

Supplementary Information for

Leptin signaling impairs macrophage defenses against *Salmonella* Typhimurium

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Figs. S1 to S6

Fig-S1

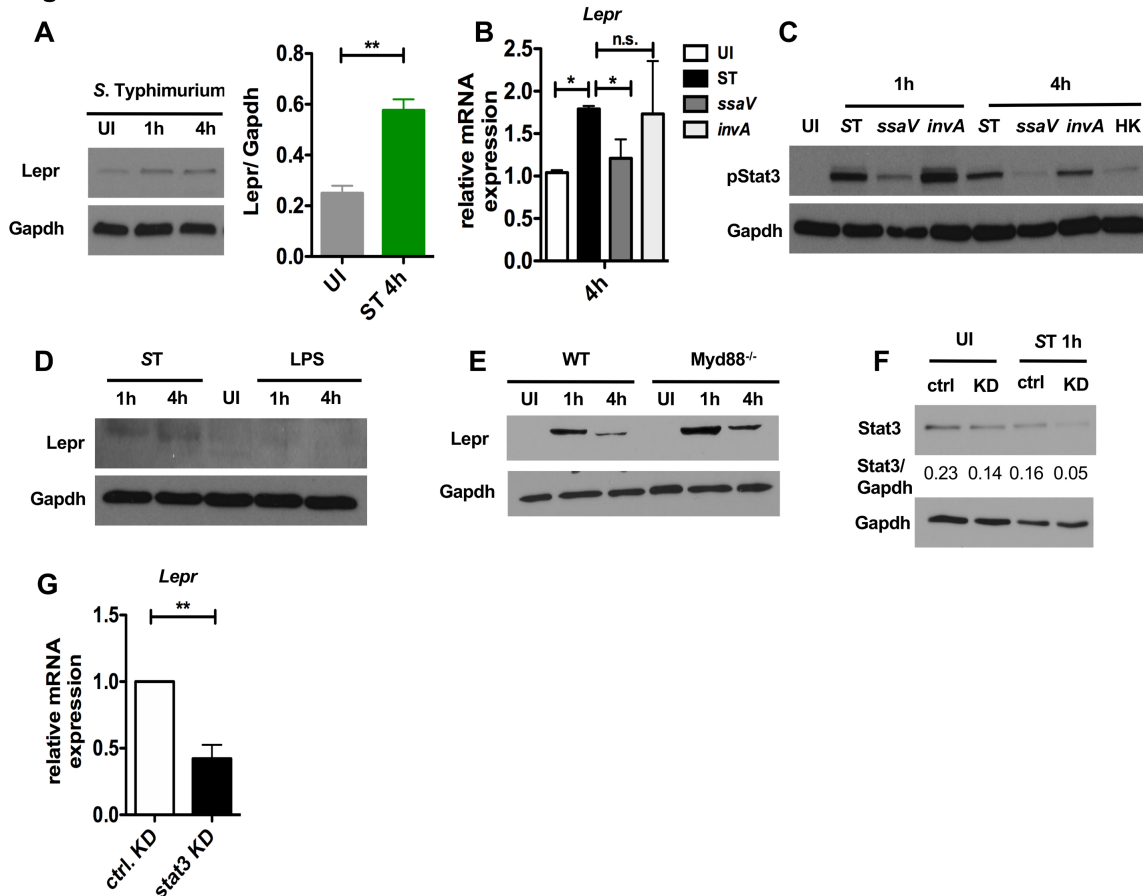


Fig. S1.

(A) Immunoblot analysis of Lepr expression in BMDMs and blots quantified using Image-Jâ. (B) Relative Lepr mRNA expression to Hprt in WT BMDMs 4h post *S. Typhimurium*, $\Delta ssaV$ and $\Delta invA$ infection. (C) Immunoblot analysis of phospho-Stat expression in WT BMDMs infected with *S. Typhimurium*, $\Delta ssaV$ and $\Delta invA$ mutant strains. (D) Westernblot analysis of Lepr expression in WT BMDMs infected with ST, heat-killed *S. Typhimurium* (HK). (E) Immunoblot analysis of Lepr in uninfected and infected BMDMs from WT and Myd88^{-/-} mice. (F) Immunoblot analysis of Stat3 siRNA mediated knock-down. (G) Relative Lepr mRNA expression in uninfected control siRNA and Stat3 siRNA mediated knock-down (n=2). (Data are shown as mean \pm S.E.M. and statistical significance calculated using student t-test and represented as *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$).

Fig-S2

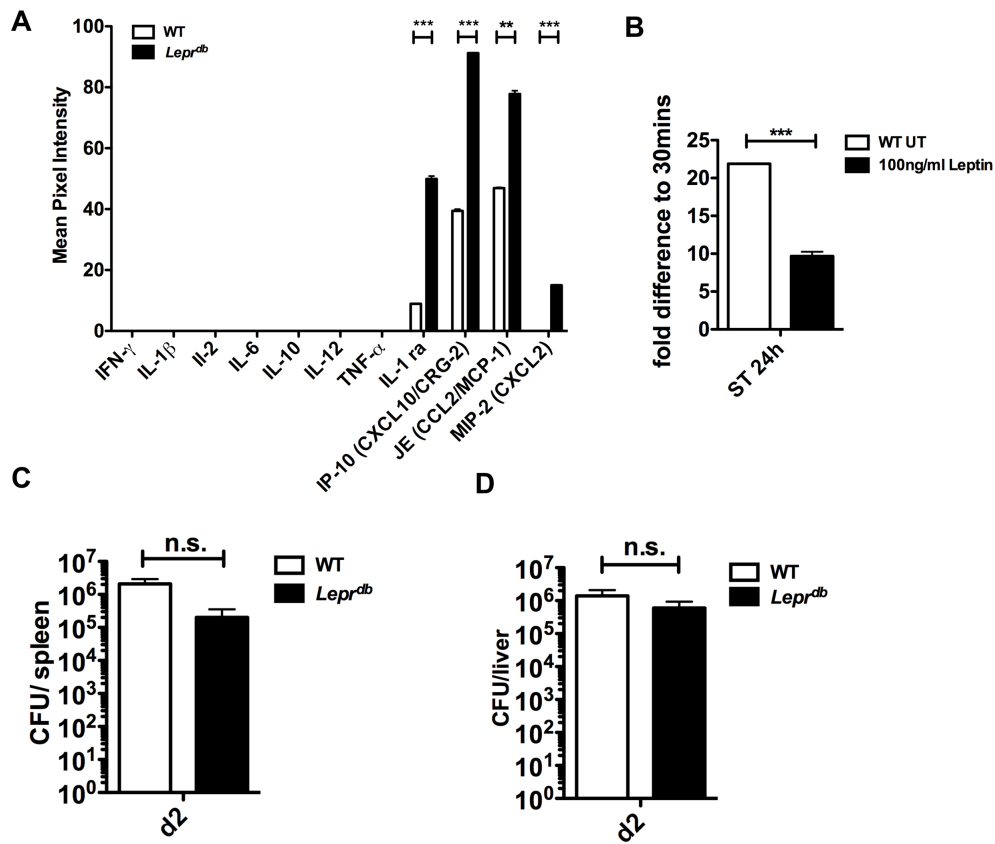


Fig. S2.

(A) Analysis of cytokine array in uninfected WT and *Leprd* BMDMs (n=1). (B) Bacterial burden in untreated and Leptin 100ng/ml treated BMDMs (n=2). (C) Bacterial load in spleen from WT and *Leprd* mice 2days post *S. Typhimurium* infection (n=1). (D) Bacterial load in liver from WT and *Leprd* mice 2days post *S. Typhimurium* infection (n=1).

Fig-S3

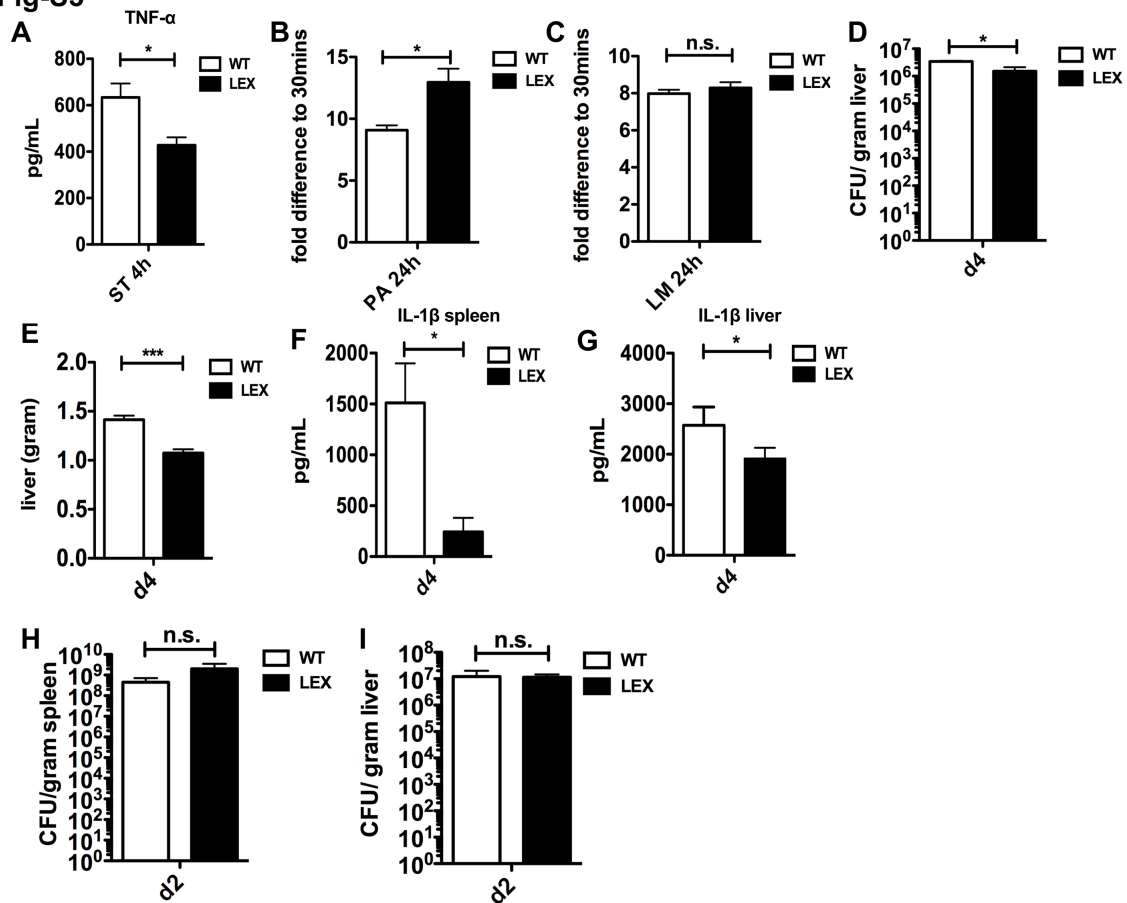


Fig. S3.

(A) TNF- α measured in the supernatants of 4h *S. Typhimurium* infected WT and LEX BMDMs (n=3). (B) Bacterial burden in WT versus LEX BMDMs infected with *P. aeruginosa* (PA) 24h post infection. (C) Bacterial burden in WT versus LEX BMDMs infected with *L. monocytogenes* (LM) 24h post infection. (D) Bacterial load in the liver from LEX compared to WT mice 4 days post intravenous infection (n=3). (E) Weight of liver from LEX compared to WT mice 4 days post intravenous infection (n=3). (F) IL-1 β measured by ELISA from WT and LEX spleens 4 days post infection. (G) IL-1 β measured by ELISA from WT and LEX spleens 4 days post infection. (H) Bacterial load in spleen from WT and LEX mice 2days post *S. Typhimurium* infection (I) Bacterial load in liver from WT and LEX mice 2days post *S. Typhimurium* infection. Data are shown as mean \pm S.E.M. and statistical significance calculated using student t-test and represented as *= p <0.05; **= p <0.01; ***= p <0.001.

Fig-S4

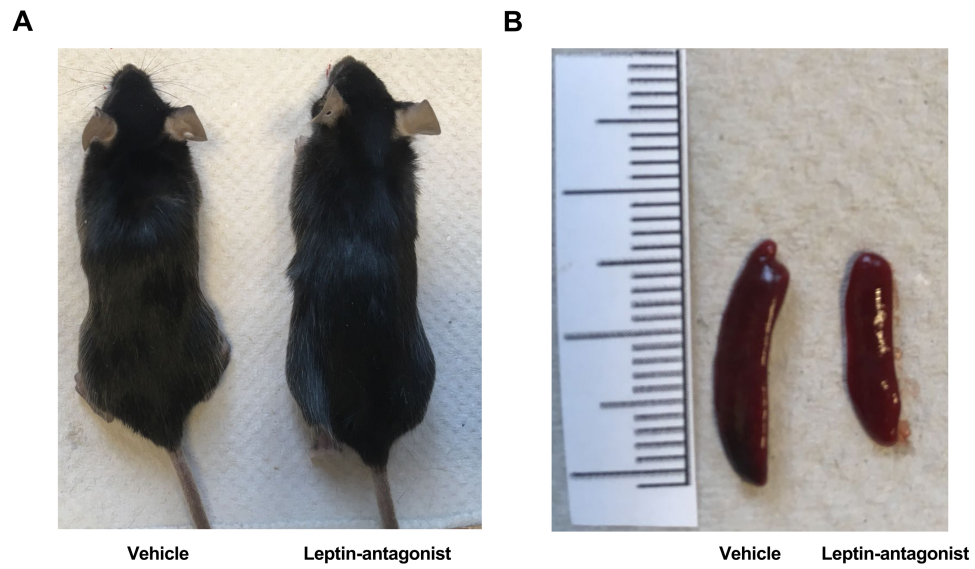


Fig. S4.

(A) Leptin-antagonist and vehicle treated-WT mice infected with *S. Typhimurium* on day 4 post infection (n=1). (B) Spleen size on day4 post-infection (n=1).

Fig-S5

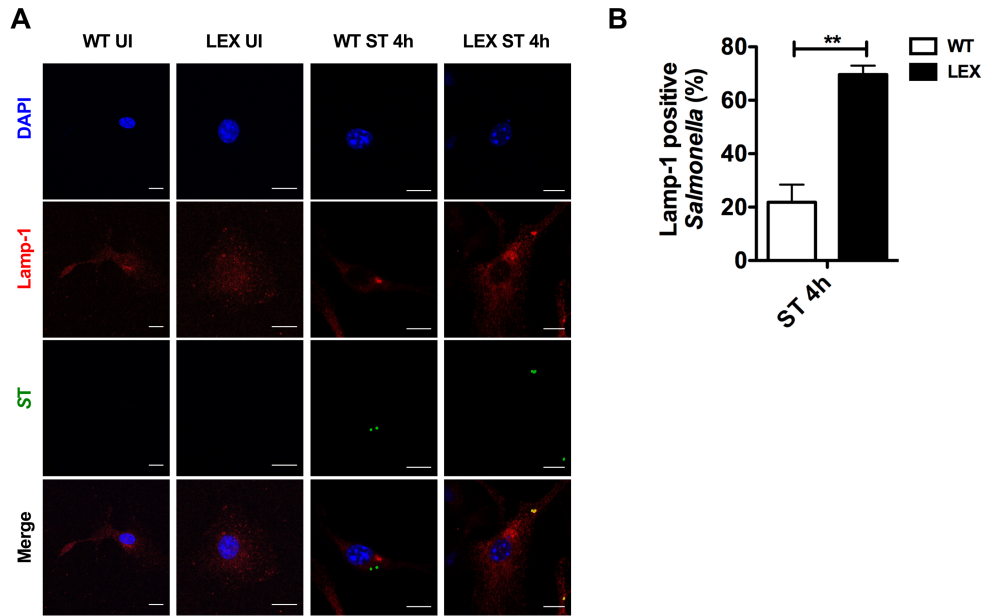


Fig. S5.

(A) Confocal microscopy analysis of UI and 4h infected WT and LEX BMDMs stained for *S. Typhimurium* LPS and Lamp-1 (n=1). (B) Bar graph shows *S. Typhimurium*-Lamp-1 colocalization in WT and LEX BMDMs 4h post infection.

Fig-S6

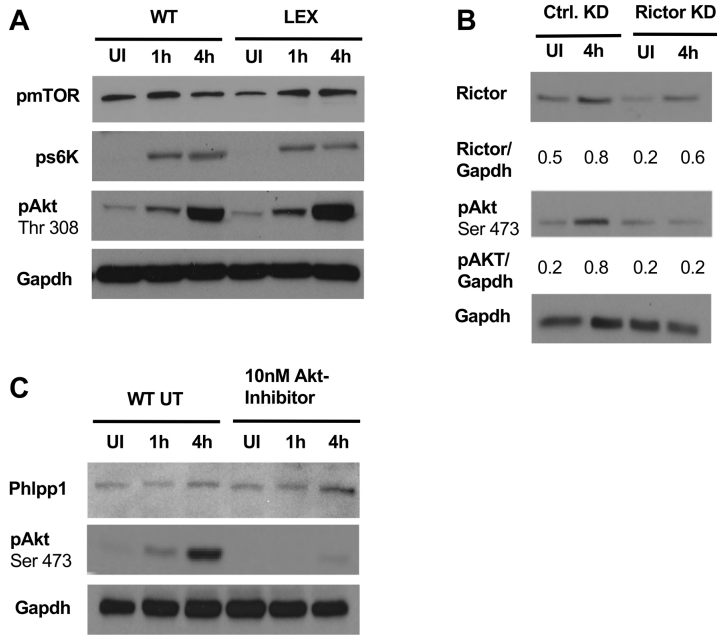


Fig. S6.

(A) Immunoblot analysis of mTORC1 pathway in WT and LEX BMDMs infected with *S. Typhimurium* for 1h and 4h compared to UI (n=3). (B) Immunoblot analysis of Rictor siRNA transfected WT BMDMs versus control siRNA transfected cells infected with *S. Typhimurium* for 4h for Rictor and phospho-Akt Ser 473 (n=3). (C) Immunoblot of Akt-Inhibitor treated and untreated WT BMDMs 1h and 4h post infection compared to UI analyzed for Phlpp1 and phospho-AKT-Ser 473 (n=3).