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Supplemental information

SARS-CoV-2 S1 protein causes brain inflammation by reducing intracerebral acetylcholine production

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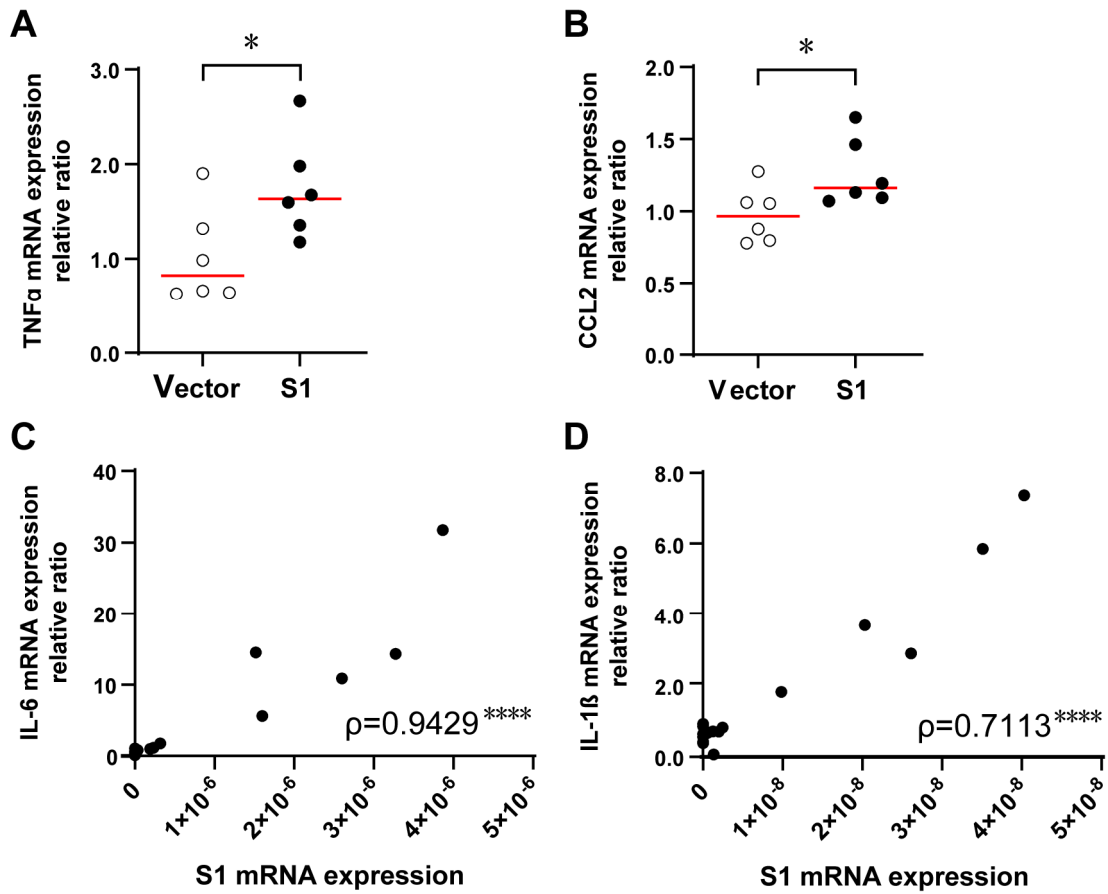


Figure S1. Creation of COVID-19 encephalopathy model using S1 protein and analysis of S1 protein function, related to Figure 1.

(A) Enhanced TNF α expression in S1 mouse brain tissue (vector control, n=6; S1 mouse, n=6; Mann-Whitney U-test; median values; *, p < 0.05).

(B) Enhanced CCL-2 expression in S1 mouse brain tissue (vector control, n=6; S1 mouse, n=6; Mann-Whitney U-test; median values; *, p < 0.05).

(C) Correlation of S1 mRNA expression and IL-6 mRNA expression in lung (n=22; Spearman's rank correlation test; $\rho=0.9429$, ****, p < 0.0001).

(D) Correlation of S1 mRNA expression and IL-1 β mRNA expression in lung (n=18; Spearman's rank correlation test; $\rho=0.7113$, ****, p < 0.0001).

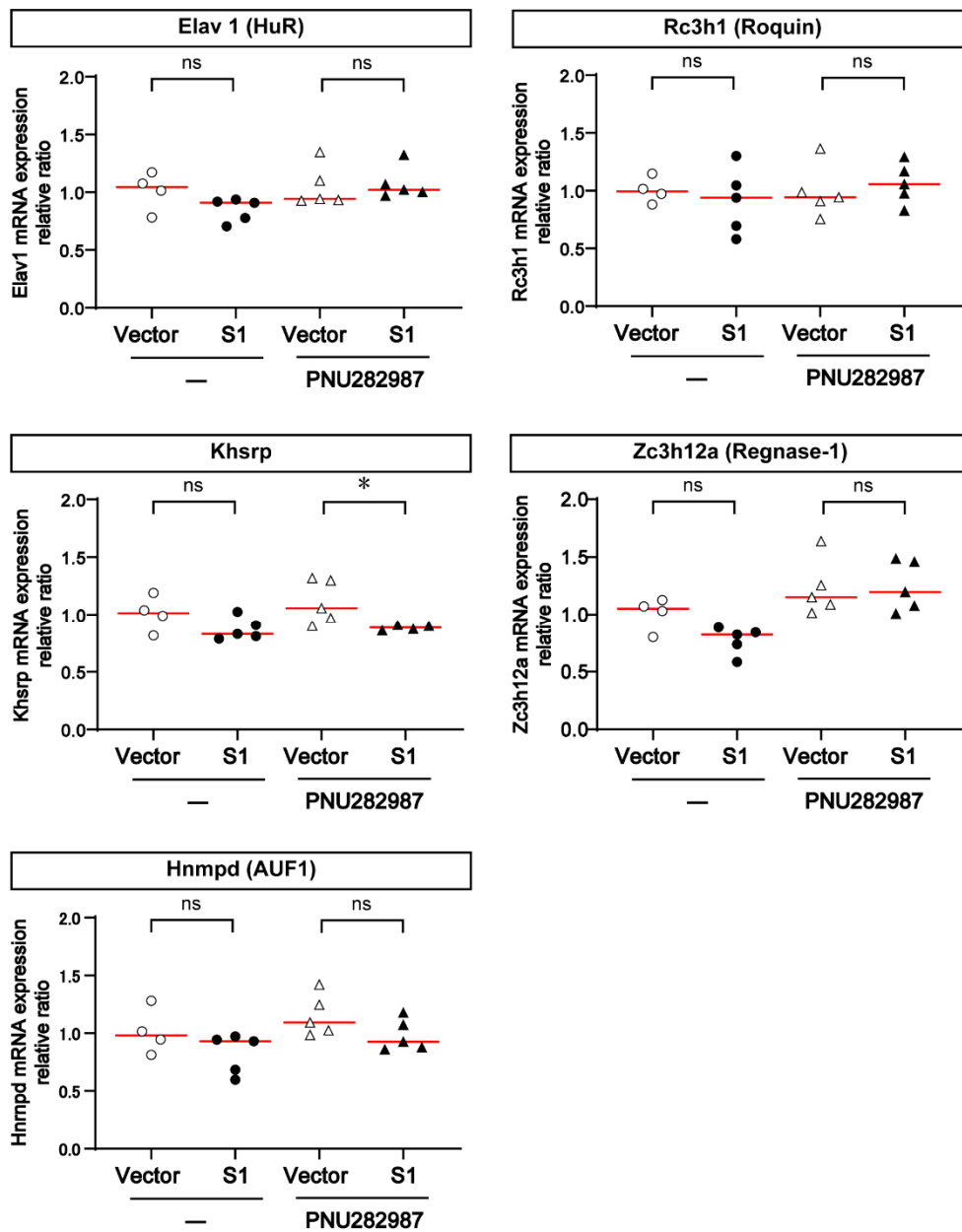


Figure S2. Expression of RNA binding protein with inflammatory cytokine mRNA degrading function, related to Figure 2.

Expression of the indicated genes was measured in brain tissue from S1 mouse and S1 mouse administered PNU282987. (no treatment; vector control, n=4, S1 mouse, n=5; Mann-Whitney U-test; median values; ns, not significant. Donepezil; vector control, n=5 S1 mouse, n=5 (Only one sample was excluded because it showed an abnormal value in the Khsrp measurement.); Mann-Whitney U-test; median values; ns, not significant; *, p < 0.05).

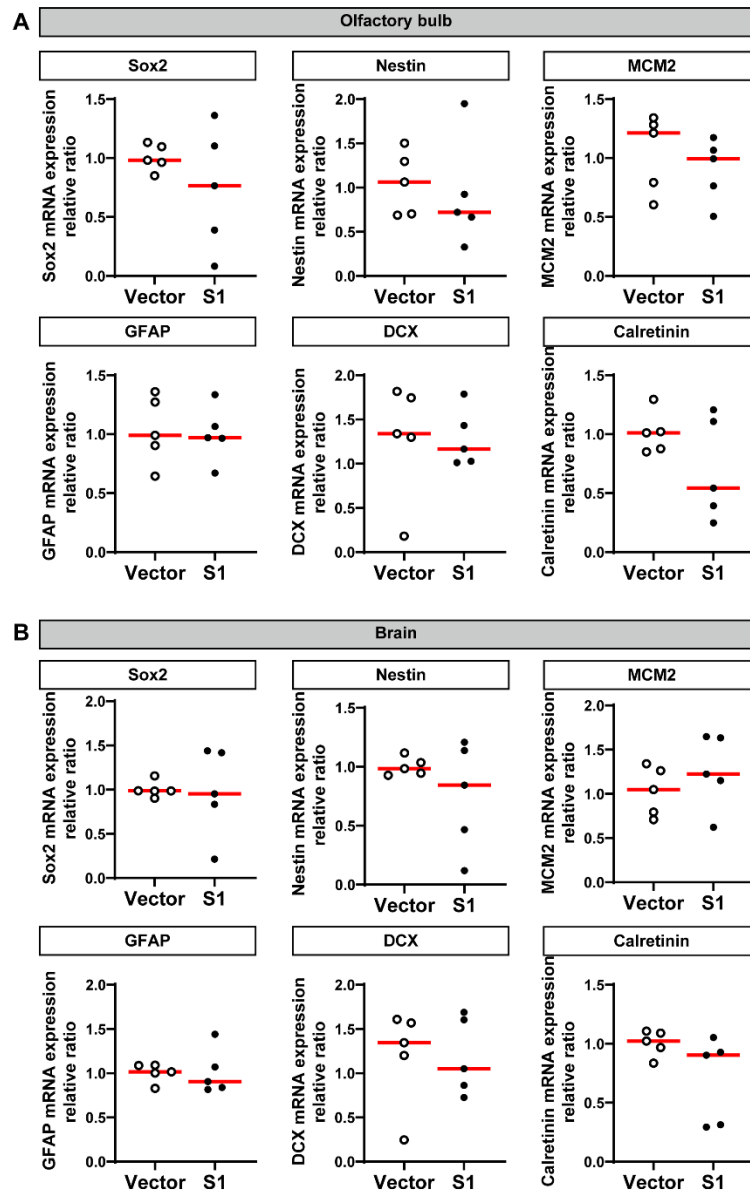


Figure S3. Changes in nerve differentiation marker expression in S1 mice additionally administered LPS, related to Figure 2.

(A) Changes in expression of indicated nerve differentiation marker genes in olfactory bulb tissue of S1 mice additionally administered LPS. (vector control, n=5 S1 mouse, n=5; Mann-Whitney U-test; median values).

(B) Changes in expression of indicated nerve differentiation marker genes in brain tissue of S1 mice additionally administered LPS. (vector control, n=5 S1 mouse, n=5; Mann-Whitney U-test; median values).

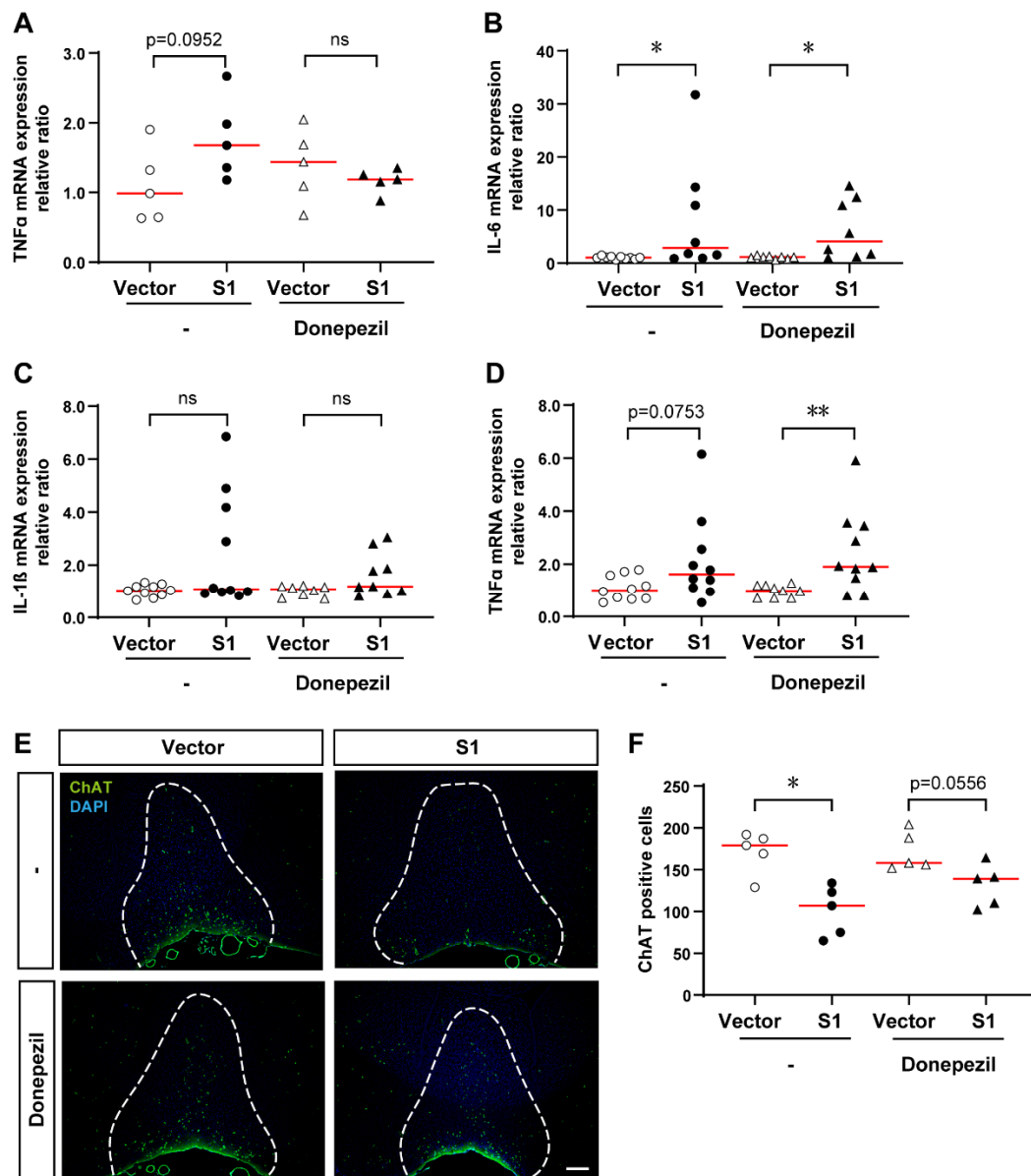


Figure S4. Mitigation of olfactory and brain dysfunction in S1 mouse due to donepezil, related to Figure 3.

(A) Enhanced TNFα expression in S1 mouse brain tissue and improvement due to donepezil (no treatment; vector control, n=5, S1 mouse, n=5; Mann-Whitney U-test; median values. Donepezil; vector control, n=5, S1 mouse, n=5; Mann-Whitney U-test; median values; ns, not significant).

(B) Enhanced IL-6 expression in S1 mouse lung tissue and effect of administering donepezil (no treatment; vector control, n=8, S1 mouse, n=8; Mann-Whitney U-test; median values; *, p <

0.05. Donepezil; vector control, n=8, S1 mouse, n=8; Mann-Whitney U-test; median values; *, p < 0.05).

(C) Enhanced IL-1 β expression in S1 mouse lung tissue and effect of administering donepezil (no treatment; vector control, n=10, S1 mouse, n=10; Mann-Whitney U-test; median values; ns, not significant. Donepezil; vector control, n=8, S1 mouse, n=8; Mann-Whitney U-test; median values; ns, not significant).

(D) Enhanced TNF α expression in S1 mouse lung tissue and effect of administering donepezil (no treatment; vector control, n=10, S1 mouse, n=10; Mann-Whitney U-test; median values. Donepezil; vector control, n=10, S1 mouse, n=10; Mann-Whitney U-test; median values; **, p < 0.01).

(E) Decreased ChAT positive cells in S1 mouse MS and DBB and effect of donepezil (green, ChAT; blue, DAPI; scale bar, 200 μ m).

(F) Decreased ChAT positive cells in S1 mouse MS and DBB and effect of donepezil (no treatment; vector control, n=5, S1 mouse, n=5; Mann-Whitney U-test; median values; *, p < 0.05. Donepezil; vector control, n=5 S1 mouse, n=5; Mann-Whitney U-test; median values).