Supplementary figures and tables

De Kleijn et al. (2019)

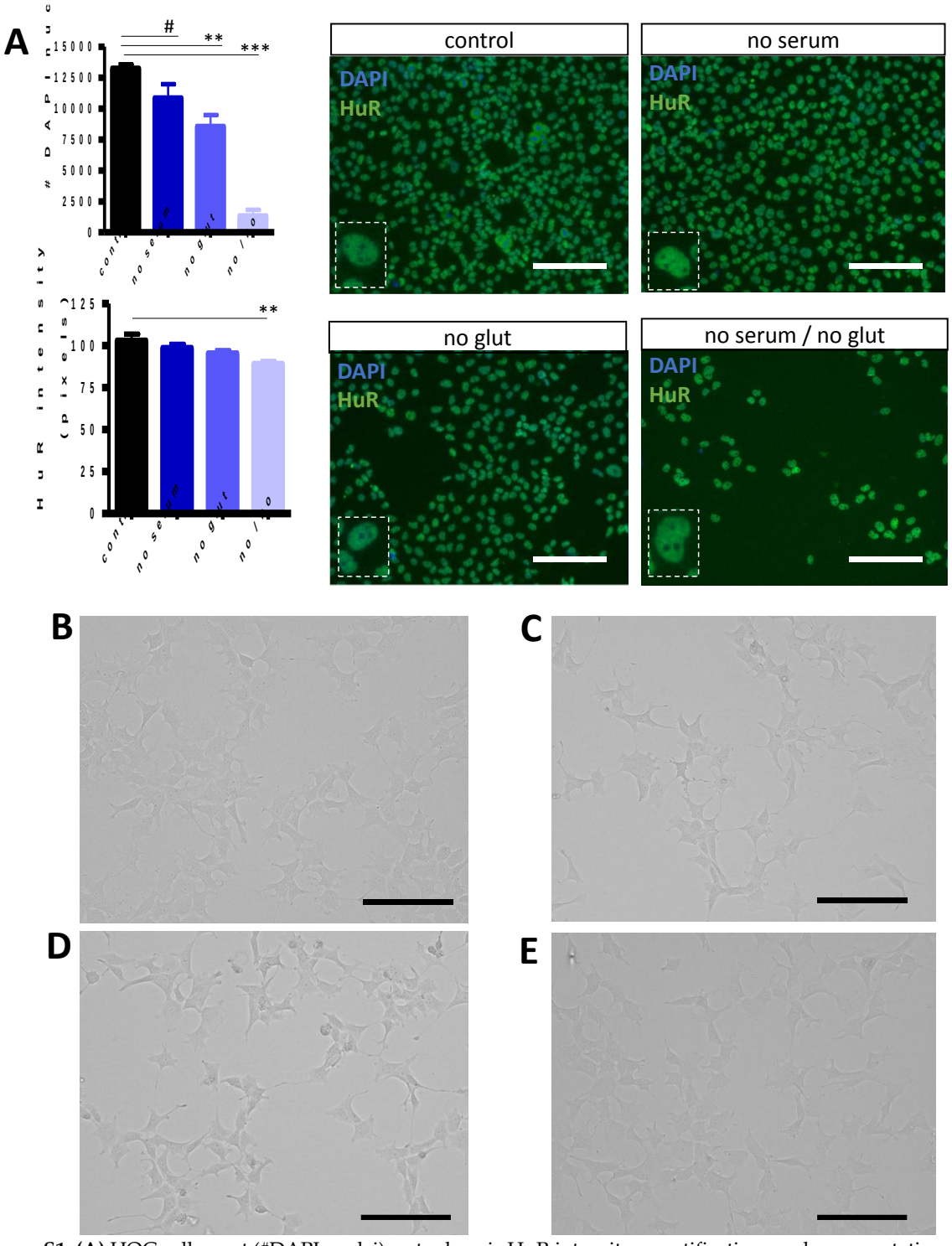

Figure S1. (A) HOG cell count (#DAPI nuclei), cytoplasmic HuR intensity quantifications and representative immunocytochemistry images of HOG cells cultured without serum, without Glutamax (glutamine) and without serum and Glutamax. Based on ANOVA with Tukey-b post-hoc comparisons on four independent experiments (n=4). P-values: * < 0.05, ** < 0.01, *** < 0.001. Morphology of **(B)** undifferentiated HOG and **(C)** HOG differentiated with N2.1 medium, **(D)** N2.2 medium and **(E)** T3. Scale bar = 50 µm.

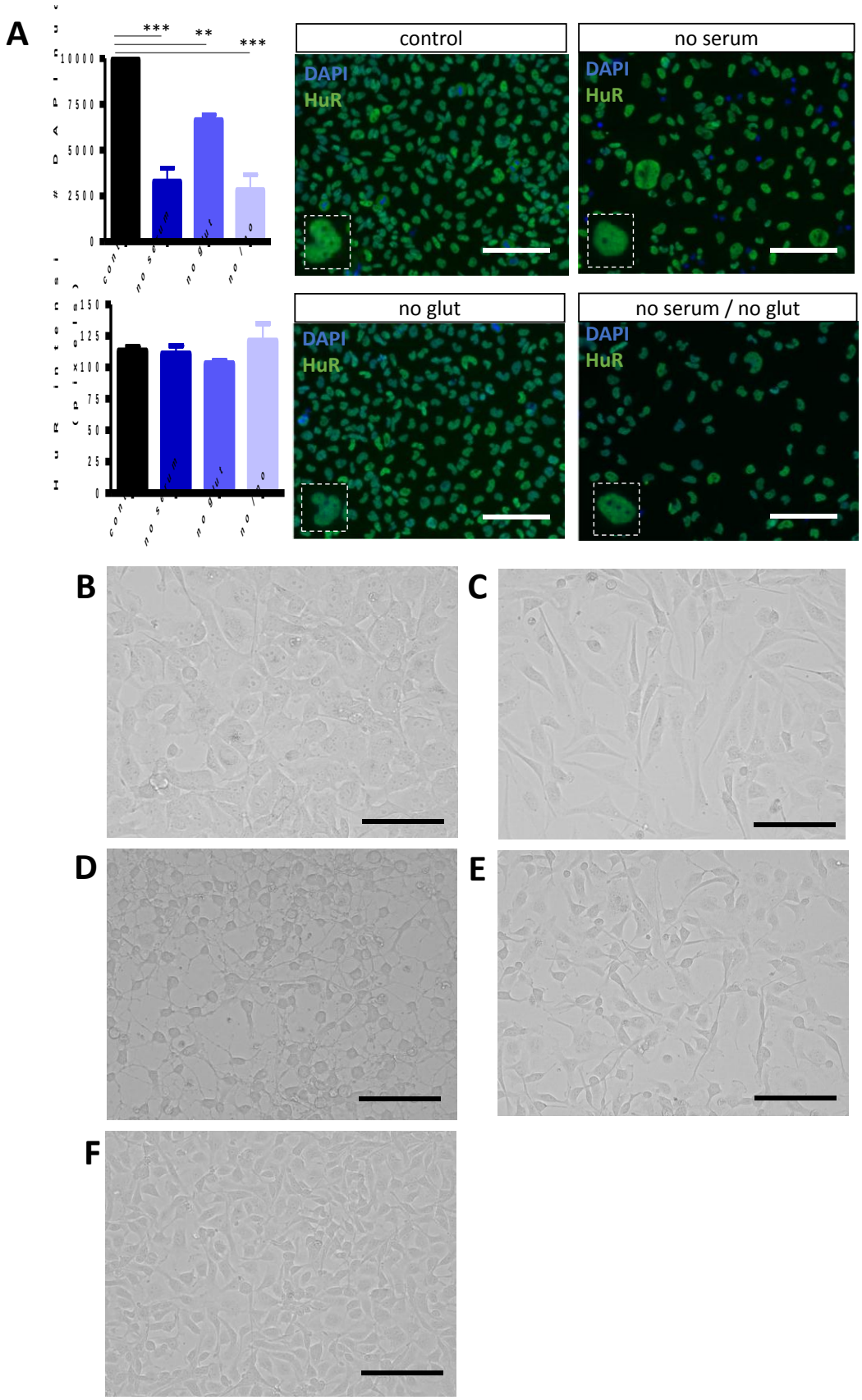

Figure S2. (A) MO3.13 cell count (#DAPI nuclei), cytoplasmic HuR intensity quantifications and representative immunocytochemistry images of MO3.13 cells cultured without serum, without Glutamax (glutamine) and without serum and Glutamax. Based on ANOVA with Tukey-b post-hoc comparisons on four independent experiments (n=4). P-values: * < 0.05, ** < 0.01, *** < 0.001. Morphology of **(B)** undifferentiated MO3.13 and differentiated with **(C)** PMA, **(D)** N2.1 medium, **(E)** N2.2 medium and **(F)** T3. Scale bar = 50 µm.

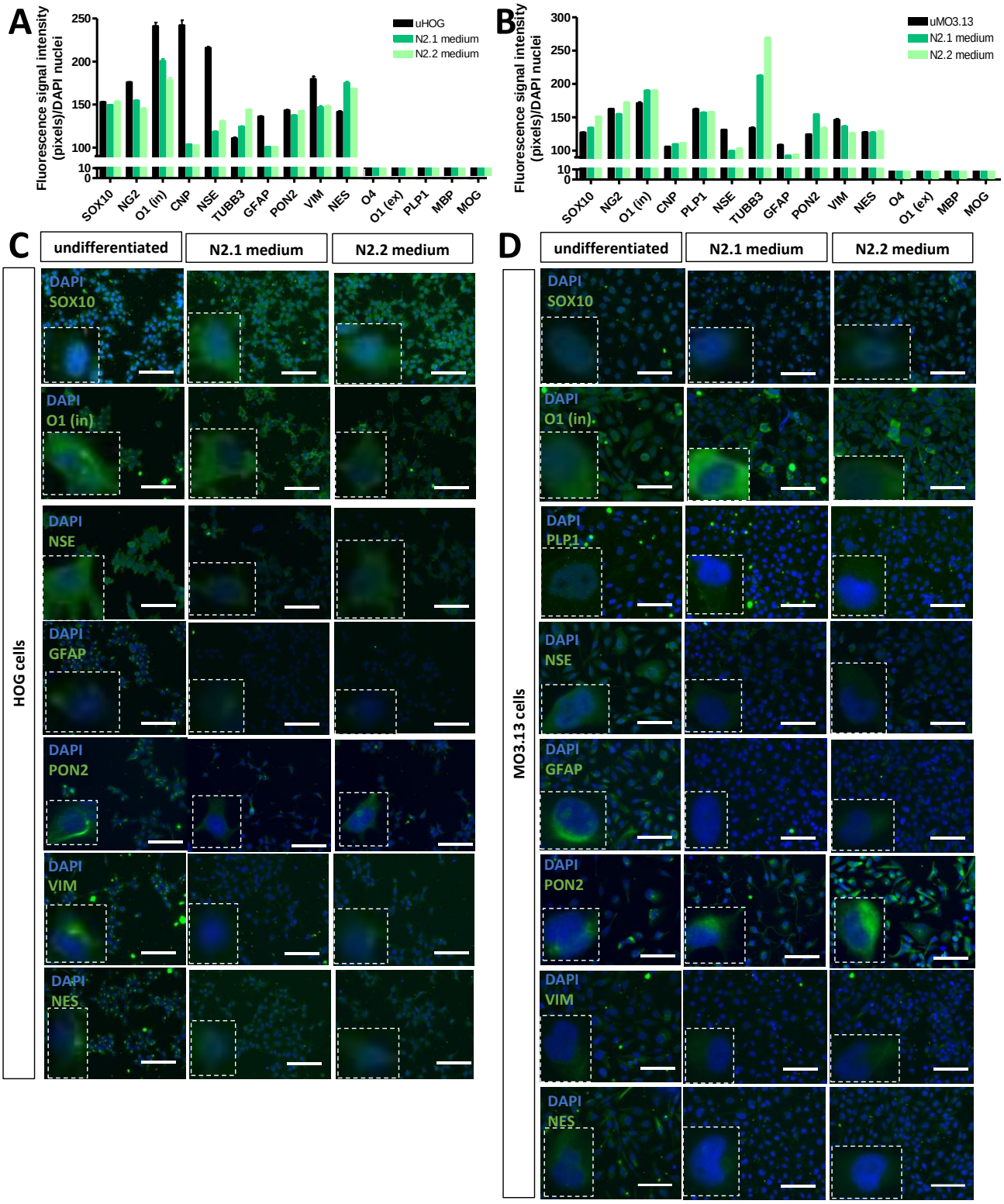

Figure S3. Immunocytochemical analyses in undifferentiated and differentiated HOG and MO3.13 cells. **(A)** Quantification of fluorescence signal in immunocytochemistry for undifferentiated HOG cells (uHOG) and HOG cells differentiated with N2.1 or N2.2 medium. Fluorescence quantification was based on one experiment (n=1), error bars = SEM of total number of DAPI-positive nuclei in one condition. **(B)** Quantification of fluorescence signal in immunocytochemistry for undifferentiated MO3.13 cells (uMO3.13) and MO3.13 cells differentiated with N2.1 or N2.2 medium. Fluorescence quantification was based on one experiment (n=1), error bars = SEM of total number of DAPI-positive nuclei in one condition. **(C)** Representative immunocytochemistry images for each marker in HOG cells and **(D)** MO3.13 cells. Scale bar = 50 μm.

Table S1. Variables and operationalisations used for SH-SY5Y and HOG or SH-SY5Y and MO3.13 co-culture experiments. OL: oligodendrocyte.

Variable	Operationalisation
Coating	- Matrigel 1:100 (Corning) - Poly-L-ornithine (Sigma) + Laminin (Sigma)
Total cell density	- $2.8 \cdot 10^5$ cells/cm^2 - $2.25 \cdot 10^5$ cells/cm^2 - $1.8 \cdot 10^5$ cells/cm^2 - $1.125 \cdot 10^5$ cells/cm^2 - $5.6 \cdot 10^4$ cells/cm^2 - $2.8 \cdot 10^4$ cells/cm^2
OL to neuron ratio	- 1:1 - 1:2 - 1:3 - 1:6 - 1:9 - 1:18
Co-culture duration	- 8 days - Longer than 8 days
Pre-differentiation of OLs in mono-culture	- Yes - No
Time-point of OL addition after neuronal differentiation initiation (day 10)	- Day 10 - Day 11 - Day 14 - Day 17 - Day 19 - Day21
Medium change mode	- 100% of medium - 50% of medium
Basal differentiation medium	- Neurobasal (Thermo Fisher) - Brainphys (STEMCELL technologies)
Medium supplements	- D-Glucose (Merck) - Sodium pyruvate (Gibco) - Glutamax (Gibco) - B27 supplement (Thermo Fisher) - SM1 supplement (STEMCELL technologies) - Culture-one supplement (Thermo Fisher)
Proteins/hormones	- Brain-derived neurotrophic factor (BDNF) (Sigma) - All-trans retinoic acid (ATRA) (Sigma) - Apo-transferrin (Sigma) - Putrescine (Sigma) - Triiodothyronine (T3) (Sigma) - Sodium selenite (Sigma) - Dibutyryl-cAMP (Sigma) - Progesterone (Sigma) - Human insulin (Sigma) - Hydrocortisone (Sigma) - D-biotin (Sigma) - L-Glutamic Acid (Sigma) - IBMX (Sigma) - Ascorbic acid (Sigma) - N-acetyl-cysteine (NAC) (Sigma) - IGF-1 (Cell Guidance Systems)

	- PMA (Sigma) - Lithium chloride (LiCl) - Sodium chloride (NaCl) - Potassium chloride (KCl)	
Small molecules	- Miconazole (Sigma) - Montelukast (Tebu-Bio) - Clobetasol (Sigma) - MDL29,951 (Tebu-Bio)	- Bradykinin (Sigma) - Prosaptide (Sigma) - Carbachol (Sigma) - ATP (Sigma

Table S2. Vendors and concentrations of antibodies used in immunohistochemistry (ICC) and Western blot (WB) experiments.

Target	**Vendor(clone)**	**Dilution for ICC**	**Dilution for WB**
CC1	Abcam (16794)	1:500	
CNPase	Aves Labs	1:250	1:500
DBH	Abcam (ab96615)	1:500	
FluoroMyelin Red	Thermo Fisher Scientific	1:100	
GAPDH	Cell Signaling Technology (14C10)		1:2000
GAP43	Aves Labs	1:250	
GFAP	UC Davis/NIH NeuroMab Facility (N206A/8)	1:500	
HuR	Santa Cruz (3A2)	1:200	
Jagged1	Santa Cruz (E-12)	1:500	
L1CAM	Santa Cruz (5G3)	1:500	
MBP	Aves labs (polyclonal)	1:500	1:2000
MBP	Millipore (SMI99; monoclonal)	1:500	1:500
MOG	Home-made antibody CRICM, Pitié-Salpêtrière, Hospital, Paris	1:50	1:1000
MPZ	Aves Labs	1:250	
NEFL	Santa Cruz (DA2)	1:500	
NES	Millipore (MAB353)	1:500	
NG2	Abcam (ab129051)	1:100	
NSE	Aves Labs	1:250	
O1	Invitrogen	1:250	
O4	Home-made antibody CRICM, Pitié-Salpêtrière, Hospital, Paris	1:2	
PLP1	Abcam (ab28486)	1:500	1:2000
PON2	Santa Cruz (C-5)	1:500	1:250
SOX10	Abcam (EPR4007)	1:100	
TH	Millipore (AB152)	1:500	
TUBB3	Biolegend (801201)	1:1000	1:2000
TUBB3	Biolegend (802001)	1:1000	
VIM	DSHB (40E-C)	1:50	

Table S3. Sequences of primers used in HOG and MO3.13 differentiation experiments. FW: forward primer, RV: reverse primer.

Gene	Sequence	Gene	Sequence
BLBP FW	5′-GAGACAAAGTGGTCATCAGGACTC	MYRF FW	5′-CTTCAGCGTGGTGTCCATGTC
BLBP RV	5′-CCATCCAGGCTAACAACAGACTTA	MYRF RV	5′-GCAGCAAAGAGGGCTGTATGC
CNP FW	5′-GGAGTACGCTCAACAAGATGTGA	NESFW	5′-CGGGCTACTGAAAAGTTCCAG
CNP RV	5′-CACAAAGAGGGCAGAGATGGT	NES RV	5′-ACATCTTGAGGTGCGCCAG
DHH FW	5′-GCCGTGCTTTGGACATCACTA	NEFL FW	5′-GACCCTGGAAATCGAAGCATG
DHH RV	5′-ATCAGCTTTGACCGACACGTG	NEFL RV	5′-TTGATCGTGTCCTGCATAGCG
EGR2 FW	5′-CACGTCGGTGACCATCTTTC	PDGFRa FW	5′-GCCCGAGGAATGGAGTTTTT
EGR2 RV	5′-ATCATGCCATCTCCGGC	PDGFRa RV	5′-GCAGAAAGGTACTGCCTTTCGA
EIF4A2 FW	5′-GGTGACATGGACCAGAAGGAGA	PLP FW	5′-GCTGATGCCAGAATGTATGGTG
EIF4A2 RV	5′-CCCCTCTGCCAATTCTGTGAA	PLP RV	5′-CAATCATGAAGGTGAGCAGGG
FGFR3 FW	5′- TCCTGCTCTGGGAGATCTTCAC	PPIA FW	5′-CAGGGTTTATGTGTCAGGGTGG
FGFR3 RV	5′- TGATCATGTACAGGTCGTGTGTG	PPIA RV	5′- CCATTTGTGTTGGGTCCAGC
GAPDH FW	5′-GTCATGGGTGTGAACCATGAGA	SLC1A3 FW	5′- CTTCTTGGTAACACGGAAAAACC
GAPDH RV	5′-GCATGGACTGTGGTCATGAGTC	SLC1A3 RV	5′- TGGGTAGGGTGGCAGAACTT
GPR17 FW	5′- GAGAGATGCTGAAACTCTCAGC	SOX10 FW	5′-AGGAGAAGGAGGTTGACTGTTGC
GPR17 RV	5′- CAGGGAGAAGTTGGTGATCAGAC	SOX10 RV	5′-AGGTGCAGCCCCTCATCTTTC
GFAP FW	5′-CCAGGACCTGCTCAATGTCAA	TUBB3 FW	5′-GGGAAGTCATCAGTGATGAGCAT
GFAP RV	5′-TCCAGGCTGGTTTCTCGAATC	TUBB3 RV	5′-GAGGCACGTACTTGTGAGAAGAGG
MAG FW	5′-CCAGGGAGCCCATCGAC	VCAN FW	5′- GAATGTCACTCTAATCCCTGTCGT
MAG RV	5′-GGTTGTCCCCTGCCGAG	VCAN RV	5′- TCACATGTCTCGGTATCTTGCTC
MOBP FW	5′-CCGTTCACCTTCCTCAATTCC	VEGF-A FW	5′-GCTCAGAGCGGAGAAAGCATTT
MOBP RV	5′-GCTGGTTCTGGTCTTCTGGC	VEGF-A RV	5′-TCGGCTTGTCACATCTGCAAG
MOG FW	5′-TTTTGATCCCCACTTTCTGAGG	VEGF-C FW	5′-GGGCCAACCGAGAATTTGA
MOG RV	5′-CGTAGCTCTTCAAGGAATTGCC	VEGF-C RV	5′-GCCGTCTGTAACAGCTGCATGT
MBP FW	5′-ACCCAAGATGAAAACCCCGTA	VIM FW	5′-GAAATGGCTCGTCACCTTCGT
MBP RV	5′-TCCGTAGCCAAATCCTGGTCT	VIM RV	5′-GGAAGAGGCAGAGAAATCCTGC
MPZ FW	5′-TGCAGAGGAGGCTCAGTGCTAT	YWHAZ FW	5′-CGCTGGTGATGACAAGAAAGG
MPZ RV	5′-CCTTGGCCTTCTTCTCACTGAC	YWHAZ RV	5′-GAAGTTAAGGGCCAGACCCAGT

Table S4. Differentiation studies in HOG and MO3.13 cells. ↓: a decreased expression relative to undifferentiated cells was reported. ↑: an increased expression relative to undifferentiated cells was reported. X: an absence of expression was reported. √: a presence of expression was reported.

	HOG		MO3.13	
	Undifferentiated	**Differentiated**	**Undifferentiated**	**Differentiated**
NG2				↓ protein [25,34]
PDGFRα		↓ protein [25]		↓ protein [25,12]
Gangliosides (A2B5)	√ lipid [24]	√ lipid [24]	√ lipid [73]	
Olig2			√ protein [59]	↑ mRNA [43] ↑ protein [43] √ protein [59]
CNPase	√ mRNA [14] √ protein [14] X protein [24]	√ mRNA [14] ↑ protein [24]	√ mRNA [14] √ protein [14,73,60] X mRNA [35]	√ mRNA [14] ↑ mRNA [35] ↑ protein [34]
GalC (O1)	√ lipid [25,14] X lipid [24]	↑ lipid [24] √ lipid [25]	√ lipid [14,25,73]	√ lipid [25]
Sulfatide (O4)			√ lipid [73]	
CC1				√ ? [49]
CGT				↑ protein [12]
FynKinase		↑ mRNA [7]		↑ mRNA [7]
OSP			√ protein [60]	
GST-p				↑ protein [53,54]
MBP	√ protein [14] X protein [24]	↑ mRNA [14] ↑ protein [14] X protein [24]	√ mRNA [14,35] √ protein [14,73,37,59]	↑ mRNA [14] ↑ mRNA [43,35] ↑ protein [27,34,73,60,43] √ protein [37,40,38,59,47,50] √ ? [49]
PLP1	X protein [18,21]	↑ protein [21,18,20]	√ protein [60]	↑ protein [34,12]
OMG		↑ mRNA [14]		
MOBP		↑ mRNA [7,14]		↑ mRNA [7]
MOG		↑ mRNA [14]		↑ mRNA [14] √ protein [40, 50]
MAG		↑ mRNA [14]	√ protein [60]	↑ protein [34]
VIM			√ protein [73]	
S100B				↓ protein [34]
GFAP	X protein [14]		X protein [14,59] √ protein [37]	↓ protein [34,37] X protein [59]

PDGFRβ				√ protein [47]
FGFR1				√ protein [47]
FGFR3				√ protein [47]
MAL2	√ protein [21]	↑ protein [21,20]		