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Adaptive immune cells are necessary for SARS-CoV-2–induced pathology

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Figs. S1 to S4

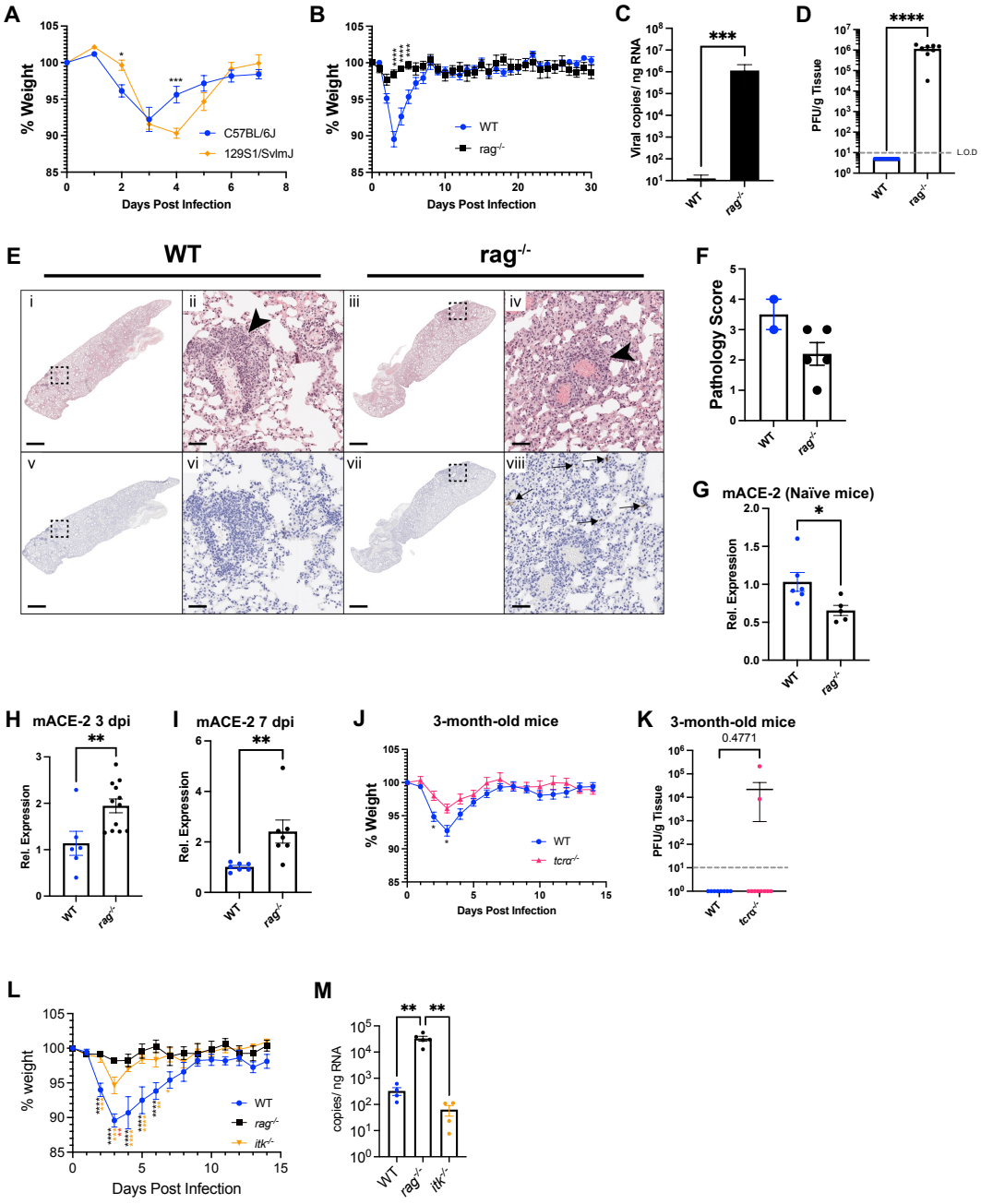

Fig. S1. Virus infection persists in *rag*$^{-/-}$ mice up to 30 days post infection. A. C57BL/6J (n=6) and 129S1/SvlmJ (n=7) mice were infected with 1X10^5 PFU/mouse and weights were monitored daily. Percent weight following infection. *p= 0.0428, ***p=0.0004, 2-way ANOVA with Sidak's multiple comparison test, experiments performed once. **B.** Percent weight loss in wild-type and *rag*$^{-/-}$ mice following infection. WT: n=11, *rag*$^{-/-}$: n=8, ****p< 0.0001, ***p=0.0005, 2-way ANOVA with Sidak's multiple comparison test. experiments performed twice. **C.** Viral loads in the lung of wild-type and *rag*$^{-/-}$ mice 30 days post infection. WT: n=11, *rag*$^{-/-}$: n=8, ***p=0.001,

2-tailed Mann-Whitney test, experiments performed twice, and 2 technical replicates were performed for each animal. **D.** Infectious virus in the lungs of wild-type and *rag*$^{-/-}$ mice 30 days post infection. WT: n=11, *rag*$^{-/-}$: n=8, ***p<0.0001, 2-tailed Mann-Whitney test, experiments performed twice, and 2 technical replicates were performed for each animal. **E.** H&E staining of wild-type and *rag*$^{-/-}$ mice lung (top row) and immunohistochemistry staining (brown stain, bottom row) against the SARS-CoV-2 MA10 nucleoprotein at day 30 post infection. WT: n=2, *rag*$^{-/-}$: n=5, experiments performed once. **F.** Histopathology scoring of wild-type and *rag*$^{-/-}$ mice at day 30 post infection. WT: n=2, *rag*$^{-/-}$: n=5, experiments performed once. **G.** mouse-ACE-2 mRNA expression from the lungs of non-infected mice (Naïve mice). WT: n=6, *rag*$^{-/-}$: n=5, *p<0.05, 2-tailed Mann-Whitney test. **H.** mouse-ACE-2 mRNA expression from the lungs of SARS-CoV-2 MA10 infected mice 3 d.p.i. WT: n=6, *rag*$^{-/-}$: n=12, **p<0.01, 2-tailed Mann-Whitney test. **I.** mouse-ACE-2 mRNA expression from the lungs of infected mice 7 d.p.i. WT: n=7, *rag*$^{-/-}$: n=7, **p<0.01, 2-tailed Mann-Whitney test. **J&K.** 3-month-old wild-type and *tcr*$\alpha^{-/-}$ mice were infected with 10^5 PFU/mouse SARS-CoV-2 MA10 and weights were monitored daily. **J.** Percent weight following infection. WT: n=8, *tcr*$\alpha^{-/-}$: n=10, * $p< 0.05$, 2-way ANOVA with Sidak's multiple comparison test. **K.** Infectious virus in the lungs of WT and *tcr*$\alpha^{-/-}$ 14 d.p.i. WT: n=8, *tcr*$\alpha^{-/-}$: n=10. **L&M.** 6-month-old wild-type and *rag*$^{-/-}$ and *itk*$^{-/-}$ mice were infected with 10^5 PFU/mouse SARS-CoV-2 MA10 and weights were monitored daily. **L.** Percent weight following infection. WT: n=8, *rag*$^{-/-}$: n=10, *itk*$^{-/-}$: n=4, * p< 0.05, ** p< 0.01, *** p< 0.001, ****p < 0.0001, 2-way ANOVA with Tukey's multiple comparisons test. **M.** viral RNA in the lungs of wild-type, *rag*$^{-/-}$ and *itk*$^{-/-}$ mice at 14 d.p.i. WT: n=4, *rag*$^{-/-}$: n=5, *itk*$^{-/-}$: n=4, ** p<0.01, 1-way ANOVA with Tukey's multiple comparisons test.

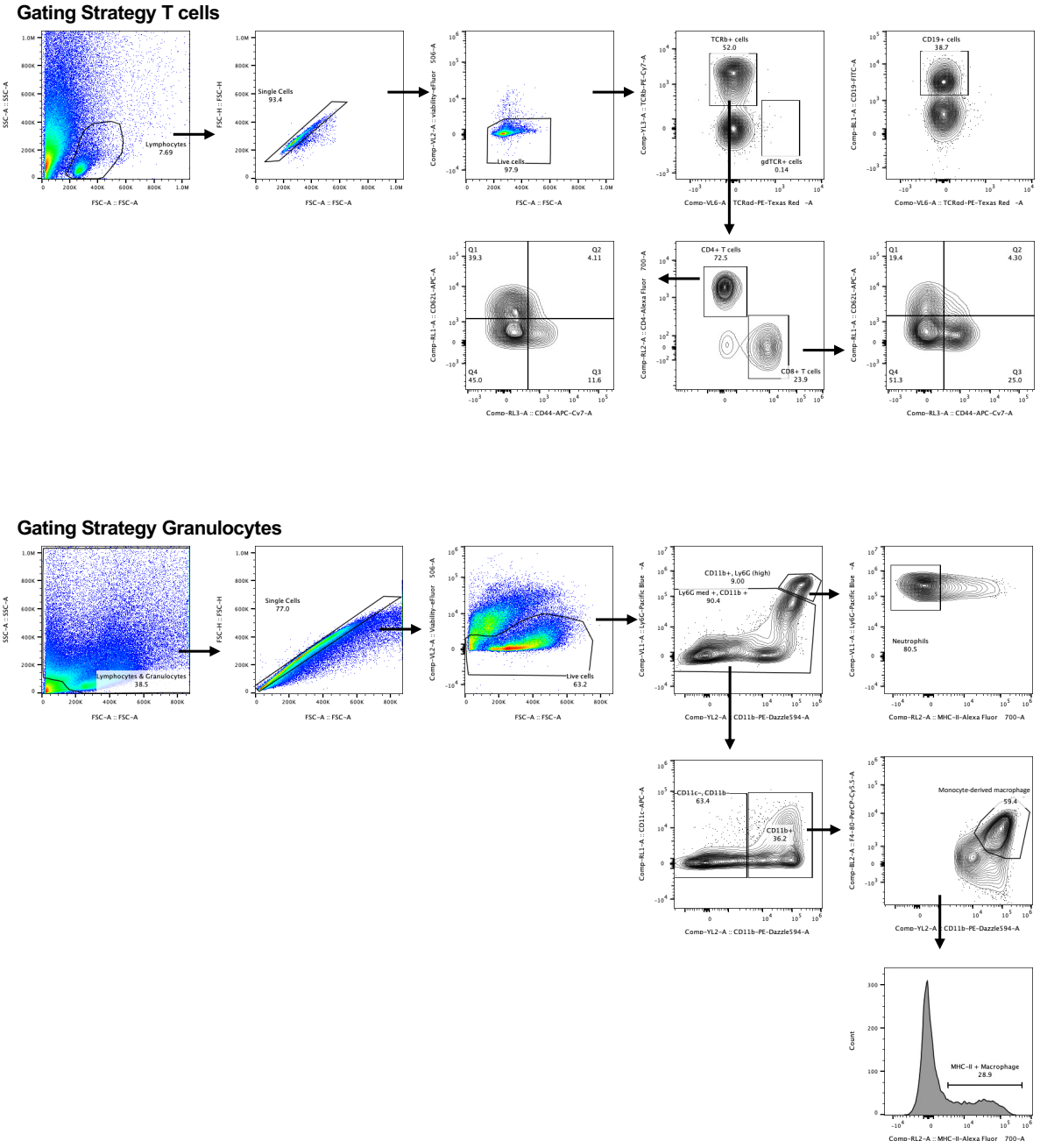

Fig. S2. Gating strategy to identify indicated cell populations in the lungs of mice.

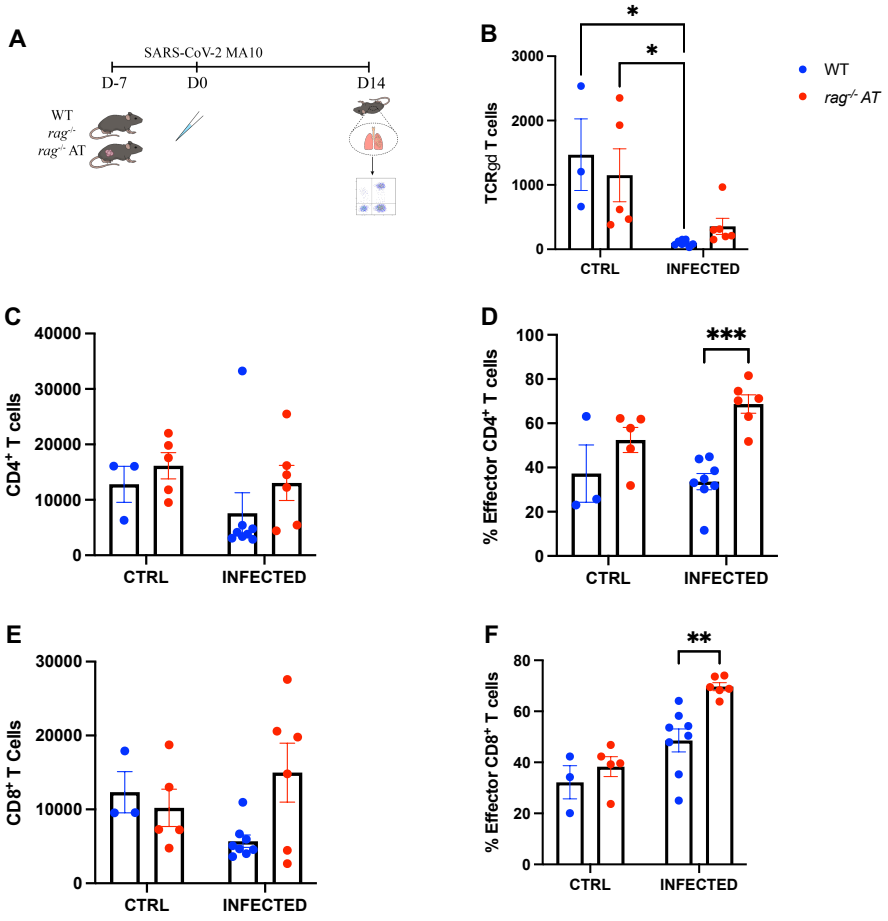

Fig. S3. *rag*$^{-/-}$ AT mice have elevated effector T cells in the lung compared to wild-type mice. **A**. On day 14 post infection (or mock infection, CTRL), lungs were collected from wild-type and *rag*$^{-/-}$ AT mice and cells were analyzed using flow cytometry. **B**. Number of γδ T cells. **C**. Pulmonary CD4^{+} T (TCRβ^{+}, CD4^{+}) cells at day 14 post infection. **D**. Percent Effector CD4^{+} T (TCRβ^{+}, CD4^{+}, CD62L^{-}, CD44^{+}) cells in the lungs at day 14 post infection. **E**. Number of CD8^{+} T (TCRβ^{+}, CD8^{+}) cells in the lungs at day 14 post infection. **F**. Percent of Effector CD8^{+} T (TCRβ^{+}, CD8^{+}, CD62L^{-}, CD44^{+}) cells in the lungs of wild-type and *rag*$^{-/-}$ mice at day 14 post infection. WT: control, n=3, infected, n=8, *rag*$^{-/-}$: control, n=5, infected, n=6, *p<0.05,**p<0.01, ***p<0.001, 2-way ANOVA with Tukey's multiple comparison test, experiments performed once.

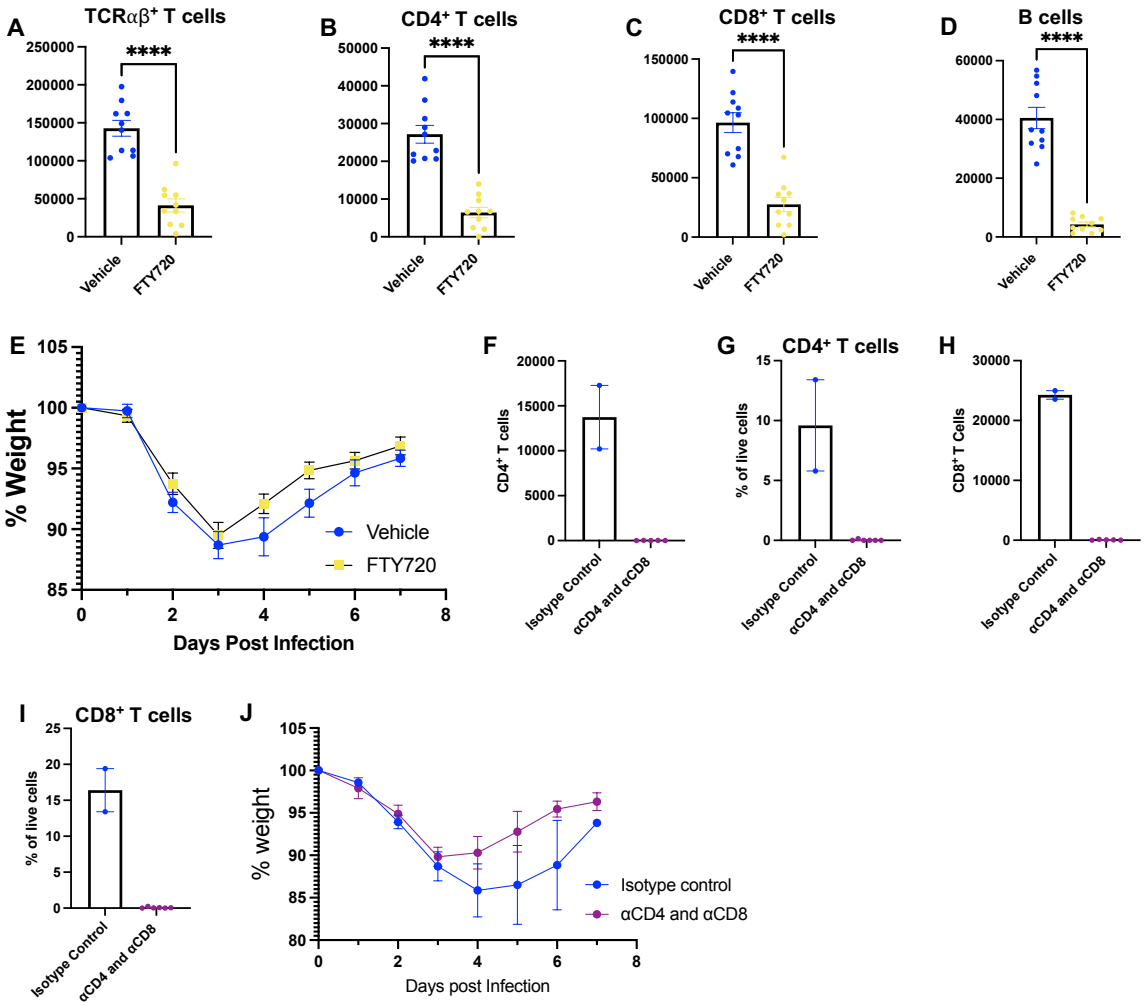

Fig. S4. Tissue resident immune cell likely drive the morbidity observed in WT mice following SARS-CoV-2 MA10 infection. WT mice treated with 1mg/kg FTY720 or vehicle (2% hydroxypropyl-beta-cyclodextrin) starting -2dpi and infected with $1X10^5$ PFU/mouse on day 0. Flow cytometry analysis was performed to measure immune cells in the lungs of FTY720 and vehicle treated mice (**A-D**). **A.** TCRβ$^+$ T cells. **B.** CD4$^+$ T cells. **C.** CD8$^+$ T cells. **D.** CD19$^+$, B220$^+$ cells. Vehicle: n=10, FTY720: n=10, ****p<0.0001, 2-tailed Mann-Whitney test, experiments performed once. **E.** Percent weight change. Vehicle: n=10, FTY720: n=10, 2-way ANOVA with Sidak's multiple comparison test, experiments performed once. WT mice were treated with either a combination of α-CD4 and α-CD8 depleting antibodies or isotype control every other starting -2 dpi followed by infection with 10^5 PFU/mouse SARS-CoV-2 MA10 on day 0. Lung T cells were analyzed by flow cytometry 7 dpi and weights monitored daily (**F-J**). **F.** CD4$^+$ T cells. **G.** Frequency of CD4$^+$ T cells as a percentage of live cells. **H.** CD8$^+$ T cells. **I.** Frequency of CD8$^+$ T cells as a percentage of live cells. **J.** Percent weight change.