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Developmental trajectories of GABAergic cortical interneurons are sequentially modulated by dynamic *FoxG1* expression levels

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GABAergic inhibitory interneurons, originating from the embryonic ventral forebrain territories, traverse a convoluted migratory path to reach the neocortex. These interneuron precursors undergo sequential phases of tangential and radial migration before settling into specific laminae during differentiation. Here, we show that the developmental trajectory of *FoxG1* expression is dynamically controlled in these interneuron precursors at critical junctures of migration. By utilizing mouse genetic strategies, we elucidate the pivotal role of precise changes in *FoxG1* expression levels during interneuron specification and migration. Our findings underscore the gene dosage–dependent function of *FoxG1*, aligning with clinical observations of *FOXG1* haploinsufficiency and duplication in syndromic forms of autism spectrum disorders. In conclusion, our results reveal the finely tuned developmental clock governing cortical interneuron development, driven by temporal dynamics and the dose-dependent actions of *FoxG1*.

cortex | development | inhibitory neuron | interneuron | gene-dosage

GABAergic interneurons within the neocortex play pivotal roles in local inhibition and have been shown to contribute to diverse cortical functions such as memory, cognition, and sociability through the regulation of rhythm generation in circuits. During development, cortical circuits are formed through the assembly of locally generated excitatory pyramidal cells and GABAergic inhibitory interneurons derived from the subpallium (1–3). Concurrent with the sequential formation of pyramidal cell layers in an inside-out manner (4, 5), migratory interneuron precursors disperse across all cortical areas and layers. During this process, interneuron precursors undergo distinct forms of tangential and radial migration following their specification in the distantly located embryonic ventral forebrain territories of the ganglionic eminences and preoptic area (6–13). Newly born interneuron precursors rapidly exit the ventral germinal zones and migrate through ventral structures such as the developing striatum to invade the neocortex (14, 15). Upon entering the cortical primordium, interneuron precursors avoid the developing cortical plate and migrate tangentially above and below this structure (16–21). At late embryonic to early postnatal stages, most interneuron precursors change their migration mode from tangential to radial in order to populate specific cortical lamina (22, 23). Thus, interneuron precursors must sequentially switch their migration mode to arrive at their correct areal and laminar location, but how these transitions are controlled during development is not well understood (24–33).

Interestingly, there are indications that the differentiation program of cortical interneurons is regulated under the tight control of an intrinsic developmental clock, mediated via sequential expression of transcription factors. For example, *Ascl1* and *Dlx1/2* genes are required for initial GABAergic specification (34–36), *Arx* and *Zeb2* facilitate postmitotic cell migration into the cortex (37–39), and later, *Lhx6*, *SatB1*, *Sox6,* and *Prox1* regulate interneuron migration within the cortex (40–46). Recently, we have shown that the dynamically regulated expression of a forkhead box-containing transcription factor *FoxG1* is essential for the proper formation of cortical lamina (47). Considering that *FoxG1* is expressed in both pyramidal neurons and interneurons, we hypothesized that *FoxG1* may play critical roles as part of an intrinsic developmental clock in coordinating the differentiation and migration of interneurons. The central role of *FoxG1* in cortical specification is well established: *FoxG1* is expressed at neural plate stages, and embryos null for *FoxG1* exhibit almost complete loss of telencephalic structures (48–54). Interestingly, *FOXG1* gene dosage itself has been found to impact human development and mental health. Either haploinsufficiency (55–58) or gene duplication (59, 60) of *FOXG1* both lead to syndromic forms of autism spectrum disorder/intellectual disability (61–63). Recently, we have demonstrated that the autism-related social behavioral impairments in human *FOXG1* haploinsufficiency and duplication cases could be recapitulated in transgenic *FoxG1* mouse models (64). Still, it is not yet known how *FoxG1* functions in a dose-dependent manner or whether

Significance

Interneurons within the neocortex inhibit circuit activity through the timely release of the neurotransmitter GABA (gamma-aminobutyric acid). In contrast to excitatory neurons, interneurons originate from the embryonic ventral forebrain and undergo an extensive migration to reach their ultimate destinations. Our findings reveal that dynamic expression of the transcription factor *FoxG1* serves as an intrinsic developmental clock in interneuron precursors. Mechanistically, *FoxG1* activity in interneuron precursors sequentially regulates GABAergic cell identity, entrance into the cortex, distribution across hippocampal and cortical territories, and allocation within the six-layered neocortex. Indeed, a stepwise decrease in gene dosage revealed the dose-dependent functions of *FoxG1*, underscoring the association of both haploinsufficiency and gene duplication forms of *FOXG1* with autism-linked neurodevelopmental disorders.

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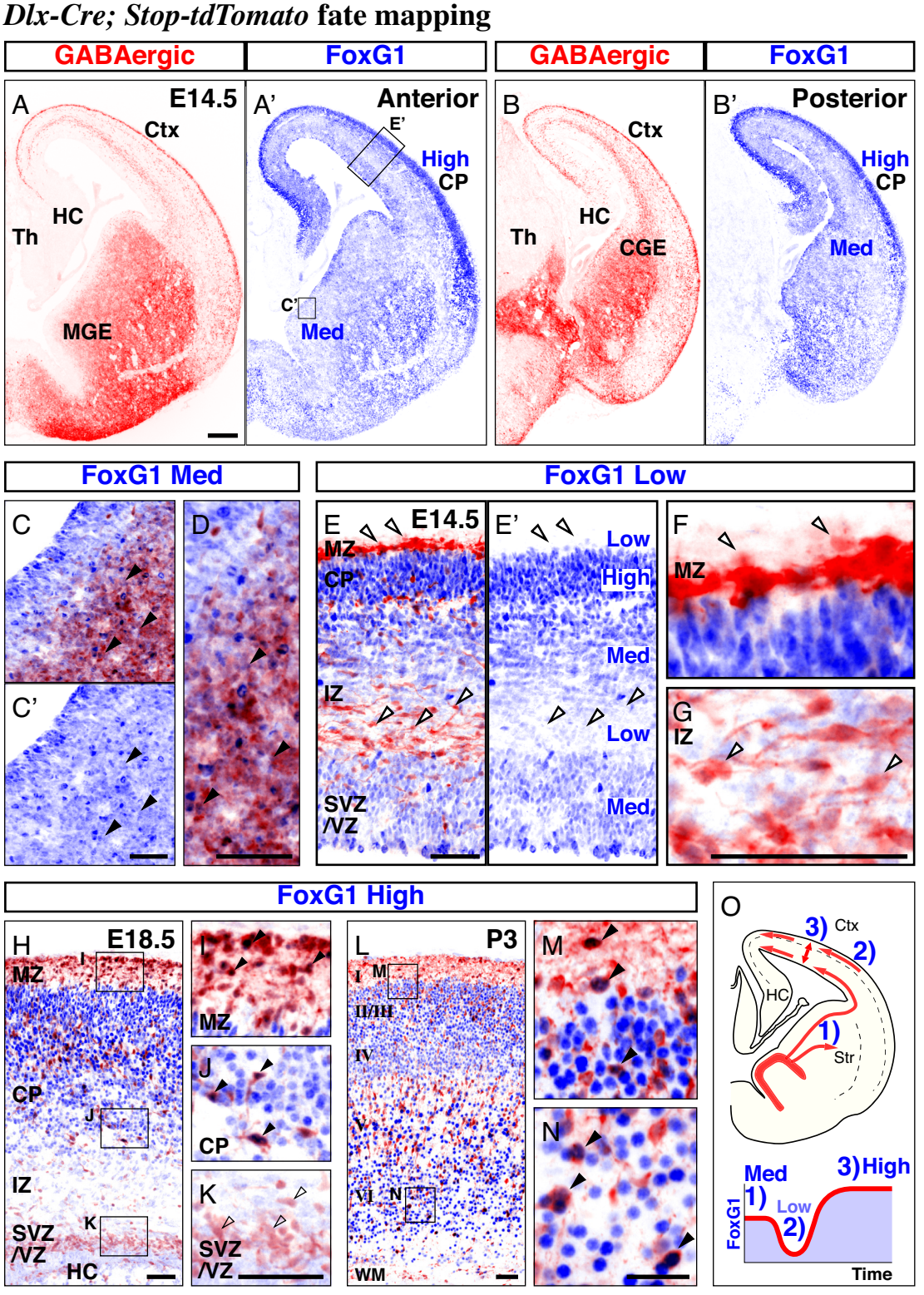

Fig. 1. FoxG1 expression is tightly correlated with the differentiation and migratory stages of GABAergic neuronal precursors. (*A*–*N*) GABAergic neuronal precursors are labeled in red using *Dlx5a-Cre*; *R26-stop-tdTomato* (*Ai9*) fate mapping, with FoxG1 immunohistochemistry shown in blue. (*A* and *B*) Anterior and posterior coronal sections of the mouse forebrain at E14.5. Medium levels of FoxG1 expression in postmitotic GABAergic neuron precursors (*A'*, Med) are evident (*C*, *C'*, and *D*, arrowheads). (*E* and *E'*) Higher magnification of the E14.5 cortical region outlined in *A'*. Within GABAergic neuronal precursors (red), FoxG1 expression (blue) is very low in the intermediate zone and is almost invisible in the marginal zone (*E* and *E'*, open arrowheads). However, FoxG1 is expressed at high levels in pyramidal neuron precursors in the cortical plate. Higher magnification of the marginal (*F*) and intermediate (*G*) zones in *E*. (*H*–*K*) At E18.5, while GABAergic cells in the marginal zone (*I*) and cortical plate (*J*) express FoxG1, the ones in the SVZ/VZ do not express at comparable levels (*K*, open arrowheads). (*L*–*N*) At P3, many GABAergic neuron precursors are undergoing radial migration and have reexpressed FoxG1. High magnification of the squared areas in *L* is shown in *M* and *N*. (*O*) A schematic drawing depicting the high, medium, and low phases of FoxG1 expression (blue) in developing cortical GABAergic interneurons (red) originating from the ventral forebrain. Each figure represents the analysis of three brains. MGE, LGE, and CGE: medial, lateral, and caudal ganglionic eminence, Ctx: cortex, HC: hippocampus, Th: thalamus, MZ: marginal zone, CP: cortical plate, IZ: intermediate zone, SVZ/VZ: subventricular/ventricular zone, WM: white matter. (Scale bars: *A* and *B* 200 μm and *C*–*N* 50 μm.)

dynamic regulation of *FoxG1* expression regulates differentiation in GABAergic lineages.

Here, we find that FoxG1 expression levels are dynamically regulated during the course of GABAergic interneuron development and that FoxG1 expression is selectively down-regulated in interneuron precursors during tangential migration. Utilizing in vivo genetic strategies to carry out *FoxG1* gain and loss-of-function (GOF and LOF), we first identified that *FoxG1* is an essential gene for GABAergic lineages and is required for their initial specification in a cell-autonomous manner. While *FoxG1* expression is required for postmitotic interneuron precursors to invade the cortical primordium, *FoxG1* must be down-regulated to facilitate tangential migration into dorsomedial regions of the developing cortex as well as the hippocampus. Subsequent to this phase of tangential migration, *FoxG1* expression needs to be reinitiated in order of cells to enter into the cortical plate and to occupy proper laminar locations. We further show that *FoxG1* functions in a gene dosage–dependent manner through generation of an allelic series of *FoxG1* mouse models. Our study provides evidence for how distinct phases of interneuron differentiation are controlled through dynamic changes in the expression of a single gene, *FoxG1*.

Results

FoxG1 Levels Are Dynamically Regulated in Developing Forebrain GABAergic Interneurons. Once specified, pyramidal neuron precursors must transiently down-regulate FoxG1 expression during the initial phases of their migration in order to reach the correct cortical lamina (47). Thus, we examined whether FoxG1 was similarly regulated in a dynamic manner in cortical GABAergic interneuron precursors during their developmental trajectory. Postmitotic GABAergic populations were fluorescently labeled in *Dlx-Cre*; *R26-stop-tdTomato* (*Ai9*) animals, and FoxG1 protein levels at various developmental time points were evaluated by immunohistochemistry (Fig. 1 *A*–*N*). The FoxG1 antibodies show minimal background outside the forebrain territories such as in the thalamus (Fig. 1*B*') as well as in null tissues (this study). At E14.5 (Fig. 1 *A*, *A*', *B*, and *B*'), FoxG1 expression is evident in postmitotic GABAergic neuronal precursors (Fig. 1 *C*, *C*', and *D*, high magnification of Fig. 1*A*', ventral) but is down-regulated during the tangential migration phase in the cortex (Fig. 1 *E*, *E*', *F*, and *G*, open arrowheads, high magnification of Fig. 1*A*' cortex). In particular, cells in the marginal zone exhibit almost no expression of FoxG1 compared to pyramidal cells within the cortical plate (Fig. 1 *E*, *E*' and *F*, MZ; Fig. 1*O* scheme, bottom: low). These data suggest that as interneuron precursors begin tangential migration, they rapidly down-regulate FoxG1 expression upon reaching the cortex. Following their tangential dispersion throughout the cortex, GABAergic interneuron precursors switch to a radial migration mode in order to reach their final laminar locations (22, 65). Interestingly, at E18.5, many GABAergic neuron precursors migrating inside the cortical plate reexpress FoxG1 at high levels, in a manner comparable to the neighboring pyramidal neurons (Fig. 1 *H* and *J*, arrowheads). In addition, GABAergic cells in the marginal zone (Fig. 1*I*) but not below the intermediate zone (Fig. 1*K*) were found to express FoxG1 at high levels. At postnatal day 3 (P3), when most GABAergic neuron precursors are commencing radial migration in the cortical plate, cells express FoxG1 at high levels (Fig. 1 *L*–*N*). These data indicate that FoxG1 expression is reinitiated upon the shift from tangential to radial migration (Fig. 1*O* scheme: high). Thus, we find that FoxG1 expression levels undergo dynamic changes that are tightly correlated with the migration phases of GABAergic interneurons (Fig. 1*O*, scheme, bottom).

***FoxG1* Is Cell Autonomously Required for the Establishment of GABAergic Neuronal Identity.** To examine how dynamic changes in FoxG1 expression regulate the formation of inhibitory circuits, we removed (LOF) or increased (GOF) *FoxG1* expression during progressive stages of GABAergic cell development and followed the fate of targeted cells. First, we examined the roles of *FoxG1* in the progenitor domains of interneurons. Complete loss of GABAergic neuron production in the *FoxG1*-null animals has been reported in the first *FoxG1* LOF mutant study (49). This could simply reflect the lack of ventral structures in *FoxG1* mutants through patterning defects. Alternatively, *FoxG1* may play roles in specifying GABAergic neuronal precursors. In order to test this, we performed mosaic *FoxG1* LOF experiments using *R26-CreER* to conditionally remove *FoxG1* expression by utilizing a floxed-*FoxG1* allele (47). It has been shown that the ligand tamoxifen-mediated CreER activation takes place for an approximately 1-d period (66). Administration of low doses of tamoxifen at E8.5 induced patches of recombination of the conditional *FoxG1* allele(s) in control (Fig. 2*A*) and mosaic *FoxG1* LOF embryos by E11.5 (Fig. 2*B*). In *FoxG1*-null cell patches located in the ventral forebrain (Fig. 2 *B* and *B*'), Nkx2-1 expression was lost, and conversely, Pax6 was ectopically expressed (Fig. 2*C*). This demonstrates that *FoxG1* is required in a cell-autonomous manner to establish Nkx2-1-positive neural progenitors within the Pax6+ neural tube (67). Interestingly, when tamoxifen administration was performed 1 d later at E9.5, removal of *FoxG1* did not affect Nkx2-1 expression in a similar manner and some *FoxG1*-null cell patches properly expressed Nkx2-1 (Fig. 2 *D* and *D*', arrowhead). This indicates that there is a narrow time window during early forebrain development where *FoxG1* expression is required for the acquisition of ventral telencephalic GABAergic identity, but once specified, *FoxG1* is not required to maintain Nkx2-1 expression. In order to directly test this possibility, we next removed *FoxG1* by using the *Nkx2-1BAC-Cre* driver, which expresses Cre only after the cells have initiated *Nkx2-1* expression (Fig. 2 *E*–*L*). The *Nkx2-1* BAC transgenic lines have been demonstrated to faithfully recapitulate endogenous *Nkx2-1* expression in the MGE (Medial Ganglionic Eminence) domain, with the exception of the most dorsal regions (12, 68). We found that *FoxG1*-null cells (Fig. 2*F*, open arrowhead) maintain Nkx2-1 expression (Fig. 2 *G* and *H*) and do not ectopically express Pax6 (Fig. 2 *I* and *J*), unlike the earlier removal (Fig. 2*C*). We further found that the ventrally expressed proneural gene Ascl1/Mash1 (34, 36) shows no changes in the ventricular zone of these territories (Fig. 2 *K* and *L*). Altogether, we conclude that while *FoxG1* is initially cell autonomously required for GABAergic cell specification in progenitors, once specified, *FoxG1* is not required to maintain GABAergic neuronal identity.

***FoxG1* Facilitates GABAergic Neuron Entrance into the Cortex and Striatum.** We next tested whether *FoxG1* plays any role in the migration of GABAergic neuron precursors once they have been specified. For this, we again utilized the *Nkx2-1-Cre* driver to remove *FoxG1* in GABAergic cell precursors (1st phase, Fig. 1*O*) and followed their fate with a *tdTomato* or an *EGFP* reporter (*RCE:loxP*). We observed an almost complete failure of *FoxG1*-null cells to migrate into the hippocampus and cortex (Fig. 3 *A* and *B*), even by birth (Fig. 3 *C* and *D*). Since the *Nkx2-1-Cre* driver spares the LGE-derived GABAergic populations that are necessary to support the migration of MGE-derived interneurons (14), these data strongly suggest that *FoxG1* plays direct roles in cortical migration of GABAergic precursors. Consistent with this, when we analyzed the expression of *Lhx6*, a gene downstream of *Nkx2-1* and maintained in MGE-derived cortical interneuron lineages (Fig. 3*E*), it was restricted in the ventral domains in the

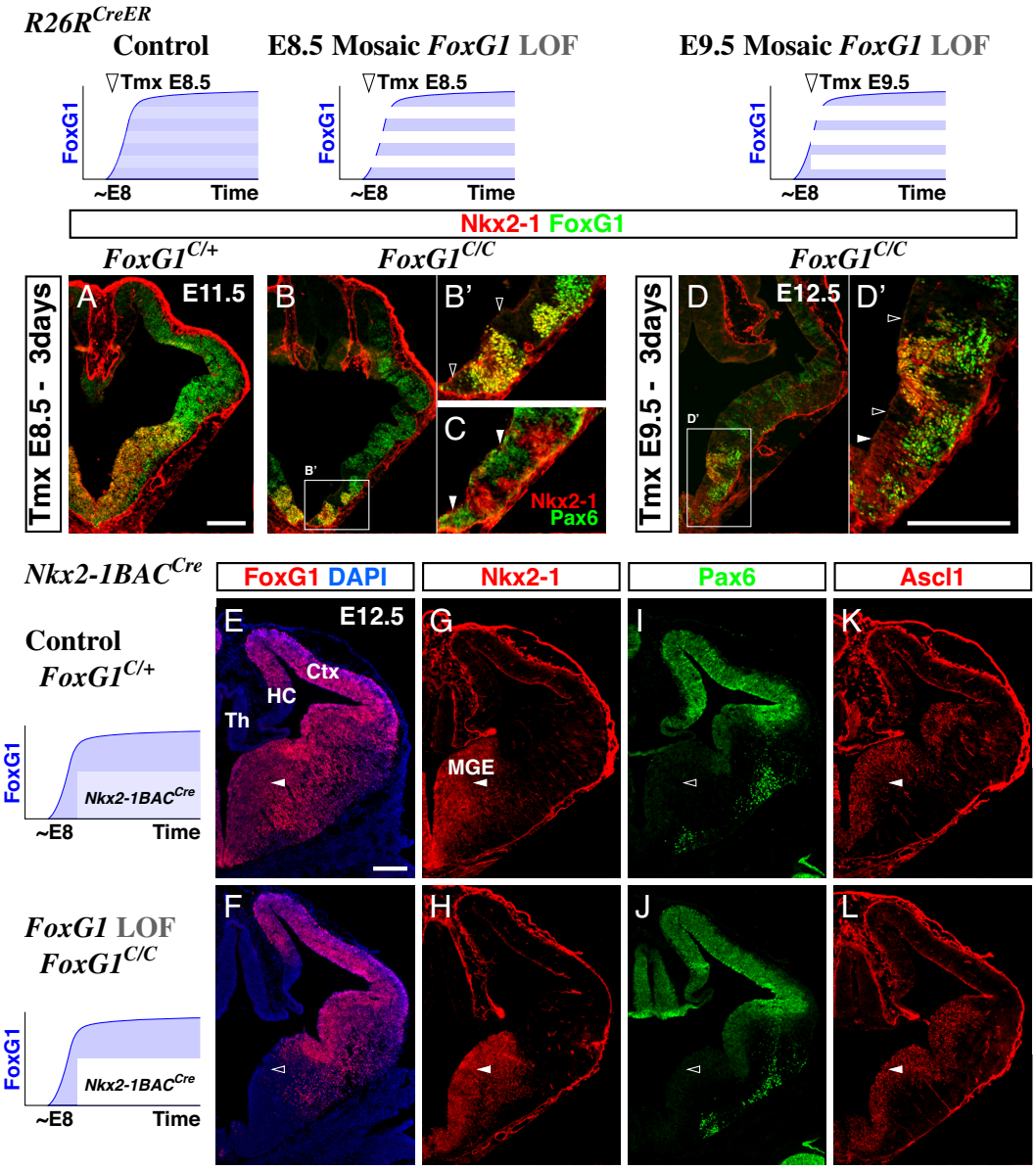

Fig. 2. *FoxG1* is cell autonomously required to establish GABAergic neuronal identity but is not required for its maintenance. (*A–D*) A cell-autonomous role of *FoxG1* was analyzed by mosaic LOF (schemes on the *Top*) by combining *R26-CreER* and conditional *FoxG1* alleles and by activating *CreER* with tamoxifen administration. (*A* and *B*). Compared to the E11.5 control tissue (*A*, *FoxG1-C/+*) and neighboring control cells (*B*, *FoxG1-C/C* without homozygous recombination) with FoxG1 expression (green), LOF cells (no green signals, *FoxG1-C/C* with recombination) failed to express Nkx2-1 (*B* and *B'*) upon E8.5 tamoxifen administration. In the neighboring section of *B'*, Pax6 expression was complementary to Nkx2-1 (*C*), indicating that *FoxG1* is cell autonomously required to acquire ventral GABAergic identity. When similar mosaic LOF was carried out 1 d later at E9.5, some *FoxG1*-null cells similarly failed to express Nkx2-1 (*D* and *D'*, open arrowheads), but some did not (arrowheads). (*E–L*) In order to test the roles of *FoxG1* in cells which have already expressed *Nkx2-1*, *Nkx2-1BAC-Cre*-mediated *FoxG1* LOF was carried out, and adjacent sections were compared (*E* and *F*, compare the filled and open arrowheads). In the ventral MGE progenitor domain, no obvious change in Nkx2-1 expression was observed in the *FoxG1* LOF territories compared to the control (*G* and *H*, arrowheads). Consistent with this notion, Pax6 expression was not changed in these domains (*I* and *J*, open arrowheads). The proneural gene *Ascl1* (*Mash1*), which shows ventral-specific expression during early telencephalic development, is also not changed in the Cre-recombined domains (*K* and *L*, arrowheads), although the VZ/SVZ (marked by Ascl1 expression) is thinner in the *FoxG1* LOF compared to the control due to decreased cell proliferation. Each figure represents the analysis of three brains. Ctx: cortex, HC: hippocampus, Th: thalamus, MGE: medial ganglionic eminence. (Scale bars: 200 μm.)

reporter-positive *FoxG1* LOF cells (Fig. 3*F*). Interestingly, the *ErbB4* receptor, which has been shown to regulate interneuron migration (14), was severely down-regulated in these labeled *FoxG1* LOF cells (Fig. 3 *G* and *H*). These results indicate that *FoxG1* expression is autonomously required in GABAergic interneuron precursors to invade cortical territories.

In addition to the loss of cortical GABAergic cells, reporter-positive cells fail to form the globus pallidus (GP) structure in the *FoxG1* LOF brain (Fig. 3 *C* and *D*, GP). We thus analyzed the expression of Nkx2-1, which rapidly shuts off in cortical interneurons but is maintained in ventral lineages (69). Indeed, the reporter and Nkx2-1 double-positive population is missing in the *FoxG1* mutants (Fig. 3 *K* and *L*), suggesting that *FoxG1* is required for the proper formation of the GP. Consistent with this idea, at more anterior regions of the forebrain (Fig. 3 *I* and *J*), the Nkx2-1-positive EGFP-expressing population is found ectopically located medioventrally to the striatum. In order to test whether *FoxG1* is generally required for migration through the striatum, we performed *FoxG1* LOF in the neural progenitors at E11.5 by using a *Nestin-CreER* driver (70) and followed their fate

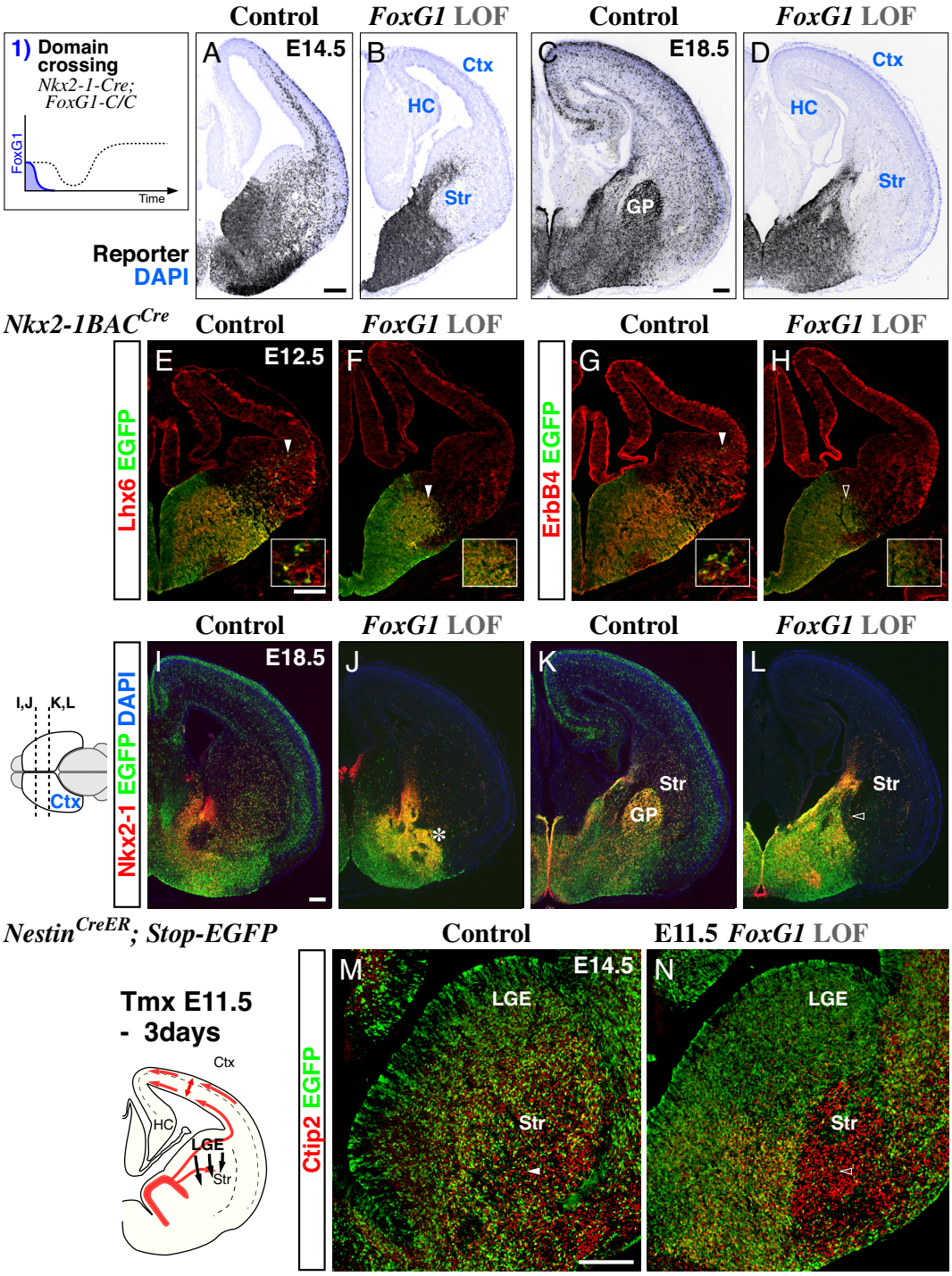

Fig. 3. GABAergic neuron precursors require *FoxG1* expression to enter into the striatum and the neocortex. (*A–L*) *FoxG1* LOF during the 1st phase (*Left* scheme, Fig. 1*O*) by using a *Nkx2-1BAC-Cre* transgenic driver prevents reporter-labeled GABAergic cells from entering the hippocampus, cortex, and striatum at E14.5 (*A* and *B*) and also later at E18.5 (*C* and *D*). (*E–H*) The *FoxG1* LOF cells remaining in the vicinity of the MGE still retained expression of Lhx6 (*E* and *F* arrowheads), a downstream target of *Nkx2-1*. However, these LOF cells significantly down-regulated the expression of ErbB4 (*G* arrowhead and *H* open arrowhead), a transmembrane receptor that is critically required for MGE-derived cells to migrate into the cortex. (*I–L*) Fluorescent images of figures *C* and *D* with Nkx2-1 expression (*K* and *L*) and rostral forebrain regions (*I* and *J*). Even in anterior forebrain regions, *FoxG1* LOF cells are not able to enter into the cortex and the striatum (*I* and *J*). The globus pallidus (GP) is a structure located medially to the striatum (Str) that normally maintains Nkx2-1 expression into mature stages (*K*, GP). While the globus pallidus structure is largely absent in the *FoxG1* LOF forebrain (*L*, open arrowhead), a population strongly maintaining Nkx2-1 can be found in anterior regions (*J*, asterisk), suggesting that *FoxG1* is required for globus pallidus cell migration. (*M* and *N*) In order to characterize the migration of LGE-derived medium spiny neuron precursors (*Left* panel of *M* and *N*), *Nestin-CreER* driver and an *EGFP* reporter are combined in the conditional *FoxG1* background, and tamoxifen administration was carried out at E11.5. The control EGFP-labeled cells born after E11.5 and coexpressing Ctip2 successfully entered into the striatal domain (*M*, arrowhead) and relatively homogeneously intermingled with Ctip2 non-EGFP cells, most of which are likely born prior to E11.5. However, *FoxG1* LOF cells labeled with both EGFP and Ctip2 failed to mix with Ctip2/non-EGFP cells (*N*, open arrowhead), indicating that *FoxG1* is required for LGE-derived medium spiny cells to migrate within the striatum. Each figure represents the analysis of three brains. (Scale bars: 200 μm.)

with an EGFP reporter. Distinct from the EGFP-labeled control cells that are intermingled with the medium spiny cells labeled by Ctip2 (71), *FoxG1* LOF cells are located mostly outside the striatum (Fig. 3 *M* and *N* open arrowhead). Taken together, we find that *FoxG1* facilitates the migration of GABAergic neuronal precursors into the cortex and the striatum.

***FoxG1* Downregulation during Tangential Migration is Required for the GABAergic Cell Dispersion into Distant Cortical Territories.** Interestingly, despite the initial requirement for *FoxG1* for their entrance into the cortex, GABAergic interneuron precursors down-regulate *FoxG1* expression during cortical tangential migration (Fig. 1 *E* and *E'*). To explicitly test the importance of this downregulation, we carried out *FoxG1* GOF during this migration phase by combining the *Dlx-Cre* driver with a conditional transactivator line (*R26-stop-tTA*) and a transactivator-dependent *FoxG1* expression allele (*TRE-FoxG1*) (2nd phase, Fig. 1*O*) (64). At E14.5, the overall migration pattern of the reporter-positive cells in the cortex was comparable between the control (Fig. 4*A*) and *FoxG1* GOF cells (Fig. 4*B*). Notably by P7, *FoxG1* augmentation resulted in a significant decrease in the number of labeled GABAergic cells in the hippocampus and

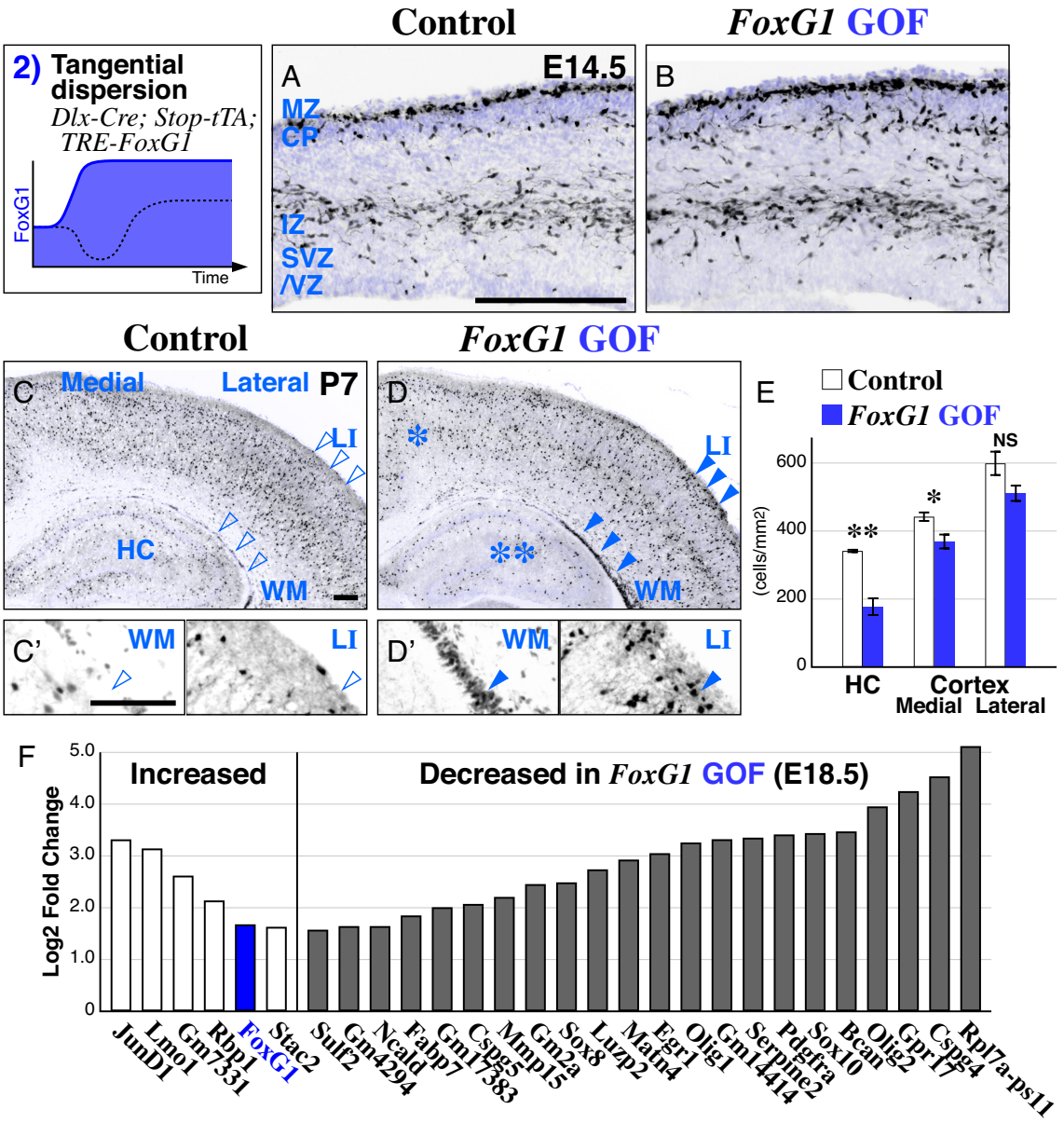

Fig. 4. GABAergic neuron precursors require *FoxG1* downregulation to maintain tangential migration and to reach distant cortical territories including the hippocampus. *FoxG1* GOF was carried out (*Dlx-Cre*; *R26-stop-tTA*; *TRE-FoxG1*), and recombined cells were visualized with *tdTomato* (*Ai9* reporter) during the 2nd phase (*Left* scheme, Fig. 1*O*) of GABAergic interneuron migration. Control littermates do not carry the *TRE-FoxG1* allele. (*A* and *B*) No clear difference was observed in the E14.5 cortex between control and *FoxG1* GOF experiments. (*C* and *D*) Distribution of *FoxG1* GOF cells was analyzed at P7. Higher-magnification views of the white matter (WM) and layer 1 (LI) in *C* and *D* are shown in *C'* and *D'*. *FoxG1* GOF resulted in reduced interneuron numbers in the hippocampus (HC) and the medial cortex (Ctx, asterisk in *D*). Ectopically located GOF cells were found in the white matter and layer 1, particularly in the lateral part of the cortex (*D* and *D'*, arrowheads), corresponding to the major tangential migration routes (intermediate and marginal zones, respectively) at earlier time points (*C* and *C'*, open arrowheads). (*E*) Comparison of the labeled cell density in the hippocampus (HC), medial (retrosplenial), and lateral (barrel) part of the cortex (n = 3 each). Two-tailed *t* test: *P* = 0.00272**(HC), *P* = 0.0379*(medial), and *P* = 0.0992(lateral). (*F*) Bulk RNA sequencing analysis of FACS-purified cortical interneurons from five control and eight FoxG1 GOF brains revealed 5 increased and 22 decreased genes upon *FoxG1* GOF (increased ×3.2) at E18.5. MZ: marginal zone, CP: cortical plate, IZ: intermediate zone, SVZ/VZ: subventricular/ventricular zone, WM: white matter, HC: hippocampus. (Scale bars: 50 μm.)

the medial part of the cortex (Fig. 4 *C* and *D*, asterisks), with ectopic clusters of GABAergic interneurons stranded along the earlier tangential migration routes (Fig. 4*C*, open arrowheads and Fig. 4*D*, arrowheads). Thus, preventing the downregulation of *FoxG1* in tangentially migrating GABAergic interneurons disrupts their ability to disperse into the hippocampus and medial cortical areas (Fig. 4*E*, bar graphs), with many cells remaining within the white matter and layer 1 of the lateral part of the cortex (Fig. 4 *C'* and *D'*). These results led us to investigate the molecular mechanisms downstream of *FoxG1* underlying the attenuation in GABAergic tangential migration. We carried out RNA sequencing analysis to compare the gene-expression profiles of the control and *FoxG1* GOF EGFP-labeled cells which were FACS (fluorescence-activatedcell sorting) purified from the E18.5 cortex, prior to overt cell migration alterations (Fig. 4*F*). First, we confirmed the efficacy of our GOF strategy, with *FoxG1* mRNA levels being 3.2× greater in the *Dlx-Cre*-labeled cortical cell cohorts at E18.5 (Fig. 4*F*, blue bar). Consistent with the notion that *FoxG1* mainly functions as a repressor (47, 72), we found more significantly down-regulated genes (22) than up-regulated (5). Surprisingly, we found many oligodendrocyte precursor genes such as *Olig1/2*, *Sox10*, *Pdgfra*, *Gpr17*, and *Cspg4*(*NG2*) (73) to be down-regulated in *FoxG1* augmented GABAergic cell lineages. This suggests that GABAergic and oligodendrocyte precursors, both of which migrate dorsally from the ventral forebrain into the cortex, share some fundamental genetic programs required for tangential dispersion throughout the cortex. We also found genes such as *JunD1*, *Lmo1,* and *Rbp1* to be highly up-regulated in *FoxG1* GOF cells, suggesting that these genes are normally suppressed during tangential migration. Interestingly, in our previous microarray analysis comparing control vs. *FoxG1* LOF pyramidal neuron precursors (47), *Lmo1* (×0.48) and *Rbp1* (×0.47) were found to be decreased. This strongly suggests that these two factors have important roles in regulating migration modes in both glutamatergic and GABAergic neuron precursors. We conclude that, although *FoxG1* expression is required for GABAergic interneurons to reach the cortex, upon entry, downregulation of *FoxG1* expression is necessary in order to maintain tangential migration into hippocampal and medial cortical territories.

***FoxG1* Upregulation during Radial Migration Directs GABAergic Interneurons to Specific Cortical Layers.** Having observed that FoxG1 is robustly up-regulated during the transition from tangential to radial migration (Fig. 1*O*, 3rd phase), we next tested the importance of *FoxG1* activity specifically during this stage. To this end, we carried out *FoxG1* LOF in a subset of GABAergic interneurons by using the *Sst-Cre* driver, whose expression commences during tangential migration. Approximately 30% of cortical interneurons are *Sst*-positive cells (11, 26), a population primarily located in deeper layers and largely excluded from layer 1 (Fig. 5*A*). Strikingly, *FoxG1*-null Sst cells were distributed to more superficial layers, with a marked decrease in layer 6 and concomitant increases in layers 1 to 3 (Fig. 5 *B* and *C*), including ectopically located interneurons in layer 1 (Fig. 5*B*, arrowhead). These data strongly suggest that *FoxG1* reinitiation during the transition from tangential to radial migration modes is critical for achieving the correct laminar distribution of GABAergic interneurons.

To test whether *FoxG1* upregulation during radial migration is further required for interneuron maturation beyond positioning, we analyzed intrinsic firing properties by carrying out whole-cell patch clamp in 3-wk-old acute brain slices. Consistent with what has been shown (10, 74), the *Sst-Cre*-labeled control and *FoxG1* LOF cells showed regular- or low threshold-spiking properties (Fig. 5 *D* and *F*). Furthermore, control and *FoxG1*-null cells were indistinguishable with regard to frequency (Fig. 5 *E*, *Left*) and other properties (Fig. 5 *F–I*) besides the time constant (Fig. 5*J*). Within the *FoxG1* LOF interneuron group, we further compared ectopically located layer 1 cells to the layer 2/3 interneurons and, to our surprise, their intrinsic firing properties were comparable (Fig. 5 *E, Right* and *F–J*, *Lower*). This suggests that GABAergic interneurons possess an intriguing ability to acquire mature physiological properties even in ectopic laminar locations. In sum, we find that precisely regulated up- and downregulation of *FoxG1* expression in migrating interneuron precursors critically regulates the positions of cortical interneurons.

***FoxG1* Functions in a Gene Dosage–Dependent Manner in Forebrain Patterning.** Having found that dynamic changes in *FoxG1* expression levels critically regulate the allocation of GABAergic cortical interneurons, we next investigated whether *FoxG1* functions in a gene dosage–dependent manner. *FOXG1* gene dosage has been shown to be critical for human mental health as both haploinsufficiency and gene duplication can result in FOXG1 syndrome, an autism spectrum disorder-related condition. We thus developed a genetic strategy to systematically reduce *FoxG1* expression in a stepwise manner through the generation of a hypomorphic allelic series. These alleles are the control (homozygous for floxed conditional allele), heterozygous (floxed allele/*LacZ* knock-in null), hypomorphic (floxed allele with a *Neo* cassette remaining in downstream 3′UTR/null), and null (floxed allele/*Cre* knock-in null). We first compared *FoxG1* +/+ and +/– alleles to the floxed-*FoxG1* alleles (C/+ and C/–) to confirm that the gene targeting has a minimal impact on *FoxG1* gene dosage (*SI Appendix, Supplementary Figure*). We found that progressive reduction in *FoxG1* levels (Fig. 6 *A–D*, top schematics) results in increasingly severe alterations in telencephalic size and morphology at E12.5. By comparing the expression of homeodomain transcription factors Pax6 (Fig. 6 *A–D*) and Nkx2-1 (Fig. 6 *A'–D'*), we found that when *FoxG1* levels were reduced by >50%, the Nkx2-1-positive domain, which encompasses the medial ganglionic eminence—the primary source of cortical GABAergic interneurons—completely disappeared (Fig. 6 *B'* and *C'*). This indicates that there is a critical threshold for *FoxG1* expression in the establishment of *Nkx2-1* lineages during early embryonic forebrain patterning and that GABAergic lineages are particularly sensitive to *FoxG1* gene dosage in comparison to pyramidal neurons.

Discussion

In sum, we have demonstrated an intriguing developmental mechanism utilized by interneuron precursors for their transition in migratory phases. We show that precisely regulated dynamic expression of *FoxG1* during the entire developmental trajectories of GABAergic interneurons, from early specification to cortical invasion, areal distribution, and laminar positioning, is essential for the proper assembly of cortical inhibitory circuits. Given that even relatively subtle changes in its expression can impair the formation of both excitatory (47) and inhibitory circuits (this study), it begins to be evident why precise regulation of FoxG1 levels and by proxy *FoxG1* gene dosage is so critical for normal cortical development.

We find that loss of *FoxG1* in newly specified GABAergic neuron precursors prevents them from entering into the striatum and the cortex (Fig. 3). This is consistent with our previous findings of *FoxG1*-null pyramidal cells failing to enter into the cortical plate (47). Our current hypothesis is that without *FoxG1*, cells are not permitted to cross into these territories of the cortex and the

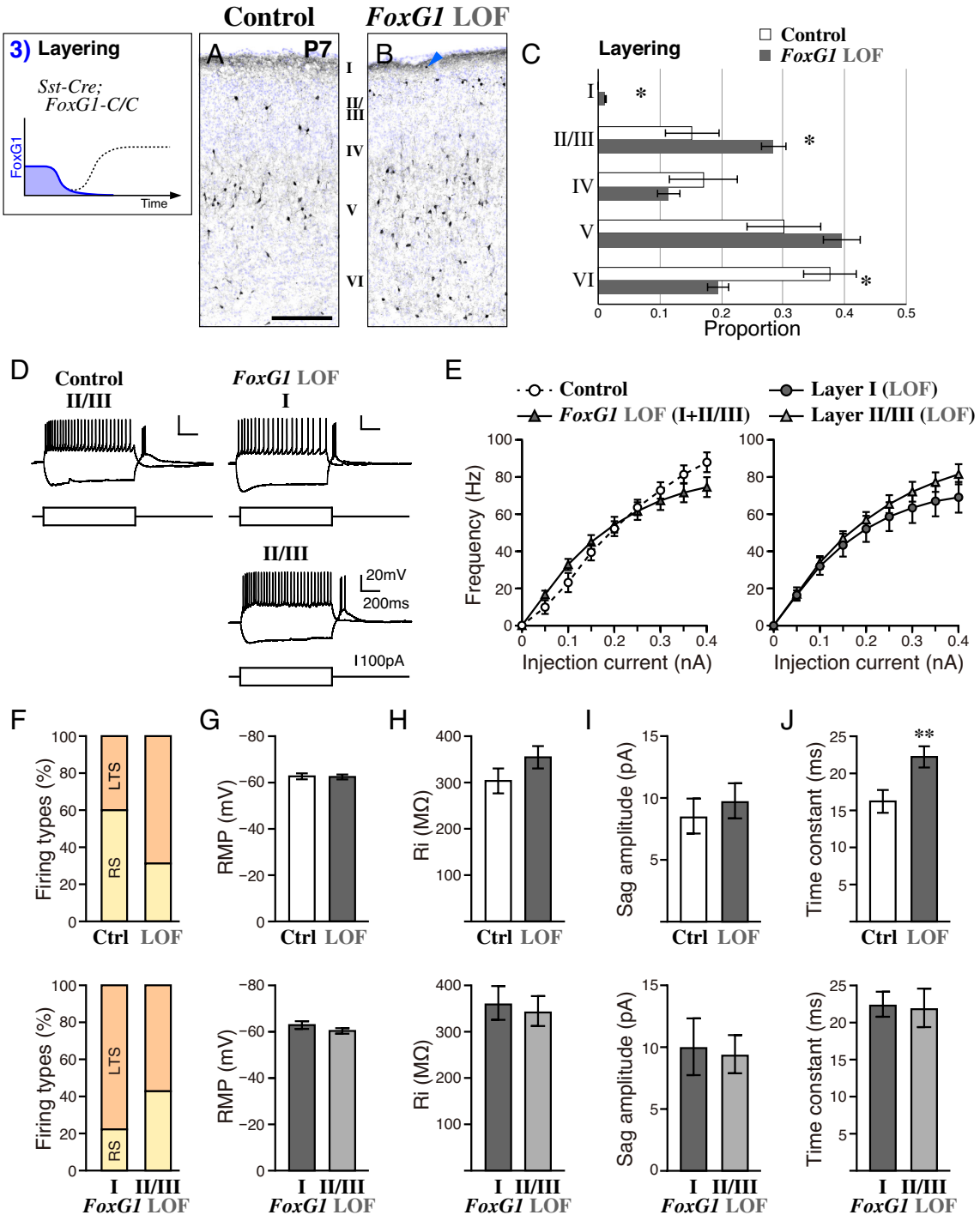

Fig. 5. *FoxG1* upregulation during radial migration is required for the positioning but not the maturation of interneuron precursors. *FoxG1* LOF was carried out during tangential migration prior to the 3rd phase (Fig. 1*O*) by using a *Sst-Cre* driver. Control (*Sst-Cre*; *FoxG1-C/+*) and *FoxG1* LOF (*Sst-Cre*; *FoxG1-C/C*) cells were compared. Approximately 30% of cortical interneurons are *Sst* positive, and they mostly occupy deep layers (*A*). At P7, the positions of LOF cells were shifted more superficially (*B* and *C*), and some were ectopically located in layer 1 (*B*, arrowhead). Error bars are ± SEM, two-tailed *t* test: $P = 0.0127$*(I), $P = 0.0376$*(II/III), $P = 0.269$ (IV), $P = 0.0880$(V), $P = 0.0366$*(VI). (*D–J*) Intrinsic electrophysiological properties of *FoxG1* LOF cells were analyzed. For this, *tdTomato*-labeled cells (*Ai9*) at postnatal 3 wk were compared by whole-cell patch clamp analysis in acute brain slices. (*D*) Responses to depolarizing or hyperpolarizing current injection of *Sst* interneurons in the superficial layers of the S1 barrel field. Similar to control layer 2/3 *Sst* interneurons, *FoxG1* LOF cells in layer 2/3 as well as the ones ectopically located in layer 1 showed low threshold spike (LTS) firing properties. (*E*) Frequency–current relationships were found to be similar between control (n = 10) and *FoxG1* LOF (n = 16) *Sst* interneurons (*E*, *Left*). Ectopically located layer 1 (n = 9) and layer 2/3 (n = 7) *FoxG1* LOF cells were both indistinguishable from the wild type (*E*, *Right*). (*F–J*) Intrinsic firing properties of *Sst* interneurons were found to be largely unaffected by *FoxG1* LOF. (*Top*) Data comparing control vs. *FoxG1* LOF, and (*Bottom*) data comparing layer 1 vs. layer 2/3 *FoxG1* LOF interneurons. (*F*) Intrinsic firing properties of *Sst* interneurons, which show either LTS or regular spiking (RS) features. The proportion of LTS and RS *Sst* types was similar between control vs. *FoxG1* LOF (*F*, *Top*) and L1 vs. L2/3 FoxG1 LOF cells (*F*, *Bottom*; not significant by Chi-square test). While resting membrane potential (RMP, *G*, *Top*), input resistance (Ri, *H*, *Top*) and voltage sag amplitude in response to a hyperpolarizing current injection (−0.2 nA, 1 s) were indistinguishable (*I*, *Top*), *FoxG1* LOF cells exhibited a longer time constant compared to control *Sst* interneurons ($P < 0.01$, Welch's *t* test) (*J*, *Top*). Note that *FoxG1* LOF interneurons in layers 1 and 2/3 have similar intrinsic electrophysiological properties (*G–J*, *Bottom*). (Scale bars: 50 μm.)

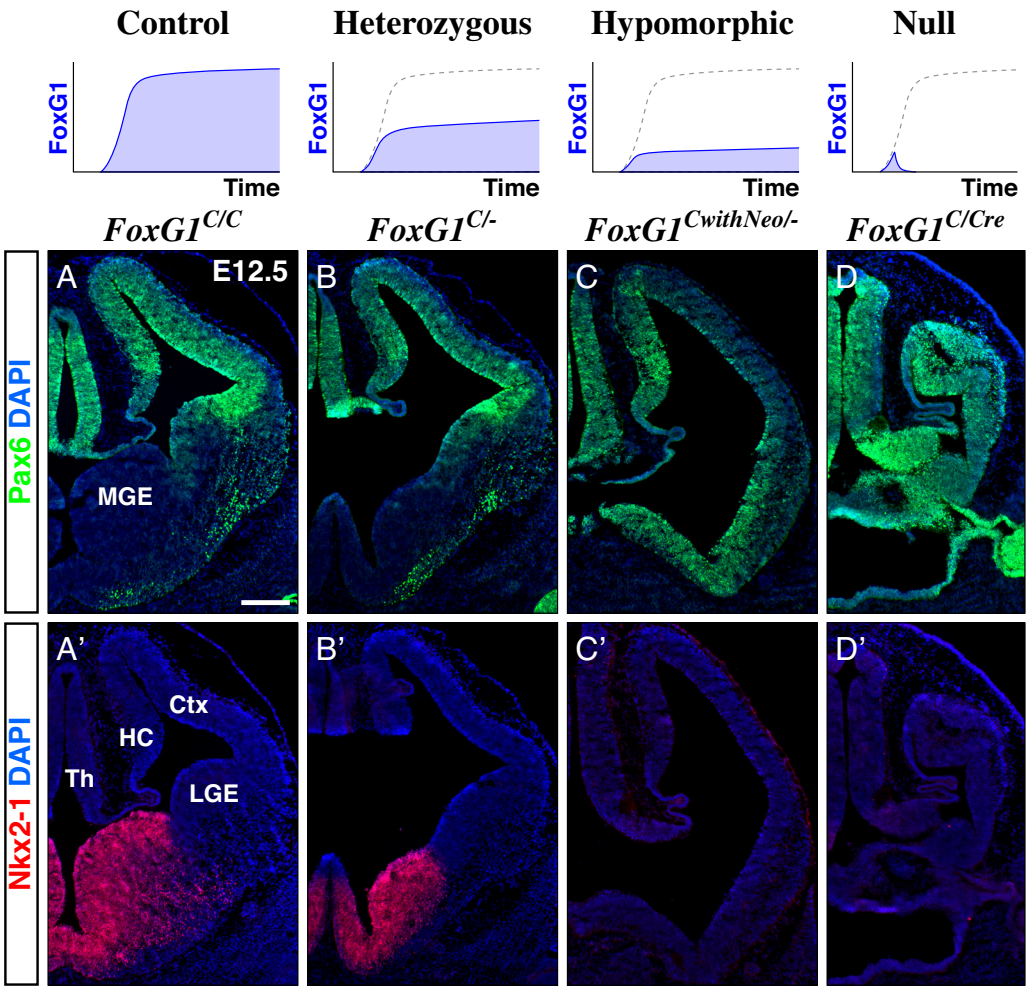

Fig. 6. *FoxG1* functions in a gene dosage–dependent manner in forebrain patterning. Control (homozygous for floxed conditional allele), heterozygous (floxed allele/*LacZ* knock-in null), hypomorphic (floxed allele with a *Neo* cassette remaining in downstream 3′UTR/*LacZ* knock-in null), and null (floxed allele/*Cre* knock-in null) brains were compared at E12.5. Immunohistochemistry is shown for Pax6 (*A*–*D*) and Nkx2-1 (*A*′–*D*′), with nuclear counterstaining (DAPI) in blue. As *FoxG1* gene dosage decreases (top scheme), the Nkx2-1 positive domain disappears between the heterozygous (*B*′) and hypomorphic (*C*′) models. Refer to *SI Appendix, Supplementary Figure* for the FoxG1 +/+ and +/− genotypes. (Scale bars: 200 μm.)

striatum. This is in contrast to scenarios where the general migration machinery is attenuated and GABAergic cell numbers are reduced in most regions and not specifically excluded from particular domains (39, 75). In the case of *Zeb2* (*Zfhx1b*, *Sip1*), this gene regulates cortical vs. striatal fate of GABAergic precursors, and thus, GABAergic cell migration in the cortex is severely reduced, with increased numbers of cells found ectopically in the striatum (37, 38). Instead, in our *FoxG1* mutant study, GP cells maintaining Nkx2-1 expression are excluded from the striatum and ectopically found ventromedially to the striatum. In contrast to the use of pan-GABAergic *Dlx-Cre* line (54), our method utilizing *Nkx2-1-Cre* driver spares LGE-derived populations, which are required to support interneuron migration from the MGE into the cortex (14). Thus, our study reveals the fundamental roles of *FoxG1* in actively promoting cell migration into distinct forebrain territories of the cortex and the striatum.

Pyramidal neuron precursors show tightly regulated transitions from a multipolar cell tangential phase into a radial migration phase prior to entering into the cortical plate (76–78). Compared to this, entrance of interneuron precursors into the cortical plate occurs in a rather permissive manner (21). Still, the mechanisms to maintain the tangential migration of interneuron precursors above and below the cortical plate play essential roles in nascent inhibitory circuit formation. The expression of chemokine ligand *Cxcl12* both above and below the cortical plate facilitates the tangential migration of interneuron precursors, which express the receptor *Cxcr4/7* (18, 19, 79). Intriguingly, pyramidal neuron precursors facilitate the tangential migration of interneuron precursors by transiently expressing *Cxcl12* specifically during the multipolar cell phase (17, 20). Recently, JNK signaling has been shown to be important for the transition from tangential to radial migration of interneuron precursors (80). Aside from these overall mechanisms, the migration of specific subtypes of interneuron precursors is under the control of selective genetic programs. Approximately 70% of interneurons are derived from the MGE and their transition to radial migration is controlled by *Sox6*, which when mutated, results in the relocation of *Pvalb*-expressing basket cells ectopically into layer 1 (43). In CGE (Caudal Ganglionic Eminence)-derived lineages, the *Prox1* transcription factor facilitates migration of interneuron precursors from the SVZ/IZ into the cortical plate and marginal zone tangential paths (44). Thus, loss of *Prox1* displaces CGE-derived interneurons which are normally mostly found in the superficial layers into deep locations. In contrast to these molecular pathways, *FoxG1*, by dynamically changing its expression levels, coordinates the phase transitions of tangential and radial migrations. In a manner distinct from the pan-GABAergic or subtype-specific molecules

required for the migration of interneuron precursors, we find that a decrease in *FoxG1* levels facilitates tangential migration allowing interneuron precursors to reach distant cortical locations, whereas an increase in *FoxG1* expression facilitates radial migration into appropriate laminar locations.

Intriguingly, we observe that *FoxG1* expression is consistently down-regulated in a diverse set of forebrain neuronal precursors specifically during the phase of tangential migration. These populations include multipolar pyramidal neuron precursors in the intermediate zone (47), Cajal–Retzius cells in the marginal zone (48), and GABAergic interneuron precursors in both intermediate and marginal zones (this study). Thus, it may be interesting in the future to establish a system for monitoring in vivo FoxG1 protein levels in migrating interneuron precursors by targeting the *FoxG1* allele to generate a fusion protein with a rapidly maturing fluorescent protein, such as Achilles (81). Considering that failure to down-regulate *FoxG1* disrupts tangential migration [(47); this study], it is tempting to speculate that there is a shared mechanism utilized across different cell types within the telencephalon to down-regulate *FoxG1* specifically during this migration phase. Consistent with this idea, we found two genes (*Lmo1* and *Rbp1*) that are down-regulated in the pyramidal cell precursors upon *FoxG1* LOF (47) and are up-regulated in GABAergic precursors upon *FoxG1* GOF (this study). Thus, it may be interesting in the future to test the roles of these genes in cell migration, particularly *Lmo1*, which has been shown to be downstream of *Arx* (82–84). In pyramidal neuron precursors, transient upregulation of *Unc5D* expression during the multipolar cell phase of tangential migration through *FoxG1* downregulation facilitates cell entrance into the cortical plate (47). Indeed, this transition phase plays essential roles in the fate determination of pyramidal cells (47, 85). *Unc5D* is not expressed in GABAergic neuron precursors, at least not at comparable levels as pyramidal lineages, and thus, it is likely that while *FoxG1* downregulation is universally required in tangentially migrating cortical neuron precursors, *FoxG1* regulates distinct sets of genes according to the particular cell type.

How does gene dosage affect the functionality of *FoxG1*-dependent processes? Alterations in gene dosage of secreted morphogens/patterning molecules are known to affect early patterning of the brain. For example, studies on an allelic series of *Fgf8* mutants have revealed the dose-dependent function of this gene in forebrain development (86). *Fgf* is a molecule secreted by patterning centers and thus reduced dosage results in a spatiotemporal decline in *Fgf8*-mediated signaling and disruption of forebrain arealization. Dosage-dependent effects on disease phenotypes have also been studied by developing allelic series, e.g., for the genes *CHD8* and *TSC*, both of which are implicated in syndromic forms of autism spectrum disorder (87, 88). In our recent study, we found that FoxG1 protein levels detected by western blotting are reduced to approximately half of that of the controls in the heterozygous and neuron-specific (*Nex-Cre* plus *Dlx-Cre*) conditional heterozygous models (64). We find that there is a gene dosage threshold for *FoxG1* and at least one intact copy of this gene is required for the specification of ventral GABAergic progenitor domains. It will be interesting in the future to uncover the molecular pathways downstream of *FoxG1* that are mediating this effect. Together with our previous study (47), this work provides a comprehensive picture of how dynamic regulation of *FoxG1* expression is required during excitatory and inhibitory neuron specification, migration, and circuit integration. Our findings reveal that relatively subtle changes in *FoxG1* expression levels during development can impair the formation of inhibitory circuits and thus illuminate how perturbations in *FoxG1* regulation could contribute to the etiology of neurodevelopmental disorders such as schizophrenia and autism.

Materials and Methods

In Vivo Mouse Genetics. All animal handling and experiments were performed in accordance with protocols approved by the respective Institutional Animal Care and Use Committees of the NYU School of Medicine and Tokyo Women's Medical University. Animal cages are maintained at 22 °C ± 1 °C, 50 ± 15% humidity with a 12 h light/dark cycle. A hypomorphic *FoxG1* allelic series was generated from combinations of the *FoxG1* conditional allele (*C/+*: foxed-*FoxG1*, *SI Appendix, Supplementary Figure*), *FoxG1* conditional allele still harboring a *Neo* cassette (47), *LacZ* knock-in null allele (49), or *Cre* knock-in null allele (89). GABAergic populations were visualized by combining the *Dlx5a-Cre* driver (90) (Jackson Laboratories stock #008199) with the red-fluorescent protein (tdTomato) reporter line *Ai9* (Jackson Laboratories stock #007909) or the *R26-CAG-loxPstop-EGFP* reporter (11, 91) (*RCE:loxP*; Jackson Laboratories stock #10701). For the LOF experiments, males with *FoxG1-C/+* and either *Nkx2-1BAC-Cre* (68) (Jackson Laboratories stock #008661), *Sst-IRES-Cre* (92) (Jackson Laboratories stock #013044) or *Nestin-CreER* (70) were crossed to *FoxG1-C/C*; *Ai9* homozygous or *FoxG1-C/C*; *RCE:loxP* homozygous females. For the GOF experiments (64), *Dlx5a-Cre*; *Ai9* homozygous males were crossed to *R26-stop-tTA* homozygous (93) (Jackson Laboratories stock #008600); *TRE-FoxG1* heterozygous (94) females. The *TRE-FoxG1* allele contains an *IRES-LacZ* cassette downstream of the *FoxG1* coding sequence, and thus, the corresponding *FoxG1* GOF population can be visualized based on X-gal staining or by using anti-beta-galactosidase immunohistochemistry.

Tissue Preparation and Immunohistochemistry. Detailed procedures for tissue preparation and immunohistochemistry are provided in *SI Appendix*. All of the fluorescent images were captured with a Zeiss AxioScope.A1 (Carl Zeiss) by using a cooled-CCD camera (Princeton Scientific Instruments) with Metamorph software (Universal Imaging, Downingtown, Pennsylvania) or by using a QSI RS 6.1s cooled-CCD camera (2,758 × 2,208 pixels, QSI) with micromanager (open source software). In order to facilitate the visualization of gene expression, acquired images were inverted in Adobe Photoshop and subsequently combined to generate the figures. For example, tdTomato fluorescent images were placed into both the green and blue channels of the RGB format file, and then, all color channels were simultaneously inverted to generate a figure with red signals in the white background. For a multicolor presentation, layers including different color signals were assembled by using the function of multiply layers.

Areal and Layer Analysis of Cortical Interneuron Distribution. For the P7 FoxG1 GOF analysis, pictures were taken from three representative hemispheric fields of control and GOF sections. Image acquisition was carried out with the ×2.5 lens. Using Adobe Photoshop, the DAPI channel was placed in a different picture layer, areal borders of the hippocampus and medial and lateral cortex were drawn using the pen tool based on the DAPI signal, and then, the DAPI layer was made invisible. Cell numbers were tabulated for each specific area. At the end of the cell counting, the total pixel numbers of the counted area were analyzed and then converted to surface area (313.6k pixels = 1 mm^2). Cell numbers for each area were converted to cell density (cells/1 mm^2) for each section, and the values from the three fields were averaged and final results were shown as the average ± SEM. Similar analysis was carried out for the P7 *Sst-Cre*-mediated *FoxG1* LOF experiments, using layer borders drawn based on DAPI signals to determine the fluorescently labeled cell numbers within each specific layer.

RNA Sequencing Analysis. For the comparison of genes selectively expressed in GABAergic neuron precursors upon *FoxG1* GOF, we compared *Dlx5a-Cre*; *RCE:loxP*; *R26-stop-tTA* embryos with (GOF) or without (control) *TRE-FoxG1* at E18.5. Detailed procedures for tissue dissociation to obtain single cells (44, 47, 64) are provided in *SI Appendix*. These collected cells were subjected to FACS (Aria cell sorter, Becton Dickinson), and EGFP-positive cells were collected into numbered tubes containing 250 µL of ice-cold HBSS. Immediately after collecting the cells, 250 µL of Trizol reagent (15596026, Invitrogen, Thermo Fisher) was added into the tube. Cell numbers were typically between 15,000 and 30,000 cells/brain. These tubes were vortexed and then stored at –80 °C. After genotyping, samples were combined for each genotype resulting in control (114,200 cells) and FoxG1 GOF (148,700 cells) solution and kept at –80 °C. These samples were shipped on dry ice, and further mRNA extraction and subsequent sequencing were performed at the GENEWIZ Japan corporation in Saitama.

Total reads/mapped for the five control and eight FoxG1 GOF brains were 50,716,404/46,347,154 and 60,397,698/56,014,194, respectively. The results from cuffdiff analysis were further analyzed to determine genes with significant differential expression according to the criteria of fold change greater than 2 and FDR less than 0.05. The number of up- and down-regulated genes in the FoxG1 GOF is 15 and 25, respectively. We excluded the candidate genes with zero values for FPKM in either control or FoxG1 GOF resulting in 7 up- and 22 down-regulated genes in the FoxG1 GOF. All of the values of these genes are shown in Fig. 4*F*.

In Vitro Electrophysiological Recordings. Detailed procedures for whole-cell patch in postnatal 3-wk animals are provided in *SI Appendix*. Recordings were amplified with a Multiclamp 700A amplifier (Molecular Devices, LLC, Sunnyvale, CA), digitized at 10 kHz using a Digidata 1322A apparatus (Molecular Devices, LLC), and collected with pClamp 8.2 software (Molecular Devices, LLC). Statistical analyses were performed as follows using GraphPad Prism 7.05 software (GraphPad Software, San Diego, CA): Two-way ANOVA and post hoc Tukey's test were used for comparisons of current–frequency relationships; Welch's *t* test was used for two-group comparisons; and the chi-square test was used for comparison of firing type composition.

Data, Materials, and Software Availability. All unique materials used here are readily available from the authors or from Jackson Labs (mouse lines). RNA-seq data are deposited in the NIH SRA database under experiment accession number PRJNA1072317, with IDs SRX23547063 and SRX23547064 RNA-Seq (95).

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1. D. V. Hansen *et al.*, Non-epithelial stem cells and cortical interneuron production in the human ganglionic eminences. *Nat. Neurosci.* **16**, 1576–1587 (2013).
2. T. Ma *et al.*, Subcortical origins of human and monkey neocortical interneurons. *Nat. Neurosci.* **16**, 1588–1597 (2013).
3. S. A. Anderson, D. D. Eisenstat, L. Shi, J. L. Rubenstein, Interneuron migration from basal forebrain to neocortex: Dependence on Dlx genes. *Science* **278**, 474–476 (1997).
4. P. Rakic, Neurons in rhesus monkey visual cortex: Systematic relation between time of origin and eventual disposition. *Science* **183**, 425–427 (1974).
5. J. B. J. Angevine, R. L. Sidman, Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192**, 766–768 (1961).
6. D. M. Gelman *et al.*, The embryonic preoptic area is a novel source of cortical GABAergic interneurons. *J. Neurosci.* **29**, 9380–9389 (2009).
7. D. Gelman *et al.*, A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. *J. Neurosci.* **31**, 16570–16580 (2011).
8. L. Sussel, O. Marin, S. Kimura, J. L. Rubenstein, 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 (1999).
9. S. Nery, G. Fishell, J. G. Corbin, The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nat. Neurosci.* **5**, 1279–1287 (2002).
10. G. Miyoshi, S. J. Butt, H. Takebayashi, G. Fishell, Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. *J. Neurosci.* **27**, 7786–7798 (2007).
11. G. Miyoshi *et al.*, Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. *J. Neurosci.* **30**, 1582–1594 (2010).
12. M. Fogarty *et al.*, Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. *J. Neurosci.* **27**, 10935–10946 (2007).
13. H. Taniguchi, J. Lu, Z. J. Huang, The spatial and temporal origin of chandelier cells in mouse neocortex. *Science* **339**, 70–74 (2013).
14. N. Flames *et al.*, Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. *Neuron* **44**, 251–261 (2004).
15. O. Marin, M. Valiente, X. Ge, L. H. Tsai, Guiding neuronal cell migrations. *Cold Spring Harb. Perspect. Biol.* **2**, a001834 (2010).
16. D. H. Tanaka, K. Maekawa, Y. Yanagawa, K. Obata, F. Murakami, Multidirectional and multizonal tangential migration of GABAergic interneurons in the developing cerebral cortex. *Development* **133**, 2167–2176 (2006).
17. M. C. Tiveron *et al.*, Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/CXCR4 signaling in the cortical subventricular zone/intermediate zone. *J. Neurosci.* **26**, 13273–13278 (2006).
18. G. Li *et al.*, Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. *J. Neurosci.* **28**, 1085–1098 (2008).
19. G. Lopez-Bendito *et al.*, Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons. *J. Neurosci.* **28**, 1613–1624 (2008).
20. A. Sessa *et al.*, Tbr2-positive intermediate (basal) neuronal progenitors safeguard cerebral cortex expansion by controlling amplification of pallial glutamatergic neurons and attraction of subpallial GABAergic interneurons. *Genes Dev.* **24**, 1816–1826 (2010).
21. D. Tanaka, Y. Nakaya, Y. Yanagawa, K. Obata, F. Murakami, Multimodal tangential migration of neocortical GABAergic neurons independent of GPI-anchored proteins. *Development* **130**, 5803–5813 (2003).
22. G. Miyoshi, G. Fishell, GABAergic interneuron lineages selectively sort into specific cortical layers during early postnatal development. *Cereb. Cortex* **21**, 845–852 (2011).
23. J. C. Wester *et al.*, Neocortical projection neurons instruct inhibitory interneuron circuit development in a lineage-dependent manner. *Neuron* **102**, 960–975.e6 (2019).
24. K. T. Sultan, S. H. Shi, Generation of diverse cortical inhibitory interneurons. *Wiley Interdiscip. Rev. Dev. Biol.* **7**, 10.1002/wdev.306 (2018).
25. G. Miyoshi, R. P. Machold, G. Fishell, "Specification of GABAergic neocortical interneurons" in *Cortical Development, Neural Diversity and Neocortical Organization*, R. Kageyama, T. Yamamori, Eds. (Springer, Japan, 2013), chap. 5, pp. 89–126, 10.1007/978-4-431-54496-8_5.
26. G. Miyoshi, Elucidating the developmental trajectories of GABAergic cortical interneuron subtypes. *Neurosci. Res.* **138**, 26–32 (2019).
27. S. J. Butt, J. A. Stacey, Y. Teramoto, C. Vagnoni, A role for GABAergic interneuron diversity in circuit development and plasticity of the neonatal cerebral cortex. *Curr. Opin. Neurobiol.* **43**, 149–155 (2017).
28. I. Evsyukova, C. Plestant, E. S. Anton, Integrative mechanisms of oriented neuronal migration in the developing brain. *Annu. Rev. Cell Dev. Biol.* **29**, 299–353 (2013).
29. J. S. Hu, D. Vogt, M. Sandberg, J. L. Rubenstein, Cortical interneuron development: A tale of time and space. *Development* **144**, 3867–3878 (2017).
30. S. Lodato, P. Arlotta, Generating neuronal diversity in the mammalian cerebral cortex. *Annu. Rev. Cell Dev. Biol.* **31**, 699–720 (2015).
31. B. Wamsley, G. Fishell, Genetic and activity-dependent mechanisms underlying interneuron diversity. *Nat. Rev. Neurosci.* **18**, 299–309 (2017).
32. L. Lim, D. Mi, A. Llorca, O. Marin, Development and functional diversification of cortical interneurons. *Neuron* **100**, 294–313 (2018).
33. C. G. Silva, E. Peyre, L. Nguyen, Cell migration promotes dynamic cellular interactions to control cerebral cortex morphogenesis. *Nat. Rev. Neurosci.* **20**, 318–329 (2019).
34. S. Casarosa, C. Fode, F. Guillemot, Mash1 regulates neurogenesis in the ventral telencephalon. *Development* **126**, 525–534 (1999).
35. C. Fode *et al.*, A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev.* **14**, 67–80 (2000).
36. K. Yun *et al.*, Modulation of the notch signaling by Mash1 and Dlx1/2 regulates sequential specification and differentiation of progenitor cell types in the subcortical telencephalon. *Development* **129**, 5029–5040 (2002).
37. G. L. McKinsey *et al.*, Dlx1&2-dependent expression of Zfhx1b (Sip1, Zeb2) regulates the fate switch between cortical and striatal interneurons. *Neuron* **77**, 83–98 (2013).
38. V. van den Berghe *et al.*, Directed migration of cortical interneurons depends on the cell-autonomous action of Sip1. *Neuron* **77**, 70–82 (2013).
39. K. Kitamura *et al.*, Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat. Genet.* **32**, 359–369 (2002).
40. J. Close *et al.*, Satb1 is an activity-modulated transcription factor required for the terminal differentiation and connectivity of medial ganglionic eminence-derived cortical interneurons. *J. Neurosci.* **32**, 17690–17705 (2012).
41. M. Denaxa *et al.*, Maturation-promoting activity of SATB1 in MGE-derived cortical interneurons. *Cell Rep.* **2**, 1351–1362 (2012).
42. E. Azim, D. Jabaudon, R. M. Fame, J. D. Macklis, SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. *Nat. Neurosci.* **12**, 1238–1247 (2009).
43. R. Batista-Brito *et al.*, The cell-intrinsic requirement of Sox6 for cortical interneuron development. *Neuron* **63**, 466–481 (2009).
44. G. Miyoshi *et al.*, Prox1 regulates the subtype-specific development of caudal ganglionic eminence-derived GABAergic cortical interneurons. *J. Neurosci.* **35**, 12869–12889 (2015).
45. P. Liodis *et al.*, Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. *J. Neurosci.* **27**, 3078–3089 (2007).
46. Y. Zhao *et al.*, Distinct molecular pathways for development of telencephalic interneuron subtypes revealed through analysis of Lhx6 mutants. *J. Comp. Neurol.* **510**, 79–99 (2008).
47. G. Miyoshi, G. Fishell, Dynamic FoxG1 expression coordinates the integration of multipolar pyramidal neuron precursors into the cortical plate. *Neuron* **74**, 1045–1058 (2012).

48. C. Hanashima, S. C. Li, L. Shen, E. Lai, G. Fishell, Foxg1 suppresses early cortical cell fate. *Science* **303**, 56–59 (2004).
49. S. Xuan *et al.*, Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. *Neuron* **14**, 1141–1152 (1995).
50. B. Martynoga, H. Morrison, D. J. Price, J. O. Mason, Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis. *Dev. Biol.* **283**, 113–127 (2005).
51. L. Muzio, A. Mallamaci, Foxg1 confines Cajal–Retzius neuronogenesis and hippocampal morphogenesis to the dorsomedial pallium. *J. Neurosci.* **25**, 4435–4441 (2005).
52. G. Godbole *et al.*, Hierarchical genetic interactions between FOXG1 and LHX2 regulate the formation of the cortical hem in the developing telencephalon. *Development* **145**, dev154583 (2018).
53. M. Manuel *et al.*, The transcription factor Foxg1 regulates the competence of telencephalic cells to adopt subpallial fates in mice. *Development* **137**, 487–497 (2010).
54. Y. Yang *et al.*, Impaired interneuron development after Foxg1 disruption. *Cereb Cortex* **27**, 793–808 (2017).
55. F. Ariani *et al.*, FOXG1 is responsible for the congenital variant of Rett syndrome. *Am. J. Hum. Genet.* **83**, 89–93 (2008).
56. N. Bahi-Buisson *et al.*, Revisiting the phenotype associated with FOXG1 mutations: Two novel cases of congenital Rett variant. *Neurogenetics* **11**, 241–249 (2010).
57. F. Kortum *et al.*, The core FOXG1 syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *J. Med. Genet* **48**, 396–406 (2011).
58. S. Takahashi *et al.*, FOXG1 mutations in Japanese patients with the congenital variant of Rett syndrome. *Clin. Genet.* **82**, 569–573 (2012).
59. N. Brunetti-Pierri *et al.*, Duplications of FOXG1 in 14q12 are associated with developmental epilepsy, mental retardation, and severe speech impairment. *Eur. J. Hum. Genet.* **19**, 102–107 (2010).
60. A. Yeung *et al.*, 4.45 Mb microduplication in chromosome band 14q12 including FOXG1 in a girl with refractory epilepsy and intellectual impairment. *Eur. J. Med. Genet.* **52**, 440–442 (2009).
61. C. Florian, N. Bahi-Buisson, T. Bienvenu, FOXG1-related disorders: From clinical description to molecular genetics. *Mol. Syndromol.* **2**, 153–163 (2012).
62. L. E. Seltzer *et al.*, Epilepsy and outcome in FOXG1-related disorders. *Epilepsia* **55**, 1292–1300 (2014).
63. E. Brimble *et al.*, Expanding genotype-phenotype correlations in FOXG1 syndrome: Results from a patient registry. *Orphanet J. Rare Dis.* **18**, 149 (2023).
64. G. Miyoshi *et al.*, FoxG1 regulates the formation of cortical GABAergic circuit during an early postnatal critical period resulting in autism spectrum disorder-like phenotypes. *Nat. Commun.* **12**, 3773 (2021).
65. E. Yamasaki, D. H. Tanaka, Y. Yanagawa, F. Murakami, Cortical GABAergic interneurons transiently assume a sea urchin–like nonpolarized shape before axon initiation. *J. Neurosci.* **30**, 15221–15227 (2010).
66. M. Zervas, S. Millet, S. Ahn, A. L. Joyner, Cell behaviors and genetic lineages of the mesencephalon and rhombomere 1. *Neuron* **43**, 345–357 (2004).
67. J. G. Corbin, M. Rutlin, N. Gaiano, G. Fishell, Combinatorial function of the homeodomain proteins Nkx2.1 and Gsh2 in ventral telencephalic patterning. *Development* **130**, 4895–4906 (2003).
68. Q. Xu, M. Tam, S. A. Anderson, Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. *J. Comp. Neurol.* **506**, 16–29 (2008).
69. P. Flandin, S. Kimura, J. L. Rubenstein, The progenitor zone of the ventral medial ganglionic eminence requires Nkx2-1 to generate most of the globus pallidus but few neocortical interneurons. *J. Neurosci.* **30**, 2812–2823 (2010).
70. F. Balordi, G. Fishell, Mosaic removal of hedgehog signaling in the adult SVZ reveals that the residual wild-type stem cells have a limited capacity for self-renewal. *J. Neurosci.* **27**, 14248–14259 (2007).
71. P. Arlotta, B. J. Molyneaux, D. Jabaudon, Y. Yoshida, J. D. Macklis, Ctip2 controls the differentiation of medium spiny neurons and the establishment of the cellular architecture of the striatum. *J. Neurosci.* **28**, 622–632 (2008).
72. J. Yao, E. Lai, S. Stifani, The winged-helix protein brain factor 1 interacts with groucho and hes proteins to repress transcription. *Mol. Cell Biol.* **21**, 1962–1972 (2001).
73. B. Elbaz, B. Popko, Molecular control of oligodendrocyte development. *Trends Neurosci.* **42**, 263–277 (2019).
74. Y. Kawaguchi, Y. Kubota, Physiological and morphological identification of somatostatin- or vasoactive intestinal polypeptide-containing cells among GABAergic cell subtypes in rat frontal cortex. *J. Neurosci.* **16**, 2701–2715 (1996).
75. S. A. Anderson *et al.*, Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. *Neuron* **19**, 27–37 (1997).
76. H. Tabata, K. Nakajima, Multipolar migration: The third mode of radial neuronal migration in the developing cerebral cortex. *J. Neurosci.* **23**, 9996–10001 (2003).
77. S. C. Noctor, V. Martinez-Cerdeno, L. Ivic, A. R. Kriegstein, Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* **7**, 136–144 (2004).
78. M. Torii, K. Hashimoto-Torii, P. Levitt, P. Rakic, Integration of neuronal clones in the radial cortical columns by EphA and ephrin-A signalling. *Nature* **461**, 524–528 (2009).
79. J. A. Sanchez-Alcaniz *et al.*, Cxcr7 controls neuronal migration by regulating chemokine responsiveness. *Neuron* **69**, 77–90 (2011).
80. A. K. Myers *et al.*, JNK signaling is required for proper tangential migration and laminar allocation of cortical interneurons. *Development* **147**, dev180646 (2020).
81. K. Yoshioka-Kobayashi *et al.*, Coupling delay controls synchronized oscillation in the segmentation clock. *Nature* **580**, 119–123 (2020).
82. C. T. Fulp *et al.*, Identification of Arx transcriptional targets in the developing basal forebrain. *Hum. Mol. Genet.* **17**, 3740–3760 (2008).
83. G. Friocourt, J. G. Parnavelas, Identification of Arx targets unveils new candidates for controlling cortical interneuron migration and differentiation. *Front. Cell Neurosci.* **5**, 28 (2011).
84. G. Colasante *et al.*, Arx acts as a regional key selector gene in the ventral telencephalon mainly through its transcriptional repression activity. *Dev. Biol.* **334**, 59–71 (2009).
85. P. S. Hou, G. Miyoshi, C. Hanashima, Sensory cortex wiring requires preselection of short- and long-range projection neurons through an Egr-Foxg1-COUP-TFI network. *Nat. Commun.* **10**, 3581 (2019).
86. E. E. Storm *et al.*, Dose-dependent functions of Fgf8 in regulating telencephalic patterning centers. *Development* **133**, 1831–1844 (2006).
87. E. Yuan *et al.*, Graded loss of tuberin in an allelic series of brain models of TSC correlates with survival, and biochemical, histological and behavioral features. *Hum. Mol. Genet.* **21**, 4286–4300 (2012).
88. S. Hurley *et al.*, Distinct, dosage-sensitive requirements for the autism-associated factor CHD8 during cortical development. *Mol. Autism* **12**, 16 (2021).
89. J. M. Hebert, S. K. McConnell, Targeting of cre to the Foxg1 (BF-1) locus mediates loxP recombination in the telencephalon and other developing head structures. *Dev. Biol.* **222**, 296–306 (2000).
90. K. Monory *et al.*, The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* **51**, 455–466 (2006).
91. V. H. Sousa, G. Miyoshi, J. Hjerling-Leffler, T. Karayannis, G. Fishell, Characterization of Nkx6-2-derived neocortical interneuron lineages. *Cereb. Cortex* **19** (suppl. 1), i1–10 (2009).
92. H. Taniguchi *et al.*, A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* **71**, 995–1013 (2011).
93. L. Wang *et al.*, Restricted expression of mutant SOD1 in spinal motor neurons and interneurons induces motor neuron pathology. *Neurobiol. Dis.* **29**, 400–408 (2008).
94. C. Hanashima, L. Shen, S. C. Li, E. Lai, Brain factor-1 controls the proliferation and differentiation of neocortical progenitor cells through independent mechanisms. *J. Neurosci.* **22**, 6526–6536 (2002).
95. G. Miyoshi, DlxCreFoxG1GOF E18 cortex. NIH SRA database. https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1072317. Deposited 2 February 2024.