Nicotinamide Riboside Enhances Mitochondrial Bioenergetics and Dopaminergic Signaling Independent of Neuron Survival in a Double-Hit Parkinson's Model

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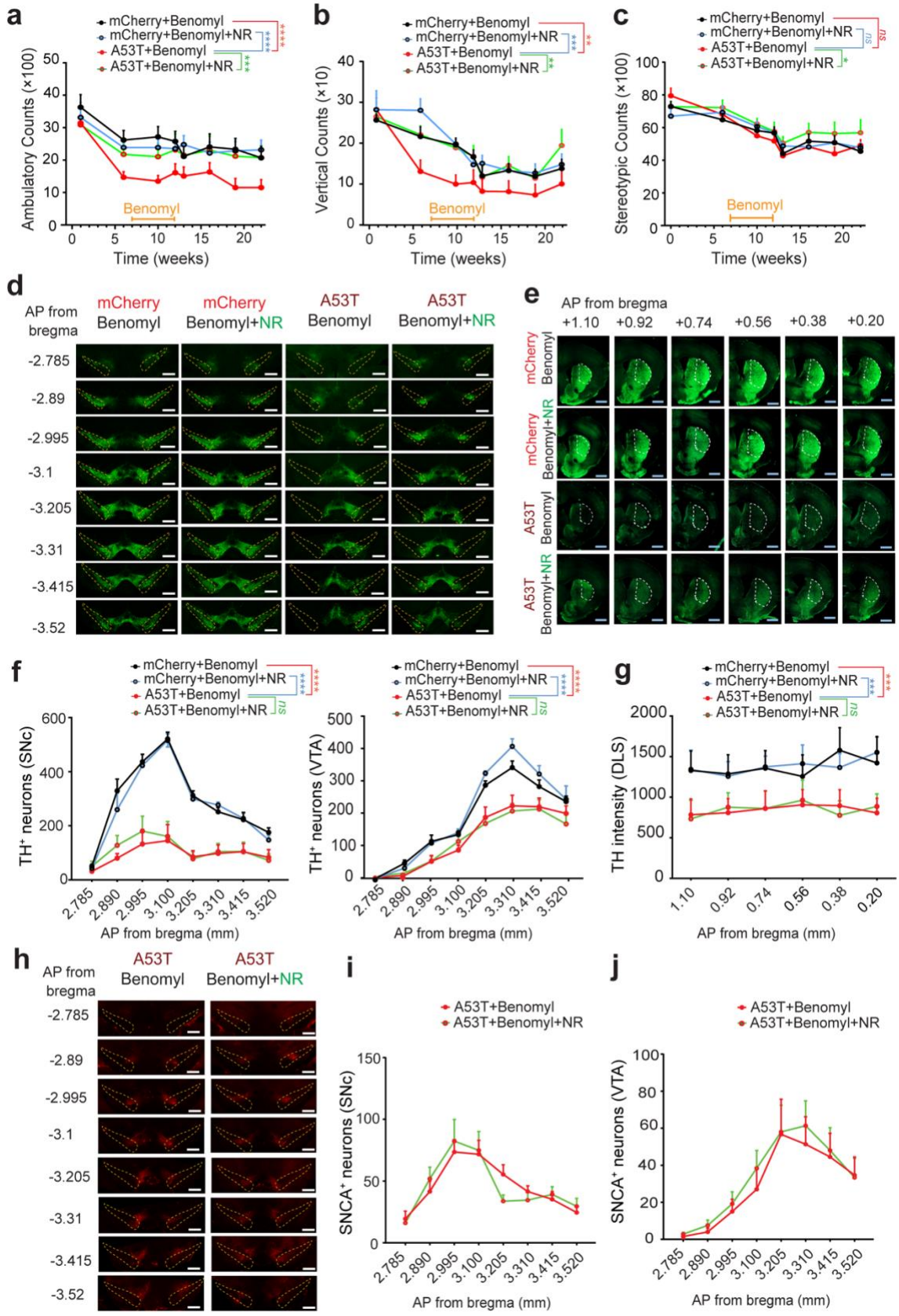
Supplementary Figures


a
mCherry+Benomyl
mCherry+Benomyl+NR
A53T+Benomyl
A53T+Benomyl+NR
Ambulatory Counts (×100)
Benomyl
Time (weeks)
b
Vertical Counts (×10)
c
Stereotypic Counts (×100)
d
AP from bregma
mCherry Benomyl
mCherry Benomyl+NR
A53T Benomyl
A53T Benomyl+NR
-2.785
-2.89
-2.995
-3.1
-3.205
-3.31
-3.415
-3.52
e
AP from bregma
+1.10
+0.92
+0.74
+0.56
+0.38
+0.20
f
TH⁺ neurons (SNc)
TH⁺ neurons (VTA)
AP from bregma (mm)
g
TH intensity (DLS)
h
i
SNCA⁺ neurons (SNc)
j
SNCA⁺ neurons (VTA)

Supplementary Figure 1. NR ameliorates behavioral deficiency in PD mice but does not rescue dopamine neuron loss.

(a–c) Line graph showing total ambulatory counts, vertical counts and stereotypic counts respectively, in open field test in different subgroups of experimental mice and the positive effect of chronic NR on increasing locomotory movement counts. Data are presented as mean ± SEM from 8 mice/group (n=8). Significance (*$P < 0.05$) was determined by two-way ANOVA followed by Tukey's multiple comparisons test.

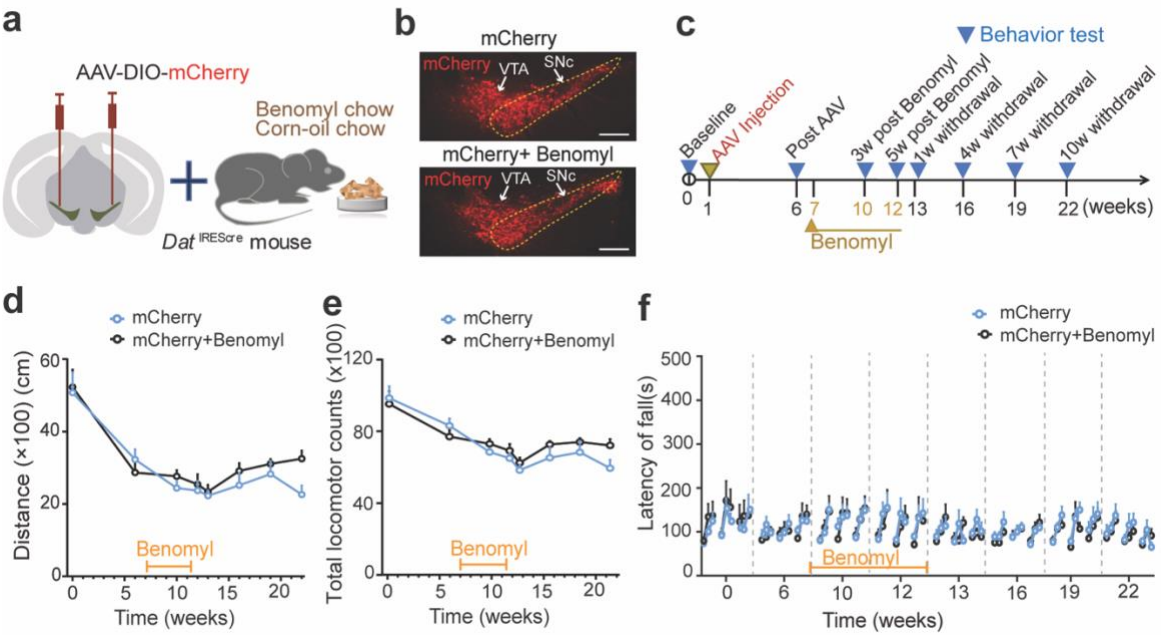
(d-e) Confocal images portraying serial immunostaining for TH (green) in SNc. and TH (green) nigrostriatal DA neuron projections to the DLS. Scale bar: 200 μm (SNc) and 500 μm (DLS).

(f) Summary statistics of consecutive quantification of total TH+ DA neurons in SNc and VTA. Data are presented as mean ± SEM from 6 mice/group (n=6). Significance (*$P < 0.05$) was determined by two-way ANOVA followed by Tukey's multiple comparisons test.

(g) Sequential quantification of TH+ terminal density expression in DLS. Data are presented as mean ± SEM from 6 mice/group (n=6). Significance (*$P < 0.05$) was determined by two-way ANOVA followed by Tukey's multiple comparisons test.

(h) Confocal images portraying serial sectioning and immunostaining for accumulation of α-synuclein (SNCA+) in SNc in AAV A53T [AAV1 serotype (pAAV-EF1α-DIO-SNCA-hGHpA] injected PD mice.

(i-j) Summary statistics of serial quantification of A53T + DA neurons in SNc and VTA. Data are presented as mean ± SEM from 6 mice/group (n=6).



Supplementary Figure 2: Sublethal benomyl doesn't cause any PD like symptoms in control mice.

(a) Schematic representation of Control mCherry AAV IC administration and benomyl exposure in DAT^{IRESCre}.

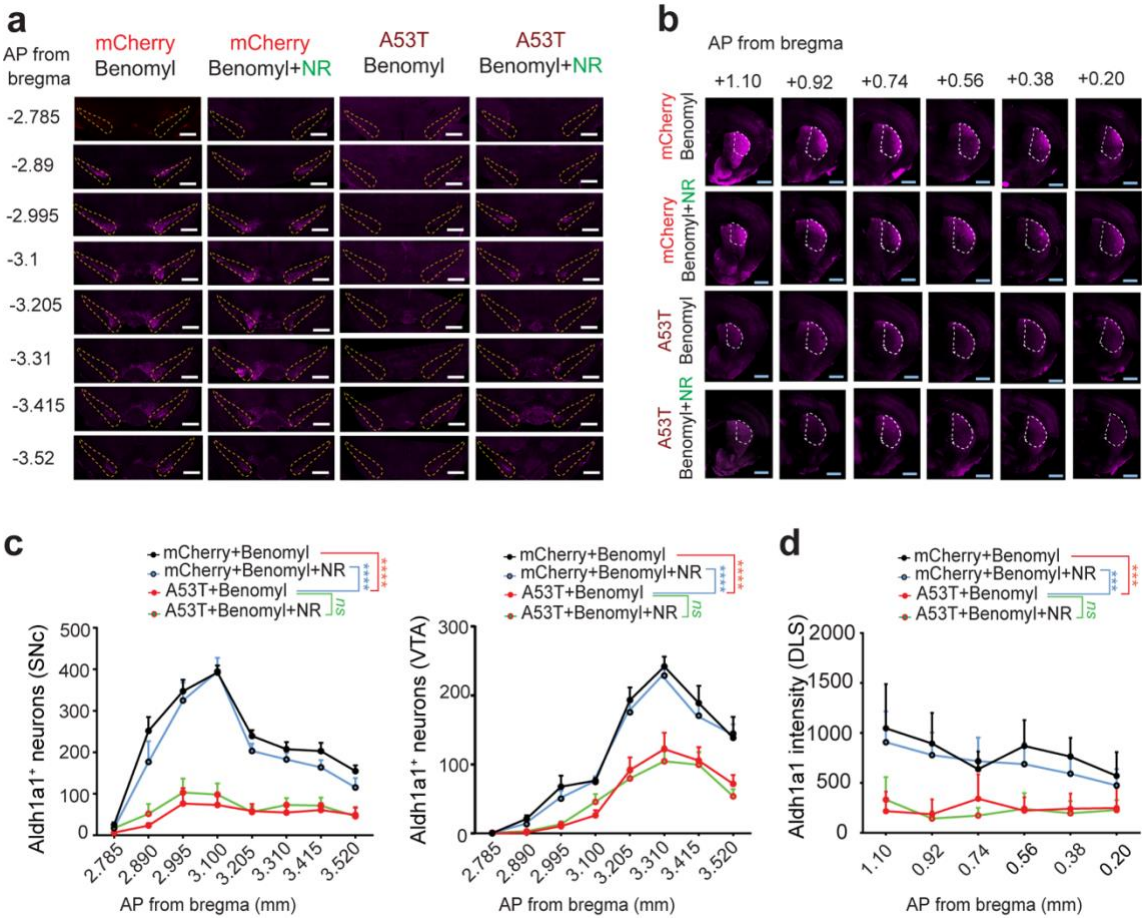
(b) Confocal images portraying immunostaining for control mCherry (red), Scale bar: 200 µm.

(c) Timeline of the AAV injection, longitudinal behavior measurement, and benomyl exposure.

(d-e) Line graph showing no changes in total ambulatory distance covered in cm and total locomotory counts by open field test between the two subgroups of experimental mice. Data are presented as mean ± SEM from 9 mice (mCherry group). (*n*=9) and 7 mice (mCherry+Benomyl group) (n=7). Significance (*$P < 0.05$) was determined by paired t-test.

(f) Line graph showing no changes in latency of fall in seconds examined by rotarod analysis between the two subgroups of experimental mice. Data are presented as mean ± SEM from 9

mice (mCherry group). (*n*=9) and 7 mice (mCherry+Benomyl group) (n=7). Significance (*P < 0.05) was determined by paired t-test.



Supplementary Figure 3. NR does not rescue the loss of ALDH1A1$^+$ neurons.

(a-b) Confocal images portraying serial immunostaining for ALDH1A1 (magenta) in SNc and for aldh1a1(magenta) nigrostriatal DA neuron projections to the DLS. Scale bar: 200 µm (SNc) and 500 µm (DLS).

(c) Summary statistics of consecutive quantification of total ALDH1A1^{+} DA neurons in SNc and VTA. Data are presented as mean ± SEM from 6 mice/group (n=6). Significance (*P < 0.05) was determined by two-way ANOVA followed by Tukey's multiple comparisons test.

(d) Sequential quantification of ALDH1A1^{+} terminal density expression in DLS. Data are presented as ± SEM from 4 mice/group. (*n*=4). Significance (**P* < 0.05) was determined by two-way ANOVA followed by Tukey's multiple comparisons test.