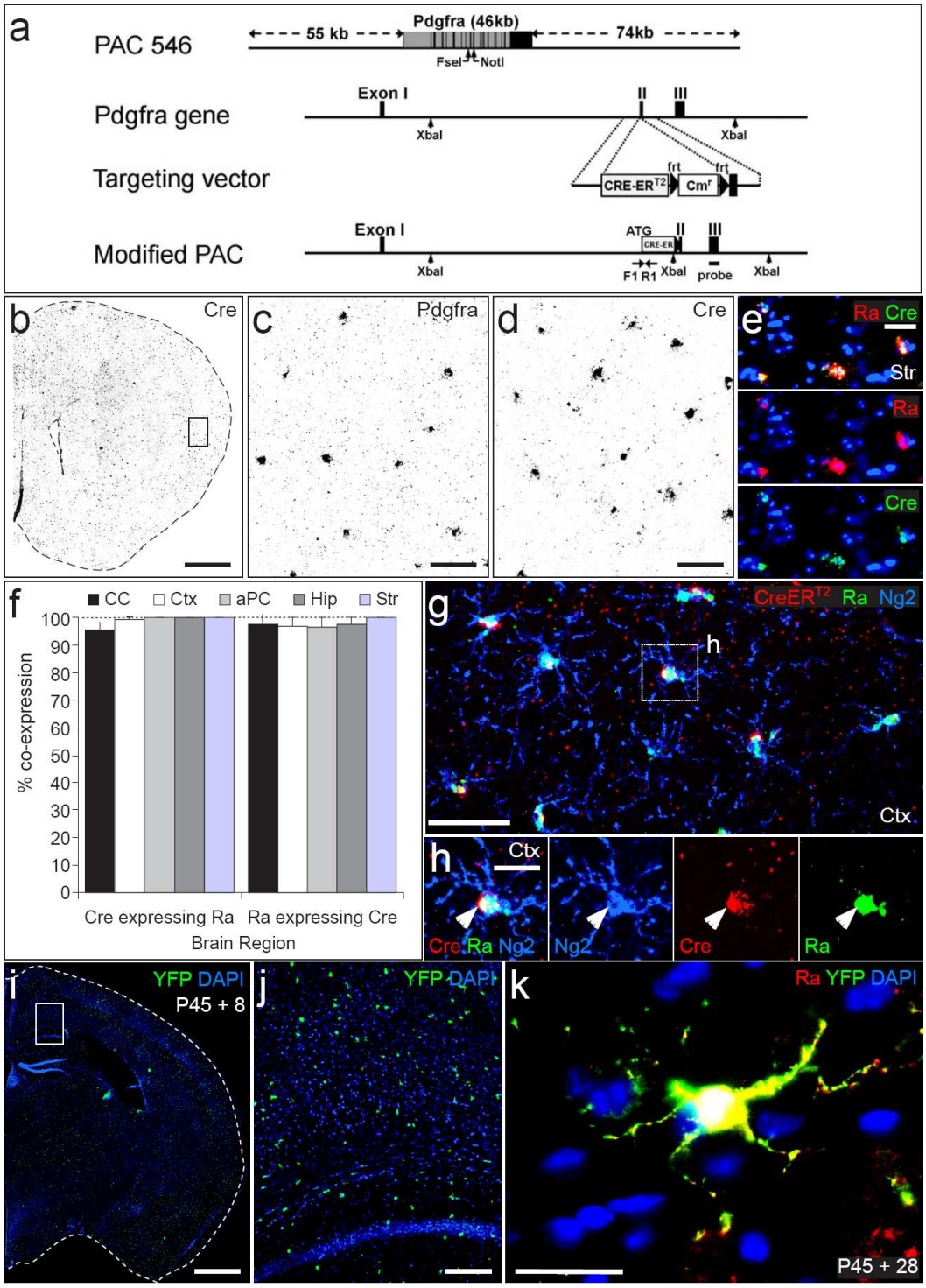

Supplementary Figure 1. Generation and characterization of Pdgfra-CreER^{T2} transgenic mice.

The mouse *Pdgfra* gene consists of a non-coding exon 1 plus 22 coding exons, distributed over 46kb. A mouse genomic PAC library (RPCI 21 from the UK Human Genome Mapping Project Resource centre) was screened with a PCR-generated probe spanning exon 3 of *Pdgfra* (forward primer: 5'-CTCCTGCCAGCTCTTATTACCC-3', reverse primer: 5'-CCTGCCTTCGATCTCACTCTCA-3'). One

clone (546-M3), which contained a genomic fragment ~175 kb in length, was selected for modification and transgenic mouse production.

The targeting vector used to modify PAC 546-M3 is illustrated (**a**). The construct was designed to insert the tamoxifen-inducible form of Cre recombinase (CreER^{T2}) (Indra et al., 1999) into the first coding exon of the *Pdgfra* gene (exon 2). Homology regions 0.5 kb in length were amplified by PCR from the genomic PAC using Expand High Fidelity *Taq*I DNA Polymerase (Roche). The coding sequence of CreER^{T2} was fused to the initiation codon of *Pdgfra* via a *Bsa*I restriction site by a PCR-based approach. A chloramphenicol resistance (*Cm^R*) cassette flanked by *frt* sites was inserted between *CreER^{T2}* and the 3′ homology sequence to allow selection of correctly recombined clones. PAC recombination and removal of the *Cm^R* cassette was carried out in a bacterial system as previously described (Lee et al., 2001). The *SV40* promoter*, blasticidin-8-methylase* gene and *SV40 polyA* site, as well as the downstream *CMV* promoter and the *loxG* site that are present on the pPAC4 vector backbone were removed by homologous recombination. The modified PAC was linearized with *Asc*I, purified by PFGE and transgenic mice generated by pronuclear injection. Genotyping was by PCR using a forward primer spanning the initiation codon (F1:CAGGTCTCAGGAGCTATGTCCAATTTACTGAACGTA) and a reverse primer in *CreER^{T2}* (R1:GGTGTTATAAGCAATCCCCAGAA), yielding a 525 bp product.

We generated eleven independent founders, two of which expressed *CreER* in the pattern expected for *Pdgfra*+ OLPs in the postnatal CNS. The more strongly-expressing of these two was used for the experiments in the present paper. In this line of *Pdgfra-CreER^{T2}* mice, both *Pdgfra* mRNA (**c**) and *Cre* mRNA (**d**) were found in cells scattered through the brain, as expected for PDGFRA-positive OLPs (**c** and **d** are taken from the lateral cortex in coronal forebrain sections, indicated by the small rectangle in **b**). A survey by double in situ hybridization demonstrated that *Pdgfra* and *Cre* mRNA was in the same cells in striatum (**e**), neocortex (**g**, **h**), CC, hippocampus (Hip) and anterior piriform cortex (aPC) (not shown). Images **g**, **h** combine NG2 immunolabelling with double in situ hybridization for *Pdgfra* and *Cre.* Greater than 99% of *Cre*+ cells were also *Pdgfra*+ in all regions examined except the CC, where the figure was >95% (**f**). In the anterior piriform cortex (aPC), the figure was 99.9 ± 0.2% (one single *Cre*+, *Pdgfra*-negative cell found among ~300 *Cre*+ cells). >96% of *Pdgfra*+ cells were *Cre*+. When tamoxifen was administered at P45 to *Pdgfra-CreER^{T2}* / *Rosa26-YFP* double-transgenic mice and forebrain sections analyzed at short times post-tamoxifen (e.g. P45+8), most YFP-labelled cells were also PDGFRA+ (**i-k**). 45-50% of PDGFRA+ cells with typical OLP morphology (**k**) became YFP-labelled in the CC and cerebral cortex (also see main text and **Fig. 3a-d**). Sections were post-stained with DAPI to reveal cell nuclei. *Scale bars*: 500 μm (**b**, **i**), 25 μm (**c-e**, **g**, **h**, **k**), 200 μm (**j**).

In situ hybridization

Coronal brain cryo-sections (20 μm) were collected into DEPC-treated PBS. Sections were transferred to glass slides, allowed to dry and hybridized either with a *Cre*-DIG RNA probe, a *Pdgfra*–DIG probe or *Cre*-DIG/*Pdgfra*–FITC or *Cre-FITC/Pdgfra-DIG* together. The probes were detected with alkaline phosphatase (AP)- or horseradish peroxidase (POD)-conjugated anti-DIG (1:1000) (or anti–FITC, 1:500) Fab Fragments (Roche). For double-labelling, AP was detected using Fast Red (Roche; one tablet dissolved in 2 ml of 0.1M Tris pH8. 0.4M NaCl) and POD was detected with fluorescein amplification reagent (Perkin Elmer). Detailed protocols are at http://www.ucl.ac.uk/~ucbzwdr/MandM.htm. After developing the in situ colour reagents sections were washed with PBS containing Hoechst 33258 dye (Sigma, 10^4 dilution) and sometimes NG2 immunolabelling was performed - in which case the primary antibody was detected with Alexa Fluor 647-conjugated secondary antibody (see main text for details of immunohistochemistry).

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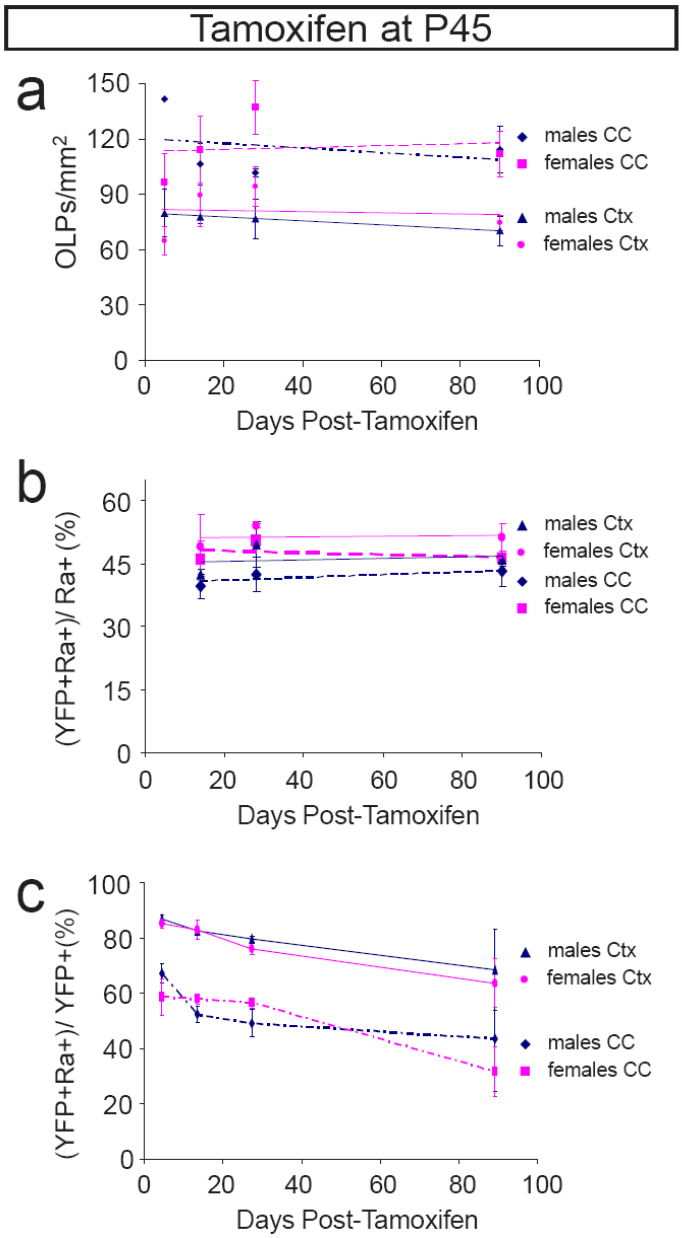
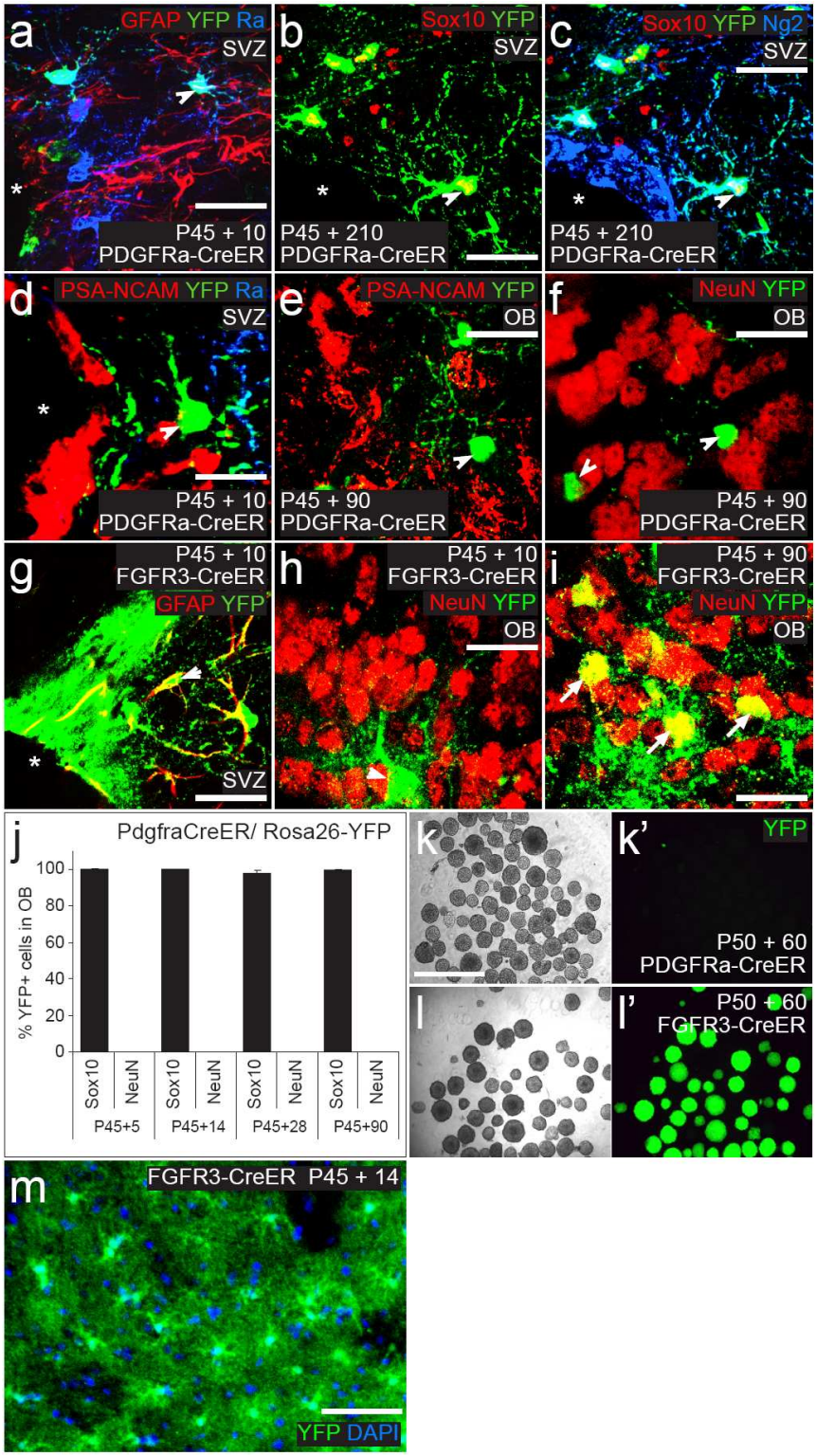


Supplementary Figure 2. Comparison of male and female Pdgfra-CreER^{T2}/Rosa26-YFP mice.

Graph **a** shows numbers of PDGFRA+ (immunolabelled) OLPs in the CC and motor cortex of *Pdgfra-CreER^{T2}/ Rosa26-YFP* mice at different times post-tamoxifen (one treatment per day for four days). The number of OLPs per mm^2 (14 µm sections) did not change after tamoxifen treatment or with age up P45+90. The density of OLPs was slightly less in the cortex versus CC but there was no significant difference between males and females. Graph **b** shows the fraction (percent) of PDGFRA+ OLPs that became YFP-labelled at different times post-tamoxifen. There were no significant differences between males and females at steady state (P45+14 and later). We can conclude from these data that maximum recombination takes > 5 days after the first dose of tamoxifen (one day after the final dose). Graph **c** shows the fraction (percent) of YFP+ cells that were PDGFRA+ at different times post-tamoxifen. As expected, the fraction fell with time as (YFP+, PDGFRA+) OLPs differentiated into YFP+, PDGFRA-negative cells, but there was no significant difference in the rate of differentiation of males versus females. Error bars indicate s.d. (cells scored in three sections from each of three mice).



Supplementary Figure 3. Pdgfra-CreER^{T2} is not active in SVZ stem cells: comparison of Pdgfra-CreER^{T2} and Fgfr3-iCreER^{T2} transgenic mice.

(**a**) Sections of tamoxifen-induced *Pdgfra-CreER^{T2} / Rosa26-YFP* forebrain (P45+10) were immunolabelled for YFP, GFAP and PDGFRA. No (YFP+, GFAP+) type-B stem cells were observed within the subventricular zone (SVZ) (asterisks in this and subsequent panels marks the lateral

ventricle). **Arrowhead** in (**a**) indicates a (YFP+, PDGFRA+) cell (green/blue) that is GFAP-negative - presumably an OLP that was either formed within the SVZ or migrated in from outside. The YFP+, PDGFRA-negative (green) cells in (**a**) are presumably recently differentiated from (YFP+, PDGFRA+) cells because all YFP+ cells in the SVZ were SOX10+ (**b**, **c**). (**d**) YFP+ cells in the SVZ did not co-label for PSA-NCAM, a marker of migratory neuroblasts (type-A cells). (**e**, **f**) At P45+90, long enough post-tamoxifen for migratory neuroblasts to have traversed the rostral migratory stream to the olfactory bulb, there were no YFP+, PSA-NCAM+ neuroblasts (**e**) or YFP+, NeuN+ interneurons (**f**) in the olfactory bulb. (**g**) Sections of tamoxifen-induced *Fgfr3-iCreER^{T2}* / *Rosa26-YFP* forebrain (P45+10) were immunolabelled for YFP and GFAP. All GFAP+ cells within the SVZ were YFP-labelled (small arrows in **g**; single confocal scan), including all (YFP+, GFAP+) type-B stem cells. At P45+10 there were no (YFP+, NeuN+) neurons in the olfactory bulb (**h**; **arrowhead** indicates YFP+, NeuN- cell) but by P45+90 many (YFP+, NeuN+) neurons had developed (**arrows** in **i**). (**j**) Quantification of YFP-labelled cells in the olfactory bulbs of *Pdgfra-CreER^{T2} / Rosa26-YFP* mice that express SOX10 or NeuN indicate that, even three months post-tamoxifen, all YFP-positive cells express SOX10 and therefore belong to the oligodendrocyte lineage. (**k**, **l**). Neurospheres were generated from the SVZ of (P50+60) *Pdgfra-CreER^{T2}* / *Rosa26-YFP* mice (**k**, phase; **k'**, YFP fluorescence) or *Fgfr3-iCreER^{T2}* / *Rosa26-YFP* mice (**l**, phase; **l'**, YFP fluorescence). *Pdgfra-CreER^{T2}* never induced YFP-labelling in neurosphere-forming stem/progenitor cells. (**m**) Many YFP+ protoplasmic astrocytes were present throughout the forebrain of *Fgfr3-iCreER^{T2} / Rosa26-YFP* mice (image shown is of striatum). These had many more and finer processes than OLPs, surrounding the central cell body like a cloud. *Scale bar*s: 15 µm (**a**-**i**), 25 µm (**m**), 400 µm (**k**, **l**).

Generation of Fgfr3-iCreER^{T2} mice

The RPCI 21 library was screened using a rat *Fgfr3* partial cDNA including most of the extracellular protein-coding domain as probe. One clone (608P12) was selected for modification and transgenic mouse generation. It contained an insert of ~180 kb in length, including 37 kb upstream and 130 kb downstream of the *Fgfr3* gene. The targeting vector was designed to insert an *iCreER^{T2}-SV40polyA* cassette into the first coding exon of the *Fgfr3* gene (exon 2), fusing it to the endogenous initiation codon and deleting 58 bp immediately downstream (Young et al., manuscript in preparation). *iCreER^{T2}* is a fusion between *iCre* (excluding the nuclear localization signal) (Shimshek et al., 2002) and the *ER^{T2}* component of *CreER^{T2}* (Indra et al., 1999). The PAC was modified as described in **Supplementary Fig. 1**. Transgenic mice were generated by pronuclear injection of *Sgf*I -linearized PAC DNA and genotyped by PCR using primers iCre250 (GAGGGACTACCTCCTGTACC) and iCre880 (TGCCCAGAGTCATCCTTGGC), which amplify a 630 bp fragment.

Neurosphere cultures

Pdgfra-CreER^{T2}/ Rosa26-YFP or *Fgfr3-iCreER^{T2}/ Rosa26-YFP* mice were induced with tamoxifen (250 mg/Kg body weight) on five consecutive days, starting on P50. After one or eight weeks the forebrain SVZ was micro-dissected and clonal density neurosphere cultures established by culturing in serum-free medium (Stem Cell Technologies) containing the mitogens EGF (Sigma) and bFGF (Roche) as described previously (Young et al., 2007). To check Cre induction, brain tissue caudal to the optic chiasm was taken at the time of culturing and immunolabelled for YFP as described above. YFP-positive neurospheres were counted in an inverted fluorescence microscope at seven days post-plating.

References

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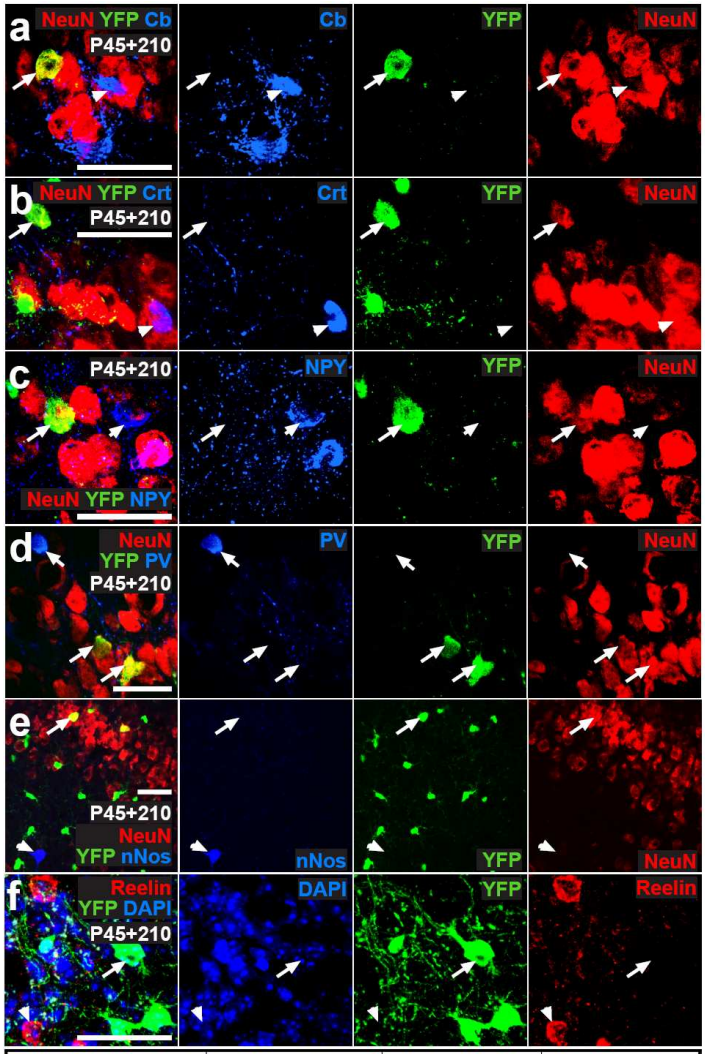

g

Interneuron Marker	P45 + 210 days PDGFRa-CreER^{T2}	YFP+NeuN+ cells in the PC that are interneuron +	YFP+ cells in the PC that are interneuron +
Calbindin (Cb)	n = 3	0 / 125	0 / 500
Calretinin (Crt)	n = 3	0 / 166	0 / 500
Neuropeptide Y (NPY)	n = 3	0 / 172	0 / 500
Parvalbumin (PV)	n = 3	0 / 129	0 / 500
Neuronal Nitric Oxide Synthase (nNOS)	n = 3	0 / 92	0 / 500
Reelin	n = 3	0 / 85	0 / 500

Supplementary Figure 4. Adult-born piriform neurons do not express interneuron markers.

Images in **a**-**d** show layer 2 of the piriform cortex immunolabelled for YFP, NeuN and one of the interneuron markers (**arrowheads**) Calbindin (Cb), Calretinin (Crt), Neuropeptide-Y (NPY) or Parvalbumin (Pv). (YFP+, NeuN+) neurons are indicated by **arrows**. No immunolabelled interneurons were YFP+. Two other interneuron markers, Tyrosine Hydroxylase and Somatostatin were also tested but no immuno-positive interneurons were detected, either YFP-positive or –negative, in this part of the piriform cortex. YFP+ neurons (NeuN+ or Sox10-) did not label for Nitric Oxide Synthase (nNOS) (**e**) or Reelin (**f**). Numbers of cells scored are tabulated in **g**. *Scale bars:* 35 μm (**a**, **b**, **c**, **f)**, 60 μm (**d**), 80 μm (**e**).