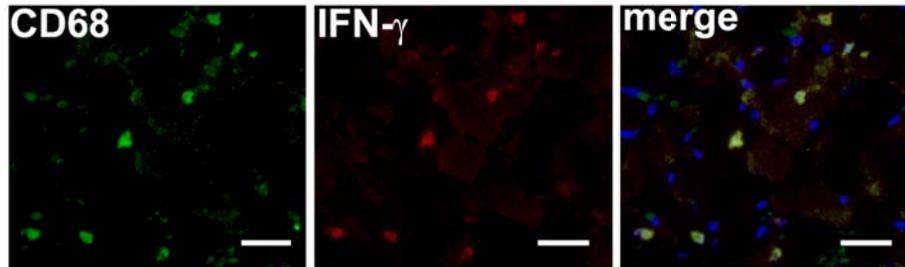


# Supplemental Material

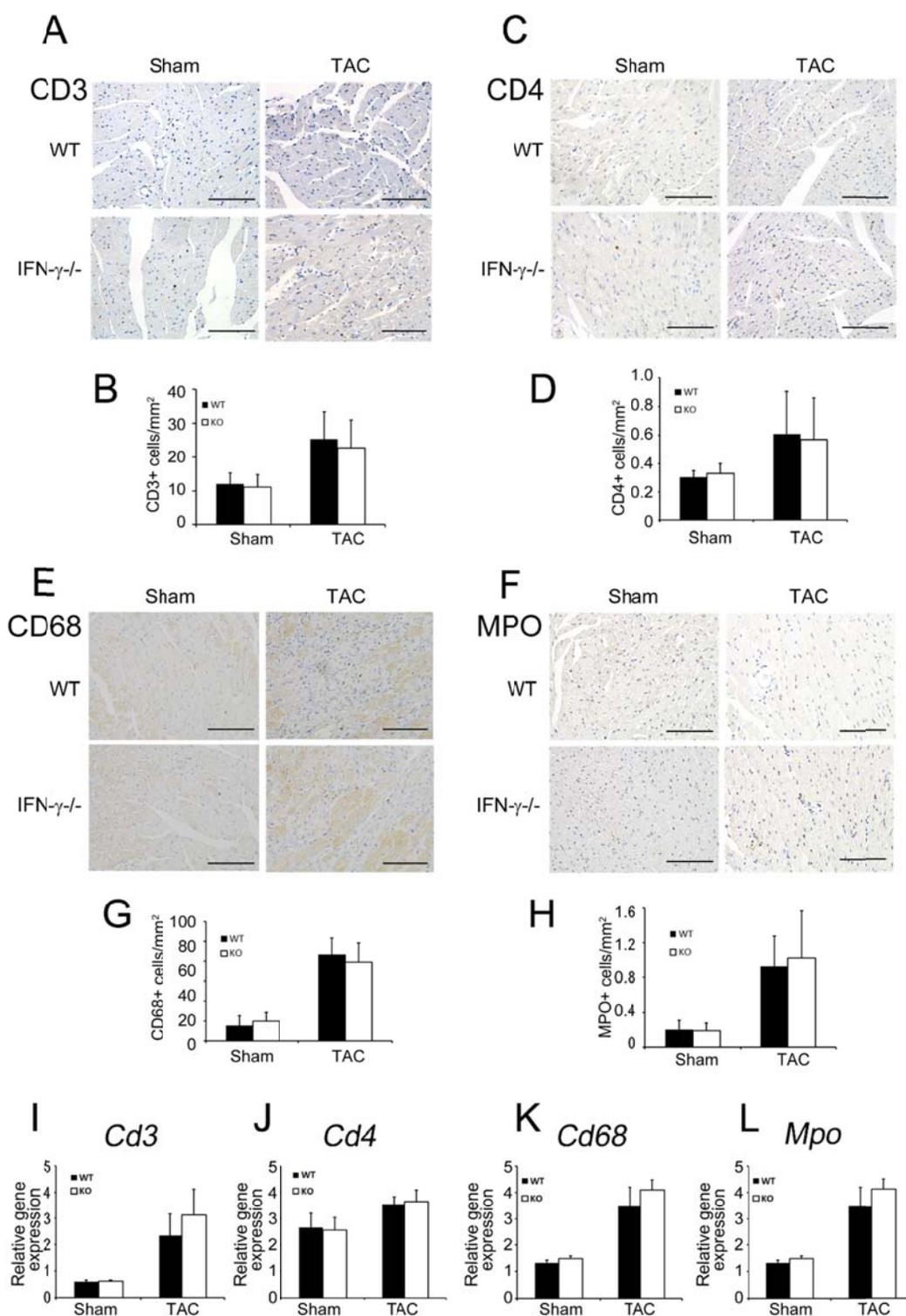
**Supplemental table I. Sequences of the primers used for RT-PCR**

Transcript	Sequence
GAPDH (mouse)	(F) 5'- GCACCGTCAAGGCTGAGAAC-3'
	(R) 5'- TGGTGAAGACGCCAGTGG-3'
GAPDH (rat)	(F) 5'- GACATGCCGCCTGGAGAAAC
	(R) 5'-AGCCCAGGATGCCCTTTAGT
IFN- $\gamma$ (mouse)	(F) 5'- CGGCACAGTCATTGAAAGCCTA -3'
	(R) 5'- GTTGCTGATGGCCTGATTGTC 3'
NPPB (mouse)	(F) 5'- ATCGGATCCGTCAGTCGTTTG
	(R) 5'-CCAGGCAGAGTCAGAAACTGGAG
IFNGR1 (mouse)	(F) 5'-TGACGGGAGCACCTGTTACAC
	(R) 5'-TTTCGACCGTATGTTTCGTATGTAG
IFNGR1 (rat)	(F) 5'-TGTCCCGGCACTTGTGTGTTGT
	(R) 5'-AGGTTTGGTCTCGGACGTGGCA
	(F) 5'-AGGCCTGCATTGACCTCATC
	(R) 5'-TTGCCCAGGTGGTTGTTCGTA
MYH7 (mouse)	(F) 5'- ACAACCCCTACGATTATGCG
	(R) 5'- CGCCTGTCAGCTTGTAATG
MYH7 (rat)	(F) 5'- CACTCTGGGCTTGCTGATGG
	(R) 5'- TCATAGTCTGGGTTGGGAACAGG
CD3 (mouse)	(F) 5'- CAACCTGACTCTGACTCTGGACAA
	(R) 5'- AGGTAGGTCCCATCACCTCACA
CD68 (mouse)	(F) 5'- TCCAAGATCCTCCACTGTTG
	(R) 5'- ATTTGAATTTGGGCTTGGAG
MPO (mouse)	(F) 5'- CTGCCTCATTGGCACTCAGTTTA
	(R) 5'- GGTGATGCCAGTGTGTCACAG

(F) Forward primer; (R) Reverse primer

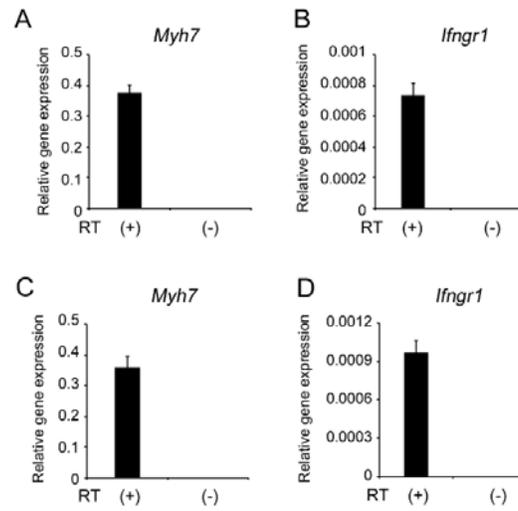


**Supplemental Figure 1.** A double-color immunofluorescence analysis was performed on the heart 1 week after TAC. The analysis was performed using anti-CD68 (FITC) and anti-IFN- $\gamma$  (Cy3), followed by observations under a fluorescence microscopy, and signals were merged digitally. Representative results from six individual animals are shown here. Original magnification,  $\times 400$  (scale bar: 100  $\mu\text{m}$ ). TAC indicates transverse aortic constriction; FITC, Fluorescein isothiocyanate; and Cy3, Cyanine3.

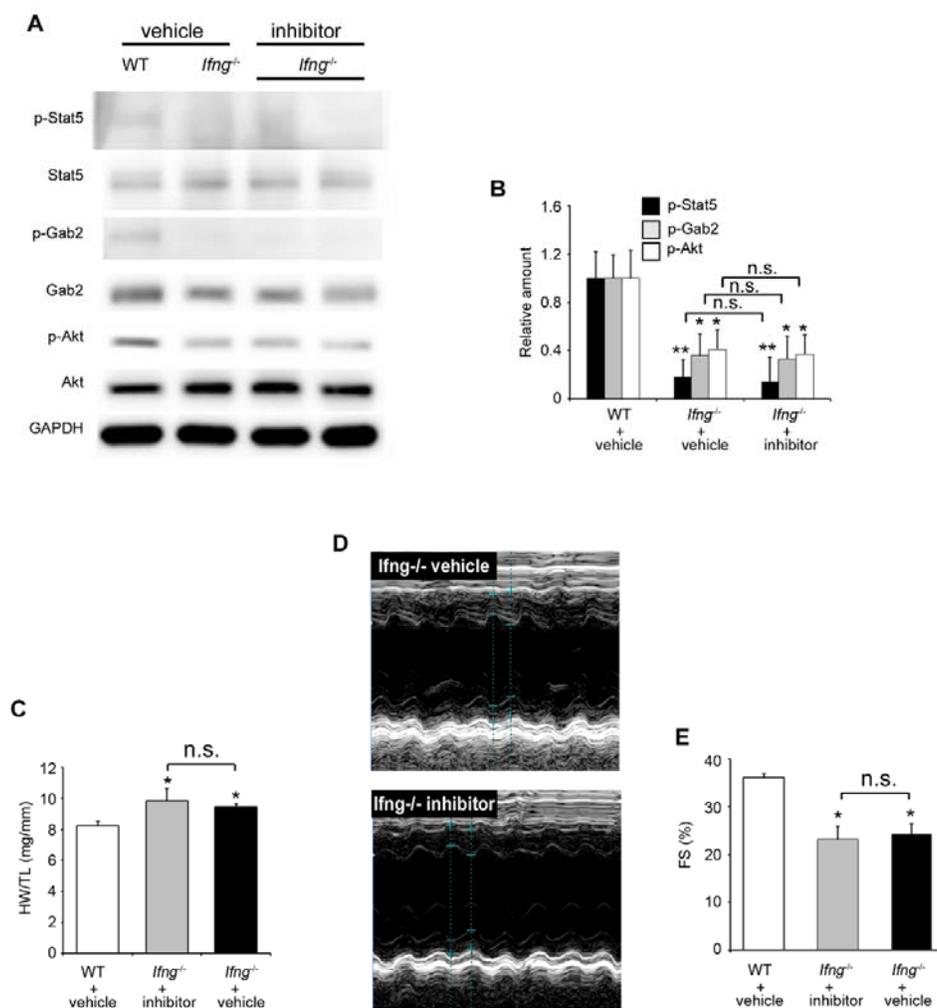


Supplemental Figure 2. Quantification of intracardiac inflammatory cells.

Representative microscopic images and quantification of CD3-positive T cell (A and B), CD4-positive T cell (C and D), CD68-positive macrophages (E and F) and MPO-positive neutrophil (G and H) in the heart paraffin sections of sham operated and TAC operated (day 3) mice. Original magnification, x 400 (scale bar: 100  $\mu$ m). All values represent means  $\pm$  SEM (n=6). Relative gene expression of *Cd3* (I), *Cd4* (J), *Cd68* (K) and *Mpo* (L) in the LV of sham operated and TAC operated mice (day 3) was analyzed by real-time RT-PCR. All values represent means  $\pm$  SEM (n=5). MPO indicates myeloperoxidase; TAC, transverse aortic constriction operated mice; and Sham, sham operated mice.



**Supplemental Figure 3.** Gene expressions of *Myh7* (A and C) and *Ifngr1* (B and D) in isolated mouse (A and B) and rat (C and D) cardiomyocytes were analyzed by real-time PCR with or without reverse transcription. All values represent means  $\pm$  SEM (n=4). *Myh7* indicates myosin heavy chain 7; and *Ifngr1*, interferon gamma receptor 1.



**Supplemental Figure 4.** Effects of a Stat5 inhibitor on TAC-induced cardiac hypertrophy and heart failure in *Ifng*<sup>-/-</sup> mice. (A and B) Phosphorylation of Akt and Gab2 in LV in mice treated with a Stat5 inhibitor or vehicle. Western blotting analysis was conducted to detect Stat5, Gab2, and Akt in LV of mice at 3 days after TAC operation. Representative results from 6 independent experiments are shown in A. Relative amounts of p-Stat5, p-Gab2 and p-Akt to GAPDH were analyzed by densitometry and calculated, and are shown in B. All values represent means  $\pm$  SEM

(n=6). \*,  $p<0.05$ , \*\*,  $p<0.01$ , vs. WT treated with vehicle in each column. (C) The HW/TL ratio in WT mice treated with vehicle and *Ifng*<sup>-/-</sup> mice treated with the Stat5 inhibitor or vehicle 3 weeks after TAC was calculated. All values represent means  $\pm$  SEM (n=6). \* $p<0.05$ . WT treated with vehicle. (D) M-mode echocardiographic images of *Ifng*<sup>-/-</sup> mice treated with a Stat5 inhibitor or vehicle, 3 weeks after TAC operation. Representative images from at least 5 animals are shown here. (E) LVFS were calculated on WT mice treated with vehicle or *Ifng*<sup>-/-</sup> mice treated with a Stat5 inhibitor or vehicle at 3 weeks after TAC surgery. All values represent means  $\pm$  SEM (n=6). \*,  $p<0.05$ . WT treated with vehicle. Stat5 indicates signal transducer and activator of transcription 5; TAC, transverse aortic constriction; *Ifng*, interferon gamma; Akt, protein kinase B; Gab2, GRB2-associated-binding protein 2; LV, left ventricle; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HW/TL ratio, heart weight/tibial length ratio; n.s., not significant; and LVFS, percent LV fractional shortening.