Supplemental data

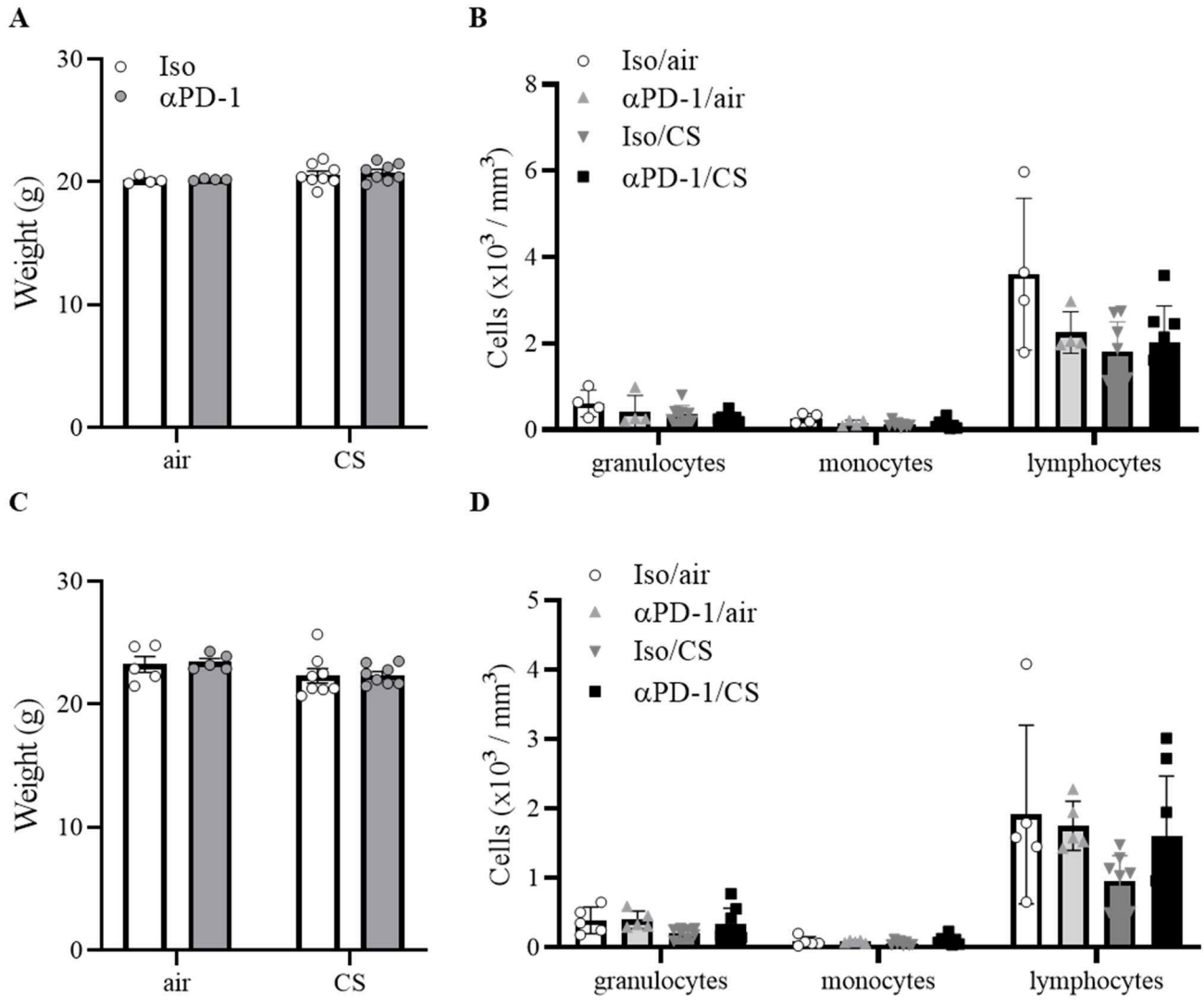

Figure S1. Anti-PD-1 treatment does not affect weight and leukocyte numbers of CS-exposed mice. C57BL/6 mice were exposed to CS for 4 (A/B) and 12 (C/D) weeks and treated with a PD-1-blocking antibody or an isotype antibody two times a week. (A/C) Weight of mice. (B/D) Leukocytes were counted and differentiated by light microscopy. Data are shown as the mean ± SEM. N=4-8 per group.

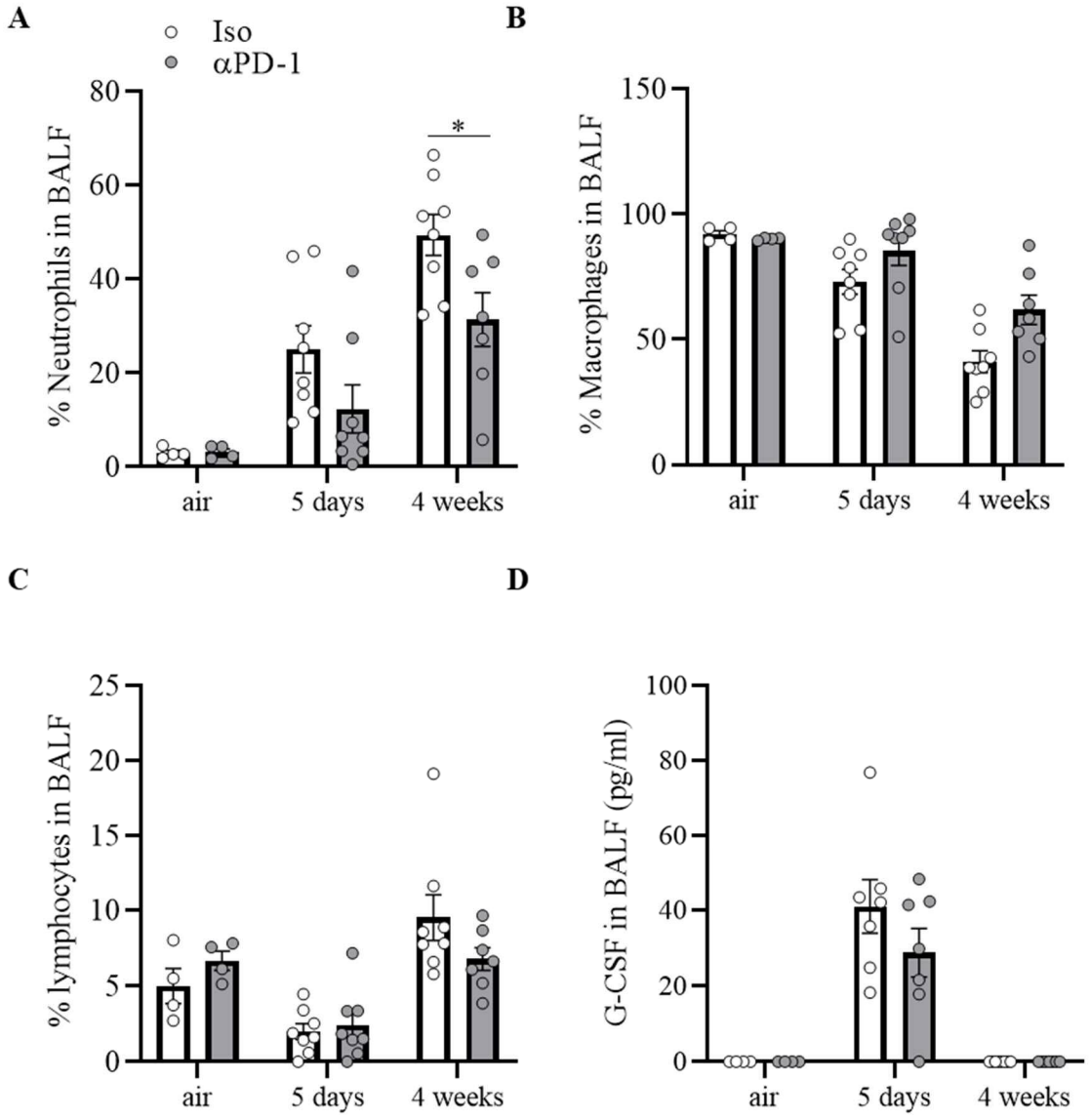

Figure S2. Anti-PD-1 treatment decreases CS-induced neutrophilic inflammation. C57BL/6 mice were exposed to CS 5 days a week for 5 days and 4 weeks and treated with a PD-1-blocking antibody or an isotype antibody two times a week. Percentage of (A) neutrophils, (B) macrophages, and (C) lymphocytes and concentrations of (D) G-CSF were determined in BAL fluids 24 hours after the final exposure to CS. N=4-8 per group. Data were compared by two-way ANOVA with Bonferroni post-test and are shown as the mean ± SEM. *$p < 0.05$.

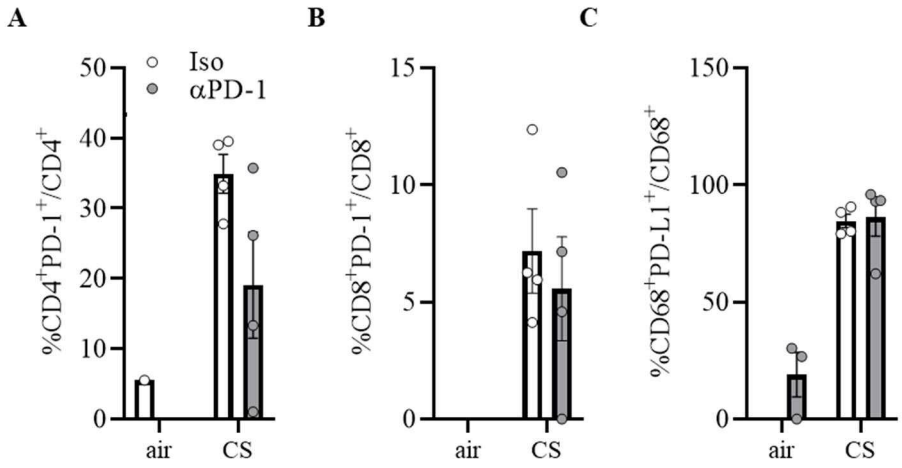

Figure S3. Percentage of PD-1-expressing $CD4^+$ and $CD8^+$ cells and PD-L1-expressing $CD68^+$ cells. C57BL/6 mice were exposed to CS 5 days a week for 12 weeks and treated with a PD-1-blocking antibody or an isotype antibody two times a week. Double immunohistochemical staining for (A) CD4/PD-1, (B) CD8 /PD-1, and (C) CD68/PD-L1 was performed and the percentage of cells positive for (A/B) PD-1 and (C) PD-L1 was calculated. N=3-4 per group. Results are presented for each mouse and are shown as the mean ± SEM.

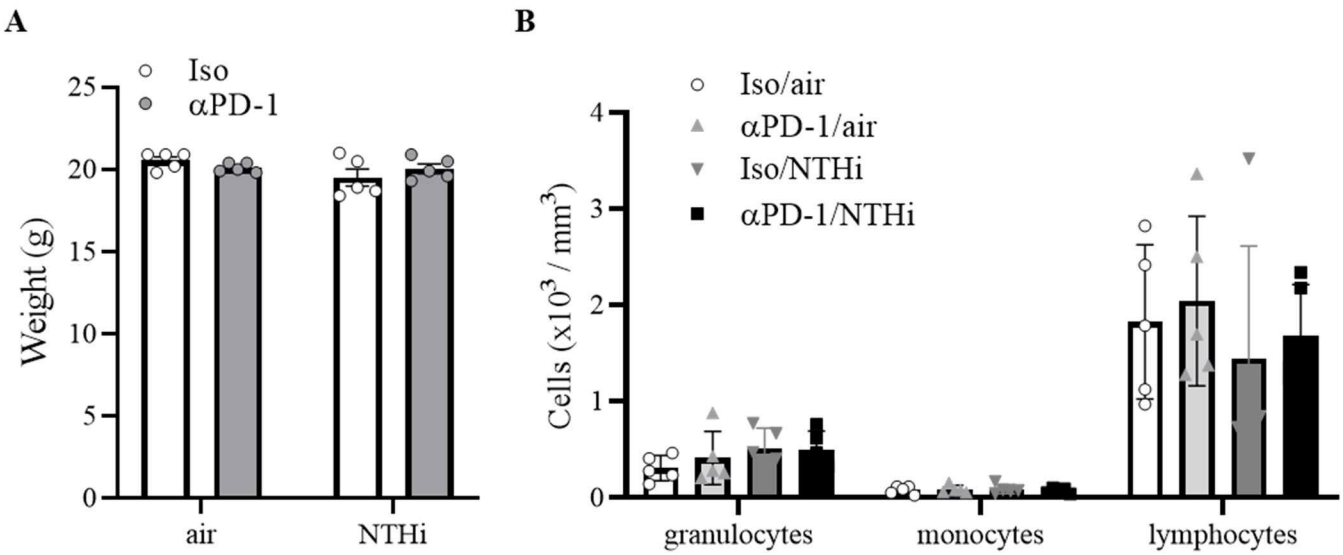

Figure S4. Anti-PD-1 treatment does not affect weight and leukocyte numbers of NTHi-exposed mice. C57BL/6 mice were exposed to NTHi 3 days a week for 4 weeks and treated with a PD-1-blocking antibody or an isotype antibody three times a week. (A) Weight of mice. (B) Leukocytes were counted and differentiated by light microscopy. N=5 per group. Data are shown as the mean ± SEM.

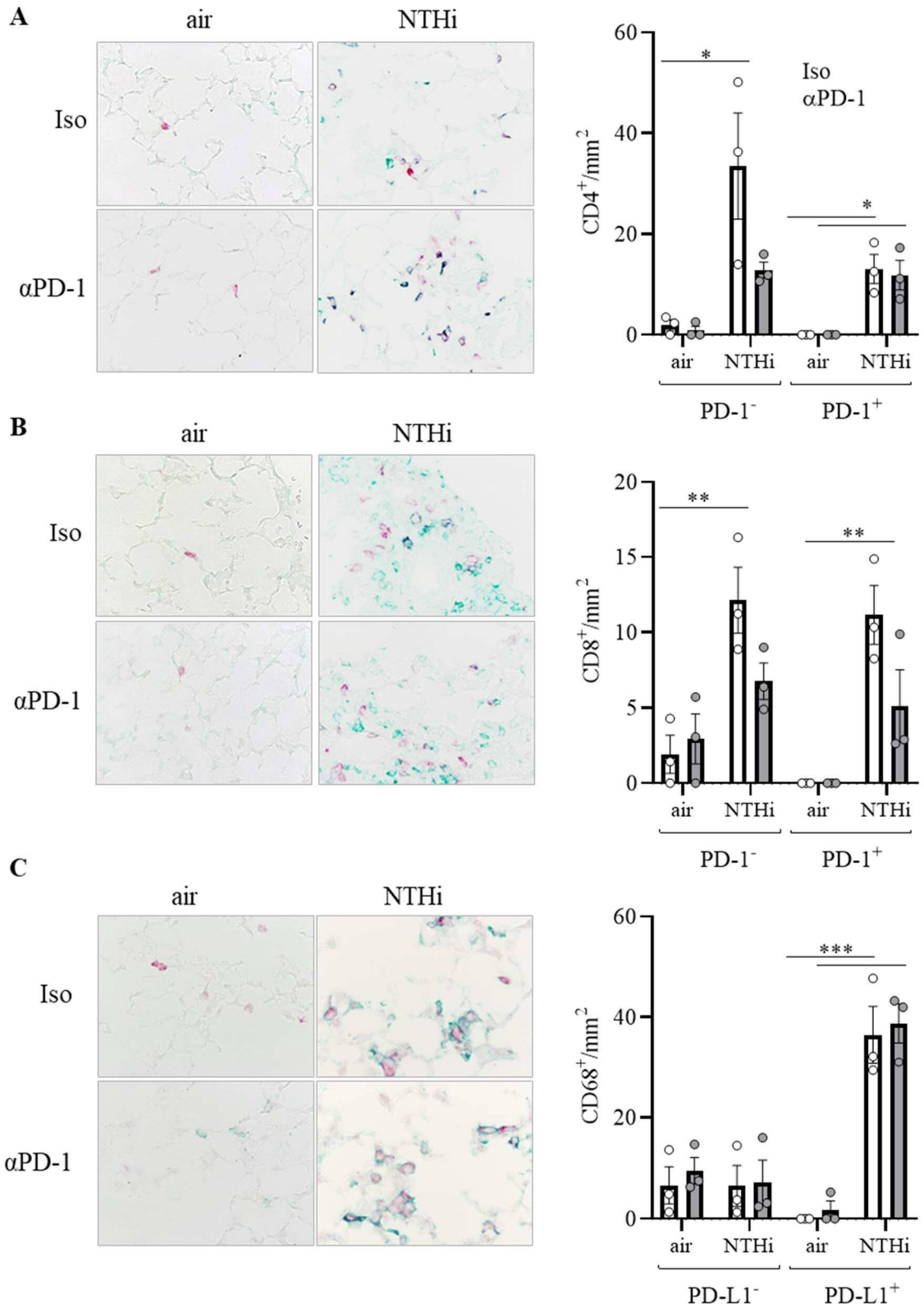

Figure S5. Numbers of PD-1-expressing $CD4^+$ and $CD8^+$ cells and PD-L1-expressing $CD68^+$ cells are increased in lungs of NTHi-exposed mice. C57BL/6 mice were exposed to NTHi 3 days a week for 4 weeks and treated with a PD-1-blocking antibody or an isotype antibody three times a week. Double

immunohistochemical staining (representative histology, scale bar: 20 μm) for (A) CD4 (red)/PD-1 (green), (B) CD8 (red)/PD-1 (green), and (C) CD68 (red)/PD-L1 (green), and quantification of the cells in lung parenchyma. N=3-4 per group. Results are presented for each mouse and were compared by two-way ANOVA with Bonferroni post-test and are shown as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$