Fig. S1

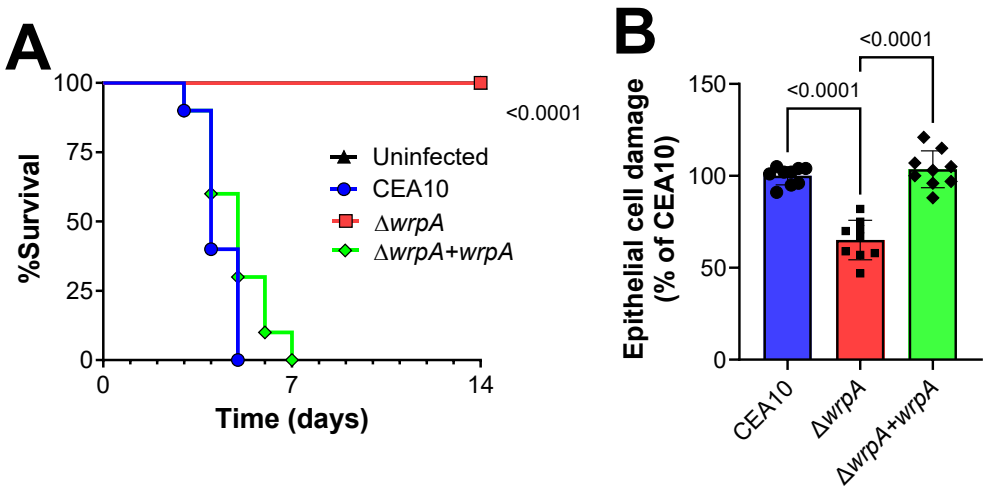


Fig. S1. (A) Survival of mice infected with a Δ*wrpA* mutant in the CEA10 strain background. Results are from a single experiment with 10 mice per *A. fumigatus* strain. Survival comparisons by the Log rank test. (B) Damage to A549 pulmonary epithelial cells at 20 h by the indicated *A. fumigatus* strains. *P* values determined by one-way ANOVA with Šídák's multiple comparisons test.

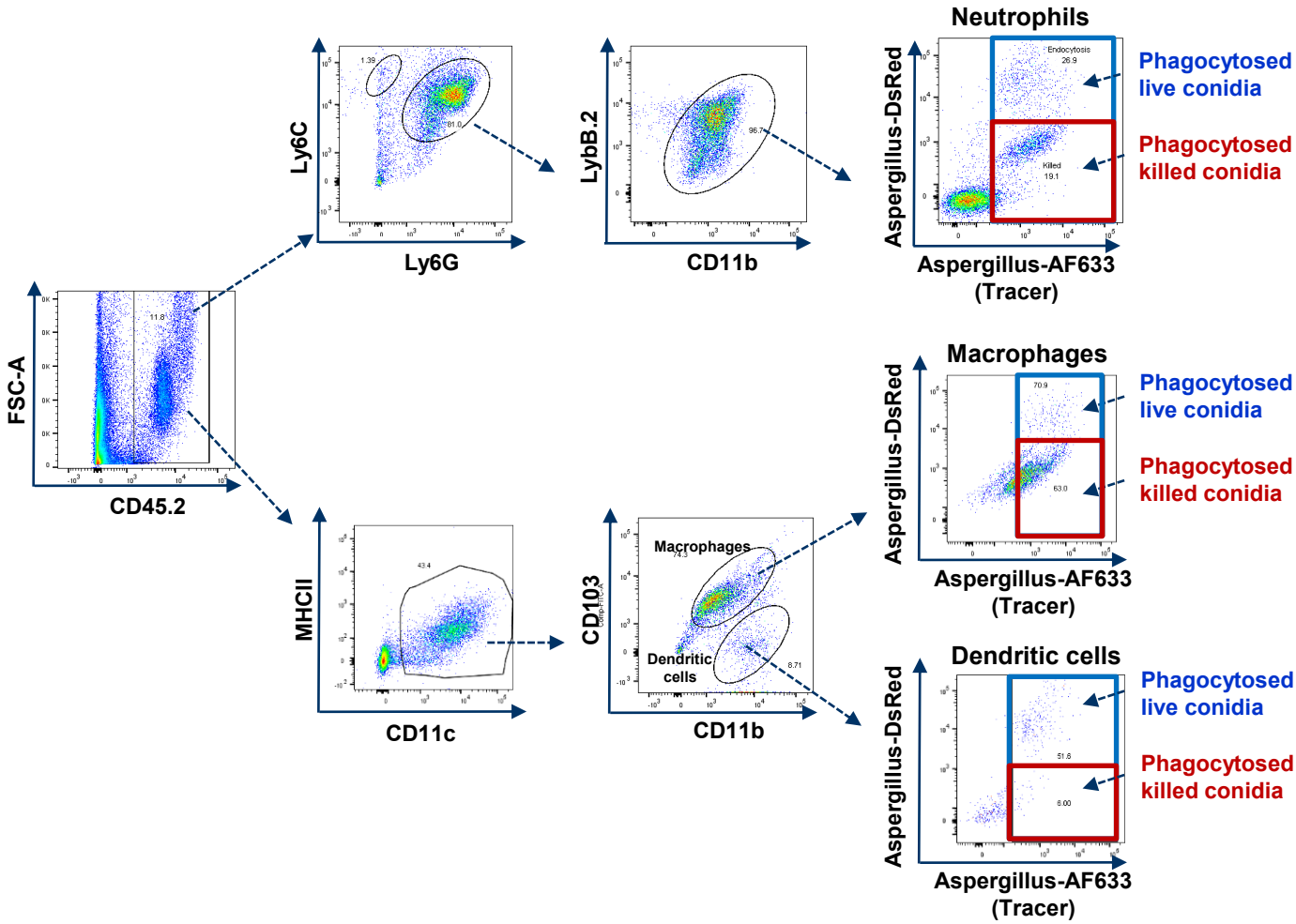


Figure S2. Gating strategy used for flow cytometric analysis of *A. fumigatus* phagocytosis and killing by immune cells. In some strains RFP was used instead of DsRed.

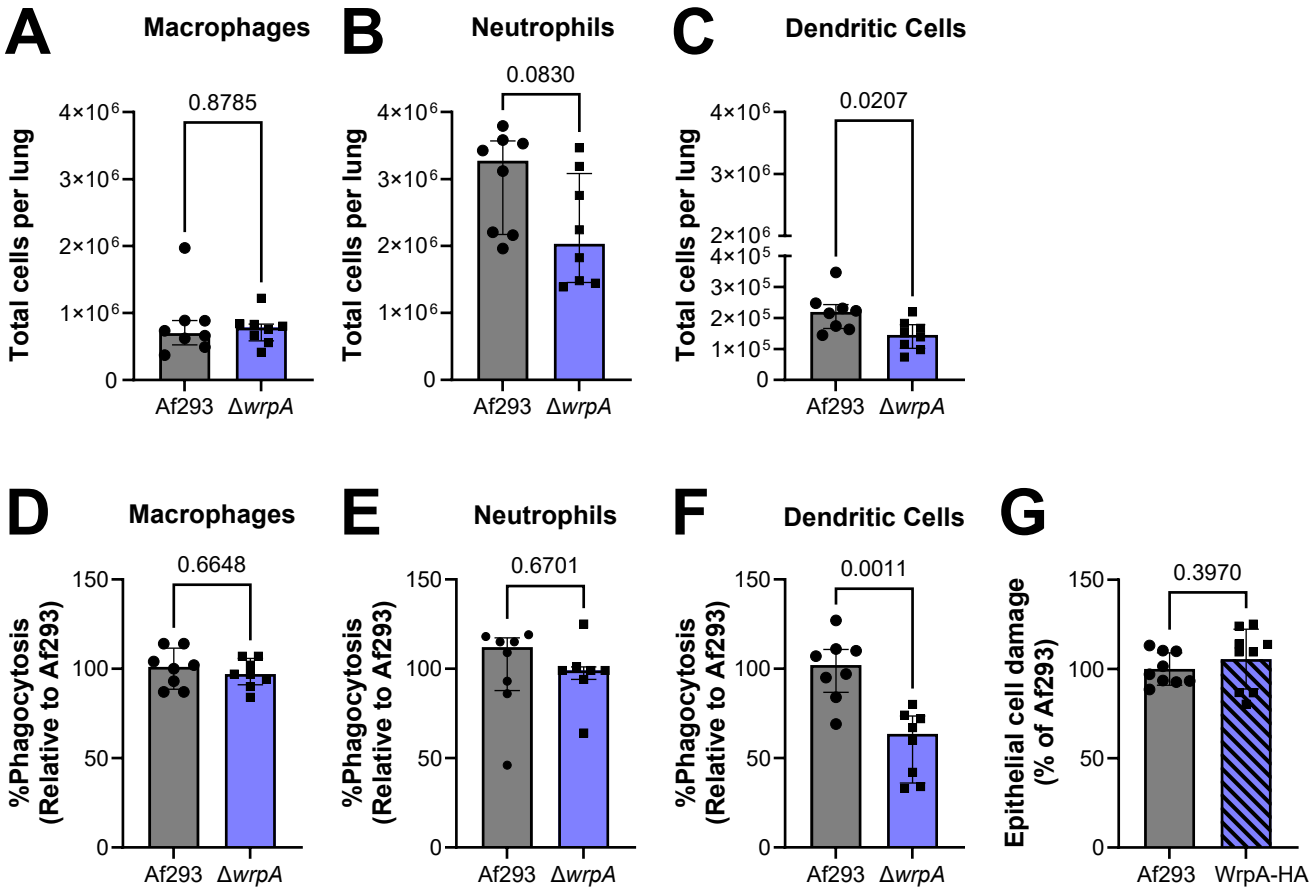


Figure S3. (A-F) Corticosteroid immunosuppressed mice were inoculated with *A. fumigatus* Af293 and the Δ*wrpA* mutant. After 18 h, the mice were sacrificed and the number of macrophages (A), neutrophils (B), and dendritic cells (C) in the lungs was determined by flow cytometry. The percentage of macrophages (D), neutrophils (E), and dendritic cells (F) containing phagocytosed Af293 and Δ*wrpA* mutant conidia was also determined. Results are median ± interquartile range of 7-8 mice per group in a single experiment. Data were analyzed using the Mann Whitney test. (G) Damage to A549 epithelial cells by the indicated strains after 18 h of infection. Results are mean ± SD of 3 experiments, each performed in triplicate. Significance determined by unpaired t-test.

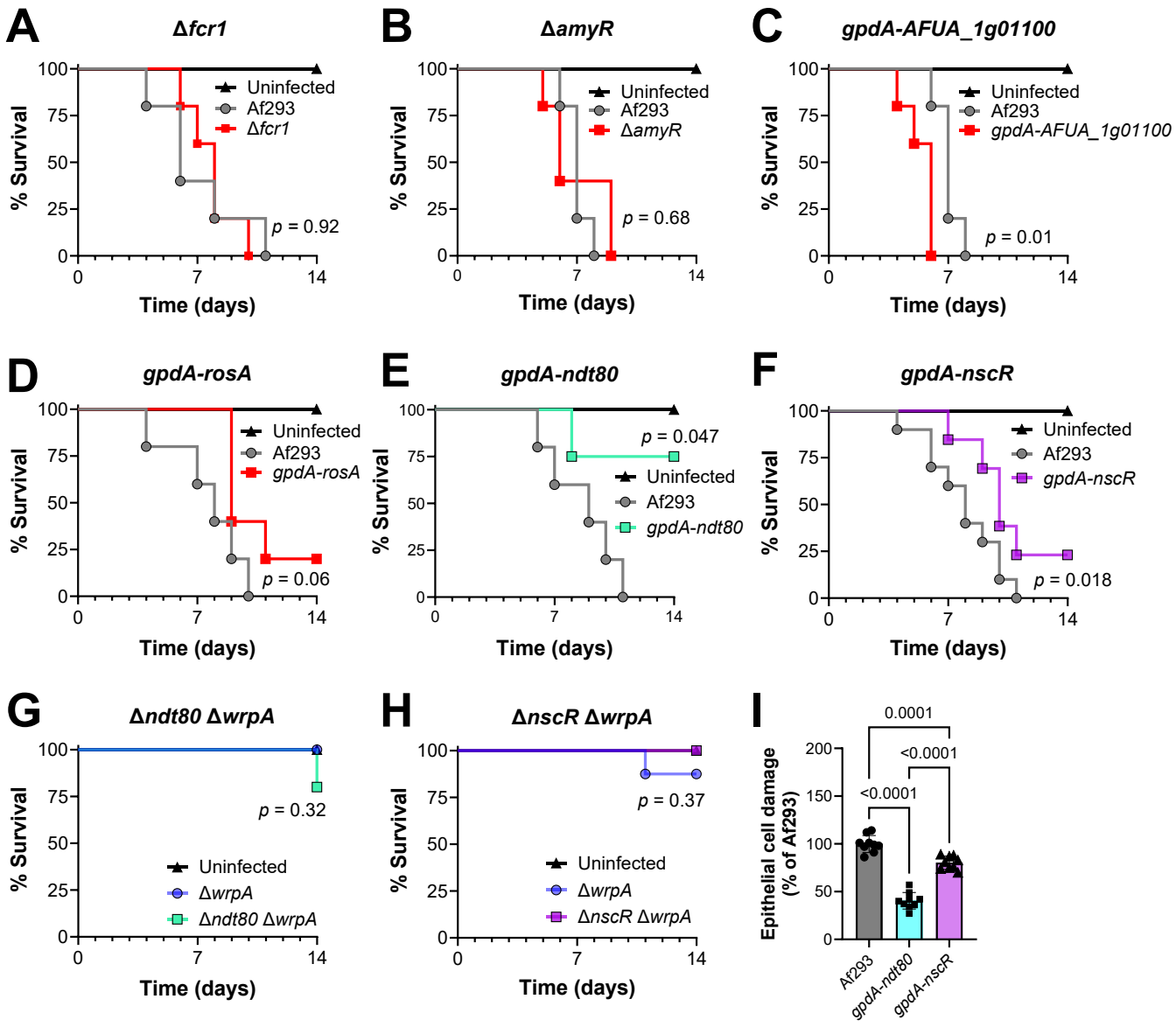


Figure S4. Survival of corticosteroid immunosuppressed mice after inoculation with the indicated *A. fumigatus* strains. (A) Δ*fcr1* mutant. (B) Δ*amyR* mutant. (C) *gpdA*-AFUA_1g01100 overexpression mutant. (D) *gpdA-rosA* overexpression mutant. (E) *gpdA-ndt80* overexpression mutant, a longer clone based on the updated annotation of Ndt80. (F) *gpdA-nscR* overexpression mutant, a second clone independent from the one in Figure 3C. (G) Δ*ndt80* Δ*wrpA* mutant. (H) Δ*nscR* Δ*wrpA* mutant. Results in (A-E and G) are from 5 mice per strain in single experiments. Results in (F and H) are from 8 mice per strain in single experiments. Survival data were analyzed using the Log rank test. (I) Damage to A549 pulmonary epithelial cells after infection with the indicated strains for 20 h. The *gpdA-ndt80* clone was the same one used in Figure S. 4E. Results are mean ± SD of three experiments, each performed in triplicate. *P* values were determined by one-way ANOVA with Šídák's multiple comparisons test.

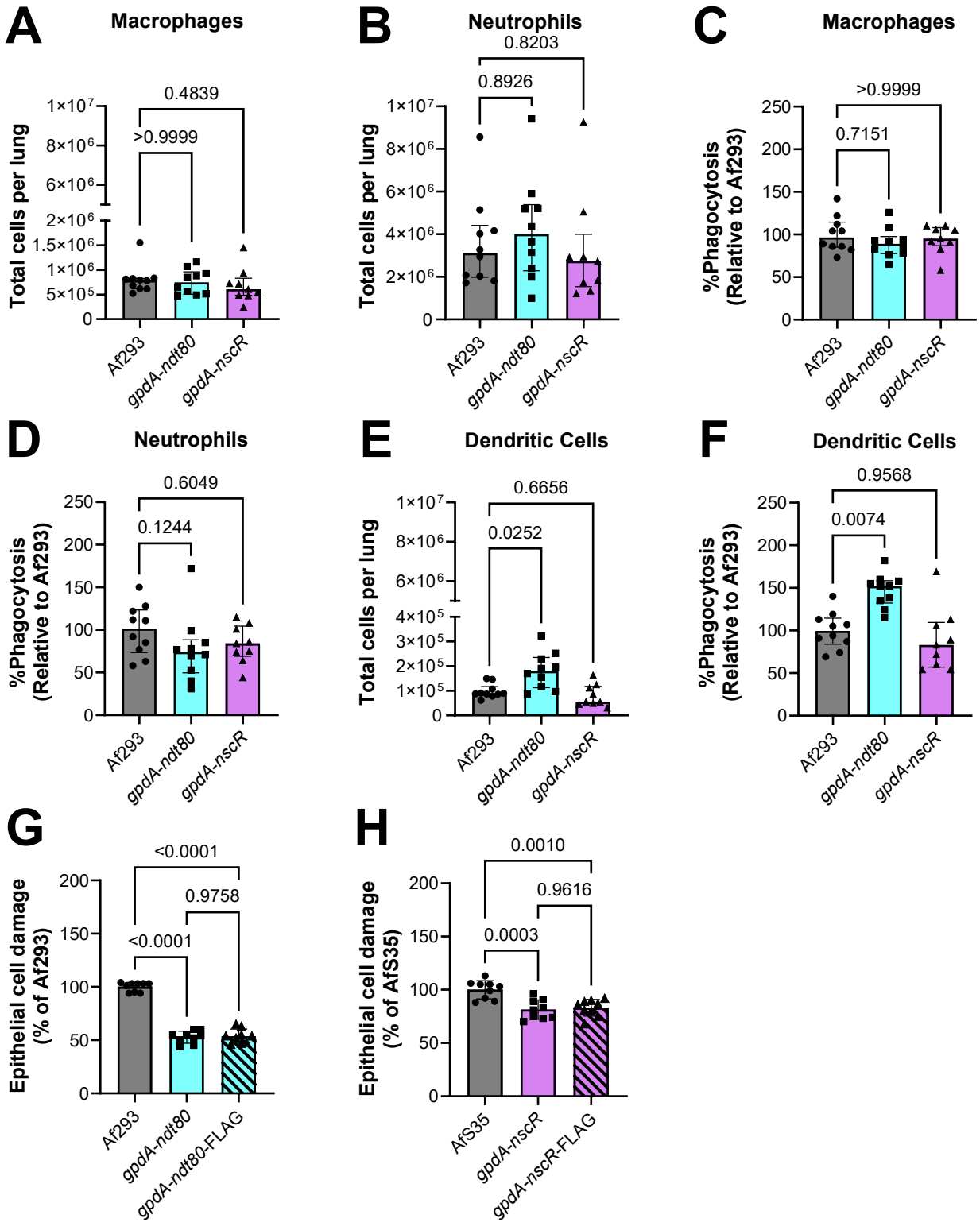


Figure S5. (A-F) Corticosteroid immunosuppressed mice were inoculated with the indicated *A. fumigatus* strains. After 18 h, the mice were sacrificed and the lungs were analyzed by flow cytometry. The number of macrophages (A) and neutrophils (B) in the lungs and the percentage of macrophages (C) and neutrophils (D) containing phagocytosed Af293 and mutant conidia were determined, as were the number of dendritic cells (E) and the percentage of dendritic cells containing phagocytosed conidia (F). Results are median ± interquartile range of 9-10 mice per group in a single experiment. Statistics were determined by the Kruskal-Wallis test with Dunn's multiple comparisons correction. (G and H) A549 epithelial cell damage caused by mutants that overexpressed FLAG-tagged alleles of *ndt80* (G) and *nscR* (H). Results are mean ± SD of 3 experiments, each performed in triplicate. Data were analyzed by one-way ANOVA with Šídák's multiple comparisons test.

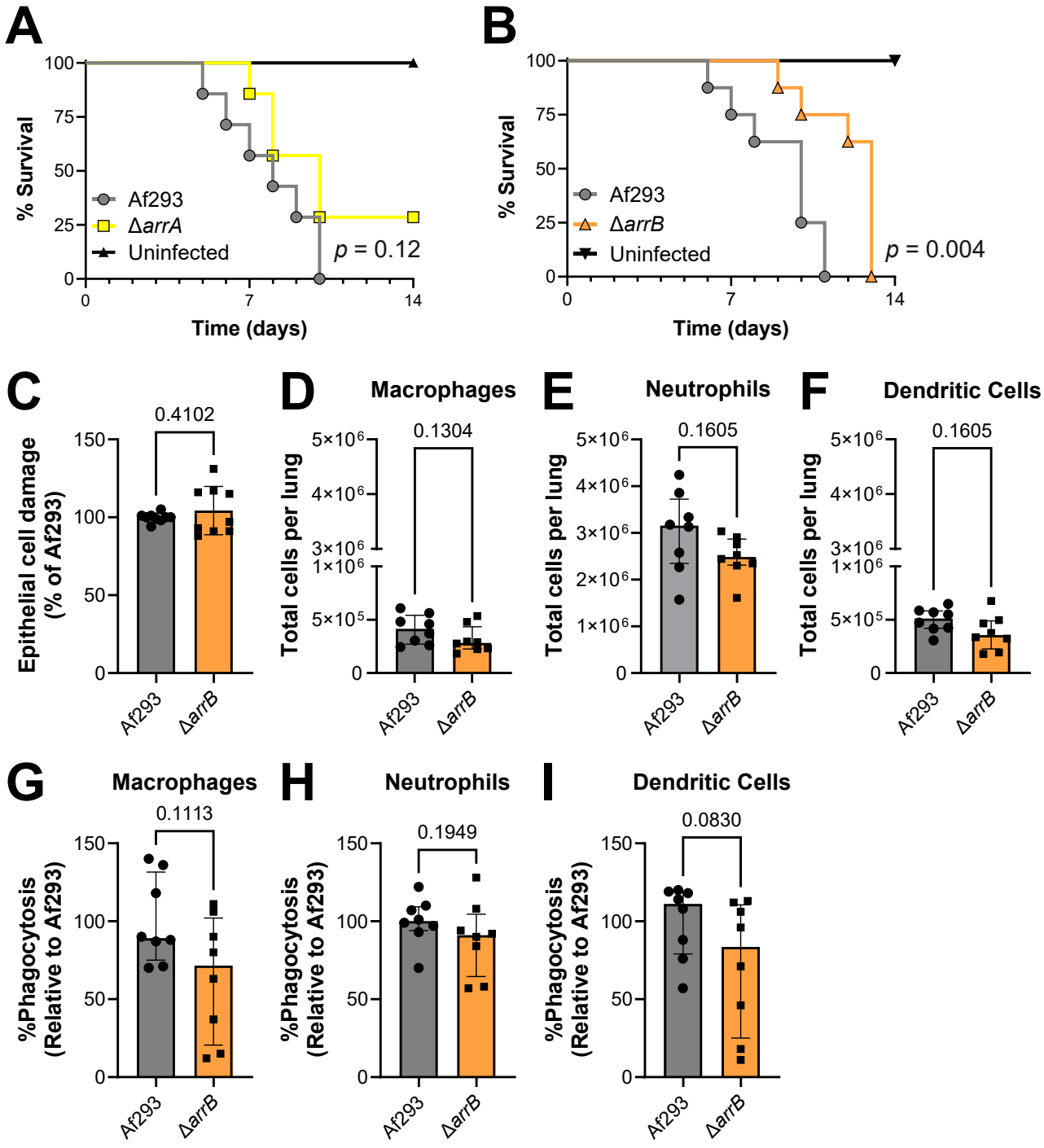


Figure S6. (A) Survival of corticosteroid immunosuppressed mice inoculated with the Δ*arrA* mutant. (B) Survival of mice inoculated with a second, independent Δ*arrB* mutant. Results are from single experiments with 7 mice per strain in (A) and 8 mice per strain in (B). Survival data were analyzed using the Log rank test. (C) Damage to A549 pulmonary epithelial cells caused by the Δ*arrB* mutant. Results are mean ± SD of 3 experiments, each performed in triplicate, analyzed by one-way ANOVA with Šídák's multiple comparisons test. (D-I) Corticosteroid immunosuppressed mice were inoculated with Af293 and the Δ*arrB* mutant. After 18 h, the mice were sacrificed and the number of macrophages (D), neutrophils (E), and dendritic cells (F) in the lungs was determined by flow cytometry, as was the percentage of macrophages (G), neutrophils (H), and dendritic cells (I) containing phagocytosed conidia. Results are median ± interquartile range of 9-10 mice per group in a single experiment. Data were analyzed using the Kruskal-Wallis test with Dunn's multiple comparisons test.