Supplementary results corresponding to: CB1 receptor expression and signaling are required for dexamethasone-induced aversive memory consolidation.

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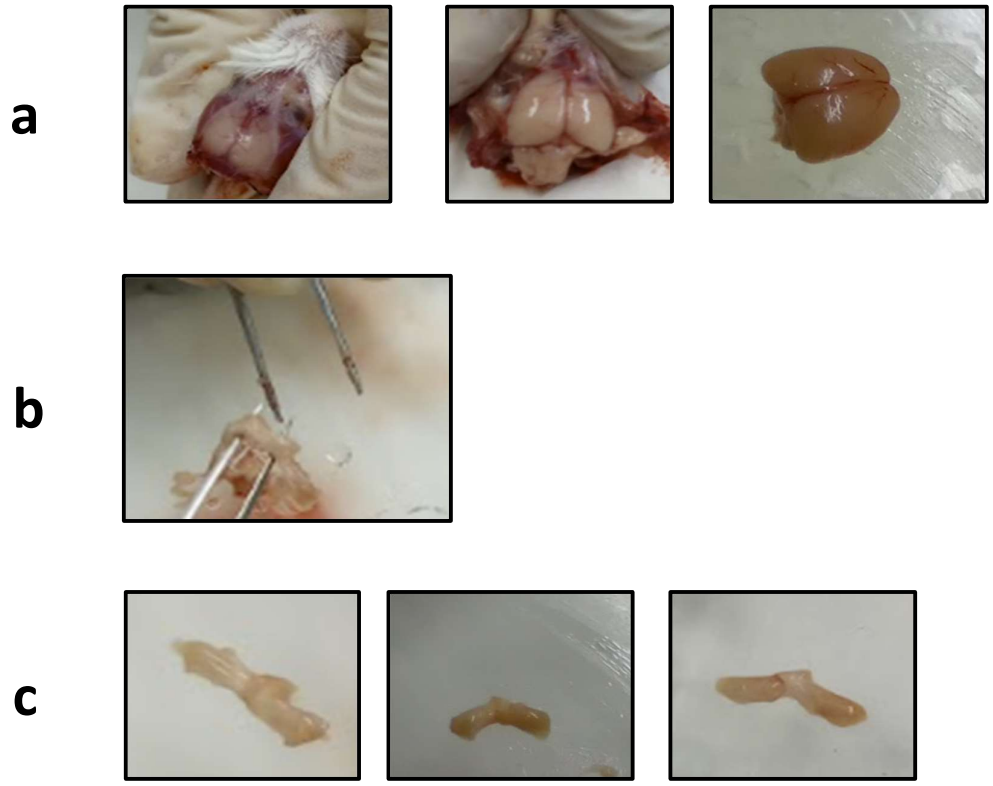
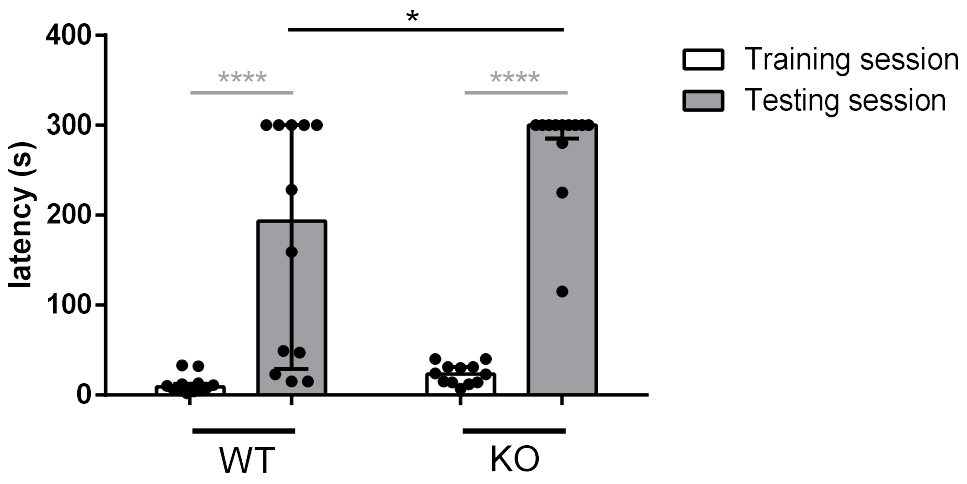
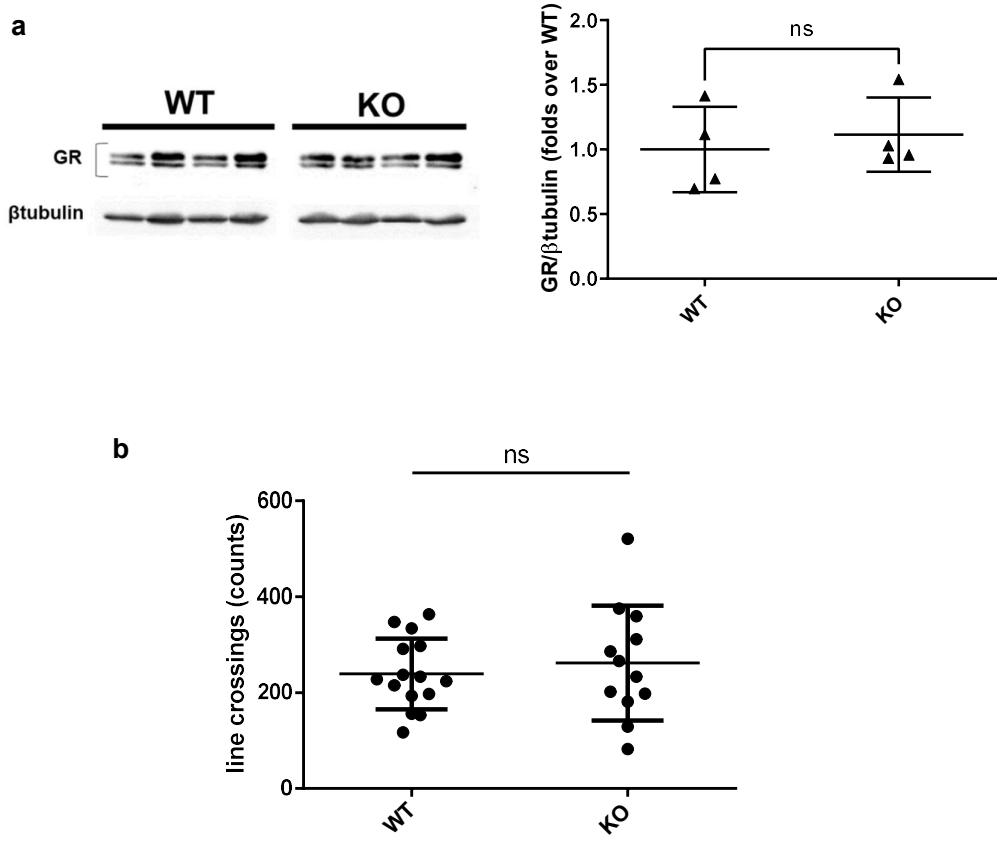


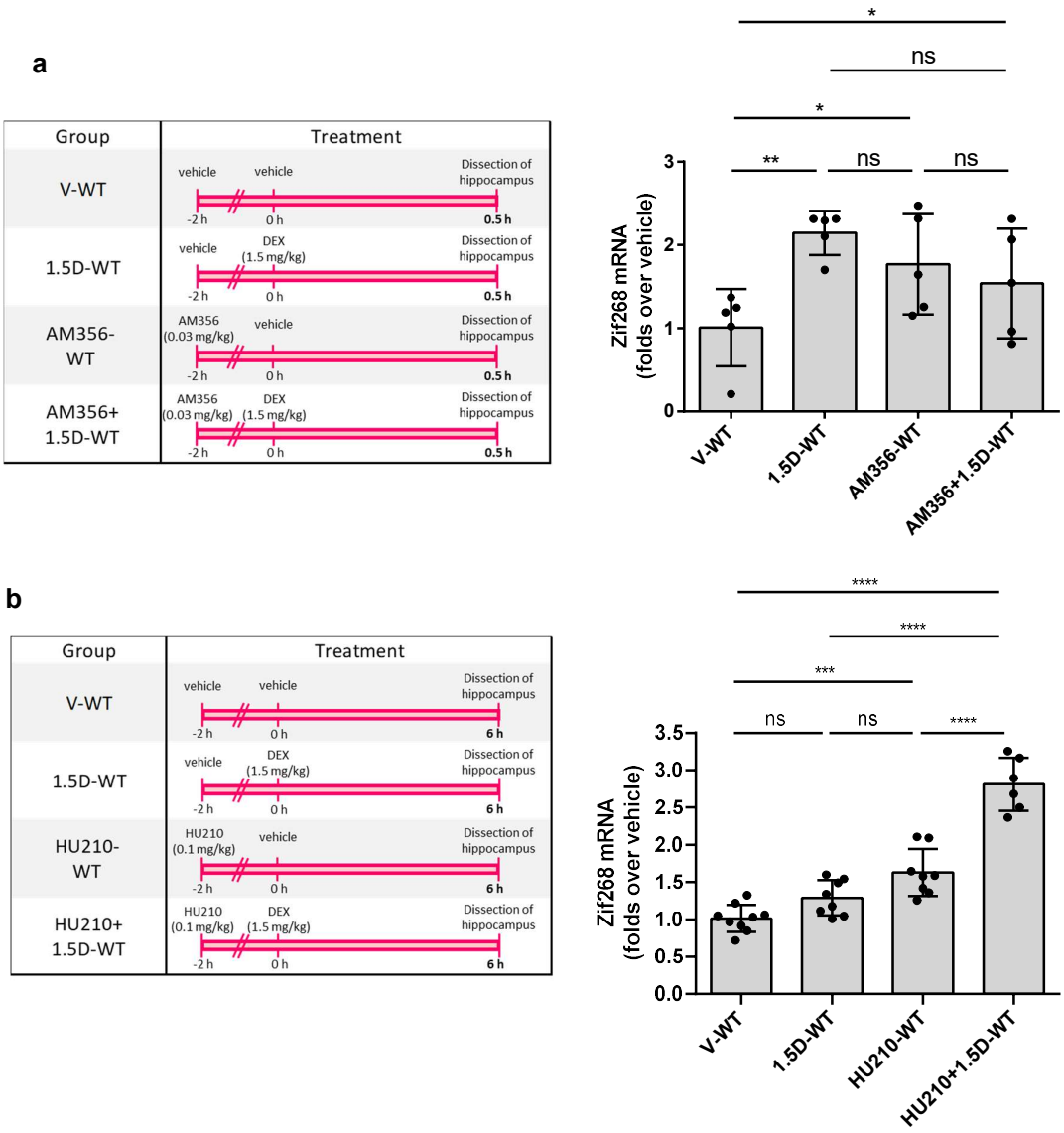
Supp Figure 1. Dissection of the hippocampus. (a) The brain was carefully removed from the skull by making small bilateral incisions on the skull bones while ensuring the preservation of the underlying brain tissue. A second cut was made across the bregma and lambda landmarks to facilitate skull detachment. The skull was gently lifted and detached from the surrounding brain regions using forceps. Subsequently, the brain was then delicately transferred to a cold Petri dish using a spatula. **(b)** To isolate the hippocampus, the cortex was gently removed using a spatula until the hippocampus could be identified. Fine-tipped forceps were then used to gently slide under the hippocampus from both sides, gradually lifting it away from the surrounding tissue. **(c)** Representative images of dissected hippocampus from mice. The images demonstrate the isolation of the hippocampus using the described dissection technique.



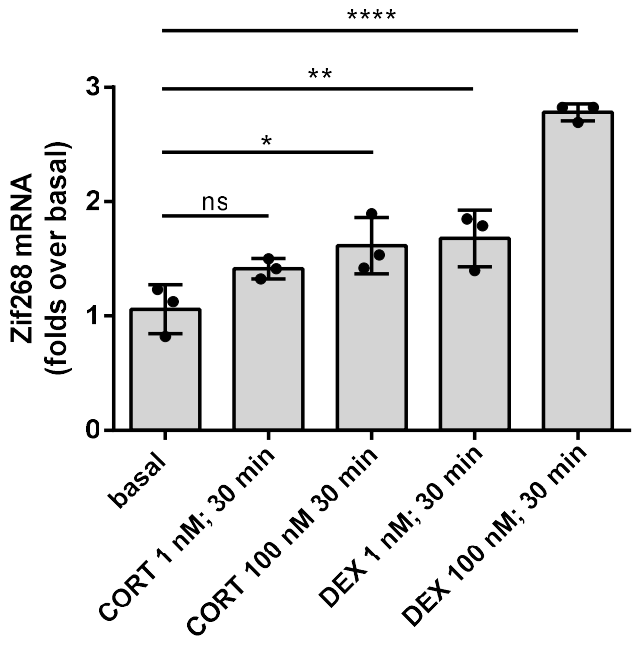
Supp Figure 2. CB1-deficient mice exhibit enhanced memory retention in an inhibitory avoidance paradigm when subjected to a high-intensity shock (0.35 mA). WT and CB1 KO mice were trained in an inhibitory avoidance task with a 0.35 mA shock. 48 h later, animals were tested to assess memory retention. The latency to enter the dark compartment was measured for both training and testing sessions. Data represent the median and interquartile range of at least 10 animals. Statistical analysis was performed with the nonparametric analysis of variance of Kruskal–Wallis and differences between groups were estimated by individual Mann–Whitney U tests (two-tailed). *p<0.05; ****p<0.001.



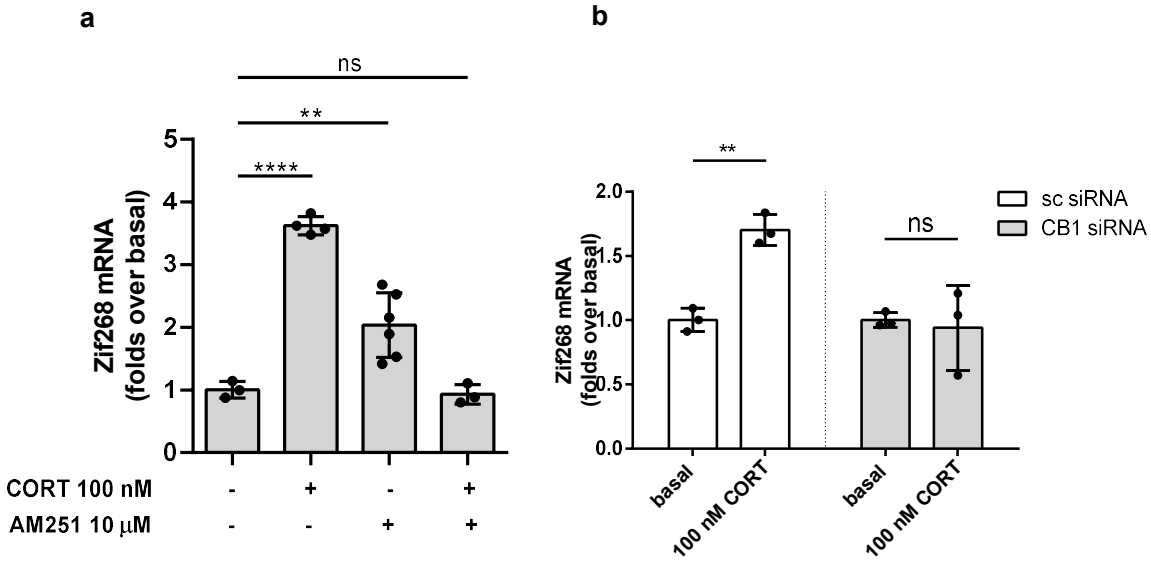
Supp Figure 3. Differences in GR expression levels and locomotor activity between WT and CB1 KO mice. (a) WT (n=4) and CB1 KO (n=4) mice were sacrificed under basal conditions. GR and βtubulin protein expression in hippocampal extracts were analyzed by western blot, and the corresponding images were quantified by densitometry (right panel). **(b)** WT and CB1 KO mice were placed in an open field for 5 minutes, during which their behavior was recorded. The recorded data were subsequently analyzed to quantify the instances of line crossings by the mice, where in all four paws crossed grid lines. Data are mean ± SD of at least 10 animals. Statistical analysis was performed by *t-test*. ns $p > 0.05$.



Supp Figure 4. **CB1 agonists AM356 and HU210 enhances Zif268 mRNA expression. (a)** Left panel: Schematic representation of the drug administration protocol: WT mice were pre-treated with the CB1 agonist AM356 (0.03 mg/kg, *i.p.*) or saline solution (vehicle) for a duration of 2 hours. Immediately following pre-treatment, the animals were administered DEX (1.5 mg/kg, *i.p.*) or vehicle. 0.5 hours mice were sacrificed, and the hippocampus was dissected to quantify Zif268 mRNA levels by qPCR (right panel), as described in the Methods section. **(b)** Left panel: Schematic representation of the drug administration protocol: WT mice were pre-treated with the CB1 agonist HU210 (0.1 mg/kg, *i.p.*) or saline solution (vehicle) for a duration of 2 hours. Immediately following pre-treatment, the animals were administered DEX (1.5 mg/kg, *i.p.*) or vehicle. After 6 hours mice were sacrificed, and the hippocampus was dissected to quantify Zif268 mRNA levels by qPCR (right panel), as described in the Methods section. Statistical analysis was performed using one-way ANOVA, with Tukey's test for comparisons between all groups. Data represents the mean ± SD of at least 5 animals. Statistical significance is indicated as ** p<0.05; **p<0.01; ***p<0.005; and ****p<0.0001. ns p>0.05.*



Supp Figure 5. Corticosterone and dexamethasone effect on Zif268 mRNA in HT22 cell line. HT22 cells were treated for 30 min with different concentrations of corticosterone or dexamethasone as indicated. Statistical analysis was performed by one-way ANOVA and comparisons between all groups were made with Tukey's test. Zif268 mRNA levels were quantified by qPCR as described in the methods section. Data are the individual data points and the mean ± SD of three independent experiments. Each experiment was performed and measured in triplicates. **p<0.05; **p<0.01; **** p<0.0001. ns p>0.05.*



Supp Figure 6: **Inhibition of CB1 receptor signaling disrupts CORT-induced Zif268 gene expression. (a)** HT22 cells were treated for 30 minutes with 10 µM AM251, 100 nM corticosterone (CORT), or a combination of both ligands. Zif268 mRNA levels were quantified by qPCR as described in the methods section. Statistical analysis was performed by two-way ANOVA and comparisons between all groups were made with Tukey's test. **(b)** HT22 cells transfected with scrambled siRNA or CB1R-directed siRNA were incubated with 100 nM corticosterone (CORT) for 30 min. Zif268 mRNA levels were quantified by qPCR as described in the methods section. Statistical analysis was conducted using a two-way analysis of variance (ANOVA), followed by pairwise comparisons between each treatment and the corresponding group's baseline using Dunnett's test. Data are mean ± SD of three independent experiments performed in triplicates. ***p<0.01; **** p<0.0001. ns p>0.05.*