Table 1. Rapamycin does not decrease the half-life of RTP801. Neuronal PC12 cells were exposed to rapamycin for 1 hr where indicated and then to MPP+ for 8 hr where indicated. The half-life of RTP801 was determined under each condition as determined in Materials and Methods. n = number of cultures evaluated. r = regression coefficient.

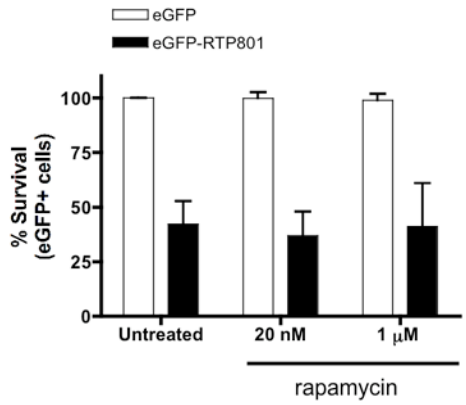
TREATMENT	T1/2(min)	n	r
CONTROL	5.1	6	0.92
RAPA	10.6	2	0.99
MPP+	7.4	5	0.96
RAPA+MPP+	9.6	5	0.96

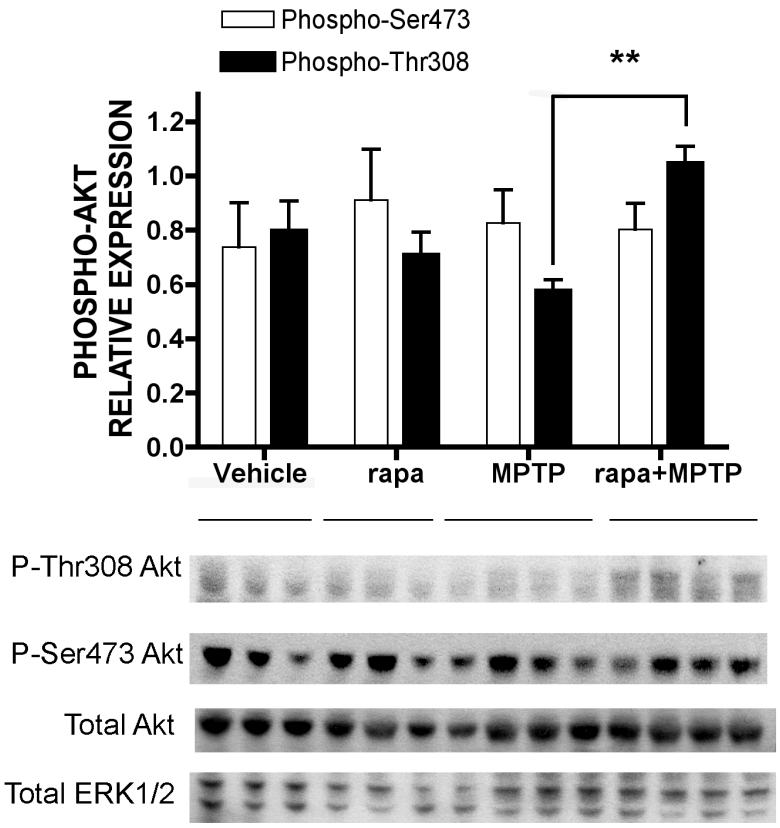
SUPPLEMENTAL FIGURE 1. Rapamycin does not protect from exogenous RTP801 expression. Neuronal PC12 cells were transfected with empty vector pCMS-eGFP or pCMS-eGFP-RTP801. Immediately after transfection, cultures were treated with or without 20 nM or 1 μM rapamycin. Viability of transfected cells was scored under fluorescence microscopy 48 hours later. Values represent mean ± SEM of at least three independent experiments in triplicate in each condition.

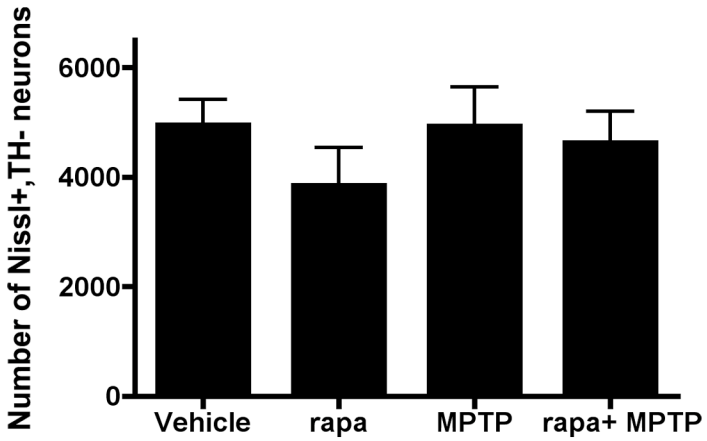
SUPPLEMENTAL FIGURE 2. Effects of rapamycin on Akt phosphorylation in the MPTP-injected mouse model.

Mice were injected with either vehicle, rapamycin, MPTP or rapamycin + MPTP, as described in Methods for the acute regimen, and sacrificed after 24 hours. Ventral midbrains were dissected, homogenized in lysis buffer and the homogenates subjected to SDS-PAGE and Western immunoblotting. Membranes were probed with Phospho-Thr308-Akt and Phospho-Ser473-Akt antibodies and reprobed with antibodies for total Akt and ERK1/2 as loading controls. Films resulting from the immunoblot were scanned and relative densities of Phospho-Akt bands were normalized in each case to the densities of the corresponding total Akt signals. Values (in arbitrary units) for Phospho-Ser473 and Phospho-Thr308 Akt are expressed as means ± SEM for at least three mice per condition. **p<0.01 vs mice injected with MPTP.

SUPPLEMENTAL FIGURE 3. Numbers of TH-negative, Nissl-positive neurons in the SNpc of mice treated with vehicle, rapamycin, MPTP or rapamycin + MPTP. Mice subjected to the acute regimen as described in the text were sacrificed 7 days after the last MPTP injection. Midbrains were cryo-sectioned, immunostained with tyrosine hydroxylase antibody and subjected to Nissl staining with cresyl violet. Unbiased stereology was used to determine the total numbers of TH-negative, Nissl-positive neurons in the substantia nigra pars compacta. There were no statistically significant differences between the various conditions.


eGFP
eGFP-RTP801
% Survival
(eGFP+ cells)
100
75
50
25
0
Untreated
20 nM
1 μM
rapamycin


Phospho-Ser473
Phospho-Thr308
**
PHOSPHO-AKT
RELATIVE EXPRESSION
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
rapa
MPTP
rapa+MPTP
P-Thr308 Akt
P-Ser473 Akt
Total Akt
Total ERK1/2


Number of Nissl+,TH- neurons
6000
4000
2000
0
Vehicle
rapa
MPTP
rapa+ MPTP