

**Supplemental Digital Content:**

**SUPPLEMENTAL TABLE 1:** List of SYBR green (A) and TaqMan (B) primers used in this paper.

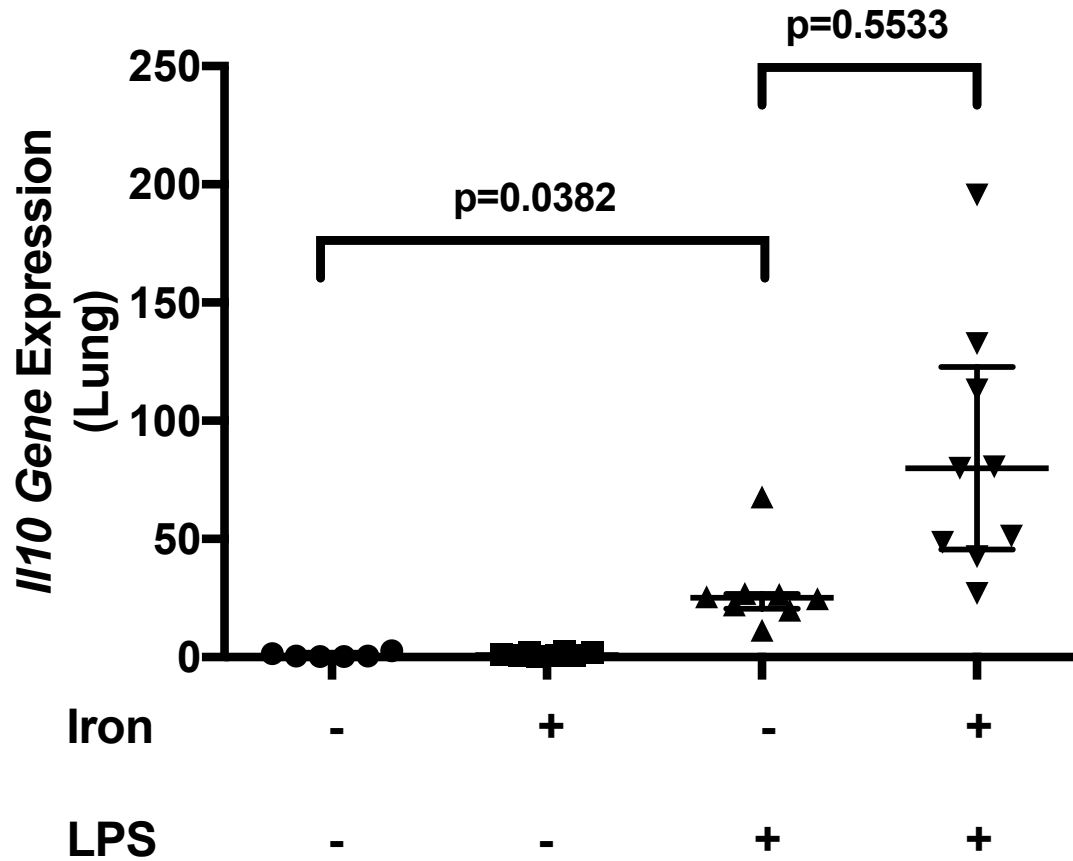
**(A)**

<b>SYBR Green primers</b>	
Genomic 18S DNA	Fwd: 5' – TAGAGGGACAAGTGGCGTTC – 3' Rev: 5' – CGCTGAGCCAGTCAGTGT – 3'
Mitochondrial CO1 DNA	Fwd: 5' – GCCCCAGATATAGCATTCCC – 3' Rev: 5' – GTTCATCCTGTTCTGCTCC – 3'
<i>Il10</i>	Fwd: 5' – GCTCTTACTGACTGGCATGAG – 3' Rev: 5' – CGCAGCTCTAGGAGCATGTG – 3'

**(B)**

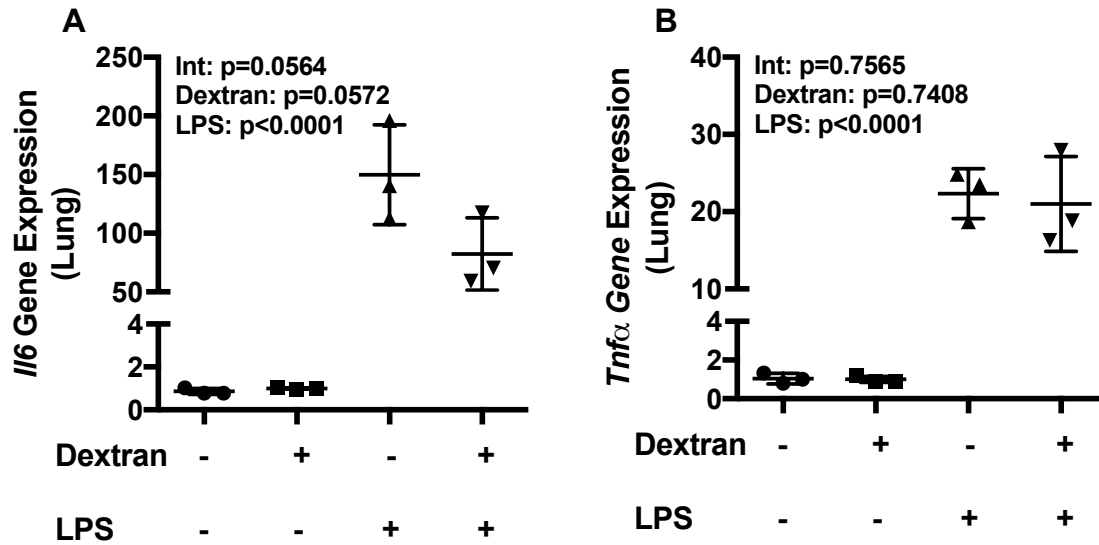
<b>TaqMan Primers</b>	
<i>Rn18S</i>	Mm03928990_g1
<i>Hamp</i>	Mm04231240_s1
<i>Il6</i>	Mm00446190_m1
<i>Tnfα</i>	Mm00443260_g1
<i>Mcp1</i>	Mm00441242_m1
<i>Lc3b</i>	Mm00782868_sH
<i>Ampk</i>	Mm01264789_m1
<i>Pgc-1α</i>	Mm01208835_m1

**SUPPLEMENTAL FIGURE 1:**



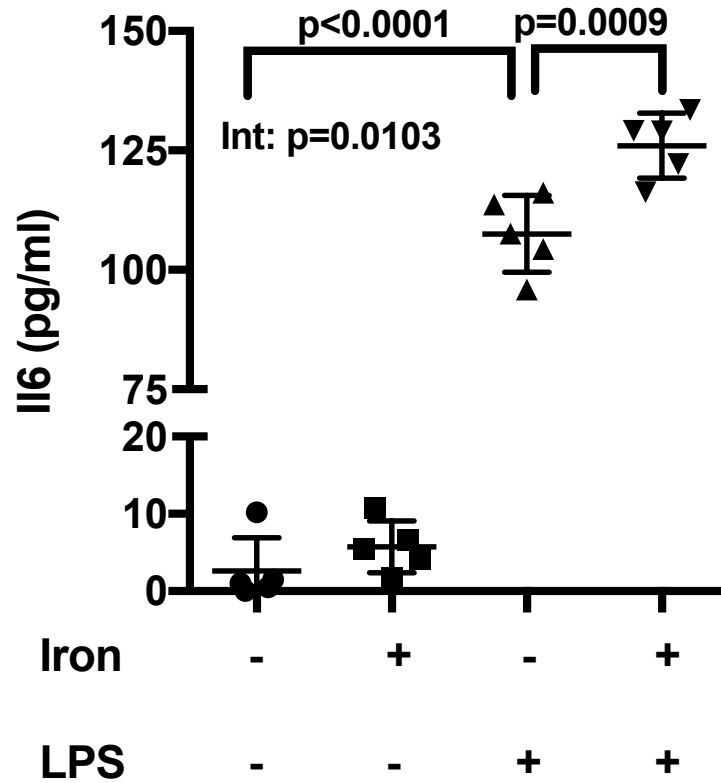
**SUPPLEMENTAL FIGURE 1: Iron loading does not result in a decrease in *Il10* gene expression *in vivo*:** WT C57Bl6 mice were treated with iron and/or LPS as mentioned earlier, and Lung IL6 mRNA levels were examined. LPS alone induced *Il10* gene expression in mouse lungs. The mice pretreated with iron did not demonstrate a reduced *Il10* response, unlike in RAW cells. N = 7-9 mice per group. (One-way Kruskal Wallis with Dunn's post hoc tests, p values adjusted for all possible comparisons; bars indicate median and IQR).

**SUPPLEMENTAL FIGURE 2:**



**SUPPLEMENTAL FIGURE 2: Dextran pre-treatment does not alter the in vivo response to a subsequent LPS stimulation.** WT C57Bl/6 mice were injected with 7.5% dextran intraperitoneally at a dose of 10  $\mu$ L/g or an equal volume of normal saline. Three days later the mice were injected with 5 mg/kg LPS or an equal volume of saline. Six hours after injection the mice were sacrificed and organs harvested. A and B show *Il6* and *Tnfα* gene expression in the lungs, respectively. N = 3 mice per group (two-way ANOVA, interaction between iron and LPS not significant, therefore only main effects reported).

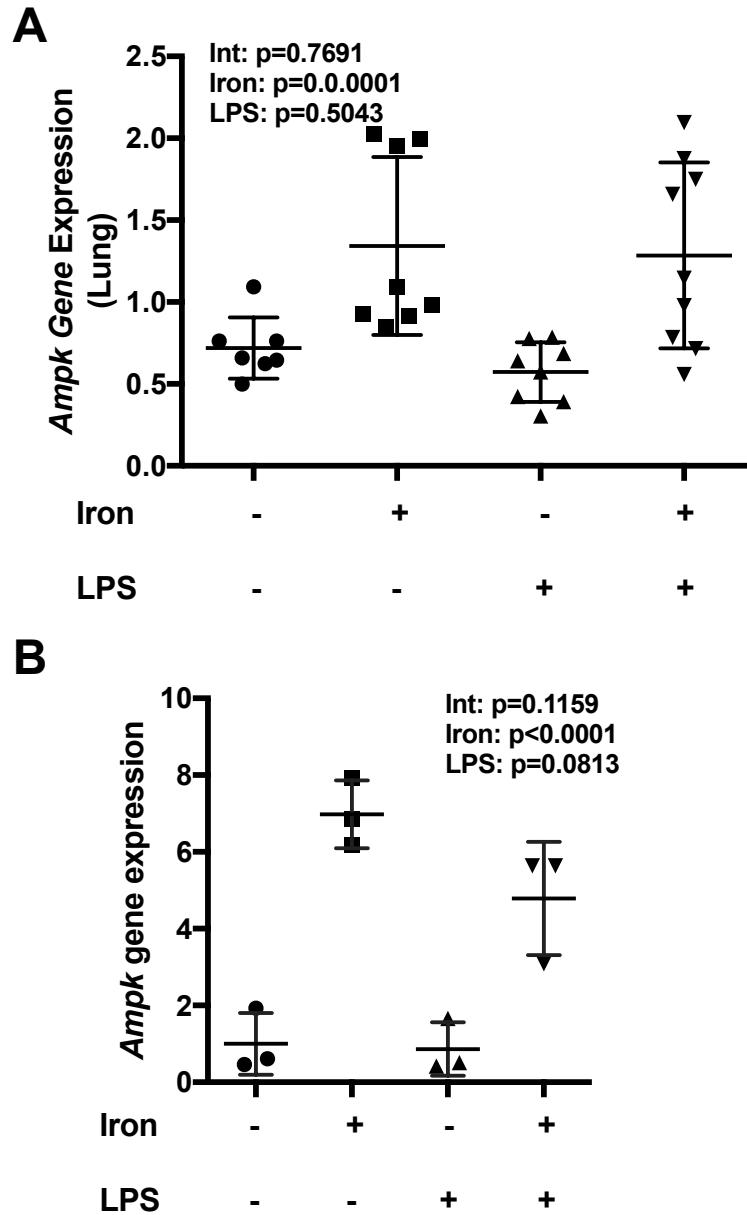
**SUPPLEMENTAL FIGURE 3:**



**SUPPLEMENTAL FIGURE 3: Iron loading on RAW 264.7 cells *in vitro* increases**

**IL6 protein production:** RAW cells were incubated with iron (200  $\mu$ M) or control overnight, and then stimulated overnight with LPS (150 ng/ml). IL6 protein levels were assessed in the supernatant by ELISA. LPS alone induced IL6 production. Iron loading significantly increased IL6 levels in response to LPS (two-way ANOVA with Bonferroni's post hoc tests, p values adjusted for all possible comparisons; Int: Interaction p value, n = 5 replicates/condition).

**SUPPLEMENTAL FIGURE 4:**



**SUPPLEMENTAL FIGURE 4: Iron loading increases Ampk gene expression in mouse lungs *in vivo* and RAW cells *in vitro*:** (A) C57Bl/6 mice were treated with iron and/or LPS, N = 7-9 mice per group. Total RNA was extracted from mouse lung and RT-qPCR was performed using primers *Ampk* (a mitochondrial biogenesis associated gene,

LPS alone decreases *Ampk* mRNA levels. Iron-loaded mice showed an increase in *Ampk* mRNA levels. **(B)** RAW cells treated with iron showed an increase in *Ampk* mRNA levels as well (two-way ANOVA, interaction between iron and LPS not significant, therefore only main effects reported, n = 3 replicates/condition).