Supplemental Data:

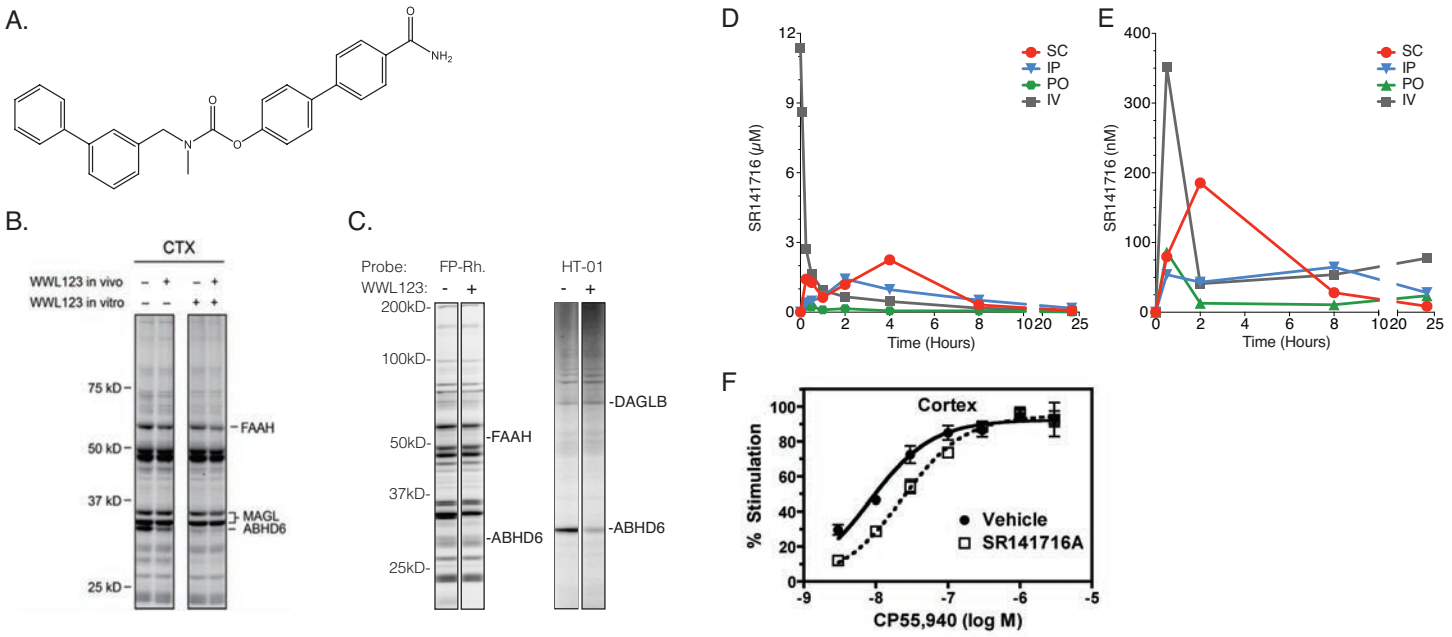

Figure S1, related to Figure 1: *Pharmacokinetics and functional validation of WWL123 and SR1.* (A) The chemical structure of WWL123 is shown. (B) Four hours after i.p. administration of 10 mg/kg WWL123 (n=4) or vehicle (n=4), cortical homogenates were prepared and activity based protein profiling was used to demonstrate selective inhibition of ABHD6 activity, but not MGL or FAAH activity. (B) Four hours after i.p. administration of 10 mg/kg WWL123 (n=3) or vehicle (n=3), whole-brain homogenates were prepared as previously described (Hsu et al., 2012) and membrane proteomes were analyzed by gel-based ABPP using the tailored or broad-spectrum activity-based probes HT-01 or FP-Rh (1 μM probe), respectively (Hsu et al., 2013a; 2013b). We conclude that WWL123 crosses the blood brain barrier, and selectively inactivates ABHD6 at a dose of 10 mg/kg i.p. (D) Blood levels of SR141716 (SR1) after i.v, i.p., p.o., or s.c. administration (4 mice per time point, per dosing regimen, see Table S1 for detailed results). Subcutaneous administration resulted in a prolonged rise in SR1 levels that peaked at 4 hours (C_{max} = 2.25μM). (E) Brain levels of SR1 were highest for i.v. injection at 30 minutes (C_{max} = 350nM), but s.c. administration resulted in similar levels (C_{max} = 190nM) which were more broadly sustained. (F) Dose-response of GTPγS stimulation by CP55940 performed on homogenized cortical tissue demonstrated that 10mg/kg s.c. SR1 (n=5, both SR1 and vehicle) results in functional CB_1 antagonism in brain two hours after administration. Error bars show SEM.

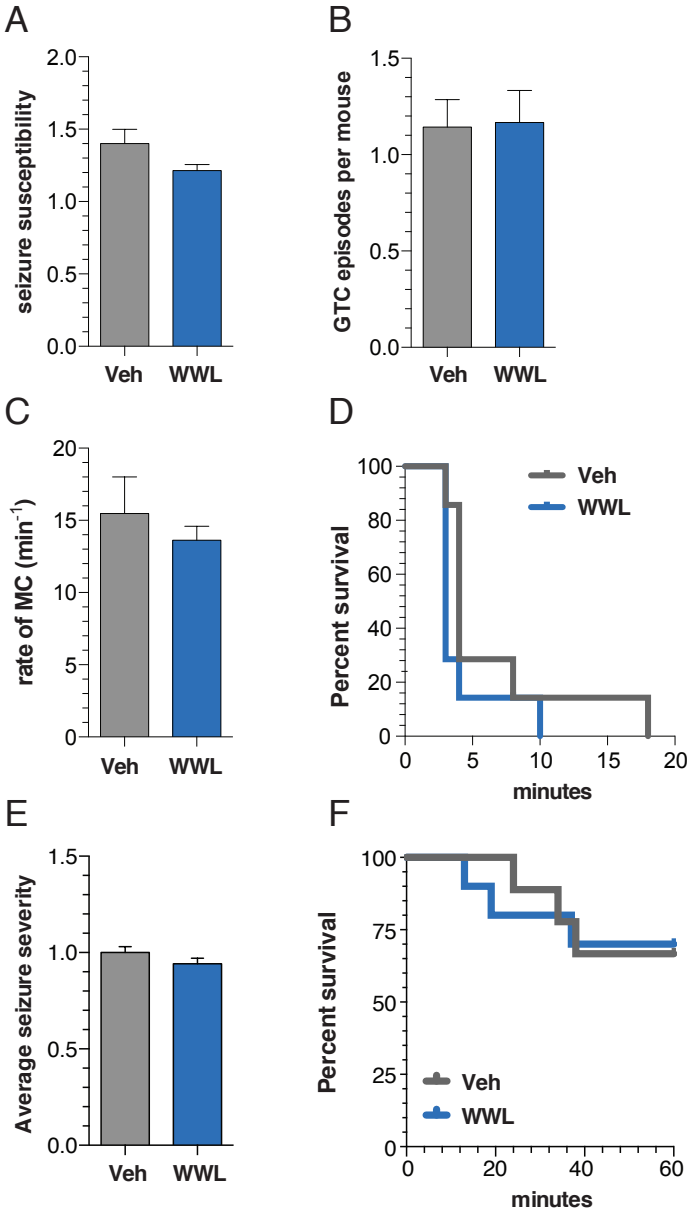

Figure S2, related to Figure 1: *WWL123 is not protective against a higher dose of PTZ or against kainate-induced seizures.* We pretreated animals with vehicle (n=6) or 10 mg/kg WWL123 (n=6), and four hours later induced epileptiform seizures by administering 70 mg/kg PTZ (i.p.). We measured no difference between treated and untreated mice in epileptiform seizure susceptibility (A), GTCs per mouse (B), MC (C), or mortality (D). Similarly, we tested the effect of WWL123 on kainate-induced epileptiform seizures. We pretreated mice with vehicle (n=7) or 10 mg/kg WWL123 (n=8), and then induced seizures by administering 30 mg/kg kainate (i.p.). We measured no difference between treated and untreated mice in seizure severity (E,) or mortality (F). Error bars depict SEM.

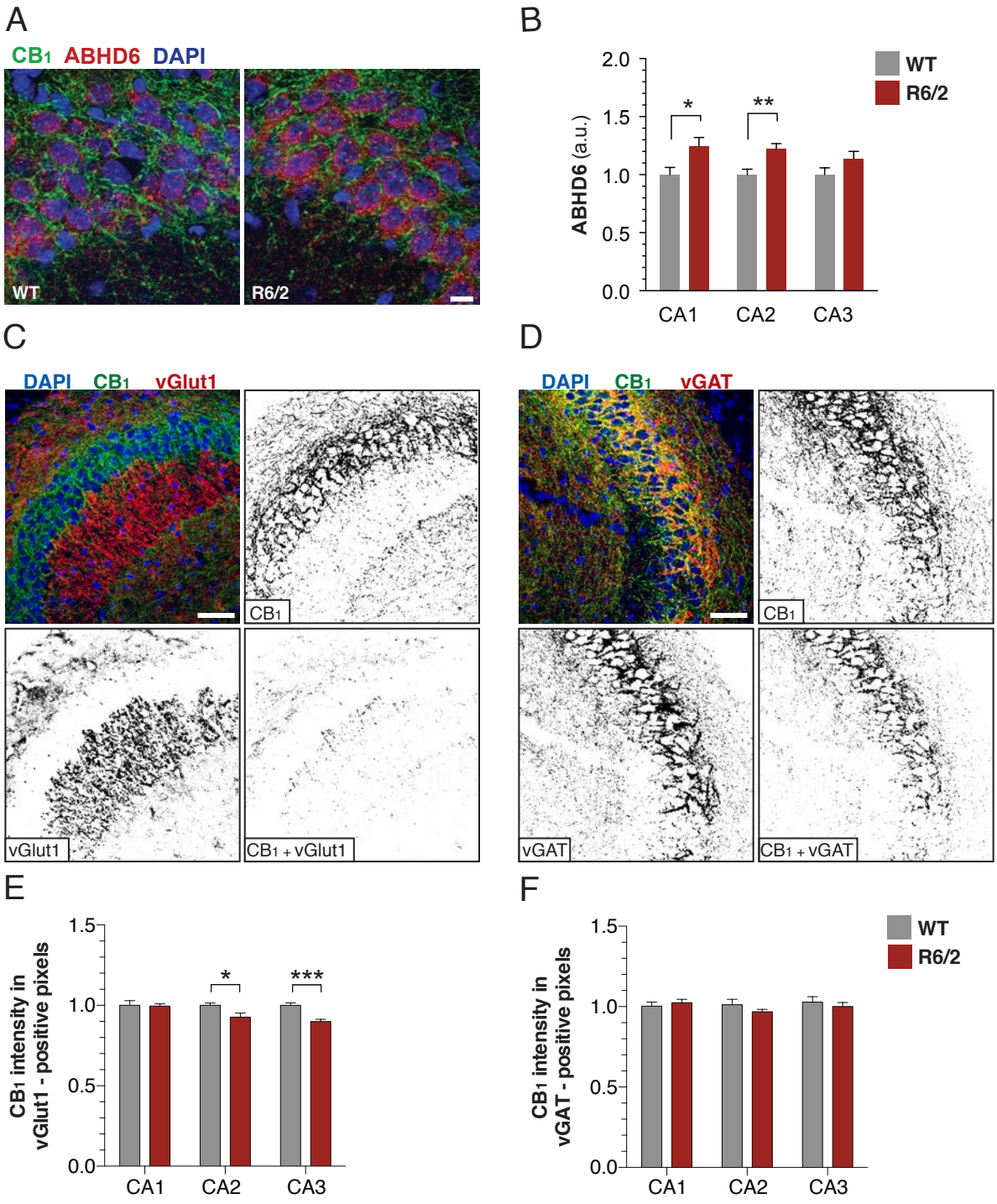

Figure S3, related to Figure 4: *ABHD6 and CB_1 immunoreactivity in R6/2 hippocampus.* (A) Representative images are presented for CB_1 receptor and ABHD6 staining in WT (n=5) and R6/2 mice (n=7), scale bars depict 10 µm. After thresholding to mean + SD, average intensity of ABHD6 staining was quantified in the remaining pixels (B). We found a significant increase in ABHD6 immunoreactivity in the CA1 (one-way ANOVA, p=0.04) and CA2 (one-way ANOVA, p=0.006) regions of R6/2 mice compared to wild-type mice.

Representative images and mean+SD masks are presented for CB_1/vGLUT1 (A) and CB_1/vGAT (B) costaining (Scale bars depict 50 μm). Masks were generated in ImageJ and used to perform a Boolean operation to generate a map of pixels that passed threshold for both channels (ex. CB_1 + vGLUT1). These combined masks were used to measure CB_1 intensity in only those pixels whose intensity is greater than mean+SD in both channels, to generate the final intensity measures in glutamatergic (C) and GABAergic (D) synapses. There was a significant effect of genotype in glutamatergic synapses (one way, $p<0.001$; n=5 WT, n=7 R6/2), but not at GABAergic synapses (one way, $p=0.432$; n=5 WT, n=8 R6/2). Error bars show SEM, and post-hoc tests were conducted with Fisher's T test, with $*P<0.05$, $**P<0.01$, $***P<0.001$.

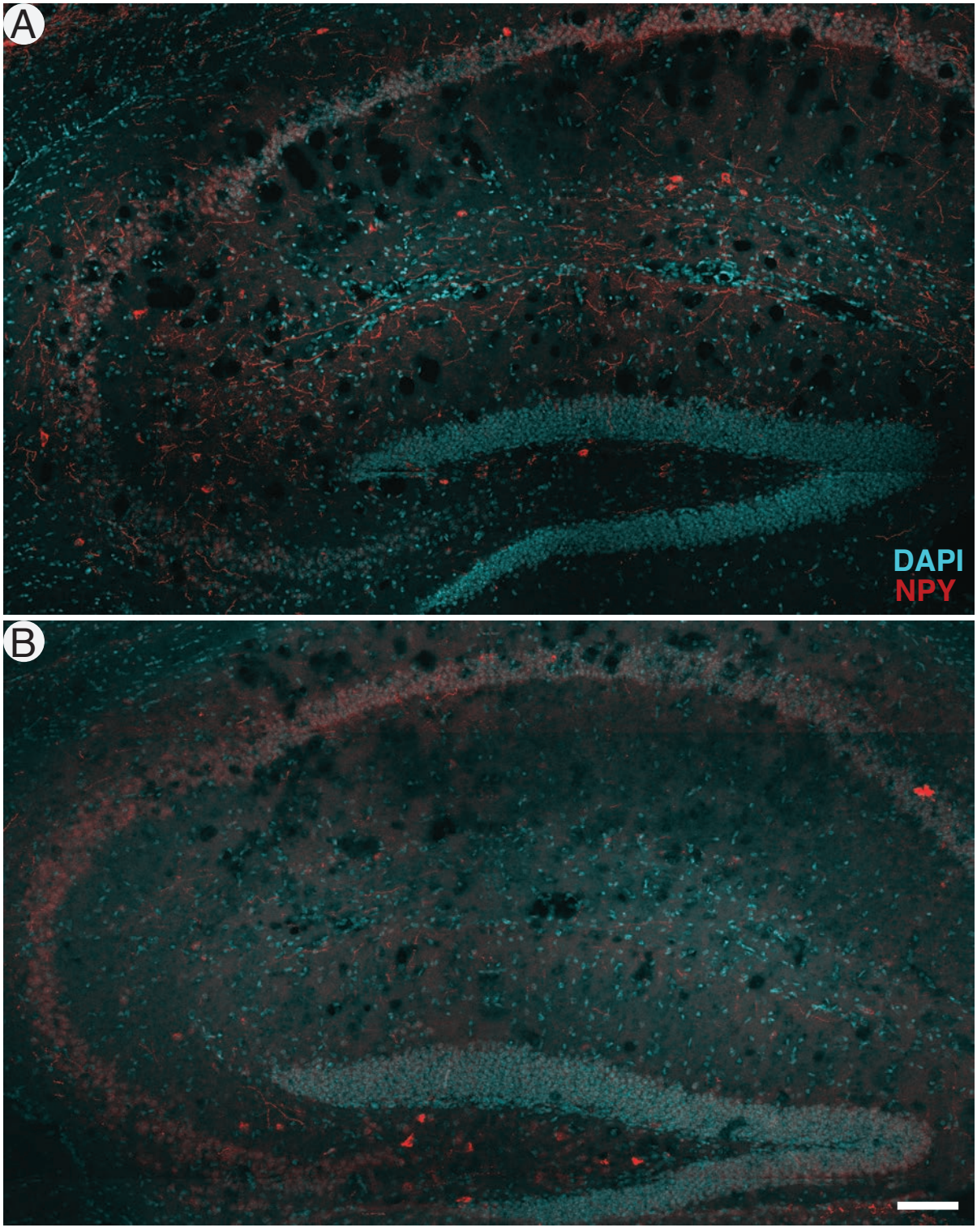

Figure S4, related to Figure 4: *Loss of NPY staining in R6/2 hippocampus*. Montages of hippocampal NPY immunostaining are presented for visualization of detail. Compared to WT (A), a notable loss of NPY interneuron branches and staining intensity is observed in R6/2 mice (B). Scale bar depicts 100 μm.

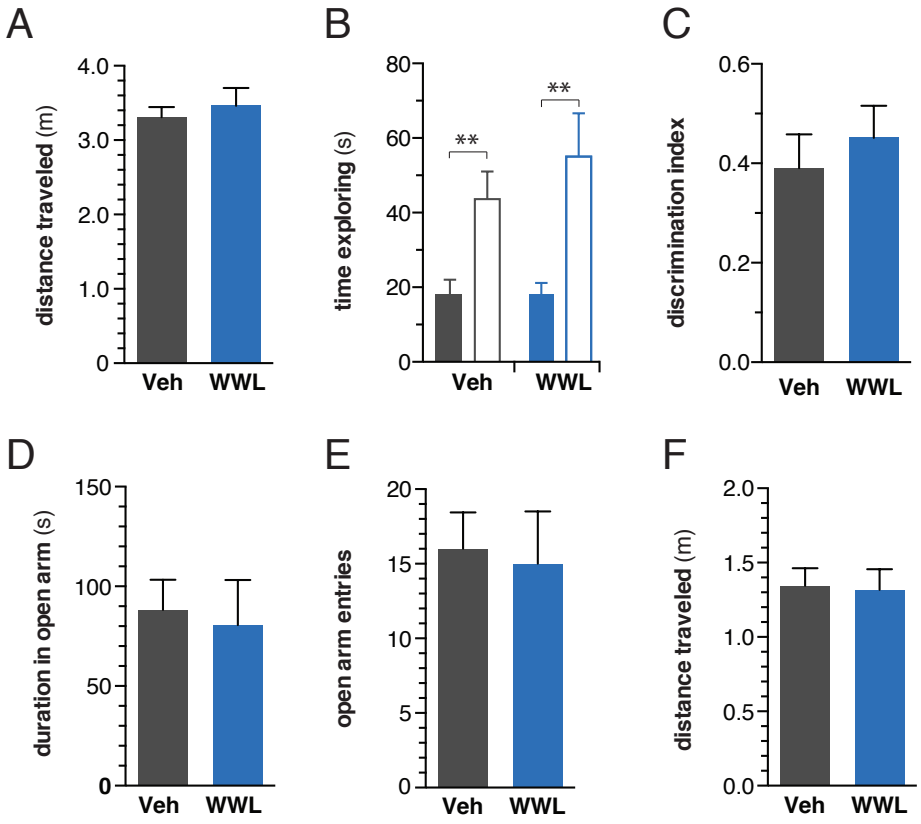

Figure S5, related to Figure 5: *WWL123 does not cause psychomotor impairment.* To test whether WWL123 causes psychomotor impairment, we measured locomotion, memory retention, and anxiety-like behavior. (A) Four hours after vehicle (n=6) or 10 mg/kg WWL (n=6), WT mice were placed in an open field chamber for 10 minutes, and movement was video-recorded and analyzed in Ethovision. Memory impairment was tested by novel object recognition: animals (n=11 vehicle, n=14 WWL) were habituated to two identical objects, and then after one-hour were reintroduced to the chamber with one familiar and one novel object. (B) Behavior was video-recorded and analyzed in Ethovision for time spent exploring (C) either the familiar (closed bars) or novel (open bars) object and for object recognition index (two-way ANOVA, object: $p<0.001$, treatment: $p=0.496$, interaction: $p=4.33$). To measure anxiety, animals were placed in an elevated plus maze, and were analyzed for duration (D) or number of entries (E) in the open arms. Similar to what we observed in the open field assay, there was no difference in locomotion between vehicle or WWL123-treated mice on elevated plus maze (F). Statistical testing was performed with Fisher's T-test, with *$P<0.05$, **$P<0.01$. Error bars show SEM.