Supplementary Material

Supplementary figures

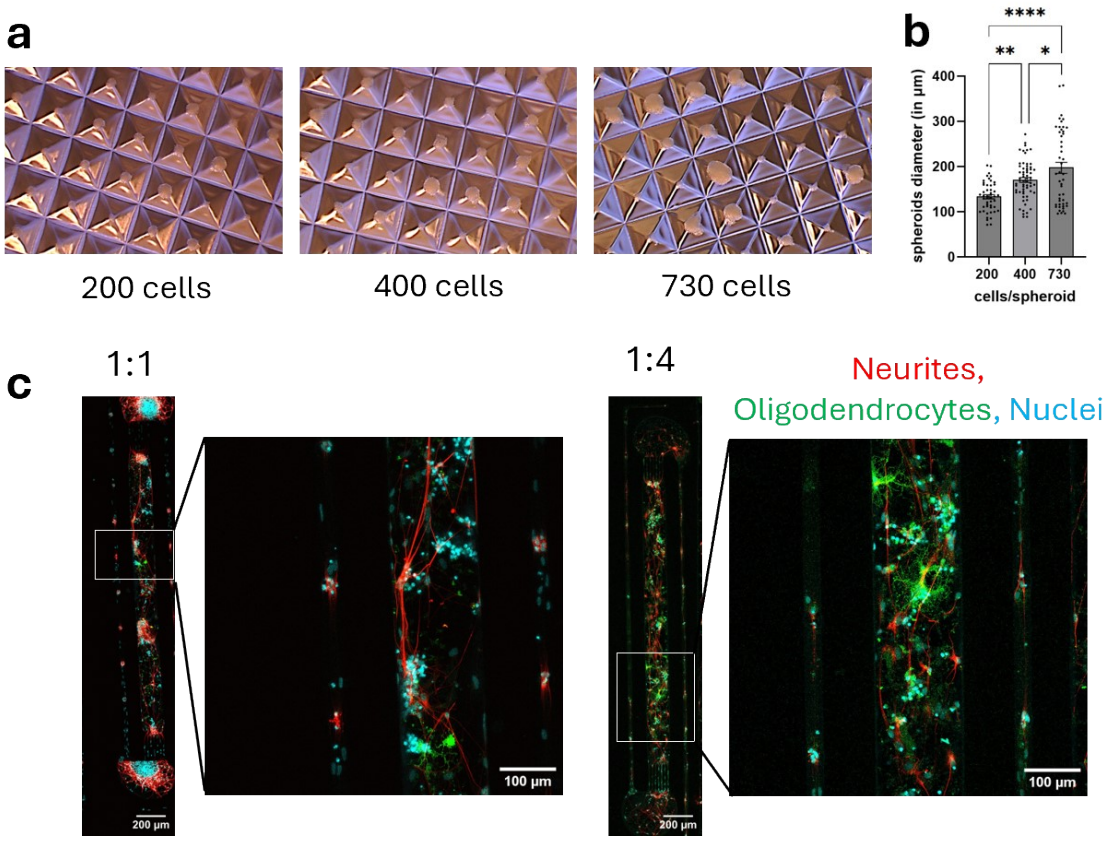

Figure S1: Oligodendrocytes preferentially grow and survive in a soft matrix. a-b Brightfield images and quantification of neuronal spheroid diameters generated from 200, 400, and 730 cells per spheroid. **c** Immunofluorescence images of MyeliMAP with 1:1 and 1:4 Matrigel dilutions showing improved oligodendrocyte (MBP+) survival in the softer (1:4) condition along with neurites (NF+). Hoechst (nuclei) images are shown for context. Data shown as mean ± SEM. Statistical analysis was performed by one-way ANOVA with Turkey Multiple Comparison Test (b). *p< 0.05, **p< 0.01, ****p< 0.0001. N = 1 spheroid generation experiment with n=47-63 spheroids (b) and N = 1 MyeliMAP microstructure with n=3 PDMS microfluidic networks (a-c).

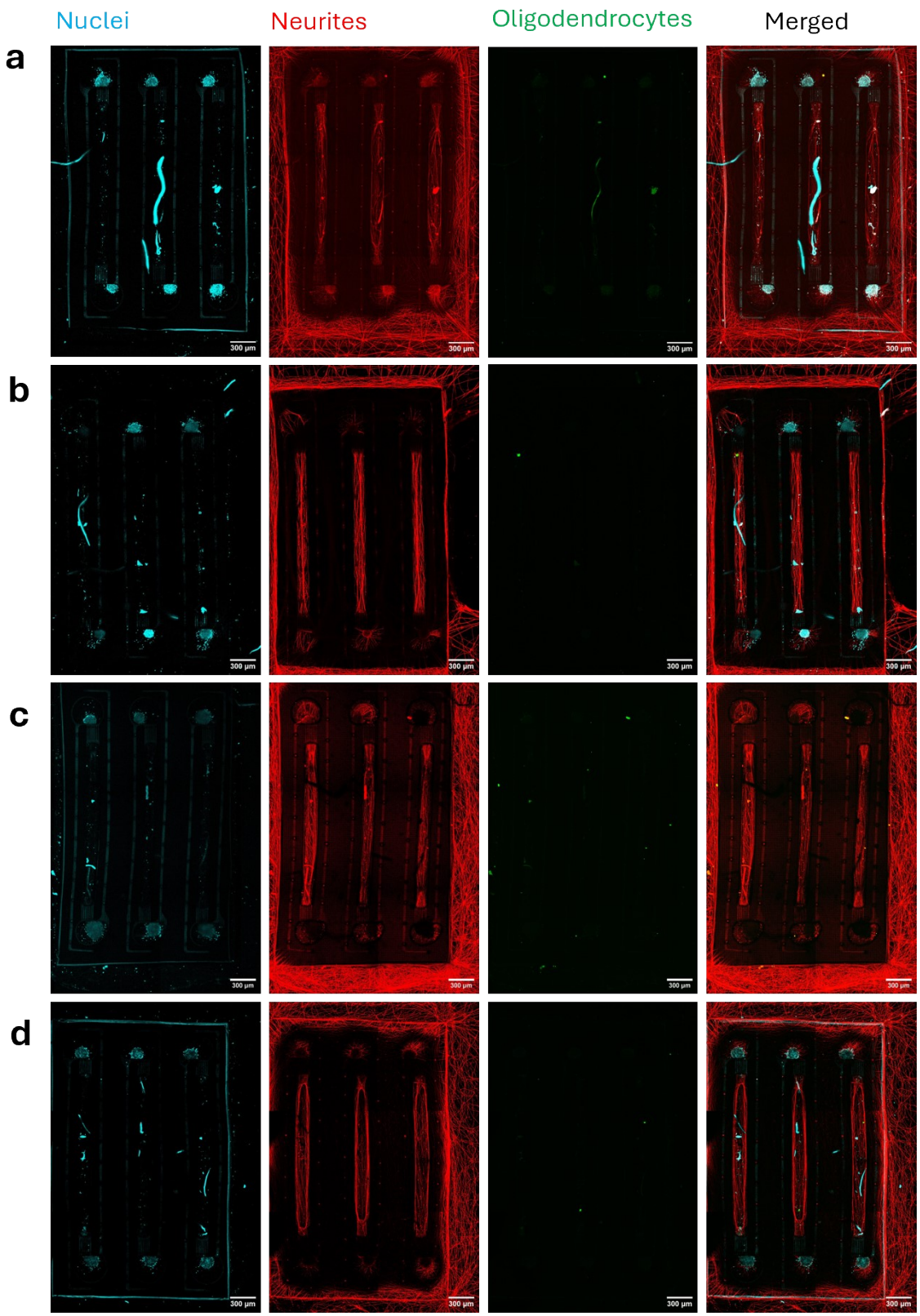

Figure S2: Immunofluorescent staining of MyeliMAPs with neuron monocultures on MyeliMAP-HD-MEA platforms. a-d Representative low magnification immunofluorescence images of neuronal monocultures on

MyeliMAP-HD-MEA platform after 9 weeks in culture reveals neurites (NF+) from peripheral seeding wells to central axonal chamber where oligodendrocytes (MBP+) are absent. Higher magnification immunofluorescent image from one MyeliMAP-HD-MEA platform (a) showing absence of oligodendrocytes and thus no neuron-oligodendrocyte alignment, shown in Figure 4h. Hoechst (nuclei) images are shown for context. Neuronal extensions are also seen on top and outside of the MyeliMAP microstructure indicating neuronal overgrowth due to presence of 3D extracellular matrix. Scale bar: 300µm. N=4 independent experiments (HD-MEA chips) with n=12 PDMS microfluidic networks.

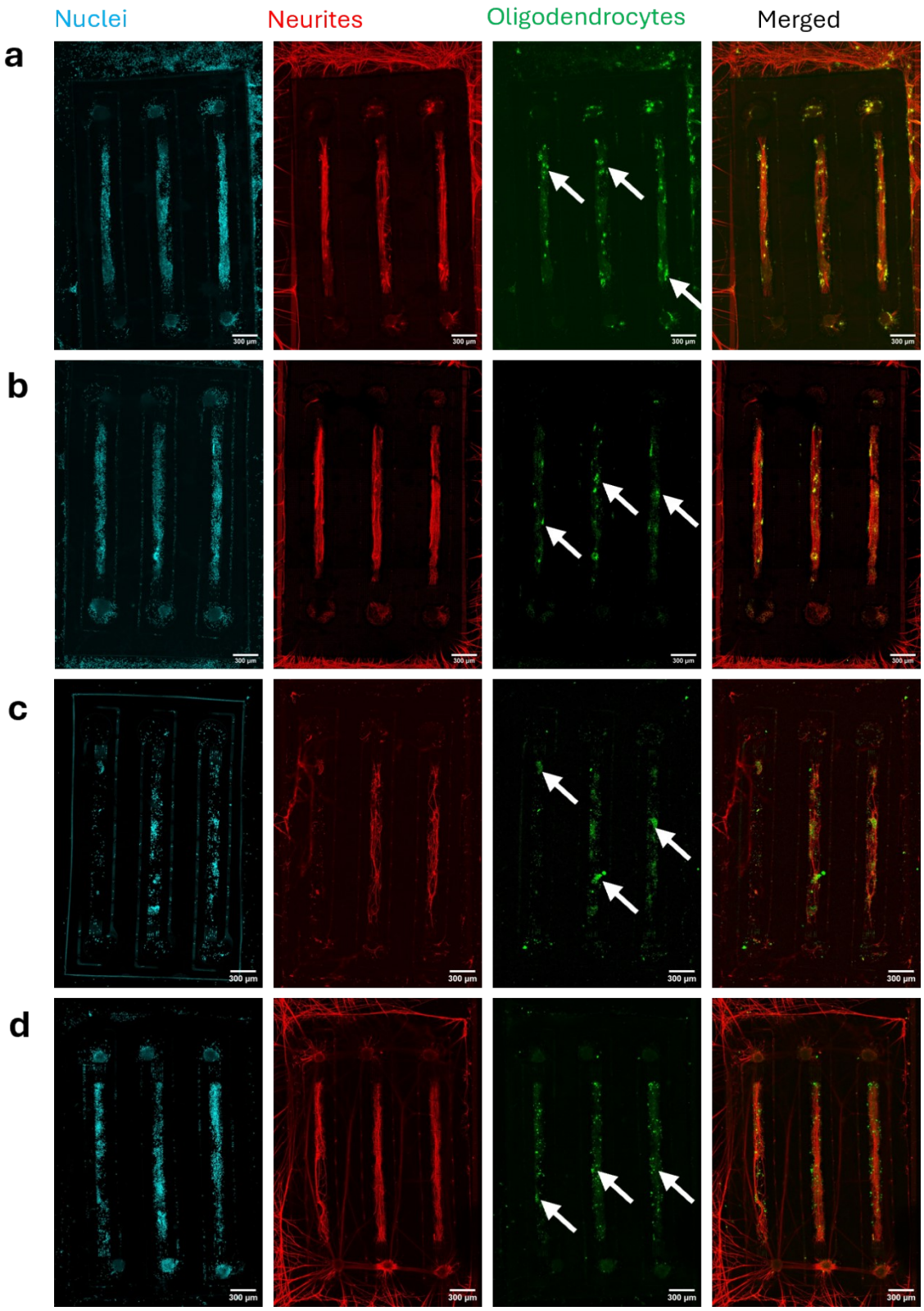

Figure S3: Immunofluorescent staining of MyeliMAPs with neuron-oligodendrocyte cocultures on MyeliMAP-HD-MEA platforms. a-d Representative low magnification immunofluorescence images of neuron-oligodendrocyte

cocultures grown on MyeliMAP-HD-MEA platforms after 9 weeks in culture reveal neurites (NF+) from peripheral seeding wells to central axonal chamber where they interact with oligodendrocytes (MBP+, indicated with white arrows) to enable neuron myelination. Higher magnification immunofluorescent image from MyeliMAP-HD-MEA platform (a) showing neuron-oligodendrocyte alignment shown in Figure 4h. Hoechst (nuclei) images are shown for context. Neuronal extensions are also seen on top and outside of the MyeliMAP microstructure indicating neuronal overgrowth due to presence of 3D extracellular matrix. Scale bar: 300µm. N=4 independent experiments (HD-MEA chips) with n=12 PDMS microfluidic networks.

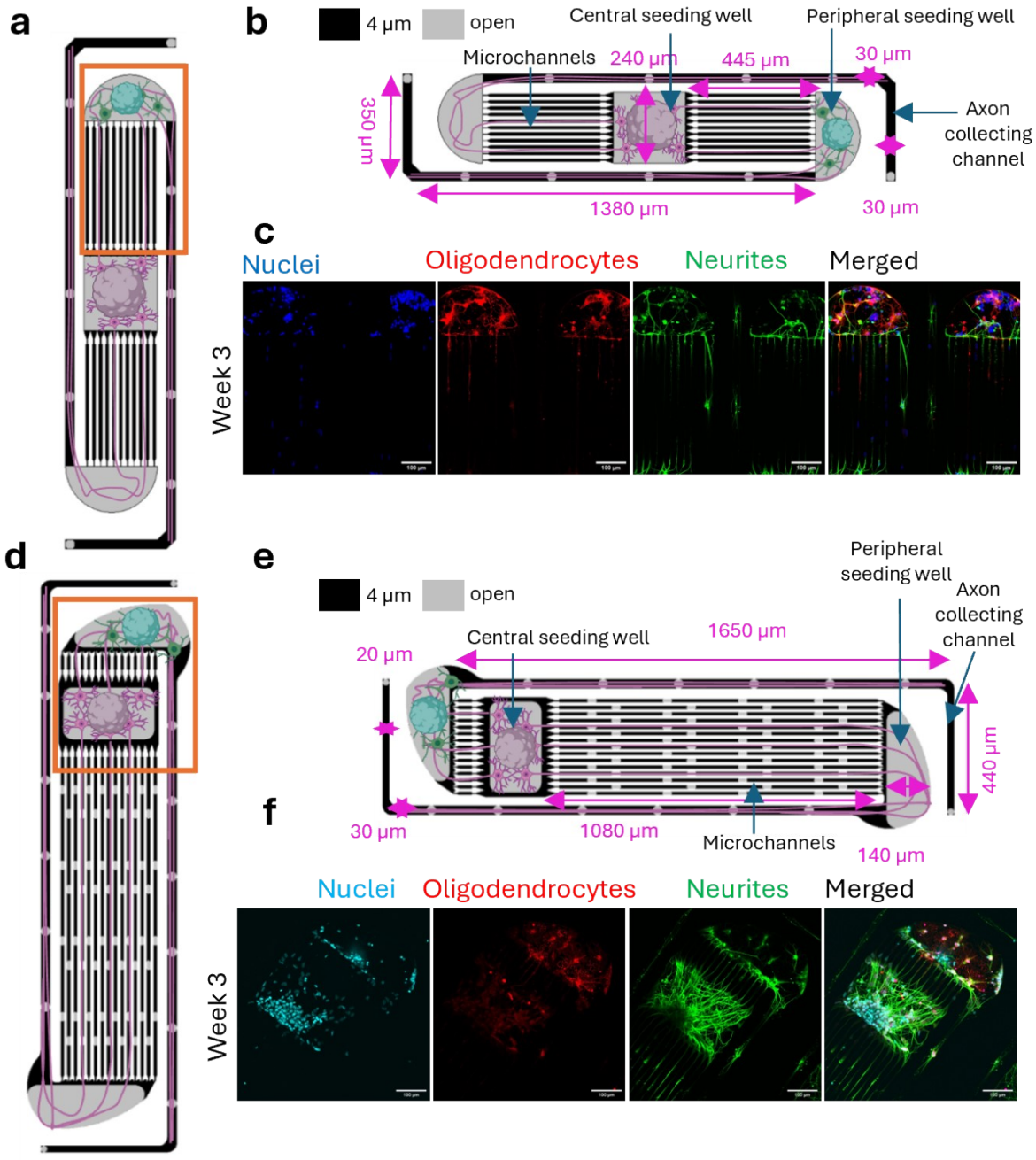

Figure S4. Oligodendrocytes preferentially remain in seeding wells rather than migrating into axon collecting channel for axon myelination. a Schematic of the 2D nerve PDMS microfluidic network containing one central and

two peripheral seeding wells (grey, open for cell loading) connected by eleven microchannels to an axon-collecting channel (black, 4 µm height with underlying electrodes). iNGN2 neurons seeded in the central well extend neurites toward the peripheral wells containing iSOX10 OPCs. **b** Dimensions of the 2D nerve PDMS microfluidic network. **c** Representative immunofluorescence images of neuron-oligodendrocyte cocultures after 3 weeks show neurites (NF+) projecting from the central to peripheral wells, where they interact with oligodendrocytes (MBP+); OPCs largely remain in the peripheral wells and do not migrate toward the axon-collecting channel. Hoechst (nuclei) images are shown for context. **d** Schematic of the modified 2D long-nerve PDMS microfluidic network with displaced central seeding to generate short and long microchannels across different peripheral wells. **e** Dimensions of the modified 2D long-nerve PDMS microfluidic network. **f** Representative immunofluorescence images after 3 weeks of coculture show neurite (NF+) extension and interaction with oligodendrocytes (MBP+), which similarly remain confined to the peripheral wells and do not migrate into the axon-collecting channel. Hoechst (nuclei) images are shown for context. Scale bar: 100µm. N=3 independent microstructures with n=12 PDMS microfluidic networks.

Supplementary videos

Video S1: Baseline spontaneous electrophysiological activity recorded from integrated MyeliMAP-HD-MEA containing neuron-oligodendrocyte cocultures at week 4.

Video S2: Stimulation-induced electrophysiological activity recorded from integrated MyeliMAP-HD-MEA containing neuron-oligodendrocyte cocultures at week 4.

Supplementary tables

Table S1: Medium composition used in the study.

Medium	Composition
Proliferation Medium	DMEM/F12 with Glutamax (Lifetech 31331-028), 1x B27 with Vit. A [Life technologies, cat 17504-044], 1x N2 supplements [Invitrogen, cat 17502-048], 10 ng/ml hEGF (Invitrogen, cat PHG0315), 10 ng/ml hFGF (Gibco CTP0263), Pen/Strep (Lifetech 15070-063), Doxycyclin 1ug/ml (Clontech Cat No 631311)
Neuronal Maintenance Medium (NMM)	NMM comprises of 1:1 mixture of Neurobasal medium (Gibco) and DMEM/F-12 Glutamax™ complimented with 0.5X Glutamax™ (Gibco), 50 U/mL Penicillin-Streptomycin, 0.5X B27 (Gibco), 0.5X N2 (Gibco), 0.5X MEM-NEAA (Gibco), 0.5X sodium pyruvate, 0.025 % human insulin (Sigma) and 50 µM β-mercaptoethonal (Gibco)
Oligodendrocyte Maturation Medium (OMM)	NMM supplemented with 10 ng/mL insulin growth factor-1 (IGF1), 5 ng/mL hepatocyte growth factor (HGF, Peprotech), 10 ng/mL neurotrophin-3 (NT3, Peprotech), 60 ng/mL thriiodothyronine (T3, Sigma), 100 ng/mL Biotin (Sigma), 1 µM cyclic adenosine monophosphate (cAMP, Sigma), and 5 µg/mL doxycycline (Sigma)
Oligodendrocyte Induction Medium (OIM)	Neurobasal medium supplemented with 0.1 µM retinol acid (RA) (Sigma), 10 µM SB431542, and 1 µM LDN-193189

Basal medium	Neurobasal medium supplemented with 10 ng/mL platelet-derived growth factor AA (PDGF-aa, Peprotech), 10 ng/mL insulin growth factor-1 (IGF1), 5 ng/mL hepatocyte growth factor (HGF, Peprotech), 10 ng/mL neurotrophin-3 (NT3, Peprotech), 60 ng/mL thriiodothyronine (T3, Sigma), 100 ng/mL Biotin (Sigma), 1 µM cyclic adenosine monophosphate (cAMP, Sigma), and 5 µg/mL doxycycline (Sigma)

Table S2: List of primary and secondary antibodies used in the study.

Antibody	Company	Cat. No.	Dilution factor
Chicken- Neurofilament H	Synaptic System	#171 106	1/1000
Rabbit- TUJ1	Merck	#AB18207	1/100
Rat- MBP	Sigma Aldrich	#M9434	1/500
Mouse- CASPR	NeuroMAB	#AB2083496	1/200
Goat anti-mouse Alexa Fluorophore 647	ThermoFisher	#A-21235	1/1000
Goat anti-chicken Alexa Fluorophore 555	ThermoFisher	#A-11039	1/1000
Goat anti-rabbit Alexa Fluorophore 488	ThermoFisher	#A-11008	1/1000
Goat anti-rat Alexa Fluorophore 488	ThermoFisher	#A-11006	1/1000
Goat anti-rat Alexa Fluorophore 555	ThermoFisher	#A-21434	1/1000