Electronic Supplementary Material (ESI) for Analyst.

Supplementary material

Cell-free hemoglobin is a marker of systemic inflammation in mice models of sepsis: A Raman spectroscopic study

Taru Verma[1], Shamik Majumdar[2], Shikha Yadav[2], Syed Moiz Ahmed[2], Siva Umapathy[1,3]* and Dipankar Nandi[1,2]*

[1]Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore
[2] Department of Biochemistry, Indian Institute of Science, Bangalore
[3]Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore

*corresponding authors

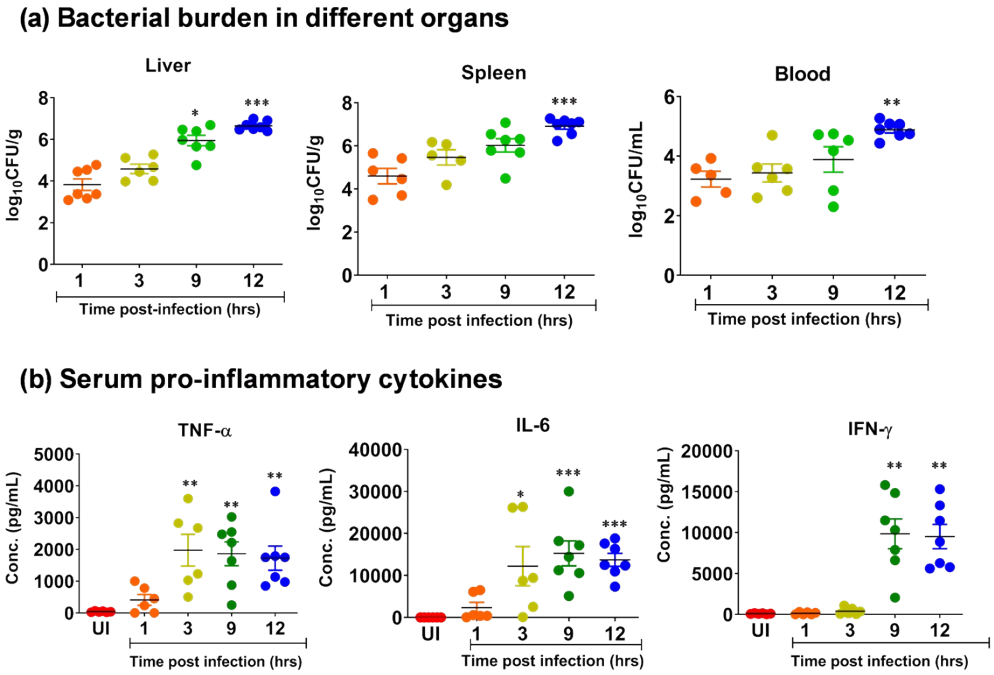

Figure S1: *S.* Typhimurium injected intra-peritoneally in mice results in rapid increase in CFU and cytokines. (a) Estimation of the bacterial burden in liver, spleen and blood at the indicated time points post infection; (b) Determination of the pro-inflammatory cytokine burst in the blood sera of uninfected (UI) and infected mice. The data are representative of two independent experiments with 3-4 mice per group per experiment and are presented as mean ± S.D. Each dot represents one mouse. Statistical analysis was done using the non-parametric Kruskal Wallis test where * represents $p< 0.05$, ** $p<0.01$, ***$p<0.001$ and **** represents $p<0.0001$

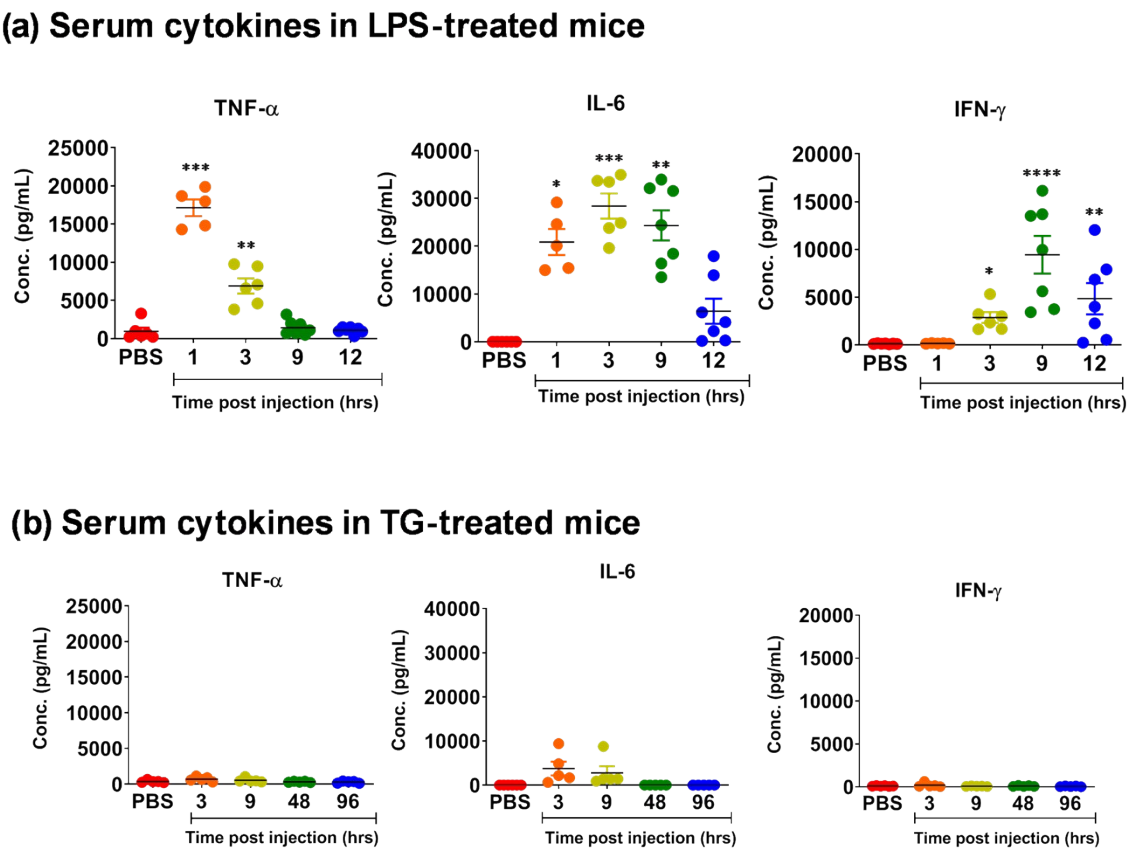

Figure S2: Pro-inflammatory cytokines are induced in a kinetic manner in the sera of LPS-treated mice but not in the TG-treated mice. Estimation of the cytokines in the blood sera of (A) LPS-treated and (B) TG-treated mice was performed at the indicated time-points using ELISA. The data are representative of two independent experiments with 2-3 mice per group per experiment and are presented as mean ± S.D. Each dot represents one mouse. Statistical analysis was done using the non-parametric Kruskal Wallis test where * represents $p< 0.05$, ** $p<0.01$, ***$p<0.001$ and **** represents $p<0.0001$

Concentration of hemoglobin in the sera of uninfected and infected mice

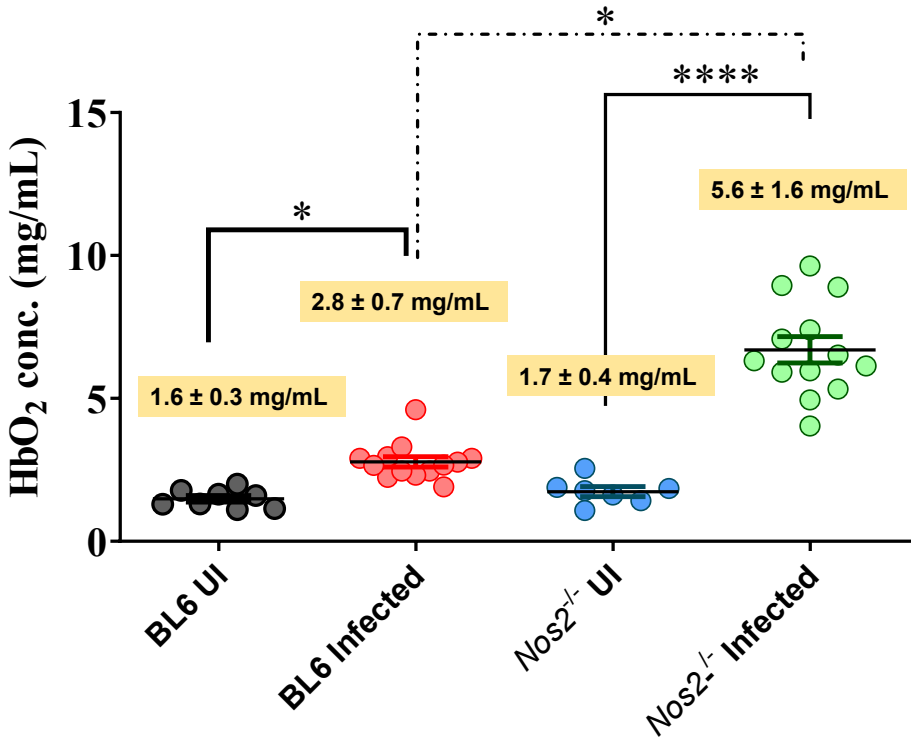

Figure S3: Higher cell-free hemoglobin is detected in the sera of infected mice when compared to the uninfected mice. HbO_2 concentration in mg/mL was determined using the Harboe method in the sera of uninfected (UI) and *Salmonella* Typhimurium-infected mice 12 hrs post infection, for both the C5BL/6 (BL6) and the *Nos2*$^{-/-}$ mice. The data are representative of two independent experiments with 5-6 mice per group per experiment and are presented as mean ± S.D with the values mentioned in the yellow boxes. Each dot represents one mouse. Statistical analysis was done using the non-parametric Kruskal Wallis test where * represents $p< 0.05$, ** $p<0.01$, ***$p<0.001$ and **** represents $p<0.0001$

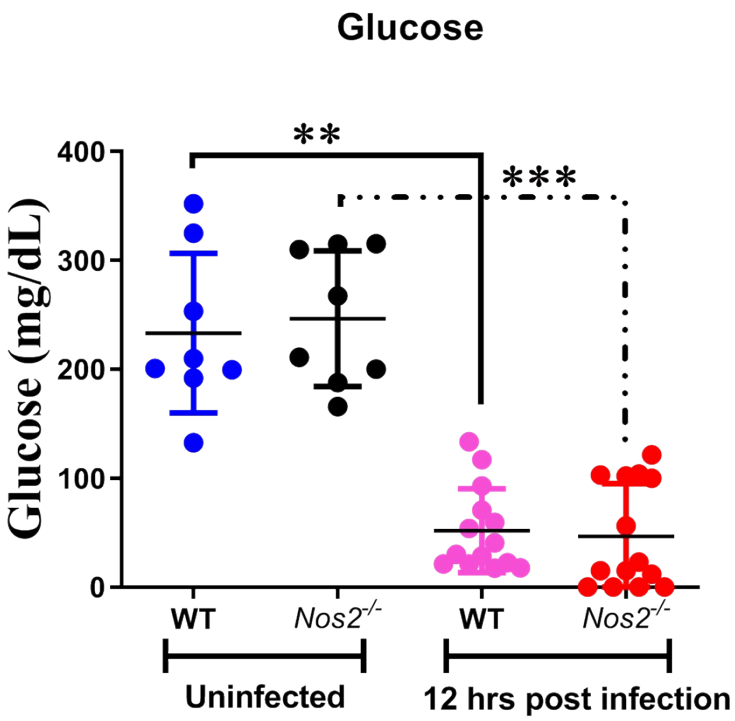

Figure S4: Sera glucose amounts are reduced in both WT and *Nos2*$^{-/-}$ mice during sepsis. WT and the *Nos2*$^{-/-}$ mice were sacrificed at 12 hrs post *Salmonella* Typhimurium-infection. The blood was collected, and serum was isolated for performing the glucose estimation assay. Each dot represents one mouse. The data are representative of two independent experiments and are presented as mean ± S.D. Statistical analysis was done using the non-parametric Kruskal Wallis test where * represents p< 0.05, ** p<0.01, ***p<0.001 and **** represents p<0.0001