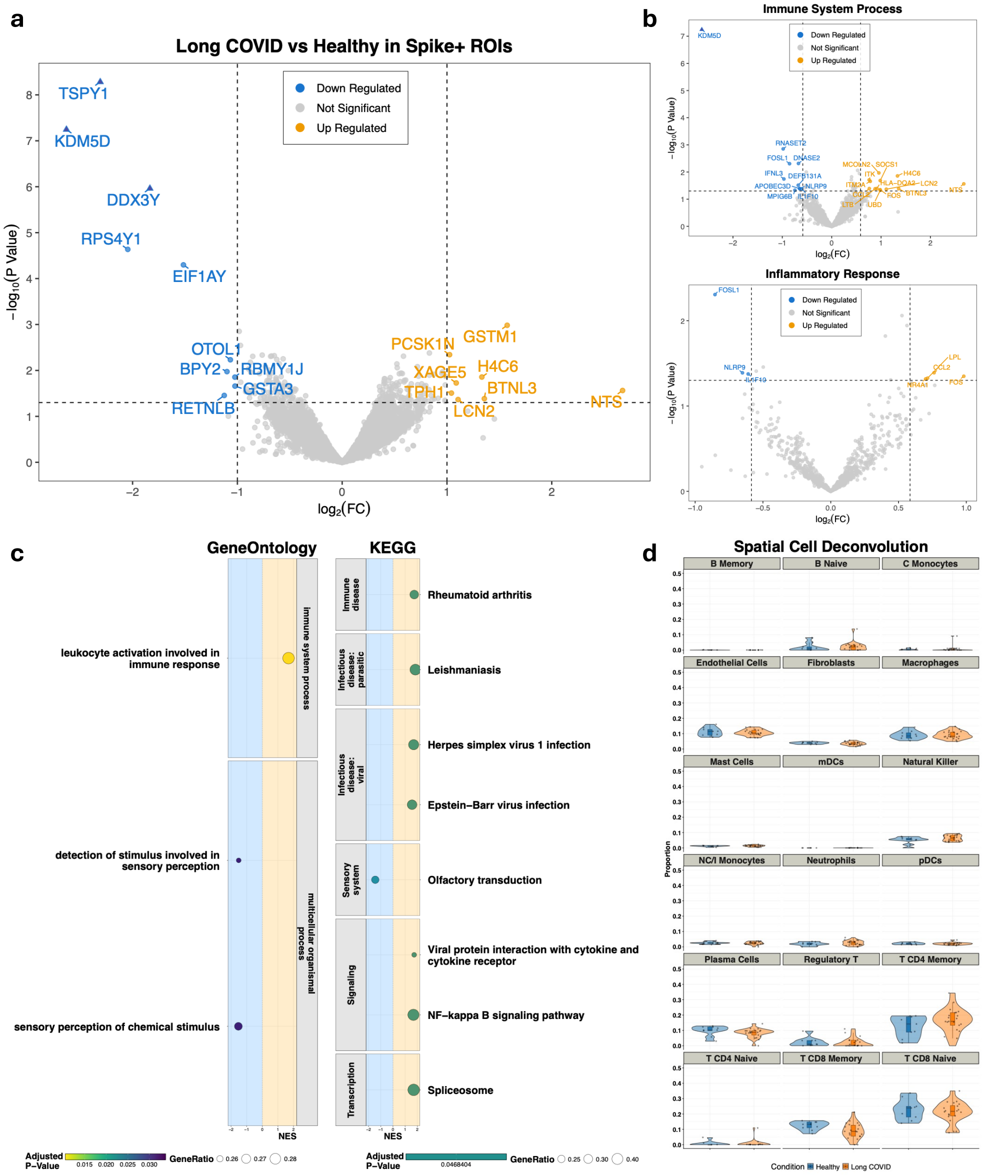
Supplementary Figures

Supplementary Table 1. Demographics of Patients by Condition

Variable	Control (n=5)[1]	Long COVID (n=8)[1]	p-value[2]
Tissue Collected			
Colon & Ileum	5/5 (100%)	7/8 (88%)	
Colon Only	0/5 (0%)	1/8 (13%)	
Age (years)	48 (18)	45 (12)	0.94
Sex			0.10
Female	1/5 (20%)	6/8 (75%)	
Male	4/5 (80%)	2/8 (25%)	
BMI (kg/m²)	28.2 (4.1)	24.4 (5.0)	0.12

[1] n / N (%); Mean (SD)

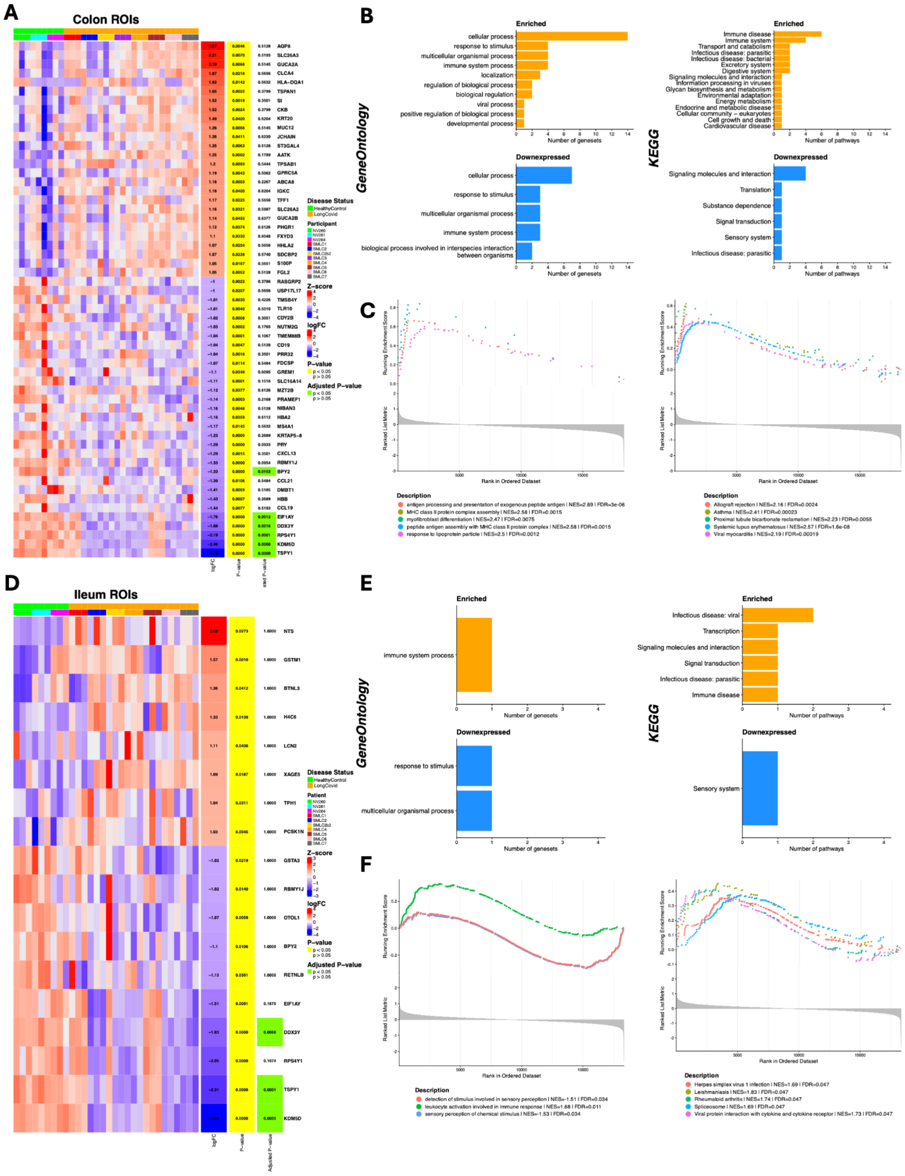
[2] Wilcoxon rank sum test; Fisher’s exact test; Wilcoxon rank sum exact test



Supplementary Figure 1. Differential Expression of Long COVID and Healthy Spike+ ROIs in the Ileum by Spatial Transcriptomics. (A) A total of 33 ROIs were analyzed between 8 Long COVID (24 ROIs) and 3 healthy (9 ROIs) participants in the region positive for SARS-CoV-2 Spike antigen. Differential analysis was performed using the limma-voom pipeline, correcting for replicate samples and variation in the frequency of detected SARS-CoV-2 Spike by ROI. 18,581 transcript targets were plotted. The main volcano plot highlights 8 and 10 genes that are upregulated and downregulated, respectively, at an unadjusted *p*-value threshold of 0.05 and an absolute $\log_2$FC > 1. Points with a triangular shape passed the adjusted p-value threshold based on a Benjamini-Hochberg correction. (B) Genes involved in *GeneOntology* (GO) (GO:0002376 & GO:0006954) parent category were filtered and plotted into each respective volcano plot with an unadjusted *p*-value threshold of 0.05 and an absolute FC > 1.5. (C) Gene-Set Enrichment Analysis was performed on a ranked list of $\log_2$FC values, resulting in 4 *GeneOntology* gene sets (2 enriched and 2 downexpressed) and 8 *KEGG* pathways (7 enriched and 1 downexpressed) passing the adjusted p-value threshold of 0.05. Representative categories were selected, and pathways/ gene sets were plotted using the normalized enrichment score (NES) alongside the ratio of genes in the leading edge to total genes in the gene set

(GeneRatio) and the adjusted p-value. (D) Spatial deconvolution was performed using a safeTME reference expression profile. Statistical significance was assessed using the propeller framework, a moderated two-tailed Mann-Whitney U test with BH FDR correction (*FDR* ≤ 0.05 (*), *FDR* ≤ 0.01 (**), *FDR* ≤ 0.001 (***)). No statistical significance was found in any cell type compared.

Supplementary Figure 2.



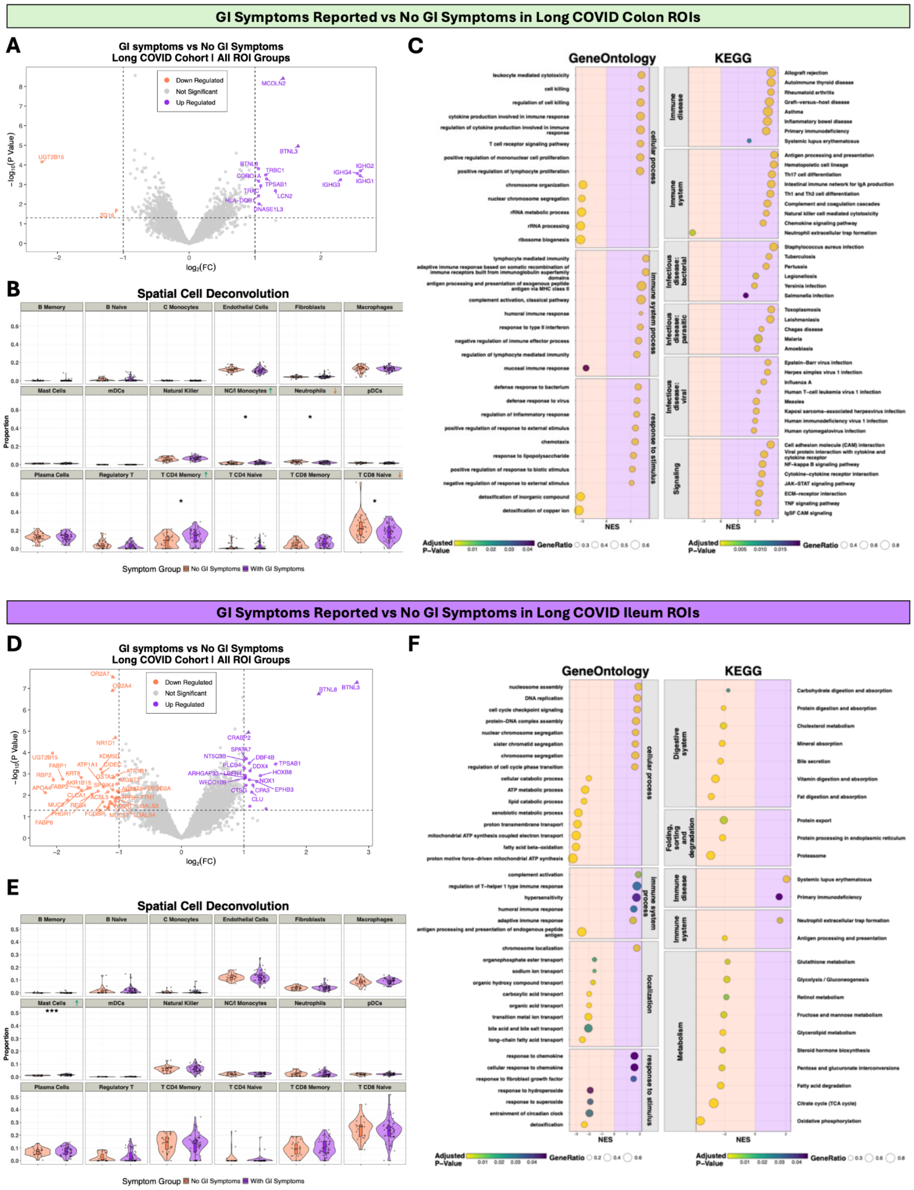
Supplementary Figure 2. GSEA enrichment hierarchy count and top leading-edge. (A) (D) heatmap of differentially expressed genes (by p-value < 0.05) showing row-wise z-scored log-normalized expression scores organized by condition then participant. (B) (E) Count of genesets/ pathways in GeneOntology (BP) and KEGG by major category produced from GSEA (BH adjusted p-value < 0.05). (C) (F) The top 5 pathways by absolute NES were selected and plotted in a leading-edge enrichment plot with rank list metrics.

Supplementary Table 2. Patient Comorbidities and Medications.

Patient ID	Condition	Comorbidities	Medications
SMLC001	Long COVID	allergic rhinitis; hyperlipidemia; vitamin D deficiency; colon polyps	aspirin; lactobacillus 3/FOS/pantethine; quercetin; alpha-lipoic acid; American ginseng root; bromelains; cholecalciferol; coenzyme Q; famotidine; fexofenadine; levocarnitine; montelukast; omega-3 DHA/EPA fish oil; turmeric; vitamin B complex capsule
SMLC002	Long COVID	appendicitis; GERD; Hashimoto's thyroiditis; hemorrhoids; colon polyps	rimegepant; nebivolol; candesartan; ondansetron; spironolactone; levothyroxine
SMLC003	Long COVID	dysautonomia	none
SMLC004	Long COVID	proctitis/periappendical inflammation	none
SMLC002B2	Long COVID	appendicitis; GERD; Hashimoto's thyroiditis; hemorrhoids; colon polyps; mildly active nonspecific colitis (7/27/2023)	rimegepant; nebivolol; candesartan; ondansetron; spironolactone; levothyroxine
SMLC005	Long COVID	hemorrhoids	acetylcysteine; cyanocobalamin; guanfacine; pyridoxine; pyridostigmine; valganciclovir; finasteride
SMLC006	Long COVID	hemorrhoids; anxiety; depression	none
SMLC007	Long COVID	PCOS; severe obesity; menorrhagia; oligomenorrhea; PTSD; ADHD; dysautonomia	tizanidine; gabapentin; propranolol; cholecalciferol; alprazolam
NV260	Control	anxiety; low blood pressure; thyroid disease	none
NV261	Control	heart failure due to valvular disease; hyperlipidemia; hypertriglyceridemia	none
NV264	Control	dyslipidemia; vitamin D deficiency; peptic duodenitis	none
NV362	Control	colon polyps	famotidine; valsartan–hydrochlorothiazide
NV363	Control	colon polyps; breast cancer; hypertension; osteoarthritis	memantine; losartan

Supplementary Table 3. Date of Last COVID-19 Infection and Vaccination History.

Patient ID	Condition	Date of Biopsy	Date of Last Infection	Vaccination History
SMLC001	Long COVID	11/11/22	4/16/22	COVID-19 Moderna (12Y+, 0.5 mL) – 10/23/2021, 05/28/2022 COVID-19 Ad26 (Janssen, Johnson & Johnson) – 4/2/2021
SMLC002	Long COVID	11/15/22	2/8/21	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 12/22/2021, 4/21/2021, 3/31/2021 Pfizer booster (30 mcg/0.3 mL, 12Y+) – 10/14/2022
SMLC003	Long COVID	3/28/23	~07/22 (reported)	No vaccine record
SMLC004	Long COVID	5/23/23	~02/22 (reported)	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 03/19/2021, 02/26/2021 Pfizer booster (30 mcg/0.3 mL, 12Y+) – 9/23/2022
SMLC002B2	Long COVID	7/27/23	2/8/21	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 12/22/2021, 4/21/2021, 3/31/2021 Pfizer booster (30 mcg/0.3 mL, 12Y+) – 10/14/2022
SMLC005	Long COVID	10/23/23	1/7/22	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 2/25/2021, 03/19/2021, 09/29/2021 Pfizer booster (30 mcg/0.3 mL, 12Y+) – 9/9/2022 COMIRNATY 2023–24 (30 mcg/0.3 mL, 12Y+) – 9/29/2023
SMLC006	Long COVID	10/23/23	~07/22 (reported)	Janssen – 03/01/2021 Moderna – 11/24/2021
SMLC007	Long COVID	12/7/23	6/9/22	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 12/16/2021 Novavax (NVX-CoV2373, 0.5 mL) – 4/25/2023
NV260	Control	6/20/23	5/18/22	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 01/27/2021, 02/17/2021, 12/18/2021
NV261	Control	6/20/23	NA	Moderna mRNA (100 mcg/0.5 mL) – 1/9/2021, 2/6/2021
NV264	Control	7/25/23	NA	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 12/8/2021
NV362	Control	6/26/25	NA	No vaccine record
NV363	Control	6/26/25	~03/20 (reported)	Pfizer mRNA (30 mcg/0.3 mL, 12Y+) – 2/3/2021, 2/24/2021, 9/27/2021



Supplementary Figure 3. Differential Analysis of the Colon and Ileum between Long COVID Patients with and Without GI Symptoms. (A) Differential expression of Colon ROIs for Long COVID participants with GI symptoms (5 patients) and without GI symptoms (3 patients) produced 14 upregulated and 2 downregulated statistically significant genes. (B) Spatial cell deconvolution was performed using safeTME as the reference dataset; no statistically significant (BH adjusted p-value < 0.05) comparisons were found. (C) GSEA was performed on a ranked list of log$_2$FC values, resulting in 341 *GeneOntology* genesets (250 enriched and 91 downexpressed) and 119 *KEGG* pathways (86 enriched and 33 downexpressed) passing the BH-adjusted p-value threshold of 0.05. Representative categories were selected, and pathways/ gene sets were plotted using the normalized enrichment score (NES) alongside ratio of genes in the leading edge to total genes in the gene set (GeneRatio) and the adjusted p-value. (D) Differential expression of Ileum ROIs for Long COVID participants with GI symptoms (4 patients) and without GI symptoms (2 patients) produced 20 upregulated and 40 downregulated statistically significant genes. (E)

Spatial cell deconvolution was performed using safeTME as the reference dataset; no statistically significant (BH adjusted p-value < 0.05) comparisons were found. (F) GSEA was performed on a ranked list of $\log_2$FC values, resulting in 171 *GeneOntology* genesets (55 enriched and 116 downexpressed) and 70 *KEGG* pathways (10 enriched and 60 downexpressed) passing the adjusted p-value threshold of 0.05.