Human coronavirus OC43-elicited CD4$^+$ T cells protect against SARS-CoV-2 in HLA transgenic mice

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TABLES

Table S1. Whole-genome sequence homology between SARS-CoV-2 (MT786327) and the 6 other HCoVs[35].						
	SARS-CoV (NC_004718)	**MERS-CoV** (NC_019843)	**OC43** (NC_006213)	**HKU1** (NC_006577)	**NL63** (NC_005831)	**229E** (NC_002645)
SARS-CoV-2	86.85	81.25	79.34	81.58	80.09	78.40

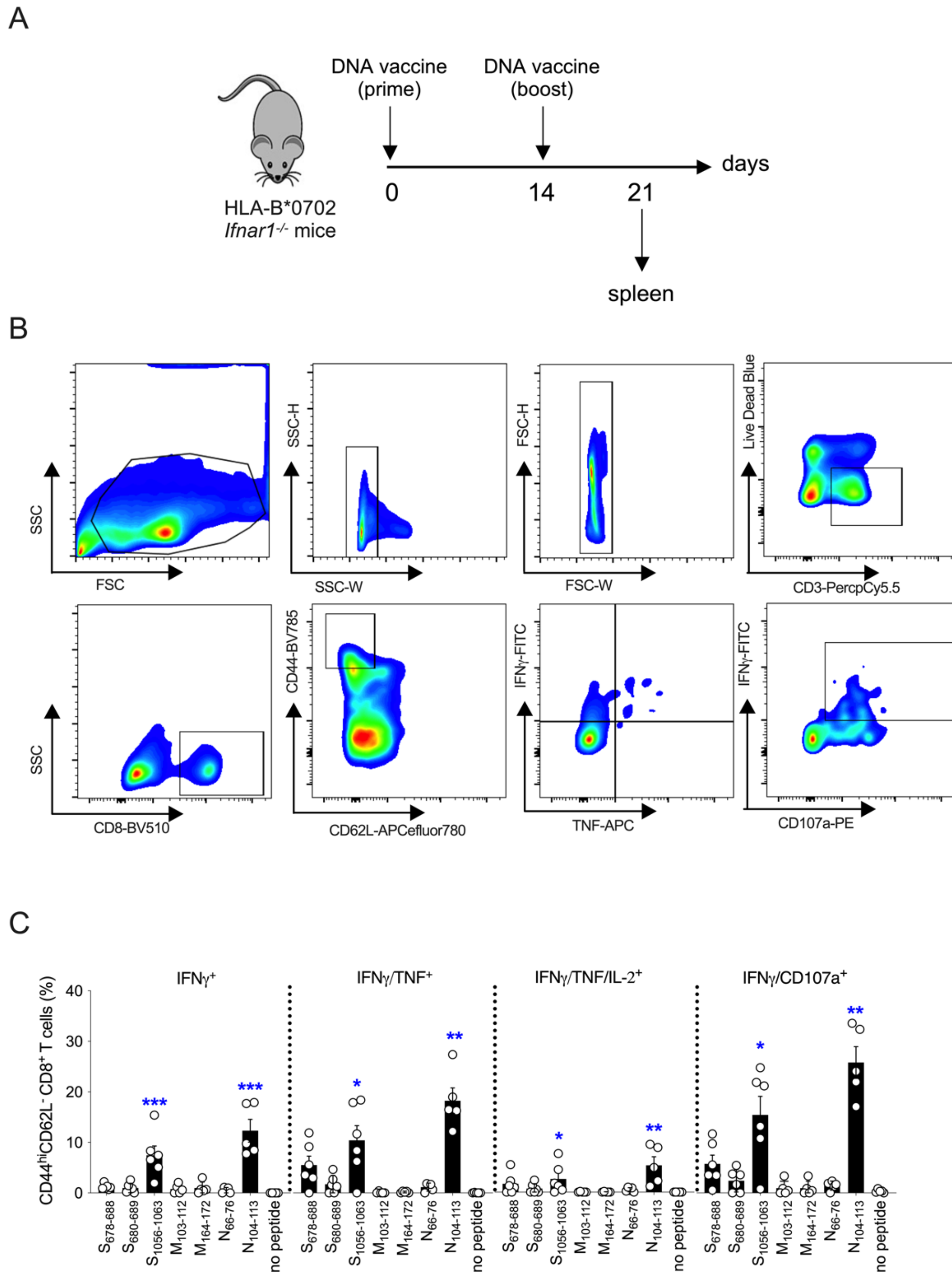


Figure S1 (related to Figure 1). Validation of SARS-CoV-2 S, N, and M protein-derived epitopes in vaccinated HLA-B*0702 *Ifnar1*$^{-/-}$ mice

(A) Experimental protocol. HLA-B*0702 *Ifnar1*$^{-/-}$ mice were injected with 25 µg S-, N-, or M-based DNA vaccine (*vs* saline) via intramuscular electroporation on days 0 and 14 and spleens collected 7 days later.

(B) Gating strategy used to analyze activated ($CD44^+$ $CD62L^−$) $CD8^+$ T cells producing cytokines (IFNγ, TNF, IL-2) and a degranulation marker (CD107a) after stimulation of splenocytes with SARS-CoV-2-derived peptides. Cells producing $IFN\gamma^+/TNF^+/IL\text{-}2^+$ were identified from the $IFN\gamma^+/TNF^+$ population using a Boolean algorithm.

(C) ICS analysis of activated $CD8^+$ T cells in splenocytes stimulated for 6 h with 1 of 7 SARS-CoV-2 S, M, or N protein-derived peptides (*vs* no peptide), immunolabeled for cell surface markers, cytokines, and CD107a, and analyzed by flow cytometry. Data are presented as the mean ± SEM. Circles, individual mice; N=5-6 mice/group pooled from 2 independent experiments. Peptide *vs* control were compared using the Kruskal-Wallis test. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

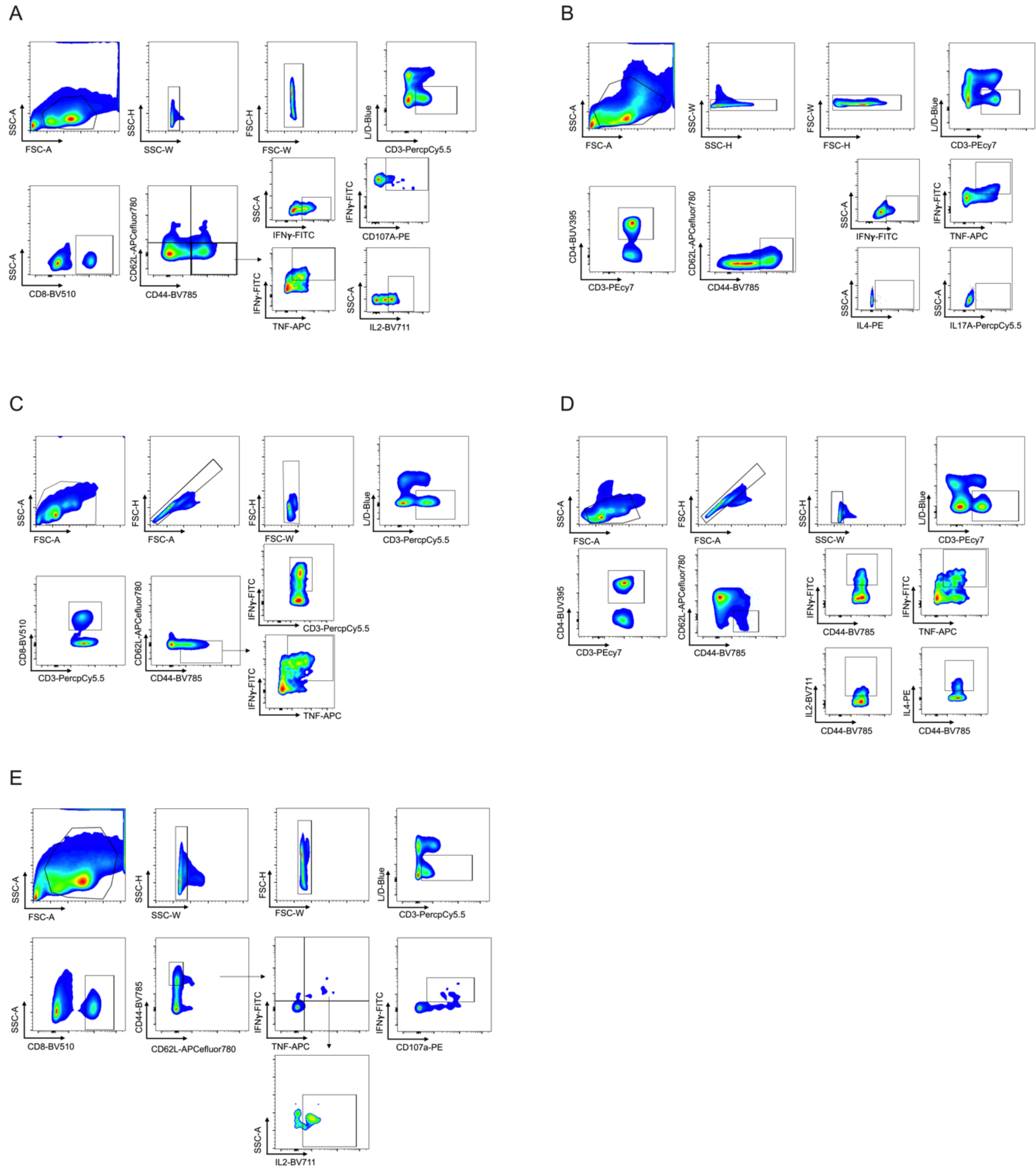
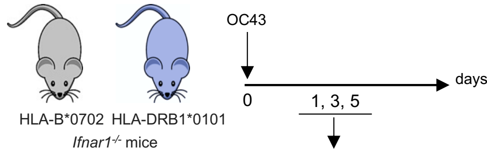


Figure S2 (related to Figures 2, 3, 4, and 5). Gating strategies used to analyze T cell responses.

The lymphocyte gate was selected based on size and granularity (FSC and SSC), and double events were excluded using SSC/ FSC height and width. Lymphocytes were selected first for viable $CD3^+$ cells, second for $CD8^+$ or $CD4^+$ subset, and finally for effector memory cells ($CD44^+CD62L^-$). In the effector memory population, polyfunctional T cells were examined for

expression of different cytokines (IFNγ, TNF, IL-2, IL-4, IL-17A) and degranulation marker (CD107a). For each sample, no less than 50,000 events were acquired within the region of viable $CD3^+$ lymphocytes.

A



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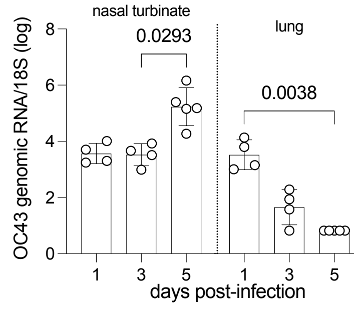

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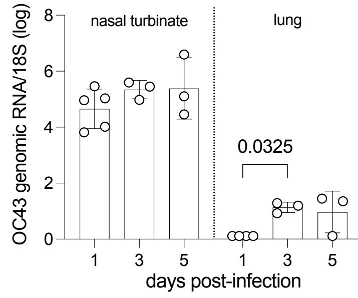

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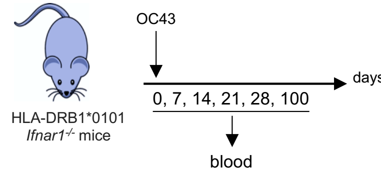

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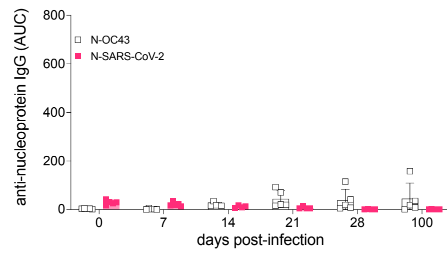

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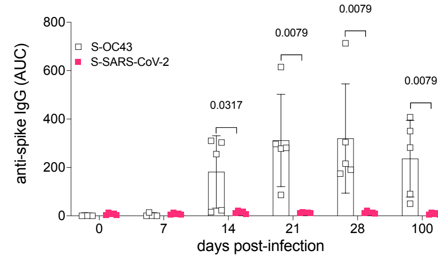

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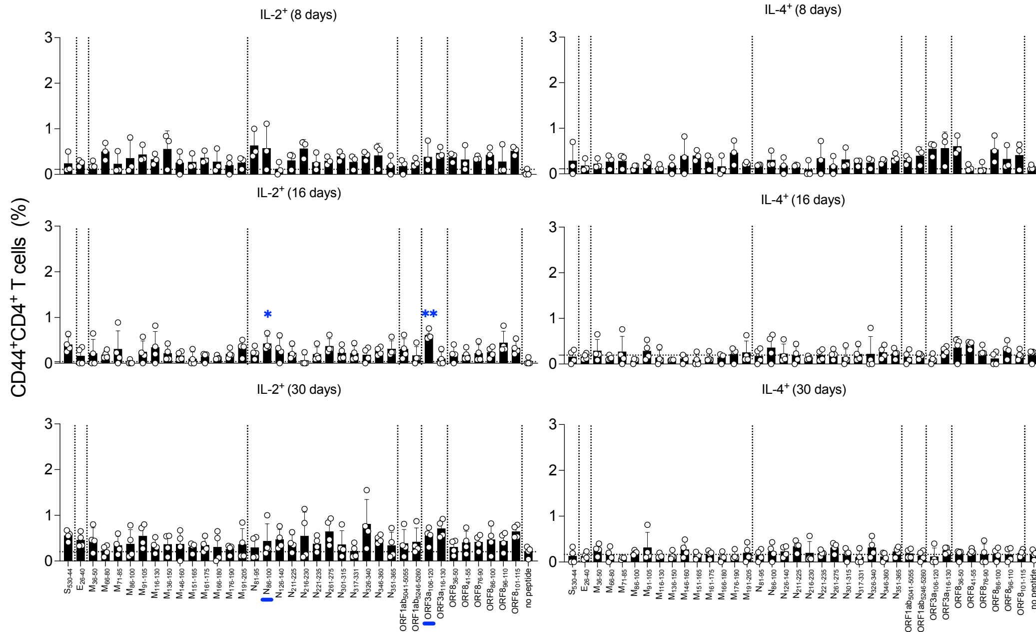

Figure S3 (related to Figures 3 and 4). OC43 infection in mice.

(A) Experimental protocol for B and C. HLA-B*0702 and HLA-DRB1*0101 *Ifnar1*$^{-/-}$ mice were infected with OC43 (10^9 GE, IN) and nasal turbinates and lungs collected on days 1, 3, and 5 post-infection.

(B and C) RT-qPCR analysis of genomic OC43 RNA in tissues of HLA-B*0702 *Ifnar1*$^{-/-}$ (B) and HLA-DRB1*01010 *Ifnar1*$^{-/-}$ (C) mice. N=3-5 mice/group. Data were compared using the nonparametric Kruskal-Wallis test.

(D) Experimental protocol for E and F. HLA-DRB1*0101 *Ifnar1*$^{-/-}$ mice were infected with OC43 (10^9 GE, IN), and blood samples were collected immediately prior to infection, and at 5 time points post-infection.

(E and F) ELISA analysis of IgG against S protein (E) and N protein (F) of OC43 and SARS-CoV-2. N=4-5 mice/group. IgG levels against antigens from SARS-CoV-2 *vs* OC43 at each time point were compared using the nonparametric Mann-Whitney test.

(G) ICS analysis of activated CD4$^+$ T cells. HLA-DRB1*0101 *Ifnar1*$^{-/-}$ mice were infected with OC43 (10^9 GE, IN), and spleens were collected on day 8 (N=3), 16 (N=4), or 30 (N=4) post-infection. Splenocytes were stimulated for 6 h with 37 SARS-CoV-2 peptides (Table 5) *vs* no peptide, immunolabeled for cell surface markers and intracellular cytokines, and analyzed by flow cytometry. Peptide *vs* control data were compared using the nonparametric Kruskal-Wallis test. Blue bars on the x-axis, peptides that significantly stimulated cells at 1 or more time points.

(B,C,E-G) Data, pooled from 2 independent experiments, are presented as the mean ± SEM. *, $P < .05$; **, $P < .01$. Circles, individual mice.

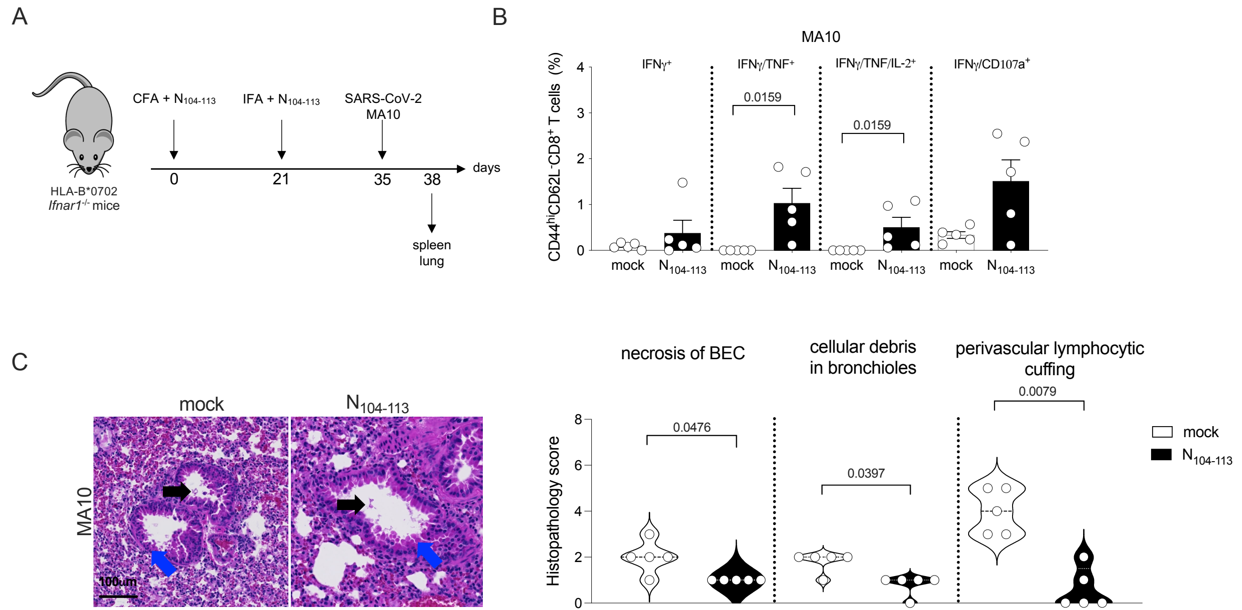

Figure S4 (related to Figure 5). SARS-CoV-2 $N_{104-113}$ immunization is protective against SARS-CoV-2-induced lung disease in HLA-B*0702 *Ifnar1*$^{-/-}$ mice challenged with SARS-CoV-2 MA10.

(A) Experimental protocol for B and C. HLA-B*0702 *Ifnar1*$^{-/-}$ mice were injected with SARS-CoV-2 $N_{104-113}$ *vs* DMSO (mock) on days 0 (complete Freund's adjuvant, CFA) and 21 (incomplete Freund's adjuvant, IFA); 2 weeks later, mice were challenged with SARS-CoV-2 MA10 (10^5 PFU, IN) and tissues collected 3 days later.

(B) ICS analysis of activated $CD8^+$ T cells. Splenocytes were stimulated for 6 h with SARS-CoV-2 $N_{104-113}$ peptide, immunolabeled for cell surface markers, intracellular cytokines, and CD107a, and analyzed by flow cytometry.

(C) Representative H&E-stained sections of lungs. Blue arrows, bronchiolar epithelial cells (BEC); black arrows, epithelial cells in bronchioles. Sections were scored from 0 (least severe) to 5 (most severe) for standard histopathological features of SARS-CoV-2-induced lung disease.

(B,C) Data, pooled from 2 independent experiments, are presented as the mean ± SEM. Circles, individual mice; N=5 mice/group. Groups were compared by the Mann–Whitney test.

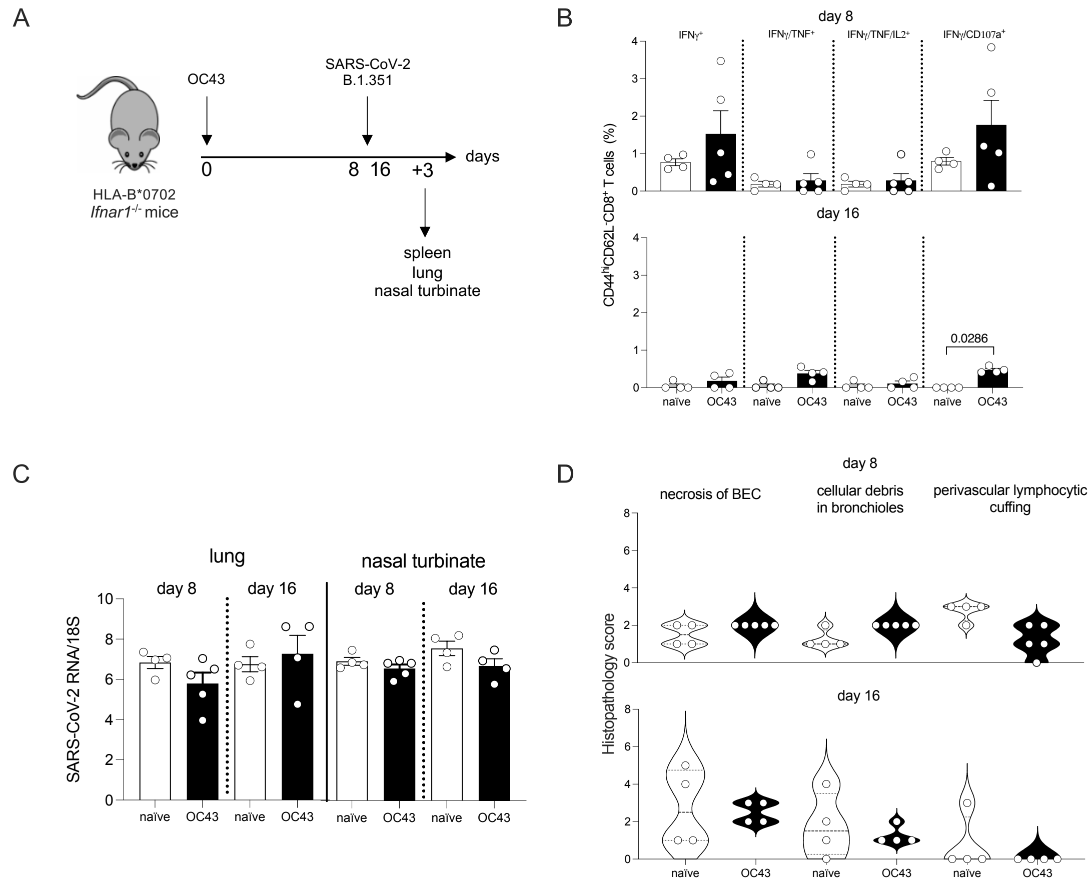

Figure S5 (related to Figure 5). SARS-CoV-2 RNA load and lung pathology of OC43-infected HLA-B*0702 *Ifnar1*$^{-/-}$ mice challenged with SARS-CoV-2 B.1.351.

(A) Experimental protocol. Mice were infected with OC43 (10^9 GE, IN) *vs* medium (naïve), challenged with SARS-CoV-2 B.1.351 (10^5 PFU, IN) 8 or 16 days later, and tissues were harvested at 3 days post-challenge.

(B) RT-qPCR of genomic OC43 RNA.

(C) H&E-stained lung sections were scored from 0 (least severe) to 5 (most severe) for standard histopathological features of SARS-CoV-2-induced lung disease.

(D) ICS analysis of OC43-elicited activated CD8$^+$ T cells. Splenocytes were stimulated for 6 h with SARS-CoV-2 $N_{104-113}$ peptide, immunolabeled for cell surface markers, intracellular cytokines, and CD107a, and analyzed by flow cytometry.

(B-D) Data, pooled from 2 independent experiments, are presented as the mean ± SEM. Circles, individual mice; n=4-5 mice/group. Groups were compared using the Mann–Whitney test.

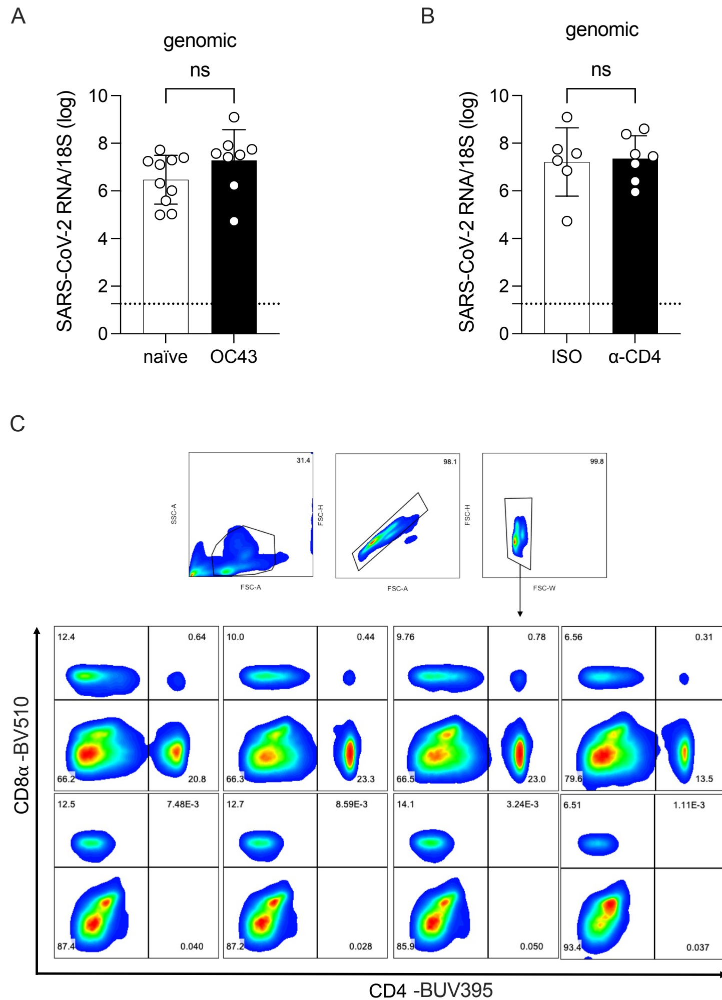

Figure S6 (related to Figure 6). SARS-CoV-2 challenge of HLA-DRB1*0101 *Ifnar1*$^{-/-}$ mice pre-exposed to OC43.

Experimental protocols are described in Figures 6A (A) and 6E (B,C).

(A,B) RT-qPCR of SARS-CoV-2 genomic RNA in nasal turbinates. Data are presented as the mean ± SEM. Circles, individual mice. Mice/group: A, 8 (OC43-infected) and 10 (naïve); B, 6-7. Group differences were analyzed by the Mann–Whitney test. Horizontal dotted line, the limit of detection.

(C) Gating strategy used to analyze CD4^{+} T cells in the blood of mice treated with a CD4^{+} T cell-depleting antibody (α-CD4) vs isotype control. Each dot plot represents a mouse.