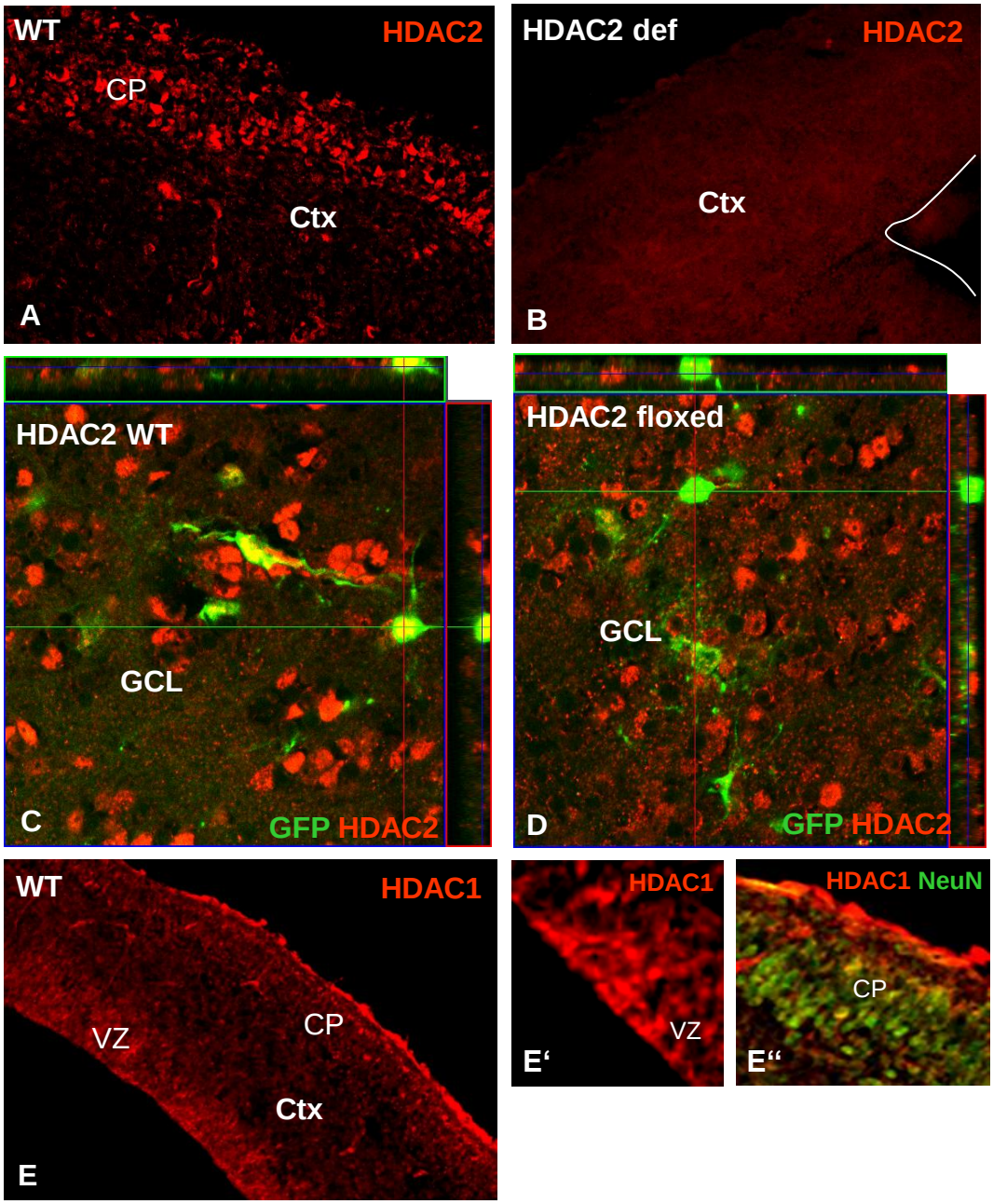
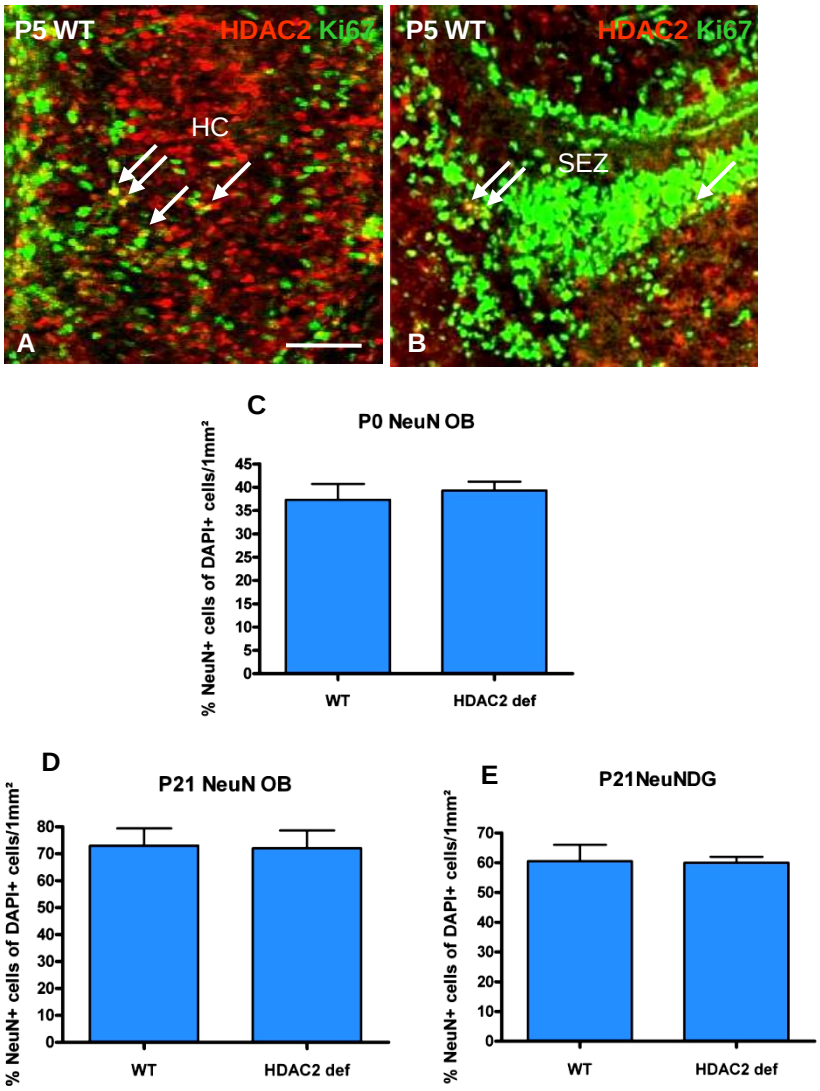
Supplemental Figure 1



Supplemental Figure 1. HDAC1 and 2 protein localisation in the embryonic telencephalon

Fluorescence micrographs depict immunostainings for HDAC2 (A,B) in the embryonic day (E) 14 telencephalon of WT (A) and HDAC2-deficient (B) mice. Note the absence of HDAC2-immunoreactivity in HDAC2-deficient brains (B) that lack the immunogenic site of the HDAC2 protein located at the C-terminal end. HDAC2 protein is concentrated in neurons located in the cortical plate (CP, A). (C,D) depict fluorescence micrographs of HDAC2 and GFP immunostainings in sections of the olfactory bulb (OB) from WT (C) and HDAC2 homozygous floxed (D) mice after Cre recombination (GFP depicts recombined cells). Panel D depicts HDAC2-staining of HDAC2 depleted cells underlining further the specificity of the staining. HDAC1-immunoreactivity localizes to the ventricular zone (VZ= progenitor cells, E, E') and is absent from neurons labelled by NeuN in the cortical plate (CP, E''). White line in B marks the ventricle. CP= cortical plate, Ctx= cortex, GCL= granule cell layer, VZ= ventricular zone. White line in D depicts the ventricle. Scale bars: E 50µm, A, B, C, D, F 20µm.

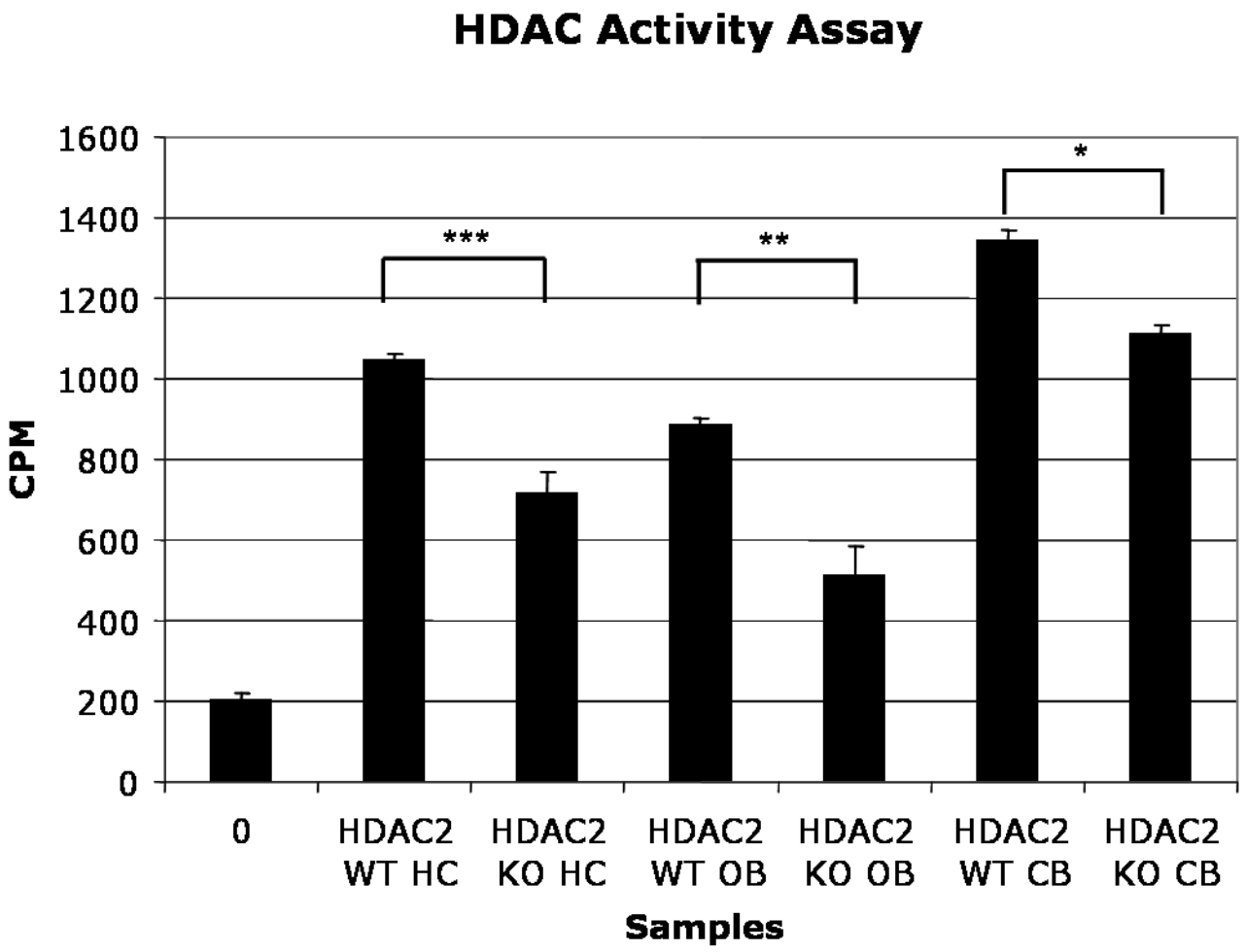
Supplemental Figure 2



Supplemental Figure 2. Expression of HDAC2 in neuronal progenitors at postnatal day 5 and neuronal differentiation during different stages of brain development

Fluorescence micrographs (A,B) show HDAC2-immunoreactivity in Ki67+ progenitor cells in the neurogenic regions of the HC (A) and the SEZ (B). Histograms in (C-E) depict the comparable percentage of neurons generated during development at PO in the OB (C) and at P21 in the OB (D) and in the DG (E). HC= Hippocampus, SEZ= subependymal zone. n= 3 animals each. Arrows demark double-positive cells. Scale bar: 20µm

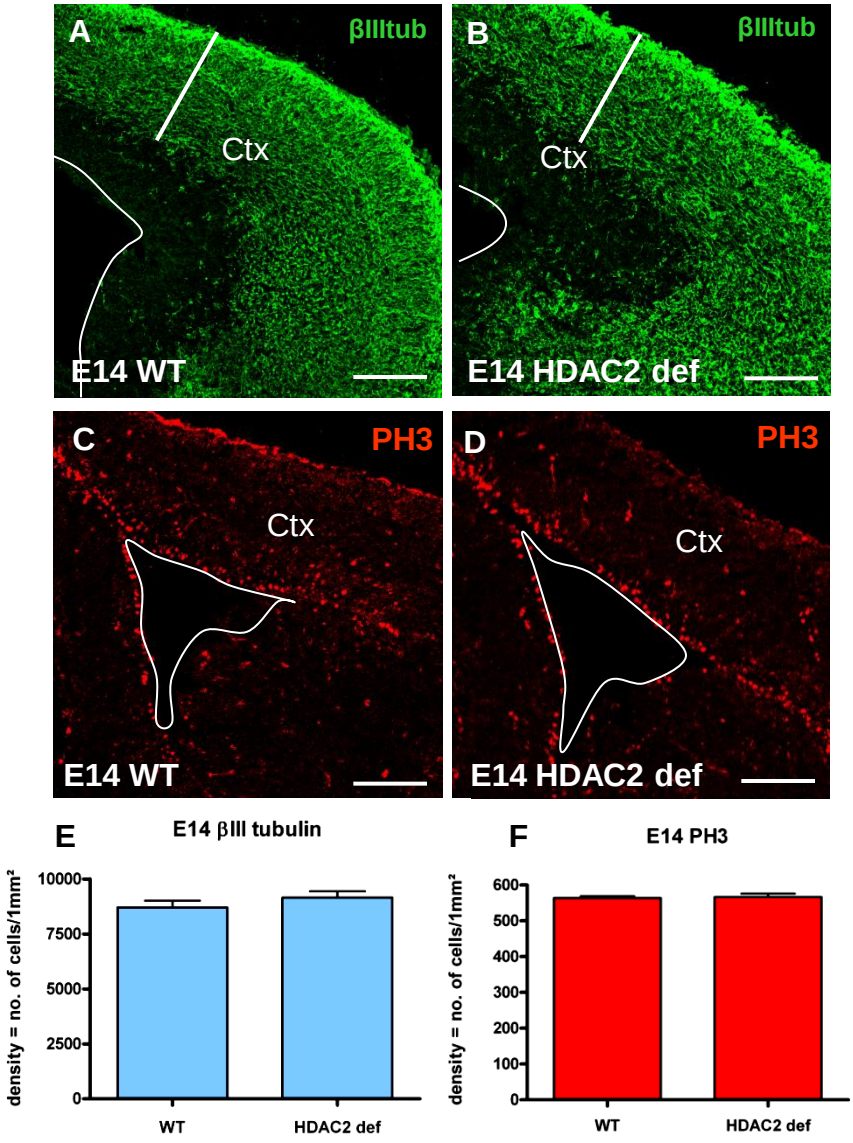
Supplemental Figure 3



Supplemental Figure 3. HDAC activity assay

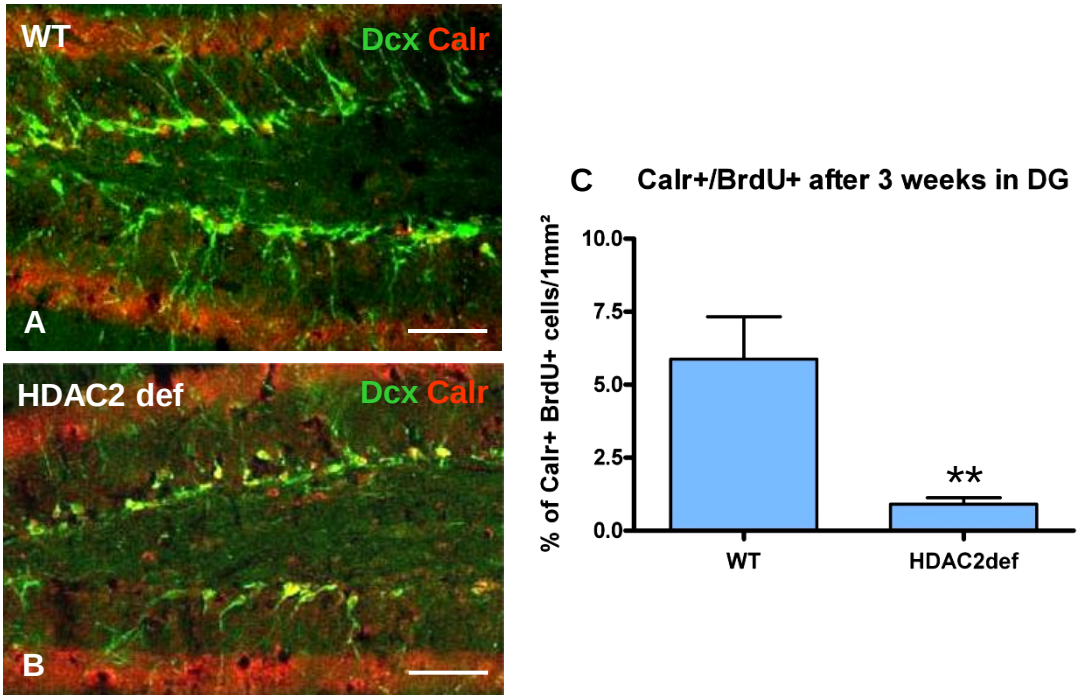
The activity of the HDACs was measured in different WT and HDAC2-deficient brain regions.
Note the significant reduction of HDAC activity measured by counts per minute (CPM) in all HDAC2-deficient brain areas compared to WT. HC= hippocampus, OB= olfactory bulb, CB= cerebellum. n= 3 animals each. Error bars depict SEM and *, **, *** depict p ‹ 0.05, 0.01, 0.001 determined by unpaired T-test respectively.

Supplemental Figure 4



Supplemental Figure 4. Normal development of the HDAC2-deficient telencephalon
(A,B) depict fluorescence micrographs of neurons labeled with βIIItubulin in embryonic day (E)14 WT and HDAC2-deficient telencephalon. Note the comparable size of WT and HDAC2-deficient cortices and the comparable thickness of the neuronal layers (white bars, 0,8 cm). (C,D) depict cells in M-phase (PH3) lining the ventricle (white line in A-D) in WT (C) and HDAC2-deficient (D) brains at E14. Histograms in (E,F) depict numbers of neurons (E) and proliferating cells (F) in the E14 cerebral cortex of both genotypes. Ctx= cortex. n= 3 animals. Scale bars: A-D 50µm.

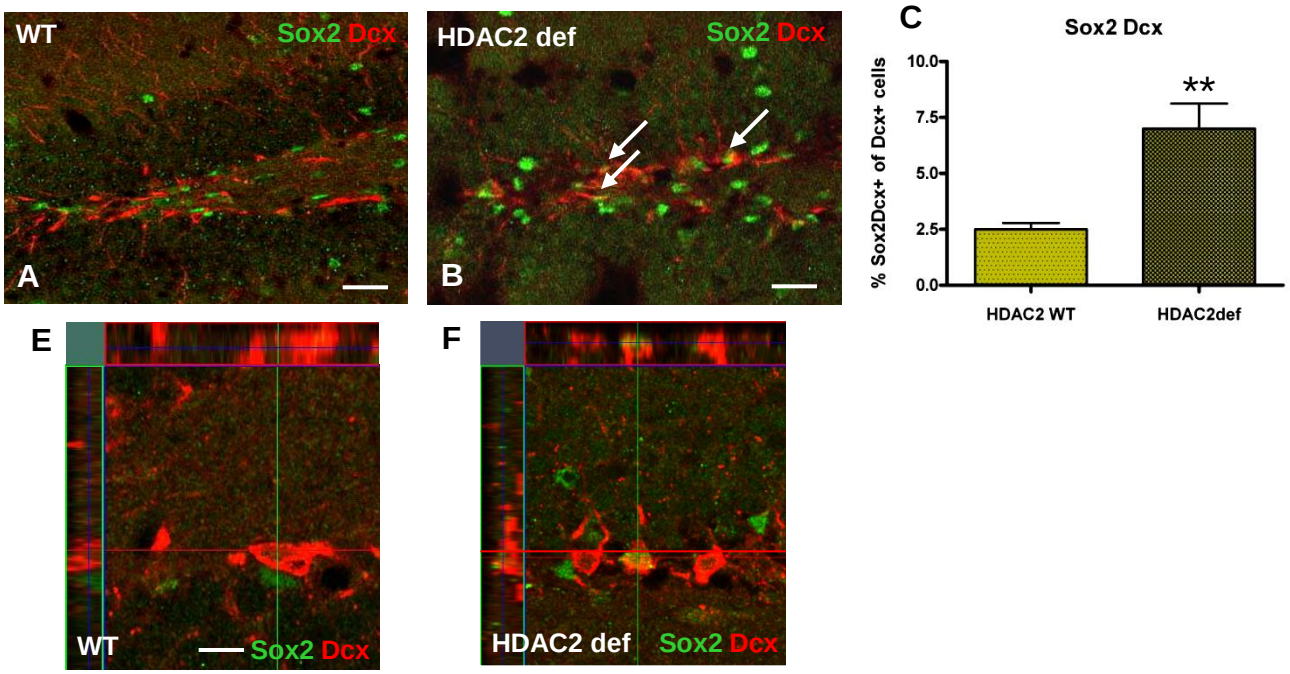
Supplemental Figure 5



Supplemental Figure 5. Neuronal differentiation of Calretinin+ neurons is impaired in HDAC2-deficient DG

(A-C) The decrease in maturating neurons that are Calretinin+ is prominent in cells born 1-3 weeks before (BrdU) in the DG. n= 3 animals. Error bar depicts SEM and ** depicts p ‹ 0.01 determined by unpaired T-test respectively. Scale bars: 50µm.

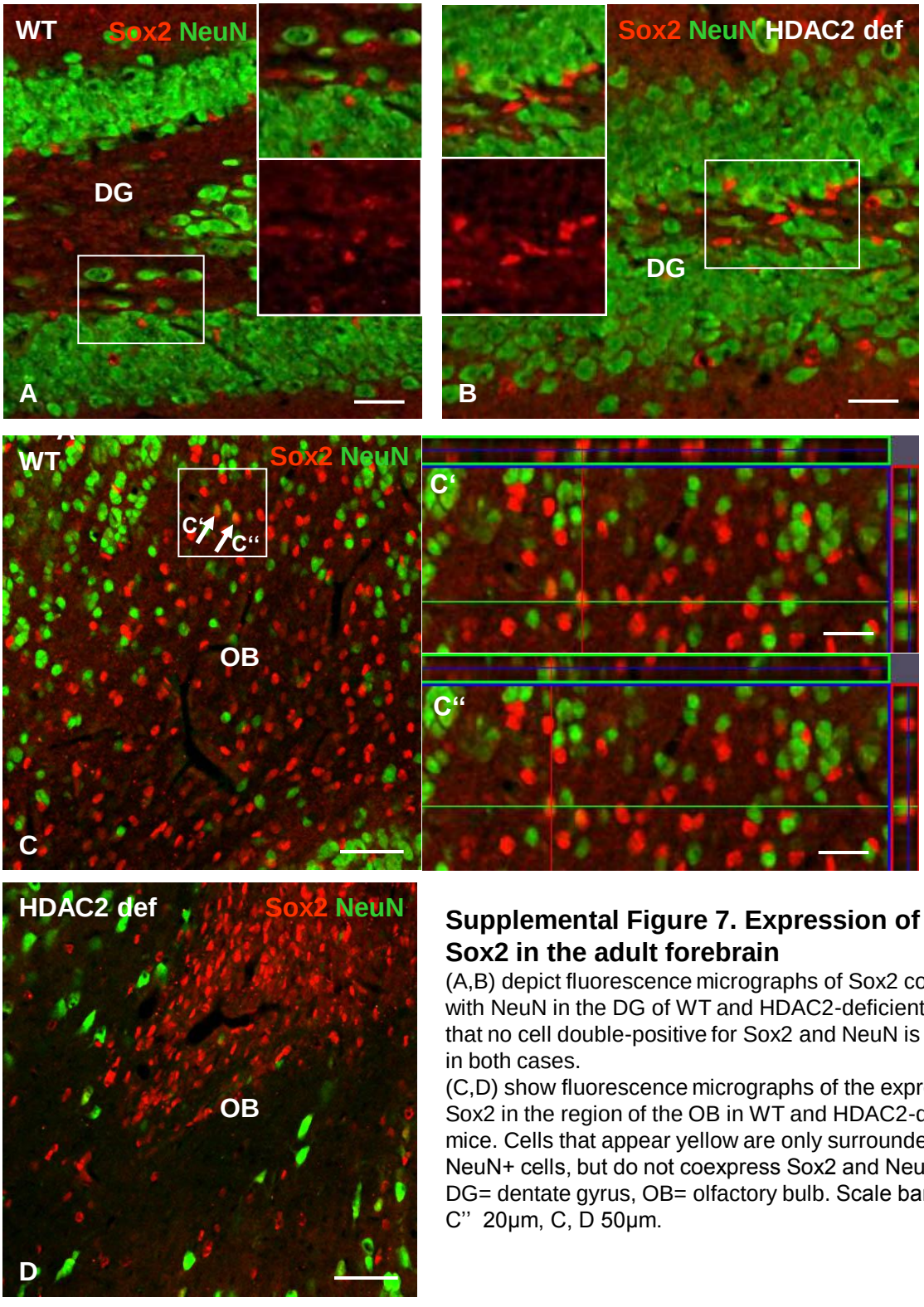
Supplemental Figure 6



Supplemental Figure 6. Upregulation of Sox2 in HDAC2 deficient DG

Fluorescent micrographs in (A,B) show Sox2 expressing cells in the subgranular zone that are mostly Dcx-negative in WT, while some double-positive cells are present in the HDAC2-deficient DG. Histogram in (C) depicts the percentage of Sox2+ cells that co-express Dcx (C). Fluorescent micrographs in (E,F) depict higher magnification of a cell either positive for Sox2 or Dcx (E) compared to a cell positive for Sox2 and Dcx in the HDAC2-deficient DG (F). n= 3 animals. Error bar depicts SEM and ** depicts p ‹ 0.01 determined by unpaired T-test respectively. Scale bars: A,B 20µm; E,F 10µm.

Supplemental Figure 7

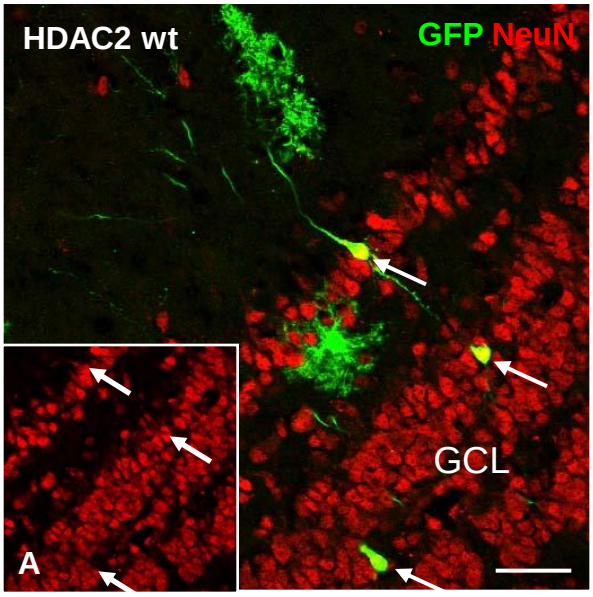


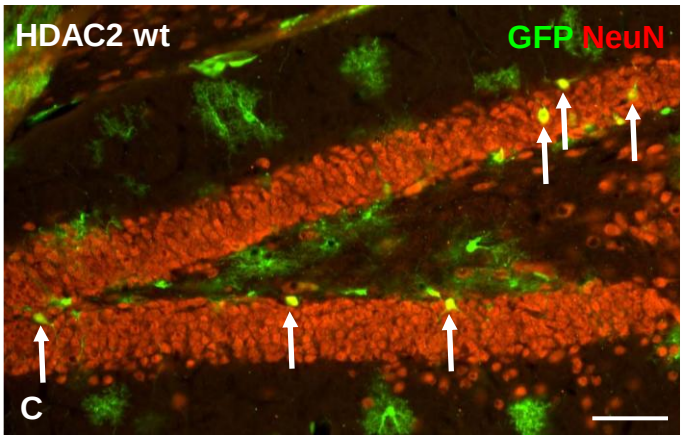
Supplemental Figure 7. Expression of Sox2 in the adult forebrain

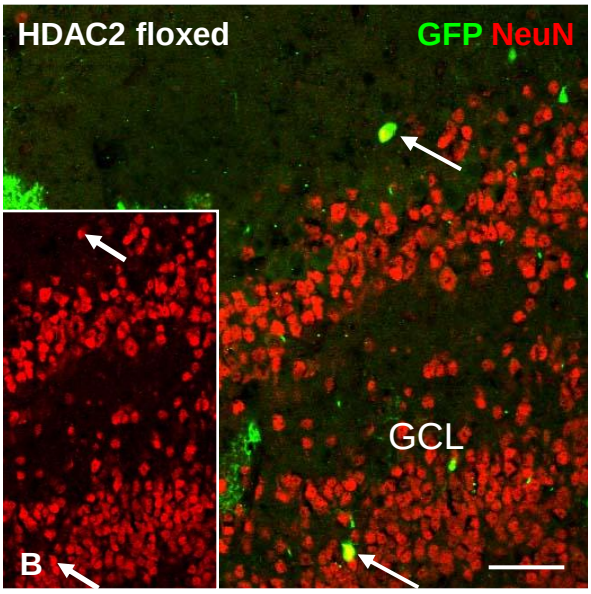
(A,B) depict fluorescence micrographs of Sox2 colabelled with NeuN in the DG of WT and HDAC2-deficient mice. Note that no cell double-positive for Sox2 and NeuN is detectable in both cases.

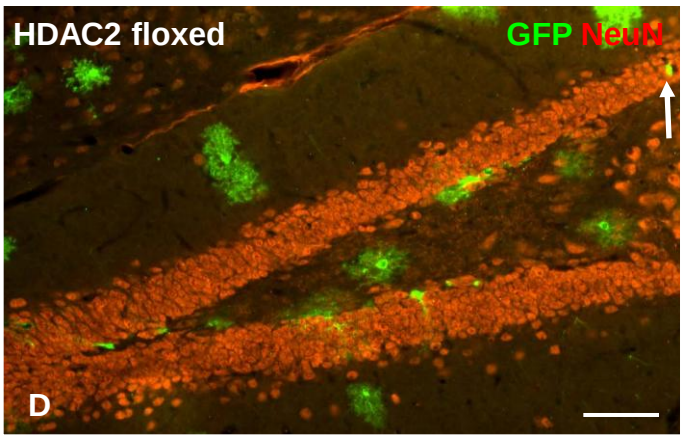
(C,D) show fluorescence micrographs of the expression of Sox2 in the region of the OB in WT and HDAC2-deficient mice. Cells that appear yellow are only surrounded by NeuN+ cells, but do not coexpress Sox2 and NeuN (C', C''). DG= dentate gyrus, OB= olfactory bulb. Scale bars: A,B, C', C'' 20µm, C, D 50µm.

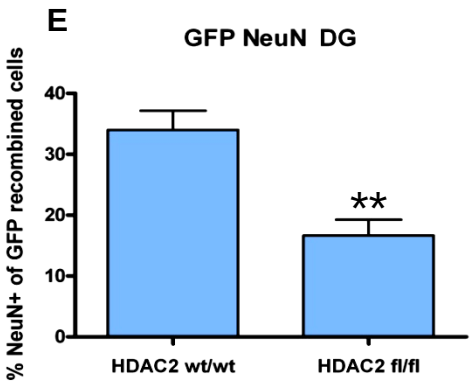
Supplemental Figure 8







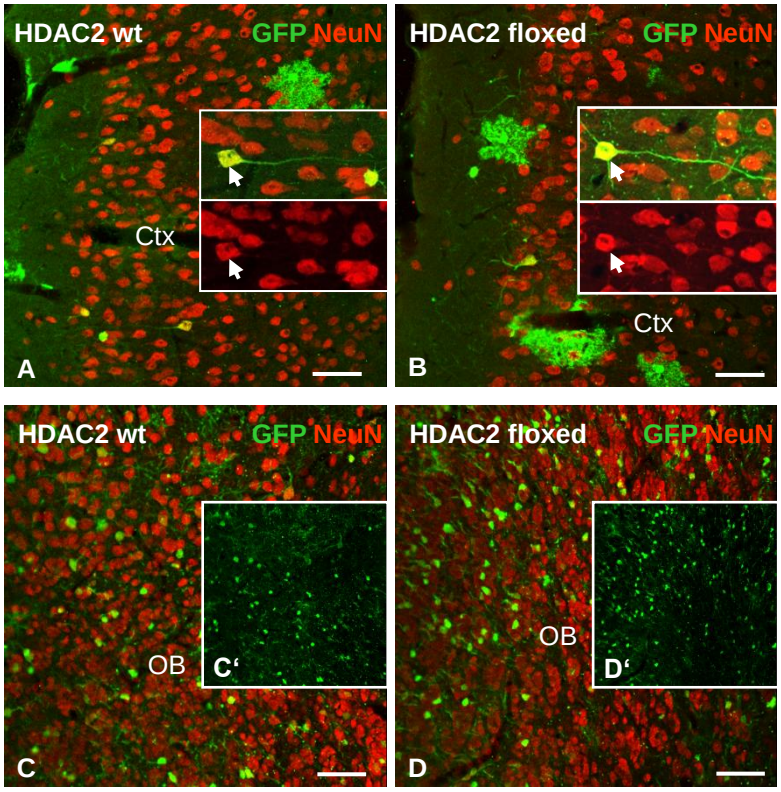


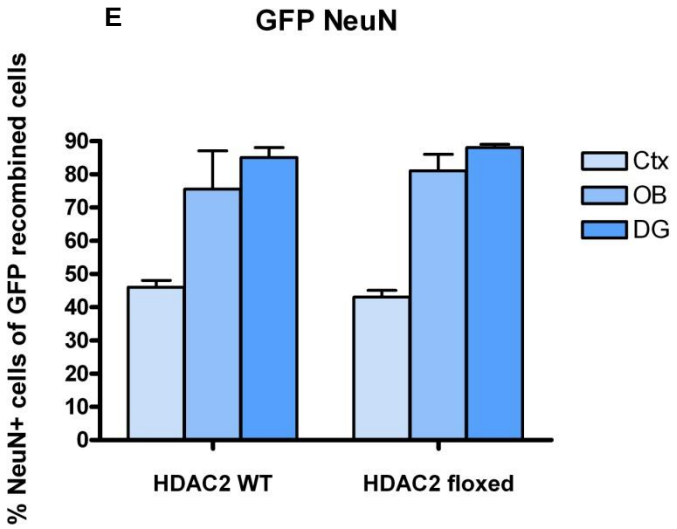


Supplemental Figure 8. Adult born NeuN+ neurons after conditional deletion of HDAC2 in adult neural stem cells

(A,B) depict fluorescence micrographs of GFP recombined cells 3 weeks after tamoxifen in the OB that express NeuN. Note the defective maturation of GFP+ cells in the GFP+ cells homozygous for floxed HDAC2 (B, HDAC2-floxed). (C,D) show fluorescence micrographs of GFP+ cells in the DG of WT and homozygous HDAC2 floxed mice. Note the reduction of GFP recombined cells in HDAC2 floxed DG. Histogram in (E) depicts the significant reduction of GFP+NeuN+ cells in the DG of HDAC2 floxed mice, comparable to results in the OB (Figure 7I). Arrows demark double-positive cells. GCL= granular cell layer. n= 3 animals. Error bar depicts SEM and ** depicts p ‹ 0.01 determined by unpaired T-test respectively. Scale bars: A-D 50µm.

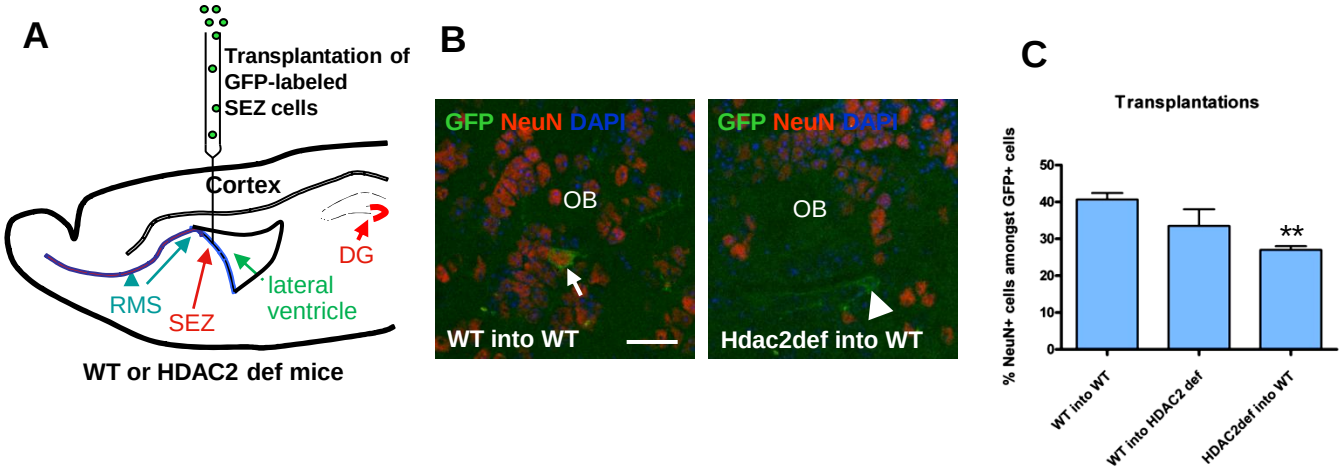
Supplemental Figure 9





Supplemental Figure 9. Conditional deletion of HDAC2 in embryonic neural progenitors *in vivo*.
(A-D) Fluorescence micrographs show GFP+ neurons and glial cells in cortices of mice of control and fl/fl genotypes 4 weeks after birth tamoxifen induced at E14. Histogram in (E) shows the comparable percentage of neurons amongst GFP-reporter-positive cells in control and HDAC2 floxed cortices, OB and DG. Ctx= cortex, OB= olfactory bulb. n= 2 animals. Arrows demark double-positive cells. Scale bars: 50µm.

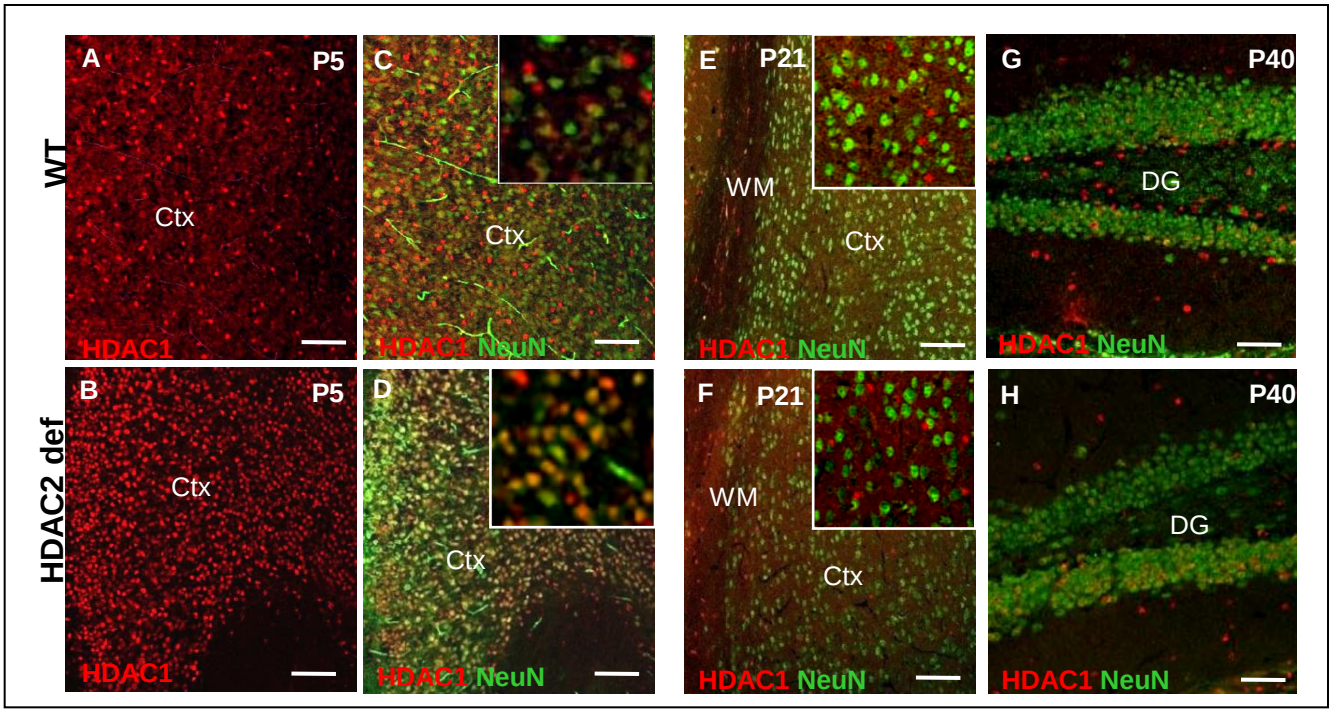
Supplemental Figure 10



Supplemental Figure 10. Transplantation experiments of GFP-labelled WT SEZ cells in the HDAC2 deficient background

Schematic drawing in (A) depicts the technique of GFP-labeled cell transplantation into the SEZ of WT and HDAC2-deficient. (B) depicts an example of a transplanted cell (GFP+) positive for NeuN showing a normal neuronal differentiation of WT SEZ cells in a WT background and an HDAC2-deficient transplanted GFP+ cell lacking NeuN expression in the OB 21 days after transplantation. Histogram in (C) depicts the proportion of transplanted WT and HDAC2-deficient cells differentiating into neurons (positive for NeuN) after 21 days in WT or HDAC2 deficient environment. Note the reduction in the percentage of NeuN+ cells when HDAC2-deficient cells were transplanted in WT background while the percentage of NeuN+ cells amongst GFP+ transplanted cells remains normal when WT cells were transplanted into HDAC2-deficient background compared to WT tranplantation. OB = olfactory bulb. n= 3 animals for the “WT into WT group”, n= 2 for the “WT into HDAC2 def” and “HDAC2def into WT” group each. Arrow demarks a double-positive cell, arrowhead demarks a double-negative cell. Error bar depicts SEM and ** depicts p ‹ 0.01 determined by unpaired T-test respectively. Scale bar 20µm.

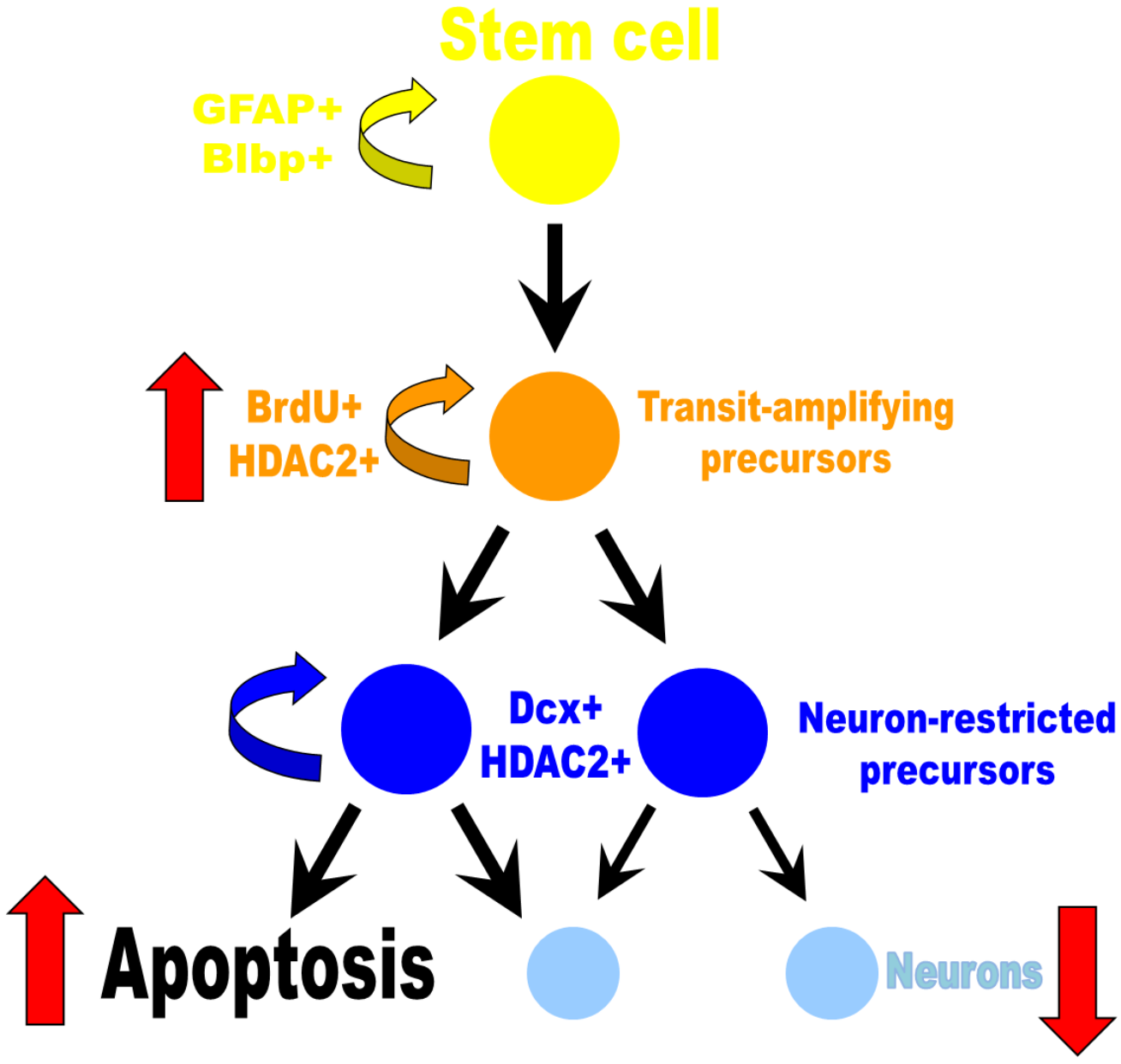
Supplemental Figure 11



Supplemental Figure 11. HDAC1 immunoreactivity during development in WT and HDAC2-deficient mice

(A,B) In WT sections at P5 and P21 (E) HDAC1 expression is restricted to non-neuronal cells and shows no overlap with NeuN expression while HDAC1 was detectable in NeuN+ neurons in HDAC2-deficient brains (C,D). At P21 HDAC1 is not expressed in NeuN+ neurons and declines to normal levels (F). (G,H) The expression of HDAC1 in adult neurogenic regions is comparable between WT and HDAC2-deficient mice. Ctx= cortex, DG= dentate gyrus, WM= white matter. Scale bars: 50µm.

Supplemental Figure 12



Supplemental Figure 12. Summary of HDAC2 function in the brain.
Schematic drawing summarizing the expression of HDAC2 in neurogenesis during development and adulthood and the defects observed upon lack of HDAC2 function in the adult neurogenesis.