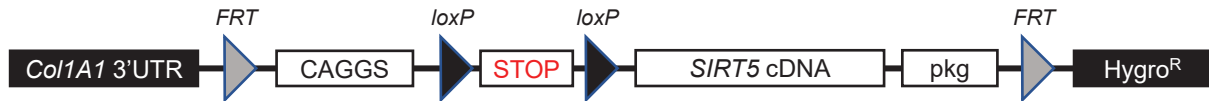


Supplemental Figure 1

A



B

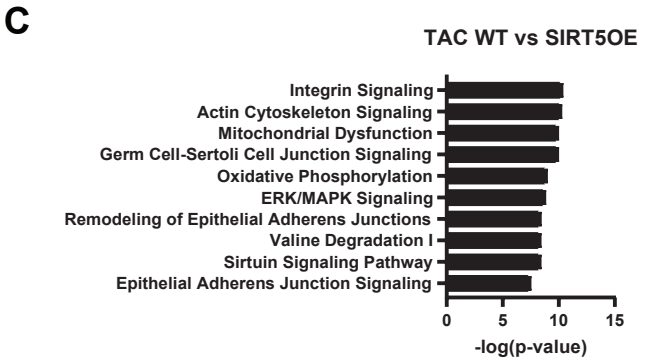
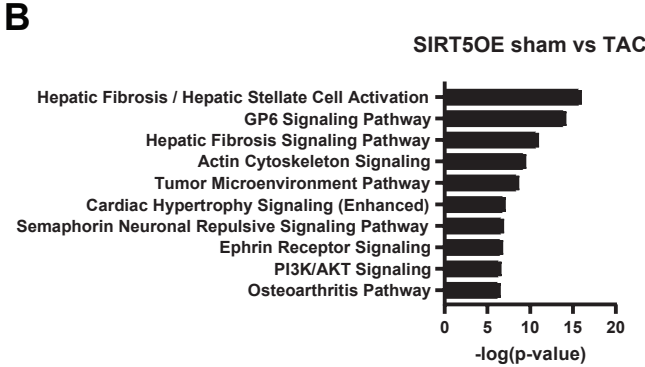
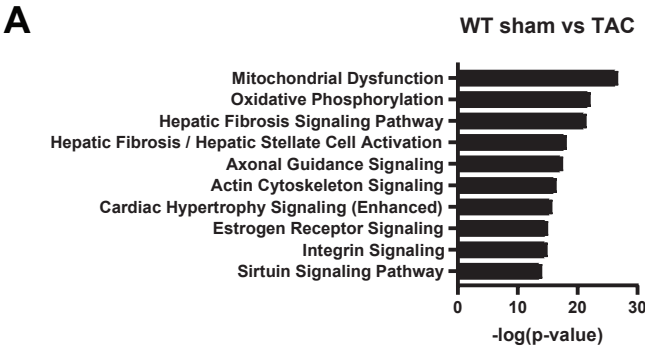
WT male, SIRT5OE female					
	WT	SIRT5OE	Total	χ^2	p-value
Males	73	77	150	2.09	0.55
Females	74	61	135		
Total	147	138	285		

SIRT5OE male, WT female					
	WT	OE	Total	χ^2	p-value
Males	38	25	63	5.07	0.17
Females	42	41	83		
Total	80	66	146		

Combined					
	WT	SIRT5OE	Total	χ^2	p-value
Males	111	102	213	1.32	0.72
Females	116	102	218		
Total	227	204	431		

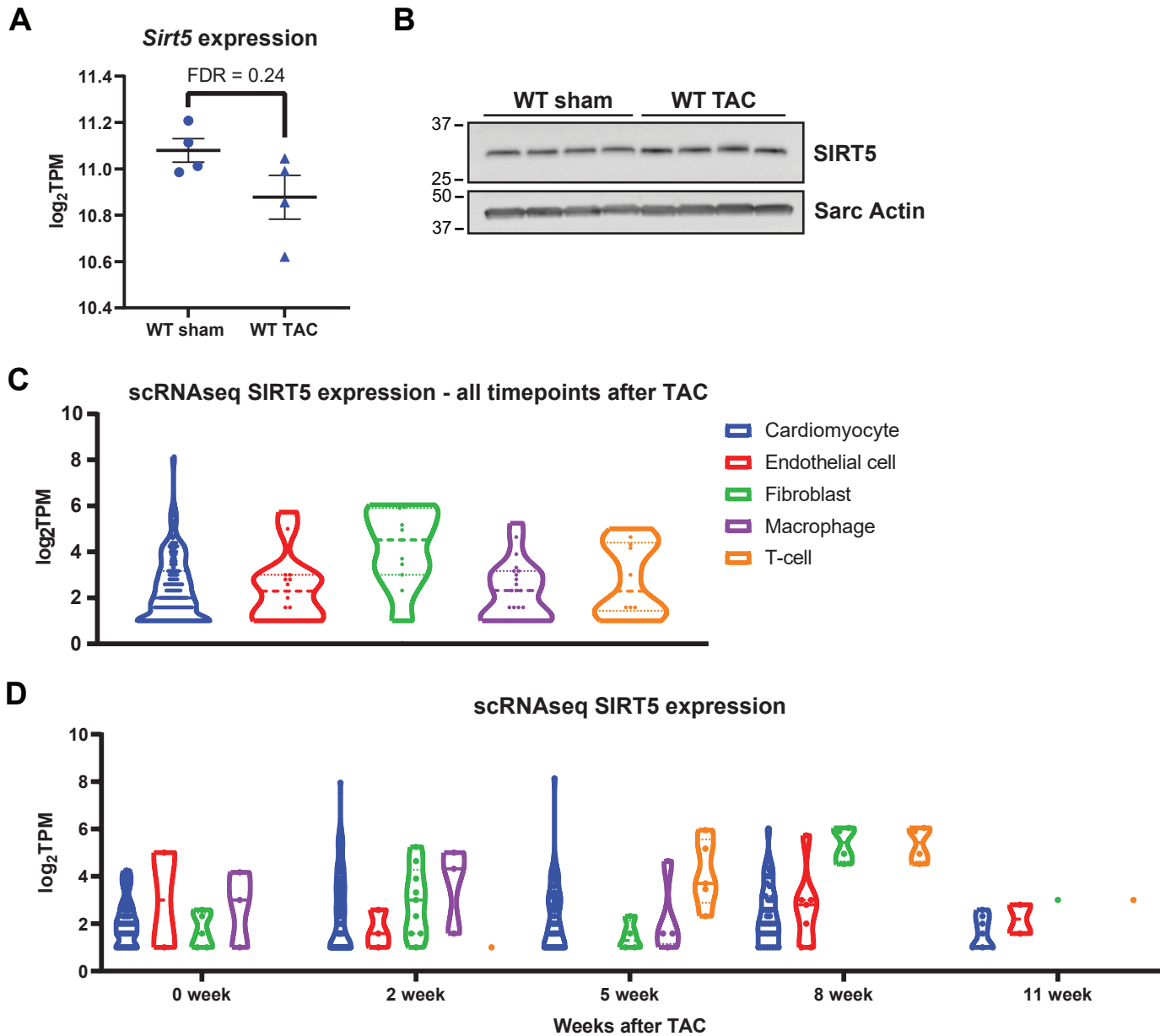
Supplemental Figure 1. Generation of SIRT5OE mice. A, cassette containing a constitutive CAGGS promoter, a transcriptional flox-STOP-flox, followed by the SIRT5 cDNA was inserted into the collagen1 A1 (Col1A1) 3'UTR by FLP recombination. B, number of male and female mice born per WT or SIRT5OE litter.

Supplemental Figure 2



Supplemental Figure 2. Top 10 IPA pathways, sorted on significance, enriched by differentially expressed genes in each labelled comparison.

Supplemental Figure 3



Supplemental Figure 3. SIRT5 expression in bulk heart tissue and specific cardiac cell populations. A, *Sirt5* expression in bulk heart WT sham and WT TAC samples. B, SIRT5 protein levels in WT sham and TAC heart lysates. C, *Sirt5* expression in five major cardiac cell types using single-cell data generated by Ren et. al. Only cells with at least 2 reads of *Sirt5* were graphed. D, *Sirt5* expression in single-cell RNA-seq across 5 different time-points through 11 weeks of pressure overload.

Supplemental Table 1. Two-way ANOVA analyses of metabolites in Figure 6B-D			
	Genotype Effect	TAC Effect	Interaction Effect
NAD⁺	0.136	0.005*	0.740
NADH	0.044*	0.522	0.275
NAD⁺/NADH	0.074 ⁺	0.002*	0.599
Glucose 6-phosphate	0.584	0.888	0.705
Fructose 6-phosphate	0.694	0.945	0.712
Fructose 1,6-bisphosphate	0.504	0.598	0.850
Dihydroxyacetone phosphate	0.326	0.555	0.321
Glyceraldehyde 3-phosphate	0.197	0.235	0.548
2-phosphoglycerate	0.133	0.940	0.306
Phosphoenolpyruvate	0.825	0.696	0.832
Pyruvate	0.399	0.962	0.826
Citrate/Isocitrate	0.532	0.345	0.441
Aconitate	0.840	0.005*	0.263
α-ketoglutarate	0.460	<0.001*	0.065 ⁺
Succinate	0.069 ⁺	0.002*	0.183
Malate	0.058 ⁺	<0.001*	0.638
FAD	0.049*	<0.001*	0.940

Supplemental Table Legends

Supplemental Table 1. Two-way ANOVA analyses results of all metabolites plotted in Figure 6B-D. Significance markers: (*) $p < 0.05$, (+) $p < 0.1$.

Supplemental Table 2. Median-centered data from LC-MS/MS metabolite profiling in WT sham, WT TAC, SIRT5OE sham, and SIRT5OE TAC hearts. P-value was calculated using a two-tailed t-test.

Supplemental Table 3. DESeq2 output of differential gene expression of the RNA-seq data. Samples in each comparison are labelled on the spreadsheet name. Padj (p-value adjusted for multiple comparisons) were used to determine significance.