Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Qin Yao, Máire E. Doyle, Qing-Rong Liu, et al. Long-Term Dysfunction of Taste Papillae in SARS-CoV-2. NEJM Evid. DOI: 10.1056/ EVIDoa2300046

Supplementary Appendix

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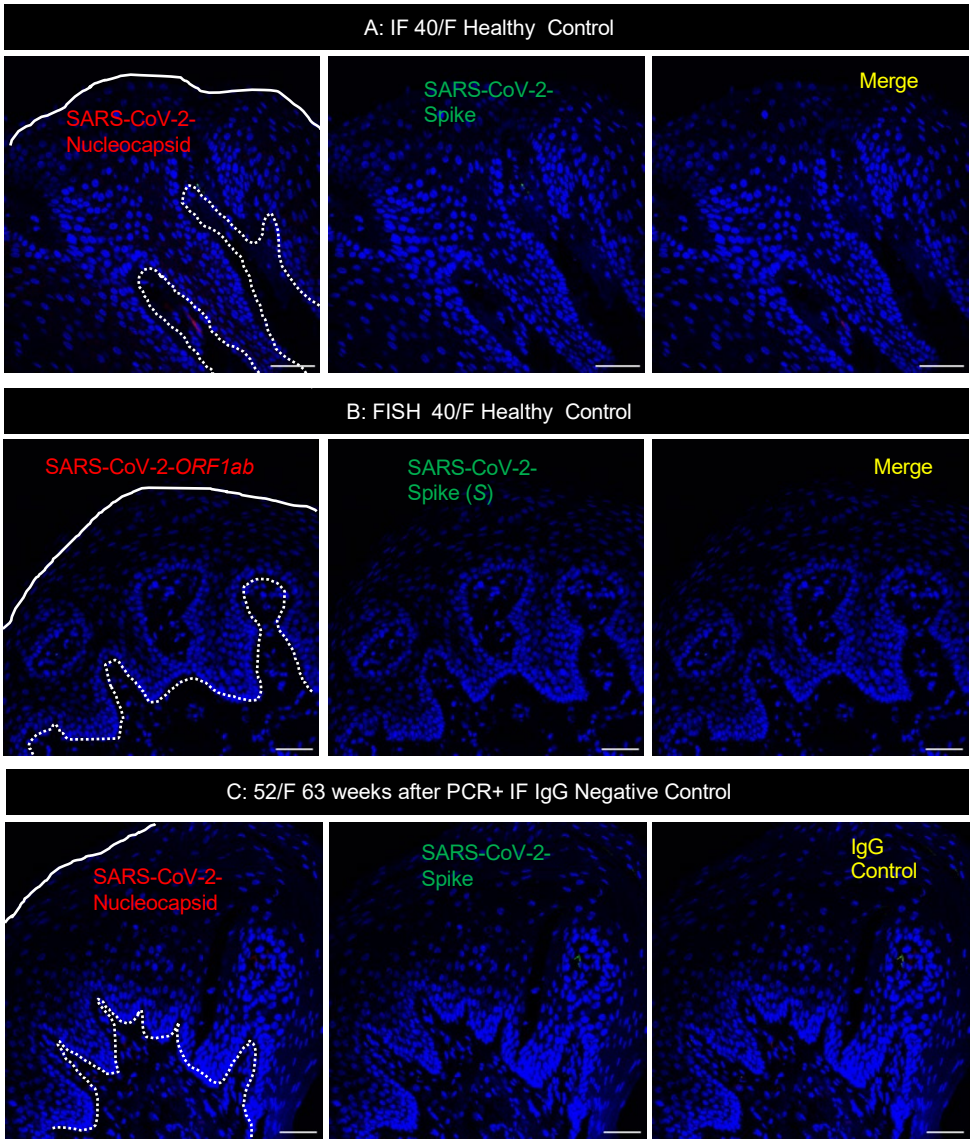
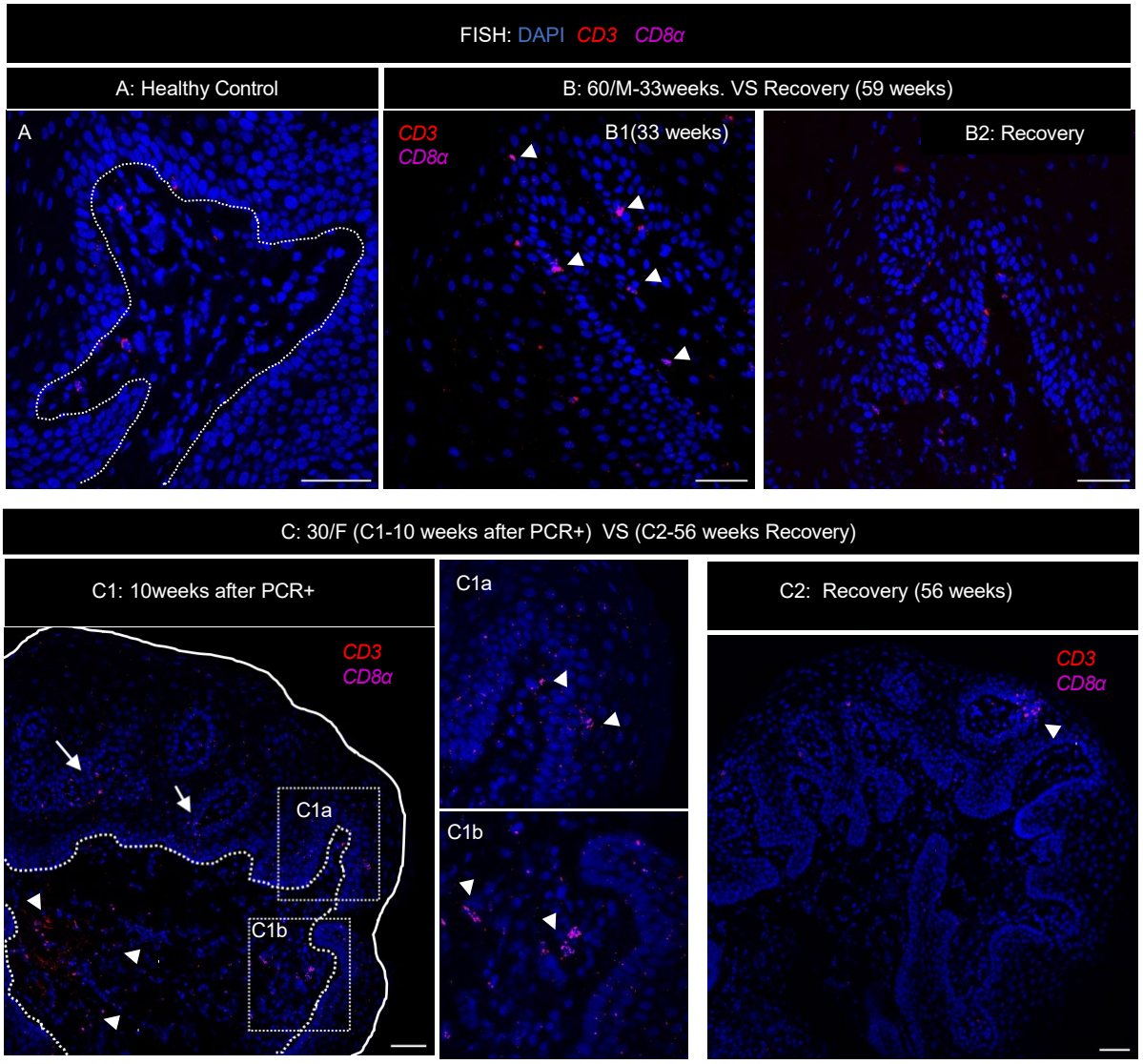
Figure S1: Controls Used to Verify SARS-CoV-2 IF and FISH Staining.



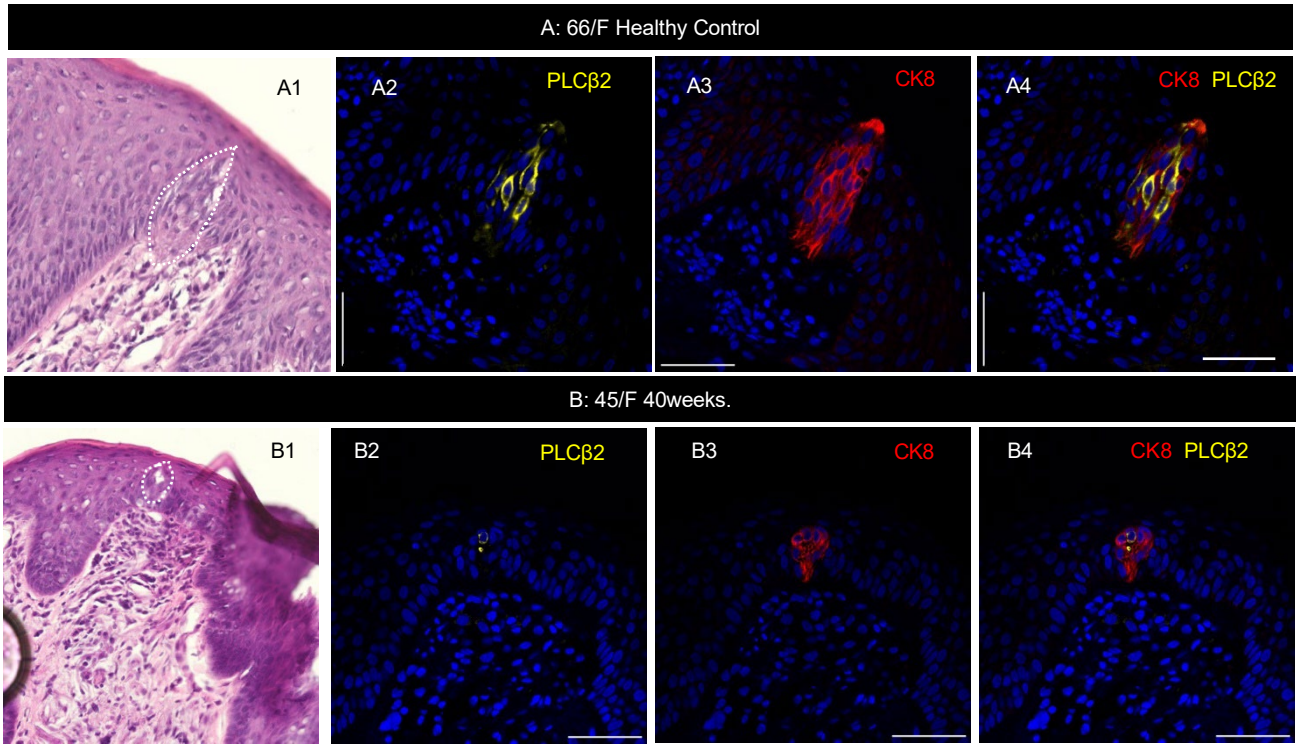
Panel A shows IF staining for SARS- CoV-2 nucleocapsid (left panel) and spike protein (middle panel) in 40-year-old female control fungiform papilla, confirming the absence of virus in a healthy control. Panel B shows FISH staining for the *ORF1ab* (left panel) and Spike (*S*) (middle panel) probes in 40-year-old female control fungiform papilla, confirming the absence of virus and replication of virus in a healthy control. Panel C shows a 52-year- old female PASC (63 weeks after PCR+) fungiform papilla lacking SARS-CoV-2 nucleocapsid (left panel) and spike protein (middle panel) IF signals when mouse and rabbit IgGs are substituted for primary antibodies. In panels A-C, the solid white line indicates FP surface, the white dotted line demarcates the epithelial layer from the lamina propria. Scale bars are 50 µm.

Figure S2 Pathology of FP from PASC Patients with Long-Term Taste Deficits Before and After Recovered.



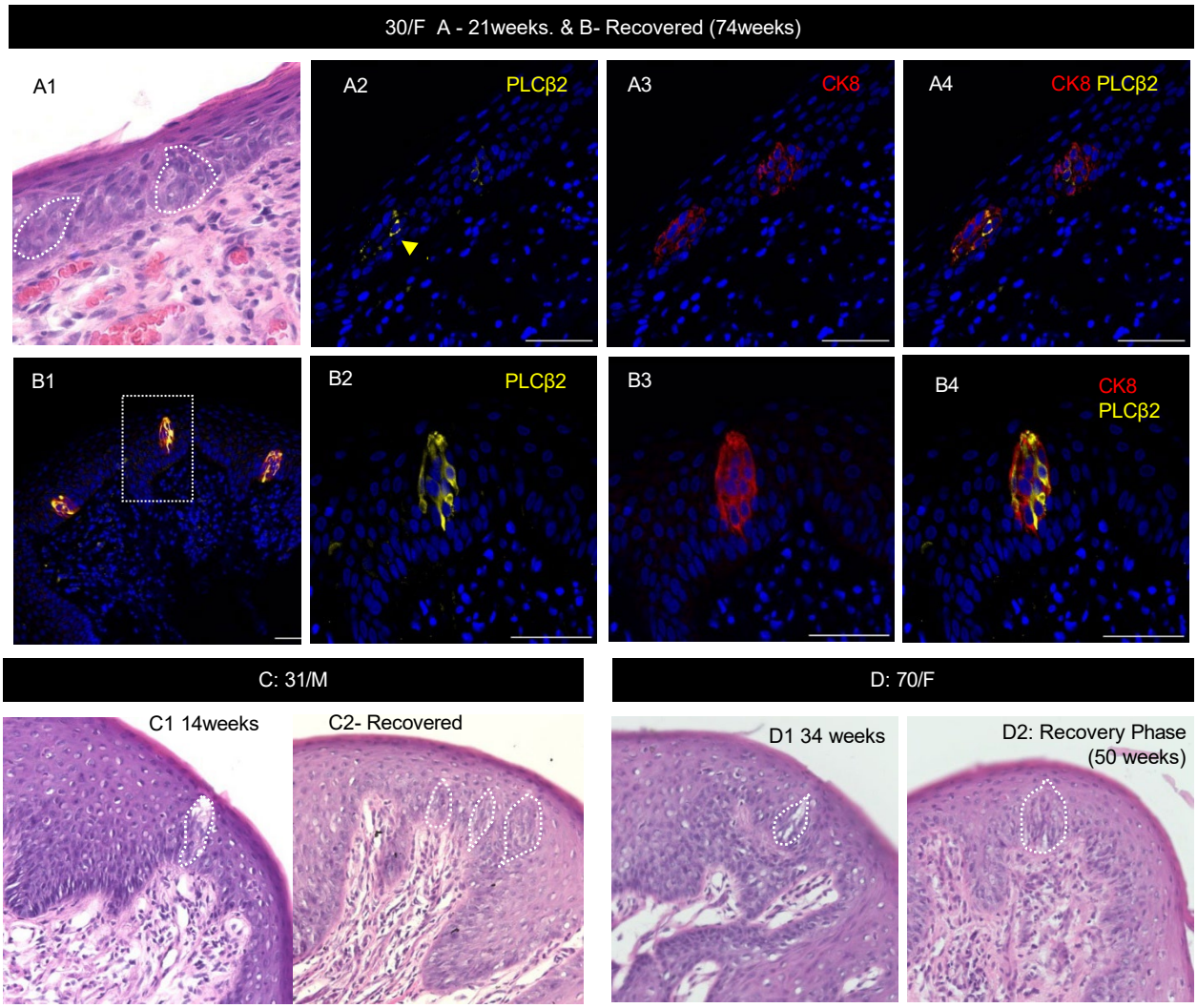
Panels A, B and C show FISH using probes for *CD3* (red dots) and *CD8α* (purple dots) in FP. Panel A shows normal healthy control (40-year-old female) fungiform papilla morphology and no CD8 T cells. Panel B shows multiple CD8 T cells (white arrows) in 60-year-old male 33 weeks after PCR+ fungiform papilla (B1) compared to fungiform papilla from the same individual upon taste perception recovery at 59 weeks (B2). The white dotted line demarcates the epithelium from the lamina propria. Panel C shows CD8 T cells in fungiform papilla from a 30-year-old female with taste disturbances at 10 weeks after PCR+ (C1) compared to fungiform papilla from the same patient upon taste perception upon recovered at 56 weeks (C2). The solid white line indicates the surface of the FP, the dotted white line demarcates the separation of the epithelium from the lamina propria, and the white arrows indicate the presence of CD8 T cells. C1a and C1b show a higher magnification of the areas marked by rectangles in C1. Scale bars are 50 μm.

Figure S3 The Morphology of a Healthy Taste Bud/Fungiform Papilla and Morphological changes in Taste Bud/FP of PASC Patients with Long-Term Taste Disturbances.



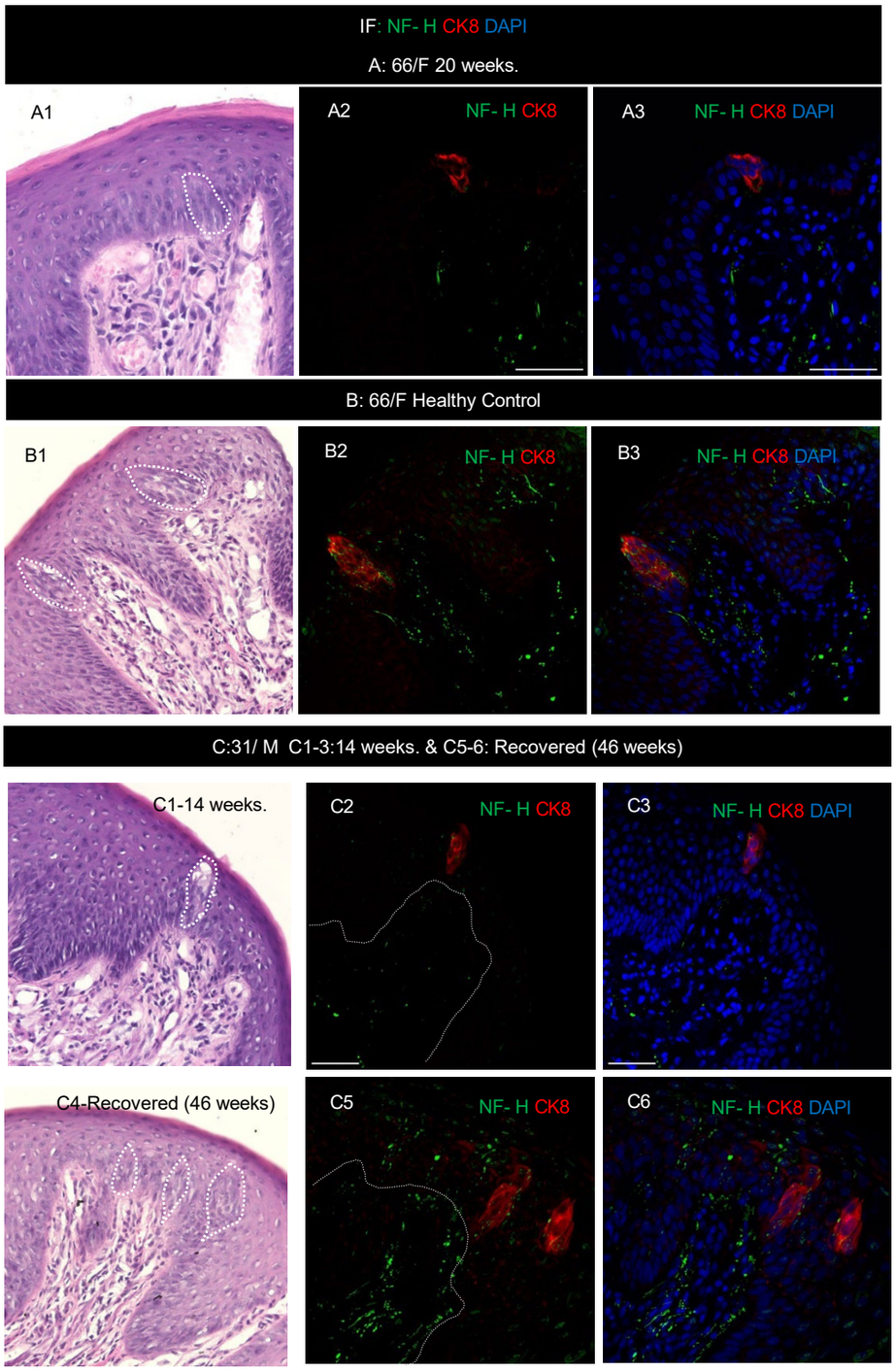
Panel A shows consecutive sections of FP from a healthy 66-year-old female. The dotted white lines outline a taste bud in a H&E-stained section (A1). This same taste bud can also be seen in the adjacent sections stained by IF for PLCβ2 (yellow, A2) and CK8 (red, A3). Note that all the taste receptor cells in the taste bud are positive for CK8 and most of them stain robustly for PLCβ2 (A4).
Panel B shows consecutive sections of FP from a 45-year-old female 40 weeks after PCR+. The dotted white lines outline a taste bud in a H&E-stained section (B1). This same taste bud can also be seen in the adjacent sections stained by IF for PLCβ2 (yellow, B2) and CK8 (red, B3). Note the weak staining for the taste cell marker CK8 and only one cell appears to be positive for PLCβ2 (B2 and B4, yellow).
Scale bars are 50 μm.

Figure S4 Morphological Changes in Taste Buds and FP of PASC Patients with Long-Term Taste Disturbances is Resolved Upon Spontaneous Recovered.



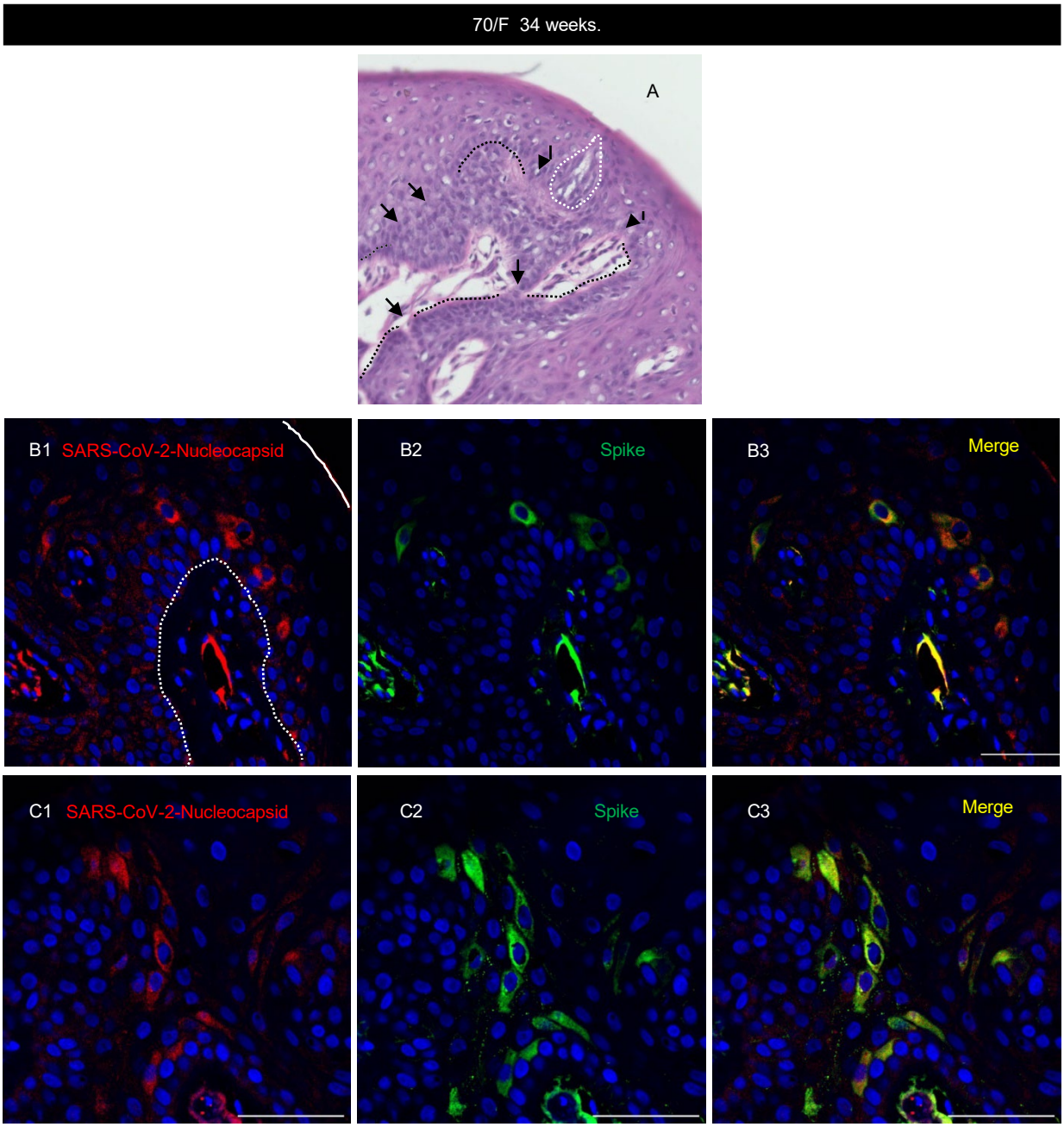
We followed a 30-year-old female with multiple biopsies from 21 weeks after PCR+ to full taste perception recovered at 74 weeks. Panels A1-A4 show consecutive sections of FP from this patient at 21 weeks after PCR+. The white dotted lines outline two malformed taste buds in the H&E-stained section (A1), while the adjacent sections are IF stained for PLCβ2 (yellow, A2) and CK8 (red, A3). Note only one taste receptor cell appears to be positive for PLCβ2 (A2, yellow arrow) and the weak staining for the taste receptor cell marker CK8. Panel B shows IF for PLCβ2 (yellow) and CK8 (red) in FP taken from the same individual at 74 weeks after PCR+ when her taste perception had recovered. Note three more normally formed taste buds now each containing robust signal for PLCβ2 (B1). Panels B2-B4 show higher magnification of the rectangles marked in B1. Panel C shows representative images of an infected (C1 and D1) and healthy (C2 and D2) FP from two different individuals who underwent spontaneous recovery from PASC associated taste disturbances. White dotted lines outline the taste buds. Note the infected FPs only have one small taste (C1 and D1). Upon recovery there more taste buds (C2) that are more normal in size and shape (C2 and D2) in each fungiform papilla. Scale bars are 50 μm.

Figure S5 Taste Bud and FP Neurites are Disrupted in PASC with Long-Term Taste Disturbances and Resolved Upon Spontaneous Recovered.



Representative images are shown of FP from a PASC patient (Panel A), healthy control (Panel B) and PASC patient (Panels C1-C3) who subsequently recovered (Panels C4-C6). Left panel in each case shows H&E staining in which the taste buds are outlined by white dotted lines. The middle and right panels show IF staining for CK8 (red) and the neurites marker neurofilament heavy NF-H (green) in the adjacent section in each individual's FP. White dotted line delineates the epithelium from the lamina propria. Note that the taste bud neurites are sparse and not well connected in the patients' FP (A1-A3 and C1-C3). By contrast both the healthy FP (B1-B3) and the FP taken from the individual when their taste perception had returned at 46 weeks (C4-C6) show multiple taste buds and the presence of afferent fibers. Scale bars are 50 μm.

Figure S6: SARS-CoV-2 Persists in FP from PASC Patient at 34 Weeks After PCR+.



This figure shows H&E and IF of sections of FP biopsied from a 70-year-old female at 34 weeks after PCR+ when she was still experiencing taste disturbances. Panel A shows H&E staining of a fungiform papilla section with the malformed taste bud outlined by the white dotted line, and disruption of the continuity of the basal cell layer at the points indicated by the black arrows. Panels B & C show IF staining for the SARS-CoV-2 nucleocapsid (red, B1 and C1) and spike protein (green, B2 and C2) in the epithelium of two FP. The solid white line indicates the surface of FP, and the white dotted lines demarcate the epithelium layer ends from the lamina propria. Note the virus was still present in her FP at 34 weeks after PCR+. Scale bars are 50 μm.

Supplemental tables:

Table S1: Characteristics of the Participants

Identifiers		Baseline characteristics					Laboratory findings in FP				
No.	Race	PASC/ Acute/ Control	Age/ Gender	Visit (FPs biopsy) : Weeks After PCR+	Symptoms When Biopsied	Date of COVID19 Diagnosis	Taste Buds Present	SARS-CoV-2-Spike Protein	SARS-CoV-2-Nucleocapsid Protein	Spike(*S*)	*ORF1ab*
1	N/A	PASC	20/F	V1:21	Hypogeusia & Hyposmia	12/02/2020	+	+	+	+	N/A
2	Cauca	PASC	30/F	V1:21	Hypogeusia & Hyposmia	11/09/2020	+	+	N/A	+	N/A
				V2:25	Improved		-	N/A	N/A	N/A	N/A
				V3:74	Recovered		+	-	-	N/A	N/A
3	AA	PASC	30/F	V1:3	Ageusia	09/02/2021	-	N/A	N/A	N/A	N/A
				V2:10	Hypogeusia & no bitter taste		+	+	+	N/A	N/A
				V3:22	Improved		+	-	-	N/A	N/A
				V4:56	Recovered		+	-	-	N/A	N/A
4	Cauca	PASC	31/M	V1:7	Hypogeusia & Hyposmia	2/24/2021	+	+	+	+	+
				V2:14	Not improved		-	+	+	+	+
				V3:18	Not improved		-	N/A	N/A	N/A	N/A
				V4:46	Recovered		+	-	-	N/A	N/A
5	Cauca	PASC	32/F	V1:40	Hypogeusia	06/02/2020	-	-	-	N/A	N/A
6	AA	PASC	37/F	V1:17	Hypogeusia & Hyposmia	01/27/2021	+	+	N/A	+	+
				V2:34	Hypogeusia & No sweet taste		-	+	+	+	+
				V3:42	No change		-	+	+	+	+
				V4:67	Recovered		+	-	-	N/A	N/A
7	Cauca	PASC	45/F	V1:25	Ageusia	12/14/2020	-	N/A	N/A	N/A	N/A
				V2:40	Hypogeusia		+	+	+	+	+
				V3:57	Dysgeusia		+	+	+	N/A	N/A
8	Cauca	PASC	49/F	V1:20	Hypogeusia & Hyposmia	July/2021	-	+	N/A	N/A	N/A
9	Cauca	PASC	52/F	V1:31	Hypogeusia	09/27/2020	+	+	+	+	N/A
				V2:63	Improved, weak sweet taste		+	+	+	N/A	N/A
10	Cauca	PASC	60/M	V1:6/21/19	Pre-COVID	Pre-COVID	+	-	-	-	N/A
				V2:8	Hypogeusia & Hyposmia	07/01/2020	-	+	N/A	+	N/A
				V3:13	Hypogeusia & Hyposmia		-	+	N/A	+	N/A
				V4:33	Improved		-	+	N/A	+	N/A
				V5:59	Recovered		+	-	-	-	N/A
11	AA	PASC	63/F	V1:19	Hypogeusia & Hyposmia No sweet taste	01/28/2021	-	+	N/A	N/A	N/A
12	Cauca	PASC	66/F	V1:20	Ageusia & Phantosmia	11/17/2020	+	+	+	+	N/A
				V2:83	Recovered		+	-	-	N/A	N/A
13	Cauca	PASC	68/F	V1:16	Hypogeusia	11/22/2020	-	-	-	N/A	N/A
				V2:59	Hypogeusia		-	-	-	N/A	N/A
14	Cauca	PASC	69/F	V1:63	Hypogeusia & No bitter and sour taste	9/14/2020	-	+	+	N/A	N/A
15	Cauca	PASC	69/F	V1:49	Dysgeusia	July/2021	-	+	N/A	N/A	N/A
16	Cauca	PASC	70/F	V1:34	Ageusia & Phantosmia	11/29/2020	+	+	+	+	+
				V2:50	Improved		+	+	+	N/A	N/A
17	Cauca	Acute	31/M	V1:2	Hypogeusia & Hyposmia	11/30/2021	-	+	+	N/A	N/A
18	Cauca	Acute	31/F	V1:2	Hypogeusia & Hyposmia	12/24/2021	-	+	+	N/A	N/A
19	Cauca	Acute	45/F	V1:2	Hypogeusia & Hyposmia	08/12/2020	+	+	+	+	+
20	N/A	Control	23/M	-	N/A	N/A	+	-	-	N/A	N/A
21	Cauca	Control	24/F	-	N/A	N/A	+	-	-	N/A	N/A
22	Cauca	Control	27/M	-	N/A	N/A	+	-	-	N/A	N/A
23	Cauca	Control	32/M	-	N/A	N/A	+	-	N/A	N/A	N/A
24	Hisp	Control	33/F	-	N/A	N/A	+	-	-	-	N/A
25	Hisp	Control	35/M	-	N/A	N/A	+	-	-	-	N/A
26	Cauca	Control	40/F	-	N/A	N/A	+	-	-	-	-
27	Cauca	Control	67/F	-	N/A	N/A	+	-	N/A	N/A	N/A
28	Cauca	Control	91/F	-	N/A	N/A	+	-	-	N/A	N/A
29	Cauca	Control	33/M	V1:10	NO taste/smell issues	8/28/2021	+	-	-	-	N/A
30	Cauca	Control	44/F	V1:7	NO taste/smell issues	9/10/22	+	-	-	N/A	N/A
31	Cauca	Control	47/F	V1:7	NO taste/smell issues	3/3/2022	+	-	-	N/A	N/A
32	Cauca	Control	66/F	V1:11	NO taste/smell issues	March/2022	+	-	-	N/A	N/A

(Cauca: Caucasian; AA: African American; Hisp: Hispanic; + positive; - negative, N/A not available.1-16 PASC patients, 17-19 Acute infection patients, 20-28 Healthy Control with no infection, 29-32 PCR+ with no long-lasting taste and/or smell symptoms. Ageusia: complete loss of taste; Hypogeusia: partial loss of taste; Dysgeusia: distorted sense of taste)

Table S2.: **Primary Antibodies**

Antigen	Source Species	Dilution	Manufacturer; Catalog #, RRID	Reference
SARS-CoV / SARS-CoV-2 (COVID-19) spike antibody [1A9]	Mouse	1:100	GeneTex; GTX632604, AB_2864418	1-3
SARS/SARS-CoV-2 Nucleocapsid Monoclonal Antibody (E16C)	Mouse	1:100	Thermo Fisher; MA1-7403, AB_1018420	1,4,5
SARS-CoV-2 (COVID-19) nucleocapsid antibody	Rabbit	1:100	GeneTex; GTX135361, AB_2887484	1,6,7
SARS-CoV-2 Spike (RBD) Antibody (T01KHuRb)	Rabbit	1:200	Invitrogen; 703959, AB_2866478	8,9
SARS-CoV-2 Spike (RBD) Antibody (T01KHu)	Human	1:200	Invitrogen; 703958, AB_2866477	10
NF-H	Rabbit	1:1000	Novus Biologicals; NB300-135, AB_10001839	11.12
PLCβ2	Rabbit	1:100	Santa Cruz Biotechnology; sc-206, AB_632197	13-16
CK8(cytokeratin8)	Rat	1:200	DSHB; TROMA-I, AB_531826	16

Table S3: Secondary Antibodies

Antigen	Source Species	Dilution	Manufacturer; Catalog #, RRID
Rabbit IgG	Goat (AlexaFluor 488)	1:1000	Invitrogen; A27034, AB_2536097
Rabbit IgG	Goat (AlexaFluor 568)	1:1000	Invitrogen; A11036, AB_10563566
Human IgG	Goat (AlexaFluor 488)	1:2000	Invitrogen; A-11013, AB_2534080
Mouse IgG	Goat (AlexaFluor 488)	1:1000	Invitrogen; A28175, AB_2536161
Mouse IgG	Goat (AlexaFluor 568)	1:1000	Invitrogen; A-11004, AB_2534072
Mouse IgG	Goat (AlexaFluor 647)	1:1000	Invitrogen; A-21235, AB_2535804
Rat IgG	Goat (AlexaFluor 568)	1:1000	Invitrogen; A-11077, AB_2534121
Rat IgG	Goat (AlexaFluor 647)	1:1000	Invitrogen; A21247, AB_141778

Table S4: IgG Controls

Antigen	Manufacturer; Catalog #, RRID
Human IgG control	R & D Systems; 1-001-A, AB_907192
Mouse IgG1 control	Thermo Fisher Scientific; 14-4714-82, AB_470111
Mouse IgG2a control	R & D Systems; MAB003, AB_357345
Mouse IgG2b control	Thermo Fisher Scientific; 14-4732-82, AB_470117
Rabbit IgG control	R & D Systems; AB-105-C, AB_354266
Rat IgG control	BioLegend; 400526, AB_2864284

Table S5: **FISH Probes**

Gene Symbol	FISH probe	Catalog #	Accession number	ZZ probe pairs	Nucleotide position
SARS-CoV-2 Spike	Probe - V-nCoV2019-S	848561-C1	NC_045512.2	20	21,631 - 23,303
SARS-CoV-2 ORF1ab	Probe - V-nCoV2019-orf1ab-sense	859151-C2	NC_045512.2	40	1583 -4388
IL1B	Probe-Hs-IL1B	310361-C2	NM_000576	20	2 - 1319
CD3 Pool (CD3D, CD3E, CD3G)	Probe -Hs-CD3-pool-C2	426621-C2	N/A	11 (Hs-CD3D). 20 (Hs-CD3E). 18 (Hs-CD3G)	180 – 743 (Hs-CD3D); 26 – 1288 (Hs-CD3E). 40 – 911 (Hs-CD3G)
CD8A	Probe -Hs-CD8A-C3 Probe -Hs-CD8A-C2	560391-C3 560391-C2	NM_001768.6	20	971 - 2342
Perforin (PRF1)	Probe - Hs-PRF1	407381-C1	M28393.1	19	7 - 1587
GZMB	Probe - Hs-GZMB	445971-C1	NM_004131.4	11	3 - 912

List of Non-Standard Abbreviations

ACE2	Angiotensin-Converting Enzyme-2
COVID-19	Coronavirus Disease 2019
CD	Cluster of Differentiation
FP	Fungiform Papillae
FISH	In-Situ Hybridization
H&E	Hematoxylin and eosin
IF	Immunofluorescence
NF-H	Neurofilament-heavy chain
ORF1ab	Open Reading Frame 1ab
PASC	Post-Acute Sequelae of COVID-19
PLCβ2	Phospholipase C β 2
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TRC	Taste Receptor Cell

AUTHORSHIP

QY, MED, and JME designed the study; QY and JME gathered, and analyzed the data, wrote the first draft of manuscript; NPW provided the immunological expertise and edited the immunology data; MED, QRL, AA and JFOC were involved in sectioning, staining, and analyzing the findings; all contributed to the preparation and development of this manuscript and approved the final draft before submission.

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