Supplemental Methods

Immunohistochemistry and confocal microscopy

Neonatal and adult animals were perfused with 4% paraformaldehyde (PFA) with (<P12) or without (>P12) 4% sucrose in 0.1 M PB. The brains were removed, post-fixed for 18 hr at 4°C, and sectioned at 50μm thickness. Embryos were removed from pregnant females and processed according to age. For embryos younger than E14, brains were exposed by opening the upper skull, and post-fixed within the head in 4% PFA with 4% sucrose at 4°C overnight; for embryos older than E14, brains were removed from the head and post-fixed under the same condition. Fixed tissues were embedded in 1.5% low melting temperature agarose and sectioned at 50 μm. Sections were blocked with 10% normal goat serum (NGS) or 10% normal donkey serum, and 0.1% Triton in PBS, and then incubated with the following primary antibodies at 4°C overnight: GFP (rabbit polyclonal; 1:800; Rockland, chicken polyclonal antibody; 1:1000; abcam), RFP (rabbit polyclonal; 1:1000; Rockland), Nkx2.1 (rabbit polyclonal; TTF-1 H-190; 1:500; Sanra Cruz biotechnology, inc.), BrdU (rat monoclonal; 1:1000; Accurate Chemical, inc.), parvalbumin (Pv, mouse monoclonal; 1:1000; Sigma, St. Louis, MO), somatostatin (SST; rat monoclonal; 1:300; Millipore), calretinin (CR; goat polyclonal; 1: 1000; Millipore, rabbit polyclonal; 1:1000; Swant), VIP (rabbit polyclonal; 1:600; Immunostar), nNOS (rabbit polyclonal; 1:500; Zymed). Sections were then incubated with appropriate Alexa fluor dye-conjugated IgG secondary antibodies (1: 400; Molecular Probes) or DyLight –conjugated IgG secondary antibodies (1:400; Jackson ImmunoResearch) and mounted in Fluoromount-G (SouthernBiotech). In some experiments sections were incubated with TOTO-3 (1:3000; Molecular Probes) together with secondary antibodies to visualize nuclei. For immunostaining against Gad67 (monoclonal; 1:800; Millipore), no detergent was added in any step, and incubation was done at room temperature for 2 days at the primary antibody step. For BrdU immunostaining, sections were denatured in HCl (2N) at 37°C for 45 min, then neutralized in borate (0.1M; PH8.5) twice for 10min at room temperature and washed in PBS, followed by the blocking reaction.

Electrophysiology in cortical slice

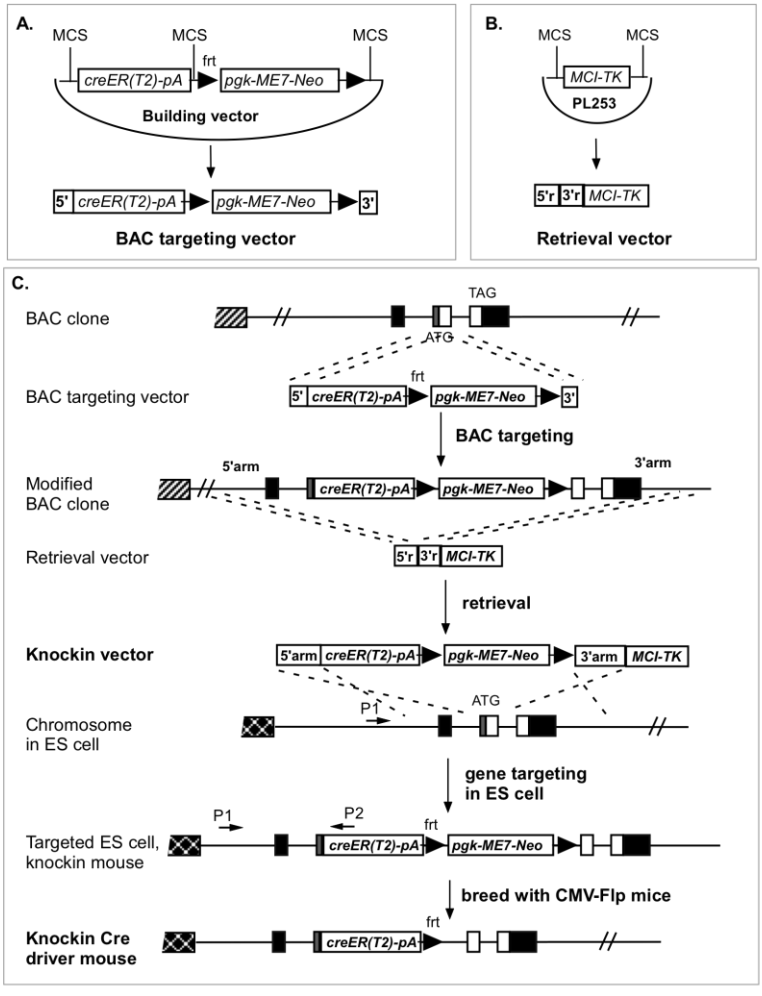
Mice (P30-34) were quickly decapitated, and the brains were dissected and transferred into ice-cold oxygenated artificial cerebrospinal fluid (ACSF composition in mM: 110 choline-Cl, 10 Na-ascrobate, 3.1 Na-pyruvate, 2.5 KCl, 4 $MgCl_2$, 1 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 11 D-glucose, pH 7.35, ~300 mOsm). Transverse slices (300-350 μm) containing visual cortex were prepared with a vibratome at 0-2 °C and further incubated with oxygenated ACSF at 34 °C for 30 min, and then transferred to ACSF at room temperature (25 °C) for > 30 min. For experiments, slices were transferred to the recording chamber and perfused with oxygenated ACSF at 32-34 °C. Whole-cell recording was made with Axopatch 700B amplifiers (Molecular Devices, Union City, CA), using an upright microscope (Olympus, BX51) equipped with infrared-differential interference contrast (IR-DIC) optics and fluorescence excitation source. Both IR-DIC image and fluorescence image are captured with a digital camera (Microfire, Optronics, CA). The internal solution of the recording pipette contained (in mM): 130 K-gluconate, 15 KCl, 10 Na-phosphocreatine, 10 HEPES, 4 ATP-Mg, 0.3 GTP and 0.3 EGTA, adjusted to pH 7.3 with KOH and to ~300 mOsmol. The pipette resistance was 3 - 5 MΩ. Signals were recorded and filtered at 2 kHz, digitalized at 10 or 20 kHz (DIGIDATA 1322A, Molecular Devices) and further analyzed using the pClamp 9.0 software (Molecular

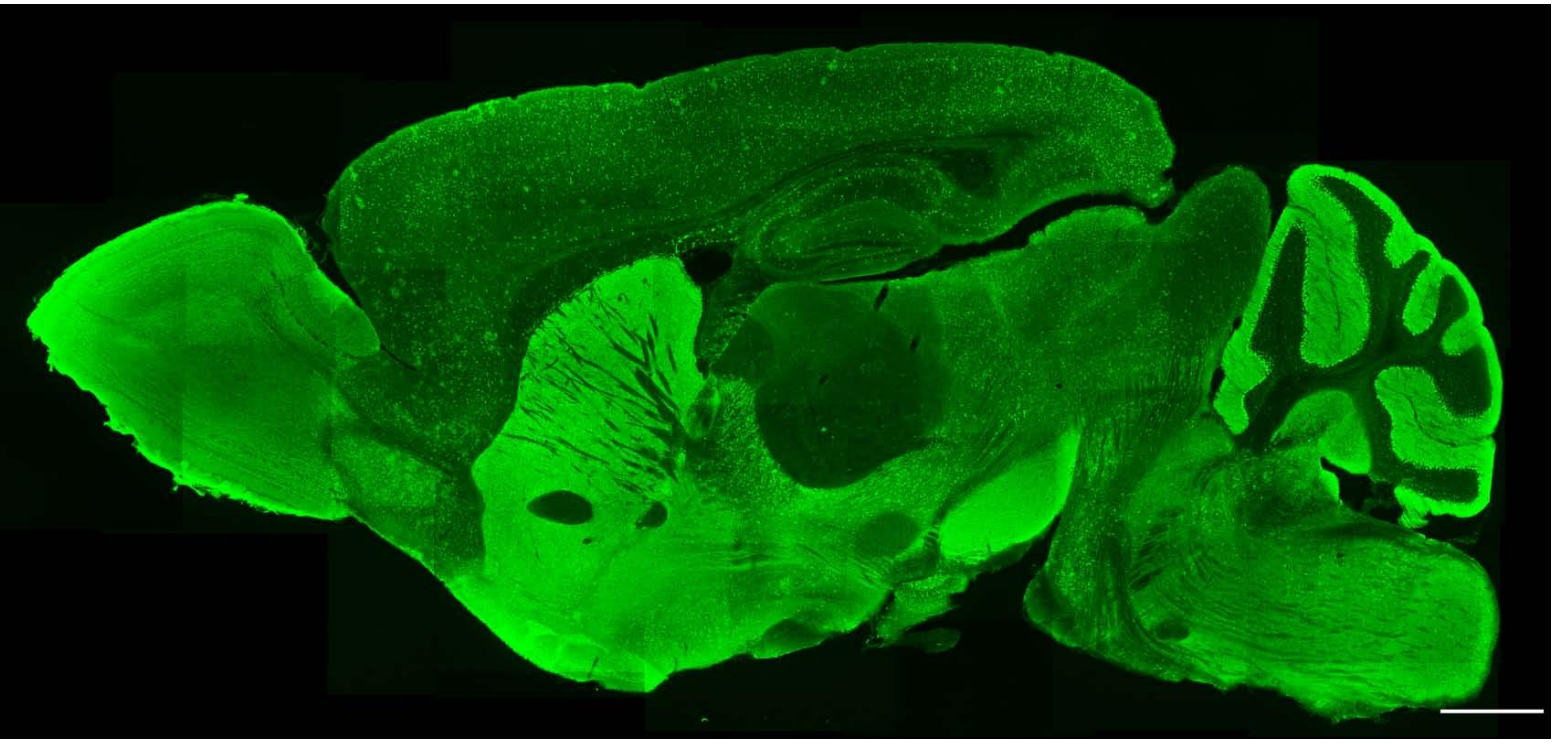
Devices) for intrinsic properties. Data are presented as mean ± SEM. The comparison of results before and after treatments was done by Mann-Whitney test.

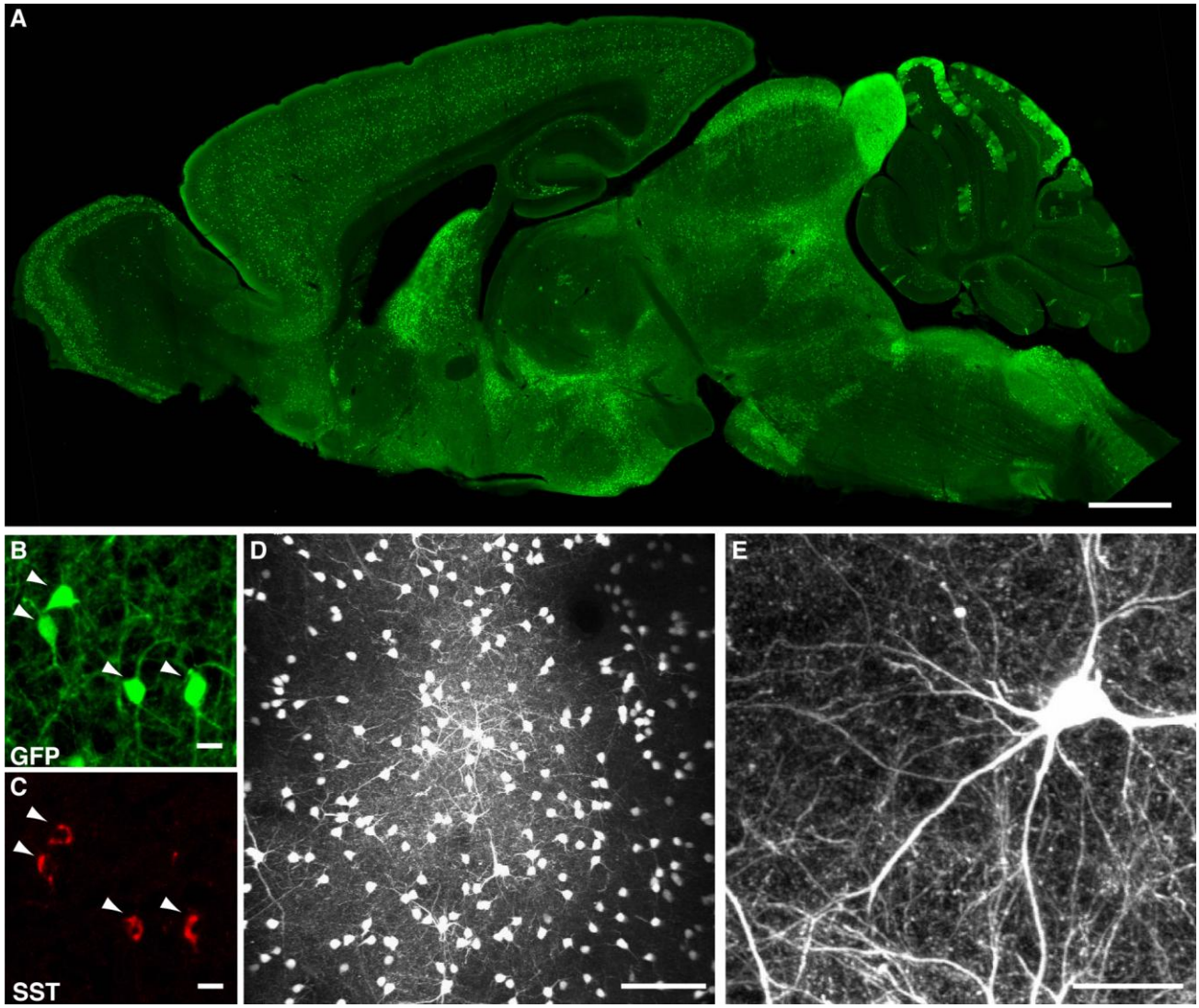
***In vivo* 2-photon imaging**

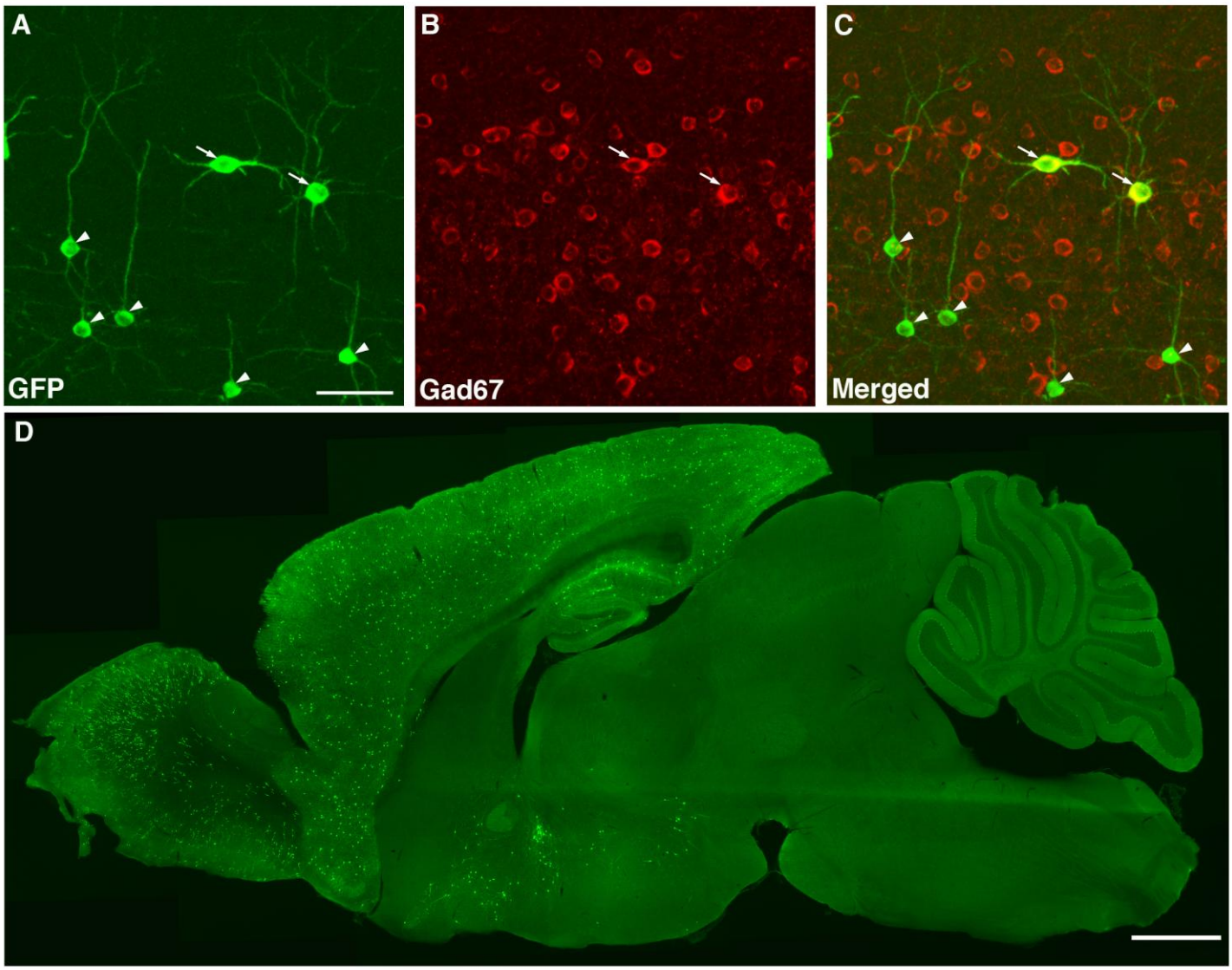
Mice were anaesthetized with intraperitoneal injection of ketamine (0.13mg per g body weight) and xylazine (0.01mg/g). A 5X5mm^2 region of the skull was removed to expose the dura. An optical chamber was constructed by covering the intact dura with agarose and cover glass. Mice were transferred to be imaged with a custom-built 2-photon scanning microscope with a Chameleon UltaII laser (Coherent). In vivo imaging of neurons expressing TdTomato was acquired by using a 20X 1.0 numerical aperture lens (Olympus) and custom image-acquisition software (LabView). The images were processed with ImageJ.

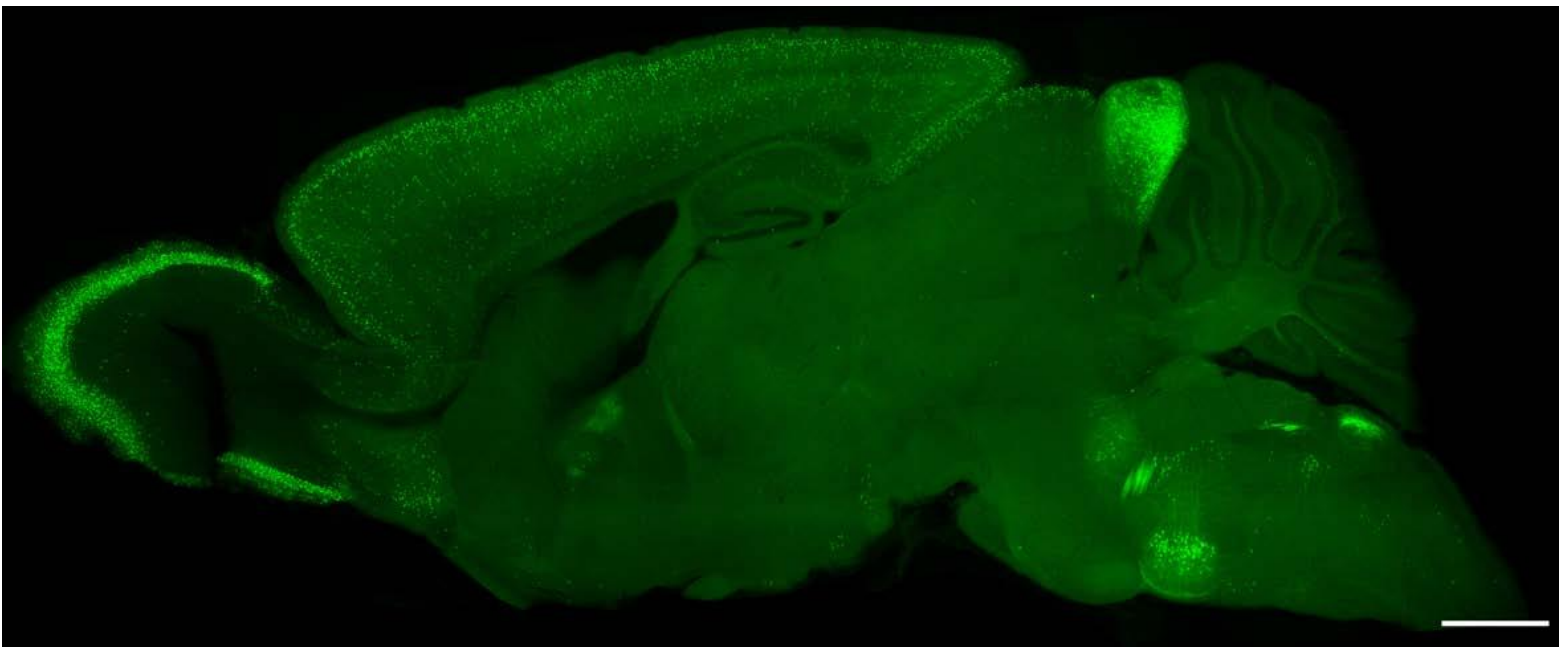
Supplemental Text and Figures

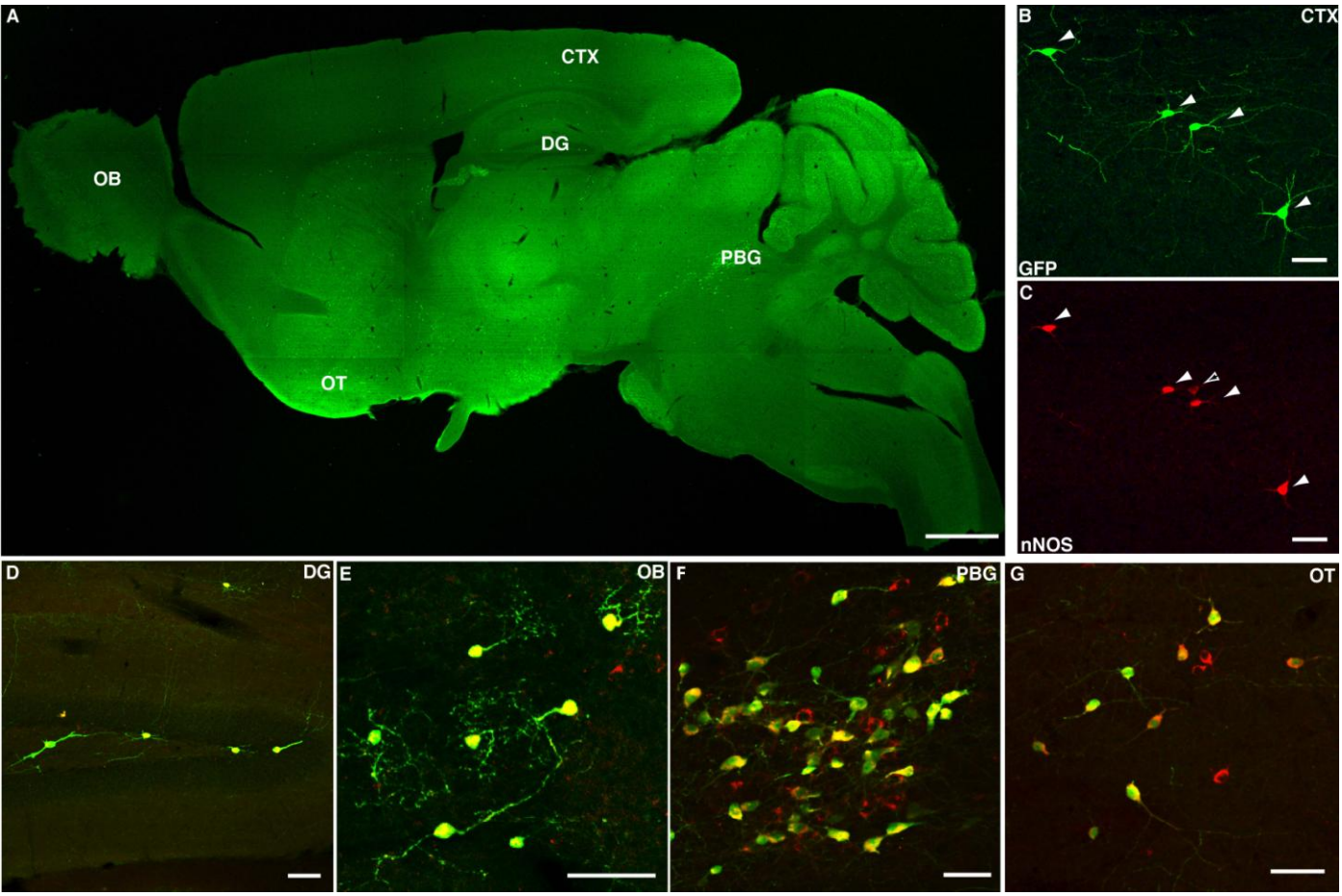


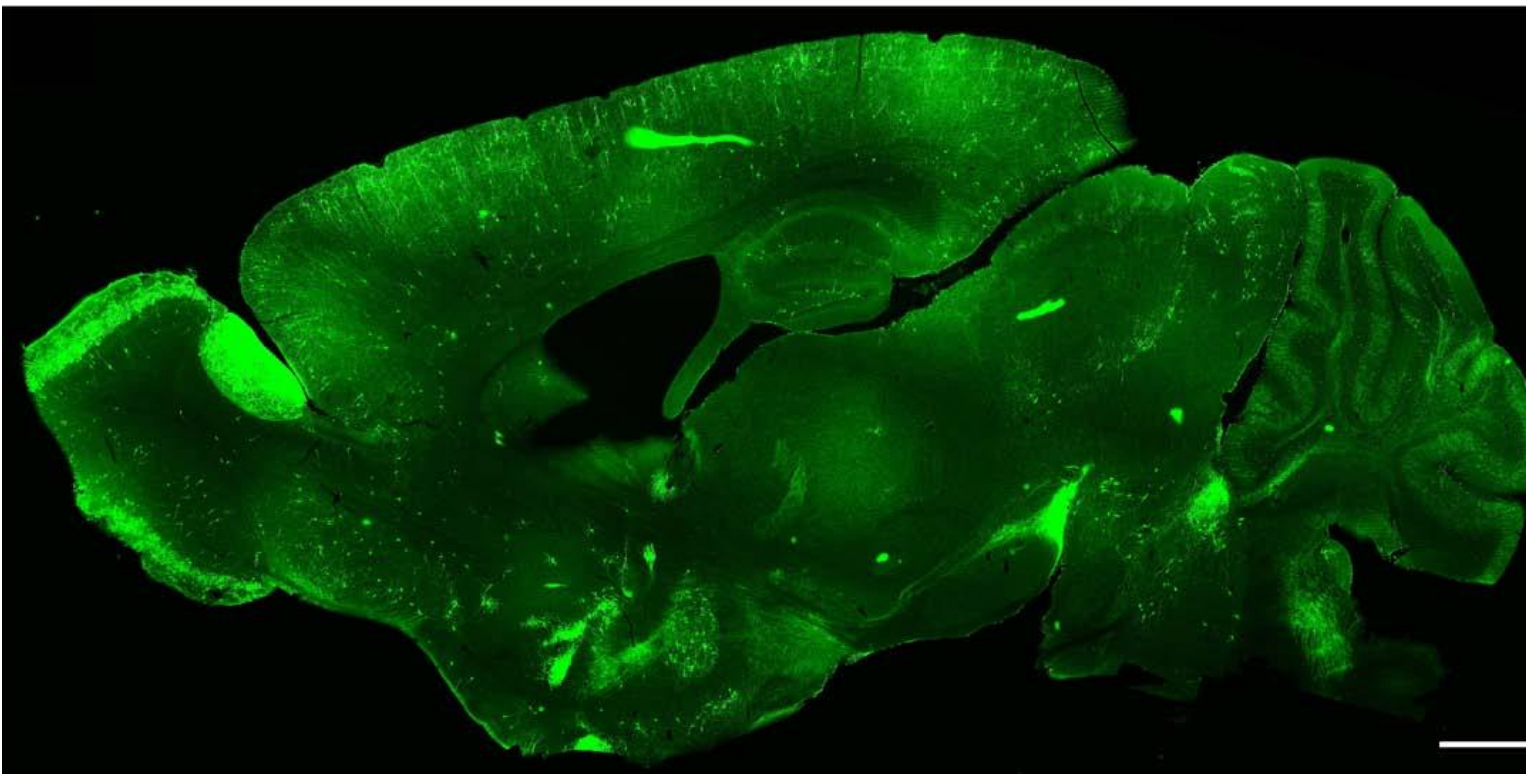


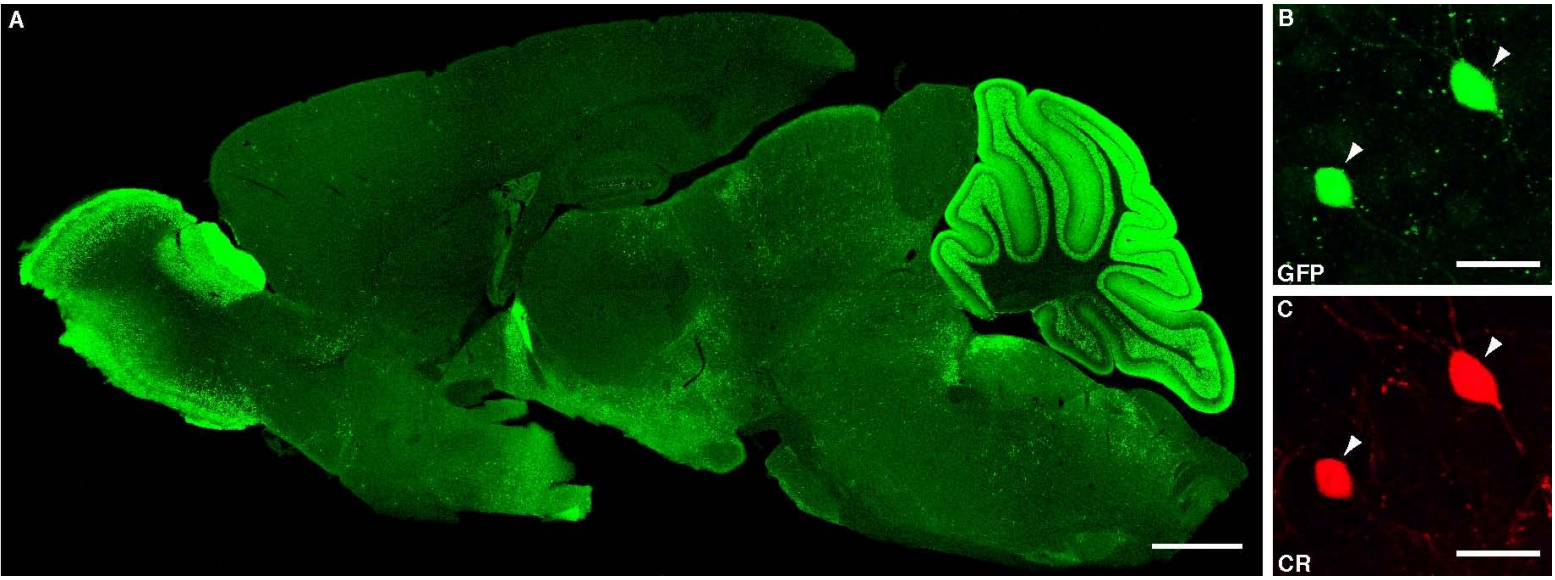

A
B
GFP
C
SST
D
E


A
GFP
B
Gad67
C
Merged
D




A
CTX
DG
OB
PBG
OT
B
CTX
GFP
C
nNOS
D
DG
E
OB
F
PBG
G
OT




A
B
GFP
C
CR

Supplemental Figures

Sppl Figure 1

Scheme for the generation of GABA Cre drivers through recombineering and gene targeting.
(A) Example of a CreER^{T2} building vector and the generation of BAC targeting vector. pA: polyadenylation sequence; *pgk-ME7-Neo*: pgk and ME7 promoters driving the Neo gene; black arrow: frt site; MCS: multi-cloning sites; 5': 5' recombination arm; 3': 3' recombination arm. (B) Generation of a retrieval vector. *MC1-TK*: MC1 promoter driving the TK gene. 5r': 5' retrieval arm; 3r': 3' retrieval arm. (C) Recombineering scheme for generating a CreER^{T2} knockin vector. A CreER^{T2} expression cassette from the BAC targeting vector in (A) is first targeted into a selected BAC clone at the translation initiation site. This modified BAC is then used to generate a knockin vector by retrieving from it a DNA segment with defined 5' homology, CreER^{T2} expression cassette, and 3' homology into the retrieval vector in (B). Knockin vector is used to target ES cells and generate knockin mice. The final Cre knockin driver is generated by breeding with *CMV-Flp* transgenic mice to

remove the *pgk-EM7-Neo* cassette from the germ line.

Sppl Figure 2

The *Gad2-ires-Cre* driver allows genetic access to GABAergic neurons throughout the brain.
GFP immunostaining of a sagittal section from an adult *Gad2-ires-cre::RCE-LoxP* mouse brain. Scale bar: 1mm. Note that a few astrocytes in cortex are labeled; this may reflect developmental expression of Gad2 in these cells.

Sppl Figure 3

The *SST-ires-Cre* driver allows genetic access to somatostatin expressing neurons.
(A) GFP immunostaining of a sagittal section from an adult *SST-ires-cre::RCE-LoxP* mouse brain. Scale bar: 1mm. (B, C) Immunostaining shows colocalization of GFP and somatostatin (red) in cortical neurons. Arrowheads: GFP and SST double positive cells. Scale bar: 10 μm. (D,E) Projections of in vivo 2-photon image stacks from somatosensory cortex of an anesthetized *SST-ires-Cre::Ai9* mouse. A movie file from the stacked images is available as sppl movie 1. Scale bars: 100 μm in (D) and 20μm in (E).

Sppl Figure 4

The *CCK-ires-Cre* driver captures cholecystokinin expressing GABA neurons through intersection with *Dlx5/6-Flp*.
(A-C) Confocal images of an adult *CCK-CreER::RCE-LoxP* mouse cortex induced in the adult stage; note that both pyramidal (arrow heads) and GABA (arrows) neurons are labeled. (A) GFP. (B) Gad67. (C) A merged image. Scale bar: 50 μm. (D) GFP expression in a sagittal section from an adult *CCK-ires-cre::Dlx5/6-Flp::RCE-dual* mouse brain. This intersection strategy targets highly selective subsets of GABAergic neurons in the brain. Scale bar: 1mm.

Sppl Figure 5

The *VIP-ires-Cre* driver allows access to vasoactive intestinal peptide expressing cells in the brain.
Native RFP signal from a sagittal section of an adult *VIP-ires-cre::Ai9* mouse brain. Scale bar: 1mm.

Sppl Figure 6
Tamoxifen administration efficiently induces Cre activity in nNOS expressing neurons.
(A) GFP immunostaining of a sagittal section from an induced adult *nNOS-CreER::RCE-LoxP* mouse brain. Tamoxifen was administrated by intraperitoneal injection once at 8mg/d dosage. Mouse was perfused 9 days after injection Brain regions with high number of GFP positive cells are indiated. OB: olfactory bulb. CTX: cortex. DG: Denate gyrus. PBG: Parabigemenal neuclus. OT: Olfactory tubercle. Scale bar: 1mm. (B, C) Immunostaining shows colocalization of GFP and nNOS in induced neurons. Arrow heads: GFP and nNOS double positive cells. Scale bar: 20 μm.(B-G) Higher magnification image of labeled cells in different brain region as indicated in (A). Scale bar: 50 μm.

Sppl Figure 7
The *CRH-ires-Cre* driver allows genetic access to corticotropin-releasing hormone expressing cells throughout the brain.
GFP immunostaining of sagittal section from an adult *CRH-ires-cre::RCE-LoxP* mouse brain. Scale bar: 1mm.

Sppl Figure 8
Tamoxifen administration induces moderate level of recombination in the *CR-CreER* driver.
(A) GFP immunostaining of a sagittal section from an induced adult *CR-CreER::RCE-LoxP* mouse brain. Tamoxifen was administrated by intraperitoneal injection every other day for 5 days at 8mg/d dosage. Mouse was perfused 9 days after last injection. Scale bar: 1mm. (B,C) Co-localization of GFP and calretinin immune- fluorescence (red) in induced neurons. Arrowheads: GFP and CR double positive cells. Scale bar: 20 μm.

Supplemental Table 1. List of building vectors

Building vector	Purpose
CreER^{T2}pAfrt-Neo-frt	Target ATG when it is in the 2nd or 3rd exon
CreER^{T2}frt-Neo-frt	Target ATG when it is in 1st exon; use endogenous intron, polyA
CrepAfrt-Neo-frt	Target ATG when it is in the 2nd or 3rd exon
Crefrt-Neo-frt	Target ATG when it is in 1st exon; use endogenous intron, polyA
iresCrefrt-Neo-frt	Inserted after STOP codon in order not to disrupt coding region
iresCreER^{T2}frt-Neo-frt	Inserted after STOP codon in order not to disrupt coding region