## 3D co-culture platform to study myelination deficits in schizophrenia using hiPSC-derived neurons and oligodendrocyte lineage cells

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Schizophrenia is a neurodevelopmental disorder that affects about 1% of the population globally. Recent brain imaging studies have shown decreased myelin integrity in white matter tracts of schizophrenia patients. However, the molecular pathways behind the hypomyelination pathology are still unknown.

To unravel the mechanisms of hypomyelination in schizophrenia, we focus on neurons and oligodendrocyte lineage cells. Therefore, we differentiated human induced pluripotent stem cells (hiPSCs) derived from six healthy controls and six schizophrenia patients into neurons and oligodendrocyte lineage cells on a 3D nanofiber network and performed differential gene expression analysis.

To further study the interaction of neurons and oligodendrocytes, in particular the process of myelination, we established a controlled direct co-culture system of hiPSC-derived neurons and oligodendrocyte lineage cells in a 3D micro-environment. This co-culture system provides an *in vitro* assay to study myelination biology in health and disease on a microscopic and ultrastructural level.

Cellevat3d<sup>™</sup> NanoMatrice<sup>™</sup> – 24-well plate insert

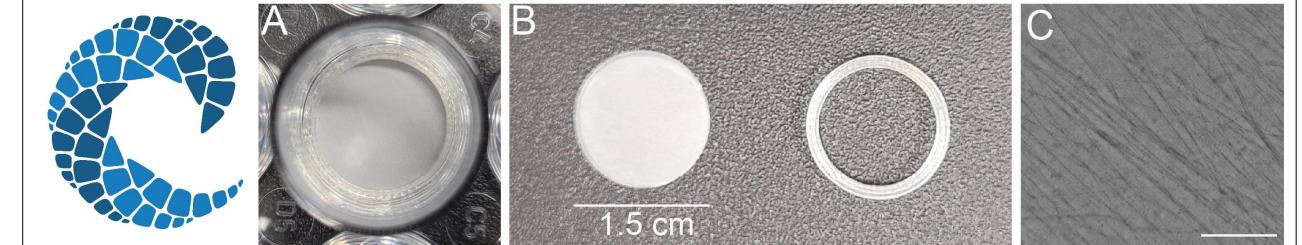
Establishment of a direct co-culture system with hiPSC-derived neurons

BioMed X

**Cellevate** 

Boehringer Ingelheim

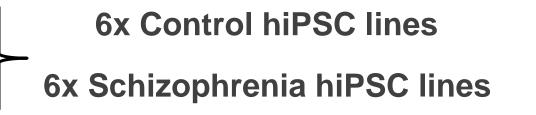
McLean

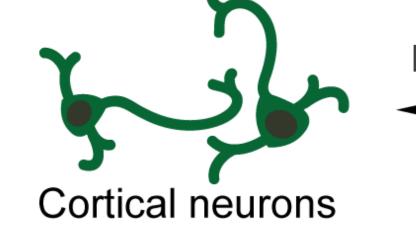


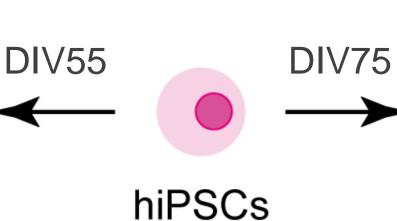
(A) Image of one well of a 24-well plate (Cellevat3d<sup>™</sup> NanoMatrice<sup>™</sup>) showing the insert containing the 3D nanofiber network on the bottom of the well and the ring on top protecting the network.(B) Image of the 24-well plate insert containing the 3D nanofiber network, diameter: 1.5 cm (left) and the ring (right) outside of the well. (C) Image of nanofibers in brightfield channel. Scale bar: 100 µm.

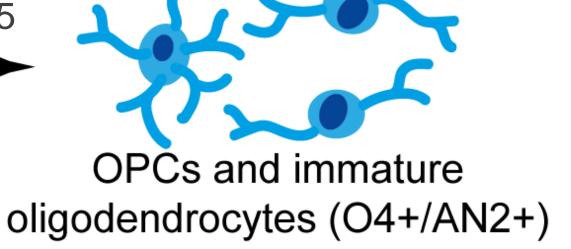
Target identification – RNA Sequencing of hiPSC-derived cortical neurons and oligodendrocyte lineage cells

hiPSCs derived from Schizophrenia patients showing decreased white matter volume in MRI scan





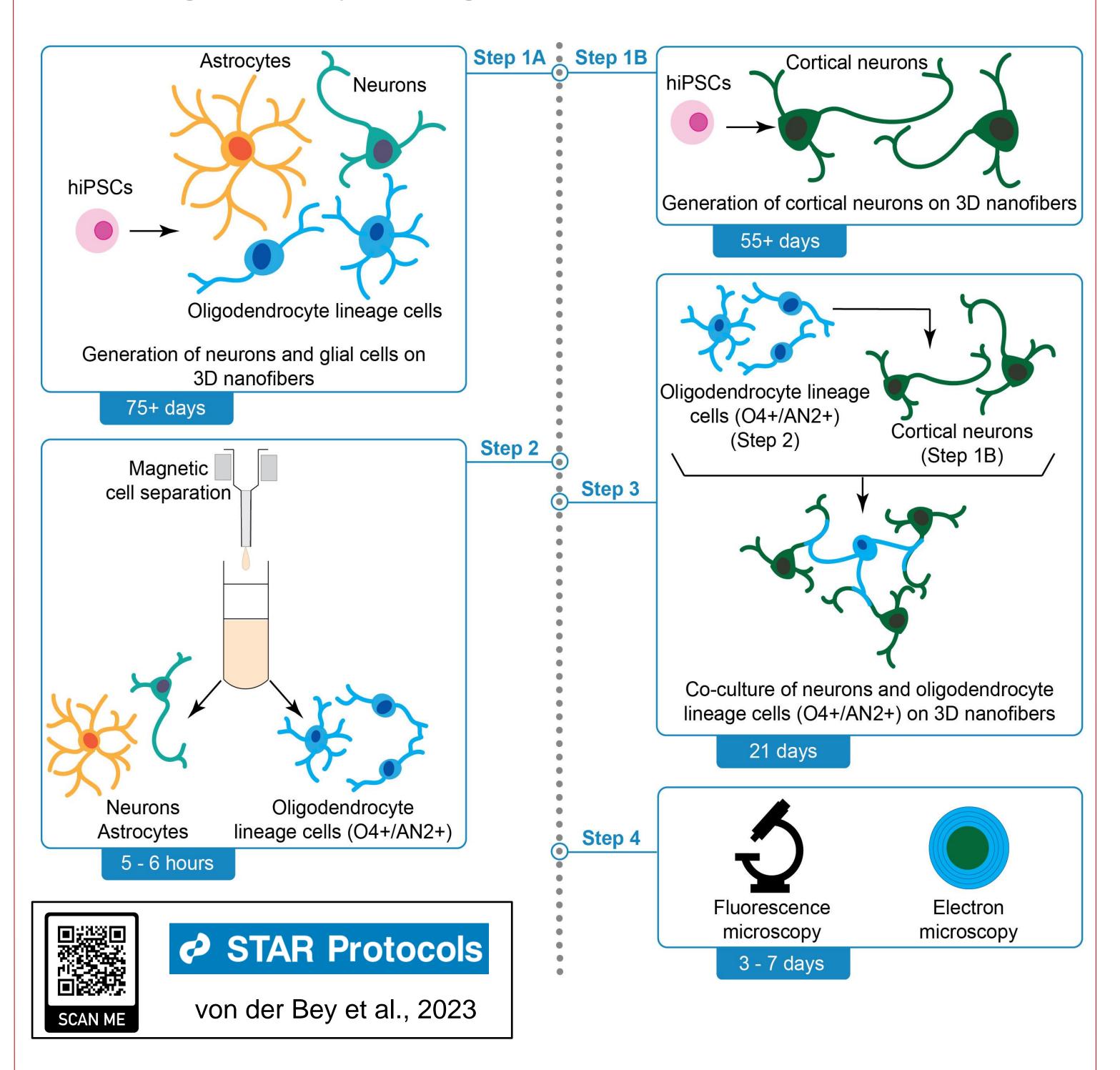


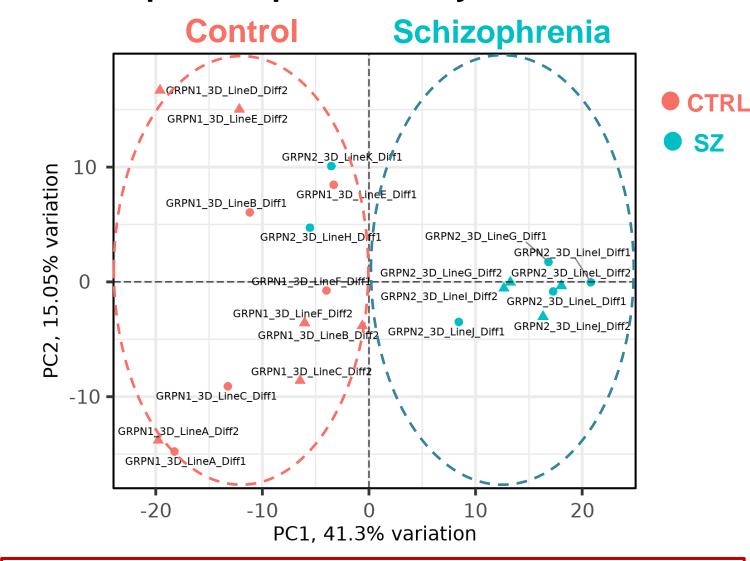


**Principal Component Analysis – Neurons** 

**Principal Component Analysis** 

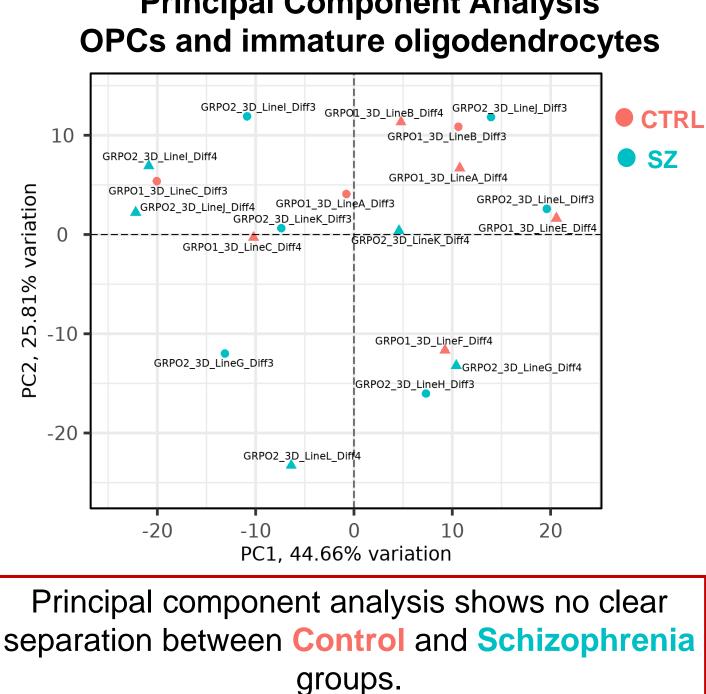
and oligodendrocyte lineage cells on Cellevat3d<sup>™</sup> NanoMatrice<sup>™</sup>



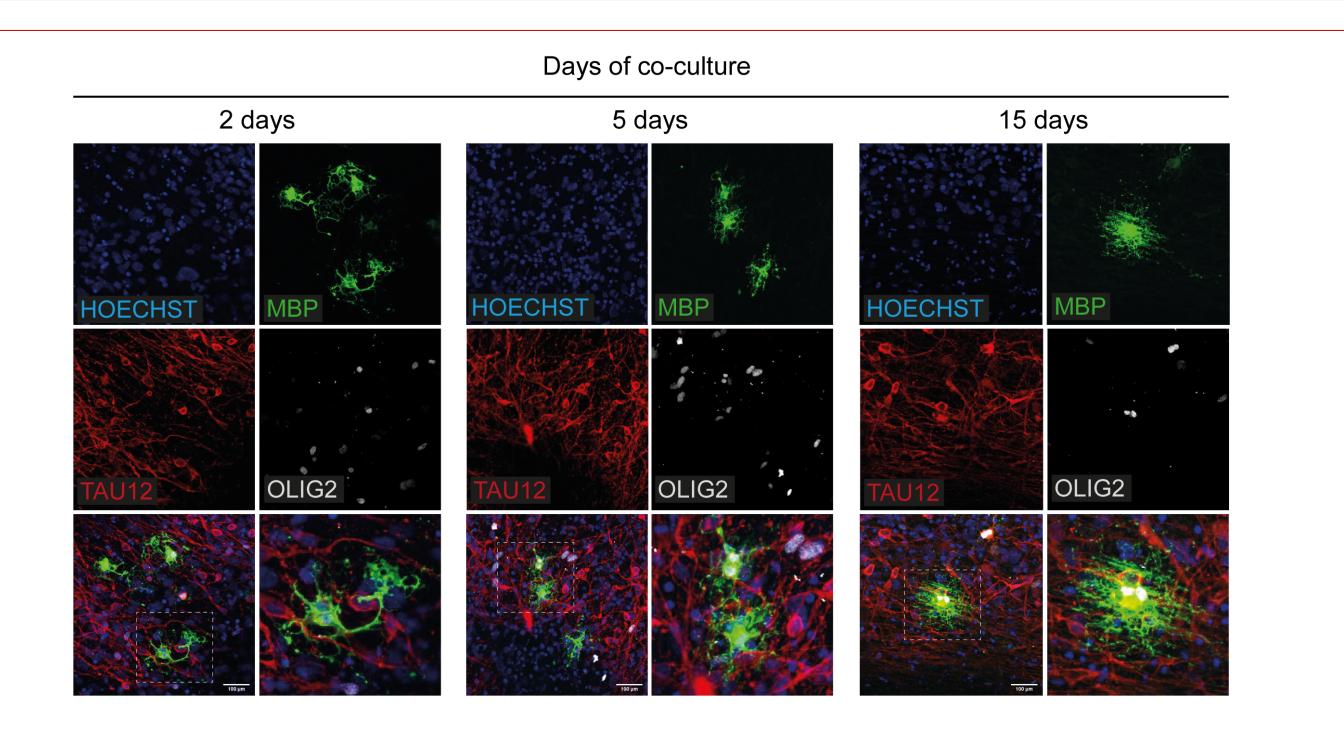


Principal component analysis shows the separation of **Control** and **Schizophrenia** groups.

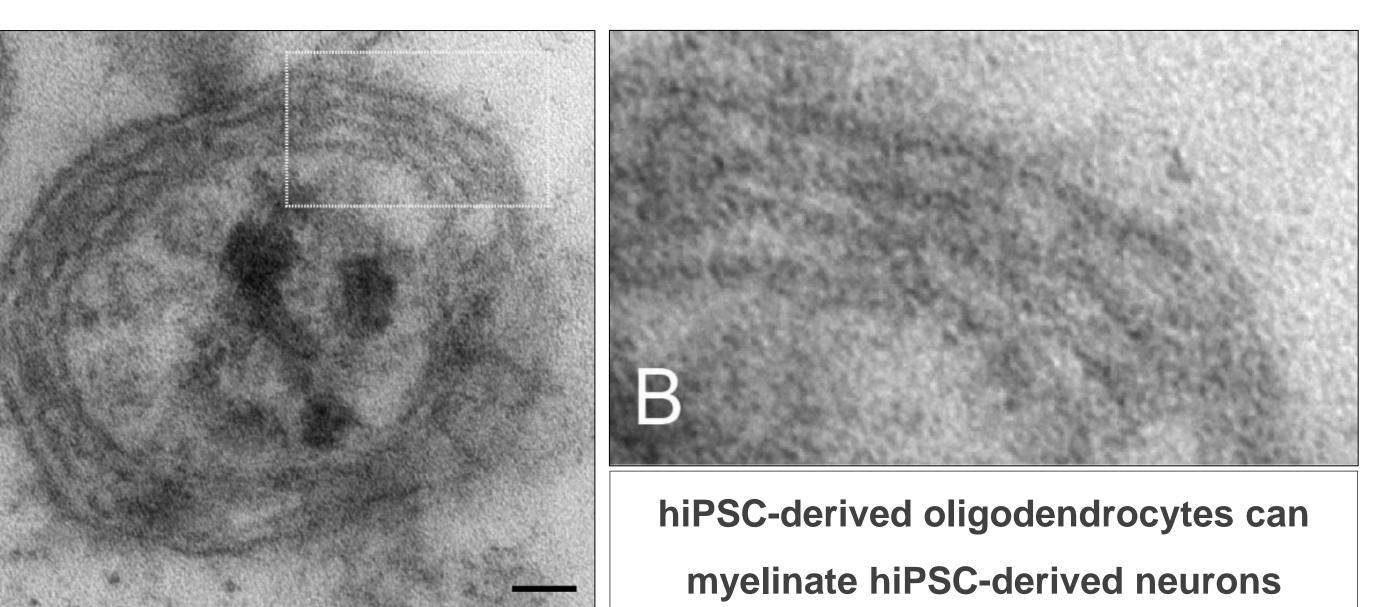
The differential gene expression analysis comparing SZ vs CTRL groups led to 183 increased and 394 decreased transcripts of genes (|log2 fold-change| > 1, FDR < 5%)



The differential gene expression analysis comparing SZ vs CTRL groups led to 35 increased and 12 decreased transcripts of genes (|log2 fold-change| > 1, FDR < 5%)



**Transmission Electron Microscopy (TEM) – 21 days of co-culture:** 



Transmission electron microscopy of hiPSC-derived neurons (DIV108) and oligodendrocytes (DIV96) cocultured for 21 days on 3D nanofibers: (A) Representative image of a cross section of a myelinated axon. (B) Zoom in of the white dashed rectangle showing four myelin sheath wraps. Image acquisition with JEOL JEM-1400 electron microscope. Scalebar: 100 nm.

Co-culture of hiPSC-derived cortical neurons (DIV89 – 102) and oligodendrocytes (DIV77 – 90): Representative immunofluorescence images of hiPSC-derived neurons (TAU12+) and oligodendrocytes (MBP+, OLIG2+) after 2, 5 and 15 days of co-culture on 3D nanofibers. Nuclei were stained with Hoechst 33342. Image acquisition with Nikon A1R confocal microscope. MBP: Myelin basic protein, TAU12: Microtubule-associated protein Tau, OLIG2: Oligodendrocyte transcription factor 2. Scale bar: 100 µm.

Our 3D co-culture system provides an *in vitro* assay to

study myelination biology in health and disease.

**Outlook:** Using 3D culture system of hiPSC-derived neurons and

oligodendrocyte lineage cells for the validation of candidate targets

**References:** von der Bey et al., 2023

