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CD3(bright) signals on $\gamma\delta$ T cells identify IL-17A-producing Vγ6Vδ1(+) T cells.

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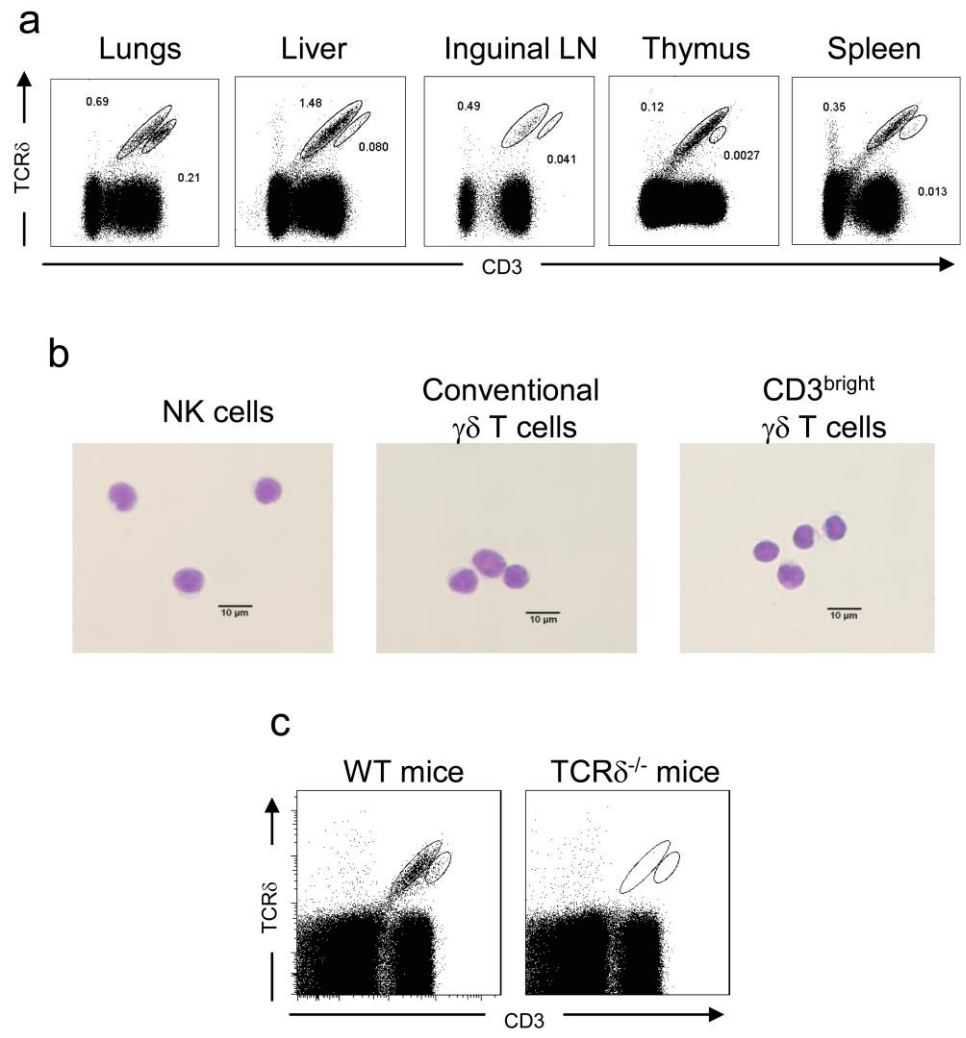
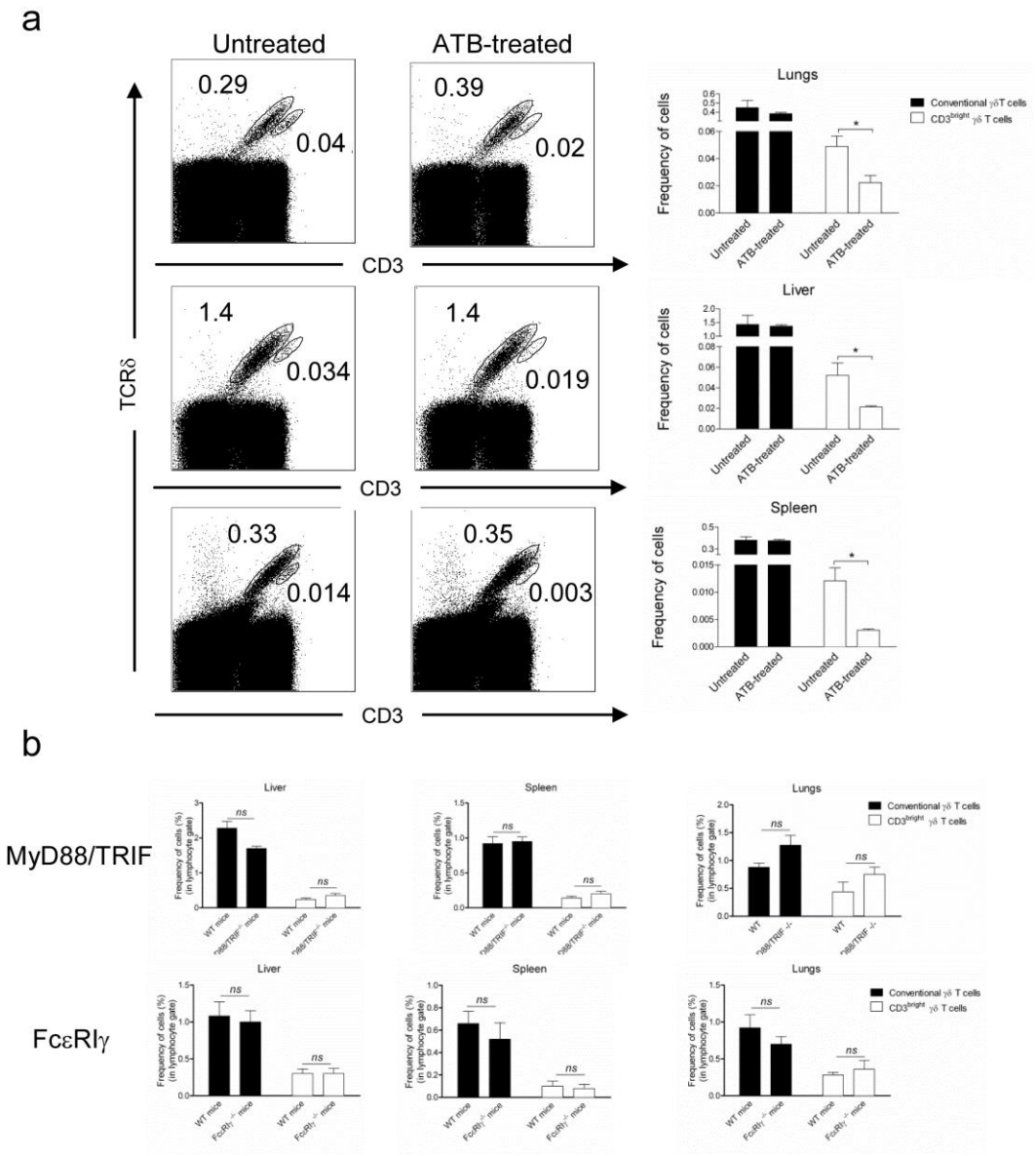
Supplementary materials:



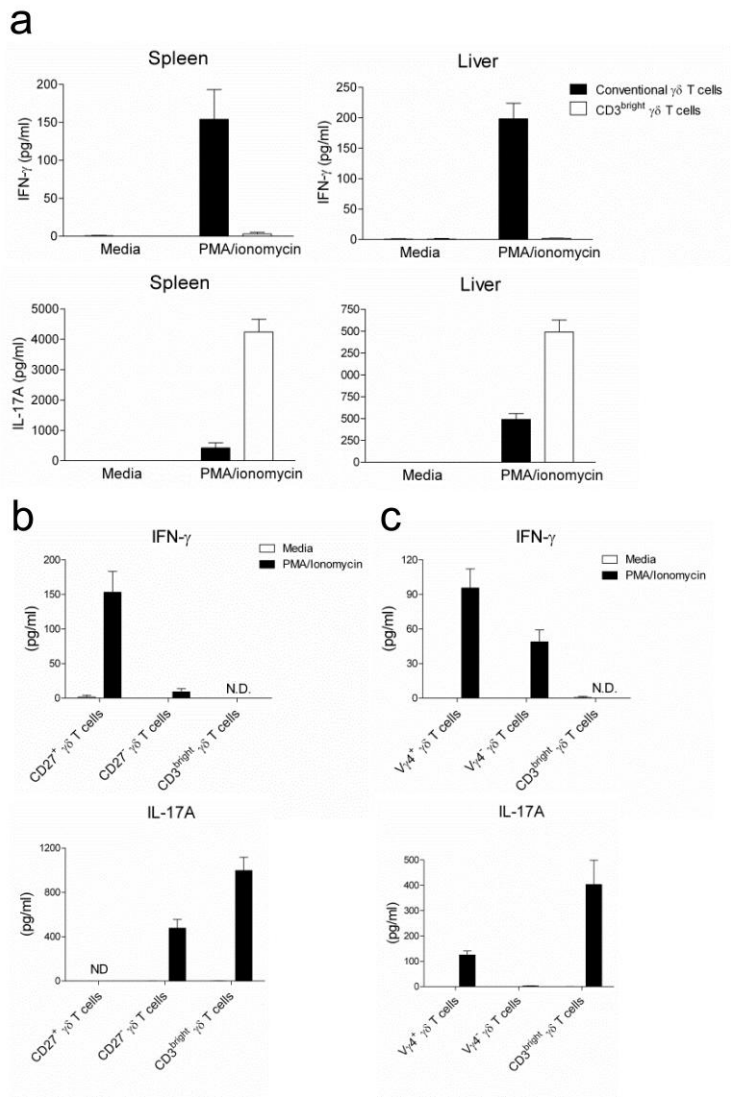
Supplemental Figure 1: CD3^{bright} γδ T cells are authentic γδ T cells and conserved in different mouse backgrounds.

a, Cells from naïve BALB/c WT mice were analyzed by flow cytometry based on CD3 and TCRδ expression. *b,* Morphology and Giemsa staining of cytospun FACS-sorted C57BL/6 WT lymphocyte populations. *c,* Cells from naïve C57BL/6 WT and TCRδ$^{-/-}$ mice were analyzed by flow cytometry based on CD3 and TCRδ expression.



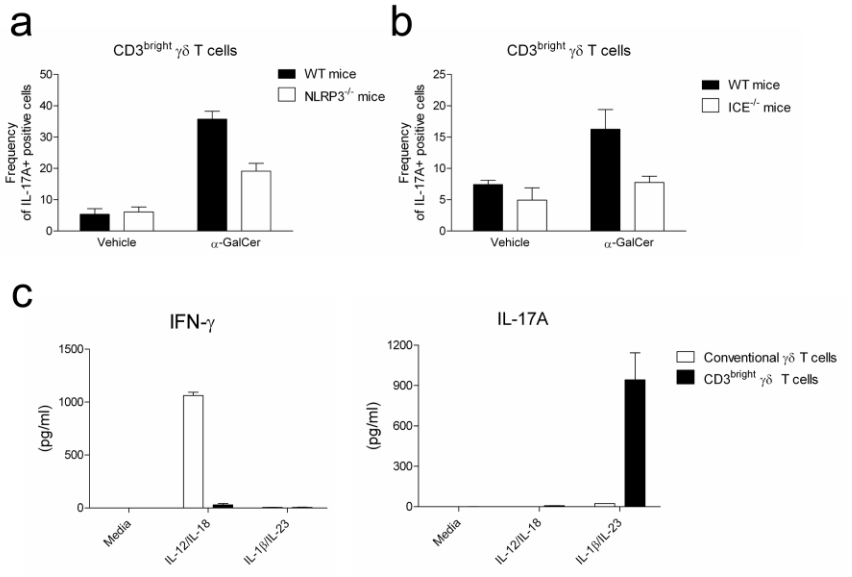
Supplemental Figure 2: Factors influencing CD3^{bright} γδ T cells homeostasis.

a, Effect of chronic exposure to antibiotics on CD3^{bright} γδ T cells. 5-week-old C57BL/6 mice were exposed three weeks with or without neomycin in the drinking water. Frequency of CD3^{bright} and conventional γδ T cells in each group was evaluated by flow cytometry in different organs. Of note, antibiotic exposure did not impact on the total number of cells in each organ. *b*, TLR-signalling and FcεRIγ are not involved in CD3^{bright} γδ T cell homeostasis. Frequency of γδ T subsets was analyzed by flow cytometry in WT *vs* MyD88/TRIF$^{-/-}$ mice. The percentage of γδ T cell subsets is shown. The mean ± SEM of γδ T cell subset frequencies of one representative experiment out of two is shown (n = 5-6).



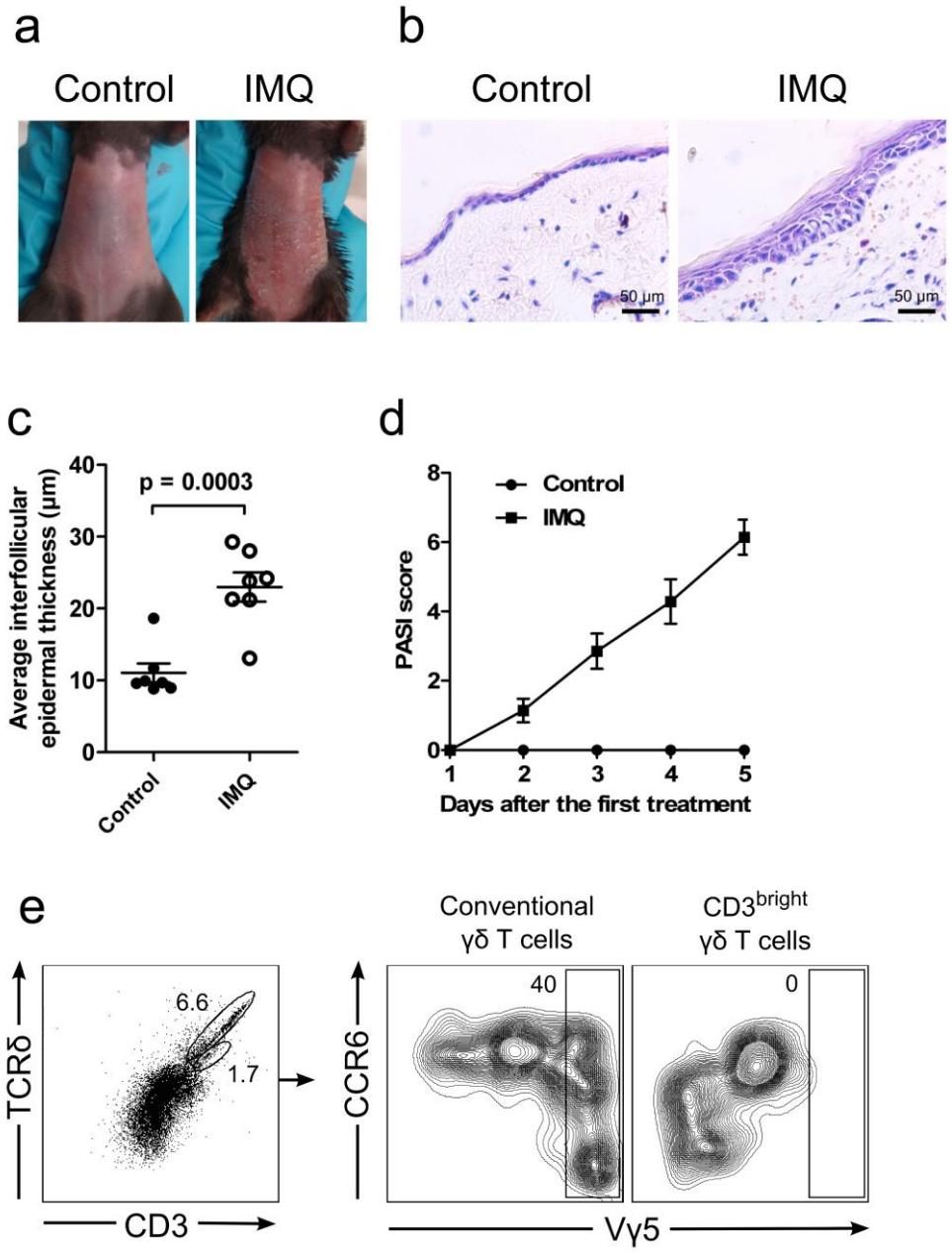
Supplemental Figure 3: Capacity of CD3^{bright} γδ T cells *vs* other γδT17 populations to produce IL-17A.

a, FACS-sorted splenic or hepatic γδ T cell subsets were incubated with PMA (10 ng/ml) and ionomycin (1 μg/ml). Data represent the pooled mean ± SEM of two independent experiments performed at least in duplicate. *b and c*, FACS-sorted pulmonary γδ T cell subsets were incubated as in panel *a*. Cytokine production in the supernatants was measured by CBA. Data represent the pooled mean ± SEM of two independent experiments performed at least in duplicate.



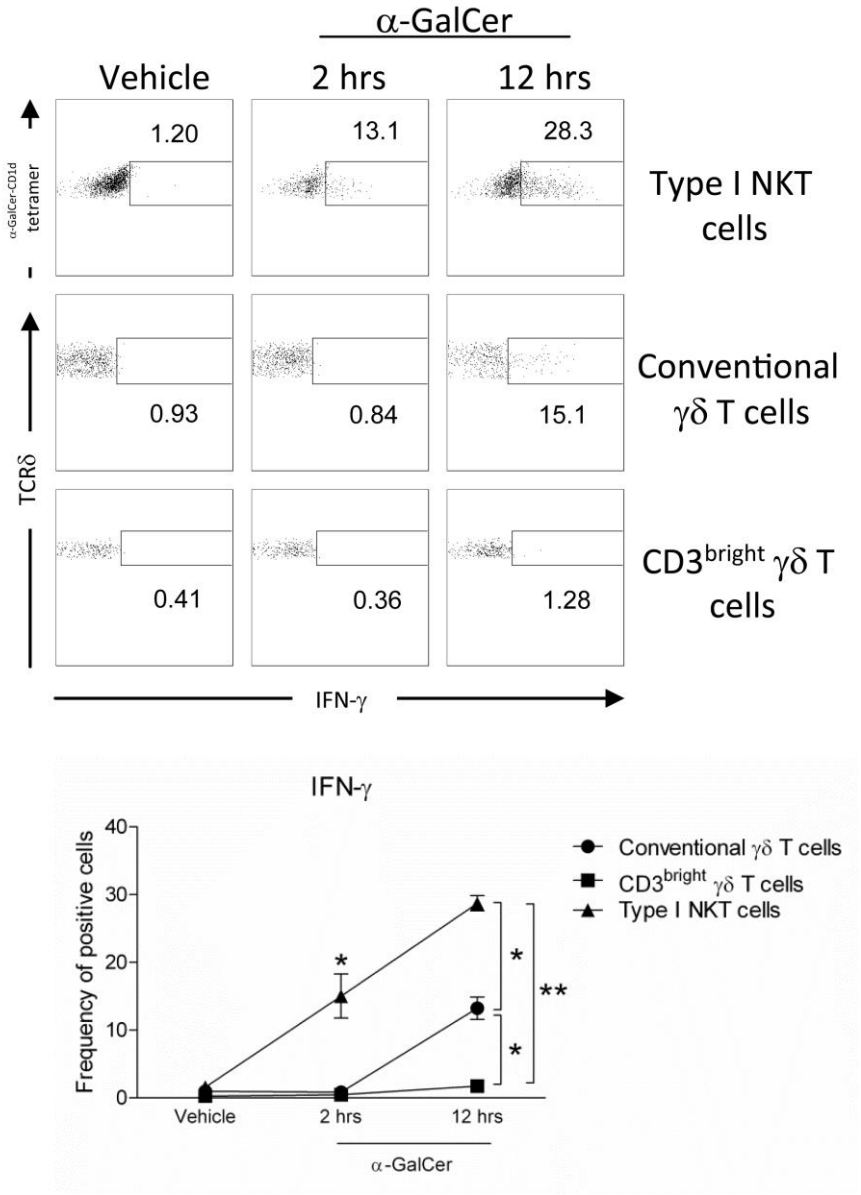
Supplemental Figure 4: IL-17 production by CD3^{bright} γδ T cells requires NLRP3 inflammasome components *in vivo*.

a-b, WT, NLRP3$^{-/-}$ (*a*) or ICE$^{-/-}$ (*b*) mice were injected i.p. with vehicle or α-GalCer (2 μg/mouse) and were sacrificed after 2 hrs. Liver cells were treated with GolgiStop for another 2 hrs. Gated CD3^{bright} γδ T cells were screened for IL-17A production. The pooled mean ± SEM of two independent experiments for IL-17A-positive cells subsets is shown (*n* = 5-6). *c*, FACS-sorted γδ T cell subsets were incubated or not with IL-12/IL-18 (50 pg/ml and 1 ng/ml respectively) or IL-1β/IL-23 (1 ng/ml for both) for 20 hrs. Cytokine production in the supernatants was measured by CBA. Data represent the mean ± SEM of two experiments performed in duplicate.



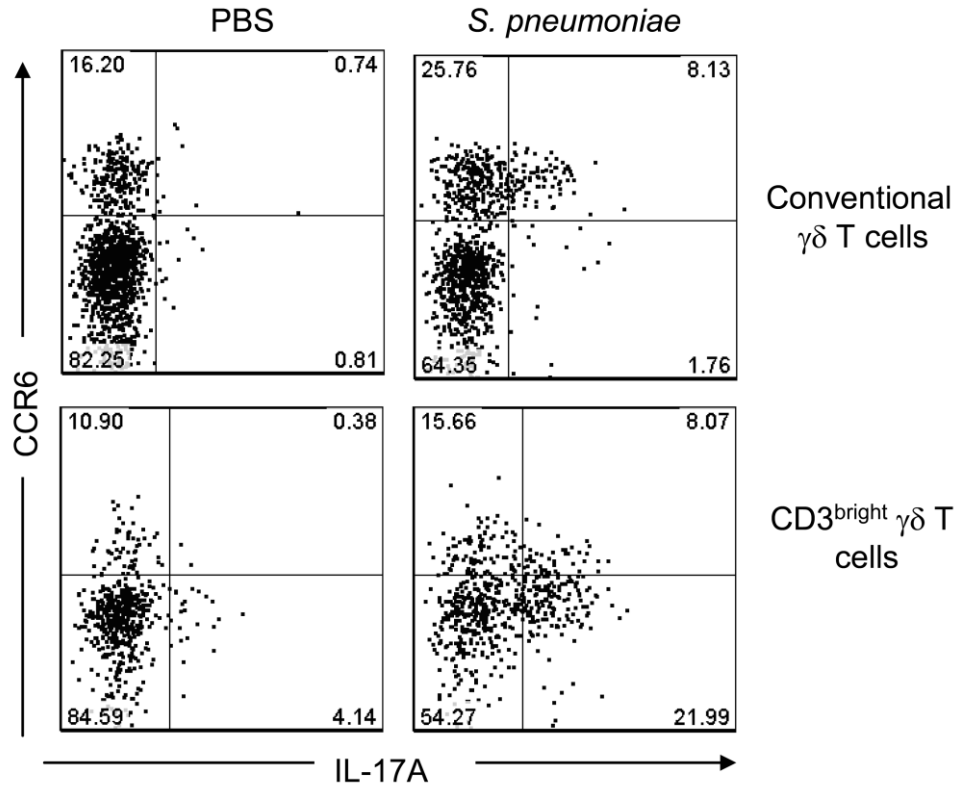
Supplemental Figure 5: Psoriasis-like Mouse Model

Representative skin morphology (*a*) and Giemsa-stained sections (*b*) and average interfollicular epidermal thickness (*c*) of the abdominal skin of C57BL/6 WT mice treated with a daily topical dose of IMQ cream (IMQ) or control "Lanette" cream (Control) during 5 days: presented data correspond 5 days after the first application of the cream. Scale bar corresponds to 50 µm. (*d*) PASI score was estimated at the 1st to 5th days during treatment with IMQ cream (IMQ) or control "Lanette" cream (Control). Data are shown as mean ± SEM, n = 7 mice per group. Statistical differences between groups were analysed by unpaired two-tailed *t*-test. (*e*) DETC Vγ5^{+} T cells are part of the skin conventional γδ T cell population.



Supplemental Figure 6: CD3^{bright} γδ T cells do not produce IFN-γ following intranasal instillation of α-GalCer.

WT mice were injected i.n. with vehicle or α-GalCer (500 ng/mouse) and were sacrificed at indicated times. Pulmonary cells were treated with GolgiPlug for another 2 hrs. Gated innate-like T cells were screened for IFN-γ production. One representative experiment out of three is shown (*upper panel*). The pooled mean ± SEM of three independent experiments for IFN-γ-positive cells subsets is shown (6 mice/group/experiment) in the lower panel. *, $p < 0.05$ and **, $p < 0.01$.



Supplemental Figure 7: CCR6 expression on conventional, but not on CD3^{bright} γδ T cells correlated with their ability to produce IL-17.

WT mice were infected i.n. with *S. pneumoniae* serotype 1 (2 x 10^6 bacteria) and were sacrificed at indicated time points. Gated pulmonary γδ T cell subsets were screened for IL-17A production based on their CCR6 expression. One representative experiment out of two is shown. Gates were set based on the staining with isotype control.