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The neurogenic basic helix–loop–helix transcription factor NeuroD6 concomitantly increases mitochondrial mass and regulates cytoskeletal organization in the early stages of neuronal differentiation

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SUPPLEMENTARY ONLINE DATA

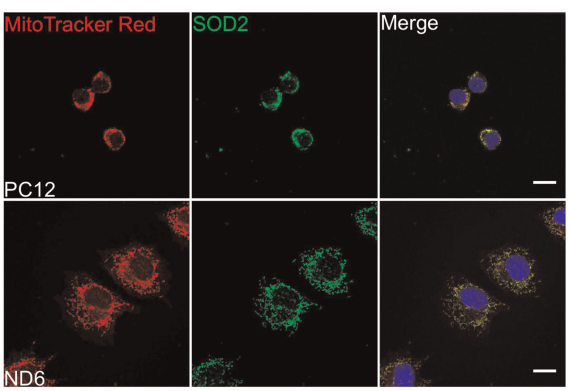

Figure S1 MTR labels the entire mitochondrial population of PC12 and PC12-ND6 cells

Control PC12 and PC12-ND6 cells were stained with MTR (red), anti-SOD2 antibody (green) and TO-PRO®-3 (blue). The corresponding merged images demonstrate perfect overlapping expression pattern between the mitochondrial protein SOD2 and MTR in both PC12 and PC12-ND6 cells. Scale bar, 10 μm.

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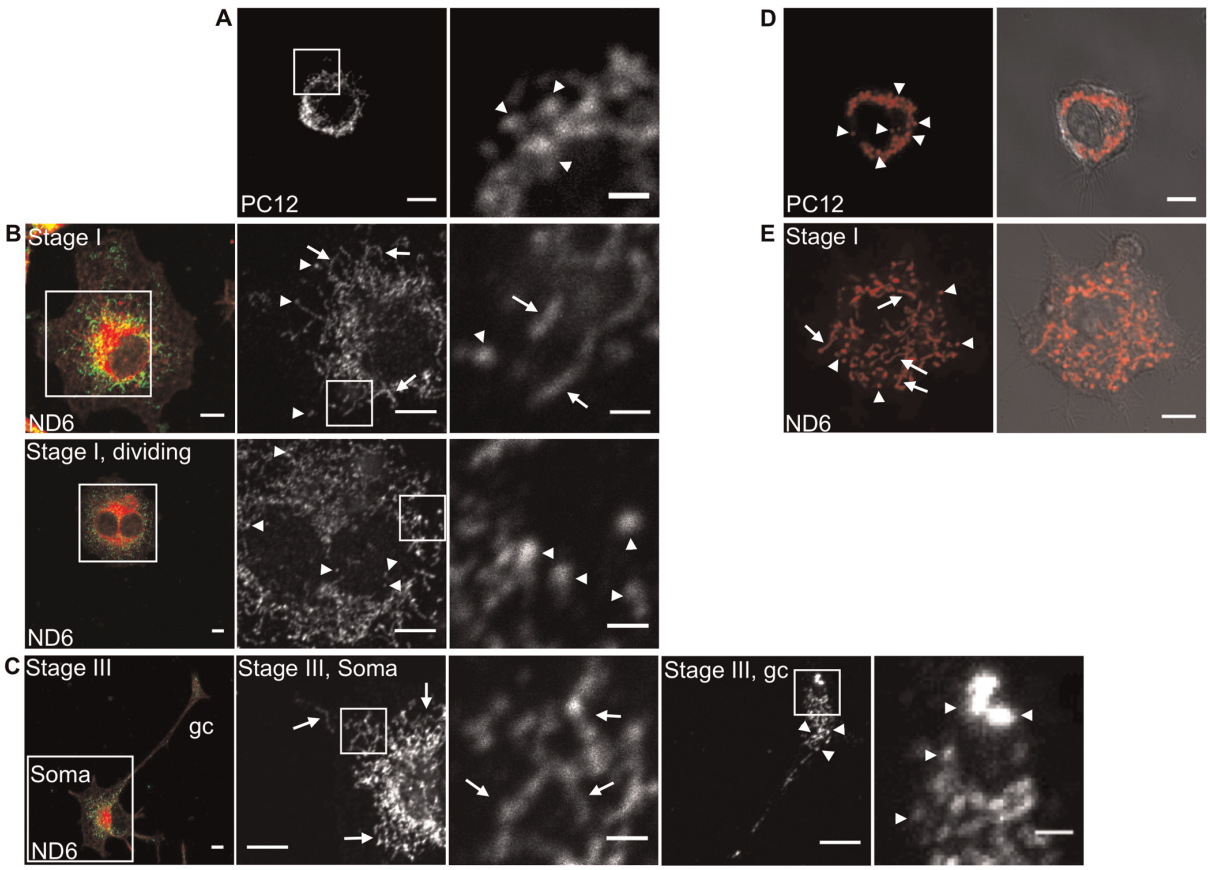

Figure S2 **Mitochondria display distinct morphology at specific stages of neuronal differentiation in PC12-ND6 cells**

Arrowheads indicate short and punctate mitochondria, whereas arrows indicate elongated and tubular mitochondria. (**A–C**) Untreated PC12 and PC12-ND6 cells were fixed and labelled with antibodies against SOD2 (green) and β-III-tubulin (red) to examine mitochondrial morphology and delineate the overall shape of the cells by confocal fluorescence microscopy. (**A**) Naïve PC12 cells (left-hand panel; scale bar, 5 μm) with corresponding high magnification of the boxed area (right-hand panel) illustrating the short and punctate morphology of mitochondria (arrowheads; scale bar, 1 μm). (**B**) Stage I non-dividing PC12-ND6 cells (top row; scale bar, 5 μm) and stage I dividing PC12-ND6 cells (bottom row; scale bar, 5 μm) with corresponding high magnification images of the boxed region illustrating the morphology of mitochondria (right-hand panels; scale bars, 1 μm); far right images show high magnification of mitochondria (arrowheads for short mitochondria and arrows for elongated mitochondria). (**C**) Stage III PC12-ND6 cells (left-hand panel; scale bar, 5 μm), with corresponding magnification images from the soma (second panel; scale bar, 5 μm) and the growth cone (third panel; gc; scale bar, 5 μm). The images to the right of each subcellular location show high magnification of mitochondria corresponding to that subcellular region (arrowhead, short mitochondria; arrow, elongated mitochondria; scale bar, 1 μm). Images are reflective of the predominant mitochondrial morphology observed a minimum of 25 cells from three independent experiments. (**D** and **E**) Live-cell confocal imaging on PC12 (**D**) and stage I PC12-ND6 (**E**) cells labelled with MTR. The right-hand panels show the merge with the corresponding DIC (differential interference contrast) pictures (scale bar, 5 μm).

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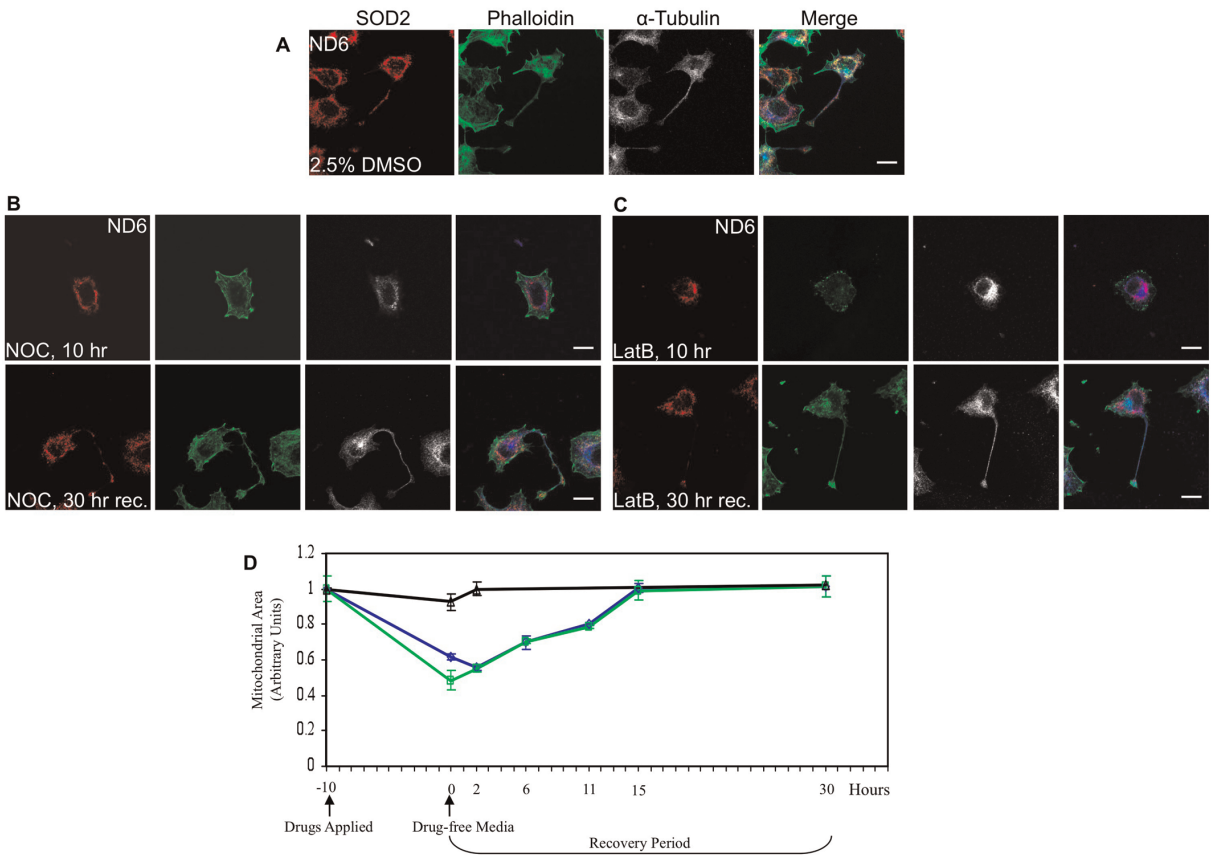

Figure S3 **Nocodazole and latrunculin B treatment results in decreased mitochondrial area in PC12-ND6 cells**

(**A**) PC12-ND6 cells were treated with 2.5% DMSO for 10 h and subsequently labelled with anti-SOD2 antibody (red), Alexa Fluor® 488–phalloidin (green) and anti-α-tubulin antibody (grey, and blue in merge panels). Scale bar, 10 μm. (**B**) Reversible effect of nocodazole exposure on the mitochondrial area of PC12-ND6 cells. The top row shows PC12-ND6 cells treated with 10 μg/ml nocodazole (NOC) for 10 h and subsequently labelled with anti-SOD2 antibody (red), Alexa Fluor® 488–phalloidin (green) and anti-α-tubulin antibody (grey, and blue in merge panels). After 10 h of treatment, PC12-ND6 cells were transferred to drug-free medium for the indicated recovery period (bottom row). Scale bar, 10 μm. (**C**) Reversible effect of latrunculin B exposure on the mitochondrial area of PC12-ND6 cells. The top row shows PC12-ND6 cells treated with 15 μM latrunculin B (LatB) for 10 h and subsequently labelled with anti-SOD2 antibody (red), Alexa Fluor® 488–phalloidin (green) and anti-α-tubulin antibody (grey, and blue in merge panels). After 10 h of treatment, PC12-ND6 cells were transferred to drug-free medium for the indicated recovery period (bottom row). Scale bar, 10 μm. (**D**) Quantification of mitochondrial area in PC12-ND6 cells before, during and after treatment with nocodazole or latrunculin B. Control (2.5% DMSO), black; latrunculin B, green; nocodazole, blue. Arrows indicate time of drug application and removal. The graph is representative of three independent experiments, and results are means ± S.D. from $n=150$ cells per treatment.

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