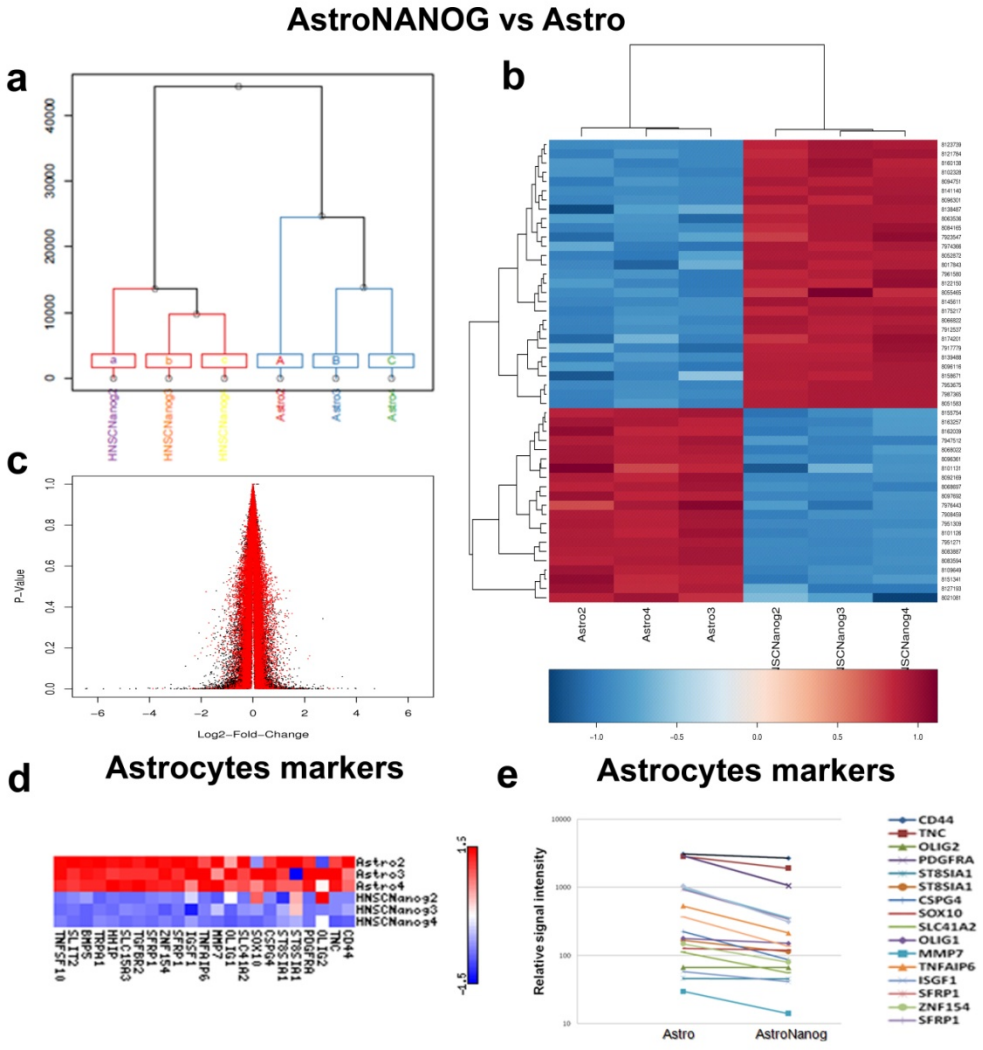
Supplementary Figures

Supplementary Figure 1

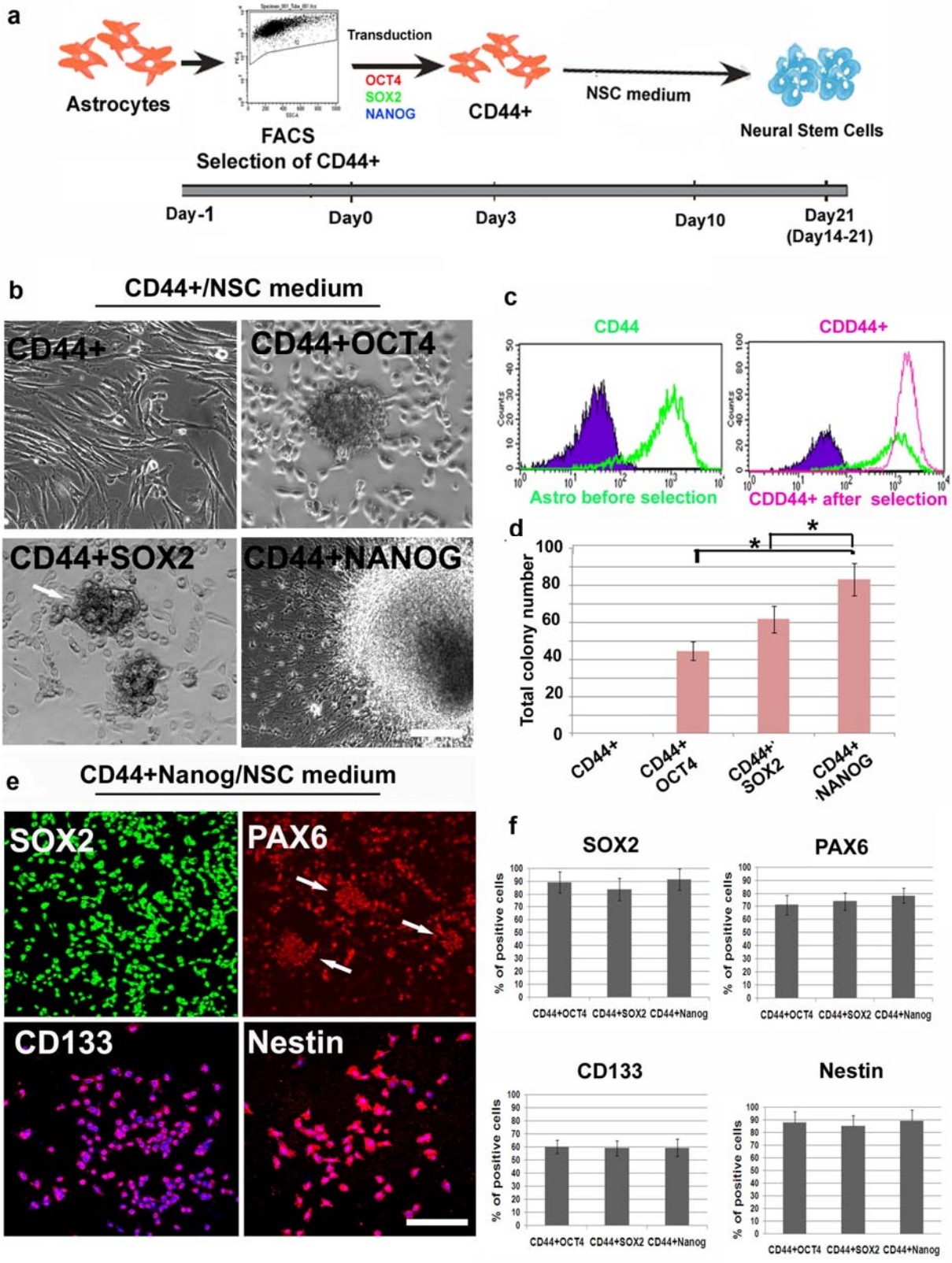


Supplementary Figure 1

Global gene expression analyses of AstroNANOG vs parental astrocytes

a) Pearson correlation analyses of global gene expression in AstroNANOG vs astrocytes, demonstrating that the profile of AstroNANOG cells was significantly different relative to that of parental cells. b) Heat map analysis demonstrated differential gene expression between AstroNANOG and parental astrocytes. Blue = low expression; red = high expression. c) Volcano-plot t-test representation of AstroNANOG vs astrocyte gene expression data. d) Heat map analysis of selected astrocyte markers demonstrating their reduction in AstroNANOG relative to parental astrocytes. Blue = low expression; red = high expression. e) Astrocytic marker intensity level comparison between primary astrocytes and AstroNANOG clones.

Supplementary Figure 2

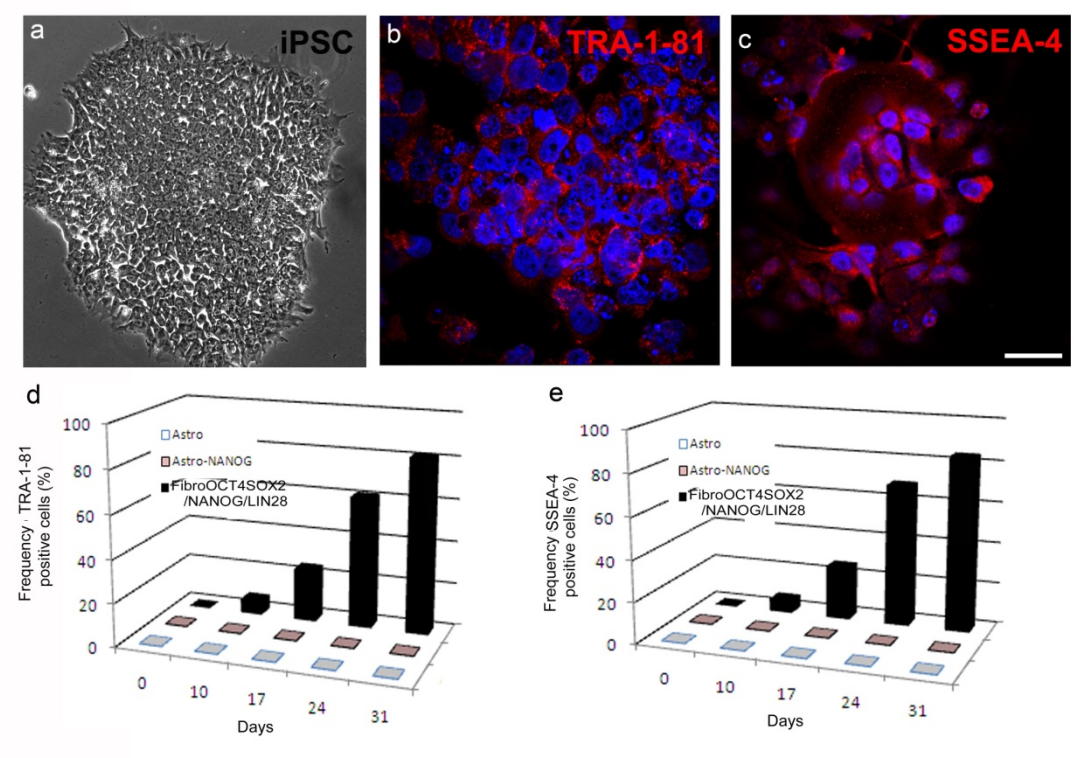


Supplementary Figure 2

OCT4/SOX2/NANOG-transduced human CD44+ astrocytes generate neural stem cell (NSC) colonies

a) Schematic illustration of NSC generation from CD44+ purified human astrocytes (CD44). Human astrocytes were purified by FACS for expression of CD44 and transfected with SOX2 or OCT4 or NANOG. The cells were cultured in ESC/iPSC or NSC media. NSC colonies were observed between days 14 and 21 post-transfection. b) Bright-field images of human untransduced CD44+-selected astrocytes (CD44+) and OCT4- (CD44+OCT4), SOX2- (CD44+SOX2), or NANOG- (CD44+NANOG) transduced astrocytes colonies (arrows indicate the spheres) (n = 12) in NSC medium. Scale bar: 150 μm. c) Representative FACS analysis of CD44 (astrocytic marker) levels in astrocytes before (left) and after (right) FACS selection. d) Quantification of colonies in human CD44+ astrocytes after transduction in NSC medium at 21 days (12 biological replicates; error bars, s.d.; *P < .00001). e) Immunohistochemical expression of SOX2, PAX6, CD133, and nestin in CD44+-NANOG colonies. Similar results were obtained with CD44+-OCT4 and CD44+SOX2 colonies. f) Quantification of the NSC marker expression as a percentage of positive cells in AstroOCT4, AstroSOX2, and AstroNANOG colonies. Scale bar: 150 μm.

Supplementary Figure 3

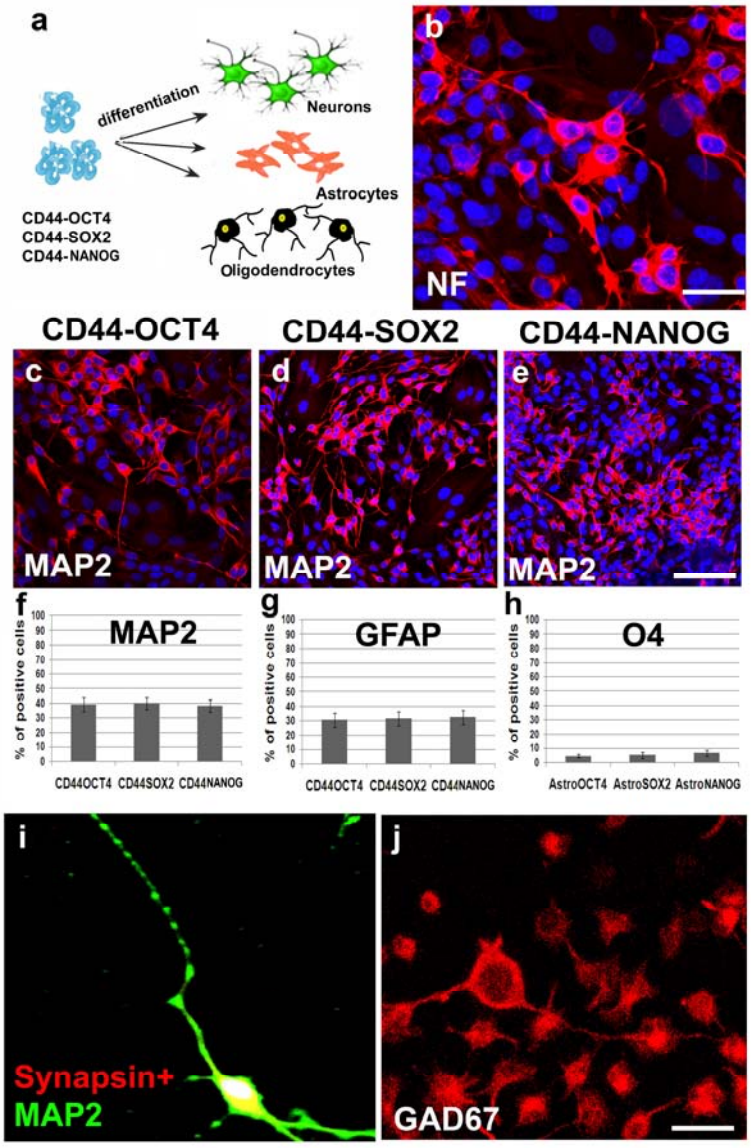


Supplementary Figure 3

Astrocyte reprogramming into NSCs by OCT4/SOX2 or NANOG does not require the pluripotent state

a–c) Contrast-phase analysis of an iPSC colony obtained from fibroblasts by contemporaneous transfection of OCT4/SOX2/NANOG/LIN28. iPSCs were positive for pluripotency markers TRA-1-81 and SSEA4. Scale bar: a: 150 μm; b, c: 50 μm. d, e) Quantitative analysis of TRA-1-81 and SSEA-4 in Astro, AstroNANOG, and human fibroblasts transduced with OCT4, SOX2, NANOG, and LIN28, over the human iPSC derivation timeline.

Supplementary Figure 4



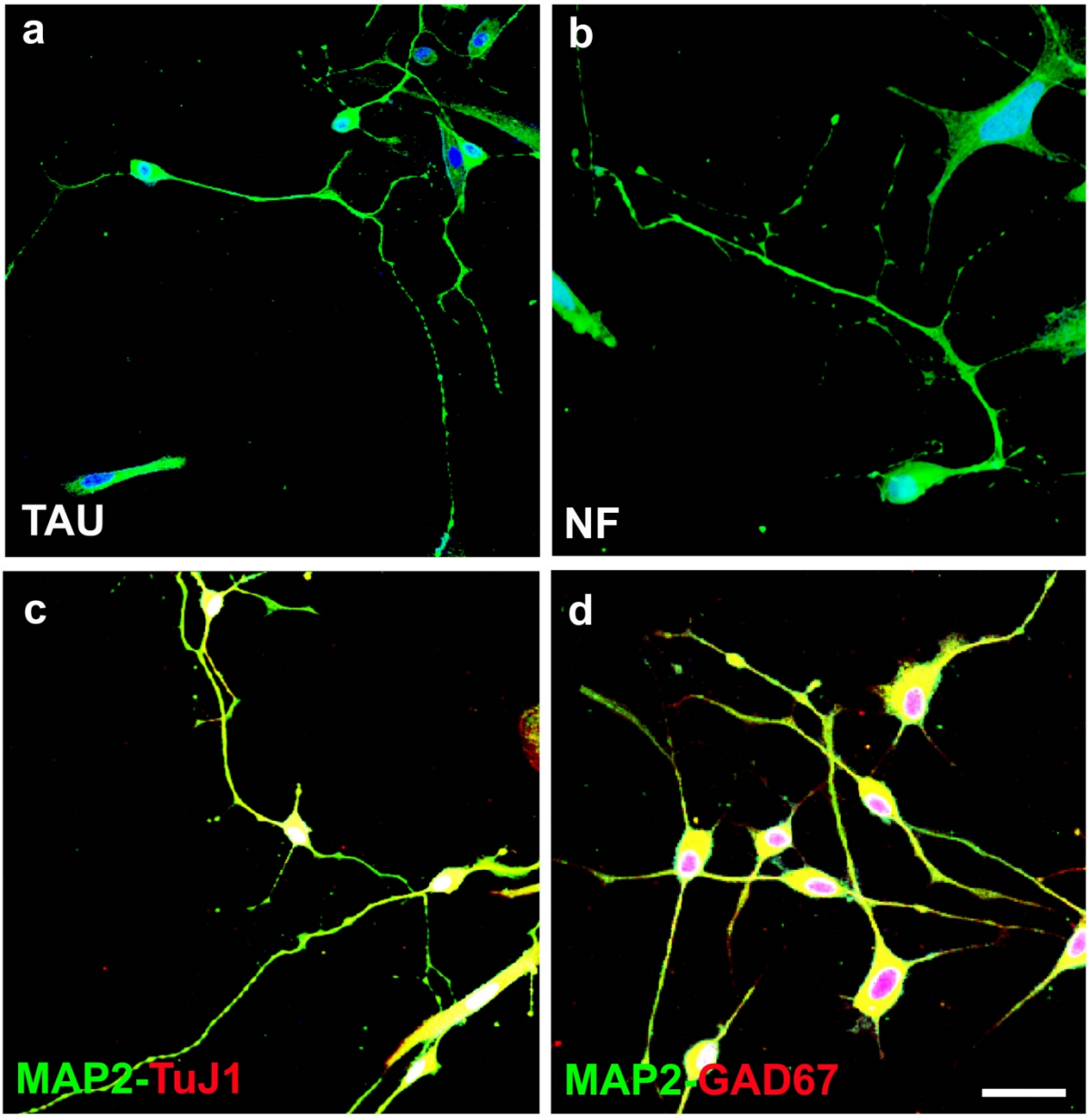
Supplementary Figure 4

In vitro differentiation of CD44+OCT4/SOX2 or NANOG

a) Schematic illustration of CD44+NSC differentiation into neurons, astrocytes, and oligodendrocytes. AstroNSCs were cultured in human neuronal differentiation medium. The cell phenotype was determined after 15 days of differentiation. b–e) Differentiation of CD44+NANOG cells into neurons positive for NF (b) and MAP2 (c–e). f–h) Quantification of the differentiation efficiency into neurons (MAP2), astrocytes (GFAP), and O4 for the three conditions (CD44+/NANOG, OCT4, or SOX2). i,j) The CD44-NSC–derived neurons showed a complex mature phenotype and were positive for synapsin (i, shown here in red as double staining with MAP2, green) and neurotransmitters like GABA (GAD67) (j), supporting correct functional properties.

Scale bar: b, 70 μm; c–e: 150 μm; i, j: 50 μm.

Supplementary Figure 5



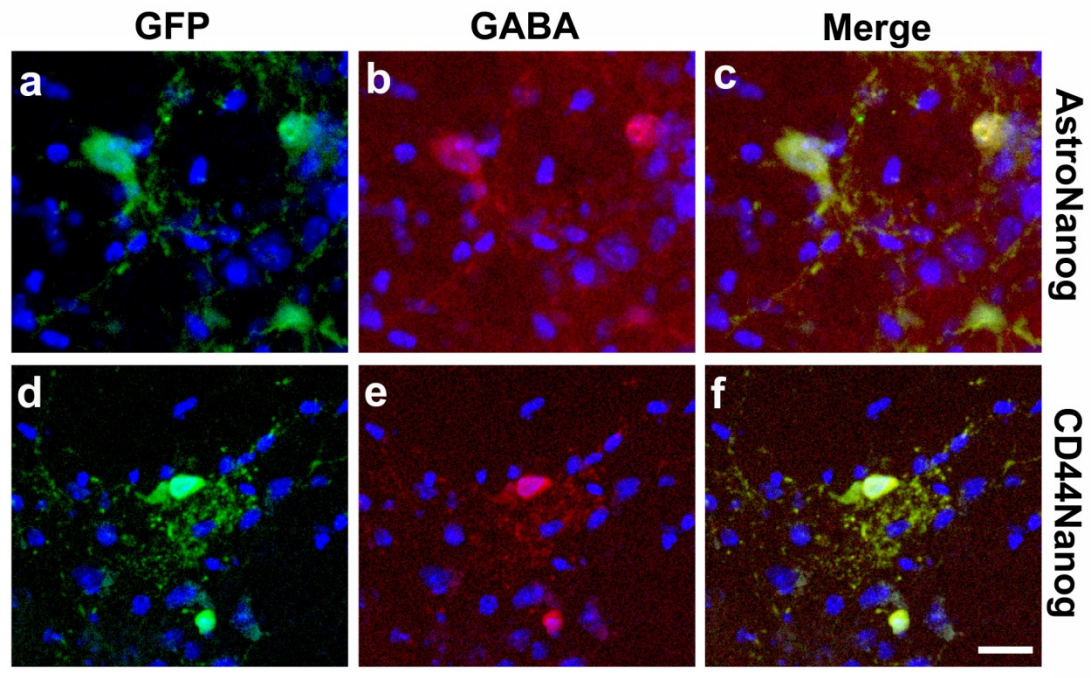
Supplementary Figure 5

AstroNANOG-ASCL1–derived neurons in vitro

a, b) Neurons differentiated from AstroNANOG-ASCL and CD44-NANOG-ASCL1 showed complex mature morphology with dendrites and long axons shown here with immunocytochemistry for TAU and NF. c, d) These neurons were positive for GAD67 (shown here in red as double staining with MAP2) and Tuj1.

Scale bar: a: 150 μm, b: 100 μm; c: 150 μm; h: 50 μm.

Supplementary Figure 6



Supplementary Figure 6

AstroNSC-derived neurons express neurotransmitters in vivo

a–f) AstroNANOG and CD44-NANOG–derived neurons (GFP+: a, d) were positive for GAD67 (b–e). c–f: merged images.

Scale bar: a–d: 50 μm; d–f: 70 μm.

Supplementary Video 1

Contrast-phase time-lapse acquisition of AstroNSCs in culture (24 hours of recording). The movie shows the small round morphology of the cells and their proliferative capacity.