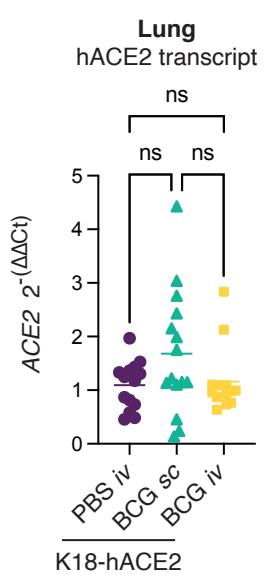


Supplemental Figures for:

Intravenous administration of BCG protects mice against lethal SARS-CoV-2 challenge

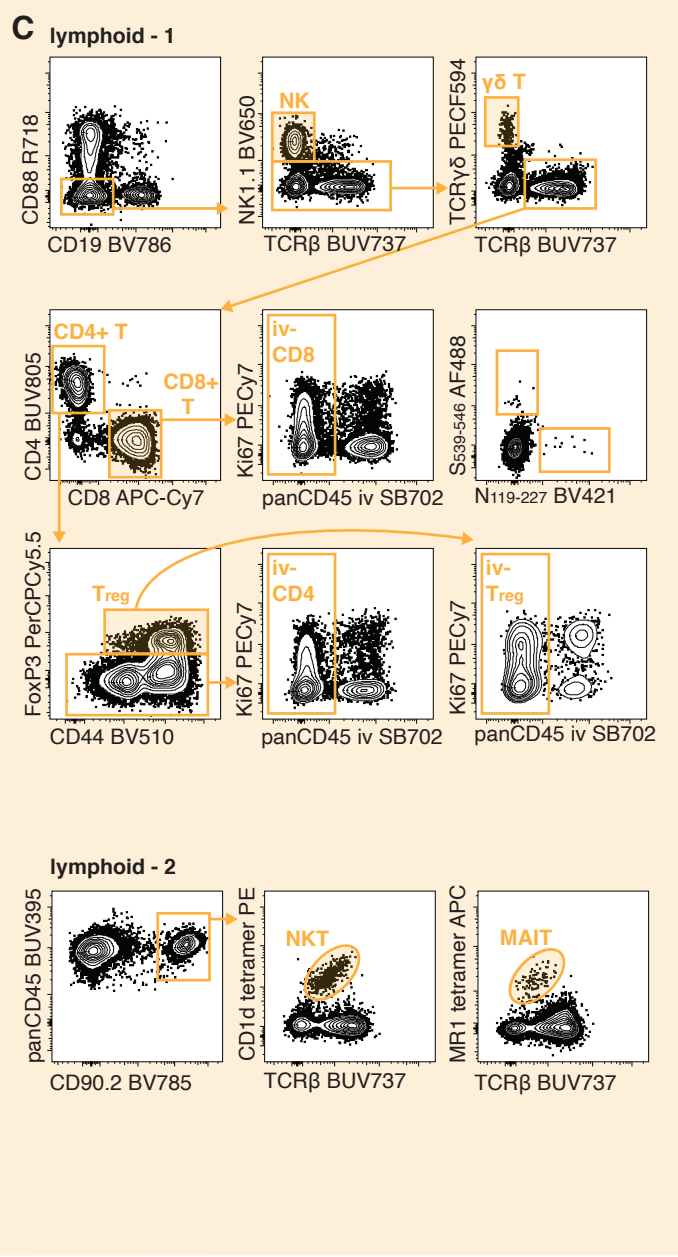
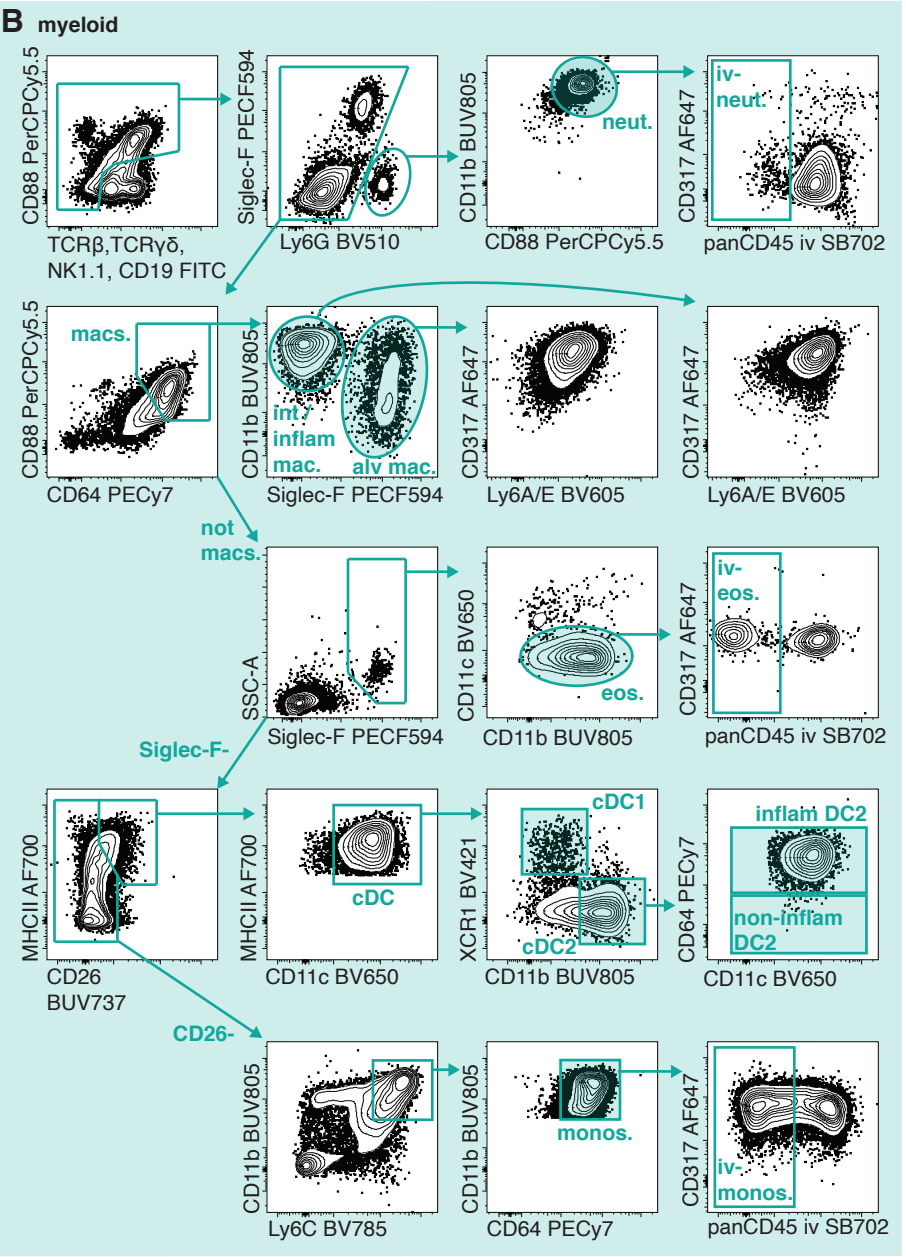
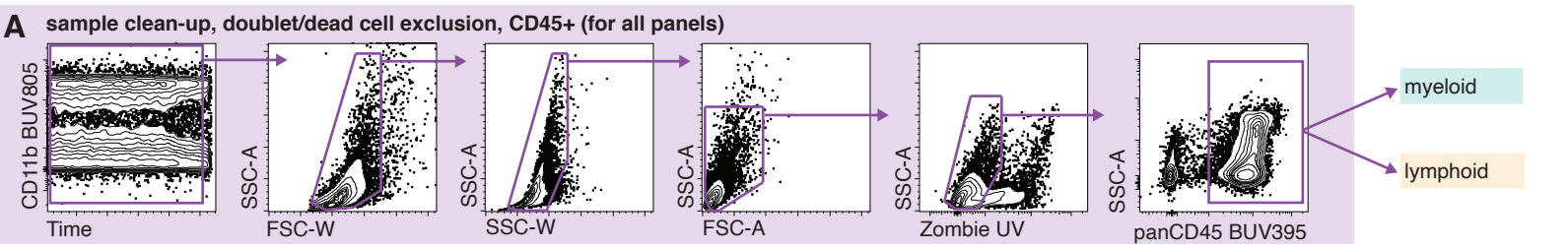
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Supplementary Fig 1: Prior *iv* BCG inoculation does not impact expression of hACE2.

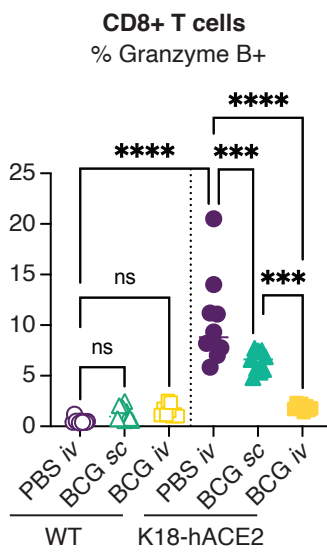
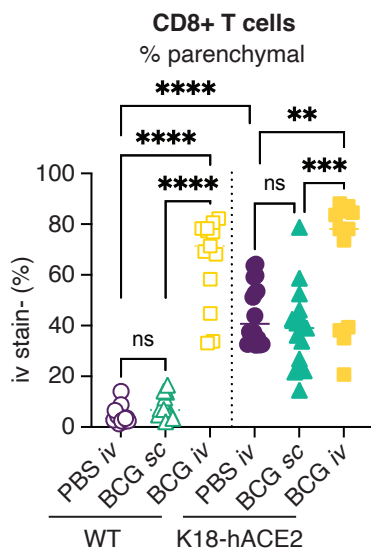
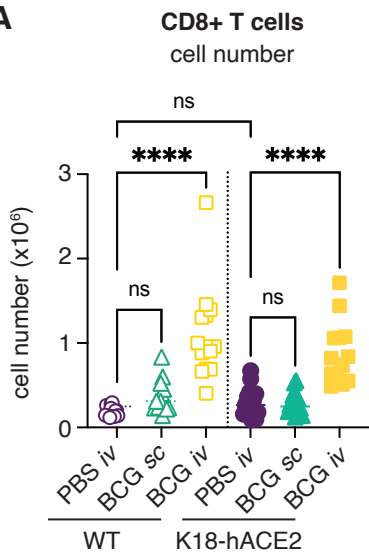
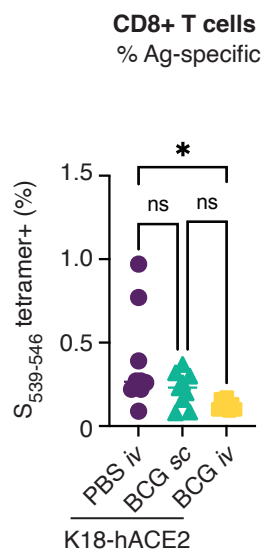
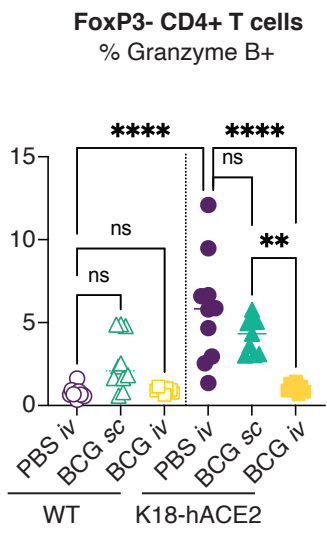
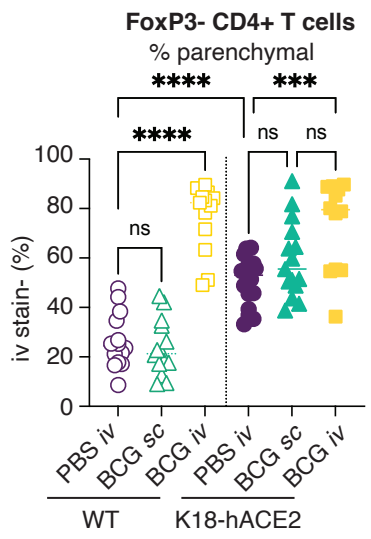
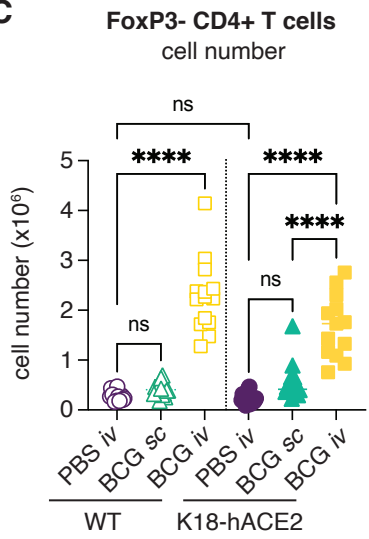
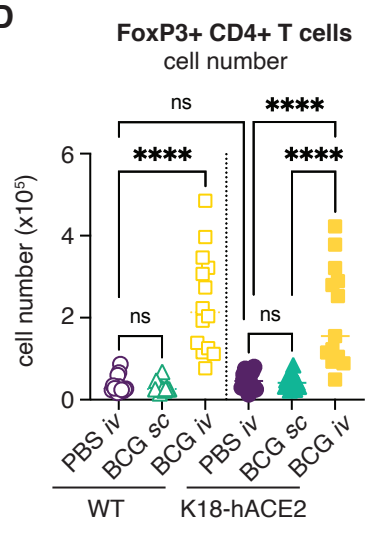
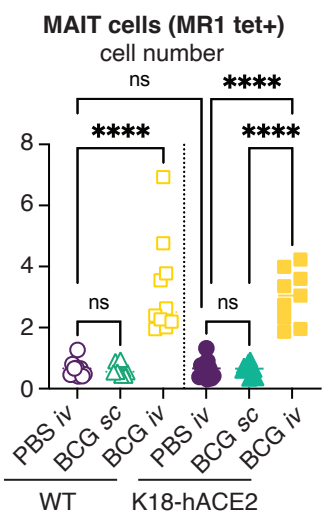
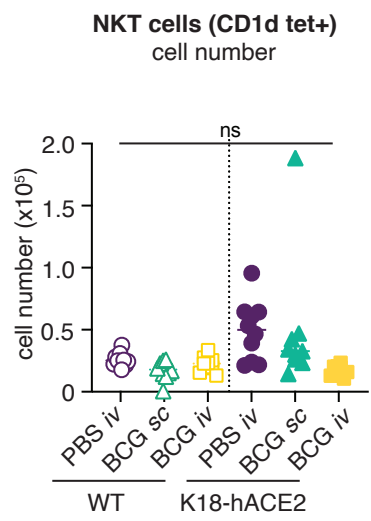
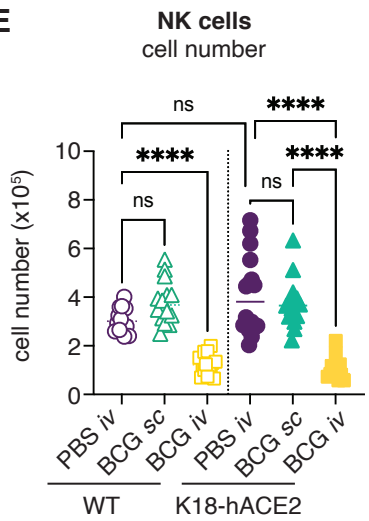
K18-hACE2 mice were inoculated with 10^6 CFU BCG Pasteur by subcutaneous (*sc*) or intravenous (*iv*) injection. Control animals received the same volume of PBS *iv*. At 42 days post BCG administration, mice were infected with 10^3 TCID₅₀ SARS-CoV-2 (WA1/2020) by intranasal instillation. Lungs were collected 5 days after viral challenge and assessed for expression of human ACE2 by RT-qPCR.

Statistical significance was assessed by One-Way ANOVA with Tukey post-test. ns $p > 0.05$. Data are pooled from 3 independent experiments each with 4-5 mice per group.



Supplementary Fig 2: Gating strategies for identifying cell types by flow cytometry.

(A-C) Single cell suspensions were prepared from the lungs of animals intravenously injected with a fluorescently labeled panCD45 antibody 3 minutes prior to euthanasia to enable identification of cells located within the pulmonary vasculature. Cell suspensions were stained with cocktails of fluorescent antibodies and analyzed by flow cytometry. **(A)** Gating strategy to identify CD45⁺, live, singlets. **(B)** Gating strategy to identify myeloid subsets by flow cytometry. **(C)** Gating strategies to identify lymphoid subsets by flow cytometry.

A**B****C****D****E**

Supplementary Fig 3: Prior *iv* BCG administration limits bystander cytotoxic responses in SARS-CoV-2 challenged mice.

(A-E) K18-hACE2 mice or non-transgenic littermate controls (WT) were inoculated with 10^6 CFU BCG Pasteur by subcutaneous (*sc*) or intravenous (*iv*) routes. Control animals received the same volume of PBS *iv*. At 42 days post BCG administration, mice were infected with 10^3 TCID₅₀ SARS-CoV-2 (WA1/2020) by intranasal instillation. Lungs were collected 5 days after viral challenge and assessed by flow cytometry. Gating strategy is shown in Supplementary Fig 2. **(A)** Number of CD8⁺ T cells, frequency of CD8⁺ T cells negative for *iv* stain and frequency of CD8⁺ T cells expressing Granzyme B. **(B)** Frequency of CD8⁺ T cells positive for the S539-546 tetramer. **(C)** Number of FoxP3⁻ CD4⁺ T cells, frequency of FoxP3⁻ CD4⁺ T cells negative for *iv* stain and frequency of FoxP3⁻ CD4⁺ T cells expressing Granzyme B. **(D)** Number of FoxP3⁺ CD4⁺ T cells. **(E)** Number of NK, NKT and MAIT cells. Statistical significance was assessed by One-Way ANOVA with Tukey post-test. ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$. Data are pooled from 3 independent experiments each with 4-5 mice per group.