SUPPLEMENTARY INFORMATION

Engrailed protects mouse midbrain dopaminergic neurons against mitochondrial complex I insults

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**Supplementary Fig. 1** Engrailed treatment has no effect on DAT activity.

DAT-activity was measured by the uptake of $[^3]$H-dopamine in mesencephalic cultures treated or not with Engrailed (3 nM). The cultures were preincubated for 10 min with 500 µl PBS, 5 µM glucose and 100 µM ascorbic acid prior to the addition of 50 nM $[^3]$H-dopamine (30 mCi/mmol). The incubation was stopped after 15 min by two rapid washes with cold PBS. The cells were scraped off and radioactivity was measured in a liquid scintillation spectrophotometer. Blank values, determined with 5 µM of the dopamine uptake inhibitor GBR-12909, represented less than 10% of total uptake in control conditions. Data are expressed in percentage of control values, which corresponds to 24,400 cpm.
Supplementary Fig. 2 Quantification of neurons and astrocytes in mesencephalic cultures

Neuronal cultures were fixed after 5 days in vitro and stained with Tuj1, a neuronal marker, and S100 beta, a glial marker. Scale bar, 100 µm. The graph depicts the percentage of each cell type.
**Supplementary Fig. 3** Engrailed does not affect the synthesis of complex II or complex V subunits

Metabolic labeling of midbrain synaptoneurosomes followed by complex II and complex V immunocapture and SDS-PAGE does not reveal any change upon Engrailed treatment.
Supplementary Fig. 4 Ndufs3 expression in mDA neurons in the VTA of wt and En1<sup>+/−</sup> mice

Ndufs3 levels in TH positive neurons in the VTA area were quantified as for mDA neurons in the SNpc area using the same sections as described in the legend of Fig. 4. The values for wt and En1<sup>+/−</sup> mice are not significantly different.
Supplementary Fig. 5 The number of TH-negative neurons remains constant upon MPTP treatment

The non-infused side of control mice injected with saline was set as 100% of TH-negative, Nissl-positive cells and used for comparison with all other conditions. As expected MPTP did not affect the number of TH-negative neurons indicating that differences seen in TH-positive neurons are true cell loss and not to a reduction in TH immunostaining. No conditions showed a significant difference compared to controls. Data are expressed in percentage of control, n = 6-10 animals/group. c, contralateral, non-infused side; S, sham-infused side; En, Engrailed-infused side.
Supplementary Fig. 6 Verification of siRNA used against Ndufs1

(a) Western blot analysis of siRNA infused ventral midbrain extracts. Penetratin-coupled siRNA against Ndufs1 or control siRNA were infused for three days with a minipump into a region dorsal to the substantia nigra. The Western blots shows decreased amounts of Ndufs1 as compared to a control siRNA. (b) Quantification of Western blot analysis. The average ipsilateral versus contralateral ratio of Ndufs1 levels normalized to actin was calculated for each condition. Ndufs1 siRNA decreased Ndufs1 protein level on the infused side as compared to the contralateral side by about 30%. * P<0.05 (n = 3-4).