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Supplemental figures

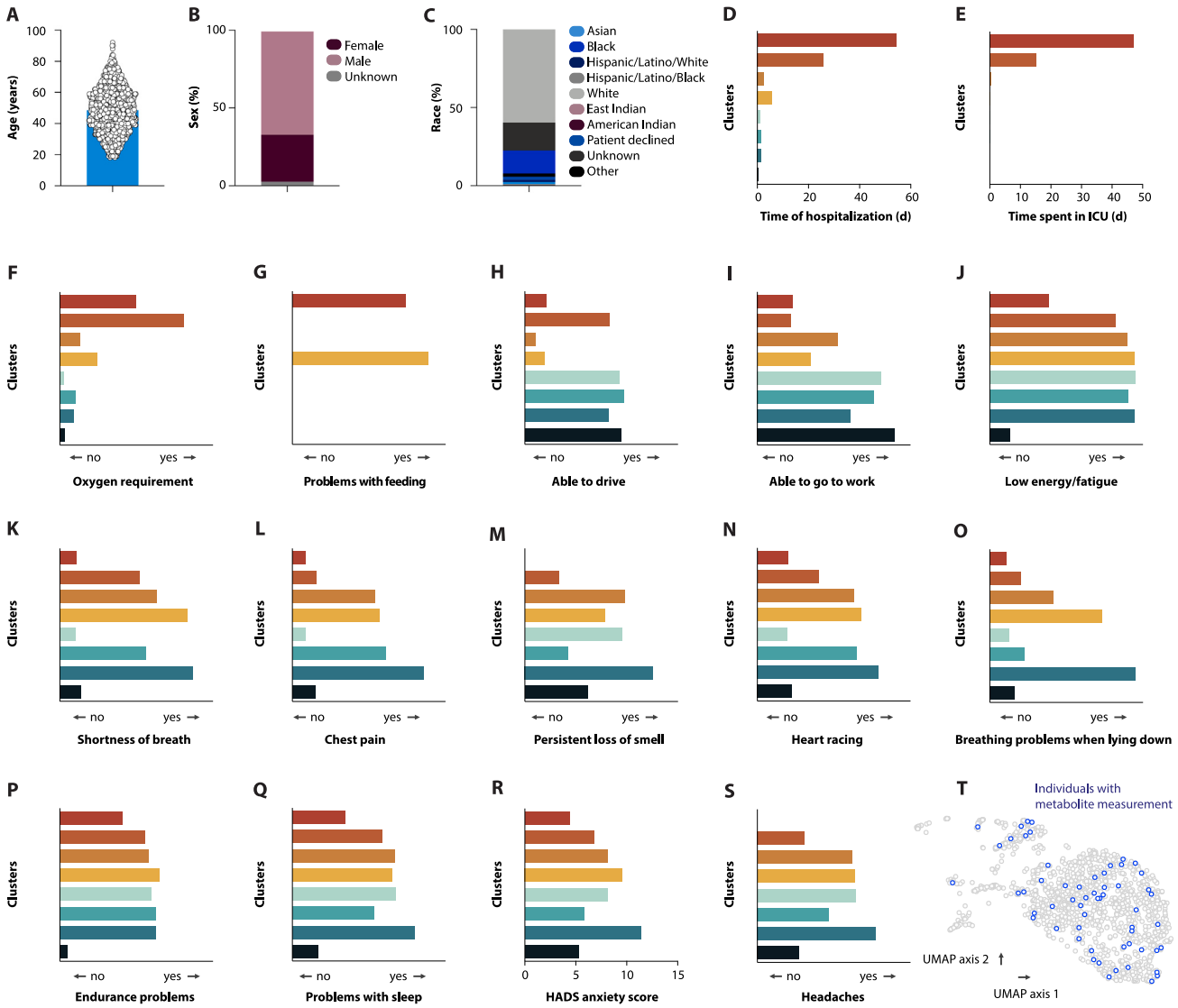

Figure S1. Symptom clusters in a cohort of 1,540 individuals with PASC, related to Figure 1

(A–C) Age (A), sex (B), and race (C) in the UPenn PASC cohort.

(D–S) Symptom distribution in PASC cohort clusters.

(T) UMAP clusters of symptom presentation in UPenn PASC cohort. Highlighted are PASC patients whose circulating metabolite levels were determined.

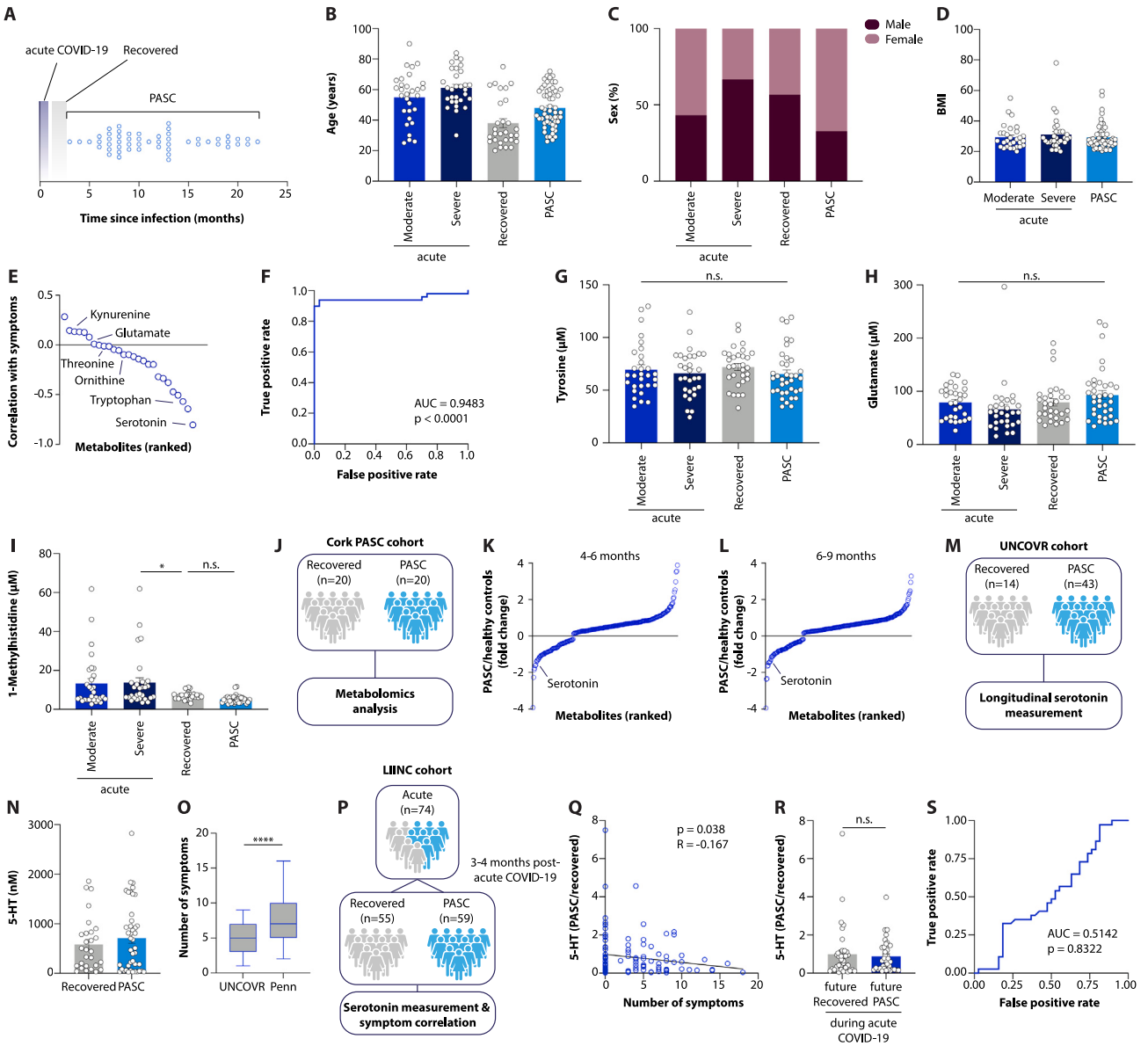

Figure S2. Metabolite changes in PASC, related to Figure 1

(A–D) Time after acute infection (A), age (B), sex (C), and BMI (D) of patients from acute COVID-19, recovered, and PASC cohorts.

(E) Metabolites ranked by their correlation with acute COVID-19 or PASC.

(F) Receiver operating characteristic (ROC) curve of PASC classification based on serotonin measurements.

(G–I) Plasma tyrosine (G), glutamate (H), and 1-methylhistidine (I) levels in acute COVID-19, recovered, and PASC patients.

(J–L) Study schematic (J) and metabolites ranked by differential abundance in PASC compared to healthy controls at 4–6 months (K) and 6–9 months (L) after acute COVID-19 in the Cork PASC cohort.[12]

(M–O) Study schematic (M), serotonin levels of patients recovered from COVID-19 (0 symptoms) and patients with PASC (≥2 symptoms) (N), and average symptom number (O) in the UNCOVR cohort.[13]

(P and Q) Study schematic (P) and correlation between the number of PASC symptoms and plasma serotonin level in patients recovered from COVID-19 (symptoms = 0) and patients with PASC (symptoms ≥2) 3–4 months following acute COVID-19 (Q) of the UCSF LIINC cohort.[14]

(R and S) Serotonin levels during acute COVID-19 in patients who will fully recover versus those who go on to develop PASC (R) and ROC curve of PASC classification based on serotonin levels during acute COVID-19 (S).

Plotted are means ± SEM or mean ± min. and max. values (O). n.s. $p > 0.05$, *$p < 0.05$, ****$p < 0.0001$.

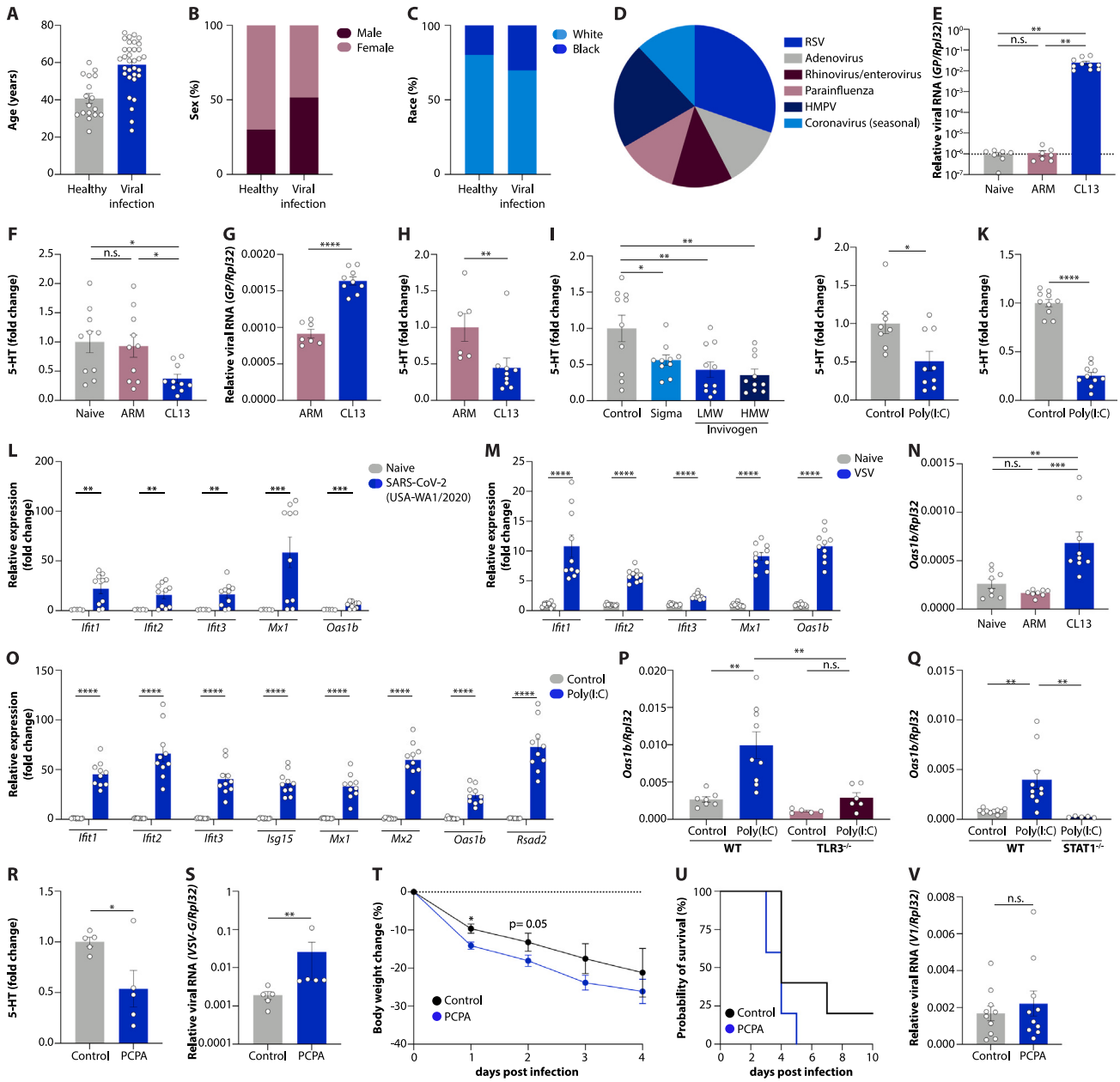

Figure S3. Characteristics of viral inflammation, related to Figure 2

(A–D) Age (A), sex (B), race (C), and source of viral infection (D) of participants with systemic viral infection.

(E–H) Relative viral RNA load in the ileum (E and G) and platelet serotonin levels (F and H) in mice infected with LCMV Armstrong (ARM) or LCMV Clone 13 (CL13) for 30 days (E and F) or 60 days (G and H).

(I) Serotonin levels in mice treated with low-molecular-weight (LMW) or high-molecular-weight (HMW) poly(I:C) from the indicated vendors.

(J and K) Plasma (J) and platelet (K) serotonin levels in mice treated with poly(I:C).

(L–Q) Relative expression of interferon-stimulated genes (ISGs) in lungs of SARS-CoV-2 (USA-WA 1/2020)-infected mice (L); spleens of VSV-infected mice (M); ileum of mice infected with LCMV ARM or LCMV CL13 for 15 days (N); and ileum of poly(I:C)-treated wild-type (O), TLR3$^{-/-}$ (P), and STAT1$^{-/-}$ mice (Q).

(R–U) Platelet serotonin levels (R), viral RNA load (S and V), weight loss (T), and survival curve (U) in PCPA-treated mice infected with VSV for 48 h (S–U) or SARS-CoV-2 (B.1.351) for 7 days (V).

Plotted are means ± SEM. n.s. $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

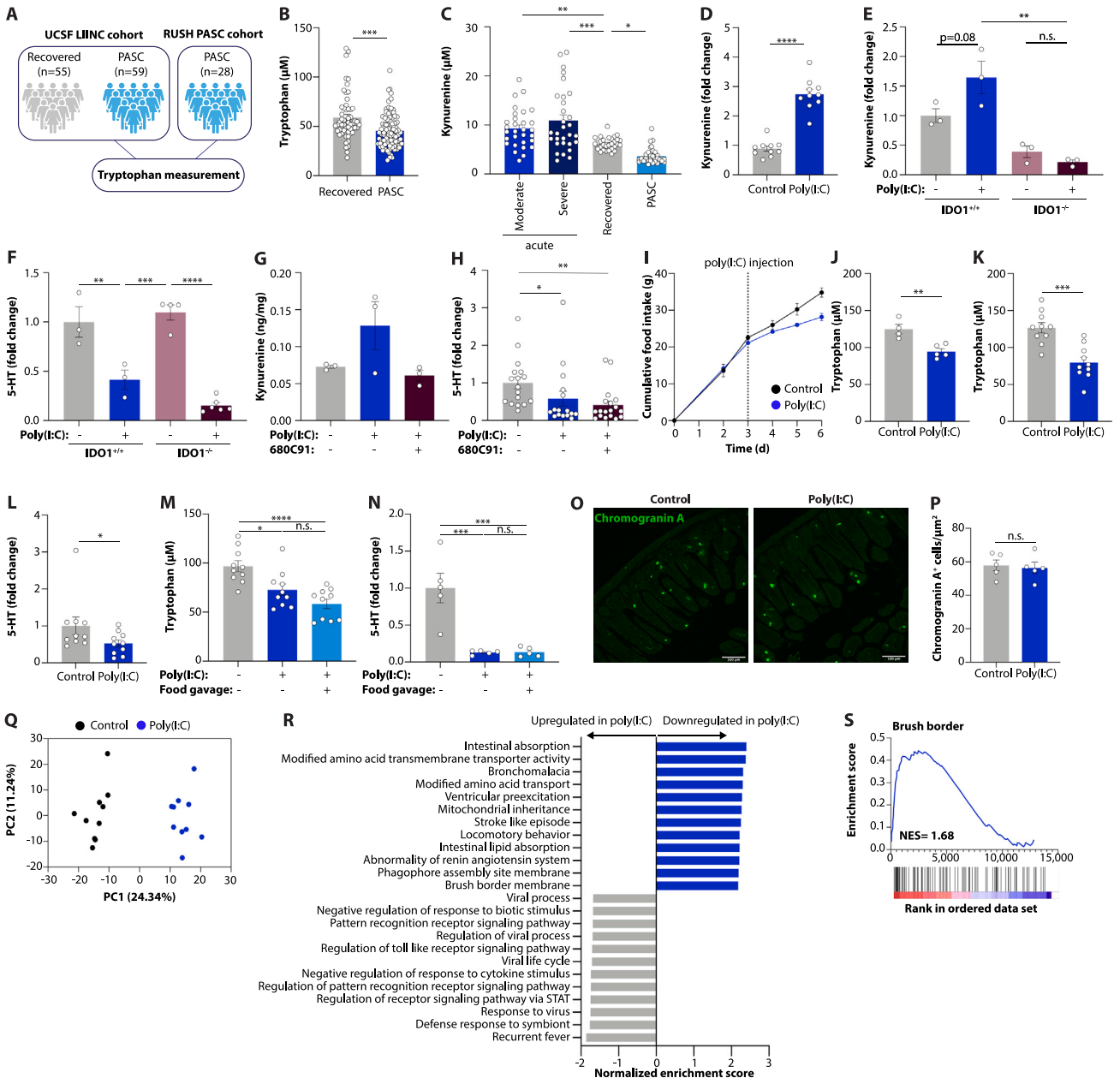

Figure S4. The impact of viral inflammation on metabolite abundances, related to Figure 3

(A and B) Study schematic (A) and plasma tryptophan levels in recovered individuals or PASC patients 3–4 months after acute infection (B) in the UCSF LIINC and RUSH PASC cohorts.[14,27]

(C and D) Plasma kynurenine levels in acute COVID-19 and after recovery in individuals with PASC (C) and in poly(I:C)-treated mice (D).

(E–H) Plasma kynurenine (E), platelet serotonin (F), liver kynurenine (G), and plasma serotonin (H) in poly(I:C)-treated IDO1$^{+/+}$ and IDO$^{-/-}$ mice (E and F) or mice receiving the TDO2 inhibitor 680C91 (G and H).

(I) Cumulative food intake in mice treated with poly(I:C) or vehicle control. Dotted line indicates the beginning of daily injections.

(J–N) Plasma tryptophan (J, K, and M) and platelet serotonin (L and N) levels in poly(I:C)-treated mice after 36 h of fasting (J), paired feeding with control mice (K and L), or food gavage (M and N).

(O and P) Representative images (O) and quantification (P) of chromogranin A staining in the ileum of poly(I:C)-treated mice. Scale bars, 100 μm.

(Q–S) PCA plot of global gene expression (Q), enriched gene sets (R), and GSEA plot of gene set negatively correlated with poly(I:C) treatment (S) in ileal tissue.

Plotted are means ± SEM. n.s. $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

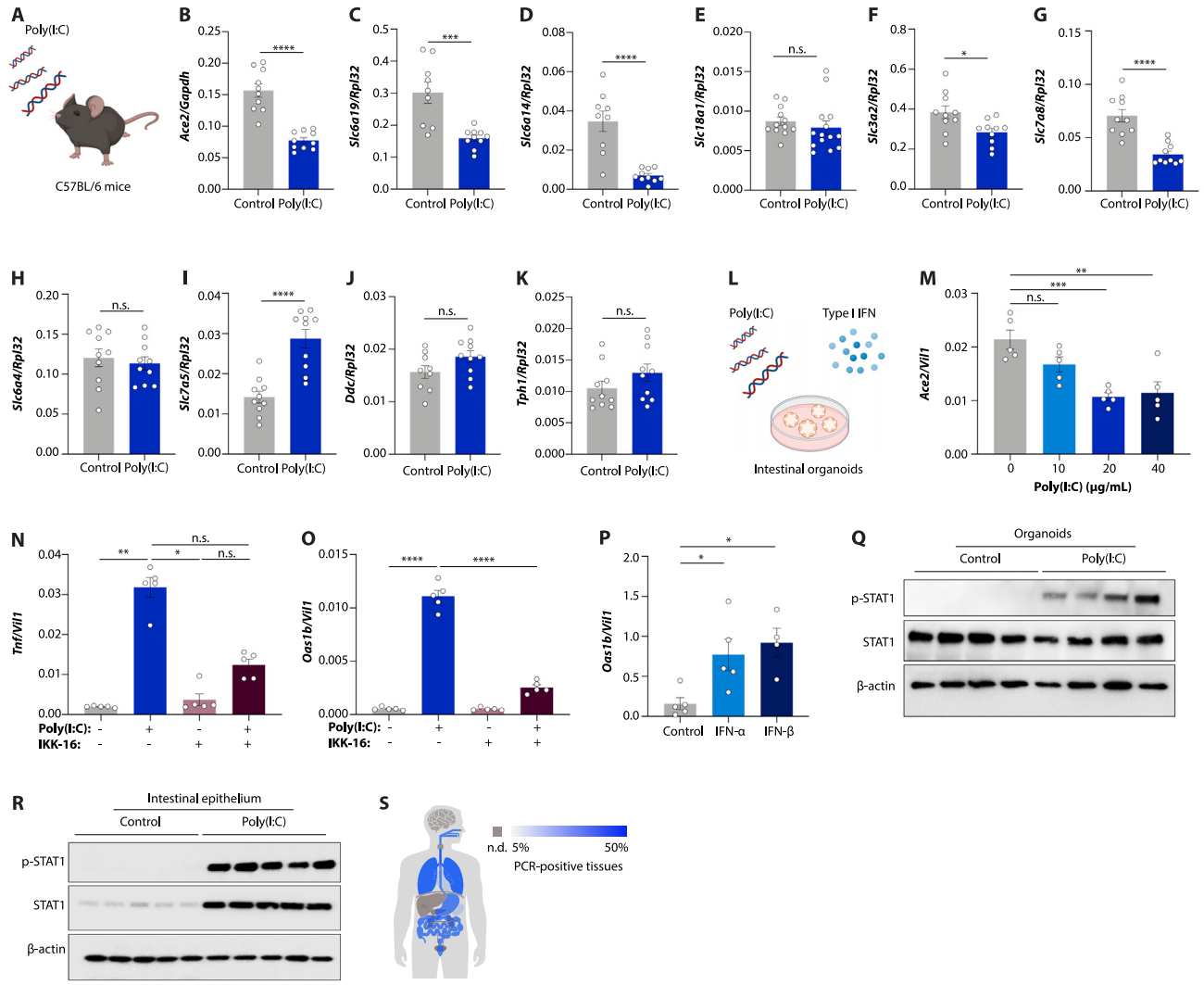

Figure S5. The transcriptional impact of viral inflammation, related to Figure 4

(A) Schematic of the experimental setup for poly(I:C) treatment of mice.

(B–K) qPCR expression of *Ace2* (B), *Slc6a19* (C), *Slc6a14* (D), *Slc18a1* (E), *Slc3a2* (F), *Slc7a8* (G), *Slc6a4* (H), *Slc7a5* (I), *Ddc* (J), and *Tph1* (K) in the ileum of poly(I:C)-treated mice.

(L) Schematic of the experimental setup for treatment of organoids with poly(I:C) or type I IFNs.

(M) *Ace2* expression in murine small intestinal organoids treated with different concentrations of poly(I:C) for 4 h.

(N and O) *Tnf* (N) and *Oas1b* (O) expression levels in small intestinal organoids treated with poly(I:C) and IKK-16.

(P) *Oas1b* expression levels in small intestinal organoids with or without IFN-α and IFN-β.

(Q and R) Western blots of intestinal organoids (Q) or ileal epithelial cells from mice (R) treated with poly(I:C).

(S) Schematic indicating the percentage of SARS-CoV-2 RNA-positive tissues obtained from autopsies >2 weeks after acute infection.

Plotted are means ± SEM. n.s. $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

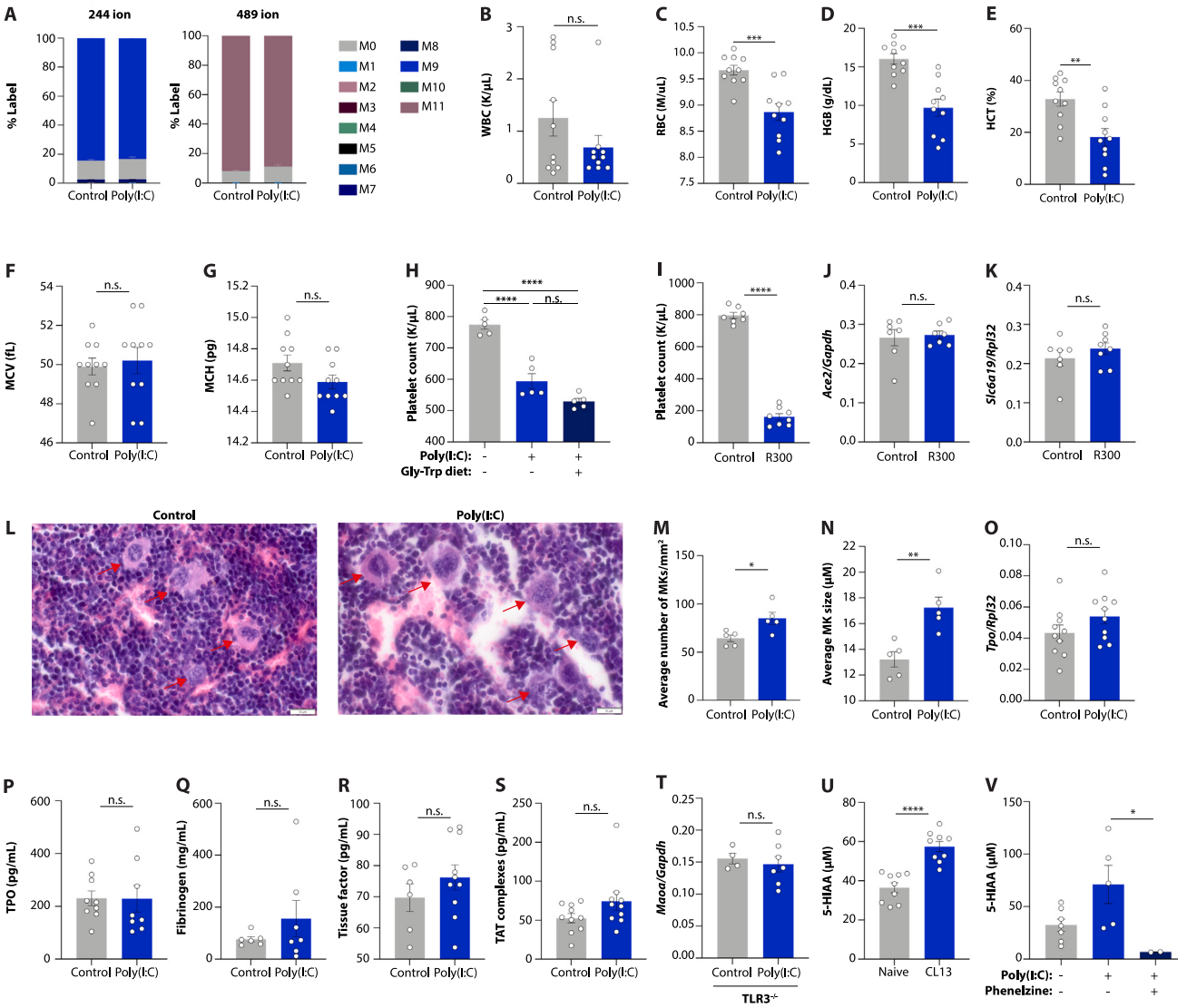

Figure S6. Blood parameters during viral inflammation, related to Figure 6

(A) Fractional labeling of circulating tryptophan after oral gavage in poly(I:C)-treated and control mice.

(B–G) White blood cell (WBC) counts (B), red blood cell (RBC) counts (C), hemoglobin (HGB) levels (D), percent hematocrit (HCT) (E), mean corpuscular volume (MCV) (F), and mean corpuscular hemoglobin (MCH) values (G) in poly(I:C)-treated mice.

(H) Platelet counts in poly(I:C)-treated mice fed a Gly-Trp dipeptide diet.

(I–K) Platelet counts (I), *Ace2* expression (J), and *Slc6a19* expression (K) in mice treated with a platelet-depleting antibody (R300) or isotype control for 24 h.

(L–N) Representative images (L) and average number (M) and size (N) of megakaryocytes in femur bone marrow of poly(I:C)-treated mice. Arrows indicate megakaryocytes. Scale bars, 10 μm.

(O) Relative *Tpo* expression in livers of control and poly(I:C)-treated mice.

(P–S) Plasma thrombopoietin (TPO) levels (P), plasma fibrinogen (Q), tissue factor (R), and thrombin-antithrombin (TAT) complex (S) in poly(I:C)-treated mice.

(T) *Maoa* expression in the ileum of TLR3$^{-/-}$ mice with or without poly(I:C) treatment.

(U and V) 5-HIAA levels in urine of mice infected with LCMV CL13 for 30 days (U) and of poly(I:C)-treated mice receiving the MAO inhibitor phenelzine (V).

Plotted are means ± SEM. n.s. $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

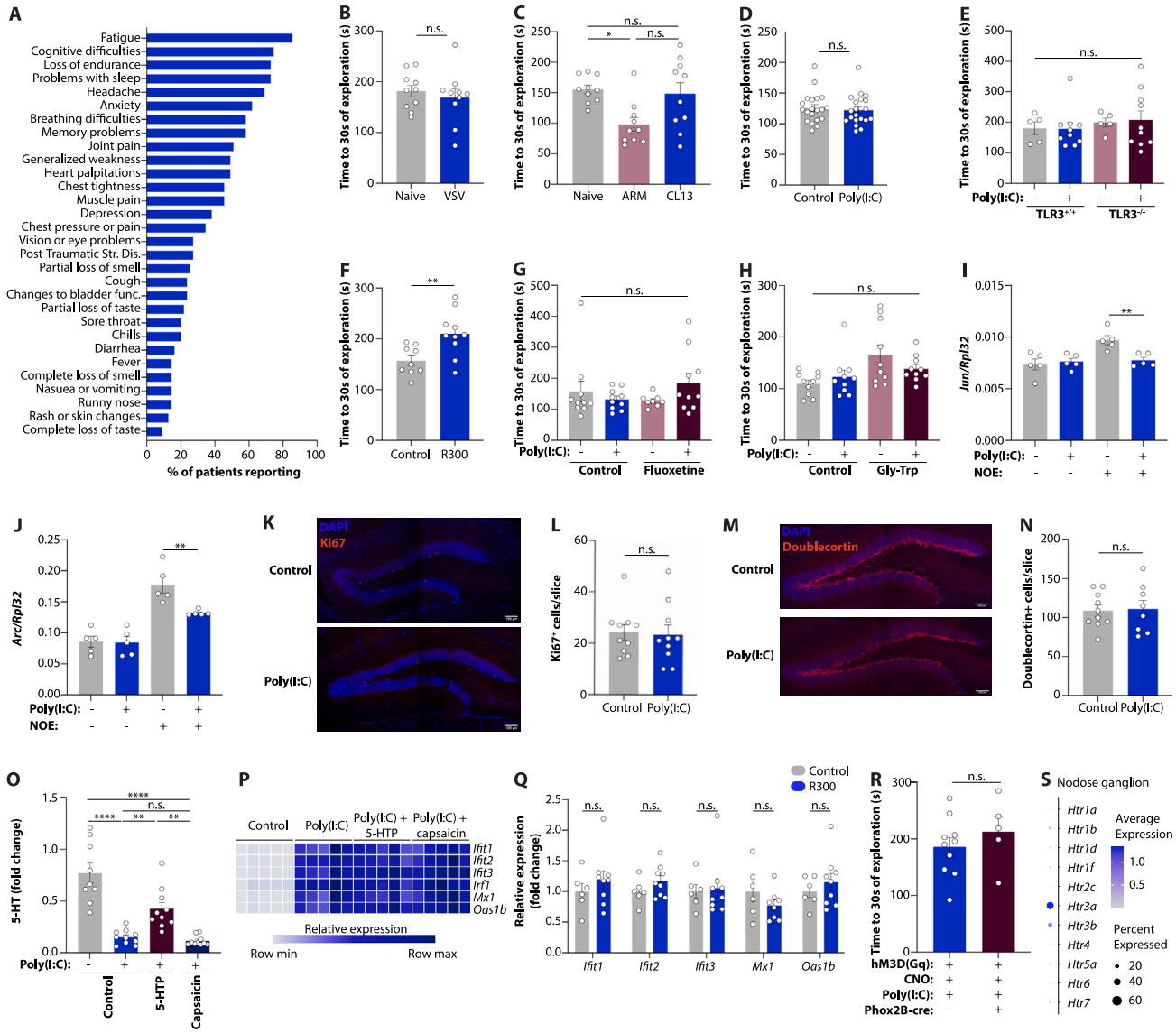

Figure S7. Cognitive performance during viral inflammation, related to Figure 7

(A) Symptoms most commonly reported by patients in the UPenn PASC cohort with targeted metabolomics data.

(B–H) Total time to reach 30 s of exploration between familiar and novel objects in mice infected with VSV for 24 h (B), mice infected with LCMV ARM or LCMV CL13 for 14 days (C), poly(I:C)-treated mice (D), poly(I:C)-treated TLR3$^{-/-}$ mice (E), mice treated with a platelet-depleting antibody (R300) or isotype control for 24 h (F), poly(I:C)-treated mice treated with fluoxetine (G), and poly(I:C)-treated mice fed a Gly-Trp dipeptide diet (H).

(I and J) Expression of *Jun* (I) and *Arc* (J) in the hippocampus of poly(I:C)-treated mice with or without NOE.

(K–N) Representative images (K and M) and quantification (L and N) of Ki67-positive cells (K and L) and doublecortin-positive cells (M and N) in the dentate gyrus of poly(I:C)-treated mice. Scale bars, 100 μm.

(O and P) Serotonin levels (O) and relative expression of ISGs in the hippocampus (P) of poly(I:C)-treated mice receiving 5-HTP or capsaicin.

(Q) Relative expression of ISGs in hippocampi of mice treated with a platelet-depleting antibody (R300) or isotype control for 48 h.

(R) Total time to reach 30 s of exploration between familiar and novel objects in Phox2b-cre mice injected with AAV-hM3Dq, CNO, and poly(I:C).

(S) Dot plots of serotonin receptor expression in single-cell RNA-seq data from mouse nodose ganglia.[52]

Plotted are means ± SEM. n.s. $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.