

**CD3(bright) signals on $\gamma\delta$ T cells identify
IL-17A-producing V γ 6V δ 1(+) T cells.**

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Christophe Paget, M T Chow, N A Gherardin, P A Beavis, a P Uldrich, et al.. CD3(bright) signals on $\gamma\delta$ T cells identify IL-17A-producing V γ 6V δ 1(+) T cells.. Immunology and Cell Biology, Nature Publishing Group, 2015, 93 (2), pp.198-212. 10.1038/icb.2014.94 . inserm-01116326

HAL Id: inserm-01116326

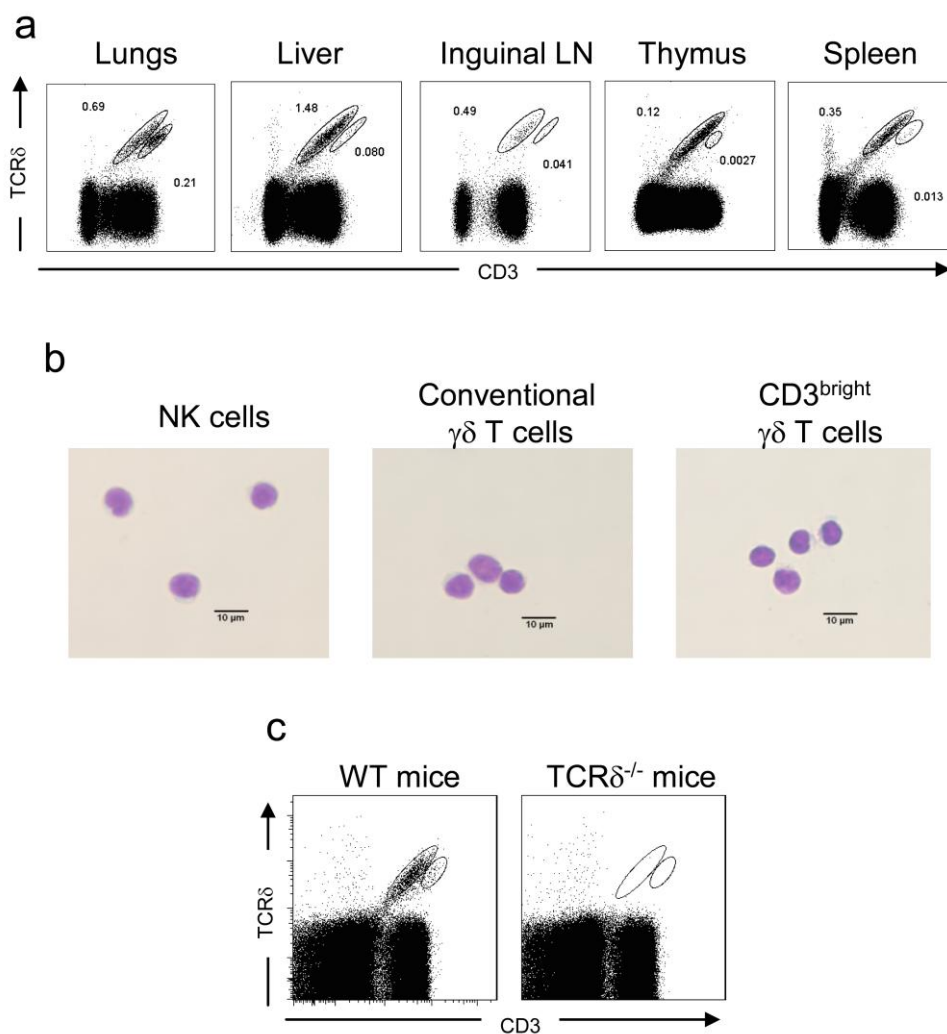
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Submitted on 13 Feb 2015

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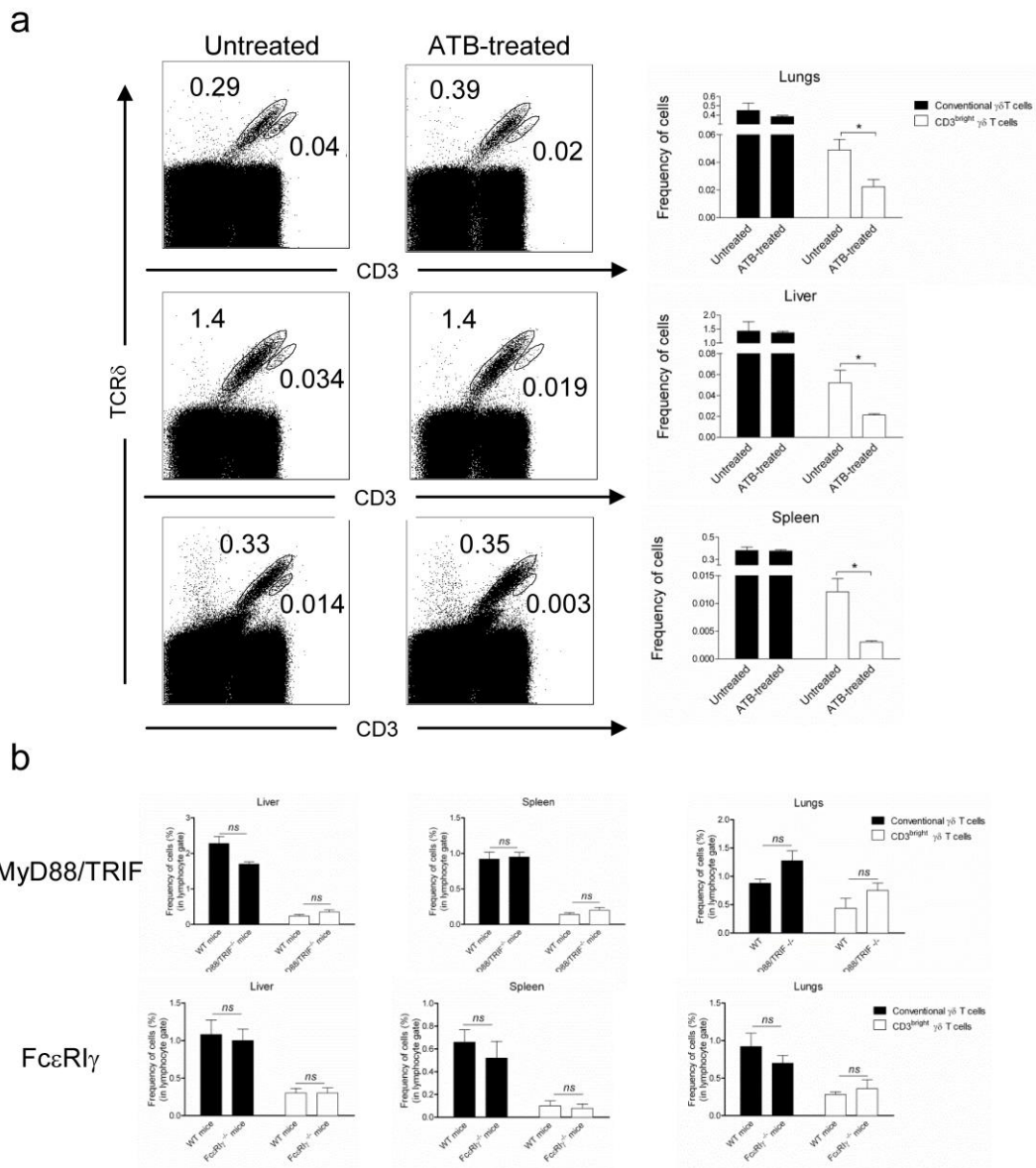
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Supplementary materials:



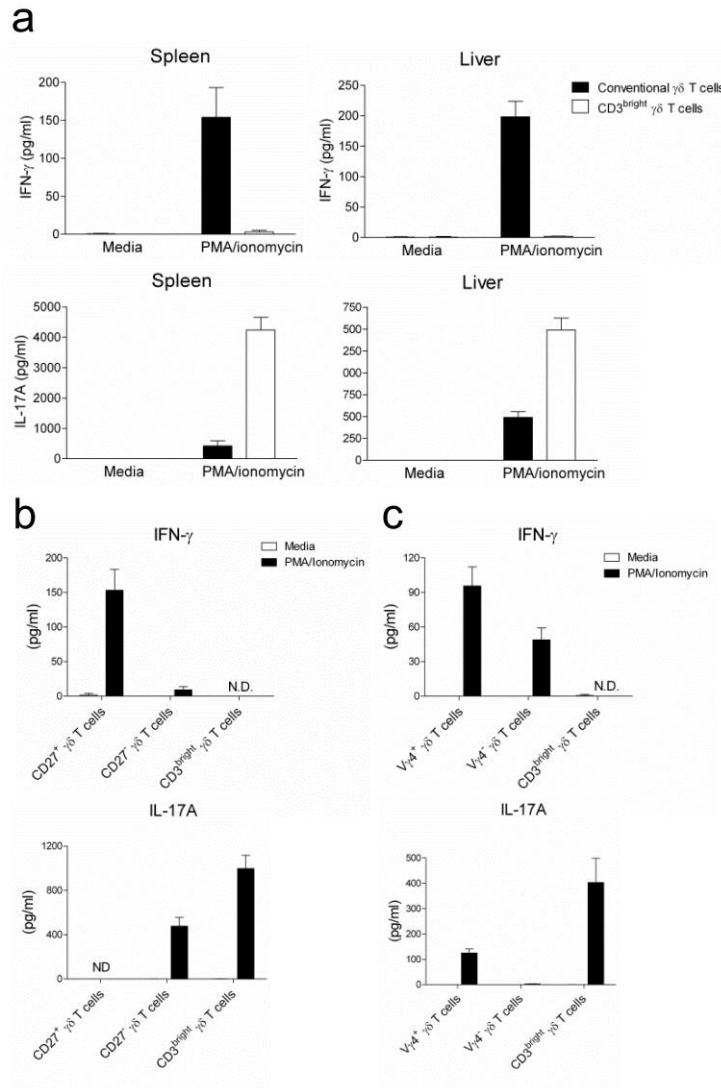
Supplemental Figure 1: CD3^{bright} $\gamma\delta$ T cells are authentic $\gamma\delta$ T cells and conserved in different mouse backgrounds.

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



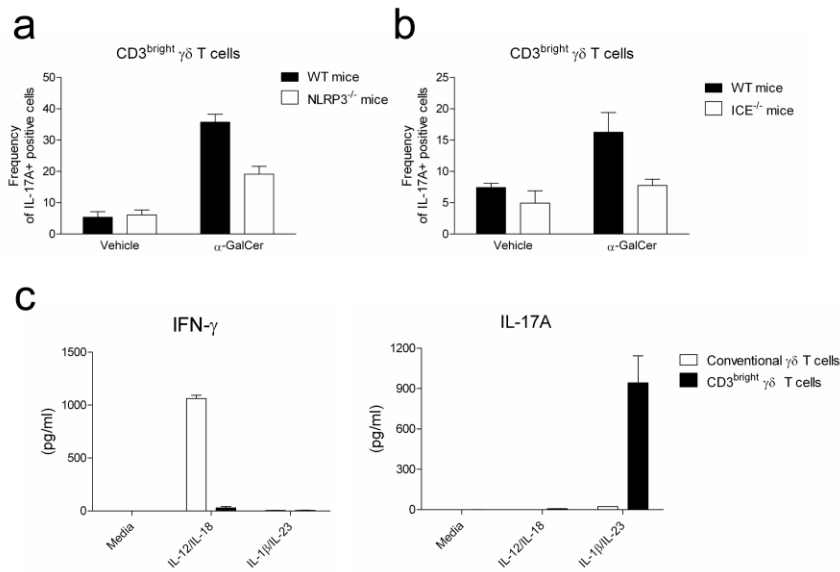
Supplemental Figure 2: Factors influencing CD3^{bright} $\gamma\delta$ T cells homeostasis.

a, Effect of chronic exposure to antibiotics on CD3^{bright} $\gamma\delta$ 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 $\gamma\delta$ 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 Fc ϵ RI γ are not involved in CD3^{bright} $\gamma\delta$ T cell homeostasis. Frequency of $\gamma\delta$ T subsets was analyzed by flow cytometry in WT vs MyD88/TRIF^{-/-} mice. The percentage of $\gamma\delta$ T cell subsets is shown. The mean \pm SEM of $\gamma\delta$ T cell subset frequencies of one representative experiment out of two is shown ($n = 5-6$).



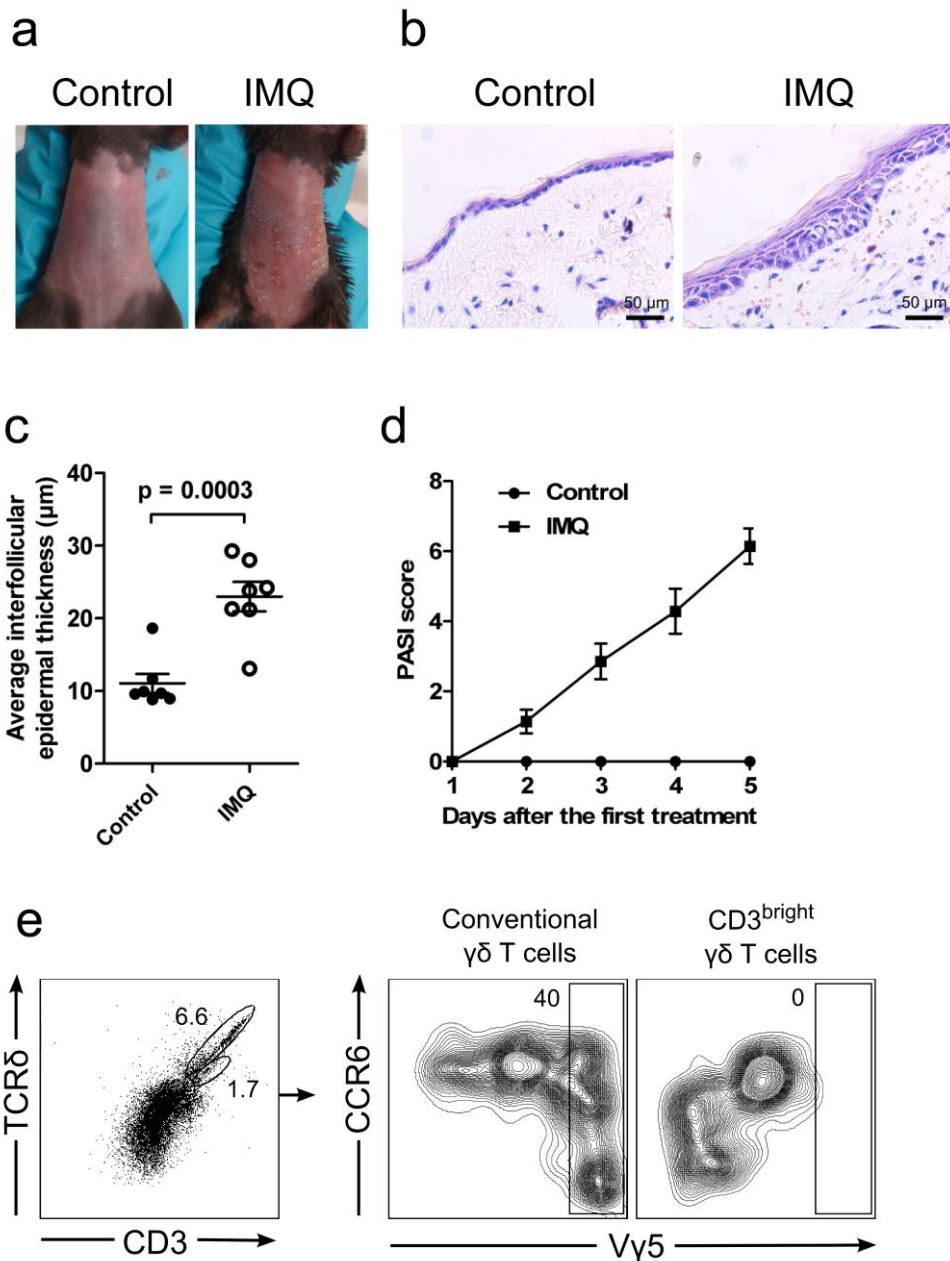
Supplemental Figure 3: Capacity of CD3^{bright} $\gamma\delta$ T cells vs other $\gamma\delta$ T17 populations to produce IL-17A.

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



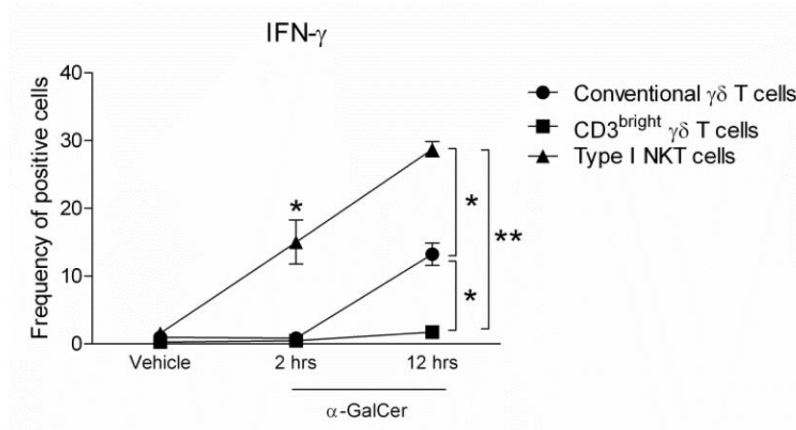
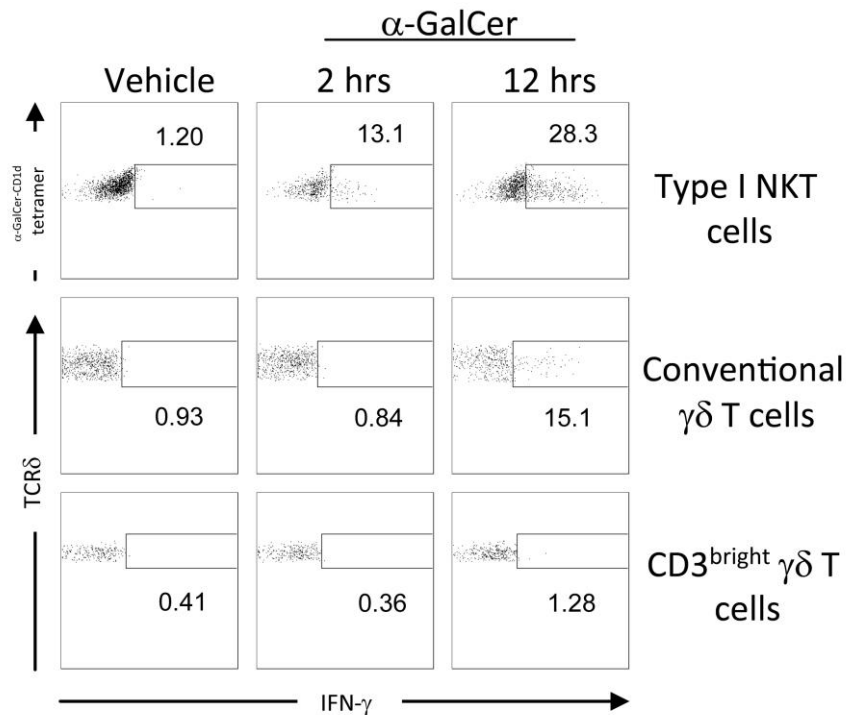
Supplemental Figure 4: IL-17 production by CD3^{bright} γδ T cells requires NLRP3 inflammasome components *in vivo*.

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.



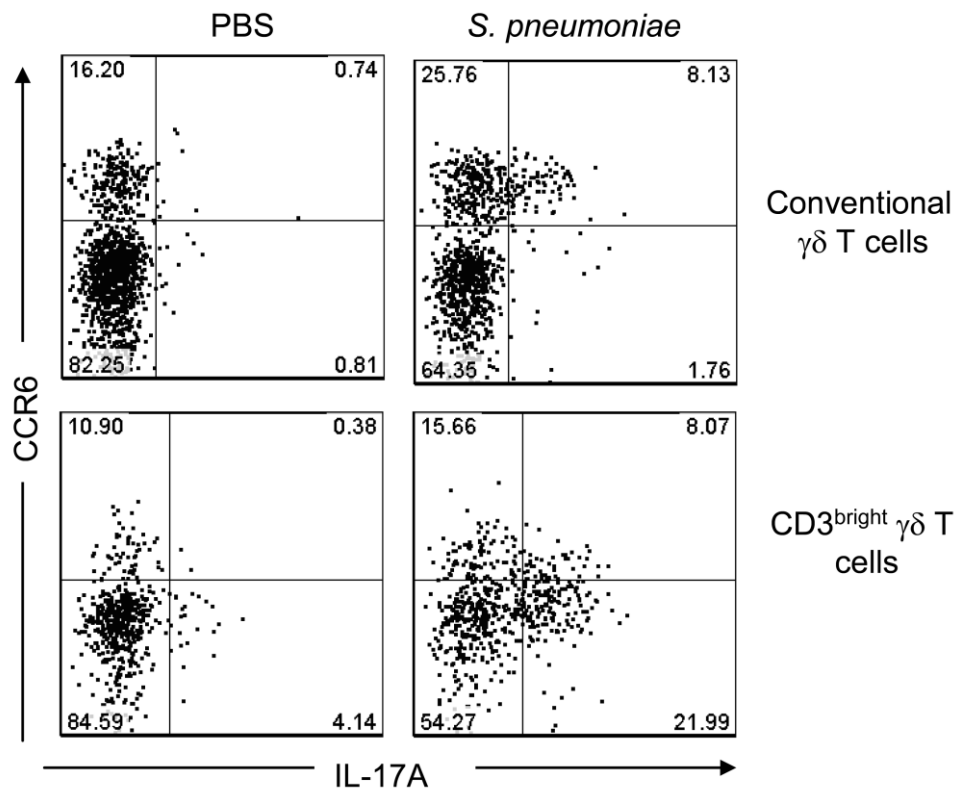
Supplemental Figure 5: Psoriasis-like Mouse Model

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 \pm SEM, $n = 7$ mice per group. Statistical differences between groups were analysed by unpaired two-tailed t -test. (e) DETC V γ 5⁺ T cells are part of the skin conventional $\gamma\delta$ 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} $\gamma\delta$ T cells correlated with their ability to produce IL-17.

WT mice were infected i.n. with *S. pneumoniae* serotype 1 (2×10^6 bacteria) and were sacrificed at indicated time points. Gated pulmonary $\gamma\delta$ 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.