SUPPORTING INFORMATION

Carboxylesterase 1d inactivation augments lung inflammation in mice

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Figure S1

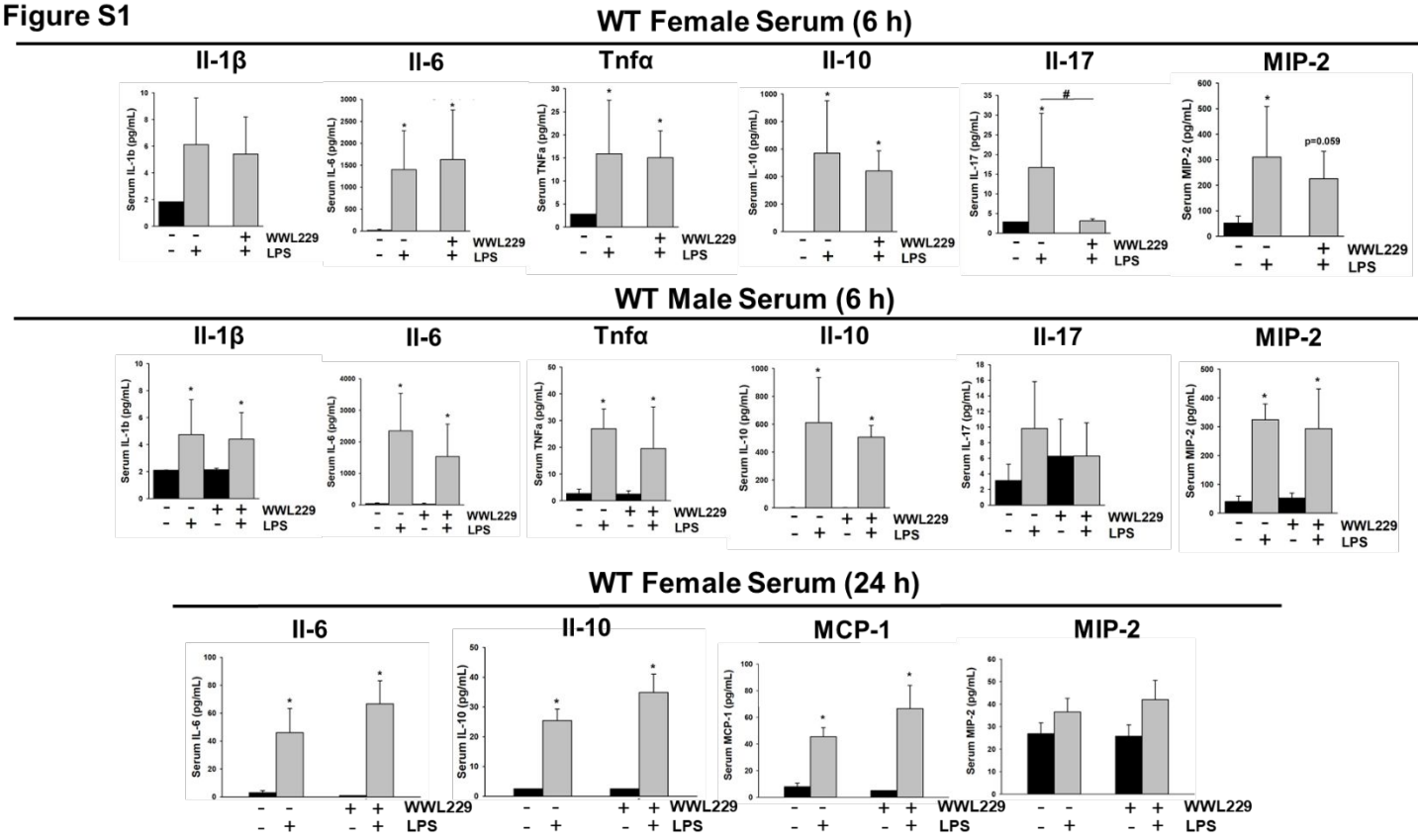

Figure S1. Serum cytokine/chemokine levels in WT mice treated with WWL229 and LPS were determined using a multiplex assay. IL-1β, IL-6, TNF-α, IL-10, IL-17, MCP-1, and MIP-2 protein levels were measured in the serum of male and female mice treated with vehicle or WWL229 in the presence or absence of LPS (6 h after LPS challenge, female and male; 24 h after LPS challenge, female only). Data represent the mean ± SD (n=5 mice/group; measurement of each cytokine/chemokine was performed in duplicate). One-way or two-way ANOVA was used to assess significance. *$p<0.05$ for treatments vs. vehicle control; #$p<0.05$ for the indicated comparison.

Figure S2

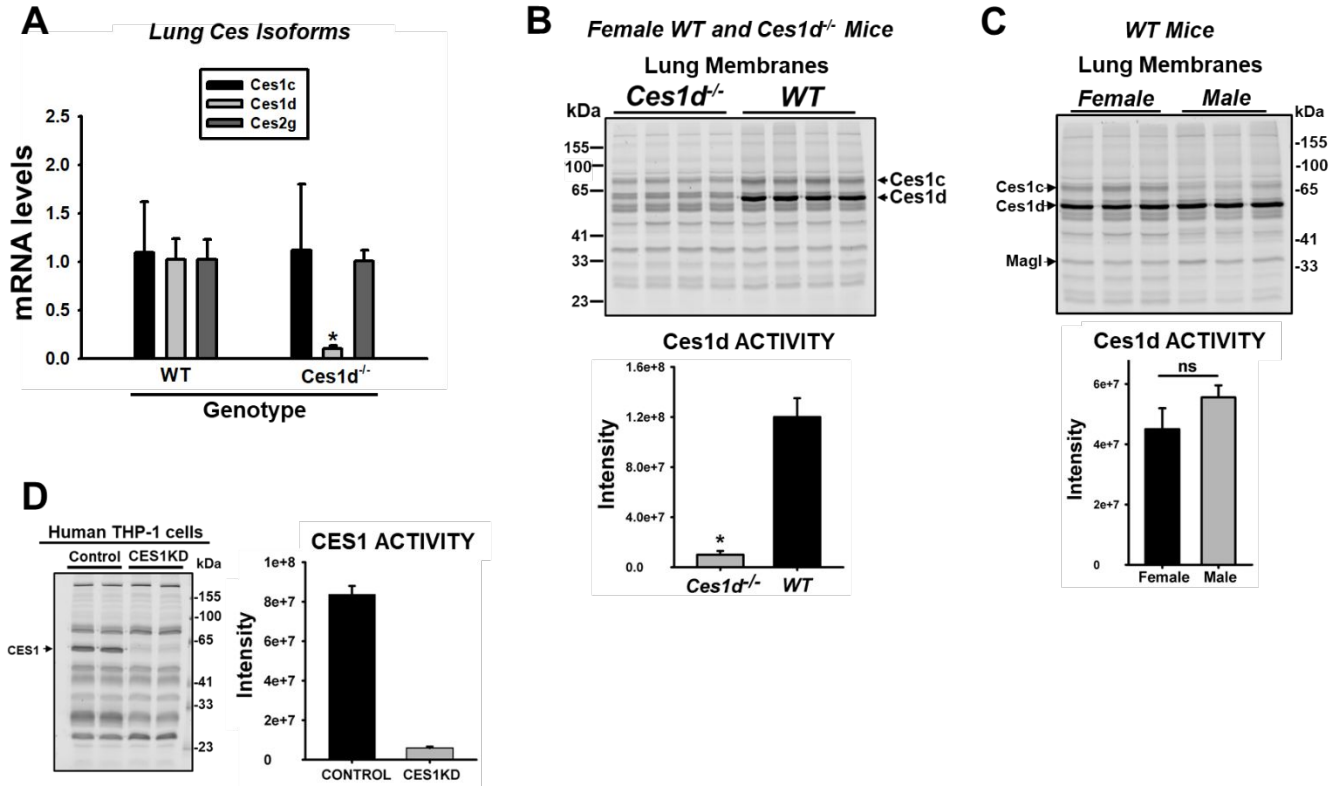

Figure S2. Ces1d is expressed/active in WT mouse lung (A-C), and CES1 activity in human THP-1 monocytes/macrophages (D). (A) mRNA levels of *Ces1c*, *Ces1d*, and *Ces2g* genes in naïve adult WT and *Ces1d*$^{-/-}$ mouse lung. mRNA quantity in WT lung was normalized to 1 for each Ces isoform. Importantly, the knockout of *Ces1d* did not alter the levels of either *Ces1c* or *Ces2g* compared to WT controls. (B) Gel-based ABPP of lung membranes from adult female WT and *Ces1d*$^{-/-}$ mice. The absence of a 60-kDa band in the *Ces1d*$^{-/-}$ lung, which was prominent in the WT lung, is consistent with Ces1d abrogation. Quantitation of the bands designated Ces1d is indicated. (C) Gel-based ABPP of lung membranes from adult female and male WT mice. Comparable levels of Ces1d enzyme activity are noted in females and males. Females appear to have more Ces1c than males, but this may be due to differences in plasma Ces1c contamination in the membranes. (D) Gel-based ABPP of human THP-1 cell lysates (Control cells and CES1KD cells) demonstrating efficient lentiviral-based knockdown of CES1. Data represents the mean ± SD (n=5 mice/group, A; n=4 mice/group, B; n=3 mice/sex, C; n=2 technical replicates for each cell type, D). *$p<0.05$ for *Ces1d*$^{-/-}$ vs. WT; ns, not significant.

Figure S3

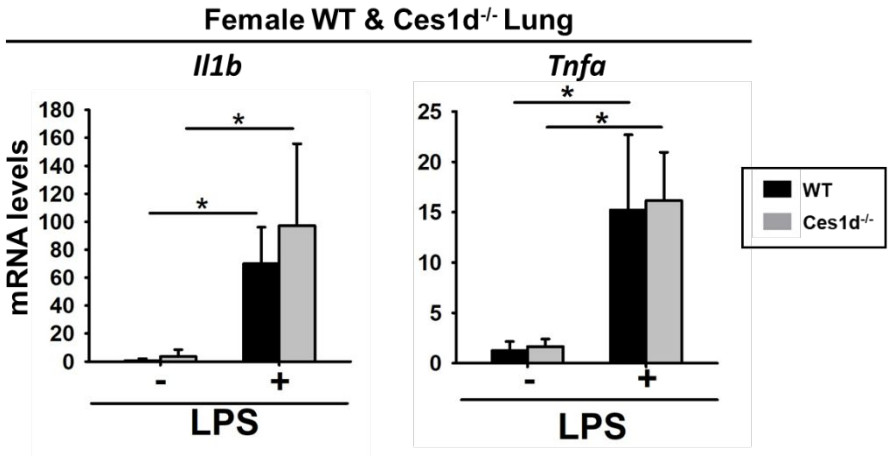

Figure S3. *Il1b* and *TNFa* mRNA levels in female WT and *Ces1d*$^{-/-}$ mouse lungs at 6 h following LPS challenge. Two-way ANOVA was used to analyze data; *$p<0.05$ compared to saline control (-LPS).

Figure S4

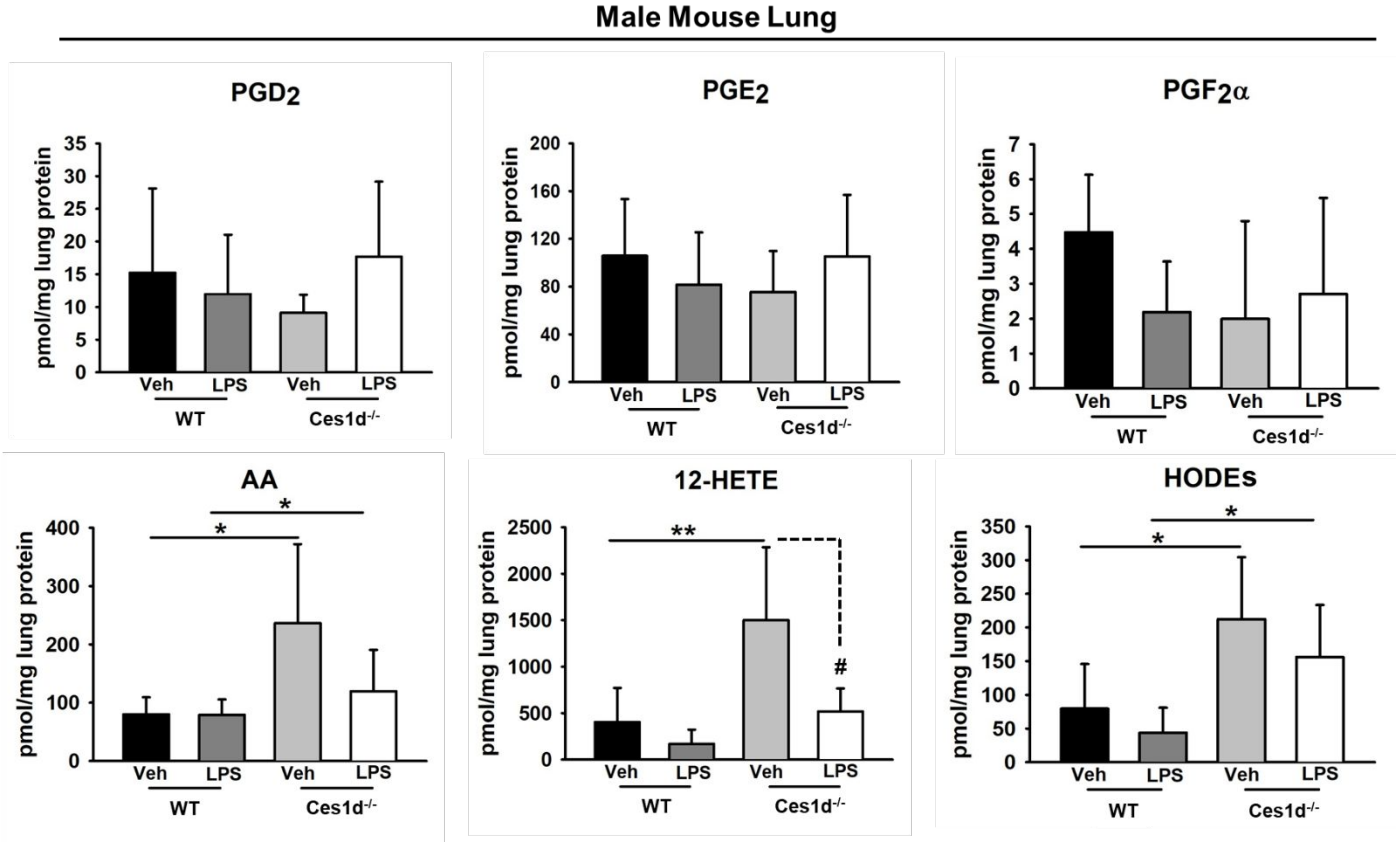

Figure S4. Eicosanoids were quantified in lungs from male WT and *Ces1d*$^{-/-}$ mice at 6 h after treatment with saline (Veh) or LPS. HODEs represent the combined levels of 9-HODE and 13-HODE. Data are expressed as mean ± SD (n=5 mice/group). Two-way ANOVA was used to assess significant differences between groups. *, # $p<0.05$ and **$p<0.01$ for the indicated comparisons.

Figure S5

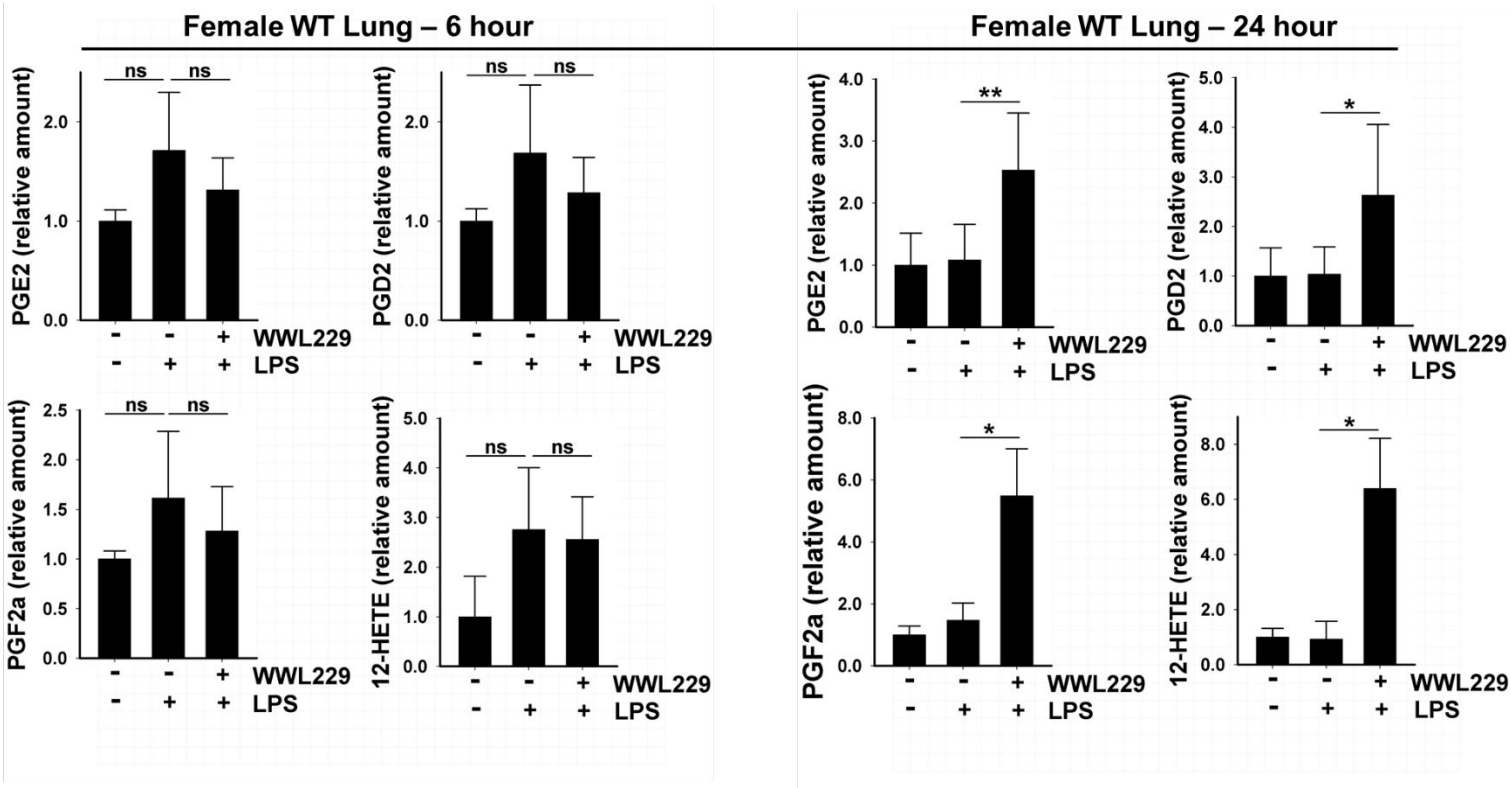

Figure S5. Eicosanoids were quantified in lungs from female WT mice that had been treated with LPS in the presence or absence of WWL229. The Ces1d inhibitor augmented the levels of PGE2, PGD2, PGF2a, and 12-HETE in lungs at 24 h after the LPS challenge, but not 6 h after LPS. Data are expressed as mean ± SD (n=5 mice/group). One-way ANOVA was used to assess significant differences between groups. *$p<0.05$ and **$p<0.01$ for the indicated comparisons; ns, not significant.

Figure S6

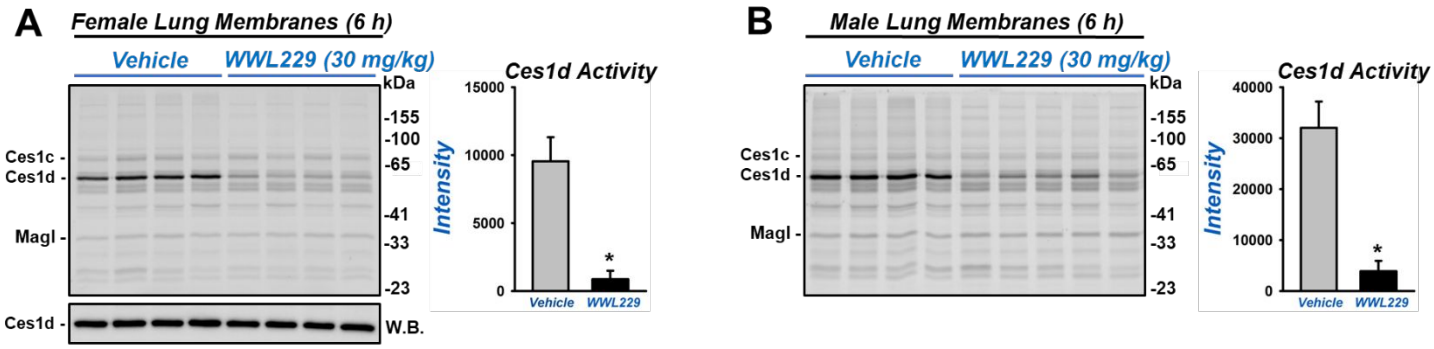

Figure S6. WWL229 inhibited lung Ces1d in female and male WT mice at 6 h after treatment. Female (A) and male (B) lung membranes from vehicle- and drug-treated mice were obtained and treated with FP-TAMRA, then subjected to gel electrophoresis. Densitometric analyses of Ces1d bands are indicated. The total proteins in the gel shown in (A) were transferred to a polyvinylidene difluoride (PVDF) membrane and probed with an anti-human CES1 antibody, which cross-reacts with mouse Ces1d protein. The resulting Western blot (W.B.) of Ces1d in the female membranes is shown below the ABPP gel and indicates that protein abundance was unaltered.

Figure S7

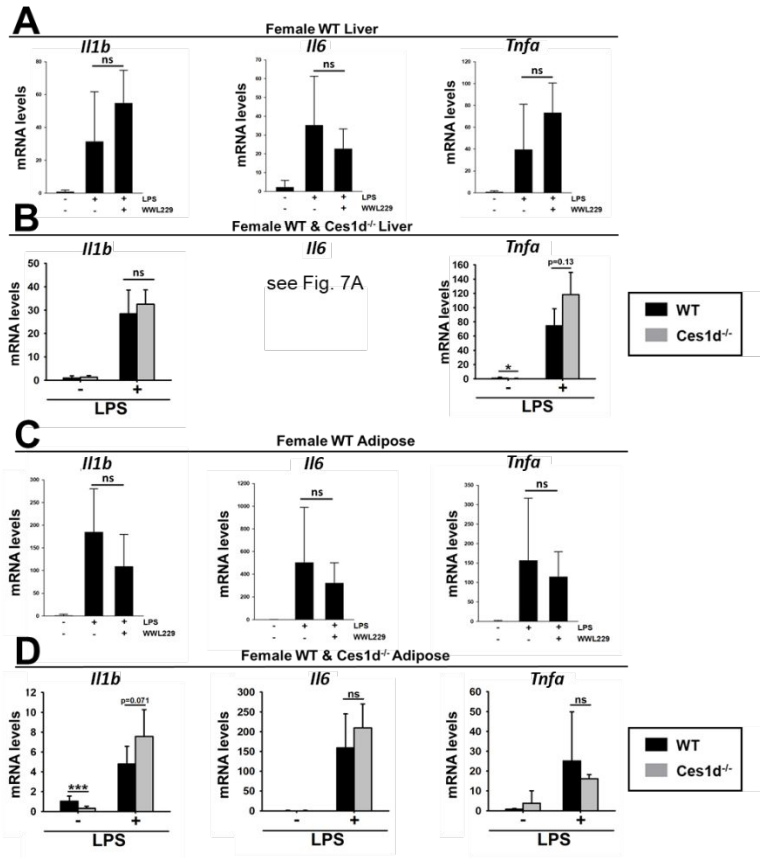

Figure S7. Cytokine mRNA levels in female mouse liver and adipose: Ces1d inhibition (WWL229) or genetic knockout of Ces1d (*Ces1d*$^{-/-}$). (A) *Il1b*, *Il6*, and *Tnfa* mRNA in WT liver 6 h following LPS challenge in the presence or absence WWL229. (B) *Il1b* and *Tnfa* mRNA in livers of female WT and *Ces1d*$^{-/-}$ mice. Results for *Il6* are shown in Figure 7A. (C) *Il1b*, *Il6*, and *Tnfa* mRNA in WT adipose 6 h following LPS challenge in the presence or absence WWL229. (D) *Il1b*, *Il6*, and *Tnfa* mRNA in adipose of female WT and *Ces1d*$^{-/-}$ mice. Data represent the mean ± SD (n=6 mice/group). Two-way ANOVA was used to analyze data; *p<0.05 and ***p<0.001 for the indicated comparisons; ns, not significant.

Figure S8

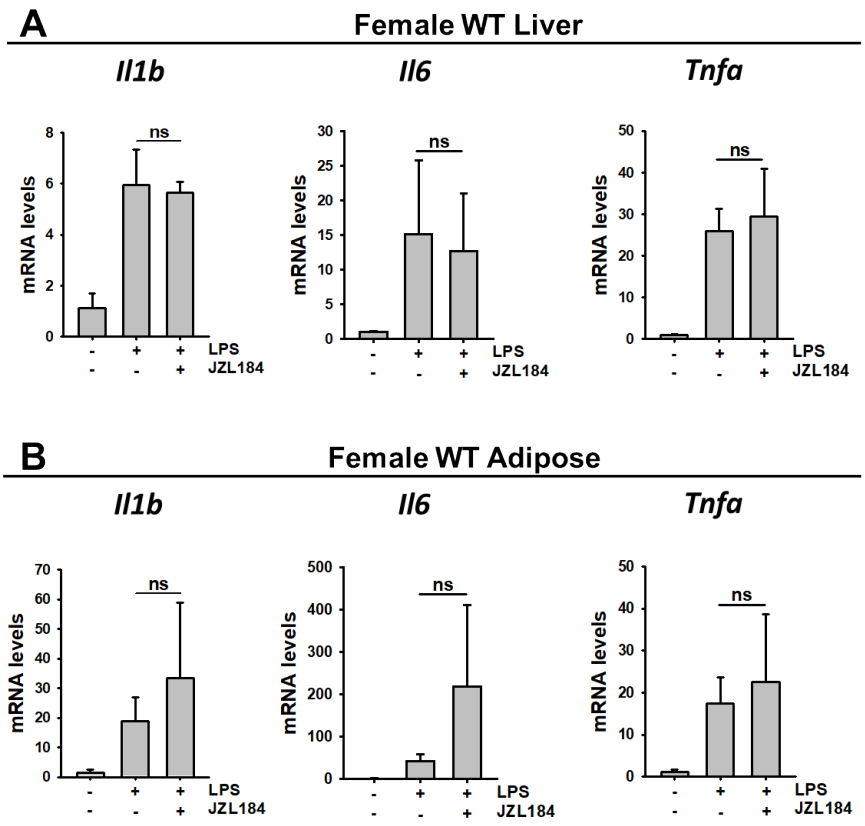

Figure S8. Cytokine mRNA levels in female mouse liver and adipose: Magl inhibition (JZL184). (A) *Il1b*, *Il6*, and *Tnfa* mRNA in WT liver 6 h following LPS challenge in the presence or absence JZL184. (B) *Il1b*, *Il6*, and *Tnfa* mRNA in WT adipose 6 h following LPS challenge in the presence or absence JZL184. Data represent the mean ± SD (n=6 mice/group). Two-way ANOVA was used to analyze data; ns, not significant.