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## Supplemental Information

# Striatal Projection Neurons Require Huntingtin for Synaptic Connectivity and Survival

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# Figure S1
# (Related to Figure 1)

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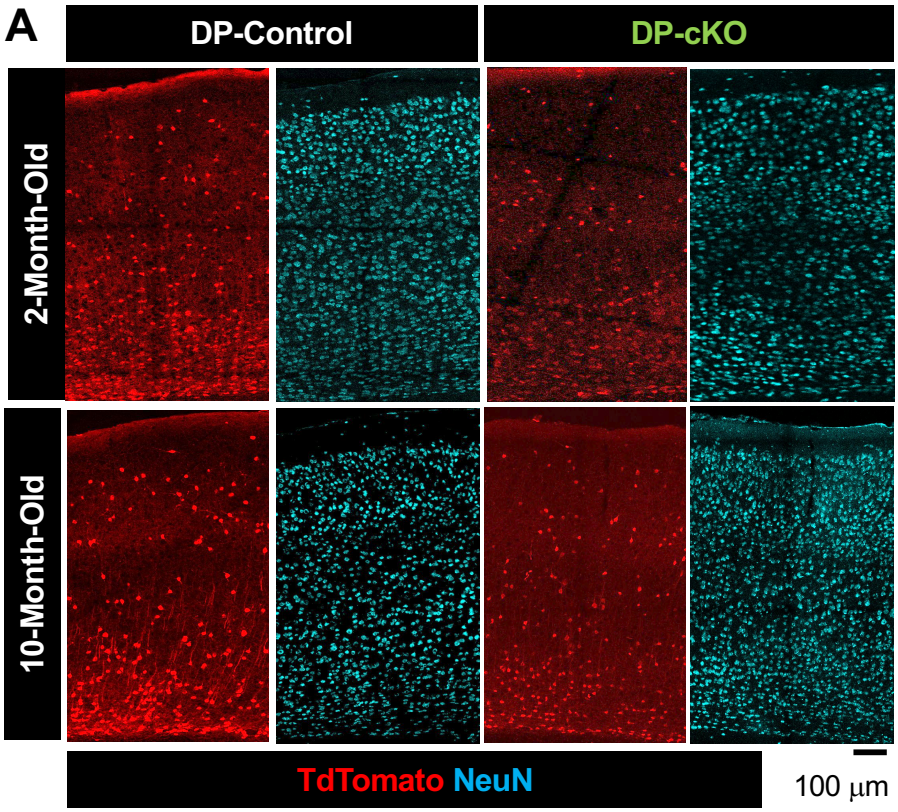


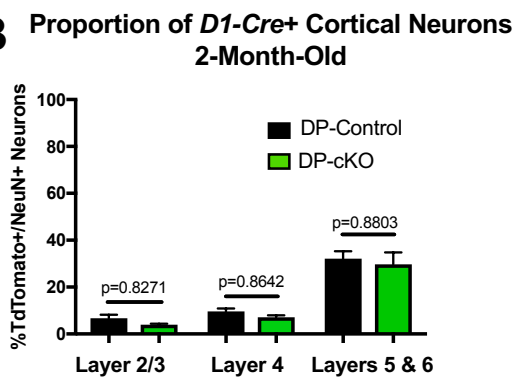


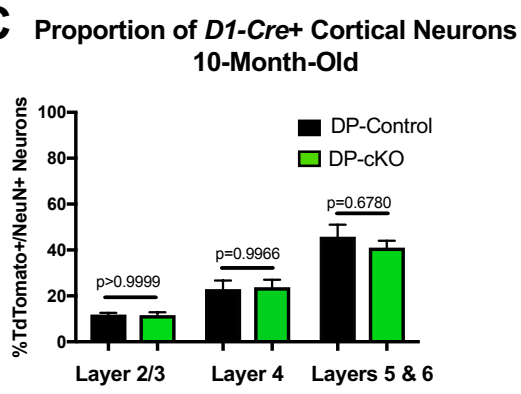


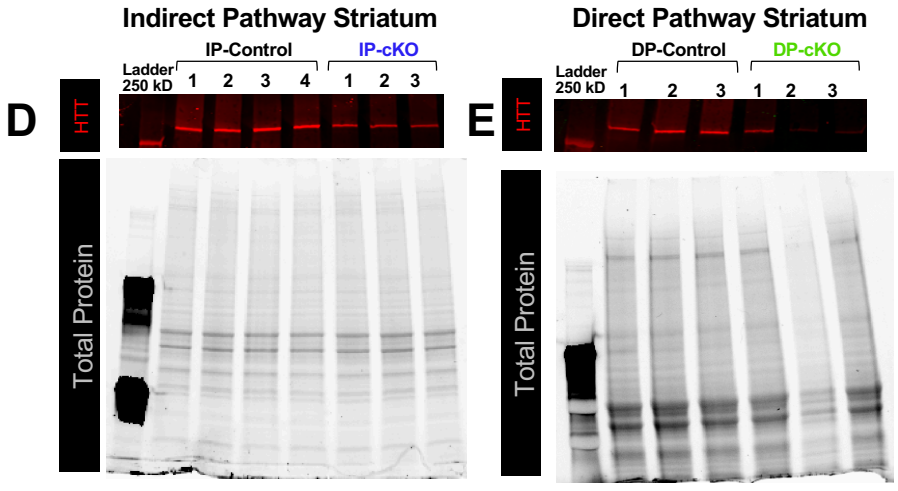


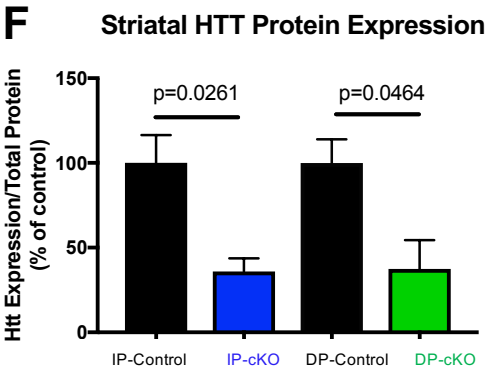


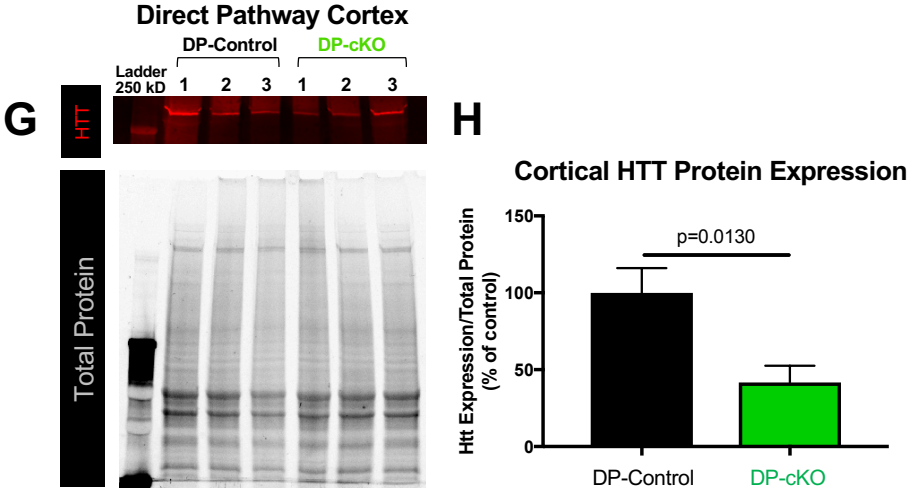

**Figure S1: *A2A-Cre* and *D1-Cre* drive significant reduction of HTT protein expression, and *D1-Cre* is expressed in a subset of cortical neurons (Related to Figure 1).**

A. Representative images of 2- and 10-m/o DP-Control and DP-cKO M1 motor cortex stained for TdTomato (*D1-Cre*) and NeuN.
B. Quantification of the percentage of NeuN+ cortical neurons expressing *D1-Cre* in 2-m/o mice (n=2-3 images per mouse, 3 mice/genotype, one-way ANOVA $F(5,42)=25$, Sidak's multiple comparisons test for within-region comparisons, p-values displayed on graph).
C. Quantification of the percentage of NeuN+ cortical neurons expressing *D1-Cre* in 10-m/o mice (n=2-3 images per mouse, 3 mice/genotype, one-way ANOVA $F(5,42)=19.51$, Sidak's multiple comparisons test for within-region comparisons, p-values displayed on graph).
D. Top: Western blot of HTT protein in lysates from adult IP-Control and IP-cKO striatum. Bottom: corresponding activated stain-free gel total protein loading control.
E. Top: Western blot of HTT protein in lysates from adult DP-Control and DP-cKO striatum. Bottom: corresponding activated stain-free gel total protein loading control.
F. Quantification of striatal HTT protein levels in cKOs compared to controls. Li-Cor fluorescence of each HTT band was normalized to total protein per lane, and relative HTT expression in cKOs was then normalized to their controls (unpaired two-way t-test, n=3-4 mice/genotype, IP-Control vs. IP-cKO: $t=3.124$, $df=5$, $p=0.0261$; DP-Control vs. DP-cKO: $t=2.85$, $df=4$, $p=0.0464$).
G. Top: Representative Western blot of HTT protein in lysates from adult DP-Control and DP-cKO cortex. Bottom: corresponding activated stain-free gel total protein loading control.
H. Quantification of cortical HTT protein levels in DP-cKOs compared to DP-Controls. Li-Cor fluorescence of each HTT band was normalized to total protein per lane, and relative HTT expression in DP-cKOs was then normalized to DP-Controls (unpaired two-way t-test, n=3 mice/genotype, samples run in duplicate, $t=3.017$, $df=10$, $p=0.0130$).

# Figure S2
# (Related to Figure 1)

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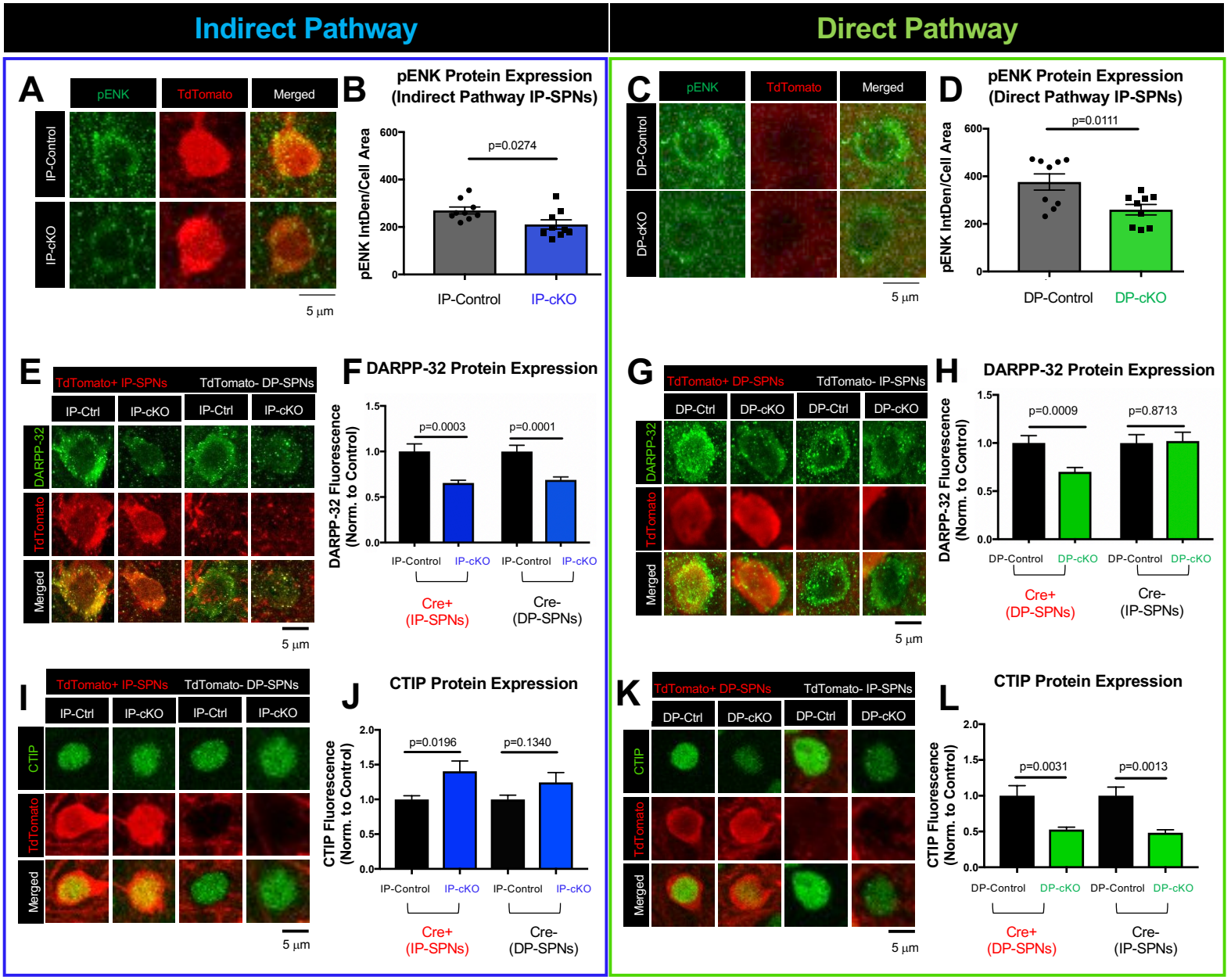

**Figure S2: *Htt* cKO alters SPN gene expression (Related to Figure 1).**

A. Representative images of pENK (green) expression within IP-SPNs (TdTomato+) from the dorsal striatum of 2-m/o IP-Control and IP-cKO mice.
B. pENK fluorescence intensity per cell was reduced in IP-cKO IP-SPNs compared to IP-Controls (n=3 mice/genotype, 3 images/mouse, unpaired two-way t-test t=2.428, df=16, p=0.0274).
C. Representative images of pENK expression (green) within IP-SPNs (TdTomato-) from the dorsal striatum of 2-m/o DP-Control and DP-cKO mice.
D. pENK fluorescence intensity was reduced in DP-cKO IP-SPNs compared to DP-Controls (n=3 mice/genotype, 3 images/mouse, unpaired two-way t-test t=2.872, df=16, p=0.0111).
E. Representative images of DARPP-32 (green) expression within Cre+ and Cre- SPNs from the dorsal striatum of 2-m/o IP-Control and IP-cKO mice.
F. DARPP-32 fluorescence intensity per SPN was reduced in both IP-cKO Cre+ IP-SPNs (n>22 cells per genotype, unpaired two-way t-test, t=3.91, df=54, p=0.0003) and neighboring Cre- DP-SPNs (n>22 cells per genotype, unpaired two-way t-test, t=4.214, df=44, p=0.0001) compared to IP-Controls.
G. Representative images of DARPP-32 (green) expression within Cre+ and Cre- SPNs from the dorsal striatum of 2-m/o DP-Control and DP-cKO mice.
H. DARPP-32 fluorescence intensity per SPN was reduced in DP-cKO Cre+ DP-SPNs versus DP-Control DP-SPNs (n>19 cells per condition, unpaired two-way t-test, t=3.574, df=44, p=0.0009), but not in DP-cKO Cre- IP-SPNs compared to DP-Control IP-SPNs (n>14 cells per genotype, unpaired two-way t-test, t=0.1633, df=31, p=0.8713).
I. Representative images of CTIP (green) expression within Cre+ and Cre- SPNs from the dorsal striatum of 2-m/o IP-Control and IP-cKO mice.
J. CTIP fluorescence intensity per SPN was increased in IP-cKO Cre+ IP-SPNs compared to IP-Control IP-SPNs, but was not significantly different in IP-cKO Cre- DP-SPNs compared to IP-Control DP-SPNs (n=3 mice/genotype, 3 images/mouse, one-way ANOVA with Sidak's multiple comparisons test, F(3,32)=3.266, p-values displayed above).
K. Representative images of CTIP (green) expression within Cre+ and Cre- SPNs from the dorsal striatum of 2-m/o DP-Control and DP-cKO mice.
L. CTIP fluorescence intensity per SPN was significantly reduced in both DP-cKO Cre+ DP-SPNs compared to DP-Control DP-SPNs and DP-cKO Cre- IP-SPNs compared to DP-Control IP-SPNs (n=3 mice/genotype, 3 images/mouse, one-way ANOVA with Sidak's multiple comparisons test, F(3,32)=8.783, p-values displayed above.)

# Figure S3 (Related to Figure 3)

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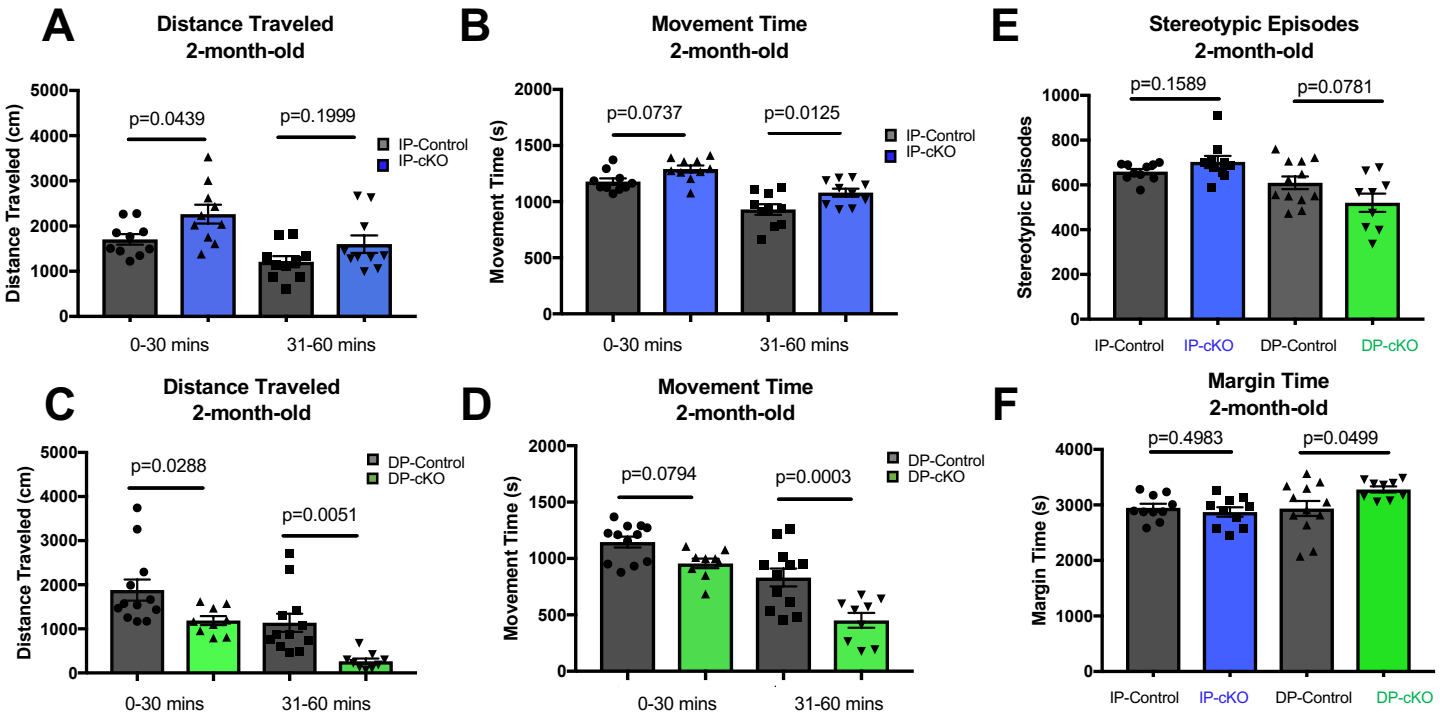


**Figure S3: Stereotypic episodes and margin time analysis of 2-m/o mice (Related to Figure 3)**

A. 2-m/o IP-cKOs traveled significantly farther than IP-Controls during the first 30 minutes of the Open Field Test (OFT), and displayed a trending increase in distance traveled during the last 30 minutes (one-way ANOVA $F(3,36)=6.902$, $p=0.0009$, Sidak's multiple comparisons test p-values displayed on graph).

B. 2-m/o IP-cKOs displayed a trending increase in movement time compared to IP-Controls during the first 30 minutes of the OFT, and moved significantly more than controls during the last 30 minutes (one-way ANOVA $F(3,36)=17.49$, $p<0.0001$, Sidak's multiple comparisons test p-values displayed on graph).

C. 2-m/o DP-cKOs traveled a significantly shorter distance than DP-Controls during the first 30 minutes as well as the last 30 minutes of the OFT, although this difference was more pronounced during the last 30 minutes (one-way ANOVA $F(3,38)=11.93$, $p<0.0001$, Sidak's multiple comparisons test p-values displayed on graph).

D. 2-m/o DP-cKOs had a trending decrease in movement time compared to DP-Controls during the first 30 minutes of the OFT, and moved significantly less than controls during the last 30 minutes of the OFT (one-way ANOVA $F(3,38)=20.77$, $p<0.0001$, Sidak's multiple comparisons test p-values displayed on graph).

E. Neither 2-m/o IP-cKOs nor DP-cKOs differed significantly from relevant controls on OFT stereotypic episodes, although DP-cKOs displayed a trending reduction in the number of episodes performed (IP-Control vs IP-cKO: n=10 animals/genotype, unpaired two-way t-test $t=1.47$, $df=18$, $p=0.1589$; DP-Control vs. DP-cKO: n=9-12 animals per genotype, unpaired two-way t-test $t=1.862$, $df=19$, $p=0.0781$).

F. 2-m/o IP-cKOs did not differ from IP-Controls on time spent in OFT margins (n=10 mice/genotype, unpaired two-way t-test $t=0.6912$, $df=18$, $p=0.4983$), whereas 2-m/o DP-cKOs spent more time in the OFT margins compared to DP-Controls (n=9-12 mice/genotype, unpaired two-way t-test $t=2.094$, $df=19$, $p=0.0499$).

# Figure S4 (Related to Figure 4) Burrus *et al.*

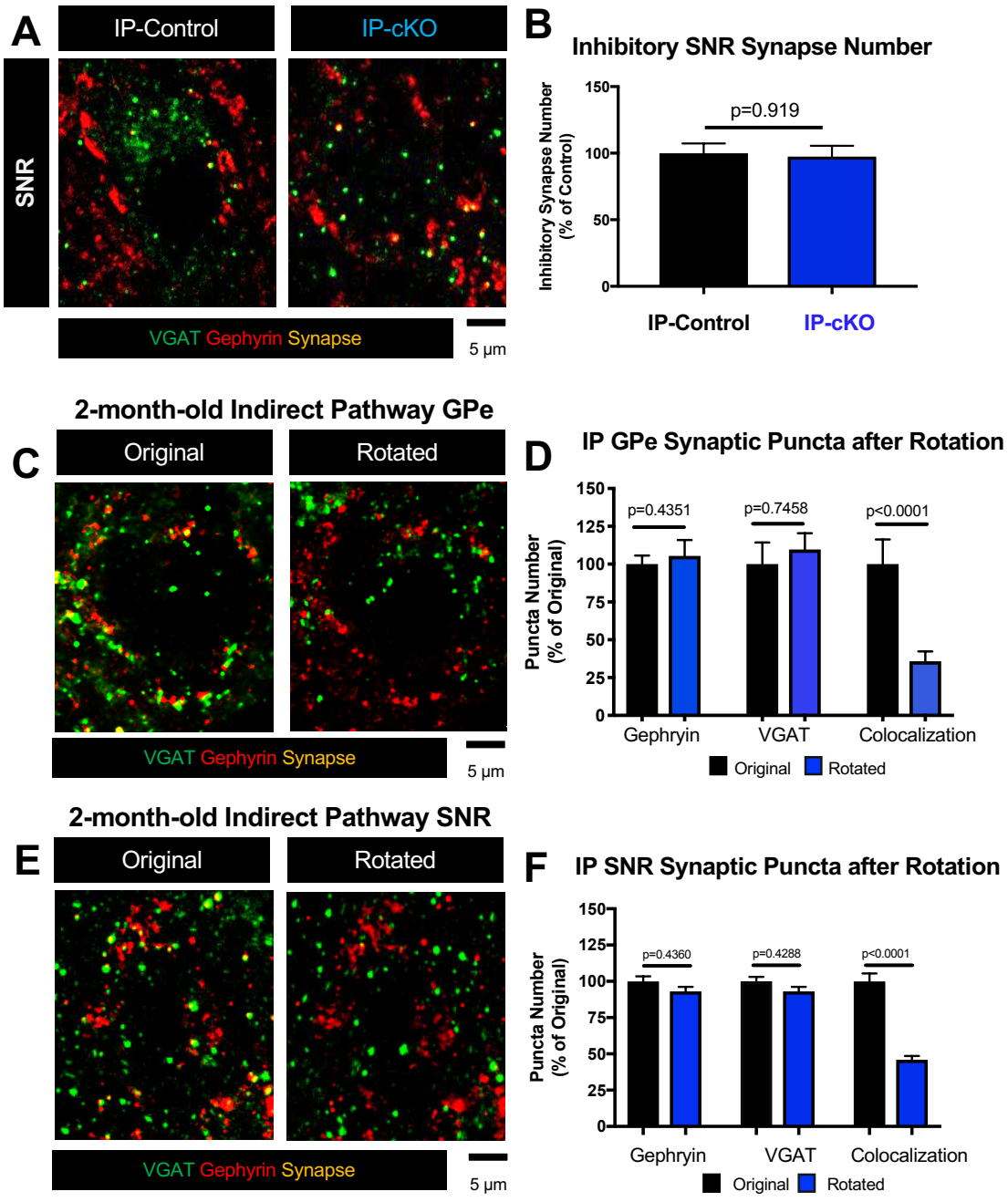

**Figure S4: Inhibitory synapse number in the SNR is unchanged by IP-cKO, and colocalization of VGAT and gephyrin puncta in GPe is not random (Related to Figure 4).**

A. Representative images of inhibitory synapses in the SNR of 2-m/o IP-Controls and IP-cKOs, stained for VGAT (green) and gephyrin (red).
B. Inhibitory synapse numbers are not changed in the SNR of 2-m/o IP-cKO mice compared to controls (n=3 replicates/mouse, 2 mice/genotype, Nested ANOVA by genotype $F(1,11)=0.01$, $p=0.919$).
C. Representative images of original and gephyrin-rotated images from 2-m/o IP-Control GPe. Puncta location was randomized by rotating gephyrin channel 90° relative to VGAT channel.
D. While gephyrin or VGAT puncta number do not differ, there is a significant reduction in the number of colocalized puncta in the rotated images compared to the originals (n = 3 replicates/mouse, 15 images/mouse, unpaired two-way t-tests, p-values displayed on graph).
E. Representative images of original and gephyrin-rotated 2-m/o IP-Control SNR images. Puncta location was randomized by rotating gephyrin channel 90° relative to VGAT channel.
F. Quantification of SNR rotated puncta number and colocalized synapses normalized to original images. The number of colocalized puncta is significantly reduced in the rotated images compared to the originals (original vs. rotated: n=3 replicates/mouse, 3 mice/condition, unpaired two-way t-test, p-values displayed on graph).

# Figure S5 (Related to Figure 5) Burrus *et al.*

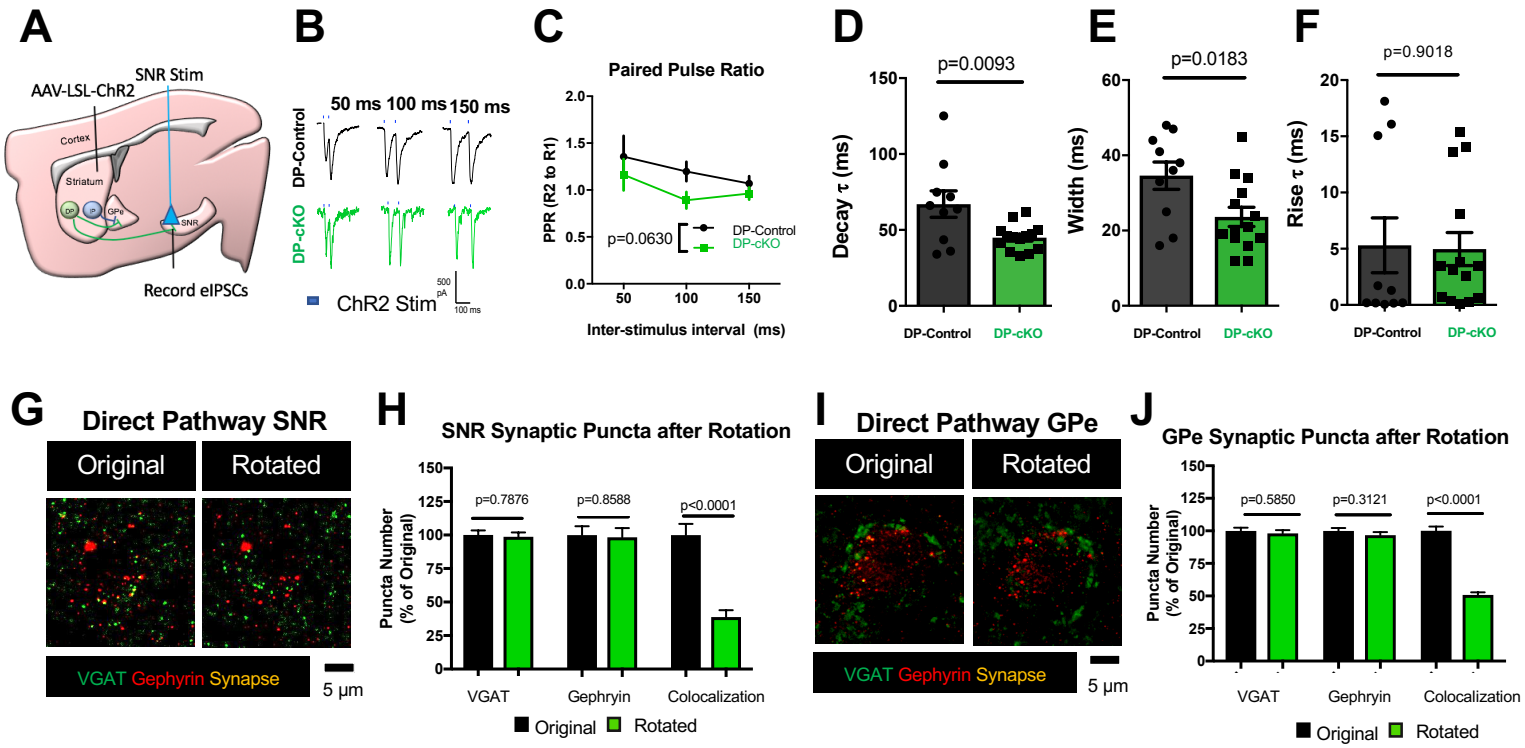


**Figure S5: DP-cKO alters the kinetics of synaptic transmission in the SNR (Related to Figure 5).**

A. Diagram of optogenetics experiment strategy. AAV-LoxStopLox-ChR2 virus was injected into the dorsal striatum of 2-m/o DP mice. After 3 weeks, sagittal acute sections containing the SNR were prepared and blue light bursts were applied to activate SNR terminals. eIPSCs were recorded from SNR neurons.

B. Representative traces of SNR eIPSCs produced in response to blue light stimulation.

C. Quantification of paired pulse ratio of SNR eIPSCs in response to blue light at 50 ms, 100 ms, or 150 ms intervals (two-way ANOVA, genotype effect $F(1,66)=3.577$, $p=0.0630$, Graph represents mean ± SEM).

D. Quantification of decay time (ms) of SNR eIPSCs (n=10-14 cells per genotype, unpaired two-way t-test, $p=0.0093$, $t=2.850$, $df=22$, Bar graph displays mean ± SEM, individual data points are overlaid).

E. Quantification of SNR eIPSC width (ms) (n=10-14 cells per genotype, unpaired two-way t-test, $p=0.0183$, $t=2.549$, $df=22$, Bar graph displays mean ± SEM, individual data points are overlaid).

F. Quantification of SNR eIPSC rise time (ms) (n=10-14 cells per genotype, unpaired two-way t-test, $p=0.9018$, $t=0.1249$, $df=22$, Bar graph displays mean ± SEM, individual data points are overlaid).

G. Representative images of original and gephyrin-rotated SNR images from the SNR of 2-m/o DP-Control mouse.

H. Quantification of SNR puncta number and colocalized puncta normalized to original images. Rotated images from DP-Control and DP-cKO SNR were analyzed (synapses original vs. rotated: n=1-2 replicates/mouse, 3 mice/condition, unpaired two-way t-test, $t=6.169$, $df=56$, $p<0.0001$).

I. Representative images of original and gephyrin-rotated GPe images from 2-m/o DP-Control mouse.

J. Quantification of GPe rotated puncta number and co-localized puncta normalized to original images. Rotated images from DP-Control and DP-cKO GPe were analyzed (synapses original vs. rotated: n=3 replicates/animal, 3 animals/condition, unpaired two-way t-test, $t=12.64$, $df=178$, $p<0.0001$).

# Figure S6 (Related to Figure 6) Burrus *et al.*

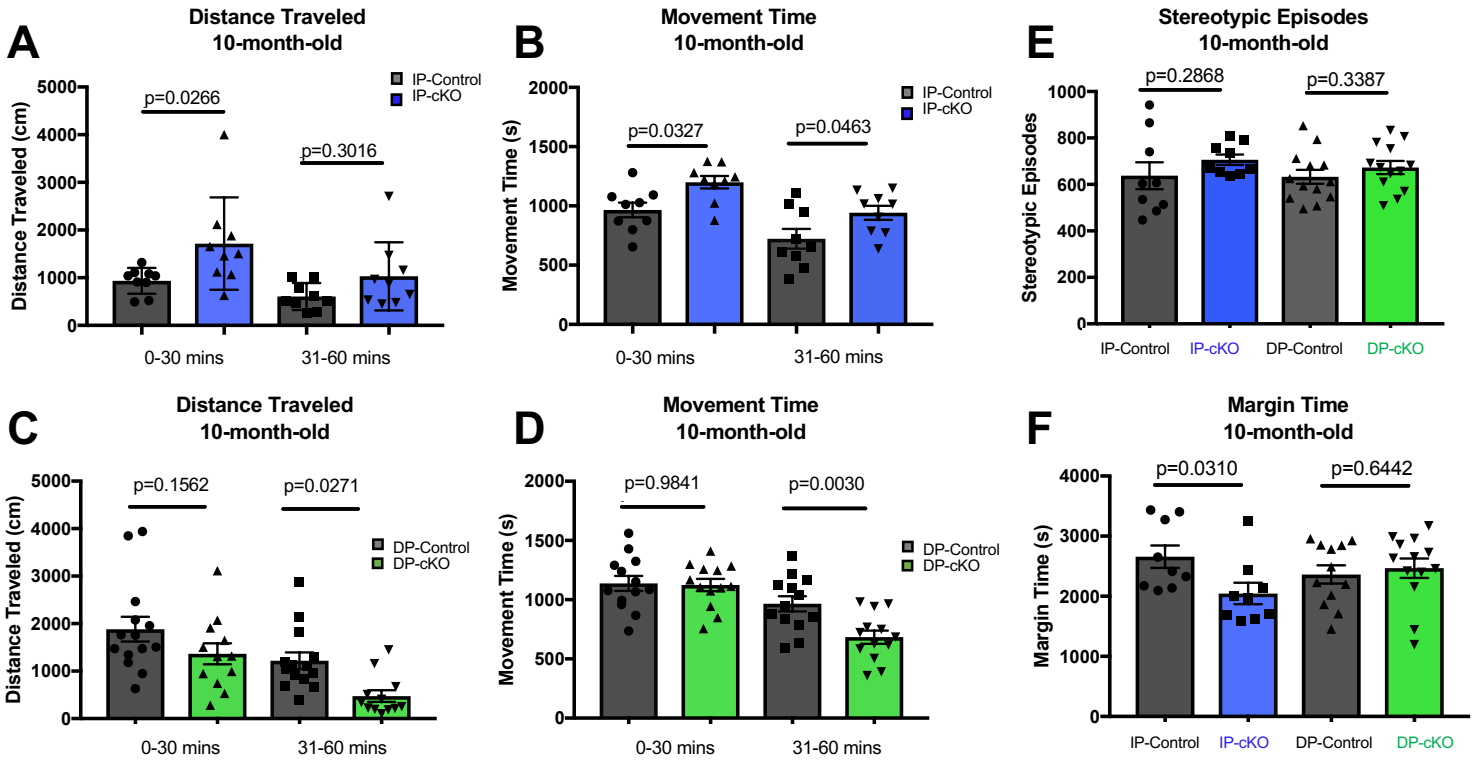


**Figure S6: Stereotypic episodes and margin time analysis of 10-m/o mice (Related to Figure 6).**

A. 10-m/o IP-cKOs traveled significantly farther than IP-Controls during the first 30 minutes of the open field test, but not during the last 30 minutes of the test (one-way ANOVA $F(3,32)=4.892$, $p=0.0065$, Sidak's multiple comparisons test p-values displayed on graph).

B. 10-m/o IP-cKOs spent more time moving than IP-Controls during the first 30 minutes and last 30 minutes of the open field test, (one-way ANOVA $F(3,32)=8.953$, $p=0.0002$, Sidak's multiple comparisons test p-values displayed on graph).

C. 10-m/o DP-cKOs traveled a significantly shorter distance compared to DP-Controls only during the final 30 minutes of the open field test (one-way ANOVA $F(3,48)=7.909$, $p=0.0002$, Sidak's multiple comparisons test p-values displayed on graph).

D. 10-m/o DP-cKOs spent significantly less time moving compared to DP-Controls only during the final 30 minutes of the open field test (one-way ANOVA $F(3,32)=12.76$, $p<0.0001$, Sidak's multiple comparisons test p-values displayed on graph).

E. 10-m/o IP-cKO and DP-cKO mice did not differ from their relevant controls on OFT stereotypic episodes (IP-Control vs IP-cKO: n=9 mice/genotype, unpaired two-way t-test $t=1.102$, $df=16$, $p=0.2868$; DP-Control vs. DP-cKO: n=13 mice/genotype, unpaired two-way t-test $t=0.9763$, $df=24$, $p=0.3387$).

F. 10-m/o IP-cKOs, but not DP-cKOs, spent less time in the OFT margins compared to relevant controls (n=9 mice/genotype, unpaired two-way t-test $t=2.366$, $df=16$, $p=0.0310$; DP-Control vs. DP-cKO: n=12-13 mice/genotype, unpaired two-way t-test $t=0.4680$, $df=23$, $p=0.6442$).

# Figure S7 (Related to Figure 7)

**Burrus *et al.***

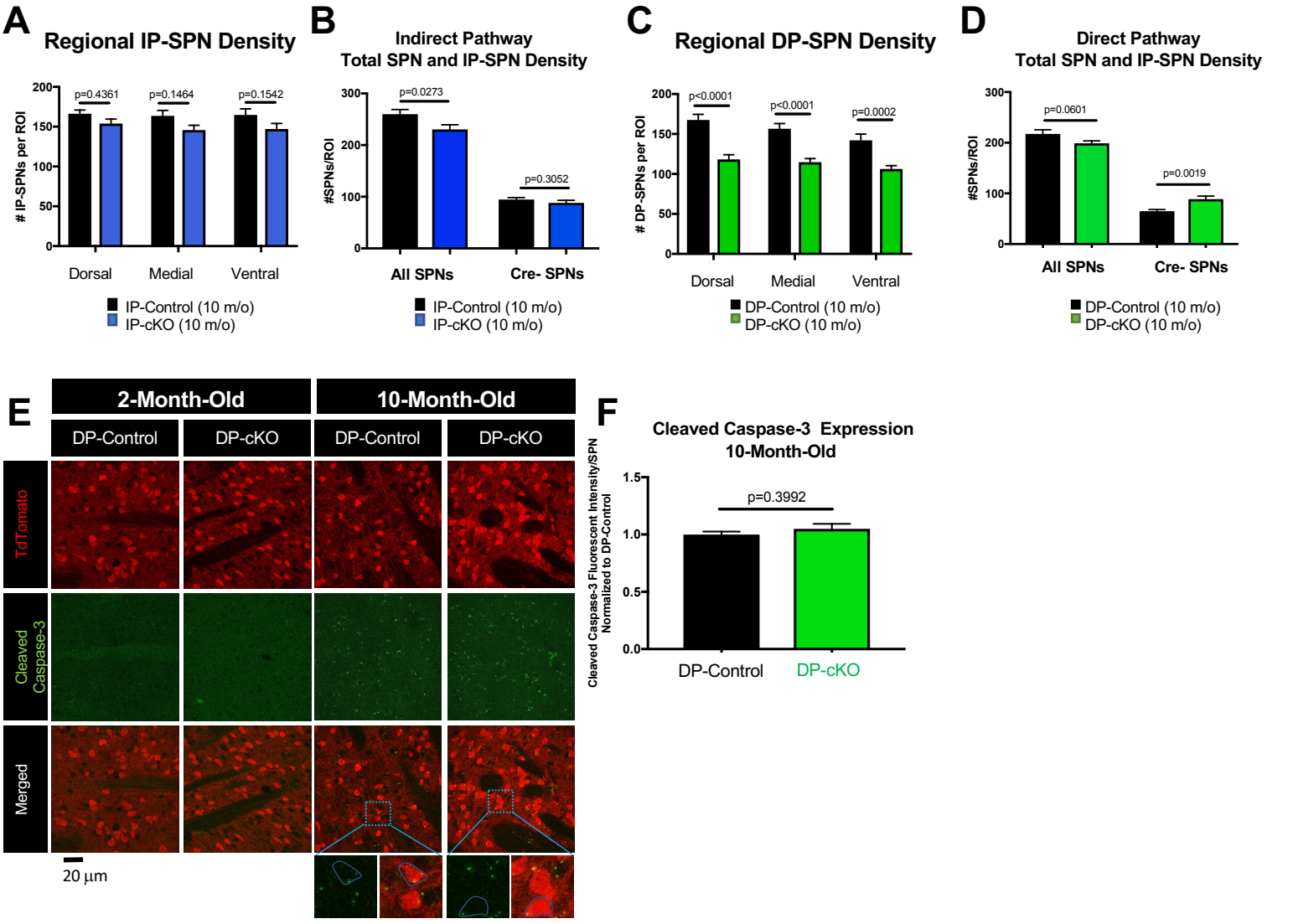


**Figure S7: Htt cKO does not enhance apoptosis but does lead to reactive gliosis (Related to Figure 7).**

A. There is no effect of region on IP-SPN density in 10-m/o IP-cKOs versus IP-Controls (n=6 images/animal, 3 animals/genotype, two-way ANOVA genotype x region interaction $F(2,102)=0.1160$, $p=0.8906$, Sidak's multiple comparisons test used to compare within region, p-values displayed above).

B. The total density of SPNs (DARPP-32+ cells) in IP-cKOs is reduced compared to controls (n=2 mice/genotype, 6 images/mouse, unpaired two-way t-test $t=2.307$, $df=34$, $p=0.0273$), but there is no difference in the density of Cre-/DARPP-32+ SPNs (n=2 mice/genotype, 6 images/mouse, unpaired two-way t-test $t=1.041$, $df=34$, $p=0.3052$).

C. There is no effect of region on DP-SPN density in 10-m/o DP-cKOs versus DP-Controls, although there is a significant reduction in DP-SPN density within each region (n=6 images/mouse, 3 mice/genotype, two-way ANOVA genotype x region interaction $F(2,102)=0.5959$, $p=0.5530$, Sidak's multiple comparisons test used to compare within region, p-values displayed above).

D. There is a trending reduction in the total density of SPNs (DARPP-32+ cells) in DP-cKOs compared to controls (n=2 mice/genotype, 6 images per mouse, unpaired two-way t-test $t=1.945$, $df=34$, $p=0.0601$), along with an increase in the density of Cre-/DARPP-32+ SPNs (n=2 mice/genotype, 6 image/mouse, unpaired two-way t-test $t=3.371$, $df=34$, $p=0.0019$).

E. Representative images of 2-m/o and 10-m/o DP-Control and DP-cKO dorsal striatum stained for TdTomato (red, DP-SPNs) and cleaved caspase-3 (green). Insets are provided to demonstrate the presence of cleaved caspase-3 puncta within 10-m/o DP-SPNs of both genotypes.

F. There is no difference in the expression of cleaved caspase-3 (integrated density/SPN area) within DP-SPNs of 10-m/o DP-cKO mice compared to controls (n= 2-3 mice/genotype, 6 images/mouse, unpaired two-way t-test $t=0.8561$, $df=28$, $p=0.3992$).