Supplementary Information:

Supplementary Material and Methods

Isolation of brain mitochondria

Isolation of non-synaptosomal brain mitochondria from adult males or females (12- to 20-week-old) was performed as described elsewhere.[1] Briefly, brains were homogenized in isolation buffer (225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 5 mM HEPES, and 2 mg/ml fat-free BSA). The homogenate was centrifuged at 1,000 *g* for 10 minutes, and the resulting supernatant was layered onto a gradient of Ficoll medium (5 – 10% Ficoll in a medium containing 0.3 M sucrose, 50 µM EGTA, and 10 mM HEPES), centrifuged at 79,000 *g* for 30 minutes and the mitochondrial pellet resuspended in isolation buffer. Protein concentrations were determined by the bicinchoninic assay method (Thermo Scientific, Rockford, IL, USA) with BSA as a standard protein.

Oxygen consumption

Isolated brain mitochondria (300 µg of protein) were resuspended in 1 ml of respiration buffer consisting of 225 mM mannitol, 75 mM sucrose, 10 mM KCl, 5 mM HEPES, 5 mM K_2HPO_4, and freshly added 1 mg/ml defatted BSA at 30°C.[2] All mitochondria preparations had an average respiratory control ratio of at least 5 when 10 mM glutamate and 5 mM malate were used as NADH-linked substrates. 1.5 mM ADP was added to stimulate oxygen consumption and mitochondria were incubated for different lengths of time (from 15 to 60 min) with the complex I inhibitor 1-methyl-4-phenylpyridinum ion (MPP^+). Oxygen-consumption rates were measured in a closed chamber cuvette with

a mini–stirring bar using a Clark- type electrode (Hansatech Instruments Ltd., King's Lynn, United Kingdom).

H_2O_2 production

Isolated brain mitochondria (500 µg of protein) were incubated in 125 mM KCl, 20 mM HEPES (pH 7.0), 2 mM KH_2PO_4, 4 mM ATP, 5 mM $MgCl_2$, 1 µM Amplex Red, 5 U/mL horseradish peroxidase superoxide dismutase (HRP) and 40 U/mL Cu,Zn SOD. A change in the concentration of H_2O_2 in the medium was detected by fluorescence of the oxidized Amplex Red product using excitation and emission wavelengths of 550 and 585 nm respectively, as indicated previously.[2]

Real-time qPCR

Ventral mesencephalons from 14- to 24-week-old C57BL6/J wild type and mBACtg*Dyrk1a* male mice were homogenized with a Polytron and total RNA was extracted with Tripure Isolation Reagent (Roche, Madrid, Spain) according to the manufacturer's instructions. cDNAs were synthesized from 1 µg of total RNA using Superscript II retrotranscriptase (Life Technologies S.A.) and random examers. Real-time qPCR was carried out with the Lightcycler 480 platform (Roche), using SYBR Green I Master Kit (Roche). Beta-actin (Actb) was used as reference gene for data normalization. The PCR primers used were: Cox7b, 5'-CTAAGCCGTCTCCAAGTTCG-3' and 5'-AAGATGGCTCCACCTGCTAA-3'; Cox11, 5'-GAGAACATGGTGCCTGTCAA-3' and 5'-ATGCCAGTGCAGTCTCTCCT-3; Actb, 5'-CAACGGCTCCGGCATGTGC-3' and 5'-CTCTTGCTCTGGGCCTCG-3.

Supplementary Table S1: Antibodies used for immunohistochemistry

Antibody	Dilution	Provider
Primary		
α-TH	1:3,000** 1:10,000	Sigma-Aldrich
α-Act-Casp3	1:1,000*	BD Pharmigen (Franklin Lakes, NJ, USA)
α-Act-Casp9 (Asp353)	1:500*	Cell Signaling Technology (Billerica, MA, USA)
α-Dyrk1A	1:250	Abnova Corporation (Taipei City, Taiwan)
α -Nurr1	1:300	Santa Cruz Biotechnology (Dallas, TX, USA)
α -DAT	1:1,000	Merck-Millipore (Mollet del Vallès, Spain)
Secondary		
Biotinilated α-mouse IgG	1:1,000	Vector Labs (Burlingame, CA, USA)
Biotinilated α-rabbit IgG	1:1,000	Vector Labs
α-mouse Alexa 488	1:1,000	Life Technologies S.A.
α-rabbit Alexa 555	1:1,000	Life Technologies S.A.

* Antigen retrieval was preformed with these antibodies

** Antigen retrieval was preformed with anti-TH antibody (1:3,000) in tissue samples from embryonic and P0 brains.

Supplementary Table 2: Antibodies used for Western blotting

Antibody	Dilution	Provider
Primary		
α-Dyrk1a	1:250	Abnova Corporation
α-Casp9-Pi-Thr125	1:500	BIOSS (Woburn, MA, USA)
α-Pro-casp9	1:500	Cell Signaling Technology
α-Actin	1:5,000	Sigma-Aldrich
α-Vinculin	1:20,000	Sigma-Aldrich
α-Hsp60	1:1,000	Santa Cruz Biotechnology
α-Vdac	1:3,000	Abcam (Cambridge, United Kingdom)
α-Mtfam	1:1,000	Abcam
α-CoxIV subunit I	1:1,000	Life Technologies
α-CoxIV subunit IV	1:1,000	Life Technologies
α-Sod2	1:1,000	Abcam
α-Gpx	1:1,000	Abcam
α-Catalase	1:1,000	Merck-Calbiochem
Secondary		
α-mouse IgG IRDye-800CW	1:5,000	LI-COR ODYSSEY
α-rabbit IgG IRDye-680CW	1:5,000	LI-COR ODYSSEY
α-anti-mouse-HRP	1:1,000	Dako (Sant Just Desvern, Spain)
α-anti-rabbit-HRP	1:1,000	Dako

Supplementary References

1. Tieu K, Perier C, Caspersen C, Teismann P, Wu DC, Yan SD*, et al.* D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *J Clin Invest* 2003; **112:** 892-901.
2. Starkov AA, Polster BM, Fiskum G. Regulation of hydrogen peroxide production by brain mitochondria by calcium and Bax. *J Neurochem* 2002; **83:** 220-228.

Supplementary Figures

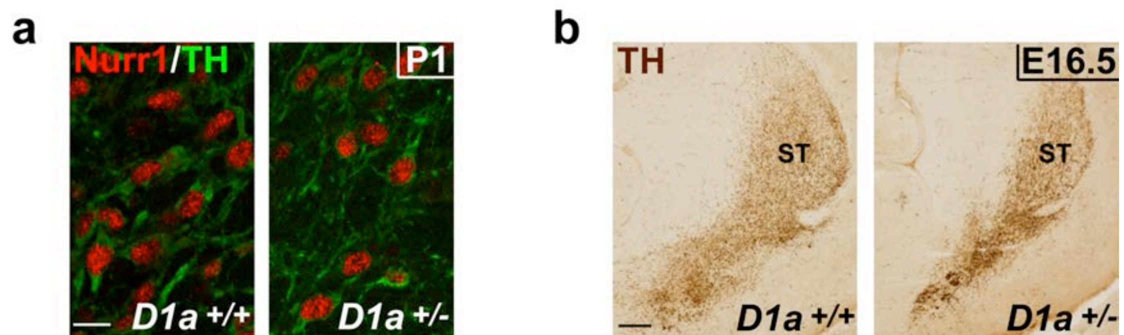

Figure S1. Brain sections at the level of the ventral mesencephalon (in **a**) or at the level of the striatum (in **b**) showing the expression of DA specific cell markers. (**a**) Confocal images showing co-localization of the DA markers TH (green) and Nurr1 (red) in P1 *Dyrk1a*$^{+/+}$ (*D1a*$^{+/+}$) and *Dyrk1a*$^{+/-}$ (*D1a*$^{+/-}$) mice. Note that all TH^{+} neurons express Nurr1 in both genotypes. (**b**) TH immunohistochemistry showing dopaminergic fibers innervating the striatum (ST) in E16.5 *D1a*$^{+/+}$ and *D1a*$^{+/-}$ embryos. Bar: 100 *μ*m.

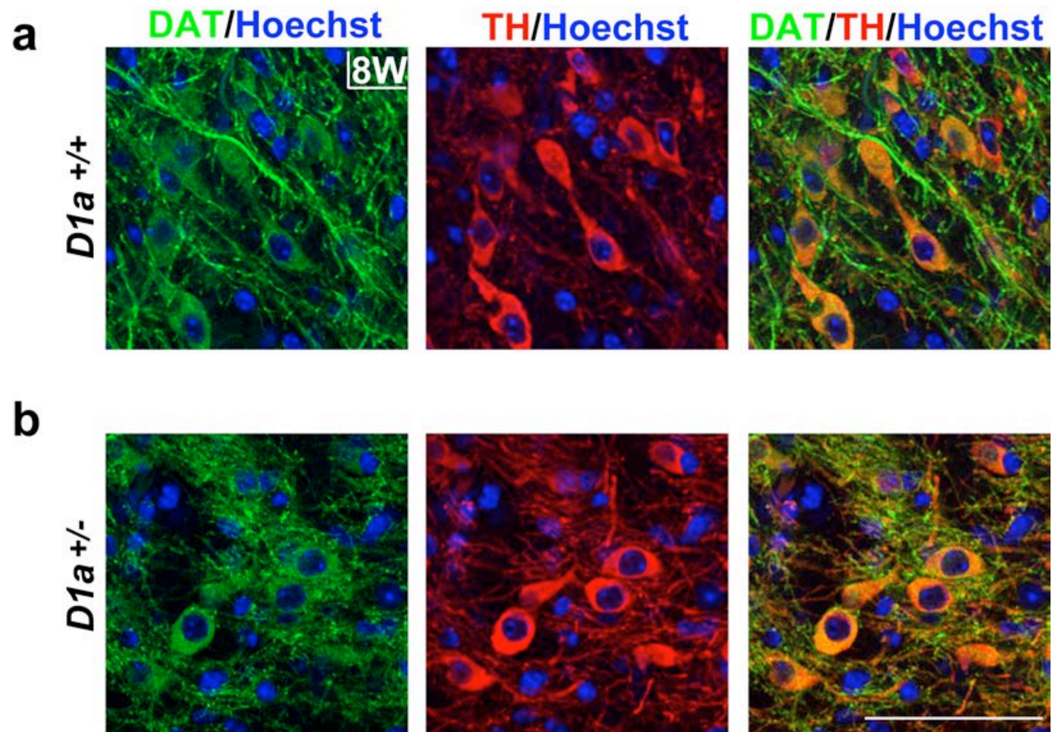

Figure S2. Confocal pictures from brain sections at the level of the ventral mesencephalon obtained from of 8-week-old (8W) *Dyrk1a*$^{+/+}$ (*D1a*$^{+/+}$, **a**) and *Dyrk1a*$^{+/-}$ (*D1a*$^{+/-}$, **b**) mice. Sections were immunostained for TH (red) and the dopamine transporter (DAT, green), and the nuclei visualised with Hoechst staining (blue). Note that all DAT^{+} neurons also express TH in both, *Dyrk1a*$^{+/+}$ and *Dyrk1a*$^{+/-}$ mice. Bar: 50 μm.

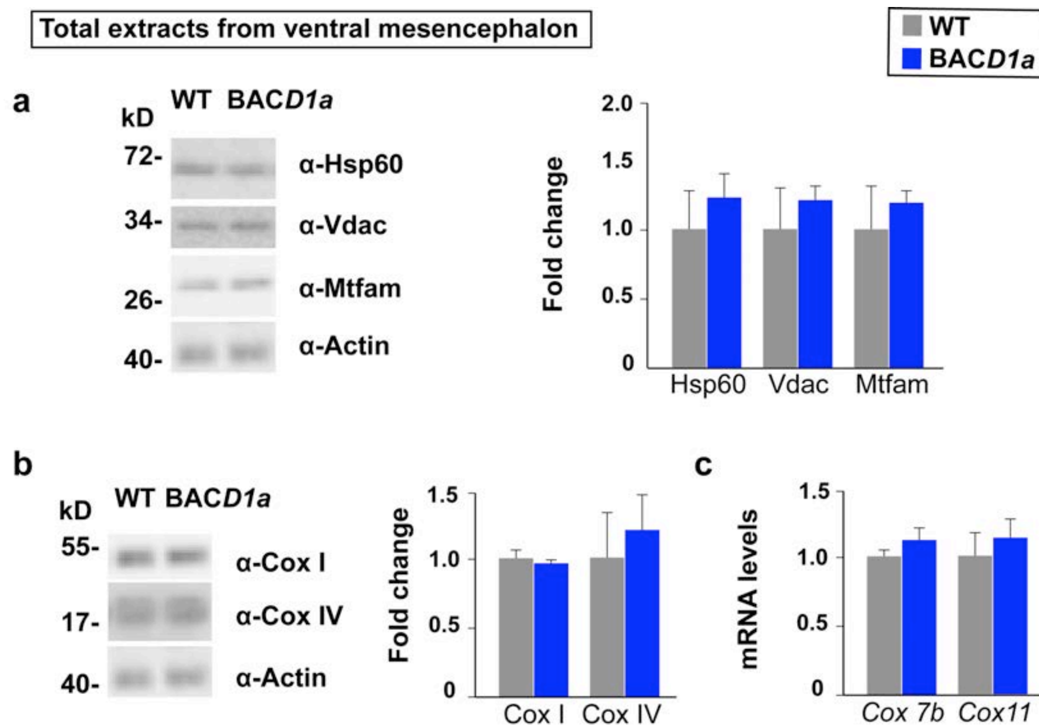

Figure S3. Relative levels of mitochondrial proteins in total extracts prepared from the ventral mesencephalon of 12- to 20-week-old wild type (WT) and mBACtg*Dyrk1a* (BAC*D1a*) mice. (**a**, **b**) Representative Western blots probed with the indicated antibodies and histograms showing the protein levels in BAC*D1a* mice normalized to actin levels and expressed relative to the wild types. (**c**) Relative mRNA expression of *Cox7b* and *Cox11* determined by RT-PCR on mRNA obtained from the ventral mesencephalon of wild type and BAC*D1a* adult mice. Histogram values are the mean ± S.E.M. (*n* = 5 animals per genotype) and the differences between genotypes were not statistically significant: *P*-values >0.05 in a two-tailed Student's t-test for all the analyzed proteins. Hsp60: Heat shock protein 60; Vdac: Voltage-dependent anion channels; Mtfam: Mitochondrial transcription factor; Cox: Cytochrome c oxidase subunit. The sequence of PCR primers and details on the antibodies

used for Western blotting are provided in Supplementary Materials and Methods and in Supplementary Table 2.

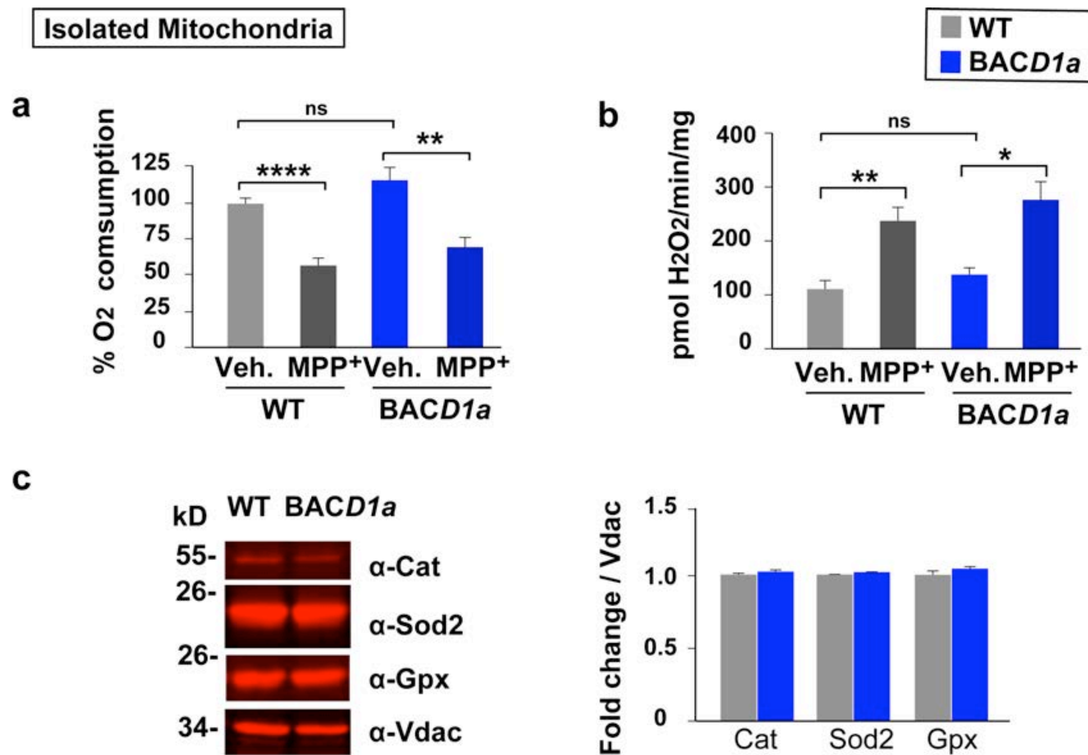

Figure S4. Effect of MPP$^+$ on complex I-driven mitochondrial respiration (**a**) and production of H_2O_2 (**b**), and relative levels of antioxidant proteins (**c**) in non-synaptosomal brain mitochondria isolated from adult wild type (WT) and mBACtg*Dyrk1a* (BAC*D1a*) mice. Isolated mitochondria in **a** and **b** were incubated in the presence or absence (Veh.) of MPP$^+$ (100 μM). (**c**) Representative Western blot of mitochondrial protein extracts probed with the indicated antibodies and histograms showing the protein levels in BAC*D1a* mice normalized to Vdac (Voltage-dependent anion channels) levels and expressed relative to the wild types. Cat: Catalase; Sod2: Superoxide dismutase 2; Gpx: Glutathione peroxidase. The data in **a** (*n* = 9 animals per group) and **b** (*n* = 6 animals per group) were analyzed by ANOVA followed by a two-tailed Student's t-test for matched pairs and are presented as the mean ± S.E.M. Note that MPTP treatment has similar effects in WT and BAC*D1a*

mitochondria and that differences between genotypes are not statistically significant (ns: $p > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$). Histogram values in **c** are the mean ± S.E.M. ($n = 6$ animals per genotype) and the differences between genotypes were not statistically significant: *P*-values > 0.05 in a two-tailed Student's t-test for all the analyzed proteins.

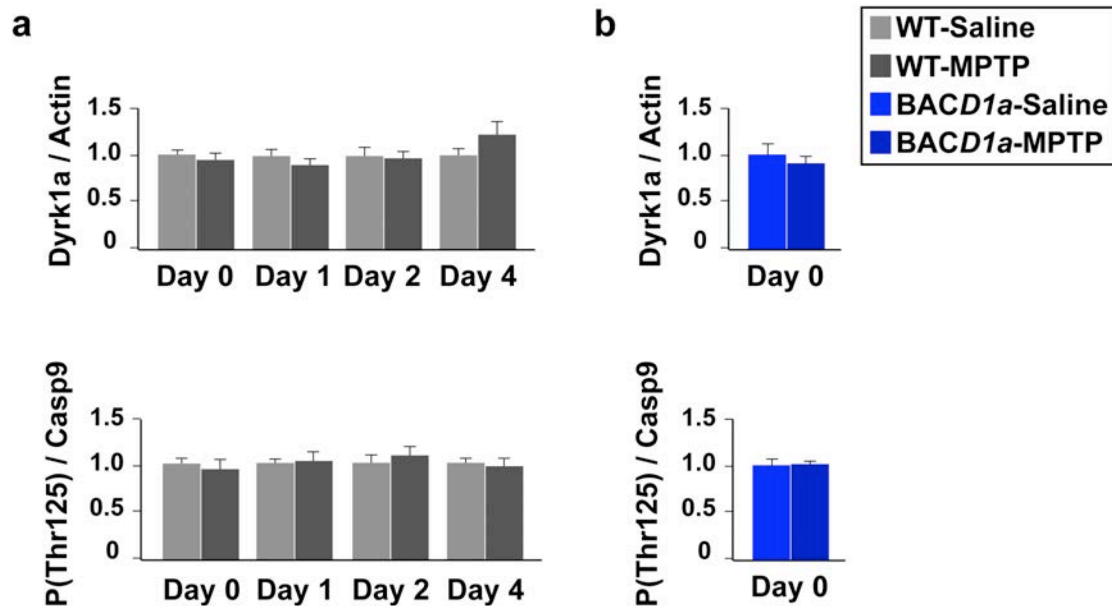

Figure S5. Effect of MPTP treatment on Dyrk1A protein levels and on Thr125 phosphorylated Casp9 (P(Thr125)) in the ventral mesencephalon of 12-week-old C57BL6/J wild-type (**a**) and mBACtg*Dyrk1a* (BAC*D1a*) mice (**b**). Mice were sacrificed on the days indicated after the last injection with saline (WT-Saline and BAC*D1a*-Saline) or MPTP (WT-MPTP and BAC*D1a*-MPTP) treatment and protein levels determined by Western blot in total extracts. Dyrk1a and Thr125 phosphorylated Casp9 were normalized to the actin or pro-Casp9 content respectively and expressed relative to the values in the untreated group (saline). The data are presented as the mean ± S.E.M. (n = 4 animals per condition) and the differences between untreated and MPTP-treated mice were not statistically significant: P-values > 0.05 in a two-tailed Student’s t-test at all time points.

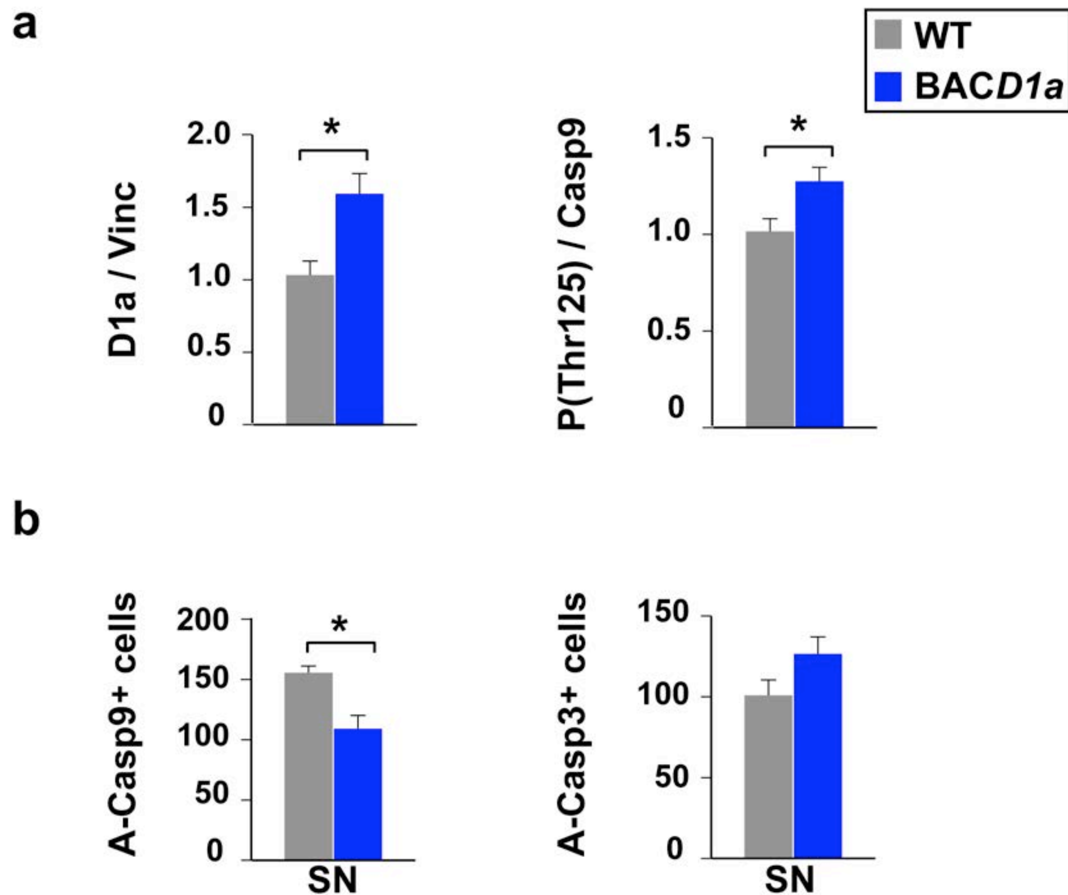

Figure S6. Quantifications showing Dyrk1a (D1a) protein normalized to the amount of Vinculin (Vinc), and the proportion of Casp9 that is Thr125 phosphorylated (P(Thr125)) in total mesencephalic extracts of P14 wild type (WT) and mBACtg*Dyrk1a* (BAC*D1a*) mice (**a**), and total numbers of active-Casp9 (A-Casp9) or active-Casp3 (A-Casp3) in the Substantia Nigra (SN) of these mice (**b**). Histogram values are the mean ± S.E.M. $^{*}P \leq 0.05$ (n = 4 in **a**; n = 3 in **b**).