Supplementary Results

This section contains 10 figures and associated text and figure legends of supplementary results.

Lhx2 specifies regional fate in Emx1 lineage of telencephalic progenitors generating cerebral cortex

Shen-Ju Chou, Carlos G. Perez-Garcia, Todd T. Kroll, and Dennis D.M. O'Leary[1]

Molecular Neurobiology Laboratory
The Salk Institute
10010 North Torrey Pines Road
La Jolla, CA 92037, USA

[1]Corresponding Author:
Dennis D.M. O'Leary
Tel: 858-453-4100 x1415
Fax: 858-558-6207
Email: doleary@salk.edu

Putative patterning centers are not affected in Lhx2 cKO-E mice

Deletion of Lhx2 from progenitors of the Emx1 lineage in Lhx2 cKO-E ($Lhx2^{fl/-}$:Emx1-Cre) results in an expansion of paleocortex and a restriction of neocortex (Figure 2). To address whether this change in telencephalic patterning is due to a direct effect of Lhx2 on progenitors of the Emx1 lineage, or if the effect is more indirect and possibly due to changes in putative patterning centers, we used in situ hybridization with specific markers to assess the integrity and positioning of the hem[13], a dorsomedial patterning center, and the anti-hem[14], a putative ventrolateral patterning center positioned coincident with the pallium-subpallium boundary (PSB). We find that the expression patterns of representative markers for hem and choroid plexus, Wnt3a and BMP7 [S1], and anti-hem / PSB, Sfrp2[S2] and Er81[26], in the Lhx2 cKO-E mice closely resemble wild-type (WT) (Supplementary Figure 1).

Expression of LGE-specific marker genes is not changed in Lhx2 cKO-E mice

We analyzed the expression of transcription factors in the cortical ventricular zone (VZ) of the Lhx2 cKO-E mice at embryonic stages during cortical neurogenesis. Because the lateral ganglionic eminence (LGE) has been reported to contribute neurons of a Dlx2 lineage to the piriform cortex (PC) [S3,26], we analyzed expression of the transcription factors, Dlx2, Dlx5, Gsh2, Mash1, and Arx, which are normally expressed in the LGE with roles in specifying fates of LGE-derived neurons[15-18], to determine if their expression might expand into the VZ of dorsal telencephalon (dTel) to parallel the dorsal expansion of the paleocortical marker Nrp2. However, we find that these transcription factors retain their normal expression pattern within LGE with no evidence for ectopic expression in the dTel VZ (Supplementary Figure 2).

Ectopic PC is located dorsal to the rhinal fissure where lateral neocortex is positioned in WT

The rhinal fissure is an anatomical landmark formed by a morphogenetic event and common to all mammals that separates the neocortex, located dorsal to the rhinal fissure, and the PC, positioned ventral to it[25]. In the Lhx2 cKO-E, the ectopic PC (ePC) is always dorsal to the rhinal fissure throughout the entire anterior-posterior (A-P) axis, where lateral neocortex should be located as in WT (Supplementary Figure 3). This positioning of the ePC argues that it is generated by dTel progenitors of the Emx1 lineage that would normally generate lateral neocortex but are refated following early deletion of Lhx2 by Emx1-Cre to generate ePC. Because they are part of the pool of progenitors that would normally generate neocortex, albeit refated, their progeny migrate to the position dorsal to the rhinal fissure normally occupied by lateral neocortical neurons and form a continuous structure with dorsomedial neocortex, with a smooth transition zone between the two

characterized by a mixing of neurons of PC and neocortical fates (see Supplementary Figure 8). This contrasts with the wild type PC (wtPC), which is generated by a subset of dTel progenitors of the Emx1 lineage distinct from that which generates neocortex[8,26], whose progeny migrate along distinct radial glial fibers, including the glial palisade that originates from the PSB[27] (See Supplementary Figure 5), and establish the wtPC ventral to the rhinal fissure.

The ectopic PC in Lhx2 cKO-E mice is generated by a uniform population of cortical progenitors

The ePC in Lhx2 cKO-E mice is significantly larger than the PC in WT (Figure 6). The mechanism that best explains the data is that the ePC is generated by refated neocortical progenitors. The alternative explanation requires that the progenitors that normally generate the PC, which are localized to the dTel VZ in the vicinity of the PSB, undergo a substantial increase in proliferation in Lhx2 cKO-E mice to generate a substantially greater number of progeny that form the ePC, which is significantly larger than the wtPC. In addition, for changes in progenitor proliferation to be a realistic alternative to refating of neocortical progenitors requires a decrease in proliferation of lateral neocortical progenitors, as their progeny no longer exist in the Lhx2 cKO-E mice and are replaced by PC neurons. Thus, this alternative mechanism to refating would require a very non-uniform population of active progenitors in the dTel VZ, characterized by an abnormally high density of active progenitors around the PSB, and just dorsal to it a domain of quiescent progenitors over roughly half of the dTel VZ, followed dorsal to the quiescent zone, a zone of active progenitors with a roughly normal density. This scenario of a sequence of a "hotspot", "coldspot", and normal density of active progenitors as one progresses from ventral to dorsal in the dTel VZ would be readily visible by visual inspection. However, as described below and illustrated in Supplementary Figure 4, we find a uniform population of active progenitors in the dTel VZ, ruling out the possibility that this mechanism is a viable alternative to refating.

To address this issue, we assessed the distribution and relative densities of active progenitors using BrdU-pulse labeling during the period of PC and neocortical neurogenesis, E11.5, E13.5 and E15.5. For this mechanism to be even remotely feasible as an alternative to the refating of progenitors would require that beginning soon after the deletion of Lhx2 on E10.5, the population of dTel progenitors would abruptly undergo a very substantial changes in proliferation as described above relative to WT. However, at each embryonic age analyzed, we find no evidence for either of these aberrant labeling patterns, let alone the disparate changes in active progenitors juxtaposed within the VZ. Instead, we find that the distribution and density of the BrdU labeled progenitors is similar between WT and Lhx2 cKO-E embryos, and that in both the density of labeled progenitors is relatively uniform across the entire dTel VZ including the VZ domain that

generates lateral neocortex and the domain around the PSB that generates the PC[26] (Supplementary Figure 4 and data not shown). In summary, we find no indications of the substantial changes in labeling that would be required for this mechanism of substantial and rapid changes in proliferation to be a feasible alternative to progenitor refating.

In P7 WT mice exposed to BrdU at E13.5, the most heavily labeled cells, and therefore the ones generated shortly after the BrdU injection, are found in the deeper layers, primarily layer 6. In contrast, in their Lhx2 cKO-E littermates, within the ePC in the lateral neocortex, the most heavily labeled cells are found in a compact band within layer 2, whereas within dorsomedial neocortex, the most heavily labeled cells are found in the deeper layers, but they exhibit a broad, relatively non-specific laminar distribution compared to WT (Supplementary Figure 4).

In summary, we find no differences in the distribution or density of progenitors in the dTel VZ of Lhx2 cKO-E mice that could be an alternative mechanism to progenitor refating to account for the expanded ePC, nor the accompanying lack of generation of the lateral neocortex. These findings rule out the possibility that changes in proliferation within the Emx1 progenitor population in Lhx2 cKO-E mice account for the ePC, neither its dramatic size increase compared to WT nor its ectopic position, and therefore provide an additional argument that the expanded ePC is produced by progenitors that normally generate lateral neocortex but following an early conditional deletion of Lhx2 undergo a fate change and generate an ePC.

Relationship between the PSB glial palisade and the PC

Radial glia in the dTel VZ are the progenitors of the Emx1 lineage that generate the cerebral cortex [S4,S5]. Radial glial processes extend from the VZ to the pial surface and guide neurons generated by VZ progenitors from the VZ to developing layers of the neocortex and PC. An especially dense palisade of radial glial processes originating from the PSB VZ extends to the pial surface at the ventral-most position in the anlagen of the cerebral cortex, where the wtPC forms[27]. To determine the relationship in Lhx2 cKO-E mice between the glial palisade and cells generated by dTel progenitors of the Emx1 lineage, in particular those that form the ePC and the wtPC , we performed immunostaining using the radial glial marker BLBP on coronal sections of E15.5 WT ($Lhx2^{fl/+}$:Emx1-Cre:R26R) and Lhx2 cKO-E ($Lhx2^{fl/-}$:Emx1-Cre:R26R) brains. In Lhx2 cKO-E mice, as in WT, the BLBP-immunolabeled PSB glial palisade extends from the VZ to the ventral-most position in the developing cerebral cortex where the developing wtPC is identified by the patterned distribution of β-gal labeled cells of the Emx1 lineage. In both WT and Lhx2 cKO-E mice, the ventral-most portion of the glial palisade is coincident with the ventral-most distribution of β-gal labeled cells of the Emx1 lineage, which form the wtPC. In WT mice, the developing lateral neocortex is also marked by β-gal labeled cells and is positioned dorsal to the palisade and the

wtPC. In Lhx2 cKO-E mice, the developing ePC is positioned in place of lateral neocortex dorsal to the glial palisade and the wtPC. These findings provide further evidence that the ePC is not simply the wtPC that shifts to an aberrant dorsal position due to the reduced size of the cortex in Lhx2 cKO-E mice, and that it develops dorsal to the wtPC and is generated by progenitors that would normally generate lateral neocortex, but are refated due to early deletion of Lhx2 by Emx1-Cre.

Lhx2 regulates a fate decision between neocortex and piriform cortex in the progenitor cells in the Emx1 lineage

Because Lhx2 is expressed by both progenitors of the Emx1 lineage and their post-mitotic neuronal progeny that form neocortex and wtPC[2], a question is whether the elimination of wtPC neurons in Lhx2 cKO-E mice is due to either a defect in their progenitors following early Lhx2 deletion by Emx1-Cre that is inherited by PC neurons or alternatively to a later function of Lhx2 in PC neurons. To distinguish between these alternatives, we crossed the floxed Lhx2 mice with a Nex-Cre line of mice[10]. In dTel of Nex-Cre mice, Cre recombinase is only expressed in post-mitotic neurons and is absent from progenitors. The size and the location of the PC in Lhx2 cKO-X ($Lhx2^{fl/-}$:Nex-Cre) mice is indistinguishable from WT, indicating that the function of Lhx2 in determining the viability of wtPC neurons in Lhx2 cKO-E mice is in the progenitor cells of the Emx1 lineage and not in the differentiating neurons (Supplementary Figure 6). Thus, the elimination of wtPC in Lhx2 cKO-E mice is due to a defect resulting from deletion of Lhx2 from progenitors that is inherited by wtPC neurons.

Nestin-Cre mice promote robust recombination of floxed alleles on E11.5, one day after Emx1-Cre mice

When Lhx2 is deleted by Emx1-Cre from dTel progenitors of the Emx1 lineage they are refated to generate an ePC in place of lateral neocortex. However, when Lhx2 is deleted by Nestin-Cre in Lhx2 cKO-N mice[9], which also deletes Lhx2 from progenitors of the Emx1 lineage, an ePC is not generated, and instead the cerebral cortex is relatively normally patterned, albeit reduced in size, with a normally positioned wtPC, as well as lateral and dorsomedial neocortex. The critical distinction between the Emx1-Cre and the Nestin-Cre must be the timing of effective recombination produced by Cre. We used the ROSA26 reporter line[24] to show that robust recombination indicated by Cre-dependent β-gal expression is evident at E10.5 when crossed with the Emx1-Cre knockin mice, and at E11.5 when crossed with the Nestin-Cre transgenic mice used in this study (Supplementary Figure 7). When the distinct phenotypes of Lhx2 cKO-E and cKO-N mice are interpreted within the context of these findings on timing of recombination, they indicate that the critical period for Lhx2 regulation of the regional fate decision to generate lateral neocortical

neurons or PC neurons exhibited by dTel progenitors of the Emx1 lineage is open at E10.5, but is closed by E11.5.

A unique transition zone is present between the ectopic piriform cortex and the neocortex in Lhx2 cKO-E mice

The early critical period superimposed upon the rostrolateral to caudomedial temporal gradient of corticogenesis across the neocortical axes leads to a prediction of a transition zone between the neocortex and the ePC. Such a transition zone would have a mis-match in the layer-specific expression pattern of markers for PC and neocortical fate. To address this issue, we examined the expression of Ctip2[22] relative to the neocortical marker Satb2[11]. In WT, Ctip2 is preferentially expressed by layer 5 neurons within the neocortex, and strongly labels layer 2 of the PC and the olfactory tubercle[22]. In Lhx2 cKO-E mice, Ctip2 is expressed in layer 2 of the ePC, while retaining a WT-like expression pattern in dorsomedial neocortex, albeit with some laminar defects (Figure 4). Progressive, layer-distinct changes in the expression of these markers from neocortex into ePC demonstrate the predicted transition zone, revealed by layer 6 expression of Satb2 characteristic of neocortex, and directly overlying it, layer 2 expression of Ctip2 characteristic of PC (Supplementary Figure 8). A similar transition zone is not seen between neocortex and wtPC in WT mice because a temporal refating does not establish PC in WT. In summary, the presence in the Lhx2 cKO-E cortex of a unique transition zone of mixed neocortical and PC fates is consistent with an early critical period for Lhx2 regulation of the regional fate decision by lateral neocortical progenitors of the Emx1 lineage to generate neurons of either a neocortical or PC fate.

Genetic changes in cortical progenitors in Lhx2 cKO mice

To begin to assess the genetic hierarchy that accounts for the refating of cortical progenitors following deletion of Lhx2, we have analyzed the expression of other transcription factors in the dTel VZ, including Emx1, Pax6 and Ngn2, which are involved in cell specification and patterning in the forebrain[29,S6]. Because progenitors of the Emx1 lineage exhibit a fate change in Lhx2 cKO-E mice but not in Lhx2 cKO-N mice, comparing changes in the expression of these transcription factors between the two Lhx2 cKO lines would yield valuable insights, with those transcription factors that exhibit changes uniquely in the Lhx2 cKO-E mice being better candidates for prominent involvement in the neocortical versus PC fate decision. However, each of these transcription factors is substantially down-regulated in the VZ of Lhx2 cKO-E mice in similar manners (Supplementary Figure 9), suggesting that they are unlikely to have a significant instructive role in mediating the regional-fate decision within the Emx1 lineage of dTel progenitors.

Summary of experimental design and major findings supporting refating of dTel

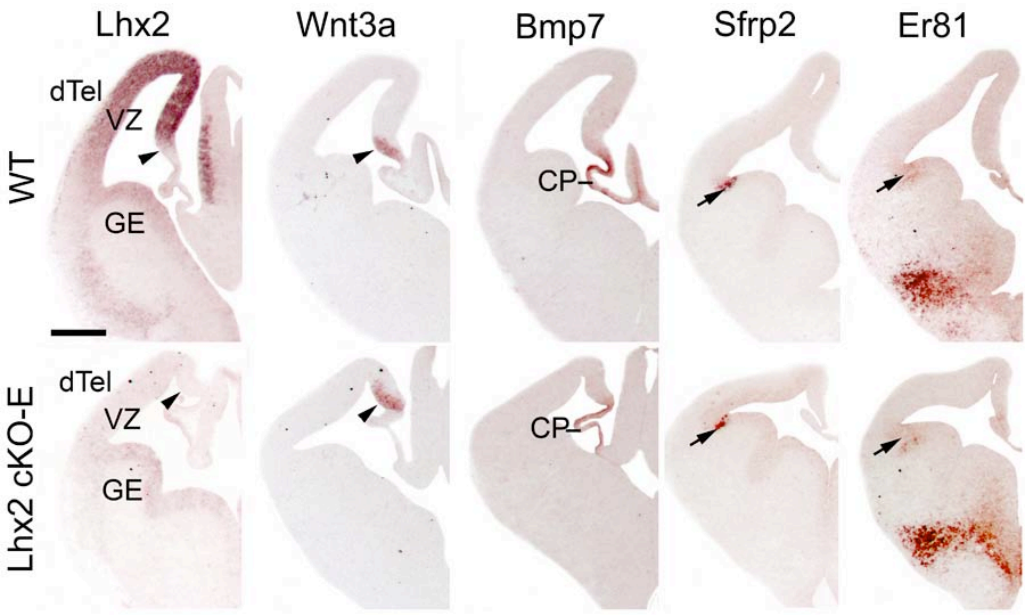
progenitors of the Emx1 lineage following early deletion of Lhx2

Supplementary Figure 10 is a schematic depicting the experimental design and major findings in the Lhx2 cKO-E mice generated by crossing the floxed Lhx2 mice with the Emx1-Cre knockin line[8]. Several lines of evidence conclusively demonstrate that the ePC in the Lhx2 cKO-E mice is generated by progenitors normally fated to generate lateral neocortex, but are refated following early deletion of Lhx2 to generate a cortical structure that phenocopies the PC. These lines of evidence include: (1) Fate mapping demonstrates that both the wtPC as well as the ePC are generated by dTel progenitors of the Emx1 lineage in the Lhx2 cKO-E mice. This finding definitively demonstrates that the ePC positioned in lateral neocortex is not wtPC but instead is a distinct structure that must be generated by refated neocortical progenitors. (2) Expression of Satb2[11], a neocortical marker, is retained throughout the ePC, albeit at a diminished level compared to WT neocortex, showing that the ePC retains some neocortical traits, consistent with its origin from progenitors originally fated to generate lateral neocortex, rather than progenitors originally fated to generate PC because wtPC does not express Satb2. (3) The ePC in the Lhx2 cKO-E mice is positioned dorsal to the rhinal fissure, a morphogenetic landmark that forms between neocortex and PC[25], in the location of lateral neocortex and as a continuous sheet of cells with the remainder of the neocortex, whereas wtPC is positioned ventral to the rhinal fissure. This finding strongly argues that the ePC is generated by progenitors that would normally generate neocortex, and remain part of that pool of neocortical progenitors albeit refated due to Lhx2 deletion. (4) The relationship in WT and Lhx2 cKO-E mice between the processes of radial glia in the dTel VZ (which are the progenitors of the Emx1 lineage), in particular those that form the glial palisade originating from the PSB[27], with cells of the Emx1 lineage, demonstrate that the transient wtPC is positioned at the distal end of the glial palisade as in WT[27], and that the ePC is positioned in place of lateral neocortex at the end of glial processes dorsal to the glial palisade and the wtPC. These findings are evidence that the ePC is generated by progenitors that would normally generate lateral neocortex, those positioned within the VZ dorsal to the progenitors that generate wtPC that are localized to the PSB. (5) Consistent with these interpretations, we observe between ePC and neocortex a transition zone of progressive change from neocortical to PC properties not observed between wtPC and neocortex, again arguing that the ePC and neocortex are both generated by progenitors that would normally generate only neocortex. (6) The ePC in Lhx2 cKO-E mice receives input from the OB to layers 1 and 3 similar to wtPC in WT[23]. However, in contrast to WT, in Lhx2 cKO-E mice, the OB projection continues beyond ePC and aberrantly projects within layer 1 throughout much of neocortex, consistent with lateral neocortex being refated into ePC and the ePC and dorsomedial neocortex being a continuous sheet of cells, like lateral and dorsomedial neocortex in WT, with a gradual transitioning of ePC into neocortex. (7) The ePC is located where

lateral neocortex is normally found, and its position parallels that of lateral neocortex along not only the D-V cortical axis, but also the entire A-P cortical axis, extending well beyond the A-P extent of wtPC. (8) The ePC is 400% larger in relative size and over 200% of the absolute size of wtPC. These findings cannot be explained by a dorsal shift of wtPC to the ectopic location, as the ePC then should be no larger than wtPC. Nor can they be explained by the required combination of substantially increased proliferation of PC progenitors and virtual cessation of proliferation of lateral neocortical progenitors beginning after Lhx2 is deleted at E10.5, because proliferation remains relatively uniform throughout the Emx1 lineage of progenitors in the dTel VZ in Lhx2 cKO-E mice. (9) Reduced cortical size does not lead to an aberrant dorsal shift of wtPC, shown by analysis of Lhx2 cKO-N mice in which Lhx2 is conditionally deleted with a Nestin-Cre. Lhx2 cKO-N mice have a cortex of a reduced size similar to Lhx2 cKO-E mice, but in contrast, Lhx2 cKO-N mice do not have an ePC and instead have a normally sized PC positioned at its normal ventrolateral location. These findings rule out the possibility that the ePC is formed entirely, or in part, by the wtPC that has aberrantly shifted dorsally, above the rhinal fissure, as a consequence of the reduced cortical size. (10) Our demonstration of a critical period for the formation of an ePC following Lhx2 deletion itself strongly argues for refating: the ePC is identified in Lhx2 cKO-E mice following Lhx2 deletion on E10.5, but not in Lhx2 cKO-N mice following Lhx2 deletion on E11.5. This can only be explained as a fate restriction in dTel progenitors of the Emx1 lineage occurring between E10.5 and E11.5, after which deletion of Lhx2 no longer leads to refating.

Several of these findings considered individually, and certainly when they are considered as a group, conclusively demonstrate that the ePC is generated by dTel progenitors of the Emx1 lineage normally fated to generate lateral neocortex but are refated to generate PC following deletion of Lhx2 during an early critical period. In conclusion, these findings provide definitive evidence for refating and establish a mechanism for determining regional fate of dTel progenitors of the Emx1 lineage.

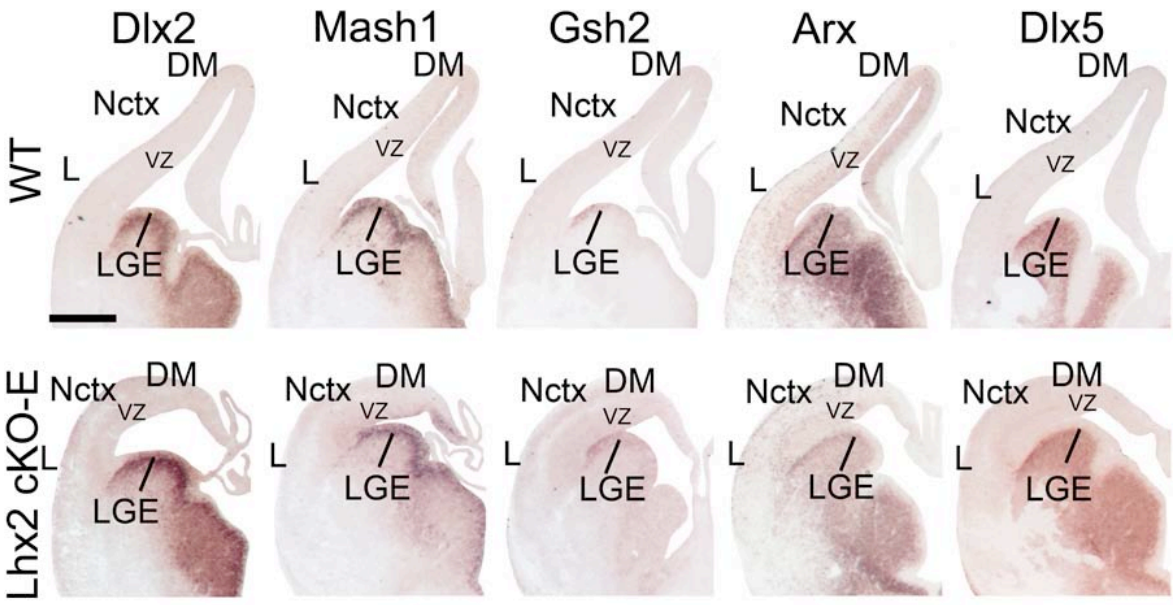
Supplementary Figure 1



Supplementary Figure 1. The size and location of the dorsal signaling centers and pallium-subpallium boundary are not changed in Lhx2 cKO-E mice.

In situ hybridization for dorsal signaling center markers, Wnt3a and Bmp7, and pallium-subpallium boundary (PSB) markers, Sfrp2 and Er81, on coronal sections of E12.5 WT ($Lhx2^{fl/+}$) and Lhx2 cKO-E ($Lhx2^{fl/-}$:Emx1-Cre). In WT, Lhx2 is strongly expressed in the ventricular zone (VZ) of dorsal telencephalon (dTel) but is not expressed in the cortical hem (arrowhead). Expression of Lhx2 is genetically deleted from the ventricular zone (VZ) of the dorsal telencephalon in Lhx2 cKO-E mice. In both WT and Lhx2 cKO-E mice, Wnt3a is expressed in the cortical hem (arrowheads) and Bmp7 is expressed in the choroid plexus (CP). In addition, in Lhx2 cKO-E mice, the expression of Sfrp2 and Er81 is maintained in the PSB (arrows) and has not spread to the dorsal telencephalic ventricular zone. Scale bar: 0.3 mm. GE, ganglionic eminence.

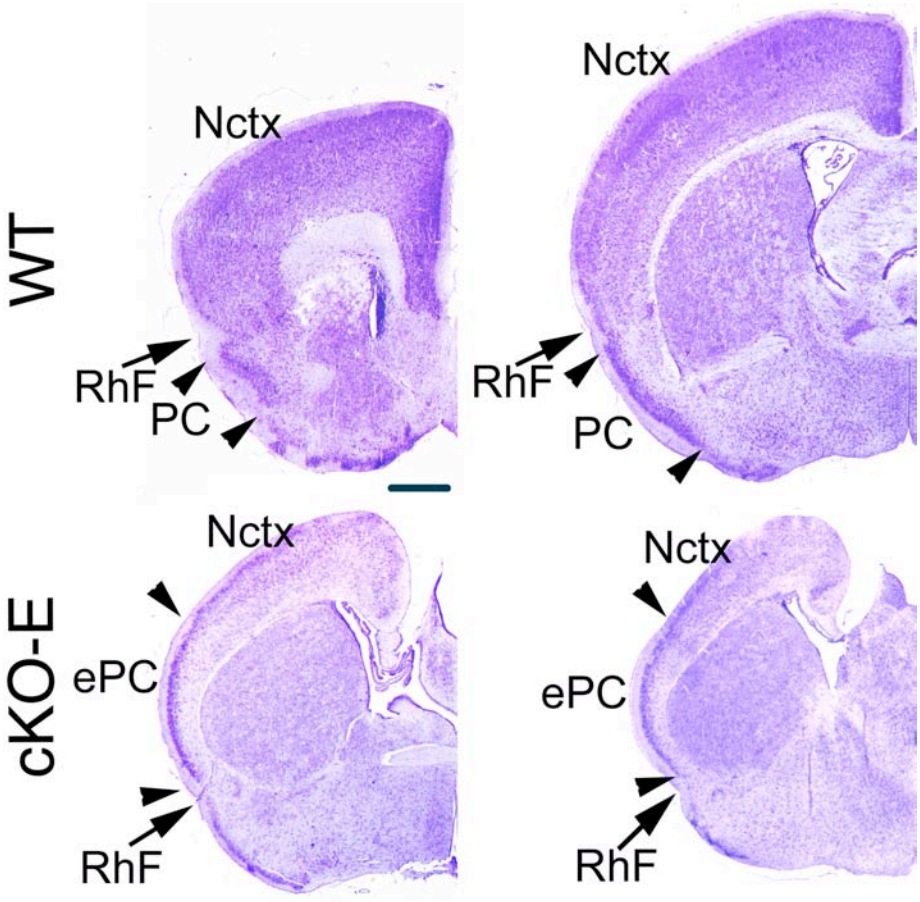
Supplementary Figure 2



Supplementary Figure 2. Cortical ventricular zone of Lhx2 cKO-E mice does not acquire properties of the lateral ganglionic eminence.

In situ hybridization for the lateral ganglionic eminence (LGE) markers, Dlx2, Mash1, Gsh2, Arx and Dlx5, on coronal sections of E13.5 WT ($Lhx2^{fl/+}$) and Lhx2 cKO-E ($Lhx2^{fl/-}$:Emx1-Cre). In both WT and Lhx2 cKO-E mice the expression of Dlx2, Mash1, Gsh2, Arx and Dlx5 is maintained in the LGE and does not spread to the dorsal telencephalic ventricular zone (VZ). Scale bar: 0.3 mm. DM, dorsomedial neocortex; L, lateral neocortex; Nctx, neocortex.

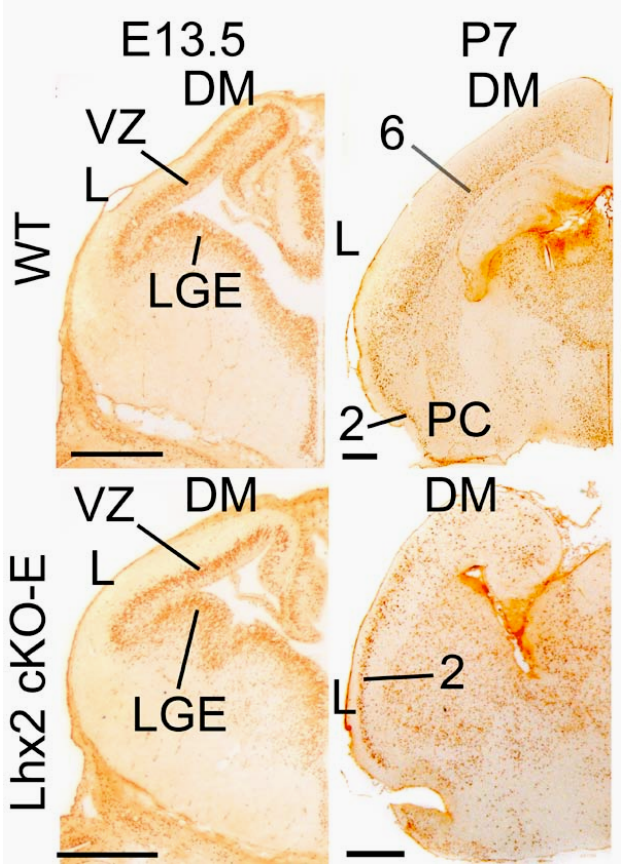
Supplementary Figure 3



Supplementary Figure 3. The ectopic piriform cortex is positioned dorsal to the rhinal fissure at the wild type location of lateral neocortex.

Nissl staining of P7 coronal sections from WT (top panel) and Lhx2 cKO-E mice (bottom panel) at two different rostral-caudal levels (more rostral level to the left) to show the positioning of neocortex (Nctx), ectopic piriform cortex (ePC) and PC relative to the rhinal fissure (RhF, arrow), a morphogenetic landmark found in all mammals that is positioned between Nctx and paleocortex. In WT, the Nctx is dorsal to the RhF and the PC is ventral to it. However, in Lhx2 cKO-E mice, the ePC is located dorsal to the RhF where lateral Nctx is in WT. Arrowheads mark the dorsal-ventral extent of PC and ePC. Scale bar: 0.1 mm.

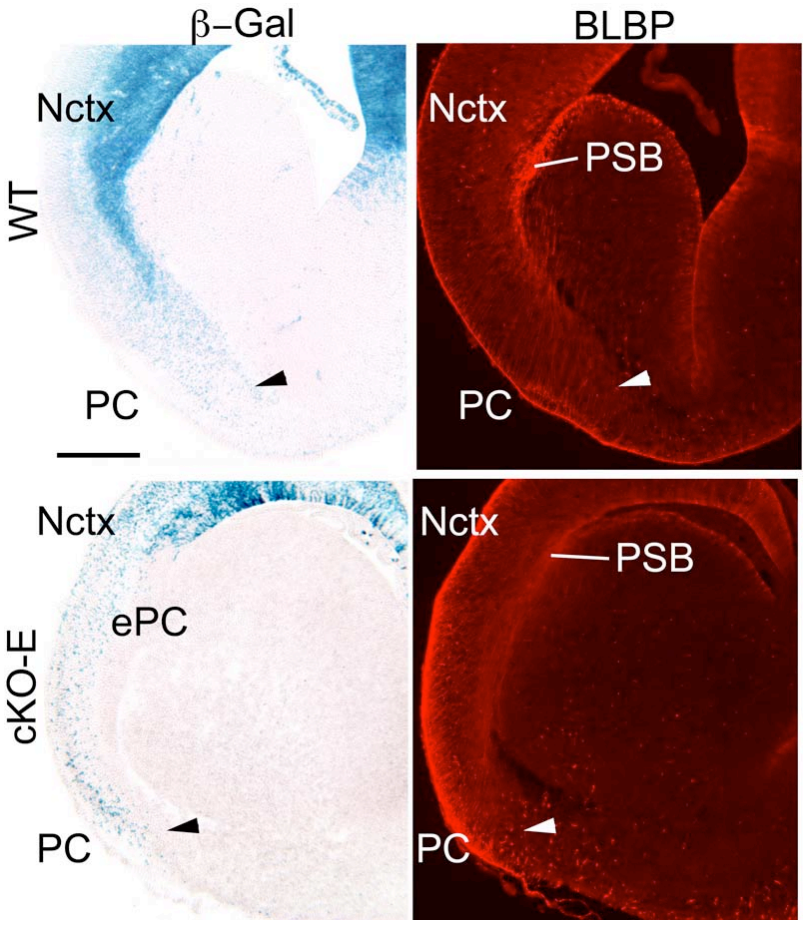
Supplementary Figure 4



Supplementary Figure 4. The ectopic piriform cortex in Lhx2 cKO-E mice is produced from a uniform population of cortical progenitors.

Coronal sections of WT (Lhx2$^{fl/+}$:Emx1-Cre) and Lhx2 cKO-E (Lhx2$^{fl/-}$:Emx1-Cre) at E13.5 and P7 stained with an anti-BrdU antibody. BrdU was administered at E13.5 and brains were collected either 1hr after the BrdU injection (left) or at P7 (right). The density of the BrdU labeled progenitors in the Emx1 lineage is relatively uniform across the neocortex in both WT and Lhx2 cKO-E mice at E13.5 (left). At P7, the majority of BrdU labeled cells in the neocortex in the WT and in dorsomedial neocortex (DM) of Lhx2 cKO-E mice are in deep layers, especially layer 6 (6). In contrast, in the lateral neocortex (L) in Lhx2 cKO-E mice, most of the labeled cells are in layer 2 (2), similar to the piriform cortex (PC) in WT (right panel). Scale bar: 0.5 mm. LGE, lateral ganglionic eminence; VZ, ventricular zone of dorsal telencephalon.

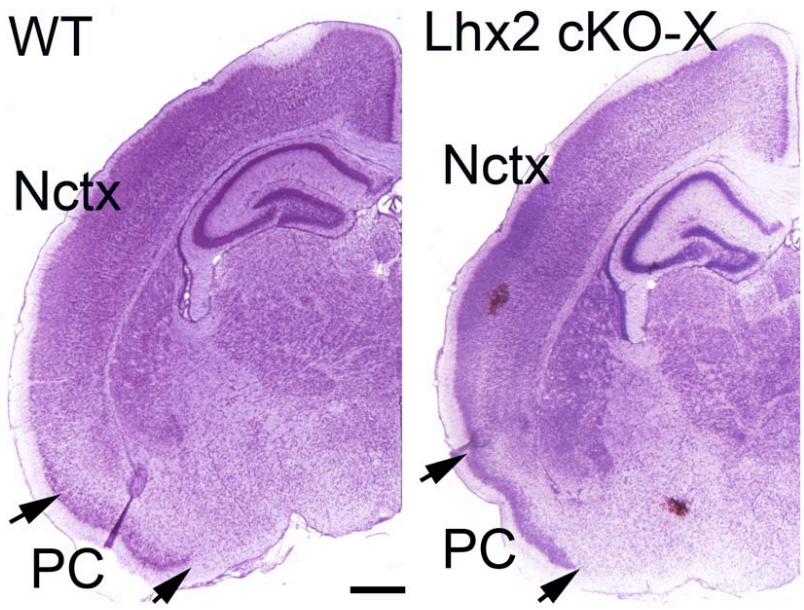
Supplementary Figure 5



Supplementary Figure 5. The wtPC in both WT and Lhx2 cKO-E mice is formed at the end of a dense bundle of radial glial fibers from the pallium-subpallium boundary.

β-gal staining (blue) of cells of the Emx1 lineage on coronal sections of E15.5 WT (Lhx2$^{fl/+}$:Emx1-Cre:R26R) and Lhx2 cKO-E (Lhx2$^{fl/-}$:Emx1-Cre:R26R) brains. Cells of the Emx1 lineage give rise to neocortex (Nctx) and piriform cortex (PC). Immunostaining for the radial glial marker, BLBP, labels a palisade of radial glial fibers that connect the ventricular zone at the Pallium-Subpallium Boundary (PSB) to the pial surface. In WT, β-gal labeled PC neurons are detected at the ventral-most group of radial glial fibers, which originate in the PSB (arrowhead). In Lhx2 cKO-E mice, β-gal labeled wtPC neurons are present at a similar position relative to the radial glial fibers, coincident with the ventral-most group of fibers that originate in the PSB (arrowhead), whereas the ePC is formed dorsal to it. Scale bar: 0.5mm.

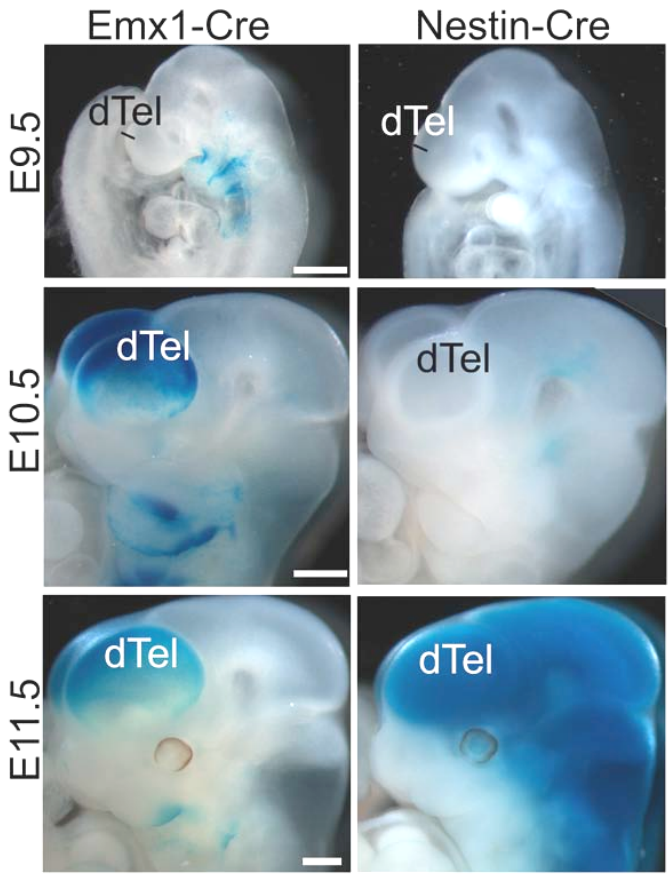
Supplementary Figure 6



Supplementary Figure 6. The location and size of neocortex and piriform cortex are similar to WT when Lhx2 is deleted from postmitotic neurons.

Cytoarchitecture of the cerebral cortex of WT ($Lhx2^{fl/-}$) and Lhx2 cKO-X ($Lhx2^{fl/-}$:Nex-Cre) brains at P7 revealed by Nissl staining of coronal sections. Indistinguishable from WT, in Lhx2 cKO-X mice the neocortex (Nctx) has a six-layer cytoarchitecture and the piriform cortex (PC, between arrows) is located at the base of the telencephalon ventral to the neocortex. Scale bar: 0.5 mm.

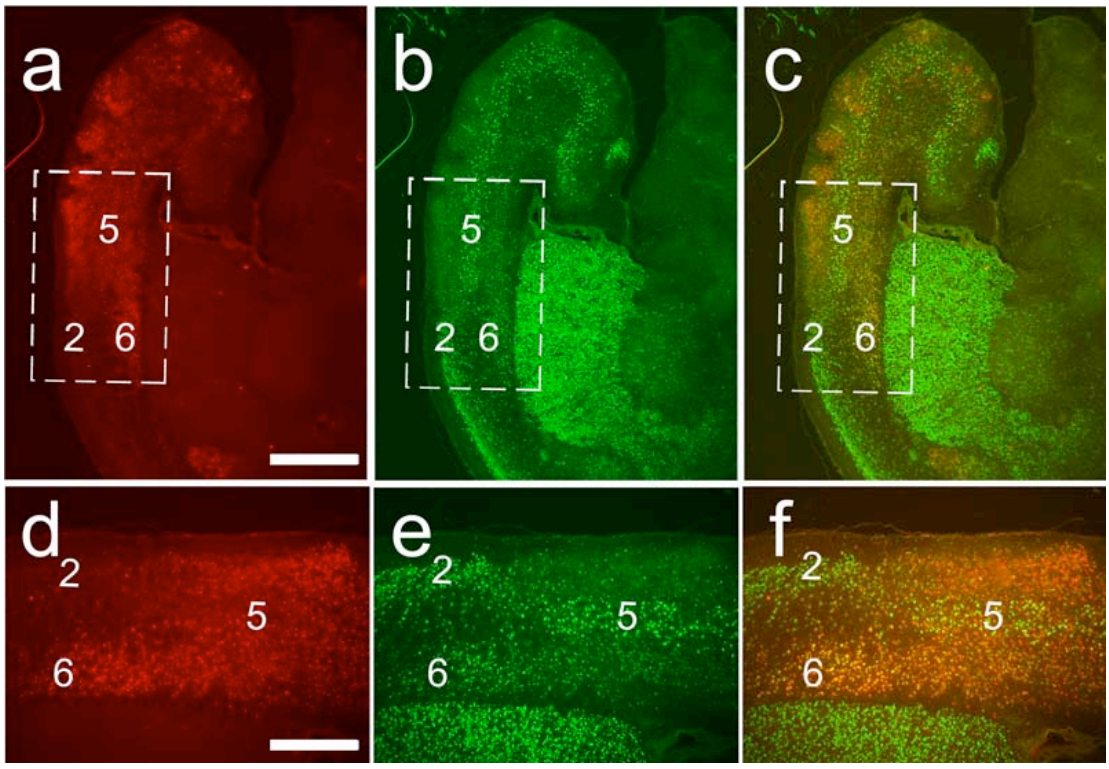
Supplementary Figure 7



Supplementary Figure 7. Emx1-Cre is activated at E10.5, one day prior to Nestin-Cre on E11.5.

Cre-mediated recombination in the embryonic brain was observed by β-gal staining of whole mount embryos of Emx1-Cre-R26R (left panels) and Nestin-Cre-R26R (right panels) at the indicated ages. In Emx1-Cre-R26R embryos, robust β-gal staining is first detectable in the dorsal telencephalon (dTel) at E10.5. In Nestin-Cre-R26R embryos, robust β-gal staining is first detectable in the dTel one day later at E11.5. Scale bar: 0.5 mm.

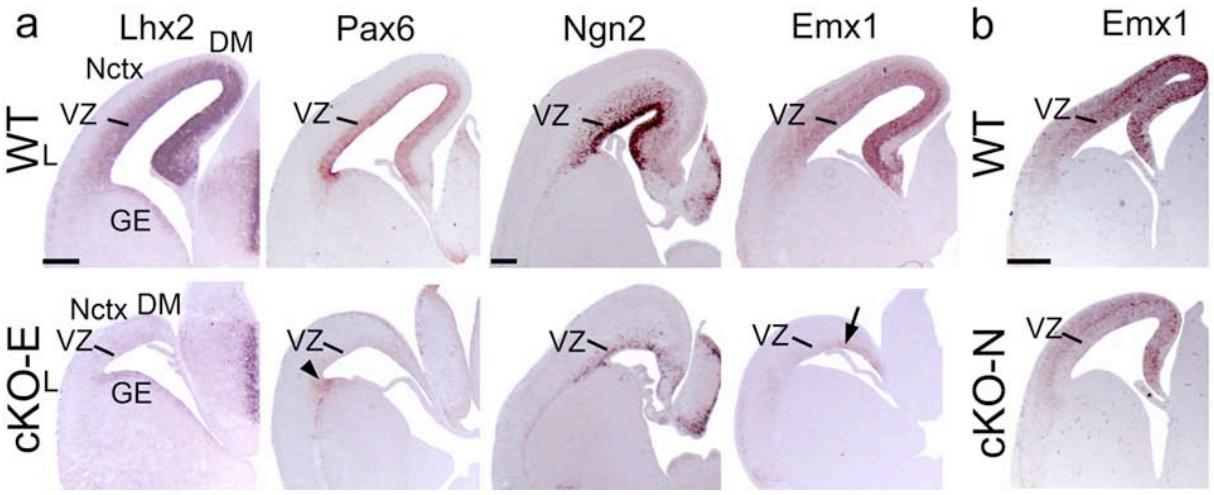
Supplementary Figure 8



Supplementary Figure 8. Unique transition between the ectopic piriform cortex and the neocortex in Lhx2 cKO-E mice.

Immunostaining for the markers Satb2 (a) and Ctip2 (b) in adjacent coronal sections of P7 Lhx2 cKO-E brain. Panel c is an overlay of panels a (Satb2; red) and b (Ctip2; green). In WT (Fig.4 of main text) and dorsal neocortex in Lhx2 cKO-E mice, Satb2 labels all neocortical layers. Ctip2 labels layer 5 in neocortex and layer 2 in piriform cortex. Dashed boxes in panels a and b highlight the transition zone within the neocortex of Lhx2 cKO-E mice between the ePC positioned in lateral neocortex and adjacent dorsomedial neocortex, and are shown at higher power in panels d-e. (d-e) High magnification views of the boxed areas in panels a and b, showing Satb2 (d), Ctip2 (e), and an overlay of the two (f). (d) Ctip2 labeling abruptly changes from layer 5 (where it is expressed in WT neocortex) to layer 2 (where it is expressed in wtPC) as dorsomedial neocortex transitions into the PC located in the lateral neocortex. (d-f) Satb2 labeling continues into the ePC, particularly in layer 6 where it extends ventrally beneath the Ctip2 labeling in layer 2, forming the transition zone. Scale bars: a-b: 200 μm; c-e: 25 μm.

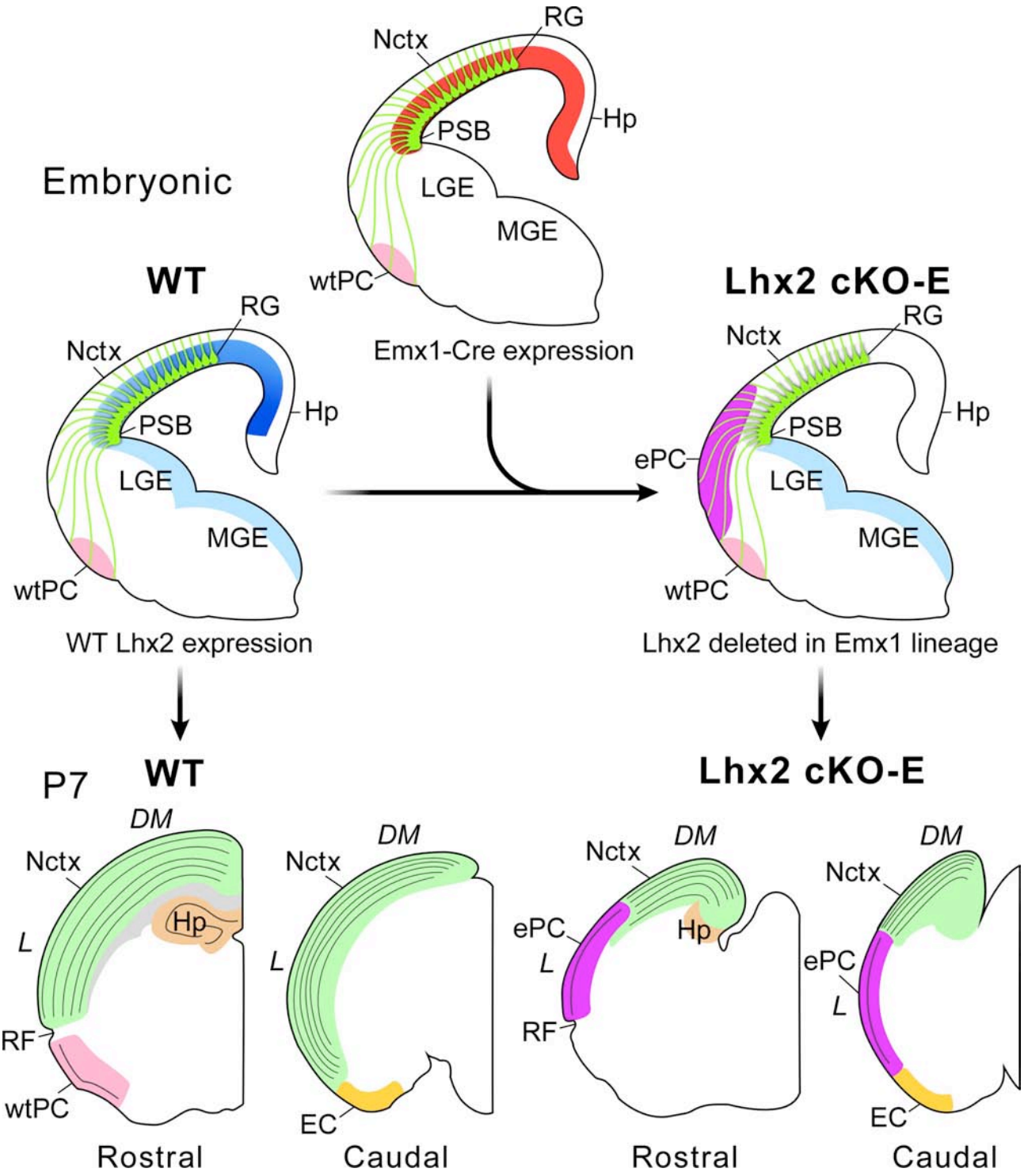
Supplementary Figure 9



Supplementary Figure 9. Altered expression of transcription factors implicated in cortical patterning in the dorsal telencephalic ventricular zone of Lhx2 cKO mice.

(a, b) Expression of Lhx2, Emx1, Pax6 at E14.5 and of Ngn2 at E16.5, analyzed on coronal sections of WT ($Lhx2^{fl/+}$), Lhx2 cKO-E ($Lhx2^{fl/-}$:Emx1-Cre), and Lhx2 cKO-N ($Lhx2^{fl/-}$:Nestin-Cre) brains processed for in situ hybridization. (a) Expression of Lhx2 is genetically deleted from the ventricular zone (VZ) of the dorsal telencephalon in Lhx2 cKO-E mice, but is retained in the ganglionic eminence (GE). Pax6 expression is dramatically down-regulated in the dorsal telencephalic VZ of Lhx2 cKO-E mice but is maintained in the pallium-subpallium boundary (arrowhead). Ngn2 expression is also diminished in the dorsal telencephalic VZ of Lhx2 cKO-E mice. Emx1 expression is diminished in dorsal telencephalic VZ of Lhx2 cKO-E mice, although in dorsomedial neocortex (DM), the VZ retains modest expression (arrow). (b) In Lhx2 cKO-N mice, Emx1 expression is also down-regulated in the cortical VZ, as are the other transcription factors (data not shown). Scale bars: 0.2 mm. L, lateral neocortex; Nctx, neocortex.

Supplementary Figure 10



Supplementary Figure 10. Summary of experimental design and major findings from conditional deletion of Lhx2 from telencephalic progenitors of the Emx1 lineage.

Supplementary Figure 10. Summary of experimental design and major findings from conditional deletion of Lhx2 from telencephalic progenitors of the Emx1 lineage.
(Top panels) Mice with floxed alleles of the LIM homeodomain transcription factor Lhx2 were made and crossed with three distinct Cre lines, Emx1-Cre, Nestin-Cre and Nex-Cre, to conditionally delete Lhx2 from progenitors of the Emx1 lineage that generate the majority of neurons that form the regions of cerebral cortex, including neocortex (Nctx), the paleocortical piriform cortex (wild-type piriform cortex, wtPC), and the archicortical hippocampus (Hp). Results summarized here are from Lhx2 cKO-E mice, obtained from the cross with the Emx1-Cre line (red indicates Emx1-Cre expression). In embryonic mice, Lhx2 (blue) is expressed in a high dorsal (medial) to low ventral (lateral) gradient in the ventricular zone (VZ) of the dorsal telencephalon (dTel). Radial glia (RG) are the progenitors of the Emx1 lineage, and both generate neurons of the cerebral cortex and provide a migrational guide for them by means of a long process that they extend from the VZ to the pial surface. wtPC progenitors are located in the vicinity of the pallium-subpallium boundary (PSB) and their neuronal progeny migrate along a dense collection of RG processes originating from these progenitors at the PSB VZ to their far ventral destination. Crossing of floxed-Lhx2 and Emx1-Cre mice to generate Lhx2 cKO-E mice, results in the selective deletion of Lhx2 from dTel progenitors of the Emx1 lineage and results in the a refating of progenitors that would normally generate lateral neocortex and instead generate an ectopic PC (ePC). The wtPC is also generated in Lhx2 cKO-E mice but is subsequently eliminated. **(Bottom Panels)** In WT at P7, the wtPC is located ventral to the rhinal fissure (RF), and the neocortex (Nctx) is positioned dorsal to it. In P7 Lhx2 cKO-E mice, the neocortex has two distinct architectures: dorsomedially (DM), the neocortex has a six-layer architecture and marker expression typical for neocortex, albeit with some defects, whereas lateral neocortex (L) has a three-layer pattern and phenocopies the architecture, marker expression and connectivity of the PC. This ePC is generated by progenitors of the Emx1 lineage that would normally generate lateral neocortex. The ePC is located dorsal to the rhinal fissure, is significantly larger than wtPC, and extends well caudal to it, paralleling the entire normal rostral-caudal extent of lateral neocortex. At caudal positions in WT, wtPC is not found ventral to neocortex and instead entorhinal cortex (EC) is ventral, which is also ventral to the ePC in Lhx2 cKO-E mice. These findings, and others described in the main text, demonstrate that Lhx2 regulates a fate decision among dTel progenitors of the Emx1 lineage to generate phylogenetically distinct telencephalic regions, lateral neocortex or paleocortical PC, and is required for progenitors of lateral neocortex and their progeny to acquire a neocortical fate. As described in the main text, Lhx2 regulates this fate decision within a critical period that closes with differentiation of neuronogenic radial glia and onset of cortical neurogenesis. These findings establish a genetic mechanism for determining regional

fate of dTel progenitors of the Emx1 lineage that generate cerebral cortex. LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence.

Supplementary References

S1. Shimogori, T., Banuchi, V., Ng, H. Y., Strauss, J. B. & Grove, E. A. Embryonic signaling centers expressing BMP, WNT and FGF proteins interact to pattern the cerebral cortex. Development 131, 5639-47 (2004).

S2. Kim, A. S., Anderson, S. A., Rubenstein, J. L. R., Lowenstein, D. H. & Pleasure, S. J. Pax-6 regulates expression of SFRP-2 and Wnt-7b in the developing CNS. J Neurosci 21, RC132 (2001).

S3. Carney, R. S., Cocas, L. A., Hirata, T., Mansfield, K. & Corbin, J. G. Differential regulation of telencephalic pallial-subpallial boundary patterning by pax6 and gsh2. Cereb Cortex 19, 745-59 (2009).

S4. Malatesta, P. et al. Neuronal or glial progeny: regional differences in radial glia fate. Neuron 37, 751-64 (2003).

S5. Anthony, T. E., Klein, C., Fishell, G. & Heintz, N. Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron 41, 881-90 (2004).

S6. Nakagawa, Y. & O'Leary, D. D. M. Combinatorial expression patterns of LIM-homeodomain and other regulatory genes parcellate developing thalamus. J Neurosci 21, 2711-25 (2001).