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Supplemental information

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SUPPLEMENTAL INFORMATION

Inhibitory co-transmission from midbrain dopamine neurons relies on presynaptic GABA uptake

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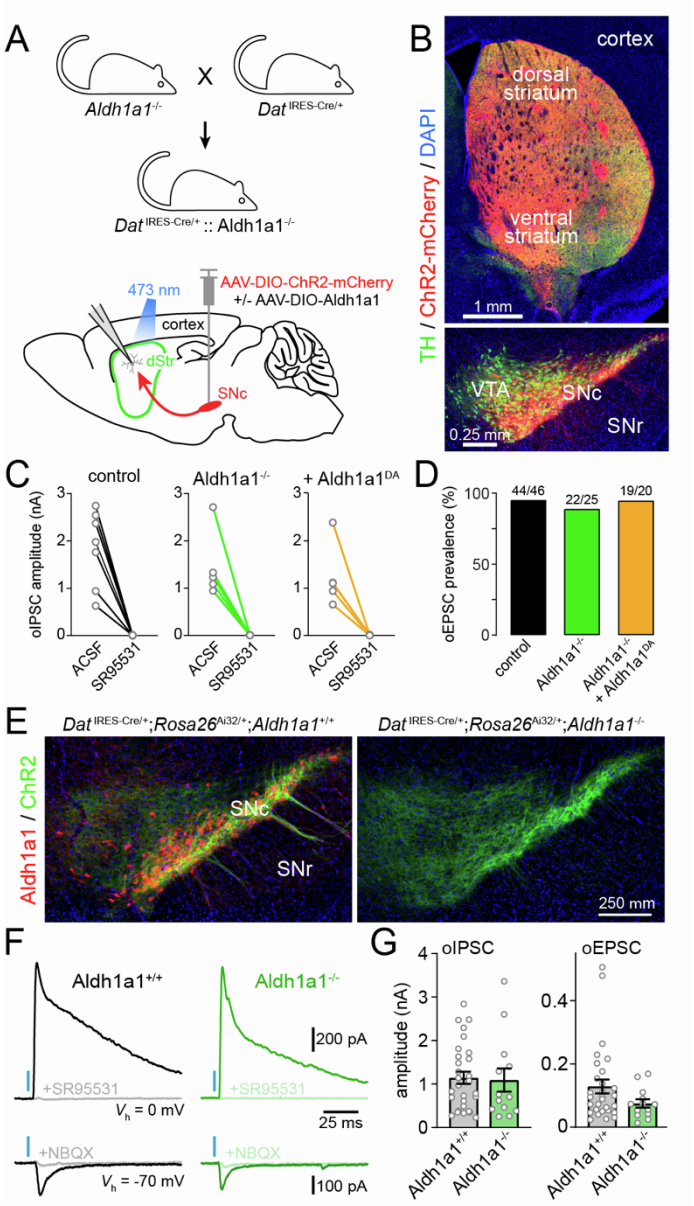

Figure S1. Aldh1a1 is dispensable for inhibitory co-transmission. Related to Figure 1.

A) Diagram of strategy to generate mice in which the gene encoding Aldh1a1 is intact or knocked out in all cells, while gaining genetic access to SNc^{DA} neurons using intracranial injection of AAVs encoding Cre-dependent (DIO) ChR2-mCherry and Aldh1a1. **B)** Coronal forebrain (top) and ventral midbrain (bottom) sections from a control *Dat*$^{IRES\text{-}Cre/+}$ mouse expressing Cre-dependent ChR2-mCherry in midbrain DA neurons for 3 weeks immunolabeled for tyrosine hydroxylase (TH, green) and stained with DAPI (blue). ChR2-mCherry expression is limited to TH-positive DA neurons in SNc/VTA and distributes throughout axons in striatum. **C)** Amplitude of oIPSCs recorded from SPNs before (ACSF) and after bath application of the $GABA_A$ receptor antagonist SR95531 (10 µM). **D)** Prevalence of oEPSCs recorded from SPNs that exceed our detection threshold across conditions. **E)** Coronal sections of ventral midbrain from mice expressing ChR2-EYFP genetically in all DA neurons with Aldh1a1 expression either intact (left) or constitutively knocked out (right) immunolabeled for Aldh1a1 (red) and stained with DAPI (blue). **F)** Example oIPSCs (top) and oEPSCs (bottom) recorded from SPNs in *Dat*$^{IRES\text{-}Cre/+}$;*Rosa26*$^{Ai32/+}$;*Aldh1a1*$^{+/+}$ (black) and *Dat*$^{IRES\text{-}Cre/+}$:*Rosa26*$^{Ai32/+}$;*Aldh1a1*$^{-/-}$ mice (green) before and after bath application of SR95531 (10 *µ*M) or NBQX/CPP (10 *µ*M). **G)** Mean amplitude of oIPSCs (left) and oEPSCs (right) recorded in *Aldh1a1*$^{+/+}$ (n = 29 SPNs from 8 mice) and *Aldh1a1*$^{-/-}$ (n = 13 SPNs from 3 mice) littermates (oIPSCs: p = 0.63; oEPSCs: p = 0.17, Mann-Whitney). Individual values are shown along with population mean ± s.e.m.

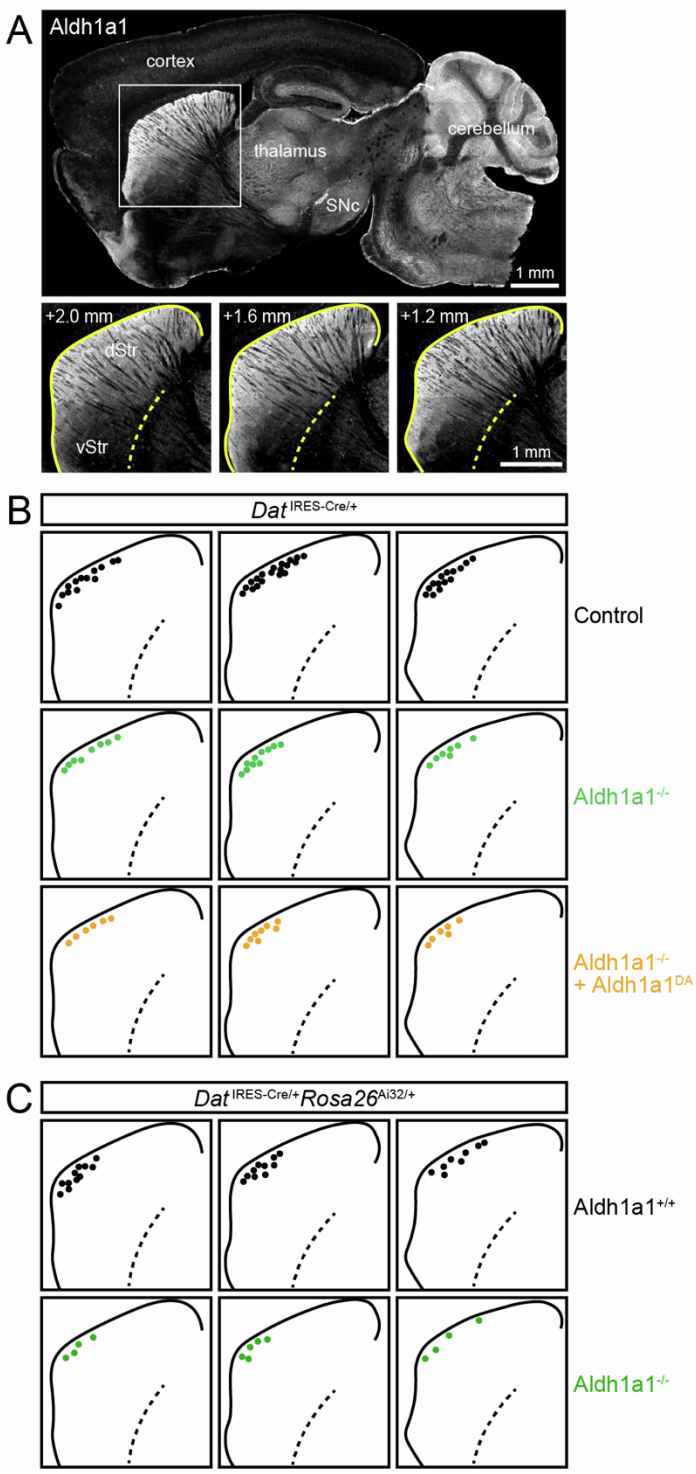

Figure S2. Physical location of recorded SPNs. Related to Figure 1.

A) *Top:* Sagittal brain section from a control mouse immunolabeled for Aldh1a1. The white box outlines striatum. *Bottom:* Detail of striatum at 3 distinct planes along the medio-lateral axis where most brain slices were obtained to record from SPNs. dStr: dorsal striatum, vStr: ventral striatum. Note that Aldh1a1 expression is uniformly strong in dorsal striatum in all 3 planes. **B)** Location of recorded SPNs in each of the 3 experimental conditions shown in Figure 1. Each dot represents one SPN **C)** Same as **(B)** for SPN recordings that make up Figure S1E-G.

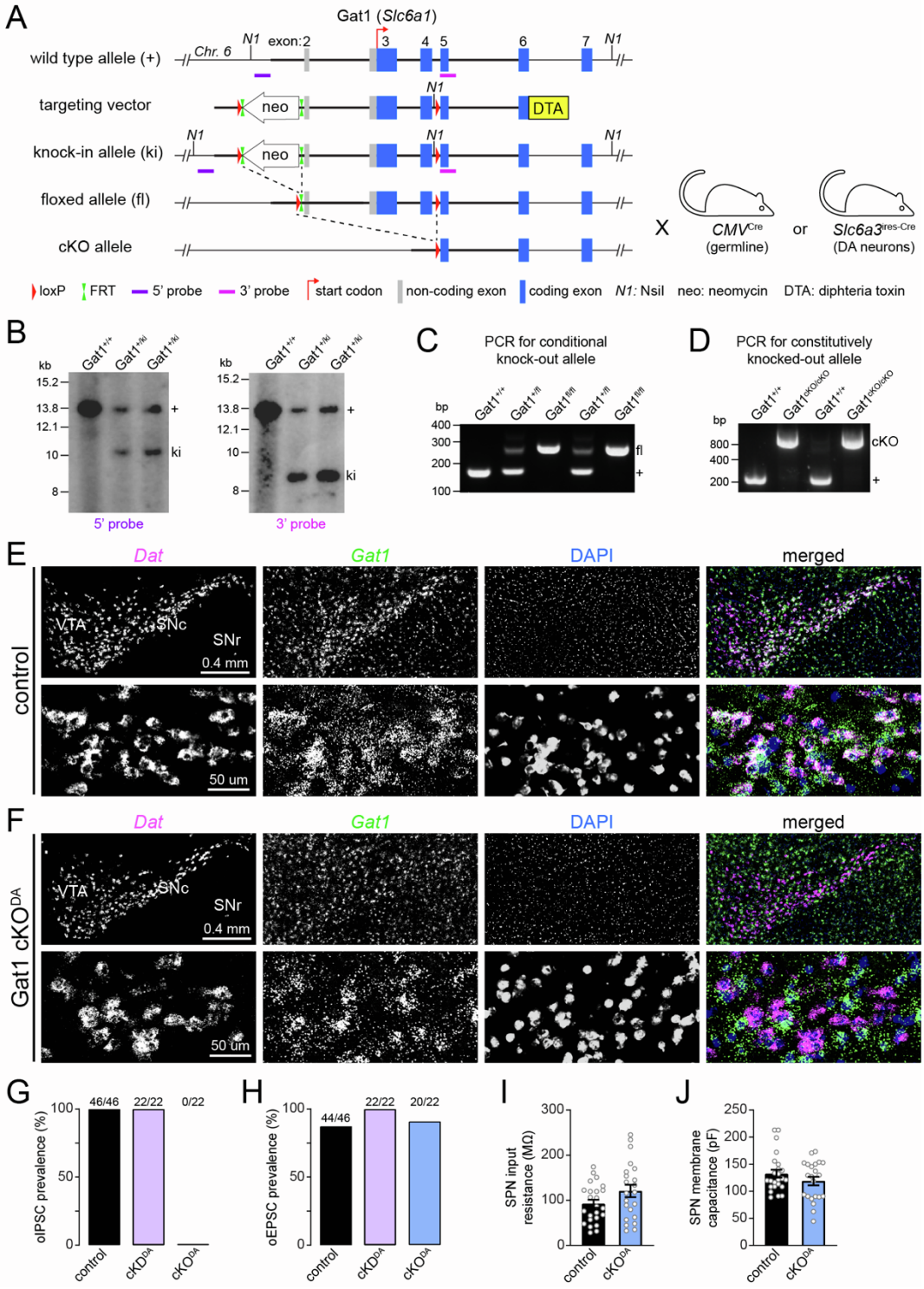

Figure S3. Generation and validation of Gat1 conditional knockout mice. Related to Figure 2.

A) Schematic of targeting strategy (not drawn to scale). Solid horizontal line represents relevant portion of *Slc6a1* genomic locus on mouse chromosome 6. Rectangles represent Slc6a1 exons (numbered), with coding sequences highlighted in blue and non-coding exons in gray (the initiation (ATG) codon is highlighted with red angled arrow). Thick horizontal lines depict intronic sequences included in targeting vector for homologous recombination. Targeting vector contains a neomycin positive selection cassette flanked by FRT sites, a diphtheria toxin negative selection marker on 3' end, two LoxP sites (one between exons 1 and 2, and another between exons 4 and 5) and a novel NsiI restriction site. ES cell clones with correct 5' and 3' recombination events (i.e. knockin allele) were injected into blastocyst-stage embryos and implanted in pseudo-

pregnant females to generate chimeric males, which were bred to Flp-deleter mice to excise the Neomycin cassette and generate mice heterozygous for the conditional knockout allele. Cre recombinase subsequently excises exons 2-4 to functionally delete Slc6a1 only in Cre-expressing cells. **B)** Southern blot analysis for correct 5' (left) and 3' (right) homologous recombination in ES cells. NsiI-digested genomic DNA from two ES cell clones with recombined ki alleles show expected restriction fragments for one wild-type allele (13.7 kb) and one ki allele (10.2 kb for 5' hybridization probe and 8.3 kb for 3' hybridization probe). Wild-type DNA is shown in first lane for comparison. **C)** Example PCR gel for Gat1 conditional knockout mice (prior to Cre-mediated recombination) with GAT1-FloxF and GAT1-FloxR primers yielding expected wild-type (170 bp) and floxed-allele (276 bp) bands in 5 littermates **D)** Same as **C** for $Gat1^{fl}$ mice after germline Cre-mediated recombination (using CMV^{Cre} mice) and $Gat1^{fl}$ mice, which effectively yields a conventional constitutive Gat1 knockout allele, using GAT1-Flox14F, GAT1-Flox18F and GAT1-Flox15R primers to obtain expected wild-type (211 bp) wild-type and recombined/knockout (818 bp) bands. **E)** Representative low (top) and high magnification (bottom) epifluorescence images of fluorescence *in situ* hybridization for *Dat* (magenta) and *Gat1* (green) in coronal section of ventral midbrain of a control mouse ($Dat^{IRES\text{-}Cre/+}$;$Gat1^{+/+}$). SNc is visible with *Gat1* antisense probe alone. Colocalization with DA cell bodies appears white in merged images. **F)** Same as **E** for Gat1 cKO^{DA} mouse ($Dat^{IRES\text{-}Cre/+}$;$Gat1^{fl/fl}$). Note how *Gat1* signal remains intact everywhere except for DA neurons; SNc is no longer visible with *Gat1* antisense probe alone and colocalization is no longer evident in merged images. **G–H)** Percentage of all recordings showing a ChR2-evoked IPSC (**G**) or EPSC (**H**) from SNc^{DA} axons in dorsal striatum SPNs in control, Gat1 cKD^{DA} and Gat1 cKO^{DA} slices. Number of recordings per condition is indicated above each bar. **I)** Input resistance of SPNs recorded in control and Gat1 cKO^{DA} slices ($p = 0.15$, Mann–Whitney). Data show Individual values along with mean ± s.e.m. **J)** Same as **I** for membrane capacitance ($p = 0.42$, Mann–Whitney).

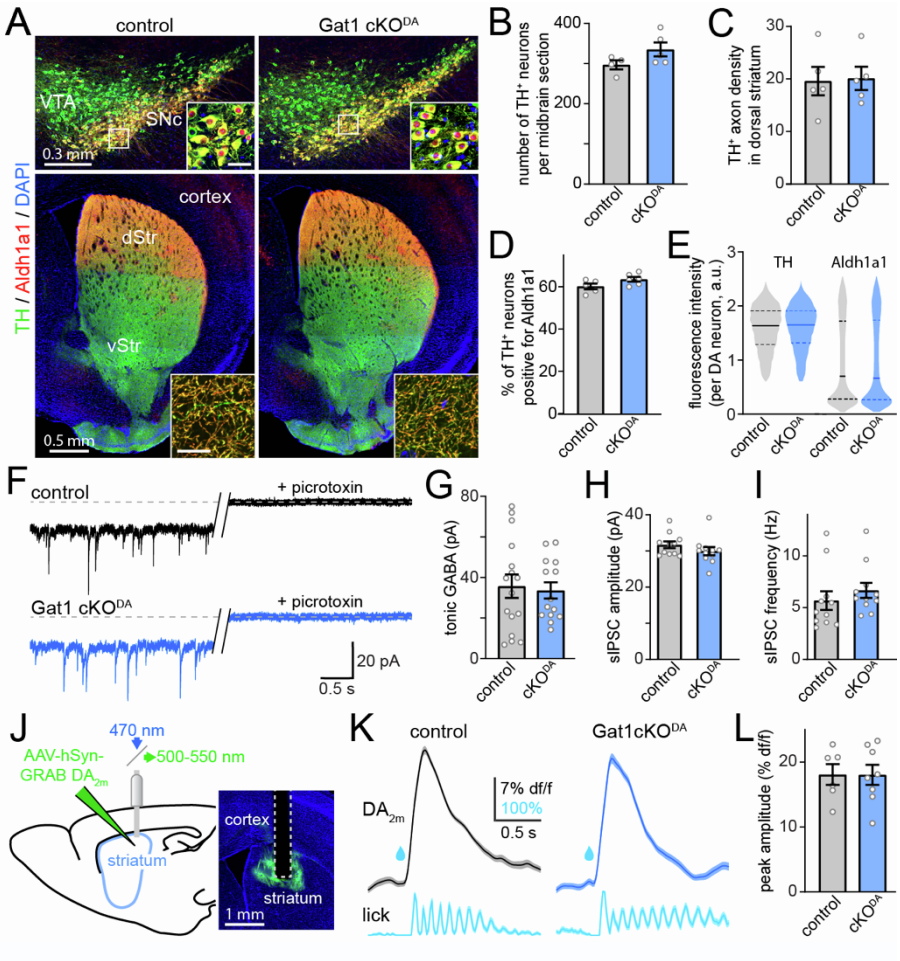

Figure S4. Nigrostriatal development and function are largely intact in Gat1 cKO^{DA} mice. Related to Figure 2.

A) Coronal brain sections from control *Dat*$^{IRES\text{-}Cre/+}$ (left) and *Dat*$^{IRES\text{-}Cre/+}$;Gat1$^{fl/fl}$ mouse (Gat1 cKO^{DA}; right) mice immunolabeled for tyrosine hydroxylase (TH, green), Aldh1a1 (red) and DAPI (blue). Top row: confocal images of ventral midbrain with white box show magnified in inset (scale bar inset: 25 µm). Bottom row: Epifluorescence image of striatum. Inset: confocal image of dorsal striatum (scale bar: 20 µm). **B)** Average number of TH$^+$ DA neurons identified across 3 coronal sections of SNc in control (n = 4 mice) and Gat1 cKO^{DA} mice (n = 5 mice; p = 0.17 vs. control, Mann–Whitney). **C)** Mean density of TH$^+$ axons imaged in dorsal striatum of control (n = 5 mice) and Gat1 cKO^{DA} mice (n = 5 mice; p = 0.99 vs. control, Mann–Whitney). **D)** Percentage of TH$^+$ DA neurons positive for Aldh1a1 in control (n = 4 mice) and Gat1 cKO^{DA} mice (n = 5 mice; p = 0.12, Mann–Whitney). **E)** Violin plot of TH and Aldh1a1 relative fluorescence intensity per TH$^+$ DA neuron in control (n = 7714 cells from 4 mice) and Gat1 cKO^{DA} mice (n = 9740 cells from 5 mice). Filled line indicates median, and dashed lines first and third quartiles. **F)** Example whole-cell voltage-clamp recordings (V_h = –70 mV) from SPNs in control (black) and Gat1 cKO^{DA} slices (blue) before and after the application of the GABA$_A$ receptor blocker picrotoxin (100 µM). Slices were continuously bathed in NBQX (10 µM), R-CPP (10 µM), and CGP55845 (5 µM) to block ionotropic glutamate and metabotropic GABA$_B$ receptors. The shift in picrotoxin-evoked holding current highlights the inward tonic GABAergic current at baseline. **G)** Quantification of picrotoxin-evoked shift in holding currents recorded in control (n = 16 SPNs from 6 mice) and Gat1 cKO^{DA} slices (n = 14 SPNs from 5 mice; p = 0.92 vs. control, Mann–Whitney). **H)** Mean amplitude of spontaneous IPSCs recorded from SPNs in control (n = 11 SPNs from 4 mice) and Gat1 cKO^{DA} slices (n = 11 SPNs from 4 mice; p = 0.24 vs. control, Mann–Whitney). **I)** same as **H** for sIPSC frequency (p = 0.10, Mann–Whitney). **J)** Left: schematic of photometry experiment. Right: example coronal section showing fiber tract in dorsal striatum expressing the fluorescent DA sensor GRAB-DA$_{2m}$. **K)** Mean (± s.e.m.) GRAB-DA$_{2m}$ fluorescence (top) and lick probability (bottom) following water delivery (drop) in control (left) and Gat1 cKO^{DA} mice (right). **L)** Mean reward-evoked GRAB-DA$_{2m}$ peak fluorescence in control (n = 6 imaging sessions from 3 mice) and Gat1 cKO^{DA} mice (n = 8 sessions from 4 mice; p = 0.95 vs. control, Mann–Whitney). Data in **B-E**, **G-I** and **L** show Individual values along with mean ± s.e.m.

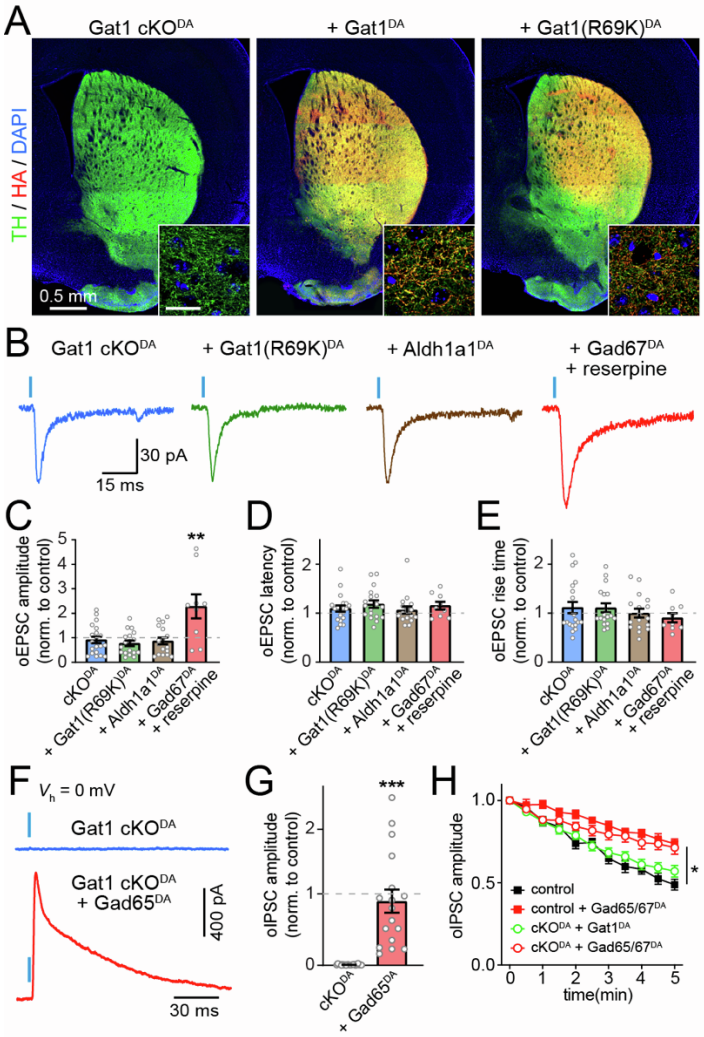

Figure S5. Controls for ChR2-mediated activation of SNc^{DA} axons. Related to Figure 3.

A) Epifluorescence images of coronal forebrain sections immunostained for TH (green) to label DA axons, HA (red) to label constructs exogenously-expressed in DA neurons, and DAPI (blue) to label nuclei in Gat1 cKO^{DA} mice (*Dat*$^{IRES\text{-}Cre/+}$;*Gat1*$^{fl/fl}$) injected in ventral midbrain with AAVs encoding Cre-dependent ChR2-mCherry alone (left), ChR2-mCherry + Gat1-HA (middle), and ChR2-mCherry + Gat1(K69K)-HA (right). Insets: high magnification view in dorsal striatum (scale bar: 20 µm). Note Gat1's distribution in axons. **B)** Example EPSCs evoked by optogenetic stimulation of SNc^{DA} axons in dorsal striatum SPNs in slices from Gat1 cKO^{DA} mice (blue) expressing Gat1(R69K) (green), Gad67 in presence of reserpine (red), or Aldh1a1 (brown) in DA axons. **C–E)** Quantification of oEPSC amplitude (**C**), latency (**D**) and rise time (**E**) normalized to *Dat*$^{IRES\text{-}Cre/+}$ controls. oEPSCs are significantly larger in amplitude in reserpine-treated Gat1 cKO mice expressing Gad67 ($p = 0.004$ vs. control, Mann–Whitney). **F)** IPSCs recorded from Gat1 cKO^{DA} SPNs held at 0 mV upon optogenetic stimulation of DA axons overexpressing Gad65 (red) or not (blue). **G)** oIPSC amplitude normalized to Dat$^{IRES\text{-}Cre/+}$ controls (Gat1 cKO^{DA}+Gad65^{DA}: n = 17 SPNs from 5 mice; $p = 0.99$ vs. control, $p = 3.3 \times 10^{-11}$ vs. Gat1 cKO^{DA}; Dunn's Multiple Comparison). **H)** Quantification of oIPSC amplitude vs. time in control slices (filled squares) and Gat1 cKO^{DA} (empty circles) expressing either Gat1 (green) or Gad65/Gad67 (red) in SNc^{DA} neurons. Gat1 cKO^{DA} + Gat1^{DA} (n = 14 SPNs in 4 mice), Gat1 cKO^{DA} + Gad65/67DA (n = 25 SPNs in 8 mice). In control mice, the amplitude of oIPSCs decreased significantly less when SNc^{DA} neurons expressed Gad65 or Gad67 (control: n = 22 SPNs from 9 mice; Gad65/67: n = 17 SPNs from 6 mice; two-way ANOVA group x time: $F_{10,370} = 10.55$, $p = <0.0001$). In Gat1 cKO^{DA} mice too, oIPSCs were less prone to rundown when SNc^{DA} express Gad65 or Gad67 (n = 25 SPNs from 8 mice) vs. Gat1 (n = 14 SPNs from 4 mice; two-way ANOVA group x time: $F_{10,365} = 3.15$, $p = 7 \times 10^{-4}$).

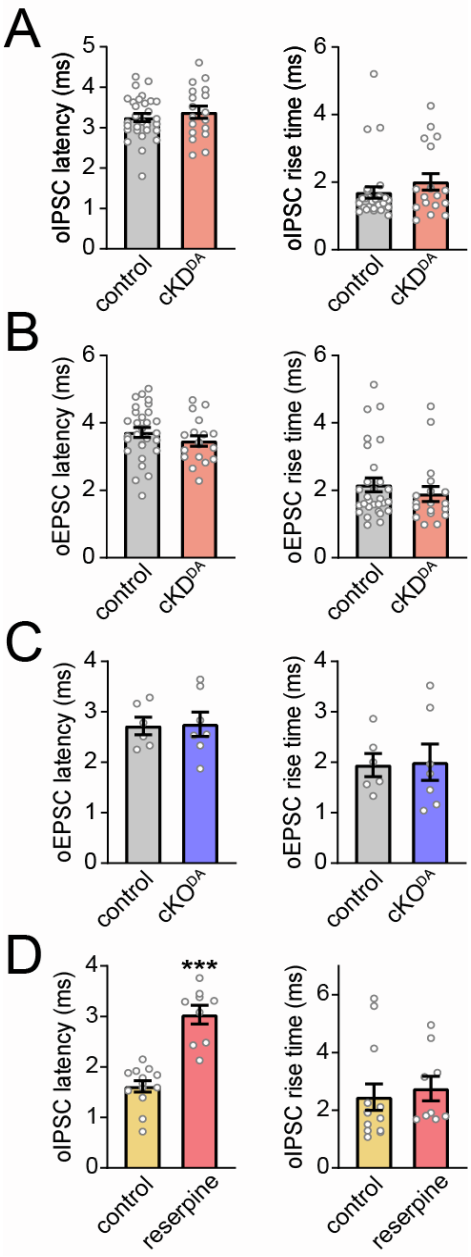

Figure S6. Controls for ChR2-mediated activation of SNc^{DA} axons. Related to Figure 4.

A) Mean latency (left) and 10-90% rise time (right) of oIPSCs recorded from SPNs in control (*Dat*$^{IRES\text{-}Cre/+}$;*Rosa26*$^{Ai32/-}$;*Vmat2*$^{+/+}$; n =29) or Vmat2 cKD^{DA} (*Dat*$^{IRES\text{-}Cre/+}$;*Rosa26*$^{Ai32/-}$;*Vmat2*$^{fl/+}$; n = 18) littermates (latency: p = 0.52; rise time: p = 0.24, Mann-Whitney). Individual values are shown along with population mean ± s.e.m. **B)** Same as **A** for oEPSCs (latency: p = 0.19; rise time: p = 0.35, Mann-Whitney). **C)** Same as **B** for SPNs recorded in *Dat*$^{IRES\text{-}Flpo/+}$;*Vmat2*$^{+/+}$ (control; n = 6) and *Dat*$^{IRES\text{-}Flpo/+}$;*Vmat2*$^{fl/fl}$ (Vmat2 cKO^{DA}; n = 7) littermates transduced with AAVs encoding Flp-dependent Cre and Cre-dependent ChR2 in SNc (latency: p = 0.76; rise time: p = 0.83, Mann-Whitney). **D)** Same as **A** for SPNs recorded in Gat1 cKO^{DA} mice expressing Gad67 exogenously in SNc^{DA} neurons without (yellow; n = 13) or with (red; n = 9) pharmacological block of Vmat2 using reserpine (latency: p = 8.04 x 10^{-6}; rise time: p = 0.44, Mann-Whitney).

Supplemental Table 1. AAV titers and dilutions used to transduce SNc^{DA} neurons. Related to Star Methods.

	Titer (GC/ml)	Volume (μl)
Figure 1		
control / Aldh1a1$^{-/-}$		
AAV.DIO.ChR2	1x10^{12}	0.5
saline		0.5
Total		**1**
Aldh1a1$^{-/-}$ + Aldh1a1^{DA}		
AAV.DIO.ChR2	1x10^{12}	0.5
AAV.DIO.Aldh1a1	2.4x10^{10}	0.5
Total		**1**
Figure 2		
control / cKD^{DA}/ cKO^{DA}		
AAV.DIO.ChR2	1x10^{12}	0.5
saline		0.5
Total		**1**
Figures 3 and Supplemental Figure 5		
control		
AAV.DIO.ChR2	1x10^{12}	0.5
saline		0.5
Total		**1**
cKO^{DA} + Gat1^{DA}		
AAV.DIO.ChR2	1x10^{12}	0.5
AAV.DIO.Gat1	7.5x10^{11}	0.5
Total		**1**
cKO^{DA} + Gat1(R69K)^{DA}		
AAV.DIO.ChR2	1x10^{12}	0.5
AAV.DIO.Gat1(R69K)	5.7x10^{11}	0.5
Total		**1**
cKO^{DA} + Gad67^{DA}		
AAV.DIO.ChR2	1x10^{12}	0.5
AAV.DIO.Gad67	3.2x10^{12}	0.5
Total		**1**
cKO^{DA} + Aldh1a1^{DA}		
AAV.DIO.ChR2	1x10^{12}	0.5
AAV.DIO.Aldh1a1	2.4x10^{10}	0.5
Total		**1**
cKO^{DA} + Gad65^{DA}		
AAV.DIO.ChR2	1x10^{12}	0.5
AAV.DIO.Gad65	1.1x10^{12}	0.5
Total		**1**
Figure 4 and Supplemental Figure 6		
control / cKO^{DA}		
AAV.fDIO.Cre	4.2x10^{11}	0.5
AAV.DIO.ChR2	1x10^{12}	0.5
Total		**1**