**A robust and high-throughput Cre reporting and characterization system for the whole mouse brain**

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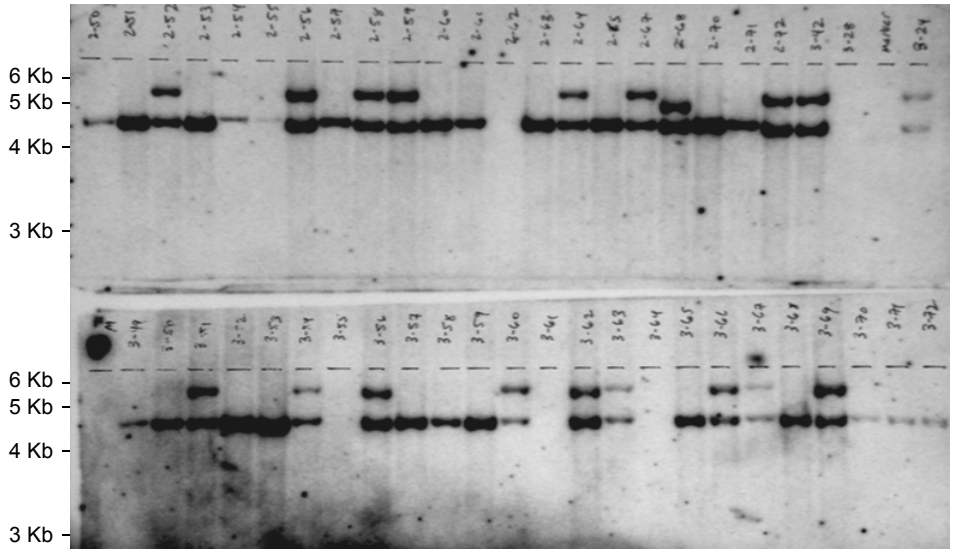
**Supplementary Information**

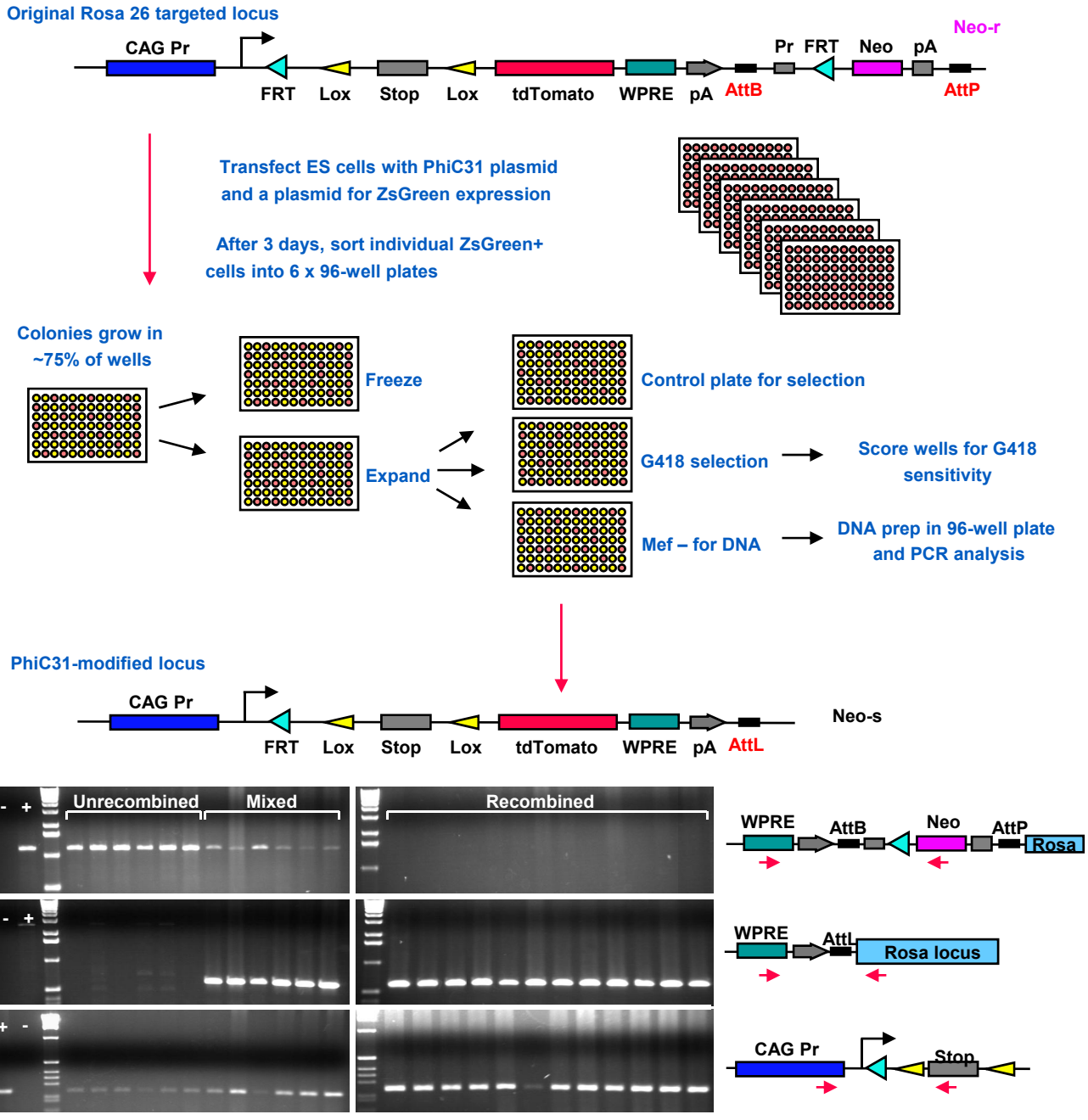
## Supplementary Figure 1




**Supplementary Figure 1.** Full Southern blot image for a screening of homologously recombined (HR) ES clones at the Rosa26 locus. The screening uses *HindIII* digested genomic DNA and the probe indicated in Fig. 1a, which results in a 4.5 kb band for the wild-type locus and a 5.6 kb band for the HR locus.

Nature Neuroscience: doi:10.1038/nn.2467

# Supplementary Figure 2

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**Supplementary Figure 2.** Deletion of the PGK-Neo selection marker cassette by PhiC31 transfection. The Ai9 targeted ES clone was co-tranfected by electroportation with 40 μg pPGKPhiC31obpA (Addgene) and 5 μg pZsGreen1-N1 (Clontech). Three days after transfection, individual cells that were transiently expressing ZsGreen (thus, likely took up the pPGKPhiC31obpA plasmid DNA as well) were sorted into 6 x 96-well plates containing feeders and were cultured in non-selective ES media with media exchange every few days. Within 12 days, approximately 75% of the wells had grown to near confluency. Cells from each 96-well plate were split onto 2 plates, one to be used for freezing and the other for cell expansion. When confluent, each expansion plate was again split into 3 plates; plate 1 was grown in control media, plate 2 was grown in G418-containing media, and plate 3 was used for DNA isolation. Cells that grew in control, but not in G418-containing media, were analyzed by PCR using primer sets designed to confirm loss of the Neomycin gene (top and middle gel images) and integrity of the 5' end of the construct (bottom gel image). Our analysis demonstrated that complete deletion of the PGK-Neo marker had occurred in over half of the PCR-screened clones. We further found that 5 of 6 Neo-deletion clones injected into blastocysts gave rise to high-percentage chimeras, demonstrating that they retained the strong germline transmission capability of the parental Ai9 clone. One Neo-deleted clone tested has been used successfully in an additional round of gene targeting with a Cre-driver construct. Correctly retargeted ES cells have given rise to high-percentage chimeras, allowing us to directly characterize a driver and reporter containing mouse, without additional breeding (data not shown). The Neo-deleted mouse line has been renamed Ai14.

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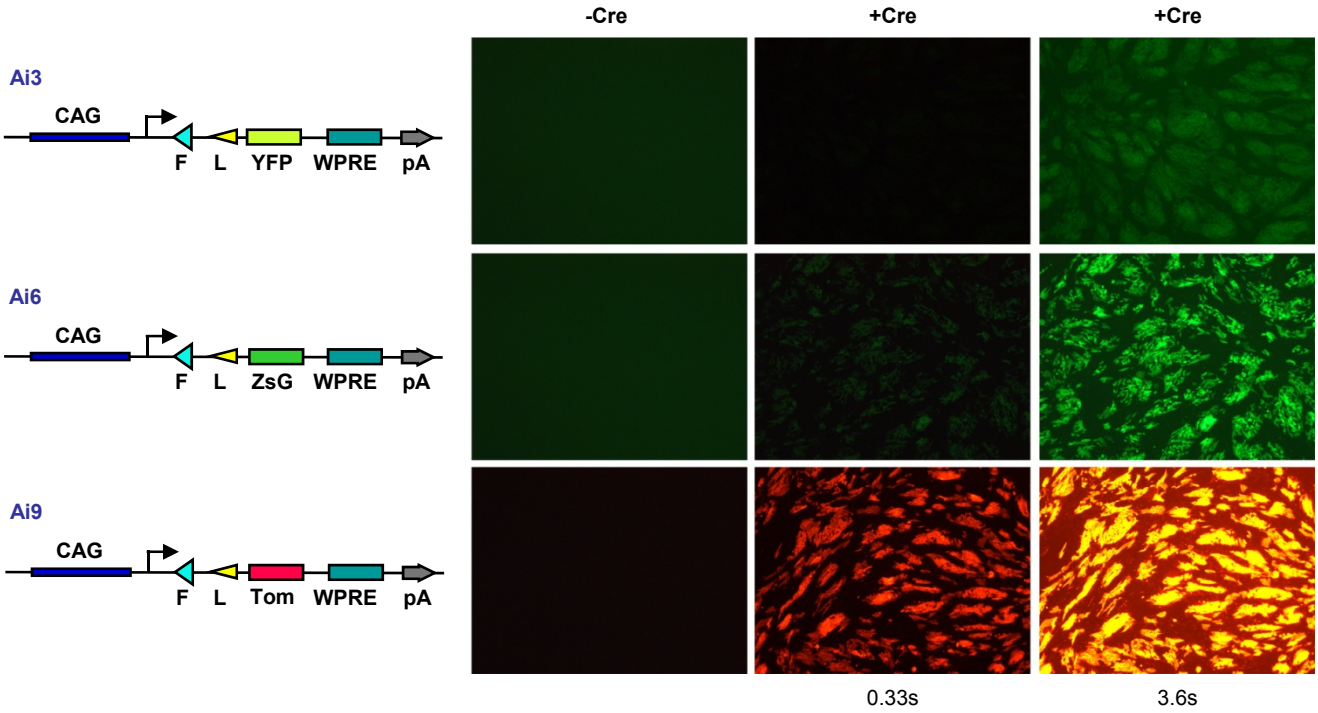
# Supplementary Figure 3




**Supplementary Figure 3.** Comparison of expression of different fluorescent reporter proteins in the targeted Ai3, Ai6 and Ai9 ES clones before and after removal of the floxed-Stop cassette by Cre transfection. Left panels: -Cre, ES cells before Cre transfection. Center and right panels: +Cre, ES cells after Cre transfection, fluorescence-activated cell sorting and replating. Exposure times for image acquisition are shown at the bottom.

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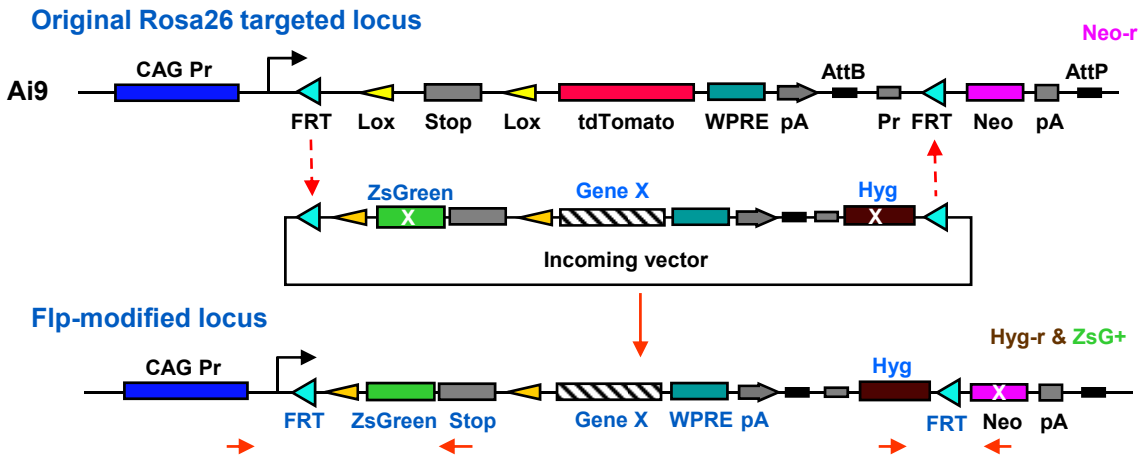
# Supplementary Figure 4




**Supplementary Figure 4.** The Flp-in strategy used to rapidly shuffle reporter components *in vitro*. To test whether Flp-mediated recombination could be used to swap other genes into the same locus, the Ai9 ES clone was co-transfected with a Flp-expressing plasmid and an incoming vector that had been designed to allow a properly recombined locus to be selected for following a double-selection strategy. The starting ES clone (*e.g.* Ai9) is Neo-resistant (Neo-r). After co-transfection of a Flp-expressing plasmid and an incoming vector, cells were selected for hygromycin-resistance (Hyg-r). Upon proper recombination, the polyA-less Hyg gene from the incoming vector becomes linked to the polyA (after Neo) in the targeted locus at the 3' FRT site, conferring hygromycin resistance. Similarly, the promoter-less ZsGreen in the floxed-Stop cassette (in front of the Stop codons) from the incoming vector becomes linked to the CAG promoter in the targeted locus through proper recombination at the 5' FRT site, giving rise to green fluorescence. Hygro-resistant colonies that were also ZsGreen-positive (ZsG+) were picked and further screened by PCR using primers across the 5' and 3' Flp-recombination junctions (blue arrows). Using this strategy, we were able to obtain ES clones containing replacement genes at high efficiency (>90% clones screened had correct Flp-in events).

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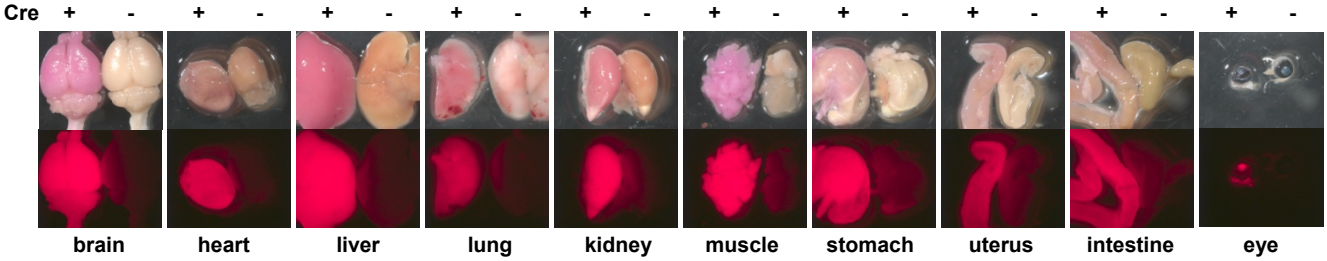
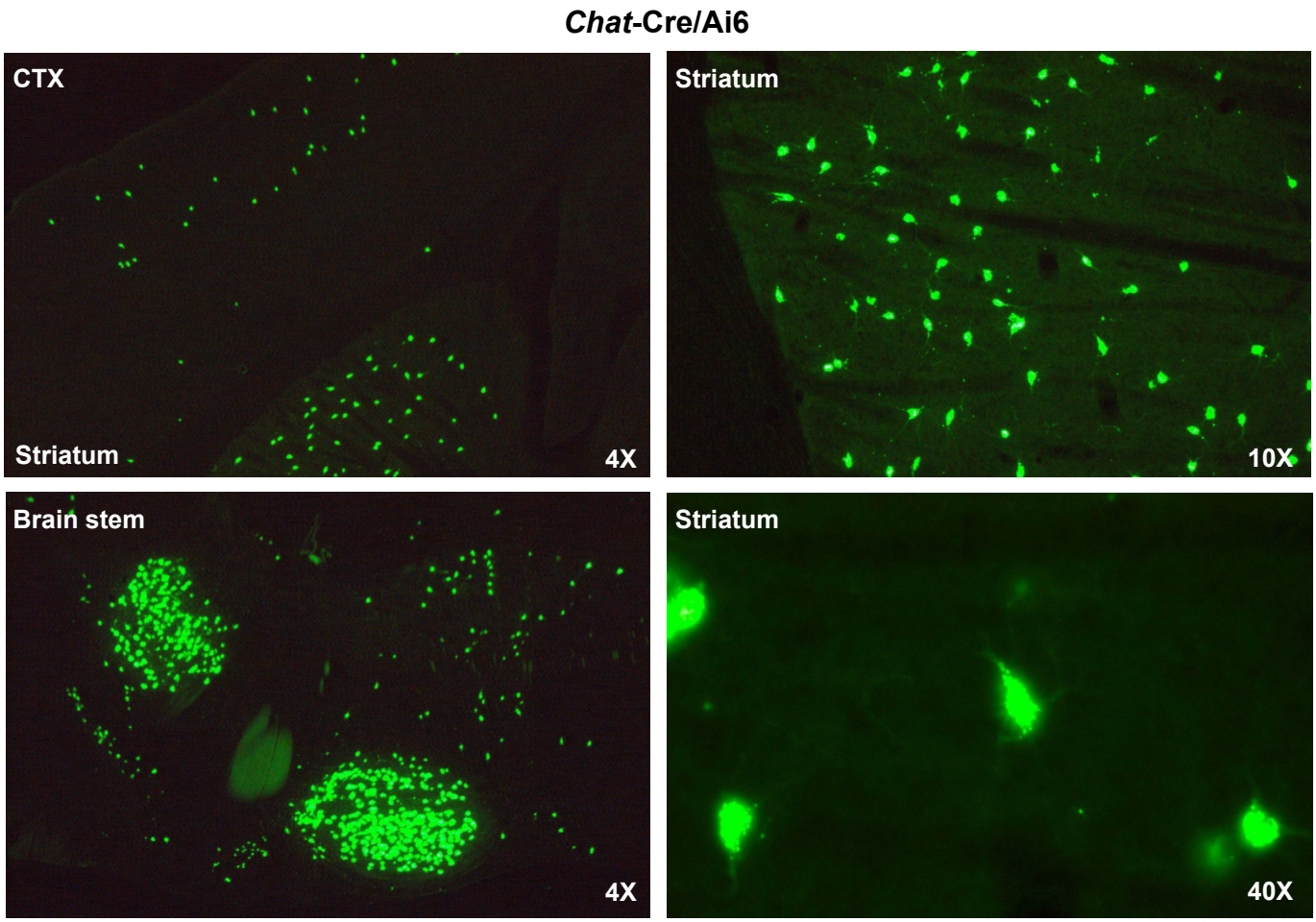
# Supplementary Figure 5




**Supplementary Figure 5.** Strong Cre-induced fluorescence in the new Cre-reporter lines by comparison of fluorescence in various tissues from an EIIa-Cre/Ai14 mouse (left, Cre+) with that in tissues from a control Ai14 mouse (right, Cre-). EIIa-Cre (adenovirus EIIa promoter-driven Cre) induces recombination during early embryonic development, thus tdTomato expression is turned on throughout the body. Notice that the tdTomato expression was so robust that even under white light (upper panels) all EIIa-Cre/Ai14 tissues appeared more pinkish than those from Ai14 (except eyes, which are black). In the lower panels, all EIIa-Cre/Ai14 tissues showed strong red fluorescence, whereas no fluorescence was observed in Ai14 tissues.

Nature Neuroscience: doi:10.1038/nn.2467

# Supplementary Figure 6




**Supplementary Figure 6.** ZsGreen fluorescence from Ai6 is very bright, but fluorescence is mostly confined within cell bodies and appears to be aggregated. An example of this is shown for the *Chat*-Cre/Ai6 mouse, in which individual ZsGreen-positive neurons are bright even under low magnification (4x). At 40x magnification, aggregates are seen within the soma.

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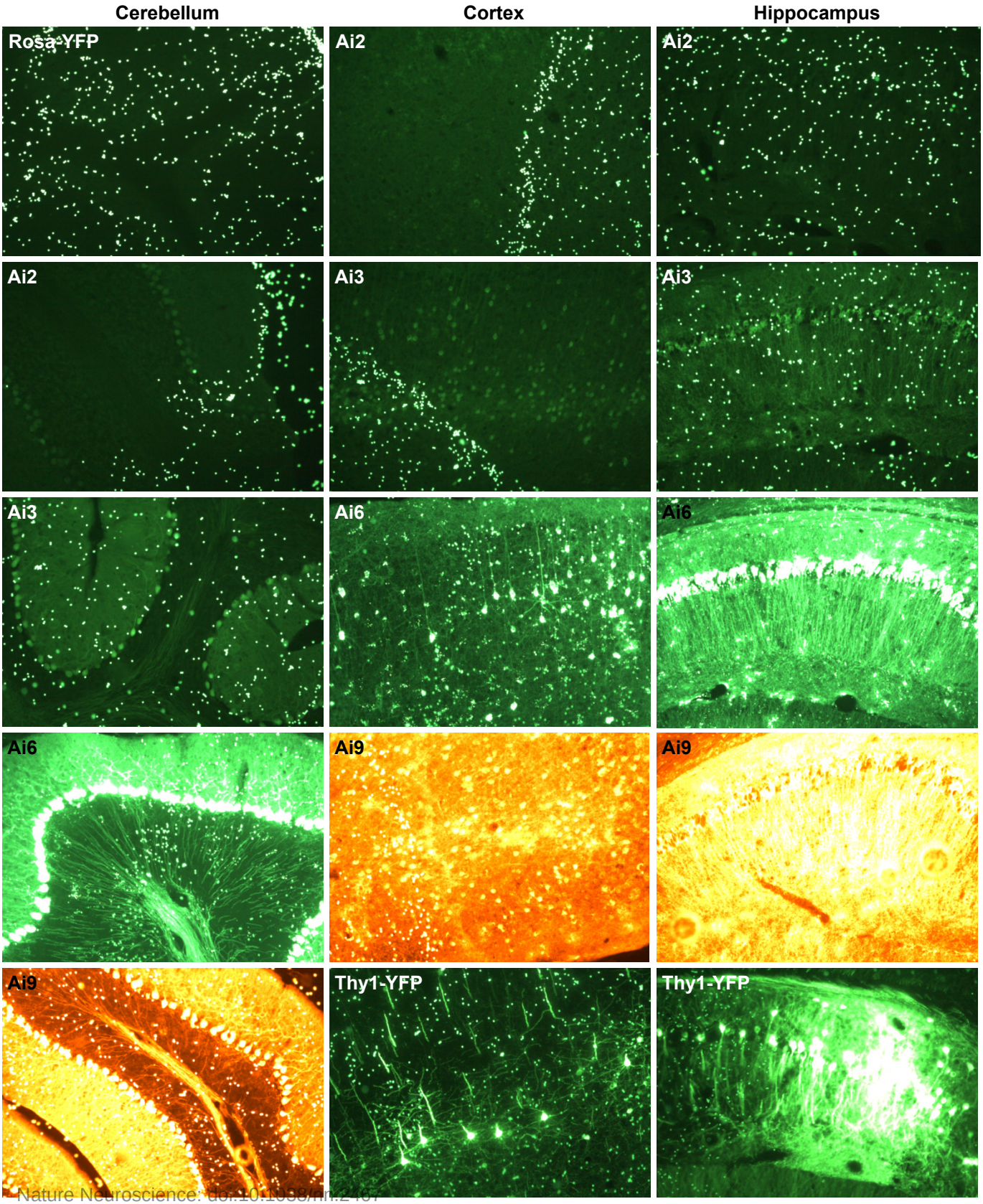
# Supplementary Figure 7

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**Supplementary Figure 7.** Comparison of relative fluorescence intensity of different fluorescent reporter labeled cells using TetraSpeck microspheres (4.0 μm) (Invitrogen) as a standard. These fluorescent beads display 4 separate colors – blue, green, orange and dark red. The beads were loaded directly onto the tape transfer XFP sections, and the slides were then mounted for taking images of the labeled cells together with the beads. Images shown here were taken from 3 regions, cerebellum, cortex and hippocampus, of mice in which each reporter was crossed to *Pcp2*-Cre – the *Pcp2*-Cre/Rosa26-EYFP, *Pcp2*-Cre/Ai2, *Pcp2*-Cre/Ai3, *Pcp2*-Cre/Ai6, and *Pcp2*-Cre/Ai9 mice, plus the *Thy1*-YFP mouse as a positive control. The beads can be seen as the scattered dots on each image. Images were taken from each section using the same exposure time, 400 ms, to allow visualization of weak labeling in Ai2. With this exposure time, signals from Ai6, Ai9 and *Thy1*-YFP are saturated. However, quantitative comparison results of YFP fluorescence intensity among Rosa26-YFP, Ai2 and Ai3 were similar to those shown in **Figure 2c** (from images with 140-ms exposure time).

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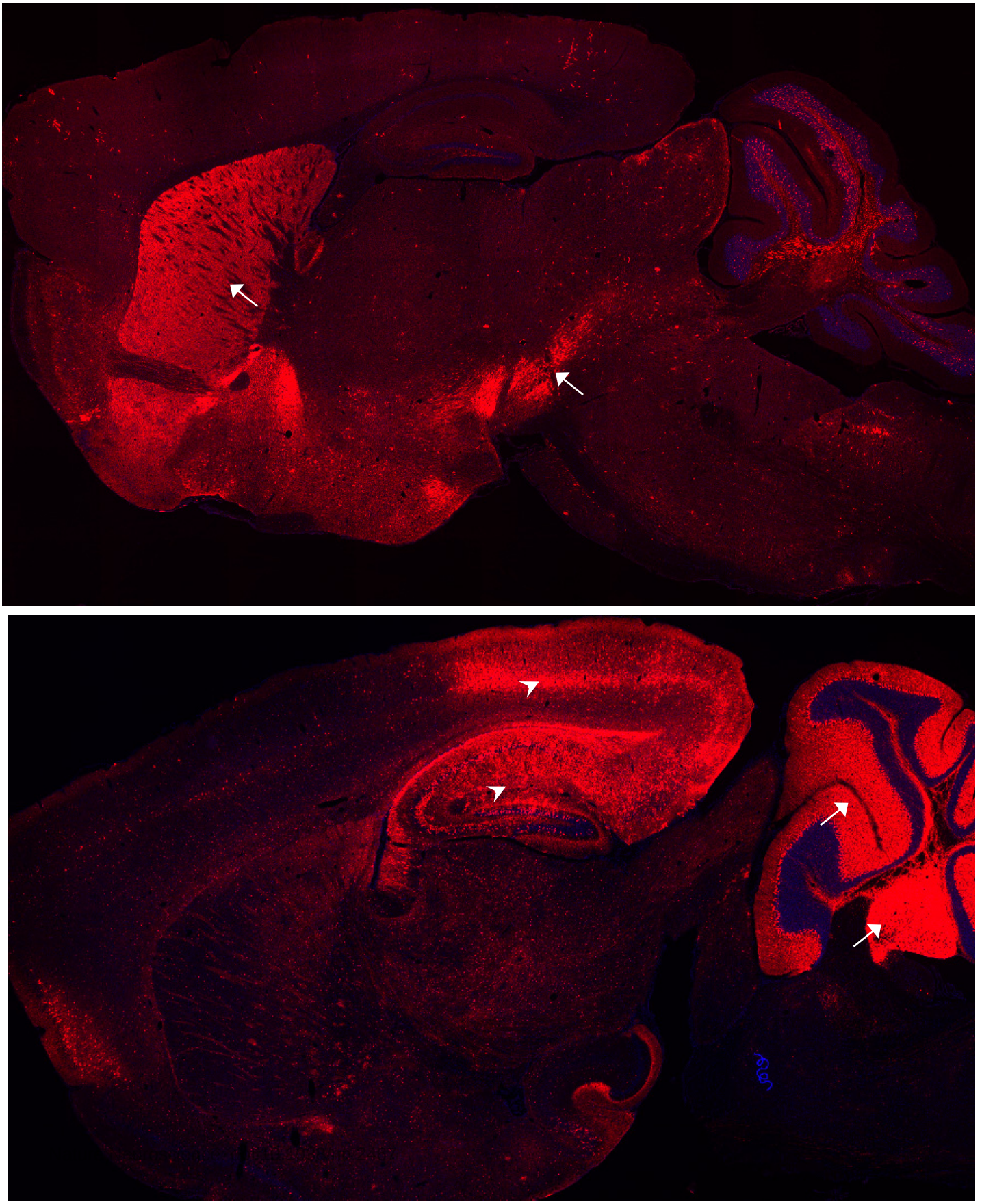
# Supplementary Figure 8



**Supplementary Figure 8.** More recombined cells than expected were detected with the new Cre reporter lines. **(a)** Enlarged images of *Slc6a3*-Cre controlled reporter gene expression showing sparsely scattered Cre-recombination in addition to recombination expected in dopamine neurons. Expression of tdTomato in dopamine neurons in the ventral tegmental area (VTA) and substantia nigra and their projections into the striatum is brightly labeled (arrows). In addition, scattered cells expressing tdTomato are seen throughout the brain. **(b)** Enlarged images of *Pcp2*-Cre controlled reporter gene expression showing extensive and widespread Cre-recombination in addition to that expected in cerebellar Purkinje cells. Expression of tdTomato in the Purkinje cells and their axon bundles is brightly labeled (arrows). In addition, scattered cells expressing tdTomato are seen throughout the brain, especially in the visual cortex and hippocampus (arrowheads). DAPI staining is shown in blue.

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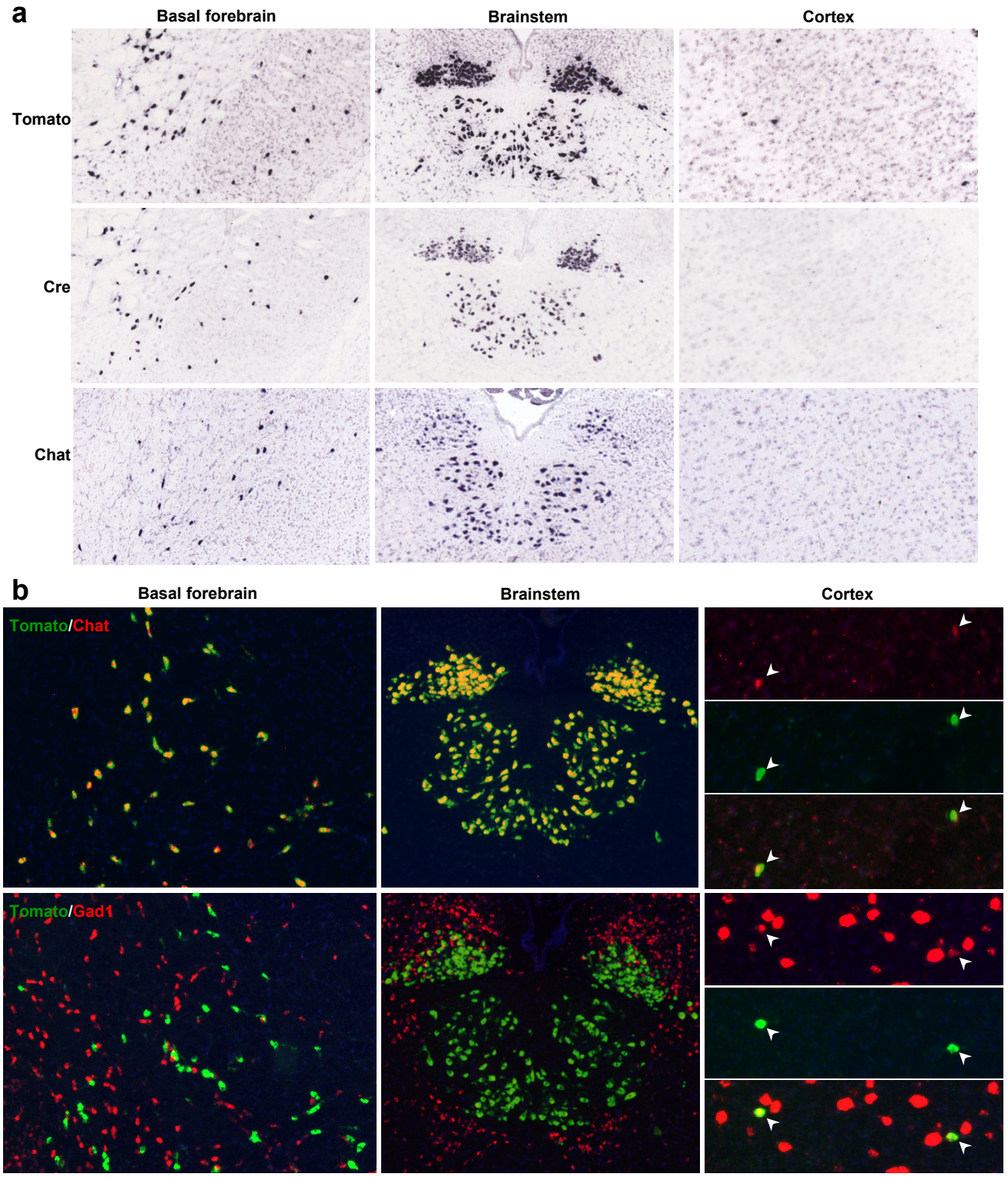
# Supplementary Figure 9

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**Supplementary Figure 9.** Colorimetric ISH and DFISH characterization of the identity of Cre-recombined cells in *Chat*-Cre mice, of which the original targeted gene *Chat*, the choline acetyltransferase, is expressed in all cholinergic neurons. **(a)** Comparison of the expression of the tdTomato reporter, Cre and the original targeted gene, *Chat*, in the *Chat*-Cre/Ai9 mouse by colorimetric ISH. All 3 genes showed similar expression patterns in the striatum/basal forebrain (left panels) and brainstem (middle panels). In the cortex (right panels), tdTomato was expressed in a sparse population of cells, whereas expression of both *Chat* and Cre was barely detectable. **(b)** DFISH characterization of Cre-recombined cells from the *Chat*-Cre/Ai9 mouse through co-labeling of tdTomato (green) with *Chat* (red, upper panels) or the interneuron marker *Gad1* (red, lower panels). In the basal forebrain (left panels) and brainstem (middle panels), tdTomato was almost always co-localized with *Chat*, but not with *Gad1*, indicating that the labeled cells were cholinergic but not GABAergic. In the cortex (right panels), a very sparse population of cells were tdTomato-positive (arrowheads); the red channels for both *Chat* and *Gad1* were adjusted to near saturation, to show that these cells might also express both *Chat* and *Gad1* at low levels. DAPI staining is shown in blue. The tdTomato-positive cells in the cortex had mostly bipolar dendrites. Although suspected to be interneurons, it was previously found that these bipolar neurons do not exert inhibitory effects on nearby neurons, nor do they have robust cholinergic outputs. Consistent with this, the cortical tdTomato-positive cells did not co-localize with the strongly *Gad1*-positive interneuron populations, but rather express *Chat* and *Gad1* at only marginal levels (right panels).

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