Sustained Stimulation of β2- and β3-adrenergic Receptors Leads to Persistent Functional Pain and Neuroinflammation

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Supplementary Figures

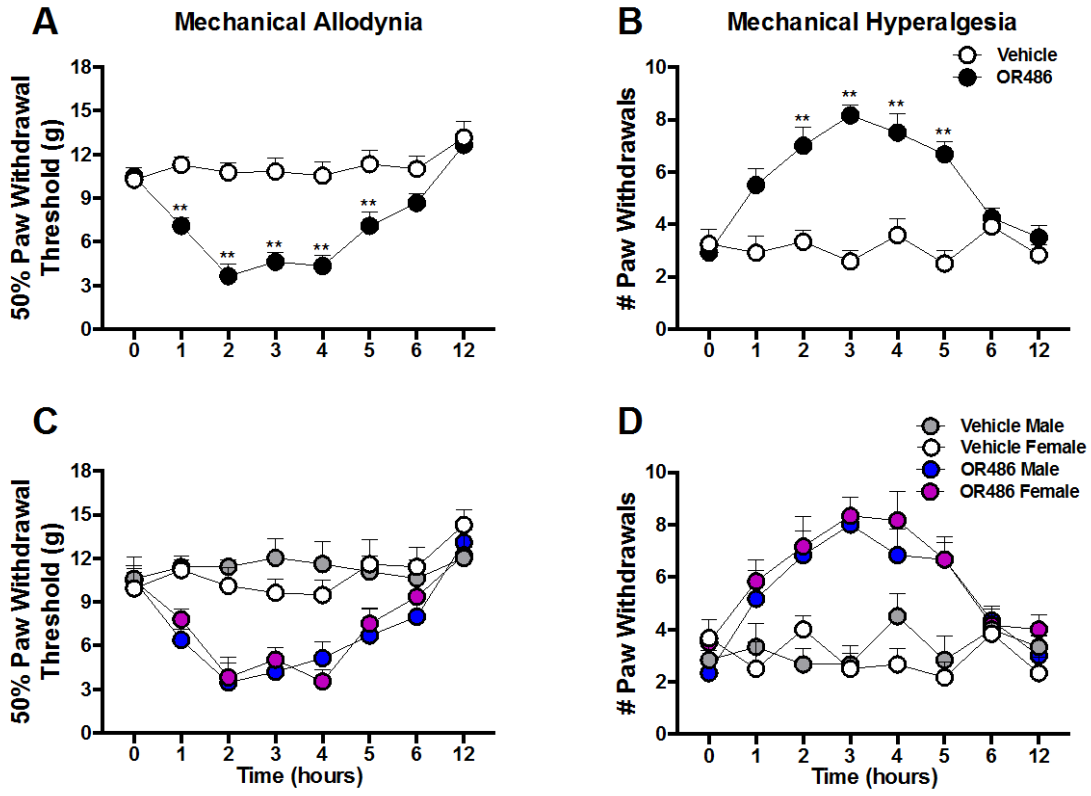

Figure 1S. Acute COMT-dependent mechanical pain resolves after 5 hours, and is consistent between male and female rats. Compared to vehicle, a single dose of OR486 (30mg/kg, ip) produces (A) mechanical allodynia and (B) mechanical hyperalgesia for up to 5 hours. No significant differences in (C) mechanical allodynia or (D) mechanical hyperalgesia are observed between male and female rats. N=6 (3 males + 3 females) per group. Data are mean ± SEM. $^{**}P < 0.01$ *vs* vehicle.

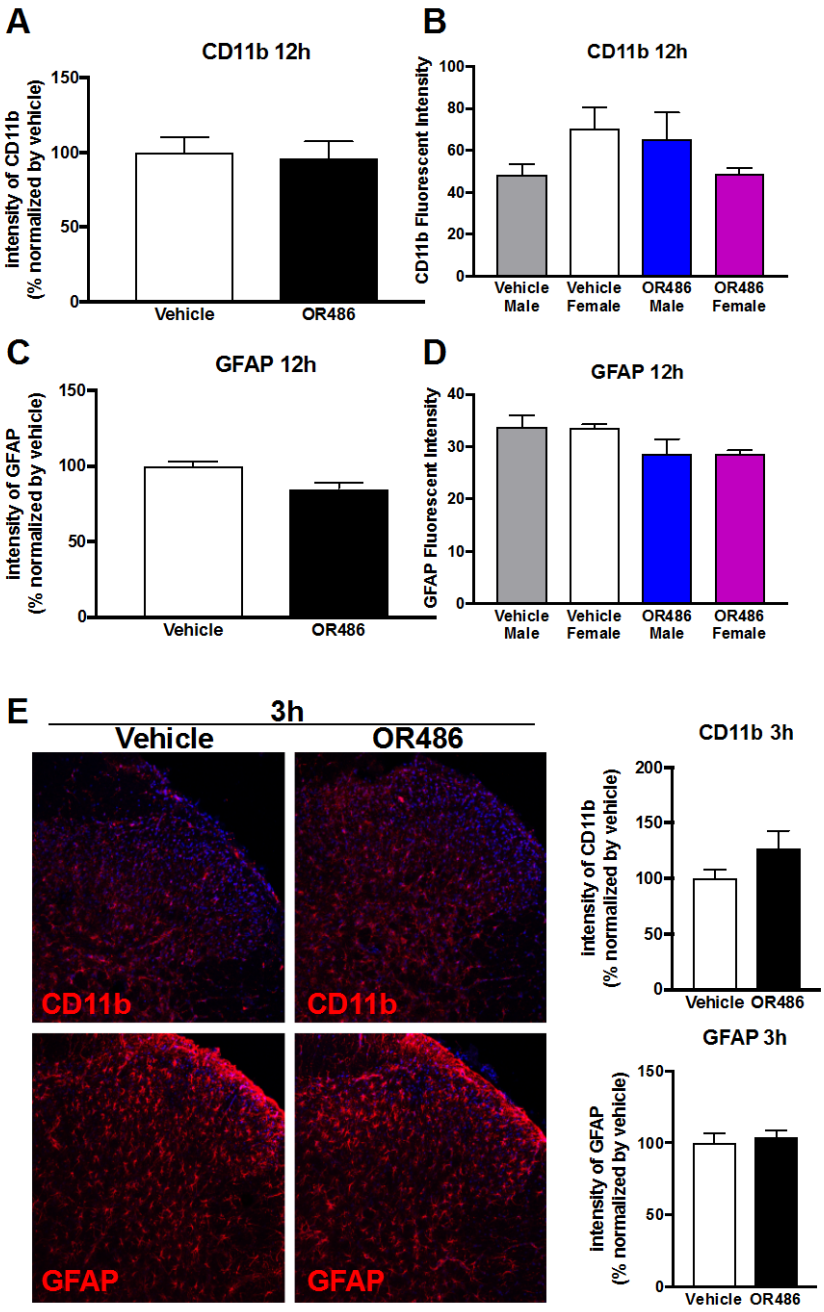

Figure 2S. Acute OR486 delivery does not alter glial activation. Quantitative analysis of immunofluorescence intensity demonstrates that a single dose of OR486 does not alter (A,B) microglia or (C,D) astrocyte activation at 12 hours, which is consistent between male and female rats. N=6 (3 males and 3 females) per group. Further, immunohistochemical staining and quantitative analysis of immunofluorescence intensity shows that a single dose of OR486 does not alter (E) microglia or astrocyte activation at 3 hours. N=3 males per group. Data are mean ± SEM.

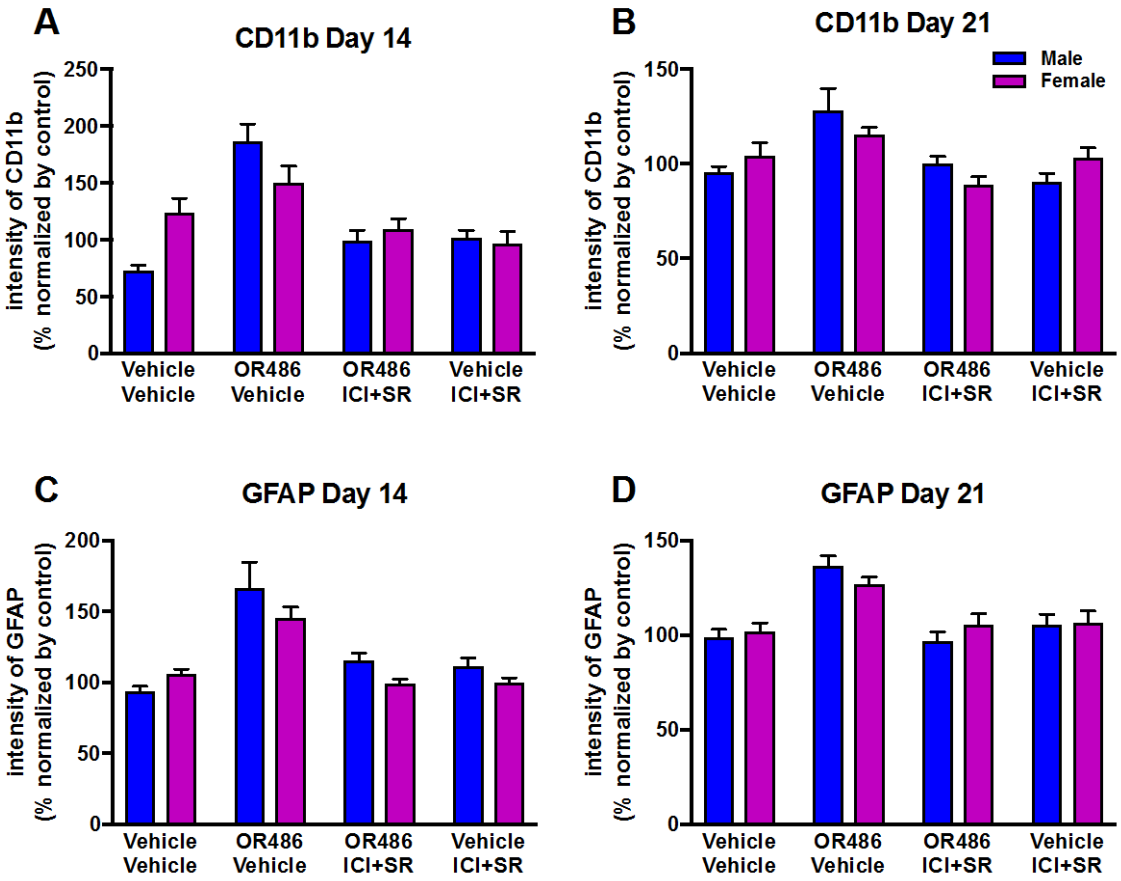

Figure 3S. COMT-dependent glial activation is consistent between male and female rats. Quantitative analysis of immunofluorescence intensity demonstrates that male and female rats exhibit similar levels of (A, B) microglia and (C, D) astrocyte activation following sustained 14-day systemic delivery of OR486. Day 14 groups: Vehicle/Vehicle n=5 (3 males + 2 females); OR486/Vehicle n=6 (3 males + 3 females); OR486/ICI&SR n=6 (3 males + 3 females); Vehicle/ICI&SR n=5 (3 males + 2 females). Day 21 groups: Vehicle/Vehicle n=6 (3 males + 3 females); OR486/Vehicle n=7 (4 males + 3 females); OR486/ICI&SR n=7 (4 males + 3 females); Vehicle/ICI&SR n=6 (3 males + 3 females). Data are mean ± SEM.

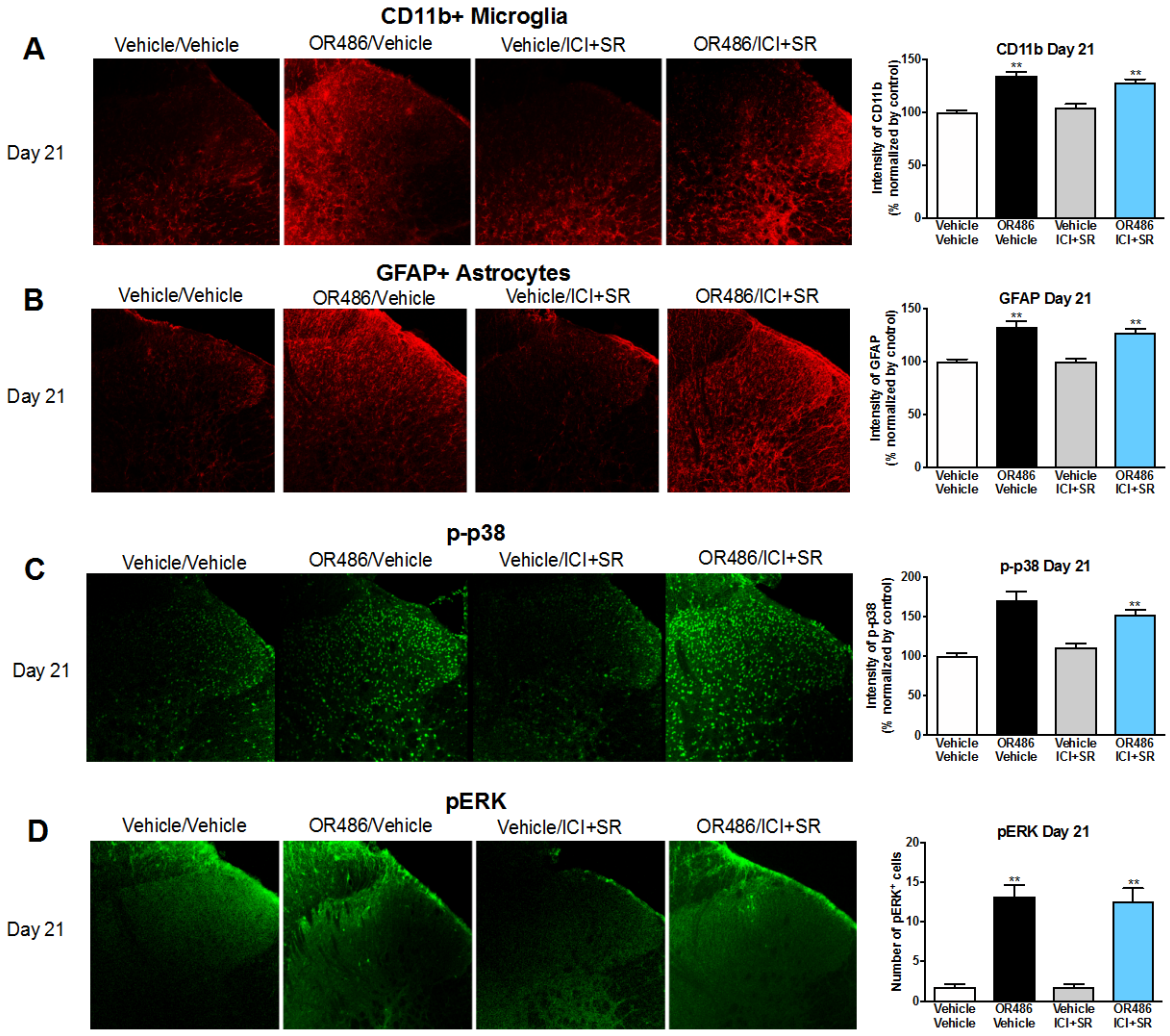

Figure 4S. COMT-dependent glial activation and MAPK phosphorylation remain unaltered following systemic delivery of β_2- and β_3AR antagonists. Immunohistochemical staining and quantitative analysis of immunofluorescence intensity show that systemic delivery of ICI118,551+SR59230A for 14 days beginning on day 7 does not change activation of (A) microglia, (B) astrocytes, (C) p38, and (D) ERK measured on day 21 following sustained 14-day systemic delivery of OR486 or vehicle. Vehicle/Vehicle n=6 (3 males + 3 females); OR486/Vehicle n=7 (4 males + 3 females); OR486/ICI&SR n=7 (4 males + 3 females); Vehicle/ICI&SR n=6 (3 males + 3 females). Data are mean ± SEM. $^{**}P < 0.01$ *vs* vehicle/vehicle.

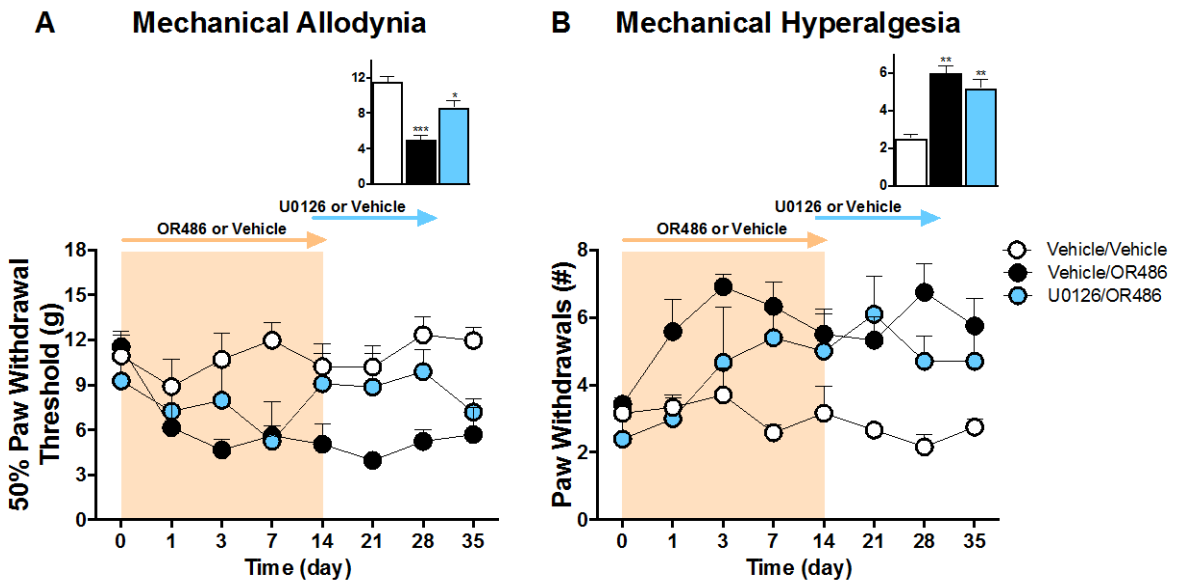

Figure 5S. COMT-dependent hyperalgesia remains unaltered following ERK inhibition. Intrathecal administration of the ERK inhibitor U0126 for 14 days beginning on day 14 does not reverse OR486-induced (B) mechanical hyperalgesia. Insets represent average pain behavior following inhibitor delivery on days 21-35. Vehicle/Vehicle n=7, OR486/Vehicle n=7, OR486/U0126 n=6 males per group. Data are mean ± SEM. ****P* < 0.001, ***P* < 0.01, **P* < 0.05 *vs* vehicle/vehicle.

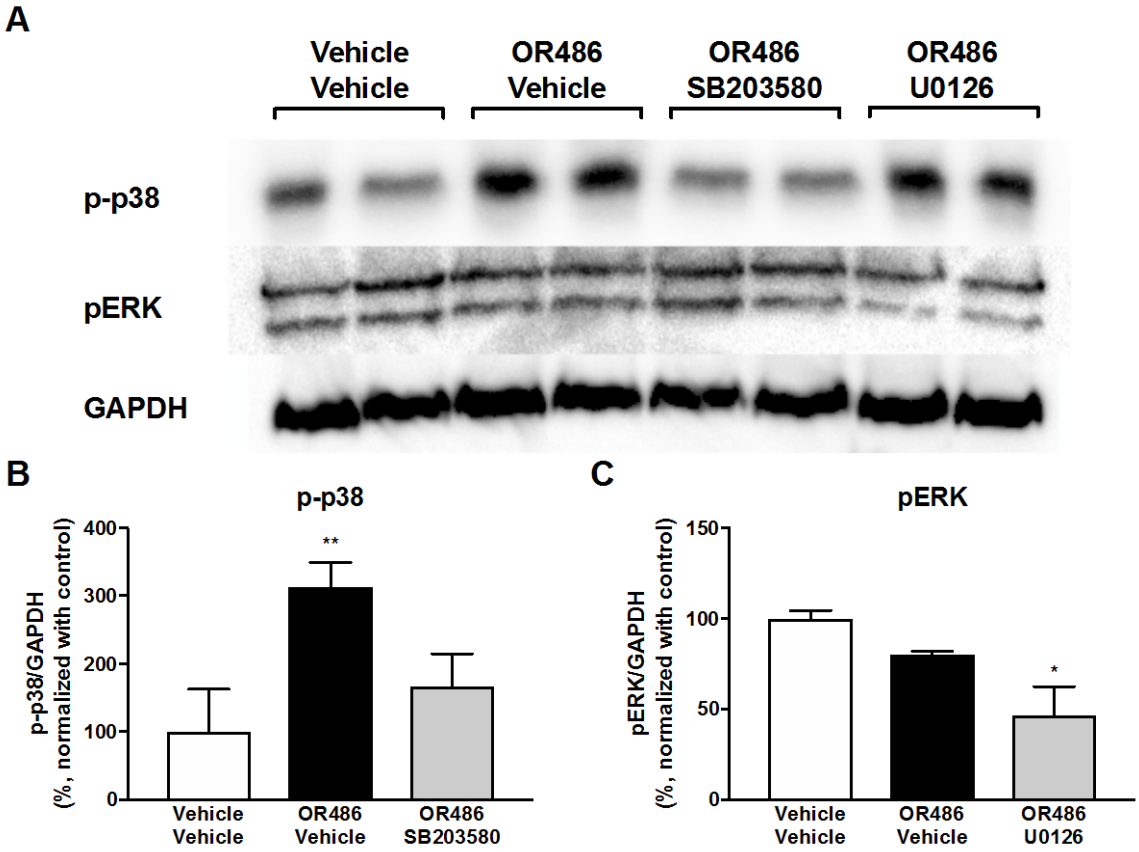

Figure 6S. SB203580 and U0126 effectively reduce p-p38 and pERK expression in the spinal cord. Quantitative analysis of (A) western blot bands demonstrates that (B) SB203580 delivery for 14 days beginning on day 14 reduces sustained OR486-induced increases in spinal p-p38 expression measured on day 35. (C) Similarly, U0126 delivery for 14 days beginning on day 14 reduces spinal pERK expression measured on day 35. N=4 males per group. Data are mean ± SEM, **P* < 0.05, ***P* < 0.01 *vs* vehicle/vehicle.