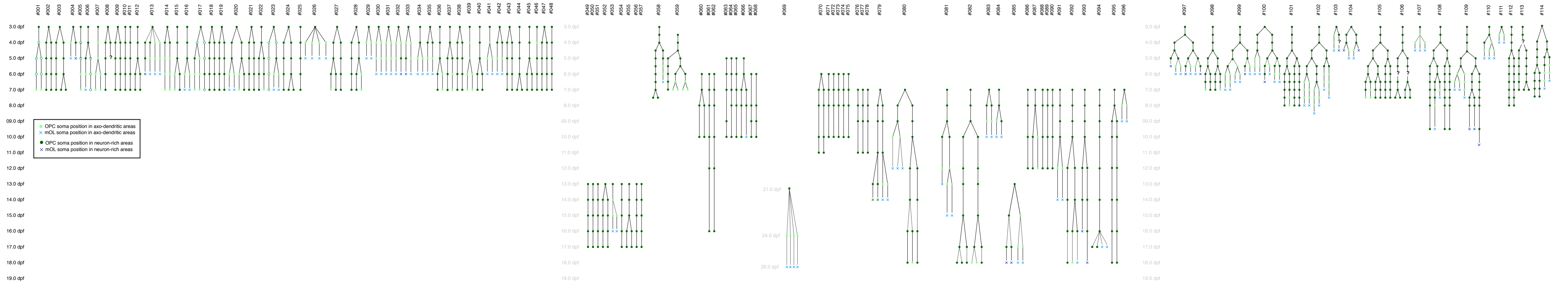


**Supplementary Figure 1: Bioinformatic analysis of sequencing clusters**

- a)** TSNE plot at resolution 1 showing clusters identified from all sequenced olig1:memEYFP cells. Total sample size  $n=310$  cells (OPC #1=110, OPC #2=28, OPC #3=33, OPC #4=18, mOL=19, VLMC1=58, VLMC2=44). This set of cells is the basis for panels a-i.
- b)** TSNE plot at resolution 0.1 of all cells with OPC identity.
- c)** TSNE plot at resolution 2 of all cells with OPC identity.
- d)** TSNE plot at resolution 1 of all cells with oligodendrocyte lineage identity with regression of cell cycle genes.
- e)** Principal component (PC) analysis of olig1:memEYFP-sorted cells considering only cell cycle genes.
- f)** Frequency of markers for cell cycle stages in different clusters of olig1:memEYFP sorted cells.
- g)** Principal component (PC) analysis of olig1:memEYFP-sorted cells after removing the cell cycle PC.
- h)** Heatmap of expression of cell cycle genes considered for regression analysis in the different clusters.
- i)** Principal component analysis of olig1:memEYFP-sorted cells, and with regression of cell cycle genes.



**Supplementary Figure 2: Fate analysis of OPCs with the soma in neuron-rich areas.**

Clonal trees of 114 individually labelled olig1:memEYFP cells positioned with their soma in neuron-rich areas were selected as starting point (from 45 animals in 9 experiments).

**Supplementary Table 1: List of Top 30 Differentially Regulated Genes in OPC clusters #1-#4.**

Total sample size was n=212 cells (#1=110, #2=28, #3=33, #4=18). Differential gene expression was calculated with Seurat FindAllMarkers using the two-tailed Wilcoxon Rank Sum test.

OPC #1		
gene name	avg_logFC	p-value
glula	1.66	3.92E-28
aplnrb	1.58	1.80E-28
gria1a	1.50	6.77E-30
slc1a2b	1.48	3.06E-25
gria2b	1.47	1.33E-30
atcaya	1.42	3.76E-29
wscd1b	1.41	3.57E-24
ncalda	1.36	2.08E-24
mdkb	1.36	9.95E-28
gpm6aa	1.36	2.39E-31
scg2b	1.35	1.29E-27
slc6a1a	1.33	1.34E-24
nrxn1a	1.32	2.07E-24
pcdh2ab9	1.31	1.81E-25
ckbb	1.31	2.66E-28
grin1b	1.31	3.52E-24
tnmem178b	1.30	5.49E-28
camk2n1a	1.30	1.62E-27
olig1	1.29	4.06E-24
nlg3a	1.29	3.46E-27
cspg5a	1.26	2.50E-27
sema5a	1.25	6.44E-28
lrm1	1.22	2.75E-22
fabp7a	1.22	3.72E-24
ndrg3a	1.21	3.51E-25
rassf2a	1.21	3.74E-26
ttyh3b	1.20	6.44E-25
spock3	1.18	1.51E-26
kiaa1549la	1.18	9.75E-24
pygmb	1.17	1.86E-23
cspg4	0.80	2.15E-13

OPC #2		
gene name	avg_logFC	p-value
pcna	2.72	1.38E-20
rpa1	1.99	5.62E-22
slc29a2	1.96	9.20E-17
fen1	1.84	7.88E-26
rrm2	1.83	6.03E-19
dnmt1	1.78	7.24E-19
zgc:110540	1.72	1.92E-25
stmn1a	1.63	9.53E-16
mcm5	1.60	6.46E-09
dnajc9	1.60	2.35E-18
rrm1	1.58	2.97E-23
rbbp4	1.53	3.34E-16
slbp	1.52	5.10E-31
lig1	1.50	6.27E-26
rrm2	1.47	9.29E-14
rpa2	1.44	3.26E-22
mcm2	1.44	4.20E-12
chaf1a	1.41	4.54E-22
nasp	1.41	2.64E-18
atad2	1.40	1.41E-21
mcm3	1.40	4.82E-18
mibp	1.33	2.78E-10
rfc5	1.32	1.33E-17
mcm4	1.30	2.67E-08
sumo3b	1.29	1.29E-14
tubb2b	1.28	2.35E-12
asf1ba	1.21	1.97E-12
dck	1.20	3.25E-18
atad5a	1.20	1.05E-20
cdca7a	1.20	5.14E-11

OPC #3		
gene name	avg_logFC	p-value
kpna2	2.86	1.92E-23
cenpf	2.62	1.50E-27
aspm	2.55	3.40E-28
tuba8l	2.48	2.53E-16
kif11	2.45	1.41E-29
plk1	2.36	5.53E-27
ccnb1	2.23	7.20E-31
tpx2	2.21	2.10E-31
top2a	2.17	1.68E-25
cdk1	2.16	1.42E-26
nusap1	2.15	1.26E-28
ube2c	2.10	4.63E-29
mki67	2.10	2.11E-26
tacc3	2.08	1.44E-34
cdc20	2.06	8.57E-36
cdc14b	2.03	6.80E-23
prc1b	1.94	4.04E-34
kifc1	1.92	2.26E-29
prc1a	1.92	1.75E-27
g2e3	1.91	1.28E-30
ccna2	1.86	9.00E-25
cenpe	1.77	3.17E-29
cdca8	1.75	2.37E-30
ccnf	1.74	2.36E-31
anln	1.73	5.74E-31
masl1	1.73	2.08E-28
mad2l1	1.71	2.13E-31
ccnb3	1.71	9.96E-33
rtkn2a	1.70	2.49E-31
kif2c	1.70	6.93E-27

OPC #4		
gene name	avg_logFC	p-value
gpr17	1.94	8.34E-25
prdm8	1.85	4.93E-13
draxin	1.82	4.46E-12
hmg2	1.81	1.55E-11
hmg2a	1.70	6.68E-11
lmnb1	1.67	7.53E-12
cdk1	1.60	7.18E-13
mki67	1.56	4.63E-13
top2a	1.52	5.23E-11
plk1	1.50	5.03E-12
aspm	1.50	6.28E-13
hmg1a	1.50	3.68E-10
lbr	1.47	3.99E-12
nusap1	1.45	3.37E-13
ccnb1	1.45	3.09E-14
sox9b	1.44	3.71E-10
tuba8l	1.44	9.82E-11
cenpe	1.38	4.52E-15
hmg2b	1.36	9.28E-11
kpna2	1.35	4.94E-09
zeb2a	1.34	9.61E-11
tnr	1.34	9.69E-09
marcksb	1.32	8.60E-11
cenpf	1.31	1.47E-11
sox8b	1.30	8.16E-18
kif11	1.30	1.19E-12
mycb	1.29	1.76E-13
tuba8l	1.27	7.19E-08
wu:fb44b02	1.27	7.67E-16
tpx2	1.27	2.35E-13
myrf	0.97	1.96E-10

**Supplementary Table 2: Primers used for molecular cloning**

Primer name	Sequence
attB1_GCaMP6m_F attB2R_GCaMP6m_R CAAX-GCaMP6m_R attB2R_CAAX_R	GGGGACAAGTTTGTACAAAAAGCAGGCTGCCACCATGGTTCTCATC GGGGACCACCTTTGTACAGAAAGCTGGGTCTCACTTCGCTGTCACTTTGTA TCAGGAGAGCACACACTTGCAGCTCATGCAGCCGGGGCCACTCTCATCAGGAGGTTTCAGCTTCACTTCGCTGTCACTTTGTAC GGGGACCACCTTTGTACAGAAAGCTGGGTCTCAGGAGAGCACACACTTGC
attB1_mCherry_F attB2R_CalEx-R	GGGGACAAGTTTGTACAAAAAGCAGGCTGCCACCATGGTGAGCAAGGGCGAG GGGGACCACCTTTGTACAGAAAGCTGGGTCTCAAGCGACGTCTCCAG
attB4_mfap4 attB1R_mfap4	GGGGACAAGTTTGTATAGAAAAGTTGGCGTTTCTTGGTACAGCTGG GGGGACTGCTTTTTTGTACAACTTGCTTCTCACTCTCTCTCAAC
attB1_synaptophysin_F attB2R_synaptophysin_nostop_R	GGGGACAAGTTTGTACAAAAAGCAGGCTGCCACCATGGATGTTGCC GGGGACCACCTTTGTACAGAAAGCTGGGTCACTTCGTTGGAGAGGATG

**Supplementary Table 3: List of Antibodies and Dyes Used**

Name	Organism / type	Dilution	Company	Catalog number
anti-DSRed	polyclonal rabbit IgG	1:1000	Takara/Clontech	632496
anti-mCherry	polyclonal chicken IgY	1:1000	Novus Biologicals	NBP2-25158
anti-GFP	polyclonal chicken IgY	1:2000	Abcam	ab13970
3A10	monoclonal mouse IgG1	1:10	DHSB	AB_531874
anti Sox10	polyclonal rabbit IgG	1:2000	GeneTex	GTX128374
anti-acetylated tubulin	monoclonal mouse IgG2b	1:2000	Sigma-Aldrich	T7451
anti-MAP2	monoclonal mouse IgG1	1:5000	Abcam	Ab11268
anti-gephyrin	monoclonal mouse IgG1	1:500	Synaptic systems	147 011
anti-chicken Alexa Fluor 488	polyclonal goat IgG	1:1000	Invitrogen	A-11039
anti-chicken Alexa Fluor 555	polyclonal goat IgG	1:1000	Invitrogen	A-32932
anti-rabbit AlexaFluor 555	polyclonal goat IgG	1:1000	Invitrogen	A-21428
anti-rabbit Alexa Fluor 633	polyclonal goat IgG	1:1000	Invitrogen	A-21071
anti-mouse Alexa Fluor 633	polyclonal goat IgG	1:1000	Invitrogen	A-21052
anti-mouse IgG1 Alexa Fluor 555	polyclonal goat IgG	1:1000	Invitrogen	A-21127
anti-mouse IgG2b Alexa Fluor 633	polyclonal goat IgG	1:1000	Invitrogen	A-21146
BODIPY 630/650-X	n/a	1:200	Thermo Fisher	D10000
Opal 650	n/a	1:1000	Perkin Elmer	FP1496001KT