

# Cultured Mesenchymal Cells from Nasal Turbinate as a Cellular Model of the Neurodevelopmental Component of Schizophrenia Etiology

1 **Victoria Sook Keng Tung<sup>1</sup>, Fasil Mathews MD<sup>2</sup>, Marina Boruk<sup>2</sup>, Gabrielle Suppa<sup>1</sup>, Robert**  
2 **Foronjy<sup>1</sup>, Michele Pato<sup>3</sup>, Carlos Pato<sup>3</sup>, James A. Knowles<sup>4</sup>, Oleg V. Evgrafov<sup>1\*</sup>**

3 <sup>1</sup>Department of Cell Biology, State University of New York at Downstate, Brooklyn, NY, USA.

4 <sup>2</sup>Department of Otolaryngology, State University of New York at Downstate, Brooklyn, NY, USA.

5 <sup>3</sup>Department of Psychiatry, Rutgers University

6 <sup>4</sup>Human Genetics Institute of New Jersey, Rutgers University, Piscataway, NJ, USA

7 **\* Correspondence:**

8 Oleg V. Evgrafov

9 oleg.evgrafov@downstate.edu

10 **Keywords: schizophrenia, neural progenitors, mesenchymal cells, scRNA-seq, middle**  
11 **turbinate. (Min.5-Max. 8)**

## 12 **Abstract**

13 Study of the neurodevelopmental molecular mechanisms of schizophrenia requires the development  
14 of adequate biological models such as patient-derived cells and their derivatives. We previously used  
15 cell lines with neural progenitor properties (CNON) derived from superior or middle turbinates of  
16 patients with schizophrenia and control groups to study gene expression specific to schizophrenia.

17 In this study, we compared single cell-RNA seq data from two CNON cell lines, one derived from an  
18 individual with schizophrenia (SCZ) and the other from a control group, with two biopsy samples  
19 from the middle turbinate (MT), also from an individual with SCZ and a control. In addition, we  
20 compared our data with previously published data from olfactory neuroepithelium (1). Our data  
21 demonstrated that CNON originated from a single cell type which is present both in middle turbinate  
22 and olfactory neuroepithelium. CNON express multiple markers of mesenchymal cells. In order to  
23 define relatedness of CNON to the developing human brain, we also compared CNON datasets with  
24 scRNA-seq data of embryonic brain (2) and found that the expression profile of CNON very closely  
25 matched one of the cell types in the embryonic brain. Finally, we evaluated differences between SCZ  
26 and control samples to assess usability and potential benefits of using single cell RNA-seq of CNON  
27 to study etiology of schizophrenia.

## 28 **Introduction**

29 Schizophrenia (SCZ) is a brain disease with a complex etiology that commonly develops during  
30 adolescence or early adulthood. It is widely considered that alterations in brain development play a  
31 significant role in the etiology of the disease (3). While post-mortem brain samples can be used to  
32 investigate epigenetic, transcriptomic, and proteomic alterations in SCZ patients' brains, disease-

33 specific alterations in neuronal cells during embryonic and fetal brain development requires the use  
34 of cellular models.

35 Patient-derived induced pluripotent stem cells (iPSCs) and iPSC-derived neural progenitors or  
36 neurons are often used as cellular models to study the neurodevelopmental aspects of SCZ and other  
37 disorders. However, iPSCs-derived cellular models represent *in vitro* induced neurodevelopment  
38 from cells regaining stemness properties after genetic alterations. They more likely reproduce very  
39 early events of neurodevelopment and may not capture specifics of neurodevelopment in complex  
40 biological system in sufficient detail, especially at later stages.

41 An alternative approach is to use cells derived from olfactory neuroepithelium (ON) where  
42 neurogenesis occurs throughout life. The brain and ON develop from neighboring ectoderm regions  
43 with some contributions from the neural crest (4,5). ON develops from ectoderm during the 5th week  
44 of post-fertilization age (PFA) (4). Although the brain undergoes a more advanced developmental  
45 process, we may expect that the brain at *early* stages of development will still share some of the cell  
46 types of ON due to a common origin from neuroepithelium. While there is no specific data about the  
47 degree of relatedness between developed nasal turbinate and embryonic brain, the ability of ON to  
48 produce neuronal cells suggests that the similarity between them could be substantial, at least in some  
49 cell types.

50 The hypothesis that ON could serve as a model to study SCZ is further supported by high correlation  
51 between SCZ and anosmia, affecting neuronal functions in brain and ON respectively and findings of  
52 dysregulation of olfactory neuron lineages in SCZ (6).

53 Several cellular models have been developed using different protocols (7–12). In our previous  
54 studies, we used a protocol for developing cell cultures from ON which was originally proposed by  
55 Wolozin et al. (7). The key element of this protocol is to cover small pieces of ON with Matrigel and  
56 propagate only those cells that penetrate through the gel (see protocol details in (13)). We named  
57 these cells CNON, Cultured Neuronal Cells derived from Olfactory Neuroepithelium, and considered  
58 them neural progenitors, although their ability to differentiate into neurons *in vivo* has not been  
59 proven. It was subsequently discovered that the same cell type could be generated from both superior  
60 and middle turbinate (14).

61 We have previously developed CNON from 256 individuals including 144 patients with  
62 schizophrenia (SCZ) and demonstrate the robustness of CNON development and consistency of  
63 expression profiles during growth in culture and between individuals (13). Using single-cell  
64 transcriptomics, we identified a cell type in middle turbinate (MT) with an expression profile  
65 corresponding to CNON (15), confirming that CNON is not a mixture of cell types but instead a  
66 single cell type with a specific gene expression profile. These properties resulted in lower biological  
67 noise as compared to cellular models with higher heterogeneity. It warrants higher statistical power  
68 and requires a smaller number of samples to identify the signal. With advances in single-cell  
69 transcriptomics, it becomes possible to assess the similarity of CNON to cells in the brain in more  
70 detail and evaluate the relevance of this model to study brain disorders.

## 71 **Article types**

72 Original Research

73 **Manuscript Formatting**

## 74 **Materials and Methods**

75 **Biopsy collection and sample preparation.** Biopsies were obtained from patients without any  
76 history of sinonasal disease or surgery or immunocompromise. Tissue samples were obtained from  
77 the superior-medial region of the head of the middle turbinate; laterality was determined by ease of  
78 access. The mucosa was anesthetized and decongested with topical 1% lidocaine and 0.05%  
79 oxymetazoline. After 5 minutes, 0.3 ml of 1% lidocaine with 1:100,000 epinephrine was injected into  
80 the targeted mucosal site under visualization. 2mm cupped forceps were used to obtain biopsy  
81 specimens. These samples were immediately transported to the lab in Leibovitz's L-15 Medium  
82 (ThermoFisher) supplemented with Antibiotic-Antimycotic solution (ThermoFisher) and processed  
83 to prepare single-cell suspension. Biopsy pieces were minced with two scalpels in a Petri dish with a  
84 small amount of cold Hank's buffer without magnesium or calcium to prevent drying and then  
85 transferred to a 1.5 ml Eppendorf tube and washed twice with 1 ml cold Hank's buffer. Cells were  
86 dissociated in 250  $\mu$ l of 0.25% Trypsin-0.02% EDTA (VWR) at 37°C while shaking at 500 rpm.  
87 After 10 minutes, the suspension was mixed by pipetting and returned to thermomixer for another 10  
88 minutes. After another mixing, the cells were sedimented by centrifugation at 300 relative centrifugal  
89 force for 5 minutes, resuspended in 500  $\mu$ l of cold Hank's solution (ThermoFisher) with BSA,  
90 filtered using 40  $\mu$ m FLOWMI cell strainer (Bel-Art) cell suspension into a 1.5 ml Eppendorf tube,  
91 centrifuged again, and finally resuspended in 50  $\mu$ l of Hank's with 0.04% BSA (ThermoFisher).

92  
93 **CNON cell culture.** Protocols for developing CNON cell cultures from middle or superior turbinate  
94 biopsies have been previously described (13). In brief, each biopsy sample was dissected into 3-4  
95 pieces approximately 1 mm<sup>3</sup> in size, and each piece was placed onto the surface of a 60 mm tissue  
96 culture dish coated with 25  $\mu$ l of Matrigel Basement Membrane (Corning) reconstituted in F12  
97 Coon's medium (Sigma), and then covered by 15  $\mu$ l of full-strength Matrigel. After Matrigel  
98 gelatinizes, 5 ml of medium 4506 (16) was added. Medium 4506 is based on F12 Coon's medium  
99 (Sigma) supplemented with 6% of FBS (KSE scientific), 5  $\mu$ g/ml human Gibco transferrin (Fisher  
100 Scientific), 1  $\mu$ g/ml human insulin (Sigma), 10 nM hydrocortisone (Sigma), 2.5 ng/ml sodium  
101 selenite (Sigma), 40 pg/ml thyroxine (Sigma), 1% Gibco Antibiotic-Antimycotic (Fisher Scientific),  
102 150  $\mu$ g/ml Bovine hypothalamus extract (Millipore-Sigma) and 50  $\mu$ g/ml Bovine pituitary extract  
103 (Sigma). Within 1-4 weeks of incubation, neuronal cells CNON cells were observed to grow out of  
104 the embedded pieces of tissue. Due to unique ability to grow through Matrigel, neural progenitors  
105 often populate large areas without presence of other cell types. Outgrown cells with a neuronal  
106 phenotype were then physically isolated using cloning cylinders and dislodged using 0.25% Trypsin-  
107 0.02% EDTA (VWR) and transferred into a new Petri dish for further cultivation.

108  
109 **Single cell preparation from CNON.** Cells at ~80% monolayer on a 6 cm Petri dish were dislodged  
110 with 1 ml of 0.25% Trypsin-0.02% EDTA solution, and 3 ml 4506 culture medium was added to stop  
111 digestion. Cells were then gently and thoroughly mixed to break up clumps of cells, spun at 300 rcf  
112 for 5 min, and resuspended in a 3 ml culture medium. After gentle mixing by pipetting, the cell  
113 suspension was filtered using a 40  $\mu$ m Flowmi™ cell strainer into a 15 ml tube. Cells were washed  
114 with 1x DPBS (ThermoFisher) with 0.04% BSA, then 1X PBS (ThermoFisher) with 0.04% BSA, re-  
115 suspended in 500  $\mu$ l of PBS, and filtered using Flowmi™ Tip Strainer. After counting, cell  
116 concentration was adjusted to 700 cells/ $\mu$ l.

117  
118 **scRNA-seq.** The concentration and viability of cells were determined using a hemocytometer and  
119 Trypan blue. After counting, single-cell libraries were prepared according to the 10x Genomics  
120 protocol CG000183 on Chromium controller (10x Genomics) and sequenced on NovaSeq6000 as  
121 paired end 28 + 90 bp reads plus two indexing reads.

122  
123 **scRNA-seq analysis** Raw sequencing data were processed using `bcl2fastq2 v2.20` to convert BCL  
124 files to fastq files while simultaneously demultiplexing. Fastq files were processed using *TrimGalore*  
125 v. 0.6.5 to automate quality, adapter trimming, and perform quality control.

126 We used Cell Ranger v. 6.1.2 (10x Genomics) to generate raw gene-barcode matrices from the reads,  
127 which were aligned to the **GRCh38 Ensembl v93**-annotated genome. The following analysis was  
128 done using R package Seurat v.4.10 (17). First, cells with low number of detected RNA molecules or  
129 genes were removed from the data and gene expressions were normalized and scaled using  
130 *Scran*. We then applied a graph-based clustering algorithm in order to group cells into distinct  
131 clusters.

132 To account for any potential variability due to cell-cycle phase, we calculated cell-cycle phase scores  
133 using Seurat's built-in lists of cell-cycle genes. We then regressed out these scores from the dataset  
134 during normalization in order to reduce the effect of cell cycle heterogeneity on our analysis using  
135 Seurat algorithm ([https://satijalab.org/seurat/articles/cell\\_cycle\\_vignette.html](https://satijalab.org/seurat/articles/cell_cycle_vignette.html)). After analysis of  
136 cluster trees (*clustree*) we selected an optimal resolution parameter (0.5 for MT studies) for cluster  
137 analysis. We annotated the clusters using known markers and data from relevant single-cell studies,  
138 and generated UMAP plots for visualization.

139 To identify genes that are differentially expressed between the cell clusters in scRNA-seq data, we  
140 used the *FindAllMarkers()* function in Seurat. This function compares the expression of each gene in  
141 each cluster using a Wilcoxon rank-sum test and returns a list of differentially expressed genes. We  
142 set the `min.pct` argument to 0.25 (to include only genes with at least 25% difference in mean  
143 expression) and the `logfc.threshold` argument to 0.25 (log fold change of at least 0.25 between the  
144 clusters).

145 To visualize the DE results, we used the *DotPlot()* function in Seurat, which plots the fold change in  
146 gene expression using the color scale and the size of the circle as percentage of cells expressing the  
147 gene. We also created a table of these DE genes using the data from the dot plot. This table allowed  
148 us to more easily review and analyze the DE genes identified by the *FindAllMarkers()* function.

149 To compare expression profiles of CNON cells with single cell data from tissues (middle turbinate,  
150 olfactory neuroepithelium and embryonic brain), we performed reference mapping using Seurat,  
151 annotating the query CNON datasets on a cell-type labeled reference datasets and evaluating the  
152 mapping of predicted cell-type annotations in the query datasets to the reference dataset using the  
153 *TransferData()* function.

154  
155 ScRNA-seq data from embryonic brain was obtained from the UCSC Cell Browser (matrixes from  
156 combined samples) and the NeMO repository (<https://assets.nemoarchive.org/dat-0rsydy7>) where  
157 individual sample matrixes were available.

158 ScRNA-seq data from olfactory neuroepithelium was obtained from Gene Expression Omnibus  
159 under accession code **GSE139522**.

160

## 161 **Results**

162 To assess potential utility of cultured cells derived from nasal turbinates to study the developmental  
163 mechanisms of schizophrenia, we performed scRNA-seq of cultures derived from a patient with SCZ  
164 (CNON-SCZ) and from an individual from the control group (CNON-CTRL). To identify parental

165 cell types, we compared this with scRNA-seq data from two middle turbinate biopsy samples, taken  
166 from a patient with SCZ (MT-SCZ) and control (MT-CTRL). Lastly, CNON datasets were compared  
167 with single cell transcriptome data from the embryonic brain (2).

168 For comparison we employed *reference mapping* from Seurat, which mapped query datasets  
169 (CNON) to reference datasets, which were either MT or embryonic brain data. Transcriptome of each  
170 CNON cell was placed in 3-dimensional space with PC coordinates of reference dataset, and assessed  
171 if it could be assigned to any cluster of reference dataset, associated with particular cell type. The  
172 quality of assigned cell type of each query cell was evaluated by calculated *prediction score*.

173 Such reference mapping showed that the vast majority of CNON-CTRL cells (11741, 96%) mapped  
174 to the cluster corresponding to the Mesenchymal cell (MC) cluster of the MT-CTRL, with an average  
175 prediction score of 0.96. However, cells from MC cluster are not proliferating (do not express cell  
176 cycle-specific genes, such as *MKI67*), and the effect of cell culture genes may reduce the quality of  
177 mapping to non-proliferating cells of the same type. To mitigate the effects of CNON proliferation  
178 we assigned cells with a cell-cycle score based on the expression of canonical cell-cycle genes,  
179 applied these scores to model the relationship between gene expression and cell-cycle score and  
180 removed this cell-cycle phase variability from our data using regression. These procedures can be  
181 performed using functions in Seurat. Indeed, regressing cell-cycle score resulted in accurate mapping  
182 of all CNON-CTRL cells to MC cluster with a perfect prediction score of 1 (**Table 1**).

183 **Figure 1a and b** show results of mapping of CNON data from both samples onto two MT datasets  
184 after Uniform Manifold Approximation and Projection (UMAP) for dimension reduction (18); all six  
185 figures were presented using the same axes and on the same scale. Cell types in MT-CTRL were  
186 annotated based on marker genes specific for every cluster. Such genes were identified for each  
187 cluster by differentially gene expression analysis with all other clusters. The heatmap (**Figure 1g**) of  
188 MT-CTRL reveals a clear distinction between various cell types. While **Figure 1a** and **Figure 1b**  
189 demonstrate allocation of CNON to cluster MC in UMAP coordinates, **Table 1** provides numbers of  
190 cells mapped to specific cluster with corresponding prediction scores, supporting our previous  
191 findings that CNON developed from one cell type.

192 Transcripts of MC markers are found in the vast majority of CNON cells. Meanwhile, the expression  
193 of prominent markers of other middle turbinate cell types: basal (*SERPINB3*, *KRT5*), endothelial  
194 (*CCL14*, *VWF*), serous (*DMBT1*), club (*LYPD2*, *SCGB1A1*), ciliated (*SNTN*), goblet cells (*MUC5B*)  
195 and ionocytes (*CFTR*) were either not present or insignificant as they were identified in less than 1%  
196 of CNON. Expression of all these genes was also low in bulk RNA-seq from CNON (**Table 2**).

197  
198 We also mapped CNON data to single cell datasets from olfactory neuroepithelium (1). It contains  
199 data from four patients. We focus our investigation on reference mapping CNON cells to Patient 2, as  
200 it has the most neuronal cells per sample and we believe that it is most accurately representing  
201 olfactory neuroepithelium. The mapping result showed that CNON-CTRL exclusively mapped to a  
202 single cluster in the data of Patient 2 (**Figure 1c**) and this cluster expresses markers similar to MC  
203 cluster in MT-CTRL and MT-SCZ. The majority of cells in CNON-SCZ also map to this same  
204 cluster. Additionally, we compared our CNON cells with integrated data from all 4 patients, and  
205 most CNON cells correspond to a single cell cluster that exhibits the same gene expression profile  
206 (**Figure 1c&d**).

207  
208 We then compared CNON expression profile with a large dataset of brains at several embryonic  
209 stages of development (Carnegie stages 13-22) (2). Dataset from early stages of development was

210 chosen for our comparison as we believe that the embryonic brain was more likely to contain CNON-  
211 like cells than fetal brain at later stages of development, due to a closer relationship to a common or  
212 similar ancestors.

213  
214 Comparing CNON single-cell data with scRNA-seq in this study with embryonic brain at different  
215 stages of development shows similarity with cluster 47, described by the authors in supplementary  
216 data (2). Most cells from cluster 47 were sourced from one sample designated as CS14\_3, and we  
217 performed reference mapping of CNON data to this sample alone. 99.99% of CNON-CTRL (all  
218 except one cell) mapped to a single cluster 9 of CS14\_3 (**Figure 1e, Table 1**), with an average  
219 prediction score of 0.995. This cluster corresponds to cluster 47 in the analysis of data from all  
220 embryonic samples (19). Similarly, reference mapping of a smaller dataset of CNON-SCZ resulted in  
221 3297 cells (98.5%) annotated as cluster 9 with an average prediction score of 0.985, while 6 cells  
222 were assigned to cluster 0 with a prediction score of only 0.015 (**Table 1**).

223  
224 Our findings indicate that CNON exhibits a highly comparable expression profile with one cell type  
225 found in the developing brain. In the article describing the embryonic brain dataset (2), cluster 47  
226 was classified as “others” and it was distinctly different from explicitly classified cell types such as  
227 neurons, radial glia, neuroepithelial, intermediate progenitors, and mesenchymal cells. In sample  
228 CS14\_3 this cell type accounted for about 3.5% of the total population of cells, while in other  
229 samples, including those from earlier CS13, another sample from CS14, and samples from later  
230 stages CS15, CS20 and CS22 their proportion was lower. According to our calculation these cells  
231 make up 0.87% of all cells, while the authors estimate their fraction even higher at 1.1%.

232  
233 To better characterize this MC cell type, we performed gene ontology enrichment analysis of genes  
234 differentially expressed (DEX) in MC cells (adjusted P-value < 0.05) compared to all other cell types  
235 in the middle turbinate. The analysis revealed significant enrichment in multiple biological processes  
236 related to development (**Supplemental Table 1**). Other noteworthy biological processes are those  
237 related to cell adhesion, cell migration, and mesenchymal cell maintenance (mesenchymal  
238 development, mesenchymal cell differentiation, regulation of epithelial to mesenchymal transition,  
239 positive regulation of epithelial to mesenchymal transition, epithelial to mesenchymal transition).  
240 While there is an enrichment of genes involved in neurogenesis (GO:0022008) and related processes  
241 (generation of neurons (GO:0048699), regulation of neuron projection development (GO:0010975),  
242 neuron projection development (GO:0031175), neuron projection morphogenesis (GO:0048812),  
243 etc.), processes involved in the development of some non-neuronal tissues and organs are also  
244 present.

245  
246 Overall, the development of the human body is driven by the stem and progenitor cells, which divide,  
247 migrate, differentiate and shape different organs. There are several known sets of genes supporting  
248 stem cell properties, such as those described in embryonic, neural, intestinal, adipose-derived stem  
249 cells, mesenchymal stem cells in different tissues, and cancer stem cells.

250  
251 Finally, we compared single cell gene expression data between CNON cells (CNON-CTRL and  
252 CNON-SCZ) to assess potential usability of scRNA-seq to study schizophrenia. CNON-SCZ sample  
253 was selected for this study because cells from CNON-SCZ have a low growth rate and extreme cell  
254 cycle eigengene value in our previous study (20). It was located on the periphery of PCA1/PCA2  
255 map based on all or only DEX genes, suggesting that this sample may reveal schizophrenia-specific  
256 differences in expression profiles in single cell data despite the high genetic and transcriptomic  
257 heterogeneity of both CNON samples. Most of DEX genes identified in our previous study had low  
258 expression, with only 21 of them with transcripts per million transcripts (TPM) more than 10, and

259 only 5 genes with TPM>100. In single cells, we found 15 DEX genes with detected expression in  
260 more than 50% of cells. Eleven of them showed difference in expression between control and SCZ  
261 sample of more than 50%, all of them in the same direction as it was assumed by DEX analysis in  
262 bulk RNA-seq study. We initially assumed that these alterations in gene expression could explain the  
263 dramatic reduction in proliferation rate, but single cell data revealed a different picture. The  
264 percentage of cells in G2-M and S cycle stage in the slow-growing CNON-SCZ sample was higher  
265 than in the fast-growing CNON-CTRL. Instead, we found that a substantially larger fraction of cells  
266 from SCZ sample was associated with a cluster of cells with elevated level of mitochondrial gene  
267 expression, often characteristic of apoptotic processes (**Table 3**). Given that apoptosis is relatively  
268 rapid process (21), slow growth of SCZ sample may potentially be explained by a higher apoptosis  
269 rate.

## 270 Discussion

271 Cells in CNON lack prominent markers of pluripotency, such as *POU5F1* and *NANOG*, which are  
272 the hallmarks of embryonic stem cells and iPSCs. *PROM1* (CD133), a marker used for the  
273 purification of neural stem cells (22), is also not expressed in CNON. Currently, the literature on  
274 neural progenitor cells in the brain is less well described. One of the better-known types is radial glia  
275 (a cell that differentiates into outer radial glia and ventricular radial glia at later stages of brain  
276 development), which can directly differentiate into neurons or produce intermediate progenitor cells  
277 (IPCs). However, the expression profile of CNON does not fit into the radial glial gene expression  
278 pattern; in particular, CNON does not express radial glial markers *SOX2*, *HES5*, *PAX6*, or *GFAP*.  
279 CNON also does not express *EOMES*, a marker of IPCs, which play an important role in producing  
280 neurons after gestation week 8 (~PFA 6 weeks).

281  
282 Neuroepithelium such as ON and epithelium of middle turbinate originates mostly from ectoderm,  
283 while cells from the MC cluster express multiple markers of mesenchymal cells, or mesenchymal  
284 stem cells, which are multipotent cells of mesodermal origin (**Table 4**). Precursors of these  
285 mesenchymal cells likely originate from neural crest, from which they migrate to different locations  
286 of the fetus to establish mesenchymal cell populations in bone marrow (23), adipose tissue (24), oral  
287 mucosa lamina propria (25), and several other locations (see (26) for review), including the brain  
288 (27). Expectedly, cluster 9 of embryonic brain sample CS14\_3 also expressed multiple mesenchymal  
289 markers.

290  
291 Despite the characteristic similarities in the expression of specific marker genes, mesenchymal cells  
292 from different tissues may differ in the expression of some genes and their ability to differentiate into  
293 particular cell types in a specific environment. For example, mesenchymal-like stem cells residing in  
294 the olfactory mucosa demonstrated the promyelination effect on oligodendrocyte precursor cell,  
295 while similar mesenchymal cells derived from bone marrow do not enhance myelination (28).  
296 Distinctions between MSC from different tissues define their specific use in regenerative medicine  
297 (29,30).

298  
299 Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has  
300 developed a set of minimum criteria for defining multipotent mesenchymal stromal cells: **(a)** on the  
301 expression of certain proteins/genes, **(b)** the ability to adhere to plastic and **(c)** differentiate *in*  
302 *vitro* into osteoblasts, adipocytes, chondroblasts (31). CNON satisfied the first two criteria of  
303 expressing specific genes and their ability to adhere to plastic (**Figure 2b**), but we have yet to  
304 investigate the multipotency of CNON.

305

306 In previous studies, WNT signaling has been shown to play an important role in controlling both the  
307 maintenance and differentiation of mesenchymal stem cells (32–35). The CNON expression profile  
308 shows a robust expression of *Wnt5A*, *Wnt5B* and *Notch2* ligands, accompanied by genes involved in  
309 corresponding signaling pathways. For example, genes for frizzled receptors *FZD2* and *FZD7*, co-  
310 receptors *ROR2*, *LPR5*, *LPR6*, and secreted frizzled receptors *SFRP1* and *SFRP2*, as well  
311 as *CTNNB1* ( $\beta$ -catenin), are robustly expressed in CNON to support both canonical and non-  
312 canonical signaling (**Table 5**). Similarly, *JAG1*, presenilins *PSEN1* and *PSEN2*, *ADAMS17*,  
313 *PSENE1*, and *APH1A* ( $\gamma$ -secretase subunits) are also expressed in CNON (**Table 5**). This suggests  
314 that CNON can regulate differentiation and function in an autocrine or paracrine manner.

315  
316 Other studies also reported that cells with mesenchymal properties in the ON (ecto-mesenchymal  
317 stem cells) (29) and were able to culture them *in vitro* albeit using different cell culture method (36).  
318 As we demonstrated in *Results* section, single cell data from olfactory neuroepithelium (1) shows  
319 large group of cells with expression profile corresponding to CNON, which express multiple  
320 mesenchymal markers. Recent single cell study of cell cultures derived from olfactory mucosa also  
321 showed a large group of cells referred as “fibroblast/stromal”. However, at the time of writing, we are  
322 unable to gain access to the data to assess expression of mesenchymal markers. Notably, the study  
323 did not utilize selection for cells penetrating Matrigel, and resulting culture consists of multiple cell  
324 types, which were described as fibroblast/stromal, GBC and myofibroblasts (37).

325  
326 Another study suggests that mesenchymal cells from olfactory mucosa possess multipotency and can  
327 form neurospheres different from those produced by horizontal epithelial global cells (38).

328  
329 Therefore, the similarity of mesenchymal properties between these cells and CNON suggests that  
330 CNON is likely a derivative of ecto-mesenchymal stem cells of ON. CNON is a single cell type due  
331 to the specific way of developing cell cultures, while other methods without using Matrigel for cell  
332 type selection resulted in at least three different cell types growing in culture (37).

333  
334 It should be noted that CNON have a more pronounced gene expression profile of mesenchymal cell  
335 markers compared to MC cluster of MT or cluster 9 from embryonic brain sample. For example, cells  
336 from MC in MTs express HLA-DR genes but their expression is halted when culturing them *in vitro*  
337 under our conditions. It is not unexpected, as the definition of mesenchymal stem cells is based on  
338 assays of cells in culture, and for a long time there was a hypothesis that MSC is an artifact of  
339 culturing cells *in vitro* (as discussed, for example, in (39)). We attribute it to plasticity of  
340 mesenchymal cells, changing phenotype and gene expression according to environment.

341  
342 Indeed, cells in MC cluster in turbinates are not dividing; in Matrigel they change phenotype, divide  
343 and migrate (**Figure 2A**) and then change their phenotype again to classical mesenchymal phenotype  
344 when grown in 2D (**Figure 2b**). However, when placed inside Matrigel after multiple passages in 2D,  
345 cells reverted to a phenotype with multiple elongated branches and organizing complex  
346 interconnected cell structures resembling a neuronal network (**Figure 2c**).

347  
348 Similar complex cellular structures have been previously observed when human mesenchymal  
349 stromal cells derived from bone marrow grew in diluted Matrigel, which the authors attributed to  
350 formation of capillary network (40). It may indicate versatility and plasticity of mesenchymal cells  
351 exploiting similar architectural solutions for different biological purposes.

352  
353 The role of cells with mesenchymal gene expression signatures at the early stages of development of  
354 the brain is not clear. Many noticed similarities of MSC with pericytes and suggest the involvement



355 of MSC in making a brain barrier and vascular systems. There is also an appreciation of their  
356 paracrine function in a neurovascular niche context involving in orchestrating the complex  
357 development of brain structures (27). Ability of MSC to differentiate into neurons *in vitro* and  
358 engrafted in the brain also suggest involvement of these cells in neurogenesis. Transplantation of  
359 MSC in brain lesion models showed therapeutic effects (41), although it is not yet clear if it is caused  
360 by MSC differentiation into neurons or by a paracrine effect or both. Anyway, the important role of  
361 MSC in the brain is well recognized, and the hypothesis that alterations in gene expression in MSC  
362 cause SCZ is well justified.

363  
364 The role of mesenchymal cells in turbinates is not known. Although they have a capability to  
365 differentiate into neurons, it is not clear if they realize this potential in olfactory mucosa either during  
366 development or injuries or regular replenishment of olfactory neurons. The striking similarity of  
367 CNON to cells in embryonic brain is probably a reflection of similarities of MSC among tissues. MT  
368 belongs to the respiratory system, where the role of mesenchymal cells is quite pronounced.  
369 Mesenchymal cells are involved in lung development and responsible for homeostasis and tissue  
370 repair in the lung (42). If alterations in gene expression of mesenchymal cells contribute to the  
371 etiology of schizophrenia, we should expect that the same changes in MSC properties can affect other  
372 organs with substantial presence of MSC. Indeed, a comorbidity between SCZ and lung diseases  
373 have been reported, and in both directions: schizophrenia was found to be associated with impaired  
374 lung function (43), and patients with COPD have 10 times higher risk of psychiatric comorbidities  
375 (44). There are reports that olfactory deficits known to be prevalent in SCZ are also found in most  
376 COPD patients (45). We hypothesize that alterations in the properties of mesenchymal cells may  
377 contribute to a range of “mesenchymal” disorders, which include some subtypes of schizophrenia and  
378 lung diseases.

379  
380 Our findings made on analysis of CNON that WNT signaling and regulation of WNT production  
381 (and specifically WNT5A), are involved in etiology of SCZ (19) fits this hypothesis well. These  
382 pathways are central for self-renewal and differentiation of MSC (33), and they are also play a  
383 critical role in lung development (46), tissue regeneration (47) and etiology of COPD (48). Thus,  
384 alterations in these pathways could be one of common mechanisms of “mesenchymal” disorders.

385  
386

## 387 **Conclusion**

388 Using scRNA-seq we confirmed that CNON cells originate from a single cell type of middle  
389 turbinate or olfactory neuroepithelium. The expression profile of CNON closely matches those of  
390 mesenchymal stem cells. Although we have not tested multipotency of these cells in this study, other  
391 studies suggest that mesenchymal cells of olfactory mucosa are able to differentiate into multiple  
392 lineages, including neurons.

393 Our analysis of gene expression in embryonic brain (2) identified a cell type that closely matches to  
394 CNON cells by expression profiling. Cell type homogeneity of CNON, stability of their expression  
395 profiles in cell culture during multiple passages, and high similarity to one of cell type in the  
396 embryonic brain providing support that CNON is a promising cellular model of neurodevelopmental  
397 disorders.

## 398 **Conflict of Interest**

399 *The authors declare that the research was conducted in the absence of any commercial or financial*  
400 *relationships that could be construed as a potential conflict of interest.*

#### 401 **Author Contributions**

402 VSKT performed most of data analysis and made substantial contribution to manuscript preparation.  
403 MB performed biopsies. MB, FM, and RF provided expertise in respiratory epithelium physiology  
404 and cell biology. GS worked with cell cultures, performed single cells library preparation and  
405 sequencing. JK contributed to discussion and helped with organisation of the study. CP and MP  
406 organized recruitment of participants and contributed to discussion. OE designed and supervised all  
407 stages of the project, performed data analysis with VSTG and contributed to manuscript preparation.

#### 408 **Funding**

409 The study was funded by the National Institute of Mental Health (Grant MH086874) [to OVE] and  
410 start-up funding provided by SUNY Downstate Health Sciences University [to OVE].

#### 411 **Acknowledgments**

412

#### 413 **1 Data Availability Statement**

414 The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (49)  
415 and are accessible through GEO Series accession number GSE219165  
416 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE219165>). Original R scripts are available from  
417 the corresponding author, VSKT, upon reasonable request.

#### 418 **References**

- 419 1. Durante MA, Kurtenbach S, Sargi ZB, Harbour JW, Choi R, Kurtenbach S, et al. Single-cell  
420 analysis of olfactory neurogenesis and differentiation in adult humans. *Nat Neurosci* [Internet].  
421 2020;23(3):323–6. Available from: <http://dx.doi.org/10.1038/s41593-020-0587-9>
- 422 2. Eze UC, Bhaduri A, Haeussler M, Nowakowski TJ, Kriegstein AR. Single-cell atlas of early  
423 human brain development highlights heterogeneity of human neuroepithelial cells and early  
424 radial glia. *Nat Neurosci*. 2021;24(4):584–94.
- 425 3. Murray RM, Bhavsar V, Tripoli G, Howes O. 30 Years on: How the Neurodevelopmental  
426 Hypothesis of Schizophrenia Morphed into the Developmental Risk Factor Model of  
427 Psychosis. *Schizophr Bull*. 2017;43(6):1190–6.
- 428 4. Som PM, Naidich TP. Illustrated Review of the Embryology and Development of the Facial  
429 Region, Part 1: Early Face and Lateral Nasal Cavities. *AJNR Am J Neuroradiol*.  
430 2013;34:2233–2240.
- 431 5. Kaltschmidt B, Kaltschmidt C, Widera D. Adult Craniofacial Stem Cells: Sources and  
432 Relation to the Neural Crest. *Stem Cell Rev Reports*. 2012;8(3):658–71.
- 433 6. Arnold SE, Han LY, Moberg PJ, Turetsky BI, Gur RE, Trojanowski JQ, et al. Dysregulation  
434 of olfactory receptor neuron lineage in schizophrenia. *Arch Gen Psychiatry* [Internet]. 2001

- 435 Sep;58(9):829–35. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11545665>
- 436 7. Wolozin B, Bacic M, Merrill MJ, Lesch KP, Chen C, Lebovics RS, et al. Continuous culture  
437 of neuronal cells from adult human olfactory epithelium. *J Mol Neurosci*. 1992;3(3):137–146.
- 438 8. Horiuchi Y, Kondo MA, Okada K, Takayanagi Y, Tanaka T, Ho T, et al. Molecular signatures  
439 associated with cognitive deficits in schizophrenia: a study of biopsied olfactory neural  
440 epithelium. *Transl Psychiatry* [Internet]. 2016;6(10):e915. Available from:  
441 <http://www.nature.com/doi/10.1038/tp.2016.154>
- 442 9. Zhang X, Klueber KM, Guo Z, Lu C, Roisen FJ. Adult human olfactory neural progenitors  
443 cultured in defined medium. *Exp Neurol* [Internet]. 2004;186(2):112–123. Available from:  
444 <http://linkinghub.elsevier.com/retrieve/pii/S0014488603005545>
- 445 10. Gomez G, Rawson NE, Hahn CG, Michaels R, Restrepo D. Characteristics of odorant elicited  
446 calcium changes in cultured human olfactory neurons. *J Neurosci Res* [Internet].  
447 2000;62(5):737–49. Available from:  
448 <http://www3.interscience.wiley.com/journal/76500345/abstract>
- 449 11. Lavoie J, Sawa A, Ishizuka K. Application of olfactory tissue and its neural progenitors to  
450 schizophrenia and psychiatric research. *Curr Opin Psychiatry*. 2017;30(3):176–83.
- 451 12. Mackay-Sim A. Concise review: Patient-derived olfactory stem cells: New models for brain  
452 diseases. *Stem Cells*. 2012;30(11):2361–5.
- 453 13. Evgrafov O V, Wrobel BB, Kang X, Simpson G, Malaspina D, Knowles J a. Olfactory  
454 neuroepithelium-derived neural progenitor cells as a model system for investigating the  
455 molecular mechanisms of neuropsychiatric disorders. *Psychiatr Genet* [Internet]. 2011 Oct  
456 [cited 2011 Dec 20];21(5):217–28. Available from:  
457 <http://www.ncbi.nlm.nih.gov/pubmed/21451437>
- 458 14. Wrobel BB, Mazza JM, Evgrafov O V., Knowles JA. Assessing the efficacy of endoscopic  
459 office olfactory biopsy sites to produce neural progenitor cell cultures for the study of  
460 neuropsychiatric disorders. *Int Forum Allergy Rhinol*. 2013;3(2):133–8.
- 461 15. Mathews F, Tung VSK, Foronjy R, Boruk M, Knowles JA, Evgrafov O V. Cell type catalog of  
462 middle turbinate epithelium. *bioRxiv*. 2022;
- 463 16. Wolozin B, Sunderland T, Zheng B, Resau J, Dufy B, Barker J, et al. Continuous culture of  
464 neuronal cells from adult human olfactory epithelium. *J Mol Neurosci* [Internet].  
465 1992;3(3):137–146. Available from:  
466 <http://www.springerlink.com/index/E2M2038GW172WM54.pdf>
- 467 17. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis  
468 of multimodal single-cell data. *Cell* [Internet]. 2021;184(13):3573–3587.e29. Available from:  
469 <https://doi.org/10.1016/j.cell.2021.04.048>
- 470 18. McInnes L, Healy J, Melville J. UMAP: Uniform Manifold Approximation and Projection for  
471 Dimension Reduction [Internet]. <http://arxiv.org/abs/1802.03426>. 2020. Available from:  
472 <http://arxiv.org/abs/1802.03426>

- 473 19. Evgrafov O V., Armoskus C, Wrobel BB, Spitsyna VN, Souaiaia T, Herstein JS, et al. Gene  
474 Expression in Patient-Derived Neural Progenitors Implicates WNT5A Signaling in the  
475 Etiology of Schizophrenia. *Biol Psychiatry* [Internet]. 2020;88(3):236–47. Available from:  
476 <https://doi.org/10.1016/j.biopsych.2020.01.005>
- 477 20. Evgrafov O V., Armoskus C, Wrobel BB, Spitsyna VN, Souaiaia T, Herstein JS, et al. Gene  
478 Expression in Patient-Derived Neural Progenitors Implicates WNT5A Signaling in the  
479 Etiology of Schizophrenia. *Biol Psychiatry* [Internet]. 2020;(February):1–12. Available from:  
480 <https://doi.org/10.1016/j.biopsych.2020.01.005>
- 481 21. Elmore S. Apoptosis: A Review of Programmed Cell Death. *Toxicol Pathol.* 2007;35(4):495–  
482 516.
- 483 22. Beckervordersandforth R, Tripathi P, Ninkovic J, Bayam E, Lepier A, Stempfhuber B, et al. In  
484 vivo fate mapping and expression analysis reveals molecular hallmarks of prospectively  
485 isolated adult neural stem cells. *Cell Stem Cell.* 2010;7(6):744–58.
- 486 23. Charbord P. Bone marrow mesenchymal stem cells: Historical overview and concepts. *Hum*  
487 *Gene Ther.* 2010;21(9):1045–56.
- 488 24. Si Z, Wang X, Sun C, Kang Y, Xu J, Wang X, et al. Adipose-derived stem cells: Sources,  
489 potency, and implications for regenerative therapies. *Biomed Pharmacother* [Internet].  
490 2019;114(March):108765. Available from: <https://doi.org/10.1016/j.biopha.2019.108765>
- 491 25. Davies LC, Locke M, Webb RDJ, Roberts JT, Langley M, Thomas DW, et al. A multipotent  
492 neural crest-derived progenitor cell population is resident within the oral mucosa lamina  
493 propria. *Stem Cells Dev.* 2010;19(6):819–30.
- 494 26. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. *Cell Transplant.* 2011;20(1):5–14.
- 495 27. Pombero A, Garcia-Lopez R, Martinez S. Brain mesenchymal stem cells: physiology and  
496 pathological implications. *Dev Growth Differ.* 2016;58(5):469–80.
- 497 28. Lindsay SL, Johnstone SA, Mountford JC, Sheikh S, Allan DB, Clark L, et al. Human  
498 mesenchymal stem cells isolated from olfactory biopsies but not bone enhance CNS  
499 myelination in vitro. *Glia* [Internet]. 2013 Mar [cited 2023 Jan 11];61(3):368–82. Available  
500 from: <https://pubmed.ncbi.nlm.nih.gov/23281012/>
- 501 29. Delorme B, Nivet E, Gaillard J, Häupl T, Ringe J, Devèze A, et al. The human nose harbors a  
502 niche of olfactory ectomesenchymal stem cells displaying neurogenic and osteogenic  
503 properties. *Stem Cells Dev.* 2010;19(6):853–66.
- 504 30. Lim JY, In Park S, Park SA, Jeon JH, Jung HY, Yon JM, et al. Potential application of human  
505 neural crest-derived nasal turbinate stem cells for the treatment of neuropathology and  
506 impaired cognition in models of Alzheimer’s disease. *Stem Cell Res Ther.* 2021;12:402.
- 507 31. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al.  
508 Minimal criteria for defining multipotent mesenchymal stromal cells. The International  
509 Society for Cellular Therapy position statement. *Cytotherapy* [Internet]. 2006;8(4):315–7.  
510 Available from: <http://dx.doi.org/10.1080/14653240600855905>

- 511 32. Harrison-Uy SJ, Pleasure SJ. Wnt signaling and forebrain development. *Cold Spring Harb*  
512 *Perspect Biol.* 2012;4:a008094.
- 513 33. Ling L, Nurcombe V, Cool SM. Wnt signaling controls the fate of mesenchymal stem cells.  
514 *Gene* [Internet]. 2009;433(1–2):1–7. Available from:  
515 <http://dx.doi.org/10.1016/j.gene.2008.12.008>
- 516 34. Lasky JL, Wu H. Notch Signaling, Brain Development, and Human Disease. *Pediatr Res.*  
517 2005;57(5):104R-109R.
- 518 35. Nian, F. S., & Hou PS. Evolving Roles of Notch Signaling in Cortical Development. *Front*  
519 *Neurosci* [Internet]. 2022;16:844410. Available from:  
520 <https://doi.org/10.3389/fnins.2022.844410>
- 521 36. Girard SD, Devéze A, Nivet E, Gepner B, Roman FS, Féron F. Isolating Nasal Olfactory Stem  
522 Cells from Rodents or Humans. *J Vis Exp.* 2011;(August):1–5.
- 523 37. Lampinen R, Fazaludeen MF, Avesani S, Örd T, Penttilä E, Lehtola JM, et al. Single-Cell  
524 RNA-Seq Analysis of Olfactory Mucosal Cells of Alzheimer’s Disease Patients. *Cells.*  
525 2022;11(4):1–20.
- 526 38. Tomé M, Lindsay SL, Riddell JS, Barnett SC. Identification of nonepithelial multipotent cells  
527 in the embryonic olfactory mucosa. *Stem Cells.* 2009;27(9):2196–208.
- 528 39. Murray IR, Péault B. Q&A: Mesenchymal stem cells - where do they come from and is it  
529 important? *BMC Biol.* 2015;13(1):4–9.
- 530 40. Janeczek Portalska K, Leferink A, Groen N, Fernandes H, Moroni L, van Blitterswijk C, et al.  
531 Endothelial Differentiation of Mesenchymal Stromal Cells. *PLoS One.* 2012;7(10).
- 532 41. Hernández R, Jiménez-Luna C, Perales-Adán J, Perazzoli G, Melguizo C, Prados J.  
533 Differentiation of human mesenchymal stem cells towards neuronal lineage: Clinical trials in  
534 nervous system disorders. *Biomol Ther.* 2020;28(1):34–44.
- 535 42. Nasri A, Foisset F, Ahmed E, Lahmar Z, Vachier I, Jorgensen C, et al. Roles of mesenchymal  
536 cells in the lung: From lung development to chronic obstructive pulmonary disease. *Cells.*  
537 2021;10(12):1–18.
- 538 43. Partti K, Vasankari T, Kanervisto M, Perälä J, Saarni SI, Jousilahti P, et al. Lung function and  
539 respiratory diseases in people with psychosis: Population-based study. *Br J Psychiatry.*  
540 2015;207(1):37–45.
- 541 44. Chaudhary SC, Nanda S, Tripathi A, Sawlani KK, Gupta KK, Himanshu D, et al. Prevalence  
542 of psychiatric comorbidities in chronic obstructive pulmonary disease patients. *Lung India.*  
543 2016;33(2):174–8.
- 544 45. Thorstensen WM, Øie MR, Dahlslett SB, Sue-Chu M, Steinsvåg SK, Helvik AS. Olfaction in  
545 COPD. *Rhinology.* 2022;60(1):47–55.
- 546 46. Foronjy R, Imai K, Shiomi T, Mercer B, Sklepkiwicz P, Thankachen J, et al. The divergent

- 547 roles of Secreted Frizzled Related Protein-1 (SFRP1) in lung morphogenesis and emphysema.  
548 Am J Pathol [Internet]. 2010;177(2):598–607. Available from:  
549 <http://dx.doi.org/10.2353/ajpath.2010.090803>
- 550 47. Foronjy RF, Majka SM. The potential for resident lung mesenchymal stem cells to promote  
551 functional tissue regeneration: Understanding microenvironmental cues. Cells. 2012;1(4):874–  
552 85.
- 553 48. Baarsma HA, Spanjer AIR, Haitzma G, Engelbertink LHJM, Meurs H, Jonker MR, et al.  
554 Activation of WNT/  $\beta$ -catenin signaling in pulmonary fibroblasts by TGF- $\beta$  1 is increased in  
555 chronic obstructive pulmonary disease. PLoS One. 2011;6(9).
- 556 49. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and  
557 hybridization array data repository. Nucleic Acids Res. 2002;30(1):207–10.
- 558
- 559

560 **Table 1.** Mapping CNON cells onto various reference datasets. Number of cells from CNON-CTRL  
 561 and CNON-SCZ mapped onto different cell types or clusters found in MT-CTRL (after cell-cycle  
 562 score regression), MT-SCZ (after cell-cycle score regression), CS14\_3, ON-Patient 2, and ON-  
 563 Integrated, and average predicted identity scores for each cell cluster mapping.

		Predicted Number of Mapped Cells		Average predicted ID score	
		CNON-CTRL	CNON-SCZ	CNON-CTRL	CNON-SCZ
MT-CTRL Cell-Cycle Regressed	MC	12234	3303	1	1
	Basal	0	35	0	0.035
MT-SCZ Cell-Cycle Regressed	MC	12234	3268	1	0.964
	MC	11425	2428	1	0.853
ON-Patient 2	Vascular Smooth Muscle	0	127	0	0.117
	MC	10901	1868	0.986	0.864
ON-Integrated	Vascular Smooth Muscle	78	207	0.014	0.136
	Cluster 0	1	6	0.005	0.015
CS14_3	Cluster 9	12233	3297	0.995	0.985

564

565 **Table 2.** Expression of marker genes of major respiratory epithelial cell types in CNON (CNON-  
 566 CTRL and CNON-SCZ), percentage of CNON cells and bulk CNON (average Transcripts per  
 567 Million transcripts from 255 CNON samples) expressing these markers.

		CNON-CTRL		CNON-SCZ		Bulk CNON
		Average Expression	Percentage of Cells Expressing Gene	Average Expression	Percentage of Cells Expressing Gene	TPM
Housekeeping	ACTB	2.456	100	2.456	100	1540.14
	GAPDH	1.697	100	3.4082	100	1153.02
Basal	SERPINB3	0.00375	0.0172	0	0	0.01
	KRT5	0	0	0.0005	0.303	0.21
Endothelial	CCL14	0	0	0	0	0.20
	VWF	0.0009	0.058	0.0031	0.182	0.09

<b>Serous</b>	<b>DMBT1</b>	0.0001	0.0007	0.006	0.394	0.04
<b>Club</b>	<b>LYPD2</b>	0	0	0	0	0.02
	<b>SCGB1A1</b>	0	0	0	0.003	0.00
<b>Ciliated</b>	<b>SNTN</b>	0.0005	0.0327	0.004	0.272	0.23
<b>Goblet</b>	<b>MUC5B</b>	0	0	0.001	0.0606	0.06
<b>Ionocytes</b>	<b>CFTR</b>	0	0	0.0005	0.0303	0.07

568

569 **Table 3.** Number and percentage of cells in G0, G1, S, G2/M and apoptotic clusters in CNON-CTRL  
570 and CNON-SCZ cell cultures.

	CNON-CTRL		CNON-SCZ	
	Number of Cells	Percentage of Cells	Number of Cells	Percentage of Cells
G0	10854	88.7	2553	77.3
G1	634	5.2	348	10.5
S	556	4.5	242	7.3
G2-M	143	1.2	106	3.2
Apoptotic	47	0.4	54	1.6

571

572 **Table 4.** Expression of mesenchymal markers in the MC cluster (MT-CTRL), CNON (CNON-  
573 CTRL) and cluster 9 of embryonic brain (CS14\_3, (19)) and bulk CNON (average Transcripts per  
574 Million transcripts from 255 CNON samples). According to the Mesenchymal and Tissue Stem Cell  
575 Committee of the International Society for Cellular Therapy (31), “MSC must express CD105, CD73  
576 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR  
577 surface molecules”. Other prominent markers of mesenchymal cells are also included in the table.

578

	Gene	Percentage of Cells Expressing gene in the MC cluster	Percentage of Cells Expressing gene in CNON-CTRL	Percentage of Cells Expressing gene in cluster 9, CS14_3	Bulk CNON, TPM
<b>Must be expressed</b>	<b>NT5E (CD73)</b>	7.20	68.8	32.62	299.07
	<b>THY1 (CD90)</b>	47.88	100	20.92	1347.66
	<b>ENG (CD105)</b>	10.59	77	25.89	16.52
<b>Must NOT be expressed</b>	<b>CD34</b>	0	1.64	1.06	1.12
	<b>PTPRC (CD45)</b>	2.54	0.43	0	0.59



	<b>HLA-DRA</b>	49.58	0.07	0.35	0.10
	<b>HLA-DRB1</b>	44.07	0.07	0.35	0.03
	<b>HLA-DRB5</b>	0	0	0	0.01
<b>One of them must NOT be expressed</b>	<b>CD14</b>	4.66	32.17	0	9.00
	<b>ITGAM (CD11b)</b>	0.42	0	0	0.06
<b>One of them must NOT be expressed</b>	<b>CD79A</b>	0.42	0.70	0	0.16
	<b>CD19</b>	0	0.02	0	0.04
<b>Other mesenchymal markers</b>	<b>CD44</b>	17.37	98.2	15.96	2296.28
	<b>VIM</b>	99.15	100	100	3869.50
	<b>ALCAM (CD166)</b>	19.49	90.3	29.08	555.48
	<b>HSPA8 (STRO-1)</b>	82.63	99.4	52.13	495.22
	<b>ANPEP (CD13)</b>	2.966	59.0	5.67	125.92
	<b>LUM</b>	36.86	11.4	12.77	87.47

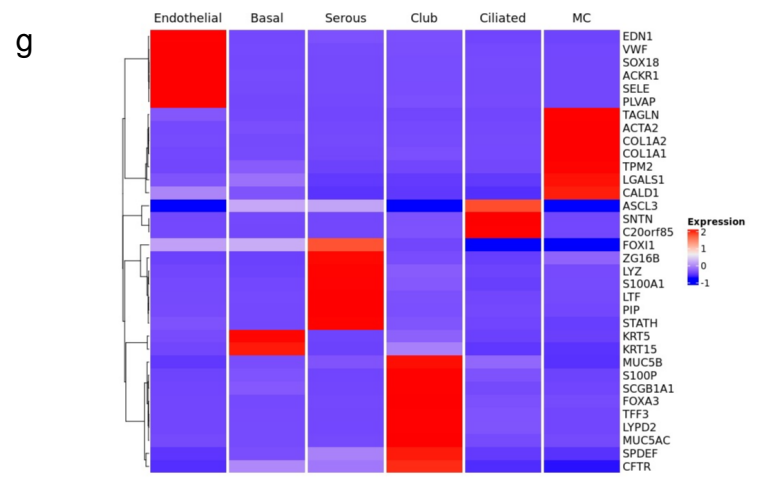
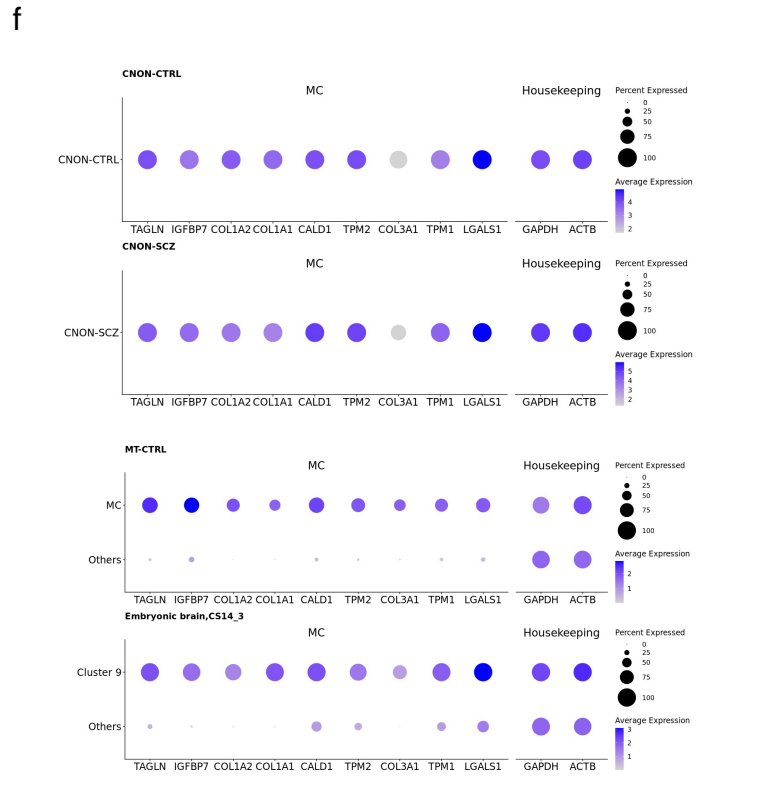
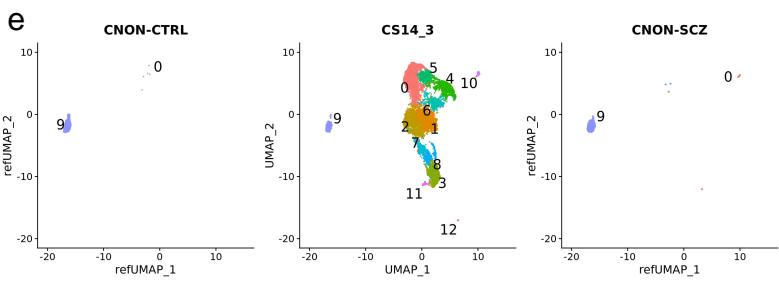
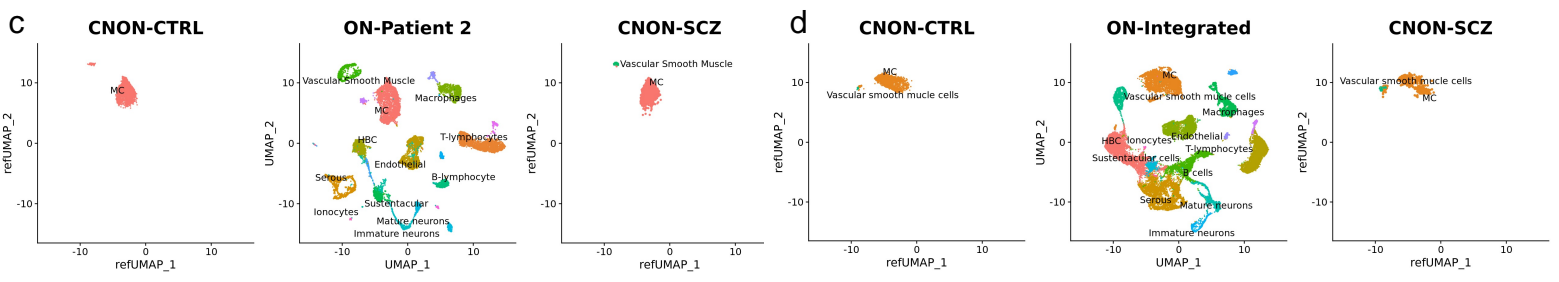
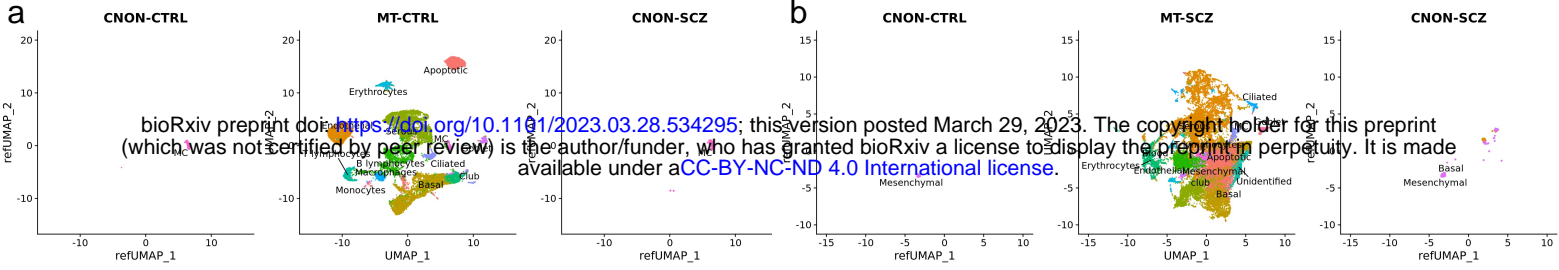
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580

581 **Table 5.** Expression of Wnt- and Notch- pathways genes in CNON (SEP036), the MC cluster of MT  
 582 (SEP310), cluster 9 (CS14\_3) of embryonic brain and bulk CNON (average Transcripts per Million  
 583 transcripts from 255 CNON samples).

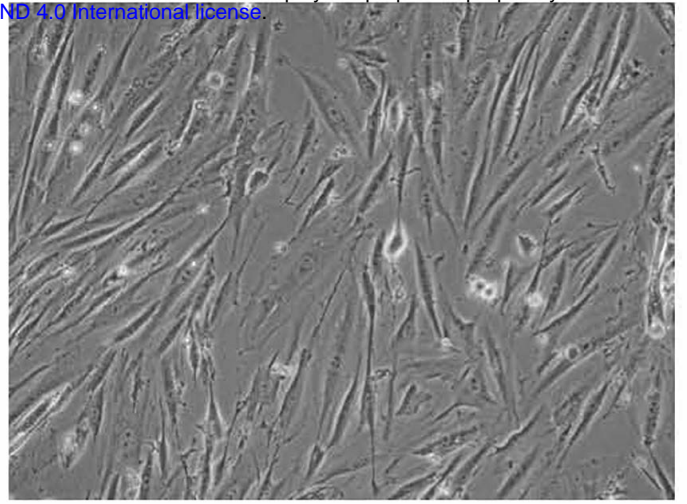
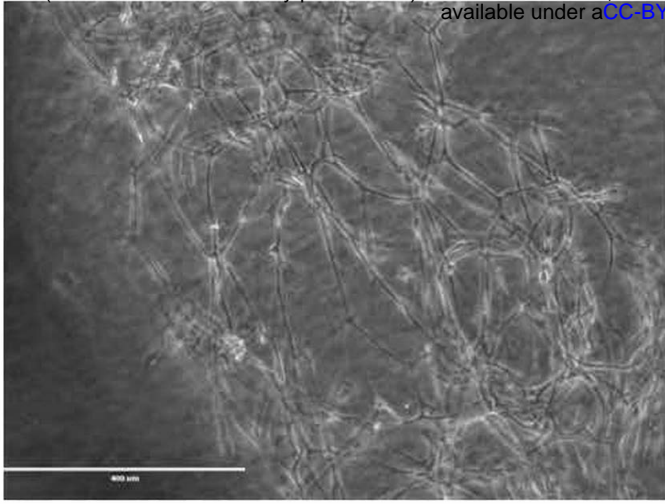
	<b>Gene</b>	<b>Percentage of Cells Expressing Gene in CNON-CTRL</b>	<b>Percentage of Cells Expressing Gene in the MC cluster of MT-CTRL</b>	<b>Percentage of Cells Expressing Gene in Cluster 9 (CS14_3)</b>	<b>Bulk CNON, TPM</b>
<b>Notch pathway</b>	NOTCH1	3.96	16.10	4.96	1.21
	NOTCH2	45.98	11.02	15.60	96.47
	NOTCH3	4.66	16.10	34.04	2.22
	JAG1	14.09	28.39	53.55	11.15
	PSEN1	19.13	5.08	3.9	85.09
	PSEN2	10.89	3.81	2.13	11.30

	PSENN	32.52	5.51	17.73	23.58
	APH1A	45.28	11.86	19.86	140.18
	ADAM17	27.96	5.51	4.26	48.05
	HES1	7.52	32.63	22.7	2.15
WNT pathway	WNT3	2.337	2.12	0.35	5.65
	WNT5A	39.99	21.61	0.35	185.16
	WNT5B	67.39	2.12	7.8	150.35
	WNT6	0	4.66	0	0.16
	ROR2	7.77	7.2	0	3.44
	LRP5	20.9	4.24	2.13	7.60
	LRP6	21.6	16.1	3.9	18.00
	AXIN1	7.34	2.12	2.48	9.21
	AXIN2	1.01	6.36	0	1.46
	FZD1	6.89	6.36	2.13	5.82
	FZD2	45.75	9.32	8.16	23.82
	FZD3	1.78	1.27	1.06	0.38
	FZD4	6.12	4.24	1.77	3.04
	FZD5	4.15	3.81	0	1.08
	FZD6	8.37	1.69	1.06	33.64
	FZD7	16.54	6.36	11.7	17.72
	FZD8	1.99	1.69	0.71	3.96
	CTNNB1	46.28	18.64	16.31	308.46
	SFRP1	93.86	44.49	1.42	241.89
	SFRP2	82.61	59.32	0.71	27.51

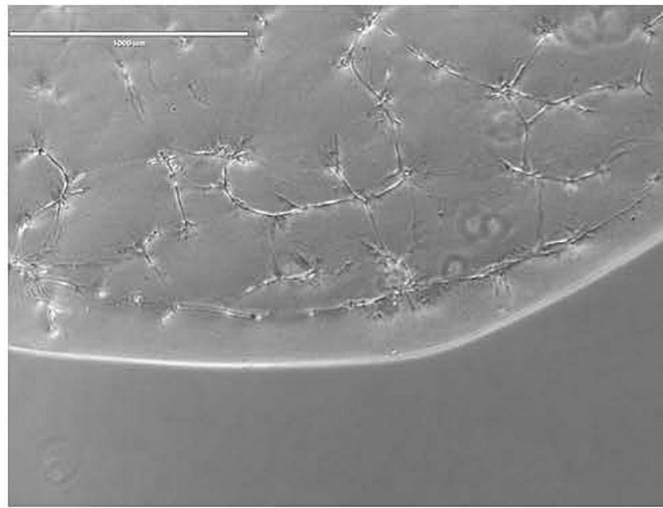


**Figure 1.** Single cell reference mapping and gene expression comparison of CNON datasets ((CNON-CTRL and CNON-SCZ) to human middle turbinate (MT-CTRL & MT-SCZ) and embryonic brain at Carnegie stage 14 (CS14\_3) datasets. (A) Central panel shows UMAP dimensionality reduction plot of 21,565 human middle turbinate cells (MT-CTRL) with 13 cell types annotated. Left panel: UMAP dimensionality reduction plot of 12,234 CNON cells (CNON-CTRL). Right panel: UMAP dimensionality reduction plot of 3303 CNON cells (CNON-SCZ). All cells from both CNON-CTRL and CNON-SCZ map to the MC cluster location in MT-CTRL (B) Central panel shows UMAP dimensionality reduction plot of 28,140 human middle turbinate cells (MT-CTRL) with 13 cell types annotated. Left panel: UMAP dimensionality reduction plot of 12,234 CNON cells (CNON-CTRL), all of them mapped to MC cluster. Right panel: UMAP dimensionality reduction plot of 3303 CNON cells (CNON-SCZ). 3,268 of them map to the MC cluster location in MT-CTRL, while 35 cells mapped to Basal cluster. (C) Single cell reference mapping of CNON datasets to olfactory neuroepithelium, Patient 2. Central panel shows UMAP dimensionality reduction plot of cells from olfactory neuroepithelium of Patient 2 (1) with 13 cell types annotated. Left panel: UMAP dimensionality reduction plot of 11,425 CNON cells (CNON-CTRL); all cells mapped to Mesenchymal cell type. Right panel: UMAP dimensionality reduction plot of 2547 CNON cells (CNON-SCZ). The majority of cells (2420 CNON-SCZ cells) map to Mesenchymal cell type and 127 CNON-SCZ cells to Vascular Smooth Muscle cell clusters. (D) Single cell reference mapping of CNON datasets to olfactory neuroepithelium (integration of 4 patient samples data). Central panel shows UMAP dimensionality reduction plot of cells from olfactory neuroepithelium, integrated data from 4 patients (1) with 13 cell types annotated. Left panel: UMAP dimensionality reduction plot of 10,979 CNON cells (CNON-CTRL); 10901 cells mapped to Mesenchymal cell type, while 78 cells mapped to Vascular Smooth Muscle cells cluster. Right panel: UMAP dimensionality reduction plot of 2075 CNON cells (CNON-SCZ). The majority of cells (1,868 CNON-SCZ cells) map to Mesenchymal cell type and 207 CNON-SCZ cells to Vascular Smooth Muscle cells clusters. (E) Single Cell Reference Mapping of CNON datasets to embryonic brain (CS14\_3). Central panel: UMAP dimensionality reduction plot of CS14\_3 with 13 clusters. Left panel: UMAP dimensionality reduction plot of 12,234 CNON cells (CNON-CTRL); 12,233 cells mapped to to Cluster 9, 1 cell maps to cluster 0. Right panel: UMAP dimensionality reduction plot of 3303 CNON cells (CNON-SCZ). 3297 cells map to cluster 9 and 6 cells map to cluster 0. (F) Average gene expression of selected cell markers in CNON. A gene considered MC marker gene if it is expressed in the MC cluster higher than in all other clusters with (a) statistical significance, (b)  $\log_2(\text{FoldChange}) > 2$ , and (c) expressed in at least 50% of cells of MC. MC markers: *TAGLN*, *COL1A2*, *COL1A1*, *CALD1*, *TPM2*, *COL3A1*, *TPM1*, and *LGALS1*; Housekeeping markers: *GAPDH* and *ACTB*; Neural stem cell marker: *ITGB1* are shown in CNON-CTRL, CNON-SCZ, MT-CTRL, and Embryonic brain (sample CS14\_3). The size of the dot represents the percentage of cells expressing the gene, and the colors represent the average expression level of each gene.

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**Figure 2.** Microscopic images of CNON cell (A) outgrowing from biopsy piece in Matrigel, (B) growing in 2D culture, and (C) growing in Matrigel after culturing in 2D for several passages.