to 70,000 cal yr B.P.). It remained partly emergent during the warm Karginsk/MW Interval (23,000 to 50,000 cal yr B.P.). Paleo valleys of several major rivers (Lena, Yana, and others) can be traced on the Arctic shelf to a depth of 50 m at this time (*23*).

Yana RHS offers bifacial technology with no sign of blade making. By contrast, the Dyuktai culture combines bifaces with a blade industry based on wedge-shaped cores (*3*). Its earliest appearance in the Bering Land Bridge region dates to about 12,000 to 11,000 cal yr B.P. (*24*). Yana is 27,000 radiocarbon yr B.P. In theory, the Yana people may have crossed over the land bridge toward the end of Karginsk Interval.

It is difficult to assess similarities between Yana RHS and Clovis foreshafts. Thousands of kilometers and roughly 16,000 years separate them. Their similarity is intriguing, and they both have bifacial industries. Although a direct connection remains tenuous, the Yana RHS site indicates that humans extended deep into the Arctic during colder Pleistocene times.

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14. Apparently, these terraces present Terrace 3 (the higher one, 35 to 40 m a.w.l.) and Terrace 2 (lower terrace, 16 to 18 m a.w.l.). Terrace 1 is not presented within the bluff. Presumably, it is totally eroded here. The floodplain terrace, or Terrace 0, exists here in the form of small sections.
15. First ^{14}C dates for the site obtained by L. D. Sulerzhitsky (Geological Institute, Russian Academy of Sciences, Moscow).
16. Species identification for ^{14}C samples provided by E. A. Vanghenheim (Geological Institute, Russian Academy of Sciences, Moscow).
17. The Yana RHS bone collection offers the full range of carnivore species present at the end of the Karginsk Interval. Carnivore bones, especially those of the large species, rarely appear in natural exposures. The appearance of so many bones in one place suggests human activity in the past.
18. Species identification provided by P. A. Nikolsky (Geological Institute, Russian Academy of Sciences, Moscow).
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25. The Zhokhov-Yana Project, a long-term Russian-American effort, is directed by V. Pitulko and financed by a private foundation in New York. Unlimited thanks to M. Dashtzeren, who guided us to Yana RHS; to the Lena-Delta Wildlife Reserve, Tiksi; to E. Savchenko, VICAAR (Victory Arctic & Antarctic Research, Ltd., St. Petersburg), for logistic skills; and to L. Sulerzhitsky (Geological Institute, Moscow), who provided ^{14}C dates when we were in the field. Unlimited thanks to the 28 people who, to date, worked at the Yana site.

Supporting Online Material
www.sciencemag.org/cgi/content/full/303/5654/52/DC1
Figs. S1 to S9
Table S1

2 April 2003; accepted 14 November 2003

Foxg1 Suppresses Early Cortical Cell Fate

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During mammalian cerebral corticogenesis, progenitor cells become progressively restricted in the types of neurons they can produce. The molecular mechanism that determines earlier versus later born neuron fate is unknown. We demonstrate here that the generation of the earliest born neurons, the Cajal-Retzius cells, is suppressed by the telencephalic transcription factor Foxg1. In *Foxg1* null mutants, we observed an excess of Cajal-Retzius neuron production in the cortex. By conditionally inactivating *Foxg1* in cortical progenitors that normally produce deep-layer cortical neurons, we demonstrate that *Foxg1* is constitutively required to suppress Cajal-Retzius cell fate. Hence, the competence to generate the earliest born neurons during later cortical development is actively suppressed but not lost.

In both invertebrate (*1*, *2*) and vertebrate (*3*, *4*) central nervous system development, neuronal progenitors produce specific cell types in a characteristic temporal order. Analysis in the mammalian brain (*5–7*) and retina (*8–10*) suggests a general rule governing this process: Neural progenitors can produce cells characteristic of later but not earlier points in development. The mechanism behind this progressive restriction in progenitor potential is not understood. The laminar cell fate in the mammalian cortex provides an excellent model for studying these changes in progenitor potential. The mammalian cerebral cortex comprises six layers of neurons that are generated in an orderly sequence during development (*11*, *12*). With the exception of the Cajal-Retzius (CR) cells, which reside in layer 1, the cerebral cortex is produced in an inside-out manner. The deeper layer cells exit the ventricular zone (VZ) first, followed by more superficial cells at later periods. Hence, the birthdate of a cortical neuron is predictive of its fate (*13–15*). Furthermore, cell transplantation studies suggest that early-born classes of neurons can adopt later cell fates but not the converse (*5–7*). Thus, during cortical development, there appears to be a ratcheting mechanism by which the potential of early progenitors is progressively restricted.

The first restriction in the neuronal cell types that cortical progenitors can generate is the transition from the production of CR cells to the production of deep-layer neurons. CR cells, in addition to being the first postmitotic population, are of particular importance for the development of a properly organized cerebral cortex (*16*, *17*). CR cells reside in the subpial region of layer 1 and secrete the extracellular glycoprotein Reelin (*18*, *19*), which provides a critical signal for the guidance of later born cells that populate the cortical laminae. One of the few genes known to affect this early phase of cortical development is *Foxg1*, which encodes a winged helix transcriptional repressor (*20–22*). *Foxg1* controls the number of cells produced in the cortex and the loss of this gene results in hyp-

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oplasia of the cerebral hemispheres (Fig. 1A) (*23*, *24*). However, the principal mechanism by which *Foxg1* regulates this early step in cortical development has remained elusive.

Most cortical neurons in *Foxg1* null mutants become CR cells. To address which cortical cell types are generated in the *Foxg1*$^{-/-}$ mutants, we examined the expression of layer-specific markers at embryonic day 18.5 (E18.5), the latest time point at which these mutants are viable. In the E18.5 wild-type cortex, only the deeper layers (layers 4 to 6) have achieved their mature laminar organization. These layers are characterized by the expression of *ER81*, *Otx1*, *Foxp1*, *Foxp2*, and *ROR*β (Fig. 1C) (*25*–*28*). In situ analysis of the telencephalon revealed that the *Foxg1*$^{-/-}$ mice failed to express any of these genes, demonstrating that these cortical laminae are absent in these mutants.

In contrast, we observed that the earliest born CR neurons were not only present but supernumerary in these mice (Fig. 1C, bottom right panel). CR neurons can be identified by a number of criteria. Foremost among these is their expression of *Reelin*. In addition, CR cells express both calretinin (*29*) and CXCR4 (*30*). Consistent with the *Foxg1* telencephalon's possessing increased numbers of CR cells, we observed the widespread expression of both of these markers in the cortex of the mutant mice (fig. S1).

To determine how this overproduction of CR neurons occurs, we examined progressive stages of corticogenesis in these mutants. The cortex of *Foxg1*$^{-/-}$ mice appeared phenotypically indistinguishable from wild-type animals from E8.5 through E10.5. Furthermore, at E10.5, when the normal production of CR cells is occurring, we observed that the distribution and number of these cells is comparable in *Foxg1* null mice compared with wild-type littermates. During normal cortical development, although all postmitotic cells express microtubule-associated protein 2 (MAP2), only those cells in the most superficial layer of the cortex express CR-50. In contrast, in the *Foxg1* null mutant cortex, the entire MAP2 population appears to express CR-50 (fig. S2 and Fig. 2).

***Foxg1* cell-autonomously represses CR cell identity.** To evaluate whether the repression of CR cell production is cell-autonomously regulated by *Foxg1*, we assessed the normal expression of *Foxg1* in this population with a *Foxg1*$^{lacZ/+}$ transgenic. Except for the presence of β-galactosidase–positive cells, *Foxg1*$^{lacZ/+}$ heterozygous mice are phenotypically indistinguishable from wild-type mice (*23*). Previous studies have demonstrated that *Foxg1* is expressed in progenitor cells (*20*). We found that expression persisted in postmitotic neurons in the cortical plate (Fig. 3A). However, we observed that *LacZ* expression was excluded from the early-born CR neurons. At E18.5, individual CR cells were seen within the marginal zone (MZ), a region only sparsely populated with cells (Fig. 3C). Although a few LacZ-positive cells were also detected in the MZ of *Foxg1*$^{lacZ/+}$, these likely represent cells that migrated from the ventral telencephalon during later neurogenesis (*31*). Consistent with this notion, these cells never coexpressed CR-50 (Fig. 3C).

Our results suggest that *Foxg1* cell-autonomously represses CR cell identity. Conversely, we found evidence that *Foxg1* expression was repressed in CR cells. Specifically, the complementary expression of LacZ and CR-50 cells persisted in *Foxg1*$^{lacZ/lacZ}$ null cortex. In these mice, *LacZ* was expressed in neural progenitor cells before CR cell differentiation but was absent in mature CR cells in the cortex (fig. S3). Taken together, these results suggest that early neuronal fate is suppressed by *Foxg1* in later progenitors in the wild-type cortex.

The absence of *Foxg1* expression in CR cells in the cortex raises the question of whether these two populations are segregated from the onset of neural development. To address this issue, we crossed *Foxg1*$^{Cre/+}$ mice (*32*) onto a ROSA26 reporter (R26R) line (*33*). This allowed us to identify cells

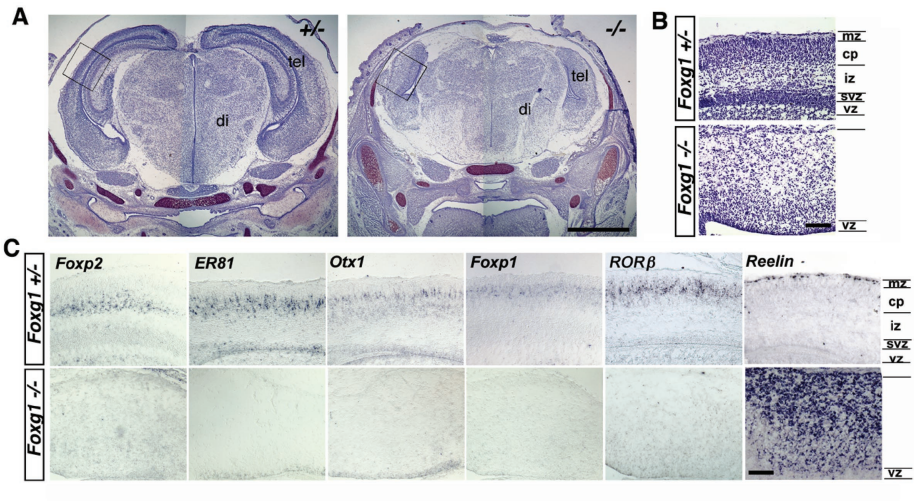

Fig. 1. Laminar specification is lost in the *Foxg1*$^{-/-}$ cortex. (**A**) Coronal sections through the forebrain stained with cresyl violet of E18.5 *Foxg1*$^{+/-}$ heterozygote (left) and *Foxg1*$^{-/-}$ (right) mice. The boxed regions in the upper left of each panel indicate the regions shown in (**B**) [left panel in (A) corresponds to top panel in (B); right panel in (A) corresponds to bottom panel in (B)]. The *Foxg1*$^{-/-}$ mutant telencephalon is substantially reduced in size, but appears thicker in lateral cortical regions as a result of the accelerated neuronal differentiation. tel, telencephalon; di, diencephalon. Scale bar, 1 mm. The MZ (mz), cortical plate (cp), intermediate zone (iz), subventricular zone (svz), and VZ (vz) of *Foxg1*$^{+/-}$ animals are morphologically indistinguishable by Nissl staining from wild-type mice. In the *Foxg1*$^{-/-}$ cortex, the VZ appears thinner and the cortical cytoarchitecture is disrupted. (**C**) Expression of the layer-specific markers (*Foxp2*, *ER81*, *Otx1*, *Foxp1*, *ROR*β, *Reelin*) in the cortex (top panels are *Foxg1*$^{+/-}$; bottom panels are *Foxg1*$^{-/-}$). Markers are aligned from the deep layers (*Foxp2*) to superficial layers (*Reelin*). In *Foxg1*$^{-/-}$ mutants, all examined markers of cortical laminae are lost. However, the number of *reelin*-expressing CR neurons in the telencephalon of *Foxg1*$^{-/-}$ mice is increased, and CR neurons are dispersed throughout the cortex. Scale bar, 100 μm.

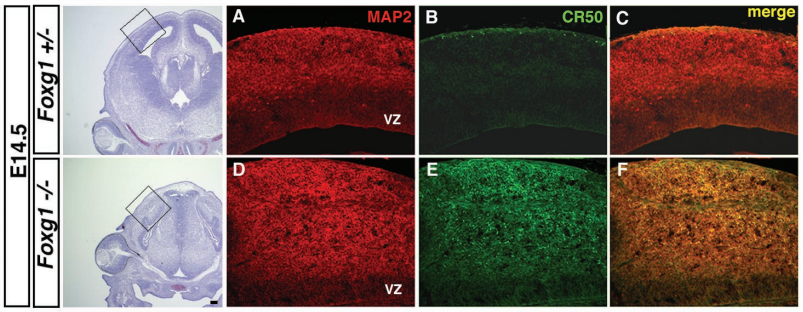

Fig. 2. Supernumerary production of CR neurons in the *Foxg1*$^{-/-}$ cortex. Boxed areas in the cresyl violet–stained coronal sections (panels on the left) represent the regions enlarged in (**A** to **C**) and (**D** to **F**), respectively. The CR cell population is visualized by CR-50 immunoreactivity (green). At E14.5, a substantial population of MAP2-positive neurons (red) has been generated in both *Foxg1*$^{+/-}$ (A) and *Foxg1*$^{-/-}$ cortex (D). CR cells are restricted to the superficial layer in the *Foxg1*$^{+/-}$ heterozygotes (B). In *Foxg1* mutant mice, all postmitotic cells appear to coexpress CR-50 and MAP2 (F). These results indicate that CR cell production progresses concomitantly with neurogenesis in the absence of *Foxg1* gene.

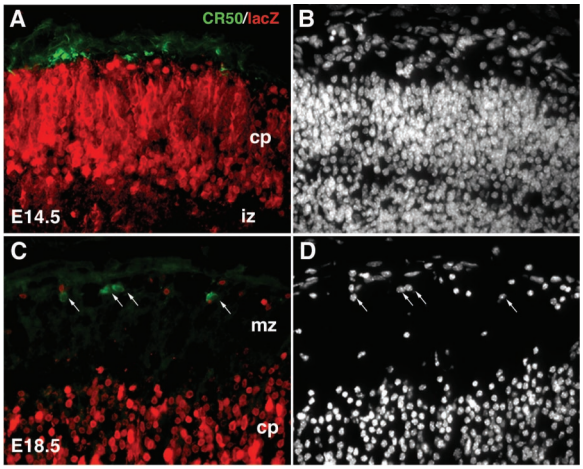

Fig. 3. *Foxg1-LacZ* expression is absent in CR cells. Coronal sections of the lateral cortex at E14.5 (**A** and **B**) or E18.5 (**C** and **D**) double-labeled to visualize CR-50 (green) and LacZ (red) [(A) and (C)] and corresponding 4′,6′-diamidino-2-phenylindole nuclear stain [(B) and (D)] in the *Foxg1*$^{lacZ/+}$ cortex. *Foxg1-lacZ* is expressed in the majority of the cells in the cortical plate (cp) but is excluded from both E14.5 (A) and E18.5 (C) MZ (mz) CR cells. Arrows in (C) and (D) indicate CR cells in the MZ in the E18.5 cortex, all of which are LacZ-negative.

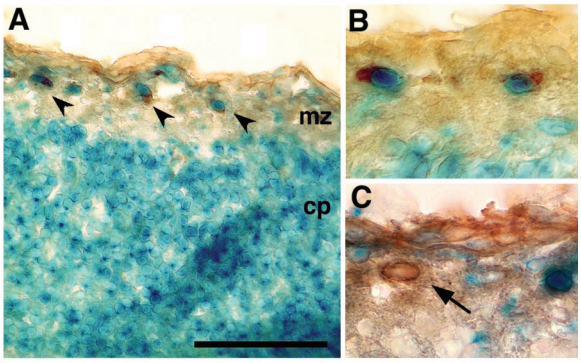

Fig. 4. *LacZ* expression in the *Foxg1*$^{Cre/+}$:R26R cortex at postnatal day 0. Double-labeling of CR-50 (brown) with β-galactosidase staining (blue) demonstrates co-labeled CR neurons [arrowheads in (**A**)] in the MZ. Scale bar, 100 μm. (**B** and **C**) Enlarged views of the cortex reveal that the majority (~90%) of CR cells express *LacZ* (B). *LacZ*-negative CR cells [arrow in (C)] were, however, occasionally detected in the MZ, indicating that small numbers of CR cells never express *Foxg1*.

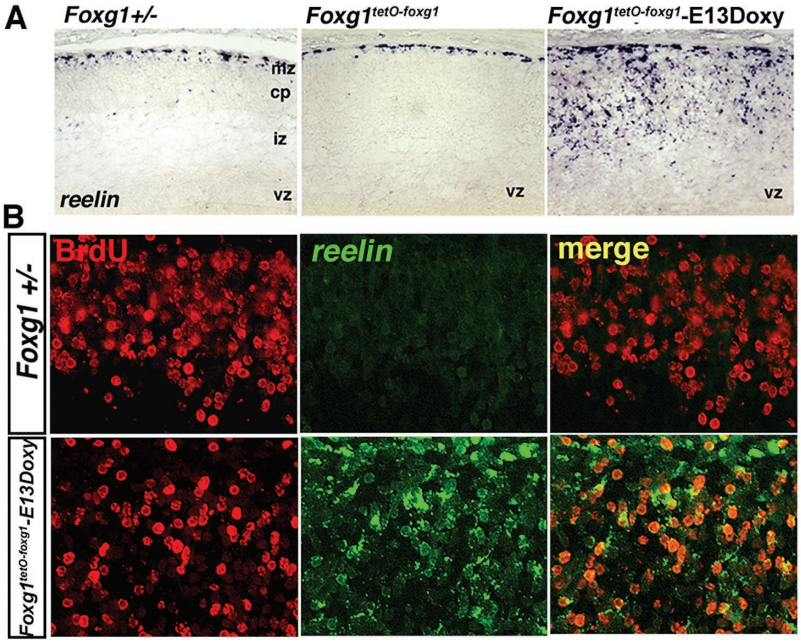

Fig. 5. CR cell production is reinitiated in *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mice (i.e., mice in which *Foxg1* gene function is removed at E13). (**A**) The expression of *reelin* in the E16.5 cortex of *Foxg1*$^{+/-}$ heterozygotes, *Foxg1*$^{tetO\text{-}foxg1}$, and *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mice. Expression is restricted to the marginal layer in *Foxg1*$^{tetO\text{-}foxg1}$ cortex [center panel in (A)]. Ectopic and supernumerary CR cells in the cortex are observed in *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mice [right panel in (A)] (fig. S5). (**B**) *Reelin* (green) and BrdU (red) double-labeling demonstrates coexpression of *reelin* in BrdU-labeled cells in *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mutants, indicating that the ectopic CR cells are newly born. In the *Foxg1*$^{+/-}$ control littermates, which were fed with doxycycline, no *reelin*-expressing cells were detected in the cortical plate.

that expressed *Foxg1* at any point of their development by evaluating whether β-galactosidase was produced. Examination of the *Foxg1*$^{Cre/+}$:R26R cortex revealed that the majority of the CR cells were *LacZ*-positive (Fig. 4, A and B). This suggests that most CR cells express *Foxg1* at some point during their development. We envisioned two scenarios: (i) The total complement of CR cells may be committed before the onset of *Foxg1* expression, making them refractory to the effects of *Foxg1*; or (ii) continued *Foxg1* expression may be required to suppress neurons born later in cortical development from adopting an early neuronal fate. This raises the question of whether the inability of later progenitors to give rise to CR cells reflects a loss of competence or suppression by *Foxg1*.

To differentiate between these possibilities, we used the tet-transactivator (tTA) system (*24*) to conditionally remove the *Foxg1* gene function in the progenitor cells after the normal birthdate of CR cells had passed. To achieve this, we replaced the endogenous *Foxg1* gene with a tTA-regulated *Foxg1* gene by generating *Foxg1*$^{lacZ/tTA}$:tet*OFoxg1*-IRES*lacZ* mice (*Foxg1*-rescued mice, designated as *Foxg1*$^{tetO\text{-}foxg1}$; IRES, internal ribosome entry site). We confirmed that in mice where *Foxg1* expression was rescued, cortical lamination was restored (fig. S4) and the CR neurons were confined to the superficial layer as in wild-type animals.

Removal of *Foxg1* function at E13 reinitiates CR cell production. We next selectively removed *Foxg1* gene function in these mice at E13 by administering doxycycline (2 mg/ml daily in drinking water). We refer to these mice as *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy. In wild-type mice (and in *Foxg1*$^{tetO\text{-}foxg1}$ mice), this is the time point when layer 5 neurons are being generated. Interestingly, the removal of *Foxg1* expression at E13 resulted in the resumption of CR cell production in the cortex (Fig. 5A, compare the left and middle panels with the right panel). Indeed, the number of CR cells in the MZ of these animals was increased by over 50% on the basis of the numbers of Reelin and CXCR4 cells in this region (fig. S5).

Newborn E13 neurons become CR cells in the absence of *Foxg1* gene function. To determine whether CR cells in *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mice arose from newborn neurons, we used 5-bromo-2′-deoxyuridine (BrdU) to pulse-label dividing cells in these mice subsequent to the administration of doxycycline. The majority of BrdU-labeled cells in these animals expressed *reelin* (Fig. 5B, bottom right panel), indicating that these CR neurons likely represent cells that exited the cell cycle subsequent to removal of *Foxg1* gene function. These results suggest that in *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mutants, neural progenitors that are normally committed to generating deeper layer neurons revert to pro-

ducing the earliest born CR neurons. To confirm this, we followed the fate of deep-layer neurons in *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mice. We found that fewer cells expressed *ER81*, and those that were generated were not colabeled with BrdU (fig. S6). This suggests that *ER81* neurons observed in these animals were born before doxycycline administration. In control mice, many *ER81* cells were BrdU-positive, showing that under normal conditions they are generated at this stage. Moreover, *ER81* cells and *reelin* cells never overlapped in the *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mutants (fig. S6). This suggests that the neurons rising from progenitor cells in which *Foxg1* gene function is removed do not adopt a hybrid deep-layer/CR cell fate.

In summary, we have provided evidence that *Foxg1* functions cell-autonomously during early phases of cortical neurogenesis to prevent the generation of CR neurons after their normal birthdate. Hence, although cortical progenitor cells are competent to produce CR neurons during later neurogenesis, *Foxg1* normally acts to suppress this early-born cell fate during the subsequent generation of the cortical laminae. It will be interesting to assess at which time point during development the progenitor cells lose their competence to revert to earliest born neurons in the absence of *Foxg1*.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/303/5654/56/DC1
Materials and Methods
Figs. S1 to S6
References

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REPORTS

The Galactic Habitable Zone and the Age Distribution of Complex Life in the Milky Way

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We modeled the evolution of the Milky Way Galaxy to trace the distribution in space and time of four prerequisites for complex life: the presence of a host star, enough heavy elements to form terrestrial planets, sufficient time for biological evolution, and an environment free of life-extinguishing supernovae. We identified the Galactic habitable zone (GHZ) as an annular region between 7 and 9 kiloparsecs from the Galactic center that widens with time and is composed of stars that formed between 8 and 4 billion years ago. This GHZ yields an age distribution for the complex life that may inhabit our Galaxy. We found that 75% of the stars in the GHZ are older than the Sun.

As we learn more about the Milky Way Galaxy, extrasolar planets, and the evolution of life on Earth, qualitative discussions of the prerequisites for life in a Galactic context can become more quantitative (*1*–*3*). The Galactic habitable zone (GHZ) (*4*), analogous to the concept of the circumstellar habitable zone (*5*), is an annular region lying in the plane of the Galactic disk possessing the heavy elements necessary to form terrestrial planets and a sufficiently clement environment over several billion years to allow the biological evolution of complex multicellular life. In order to more quantitatively estimate the position, size, and time evolution of the GHZ, we combined an updated model of the evolution of the Galaxy (*6*) with metallicity constraints derived from extrasolar planet data (*7*).

Of the factors that determine the location of the GHZ, the abundance of elements heavier than hydrogen and helium (metallicity) is particularly crucial because these elements are what terrestrial planets are composed of. The current metallicity of the Galaxy can be directly measured. However, modeling is needed to identify the metallicity distribution throughout the history of the Milky Way.

We simulated the formation of the Galaxy with the use of two overlapping episodes of accretion that correspond to the buildup of the halo and disk. The gas accretion rate falls off exponentially on a small [~1 Gyear (Gy)] time scale for the first phase and a longer time scale (~7 Gy) for the second phase. Although there is a 1-Gy delay between the onset of halo formation and the onset of thin disk formation, the formation of these two components overlaps in time. In our model, we monitor the creation of heavy elements and the exchange of matter between stars and gas. Model parameters have been chosen to reproduce the key observational constraints, namely, the radial distribution of stars, gases, and metals; the metallicity

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Supporting Online Material

Includes: Materials and Methods

Figs. S1 to S6

References

Materials and Methods

Animals

Foxg1$^{-/-}$ null mice were obtained by intercrossing *Foxg1*$^{+/-}$ mice as described previously (*S1*). Mice in which *Foxg1* expression was rescued were generated by cloning the *Foxg1* sequence into pUHD10.3 plasmid containing multiple tet-Operator sites and the IRES*lacZ* reporter cassette. The eight transgenic lines generated with this transgene were crossed with *Foxg1*$^{tTA/+}$ mice (*S2*) to examine these animals for their expression of *Foxg1* and the extent to which they compensated for the loss of this gene. Of these, 4 lines were found to have moderate expression levels of *Foxg1*. These lines were then mated with *Foxg1*$^{lacZ/+}$ to obtain the *Foxg1*$^{lacZ/+}$;tet*OFoxg1*-IRESlacZ mice. These in turn were crossed with the *Foxg1*$^{tTA/+}$ mice to generate *Foxg1*$^{lacZ/tTA}$;tetO*Foxg1*-IRESlacZ embryos, resulting in mice whose cerebral cortex resembled wild type animals (*S2*). We refer to these embryos as *Foxg1*-rescued animals (for simplicity these are designated as *Foxg1*$^{tetO\text{-}foxg1}$*)*. These embryos lack the endogenous *Foxg1* gene but express *Foxg1* under the control of tTA activation. *Foxg1* expression was abolished in these embryos when pregnant mothers were fed 0.2μg/ml or higher doxycyline concentrations in the drinking water (which we designate as *Foxg1*$^{tetO\text{-}foxg1}$-EXDoxy, where X refers to the date of doxycycline administration*)*. Embryos of this genotype were obtained from timed pregnancies where

noon of the plug date was designated as E0.5. These embryos were fixed in 4% paraformaldehyde and embedded in OCT. ß-gal staining on 10μm frozen sections was performed as described (*S2*).

In situ hybridization and Immunohistochemistry

In situ hybridization using digoxigenin-labeled probes was performed as previously described *(S2)*. *In situ* cDNA probe templates were kindly provided by T. Curran (*reelin*), T. Jessell (*ER81*), E.Morrisey (*Foxp1, Foxp2),* A. Simeone *(Otx1),* R. Slack *(ROR)*.

Double-labeling of CR-50 and MAP2 or CR-50 and LacZ immunoreactivity was performed using the mouse MOM kit (Vector Labs). Sections were incubated overnight with mouse monoclonal CR-50 antibody (1:50) (*S3*). CR-50 immunoreactivity was visualized using biotinylated -mouse antibody (1:500) followed by fluorescein-conjugated streptavidin or Texas Red-conjugated streptavidin (Vector). Sections were then incubated with -MAP2 antibody (1:250 Sigma) or --gal antibody (1:100 Biogenesis) for 1 hr followed by the biotinylated -mouse antibody or Cy3-conjugated donkey -goat antibody.

Double-labeling of LacZ and CR-50 was performed by first staining the sections with X-gal (5-bromo-4-chloro-3-indolyl--D-galactoside) for 3 hours (*S1*) and subsequently processing for CR-50 immunohistochemistry as described above. CR-50 immunoreactivity was visualized using the Vector ABC system (Vector).

BrdU labeling

For BrdU labeling, pregnant dams were given a single injection of BrdU (30mg/kg body weight, i.p.). Embryos were harvested at a given date and fixed and processed for BrdU immunohistochemistry as described previously (*S2*). Double-labeling of reelin and BrdU or ER81 and BrdU was performed by first amplifying Reelin and ER81 signals with biotinylated-tyramide followed by fluorochrome-conjugated streptavidin, treated with 1N HCl for 10min at 55ºC, then processed for BrdU immunohistochemistry as above.

Doxycycline administration

To conditionally knockout the *Foxg1* gene function, pregnant females from *Foxg1*$^{lacZ/+}$:tet*OFoxg1*-IRES *lacZ* and *Foxg*$^{1rTA/+}$ matings (i.e. *Foxg1*$^{tetO\text{-}foxg1}$*)* were fed daily from E13 onward 2mg/ml doxycycline in their drinking water with 5% sucrose (which we designate as *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy). BrdU (30mg/kg body weight) was injected 6 hrs later to label newly born cells subsequent to doxycycline administration. Embryos were harvested at E16.5 and processed as above.

FIGURE LEGENDS

Fig. S1: Increased CXCR4 and calretinin expressing cells in the *Foxg1*$^{-/-}$ null cortex. CXCR4 expression is confined to the superficial layer of the cortex, whereas calretinin is expressed in both the MZ and subplate cells in *Foxg1*$^{+/-}$ heterozygotes. In *Foxg1*$^{-/-}$ mutants, both markers are expressed throughout the cortex.

Fig. S2: Supernumerary production of CR neurons in the *Foxg1*$^{-/-}$ cortex. Boxed areas in the cresyl violet stained coronal sections panels on the left represent the regions enlarged in A-C and D-F respectively. (B) The CR cell population in the *Foxg1*$^{+/-}$ telencephalon at E11.5, visualized by CR-50 immunoreactivity (green), represents a subpopulation of the MAP2 neuronal population (A). In *Foxg1*$^{-/-}$ cortex the entire MAP2 population (D) co-expresses CR-50 (E and F).

Fig. S3: *Foxg1* expression is repressed in CR cells. Double-labeling of CR-50 (green) and lacZ (red) immunoreactivity in *Foxg1*$^{lacZ/lacZ}$ null mutants have a complementary expression pattern. *LacZ* is expressed in the VZ progenitor cells in the *Foxg1*$^{lacZ/lacZ}$ telencephalon but is excluded from mature CR cells expressing CR-50. The boxed region in the top right panel indicates an enlarged view, demonstrating that L*acZ* and CR-50 populations are mixed but distinct.

Fig. S4: Cortical lamination is normal in *Foxg1*$^{tetO\text{-}foxg1}$ (i.e. *Foxg1*-rescued animals). Expression of deep-layer cortical markers (*Otx1, Foxp2, ER81, Foxp1*) is restored in the E18.5 *Foxg1*$^{tetO\text{-}foxg1}$ telencephalon. *Reelin*-expressing CR neurons are restricted to the marginal zone in *Foxg1*$^{tetO\text{-}foxg1}$ animals and resemble that seen in heterozygote littermates.

Fig. S5: Increased numbers of CR cells are observed in the MZ of *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mice. The number of *Reelin* and *CXCR4* expressing cells is increased in the MZ of *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mutant mice (right panel) compared to the doxycycline fed heterozygote littermates (left panel). Quantitation of number of cells in the MZ of these mice showed an increase of ~50% in mutant versus heterozygote littermates. Single (P<0.01) and double (P<0.001) asterisks indicate statistical significance.

Fig. S6: Newly born cells in the *Foxg1*$^{tetO\text{-}foxg1}$-E13Doxy mutant cortex do not adopt a deep-layer fate. *ER81* (green) and BrdU (red) double-label shows that newborn cells are *ER81*-negative (middle row). In the heterozygote controls, these two populations partially overlap (top row). The newly born *reelin*-positive cells (red) do not co-express *ER81* (bottom row) suggesting that the neurons rising from progenitor cells in which *Foxg1* gene function is removed do not adopt a hybrid deep-layer/CR cell fate.

Supporting References

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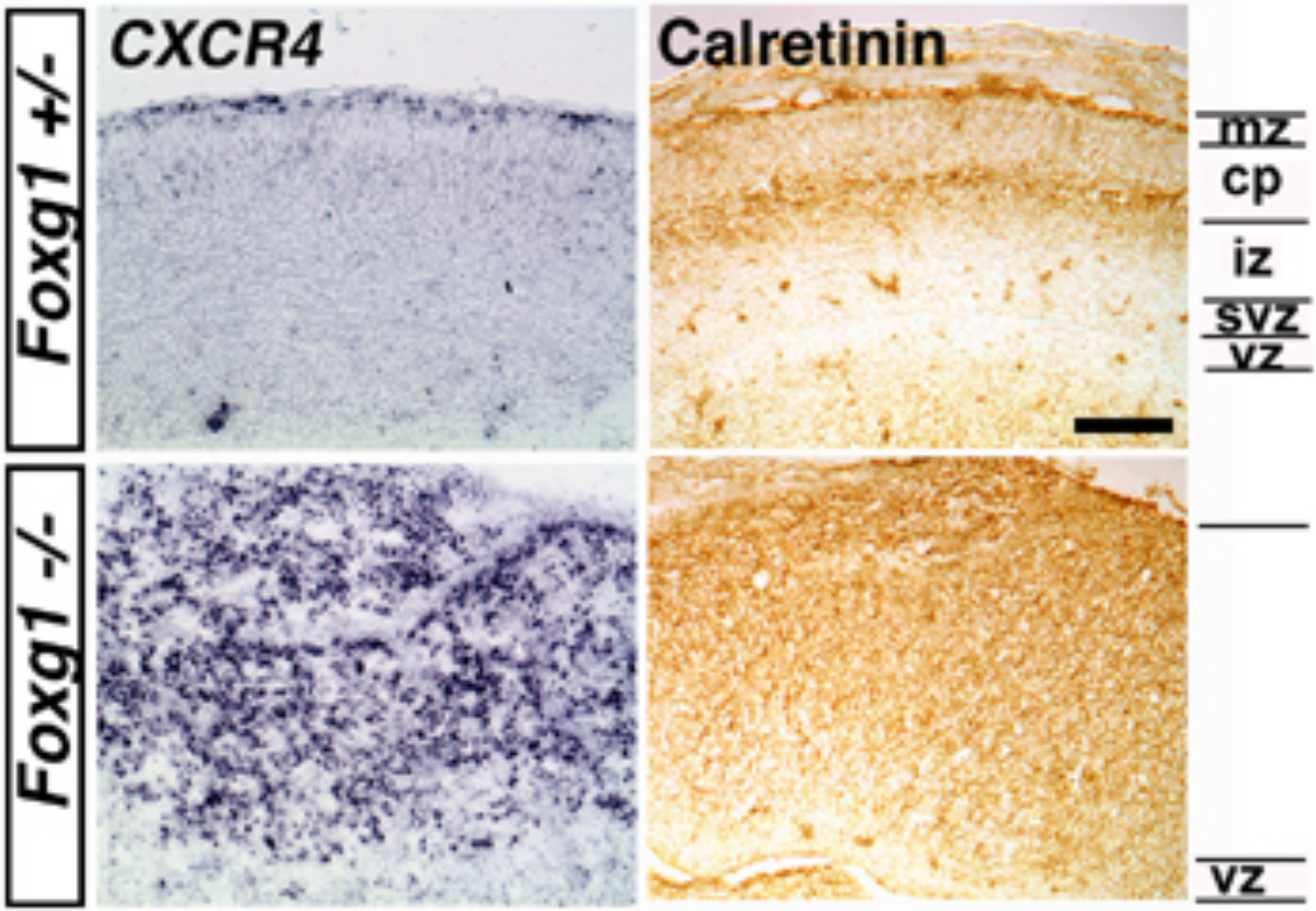
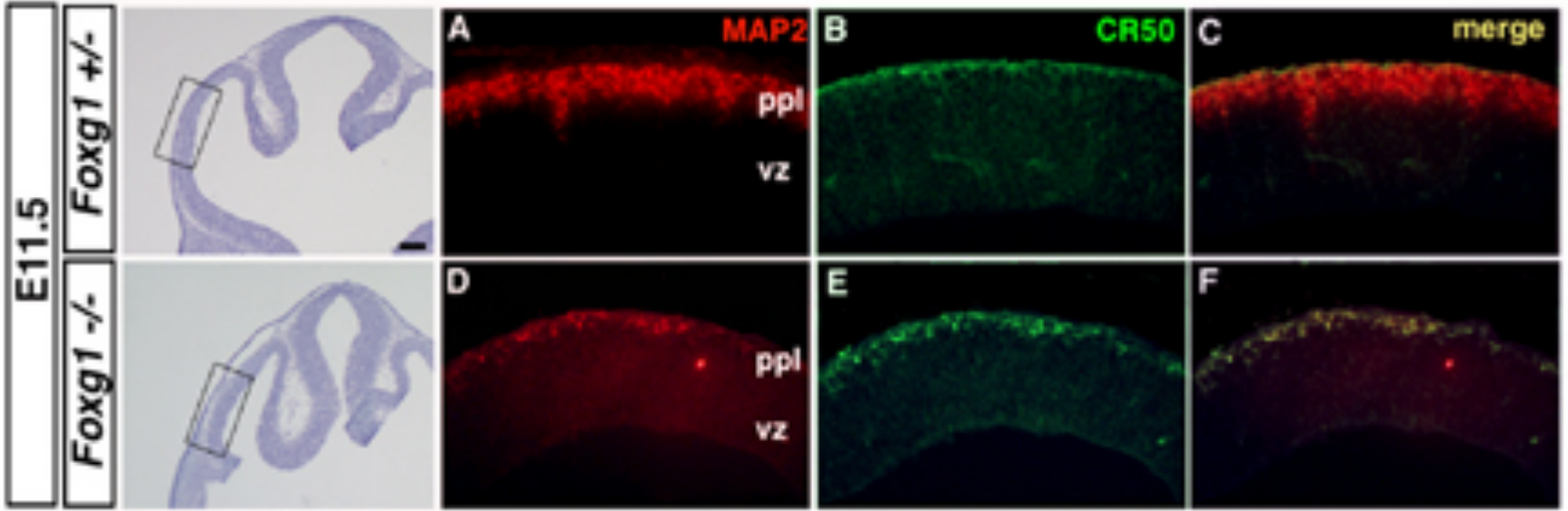
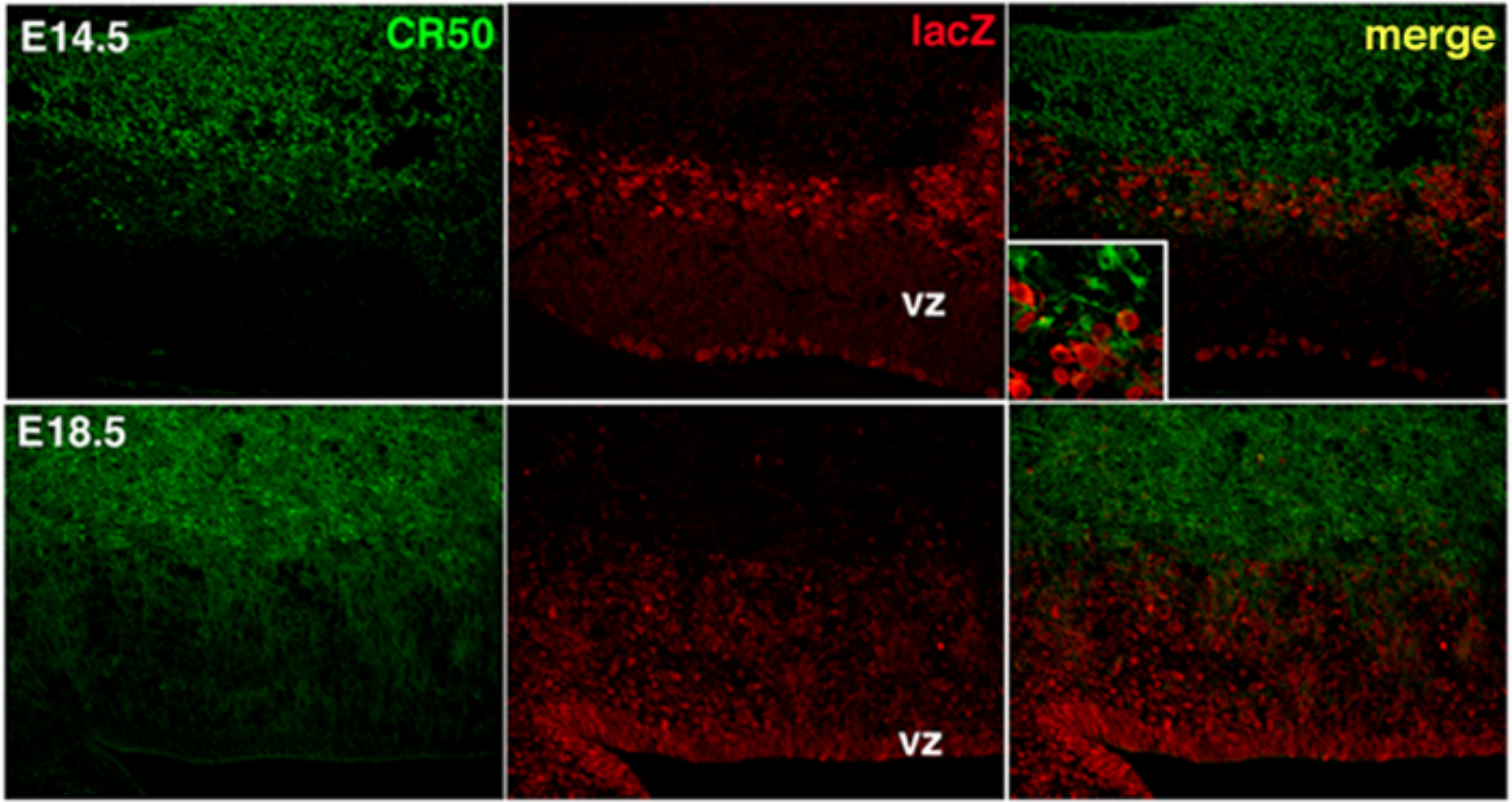
S3. M. Ogawa *et al.*, *Neuron* **14**, 899-912 (May, 1995).


Foxg1 +/-
CXCR4
Calretinin
mz
cp
iz
svz
vz
Foxg1 -/-
vz


E11.5
Foxg1 +/-
Foxg1 -/-
A
MAP2
ppl
vz
B
CR50
C
merge
D
ppl
vz
E
F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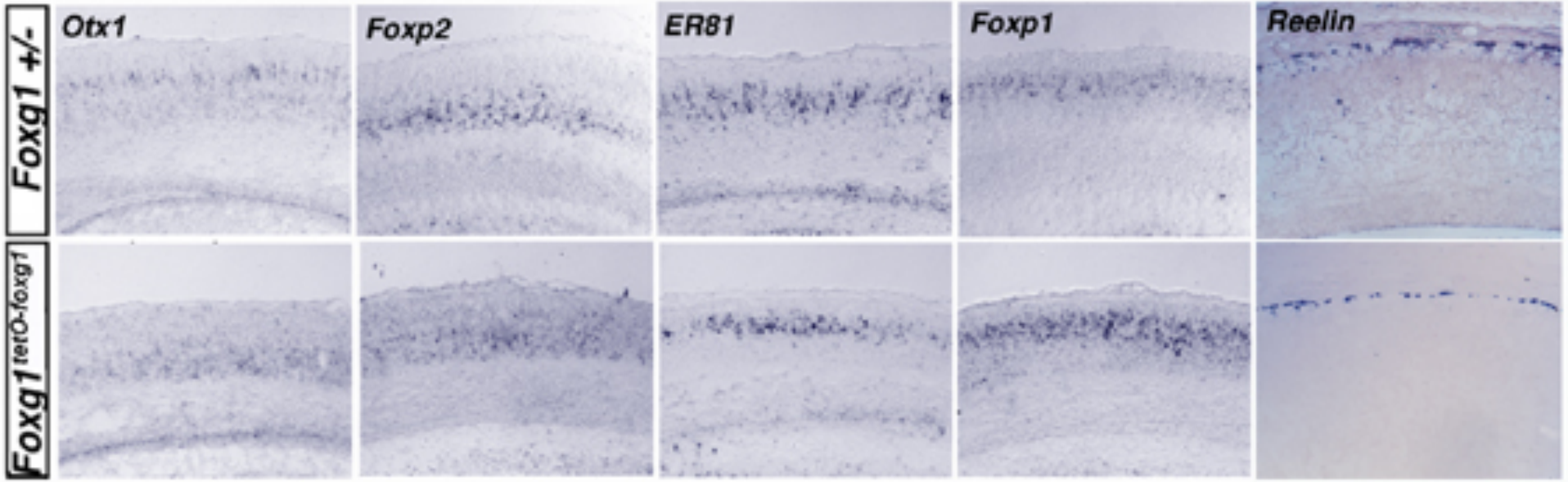
CR50
lacZ
merge
VZ
E18.5
VZ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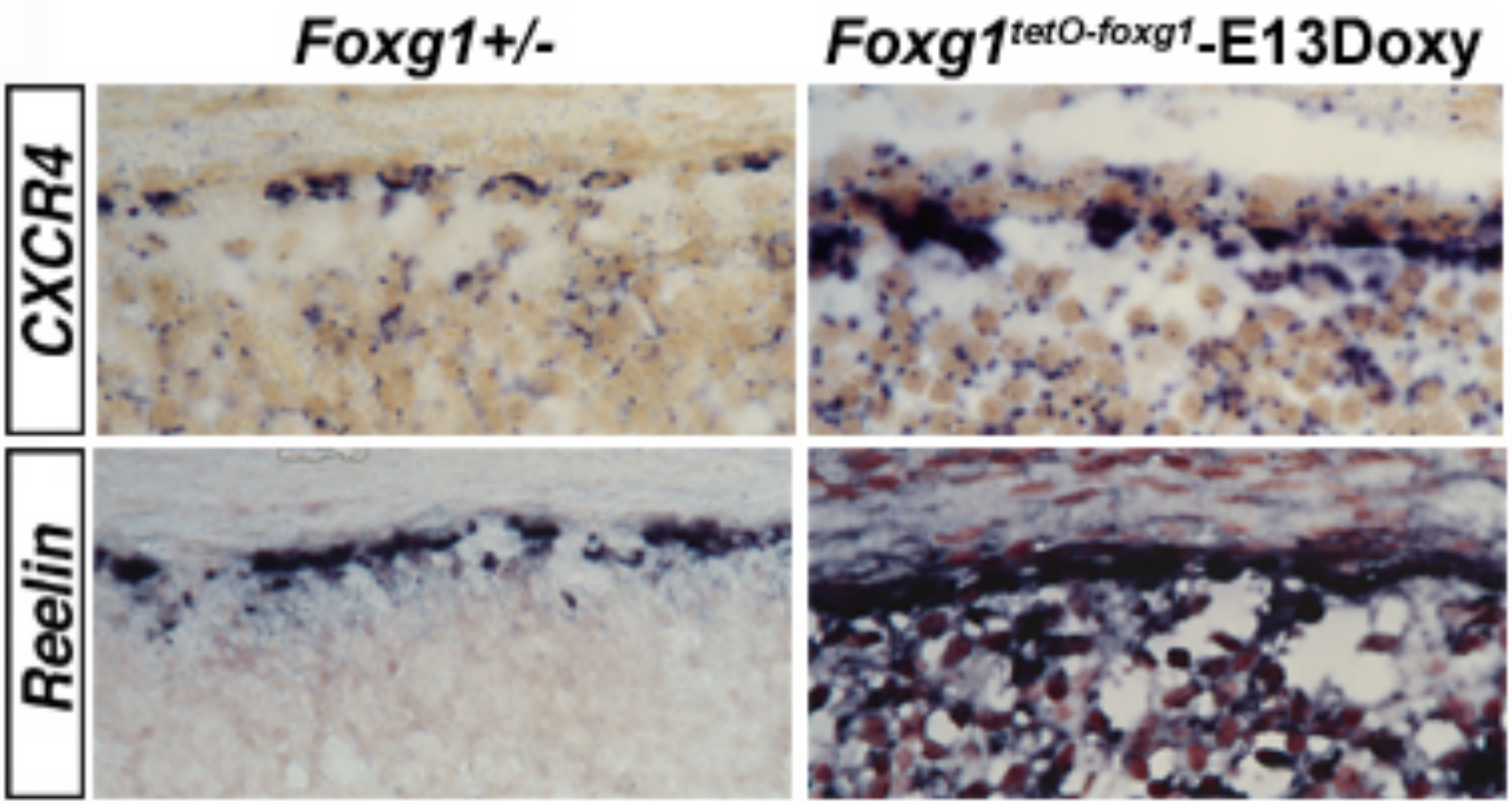
Otx1
Foxp2
ER81
Foxp1
Reelin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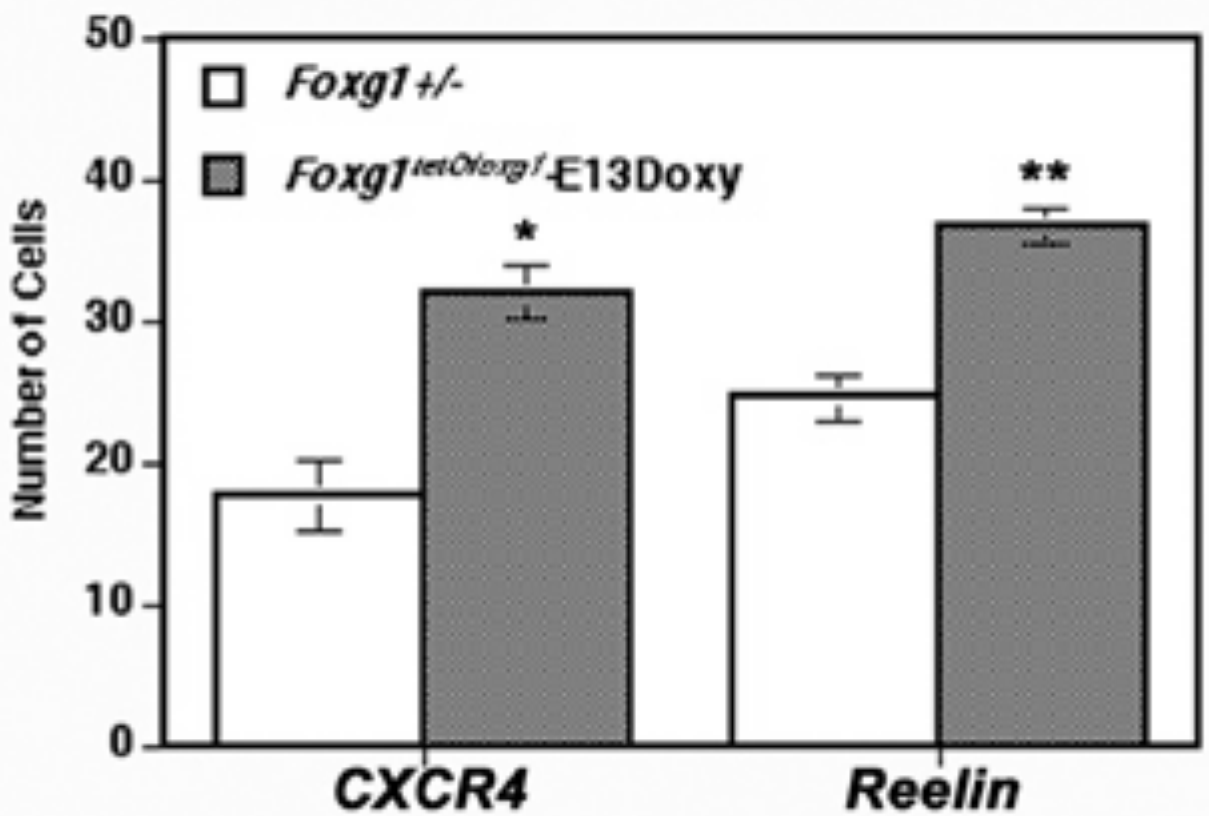
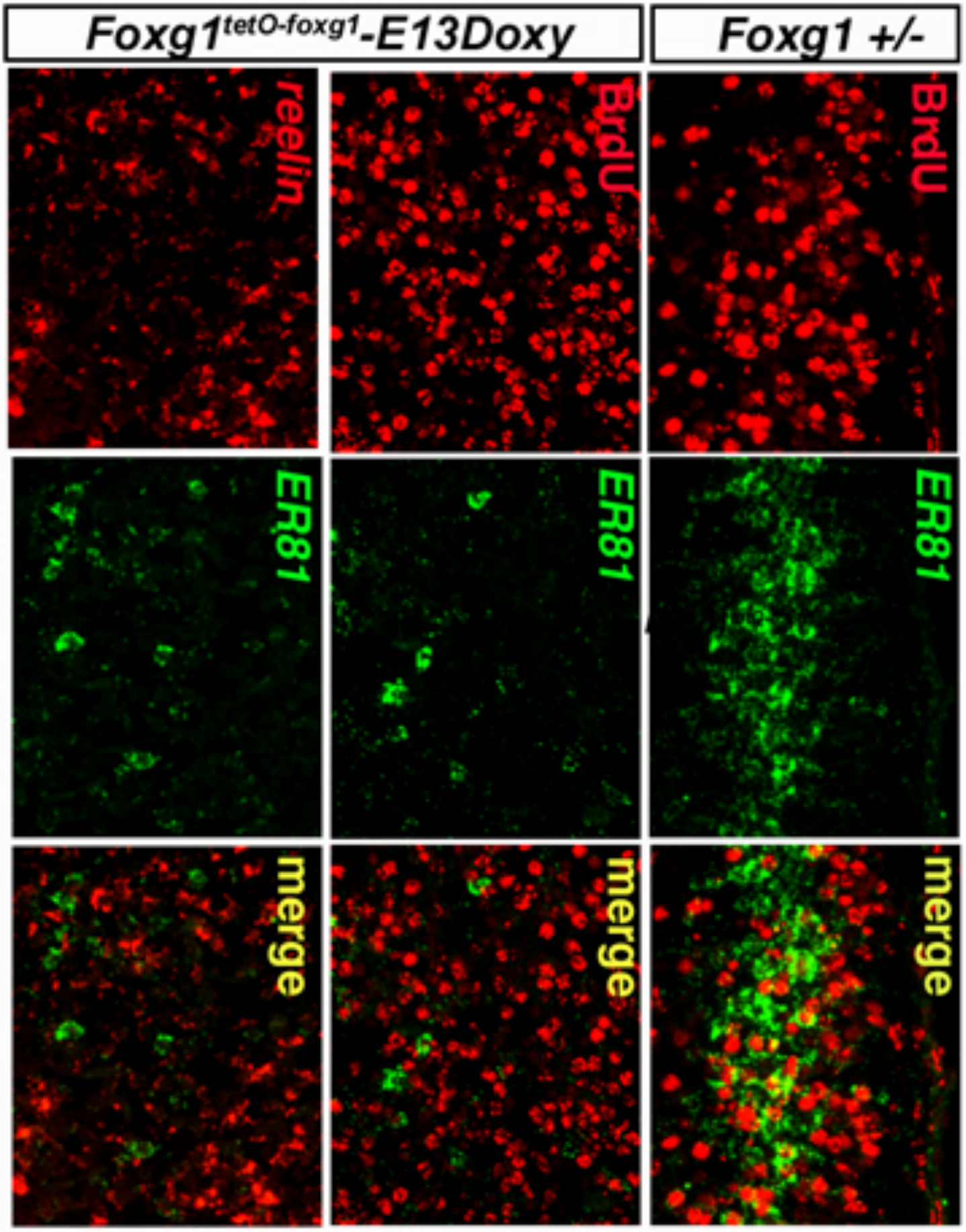
Foxg1tetO-foxg1-E13Doxy
CXCR4
Reelin


Foxg1+/-
Foxg1tetOfoxg1-E13Doxy
Number of Cells
50
40
30
20
10
0
*
**
CXCR4
Reelin


Foxg1tetO-foxg1-E13Doxy
Foxg1 +/-
reelin
BrdU
BrdU
ER81
ER81
ER81
merge
merge
merge