

Figure S1

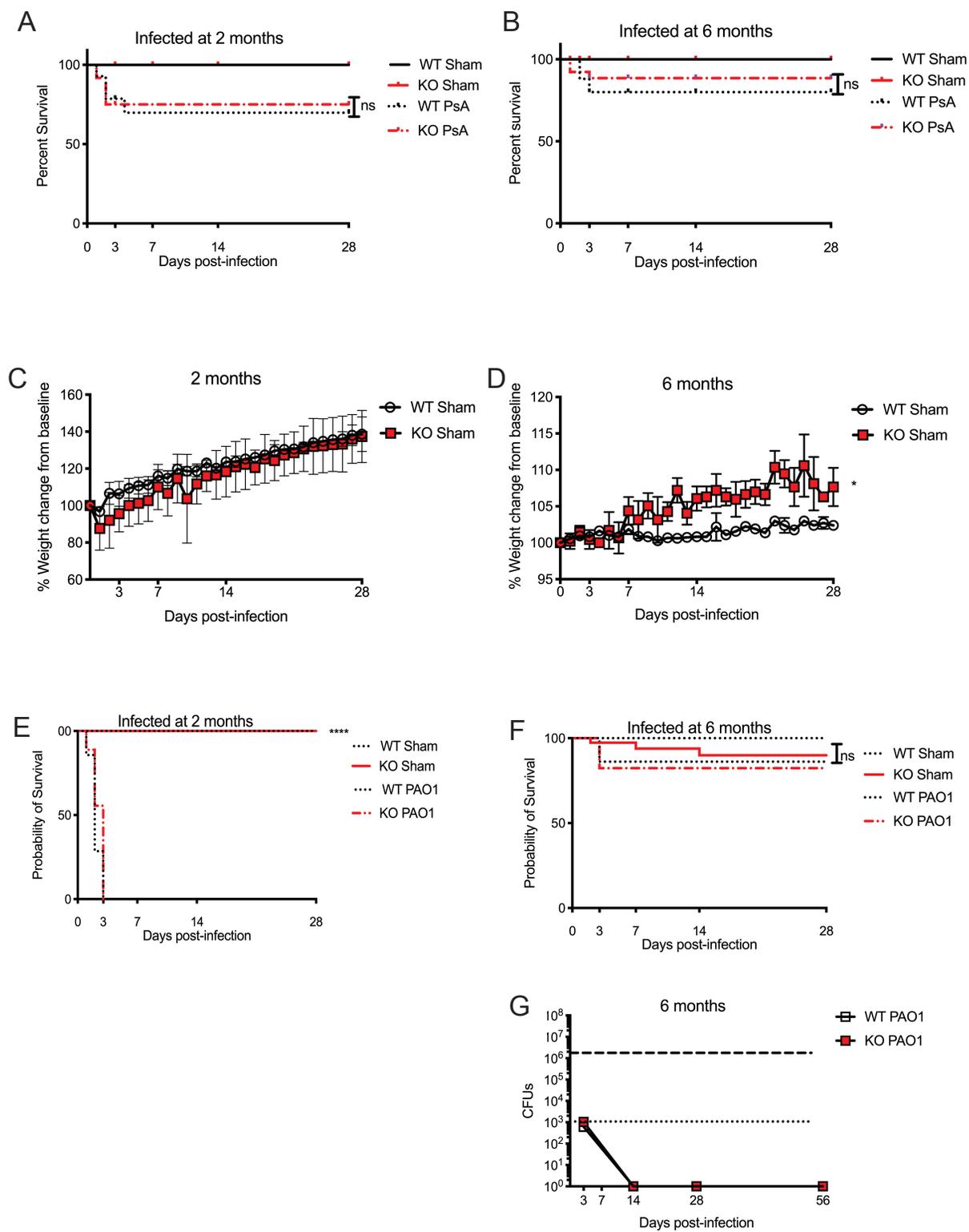


Figure S2

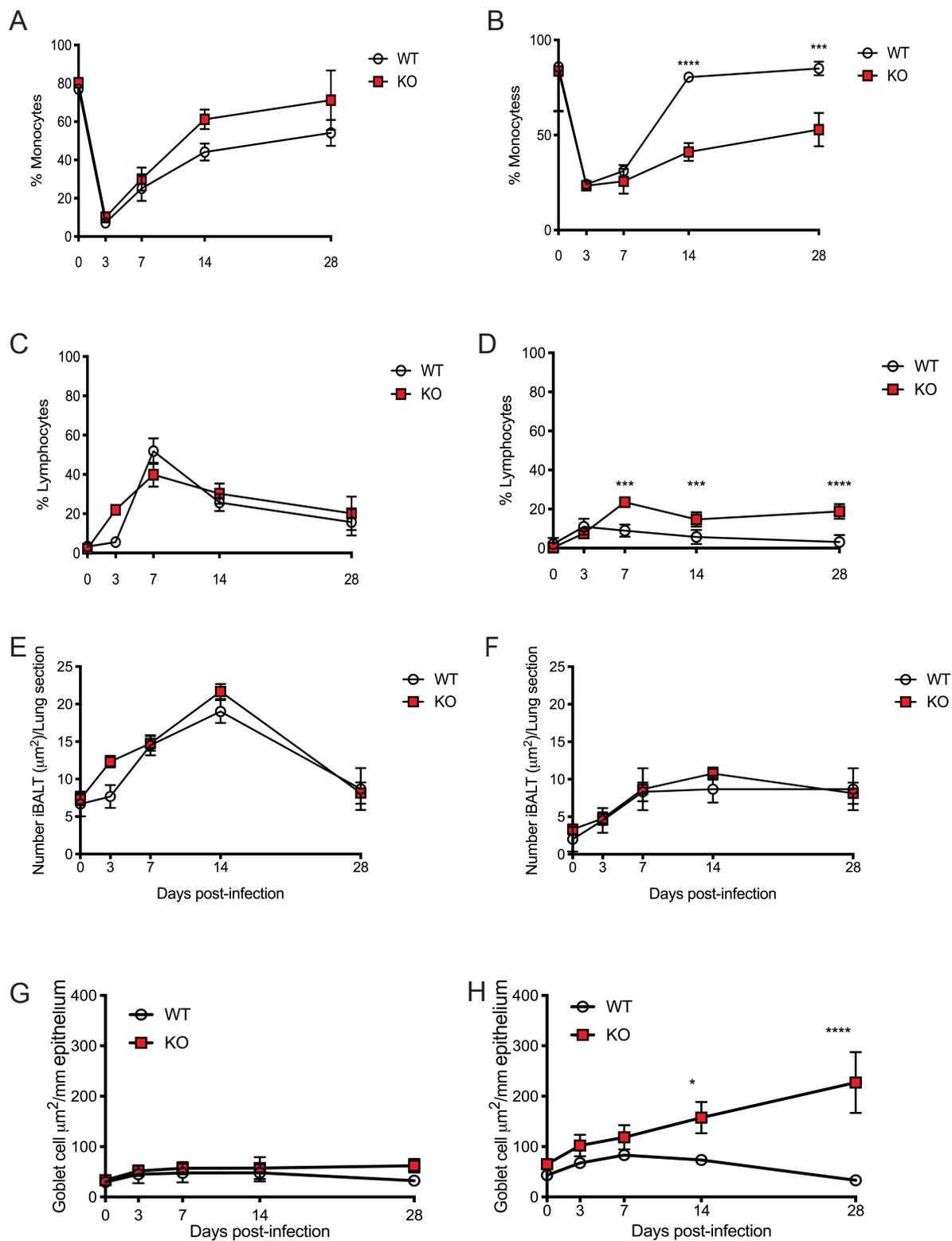


Figure S3

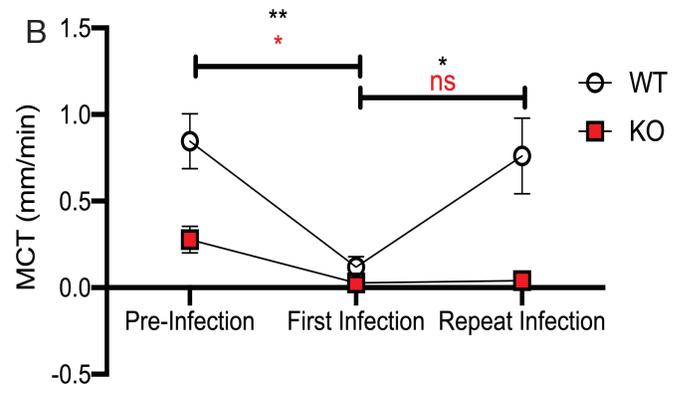
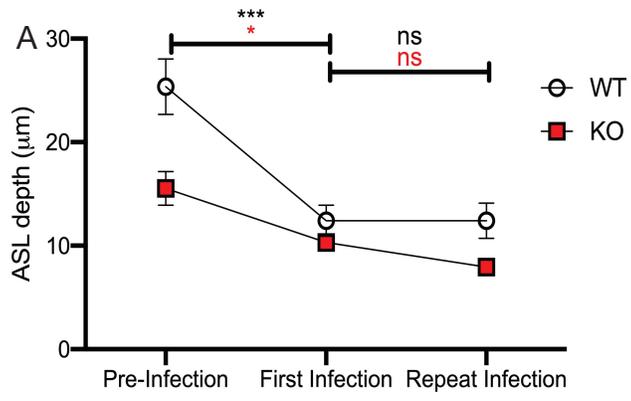
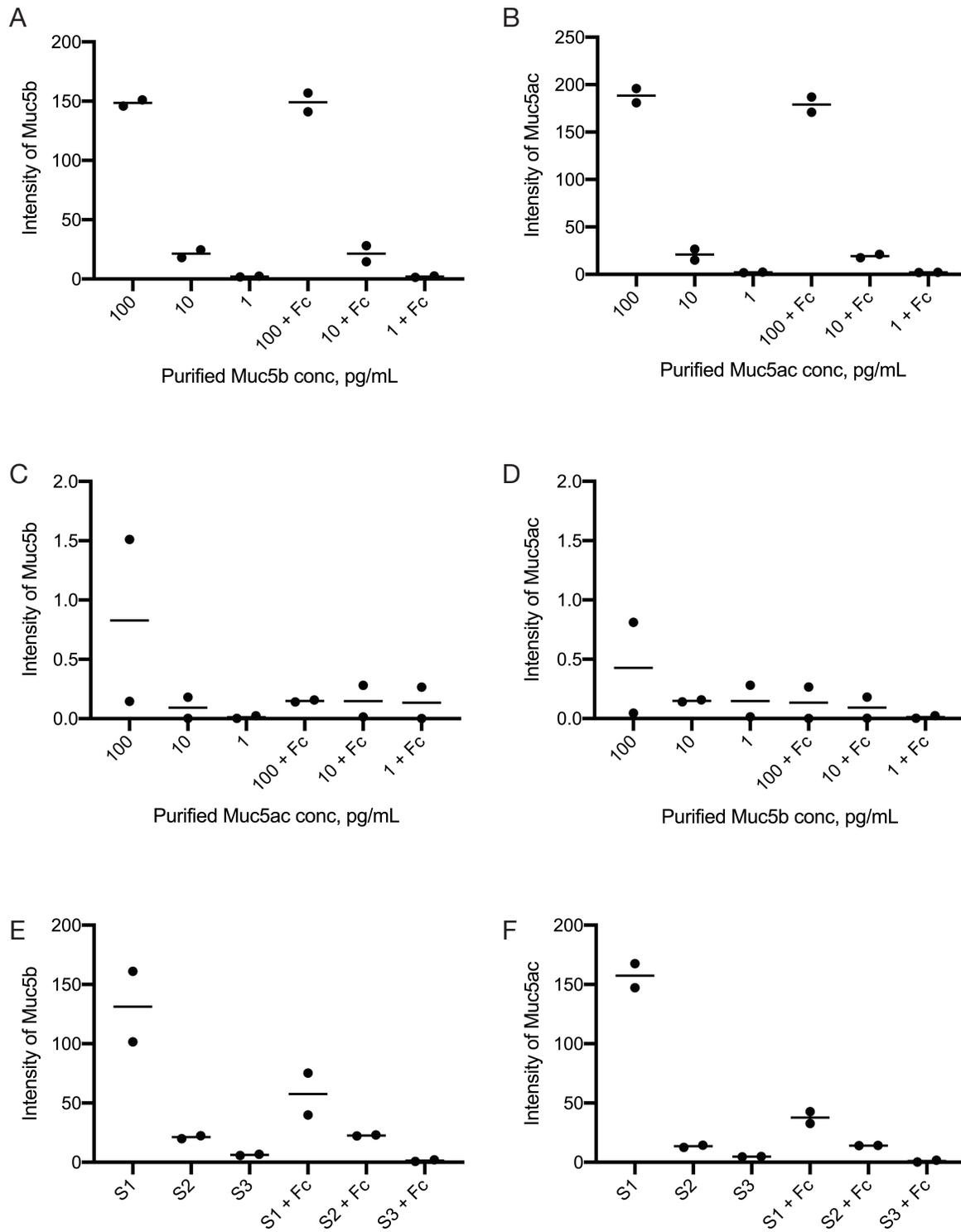


Figure S4



**Figure S1.** A) Survival curves of 2 month old WT and KO rats infected with  $3 \times 10^6$  CFUs of mucoid *P. aeruginosa* PAM57-15. B) Survival curves of 6 month old WT and KO rats infected with  $3 \times 10^6$  CFUs of mucoid *P. aeruginosa*. Survival curves are presented as total percent survival and were analyzed by Mantel-Cox log-rank test. C) Weight change from day 0 in 2 month old WT and KO that received sham infection. D) Weight change from day 0 in 6 month old WT and KO that received sham infection. E) Survival curves of 2 month old WT and KO rats infected with  $3 \times 10^6$  CFUs of PAO1, a non-lung adapted strain. F) Survival curves of 6 month old WT and KO rats infected with  $3 \times 10^6$  CFUs of PAO1. G) CFUs collected from 6 month old WT and KO rats infected with  $3 \times 10^6$  CFUs of PAO1. None of the 2 month old rats infected survived to collection. Data are presented as mean  $\pm$  SEM and were analyzed via two-way ANOVA. \* $P < 0.05$ , \*\*\*\* $P < 0.0001$ . n = 6 - 8/ group.

**Figure S2.** Cells were collected from the airways of WT and KO rats via bronchoalveolar lavage and counted with differentiation. Percentages of monocytes from A) 2 month old and B) 6 month old rats and percentages of lymphocytes from C) 2 month old and D) 6 month old rats are reported. Morphometric analysis of the H&E slides yielded a number of induced bronchus associated lymphoid tissue (iBALT) per lung section in E) 2 month old and F) 6 month old rats. Morphometric analysis of the AB-PAS slides yielded a goblet cell area per length of basal lamina from G) 2 and H) 6 month old rats. Data are presented as mean  $\pm$  SEM and were analyzed via two-way ANOVA with Sidak post-test. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001. n = 5 - 6/ group.

**Figure S3.** From the re-infection experiment, A) ASL and B) MCT rates were measured in tracheae using  $\mu$ OCT imaging. Data are presented as mean  $\pm$  SEM. All data were analyzed via one-way ANOVA, with Tukey's post-test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ . n = 5 - 6/ group.

**Figure S4.** Mucin antibody validation. Recombinant purified rat Muc5b and rat Muc5ac were reconstituted at concentrations of 100, 10, and 1 pg/mL. Dot blots were performed in the presence and absence of Fc blocker to reduce potential non-specific staining. A) The anti-Muc5b primary antibody showed a dose-dependent signal, that was not changed in the presence of Fc block. B) The anti-Muc5ac primary antibody showed a dose-dependent signal, that was not changed in the presence of Fc block. C) Anti-Muc5b primary showed minimal signal against Muc5ac, while D) anti-Muc5ac primary antibody has similarly minimal signal against anti-Muc5b. To test the specificity of primary antibodies against more complex bronchoalveolar lavage, samples from 3 different *Pseudomonas aeruginosa*-infected rats were tested with and without Fc block. In both E) Muc5b signal and F) Muc5ac signal, Fc block was most effective at eliminating non-specific signal in the samples with the highest amount of protein content (S1). Data are presented as mean  $\pm$  SEM.