Supplementary Figures:

Supplemental Figure 1. Schematic representation of the Nurr1 wild-type locus and targeting vector. Untranslated and translated exons are indicated by open and shadowed boxes, respectively. The loxP sites (closed triangle) and the floxed neomycin cassette are indicated. Restriction endonucleases are *EcoRI* (E), *BamHI* (B), *BglII* (Bg), *SalI* (S), *MunI* (M).

Supplemental Figure 2. Nurr1 expression is lost from E15.5 in cNurr1^{DATCre} mice. Micrographs show confocal Nurr1 immunofluorescence in the ventral midbrain at E10.5, E11.5, E12.5, E15.5 and P1, as indicated. Scale bars: A-F 100 μm; G-J 200 μm.

Supplemental Figure 3. Viability, body weight and rearing in control and cNurr1^{DATCre} mice. Approximately 50% (n=6) of cNurr1^{DATCre} mice died within the first 20 weeks if not separated from their littermates (A). Body weight was lower in surviving animals (n=7) as compared to controls. Moreover, at both 4 (n=8) and 14 (n=5) months rearing was diminished in cNurr1^{DATCre} mice when placed in a novel environment (see Methods). At 14 months locomotor activity appeared to be increased in cNurr1^{DATCre} mice; however, the increase was not statistically significant.

Supplemental Figure 4. Micrographs showing TH immunohistochemistry in cNurr1^{DATCre} mice. A densely immunoreactive fiber network is apparent in control animals (A and C). TH positive fibers are almost completely absent in the dorsolateral striatum while a fiber network remains ventro-medially (B) in cNurr1^{DATCre}

mice. Moreover, in largely TH-depleted striatal areas the appearance of ectopic TH-positive cell bodies is evident (B). Examples of such cells are shown in higher magnifications in D, E and F. These cells resemble TH-positive neurons appearing after DA-depletion by 6-hydroxy dopamine in rats and primates. Most ectopic neurons are bipolar and probably derived by a shift in phenotype from existing medium-sized aspiny striatal interneurons (reviewed in Hout and Parent, J. Neurochem., 2007, 101:1441-1447).

Supplemental Figure 5. Micrographs showing DAT immunohistochemistry in mice heterozygous for the DAT-Cre allele. The section shows that staining within both SNc and VTA is roughly equal in both genotypes.

Supplemental Figure 6. Diagram displaying the per cent of cells that are double positive for both TH and DAT within control and cNurr1^{DATCre} P15 VTA, respectively. Cell counting was performed by counting all cells in four non-consecutive sections in each animal. Note that the total number of TH-positive cells was considerably lower in cNurr1^{DATCre} mice (see Results section). Of these few TH-positive cells a remarkably low frequency (approximately 3%) were double-positive showing that most of the remaining cells were defective for DAT expression.

Supplemental Figure 7. To assess the efficiency of the AAV-Cre vector, 1 µl of the vector was injected over the SN (as shown in A) into reporter mice in which the ROSA26 locus is targeted with a LacZ reporter gene41. Three weeks later the brains were perfused and stained for Cre (B) and X-gal (C) on adjacent sections.

Supplemental Figure 8. Non-fluorescent 3,3'-diaminobenzidine (DAB) TH immunostaining at the level of striatum of sections from 0.5, 1.5 and 4 months (mo; as indicated) old wt^{AAVCre} mice. Results show that staining is equal in both sides corresponding to both the injected and non-injected SNc. B, . Images of the striatum were stained for TH with DAB. Mean optical density of the TH-positive fiber staining in the straitum was measured by using the Image J software. Measurements were from animals sacrificed at 1.5 months after AAV-vector injection.

Supplemental Figure 9. Decreased level of DAT in the VTA at 1.5 and 4 months in *cNurr1*^{AAVCre} mice. ***A-H,*** DAT immunostaining, is clearly reduced in cNurr1^{AAVCre} mice at both 1.5 and 4 months (***A-D***); however, Hu staining persists even at late stages after ablation (***E-H***).

Supplemental Figure 10. A., Amphetamine rotations were measured in rotation chambers specially modified for mice. Mice were habituated for 10 minutes prior to treatment with either amphetamine (5 mg/kg) or saline control administered by subcutaneous injections. Mice were left in rotation chambers for 60 minutes and rotations were measured for 1 minute every 5 minutes. B., The corridor test is a drug-independent test for unilateral DA leisions measuring pellett retrievals as hungry animals move along a corridor with an equal number of pellets placed on each side (see Dowd, E., Monville, C., Torres, E.M., Dunnett, S.B. The Corridor Task: a simple test of lateralised response selection sensitive to unilateral dopamine deafferentation and graft-derived dopamine replacement in the striatum. Brain Res Bull. 2005 Dec 15;68(1-2):24-30). A reduced number of eaten pellets on the right side would be consistent with a unilateral mDA neuron deficiency. Although there is a trend for

lower scores of cNurr1^{AAVCre} mice at 3 and 4 months the difference relative to controls was not significant.

Supplementary Tables 1 to 4

Levels of DA, DOPAC, HVA and 5-HT as determined by HPLC. Striatal and cortical levels are expressed as pmol/mg of wet tissue. Number of animals (n) per group are indicated. Note the increased ratio of HVA to DA at P14 of cNurr1^{DATCre} mice indicating increased DA turnover (HVA/DA increases from 0.53 to 0.89 in caudatus putamen and from 0.82 to 3.0 in the substantia nigra). Tables S1 to S3: In brackets percentage value in cNurr1^{DATCre} versus wt^{DATCre}. Table S4: In brackets percentage value in injected versus non-injected sides.

$*$ = different from controlateral side, $p<0.05$ in the paired t test.

$**$ = different from controlateral side, $p<0.01$ in the paired t test.

+ = different from wt, $p<0.05$ in the paired t test.

++ = different from wt, $p<0.01$ in the paired t test.

Figure S1

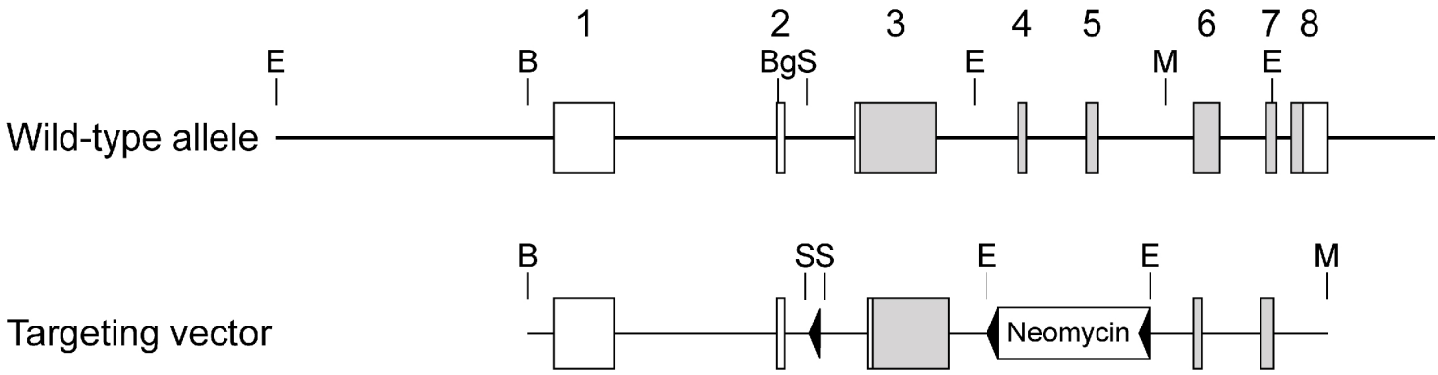
1
2
3
4
5
6
7
8
E
B
BgS
E
M
E
Wild-type allele
B
SS
E
E
M
Neomycin
Targeting vector

Figure S2

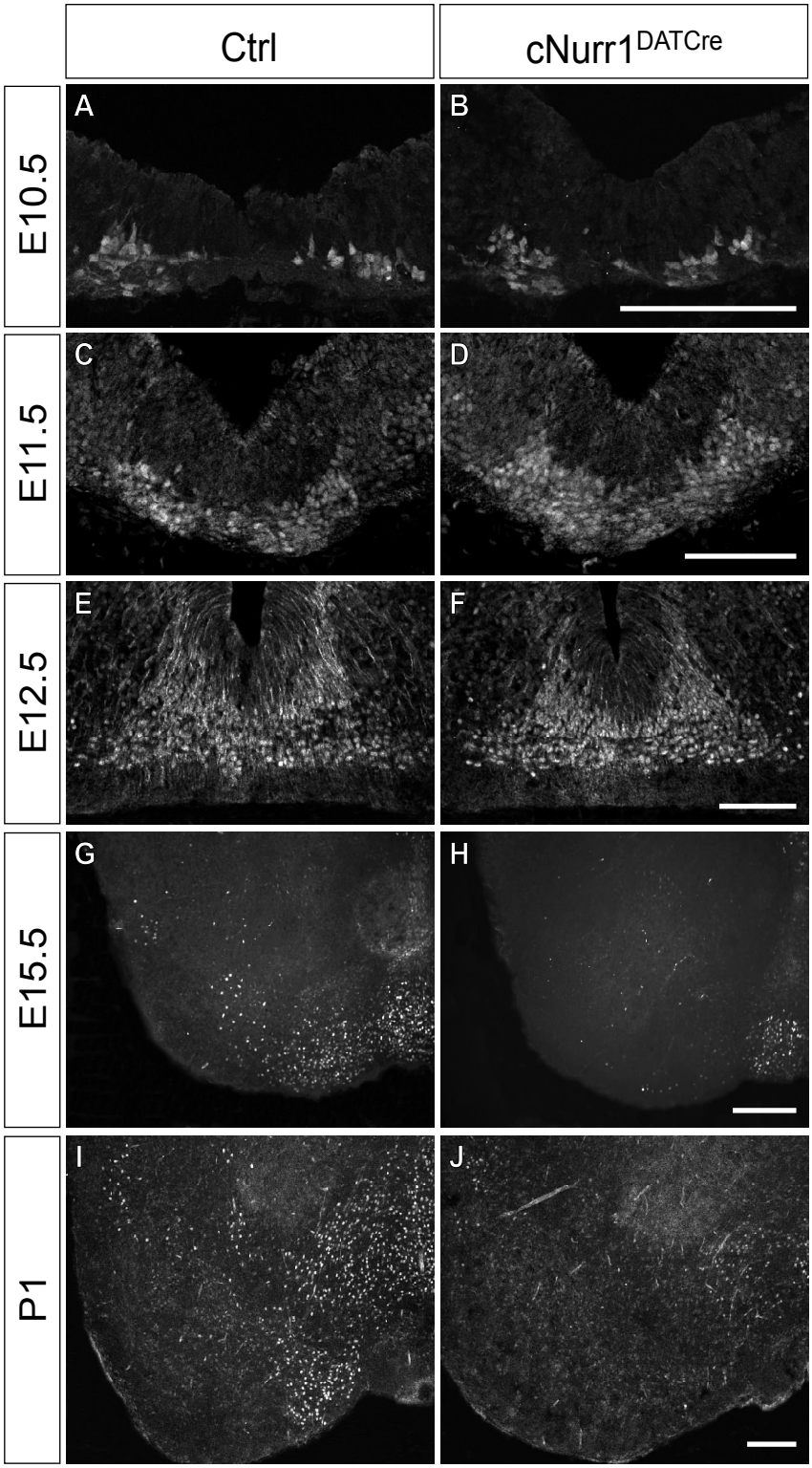

Figure S3

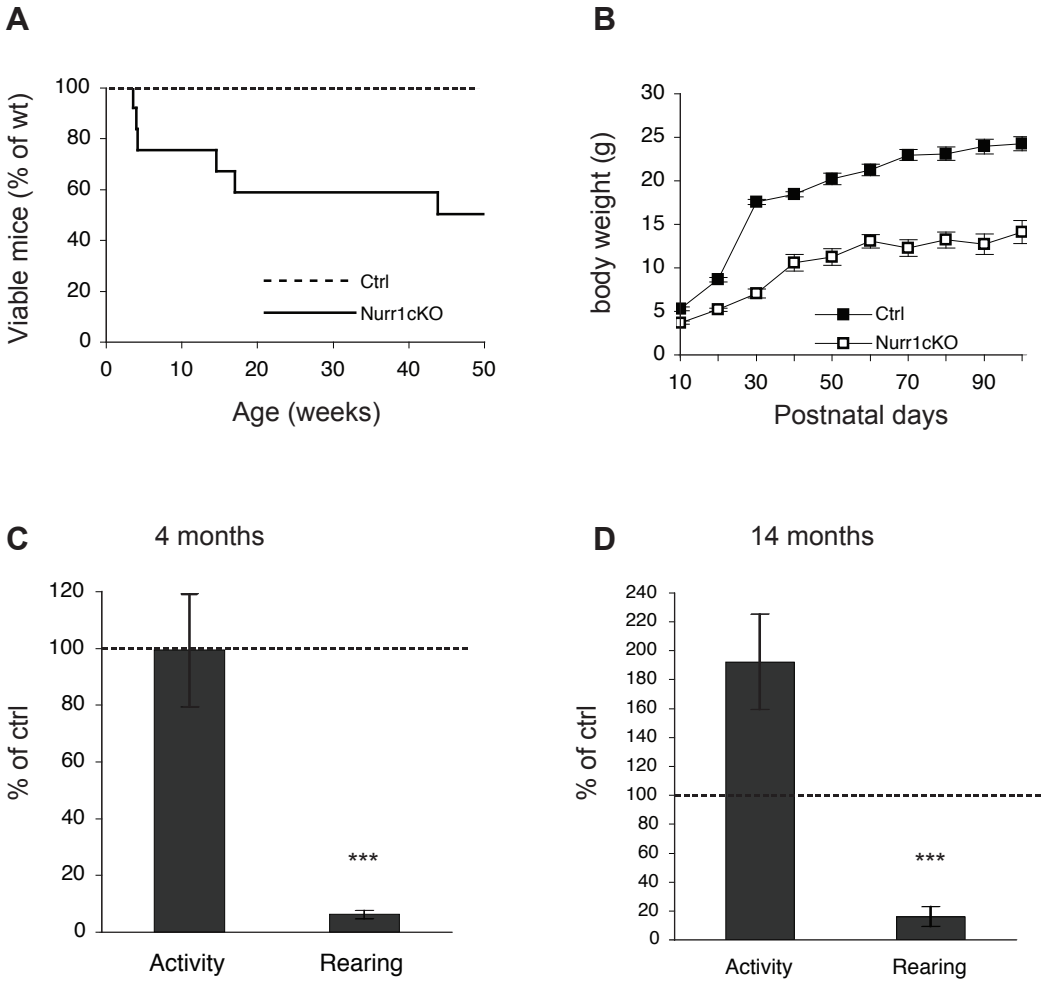
A
Viable mice (% of wt)
100
80
60
40
20
0
0
10
20
30
40
50
Age (weeks)
Ctrl
Nurr1cKO
B
body weight (g)
30
25
20
15
10
5
0
10
30
50
70
90
Postnatal days
Ctrl
Nurr1cKO
C
4 months
% of ctrl
120
100
80
60
40
20
0
***
Activity
Rearing
D
14 months
% of ctrl
240
220
200
180
160
140
120
100
80
60
40
20
0
***
Activity
Rearing

Figure S4

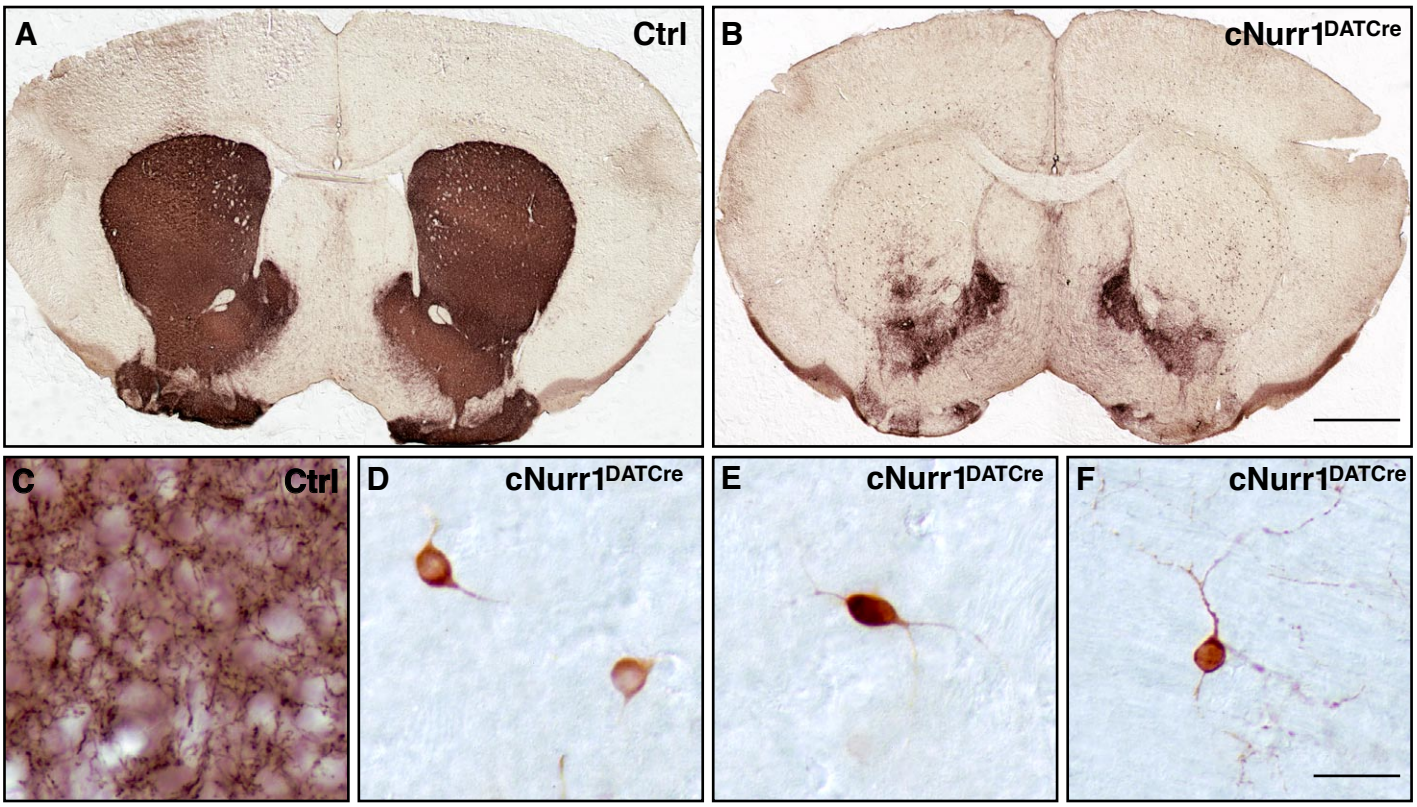
A
Ctrl
B
cNurr1DATCre
C
Ctrl
D
cNurr1DATCre
E
cNurr1DATCre
F
cNurr1DATCre

Figure S5

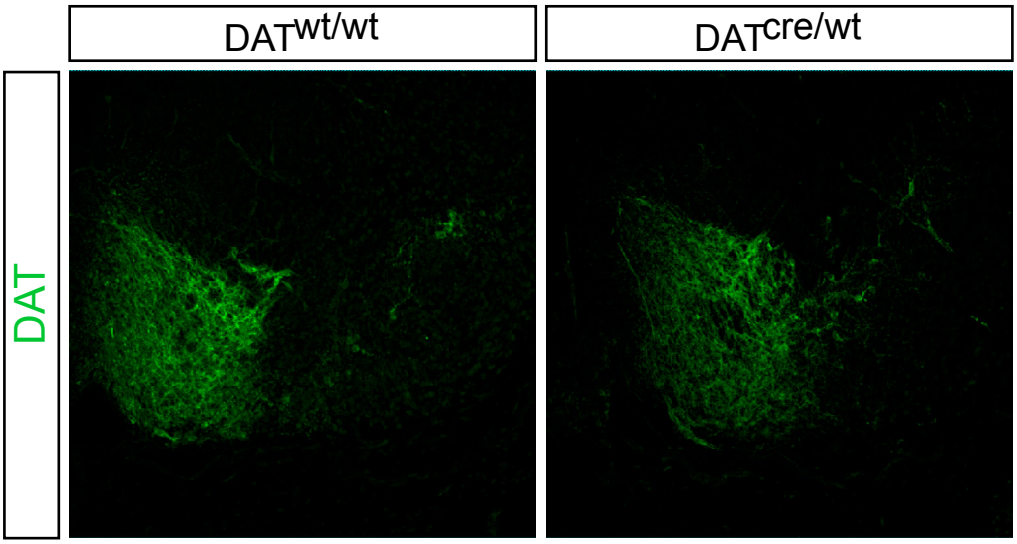

Figure S6

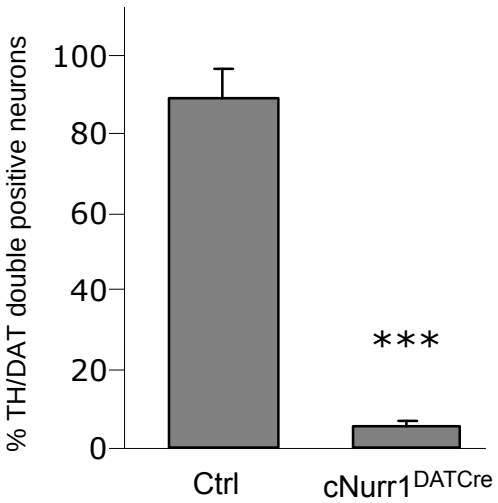
% TH/DAT double positive neurons
100
80
60
40
20
0
***
Ctrl
cNurr1DATCre

Figure S7

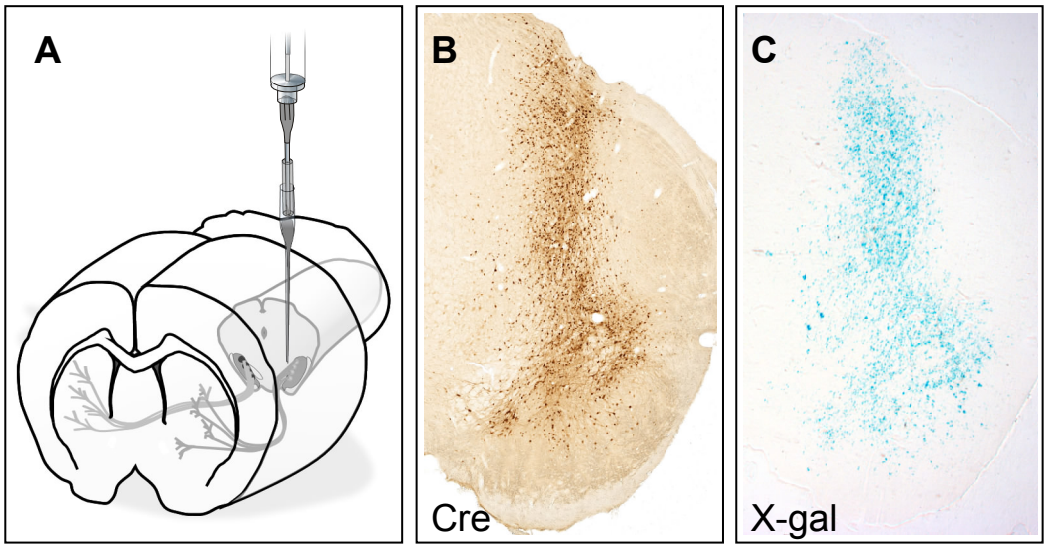

Figure S8

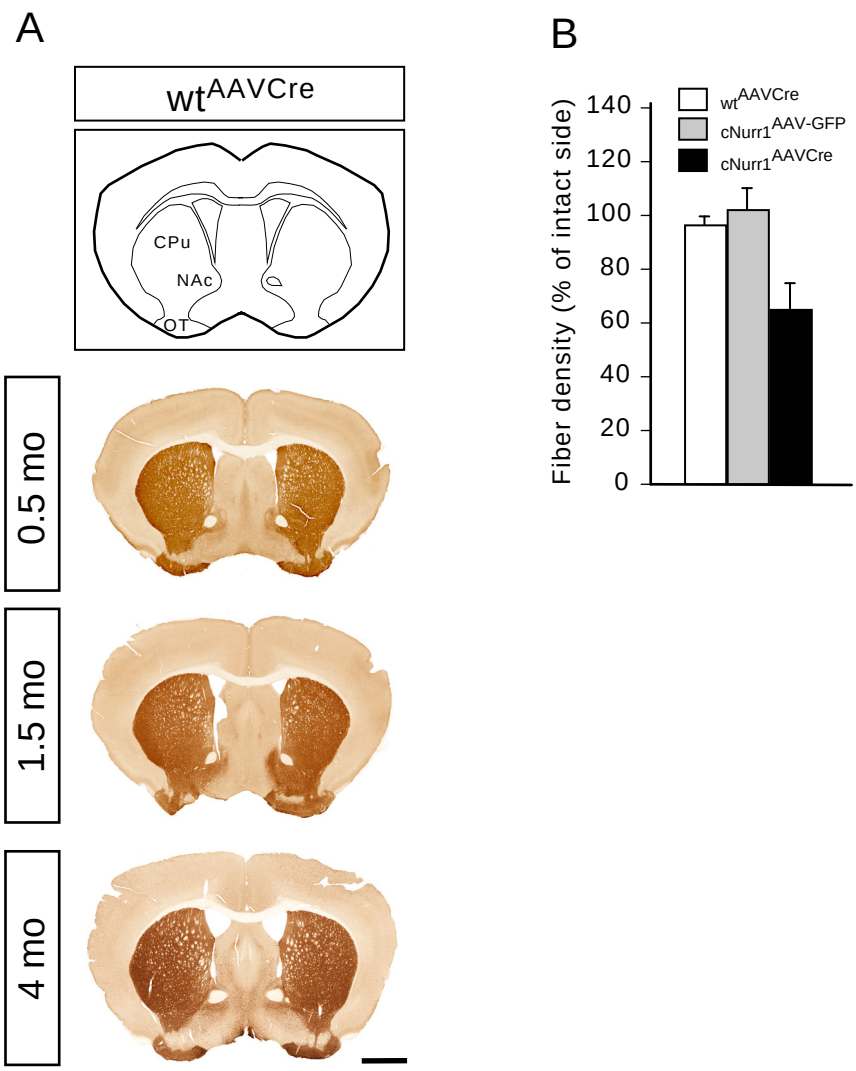
A
wtAAVCre
CPu
NAc
OT
0.5 mo
1.5 mo
4 mo
B
Fiber density (% of intact side)
140
120
100
80
60
40
20
0
wtAAVCre
cNurr1AAV-GFP
cNurr1AAVCre

Figure S9

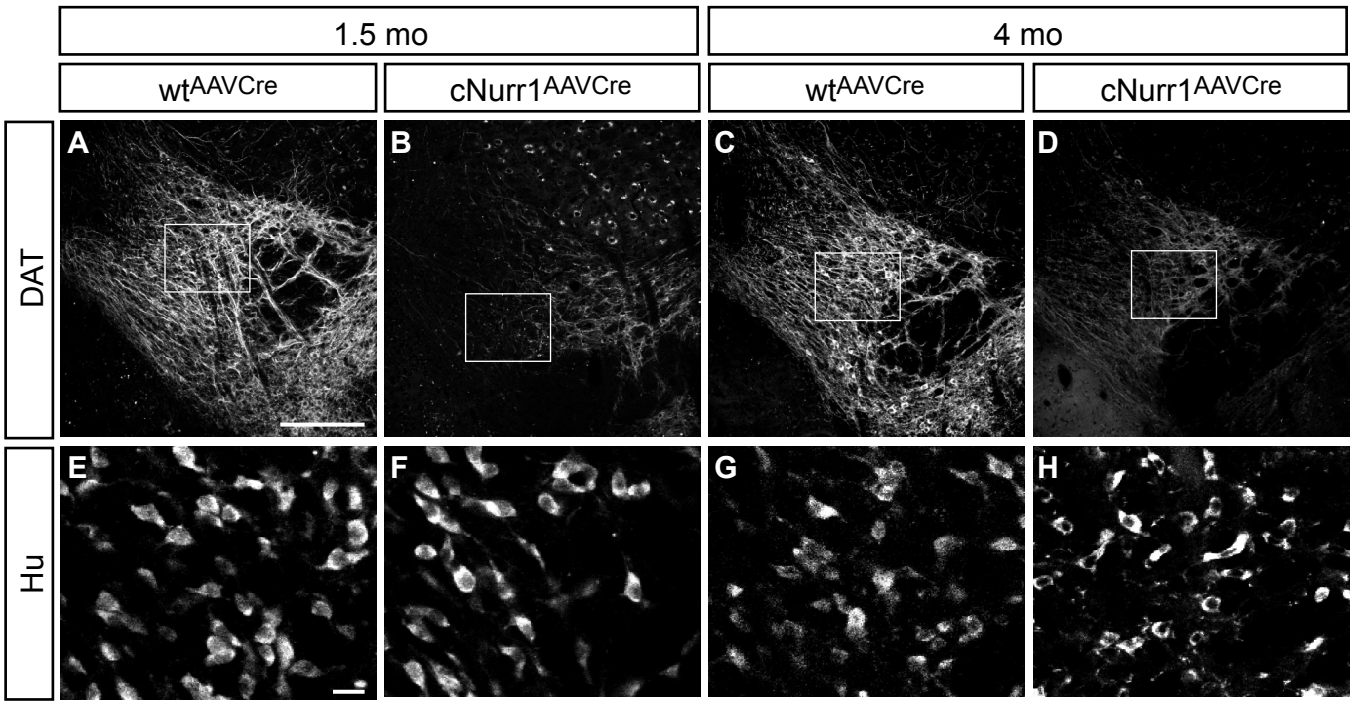
1.5 mo
4 mo
wtAAVCre
cNurr1AAVCre
wtAAVCre
cNurr1AAVCre
DAT
Hu
A
B
C
D
E
F
G
H

Figure S10

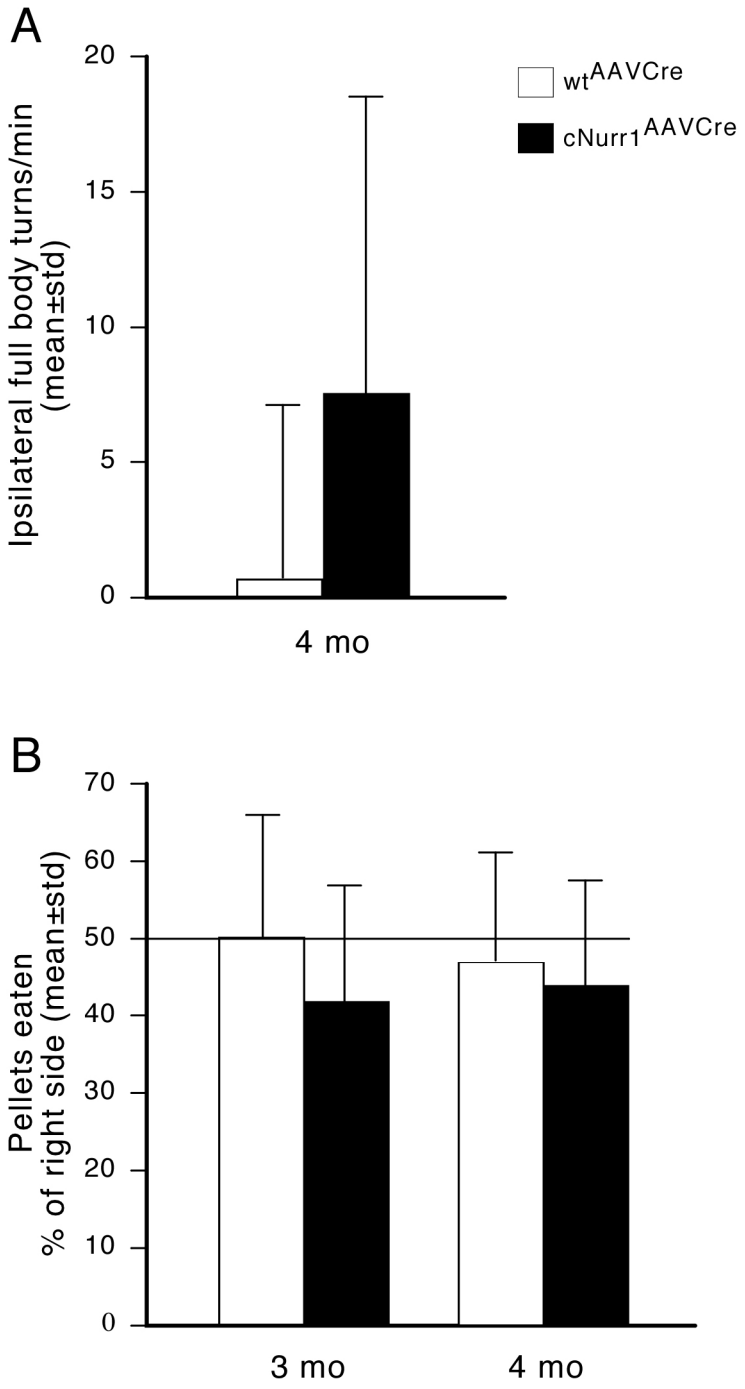
A
Ipsilateral full body turns/min
(mean±std)
20
15
10
5
0
wtAAVCre
cNurr1AAVCre
4 mo
B
Pellets eaten
% of right side (mean±std)
70
60
50
40
30
20
10
0
3 mo
4 mo

Table S1

Dopamine and dopamine metabolites and serotonin in cNurr1^{DATCre} vs wt^{DATCre} at P1

Metabolites / Brain region	DA	DOPAC	HVA	5-HT
Caudate Putamen wt^{DATCre}	8.99±2.87	6.58±2.94	2.54±0.99	2.07±0.61
Caudate Putamen cNurr1^{DATCre}	1.25±0.44* (13.95%)	0.47±0.19 (7.08%)	0±0*	2.64±1.08 (127.28%)
Substantia Nigra wt^{DATCre}	1.82±0.39	2.40±0.20	1.26±0.14	2.19±0.49
Substantia Nigra cNurr1^{DATCre}	0.40±0.09** (22.00%)	0.48±0.12*** (19.87%)	0.12±0.12*** (9.18%)	2.12±0.33 (96.83%)

cNurr1^{DATCre} n=7
wt^{DATCre} n=9

Table S2

Dopamine and dopamine metabolites and serotonin in cNurr1^{DATCre} vs wt^{DATCre} at P14

Metabolites / Brain region	DA	DOPAC	HVA	5-HT
Caudate Putamen wt^{DATCre}	12.40±1.66	18.66±1.94	6.69±0.99	1.61±0.21
Caudate Putamen cNurr1^{DATCre}	1.37±0.78*** (11.08%)	0.81±0.19*** (4.33%)	1.23±0.18*** (18.33%)	2.42±0.40 (150.45%)
Substantia Nigra wt^{DATCre}	4.29±0.79	5.10±0.43	3.52±0.66	5.77±1.72
Substantia Nigra cNurr1^{DATCre}	0.32±0.08*** (7.39%)	0.588±0.003*** (11.53%)	0.96±0.13*** (27.14%)	4.12±0.30* (71.33%)
Cortex wt^{DATCre}	0.18±0.02	0.44±0.04	0.58±0.06	0.91±0.10
Cortex cNurr1^{DATCre}	0.14±0.03 (74.99%)	0.33±0.04 (76.83%)	0.22±0.02*** (38.64%)	0.78±0.06 (85.30%)

n=11 per group

Table S3

Dopamine and dopamine metabolites and serotonin in cNurr1^{DATCre} vs wt^{DATCre} at P60

Metabolites / Brain regions	DA	DOPAC	HVA	5-HT
Caudate Putamen wt^{DATCre}	57.43±9.30	4.88±0.78	4.48±0.82	3.84±0.44
Caudate Putamen cNurr1^{DATCre}	0.53±0.12** (0.92%)	0.10±0.02** (2.02%)	0.053±0.036** (1.18%)	6.33±0.37*** (164.84%)
N Accumbens wt^{DATCre}	28.80±5.57	3.53±0.68	2.46±0.41	6.36±0.24
N Accumbens cNurr1^{DATCre}	1.57±0.12** (85.45%)	0.31±0.03** (8.82%)	0.25±0.04** (10.24%)	8.59±0.33*** (134.92%)
Substantia Nigra wt^{DATCre}	2.63±0.39	1.06±0.14	0.98±0.11	9.99±1.16
Substantia Nigra cNurr1^{DATCre}	0.27±0.02** (10.29%)	0.15±0.01** (13.75%)	0.064±0.042*** (6.55%)	9.45±0.83 (94.59%)
VTA wt^{DATCre}	10.07±0.01	5.47±0.01	3.52±0.01	22.32±0.01
VTA cNurr1^{DATCre}	1.40±0.13*** (16.49%)	0.53±0.10*** (12.94%)	0.53±0.12*** (18.08%)	18.97±1.74 (98.68%)
Cortex wt^{DATCre}	0.24±0.02	0.12±0.02	0.24±0.04	2.56±0.32
Cortex cNurr1^{DATCre}	0.054±0.006*** (22.04%)	0.019±0.07*** (16.23%)	0.009±0.009** (3.69%)	2.98±0.28 (116.39%)

n=12 per group

Table S4

Dopamine and dopamine metabolites and serotonin in cNurr1^{AAVCre} vs wt^{AAVCre} at 1.5 months

Metabolites / Brain region	DA		DOPAC		HVA		5-HT	
	Non-inj side	inj side	Non-inj side	inj side	Non-inj side	inj side	Non-inj side	inj side
Dorsal STR wt^{AAVCre}	76.65±3.83	62.50±5.09 $^{+}$ (81.5%)	3.40±0.20	2.81±0.23 $^{+}$ (82.6%)	4.89±0.39	4.95±0.66 (101%)	1.83±0.11	1.62±0.17 (88.5%)
Dorsal STR cNurr1^{AAVCre}	65.51±4.35	28.88±5.00 $^{**++}$ (44.1%)	2.96±0.16	1.22±0.19 $^{**++}$ (41.2%)	4.14±0.30	2.50±0.31 $^{**++}$ (60.4%)	1.59±0.05	1.74±0.16 (109.5%)
Ventral STR wt^{AAVCre}	64.15±5.12	47.06±5.41 $^{++}$ (73.4%)	3.23±0.27	2.52±0.20 $^{+}$ (78%)	5.25±0.29	4.71±0.43 (89.7%)	2.05±0.15	2.28±0.19 (111.2%)
Ventral STR cNurr1^{AAVCre}	60.97±4.02	29.39±4.72 $^{*++}$ (48.2%)	3.17±0.26	1.24±0.19 $^{**++}$ (39.1%)	4.96±0.31	2.80±0.37 $^{**++}$ (56.5%)	2.15±0.09	2.60±0.20 (120.9%)
Cortex wt^{AAVCre}	1.78±0.24	1.83±0.38 (103%)	0.35±0.03	0.33±0.05 (94.3%)	0.82±0.11	0.78±0.13 (95.1%)	2.09±0.07	1.99±0.14 (95.2%)
Cortex cNurr1^{AAVCre}	1.26±0.20	0.66±0.11 $^{*+}$ (52.4%)	0.28±0.02	0.14±0.02 $^{**++}$ (50%)	0.66±0.05	0.35±0.04 $^{**+}$ (53%)	2.22±0.08	2.00±0.14 (90.1%)

n=7 per group