Expanded View Figures

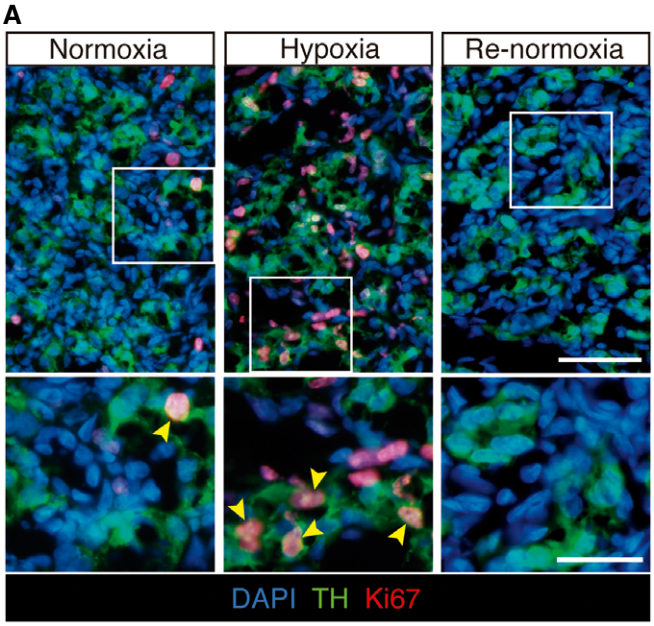

Figure EV1. Proliferation of CB TH-positive glomus cells in response to hypoxia.

A Immunohistochemical detection of TH (green) and Ki67 (red) in carotid body (CB) of normoxic, hypoxic (7d), and re-normoxic rats (7d).

B Percentage of positive cells versus total cells for the indicated markers in the three experimental groups of animals shown in (A) (n = 2–3 rats per group).

Data information: Scale bars: 100 μm in (A; top), and 50 μm in (A; bottom). Data in bar graph are represented as mean ± SEM. *P < 0.05, ***P < 0.001 (Student's t-test).
Source data are available online for this figure.

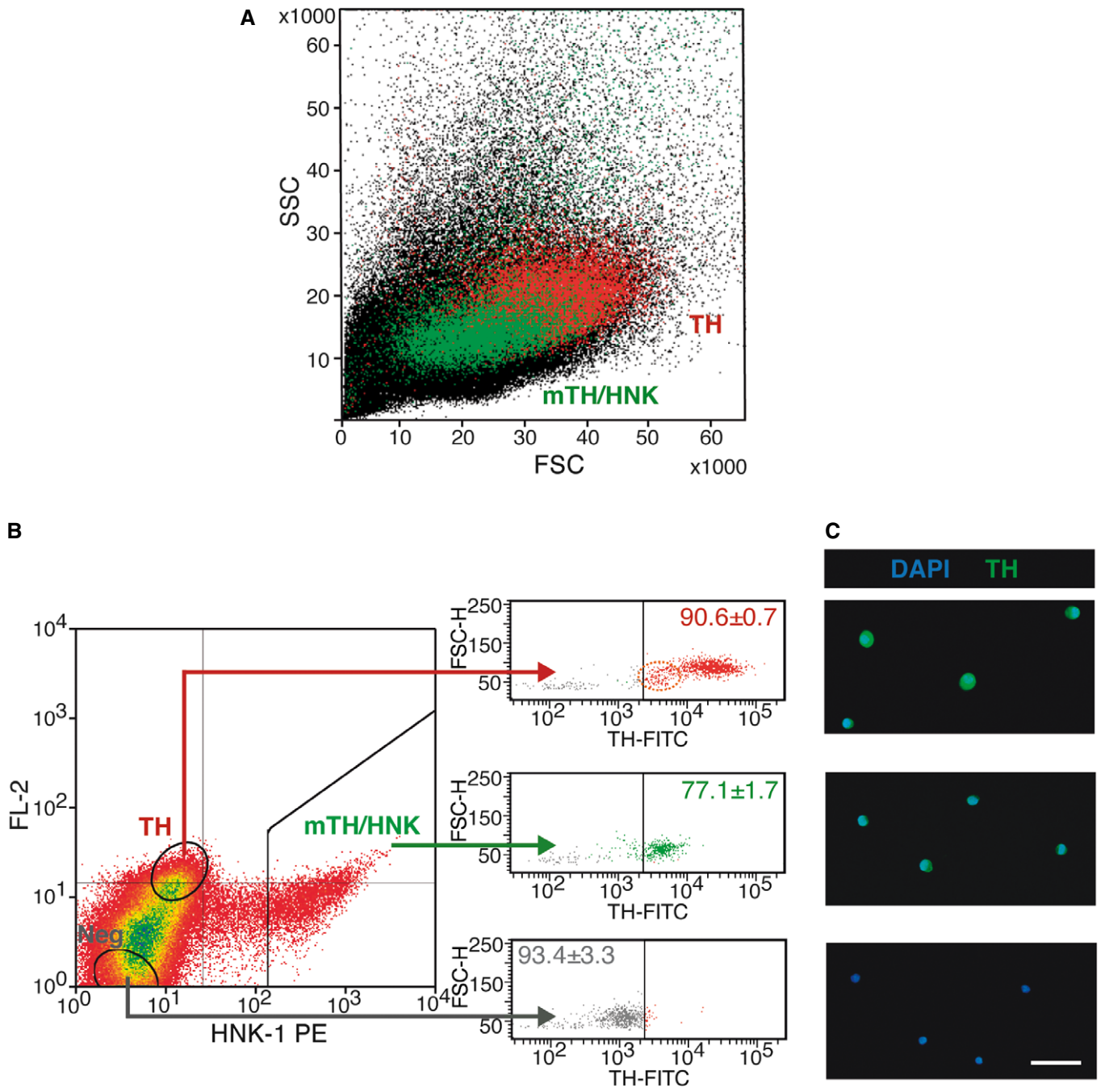

Figure EV2. Enrichment and sorting of live mature and immature CB glomus cells.

A Forward (FSC) versus side (SSC) scatter plot of CB dispersed cells, illustrating the difference in size and complexity between mature (TH, red dots) and immature (mTH/HNK, green dots) CB glomus cells.

B FACS plot representing HNK-1 staining versus FL-2 channel autofluorescence, with sorting gates to enrich as mature (TH; which are HNK^- and autofluorescent) and immature (mTH/HNK; which are HNK^+) CB glomus cells. Bulk CB-negative (Neg) cells are also sorted as a control. The right panel shows TH expression in the different sorted cells, as revealed by intracellular staining of fixed cells, confirming the nature of the different sorted populations. The percentages inside the plots refer to the proportion of cells within that particular side of the plot with regard to TH expression (n = 3 independent experiments with a total of 11 rats). Note the presence of a small subpopulation of TH low/HNK-1-negative cells (dashed orange ellipse in the upper right panel), which might constitute neuroblasts that lost HNK-1 expression due to tissue dispersion, or a subpopulation of intermediate cells between neuroblasts and mature glomus cells. In the last case, these cells might account for the basal proliferation observed in the TH^+ population (see Fig 2C).

C Microscopy images of the different sorted cells shown in (B), confirming their differential expression of the neuronal marker TH. Scale bar: 25 μm.

Source data are available online for this figure.

Figure EV3. Maturation of mTH/HNK cells under hypoxia entails an increase in cell size and a decrease in HNK-1 expression levels, with stabilization of Hif2α.

A Immunofluorescent images showing detection of tyrosine hydroxylase (TH; red) in mTH/HNK sorted cells from carotid bodies of normoxic rats. Cells were cultured on adherent substrate either in normoxic conditions (21% O_2; Normoxia) or in hypoxic conditions (3% O_2; Hypoxia) for 4 days.

B Quantification of cell size from the experiment shown in (A) (n = 191 cells from three independent cultures with a total of 12 rats).

C Quantification of the percentage of HNK-1-positive cells from the experiment shown in (A). The data show a clear decrease in the number of HNK-1-positive cells in cultures exposed to hypoxia (n = 182 cells from three independent cultures with a total of 12 rats).

D–F Immunocytochemical pictures showing stabilization of Hif2α (Epas1) in mTH/HNK sorted cells after 3 days cultured in hypoxic conditions (3% O_2) (D), as compared to normoxic conditions (21% O2) (E). Pictures were taken in parallel, with the same exposure time and conditions, in order to see the differences in Epas1 and TH staining intensities between normoxia and hypoxia. Negative cells (sorted as shown in Fig EV2B), cultured in parallel conditions, are not stained by the anti-Epas1 antibody (F).

Data information: Scale bars: 10 μm in (A) and 20 μm in (D), (E), and (F). Data in bar graphs are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$ (Student's t-test).
Source data are available online for this figure.

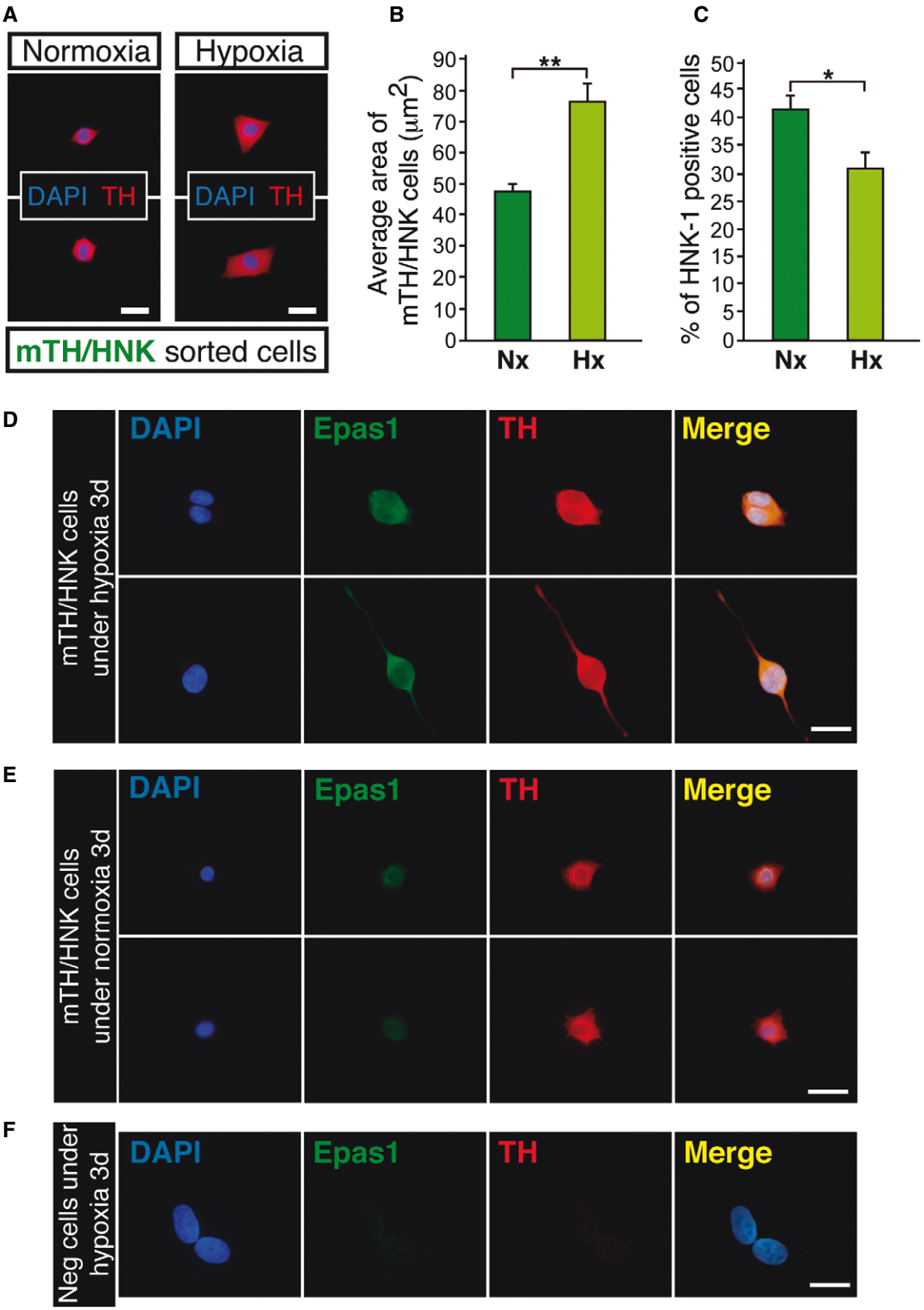
A
Normoxia
Hypoxia
DAPI TH
DAPI TH
mTH/HNK sorted cells
B
Average area of mTH/HNK cells (μm2)
90
80
70
60
50
40
30
20
10
0
**
Nx
Hx
C
% of HNK-1 positive cells
50
45
40
35
30
25
20
15
10
5
0
*
Nx
Hx
D
mTH/HNK cells under hypoxia 3d
DAPI
Epas1
TH
Merge
E
mTH/HNK cells under normoxia 3d
DAPI
Epas1
TH
Merge
F
Neg cells under hypoxia 3d
DAPI
Epas1
TH
Merge

Figure EV3.

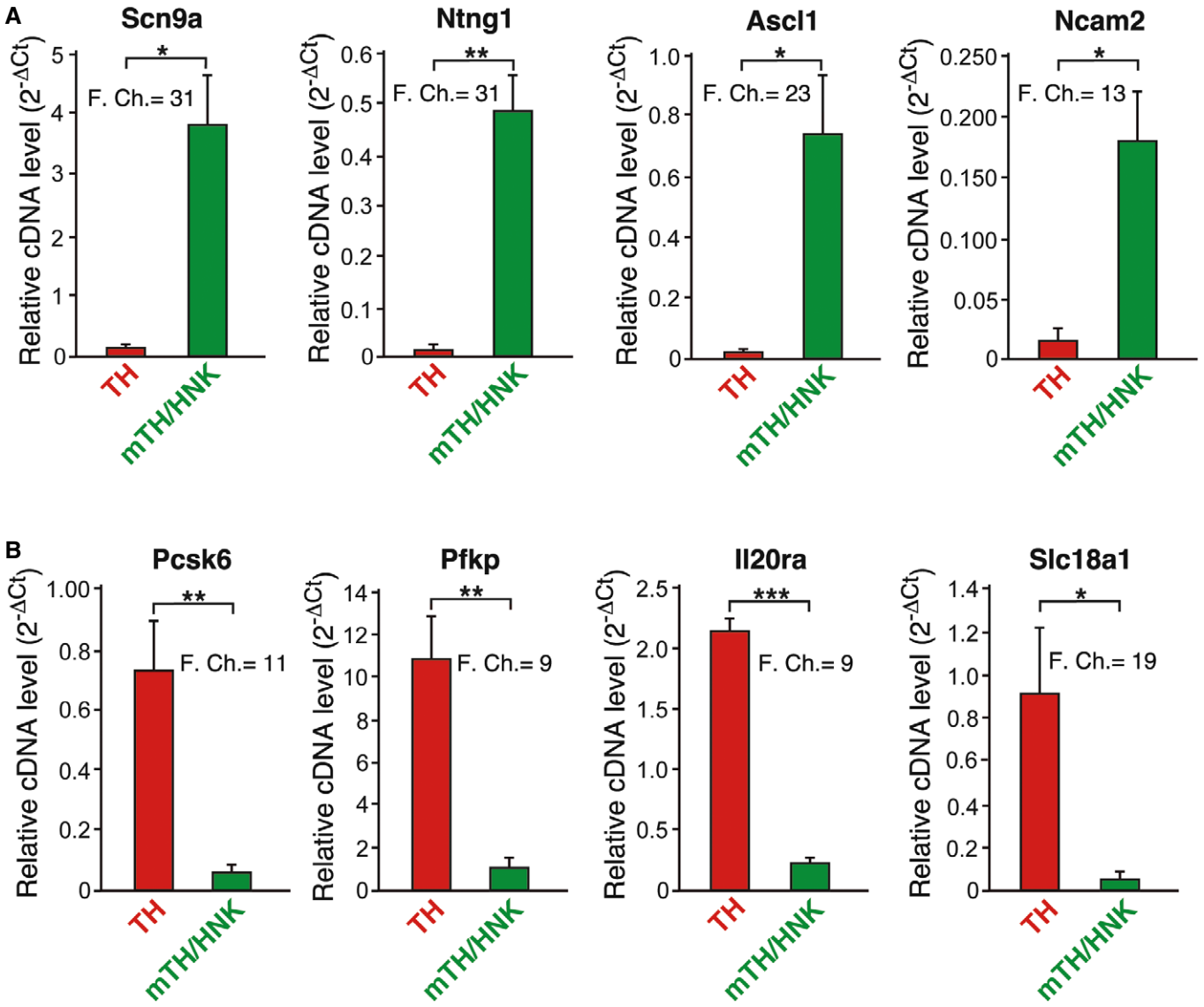

Figure EV4. Differential gene expression between TH and mTH/HNK cells.

A Graphs showing relative cDNA levels, as a measure of mRNA, and fold changes of genes preferentially expressed in mTH/HNK cells. These cells show upregulation in some genes typically expressed in neuroblastic and immature cells, such as Ascl1, Ncam2, Scn9a, and Ntng1 (n = 3 independent mRNA samples from four animals each).

B Graphs showing relative cDNA levels, as a measure of mRNA, and fold changes of genes preferentially expressed in TH cells. These cells display a typical neuroendocrine mature cell profile, with high expression of genes related to transport of vesicles and signaling, such as Slc18a1 and Il20ra, and genes related to metabolism, such as Pfkp. Interestingly, Pcsk6, which encodes for a negative regulator of Ascl1, is also upregulated [27], (n = 3 independent mRNA samples from four animals each).

Data information: F.Ch. = Fold Change. Data in bar graphs are represented as mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001 (Student's t-test).
Source data are available online for this figure.

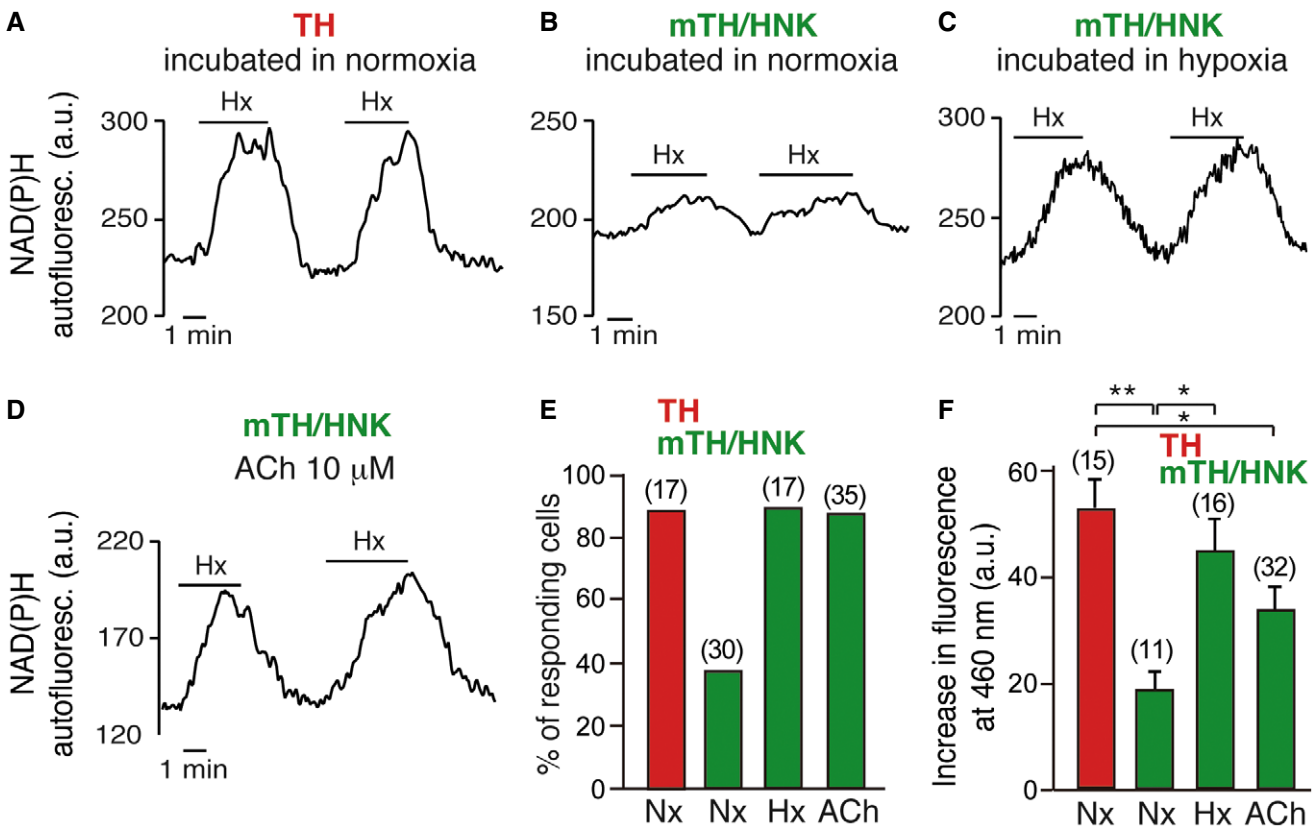

Figure EV5. Hypoxia and cholinergic signaling induce neuroblast maturation.

A–D Increase in NAD(P)H autofluorescence in response to hypoxia in mature glomus cells (TH) after 24–48 h in normoxia (A), in immature neuroblasts (mTH/HNK) after 24–48 h in normoxia (B), in immature neuroblasts after 24–48 h in hypoxia (C), and in immature neuroblasts after exposure to acetylcholine (ACh) for 24–48 h (D).

E Quantification of hypoxia-responsive cells, as measured by NAD(P)H autofluorescence, under the indicated conditions. Number of total cells studied is indicated between brackets.

F Quantification of the magnitude of the response to hypoxia in the different cells under the indicated conditions. Number of total cells studied is indicated between brackets.

Data information: Data in bar graph of panel (E) are presented as the sum of responding cells among total cells studied. Data in bar graph of panel (F) are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$ (Student's t-test). Cells used for the analysis in this figure were obtained in eight independent experiments with four rats each.
Source data are available online for this figure.