Radial glia require PDGFD–PDGFRβ signalling in human but not mouse neocortex

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Evolutionary expansion of the human neocortex underlies many of our unique mental abilities. This expansion has been attributed to the increased proliferative potential of radial glia (RG; neural stem cells) and their subventricular dispersion from the periventricular niche during neocortical development. Such adaptations may have evolved through gene expression changes in RG. However, whether or how RG gene expression varies between humans and other species is unknown. Here we show that the transcriptional profiles of human and mouse neocortical RG are broadly conserved during neurogenesis, yet diverge for specific signalling pathways. By analysing differential expression between human and mouse neocortical RG, we demonstrate that the growth factor PDGFD is specifically expressed by RG in human, but not mouse, corticogenesis. We also show that the expression domain of PDGFRβ, the cognate receptor for PDGFD, is evolutionarily divergent, with high expression in the germinal region of dorsal human neocortex but not in the mouse. Pharmacological inhibition of PDGFD–PDGFRβ signalling in slice culture prevents normal cell cycle progression of neocortical RG in human, but not mouse. Conversely, injection of recombinant PDGFD or ectopic expression of constitutively active PDGFRβ in developing mouse neocortex increases the proportion of RG and their subventricular dispersion. These findings highlight the requirement of PDGFD–PDGFRβ signalling for human neocortical development and suggest that local production of growth factors by RG supports the expanded germinal region and progenitor heterogeneity of species with large brains.

Radial glia are the physical substrate and progenitor population that underlie production of most cells in human neocortex. We sought to determine a general transcriptional ‘signature’ of human neocortical RG (hRG) as a starting point for identifying genes that may regulate human aspects of cortical development. We and others have previously shown that gene co-expression analysis of heterogeneous tissue samples can deconvolve transcriptional signatures of distinct cell types without cell isolation or purification. Because prenatal samples of human neocortex are scarce, we developed a novel strategy called Gene Co-expression Analysis of Serial Sections (GCASS) that exploits variation in cellular abundance across serial sections of a single tissue sample to reveal cell-type-specific patterns of gene expression (Fig. 1a–c and Extended Data Fig. 1; see Supplementary Information for methods, rationale and further discussion). We applied GCASS to 87 150-μm sections of a single human cortical sample from gestational week 14.5 (GW14.5, corresponding to peak layer V neurogenesis); Supplementary Table 1) and identified 55 modules of co-expressed genes. Six modules overlapped significantly with a set of genes that we determined were expressed significantly higher in fluorescence-activated cell sorting (FACS)-sorted mouse RG (mRG) versus intermediate progenitor cells (FACS mRG; Extended Data Fig. 1 and Supplementary Table 2), suggesting that they might represent transcriptional signatures of hRG (Fig. 1d). Analysis of laser-microdissected samples from three independent transcriptomic data sets confirmed that genes in these modules are highly expressed in the ventricular zone (VZ) and subventricular zone (SVZ) of developing human neocortex, where both ventricular (vRG) and outer subventricular (oRG) subtypes of RG reside (Extended Data Fig. 2).

To produce a consensus transcriptional signature for GW14.5 hRG, we first summarized each of these six modules by its first principal component/module eigengene (ME) and calculated the Weighted Gene Co-expression Network Analysis measure of intramodular gene connectivity, kME (see Fig. 1c). kME quantifies the extent to which a gene might represent transcriptional signatures of hRG (Fig. 1d). Analysis of mouse RG (mRG) versus intermediate progenitor cells (FACS mRG; 87,150 μm sections of a single tissue sample) showed that gene co-expression analysis of heterogeneous tissue samples significantly with a set of genes that we determined were expressed significantly higher in fluorescence-activated cell sorting (FACS)-sorted mouse RG (mRG) versus intermediate progenitor cells (FACS mRG; Extended Data Fig. 1 and Supplementary Table 2), suggesting that they might represent transcriptional signatures of hRG (Fig. 1d). Analysis of laser-microdissected samples from three independent transcriptomic data sets confirmed that genes in these modules are highly expressed in the ventricular zone (VZ) and subventricular zone (SVZ) of developing human neocortex, where both ventricular (vRG) and outer subventricular (oRG) subtypes of RG reside (Extended Data Fig. 2).

Figure 1| GCASS identifies a transcriptional signature of radial glia in human neocortex. Conceptual framework is indicated in a–c. a, Transcriptional profiling of serial sections (n = 87, Illumina HT-12 v4 microarrays) from a GW14.5 human neocortical specimen. Scale bar, 2.5 mm. b, Genes with similar expression patterns are grouped into modules, which may reflect cell-type-specific gene expression. c, The transcriptional signature of a module is defined as a list of genes ranked by their correlation to the module eigengene. d–f, Finding human RG modules. d, Six out of fifty-five modules were significantly enriched (Fisher’s exact test) with the FACS mRG gene set. Blue line, P = 0.05; red line, P = 9.1 × 10−4 (Bonferroni correction). e, Genome-wide distribution of predicted GW14.5 neocortical RG expression specificity (ZGW14.5) based on enriched modules in d. f, ISH confirms RG expression specificity for novel RG genes from e. Scale bar, 300 μm. CP, cortical plate; ISVZ, inner subventricular zone; OSVZ, outer subventricular zone; SP, subplate. Sample size was determined on the basis of statistical and technical considerations (see Supplementary Information).
conforms to the characteristic expression pattern of a module and can predict gene expression specificity for individual cell types. Genes with high $Z_{HRG}$ values included markers of neocortical RG such as SLC1A3 (GLAST1), VIM, SOX2, NOTCH1 and PAX6 (Fig. 1e, blue lines). Genes with low $Z_{HRG}$ values included markers of committed neuronal lineages such as TBR1, FEZ2, RELN and SATB2 (Fig. 1e, black lines). We performed in situ hybridization (ISH) and immunostaining on independent prenatal human neocortical samples for genes with high $Z_{HRG}$ values that have not, to our knowledge, previously been implicated in RG biology (Fig. 1e, red lines and Extended Data Fig. 3). In all cases, expression of these genes was restricted to the VZ/SVZ (Fig. 1f and Extended Data Fig. 3). These results indicate that GCASS can discern a general transcriptional signature of hRG from a single, heterogeneous tissue sample without cell labelling, isolation, or purification. Moreover, because the sample derives from a single individual, this strategy implicitly controls for genotype and developmental stage and has broad implications for the molecular analysis of rare tissue samples.

To establish the robustness of the hRG transcriptional signature, we analysed four additional prenatal human cortex gene expression data sets (see also http://www.brainspan.org/rnaseq/search/index.html) that were generated with diverse sampling strategies and technology platforms (Extended Data Table 1). In parallel, we also analysed three embryonic mouse cortex gene expression data sets (largely embryonic day (E)14–14.5, corresponding to peak layer V neurogenesis11; Extended Data Fig. 3). These results indicate that GCASS can discern a general transcriptional signature of hRG from a single, heterogeneous tissue sample without cell labelling, isolation, or purification. Moreover, because the sample derives from a single individual, this strategy implicitly controls for genotype and developmental stage and has broad implications for the molecular analysis of rare tissue samples.

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knowledge, previously been implicated in cortical development of any species. Compared to genes with the highest RG PR (conserved in both species), PDGFD expression was highly correlated in human, but not mouse (Fig. 3a, b). ISH confirmed that PDGFD was expressed by RG throughout the VZ of GW14.5 human neocortex (Fig. 3c). In contrast, Pdgfd was not detected in RG (or any cell type) in E15.5 mouse neocortex (Fig. 3d). These expression differences were consistent for multiple ages in human (GW14.5–GW18.2) and mouse (E14.5–E17.5) prenatal cortex (Extended Data Fig. 7).

The effects of PDGFD are specifically mediated by the PDGFRβ receptor, which upon phosphorylation can trigger signalling pathways that promote cell proliferation. Although PDGFRB did not meet the same stringent criteria as PDGFD, its location on the DS/DE plot was proximal to PDGFD (Fig. 2d (right panel), red square), also suggesting species differences in PDGFD expression. Compared to genes with the highest RG PR, PDGFRB expression was moderately correlated in human, but not mouse (Fig. 3e, f). Immunostaining for PDGFRβ in GW14.5 human brain revealed strong expression throughout the telencephalic germinal zones (VZ/SVZ) and in vascular pericytes, with highest levels in dorsolateral cortical progenitors and the lateral ganglionic eminence (Fig. 3g). In contrast, immunostaining for PDGFRβ in E15.5 mouse brain revealed expression in lateral ganglionic eminence progenitors but no evidence of expression in cortical progenitors (Fig. 3h). However, we did observe very low levels of Pdgfrb transcript in the VZ of lateral mouse cortex (Extended Data Fig. 8), leaving open the possibility of modest, region-specific function. Collectively, these results indicate that expression patterns of PDGFD and PDGFRβ in developing neocortex have diverged considerably during human and mouse evolution, despite retaining amino acid sequences that are ~85% identical between the species.

We tested the requirement of PDGFD–PDGFRβ signalling for hRG proliferation in GW17.5 human neocortical slice cultures, screening four chemical inhibitors of PDGFRβ signalling (Sutent, tivozanib, imatinib and CP673451). Three out of four PDGFRβ inhibitors reduced the percentage of SOX2 progenitors (RG) that incorporated 5-bromodeoxyuridine (BrdU) over 2 days in slice culture (Extended Data Fig. 9). For replication we focused on CP673451, which exhibits the greatest selectivity for PDGFRβ over other receptors and caused the greatest reduction in progenitors (RG) that incorporated 5-bromodeoxyuridine (BrdU) over 2 days in slice culture (Extended Data Fig. 9).
in SOX2\(^+\) BrdU\(^+\) cells among tested inhibitors (Extended Data Fig. 9). PDGFRβ inhibition by CP673451 in GW17.5 human neocortical slice cultures reduced the number of RG and intermediate progenitors that incorporated BrdU by \(\geq 50\%\), affecting progenitors in the VZ and SVZ (Fig. 4a, b). The percentage of progenitor cells co-staining with cleaved caspase-3, an apoptosis marker, was slightly elevated by CP673451, but sufficiently low to attribute reduced BrdU incorporation to cell cycle dysregulation rather than cell death (Fig. 4b). Furthermore, CP673451 treatment of E13.5 mouse cortical slice cultures did not decrease BrdU incorporation or the cycling proportion (Ki67\(^+\)) of RG or intermediate progenitor populations over multiple time points (Fig. 4b and Extended Data Fig. 9). These results indicate that PDGFRβ signalling is required for hRG but not mRG to progress through the cell cycle and expand at a normal rate.

We next investigated whether introducing PDGFD into embryonic mouse cortex, where it is normally absent, could promote mRG proliferation. We injected recombinant PDGFD-D protein into the lateral ventricles of E13.5 mouse embryos, bypassing the need for PDGFD to be generated and dimerized in vivo, and analysed the number and spatial distribution of SOX2\(^+\) progenitors at E15.5 in lateral cortex. Relative to vehicle, PDGFD-D increased the proportion of RG (SOX2\(^+\)DAPI\(^-\)) in lateral cortex by \(\sim 10\%\) (Fig. 4c, d). Furthermore, PDGFD-D induced a modest subventricular shift in the distribution of mRG in the developing cortical wall (Fig. 4d). These effects were not observed in dorsomedial and lateral cortex (at least \(n = 3\) slices per embryo from five litters/experiments (lateral: \(n = 49\) vehicle; \(n = 47\) PDGFD-D; dorsomedial: \(n = 45\) vehicle; \(n = 39\) PDGFD-DD)). The distribution of RG in the cortex (from ventricle to pia) was quantified; grey band delineates 95% confidence interval for test of equal univariate densities (\(n = 10,000\) permutations). e, In utero electroporation of constitutively active PDGFRβ(D850V)\(^2\) (mouse E13.5–E15.5). Cortex was stained for SOX2; white arrowheads indicate co-labelling with electroporated GFP cells (quantified in f; at least \(n = 3\) slices per embryo from two litters; \(n = 15\) control, \(n = 18\) (PDGFRβ(D850V)); scale bar, 50 \(\mu\)m). Note disrupted epithelial structure of VZ. Error bars indicate mean ± s.e.m. Statistical significance for treatment was determined by ANOVA of multiple linear regression after controlling for individual (b) or litter (d, f) NS (not significant), \(P \geq 0.05\); *\(P \leq 0.05\); **\(P \leq 0.01\); ***\(P \leq 0.001\), ****\(P \leq 0.0001\). g, Schematic summarizing experimental manipulations and results. GOF, gain-of-function; LOF, loss-of-function.
cortex, where PDGFRβ was not detected in RG (Fig. 4c, d and Extended Data Fig. 8).

To test whether mRG can respond to PDGFRβ activation, we ectopically expressed two forms of constitutively active PDGFRβ (PDGFRβ(D850V) (homologous to human D849V (ref. 22)) or the TEL–PDGFRβ (ref. 23) fusion protein) in mRG by *in utero* electroporation at E13.5. By E15.5, expression of PDGFRβ(D850V) approximately doubled the proportion of SOX2+ or Ki67+ progenitors among electroporated (GFP+) cells and markedly dispersed SOX2+ progenitors in the basal direction (Fig. 4e, f and Extended Data Fig. 9). Similar but less marked effects were observed after TEL–PDGFRβ electroporation (Extended Data Fig. 9).

These results indicate that PDGFRβ signalling in mRG can function analogously to its known role as an oncogenic pathway that promotes proliferation and epithelial–mesenchymal transition10,11. We therefore propose that physiological levels of PDGF–PDGFRβ signalling in hRG may contribute to the proliferation and subventricular dispersion of neural progenitors that characterize OSVZ formation4,5,12.

Mouse studies have demonstrated that the size and shape of cerebral cortex depend on precise regulation of molecular pathways controlling RG proliferation and differentiation12–14,16,17. While many of these pathways are probably conserved in humans, mouse studies alone cannot reveal uniquely human aspects of cortical development14–22. By analysing human tissue as a starting point, we found that PDGFD–PDGFRβ signalling is required for normal RG proliferation in developing human but not mouse neocortex, and is sufficient to promote some ‘humanizing’ characteristics in mouse. Our analysis has also identified other genes that probably contribute to differences between human and mouse cortical development. BMP7, another secreted growth factor, was predicted and validated to be expressed by hRG but not mRG (Fig. 2d and Extended Data Fig. 6), raising the possibility that local production of growth factors by hRG may be necessary to support the expanded germinal region and progenitor heterogeneity of developing human neocortex. We anticipate that the analytical and experimental strategies described here will help determine the extent to which these and other pathways are shared among primates or uniquely required for human cortical development.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** M.C.O. conceived the GCASS strategy and J.H.L generated the GCASS data set. A.J.L generated the FACs mRG data set. M.C.O conceived, designed, and performed the bioinformatic analyses. J.H.L, T.J.N. and A.A.P. designed and performed the experiments leading up to the prioritization of PDGFD as the focus of this study. T.J.N. performed the majority of the in situ hybridizations and the in vivo mouse experiments. J.H.L performed the human and mouse slice culture experiments, as well as all of the immunostaining, imaging and image analysis in the study. M.C.O and J.H.L wrote the manuscript, which was edited by all the authors. M.C.O. and A.A.P provided conceptual guidance at every stage of the project.

**Author Information** Microarray data from the GCASS dataset have been deposited in Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE20646. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.C.O. (olechristm@stemcell.ucsf.edu) or A.A.P. (kriegstein@stemcell.ucsf.edu).

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**Extended Data Figure 1** | Human brain dissection for GCASS and schematic for generation of FACS mRG data set.  

**a**, Top: to generate the GCASS data set, an almost-intact prenatal human telencephalic hemisphere (GW14.5) was microdissected to separate the dorsal telencephalon from the ventral telencephalon (including medial and lateral ganglionic eminences). Bottom: the dorsal fragment was flash-frozen and serially sectioned (150 μm) for transcriptional profiling with Illumina HT-12 v4 Beadchip microarrays.

**b**, To generate the FACS mRG data set, dorsal neocortices of Eomes–GFP mouse embryos were microdissected, pooled (n = 3 litters, 5–8 pooled embryos per litter), dissociated and FACS-sorted according to the gating scheme depicted to isolate RG and intermediate progenitor cells. Transcriptional profiling of the resultant populations was performed using Illumina mouseRef-8 v1.0 Beadchip microarrays.
Extended Data Figure 2 | Genes comprising the six RG co-expression modules identified by GCASS are expressed in a manner consistent with the known distribution of RG in developing human neocortex. Six candidate hRG gene co-expression modules (Fig. 1d) were superimposed on three independent gene expression data sets generated from laser-microdissected samples from prenatal human cortex: ABI.1 (ref. 13) (GW17), ABI.2 (ref. 13) (GW18), and Fietz et al. (ref. 12) (GW15–18) (as listed in Extended Data Table 1). The characteristic expression patterns of the superimposed modules were summarized by singular value decomposition; the first principal component (PC1) for each module in each data set is shown. In all cases, PC1 revealed substantially higher expression levels for these genes in germinal zones (VZ, ISVZ, and OSVZ, highlighted in red) versus non-germinal zones (IZ, SP, ICP, OCP, MZ, SG). Permutation analysis indicated that the per cent variance explained (VE) by PC1 of each superimposed module was significantly greater than expected by chance ($n = 10,000$ permutations).

CP, cortical plate; CTX, cortex; GW, gestational week; ICP, inner cortical plate; ISVZ, inner subventricular zone; IZ, intermediate zone; MZ, marginal zone; OCP, outer cortical plate; OSVZ, outer subventricular zone; SG, subpial granular layer; SP, subplate; VZ, ventricular zone.
Extended Data Figure 3 | GCASS successfully predicts novel markers of neocortical hRG. a, Genome-wide distribution of predicted GW14.5 neocortical hRG expression specificity ($Z_{\text{hRG}}$). Red lines indicate predicted RG genes (validated in b, c). b, c, Immunostaining and in situ hybridization in GW14.5 human neocortex confirms RG expression specificity for novel candidate markers predicted in a (b, scale bar, 50 μm; c, scale bar, 100 μm). Analysed tissue sections were independent from the sample used for microarray analysis (Fig. 1a). ISVZ, inner subventricular zone; OSVZ, outer subventricular zone; VZ, ventricular zone.
Extended Data Figure 4 | Workflow of bioinformatic procedures and experimental rationale for the entire study. The bioinformatic component of this study sought to identify a homologous gene co-expression signature for human and mouse RG that is robust across multiple sampling strategies/technology platforms and can be normalized to facilitate comparisons within and between species. This pipeline illustrates the steps that were taken to identify, integrate and compare RG gene co-expression signatures in eight transcriptomic data sets generated from prenatal human and mouse neocortex.
Extended Data Figure 5 | Genome-wide predictions of expression specificity for hRG and mRG are robust across independent data sets.

a, b, Heat maps of Spearman correlation coefficients for predicted RG expression specificity (RG PR) over 10,929 genes present in all five human data sets (a) and 10,649 genes present in all three mouse data sets (b) (as indicated in columns BE and BI in Supplementary Table 3). Data sets are denoted by the sample ages listed in Extended Data Table 1, although factors besides age also contribute to the observed correlations (for example, choice of technology platform, sample preparation strategy). E, embryonic; GW, gestational week.
Extended Data Figure 6 | In situ hybridization (ISH) validates predicted presence or absence of gene expression in hRG and mRG. a, Pink box: human in situ probes for six genes predicted to be expressed by hRG but not mRG were generated and hybridized in GW15 human neocortical tissue to validate predicted hRG expression (human scale bar, 200 μm). Blue box (Eurexpress20: http://www.eurexpress.org/ee/): in situ hybridizations for 13 genes predicted to be expressed by hRG but not mRG reveal no expression by mRG in E14.5 mouse cortex. Green box: mouse in situ probes for three genes predicted to be expressed by hRG but not mRG were generated and revealed no expression by mRG (E13.5). Positive control expression in cells other than RG are labelled in red. b, Expression patterns of genes predicted to be expressed by mRG (that is, those with the highest mRG PR values in Supplementary Table 3) are shown as further validation (blue (E14.5, Eurexpress20: http://www.eurexpress.org/ee/); orange (E14.5, GenePaint: http://www.genepaint.org); mouse scale bars, ~500 μm). One other gene in the top 15, Cks2, is not shown, but has been validated previously30.

30. Ajioka, I., Maeda, T. & Nakajima, K. Identification of ventricular-side-enriched molecules regulated in a stage-dependent manner during cerebral cortical development. Eur. J. Neurosci. 23, 296–308 (2006).
Extended Data Figure 7 | PDGFD is expressed by neocortical RG during neurogenesis in humans but not mice. a, In situ hybridization of PDGFD in GW14.5, 16.5, 17.3 and 18.2 human neocortex demonstrates consistent expression in RG across multiple ages (scale bar, 200 μm). b, In situ hybridization of Pdgfd in E14.5 mouse (Eurexpress®: http://www.eurexpress.org/ee/) demonstrates lack of expression (scale bar, 500 μm). c, To demonstrate the lack of Pdgfd expression in mouse neocortex across multiple ages, a pCAG-PDGFD-IG expression plasmid was electroporated into the mouse VZ at E13.5 as an internal positive control and harvested at E14.5, E15.5 or E17.5. At E14.5 and E15.5, Pdgfd (blue signal) is seen only in the electroporated region in the ventricular zone (scale bar, 200 μm; inset scale bar, 50 μm). At E17.5, Pdgfd is in the cortical plate and not in the ventricular zone or anywhere else (scale bar, 500 μm, inset scale bar, 50 μm).
Extended Data Figure 8 | *Pdgfrb* is strongly expressed by ventral RG and weakly expressed by lateral RG in mice. *In situ* hybridization of *Pdgfrb* in sagittal sections through the mouse forebrain (E14.5) across a medial–lateral axis (Eurexpress<sup>®</sup>: http://www.eurexpress.org/ee/) demonstrates progenitor expression in the ventral germinal regions. This expression extends into the dorsal cortex in the lateral aspect of the brain, but is not widespread. In contrast, no progenitor expression is detected in dorsomedial cortex (scale bar, 500 μm; inset scale bar, 100 μm). Expression is also detected in the pia and vascular pericytes.
Extended Data Figure 9 | Manipulation of PDGFRβ signalling in human and mouse neocortex. a–d. Chemical blockade of PDGFRβ signalling in cultured slices of GW14.5 human neocortex impairs RG cell cycle progression. Four pharmacological inhibitors of PDGFRβ signalling were screened at different concentrations to determine their effects on RG proliferation in cultured slices of GW17.5 human neocortex (2 days). Slices were treated with BrdU for the duration of the experiment and RG proliferation was quantified as the fraction of SOX2+ cells that incorporated BrdU after treatment with inhibitor or vehicle. Statistical significance was assessed with the Wilcoxon rank sum test using the wilcox.test R function with default settings. Images are derived from 3 slices in each condition. Control 1DMSO, n = 18; control no DMSO, n = 9; CP673451 all concentrations, n = 9; Sutent all concentrations, n = 6; imatinib (0.1 μM, 10 μM), n = 9 (100 μM), n = 6; tivozanib (1 μM, 10 μM), n = 9 (0.1 μM), n = 6. Significance indicated by: n.s. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. e. Slice cultures of E13.5 mouse neocortex were treated with BrdU and DMSO (control) or a pharmacological inhibitor of PDGFRβ signalling (CP673451) for 1 or 2 days (slices from at least three independent litters). RG (SOX2+) or intermediate progenitor (TBR2+) cell proliferation was assessed as the fraction of each population that incorporated BrdU or was Ki67+ (1d: n = 10 (DMSO) versus n = 9 (CP673451); 2d: n = 11 (DMSO) versus n = 9 (CP673451)). This experiment serves as a negative control to compare with the human. f. Ectopic PDGFRβ signalling promotes RG identity in E13.5 mouse neocortex. In utero electroporation of constitutively active TEL–PDGFRβ (ref. 23) was compared with control (mouse E13.5–E15.5) and assessed for the proportion and distribution of SOX2+ RG cells or Ki67+ progenitors (out of GFP+) in the cortical wall (quantified in g: at least n = 3 slices per embryo from two independent litters, n = 15 (control); n = 18 (TEL–PDGFRβ) or (PDGFRβ(D850V)); scale bar, 50 μm). Ki67+ GFP+ cell quantification following PDGFRβ(D850V)2 electroporation was performed in a similar fashion and is also shown. The spatial distributions of RG (GFP+ SOX2+) in the cortical wall were assessed by quantitative image analysis (spanning ventricle to pia). The grey band delineates a 95% confidence interval for a test of equal univariate densities based on 10,000 permutations. All error bars represent mean ± s.e.m. Statistical significance for the effects of treatment was calculated by ANOVA of multiple linear regression while controlling for individual (e) and litter (f) variability (significance indicated by: n.s. P > 0.05, *P ≤ 0.05, ***P ≤ 0.001, ****P ≤ 0.0001).
### Extended Data Table 1 | Characteristics of additional gene expression data sets analysed in the present study

| Dataset          | Platform | Species   | CTX prep | Age  | Samples | mRG enrichment P value | OR      |
|------------------|----------|-----------|----------|------|---------|------------------------|---------|
| ABI 1\(^{19}\)   | Agilent  | Human     | LMD      | GW17 | 179     | 2.6e-39                | 7.2 [5.5-9.5] |
| ABI 2\(^{19}\)   | Agilent  | Human     | LMD      | GW18 | 168     | 9.7e-35                | 7.5 [5.6-10.0] |
| BrainSpan        | RNA-seq  | Human     | Macro    | GW10-18 | 107     | 2.0e-47                | 9.2 [0.9-12.1] |
| Fietz et al\(^{12}\) | RNA-seq  | Human     | LMD      | GW15-18 | 24      | 1.3e-39                | 8.4 [6.3-11.1] |
| Fietz et al\(^{12}\) | RNA-seq  | Mouse     | LMD      | E14.5 | 15      | 1.3e-73                | 12.5 [9.6-16.4] |
| Kawaguchi et al.\(^{17}\) | Affymetrix | Mouse | Single cell | E14 | 70      | 3.1e-34                | 59.0 [34.2-100.6] |
| Pinto et al.\(^{18}\) | Affymetrix | Mouse | FACS | E14-16 | 17 | 7.8e-46                | 20.7 [14.7-29.0] |

Unsupervised gene co-expression analysis was performed for each data set using the same parameters, followed by unbiased enrichment analysis with the FACS mRG gene set (Supplementary Table 2 and Extended Data Fig. 1). For BrainSpan RNA-seq data see also (http://www.brainspan.org/rnaseq/search/index.html). 'mRG enrichment P value' denotes the P value (Fisher’s exact test) for the module with the most significant enrichment in each data set. ABI: Allen Institute for Brain Science; CTX, cortex; E, embryonic; FACS, fluorescence-activated cell sorting; GW, gestational week; LMD, laser-microdissection; Macro, macrodissection; OR, odds ratio (95% confidence interval).