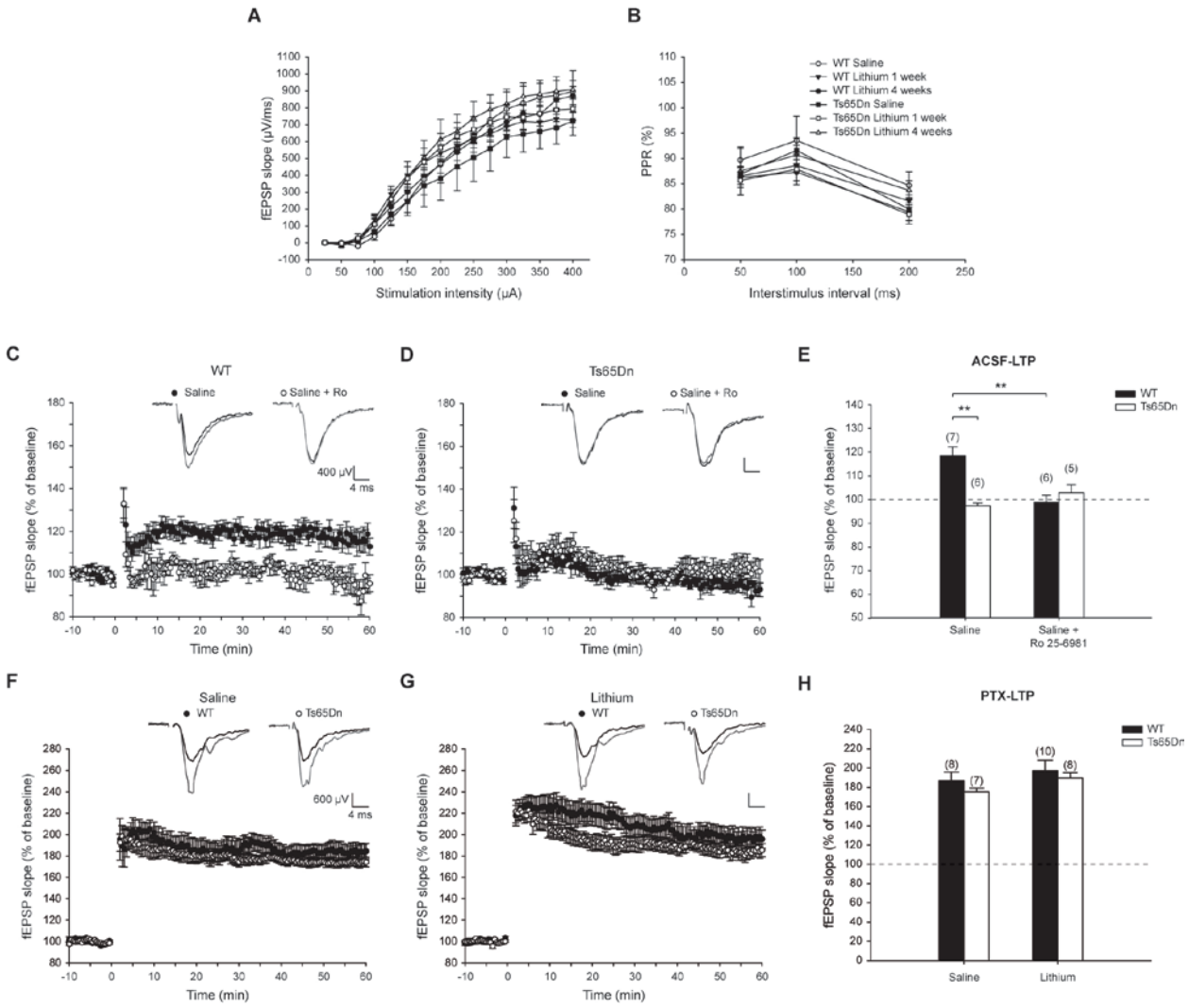
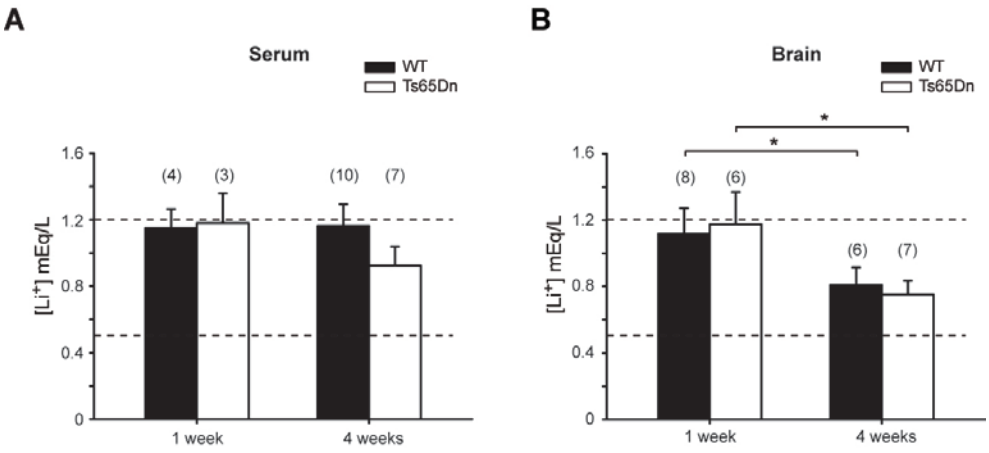
Supplemental Figure 1



Supplemental Figure 1. – (A-B) Basal synaptic transmission was similar in the DG of saline- or lithium-treated Ts65Dn and WT mice. (A) Input-output (I/O) relationships were similar across experimental groups for stimulus intensities ranging from 25 to 400 μA (25 μA step), indicating similar pre-synaptic recruitment of afferent fibers and comparable post-synaptic responses. 2-way ANOVA F ratio and P values were: genotype [$F_{1,34}$=1.739, P=0.196] at 50 μA, treatment [$F_{2,34}$=1.745, P=0.189] at 125 μA, genotype x treatment [$F_{2,34}$=2.458, P=0.101] at 350 μA, or higher. **(B)** The paired-pulse ratio (PPR) was not significantly different across groups for inter-stimulus intervals of 50, 100 and 200 msec. 2-way ANOVA, 50 msec: genotype [$F_{1,34}$=0.122 , P=0.729], treatment [$F_{2,34}$=0.265, P=0.769], genotype x treatment [$F_{2,34}$=0.259, P=0.774]. 2-way ANOVA, 100 msec: genotype [$F_{1,34}$=0.014 , P=0.907], treatment [$F_{2,34}$=1.417, P=0.256], genotype x treatment [$F_{2,34}$=0.667, P=0.520]. 2-way ANOVA, 200 msec: genotype [$F_{1,34}$=0.375 , P=0.544], treatment [$F_{2,34}$=0.475, P=0.626], genotype x treatment [$F_{2,34}$=2.862, P=0.071]. **(C-E) The NR2B antagonist Ro25-6981 inhibited LTP induction in the DG of saline-treated WT mice.** Tetanic stimulation induced LTP in slices obtained from saline-treated WT (**C**, p<0.001 vs baseline), but not Ts65Dn mice (**D**; p=0.190 *vs* baseline). Application of the NR2B antagonist Ro25-6981 (1.5 μM)

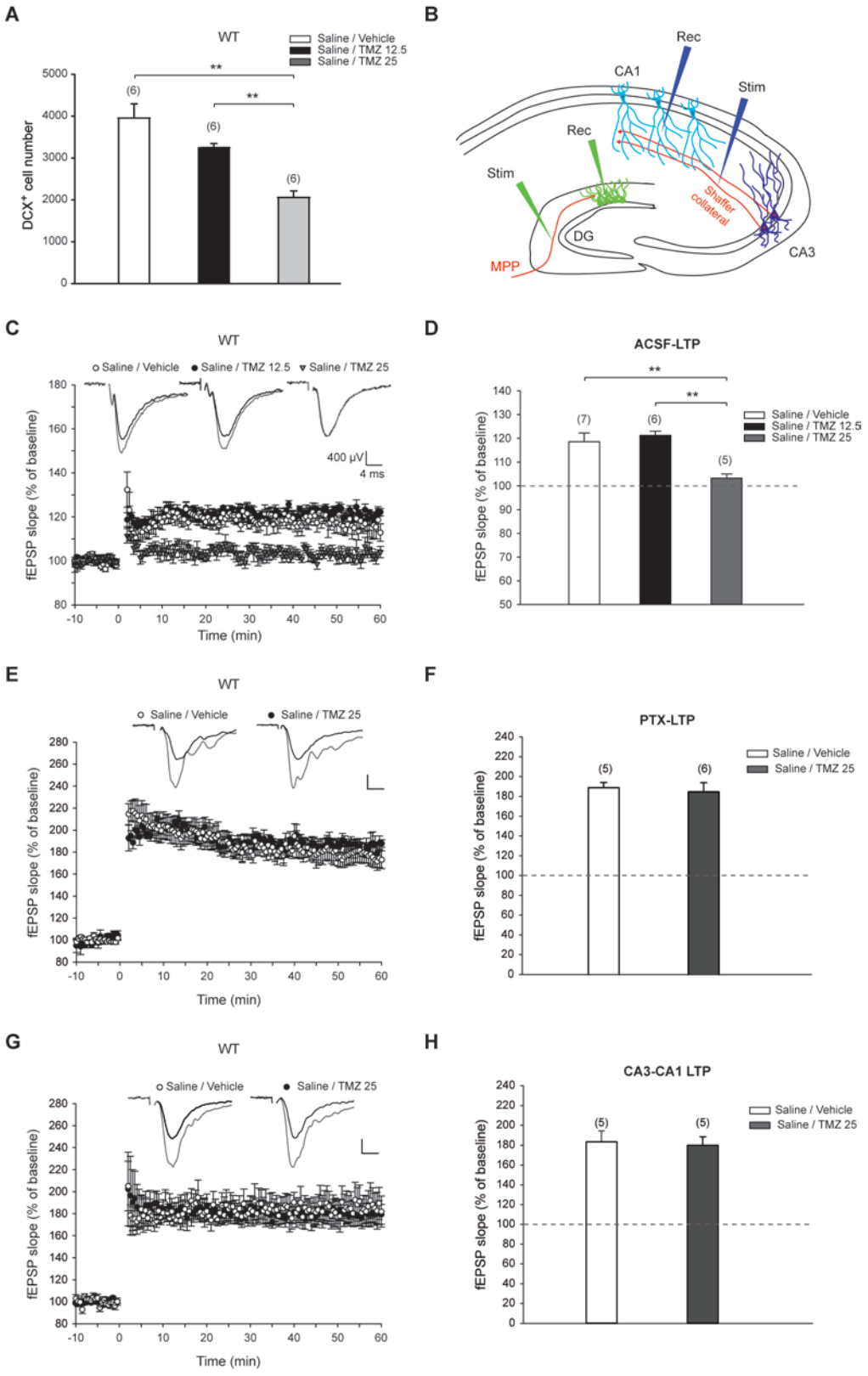
prevented ACSF-LTP induction in WT slices (**C**; p=0.708 *vs* baseline), but was ineffective in Ts65Dn slices (**D**; p=0.690 *vs* baseline). **(E)** Quantification of ACSF-LTP induction in the DG at 40-45 min after tetanic stimulation. 2-way ANOVA: genotype [$F_{1,23}$=8.199, P=0.001], treatment [$F_{1,23}$=5.516, P=0.029], genotype x treatment [$F_{1,23}$=17.443, P<0.001]. **p<0.01 Tukey *post hoc* test. **(F-H) LTP induction in presence of PTX was similar in Ts65Dn and WT mice.** fEPSPs were recorded in presence of PTX (100 µM) in slices from Ts65Dn and WT mice fed either with normal or lithium-containing diet for 4 weeks. Tetanic stimulation in presence of PTX elicited LTPs of similar amplitude in both saline-treated (**F**) and lithium-fed (**G**) Ts65Dn mice and WT littermates (**F-G**; p<0.001 *vs* baseline) **(H)** Quantification of PTX-LTP induction at 40-45 min after tetanic stimulation. 2-way ANOVA: genotype [$F_{1,29}$=1.346, P=0.256], treatment [$F_{1,29}$=2.197, P=0.149], genotype x treatment [$F_{1,29}$=0.0542, P=0.818]. The numbers in parentheses indicate the number of brain slices recorded for each experimental group. Insets in panels C-D and F-G show representative fEPSPs traces recorded 5 min before (black) and 45 min after (gray) LTP induction.

Supplemental Figure 2



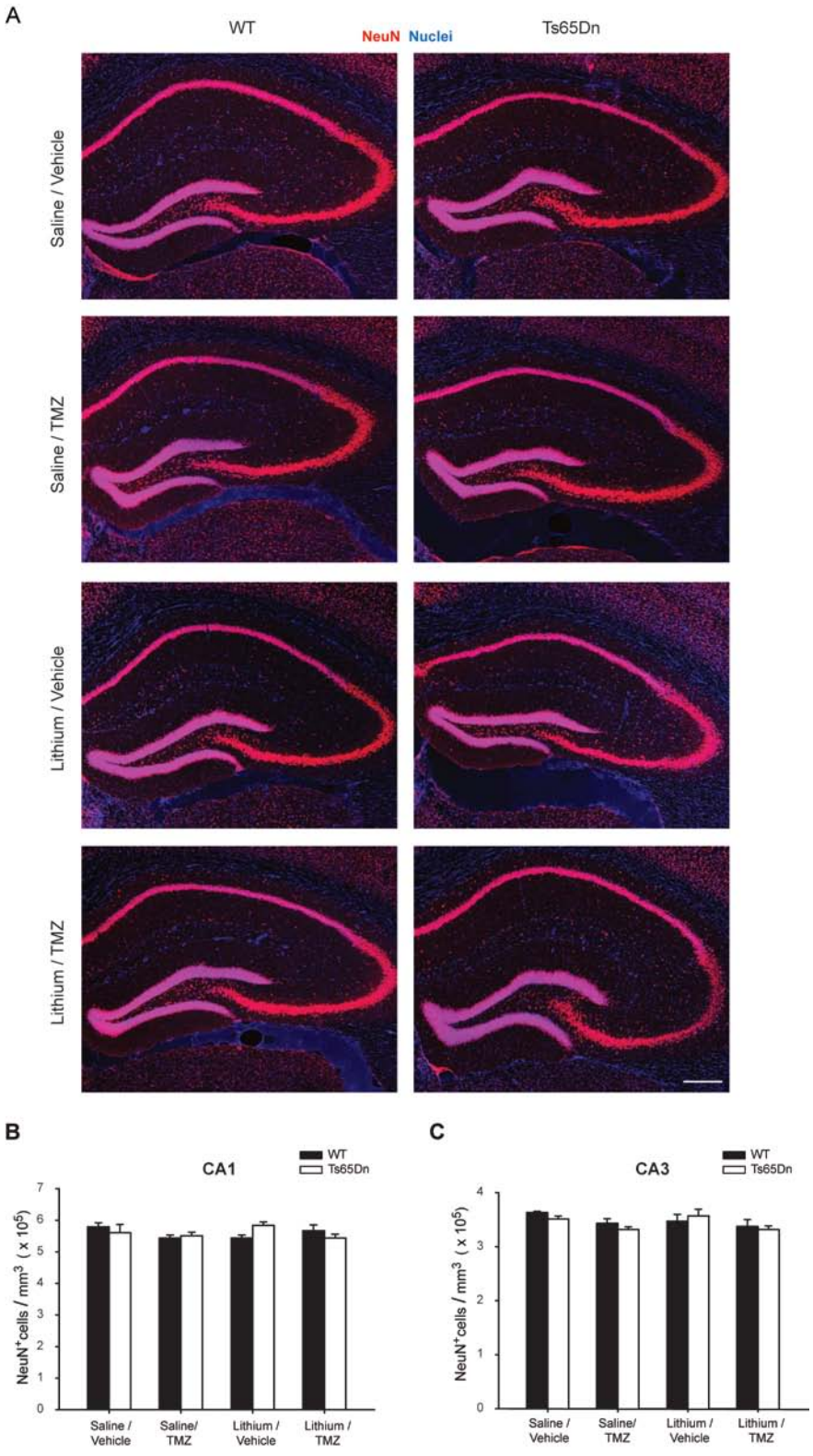
Supplemental Figure 2. – Serum and brain lithium concentrations in WT and Ts65Dn mice. (A) The serum concentration of lithium was assessed after 1 or 4 weeks of treatment. No statistical difference was detected between groups. 2-way ANOVA: genotype [$F_{1,20}$=0.412, P=0.528], treatment [$F_{1,20}$=0.554, P=0.466], genotype x treatment [$F_{1,20}$=0.686, P=0.417]. **(B)** The brain concentration of lithium was assessed after 1 or 4 weeks of treatment. Lithium concentration was slightly decreased in both WT and Ts65Dn brains after 4 weeks in comparison with 1 week of treatment, but remains in the range achieved by lithium therapy in humans (0.5-1.2 mEq/L; dashed lines in both A and B panels). 2-way ANOVA: genotype [$F_{1,23}$>0.001, P=0.999], treatment [$F_{1,23}$=6.841, P=0.015], genotype x treatment [$F_{1,23}$=0.161, P=0.692]. *p<0.05 Tukey *post hoc* test. The numbers in parentheses indicate the number of samples analyzed for each experimental group.

Supplemental Figure 3



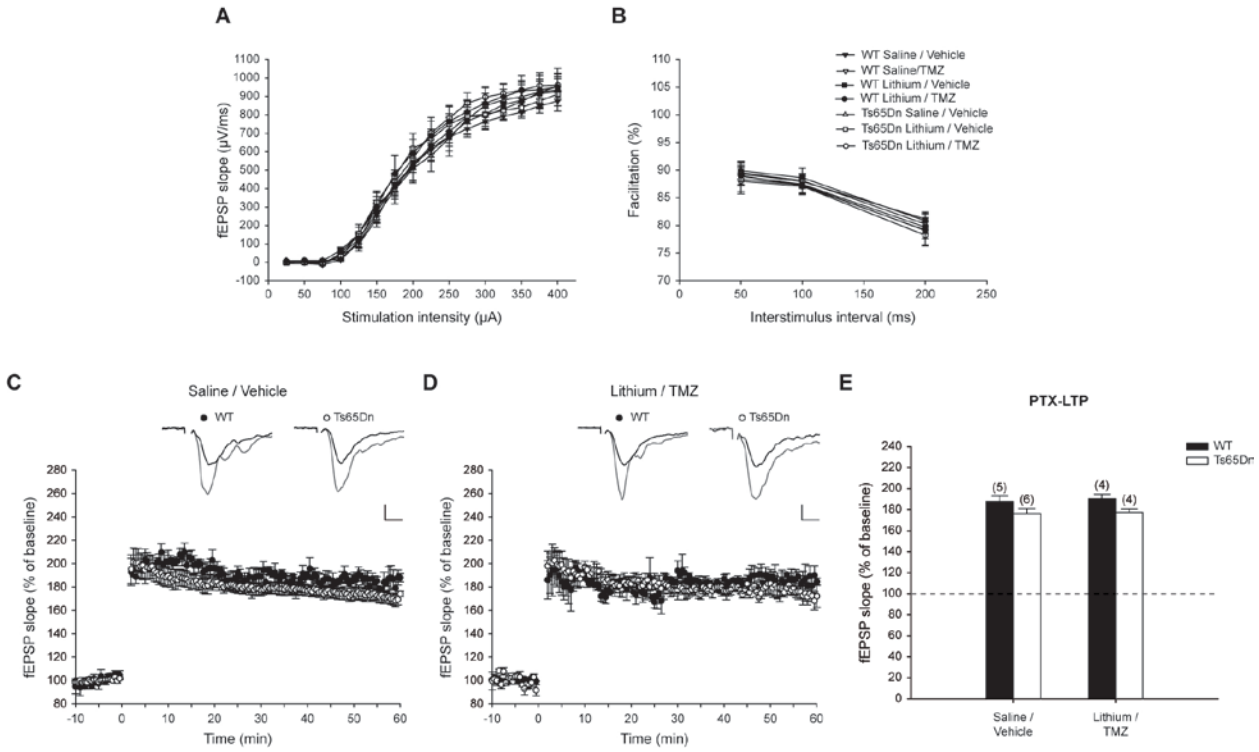
Supplemental Figure 3. – TMZ reduced the number of newborn neurons and blocked neurogenesis-dependent plasticity in the DG of WT mice without altering the functional properties of other hippocampal neurons. (A) Quantitative analysis of DCX^+ newborn neurons in the DG of WT mice treated with 12.5 or 25 mg/Kg of TMZ (see Methods). 1-way ANOVA: [$F_{2,15}$=18.210, P<0.001], **p<0.01 Tukey *post hoc* test. **(B)** Position of the stimulating and recording electrodes for LTP induction in the DG (green) and CA3-CA1 region (blue). **(C)** TMZ prevented neurogenesis-dependent LTP (ACSF-LTP) induction in slices obtained from WT mice treated with 25 mg/Kg (p=0.141 *vs* baseline), but not with 12.5 mg/Kg of TMZ (p<0.001) or saline/vehicle (p<0.001). **(D)** Quantification of the extent of ACSF-LTP elicited in the DG of mice treated with or without TMZ. 1-way ANOVA: [$F_{2,15}$=10.869, P=0.001], **p<0.01 Tukey *post hoc* test. **(E)** Potentiation of mature DG neurons (PTX-LTP) was similar in slices from WT mice treated with either vehicle or 25 mg/Kg TMZ (p<0.001 *vs* baseline for each group). **(F)** Quantification of PTX-LTP induction 40-45 min after tetanic stimulation in the DG. P=0.724 Student t-test. **(G)** LTP at Shaffer collateral-CA1 synapses (CA3-CA1 LTP) was similar in slices from WT mice treated with vehicle or 25 mg/Kg TMZ (p<0.001 *vs* baseline for each group). **(H)** Quantification of CA3-CA1 LTP induction 40-45 min after stimulation. P=0.821 Student t-test. The numbers in parentheses indicate the number of brain slices recorded for each experimental group. Insets in panels C, E and G show representative fEPSPs traces recorded 5 min before (black) and 45 min after (gray) LTP induction.

Supplemental Figure 4



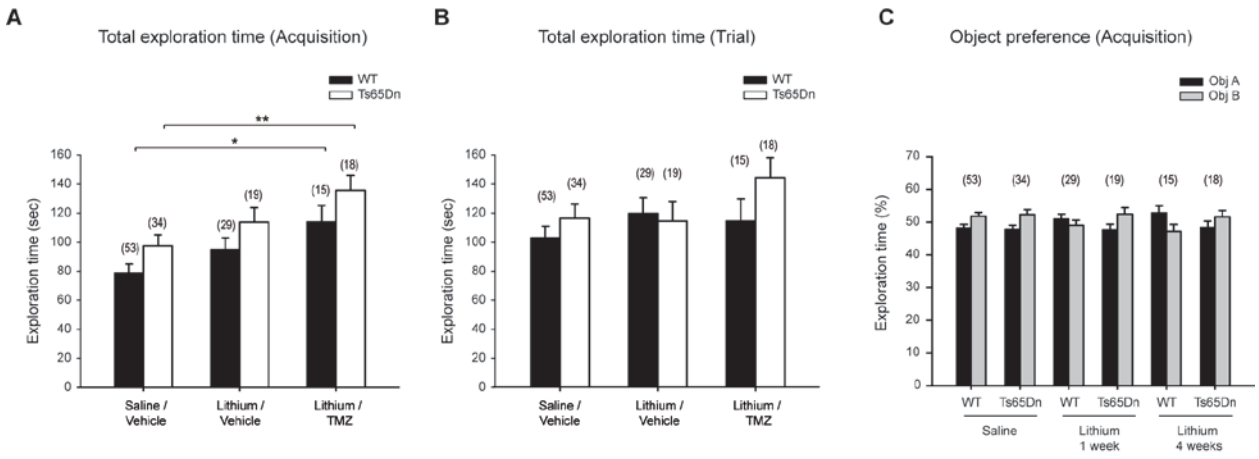
Supplemental Figure 4. – TMZ treatment did not alter neuronal density in non-neurogenic hippocampal regions (A) Representative confocal images showing NeuN immunoreactivity (red). Nuclei were counterstained with Hoechst-33342 (blue). Scale bar, 250 μm. **(B)** Neuronal density in the CA1 region was similar in all experimental groups. 2-way ANOVA: genotype [$F_{1,24}$=0.011, P=0.917], treatment [$F_{3,24}$=0.892, P=0.459], genotype x treatment [$F_{3,24}$=1.919, P=0.153]. **(C)** Neuronal density in the CA3 region was similar in all experimental groups. 2-way ANOVA: genotype [$F_{1,24}$=0.725, P=0.403], treatment [$F_{1,24}$=3.652, P=0.027], genotype x treatment [$F_{2,24}$=0.783, P=0.515].

Supplemental Figure 5



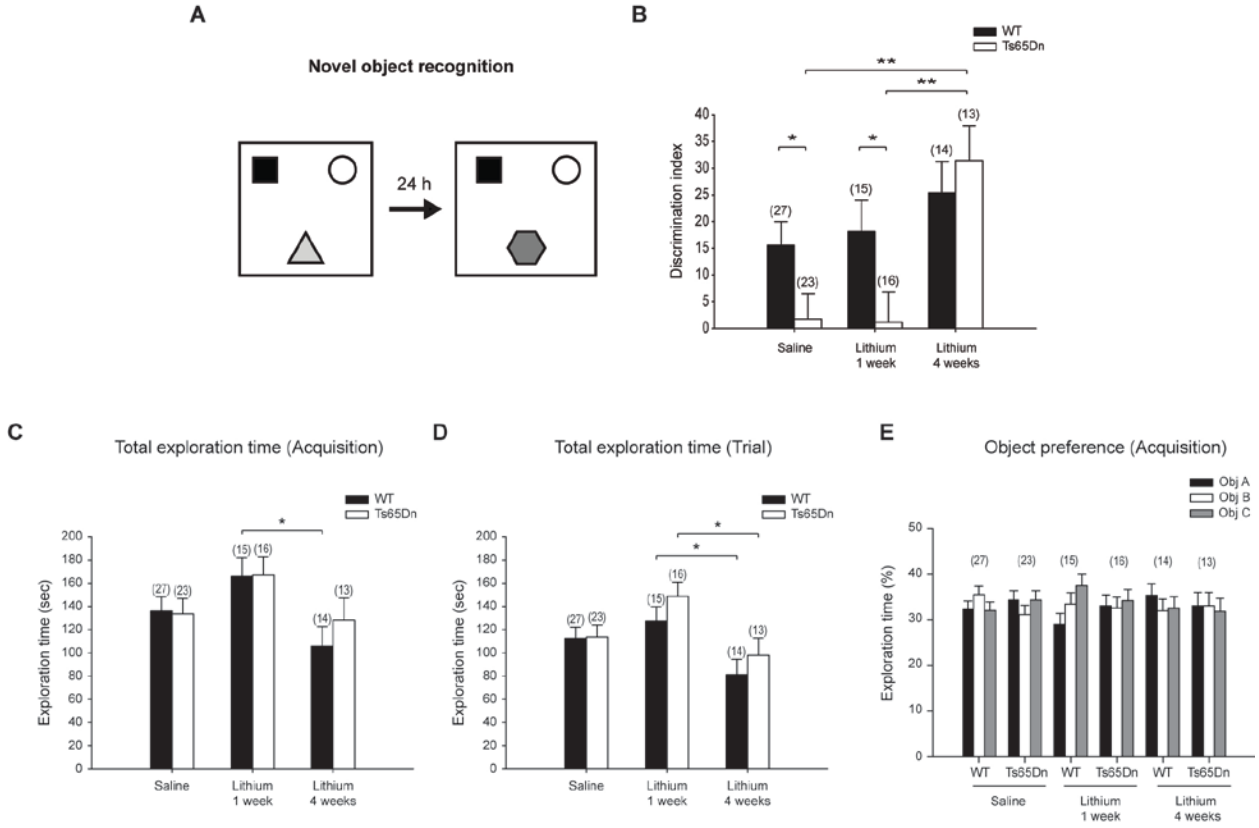
Supplemental Figure 5. - **(A-B) Basal synaptic transmission was not altered by TMZ treatment**. fEPSPs were elicited by stimulation of the MPP. **(A)** I/O relationships were similar across all groups for stimulus intensities ranging from 25 to 400 µA (25 µA step). 2-way ANOVA F ratio and P values were: [$F_{1,36}$=1.687 , P=0.203] at 50 µA, treatment [$F_{2,36}$=2.234, P=0.123] at 75 µA, genotype x treatment [$F_{2,36}$=1.419, P=0.256] at 100 µA or higher. **(B)** PPR was not significantly different across groups at inter-stimulus intervals of 50, 100 and 200 msec. 2-way ANOVA for 50 msec: genotype [$F_{1,36}$=0.215 , P=0.646], treatment [$F_{2,36}$=0.0.81, P=0.992], genotype x treatment [$F_{2,36}$=0.150, P=0.861]. 2-way ANOVA for 100 msec: genotype [$F_{1,36}$=0.173 , P=0.680], treatment [$F_{2,36}$=0.091, P=0.913], genotype x treatment [$F_{2,36}$=0.185, P=0.832]. 2-way ANOVA for 200 msec: genotype [$F_{1,36}$=1.411 , P=0.243], treatment [$F_{2,36}$=0.496, P=0.613], genotype x treatment [$F_{2,36}$=0.303, P=0.740]. **(C-E) LTP of mature DG neurons (PTX-LTP) was unchanged after TMZ treatment.** Tetanic stimulation of slices in presence of PTX (100 µM) elicited similar LTP in either WT or Ts65Dn ($p<0.001$ *vs* baseline for each group) mice treated with vehicle (C) or co-administered with lithium and TMZ **(D)**. Insets in C and D show representative fEPSP traces recorded 5 min before (black) and 45 min after (gray) LTP induction. **(E)** Quantification of LTP induction in presence of PTX at 40-45 min after tetanic stimulation. 2-way ANOVA: genotype [$F_{1,15}$=6.743, P=0.020], treatment [$F_{1,15}$=0.160, P=0.695], genotype x treatment [$F_{2,15}$=0.016, P=0.900]. The numbers in parentheses indicate the number of brain slices recorded for each experimental group.

Supplemental Figure 6



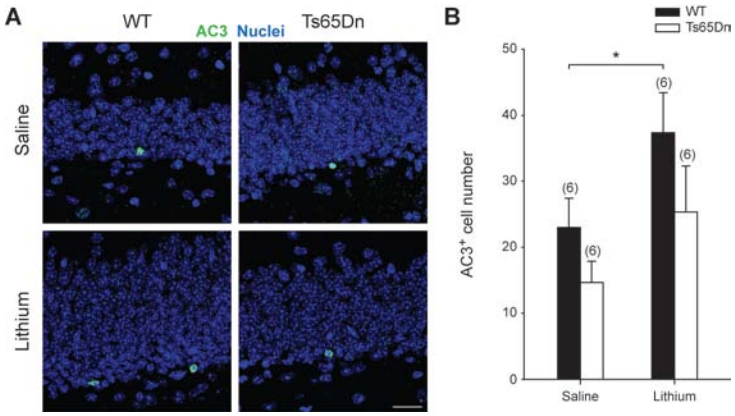
Supplemental Figure 6. - Total object exploration time and object preference in the object location (OL) test. Ts65Dn and WT mice were fed with either normal or lithium-containing diet for 4 weeks and concomitantly treated with TMZ or vehicle. **(A)** The total exploration time during the acquisition phase of the OL test was similar in saline/vehicle and saline/lithium treated Ts65Dn and WT mice, but was increased in both WT and Ts65Dn mice treated with TMZ. 2-way ANOVA: genotype [$F_{1,162}$=7.213, P=0.008], treatment [$F_{2,1162}$=8.636, P<0.001], genotype x treatment [$F_{2,162}$=0.017, P=0.983]. *p<0.05, **p<0.01 Tukey *post hoc* test. **(B)** The total object exploration time in the OL trial phase was not significantly different across genotype and treatment. 2-way ANOVA: genotype [$F_{1,162}$=1.659, P=0.200], treatment [$F_{2,162}$=1.373, P=0.256], genotype x treatment [$F_{2,162}$=0.883, P=0.415]. **(C)** The percentage of time spent exploring the two objects was not statistically different across groups. 2-way ANOVA: genotype [$F_{1,324}$=0.000, P=1.000], treatment [$F_{5,324}$=2.334, P=0.042], genotype x treatment [$F_{5,324}$=1.724, P=0.129]. In all panels, the numbers in parentheses indicate numbers of animals per group.

Supplemental Figure 7



Supplemental Figure 7. - Lithium Treatment restores novel object recognition in Ts65Dn mice. (A) Schematic representation of the Novel object recognition (NOR) test**.** Ts65Dn mice and WT littermates were fed with either normal or lithium-containing diet for 1 or 4 weeks and then tested in the NOR test. **(B)** Lithium administration for 4 weeks, but not for 1 week, restored novelty discrimination in Ts65Dn mice. 2-way ANOVA: genotype [$F_{1,102}$=3.322, P=0.071], treatment [$F_{2,102}$=7.311, P=0.001], genotype x treatment [$F_{2,102}$=2.198, P=0.116]**. (C, D)** The total exploration time during the acquisition (C) and trial phases (D) was similar in saline-treated Ts65Dn and WT mice. Total exploration time was slightly increased during both phases after 1 week of lithium treatment in both Ts65Dn and WT littermates, but was unchanged after 4 weeks of treatment. Acquisition phase 2-way ANOVA: genotype [$F_{1,95}$=0.293, P=0.590], treatment [$F_{2,95}$=4.474, P=0.014], genotype x treatment [$F_{2,95}$=0.334, P=0.717]. *p<0.05, Tukey *post hoc* test. Trial phase 2-way ANOVA: genotype [$F_{1,95}$=1.710, P=0.194], treatment [$F_{2,95}$=6.705, P=0.002], genotype x treatment [$F_{2,95}$=0.469, P=0.627]. *p<0.05, Tukey *post hoc* test. **(E)** The percentage of time spent exploring the three objects was not statistically different across groups. 2-way ANOVA: genotype [$F_{1,300}$<0.001, P=1.000], treatment [$F_{8,300}$=0.563, P=0.808], genotype x treatment [$F_{8,300}$=0.895, P=0.521]. In all panels, the numbers in parentheses indicate numbers of animals per group.

Supplemental Figure 8



Supplemental Figure 8. - Apoptotic cell death was not altered in saline- and lithium-treated Ts65Dn mice (A-B). 5-6 month old Ts65Dn mice and WT littermates were fed with either lithium-containing or normal diet for 4 weeks. Apoptotic cells were identified by immunoreactivity for active Caspase-3 (AC3). **(A)** Representative confocal z-stack maximal projection images showing AC3 immunostaining (green). Nuclei are counterstained with Hoechst-33342 (blue). Scale bar, 25 μm. **(B)** Quantification of $AC3^+$ apoptotic cells. The number of $AC3^+$ cells was similar in saline-treated WT and Ts65Dn mice. $AC3^+$ cells were significantly increased in WT, but not in Ts65Dn mice upon lithium treatment. 2-way ANOVA: genotype [$F_{1,20}$=4.286, P=0.052], treatment [$F_{1,20}$=6.299, P=0.021], genotype x treatment [$F_{1,20}$=0.225, P=0.640]. *p<0.05 Tukey *post hoc* test. The numbers in parentheses indicate numbers of animals per group