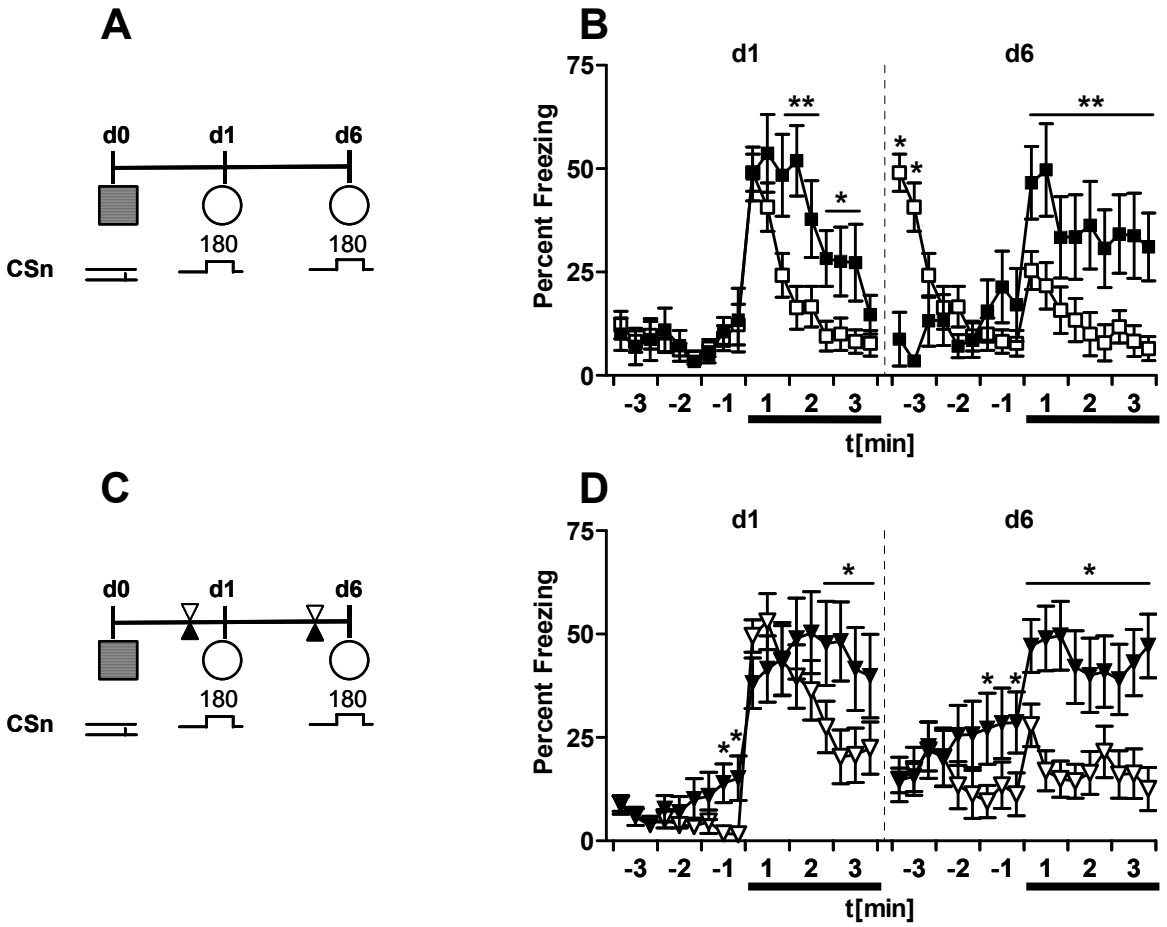

Supplementary Figure 3: Differential involvement of CB1 in regulation of pre-tone and tone freezing following sensitization procedures.

(A) Experimental procedure: Mice with genetic ablation of CB1 ($CB1^{-/-}$, ■, n = 10; $CB1^{+/+}$, □, n = 15; cf. Fig. 3A) and **(C)** inbred C57BL/6N mice with acute pharmacological blockade of CB1 (3mg/kg SR141716A, SR; ▼, n = 17; vehicle, V; ▽, n = 17; cf. Fig. 3D) before tone presentation were exposed to a 180-s tone one and six days after sensitization. **(B)** Genetic inactivation of CB1 failed to affect pre-tone freezing to the test contex (day 1; *Genotype x Interval:* $F_{8,184}$ = 0.26, P = 0.978), but caused a sustained freezing response to the subsequently presented tone (black bar indicates duration of tone presentation). Because of yet unknown reasons, $CB1^{+/+}$ showed a more pronounced pre-tone freezing before the second tone presentation than $CB1^{-/-}$ (day 6; *Genotype x Interval:* $F_{8,176}$ = 3.57, P < 0.001). **(D)** Mice with pharmacological blockade of CB1 showed a slight increase in freezing towards the onset of tone presentation both at day 1 (*Drug x Interval:* $F_{8,256}$ = 2.67, P = 0.007) and at day 6 (*Drug x Interval:* $F_{8,256}$ = 2.71, P = 0.007). Tone presentation (black bar indicates duration of tone presentation) caused a sharp increase in freezing in both antagonist and vehicle treated mice. 2-way ANCOVAs [(*Drug, Interval*) for repeated measures (*Interval*)] with the covariate *Freezing before tone* still revealed a significant *Drug x Interval* interaction at day 1 ($F_{8,256}$ = 5.5, P < 0.0001) as well as a significant effect of *Drug* at day 6 ($F_{1,31}$ = 5.8, P = 0.021), even if the last 20-s before tone presentation were considered as covariate, indicating that pharmacological blockade of CB1 caused a sustained freezing response to the tone independently of potential influences on pre-tone behavior. Data were normalized to 20-s observation intervals. * P < 0.05, ** P < 0.01 vs. other genotype. Together these data indicate that genetic ablation and pharmacological blockade of CB1 have inconsistent effects on pre-tone freezing but consistently cause a sustained freezing response to the tone. Apparently, CB1 differentially affects pre-tone and tone freezing with the consequence that context generalization unlikely accounts for the sustained freezing response to the tone.