Supporting Information

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SI Materials and Methods

Generation of the Lhx7^{fl} Conditional Knockout Animals. To generate the targeting construct, a fragment of *Lhx7* genomic DNA extending from a NotI site in intron 1 to an EcoRV site in exon 6 was modified. A floxed neomycin (neo) resistance cassette was introduced into the BamHI site in the third intron, and a LoxP sequence with a BamHI restriction site was introduced into the fourth intron (Fig. 1*A*). The construct was linearized and electroporated into E14Tg2A ES cells. Neomycin-resistant clones were chosen and tested by Southern blot with 3′ and 5′ external probes. Positive clones were injected into C57BL/6 blastocysts. The neomycin resistance cassette was deleted by crossing to male germ-line-specific PC3-Cre line (1), which generates all possible recombinations of the three loxP sites. Animals that retained loxP sites flanking exon 4 but had lost the neocassette were selected by PCR and used to establish the *Lhx7^{fl}* line. For genotyping the *Lhx7^{fl}* allele, the following PCR primers were used: 5′-AGCACTGCTTTAATTGTGGTC-3′ and 5′-ATCAGATG-CCCTGCTCTGTC-3′. PCR was followed by BamHI digestion generating a 596-bp band for the wild-type allele and two bands of 281 and 315 bp for the floxed allele.

ISH and Immunohistochemistry. Embryonic brains were dissected and fixed in 4% PFA (wt/vol) for 2 h. The brains were cryoprotected in 30% sucrose (wt/vol), embedded in 15% sucrose and 7.5% gelatin (wt/vol) PBS, and stored at −80 °C until cryosectioning at 12 μm. For immunostaining, the cryosections were blocked in 0.15% glycine, 1% BSA, and 0.1% in PBT (0.1% Triton X-100 in PBS) for 1 h at room temperature (RT) and then incubated overnight at 4 °C in primary antibody diluted in the same solution. After washing with PBT (three times; 10 min), the sections were incubated in secondary antibodies (1 h at RT). The sections were washed and mounted with Vectashield mounting medium.

Adult animals were perfused intracardially with 0.9% NaCl, followed by 4% PFA. The brains were dissected and fixed for 2 h in 4% PFA. The brains were embedded in 3.5% low-melting-point agarose (wt/vol) (Invitrogen) and sectioned in a vibratome at 50 μm. Floating vibratome sections were permeabilized in 1% PBT (1 h at RT), blocked in 10% FCS (vol/vol) and 1% BSA in PBT (1 h at RT), and incubated in primary antibodies diluted in 1% FCS and 0.1% BSA in PBT (overnight at RT). The sections were washed in PBT (four times; 10 min) and incubated in secondary antibodies (1 h at RT). Sections were washed (four times; 10 min) and mounted with Vectashield mounting medium. When the Cyanine-3 TSA amplification kit (Perkin-Elmer) was used, the sections were permeabilized as described previously, and the endogenous peroxidase activity was quenched by incubation in 10% (vol/vol) methanol and 3% hydrogen peroxide (vol/vol) in PBS (30 min at RT). Sections were blocked with the blocking reagent from the TSA kit and incubated in primary antibodies as described previously. After washes with PBT, the sections were incubated with a biotinylated secondary antibody against guinea pig (for Lhx7) or rabbit (Isl1), and then a streptavidin-HRP amplification step and the Cy3 tyramide reaction step were performed as described in the TSA kit protocol.

For 3′-diaminobenzidine (DAB) immunostaining, the sections were incubated with 10% methanol (vol/vol) and 3% hydrogen peroxide (vol/vol) (30 min at RT) and then blocked and incubated with primary antibody as described previously. The sections were incubated with biotin-conjugated secondary antibody and a streptavidin-HRP signal amplification step was performed using the ABC kit (Vectastain) according to the instructions of the manufacturer. The sections were incubated in DAB solution (Sigma) until a specific signal was visible. The reaction was stopped by changing the solution to PBS.

The sections were washed and incubated with secondary antibodies for the other primary antibodies used (1 h at RT) and then washed and mounted as usual.

The following antibodies were used: goat anti-ChAT (Chemicon; 1:500); rabbit anti-Lhx6 (1:1,000) (2); mouse anti-β-gal (Promega; 1:1,000); rabbit anti-β-gal (Cappel; 1:1,000); rabbit anti-GFP (Invitrogen; 1:1,000); rat anti-GFP (1:500; Nacalai Tesque); rabbit anti-Isl1 (1:2,000) (3); Guinea pig anti-Lhx7 (1:200) (4); mouse anti-Ki67 (1:200; BD Pharmingen); rabbit anti-Cre (1:750; Covance Research Products); mouse anti-parvalbumin (1:750; Chemicon); rat anti-somatostatin (1:1,000; Chemicon); guinea pig anti-VAChT (1:150; Chemicon); rabbit anti-TrkA (1:200; Chemicon); donkey anti-goat Cy5 (1:250; Jackson ImmunoResearch); donkey anti-mouse Cy5 (1:250; Jackson ImmunoResearch); donkey anti-rabbit Cy5 (1:500; Jackson ImmunoResearch); donkey anti-guinea pig Cy3(1:500; Jackson ImmunoResearch); donkey anti-rabbit Cy3 (1:500; Jackson ImmunoResearch); donkey anti-mouse Cy3 (1:500; Jackson ImmunoResearch); donkey anti-goat Cy3 (1:500; Jackson ImmunoResearch); donkey anti-rat Alexa Fluor 488 (1:500; Invitrogen); donkey anti-goat Alexa Fluor 488 (1:500; Invitrogen); donkey anti-rabbit Alexa Fluor488 (1:500; Invitrogen); donkey anti-mouse Alexa Fluor 488 (1:500; Invitrogen); and donkey anti-mouse Biotin (1:1,000; Jackson ImmunoResearch).

Confocal Microscopy. Colocalization of different markers was evaluated in confocal images of the striatum collected using a Leica MPSP5 confocal microscope with a 20× magnification objective.

For electroporated embryonic brain sections, a series of five optical sections, 1.6 μm apart, was collected in one field of all of the sections that had GFP fluorescence.

For adult brains, a series of 8–10 optical sections covering a total depth of 20–24 μm was taken in a minimum of 32 fields (each field being 775 × 775 μm) along different rostrocaudal levels of the striatum. A minimum of seven different rostrocaudal sections extending from the most rostral to the most caudal section containing the striatum was included in each quantification. The number of fields, number and depth of optical sections, and number and bregma position of rostrocaudal were constant for all animals analyzed in a given quantification.

For measurements of nuclear size and fluorescence intensity, a series of confocal images were taken at nine different rostrocaudal levels. For each level, the whole area of the striatum was scanned and reconstructed by joining the acquired fields in the *x–y* axis using a tiling option in the Leica LAS acquisition software; for each field, eight z stacks spaced by 3 μm were acquired and a z-stack projection was created. Data were analyzed with ImageJ and Igor Pro (Wavemetrics).

In Vitro Focal Electroporation and Organotypic Slice Culture. Organotypic slice culture and focal electroporation were performed as described previously (5) with some modifications. Briefly, brains of E14.5 mouse embryos were dissected in cold Krebs buffer, embedded in 3% low-melting-point agarose (wt/vol) in PBS, and subsequently vibratome-sectioned into 300-μm-thick coronal slices. Slices were collected in cold Krebs buffer and transferred into sterile Krebs buffer (filtered Krebs with 10 mM Hepes, penicillin, streptomycin, and gentamicin). Slices with a clearly distinguished MGE were chosen and transferred to polycarbonate culture

membranes (Whatman Nucleopore; 13-mm diameter; 8-μm pore size) and placed into organ culture dishes (Falcon) containing 750 μL of medium (Gibco MEM Alpha with glutamine; 10% FCS (vol/vol), penicillin/streptomycin). Slices were allowed to recover for 1 h (37 °C; 5% CO_2) before electroporation. Plasmids encoding GFP or Isl1-IRES-GFP [1.0 μg/μL each (6)] were focally injected into the MGE of coronal slice cultures using pulled borosilicate needles (Harvard Apparatus) and a Femtojet microinjector (Eppendorf). Slices were electroporated (2 pulses of 15 ms and 125 V, separated by a 500-ms interval) within a setup of two horizontally oriented platinum electrodes (Protech International) powered by a ECM830 electroporator (BTX).

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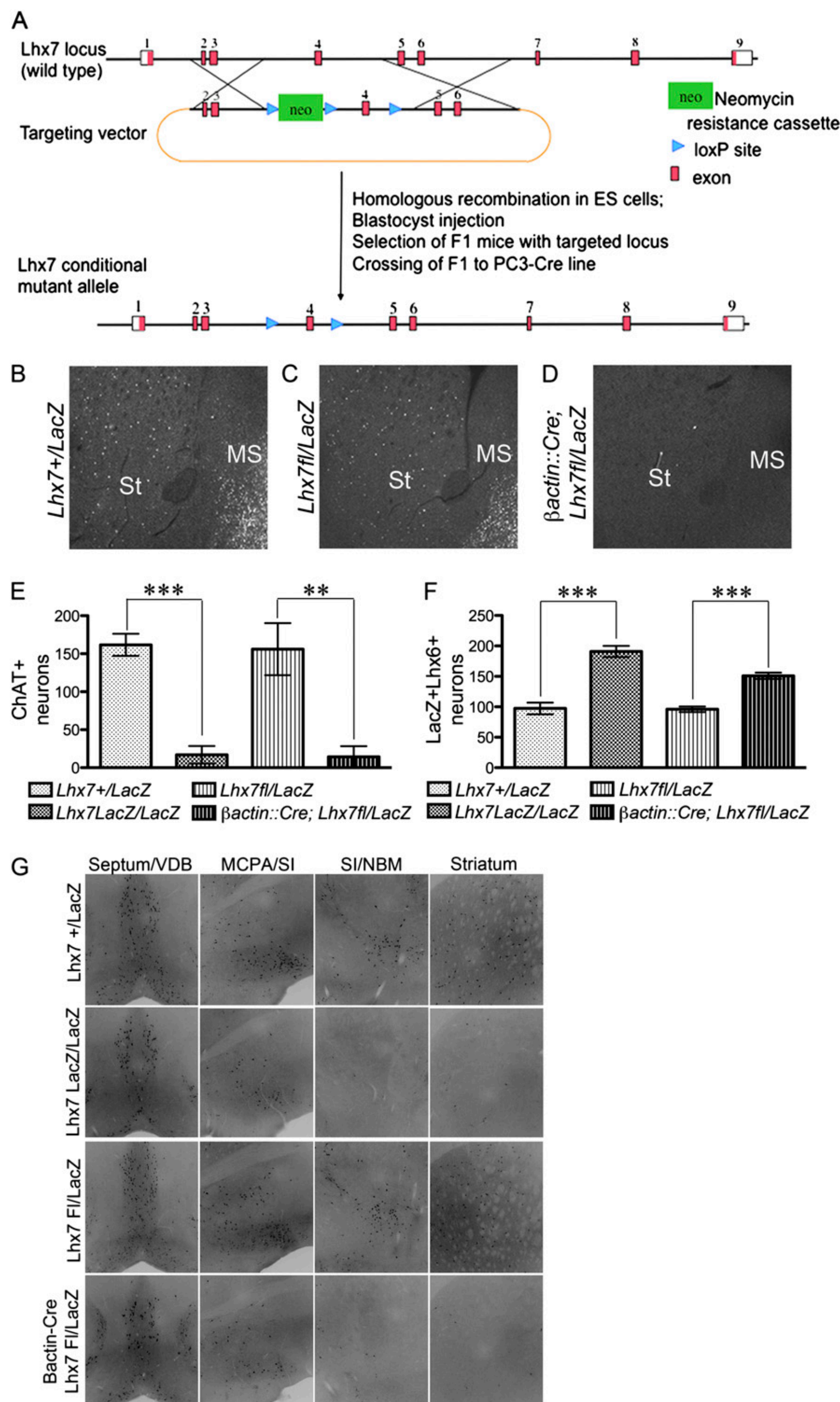

Fig. S1. The Lhx7fl conditional mutant line shows normal Lhx7 function in the absence of Cre-mediated recombination but recapitulates the phenotype of *Lhx7LacZ* null mice when crossed to the *β-actin::Cre* line. (*A*) Schematic representation of the strategy used to generate an *Lhx7* conditional mutant allele. The *Lhx7* locus was targeted in ES cells to introduce loxP sites flanking exon4. (*B–D*) Lhx7 immunofluorescence in adult forebrain sections showing the striatum (St) and medial septum (MS) areas. $Lhx7^{fl/LacZ}$ (*B*) show identical pattern and levels of *Lhx7* expression as $Lhx7^{+/LacZ}$ heterozygous controls (*C*). In contrast, *β-actin::Cre;* $Lhx7^{fl/LacZ}$ (*D*) conditional mutants do not express *Lhx7*. (*E* and *F*) Quantification of the loss of cholinergic neurons (*E*) and increase in the number of GABAergic neurons (*F*) in the adult striatum of Lhx7 null ($Lhx7^{LacZ/LacZ}$) and conditional mutants (*β-actin::Cre;* $Lhx7^{fl/LacZ}$) compared with the respective controls ($Lhx7^{+/LacZ}$ *and* $Lhx7^{fl/LacZ}$). ChAT was used as a marker of cholinergic interneurons (*E*). Coexpression of β-gal and Lhx6 was used to mark GABAergic interneurons of Lhx7 lineage (*F*). Bars represent means ± SD, and significance levels are indicated by $*P < 0.5$, $**P < 0.05$, or $***P < 0.005$. (*G*) Immunostaining for ChAT shows loss of cholinergic neurons in all of the cholinergic groups of the ventral forebrain in null mutants ($Lhx7^{LacZ/LacZ}$) and conditional mutants (*β-actin::Cre:*$Lhx7^{fl/LacZ}$) compared with their respective controls ($Lhx7^{+/LacZ}$ *and* $Lhx7^{fl/LacZ}$). DB, diagonal band; MCPA, magnocellular preoptic area; NBM, nucleus basalis Meynert; SI, substantia innominata.

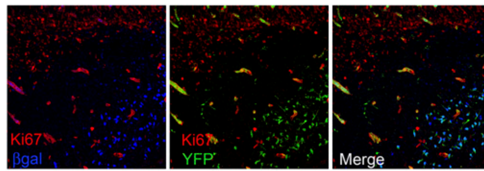

Fig. S2. ChAT-Cre activates recombination in postmitotic Lhx7^{+} precursor neurons. Immunostaining for Ki67, β-gal, and YFP in the MGE of E14.5 *ChAT::Cre*; *Lhx7*$^{+/LacZ}$;*R26*$^{+/YFP}$ control animals shows no colocalization between β-gal and Ki67 or between YFP and Ki67.