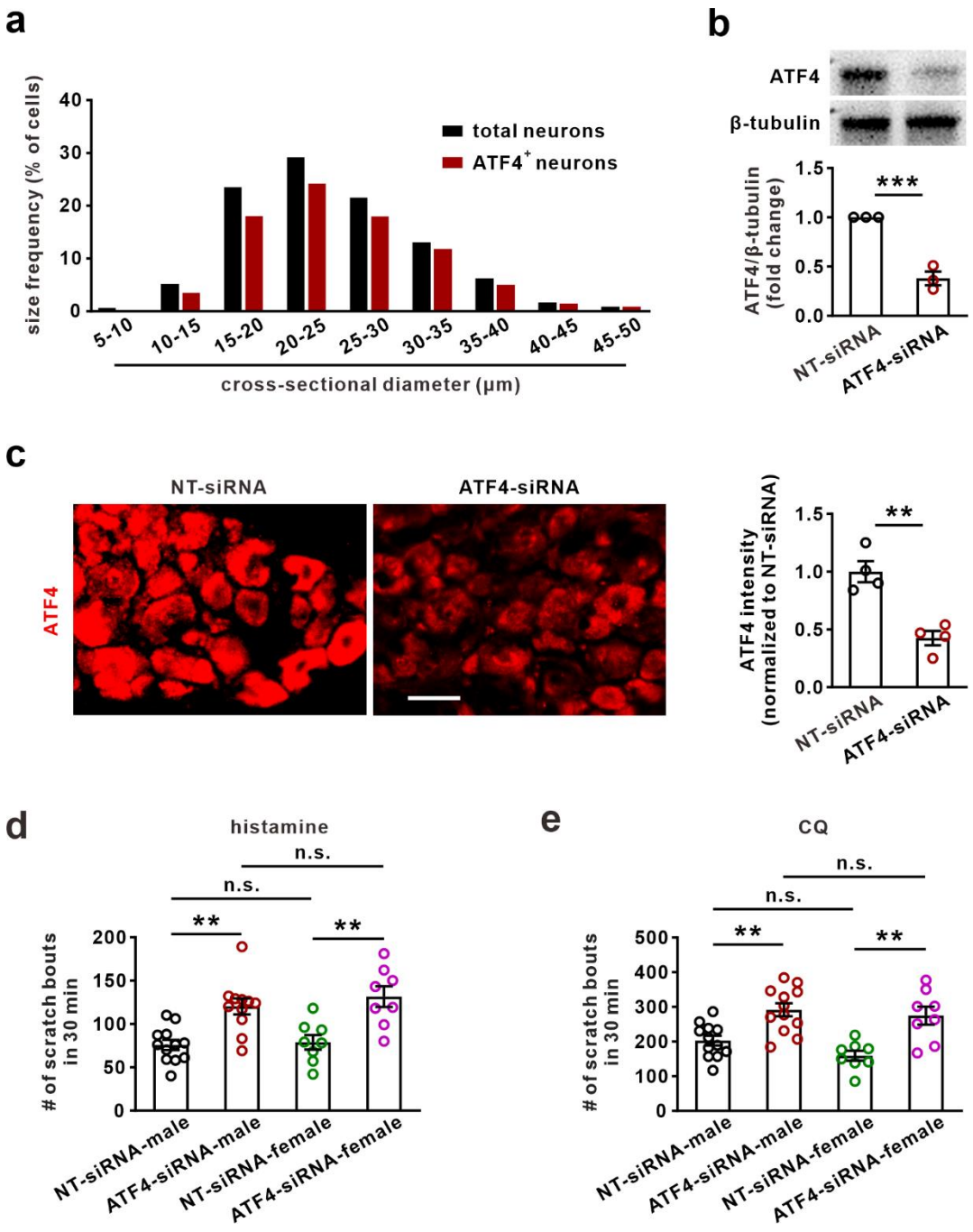
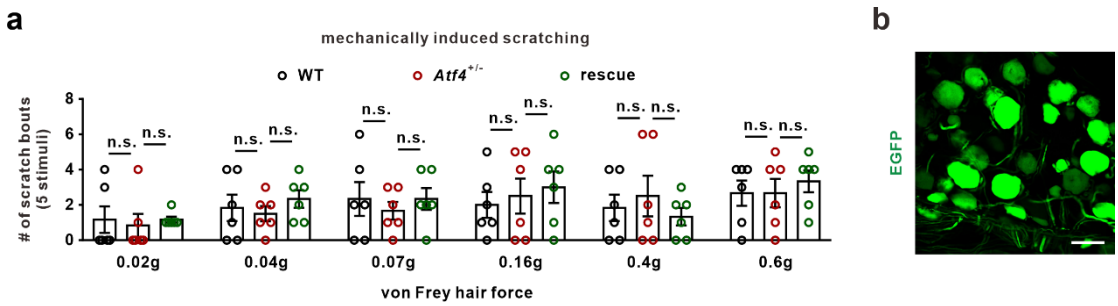
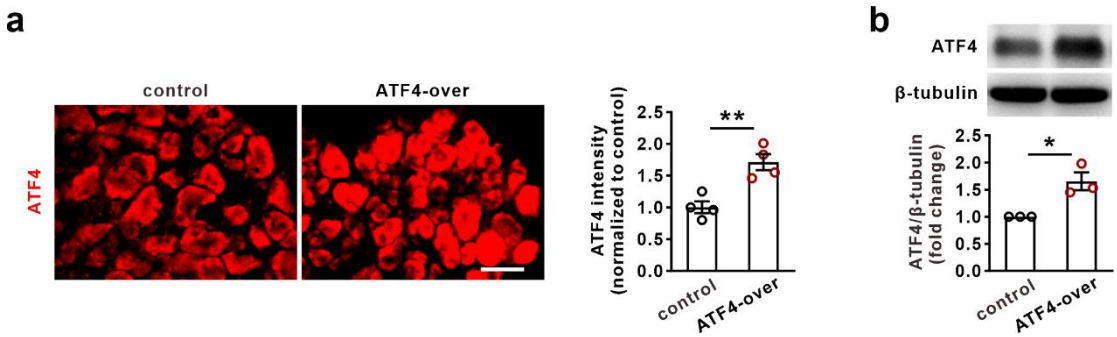


Supplementary Fig. 1 Intrathecal injection of ATF4-siRNA to knockdown the expression of ATF4 in DRG tissues. (**a**) Size frequency distribution of ATF4-positive ($ATF4^+$) and total neurons in the mouse cervical DRGs. A total of 1,321 neurons from 4 mice were analyzed. (**b**) The expression of ATF4 in cervical DRGs of mice by immunoblotting after intrathecal injection with ATF4-siRNA in 2 days. n = 3 samples per group (2 mice mixed into a sample). (**c**) The expression of ATF4 in cervical DRGs

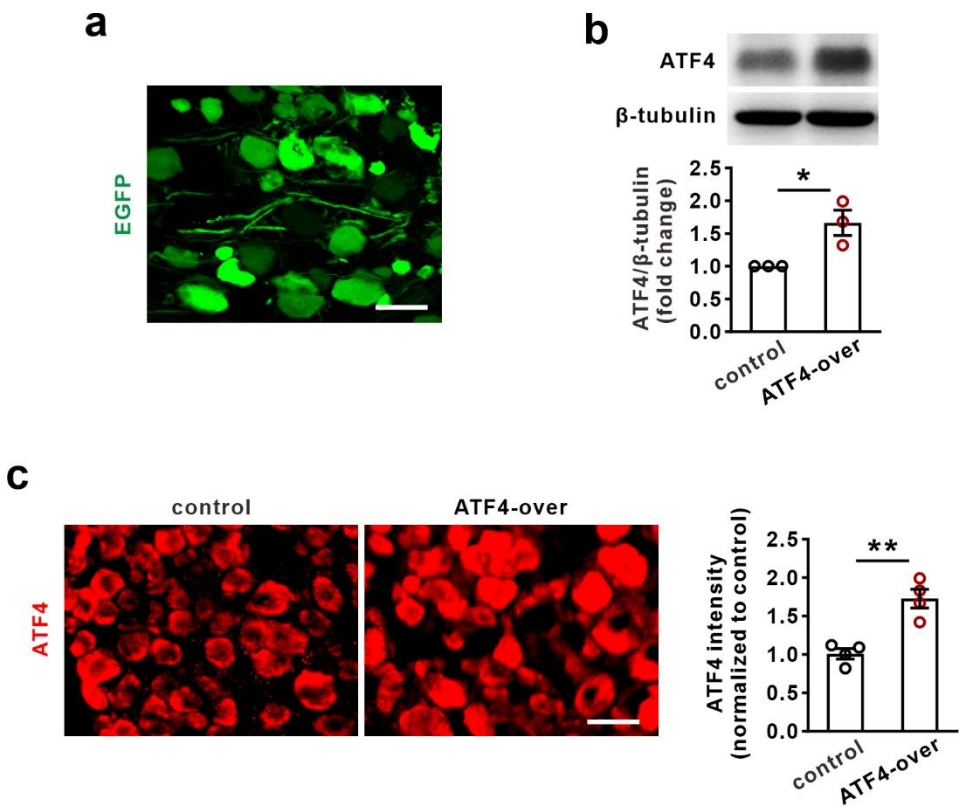
of mice by immunostaining after intrathecal injection with ATF4-siRNA in 2 days. n = 4 mice per group. Scale bar, 50 μm. (**d and e**) The effect of intrathecal injection of ATF4-siRNA or NT-siRNA on the acute itch induced by histamine and CQ in male and female mice. n = 8-12 mice per group. **b**, **c**, Two-tailed Independent Student's *t* test; **d**, **e**, One-way ANOVA followed by Tukey's multiple comparisons test. $^{**}P < 0.01$, $^{***}P < 0.001$, n.s. means not significant. The error bars indicate the SEMs.



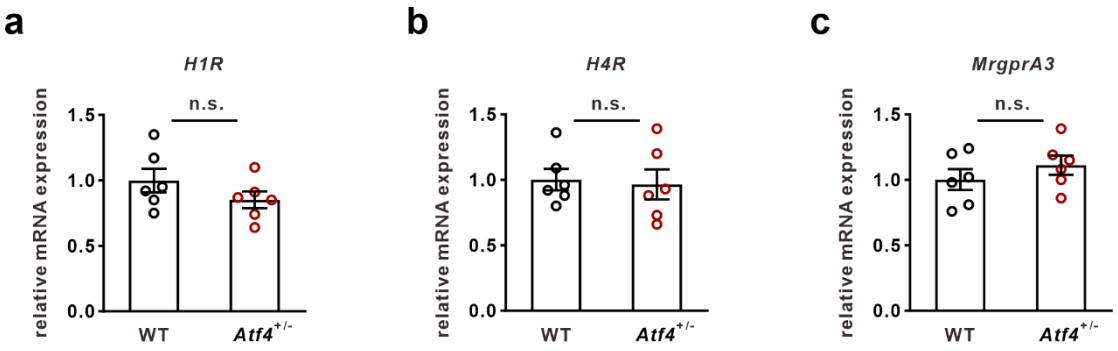
Supplementary Fig. 2 The expression of EGFP in DRG neurons after intrathecal injecting AAV expressing ATF4 (rAAV-hSyn-*Atf4*-2A-EGFP) in *Atf4*$^{+/-}$ mice. (**a**) The effect of deletion and re-expression of ATF4 on mechanical itch. n = 6 mice per group. (**b**) The expression of EGFP in cervical DRG neurons of *Atf4*$^{+/-}$ mice in 21 days after intrathecal injecting rAAV-hSyn-*Atf4*-2A-EGFP. Scale bar, 50 μm. **a**, One-way ANOVA followed by Tukey's multiple comparisons test. n.s. means not significant. The error bars indicate the SEMs.



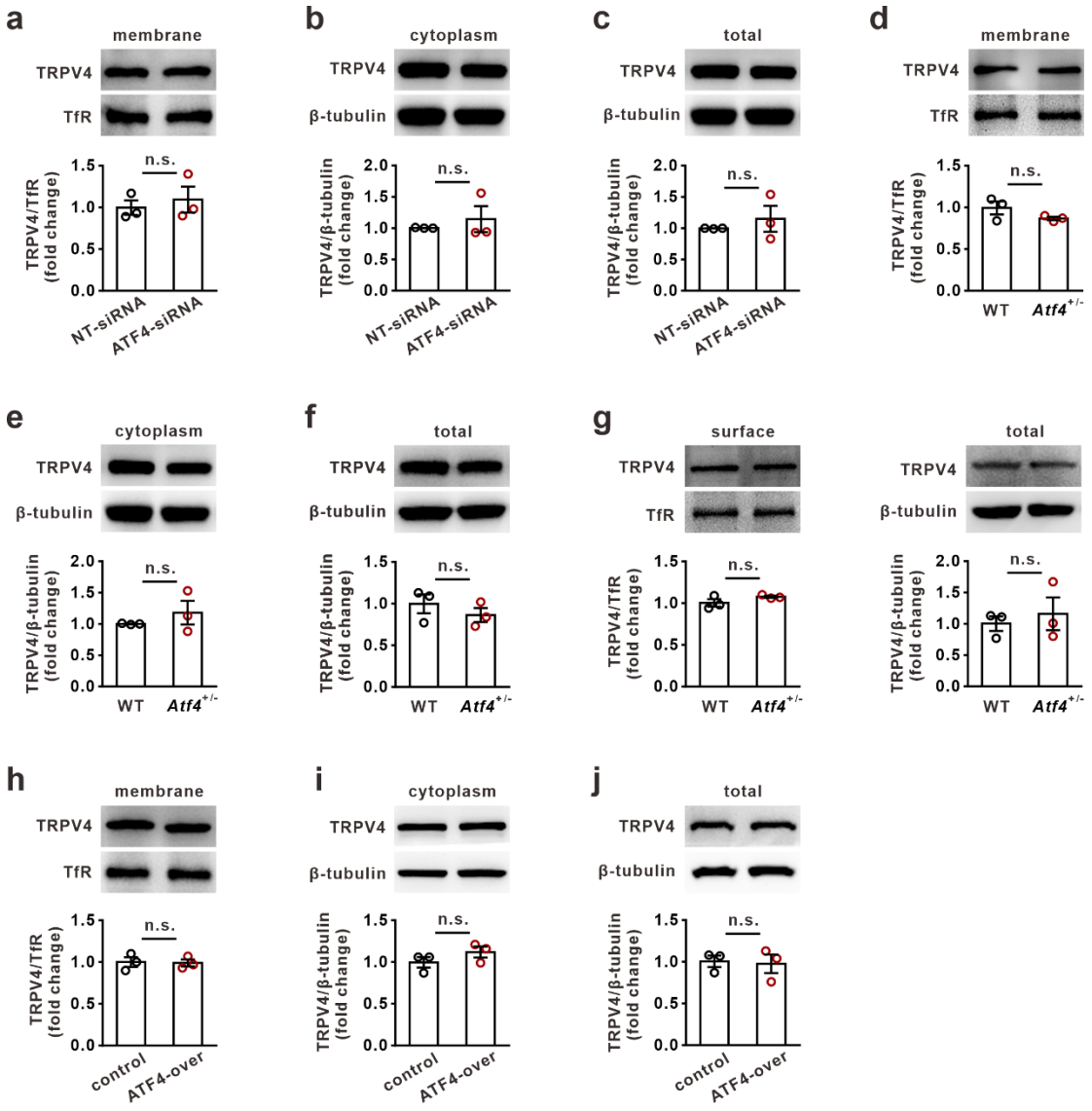
Supplementary Fig. 3 Intrathecal injection of AAV expressing ATF4 (rAAV-hSyn-*Atf4*-2A-EGFP) to overexpress ATF4 in DRGs of WT mice. (**a**) The expression of ATF4 in cervical DRGs of WT mice by immunostaining after intrathecal injection with rAAV-hSyn-*Atf4*-2A-EGFP in 21 days. n = 4 mice per group. Scale bar, 50 μm. (**b**) The expression of ATF4 in cervical DRGs of WT mice by immunoblotting after intrathecal injection with rAAV-hSyn-*Atf4*-2A-EGFP in 21 days. n = 3 samples per group (2 mice mixed into a sample). **a**, **b**, Two-tailed Independent Student's *t* test. **P* < 0.05, ***P* < 0.01. The error bars indicate the SEMs.



Supplementary Fig. 4 Spinal nerves local injecting AAV expressing ATF4 (rAAV-hSyn-*Atf4*-2A-EGFP) to over-express ATF4 in DRG neurons of mice. (**a**) The expression of EGFP in cervical DRGs of mice in 21 days after spinal nerves injecting rAAV-hSyn-*Atf4*-2A-EGFP. Scale bar, 50 μm. (**b**) The expression of ATF4 in cervical DRGs of mice by immunoblotting after spinal nerves injection with rAAV-hSyn-*Atf4*-2A-EGFP in 21 days. n = 3 samples per group (2 mice mixed into a sample). (**c**) The expression of ATF4 in cervical DRGs of mice by immunostaining after spinal nerves injection with rAAV-hSyn-*Atf4*-2A-EGFP in 21 days. n = 4 mice per group. Scale bar, 50 μm. **b**, **c**, Two-tailed Independent Student's *t* test. **P* < 0.05, ***P* < 0.01. The error bars indicate the SEMs.

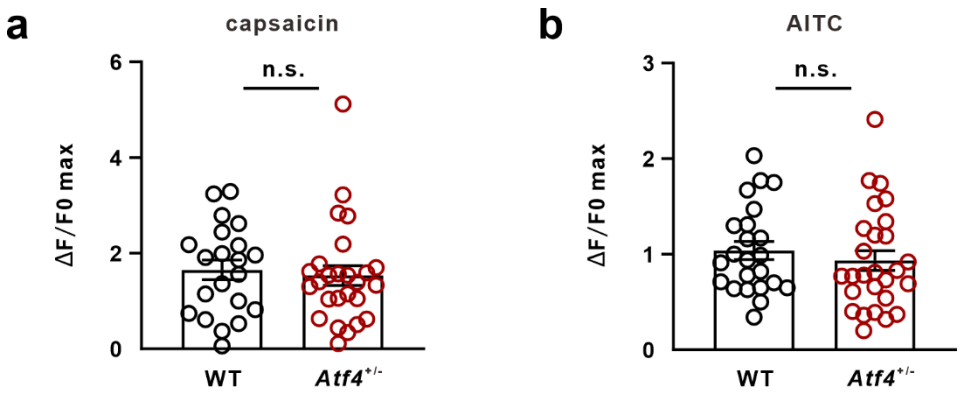


Supplementary Fig. 5 ATF4 does not alter the expression of H1R, H4R, and MrgprA3 in DRGs. (**a-c**) The mRNA expression levels of *H1R*, *H4R*, and *MrgprA3* in WT and *Atf4*$^{+/-}$ mouse DRGs. n = 6 mice per group. **a-c**, Two-tailed Independent Student's *t* test. n.s. means not significant. The error bars indicate the SEMs.



Supplementary Fig. 6 ATF4 does not change the expression and trafficking of TRPV4 in sensory neurons. (**a-c**) The effect of ATF4 knockdown on the membrane (**a**), cytoplasm (**b**) and total (**c**) expression of TRPV4 in DRGs. n = 3 samples per group (2 mice mixed into a sample). (**d-f**) The expression of TRPV4 in the DRG membrane fraction (**d**), cytoplasm fraction (**e**) and total lysate (**f**) from WT and *Atf4*$^{+/-}$ mice. n = 3 samples per group (2 mice mixed into a sample). (**g**) TRPV4 surface levels were measured in cultured DRG neurons prepared from WT and *Atf4*$^{+/-}$ mice using a surface biotinylation assay. n = 3 repeats per group. (**h-j**) The effect of overexpressing ATF4 on

the membrane (**h**), cytoplasm (**i**) and total (**j**) expression of TRPV4 in DRGs. n = 3 samples per group (2 mice mixed into a sample). **a-j**, Two-tailed Independent Student's *t* test. n.s. means not significant. The error bars indicate the SEMs.



Supplementary Fig. 7 ATF4 does not regulate TRPV1 and TRPA1 channels in sensory neurons. (**a and b**) The peak Ca^{2+} responses in DRG neurons, triggered by capsaicin (a TRPV1 agonist at 100 nM) and AITC (a TRPA1 agonist at 200 μM), were compared between WT and *Atf4*$^{+/-}$ mice. n = 21-27 neurons per group. **a**, **b**, Two-tailed Independent Student's *t* test. n.s. means not significant. The error bars indicate the SEMs.