

Expanded View Figures

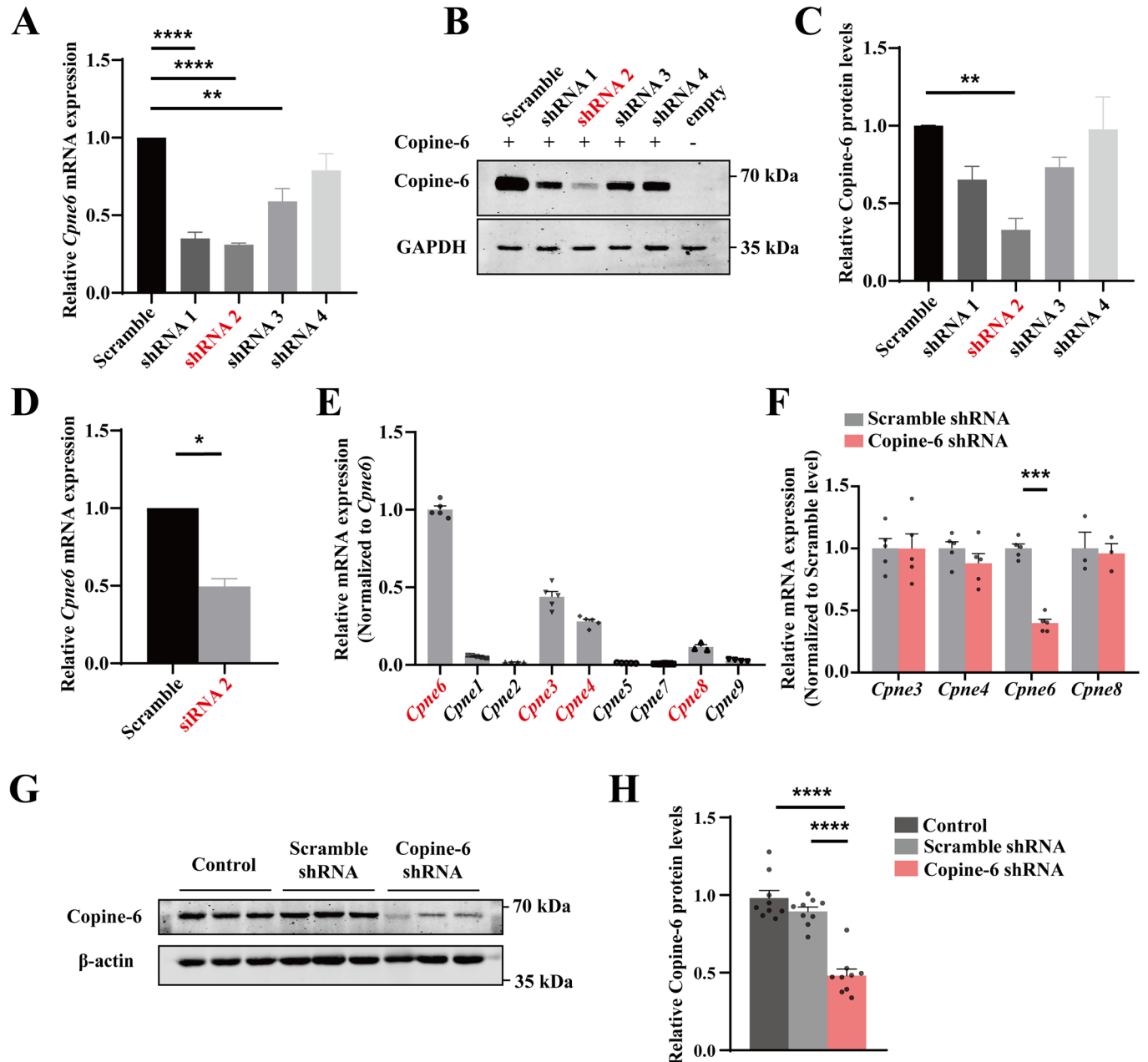
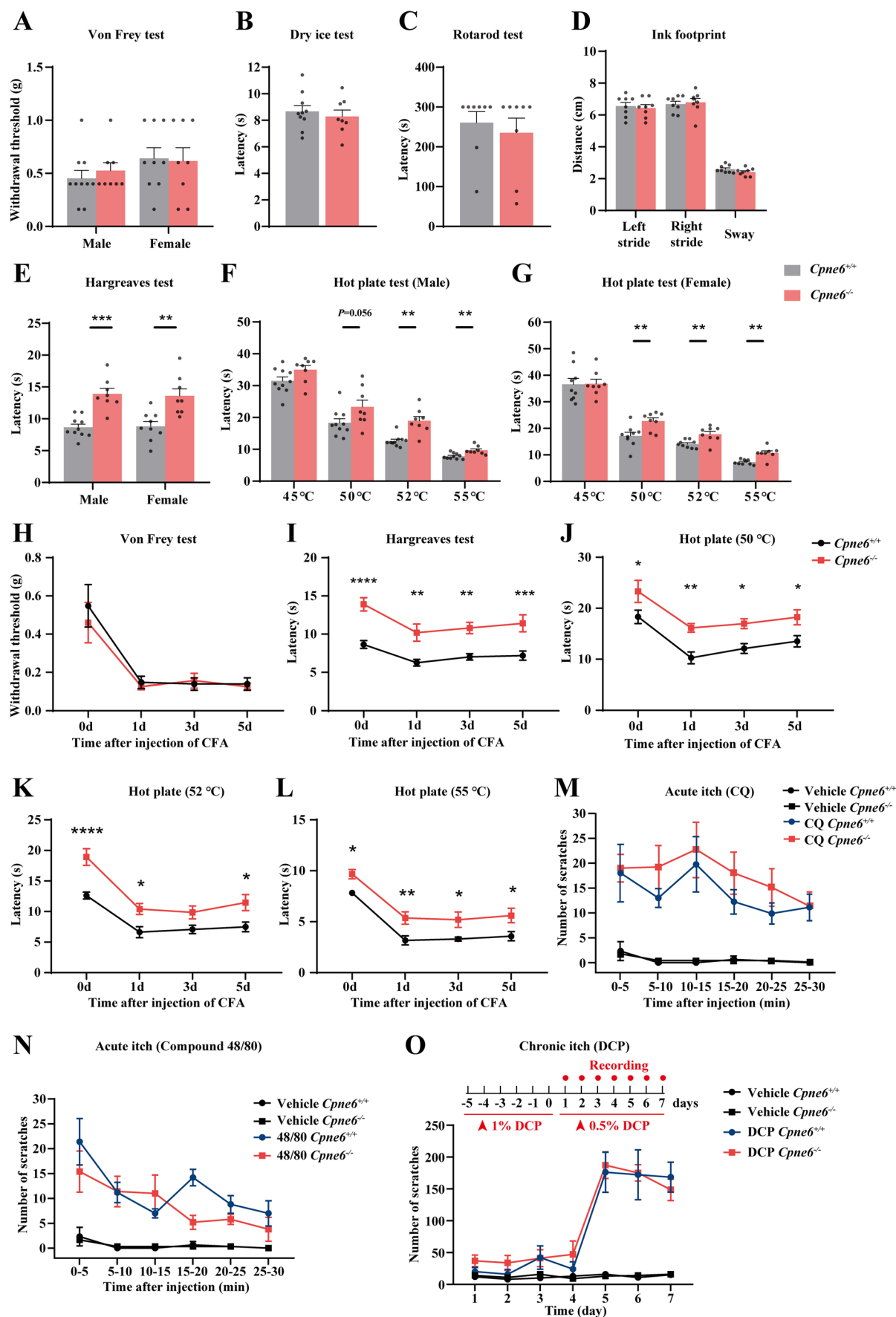


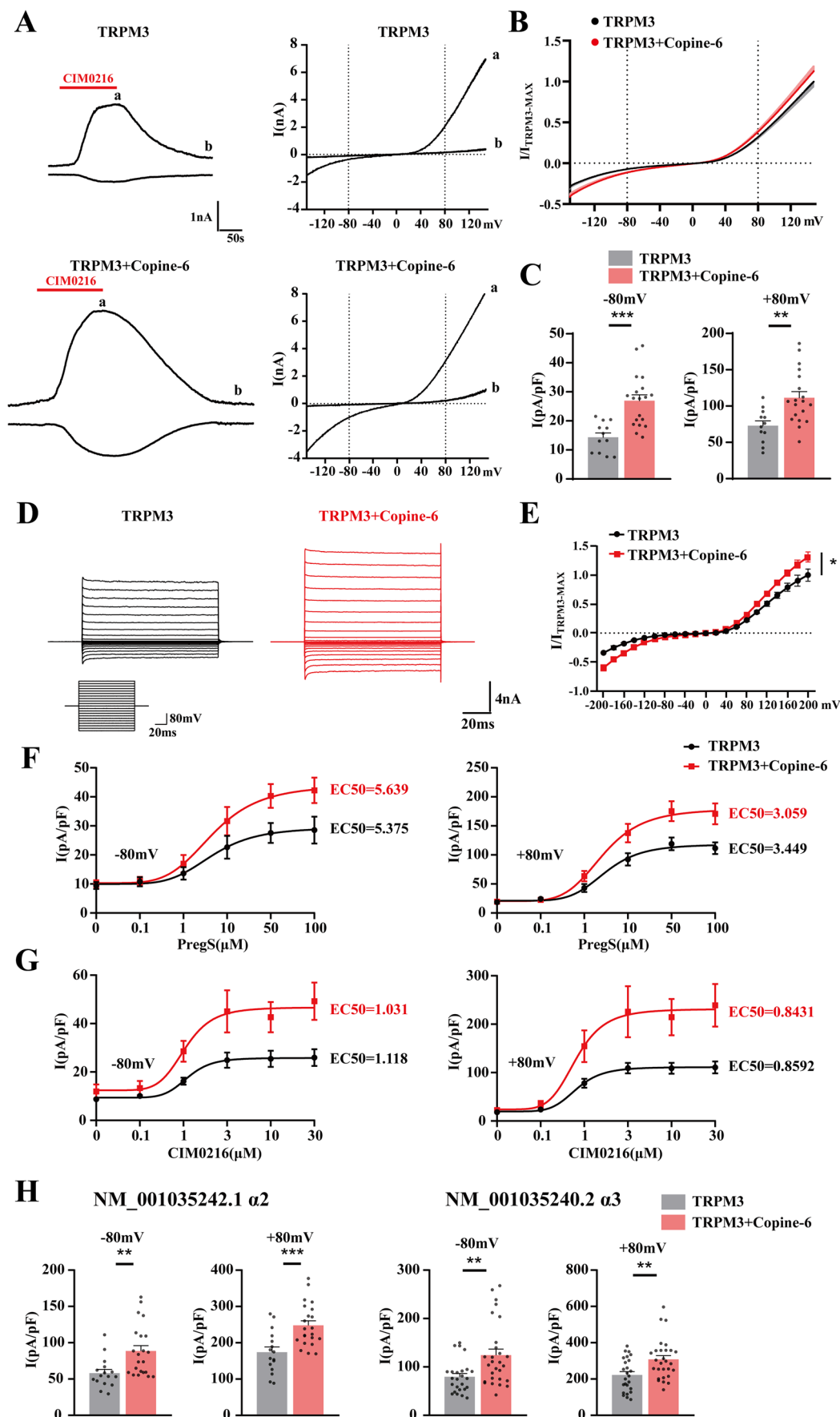
Figure EV1. Validation of *Cpn6* knockdown efficiency in vitro and in vivo in the rat.

(A) Summary data of *Cpn6* mRNA knockdown by four *Cpn6*-targeting shRNAs in HEK293T cells overexpressing *Cpn6*; $n = 4$; Data are shown as mean $\pm$ SEM; Scramble vs. shRNA1, **** $P < 0.0001$; Scramble vs. shRNA2, **** $P < 0.0001$; Scramble vs. shRNA3, ** $P = 0.0014$ (one-way ANOVA followed by Bonferroni multiple comparisons test). (B) Example of western blot assay for Copine-6 expression in HEK293T cells co-transfected with Copine-6 and four shRNA constructs used. (C) Quantification of the Copine-6 protein expression knockdown by Copine-6 shRNAs in HEK293T cells (from the experiments as these shown in (B)); $n = 4$; Data are shown as mean $\pm$ SEM; ** $P = 0.0024$ (one-way ANOVA followed by Bonferroni multiple comparisons test). (D) Knockdown of *Cpn6* mRNA by siRNA2 in cultured DRG neurons; $n = 4$; Data are shown as mean $\pm$ SEM; * $P = 0.029$ (Mann-Whitney U test). (E) The mRNA expression levels of *Cpn1-9* genes in the whole DRG of rats, relative to *Cpn6*. Genes displaying considerable expression are highlighted in red. *Cpn1*, 3-6, $n = 5$; *Cpn2*, 9, $n = 4$; *Cpn7*, $n = 8$; *Cpn8*, $n = 3$; Data are shown as mean $\pm$ SEM. (F) qPCR analysis of the *Cpn3*, 4, 6 and 8 mRNA in the whole DRG of rats injected with AAV9-U6-Copine-6-shRNA-EGFP or AAV9-U6-Scramble-shRNA-EGFP (45 days after viral injection into the DRG); *Cpn3*, 4, 6, $n = 5$; *Cpn8*, $n = 3$. Data are shown as mean $\pm$ SEM. *** $P < 0.001$ (two-tailed independent t test). (G) Example of western blot assay for Copine-6 expression in the DRGs of rats with viral DRG-targeted shRNA knockdown of *Cpn6*. (H) Quantification of the Copine-6 protein expression in the DRG (from the experiments as these shown in (G)); $n = 9$; Data are shown as mean $\pm$ SEM; **** $P < 0.0001$ (one-way ANOVA followed by Bonferroni multiple comparisons test). Source data are available online for this figure.



◀ **Figure EV2. Additional behavioral characterization of *Cpne6*^{-/-} mice.**

(A–F) The somatosensory and motor coordination tests were performed on *Cpne6*^{+/+} and *Cpne6*^{-/-} mice, as follows: Von Frey test (A), dry ice test (B), rotarod test (C), ink footprint test (D), Hargreaves test (E), hot plate test (F, G). (A, E–G): *n* = 10 mice in male *Cpne6*^{+/+} group, *n* = 9 mice in female *Cpne6*^{+/+} group and *n* = 8 mice in *Cpne6*^{-/-} groups of either gender. (B) *n* = 10 mice in *Cpne6*^{+/+}, *n* = 8 mice in *Cpne6*^{-/-} group. (C, D) *n* = 8 mice in both groups. Data are shown as mean ± SEM. (E) Male, ****P* < 0.001; Female, ***P* = 0.003. (F) 50 °C, *P* = 0.056; 52 °C, ***P* = 0.001; 55 °C, ***P* = 0.002. (G) 50 °C, ***P* = 0.007; 52 °C, ***P* = 0.005; 55 °C, ***P* = 0.001 (two-tailed independent *t* test). (H–L) Knockout of *Cpne6* reduced CFA-induced heat hyperalgesia (I–L) but not mechanical allodynia (H); *n* = 10 mice in all *Cpne6*^{+/+} groups and *n* = 8 mice in all *Cpne6*^{-/-} groups. Data are shown as mean ± SEM. I, 0 d, *****P* < 0.0001; 1 d, ***P* = 0.0012; 3 d, ***P* = 0.0019; 5 d, ****P* = 0.005. J, 0 d, **P* = 0.0308; 1 d, ***P* = 0.0078; 3 d, **P* = 0.038; 5 d, **P* = 0.0465. (K) 0 d, *****P* < 0.0001; 1 d, **P* = 0.025; 5 d, **P* = 0.0168. (L) 0 d, **P* = 0.0406; 1 d, ***P* = 0.0099; 3 d, **P* = 0.0334; 5 d, **P* = 0.0198. Two-way ANOVA with Bonferroni multiple comparisons test. (M–O) Acute and chronic itch tests. (M) Histamine-independent acute itch model. Chloroquine (CQ) (200 µg/50 µL, vehicle: Saline) was intradermally injected into dorsal neck area and number of scratching attempts quantified during 30 min period. *n* = 3 mice in each vehicle group and *n* = 8 mice in each CQ group. Data are shown as mean ± SEM. (N) Histamine-dependent acute itch model. Compound 48/80 (100 µg/50 µL, vehicle: Saline) was intradermally injected into dorsal neck area and number of scratching attempts quantified during 30 min period. *n* = 3 mice in each vehicle group and *n* = 5 mice in each 48/80 group. Data are shown as mean ± SEM. (O) Chronic itch model. Shown on the top is the experimental timeline. Diphenylcyclopropenone (DCP, 1%, then 0.5% in acetone, as indicated) was applied to the dorsal neck area (see “Methods”) and scratching behavior recorded for 30 min after each application during seven consecutive days; vehicle: acetone; *n* = 3 mice in each vehicle group and *n* = 5 mice in each DCP group. Data are shown as mean ± SEM. Source data are available online for this figure.



◀ **Figure EV3. Expression of Copine-6 increases TRPM3-mediated currents.**

(A) Time course (left) and I-V relationship (right) of whole-cell currents induced by CIM0216 (5 μ M) in CHO cells expressing TRPM3 (top) or TRPM3 + Copine-6 (bottom). (B) Voltage dependence of CIM0216-induced currents in TRPM3-expressing CHO cells with (red) and without (black) Copine-6 co-expression. Currents were induced by voltage ramps from -150 to $+150$ mV in 900 ms. Current-voltage relationships normalized to I_{TRPM3} at $+150$ mV are shown. TRPM3, $n = 12$; TRPM3 + Copine-6, $n = 19$. (C) Comparison of 5 μ M CIM0216-induced current densities in CHO cells expressing TRPM3 (NM_177341.4/y3), either alone or together with Copine-6 at -80 or $+80$ mV. TRPM3, $n = 12$; TRPM3 + Copine-6, $n = 19$; Data are shown as mean $\pm$ SEM; -80 mV, $***P < 0.001$; $+80$ mV, $**P = 0.003$ (two-tailed independent t test). (D) Current traces obtained with a voltage step protocol (inset on the bottom-left) in CHO cells transfected with TRPM3 alone (black) or co-transfected with Copine-6 (red) after stimulation by 5 μ M CIM0216. (E) Voltage dependence of CIM0216-induced currents in TRPM3-expressing CHO cells with (red) and without (black) Copine-6 co-expression. Current-voltage relationships normalized to I_{TRPM3} at $+200$ mV are shown. TRPM3, $n = 13$; TRPM3 + Copine-6, $n = 19$; Data are shown as mean $\pm$ SEM; $*P = 0.0188$ (two-way ANOVA followed by Bonferroni multiple comparisons test). (F) Concentration-response curves for Pregnenolone monosulfate sodium (PregS) in CHO cells transfected with TRPM3 alone (black) or co-transfected with Copine-6 (red) at -80 mV (left) and $+80$ mV (right); recordings as these shown in (A). The EC_{50} values are shown next to the curves. Current densities (pA/pF) represent the peak values of the PregS-induced currents. The number of recordings, n (from low to high concentration of PregS, respectively): 22, 18, 17, 20, 22, and 20 for cells expressing TRPM3 alone (black); 20, 20, 20, 20, 19 and 18 for cells expressing TRPM3 and Copine-6 (red). Data are shown as mean $\pm$ SEM. All curves were fitted with Four-Parameter Logistic function. (G) Similar to (F), but CIM0216 concentration-response curves are plotted. The number of recordings, n (from low to high concentration of CIM0216, respectively): 17, 16, 16, 17, 15, and 12 for cells expressing TRPM3 alone (black); 17, 17, 17, 14, 13 and 13 for cells expressing TRPM3 and Copine-6 (red). Data are shown as mean $\pm$ SEM. (H) Comparison of 5 μ M CIM0216-induced current densities in CHO cells expressing TRPM3 splice variants (NM_001035242.1/a2 or NM_001035240.2/a3), either alone or together with Copine-6 at -80 or $+80$ mV. NM_001035242.1/a2: TRPM3, $n = 16$; TRPM3 + Copine-6, $n = 22$; NM_001035240.2/a3: TRPM3, $n = 25$; TRPM3 + Copine-6, $n = 28$. Data are shown as mean $\pm$ SEM. NM_001035242.1/a2: -80 mV, $**P = 0.002$; $+80$ mV, $***P < 0.0001$. NM_001035240.2/a3: -80 mV, $**P = 0.003$; $+80$ mV, $**P = 0.0003$ (two-tailed independent Student's t -test). Source data are available online for this figure.

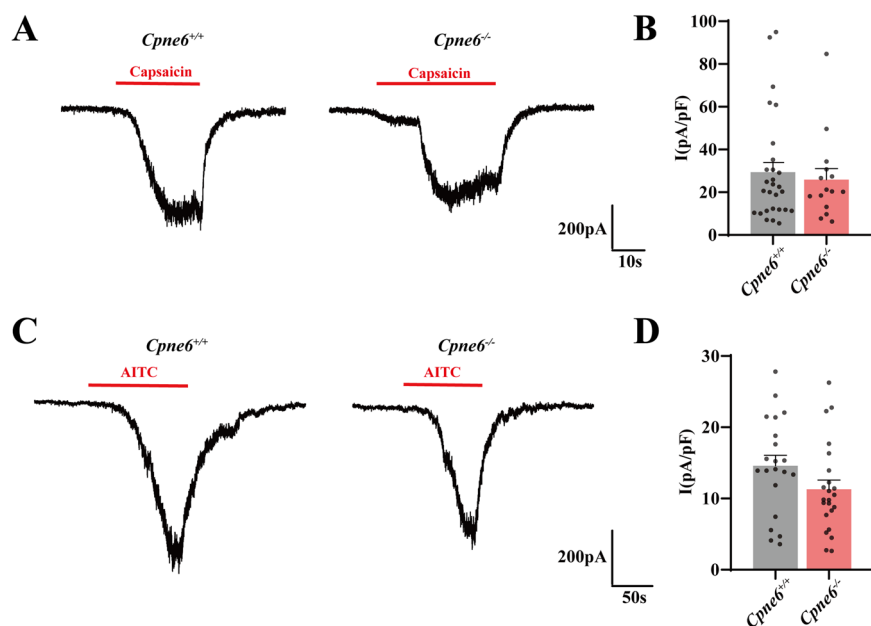


Figure EV4. TRPV1 and TRPA1 currents in DRG neurons of *Cpne6*^{+/+} and *Cpne6*^{-/-} mice.

(A) Example current traces evoked by capsaicin (1 μ M) in DRG neurons from *Cpne6*^{+/+} (left) or *Cpne6*^{-/-} (right) mice. Recordings were performed using a gap-free protocol at a holding potential of -60 mV. (B) Comparison of capsaicin-evoked current densities in DRG neurons from *Cpne6*^{+/+} ($n = 28$) and *Cpne6*^{-/-} ($n = 15$) mice. Data are shown as mean $\pm$ SEM. (C) Example currents evoked by AITC (100 μ M) in DRG neurons from *Cpne6*^{+/+} (left) or *Cpne6*^{-/-} (right) mice. Recordings were performed as in (A). (D) Comparison of AITC-evoked current densities in DRG neurons from *Cpne6*^{+/+} ($n = 21$) and *Cpne6*^{-/-} ($n = 23$) groups. Data are shown as mean $\pm$ SEM. Source data are available online for this figure.

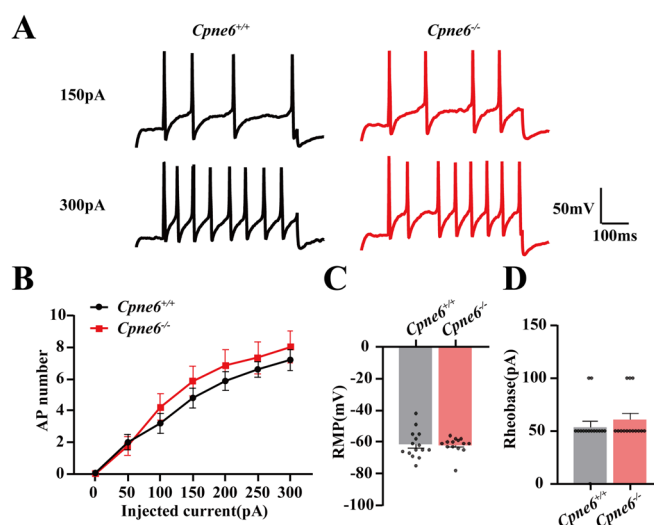


Figure EV5. Copine-6 deficiency has no impact on the general excitability of DRG neurons.

(A) Representative current clamp recordings from dissociated DRG neurons from *Cpne6*^{+/+} (left) or *Cpne6*^{-/-} (right) mice. Firing is induced by 500 ms injections of either 150 (top) or 300 pA (bottom) depolarizing currents. (B) Summary of the numbers of action potentials elicited by depolarizing current steps from 0 to 300 pA (in 50 pA increments) elicited in DRG neurons from *Cpne6*^{+/+} (*n* = 15) or *Cpne6*^{-/-} (*n* = 14) mice; Data from 3 independent preparations. Recordings were made at room temperature. Data are shown as mean ± SEM. (C, D) Bar graphs and scatter plots showing the resting membrane potential (RMP; C) and rheobase (D) of DRG neurons from *Cpne6*^{+/+} (*n* = 15) or *Cpne6*^{-/-} (*n* = 14) mice; data from 3 independent preparations. Data are shown as mean ± SEM. Source data are available online for this figure.