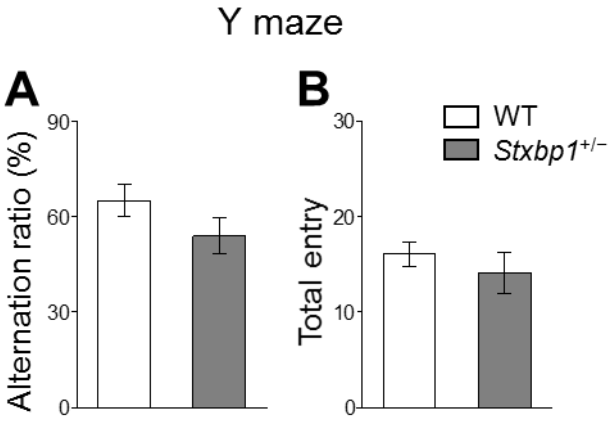
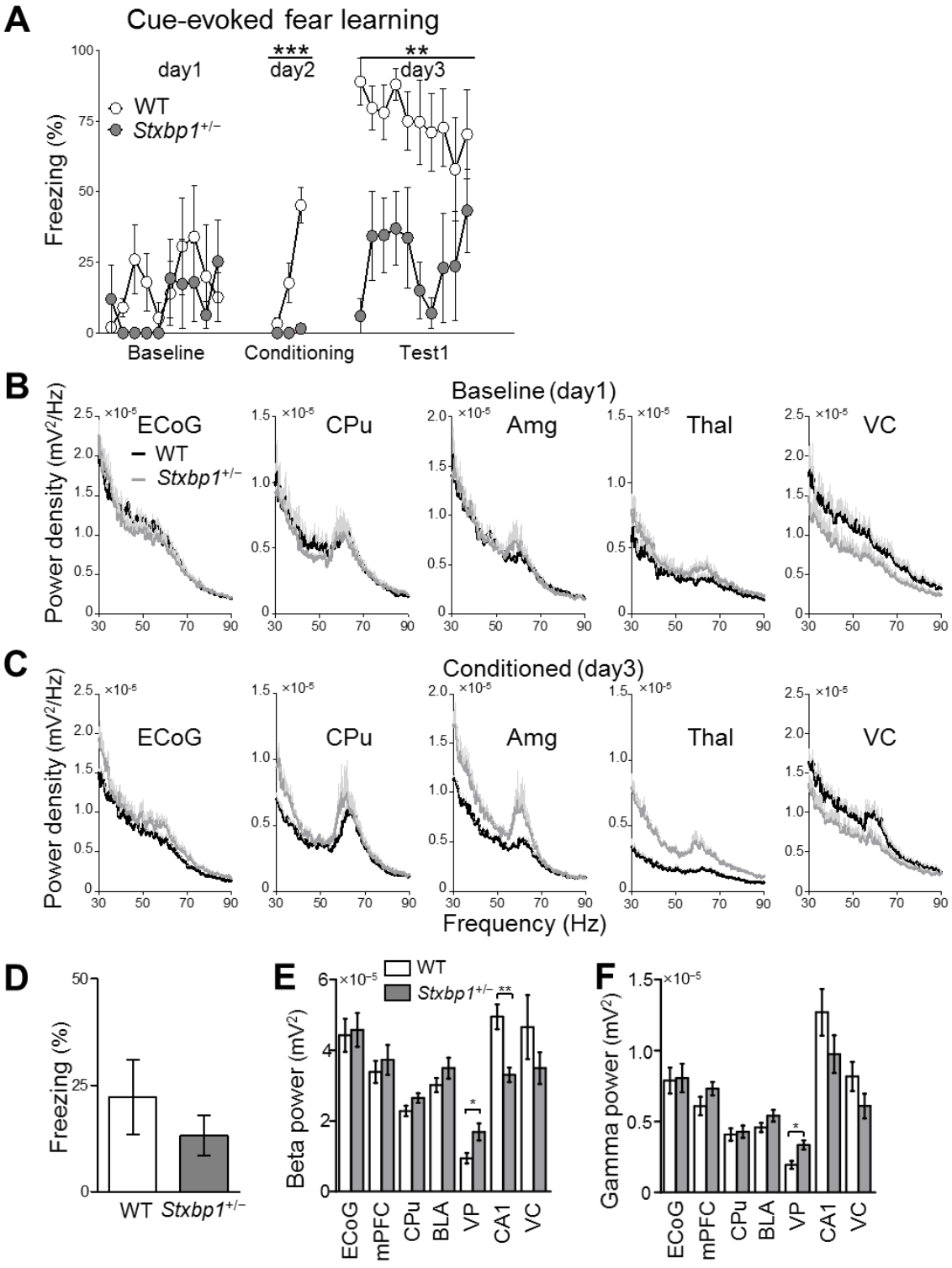


Supplementary Figure S1. ***Stxbp1*$^{+/-}$ mice retained normal short-term memory on the Y-maze test**

(A) Alternation rate during the test (10 min). Alternation rate is the frequency of entry into another arm different ("correct response"). WT (n = 10) *vs.* ***Stxbp1*$^{+/-}$** mice (n = 9), male, 9 months old, *t*-test, $t_{17} = 1.458$, $p = 0.1632$.

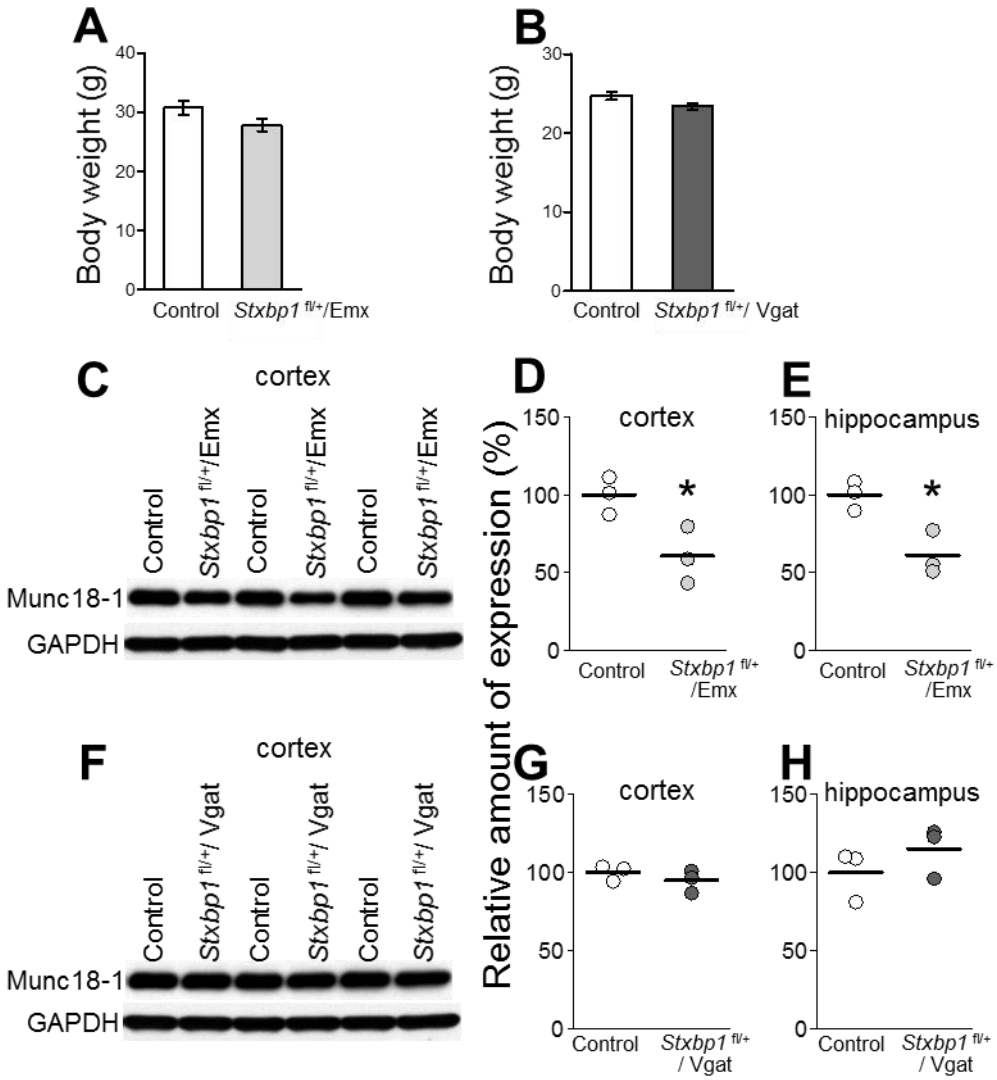
(B) The number of total entries into the arms. *t*-test, $t_{17} = 0.8217$, $p = 0.4226$.



Supplementary Figure S2. Impaired fear conditioning of naive *Stxbp1*$^{+/-}$ mice and their LFP recordings

(A) Time course of freezing responses (30-sec bin) in the tone-evoked fear conditioning concurrent with LFP recordings (see Figure 3). Two-way repeated measures ANOVA, WT (n = 5) vs. *Stxbp1*$^{+/-}$ (n = 5), baseline: $F_{1,72} = 0.72$, $p = 0.4207$; conditioning: $F_{1,16} =$

56.87, ***$p < 0.0001$; test1: $F_{1,72} = 14.83$, **$p = 0.0049$.
(B) Power spectra of brain regions before fear conditioning (baseline, day1) in WT (n = 5) and *Stxbp1*$^{+/-}$ (n = 5) mice (5 months of age).
(C) Power spectra of brain regions 24 hours after fear conditioning (conditioned, day 3). LFP recordings (1 amygdala and 1 CPu from 1 *Stxbp1*$^{+/-}$ mouse) were excluded from the analysis due to recording problems (B, C).
(D) Group analysis of freezing before tone presentations 24 hours after fear conditioning (Fig.3C, 0–180 sec). *t*-test, $t_{18} = 0.9075$, $p = 0.3761$.
(E) Regional beta band activity (10–30 Hz) before tone presentations 24 hours after fear conditioning (related to Fig. 3E–J and Supplementary Figure S3A–C). LFP recordings (1 amygdala and 1 CPu from 1 *Stxbp1*$^{+/-}$ mouse) were excluded from the analysis due to recording problems. WT *vs. Stxbp1*$^{+/-}$ mice, *t*-test, S1 ECoG: $t_8 = 0.2307$, $p = 0.8234$; mPFC: $t_8 = 0.6474$, $p = 0.5355$; CPu: $t_7 = 1.774$, $p = 0.1194$; BLA: $t_7 = 1.405$, $p = 0.2029$; VP: $t_8 = 2.623$, *$p = 0.0305$; CA1: $t_8 = 4.041$, **$p = 0.0037$; VC: $t_8 = 1.148$, $p = 0.2841$. Two-way repeated measures ANOVA, genotype: $F_{1,42} = 0.00$, $p = 0.9895$; region: $F_{6,42} = 21.11$, $p < 0.0001$; interaction: $F_{6,42} = 3.39$, $p = 0.0081$; Bonferroni post-hoc test, not significant.
(F) Regional gamma band activity (50–70 Hz) before tone presentations 24 hours after fear conditioning (related to Fig. 3E–J and Supplementary Figure S3A–C). LFP recordings (1 amygdala and 1 CPu from 1 *Stxbp1*$^{+/-}$ mouse) were excluded from the analysis due to recording problems. WT *vs. Stxbp1*$^{+/-}$ mice, *t*-test, S1 ECoG: $t_8 = 0.1343$, $p = 0.8965$; mPFC: $t_8 = 1.531$, $p = 0.1643$; CPu: $t_7 = 0.3250$, $p = 0.7547$; BLA: $t_7 = 1.607$, $p = 0.1520$; VP: $t_8 = 3.306$, *$p = 0.0108$; CA1: $t_8 = 1.394$, $p = 0.2009$; VC: $t_8 = 1.549$, $p = 0.1599$. Two-way repeated measures ANOVA, genotype: $F_{1,42} = 0.00$, $p = 0.9958$; region: $F_{6,42} = 32.54$, $p < 0.0001$; interaction: $F_{6,42} = 1.79$, $p = 0.1246$; Bonferroni post-hoc test, not significant.



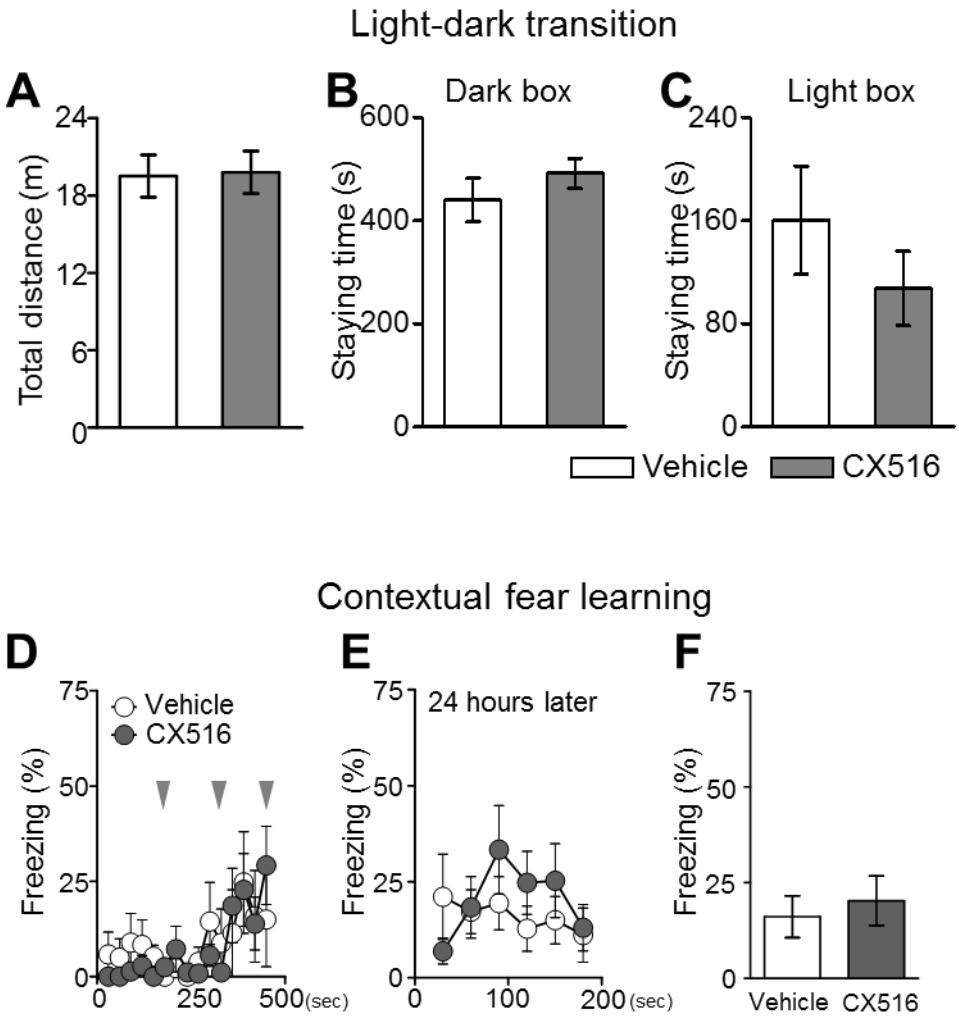
Supplementary Figure S3. **Munc18-1 expressions in *Stxbp1* conditional knockout mice**

(A) Body weights of *Stxbp1*$^{fl/+}$/*Emx* (n = 6) and control (5 *Stxbp1*$^{fl/+}$ and 1 *Stxbp1*$^{+/+}$/*Emx*, n = 6) mice (3 months of age). There is no statistically significant difference between genotypes (t_{10} = 1.791, p = 0.1036). *Emx1*-Cre is specifically expressed in dorsal-telencephalic excitatory neurons, namely in the cerebral cortex, hippocampus, amygdala, and olfactory bulb, but not the basal ganglia and thalamus (25,26). *Vgat*-Cre is specifically expressed in global inhibitory neurons (26).

(B) Body weights of *Stxbp1*$^{fl/+}$/*Vgat* (n = 8) and control (*Stxbp1*$^{fl/+}$, n = 8) mice (2 months of age). There is no statistically significant difference between genotypes (t_{14} = 2.028, p = 0.0620).

(C–E) Western blots of cerebral cortex samples of *Stxbp1*$^{fl/+}$/*Emx* (n = 3) and control (2 *Stxbp1*$^{fl/+}$, 1 *Stxbp1*$^{+/+}$/*Emx*, n =3) mice (3–4 months of age) probed with anti-Munc18-1 or anti-GAPDH antibodies (C). Quantification of Munc18-1 protein expression in the neocortex (D) and the hippocampus (E). Control *vs.* *Stxbp1*$^{fl/+}$/*Emx* mice, *t*-test, cortex: t_4 = 3.116, *p = 0.0357; hippocampus: t_4 = 3.970, *p = 0.0165.

(F–H) Western blots of cerebral cortex samples of *Stxbp1*$^{fl/+}$/*Vgat* (*n* =3) and control (1 WT, 1 *Stxbp1*$^{fl/+}$, 1 *Stxbp1*$^{+/+}$/*Vgat*, *n* =3) mice (3 months of age) probed with anti-Munc18-1 or anti-GAPDH antibodies (F). Quantification of Munc18-1 protein expression in the neocortex (G) and the hippocampus (H). Control *vs. Stxbp1*$^{fl/+}$/*Vgat* mice, *t*-test, cortex: $t_4 = 1.026$, $p = 0.363$; hippocampus: $t_4 = 1.107$, $p = 0.3302$.



Supplementary Figure S4. The effects of CX516 on anxiety and fear learning in *Stxbp1*$^{+/-}$ mice

(A–C) The effect of CX516 on light-dark transition test in *Stxbp1*$^{+/-}$ (vehicle: $n = 6$, CX516: $n = 6$) male mice (2–3 months of age). Total distances traveled (A) (*t*-test, $t_{10} = 0.1234$, $p = 0.9043$), time spent in the dark box (B) (*t*-test, $t_{10} = 1.008$, $p = 0.3373$) and the light box (C) (*t*-test, $t_{10} = 1.032$, $p = 0.3263$) did not differ significantly between vehicle and CX516 group. Vehicle or CX516 (40 mg/kg) was intraperitoneally given 10 min before the test.

(D–F) The effect of CX516 on contextual fear memory test in *Stxbp1*$^{+/-}$ (vehicle: $n = 6$, CX516: $n = 6$) male mice (2–3 months of age). Conditioned fear responses (D), fear expression 24 hours after fear conditioning (E) did not differ between vehicle and CX516 group. Two-way repeated measures ANOVA, vehicle *vs.* CX516, conditioning: $F_{1,140} = 0.05$, $p = 0.8330$; testing: $F_{1,50} = 0.24$, $p = 0.6325$. Averaged freezing time expressed as percent of recording time (30-sec bin). (F) Averaged freezing time (%) during the test, vehicle *vs.* CX516, *t*-test, $t_{10} = 0.4933$, $p = 0.6325$. Vehicle or CX516 (40 mg/kg) was intraperitoneally given 10 min before the fear conditioning (D).

Supplementary Movie S1
Resident-intruder test of a male *Stxbp1*$^{+/-}$ mouse (5 months old, housed individually over one week, black). Vehicle (150 μL saline) was intraperitoneally injected 10 min before the test. A male, white BALB/c mouse (5 weeks) was introduced to the resident's home cage as an intruder just after starting the recording (movie length: 24 sec).

Supplementary Movie S2
Resident-intruder test of the same *Stxbp1*$^{+/-}$ mouse (Supplementary Movie 1) on the next day. CX516 (100 mg/kg) was intraperitoneally injected 10 min before the test. No aggressive behavior was observed (movie length: 59 sec).

Supplementary Movie S3
One hundred minutes after CX516 injection, the *Stxbp1*$^{+/-}$ mouse recovered their aggressiveness (movie length: 36 sec).