Supporting Information

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SI Materials and Methods

Animals. Mice were maintained on a 12-h/12-h light/dark cycle with food and water ad libitum. We used PCR to determine genotypes of mice. The following PCR primers were used: 5′-ggatgaggttcgcaagaacc-3′ and 5′-ccatgagtgaacgaacctgg-3′ for the presence of a *Cre* transgene; 5′-actgacatccgtaagccagt-3′ and 5′-atgtcgccctggctgaagtg-3′ for the *fB* allele with PCR products of 369 bp for the WT *TrkB* allele and ∼450 bp for the *fB* allele; 5′-aagttcatctgcaccaccg-3′ and 5′-tccttgaagaagatggtgcg-3′ for the presence of the *D2-EGFP* transgene; and 5′-cttctgaggcggaaa-gaacc-3′ and 5′-tttctgattgagagcattcg-3′ for the presence of the *Drd1a-tdTomato* transgene. The Georgetown University Animal Care and Use Committee approved all animal procedures.

Stereology. We used Stereo Investigator software (MicroBright-Field Inc.) to calculate striatal volume and neuronal number as described previously (1). The following morphological criteria were used consistently in all mice to determine the boundary of the striatum: the superior boundary was defined by the corpus callosum; the lateral boundary by the external capsule; the medial boundary by the lateral ventricle and the corpus callosum; and the ventral boundary by the anterior commissure. The nucleus accumbens was excluded from the calculation. Measurements were performed on every sixth Nissl-stained coronal sections, extending from the most-rostral to the most-caudal parts of the striatum (8–10 histological sections per brain). Both hemispheres of a given brain were analyzed separately. Striatal volume was quantified according to a previously described method (2). The number of neurons in each striatum was estimated using a fractionator sampling method. For each stereological probe (16–20 probes for each brain), striatal neurons were counted within a counting frame of 25 × 25 μm (a sampling site), and 10–15 sampling sites were randomly picked by the software within the outlined area. The counts were then extrapolated to estimate the total number of neurons in the striatum. For all probe runs the coefficient of error (CE Scheaffer) was <1. The following criteria were used to distinguish neurons from glial cells: cell body diameter >5 μm, light staining of the nucleus, and the presence of Nissl bodies.

Generation of a Floxed *TrkB* Allele. An 11-kb genomic fragment covering the first coding exon (exon S) was used to create the *TrkB* targeting construct (Fig. S2*A*). Exon S covers a 346-bp 5′ untranslated region and a 211-bp coding region, including 31 codons for the signal peptide. A ClaI site that is located at the 19th bp of exon S and a KpnI site that is 112 bp downstream of exon S were two critical restriction sites for generation of the targeting construct. The targeting construct was generated by the insertion of a *loxP* site into the ClaI site and a sequence containing a *PGKneo* expression unit, two *Frt* sites, and one *loxP* site into the KpnI site (Fig. S2*A*). The targeting construct contains 7 kb of homologous DNA (left arm) upstream of the first *loxP* site and 2.5 kb of homologous DNA (right arm) downstream of the second *loxP* site.

The linearized targeting construct was electroporated into JM1 embryonic stem cells grown on mitotically inactivated STO cells. After 8–10 d in selective medium (300 μg/mL G418), colonies were picked, expanded, and screened for homologous recombination by Southern blotting using probe A and probe B as depicted in Fig. S2*A*. The targeted ES cells with one fB^{neo} locus were injected into C57BL/6J blastocysts. Chimeric male mice were mated to C57BL/6J females to obtain fB^{neo}/+ mice. To remove the *PGKneo* expression unit from the fB^{neo} locus, we crossed fB^{neo}/+ mice to transgenic mice containing the *FLPe* coding sequence under the control of the promoter for human β-actin (*actin-FLPe*; Jackson Laboratory). FLPe recombinase expressed from the *actin-FLPe* transgene deleted the *PGKneo* expression unit by mediating recombination between the two *Frt* sites and thus converted the fB^{neo} locus to an *fB* locus.

Generation of $Bdnf^{LacZ/+}$ Mice. The *Bdnf* lox strain generated by Gorski et al. (3) harbors the *LacZ* gene downstream of the *Bdnf* coding region that is flanked by two *loxP* sites. When the floxed region is deleted by Cre-mediated recombination, the *LacZ* gene is put under the control of the *Bdnf* promoters, thus generating a $Bdnf^{LacZ}$ allele. We generated $Bdnf^{LacZ/+}$ mice by crossing the *Bdnf* lox strain to mice expressing Cre recombinase in male germline cells.

Histology. Mice at P10 or older ages were anesthetized with avertin and transcardially perfused with PBS and 4% paraformaldehyde (PFA). Brains were removed from the skull, postfixed overnight, transferred to 30% sucrose solution for 3–5 d, and sectioned at 50 μm using a sliding microtome. Brains from newborn mice (P0) were removed from the skull without perfusion, fixed in 4% PFA overnight, transferred to a solution containing 4% PFA and 20% sucrose for 24 h, and stored in 30% sucrose before sectioning. Immunohistochemistry was performed as described previously (4). Sources and dilution of primary antibodies were as follows: DARPP-32 (Cell Signaling; 1:200), enkephalin and substance P (Immunostar Inc.; 1:1,000), EGFP (abcam; 1:1,000; Clontech Laboratories; 1:10,000), DsRed (Clontech Laboratories; 1:1,000), DRD1a (Chemicon; 1:1,000), parvalbumin and NPY (Sigma; 1:2,000), β-galactosidase (Promega; 1:300; Cappel; 1:2,000), BrdU (Sigma; 1:1,000), and activated caspase-3 (Cell Signaling; 1:200). Fluorescent dye-conjugated secondary antibodies were obtained from Vector Labs or the Jackson ImmunoResearch Laboratories and used according to the manufacturer's instruction. X-gal staining was performed as described previously (5).

Immunoblotting. Immunoblotting was performed as described previously (4). Antibodies used are against the TrkB extracellular domain (affinity-purified rabbit IgG, 1:1,000) (6), DARPP-32 (Cell Signaling; 1:200), and α-tubulin (Sigma; 1:7,500).

In Situ Hybridization. To generate antisense riboprobes, mouse cDNA sequences for *Drd1a* (GenBank accession no. NM_010076; nucleotides 896–1337), *Drd2* (NM_010077; nucleotides 551–1350), *SP* (NM_009311; nucleotides 50–540), and *Enk* (NM_001002927; nucleotides 301–800) were amplified by PCR and cloned into the pBluscript II KS (−) plasmid (Stratagene). Antisense cRNAs for the gene of interest were synthesized from linearized plasmids using T3 RNA polymerase (Promega), [α-^{35}S]-CTP, and [α-^{35}S]-UTP (Amersham Biosciences). In situ hybridization of brain sections was performed as previously described (5). X-ray films were scanned into digital images and in situ hybridization signals were quantified using Image J software (National Institutes of Health, Bethesda). After background subtraction, signal densities obtained from 8–10 sections were averaged for each animal.

BrdU Labeling. To label newborn cells, pregnant dams were administered BrdU (Roche) by i.p. injection at a dose of 50 mg/kg in PBS. To achieve short-term labeling, the dams were killed 2 h after injection, and embryos were harvested. Brains were removed from the embryos, fixed in 4% PFA overnight, and transferred to

30% sucrose before sectioning. Coronal sections (50 μm) were cut using a sliding microtome, treated with 2N HCl for 30 min at 37 °C, rinsed in PBS three times, and then processed for BrdU immunohistochemistry as described above.

1. Xie Y, Hayden MR, Xu B (2010) BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. *J Neurosci* 30:14708–14718.
2. Regeur L, Pakkenberg B (1989) Optimizing sampling designs for volume measurements of components of human brain using a stereological method. *J Microsc* 155:113–121.
3. Gorski JA, Zeiler SR, Tamowski S, Jones KR (2003) Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. *J Neurosci* 23:6856–6865.
4. Gharami K, Xie Y, An JJ, Tonegawa S, Xu B (2008) Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington's disease phenotypes in mice. *J Neurochem* 105:369–379.
5. Xu B, et al. (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat Neurosci* 6:736–742.

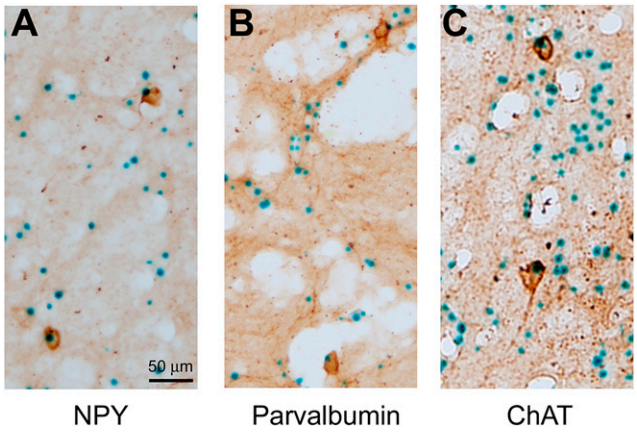

Fig. S1. *Dlx5/6-Cre*-mediated recombination in striatal interneurons. X-gal staining and immunohistochemistry were performed on brain sections obtained from *Rosa26/+;Dlx5/6-Cre* mice in which expression of β-galactosidase serves as an indicator of Cre-mediated recombination. The activity of β-galactosidase (blue dots) was present in striatal interneurons expressing NPY (*A*), parvalbumin (*B*), or ChAT (*C*). (Scale bar, 50 μm.)

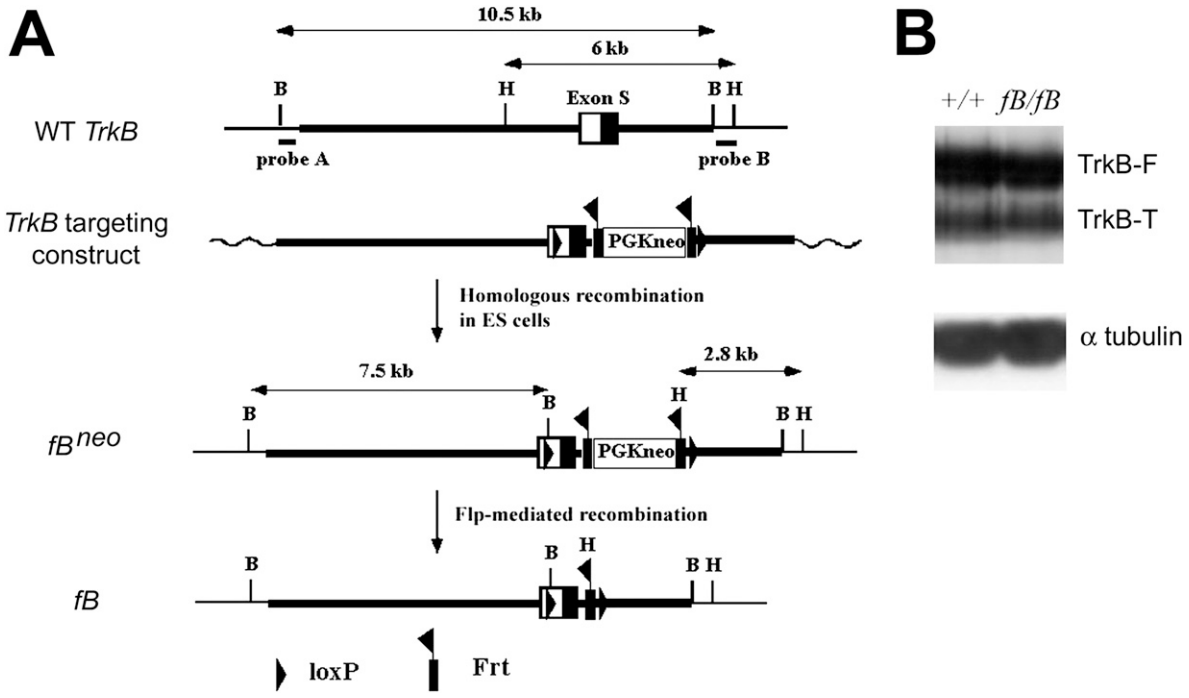

Fig. S2. Generation of a floxed *TrkB* allele. (*A*) Schematic diagrams of the *TrkB* gene, the targeting construct, and the targeted *TrkB* locus. Two homology arms are represented in thick lines. The targeting construct included one *loxP* site in the 5′ untranslated region of exon S that encodes the signal peptide for the TrkB receptor and the other *loxP* site in the intron downstream of exon S. A *PGKneo* expression unit flanked by two *frt* sites served as a selectable marker and could be removed by Flp-mediated recombination to obtain the *fB* allele. The targeting construct was used to produce an fB^{neo} allele. Once the PGKneo sequence was deleted by Flp recombinase, the *fB* allele was generated. B, *Bam* HI; H, *Hind* III; Flp, a yeast recombinase that mediates recombination between two *Frt* sites. (*B*) Western blot analysis of TrkB expression showing that the *fB* allele expresses normal amounts of the TrkB receptors. Protein extracts were prepared from the brains of newborn wild-type (+/+) and *fB/fB* homozygous mice. Fifty micrograms of protein was loaded onto each lane.

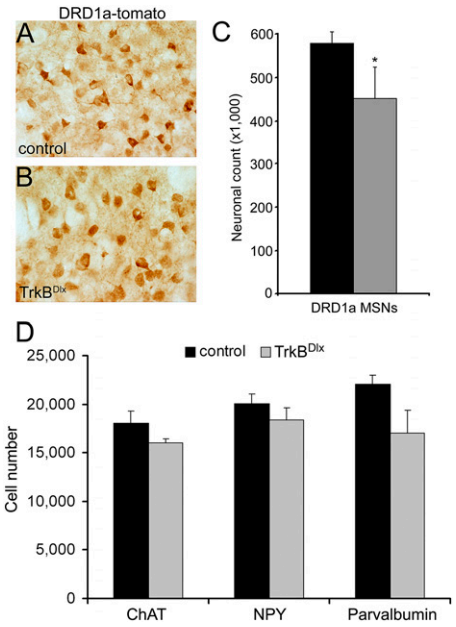

Fig. S3. Numbers of DRD1a MSNs and striatal interneurons in *TrkB*^{Dlx} mice. (*A* and *B*) Immunohistochemistry of tdTomato on brain sections from *fB/fB;Drd1a-tdTomato* (*A*) and *Dlx5/6-Cre;fB/fB;Drd1a-tdTomato* (*B*) mice at P21. (*C*) Counts of tdTomato-expressing striatal neurons in control (black bar) and mutant (gray bar) mice at P21. Student's *t* test: **P* < 0.05 (*n* = 3 mice per genotype). (*D*) Numbers of striatal interneurons. Coronal brain sections of *TrkB*^{Dlx} and control mice at 21 d of age were processed for immunohistochemistry using antibodies against markers for striatal interneurons. Unbiased stereological cell counting revealed normal numbers of striatal interneurons expressing NPY, parvalbumin, or ChAT (*n* = 4 mice each). Error bars represent SEM.

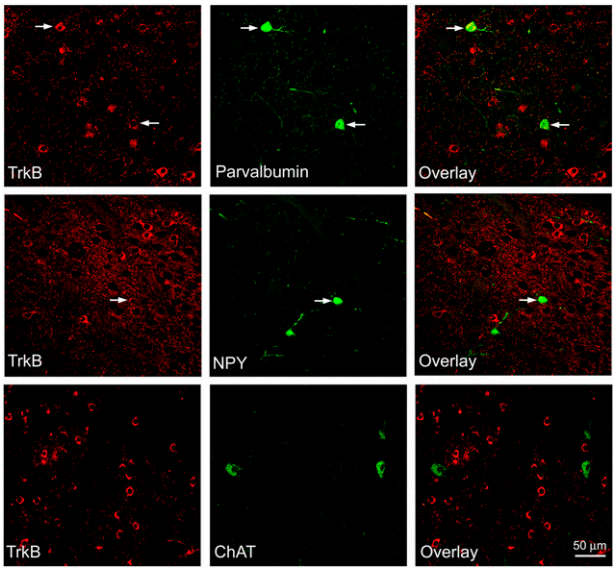

Fig. S4. TrkB expression in striatal interneurons. Immunohistochemistry was performed on brain sections obtained from *TrkB*$^{LacZ/+}$ mice using antibodies to β-galactosidase and interneuron markers. Representative images show colocalization of TrkB with parvalbumin, NPY, or ChAT. Analyses of many images indicate that 69% (9/14) of NPY neurons, 64% (9/13) of parvalbumin neurons, and 5% (1/21) of ChAT neurons express TrkB. (Scale bar, 50 μm.)

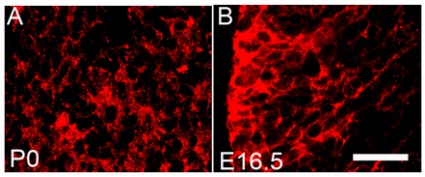

Fig. S5. TrkB expression in striatal VZ/SVZ at P0 and E16.5. Fluorescent immunohistochemistry against β-galactosidase was performed on brain sections obtained from *TrkB*$^{LacZ/+}$ mice at P0 (*A*) and E16.5 (*B*). (Scale bar, 25 μm.)