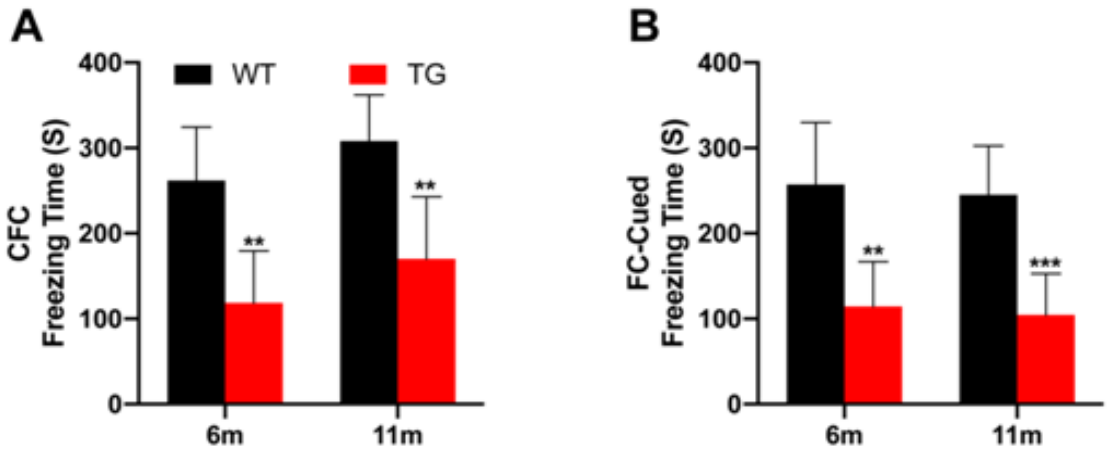
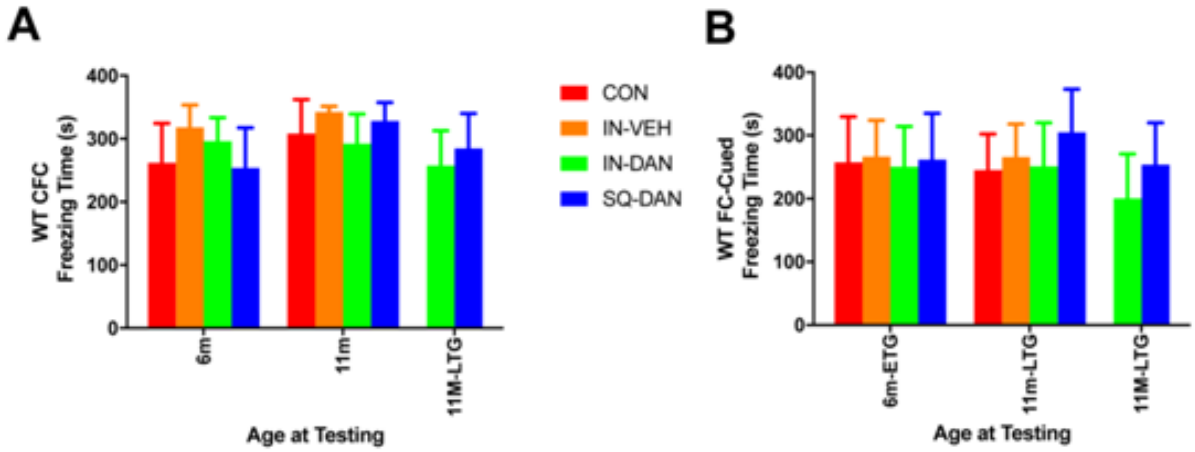
Supplementary Material

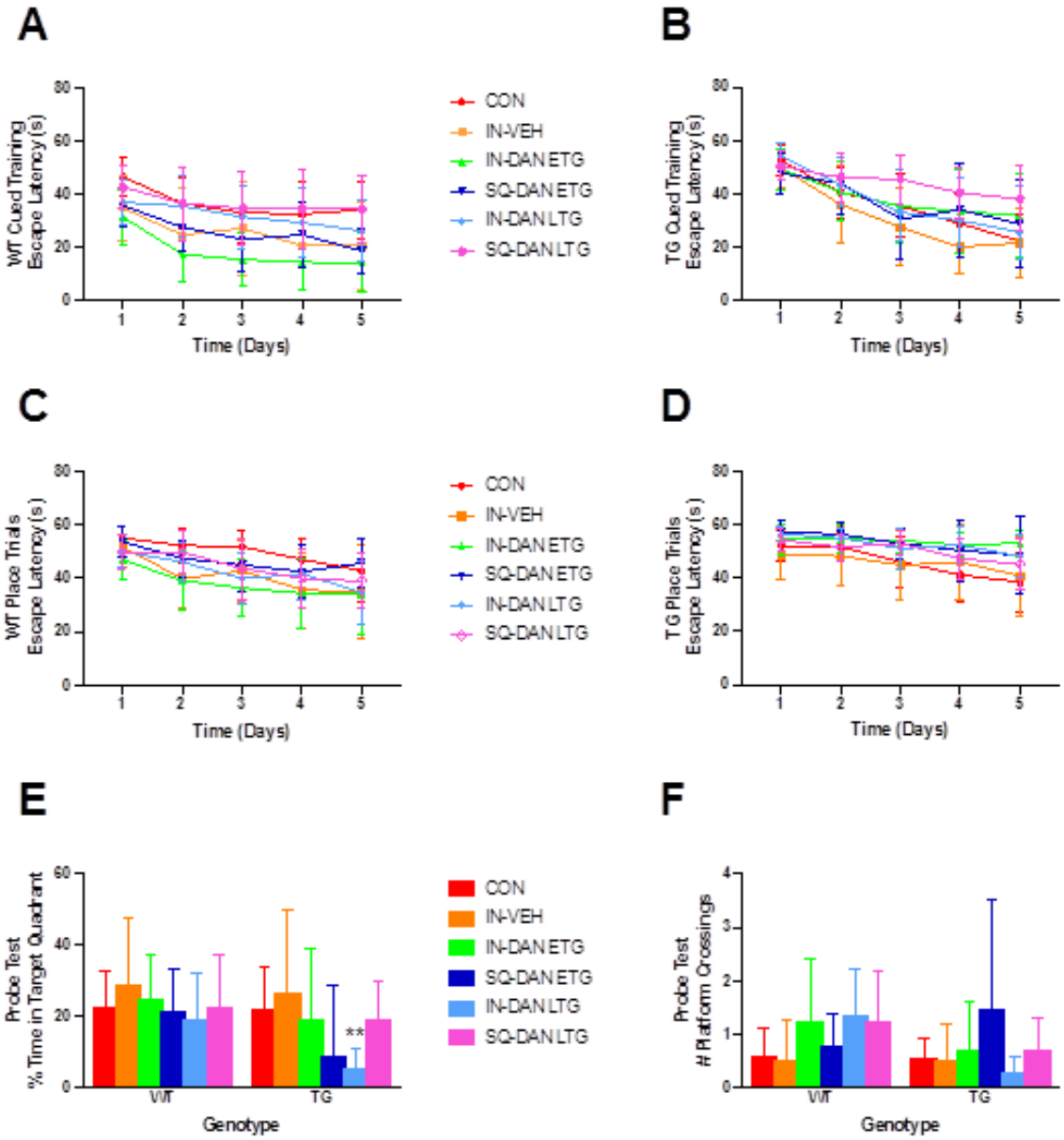
Intranasal Dantrolene as a Disease-Modifying Drug in Alzheimer 5XFAD Mice



Supplementary Figure 1. Memory impairment in 5XFAD mice. Both contextual fear conditioning (CFC, hippocampal-dependent) and cued fear conditioning (FC-cued, hippocampal-independent) memory were assessed with fear conditioning tests (FC). A) For CFC, hippocampal-dependent memory was significantly impaired in 5XFAD control (CON) mice compared to WT-CON mice at 6 and 11 months of age ($p=0.0015$, $p=0.0033$, respectively), which was analyzed using unpaired t-test. Genetics (WT versus TG) were found a significant source of variation for impaired memory ($p<0.0001$, $F(1,47)=23.44$). B) Similarly, hippocampal-independent memory was significantly impaired in the 5XFAD-CON mice compared to WT-CON mice at 6 and 11 months of age ($p=0.0019$, $p=0.0004$, respectively), analyzed using unpaired t-test. Genetics (WT versus TG) were found to be a significant source of variation for impaired memory ($p<0.0001$, $F(1,47)=27.94$). Data are presented as Mean with 95% CI. (WT: 6M n=13, 11M n= 12; TG: 6M n=13, 11M n=13).

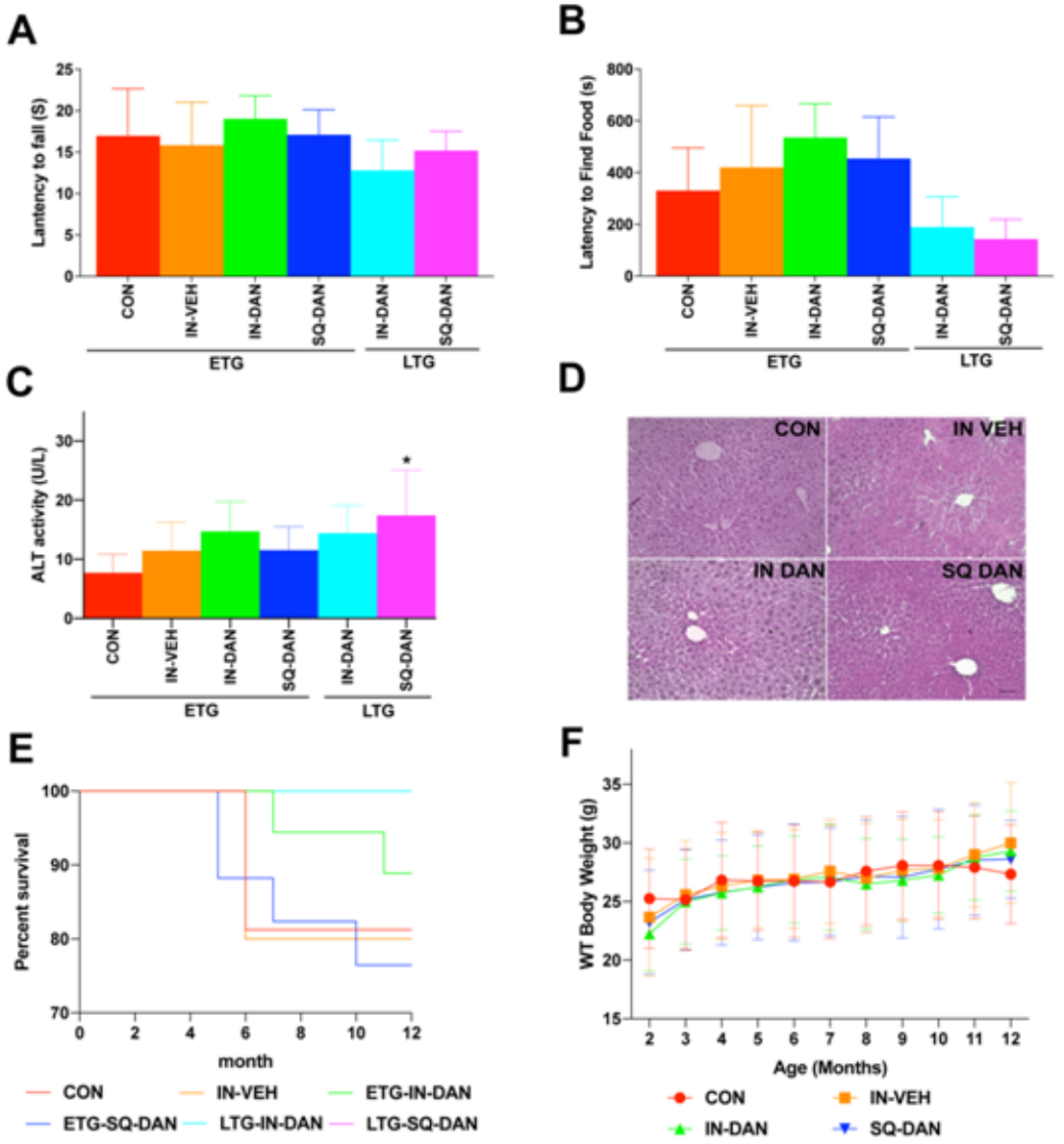


Supplementary Figure 2. Memory in WT groups. Memory was assessed with both contextual fear conditioning (CFC; hippocampal-dependent) and cued fear conditioning (FC-cued; hippocampal-independent) tests. The test was performed after 4 and 9 months of treatment, at 6 (6M) and 11 (11M) months of age, respectively, for the Early Treatment Group (ETG) and after 5 months of treatment at 11 months of age for the Late Treatment group (LTG). A) With the CFC test, at both 6 and 11 months of age, no significant differences were detected in all ETG and LTG groups, including intranasal administration of vehicle (IN-VEH), dantrolene (IN-DAN), and subcutaneous injection of dantrolene (SQ-DAN) compared to the untreated controls. The ETG at these 2 ages were analyzed using the ordinary 2-way ANOVA with Dunnett's multiple comparison test (MCT). The LTG data at 11 months of age were analyzed using ordinary 1way-ANOVA with Tukey's MCT. B) Similarly, no significant differences were detected in all ETG and LTG groups in hippocampal-independent memory (FC-cued) at both ages. The ETG at these 2 ages were analyzed using the ordinary 2-way ANOVA with Dunnett's multiple comparison test (MCT). The LTG data at 11 months were analyzed using ordinary 1way-ANOVA with Tukey's MCT. All data are presented as Mean with 95% CI. Animal numbers, ETG 6M: CON n=13, IN-VEH n=15, IN-DAN n=18, SQ-DAN n=15; ETG 11M: CON n=12, IN-VEH n=12, IN-DAN n=17, SQ-DAN n=13; LTG 11M: IN-DAN n=13, SQ-DAN n=13.



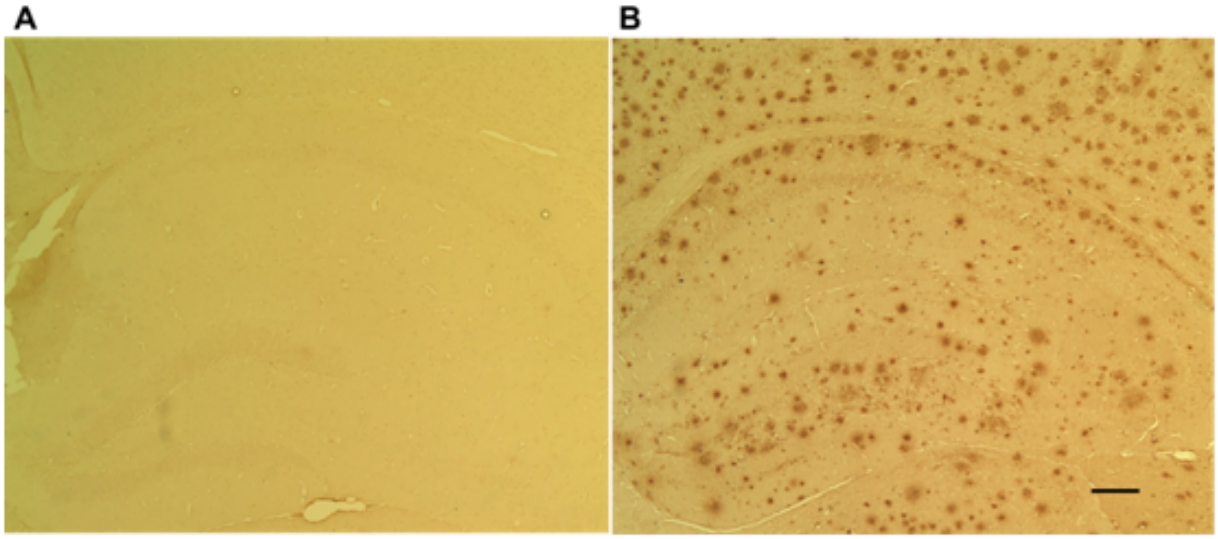
Supplementary Figure 3. Morris Water Maze learning and memory test. Hippocampal-dependent learning and memory were examined with the Morris Water Maze at 10 months of age for wild type (WT) and 5XFAD transgenic (TG) mice in early treatment (ETG) and late treatment (LTG) groups. A) The 5-day cued training detected no significant differences in the time to locate the platform (escape latency) for the WT groups (p=0.7062) or B) for the TG groups (p=0.1209), analyzed with the repeated measures 2-way ANOVA (time x treatment). C) No significant differences were found in the escape latency for the place trials (spatial learning) in the WT groups (adjusted p=0.7898) or D) for the TG groups (adjusted p=0.9068) with repeated measures 2-way ANOVA. E) There were no significant differences in the probe trial (percent time spent in the target quadrant) for WT groups (p=0.9139), though there was a significant difference in the probe trial for the TG groups (p=0.0254), analyzed with the Kruskal-Wallis test. Dunn's multiple comparison tests detected a significant decrease in the time spent in the target quadrant for the transgenic IN-DAN LTG compared to controls (adjusted p=0.0074).

F) Analysis of the number of platform crossings during the probe test for both genotypes found no significant differences for either the WT ($p=0.6492$) or the TG ($p=0.9004$) groups with the Kruskal-Wallis test. All data are presented as Mean with 95% CI, $^{**}p<0.01$. Animal numbers, WT groups: ETG: CON n=14, IN-VEH n=8, IN-DAN n=12, SQ-DAN n=13, LTG: IN-DAN n=14, SQ-DAN n=13; TG groups: ETG: CON n=13, IN-VEH n=10, IN-DAN n=10, SQ-DAN n=9, LTG: IN-DAN n=14, SQ-DAN n=14. CON (Control), IN-VEH (intranasal vehicle), IN-DAN (intranasal dantrolene), SQ-Dan (subcutaneous dantrolene).

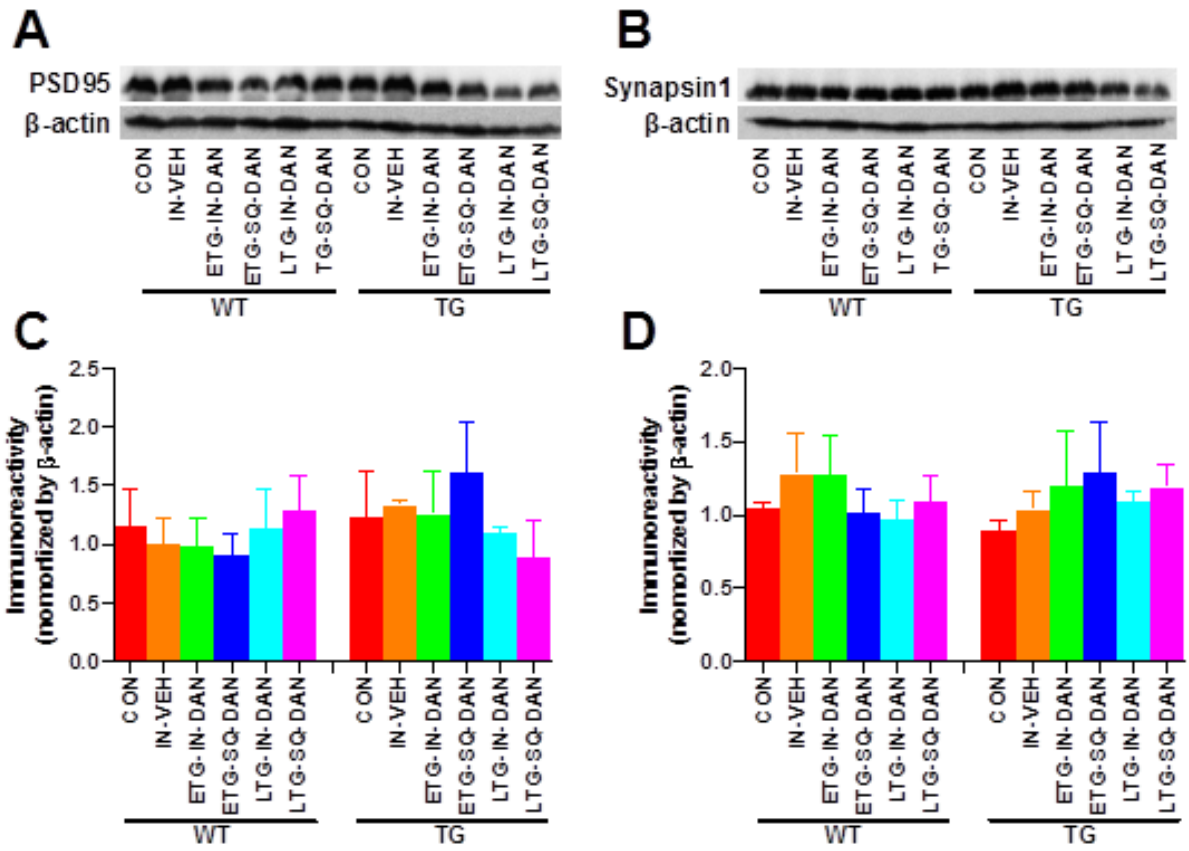


Supplementary Figure 4. Side effects after long-term dantrolene treatment in WT groups. Intranasal administration of dantrolene (IN-DAN) or vehicle (IN-VEH) and subcutaneous administration of dantrolene (SQ-DAN) were administered 3x/ week starting at 2 months of age for the early treatment group (ETG) or at 6 months of age for the late treatment group (LTG). A) Motor function was measured using the rotarod test for all groups at 10 months of age. No significant differences were detected between the treatment and control groups with the ordinary one-way ANOVA and Dunnett's multiple comparison test (MCT). (ETG: CON n=14, IN-VEH n=12, IN-DAN n=17, SQ-DAN n=14; LTG: IN-DAN n=15, SQ-DAN n=13.) B) Olfaction was measured using the food buried test for all groups at 10 months of age. No significant differences were found with the Kruskall-Wallis test for nonparametric data and Dunn's MCT. (ETG: CON n=14, IN-VEH n=8, IN-DAN n=12, SQ-DAN n=14; LTG: IN-DAN n=15, SQ-DAN n=13.) C) Liver function was evaluated for the ETG and LTG by measuring plasma alanine aminotransferase (ALT) activity. ALT was significantly increased after a 6-month subcutaneous

treatment in LTG compared with the control group using ordinary one-way ANOVA with Dunnett's MCT (p=0.0142). No significant difference was detected in other treatment groups compared with the control group with ordinary one-way ANOVA with Dunnett's MCT. (ETG: CON n= 7, IN-VEH n=8, IN-DAN n=8, SQ-DAN n=9, LTG: IN-DAN n=8, SQ-DAN n=7). All data are presented as Mean with 95% CI. D) Hepatic pathology was examined at 11 months of age in H&E stained sections of the ETG mice. No gross differences were observed between ETG groups (3 sections/animal: CON n= 3, IN-VEH n=3, IN-DAN n=3, SQ-DAN n=3, bar=50 μm). E) Mortality after chronic treatment of dantrolene (IN-DAN, SQ-DAN) and vehicle (IN-VEH) was compared to WT CON using Log-rank (Mantel-Cox) test and there were no significant differences (p=0.2388). F) No significant differences were detected in the growth curve in all WT groups with repeated measures Two-way ANOVA. (ETG: CON n=13, IN-VEH n=12, IN-DAN n=16, SQ-DAN n=13; LTG: IN-DAN n=13, SQ-DAN n=13.) All data are presented as Mean with 95% CI.



Supplementary Figure 5. Amyloid plaques levels in WT-CON and TG-CON mice. Representative micrographs of 6E10 immunoreactivity in the hippocampus and cortex of wild type (WT) (A) and 5xfAD (TG) (B) control animals are presented (bar=100 μm).



Supplementary Figure 6. Synaptic proteins expression in WT and TG mice. A, B) Synaptic proteins were determined by representing the expression of PSD95 (A) and synapsin1 (B) using western blot. C, D) Statistical analysis on PSD95 (C) or synapsin1 (D) protein expression. No significant differences were detected in all groups compared with controls with the ordinary 1way ANOVA and Dunnett's MCT. N=3 in each group. All data are presented as Mean with 95% CI.