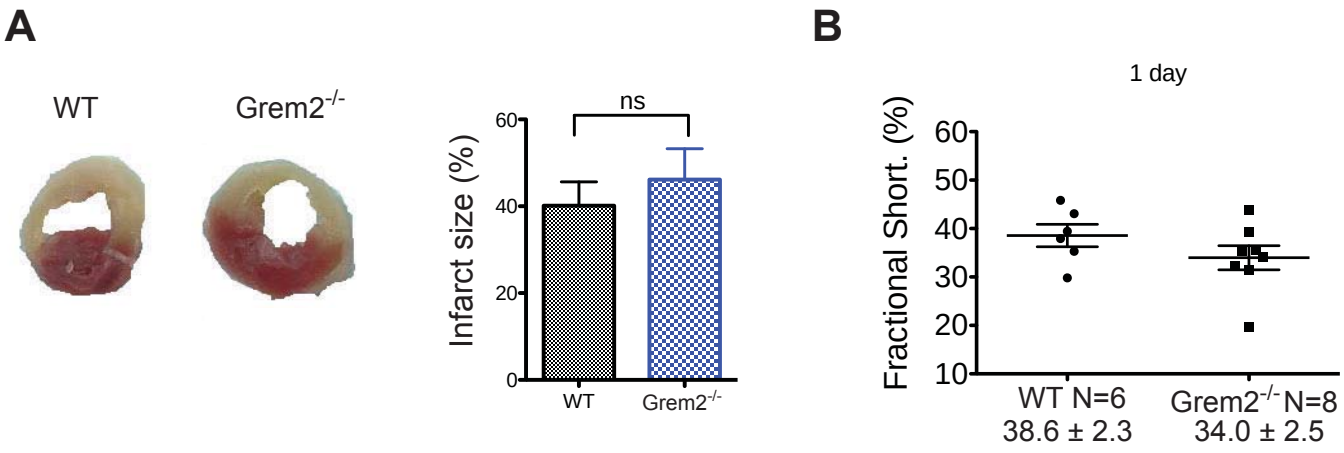
Online Figure II. *Grem2* gene inactivation by homologous recombination. (A) qPCR analysis of whole heart RNA samples isolated from *WT*, *Grem2*$^{+/-}$ and *Grem2*$^{-/-}$ mice shows that *Grem2* baseline gene expression levels are approximately halved in *Grem2*$^{+/-}$ mice and completely absent in *Grem2*$^{-/-}$ mice. * $P < 0.05$. Student's two-tailed unpaired *t*-test. N=3 for all groups. All data are means ± SEM. **(B)** Schematic drawing to mark the location of the primer pairs used to genotype *WT*, *Grem2*$^{+/-}$ and *Grem2*$^{-/-}$ mice. Primer pairs 1,2 and 4,6 are specific to the endogenous *WT* locus, whereas 1,3 and 5,6 amplify DNA fragments generated after homologous recombination. **(C)** Example of conventional PCR results using genomic DNA isolated by mouse tail tip clipping. Mice a,b are *WT*, c,d are *Grem2*$^{-/-}$. The expected size of the amplicons is indicated below. *Gapdh* primers served as controls. L: DNA ladder marker. **(D, E)** *Grem2*$^{-/-}$ hearts appear morphologically normal**. (D)** Whole mount images of 12-week old *WT* and *Grem2*$^{-/-}$ hearts show no differences in morphology and size between the two genotypes. **(E)** Hematoxylin & Eosin stained cardiac sections from the left ventricle of *WT* and *Grem2*$^{-/-}$ show no apparent cellular and tissue abnormalities in *Grem2*$^{-/-}$ hearts. Scale bar, 10 µm.



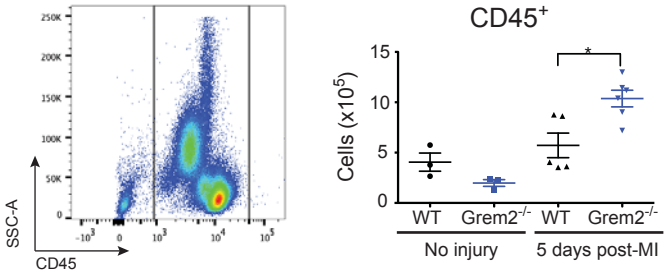
Online Figure III. Chemokine expression levels after MI are comparable among *WT* and *Grem2* mouse lines. qPCR analysis of whole heart RNA samples isolated from *WT*, *Grem2*$^{-/-}$, and *TG*^{Grem2} mice at days 0, 2, and 7 post-MI. Relative expression levels of chemokines in *Grem2*$^{-/-}$ **(A)** and *TG*^{Grem2} **(B)** mice before and after MI are comparable to *WT* with the exception of a moderate increase in *Ccl2* in *Grem2*$^{-/-}$ mice. Student's two-tailed unpaired *t*-test between genotypes at various time points. N=3 per group for all time points. All data are means ± SEM.



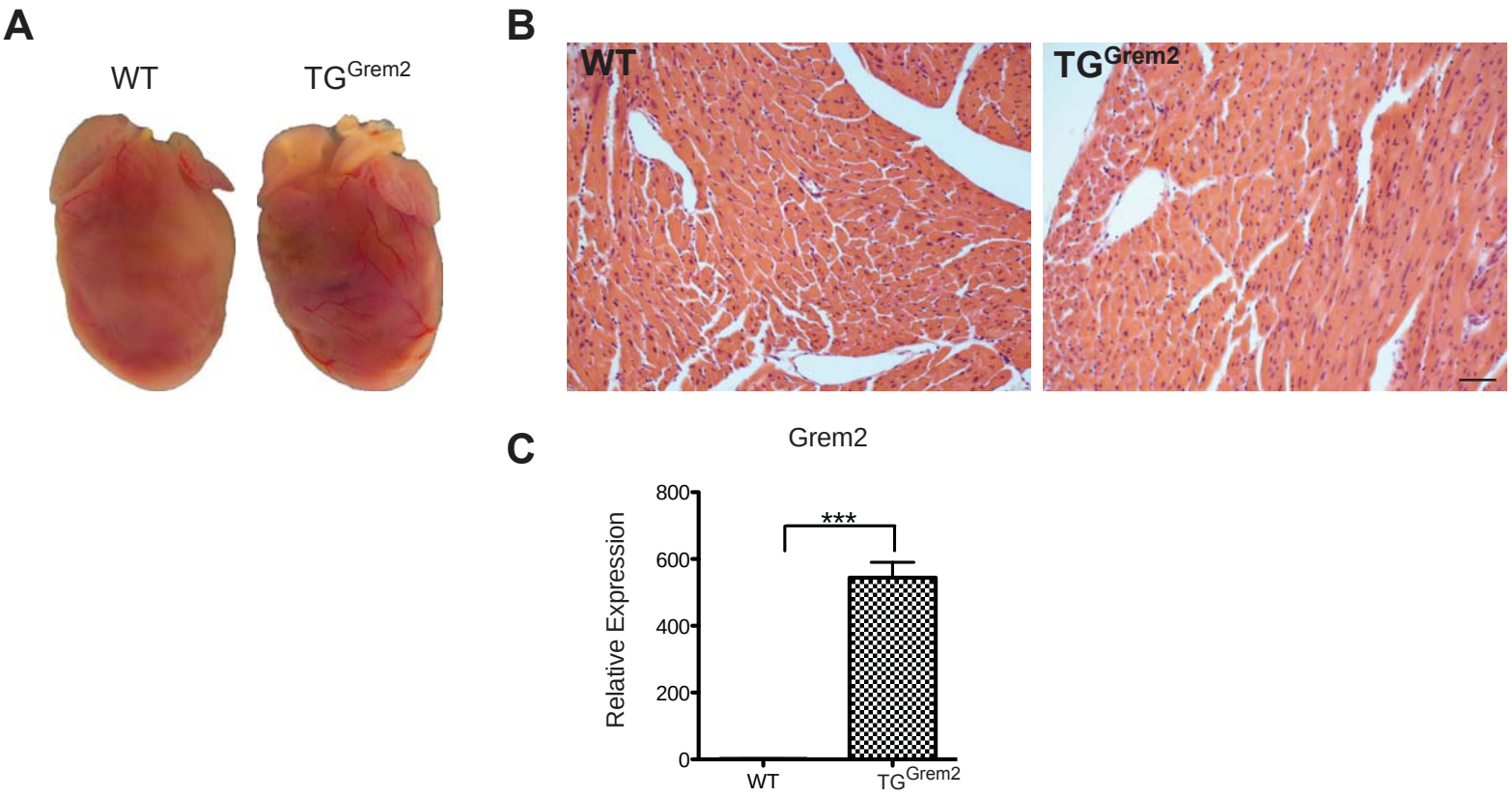
Online Figure IV. Flow cytometry gating strategy. (A) Representative graphs of the flow cytometry analysis of non-cardiomyocyte cells isolated from whole hearts showing the gating strategy, where we gated out debris first, followed by gating for live cells and then singlets. SSC=side scatter, FSC= forward side scatter, 7AAD= 7-Aminoactinomycin D**. (B)** Representative graphs of the flow cytometry analysis of non-cardiomyocyte single cells from a *WT* mouse. $CD45^+$ cells, which represent all leukocytes, were then gated for antibodies used to identity various inflammatory cell subpopulations: $Ly6C^{hi}$ that marks primarily monocytes, Ly6G for neutrophils, CD3 for T-cell lymphocytes, and F4/80 for macrophages. Inflammatory macrophages within the F4/80 population were further characterized as $Ly6C^{hi}$ and/or $Ccr2^+$.



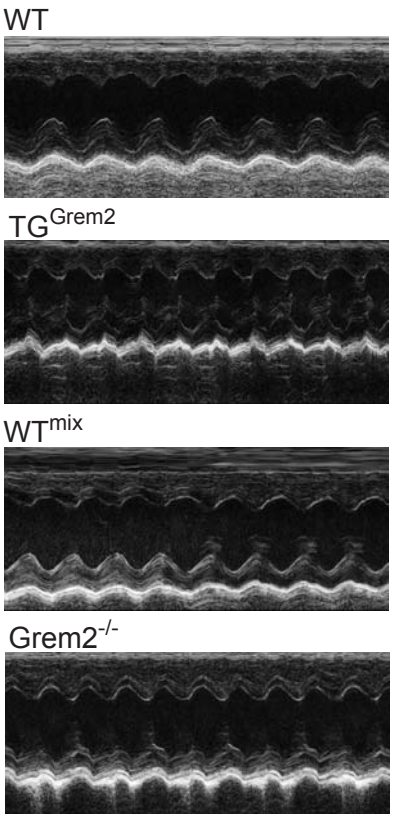
Online Figure V. Infarct sizes are comparable in WT and Grem2 mice. (A) TTC staining on *WT* and *Grem2*$^{-/-}$ mouse hearts at day 1 post-MI illustrates no significant differences in initial infarct sizes. N=6-7 per group. All data are means ± SEM. **(B)** Echocardiography measurements conducted demonstrate comparable drop in %FS at 1 day post-MI, further supporting that the initial injury in these mice are comparable. N=6-8 per group. All data are means ± SEM.



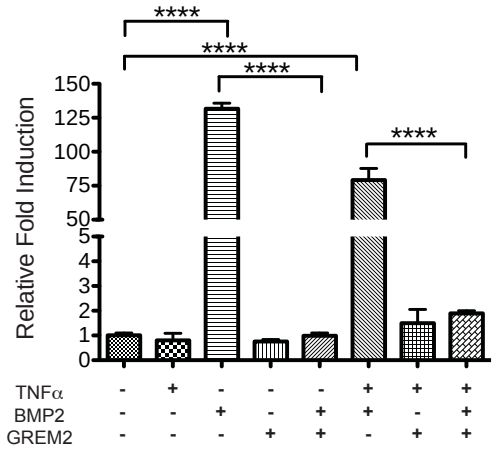
Online Figure VI. Flow cytometry of circulating leukocytes. CD45^{+} cells in blood samples from *WT* and *Grem2*$^{-/-}$ mice demonstrate that there are no significant differences in circulating leukocytes before injury. There is a ~2-fold increase in the level of leukocytes in *Grem2*$^{-/-}$ mice compared to *WTs* at day 5 post-MI. * $P < 0.05$. Student's two-tailed unpaired *t*-test. No injury N=3 per group, post-MI N=5-6. All data are means ± SEM.



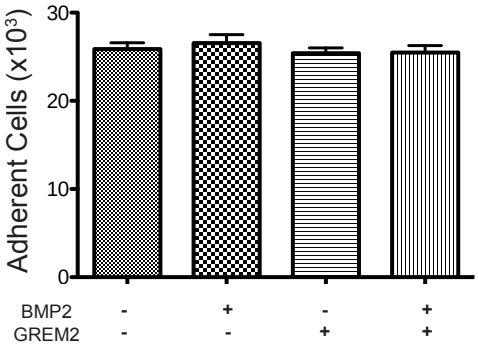
Online Figure VII. *TG^{Grem2}* hearts appear morphologically normal. (A) Whole mount images of 12-week old *WT* and *TG^{Grem2}* hearts show no differences in morphology and size between the two genotypes. **(B)** Hematoxylin & Eosin stained cardiac sections from the left ventricle of *WT* and *TG^{Grem2}* hearts show no apparent cellular and tissue abnormalities. Scale bar, 10 μm. **(C)** qPCR analysis of whole heart RNA samples isolated from *WT* and *TG^{Grem2}* mice shows a significant increase in *Grem2* expression in *TG^{Grem2}* compared to *WT*. *** *P < 0.001*. Student's two-tailed unpaired *t*-test. N=3 for all groups. All data are means ± SEM.



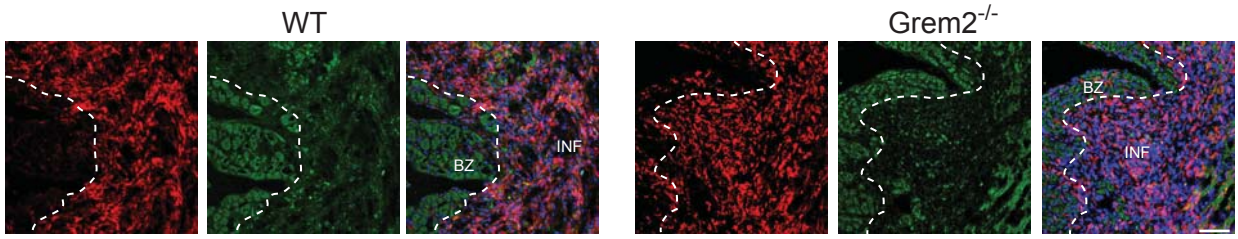
Online Figure VIII. Representative M-mode images from *WT*, *TG*^{Grem2}, *WT*^{mix} and *Grem2*$^{-/-}$ mice 21 days post-MI.



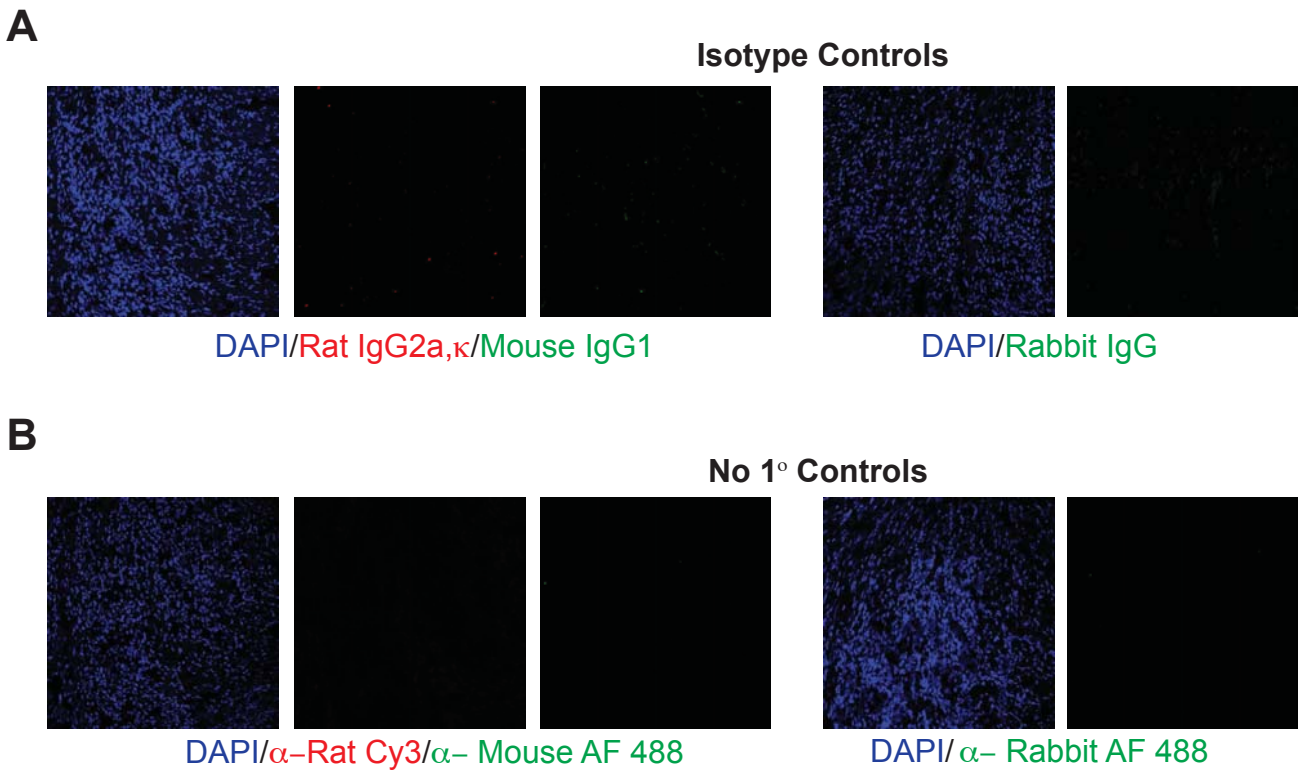
Online Figure IX. Grem2 inhibits canonical BMP signaling in endothelial cells. *ID2* induction in endothelial cells is completely inhibited by Grem2. TNFα has a moderate effect on BMP2-mediated *ID2* induction. ***** P < 0.0001*. One-way ANOVA with Dunnett's multiple comparisons test. N=3 for all treatments. All data are means ± SEM.



Online Figure X. Grem2 acts specifically on endothelial cells. Incubation of human monocytes (THP-1 cells) with BMP2, Grem2, or in combination did not alter binding to endothelial cells (HMEC). N=12. All data are means ± SEM.



Onlline Figure XI. Canonical BMP signaling is not active in infiltrating leukocytes. IF analysis of cardiac tissue sections 5 days post-MI using antibodies recognizing p-Smad1/5/8 (green) and CD45, (red) shows that BMP signaling is not active in infiltrating inflammatory cells of *WT* and *Grem2*$^{-/-}$ hearts. DAPI marks cellular nuclei. Scale bars, 100 μm. BZ=infarct border zone; INF=infarct.



Online Figure XII. Control antibody analyses for immunofluorescence staining. (A) IF using non-immune immunoglobulins (isotype controls) of same species and same concentration as the corresponding primary antibodies used on cardiac tissue sections post-MI. Rat and mouse isotypes controls are shown in combination (left) and the rabbit isotype control is shownindependently (right). **(B)** IF staining with respective secondary antibody-only of the sections in the absence of primary antibodies.

	Body Weight (g)	LV Mass (mg)	HR (bpm)	
WT	26.0 ± 0.2	87.1 ± 10.0	513.8 ± 78.7	
Grem2$^{-/-}$	26.0 ± 2.0	78.7 ± 13.0	627.0 ± 59.4	
P Value	ns	ns	p<0.01	

	IVS;d (mm)	IVS;s (mm)	LVPW;d (mm)	LVPW;s (mm)
WT	0.9 ± 0.07	1.3 ± 0.1	0.8 ± 0.1	1.2 ± 0.1
Grem2$^{-/-}$	1.0 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	1.2 ± 0.1
P Value	ns	ns	ns	ns

	LVID;d (mm)	LVID;s (mm)	LV vol;d (µl)	LV vol;s (µl)
WT	3.5 ± 0.1	1.8 ± 0.1	53.1 ± 3.3	10.6 ± 0.8
Grem2$^{-/-}$	3.3 ± 0.1	1.7 ± 0.1	45.8 ± 2.5	8.5 ± 0.7
P Value	ns	ns	ns	ns

	EF	FS (%)	SV (µL)	CO (ml/min)
WT	0.8 ± 0.01	48.0 ± 0.8	41.8 ± 7.9	21.0 ± 2.3
Grem2$^{-/-}$	0.8 ± 0.01	49.1 ± 1.2	36.6 ± 5.3	23.0 ± 2.6
P Value	ns	ns	ns	ns

Online Table I. Physiological and cardiac functional parameters of *Grem2$^{-/-}$* mice and *WT* siblings. Body weight, left ventricle (LV) mass, heart rates, LV wall and cavity dimensions, including calculated functional parameters (measured by echocardiography), are comparable in *WT^{mix}* and *Grem2$^{-/-}$* adult mice at baseline, with the exception of heart rate. HR=heart rate; IVS=interventricular septum; LVPW=Left Ventricle Posterior Wall; LVID=Left Ventricle Internal Dimension; vol=volume, EF=ejection fraction, FS=fractional shortening; SV=stroke volume; CO=cardiac output; d=dystole; s=systole.ns=not significant. Student's two-tailed unpaired *t*-test. *WT* N=9, *Grem2$^{-/-}$* N=8. All data represent means ± S.D.

	Body Weight (g)	LV Mass (mg)	HR (bpm)	
WT	24.5 ± 0.4	75.6 ± 10.4	619.3 ± 26.8	
TG^{Grem2}	24.3 ± 0.5	60.7 ± 6.6	645.6 ± 35.9	
P Value	ns	ns	ns	
	IVS;d (mm)	**IVS;s (mm)**	**LVPW;d (mm)**	**LVPW;s (mm)**
WT	0.9 ± 0.08	1.1 ± 0.06	0.8 ± 0.07	1.2 ± 0.03
TG^{Grem2}	0.8 ± 0.04	1.1 ± 0.04	0.8 ± 0.04	1.1 ± 0.1
P Value	ns	ns	ns	ns
	LVID;d (mm)	**LVID;s (mm)**	**LV vol;d (µl)**	**LV vol;s (µl)**
WT	3.2 ± 0.1	1.5 ± 0.2	40.6 ± 4.2	7.7 ± 1.4
TG^{Grem2}	3.2 ± 0.1	1.5 ± 0.1	37.7 ± 2.1	7.2 ± 0.6
P Value	ns	ns	ns	ns
	EF	**FS (%)**	**SV (µL)**	**CO (ml/min)**
WT	0.8 ± 0.01	46.2 ± 0.6	33.6 ± 7.2	20.7 ± 3.8
TG^{Grem2}	0.8 ± 0.01	47 .7 ± 0.5	29.0 ± 5.1	18.8 ± 2.9
P Value	ns	ns	ns	ns

Online Table II. Physiological and cardiac functional parameters of *TG^{Grem2}* mice and *WT* siblings.
Body weight, left ventricle (LV) mass, heart rates, LV wall and cavity dimensions, including calculated functional parameters (measured by echocardiography), are comparable in *WT* and *TG^{Grem2}* adult mice at baseline. HR=heart rate; IVS=interventricular septum; LVPW=Left Ventricle Posterior Wall; LVID=LeftVentricle Internal Dimension; vol=volume, EF=ejection fraction, FS=fractionalshortening; SV=stroke volume; CO=cardiac output; d=dystole; s=systole. ns=not significant. Student's two-tailed unpaired *t*-test. Body weight, LV mass, HR, IVSd, IVSs, LBPWd, LVPWs, LVvold, LVvols, SV, CO N=4 per group; LVIDd, LVIDs, EF, FS *WT* N=6, *TG^{Grem2}* N=10. All data representmeans ± S.D.

Online Table III. Primer Sequences used in qPCR analyses and *Grem2*$^{-/-}$ mouse genotyping

Mouse primers qPCR:

Bmp2	5’	GCTGTCTTCTAGTGTTGCTGCTT
	3’	GGGACAGAACTTAAATTGAAGAAGA
Bmp4	5’	ATGATTCCTGGTAACCGAATGCTG
	3’	CTTCGTGATGGAAACTCCTC
Bmp6	5’	AACGCCCTGTCCAATGACG
	3’	ACTCTTGCGGTTCAAGGAGTG
Bmp7	5’	ACGGACAGGGCTTCTCCTAC
	3’	ATGGTGGTATCGAGGGTGGAA
Bmp10	5’	AAATTCGCCACAGACCGGAC
	3’	GGTGAGGGATAGACACATTGAAG
Ccl2	5’	ACCTGCTGCTACTCATTCACC
	3’	CACTGTCACACTGGTCACTCC
Chordin	5’	CTAGGAAATGGCTCCCTTATCTATC
	3’	TGTAAGTGACAATGTGTATCCAAGG
Dand5	5’	CTGTCCTTTGTTCAGGTGATCTC
	3’	CCGAGGGGAGGCTAATTGG
Dan	5’	CTAGGACAATGCTTCAGTTACAGC
	3’	CTTCAGATCTCCATGACAACCAG
E-selectin	5’	GAGCACAGCTTGGTACTACAATGC
	3’	GGTGGCACTTGCAGGTGTAAC
Gapdh	5’	CTCACTCAAGATTGTCAGCAATG
	3’	GAGGGAGATGCTCAGTGTTGG
Grem1	5’	GGAA/TTCTGCAAGCCCAAGAAGTTCACCAC
	3’	CGGGA/TCCTCTGTCCCGTTTGCCATCAC
Grem2[1,2]	5’	CCTGTCATTCACAGAGAGGA
	3’	CATTCGAGCTCTACGATGAC
Icam1	5’	GGAGACGCAGAGGACCTTAACAG
	3’	CATCTCCTGTTTGACAGACTTCACC
Id2	5’	CGACCCGATGAGTCTGCTCTACAAC
	3’	GTGTTCTCCTGGTGAAATGGCTGATAAC
Il-8	5’	CACCTCAAGAACATCCAGAGCT
	3’	CAAGCAGAACTGAACTACCATCG
Il-10	5’	GACCAGCTGGACAACATACTGC
	3’	CCAGCAGACTCAATACACACTGC
Il-1β	5’	TTTGACCTGGGCTGTCCTGATG
	3’	CATATGGGTCCGACAGCACGAG
Noggin	5’	GCCAGCACTATCTACACATCC
	3’	GCGTCTCGTTCAGATCCTTCTC
αSma	5’	CCACGAAACCACCTATAACAGCATC
	3’	GTCGTATTCCTGTTTGCTGATCCAC
Sost	5’	AGCCTTCAGGAATGATGCCAC
	3’	CTTTGGCGTCATAGGGATGGT
Tgfβ1	5’	AGATTAAAATCAAGTGTGGAGCAAC
	3’	GTCCTTCCTAAAGTCAATGTACAGC
Tnfα	5’	CTACTGAACTTCGGGGTGATCGGTCC
	3’	CCTTCATCTTCCTCCTTATCTCTCATGCC

Twsg1	5’	TCTAGCCTCCCTGACGTTCC
	3’	CACATACCGACACAGTCGC
Vcam1	5’	AGAGAAACCATTTATTGTTGACATCTCCC
	3’	CAAGTGGCCCACTCATTTTAATTACTGG

Human primers qPCR:

BMP2	5’	ACCCGCTGTCTTCTAGCGT
	3’	TTTCAGGCCGAACATGCTGAG
E-SELECTIN	5’	GCTGGACTCTCCCTCCTGACATTAGC
	3’	CATAAAGGCATCTGGCATAGTAGGCAAG
GAPDH	5’	AAGGTGAAGGTCGGAGTCAAC
	3’	GGGGTCATTGATGGCAACAATA
GREM2	5’	ATCCCCTCGCCTTACAAGGA
	3’	TCTTGCACCAGTCACTCTTGA
ID2	5’	GCATCCCCCAGAACAAGAAGGTGAG
	3’	CGCTTATTCAGCCACACAGTGCTTTG

Genotyping Primers:

Primer 1	5’	CTGTGCAGCAGAGAAAGCTG
Primer 2	3’	TGGCAATGTACCTCATCTCA
Primer 3	3’	CTGTCCATCTGCACGAGACT
Primer 4	5’	TCTGGTACCCACGAGGACAAGC
Primer 5	5’	GTCTGAGTAGGTGTCATTCTA
Primer 6	3’	CACAGATCACTCGATGCTCT