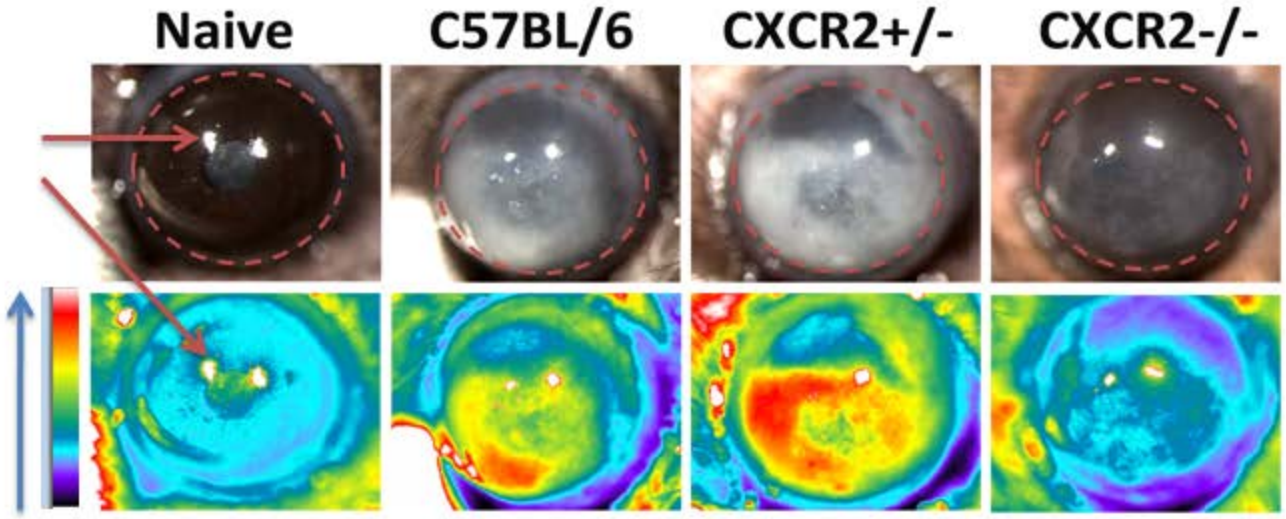
Figure S1



Percent corneal opacity:	**100%**	**98.5%**	**49.5%**
Integrated pixel intensity/ corneal opacity:	1.1 x 10^8	1.5x 10^8	3.8 x 10^7

Figure S1. Quantification of corneal opacification

Metamorph Imaging software (Molecular Devices, Downington PA) was used to determine the area of opacity and integrated corneal opacity. A circular region of constant area was centered on the images of corneas (upper panels). In order to standardize analysis of images within a given data set, 4 images of corneas with distinct regions of corneal opacification and no disease within the same eye were chosen. These standard eye images were converted into pseudo-color images to aid in opacity visualization (See panel above), Subsequently, the minimum threshold pixel intensity corresponding to opaque regions of the cornea were determined for each eye and averaged. All subsequent images within the data set were analyzed using the average minimum threshold pixel intensity value obtained during standardization. The threshold areas are clearly seen in the pseudo-color images (lower panels) with a scale shown of increasing pixel number from purple to red that correspond to areas of corneal opacity. Areas of glare (red arrows) were also demarcated and then set to zero, thereby eliminating glare from the subsequent analysis. To determine the area of opacity, the total area above the threshold within the circle was calculated and converted to percentage of total area of the circle minus areas of glares (i.e. Percent Corneal Opacity = (Threshold Area/ (Circle Area- Glare Area)*100). A second measure of corneal opacity was determined from the integrated intensity values, which record the total pixel numbers above the threshold. The more opaque corneas displayed a greater integrated intensity value (C57BL/6; CXCR2+/-) compared with less opaque corneas (CXCR2-/-),and are represented in the data as Integrated Corneal Opacity. Upper panels: Naïve mice, C57BL/6 corneas , CXCR2+/-, and CXCR2-/- corneas infected with Af dsRed as shown in Figure 2. Lower panels: pseudocolor images generated by Metamorph.

Figure S2

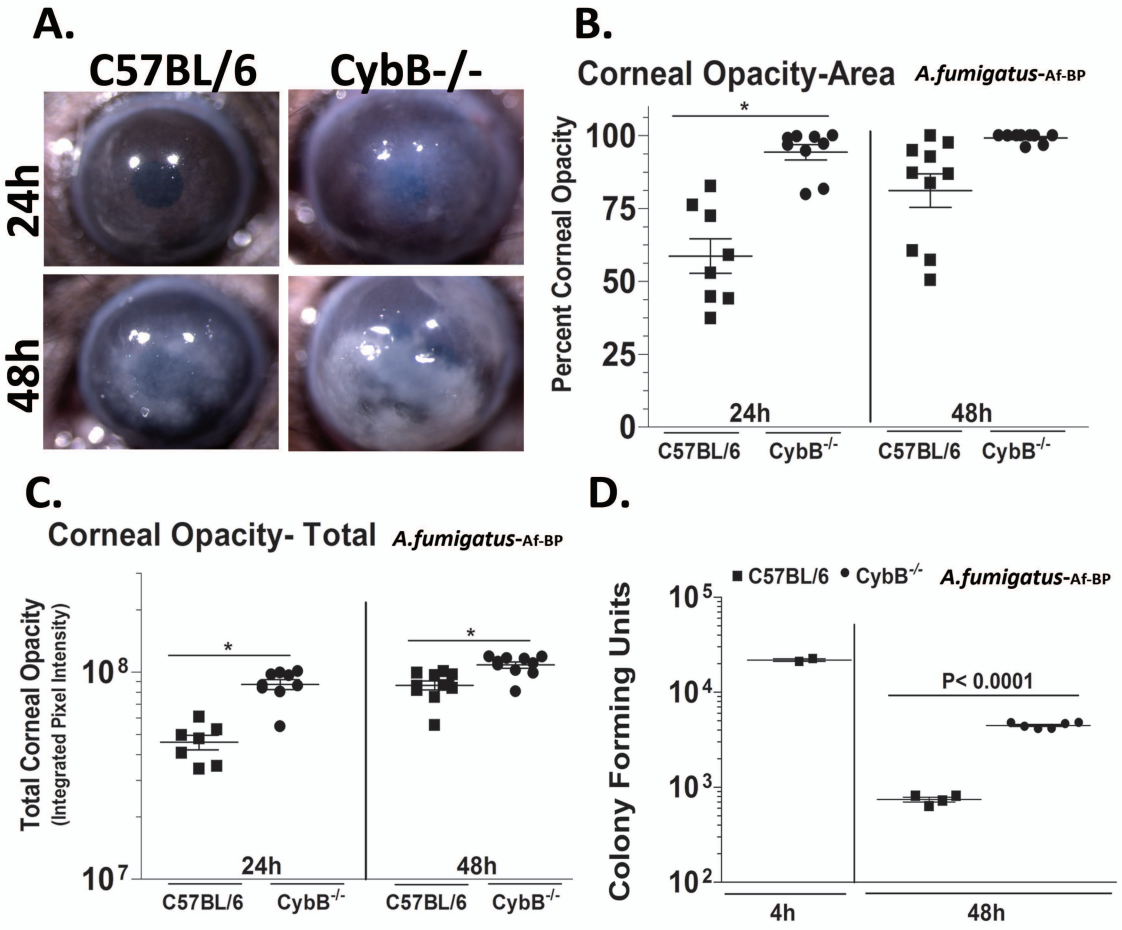


Figure S2. NOX is required to control the growth of A.fumigatus clinical isolate Af-BP during corneal infection A. C57BL/6 mice and CybB-/- mice were infected with 105 conidia from the A.fumigatus keratitis clinical isolate strain Af-BP. B. Corneal opacity area, C. total cornea opacity, and D. CFU were quantified in infected corneas post-infection.

Figure S3

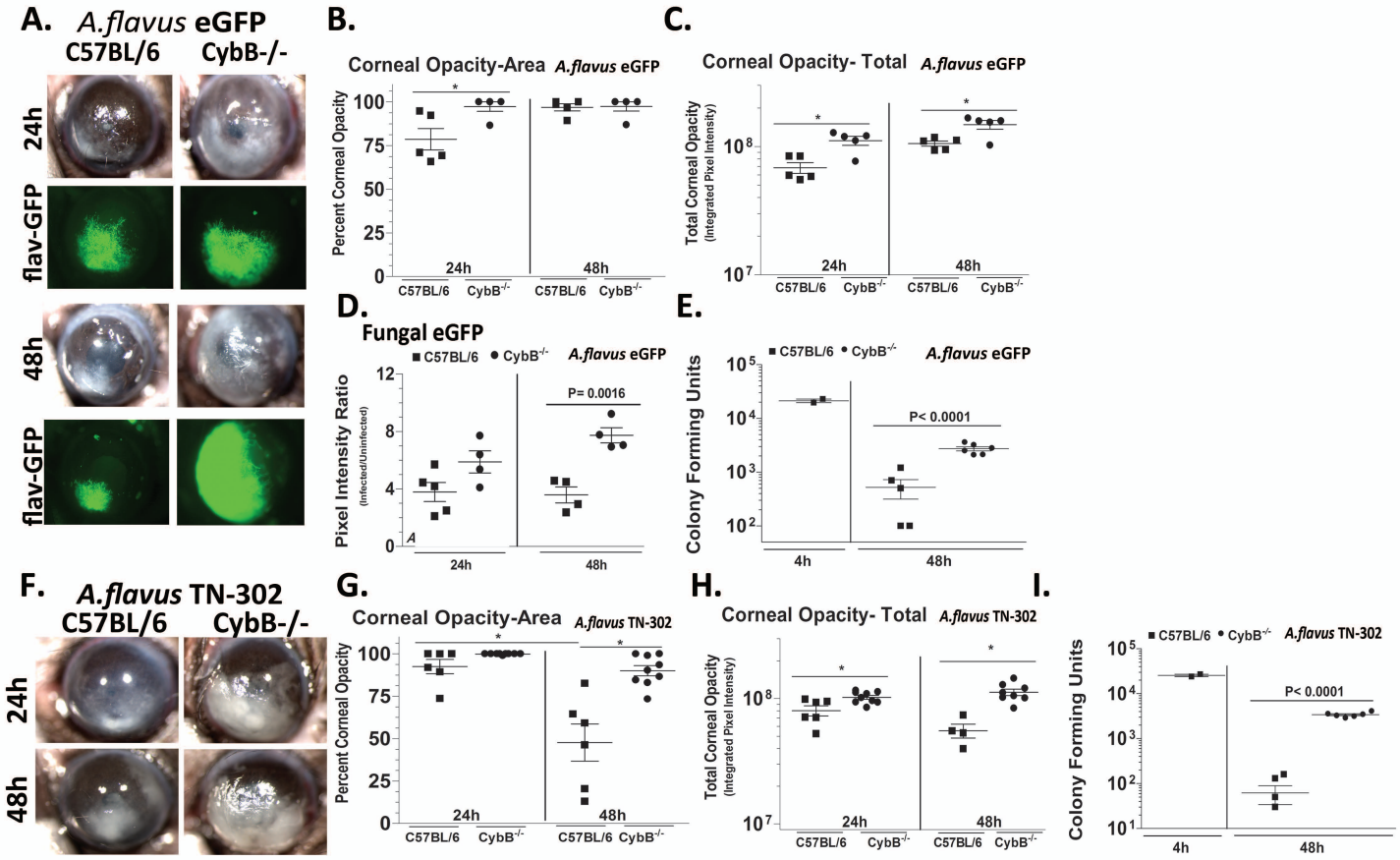


Figure S3. NOX is required to control the growth of A.flavus during corneal infection

A. C57BL/6 mice and CybB-/- mice were infected with 40,000 A.flavus 70-GFP conidia. Eyes were imaged at 24h and 48h post-infection for corneal opacity and fungal eGFP expression. B. Corneal opacity area, C. total cornea opacity, D. fungal eGFP expression, and E. CFU were quantified in infected corneas post-infection. F. C57BL/6 mice and CybB-/- mice were infected with 40,000 conidia from the A.flavus clinical isolate strain TN-302 and eyes were imaged at 24h and 48h post-infection for corneal opacity. G. Corneal opacity area, H. total cornea opacity, and I. CFU were quantified in infected cor-

Figure S4

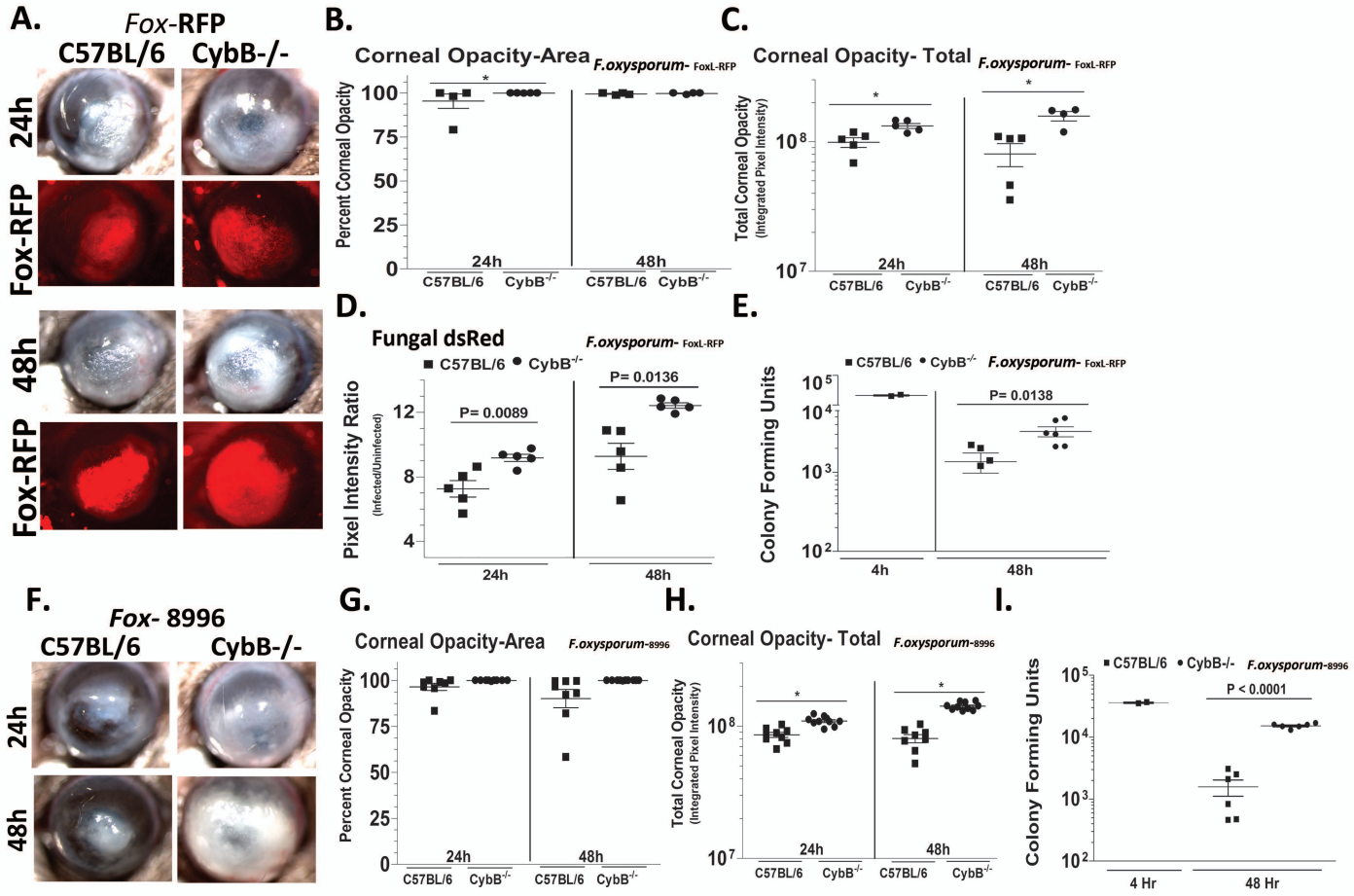


Figure S4. NOX is required to control the growth of F.oxysporum during corneal infection
A. C57BL/6 mice and CybB-/- mice were infected with 000 F.oxysporum lysopersicum-RFP conidia. Eyes were imaged at 24h and 48h post-infection for corneal opacity and fungal dsRed expression. B. Corneal opacity area, C. total cornea opacity, D. fungal dsRed expression, and E. CFU were quantified in infected corneas post-infection. F. C57BL/6 mice and CybB-/- mice were infected with 50,000 conidia from the F.oxysporum clinical isolate strain 8996 and eyes were imaged at 24h and 48h post-infection for corneal opacity. G. Corneal opacity area, H. total cornea opacity, and I. CFU were quantified in infected corneas.

Figure S5

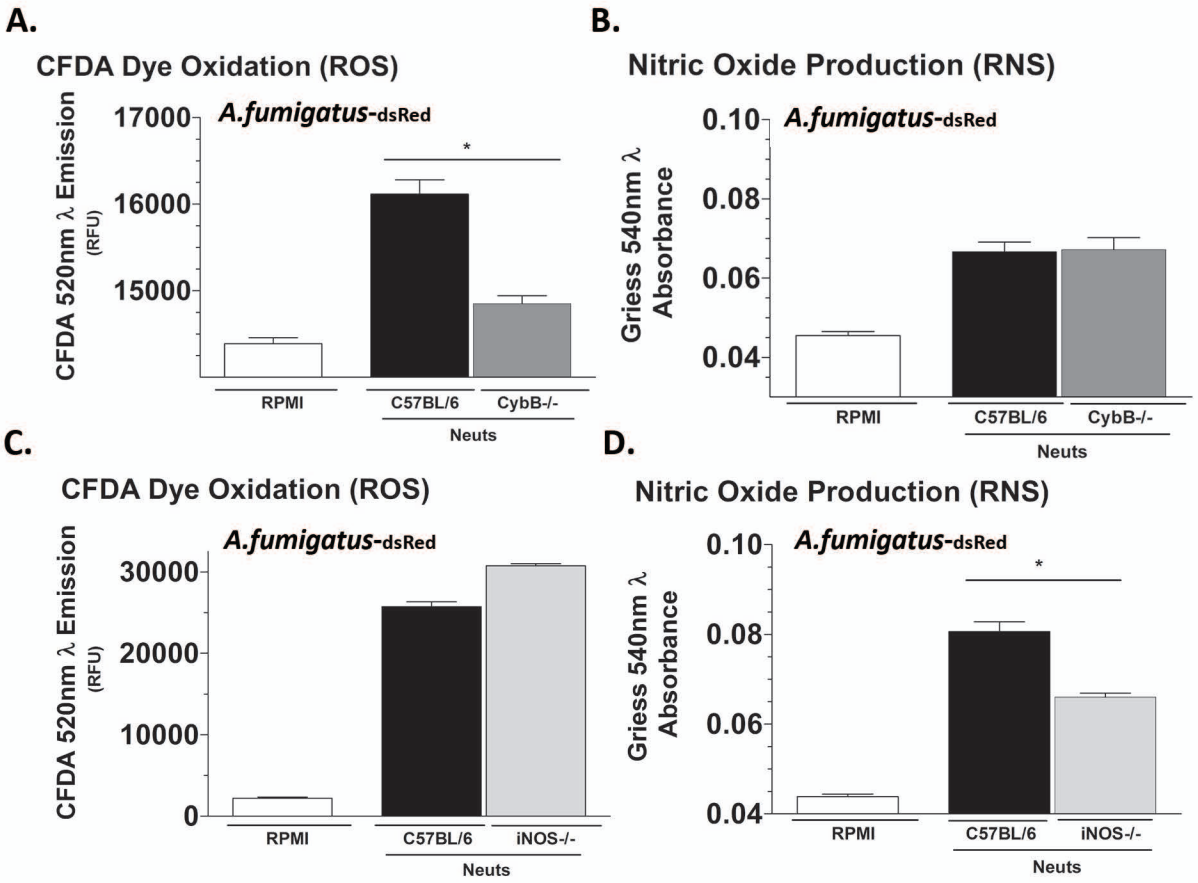


Figure S5. CybB-/- but not iNOS-/- neutrophils exhibit reduced ROS production upon exposure to fungal hyphae A.fumigatus strain Af-dsRed conidia (12,500/well) were cultured for 6h in black-walled, clear-bottom 96 well-plates before addition of purified thioglycolate-elicited peritoneal neutrophils from C57BL/6, CybB-/- iNOS-/- . At 2h post-infection, A. ROS and B. NO release by C57BL/6 and CybB-/- neutrophils into neutrophil-fungal coincubation supernatants were measured via fluorometry (CFDA dye oxidation) and spectrophotometry (Nitrosylation of Griess reagent), respectively. Similarly, At 2h post-infection C. ROS and D. NO release by C57BL/6 and iNOS-/- neutrophils into neutrophil-fungal coincubation superna-

Figure S6

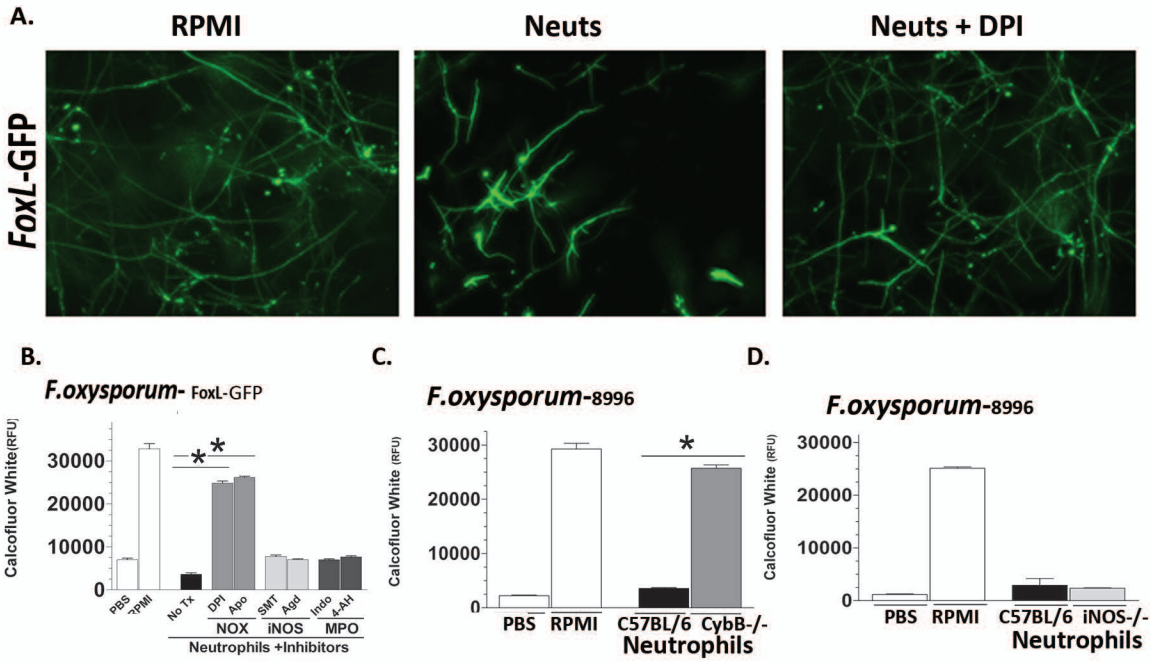


Figure S6. Neutrophil NADPH oxidase is required to control the growth of F.oxysporum
A. F.oxysporum (FoxL-GFP).was cultured either alone in PBS or RPMI or coincubated with 2x10^5 human neutrophils in RPMI or the same number of neutrophils in RPMI + the NOX inhibitor DPI, and fungal GFP expression was imaged at 16h post incubation, and B. chitin content was quantified. C. To examine the role of NOX on mouse neutrophil-mediated killing of fungal hyphae we grew F.oxysporum conidia as described above for 6h and co-incubated them with thioglycolate-elicited peritoneal neutrophils from WT C57BL/6 and CybB-/- mice or D. iNOS -/- mice and fungal chitin content was determined via fluorometry.

Figure S7

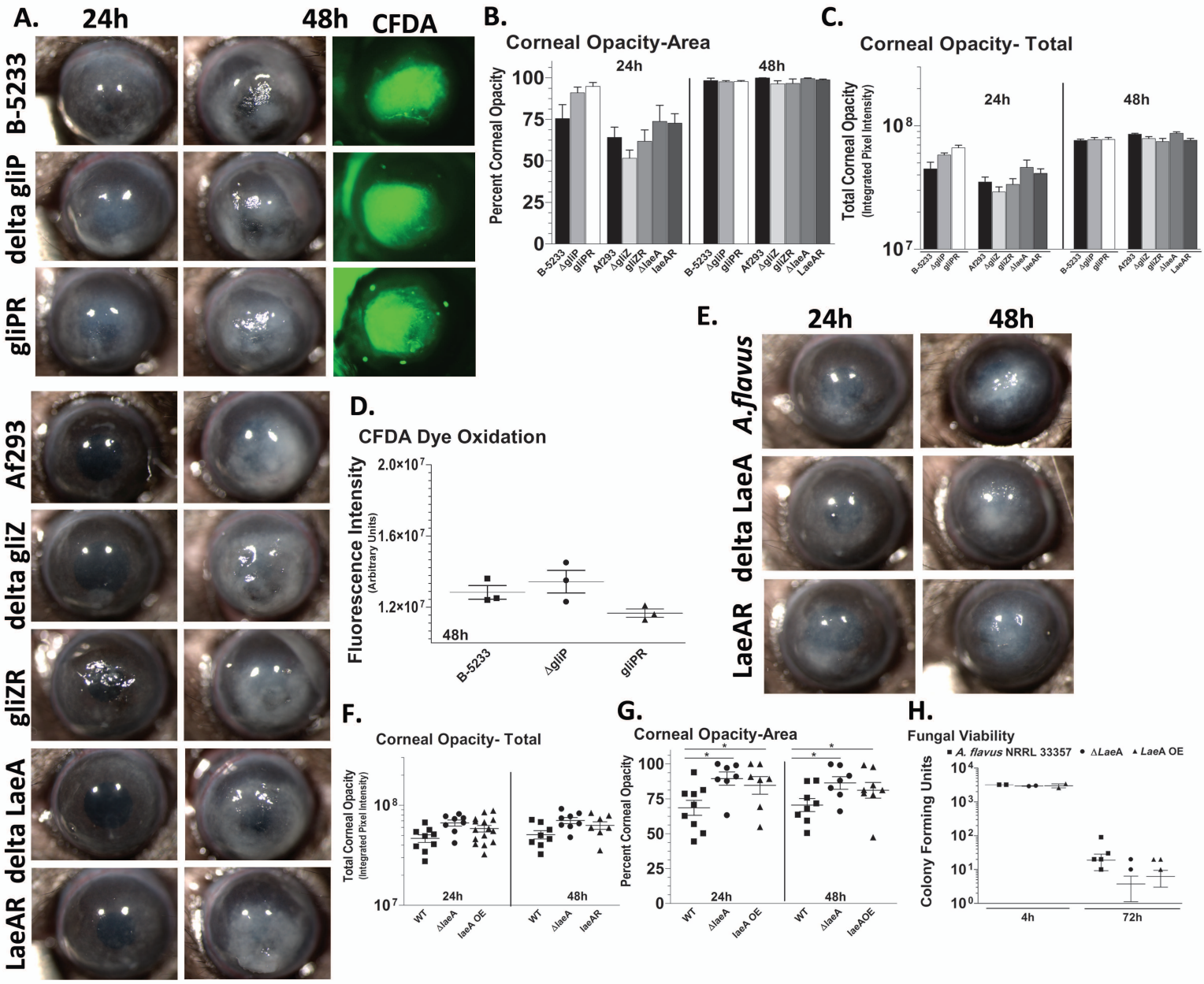


Figure S7. Gliotoxin and LaeA-regulated 2° metabolites do not mediate fungal survival during corneal infection
A. C57BL /6 mice were infected with 50,000 A.fumigatus conidia isolated from either ΔgliZ, gliZ-R, ΔlaeA, laeA-R, WT-Af293 or ΔgliP, gliP-R, WT-B-5233, and eyes were imaged at 24h and 48h post-infection.
In addition, at 48h post-infection CFDA dye was injected into 48h ΔgliP, gliP-R, or WT-B-5233- infected corneas and eyes imaged (GFP filter) to detect ROS- mediated oxidation of CFDA in infected corneas. B. Corneal opacity area, C. total cornea opacity, and D. ROS-mediated CFDA dye oxidation were quantified in infected corneas post-infection. E. C57BL/6 mice were infected with 50,000 conidia isolated from the A.flavus strains ΔlaeA, laeA-R, WT-NRRL and eyes were imaged at 24h and 48h post-infection. F. Corneal opacity area, G. total cornea opacity, and H. CFU were quantified in infected corneas post-infection.