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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	QCapture (QImaging), Olympus VS-ASW (Olympus, v2.9)
Data analysis	Numbers (Apple;v3.6.2), Prism (Graphpad; v5.0f), Photoshop (Adobe; CC2018), Illustrator (Adobe; CC2018), Fiji (1.48c)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All newly generated mouse lines will be made available upon request. The original axonal tracing data that support the findings depicted in Figures 3 to 5 are available online (labs.feinberg.northwestern.edu/awatramani/data/poulin-et-al-2018.html). Additional data are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We did not employ a statistical method to determine sample-size. We aimed at providing a n ≥ 3 for each experiment, a sample size we've judged sufficient because of the low variability between the labeling observed in mice with a the same genotype. In addition, we use more than one approach for the majority of Cre lines.
Data exclusions	No data have been excluded from analysis in this study.
Replication	We aimed at providing a n ≥ 3 for each experiment, and to employ more than one approach for each Cre line (all summarized in Table S1). No results are included that were not observed in multiple animals. No finding could not be replicated.
Randomization	For each liter, mice with the same genotype that received a surgery, or were processed for imaging, were determined arbitrarily.
Blinding	Data were processed by an experimenter not blind to experimental condition, using pre-established image processing parameters which aimed at displaying projections without experimental biases (Fig. S4)

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials will be made readily available to the scientific community.

Antibodies

Antibodies used	1) Rabbit anti-Aldh1a1 (Abcam; Cat# ab23375; lot#1158940; polyclonal) 2) Goat anti-Bgal (Biogenesis; Cat# 4600-1409; lot# 5363KC1; polyclonal) 3) Rabbit anti-Bgal (Cappel; Cat# 55976; lot# 04866; polyclonal) 4) Goat anti-FOXA2 (Santa Cruz; Cat# sc-6554; lot# I1715; polyclonal) 5) Chicken anti-GFP (Abcam; Cat# ab13970; lot# GR89472-16; polyclonal) 6) Rabbit anti-NURR1 (Santa Cruz; Cat# sc-990; lot# H0305; polyclonal) 7) Goat anti-Otx2 (Neuromics; Cat# GT15095; lot#401947; polyclonal) 8) Rabbit anti-Sox6 (Abcam; Cat# ab30455; lot# GR78156; polyclonal) 9) Rabbit anti-Th (Pel-Freez; Cat# P40101; lot# 34133; polyclonal) 10) Sheep anti-Th (Pel-Freez; Cat# P60101; lot# 04233; polyclonal)
Validation	1) Rabbit anti-Aldh1a1: no staining in Aldh1a1 KO mice (unpublished observation) 2) Goat anti-Bgal: no staining observed in wildtype mice (unpublished observation) 3) Rabbit anti-Bgal: no staining observed in wildtype mice (unpublished observation)

4) Goat anti-FOXA2: no staining in Foxa1/2 KO Pristerà et al. (2015). PNAS. 112, E4929-38.
5) Chicken anti-GFP: no staining observed in wildtype (unpublished observation)
6) Rabbit anti-NURR1: Present the correct cell distribution in the midbrain and co-localize with known marker (Lmx1 and Pitx3).
7) Goat anti-Otx2: no staining in DA neurons in Dat-Cre/Otx2 cKO (Di Salvio al. (2010). Nature Neuroscience, 13(12), 1481–1488.
8) Rabbit anti-Sox6: Shows specific and expected neuronal distribution in Poulin et al.
(2014, Cell Reports, 9, 930-943) and Panman et al. (2014, Cell Reports, 8, 1018-1025).
9) Rabbit anti-Th: no staining observed in Dat-Cre/ Th cKO mice (unpublished observation)
10) Sheep anti-Th: no staining observed in the DAT+ midbrain DA neurons in Dat-Cre/ Th cKO mice (unpublished observation)

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	All mice used in this manuscript are listed in Table S1. The origin of the mice used in this study, or how the mice were generated, are described in the Method section. All mice were maintained on C57Bl6 background. Adults (2-8 months of age) males and females were used.
Wild animals	No wild animal were used in this study.
Field-collected samples	No sample were field-collected.