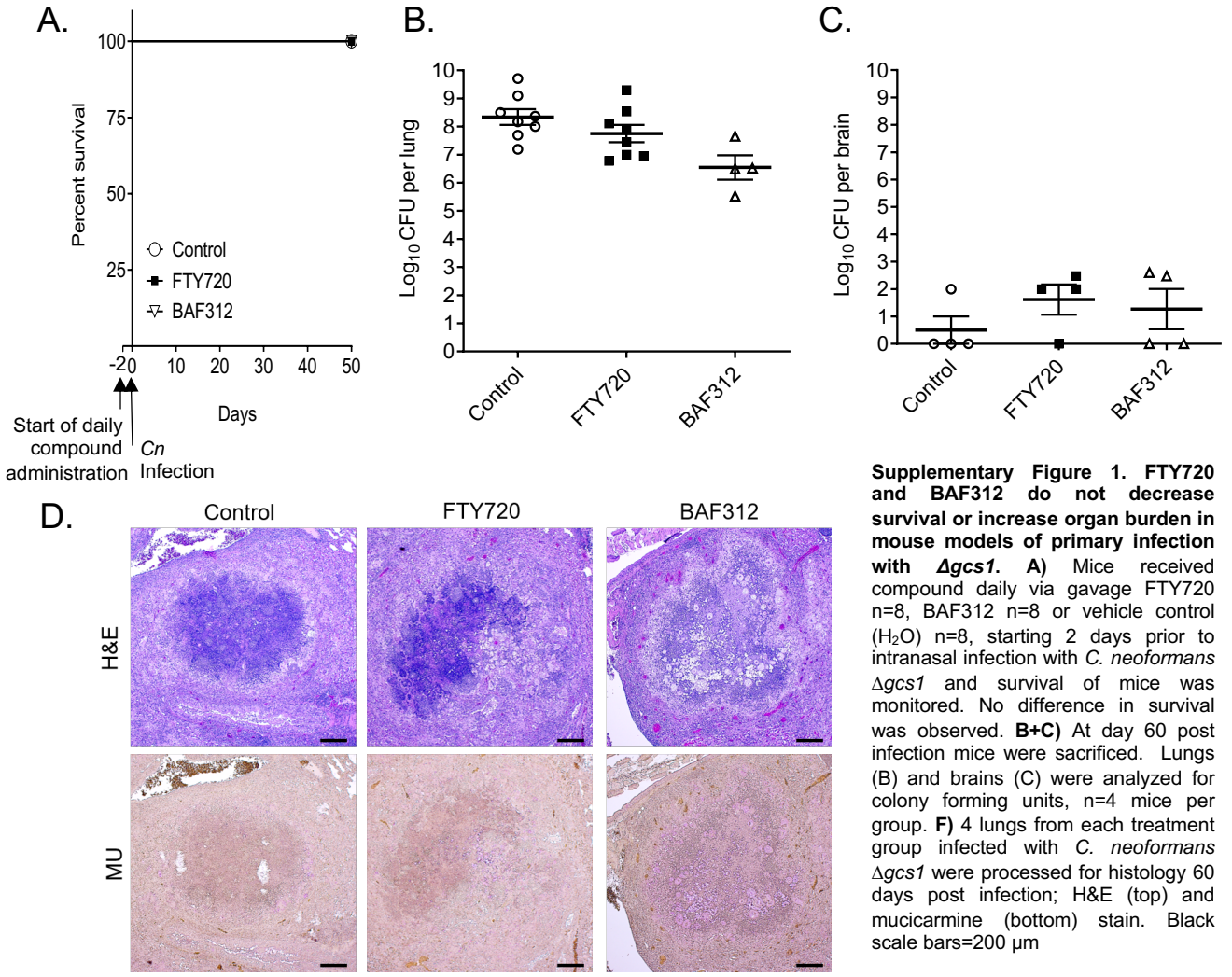
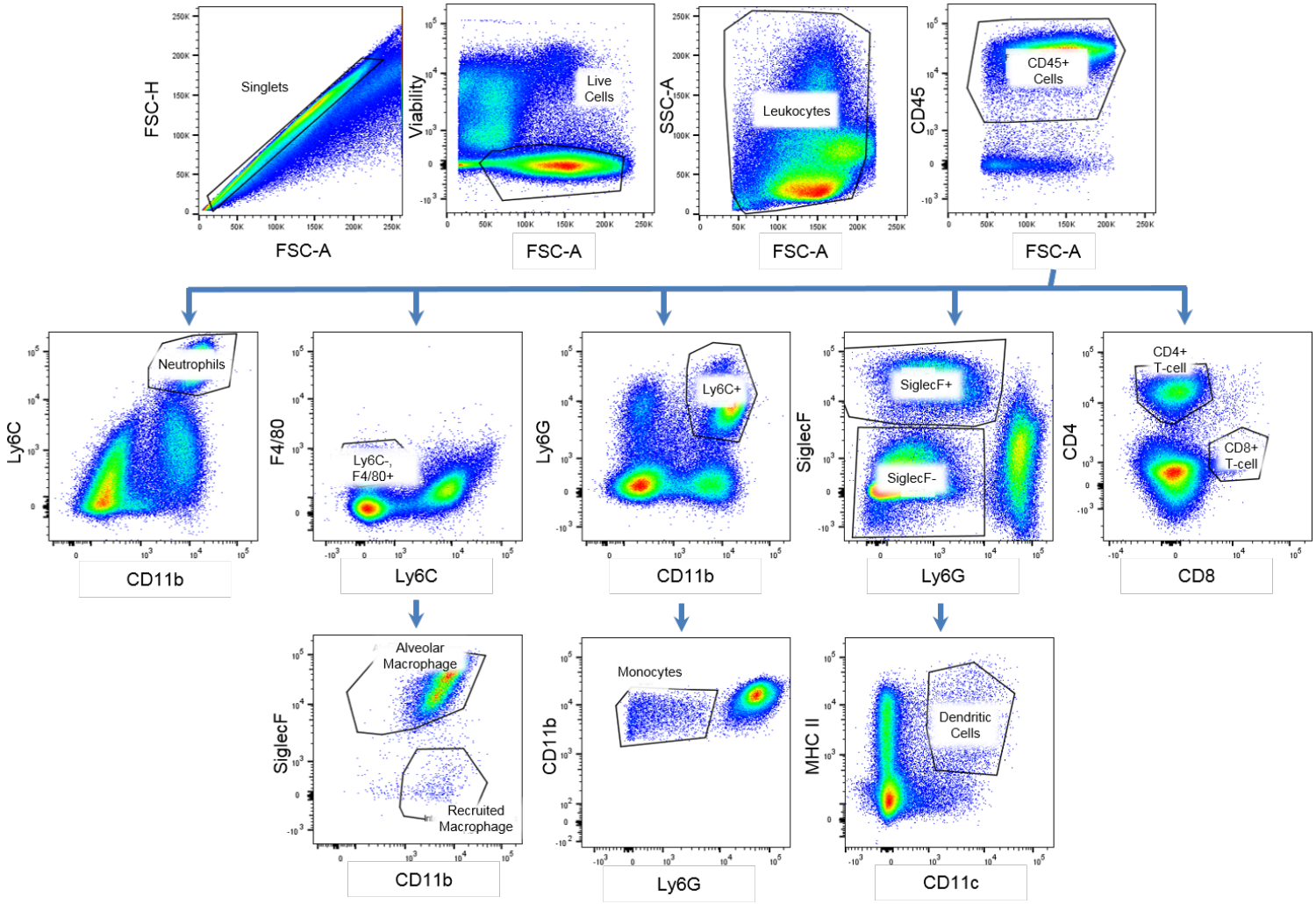


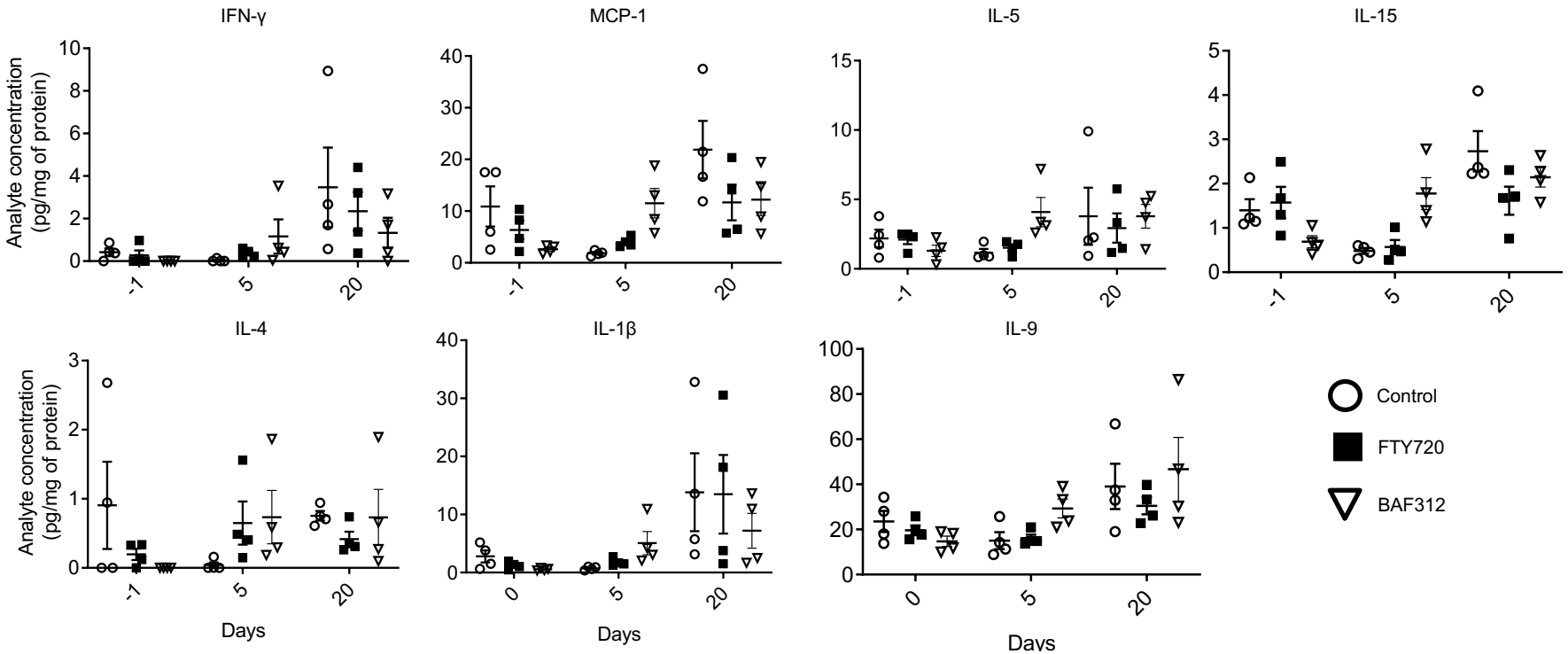
Supplementary Figure 1. FTY720 and BAF312 do not decrease survival or increase organ burden in mouse models of primary infection with *Δgcs1*. A) Mice received compound daily via gavage FTY720 n=8, BAF312 n=8 or vehicle control (H_2O) n=8, starting 2 days prior to intranasal infection with *C. neoformans* Δ*gcs1* and survival of mice was monitored. No difference in survival was observed. **B+C)** At day 60 post infection mice were sacrificed. Lungs (B) and brains (C) were analyzed for colony forming units, n=4 mice per group. **F)** 4 lungs from each treatment group infected with *C. neoformans* Δ*gcs1* were processed for histology 60 days post infection; H&E (top) and mucicarmine (bottom) stain. Black scale bars=200 μm



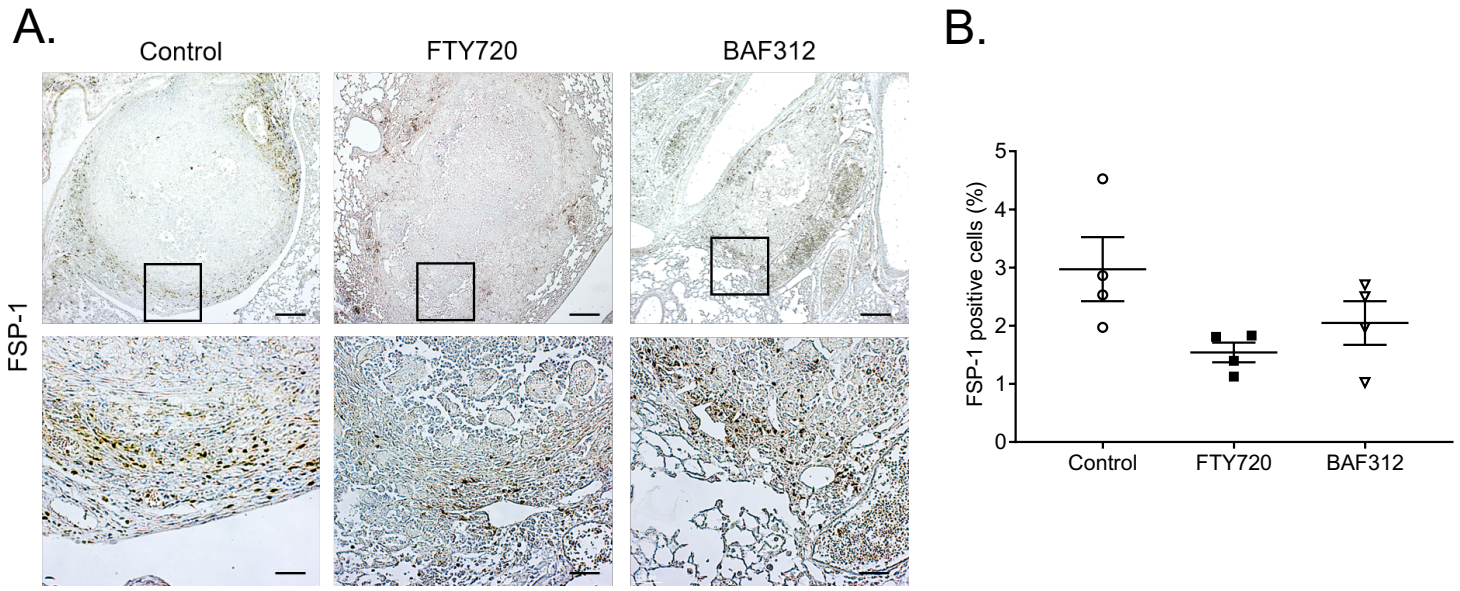
Supplementary Figure 2. Gating strategy used for flow cytometry. Cell populations were identified as follows, singlets were gated to remove clumped cells using Forward scatter A vs Forward scatter H. Live cells were identified by gating for Alexa Fluor 700 Carboxylic Acid, Succinimidyl Ester low cells. Leukocytes were identified by size and granularity in Forward and Side scatter. CD45 positive cells of the correct size were then selected. When intravascular staining was used, tissue cells were gated as intravascular CD45+ negative after the "leukocytes" gate. The following populations were then identified from this CD45+ subset: neutrophils (Ly6G+, CD11b+), alveolar macrophages (Ly6C-, F4/80+, SiglecF-), recruited macrophages (Ly6C-, F4/80+, SiglecF-), monocytes (Ly6C+, CDllb+, Ly6G), dendritic cells (Ly6G-, SiglecF-, MHCII+, CD11c+), CD4+ T cells (CD4+, CD8-), and CD8+ T cells (CD8+, CD4-).

Cell Population	Time point	Comparison	Adjusted P-value
Blood CD4+ T cells (CD4+/CD8-)	Day 5	Control vs. BAF312	<0.0001
		Control vs. FTY720	<0.0001
	Day 20	Control vs. BAF312	0.0001
		Control vs. FTY720	0.0002
Blood CD8+ T cells (CD4-/CD8+)	Day 5	Control vs. BAF312	<0.0001
		Control vs. FTY720	<0.0001
	Day 20	Control vs. BAF312	0.0001
		Control vs. FTY720	0.0003
Lung CD4+ T cells (CD4+/CD8-)	Day 5	Control vs. BAF312	0.0446
		Control vs. FTY720	0.0154
Lung CD8+ T cells (CD4-/CD8+)	Day 5	Control vs. BAF312	0.0001
		Control vs. FTY720	0.0001
	Day 20	Control vs. BAF312	0.0058
		Control vs. FTY720	0.0095
Lung Recruited Macrophages (F4/80+/CD11b+/Ly6C-/SiglecF-)	Day 5	Control vs. BAF312	0.0050
		Control vs. FTY720	0.0080
Lung Alveolar Macrophages (F4/80+/CD11b+/Ly6C-/SiglecF+)	Day 5	Control vs. BAF312	0.0483
		Control vs. FTY720	0.0436
	Day 20	Control vs. BAF312	0.0179
Lung Dendritic Cells (Ly6G-, SiglecF-, MHCII+, CD11c+)	Day 5	Control vs. FTY720	0.0428

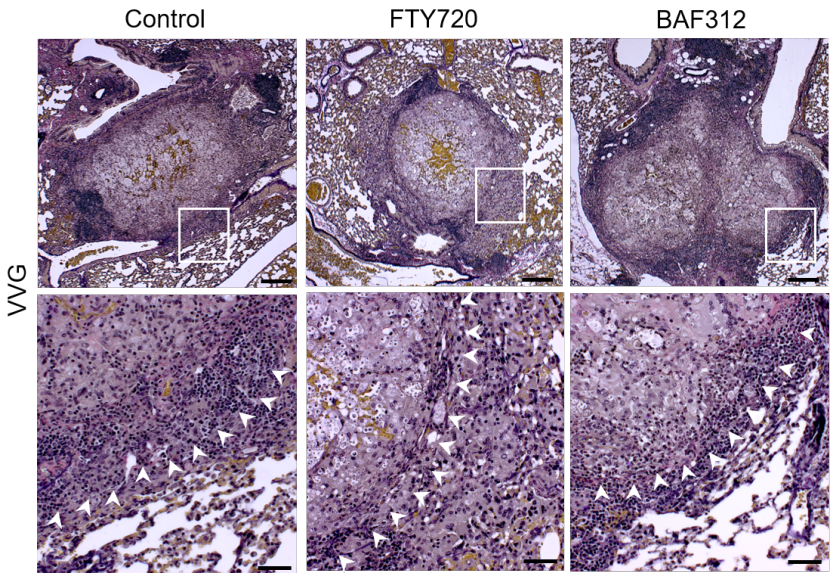
Supplementary Table 1. Adjusted P values for flow cytometry analysis. Flow cytometry data was analyzed as follows; two-way repeated measures ANOVA was performed with the Bonferroni multiple comparisons post-hoc test. P values were corrected for multiplicity using the Bonferroni adjustment.



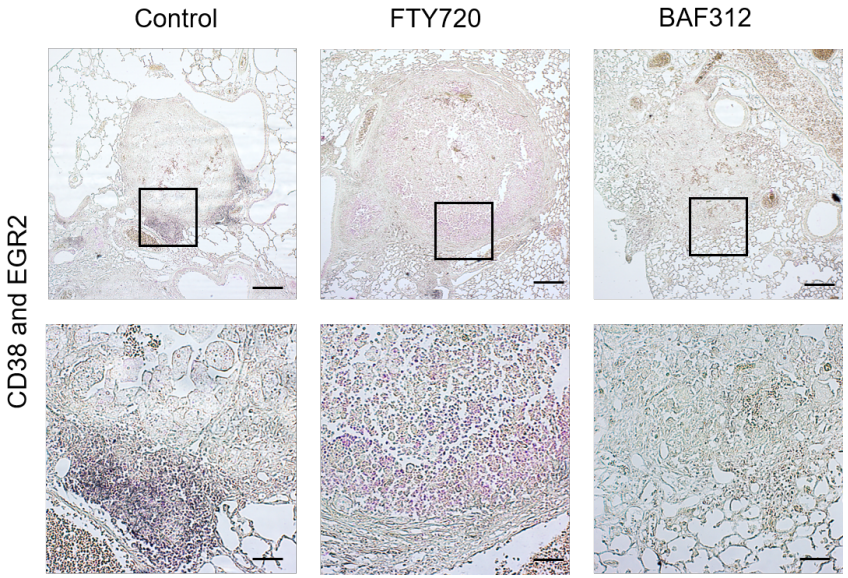
Supplementary Figure 3. FTY720 does not induce a major change in cytokine profile compared to BAF312. Mice were infected for thirty days with *C. neoformans* Δ*gcs1* prior to FTY720, BAF312, or vehicle control (H_2O) administration. For the lung analysis, n=4 mice per group at each time point (prior to compound administration [Day -1], Day 5, Day 20, and Day 60 post compound administration). Cytokine levels (pg/ml) were normalized to protein concentration (mg/ml) determined by the Bradford assay. All error bars represent SEM



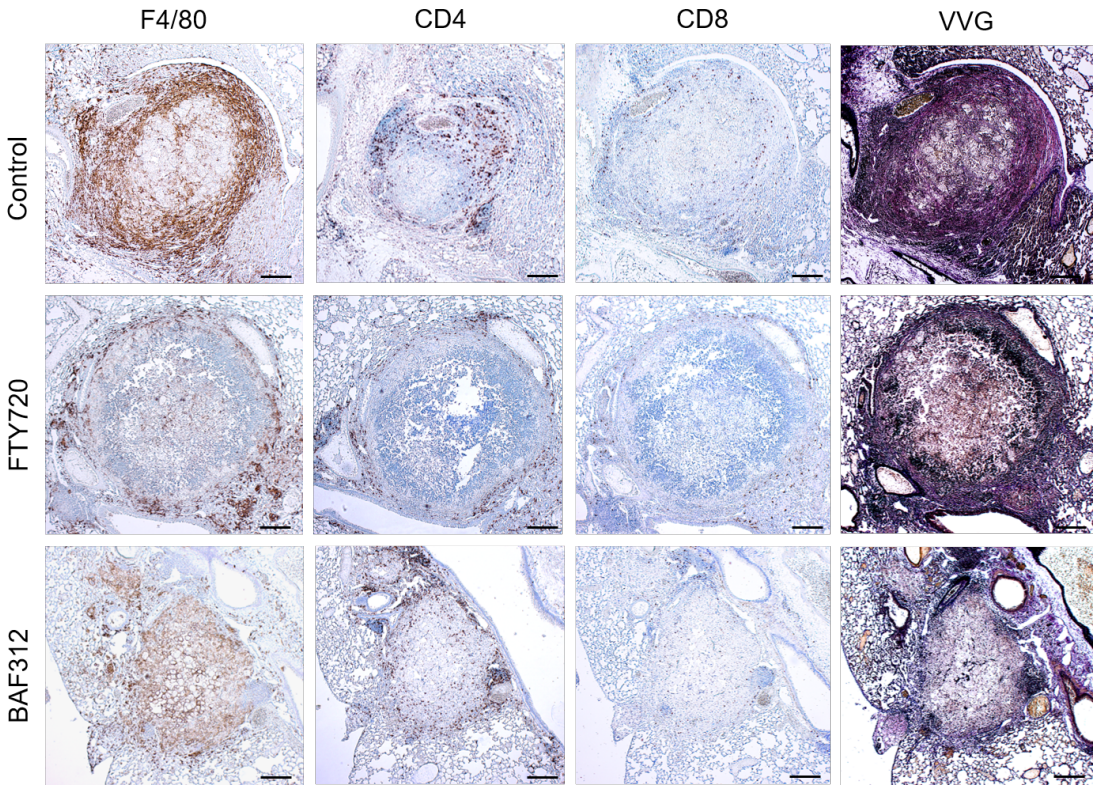
Supplementary Figure 4. Presence of fibroblasts is not altered upon FTY720 or BAF312 administration during the reactivation model. A) Mice were infected for thirty days with *C. neoformans* Δ*gcs1* prior to daily compound administration**.** At day 60 post compound administration lungs were processed for FSP-1 immunohistochemistry. Scale bars represent 200 μm (top) or 50 μm (bottom). Black box indicates enlarged area. **B)** The mean percentage of FSP-1 positive cells was quantified using Image J software, n=4. All error bars represent SEM.



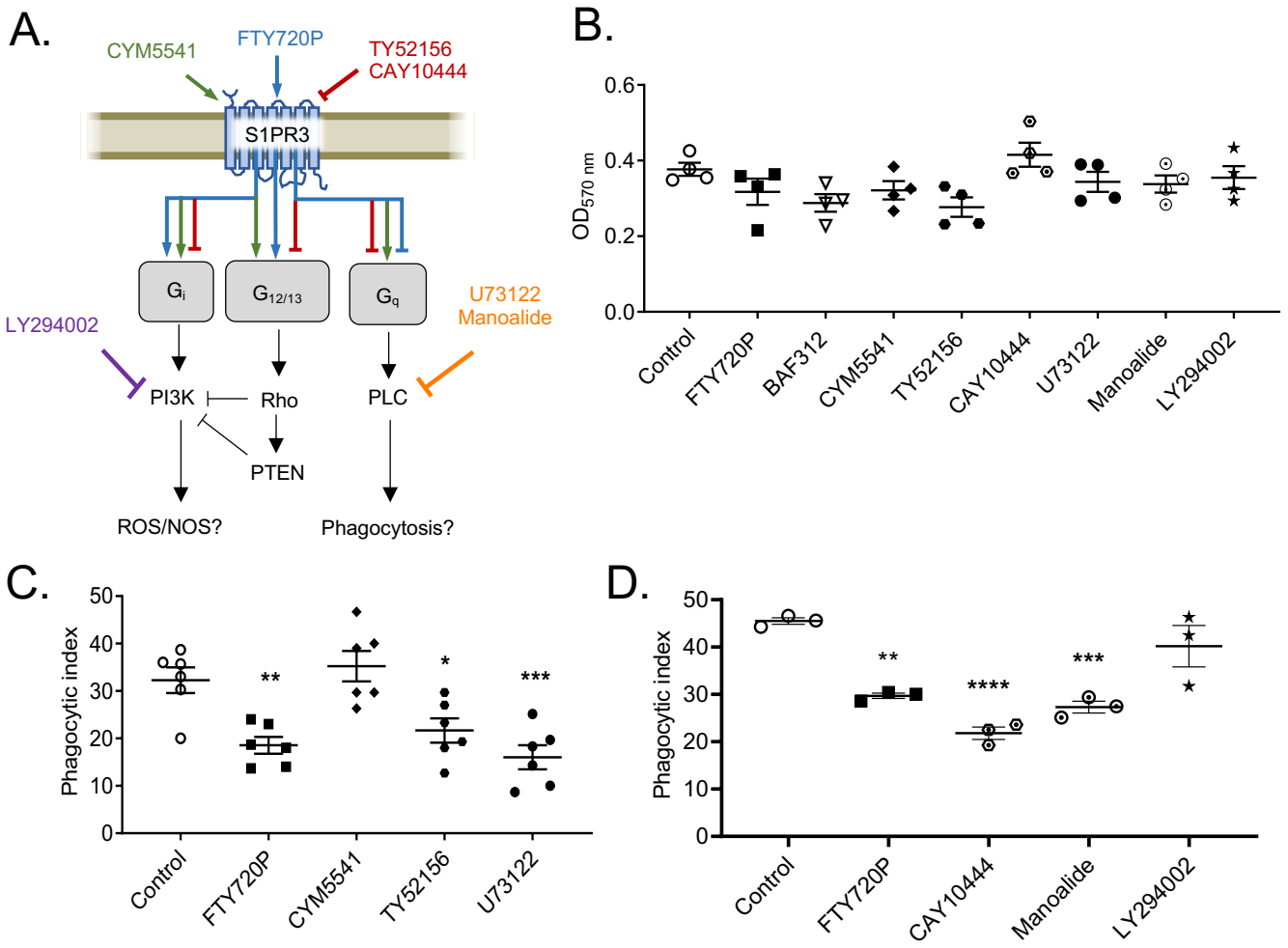
Supplementary Figure 5. Elastin and collagen deposition is not altered upon FTY720 or BAF312 administration during the reactivation model. At day 50 post compound administration, lungs were processed for histology and VVG stain. For the top row the scale bar=200 µm. For the bottom row the scale bar=50 µm. The white boxes indicate enlarged area and the white arrowheads denote the area of collagen and elastin deposition.



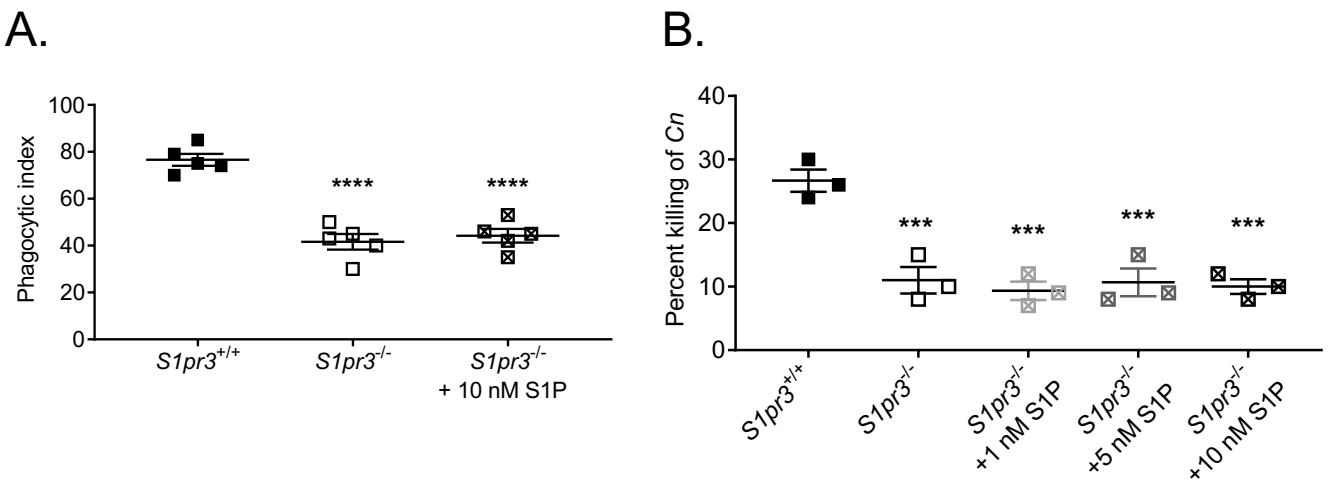
Supplementary Figure 6. Treatment with FTY720, but not BAF312 induces M2 polarization in the granuloma structure. A) Mice were infected for thirty days with C. neoformans Δ*gcs1* prior to daily compound administration. At day 60 post compound administration lungs were processed for CD38 (gray) and EGR2 (magenta) immunohistochemistry.



Supplementary Figure 7. T cells remain present in lung granulomas after FTY720 and BAF312 administration. Mice were infected for thirty days with *C. neoformans* Δ*gcs1* prior to daily compound administration**.** At day 60 post compound administration lungs were processed for F4/80, CD4, or CD8 immunohistochemistry and VVG staining. Scale bars represent 200 μm.



Supplementary Figure 8. FTY720 impairs phagocytosis in macrophages mediated by S1Pr3/Gq signaling pathway A) A schematic of pathways downstream of S1PR3 and the potential downstream pathways in macrophages as well as the reported activity of the compounds used in Supplementary Figure 8 are shown. **B)** Alveolar macrophage cell line MH-S was treated with 1 μM of indicated compound for 1 hour, n=4. In vitro cytotoxicity was evaluated using the MTT cell viability assay. Experiment was conducted 2 times. **C+D)** MH-S cells were treated with 1 μM of indicated compound for 1 hour (n=6 for C and n=3 for D) and subsequently co-incubated with opsonized *C. neoformans* Δ*gcs1* and phagocytic index was calculated by microscopic observation. For D, experiment was conducted 2 times. All error bars represent SEM and statistical comparisons were done using the one-way ANOVA with Bonferroni's multiple comparisons post-hoc test. P-values were corrected for multiplicity using the Bonferroni adjustment.(C: ***P=0.0039* for control vs. FTY720P, **P=0.0318* for control vs. TY52156, and ****P=0.0007* for control vs. U73122. D: ***P=0.0016* for control vs. FTY720P, *****P=<0.0001* for control vs. CAY10444, and ****P=0.0005* for control vs. Manoalide).



Supplementary Figure 9. S1P/S1Pr3 signaling is required for phagocytosis and intracellular killing of *C. neoformans H99* A) Primary alveolar macrophages isolated from wild type and S1Pr3 deficient mice with and without S1P supplementation were co-incubated with opsonized *C. neoformans H99* and phagocytic index was calculated by microscopic observation (n=5 each). Experiment was conducted 3 times. **B)** Primary alveolar macrophages isolated from mice of indicated genotype (n=3) with and without S1P supplementation were co-incubated with opsonized *C. neoformans* Δ*gcs1.* After incubation, culture media were plated onto YPD agar. Percent killing was calculated as the difference in colony forming units (CFU's) of *H99* incubated with vs. without macrophages. Experiment was conducted 5 times. All error bars represent SEM and statistical comparisons were done using one-way ANOVA with Bonferroni's multiple comparisons post-hoc test. P-values were corrected for multiplicity using the Bonferroni adjustment (B: ****P=<0.0001* compared to the control, C: ****P=<0.001* compared to the control).