Supplemental Figure 1

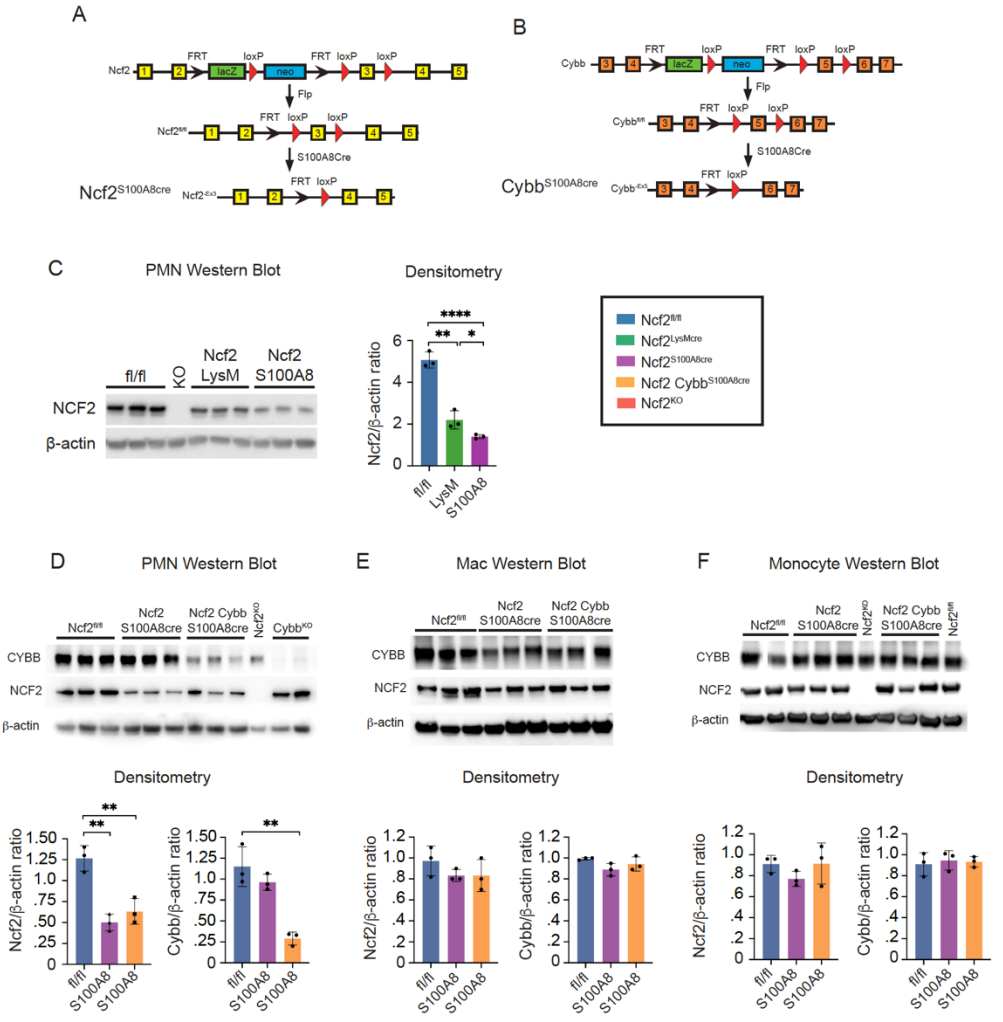

Fig. S1. Conditional deletion of *Ncf2* and *Cybb* and analysis of NCF2 and CYBB expression. A) Schematic representation of *Ncf2*$^{tm1a(EUCOMM)Wtsi}$, the *Ncf2*$^{fl/fl}$ allele generated after Flp-mediated excision of the region between the 5' and 3' FRT sites, and the exon 3-deleted allele in *Ncf2*^{S100A8Cre} mice. (B) Schematic representation of Cybb$^{tm2a(KOMP)Wtsi}$, the *Cybb*$^{fl/fl}$ allele generated after Flp- mediated excision of the region between the 5' and 3' FRT sites, and the exon 5-deleted allele in *Cybb*^{S100A8Cre} mice. (C – F) Western blots and densitometry for expression of NCF2 and CYBB in bone marrow neutrophils (PMN), resident peritoneal macrophages (Mac) and bone marrow monocytes from mice with wild-type NOX2 (*Ncf2*$^{fl/fl}$), global NOX2 knockout (*Ncf2*^{KO}), or conditional deletion of NCF2 and CYBB NOX2 subunits (*Ncf2*^{LysMCre}, *Ncf2*^{S100A8Cre}, *Ncf2Cybb*^{S100A8Cre}), as indicated. Actin was used as a loading control, and representative blots are shown. Quantification of NCF2 and CYBB levels were assessed by ImageJ in three independent samples from each group of blots, and shown as mean ± SD. Student's 't' test was performed for comparisons between 2 groups and *P<0.05, **P<0.01, and ****P<0.0001 were considered as significant.

Supplemental Figure 2

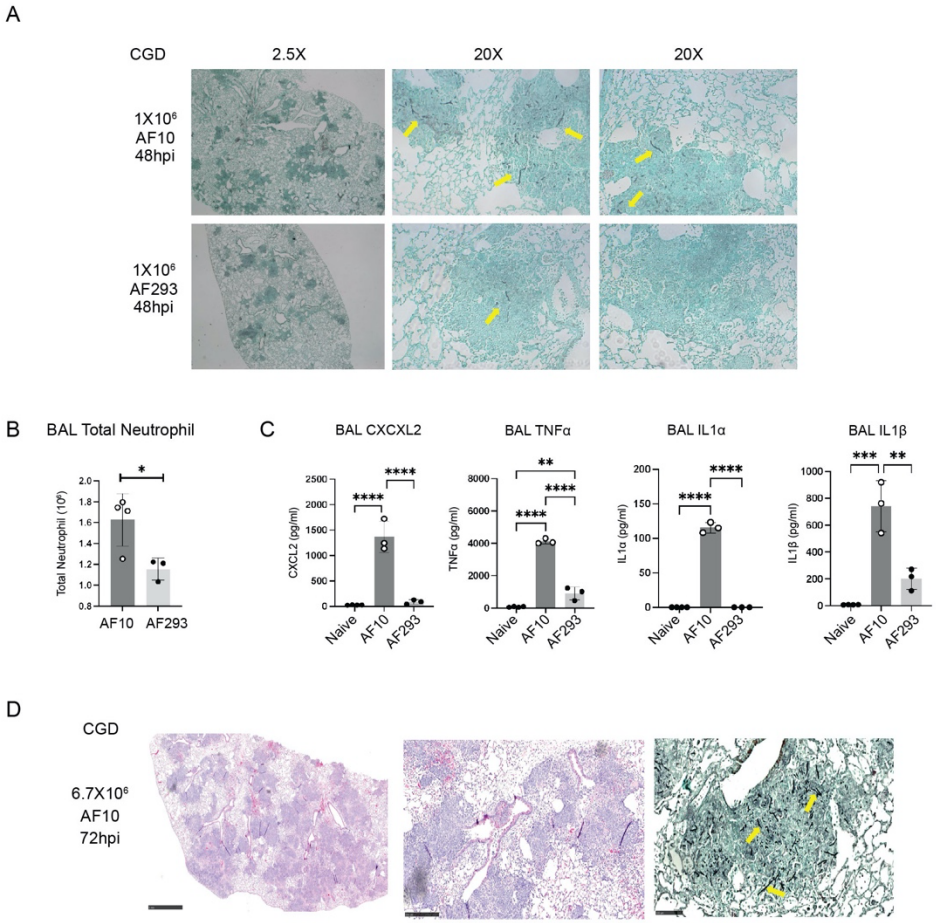

Fig. S2. *Aspergillus fumigatus* pneumonia in CGD mice following inhalation of AF10 or AF293 conidia

(A) Histology of GMS-stained lung sections for CGD (*Ncf2*^{KO}) mice at 48 hpi following inhalation of one million conidia of either AF10 or AF293 strains. Photomicrographs show a low power view (2.5X) and two different higher power (20X) sections from the same mouse. Arrows indicate hyphae. Data is representative of at least 3 mice for each AF strain.
(B) Total neutrophil counts from 1 ml BAL fluid, calculated from the total leukocyte count and percentage of neutrophils identified by cytospin. (C) Concentrations of indicated cytokines in 1 ml BAL fluid obtained from naive CGD mice or at 48 hpi with one million conidia of either AF10 or AF293 strains. Cytokines were measured using ProcartaPlex panels. (D) Representative histology of lung sections from CGD (*Ncf2*^{KO}) at 72hpi following challenge with 6 million AF10 conidia. From right to left, H&E (bar 1 mm), H&E (bar 250 µm), and GMS (bar 100 µm). Arrows indicate hyphae. Graphical data shown as mean ± SD. Student's 't' test was performed for comparisons between 2 groups for (B) and one-way Anova with Tukey's multiple comparisons for (C). P<0.05, **P<0.01, ***P<0.001 ****P< 0.0001 were considered as significant.

Idol et al. Neutrophil and macrophage NADPH oxidase 2 (NOX2) differentially control responses to inflammation and to *Aspergillus fumigatus* in mice

Supplemental Figure 3

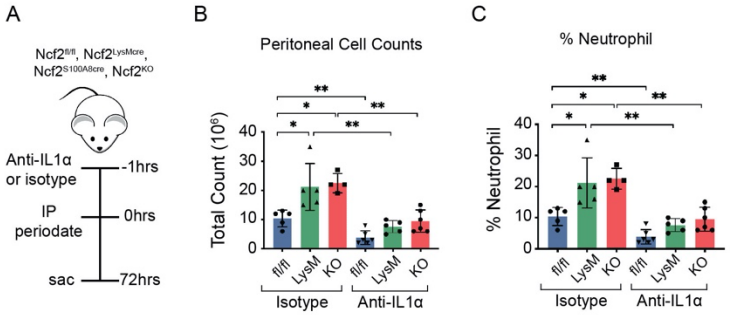

Fig. S3. IL-1α antibody treatment in mice with periodate-induced peritonitis

A) Schema for periodate-induced peritonitis in presence of isotype or anti-IL-1α in *Ncf2*$^{fl/fl}$, *Ncf2*^{LysMCre}, and *Ncf2*^{KO} mice. Mice received either isotype or anti-Il-1α by retroorbital injection 1 hour before IP injection of periodate. (B) Peritoneal lavage cell counts and % neutrophils at 72 hours in the indicated groups of isotype or anti-IL-1α-treated mice.

Data represents 2 independent sets of experiments with 4 – 6 mice for each genotype and treatment. Graphical data shown as mean ± SD. Student's 't' test was performed for comparisons between 2 groups and *P<0.05, **P<0.01 were considered as significant.