NLRP3 inflammasome triggers Th2-biased adaptive immunity that promotes leishmaniasis

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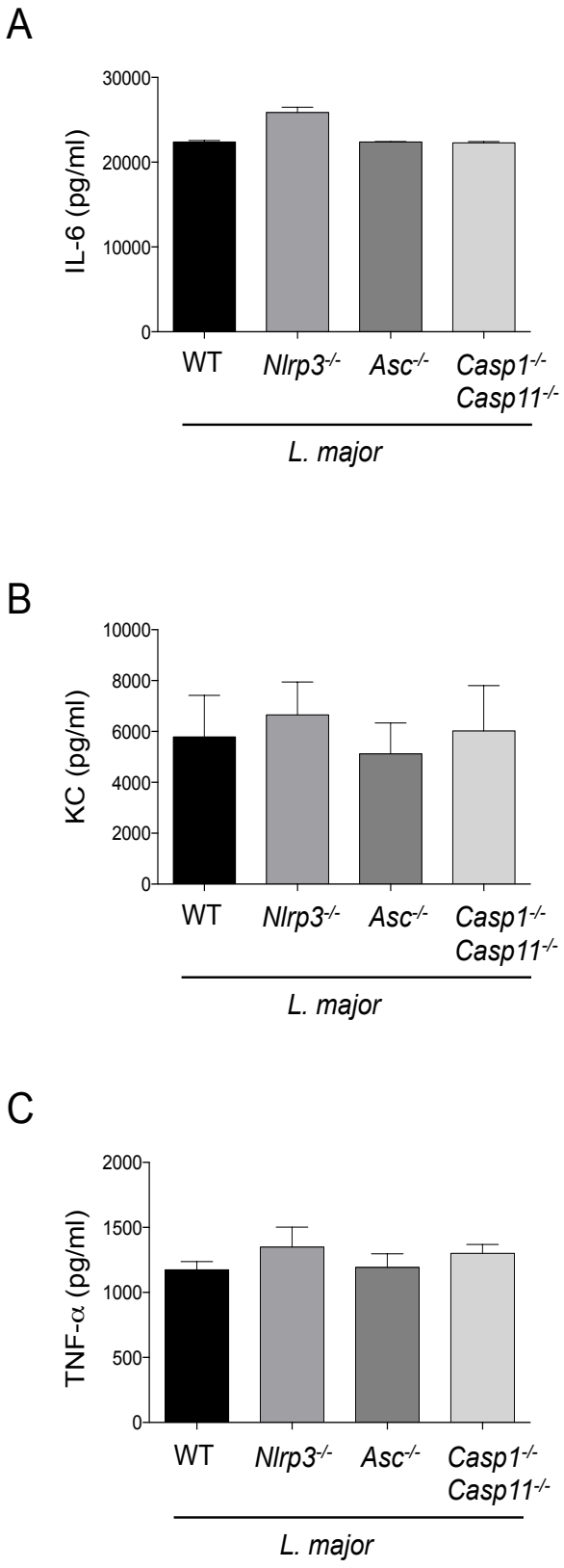
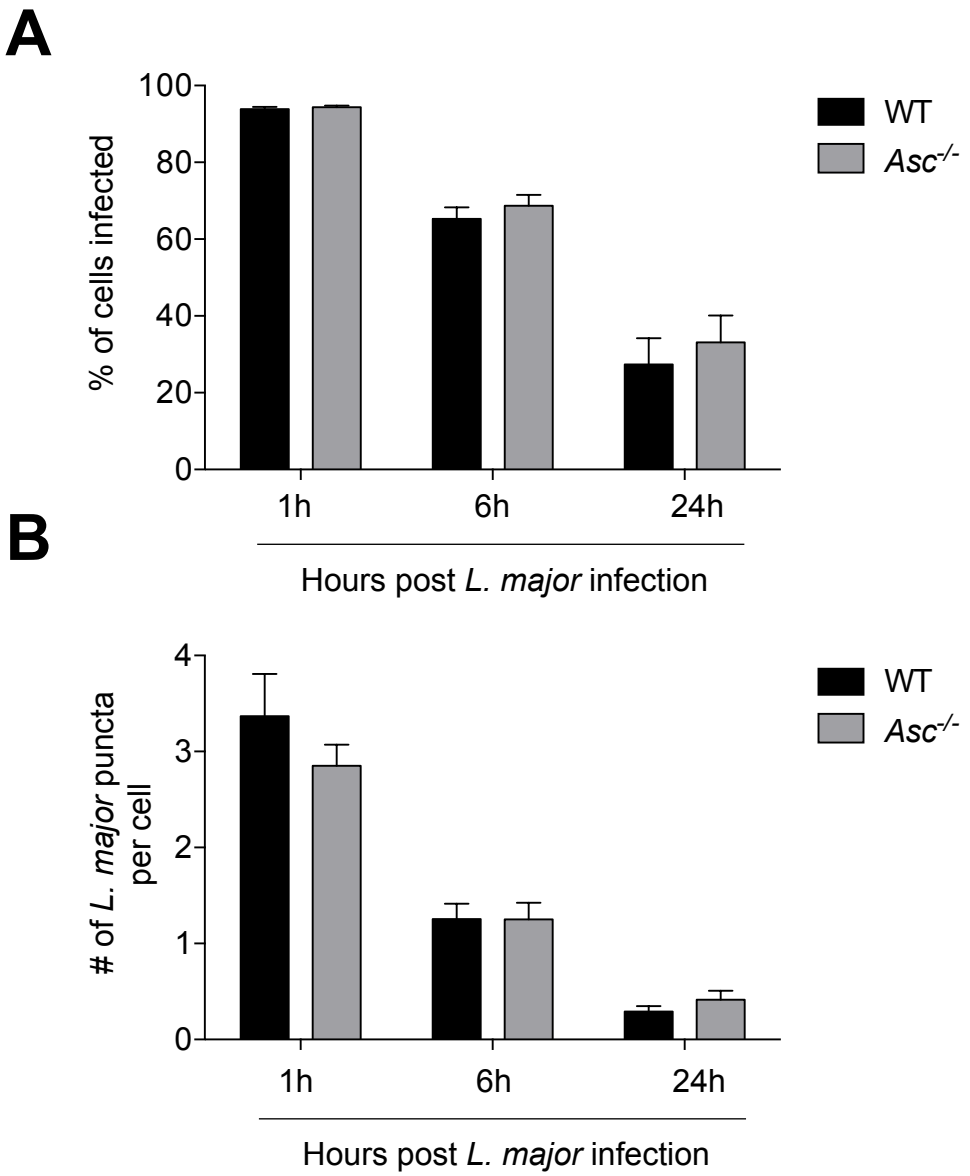
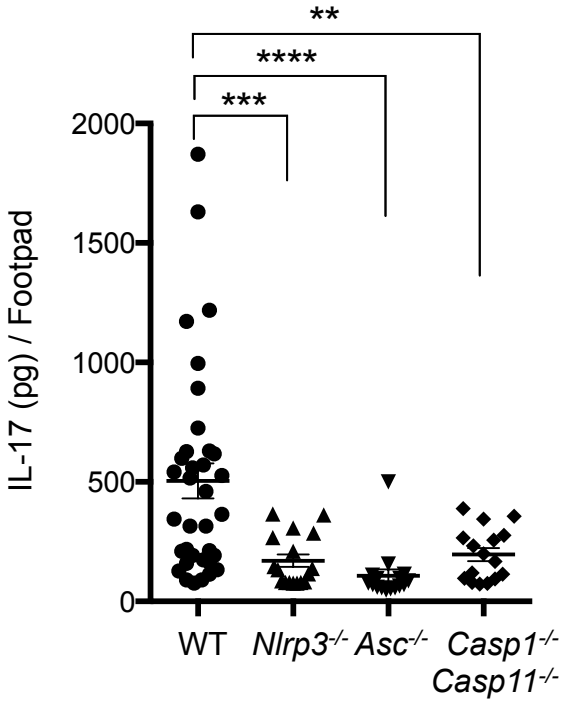


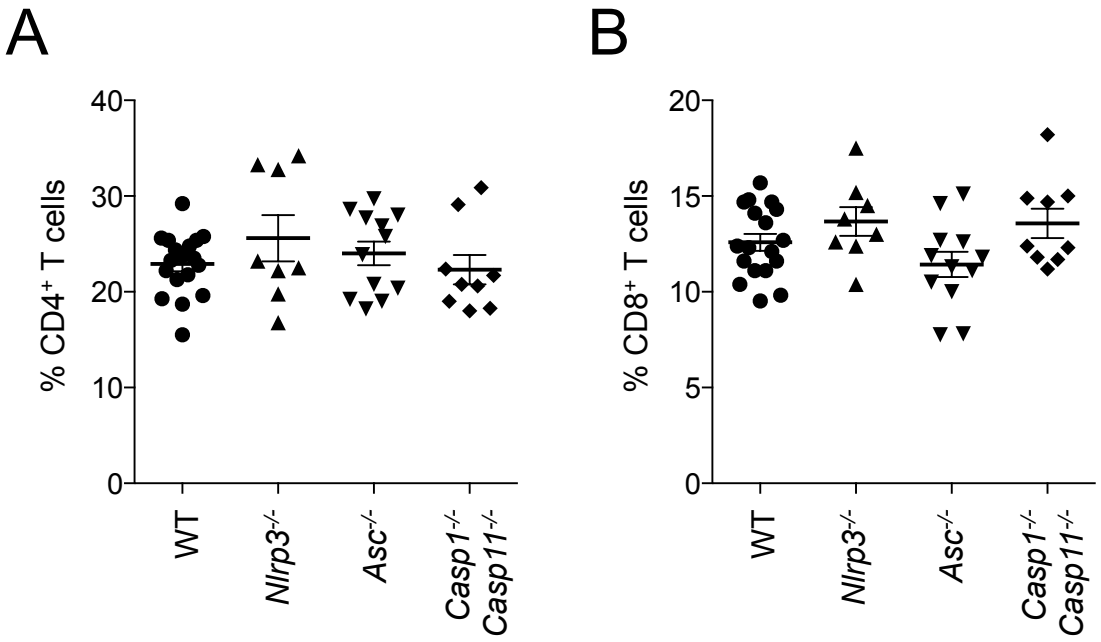
Supplemental Figure 1. Inflammasome signaling does not affect *L. major*-induced production of IL-6, KC and TNF-α production by BMDM *in vitro*. WT, *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ BMDMs were stimulated with 500 ng/ml LPS for 6 hours followed by *L. major* infection (20 m.o.i.) for 48 hours. IL-6 **(A)**, KC **(B)** and TNF-α **(C)** cytokine levels in the supernatants were determined by ELISA. Data are presented as mean ± s.e.m. and are representative of at least three independent experiments.



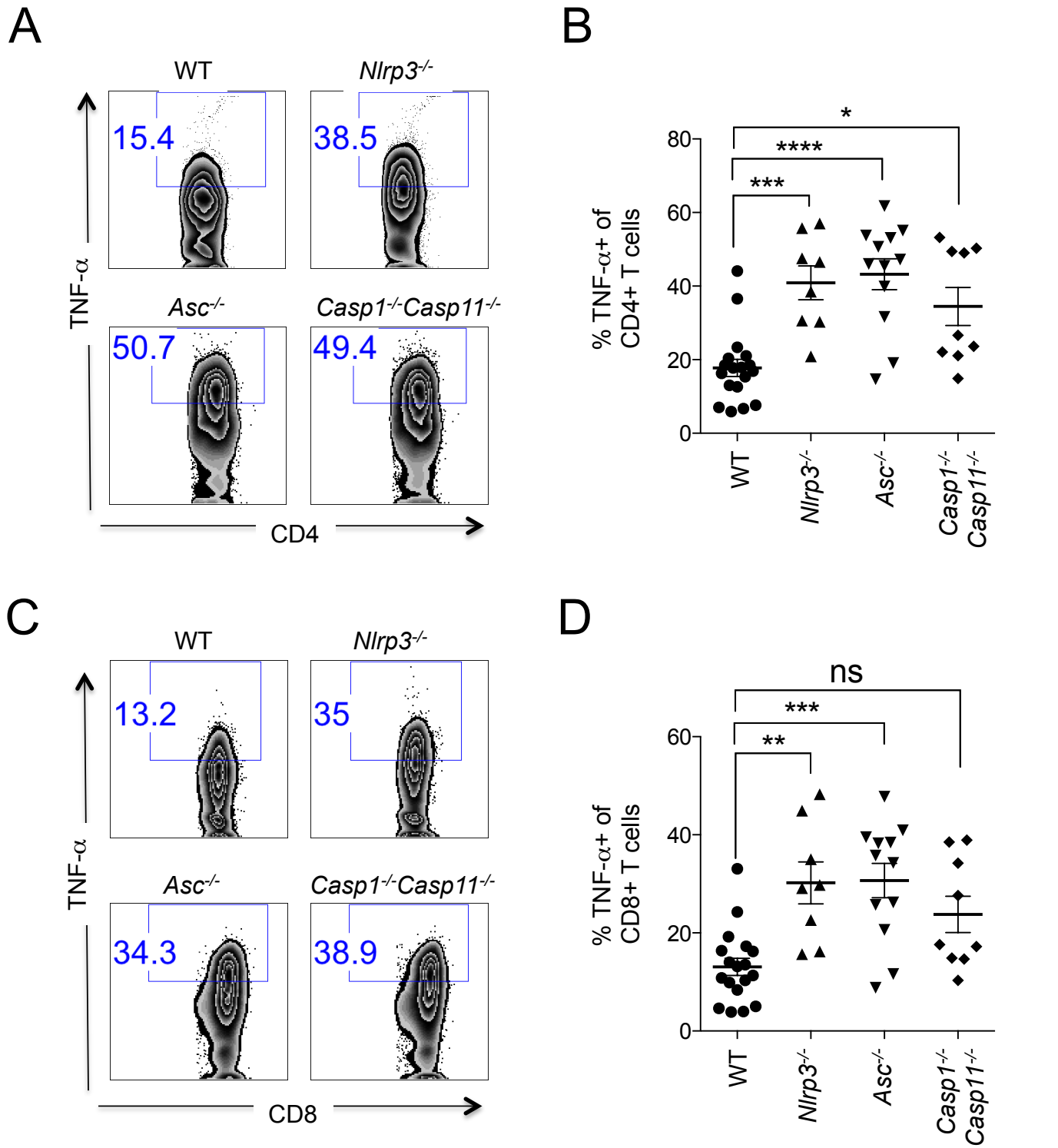
Supplemental Figure 2. ASC deficiency does not alter phagocytosis and clearance of *L. major* by BMDM *in vitro*. WT and *Asc*$^{-/-}$ BMDMs were infected with 20 m.o.i. *L. major* for 1h. Infected BMDMs were washed to remove excess *L. major* and supplemented with fresh media. BMDM were then stained with giemsa immediately to determine phagocytosis of *L. major* (1 hour). Other groups of BMDMs were incubated for additional 6 and 24 hours to determine clearance of phagocytosed *L. major* before staining the cells with giemsa stain (6 hour and 24 hour time points). Giemsa stained cells were examined under light microscope to visualize *L. major* phagocytosis and clearance. **(A)** Total number of cells that were infected in independent 40x areas were counted and represented as "% of infected cells". **(B)** In the same 40x area, total numbers of *L. major* puncta were counted at each time point and represented as "# of *L. major* puncta per cell". Data are presented as mean ± s.e.m. and are representative of at least three independent experiments.



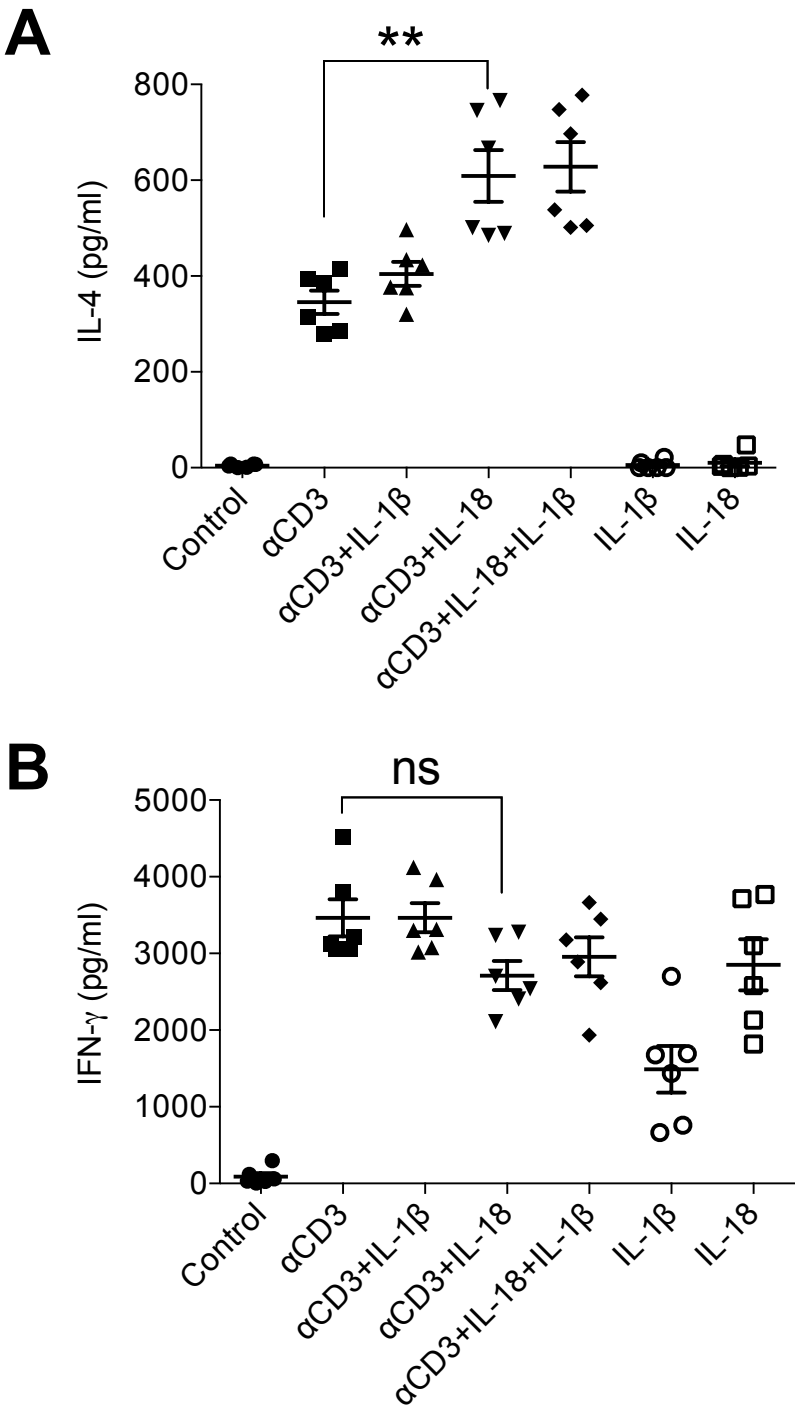
Supplemental Figure 3. NLRP3 inflammasome deficiency results in reduced IL-17 levels following *L. major* infection *in vivo*. Footpads from WT (n=35), *Nlrp3*$^{-/-}$ (n=17), *Asc*$^{-/-}$ (n=16) and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ (n=16) mice infected with 10^6 *L. major* promastigotes were harvested on day 28 and IL-17 cytokine in the footpads of infected mice were determined by ELISA. Ordinary one-way ANOVA (Dunnett's multiple comparisons test) was used to determine significance among groups. Data are presented as mean ± s.e.m. ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$



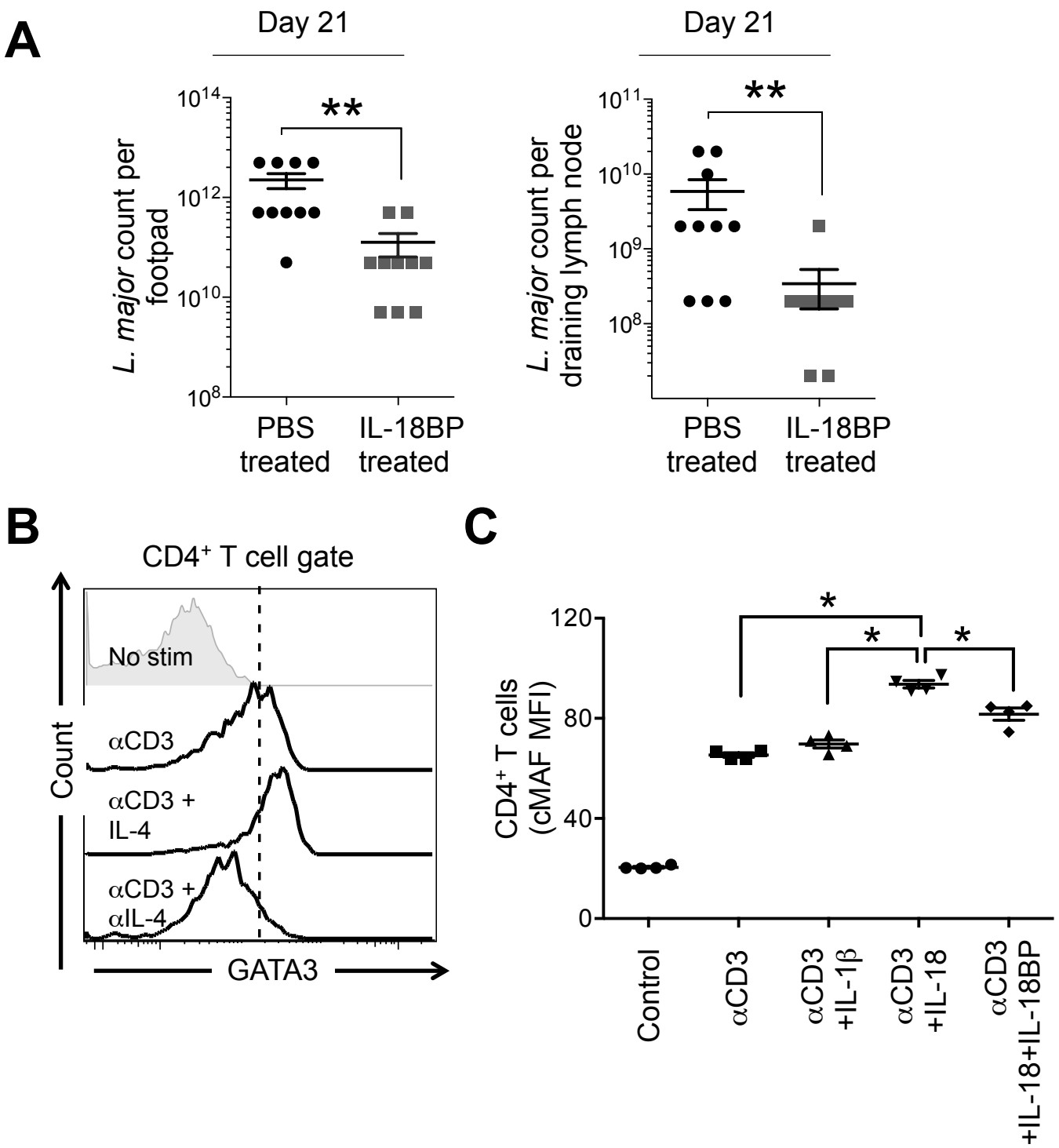
Supplemental Figure 4. Frequencies of CD4$^+$ and CD8$^+$ T cells in the draining popliteal lymph nodes of 28 day infected mice. WT (n=18), *Nlrp3*$^{-/-}$ (n=8), *Asc*$^{-/-}$ (n=12) and *Casp1*$^{-/-}$ *Casp11*$^{-/-}$ (n=9) mice were infected with 10^6 *L. major* promastigotes. Infected mice were euthanized 28 days later and draining popliteal lymph nodes were harvested. Single cell suspensions of popliteal lymph node cells were stained with anti-CD4 and anti-CD8 monoclonal antibodies. Frequency of CD4$^+$ **(A)** and CD8$^+$ T cells **(B)** were analyzed by flow cytometry. Data are presented as mean ± s.e.m.



Supplemental Figure 5. Inflammasome deficient CD4⁺ and CD8⁺ T cells produce higher levels of TNF-α during *L. major* infection. (A-D) WT (n=18), *Nlrp3*$^{-/-}$ (n=8), *Asc*$^{-/-}$ (n=12) and *Casp1*$^{-/-}$*Casp11*$^{-/-}$ (n=9) mice were infected with 10^6 *L. major* promastigotes. Infected mice were euthanized 28 days later and draining popliteal lymph nodes were harvested. Single cell suspensions of popliteal lymph node cells were stimulated anti-CD3/anti-CD28 for 4 hours and TNF-α production by CD4⁺ **(A and B)** and CD8⁺ **(C and D)** T cells were determined by flow staining and flow cytometry. Groups were tested for significance using Dunnett's multiple comparisons test. Data are presented as mean ± s.e.m. ns= not significant, *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.



Supplemental Figure 6. IL-1β and IL-18 stimulation alone are sufficient to induce IFN-γ production but not IL-4. WT splenocytes were stimulated with anti-CD3 Ab in the presence of IL-1β and IL-18. In some settings splenocytes were stimulated with IL-1β or IL-18 alone. After 72 hours of stimulation, supernatants were collected and analyzed for IL-4 **(A)** and IFN-γ **(B)** by ELISA. Data are presented as mean ± s.e.m. and are representative of at least three independent experiments. Significance between aCD3 and aCD3+IL-18 were determined using Mann Whitney test. ns=not significant and **=p<0.01.



Supplemental Figure 7. IL-18 attenuates *L. major* infection *in vivo* and IL-4 enhances GATA3. (A) WT BALB/c mice were treated with PBS (n=10) or IL-18BP (n=10) at day -1, 0, 1, 3, 7 and 14. On day 21 *L. major* titers in the footpads and draining lymph nodes of infected mice were determined using limiting dilution assay as described in the methods section. **(B and C)** Single cell suspension of splenocytes from WT BALB/c mice were stimulated with anti-CD3 Ab in the presence of rIL-4, anti-IL4 Ab, IL-1β or IL-18 for 48 hours. **(B)** Representative histograms for GATA3 expression during rIL-4 or anti-IL4 treatment are shown. **(C)** cMAF MFI of CD4+ T cells during IL-1β and IL-18 treatment are shown. Mann Whitney test used for significance test. Data are shown at mean ± s.e.m. **B** and **C** are representative of at least two independent experiments. *=p<0.05, **=p<0.01.