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Misexpression of *Gbx2* throughout the mesencephalon by a conditional gain-of-function transgene leads to deletion of the midbrain and cerebellum in mice

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Summary

The mouse homeobox gene, *Gbx2*, is expressed in discrete domains in the neural tube and plays a key role in forebrain and hindbrain development. Previous studies have demonstrated that mutual inhibition between *Gbx2* and *Otx2*, which are respectively expressed in the anterior and posterior parts of the neural plate, positions the prospective midbrain-hindbrain junction. We describe here a conditional *Gbx2* gain-of-function transgenic mouse line, *Gbx2*-GOF, which expresses *Gbx2* and red fluorescence protein, mCherry, upon Cre-mediated recombination. In the absence of Cre, β -galactosidase is broadly expressed in mouse embryos and adult brains carrying the transgene. By combining *Gbx2*-GOF and *En1*^{Cre} knock-in allele, we activated expression of *Gbx2* and mCherry throughout the mesencephalon (mes) and rhombomere 1 (r1). The ectopic expression of *Gbx2* causes an anterior shift of the mes/r1 junction at embryonic day 10.5. Interestingly, we found that persistent expression of *Gbx2* throughout the mes/r1 region largely abolishes expression of the isthmus organizer gene *Fgf8*, leading to deletion of the midbrain and cerebellum at later stages. Our data suggest that the juxtaposition of the expression domains of *Gbx2* and *Otx2* within the mes/r1 area is essential for the maintenance of *Fgf8* expression. Furthermore, the *Gbx2*-GOF transgenic line is suitable for functional study of *Gbx2* during development.

Keywords

Gbx2; *Fgf8*; transcription factor; Cre; conditional gain of function

The mouse homeobox gene, *Gbx2* (gastrulation and brain homeobox gene) displays a complex and dynamic expression profile in the posterior mesoderm, limb buds and the neural tube during development (Bouillet et al., 1995; Bulfone et al., 1993; Chen et al., 2009). *Gbx2* is initially expressed in the posterior part of the mouse embryo during gastrulation (Bouillet et al., 1995; Wassarman et al., 1997). Genetic studies have demonstrated that mutual inhibition between *Gbx2* and *Otx2*, which is expressed in the anterior part of the embryo, positions a signaling center, known as the isthmus organizer, at the prospective mid-hindbrain junction (Joyner et al., 2000). Mutation of *Gbx2* leads to a posterior expansion of the expression domain of *Otx2* and deletion of the entire cerebellum (Li and Joyner, 2001; Martinez-Barbera et al., 2001; Millet et al., 1999; Wassarman et al., 1997). Conversely, ectopic expression of *Gbx2* in the mesencephalon (mes) in chick embryos by electroporation, or in mouse embryos by a transgene driven by regulatory elements of *Wnt1*, which is expressed in the mes, results in an anterior shift of the isthmus (Katahira et al., 2000; Millet et al., 1999). At later stages, however, the formation of the

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midbrain and cerebellum are relatively normal in the above *Gbx2* misexpression experiments (Katahira et al., 2000; Millet et al., 1999). By contrast, ectopic expression of *Otx2* in r1 leads to complete transformation of r1 into a midbrain fate (Broccoli et al., 1999; Katahira et al., 2000). The ectopic expression of *Gbx2* by electroporation or using a *Wnt1-Gbx2* transgene is transient (Katahira et al., 2000; Millet et al., 1999), which precludes an assessment of whether *Gbx2* is sufficient to respecify the mes. Therefore, it remains unclear whether persistent expression of *Gbx2* is required to respecify the mes into a cerebellar fate.

In order to further study *Gbx2* function and develop a tool useful for controlled spatiotemporal expression of *Gbx2*, we generated a transgenic mouse line, called *Gbx2-GOF*. The transgene is driven by the *CAGGS* promoter, which contains a chicken β -actin promoter with upstream cytomegalovirus enhancer (Niwa et al., 1991). In transgenic mice, transgene-expressing cells produce β -geo, a fusion protein with neomycin-resistance and β -galactosidase (β -gal) activity (Friedrich and Soriano, 1991). Cre-mediated recombination will remove the β -geo and render cells able to express a bicistronic mRNA producing both *Gbx2* and red fluorescent protein (RFP), mCherry (Shaner et al., 2004) (Fig. 1A). In mice carrying the *Gbx2-GOF* transgene, β -gal activity was initially detected at embryonic day 9.5 (E9.5) (data not shown). Broad and robust expression of β -gal was seen throughout the embryo at E10.5 and in the central nervous system (CNS) at E18.5 and adult stages (Fig. 1B, C and 4D). Therefore, the *Gbx2-GOF* transgene is widely expressed in the CNS of embryos and adult mice. The transgenic mice are viable and display no detectable abnormality.

To assess the function of the recombined *Gbx2-GOF* transgene, we crossed mice carrying *Gbx2-GOF* and *En1^{Cre}*, an *En1* knock-in (KI) allele containing Cre (Kimmel et al., 2000). Using an EGFP reporter line, ZEG (Novak et al., 2000), to monitor the Cre activity from the *En1^{Cre}*, we showed that the entire mes/r1 region is marked by EGFP (Fig. 1D). In *En1^{Cre/+}; Gbx2-GOF* embryos at E10.5, RFP was detected in the mes/r1 area (Fig. 1E), consistent with previous reports (Li et al., 2002; Zervas et al., 2004). However, the RFP+ domain in *En1^{Cre/+}; Gbx2-GOF* embryos was significantly smaller than the GFP+ domain in *En1^{Cre/+}; ZEG* embryos, suggesting a reduction in the size of the mes/r1 region due to *Gbx2* misexpression (Fig. 1D–E). Compared with that in control embryos, the isthmus constriction, which demarcates the prospective midbrain-hindbrain junction, was less prominent and apparently shifted rostrally in *En1^{Cre/+}; Gbx2-GOF* embryos (Fig. 1D and E). These results demonstrate that Cre-mediated recombination leads to specific expression of *Gbx2* and *RFP* in the mes/r1 region in *En1^{Cre/+}; Gbx2-GOF* embryos. The anterior displacement of the isthmus observed in *En1^{Cre/+}; Gbx2-GOF* embryos is in agreement with previous findings that ectopic expression of *Gbx2* in the mes leads to an anterior shift of the isthmus (Katahira et al., 2000; Millet et al., 1999).

Given the apparent translocation of the isthmus in *En1^{Cre/+}; Gbx2-GOF* embryos at E10.5, we expected that the misexpression of *Gbx2* would enlarge the cerebellum at the expense of the midbrain. Surprisingly, both the midbrain and cerebellum were found truncated in *En1^{Cre/+}; Gbx2-GOF* embryos at E18.5, although the extent of deletion varies among embryos (Fig. 2B and C). Based on the severity of the phenotype, the mutant embryos could be classified into two groups: group I (n=4/6) displayed almost complete deletion of the dorsal midbrain and the cerebellum (Fig. 2A–B and 2D–E); group II (2/6) exhibited deletion of the inferior colliculus, isthmus and the vermis of the cerebellum, while the superior colliculus and cerebellar hemispheres were largely unaffected (Fig. 2F and I). The variable severity of tissue loss may be caused by variations in the onset or levels of the expression of the *Gbx2-GOF* transgene on different genetic backgrounds. To examine the development of the ventral midbrain, we performed immunohistochemical analysis of tyrosine hydroxylase (TH), which labels dopaminergic neurons in the substantia nigra and ventral tegmental area (Fig. 2G). TH-positive neuron clusters were detected in the ventral midbrain in both classes

of mutants, suggesting that the ventral midbrain is less affected compared with the dorsal structures following misexpression of *Gbx2* (Fig. 2H–I).

We next investigated the molecular basis of the loss of the midbrain and cerebellum in *En1^{Cre/+}; Gbx2-GOF* embryos. As the phenotype of *En1^{Cre/+}; Gbx2-GOF* embryos resembles that found in embryos with loss or reduction of Fgf8 signaling (Basson et al., 2008; Chi et al., 2003; Trokovic et al., 2003), we examined if the misexpression of *Gbx2* disrupts *Fgf8* expression. At E10.5, *Fgf8* expression is localized in a transverse band corresponding to the isthmus (Fig. 3A). By contrast, in *En1^{Cre/+}; Gbx2-GOF* embryos at E10.5, *Fgf8* transcripts were only detected in a few clusters of cells in the mes/r1 area (Fig. 3B). Therefore, anterior expansion of *Gbx2* expression leads to a failure to maintain *Fgf8* expression in the isthmus. The disruption of *Fgf8* expression may contribute to the deletion of the midbrain and cerebellum in *En1^{Cre/+}; Gbx2-GOF* embryos.

Math1 encodes a basic helix-loop-helix transcription factor and plays an essential role in the development of the external granular layer of the cerebellum (Wang et al., 2005). At E10.5, *Math1* is expressed in the rhombic lip of r1 and the posterior hindbrain, whereas *Gbx2* is expressed in the ventricular zone of r1 outside of the *Math1*-positive domain (Fig. 3C and data not shown), suggesting that *Gbx2* may normally restrict the expression of *Math1* to the rhombic lip. We thus examined if ectopic expression of *Gbx2* in the entire r1 abolishes *Math1* expression and disrupt formation of the cerebellum in *En1^{Cre/+}; Gbx2-GOF* embryos. Interestingly, we found that the expression of *Math1* is maintained in the rhombic lip of r1 in *En1^{Cre/+}; Gbx2-GOF* embryos at E10.5 (Fig. 3D). Furthermore, *Math1* expression is significantly expanded anteriorly, indicating an anterior shift of the mes/r1 border in *En1^{Cre/+}; Gbx2-GOF* embryos at E10.5 (Fig. 3D). Together, our results demonstrate that misexpression of *Gbx2* throughout the mes/r1 leads to anterior shift of the isthmus, and *Gbx2* does not repress *Math1* expression.

To rule out the possibility that midbrain and cerebellum loss in *En1^{Cre/+}; Gbx2-GOF* mice results from nonspecific cell death due to permanent expression of *Gbx2*, we mated *Gbx2-GOF* transgenic mice with *Nkx2.1-Cre* BAC transgenic mice, in which Cre is broadly expressed in the ventral telencephalon (Xu et al., 2008). As described previously (Xu et al., 2008), Cre activity was detected in the medial ganglionic eminence (MGE), anterior endopeduncular/preoptic area and cells originating from these structures in *Nkx2.1-Cre* embryos at E13.5 (Fig. 4A). In *Nkx2.1-Cre; Gbx2-GOF* embryos at E13.5, ectopic expression of *Gbx2* was clearly seen in the ventricular zone of the MGE, in addition to the endogenous expression of *Gbx2* in the thalamus and MGE mantle zone (Fig. 4B and C). In *Nkx2.1-Cre; Gbx2-GOF* mice on postnatal day 25, there was a significant reduction in the percentage of X-gal positive cells in the globus pallidus and hypothalamus, to which descendants of the *Nkx2.1* lineage heavily contribute (Xu et al., 2008), thus confirming Cre-mediated recombination in these structures (Fig. 4D and E). Importantly, no tissue loss was detected in the forebrain of *Nkx2.1-Cre; Gbx2-GOF* mice, and these double transgenic mice were viable with no detectable gross defects. These data suggest that Cre-mediated activation of the *Gbx2-GOF* transgene does not lead to nonspecific cell death.

The phenotype of *En1^{Cre/+}; Gbx2-GOF* embryos is clearly different from the results of transient misexpression of *Gbx2* in the mes in chick or mouse embryos as described previously (Katahira et al., 2000; Millet et al., 1999). In those studies, particularly the one by Millet et al., transient misexpression of *Gbx2* resulted in aberrant early patterning of the mes/r1 region but no obvious morphological changes at E12.5 and in the adult. Downregulation of the *Wnt1-Gbx2* transgene likely led to a recovery of normal *Gbx2/Otx2* limits with concomitant recovery of the normal MHB. In contrast to the persistent expression of *Fgf8* in the translocated isthmus in embryos with transient *Gbx2*

misexpression, sustained ectopic expression of *Gbx2* in *En1^{Cre/+}*; *Gbx2-GOF* embryos largely abolishes *Fgf8* expression in the isthmus, which likely contribute to deletion of the midbrain and cerebellum. Our results suggest that the juxtaposition of the expression domains of *Gbx2* and *Otx2* within the mes/r1 area is essential for the maintenance of *Fgf8* expression and subsequent patterning and growth of the midbrain and cerebellum.

In summary, we here report generation of a novel conditional *Gbx2* gain-of-function transgenic mouse line. We demonstrate that the *Gbx2-GOF* transgene is transcribed broadly from E10.5 onwards, making it particularly suitable for studies of CNS development. The transgene expresses *Gbx2* in the CNS upon Cre-mediated recombination, and we show that Cre-induced expression of *Gbx2* in the entire mes/r1 region results in loss of the midbrain and cerebellum. Therefore, our genetic study clearly demonstrates that the *Gbx2-GOF* transgene is functional. Additionally, our study provides insight into the maintenance of the mid-hindbrain border by *Gbx2* and *Otx2* interaction. The *Gbx2-GOF* mouse line will be an invaluable tool for further analysis of *Gbx2* function in mouse development.

Materials and Methods

Mouse breeding and genotyping

Animals were housed in a facility with a 12-hour light/dark cycle (7am to 7pm) and allowed access to food and water *ad libitum*. All mutant mouse strains were maintained on a mixed genetic background. Noon of the day on which the vaginal plug was detected was designated as E0.5 in staging of embryos. Genotypes were determined by PCR analysis of embryonic yolk sac or tail DNA.

Generation of *Gbx2*-mCherry transgenic mice

To generate the *Gbx2*-GOF transgene, an *ires-mCherry* cassette was inserted into the *XhoI/NotI* sites of *pCALL2* plasmids (a gift from Drs. Corrine Lobe and Caiyin Guo), and a *Gbx2* full-length cDNA was inserted into the *BglII/XhoI* sites upstream of the *ires-mCherry*. Transgenic mice carrying the *Gbx2*-GOF were generated using standard protocol (Nagy, 2003). Four transgenic lines were analyzed for *lacZ* expression before Cre-mediated recombination, and one transgenic line that displayed broad expression of *lacZ* in the neural tube during embryogenesis was used in this study.

Histochemical and immunohistochemical analyses

Embryos were recovered in PBS and fixed immediately in 4% paraformaldehyde (PFA) at 4°C. Postnatal mice were deeply anesthetized and transcardially perfused with 4% PFA. Brains were postfixed in the same fixative overnight at 4°C. For frozen tissue sections, brains were cryoprotected in 30% sucrose in PBS before rapid freezing in OCT compound (TissueTek) and cryosectioned. Detailed protocols for RNA in situ hybridization in wholemount embryos, immunohistochemical assays, and β -gal activity analysis are described on the Li Lab website (<http://www.genetics.uhc.edu/lilab/Pages/Protocols.html>). The primary antibody was rabbit anti-TH (Pel-Freez Biologicals), and bound primary antibody was detected by Vectastain ABC kit (Vector Laboratory).

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References

- Basson AE, Ntsala M, Martinson N, Tlale E, Corrigan GE, Shao X, Gray G, McIntyre J, Puren A, Morris L. Development of phenotypic HIV-1 drug resistance after exposure to single-dose nevirapine. *J Acquir Immune Defic Syndr*. 2008; 49:538–543. [PubMed: 18989222]
- Bouillet P, Chazaud C, Oulad-Abdelghani M, Dolle P, Chambon P. Sequence and expression pattern of the *Stra7* (*Gbx-2*) homeobox-containing gene induced by retinoic acid in P19 embryonal carcinoma cells. *Dev Dyn*. 1995; 204:372–382. [PubMed: 8601031]
- Broccoli V, Boncinelli E, Wurst W. The caudal limit of *Otx2* expression positions the isthmus organizer. *Nature*. 1999; 401:164–168. [PubMed: 10490025]
- Bulfone A, Puelles L, Porteus MH, Frohman MA, Martin GR, Rubenstein JL. Spatially restricted expression of *Dlx-1*, *Dlx-2* (*Tes-1*), *Gbx-2*, and *Wnt-3* in the embryonic day 12.5 mouse forebrain defines potential transverse and longitudinal segmental boundaries. *J Neurosci*. 1993; 13:3155–3172. [PubMed: 7687285]
- Chen L, Guo Q, Li JY. Transcription factor *Gbx2* acts cell-nonautonomously to regulate the formation of lineage-restriction boundaries of the thalamus. *Development*. 2009; 136:1317–1326. [PubMed: 19279136]
- Chi CL, Martinez S, Wurst W, Martin GR. The isthmus organizer signal *FGF8* is required for cell survival in the prospective midbrain and cerebellum. *Development*. 2003; 130:2633–2644. [PubMed: 12736208]
- Friedrich G, Soriano P. Promoter traps in embryonic stem cells: a genetic screen to identify and mutate developmental genes in mice. *Genes Dev*. 1991; 5:1513–1523. [PubMed: 1653172]
- Joyner AL, Liu A, Millet S. *Otx2*, *Gbx2* and *Fgf8* interact to position and maintain a mid-hindbrain organizer. *Curr Opin Cell Biol*. 2000; 12:736–741. [PubMed: 11063941]
- Katahira T, Sato T, Sugiyama S, Okafuji T, Araki I, Funahashi J, Nakamura H. Interaction between *otx2* and *gbx2* defines the organizing center for the optic tectum [In Process Citation]. *Mech Dev*. 2000; 91:43–52. [PubMed: 10704829]
- Kimmel RA, Turnbull DH, Blanquet V, Wurst W, Loomis CA, Joyner AL. Two lineage boundaries coordinate vertebrate apical ectodermal ridge formation. *Genes Dev*. 2000; 14:1377–1389. [PubMed: 10837030]
- Li JY, Joyner AL. *Otx2* and *Gbx2* are required for refinement and not induction of mid-hindbrain gene expression. *Development*. 2001; 128:4979–4991. [PubMed: 11748135]
- Li JY, Lao Z, Joyner AL. Changing requirements for *Gbx2* in development of the cerebellum and maintenance of the mid/hindbrain organizer. *Neuron*. 2002; 36:31–43. [PubMed: 12367504]
- Martinez-Barbera JP, Signore M, Boyl PP, Puelles E, Acampora D, Gogoi R, Schubert F, Lumsden A, Simeone A. Regionalisation of anterior neuroectoderm and its competence in responding to forebrain and midbrain inducing activities depend on mutual antagonism between *OTX2* and *GBX2*. *Development*. 2001; 128:4789–4800. [PubMed: 11731459]
- Millet S, Campbell K, Epstein DJ, Losos K, Harris E, Joyner AL. A role for *Gbx2* in repression of *Otx2* and positioning the mid/hindbrain organizer. *Nature*. 1999; 401:161–164. [PubMed: 10490024]
- Nagy A, Gertsenstein M, Vintersten K, Behringer R. *Manipulating the Mouse Embryo*. 3. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 2003.
- Niwa H, Yamamura K, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene*. 1991; 108:193–199. [PubMed: 1660837]
- Novak A, Guo C, Yang W, Nagy A, Lobe CG. *Z/EG*, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis*. 2000; 28:147–155. [PubMed: 11105057]
- Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat Biotechnol*. 2004; 22:1567–1572. [PubMed: 15558047]
- Trokovic R, Trokovic N, Hernesniemi S, Pirvola U, Vogt Weisenhorn DM, Rossant J, McMahon AP, Wurst W, Partanen J. *FGFR1* is independently required in both developing mid- and hindbrain for sustained response to isthmus signals. *Embo J*. 2003; 22:1811–1823. [PubMed: 12682014]

- Wang VY, Rose MF, Zoghbi HY. Math1 expression redefines the rhombic lip derivatives and reveals novel lineages within the brainstem and cerebellum. *Neuron*. 2005; 48:31–43. [PubMed: 16202707]
- Wassarman KM, Lewandoski M, Campbell K, Joyner AL, Rubenstein JL, Martinez S, Martin GR. Specification of the anterior hindbrain and establishment of a normal mid/hindbrain organizer is dependent on Gbx2 gene function. *Development*. 1997; 124:2923–2934. [PubMed: 9247335]
- Xu Q, Tam M, Anderson SA. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. *J Comp Neurol*. 2008; 506:16–29. [PubMed: 17990269]
- Zervas M, Millet S, Ahn S, Joyner AL. Cell behaviors and genetic lineages of the mesencephalon and rhombomere 1. *Neuron*. 2004; 43:345–357. [PubMed: 15294143]

Figure 1. Generation and characterization of a conditional *Gbx2* gain-of-function transgenic mouse line

(A) Schematic representation of the *Gbx2-GOF* transgene. The CAGG promoter drives expression of the β -*geo* gene, which is followed by a triple polyadenylation sequence (3xpA). Cre-mediated recombination deletes β -*geo*, allowing expression of both *Gbx2* and red fluorescent protein, mCherry (RFP) from a bicistronic mRNA containing an internal ribosome entry site (ires). (B–C) Histochemical analysis of β -gal activity by X-gal in E10.5 embryos (B) or sagittal sections of E18.5 brains (C) hemizygous for *Gbx2-GOF*. (D) Analysis of Cre activity of *En1^{Cre}* by green fluorescent protein (GFP) using a Cre-reporter line, ZEG. Bracket marks the mesencephalon. (E) Analysis of RFP fluorescence following Cre-mediated recombination in *En1^{Cre/+}; Gbx2-GOF* embryos at E10.5 (right). No fluorescence is present in embryos carrying only the *Gbx2-GOF* transgene (left). The isthmus and the caudal limit of r1 are marked by red and white arrowheads, respectively. Note that the isthmic constriction is shifted rostrally in *En1^{Cre/+}; Gbx2-GOF* embryos, and the mesencephalon (bracket) is significantly reduced. The isthmic constriction is more evident in controls. Abbreviations: Ctx, cortex; Hip, hippocampus; Mes, mesencephalon; Ot, otic vesicle; r1, rhombomere 1; Str, striatum; Th, Thalamus.

Figure 2. Misexpression of *Gbx2* in the mes/r1 region results in deletion of the midbrain and cerebellum

(A–C) Dorsal views of E18.5 brains of the genotypes indicated. Insets in B and C show mCherry fluorescence in residual midbrain-hindbrain tissue. (D–F) Nissl staining of sagittal sections of E18.5 brains of the genotypes denoted. Inset in D shows X-gal labeling of a sagittal section of the brain of an E18.5 *En1^{Cre/+}; R26R^{+/-}* embryo. Note that in the *En1^{Cre/+}; R26R^{+/-}* line, the midbrain and the anterior hindbrain, including the cerebellum and pons, are marked by X-gal. Inset in E shows X-gal analysis of a section adjacent to E. Note that in the *En1^{Cre/+}; Gbx2-GOF* embryos, X-gal staining is ubiquitous except in the region of *Cre* activity. Thus, the mid-hindbrain, which is delineated with dashed lines, is negative for X-gal stain (blue) and is significantly reduced in size in *En1^{Cre/+}; Gbx2-GOF* embryos. (G–I) Tyrosine hydroxylase (TH) immunohistochemistry to mark dopaminergic neurons in the ventral tegmental area (VTA) on adjacent sections of D, E, and F, respectively. Sections in H and I are also stained with X-gal. Abbreviations: Cb, cerebellum; Ctx, cortex; fr, fornix; IC, inferior colliculus; SC, superior colliculus.

Figure 3. The expression of *Fgf8* in the isthmus is lost, and the roof plate of r1 is expanded due to misexpression of *Gbx2* throughout the mes/r1 region

(A–D) RNA in situ hybridization of *Fgf8* (A–B) and *Math1* (C–D) in controls and *En1^{Cre/+}; Gbx2-GOF* embryos at E10.5. The genotypes are as indicated at the top of the panels. Insets in C and D are dorsal views of the embryos in C and D, respectively. The isthmus constriction (arrow) is shifted anteriorly and the roof plate of r1 is abnormally enlarged in *En1^{Cre/+}; Gbx2-GOF* embryos. Red arrowhead indicates clusters of *Fgf8*-expressing cells.

Figure 4. Misexpression of *Gbx2* in the ventral telencephalon does not result in gross defects in neurons derived from the *Nkx2.1* lineage

(A) X-gal histochemistry of coronal sections of *Nkx2.1-Cre; R26R* embryos at E12.5. Note that *Nkx2.1*-expressing cells and their descendants contribute widely to the ventral telencephalon and the cortex (arrowheads). (B–C) In situ hybridization of *Gbx2* on coronal sections of control (B) and *Nkx2.1-Cre; Gbx2-GOF* double transgenic (C) embryos at E12.5. Dashed circle marks the endogenous *Gbx2* expression in MGE mantle zone; asterisk indicates ectopic expression of *Gbx2* in MGE ventricular zone. (D–I) X-gal histochemical analysis on coronal sections of adult *Gbx2-GOF* hemizygous mice with and without the *Nkx2.1-Cre* transgene as indicated. Boxed areas in D and E corresponding to the globus pallidus and hypothalamus are magnified in F–I.