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Supplemental information

Transcriptome profiling of the Olig2-expressing astrocyte subtype reveals their unique molecular signature

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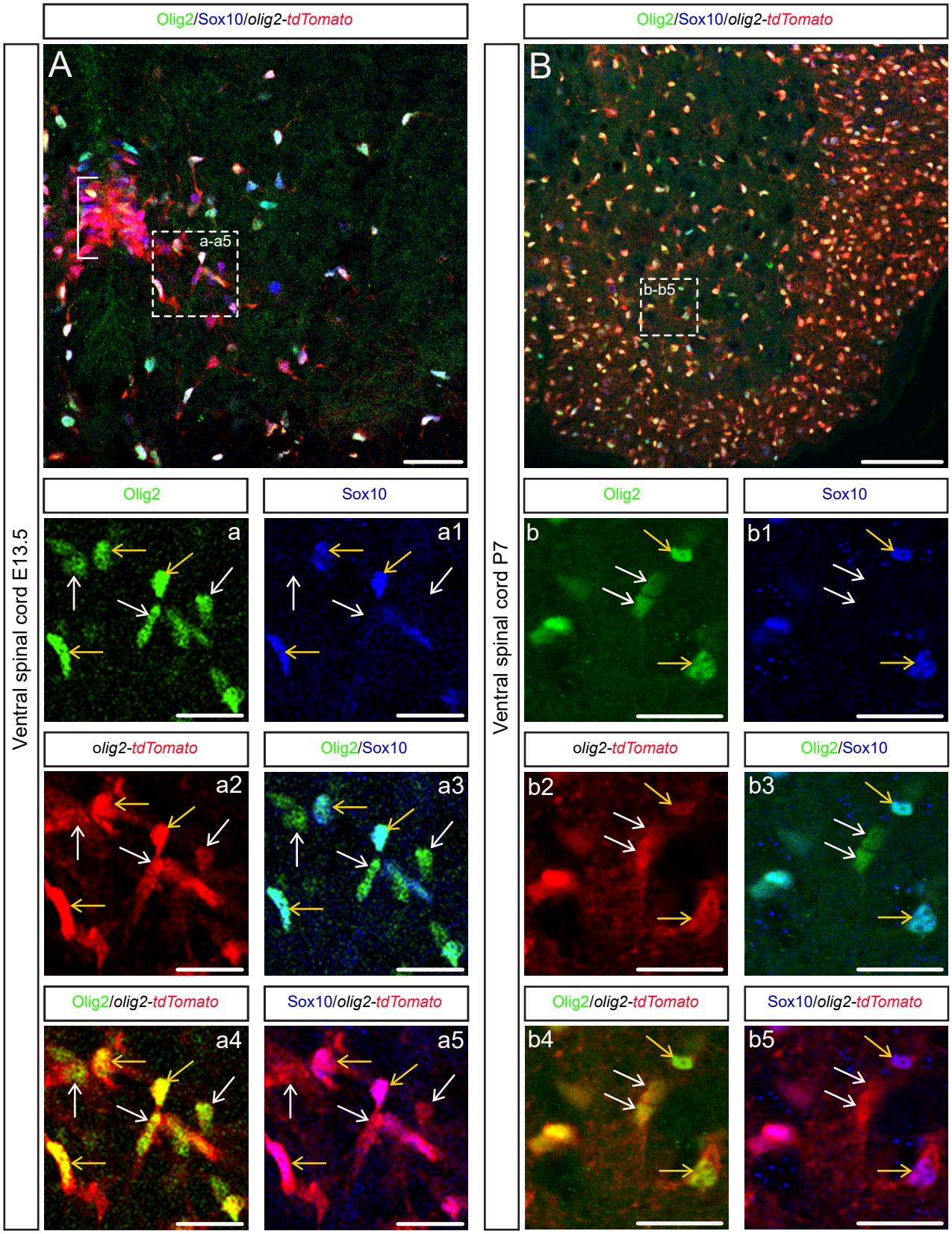

Olig2/Sox10/olig2-tdTomato
A
a-a5
Olig2/Sox10/olig2-tdTomato
B
b-b5
Ventral spinal cord E13.5
Ventral spinal cord P7
Olig2
Sox10
a
a1
b
b1
olig2-tdTomato
Olig2/Sox10
a2
a3
b2
b3
Olig2/olig2-tdTomato
Sox10/olig2-tdTomato
a4
a5
b4
b5

Figure S1

Figure S1: Validation of *olig2*-tdTomato transgenic mouse line by immunodetection of Olig2, Related to Figure 1

A-b5: Combined immunodetection of Olig2 (blue) and Sox10 (green) on *olig2*-tdTomato transgenic mice at E13.5 (A-a5) and P7 (B-b5). Images a-a5 and b-b5 show higher magnification of the areas framed in A and B, respectively, and show successively detection of Olig2 (a, b), Sox10 (a1, b1), tdT (a2, b2), double detection of Olig2 and Sox10 (a3, b3), double detection of Olig2 and tdT (a4, b4) and double detection of Sox10 and tdT (a5, b5). Coloured arrows point to Olig2-AS (white) and OPC/OL (yellow). Bracket in A represents the pMN progenitor domain. Scale bars = 100 µm in B, 50 µm in A and 25 µm in a-a5 and b-b5.

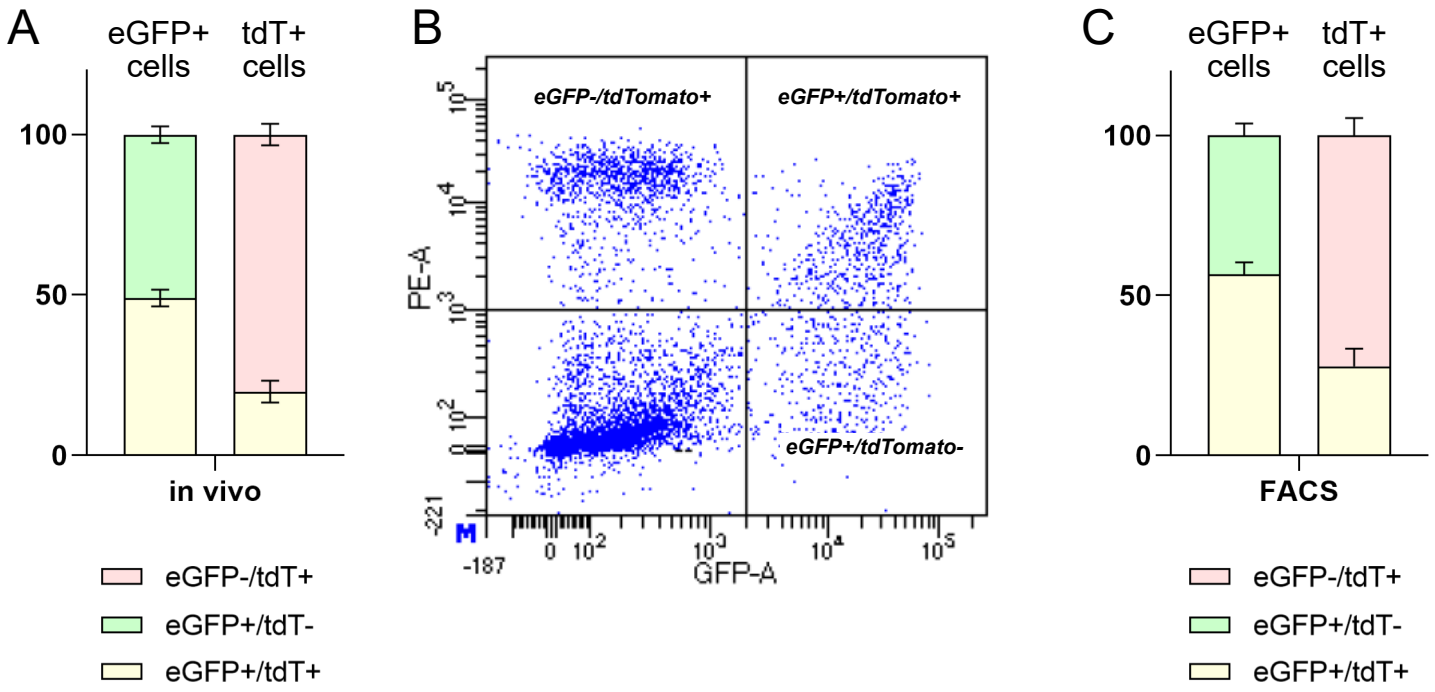
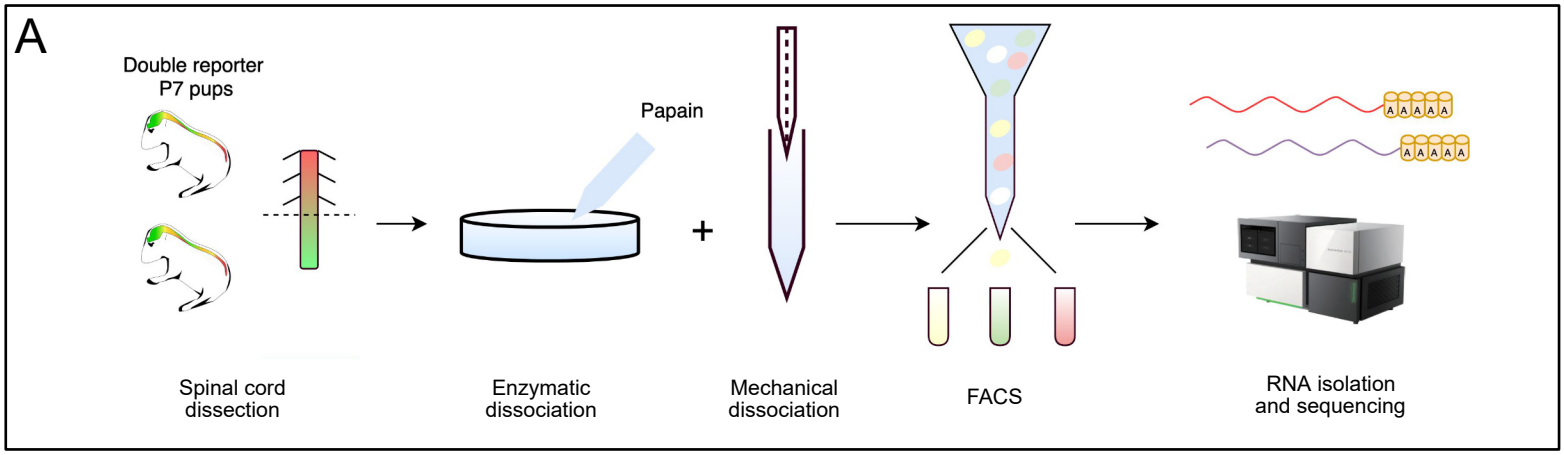
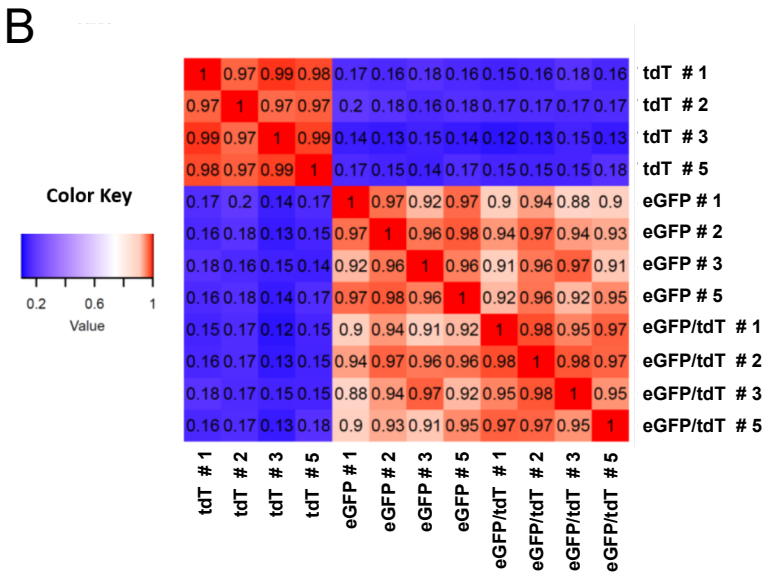

A
eGFP+ cells
tdT+ cells
100
50
0
in vivo
eGFP-/tdT+
eGFP+/tdT-
eGFP+/tdT+
B
eGFP-/tdTomato+
eGFP+/tdTomato+
eGFP+/tdTomato-
PE-A
GFP-A
-221
-187
0
C
eGFP+ cells
tdT+ cells
100
50
0
FACS
eGFP-/tdT+
eGFP+/tdT-
eGFP+/tdT+

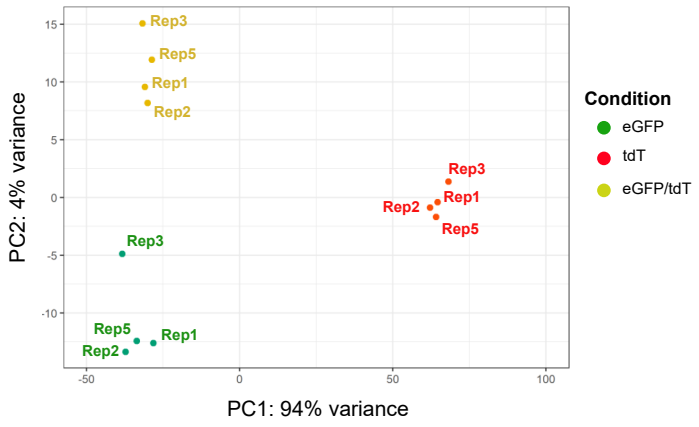
Figure S2

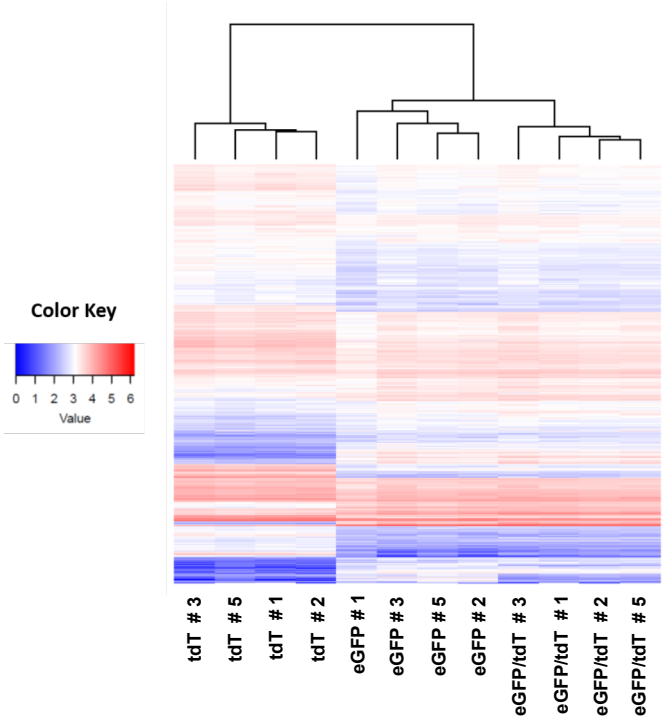
Figure S2: Representativeness of glial cell populations in the P7 spinal cord and after FACS sorting, Related to Figure 1

A: Proportion of Olig2AS (eGFP+/tdT+, green), nonOlig2AS (eGFP+/tdT-, red) and OPC/OL (eGFP-/tdT+, yellow) on the entire population of astrocytes (eGFP+) and td-Tomato positive cells (tdT+) calculated from cell counting performed on P7 spinal cord sections (in vivo, n=4). **B**: FACS dot-plot of a reprsentating experiment showing distribution of the three cell populations. **C:** Proportion of Olig2AS (eGFP+/tdT+, green), nonOlig2AS (eGFP+/tdT-, red) and OPC/OL (eGFP-/tdT+, yellow) on the entire population of astrocytes (eGFP+) and td-Tomato positive cells (tdT+) calculated on ten separated experiments including the ones used for cell sorting. Each experiment corresponds to one spinal cord harvested from a P7 *aldh1L1*-GFP/*olig2*-tdTomato pup. (n=10 animals). Data are presented as mean percentage of cells ± SEM.


A
Double reporter
P7 pups
Papain
+
Spinal cord
dissection
Enzymatic
dissociation
Mechanical
dissociation
FACS
RNA isolation
and sequencing


B
Color Key
0.2
0.6
1
Value
tdT # 1
tdT # 2
tdT # 3
tdT # 5
eGFP # 1
eGFP # 2
eGFP # 3
eGFP # 5
eGFP/tdT # 1
eGFP/tdT # 2
eGFP/tdT # 3
eGFP/tdT # 5


C
Rep3
Rep5
Rep1
Rep2
Rep3
Rep1
Rep2
Rep5
Rep3
Rep5
Rep2
Rep1
PC2: 4% variance
PC1: 94% variance
Condition
eGFP
tdT
eGFP/tdT


D
Color Key
0 1 2 3 4 5 6
Value
tdT # 3
tdT # 5
tdT # 1
tdT # 2
eGFP # 1
eGFP # 3
eGFP # 5
eGFP # 2
eGFP/tdT # 3
eGFP/tdT # 1
eGFP/tdT # 2
eGFP/tdT # 5

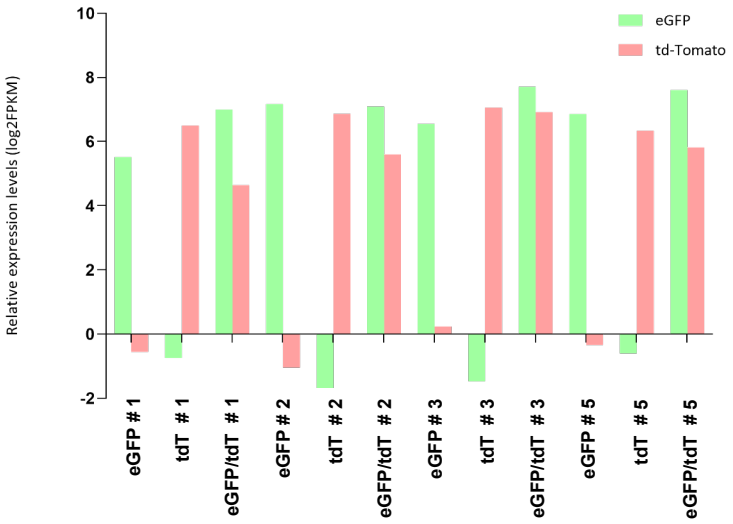

E
eGFP
td-Tomato
Relative expression levels (log2FPKM)
eGFP # 1
tdT # 1
eGFP/tdT # 1
eGFP # 2
tdT # 2
eGFP/tdT # 2
eGFP # 3
tdT # 3
eGFP/tdT # 3
eGFP # 5
tdT # 5
eGFP/tdT # 5

Figure S3

Figure S3: Cell isolation strategy and validation of the transcriptome profiling, Related to Figure 2

A: Schematic of the experimental procedure from tissue dissection to RNA sequencing. **B**: Heatmap of Pearson correlation between each sample. Note high correlation coefficients between samples of the same group. **C**: Principal component analysis (PCA) on all detected mRNAs normalized read count, showing a clear distinction between oligodendrocytes and astroglial subpopulations for mRNA based on the first principal components (PC1). Grouping of the messenger RNA dots suggests separation by cell type. **D**: Euclidian unsupervised hierarchical clustering of the standard deviation on normalized expression levels from the 20% most variable mRNAs. **E:** Comparison of eGFP and tdTomato gene expression levels (by log2FPKM values) for each replicate of the three cell populations. All heatmaps are presented as z-score.

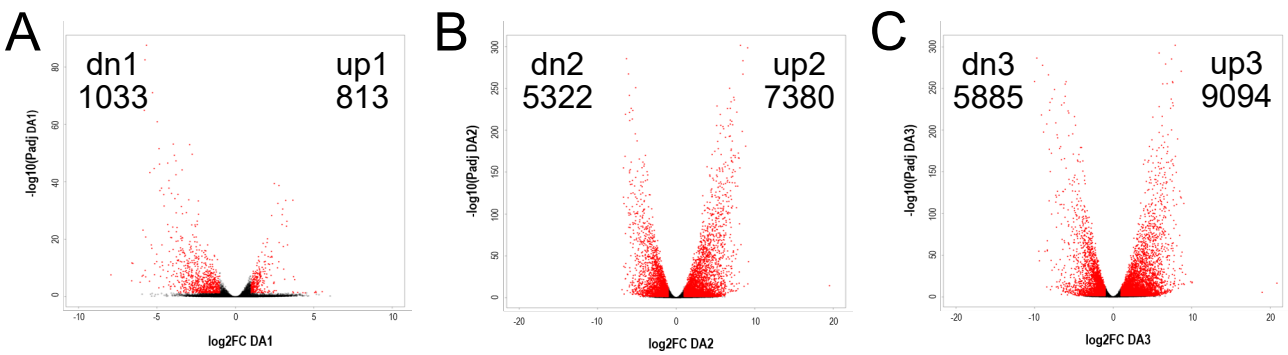

A
dn1
1033
up1
813
-log10(Padj DA1)
log2FC DA1
B
dn2
5322
up2
7380
-log10(Padj DA2)
log2FC DA2
C
dn3
5885
up3
9094
-log10(Padj DA3)
log2FC DA3

Figure S4

Figure S4: Differential analysis between Olig2-AS, nonOlig2-AS and OPC/OL, Related to Figure 3 **A-C**: Volcano plots of differentially expressed genes representing the log2FC and –log10Padj from the differential analysis DA1 (A), DA2 (B) and DA3 (C). Red dots represent transcripts with a significant padj<0.05 and a log2FC>0 or a log2FC<0.

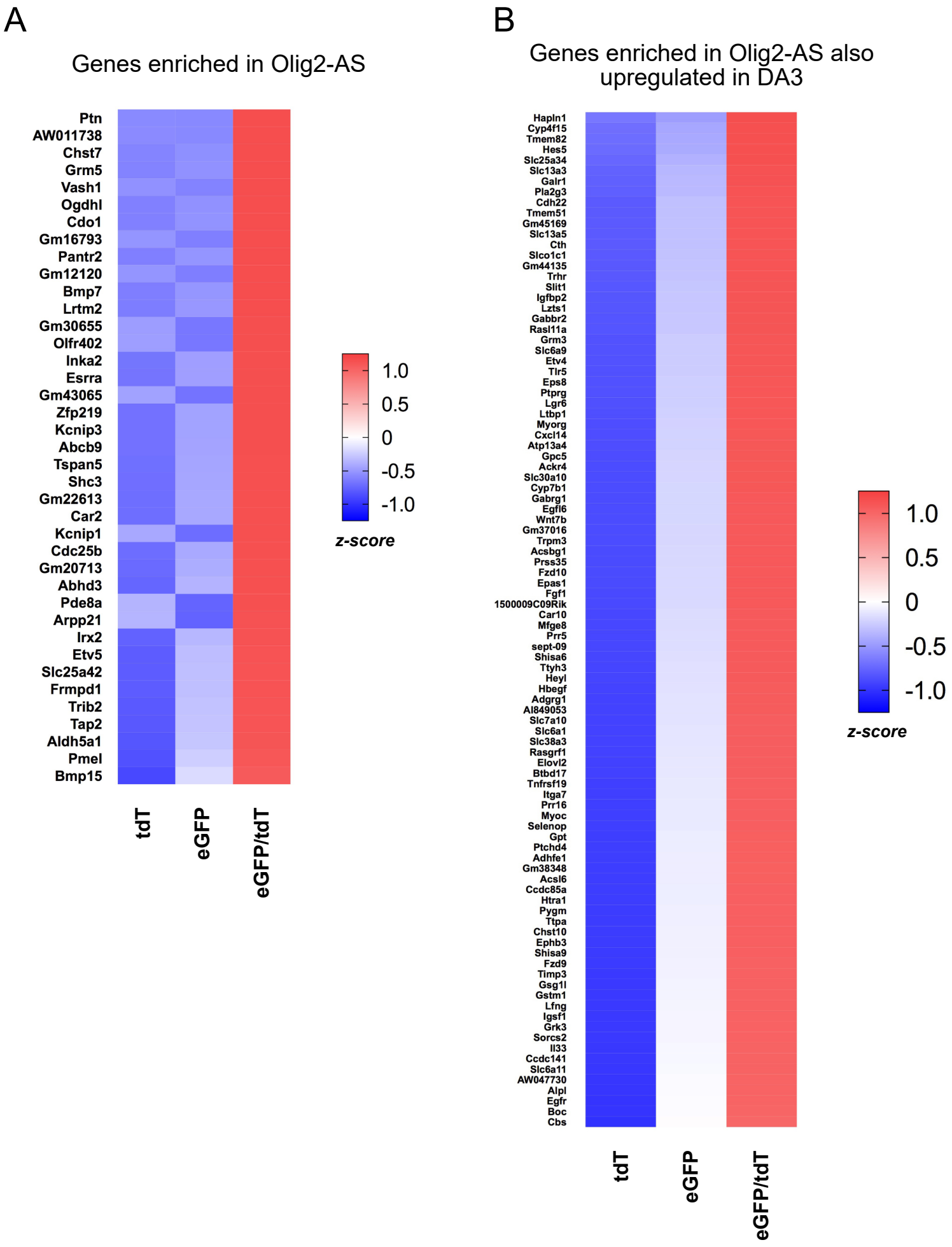

A
Genes enriched in Olig2-AS
Ptn
AW011738
Chst7
Grm5
Vash1
Ogdhl
Cdo1
Gm16793
Pantr2
Gm12120
Bmp7
Lrtm2
Gm30655
Olfr402
Inka2
Esrra
Gm43065
Zfp219
Kcnip3
Abcb9
Tspan5
Shc3
Gm22613
Car2
Kcnip1
Cdc25b
Gm20713
Abhd3
Pde8a
Arpp21
Irx2
Etv5
Slc25a42
Frmpd1
Trib2
Tap2
Aldh5a1
Pmel
Bmp15
tdT
eGFP
eGFP/tdT
1.0
0.5
0
-0.5
-1.0
z-score
B
Genes enriched in Olig2-AS also upregulated in DA3
Hapln1
Cyp4f15
Tmem82
Hes5
Slc25a34
Slc13a3
Galr1
Pla2g3
Cdh22
Tmem51
Gm45169
Slc13a5
Cth
Slco1c1
Gm44135
Trhr
Slit1
Igfbp2
Lzts1
Gabbr2
Rasl11a
Grm3
Slc6a9
Etv4
Tlr5
Eps8
Ptprg
Lgr6
Ltbp1
Myorg
Cxcl14
Atp13a4
Gpc5
Ackr4
Slc30a10
Cyp7b1
Gabrg1
Egfl6
Wnt7b
Gm37016
Trpm3
Acsbg1
Prss35
Fzd10
Epas1
Fgf1
1500009C09Rik
Car10
Mfge8
Prr5
sept-09
Shisa6
Ttyh3
Heyl
Hbegf
Adgrg1
AI849053
Slc7a10
Slc6a1
Slc38a3
Rasgrf1
Elovl2
Btbd17
Tnfrsf19
Itga7
Prr16
Myoc
Selenop
Gpt
Ptchd4
Adhfe1
Gm38348
Acsl6
Ccdc85a
Htra1
Pygm
Ttpa
Chst10
Ephb3
Shisa9
Fzd9
Timp3
Gsg1l
Gstm1
Lfng
Igsf1
Grk3
Sorcs2
Il33
Ccdc141
Slc6a11
AW047730
Alpl
Egfr
Boc
Cbs
tdT
eGFP
eGFP/tdT
1.0
0.5
0
-0.5
-1.0
z-score

Figure S5

Figure S5: List of genes found up-regulated in Olig2-AS, Related to Figure 3

A, B: Heatmaps depicting the abundance of mRNA corresponding to the 135 genes enriched in Olig2-AS, distinguishing the 39 genes specifically enriched in Olig2-AS (A) and the 96 genes as well enriched in Olig2-AS but also found up-regulated in nonOlig2-AS compared to OPC/OL (DA3). All heatmaps are presented as z-score.

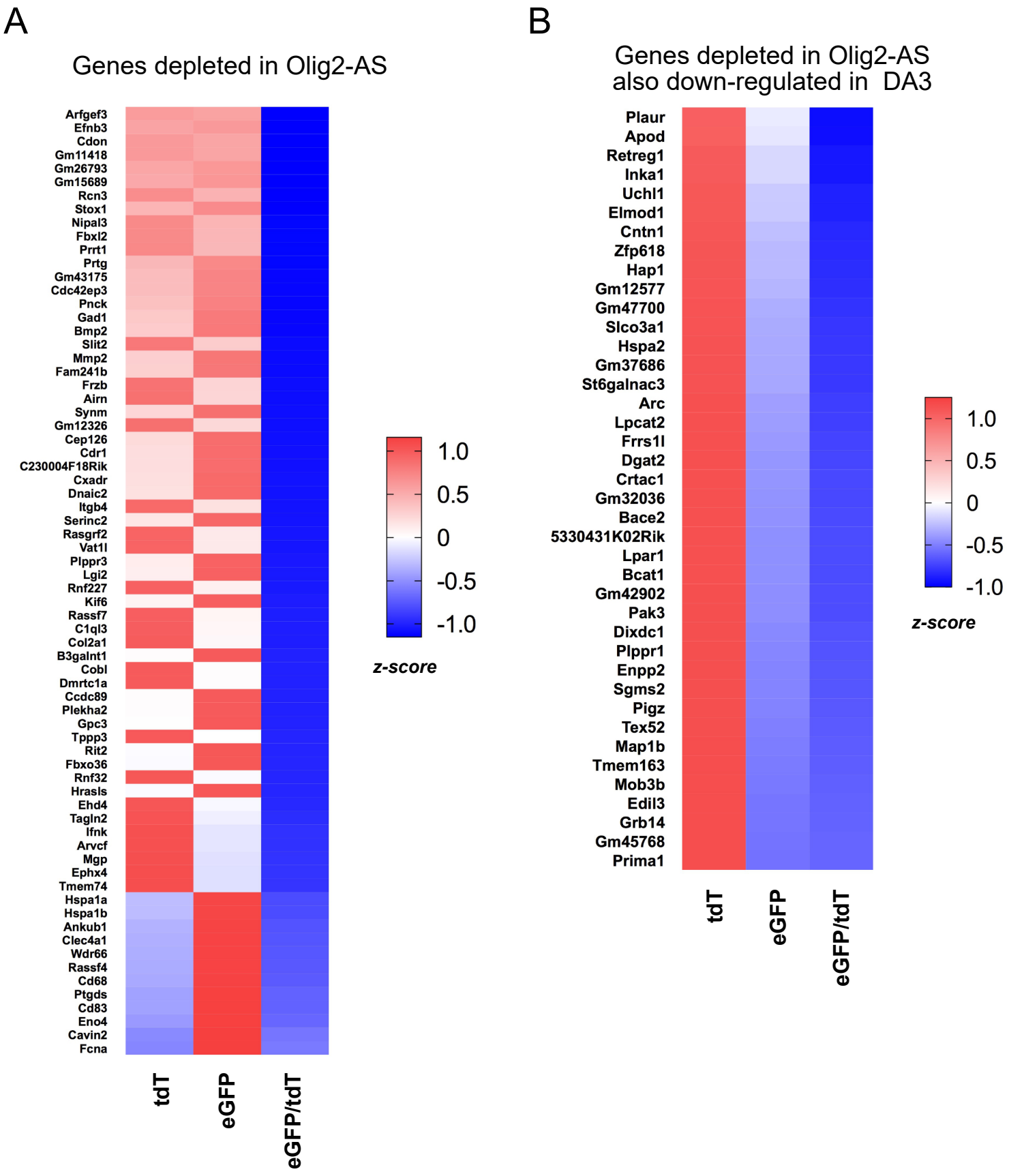

A
Genes depleted in Olig2-AS
Arfgef3
Efnb3
Cdon
Gm11418
Gm26793
Gm15689
Rcn3
Stox1
Nipal3
Fbxl2
Prrt1
Prtg
Gm43175
Cdc42ep3
Pnck
Gad1
Bmp2
Slit2
Mmp2
Fam241b
Frzb
Airn
Synm
Gm12326
Cep126
Cdr1
C230004F18Rik
Cxadr
Dnaic2
Itgb4
Serinc2
Rasgrf2
Vat1l
Plppr3
Lgi2
Rnf227
Kif6
Rassf7
C1ql3
Col2a1
B3galnt1
Cobl
Dmrtc1a
Ccdc89
Plekha2
Gpc3
Tppp3
Rit2
Fbxo36
Rnf32
Hrasls
Ehd4
Tagln2
Ifnk
Arvcf
Mgp
Ephx4
Tmem74
Hspa1a
Hspa1b
Ankub1
Clec4a1
Wdr66
Rassf4
Cd68
Ptgds
Cd83
Eno4
Cavin2
Fcna
tdT
eGFP
eGFP/tdT
1.0
0.5
0
-0.5
-1.0
z-score
B
Genes depleted in Olig2-AS
also down-regulated in DA3
Plaur
Apod
Retreg1
Inka1
Uchl1
Elmod1
Cntn1
Zfp618
Hap1
Gm12577
Gm47700
Slco3a1
Hspa2
Gm37686
St6galnac3
Arc
Lpcat2
Frrs1l
Dgat2
Crtac1
Gm32036
Bace2
5330431K02Rik
Lpar1
Bcat1
Gm42902
Pak3
Dixdc1
Plppr1
Enpp2
Sgms2
Pigz
Tex52
Map1b
Tmem163
Mob3b
Edil3
Grb14
Gm45768
Prima1
tdT
eGFP
eGFP/tdT
1.0
0.5
0
-0.5
-1.0
z-score

Figure S6

Figure S6: List of genes found down-regulated in Olig2-AS, Related to Figure 3

A, B: Heatmaps depicting the abundance of mRNA corresponding to the 110 genes down-regulated in Olig2-AS, distinguishing the 70 genes specifically depleted in the Olig2-AS (A) and the 40 genes as well decreased in Olig2-AS but also found down-regulated in nonOlig2-AS compared to OPC/OL (DA3). All heatmaps are presented as z-score.

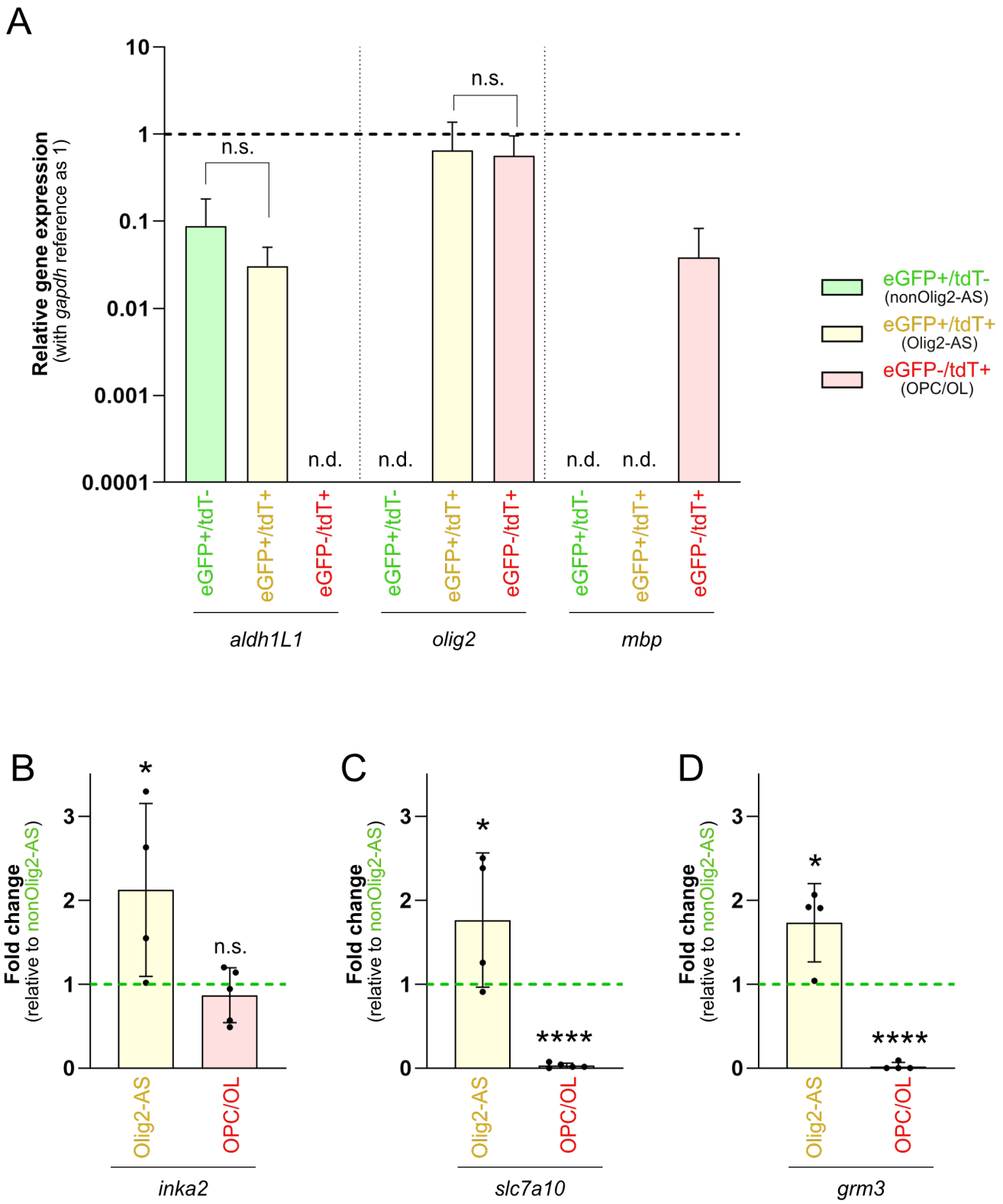

A
Relative gene expression
(with gapdh reference as 1)
10
1
0.1
0.01
0.001
0.0001
n.s.
n.s.
n.d.
n.d.
n.d.
n.d.
eGFP+/tdT-
eGFP+/tdT+
eGFP-/tdT+
aldh1L1
olig2
mbp
eGFP+/tdT-
(nonOlig2-AS)
eGFP+/tdT+
(Olig2-AS)
eGFP-/tdT+
(OPC/OL)
B
C
D
Fold change
(relative to nonOlig2-AS)
*
n.s.
****
Olig2-AS
OPC/OL
inka2
slc7a10
grm3

Figure S7

Figure S7: Validation of a selection of Olig2-AS enriched genes by qRT-PCR, Related to Figure 3 **A**: Quantitative RT-PCR of known glial markers *aldh1L1*, *olig2* and *mbp* among the three FACS-sorted cell populations from P7 *aldh1L1*-GFP/*olig2*-tdTomato mice. The qRT-PCR results are presented as relative gene expression related to *gapdh* expression reference value as one, over a logarithmic scale. p value for each glial marker gene was determined using one-way ANOVA and a Tukey post hoc test. **B-D**: Quantitative RT-PCR of *inka2* (B), *slc7a10* (C) and *grm3* (D) among the three FACS-sorted cell populations. The qRT-PCR results are presented as the fold-change relative to the eGFP+/tdT- population (green dotted line) using the $2^{-\Delta\Delta Ct}$s method. Each dot represents the analysis of two pooled P7 pups prior to FACS sorting. p values were determined using Student's t test. (n = 4-5, two pooled individual FACS samples)

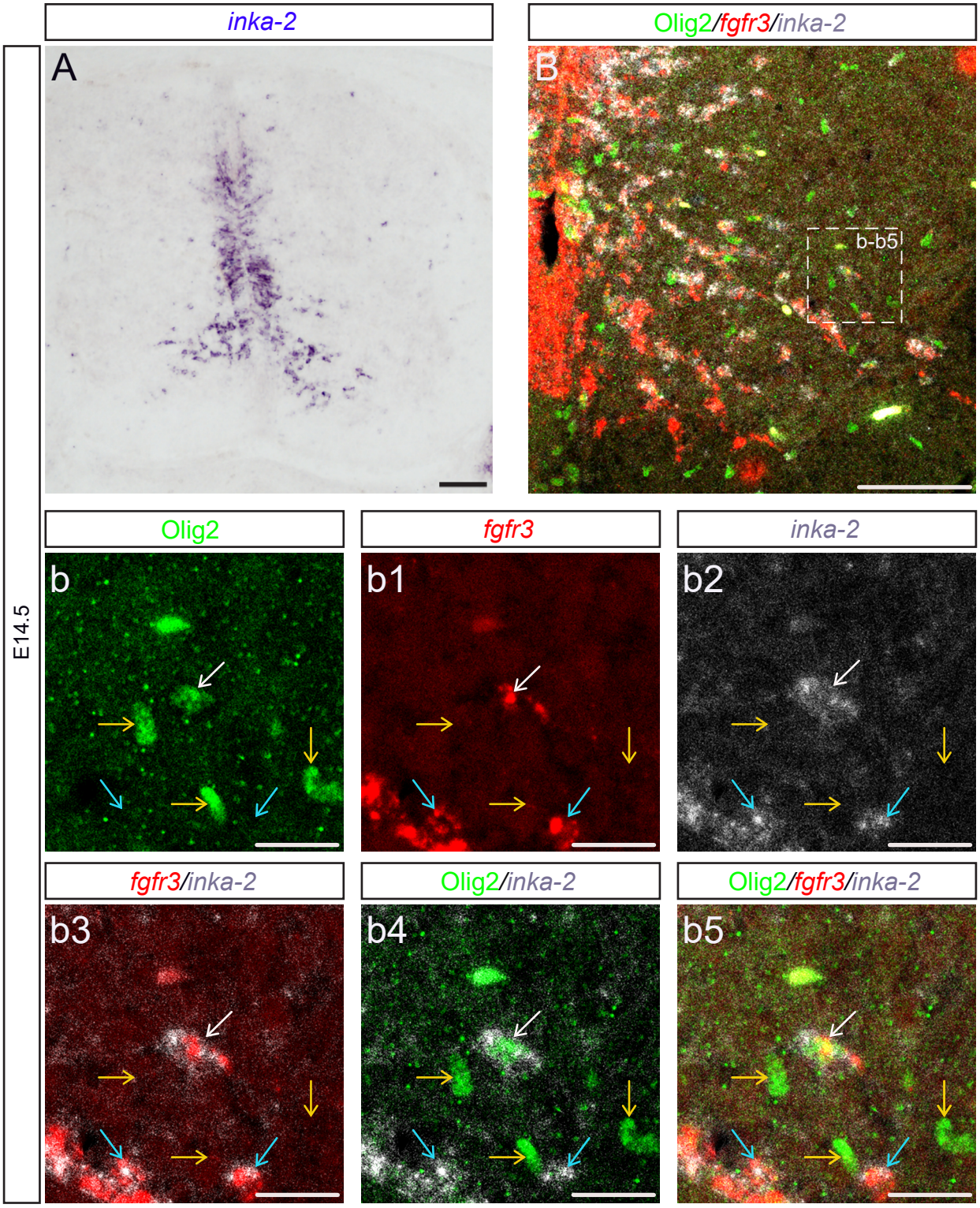

E14.5
inka-2
A
Olig2/fgfr3/inka-2
B
b-b5
Olig2
b
fgfr3
b1
inka-2
b2
fgfr3/inka-2
b3
Olig2/inka-2
b4
Olig2/fgfr3/inka-2
b5

Figure S8

Figure S8: Expression of *inka2* specifically marks astrocyte precursor cells of the developing spinal cord, Related to Figure 4

A: Expression profile of *inka2* mRNA at E14.5. **B-b5:** Immunodetection of Olig2 (green) combined with detection of *inka2* (gray) and *fgfr3* (red) mRNAs in E14.5 embryonic spinal cord. Images b-b5 show higher magnification of the areas framed in B and show successively Olig2 immunostaining (b), *fgfr3* mRNA staining (b1), *inka2* (b2) mRNA detection, combined *fgfr3* and *inka2* (b3) mRNA detection, combined Olig2 and *inka2* (b4) detection and the merged images (b5). Coloured arrows point to OPC/OL (yellow), Olig2-AS (white) and nonOlig2-AS (blue). Scale bars = 100 µm in A and B and 25 µm in b-b5.

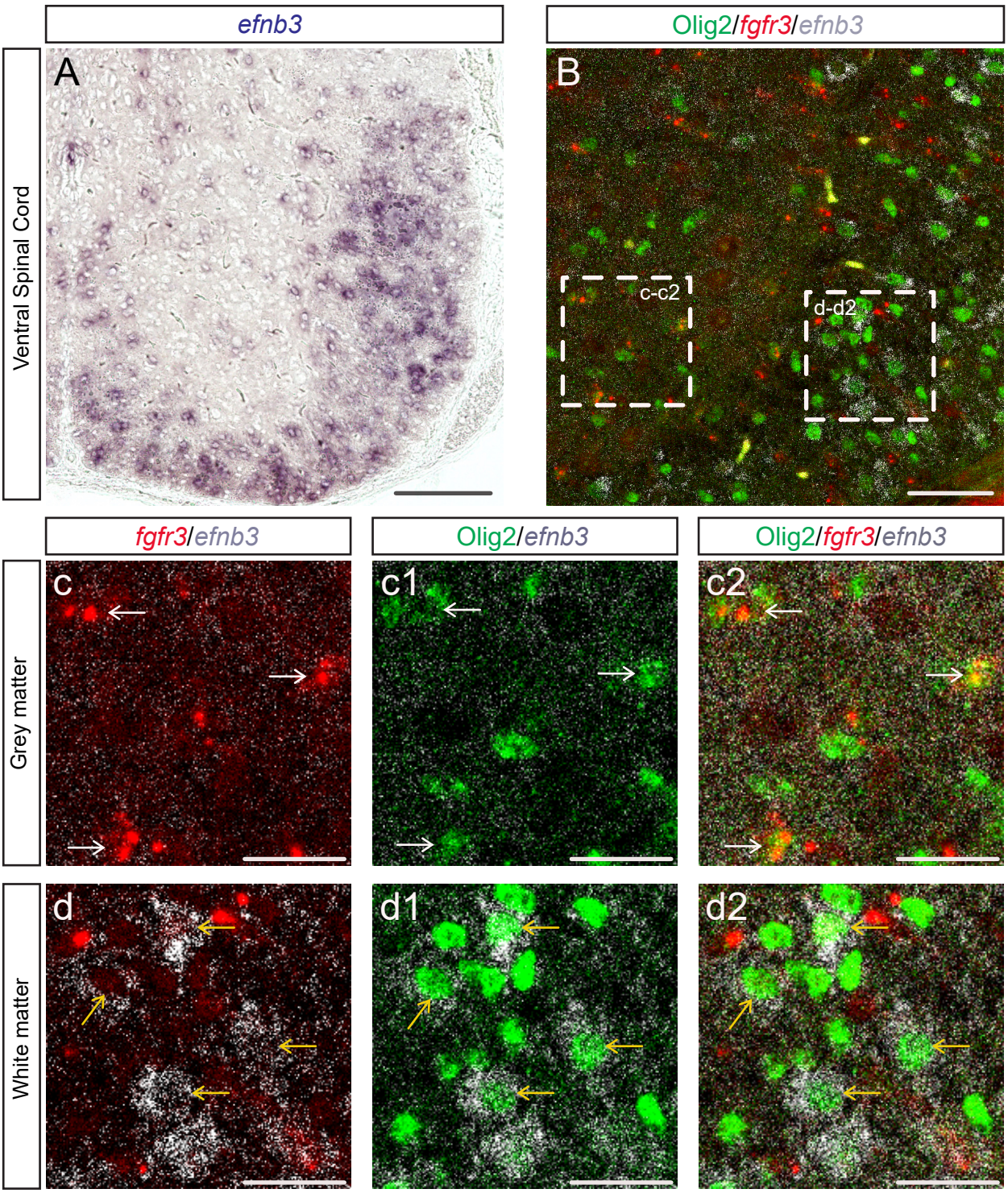

efnb3
Olig2/fgfr3/efnb3
Ventral Spinal Cord
A
B
c-c2
d-d2
fgfr3/efnb3
Olig2/efnb3
Olig2/fgfr3/efnb3
Grey matter
c
c1
c2
White matter
d
d1
d2

Figure S9

Figure S9: Expression pattern of *efnb3*, Related to Figure 4

A: Expression profile of *efnb3* mRNA at P7. **B-d2**: Immunodetection of Olig2 (green) combined with detection of *fgfr3* (red) and *inka2* (gray) mRNAs. Images c-c2 and d-d2 show higher magnification of the areas framed in B in the gray matter (c-c2) and in the white matter (d-d2) and show successively combined detection of *fgfr3* and *efnb3* (c, d) mRNAs, combined Olig2 and *efnb3* staining (c1, d1) and the merged images (c2, d2). Coloured arrows point to OPC/OL (yellow) and Olig2-AS (white). Scale bars = 100 µm in A; 50 µm in B and 25 µm in c-c2 and d-d2.

Table S5: List of qRT-PCR primers, Related to STAR Methods

Gene symbol	Sequence	
gapdh	Forward	CTGTTGCTGTAGCCGTATTCA
	Reverse	GGCCTTCCGTGTTCCTAC
olig2	Forward	CCCTCCTGTTGTCTCTCCTG
	Reverse	TAACGGTGGCAAAAGGTGTG
aldh1L1	Forward	CCAGCCTCCCAGTTCTTCAA
	Reverse	GGACATTGGGCAGAATTCGC
mbp	Forward	CCCTCAGAGTCCGACGAGCTT
	Reverse	TCCCTTGTGAGCCGATTTAT
inka2	Forward	CCTTCCTTCCTTCCTTCCTTTC
	Reverse	CAGCTTTGTACTGTGTGACCT
slc7a10	Forward	CCTTCATCAACTACCTCTGCTAC
	Reverse	GAACGAGGAGGTTCACCTTAAT
grm3	Forward	AGCACTTCGTCTAACAGCCTATA
	Reverse	CGACCACATATTCTCAGTCCTCT