Supplemental material

Materials and Methods

Study cohort. We obtained tissue and blood specimens from five patients who had confirmed COVID-19 infection and subsequently underwent surgery for unrelated conditions (Supplementary Table 1). The age of the patients ranged from 28 to 65 years. All patients were confirmed COVID-19 negative by two consecutive PCR nasal pharyngeal swabs at the time of surgery. This study was approved by the SingHealth Centralised Institutional Review Board (reference number: 2018/3045 and 2019/2653).

Specimen collection. The type of fresh tissues obtained from the patients is included in Supplementary Figure 1. Tissues from more than 20 non-COVID-19 patients were obtained in 2018 or earlier to be used as negative controls. All the fresh tissues taken were sent to the Department of Anatomical Pathology, Singapore General Hospital for further formalin-fixed paraffin-embedded (FFPE) processing and analysis.

Immunohistochemistry. Immunohistochemistry was performed on FFPE tissue samples as previously described.[1, 2, 3] In brief, FFPE tissue sections (4-µm thick) were labelled with antibodies targeting the SARS-CoV-2 nucleocapsid (Novus Biologicals, Cat# NB100-56576, Polyclonal) and spike protein (GeneTex, Cat# GTX632604, 1A9). Appropriate positive and negative controls were included. The nuclei were counterstained with haematoxylin. Images were captured using an IntelliSite Ultra-Fast Scanner (Philips, Netherlands).

Multiplex immunohistochemistry. mIHC was performed using an Opal Multiplex fIHC kit (Akoya Biosciences, USA), as previously described.[4, 5, 6] In brief, FFPE tissue sections were cut onto Bond Plus slides (Leica Biosystems, Richmond) and heated at 60°C for 20 min.[7] The tissue slides were then subjected to deparaffinization, rehydration and heat-induced epitope retrieval using a Leica Bond Max autostainer (Leica Biosystems, Melbourne) before

endogenous peroxidase blocking (Leica Biosystems, Newcastle). Next, the slides were incubated with primary antibodies against SARS-CoV-2 NP, ACE2 (Abcam, Cat# Ab108252, EPR4435(2)) and CD68 (Dako, Cat# DKO.M087601, PG-M1), followed by incubation with polymeric HRP-conjugated secondary antibodies (Leica Biosystems, Newcastle). Then, the samples were incubated with Opal fluorophore-conjugated tyramide signal amplification (TSA) (Akoya Biosciences, USA) at a 1:100 dilution. The slides were rinsed with wash buffer (BOND Wash Solution 10X Concentrate) after each step. Following TSA deposition, the slides were again subjected to heat-induced epitope retrieval to strip the tissue-bound primary/secondary antibody complexes prior to further labelling. These steps were repeated until the samples were labelled with all six markers and spectral DAPI (Akoya Biosciences, USA) at a 1:10 dilution. Finally, the slides were mounted in ProLong Diamond Anti-fade Mountant (Molecular Probes, Life Technologies, USA) and developed in the dark at room temperature for 24 h. Images were captured for each case under a Vectra 3 pathology imaging system microscope (Akoya Biosciences, USA), and then analysed and scored by a pathologist using inForm software (version 2.4.2; Akoya Biosciences) and HALO™ (Indica Labs).

RNA extraction and RT-PCR. Total RNA was extracted using an AllPrep FFPE DNA/RNA Kit (Qiagen, Germany) according to the manufacturer's protocol. Extracted RNA was tested for SARS-CoV-2 envelope (*E*) and spike (*S*) genes using the RealStar® SARS-Cov-2 RT-PCR kit RUO (Altona Diagnostics, Germany) following the manufacturer's instructions[8] on the CFX96 (BioRad) real time thermal cycler. Each RT-PCR reaction (total volume 30 µl) comprised 20 µl master mix from the kit and 10 µl template. Fluorescent-labelled probes were used to enable parallel identification and detection of target RNA. Samples in which both *S* and *E* genes showed positive amplification signal above the baseline within 45 cycles were considered positive. Amplification below the baseline were marked as 'Undetectable', and thus negative for the *S* and *E* genes. If positive amplification signal was only displayed for one of

the two targets, it was reported as a 'Presumptive positive'. The limit of detection of the Altona RealStar® SARS-CoV-2 assay based on the *E* gene at 95% confidence was determined to be 14.85 copies/ml using Probit analysis. Each run contained a positive control for *E* and *S* genes, and a non-template control. The cycling conditions were according to the manufacturer's instruction with a total of 45 amplification cycles.

RNAscope. RNAscope in situ hybridisation (Advanced Cell Diagnostics, USA) assay was performed according to standard manufacturer protocol,[9] on FFPE tissue sections. Deparaffinised tissues were subjected to peroxidase inhibition and pre-treatments, followed by incubation with specific probes (ACE2, Cat# 848151; CD68, Cat# 560591-C2; SARS-CoV2-S, Cat# 848561-C3) and Opal dyes prior to DAPI counterstain. RNAscope Multiplex Fluorescent Reagent Kit v2 (Cat# 323100) and Opal TSA Plus fluorophores (Akoya Biosciences, USA) were used for detection. Appropriate positive and negative controls were included in accordance to manufacturer's recommendation. Images were acquired using a Vectra 3 pathology imaging system (Akoya Biosciences, USA).

Ex vivo peptide stimulation assay. To simulate SARS-CoV-2 infection and to examine SARS-CoV-2-specific immune cells, SARS-CoV-2 PepTivator peptide pools (Miltenyi Biotec, Germany) were used. Lyophilised peptide pools were reconstituted as per the manufacturer's instructions. Blood and tissue samples were prepared as previously described.[5] A total of $1x10^6$ cells were stimulated with 1 μg/mL peptides for 16 h at 37°C in 5% CO_2 in RPMI 1640 media (Gibco, USA) supplemented with 10% FBS (Hyclone) and 1% Penicillin-Streptomycin-Glutamine (Gibco, USA).[10, 11] Negative controls were left unstimulated. Brefeldin A (1 μg/ml, Sigma Aldrich, Germany) was added 2 h into the stimulation assay.

Flow cytometry and Analysis. Cells were stained with Zombie NIR Fixable Viability dye (BioLegend, USA) for 10 min at 4°C in the dark, washed with PBS at 300 *g* for 5 min at 4°C,

and blocked with Human TruStain FcX (BioLegend, USA) for 10 min at room temperature prior to staining with fluorescent-conjugated antibodies. Then the cells were surface stained with an antibody cocktail containing Pacific Orange-anti CD45 (Invitrogen, Cat# MHCD4530, HI30), BV605-anti CD103 (BD Biosciences, Cat# 743652, Ber-ACT8), BV750-anti CD4 (BD Biosciences, Cat# 566355, SK3), Alexa 532-anti CD3 (Invitrogen, Cat# 58-0038-42, UCHT1), PerCP-eFluor 710-anti CD38 (Invitrogen, Cat# 46-0388-42, HB7) and PE-CF594-anti CD39 (BioLegend, Cat# 563678, TU66) in Brilliant Stain Buffer (BD Biosciences, USA), and incubated for 30 min at 4°C in the dark.

Prior to intracellular staining, the cells were fixed and permeabilised with BD Cytofix/Cytoperm™ (BD Biosciences, USA). The cells were then stained with PE-Cy5.5-anti Granzyme B (Invitrogen, Cat# GRB18, GB11) in Brilliant Stain Buffer (BD Biosciences, USA), for 30 min at 4°C in the dark. Finally, the cells were washed with 1x BD Perm/Wash Buffer™ (BD Biosciences, USA) and resuspended in PBS containing 2% FBS for flow cytometry using Cytek Aurora spectral flow cytometer (Cytek Biosciences, USA). Data analysis was performed using FlowJo V.10 software (FlowJo LLC, USA).

Supplementary Table 1. Cohort characteristics.

Patient Profile	**ID**	1	2	3	4	5
	Age/Sex	45/Male	49/Male	28/Male	65/Female	32/Male
	Race	South Asian	South Asian	South Asian	South East Asian	South East Asian
	Pertinent medical history / comorbidities	Caecal adeno-carcinoma	Hepatocellular carcinoma, HBV, TB	Appendicitis	Chronic cholecystitis, hepatocellular carcinoma	Hemorrhoid
COVID-19 History	**Hospitalisation (Y/N)**	Y	Y	Y	Y	Y
	ICU admission (Y/N)	N	N	N	N	N
	Presence of COVID-19 associated GI symptoms (Y/N)	N	N	N	N	N
Surgical History	**Type of Surgery**	Laparoscopic right hemicolectomy	Liver segment VII resection	Appendectomy	Cholecystectomy, liver segment IV resection	Hemorrhoidectomy
	Number of days from negative COVID-19 PCR to surgery (days)	9	85	45	180	145
Sample Collection	**Fresh tissue obtained**	Colon, ileum, appendix, lymph node	Liver	Appendix	Gallbladder, liver	Hemorrhoid
	Number of days from negative COVID-19 RT-PCR to tissue collection (days)	9	85	45	180	145
	Stool obtained (Y/N)	N	N	N	N	N
	Blood obtained (Y/N)	Y	Y	N	N	N
	Number of days from negative COVID-19 RT-PCR to blood collection (days	9	85	N/A	N/A	N/A
Investigation and Result	**Presence of SARS-CoV-2 IgG antibody (Y/N)**	Y	Y	N/A	N/A	N/A
	Number of days from negative COVID-19 to IgG detection (days)	9	85	N/A	N/A	N/A
	RNAscope for SARS-CoV-2 (+/-)	+ (all tissues)	-	-	- (all tissues)	+
	IHC for SARS-CoV-2 (+/-)	+ (all tissues)	+	+	+ (all tissues)	+
	Multiplex IHC for SARS-CoV-2 (+/-)	+ (all tissues)	+	+	+ (all tissues)	+
	RT-PCR for SARS-CoV-2 (+/-)	+ (only performed with colon due to the availability of the tissue)	-	-	-	-

References

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