

SUPPLEMENTAL DATA

TGF β -activated kinase 1 signaling controls acquisition of the inflammatory fibroblast phenotype and regulates cardiac remodeling after myocardial infarction

Daniel C. Nguyen^{1,2}, Jonah K. Stephan^{1,3}, Robert E. Brainard², Kenneth R. Brittian¹, Lianay Gutierrez Luque^{1,3}, Collin K. Wells^{1,3}, Madison S. Taylor¹, Yania Martinez-Ondaro¹, Kara R. Gouwens^{1,2}, Danielle T. Little^{1,3}, Nolan Boyd¹, Richa A. Singhal¹, Jason Hellmann¹, Marcin Wysoczynski¹, and *Bradford G. Hill¹

Affiliations:

¹Center for Cardiometabolic Science, Christina Lee Brown Envirome Institute, Division of Environmental Medicine, Department of Medicine, University of Louisville, Louisville, KY

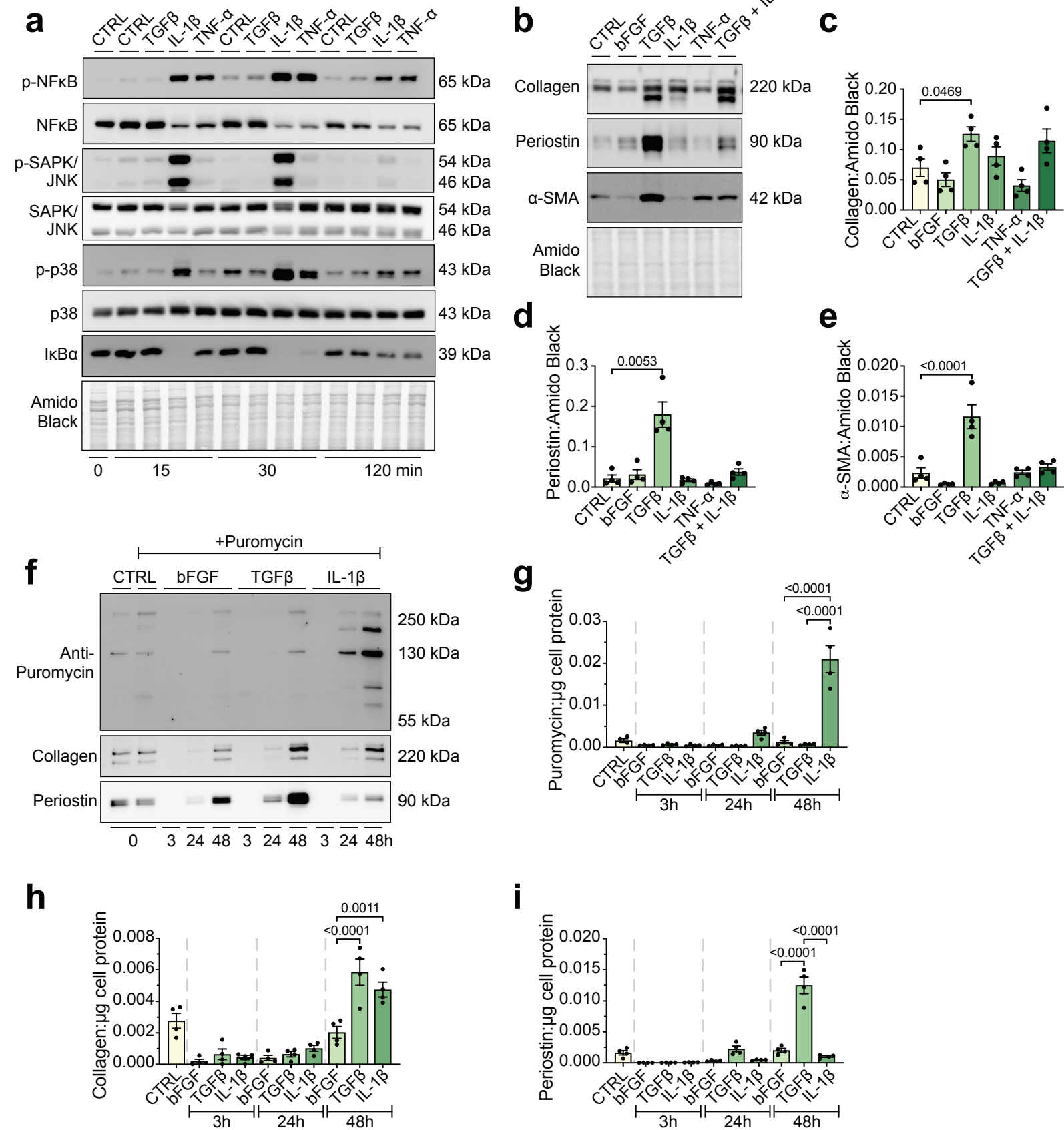
²Department of Physiology, University of Louisville, Louisville, KY

³Department of Biochemistry and Molecular Genetics, University of Louisville, Louisville, KY

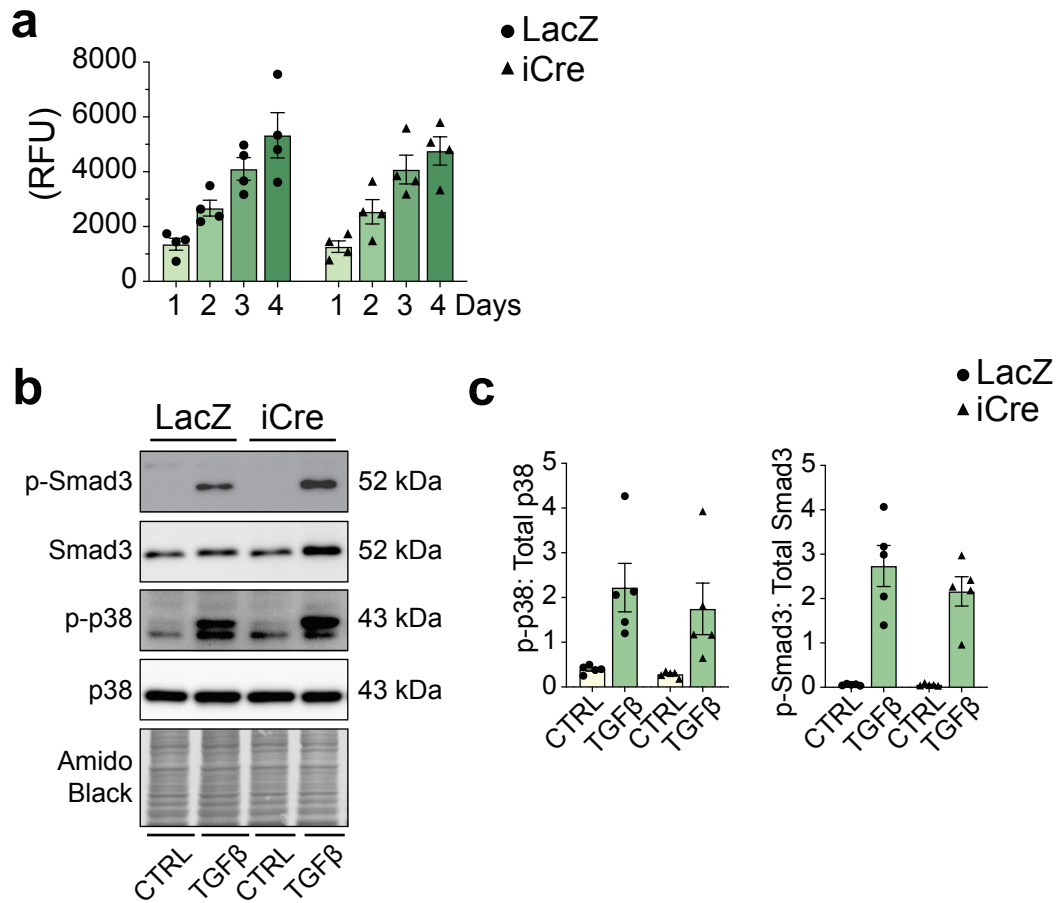
***Correspondence:**

Bradford G. Hill, Ph.D., Center for Cardiometabolic Science, Christina Lee Brown Envirome Institute, Division of Environmental Medicine, Department of Medicine, University of Louisville, 580 S. Preston St., Rm 321E, Louisville, KY 40202; Tel: (502) 852-1015, E-mail: bradford.hill@louisville.edu

Suppl. Fig. 1

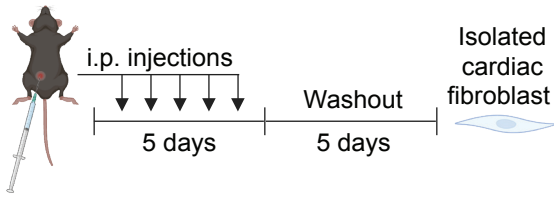


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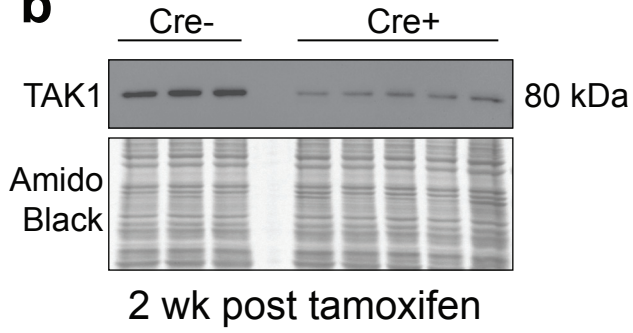


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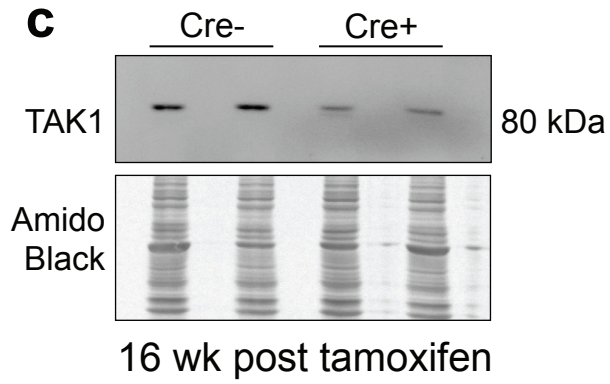
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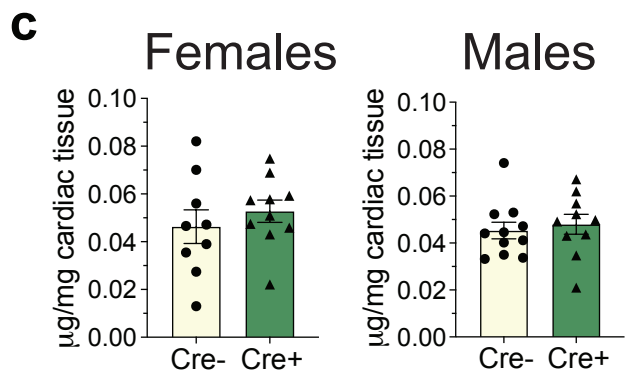
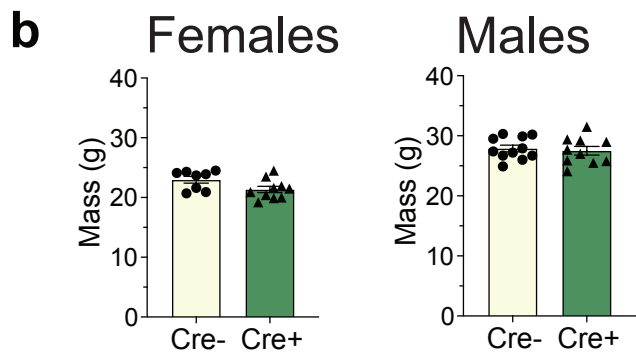
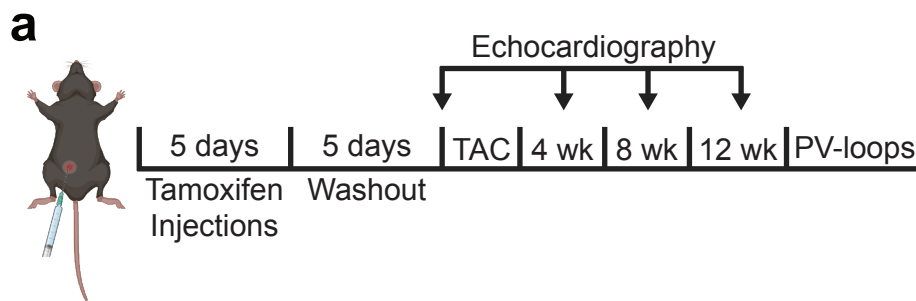
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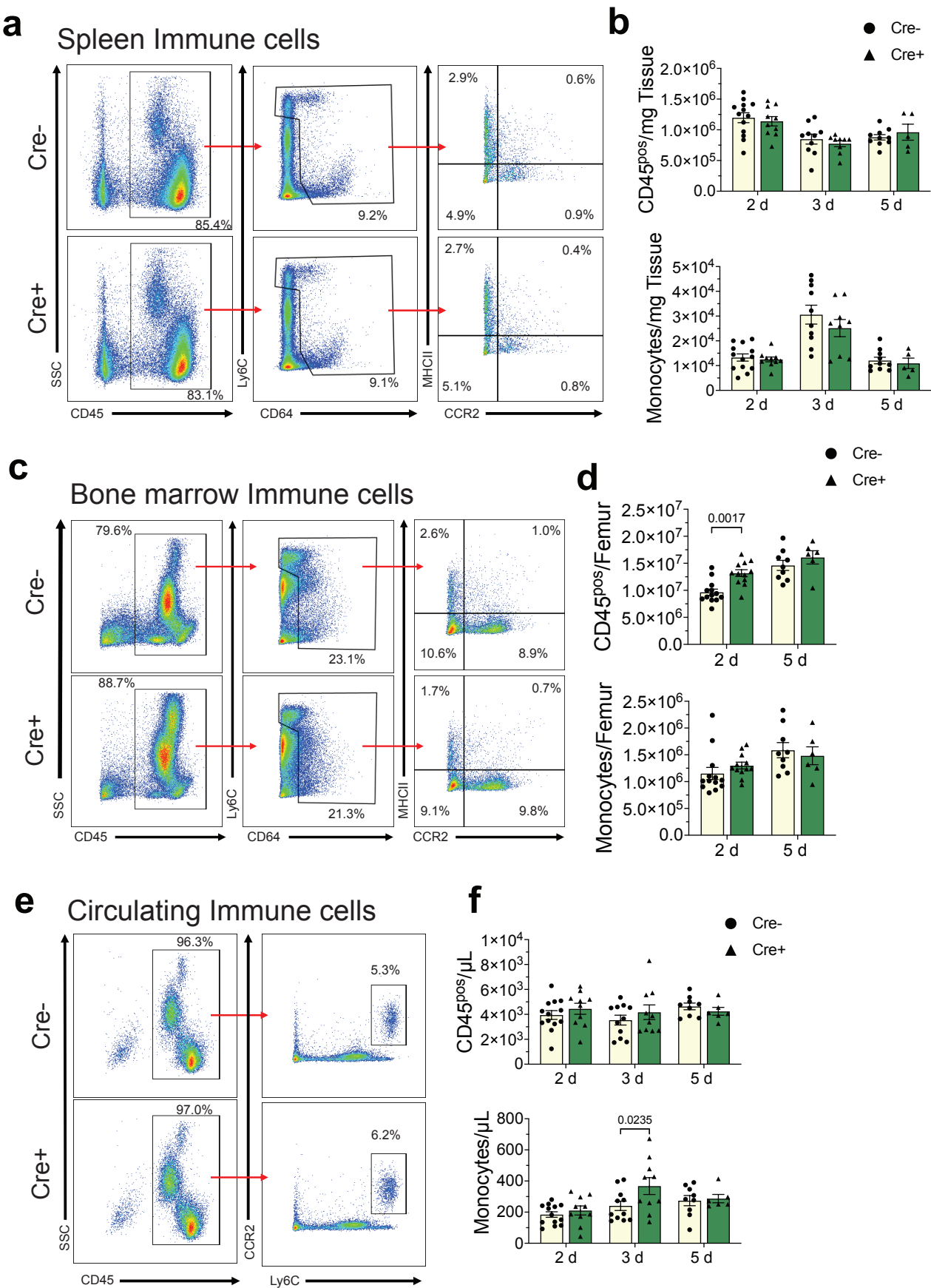
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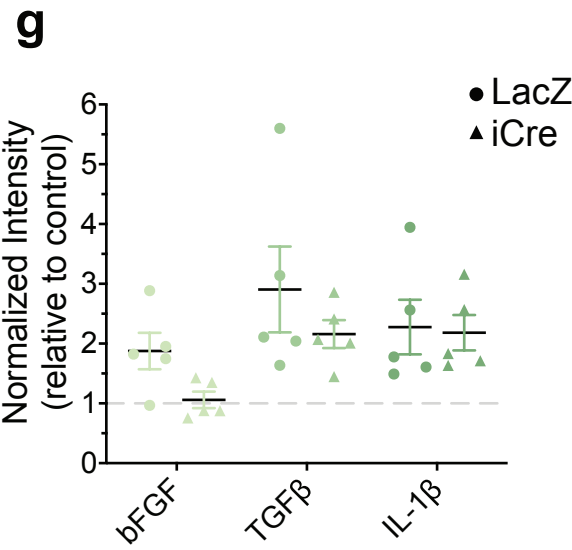
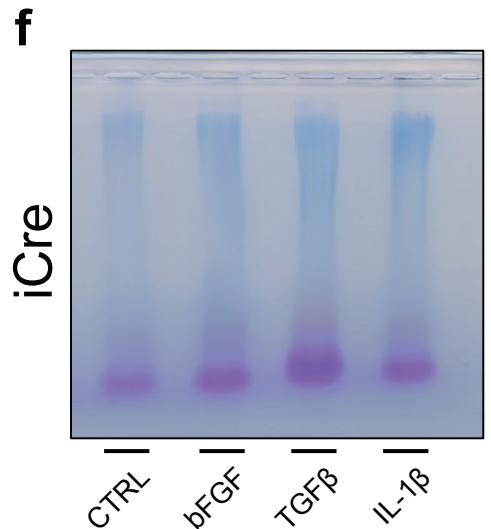
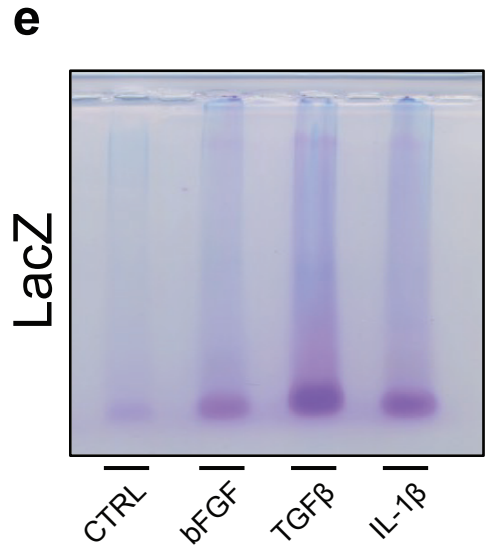
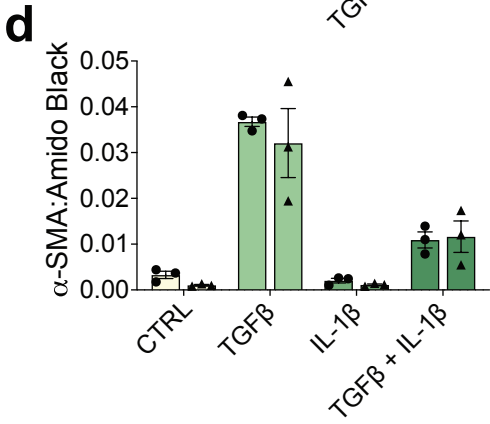
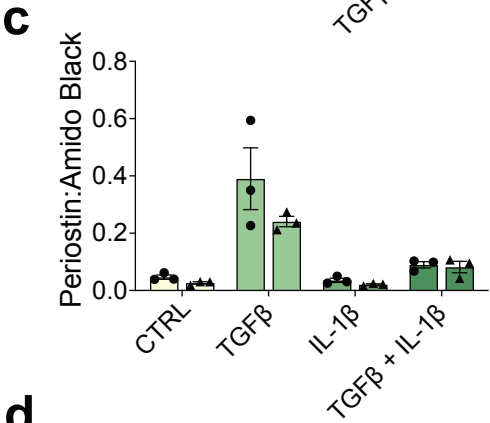
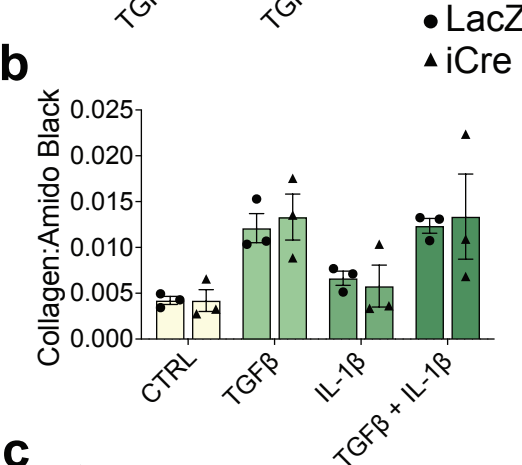
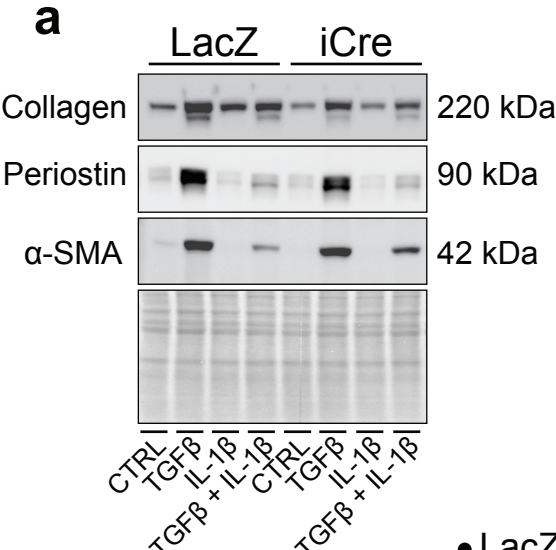
Suppl. Fig. 4



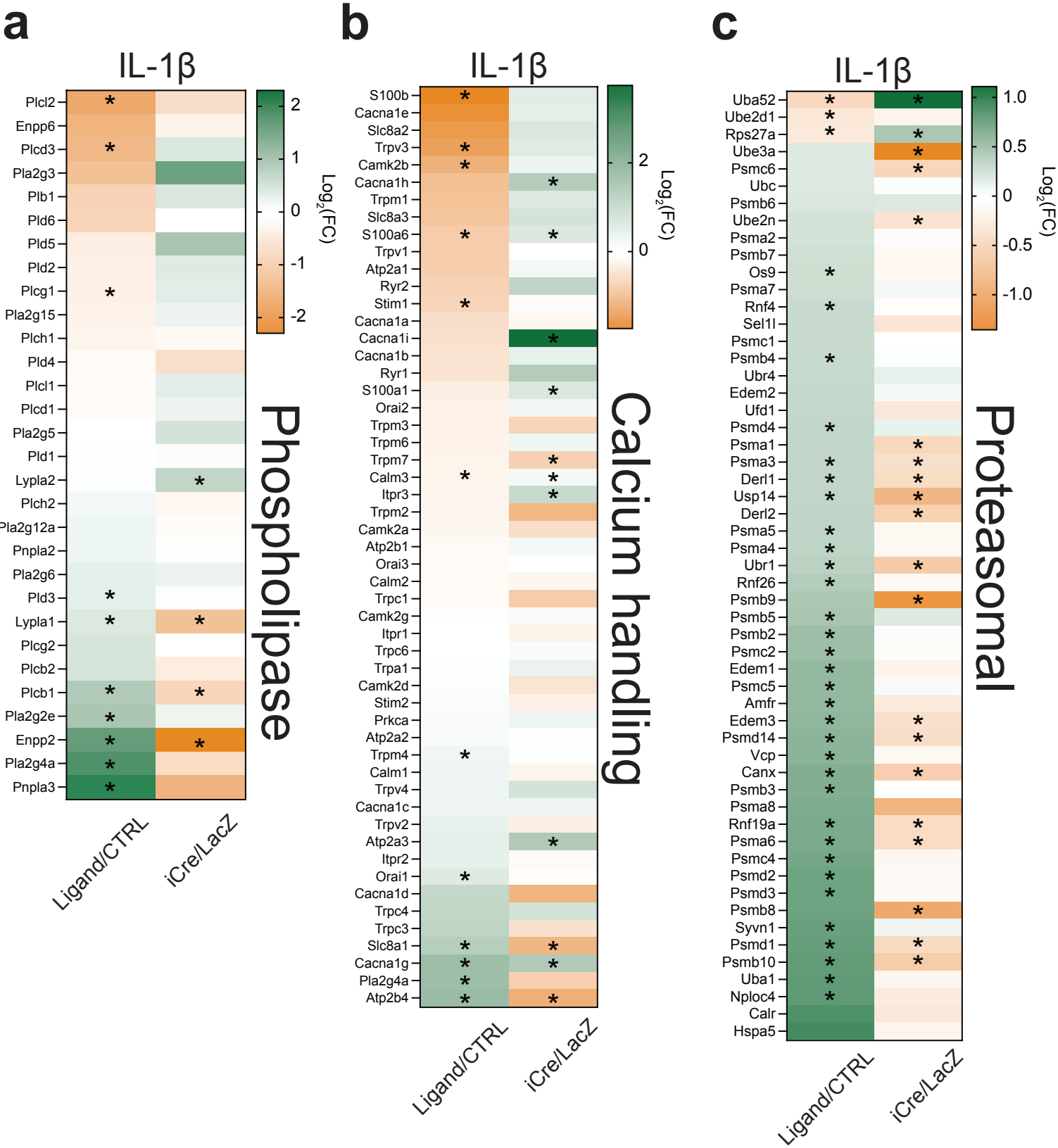
Suppl. Fig. 5



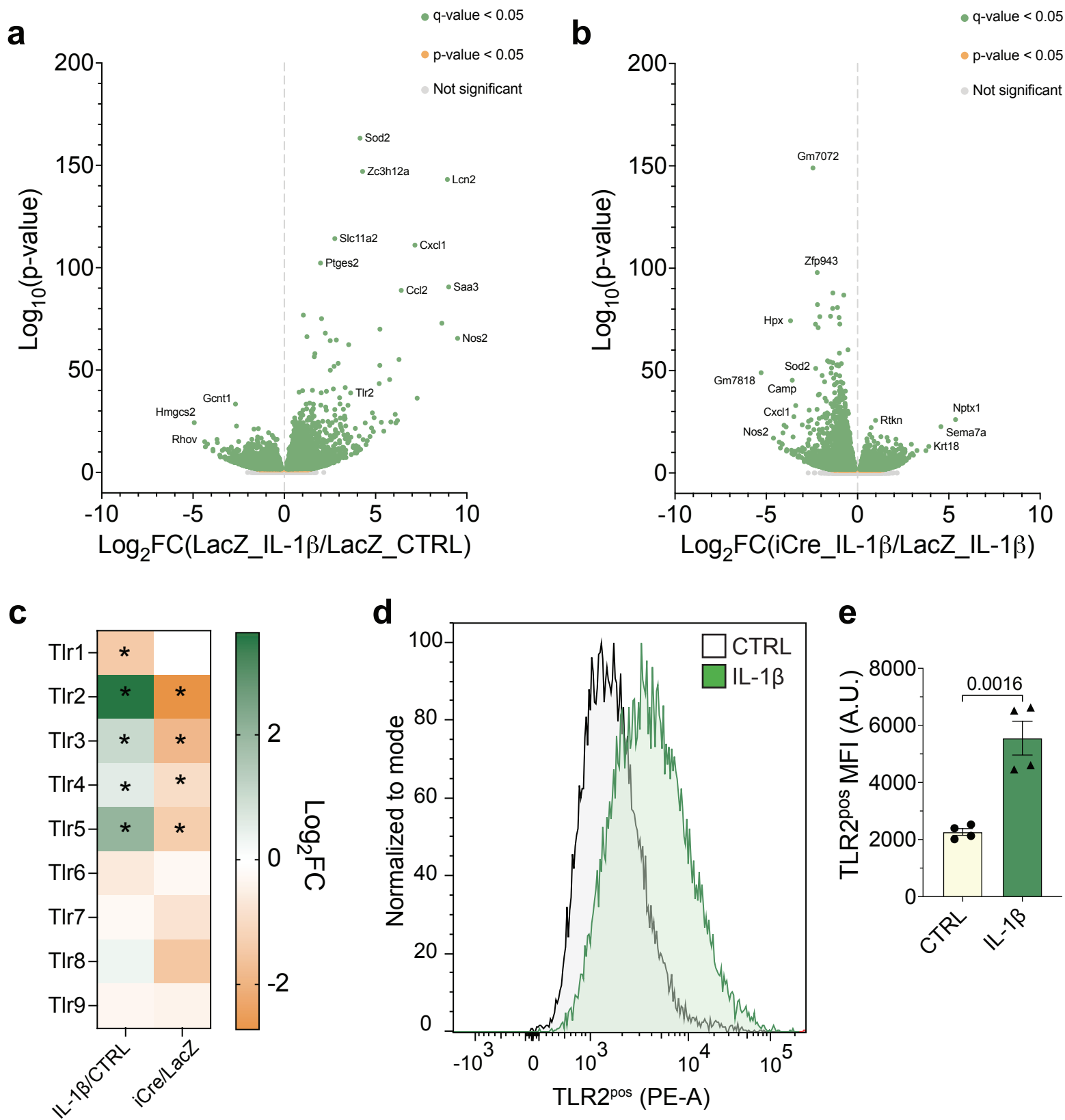
Suppl. Fig. 6



Suppl Fig. 7



Suppl. Fig. 8



Supplementary Figure Legends:

Supplemental Figure 1: IL-1 β increases proinflammatory signaling and enhances fibroblast protein translation and secretion but does not promote myofibroblast differentiation. **a**, Isolated cardiac fibroblasts stimulated with different ligands for up to 120 min prior to protein harvest and immunoblot visualization of inflammatory and stress signaling proteins. $n = 1$ independent biological replicate per group. **b–e**, Representative immunoblots (**b**) and densitometric quantification (**c–e**) of Collagen, Periostin, and α -SMA in fibroblast following 48 h of ligand stimulation. **f–i**, Conditioned medium from fibroblasts treated with ligands for up to 48 h, followed by a 30-min puromycin pulse, was collected for immunoblot visualization (**f**) and quantification of secreted puromycin-labeled proteins (**f**), collagen (**h**), and periostin (**i**). Data are mean $\pm$ SEM. Proteins were normalized to amido black signal from stained membranes (**c–e**) or total μ g cell protein (**g–i**). Experiments and blots were processed in parallel and normalized to an anchor protein. $n = 4$ biological replicates per group. Statistical procedures: (**c–e**) ordinary one-way ANOVA with Dunnett post-hoc test; (**g–i**) two-way ANOVA with Šidák's post-hoc test.

Supplemental Figure 2: Deletion of TAK1 does not affect cell proliferation or TGF β -mediated p38 or Smad3 signaling. **a**, Proliferation and viability of isolated cardiac TAK1^{fl/fl} fibroblasts transduced with LacZ or iCre were assessed for up to 4 days using PrestoBlue assay. $n = 4$ independent biological replicates per group. **b,c**, TAK1^{fl/fl} and TAK1 Δ fibroblasts were treated with TGF β for 30 min prior to protein harvest for immunoblot visualization (**b**) and densitometry quantification of p-p38 and p-Smad3 (**c**). Data are mean $\pm$ SEM. Experiments and blots were processed in parallel and normalized to an anchor protein. $n = 5$ biological replicates per group. Statistical procedure: two-way ANOVA with Šidák's post-hoc test.

Supplemental Figure 3: Tamoxifen-inducible TAK1 knockdown persists in cardiac fibroblasts for up to 16 weeks. **a**, Schematic of tamoxifen injection strategy. **b,c**, Immunoblots of isolated cardiac fibroblasts from Cre⁻ and Cre⁺ TAK1^{fl/fl} mice 2 wk (**b**) and 16 wk (**c**) following tamoxifen administration. $n = 2$ –5 independent biological replicates per group.

Supplemental Figure 4: Fibrotic progression in a transverse aortic constriction (TAC) model is not mediated by fibroblast TAK1 signaling. **a**, Experimental scheme of mice subjected to TAC surgery with echocardiography acquisition at baseline, and at 4, 8, and 12 wk post-TAC, followed by PV-loops. **b**, Body mass taken at the conclusion of the study period. **c**, Quantification of myocardial collagen content using hydroxyproline assay: Data were normalized to mg cardiac tissue. $n = 8$ –11 biological replicates per group. Statistical procedures: Normality was assessed via Shapiro-Wilk test. Parametric unpaired student's t-test or non-parametric Mann-Whitney U test were used as appropriate. Schematics in **a** were created using BioRender (<https://biorender.com>).

Supplemental Figure 5: Fibroblast TAK1 knockdown increases monocytes in bone marrow and the peripheral circulation post-MI. **a,c,e**, Flow cytometric gating strategy for identification of immune cell populations in spleen (**a**), bone marrow (**c**), blood (**e**) at 2, 3, and 5 d post-MI. **b, d, f**, Populations were quantified and normalized to cells/mg splenic tissue (**b**), cells/femur (**d**), cells/ μ l blood (**f**). $n = 5$ –13 male biological replicates per group. Data are shown as mean $\pm$ SEM. Statistical procedures: two-way ANOVA with Šidák's post-hoc.

Supplemental Figure 6: TGF β -mediated myofibroblast differentiation is independent of TAK1 signaling. **a**, Representative immunoblots from TAK1^{fl/fl} and TAK1 Δ cardiac fibroblasts following 48 h of ligand stimulation. **b–d**, Densitometry quantification of Collagen (**b**), Periostin

(c), and α -SMA (d) normalized to amido black signal from stained membranes. n = 3 biological male replicates per group. e–g, Evaluation of secreted hyaluronan in conditioned medium from LacZ (e) or iCre (f) treated fibroblasts following 48 h of ligand stimulation and quantification normalized relative to serum-starved control (g). n = 5 biological replicates per group. Data are mean $\pm$ SEM. Statistical procedure: two-way ANOVA with Šidák's post-hoc test

Supplemental Figure 7: TAK1 signaling controls IL-1 β -induced genes involved in key cellular regulatory processes. a, b, c, Heatmap illustration of log₂FC values for genes associated with phospholipase (a), calcium handling (b), and proteasomal processing (c) from TAK1^{fl/fl} and TAK1 Δ cardiac fibroblasts treated with IL-1 β for 24 h. *q < 0.01, n = 4 biological replicates per group.

Supplemental Figure 8: Fibroblast TAK1 activity regulates IL-1 β -mediated TLR expression in cardiac fibroblasts. a,b, Volcano plot visualization of gene expression fold change comparisons between LacZ_CTRL vs LacZ_IL-1 β (a) and LacZ_IL-1 β vs iCre_IL-1 β (b). c, Heat map of *Tlr* gene expression. *q < 0.01. n = 4 biological replicates per group. d,e, TLR2 surface expression in isolated cardiac fibroblast following 48 h of IL-1 β stimulation examined by flow cytometry. n = 4 biological replicates per group. Data are mean $\pm$ SEM. Statistical procedures: Normality was assessed via Shapiro-Wilk test followed by parametric unpaired student's t-test.

Supplemental Table 1: Gravimetric and echocardiography measurements of cardiac function in unstressed Cre⁻ and Cre⁺ TAK1^{fl/fl} male mice.

Males Cre-								
	Baseline		2-weeks		4-weeks		16-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	529.3	26.7	589.4	12.9	559.1	21.4	505.0	28.1
LVESV (uL)	9.4	1.7	10.2	1.7	11.8	2.4	9.2	1.3
LVEDV (uL)	35.4	3.8	39.0	3.3	39.6	6.0	37.1	4.5
IVRT (ms)	12.9	2.0	12.6	1.0	13.5	0.9	11.2	0.8
FS (%)	48.8	5.2	51.9	2.4	51.5	3.2	45.2	4.8
Ejection Fraction (%)	73.5	4.6	74.2	3.1	70.4	3.0	75.2	2.3
Cardiac Output (mL/min)	13.8	1.8	17.0	1.1	15.5	1.9	14.1	1.9
SV (uL)	26.1	3.6	28.9	2.2	27.9	4.1	27.9	3.6
LVAW;d (mm)	1.2	0.1	1.1	0.1	1.1	0.1	1.2	0.1
LVAW;s (mm)	1.8	0.2	1.8	0.1	1.8	0.1	1.8	0.1
LVID;d (mm)	3.3	0.1	3.4	0.2	3.6	0.2	3.2	0.2
LVID;s (mm)	1.7	0.2	1.6	0.1	1.7	0.2	1.8	0.2
LVPW;d (mm)	1.0	0.2	0.9	0.1	0.9	0.2	1.0	0.2
LVPW;s (mm)	1.6	0.2	1.6	0.1	1.6	0.2	1.6	0.3
Males Cre+								
	Baseline		2-weeks		4-weeks		16-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	526.6	26.8	565.9	18.8	572.5	11.8	538.2	51.6
LVESV (uL)	8.5	4.2	11.7	8.2	10.4	7.5	9.4	5.7
LVEDV (uL)	33.2	11.7	37.7	12.9	34.6	14.1	36.1	12.2
IVRT (ms)	11.5	0.8	13.3	1.4	13.6	1.5	11.4	0.9
FS (%)	49.7	6.9	51.0	10.4	51.8	7.6	46.3	5.1
Ejection Fraction (%)	75.1	6.0	71.1	9.7	71.8	11.2	75.4	6.9
Cardiac Output (mL/min)	12.9	3.9	14.7	2.8	13.9	4.7	14.2	3.1
SV (uL)	24.7	7.8	26.0	5.6	24.2	8.1	26.6	6.9
LVAW;d (mm)	1.2	0.1	1.1	0.1	1.2	0.2	1.2	0.1
LVAW;s (mm)	1.8	0.2	1.7	0.3	1.8	0.3	1.8	0.2
LVID;d (mm)	3.2	0.4	3.3	0.5	3.3	0.4	3.3	0.4
LVID;s (mm)	1.6	0.3	1.7	0.6	1.6	0.4	1.8	0.4
LVPW;d (mm)	1.0	0.2	0.9	0.2	1.1	0.3	1.0	0.2
LVPW;s (mm)	1.6	0.2	1.5	0.2	1.7	0.3	1.5	0.3
Morphometrics	Cre-		Cre+					
	Mean	± SD	Mean	± SD				
Body mass (g)	31.3	2.8	28.7	2.2				
Heart weight/tibial length (mg/mm)	6.6	0.5	6.2	0.8				
Wet/dry lung ratio (mg/mg)	4.0	0.3	4.0	0.2				

Cre⁻ and Cre⁺ TAK1^{fl/fl} male mice underwent echocardiography prior to (baseline) as well as 2-, 4-, and 16-weeks following tamoxifen administration. Organs were harvested and body mass taken at the conclusion of the study, n = 6 – 8 male mice per group. Statistics: two-way ANOVA with Šidák's post-hoc or unpaired 2-tailed t test as appropriate.

Supplemental Table 2: Gravimetric and echocardiography measurements of cardiac function in unstressed Cre⁻ and Cre⁺ TAK1^{fl/fl} female mice.

Females Cre-								
	Baseline		2-weeks		4-weeks		16-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	520.1	6.8	560.1	28.3	565.9	33.3	534.2	49.8
LVESV (uL)	7.3	2.0	14.5	8.0	13.7	10.0	9.2	4.5
LVEDV (uL)	31.7	9.2	40.2	9.4	37.3	14.8	35.1	10.4
IVRT (ms)	12.0	0.5	14.6	1.9	12.6	1.3	11.0	0.6
FS (%)	47.4	6.0	47.1	5.8	49.3	6.4	46.2	7.2
Ejection Fraction (%)	76.7	4.5	65.3	15.1	66.1	10.1	74.6	5.2
Cardiac Output (mL/min)	12.7	4.1	14.4	3.4	13.2	2.1	13.6	1.9
SV (uL)	24.4	7.7	25.8	6.8	23.5	4.8	25.8	6.0
LVAW;d (mm)	1.0	0.0	1.0	0.1	1.0	0.1	1.1	0.1
LVAW;s (mm)	1.6	0.1	1.6	0.1	1.7	0.2	1.6	0.2
LVID;d (mm)	3.3	0.2	3.6	0.2	3.5	0.4	3.2	0.3
LVID;s (mm)	1.7	0.2	1.9	0.3	1.8	0.5	1.7	0.4
LVPW;d (mm)	1.0	0.1	0.9	0.1	0.9	0.2	1.0	0.2
LVPW;s (mm)	1.6	0.1	1.5	0.1	1.6	0.2	1.6	0.2
Females Cre+								
	Baseline		2-weeks		4-weeks		16-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	527.9	17.7	552.1	28.0	553.4	14.4	540.9	33.0
LVESV (uL)	8.9	3.3	12.7	7.0	8.2	3.2	8.2	3.2
LVEDV (uL)	33.0	6.9	36.3	12.7	31.1	10.3	32.5	6.4
IVRT (ms)	12.8	1.0	12.6	1.9	13.5	1.4	11.4	0.6
FS (%)	48.5	5.7	48.9	9.7	52.7	11.1	51.1	6.0
Ejection Fraction (%)	73.7	4.5	67.3	10.5	73.8	4.4	75.4	5.8
Cardiac Output (mL/min)	12.7	2.2	12.9	3.3	12.6	3.9	13.0	1.6
SV (uL)	24.1	3.9	23.6	6.8	22.9	7.4	24.3	4.0
LVAW;d (mm)	1.1	0.1	1.0	0.1	1.1	0.3	1.0	0.1
LVAW;s (mm)	1.6	0.2	1.5	0.2	1.7	0.3	1.6	0.1
LVID;d (mm)	3.2	0.3	3.2	0.6	3.3	0.5	3.2	0.5
LVID;s (mm)	1.7	0.3	1.7	0.6	1.6	0.5	1.6	0.4
LVPW;d (mm)	0.9	0.2	0.9	0.2	1.1	0.3	1.1	0.3
LVPW;s (mm)	1.4	0.3	1.4	0.3	1.6	0.3	1.7	0.3
Morphometrics	Cre-		Cre+					
	Mean	± SD	Mean	± SD				
Body mass (g)	24.6	2.3	23.5	1.3				
Heart weight/tibial length (mg/mm)	5.6	1.1	5.5	0.6				
Wet/dry lung ratio (mg/mg)	4.0	0.1	4.0	0.2				

Cre⁻ and Cre⁺ TAK1^{fl/fl} female mice underwent echocardiography prior to (baseline) as well as 2-, 4-, and 16-weeks following tamoxifen administration. Organs were harvested and body mass taken at the conclusion of the study, n = 6 – 8 male mice per group. Statistics: two-way ANOVA with Šidák's post-hoc or unpaired 2-tailed t test as appropriate.

Supplemental Table 3: Echocardiography measurements of cardiac function, gravimetric, and mortality in Cre⁻ and Cre⁺ TAK1^{fl/fl} male mice following TAC.

Males Cre-								
	Baseline		4-weeks		8-weeks		12-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	541.4	29.1	534.6	53.0	556.2	24.8	551.0	29.8
LVESV (uL)	8.3	1.9	16.5	6.8	20.8	10.6	26.9	22.9
LVEDV (uL)	32.8	5.6	39.3	6.9	42.5	10.8	49.2	20.4
IVRT (ms)	12.3*	0.9	11.6	2.6	15.1	2.1	15.5	2.4
FS (%)	50.1	5.8	38.4	9.1	35.9	13.1	32.3	13.9
Ejection Fraction (%)	74.7	3.5	59.0	10.8	54.1	15.1	51.9	19.8
Cardiac Output (mL/min)	13.3	2.3	12.0	1.3	12.1	2.0	12.2	2.5
SV (uL)	24.5	4.0	22.8	3.9	21.7	3.6	22.3	4.8
LVAW;d (mm)	1.1	0.1	1.3	0.1	1.3	0.2	1.3	0.2
LVAW;s (mm)	1.7	0.1	1.8	0.1	1.8	0.3	1.8	0.2
LVID;d (mm)	3.2	0.2	3.5	0.3	3.6	0.5	3.8	0.6
LVID;s (mm)	1.6	0.2	2.2	0.5	2.4	0.7	2.7	0.9
LVPW;d (mm)	0.8	0.2	1.0	0.2	1.1	0.1	1.2	0.3
LVPW;s (mm)	1.5	0.2	1.5	0.1	1.5	0.2	1.5	0.2
LV Mass AW (corrected) (mg)	88.2	11.2	129.0	31.1	144.4	37.7	163.4	58.5
Males Cre+								
	Baseline		4-weeks		8-weeks		12-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	518.3	29.2	564.5	27.4	564.5	27.5	571.1	38.9
LVESV (uL)	10.7	3.5	18.6	14.8	26.1	12.8	26.0	15.7
LVEDV (uL)	39.3	8.5	39.3	17.6	46.2	14.3	48.4	20.6
IVRT (ms)	11.2*	0.6	12.4	2.3	15.5	0.7	14.4	2.8
FS (%)	47.3	5.0	35.7	14.7	25.9	6.6	30.3	10.7
Ejection Fraction (%)	73.3	4.7	58.8	17.6	46.8	13.5	50.4	12.9
Cardiac Output (mL/min)	14.8	2.5	11.6	2.2	11.4	2.1	12.8	3.4
SV (uL)	28.6	5.6	20.6	4.2	20.1	3.7	22.4	6.0
LVAW;d (mm)	1.1	0.1	1.3	0.2	1.3	0.2	1.3	0.1
LVAW;s (mm)	1.7	0.1	1.7	0.3	1.7	0.3	1.7	0.2
LVID;d (mm)	3.5	0.3	3.5	0.6	3.9	0.5	3.8	0.7
LVID;s (mm)	1.8	0.3	2.3	0.8	2.9	0.5	2.7	0.8
LVPW;d (mm)	0.9	0.2	1.1	0.2	1.1	0.3	1.2	0.3
LVPW;s (mm)	1.4	0.2	1.6	0.2	1.5	0.4	1.5	0.3
LV Mass AW (corrected) (mg)	99.4	19.3	139.9	36.2	165.2	39.1	167.9	48.7
Morphometrics	Cre-		Cre+					
	Mean	± SD	Mean	± SD				
Heart weight/tibial length (mg/mm)	9.2	2.1	10.3	2.5				
Wet/dry lung ratio (mg/mg)	4.1	0.1	4.0	0.3				
Mortality	Total		Survived		Mortality			
Cre-	14		11		3			
Cre+	13		10		3			

Cre⁻ and Cre⁺ TAK1^{fl/fl} male mice underwent echocardiography prior to (baseline) as well as 4-, 8-, and 12-weeks following TAC. Organs were harvested at the conclusion of the study, n = 13-14 male mice per group. Statistics: two-way ANOVA with Šidák's post-hoc or unpaired 2-tailed t test as appropriate, * p < 0.05, Cre⁻ vs Cre⁺.

Supplemental Table 4: Echocardiography measurements of cardiac function, gravimetric, and mortality in Cre⁻ and Cre⁺ TAK1^{fl/fl} female mice following TAC.

Females Cre-								
	Baseline		4-weeks		8-weeks		12-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	532.8	27.5	552.1	28.5	552.1	36.1	562.8	28.9
LVESV (uL)	7.4	1.7	14.4	9.9	14.7	10.6	16.9	13.4
LVEDV (uL)	30.9	5.0	33.6	9.8	36.8	9.8	34.5	11.3
IVRT (ms)	11.4	0.9	11.7	0.9	15.3	1.1	15.1	1.3
FS (%)	50.5	4.4	39.8	12.0	40.8	13.8	37.7	12.0
Ejection Fraction (%)	76.1	2.3	60.8	14.3	63.6	15.1	56.2	18.8
Cardiac Output (mL/min)	12.5	2.0	10.6	1.0	12.2	1.2	9.9	1.6
SV (uL)	23.5	3.5	19.2	1.5	22.1	2.0	17.7	3.0
LVAW;d (mm)	1.0	0.1	1.2	0.1	1.3	0.1	1.3	0.2
LVAW;s (mm)	1.6	0.1	1.8	0.2	1.8	0.2	1.8	0.2
LVID;d (mm)	3.2	0.2	3.4	0.4	3.3	0.4	3.3	0.5
LVID;s (mm)	1.6	0.2	2.1	0.7	2.0	0.8	2.1	0.8
LVPW;d (mm)	0.8	0.1	1.1	0.2	1.1	0.2	1.1	0.2
LVPW;s (mm)	1.4	0.1	1.5	0.2	1.6	0.2	1.5	0.2
LV Mass AW (corrected) (mg)	77.1	10.5	124.3	33.9	127.7	26.8	133.1	30.9
Females Cre+								
	Baseline		4-weeks		8-weeks		12-weeks	
Echocardiography	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Heart Rate (BPM)	501.3	29.1	536.5	34.9	548.3	19.9	557.1	22.2
LVESV (uL)	7.9	2.5	15.0	8.6	15.3	8.0	23.0	14.8
LVEDV (uL)	31.0	6.4	32.5	10.1	35.3	10.5	41.4	17.7
IVRT (ms)	12.8	0.8	13.1	1.8	15.7	1.1	16.0	1.0
FS (%)	49.2	5.4	37.8	9.6	39.0	9.8	30.6	9.7
Ejection Fraction (%)	75.0	3.8	56.6	11.3	58.9	12.1	48.1	11.0
Cardiac Output (mL/min)	11.5	2.0	9.3	1.2	10.9	2.3	10.2	1.8
SV (uL)	23.1	4.2	17.5	2.7	20.0	4.4	18.5	3.6
LVAW;d (mm)	1.0	0.1	1.2	0.1	1.3	0.1	1.4	0.1
LVAW;s (mm)	1.6	0.2	1.6	0.2	1.8	0.2	1.7	0.1
LVID;d (mm)	3.2	0.3	3.4	0.4	3.3	0.4	3.6	0.6
LVID;s (mm)	1.6	0.3	2.1	0.5	2.0	0.5	2.5	0.7
LVPW;d (mm)	0.9	0.1	1.0	0.3	1.1	0.2	1.1	0.2
LVPW;s (mm)	1.4	0.1	1.5	0.3	1.5	0.2	1.5	0.2
LV Mass AW (corrected) (mg)	84.3	10.6	115.0	27.1	126.2	22.8	152.9	46.7
Morphometrics								
	Cre-		Cre+					
	Mean	± SD	Mean	± SD				
Heart weight/tibial length (mg/mm)	8.0	1.7	7.7	1.1				
Wet/dry lung ratio (mg/mg)	3.8	0.3	4.2	0.2				
Mortality								
	Total		Survived		Mortality			
Cre-	11		8		3			
Cre+	11		10		1			

Cre⁻ and Cre⁺ TAK1^{fl/fl} female mice underwent echocardiography prior to (baseline) as well as 4-, 8-, and 12-weeks following TAC. Organs were harvested at the conclusion of the study, n = 11 female mice per group. Statistics: two-way ANOVA with Šidák's post-hoc or unpaired 2-tailed t test as appropriate, * p < 0.05, Cre⁻ vs Cre⁺.

Supplemental Table 5: Left ventricular pressure-volume analysis in Cre⁻ and Cre⁺ TAK1^{fl/fl} mice following TAC.

Males				
	Cre-		Cre+	
	Mean	±SD	Mean	±SD
EDPVR (mmHg/ml)	0.061	0.040	0.096	0.101
ESPVR (mmHg/ml)	7.4	4.4	9.8	8.5
Heart rate (bpm)	389.0	39.8	369.5	34.8
ESP (mmHg)	130.4	30.4	133.8	26.0
EDP (mmHg)	8.5	3.6	8.9	3.6
dPmax (mmHg/sec)	6263.8	557.3	6429.5	971.8
dPmin (mmHg/sec)	-7267.6	875.4	-7169.9	1299.9
ESV (μl)	19.8	11.4	28.2	17.9
EDV (μl)	42.8	11.6	48.1	18.6
SV (μl)	23.0	6.3	19.9	3.5
CO (μl/min)	8910.7	2528.5	7464.3	1471.2
EF (%)	55.5	16.6	46.7	19.8
SW (mJoules)	0.457	0.158	0.407	0.133
Tau Weiss (msec)	7.2	0.6	7.1	0.9
Females				
	Cre-		Cre+	
	Mean	±SD	Mean	±SD
EDPVR (mmHg/ml)	0.053	0.019	0.097	0.064
ESPVR (mmHg/ml)	12.4	14.8	7.6	4.6
Heart rate (bpm)	400.0	23.2	402.8	31.8
ESP (mmHg)	137.3	24.2	141.2	32.2
EDP (mmHg)	10.8	5.0	8.6	3.6
dPmax (mmHg/sec)	7104.2	2056.1	7095.5	1635.0
dPmin (mmHg/sec)	-7759.0	1866.1	-8021.4	1421.0
ESV (μl)	22.9	11.0	27.2	4.7
EDV (μl)	40.1	12.7	42.7	6.4
SV (μl)	17.1	3.5	15.5	2.0
CO (μl/min)	6865.1	1441.6	6283.9	1131.4
EF (%)	46.7	17.2	36.4	2.6
SW (mJoules)	0.348	0.146	0.356	0.087
Tau Weiss (msec)	7.0	1.1	6.5	0.3

Cre⁻ and Cre⁺ TAK1^{fl/fl} mice underwent PV-loop analysis at 12-weeks following TAC, n = 6 mice per group. Statistics: unpaired 2-tailed t test, * p < 0.05, Cre⁻ vs Cre⁺.

Supplemental Table 6: Echocardiography measurements of cardiac function, gravimetric, and mortality in Cre⁻ and Cre⁺ TAK1^{fl/fl} mice following MI.

Males								
	Cre -				Cre +			
	7 d		28 d		7 d		28 d	
Echocardiography	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Heart Rate (BPM)	582.4	22.9	558.9	30.1	574.4	22.4	570.6	25.5
ESV (μl)	92.8	34.6	87.5	32.3	67.9	44.0	69.1	40.2
EDV (μl)	117.3	32.2	120.2	31.0	100.0	40.5	108.1	42.3
SV (μl)	24.5	6.6	32.7	8.2	32.1	8.9	39.0	8.5
IVRT (ms)	15.5	2.2	15.7	2.1	14.5	1.9	14.1	1.5
FS (%)	11.7	6.7	14.7	6.1	17.4	11.6	19.3	12.7
LVAW;d (mm)	0.59	0.17	0.44*	0.17	0.78	0.26	0.80*	0.53
LVAW;s (mm)	0.67	0.21	0.49*	0.20	0.92	0.37	0.99*	0.68
LVID;d (mm)	4.79	0.62	4.89	0.86	4.48	0.79	4.49	1.12
LVID;s (mm)	4.26	0.83	4.22	1.07	3.78	1.14	3.73	1.44
LVPW;d (mm)	0.84	0.25	0.97	0.35	0.89	0.29	0.96	0.38
LVPW;s (mm)	1.06	0.36	1.26	0.46	1.20	0.41	1.25	0.54
Morphometric	Cre -		Cre +					
	Mean	±SD	Mean	±SD				
Body mass (g)	26.2 [#]	1.0	24.8 [#]	1.2				
HW/TL (mg/mm)	7.6	0.7	7.3	0.6				
Wet/dry lung ratio	4.2	0.1	4.2	0.2				
Mortality	Survived		Fatality		Known ruptures			
Cre -	14/25		11/25		3/11			
Cre +	10/24		14/24		4/14			
Females								
	Cre -				Cre +			
	7 d		28 d		7 d		28 d	
Echocardiography	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Heart Rate (BPM)	571.8	29.4	552.1	45.0	586.7	26.9	573.6	27.0
ESV (μl)	62.3	30.6	68.7	39.1	77.4	27.2	91.3	31.5
EDV (μl)	85.1	26.9	98.3	36.6	101.1	24.3	121.7	28.2
SV (μl)	22.8	5.1	29.6	5.9	23.7	6.9	30.4	6.9
IVRT (ms)	14.7	2.2	15.4	2.1	14.8	2.0	14.4	1.9
FS (%)	17.0	12.3	17.0	11.7	14.3	7.9	13.6	6.6
LVAW;d (mm)	0.77	0.32	0.64	0.37	0.78	0.40	0.77	0.33
LVAW;s (mm)	0.91	0.43	0.77	0.50	0.95	0.51	0.95	0.47
LVID;d (mm)	4.09	0.61	4.39	0.93	4.31	0.60	4.74	0.70
LVID;s (mm)	3.45	0.94	3.73	1.24	3.72	0.79	4.13	0.90
LVPW;d (mm)	0.91	0.22	0.93	0.28	0.91	0.30	1.03	0.28
LVPW;s (mm)	1.15	0.33	1.17	0.36	1.15	0.38	1.17	0.30
Morphometric	Cre -		Cre +					
	Mean	±SD	Mean	±SD				
Body mass (g)	20.8	0.9	20.3	1.0				
HW/TL (mg/mm)	6.8	1.0	7.0	0.8				
Wet/dry lung ratio	4.2	0.1	4.2	0.1				
Mortality	Survived		Fatality		Known ruptures			
Cre -	17/22		5/22		2/5			
Cre +	12/25		13/25		4/13			

Cre⁻ and Cre⁺ TAK1^{fl/fl} mice underwent echocardiography acquisition at 7- and 28-days following MI. Organs were harvested and body mass taken at the conclusion of the study, n = 22-25 mice per group. Statistics: *two-way ANOVA with Šidák's post-hoc or [#]unpaired 2-tailed t test as appropriate, Cre⁻ vs Cre⁺, ^{**}p < 0.05.

Supplemental Table 7. Transcriptional characterization of isolated cells

Cell Type	Gene	Modeled Mean	Gene Length	Normalized (Modeled Mean/ Gene Length)
Fibroblasts	<i>Col1a1</i>	333299.4	5946	56.054
	<i>Col1a2</i>	292003.2	5222	55.918
	<i>Postn</i>	39312.8	3406	11.542
	<i>Ddr2</i>	21437.2	8161	2.627
	<i>Pdgfra</i>	41352.9	6700	6.172
	<i>Vim</i>	107847.1	1834	58.804
	<i>Sparc</i>	317113.3	2638	120.210
Endothelial	<i>Pecam1</i>	28.8	3030	0.009
	<i>Vwf</i>	34.3	8834	0.004
	<i>Kdr</i>	39.9	5732	0.007
	<i>Cldn5</i>	1.0	1420	0.001
	<i>Nos3</i>	12.1	4081	0.003
	<i>Esam</i>	12.0	1859	0.006
	<i>Ecscr</i>	769.4	975	0.789
Macrophages	<i>Cd68</i>	136.7	1263	0.108
	<i>Adgre1</i>	107.0	3229	0.033
	<i>Mrc1</i>	81.7	5322	0.015
	<i>Ccr2</i>	0.3	2206	0.000
	<i>Csf1R</i>	299.4	3875	0.077
	<i>Lyz1</i>	13.9	1282	0.011
	<i>Il1b</i>	0.7	1883	0.0004

Bulk-RNAseq gene expression profile of serum-starved isolated cardiac fibroblast was described using calculated modeled mean normalized to gene length.