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## Supplemental information

# BCG administration promotes the long-term protection afforded by a single-dose intranasal adenovirus-based SARS-CoV-2 vaccine

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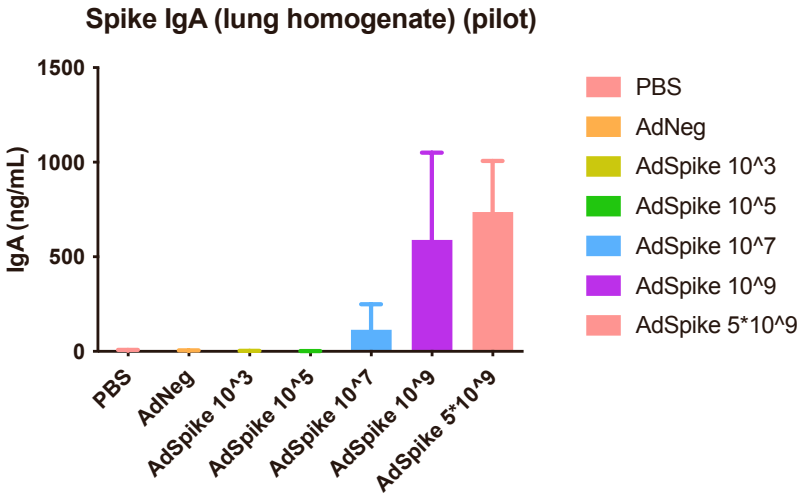
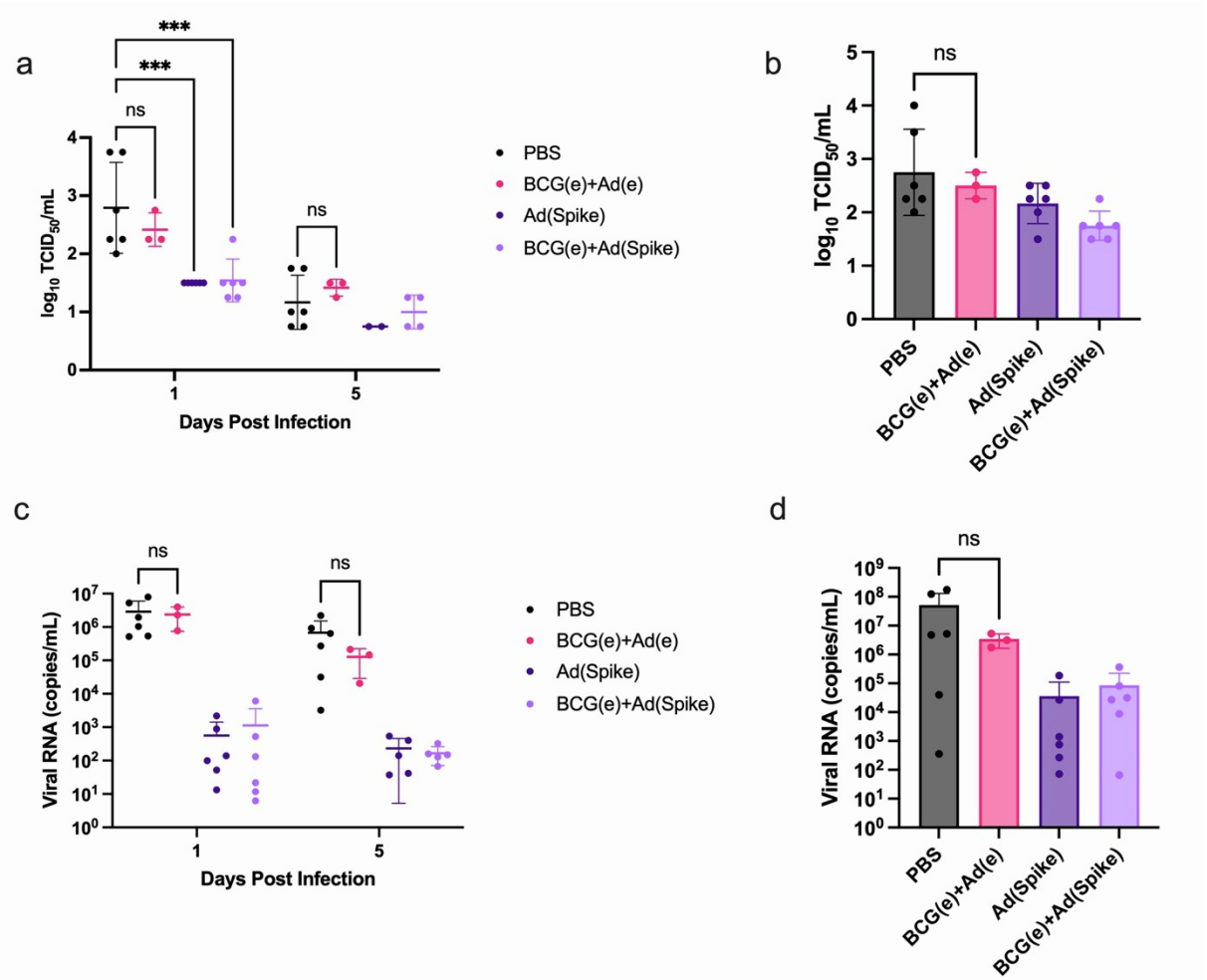
## Supplemental Figures



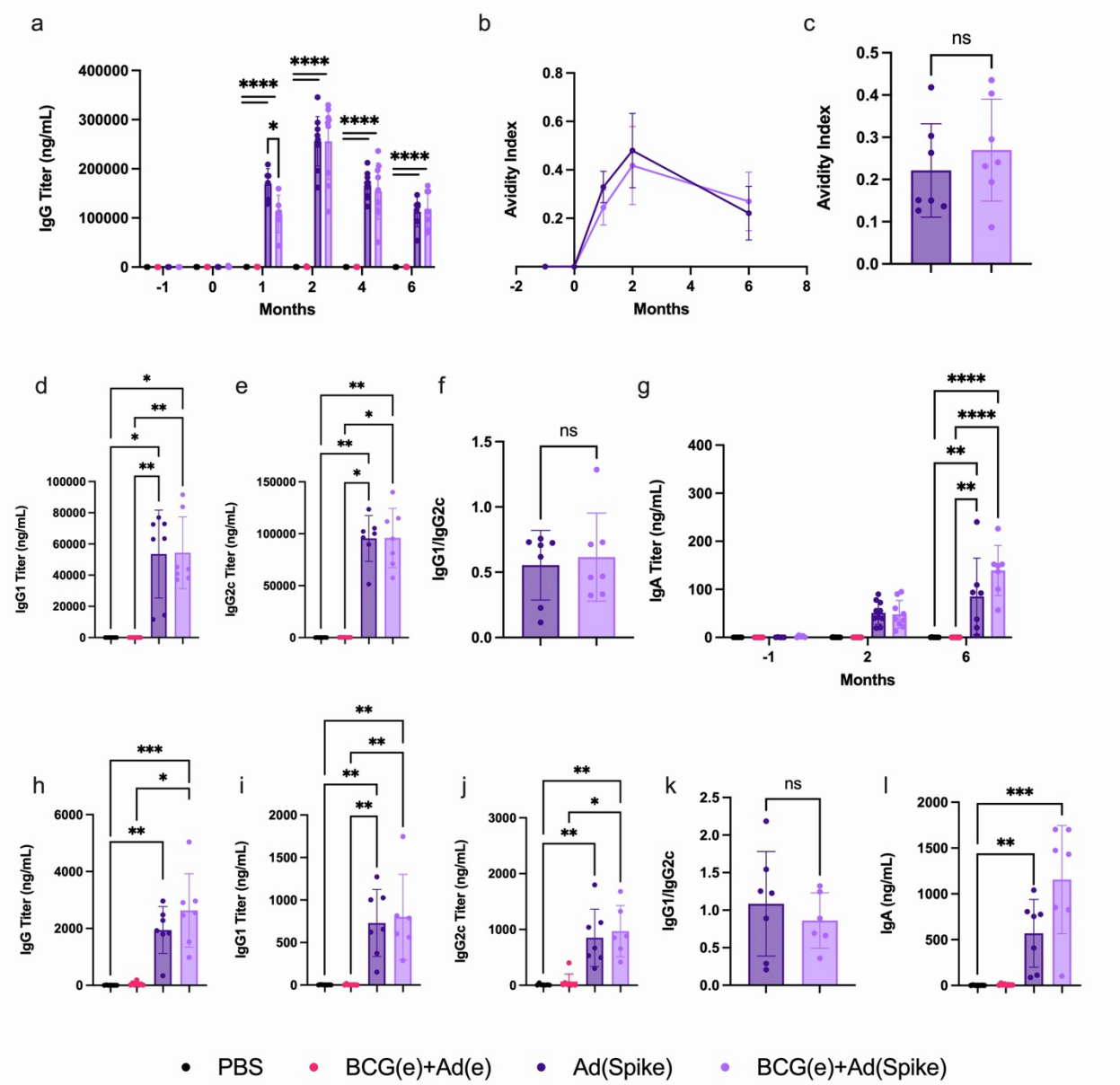

**Supplemental Figure 1. Induction of spike-specific lung IgA based on Ad(Spike) dosage (Related to Figure 1)**

A pilot study was run to determine optimal dosage of Ad(Spike) for intranasal administration. Nine weeks after immunization, animals were sacrificed, and spike-specific IgA was calculated in lung homogenates by ELISA. AdNeg=human adenovirus serotype 5 containing an empty gene cassette administered at $10^9$ $TCID_{50}$. All other doses are given in $TCID_{50}$. N=5. Data points represent individual mice, means ± SD are shown.

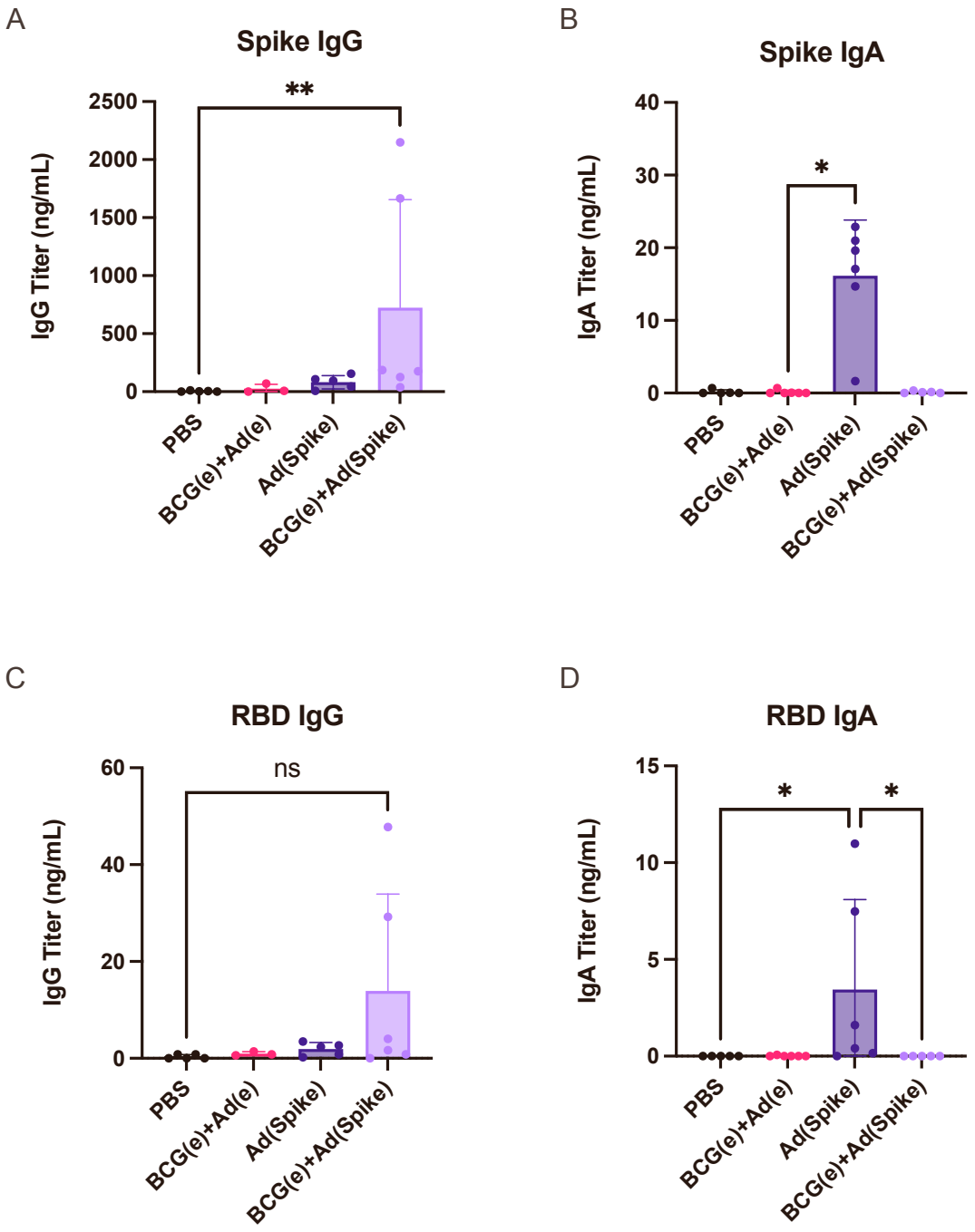



**Supplemental Figure 2. BCG(e) alone does not provide protection from SARS-CoV-2 infection in C57BL/6 mice (Related to Figure 2)**

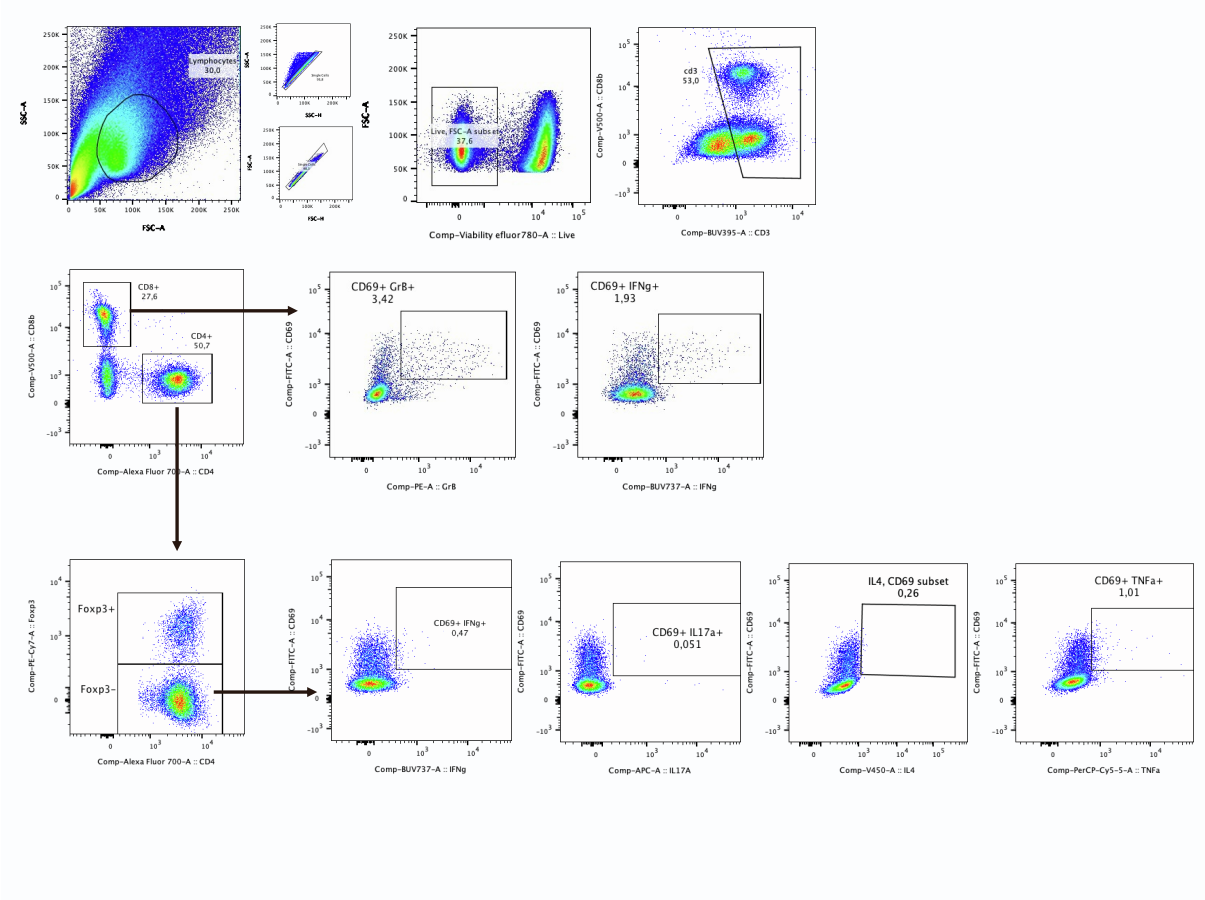
Mice were pre-immunized with $10^6$ BCG(e) or PBS i.p. at -1 month and immunized with either Ad(e) or Ad(Spike) i.n. at 0 months. Two months post-vaccination animals were infected with $10^6$ $TCID_{50}$ SARS-CoV-2 South African strain (B.1.351). Infectious viral load ($TCID_{50}$) in (a) oral swabs at 1 and 5 dpi, and (b) lungs at 5 dpi, quantified by the Spearman–Kärber method. Viral RNA in (c) oral swabs at 5 dpi and (d) lungs at 5 dpi. N=3-6; note that 3 animals died within the BCG(e)+Ad(e) group post-infection. Data points represent individual mice, means ± SD are shown. For (a), (c), Two-way ANOVA with Tukey's multiple comparisons: ***$p<0.001$; ns = not significant. For (b), (d), Kruskal-Wallis test with Dunn's multiple comparisons: ns = not significant.



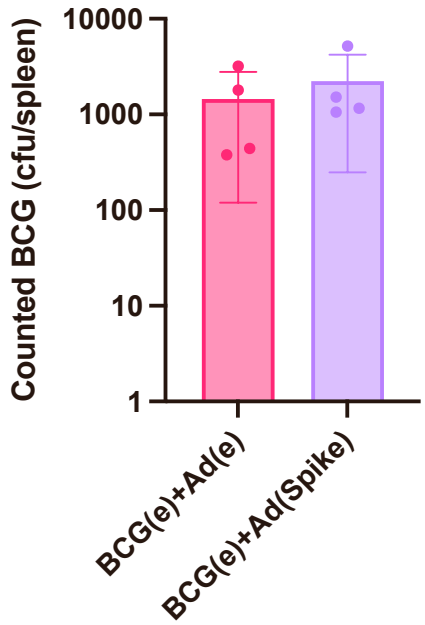

**Supplemental Figure 3. RBD-specific antibody response (Related to Figure 4)**
RBD-specific antibodies in the (a)-(g) serum and (h)-(l) bronchoalveolar lavage fluid (BALF). (a) IgG titers in mouse sera throughout the study schedule determined by ELISA. (b) IgG avidity index at -1, 0-, 1-, 2-, and 6-months post vaccination with 6 months shown in (c). (d) IgG1 and (e) IgG2c at 6 months post vaccination. The ratio of RBD-specific IgG1/IgG2c at 6 months post vaccination is given in (f). (g) IgA titers in mouse sera calculated at 0-, 3-, and 6-months post vaccination. N=7-10. Spike-specific (h) IgG, (i) IgG1, (j) IgG2c, (l) IgA in BALF at 6 months post vaccination calculated by ELISA with the ratio of IgG1/IgG2c given in (k). N=7. Data points represent individual mice, means ± SD are shown. For (a), (g), Two-way ANOVA with Tukey's multiple comparisons: *p<0.05;**p<0.01; ****p<0.0001. For (c), (k), One-way ANOVA with Tukey's multiple comparisons: ns = not significant. For (d)-(f), (h)-(j), (l), Kruskal-Wallis test with Dunn's multiple comparisons: *p<0.05; **p<0.01; ***p<0.001; ns = not significant.




**Supplemental Figure 4. Mouse turbinate immunoglobulins (Related to Figure 4)**
Mouse nares were washed and assessed for the presence of Spike-specific (a) IgG, and (b) IgA 6-months post vaccination, by ELISA. Washes were also assessed for the presence of RBD-specific (c) IgG, and (d) IgA. N=3-6. Data points represent individual mice, means ± SD are shown. For (a)-(d), Kruskal-Wallis test with Dunn's multiple comparisons: *p<0.05; **p<0.01; ns = not significant.

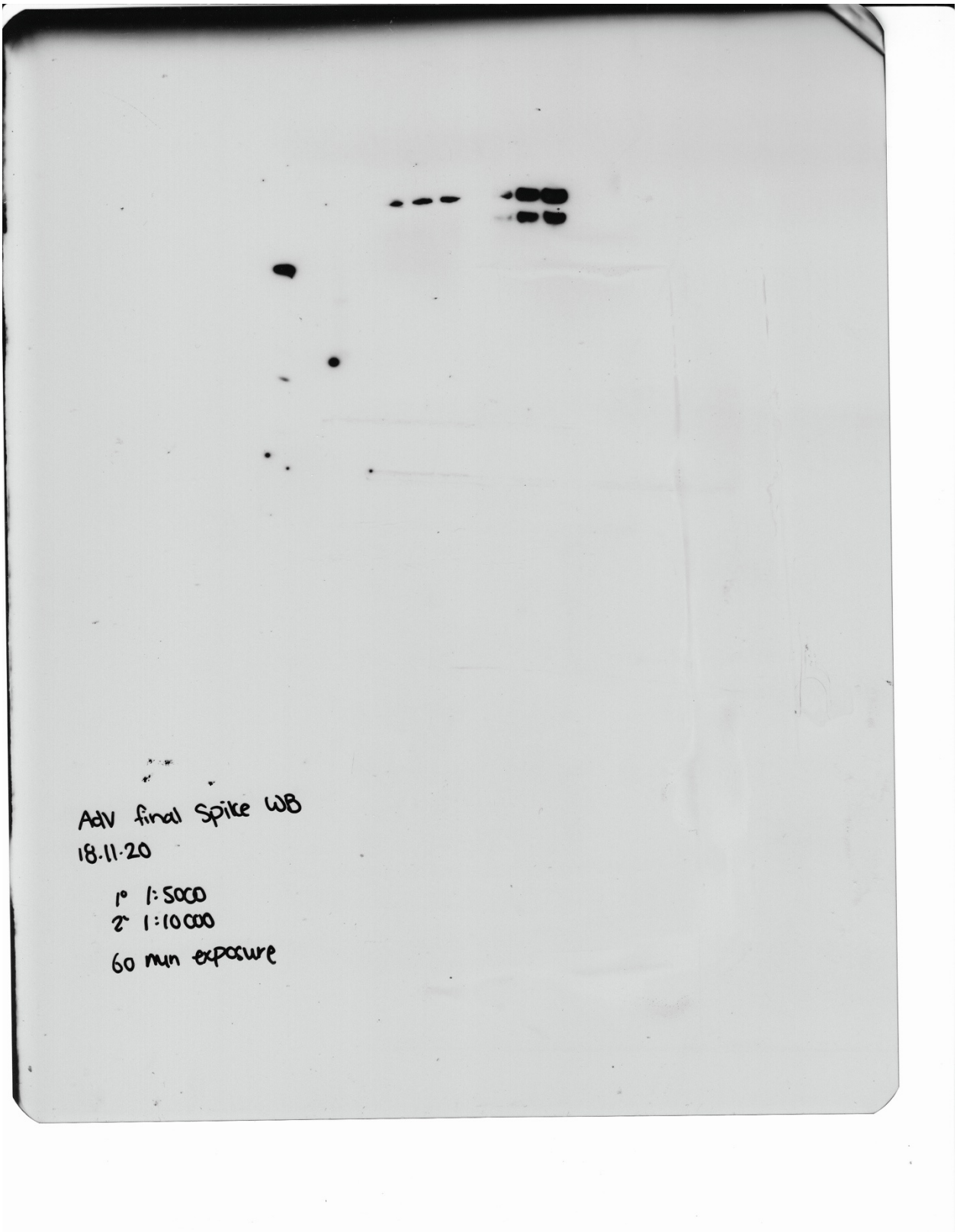



**Supplemental Figure 5. Flow cytometry gating strategy (Related to Figure 5)**
Lung cells were isolated 6 months after immunization with Ad(Spike) before restimulation with a pool of SARS-CoV-2 Spike CD8+ epitopes for 96 hours. During acquisition on a flow cytometer, cells were gated on lymphocytes, and then single cells. The live population was chosen and gated on CD3+ cells. CD4+ and CD8+ T cells were gated and CD8+ T cells were assessed on their expression of CD69+GrB+ and CD69+IFNg+. The CD4+ cells were gated as FoxP3- and assessed on their expression of CD69+IFNg+, CD69+IL17a+, CD69+IL4+, CD69+TNFa+. (IFNg=IFNɣ, TNFa=TNFα, GrB=Granzyme B).




**Supplemental Figure 6. BCG persists throughout long term challenge (Related to Figure 2 and Discussion)**

Animals which were pre-immunized with BCG(e) (-1 months) and vaccinated with either Ad(e) or Ad(Spike) (0 months) were euthanized 6 months post-vaccination. Upon necropsy, spleens were isolated, homogenized and then plated on Middlebrook 7H11/OADC for BCG quantification. Both groups show viable BCG present in the spleens. Data is represented as means ± SD with each point being an individual mouse. N=4.




**Supplemental Figure 7. Full Ad(Spike) Western Blot (Related to Figure 1)**

Western blot conducted against cell lysates from Ad(Spike) and a second adenovirus, containing a mutated version of the S-protein (not used in this study). This western blot was run with a protein ladder and negative control: adenovirus containing an empty gene cassette, termed Ad(e).