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Supplemental Information

SHP-1 Regulates Antigen Cross-Presentation

and Is Exploited by *Leishmania* to Evade Immunity

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Figures S1-S4

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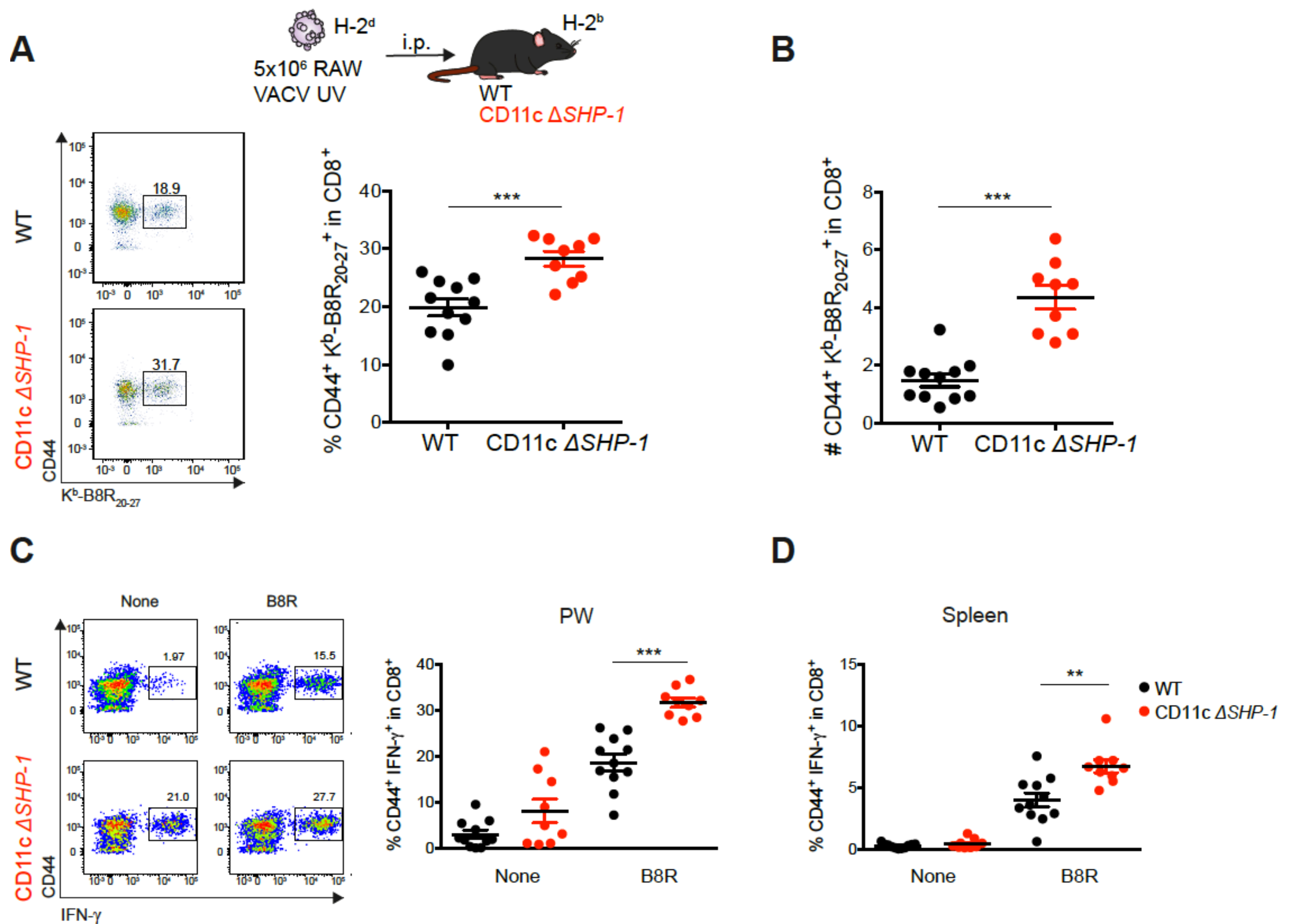
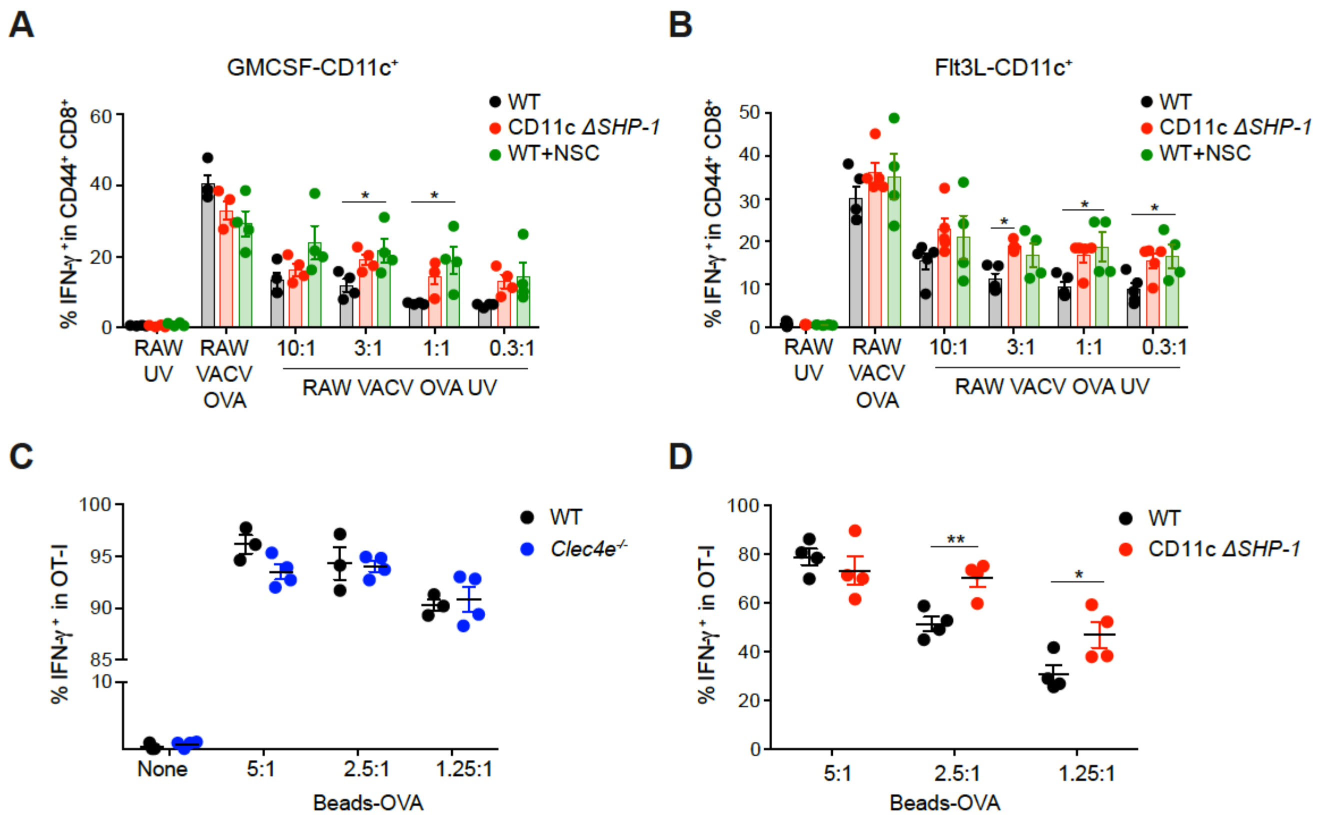
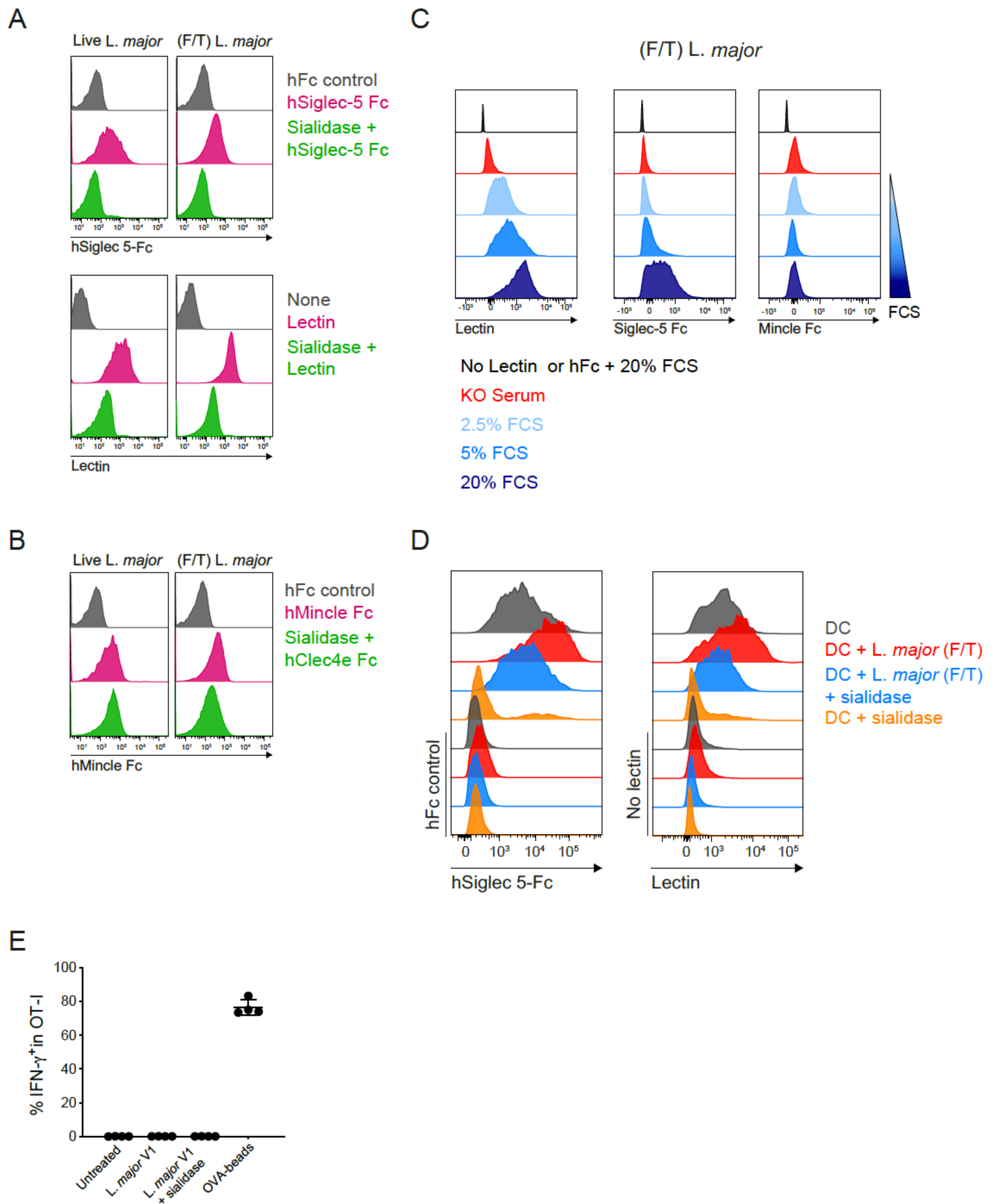


Supplementary Figure 1. Related to Figure 1. Increased CD8^{+} T cell priming in CD11cΔ*SHP-1* mice. RAW cells were infected with VACV and treated with UV to inactivate the virus. Cells were inoculated i.p. 16h after irradiation to mice of the indicated genotype. Mice were euthanized 6 days later and cells from spleens and peritoneal washes (PW) were stained with VACV B8R specific tetramer (K^{b}-B8R$_{20-27}$). (A) Left: Representative dot plots of CD44^{+} B8R-specific CD8^{+} T cells. Right: Percentages and numbers (B) of B8R-specific CD8^{+} T in the peritoneal wash (PW). Cells from PW (C) and spleen (D) were restimulated with the VACV B8R peptide and intracellular IFN-γ was determined by flow cytometry. (C) Left: Representative dot plots are shown. Right: percentage of cells producing IFN-γ. Individual data of 2 pooled experiments shown as mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Student's t test.

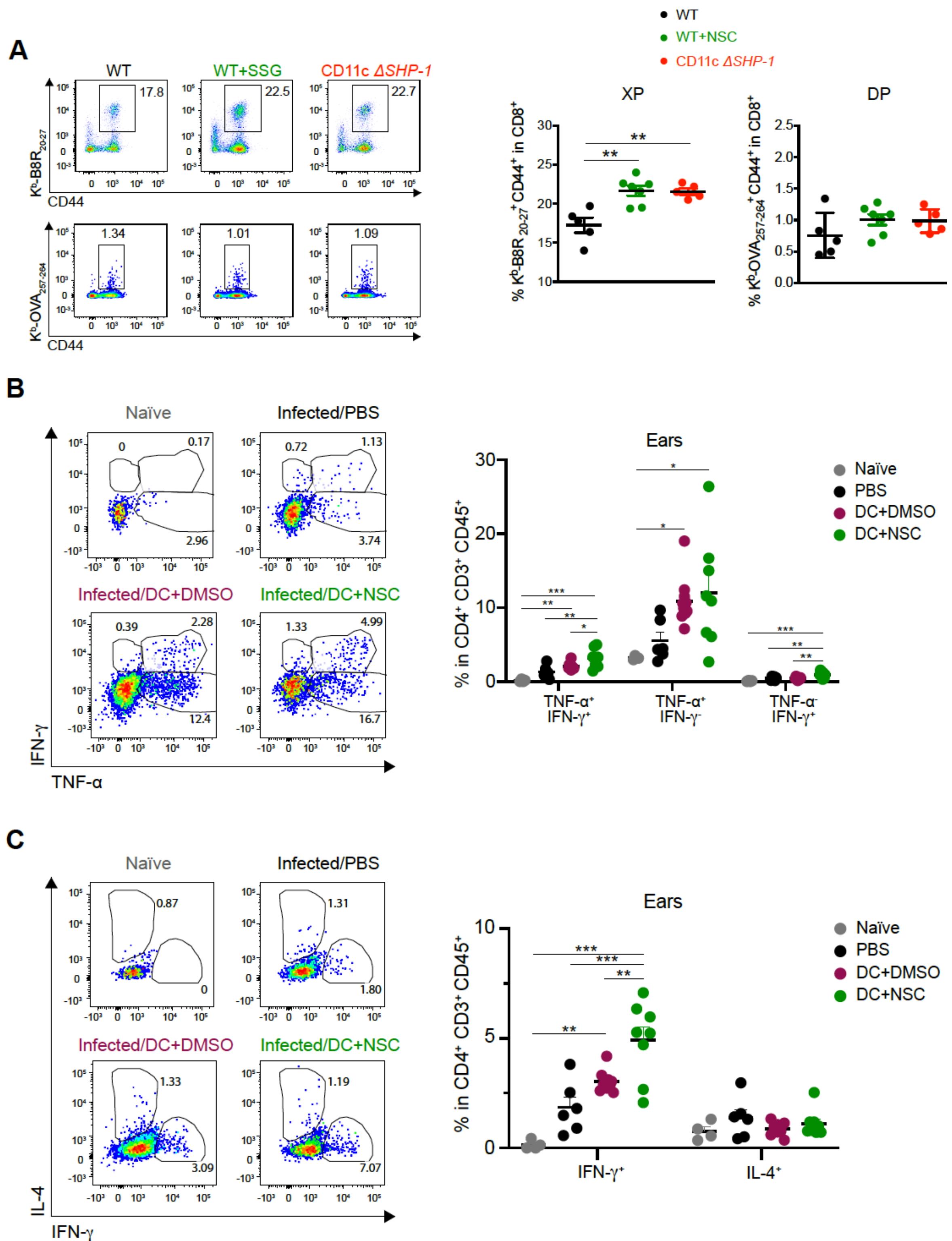


Supplementary Figure 2. Related to Figure 2. SHP-1 deficiency improves antigen cross-presentation from different sources. (A- B) CD11c$^+$ DCs purified from GM-BM (A) or Flt3L BMDC (B) of the indicated genotype were exposed to titrated RAW cells treated with UV 16h before (RAW-UV), or RAW cells infected with VACV-OVA not treated (RAW-VACV-OVA) or treated with UV (RAW-VACV-OVA-UV) to inactivate the virus. Where indicated, cells were treated for 30 minutes with NSC-87877, before adding the antigens. As a readout for antigen presentation, IFN-γ production was measured in CD8$^+$ T cells from lymphoid organs of wild type mice infected with VACV WR, after 6h of co-culture, in the presence of BFA for the last 4 hours. Cultures were stained for CD8, CD44 and intracellular IFN-γ. (C-D) CD11c$^+$ DCs were purified from GM-BM of the indicated genotype and exposed for 4 hours to titrated OVA-coupled latex beads. Thereafter, cells were co-cultured for 6h, in the presence of BFA for the last 4 hours, with *in vitro* expanded OT-I T cells. Cultures were stained for CD8 and intracellular IFN-γ. Production of IFN-γ as mean ± SEM from 2 pooled experiments is shown. * $p<0.05$; **$p<0.01$, *** $p<0.001$. (A, B) One-way ANOVA with Tukey post-hoc test. (C, D) Student's t test.



Supplementary Figure 3. Related to Figure 3. (A-C) *L. major* F/T parasites contains sialic acid on their surface that is acquired from the serum. (A-B) Live or F/T *L. major* V1 promastigotes pretreated or not with sialidase for 1 hour (Neuraminidase, 0.1 U/mL) were stained with *Sambucus nigra* lectin (A, lower histogram panel) or hFc (hFc control) and hSiglec 5-Fc chimera (A, upper histogram panel) or hMincle-Fc chimera (B).

(C) F/T *L. major* V1 extracts were prepared from promastigotes grown in the concentrations of serum indicated in the figure, or with a serum replacement (KO serum) and were stained with *Sambucus nigra* lectin and hSiglec 5-Fc chimera, hFc (hFc control), or hMincle-Fc chimera as in B. **(D-E) F/T parasites treated with sialidase do not affect sialic acid abundance on DC or induce antigen-unspecific T cell activation.** (D) WT CD11c$^+$ cells purified from WT GM-BM were exposed F/T *L. major*-OVA extracts pretreated or not with sialidase for 1 hour (Neuraminidase, 0.1 U/mL) as in figure 3A, or directly with sialidase. Cells were washed and further stained with *Sambucus nigra* lectin and hSiglec 5-Fc chimera or hFc (hFc control). (E) CD11c$^+$ cells were exposed for 4 hours to titrated F/T *L. major* V1 extracts pretreated or not with sialidase for 1 hour or OVA-coupled latex beads. Thereafter, cells were co-cultured for 6h, in the presence of BFA for the last 4 hours, with *in vitro* expanded OT-I T cells. Cultures were stained for CD8 and intracellular IFN-γ as in figure 3A-B.



Supplementary Figure 4. Related to Figure 4. SHP-1 inhibition improves GM-BM vaccination and promotes protection to *L. major* challenge. (A) SHP-1 inhibition improves cross-priming of CD8$^+$ T cells upon GM-BM immunization. WT GM-BM, treated or not with Sodium Stibogluconate (SSG), or SHP-1 deficient GM-BM were

exposed for 4h to RAW-VACV-UV as in figure 4A. Next, GM-BM were additionally loaded with an excess of $OVA_{257-264}$ peptide for 30 min and inoculated i.v. into mice. Left: representative plots of $CD44^+$ and K^b-$B8R_{20–27}$ tetramer$^+$ or K^b-$OVA_{257-264}$ dextramer$^+$ Right: frequency of $CD44^+$ and K^b-$B8R_{20–27}$ tetramer$^+$ or K^b-$OVA_{257-264}$ dextramer$^+$ in the spleen, seven days after inoculation. (B-C) SHP-1 inhibition during vaccination with *Leishmania*-loaded GM-BM induces IFN-γ-producing $CD4^+$ T cells. GM-BM were treated an inoculated i.d. in the ears of BALB/c mice as in figure 4C. Six days after challenge with $5x10^3$ *L. major* V1 parasites, ear cell suspensions from the indicated mice were co-cultured with GM-BM loaded with *L. major* V1 F/T for 6h, in the presence of BFA for the last 4 hours. Cells were stained for CD45, CD3 and CD4 and intracellular IFN-γ, TNF-α and IL-4. Left: Representative dot plots of IFN-γ and TNF-α-producing $CD4^+$ T cells (B) or IFN-γ and IL-4-producing $CD4^+$ T cells (C). Right: Quantification of the percentages of IFN-γ^+, TNF-α^+ or double positive $CD4^+$ T cells (B), or IFN-γ^+ and IL-4^+ $CD4^+$ T cells (C). Individual data of a representative experiment of three performed is shown as mean ± SEM. (A-C) Individual data of a representative experiment of two performed shown as mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. One-way ANOVA with Tukey post-hoc test.