

Supplemental Fig S1. Splenic Th1 and Th17 cells in *Aspergillus* **and** *Fusarium* **immunized mice.** C57BL/6 mice received subcutaneous immunization of heatkilled, swollen *Aspergillus* or *Fusarium* conidia on days -10 and -3. On day 0, splenocytes were collected, stimulated with *Aspergillus* or *Fusarium* hyphal extract for 3h, and incubated with anti-mouse CD4, a-CD44, and intracellular IL-17 and IFN-γ antibodies for flow cytometry analysis. Flow cytometry analysis of all splenocytes (with no gates) was performed to detect CD4+ IFN-γ+ cells **(A)** and CD4+ IL-17+ cells **(B)**. Total CD4+ cells were then gated and CD44 positivity was analyzed by flow cytometry and shown as histograms.

Supplemental Figure S1

Supplemental Figure S2



Unimmunized, Aspergillus infected cornea



Immunized, Aspergillus infected cornea



Supplemental Fig S2. Corneal opacity in Aspergillus infected corneas

imaging and quantification. Corneal opacity was visualized in the intact cornea using a highresolution stereo MZFLIII microscope (Leica Micro-systems), images were captured, and Metamorph imaging software (Molecular Devices, Downington, PA) was used to quantify percent and total corneal opacity. Details of this approach are described elsewhere (Leal et al., 2010). Briefly, images were converted to pseudo-colors (purple = no opacity and red = maximum opacity) that correlated to pixel intensity levels, the threshold for minimum intensity was set using images of transparent, uninfected eyes, and areas of glare (pixel intensity = 249-255) were set to zero. Percent of corneal opacity = (area over transparent threshold)/ (total area of circlearea of glare) X 100. Total corneal opacity = sum of the intensity of all pixels within the circle above the minimum opacity threshold and below glare intensity calculated by Metamorph.



Supplementary Fig S3. IL-17 producing NK and $\gamma\delta$ T cells in Aspergillus or Fusarium infected corneas of immunized mice.

C57BL/6 mice were given a subcutaneous immunization of heat-killed, swollen *Aspergillus* or *Fusarium* conidia on d-10 and d-3 prior to corneal infection with live conidia. 24, 48, and 72 hours post infection, corneas were excised, cells collected, and incubated with anti-mouse $\gamma\delta$ TCR (**A**), or NK1.1 (**B**), and intracellular IL-17 antibodies for flow cytometry analysis. NK1.1 and $\gamma\delta$ TCR positivity was confirmed in splenocytes (data not shown).







D Corneal opacity in Fusarium infected, α - IFN- γ treated mice

Α

Ε



Corneal opacity in neutrophil depleted, Aspergillus and Fusarium infected C57BL/6 mice



Supplementary Fig S4. Effect of IL-17, IFN- γ , and neutrophils on corneal opacity caused by Aspergillus and Fusarium infection. Immunized C57BL/6 mice were given a sub-conjunctival injection of neutralizing α - IL-17A or α - IFN- γ prior to infection with Aspergillus or Fusarium conidia. IL-17A^{-/-} mice were also immunized and infected. Alternatively, immunized mice were given anti- neutrophil Ab NIMP-R14 through IP injections, prior to corneal infection. Quantification of percent and total corneal opacity was assessed using Metamorph (as described in Supplementary Figure S1) Data points represent individual corneas. Corneas of IL-17 neutralized and IL-17-/- mice infected with (A) Aspergillus or Fusarium (B). Corneal opacity of Fusarium (C) or Aspergillus (D) infected corneas after IFN- γ neutralization. (E) Corneal opacity after fungal infection in neutrophil depleted, immunized mice.