Supplementary methods

Drug discrimination

Mice were housed individually in clear plastic cages in a temperature-controlled (20–22 °C) vivarium. Water was available ad libitum except while the mice were in the operant chambers. Training and test sessions were conducted at similar times during the light phase of a 12 h light/dark cycle. Mice were maintained at 85–90% of free-feeding body mass, which was initially calculated based on free feeding body mass before start of deprivation at 11 weeks, and again following each free feeding period that occurred every six months. Eight standard mice operant conditioning chambers that were sound- and light-attenuated (MED Associates, St. Albans, VT) were used for behavioral training and testing. Each operant conditioning chamber (18 x 18 x 18 cm) was equipped with a house light, two nose poke apertures (left and right), and a recessed well centered between the two apertures. A sweetened pellet served as reinforcement and was delivered to the recessed well according to the reinforcement schedule. Fan motors provided ventilation and masking noise for each chamber. Houselights were illuminated during all operant sessions. A computer with Logic ‘‘1’’ interface and MED-PC software (MED Associates) was used to control schedule contingencies and to record data. Mice were trained to respond in one aperture following administration of 0.1 mg/kg CP55,940 and to respond in the opposite aperture following vehicle administration according to a FR10 schedule of reinforcement. Each incorrect response reset the response requirement. Daily injections were administered on a double alternation sequence of drug or vehicle (e.g., drug, drug, vehicle, vehicle). Daily 15-min training sessions were held Monday–Friday until the mice had met two criteria during nine of 10 consecutive sessions: (*i*) correct completion of the first FR10 (e.g., first 10 consecutive responses on condition appropriate aperture) and (*ii*) $\geq$80 % of the total condition appropriate responding. When these two criteria were met, acquisition of the discrimination was established and substitution testing began. Stimulus substitution tests were conducted on Tuesdays and Fridays during 15-min test sessions and training continued on all other days. Thus, test days allowed at least a 72 h washout period from the previous injection of the MAGL inhibitor. During test sessions, responses in either aperture delivered reinforcement according to an FR10 schedule. To qualify for testing, mice must have completed the first FR10 on the correct aperture and $\geq$80 % of the total condition-appropriate responding on the preceding drug and vehicle sessions.

Triad assay

Catalepsy was assessed on a bar 0.7 cm in diameter placed 4.5 cm off of the ground. The mouse was placed with its front paws on the bar and a timer (Timer #1) was started. A second timer (Timer #2) was turned on only when the mouse was immobile on the bar, with the exception of respiratory movements. If the mouse moved off the bar, it was placed back on in the original position. The assay was stopped when either Timer #1 reached 60 s, or after the fourth time the mouse moved off the bar, and the cataleptic time was scored as the amount of time on Timer #2. Nociception was then assessed in the tail immersion assay. The mouse was placed head first into a small bag fabricated from absorbent under pads (VWR Scientific Products; 4 cm diameter, 11 cm length) with the tail out of the bag. Each mouse was hand-held and 1 cm of the tail was submerged into a 52 °C water bath. The latency for the mouse to withdraw its tail within a 10 s cut off time was scored. Rectal temperature was assessed by inserting a thermocouple probe 2 cm into the rectum, and temperature was determined by telethermometer (BAT-10 Multipurpose Thermometer, Clifton, NJ, USA). Locomotor activity was assessed 120 min after treatment, for a 60 min period in a Plexiglas cage (42.7 x 21.0 x 20.4 cm) and Anymaze (Stoelting, Wood Dale, Illinois) software was used to determine the percentage of time spent immobile, mean speed and distance travelled.

Measurement of brain endocannabinoid levels

On the day of processing, tissues were weighed and homogenized with 1.4 ml of chloroform/methanol (2:1 v/v containing 0.0348 g of phenylmethylsulfonyl fluoride/ml) after the addition of internal standards to each sample (4 pmol of AEA-d8, 1 nmol of 2-AG-d8, 3.3 nmol PEA-d4, 3 nmol OEA-d4 and 1 nmol AA-d8, Cayman Chemical). Homogenates were then mixed with 0.3 ml of 0.73% w/v NaCl, vortexed, and then centrifuged for 10 min at 3220 × g (4 ^{0}C). The aqueous phase plus debris were collected and extracted two more times with 0.8 ml of chloroform. The organic phases from the three extractions were pooled and the organic solvents were evaporated under nitrogen gas. Dried samples were reconstituted with 0.1 ml of chloroform and mixed with 1 ml of ice-cold acetone. The mixtures were then centrifuged for 5 min at 1811 × g and 4 ^{0}C to precipitate the proteins. The upper layer of each sample was collected and evaporated under nitrogen. Dried samples were reconstituted with 0.1 ml of methanol and placed in autosample vials for analysis.

Liquid chromatography-tandem mass spectrometry with an electrospray ionization source was used to identify and quantify the 2-AG, arachidonic acid, AEA, OEA, and PEA. The chromatographic

separation was performed using a Discovery HS C18, 2.1 × 150 mm, 3 μm (Supelco, Bellefonte, PA). The mobile phase consisted of water/methanol (10:90) with 0.1% ammonium acetate and 0.1% formic acid. The following ions were monitored in a multiple-reaction-monitoring positive mode: (348 > 62) and (348 > 91) for AEA; (356 > 62) for AEA-d8; (379 > 287) and (379 > 269) for 2-AG; (387 > 96) for 2-AG-d8 ; (300 > 62) and (300 > 283) for PEA; (304 > 62) for PEA-d4; (326 > 62) and (326 > 309) for OEA; and (330 >66) for OEA-d4 and in negative mode: (303 > 259) and (303 > 59) for arachidonic acid and (311 > 267) for arachidonic acid-d8. A calibration curve was constructed for each assay based on linear regression with use of the peak area ratios of the calibrators. The extracted standard curves ranged from 0.039 to 40 pmol for AEA and from 0.0625 to 64 nmol for 2-AG, from 0.039 nmol to 1.25 for PEA and OEA and from 1 nmol to 32 nmol for arachidonic acid.

Supplementary results

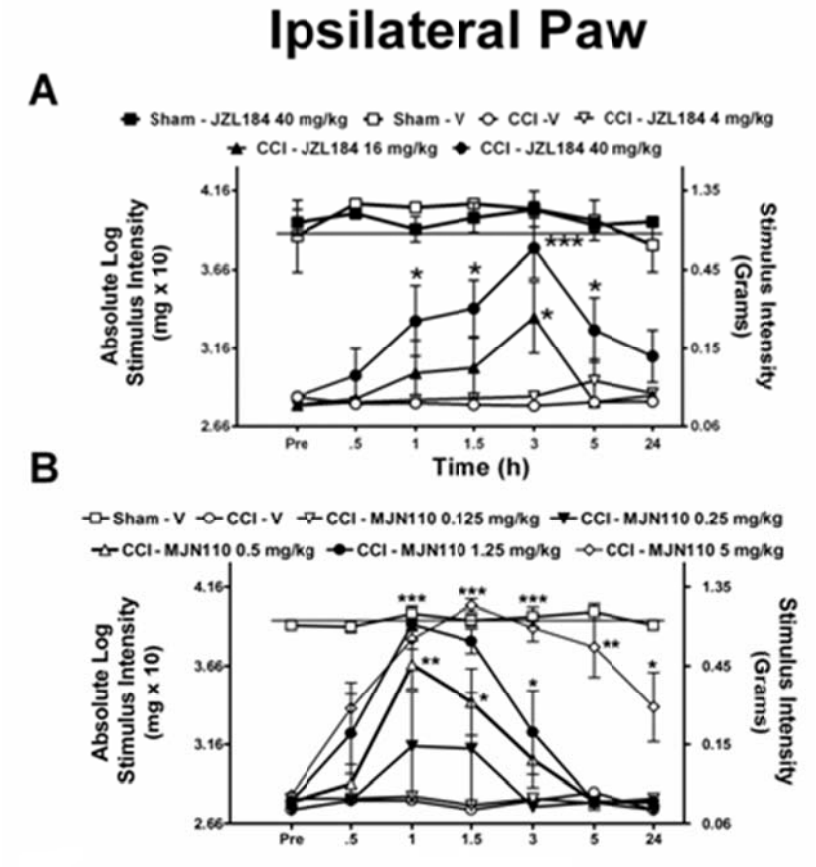
MAGL Inhibitors reverse CCI-induced mechanical allodynia and thermal hyperalgesia

JZL184 and MJN110 reversed CCI-induced bilateral mechanical allodynia in dose-related and time-dependent manners (ipsilateral paw: Figure 1A-C; complete time course of each dose shown in Supplemental Figures 1A-C for ipsilateral paw; and Supplemental Figures 2A-C for contralateral paw). The effect of JZL184 persisted for 5 h in the ipsilateral [$F(3,27) = 3.50$, $p < 0.05$] and contralateral [$F(3,27) = 6.90$, $p < 0.001$] paws. MJN110 had a longer overall duration of action than JZL184. Anti-allodynic effects were detected at 1 h post administration in ipsilateral [$F(5,41) = 16.6$, $p < 0.001$] and contralateral [$F(5,41) = 34.3$, $p < 0.001$] paws, and significant differences were still observed by 24 h in either ipsilateral paws. By 24 h post-injection, MJN110 still produced anti-allodynic effects [$F(5,41) = 8.76$, $p < 0.001$] and [$F(5,41) = 6.955$, $p < 0.001$]. Also, neither MAGL inhibitor altered paw withdrawal thresholds in sham mice at any time point (Supplemental Figures 1 and 2).

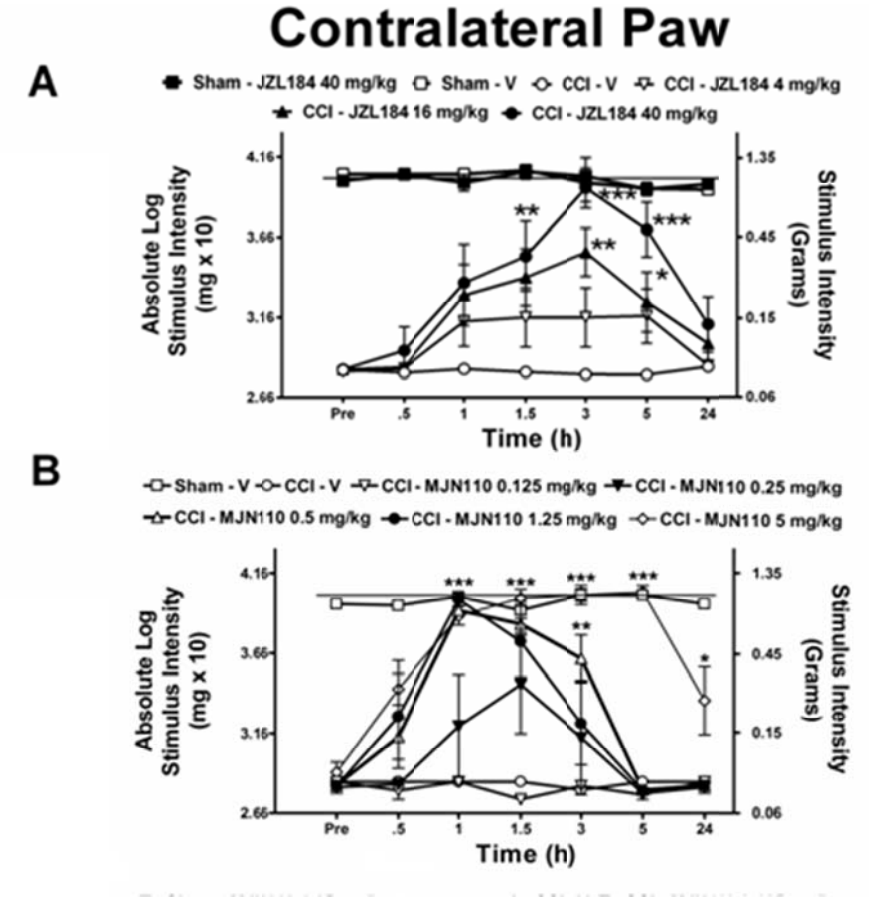
Each MAGL inhibitor also significantly reversed CCI-induced thermal hyperalgesia in dose-related and time-dependent manners (Figure1D-F; Supplemental Figures 3A-C). Prior to injection of vehicle or drug, all CCI mice displayed comparable levels of thermal hyperalgesia [$p = 0.9$]. JZL184 significantly reversed thermal hyperalgesia to varying degrees at 1 h [$F(3,27) = 3.75$, $p < 0.05$] and 5 h [$F(3,27) = 13.6$, $p < 0.0001$]. MJN110 produced anti-thermal hyperalgesic effects at 1 h [$F(5,41) = 3.72$, $p < 0.05$], 5 h [$F(5,41) = 6.96$, $p < 0.05$], and 24 h [$F(5,41) = 5.74$, $p < 0.05$]. Neither drug altered hot plate latencies in sham mice (Supplemental Figure 3).

Locomotor Activity

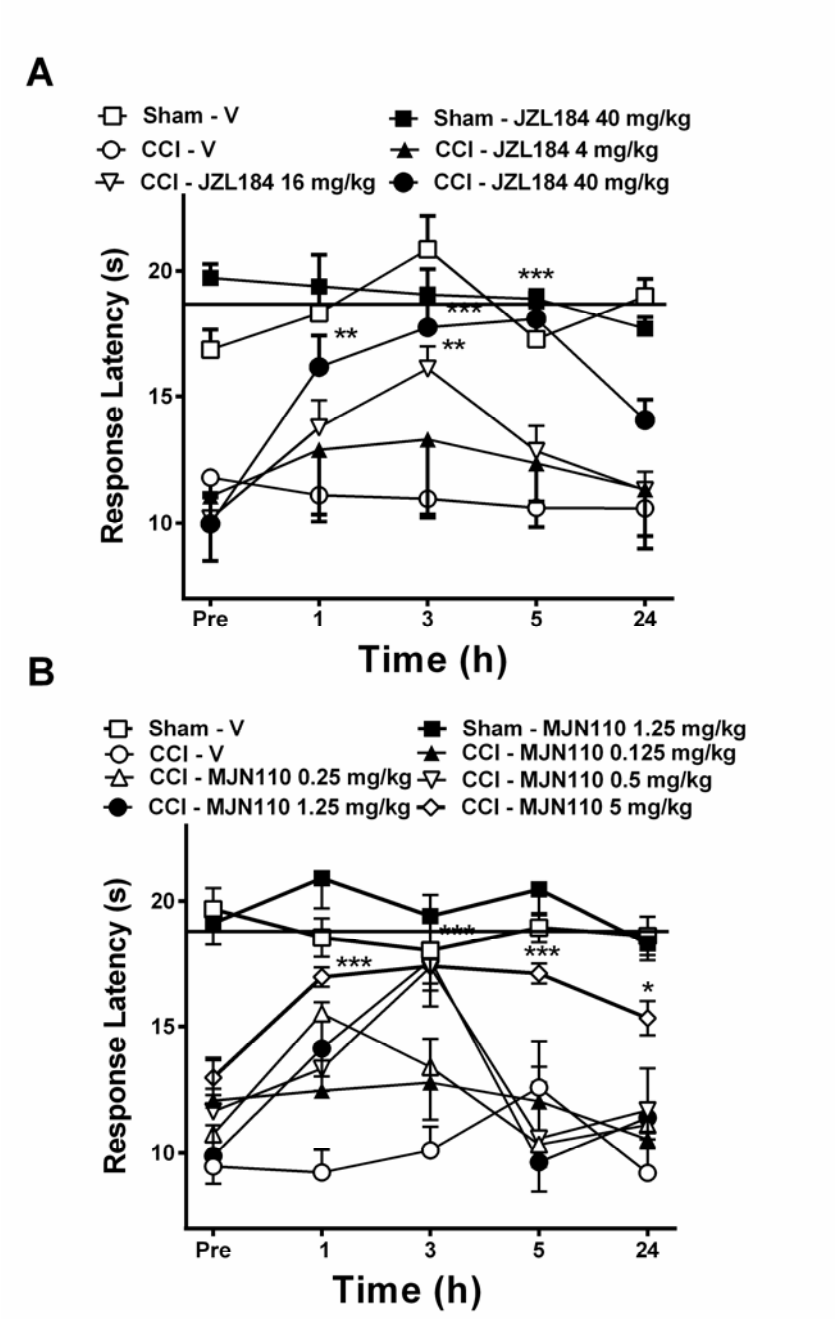
JZL184 and MJN110 produced differential effects on locomotor activity (Supplementary Figure 5). JZL184 (16 and 40 mg/kg) produced profound decreases in locomotor activity, as reflected by reductions in distance travelled [dose x time interaction: $F(15,150) = 2.8$; $p < 0.001$] and time spent mobile [dose x time interaction: $F(15,150) = 5.6$; $p < 0.0001$]. The mean running speed of animals was not affected by JZL184 at any dose ($p = 0.8$). MJN110 (1.25 and 2.5 mg/kg), produced significant increases in running speed [main effect of dose: $F(5,150) = 6.7$; $p < 0.01$, dose x time interaction: $p = 0.08$] and distance travelled [dose x time interaction: $F(5,150) = 2.19$; $p < 0.01$], but did not affect mobility time. No other changes in locomotor behavior were found following MJN110 administration (Supplementary Figure 5 G, H, I).



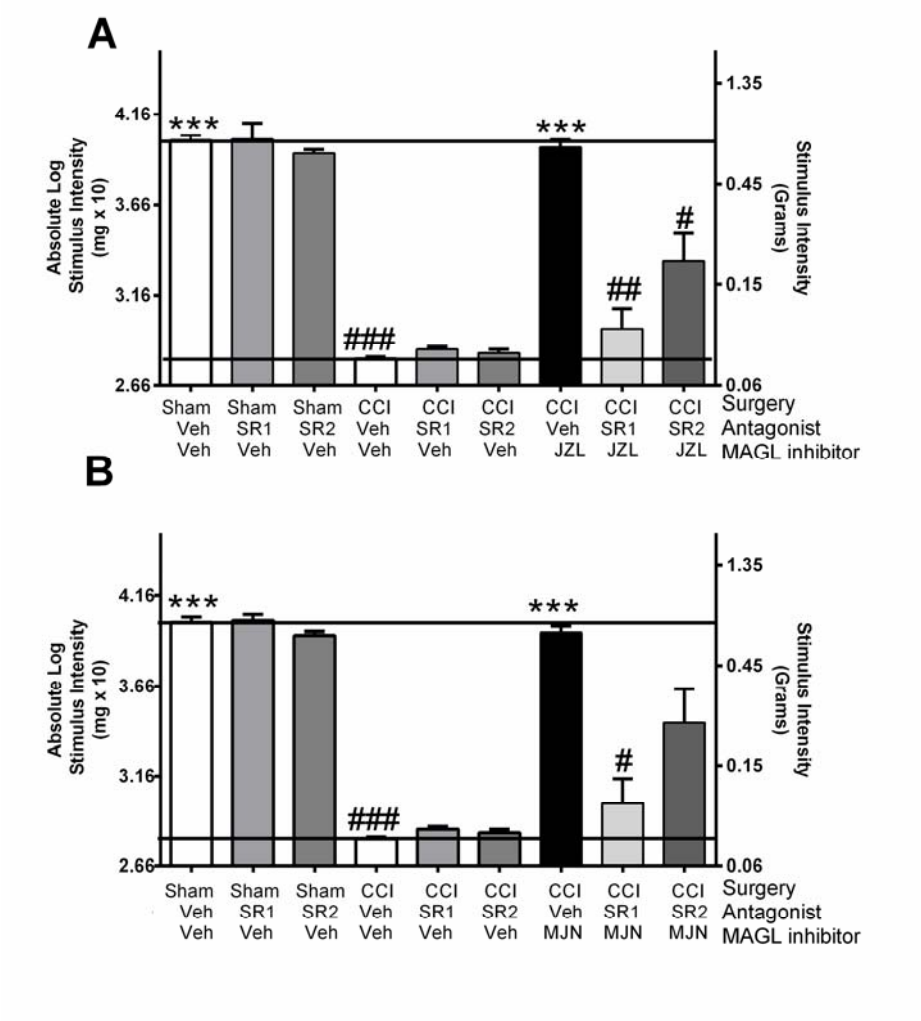
Supplemental Figure 1: MAGL inhibitors reverse CCI-induced mechanical allodynia in the ipsilateral paw in a dose- and time-related manner. (**A)** Dose response and time course of JZL184 (4, 16, 40 mg/kg i.p.) reversal of CCI-induced allodynia. (**B**) Dose response and time course of MJN110 (0.125, 0.25, 0.5, 1.25, 5 mg/kg i.p) reversal of CCI-induced allodynia. *** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$ vs. CCI vehicle. Data reflect mean ± SEM, n = 5-7 mice per group. Horizontal lines spanning across the ordinates reflect sham von Frey values (top lines).



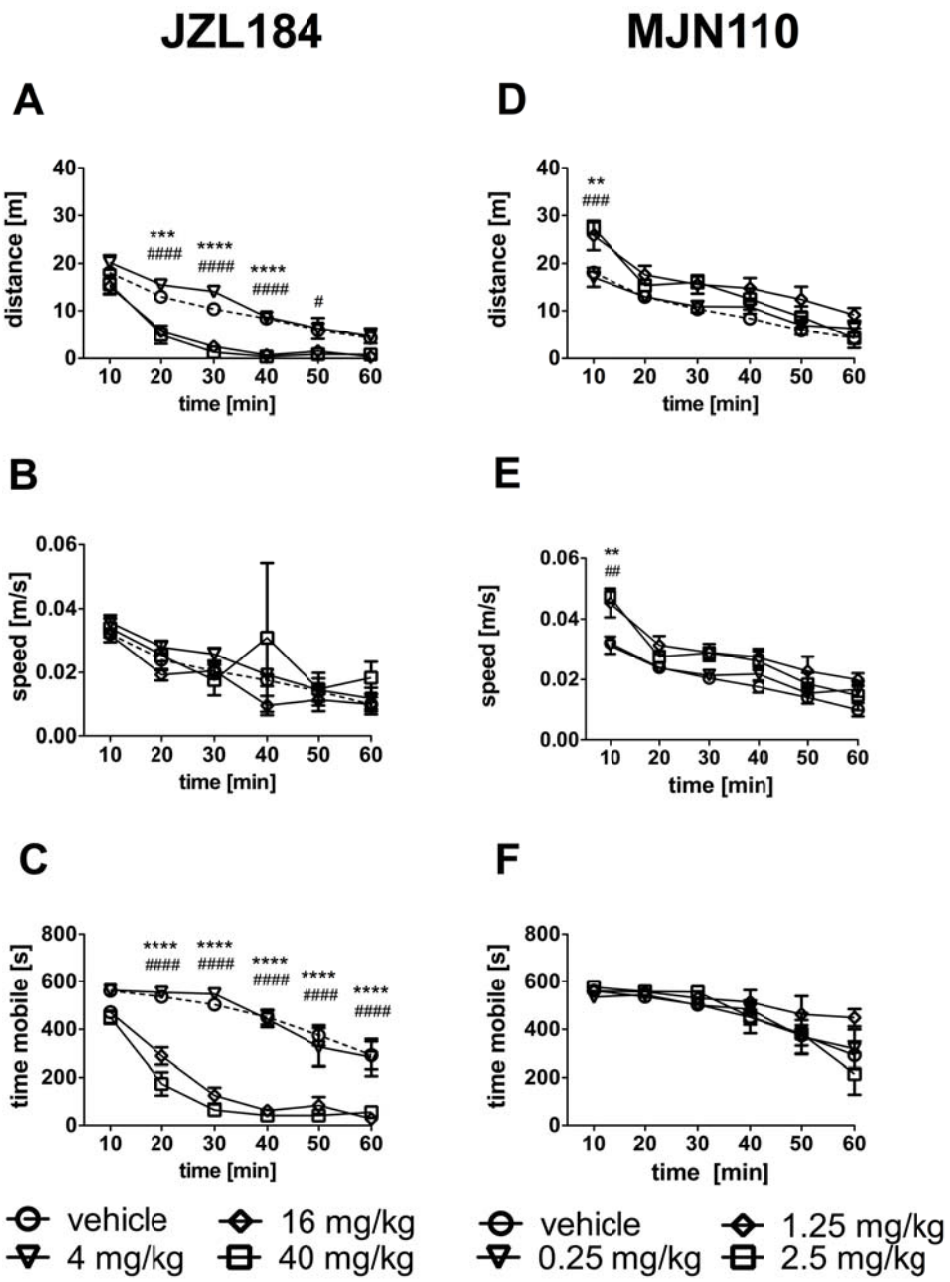
Supplemental Figure 2: MAGL inhibitors reverse CCI-induced mechanical allodynia in the contralateral paw in a dose- and time-related manner. (**A)** Dose response and time course of JZL184 (4, 16, 40 mg/kg i.p.) reversal of CCI-induced allodynia in contralateral paws. (**B**) Dose response and time course of MJN110 (0.125, 0.25, 0.5, 1.25, 5 mg/kg i.p) reversal of CCI-induced allodynia in contralateral hindpaws. *** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$ vs. CCI vehicle. Data reflect mean ± SEM, n=5-7 mice per group. Horizontal lines spanning across the ordinates reflect sham von Frey values (top lines).



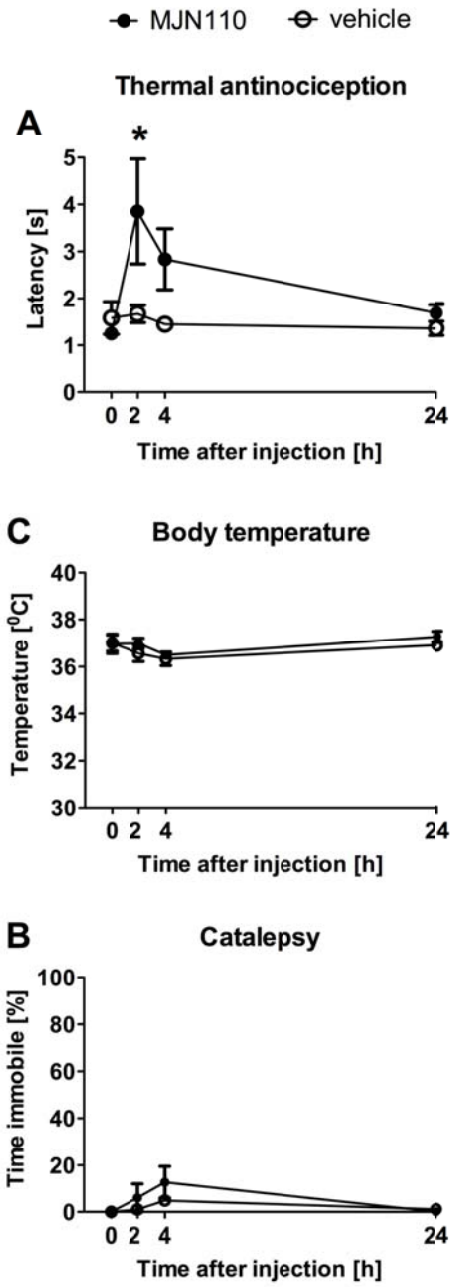
Supplemental Figure 3: MAGL inhibitors reverse CCI-induced thermal hyperalgesia in a dose- and time-related manner. (**A)** Dose response and time course of JZL184 (4, 16, 40 mg/kg i.p.) in reversal of CCI-induced thermal hyperalgesia. (**B**) Dose response and time course of MJN110 (0.125, 0.25, 0.5, 1.25, 5 mg/kg i.p) in reversal of CCI-induced thermal hyperalgesia. *** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$ vs. CCI vehicle. Data reflect mean ± SEM, n = 5-7 mice per group. Horizontal lines spanning across the ordinates reflect sham values (top lines).



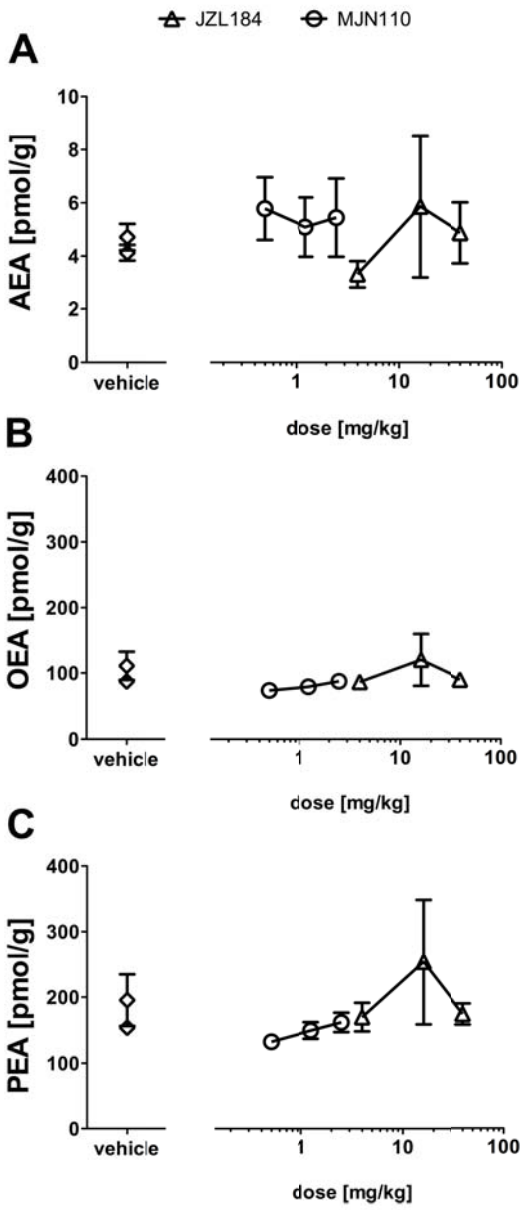
Supplemental Figure 4: Evaluation of CB_1 and CB_2 receptors in the anti-allodynic effects of MAGL inhibitors on the contralateral paw. Rimonabant (SR1) and SR144528 (SR2) block anti-allodynic effects of (**A**) JZL184 (40 mg/kg), (**B**) MJN110 (1.25 mg/kg). *** $p < 0.0001$ vs. CCI vehicle+vehicle; # $p<0.05$; ## $p < 0.001$, ### $p < 0.001$ vs. CCI vehicle+MAGL inhibitor. Data reflect mean ± SEM, n = 5-7 mice per group. Horizontal lines spanning across the ordinates reflect sham von Frey values (top lines) and CCI von Frey values (bottom lines).



Supplementary Figure 5. Time course of locomotor effects of JZL184 and MJN110. JZL184 (16 and 40 mg/kg) produced profound decrease in distance travelled **(A)** and time spent mobile (**C**), but did not affect average running speed MJN110 at 1.25 and 2.5 mg/kg also increased speed (**E**) and distance travelled (**D**) within first 10 minutes of test, while time mobile was unaffected (**F**). *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$ vehicle vs high dose; #$p < 0.05$, ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$ vehicle vs medium dose of inhibitor. Data shown as mean ± SEM, n = 13 in vehicle-treated group and n = 7 mice in each drug-treated group.



Supplementary figure 6. MJN110 (5 mg/kg) produced significant antinociceptive effect in the tail withdrawal test at 2 h following treatment (**A**) without occurrence of hypothermia (**B**) or catalepsy (**C**). Data shown as mean ± SEM, n = 6-7 per group,*$p<0.05$.



Supplementary Figure 7. Neither JZL184 (4, 16, 40 mg/kg) nor MJN110 (0.25, 1.25, 2.5 mg/kg) affected (**A**) anandamide (AEA) ($p = 0.6$, $p = 0.7$, $p = 0.1$), (**B**) oleoylethanoloamide (OEA) ($p = 0.7$, $p = 0.7$, $p = 0.2$), or (**C**) palmitoyloethanoloamide (PEA) ($p = 0.7$, $p = 0.7$, $p = 0.3$) concentrations in the whole brain of mice. Brains were harvested 3 h after i.p. injections. Data shown as mean ± SEM, n = 7-8 per group.