Supplemental Data

Concentration Determination of Tsp1a-IR800P

Concentration determination: An aliquot of solid Tsp1a-IR800P was dissolved in PBS. 1 µL of this solution was diluted with 99 µL PBS and the absorbance was measured on Tecan Safire (Zurich, Switzerland) microplate reader from 200-850 nm in 5 nm intervals to measure the maximum absorption at 780 nm. The following equations were utilized to determine the concentration of the Tsp1a-IR00P solution:

$c = AC\ cm^{-1} / 242000\ L*mol^{-1}*cm^{-1}$

$m = c * M * V * DF = c * 4683\ g*mol^{-1} * 0.0001\ L * DF$

DF = dilution factor (here: 100), c = concentration, AC = absorption coefficient

Mice infected with SARS-CoV-2

The protocol has been described by Ordonez et al. (*1*). Briefly, heterozygous K18-hACE2 C57BL/6J 8-week-old male mice (strain: 2B6.Cg-Tg, K18-ACE)2Prlmn/J, Jackson Laboratory, Maine), received 8.4×10^5 TCID50 of SARS-CoV-2/USA/WA1/2020 intranasally, after induction of anesthesia with ketamine hydrochloride and xylazine. Six days after infection, the animals were sacrificed with isofluorane overdose. The tissues were harvested and fixed in 10% formalin for 48 hours. 6-micron slides were made and submitted for $Na_V1.7$ immunohistochemistry (IHC).

Hamsters

The protocol has been described by Zazhytska et al. (*2*). Briefly, heterozygous, LVG Golden Syrian hamsters (Mesocricetus auratus) were treated and euthanized in

compliance with the rules and regulations of IACUC under protocol number PROTO202000113-20-0743. All experiments were performed on dissociated cells prepared from whole olfactory epithelium tissue.

Single cell RNA-seq

Cells were dissociated according to the Worthington Papain Dissociation System by incubating fresh olfactory tissue with papain for 40 min at 37°C. Library preparation was performed accordingly to Chromium Single Cell 5' v.2 Protocol and sequenced on NextSeq550. Cell Ranger pipelines were used to generate fastq files which subsequently were aligned against MesAur1.0/WuhCor1 genomes. Systemic biases and background noise were removed with Cellbender; resulted h5 matrixes were loaded and analyzed with Seurat. Cells with more than 400 UMIs, expressed 500 and 6000 genes and less than 5% of mitochondrial genes were kept for further analysis. Identified 13 clusters were visualized with UMAP and annotated using known marker genes for each cell type. Differential expression analysis was performed using the default two-sided non-parametric Wilcoxon rank sum test with Bonferroni correction using all genes in the dataset.

Bulk RNAseq from human olfactory epithelium

RNA was extracted using Direct-zol RNA kits from Zymo Research. 50 ng - 1 ug of total RNA was used to prepare DNA libraries with Truseq RNA Library Prep Kit v2 followed by 75 HO paired-end and multiplexed sequencing. Reads were aligned to human genome

(hg38), *Mesocricetus auratus* (MesAur1.0) and SARS-CoV-2 (wuhCor1) using subread and the raw read counts were assembled using feature counts pipeline.

Behavioral experiment

The buried food test was performed as previously described (*3*). Briefly, mice were fasted for 18–24 h before the experiment. On the day of the experiment, the mice were transferred to a clean cage containing 3 cm bedding, with a graham cookie hidden in one of the corners of the cage (random selection, 2 cm deep into the bedding). The observer then started the timer and stopped when the subject mouse found the buried food (Fig. 4A). The cookie was considered uncovered when the animal started to eat it, usually holding food with the forepaws.

Immunohistochemistry and quantification of $Na_V1.7$ expression

$Na_V1.7$ staining was performed by the Molecular Cytology Core Facility of MSK using a Discovery XT processor (Ventana Medical System, Tucson, AZ), according to our previously described work using anti-$Na_V1.7$ antibody [N68/6] (NeuroMab) that binds to both human and mouse $Na_V1.7$ (0.5 μg/mL). Paraffin-embedded formalin-fixed 4 μm sections were deparaffinized with EZPrep buffer. For IHC, a 3,3′-diaminobenzidine (DAB) detection kit (Ventana Medical Systems, Tucson, AZ) was used according to the manufacturer's instructions. In addition, these sections were counterstained with hematoxylin and eosin (H&E) and coverslip using Permount (Fisher Scientific, Pittsburgh, PA).

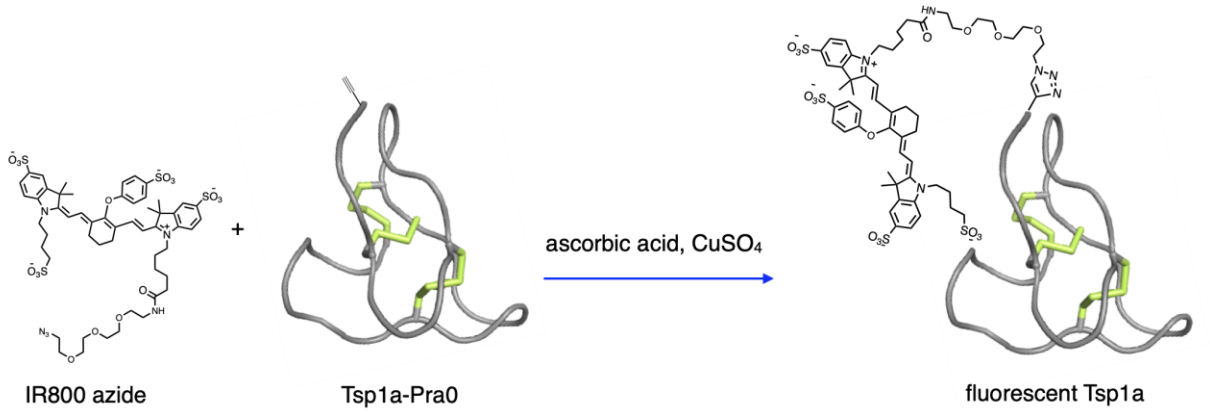
Sodium channel $Na_V1.7$ quantification was performed on digitalized slides. The threshold for signal intensity in the DAB (brown) and H&E (blue, representing all tissue area) channels was determined via an automated script using ImageJ analysis software. Color deconvolution was used to separate blue and brown signals and the threshold values were kept constant: 0–114 for DAB and 0–235 for H&E. The relative $Na_V1.7$ positive area in % was calculated by dividing the brown (DAB) area by the blue (total tissue area) and multiplied by 100. At least three slides from the same animal were used to calculate the mean value.

Obtaining human cadaveric specimens

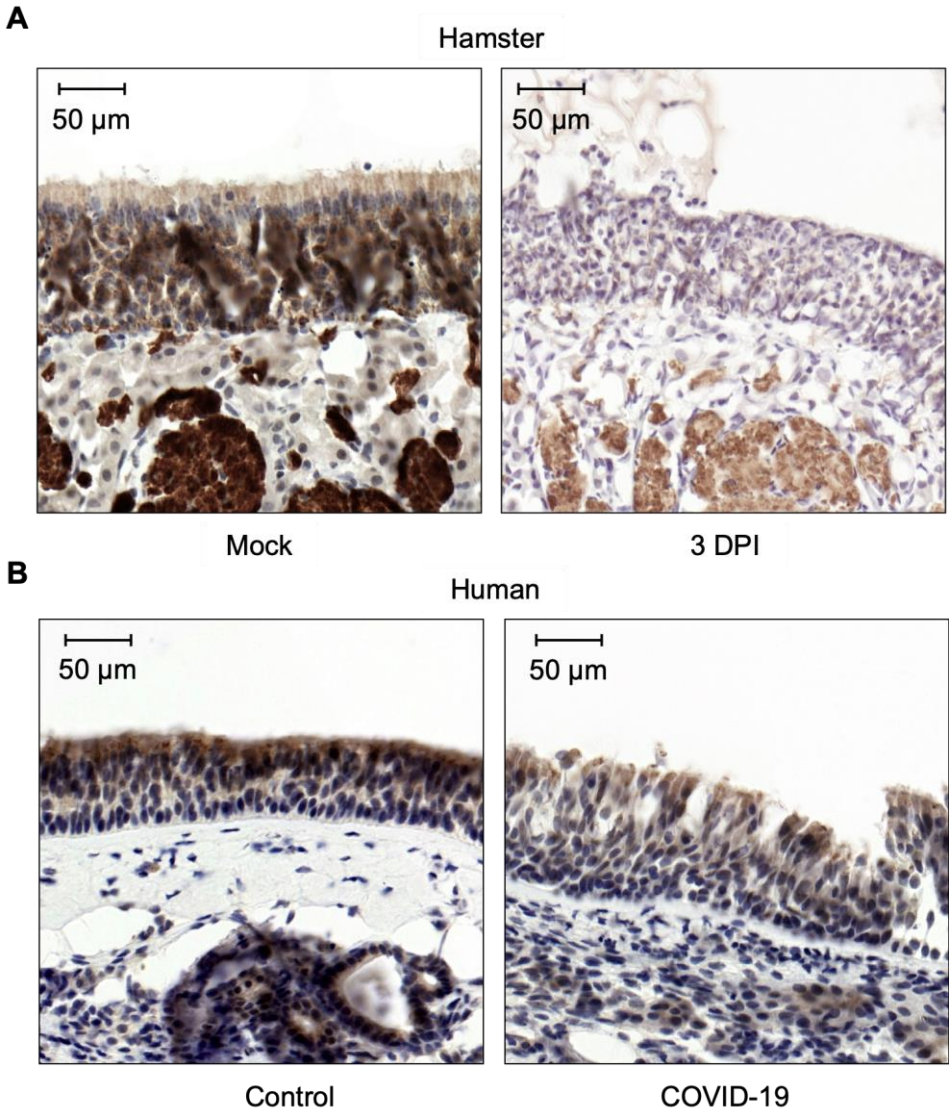
This study conducted at Columbia University Irving Medical Center in New York, USA, involved 25 patients who had been previously diagnosed with COVID-19 by SARS-CoV-2 RT-PCR analysis and underwent full body autopsy. The study was approved by the Ethics and Institutional Review Board of Columbia University Medical Center (IRB AAAT0689, AAAS7370).

Samples that contained metastatic cancer and non-SARS coronavirus were removed from further analysis. Brain tissue and nasal epithelium, including the olfactory region, were retrieved using separate surgical instruments to prevent cross-contamination and were preserved for histological, molecular, and microscopic evaluation. Additionally, nasal tissues, including olfactory and respiratory epithelium, were harvested from 7 control specimens of deceased individuals who had no clinical history of COVID-19 and had negative SARS-CoV-2 PCR. The olfactory epithelium was isolated from the olfactory

cleft, spanning turbinate and adjacent septal mucosa, and was preserved for HiC, RNA ISH/IF, or RNA-seq and IHC using different preservation methods.



Supplemental Figure 1. Chemical Synthesis of Tsp1a-IR800P. The chemical synthesis of Tsp1a-IR800P was successfully obtained in a buffer by a reaction that occurred in 4 h in the dark. The IR800 fluorophore with an attached azido group reacted with the alkyne group on the Tsp1a-Pra0 peptide to yield the fluorescent imaging agent that was used in in-vivo experiments.



Supplemental Figure 2. IHC slides of the olfactory epithelium of control and SARS-CoV-2 infected hamsters and human cadaver specimens. A) IHC slides of NaV1.7 expression in mock and infected hamster's olfactory epithelium. B) IHC slides of NaV1.7 expression in control and infected human olfactory epithelium.

References

1. Ordonez AA, Bullen CK, Villabona-Rueda AF, et al. Sulforaphane exhibits antiviral activity against pandemic SARS-CoV-2 and seasonal HCoV-OC43 coronaviruses in vitro and in mice. *Communications Biology.* 2022;5.

2. Zazhytska M, Kodra A, Hoagland DA, et al. Non-cell-autonomous disruption of nuclear architecture as a potential cause of COVID-19-induced anosmia. *Cell.* 2022;185:1052-1064.e1012.

3. Yang M, Crawley JN. Simple behavioral assessment of mouse olfaction. *Curr Protoc Neurosci.* 2009;Chapter 8:Unit 8.24.