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Supplemental Information

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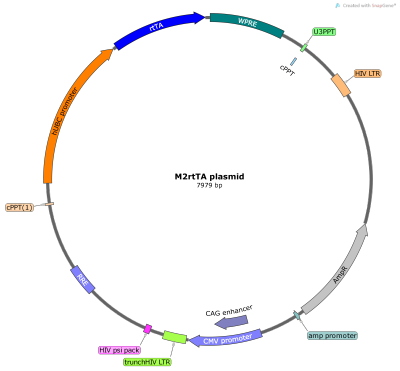
Inventory of Supplemental Information:

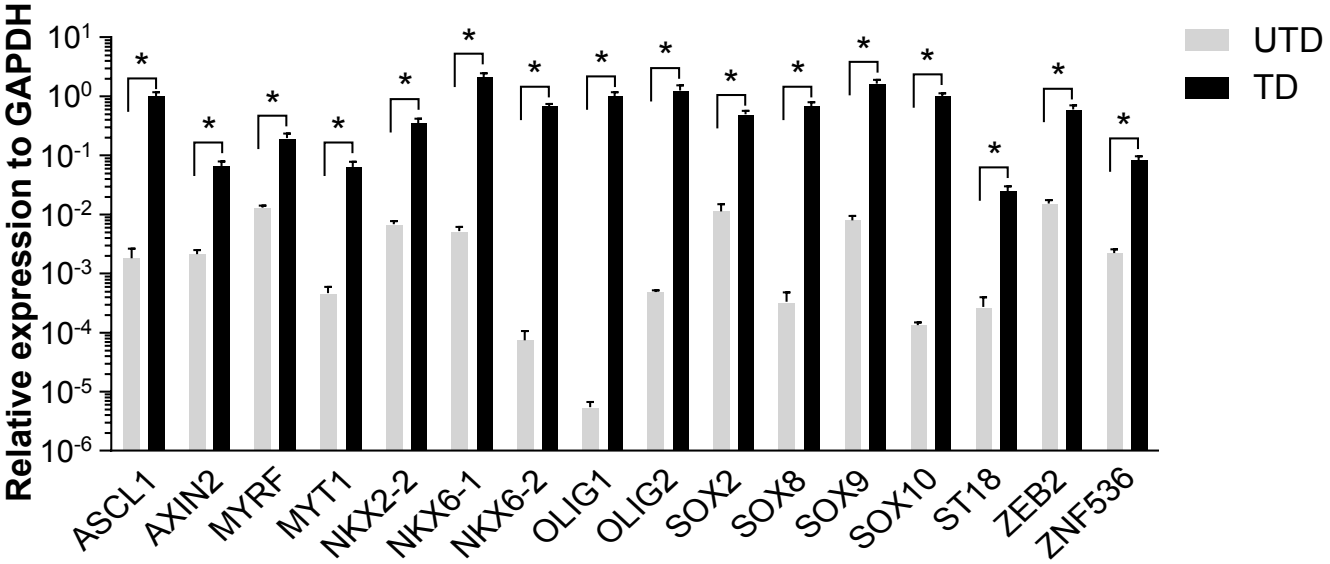
A



TF selected

ASCL, AXIN2, MYRF, MYT1, NKX2-2, NKX6-1, NKX6-2, OLIG1, OLIG2, SOX2, SOX8, SOX9, SOX10, ST18, ZEB2, ZNF536

B



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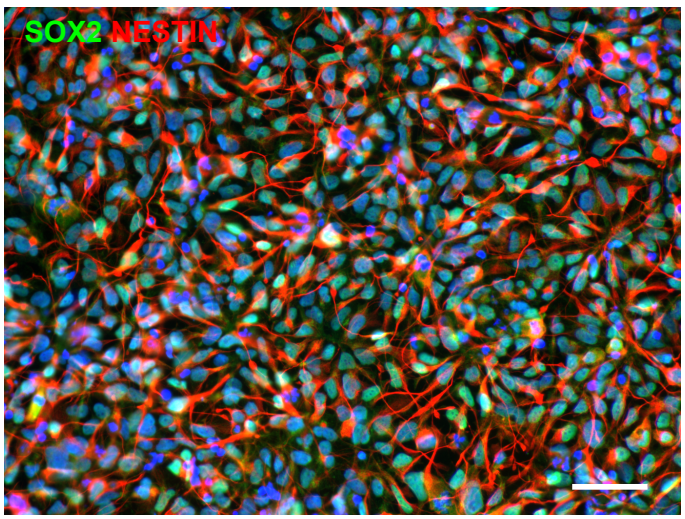
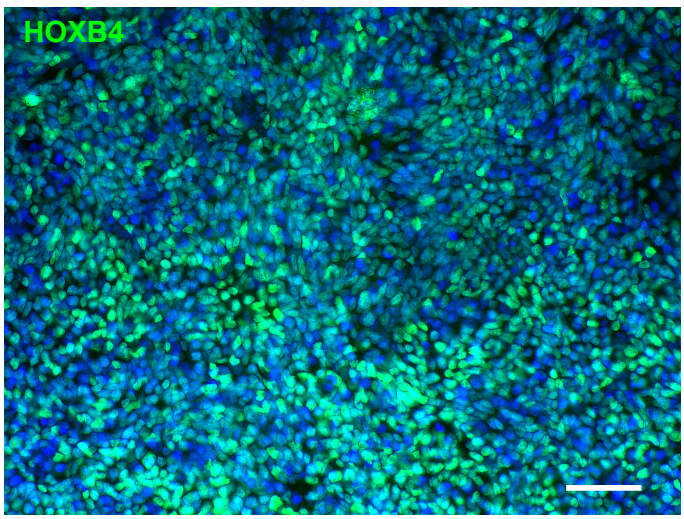
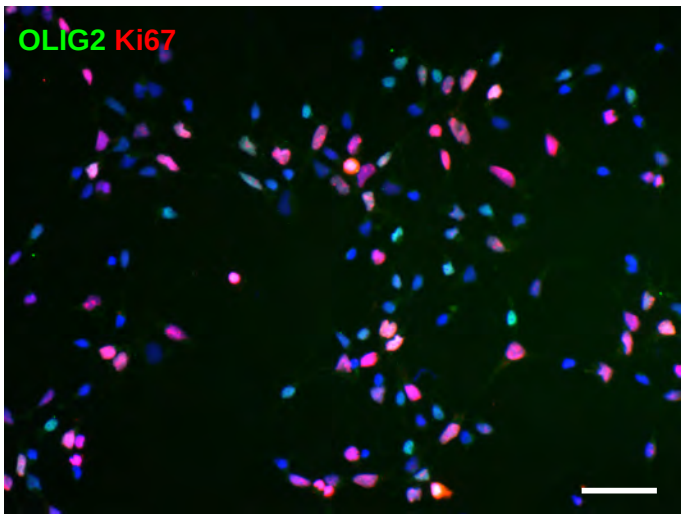




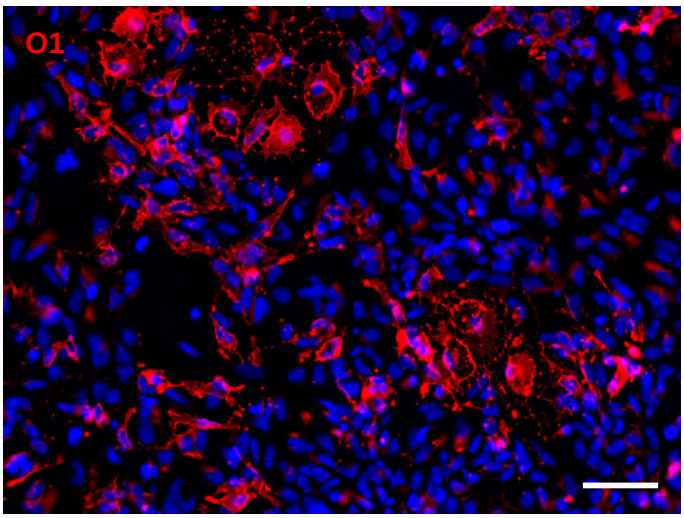
Day 0

A



Day 10

B



Primary murine Schwann cells

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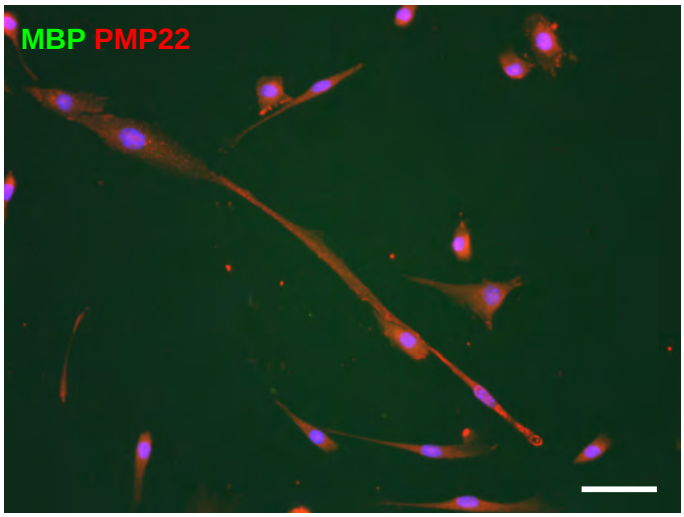
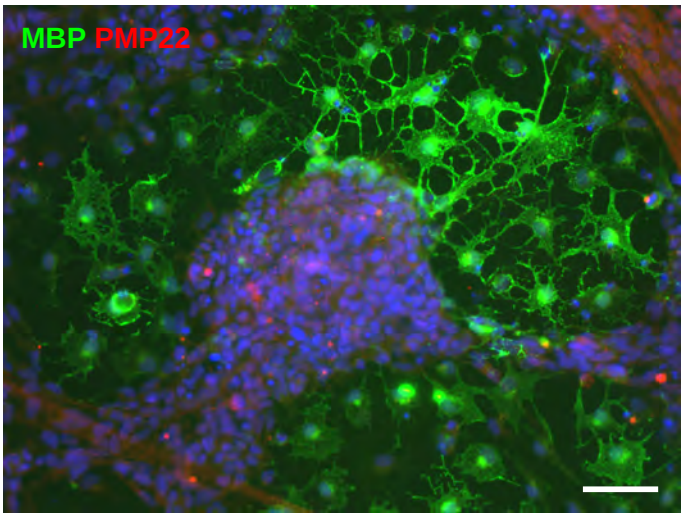


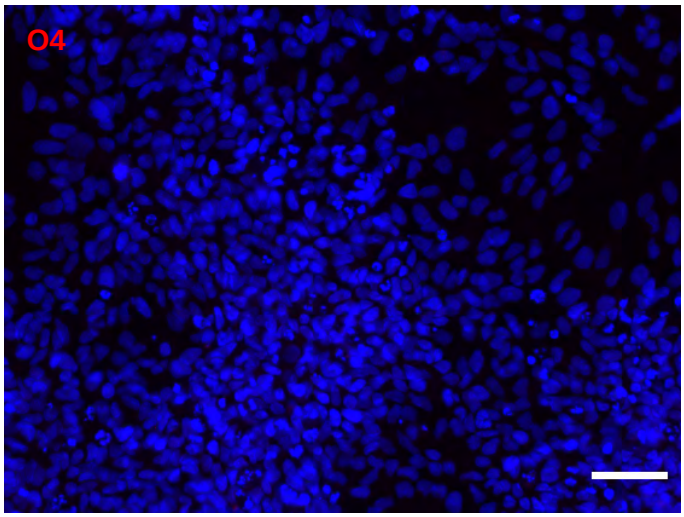
SOX10-transduced cells Day 10

D



eGFP-transduced cells Day 10

E



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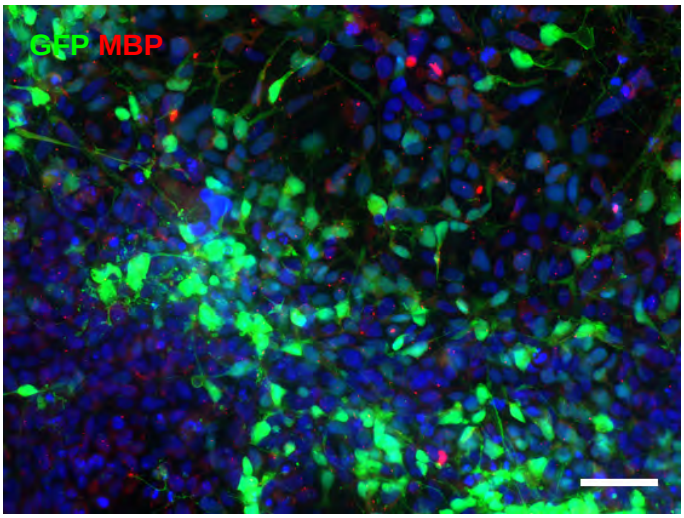

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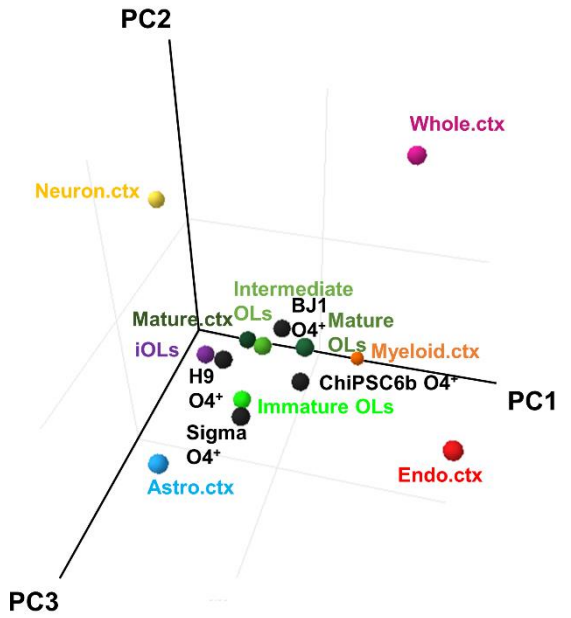
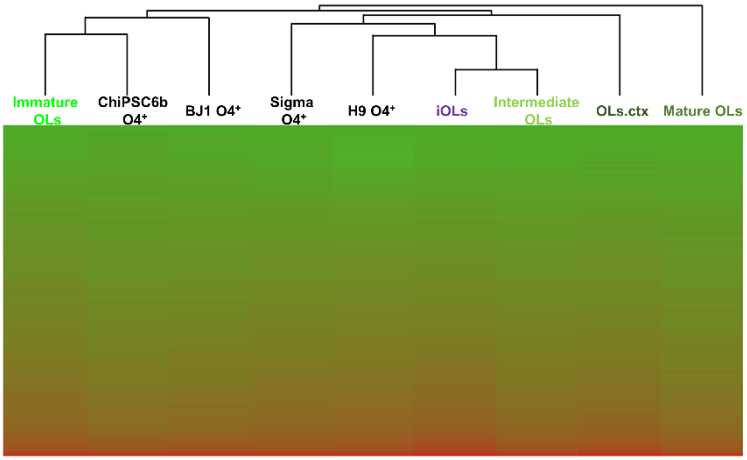
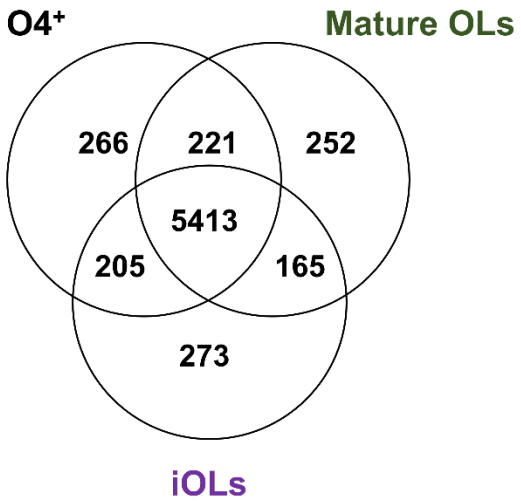
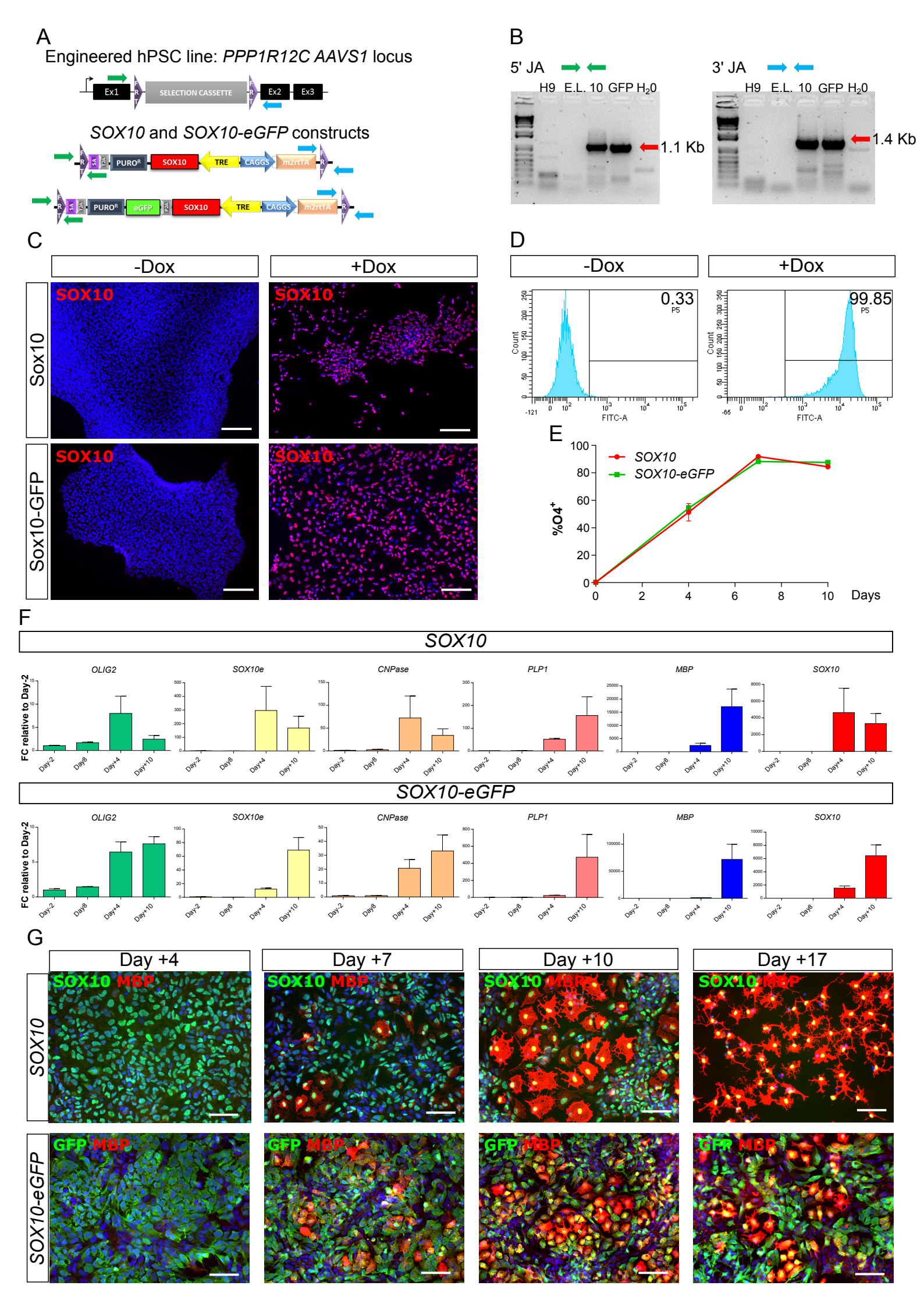

PC2
Whole.ctx
Neuron.ctx
Intermediate OLs
BJ1 O4+
Mature.ctx
Mature OLs
iOLs
Myeloid.ctx
H9 O4+
ChiPSC6b O4+
PC1
Immature OLs
Sigma O4+
Endo.ctx
Astro.ctx
PC3

B

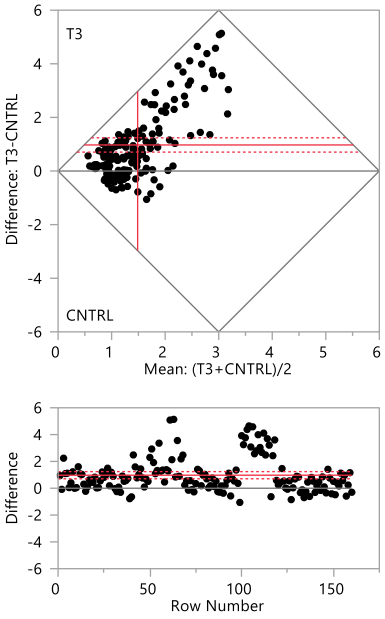

Immature OLs
ChiPSC6b O4+
BJ1 O4+
Sigma O4+
H9 O4+
iOLs
Intermediate OLs
OLs.ctx
Mature OLs

C


O4+
Mature OLs
266
221
252
5413
205
165
273
iOLs

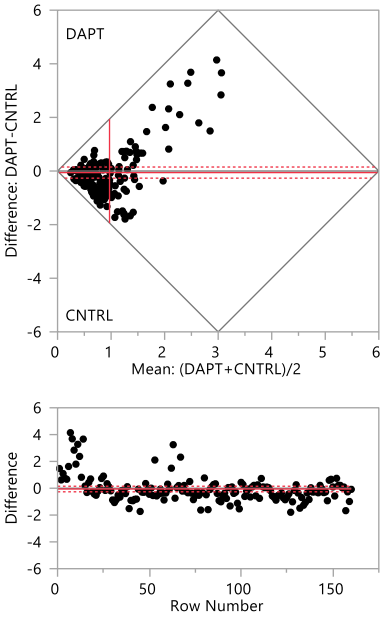

A
Engineered hPSC line: PPP1R12C AAVS1 locus
Ex1
SELECTION CASSETTE
Ex2
Ex3
SOX10 and SOX10-eGFP constructs
PURO^R
SOX10
TRE
CAGGS
m2rtTA
eGFP
B
5' JA
3' JA
H9 E.L. 10 GFP H2O
1.1 Kb
1.4 Kb
C
-Dox
+Dox
Sox10
Sox10-GFP
SOX10
D
0.33
99.85
P5
Count
FITC-A
E
SOX10
SOX10-eGFP
%O4+
Days
F
SOX10
SOX10-eGFP
OLIG2
SOX10e
CNPase
PLP1
MBP
SOX10
FC relative to Day-2
Day-2
Day8
Day+4
Day+10
G
Day +4
Day +7
Day +10
Day +17
SOX10 MBP
GFP MBP

Difference: T3-CNTRL



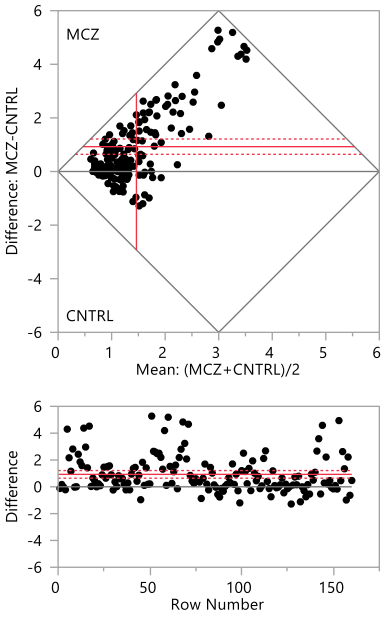
T3	1.97218
CNTRL	1.00
Mean Difference	0.97218
Std Error	0.10229
Upper 99%	1.23886
Lower 99%	0.70552
N	160
Correlation	-0.0647
Prob > |t|	<.0001*
Prob > t	<.0001*
Prob < t	1.0000

Difference: DAPT-CNTRL



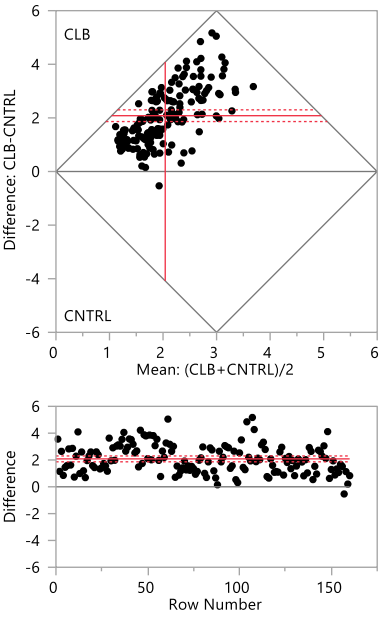
DAPT	0.94134
CNTRL	1.00
Mean Difference	-0.0587
Std Error	0.07965
Upper 99%	0.14901
Lower 99%	-0.2663
N	160
Correlation	0.13922
Prob > |t|	0.4626
Prob > t	0.7687
Prob < t	0.2313

Difference: MCZ-CNTRL



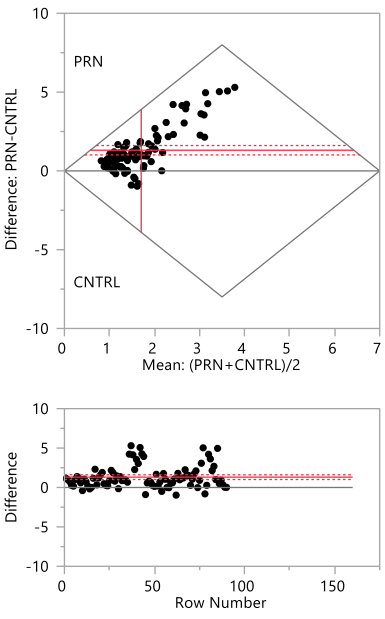
MCZ	1.92664
CNTRL	1.00
Mean Difference	0.92664
Std Error	0.10953
Upper 99%	1.21222
Lower 99%	0.64109
N	160
Correlation	-0.0754
Prob > |t|	<.0001*
Prob > t	<.0001*
Prob < t	1.0000

Difference: CLB-CNTRL



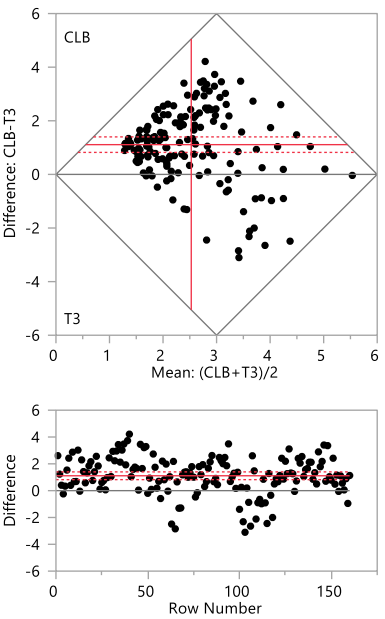
CLB	3.08201
CNTRL	1.00
Mean Difference	2.08201
Std Error	0.08382
Upper 99%	2.30056
Lower 99%	1.86349
N	160
Correlation	0.07246
Prob > |t|	<.0001*
Prob > t	<.0001*
Prob < t	1.0000

Difference: PRN-CNTRL



PRN	2.36116
CNTRL	1.00
Mean Difference	1.36116
Std Error	0.1497
Upper 99%	1.61004
Lower 99%	1.01514
N	90
Correlation	-0.0319
Prob > |t|	<.0001*
Prob > t	<.0001*
Prob < t	1.0000

Difference: CLB-T3



CLB	3.08201
T3	1.97218
Mean Difference	1.10983
Std Error	0.11095
Upper 99%	1.39909
Lower 99%	0.82058
N	160
Correlation	0.18061
Prob > |t|	<.0001*
Prob > t	<.0001*
Prob < t	1.0000

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. **Lentiviral inducible overexpression system used for the initial screening with 16 TFs, and characterization of NPCs. Related to Figure 1.**

(A) Scheme of the lentiviral vector system used for the overexpression of the 16 TFs selected. (B) Expression levels of each TF in untransduced (UTD) *vs.* transduced (TD) conditions. (C) Phenotypic characterization of NPCs generated for the transduction with each TF. Gene expression levels normalized to *GAPDH*. Hoechst 33258 (blue) was used as nuclear marker. Scale bar: 50 µm. Data is represented as Mean ± SEM of N=3 independent experiments. *p<0.05.

Supplementary Figure 2. **Phenotypic characterization of OPC/OLs generated by *SOX10* overexpression. Related to Figure 3.**

(A) Immunostaining for the early OPC marker OLIG2 and Ki67 proliferation marker in NPCs before transduction. (B) Immunostaining for the ganglioside marker O1 in *SOX10*-induced cells. (C-D) Generated OPC/OLs are morphologically different from Schwann cells (SCs) and do not express typical SC markers. (C) Primary Schwann cells obtained from postnatal murine tissue express PMP22 but not MBP. (D) *SOX10*-induced OPC/OLs do not express the Schwann cell marker PMP22. No expression of O4 (E) or MBP (F) is observed for *eGFP*-transduced NPCs after 10 days of induction. Hoechst 33258 (blue) was used as nuclear marker. Scale bar: 50 µm.

Supplementary Figure 3. **Global transcriptome comparison of *SOX10*-induced O4$^+$ cells (derived from H9, ChiPSC6b, Sigma-IPSC0028 and BJ1 PSC lines, n=12) with different samples including TF-induced human OLs from the study of Ehrlich *et al.* (Ehrlich *et al.*, 2017). Related to Figure 4.**

(A) Principal component analysis (PCA) of the different samples analyzed. (B) Hierarchical clustering of whole-genome expression profile of *SOX10*-induced O4$^+$ cells (black), different primary OL samples (green), and iOLs from Ehrlich et *al.* (purple). (C) Differentially expressed genes identified from sequence alignment map (SAM) using FDR ≤ 0.05 and >2 fold change among PSC-O4$^+$ cells, primary mature OLs and iOLs.

Supplementary Figure 4. **Generation of hPSC lines endogenously overexpressing the *SOX10* TF.**

(A) Schemes of the targeting of the endogenous *AAVS1* locus and of the constructs created for inducible overexpression of *SOX10*. (B) 5' and 3' junction assay (JA) PCRs to check for the insertion of *SOX10* and *SOX10-eGFP*-containing selection cassettes at correct genomic location and graphical depiction of the primers used (H9: untargeted cells, E.L.: engineered line with selection cassette inserted in *AAVS1* locus, 10: engineered line with SOX10-expressing cassette inserted, GFP: engineered line with SOX10-eGFP-expressing cassette inserted). (C) Doxycycline inducible expression of *SOX10* in both lines generated. (D) Doxycycline inducible expression of GFP after successful generation of the *SOX10-GFP* line measured by FACS. (E) O4 expression along the days after doxycycline induction in both *SOX10* and *SOX10-GFP* lines. (F) Gene expression levels of OPC/OL markers along the days in both *SOX10* and *SOX10-GFP* lines. SOX10e is referred to the endogenous expression of this gene. Gene expression levels normalized to *GAPDH*. (G) Representative images of the phenotypic characterization performed along differentiation of both lines. Hoechst 33258 (blue) was used as nuclear marker. Scale bar: 50 µm. Data is represented as Mean ± SEM of N=3 independent experiments. *p<0.05.

Supplementary Figure 5. **Results of matched-pair analysis of the total area identified as MBP^+ expression (MBP^+ area) relative to control condition (0.1% DMSO) of the different tested compounds/drugs affecting myelination. Related to Figure 7.**

The results show differences in MBP^+ area of the tested compound compared to control condition (N=160 for all conditions except for pranlukast, where N=90). For each comparison, the first graph is the difference plot which shows the mean of each response, the difference of the means (shown as the horizontal line), and the 95% confidence interval (above and below shown as dotted lines). The mean of pairs is shown by the vertical line. The second graph is the Tukey mean-difference plot and represents the differences by each row of the comparison. The following comparisons were performed: (A) T3-control, (B) DAPT-control, (C) Miconazole (MCZ)-control, (D) Clobetasol propionate (CLB)-control and (E) Pranlukast (PRN)-control. As can be observed, all compounds showed significant higher expression of MBP^+ area compared to control except DAPT. Comparison of all compounds among themselves was also performed, with Clobetasol propionate (CLB) being the only one showing significant higher MBP^+ expression compared to T3 (F) ($p = 0.0001$; paired *t* test). $P< 0.05$ (Prob > |t|) indicates difference is statistically significant.

Supplementary Table 3: List of primer sequences used for PCR amplification of the CDS of the selected transcription factors for cloning into the FUW lentiviral vector.

Genes	Primer sequences (5' to 3')	Type
ASCL1	cgattGAATTCATGGAAAGCTCTGCCAAGAT	Forward
	cgattGAATTCTCAGAACCAGTTGGTGAAGTC	Reverse
AXIN2	gtacgCAATTGATGAGTAGCGCTATGTTGGTGAC	Forward
	gtacgCAATTGTCAATCGATCCGCTCCAC	Reverse
MYRF	cagcaCAATTGATGCACTGGCTTCCAGCA	Forward
	tgaaCAATTGTCAGTCACACAGGCGGTAGAAGT	Reverse
MYT1	gtatcGAATTCATGAGCTTAGAAAATGAAGACAAGC	Forward
	gtatcGAATTCCTAGACCTGGATGCCCCTC	Reverse
NKX2-2	tcagtGAATTCATGTCGCTGACCAACACAAA	Forward
	tcagtGAATTCTCACCAAGTCCACTGCTGG	Reverse
NKX6-1	tactgGAATTCATGTTAGCGGTGGGGGCAAT	Forward
	tactgGAATTCTCAGGATGAGCTCTCCGGCT	Reverse
NKX6-2	tcagaGAATTCATGGACACTAACCGCCCG	Forward
	tcagaGAATTCTCACAAGGCGTCCCCCG	Reverse
OLIG1	tacgtGAATTCATGTACTATGCGGTTTCCCAG	Forward
	tacgtGAATTCTCACTTGGAGAATTGCGC	Reverse
OLIG2	tcaagGAATTCATGGACTCGGACGCCAG	Forward
	tcaagGAATTCTCACTTGGCGTCGGAGG	Reverse
SOX2	gatcgGAATTCATGTACAACATGATGGAGACGGA	Forward
	gatcgGAATTCTCACATGTGTGAGAGGGGC	Reverse
SOX8	gttcaGAATTCATGCTGGACATGAGCGAGG	Forward
	gttcaGAATTCTCAGGGCCTGGTCAGGGT	Reverse
SOX9	tagctGAATTCATGAATCTCCTGGACCCCTT	Forward
	tagctGAATTCTCAAGGTCGAGTGAGCTGTG	Reverse
SOX10	catgaGAATTCATGGCGGAGGAGCAGGAC	Forward
	catgaGAATTCTTAGGGCCGGGACAGTGTC	Reverse
ST18	gtcaaGAATTCATGGATGCAGAGGCTGAAGA	Forward
	gtcaaGAATTCCTACACATGGATACCCTTCACTGC	Reverse
ZEB2	agcaCAATTGATGAAGCAGCCGATCATGGC	Forward
	tgaaCAATTGTTACATGCCATCTTCCATATTGTCTTCC	Reverse
ZNF536	gatcgGAATTCATGGAAGAAGCGAGCCTGTGCCTT	Forward
	gatcgGAATTCTCACTTACCACACAAGTCTGGCCTCTTG	Reverse
eGFP	TAGTGAATTCATGGTGAGCAAGGGCGAG	Forward
	TAGTGAATTCTTACTTGTACAGCTCGTCCATGC	Reverse

*The underlined sequences denote the EcoRI restriction sites. For MYRF,ZEB2 and AXIN2, MfeI restriction sites were used.

Supplementary Table 4: List of primers and conditions used for junction assay PCRs.

Symbol	Target	Amp. size	Sequence	Orientation	PCR Protocol
	5’ JA	1028 bp	CACTTTGAGCTCTACTGGCTTC	Forward	95 °C/5 min, [95 °C/30 sec, 68 °C (-0.5 °C/cycle) /90 sec] x 15, [95 °C/30 sec, 58 °C/30 sec, 72 °C/90 sec] x 25, 72 °C/5 min
			CATGTTAGAAGACTTCCTCTGC	Reverse	
	3’ JA	1384 bp	ACCTTGATATGCTGCCTGCT	Forward	
			AAGGCAGCCTGGTAGACA	Reverse	

Supplementary Table 5: List of primer sequences used for gene expression analysis by QPCR.

Genes	Primer sequences (5' to 3')	Type
ASCL1	CCCCCAACTACTCCAACGAC	Forward
	TGAAGTCGAGAAGCTCCTGC	Reverse
AXIN2	TCATTTCCCGAGAACCCACC	Forward
	TCGGAGCCCTCTCTCTCTTC	Reverse
MYRF	TAACTACAAGGAGCTGCCCATG	Forward
	TGGTTCTTCTTCTGGCACAC	Reverse
MYT1	AAGAGCTGAGAAGCGTGAGATC	Forward
	CATGCATGGCTAAGATCTCTGG	Reverse
NKX2-2	GAACCCCTTCTACGACAGCA	Forward
	AGACCGTGCAGGGAGTACT	Reverse
NKX6-1	CCATCTTCTGGCCCGGAGTGA	Forward
	CTTCCCGTCTTTGTCCAACAA	Reverse
NKX6-2	CCAGGTGAAGGTCTGGTTCC	Forward
	GTATTCGTCGTCGTCCTCCG	Reverse
OLIG1	GCATGCAGGACCTGAACCT	Forward
	TATCTTGGAGAGCTTGCGGC	Reverse
OLIG2	GACAAGCTAGGAGGCAGTGG	Forward
	TCCGGCTCTGTCATTTGCTT	Reverse
SOX2	GAGTGGAAACTTTTGTCGGAGA	Forward
	AGCGTGTACTTATCCTTCTTCAT	Reverse
SOX8	CCCGACTACAAGTACCAGCC	Forward
	GGTCTGCCCTGTGTGGTC	Reverse
SOX9	GAACAAGCCGCACGTCAAG	Forward
	TCGCTCTCGTTCAGAAGTCTC	Reverse
SOX10	CTTCATGGTGTGGGCTCAGG	Forward
	CACTTTCGTTCAGCAGCCTC	Reverse
ST18	CTCTACCGCAGAGGAAATCATG	Forward
	AAGACTTGACCATGAGCTCTTG	Reverse
ZEB2	AGGGACAGATCAGCACCAAATG	Forward
	GGGCACTCGTAAGGTTTTTCAC	Reverse
ZNF536	ATGAAGGACTGCCCGTACTG	Forward
	GGTTTCTCACCTGTGTGTATCC	Reverse
GAPDH	TCAAGAAGGTGGTGAAGCAGG	Forward
	ACCAGGAAATGAGCTTGACAAA	Reverse
Sox2e	TGGCGAACCATCTCTGTGGT	Forward
	CCAACGGTGTCAACCTGCAT	Reverse
Olig2e	CACAGAGCAGTGGGGAGTG	Forward
	GCACACAGCGGTACCTTTTC	Reverse
Ascl1e	GAGCAACTGGGACCTGAGTC	Forward
	TCAGCTGTGCGTGTTAGAGG	Reverse
Sox10e	TTCTGAAGGCAGGAAGGAGTTG	Forward
	ATGCGTCTCAAGGTCATGGAG	Reverse
CSPG4	TCAGGCAGAGGTCTACGCT	Forward
	TAGGGTATCATGGGCCTCCC	Reverse
GalC	TGTCGTGACCTGGATTGTGG	Forward
	TGACCTCTCATTCCAAATTCCA	Reverse
MBP	AGGCAGAGCGTCCGACTATA	Forward
	CGACTATCTCTTCCTCCCAGC	Reverse
CNPase	ATGGTCAGCGTGAAGGC	Forward
	CAACCAAGTTTTGTGACTACGG	Reverse

PLP1	GGTTTCCCTGCTCACCTTCA	Forward
	TCAGAACTTGGTGCCTCGG	Reverse
OMG	GTGCATATACATTGGAGAGGACA	Forward
	AGATGCTGATGTTGAAGACGA	Reverse
MOBP	GTGTTCGGAGTACTTCTGGTT	Forward
	GCAAATGATACCAAGACAAGCTC	Reverse
GPR17	GATGAAGTCGTTGAGTGTCTAGG	Forward
	CAGATGCCCACCATTTCTCTA	Reverse

*Those genes followed by *e* denotes primers designed to distinguish endogenous from transgene expression of a certain factor.

Supplementary Table 6: List of primary antibodies used for immunostainings.

Antigen	Dilution	Host and serotype	Reference
A2B5	1/500	Mouse IgM	Millipore MAB312
O4-APC (FACS)	1/20	Mouse IgM	Miltenyi 130-095-891
O4 (immunostaining)	1/300	Mouse IgM	R&D MAB1326
O1	1/300	Mouse IgM	R&D MAB1327
Olig2	1/100	Goat polyclonal IgG	Santa cruz SC-19969
MBP	1/75 1/200 (brain slices)	Chicken IgY	Millipore AB9348
MOG	1/100	Mouse IgG1	Millipore MAB 5680
PLP	1/100	Rabbit IgG	Abcam ab28486
hNA	1/200	Mouse IgG1	Millipore MAB1281
NF200	1/200	Rabbit IgG	Sigma N4142
TUJI (beta 3 tubulin)	1/1000	Rabbit polyclonal	Synaptic systems 302302
SOX10	1/100	Goat polyclonal IgG	R&D AF 2864
Ki67	1/200	Mouse IgG1	BD 556003
SOX2	1/200	Rabbit IgG	Millipore Ab5603
NESTIN	1/200	Mouse IgG1	Covance 656802
HOXB4	1/50	Rat IgG	DSHB 112 anti-Hoxb4
PMP22	1/100	Rabbit IgG	Novus biotechne NBP1-67670
GFP	1/500	Goat IgG	Abcam ab5450
GFAP	1/200	Rabbit polyclonal	Dako z 0334
Hoechst 33258	1/2000		Sigma

Supplementary Table 7: List of secondary antibodies used for immunostainings.

Type	Fluorochrome	Dilution	Provider
Goat anti mouse IgM	AF-555	1/500	Life technologies
Rabbit anti mouse IgM	FITC	1/500	Life technologies
Donkey anti-Goat IgG	AF-555	1/500	Life technologies
Donkey anti-Goat IgG	AF-488	1/500	Life technologies
Goat anti chicken IgY	AF-488	1/500	Life technologies
Goat anti chicken IgY	AF-555	1/500	Life technologies
Donkey anti chicken IgY	AF-488	1/200	Jackson laboratories
Goat anti mouse IgG	AF-555	1/500	Life technologies
Goat anti Rabbit IgG	AF-488	1/500	Life technologies
Goat anti Rabbit IgG	AF-555	1/500	Life technologies
Donkey anti Rabbit IgG	AF-647	1/500	Life technologies
Donkey anti Goat IgG	AF-555	1/500	Life technologies
Donkey anti Goat IgG	AF-488	1/500	Life technologies
Donkey anti Rabbit IgG	AF-488	1/500	Life technologies
Donkey anti Rabbit IgG	AF-555	1/500	Life technologies
Donkey anti Rat IgG	AF-488	1/500	Life technologies
Donkey anti Chicken IgY	Cy3	1/500	Life technologies

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation of lentiviral vectors

We selected 16 human transcription factors (TFs) involved in OL differentiation: *ASCL1, AXIN2, MYRF, MYT1, OLIG1, OLIG2, NKX2-2, NKX6-1, NKX6-2, SOX2, SOX8, SOX9, SOX10, ST18, ZEB2* and *ZNF536* (Cahoy JD *et al*., 2008, Liu J *et al*., 2010, Pozniak CD *et al*., 2010, Weng Q *et al*., 2012, Najm FJ *et al*., 2013, Yang N *et al*., 2013). The coding sequences (CDS) of these genes were PCR amplified (Phusion® High-Fidelity DNA Polymerase, NEB, England) either from total human brain cDNA, from plasmids containing the CDS or from hESC-derived OPCs (generated by previously established protocols) (Douvaras P *et al*., 2015). The primers contained EcoRI restriction sites (sequence of primers used for the amplification of the TFs are specified on Supp. Table 3) to allow easy integration in the lentiviral vector FUW-OSKM from which the OSKM was excised (a gift from Rudolf Jaenisch, Addgene plasmid # 20328) (Carey BW *et al*., 2009) (Figure S1). Restriction digestion pattern and DNA sequence analysis were performed to demonstrate the integrity and correct sequence of the cloned TF CDS.

Each TF containing lentiviral vector, as well as the lentiviral plasmid FUW-M2rtTA (Addgene 20342) expressing the reverse tetracycline transactivator (rtTA), were co-transfected with the packaging (psPAX2, Cat No. 12260, Addgene, Cambridge, USA) and the envelope (pMD2. G, Cat No. 12259 Addgene, Cambridge, USA) plasmids into the Lenti-X™ 293T cell line (Cat No. 632180, Clontech, CA, USA) using Fugene transfection reagent (Cat No. E2311, Promega, Madison, WI, USA). Supernatants containing the lentiviral particles were collected after 48hr, filtered through a 0.45μm filter (Millipore) and stored at -80 °C for future use.

The lentiviral reporter system *EF1a-mCherry/MCS5-Sox10-eGFP* was a gift from Prof. Fraser Sim, University of Buffalo, and was produced as the vectors described above. This construct was validated in positive (hESC-derived OPCs, generated as previously described) (Douvaras P *et al*., 2015) and negative (human fibroblast line BJ1) cell lines (data not shown).

Human ESC/iPSC lines and culture conditions

The following hPSC lines were used in this study: the hESC line H9 (WA09, purchased from WiCell Research Institute), the hiPSC ChiPSC6b line (purchased from Takara Bio Inc.), the hiPSC BJ1 line (generated in our lab (Raitano S *et al*., 2015) from BJ fibroblasts, ATCC® CRL-2522™), the hiPSC Sigma line (iPSC EPITHELIAL-1 IPSC0028, purchased from Sigma-Aldrich, USA) two iPSC lines derived from primary progressive multiple sclerosis patients (MS-1001-10006-102/-104, purchased from New York Stem Cell Foundation) and two iPSC lines derived from patients with familial forms of amyotrophic lateral sclerosis with the mutations *SOD1-A4V* (purchased from Coriell Institute) and *C9ORF72* (C9 24-4, Guo W *et al*., 2017). hPSCs were maintained in feeder free conditions using mTeSR1 medium (Stemcell Technologies) on hESC-qualified matrigel (Becton Dickinson), splitting twice a week using EDTA (Lonza).

Generation of neural precursor cells (NPCs) and oligodendrocyte lineage cells from hPSCs

hPSCs were dissociated into single cells (accutase, Sigma), seeded at 20.000 cells/cm^2 on human matrigel-coated plates and cultured in mTESR1 medium containing 1:100 of Rock inhibitor analog (Revitacell, Life Technologies) for 2 days. On day 0, medium was switched to N2B27 medium (DMEM/F12 supplemented with N2 (1:100), B27 (1:50), glutamax (1:100), NEAA (1:100), beta-mercaptoethanol (1:1000), Pen/Strep (1:100), (all from Life Technologies) and 25 μg/ml insulin

(Sigma)), supplemented with the small molecules SB431542 10µM (Tocris) and LDN193189 1µM (Miltenyi Biotec) and 100nM retinoic acid (RA; Sigma). Medium was changed daily until day 8, at which time SB431542 and LDN193189 were withdrawn and 1 µM Smoothened agonist (SAG; EMD Millipore) was added to the medium. On day 12, cells were dissociated and plated for further differentiation.

For the initial screen of the 16 TFs, NPCs were expanded for 4-5 passages in N2B27 media with addition of 0.1 µM RA, 1 µM SAG and 20 ng/µl bFGF (Peprotech). For experiments performed with the 6 selected TFs or *SOX10* alone, NPCs were dissociated on day 12 and immediately subjected to OL lineage differentiation.

For OL lineage differentiation, d12 (or expanded) NPCs were plated at 50.000 cells/cm^2 on poly-L-ornithine/laminin (Sigma) coated dishes and transduced with each lentiviral vector alone, or combinations of viral vectors, at a multiplicity of infection of 1 or 1.5. The day following transduction, medium was changed to OL differentiation medium (N2B27 supplemented with 10 ng/ml of PDGFaa, 10 ng/ml IGF1, 5 ng/ml HGF, 10 ng/ml NT3 (all from Peprotech), 100 ng/ml biotin, 1 µM cAMP, and 60 ng/ml T3 (all from Sigma)) with the addition of 1 µg/ml doxycycline (Sigma). Cells were maintained in OL differentiation medium with doxycycline for 7 (initial screen) or 10 days (screen with selected TFs), changing medium every other day.

O4 purification and cryopreservation

Cells maintained for 10 days in OL differentiation media were purified using O4 microbeads (Miltenyi Biotec), used per manufacturer instructions. Purity was checked in all cases by FACS, obtaining >95% O4$^+$ purity of the isolated population. Purified O4$^+$ cells were used for transcriptome analysis or co-culture with neurons, or cryopreserved till further use. For cryopreservation, the O4$^+$ purified fraction was resuspended in OL differentiation medium and mixed 1:1 with ice-cold ProFreeze medium (Lonza) containing 15% DMSO. Cells were immediately stored in a freezing container at -80 °C overnight and transferred the next day to liquid nitrogen for long-term storage. Post-thawing viability was determined by counting the cells with trypan blue in a hemocytometer.

Generation of OLs from hESCs recombined with *SOX10* and *SOX10-GFP* constructs in the AAVS1 locus

The FRT-cassette containing H9-hESC line described in (Ordovás L *et al*., 2015) was used to recombine a *SOX10* or *SOX10-GFP* containing plasmid (Figure S4) into the *AAVS1* locus following nucleofection. After positive and negative selection using puromycin and FIAU (Ordovás L *et al*., 2015), stable cell lines expressing the *SOX10* or *SOX10-GFP* cassettes in an inducible manner were obtained. Doxycycline response experiments were performed to assess the purity and dose response of the cell lines.

Proper cassette integration was tested using junction assay PCRs using 40 ng of genomic DNA with Go Taq DNA polymerase (Promega) in 10 µl reactions. Primer sequences and PCR program conditions are described in Supp. Table 4. PCR products were loaded on 1.5% agarose (Sigma) gels and visualized with SybrSafe (Invitrogen) on a Gel Doc™ XR+ System (Bio-Rad).

To induce OL differentiation, NPC induction was performed for only 8 days (day 0-5: N2B27 medium containing 10µM SB431542, 1µM LDN193189 and 100nM RA; day 5-8: same medium with addition of 1µM SAG). On day 8, cells were dissociated and seeded at 25.000 cells/cm^2 on poly-L-ornithine/laminin coated dishes in OL differentiation medium with 2 µg/ml doxycycline (optimal concentration previously tested) and cultured for up to 17 days.

Myelination assays in shiverer mouse brain slices

Mouse organotypic cortex slices were established according to Stoppini *et al*. (Stoppini L *et al*., 1991), with modifications. All animal procedures were approved by a Dutch Ethical Committee for animal experiments. Briefly, homozygous shiverer (shi/shi) pups (C3Fe.SWV-Mbpshi, Jackson Laboratory), identified by PCR and confirmed by lack of MBP staining, were sacrificed between postnatal days 3 and 5. The brain was rapidly removed and transferred to ice cold Gey's Balanced Salt Solution (Sigma-Aldrich) containing 5.4 mg/ml glucose and 1% penicillin/streptomycin (P/S, Thermo Fisher Scientific). Fronto-parietal coronal slices (300 µm of thickness) were obtained using a tissue chopper (McIlwain). Slices were cultured on an air-fluid interface at 37°C with 5% CO, using culture plate inserts (0.4 µm pore size, 30 mm diameter, Millipore) in 50% MEMα, 25% HBSS, 25% horse serum, 6.5 mg/mL glucose, 2 mM glutamine, 1% N2 supplement and 1% P/S (all Thermo Fisher Scientific), supplemented with 100ng/ml biotin, 60ng/ml T3, 25 µg/ml insulin, 20 µg/ml ascorbic acid, 1 µg/ml of doxycycline (all Sigma-Aldrich) and 1µM cAMP (Thermo Fisher Scientific). Three days after slicing, slices of homozygous shi/shi were transplanted with 1 x 10^4 purified $O4^+$ cells in 1.0 µL PBS using a Picospritzer. $O4^+$ cells were allowed to mature into myelinating OLs for 10 days after injection, in the absence of doxycycline for the last 5 days of culture, when the slices were fixed (4% formalin in PBS) and stained for primary and secondary antibodies (diluted in 0.05 M Tris, 0.9% NaCl, 0.25% gelatin and 0.5% Triton-X-100 (pH 7.4)). Samples were imbedded in Mowiol 4-88 (Fluka) after which confocal imaging was performed with a Zeiss LSM700 confocal microscope using ZEN software (Zeiss).

Co-culture with neurons

Cortical neurons were generated from WT hPSCs based on previously described methods (Shi Y *et al*., 2012). Neuronal progenitors obtained after 3 neuronal rosette isolations steps and 4 cell passages (~50 days) were seeded at 50.000 cells/ cm^2 on poly-L-ornithine/laminin coated dishes and allowed to mature for 10-14 days in Neural Maintenance Medium (NMM, 1:1 mix of DMEM/F12 and neurobasal media, supplemented with N2 (1:200), B27 (1:100), glutamax (1:100), NEAA (1:200), beta-mercaptoethanol (1:2000), Pen/Strep (1:200), L-glutamine (1mM) (all from Life Technologies) and 2.5 µg/ml of insulin (Sigma).

$O4^+$ cells were purified by immunomagnetic beads from day 8-10 *SOX10*-transduced progeny and seeded at 50.000 cells/ cm^2 on the maturing neurons. Cultures were maintained for 20 days in co-culture media (NMM supplemented with 100 ng/ml of biotin, 1 µM of cAMP, insulin (25 µg/ml), T3 (60 ng/ml), ascorbic acid (AA, 20 µg/ml, Sigma) and 1 µg/ml doxycycline (Sigma)) with partial medium replacement every 2-3 days. Doxycycline was removed from day 10 onwards to assess OL maturation in the absence of *SOX10* overexpression.

Adaptation of co-culture for high-throughput screening:

To adapt the $O4^+$ cell-neuron culture system to 96 and 384 well plate format, purified $O4^+$ cells were seeded at 50.000 cells/cm^2 on neurons generated by culture of cortical NPCs at 50.000 cells/cm^2 and allowed to mature for 2 weeks. $O4^+$ cells were allowed to attach for 2-3 h before addition of the different compounds to the co-culture medium (as described above for co-culture media but without T3 and AA) in the presence of doxycycline. Cell culture conditions used were: control (0.1% DMSO), +T3 (60 ng/ml), +DAPT (N-[(3,5-Difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine-1,1-dimethylethyl ester, 1µM, Tocris), +Pranlukast (22.8 µM, Selleckchem), +Clobetasol propionate (5 µM, Selleckchem)

and +Miconazole (1 µM, Selleckchem). Media with corresponding compounds were exchanged twice a week.

Based on MBP expression, three variables were assessed: total MBP^+ area, number of MBP^+ cells and total intensity of the identified MBP^+ area (MBP^+ Intensity). Differences in the MBP^+ area between control and different compounds/drugs in the co-culture system were tested using matched-pairs t-tests using JMP pro12 from SAS institute (www.sas.com) (Supplementary Figure 5). Variables with p-values less than 0.05 were considered statistically significant.

Flow cytometry and cell sorting

Cells were enzymatically harvested using accutase, centrifuged and resuspended in 100 µl FACS buffer (PBS 1x, 2% fetal bovine serum and 0.02% sodium azide).

To determine O4 expression, cells were incubated with 1/20 dilution of the O4-APC antibody or isotype control (Miltenyi Biotec) for 15 min at 4 °C, washed and resuspended in 150 µl FACS buffer. Cells were analyzed on a FACS Canto flow cytometer using the FACS DIVA software (Becton & Dickinson). After exclusion of cell doublets, gates were established based on the corresponding isotype controls.

To analyze the expression of the mCherry/MCS5-SOX10-eGFP reporter, cells were analyzed on a FACS Aria III (equipped with the 561nm laser) using the FACS DIVA software (Becton & Dickinson). Thresholds were set based on untransduced cells.

$O4^+$ cells and $O4^-$ cells were FACS selected from day 10 *SOX10*-transduced progeny on a FACS Aria III using the FACS DIVA software. To isolate $O4^+$ cells for subsequent cultures, immunomagnetic separation using anti-O4 Microbeads (Miltenyi Biotec) was performed following the manufacturer's recommendations. Purity was checked afterwards by FACS, yielding always >95% of $O4^+$ cells in the purified population.

All results were analyzed using Flow Jo (FlowJo, LLC, USA) and FACS DIVA software (Becton & Dickinson). Flow cytometry and FACS sorting was performed at the KU Leuven Flow Cytometry Facility.

RNA extraction, cDNA synthesis and gene expression

Total RNA was purified using the GenElute™ Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, Saint Louis, MO, USA) and ZR RNA MicroPrep (Zymo Research, CA, USA). After concentration and integrity validation (NanoDrop 1000, Thermo Fisher Scientific, MA USA), cDNA was generated using 0.5-1µg of RNA with SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR kit (Invitrogen, CA, USA) and qRT-PCR was performed in technical triplicates on a ViiA™ 7 Real-Time PCR System with 384-well plate (Applied Biosystems, Carlsbad, CA, USA) with a Platinum® SYBR® Green qPCR SuperMix-UDG w/ROX (Invitrogen, CA, USA) and primers mix at final concentration of 250 nM.

Gene expression (Cycle threshold) values were normalized based on the *GAPDH* (Glyceraldehyde 3-phosphate dehydrogenase) housekeeping gene and the Delta CT calculated. Gene specific primers were designed in exon-exon spanning regions in common domains of all isoforms described for a given gene. To distinguish endogenous from transgene expression, primers were designed covering the 3' untranslated regions and were named as the name of the gene followed by *e* (*ASCL1e, OLIG2e, SOX2e* and *SOX10e*). The efficiency of primers was tested by serial dilutions of cDNA and by calculating the coefficient of regression (R^2). An efficiency of 90-105% with an $R^2 \geq 95\%$ was accepted (see Supp. Table 5 for a list of all qRT-PCR primers used in this study).

Transcriptome analysis by RNA sequencing

Total RNA was purified using the GenElute™ Mammalian Total RNA Miniprep Kit (Sigma). RNA concentration and purity were determined spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies) and RNA integrity was assessed using a Bioanalyzer 2100 (Agilent). Per sample, an amount of 100 ng of total RNA was used as input. Using the Illumina TruSeq® Stranded mRNA Sample Prep Kit (protocol 15031047 Rev.E "October 2013") poly-A containing mRNA molecules were purified from the total RNA input using poly-T oligo-attached magnetic beads. In a reverse transcription reaction using random primers, RNA was converted into first strand cDNA and subsequently converted into double-stranded cDNA in a second strand cDNA synthesis reaction using DNA PolymeraseI and RNAse H. The cDNA fragments were extended with a single 'A' base to the 3' ends of the blunt-ended cDNA fragments after which multiple indexing adapters were ligated, introducing different barcodes for each sample. Finally, enrichment PCR was carried out to enrich DNA fragments that had adapter molecules on both ends and to amplify the DNA in the library. Sequence-libraries of each sample were equimolar pooled and sequenced on an Illumina NextSeq 500 instrument (High Output, 75 bp, Single Reads, v2) at the VIB Nucleomics core (www.nucleomics.be).

Low quality ends and adapter sequences were trimmed off from the Illumina reads. Subsequently small reads (length < 35 bp), ambiguous reads (containing N) and low quality reads (more than 50% of the bases < Q25) were filtered. Processed reads were aligned with Tophat v2.0.8b to the Human reference genome (GRCh38), as downloaded from the Genome Reference Consortium (https://www.ncbi.nlm.nih.gov/grc/human/data). Default Tophat parameter settings were used, except for 'min-intron-length=50', 'max-intron-length=500,000', 'nocoverage-search' and 'read-realign-edit-dist=3'.

Transcriptome datasets used in the comparison were downloaded from Gene Expression Omnibus (GEO). Raw values of different brain cell types from the cortex were obtained from GSE73721 (Zhang Y *et al*., 2016). We also downloaded microarray data of different stages of oligodendrocyte maturation from GSE32589 (Abiraman K *et al*., 2015).

RNA-seq data were aligned using Tophat2 and RPKM values were computed using Cufflink. All RNA-seq data was then normalized using upper quartile normalization. Each sample in the microarray dataset was normalized using linear normalization with a mean intensity of 500. Data across the different publication datasets were then combined with our RNA-seq data based on matching ENSEMBL identifiers. Hierarchical clustering showed that data from each batch would clustered according to the study, therefore an empirical Bayes method (Johnson WE *et al*., 2007) was used to eliminate the batch effects to allow for data comparison. The batch correction was carried out in the statistical software, R treating each individual study as a batch. Principal component analysis and hierarchical clustering were performed using the OmicsOffice package built in TIBCO Spotfire v7.6. Different samples in TIBCO Spotfire were clustered by complete linkage method and their similarity was measured as city-block distance for hierarchical clustering. Visualization of the gene expression was also carried out using TIBCO Spotfire.

Differentially expressed genes were identified using the 'Samr' package in the statistical software, R. The threshold criteria of FDR > 0.05 and a fold change of two or more in gene expression among the different pair-wise comparison was used to identify the differentially expressed genes. Gene ontology

(GO) of the different differentially expressed genes were carried out using the Gene Ontology Consortium (http://www.geneontology.org/).

Immunostainings

Cells were washed with PBS 1x and fixed for 15 min at RT with 4% formaldehyde solution and washed with PBS. Preparations were permeabilized in 0.1% Triton-X-100 (Sigma) (step omitted for O4 and O1 antigens) and blocked in 10% donkey or goat serum (Dako) in PBS for 1h at RT. Primary antibodies were incubated overnight at 4 °C in 5% in donkey or goat serum in PBS, washed 3 times, and incubated for 1h at RT with fluorescently-labeled secondary antibodies diluted to 1:500. Samples were incubated for 15 min at RT with Hoechst 33258 (1:2000 dilution) for nuclear staining and mounted with ProLong® Gold Antifade Mountant (Thermo Fisher Scientific). The list of primary and secondary antibodies and the dilutions used can be found in Supp. Tables 6 and 7.

Image acquisition and analysis

The immunostained cells were examined using an Axioimager.Z1 microscope (Carl Zeiss). For quantification purposes, at least 5 independent fields per condition and per experiment were obtained. Confocal images of the neuron-OL co-cultures were obtained using a C LSM 510 Meta NLO confocal microscope (Carl Zeiss). For the initial screen using the A2B5 marker as well as for the evaluation of the miniaturized co-culture system, images were acquired on an InCell Analyzer 2000 High Content Imager (GE Healthcare), acquiring sufficient numbers of images to cover at least 70% of the well area. For A2B5, quantification was performed using the InCell image analysis software (GE Healthcare). For the co-cultures, batch analysis were performed using a developed script on ImageJ software, where MBP expression and regions of interest were elucidated based on intensity threshold and presence of staining debris were removed from the selection based on size and circularity parameters.

Preparation of transmission electron microscopy samples.

Cells were seeded on Thermanox® slides and fixed with 2% glutaraldehyde in 0.05M sodium cacodylate buffer. Following fixation, cells were postfixed in 2% osmium tetroxide for 1 hour and stained with 2% uranyl acetate in 10% acetone for 20 min. Subsequently, the cell-seeded coverslips were put through a dehydrating series of graded concentrations of acetone and embedded in araldite or durcupan resin (Sigma) according to the pop-off method (Bretschneider A *et al*., 1981). Semi-thin sections (0.5-1 µm) were performed and stained with toluidine blue for the identification of regions of interest. Ultra-thin sections (60-80 nm) were mounted on 0.7% formvar-coated grids, contrasted with uranyl acetate and lead citrate, and examined with a Philips EM 208 transmission electron microscope or JEOL JEM1400 electron microscope operated at 80 kV. Digital images were captured using a Morada camera system. For quantification purposes, images were analyzed using SIS analysis software (Germany).

Statistical analysis

Comparisons between two groups were analyzed using unpaired or paired 2-tailed Student's t test. P-values < 0.05, were considered significant (*). Data are shown as mean and error bars represent standard error of mean (SEM) of a minimum three independent experiments. Results were ploted and analyzed using GraphPad Prism 6 software.

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