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-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 ± 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 *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 ± 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-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 10⁶ 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.
S1A

Lung recall

No vaccine

Vaccine

polyclonal CD4 cells

No vaccine

Vaccine

TCR Tg CD4 cells

S1B

TCR Tg  Polyclonal

no vaccine

10⁵ yeast

10⁴ yeast

10³ yeast

#IL-17+ CD4+ cells/lung

post-infection  day 1  day 2  day 3  day 4
Figure S3. Supplement

**Transcript**

**IL-12Rβ2-/-**

**Ag-specific cytokines**

**S3A**

**ICS**

**S3B**

**S3C**

**T-bet/-**

**T-bet/Stat4-/-**
Figure S4. Supplement

Non-irradiated WT Recipients

Irradiated WT Recipients

LUNG RECALL

S4A

S4B
Coccidioidomycosis

Figure S5. Supplement

S5A Lung transcript

S5B Kinetics of lung CFU

S5C Histo Day 4 post-infection

Histoplasmosis
Figure S7 Supplement

S7A

**#IL-17+ CD4+ cells**

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![Graph showing #IL-17+ CD4+ cells](image)

1807 cells

endogenous CD4+ cells

S7B

**Lung CFU**

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![Graph showing Lung CFU](image)

1807 cells

endogenous CD4+ cells

*Significant difference