Supplemental Material **Ribot *et al.***

Adaptive versus innate receptor signals selectively control the pool sizes of murine IFN-γ- or IL-17-producing γδ T cells upon infection

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Supplemental Figures:

1. TCR stimulation favors $\gamma\delta^{27+}$ cells *in vitro* and *in vivo*.

2. TCR Vγ repertoire of γδ T cell subsets following TCR/ CD3 activation.

3. CD27 confers homeostatic expansion advantage to γδ T cells.

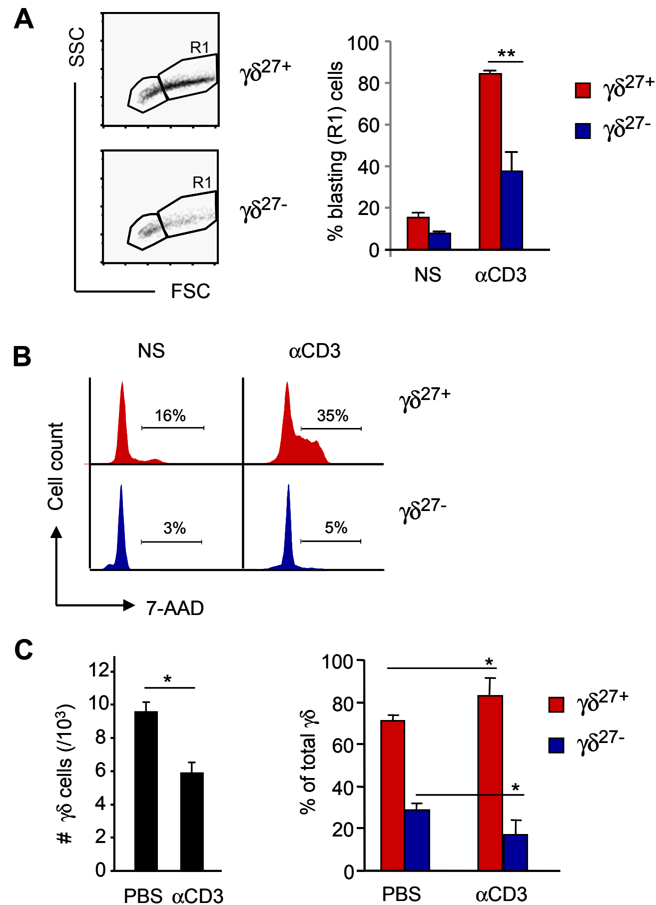
4. Stability of CD27 expression in γδ T cell subsets after malaria infection.

5. *In vivo* LPS treatment induces strong proliferation of $\gamma\delta^{27-}$ cells.

6. TLR2-dependent expansion of $\gamma\delta^{27-}$ cells upon lipoprotein administration.

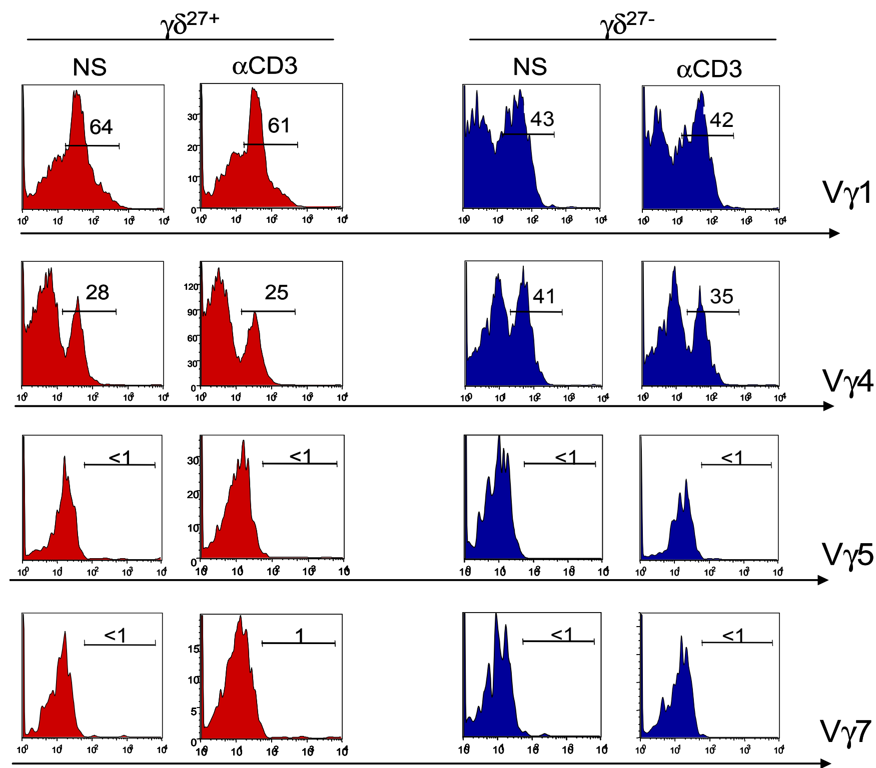
7. TLR agonists do not activate $\gamma\delta^{27-}$ lymphocytes in *cis*.

8. Inflammatory cytokines produced my myeloid cells activate IL-17-producing $\gamma\delta^{27-}$ lymphocytes in *cis*.

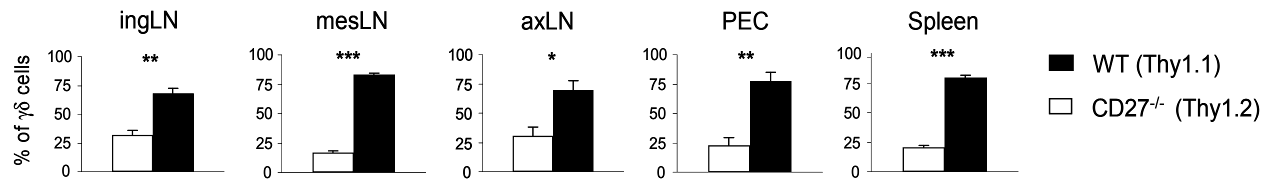


Supplemental Figure 1. TCR stimulation favors $\gamma\delta^{27+}$ cells *in vitro* and *in vivo*.

(A-B) Peripheral $\gamma\delta^{27+}$ and $\gamma\delta^{27-}$ cells were FACS-sorted from pooled spleen and lymph nodes of C57Bl/6 mice and cultured for 72 hours with anti-CD3ε mAb (αCD3) in the presence of APC. Forward Scatter (FSC)/ Side Scatter (SSC) (left panel) and percentages of cells within R1 gate ("blasting cells") (right panel) at 72 hours. Error bars represent SD (n=3, ***p*<0.01). (B) Analysis of cell cycle status at 24 hours using the DNA intercalating dye 7-AAD. Data in (A-B) are representative of two independent experiments (each involving 4-6 animals) with consistent results. (C) C57Bl/6 mice were immunized intraperitoneally with 100 µg αCD3 mAb (or PBS as control). Cells were harvested from the peritoneal cavity after 3 days, and stained for surface TCRγδ, CD3 and CD27. Absolute number of γδ T cells (left panel) and percentages of $\gamma\delta^{27+}$ and $\gamma\delta^{27-}$ cells (right panel). Error bars represent SD (n=5, **p*<0.05). Data are representative of two independent experiments (each involving 3 mice per group) with similar results.

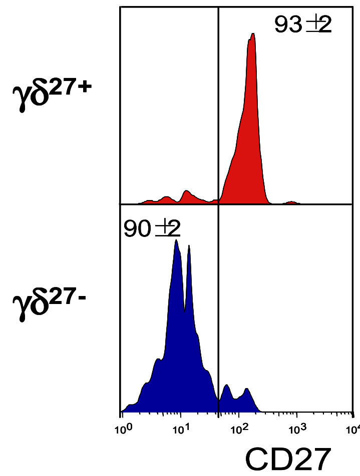


Supplemental Figure 2. TCR Vγ repertoire of γδ T cell subsets following TCR/ CD3 activation. Peripheral $\gamma\delta^{27+}$ and $\gamma\delta^{27-}$ cells were FACS-sorted from pooled spleen and lymph nodes of C57Bl/6 mice, cultured without (NS, non-stimulated) or with anti-CD3ε mAb (αCD3) for 48 hours, and then stained with mAbs specific for Vγ1, Vγ4, Vγ5 or Vγ7 TCR chains.

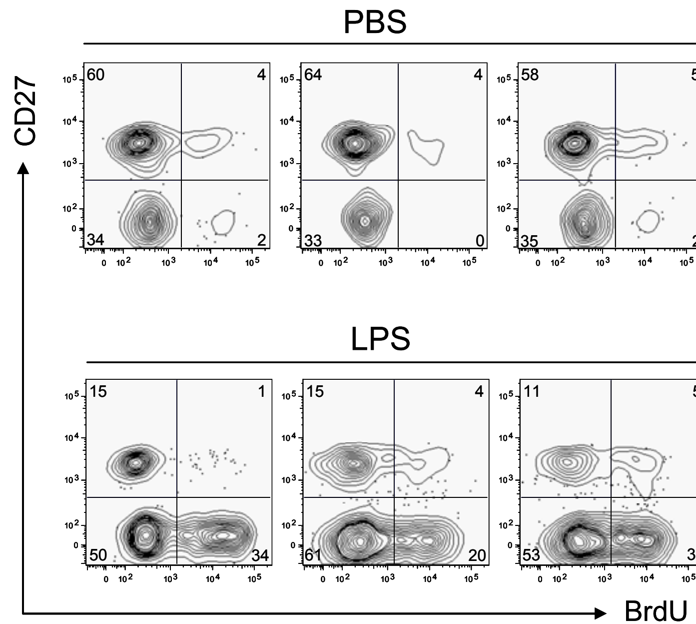


Supplemental Figure 3. CD27 confers homeostatic expansion advantage to γδ T cells.

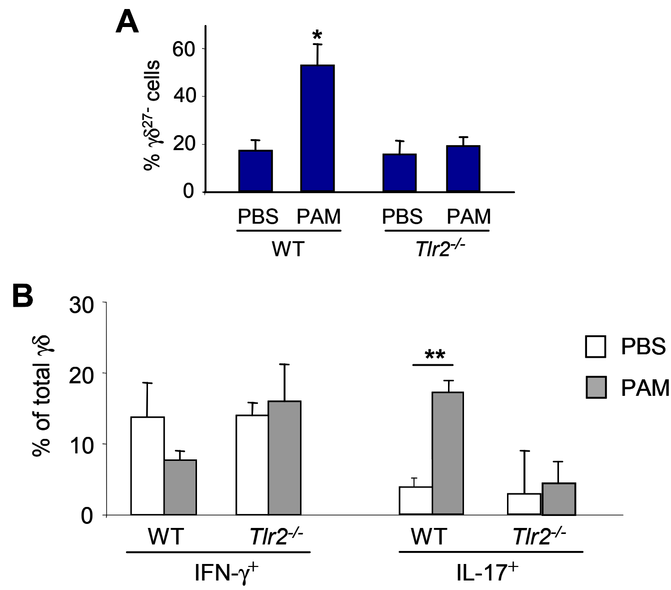
Total γδ cells were FACS-sorted from pooled spleen and LN of *Cd27*+/+.Thy1.1 (WT) and *Cd27*-/-.Thy1.2 mice. Cells were co-injected at 50:50 ratio into *TCRd*$^{-/-}$ mice, and after 4 days organs were harvested and stained for CD3ε, TCRδ and Thy1.2 to discriminate WT and *Cd27*-/- γδ cells. PEC, peritoneal cavity; LN, lymph nodes; ing, inguinal; mes, mesenteric; ax, axillary. Error bars represent SD (n=4), and significant differences between the percentages of the two populations are noted (*$p<0.05$, **$p<0.01$, ***$p<0.001$).



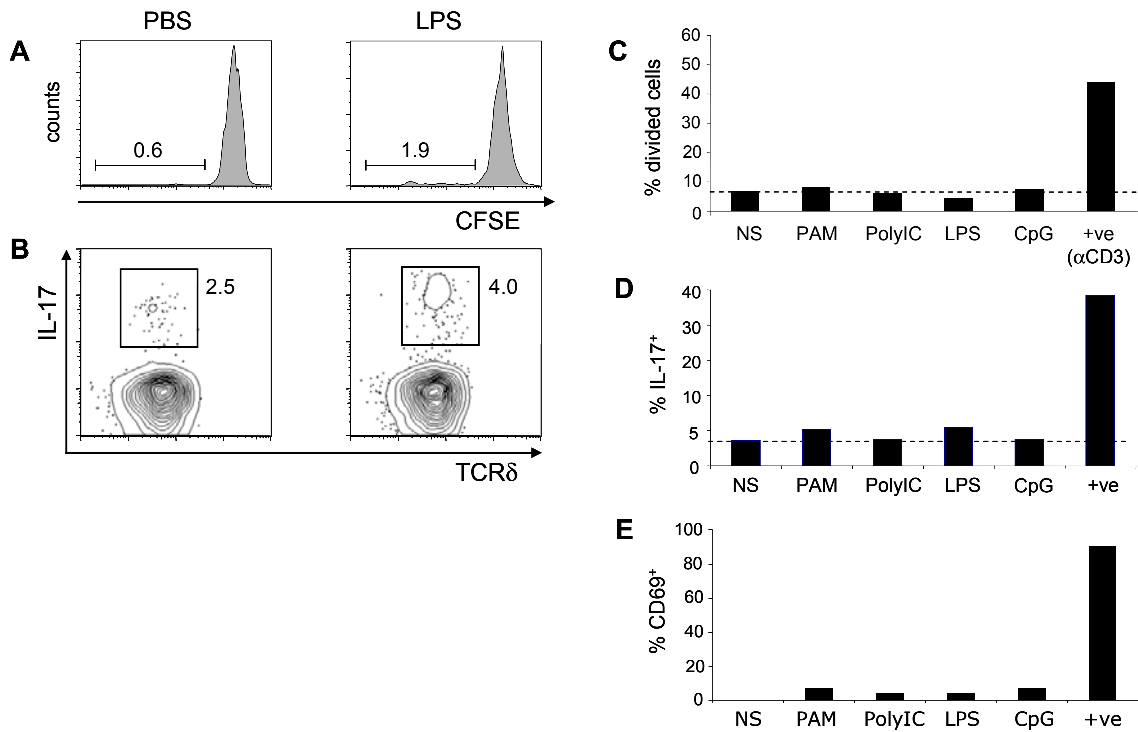
Supplemental Figure 4. Stability of CD27 expression in γδ T cell subsets after malaria infection. $\gamma\delta^{27+}$ or $\gamma\delta^{27-}$ cells were FACS-sorted from pooled spleen and lymph nodes of C57Bl/6 mice, and injected i.v. into separate Rag2-deficient mice (n=3 per group). Mice were infected with *Plasmodium berghei* on the following day, and sacrificed after 3 weeks. Splenocytes were harvested, stained for TCRδ, CD3ε and CD27, and analyzed by flow cytometry. Indicated are the percentages ± SD of cells within the original quadrants used to sort $\gamma\delta^{27+}$ or $\gamma\delta^{27-}$ cells.



Supplemental Figure 5. ***In vivo*** **LPS treatment induces strong proliferation of $\gamma\delta^{27-}$ cells.** C57Bl/6 mice were injected intra-peritoneally with 50µg LPS, or PBS as control. The thymidine analogue bromodeoxyuridine (BrdU) was administered (1mg/mouse) daily until mice were sacrificed at day 3. Cells were harvested from the peritoneal cavity and stained for TCRδ, CD3ε and CD27 expression. Cells were subsequently fixed, permeabilized and stained with FITC-labelled anti-BrdU mAb using the BrdU Flow Kit (BD Pharmingen).

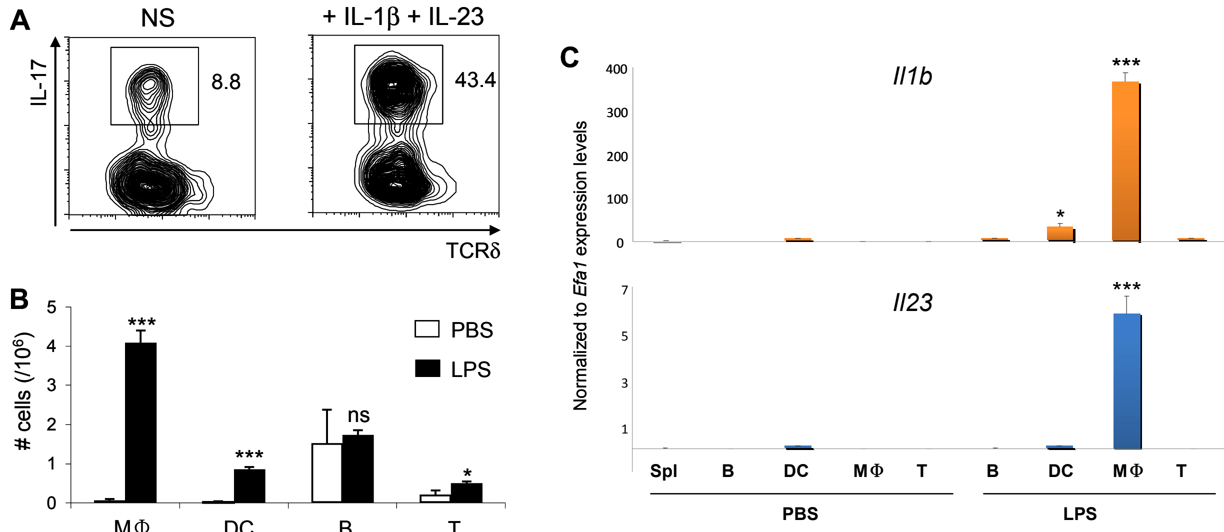


Supplemental Figure 6. TLR2-dependent expansion of $\gamma\delta^{27-}$ cells upon lipoprotein administration *in vivo*. WT and *Tlr2*$^{-/-}$ mice were immunized intra-peritoneally with 50 µg $Pam_3CysSerLys_4$ (PAM) or PBS as control, and sacrificed after 3 days. (A) Percentages of $\gamma\delta^{27-}$ cells within total γδ cells isolated from the peritoneal cavity (PEC). Error bars represent SD (n=3; *p<0.05). (B) Percentage of IFN-γ^+ or IL-17^+ cells upon intracellular cytokine staining of total γδ cells. Error bars represent SD (n=3; **p<0.01).



Supplemental Figure 7. TLR agonists do not activate $\gamma\delta^{27-}$ lymphocytes in *cis*.

(A-B) FACS-sorted and CFSE-labeled TCR$\gamma\delta^{+}$ CD3^{+} CD19^{-} CD27^{-} cells were cultured for 2 days without (non-stimulated, NS) or with 10 μg/ mL of LPS from *Salmonella* Minnesota R595. Cells were then analyzed for CFSE dilution (A) and intracellular IL-17 production (B). Data are representative of triplicate experiments with similar results. (C-D) FACS-sorted and CFSE-labeled TCR$\gamma\delta^{+}$ CD3^{+} CD19^{-} CD27^{-} cells were cultured for 2 days with Pam$_3$CysSerLys$_4$ (PAM; 3μg/mL), polyI:C (10μg/mL), LPS (10μg/mL), CpG (1.5μg/mL), or with anti-CD3ε mAb (1μg/mL, plate-bound) as positive control (+ve). Cells were then analyzed for CFSE dilution (C), intracellular IL-17 production (D) and surface expression of the activation marker CD69 (E).



Supplemental Figure 8. Inflammatory cytokines produced my myeloid cells activate IL-17-producing $\gamma\delta^{27-}$ lymphocytes in *cis*. (A) FACS-sorted TCR$\gamma\delta^{+}$ CD3^{+} CD19^{-} CD27^{-} cells were cultured for 2 days without (non-stimulated, NS) or with 10ng/mL of each IL-1β and IL-23, and then stained intracellularly for IL-17. Data are representative of duplicate experiments (each involving 4-6 animals) with similar results. (B-C) WT mice were injected intra-peritoneally with 50µg LPS or with PBS as control, and sacrificed after 3 days. CD19^{+} (B), CD11c^{high} CD11b^{low} (DC), CD11c^{low} CD11b^{high} (MΦ) or CD3^{+} (T) cells were FACS-analyzed and sorted from the peritoneal cavity (PEC). (B) Absolute numbers of cells from each PEC population. (C) Quantitative real-time PCR data for *IL1b* and *Il23*, in arbitrary units normalized to the housekeeping gene *Efa1*, in each cell population. Error bars represent SD (n=3) and significant differences between PBS and LPS treatments are noted (*p<0.05; ***p<0.001).