SUPPLEMENTAL DATA

Supplemental Table 1: Demographics and characteristics of study participants		
	Lean (n=15)	**Obese (n=12)**
Male, n (%)	3 (20%)	6 (50%)
Age, years [mean ± SD]	33.3 ± 9.5	44.8 ± 9.1
White, n (%)	13 (87%)	9 (75%)
BMI, kg/m² [mean ± SD]	21.7 ± 1.7	36.0 ± 4.8

Supplemental Table 2: Adipose tissue in lean (n=4) and obese (n=4) mice as quantified by micro-CT imaging			
Adipose Tissue, mm²	**Lean Mice (ND-fed)**	**Obese Mice (60% HFD-fed)**	**P-Value**
Thigh IMAT/PMAT	2.14 (1.7–2.5)	47.94 (28.6–65.8)	0.021
Calf IMAT/PMAT	0.18 (0.11–0.31)	8.29 (3.8–14.5)	0.043
Total IMAT/PMAT	2.3 (1.5–3.3)	56.4 (34.9–77.4)	0.021
VAT	45.3 (28.1–68.7)	288.8 (270.2–295.2)	0.021
SAT	13.8 (10.5–18.9)	125.3 (100.3–147.1)	0.021
IMAT/PMAT: intermuscular adipose tissue/perimuscular adipose tissue; VAT: abdominal visceral adipose tissue; SAT: abdominal subcutaneous adipose tissue. Values are reported as median (interquartile range). P-values were computed using the nonparametric Kruskal–Wallis test (SAS 9.3). All tests were 2-sided.			

Supplemental Figure Legends

Supplemental Figure 1: Quantitative RT-PCR analysis of inflammatory gene expression in SM. The entire quadriceps SM (including muscle fibers, IMAT, and PMAT) harvested from mice fed normal diet (ND; n=6–8 mice/group) or high-fat diet (HFD; n=8–10 mice/group) for 12 weeks were analyzed by quantitative RT-PCR. *P<0.05, **P<0.01, ***P<0.001 compared with ND group.

Supplemental Figure 2: Flow cytometric analysis of macrophages and T cells in SM. (A) Gating example of F4/80+ macrophages and F4/80+CD11c+ M1 macrophages. (B) Example of gating total CD3+, CD4+, and CD8+ T cells in SM of ND-fed mice.

Supplemental Figure 3: Immunohistochemical staining of sections of quadriceps isolated from ND-fed mice under 10x magnification

Supplemental Figure 4: Representative images of extramyocellular adipose tissue in mouse fed lower-fat diet. Cross-sectional micro-CT images of the proximal, mid, and distal thigh and midcalf for a mouse fed lower-fat diet (panels A–D) with corresponding adipose tissue superimposed in red (panels E–H).

Supplemental Figure 5: Representative images of extramyocellular adipose tissue in 60% HFD-fed mouse. Cross-sectional micro-CT images of the proximal, mid, and distal thigh and

midcalf for a mouse fed 60% HFD (panels A–D) with corresponding adipose tissue superimposed in red (panels E–H).

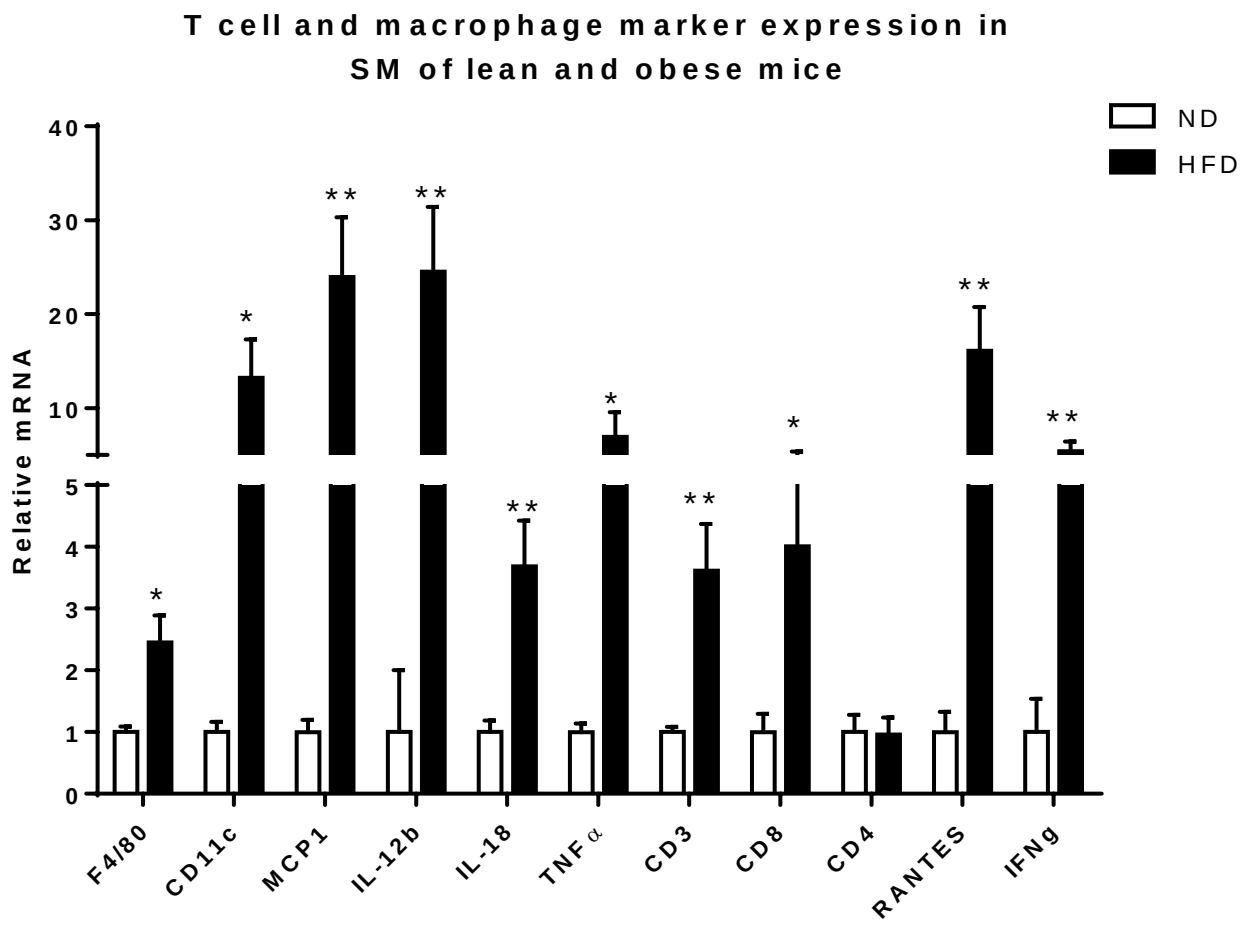
Supplemental Figure 6: Representative images of visceral adipose tissue in lean and obese mice. Cross-sectional micro-CT images of the abdomen and adipose tissue for a mouse fed lower-fat diet (panels A and B) and a mouse fed 60% HFD (panels C and D). VAT is shown in red, and SAT is shown in yellow.

Supplemental Figure 7: Obesity-induced SM and systemic insulin resistance. (A) Homeostasis model assessment of insulin resistance (HOMA-IR) in lean and 60% HFD–fed WT mice (n=4 mice/group). (B) Representative immunoblot and quantification of serine-phosphorylated Akt [P-Akt (S473)] protein expression in SM of mice fed ND or 60% HFD and injected with 1.5 U/kg body weight regular human insulin or PBS 10 minutes prior to sacrifice. The levels of P-Akt (S473) were expressed as P-Akt/Akt ratio. Insulin-stimulated P-Akt/Akt ratio was compared between lean and obese groups. *P<0.05, ***P<0.001 compared with lean controls.

Supplemental Figure 8: The effect of T_H1 cells on myotube inflammation and metabolic functions and the involvement of the JAK/STAT pathway. (A) Representative immunoblot and quantification of total STAT1 protein and tyrosine^{701} phosphorylated STAT1 (P-STAT1) protein in SM of mice fed ND or HFD for 12 weeks. (B) Quantitative RT-PCR analysis of proinflammatory cytokine and chemokine expression in differentiated C2C12 myotubes treated with T_H1 supernatant with or without antibody neutralization of IFNγ and T_H1 supernatant in the

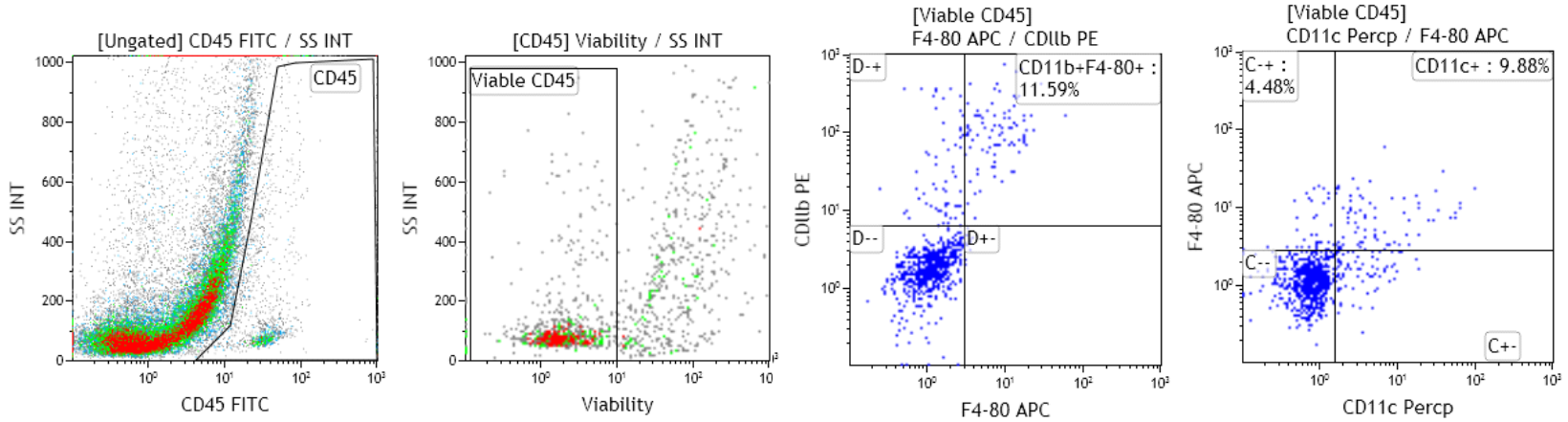
presence of JAK inhibitor I for 48 hours (n=6–10 samples/group). (C) mRNA level (n=4–6 samples/group) and the amount of P-STAT1 protein (n=5 samples/group) in C2C12 cells treated with T_H1-conditioned medium with or without antibody neutralization of IFNγ. (D) P-Akt (S473)/Akt protein levels in differentiated C2C12 cells treated with T_H1 for 48 hours and exposed to 100 nM insulin for 15 minutes (n=4–5 samples/group). Results are shown as mean ± SEM of 2 separate experiments, each with 2–3 samples/group. *P<0.05, **P<0.01, ***P<0.001 for comparison between control and each treatment group.

Supplemental Figure 1



Supplemental Figure 2

A



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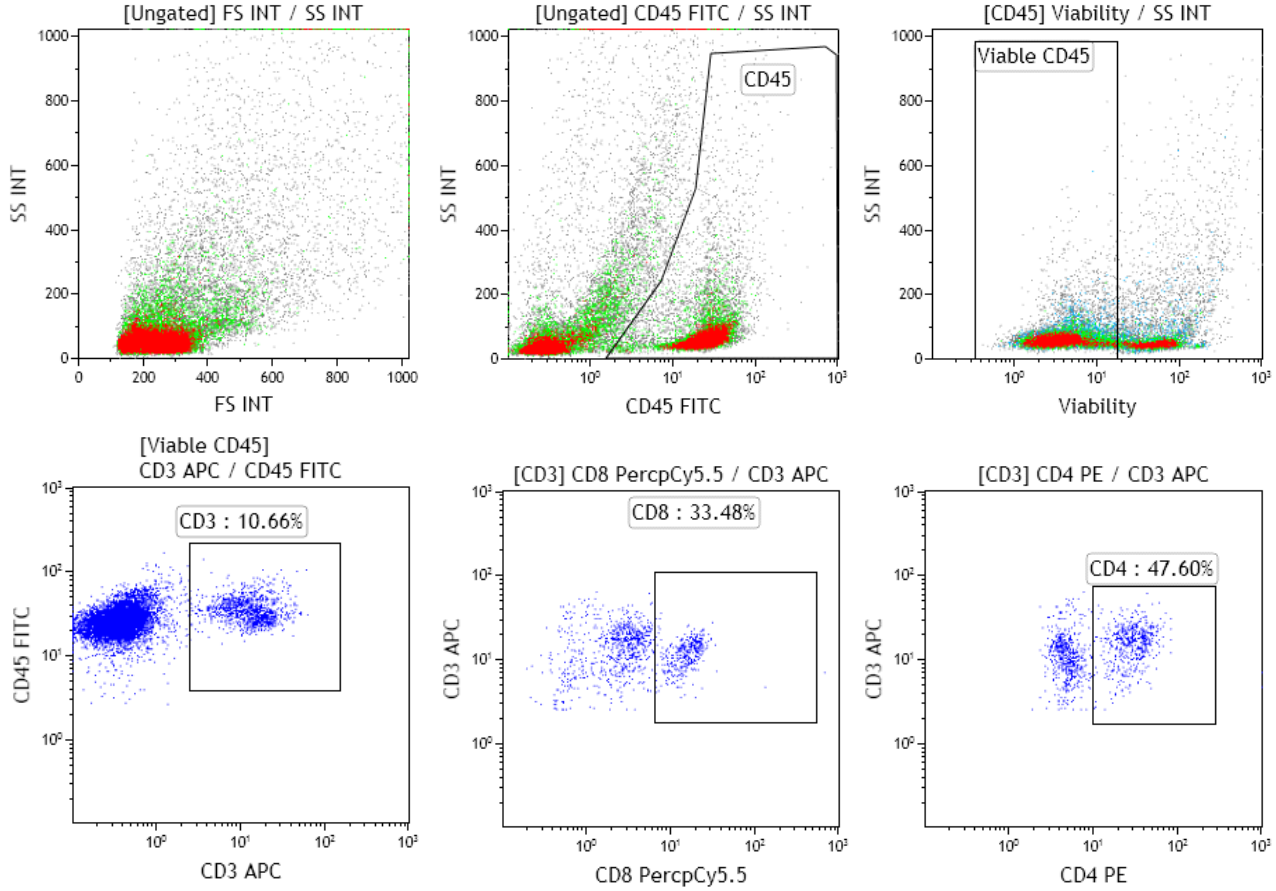
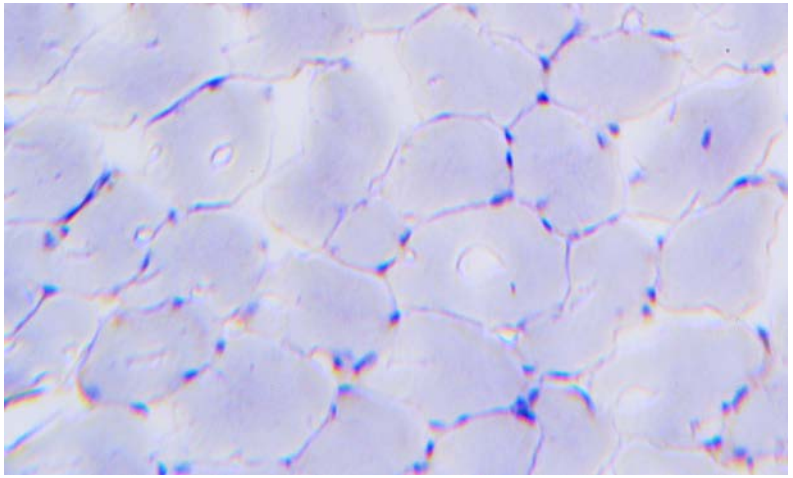
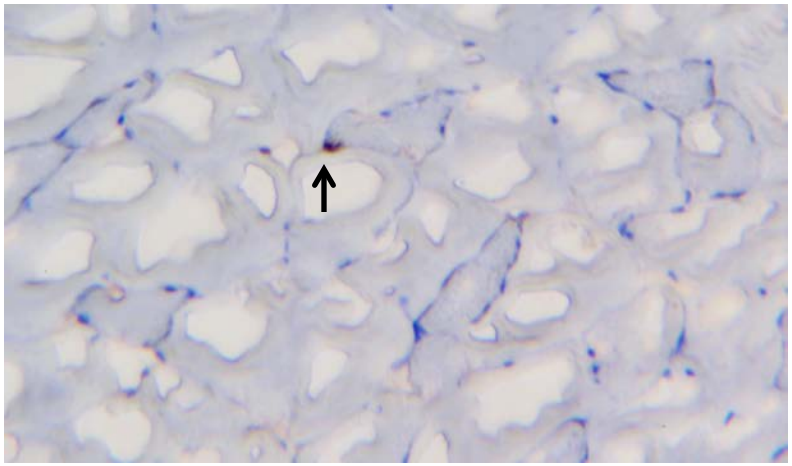


Supplemental Figure 3

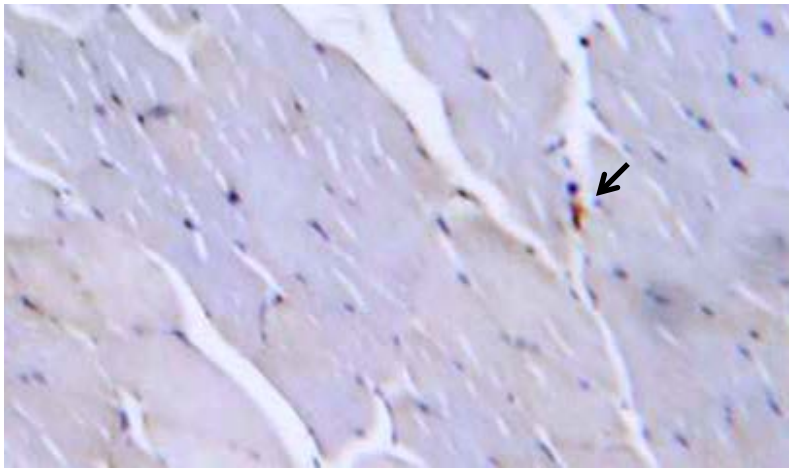
Isotype control



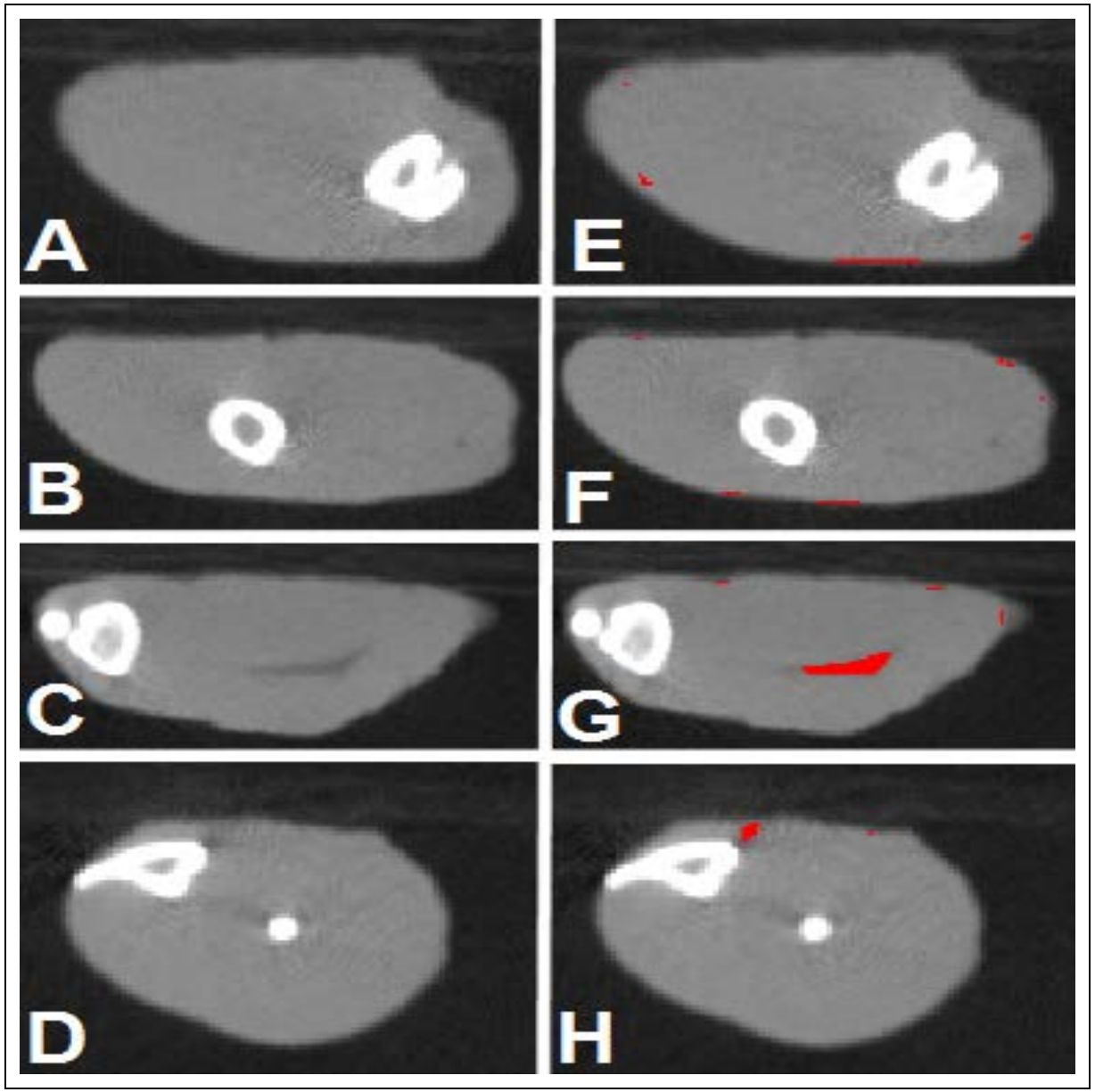
Mac3 macrophages



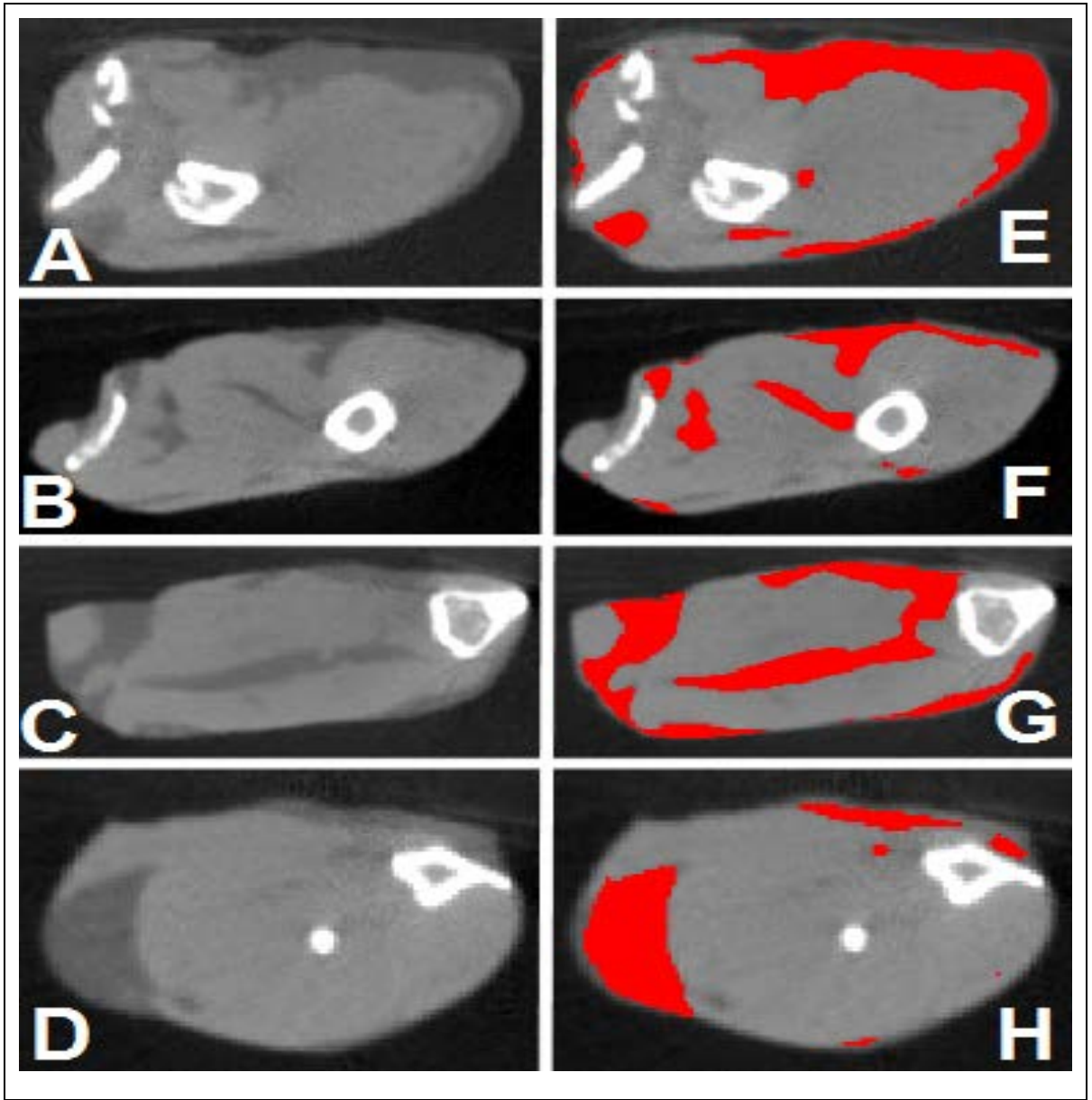
CD3 T cells



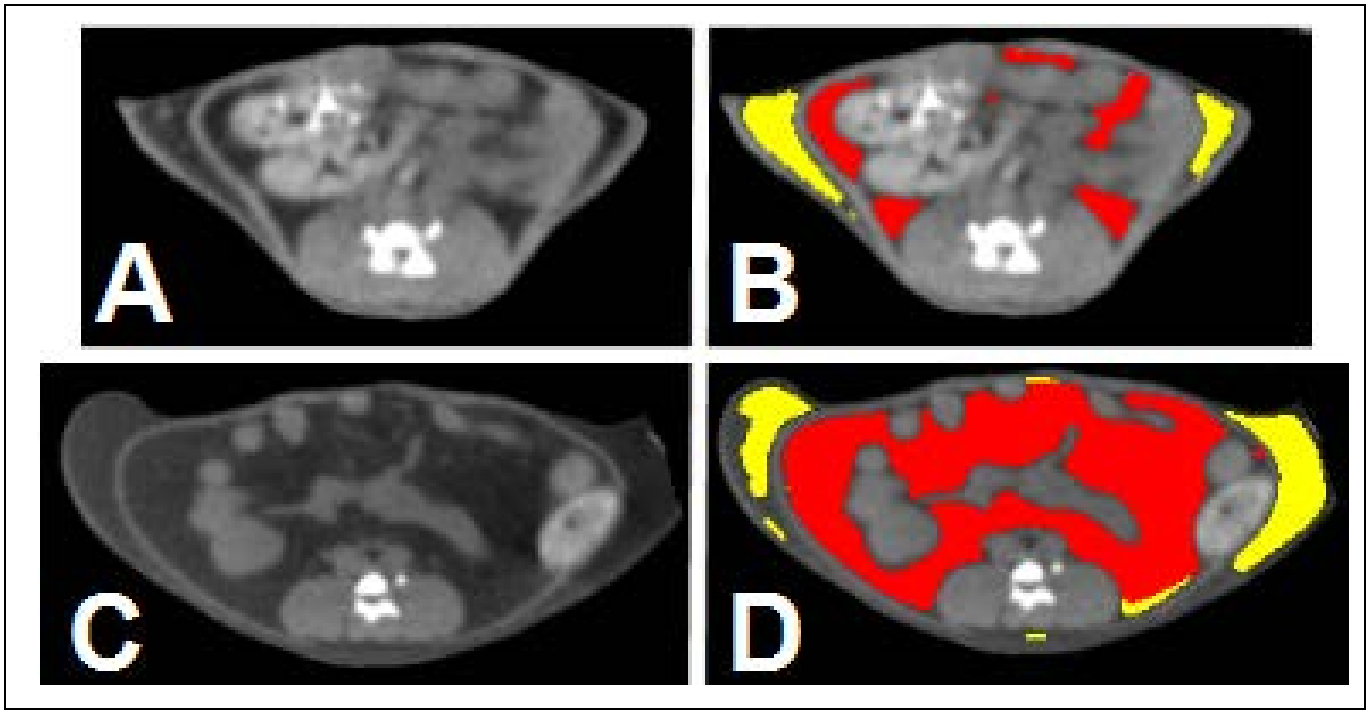
Supplemental Figure 4



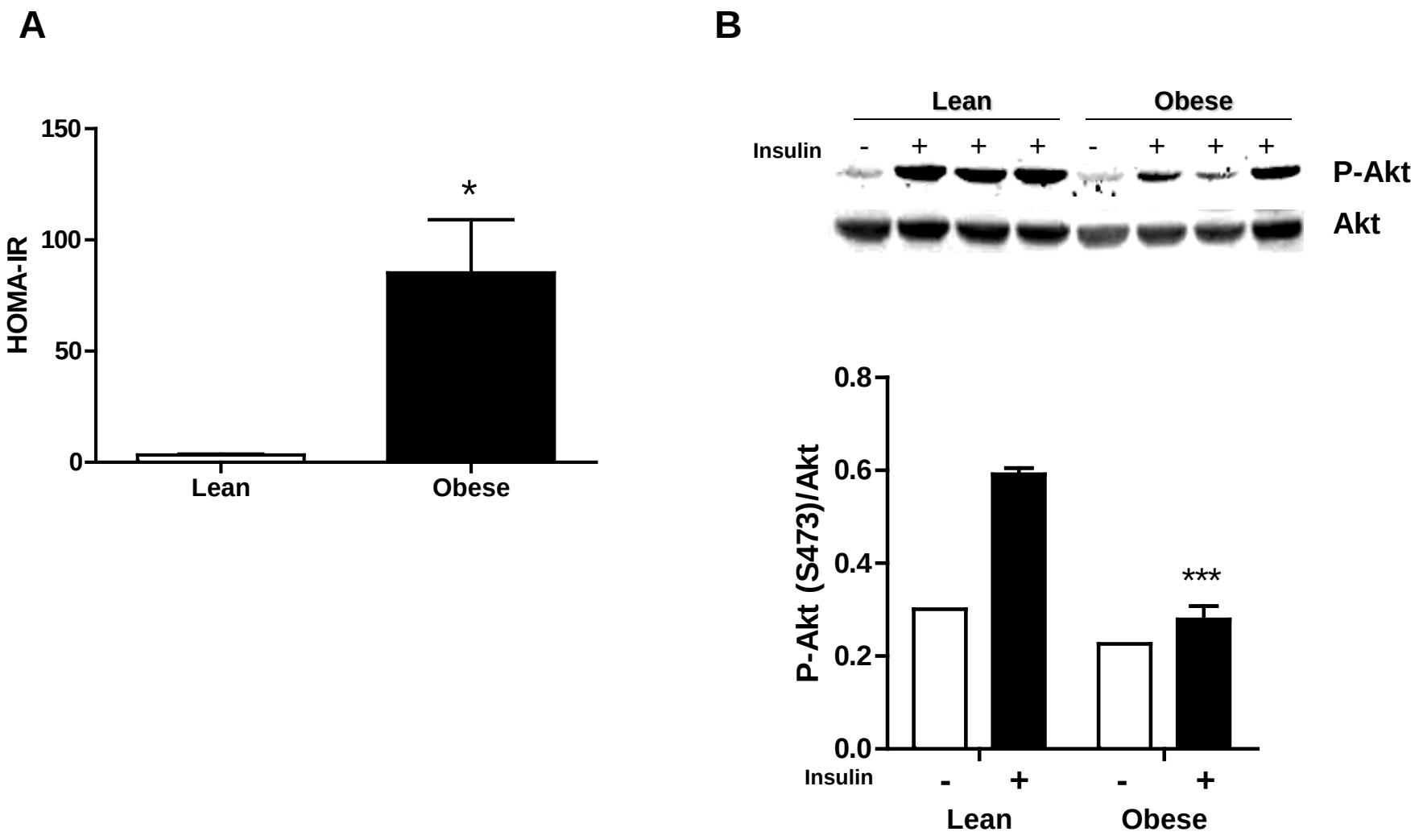
Supplemental Figure 5



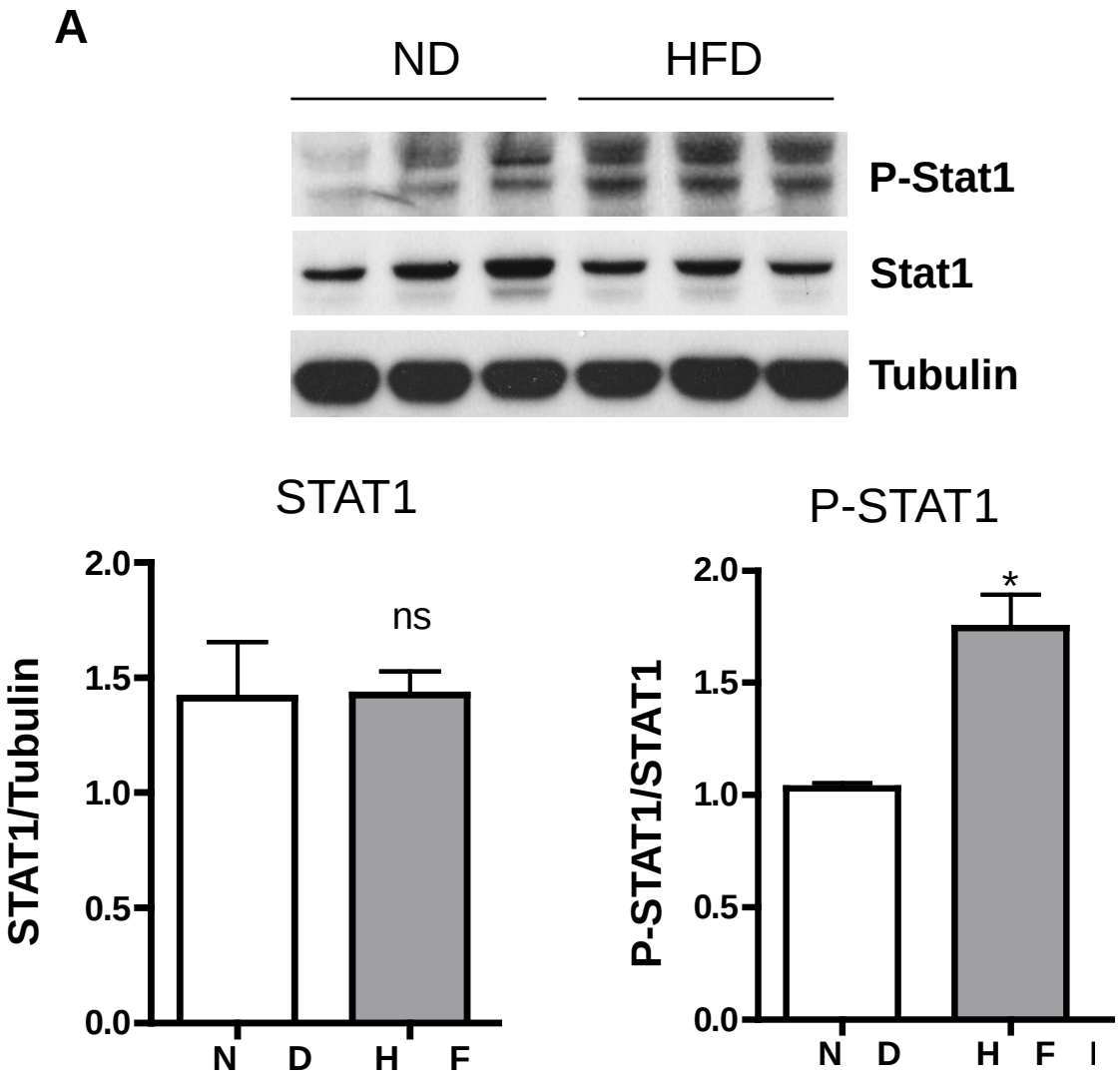
Supplemental Figure 6



Supplemental Figure 7

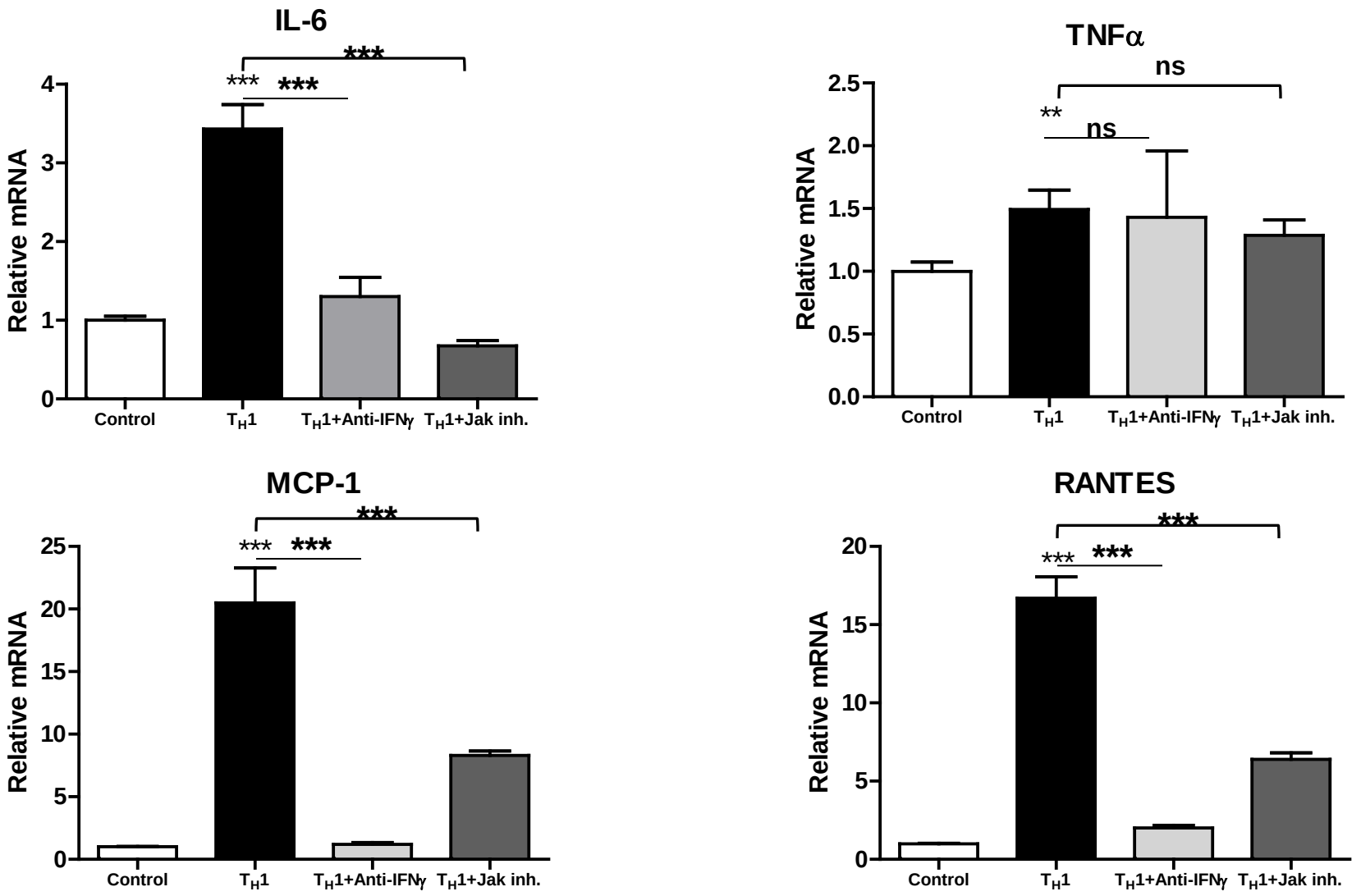


Supplemental Figure 8



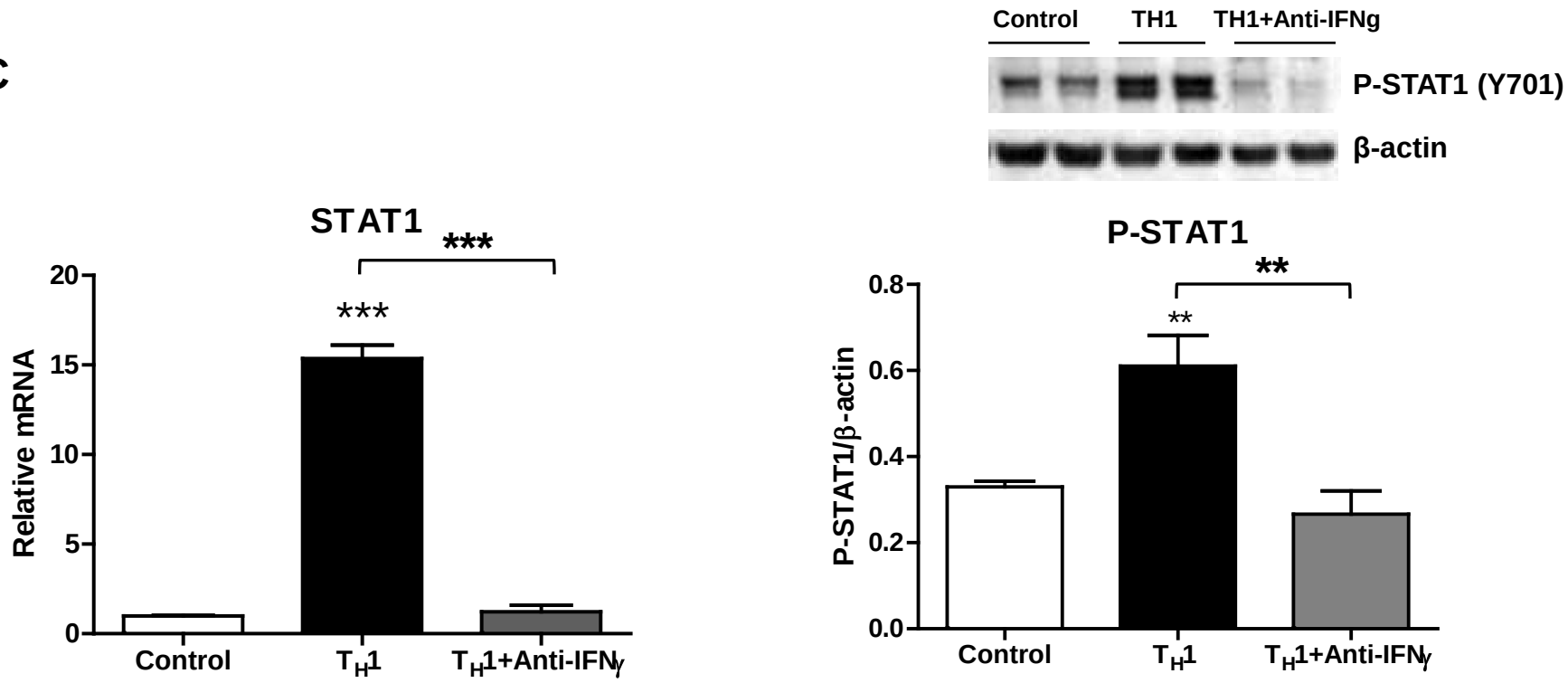
Supplemental Figure 8

B



Supplemental Figure 8

C



Supplemental Figure 8

D