Science Immunology
AAAS

Supplementary Materials for

Adaptive immune determinants of viral clearance and protection in mouse models of SARS-CoV-2

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Sci. Immunol. **6**, eabl4509 (2021)
DOI: 10.1126/sciimmunol.abl4509

The PDF file includes:

Figs. S1 to S5

Other Supplementary Material for this manuscript includes the following:

Table S1

Supplementary figures

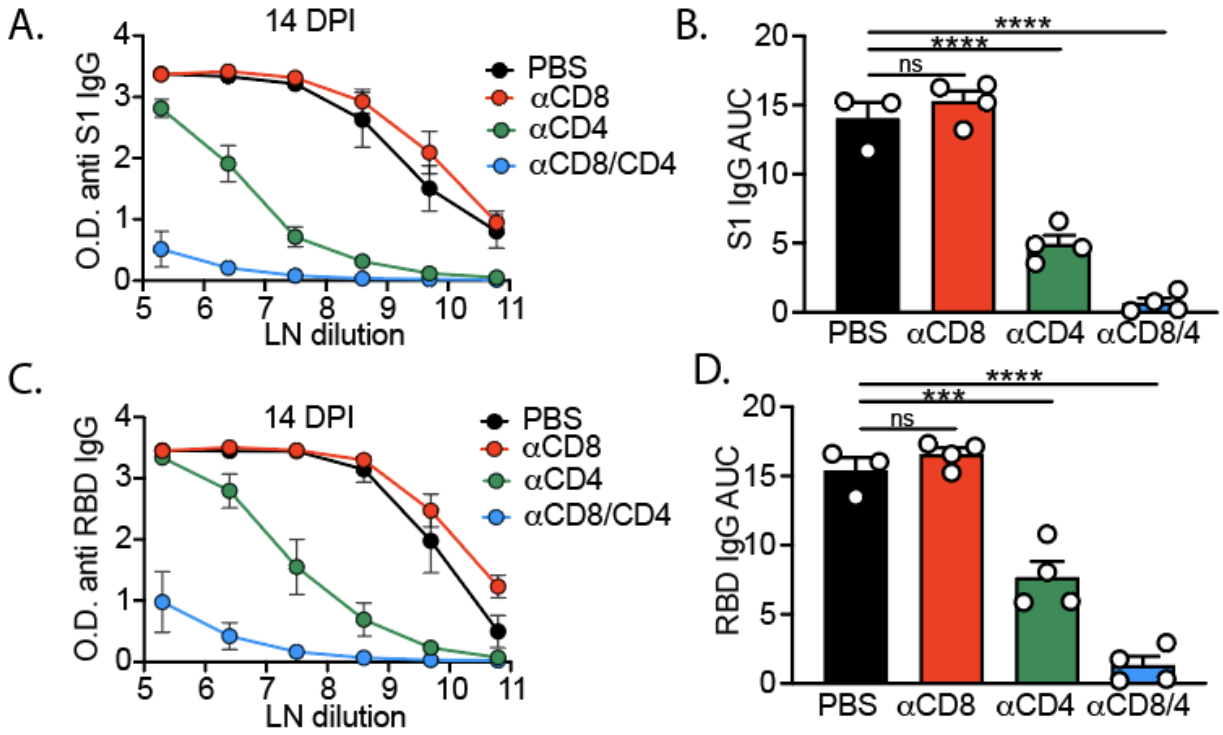

Figure S1. SARS-CoV-2 antibody production in CD4$^+$ and CD8$^+$ depleted mice

Samples from experiment in Fig 2 B, C were tested for Anti-SARS-CoV-2 antibody concentration measured from serum dilutions against (A, B) spike S1 and (C, D) receptor binding domain (RBD). (A, C) Optical density 450nm (O.D.) values are plotted against serum dilution natural log (LN) transformed noted as mean ± SEM from 3-4 samples. (B, D) Area under the curve (AUC) analysis performed on individual samples from (A) and (C). P values were calculated by one-way ANOVA with Tukey's multiple comparison. *, P< 0.05; **, P < 0.01; ***, P < 0.001, ****, P < 0.001

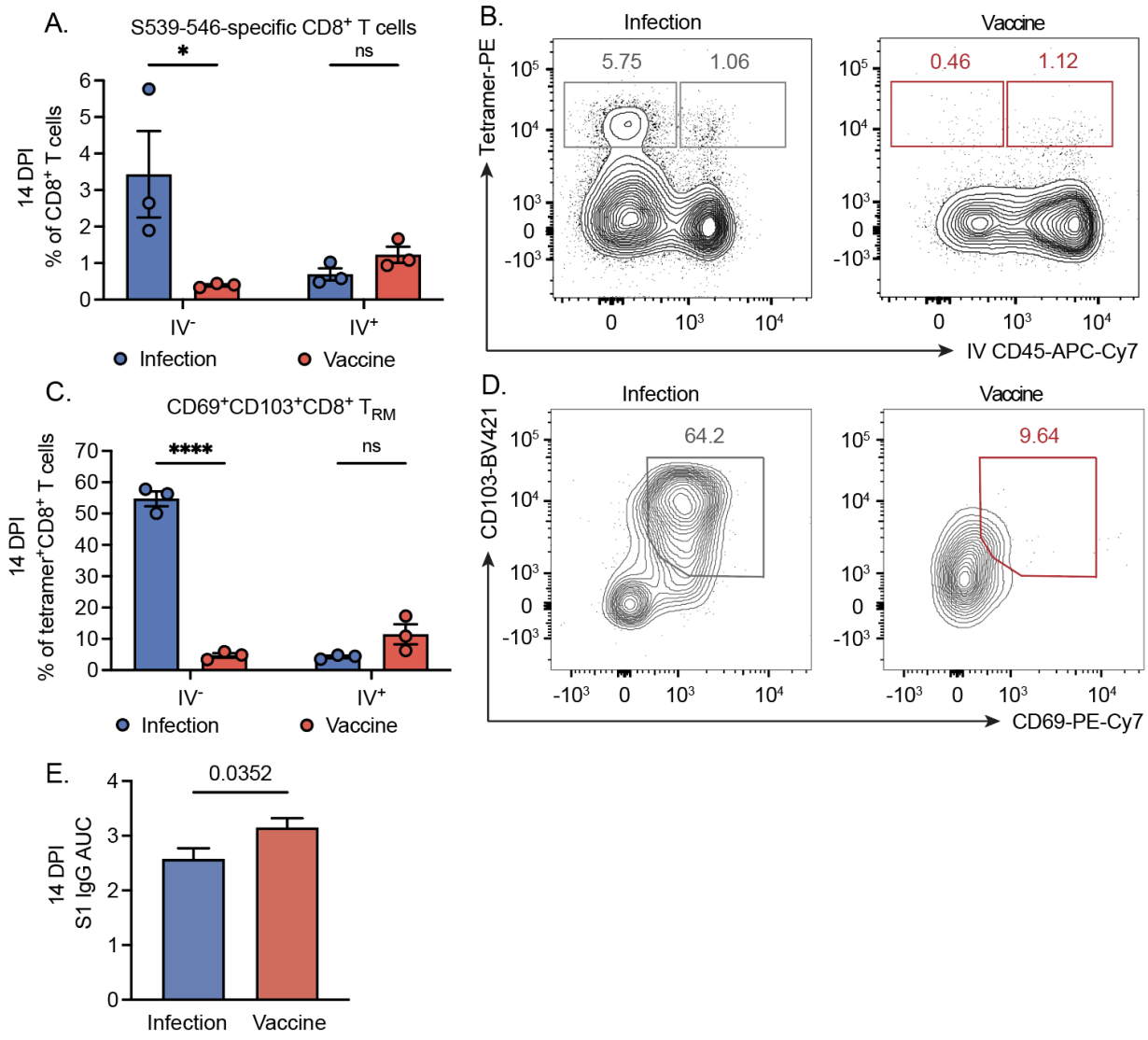

Figure S2. CD8$^+$ T_{RM} develop in infected mice, not mRNA vaccinated mice

B6.Cg-Tg(K18-ACE2)2Prlmn/J (K18-hACE2) mice received 1µg Pfizer-BioNTech mRNA vaccination via intramuscular injection (Vaccine) or were infected with 500 PFU WA1 strain SARS-CoV-2 via intranasal administration (Infection). 14 days post vaccination or infection, mice were injected intravenously (IV) with anti-CD45 labeling antibodies to distinguish circulating from tissue-resident T cell responses. (A) Lung-resident (IV$^-$) and circulating (IV$^+$) CD8 T cells specific for spike S539-546 (tetramer$^+$) were assessed by flowcytometry, with (B) representative flowcytometry plots. (C) Resident Memory CD8$^+$ T cells (T_{RM}) were assessed

using CD69 and CD103 expression on lung-resident (IV^-) and circulating (IV^+) $CD8^+$ T cells specific for spike S539-546 by flowcytometry, with (D) representative flowcytometry plots. (E) Anti-SARS-CoV-2 ELISA S1 IgG area under the curve (AUC) measurement. (A and C) Individual values noted as dots and bars represent mean ± SEM from n=3 samples, and P values were calculated by tow-way ANOVA with multiple comparison. (E) Bars represent mean ± SEM from n=18-20 samples, and P value was calculated by Student's unpaired T-test. *, $P< 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ****, $P < 0.001$

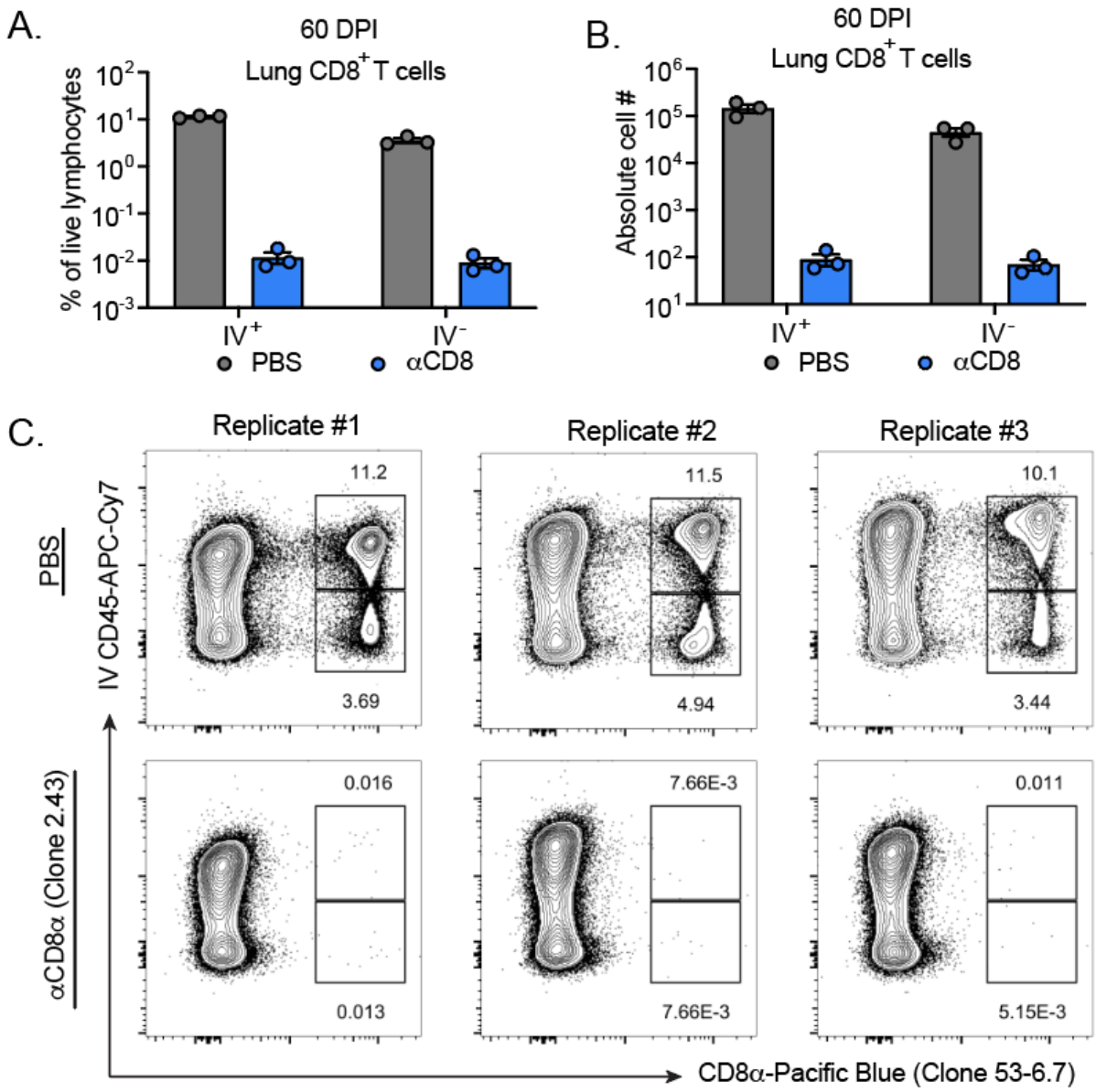

Figure S3. Depletion of CD8$^+$ T_{RM} cells in convalescent mice.

K18-hACE2 mice were infected with 500 PFU WA1 strain SARS-CoV-2 via intranasal administration once. Two months post infection mice were injected intraperitoneally with 200mg anti-mouse CD8a (clone 2.43) or PBS (control) at 3 and 1 days prior to sacrifice and received 100mg anti-mouse CD8a or PBS via intranasal administration at 1 day prior to sacrifice. On the day of sacrifice, mice were injected IV with anti-CD45 labeling antibodies to distinguish

circulating from tissue-resident T cells. Lung-resident (IV^-) and circulating (IV^+) CD8 T cells are plotted as (A) percentage of total lymphocytes or (B) total number of cells from entire lung preparation. (C) Flowcytometry plots.

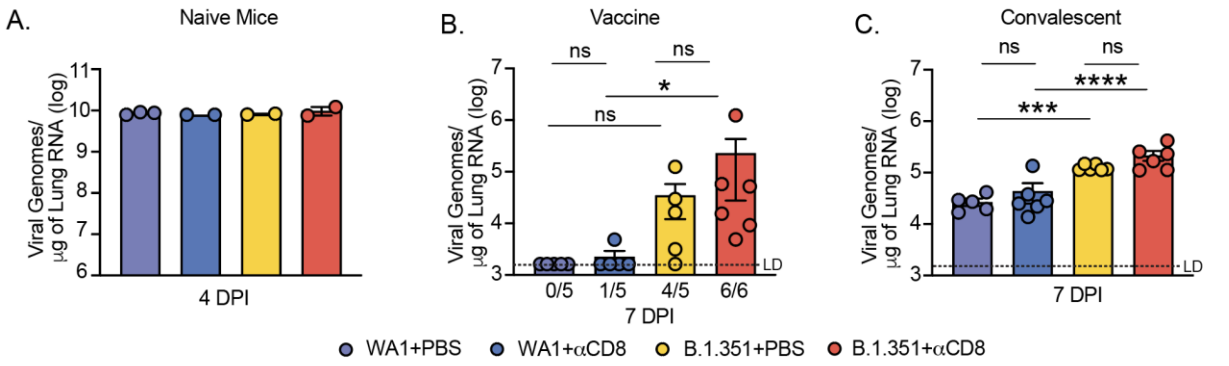

Figure S4. Naïve, vaccinated, and convalescent mice infected with WA1 or B.1.351

Mice from **Fig 5** euthanized at (A) 4 DPI naïve, (B) 7 DPI vaccinated, and (C) 7 DPI convalescent. Viral RNA was measured by quantitative PCR. Values noted below X-axis (B) indicate numbers of samples tested positive/number of samples. LD (limit of detection). Individual values noted as dots and bars as mean ± SEM from n=2-6 samples. P values were calculated by one-way ANOVA with Tukey's multiple comparison. *, $P< 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ****, $P < 0.001$

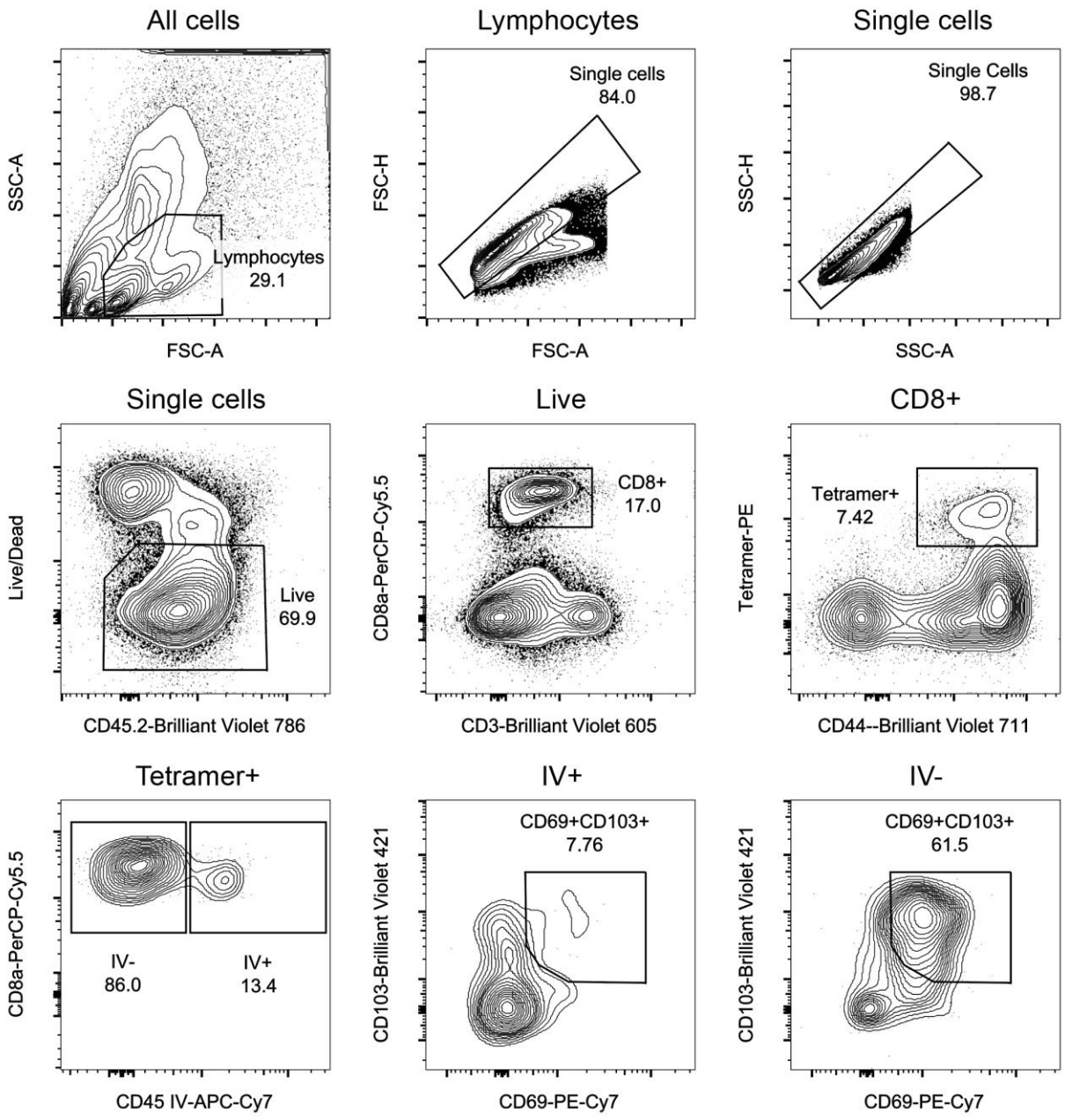

Figure S5. Assessment of CD8$^+$ T_{RM} cells

Gating strategy with representative flowcytometry plots