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Supplemental information

Loss of Tsc1 from striatal direct pathway neurons impairs endocannabinoid-LTD and enhances motor routine learning

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Supplemental Figure 1

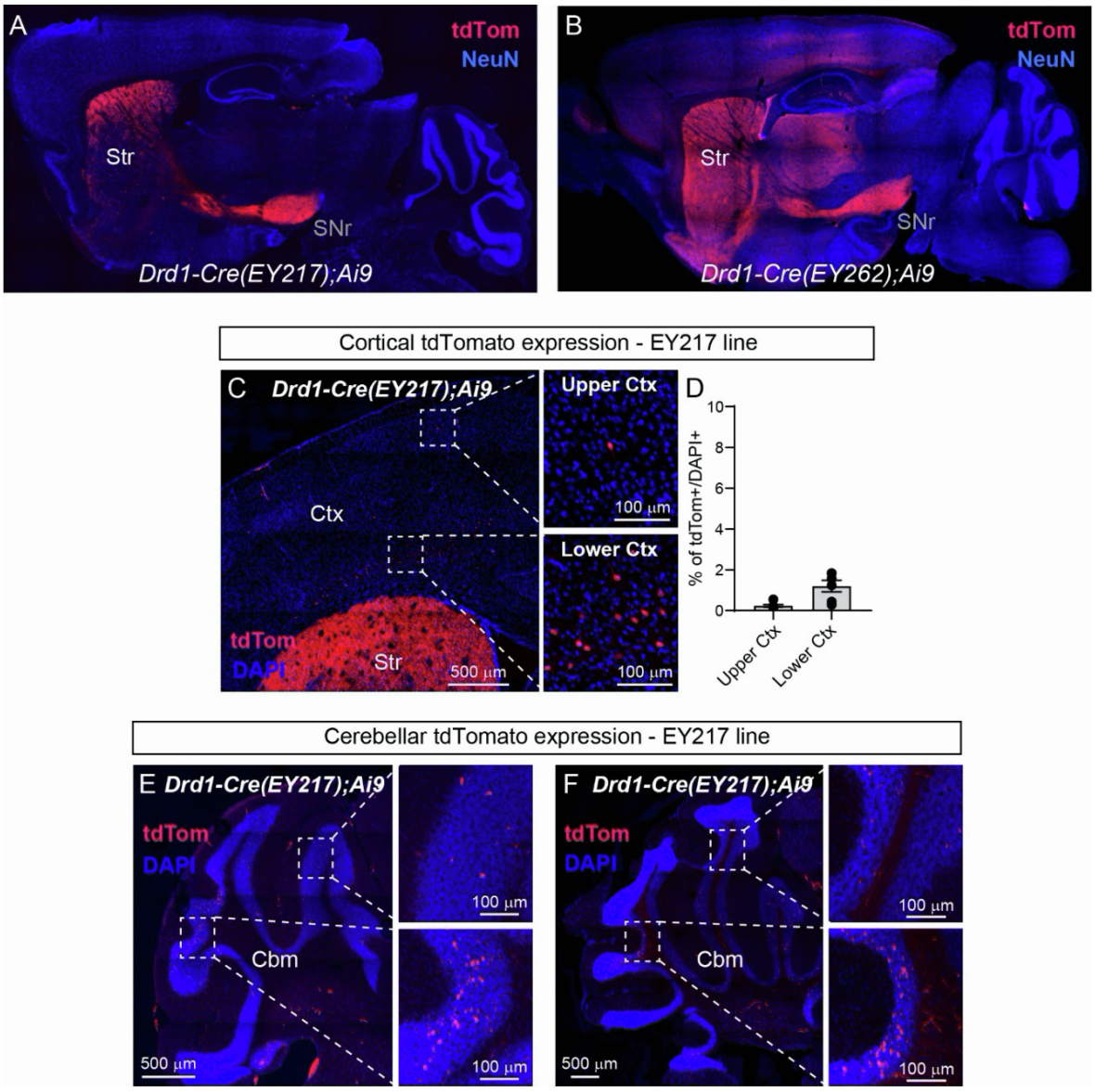

Figure S1. Cre-mediated recombination in *Drd1-Cre;Ai9* mice. Related to Figure 1.

(A-B) Images of sagittal brain sections showing the Cre-dependent tdTomato (red) expression pattern of a *Drd1-Cre(EY217);Ai9* mouse (A) and a *Drd1-Cre(EY262);Ai9* mouse (B). NeuN immunostaining (blue) labels neurons. Note the striatal-restricted tdTomato expression in the EY217 line with axon terminals in the SNr. Str=striatum, SNr=substantia nigra pars reticulata.
(C) Left panel shows an image of the motor cortex (Ctx) and dorsal striatum (Str) with tdTomato (red) expression in a sparse population of cortical cells. DAPI labeled nuclei are in blue. Right panels show higher magnification images of the boxed regions in the left panel.
(D) Quantification (mean +/- SEM) of the percentage of tdTomato+ cells/DAPI+ cells in the upper and lower layers of the cortex in *Drd1-Cre(EY217);Ai9* mice (n=2 sections from 3 mice).
(E,F) Images of tdTomato expression in a sparse population of cells in the cerebellar granule cell layer. Examples from two different mice are shown. Cbm=cerebellum. Right panels show higher magnification images of the boxed regions.

Supplemental Figure 2

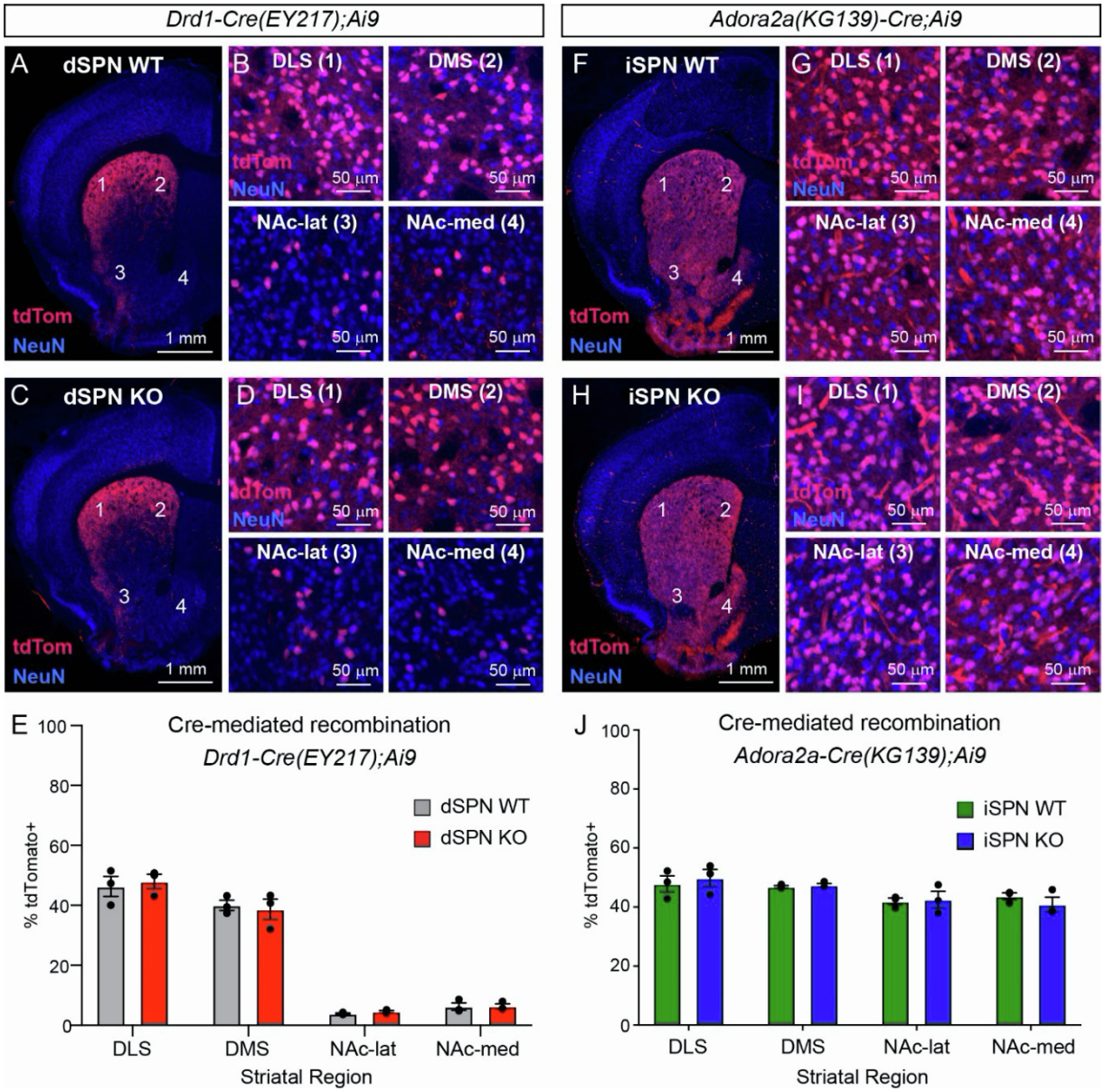

Figure S2. Cre-mediated recombination in the striatum of *Drd1-Cre;Ai9* and *Adora2a-Cre;Ai9* mice. Related to Figure 1.

(A-D) Confocal images of coronal brain sections showing Cre-dependent tdTomato expression (red) in a *Tsc1*$^{wt/wt}$*;Drd1-Cre*$^{+}$*;Ai9*$^{+}$ mouse (A,B) and a *Tsc1*$^{fl/fl}$*;Drd1-Cre*$^{+}$*;Ai9*$^{+}$ mouse (C,D). NeuN immunostaining is in blue. Higher magnification images (B,D) show region-specific expression patterns in the dorsolateral striatum (DLS, site #1), dorsomedial striatum (DMS, site #2), nucleus accumbens lateral shell (NAc-lat, site #3), and nucleus accumbens medial shell (NAc-med, site #4).

(E) Quantification of tdTomato regional expression patterns in *Tsc1*$^{wt/wt}$*;Drd1-Cre*$^{+}$*;Ai9*$^{+}$ and *Tsc1*$^{fl/fl}$*;Drd1-Cre*$^{+}$*;Ai9*$^{+}$ mice. Bars represent mean +/- SEM and dots represent individual mice. Shown is the percentage of NeuN+ cells that are tdTomato+ in a given striatal region. n=3 mice per genotype.

(F-I) Confocal images of coronal brain sections showing Cre-dependent tdTomato expression (red) in a *Tsc1*$^{wt/wt}$*;Adora2a-Cre*$^{+}$*;Ai9*$^{+}$ mouse (F,G) and a *Tsc1*$^{fl/fl}$*;Adora2a-Cre*$^{+}$*;Ai9*$^{+}$ mouse (H,I). Higher magnification images (G,I) show region- specific expression patterns.
(J) Quantification of tdTomato regional expression patterns in *Tsc1*$^{wt/wt}$*;Adora2a-Cre*$^{+}$*;Ai9*$^{+}$ and *Tsc1*$^{fl/fl}$*;Adora2a-Cre*$^{+}$*;Ai9*$^{+}$ mice. Bars represent mean +/- SEM and dots represent individual mice. Shown is the percentage of NeuN+ cells that are tdTomato+ in a given striatal region. n=3 mice per genotype.

Supplemental Figure 3

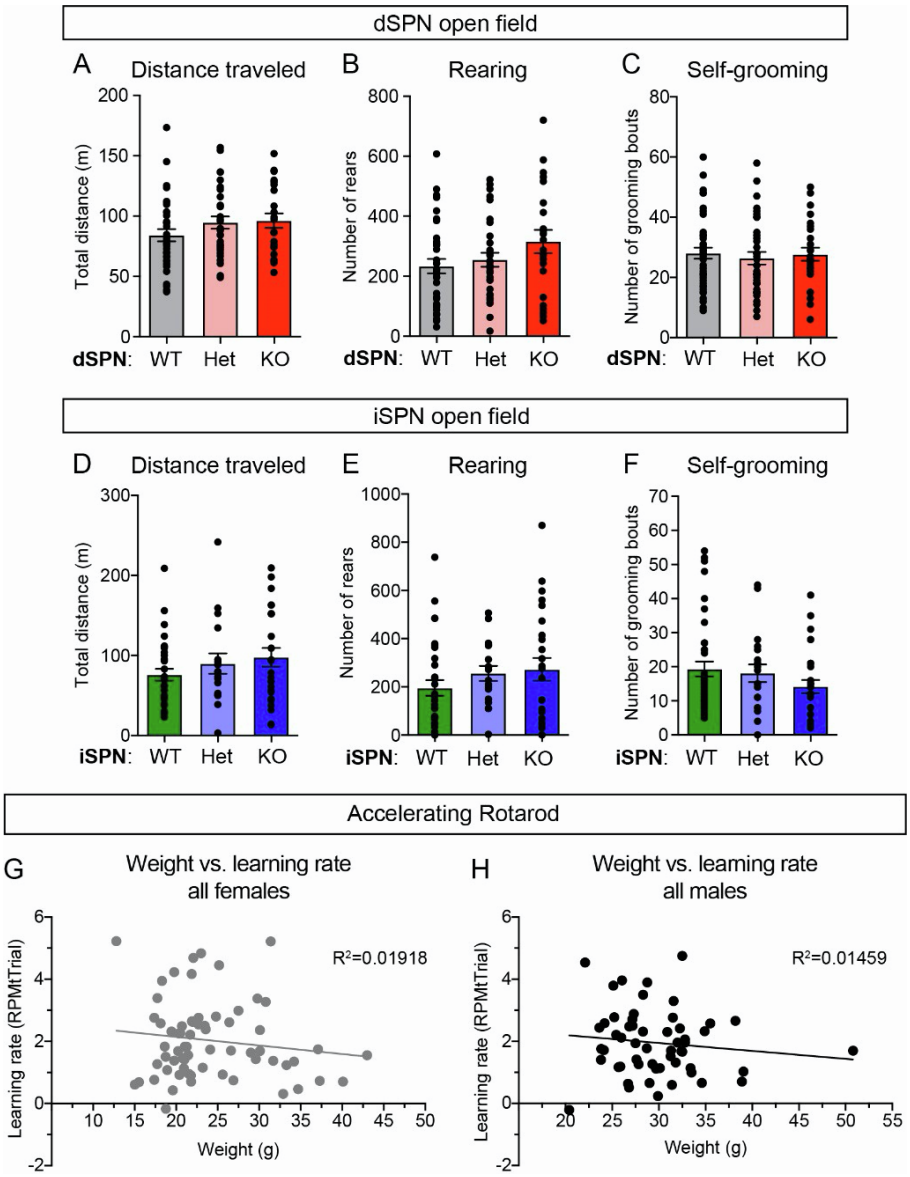

Figure S3. Open field behavior results and additional rotarod analysis. Related to Figure 2.

(A-C) Quantification of open field parameters in dSPN Tsc1-WT, Het, and KO mice. Distance traveled (A) is the total distance traveled in the open field over 60 minutes. Rearing (B) is the number of rears in 60 minutes. Self-grooming (C) is the number of grooming bouts in the first 20 minutes of the open field test. Bars represent mean +/- SEM. Dots represent individual mice. For panels A and B, n=36 dSPN-WT, 32 dSPN-Het, and 23 dSPN-KO mice. For panel C, n=44 dSPN-WT, 37 dSPN-Het, and 26 dSPN-KO mice. For panel A, p=0.2006, Kruskal-Wallis test; panel B, p=0.1252, F (2, 88)=2.128, one-way ANOVA; panel C, p=0.8112, F (2, 104)=0.2097, one-way ANOVA.
(D-F) Quantification of open field parameters in iSPN Tsc1-WT, Het, and KO mice. Bars represent mean +/- SEM. Dots represent individual mice. For panels D and E, n=32 iSPN-WT, 18 iSPN-Het, and 25 iSPN-KO mice. For panel F, n=40 iSPN-WT, 20 iSPN-Het, and 29 iSPN-KO mice. For panel D, p=0.4028, Kruskal-Wallis test; panel E, p=0.2367, Kruskal-Wallis test; panel F, p=0.2497, Kruskal-Wallis test.

(G-H) Graphs show the relationship between weight in grams (g) and overall learning rate on the rotarod test for individual female (G) and male (H) mice of all genotypes. Learning rate was calculated as the slope of the line of performance from trial 1 to trial 12. There was not a significant relationship between weight and rotarod learning rate for females (Linear regression analysis, $p=0.2674$, $F(1, 64)=1.252$, $n=66$ mice) or males (Linear regression analysis, $p=0.3796$, $F(1, 53)=0.7849$, $n=55$ mice). Mice from all strains (dSPN-Tsc1, iSPN-Tsc1, and Tsc2) and genotypes were pooled for this analysis.

Supplemental Figure 4

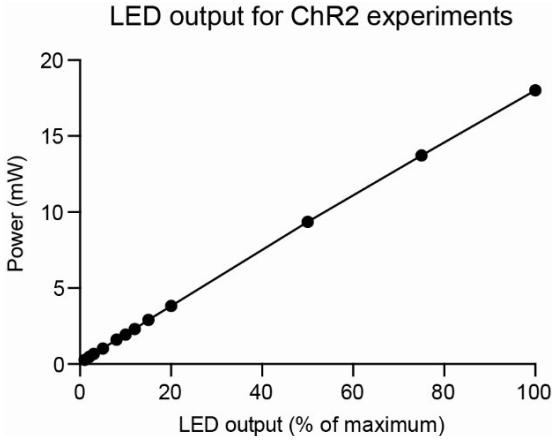

Figure S4. Light power plotted as a function of LED output. Related to Figure 3.

470 nm light power measured under the microscope objective showed a linear relationship with LED output.

Supplemental Figure 5

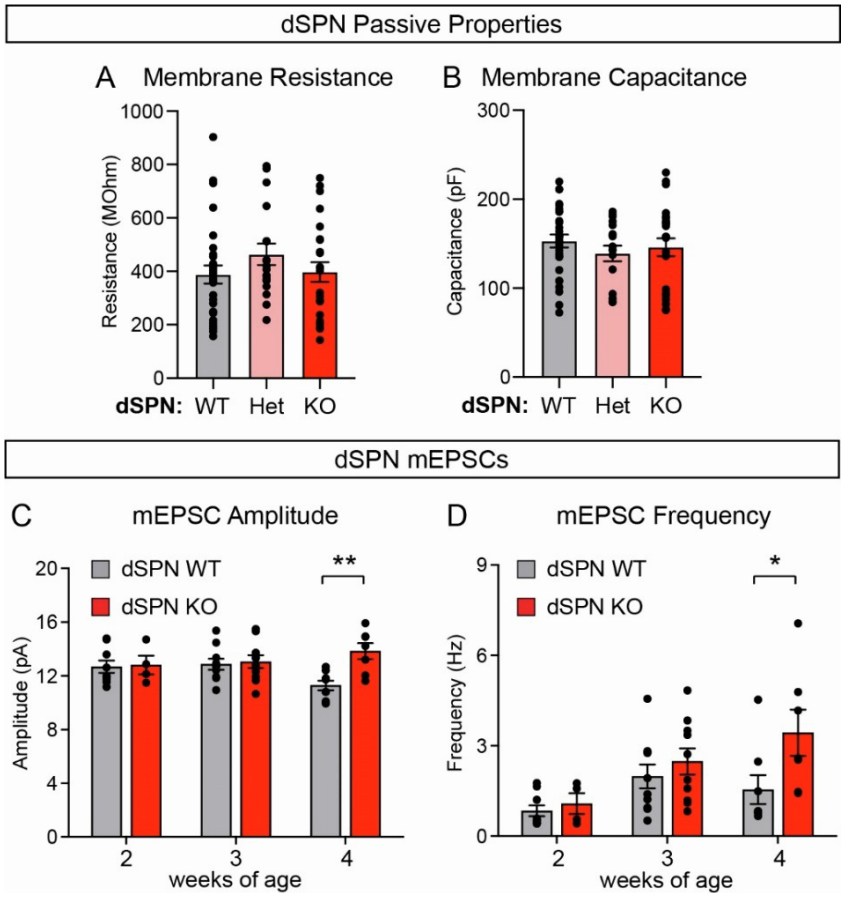

Figure S5. dSPN passive membrane properties and mEPSCs across early postnatal development. Related to Figures 3 and 4.

(A-B) Mean +/- SEM membrane resistance (Rm, A) and capacitance (Cm, B) of Tsc1-WT, Het and KO dSPNs from voltage clamp recordings. Dots represent values for individual neurons. dSPN WT n=29 neurons from 10 mice, dSPN Het n=18 neurons from 6 mice, dSPN KO n=24 neurons from 9 mice. Rm, p=0.2585, Kruskal-Wallis test; Cm, p=0.6018, Kruskal-Wallis test.
(C-D) Mean +/- SEM mEPSC amplitude (C) and frequency (D) recorded from dSPN Tsc1-WT and KO neurons at 2, 3 or 4 weeks of age. Dots represent values for individual neurons. Two weeks: n=9 dSPN WT neurons from 1 mouse and 4 dSPN KO neurons from 1 mouse. Three weeks: n=10 dSPN WT neurons from 2 mice and 10 dSPN KO neurons from 2 mice. Four weeks: n=8 dSPN WT neurons from 2 mice and 7 dSPN KO neurons from 2 mice. Comparisons were made between dSPN-WT and KO neurons at each developmental age. For panel C, 2 weeks, p=0.8252, Mann-Whitney test, 3 weeks, p=0.7741, unpaired t-test; 4 weeks, **, p=0.0093, Mann-Whitney test. For panel D; 2 weeks, p=0.7105, Mann-Whitney test; 3 weeks, p=0.4045, unpaired t-test; 4 weeks, *, p=0.0289, Mann-Whitney test.

Supplemental Figure 6

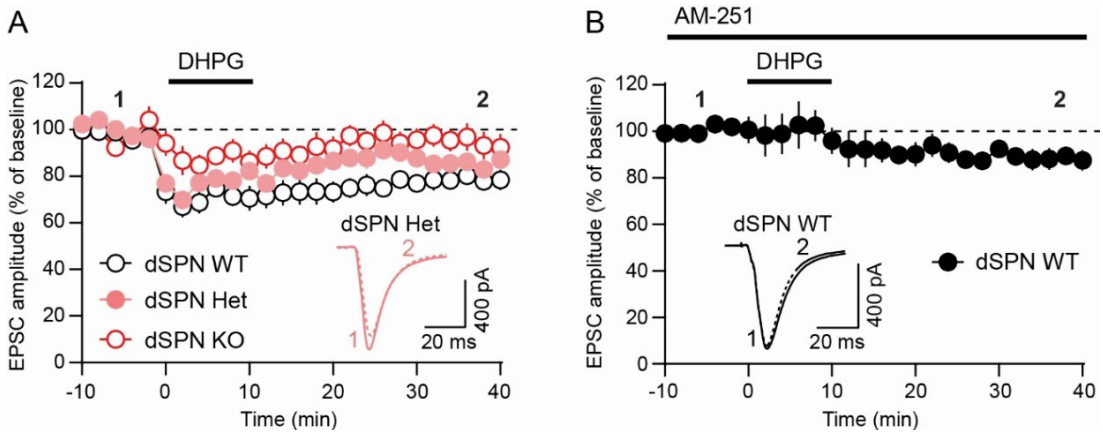

Figure S6. eCB-LTD in dSPNs. Related to Figure 5.

(A) Cortical terminals were stimulated with blue light (30s ISI) in striatal slices from *Tsc1*$^{wt/fl}$*;D1-Cre*$^{+}$*;Thy1-ChR2*$^{+}$*;Ai9*$^{+/-}$ mice to evoke EPSCs in dSPNs. eCB-LTD was induced by 10-minute bath application of the mGluR1/5 agonist DHPG (100 µM). EPSC amplitude was monitored and expressed as a percent of baseline levels and plotted versus time (mean +/- SEM). n=9 dSPN Tsc1-Het neurons from 7 mice. For reference, the dSPN WT and KO data are replotted from the analysis in Fig. 5A (open circles). Dashed line indicates 100% of baseline. Example traces show the average EPSC from the baseline period ("1", solid line) and 35-40 minutes after DHPG application ("2", dashed line) for a representative dSPN Tsc1-Het neuron.
(B) Cortical terminals were stimulated with blue light (30s ISI) in striatal slices from *Tsc1*$^{wt/wt}$*;D1-Cre*$^{+}$*;Thy1-ChR2*$^{+}$*;Ai9*$^{+/-}$ mice to evoke EPSCs in Tsc1 WT dSPNs. The CB1 receptor antagonist AM-251 (10 µM) was bath applied throughout the recording. Following a 10-minute baseline period, DHPG (100 µM) was washed on for 10 minutes. EPSC amplitude was monitored and expressed as a percent of baseline levels and plotted versus time (mean +/- SEM). n=5 neurons from 5 mice. Example traces show the average EPSC from the baseline period ("1",solid line) and 35-40 minutes after DHPG application ("2", dashed line) for a representative dSPN Tsc1-WT neuron.