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Supplemental information

Generation of a DAT-P2A-Flpo mouse line for intersectional genetic targeting of dopamine neuron subpopulations

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Supplemental Figures and Legends

Supplemental Figure 1

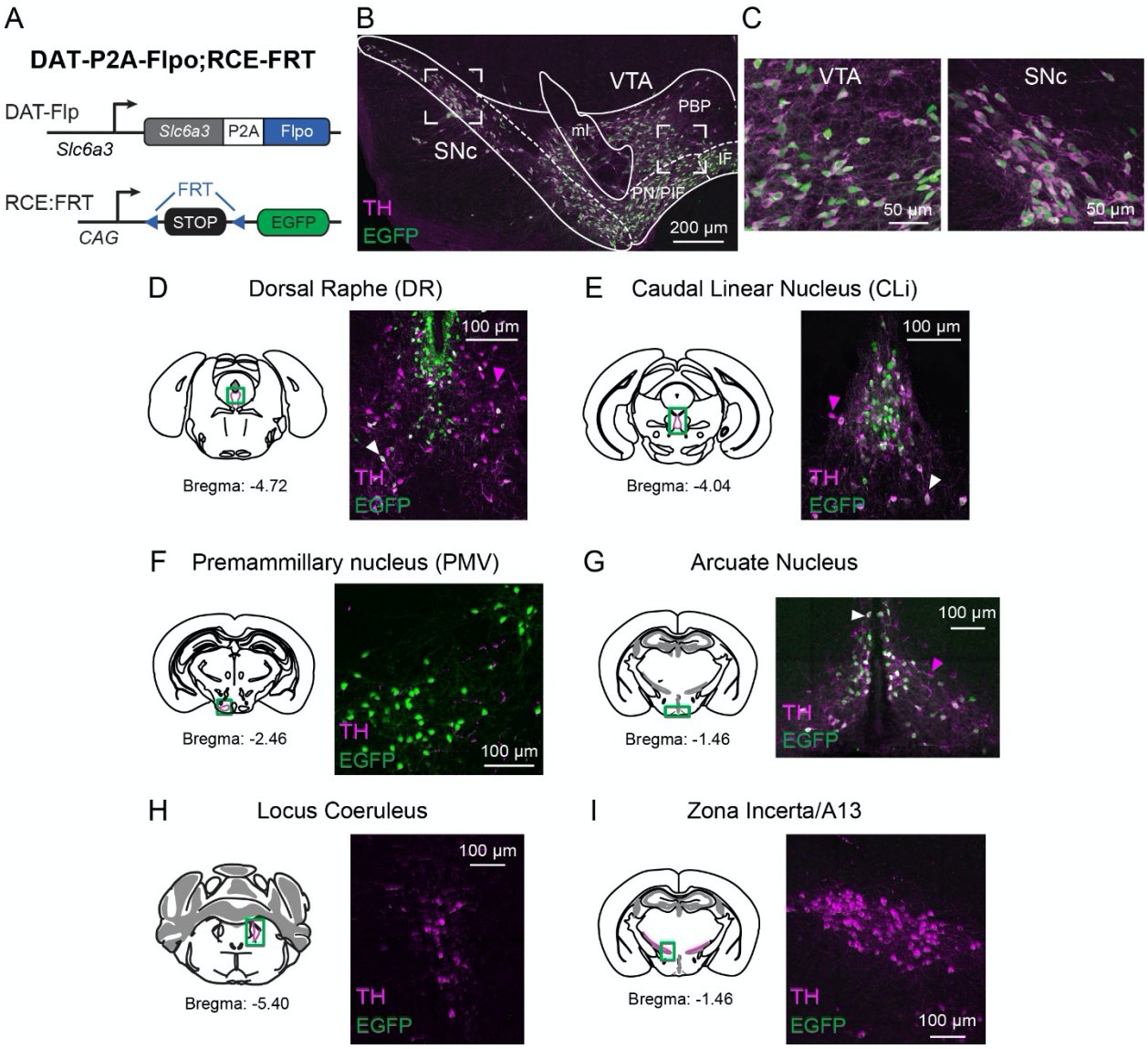

Figure S1. Flp-reporter expression in DAT-P2A-Flpo;RCE-FRT mice, Related to Figure 1.

A) Schematic of the genetic cross used to generate DAT-P2A-Flpo;RCE:FRT mice.
B) Representative confocal image of the midbrain from a DAT-P2A-Flpo;RCE-FRT mouse showing EGFP Flp reporter expression in tyrosine hydroxylase (TH) positive SNc and VTA neurons. Representative of 7 mice. SNc = substantia nigra pars compacta, VTA = ventral tegmental area, PBP = parabrachial pigmented nuclei, PN/PIF = paranigral nucleus/parainterfascicular nucleus, IF = interfascicular nucleus, ml = medial lemniscus.
C) Zoomed-in images of the VTA and SNc from the boxed regions in panel B.
D-I) Left panels show anatomical schematics of the brain regions imaged (green boxes denote imaged areas). Right panels show representative images of the Dorsal Raphe (DR, D), Caudal Linear Nucleus (CLi, E), Premammillary nucleus (PMV, F), Arcuate Nucleus (G), Locus Coeruleus (H), and Zona Incerta/A13 (I) from DAT-P2A-Flpo;RCE-FRT mice showing Flp-reporter EGFP+ cells (green) and TH immunostaining (magenta). Representative of 7 mice.

Supplemental Figure 2

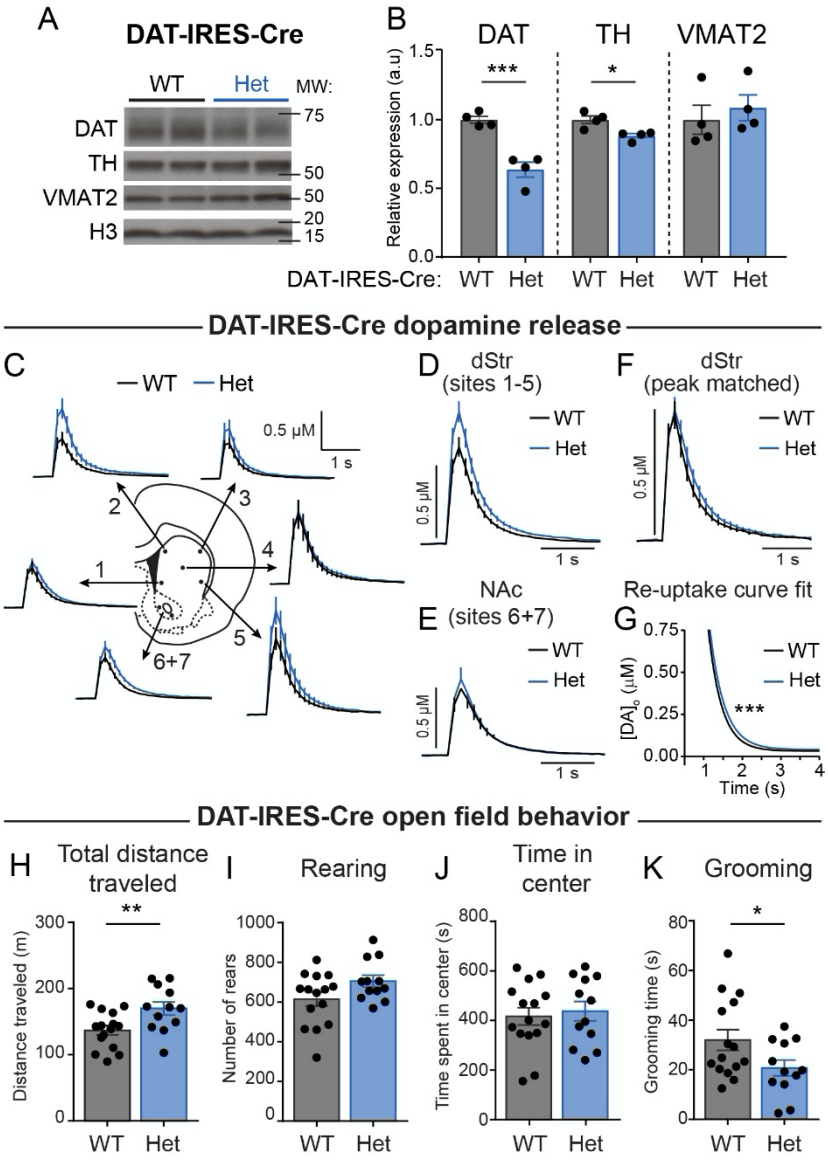

Figure S2. DAT expression and function in DAT-IRES-Cre mice, Related to Figure 2.

A) Representative western blot images of dopamine active transporter (DAT), tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), and histone H3 (H3) loading control from striatal lysates from DAT-IRES-Cre wild-type (WT) and heterozygous (Het) mice. Two samples per genotype are shown. Blots were cropped to show the relevant bands. Molecular weight (MW) in KD is indicated on the right. Representative of 4 mice.
B) Quantification of protein levels relative to histone H3, normalized to WT. Bars represent mean +/- SEM. Each dot represents the average of two striatal samples from one mouse (n=4 mice per genotype, 1 male and 3 females P100-120). DAT: p=0.0009, TH: p=0.0121, VMAT2: p=0.5655; unpaired t-tests.
C) Fast-scan cyclic voltammetry (FCV) traces of extracellular DA ($[DA]_o$) evoked by single electrical pulses in different striatal sub-regions in DAT-IRES-Cre mice. Traces are mean +/- SEM $[DA]_o$ versus time (average of 27-28 transients per site from 4 pairs of DAT-IRES-Cre WT and Het mice, 1 male pair and 3 female pairs; P100-120). 1: ventromedial striatum, 2: dorsomedial striatum, 3: dorsolateral striatum, 4: central striatum, 5: ventrolateral striatum, 6-7: nucleus accumbens.
D) Mean +/- SEM $[DA]_o$ versus time (average of 80 transients per genotype) from the dorsal striatum sites #1-5.
E) Mean +/- SEM $[DA]_o$ versus time (average of 27-28 transients per genotype) from the nucleus accumbens sites #6-7.
F) Selected region- and peak-matched mean +/- SEM $[DA]_o$ versus time from the dorsal striatum of DAT-IRES-Cre WT and Het mice (average of 33-34 transients per genotype).
G) Single exponential curve fit of the decay phase of transients in panel F. DAT-IRES-Cre heterozygous mice have significantly slower $[DA]_o$ re-uptake than WT mice (***, $p<0.0001$; WT tau=0.349, Het tau=0.377; n=33-34 transients per genotype).
H-K) Behavioral performance of DAT-IRES-Cre mice in a 60-minute open-field test. For all graphs, bars represent mean +/- SEM and dots represent values for individual mice. n= 15 WT mice; n=12 Heterozygous mice, all females; age P50-90. H) Total distance traveled in 60 minutes; p=0.0099, unpaired t-test. I) Total number of rears in 60 minutes; p=0.0668, unpaired t-test, J) Total time spent in the center of open field in 60 minutes; p=0.6976, unpaired t-test. K) Total number of grooming bouts in the first 20 minutes of open field test; p=0.0258, unpaired t-test.

Supplemental Figure 3

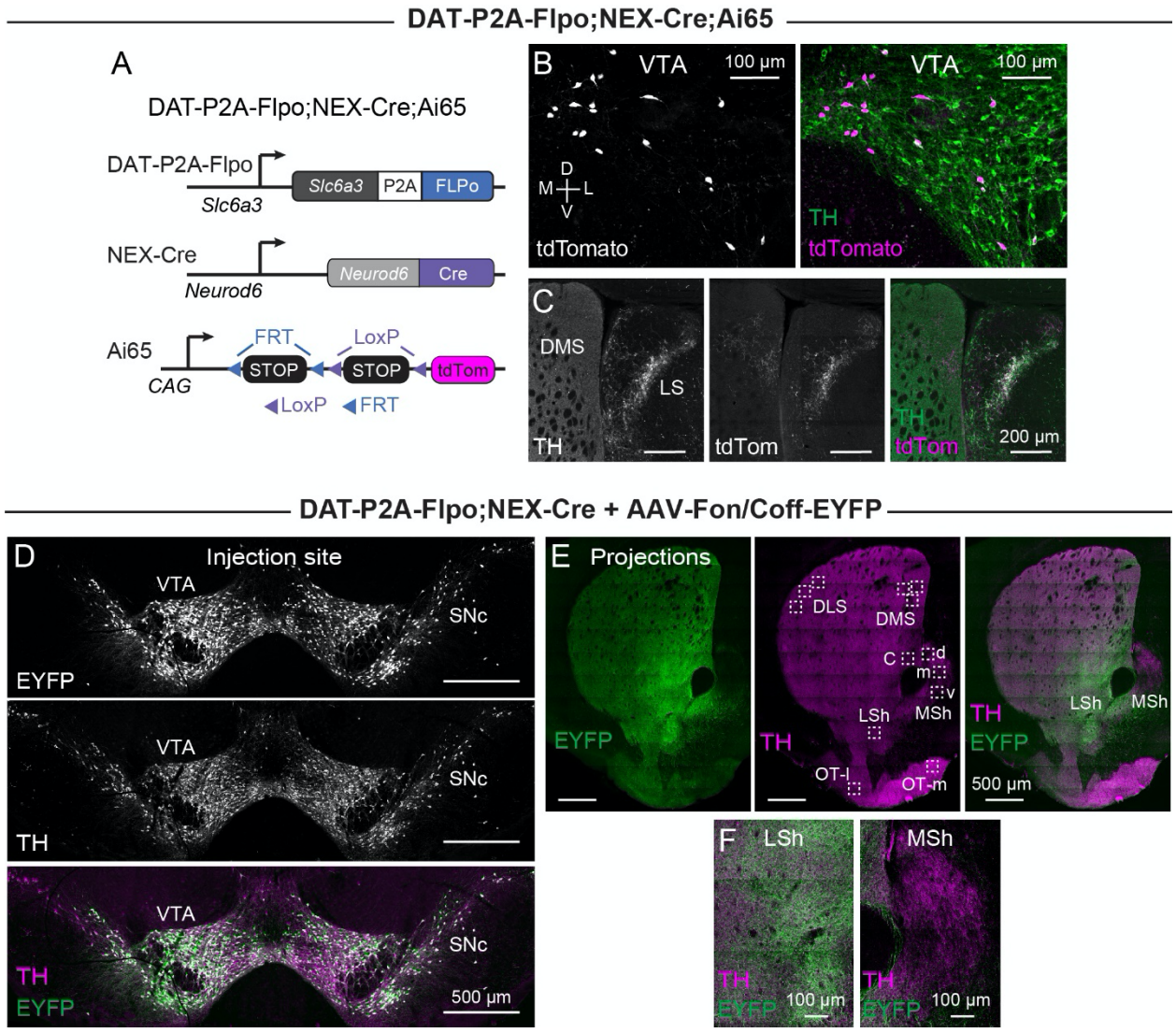

Figure S3. Intersectional labeling of neurons and projections in DAT-P2A-Flpo;NEX-Cre mice, Related to Figures 3 and 4.

A) Schematic of the triple transgenic cross to generate DAT-P2A-Flpo;NEX-Cre;Ai65 mice. In Ai65 mice, tdTomato is expressed in cells that express both Flp- and Cre-recombinase.
B) Confocal images of tdTomato+ cell bodies located in the medial ventral tegmental area (VTA) of a 15-month old male DAT-P2A-Flpo;NEX-Cre;Ai65 mouse (representative of 5 mice). TH = tyrosine hydroxylase immunostaining.
C) Some tdTomato+/TH+ projections can be observed in the lateral septum (LS) in a DAT-P2A-Flpo;NEX-Cre;Ai65 mouse. DMS = dorsomedial striatum
D) Confocal images of the midbrain of a P125 male DAT-P2A-Flpo;NEX-Cre mouse injected with AAV-Fon/Coff-EYFP and immunostained for TH (representative of 4 mice). SNc = substantia nigra pars compacta
E) Confocal images of the striatum and nucleus accumbens from a P125 male DAT-P2A-Flpo;NEX-Cre mouse injected with AAV-Fon/Coff-EYFP into the midbrain and immunostained for TH (representative of 4 mice). The white boxes in the middle panel denote the regions of interest (ROI) for the quantification shown in Fig. 4E. The 3 ROIs in the DLS and DMS were averaged to produce one value for each of these regions. DLS = dorsolateral striatum, DMS = dorsomedial striatum, C = nucleus accumbens core, LSh = nucleus accumbens lateral shell, MSh = nucleus accumbens medial shell (d, dorsal; m, medial; v, ventral), OT-m = medial olfactory tubercle, OT-l = lateral olfactory tubercle.
F) Zoomed-in images from the right merged image panel in E showing lack of EYFP signal in the NAc MSh compared to the LSh.

Supplemental Figure 4

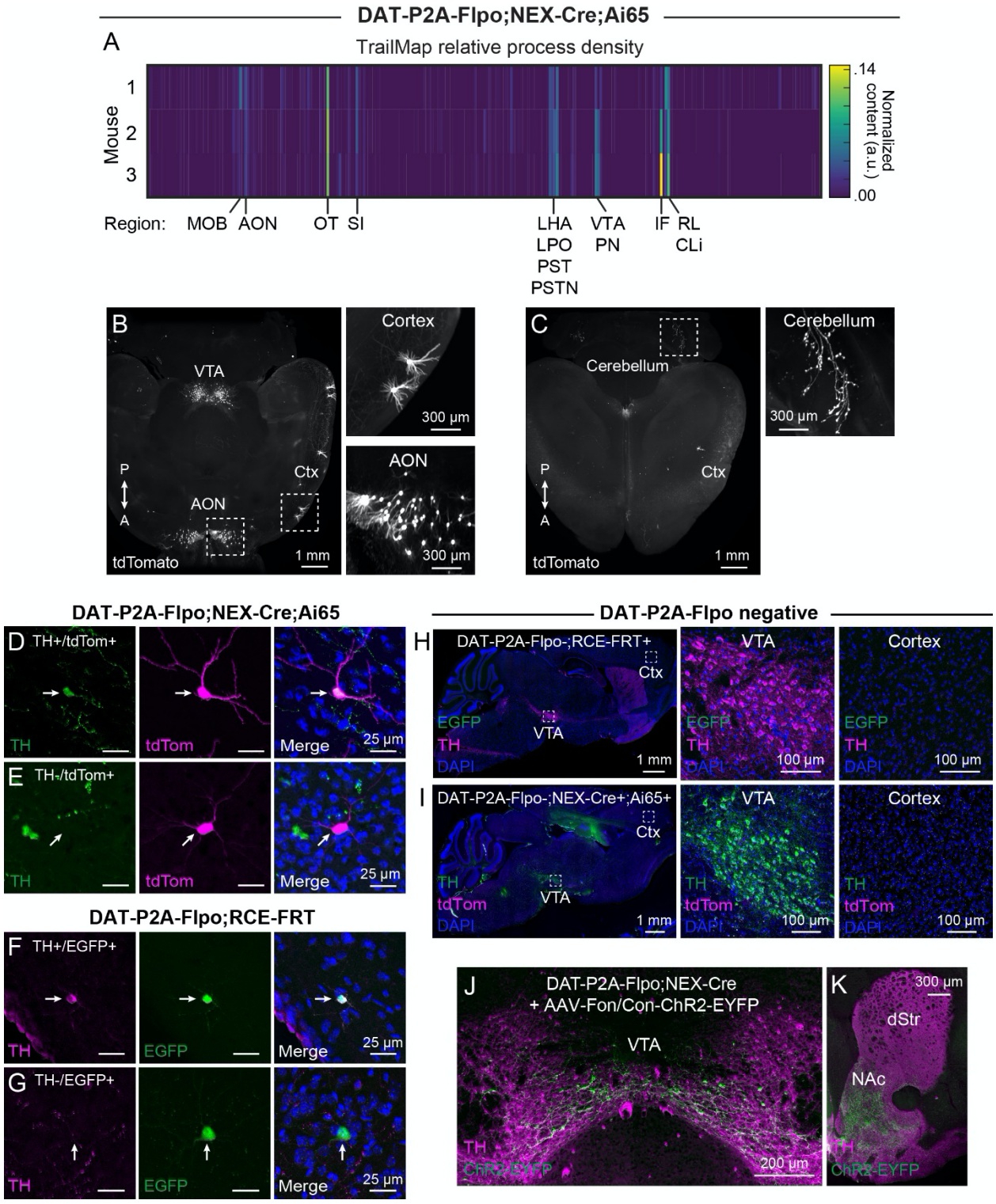

Figure S4. Additional analysis of fluorescent reporter expression patterns in DAT-P2A-Flpo;NEX-Cre;Ai65 and DAT-P2A-Flpo;RCE-FRT mice, Related to Figures 5 and 6.

A) Brains from 2 male and 1 female P120 DAT-Flp;NEX-Cre;Ai65 mice were processed with Adipo-Clear and imaged on a light sheet microscope. Projections and processes were quantified using TrailMap. Heatmap shows the total axonal/dendrite content of 277 brain regions using boundaries from the Allen Mouse Common Coordinate Framework (CCF). Values are normalized to both the region volume and total projection content per brain (taken from Supplemental Table 1 with 'root', 'fiber tracts', 'ventricular systems', and 'Nucleus accumbens' removed). The rows represent 3 independent brains. Regions with average process density values greater than 20 are labeled: MOB = Main olfactory bulb; AON = Anterior olfactory nucleus; OT = Olfactory tubercle; SI = Substantia innominata; LHA = Lateral hypothalamic area; LPO = Lateral preoptic area; PST = Preparasubthalamic nucleus; PSTN = Parasubthalamic nucleus; VTA = Ventral tegmental area; PN = Paranigral nucleus; IF = Interfascicular nucleus raphe; RL = Rostral linear nucleus raphe; CLi = Central linear nucleus raphe.
B,C) Horizontal Z-stack projections of light-sheet microscope whole brain images from a DAT-P2A-Flpo;NEX-Cre;Ai65 mouse showing tdTomato expression in subpopulations of neurons in the anterior olfactory nucleus (B, AON), cortex (B, Ctx) and cerebellum (C). Representative of 3 mice.
D-G) DAT-P2A-Flpo;Nex-Cre;Ai65 (D,E) and DAT-P2A-Flpo;RCE-FRT (F,G) mice exhibit Flp-reporter expression in a small number of cortical neurons. A subset of these neurons in 3-4 month old mice express low levels of TH by immunostaining (D, F, 22/131 neurons from 3 mice), while the majority have no detectable TH (E,G). Representative of 8 mice.
H,I) Confocal whole brain sagittal section images from DAT-P2A-Flpo negative DAT-P2A-Flpo;RCE-FRT (H) and DAT-P2A-Flpo;NEX-Cre;Ai65 (I) mice immunostained for TH. No Flp-reporter labeled cortical neurons could be found in DAT-P2A-Flpo negative mice.
J) Representative confocal image showing midbrain expression of ChR2-EYFP (green) together with TH immunostaining (magenta) in a DAT-P2A-Flpo;NEX-Cre mouse injected with AAV-Fon/Con-ChR2-EYFP into the midbrain.
K) Confocal image of ChR2-EYFP+ projections in the nucleus accumbens medial shell (NAc) from an AAV-Fon/Con-ChR2-EYFP injection into a DAT-P2A-Flpo;NEX-Cre mouse. Representative of 3 mice. dStr = dorsal striatum.
See also Supplemental Table 1 and Supplemental Videos 1-3.