Lack of Thy1 Defines a Pathogenic Fraction of Cardiac Fibroblasts in Heart Failure

Yanzhen Li[1], Daniel Song[2,3], Lan Mao[4], Dennis M. Abraham[4], and Nenad Bursac[1, 4*]

Supplementary Figures &Tables

Supplementary Figure Legends:
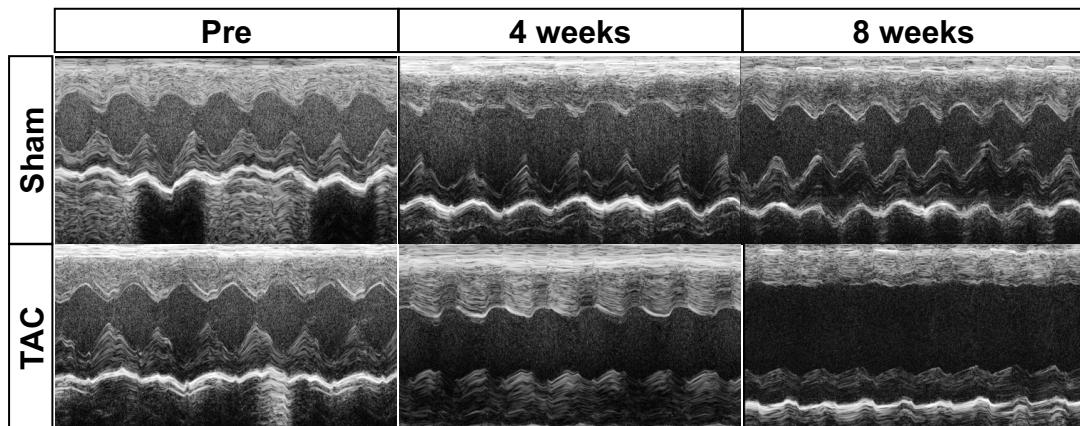
Supplementary Figure 1. Characterization of pressure overload-induced heart failure. (A) Representative left echocardiograms from transverse aortic constriction (TAC) and sham-operated (Sham) mice at pre-surgery (pre) and 4 and 8 weeks post-surgery, showing pressure-overload induced ventricular dilatation and failure. **(B)** Echocardiographic measurements of fractional shortening (FS) in TAC and Sham mice at pre-surgery (0 weeks) and 2, 4, 6, and 8 weeks post-surgery. n=20 mice per group. **(C)** Representative Masson's trichrome stains and immunostaining for filamentous actin (F-actin, green), Collagen 1 (Col1, red) or Vimentin (Vim, red), and DNA (nuclei, blue) in ventricular sections from Sham and TAC-induced heart failure (FS<30%) mice at 8 weeks post-surgery. Image quantification of the percentage of Collagen 1^+ area **(D)** and Vimentin$^+$ area **(E)** per cross-section. n=3 hearts per group. *P < 0.05, **P < 0.01. Scar bars: 100 μm in Masson's Trichrome stains and 50 μm in immunostainings.

Supplementary Figure 2. Differential gene expression in freshly isolated and 2D-cultured cardiac fibroblasts. Bar graphs showing *Thy1* **(A)**, *Postn* **(B)**, *Tgfb1* **(C)**, *Col1a1* **(D)**, *Col3a1* **(E)**, *Fn1* **(F)** and *Acta2* **(G)** gene expression in freshly isolated (P0) and cultured (passage P1 and passage P2) Thy1^{pos} and Thy1^{neg} CFs from Sham and TAC ventricles. The mRNA expression was normalized to house-keeping gene *B2M* and shown as fold change relative to P0 Sham Thy1^{pos} group. N=2 independent experiments for *Thy1*, and N=4 independent experiments for all other genes. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
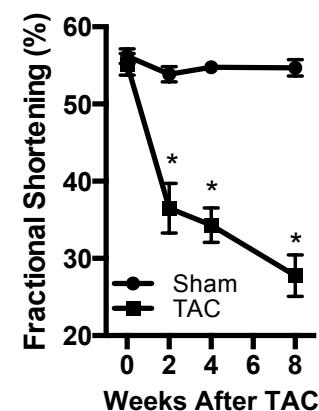
Supplementary Figure 3. The percentage of apoptotic cells in engineered cardiac bundles. (A) Representative TUNEL assay immunostaining images in bundle transverse cross-sections. **(B)** Image quantification of the percentage of TUNEL+ apoptotic nuclei per cross-section. n=2-3 bundles per group. Scar bar: 75 μm. $^\#$P < 0.05 for NRVM vs. all other groups.

Supplementary Figure 1.
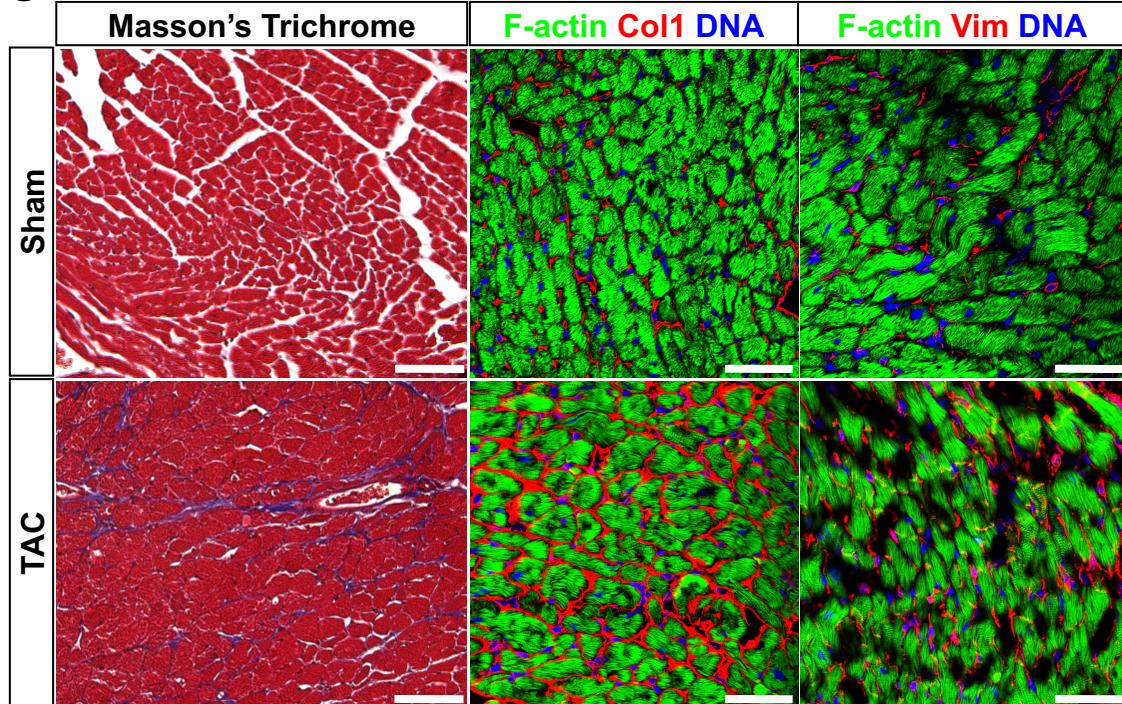
A
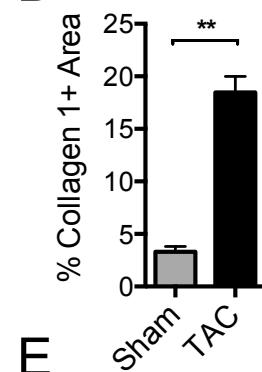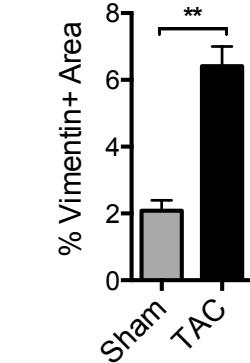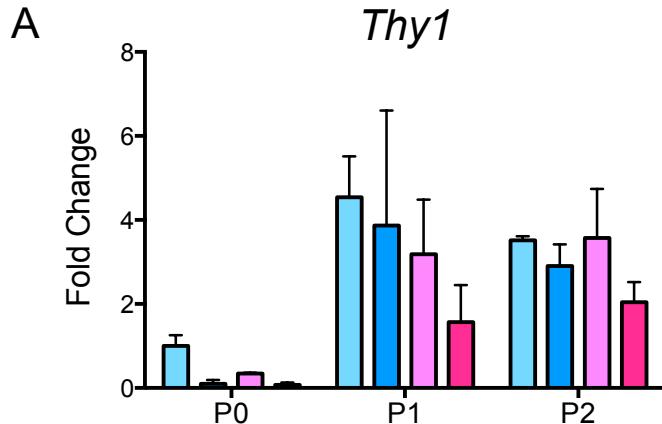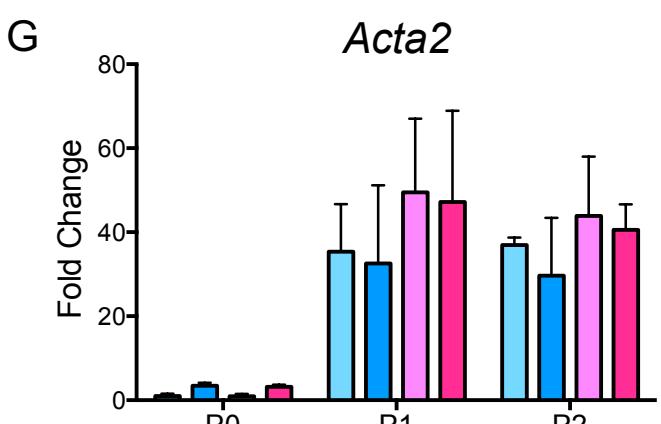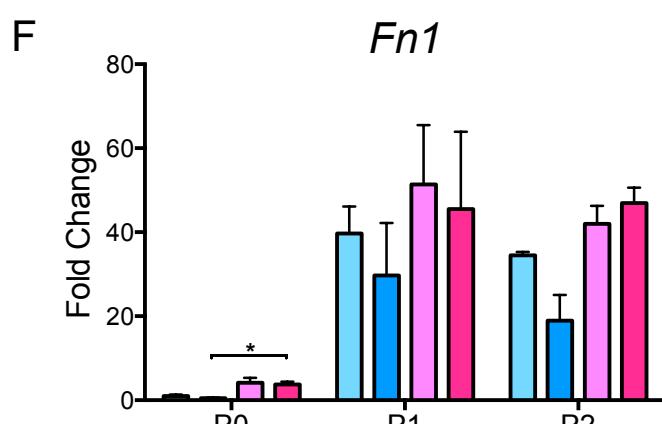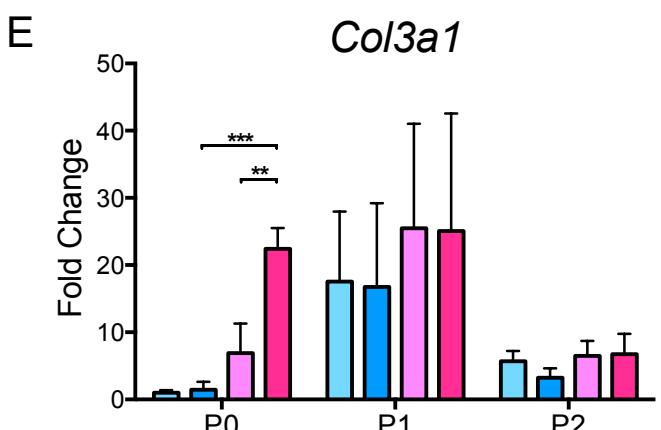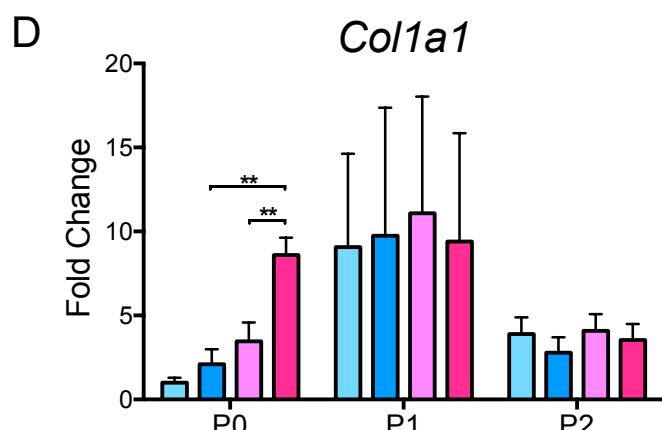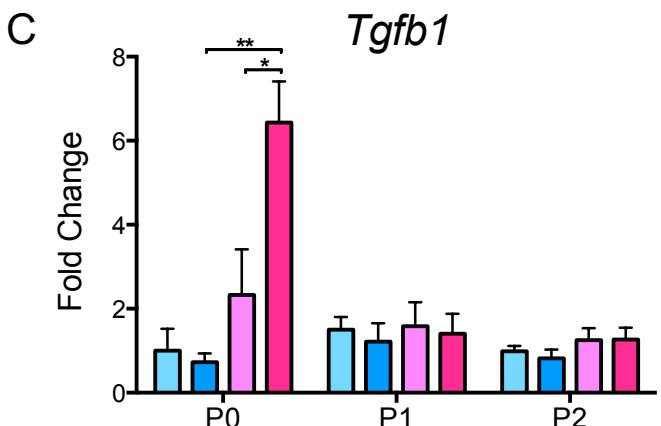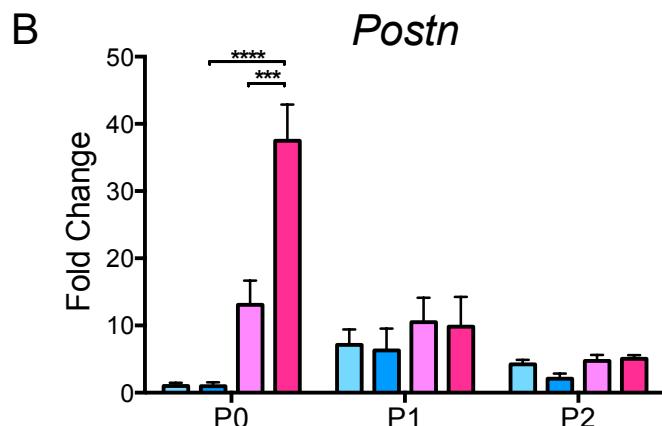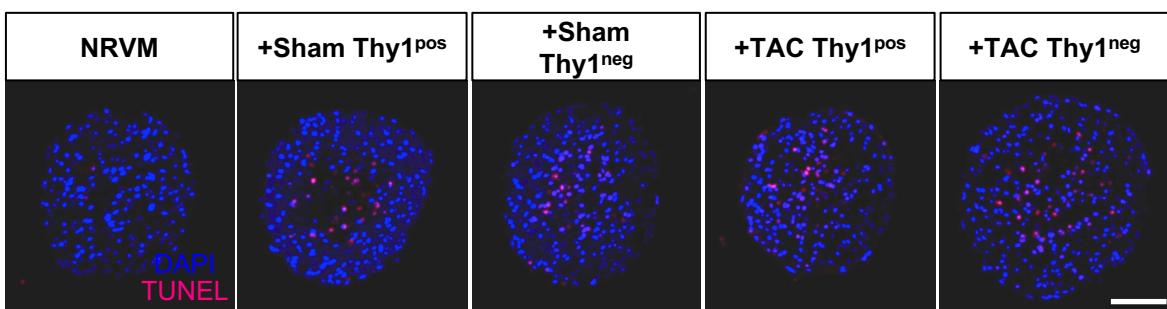
	Pre	4 weeks	8 weeks
Sham			
TAC			

B

C

	Masson's Trichrome	F-actin Col1 DNA	F-actin Vim DNA
Sham			
TAC			

D

E

Supplementary Figure 2.

Supplementary Figure 3.

A

NRVM | +Sham Thy1^{pos} | +Sham Thy1^{neg} | +TAC Thy1^{pos} | +TAC Thy1^{neg}

DAPI
TUNEL

B

Apoptosis
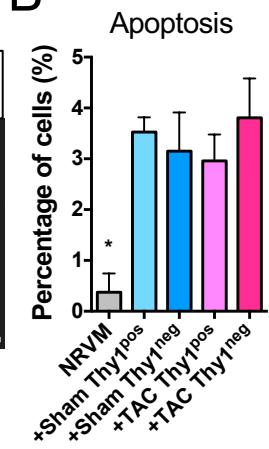
Supplementary Table 1.

Table S1: Antibodies used in FACS

Antibody	Clone	Supplier (Cat#)	Dilution	Fluorophore
CD31	390	BioLegend (102427)	1:400	Brilliant Violet 605
CD45	30-F11	BioLegend (103116)	1:200	APC/Cy7
CD90.2	53-2.1	BD (553005)	1:50	PE
MEFSK4	SK4	Miltenyi Biotec (130-102-900)	1:20	APC

Supplementary Table 2.

Table S2: Primers for qPCR

Gene	Description	Forward Primer	Reverse Primer
Acta2	Smooth Muscle actin alpha 2 (a-SMA)	TCAGCGTTCAGCCTCC	CCAGAGCCATTGTCGC
B2M	Beta-2 Microglobulin	GGTCTTTCTGGTGCTTGTCT	ACGTAGCAGTTCAGTATGTTCG
Col1a1	Collagen type I alpha 1 chain	TGGTGAAGCAGGCAAGC	GAAACCTCTCTCGCCTCTTG
Col3a1	Collagen type III alpha 1 chain	GGTGAACCTGGTCAAGCTG	TCTTCCTGACTCTCCATCCT
Fn1	Fibronectin 1	GAGCTATCCATTTCACCTTCAGA	TTGTTCGTAGACACTGGAGAC
Thy1	Thymus cell antigen 1	TGCTCTCAGTCTTGCAGGTG	TGGATGGAGTTATCCTTGGTGTT
Postn	Periostin	CCTGTAAGAACTGGTATCAAGGT	CCTTTCATCCCTTCCATTCTCA
Tgfb1	Transforming growth factor beta 1	GCGGACTACTATGCTAAAGAGG	CCGAATGTCTGACGTATTGAAGA
Wisp1	WNT1 inducible signaling pathway protein 1	AGGTACGCAATAGGAGTGTGTG	AGTTGTACCTGCAGTTGGGTTG

Supplementary Table 3.

Base	LVEDD (mm)	LVESD (mm)	SWT (mm)	PWT (mm)	AET (ms)	HR (BPM)	FS (%)	LVm (mg)	mVcFc (circ/s)
WT Sham (n=6)	3.16 ± 0.06	1.52 ± 0.07	0.85 ± 0.06	1.03 ± 0.03	38.48 ± 2.09	731.88 ± 17.6	51.94 ± 1.6	82.08 ± 4.83	3.94 ± 0.28
KO Sham (n=6)	3.38 ± 0.11	1.76 ± 0.12	0.78 ± 0.07	0.79 ± 0.03#	47.32 ± 3.62	556.3 ± 66.93#	48.09 ± 2.34	69.04 ± 2.18	3.49 ± 0.29
WT TAC (n=10)	3.17 ± 0.1	1.52 ± 0.08	0.94 ± 0.03	1.07 ± 0.04	36.84 ± 1.02	714.05 ± 13.36	52.32 ± 1.24	89.81 ± 3.36	4.14 ± 0.11
KO TAC (n=11)	3.45 ± 0.16	1.8 ± 0.12	0.87 ± 0.04	0.98 ± 0.07	46.32 ± 2.37*	447.05 ± 44.06*	48.4 ± 1.5	89.22 ± 2.94	4.01 ± 0.18

1-week	LVEDD (mm)	LVESD (mm)	SWT (mm)	PWT (mm)	AET (ms)	HR (BPM)	FS (%)	LVm (mg)	mVcFc (circ/s)
WT Sham (n=6)	2.99 ± 0.15	1.41 ± 0.07	0.85 ± 0.04	0.93 ± 0.05	39.28 ± 2.1	705.59 ± 18.5	52.65 ± 0.87	69.58 ± 5.52	3.94 ± 0.12
KO Sham (n=6)	3.2 ± 0.08	1.68 ± 0.09	0.85 ± 0.02	0.84 ± 0.08	47.54 ± 3.88	532 ± 77.26#	47.51 ± 2.37	71.2 ± 4.04	3.53 ± 0.23
WT TAC (n=10)	3.34 ± 0.16	2.15 ± 0.2	1.1 ± 0.04	1.11 ± 0.03	41.75 ± 1.18	676.08 ± 16.79	36.45 ± 3.4	112.51 ± 5.97	2.62 ± 0.25
KO TAC (n=11)	3.85 ± 0.16*	2.82 ± 0.21*	0.98 ± 0.06	1 ± 0.06	52.07 ± 1.98*	460.54 ± 44.66*	27.55 ± 3.2	121.18 ± 10.14	1.99 ± 0.25

4-week	LVEDD (mm)	LVESD (mm)	SWT (mm)	PWT (mm)	AET (ms)	HR (BPM)	FS (%)	LVm (mg)	mVcFc (circ/s)
WT Sham (n=6)	2.98 ± 0.16	1.39 ± 0.11	0.88 ± 0.04	0.9 ± 0.1	36.69 ± 1.49	743.3 ± 11.95	53.62 ± 1.95	70.58 ± 9.82	4.19 ± 0.22
KO Sham (n=6)	3.25 ± 0.22	1.63 ± 0.19	0.93 ± 0.06	1.04 ± 0.09	37.92 ± 1.92	653.89 ± 39.9	50.83 ± 2.71	90.68 ± 6.48	4.12 ± 0.29
WT TAC (n=10)	3.94 ± 0.17	2.94 ± 0.25	1.13 ± 0.05	1 ± 0.06	40.76 ± 1.21	724.09 ± 11.13	26.41 ± 3.79	136.92 ± 8.11	1.92 ± 0.31
KO TAC (n=11)	4.28 ± 0.21	3.7 ± 0.25*	1.24 ± 0.07	1 ± 0.05	49.07 ± 1.59*	535.33 ± 41.61*	14.2 ± 1.9*	170.55 ± 9.98*	0.98 ± 0.13*

Table S3: Echocardiography data from WT and Thy1KO mice displayed as mean ± SEM. LVEDD: Left Ventricular (LV) End Diastolic Dimension; LVESD: LV End Systolic Dimension; SWT: Septal Wall Thickness; PWT: Posterior Wall Thickness; AET: Aortic Ejection Time; HR: Heart Rate; BPM: Beats Per Minute; LVm: LV mass; FS: Fractional Shortening, mVcFc: Mean Velocity of Circumferential Shortening Corrected for Heart Rate calculated as VcFc = (Fractional Shortening/Aortic Ejection Time)*(60/Heart Rate)$^{0.5}$; *P < 0.05 KO TAC vs. WT TAC, #P < 0.05 KO Sham vs. WT Sham by 1-way ANOVA with Bonferroni's multiple comparisons test.