

Decode organoid complexity using single cell analysis

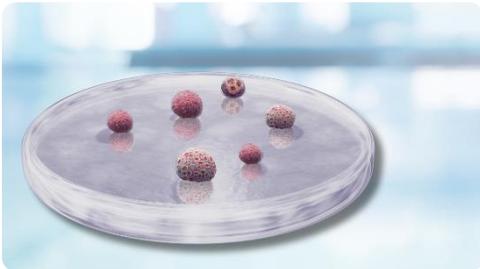


Figure 1: Organoids

Organoids are three-dimensional *in vitro* models that recapitulate many aspects of the complexity and functionality of their corresponding *in vivo* organs. Organoids are typically derived from pluripotent or tissue-resident stem cells and consist of diverse cell types with distinct functions (Zhao et al., 2022). They are a powerful tool in biomedical research allowing high-throughput screening, while ethical concerns that come along with *in vivo* studies can be omitted. A major advantage of human-derived organoids is that they allow a more accurate

modelling of human physiology and disease compared to animal models. Even disease- and patient-specific models can be achieved with organoids originating from patient-derived cells, enabling personalized medicine approaches and a better understanding of individual disease mechanisms.

Single cell sequencing provides a detailed map of the organoid inherent cellular diversity, revealing the unique gene expression signatures of each cell type within the organoid. This information enables a deeper understanding of molecular mechanisms, development, disease modeling, and therapeutic responses. Advanced bioinformatic analysis of single cell RNA sequencing (scRNAseq) data delineate differentiation paths, rank cells by pseudotime, allow clone tracing, uncover differentiation trajectory and fate potentials.

Application Areas for Single Cell Analysis of Organoids

Organoids have the potential to revolutionize biomedical research and can be applied across various fields:

Developmental Biology



- Detailed study of organ development and differentiation

Drug screening and Toxicology



- Testing of drug efficacy and toxicity
- Controlled, human-relevant environment

Regenerative Medicine



- Exploration of tissue regeneration
- Uncovering potential therapeutic applications for organ repair and transplantation

Personalized Medicine



- Development of patient-specific organoids
- Tailoring of treatments and prediction of individual responses to therapies

Model for a Spectrum of Diseases

A wide range of diseases can be accurately modelled and analyzed using single cell sequencing technologies in organoids.



Oncology: Tumor organoids from patient samples can replicate the complexity of cancer, allowing studies of tumor growth, metastasis, and drug responses.



Infectious Diseases: Study host-pathogen interactions, including infections by viruses like SARS-CoV-2 (COVID-19), bacteria, and parasites.



Neurodegenerative Diseases: Model conditions such as Alzheimer's, Parkinson's, and Huntington's diseases, providing insights into disease mechanisms and potential therapies.



Gastrointestinal Diseases: Gut organoids can be used to study conditions such as inflammatory bowel disease (IBD), Crohn's disease, and colorectal cancer.



Genetic Disorders: Model inherited diseases like cystic fibrosis or various congenital abnormalities, enabling research of underlying genetic causes and potential treatments.

End-to-End Solution: From Organoid to Publication-Ready Single Cell Data

For transcriptomic profiling of organoid-derived single cells, a full end-to-end workflow was developed based on the patented SCOPE-chip[®] technology. The initial step of the experiment is the dissociation of the organoid tissue. This step of immense importance for the entire experiment to be successful. The optimal conditions can vary immensely depending on the organoid type. While some, such as brain organoids, contain fragile cells and are recommended to be processed manually, others such as cardiac organoids might be dense or necrotic at the core, whereas digestive track organoids tend to agglomerate. This illustrates the importance of custom dissociation of your organoids.

Once a viable single cell suspension is obtained, single cells are partitioned into single microwells on the SCOPE-chip and their mRNA is labelled using barcoding beads. These magnetic beads are collected from the microwell chip and used for generation of a sequencing-ready library containing information of the whole transcriptome of the captured cells.

While all steps of the workflow can be carried out entirely instrument-free, platforms for automation can easily be implemented into the workflow to streamline the processing and increase sample throughput. For library generation the GEXSCOPE[®] Single Cell RNA Library Kit is recommended to generate a sequencing-ready NGS library that is compatible with Illumina sequencing instruments.

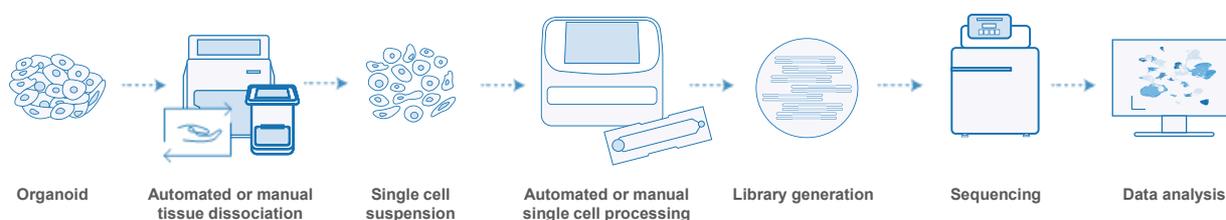


Figure 2: End-to-end workflow. Organoids require gentle yet efficient tissue dissociation into a viable single cell suspension prior to further processing. Recommendation for automated or manual processing largely depends on the organoid type. The single cell suspension is applied to the SCOPE-chip for partitioning single cells into hundreds of thousands of microwells on the chip. Barcoding beads, each coated with probes consisting of cell labels (barcodes), unique molecular identifiers (UMI), and a poly (T) probe, are added to the microwells of the chip. After cell lysis, barcoding beads capture mRNA upon binding their poly (A) tail. Barcoding beads are subsequently collected from the microwell chip and used for the subsequent library generation, sequencing, and data analysis.

Insider Knowledge Shared

Having processed nearly 300+ organoid samples in-house in our service laboratories, our staff has extensive experience with various types of organoids. Most frequent organoid types are listed below. Each of these tissue types presents its unique properties and challenges.

Hepatobiliary and pancreatic organoids	Umbilical cord mesenchymal stem cell culture
Stomach and intestinal digestive tract	Olfactory epithelial organoid
Brain organoids	Human embryonic stem cell-derived organoids (cell spheroids)
Heart/vascular organoids	Placental organoids
Kidney organoids	Adipose stem cell culture
Endometrial organoids	Organoids from mouse PDO model
Skin, melanoma	Spinal organoids
Thymoma organoids	Cartilaginous organoids
Retinal organoids	Synovial carcinoma organoids
Breast cancer organoids	Peritoneal cancer organoids

Conducting a successful single-cell RNA sequencing study using organoid tissue depends on several critical factors:

- 1) Define the optimal time point to harvest the organoid tissue:** During the growth and aging period of organoids, cells differentiate and specialize. Depending on the culture day or age of the organoid, you may encounter an increased number of necrotic cells in the organoid core. While overall cell numbers are usually not an issue, viability can become a challenge. One way to improve sample quality is to perform targeted dead cell removal. However, we recommend carefully evaluating the optimal time point for sample preparation to ensure the best outcomes.
- 2) Use mild tissue dissociation conditions:** We have optimized the buffer conditions of the sCelliVE tissue dissociation solution to achieve milder conditions and, thus, better performance. By using these milder conditions and following a custom dissociation protocol, high cellular viability rates can be maintained.
- 3) Reduce stickiness to avoid doublets:** Cells obtained from intestinal/colon organoids exhibit sticky properties, which may result in cell agglomeration making it difficult to achieve a single cell suspension. This can be mitigated by adding 0.4% BSA to the resuspension buffer, ultimately improving the overall performance of the experiment.

Singleron SCOPE-Chip Technology vs. Droplet-Based Methods

Singleron's core technology is based on microfluidic microwell chips that together with barcoding beads capture mRNA from tens of thousands of cells for processing. Performance of Singleron's SCOPE-chips was compared side-by-side with droplet-based methods using human kidney organoids.

A Sample: Human kidney organoids implanted subcutaneously to mice

Dissociation: Manual with sCellLiVE[®] tissue dissociation solution

Cell Viability: 91%

Method	Reads	Valid Reads	Uniquely Mapped Reads	Est. Cells	Fraction Reads in Cells	Mean Reads/Cell	Median UMI/Cell	Total Genes	Median Genes/Cell
Droplet	496 Mio	96.6%	92.7%	16,621	80.9%	29,854	6,205	56,694	2,300
Singleron SCOPE-chip	413 Mio	91.9%	91.9%	13,081	81.1%	29,087	7,662	59,151	2,687

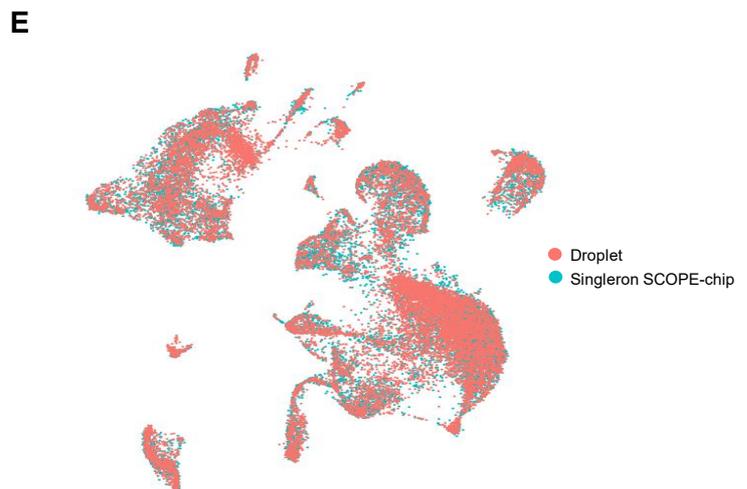
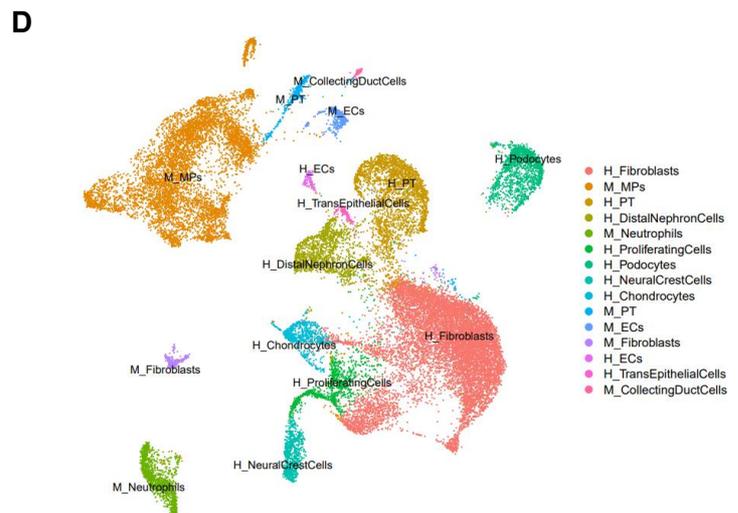
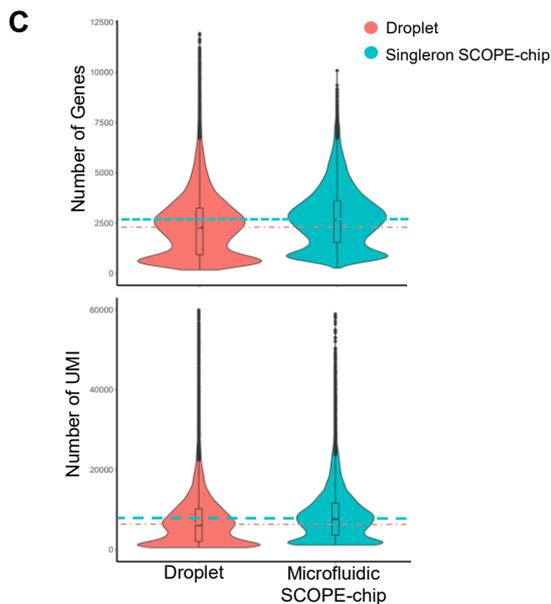
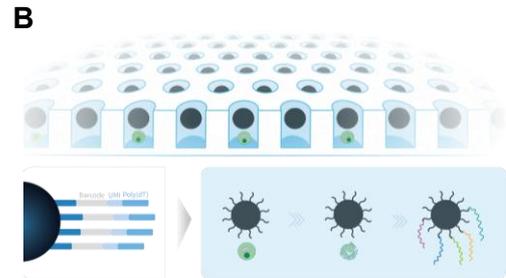


Figure 3: Human Kidney Organoid Demo Data. (A) Human kidney organoids were implanted subcutaneously to mice, grown and harvested. Tissue was dissociated manually with sCellLiVE[®] tissue dissociation solution achieving 91% cell viability. Single cell suspensions were processed in parallel on a droplet-based platform and (B) Singleron microfluidic SCOPE-chip using the GEXSCOPE[®] Single Cell RNAseq Library Kit. (C) Comparison of numbers of unique molecular identifiers (UMI) and genes between the two methods. (D) UMAP plot of cellular composition of organoid. M: Mus musculus; H: Homo sapiens (E) Comparison of cellular composition using the two different single cell technologies. UMAP plots largely overlap indicating a comparable cell type cover

Customer Publication: Parkinson's Disease

Organoid-Based Single Cell Research of Parkinson's Disease-Related Miro1 Mutation

Parkinson's disease (PD) is the fastest growing neurodegenerative disorder globally (Ou Z. et al., 2021), with most cases being sporadic and 5-15% being familial due to rare, high-penetrance mutations in single genes such as *SNCA* and *LRRK2* (Kim C. et al., 2017). Studies on these rare forms have provided significant insights into cellular mechanisms like mitochondrial dysfunction and protein misfolding. In sporadic PD, the contribution of low-frequency genetic variants is increasingly recognized. Studies identified PD patients with heterozygous mutations in the *RHOT1* gene encoding Miro1, a protein crucial for mitochondrial dynamics and calcium homeostasis, which interacts with PD-related proteins like PINK1 and α -synuclein (Berenguer-Escuder C. et al., 2019; Grossmann D. et al., 2019). Chemla A. et al. (2023), a research group from University of Luxembourg, used iPSC-derived dopaminergic neurons and 3D midbrain organoids to demonstrate that the p.R272Q Miro1 mutation increases reactive oxygen species, alters mitochondrial bioenergetics, raises α -synuclein levels, and leads to dopaminergic neuron loss. These findings suggest that mutant Miro1 is sufficient to accurately model PD *in vitro* and *in vivo*, highlighting its role in PD pathogenesis.

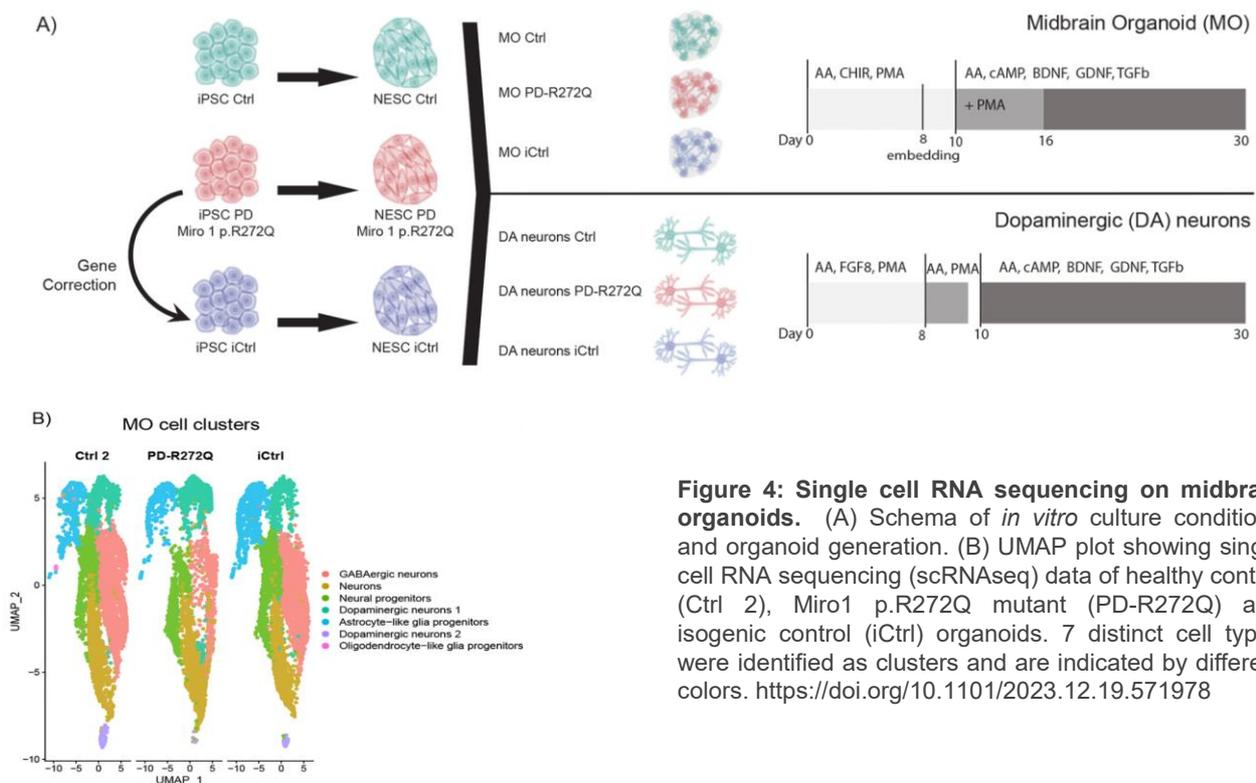


Figure 4: Single cell RNA sequencing on midbrain organoids. (A) Schema of *in vitro* culture conditions and organoid generation. (B) UMAP plot showing single cell RNA sequencing (scRNAseq) data of healthy control (Ctrl 2), Miro1 p.R272Q mutant (PD-R272Q) and isogenic control (iCtrl) organoids. 7 distinct cell types were identified as clusters and are indicated by different colors. <https://doi.org/10.1101/2023.12.19.571978>

The Miro1 p.R272Q mutation in midbrain organoids and dopaminergic neurons revealed transcriptomic deregulation of major PD-related pathways. Using iPSC-derived *in vitro* models, including midbrain organoids and 2D dopaminergic neurons from a PD patient with the *RHOT1* c.815G>A mutation (Miro1 p.R272Q), the authors observed significant transcriptomic alterations. Single-cell RNA sequencing identified seven cellular clusters in the organoids, including dopaminergic neuron clusters. Hierarchical clustering and pathway enrichment analysis highlighted significant deregulation in processes related to neurogenesis, synaptic contacts, exocytosis, ER and mitochondrial apoptosis, ROS, *HIF-1* transcription, *LRRK2* in PD neurons, and axonal transport. Furthermore, scRNAseq analysis revealed the deregulation of genes associated with apoptosis among the dopaminergic neuron clusters.

Customer Publication: Neurodegenerative Disorders

Single Cell RNAseq of midbrain organoids empowers discovery of potential new therapeutic agent for POLG-associated neurodegenerative disorders

A recent study published by the Norwegian group of Dr. Kristina Xiao Liang replicates characteristic features of POLG-related neurodegenerative disorders through employing a 3D human midbrain organoid (hMO) model, developed from induced pluripotent stem cells (iPSCs) of patients with POLG mutations (Chen A. *et al.*, 2023). The authors used single cell RNA sequencing to delineate cell type-specific transcriptomic changes between control organoids and hMOs. Analysis of the scRNAseq data revealed three DA neuronal sub-populations: DA2 neurons and ventral midbrain neurons.

Next the Norwegian group investigated the transcriptional effects of treatment with Nicotinamide Riboside (NR), an NAD⁺ precursor at single cell level. While the NR-treated organoids maintained presence of most cell populations, the frequency of DA2 neurons and ventral midbrain neurons was increased. Notably the authors found multifaceted effects on both, neuronal functions but also mitochondrial functions, which is known to play a key role for POLG-related neurodegenerative disorders. Thus, this study provides evidence for potential new therapeutic approaches using single cell RNA sequencing of organoid model systems.

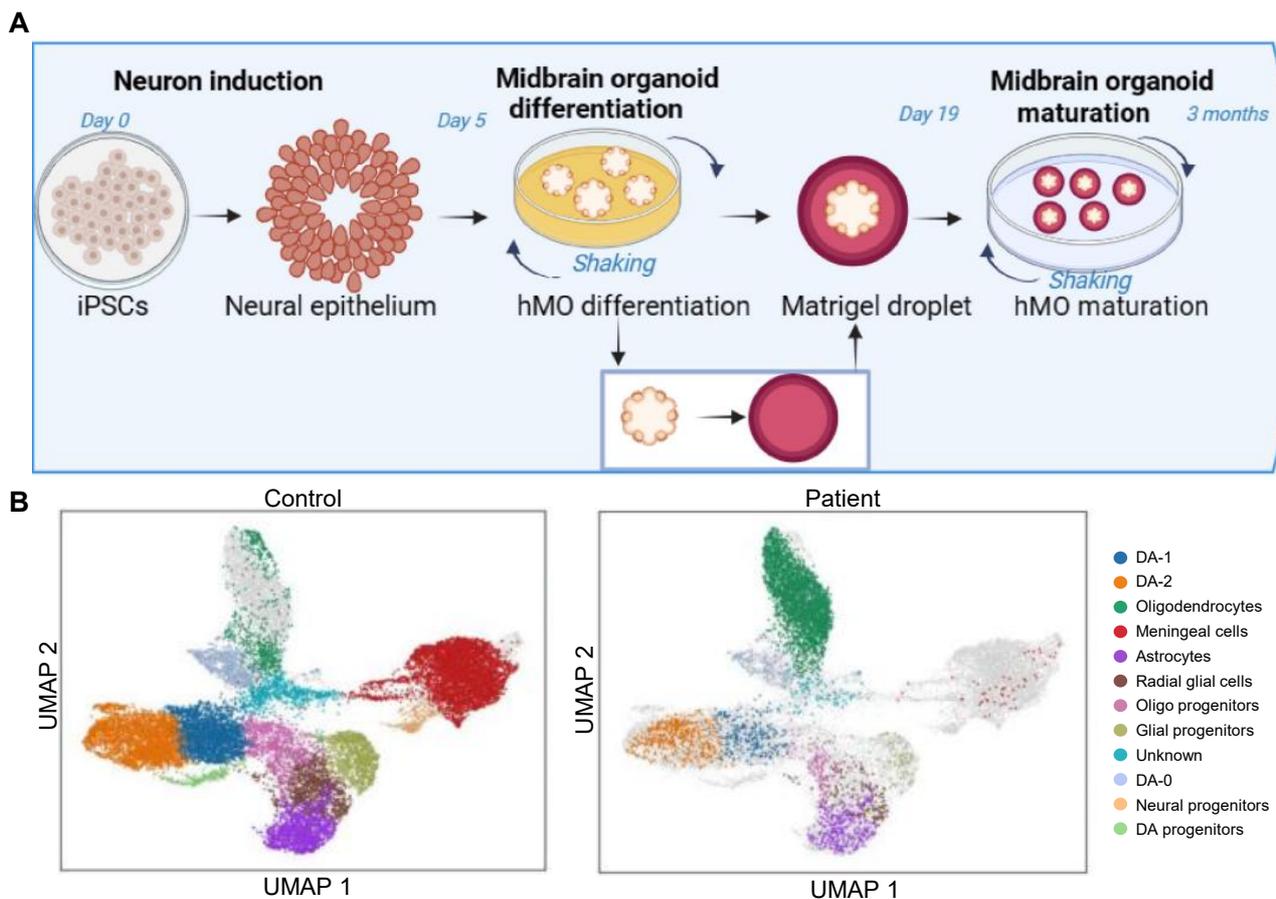
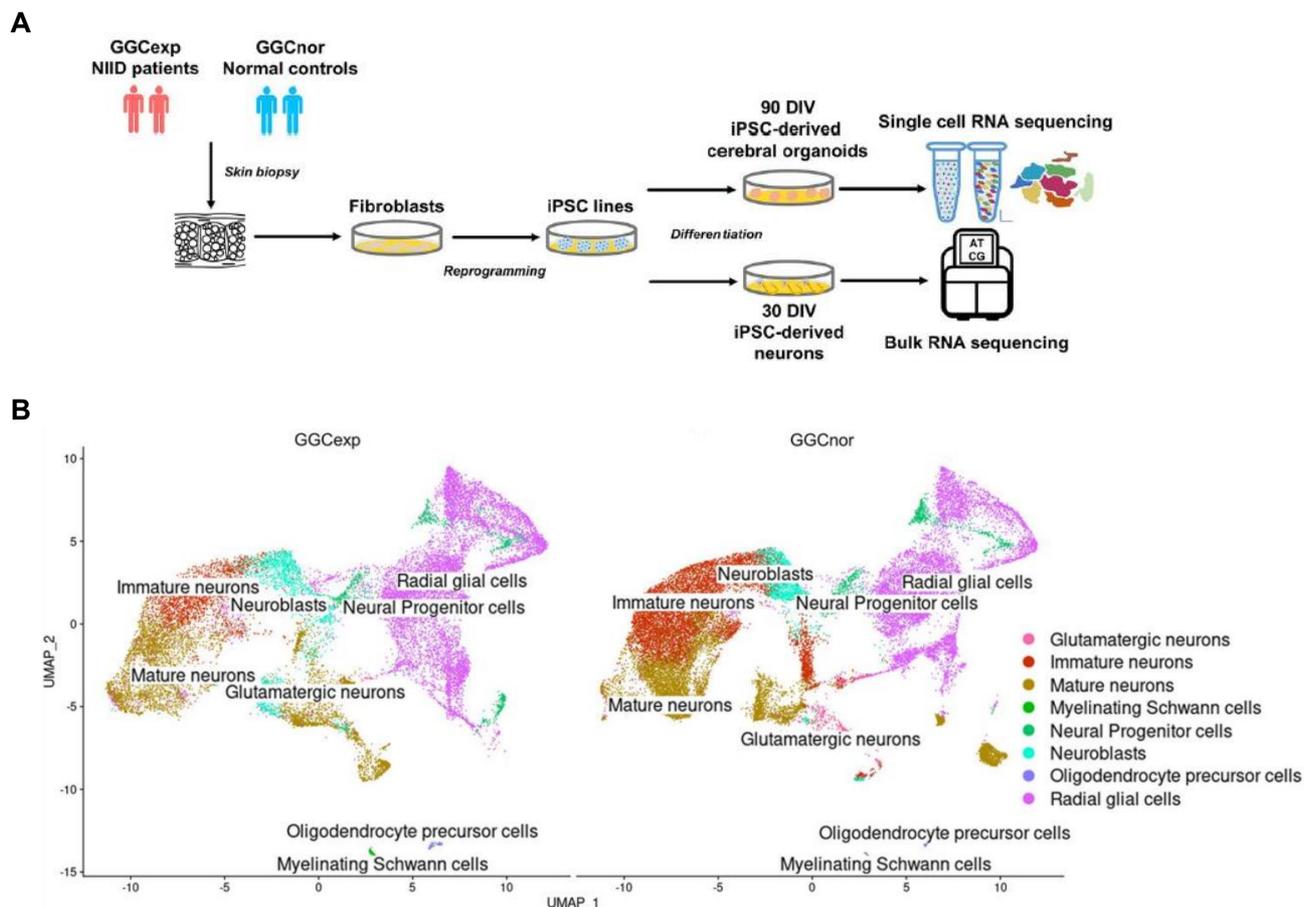


Figure 5: Single Cell Transcriptomics identify key differences in cell type representation and pave the way for novel therapeutic approaches. (A) Schema of *in vitro* culture conditions and organoid generation. (B) UMAP plot showing single cell RNA sequencing (scRNAseq) data of healthy control and patient iPSC-derived midbrain organoid. 12 distinct cell types were identified as clusters and are indicated by different colors. <https://doi.org/10.1101/2023.09.27.559684>

Customer Publication: Neurological Disorders

scRNA-seq reveals decreased frequency of immature neurons during development of organoids mimicking neuronal intranuclear inclusion disease

The NOTCH2NL families play a crucial role in brain evolution through regulating the NOTCH signaling pathway. GGC-repeats in the 5'-untranslated region of NOTCH2NLC have been associated with a wide range of neurological disorders, particularly neuronal intranuclear inclusion disease (NIID). However, their mechanism of neurotoxicity remains elusive. Fan Y, Li M, Yang J et al. employed single cell sequencing of NIID patient-specific induced pluripotent stem cell (iPSC)-derived 3D cerebral organoids (3DCOs) and cellular models to unravel the pathophysiological mechanisms of NOTCH2NLC GGC repeat expansion (Fan Y, et al., 2023). Their results demonstrate a lower frequency of immature neurons and a higher proportion of radial glial cells in the GGCexp samples compared to normal controls.



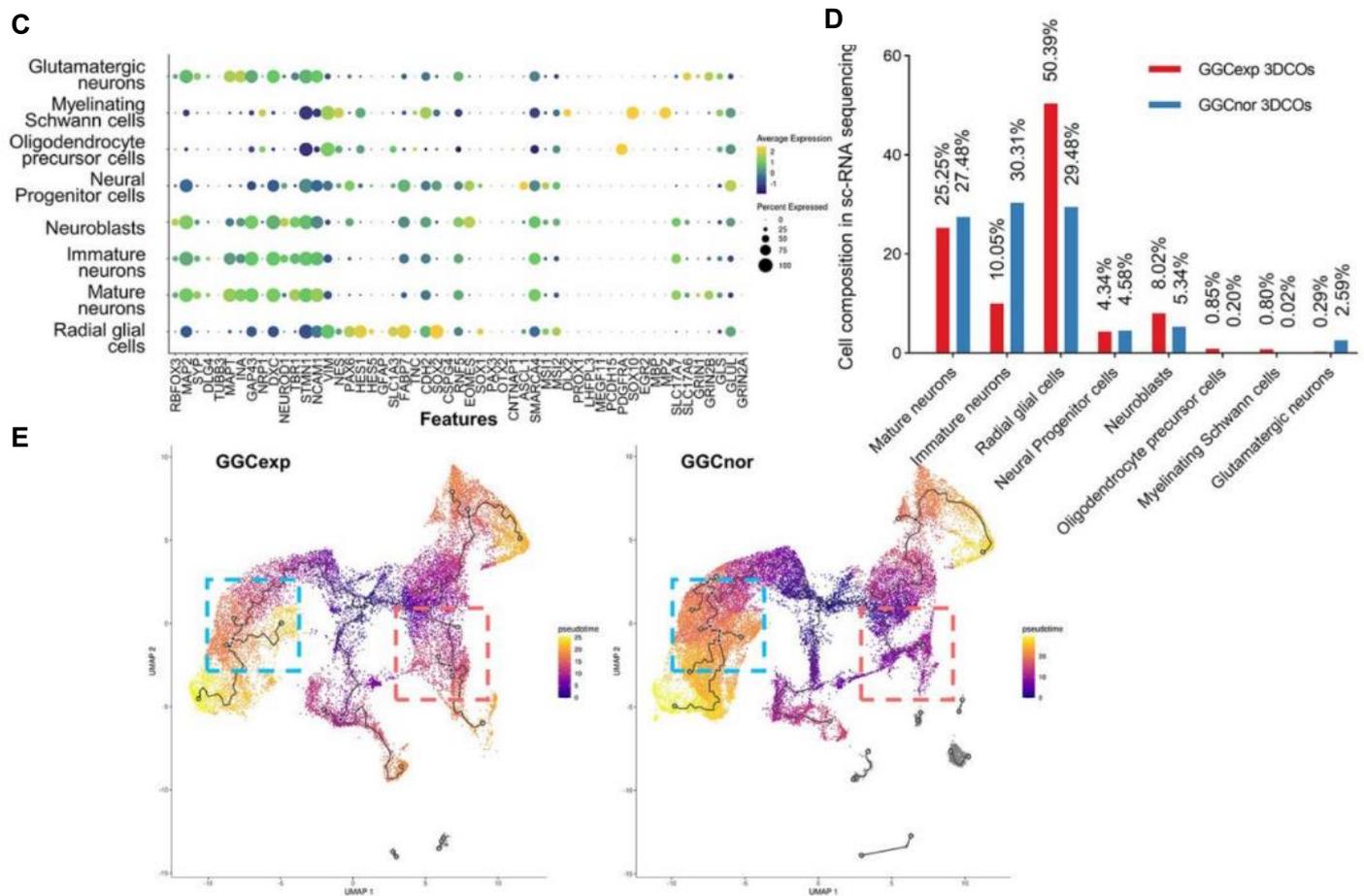


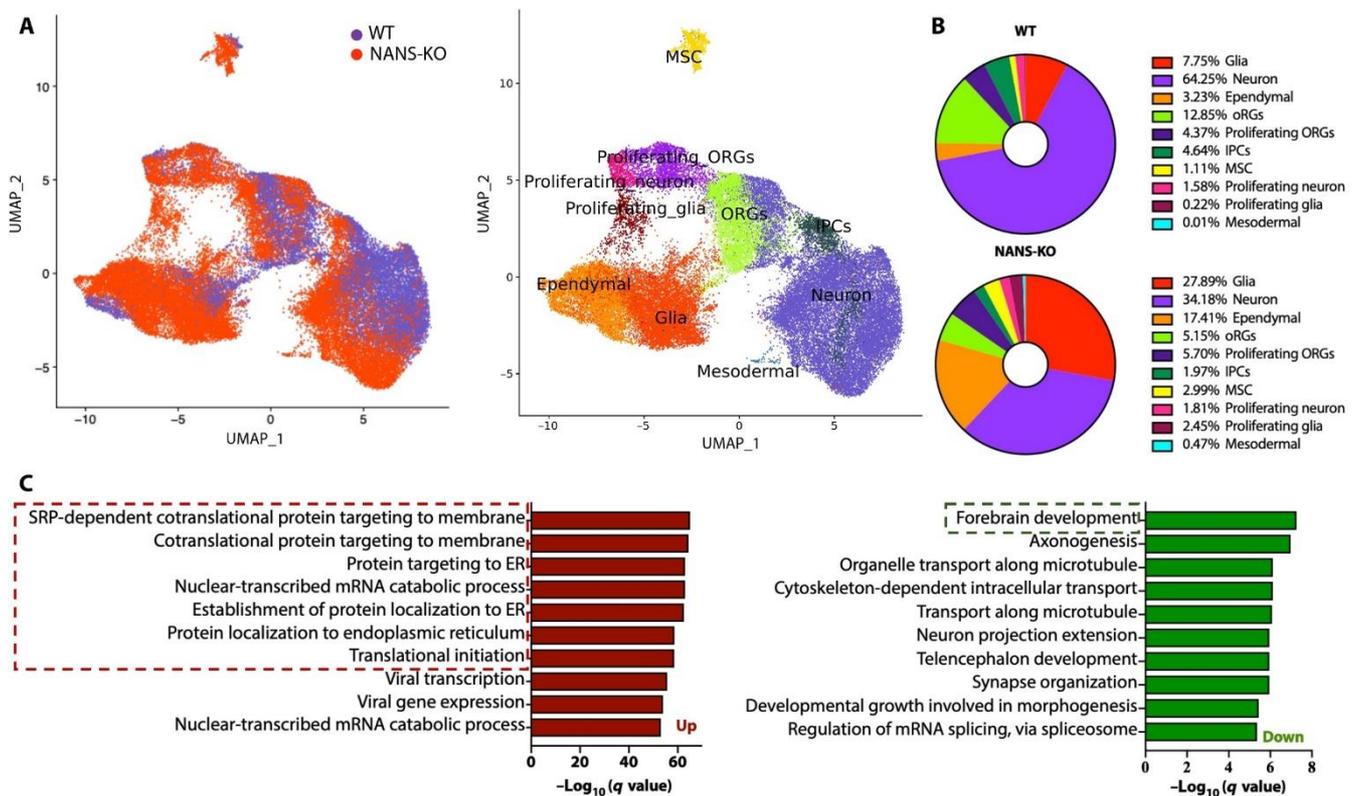
Figure 7 (cont.): Single-cell RNA sequencing of organoids derived from NIID patients with GGC-repeats unveils an increase of immature neurons. (C) Gene expression levels of marker genes used for cell type identification. (D) Overview of cellular compositions for both GGCexp 3D cortical organoids and GGCnor 3D cortical organoids. (E) Pseudotime trajectory analysis of GGCexp and GGCnor predicts the temporal sequences of cell specification throughout the organoid development.

Cell types were identified and annotated based on expression levels of marker genes (Figure 7C). While in the GGCnor sample the most frequent cell types included mature neurons, immature neurons, and radial glial cells, a skewing towards increased frequency of radial glial cells at the expense of immature neurons was detected in the GGCexp sample (Figure 7D). Pseudotime trajectory analysis was performed on the scRNA-seq data to predict the temporal sequences of cell specification. The analysis revealed that both GGCexp and GGCnor 3DCOs transitioned through neuroblasts (NBs) and immature motor neurons (iMNs) towards mature motor neurons (MNs) and other cell types, or directly towards radial glial cells (RGCs), as depicted by branching patterns. Upon comparing the reconstructed developmental paths of the 3DCOs, fewer leaves and shorter trajectories were observed for iMNs (blue box), but more complex trajectories for RGCs (red box) in GGCexp 3DCOs. These findings align with the reduced proportion of iMNs and increased proportion of RGCs in GGCexp 3DCOs, indicating that NOTCH2NLC GGC repeats may influence 3DCO development.

Customer Publication: Neurodevelopmental Disorders

Single cell transcriptomics enable discovery of neurodevelopmental defects in human cortical organoids with N-acetylneuraminic acid synthase mutation

Biallelic genetic variants of N-acetylneuraminic acid synthase (NANS) can lead to neurodevelopmental disorders. In a study using single-cell RNA sequencing, researchers found that these mutations cause a significant lack of sialic acid and protein polysialylation in brain organoids (Bu Q. et al., 2023). Single-cell RNA-seq was used to analyze 47,080 cells from WT and NANS-KO cerebral organoids, revealing clear differences between WT and NANS-KO populations in UMAP plots (Fig. 4A). In WT organoids, 64.25% of cells expressed neuronal markers and 7.75% expressed glial markers. In contrast, NANS-KO organoids had 34.18% neuronal and 27.89% glial markers (Fig. 4B). GO analysis showed that downregulated genes in NANS-KO organoids were linked to forebrain development and synaptic organization, while upregulated genes were involved in protein translation and ribosomal function (Fig. 4, C and D). Overall, NANS-KO mutations disrupted neuronal differentiation and ribosomal biogenesis in cerebral organoids.



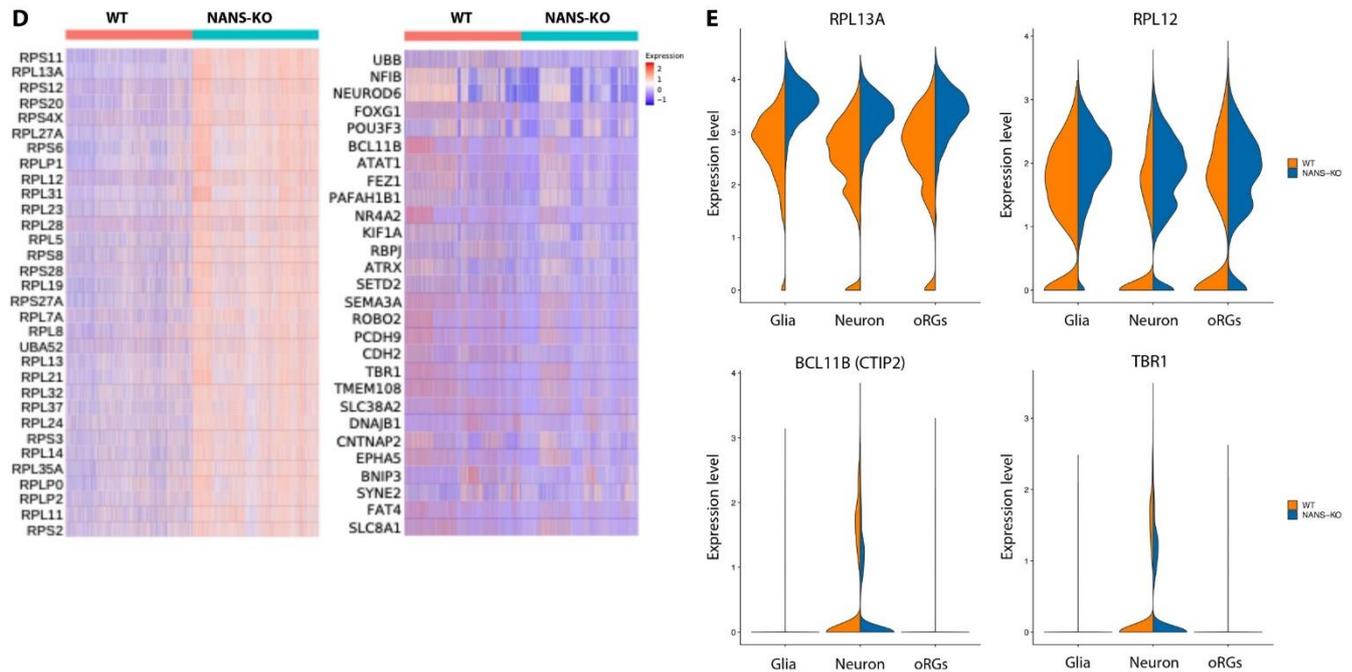


Figure 6: Single-cell RNA sequencing reveals abnormal neuron development and ribosomal protein gene expression in NANS-KO brain organoids. (A) UMAP plots illustrates cell type-specific differences between NANS-KO and wild-type (WT) organoids, identifying ten clusters by marker expression. (B) Pie charts indicate cell type composition between WT and NANS-KO organoids, specifying the percentage of each cell type. (C) Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) compares the top 10 upregulated and downregulated GO terms between WT and NANS-KO organoids. (D) Heatmap shows normalized gene expression levels for ribosomal proteins and forebrain development markers. (E) Violin plot illustrates the expression of RPL13A, RPL12, BCL11B (CTIP2), and TBR1 in glial cells, neurons, and outer radial glia (oRGs) populations between WT and NANS-KO organoids.

Conclusions

- Organoids are state-of-the-art 3D *in vitro* models that allow to mimic the complexity of and functionality of their corresponding *in vivo* organs.
- Single cell sequencing of organoids allows to investigate a range of diseases in depths to
 - reveal changes upon administration of drugs
 - discover new potential therapeutic targets
 - validate effects of drug treatment on different cell types
 - provide insights into altered pathways

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Customer Stories

“We have used the single cell and single nuclei sequencing from Singleron in several projects, all focused on human brain organoids. Working with Singleron was a great experience. I am very pleased with the quality and speed of their analysis”

- Prof. Dr. Jens Schwamborn, University of Luxembourg (LCSB)



“We performed single cell RNAseq on brain organoid using Singleron's service, and were able to discover the cell types of interests. I did not imagine that conducting single cell experiments could be made so easy, simply ship my fresh tissue in their buffer, then get fully analyzed results. Thanks to Singleron team for the continuous support.”

- Kristina Xiao Liang, DDS, PhD; University of Bergen, Norway