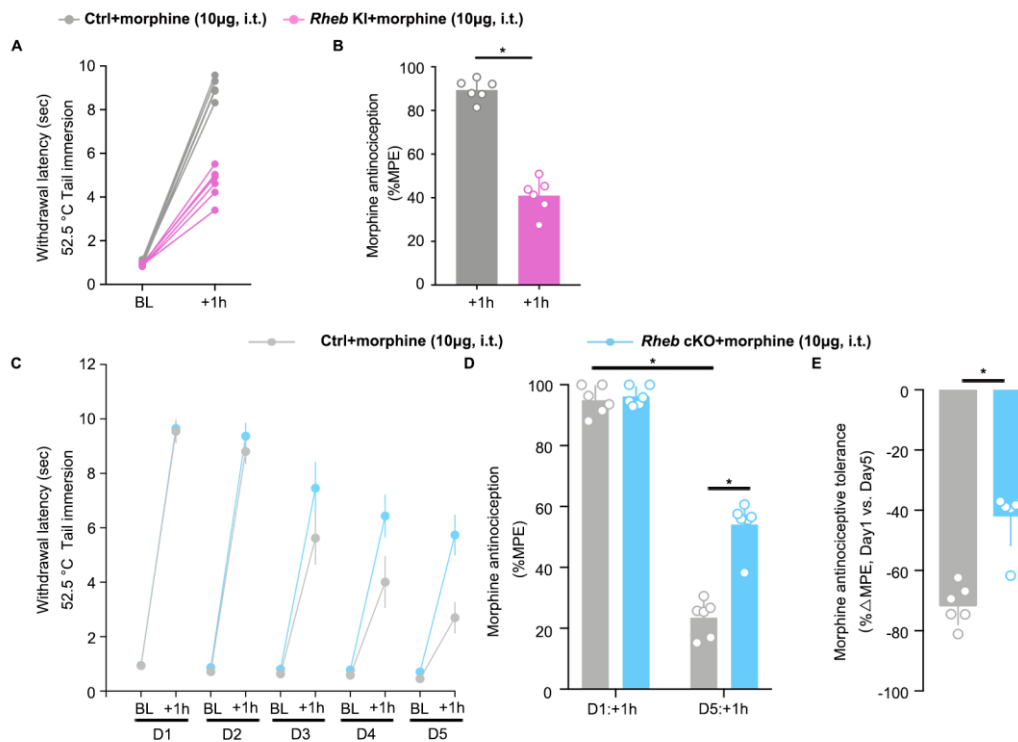


Supplementary content 1. Validation of establishment of *Rheb* S16H nestin cre mice and *Rheb*^{F/F}; CAMK II^{Cre+} mice by genotyping and western blotting analysis

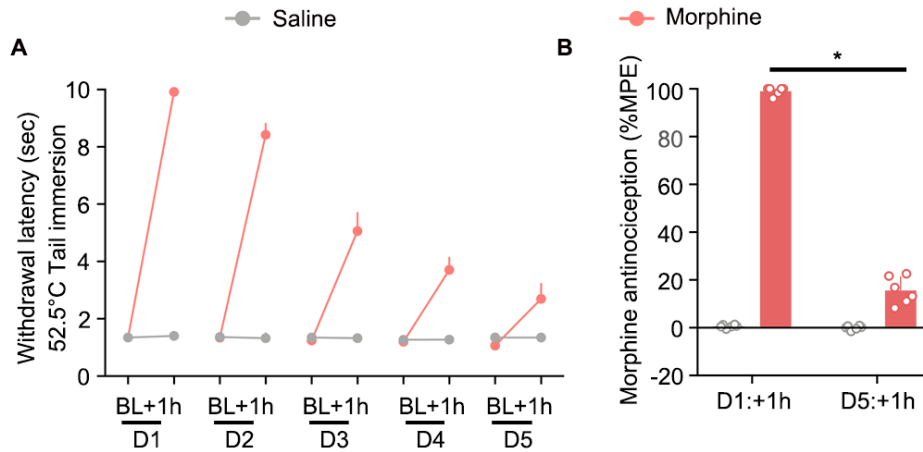
A. Genotyping of *Rheb* S16H nestin cre mice. **B.** Expression of Rheb in the spinal dorsal horn of *Rheb* S16H nestin cre mice. **C.** Phosphorylation levels of mTOR (left panel), S6 (middle panel), and 4EBP1 (right panel) in the spinal dorsal horn of *Rheb* S16H nestin cre mice. **D.** Genotyping of *Rheb*^{F/F}; CAMK II^{Cre+} mice (conditional knock out, CKO). **E.** Expression of *Rheb* in the spinal dorsal horn of *Rheb* CKO mice. Data are presented as mean \pm SD (* p <0.05) by unpaired 2-tailed Student's t test (**B**, **C** and **E**).



Supplementary content 2. Modulation of spinal Rheb expression affects acute morphine efficacy and the development of morphine-induced tolerance in female mice

A. Nociceptive behavior (pre-morphine BL timepoints) and morphine antinociception (post-morphine + 1 h timepoints) from the first administration (10 µg in 10 µL saline, intrathecal) in Rheb S16H mice. **B.** MPE for morphine antinociception from the first administration in Rheb S16H mice. **C.** Daily nociceptive behavior and morphine antinociception during a 5-day chronic morphine schedule (10 µg in 10 µL saline, intrathecal, twice daily). **D.** MPE for morphine antinociception from the first administration (Day 1: + 1 h) compared to the last administration (Day 5: + 1 h). **E.** Percent change for each subject. n=6 mice in each group Data are presented as mean ± SD (*p<0.05) by unpaired 2-tailed Student’s t test (**A**, **B** and **E**) or Repeated Measures Two-way ANOVA + Bonferroni (**C** and **D**). Overlaid points are individual animal scores. BL means baseline. +1h means 1h after morphine injection. D1 means

on Day 1.



Supplementary content 3. Validation of morphine induced-tolerance model

A. Daily nociceptive behavior and morphine antinociception during a 5-day chronic morphine schedule (10 μg in 10 μL saline, intrathecal, twice daily. **B.** MPE for morphine antinociception from the first administration (Day 1: + 1 h) compared to the last administration (Day 5: + 1 h). $n=6$ mice in each group Data are presented as mean \pm SD (* $p<0.05$) by Repeated Measures Two-way ANOVA + Bonferroni. Overlaid points are individual animal scores. BL means baseline. +1h means 1h after morphine injection. D1 means on Day 1.