Online Supplemental Information for:

Cutaneous infection with *Leishmania major* mediates heterologous protection against visceral infection with *Leishmania infantum*

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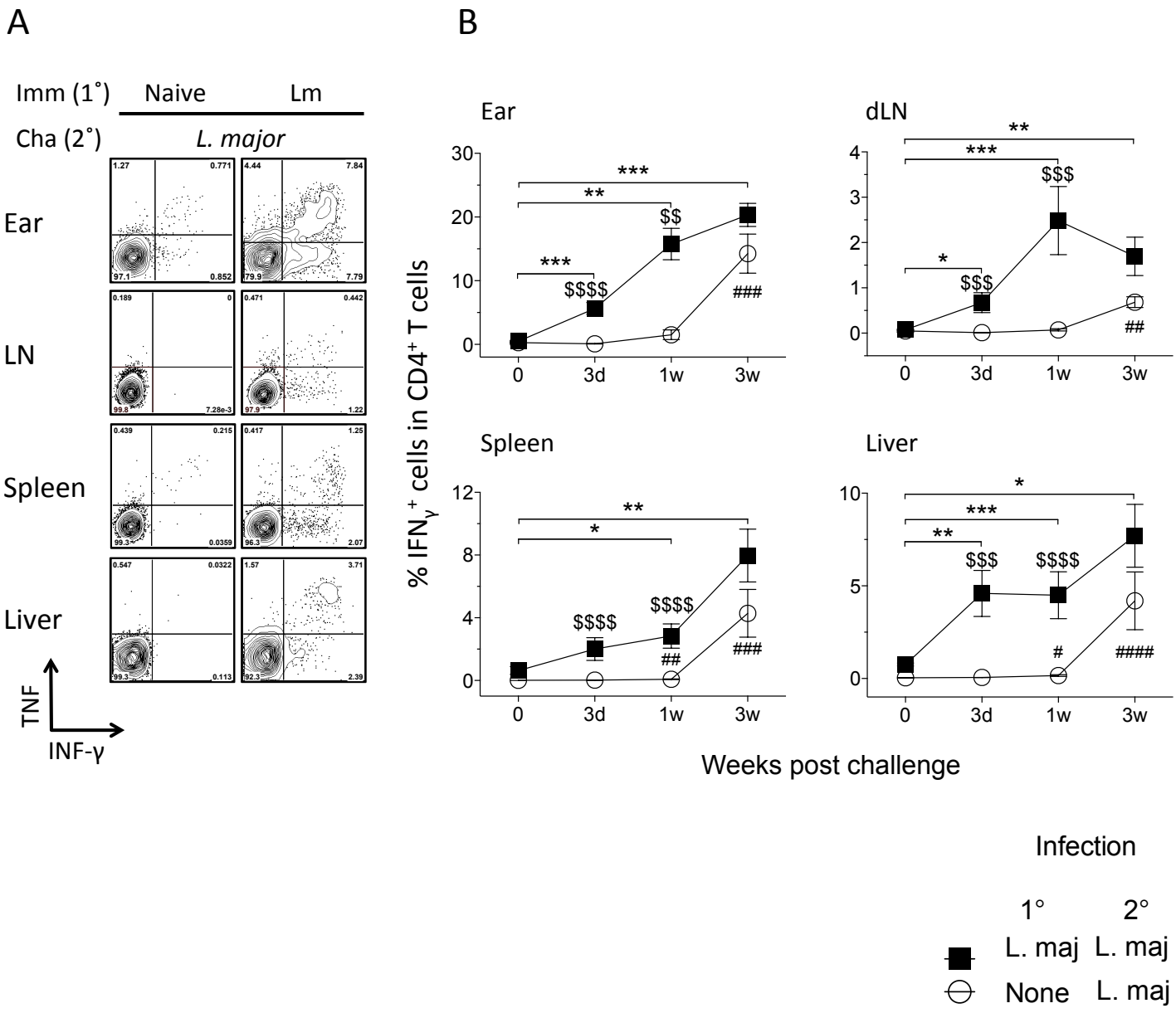
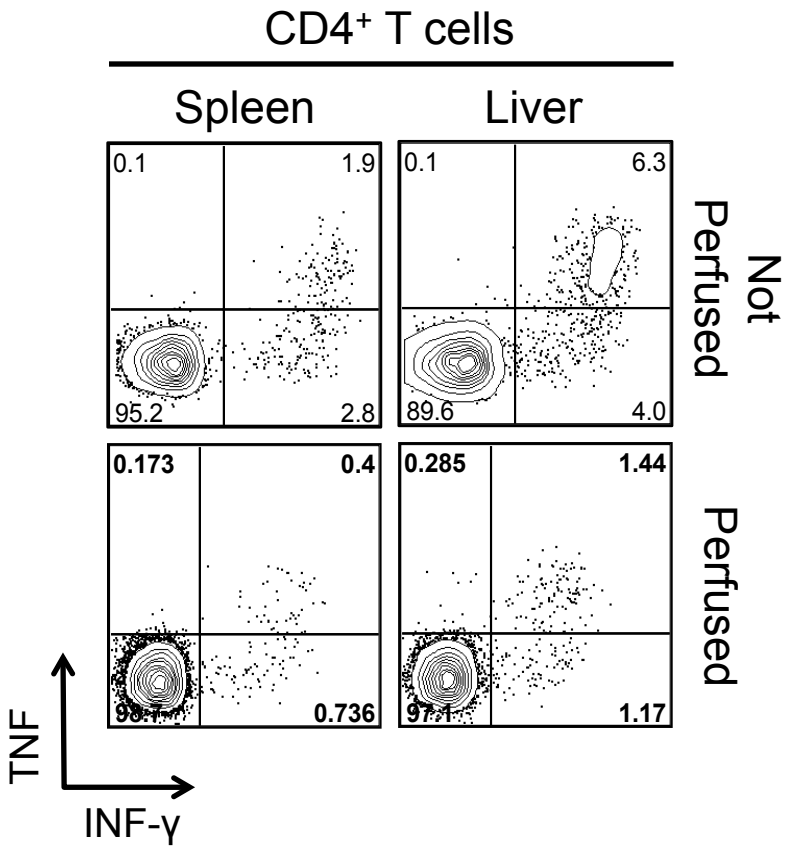
This document contains the following Figures

Supplemental Figure 1



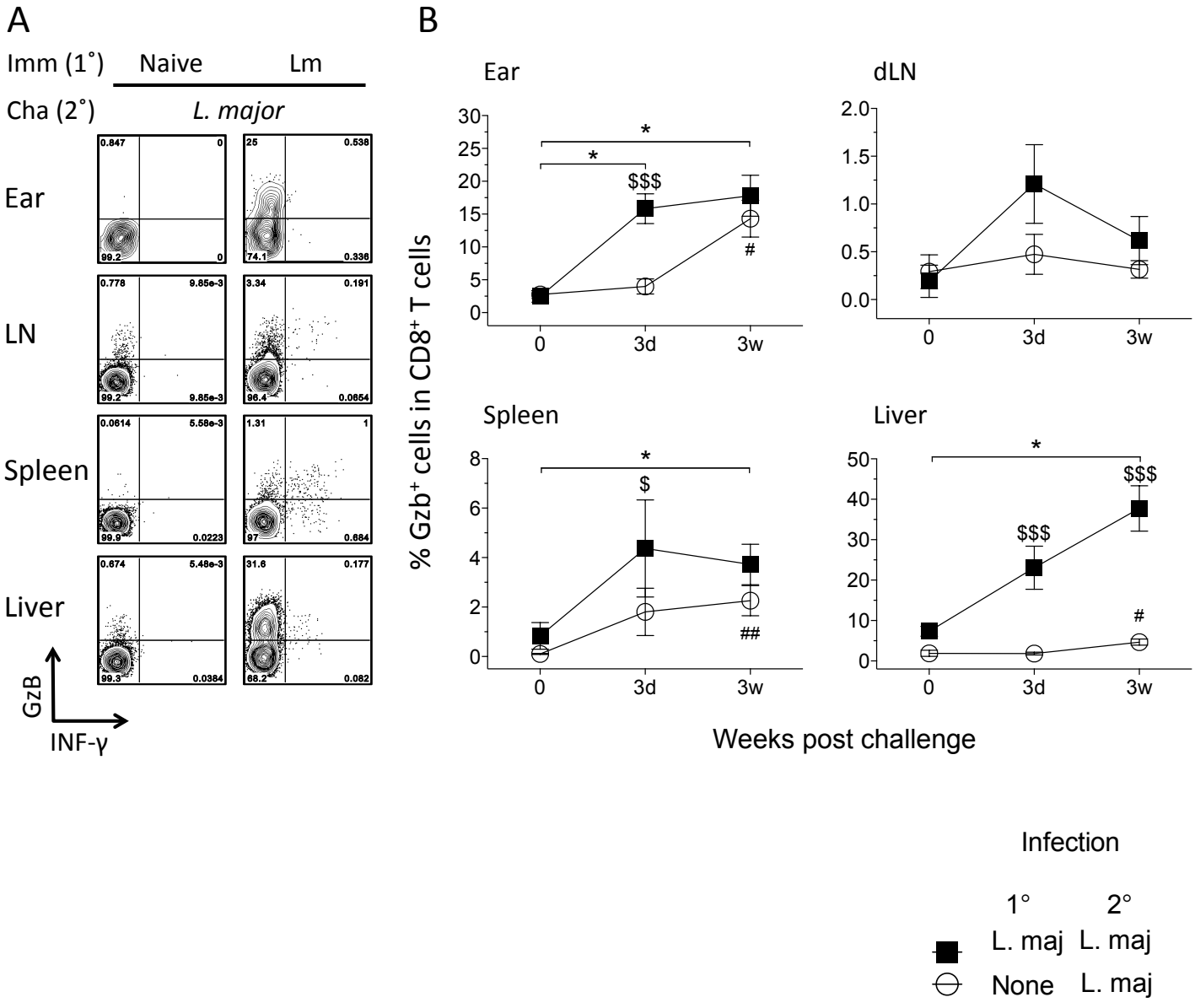
Supplementary Figure 1: Kinetic of IFN-γ producing CD4⁺ T cells in the skin and viscera after challenge infection *with L. major*.
Leishmanized or naïve mice were infected in one ear with 2x10^6 *L. major* metacyclics promastigotes. At the indicated times, cells were re-stimulated with L.m-Ags+APCs. A) Representative intracellular staining contour plots for cytokine-producing CD4⁺TCRβ⁺ T at 1 week post challenge. B) Kinetic of IFN-γ production by CD4⁺TCRβ⁺ T cells in individual ears, dLNs, spleens and livers; asterisks refers to differences between leishmanized not challenge mice vs LmLm; number symbol refers to differences between NaLm vs LmLm; currency symbol refers to differences between NaLm and naïve control mice. n=3-4 mice per group per time point. Data are the pool of 5 independent experiments including at least 2 time points (Day 3, 1 week, and/or 3 weeks) per experiment.

Supplemental Figure 2



Supplementary Figure 2: Effect of perfusion on the percentage of IFN-γ producing CD4+ T cells in the spleen and the liver of Leishmanized mice prior to challenge.
Cells were re-stimulated with APCs+ L.m-Ags and analyzed by flow cytometry. Production of TNF-α and IFN-γ by $CD4^+$ T cells in the spleen and liver of Leishmanized mice with or without cardiac and liver specific perfusion.

Supplemental Figure 3



Supplementary Figure 3: Kinetic of GzB producing CD8⁺ T cells in the skin and viscera after infection *with L. major*. Cells were re-stimulated *in vitro* with APCs plus L.m.-Ags at indicated time point and analyzed by flow cytometry. A) Intracellular staining for IFN-γ- and GzB-producing CD8⁺TCRβ⁺ T cells. B) Kinetic of GzB production by CD8⁺TCRβ⁺ T cells in individual ears, dLNs, spleens and livers; asterisks refers to differences between leishmanized not challenge mice vs LmLm; number symbol refers to differences between NaLm vs LmLm; currency symbol refers to differences between NaLm and naïve control mice. N=3-5 per group per time point Data are the pool of at least 2 independent experiments.

Supplemental Figure 4

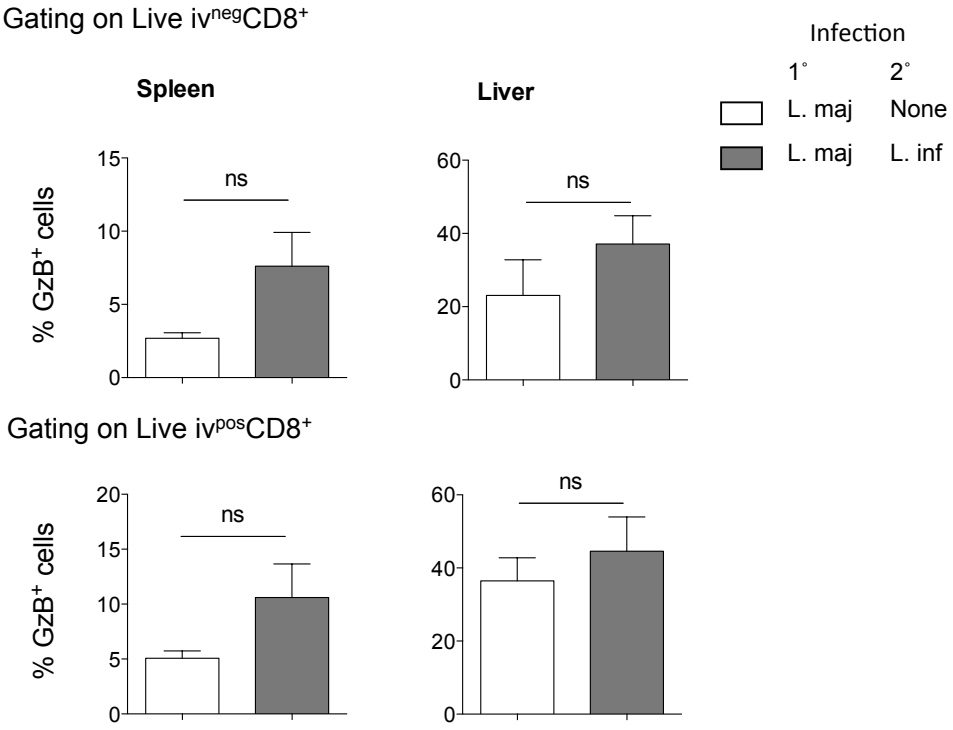


Figure S4: CD8+ T cells TCRβ positive or negative produce similar amount of GzB in the spleen and liver.

At 1 week post-challenge, cells from Leishmanized mice or Leishmanized mice challenged with *L. infantum* were re-stimulated as previously described. Percentage of GzB positive cells in $iv^{+}CD8^{+}$ T cells are shown in the upper panel and in $iv^{-}CD8^{+}$ T cells in the lower panel. n=3-5 mice per group per data point. Data are the pool of 2 independent experiments.