

Figure S1. *LdCen^{r/-}* immunization induced the expression of IL-1 β , IL-6 and inhibits the expression of IL-10 in splenic dendritic cells isolated from infected C57BI/6 mice. (A) Infected cells were sorted from the spleen of different groups of infected mice after two weeks of post- infection/ immunization with either *LdWT*-RFP or *LdCen^{-/-}*-mCherry by gating live single cells for [lineage (T cells, B cells, NK Cells)⁻ and F4/80⁻CD11b⁺CD11c⁺RFP/m-Cherry⁺] cells. (B-D) mRNA expression levels of IL-1 β , IL-6, and IL-10 were measured from infected DCs, and expressed as fold increase over naïve DCs. The data represent the mean values + SEM of results from two independent experiments. In each experiment more than eight mice splenocytes were pooled to get enough infected DCs. *, P < 0.05; **, P < 0.005.



Figure S2. LdCen^{-/-} immunized mice showed significant attenuation of IL-10 producing nTreg and Tr1 cells compared to LdWT infected mice. LdWT or LdCen^{-/-} infected mice were sacrificed five weeks of post- infection. Splenocytes were collected and cultured in presence of Leishmania Ag and PMA/Ionomycin. Flow cytometry analysis of Leishmania Ag stimulated splenocytes (A) gating strategy for IL-10 and IFN- γ secreting CD4 T cells. (B) Bar-diagram represents IL-10 secreting, (C) IL-10 and IFN γ double producing CD4 T cells. (D) Gating strategy and representative flow plots for the nTerg and Tr1 cells producing IL-10 and IFN- γ . (E) Mean percentage of CD25+FOxP3+CD4 T cells; (F) Percent of IL-10 producing nTreg and (G) percent of IL-10 producing Tr1 cells. (H) Percent of nTreg producing IL-10 and IFN- γ ; (I) Percent of Tr1 cells producing IL-10 and IFN- γ . The data represent the mean values + SEM of results from three independent experiments. *, P < 0.05 **, P< 0.005.



Figure S3. *LdCen^{-/-}* **immunization induced Th1 axis.** (A) *LdWT* or *LdCen^{-/-}* infected mice were sacrificed at two and five weeks after infection. Splenocytes were cultured and stimulated with *Leishmania* Ag. Culture supernatants were collected and level of IFN- γ was measured by the multiplex mouse cytokine ELISA kit as described in the Materials and Method section. (B) Five weeks post-immunized mice were challenged with virulent *L. donovani* parasites. At two and six weeks post challenge both immunized and non-immunized mice were euthanized, splenocytes were cultured, stimulated with *Leishmania* Ag and culture supernatants were collected and concentration of IFN- γ (B) was measured by the multiplex mouse cytokine ELISA. Flow cytometry analysis of *Leishmania* Ag stimulated splenocytes was performed. (C-D) bar diagram representing IFN- γ and IL-17 secreting CD4 and CD8 T cells in *LdWT infected* and *LdCen^{-/-}* immunized mice at indicated time points. (E-F) Bardiagram representing IFN- γ and IL-17 secreting CD4 and CD8 T cells from naïve challenged and immunized challenged mice at indicated time points. The data represent the mean values + SEM of results from two independent experiments. *, P < 0.05; **, P< 0.005. PI, Post-Immunization; PC, Post-Challenge.



Figure S4. Treatment with rIL-23 significantly enhanced the percentage of IL-17 producing CD4 and CD8 effector memory cells. Mice were immunized with *LdCen*^{-/-} and at 12 weeks post-immunization mice were sacrificed. Splenocytes were isolated and cultured in presence of exogenously added recombinant IL-23. Flow cytometry analysis of IL-17 producing CD4 and CD8 effector memory cells. (A) Gating Strategy and representative flow plots; (B-C) Bar diagram represents IL-17 secreting effector memory CD4 (CD4+CD44hiCD62L-IL-17+) and CD8T (CD8+CD44hiCD62L-IL-17+) cells. The data represent the mean values + SEM of results from two independent experiments. *, P < 0.05.