Supplemental Tables:

Table S1: Pharmacokinetics of SR141716.

PARAMETER	IV	PO	IP	SC
Dose (mg/kg)	5	10	10	10
Total Drug Exposure(0-24 hr; ng x hr/mL)	3721	628	5724	6272
Bioavailability (%)	100	8	77	84
C_{max} (ng/mL)	5277	114	665	1041
T_{max} (hrs)	0.0	0.25	2	4
Trough (8 hr)	77	29	239	144
C_{max}/C_{min}	69	4	3	7
Brain AUC (0-24 hr; ng x hr/ml)	756	196	528	525
Brain Penetrance (%)	20	31	9	8

Table S2: List of antibodies for sq-IHC, threshold, dilutions, and suppliers used.

Antibody	**Threshold**	**Dilution**	**Supplier**
NPY	Mean + (2 × SD)	1:2000	Immunostar #22940
vGAT	Mean + SD	1:1000	Synaptic Systems #131011
vGLUT1	Mean + SD	1:500	Synaptic Systems #135311
CB_1	Mean + SD	1:5000	Cayman #10006590
ABHD6	Mean + SD	1:500	Gift from Ken Mackie

Table S3: Statistical testing for experiments presented in Figure 2

Experiment	Type of ANOVA	Susceptibility	GTC	MC
Geno: WT and *cnr1*$^{-/-}$ Tx: WWL123/veh	One-way: WWL123	$F_{(1,50)} = 8.98$ $p = 0.004$	$F_{(1,50)} = 13.72$ $p = 0.001$	$F_{(1,48)} = 12.07$ $p = 0.001$
	One-way: genotype	$F_{(1,50)} = 0.34$ $p = 0.562$	$F_{(1,50)} = 3.70$ $p = 0.060$	$F_{(1,48)} = 0.06$ $p = 0.805$
	Interaction: WWL123 x genotype	$F_{(1,50)} = 0.08$ $p = 0.783$	$F_{(1,50)} = 0.79$ $p = 0.378$	$F_{(1,48)} = 0.15$ $p = 0.701$
Geno: WT and *cnr2*$^{-/-}$ Tx: WWL123/veh	One-way: WWL123	$F_{(1,38)} = 15.72$ $p = 0.0003$	$F_{(1,38)} = 1.45$ $p = 0.236$	$F_{(1,38)} = 11.70$ $p = 0.002$
	One-way: genotype	$F_{(1,38)} = 0.35$ $p = 0.560$	$F_{(1,38)} = 7.34$ $p = 0.010$	$F_{(1,38)} = 2.50$ $p = 0.123$
	Interaction: WWL123 x genotype	$F_{(1,38)} = 0.27$ $p = 0.605$	$F_{(1,38)} = 1.45$ $p = 0.235$	$F_{(1,38)} = 1.10$ $p = 0.302$
Geno: WT Tx: WWL123/veh Tx: picrotoxin/veh	One-way: WWL123	$F_{(1.62)} = 6.39$ $p = 0.015$	$F_{(1.62)} = 2.00$ $p = 0.162$	$F_{(1.62)} = 4.36$ $p = 0.042$
	One-way: picrotoxin	$F_{(1.62)} = 7.44$ $p = 0.008$	$F_{(1.62)} = 6.83$ $p = 0.011$	$F_{(1.62)} = 0.28$ $p = 0.599$
	Interaction: WWL123 x picrotoxin	$F_{(1,62)} = 4.99$ $p = 0.029$	$F_{(1,62)} = 4.99$ $p = 0.029$	$F_{(1,62)} = 4.08$ $p = 0.048$

Supplemental Experimental Procedures:

Pharmacokinetics of SR141716: To test the distribution of SR141716 (SR1) following different administration routes, C57Bl/6 mice were dosed with 10 mg/kg SR1 periorbital (p.o.), subcutaneous (s.c.), intraperitoneal (i.p.), or intravenous (i.v.). Mice were euthanized and blood and brain samples were collected (0min, 5min, 15min, 30min, 1hr, 2hr, 4hr, 8hr, and 24hr [blood]; 0min, 30min, 2hr, 8hr, and 24hr [brain]). Blood samples were extracted by an acetonitrile/protein precipitation method. Briefly, the injection sample was prepared by adding an internal standard (propranolol), sample, acetonitrile at a ratio of 1:1:1. Flash frozen brain samples were homogenized in an equal volume of acetonitrile and then centrifuged. The injection sample was prepared by adding an internal standard (propranolol), sample (1:1). SR1 concentrations were determined by LC/MS/MS. Standard curves were prepared in hemolyzed blood, brain, and PBS at concentrations of 2, 6, 50, 500 and 2000 ng/ml.

[^{35}S]GTPγS Binding Assay: Cortex was rapidly dissected on ice and snap-frozen. Frozen samples were thawed and homogenized in Tris-HCl, pH 7.4, with 2 mM $MgCl_2$ and 1 mM EGTA. The homogenate was centrifuged

at 48,000 x g for 10 minutes at 4°C, the supernatant was discarded and the pellet homogenized in Tris-HCl, pH 7.4, with 2 mM $MgCl_2$, 0.2 mM EGTA and 100 mM NaCl (assay buffer) and centrifuged again as above. The resulting pellet was homogenized in assay buffer and the protein concentration was determined. The resulting membrane homogenate was pre-incubated with adenosine deaminase (3 mU/ml) for 10 minutes at 30°C, to inactivate endogenous adenosine. For the assay, membranes (10 μg total protein) were incubated in assay buffer containing 0.1% (w/v) bovine-serum albumen, 30 μM GDP, 0.1 nM [^{35}S]GTPγS and 0.6 mU/ml adenosine deaminase, with and without 0.01-3 μM CP55,940, for 2 hours at 30°C. The incubation was terminated by vacuum filtration through GF/B glass fiber filters, followed by three washes with 3 ml ice-cold Tris-HCl, pH 7.4. Bound radioactivity was determined by liquid scintillation spectrophotometry after extraction of the filters in scintillation fluid. CP55,940 concentration-effect curves were analyzed by non-linear regression analysis to determine E_{max} and EC_{50} values and were calculated using GraphPad/Prism software.

Activity-based protein profiling (ABPP): Male C57BL/6 mice were injected with 10 mg/kg WWL123 and 4 hours later their brains were dissected and flash frozen. Cortical and striatal membrane homogenates were prepared by Dounce homogenization in phosphate-buffered saline (PBS) pH 7.5, sonication, and centrifugation (100,000 g for 45 min at 4°C). The pellet was washed and resuspended in PBS, sonicated, and saved as the membrane homogenate. The total protein concentration of each homogenate was determined using the Bio-Rad DC Protein Assay kit. Control membrane homogenates (50 μg in 50 μl PBS buffer) were pre-treated with 20 μM WWL123 or DMSO vehicle for 30 min at 25ºC followed by incubation with 1 μM of the tailored or broad spectrum activity-based probe HT-01 (Hsu et al., 2012) or FP-rhodamine, respectively, for 30 min at 37ºC (Patricelli et al., 2001). Reactions were quenched with 2x SDS/PAGE loading buffer (reducing), separated by SDS/PAGE (10% acrylamide) and visualized in-gel with a FMBio IIe flatbed fluorescence scanner (Hitachi). Rhodamine fluorescence is shown in grey scale.

Behavioral testing: Male 25g C57Bl/6-CBA mice were used for behavioral testing. For open field experiments, animals were placed in a 10”x18”x18” chamber four hours after receiving either vehicle or 10 mg/kg WWL123. Movement was videorecorded by cameras mounted above the cages, and after ten minutes, animals were removed. Novel Object Recognition testing was performed in the first two hours of the animals’ active cycle.

Animals were habituated to the testing chambers for ten minutes per day, for three consecutive days. On the fourth day, animals were given five minutes to explore the chamber with two identical objects placed at opposite ends of the chamber, and then rested for one hour in their home cage. Animals were then reintroduced to the testing environment (10''x20''x10'' chamber) where one of the familiar objects had been replaced by a novel object, and were given three minutes to explore. Behavior was tracked in by a vertically mounted camera and analyzed in real-time. Discrimination index was calculated as $((time_{novel} - time_{familiar}) / (time_{novel} + time_{familiar}))$. For elevated plus maze, animals were placed in the center of the elevated plus maze, facing the same closed arm in every trial, and videorecorded for 5 minutes by a camera mounted above the apparatus. All video recordings were analyzed in Noldus Ethovision (Wageningen, the Netherlands).

Kainate-induced seizures: Six-week old male C57Bl/6 mice were purchased from Jackson Labs (ME, USA), and were allowed to acclimate for 2 days after shipping. Mice were pretreated either with WWL123 or with vehicle, as previously described. Seizures were induced with 30 mg/kg i.p. kainate (Sigma-Aldrich, MO) formulated in saline, and mice were video recorded for one hour. Seizure behaviors were scored every minute according to criteria previously described (Giménez-Cassina et al., 2012) and validated for correspondence to EEG findings (Ferraro et al., 1997), and an average score was calculated for each mouse by dividing the scores by total time.

Supplemental References:

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