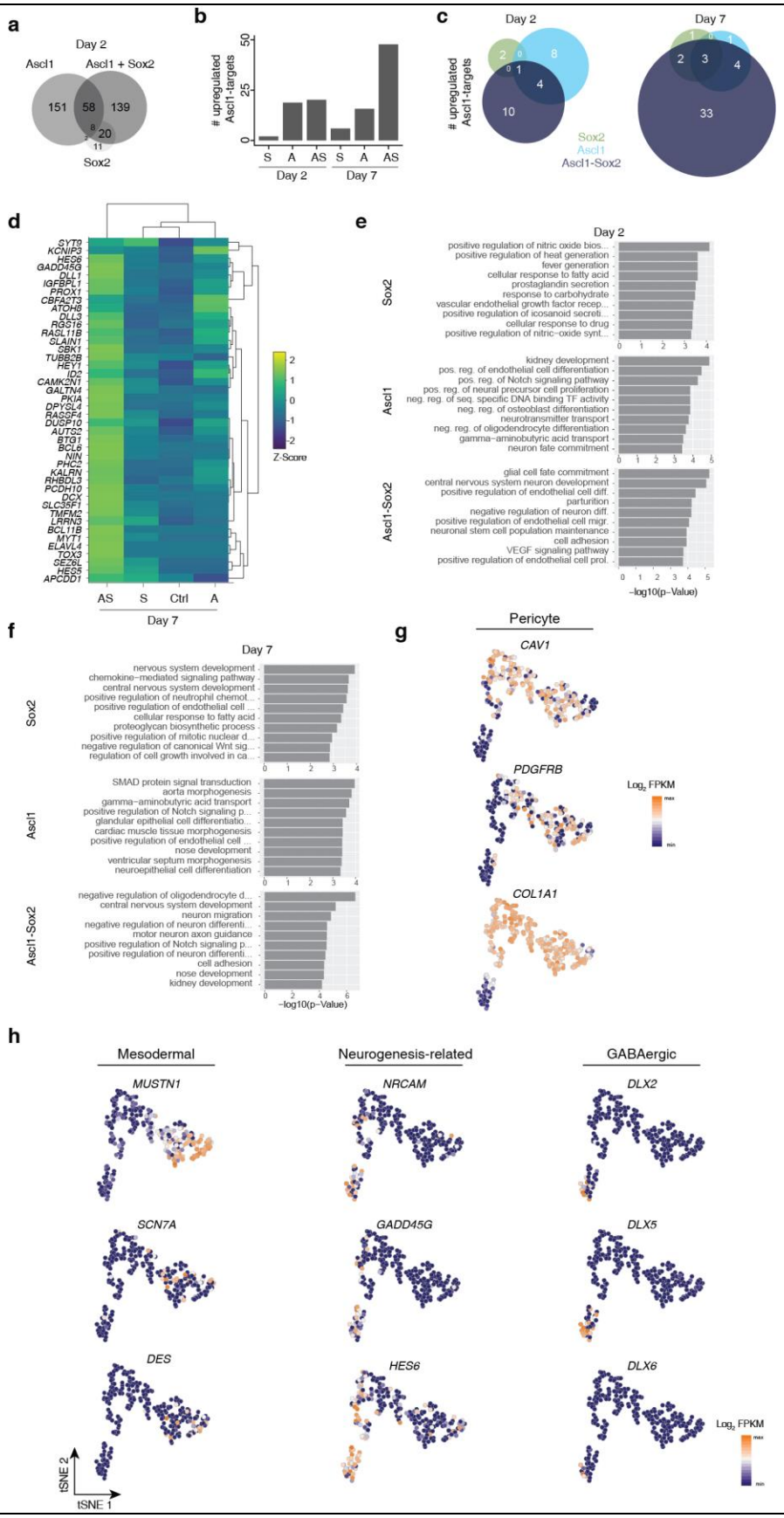
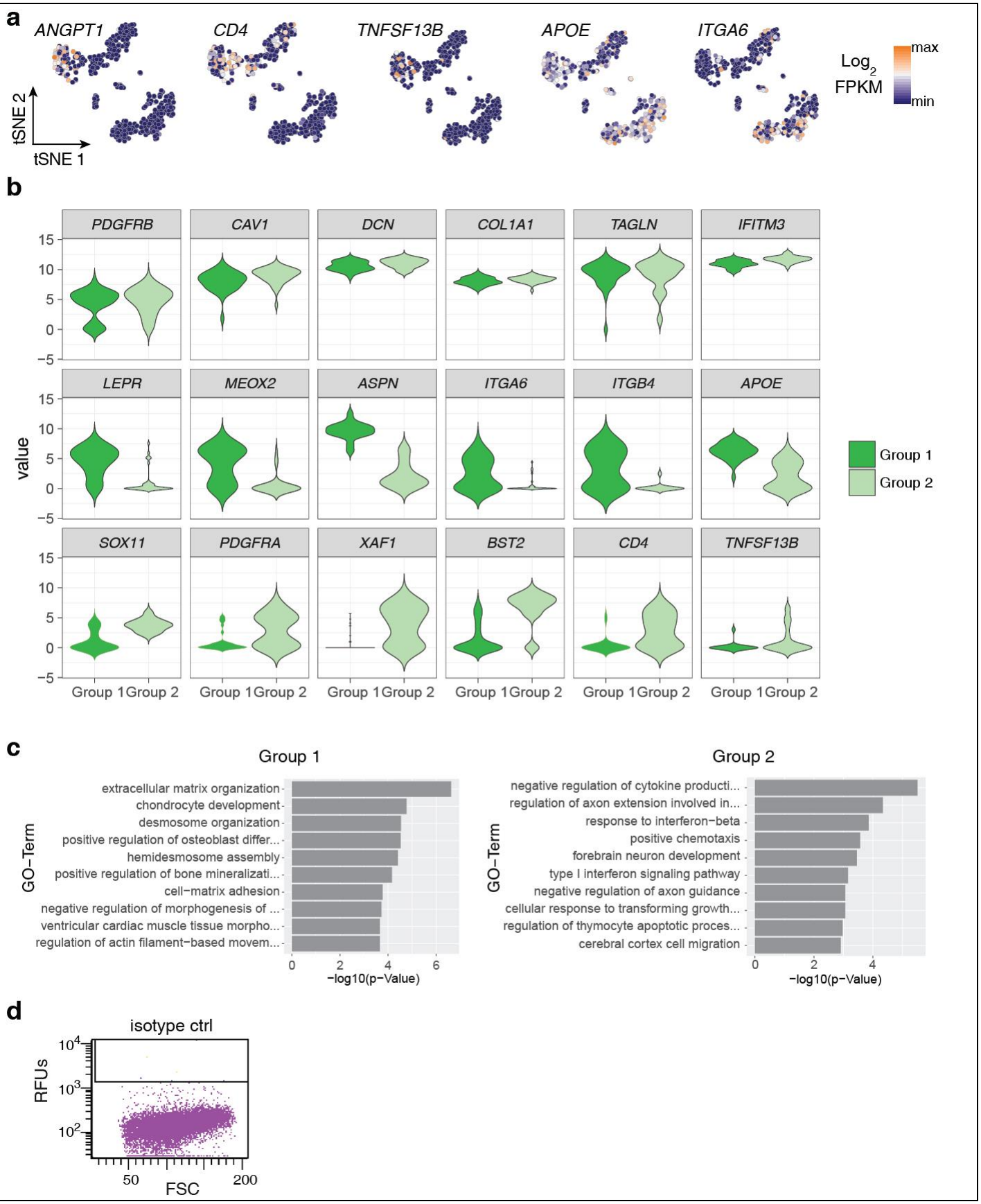

a
Day 2
Ascl1
Ascl1 + Sox2
151
58
139
8
20
2
11
Sox2
b
# upregulated Ascl1-targets
50
25
0
S
A
AS
S
A
AS
Day 2
Day 7
c
Day 2
Day 7
# upregulated Ascl1-targets
2
0
8
0
1
4
10
1
0
1
2
3
4
33
Sox2
Ascl1
Ascl1-Sox2
d
SYT9
KCNIP3
HES6
GADD45G
DLL1
IGFBPL1
PROX1
CBFA2T3
ATOH8
DLL3
RGS16
RASL11B
SLAIN1
SBK1
TUBB2B
HEY1
ID2
CAMK2N1
GALTN4
PKIA
DPYSL4
RASSF4
DUSP10
AUTS2
BTG1
BCL6
NIN
PHC2
KALRN
RHBDL3
PCDH10
DCX
SLC35F1
TMEM2
LRRN3
BCL11B
MYT1
ELAVL4
TOX3
SEZ6L
HES5
APCDD1
2
1
0
-1
-2
Z-Score
AS
S
Ctrl
A
Day 7
e
Day 2
Sox2
positive regulation of nitric oxide bios...
positive regulation of heat generation
fever generation
cellular response to fatty acid
prostaglandin secretion
response to carbohydrate
vascular endothelial growth factor recep...
positive regulation of icosanoid secreti...
cellular response to drug
positive regulation of nitric−oxide synt...
0 1 2 3 4
Ascl1
kidney development
pos. reg. of endothelial cell differentiation
pos. reg. of Notch signaling pathway
pos. reg. of neural precursor cell proliferation
neg. reg. of seq. specific DNA binding TF activity
neg. reg. of osteoblast differentiation
neurotransmitter transport
neg. reg. of oligodendrocyte differentiation
gamma−aminobutyric acid transport
neuron fate commitment
0 1 2 3 4 5
Ascl1-Sox2
glial cell fate commitment
central nervous system neuron development
positive regulation of endothelial cell diff.
parturition
negative regulation of neuron diff.
positive regulation of endothelial cell migr.
neuronal stem cell population maintenance
cell adhesion
VEGF signaling pathway
positive regulation of endothelial cell prol.
0 1 2 3 4 5
−log10(p−Value)
f
Day 7
Sox2
nervous system development
chemokine−mediated signaling pathway
central nervous system development
positive regulation of neutrophil chemot...
positive regulation of endothelial cell ...
cellular response to fatty acid
proteoglycan biosynthetic process
positive regulation of mitotic nuclear d...
negative regulation of canonical Wnt sig...
regulation of cell growth involved in ca...
0 1 2 3 4
Ascl1
SMAD protein signal transduction
aorta morphogenesis
gamma−aminobutyric acid transport
positive regulation of Notch signaling p...
glandular epithelial cell differentiatio...
cardiac muscle tissue morphogenesis
positive regulation of endothelial cell ...
nose development
ventricular septum morphogenesis
neuroepithelial cell differentiation
0 1 2 3 4
Ascl1-Sox2
negative regulation of oligodendrocyte d...
central nervous system development
neuron migration
negative regulation of neuron differenti...
motor neuron axon guidance
positive regulation of Notch signaling p...
positive regulation of neuron differenti...
cell adhesion
nose development
kidney development
0 2 4 6
−log10(p−Value)
g
Pericyte
CAV1
PDGFRB
COL1A1
Log2 FPKM
max
min
h
Mesodermal
Neurogenesis-related
GABAergic
MUSTN1
NRCAM
DLX2
SCN7A
GADD45G
DLX5
DES
HES6
DLX6
Log2 FPKM
max
min
tSNE 2
tSNE 1

Supplementary Figure 1

**Cooperative AS function is required for pericyte-to-iN conversion.**

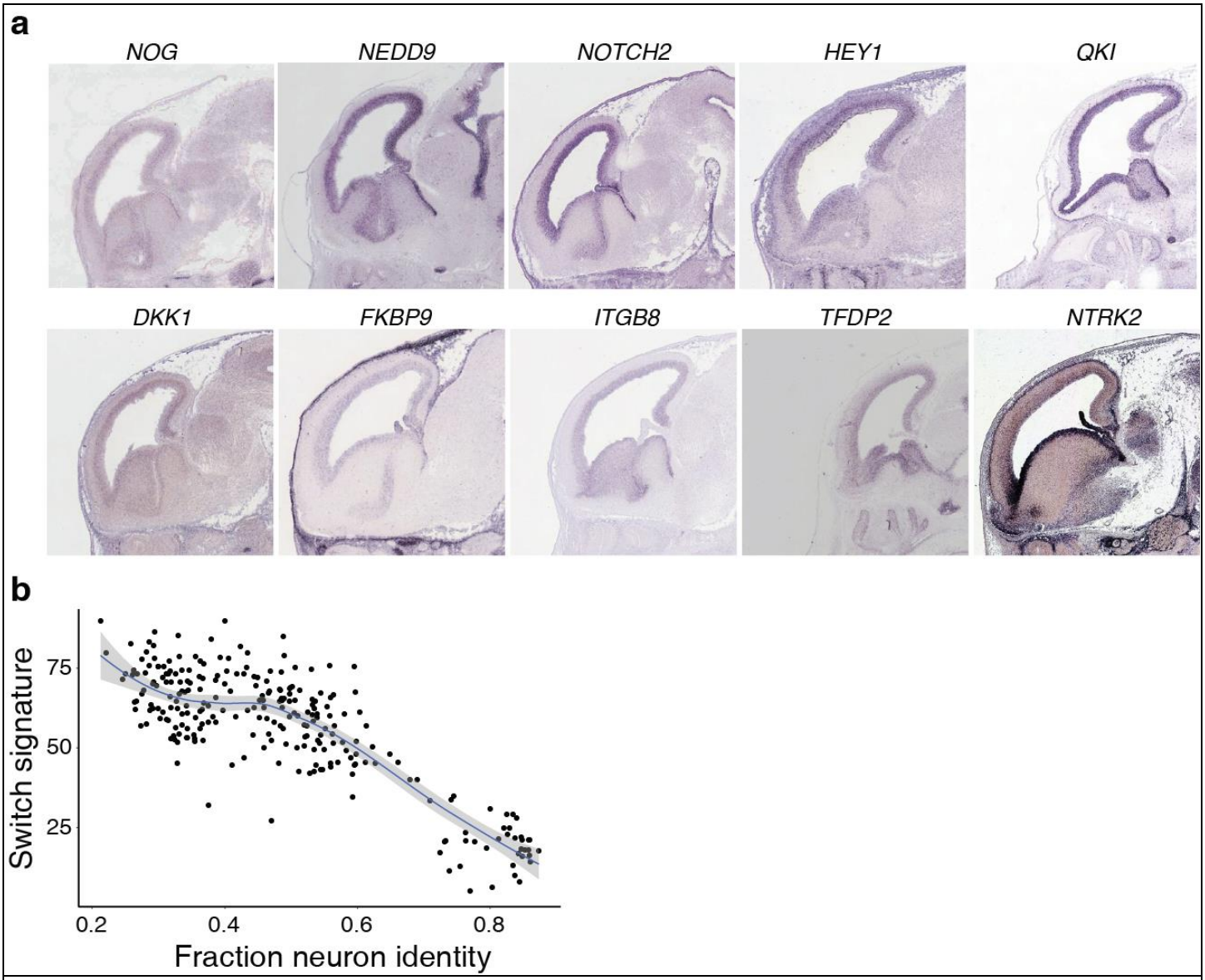
**a,** Euler diagram shows number of DE genes of each condition (Ascl1-only, Sox2-only, AS) at 2 dpi. **b,** Bar graph representing number of direct Ascl1-targets transcriptionally upregulated across different conditions and timepoints. **c,** Euler diagram showing that the majority of direct Ascl1-targets are regulated by AS synergism in human brain pericytes at 2 and 7 dpi. **d,** Heatmap shows normalized expression (Z-score) of direct Ascl1-target genes upregulated in AS-transduced cells at 7 dpi. Note that only a minor fraction of these genes is also upregulated by Ascl1 alone. **e,** GO term analysis of DE genes for each condition at 2 dpi. Shown are the 10 most significantly regulated GO terms. GO terms were ordered according to their significance as determined by Fisher´s exact test; n = 3 individual pericyte donors per experimental group. **f,** GO term analysis of DE genes for each condition at 7 dpi. Significantly regulated genes in GO categories are contained in Supplementary Table 2. GO terms were ordered according to their significance as determined by Fisher´s exact test; n = 3 individual pericyte donors per experimental group. **g,** tSNE plots from the analysis of Ascl1-only and AS transduced cells at 2 and 7 dpi from Fig. 1e are colored based on the expression of selected pericyte genes ($Log_2$ FPKM). **h,** tSNE plots from the analysis of Ascl1-only and AS transduced cells at 2 and 7 dpi from Fig. 1e are colored based on the expression of selected mesoderm, neurogenesis-related, and GABAergic signature genes ($Log_2$ FPKM).


a
ANGPT1
CD4
TNFSF13B
APOE
ITGA6
tSNE 2
tSNE 1
Log2 FPKM
max
min
b
PDGFRB
CAV1
DCN
COL1A1
TAGLN
IFITM3
LEPR
MEOX2
ASPN
ITGA6
ITGB4
APOE
SOX11
PDGFRA
XAF1
BST2
CD4
TNFSF13B
value
Group 1
Group 2
c
Group 1
extracellular matrix organization
chondrocyte development
desmosome organization
positive regulation of osteoblast differ...
hemidesmosome assembly
positive regulation of bone mineralizati...
cell−matrix adhesion
negative regulation of morphogenesis of ...
ventricular cardiac muscle tissue morpho...
regulation of actin filament−based movem...
GO−Term
−log10(p−Value)
Group 2
negative regulation of cytokine producti...
regulation of axon extension involved in...
response to interferon−beta
positive chemotaxis
forebrain neuron development
type I interferon signaling pathway
negative regulation of axon guidance
cellular response to transforming growth...
regulation of thymocyte apoptotic proces...
cerebral cortex cell migration
d
isotype ctrl
RFUs
FSC

**Supplementary Figure 2**

**Expression of pericyte heterogeneity genes.**

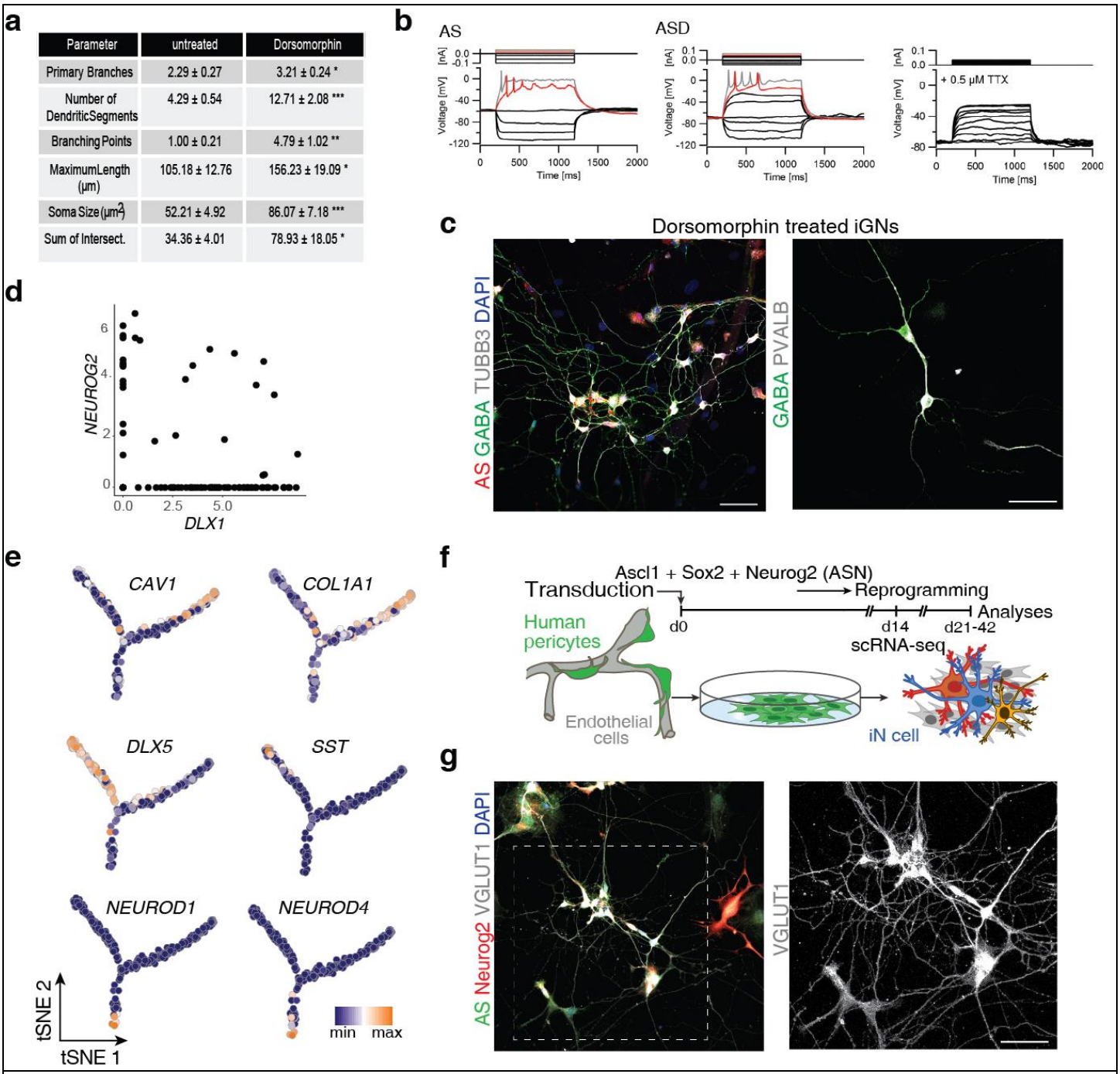
**a,** tSNE plot from Fig. 2a colored based on the expression of selected genes enriched in different pericyte groups. **b,** Violin plots show the density expression distribution of genes in pericyte group 1 (31 cells) and group 2 (44 cells). **c,** GO term analysis of pericyte heterogeneity genes from Fig. 2b; group 1, n = 31 cells; group 2, n = 44 cells; GO terms were ordered according to their significance as determined by Fisher´s exact test. **d,** Representative flow cytometry plot showing isotype control for the sorting of LEPR-positive cultured human pericytes (Fig. 2f); n = 4.




**Supplementary Figure 3**

**Switch genes are expressed in the germinal zones of the developing CNS.**

**a** In situ hybridization images (GenePaint.org) show the expression of selected switch genes in the developing mouse forebrain (E14.5). **b** Projecting the switch gene signature onto published scRNA-seq data[1] reconstructing the MEF-to-iN reprogramming path (259 cells) shows the high base level in the starting MEF population and downregulation of the switch gene signature along differentiation towards iNs. Shaded gray represents 0.95 confidence interval.



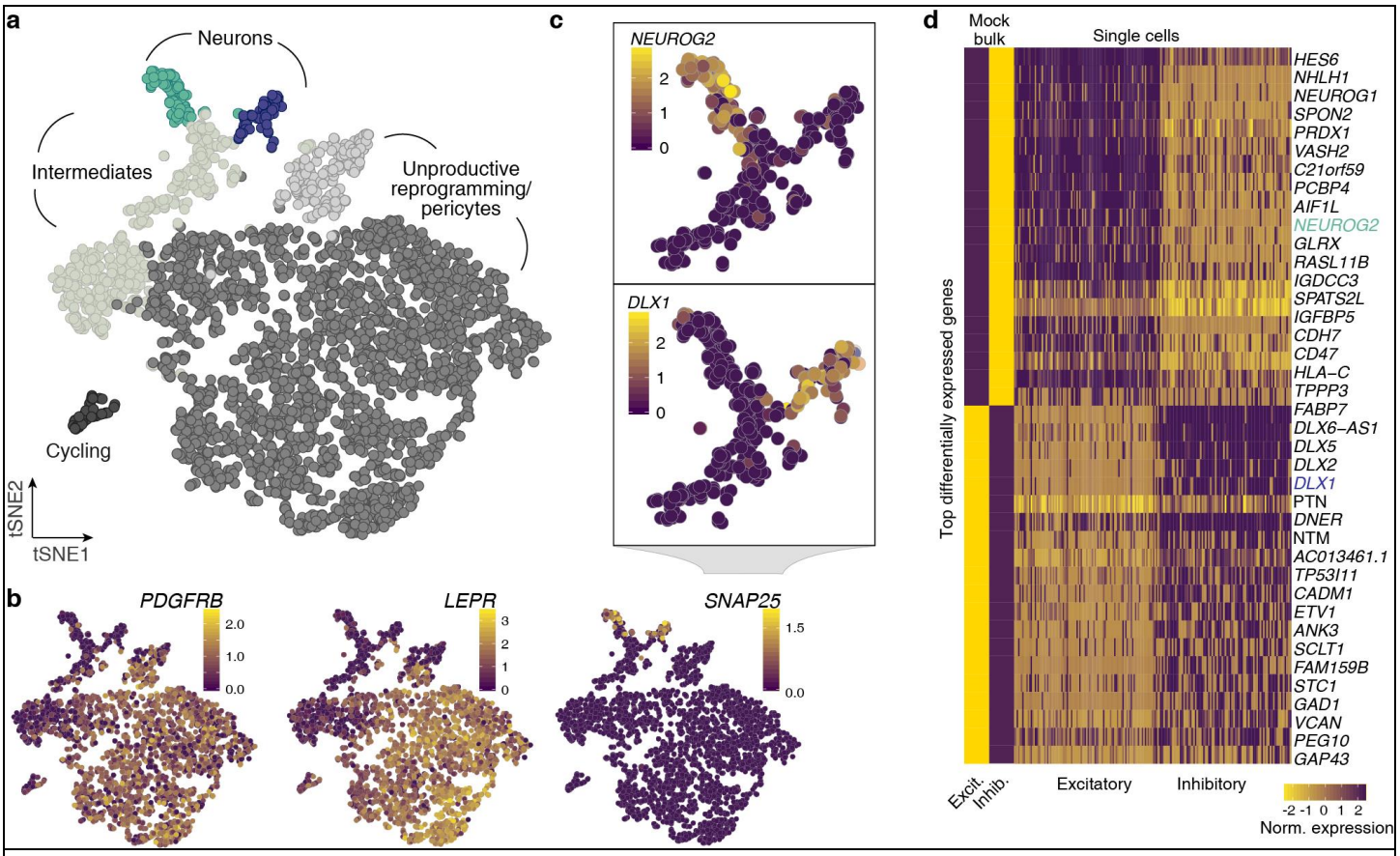

| Parameter | untreated | Dorsomorphin |
|---|---|---|
| Primary Branches | 2.29 ± 0.27 | 3.21 ± 0.24 * |
| Number of DendriticSegments | 4.29 ± 0.54 | 12.71 ± 2.08 *** |
| Branching Points | 1.00 ± 0.21 | 4.79 ± 1.02 ** |
| MaximumLength (µm) | 105.18 ± 12.76 | 156.23 ± 19.09 * |
| Soma Size (µm$^2$) | 52.21 ± 4.92 | 86.07 ± 7.18 *** |
| Sum of Intersect. | 34.36 ± 4.01 | 78.93 ± 18.05 * |

**Supplementary Figure 4**

**Characterization of ASD cells and shift towards a glutamatergic neuron phenotye by combinatorial expression of AS with Neurog2.**

**a,** Summary of morphometric analyses showing significant differences between untreated and Dorsomorphin-treated AS cells. Data are shown as mean ± SEM; (untreated, n = 14 cells of 3 independent experiments; Dorsomorphin-treated, n = 14 cells of 3 independent experiments); two-tailed unpaired Student's t test; primary branches, $P = 0.015$; number of dendritic segments, $P = 0.0005$; branching points, $P = 0.001$; maximum length, $P = 0.035$; soma size, $P = 0.0006$; *sum of intersections* $P = 0.02$; * $P < 0.05$, ** $P < 0.01$, ***$P <$

0.001. **b,** Electrophysiological assessment of AS and ASD cells. Representative traces of multiple action potential discharge after step-current injections in AS (left) and ASD (middle) cells. Action potentials could be reliably blocked by TTX [0.5 μM] bath application (right). **c,** Micrographs show iGNs treated with Dorsomorphin immunoreactive for TUBB3 and GABA which acquire highly complex neuronal morphology. ASD iGNs show overlapping immunoreactivity for parvalbumin (PVALB) and GABA (n =3). Nuclei are stained with Dapi. Scale bars = 50 μm. **d,** Biplot showing the expression of *DLX1* and *NEUROG2* in all *SNAP25*-expressing cells. **e,** Monocle plot from Fig. 4g colored based on the expression of genes that show the loss of pericyte marker gene expression along the pseudotime and the acquisition of GABAergic and glutamatergic cell fate determinants along the trajectory towards different neuronal subtypes. **f,** Schematic of ASN experiments. **g,** Micrographs show pericytes transduced with AS (green) and Neurog2 (red) that acquire VGLUT1 immunoreactivity by co-expression of these three transcription factors. Note the punctate appearance of VGLUT1 in inset (right) (n = 3). Nuclei are stained with Dapi. Scale bars = 50 μm.




**Supplementary Figure 5**

**High-throughput scRNA-seq data from a second pericyte donor confirms lineage bifurcations during iN maturation.**

**a,** scRNA-seq using a high-throughput droplet microfluidic platform (10X genomics) was performed on 3419 AS-transduced cells treated with Dorsomorphin at 14 days post infection. PCA followed by tSNE shows cell populations that maintain pericyte markers (greys) and fail to productively differentiate to a neuronal lineage, populations at intermediate stages of differentiation (light grey), and two distinct neuron populations (cyan, blue). **b,** Cells are colored in the tSNE plots based on log normalized expression of pericyte marker *PDGFRB*, group 1 marker *LEPR*, and neuronal marker *SNAP25*. **c,** The inset shows the log normalized expression of markers for distinct excitatory (*NEUROG2*) and inhibitory (*DLX1*) neuronal populations that emerge during reprogramming. **d,** Heatmap shows the scaled expression of the top 20 genes that are differentially expressed (based on average log fold change) between the two neuronal populations. Single cells are in columns, genes in rows.