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Supplemental Information

Inducing Different Neuronal Subtypes from Astrocytes in the Injured Mouse Cerebral Cortex

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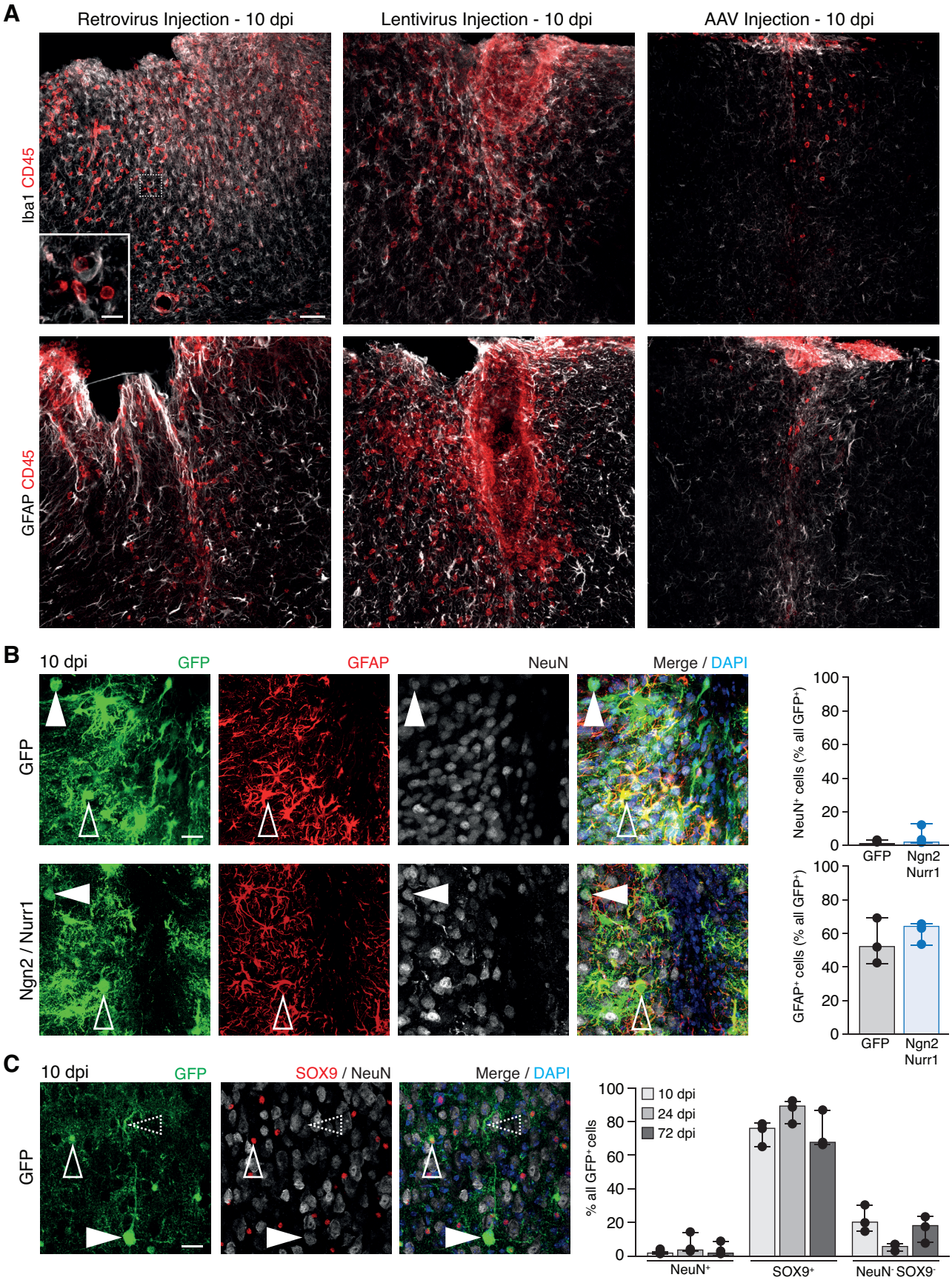
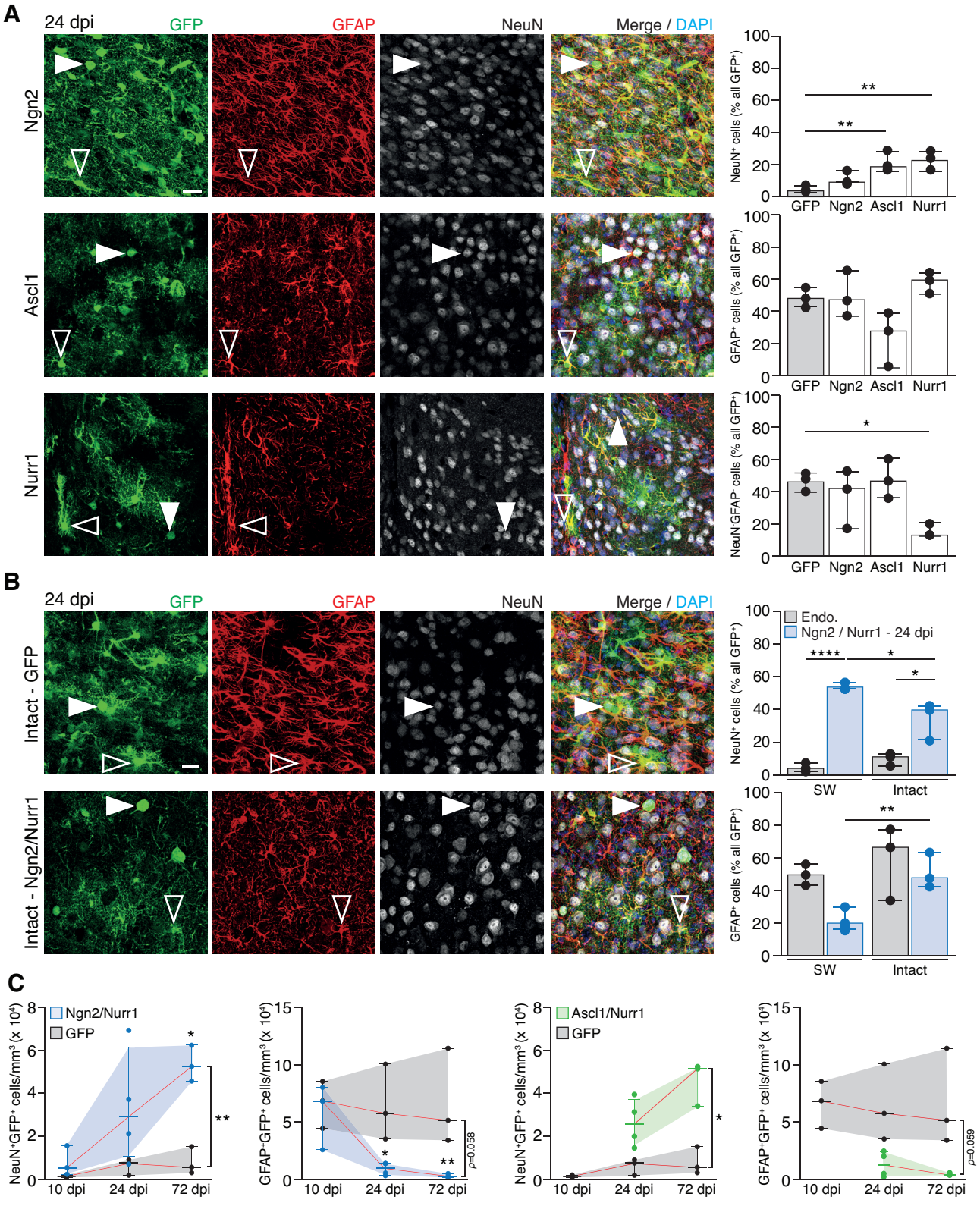
Figure S1 - Related to Figure 1



Supplementary Figure 1. Immune response to different viral vectors and identity of GFP$^+$ cells after injection of only AAV-FLEx-GFP into the cortex of mGFAP-Cre mice.

(A) Photomicrographs comparing immune cell infiltration (CD45$^+$); microglia (Iba1$^+$) and astrocyte reactivity (GFAP$^+$) in the GM of mice injected with three types of viral vectors 3 days after stab wound injury and analyzed at 10 dpi. Note that the high degree of reactive gliosis after MLV-based retrovirus (see Heinrich et al., 2014; Gascón et al., 2016) or differently pseudotyped lentivirus (see Buffo et al., 2008; Heinrich et al., 2014) versus low reactive gliosis after rAAV2/5 injection was independent from the transgene carried by the viral vectors (Ngn2 or GFP control). **(B)** Photomicrographs showing GFP$^+$ cells at 10 dpi of AAV-FLEx containig GFP or Ngn2/Nurr1. Empty arrowheads indicate GFP$^+$/GFAP$^+$ and full arrowheads indicate GFP$^+$/NeuN$^+$ neurons. Histograms show the percentage of NeuN$^+$ (top) and GFAP$^+$ (bottom) among the GFP$^+$ cells; n = 3 animals. **(C)** Photomicrographs showing GFP$^+$ cells at 10 dpi double-stained for SOX9 and NeuN. Empty arrowheads indicate GFP$^+$/SOX9$^+$ astrocytes, dashed arrowheads indicate cells with an astrocytic morphology GFP$^+$/SOX9$^-$/NeuN$^-$ and full arrowhead indicate GFP$^+$/NeuN$^+$ neurons. Histogram shows the percentage of NeuN$^+$, SOX9$^+$ and NeuN$^-$/SOX9$^-$ among the GFP$^+$ cells at different time points; n = 3 animals. Data are shown as median ± IQR. Mann-Whitney test (B) and Kruskal-Wallis test (C). dpi: days post injection. Scale bars: 50 µm (A; overview), 20 µm (B and C), and 10 µm (A; close up).

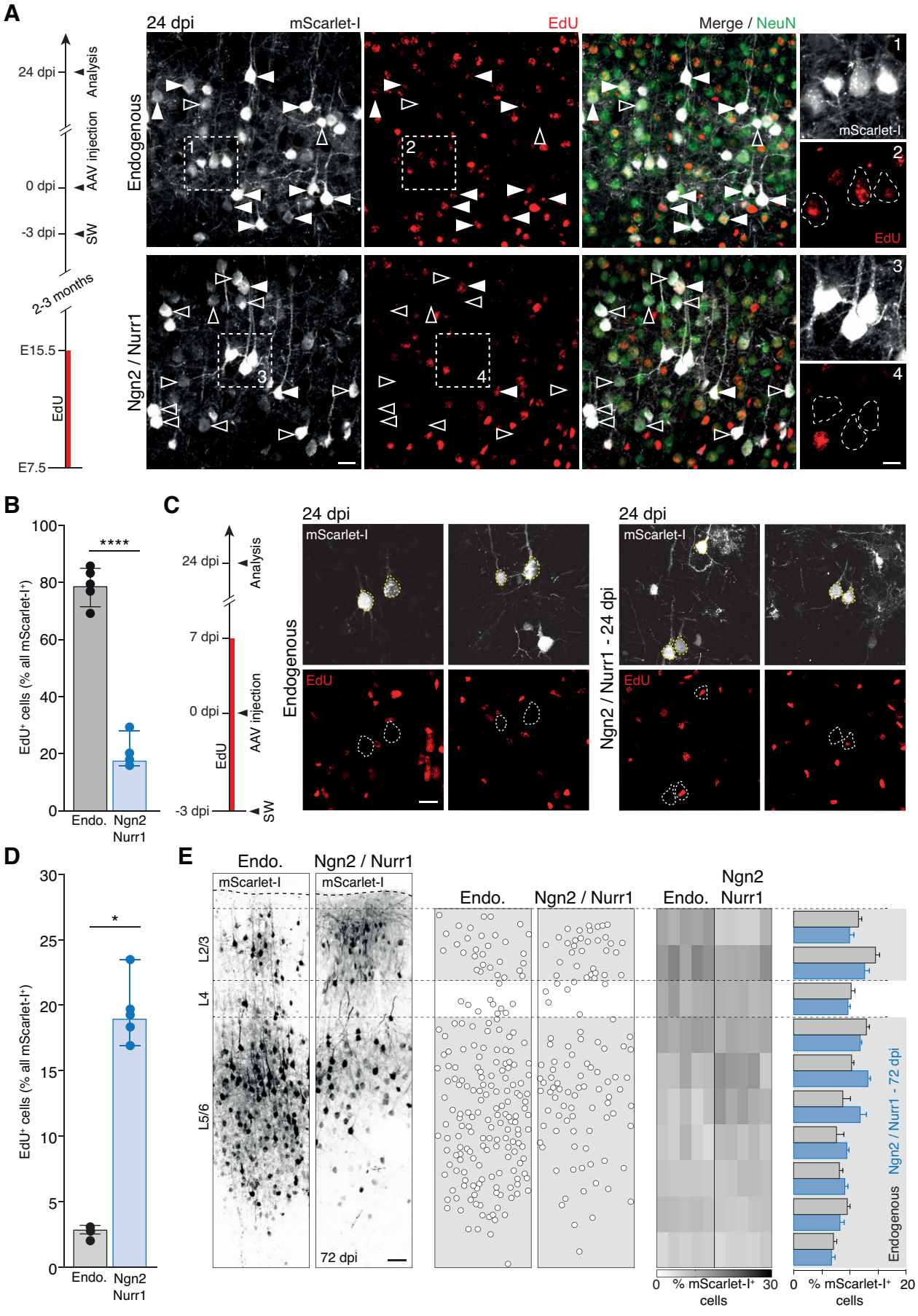
Figure S2 - Related to Figure 1



Supplementary Figure 2. Identity of GFP^+ cells in mGFAP-Cre mice at 24 dpi of FLEx-AAVs containing GFP and single neurogenic factors and reprogramming in the intact cortex.

(A) Photomicrographs show GFP^+ cells at 24 dpi of AAV-FLEx expressing single neurogenic factor: Ngn2 (top), Ascl1 (middle) and Nurr1 (bottom). Empty arrowheads indicate $GFP^+/GFAP^+/NeuN^-$ astrocytes, full arrowheads indicate $GFP^+/NeuN^+/GFAP^-$ neurons. Histograms on the right show the percentage of $NeuN^+$ (top) neurons, $GFAP^+$ astrocytes (middle) and $NeuN^-/GFAP^-$ cells (that are mostly astrocytes based on their morphology; bottom) among the GFP^+ cells; n = 3 animals. **(B)** Photomicrographs show GFP^+ cells at 24 dpi of AAV-FLEx-GFP (top) and Ngn2/Nurr1 (bottom) without stab wound injury (intact). Histograms on the right show the percentage of $NeuN^+$ (top) neurons, $GFAP^+$ astrocytes (bottom) among the GFP^+ cells with (same data as in Figure 1D) or without prior stab wound lesion; n = 3 animals. **(C)** Graphics showing the absolute number, expressed in cell per in mm^3, of $GFP^+/NeuN^+$ and $GFP^+/GFAP^+$ cells from different combination of AAV-FLEx vectors injected (GFP, Ngn2/Nurr1 or Ascl1/Nurr1) at different time points (10, 24 and 72 dpi); n = 3 or 4 animals. Data are shown as median ± IQR. One-way ANOVA (A) and Student's t-test (B and C). In C, stars above the graphs indicate the significance between the time points versus 10 dpi, while the stars in brackets show the significance between GFP and Ngn2/Nurr1 or Ascl1/Nurr1 at 72 dpi. * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$. dpi: days post injection, SW: stab wound. Scale bars: 20 µm.

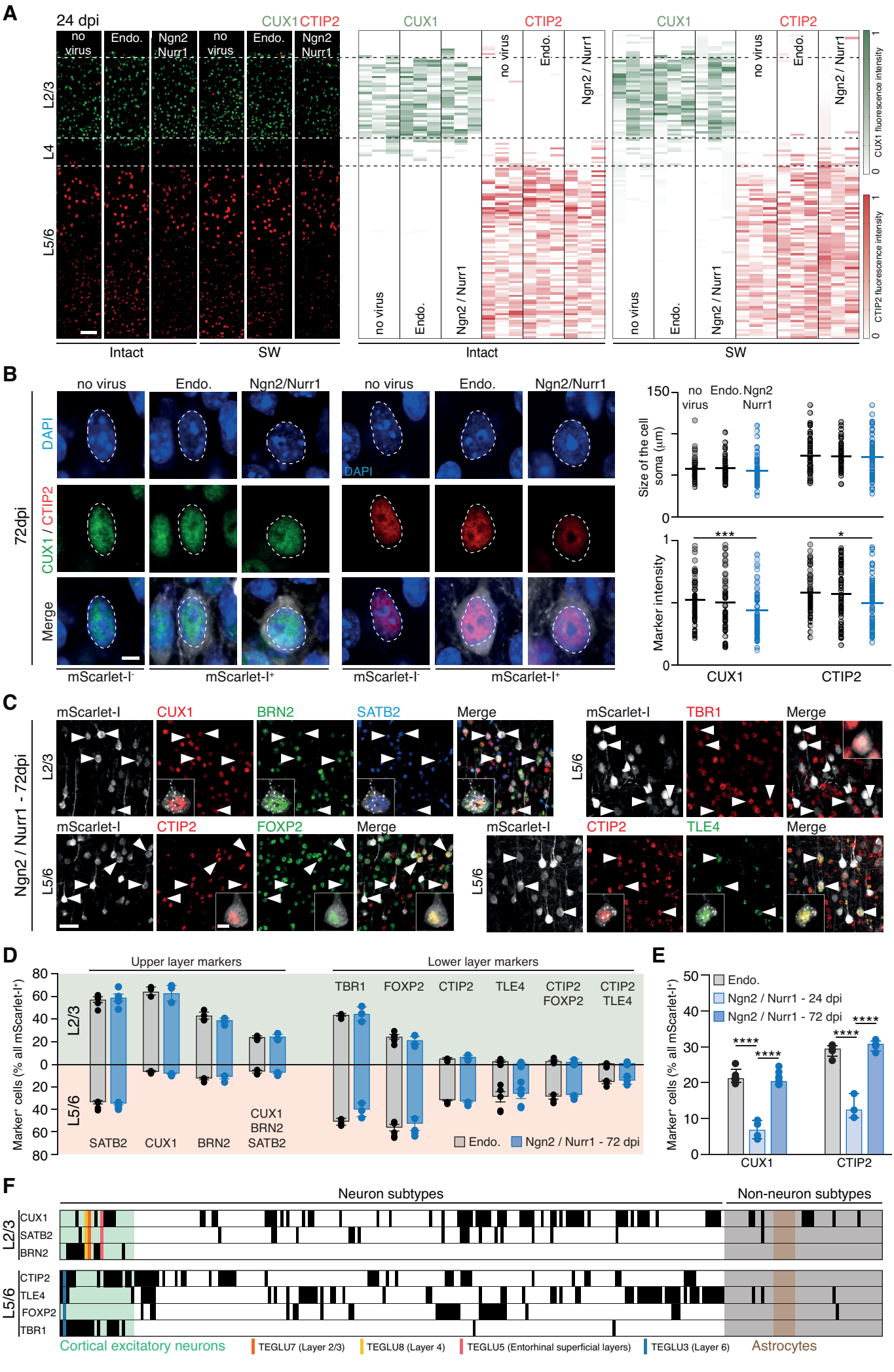
Figure S3 - Related to Figure 2



Supplementary Figure 3. Neurons induced with Ngn2/Nurr1 are EDU$^+$ after its application upon cortical injury and distribute along the entire cortical column.

(A) Photomicrographs of endogenous (top) and induced (bottom) neurons at 24 dpi of AVV-mScarlet-I and AAV-FLEx-mScarlet-I/Ngn2/Nurr1 respectively, showing the co-localization with EdU. EdU administration was performed from E7.5 to E15.5 (see time line on the left). **(B)** Quantification at 24 dpi of EdU$^+$ cells among mScarlet-I$^+$ neurons showing that only endogenous, but not induced neurons co-localized with EdU, confirming the astrocytic origin of iNs; n = 5 and 4 animals for Endo. and Ngn2/Nurr1, respectively. **(C)** Photomicrographs of endogenous (left) and induced (right) neurons at 24 dpi of mScarlet-I and mScarlet-I/Ngn2/Nurr1 respectively, showing the co-localization with EdU. EdU administration was performed from -3 dpi to 7 dpi (total 10 days after injury; see time line on the left). **(D)** Quantification at 24 dpi of EdU$^+$ cells among mScarlet-I$^+$ neurons showing that after injury mostly induced, but not endogenous neurons incorporated EdU, confirming that some iNs derive from proliferating astrocytes; n = 3 and 5 animals for Endo. and Ngn2/Nurr1, respectively. As the proportion of EdU$^+$ iNs correspond well with the overall proportion of proliferating astrocytes amongst reactive astrocytes (Buffo et al., 2008), we conclude that proliferating astrocytes are not more likely to convert into neurons than non-proliferating astrocytes. **(E)** Photomicrographs and drawings show the laminar position of endogenous and reprogrammed neurons at 72 dpi of AAV-mScarlet-I and AAV-FLEx-mScarlet-I/Ngn2/Nurr1. The heatmap shows the cell distribution in ten different bins. The color-coded columns represent the percentage of cells per bin; n = 5 animals. Histogram illustrates the average percentage of cells per bin. Data are shown as median ± IQR. Student's t-test (B and D) and one-way ANOVA (E). * $p \leq 0.05$, **** $p \leq 0.0001$. dpi: days post injection, E: embryonic day, L: layer, SW: stab wound. Scale bars: 100 µm (E), 20 µm (A and C) an 10 µm (A close up).

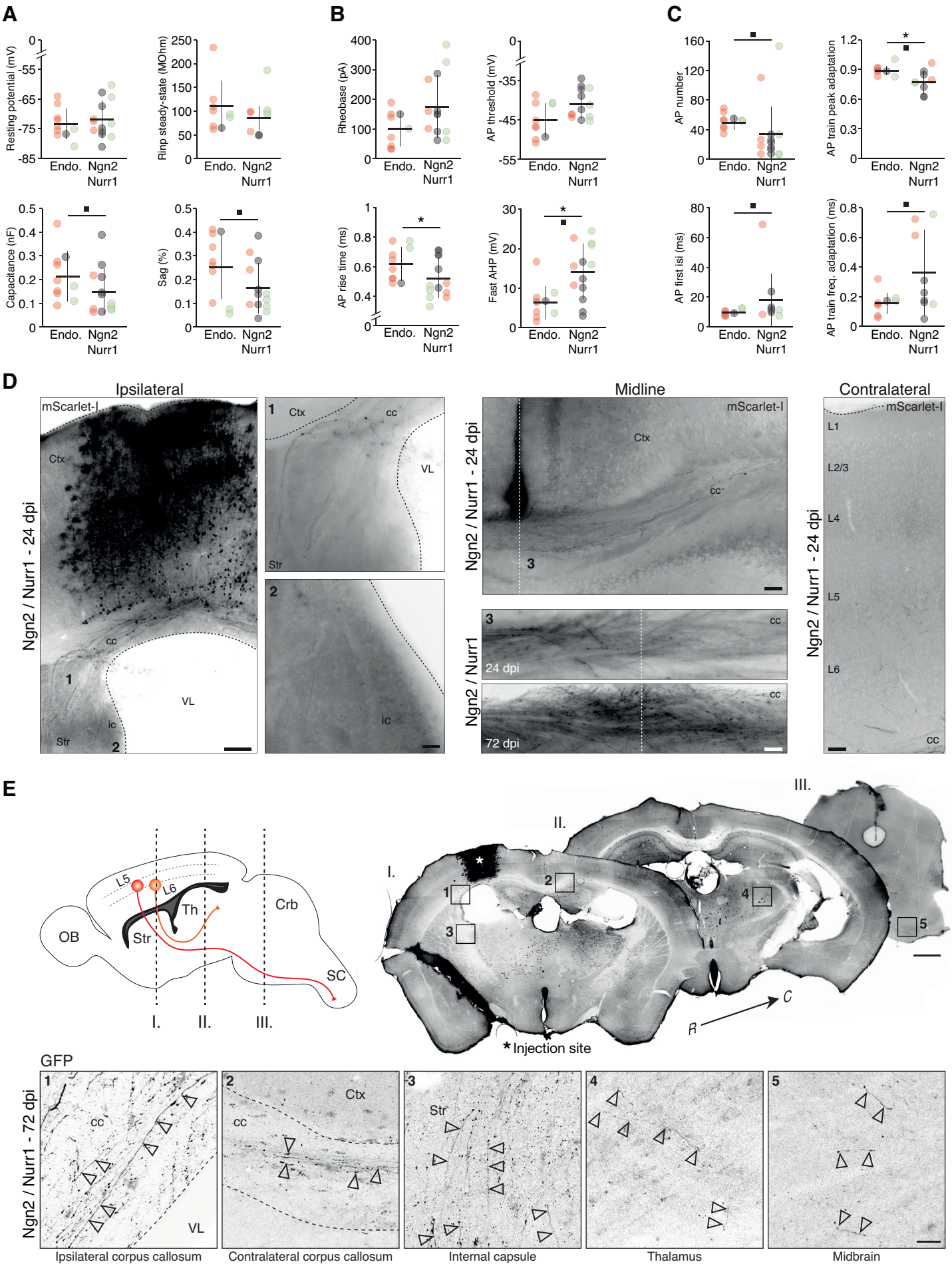
Figure S4 - Related to Figure 2



Supplementary Figure 4. Neurons induced by Ngn2/Nurr1 acquire a molecular signature according to their laminar position.

(A) Photomicrographs showing CUX1 and CTIP2 distribution in intact and lesioned (SW) cortical column. Three conditions were tested: no virus (*i.e.* no injection), Endo. (*i.e.* injection of mScarlet-I) and Ngn2/Nurr1 (*i.e.* injection of FLEx-mScarlet-I/Ngn2/Nurr1). CUX1 and CTIP2 intensity levels are layer-specific and appear not to be affected by the SW or the viral vector injection. The heatmap shows the average intensity level of CUX1 and CTIP2 in 135 bins covering the entire cortical column. CUX1 and CTIP2 intensity levels distribution does not differ between the different conditions; n = 3 animals. **(B)** Photomicrographs showing comparison between nuclear size and intensity for CUX1 (left) and CTIP2 (middle) in mScarlet-I$^-$ (*i.e.* no viral vector injection) and mScarlet-I$^+$ (*i.e.* injection of mScarlet-I or injection of FLEx-mScarlet-I/Ngn2/Nurr1) at 72 dpi. On the right histograms showing the average of the size of the cell soma (top) and the nuclear intensity for CUX1$^+$ and CTIP2$^+$ (bottom); n = 50 cells from 3 animals. **(C)** Photomicrographs showing mScarlet-I$^+$ cells at 72 dpi of FLEx-mScarlet-I/Ngn2/Nurr1 stained for various neuronal markers for upper and lower layers as indicated. **(D)** Histograms show the percentage of mentioned markers characteristic for upper and lower layer of endogenous and induced neurons (Ngn2/Nurr1) present in L2/3 and L5/6 at 72 dpi; n = 4 animals. **(E)** Histograms show the percentage of CUX1$^+$ (left) and CTIP2$^+$ cells (right) among mScarlet-I$^+$ endogenous neurons (grey) and mScarlet-I$^+$ iNs (blue) at 24 (light blue) and 72 dpi (dark blue); n = 4 animals. **(F)** Heatmap showing the expression (black line) of upper layer marker (CUX1, SATB2 and BRN2) and lower layer marker (CTIP2, TLE4, FOXP2 and TBR1) in different neuron and non-neuron subtypes (adapted from Zeisel et al., 2018). Colored lines show the cortical excitatory neurons subtypes (*i.e.* upper and lower layer excitatory neurons) generated by our reprogramming protocol at 72 dpi (*i.e.* overexpression of Ngn2/Nurr1). Cortical excitatory neurons subtypes are highlighted in green whereas astrocyte subtypes in brown. Data are shown as median ± IQR. One-way ANOVA (A, D and E) Kruskal-Wallis (B). * $p \leq 0.05$, *** $p \leq 0.001$, **** $p \leq 0.0001$. dpi: days post injection, L: layer, SW: stab wound. Scale bars: 100 µm (A), 50 µm (C; overview), 10 µm (B and C close up).

Figure S5 - Related to Figure 3



Supplementary Figure 5. Patch clamp and projections of induced neurons by Ngn2/Nurr1.
(A) Comparison of selected passive electrophysiological properties of endogenous (n = 10 cells) and induced neurons at 72 dpi (n = 13 cells). Upper and lower layer cells are represented with green and red dots, respectively. Black dots refer to cells whose position was not recorded. **(B)** Comparison of selected active electrophysiological properties and action potential parameters of endogenous (n = 10 cells) and induced neurons (n = 13 cells). iNs have on average a faster action potential rise time and a stronger fast after-hyperpolarization. **(C)** Comparison of selected firing properties of endogenous (n = 9 cells) and induced neurons (n = 8 cells). Parameters from response to 300 pA current injection. Cells that did not fire action potentials or went into saturation were not included. Note that iNs often have larger variance of the measured parameters than endogenous neurons. Data are shown as mean ± SD. Wilcoxon rank-sum test and Levene's test performed in MATLAB (A, B and C). * $p \leq 0.05$ (mean), ■ $p \leq 0.05$ (variance). **(D)** Photomicrographs illustrating axons of iNs at 24 dpi in the ipsilateral (left), midline (center) and contralateral cortical hemisphere (right). Pictures in middle panel on the bottom allow comparing the axons present at midline in the corpus callosum at 24 and 72 dpi. **(E)** Photomicrographs showing axonal processes of iNs at 72 dpi in the ipsilateral and contralateral hemisphere. Overview sections are shown on the top left with squares indicating the high-power magnifications shown in the bottom row. C: caudal, cc: corpus callosum, Crb: cerebellum, Ctx: cortex, dpi: days post injection, ic: internal capsule, L: layer, OB: olfactory bulb, R: rostral, VL: lateral ventricle, SC: spinal cord, Str: striatum Th: thalamus. Scale bars: 1 mm (E; overview), 100 µm (D; overview), 20 µm (D and E; close up).