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Supporting Information

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Lipocalin-2 ensures host defense against *Salmonella* Typhimurium by controlling macrophage iron homeostasis and immune response

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Supplementary figure legends.

Supplementary figure 1. Antibody-mediated neutralization of TNF-α does not affect the differences between *wt* and *Lcn2*$^{-/-}$ peritoneal macrophages.

Wt and *Lcn2*$^{-/-}$ peritoneal macrophages were infected with *Salmonella* Typhimurium (*S.* Tm.) at a MOI of 10:1. After 1 hr, a neutralizing antibody against TNF-α (α TNF) or an isotype control (Ctrl.) was added for additional 23 h. Thereafter, the intracellular bacterial load (A), IL-10 (B) and nitrite (C) were determined. Values are depicted as lower quartile, median and upper quartile (boxes) with minimum and maximum ranges and statistical significant differences as determined by ANOVA are indicated (n = 8-12 individual values from 4 independent experiments).

Supplementary figure 2. Comparable *Salmonella*-induced cell death in *wt* and *Lcn2*$^{-/-}$ peritoneal macrophages.

Wt (closed bars) and *Lcn2*$^{-/-}$ peritoneal macrophages (open bars) were treated with diluent (Ctrl.) or infected with *Salmonella* Typhimurium (*S.* Tm.) at a MOI of 10:1 for 24 h. The number of annexin V (A) or propidium iodide (B) single positive cells was determined by immunofluorescence. Relative values are depicted as means ± S.E.M., P values were compared by ANOVA following Bonferroni correction and statistically significant differences are indicated (n = 3 independent experiments). °, $P < 0.10$ (trend) as compared to the control of the respective genotype; +, $P < 0.05$ as compared to the respective genotype.

(C) Caspase-1 activity was determined by a specific kit (n = 6 independent experiments). +, $P < 0.05$ as compared to the respective genotype.

Supplementary figure 3. Increased bacterial loads and IL-10 production in naïve *Lcn2*$^{-/-}$ peritoneal macrophages and *Lcn2*$^{-/-}$ BMDM.

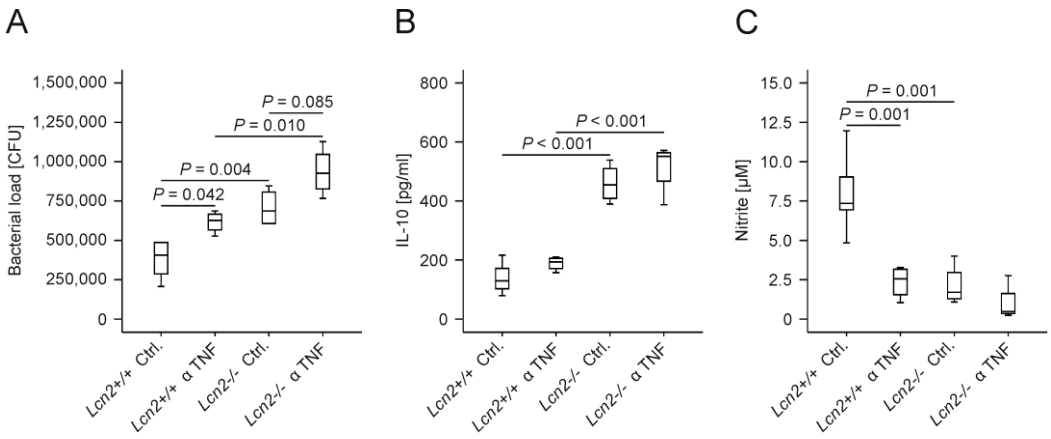
(A) Non-elicited naïve peritoneal macrophages or BMDM (C) isolated from *wt*, *IL-10*$^{-/-}$, *Lcn2*$^{-/-}$ or *Lcn2*$^{-/-}$*IL-10*$^{-/-}$ mice were infected with *Salmonella* Typhimurium (*S.* Tm.) at a MOI of 10 for 16 h and bacterial load was determined.

(B, D) IL-10 concentrations in culture supernatants of non-elicited peritoneal macrophages (B) or BMDM (D) were measured by a specific ELISA. n.d. denotes not detectable.

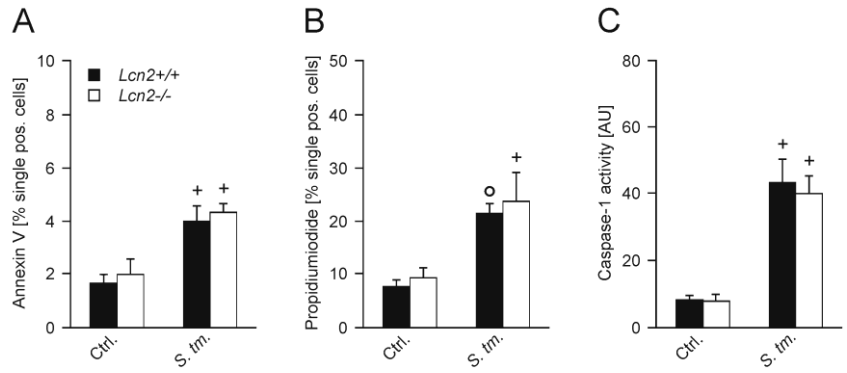
Supplementary figure 4. Increased TNF-α and IL-6 serum levels in *Lcn2*$^{-/-}$*IL-10*$^{-/-}$ mice.

TNF-α (A) and IL-6 (B) serum levels in *wt*, *Lcn2*$^{-/-}$ and *Lcn2*$^{-/-}$*IL-10*$^{-/-}$ mice were measured by specific ELISA kits (n = 8-12 individual mice).

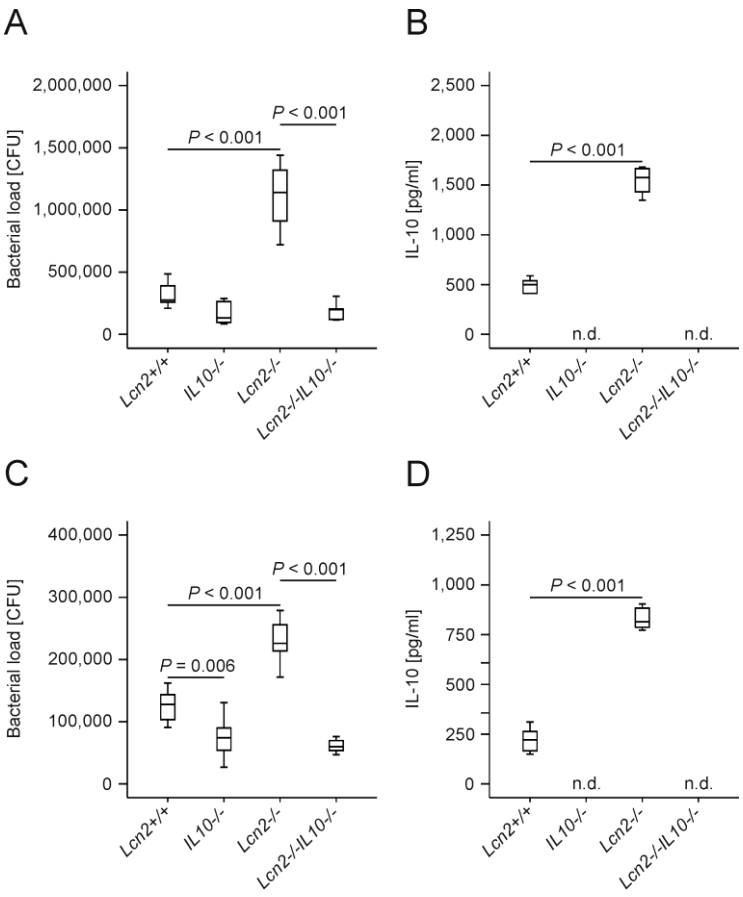
Supplementary figure 1.



Supplementary figure 2.



Supplementary figure 3.



Supplementary figure 4.