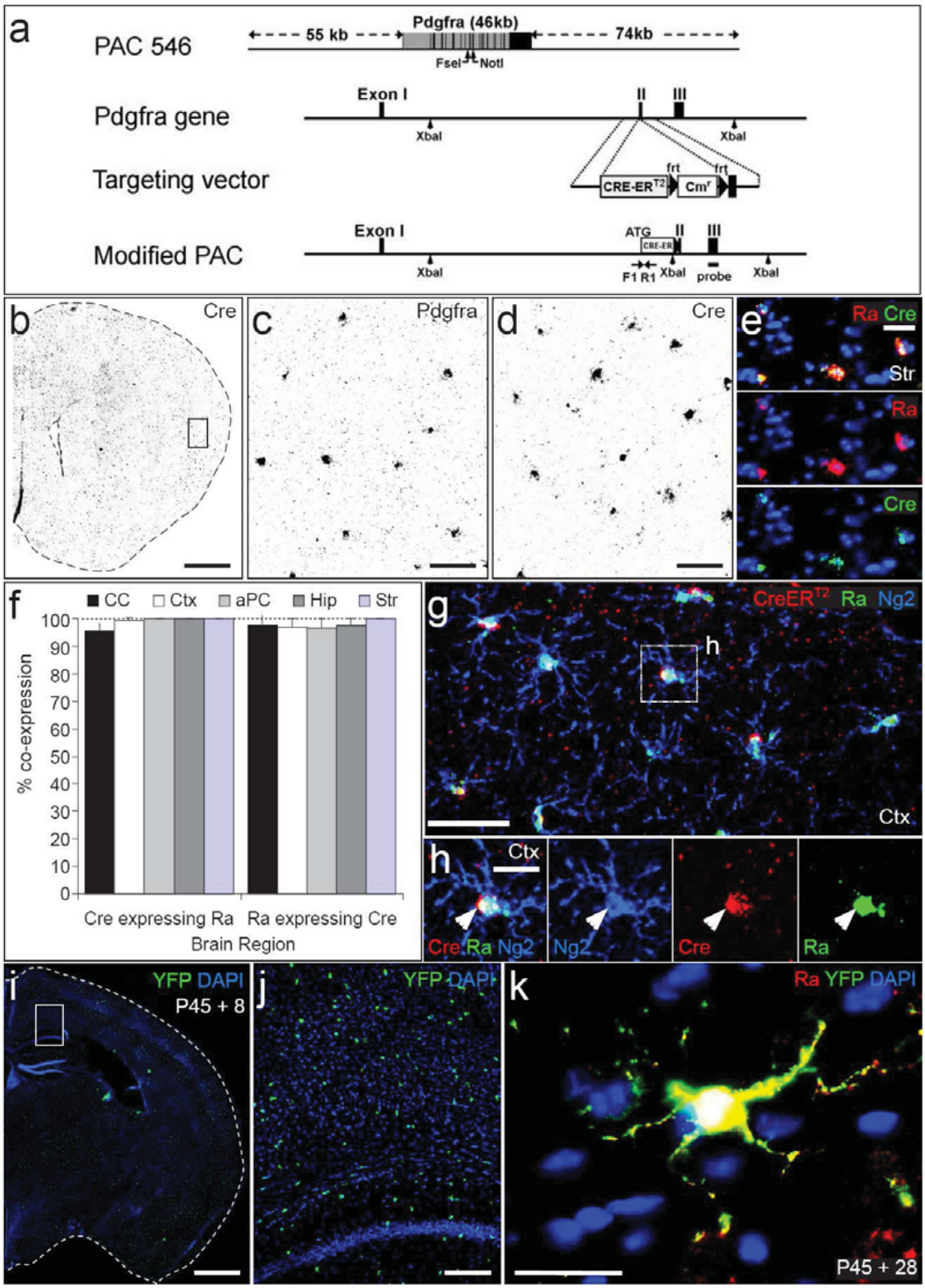
Pdgfra-CreER(T2)

Pdgfra-CreER(T2) transgenic mice

Rivers, L. E., Young, K. M., Rizzi, M., Jamen, F., Psachoulia, K., Wade, A., Kessaris, N., Richardson, W. D. (2008). PDGFRA/ NG2-positive glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice, Nature Neuroscience 11, 1392–1401.



Supplementary Figure. Generation and characterization of Pdgfra-CreERT2 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 (CreERT2) (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 TaqI DNA Polymerase (Roche). The coding sequence of CreERT2 was fused to the initiation codon of Pdgfra via a BsaI restriction site by a PCR-based approach.
A chloramphenicol resistance (CmR) cassette flanked by frt sites was inserted between CreERT2 and the 3 homology sequence to allow selection of correctly recombined clones. PAC recombination and removal of the CmR 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 AscI, 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 CreERT2 (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-CreERT2 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-CreERT2 / 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, 104 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).

References

Indra,A.K. et al. Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. Nucleic Acids Res. 27, 4324-4327 (1999).

Lee,E.C. et al. A highly efficient Escherichia coli-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. Genomics 73, 56-75 (2001).

Placing an order on XIP

To license this product, please select the **appropriate licence option** on the right-hand side of this page. Terms can be previewed from the "Preview terms" link.

MTAs require agreement between all the parties involved in supplying and receiving a product. This cannot happen instantaneously but is a controlled process, managed through XIP and should not take longer no than 10 business days in ordinary circumstances.

To place an order, please locate the Sign-in or Register options on the top right side of this page. You can either sign in to your existing account or register for a new now. **Please note that your account should be created using your academic/ institutional e-mail address.**

For additional guidance on how to create an account and place an order, refer to the **FAQs.**

References

Richardson, Young, Jamen, Psachoulia(2009),
http://www.ncbi.nlm.nih.gov/pubmed?term=Cell%20cycle%20dynamics%20of%20NG2%20cells%20ir
http://journals.cambridge.org/action/displayJournal?jid=NGB, 5, 57-67

Richardson, Kessaris, Wade, Psachoulia, Jamen, Rizzi, Young, Rivers(2008), http://www.ncbi.nlm.nih.gov/pubmed?term=%20%09PDGFRA%2F%20NG2-positive%20glia%20generate%20myelinating%20oligodendrocytes%20and%20piriform%20projection http://www.nature.com/neuro/index.html, 11, 1392-1401

Richardson, Tohyama, Attwell, Cossell, Dunn, Tripathi, Psachoulia, Young(2013), http://www.sciencedirect.com/science/article/pii/S0896627313000500, http://www.cell.com/neuron/home, 77, 873-885

https://xip.uclb.com/product/PdgfraCreERT2