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COUP-TFII controls amygdala patterning by regulating neuropilin expression

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SUMMARY

The development of the progenitor zones in the pallium, lateral ganglionic eminence (LGE) and medial ganglionic eminence (MGE) in the subpallium has been well studied; however, so far the role of the caudal ganglionic eminence (CGE), a posterior subpallial domain, in telencephalon patterning remains poorly understood. COUP-TFII, an orphan nuclear receptor, is preferentially expressed in the CGE. We generated *COUP-TFII* mouse mutants, using Rx-Cre (*RxCre;COUP-TFII$^{F/F}$*), to study its function in telencephalon development. In these mutants, we found severe defects in the formation of the amygdala complex, including the lateral (LA), basolateral (BLA) and basomedial (BMA) amygdala nuclei. Molecular analysis provided evidence that the migration of CGE-derived Pax6$^+$ cells failed to settle into the BMA nucleus, owing to reduced expression of neuropilin 1 (Nrp1) and Nrp2, two semaphorin receptors that regulate neuronal cell migration and axon guidance. Our ChIP assays revealed that *Nrp1* and *Nrp2* genes are the direct targets of COUP-TFII in the telencephalon in vivo. Furthermore, our results showed that the coordinated development between the CGE originated subpallial population (Pax6$^+$ cells) and pallial populations (Tbr1$^+$ and Lhx2$^+$ cells) was essential for patterning the amygdala assembly. Our study presented novel genetic evidence that the caudal ganglionic eminence, a distinct subpallial progenitor zone, contributes cells to the basal telencephalon, such as the BMA nucleus.

KEY WORDS: Caudal ganglionic eminence, Amygdala, Basomedial amygdala nucleus, Migration, Mouse, Nr2f2

INTRODUCTION

The embryonic telencephalon of mammals can be roughly divided into the dorsal pallium and ventral subpallium. The pallium comprises four major progenitor zones: the medial (MP), dorsal (DP), lateral (LP) and ventral (VP) pallium. The subpallium comprises three main domains: the lateral (LGE), medial (MGE) and caudal (CGE) ganglionic eminences. In past decades, the studies on the progenitor zones of the pallium and subpallial LGE and MGE reveal that each of these progenitor domains generates distinct sets of cells; some cells migrate radially and remain within the territory, whereas others migrate tangentially into adjacent and other regions (Marin and Rubenstein, 2001; Schuurmans and Guillemot, 2002; Sur and Rubenstein, 2005; Hébert and Fishell, 2008). Nevertheless, how the CGE, a subpallial progenitor zone, gives rise to the cells and nuclei in the mature brain is not clear.

The CGE was considered as a posterior extension of the LGE or MEG or both. *Gsh2* is highly expressed in the LGE, and is necessary to maintain the LGE fate (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). *Nkx2.1* is exclusively expressed and required for the MGE identities (Sussel et al., 1999). Even though the LGE, MGE and CGE share common molecular features (Wonders and Anderson, 2006; Flames et al., 2007; Long et al., 2009), studies from *Gsh2* and *Nkx2.1* mutants demonstrate that the CGE is not a simple caudal extension of the LGE and MGE (Nery et al., 2002). Studies based on gene expression profiling, in vitro migration and in vivo fate mapping imply that the CGE is the source for some specific subtypes of cortical interneurons, as well as for some nuclei in the limbic system, including the hippocampus and amygdala (Nery et al., 2002; Xu et al., 2004; Butt et al., 2005; Miyoshi et al., 2010). However, so far no genetic mutant model in which the CGE is specifically affected has been reported.

The mammalian amygdala, a complex located in the caudoventral telencephalon, comprises 11-15 nuclei, which are anatomically, genetically and functionally distinct. The amygdala plays a central role in modulating emotion and stress-related behaviors (Swanson and Petrovich, 1998; LeDoux, 2007). In the past decade, findings from mutant mouse models have begun to reveal how neuronal cell diversity is generated in the amygdala. For example, the principal neurons in the center amygdala nucleus (CeA) are derived from the ventral LGE (vLGE), and intercalated cells (ITCs) are emanated from the LGE (Waclaw et al., 2010; Wang et al., 2011). By contrast, the preoptic area (POA) gives rise to inhibitory neurons in the medial amygdala nucleus (MeA) (Hirata et al., 2009). In addition, DP contributes cells to the layer 2 nucleus of the lateral olfactory tract (nLOT2) (Remedios et al., 2007). Furthermore, OTP+ progenitors in the diencephalon migrate tangentially and colonize in the amygdala nuclei (Garcia-Moreno et al., 2010). Meanwhile, gene expression profiling analysis suggests that the lateral (LA) and basomedial (BMA) amygdala nuclei are originated from the VP, and basolateral (BLA) amygdala nucleus from the LP (Medina et al., 2004). The in vivo fate mapping in mice reveals that CGE contributes cells to the Ce, BMA and Me amygdala nuclei (Nery et al., 2002). Nonetheless, little is known regarding to the molecular and cellular mechanisms that control the development of distinct nuclei, especially the BMA nucleus located in the basolateral complex of amygdala, and how those diverse nuclei are integrated to pattern the amygdala assembly.

COUP-TFII (Nr2f2 – Mouse Genome Informatics), an orphan nuclear receptor, is essential for the morphogenesis of multiple organs (Takamoto et al., 2005a; Takamoto et al., 2005b; You et al., 2005a; You et al., 2005b; Kurihara et al., 2007; Petit et al., 2007; Li

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et al., 2009; Kim et al., 2009; Tang et al., 2010; Lin et al., 2010). In this study, we found that COUP-TFII was strongly expressed in the CGE, and we generated a conditional mutant that eliminated *COUP-TFII* function in the ventral telencephalon with *RxCre*. Analysis of *COUP-TFII* mutants reveals that *COUP-TFII* is required for patterning the amygdala complex. Our data demonstrates that CGE-derived Pax6+ cells colonized the BMA nucleus, and the migration of Pax6+ cells in the CGE might be specifically mediated by the *Nrp1* and *Nrp2* genes, which are direct targets of COUP-TFII in the forebrain. Therefore, our study provides novel genetic evidence that the CGE, a subpallial progenitor zone, populates amygdala complex.

MATERIALS AND METHODS

Animals

COUP-TFII-floxed mice, *RxCre* mice and *COUP-TFII/LacZ* knock-in (*COUP-TFII*$^{LacZ/+}$) mice used in the study were in a mixed background. All animal protocols were approved by the Animal Center for Comparative Medicine at Baylor College of Medicine. Only the littermates were compared in the experiments.

Nissl staining and immunohistochemistry

Nissl staining was performed with 0.1% Cresyl Violet for 10 minutes. Immunohistochemical assays were carried out as reported previously (Tang et al., 2010). We used the following antibodies: mouse anti-COUP-TFII (R&D, 1:2000), rabbit anti-Pax6 (Covance, 1:500), rabbit anti-Tbr1 (Abcam, 1:400), goat anti-β-galactosidase/*lacZ* (Biogenesis, 1:400), rabbit anti-Mef2C (ProteinTech, 1:400), goat anti-neuropilin 1 (A&D, 1:200) and goat anti-neuropilin 2 (A&D, 1:400).

RNA isolation and quantitative real-time PCR

Total RNA preparation, reverse-transcription PCR and quantitative real-time PCR assays were carried out as described previously (Li et al., 2009). Student's *t*-test was used to compare the means of relative mRNA levels between the control group and mutant group. Primer sequences are available upon request.

In vivo chromatin immunoprecipitation (*ChIP*) assay

Forebrains from E12.5 embryos were dissected, sliced into small pieces and transferred into a 15 ml tube containing 10 ml DMEM:F12 medium with 10% FBS. Fresh prepared 18.5% PFA (550 μl) was added into the tube, then mixed and racked for 15 minutes at room temperature. Glycine (1 ml of 10×) was added into the tube, and racked for another 15 minutes at room temperature. Tissues were collected by low-speed centrifuge. After a wash with 10 ml ice-cold 1× PBS, brain tissues were resuspended and transferred into a 1.5 ml tube with ice-cold 1× PBS. The tissues were collected by centrifugation at 800 ***g*** at 4°C for 5 minutes. From this step, ChIP assays were performed with an EZ-Magna ChIP G chromatin immunoprecipitation kit from Millipore, according to the manufacturer's protocol. Monoclonal mouse COUP-TFII antibody, polyclonal rabbit Sp1 antibody and corresponding control IgG antibodies were used in these studies. The quantitative real-time PCR assays were carried out on chromatin samples prepared above. Primer sequences are: *Nrp1-Sp1-f*, CATAGAGCGAGTTCCTTCCA; *Nrp1-Sp1-r*, TCTTCAAGGGCTTTCCAGTG; and *Nrp2-Sp1-f*, CTGCCTCTTTAATCAGGAACAC; *Nrp2-Sp1-r*, GAGCAGCATGTGGATGTCAG.

RESULTS

The expression of COUP-TFII in the CGE progenitor cells as well as the developing and mature amygdala neurons

To investigate the function of *COUP-TFII* during mouse telencephalon development, immunohistochemical assays were used to examine its expression at E12.5. In the ventricular zone, COUP-TFII was most strongly expressed in the caudoventral telencephalon, cortical hem (CH) and choroid plexus (CP) (Fig. 1A-D). In the caudal subpallium, it was highly expressed in the CGE ventricular zone (VZ), with a medial high-lateral low gradient, and in the ventral CGE, a domain that may be the anlage of the ventral hippocampus and amygdalo-hippocampal area (Fig. 1D,H). In the mantle zone, COUP-TFII was strongly expressed in the caudoventral cortex, including regions that are likely to generate pallial nuclei of the amygdala (Fig. 1C,D,G,H). Pax6, an important homeobox transcription factor for telencephalon patterning (Stoykova et al., 2000), was expressed in the dLGE derived migratory cells (open arrowheads; Fig. 1I-K), the CGE VZ and the CGE-originated migratory cells (arrowheads; Fig. 1L). The majority of Pax6$^+$ migratory cells in the CGE were co-labeled with COUP-TFII (Fig. 1P). Consistent with the previous reports (Stoykova et al., 2000; Puelles et al., 2000; Tole et al., 2005), Tbr1, a T-box transcription factor (Bulfone et al., 1995), was expressed highly in the DP originated lateral stream (sDP) and at a lower level in the parallel LP- and VP-derived medial stream (sLP/VP) at the rostral telencephalon (Fig. 1Q-S). Two unparalleled Tbr1$^+$ streams were also detected at the CGE level (Fig. 1T). For consistency, we would like to designate the medial Tbr1$^+$ stream as sLP/VP, even though the real origin of cells in the stream was still questionable. The majority of Tbr1$^+$ cells in these two streams were positive for COUP-TFII (Fig. 1X). The unique expression profiles of *Pax6* and *Tbr1* genes at the CGE were confirmed by in situ assays (supplementary material Fig. S1). Our data above reveal that COUP-TFII is most strongly expressed at the CGE at E12.5, where both the subpallial-Pax6$^+$ and pallial-Tbr1$^+$ cells are situated, indicating that the CGE is genetically distinct.

As COUP-TFII is primarily expressed in the CGE (Fig. 1) (Kanatani et al., 2008; Willi-Monnerat et al., 2008), a major source of nuclei in the amygdala (Nery et al., 2002), we next examined whether COUP-TFII is expressed in amygdala neurons. Immunostaining data revealed that COUP-TFII was expressed in developing amygdala neurons at the LA, BLA, BMA, MeA and cortical amygdala (CoA) nuclei at P0 (Fig. 1Y), and mature amygdala neurons at the LA, BLA, BMA, dorsal medial amygdala (dMeA) and ventral medial amygdala (vMeA) nuclei at 1 month (Fig. 1Z).

Generate ventral telencephalon specific *COUP-TFII* knockout mice with *RxCre*

In light of our finding that COUP-TFII is preferentially expressed in the CGE, we hypothesize that *COUP-TFII* probably plays a crucial role in the development of the ventral telencephalon. To support this hypothesis, an *RxCre* mouse model was chosen to generate *COUP-TFII* conditional knockout mice (Swindell et al., 2006). In the *COUP-TFII-floxed* allele, a *lacZ* reporting cassette was inserted behind the second *LoxP* site (Takamoto et al., 2005b). The *lacZ* cassette is silent without *COUP-TFII* deletion, and will be activated in cells where *COUP-TFII* promoter is active and *COUP-TFII* gene is deleted by Cre recombinase. Therefore, *COUP-TFII-floxed* mouse is a suitable reporter with which to assess where *COUP-TFII* gene is excised by RxCre recombinase. *COUP-TFII-floxed* mice were crossed with *RxCre* mice, and *RxCre;COUP-TFII*$^{F/+}$ embryos were collected at E12.5. Next, the activities of RxCre recombinase were assessed by co-immunostaining assays with COUP-TFII and *lacZ* antibodies. As expected, the *lacZ* signals, which represent the RxCre recombinase activity, were readily detected at the subpallial areas in the ventral telencephalon, where COUP-TFII was expressed in both pallial and subpallial populations (supplementary material Fig. S2I-L). By contrast, far fewer lacZ signals were detected in the dorsal telencephalon (supplementary material Fig. S2E-H), consistent

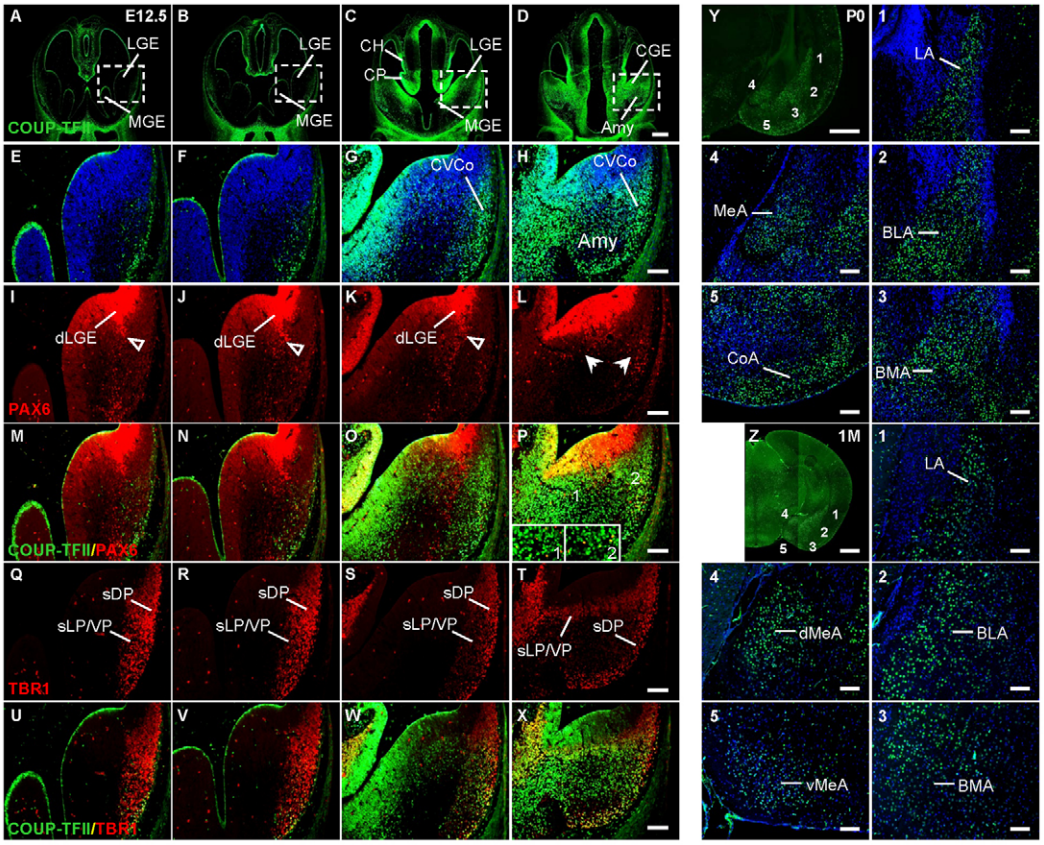

Fig. 1. COUP-TFII expression in the CGE and the developing mature amygdala neurons. (**A-H**) COUP-TFII expression at E12.5. Series of the coronal sections along the rostrocaudal axis are shown in A-D. (E-H) Higher magnification of the boxes in A-D. (**I-L**) Pax6 expression at E12.5. Open arrowheads in I-K indicate that the Pax6$^+$ cells originated from the dLGE. Arrowheads in L indicate two Pax6$^+$ migration streams derived from the CGE. (**M-P**) Double immunostaining of COUP-TFII and Pax6. Insets show the higher magnification images at regions 1 and 2 in P. (**Q-T**) Tbr1 expression at E12.5. (**U-X**) Double immunostaining of COUP-TFII and Tbr1. sLP/VP at the caudal telencephalon probably corresponds to the primordia of the pallial amygdala, including the LA and BLA nuclei. (**Y1-5**) COUP-TFII expression in the amygdala complex at P0. (**Z1-5**) COUP-TFII expression in the amygdala complex at 1 month. Amy, amygdala anlage; CGE, caudal ganglionic eminence; CH, Cortical hen; CP, choroids plexus; CVCo, mantle zone of caudoventral cortex; dLGE, dorsal lateral ganglionic eminence; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; sDP, dorsal pallium originated Tbr1+ stream; sLP/VP, lateral pallium/ventral pallium originated Tbr1+ stream. Scale bars: 200 μm in A-D; 100 μm in E-X, Y1-5 and Z1-5; 500 μm in the low-magnification image in Y; 1 mm in the montage image at 1 month (1M) in Z.

with the fact that RxCre recombinase mainly targets the *COUP-TFII* gene in the ventral forebrain. The genotype of *COUP-TFII* conditional mutant mouse is *RxCre*;*COUP-TF*$^{F/F}$ (*RxCre;F/F*); hereinafter, referred to as '*COUP-TFII* mutant' or 'mutant' mouse. In the study, no difference was observed in the *COUP-TFII*$^{F/+}$, *COUP-TFII*$^{F/F}$ or *RxCre;COUP-TFII*$^{F/+}$ (*RxCre;F/+*) littermates, suggesting that each of them is suitable for use as a control. The majority of the mutants died between P21 and P25 with the body weight only half of the control, and some escapees could survive to the adulthood (data not shown).

Development of cortical interneurons originated from the CGE is not altered in *COUP-TFII* mutant mice

The finding that CGE progenitor cells participate in the development of the interneuron (Nery et al., 2002) prompted us to assess whether COUP-TFII is expressed in the mature cortical interneuron. An immunostaining assay revealed that COUP-TFII was expressed in some cells scattered in the cortex at one month (supplementary material Fig. S3A). Our previous study has shown that *lacZ* expression recapitulates COUP-TFII expression in the *COUP-TFII*$^{LacZ/+}$ knock-in heterozygous mice (Tang et al., 2010). Therefore, double immnunostainings were carried out on samples from *COUP-TFII*$^{LacZ/+}$ mouse with *lacZ* antibody and antibodies recognizing several interneuron markers. Some *lacZ*$^+$ cells in the cortex were co-labeled with GAD67, a general interneuron marker (Tamamaki et al., 2003), indicating the interneuron identity (supplementary material Fig. S3B). Although some *lacZ*$^+$ cells co-expressed either calretinin or vasointestinal peptide (VIP), two markers of CGE originated interneurons (supplementary material Fig. S3C,D), none of the *lacZ*$^+$ cells expressed parvalbumin and somatostatin (SST), two markers of MGE-derived interneurons (supplementary material Fig. S3E,F) (Xu et al., 2004; Butt et al., 2005). To investigate whether the specification and differentiation of cortical interneurons are altered or not, the expression of interneuron markers was assessed at P25. Immunohistochemical assays revealed that the expression profiles of both calretinin and VIP were comparable between the control and mutant (supplementary material Fig. S4A,B,E,F). Furthermore, similar to the control, some *lacZ*+ *COUP-TFII*-deficient cells remained positive for either calretinin or VIP in the mutant (supplementary material Fig. S4C,D,G,H). The expression profiles of parvalbumin and SST were also normal in the mutant cortex (data

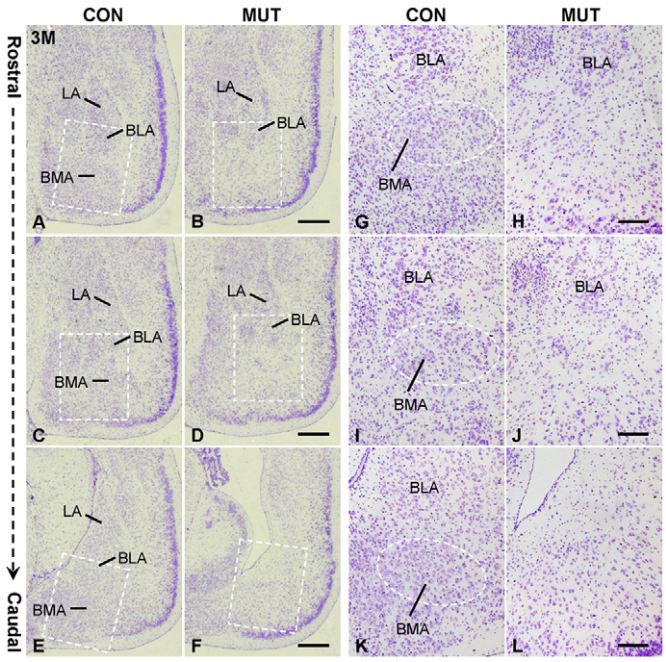

Fig. 2. The formation of the amygdala is compromised morphologically in the *COUP-TFII* mutant at 3 months of age postnatally. Nissl staining of brains sections from the control and mutant mice at 3 months of age. (**A-F**) Low-magnification images at amygdala complex from the control (A,C,E) and mutant mouse (B,D,F). (**G-L**) High-magnification images at the BMA area from the control (G,I,K) and mutant mouse (H,J,L). BLA, basolateral amygdala nucleus; BMA, basomedial amygdala nucleus; LA, lateral amygdala nucleus. Scale bars: 500 μm in A-F; 200 μm in G-L.

not shown). Thus, these findings provide evidence that the specification and basic aspects of the differentiation of the cortical interneuron is not altered in *COUP-TFII* mutant mice.

The formation of the amygdala is severely compromised in *COUP-TFII* mutant mice

Next, we analyzed the amygdala morphogenesis of *RxCre;COUP-TFII*$^{F/F}$ mutant at 3 months of age postnatally using Nissl staining. The mutant had hypoplasia, or complete loss of the MeA, amygdalo-hippocampal area and the basolateral complex (LA, BLA and BMA nuclei) (Fig. 2A-F; data not shown). Here, we concentrated on the basolateral complex. In the mutant, although LA and BLA nuclei were detected rostrally (Fig. 2B), they were barely detected at the caudal area (Fig. 2D,F). The most severe defect was observed in the BMA nucleus. From the rostral to the caudal, the cell cluster of the BMA nucleus was not clearly formed in the mutant (Fig. 2B,D,F). High magnification images confirmed that, in contrast to the control, there were much fewer cells at the mutant BMA locus (Fig. 2G-L). In order to investigate which cell type is affected in the mutant, the expression of Mef2C was used to identify BMA neurons (supplementary material Fig. S5A-D) (Waclaw et al., 2010). The majority of neurons were labeled by Glu2R, a marker of excitatory projection neuron, in the control BMA (supplementary material Fig. S5E,G), and only a few of them were positive for GAD67, a marker of inhibitory interneuron (supplementary material Fig. S5I,K). Consistent with the low number of Mef2C+ neurons observed in the mutant BMA, the number of Glu2R+ neurons was also decreased dramatically (supplementary material Fig. S5F,H), whereas the expression of GAD67 was comparable between the control and mutant (supplementary material Fig. S5I-L). Thus, all the data above suggest that the development of excitatory projection neuron is compromised in the mutant BMA.

Next, we studied the development of the basolateral complex in the early postnatal mutant using molecular markers. At P1, the expression of COUP-TFII was greatly reduced in the mutant basolateral complex (Fig. 3A′-D′). We used Mef2C as a marker for the LA and posterior BMA nuclei (Fig. 3E-H) (Waclaw et al., 2010), and Tbr1 as a marker for the basolateral complex (Fig. 3I-L) (Medina et al., 2004). Far fewer Mef2C$^+$ cells were detected in the prospected mutant BMA nucleus (Fig. 3G′,H′, insets). When compared with control (Fig. 3I-L), Tbr1$^+$ cells could not form regular basolateral complex structures in the mutant (Fig. 3I′-L′). The expression of *lacZ* mirrored that of COUP-TFII in the LA, BLA and BMA nuclei in *RxCre;F/+* heterozygous control (Fig. 3M-P). In *RxCre;F/F* mutant, a lot of *lacZ*$^+$ cells remained in the hypoplasic mutant amygdala complex, and appeared in more medial and ventral areas (Fig. 3M′-P′), suggesting that *lacZ*$^+$ *COUP-TFII*-null neurons survive, but were defective at participating in proper morphogenesis of the amygdala complex. Taken together, these results indicated that *COUP-TFII* is required for the appropriate development of the amygdala, especially for the formation of the basolateral complex.

The CGE-originated Pax6$^+$ cells colonize the BMA nucleus

To investigate the cause of defects in the amygdala, the immunohistochemical assays were carried out on serial coronal sections at the medial-caudal levels of the telencephalon from both the control and mutant at E12.5 (Fig. 4A-H). Compared with the control, the expression of COUP-TFII in the mutant ventral telencephalon was greatly reduced. As the amygdala defects observed in *COUP-TFII* mutants phenocopied *Pax6*-null mouse (Tole et al., 2005), we closely assessed the Pax6 expression patterns in *COUP-TFII* mutants. At E12.5, the Pax6$^+$ migratory stream emanating from the mutant dLGE appeared normal (Fig. 4I-L), whereas the numbers of Pax6$^+$ cells arising from the mutant CGE were greatly decreased (Fig. 4O,P,O′,P′). The expression profiles of Tbr1 were almost identical between the control and mutant (Fig. 4Q-X).

Consistent with the findings at E12.5, the expression patterns of *Pax6* and *Tbx1* genes were comparable in the rostral areas between the control and mutant at E13.5 (Fig. 5A-D,I-L). At the anterior CGE level, many Pax6$^+$ cells, which form two migratory streams, were heading ventrally to the basal telencephalon, with the medial stream to the BMA locus (Fig. 5E, open arrowheads). The BMA nucleus was first observed at the caudoventral CGE at E13.5, and many Pax6$^+$ cells colonized there (Fig. 5G) (Puelles et al., 2000). The majority of Pax6$^+$ cells in the migratory streams and BMA nucleus were COUP-TFII positive (data not shown). By contrast, far fewer Pax6+ cells were detected in the mutant medial stream (Fig. 5F); instead, some Pax6+ cells were observed at the region between VZ and prospective BMA nucleus (Fig. 5H, arrow). At the anterior CGE in the control, the Tbr1$^+$ sLP/VP became discontinued at the site of the Pax6$^+$ migratory stream shown in Fig. 5C, indicated by the asterisk (Fig. 5M). By contrast, the sLP/VP still maintained a continuing domain in the mutant (Fig. 5N). In the control, the Tbr1+ cell layer that marks the most superficial part of the ventral amygdala anlage became thinner than that at E12.5 (Fig. 5M,O, arrowheads); Tbr1$^+$ cells were excluded from the BMA area (Fig. 5O). In the mutant, by contrast, the

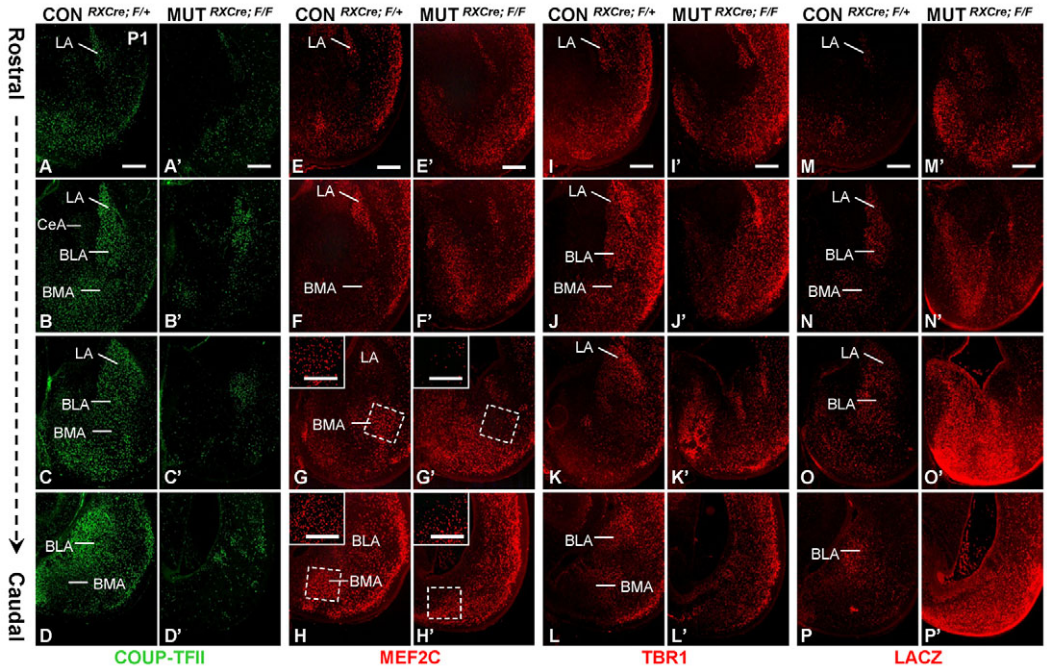

Fig. 3. The abnormal basolateral complex in the *COUP-TFII* mutant mouse at P1. Coronal sections at four planes along the rostrocaudal axis of the amygdala from the heterozygous control mouse (*RxCre;F/+*) and mutant mouse (*RxCre;F/F*) were used to perform immunohistochemical assays. (**A-D′**) COUP-TFII expression in the control (A-D) and mutant (A′-D′) at P1. (**E-H′**) Mef2C expression in the control (E-H) and mutant (E′-H′) at P1. (**I-L′**) Tbr1 expression in the control (I-L) and mutant (I′-L′) at P1. (**M-P′**) *lacZ* expression in the control (M-P) and mutant (M′-P′) at P1. BLA, basolateral amygdala nucleus; BMA, basomedial amygdala nucleus; CeA, center amygdala nucleus; LA, lateral amygdala nucleus. Scale bars: 200 μm.

superficial Tbr1$^+$ cell layer was much thicker (arrowheads, Fig. 5N,P); moreover, some Tbr1$^+$ cells were observed in the prospected mutant BMA locus (Fig. 5P, notched arrow).

At E14.5, the expression of Pax6 and Tbr1 continued appearing normal at the rostral mutant telencephalon (data not shown). At the anterior CGE, two Pax6$^+$ streams migrated ventrally in the control (supplementary material Fig. S6A). One lateral stream was originated from the CGE, and the other medial stream might be derived from the POA (Flames et al., 2007), indicated by the open arrowheads (supplementary material Fig. S6A). One Tbr1$^+$ nucleus (open arrow) was situated between two Pax6$^+$ migratory streams in the control (supplementary material Fig. S6E, open arrowheads). In the mutant, two similar Pax6$^+$ streams were observed; however, they failed to migrate into the amygdala, and rather appeared to remain in the CGE subventricular zone (supplementary material Fig. S6B, arrow). Adjacent to these Pax6$^+$ ectopic cells, the Tbr1$^+$ nucleus failed to be formed; however, an ectopic Tbr1$^+$ nucleus appeared more medially (supplementary material Fig. S6F). At the posterior CGE, Pax6$^+$ cells, which colonized the BMA nucleus in the control (supplementary material Fig. S6C), were barely

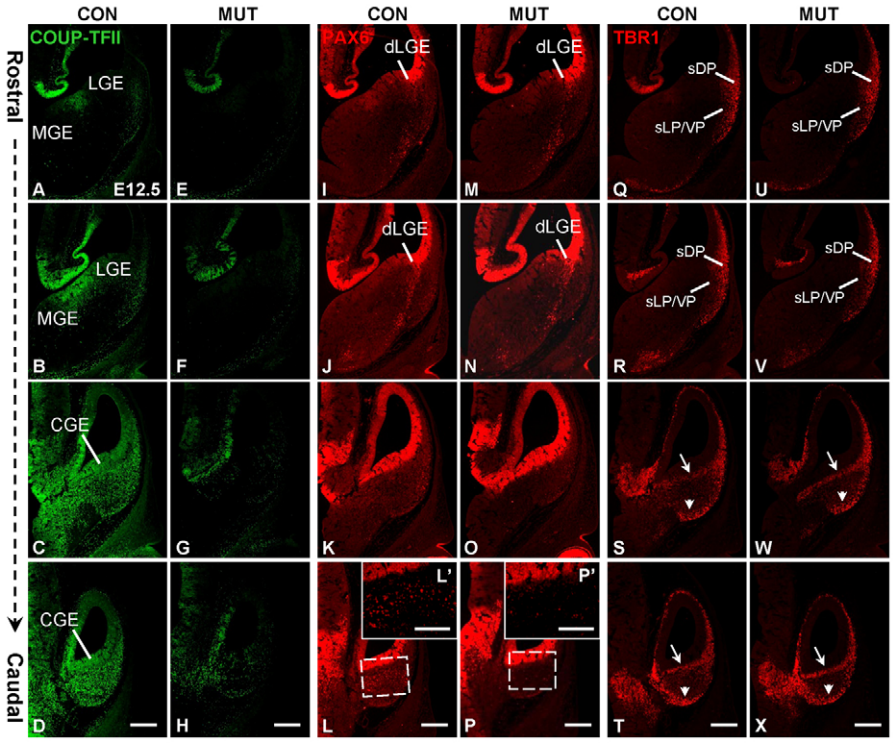

Fig. 4. The expression profiles of COUP-TFII, Pax6 and Tbr1 in the ventral telencephalon at E12.5. (**A-H**) COUP-TFII expression in the control (A-D) and mutant (E-H) at E12.5. (**I-P**) Pax6 expression in the control (I-L) and mutant (M-P) at E12.5. (L′,P′) Insets in L,P. (**Q-X**) Tbr1 expression in the control (Q-T) and mutant (U-X) at E12.5. Arrows and arrowheads indicate sLP/VP and sDP migrating streams in the CGE, respectively. CGE, caudal ganglionic eminence; dLGE, dorsal lateral ganglionic eminence; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; sDP, dorsal pallium originated Tbr1$^+$ stream; sLP/VP, lateral pallium/ventral pallium originated Tbr1$^+$ stream. Scale bars: 200 μm in A-X; 100 μm in L′,P′.

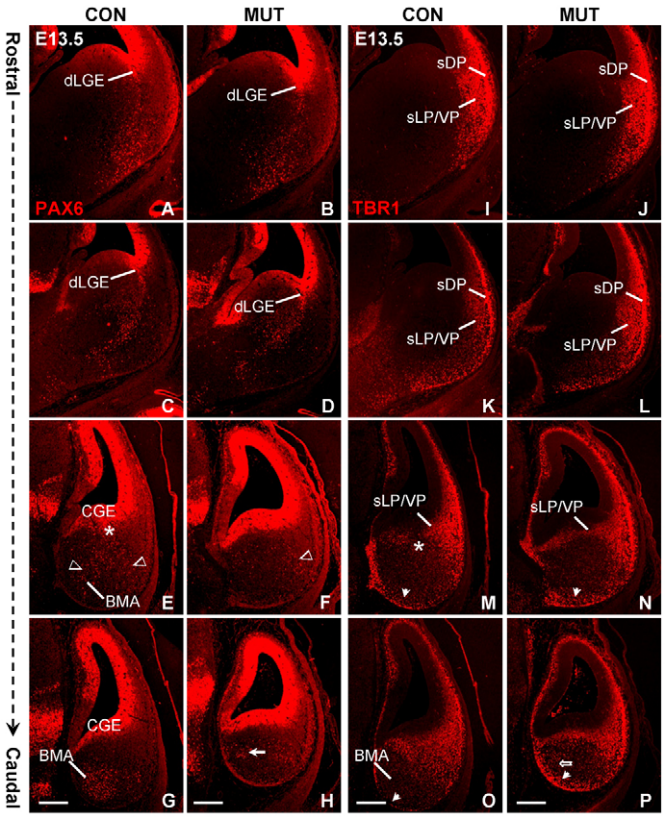

Fig. 5. The expression profiles of Pax6 and Tbr1 at the ventral telencephalon at E13.5. (**A-H**) Pax6 expression in the control (A,C,E,G) and mutant (B,D,F,H) at E13.5. Arrowheads indicate Pax6$^+$ migratory streams. Arrow indicates abnormally localized Pax6$^+$ cells in the mutant. (**I-P**) Tbr1 expression in the control (I,K,M,O) and mutant (J,L,N,P) at E13.5. Asterisks indicate Pax6$^+$ cell migration route. Arrowheads indicate multiple Tbr1$^+$ cell layers in the mutant ventral CGE. Arrow indicates ectopic Tbr1$^+$ cells in the mutant BMA locus. BMA, basomedial amgydala nucleus; CGE, caudal ganglionic eminence; dLGE, dorsal lateral ganglionic eminence; sDP, dorsal pallium originated Tbr1$^+$ stream; sLP/VP, lateral pallium/ventral pallium originated Tbr1$^+$ stream. Scale bars: 200 μm.

detected in the mutant (supplementary material Fig. S6D); meanwhile, the Tbr1$^+$ cells in the superficial zone of the ventral telencephalon continued forming a thicker layer, as at E13.5 (supplementary material Fig. S6F,H, arrowheads). Our data above reveal that the Pax6$^+$ cells derived from the CGE settle into the BMA nucleus, and the formation of the BMA nucleus is compromised in the *COUP-TFII* mutant, indicating that the CGE progenitor zone contributes cells to the BMA nucleus.

COUP-TFII mutants show no changes in apoptosis or proliferation in the CGE region

To assess whether changes in cell death and/or proliferation contributes to the phenotype, we assessed markers of apoptosis and mitosis at E12.5, E13.5 and E14.5. Immunohistochemical assays with an antibody specifically recognizing the cleaved caspase 3, an apoptotic marker, did not reveal any difference in the CGE and other subpallial regions at E12.5 (supplementary material Fig. S7A-H), E13.5 (supplementary material Fig. S7K-N) and E14.5 (supplementary material Fig. S7O,P). The positive signals detected in the somite and/or ventral spinal cord were used as the positive reference (supplementary material Fig. S7I,J,Q,R). Similarly, the cell proliferation, indicated by Ki67 and phospho-histone 3 expression, was also not altered in the mutant at E12.5, E13.5 and E14.5 (supplementary material Fig. S8; data not shown). Thus, neither apoptosis nor reduced proliferation is the reason for the defect.

Nrp1 and *Nrp2* are direct downstream targets of COUP-TFII

To investigate the molecular mechanism for the phenotypes in *COUP-TFII* mutants, total RNAs were prepared from the forebrains of the control and mutant embryos at E12.5, and the quantitative real-time PCR assays were carried out to profile the expression of genes important for the telencephalon patterning and cell migration (Fig. 6A). Among the genes examined, the expression of *Lhx2*, *Nrp1* and *Nrp2* genes were reduced significantly in the mutant group (Fig. 6A).

Lhx2, a LIM-homeodomain transcription factor, is a crucial regulator for the telencephalon patterning (Bulchand et al., 2001; Monuki et al., 2001). Lhx2 was expressed in a VP migratory stream at the rostral levels (supplementary material Fig. S9A,C) (Yun et al., 2001) and many cells at the center region of the ventral CGE in the control at E12.5 (supplementary material Fig. S9E,G,K). The expression of Lhx2 was reduced in the VP stream and ventral CGE in the mutant (supplementary material Fig. S9B,D,F,H,L). Interestingly, its expression was slightly enhanced in the CGE VZ (supplementary material Fig. S9F). Co-immunostaining with Lhx2 and Pax6 antibodies revealed that in the ventral CGE, there were more Lhx2$^+$ cells than Pax6$^+$ cells in the control at E12.5 (supplementary material Fig. S9G,I,K). One day later, many Lhx2$^+$ cells aggregated next to the Pax6$^+$ cells colonized the BMA in the control at E13.5 (supplementary material Fig. S9M,O,Q). Although there were far fewer Pax6$^+$ cells at the prospected mutant BMA (supplementary material Fig. S9P,R), the Lhx2$^+$ cells expanded medially (supplementary material Fig. S9N,R). No Lhx2/Pax6 co-labeled cells were detected at the ventral CGE in both the control and mutant at E12.5 (supplementary material Fig. S9K,L) and E13.5 (supplementary material Fig. S9Q,R), indicating that Lhx2 and Pax6 might be expressed by different populations, respectively. Therefore, the reduced *Lhx2* expression could not be the cause of the defect in Pax6$^+$ cells.

The expression of *Nrp1* and *Nrp2*, which encode two semaphorin receptors and regulate neuronal cell migration and axon guidance, was reduced 63% and 31%, respectively. Immunohistochemical assays confirmed that the expression of Nrp1 and Nrp2 was decreased in the mutant CGE at E12.5 and E13.5 (Fig. 6B,C). The expression of Nrp ligands Sema3a and Sema3f, was not altered in the mutant CGE at E13.5 (supplementary material Fig. S10A-F).

Next we examined whether Nrp genes are direct downstream targets of COUP-TFII. Chromatin immunoprecipitation analysis (ChIP) with chromatin prepared from the murine forebrain at E12.5 was carried out. It has been shown in our previous studies that COUP-TFII positively regulates target gene expression through Sp1 sites by tethering to Sp1 (Tsai and Tsai, 1997; Park et al., 2002; Pipaon et al., 1999). As three conserved Sp1 sites at the *Nrp2* promoter are used by COUP-TFII to activate the *Nrp2* expression in the lymphatic vessel (Lin et al., 2010), we asked whether the same elements of the *Nrp2* gene are targeted by COUP-TFII in embryonic forebrain. Indeed, using ChIP analysis, we found that both COUP-TFII and Sp1 were recruited to the conserved Sp1 sites in vivo (Fig. 6D). For the *Nrp1* gene, there is a Sp1 site in the 12th intron was highly conserved between mouse and human. We asked whether this site is used by COUP-TFII to regulate its expression. Therefore we

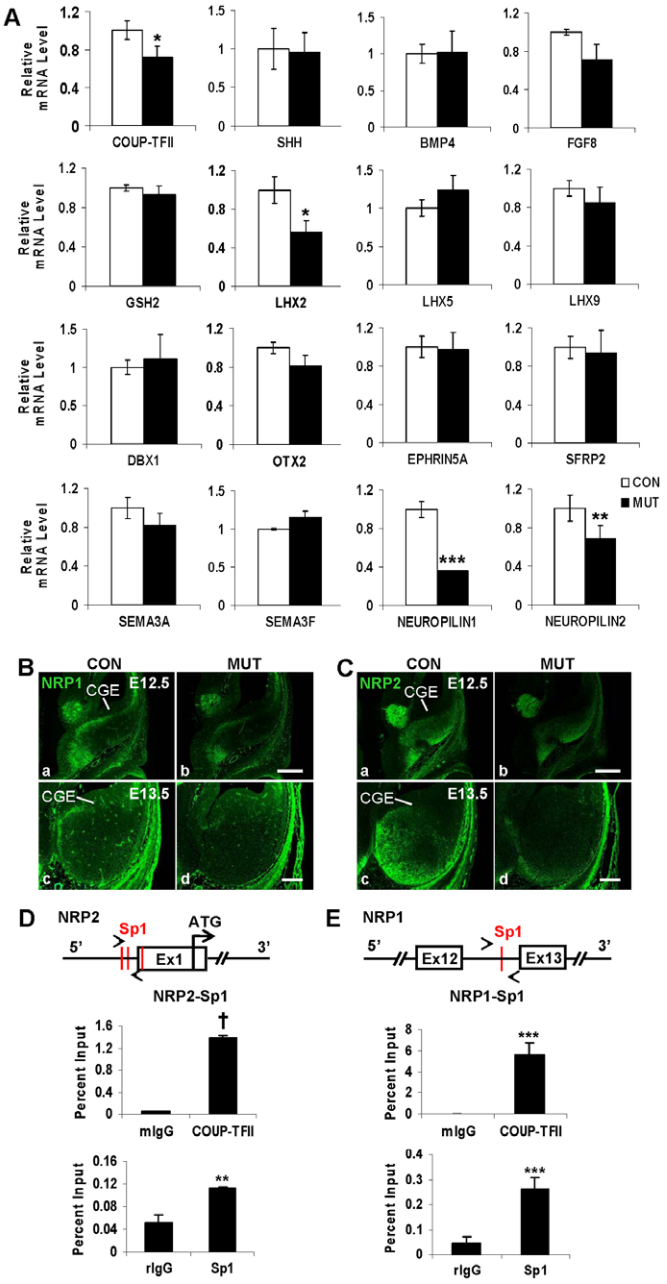

Fig. 6. *Nrp1* and *Nrp2* are direct targets of COUP-TFII in embryonic forebrain in vivo. (**A**) Quantitative real-time PCR assays were conducted to profile the expression of *COUP-TFII*, *Shh*, *Bmp4*, *Fgf8*, *Gsh2*, *Lhx2*, *Lhx5*, *Lhx9*, *Dbx1*, *Otx2*, *Epharin5a*, *Sfrp2*, *Sema3a*, *Sema3f*, *Nrp1* and *Nrp2* in the control (n=3) and mutant (*n*=4). Student's *t*-test, **P*<0.05, ****P*<0.005. (**Ba-d**) Immunohistochemical assays with Nrp1 antibody at E12.5 and E13.5. (**Ca-d**) Immunohistochemical assays with Nrp2 antibody at E12.5 and E13.5. CGE, caudal ganglionic eminence. (**D**,**E**) In vivo ChIP assays with chromatin prepared from the forebrains at E12.5. Student's *t*-test, ***P*<0.01, ****P*<0.005, †*P*<0.001. Data are mean±s.e.m. Scale bars: 300 μm in Ba,Bb,Ca,Cb; 200 μm in Bc,Bd,Cc,Cd.

generated a pair of primers to target the conserved Sp1 site of *Nrp1* for *ChIP* analysis. The real-time PCR assays revealed that both COUP-TFII and Sp1 were enriched at the site (Fig. 6E). Our data above demonstrated that COUP-TFII directly regulates the transcription of Nrp genes in the developing forebrain in vivo.

Nrp1 and Nrp2 are colocalized with the Pax6$^+$ cells in the CGE but not in the dLGE

The finding that the expression of the Nrp genes is clearly decreased in the *COUP-TFII* mutant raises an important question of why only the Pax6$^+$ cells emanating from the CGE are specifically affected in the mutant, but not Pax6$^+$ cells arising from the dLGE. To address this issue, we assessed the expression profiles of *Pax6* and Nrp genes by co-labeling Pax6/Nrp1 or Pax6/Nrp2 at E12.5.

In the subpallium of the rostral telencephalon, the expression of Nrp1 was detected in the mantle zone of the MGE and in a stream of cells that appeared to be tangentially migrating into the LGE (Fig. 7A-C). The expression profiles of Nrp2 were similar to Nrp1, with broader lateral expansion (Fig. 7A′-C′). In the caudal CGE, the expression of Nrp1 and Nrp2 was mainly complementary. Nrp1 was expressed in a thin strip of cells just below the CGE VZ with a medial high-lateral low gradient (Fig. 7D,E); and Nrp2 was expressed throughout the ventral CGE with a relatively higher level at the medial (Fig. 7D′-E′). The medial high-lateral low gradient of Nrp proteins in the CGE remained at E13.5 (Fig. 6Bc,Cc). The merged images revealed that neither Nrp1 nor Nrp2 protein was localized in Pax6$^+$ cells originating from the dLGE at the rostral telencephalon (Fig. 7K-M′). At the caudal telencephalon, many Pax6/Nrp1 co-labeling cells formed a narrow yellowish zone at the bottom of the CGE VZ, where there are pre-migratory cells exiting the cell cycle (Fig. 7N,O). At the ventral CGE, the majority of the Pax6$^+$ migratory cells were Nrp2 positive (Fig. 7N′-O′). All the observations above reveal that Nrp1 and Nrp2 are localized with only the Pax6+ pre-migratory cells or migratory cells in the CGE, but not in the dLGE, indicating that Nrp genes may specifically mediate the migration of the Pax6$^+$ cells originating from the CGE.

DISCUSSION

In our present study, we generated a ventral forebrain-specific *COUP-TFII* conditional knockout mouse to determine the contributions of the CGE to the telencephalon patterning. Our findings revealed that the CGE is the main resource of Pax6$^+$ cells in the BMA nucleus and *COUP-TFII* gene alone is a crucial regulator for the amygdala morphogenesis, but not for the development of cortical interneurons.

The CGE is genetically distinct

The subpallial progenitor zones could be delineated by the combinatorial expression of several key transcriptional factors, such as Dlx2, Gsh2, Nkx2.1, Nkx6.2 and Lhx2 (Flames et al., 2007). Our data and others revealed that COUP-TFII, a nuclear receptor, is preferentially expressed in the embryonic CGE (Fig. 1), which indicates that COUP-TFII could be used as a specific marker for the CGE. Our immunostaining and in situ results showed that the expression profiles of *Pax6*, *Tbr1* and *Lhx2* genes in the CGE are different from those at the rostral (Figs 1, 4, 5; supplementary material Figs S1, S9) (Puelles et al., 2000; Stoykova et al., 1996; Bulfone et al., 1995; Tole et al., 2005). Moreover, Nrp1 and Nrp2 generate complimentary expression patterns in the CGE (Fig. 7). The unique expression profiles of *COUP-TFII*, *Pax6*, *Tbr1*, *Lhx2*, *Nrp1* and *Nrp2* genes highly suggest that the CGE is a genetically distinct structure.

Pax6$^+$ cells colonizing the excitatory BMA nucleus are derived from the CGE

One of the main proposed functions of the CGE is involved in the amygdala formation (Nery et al., 2002; Chédotal and Rijli, 2009). It has been demonstrated that neurons in the amygdala are diverse

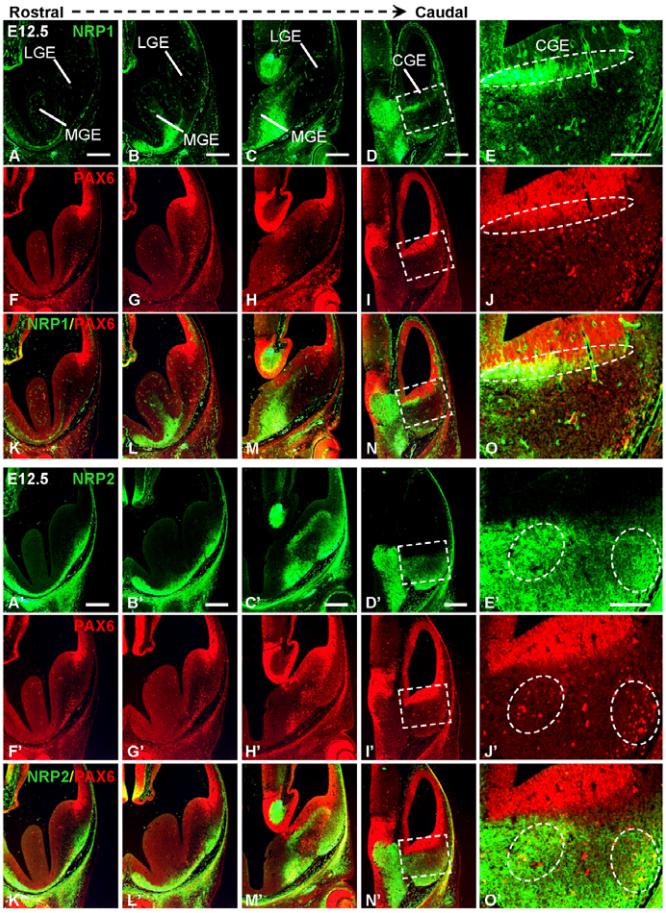

Fig. 7. Nrp1 and Nrp2 are localized in the Pax6$^+$ pre-migratory cells or migratory cells in the CGE, but not in the Pax6$^+$ migratory cells derived from the dLGE. (**A-D**) Nrp1 expression in the ventral telencephalon at E12.5. (**E**) Inset in D. (**F-I**,**F-I′**) Pax6 expression at E12.5. (**J**) Inset in I; (**J′**) inset in I′. (**K-N**) Pax6 and Nrp1 co-immunostaining. (**O**) Inset in N. (**A′-D′**) Nrp2 expression in the ventral telencephalon at E12.5. (**E′**) Inset in D′. (**K′-N′**) Pax6 and Nrp2 co-immunostaining. (**O′**) Inset in N′. CGE, caudal ganglionic eminence; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence. Scale bars: 200 μm in A-D,A′-D′,F-I,F′-I′,K-N,K′-N′; 100 μm in E,E′,J,J′,O,O′.

and contributed by both the pallium and subpallium (Swanson and Petrovich, 1998). For example, neurons in the inhibitory CeA nucleus have a subpallial origin (Waclaw et al., 2010; Wang et al., 2011). The projection neurons in the excitatory LA and BLA nuclei are derived from the VP and LP (Stenman et al., 2003; Puelles et al., 2000; Hirata et al., 2009). Neurons in the central and medial extended amygdala are originated from multiple telencephalic or extra-telencephalic progenitor zones (Bupesh et al., 2011a; Bupesh et al., 2011b). Cre-mediated fate-mapping analysis reveals the complexity of the origins for the LA, BLA and BMA nuclei (Waclaw et al., 2010). So far, how the BMA nucleus is generated remains poorly understood.

In the controls, many CGE-derived Pax6$^+$ cells migrate ventrally to the basal telencephalon at E12.5 and E13.5 (Figs 4, 5); and many Pax6$^+$ cells are settling in the BMA at E13.5 and E14.5 (Fig. 5; supplementary material Fig. S6). It indicates that the CGE VZ is a potential progenitor zone of Pax6$^+$ cells in the BMA nucleus. Indeed, while CGE-derived Pax6$^+$ cells fail to distribute in the basal telencephalon, the agenesis of the BMA nucleus is observed in the mutants (Figs 2, 3, 5; supplementary material Fig. S6). It has been proposed that the migratory cells emerging from the pallial-subpallial boundary are the sources for basal telecephalon nuclei, such as the amygdala and piriform cortex (Stoykova et al., 2000; Toresson et al., 2000; Yun et al., 2001; Carney et al., 2006). Our data further demonstrate that the CGE, a subpallial progenitor zone, contributes cells to the BMA nucleus in the amygdala and COUP-TFII is essential for this process.

The number of Glu2R$^+$ excitatory neurons is drastically reduced in the mutant BMA, whereas GAD67$^+$ interneurons remain normal (supplementary material Fig. S5), suggesting that CGE-derived Pax6$^+$ cells probably contribute mainly to the excitatory principle neurons of the BMA nucleus. In addition, it has been demonstrated that the CGE is the resource of cells in the inhibitory Ce nucleus and excitatory BMA nucleus (Nery et al., 2002). Furthermore, the Emx1$^+$ pallial populations could give rise to both excitatory and inhibitory neurons (Cocas et al., 2009). We propose that CGE might also generate both types of neurons, depending on the timing of neurogenesis and differential combinatorial gene expression in the cells.

Neuropilin genes may specifically regulate the migration of Pax6$^+$ cells originated from the CGE

The numbers of Pax6$^+$ cells decreased dramatically in the mutant ventral telencephalon (Figs 4, 5; supplementary material Figs S6, S9). Here, there are several possible mechanisms leading to the defect. First, it might be caused by either abnormal apoptosis or reduced proliferation. Our immunostaining results with antibodies that recognize cleaved caspase 3 and Ki67 or phospho-histone 3 reveal that neither apoptosis nor reduction in proliferation is the major reason (supplementary material Figs S7, S8; data not shown). Next, another possible cause is the respecification of the progenitor zone. The expression of *Tbr1* could be used as an indicator for the identity conversion between the LGE and VP (Stoykova et al., 2000; Yun et al., 2001). However, the expression patterns of *Tbr1* are identical between the control and *COUP-TFII* mutant at E12.5 (Fig. 4). Clearly, the re-specification is not the main reason for the defect. Finally, the defective cell migration could be a major contributor. Indeed, in contrast to the controls, Pax6$^+$ migratory cells were rarely detected at the ventral CGE at E12.5 (Fig. 4), and appeared to be trapped on the way to the basal telencephalon at E13.5 (Fig. 5), or mis-routed in the CGE at E14.5 in *COUP-TFII* mutants (supplementary material Fig. S6). All these findings highly suggest that the migration of Pax6$^+$ cells is compromised. Abnormal migratory phenotypes have been described in *Pax6*-null mutant (Caric et al., 1997; Chapouton et al., 1999); nevertheless, the molecular mechanisms that modulate the Pax6$^+$ cells migration are still largely unknown. Our immunohistochemical data reveal that Nrp1 and Nrp2 are specifically colocalized with Pax6 in the CGE, but not in the dLGE (Fig. 7). Consistently, although the expression of Nrp genes is greatly reduced in the mutants, it is the Pax6$^+$ cells originated from the CGE that are severely affected (Figs 4, 5; supplementary material Figs S6, S9). It has been reported that Nrp genes are required to mediate the migration of the GABAergic interneuron, cortical neuron, GnRH neuron and facial nerve neuron (Marin et al., 2001; Chen et al., 2008; Cariboni et al., 2007; Schwarz et al., 2004). In addition to the cell migration, Nrp genes also play crucial roles in axon guidance. The fornix, an axon bundle connecting the hippocampus and hypothalamus, is reduced and disorganized in the *Nrp2*-null mutant (Giger et al., 2000). In the adult *COUP-TFII*

mutants, the fornix was not detected at all (K.T. and M.-J.T., unpublished), most probably owing to the decreased expression of both *Nrp1* and *Nrp2* genes (Fig. 6; data now shown). Our in vivo ChIP assays demonstrated further that *Nrp1/2* genes are direct targets of COUP-TFII in the embryonic forebrain (Fig. 6). The results above highly suggest that COUP-TFII specifically regulate the migration of Pax6$^+$ cells in the CGE through modulating the expression of *Nrp1* and *Nrp2* genes. This conclusion is further supported by our previous findings that COUP-TFII regulates the expression of *Nrp1* and *Nrp2* during vein and lymphatic vessel development (You et al., 2005a; Lin et al., 2010). Thus, COUP-TFII-neuropilin signaling pathway seems a likely general mechanism used to mediate cell migration, axon guidance and fate specification during organogenesis.

The coordinated development between the subpallial and pallial population is essential for the morphogenesis of the amgydala

So far, how the cells emanating from the pallium and subpallium cooperate with each other to assemble the amygdala complex is still poorly understood. As early as E12.5, there are more Tbr1$^+$/Lhx2$^+$ pallial cells than Pax6$^+$ subpallial cells in the CGE (Figs 1, 4; supplementary material Fig. S9). The Pax6$^+$ subpallial population and the pallial population (Tbr1$^+$ cells and Lhx2$^+$ cells) generate complementary patterns during the development of the telencephalon (Figs 1, 4, 5; supplementary material Figs S6, S9) (Yun et al., 2001; Tole et al., 2005). In *COUP-TFII* mutants, the migration of Pax6$^+$ cells is compromised, and the formation of the Tbr1$^+$ nuclei is delayed or abolished (Fig. 5; supplementary material Fig. S6). Furthermore, while the subpallial Pax6$^+$ cells fail to settle into the mutant BMA nucleus, some pallial Tbr1$^+$/Lhx2$^+$ cells are emerging at the locus (Fig. 5; supplementary material Figs S6, S9). Our observations above reveal that there must be a yin-yang balance between the pallial and subpallial populations to coordinate the integration of the amygdala nuclei.

COUP-TFI and *COUP-TFII* genes may compensate for each other in the development of the cortical interneurons

Another important function of the CGE is related to the specification and differentiation of the cortical calretinin and VIP interneurons (Nery et al., 2002; Xu et al., 2004; Butt et al., 2005; Miyoshi et al., 2010). To our surprise, we did not observe any abnormalities in the cortical calretinin and VIP interneurons in *COUP-TFII* mutants (supplementary material Fig. S4). We asked whether the presence of *COUP-TFI* could compensate for the loss of *COUP-TFII* gene in the development of the cortical interneuron. The expression of COUP-TFI was barely detected in *lacZ*$^+$ cells in the control cortex (*RxCre;F/+*) at P25, whereas its expression was readily observed in almost every *lacZ*$^+$ *COUP-TFII*-deficient cell in the mutant (*RxCre;F/F*) (supplementary material Fig. S11). The similar phenomenon was also observed at P0 (data not shown). A recent study revealed that *COUP-TFI* is involved in the differentiation of the CGE-derived cortical interneuron (Lodato et al., 2011). All the findings above suggest that *COUP-TFI* and *COUP-TFII* may compensate for each other in the development of cortical interneuron.

In summary, our study presented direct genetic evidence that the CGE participates in the morphogenesis of the amygdala. The appropriate formation of the amygdala is the essential foundation for its crucial functions, such as the integration of external stimulating signals and coordination of behavioral outputs. Both neurodevelopmental disorders such as autism and autism spectrum disorders, and psychological disorders such as post-traumatic stress disorder are highly relevant to the amygdala dysfunction. Therefore, the findings in our present study benefit not only the understanding of telencephalon development, but also the understanding of the etiology of those disorders.

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Competing interests statement
The authors declare no competing financial interests.

Supplementary material
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References

Bulchand, S., Grove, E. A., Porter, F. D. and Tole, S. (2001). LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem. *Mech. Dev.* **100**, 165-175.
Bulfone, A., Smiga, S. M., Shimamura, K., Peterson, A., Puelles, L. and Rubenstein, J. L. (1995). T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* **15**, 63-78.
Bupesh, M., Legaz, I., Antonio Abellán, A. and Medina, L. (2011a). Multiple telencephalic and extratelencephalic embryonic domains contribute neurons to the medial extended amygdala. *J. Comp. Neurol.* **519**, 1505-1525.
Bupesh, M., Abellán, A. and Medina, L. (2011b). Genetic and experimental evidence supports the continuum of the central extended amygdala and a mutiple embryonic origin of its principal neurons. *J. Comp. Neurol.* **519**, 3507-3531.
Butt, S. J., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J. G. and Fishell, G. (2005). The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron* **48**, 591-604.
Cariboni, A., Hickok, J., Rakic, S., Andrews, W., Maggi, R., Tischkau, S. and Parnavelas, J. G. (2007). Neuropilins and their ligands are important in the migration of gonadotropin-releasing hormone neurons. *J. Neurosci.* **27**, 2387-2395.
Caric, D., Gooday, D., Hill, R. E., McConnell, S. K. and Price, D. J. (1997). Determination of the migratory capacity of embryonic cortical cells lacking the transcription factor Pax-6. *Development* **124**, 5087-5096.
Carney, R. S., Alfonso, T. B., Cohen, D., Dai, H., Nery, S., Stoica, B., Slotkin, J., Bregman, B. S., Fishell, G. and Corbin, J. G. (2006). Cell migration along the lateral cortical stream to the developing basal telencephalic limbic system. *J. Neurosci.* **26**, 11562-11574.
Chapouton, P., Gartner, A. and Gotz, M. (1999). The role of Pax6 in restricting cell migration between developing cortex and basal ganglia. *Development* **126**, 5569-5579.
Chédotal, A. and Rijli, F. M. (2009). Transcriptional regulation of tangential neuronal migration in the developing forebrain. *Curr. Opin. Neurobiol.* **19**, 139-145.
Chen, G., Sima, J., Jin, M., Wang, K. Y., Xue, X. J., Zheng, W., Ding, Y. Q. and Yuan, X. B. (2008). Semaphorin-3A guides radial migration of cortical neurons during development. *Nat. Neurosci.* **11**, 36-44.
Cocas, L. A., Miyoshi, G., Carney, R. S. E., Sousa, V. H., Hirata, T., Jones, K. R., Fishell, G., Huntsman, M. M. and Corbin, J. G. (2009). Emx1-lineage progenitors differentially contribute to neural diversity in the striatum and amygdala. *J. Neurosci.* **29**, 15933-15946.
Corbin, J. G., Gaiano, N., Machold, R. P., Langston, A. and Fishell, G. (2000). The Gsh2 homeodomain gene controls multiple aspects of telencephalic development. *Development* **127**, 5007-5020.
Flames, N., Pla, R., Gelman, D. M., Rubenstein, J. L. R., Puelles, L. and Marin, O. (2007). Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. *J. Neurosci.* **27**, 9682-9695.
García-Moreno, F., Pedraza, M., Di Giovannantonio, L. G., Di Salvio, M., López-Mascaraque, L., Simeone, A. and De Carlos, J. A. (2010). A neuronal migratory pathway crossing from diencephalon to telencephalon populates amygdala nuclei. *Nat. Neurosci.* **13**, 680-689.

Giger, R. J., Cloutier, J. F., Sahay, A., Prinjha, R. K., Levengood, D. V., Moore, S. E., Pickering, S., Simmons, D., Rastan, S., Walsh, F. S., Kolodkin, A. L., Ginty, D. D. and Geppert, M. (2000). Neuropilin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. *Neuron* **25**, 29-41.
Hébert, J. M. and Fishell, G. (2008). The genetics of early telencephalon patterning: some assembly required. *Nat. Rev. Neurosci.* **9**, 678-685.
Hirata, T., Li, P., Lanuza, G. M., Cocas, L. A., Huntsman, M. M. and Corbin, J. G. (2009). Identification of distinct telencephalic progenitor pools for neuronal diversity in the amygdala. *Nat. Neurosci.* **12**, 141-149.
Kanatani, S., Yozu, M., Tabata, H. and Nakajima, K. (2008). COUP-TFII is preferentially expressed in the caudal ganglionic eminence and is involved in the caudal migratory stream. *J. Neurosci.* **28**, 13582-13591.
Kim, B. J., Takamoto, N., Yan, J., Tsai, S. Y. and Tsai, M. J. (2009). Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) regulates growth and patterning of the postnatal mouse cerebellum. *Dev. Biol.* **326**, 378-391.
Kurihara, I., Lee, D. K., Petit, F. G., Jeong, J., Lee, K., Lydon, J. P., DeMayo, F. J., Tsai, M. J. and Tsai, S. Y. (2007). COUP-TFII mediates progesterone regulation of uterine implantation by controlling ER activity. *PLoS Genet.* **3**, 1053-1064.
LeDoux, J. (2007). The amygdala. *Curr. Biol.* **17**, R868-R874.
Li, L. P., Xie, X., Qin, J., Jeha, G. S., Saha, P. K., Yan, J., Haueter, C. M., Chan, L., Tsai, S. Y. and Tsai, M. J. (2009). The nuclear orphan receptor COUP-TFII plays an essential role in adipogenesis, glucose homeostasis, and energy metabolism. *Cell Metab.* **9**, 77-87.
Lin, F. J., Chen, X., Qin, J., Hong, Y. K., Tsai, M. J. and Tsai, S. Y. (2010). Direct transcriptional regulation of neuropilin-2 by COUP-TFII modulates multiple steps in murine lymphatic vessel development. *J. Clin. Invest.* **120**, 1694-1707.
Lodato, S., Tomassy, G. S., De Leonibus, E., Uzcategui, Y. G., Andolfi, G., Armentano, M., Touzot, A., Gaztelu, J. M., Arlotta, P., Menendez de la Prida, L. and Studer, M. (2011). Loss of COUP-TFI alters the balance between caudal ganglionic eminence- and medial ganglionic eminence-derived cortical interneurons and results in resistance to epilepsy. *J. Neurosci.* **31**, 4650-4662.
Long, J. E., Cobos, I., Greg, B., Potter, G. B. and Rubenstein, J. L. R. (2009). Dlx1&2 and Mash1 transcription factors control MGE and CGE patterning and differentiation through parallel and overlapping pathways. *Cereb. Cortex* **19**, i96-i106.
Marin, O. and Rubenstein, J. L. (2001). A long, remarkable journey: tangential migration in the telencephalon. *Nat. Rev. Neurosci.* **2**, 780-790.
Marin, O., Yaron, A., Bagri, A., Tessier-Lavigne, M. and Rubenstein, J. L. (2001). Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. *Science* **293**, 872-875.
Medina, L., Legaz, I., González, G., De Castro, F., Rubenstein, J. L. and Puelles, L. (2004). Expression of Dbx1, Neurogenin 2, Semaphorin 5A, Cadherin 8, and Emx1 distinguish ventral and lateral pallial histogenetic divisions in the developing mouse claustroamygdaloid complex. *J. Comp. Neurol.* **474**, 504-523.
Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V. H., Butt, S. J., Battiste, J., Johnson, J. E., Machold, R. P. and Fishell, G. (2010). Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. *J. Neurosci.* **30**, 1582-1594.
Monuki, E. S., Porter, F. D. and Walsh, C. A. (2001). Patterning of the dorsal telencephalon and cerebral cortex by a roof plate-Lhx2 pathway. *Neuron* **32**, 591-604.
Nery, S., Fishell, G. and Corbin, J. G. (2002). The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nat. Neurosci.* **5**, 1279-1287.
Park, J., Tsai, S. and Tsai, M. J. (2003). Molecular mechanism of chicken ovalbumin upstream promoter-transcription factor (COUP-TF) actions. *Keio J. Med.* **52**, 174-181.
Petit, F. G., Jamin, S. P., Kurihara, I., Behringer, R. R., DeMayo, F. J., Tsai, M. J. and Tsai, S. Y. (2007). Deletion of the orphan nuclear receptor COUP-TFII in uterus leads to placental deficiency. *Proc. Natl. Acad. Sci. USA* **104**, 6293-6298.
Pipaon, C., Tsai, S. Y. and Tsai, M. J. (1999). COUP-TF upregulates NGFI-A gene expression through an Sp1 binding site. *Mol. Cell. Biol.* **19**, 2734-2745.
Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., Keleher, J., Smiga, S. and Rubenstein, J. L. (2000). Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. *J. Comp. Neurol.* **424**, 409-438.
Remedios, R., Huilgol, D., Saha, B., Hari, P., Bhatnagar, L., Kowalczyk, T., Hevner, R. F., Suda, Y., Aizawa, S., Ohshima, T., Stoykova, A. and Tole, S. (2007). A stream of cells migrating from the caudal telencephalon reveals a link between the amygdala and neocortex. *Nat. Neurosci.* **10**, 1141-1150.
Schuurmans, C. and Guillemot, F. (2002). Molecular mechanisms underlying cell fate specification in the developing telencephalon. *Curr. Opin. Neurobiol.* **12**, 26-34.
Schwarz, Q., Gu, C., Fujisawa, H., Sabelko, K., Gertsenstein, M., Nagy, A., Taniguchi, M., Kolodkin, A. L., Ginty, D. D., Shima, D. T. and Ruhrberg, C. (2004). Vascular endothelial growth factor controls neuronal migration and cooperates with Sema3A to pattern distinct compartments of the facial nerve. *Genes Dev.* **18**, 2822-2834.
Stenman, J., Yu, R. T., Evans, R. M. and Campbell, K. (2003). Tlx and Pax6 co-operate genetically to establish the pallio-subpallial boundary in the embryonic mouse telencephalon. *Development* **130**, 1113-1122.
Stoykova, A., Fritsch, R., Walther, C. and Gruss, P. (1996). Forebrain patterning defects in Small eye mutant mice. *Development* **122**, 3453-3465.
Stoykova, A., Treichel, D., Hallonet, M. and Gruss, P. (2000). Pax6 modulates the dorsoventral patterning of the mammalian telencephalon. *J. Neurosci.* **20**, 8042-8050.
Sur, M. and Rubenstein, J. L. (2005). Patterning and plasticity of the cerebral cortex. *Science* **310**, 805-810.
Sussel, L., Marin, O., Kimura, S. and Rubenstein, J. L. (1999). Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359-3370.
Swanson, L. W. and Petrovich, G. D. (1998). What is the amygdala? *Trends Neurosci.* **21**, 323-331.
Swindell, E. C., Bailey, T. J., Loosli, F., Liu, C., Amaya-Manzanares, F., Mahon, K. A., Wittbrodt, J. and Jamrich, M. (2006). Rx-Cre, a tool for inactivation of gene expression in the developing retina. *Genesis* **44**, 361-363.
Takamoto, N., Kurihara, I., Lee, K., DeMayo, F. J., Tsai, M. J. and Tsai, S. Y. (2005a). Haploinsufficiency of chicken ovalbumin upstream promoter transcription factor II in female reproduction. *Mol. Endocrinol.* **19**, 2299-2308.
Takamoto, N., You, L. R., Moses, K., Chiang, C., Zimmer, W. E., Schwartz, R. J., DeMayo, F. J., Tsai, M. J. and Tsai, S. Y. (2005b). COUP-TFII is essential for radial and anteroposterior patterning of the stomach. *Development* **132**, 2179-2189.
Tamamaki, N., Yanagawa, Y., Tomioka, R., Miyazaki, J., Obata, K. and Kaneko, T. (2003). Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J. Comp. Neurol.* **467**, 60-79.
Tang, K., Xie, X., Park, J. I., Jamrich, M., Tsai, S. and Tsai, M. J. (2010). COUP-TFs regulate eye development by controlling factors essential for optic vesicle morphogenesis. *Development* **137**, 725-734.
Tole, S., Remedios, R., Saha, B. and Stoykova, A. (2005). Selective requirement of Pax6, but not Emx2, in the specification and development of several nuclei of the amygdaloid complex. *J. Neurosci.* **25**, 2753-2760.
Toresson, H., Potter, S. S. and Campbell, K. (2000). Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. *Development* **127**, 4361-4371.
Tsai, S. Y. and Tsai, M. J. (1997). Chick ovalbumin upstream promoter-transcription factors (COUP-TFs): coming of age. *Endocr. Rev.* **18**, 229-240.
Waclaw, R. R., Ehrman, L. A., Pierani, A. and Campbell, K. (2010). Developmental origin of the neuronal subtypes that comprise the amygdalar fear circuit in the mouse. *J. Neurosci.* **30**, 6944-6953.
Wang, B., Lufkin, T. and Rubenstein, J. L. R. (2011). Dlx6 regulates molecular properties of the striatum and central nucleus of the amygdala. *J. Comp. Neurol.* **519**, 2320-2334.
Willi-Monnerat, S., Migliavacc, E., Surdez, D., Delorenzi, M., Luthi-Carter, R. and Terskikha, A. V. (2008). Comprehensive spatiotemporal transcriptomic analyses of the ganglionic eminences demonstrate the uniqueness of its caudal subdivision. *Mol. Cell. Neurosci.* **37**, 845-856.
Wonders, C. P. and Anderson, S. A. (2006). The origin and specification of cortical interneurons. *Nat. Rev. Neurosci.* **7**, 687-696.
Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J. L. and Anderson, S. A. (2004). Origins of cortical interneuron subtypes. *J. Neurosci.* **24**, 2612-2622.
You, L. R., Lin, F. J., Lee, C. T., DeMayo, F. J., Tsai, M. J. and Tsai, S. Y. (2005a). Suppression of Notch signalling by the COUP-TFII transcription factor regulates vein identity. *Nature* **435**, 98-104.
You, L. R., Takamoto, N., Yu, C. T., Tanaka, T., Kodama, T., DeMayo, F. J., Tsai, S. Y. and Tsai, M. J. (2005b). Mouse lacking COUP-TFII as an animal model of Bochdalek-type congenital diaphragmatic hernia. *Proc. Natl. Acad. Sci. USA* **45**, 16351-16356.
Yun, K., Potter, S. and Rubenstein, J. L. (2001). Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development* **128**, 193-205.