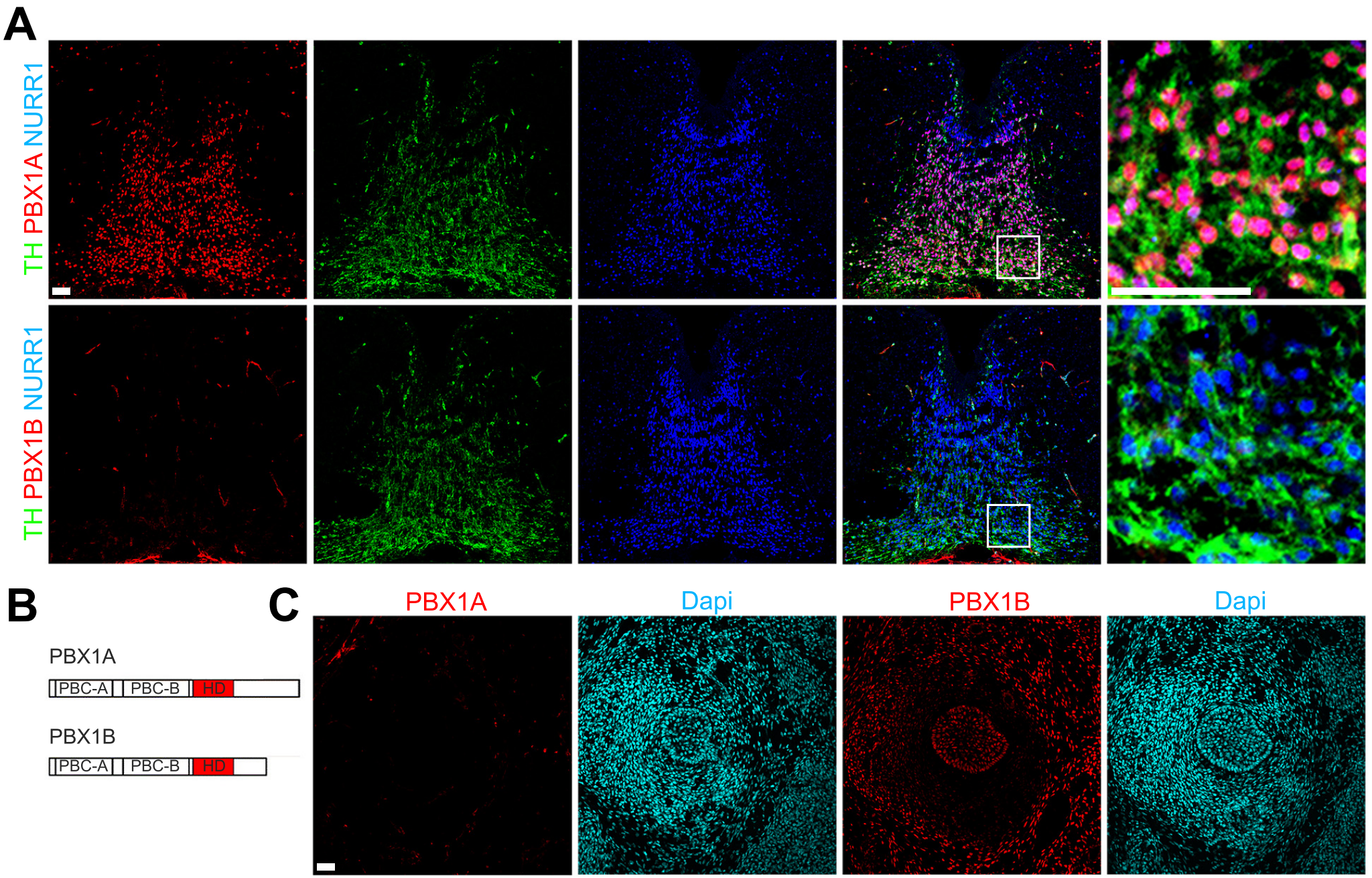
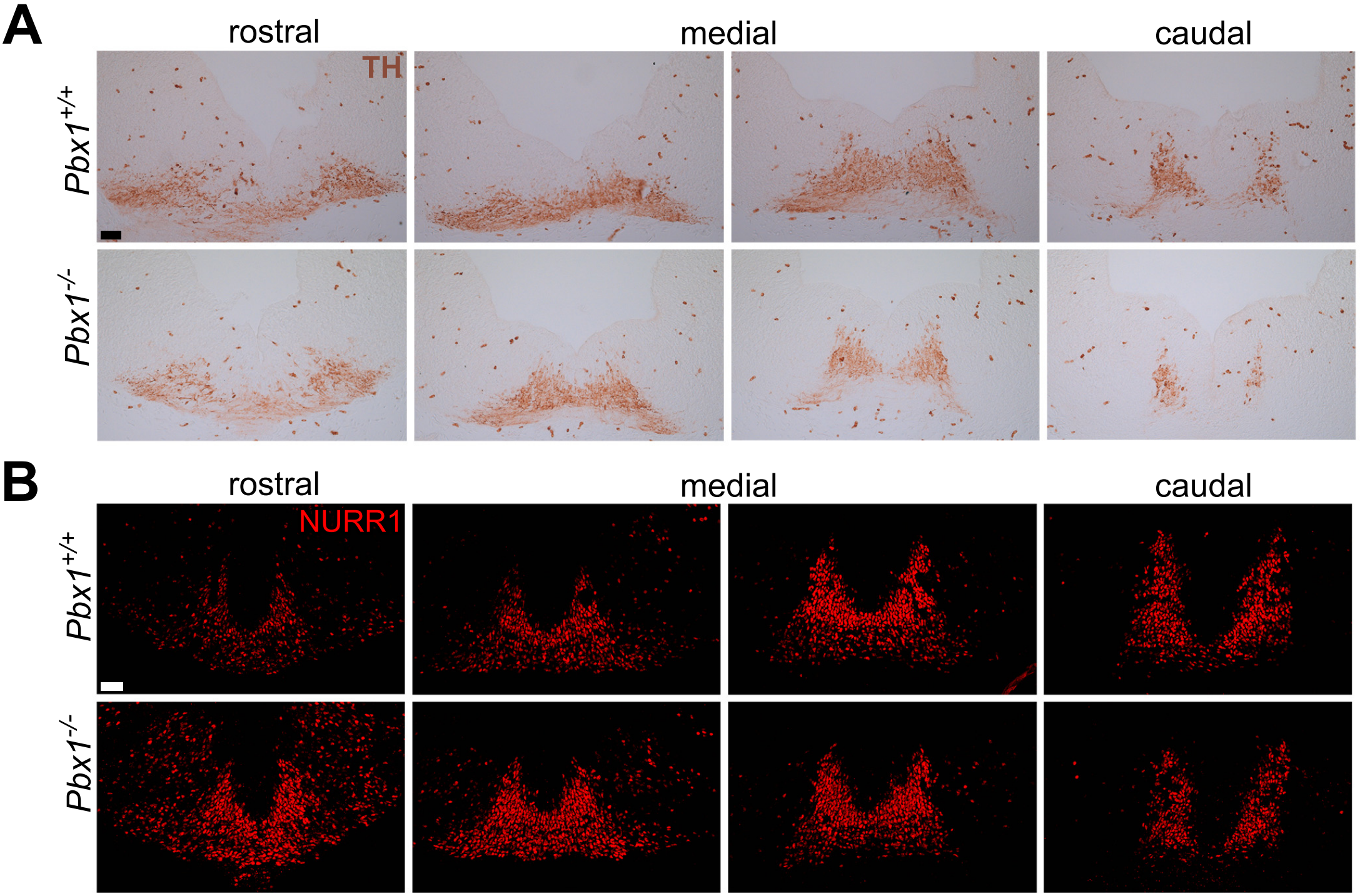
CONTENT OF APPENDIX PDF:

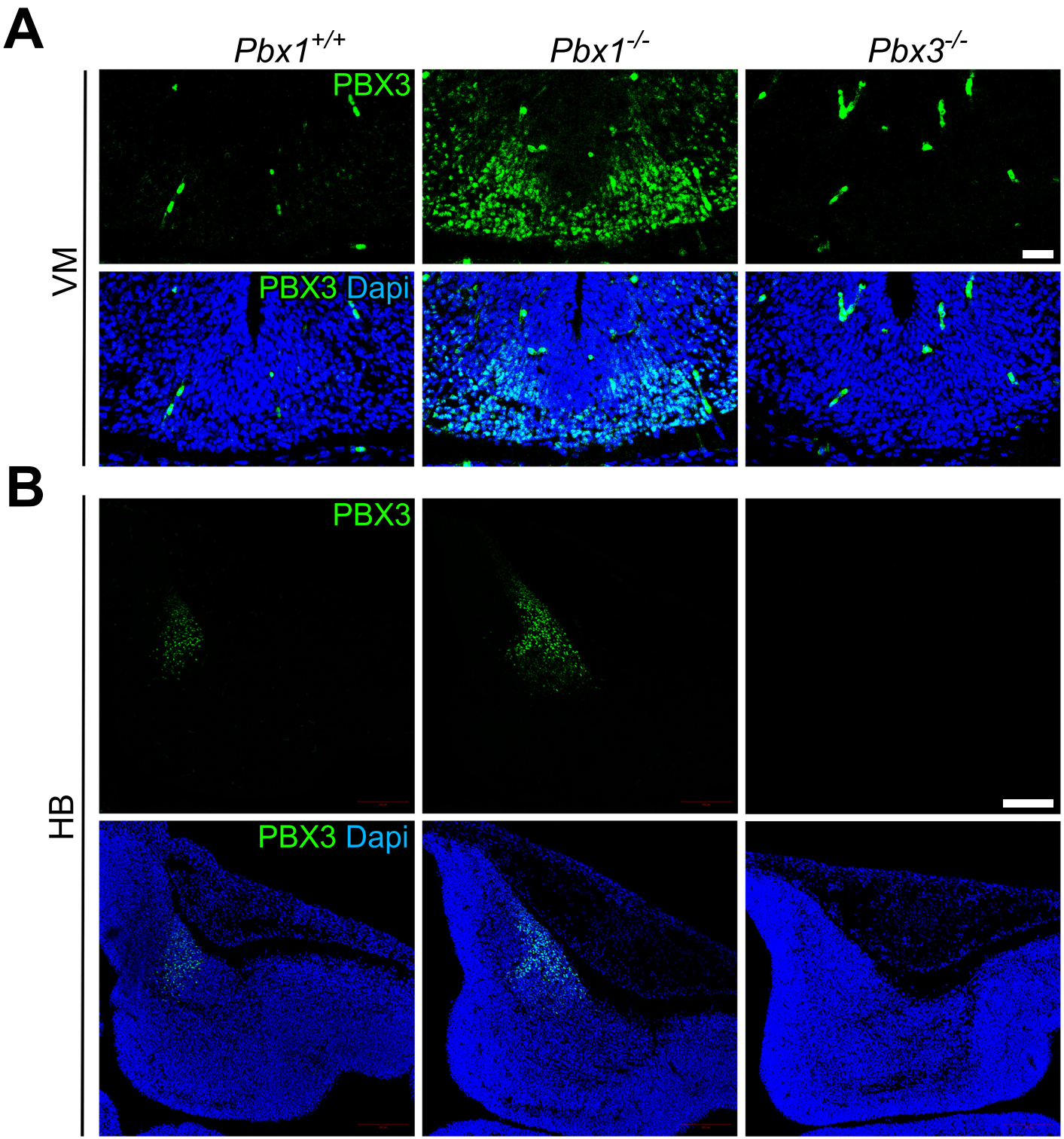
Appendix Figure S1



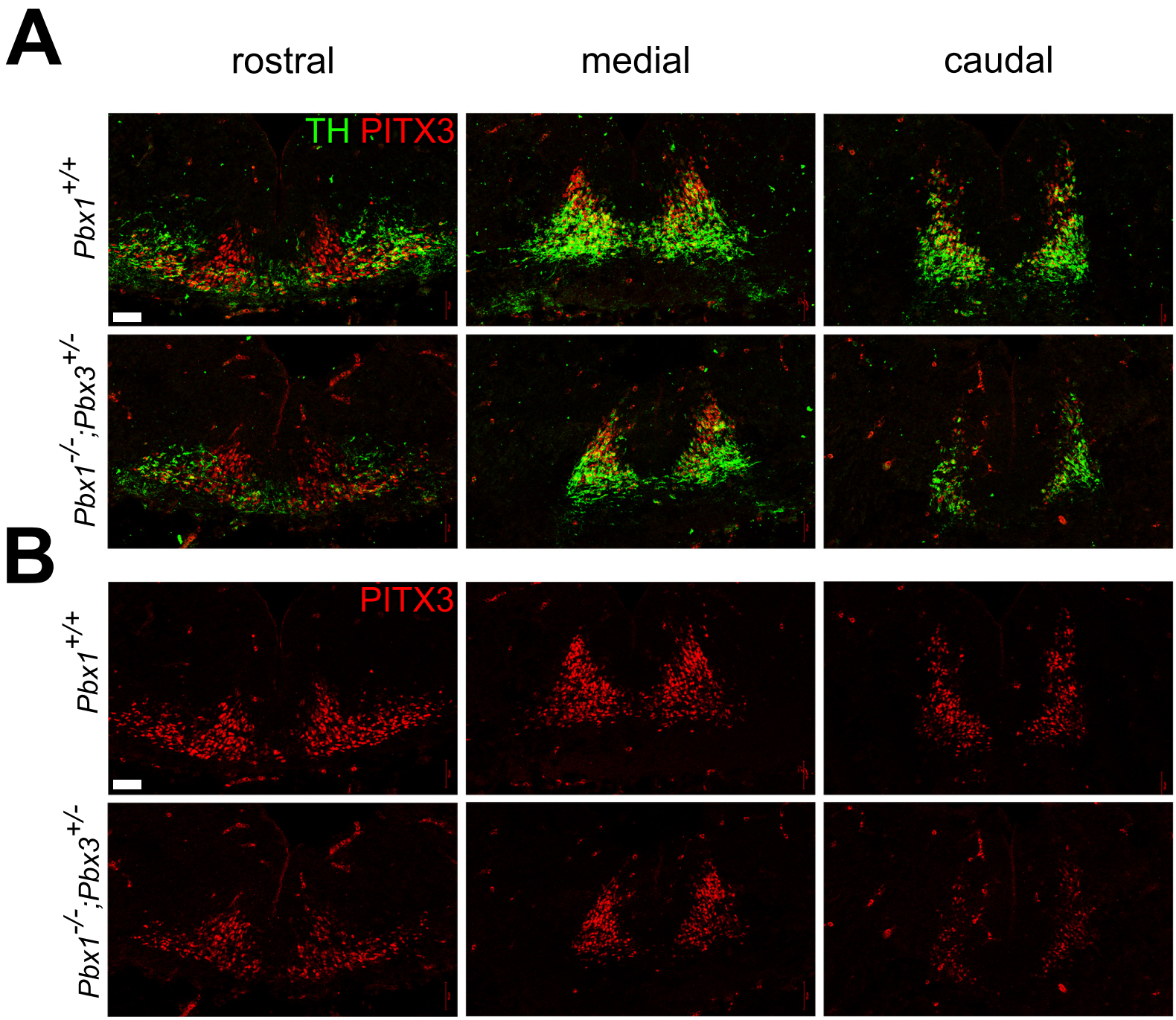
Appendix Figure S2



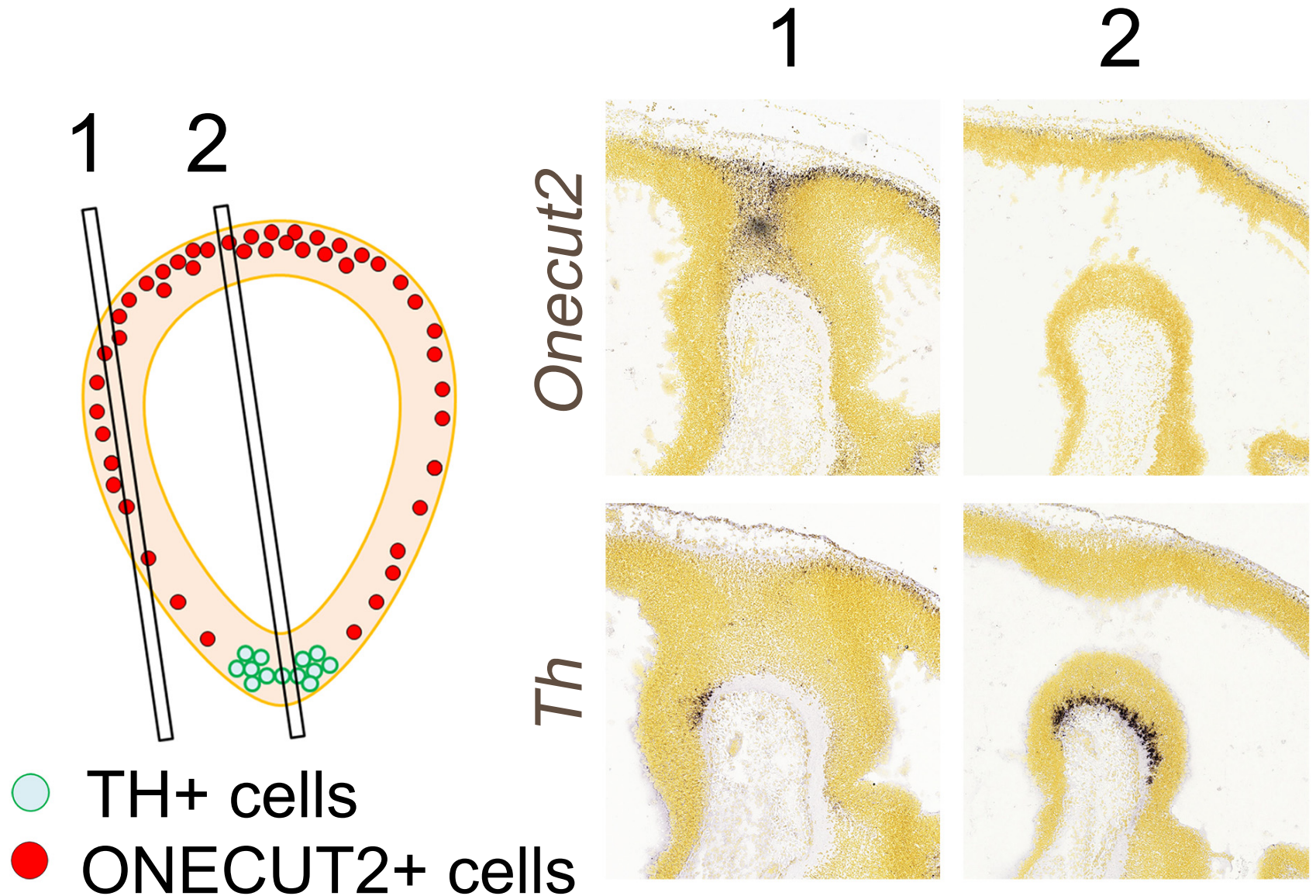
Appendix Figure S3



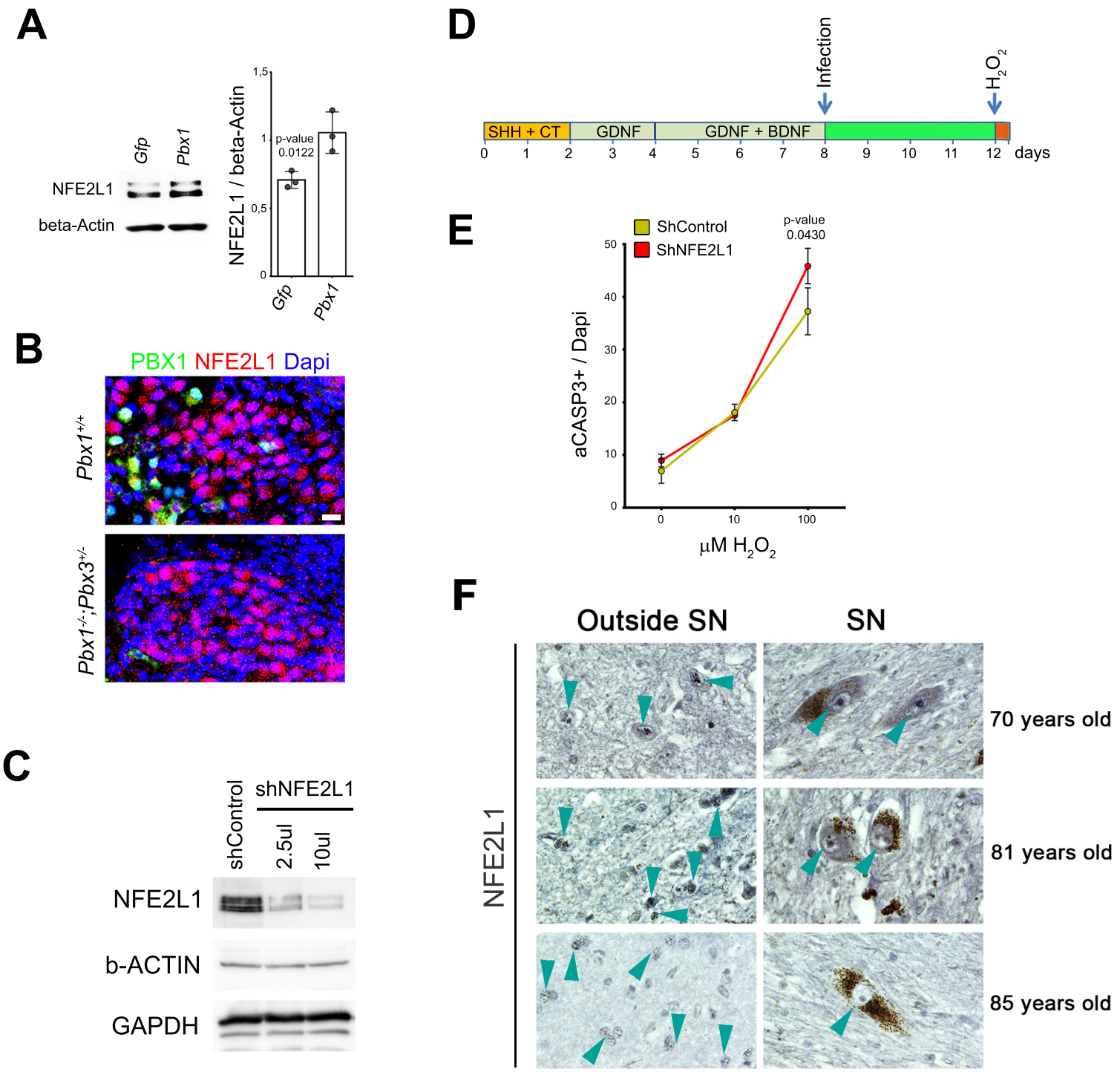
Appendix Figure S4



Appendix Figure S5



Appendix Figure S6



Appendix Figure S1 - PBX1A is the PBX isoform detected in midbrain dopaminergic neurons. (**A**) PBX1A, but not PBX1B, is detected in double TH^+NURR1^+ mDA neurons at E14.5. (**B**) Structure of the two PBX1 splice isoforms. "PBX1A" has a longer C-termini than "PBX1B" but both forms share an atypical homeodomain (HD) containing a three amino-acid loop extension between the first and second α-helices, and two highly homologous domains in their N-termini, PCB-B and PBC-A, which interacts with MEIS/PREP proteins. (**C**) PBX1B, but not PBX1A, is specifically detected in other structures such as the trigeminal ganglion. Scale bars, 40μm.

Appendix Figure S2 - Deletion of *Pbx1* reduced the number of TH^+, but not $NURR1^+$ cells. (**A**) Immunohistochemistry (DAB) for TH shows a reduced number of mDA neurons along the rostro-caudal axis in *Pbx1*$^{-/-}$ embryos at E12.5. (**B**) The total number of $NURR1^+$ cells in *Pbx1*$^{-/-}$ embryos is similar to that in wild-type mice at E12.5. Scale bars, 40μm.

Appendix Figure S3 - Deletion of *Pbx1* upregulates *Pbx3* in different brain regions. (**A**) While the PBX3 protein is undetectable in the medial VM of wt mice, high levels are detected in *Pbx1*$^{-/-}$ embryos at E12.5. The specificity of the PBX3 antibody is shown by the absence of signal in *Pbx3*$^{-/-}$ sections. (**B**) PBX3 is detected in the inferior cerebellar peduncle of wild type embryos at E12.5, is upregulated in *Pbx1*$^{-/-}$ mutant embryos and the signal is lost in *Pbx3-/-* embryos. VM, ventral midbrain; HB, hindbrain. Scale bars: a, 40μm; b, 200μm.

Appendix Figure S4 - The number of $PITX3^+$ cells is reduced in the VM of *Pbx1*$^{-/-}$*;Pbx3*$^{+/-}$ mutant embryos. (**A**) Double immunofluorescence showing a decrease in the number of $PITX3^+$ and TH^+ cells in *Pbx1*$^{-/-}$*;Pbx3*$^{+/-}$ mutant embryos at E12.5, compared to wild-type littermates. (**B**) PITX3 levels are reduced in *Pbx1*$^{-/-}$*;Pbx3*$^{+/-}$ mutant embryos and the reduction in $PITX3^+$ cells is more evident at the anterior-lateral level and in the midline. Scale bars 40μm.

Appendix Figure S5 - Expression of *Onecut2* in the developing midbrain. *In situ* hybridization of wild type mice shows *Onecut2* expression in sagittal sections throughout the lateral (level 1) and roof plate regions (level 2) of the VM at E11.5, without any overlap with *Th* expression. Images are from the Allen Developing Mouse Brain Atlas (Allen Institute for Brain Science Website ©2012, available from: http://developingmouse.brain-map.org).

Appendix Figure S6 - Reduced levels of NFE2L1 increases oxidative stress and are detected in the *substantia nigra* of PD patients. (**A**) NFE2L1 protein was increased 1.5 fold by *PBX1*-overexpression, compared to control (*Gfp*), in a mouse mDAn cell line (SN4741). (**B**) Detection of NFE2L1 in branchial arches structures in E12.5 mouse embryos. Similar levels are observed in *Pbx1*$^{-/-}$*;Pbx3*$^{+/-}$ embryos. (**C**) Western blot analysis of hNES cell extracts after infection with lentiviruses at day 8. Two different amounts of lentivirus particles shows that the NFE2L1 levels were drastically reduced compared to control (left). In contrast, the levels of beta-Actin or GADPDH did not show any variation. (**D**) Scheme of the differentiation protocol for hNES cells. The hNES (AF22 line) were differentiated for 8 days and then infected. After 4 more days, cells were treated with H_2O_2 for 12 hours and analyzed. (**E**) Differentiated hNES cells treated with *shNFE2L1*-Lentiviral particles and H_2O_2 showed increased numbers of $aCASP3^+$ cells compared to *shControl*. Error bars represent mean ± standard deviation, corresponding to 3 biological replicates. P-value (T-test) is indicated in the graph. (**F**) Chromogenic staining for NFE2L1 is present in the nuclei of some NM^- cells outside of the SN (full arrows, left) in three

representative PD patients, but it is absent in the nuclei of NM^+ cells of the SN (empty arrows, right). Scale bars 10μm.