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Supplementary Materials for

Fractionating a COVID-19 Ad5-vectored vaccine improves virus-specific immunity

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The PDF file includes:

Figs. S1 to S7

Other Supplementary Material for this manuscript includes the following:

Data files S1 to S3

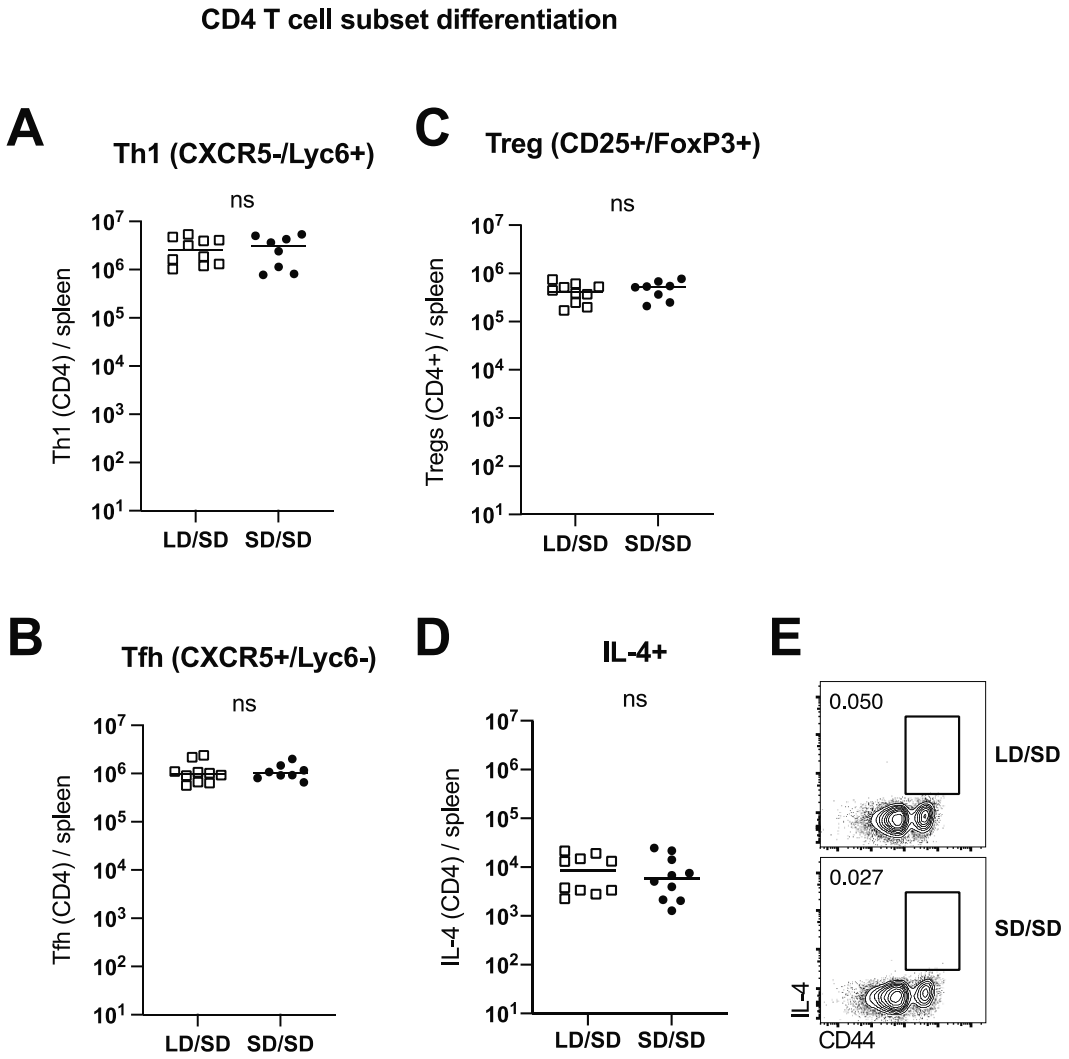

Fig. S1. CD4$^+$ T cell subset differentiation following Ad5 vaccine prime fractionation. (**A**) Summary of Th1 responses. (**B**) Summary of T follicular helper (Tfh) responses. (**C**) Summary of T regulatory (Treg) cell responses. (**D**) Summary of IL-4+ responses. (**E**) Representative FACS plots showing the frequencies of IL-4+ responses (gated from live CD4$^+$ T cells). We show FACS plots to highlight that IL-4 expression appeared to be near the limit of detection. In panels D-E, splenocytes were incubated with overlapping SARS-CoV-2 peptide pools in the presence of GolgiStop and GolgiPlug to detect IL-4+ CD4$^+$ T cell responses. All data are from spleen. Data from panels A-C are from two experiments, one with n=5 per group, and another with n=3-5 per group. Data from panels D-E are from two experiments, each with n=5 per group. Data are from day 14 post-boost. All data are shown. Not significant (ns) *P* values were determined by parametric test (unpaired t test).

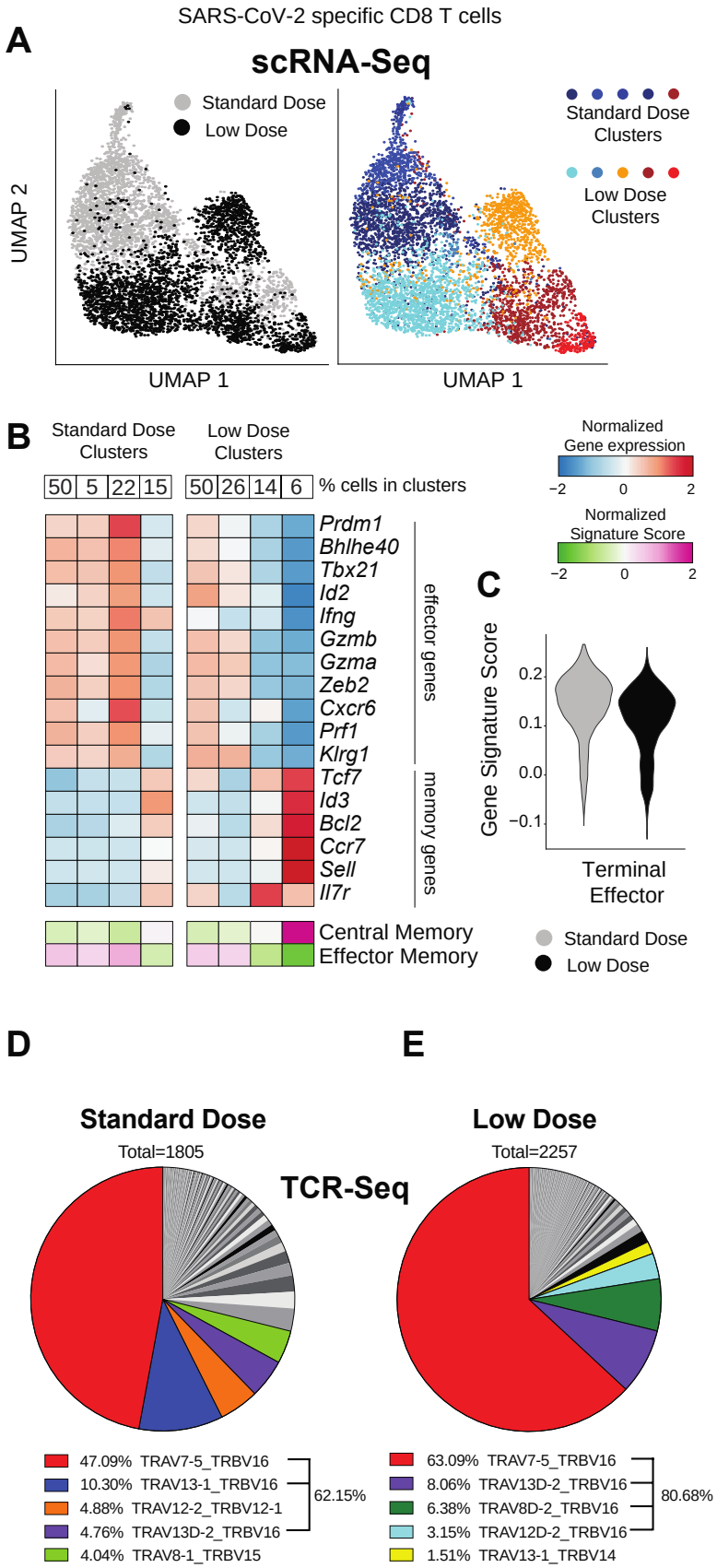

Fig. S2. Single cell RNA-seq analyses demonstrate that a low dose prime favors central memory CD8+ T cell differentiation. Mice were immunized with 10^6 or 10^9 PFU of Ad5-SARS-2 spike, and at day 28, splenic $CD8^+$ T cells were MACS-sorted. Subsequently, live, CD8+, CD44+, K^b VL8+ cells were FACS-sorted to ~99% purity for scRNA-seq (A-C) and scTCR-seq (D-E). (**A**) UMAP plots showing populations colored by regimen (left plot). Standard and low dose cells were clustered separately and UMAP (right plot) shows unsupervised cell clusters. (**B**) Heatmap showing row-standardized expression of selected effector and memory genes or gene signatures (bottom rows). For each population, percentages of cells in each cluster are indicated (top row). (**C**) Violin plot showing the normalized expression of the Terminal Effector signature in the SD and LD populations. Experiment in panels A-C was performed once with a total of 2,574 and 2,837 individual cells from a spleen per group (LD and SD, respectively). (**D**) Pie chart showing the distribution of TCRa and TCRb gene usage after SD prime. (**E**) Pie chart showing the distribution of TCRa and TCRb gene usage after LD prime. Experiment in panels D-E was performed once with a total of 2,032 and 2,431 individual cells from a spleen per group (LD and SD, respectively). Total number above the pie chart show the number of single cells selected for downstream analyses, and different colors below highlight the top 5 TCR usages and their relative proportion.

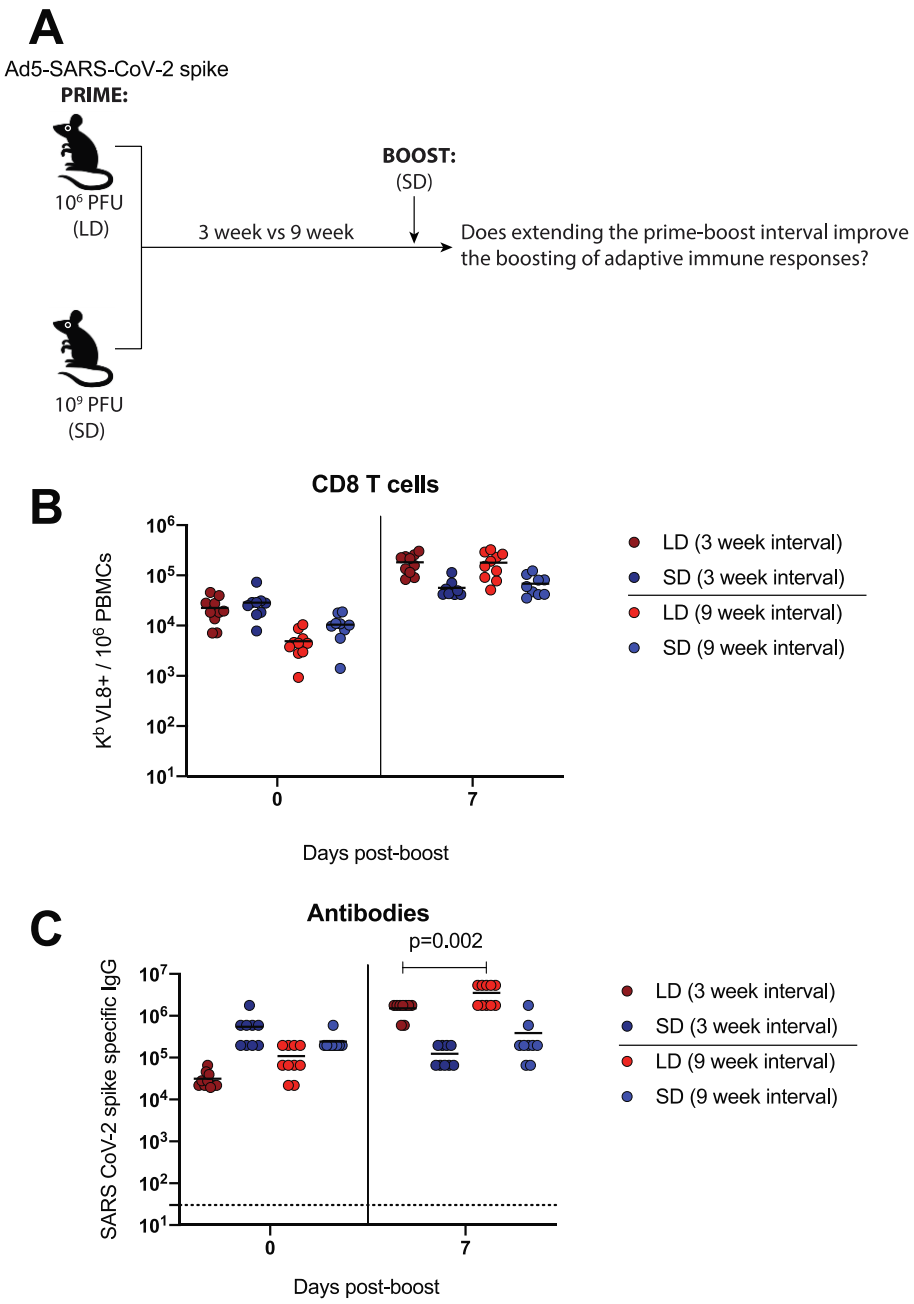

Fig. S3. Effect of extending the prime-boost interval on CD8+ T cells and antibody responses. (**A**) Experimental approach for evaluating how the prime-boost interval affects adaptive immune responses following Ad5 vector immunization. (**B**) Summary of SARS-CoV-2-specific $CD8^+$ T cell responses in PBMCs. (**C**) Summary of SARS-CoV-2 specific antibody responses in sera. Data are from two experiments, one with n=5 per group, and another with n=4-5 per group. All data are shown. Dashed line indicates limit of detection. Indicated *P* value was determined by two-way ANOVA (Dunnett's multiple comparisons test).

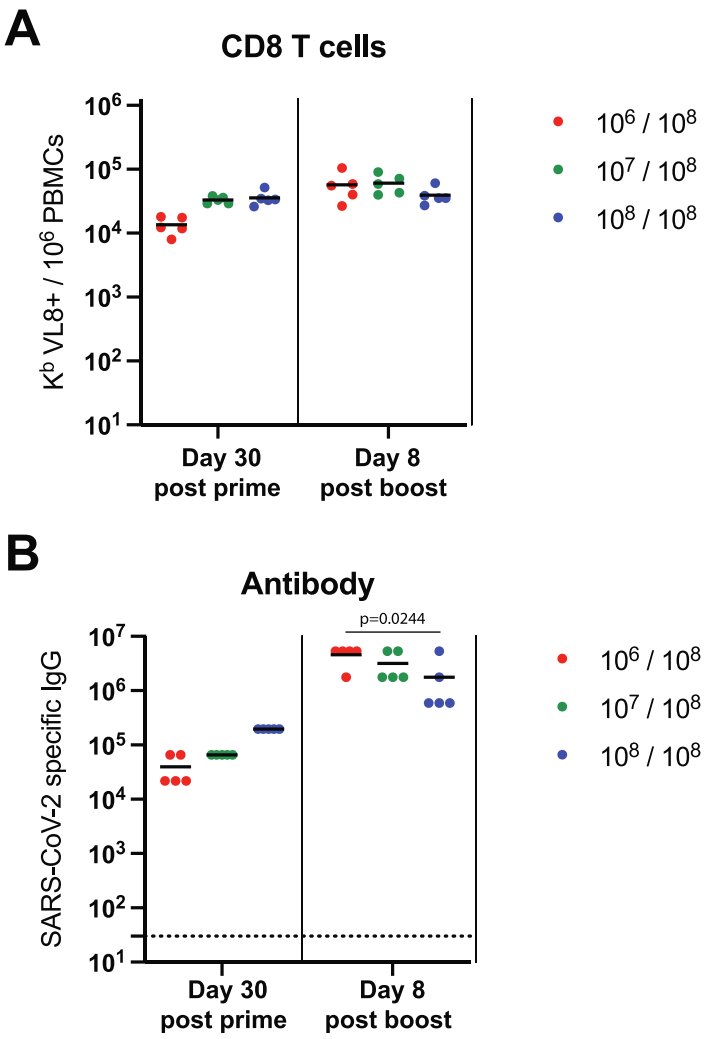

Fig. S4. Effect of "narrow" prime dose escalation on CD8+ T cells and antibody responses. (**A**) Summary of SARS-CoV-2-specific $CD8^+$ T cell responses in PBMCs. (**B**) Summary of SARS-CoV-2 specific antibody responses in sera. Mice were primed with 10^6 PFU, 10^7 PFU, or 10^8 PFU of Ad5-SARS-CoV-2 spike. All mice were boosted with 10^8 PFU of Ad5-SARS-CoV-2 spike after day 30 post-prime. Data are from one experiment with n=5 per group (three different priming groups). All data are shown. Dashed line indicates limit of detection. Indicated *P* value was determined by two-way ANOVA (Dunnett's multiple comparisons test).

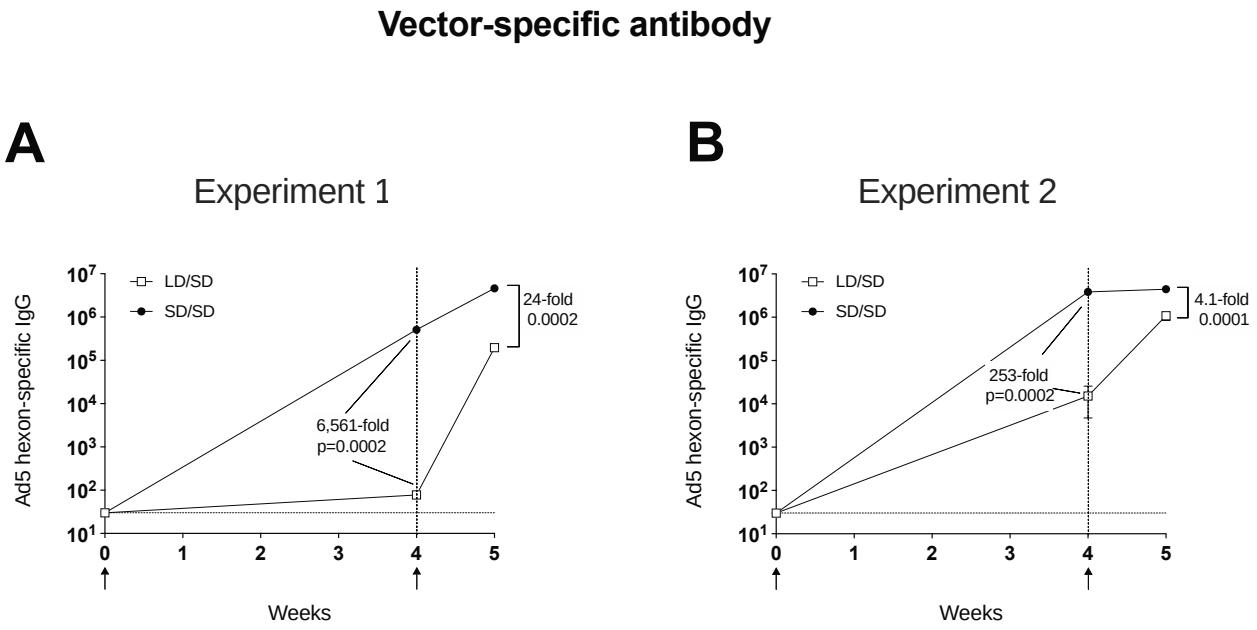

Fig. S5. Ad5 vector-specific antibody responses following Ad5 vaccine fractionation. (**A**) Summary of Ad5 hexon-specific antibody responses in sera (Experiment 1). (**B**) Summary of Ad5 hexon-specific antibody responses in sera (Experiment 2). Data are from two experiments, each with n=5 per group. All data are shown. Indicated *P* value was determined by parametric test (unpaired t test). Dashed line indicates limit of detection. Error bars represent SEM, but in these experiments little variability within groups was observed rendering some error bars small.

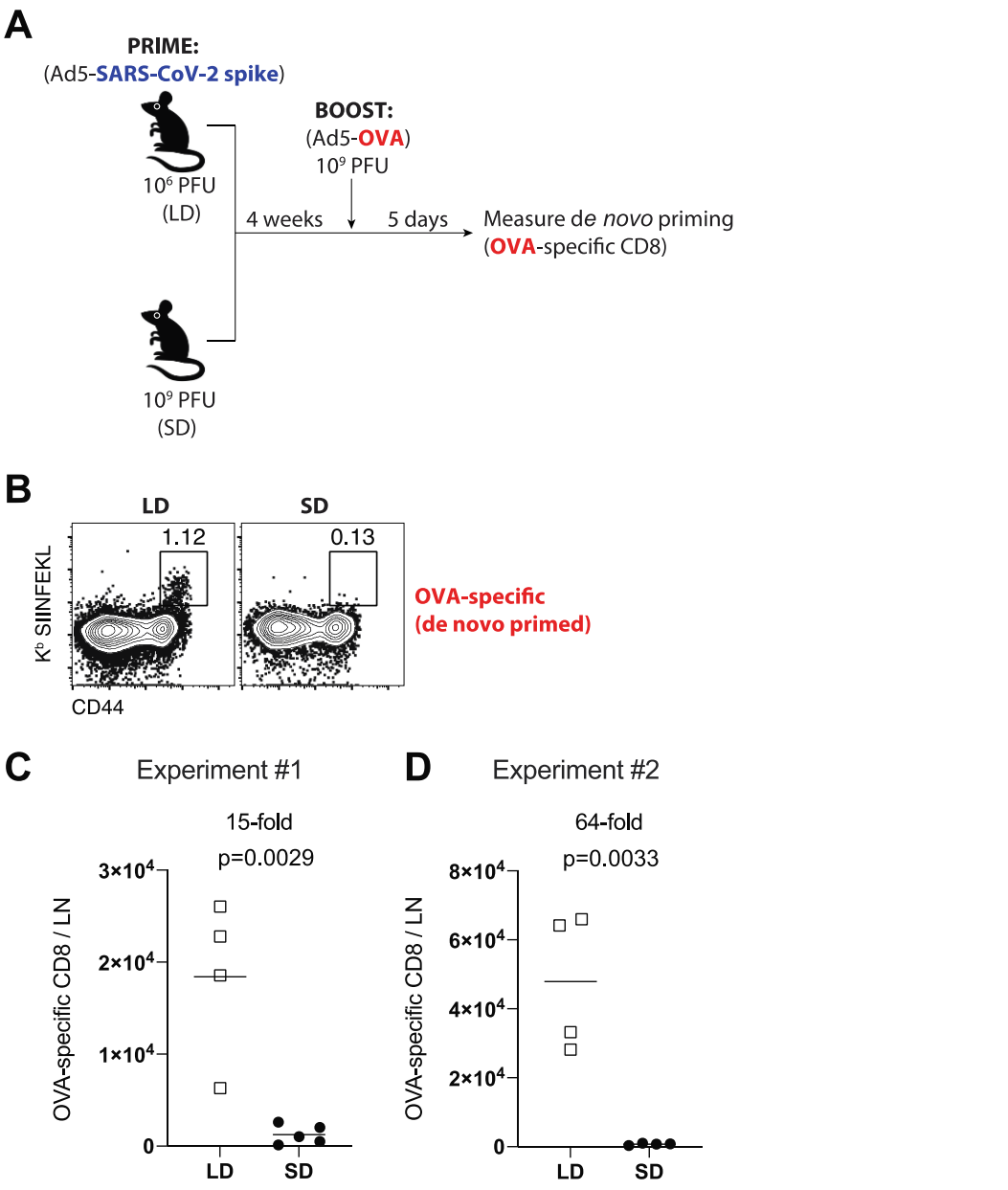

Fig. S6. Effect of an LD prime on de novo CD8+ T cell priming following a subsequent booster immunization. Effect of an LD Ad5 prime on de novo priming following a subsequent re-utilization of Ad5. (**A**) Experimental approach for evaluating how immunity raised by an LD prime affects de novo priming following subsequent re-utilization of Ad5. (**B**) Representative FACS plots showing the frequencies of OVA-specific CD8+ T cells (K^b SIINFEKL+) in draining lymph nodes. (**C**) Summary of OVA-specific CD8+ T cells in draining lymph nodes in the first experiment. (**D**) Summary of OVA-specific CD8+ T cells in draining lymph nodes in the second experiment. Data are from two experiments, each with n=5 per group. All data are shown. Indicated *P* value was determined by parametric test (unpaired t test).

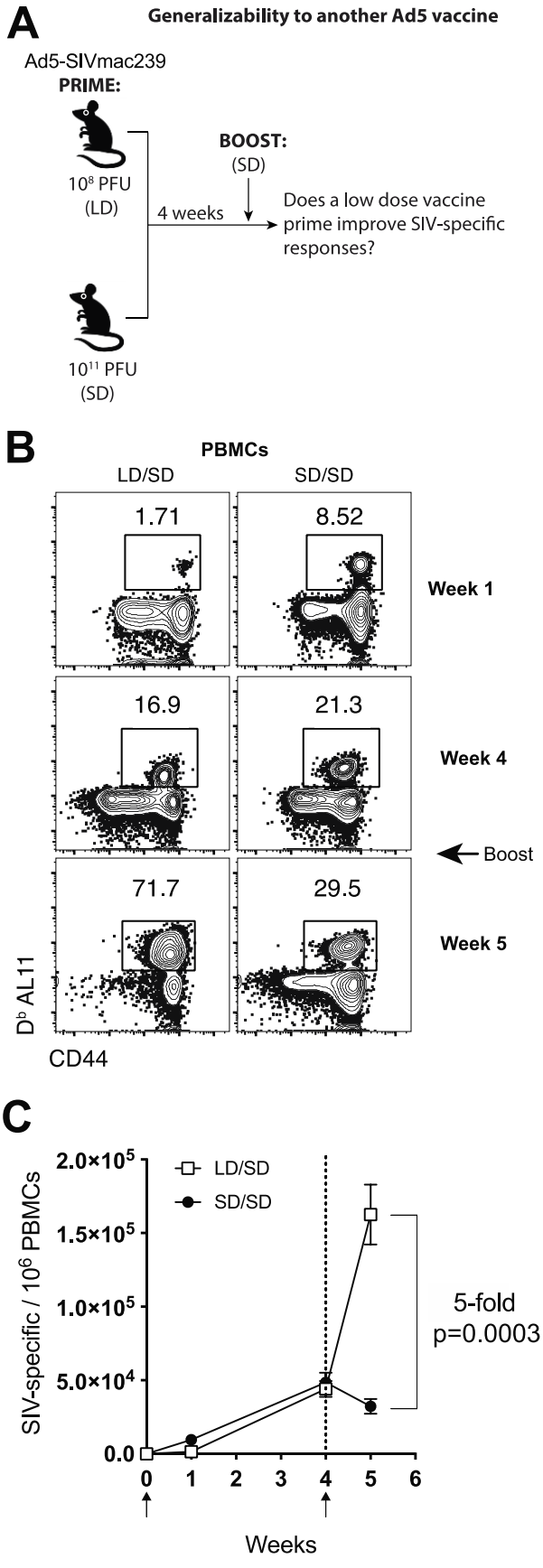

Fig. S7. Effect of LD prime on Ad5-SIV vaccination. (**A**) Experimental approach for evaluating how the priming dose of an Ad5-SIV Gag vaccine affects SIV-specific responses. (**B**) Representative FACS plots showing the frequencies of SIV Gag-specific $CD8^+$ T cells (K^b AL11+) in PBMCs. (**C**) Summary of SIV Gag-specific CD8+ T cell responses in PBMCs. The Ad5 expressed the Gag protein from SIVmac239. We used 10^8 PFU of Ad5-SIV as the LD, because this vector was not immunogenic at the $\leq 10^7$ PFU dose. Data are from one experiment with n=5 per group. All data are shown. Indicated *P* values were determined by parametric test (unpaired t test). Error bars represent SEM.

Supplementary data files

Data S1. (separate file). Average gene expression on the main four clusters of SARS-CoV-2-specific $CD8^+$ T cells from each condition (LD vs SD prime). Gene expression derived from scRNAseq. See fig. S2 for more information.

Data S2. (separate file). Overall gene expression of SARS-CoV-2-specific $CD8^+$ T cells from each condition (LD vs SD prime). Average gene expression derived from scRNAseq. See fig. S2 for more information.

Data S3. (separate file). Raw data.