Supplemental Materials

Supplemental Methods

Analysis of STAT1 phosphorylation

RAW264.7 macrophages (a gift from Dr. Douglas Green; St. Jude Children's Research Hospital) were incubated for 10 min at 37°C, 5%CO_2 with sera from naïve and LCMV-infected *Prf1*$^{-/-}$ mice that were treated or not with αIFNγ (0.5mg/mouse or 1mg/mouse intraperitoneally on days 4 and 7 post infection[p.i.]) or ruxolitinib (90mg/kg po BID on days 4 through 8 p.i.). Cells were then fixed with Fix Buffer I (BD Phosphoflow), permeabilized with Perm Buffer III (BD Phosphoflow), stained with Alexa Fluor 488 pSTAT1 (pY701) antibody (BD Phosphoflow), acquired with a BD LSRII Fortessa and analyzed using Flow Jo software (v10.1r5). STAT1 phosporylation was also assessed in CD11b+Ly6C+Ly6G- peripheral blood monocytes from naïve and LCMV-infected *Prf1*$^{-/-}$ mice that were treated or not with αIFNγ or ruxolitinib, as just described.

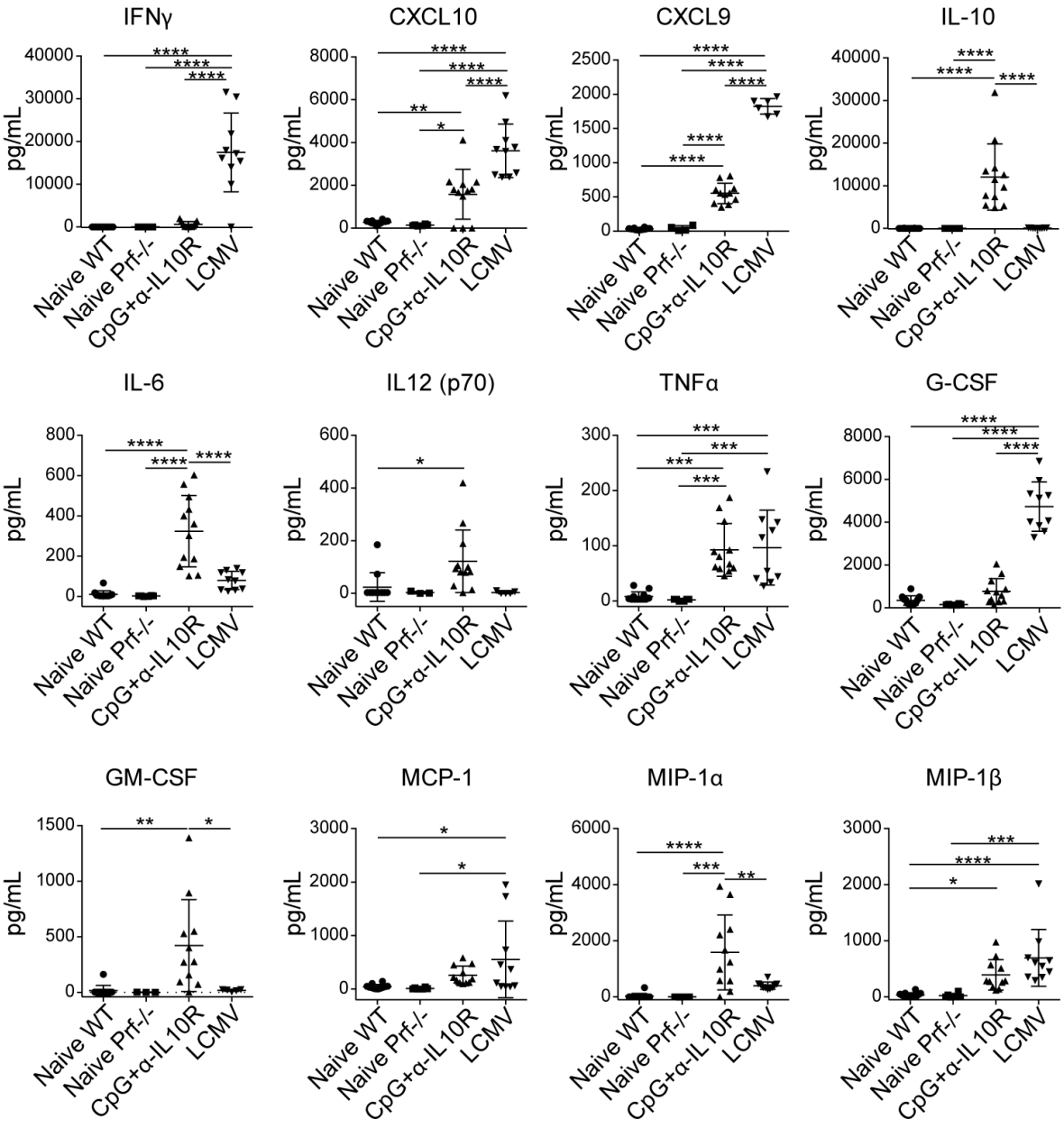
Real-time PCR analysis

RAW264.7 macrophages were incubated for 5 hours at 37°C, 5%CO_2 with sera from naïve and LCMV-infected *Prf1*$^{-/-}$ mice that were treated or not with αIFNγ or ruxolitinib. RNA was extracted using the RNAeasy Micro Kit (Qiagen) and transcribed to cDNA with Quantitect reverse transcription kit (Qiagen). Relative gene expression of *Igtp* was determined by RT-PCR and normalized to b-actin (*Actb*) expression by the ΔCT method. The reaction was set up using TaqMan gene expression assays (*Actb* Mm01205647; *Igtp* Mm0049761) and TaqMan Fast Advanced Master Mix (Applied Biosystems by Thermo Fisher Scientific). RT-PCR was performed using QuantStudio 7 (Thermo Fisher Scientific).

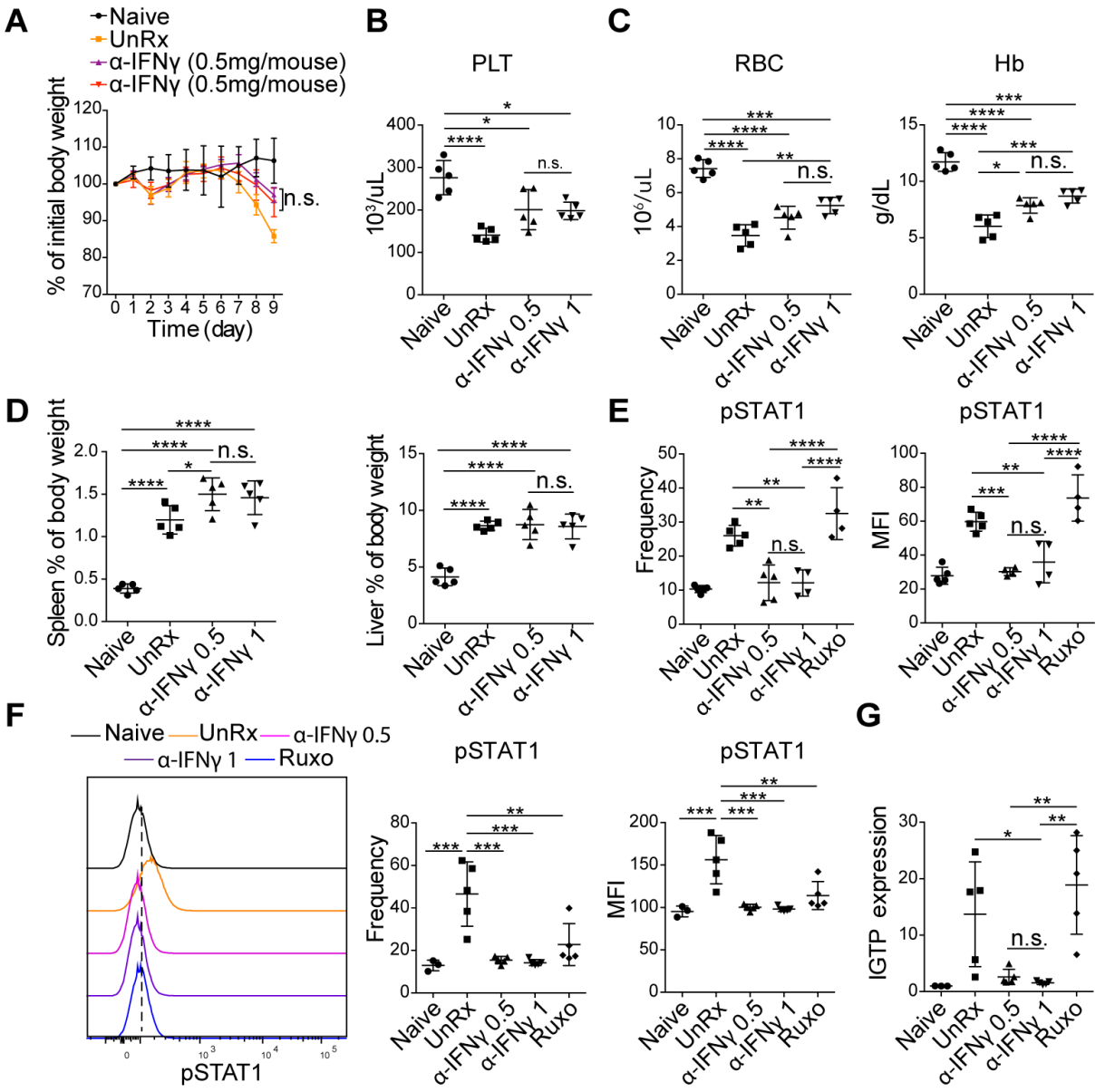
Histochemical analysis of human samples

Tissues were fixed with 10% neutral buffered formalin and embed in paraffin. De-paraffinized sections were stained with CD15 (clone MMA, Ventana Medical Systems) on the Ventana BenchMark Ultra automated staining platform. Antigen retrieval was performed using Ventana Medical System's proprietary uCC1 AR solution. Ventana's proprietary iView DAB detection kit was used for primary antibody detection. The CD15 staining for myeloid cells, including neutrophils, was compared to H&E sections to confirm the presence of neutrophils.

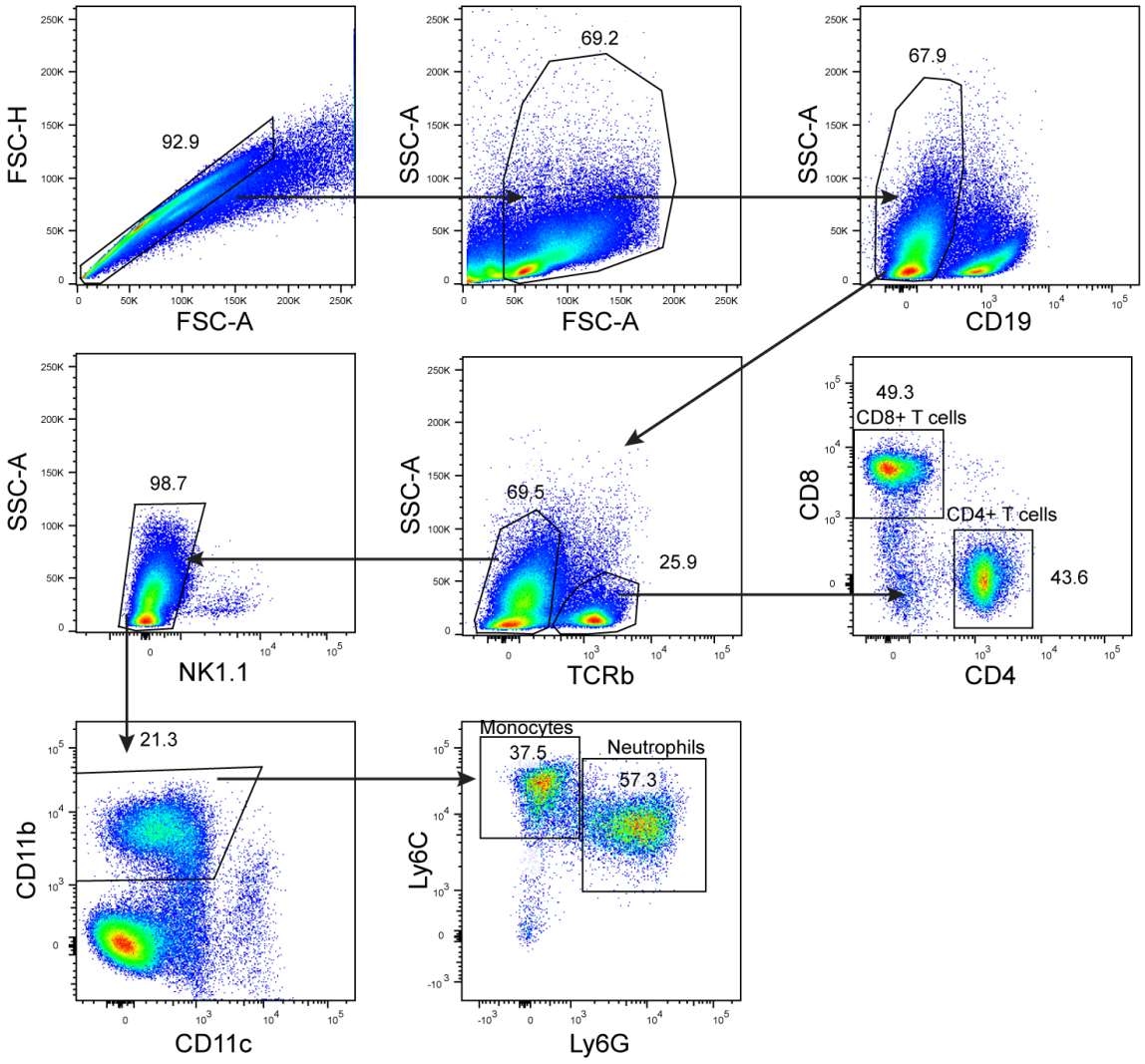
Supplemental Figures



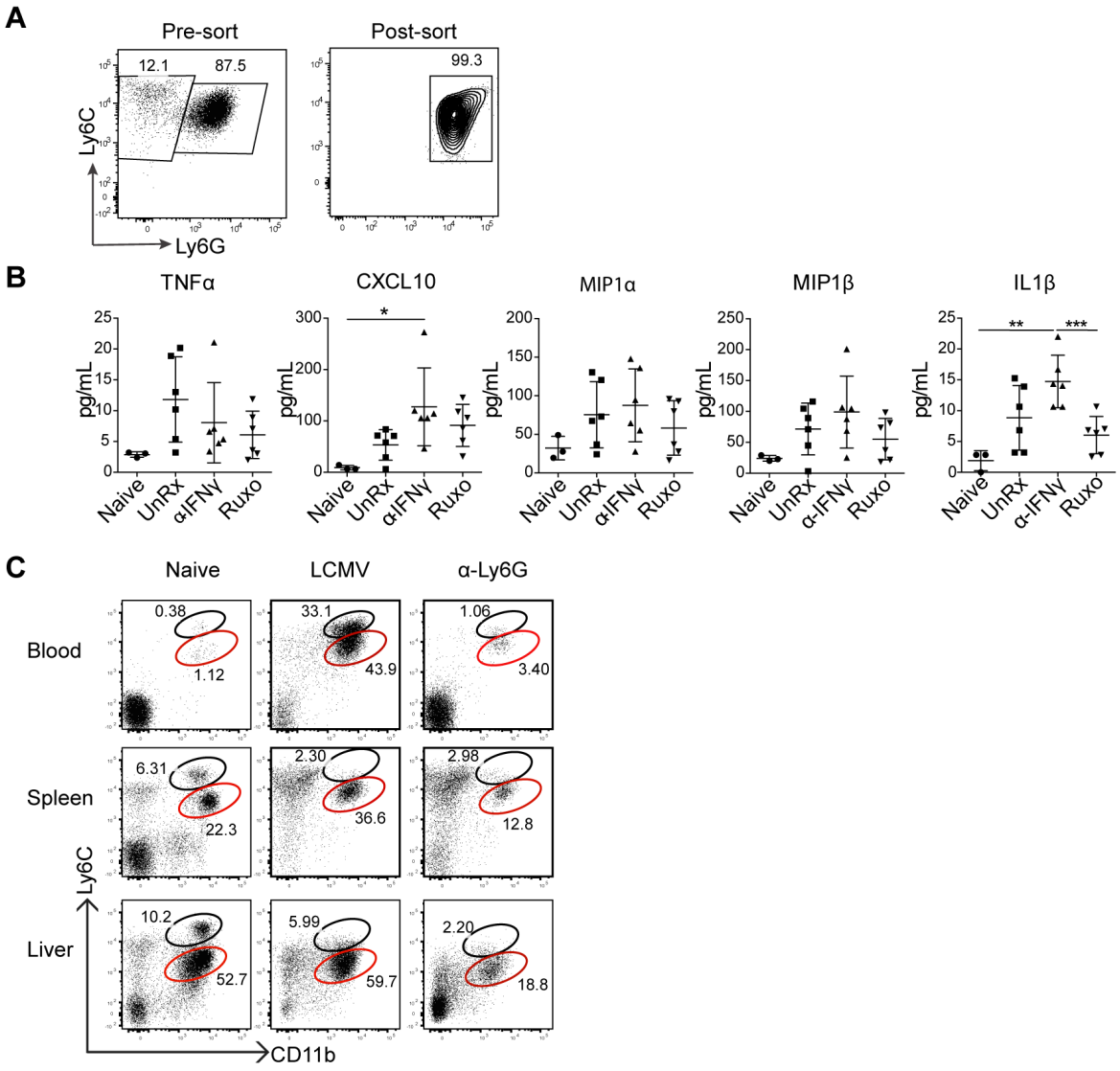
Supplemental Figure 1. Serum levels of cytokines in the two murine models of HLH. Serum levels of cytokines in naïve WT and *Prf1*-/- mice, WT mice treated with CpG plus αIL10R antibody (model of secondary HLH) or *Prf1*-/- mice infected with LCMV (model of primary HLH). Cytokine levels were obtained on day 9 post the first CpG plus αIL10R antibody treatment or day 9 post LCMV infection. Data were plotted using the values from Figures 1E and 2E of the main manuscript.



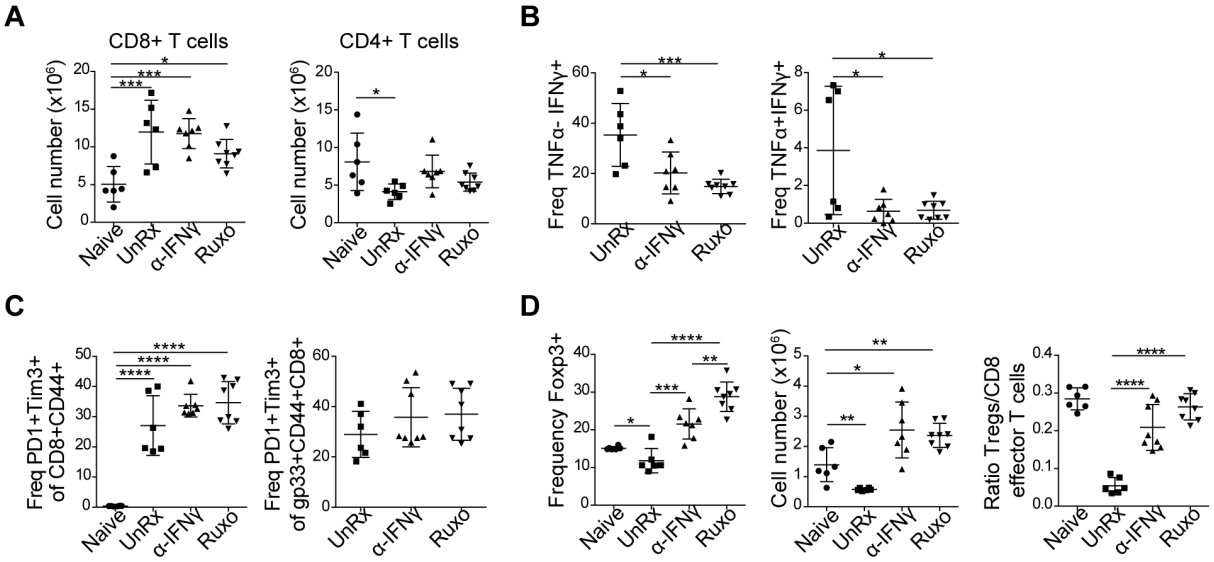
Supplemental Figure 2. Neutralization of IFNγ activity using the αIFNγ antibody. Naïve or LCMV-infected *Prf1*−/− mice were untreated (UnRx) or treated with αIFNγ antibody (0.5mg/mouse or 1mg/mouse) on days 4 and 7 p.i. Mice were followed over time for weight loss (A). On day 9, mice were examined for evidence of HLH, including thrombocytopenia (B), anemia (C), and organomegaly (D). The frequency and mean fluorescence intensity (MFI) of phospho-STAT1 (pSTAT1)-positive peripheral blood monocytes was also determined (E). pSTAT1 levels in RAW264.7 macrophages following incubation for 10 min with sera from naïve or LCMV-infected *Prf1*−/− mice that were untreated or treated with αIFNγ antibody or ruxolitinib (G). Relative expression of IFN gamma induced GTPase (IGTP) in RAW264.7 cells incubated for 5 hours with sera from the treatment groups shown in (F). n=3 - 5 mice per group. **P*<0.05, ***P*<0.01, ****P*<0.001, **** *P*<0.0001.



Supplemental Figure 3. Gating strategy of T cells, monocytes and neutrophils in spleens. Representative flow plots showing gating strategy of splenic CD8+ T cells (CD19-NK1.1-TCRb+CD8+), CD4+ T cells (CD19-NK1.1-TCRb+CD8+), monocytes (CD19-N1.1-CD11b+Ly6C^{hi}Ly6G-), and neutrophils (CD19-NK1.1-CD11b+Ly6C^{int}Ly6G+).

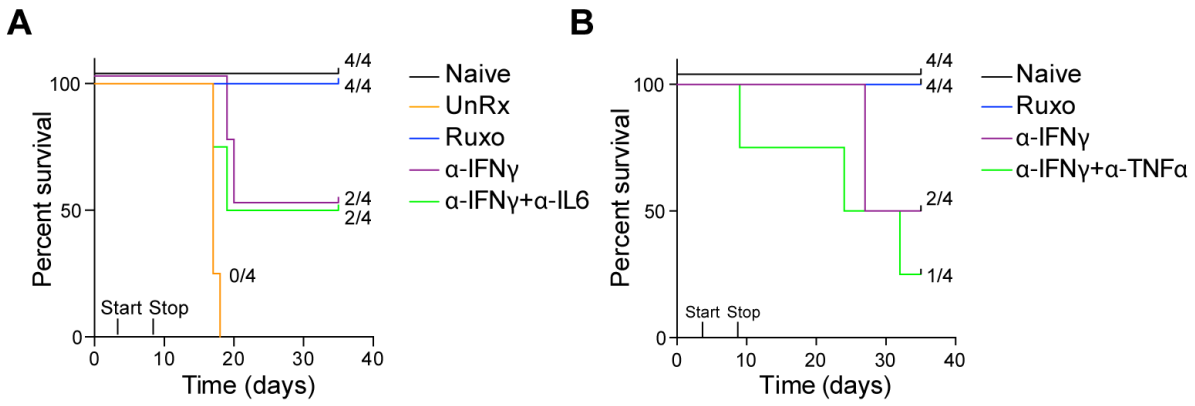


Supplemental Figure 4. Neutrophil sorting and depletion efficacy.
Representative flow plots showing (A) purity of FACS-sorted neutrophils from LCMV-infected *Prf1*−/− mice. (B) Serum cytokines from supernatants of sorted neutrophils cultured for 24 hours. Data are combined from two independent experiments. (C) Frequency of CD11b+Ly6C^{int} neutrophils (red circle) in naïve, LCMV-infected, and LCMV-infected *Prf1*−/− mice treated with αLy6G antibody on days 4, 6, and 8 p.i.

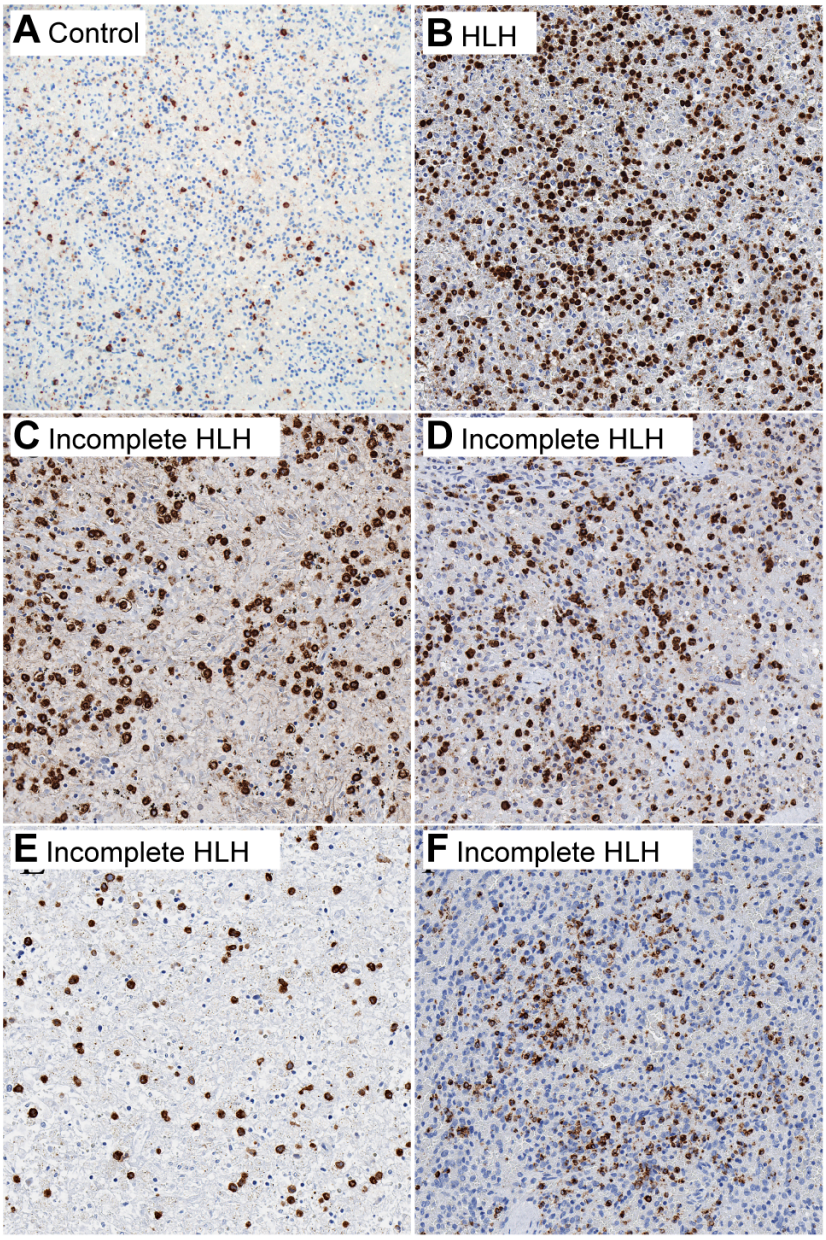


Supplemental Figure 5. CD8+ T cells in mice exposed to transient treatment are exhausted and have high numbers of regulatory T cells.

Naïve and LCMV-infected *Prf1*$^{-/-}$ mice were untreated (UnRx) or transiently exposed to αIFNγ or ruxolitinib as in Figure 7. UnRx mice were euthanized on day 9 post-infection for comparison of immune parameters (A) Numbers of splenic CD8+ and CD4+ T cells on day 20. (B) Frequency of LCMV specific (gp33+CD8$^+$CD44$^+$) T cells producing TNFα and/or IFNγ. (C) Frequency of PD1$^+$Tim3$^+$ on CD44$^+$CD8$^+$ T cells (left) and gp33-specific CD8$^+$ T cells (right). (D) Frequency (left) and number (middle) of splenic Foxp3$^+$ regulatory T cells (gated on CD4+ T cells) and ratio of the number Tregs/CD8 effector T cells (right). Total number of mice per group: naïve (n=6), UnRx (n=6), αIFNγ (n=7) and Ruxo (n=8). *P<0.05, **P<0.01, ***P<0.001, **** P<0.0001.



Supplemental Figure 6. Short-term treatment with αIFNγ in combination with αIL6 or αTNFα does not improve survival single treatment with αIFNγ alone.
(A) Survival of naïve or LCMV-infected *Prf1*−/− mice that were untreated (UnRx) or treated with αIFNγ, ruxolitinib, or αIFNγ in combination with an IL-6 blocking antibody (αIL-6) on days 4-8 p.i. followed by treatment discontinuation (same experiment as in Figure 7A). Log-rank test P<0.0006. (B) Survival of naïve or LCMV-infected *Prf1*−/− mice that were treated with αIFNγ, ruxolitinib or αIFNγ in combination with a TNFα blocking antibody (αTNFα) on days 4-8 p.i. followed by treatment discontinuation. Log-rank test P<0.0240.



Supplemental Figure 7. CD15+ cells in human tissue specimens. Immunohistochemical analysis for presence of CD15+ (brown staining) neutrophils in splenic tissue from a normal control (A), patient with secondary HLH (B) and patients who exhibited incomplete diagnostic evidence of HLH pre-mortem but showed signs of activated macrophages with hemophagocytosis in tissues from autopsy (C-F). (B-D) show increased numbers of CD15+ cells compared to the healthy control, but (E, F) do not. Original magnification (20X).