Supplemental Figures:

Figure S1

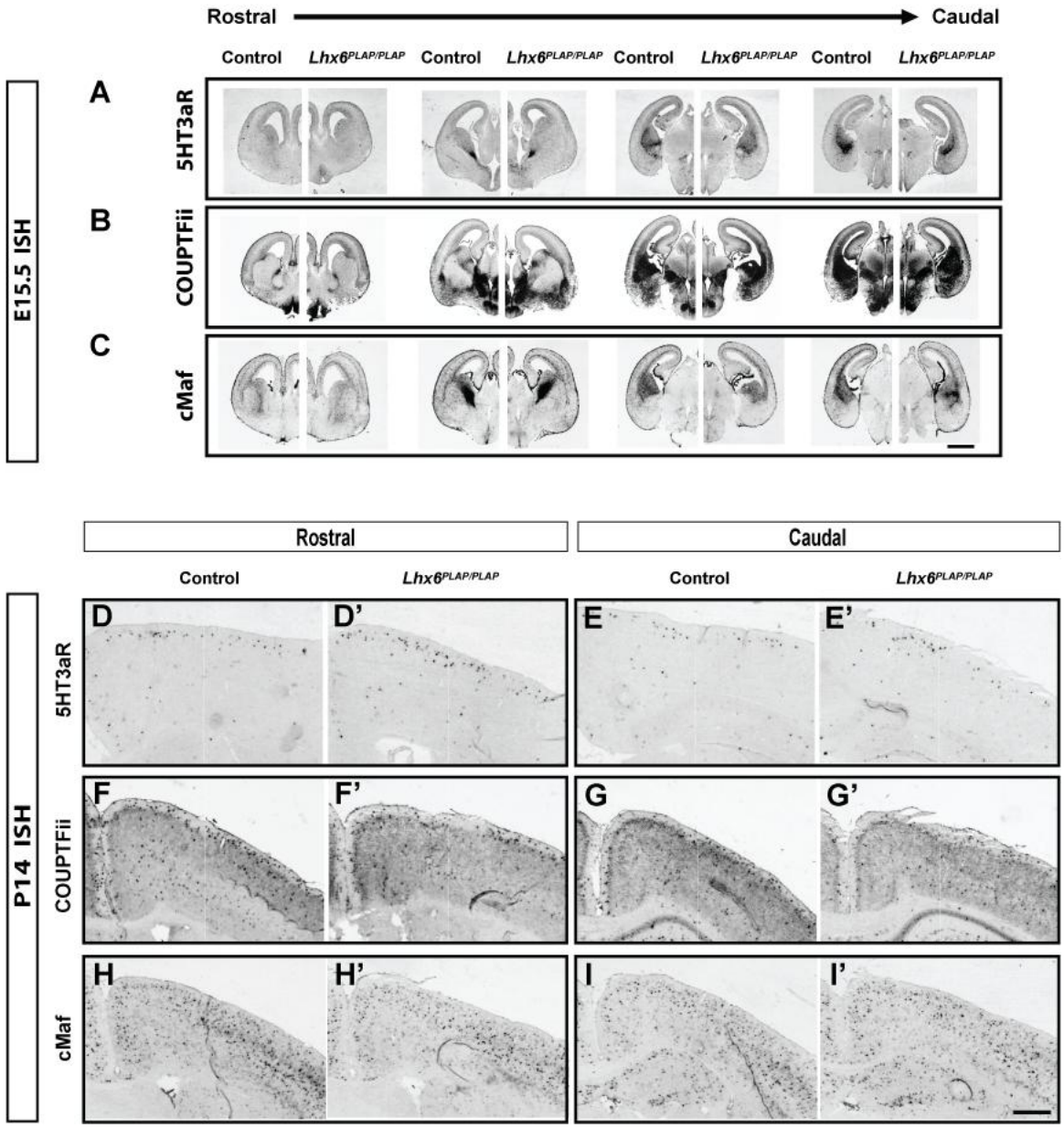


Figure S2

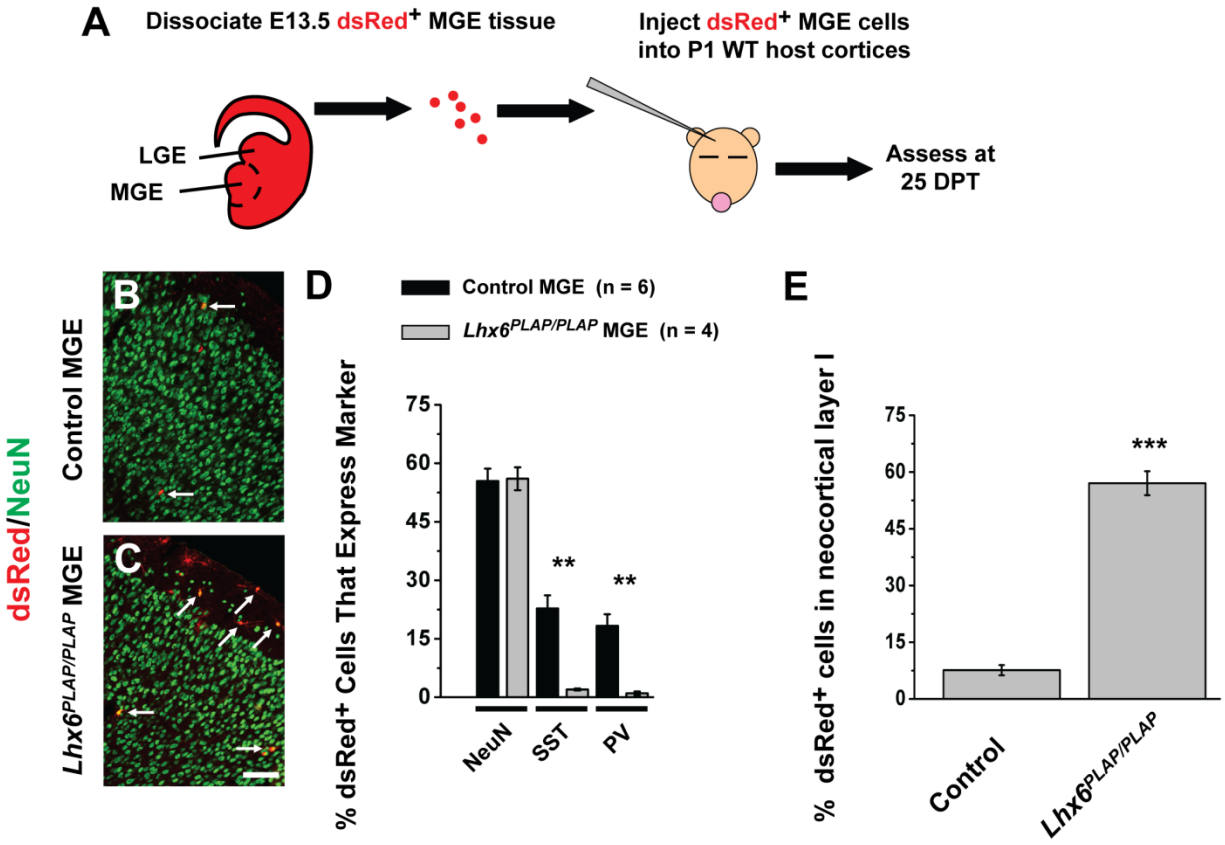

A
Dissociate E13.5 dsRed+ MGE tissue
Inject dsRed+ MGE cells into P1 WT host cortices
LGE
MGE
Assess at 25 DPT
dsRed/NeuN
Control MGE
Lhx6PLAP/PLAP MGE
B
C
D
Control MGE (n = 6)
Lhx6PLAP/PLAP MGE (n = 4)
% dsRed+ Cells That Express Marker
75
60
45
30
15
0
**
**
NeuN
SST
PV
E
% dsRed+ cells in neocortical layer I
***
Control
Lhx6PLAP/PLAP

Figure S3

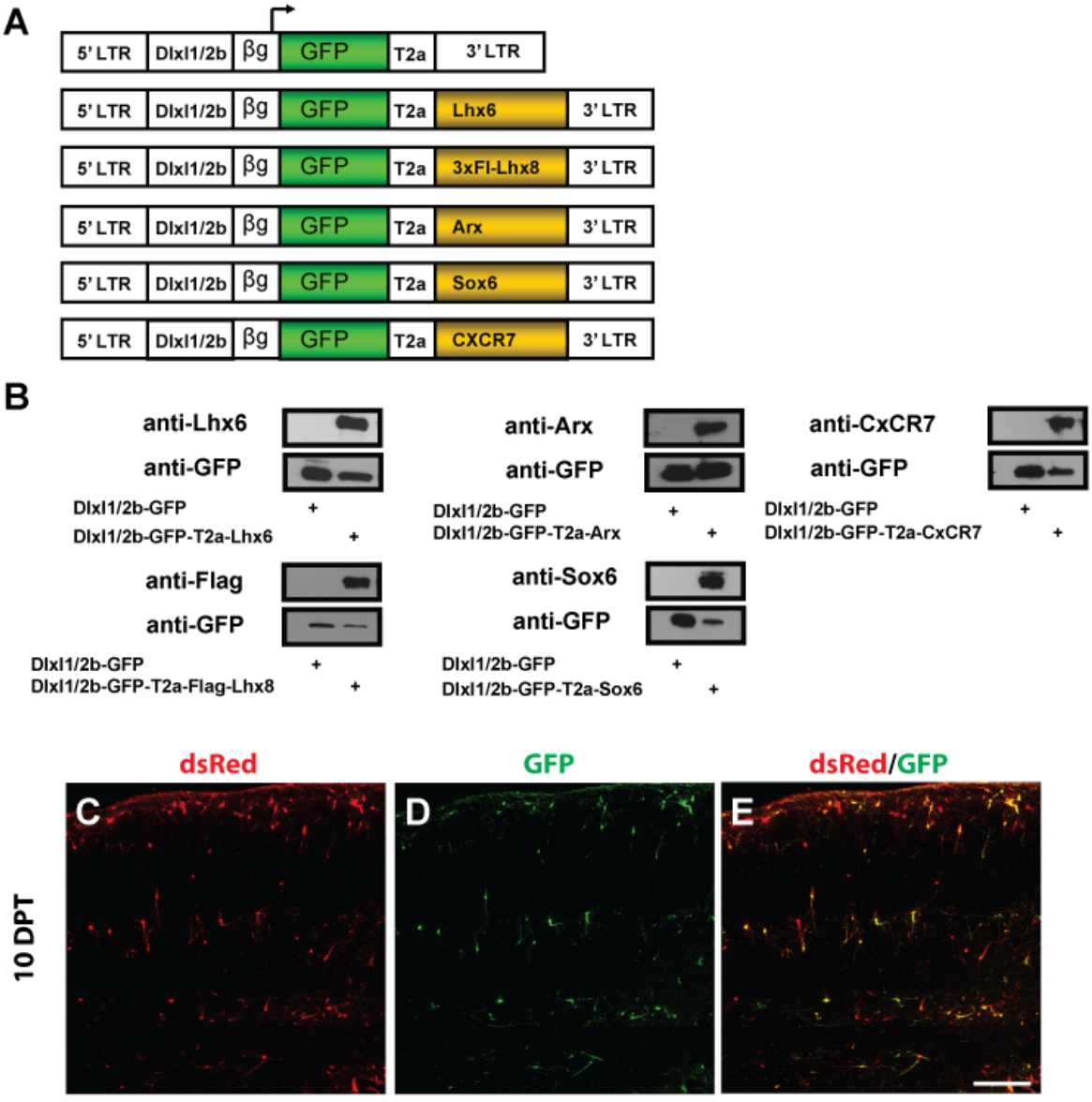
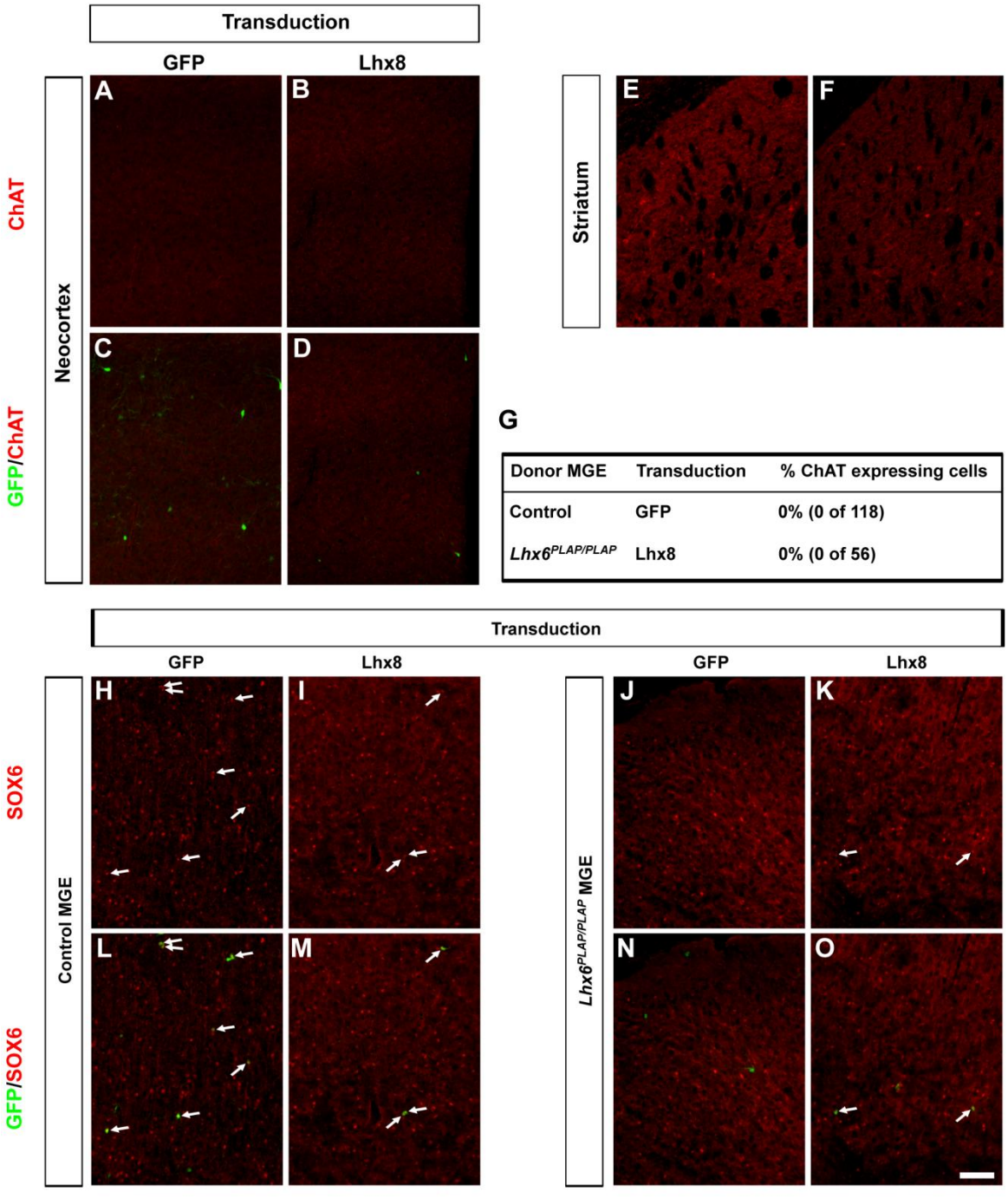

A
5' LTR
Dlxl1/2b
βg
GFP
T2a
3' LTR
Lhx6
3xFl-Lhx8
Arx
Sox6
CXCR7
B
anti-Lhx6
anti-GFP
Dlxl1/2b-GFP
Dlxl1/2b-GFP-T2a-Lhx6
anti-Arx
Dlxl1/2b-GFP-T2a-Arx
anti-CxCR7
Dlxl1/2b-GFP-T2a-CxCR7
anti-Flag
Dlxl1/2b-GFP-T2a-Flag-Lhx8
anti-Sox6
Dlxl1/2b-GFP-T2a-Sox6
dsRed
GFP
dsRed/GFP
C
D
E
10 DPT

Figure S4



G

Donor MGE	Transduction	% ChAT expressing cells
Control	GFP	0% (0 of 118)
Lhx6$^{PLAP/PLAP}$	Lhx8	0% (0 of 56)

P

Donor MGE	Transduction	% SOX6 expressing cells
Control	GFP	73.9% (85 of 115)
Control	Lhx8	87.2% (41 of 47)

Q

Donor MGE	Transduction	% SOX6 expressing cells
Lhx6$^{PLAP/PLAP}$	GFP	5.3% (4 of 75)
Lhx6$^{PLAP/PLAP}$	Lhx8	58.8% (47 of 80)

Figure S5

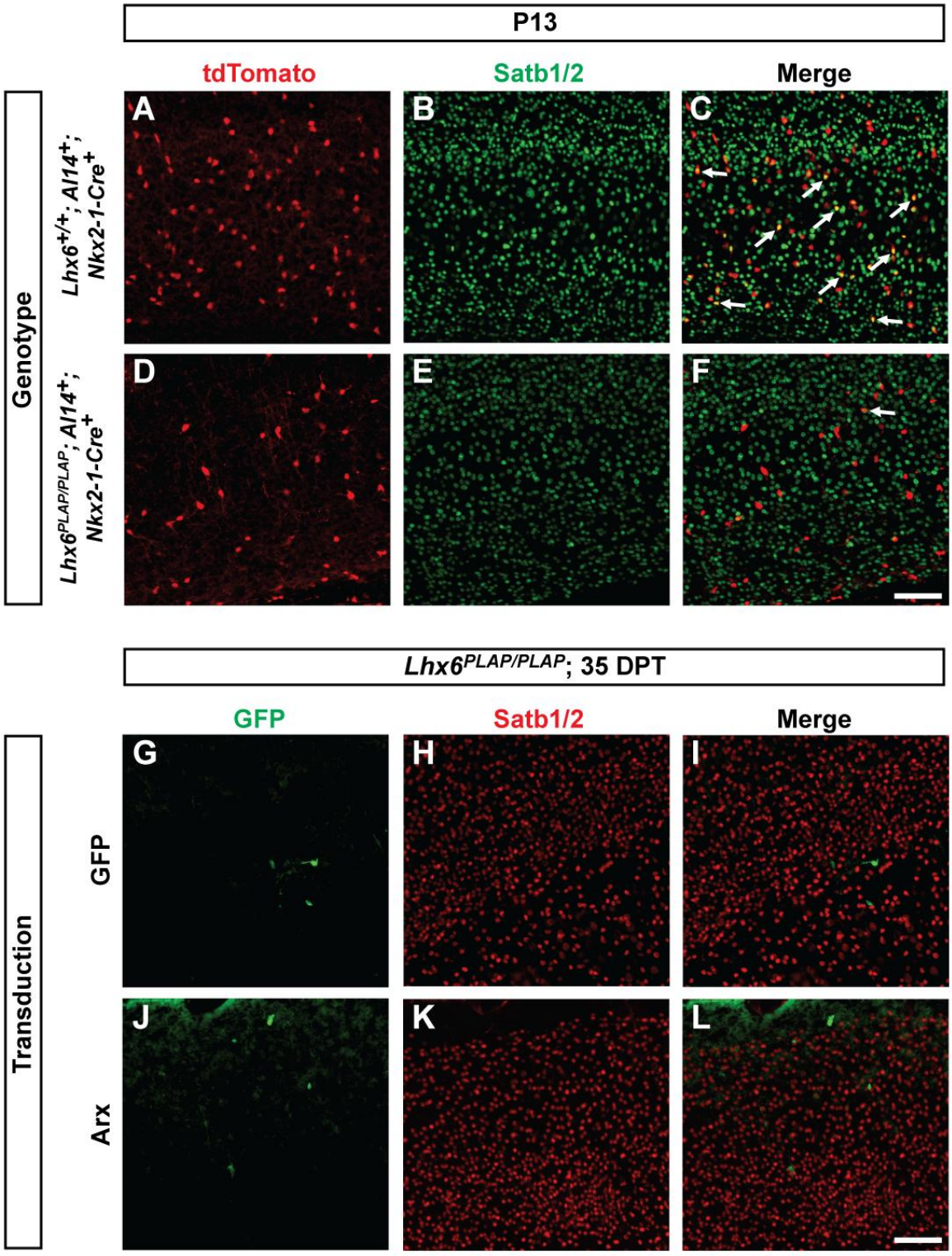

P13
tdTomato
Satb1/2
Merge
Genotype
Lhx6+/+; Al14+; Nkx2-1-Cre+
Lhx6PLAP/PLAP; Al14+; Nkx2-1-Cre+
A
B
C
D
E
F
Lhx6PLAP/PLAP; 35 DPT
GFP
Satb1/2
Merge
Transduction
GFP
Arx
G
H
I
J
K
L

Figure S6

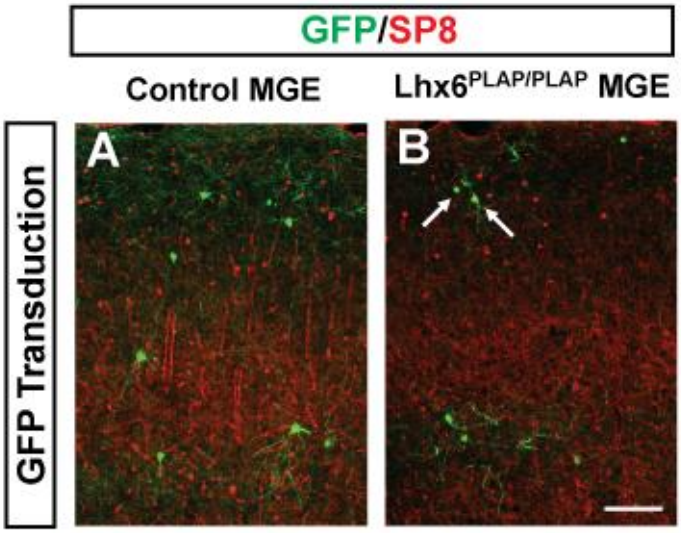


Figure S7

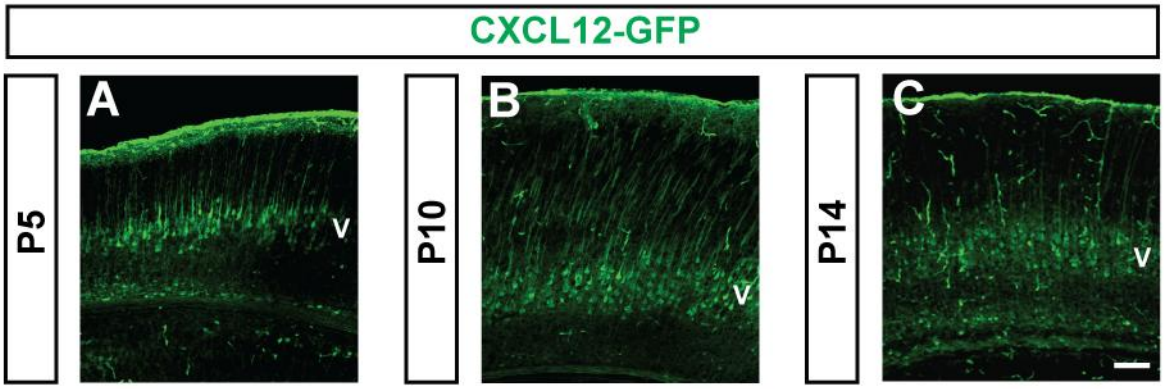


Figure S8

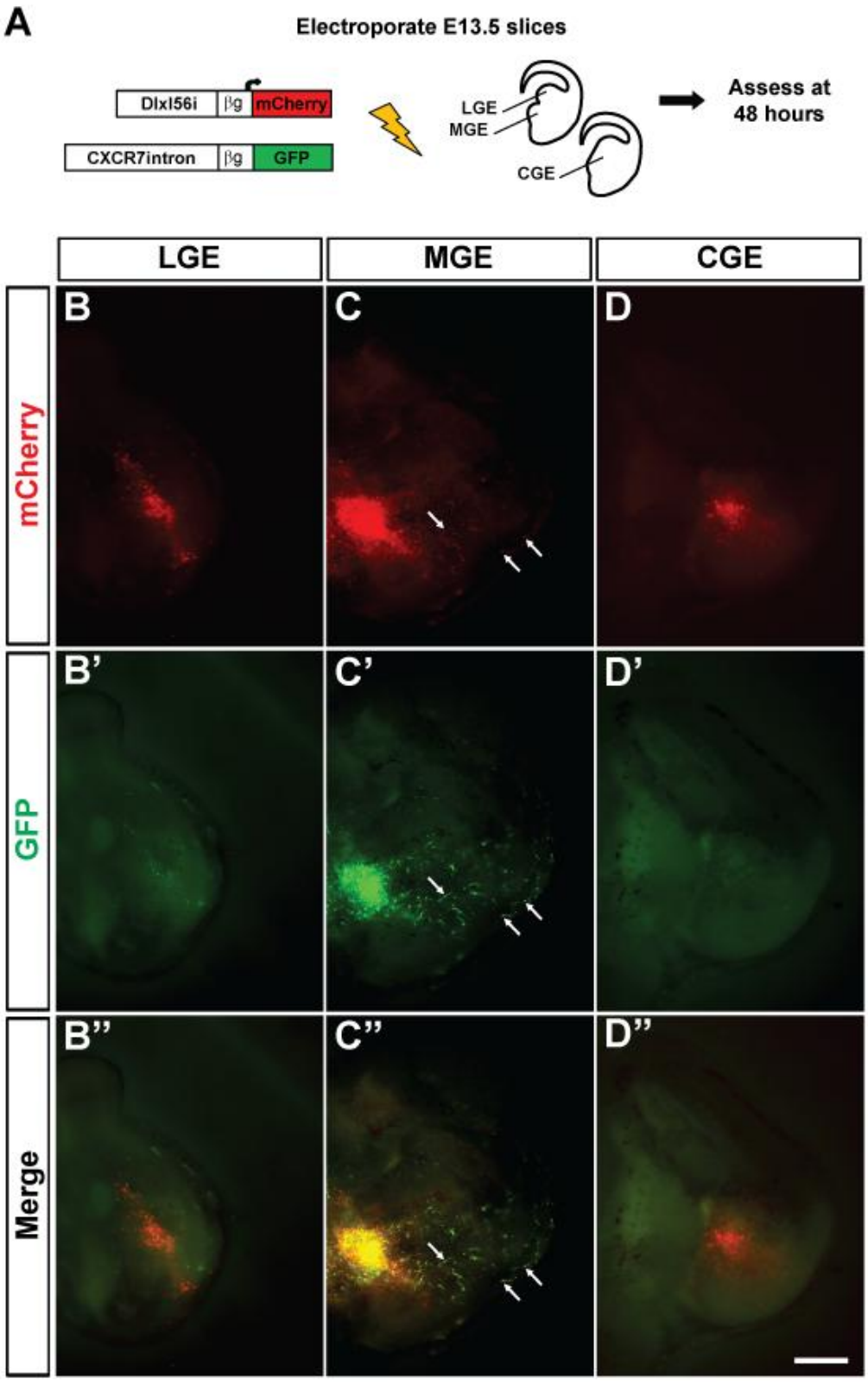

A
Electroporate E13.5 slices
Dlx1I56i
βg
mCherry
CXCR7intron
βg
GFP
LGE
MGE
CGE
Assess at
48 hours
LGE
MGE
CGE
mCherry
GFP
Merge
B
C
D
B'
C'
D'
B''
C''
D''

Table S1

		Intrinsic membrane properties							
		RMP (mV)	R input MΩ	Cm (pF)	Tm (ms)	AP Threshold	AP Amplitude	Spike Adaption	Sag Amplitude
RSNP	WT (n=12)	-57.3 ± 1.8	548.6 ± 102.8	35.9 ± 4.8	41.0 ± 8.8	-40.8 ± 1.7	50.8 ± 5.1	2.6 ± 0.7 ††	1.9 ± 0.6
	Ihx6 ko (n=15)	-56.1 ± 1.8	753.6 ± 130.4	30.0 ± 2.4	33.1 ± 6.9 †	-39.8 ± 1.3	49.2 ± 3.5	2.2 ± 0.3 ††	1.7 ± 0.5
FS	WT (n=4)	-66.8 ± 3.1	166.7 ± 61.2	31.8 ± 3.7	14.8 ± 3.9 †	-41.4 ± 8.1	51.0 ± 5.6	1.7 ± 0.1 ††	1.0 ± 0.4
	Ihx6 ko (n=3)	-66.0 ± 1.5	152.5 ± 19.9	38.0 ± 6.2	18.5 ± 7.3 †	-37.2 ± 0.8	58.9 ± 7.0	1.4 ± 0.3 ††	0.7 ± 0.4
BS	WT (n=2)	-66 .0 ± 8.0	474.6 ± 56.4	48.5 ± 18.5	108.9 ± 72.6	-48.6 ± 4.5	68.6 ± 9.4	11.1 ± 3.7	2.1 ± 0.1
	Ihx6 (n=0)								
LS	WT (n=0)								
	Ihx6 (n=7)	-69.9 ± 2.0	319.2 ± 37.0	32.4 ± 1.5	26.3 ± 8.9 †	-35.6 ± 2.8	49.7 ± 4.8	1.4 ± 0.1 ††	0.7 ± 0.4

		EPSC properties			
		FREQ	AMP	10-90RT	DTC
RSNP	WT (n=12)	5.7 ± 1.3 **	15.1 ± 2.3	1.1 ± 0.04	2.7 ± 0.2
	Ihx6 ko (n=15)	3.5 ± 0.6 **	15.1 ± 2.3	1.09 ± 0.05	2.6 ± 0.2
FS	WT (n=4)	27.3 ± 8.8	15.3 ± 2.9	0.9 ± 0.1	1.6 ± 0.3
	Ihx6 ko (n=3)	14.4 ± 6.5	17.1 ± 1.3	0.9 ± 0.1	1.5 ± 0.3
BS	WT (n=2)	6.9 ± 6.3 *	13.8 ± 2.6	1.0 ± 0.2	1.9 ± 0.3
	Ihx6 (n=0)				
LS	WT (n=0)				
	Ihx6 (n=7)	5.0 ± 0.8 **	9.1 ± 0.2	0.9 ± 0.03	1.8 ± 0.1

*	P < 0.05 versus WT FS
**	P < 0.01 versus WT FS
†	P < 0.05 versus BS
††	P < 0.01 versus BS

Supplemental Figure legends

Supplemental Figure 1: In situ analysis of 5HT3aR, COUPTFII and cMaf in control and *Lhx6*$^{PLAP/PLAP}$ brains shows no major phenotype. Related to Figure 1.

In situ hybridization (ISH) from control and *Lhx6* mutant E15.5 and P14 brains. Coronal hemisections of a rostral (left) to caudal (right) series at E15.5 is shown for 5HT3aR (A), COUPTFII (B) and cMaf (C). Rostral and caudal coronal neocortical regions at P14 for 5HT3aR, COUPTFii and cMaf in controls (D-I), and *Lhx6* mutants (D'-I'). Scale bars: (C) = 1mm; (I') = 500µm.

Supplemental Figure 2: MGE transplantation of *CAG-dsRed* control and *Lhx6*$^{PLAP/PLAP}$. Related to Figure 2.

(A) Schema depicting MGE transplantation of E13.5 control or *Lhx6*$^{PLAP/PLAP}$ expressing dsRed into P1 WT neocortex, and analysis at 25 days post transplant (DPT). (B, C) Immunofluorescent analysis of controls and mutants for NeuN and dsRed in the neocortex at 25 DPT. Arrows point to dsRed^{+} cells that express NeuN. (D) Quantification of cell fate for control and mutant cells for NeuN and the interneuron subtype markers somatostatin (SST) and parvalbumin (PV). (E) Quantification of the proportion of dsRed^{+} transplanted cells that reside in neocortical layer I normalized to all dsRed^{+} cells transplanted. Data expressed as ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$. Scale bar in (C) = 100 µm.

Supplemental Figure 3: *DlxI12b* enhancer lentiviral vectors and expression assessment. Related to Figures 3, 4 and 5.

(A) Schema depicting lentiviral vectors that utilize the *DlxI12b* enhancer and beta-globin (βg) minimal promoter to drive expression of GFP and a gene of interest separated by the T2a

peptide. (B) Western blot analysis of HEK293T cell lysates that were transfected with the lentiviral DNA vectors. Text to the left denotes the antibodies used and text below denotes which vectors were transfected into the cells. (C-E) Immunofluorescent images of transplanted E13.5 *CAG-dsRed* MGE cells that were first transduced with a *DlxI12b-GFP* virus and assessed at 10 DPT (image is from neocortex). Scale bar in (E) = 250µm.

Supplemental Figure 4: Assessment of ChAT and SOX6 after *Lhx8* transduction of transplanted MGE cells. Related to Figure 4.

(A-D) Immunofluorescent images of E13.5 control MGE cells that were transduced by *GFP* or *Lhx8*, transplanted into P1 neocortices and assessed for ChAT at 35 DPT. (E, F) ChAT staining of striatal tissue from the same sections used in (A-D). (G) Quantification of GFP^+ transduced cells that expressed ChAT in the neocortex at 35 DPT. (H-O) Immunofluorescent images of E13.5 control (H, I, L, M) or Lhx6 mutant (J, K, N, O) MGE cells that were transduced by *GFP* or *Lhx8*, transplanted into P1 neocortices and assessed for SOX6 at 35 DPT. Quantification of GFP^+ transduced cells that express SOX6 for control (P) or *Lhx6* mutant (Q) MGE transplant groups. Arrows point to cells that co-express GFP and SOX6. Scale bar in (O) = 100µm.

Supplemental Figure 5: Arx does not rescue Satb1 expression in *Lhx6* mutants. Related to Figure 4.

Immunofluorescent images of neocortex from P13 controls (A-C) and *Lhx6* mutants (D-F), stained for SATB1/2 and tdTomato. Arrows point to examples of double-positive cells. (G-L) Immunofluorescent images of E13.5 Lhx6 mutant MGE cells that were transduced by *GFP* or *GFP-T2a-Arx* lentiviruses, transplanted into P1 WT neocortices and assessed for SATB1/2 and GFP at 35 DPT. *GFP* and *Arx* transduced *Lhx6* mutant cells show little to no expression of SATB1/2 protein at 35 DPT. Scale bars in (F, L) = 100µm.

Supplemental Figure 6: Transplanted *Lhx6* mutant MGE cells express Sp8. Related to Figure 5.

Immunofluorescent images of E13.5 Lhx6 mutant MGE cells that were transduced by *GFP*, transplanted into P1 WT neocortices and assessed for Sp8 and GFP at 35 DPT. Sp8 expression was present in Lhx6 mutant transplanted MGE cells (arrows) but not in control transplants. Scale bar in (B) = 100µm.

Supplemental Figure 7: Expression of CXCL12-GFP in the postnatal neocortex. Related to Figure 6.

(A-C) Immunofluorescent images of CXCL12-GFP neocortical expression at P5, P10 and P14, (v) = neocortical layer v. Scale bar in (C) = 100 µms.

Supplemental Figure 8: The *CXCR7-intronic* enhancer is expressed in the MGE. Related to Figure 7.

(A) Schema of slice electroporation. E13.5 coronal slices were co-electroporated with *CXCR7intron*-GFP and *DlxI56i*-mCherry vectors. Native mCherry, GFP and merged fluorescent images of slices containing LGE (B,B',B''), MGE (C,C',C'') or CGE (D,D',D'') tissues 48 hours after electroporation. Arrows point to examples of GFP/mCherry colocalized cells. Scale bar in (D'') = 250µm.

Supplemental Table 1: Electrophysiological properties of transplanted MGE cells from control and *Lhx6*$^{PLAP/PLAP}$. Related to Figure 3.

Top table: intrinsic membrane properties recorded from different subgroups of neurons at 35 days after transplantation comparing control and *Lhx6* mutant cells as well as the properties between subgroups. Bottom table: Comparison of average EPSC properties received from either control or *Lhx6* mutant neurons. Abbreviations: (RSNP) regular spiking non-pyramidal,

(FS) fast spiking, (BS) burst spiking, (LS) late spiking, (Rmp) resting membrane potential, (R input) input resistaance, (Cm) whole cell capacitance, (Tm) membrane time constant, (AP) action potential, (RT) rise time, (DCT) decay time constant. OneWay ANOVA with Tukey post test was used to test significance among the groups.

Supplemental experimental procedures

Animals

CAG-dsRed mice were maintained on a CD-1 background and have previously been reported (Vintersten et al., 2004).

Antibodies and reagents

DsRed (Clontech), GFP (Aves), calretinin (Immunostar), parvalbumin (Swant Swiss Abs), somatostatin (Chemicon), VIP (Immunostar), SP8 (SantaCruz), Reelin (Millipore), NeuN (Chemicon), SatB2, cross reacts with Satb1 (abCam), Sox6 (AbCam), CxCR7 (11G8 from R&D systems), M2 anti-Flag (Sigma), Lhx6 (AbCam), Arx (SantaCruz). Alexa-conjugated secondary antibodies were from Molecular Probes. Sections were cover slipped with Vectashield containing DAPI (Vector labs). For western blots, secondary HRP antibodies were from Biorad. We generated the rabbit anti-Lhx6 polyclonal affinity-purified antibody at Genscript using Human Lhx6 amino acids 1-67 (based on Lhx6.1a numbering).

Cell counting

Sections used for all cell counting experiments were coronal and 25 µm thick. For cell density counting from transgenic crosses, 10x images were taken from the somatosensory cortex, encompassing all neocortical layers. All cells in the neocortical fields were counted, and divided by the area of the neocortex to determine cell density. For cell fate markers and lamination assessment counting of transplanted MGE cells, we assessed cells based on the following

criteria: 1) All cells in the neocortex were counted from a serial sectioned brain, as long as the cells were at least 250μms away from the injection site. 2) Transplant experiments with ~50 cells/serial section series (or greater) for each parameter were used. 3) Each parameter was normalized to either GFP or dsRed to calculate the proportion of cells that express a specific marker. 4) For lamination counts, we used DAPI to subdivide neocortical layers and counted all cells in the rostral to caudal serial sections. We did not count any cells at or within 250μm of an injection site nor cells at the midline.

Chromatin immunoprecipitation and qPCR

Basal ganglia were micro-dissected and cross-linked at room temperature for 10 minutes in 1% Formaldehyde. The cross-linked chromatin was sheared using a Bioruptor^{TM} UCD-200 for 15 rounds (1 round = 30s on/1min off at High Intensity). The sheared chromatin was incubated with primary antibody (roughly 4μg) over night at 4°C. 20-fold molar excess of blocking peptide (Lhx6 amino acids 1-67) was added to a negative control as well as a fraction of crude chromatin is saved as input. Protein/antibody complexes were collected using Dynabeads (40μl Protein A + 40μl Protein G, Invitrogen), washed, eluted and reverse cross-linked according to the Millipore-Upstate ChIP protocol. ChIP-qPCR analysis was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems) using SYBR GreenER qPCR SuperMix (Invitrogen), and qPCR data analyzed as in (Vokes et al., 2007).

The following primers were used for qPCR: Arx enhancer sites (A1: 5' ACCCAAAAGCAATCATGTCATC, 3' ATGTGCTTCTGACAGGCTCC), (A2: 5' ACAGACGCTTCCAATTCCCG, 3' TCCATTAACTTCATCATGCTCACT), (A3: 5'

TATTAGATGTTATCAGGCAGCTGGA, 3' CTCTGCAATTATGCTCGCAC), (A4: 5' TGTGCGAGCATAATTGCAGAGA, 3' TCCAGTTCTCCTCCCTTCCTG), (A5: 5' AGGACAAAAAGAAATAGCAAAACCA, 3' GCCAGCAATTTCAAACACAGT) and CXCR7 enhancer sites (C1: 5' GGTCTGACCCAAGGCACTCAGC, 3' CAGCAACGCCCGCCATGAGA), (C2: 5' GCAAGCTTGACAAACCAACATCGAA, 3' CCGGGAGTTCCTTTGAATGCCTGT), (C3: 5' GGGGTGGGGATGGATTAATTCACAG, 3' AGCAGGGGTGCCATGAAGAGA), (C4: 5' TTGAATGTTAGCTCCTCTTCATC, 3' CCAATTGAAGTTGGACAACAC), (C5: 5' TGTCTCTCCATTCATTTGCTCA, 3' TCTCAGGGATGCCACTAACC), (C6: 5' GGGCTTTGGAGGGGAGTTTATCCA, 3' CCCTTGGCCAGCTCTGGGTC), (C7: 5' TGGGAGTGCTGTCTGTGTGCAG, 3' GCCTCCTCCAGAGGCCGACT), (C8: 5' GCAGTGGCTCATTCCCTCCCC, 3' GCAGCCTCGCCTTCCTCCAG), (C9: 5' GGCTCTCTGGGAGGTCGGCA, 3' TGGACTCCAGTCCTGGACAAAGC), (C10: 5' ACGTCCAGAAGCATCTGCATTTCCA, 3' ACTGTTCAAGGGAAGACACTGACCT).

Electrophysiology

Slice preparation

Mice were deeply anesthetized by katamine/xalyazine administration and decapitated. The brain was removed and immersed in ice-cold (2–4°C) oxygenated high-sucrose artificial cerebral spinal fluid (ACSF) containing the following (in mM): 150 sucrose, 50 NaCl, 25 $NaHCO_3$, 10 dextrose, 2.5 KCl, 1 NaH_2PO_4-H_2O, 0.5 $CaCl_2$, and 7 $MgCl_2$, and equilibrated with 95% O_2–5% CO_2, pH 7.2–7.4, 300–305 mOsm/kg. Brains were blocked, glued to a sectioning stage, and 300-µm-thick coronal slices were cut in ice-cold, oxygenated high-sucrose ACSF using a Vibratome (Leica VTS1000). Slices were then transferred to a storage chamber containing oxygenated ACSF containing the following (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4-H_2O, 2

$MgSO_4$-$7H_2O$, 26 $NaHCO_3$, 10 dextrose, and 2 $CaCl_2$ (pH 7.2–7.4, 300–305 mOsm/kg), heated at 35°C for ~45 minutes in a water bath, and maintained at room temperature until use for experimentation.

Electrophysiology

After an equilibration period of at least 60 min, slices were transferred to a recording chamber on an upright, fixed-stage microscope equipped with infrared, differential interference contrast (IR-DIC) and epifluorescence optics to visualize GFP-labeled cells (Olympus BX50WI), where they were continuously perfused with warmed (34-35°C) ACSF. Patch pipettes were pulled from borosilicate glass (1.5 mm outer diameter and 0.45 mm wall thickness; World Precision Instruments) with a P-87 puller (Sutter Instrument). Open tip resistance was 3–5 MΩ. The intracellular solution contained the following (in mM): 140 K^+ gluconate, 1 NaCl, 5 EGTA, 10 HEPES, 1 $MgCl_2$, 1 $CaCl_2$, 3 KOH, 2 ATP, and 0.2% biocytin, pH 7.21. Recordings were obtained with an Axopatch 1D amplifier (Molecular Devices), filtered at 5 kHz, and recorded to pClamp 10.2 (Clampfit; Molecular Devices). After membrane rupture, cells were first voltage clamped for ~5 min at −70 mV (i.e., near resting membrane potential) to allow equilibration of intracellular and recording pipette contents. Resting membrane potentials were measured immediately after breakthrough by temporarily removing the voltage clamp and monitoring voltage. Series resistance was typically <15 MΩ and was monitored throughout the recordings. Data were only used for analysis if the series resistance remained <20 MΩ and changed by ≤20% during the recordings. Recordings were not corrected for a liquid junction potential. For voltage-clamp recordings, a 2-5 min sample recording per cell was used for measuring spontaneous excitatory postsynaptic current (sEPSC) frequency, amplitude, 10-90% rise time, and decay time constant at a holding potential of −70 mV. Events characterized by a typical fast rising phase and exponential decay phase were manually detected using MiniAnalysis (Synaptosoft). The threshold for event detection was currents with amplitudes greater than three

times the root mean square (RMS) noise level. For current-clamp recordings, cells were held at -70 mV, and intrinsic electrophysiological properties were measured in response to a series of long (1000 ms) hyperpolarizing and depolarizing current-injections (10 pA steps; range: -50 pA to 1000 pA). Input resistance (R_{input}) was measured from peak voltage responses to ±10 pA current injections (1000 ms duration). Membrane time constant (τ_m) was calculated by fitting the voltage response to a -10pA hyperpolarizing current pulse with a single exponential function ($y = y_0 + Ae^{-t/\tau_m}$, where y_0 is the y asymptote, A is amplitude, τ_m is decay time constant, and t is time). Spike accommodation was identified by examining the ratio of the interspike intervals between the last two action potentials in the train as compared to the first two at 2x spike threshold.

In situ hybridization

Coronal cryostat sections were prepared and processed as described in (Jeong et al., 2008). Briefly, either E15.5 or P14 were fixed in 4%PFA then sunk in 30% sucrose before cutting 20 µm sections. The following probes were used on E15.5 and P14 coronal serial sections from control and $Lhx6^{PLAP/PLAP}$ brains. COUPTFii (M. Tsai), 5HT3aR (B. Rudy), cMaf (McKinsey et al., 2013).

MGE slice electroporation

E13.5 brains were dissected in ice cold HBSS then embedded in 4% low-melting agarose in PBS. Next, 250µm coronal sections were made with a VT1200S vibratome (Leica). Live sections were placed atop a permeable nucleopore Track-Etch membrane (Whatman) floating in DMEM H21 + 10% FBS. Following a two hour recovery at 37ºC , slices were injected with a

DNA mixture containing CXCR7intron-GFP and DlxI56i-mCherry vectors (at a 3:1 molar ratio). Injected slices were electroporated with a BTX ECM830 electroporator equipped with two platinum electrodes; one fixed to a glass petri dish and one that is lowered onto the slice. 1% Agarose in PBS was used as the conductive surface between the electrodes and the slice. Each Slice was given three 5ms pulses at 100V. After Electroporation, the DMEM H21 + 10% FBS was replaced with serum free growth media (Neurobasal supplemented with B27, 0.5% Glucose, 1X Glutamine, 1X Penicillin-Streptomycin) and slices were cultured at 37ºC for 48 hours before assessing fluorescence activity.

MGE transplantation and transduction

E13.5 MGEs from individual embryos were dissected in ice-cold HBSS and then kept on ice in DMEM media (containing 10% fetal bovine serum). MGEs were then mechanically dissociated with a p1000 pipette tip and then either concentrated for injections or infected with lentiviruses. For lentiviral infections, dissociated MGE cells were mixed with pre-warmed media, polybrene (8 µg/ml), and about 10-20 uls of concentrated lentiviruses, and incubated at 37°C for 30 minutes, with agitation. Cells were then pelletted in a tabletop centrifuge at low speed (500xg, 3 minutes) and washed with 2-3 times with media followed by trituration to disperse cells between each wash to remove excess virus. A final cell pellet was resuspended in 2-3 ul of media, put on ice, and then remaining media was removed before loaded into the injection needle. For injections, a glass micropipette of 50 µm diameter (with a beveled tip) was preloaded with sterile mineral oil and cells were front-loaded into the tip of the needle using a plunger connected to a hydraulic drive (Narishige) that was mounted to a stereotaxic frame. Pups were anesthetized on ice for 1-2 minutes before being placed on the mold for injections. Each pup received ~3 injections of cells (~ 70 nl per site), in the right hemisphere. These sites were about 1 mm apart from rostral

to caudal and were injected into layers V-VI of the neocortex. After injections, pups were put back with the mother to recover. Mice were sacrificed between 10-35 days (depending on the assay) after transplant and transcardially perfused with PBS followed by 4% PFA. Brains were then postfixed in 4% PFA and sunk in 30% sucrose before embedding in OCT. Embedded brains were sectioned by cryostat to generate coronal serial sections of 25 µm thickness at intervals of 250 µm from rostral to caudal.

Vector generation

Reporter and transcription factor expression vectors:

The CXCR7 intron was PCR amplified (5' GAGAGGTACCCAGCTGGATACCGCAGGCAG, 3' GAGACTCGAGCCACCTCAGCCTGACCTTCA) from mouse genomic DNA (Roche), and ligated into 5' KpnI and 3' XhoI sites of the PGL4.23-luciferase (Promega). The pCAGGs-Ldb1 and CMV-Lhx6-IRES-GFP expression vectors were previously described (Flandin et al., 2011). The PGL4.23 luciferase vector was previously modified to replace the luciferase gene with a beta-globin minimal promoter and mCherry reporter (Flandin et al., 2011). To replace mCherry with GFP, a beta-globin-GFP fragment was digested from the DlxI12b-GFP lentiviral vector with 5' XmaI (cuts internal to beta-globin minimal promoter) and 3' BsrGI sites and ligated into the same sites of the PGL4.23 vector. The CXCR7-intron was then ligated into 5' KpnI and 3' XhoI sites upstream of the beta globin and GFP. The DlxI56i-beta-globin-mCherry reporter was made by replacing the luciferase gene from a PGL4.23 DlxI56i-luciferase vector with mCherry as described in (Flandin et al., 2011).

DlxI12b-GFP-T2a-mcs lentiviral vectors:

The mouse DlxI12b and beta-globin minimal promoter were amplified as a single unit by PCR (primers: 5' GAGAGGATCCACACAGCTTAATGATTATC, 3' GAGAACCGGTCGCCGCGCTCTGCTTCTGG) from a DlxI12b-beta-globin-Cre vector (Potter et al., 2009) with introduced 5’ BamHI and 3’ AgeI sites. A CMV-GFP-T2a-mcs vector was digested with BamHI and AgeI to remove a CAG enhancer and CMV promoter 5' to the GFP gene, and the DlxI12b-beta-globin was ligated in this spot. Human Lhx6, human CXCR7, and mouse Arx were PCR amplified with introduced SphI sites (Lhx6: 5' GAGAGCATGCATGGCCCAGCCAGGGTCC, 3' GAGAGCATGCGTACTGAAAAAGGATGAC; CXCR7: 5' GAGAGCATGCATGGATCTGCATCTCTTC, 3' GAGAGCATGCTCATTTGGTGCTCTGCTC; Arx: 5' GAGAGCATGCATGAGCAATCAGTACCAGG, 3' GAGAGCATGCTTAGCACACCTCCTTCCC; CXCR7: 5' GAGAGCATGCATGGATCTGCATCTCTTC, 3' GAGAGCATGCTCATTTGGTGCTCTGCTCC) and cloned in frame, 3’ to the T2a sequence of DlxI12b-GFP-T2a-mcs. human Sox6 and 3x-Flag-(human)-Lhx8 were first PCR amplified with (Sox6: 5' CCAAGAATTCATGTCTTCCAAGCAAGCC, 3' GAGACCCGGGTCAGTTGGCACTGACAGCC; 3xFlag-Lhx8: 5' GAGAGAATTCTCAGAATTAACCATGGAC, 3' GAGACCCGGGTTAGGTATGACTTATTGGC) and cloned into the EcoRI and XmaI of the CMV-GFP-T2a-mcs vector. Next, the CAG-CMV was cut out and the DlxI12b-beta-globin minimal promoter ligated into these vectors as described above.

CXCR7intron-GFP-T2a-Cre:

Cre was PCR amplified (5' GAGAGCATGCATGTCCAATTTACTGACC, 3' GAGACCATGGTCACACCGGTCCATCGCC) with 5' SphI and 3' NcoI sites and introduced in frame 3' to the T2a in the *DlxI12b-GFP-T2a-mcs* vector. Next, DlxI12b was removed from the

resulting vector with XbaI and EcoRI and the mouse CXCR7intron was PCR amplified (5' GAGATCTAGACAGCTGGATACCGCAGGCAG, 3' beta-globin described above), digested with XbaI and XmaI and ligated to the vector containing Cre to generate the final vector. All vectors were verified by sequencing.

Western blots

HEK293T cells, grown in DMEM with 10% fetal bovine serum, were transfected with the lentiviral vectors using Fugene6. Cell lysates were harvested at 48 hours post transfection with either RIPA buffer (25mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP40, 0.1% SDS. 1% sodium deoxycholate) to harvest total cell lysates or with a nuclear isolation kit (Pierce), to collect nuclear fractions. 20 µg of cell lysates were separated on SDS-PAGE gels, transferred to nitro-cellulose membranes and then probed with specific antibodies before detection by chemiluminescence.

Supplemental references

Zerucha, T., Stuhmer, T., Hatch, G., Park, B.K., Long, Q., Yu, G., Gambarotta, A., Schultz, J.R., Rubenstein, J.L., Ekker, M. (2000). A highly conserved enhancer in the Dlx5/Dlx6 intergenic region is the site of cross-regulatory interactions between Dlx genes in the embryonic forebrain. Journal of Neuroscience *20*, 709-21.