Nuclear factor one X regulates Bobby sox during development of the mouse forebrain.

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Abstract

The transcription factor Nuclear factor one X (NFIX) plays a central role during the development of the neocortex and hippocampus, through the activation of astrocyte-specific gene expression and the repression of progenitor-specific pathways. However, our understanding of transcriptional targets of NFIX during cortical development remains limited. Here we identify the transcription factor *Bobby sox (Bbx)* as a target for NFI-mediated transcriptional control. BBX is expressed within ventricular zone progenitor cells within the developing neocortex and hippocampus, and its expression is upregulated in *Nfix*$^{-/-}$ mice. Moreover, we reveal that NFIX can repress *Bbx* promoter-driven expression. Collectively, these data suggest that *Bbx* is a downstream target of NFIX during development of the forebrain.

Introduction

The transcription factors of the NFI family (NFIA, NFIB, NFIC and NFIX) are emerging as important regulators of neural development. NFIX is expressed widely within the developing brain, including within the ventricular zone and dentate gyrus of the embryonic hippocampus, and *Nfix*$^{-/-}$ mice exhibit abnormal development of the neocortex, hippocampus and cerebellum (Campbell et al., 2008; Piper et al., 2011). At a mechanistic level, NFIX has been shown to promote the expression of astrocyte-specific genes in human glioblastoma-derived cell lines (Brun et al., 2009), and to repress expression of the progenitor cell-specific gene *Sox9 in vivo* (Heng et al., 2013). As such, mice lacking *Nfix* exhibit prolonged progenitor cell self-renewal, concomitant with delayed neuronal and glial differentiation within the hippocampus and neocortex (Heng et al., 2013).

Despite these findings, our understanding of the genes regulated by NFIX during development of the forebrain remains limited. To identify further potential targets of NFIX we used a multifaceted approach. Firstly, we mined a recently published microarray performed on the hippocampus of embryonic day (E) 16 *Nfix*$^{-/-}$ mice to identify transcription factors that were misregulated in the mutant mice (Heng et al., 2013). We then ascertained if potential targets were expressed within the developing forebrain using gene expression data within the Allen Brain Atlas. Finally, we used an *in silico* bioinformatic approach to identify whether these putative NFIX target genes contained an NFI binding motif close to their transcription start site. This analysis identified *Bobby sox* (also called *Hbp2*) as a potential target downstream of NFIX. Here we reveal that BBX is expressed by neural progenitor cells within the developing neocortex and hippocampus, and that there are significantly more cells expressing BBX in the neocortical and hippocampal ventricular zone of E16 *Nfix*$^{-/-}$ mice. Finally, we demonstrate that NFIX can regulate *Bbx*-promoter driven transcriptional activity, suggestive of NFIX regulating *Bbx* transcription during forebrain development.

Methods

Mouse strains

C57Bl/6J *Nfix*$^{+/+}$ and *Nfix*$^{-/-}$ littermate mice were used in this study. Animals were bred at the University of Queensland under approval from the institutional Animal Ethics Committee.

Immunohistochemistry

Immunohistochemistry using the chromogen 3,3' diaminobenzidine on floating vibratome sections (50 μm) was performed as described previously (Heng et al., 2013) using a rabbit-anti-BBX antibody (Abcam, ab94418, 1/10,000). Immunohistochemistry was performed on 6 μm paraffin sections as described previously (Barry et al., 2008) using the anti-BBX antibody (1/1,000). For all immunohistochemical analyses, at least 5 littermate wild-type and *Nfix*$^{-/-}$ brains were analyzed. Sections from comparable positions along the rostrocaudal axis were imaged using an upright microscope (Zeiss upright Axio-Imager Z1) fitted with an Axio-Cam HRc camera. For counts of BBX-positive cells within the ventricular zone of the neocortex and hippocampus, the number of immunopositive cells per 100 μm of ventricular zone was quantified using images of immunohistochemically labelled paraffin sections at E16. For all experiments, data represent pooled results from at least 5 wild-type and 5 *Nfix*$^{-/-}$ brains. Counts were performed blind to the genotype of the sample. Statistical analyses were performed using a two-tailed unpaired *t*-test. Error bars represent the standard error of the mean.

Immunofluorescence staining

Immunofluorescence staining was performed on 6 μm paraffin sections using the BBX antibody (1/500) and a mouse-anti-nestin antibody (Developmental Studies Hybridoma Bank, rat-401, 1/100). The secondary antibodies used were a goat-anti mouse IgG Alexa 488 and a goat-anti-rabbit IgG Alexa 594 (Invitrogen; both 1/500). Images were obtained using an upright microscope (Zeiss upright Axio-Imager Z1).

Bioinformatic promoter screen

The NFI binding motif was generated as reported previously (Heng et al., 2013) from published chromatin immunoprecipitation-sequencing (ChIP-seq) data for NFI (pan-

NFI antibody used) (Pjanic et al., 2011). The DNA-binding domains of all NFI proteins are highly similar (Mason et al., 2009). In brief, we performed motif discovery using the MEME algorithm (Bailey et al., 2009) on ChIP-seq peaks redeclared using the ChIP-Peak algorithm (Schmid and Bucher, 2010) from the published ChIP-seq "tag" data for NFI. We then identified potential NFI binding sites by scanning the complete mouse genome downloaded from the UCSC Genome Browser (mm9, July 2007) (Fujita et al., 2011) using the MEME-derived motif and the FIMO motif-scanning program (Grant et al., 2011). FIMO was run on the mouse genome (without repeat masking) using a 0-order background generated on the entire mouse genome, and a pseudocount of 0.1. All potential binding sites with p-value $\leq 10^{-4}$ were reported. The putative NFI binding sites near the promoter of *Bbx* (chr16:50437453-50437467) were identified manually by viewing the FIMO output using the UCSC genome browser. We identified two potential sites upstream of the transcription start site (-4951 and -5758 relative to the transcription start site) and one downstream of the transcription start site (+222). We focussed on the first site upstream of the transcription start site for further analysis. To search for other potential transcription factor binding sites within the *Bbx* promoter, we searched the region around the transcription start site of *Bbx* (-6000 to +1000 base pairs) using FIMO and the SELEX motifs from Jolma *et al*. 2013 (Jolma et al., 2013), the UniPROBE mouse motifs (Newburger and Bulyk, 2009) and the JASPAR vertebrate motifs (Sandelin et al., 2004). Collectively these data provide a total of 1271 potential motifs. We identified 2692 predicted sites within this region at an (unadjusted) p-value threshold of 10^{-4}. To refine this list, we narrowed our screen to sites with FIMO $p < 10^{-6}$ that were within 250 base pairs either side of the putative NFI site located at -4951. This revealed two additional motifs, namely for the proteins Gm397 (-4754) and ZFP281 (-5208).

Electrophoretic mobility shift assay

Electrophoretic mobility shift assays were performed using radiolabeled annealed oligonucleotides as described previously (Heng et al., 2013). Nuclear extracts were isolated from COS cells expressing an HA-tagged NFIX expression construct or an HA-tagged AP2 expression construct. Oligonucleotide sequences were: *Bbx* upper – 5'-

CCGGGTGAGTGAGGCCTCCTTGGGCTCTTCCCCATCCACATTAACTTGTTC-

3'; *Bbx lower* 5'-CCGGGAACAAGTTAATGTGGATGGGGAAGAGCCCAAGGAGGCCTCACTCAC-3'.

Luciferase assay

The constructs used in the luciferase assay were an NFIX expression construct and a construct containing 942 base pairs of the upstream promoter of *Bbx* (from -4426 to -5368 relative to the transcription start site) that included the putative NFI binding site at -4951 cloned into the pGL4.23 vector (Promega). DNA was transfected into Neuro2A cells. A Renilla luciferase vector was added to each transfection as a normalization control. After 48 hours, luciferase activity was measured using a dual luciferase system (Promega). Within each experiment, each treatment was replicated 6 times. Each experiment was also independently replicated a minimum of three times. Statistical analyses were performed using an ANOVA with a *post hoc* Tukey's test. Error bars indicate the standard error of the mean.

Results

Microarray analysis performed on the hippocampus of littermate E16 *Nfix*$^{+/+}$ and *Nfix*$^{-/-}$ mice has previously identified over 1000 genes as being differentially expressed within the mutant, with over 65 transcription factors being upregulated (Heng et al., 2013). To determine which of these may be putative NFIX targets as assessed by being expressed in the same brain regions as NFIX, we first interrogated the mRNA expression profiles of these genes within the Allen Brain Atlas. One gene whose expression pattern within the nascent forebrain was highly specific was the transcription factor *Bbx*, a member of the SOX (Sry-related HMG box) family. *Bbx* showed a specific pattern of expression within the emerging forebrain, being expressed by progenitor cells within the ventricular zone of the embryonic neocortex and hippocampus (http://developingmouse.brain-map.org, search reference *Bbx*) at E15.5. To more clearly delineate the expression of this factor in the developing forebrain, we analysed the expression of BBX in the forebrain at a protein level using immunohistochemistry on 6 µm paraffin sections. At E14, expression of BBX was clearly evident within the nuclei of cells in the neocortical and hippocampal ventricular zone (Fig. 1***A-E***). Furthermore, co-immunofluorescence staining with the neural progenitor cell marker nestin revealed that BBX expressing nuclei within the ventricular zone were surrounded by nestin-positive fibres, suggesting that progenitor cells within the ventricular zone do indeed express BBX (Fig. 1***F-H***). At E16, expression of BBX within the hippocampus was more widespread, with ventricular zone progenitor cells and cells within the subventricular zone and intermediate zone expressing this transcription factor. Moreover, cells within the dentate migratory stream were also immunoreactive for BBX (Fig. 1***I-L***). Together, these findings suggest that BBX plays a role in forebrain development.

We next investigated the expression of BBX within the cortex of E16 *Nfix*$^{-/-}$ mice. In line with our previous microarray data, we observed using 50 µm coronal brain sections that the expression of BBX within the ventricular zone of the neocortex and hippocampus of *Nfix*$^{-/-}$ mice was elevated when compared to littermate controls (Fig. 2***A***, ***B***). To quantify this, we performed immunohistochemistry against BBX on 6 µm paraffin sections and counted the number of immunopositive cells within the ventricular zone of the neocortex and hippocampus of wild-type and mutant brains (Fig. 2***C-F***). We found significantly more neocortical and hippocampal ventricular

zone cells expressing BBX within the mutant (Fig. 2***G***), suggesting that this transcription factor is a target for transcriptional repression by NFIX during development.

To ascertain whether *Bbx* was a direct target for transcriptional regulation by NFIX, we next performed an *in silico* bioinformatic screen of the *Bbx* promoter, looking for putative NFI binding sites. This revealed three potential NFI binding sites near the *Bbx* transcription start site (Fig. 3***A***). We focussed on the first site upstream of the transcription start site, as this was predicted *in silico* to have a high probability of being a *bona fide* binding site (Fig. 3***B***). Moreover, scanning around this putative NFI site also identified potential DNA binding motifs for the transcription factors Gm397 and ZFP281 (Fig. 3***A***). The latter has been implicated in controlling the pluripotency of embryonic stem cells (Wang et al., 2008), suggestive of this region of the *Bbx* promoter being important for the expression of *Bbx in vivo*. To assess the functional relevance of the predicted NFI site at -4951, we performed electrophoretic mobility shift assays using nuclear extracts from NFIX-expressing COS cells. Oligonucleotide probes encompassing the genomic region containing the putative NFI site at -4951 were bound by a molecule within the nuclear extract (Fig. 3***C***). Using an anti-HA antibody (the NFIX expression construct carries a HA-tag), we further demonstrated a decrease in the mobility of the bound oligonucleotide in a supershift assay (Fig. 3***C***), suggesting that NFIX can directly bind to this site within the *Bbx* promoter. Finally, we performed a reporter gene assay, using a luciferase construct under the regulatory control of a 942 base pair fragment of the *Bbx* promoter that included the NFI motif at -4951. NFIX was able to repress luciferase expression controlled by the *Bbx* promoter fragment (Fig. 3***D***). Collectively, these data suggest that NFIX can repress the transcription of *Bbx*.

Discussion

NFIX has been shown to regulate multiple aspects of nervous system development, including repressing progenitor-specific genes such as *Sox9* during embryonic cortical development (Heng et al., 2013) and activating the expression of glial-specific genes such as *Gfap, brain fatty acid binding protein and Ykl-40* within cultured cells *in vitro* (Brun et al., 2009; Singh et al., 2011). Here, we demonstrate that NFIX also regulates the expression of the novel transcription factor *Bbx* during cortical development, revealing another facet of the program through which NFIX modulates corticogenesis.

BBX is strongly expressed by ventricular zone neural progenitor cells within the cortex, and significantly more cells expressing this transcription factor are found in the ventricular zone of *Nfix*$^{-/-}$ mice. An alternative explanation for the phenotype observed here is that the increase in cell numbers is the result of post-mitotic neurons failing to migrate away from the ventricular zone. However, we consider this unlikely, as our studies on different *Nfi* mutant strains has revealed that a key role for these transcription factors is to promote neural progenitor cell differentiation within the forebrain, and that in their absence, the production of post-mitotic progeny is significantly delayed (Barry et al., 2008; Piper et al., 2010). For example, we have recently shown that neural progenitor cell differentiation is delayed within the hippocampus and neocortex of *Nfix*$^{-/-}$ mice at E16, with resulting delays in forebrain neurogenesis being evident (Heng et al., 2013). Rather, our findings here point to BBX-expressing ventricular zone progenitor cells being more prevalent in the absence of NFIX. As NFIX is postulated to drive progenitor cell differentiation within the embryonic forebrain (Heng et al., 2013), these data suggest that BBX may promote progenitor cell self-renewal. However, the means by which BBX functions in this regard is currently unclear. BBX is a member of the SOX family of transcription factors, many of which have been implicated in neural progenitor cell self-renewal (Bani-Yaghoub et al., 2006; Scott et al., 2010), indicating that BBX may play a functionally analogous role developmentally. Moreover, BBX has been reported to bind DNA and to promote the G1/S phase transition in yeast (Sanchez-Diaz et al., 2001), providing further evidence of a role for this transcription factor in cell cycle progression.

Interestingly, *Bbx* knockout mice have been generated by the European Conditional Mouse Mutagenesis Program (Friedel et al., 2007). Although these mice exhibit some phenotypic abnormalities, such as altered heart weight, analysis of the nervous system of postnatal knockout mice (at 6 and 16 weeks) did not reveal any gross morphological abnormalities using cresyl violet staining (http://www.sanger.ac.uk/mouseportal/phenotyping/MAEZ/mp-report/nervous-system/). These findings do not, however, rule out a role for BBX during forebrain development, and future studies aimed at analyzing cortical neural progenitor cell self-renewal and their subsequent neuronal and glial differentiation during the period of corticogenesis may reveal deficits that will help elucidate the role of this transcription factor within the embryonic brain.

Figure Legends

Figure 1. Expression of BBX in the developing hippocampus.

A-E, Coronal paraffin section (6 μm) of an E14 brain showing the expression of BBX via immunohistochemistry (IHC). BBX was strongly expressed within the ventricular zone of the neocortex (arrowhead in ***A***) and hippocampus (arrow in ***A***). ***B,*** BBX is expressed by cells within the ventricular zone of the hippocampus (arrow in ***B***). ***C*** is a magnified view of the boxed area in ***B***, revealing the nuclear localisation of BBX within ventricular zone cells of the hippocampus. ***D,*** BBX is also expressed by ventricular zone cells within the neocortex. ***E***, At E14, there is no immunoreactivity for BBX within the developing thalamus. ***F-H,*** Co-immunofluorescence staining of a coronal 6 μm paraffin section reveals that BBX-expressing nuclei within the hippocampal ventricular zone (***F***) are surrounded by nestin-expressing fibres (***G***), suggesting that neural progenitor cells express BBX (arrowheads in ***F, H***). ***I-L***, Coronal paraffin section (6 μm) of an E16 brain showing the expression of BBX (***I***). BBX is expressed by cells within the neocortical ventricular zone (nVZ; arrowheads in ***J***) and the hippocampal ventricular zone (hVZ; arrows in ***J***) at this age, but also by cells within the dentate migratory stream (arrows in ***K***) and by cells within the subventricular zone and intermediate zone (arrows in ***L***). Scale bar (in ***L***): ***A*** 600 μm; ***B*** 300 μm; ***C-E***, 50 μm; ***F-H*** 25 μm; ***I,*** 250 μm; ***J-L***, 75 μm.

Figure 2. BBX expression is upregulated in the forebrain of *Nfix*$^{-/-}$ mice at E16.

A, B, Coronal vibratome sections (50 μm) of E16 wild-type and *Nfix*$^{-/-}$ brains, showing the expression of BBX. There were more cells in the hippocampal and neocortical ventricular zone of the mutant expressing BBX (arrows in ***B***) than within the control (arrowheads in ***A***). Coronal paraffin sections (6 μm) of wild-type (***C, E***) and *Nfix* mutant (***D, F***) brains processed for immunohistochemistry against BBX. There were significantly more cells expressing BBX within the neocortical and hippocampal ventricular zone of the mutant (arrows in ***D, F***) than the control (arrowheads in ***C, E***) at this age (***G***). VZ = ventricular zone. ** $p < 0.01$, *t*-test. Scale bar (in ***F***): ***A, B***, 600 μm; ***C-F***, 75 μm.

Figure 3. NFIX can repress *Bbx*-promoter driven transcriptional activity.

A, Schematic of the *Bbx* promoter, showing the three predicted NFI binding sites reported by FIMO. We focussed on the first site upstream of the transcription start site, at -4951. The zoomed up region of the *Bbx* promoter shown below depicts the two putative DNA binding motifs in the vicinity of the -4951 site, namely binding motifs for Gm397 and ZFP281. Also shown is the region of the promoter used in the luciferase assay (*Bbx* luc construct, grey rectangle). ***B***, Sequence of the NFI binding site reported by FIMO at -4951 base pairs upstream of the *Bbx* transcription start site. The NFI binding motif used within the genome-wide scan is also shown. ***C***, Nuclear extracts from COS cells expressing an HA-tagged AP2 construct (lanes 1 and 2) or an HA-tagged NFIX construct (lanes 3 and 4) were used in an electrophoretic mobility shift assay. The extracts were incubated with a radiolabeled probe containing the -4951 site. Whereas the non-specific transcription factor AP2 did not demonstrate any specific binding to the oligonucleotide probe, the nuclear extract from the NFIX-expressing cells exhibited significant binding to the probe (asterisk, lane 3). Moreover, the addition of an anti-HA antibody to the binding reaction depleted this complex and produced a supershifted complex (SS; lane 4). FP = free probe. ***D***, Transfection of Neuro2A cells with an *Nfix* expression vector (*Nfix* pCAGIG) elicited no luciferase activity in a reporter gene assay, whereas transfection of a luciferase construct under the control of the *Bbx* promoter element elicited robust induction of the reporter gene. Co-transfection of *Nfix* with the *Bbx* promoter reporter yielded a significantly reduced level of luciferase activity. ** $p < 0.01$, ANOVA.

Competing Interests

The authors declare that they have no conflicts of interest.

References

Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., Noble, W.S., 2009. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res 37, W202-208.

Bani-Yaghoub, M., Tremblay, R.G., Lei, J.X., Zhang, D., Zurakowski, B., Sandhu, J.K., Smith, B., Ribecco-Lutkiewicz, M., Kennedy, J., Walker, P.R., Sikorska, M., 2006. Role of Sox2 in the development of the mouse neocortex. Dev Biol 295, 52-66.

Barry, G., Piper, M., Lindwall, C., Moldrich, R., Mason, S., Little, E., Sarkar, A., Tole, S., Gronostajski, R.M., Richards, L.J., 2008. Specific glial populations regulate hippocampal morphogenesis. J Neurosci 28, 12328-12340.

Brun, M., Coles, J.E., Monckton, E.A., Glubrecht, D.D., Bisgrove, D., Godbout, R., 2009. Nuclear factor I regulates brain fatty acid-binding protein and glial fibrillary acidic protein gene expression in malignant glioma cell lines. J Mol Biol 391, 282-300.

Campbell, C.E., Piper, M., Plachez, C., Yeh, Y.T., Baizer, J.S., Osinski, J.M., Litwack, E.D., Richards, L.J., Gronostajski, R.M., 2008. The transcription factor Nfix is essential for normal brain development. BMC Dev Biol 8, 52.

Friedel, R.H., Seisenberger, C., Kaloff, C., Wurst, W., 2007. EUCOMM--the European conditional mouse mutagenesis program. Brief Funct Genomic Proteomic 6, 180-185.

Fujita, P.A., Rhead, B., Zweig, A.S., Hinrichs, A.S., Karolchik, D., Cline, M.S., Goldman, M., Barber, G.P., Clawson, H., Coelho, A., Diekhans, M., Dreszer, T.R., Giardine, B.M., Harte, R.A., Hillman-Jackson, J., Hsu, F., Kirkup, V., Kuhn, R.M., Learned, K., Li, C.H., Meyer, L.R., Pohl, A., Raney, B.J., Rosenbloom, K.R., Smith, K.E., Haussler, D., Kent, W.J., 2011. The UCSC Genome Browser database: update 2011. Nucleic Acids Res 39, D876-882.

Grant, C.E., Bailey, T.L., Noble, W.S., 2011. FIMO: scanning for occurrences of a given motif. Bioinformatics 27, 1017-1018.

Heng, Y.H., McLeay, R.C., Harvey, T.J., Smith, A.G., Barry, G., Cato, K., Plachez, C., Little, E., Mason, S., Dixon, C., Gronostajski, R.M., Bailey, T.L., Richards, L.J., Piper, M., 2013. NFIX Regulates Neural Progenitor Cell Differentiation During Hippocampal Morphogenesis. Cereb Cortex, In press, doi: 10.1093/cercor/bhs307.

Jolma, A., Yan, J., Whitington, T., Toivonen, J., Nitta, K.R., Rastas, P., Morgunova, E., Enge, M., Taipale, M., Wei, G., Palin, K., Vaquerizas, J.M., Vincentelli, R., Luscombe, N.M., Hughes, T.R., Lemaire, P., Ukkonen, E., Kivioja, T., Taipale, J., 2013. DNA-binding specificities of human transcription factors. Cell 152, 327-339.

Mason, S., Piper, M., Gronostajski, R.M., Richards, L.J., 2009. Nuclear factor one transcription factors in CNS development. Mol Neurobiol 39, 10-23.

Newburger, D.E., Bulyk, M.L., 2009. UniPROBE: an online database of protein binding microarray data on protein-DNA interactions. Nucleic Acids Res 37, D77-82.

Piper, M., Barry, G., Hawkins, J., Mason, S., Lindwall, C., Little, E., Sarkar, A., Smith, A.G., Moldrich, R.X., Boyle, G.M., Tole, S., Gronostajski, R.M., Bailey, T.L., Richards, L.J., 2010. NFIA controls telencephalic progenitor cell

differentiation through repression of the Notch effector Hes1. J Neurosci 30, 9127-9139.
Piper, M., Harris, L., Barry, G., Heng, Y.H., Plachez, C., Gronostajski, R.M., Richards, L.J., 2011. Nuclear factor one X regulates the development of multiple cellular populations in the postnatal cerebellum. J Comp Neurol 519, 3532-3548.
Pjanic, M., Pjanic, P., Schmid, C., Ambrosini, G., Gaussin, A., Plasari, G., Mazza, C., Bucher, P., Mermod, N., 2011. Nuclear factor I revealed as family of promoter binding transcription activators. BMC Genomics 12, 181.
Sanchez-Diaz, A., Blanco, M.A., Jones, N., Moreno, S., 2001. HBP2: a new mammalian protein that complements the fission yeast MBF transcription complex. Curr Genet 40, 110-118.
Sandelin, A., Alkema, W., Engstrom, P., Wasserman, W.W., Lenhard, B., 2004. JASPAR: an open-access database for eukaryotic transcription factor binding profiles. Nucleic Acids Res 32, D91-94.
Schmid, C.D., Bucher, P., 2010. MER41 repeat sequences contain inducible STAT1 binding sites. PLoS One 5, e11425.
Scott, C.E., Wynn, S.L., Sesay, A., Cruz, C., Cheung, M., Gomez Gaviro, M.V., Booth, S., Gao, B., Cheah, K.S., Lovell-Badge, R., Briscoe, J., 2010. SOX9 induces and maintains neural stem cells. Nat Neurosci 13, 1181-1189.
Singh, S.K., Bhardwaj, R., Wilczynska, K.M., Dumur, C.I., Kordula, T., 2011. A complex of nuclear factor I-X3 and STAT3 regulates astrocyte and glioma migration through the secreted glycoprotein YKL-40. J Biol Chem 286, 39893-39903.
Wang, Z.X., Teh, C.H., Chan, C.M., Chu, C., Rossbach, M., Kunarso, G., Allapitchay, T.B., Wong, K.Y., Stanton, L.W., 2008. The transcription factor Zfp281 controls embryonic stem cell pluripotency by direct activation and repression of target genes. Stem Cells 26, 2791-2799.

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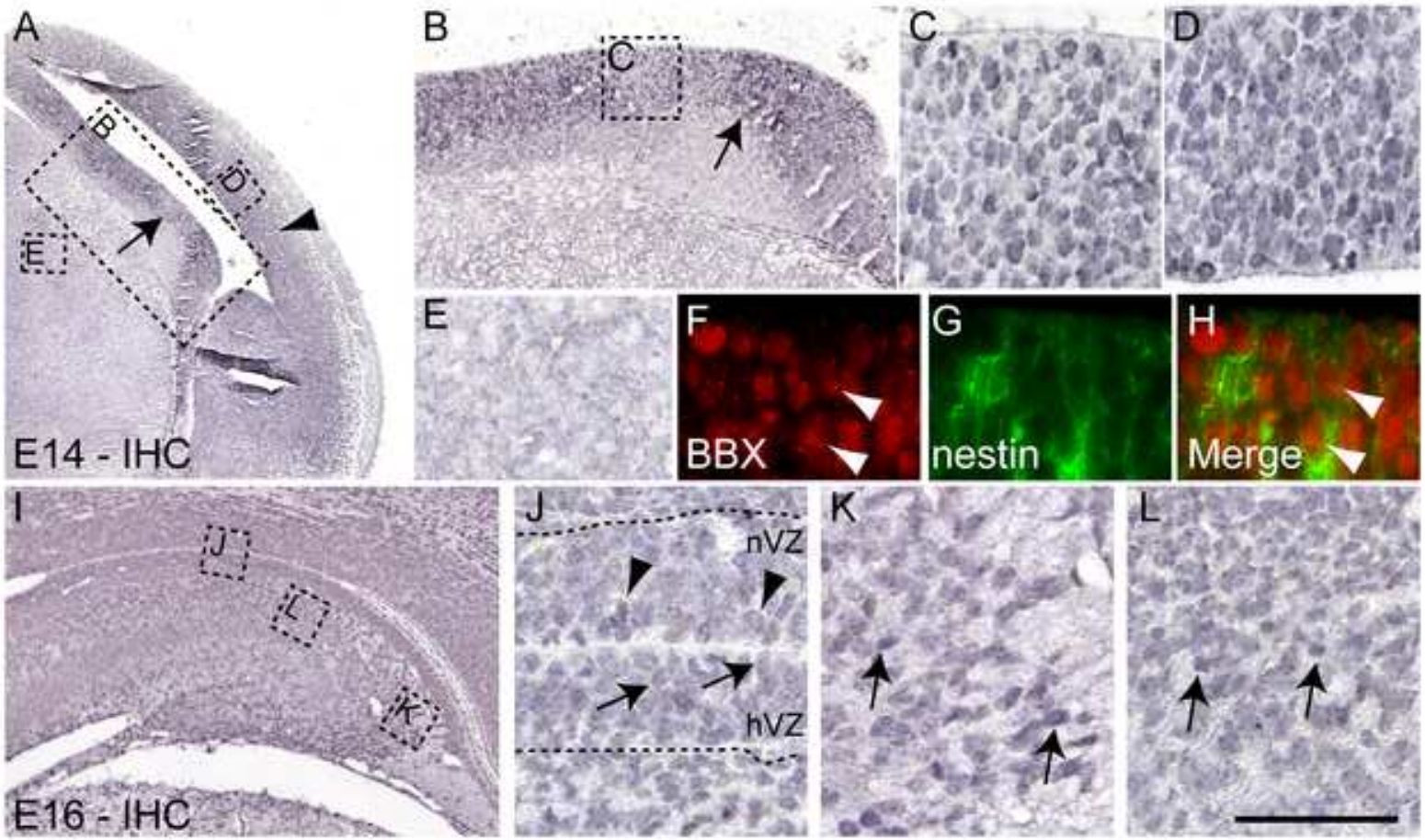

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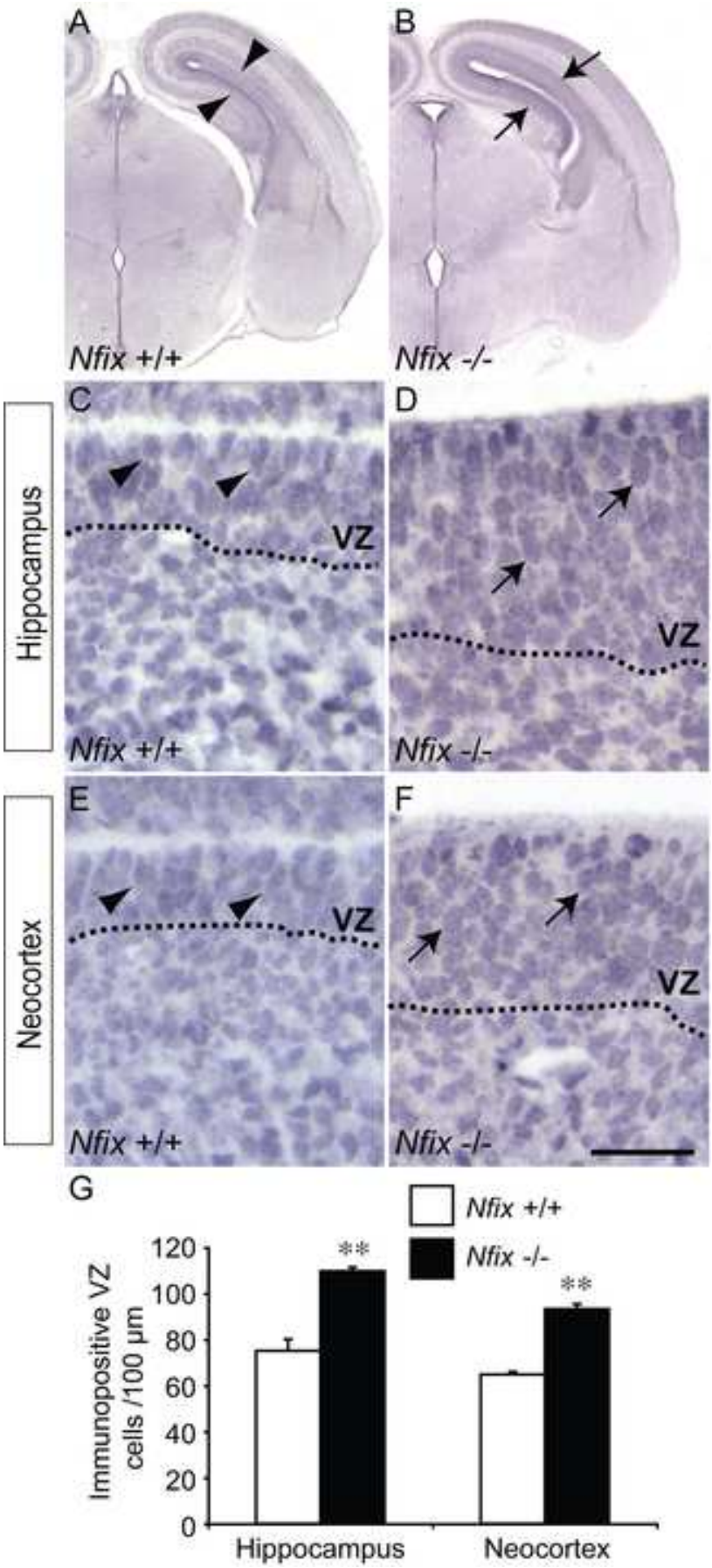

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