Supplement Figure 1. Effects of EA on the protein levels of GluR2, GluR2-p, ICA69 and PICK1 in Day1, Day3, Day5, Day7, and CFA group on the ipsilateral side of SDH, respectively. A: Representative western blot bands of GluR2, GluR2-p, ICA69 and PICK1. Quantitative analyses of ICA69 (B), GluR2-p (C), PICK1 (D) and GluR2 (E) (n = 3, group values are indicated by means ± SEM; **p < 0.01; *p <0.05). EA = electroacupuncture, CFA = complete Freund’s adjuvant, SDH = spinal dorsal horn.
Supplement Figure 2. Injecting 5µl different concentrations of ICA69 peptide into the intrathecal space of ICA69-KO mice, evaluate the analgesic effect of ICA69 peptide within 24 hours and effects of ICA69 peptide on the protein levels of GluR2, GluR2-p, ICA69 and PICK1 at 6h. A: Reduction in paw withdrawal frequency of CFA+ Peptide (10mg/ml) group compared with CFA+LP group on the ipsilateral side, this reduction was particularly obvious at 6h and 12h (n = 3; *p < 0.05, **p < 0.01). B: Representative western blot bands of GluR2, GluR2-p, ICA69 and PICK1. Quantitative analyses of ICA69 (C), GluR2-p (D), PICK1 (E) and GluR2 (F) (n = 3, group values are indicated by means ± SEM; **p < 0.01; *p < 0.05). CFA = complete Freund's adjuvant, LP= leader peptide.
Supplement Figure 3. Effects of SEA on the protein levels of GluR2, GluR2-p, ICA69 and PICK1 on the ipsilateral side of SDH at Day3, respectively. A: Representative western blot bands of GluR2, GluR2-p, ICA69 and PICK1. Quantitative analyses of ICA69 (B), GluR2-p (C), PICK1 (D) and GluR2 (E) (n = 3, group values are indicated by means ± SEM; **p < 0.01; *p < 0.05). EA = electroacupuncture, SEA = sham electroacupuncture, CFA = complete Freund’s adjuvant, SDH = spinal dorsal horn.
Supplement Figure 4. Immunohistochemical results of ICA69 in SDH in the four indicated groups: the tawny staining represents the ICA69 protein, hematoxylin staining represents the nucleus, the white arrow points to the positive cells in SDH area. Scale bars 20µm. Quantification of ICA69 per µm² in SDH (n = 3; group values are indicated by means ± SEM; **p < 0.01, *p < 0.05). EA = electroacupuncture, CFA = complete Freund’s adjuvant, SDH = spinal dorsal horn.
Supplement Figure 5. Immunofluorescence results of GluR2-p in SDH in the six indicated groups: the green staining represents the ICA69 protein, the white arrow points to the positive cells in SDH area. Scale bars 20µm. Quantification of GluR2-p per µm² in SDH (n = 3; **KO-CFA+EA vs WT-CFA+EA, p < 0.001; group values are indicated by means ± SEM; **p < 0.01; *p <0.05). EA = electroacupuncture, CFA = complete Freund’s adjuvant, SDH = spinal dorsal horn. WT = wild type, KO = knockout.