

Supplementary Data

Comprehensive Characterization of the Human Neural Stem Cell Line HNSC.100 as a Versatile Model for Neurobiological Research

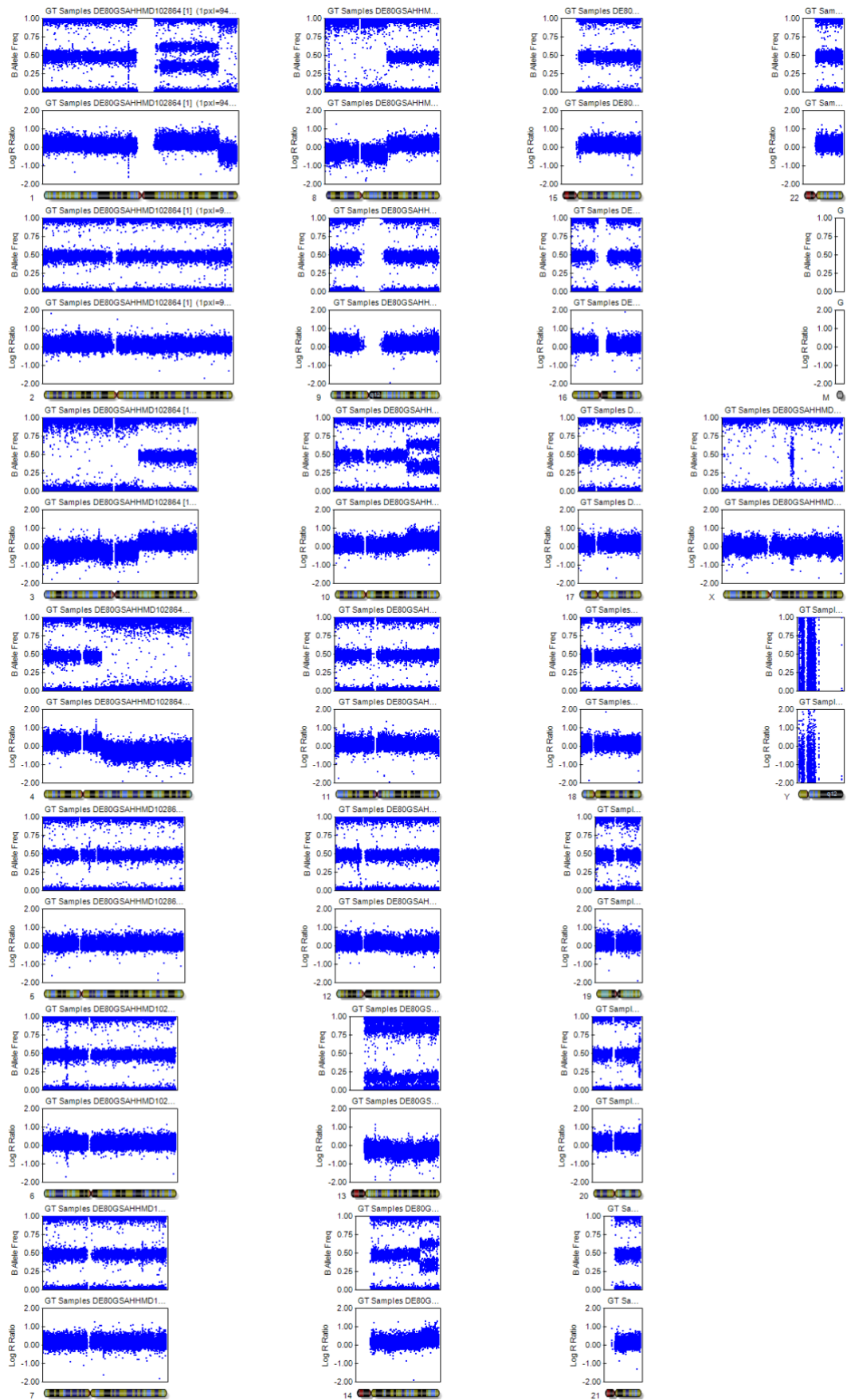
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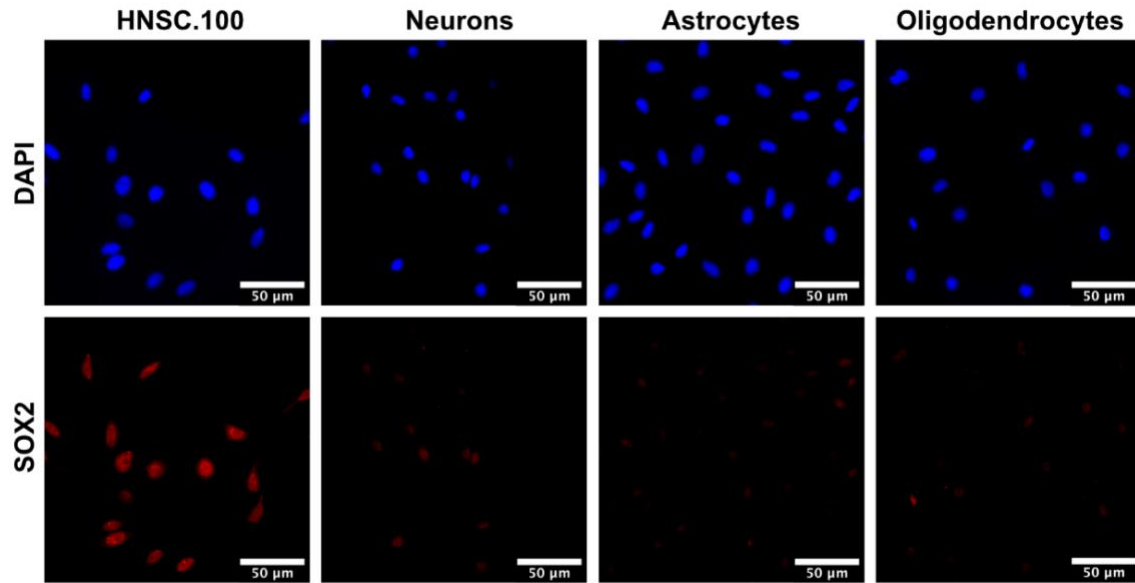
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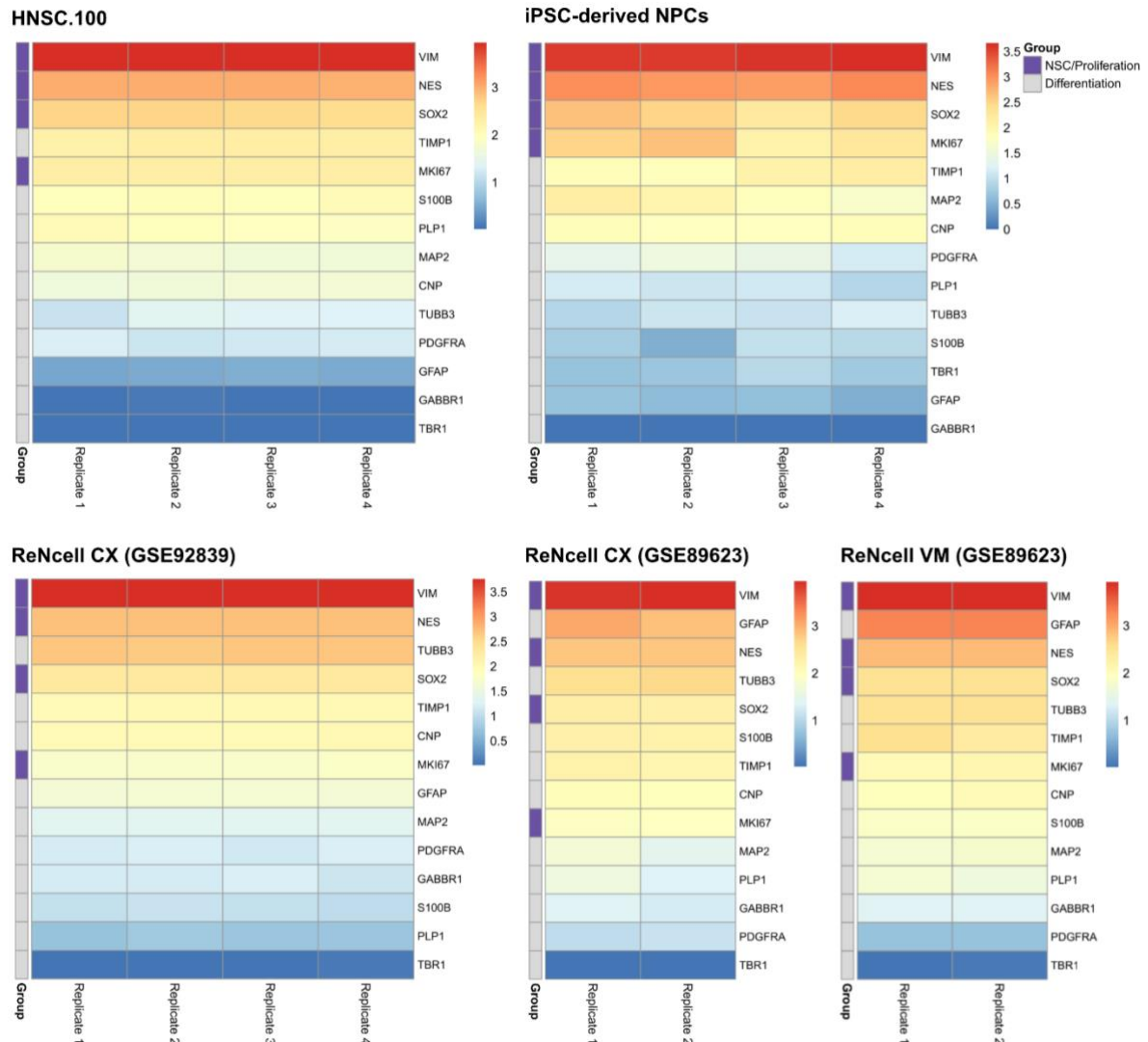
Corresponding author



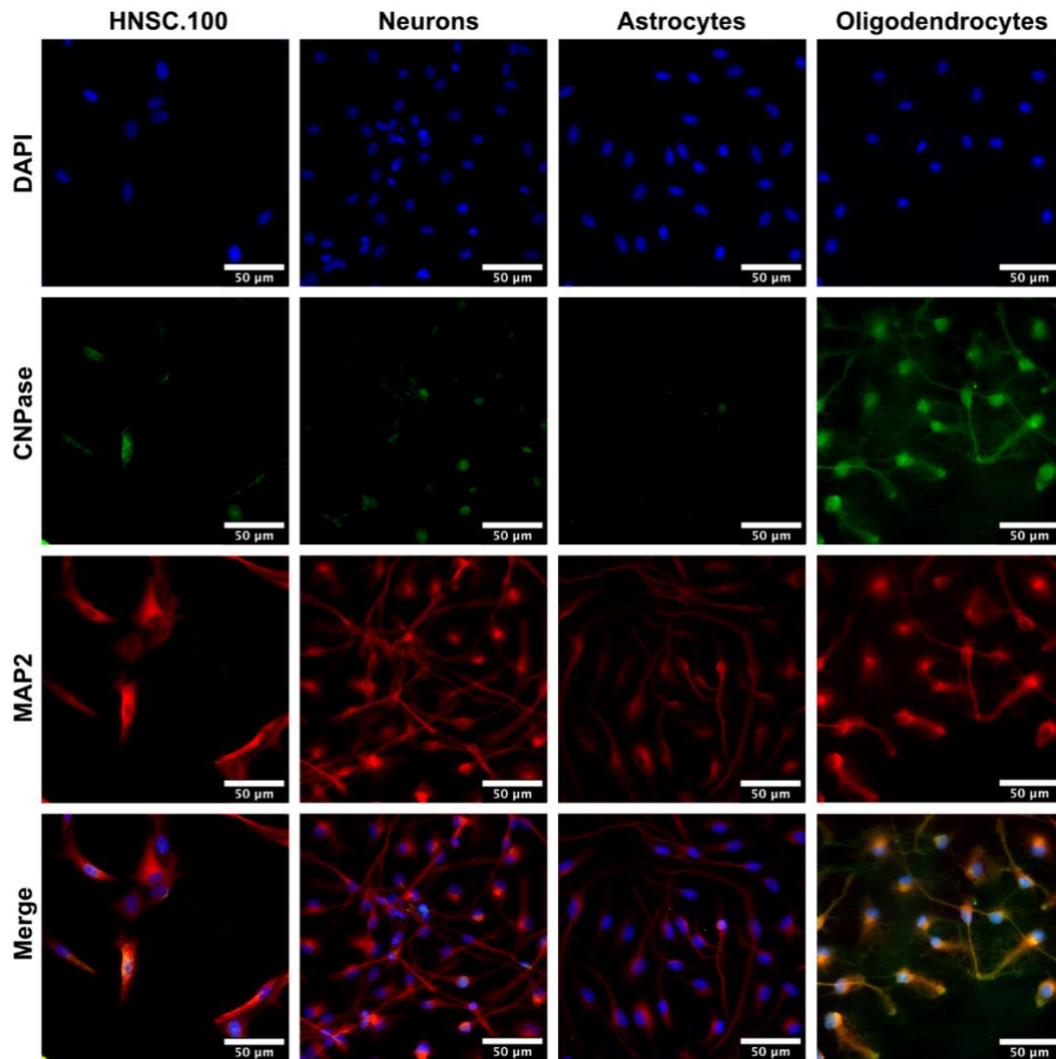
Supplementary Figure 1. Karyotype of HNSC.100 cells.



Supplementary Figure 2. Single-channel visualization of SOX2 staining in undifferentiated and differentiated HNSC.100 cells. Representative immunofluorescence images showing SOX2 (red) and DAPI (blue) channels separately for undifferentiated HNSC.100 cells, neurons, astrocytes and oligodendrocytes.



Supplementary Figure 3. Comparison of lineage marker expression across neural stem cell models. Heatmaps show relative expression of neural stem cell/ proliferation markers (purple) and differentiation markers (grey) representing neuronal, astrocytic, and oligodendrocytic lineages. Data include RNA sequencing from HNSC.100 and publicly available RNA sequencing datasets from the immortalized neural stem cell lines ReNcell CX and VM, as well as iPSC-derived neural progenitor cells (NPCs). The two different RenCell CX heatmaps correspond to independent datasets (GEO accession numbers indicated). Expression values are shown as $\log_{10}(\text{TPM} + 1)$.



Supplementary Figure 4. Co-staining of MAP2 and CNPase in undifferentiated and differentiated HNSC.100 cells. Representative immunofluorescence images showing co-staining of the neuronal marker MAP2 (red), the oligodendrocyte marker CNPase (green), and DAPI (blue) in undifferentiated HNSC.100 cells and cells differentiated into neurons, astrocytes, and oligodendrocytes.

Supplementary Table 1. Tabular representation of major chromosomal abnormalities from Supplementary Figure 1:

chromosome	region	type	copy number	ISCN
3	p26.3–q21.2	deletion	×1	del(3)(p26.3q21.2)
4	q21.1–q35.2	deletion	×1	del(4)(q21.1q35.2)
8	p23.3–q21.12	deletion	×1	del(8)(p23.3q21.12)
10	q23.33–q26.3	duplication	×3	dup(10)(q23.33q26.3)
13	q12.11–q34	deletion	×1	del(13)(q12.11q34)
14	q31.2–q32.33	duplication	×3	dup(14)(q31.2q32.33)
X	complete	deletion	×1	-X

Supplementary Table 2. Conditions for cultivation and differentiation of HNSCs

Cell type	Media reagents (Manufacturer)	Media composition	Plate coating	Differentiation period
HNSC.100	DMEM F12 (11320033, Gibco™), N2 supplement (17502048, Gibco™), BSA (P06-1403020, PAN Biotech), Streptavidin/Penicill in (P06-07100, PAN Biotech), HEPES buffer (P05-01100, PAN Biotech), FBS (A5256801, Gibco™), EGF ⁽¹⁾ FGF-2 ⁽¹⁾	Add 1% 1% 1% 0.5% 0.5% 20ng/ml 20ng/ml	10% Poly-D-lysine (A3890401, ThermoFisher) in DPBS (14190250, ThermoFisher); 30 min at RT	No applicable
Astrocytes	HNSCs medium without EGF and FGF-2	As Above without EGF and FGF-2	10% Poly-D-lysine in DPBS; 30 min at RT	16 days
Neurons ⁽²⁾	Neurobasal medium (21103049, Gibco™), B-27 supplement (17504044, Gibco™), Gluta MAX-1 (35050061, Gibco™), Anti-Anti (15240062, Gibco™)	Add 2% 1% 1%	20 µg/ml of Poly-L-ornithine (A-004-C, Sigma-Aldrich) in H ₂ O; 1h at 37°C 10 µg/ml of laminin (23017015, Gibco™) in H ₂ O; 2h at 37°C	28 days
Oligodendrocytes	<u>1st differentiation:</u> Neurobasal medium B-27 supplement Gluta-MAX-1 Anti-Anti PDGF-AA ⁽¹⁾ FGF-2 ⁽¹⁾ NT3 ⁽¹⁾	Add 2% 1% 1% 20ng/ml 10ng/ml 20ng/ml	10% Poly-D-lysine in DPBS; 30 min at RT	7 days
	<u>2nd differentiation:</u> Neurobasal medium B-27 supplement Gluta MAX-1 Anti-Anti PDGF-AA ⁽¹⁾ T3 (T5516, Sigma-Aldrich)	Add 2% 1% 1% 20ng/ml 30ng/ml	20 µg/ml of Poly-L-ornithine in H ₂ O; 1h at 37°C 10 µg/ml of laminin in H ₂ O; 2h at 37°C	28 days

(1) In-house production, also available at PAN Biotech website (FGF-2: CB-1102024, EGF: CB-1101001) and at the ThermoFisher website (NT-3: 450-03-10UG, PDGF-AA: 100-13A-10UG).

(2) Protocol was taken from:

<https://www.thermoFisher.com/de/de/home/references/protocols/neurobiology/neurobiology-protocols/differentiating-neural-stem-cells-into-neurons-and-glial-cells.html>

Supplementary Table 3: See Excel file Supplementary_Table_3.xlsx

Supplementary Table 4. Search and scoring criteria for each database with respect to neurodevelopment (DDG2P, SysNDD, DBD) and neurodegeneration (PanelApp, DisGeNET, and NHGRI-EBI GWAS Catalog). Database entries were derived from:

- G2P: <https://www.ebi.ac.uk/gene2phenotype/>
- SysNDD: <https://sysndd.dbmr.unibe.ch/>
- DBD: <https://dbd.geisingeradmi.org/>
- PanelApp: <https://panelapp.genomicsengland.co.uk/>
- DisGeNET: <https://disgenet.com/>
- NHGRI-EBI GWAS Catalog: <https://www.ebi.ac.uk/gwas/>

Database	Search Criteria	Rule	Evidence level
DDG2P	All DDG2P entries labeled as “developmental disorder (DD)”	Confidence = “Definitive”	High
		Confidence = “Strong”, “Probable”	Moderate
		Confidence = “Limited”, “Possible” or lower	Low
SysNDD	All curated monogenic NDD genes (entities_category annotated)	Category = “Definitive” and NDD phenotype = TRUE	High
		Category = “Strong” or “Moderate”	Moderate
		All others	Low
DBD	All genes with Tier 1–4 classification (de novo / inherited mutation evidence)	Tier = 1	High
		Tier = 2 or 3	Moderate
		Tier = 4 or AR (autosomal recessive only)	Low
PanelApp	Adult onset neurodegenerative disorder	Panel rating = Green	High
		Panel rating = Amber	Moderate
		Panel rating = Red	Low
DisGeNET	Major neurodegenerative disorders: Alzheimer’s, Parkinson’s, ALS/FTD, Huntington’s, spinocerebellar ataxias, prion diseases, dementia	Score ≥ 0.6	High
		$0.3 \leq \text{Score} < 0.6$	Moderate
		Score < 0.3	Low
GWAS Catalog (NHGRI-EBI)	Filtered for DISEASE/TRAIT terms: “Alzheimer”, “Parkinson”, “amyotrophic lateral sclerosis”, “frontotemporal”, “Huntington”, “prion”, “ataxia”, “dementia”, “neurodegenerative”	$P \leq 5 \times 10^{-8}$	High
		$5 \times 10^{-8} < P \leq 1 \times 10^{-5}$	Moderate
		$P > 1 \times 10^{-5}$	Low

Supplementary Table 5: See Excel file Supplementary_Table_5.xlsx

Supplementary Table 6: See Excel file Supplementary_Table_6.xlsx

Supplementary Table 7. qPCR setups for cell-type validation

Cell type	GOI-specific primers	Sequence	Fluorophore/Q uencher
HNSC.100	SOX2 FP	CTACAGCATGATGCAGGACCA	-
	SOX2 RP	TGCGAGCTGGTCATGGAGTT	-
	SOX2 Probe	TACCCGCAGCACCCGGGCCTCAAT	ROX/BMN.Q620
Astrocytes	GFAP FP	TGGAGAGGAAGATTGAGTCGC	-
	GFAP RP	GTCAAGCTCCACATGGACCT	-
	GFAP Probe	CTCCAGGAGCAGCTGGCCCGACA	ROX/BMN.Q620
	S100 β FP	TGTCTGAGCTGGAGAAGGCC	-
Neurons	S100 β RP	GCTCCTCAGTTCGGATTCT	-
	S100 β Probe	TATTCTGGAAGGGAGGGAGACAAG	ROX/BMN.Q620
	TIMP1 FP	TCCTCCAAGGCTCTGAA	-
	TIMP1 RP	TCAGGCTATCTGGGACC	-
Oligodendrocytes	TIMP1 Probe	CTTCCAGTCCCGTCACCTT	ROX/BMN.Q620
	MAP2 FP	CTGCGTCTGTCTGGAGCTA	-
	MAP2 RP	CGCATATGCAGCAATACCACT	-
	MAP2 Probe	CTCCCGGAGAAGGATTCTGCAGCA	ROX/BMN.Q620
Neurons	TUBB3 FP	ATTCTGGTGGACCTGGAA	-
	TUBB3 RP	CAAAGATGAAATTGTCAGG	-
	TUBB3 Probe	AGGGGCCTTTGGACATCTCT	ROX/BMN.Q620
	GABA-B R1 FP	ACTAACCAAGCGACTGAAAAGA	-
Oligodendrocytes	GABA-B R1 RP	GTTTCATTGCCCGGTAGATTG	-
	GABA-B R1 Probe	ACTTCAACTACAACAACCAGACCATT	ROX/BMN.Q620
	TBR1 FP	GGACGGGGTCTCTGAGCTTC	-
	TBR1 RP	GGGGTACGGGAACATGGCAC	-
Oligodendrocytes	TBR1 Probe	TCTCTCAGTCCAGCCAGCCACAGT	ROX/BMN.Q620
	PDGFRA FP	GACCATCCTGCTGTGGCA	-
	PDGFRA RP	TCAGGGACAGGGTCAATGT	-
	PDGFRA Probe	CATGCGTGTGGACTCAGACAAT	ROX/BMN.Q620
Oligodendrocytes	CNPase FP	TGAGCCCAGGGAGAAGATGG	-
	CNPase RP	ATCTTGTTGAGCGTACTCCTCT	-
	CNPase Probe	ACTTGGTCACCTACTTTGGAAAGAGA	ROX/BMN.Q620
	PLP2 FP	ACCACCTGCCAGTCTATTGC	-
Oligodendrocytes	PLP2 RP	AGCAGGGAAACCAGTGTAGC	-
	PLP2 Probe	CCAGCAAGACCTCTGCCAGTATAGGCA	ROX/BMN.Q620

Supplementary Table 8. Antibodies used for cell-type validation

Cell type	Targeted protein (host)	Dilution	Supplier	Cat. number
HNSC.100	SOX2 (rabbit)	1:500	Proteintech	11064-1-AP
Astrocytes	GFAP (rabbit)	1:500	Proteintech	16825-1-AP
Neurons	MAP2 (rabbit)	1:500	Proteintech	17490-1-AP
Oligodendrocytes	CNPase (mouse)	1:200	Proteintech	66729-1-Ig

Supplementary Table 9. Secondary antibodies used for cell-type validation

Reagent name	target	Dilution	Supplier	Cat. number
Alexa Fluor™ 647	rabbit	1:1,000	Invitrogen	A-31573
Alexa Fluor™ 488	mouse	1:1,000	Invitrogen	A-11001
Alexa Fluor™ 647	mouse	1:1,000	Invitrogen	A-21235