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Supplemental information

Nasally delivered interferon-λ protects mice against infection by SARS-CoV-2 variants including Omicron

Zhenlu Chong, Courtney E. Karl, Peter J. Halfmann, Yoshihiro Kawaoka, Emma S. Winkler, Shamus P. Keeler, Michael J. Holtzman, Jinsheng Yu, and Michael S. Diamond

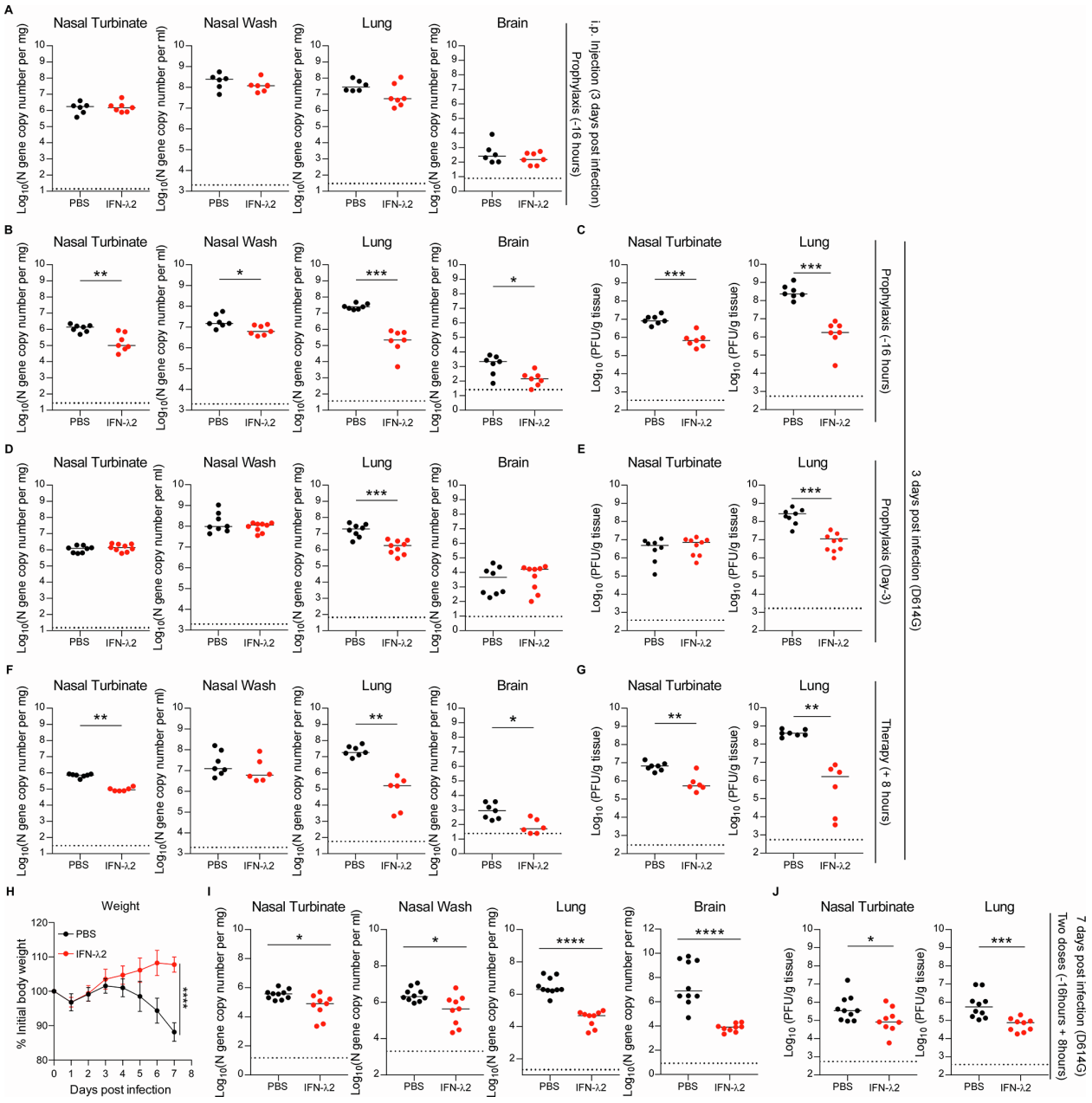

Figure S1. SARS-CoV-2 viral burden in infected K18-hACE2 mice, Related to Figure 2. (**A**) Eight-week-old female K18-hACE2 mice were inoculated by intranasal route with 10^3 FFU of WA1/2020 D614G. At -16 h before virus inoculation, mice were given 2 μg of murine IFN-λ2 or PBS by intraperitoneal injection. Viral RNA levels at 3 dpi (n = 6-7 per group, 2 experiments). (**B-G**) Eight-week-old female K18-hACE2 mice were inoculated by intranasal route with 10^3 FFU of WA1/2020 D614G. At -16 h (**B-C**), D-3 (**D-E**) or +8 h (**F-G**), mice were given 2 μg of murine IFN-λ2 or PBS by intranasal route. Viral RNA (**B, D, and F**) and infectious virus (**C**, **E,** and **G**) levels at 3 dpi (**B-C**: n = 7 per group, 2 experiments; **D-E**: n = 8-9 per group, 2 experiments; **F-G**: n = 6-7 per group, 2 experiments). (**H-J**) Eight-week-old female K18-hACE2 mice were treated with 2 μg doses of murine IFN-λ2 or PBS by intranasal route at -16 h and +8 h relative to inoculation with 10^3 FFU of WA1/2020 D614G and harvested at 7 dpi. (**H**) Weight change was

monitored daily for 7 days. (**I**) Viral RNA levels at 7 dpi. (**J**) Infectious virus levels at 7 dpi (**H-J:** n = 9-10 per group, 2 experiments). Bars (**A-G and I-J**) indicate median values. Data were analyzed by Mann-Whitney test (**A-G and I-J**) or *t* tests of the area under the curve (**H**) (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001).

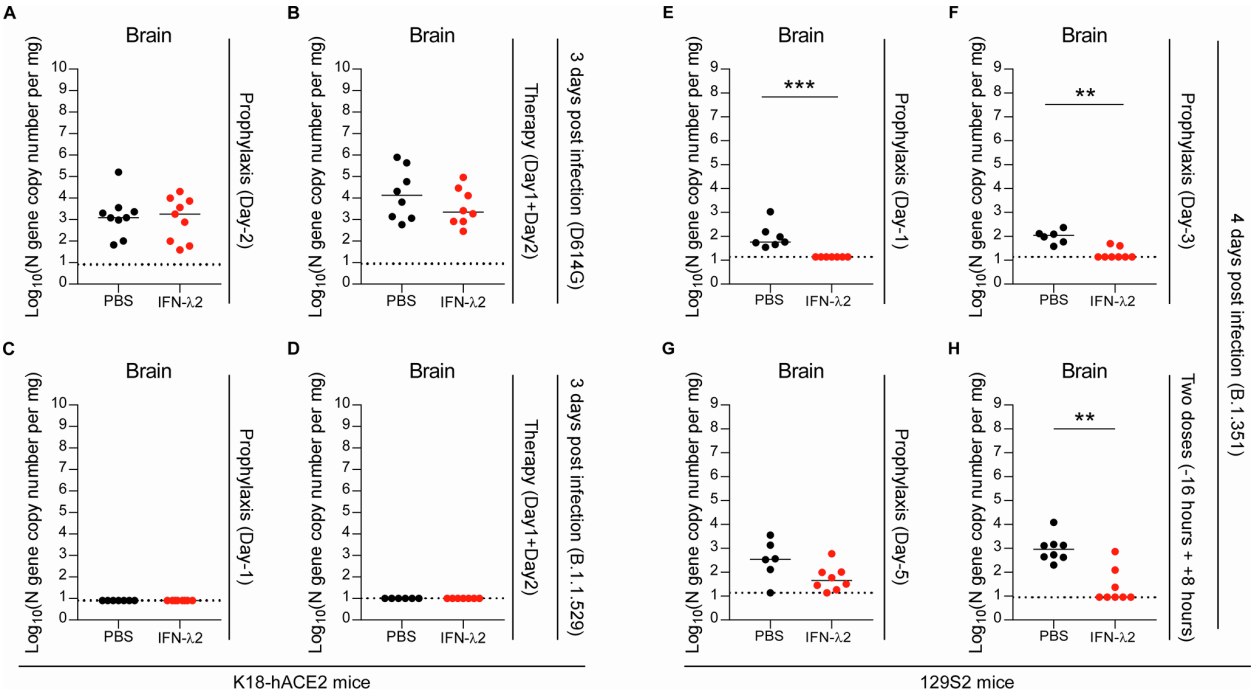

Figure S2. SARS-CoV-2 viral burden in the brains of K18-hACE2 and 129S2 mice, Related to Figures 2 and 3. (**A-D**) Eight-week-old (**A-B**) or five-month-old (**C-D**) female K18-hACE2 mice were inoculated by intranasal route with 10^3 FFU of WA1/2020 D614G (**A-B**) or B.1.1529 Omicron variant (**C-D**). At D-2 (**A**), D+1 and D+2 (**B and D**) or D-1 (**C**), mice were administered 2 µg of murine IFN-λ2 or PBS by intranasal route. Viral RNA levels from brain at 3 dpi (**A**: n = 9 per group, 2 experiments; **B**: n = 8 per group, 2 experiments; **C**: n = 7-8 per group, 2 experiments; **D**: n = 6-7 per group, 2 experiments). (**E-H**) Six-week-old female 129S2 mice were inoculated by intranasal route with 10^5 FFU of B.1.351 Beta variant. At D-1 (**E**), D-3 (**F**), D-5 (**G**) or -16 h and +8 h (**H**), mice were administered 2 µg of murine IFN-λ2 or PBS by intranasal route. Viral RNA levels from brain at 4 dpi (**E**: n = 7 per group, 2 experiments; **F**: n = 6-8 per group, 2 experiments; **G**: n = 6-8 per group, 2 experiments; **H**: n = 8 per group, 2 experiments). Bars indicate median values. Data were analyzed by Mann-Whitney test (**$P < 0.01$ and ***$P < 0.001$).

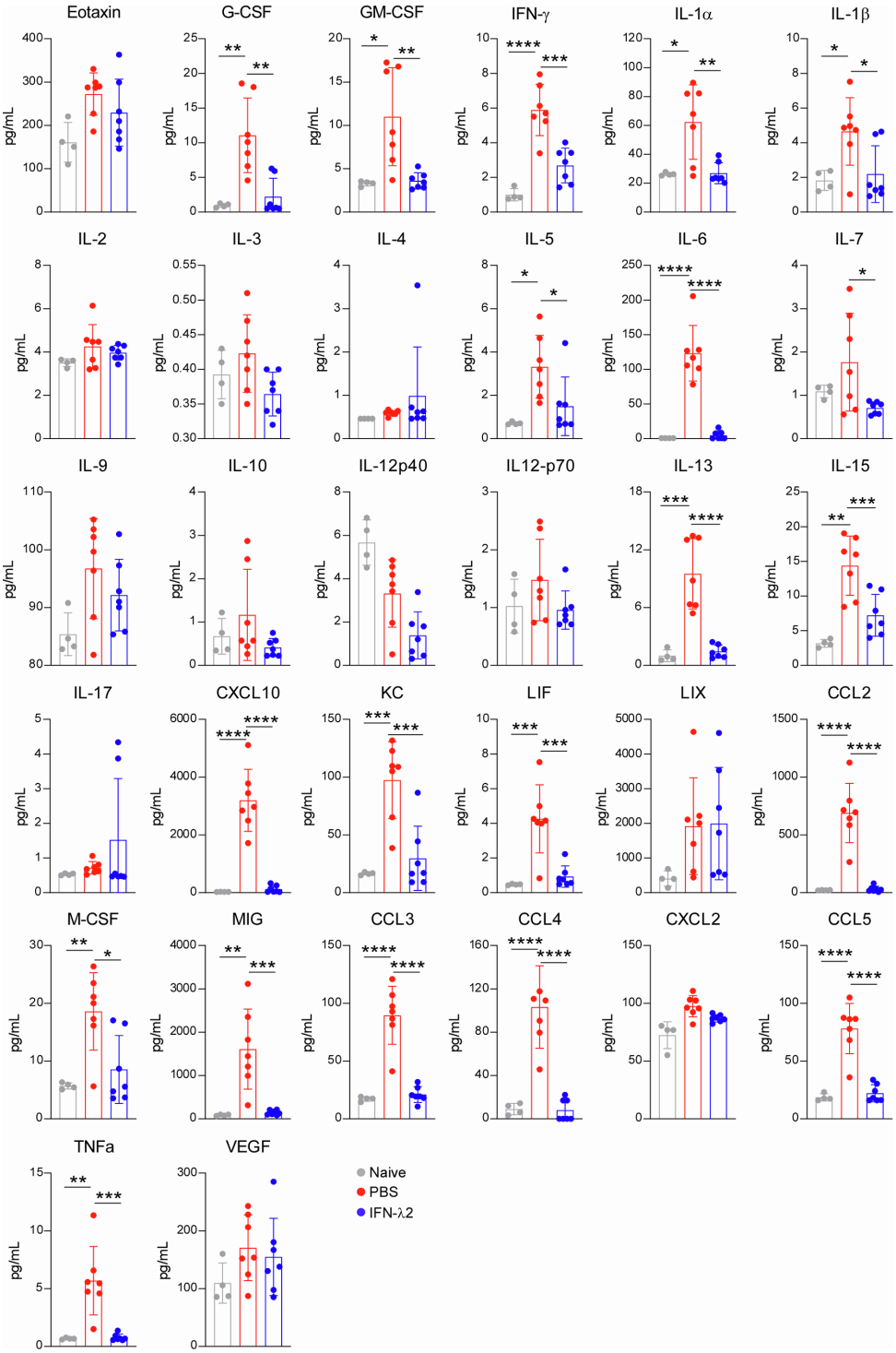

Figure S3. Cytokine responses following IFN-λ treatment and SARS-CoV-2 infection, Related to Figure 2. Eight-week-old female K18-hACE2 mice treated with 2 µg of murine IFN-λ2 or PBS at -16 h by the intranasal route were challenged with 10^3 FFU of WA1/2020 D614G. Cytokine levels in lung homogenates at 3 dpi (2 experiments, n = 7 per group except naïve n = 4). Data were analyzed by one-way ANOVA with Tukey's multiple comparison test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

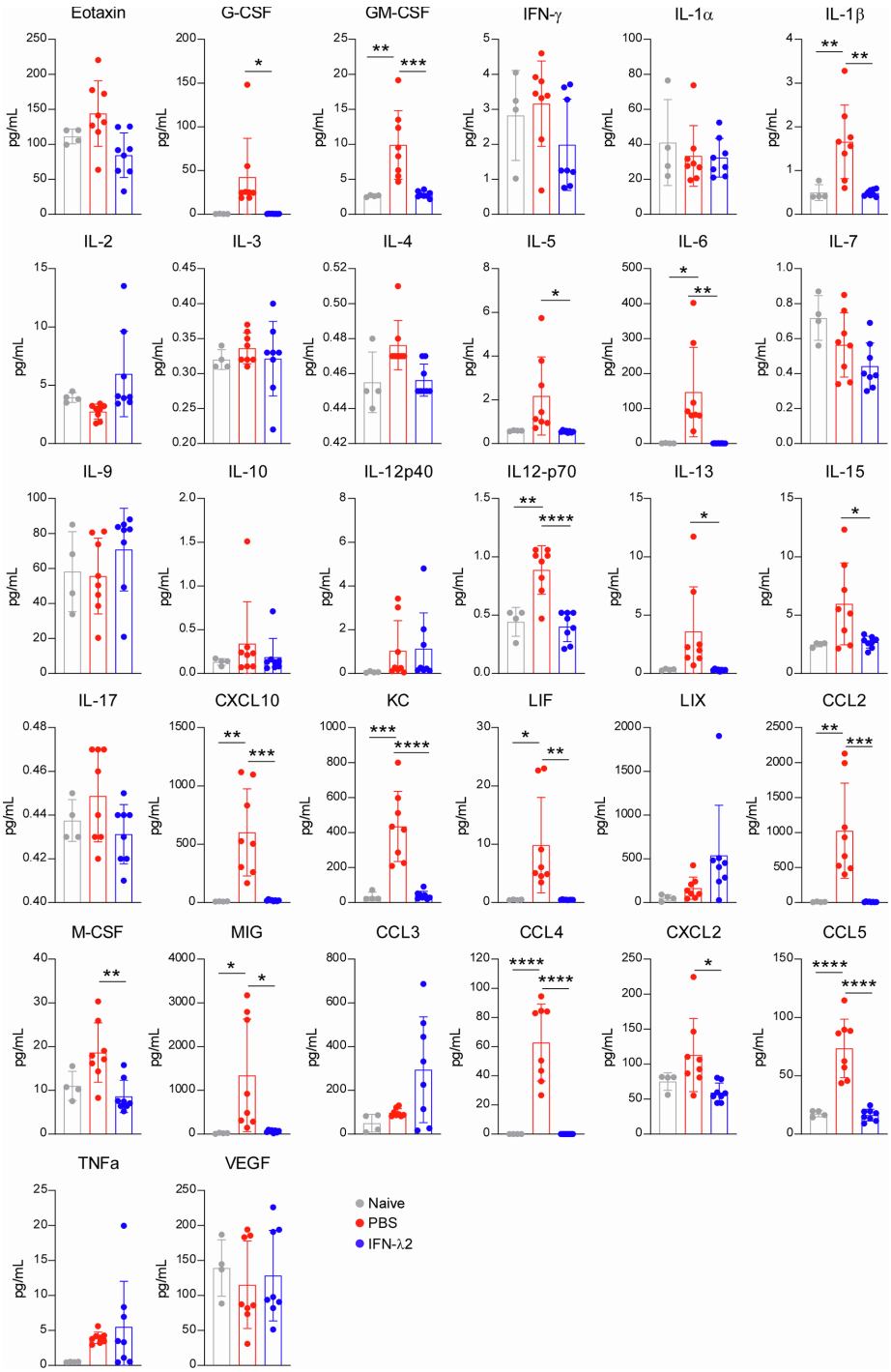

Figure S4. Cytokine induction following IFN-λ treatment and SARS-CoV-2 infection, Related to Figure 3. Six-week-old female 129S2 mice treated with two doses of 2 µg of murine IFN-λ2 or PBS at -16 h and +8 h by the intranasal route were challenged with 10^5 FFU of B.1.351 Beta variant. Cytokine levels in lung homogenates at 4 dpi (n = 7 per group except naïve n = 4, 2 experiments). Data analyzed by one-way ANOVA with Tukey's multiple comparison test (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

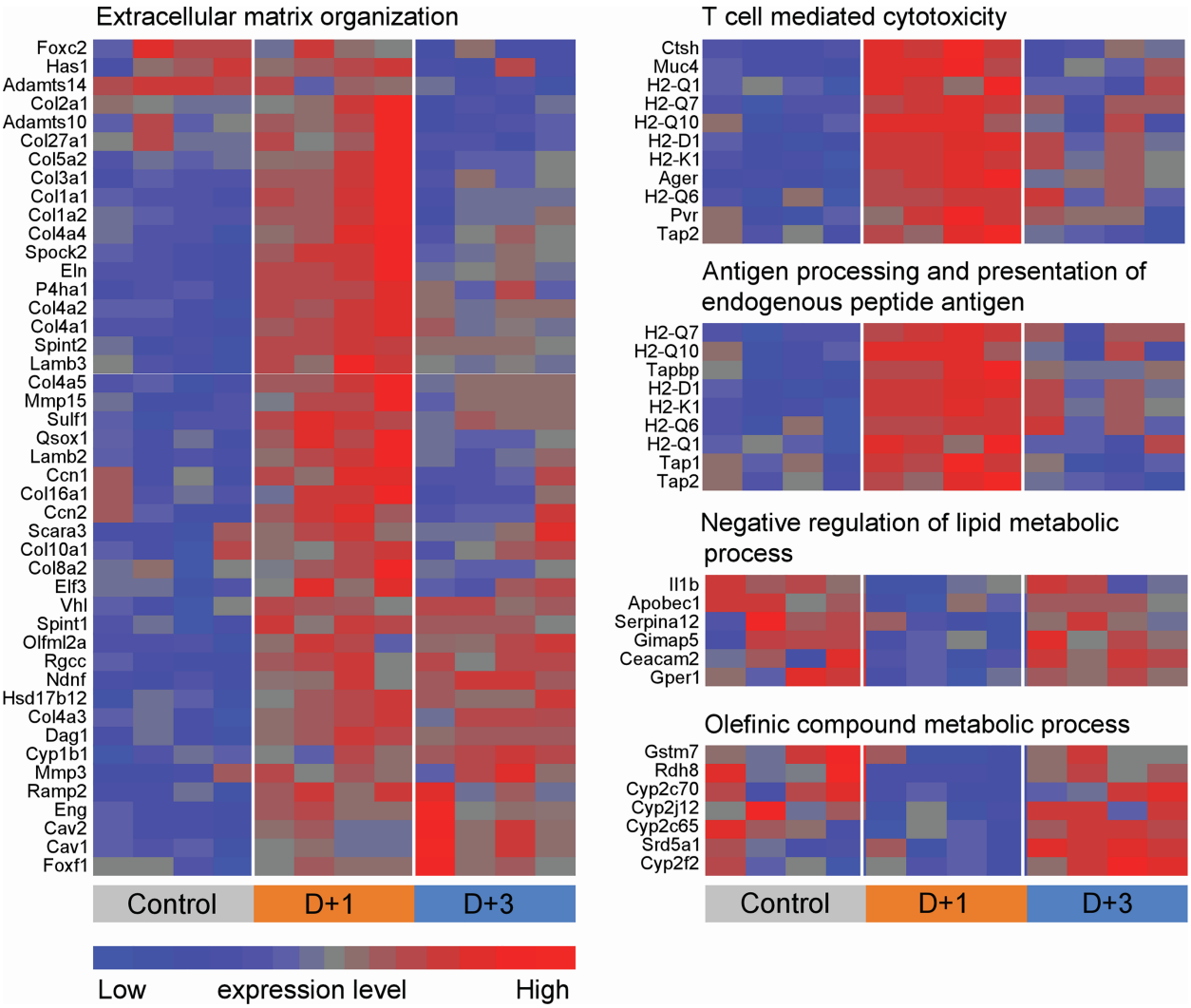

Figure S5. Heatmaps of RNA-seq data, Related to Figure 4. Heatmaps of selected significantly upregulated or downregulated gene sets corresponding with IFN-λ2 treatment identified through GO analysis. Genes shown in each pathway are the union of the differentially expressed genes (DEGs) enriched in D+1 group or D+3 group versus control group (n = 4 per group). Columns represent sample groups and rows indicate genes.

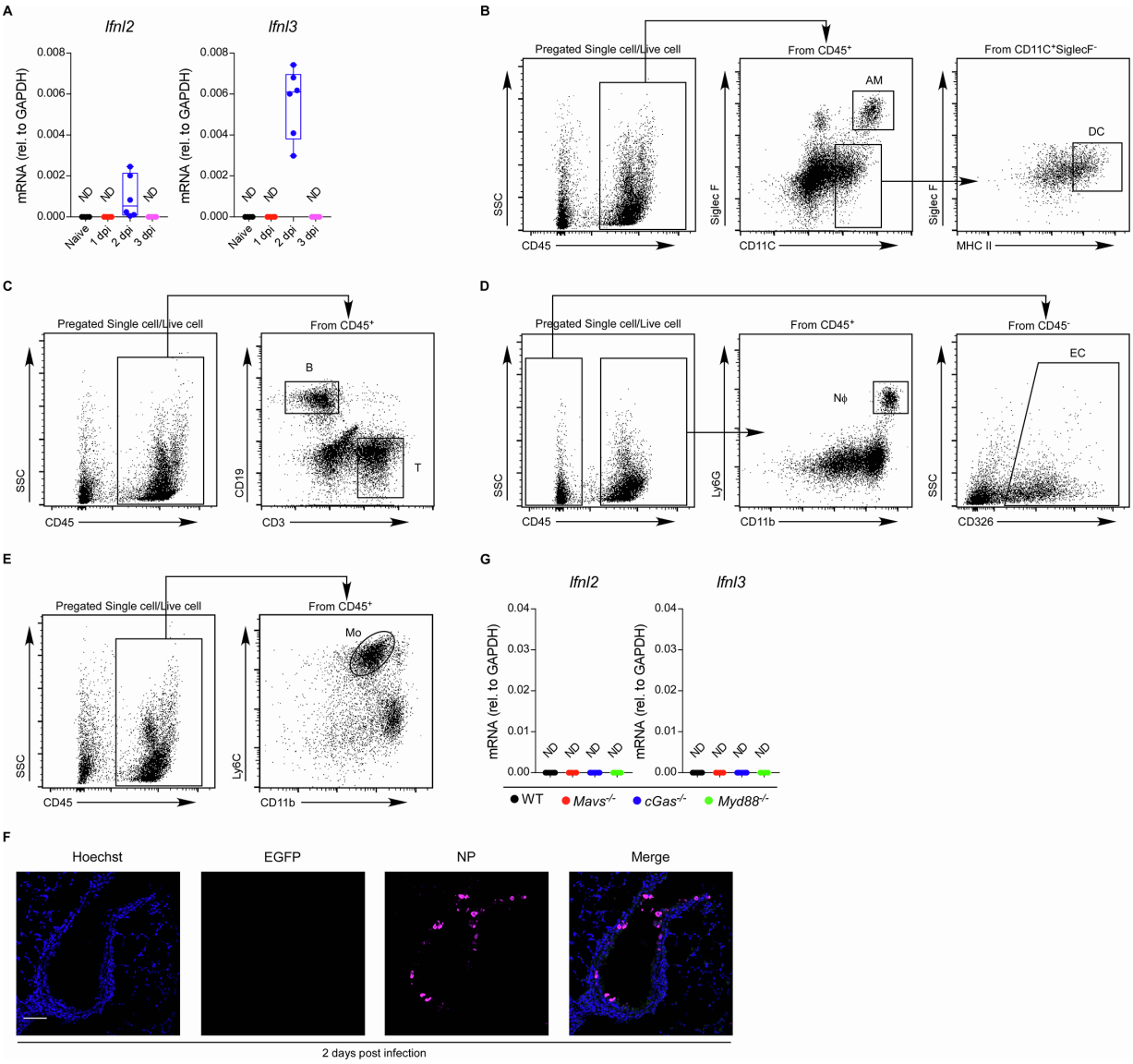

Figure S6. Flow cytometric gating strategy and staining of lung cells, Related to Figure 5. (**A**) Six-week-old male and female C57BL/6 mice were inoculated with 10^5 FFU of B.1.351 Beta variant. *Ifnl2* and *Ifnl3* mRNA levels from lungs were measured at indicated days after infection by qRT-PCR (n = 6 per group, 2 experiments) (ND, not detectable, qRT-PCR Ct value >40). (**B-E**) For lung tissues, cells were gated on single, live, $CD45^+$ and $CD45^-$ cells. Alveolar macrophages (AM) were identified as $CD45^+$ $SiglecF^{hi}$ $CD11c^{hi}$ cells, dendritic cells (DC) were identified as $CD45^+$ $SiglecF^-$ $CD11c^+$ $MHCII^+$ cells (**B**). B and T cells were identified as $CD45^+$ $CD19^+$ cells and $CD45^+$ $CD3^+$ cells, respectively (**C**). Neutrophils (Nϕ) and epithelial cells (EC) were identified as $CD45^+CD11b^+Ly6G^+$ cells and $CD45^-$ $CD326^+$ cells, respectively (**D**). Monocytes (Mo) were identified as $CD45^+$ $CD11b^+$ $Ly6C^{hi}$ cells (**E**). (**F**) Localization of EGFP and SARS-CoV-2 nucleocapsid protein (NP) in the lungs of WT C57BL/6 (non-reporter, negative control) mice at 2 dpi. Frozen sections were stained for GFP (green), NP (magenta), and Hoechst (blue). Scale bar, 50 μm. (**G**) *Ifnl2* and *Ifnl3* mRNA levels from lungs of six-week-old male and female naïve, uninfected WT, *Mavs*$^{-/-}$, *cGas*$^{-/-}$ and *Myd88*$^{-/-}$ C57BL/6 mice were measured by qRT-PCR (n = 6-8 per group, 2 experiments) (ND, not detectable, qRT-PCR Ct value > 40).

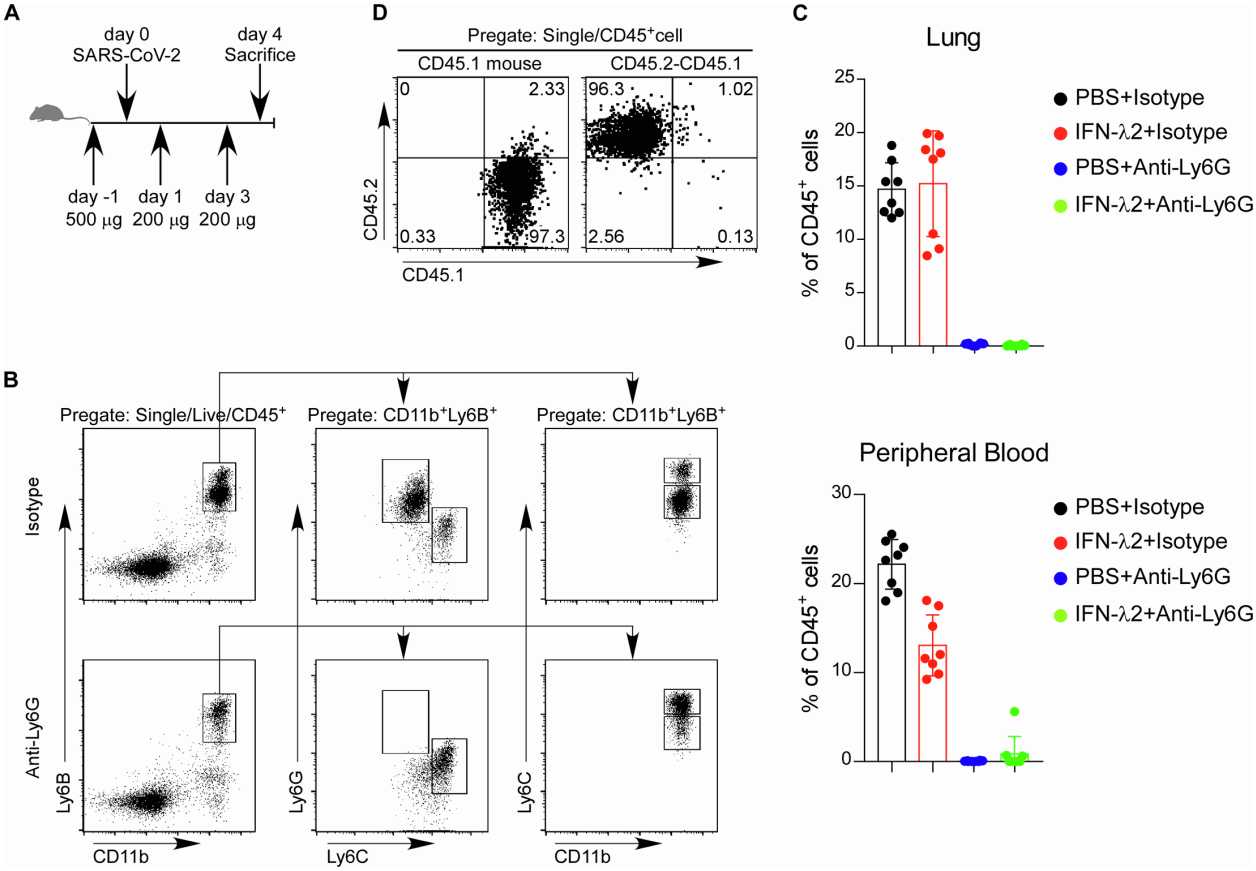

Figure S7. Flow cytometry analysis of peripheral blood and lungs from neutrophil-depleted or bone marrow chimeric mice, Related to Figure 6. (**A**) Experimental scheme of neutrophil deletion in 129S2 mice. (**B**) (*Left*) Representative flow cytometry plots of peripheral blood at D+4 following intraperitoneal injection of a depleting anti-Ly6G mAb (1A8) or isotype control mAb. (*Right*) Frequency of mature neutrophils ($CD11b^{+}Ly6B^{+}Ly6G^{+}Ly6C^{int}$) in blood are shown after antibody depletion. (**C**) Frequency of mature neutrophils ($CD11b^{+}Ly6B^{+}Ly6G^{+}Ly6C^{int}$) in lungs are shown after antibody depletion (n = 8 per group, 2 experiments). (**D**) Representative flow cytometry plots of peripheral blood at 10 weeks after irradiation and bone marrow cell transplantation of CD45.2 cells to CD45.1 recipient mice.