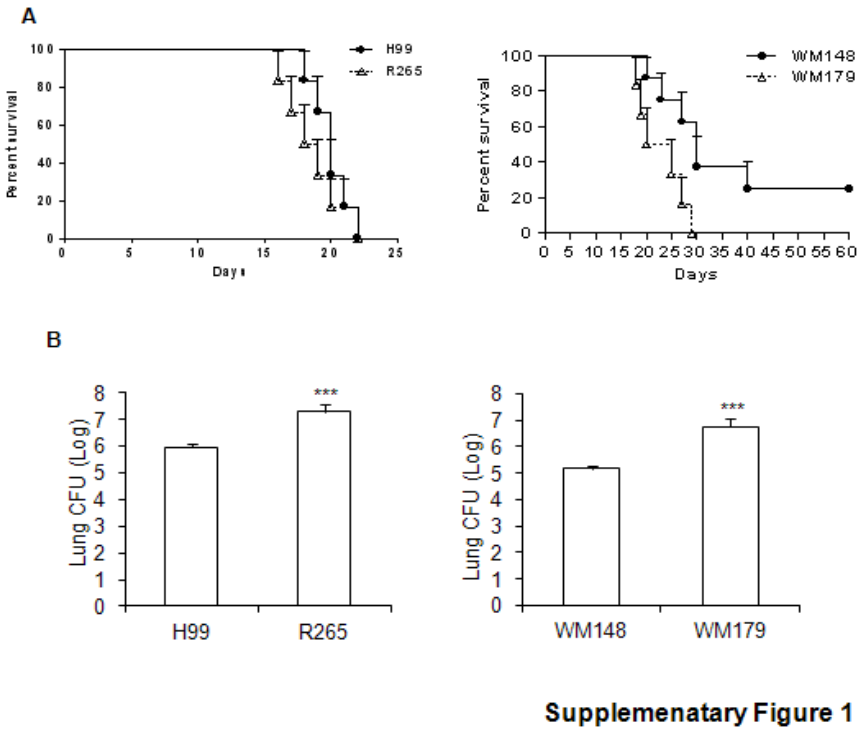
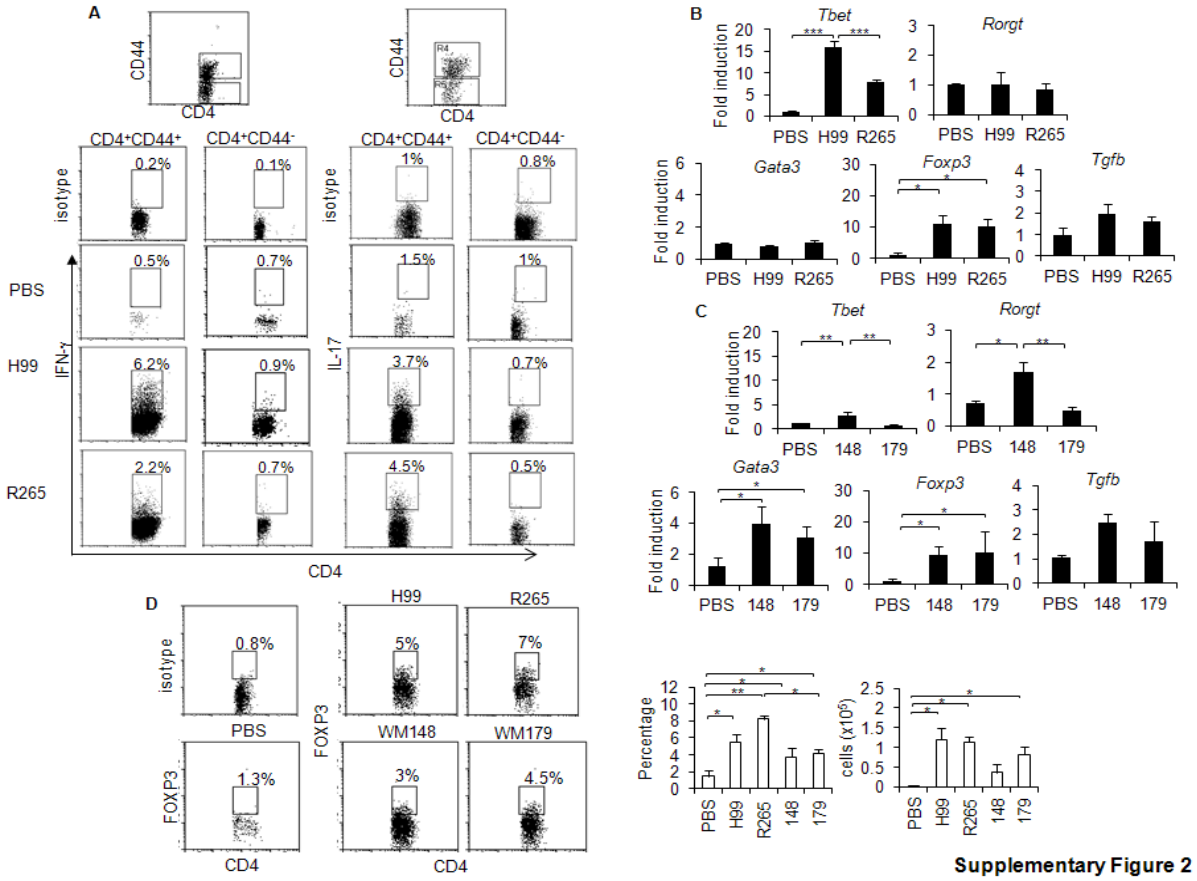


Supplemenatary Figure 1

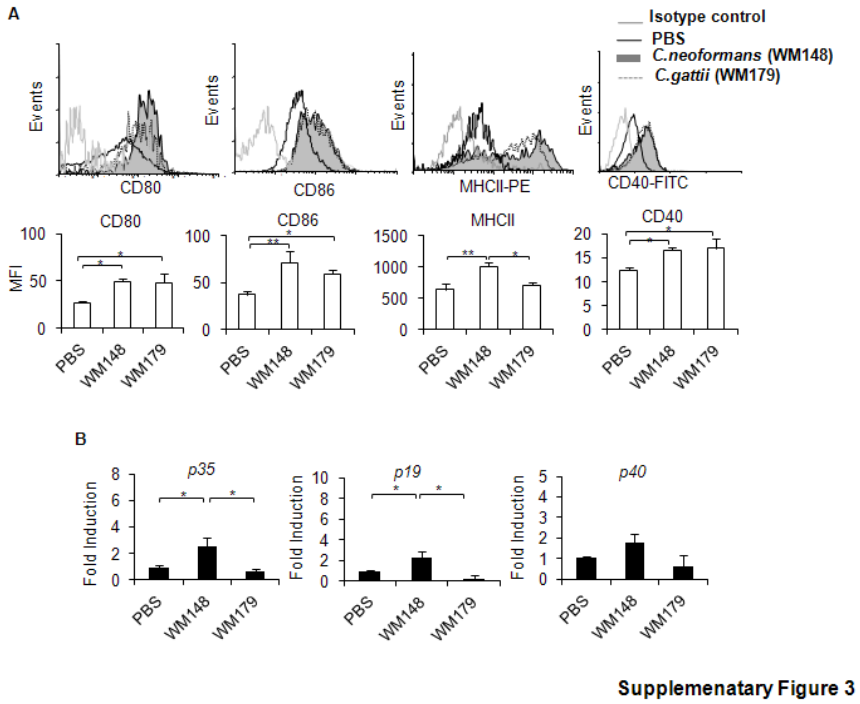
Supplementary Figure 1. Virulence of *C. neoformans* (H99, WM148) and *C. gattii* (R265, WM179)

C57Bl/6 mice were infected with the highly virulent strain of *C. neoformans* (H99) and *C. gattii* (R265); or with the less virulent strain of *C. neoformans* (WM148) and *C. gattii* (WM179). **(A)** Mice were monitored for their survival by inspection twice daily for 60 days and euthanized if they appeared to be in pain or moribund. Survival curves were generated from the results obtained with 10 mice per group and evaluated for statistical significance with Kaplan-Meier survival curves, and *P* values were obtained from a log-rank test. *C. gattii* strain WM179 was significantly more virulent than *C. neoformans* strain WM148 ($p<0.05$). (**B)** Pulmonary fungal burdens were assessed at 7 days post infection and evaluated for statistical significance with an unpaired two-tailed t test. Graphs depict mean ± SD and are representative of three experiments with five mice per group. *** $p < 0.001$.



Supplementary Figure 2. Characterization of effector Th1/Th17 cells, pulmonary T helper cell-specific transcription factors and FOXP3$^+$ regulatory T cell during *Cryptococcus* infection

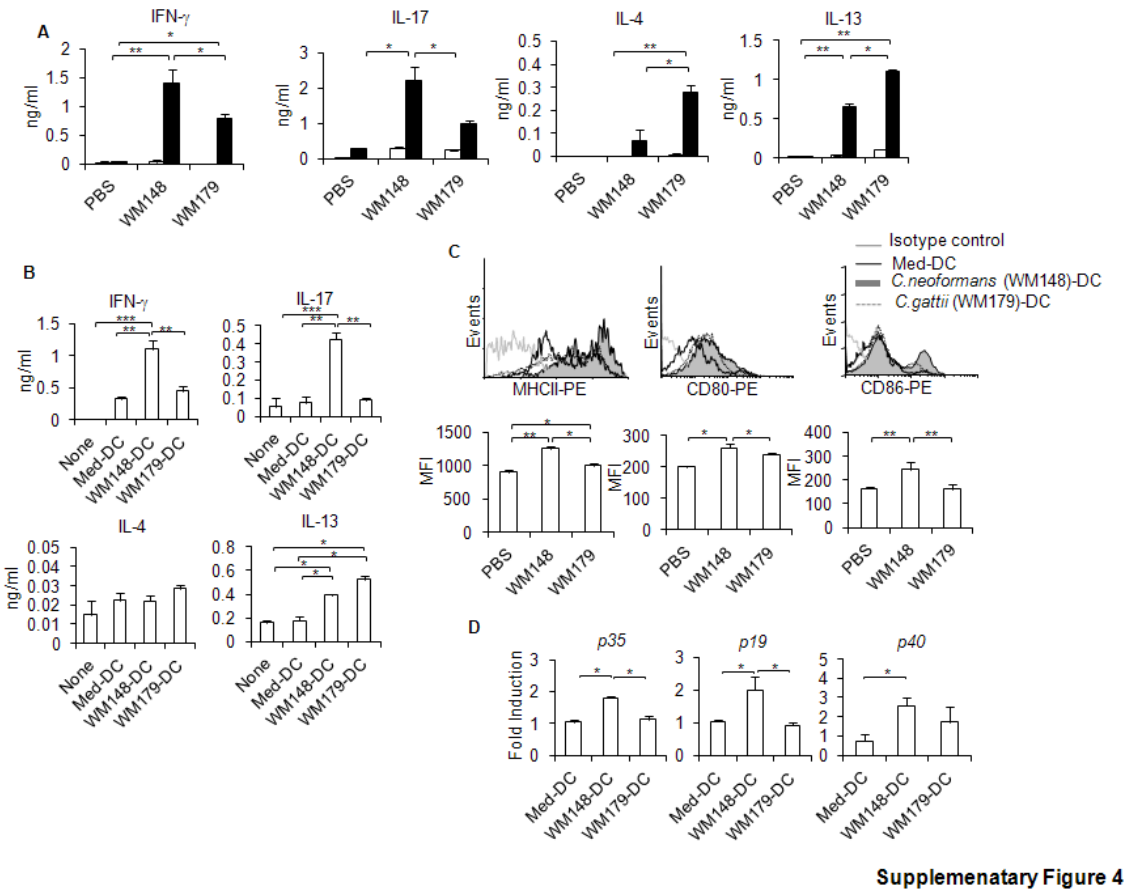
(A) C57Bl/6 mice were treated with PBS as control or infected with highly virulent strains of *C. neoformans* (H99) or *C. gattii* (R265)**.** Lungs were harvested, and analyzed for intracellular cytokine staining of IFN-γ, IL-17, and isotype matched antibody on gated CD4$^+$CD44$^+$ or CD4$^+$CD44$^-$ population. **(B-D)** C57Bl/6 mice were treated with PBS for control or infected with the highly virulent strains of *C. neoformans* (H99) or *C. gattii* (R265); or less virulent strain of *C. neoformans* (WM148) or *C. gattii* (WM179)**.** Lungs were analyzed for expression of transcription factors or the cytokine Tgfb, as specified **(B-C)** or intracellular cytokine staining of Foxp3 and isotype matched antibody on CD4$^+$CD25$^+$ cells **(D)**. Graphs depict mean ± SD and are representative of three experiments with three to four mice per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplemenatary Figure 3

Supplementary Figure 3. *C. gattii* strain WM179 infection attenuates the expression of MHC-II on lung DCs and pulmonary IL-12/IL-23 mRNA

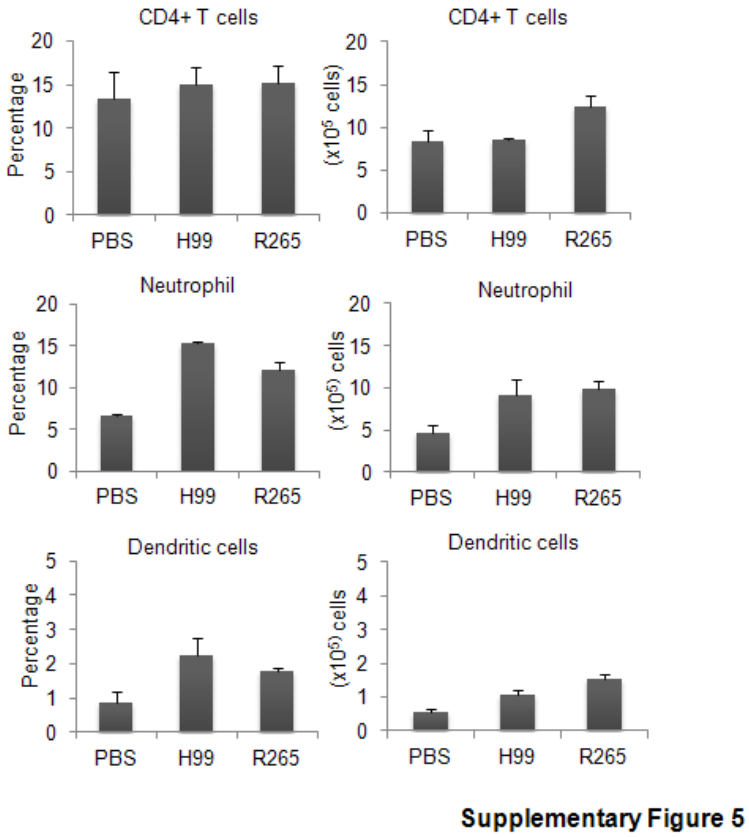
C57Bl/6 mice were treated with PBS as uninfected control or infected with less virulent strain of *C. neoformans* (WM148) and *C. gattii* (WM179). Lungs were harvested from uninfected or infected mice and analyzed for the expression of CD80, CD86, MHC-II and CD40 in gated $CD11b^{hi}CD11c^{hi}$ cells by flow cytometry **(A)** or for gene expression of IL-12 (p35/p40) and IL-23 (p19/p40) by quantitative real-time PCR **(B)**. **(A)** Flow cytometric data shown are representative histogram profiles from three independent experiments. Solid gray lines indicate the plot profiles of an isotype-matched control antibody, solid black lines show profiles of PBS-treated mice, shaded histograms represent profiles of *C. neoformans* strain WM148, and dashed lines indicate profiles of *C. gattii* strain WM179. **(B)** Total RNA of lungs was isolated and subjected to cDNA synthesis and subsequent real-time PCR analysis of cytokine IL-12p35, IL-23p19 and common subunit p40 expression. Data are expressed as fold induction over actin (*Actb*) expression, with the mRNA levels in the PBS-treated group set as 1. Graphs depict mean±SD and are a representative of at least three independent experiments with three to four mice per group. * $p < 0.05$, ** $p < 0.01$.



Supplementary Figure 4. ***C. gattii*** **strain WM179 inefficiently elicits antigen-specific Th1/Th17 cytokine production**

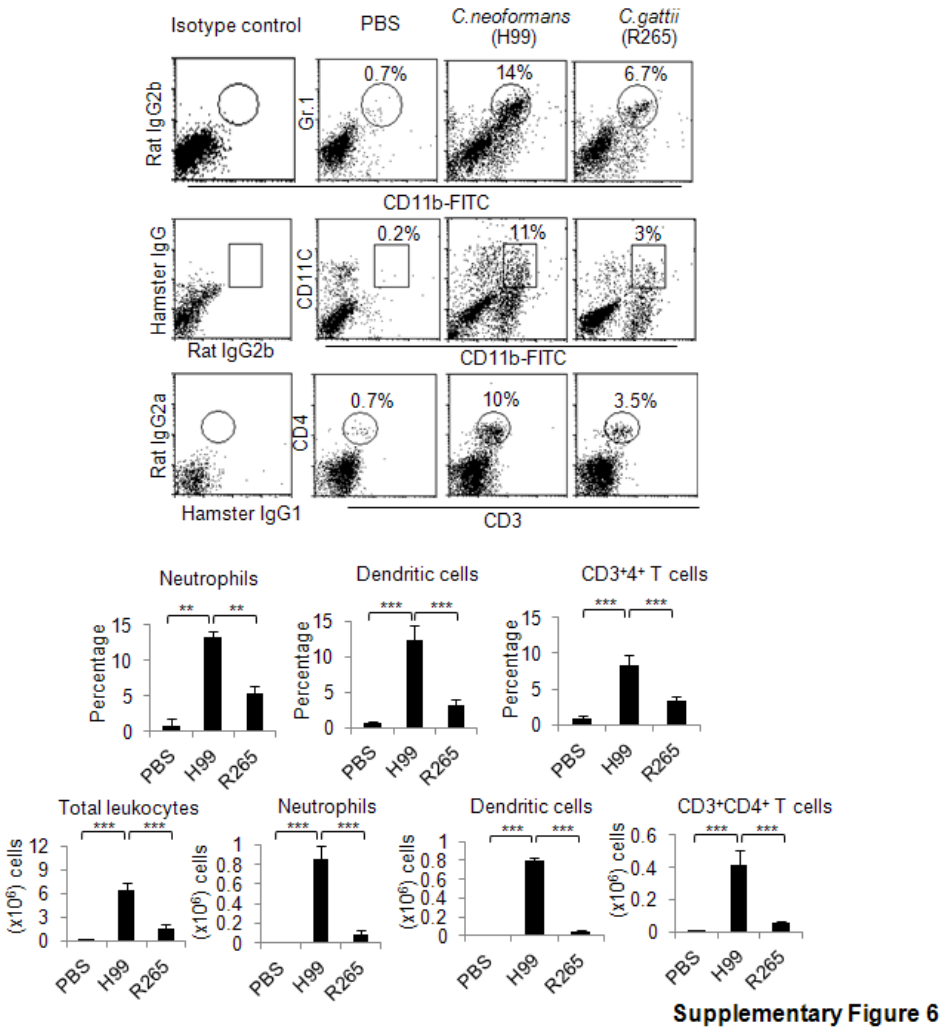
(A) C57Bl/6 mice were treated with PBS as uninfected control or infected with the less virulent strains of *C. neoformans* (WM148) or *C. gattii* (WM179). Lung-draining lymph nodes were harvested and cultured with heat-killed *Cryptococcus* cells. After for the 72 hour restimulation, supernatants were collected and analyzed for antigen-specific cytokine production by ELISA. **(B-D)** Purified bone marrow-derived dendritic cells (BM-DCs) were stimulated with heat-killed Cryptococcus (WM148, WM179). **(B)** After 24 hours, medium-treated DCs (Med-DCs) or activated BM-DCs (WM148-DC, WM179-DC) were washed and co-cultured with purified $CD4^{+}$T cells from naïve mice at the ratio of 1:2. On day 3, cultured supernatant was then collected and assayed for cytokine production by ELISA. **(C-D)** Medium-treated DCs (Med-DCs) or activated BMDCs (WM148-DC and WM179-DC) were analyzed for the expression of MHC-II, CD80, and

CD86 after 24 hours of activation by flow cytometry analysis **(C)** and cytokine gene expression by real-time PCR analysis **(D)**. **(C)** Flow cytometric data shown are a representative histogram profiles from three independent experiments. Solid gray lines indicate the plot profiles of isotype-matched control antibody, solid black lines show profiles of Medium-treated DC, shaded histograms represent profiles of *C. neoformans* strain WM148-treated DC, and dashed lines indicate profiles of *C. gattii* strain WM179-treated DC. **(D)** Total RNA was isolated and subjected to cDNA synthesis and subsequent real-time PCR analysis of cytokine IL-12p35, IL-23p19 and common subunit p40 expression. Data are expressed as fold induction over actin (*Actb*) expression, with the mRNA levels in the medium-treated DCs set as 1. Graphs depict mean ± SD and are a representative of at least three independent experiments with four mice per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 5. The effect of *C. gattii* infection on peripheral blood cells.

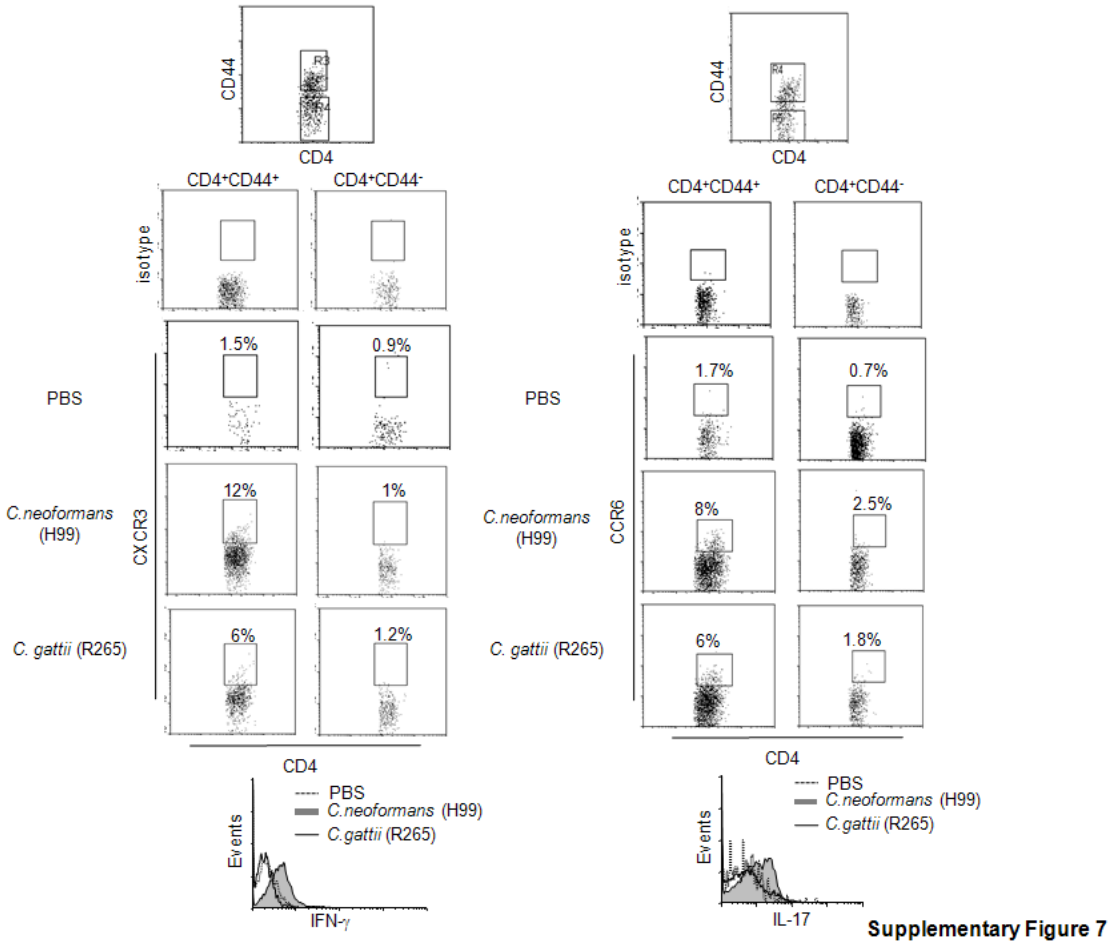
C57Bl/6 mice were treated with PBS as uninfected control or infected with the highly virulent *C. neoformans* (H99) or *C. gattii* (R265) for 7 days. Peripheral blood was collected from each animal by cardiopuncture and analyzed for the presence of neutrophils, dendritic cells, and $CD4^+$ T cells by flow cytometry. The results are presented as the percentage and absolute numbers of cells. Graphs depict mean ± SD and are representative of three experiments with four to five mice per group.



Supplementary Figure 6

Supplementary Figure 6. The effect of highly virulent *C. neoformans* and *C. gattii* infection on inflammatory cells and $CD3^+CD4^+$ cells in the lungs.

C57Bl/6 mice were treated with PBS as control or infected with highly virulent strains of *C. neoformans* (H99) or *C. gattii* (R265). At day 7 post-infection, whole lungs were harvested and analyzed for the percentage of neutrophils ($Gr.1^+CD11b^+$), dendritic cells ($CD11b^{hi}CD11c^{hi}$), and $CD3^+CD4^+$ T cells. For negative controls, lung cells were stained with isotype-matched nonspecific antibody for each population. Graphs depict mean ± SD and are representative of three experiments with three to four mice per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 7. The expression of CXCR3 and CCR6 on $CD4^+CD44^+$ T cells in lungs of mice infected with the highly virulent strains of *C. neoformans* and *C. gattii*.

C57Bl/6 mice were treated with PBS for control or infected with highly virulent strains of *C. neoformans* (H99) or *C. gattii* (R265)**.** Single cell suspensions were prepared from lungs and analyzed for the surface expression of CXCR3 and CCR6 on $CD4^+CD44^+$ or $CD4^+$ $CD44^-$ T cells, intracellular expression of the cytokines IFN-γ on gated $CXCR3^+CD4^+CD44^+$ or IL-17 on $CCR6^+CD4^+$ $CD44^+$ T cells by flow cytometric analysis. The results are presented as dot plots and the histogram plots of the cells.