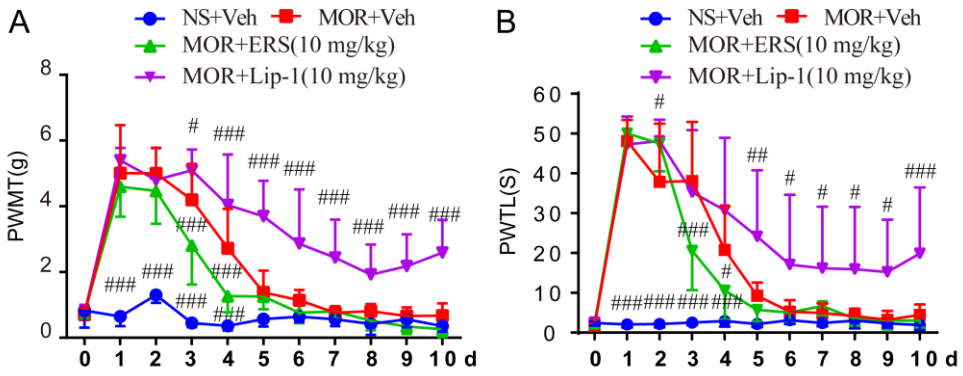

Figure S1. Erastin, a ferroptosis activator, accelerates chronic morphine tolerance. The mechanical hyperalgesia test and tail-flick test were performed to evaluate the effects of ferroptosis on chronic morphine-induced antinociception tolerance. (A) The paw withdrawal mechanic threshold. Liproxstatin-1 co-administration with morphine potentiates the morphine analgesic effect, however, Erastin accelerates the development of morphine tolerance at day 3 after morphine injection. The effect of morphine (10 mg/kg, s.c.) administered with Veh, liproxstatin-1 (10 mg/kg, i.p.) or erastin (10 mg/kg, i.p.) (MedChemExpress, Monmouth Junction, NJ, USA) is shown using the von Frey test. (B) The paw withdrawal threshold lantency. Liproxstatin-1 co-administration with morphine potentiates the morphine analgesic effect, however, Erastin accelerates the development of morphine tolerance at day 3 after morphine injection. The effect of morphine (10 mg/kg, s.c.) administered with Veh, liproxstatin-1 (10 mg/kg, i.p.) or erastin (10 mg/kg, i.p.) is shown using the tail immersion test. Data are shown as mean ± SD, n = 10 in each group and were analyzed using repeated measures two-way ANOVA with *post-hoc* Bonferroni test. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$vs. MOR + Veh group.

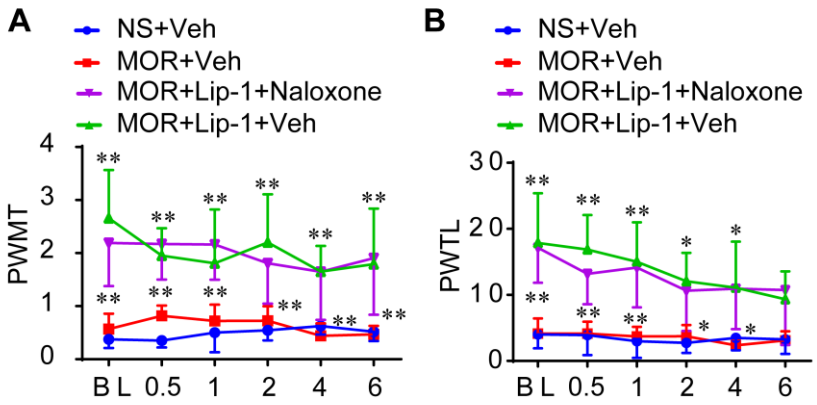

Figure S2. The effect of Naloxone on Liproxstatin-1 mediated analgesic effect.

The mechanical hyperalgesia test and tail-flick test were performed to evaluate the effects of naloxone and liproxstatin-1 on chronic morphine-induced antinociception tolerance. The paw withdrawal mechanic threshold, and the paw withdrawal threshold lantency (A and B). Liproxstatin-1 co-administration with morphine potentiates the morphine analgesic effect, however, inhibition opioid receptor with naloxone at day 10 in morphine tolerance mice, liproxstatin-1 also potentiates the morphine analgesic effect. The effect of morphine (10 mg/kg, s.c.) administered with Veh, liproxstatin-1 (10 mg/kg, i.p.) or liproxstatin-1 (10 mg/kg, i.p.) and naloxone hydrochloride (5 mg/kg, i.p.) (HuaSu, Beijing, China) is shown using the von Frey test. Data are shown as mean ± SD, n = 10 in each group and were analyzed using repeated measures two-way ANOVA with *post-hoc* Bonferroni test. *$P < 0.05$, **$P < 0.01$ vs. MOR + Veh group.