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 10^5 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 10^5 Tg cells and were vaccinated with 10^5 to 10^7 live attenuated yeast, challenged with 2 x 10^3 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 10^5, 10^6 and 10^7 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-$\gamma^{-/-}$ 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 ± 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 ± 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-12Rβ2$^{-/-}$ (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 ± 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 ± 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 *Coccidiodes* 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 *Coccidiodes* infection. Mean ± 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.

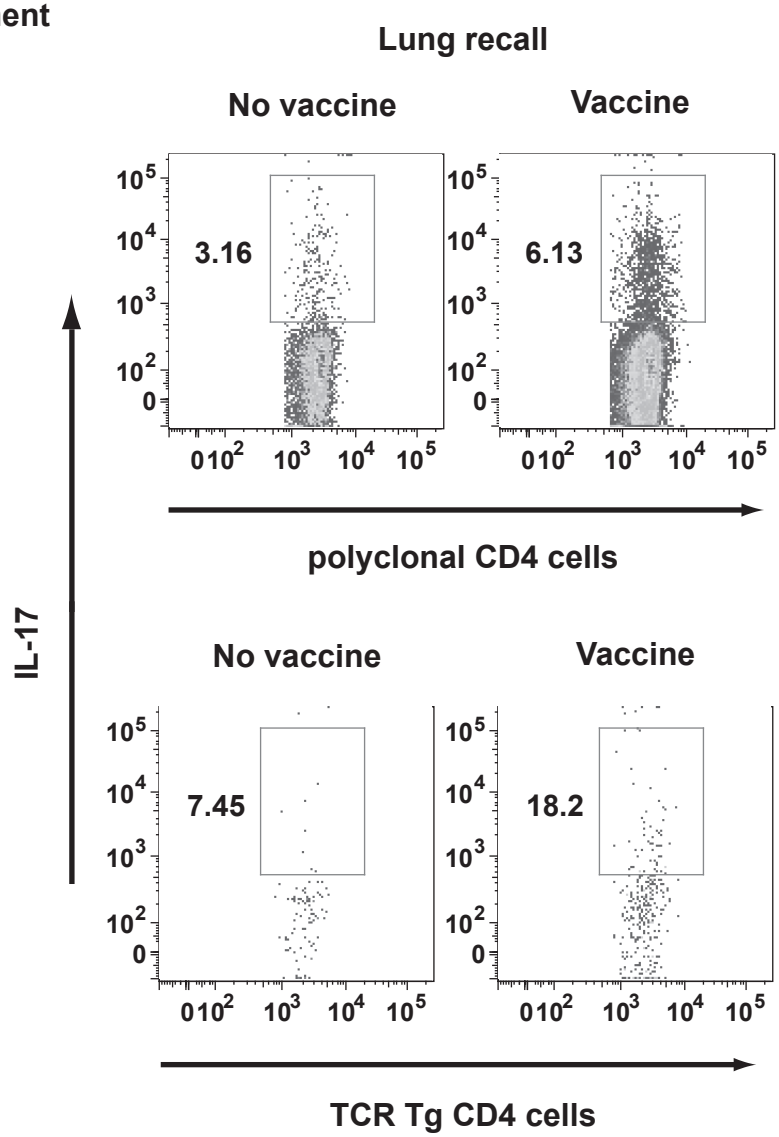
Fig. S6 (Supplement to Fig. 6). Role of phagocytes in vaccine-induced Th17 immunity to fungi. **(A)** At day 4 post-infection, CFUs from lung tissue were enumerated from vaccinated and unvaccinated wild-type and IL-$17RA^{-/-}$ mice. The data represent averages ± SEM of 8-10 mice per group. *, $p < 0.05$ 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-1R and IL-18R 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 ± SEM (n = 4); representative of 2 experiments. *, $p < 0.05$ vs. unvaccinated mice.

Figure S1. Supplement

S1A



S1B

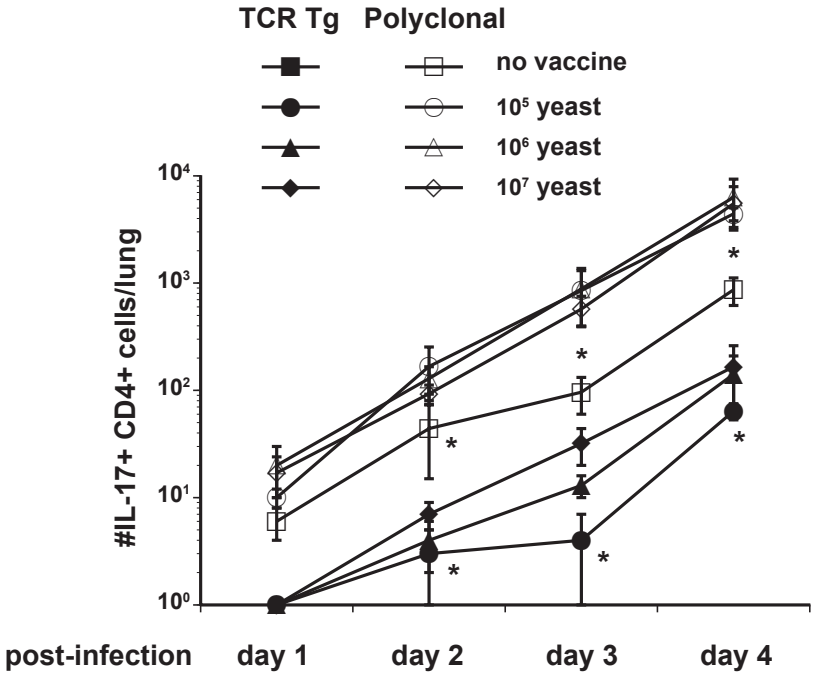
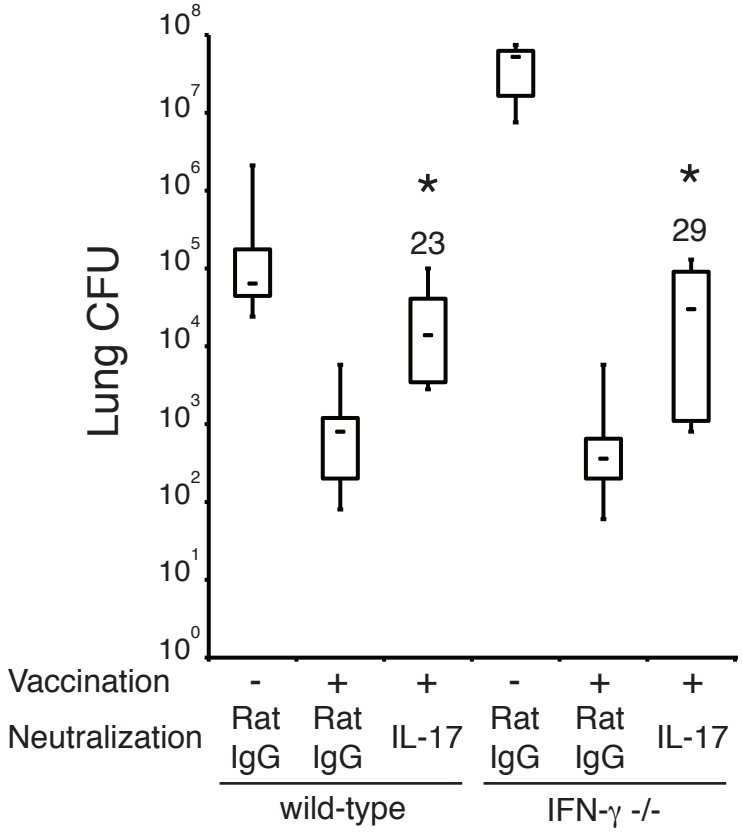
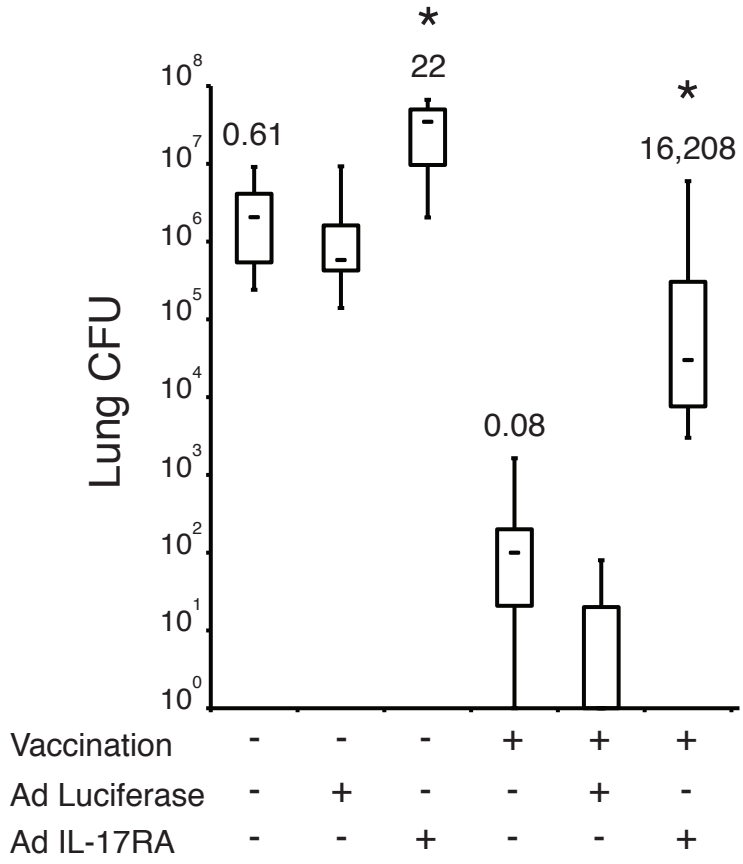


Figure S2. Supplement

S2A



S2B



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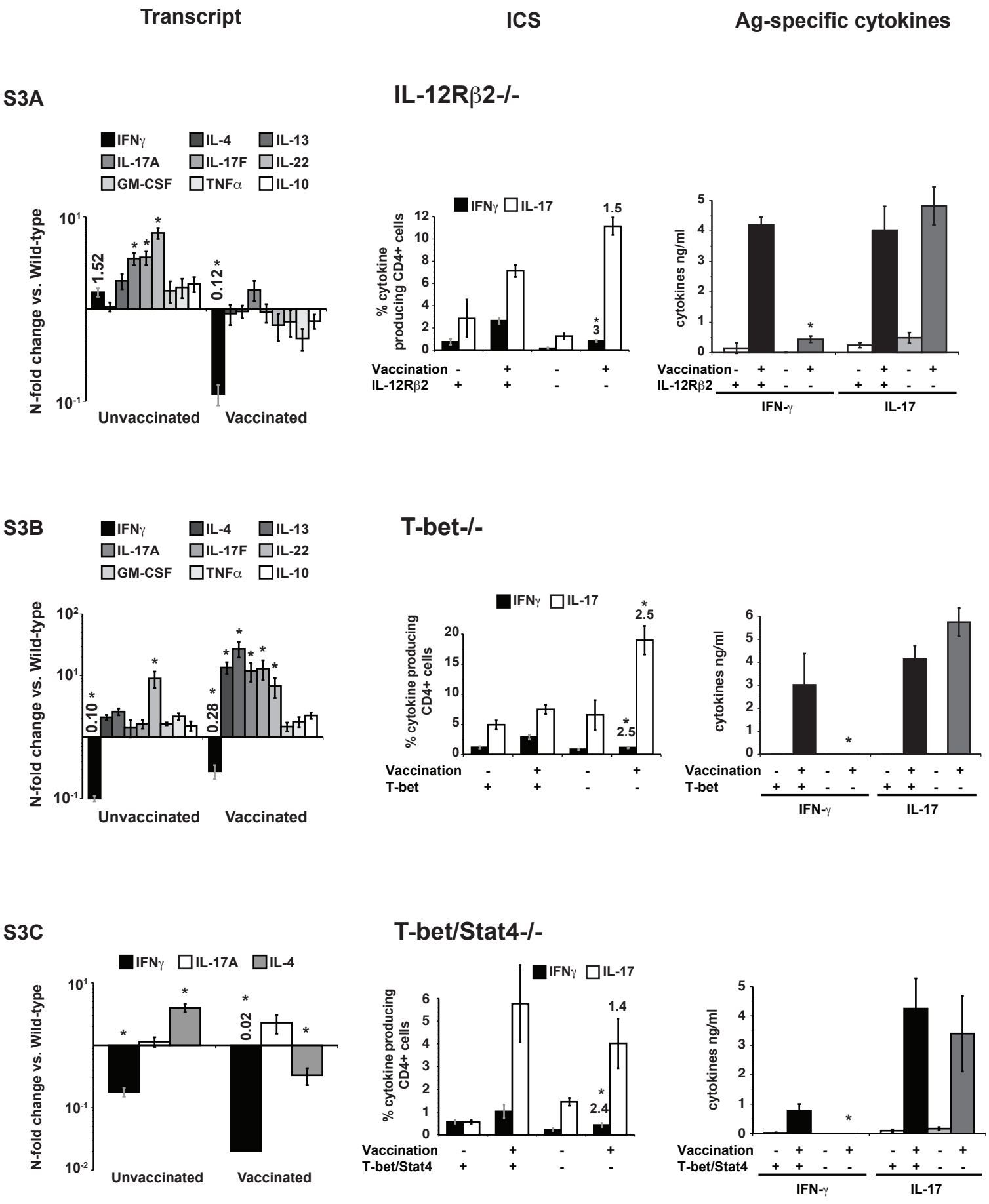

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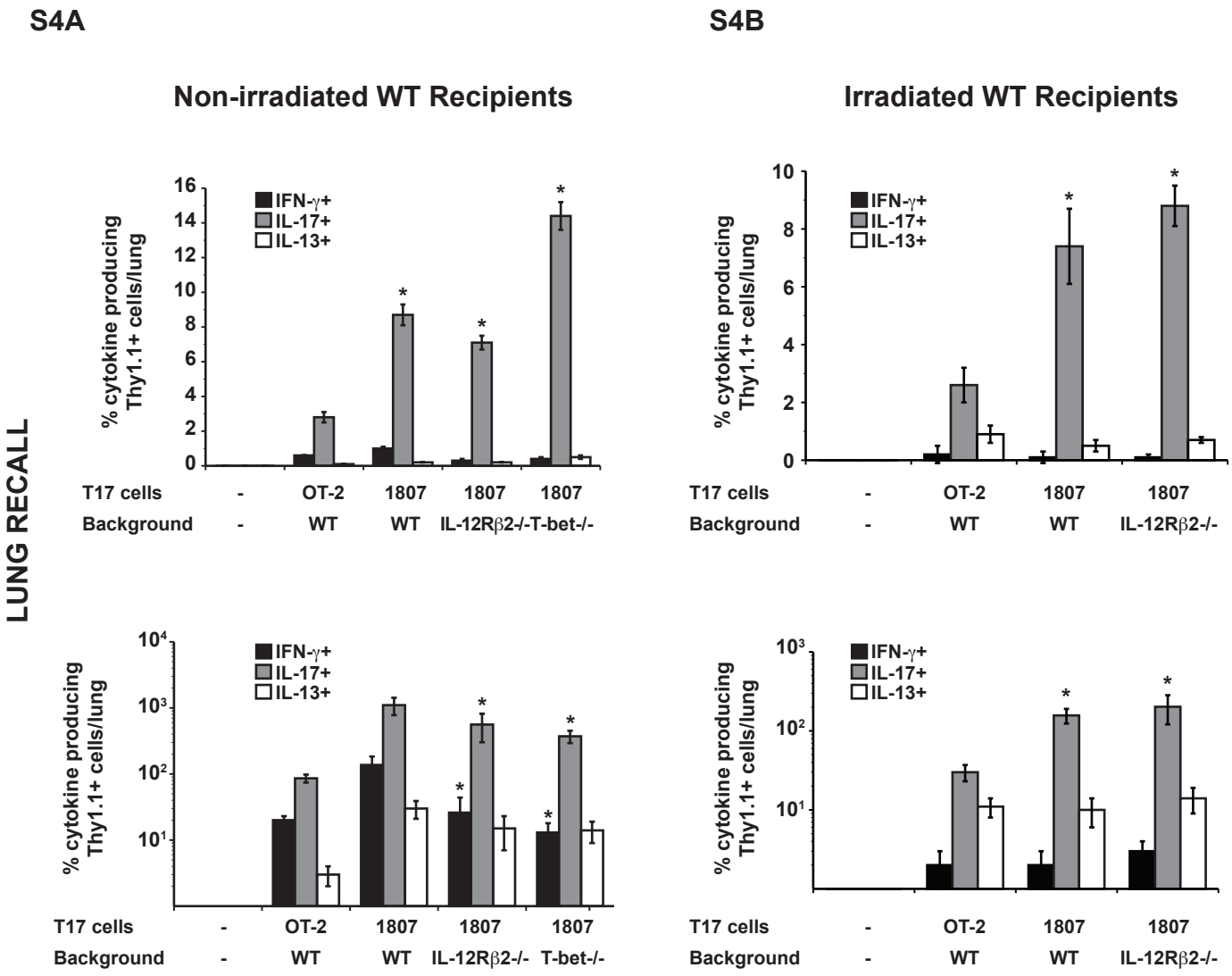
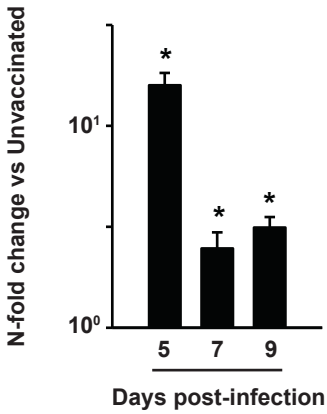


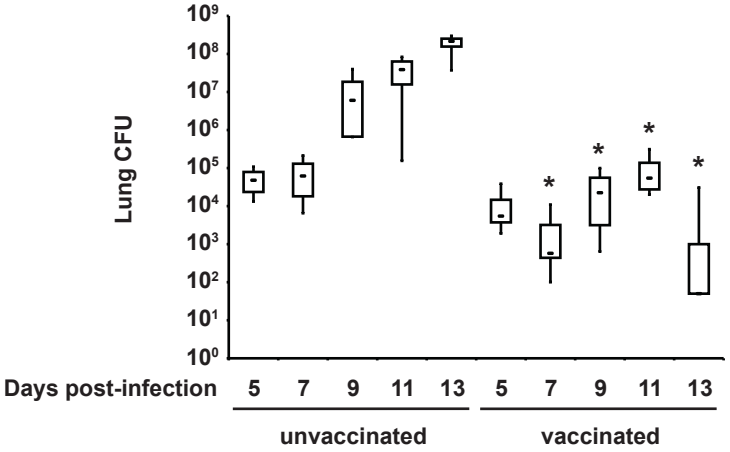
Figure S5. Supplement

Coccidioidomycosis

S5A Lung transcript



S5B Kinetics of lung CFU



Histoplasmosis

S5C Histo Day 4 post-infection

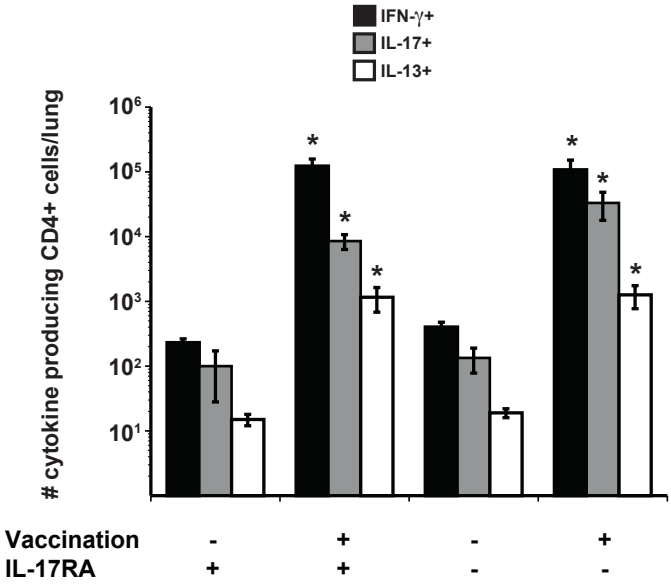
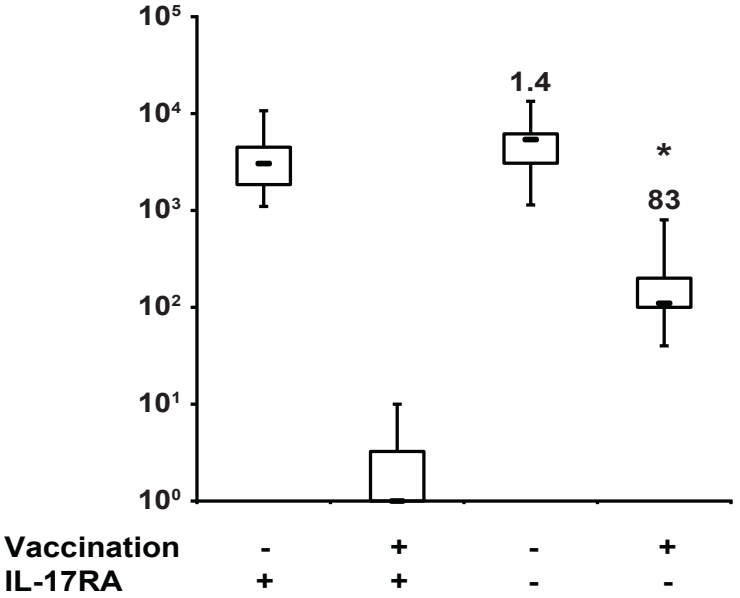


Figure S6. Supplement

S6A



S6B

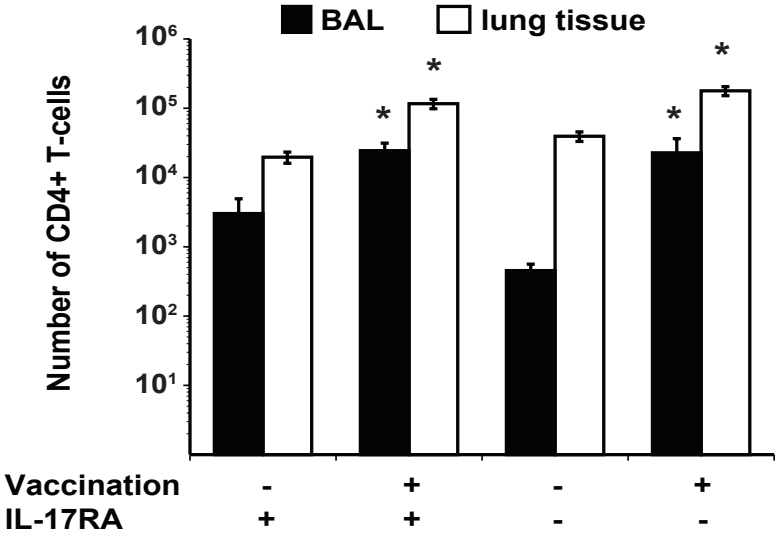
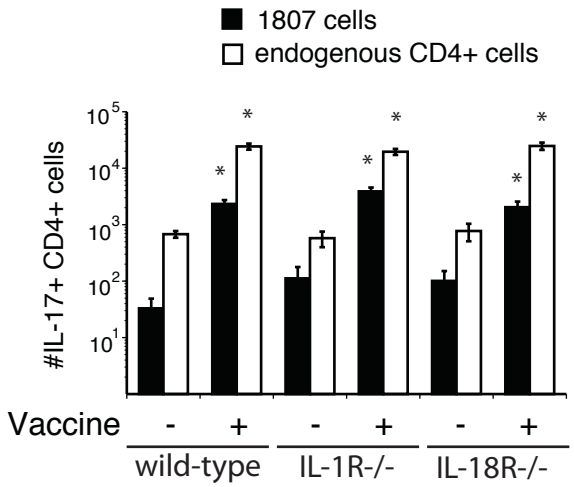


Figure S7 Supplement

S7A



S7B