Neuron, Volume *59*

Supplemental Data

Postmitotic Nkx2-1 Controls the Migration of Telencephalic Interneurons by Direct Repression of Guidance Receptors

Sandrina Nóbrega-Pereira, Nicoletta Kessaris, Tonggong Du, Shioko Kimura, Stewart A. Anderson, and Oscar Marín

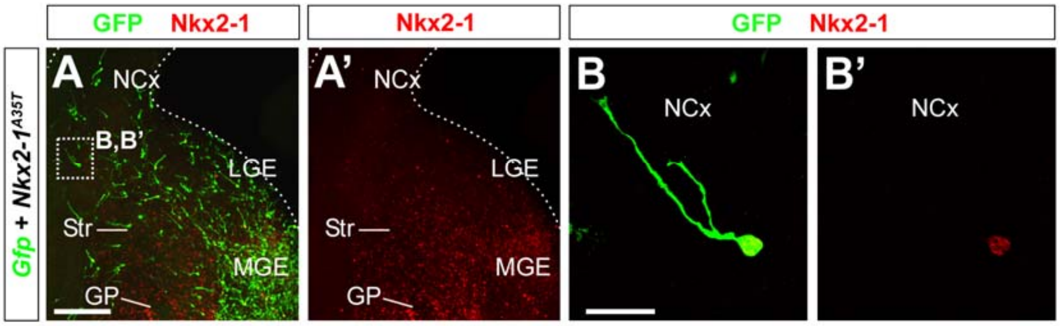


Figure S1. MGE-Derived Cells Electroporated with *Gfp* and *Nkx2-1*^{A35T} Migrate to the Cortex and Express Nkx2-1 Protein

(A and A') Expression of GFP and Nkx2-1, respectively, in MGE-derived cells electroporated with *Gfp* + *Nkx2-1*^{A35T} in organotypic slices. Dotted lines indicate the limits of the section.
(B and B') Higher magnification of the area boxed in (A) showing a cell derived from the MGE and electroporated with *Gfp* and *Nkx2-1*^{A35T}. This cell is entering the cortex and expresses a form of Nkx2-1 that is recognized by the polyclonal antibody raised against this protein.
GP, globus pallidus; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCx, neocortex; Str, striatum.
Scale bars equal 200 µm (A and A') and 20 µm (B' and B').

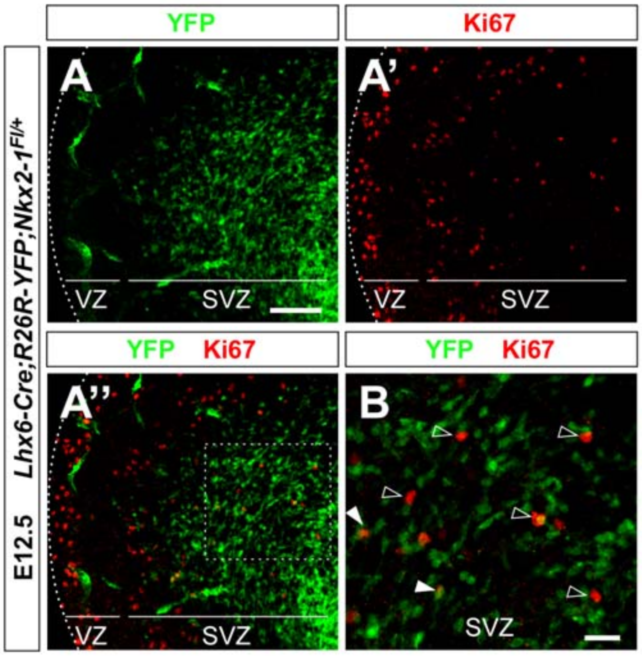


Figure S2. Recombination Driven by the *Lhx6-Cre* Transgenic Line Is Restricted Almost Exclusively to Postmitotic Cells

(A–A'') Coronal sections through the MGE of an E12.5 *Lhx6-Cre;Nkx2-1*$^{Fl/+}$*;Rosa-YFP* embryo showing that almost all YFP-expressing cells are located in the subventricular zone and do not co-label for the progenitor marker Ki67. YFP is also detected in scattered blood vessels, as previously reported (Fogarty et al., 2007).

(B) Higher magnification of the area boxed in (A'') depicting YFP-expressing cells that are negative (open arrowhead) or positive (white arrowhead) for Ki67 expression.
VZ, ventricular zone; SVZ, subventricular zone.
Scale bars equal 50 µm (A, A' and A'') and 20 µm (B).

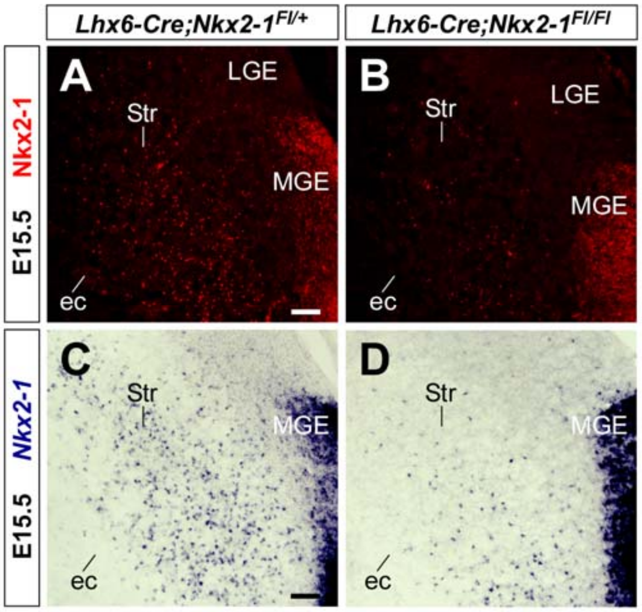


Figure S3. Nkx2-1 Expression in Control and *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$ Mutant Embryos

(A–D) Coronal sections through the telencephalon of E15.5 control (A and C) and *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$ mutant (B and D) embryos showing Nkx2-1 protein (A and B) and mRNA (C and D) expression.

ec, external capsule; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; Str, striatum.
Scale bar equals 100 µm.

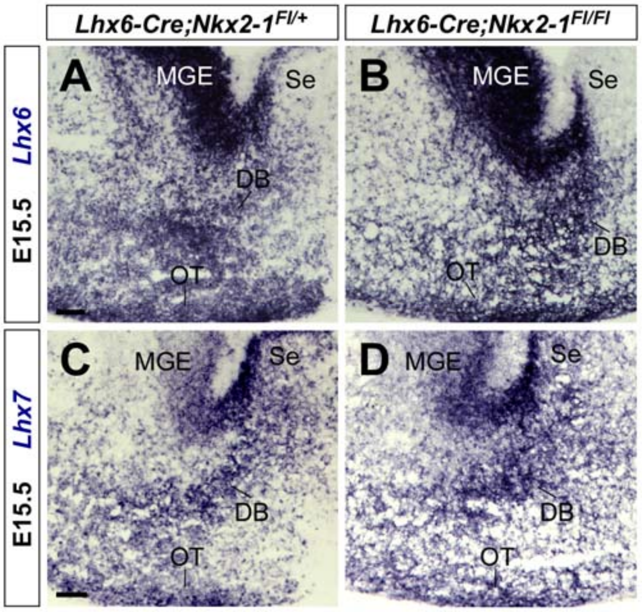


Figure S4. *Lhx6* and *Lhx7* mRNA Expression Is Unchanged in the Ventral Telencephalon of *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$ Mutant Embryos

(A–D) Coronal sections through the telencephalon of E15.5 control (A and C) and *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$ mutant (B and D) embryos showing *Lhx6* (A and B) and *Lhx7* (C and D) mRNA expression.

DB, diagonal band; MGE, medial ganglionic eminence; Se, septum; OT, olfactory tubercle. Scale bar equals 100 µm.

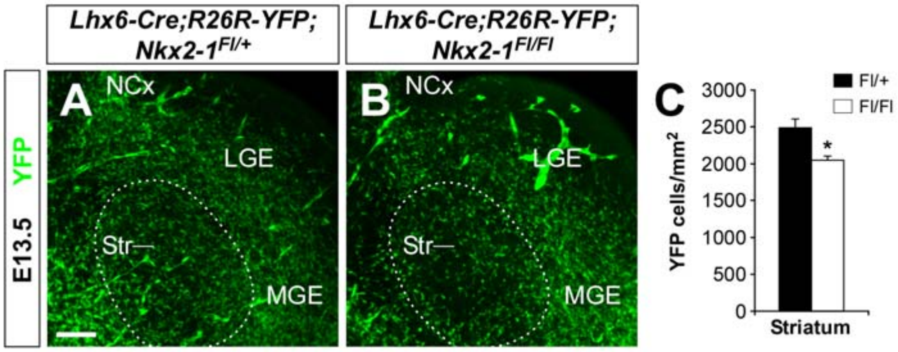


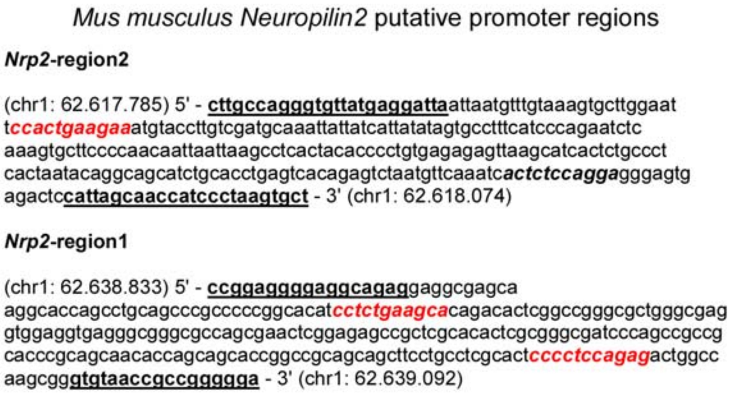
Figure S5. Tracing Experiments Reveal a Reduction in the Number of Interneurons that Invade the Embryonic Striatum upon Loss of *Nkx2-1* Function

(A and B) Coronal sections through the telencephalon of E13.5 *Lhx6-Cre;Nkx2-1*$^{Fl/+}$*;Rosa-YFP* control (A) and *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$*;Rosa-YFP* mutant (B) embryos showing YFP expression. Dotted lines indicate the limits of the developing striatum. YFP is also detected in scattered blood vessels, as previously reported (Fogarty et al., 2007).

(C) Quantification of the number of YFP-expressing cells in the striatum of E13.5 *Lhx6-Cre;Nkx2-1*$^{Fl/+}$*;Rosa-YFP* control and *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$*;Rosa-YFP* mutant embryos. Histograms show average ± s.e.m. 2488.03 ± 134.24 (YFP control); 2043.78 ± 55.54 (YFP mutant). * $p < 0.05$, *t*-test.

LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCx, neocortex; Str, striatum.

Scale bar equals 100 µm.



Mus musculus Neuropilin2 putative promoter regions

***Nrp2*-region2**

(chr1: 62.617.785) 5' - **cttgccagggtgttatgaggatta**attaatgtttgtaaagtgcttggaat
t***ccactgaagaa***atgtaccttgtcgatgcaaattattatcattatatagtgcctttcatcccagaatctc
aaagtgcttccccaacaattaattaagcctcactacacccctgtgagagagttaagcatcactctgccct
cactaatacaggcagcatctgcacctgagtcacagagtctaatgttcaaatc***actctccagga***gggagtg
agactc**cattagcaaccatccctaagtgct** - 3' (chr1: 62.618.074)

***Nrp2*-region1**

(chr1: 62.638.833) 5' - **ccggaggggaggcagag**gaggcgagca
aggcaccagcctgcagcccgcccccggcacat***cctctgaagca***cagacactcggccgggcgctgggcgag
gtggaggtgagggcgggcgccagcgaactcggagagccgctcgcacactcgcgggcgatcccagccgccg
cacccgcagcaacaccagcagcaccggccgcagcagcttcctgcctcgcact***cccctccagag***actggcc
aagcgg**gtgtaaccgccggggga** - 3' (chr1: 62.639.092)

Figure S6. Putative Promoter Regions for *Mus musculus Neuropilin-2*

Nrp2 regulatory sequences (Genbank AF022855), designated *Nrp2*-region2 (chr1: 62.617.785-62.618.074) and *Nrp2*-region1 (chr1: 62.638.833- 62.639.09), containing Nkx2-1 consensus sequences (red letters in bold and italic for 8/9 base pairs sequences and black letters in bold and italic for a 6 base sequence). The oligonucleotide primers used for PCR detection of the ChIP assays are shown in bold and underlined.

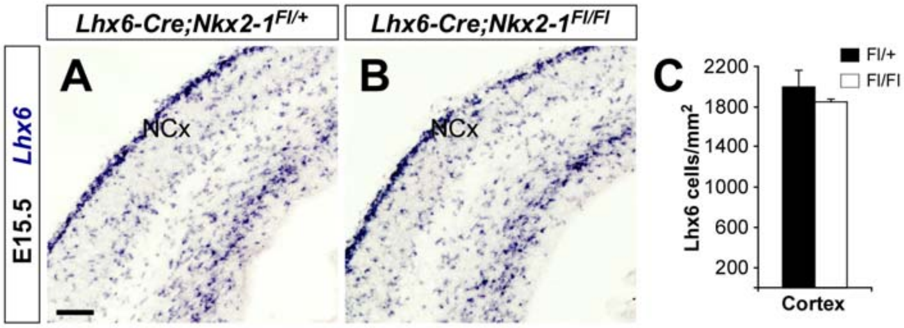
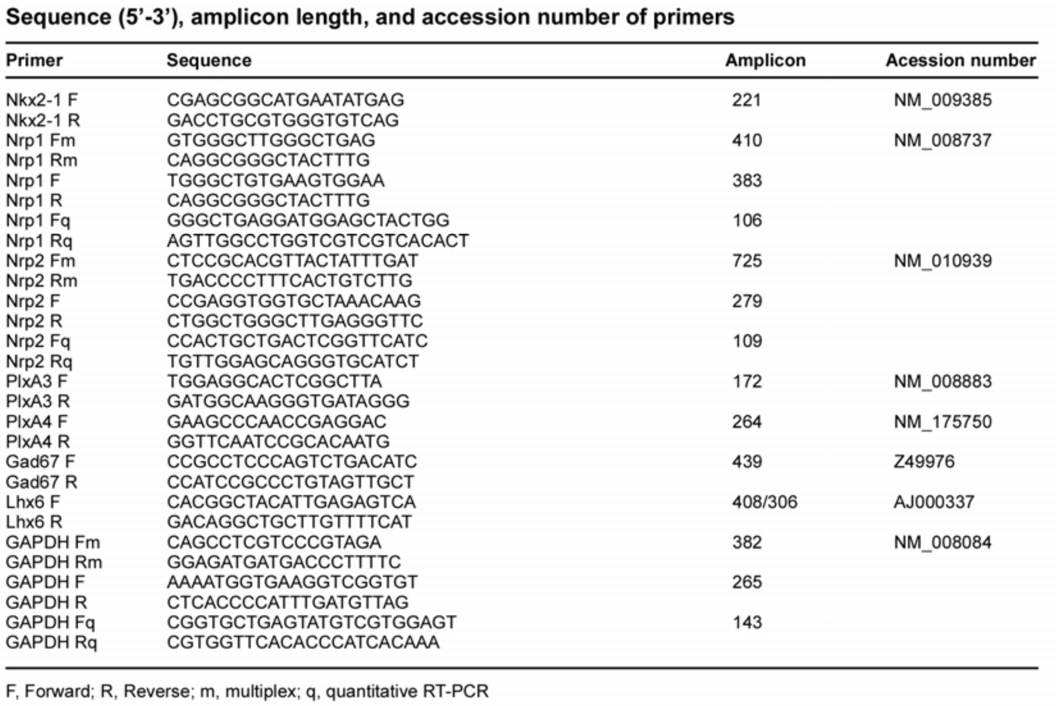


Figure S7. *Lhx6* mRNA Expression Is Unchanged in the Cortex of *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$ Mutant Embryos

(A and B) Coronal sections through the telencephalon of E15.5 control (A) and *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$ mutant (B) embryos showing *Lhx6* mRNA expression.

(C) Quantification of the number of *Lhx6*-expressing cells in the cortex of E15.5 control and *Lhx6-Cre;Nkx2-1*$^{Fl/Fl}$ mutant embryos. Histograms show average ± s.e.m. 2004.43 ± 170.50 (*Lhx6* control); 1855.71 ± 35.33 (*Lhx6* mutant).
NCx, neocortex.
Scale bar equals 100 µm.



Sequence (5'-3'), amplicon length, and accession number of primers

Primer	Sequence	Amplicon	Acession number
Nkx2-1 F	CGAGCGGCATGAATATGAG	221	NM_009385
Nkx2-1 R	GACCTGCGTGGGTGTCAG		
Nrp1 Fm	GTGGGCTTGGGCTGAG	410	NM_008737
Nrp1 Rm	CAGGCGGGCTACTTTG		
Nrp1 F	TGGGCTGTGAAGTGGAA	383	
Nrp1 R	CAGGCGGGCTACTTTG		
Nrp1 Fq	GGGCTGAGGATGGAGCTACTGG	106	
Nrp1 Rq	AGTTGGCCTGGTCGTCGTCACACT		
Nrp2 Fm	CTCCGCACGTTACTATTTGAT	725	NM_010939
Nrp2 Rm	TGACCCCTTTCACTGTCTTG		
Nrp2 F	CCGAGGTGGTGCTAAACAAG	279	
Nrp2 R	CTGGCTGGGCTTGAGGGTTC		
Nrp2 Fq	CCACTGCTGACTCGGTTCATC	109	
Nrp2 Rq	TGTTGGAGCAGGGTGCATCT		
PlxA3 F	TGGAGGCACTCGGCTTA	172	NM_008883
PlxA3 R	GATGGCAAGGGTGATAGGG		
PlxA4 F	GAAGCCCAACCGAGGAC	264	NM_175750
PlxA4 R	GGTTCAATCCGCACAATG		
Gad67 F	CCGCCTCCCAGTCTGACATC	439	Z49976
Gad67 R	CCATCCGCCCTGTAGTTGCT		
Lhx6 F	CACGGCTACATTGAGAGTCA	408/306	AJ000337
Lhx6 R	GACAGGCTGCTTGTTTTCAT		
GAPDH Fm	CAGCCTCGTCCCGTAGA	382	NM_008084
GAPDH Rm	GGAGATGATGACCCTTTTC		
GAPDH F	AAAATGGTGAAGGTCGGTGT	265	
GAPDH R	CTCACCCCATTTGATGTTAG		
GAPDH Fq	CGGTGCTGAGTATGTCGTGGAGT	143	
GAPDH Rq	CGTGGTTCACACCCATCACAAA		

F, Forward; R, Reverse; m, multiplex; q, quantitative RT-PCR

Figure S8. List of Primers Used in Semi-quantitative and Quantitative RT-PCR Experiments

Supplemental References

Fogarty, M., Grist, M., Gelman, D., Marín, O., Pachnis, V., and Kessaris, N. (2007). Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. J. Neurosci. *27*, 10935–10946.