Novel functions of LHX2 and PAX6 in the developing telencephalon revealed upon combined loss of both genes

Geeta Godbole, Achira Roy, Ashwin S. Shetty and Shubha Tole*

Abstract: Patterning of the telencephalic neuroepithelium is a tightly regulated process controlled by transcription factors and signalling molecules. The cortical primordium is flanked by two signalling centres, the hem medially, and the antihem laterally. The hem induces the formation of the hippocampus in adjacent neuroepithelium. Therefore, the position of the hem defines the position of the hippocampus in the brain. The antihem is positioned at the boundary between the dorsal and ventral telencephalon and proposed to provide patterning cues during development. LIM-homeodomain (LIM-HD) transcription factor LHX2 suppresses both hem and antihem fate in the cortical neuroepithelium. Upon loss of Lhx2, medial cortical neuroepithelium is transformed into hem, whereas lateral cortical neuroepithelium is transformed into antihem. Here, we show that transcription factor PAX6, known to regulate patterning of the lateral telencephalon, restricts this tissue from transforming into hem upon loss of Lhx2. When Lhx2 and Pax6 are both deleted, the cortical hem expands to occupy almost the complete extent of the cortical primordium, indicating that both factors act to suppress hem fate in the lateral telencephalon. Furthermore, the shift in the pallial-subpallial boundary and absence of the antihem, observed in the Pax6 mutant, are both restored in the Lhx2; Pax6 double mutant. Together, these results not only reveal a novel function for LHX2 in regulating dorsoventral patterning in the telencephalon, but also identify PAX6 as a fundamental regulator of where the hem can form, and therefore implicate this molecule as a determinant of hippocampal positioning.

Keywords: Organizer, Dorsoventral, Patterning, Craniofacial, Mediolateral, Hem

Introduction
In development, tissues are patterned by the action of morphogens secreted by signaling centers known as “organizers.” The WNT- and BMP-secreting cortical hem in the telencephalon is one such medial signaling center [1, 2]. At the lateral edge of the cortical neuroepithelium, the EGF-expressing antihem defines the boundary between the dorsal and ventral telencephalon [3].

Previously, we demonstrated that the hem is capable of organizer function, by creating embryos with multiple hems in the telencephalon, and showing that each ectopic hem induces the formation of an ectopic hippocampus [4]. The factors that act to restrict the hem to its position at the medial edge of the cortical neuroepithelium are therefore critical determinants of where the hippocampus will form in the brain. Transcription factor LHX2 is a critical suppressor of the cortical hem. In the Lhx2 null mutant, the cortical primordium cannot retain its identity, but is transformed into two alternate fates: medial cortical neuroepithelium is transformed into hem, whereas lateral cortical neuroepithelium is transformed into antihem [4, 5]. In mosaic experiments, Lhx2 null clones in the medial telencephalon display hem fate, and those in the lateral telencephalon display antihem fate, indicating a cell-autonomous requirement for LHX2 in cortical neuroepithelial cells [4]. Why loss of Lhx2 leads to two disparate fate transformations in the dorsal telencephalon is unclear.

Paired domain containing homeobox transcription factor PAX6 is required for antihem fate, which is lost in Pax6 null mutants [3]. Pax6 is expressed in a lateral(high)-medial(low) gradient, opposite to that of Lhx2 [6, 7]. We hypothesized that the presence of PAX6 may restrict the medio-lateral extent of the hem in Lhx2

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mutants. Supporting this hypothesis, in the mosaic experiments performed in Mangale et al. (2008), PAX6 was lost in medial Lhx2 null patches that formed ectopic hems, but not in lateral Lhx2 null patches that formed antihems. Here, we examined medio-lateral patterning in embryos lacking both Lhx2 and Pax6, using null mutants as well as conditional knockout strategies. We find that the hem indeed expands to encompass almost the entire dorsal telencephalon when both Lhx2 and Pax6 are lost. Concomitantly, the loss of both factors appears to partially restore dorsoventral patterning that is disrupted in the Pax6 single mutant [8]. Thus, an examination of the Lhx2;Pax6 double mutant uncovers novel functions for both transcription factors, not seen upon loss of either factor alone. Our results show LHX2 to be a regulator of dorsoventral patterning in the telencephalon. PAX6 emerges as a key player in controlling the medial position of the hippocampus, because it prevents the hippocampal organizer, the hem, from being formed at lateral positions.

Results and discussion
We used an Lhx2 null mutant line generated by targeted gene disruption [9] and the Pax6sey/sey line which has a point mutation in the Pax6 gene creating a null allele [10] to generate the Lhx2−/−;Pax6sey/sey double null mutant. This mutant suffers from gross craniofacial abnormalities including an absence of external eyes, similar to the individual null phenotypes for Lhx2−/− and Pax6sey/sey embryos, and also lacks external nostrils similar to Pax6sey/sey [9, 11]. A unique feature in the double null is the presence of cleft-lip/palate, indicating a combinatorial role for LHX2 and PAX6 in craniofacial development (Additional file 1: Figure S1). To our knowledge, this is the first characterization of an Lhx2;Pax6 double null mutant.

PAX6 and LHX2 together restrict medial cortical fate to the medial telencephalon
We analyzed Lhx2−/−;Pax6sey/sey double mutants at embryonic day (E)12.5 with markers of medial telencephalic structures and compared the results with the expression seen in the respective single null mutants. We examined medial cortical marker Wnt8b which identifies the hem and the hippocampal primordium, and septum marker Fgf17. As expected, Wnt8b expression marks the medial telencephalic primordium in the Pax6sey/sey mutant. In the Lhx2−/− mutant, Wnt8b is expressed in the expanded hem, sparing some lateral tissue. In contrast, loss of both genes results in the entire dorsal telencephalon expressing medial markers, apparently sparing no lateral cortical neuroepithelium (Fig. 1). However, the double null mutant brains were fragile and the morphology made it difficult to analyze this apparent lateral-to-medial transition properly. Furthermore, the individual null mutants of Lhx2 and Pax6 are embryonic lethal [9, 10], and both genes lie on chromosome 2, which makes it difficult to obtain homozygous Lhx2−/−;Pax6sey/sey double null mutants.

Fig. 1 Medial fates are expanded laterally upon loss of both LHX2 and PAX6. a-d E12.5 coronal sections of Control (a), Pax6sey/sey (b), Lhx2−/− (c), and Lhx2−/−;Pax6sey/sey (d) were examined for the expression of medial cortical marker Wnt8b and septum marker Fgf17, which identify adjacent, non-overlapping regions of the medial telencephalon (open arrowheads mark the boundary between the two markers). In the double mutant, these medial markers together encompass the entire dorsal telencephalon (dashed line, d). Scale bar is 200 μm.
from heterozygous crosses. Therefore, we generated
$Lhx2^{lox/lox};Pax6^{lox/lox}$ double conditional mutants and
crossed them with a ubiquitously expressed tamoxifen
inducible CreER line.

**Lhx2 and Pax6 double conditional mutants
phenocopy the patterning defects in the
corresponding double null mutants**

We tested whether administration of tamoxifen at E8.5
to CreER; $Lhx2^{lox/lox};Pax6^{lox/lox}$ animals recapitulates the
results we obtained from the respective single and double
null mutants by E12.5. For all experiments using $Lhx2$ and
$Pax6$ single or double conditional mutant embryos, we
examined the expression of the respective floxed exon(s) in
each embryo in serial sections, and only used embryos
which displayed extensive recombination for analysis. One
element of an $Lhx2^{lox/lox};Pax6^{lox/lox}$ double conditional
mutant brain is shown in Additional file 2: Figure S2. Loss
of $Pax6$ is not reported to affect patterning of the dor-
somedial telencephalon [12] and indeed, the extent of
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$Pax6$ of mutant brain is shown in Additional file 2: Figure S2. Loss
at E12.5. We used a set of three markers,
$Wnt2b$, $Wnt3a$
double conditional mutant mice and harvested the embryos
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double conditional mutants for further analysis. We ad-
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Since loss of
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conditioned mutant brains, $Wnt8b$ expression appears to extend
up to the pallial-subpallial boundary, suggesting that lateral
cortical fates are missing (Fig. 2d). We therefore proceeded with
these double conditional mutants for further analysis of
the nature of this apparent lateral-to-medial transformation
upon loss of both $Lhx2$ and $Pax6$. Furthermore,
because the boundary between the dorsal and ventral
telencephalon is difficult to identify accurately in the
double mutant by morphology alone, we used $Dbx1$
expression to identify it in subsequent experiments. $Dbx1$, a marker of the antihem [13, 14] is expressed
in a subpopulation of scattered cells in the ventricular
zone of this structure [15].

The hem expands to a greater extent upon loss of
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Since loss of $Pax6$ alone did not expand medial fates
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$Wnt8b$ expressing neuroepithelium appears comparable in
$Pax6^{lox/lox}$ and control embryos (Fig. 2a, b). In $Lhx2^{lox/lox}$ embryos, $Wnt8b$ expression appears to sparing some lateral
telencephalic tissue, which is presumably the antihem [4].
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![Image](https://example.com/image1.png)

**Fig. 2** Conditional deletion of $Lhx2$ and $Pax6$ from E8.5 recapitulates the lateralization of medial fates seen in the double null mutants. **a-d** Tamoxifen was administered at E8.5 to Control (**a**, CreER; $Pax6^{lox/lox}$ (**b**), CreER; $Lhx2^{lox/lox}$ (**c**), and CreER; $Lhx2^{lox/lox}$; $Pax6^{lox/lox}$ (**d**)) animals, and the embryos were harvested at E12.5. Coronal sections were examined for $Wnt8b$ expression at rostral, mid, and caudal levels of sectioning. $Wnt8b$ expression is restricted to the medial cortical neuroepithelium in control and $Pax6^{lox/lox}$ brains (**a**, **b**). In $Lhx2^{lox/lox}$ brains, a small region of lateral neuroepithelium remains $Wnt8b$-negative (region between open arrowhead and dashed line, **c**). In the double conditional mutants, $Wnt8b$ expression extends almost up to the pallial-subpallial boundary (dashed lines, **c, d**). Scale bar is 200 µm.

![Image](https://example.com/image2.png)

**Fig. 3** Additional file: Figure S3. Additional file 3: Figure S3. We scored rostral, medial, and caudal levels separately and found that there was no significant rostro-caudal difference in the fractional hem length in a given mutant. As is apparent from the images, the hem is longer in $Lhx2$ single conditional mutants than in control brains. The key comparison of the $Lhx2$ single with the $Lhx2;Pax6$ double conditional mutants reveals that the
Fig. 3 PAX6 restricts the lateral extent to which the hem can expand when LHX2 is lost. (a-c, f) Tamoxifen was administered at E8.5 to Control (a), CreER;Lhx2lox/lox (b), and CreER;Lhx2lox/lox;Pax6lox/lox (c, f) animals, and the embryos were harvested at E12.5. Coronal sections were examined for the expression of hem markers Wnt2b and Lmx1a. Wnt3a was examined at rostral, mid, and caudal levels of sectioning. In each condition, the length of the hem was scored as a fraction of the total length of the dorsal telencephalic neuroepithelium measured up to the ventral extent of Dbx1 expression in an adjacent section (shown in Additional file 3: Figure S3).

d) Cartoon illustrating the length measurement (yellow dotted line) of the hem (green). Blue dotted line indicates the length measurement of the dorsal telencephalic neuroepithelium measured up to the ventral extent of anti-hem (pink).
e) Quantification of the fractional length of the hem in control Lhx2 single conditional mutants, and Lhx2;Pax6 double conditional mutants, at rostral, mid, and caudal levels of sectioning. For each level, the single mutant hem was significantly longer than that in the control, and the double mutant hem was significantly longer than that in the single mutant or the control (**p < 0.005, ***p < 0.0005).
f) Adjacent sections from three different double conditional mutant embryos in which Lhx2 was incompletely recombined were examined. In each case, when Pax6 is completely removed, but Lhx2 is present in patches (open arrowheads), the hem is suppressed in Lhx2 expressing regions. A false-color overlay shows that hem (green) is not seen in patches that show Lhx2 expression (red; open arrowheads). Scale bar is 200 μm.
fractional length of the hem is significantly greater when both factors are lost compared with the loss of Lhx2 alone (Fig. 3e). This indicates that PAX6 functions to restrict the lateral expansion of the hem in an Lhx2 mutant background.

**LHX2 suppresses hem fate at the lateral extreme of the dorsal telencephalon in a Pax6 mutant background**

Tamoxifen-driven Cre recombination is sometimes incomplete and results in patches of tissue that escape recombination. When we examined the lateral telencephalon of embryos that displayed complete recombination of Pax6 but had some unrecombined patches of Lhx2, it was apparent that hem only formed where both genes were lacking (Fig. 3f). That is, the presence of Lhx2 in a Pax6 mutant background was enough to suppress hem fate. This demonstrates that LHX2 is a suppressor of hem fate both medially and laterally, the latter function being revealed only in a Pax6 mutant background.

In summary, these results demonstrate that the extreme medial fate of the cortical hem can be generated at extreme lateral positions, and that LHX2 and PAX6 each suppresses hem fate. These roles of LHX2 and PAX6 are revealed only upon analysis of the double mutants, and could not have been predicted from examining either single mutant alone.

**Absence of Lhx2 and Pax6 partially restores the pallial-subpallial boundary/antihem that is disrupted upon loss of Pax6 alone**

Finally, we examined the pallial-subpallial boundary itself, since PAX6 is known to be a regulator of the position of this border [12, 16]. We administered tamoxifen at E8.5 to conditionally remove Lhx2 alone, Pax6 alone, or both, and harvested the embryos at E12.5. The expression of both Neurog2 and Gsx2 stops at the pallial-subpallial boundary, with Neurog2 being expressed in the pallial side and Gsx2 in the subpallial side (Fig. 4, [8]). Loss of Lhx2 does not appear to alter these expression boundaries, though it

![Fig. 4](image_url)

*Fig. 4* The dorsal shift of the pallial-subpallial boundary upon loss of Pax6 is restored when Lhx2 is also removed. **a-d** Tamoxifen was administered at E8.5 to Control (**a**), CreER;Lhx2lox/lox (**b**), CreER;Pax6lox/lox (**c**), and CreER;Lhx2lox/lox;Pax6lox/lox (**d**) animals, and the embryos were harvested at E12.5. Coronal sections were examined for the expression of pallial marker Neurog2, subpallial markers Gsx2, and antihem marker Dbx1. The pallial-subpallial boundary (open arrowheads, **a-d**) appears to be normally positioned upon loss of LHX2 alone (**b**), but shifts dorsally upon loss of Pax6 alone, and Dbx1 expression is lost (**c**). Upon combined loss of both LHX2 and Pax6, the pallial-subpallial boundary appears to be restored to its normal position, together with Dbx1 expression. Loss of LHX2 results in lowered expression of Gsx2 in the subpallium compared with control brains (**a, b, d**). **e** Cartoons schematizing the results in **a-d, f** A model of the interactions between Lhx2, Gsx2, and Pax6 upon the hem and the antihem. Solid blue lines indicate regulatory functions identified in this study: LHX2 maintains Gsx2 expression levels in the ventral telencephalon, and PAX6 suppresses hem fate in the dorsolateral telencephalon. The dotted blue line indicates that regulation of Dbx1 by PAX6 is likely to be indirect. Scale bar is 200 μm.
does appear to cause an overall reduction in the level of Gsx2 expression in the subpallium (Fig. 4). In the absence of Pax6, the pallial-subpallial boundary is known to shift dorsally, with Gsx2 expression seen in the lateral region of the pallium where Neurog2 expression recedes (Fig. 4; [8, 16]). This is thought to be due to cross-repression between PAX6 and GSX2 [8, 13]. However, since LH2X appears to be required for normal Gsx2 expression, when Lhx2 and Pax6 are both lost, the expanded Gsx2 expression in the lateral neuroepithelium is no longer seen, but instead, Neurog2 is expressed there. Thus, despite the absence of Pax6, the additional loss of Lhx2 appears to restore the pallial-subpallial boundary.

This regulation of the pallial-subpallial boundary has an intriguing effect on Dbx1 expression. GSX2 is known to suppress Dbx1 and prevent it from being expressed in the subpallium, and consistent with this, Dbx1 expands into the subpallium in Gsx2 mutants [15]. Upon loss of Pax6, Gsx2 expression occupies the region of the antihem and suppresses Dbx1 expression there [15]. Thus, the loss of Dbx1 expression in the antihem of Pax6 mutants is likely to be indirect, via GSX2. LH2X also suppresses Dbx1 expression [4] and promotes Gsx2 expression (the current study). The expansion of Gsx2 into pallial territory upon loss of Pax6 is reversed upon the additional loss of Lhx2. The reappearance of Dbx1 in its apparently normal antihem location in the Lhx2lox/lox;Pax6lox/lox double conditional mutant brain may be because both its suppressors- LHX2 or GSX2- are absent in this location.

Therefore, though Dbx1 expression is undetectable in the Pax6 single mutant, it is clear from its reappearance (albeit in an ectopic location) in the Pax6seuy/seuy;Gsx2−/− double null mutant [15], and in an apparently normal location in the Lhx2lox/lox;Pax6lox/lox double conditional mutant (this study), that Dbx1 does not require Pax6, per se, for its expression. Like Dbx1, sFrp2 expression is also lost in the Pax6seuy/seuy brain [15]. However, in contrast to Dbx1, we find that sFrp2 does not reappear in the Lhx2lox/lox;Pax6lox/lox double mutant (Additional file 4: Figure S4). Since loss of Lhx2 alone does not cause loss of sFrp2 expression [17], this suggests that sFrp2 critically requires PAX6 for its expression. This indicates that different molecular features of the antihem are regulated by distinct combinations of factors, underscoring the heterogeneity of this structure.

Conclusion
This study uncovers new roles for transcription factors LHX2 and PAX6 in patterning neuroepithelial domains in the telencephalon. LH2X was known to suppress hem fate in the medial component of the dorsal telencephalon, but we find that PAX6 and LH2X together suppress hem fate in the lateral component of the dorsal telencephalon as well. We discover that hem fate can indeed arise at the lateral extreme of the dorsal telencephalon, almost up to the pallial-subpallial boundary, if both PAX6 and LHX2 are removed. Therefore, this demonstrates that PAX6 plays a role in suppressing hem fate. Finally, we show that the LH2X plays a role in regulating the pallial-subpallial boundary. This is surprising since LH2X is expressed in a gradient in the entire telencephalic ventricular zone, and does not display an expression boundary in the lateral telencephalon. This role for LH2X is partly due to its regulation of Gsx2 in the ventral telencephalon, which is a novel genetic interaction between these two developmental control molecules.

Our findings are summarized in the model in Fig. 4, which illustrates how LH2X and PAX6 interact to regulate the positioning of the hem, the expression of the antihem marker Dbx1 and also where the pallial-subpallial boundary is formed. These roles for LH2X and PAX6 are revealed only upon analysis of the double mutants, and could not have been predicted from examining either single mutant alone, thus highlighting the importance of these “classical” epistasis studies in the developing brain. More broadly, an approach that examines the functions of specific factors in the context of different mutant backgrounds may reveal unexpected spatiotemporal interactions between genes and could raise new hypotheses for the control of developmental phenomena. This may be particularly useful in understanding disorders such as autism or other neurodevelopmental diseases in which the severity of the phenotype might depend on the combinations and levels of proteins that may not appear likely to interact with each other based on standard single gene mutation studies.

Material and methods
Mice
All animal protocols were approved by the Institutional Animal Ethics Committee (Tata Institute of Fundamental Research, Mumbai, India) according to regulations devised by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The tamoxifen-inducible CreERT2 line (strain name: B6; 129-Gt(Rosa)26Sortm1(Cre/ERT2)Nat/l); stock number: 004847) was obtained from the Jackson Laboratory. The Lhx2−/− line used in this study was a kind gift from Forbes D. Porter, NIH, USA [9]. The Lhx2lox/lox line used in this study was a kind gift from Edwin Monuki, University of California, Irvine, USA [4]. The Pax6seuy/seuy line was a kind gift from David Price, University of Edinburgh [18] and the Pax6lox/lox line was a kind gift from Anastassia Stoykova, MPI Goettingen, Germany [12].

Noon of the day of vaginal plug was designated as embryonic day 0.5 (E0.5). Tamoxifen (Sigma, MO, USA) was administered to the pregnant dams at different time points as mentioned in the text and embryos were...
Sample preparation and in situ hybridization

Freshly harvested brains were fixed in 4% paraformaldehyde (PFA) (Sigma, MO, USA) overnight and equilibrated in 15% sucrose followed by 30% sucrose (SRL Chem, Mumbai, India). The brains were sectioned at 16 μm sections using a freezing microtome and mounted on Superfrost Plus slides (Electron Microscopy Sciences, Hatfield, PA, USA). Sections were post-fixed in 4% PFA, washed in Phosphate buffered Saline (PBS), and treated with protease K (Sigma, MO, USA) (1 μg/ml) at 37°C for 10 mins. One more round of post-fixing and PBS washes was performed, and then the sections were incubated in hybridization buffer (5X SSC, 50% formamide, 1% SDS) containing different antisense RNA probes at 70°C overnight.

Probes were prepared using a kit from Roche per the manufacturer’s instructions (Roche, Mannheim, Germany). The next day, after washes with solution X (2X Saline sodium citrate SSC, 50% formamide, 1%SDS) at 70°C, followed by stringent washes with 2X SSC and 0.2X SSC at room temperature, the sections were washed with TBST (10 mM Tris-HCl, pH 7.5, and 150 mM NaCl, 0.1% KCl, 0.5% Tween-20). The slides were then incubated with alkaline phosphatase conjugated anti-Digoxigenin Fab fragments (1:5000, Roche, IN, USA) for 16 h at 4°C. The slides were then washed four times with TBST and then with developing buffer NTMT (100 mM NaCl, 100 mM Tris, pH 9.5, 50 mM MgCl₂, 1% Tween-20). The color reaction had developed satisfactorily, as assessed with the intensity of signal and low background. The reaction was stopped in Tris-EDTA (10 mM Tris-HCl (pH 7.5), 10 mM EDTA (pH 8.0) and fixed 3.7% formaldehyde (PFA) (Sigma, MO, USA) overnight and equilibrated in 15% sucrose followed by 30% sucrose (SRL Chem, Mumbai, India).

For each embryo, the extent of recombination was examined by in situ hybridization for the recombined exon in one series of sections. All mutant combinations were examined in at least 4 embryos of the relevant genotype.

Image acquisition and analysis

Bright field images were acquired by Zeiss microscope Axiostar 2plus using a Nikon DS-f2 camera. Length measurements were obtained using ImageJ software from eight hemispheres (n = 8, from 4 brains) of each genotype: control, single Lhx2 conditional mutants and Lhx2;Pax6 double conditional mutants. The hem length was measured as the length of neuroepithelium expressing Wnt3a, and dorsal telencephalic neuroepithelium length was measured from the medial edge of the hem up to the ventral extent of Dbx1 expression in an adjacent section (shown in Additional file 3: Figure S3). For each hemisphere, the ratio of the hem length to the total dorsal telencephalic neuroepithelium length was determined at rostral, medial, and caudal levels of sectioning. The mean and standard deviation (SD) were calculated, and one-way ANOVA followed by the Tukey’s post-hoc test was used to determine significance. Statistical analyses were performed using Graph Pad Prism 5. Error bars in the figure indicate standard deviation (SD).

Additional file

Additional file 1: Figure S1. Constitutive loss of both Lhx2 and Pax6 results in severe cranio-facial defects. (A,B) lateral views and (C,D) dorsal views of E12.5 heads from control (AC) and Lhx2<sup>−/−</sup>;Pax6<sup>−/−</sup> double mutant (BD) embryos. A. Control embryo shows the presence of optic cups (black arrowhead) and nostrils (white arrowhead), while the double mutant lacks eyes (black asterisk) and displays two protruding structures in place of the nasal mass (open arrowheads), suggestive of an open or cleft palate. (JPEG 965 kb)

Additional file 2: Figure S2. Tamoxifen was administered at E8.5 to CreERT;Lhx2<sup>lox/lox</sup>;Pax6<sup>lox/lox</sup> animals and the embryos were harvested at E12.5. Near-complete recombination of Pax6 and Lhx2 is seen in a rostral-caudal series of sections adjacent to those examined for Wnt8b expression. (JPEG 807 kb)

Additional file 3: Figure S3. This figure shows Dbx1 expression in adjacent sections to the images shown in Fig. 3. These images were used for measurements of the hem and dorsal telencephalic neuroepithelium, taken up to the ventral extent of Dbx1 in adjacent sections in CreERT2; Lhx2<sup>lox/lox</sup> (dashed lines, B,DF) and CreERT;Lhx2<sup>lox/lox</sup>;Pax6<sup>lox/lox</sup> (H,JI) embryos. Dbx1 expression in the control brain is not shown. Scale bar is 200 μm. (JPEG 856 kb)

Additional file 4: Figure S4. Tamoxifen was administered at E8.5 to CreERT;Lhx2<sup>lox/lox</sup>;Pax6<sup>lox/lox</sup> animals and the embryos were harvested at E12.5. Fgf2 expression is not detectable in the Dbx1 expressing antherim of the double mutant embryos. (JPEG 913 kb)

Abbreviations
LIM-HD: LIM homeodomain

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**Availability of data and materials**
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

**Authors’ contributions**
GG designed the study, performed the experiments and analyses in Figs. 2, 3, and 4 and Figures S2, S3, and S4. generated the double conditional mutants; AR designed the study, performed the experiments and analyses in Figs. 2, 3, and 4 and Figures S2, S3, and S4. generated the double conditional mutants; prepared the figures. AR designed the study, performed the Lhx2lox/lox, Pax6lox/lox and the double null experiments in Fig. 1 and Additional file 1: Figure S1, and contributed to writing the manuscript. ASS conceived the project, designed the study and helped to generate the double mutants, and contributed to writing the manuscript. ST conceived the project, designed the study and helped to generate the double mutants, and contributed to writing the manuscript. ST conceived the project, designed the study, and wrote the manuscript. All authors read and approved the final manuscript.

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**Ethics approval**
All animal protocols were approved by the Institutional Animal Ethics Committee (Tata Institute of Fundamental Research, Mumbai, India) according to regulations devised by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. (Reference Approval number: TIFR/IAEC/2012–11).

**Consent for publication**
Not Applicable.

**Competing interests**
The authors declare that they have no competing interests.

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