

SUPPLEMENTAL FIGURES

Fig. S1 (Supplement to Fig.1). Recall of antigen specific Th17 cells to the lung during the vaccine efferent phase.

(A) Intracellular IL-17 production by endogenous, polyclonal and *Blastomyces*-specific TCR Tg CD4⁺ T cells. Mice received 2 x 10⁵ Tg cells and were vaccinated and challenged with *B. dermatitidis* yeast as in Methods. At day 4 post-infection, lung T cells were harvested and stimulated with anti-CD3 and anti-CD28 mAb to detect intracellular IL-17. **(B)** Kinetics of IL-17 producing CD4⁺ T cells recalled to the lungs of vaccinated mice. The number of IL-17 producing CD4⁺ T cells was calculated by multiplying the number of total lung cells by the percentage of cytokine producing CD4⁺ T cells. Mice received 2 x 10⁵ Tg cells and were vaccinated with 10⁵ to 10⁷ live attenuated yeast, challenged with 2 x 10³ wild type yeast and analyzed for the number of IL-17 producing *Blastomyces*-specific Tg (filled icons) and polyclonal (open icons) CD4⁺ T cells. *, p < 0.05 vs. mice vaccinated with 10⁵, 10⁶ and 10⁷ yeast.

Fig. S2 (Supplement to Fig. 2). IFN- γ is dispensable and IL-17 mediates vaccine immunity in BALB/c mice.

(A) Antibody neutralization of IL-17 in vaccinated IFN- γ ^{-/-} and wild-type mice after infection. Vaccinated wild-type and IFN- γ mice received anti-IL-17 or rat IgG control 2hr before challenge and every other day thereafter. Mice were challenged with yeast as in the Methods and harvested 11 days post-infection when unvaccinated mice were moribund. Lung CFU values are the mean \pm SEM of 10 mice/group. The numbers shown are the relative increase in lung CFU of mAb-treated vs. rat IgG controls. *, p < 0.001 vs. mice treated with rat IgG. **(B)** Neutralization of IL-17 by soluble IL-17 receptor (IL-17R:Fc). At day -3 and -1 before challenge, vaccinated and unvaccinated mice were infected i.v. with AdIL-17R or AdLuciferase. On day 0, mice were challenged i.t. with yeast and recombinant adenovirus. At day 12 post-infection when AdIL-17R-

treated unvaccinated mice were moribund, mice were sacrificed, and lung CFU enumerated. Values are the mean \pm SEM of 10 mice/group. The numbers shown are the fold-increase in lung CFU of AdIL-17RA vs. AdLuciferase groups. *, $p < 0.001$ vs. mice treated with AdLuciferase.

Fig. S3 (Supplement to Fig. 3). Th1 immunity in IL-12R β 2^{-/-} (A), T-bet^{-/-} (B) and T-bet^{-/-}/Stat4^{-/-} mice (C). The relative quantity of lung cytokine transcript at day 2 post-infection is shown in comparison to wild-type control mice. *, $p < 0.001$ vs. wild-type controls. The percentage of cytokine-producing CD4⁺ CD44⁺ T cells was determined by intracellular cytokine staining (ICS) and FACS at day 3 post-infection. The numbers over histogram bars indicate the relative change in IFN- γ or IL-17 in knockout mice vs. wild-type controls. *, $p < 0.05$ vs. vaccinated wild-type in panels A and B. Ag-specific IFN- γ and IL-17 production by CD4⁺ T cells were measured at day 2 post-infection. CD4⁺ T cells purified from the skin draining lymph nodes and spleen were stimulated with irradiated splenocytes and CW/M antigen for 2 days and analyzed for cytokines in the cell culture supernatants. The values are means \pm SEM of 4 mice/group for 2 independent experiments. *, $p < 0.001$ vs. IFN- γ produced by CD4⁺ cells from vaccinated wild-type mice.

Fig. S4 (Supplement to Fig. 4). T helper phenotype of polarized Th17 cells. T-helper phenotypes of adoptively transferred, polarized Th17 cells were assayed during the recall response in the lungs of non-irradiated (A) and irradiated (B) wild type recipients. Transferred TCR Tg 1807 cells harvested from lung homogenates were analyzed by intracellular cytokine staining at day 4 post-infection. The number and percentage of cytokine-producing Thy1.1⁺ Tg T-cells is illustrated. The numbers represent the means \pm SEM of 4 animals/group. In (A) *, $p < 0.05$ vs. mice that received OT2 or no cells in upper panel; and *, $p < 0.05$ vs. number of cytokine-producing Tg cells

from non-crossed 1807 Tg mice in lower panel. In (B) *, $p < 0.05$ vs. mice that received OT2 or no cells in upper and lower panels.

Fig. S5 (Supplement to Fig. 5). Cytokine producing T cells in the lungs of mice vaccinated against *Coccidioides* and *Histoplasma*. (A) The relative quantity of lung IL-17A transcript at serial time points post-infection with *Coccidioides* was determined in comparison to unvaccinated wild-type control mice. *, $p < 0.05$ vs. wild-type control. (B) Lung CFU was measured at serial time points after *Coccidioides* infection. Mean \pm SEM of 4 mice/group; representative of 2 experiments. *, $p < 0.05$ vs. unvaccinated mice. (C) The number of cytokine-producing CD4⁺ CD44⁺ T cells in the lung was determined by intracellular cytokine staining and FACS at day 4 post-infection with *Histoplasma* in unvaccinated and vaccinated wild-type and IL-17RA^{-/-} mice. *, $p < 0.001$ vs. cytokines produced by CD4 cells from unvaccinated control mice.

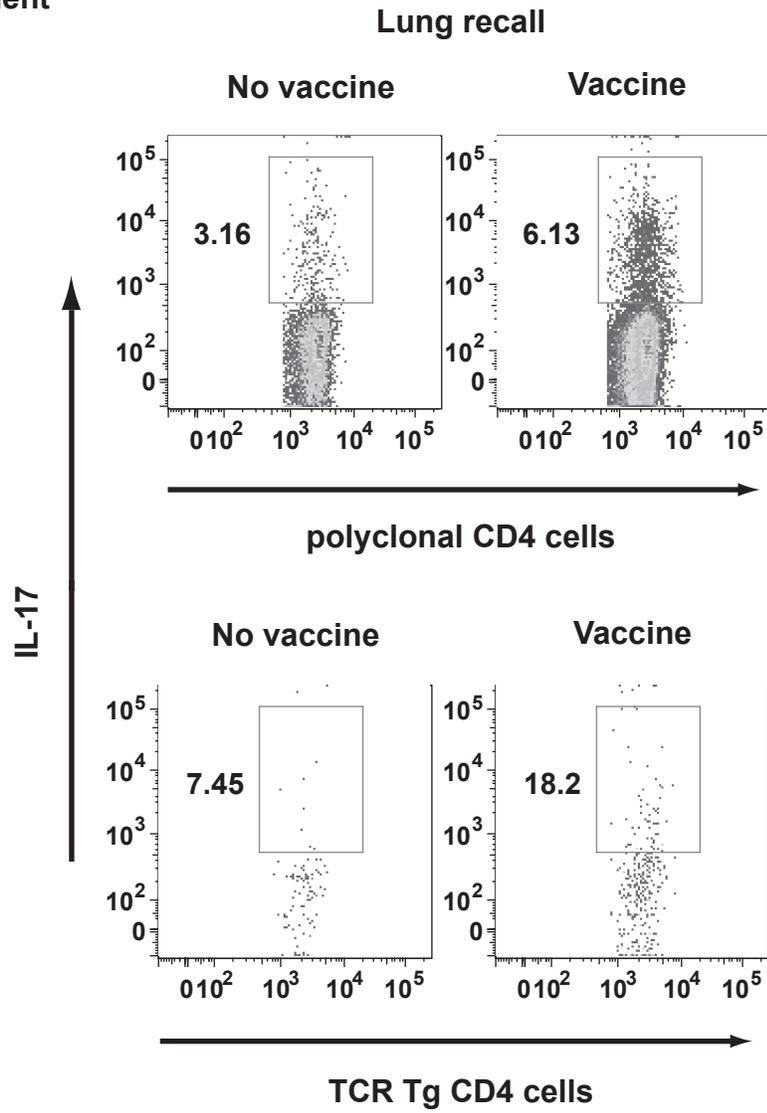
Fig. S6 (Supplement to Fig. 6). Role of phagocytes in vaccine-induced Th17 immunity to fungi. (A) At day 4 post-infection, CFUs from lung tissue were enumerated from vaccinated and unvaccinated wild-type and IL-17RA^{-/-} mice. The data represent averages \pm SEM of 8-10 mice per group. *, $p < 0.05$ vs. vaccinated wild-type mice. The numbers shown represent fold increase in IL-17RA^{-/-} vs. wild-type mice. (B) The influx of CD4⁺ T-cells into the alveolar space and lung tissue is comparable between IL-17RA^{-/-} and wild-type mice. *, $p < 0.001$ vs. numbers of CD4 cells in unvaccinated control mice.

Fig. S7 (Supplement to Fig. 7). Vaccine induced resistance and Th17 priming is independent of IL-18R but not IL-1R signaling. IL-1R^{-/-}, IL-18R^{-/-} and wild-type mice received 10^6 1807 cells

i.v. and were vaccinated with 10^6 heat-killed attenuated yeast of *B. dermatitidis*. **(A)** The number of IL-17 producing 1807 and endogenous, polyclonal CD4⁺ cells in the lung was determined by intracellular cytokine staining and FACS at day 4 post-infection in unvaccinated and vaccinated IL-1R^{-/-}, IL-18R^{-/-} and wild-type mice. *, $p < 0.001$ vs. 1807 and endogenous CD4⁺ cells in unvaccinated controls. **(B)** Lung CFU was measured at day 4 post-infection. Mean \pm SEM (n = 4); representative of 2 experiments. *, $p < 0.05$ vs. unvaccinated mice.

Figure S1. Supplement

S1A



S1B

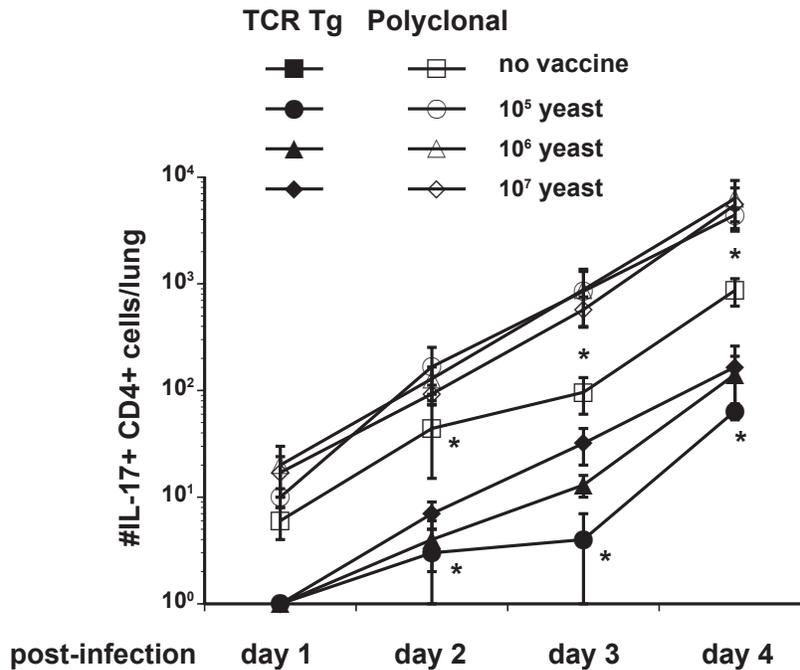
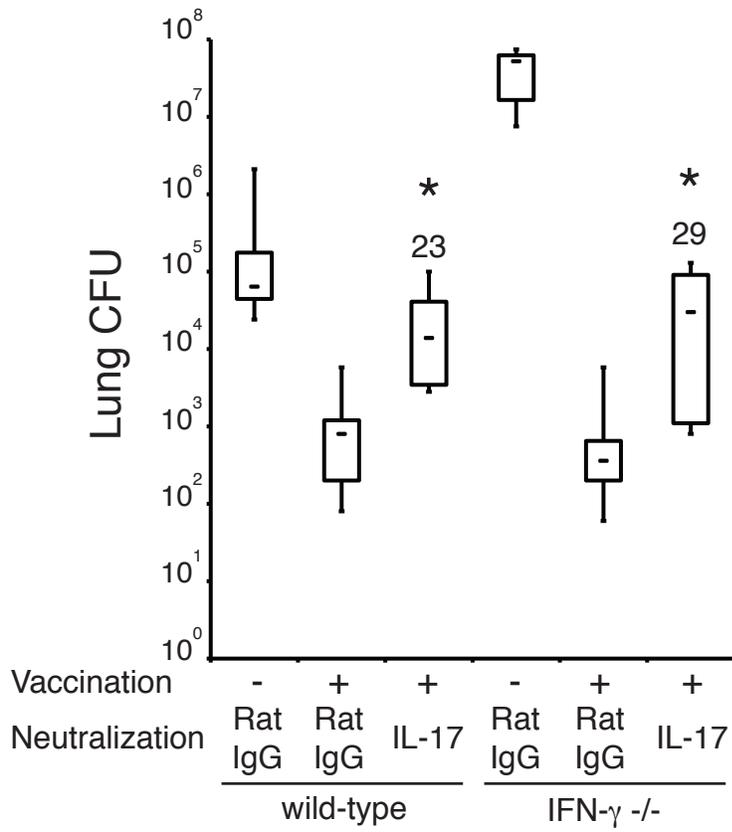


Figure S2. Supplement

S2A



S2B

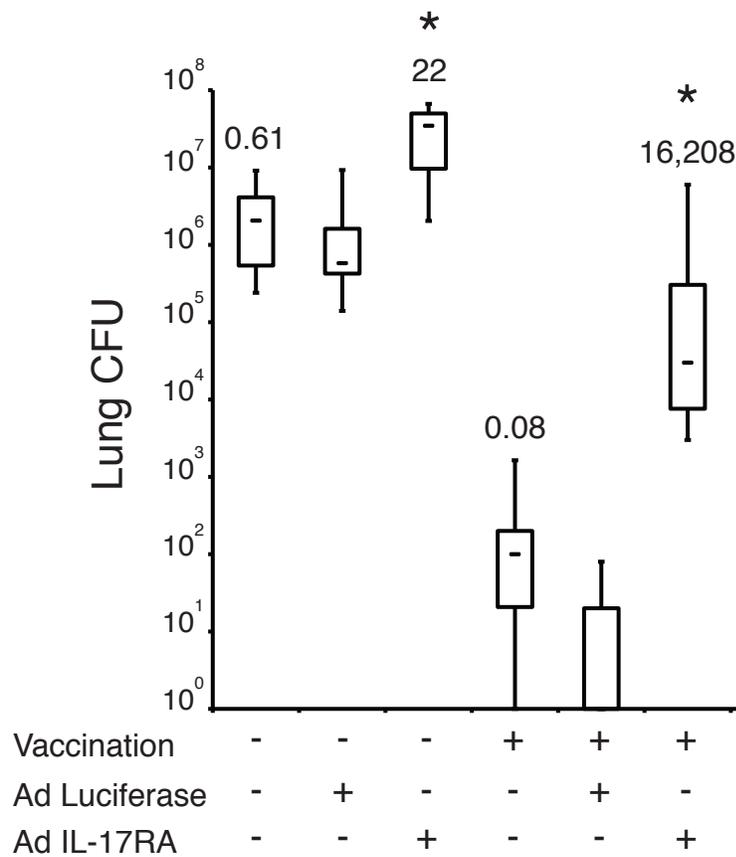


Figure S3. Supplement

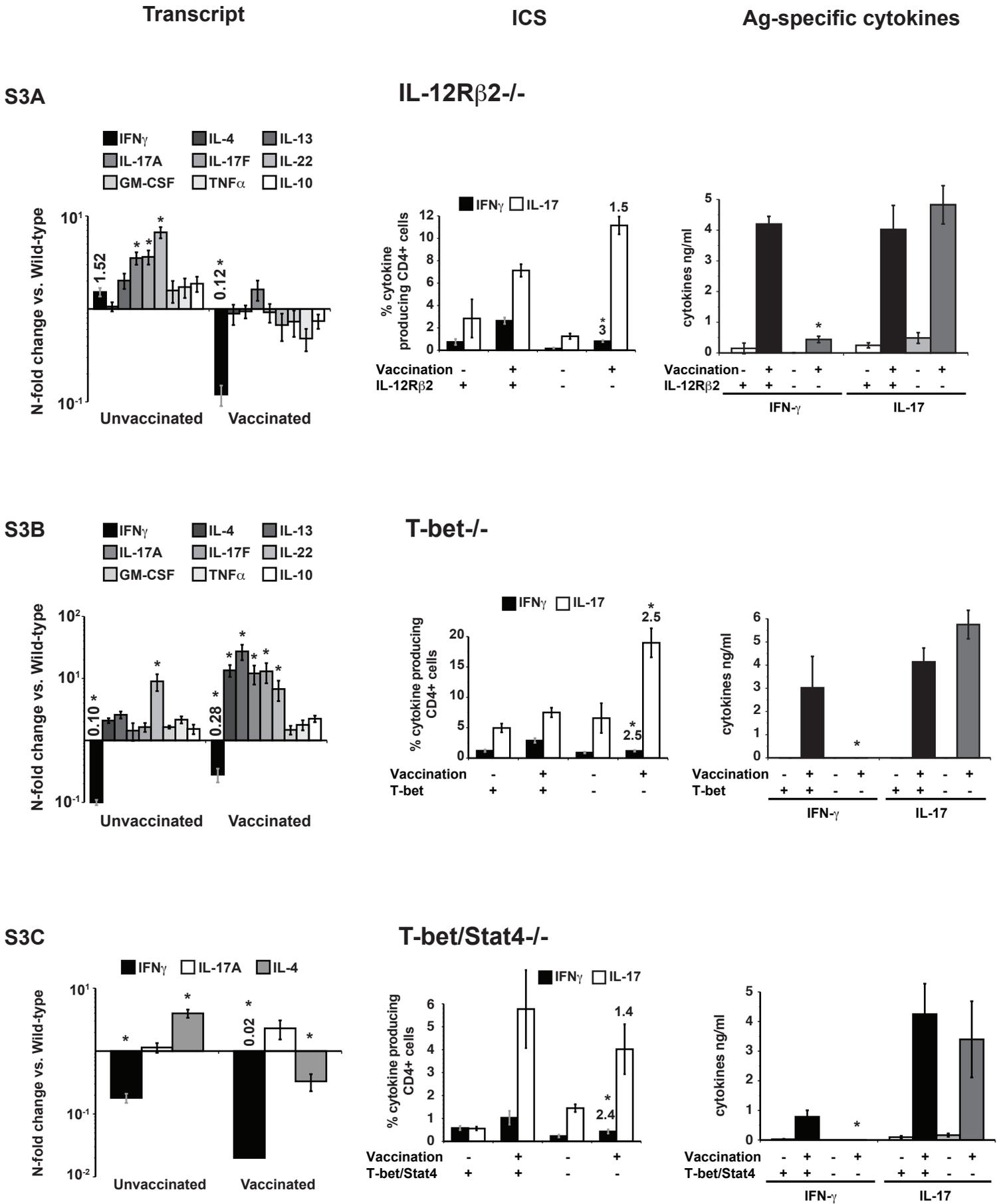
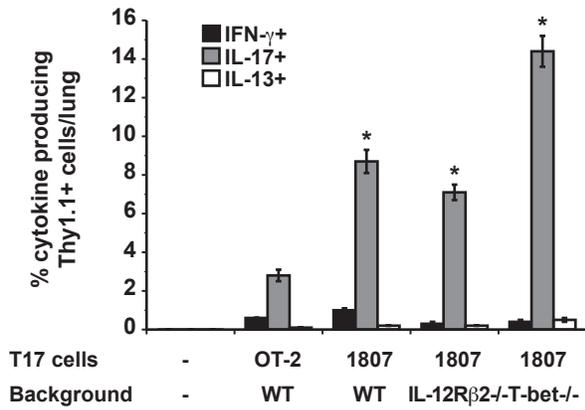


Figure S4. Supplement

S4A

Non-irradiated WT Recipients

LUNG RECALL



S4B

Irradiated WT Recipients

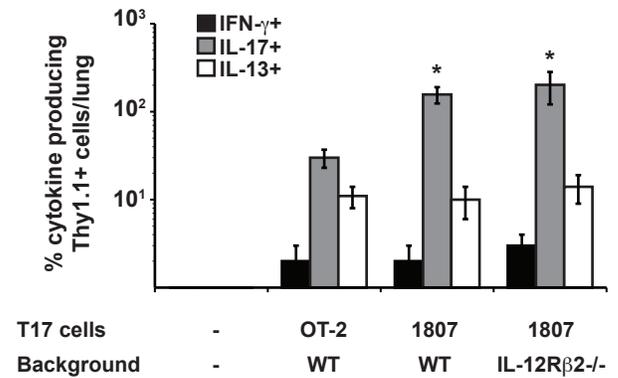
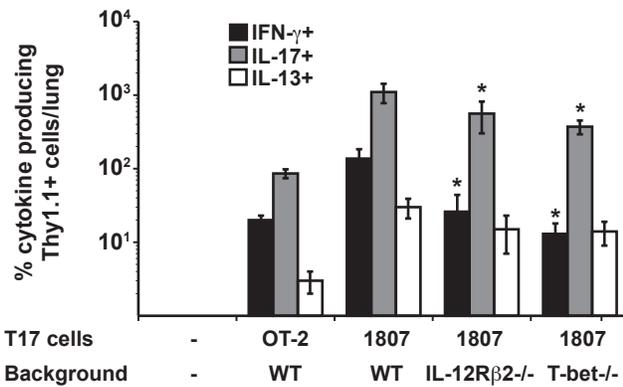
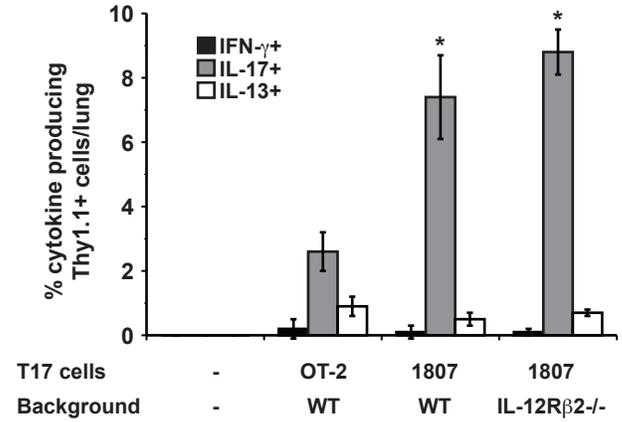
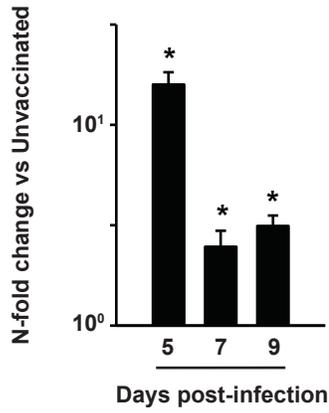


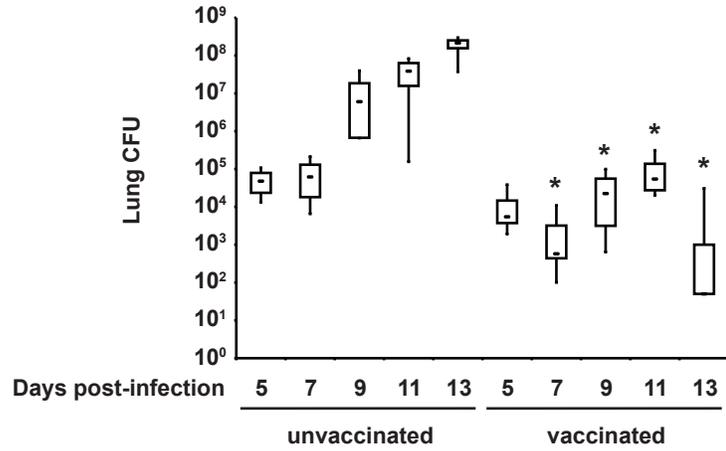
Figure S5. Supplement

Coccidioidomycosis

S5A Lung transcript



S5B Kinetics of lung CFU



Histoplasmosis

S5C Histo Day 4 post-infection

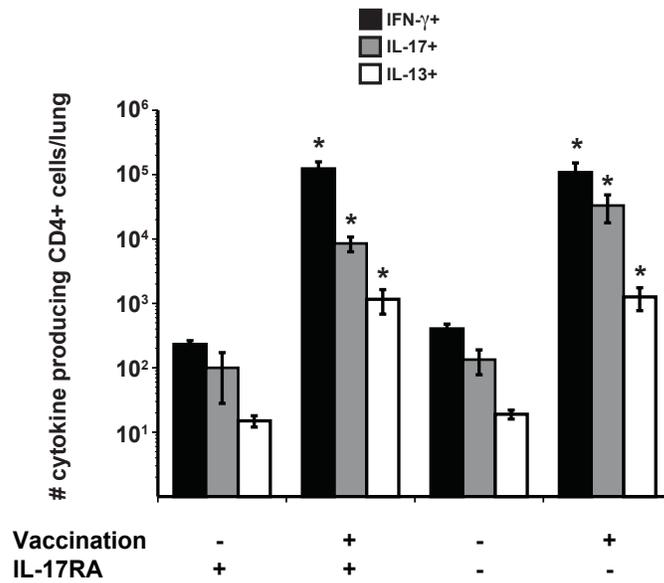
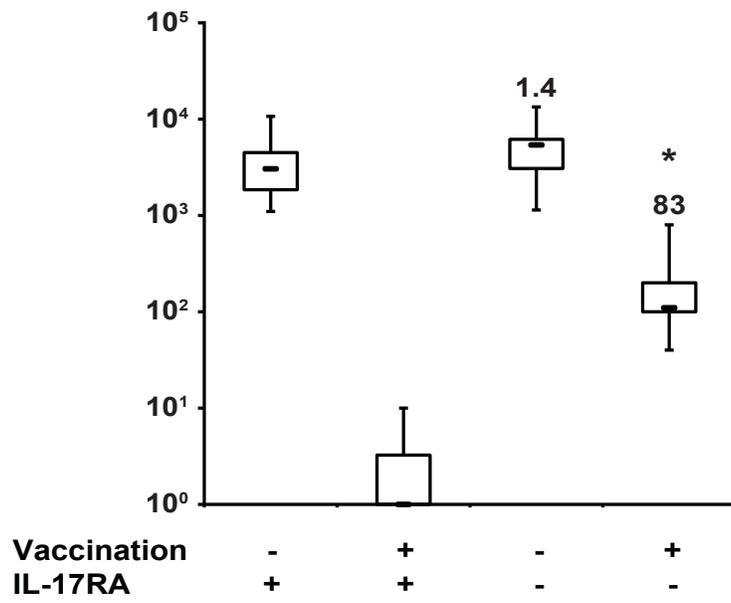


Figure S6. Supplement

S6A



S6B

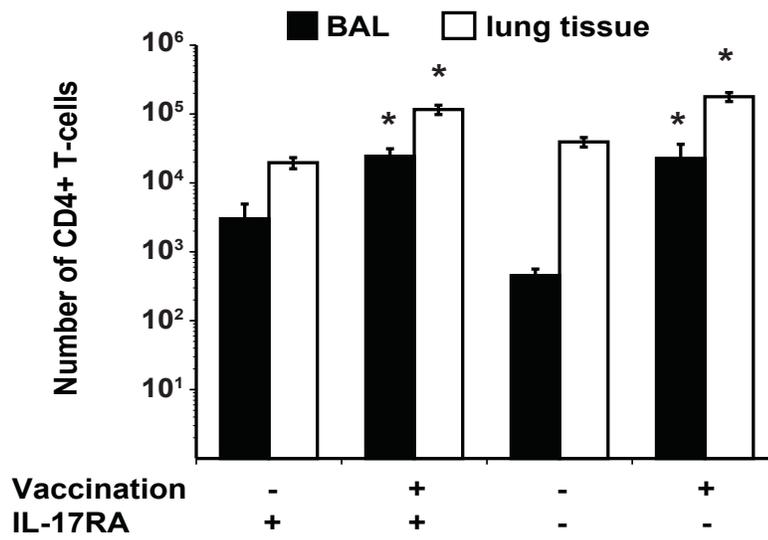


Figure S7 Supplement

