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A spatially-resolved, single-cell analysis of human olfactory cleft mucosa highlights the dysregulation of the transcriptome of sustentacular cells infected with SARS-CoV-2

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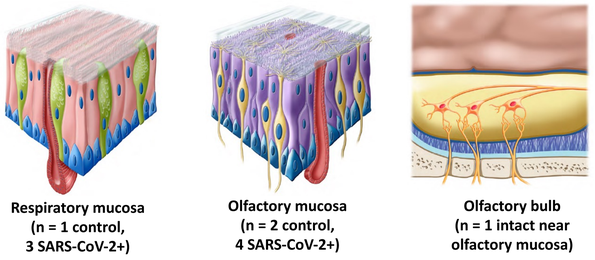
Abstract

Anosmia is a common symptom of COVID-19 and can persist well after viral clearance. This loss of smell is likely the result of effects of SARS-CoV-2 on cell types that underly olfactory function. These cell types—which include sustentacular cells and olfactory sensory neurons—exist in a well-characterized spatial landscape called the olfactory mucosa, which consists of an archipelago of islands within the olfactory cleft that are surrounded by respiratory mucosa. Our earlier work using the GeoMx® Digital Spatial Profiler characterized whole-transcriptome effects SARS-CoV-2 in the olfactory epithelium of a postmortem case at the spatial resolution of hundreds of cells and indicated that the dysregulation of the transcriptome of sustentacular cells is a likely driver of anosmia. This result raised the question of which genes in sustentacular cells are altered as a result of infection. This inherently spatial single-cell question is here being addressed using the CosMx™ Spatial Molecular Imager. Our panel consisted of 984 host targets and 9 probes for SARS-CoV-2. In total we measured 63,589,058 spatial transcripts in 401,233 cells. As a first step, we classified cells into the known cell types. We were able to classify cells in the surrounding lamina propria (Bowman's glands, pericytes) using spatially agnostic and publicly available scRNA-seq data. A semi-supervised clustering algorithm allowed us to discern more nuanced cell types of the epithelium (such as olfactory vs respiratory horizontal basal cells) and to identify cell types that were not classified in the reference data (such as suprabasal cells). Since SARS-CoV-2 infection results in degradation of host mRNAs, our approach was flexible enough to capture heavily infected cell types not adequately reflected in the reference (such as infected secretory cells and ciliated cells). Following classification, we then focused on the 725 sustentacular cells, grouped them into virus negative (535), virus low (121), and virus high (69), and found ~120 differentially expressed genes (DEGs). Differences between these groupings contain "classic" DEGs such as *TMPRSS2* and genes related to inflammatory or myeloid signaling.

Profiling key regions for SARS-CoV-2 Infection

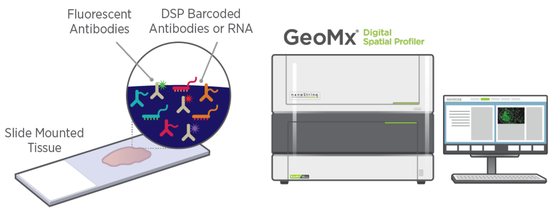


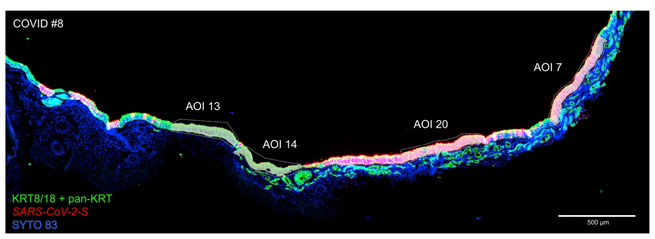
Respiratory mucosa (n = 1 control, 3 SARS-CoV-2+) | **Olfactory mucosa (n = 2 control, 4 SARS-CoV-2+)** | **Olfactory bulb (n = 1 intact near olfactory mucosa)**

Figure 1: Study design profiling various regions throughout the nasal mucosa
Ten patients (11 tissue samples) were profiled using 984 host targets and 9 probes for SARS-CoV-2, including specific probes for the Delta and Omicron variants. These were sourced from both uninfected deceased patients (3) and patients with history of and evidence of SARS-CoV-2 infection (7) at the time of death. Below is the QC table showing the profiling depth and characteristics of each class of patient samples.

	Controls	SARS-CoV-2+ Respiratory Mucosa	SARS-CoV-2+ Olfactory Mucosa
Patients	3	3	4
Viral variant	-	2 omicron, 1 delta	2 omicron, 2 delta, 1 non VoC
Total Tissue Area (mm^2)	10.3	18.63	21.45
Number of Cells Analyzed	85,426	176,455	212,061
Total Transcripts Assigned to Cells	11.7M	28.7M	33.4M
Mean Transcripts per Cell	136.8	162.9	157.4
Maximum Transcripts per Cell	1,523	2,346	2,053

GeoMx identified preferential infection of sustentacular cells





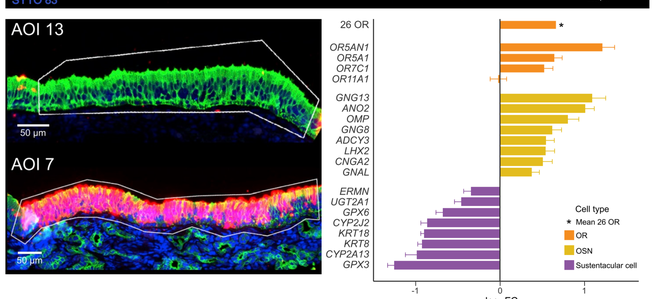


Figure 2: Transcriptional loss of sustentacular cell markers during SARS-CoV-2 Infection
Profiling of highly infected SARS-COV-2 patient with RNAscope & GeoMx Whole Transcriptome Atlas (WTA) in Khan et al [1] demonstrated preferential depletion of sustentacular cell markers from regions that were highly infected with the virus. Concurrent loss of expression of odorant receptor genes or markers of olfactory neurons was not observed.

Mapping interactions between SARS-CoV-2 infected cells and their surrounding environment

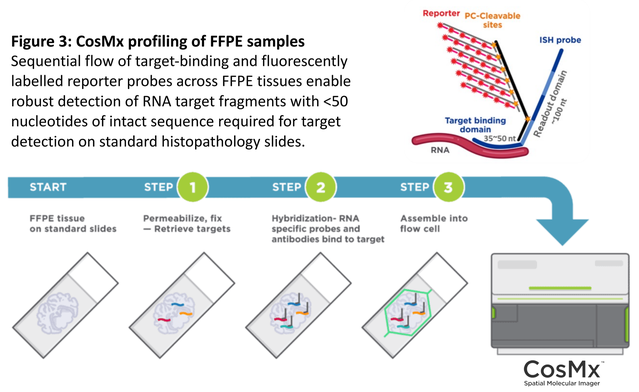


Figure 3: CosMx profiling of FFPE samples
Sequential flow of target-binding and fluorescently labelled reporter probes across FFPE tissues enable robust detection of RNA target fragments with <50 nucleotides of intact sequence required for target detection on standard histopathology slides.

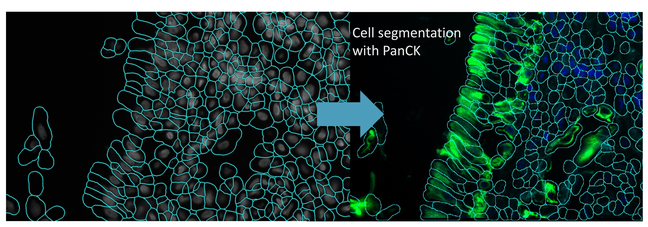


Figure 4: Segmentation of nasal epithelia for use with CosMx workflow
AI driven segmentation of epithelial cells performed with or without PanCK demonstrates improvements in segmentation of sustentacular cells.

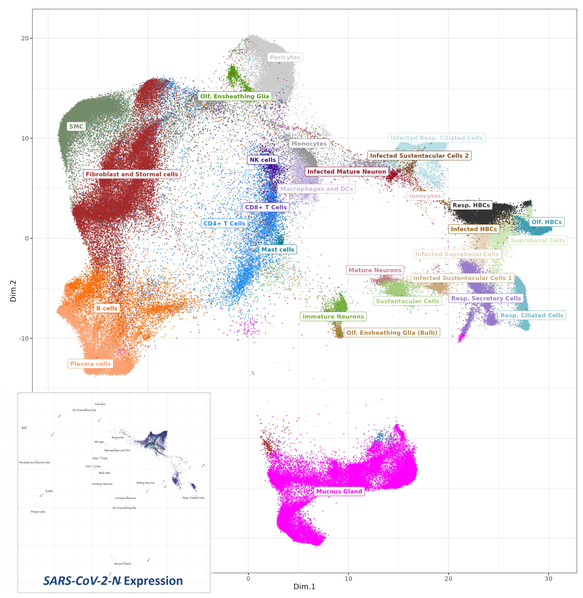


Figure 5: Atlas of SARS-CoV-2 infection in the respiratory & olfactory mucosa
UMAP shows nasal & olfactory epithelia cell types identified in Durante *et al* [2] mapped to 401,233 cells profiled with CosMx using InSituType algorithm [3]. Inset highlights infection of cells by SARS-CoV-2.

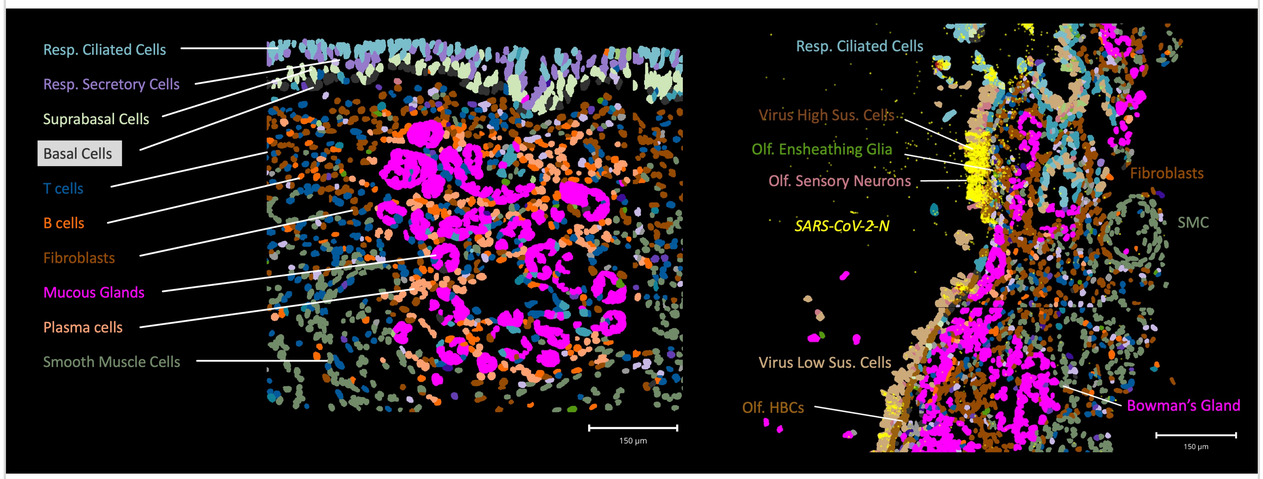
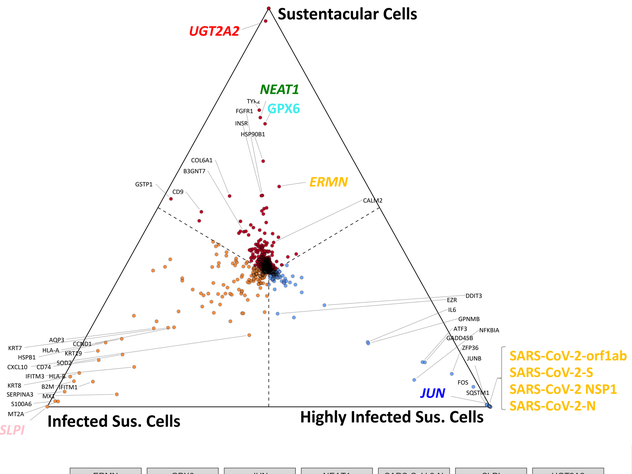


Figure 6: Mapping respiratory & olfactory epithelial cell types in uninfected deceased patients
Common cell types making up the respiratory epithelium including ciliated cells, secretory cells, suprabasal cells, as well as cells populating the lamina propria of the nasal cavity.

Figure 7: Example of infected cells in olfactory cleft mucosa
SARS-CoV-2-N (yellow points) expression localizes to infected cells making up the epithelia of the olfactory cleft preferentially. Infected cells primarily are sustentacular and ciliated cells.



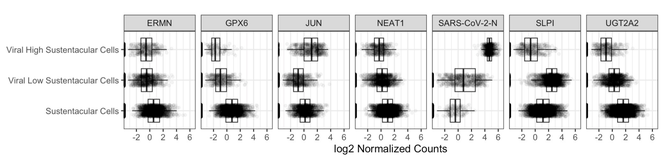
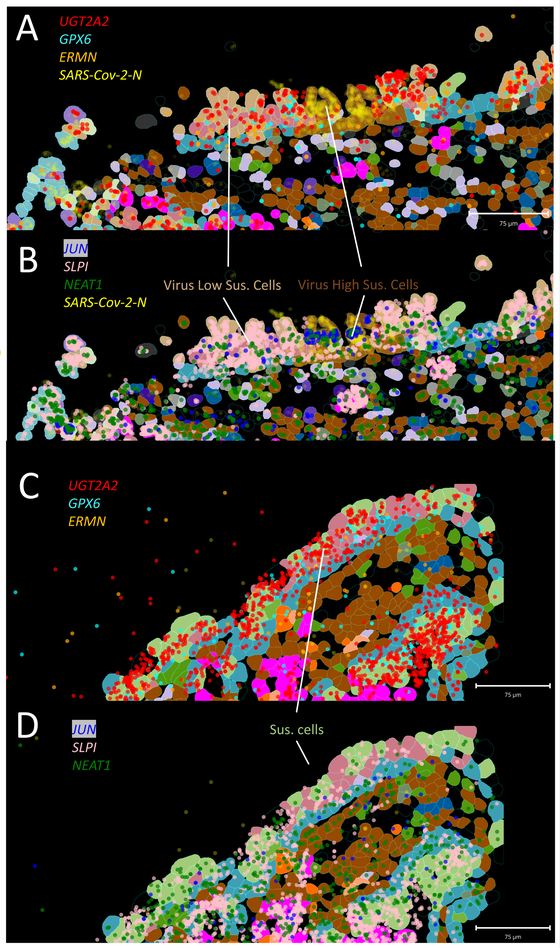


Figure 8: Differential expression in sustentacular cells
Top) 3-way ternary plot exploring relationship between genes differentially expressed between uninfected (top center), lowly infected (bottom left) and highly infected (bottom right) sustentacular cells. Labeled points show top genes in each category. Not significant points are shown in black.
Bottom) Boxplots showing the expression of marker genes and select DE genes.

Figure 9 (right): Co-expression of key targets regulating SARS-CoV-2 infection
Example images from top hits from differential expression analysis of sustentacular cells shows key markers are colocalized within infected cells. Panels A & B: COVID-19 olfactory cleft mucosa showing susentacular cell marker genes (A) and selected DE genes (B) identified from Figure 8. Panels C & D: Uninfected control olfactory cleft mucosa sample. Marker genes shown in C and DE genes from Figure 8 shown in D. *JUN* is enriched in highly infected sustentacular cells; this gene is associated with cAMP pathways that modulate MAPK signaling, which regulates cytokine production [5]. *SLPI* is enriched in virus-low sustentacular cells. The inhibitory effect of this gene product protects epithelial cells from viral infection.



Anatomical barriers to SARS-CoV-2 transmission within the nasal cavity

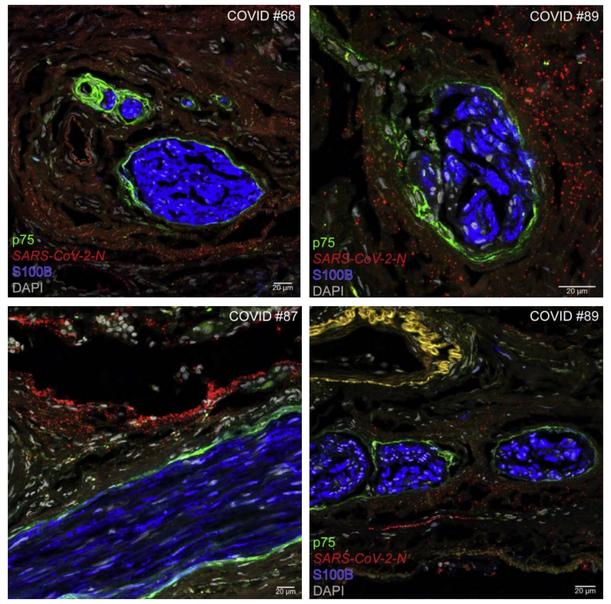


Figure 10: Anatomical barrier against SARS-CoV-2 neuroinvasion in the lamina propria of the olfactory mucosa
Further exploration with multiplexed IF & RNA Scope (Khan et al [4]) identified a novel set of fibroblasts (p75, green) associated with axon bundles (S100B, blue) in the lamina propria of the olfactory mucosa. These appear to prevent invasion of the virus (SARS-CoV-2-N red) by creating a physical barrier.

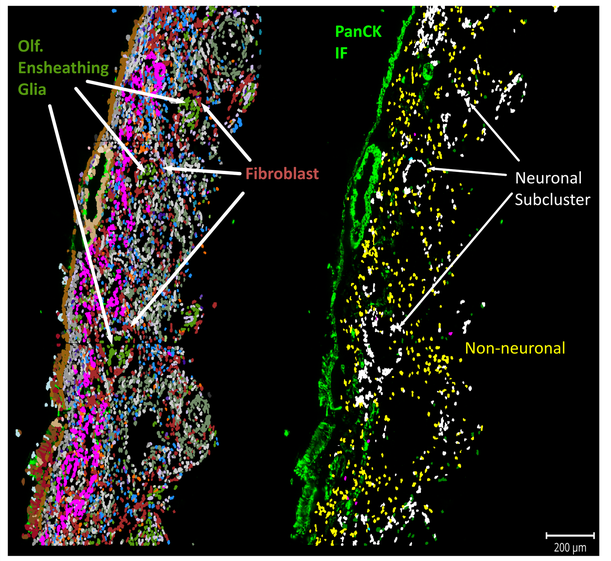


Figure 11: Identification of a novel type of barrier cells enwrapping olfactory axon fascicles of the olfactory mucosa
Cells surrounding the axon fascicles can be segregated out using CosMx SMI validating previous results suggesting their importance as well as robust characterization of their function *in situ.* Left) All cells, Right) Fibroblasts only + PanCK Immunofluorescence.

References

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3. Danaher P *et al.* Insitutype: likelihood-based cell typing for single cell spatial transcriptomics. bioRxiv. https://www.biorxiv.org/content/10.1101/2022.10.19.512902v1
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Conclusions

- **CosMx deeply characterized FFPE nasal epithelia from postmortem tissue samples using both the standard universal CosMx RNA panel and spike-ins for odorant receptor genes and viral genes**
- **Spatial profiling of nasal epithelium identifies local modifiers of SARS-CoV-2 infection and invasion**
- **Spatial cell typing identifies additional cell types not previously characterized using single-cell dissociated sequencing and consequences of viral infection**
- **120 differentially expressed genes were identified between infected and uninfected sustentacular cells**
- **A barrier to neuroinvasion was identified specifically by spatial profiling**

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