Supplemental Information

Rescuing Over-activated Microglia Restores Cognitive Performance in Juvenile Animals of the Dp(16) Mouse Model of Down Syndrome

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Figure S1. Related to Figure 1. Microglial morphology is altered in the somatosensory cortex of Dp(16) mice and is impacted by APAP treatment.

(A) Experimental protocol for APAP treatment and analysis of the morphology of microglia in the somatosensory cortex (highlighted by the dotted lines).

(B) Iba1-stained somatosensory cortices from P22 WT and Dp(16) animals treated with either vehicle or APAP. Scale bar: 20μm.

(C) Quantification of the cell body area of microglial cells. Bars represent the average of microglial cell body areas in all analyzed animals ± SEM and circles represent the single data points of the cell averages for each animal (72-126 cells/animal; 1 slice per animal). ** p<0.01, *** p<0.001; Two-Way ANOVA, F_{Interaction} (1, 19) = 13.19, p = 0.0018; Holm-Sidak post-hoc test.

(D) Binary images from fields shown in B. Scale bar: 20μm.

(E) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. Numbers in parenthesis: analyzed cells (227 vs 326), and animals (9 vs 11) for 29 different radius points (1 slice per animal). *** p<0.001; Repeated measure Two-Way ANOVA, F_{Interaction} (33, 7425) = 3.903, Holm-Sidak post-hoc test.

(F) Quantification of the average number of intersections. Bars represent the average number of intersections in all analyzed animals ± SEM, and circles represent single data points of the average number of intersections for each animal (20-41 cells/animal; 34 different 1 slice per animal). p= 0.9553; Mann-Whitney test, U=48.50.

(G) Representative skeleton images of binary images. Scale bar: 20μm.

(H) Quantification of the number of branches per microglial cell. Bars represent the average number of branches per microglial cell ± SEM in all analyzed animals, and circles represent single data points of the cell averages for each animal (20-41 cells/animal; 1 slice per animal). p= 0.4119; Mann-Whitney test, U=38.

(I) Quantification of the average length of branches per microglial cell. Bars represent the average of branch length per microglial cell ± SEM in all analyzed animals, and circles represent single data points of the cell averages for each animal (20-41 cells/animal; 1 slice per animal). ** p=0.0068; Mann-Whitney test, U=12.
Figure S2. Related to Figure 1-2. Hippocampi show increased levels of Iba1 and CD68 and decreased levels of P2Y12 in Dp(16) mice.

(A) MHCII-stained (red) hippocampal slice from P22 WT and Dp(16) animals showing no immunoreactivity. In the negative control, the primary anti-MHCII antibody was omitted. Animals treated with 1mg/kg of LPS for 3 days were used as a positive control. Scale bar: 10μm.

(B) Immunoblots on hippocampal protein lysates from P22 WT and Dp(16) mice (top). Quantification of Iba1 protein levels normalized to GADPH immunoreactivity (bottom) in WT and Dp(16) treated with vehicle or APAP. Bars represent the average percentage of Iba1 levels in Dp(16) over WT hippocampi for all the analyzed animals ± SEM, and circles represent single data points for each animal. ** p<0.01; Unpaired Two-tailed Student’s t-test, t=3.21, df=28.

(C) Microglia GFP (green) and P2Y12 (red)-stained hippocampal slices from P22 WT CX3CR1-GFP and Dp(16)CX3CR1-GFP animals. Scale bar: 20μm.

(D) Quantification of the P2Y12+ area normalized to GFP+ area of microglial cells. Bars represent the average P2Y12/GFP area for all analyzed animals ± SEM, and circles represent the average of all analyzed cells for each animal (4-9 cells/animal; 1 slice per animal). ** p=0.0049; Unpaired Two-tailed Student’s t-test, t=4.33, df=6).

(E) Iba1 (green) and CD68 (red)-stained hippocampal slices from P22 WT and Dp(16) animals. Animals treated with 1mg/kg of LPS for 3 days were used as a positive control. Scale bar: 20μm.

(F) Quantification of the CD68+ area normalized to Iba1+ area for microglial cells. Bars represent the average CD68/Iba1 area for all analyzed animals ± SEM, and circles represent the average of all analyzed cells for each animal (20 cells/animal; 1 slice per animal). * p<0.05, *** p<0.001; Two-Way ANOVA, F_{interaction} (1,16) = 10.95, p=0.0044; Holm-Sidak post-hoc test.

(G) Iba1 (green) and Lamp1 (red)-stained hippocampal slices from P22 WT and Dp(16) animals. Scale bar: 10μm.

(H) Quantification of the Lamp1+ area normalized on Iba1+ area for microglial cell. Bars represent the average Lamp1/Iba1 area for all analyzed animals ± SEM, and circles represent the average for all analyzed cells for each animal (4-9 cells/animal; 1 slice per animal). ** p=0.0038; Unpaired Two-tailed Student’s t-test, t=3.87, df=9).
Figure S3. Related to Figure 1. Cytokine levels are dysregulated and rescued by APAP treatment in Dp(16) mice.

(A) Representative cytokine arrays performed on hippocampal lysates obtained from P22 WT and Dp(16) animals. The dashed circles show the cytokines significantly dysregulated between the two groups.

(B) Quantification of the fold change of all the cytokines probed in vehicle-treated Dp(16) animals hippocampi when compared to vehicle-treated WT animals. Bars represent the average percentage of cytokine levels in Dp(16) over WT hippocampi of all independent experiments ± SEM, and circles represent single data points of each independent experiment (1 animal per experiment). Statistically significant cytokines (as indicated in A) are highlighted in red. * p<0.05, ** p<0.01, *** p<0.001; Independent One-Sample t-test against 0.

(C) Quantification of the fold change of all the cytokines probed in APAP-treated Dp(16) animals hippocampi when compared to vehicle-treated WT animals. Bars represent the average percentage of cytokine levels in APAP-treated Dp(16) over WT hippocampi of all independent experiments ± SEM, and circles represent single data points of each independent experiment (1 animal per experiment). Statistically significant cytokines are highlighted in red. * p<0.05; Independent One-Sample t-test against 0.

(D) Quantification of the fold change of all the cytokines probed in APAP-treated WT animals hippocampi when compared to vehicle-treated WT animals. Bars represent the average percentage of cytokine levels in APAP-treated WT over vehicle-treated WT animals of all independent experiments ± SEM, and circles represent single data points of each independent experiment (1 animal per experiment).
Figure S4. Related to Figure 2 and 6. The proteome of microglia is altered in Dp(16) mice.

(A) Gene ontology terms on biological processes that are significantly altered in the microglia of Dp(16) vs WT vehicle-treated mice of the same experiments as in Figure 6B. A p-value threshold of 0.05 was applied. Written in red are biological processes related to the immune system. The numbers on top of the histograms indicate the number of dysregulated proteins that generate the difference.

(B) Gene ontology terms on biological processes that are significantly altered in the dark-red cluster from experiments in Figure 6C. A p-value threshold of 0.05 was applied. The numbers on top of the histograms indicate the number of dysregulated proteins that generate the difference.

(C) Gene ontology terms on molecular functions that are significantly altered in the orange cluster from experiments in Figure 6C. A p-value threshold of 0.05 was applied. The numbers on top of the histograms indicate the number of dysregulated proteins that generate the difference.
Figure S5. Related to Figure 2. Proteomic analysis reveals alteration of biological processes related to learning and memory and Drebrin protein levels in the hippocampus of Dp(16) mice.

(A) Volcano plot depicting all the proteins that were identified in the hippocampi of P22 WT and Dp(16) mice by proteomic analysis. The red squares represent the proteins that are significantly downregulated (Student’s t test difference < 0) or upregulated (Student’s t test difference > 0) in the hippocampus of Dp(16) compared to WT mice. The data were collected from 5 animals for each group.

(B) Gene ontology terms on biological processes that are significantly altered in the hippocampi of Dp(16) vs. WT mice in the experiment in A. A p-value threshold of 0.05 was applied. The numbers on top of the histograms indicate the number of dysregulated proteins that generate the difference.

(C) Heat-map (color-coded on the left) from proteomic experiments in Dp(16) and WT mice of the peak area normalized to the maximum peak of Drebrin for the four peptides that can be ascribed to Drebrin (vertical columns). The data were collected from 7 animals per genotype (horizontal lines).

(D) Quantification of the normalized peak area for each animal from experiments in C. For each animal, the reads from each peptide were summed together. Bars represent the average sum of the peak area of all analyzed animals ± SEM, and circles represent single data points for each animal **p<0.01; Unpaired Two-tailed Student’s t-test, t=3.37, df=12.
Figure S6. Related to Figure 3. PLX3397 or APAP treatment rescues specific types of dendritic spines and in Dp(16) mice.

(A) Cartoon of immature, filopodia spines (top). Quantification of the filopodia spine density (bottom) in P22 WT and Dp16 animals treated with vehicle, PLX3397, or APAP from experiments in Figures 3B, 4E. Bars represent the average spine density of all analyzed cells ± SEM, and symbols represent single data points for each cell. p=0.431; Two-Way ANOVA, $F_{\text{Interaction}}(2, 78) = 0.949$ p = 0.431. Data were collected from 3-5 neurons from 3 different animals per condition.

(B) Cartoon of thin and stubby spines (top). Quantification of the thin or stubby spine density (bottom) in P22 WT and Dp16 animals treated with vehicle, PLX3397, or APAP from experiments in Figures 3B, 4E. Bars represent the average spine density of all analyzed cells ± SEM, and symbols represent single data points for each cell. For thin spines: ** p<0.01, *** p<0.001; Two-Way ANOVA $F_{\text{Treatment}}(2, 79) = 15.64$, p<0.0001. For stubby spines: **p<0.01; Two-Way ANOVA, $F_{\text{Treatment}}(2, 79) = 18.98$, p<0.0001; Holm-Sidak post-hoc test. Data were collected from 3-5 neurons from 3 different animals per condition.

(C) Cartoon of mushroom spines (top). Quantification of the mushroom spine density (bottom) in P22 WT and Dp16 animals treated with vehicle, PLX3397, or APAP from experiments in Figures 3B, 4E. Bars represent the average spine density of all analyzed cells ± SEM, and symbols represent single data points for each cell. * p<0.05, ** p<0.01; Two-Way ANOVA, $F_{\text{Interaction}}(2, 79) = 6.915$, p = 0.0017; Holm-Sidak post-hoc test. Data were collected from 3-5 neurons from 3 different animals per condition.

(D) Iba1 (green), Psd-95 (red)-stained and Vglut1 (blue)-stained of hippocampal slices from P22 WT and Dp(16) animals treated with either vehicle or APAP. Scale bar: 5μm.

(Е-Ф) Quantification of engulfed Psd-95 or Vglut1 puncta in Iba1 labelled microglia of P22 WT and Dp(16) animals treated with either vehicle or APAP and normalized to the volume of the microglia. Bars represent the average for all analyzed animals ± SEM, and symbols represent the average of all analyzed microglia for each animal (10-20 cells/animal; 1 slice per animal). **** p<0.0001; (F) Two-Way ANOVA, $F_{\text{Interaction}}(1,26) = 26.23$, p<0.0001; Holm-Sidak post-hoc test. (G) Two-Way ANOVA, $F_{\text{Interaction}}(1,26) = 29.47$, p<0.0001; Holm-Sidak post-hoc test. The vehicle-treated animal data (dotted circles) are from Figure 3H-J as comparison.
A

APAP Vehicle

GFP Cox2 Merge GFP Cox2 Merge

WT Dp(16)

B

Cox2/GFP(% of area)

**** ****

Vehicle APAP

WT Dp(16)

C

APAP Vehicle

lba1 PGE2 Merge GFP PGE2 Merge

WT Dp(16)

D

PGE2/lba1(% of area)

* ***

Vehicle APAP

WT Dp(16)

E

Acetaminophen (APAP) 100mg/kg

Slice Processing & Electrophysiology

P0 P20 P21 P22

Birth

F

WT Dp(16)

Vehicle APAP

G

WT Vehicle (n=126,7) Dp(16) APAP (n=62,4)

Intersections (#)

H

WT Vehicle (n=126,7) WT APAP (n=65,4)

Distance From Cell Body (μm)

J

Vehicle APAP

K

Frequency (Hz)

**** ***

Vehicle APAP

WT Dp(16)

Amplitude (pA)

** ***

Vehicle APAP

WT Dp(16)
Figure S7. Related to Figure 4. APAP treatment rescues increase of COX2, PGE2, microglial morphology and neuronal synaptic-activity in Dp(16) mice.

(A) Microglia GFP+ (green) and Cox2 (red)-stained hippocampal slices from P22 WT CX3CR1-GFP and Dp(16) CX3CR1-GFP animals. Scale bar: 50μm.

(B) Quantification of the Cox2+ area normalized to GFP+ area of microglial cells. Bars represent the average COX2/GFP area for all analyzed animals ± SEM, and circles represent the average of all analyzed cells for each animal (20 cells/animal; 1 slice per animal). **** p<0.0001; Two-Way ANOVA, F_{interaction} (1,19) = 44.23, p<0.0001; Holm-Sidak post-hoc test.

(C) Microglia GFP (green) and prostanoid PGE2 (red)-stained hippocampal slices from P22 WT CX3CR1-GFP and Dp(16) CX3CR1-GFP animals. Scale bar: 20μm.

(D) Quantification of the PGE2+ area normalized to GFP+ area of microglial cells. Bars represent the average PGE2/GFP area for all analyzed animals ± SEM, and circles represent the average of all analyzed cells for each animal (20 cells/animal; 1 slice per animal). * p<0.05, *** p<0.001; Two-Way ANOVA, F_{interaction} (1,16) = 4.596, p=0.0478; Holm-Sidak post-hoc test.

(E) Experimental protocol for morphological and electrophysiological analyses in Dp(16) and WT littermates treated with vehicle or APAP.

(F) Binary images of the fields shown in Figure 4B. Scale bar: 10μm.

(G) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. In parenthesis: analyzed cells (126 vs 62), and animals (7 vs 4) for 34 different radius points. Repeated measure Two-Way ANOVA, F_{interaction} (33, 6324) = 0.9459, p = 0.5563. The vehicle-treated WT animal data are from Figure 1E as comparison.

(H) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. In parenthesis: analyzed cells (126 vs 65), and animals (7 vs 4) for 34 different radius points. *** p<0.001; Repeated measure Two-Way ANOVA, F_{treatment} (1, 189) = 8.520, p =0.0039; Holm-Sidak post-hoc test. The vehicle-treated WT animal data were from Figure 1E as comparison.

(I) Quantification of the average number of intersections as obtained from the Sholl analysis in C-D. Bars represent the average number of intersections in all analyzed animals ± SEM, and circles represent the single data points of the average number of intersections for each animal (12-21 cells/animal; 1 slice per animal). ** p<0.01, Two-Way ANOVA, F_{interaction} (1, 17) = 14.87, p = 0.0013; Tukey’s post-hoc test). The vehicle-treated animal data (dotted circles) are from Figure 1F as comparison.
(J) Representative traces of mEPSCs recordings in CA1 hippocampal pyramidal neurons from P22 WT and Dp(16) mice treated with vehicle or APAP. Scale bars: 10pA and 1s.

(K) Quantification of the mEPSCs frequency (left) and amplitude (right) in experiments as in Figure 3D. Bars represent the average frequency and the average amplitude of mEPSCs for all analyzed cells ± SEM, and circles represent single data points for each cell (4 Dp(16) vehicle-treated animals and 3 animals per each remaining condition). *p<0.05, ** p<0.01, *** p<0.001; For frequency: Two-Way ANOVA, $F_{interaction}(1, 57) = 18.51$, p<0.001; For amplitude Two-Way ANOVA, $F_{interaction}(1, 57) = 38.21$, p<0.001; Holm-Sidak post-hoc test). The vehicle-treated animal data (dotted circles) are from Figure 3E as comparison.
Figure S8. Related to Figure 4. APAP does not significantly rescue cognitive behavior in adult Dp(16) mice.

(A) Experimental protocol for cognitive behavior before and after APAP treatment on the same Dp(16) and WT littermates.

(B) Quantification of the discrimination index in the OLT in WT and Dp(16) animals tested first in the absence of any treatment (naïve) and subsequently upon vehicle-treatment. Bars on the side represent the average discrimination index of all analyzed animals ± SEM, and symbols (circles, males; triangles, females) represent single data points for each animal. Lines connect data from a single animal before and after vehicle treatment. 

****p<0.0001; RM Two-Way ANOVA, F_{Interaction} (1, 22) = 30.88, p<0.0001; Holm-Sidak post-hoc test.

(C) Quantification of the discrimination index in the OLT in WT and Dp(16) animals tested first in the absence of any treatment (naïve) and subsequently upon APAP-treatment. Bars on the side represent the average discrimination index of all analyzed animals ± SEM, and symbols (circles, males; triangles, females) represent single data points for each animal. Lines connect data from a single animal before and after APAP treatment.

****p<0.0001; RM Two-Way ANOVA, F_{Interaction} (1, 24) = 0.1408, p=0.7108; Holm-Sidak post-hoc test.

(D) Experimental protocol for the morphology of microglia in the CA1, CA3 and DG areas of the hippocampus (highlighted by the dotted lines) and behavior.

(E) Iba1-stained WT and Dp(16) hippocampal slices from adult WT and Dp(16) animals. Scale bar: 10μm.

(F) Quantification of the cell body area of microglial cells. Bars represent the average of microglial cell body areas in all analyzed animals ± SEM and circles represent single data points of the cell averages for each animal (1 slice per animal). * p<0.05, Two-Way ANOVA, F_{Genotype} (1, 25)=15.25, p=0.0006, Holm-Sidak post-hoc test.

(G) Iba1-stained WT and Dp(16) hippocampal slice used for Sholl Analysis. Scale bar: 10μm.

(H) Sholl analysis of WT and Dp(16) microglial cells in images as in G. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. ** p<0.01, *** p<0.001, **** p<0.0001, Repeated measure Two-Way ANOVA, F_{Interaction} (99, 12022) = 1.476, p = 0.0015; Holm-Sidak post-hoc test. In parenthesis: analyzed cells, ad animals for 34 different radius points (1 slice per animal).

(I) Sholl analysis of Dp(16) treated with either vehicle or APAP microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. * p<0.05, ** p<0.01, *** p<0.001. Repeated measure Two-Way ANOVA, F_{Interaction} (99, 12022) = 1.476, p = 0.0015; Holm-Sidak post-hoc test. In parenthesis: analyzed cells, and animals for 34 different radius points (1 slice per animal).
Quantification of the discrimination index in the object location test in adult WT and Dp(16). Bars represent the average discrimination index of all analyzed animals ± SEM, and symbols (circles, males; triangles, females) represent single data points for each animal. ****p<0.0001; Two-Way ANOVA, F_{Genotype} (1, 54) = 5.329, p=0.0248; Holm-Sidak post-hoc test.
Figure S9. Related to Figure 4. Ruxolitinib treatment rescues cognitive deficits and partially rescues microglial alterations in Dp(16) mice.

(A) Experimental protocol for Ruxolitinib treatment, and for morphology of microglia and the behavior of mice.

(B) Iba1-stained hippocampal slices from P22 WT and Dp(16) animals treated with vehicle or Ruxolitinib. Scale bar: 20μm.

(C) Quantification of the cell body area of microglial cells. Bars represent the average of microglial cell body areas in all analyzed animals ± SEM and circles represent single data points of the cell averages for each animal (38-73 cells/animal; 1 slice per animal). * p<0.05, **p<0.01; Two-Way ANOVA, F_{Strain} (1, 24) = 17.35, p = 0.0003; Holm-Sidak post-hoc test. The vehicle-treated animal data (dotted circles) are from Figure 1C as comparison.

(D) Binary images of the selected fields. Scale bar: 20μm.

(E) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. * p<0.05, ** p<0.001; Repeated measure Two-Way ANOVA, F_{Treatment} (1, 210) = 2.317, p < 0.0001; Holm-Sidak post-hoc test. In parenthesis: analyzed cells (126 vs 86), and animals (7 vs 5) for 34 different radius points. The vehicle-treated WT animal data are from Figure 1E as comparison.

(F) Quantification of the average number of intersections as obtained from the Sholl analysis. Bars represent the average number of intersections in all analyzed animals ± SEM, and circles represent single data points of the average number of intersections for each animal (12-21 cells/animal; 1 slice per animal). * p<0.05, **p<0.01; Two-Way ANOVA, F_{Interaction} (1, 21) = 7.966, p = 0.0102; Holm-Sidak post-hoc test. The vehicle-treated animal data (dotted circles) are from Figure 1F as comparison.

(G) Quantification of the number of branches per microglial cell. Bars represent the average number of branches per microglial cell ± SEM of all analyzed animals, and circles represent single data points of the cell averages for each animal (12-21 cell/animal; 1 slice per animal). * p<0.05, ** p<0.01; Two-Way ANOVA, F_{Interaction} (1, 21) = 4.880 p = 0.0384, Holm-Sidak post-hoc test. The vehicle treated animals’ data are from Figure 1H as comparison.

(H) Quantification of the discrimination index in the novel object recognition and object location test in P22 WT and Dp(16) mice following vehicle or Ruxolitinib treatment. Bars represent the average discrimination index of all analyzed animals ± SEM, and symbols (circles, males; triangles, females) represent single data points for each animal. (H) **p<0.01, ***p<0.001; Two-Way ANOVA, F_{Interaction} (1, 36) = 6.785, p=0.0133; Holm-Sidak post-hoc test. (I) ****p<0.0001; Two-Way ANOVA, F_{Interaction} (1, 48) = 14.81, p=0.0004; Holm-Sidak post-hoc test.
Figure S10. Related to Figure 4. *In vivo* APAP effect on microglia and cognition is transient.

(A) Experimental protocol for drug treatment and withdrawal, morphology of microglia in the hippocampus and behavior.

(B) Iba1-stained hippocampal slices from P40 WT (blue) and Dp(16) animals treated with vehicle or APAP after withdrawal (APAP (w)). Scale bar: 10μm.

(C) Quantification of the cell body area of microglial cells. Bars represent the average of microglial cell body areas in all analyzed animals ± SEM and circles represent single data points of the cell averages for each animal (56-60 cells/animal; 1 slice per animal); * p<0.05, ** p<0.01, Two-Way ANOVA, F_{Strain} (1, 19) = 16.70, p = 0.0006; Holm-Sidak post-hoc test.

(D) Binary images of the fields. Scale bar: 10μm.

(E) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. * p<0.05, ** p<0.01, *** p<0.001; Repeated measure Two-Way ANOVA, F_{Strain} (1, 176) = 12.09, p = 0.0006; Holm-Sidak post-hoc test. In parenthesis: analyzed cells (89 vs 89), and animals (5 vs 5) for 49 different radius points (1 slice per animal).

(F) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. ** p<0.01; Two-Way ANOVA, F_{Treatment} (1,196) = 22.73, p < 0.0001, Holm-Sidak post-hoc test. In parenthesis: analyzed cells (89 vs 109), and animals (5 vs 6) for 49 different radius points (1 slice per animal).

(G) Quantification of the average number of intersections. Bars represent the average number of intersections in all analyzed animals ± SEM, and circles represent single data points of the average number of intersections for each animal (16-18 cells/animal; 1 slice per animal). * p<0.05; Two-Way ANOVA, F_{Strain} (1, 19) = 11.00, p = 0.0036; Holm-Sidak post-hoc test.

(H) Quantification of the discrimination index in the novel object recognition test in P40 WT and Dp(16) mice following vehicle or APAP treatment and withdrawal. Bars represent the average discrimination index of all analyzed animals ± SEM, and symbols (circles, males; triangles, females) represent single data points for each animal. *p<0.05; Two-Way ANOVA, F_{Strain} (1, 47) = 21.10, p < 0.0001, Holm-Sidak post-hoc test.
Figure S11. Related to Figure 4. APAP treatment rescues microglial distal ramifications and cognitive deficits in Ts65Dn mice.

(A) Experimental protocol for drug treatment, morphology of microglia in the hippocampus and behavior.

(B) Iba1-stained hippocampal slices from P22 WT (black) and Ts65Dn (green) animals treated with vehicle (solid contour) or APAP (dashed contour). Scale bar: 10μm.

(C) Quantification of the cell body area of microglial cells. Bars represent the average of microglial cell body areas in all analyzed animals ± SEM and circles represent single data points of the cell averages for each animal (56-60 cells/animal; 1 slice per animal).

(D) Binary images of the fields. Scale bar: 10μm.

(E) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. * p<0.05, ** p<0.01, *** p<0.001; Repeated measure Two-Way ANOVA, F_{Strain} (1, 177) = 49.95, p < 0.0001; Holm-Sidak post-hoc test. In parenthesis: analyzed cells (107 vs 72), and animals (6 vs 4) for 49 different radius points (1 slice per animal).

(F) Sholl analysis of microglial cells. Data are expressed as average number of intersections at each distance from the cell bodies of all analyzed cells ± SEM. * p<0.05, ** p<0.01, *** p<0.001; Repeated measure Two-Way ANOVA, F_{Treatment} (1, 213) = 3.002, p= 0.0846; Holm-Sidak post-hoc test. In parenthesis: (107 vs 108), and animals (6 vs 6) for 49 different radius points (1 slice per animal).

(G) Quantification of the average number of intersections. Bars represent the average number of intersections in all analyzed animals ± SEM, and circles represent single data points of the average number of intersections for each animal (16-18 cells/animal; 1 slice per animal). * p<0.05; Two-Way ANOVA, F_{Interaction} (1, 15) = 6.58, p = 0.02; Holm-Sidak post-hoc test.

(H) Quantification of the discrimination index in the object location test in P22 WT and Ts65Dn mice following vehicle or APAP treatment. Bars represent the average discrimination index of all analyzed animals ± SEM, and symbols (circles, males; triangles, females) represent single data points for each animal. **p<0.001, ***p<0.0001; Two-Way ANOVA, F_{Interaction} (1, 46) = 12.19, p = 0.0011, Holm-Sidak post-hoc test.
Supplementary Table 2. Related to Figure S5. Gene Ontology Biological Process Between WT and Dp(16) hippocampi.

Flavonoid and Xenobiotic Glucuronidation

| Official Gene Symbol | Gene Name                                                                 | Species       |
|----------------------|---------------------------------------------------------------------------|---------------|
| 394436               | UDP glucuronosyltransferase 1 family, polypeptide A1(Ugt1a1)              | Mus musculus  |
| 22236               | UDP glucuronosyltransferase 1 family, polypeptide A2(Ugt1a2)              | Mus musculus  |
| 394432               | UDP glucuronosyltransferase 1 family, polypeptide A7C(Ugt1a7c)            | Mus musculus  |
| 394434               | UDP glucuronosyltransferase 1 family, polypeptide A9(Ugt1a9)              | Mus musculus  |

Cell Migration

| Official Gene Symbol | Gene Name                                                                 | Species       |
|----------------------|---------------------------------------------------------------------------|---------------|
| 231841               | BRCA1-associated ATM activator 1(Brat1)                                   | Mus musculus  |
| 12476               | CD151 antigen(Cd151)                                                      | Mus musculus  |
| 12505               | CD44 antigen(Cd44)                                                       | Mus musculus  |
| 27205               | podocalyxin-like(Podxl)                                                   | Mus musculus  |
| 72508               | ribosomal protein S6 kinase, polypeptide 1(Rps6kb1)                      | Mus musculus  |
| 74392               | sperm antigen with calponin homology and coiled-coil domains 1-like(Specc1l) | Mus musculus  |

Peptidyl—Ser Phosphorylation

| Official Gene Symbol | Gene Name                                                                 | Species       |
|----------------------|---------------------------------------------------------------------------|---------------|
| 100986               | A kinase (PRKA) anchor protein (yotiao) 9(Akap9)                          | Mus musculus  |
| 12505               | CD44 antigen(Cd44)                                                       | Mus musculus  |
| 69726               | SET and MYND domain containing 3(Smyd3)                                   | Mus musculus  |
| 11606               | angiotensinogen (serpin peptidase inhibitor,clade A,8)(Agt)              | Mus musculus  |

Memory

| Official Gene Symbol | Gene Name         | Species |
|---------------------|-------------------|---------|
| 16400               | integrin alpha 3(Itga3) | Mus musculus |
| Official Gene Symbol | Gene Name | Species |
|----------------------|-----------|---------|
| 54194                | A kinase (PRKA) anchor protein 8-like (Akap8l) | Mus musculus |
| 66877                | Crn, crooked neck-like 1 (Drosophila) (Crnk11) | Mus musculus |
| 66373                | LSM5 homolog, U6 small nuclear RNA and mRNA degradation associated (Lsm5) | Mus musculus |
| 24018                | RNA guanyllytransferase and 5’-phosphatase (Rngtt) | Mus musculus |
| 83410                | cleavage stimulation factor, 3’ pre-RNA subunit 2, tau (Cstf2t) | Mus musculus |
| 19383                | hnRNP-associated with lethal yellow (Raly) | Mus musculus |
| 70650                | zinc finger, CCHC domain containing 8 (Zcchc8) | Mus musculus |

**REDOX Process**

| Official Gene Symbol | Gene Name | Species |
|----------------------|-----------|---------|
| 78330                | NADH dehydrogenase (ubiquinone) flavoprotein 3 (Ndufv3) | Mus musculus |
| 80707                | WW domain-containing oxidoreductase (Wwox) | Mus musculus |
| 102632               | acyl-Coenzyme A dehydrogenase family, member 11 (Acad11) | Mus musculus |
| 71361                | apoptosis-inducing factor, mitochondrion-associated 2 (Aifm2) | Mus musculus |
| 102115               | deoxyhypusine hydroxylase/monooxygenase (Dohh) | Mus musculus |
| 112405               | egl-9 family hypoxia-inducible factor 1 (Egln1) | Mus musculus |
| 112407               | egl-9 family hypoxia-inducible factor 3 (Egln3) | Mus musculus |
| 16828                | lactate dehydrogenase A (Ldha) | Mus musculus |
| 171580               | microtubule associated monooxygenase, calponin and LIM domain containing 1 (Mical1) | Mus musculus |
| 212503               | polyamine oxidase (exo-N4-amino) (Paox) | Mus musculus |
| 73166                | transmembrane 7 superfamily member 2 (Tm7sf2) | Mus musculus |