

Figure S1. Inhibition of Notch signaling does not significantly alter the percentage of T cells in the lungs during cryptococcal infection. (A) Schematic of gating strategy used to identify  $Tcr\beta^+ CD4^+$  and  $CD8^+ T$  cells in the perfused lungs of *C. neoformans* infected mice. (B) Percentage (of  $CD45^+$ ) of all  $Tcr\beta^+$ ,  $Tcr\beta^+ CD4^+$  and  $Tcr\beta^+ CD8^+ T$  cells in the lungs of infected mice at 4 weeks post infection. Data shown are the mean frequencies  $\pm$  SEM from 1 of 3 independent experiments with n=5-6/group and were not significantly different.



Figure S2. Inhibition of T cell restricted Notch signaling reduces spontaneous Th1 and Th2 cytokine production in the lungs of mice with cryptococcal infection in the absence of exogenous restimulation. (A-C) Leukocytes were isolated from perfused naïve and infected lungs at 4 wpi  $(5x10^{6}/ml)$  and cultured for 24 hours. Cytokine levels in supernatants were measured by ELISA and cytometric bead assays. Data are the mean ± SEM with n=4-24 mice per group. \*p<0.05 (D-G) Inhibition of T cell restricted notch signaling does not significantly reduce IL-4 and IL-10 production in the lungs of mice with cryptococcal infection, even following re-stimulation. Leukocytes were isolated from perfused naïve and infected lungs (D-E) and spleens (F-G) at 4 wpi  $(5x10^{6}/ml)$  and stimulated with heat-killed *C*. *neoformans* (10x10<sup>6</sup>/ml) at a 2:1 ratio for 24 or 48 hours, respectively. Cytokine levels in supernatants were measured by ELISA and cytometric bead assays. Data are the mean ± SEM with n=3-7 mice per group.



Figure S3. Cytokine production by T cells from the lungs of naïve mice. Lung leukocytes were isolated from perfused naïve CCD and WT mice then stimulated with plate bound anti-CD3 and anti-CD28 antibodies and analyzed for the proportion of cells producing (A) IFN- $\gamma$ , (B) IL-5, (C) IL-13 and (D) IL-17A by flow cytometry, as described in Figure 5. Representative plots shown are gated on Live, CD45<sup>+</sup>, TCR $\beta^+$  CD4<sup>+</sup> T cells and FMO controls were used to set cytokine gates. The percentages of cells in the positive gate are indicated.

Gene Name	Primer Sequences
Arg1	5'-CTAAGGACAGGCCAACAGAA-3' and 5'-CAAACTTCCATCCTCCTCCAATG-3'
Fizz1	5'-TTCTTGCCAATCCAGCTAAC-3' and 5'-GGGTTCTCCACCTCTTCATT-3'
<i>Foxp3</i>	5'- CACCCAGGAAAGACAGCAACC-3' and 5'-GCAAGAGCTCTTGTCCATTGA -3'
Gapdh	5'-TATGTCGTGGAGTCTATTGGT-3' and 5'-GAGTTGTCATATTTCTCGTGG-3'
Gata3	5'-AGAACCGGCCCCTTATCAA-3' and 5'-AGTTCGCGCAGGATGTCC-3'
Nos2 (iNos)	5'-GGCAGCCTGTGAGACCTTTG-3' and 5'-GCATTGGAAGTGAAGCGTTTC-3'
Rorc	5'-TGTGGTTGTTGGCATTGTAG-3' and 5'-CCAGCTACCAGAGGAAGTCA-3'
Tbx21 (Tbet)	5'-CAACAACCCCTTTGCCAAAG-3' and 5'-TCCCCCAAGCAGTTGACAGT-3'
Chil4 (Ym2)	5'-CAGAAGAATGGAAGAGTCAG-3' and 5'-CAGATATGCAGGGAGTCACC-3'

Supplementary Table 1. List of primers used to quantify gene expression by qRT-PCR.