

Supplemental Figures and Movies

Figure S1, related to Figures 3 and 6. Direct evidence for neuron-neuron and neuron-SGC coupling and behavioral tests of Cx43CKO mice.

(A) Confocal images obtained with the dye transfer coupling method using intracellular injection of Lucifer yellow (LY). A LY-injected DRG neuron is coupled to an adjacent neuron by dye transfer in DRG culture after CFA injection. Scale bar: 20 μm . (B) A LY-injected neuron is coupled to SGCs around the LY-injected neuron. Arrow indicates where the LY-transferred SGC is located. Blue color is DAPI labeling to confirm the SGC around the LY-injected neuron. (C) Dual patch clamp recordings of neuron-SGC pairs in acutely isolated DRG from CFA-treated mice. Top panel, the configuration of neuron and SGCs is shown. Bottom panel, holding potential -60 mV. Voltage pulses to -45 and +45 mV in one neuron of the pairs. The junctional conductance is ~ 4 nS. (D, E) Patch recording of neuron-neuron pairs in acutely isolated DRG from CFA-treated mice. Top panels, the configuration of neuron-neuron pairs are shown. Bottom panels, holding potential -60 mV. Voltage pulses to -45 and +45 mV in one neuron of the pairs. (D) Evidence for neuron-neuron electrical coupling. Note the inward currents (junctional conductance: ~ 400 pS), which characterize a neuron. (E) A neuron-neuron pair without electrical coupling. (F-I) Pain behavioral tests of Cx43CKO mice. Baseline pain sensitivity of naïve Cx43CKO mice was compared to WT littermates by hot plate (F; n=8 per genotype), tail immersion (G; n=8 WT; n=7 Cx43CKO), and von Frey filament (H; n=8 per genotype). CFA-induced thermal hyperalgesia was examined for WT and Cx43CKO mice by Hargreaves test (I; n=8 per genotype). No significant differences were found between WT and KO mice in these behavioral tests. Cx43CKO mice did not display spontaneous pain. The data are presented as mean \pm SEM.

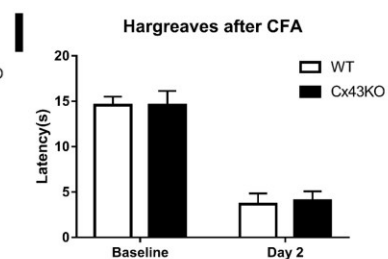
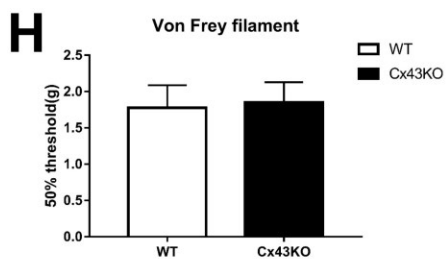
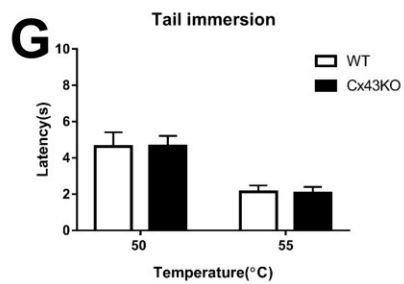
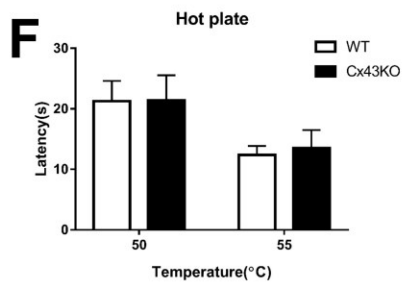
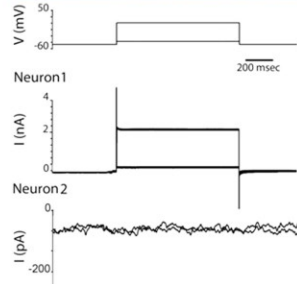
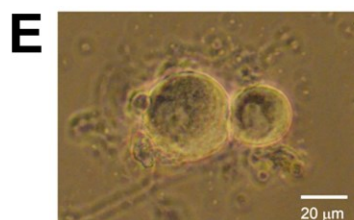
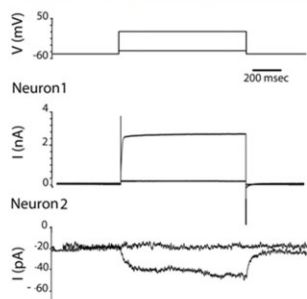
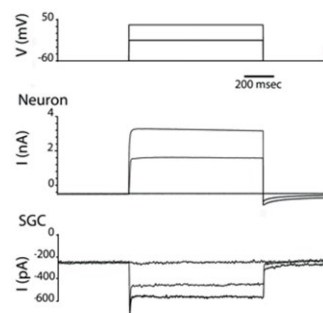
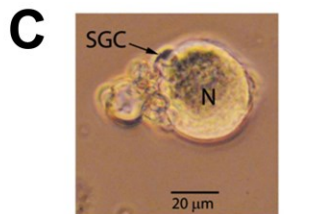
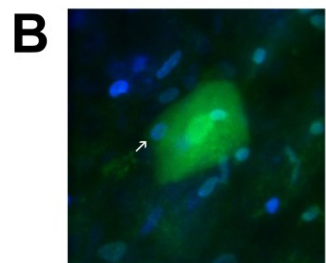
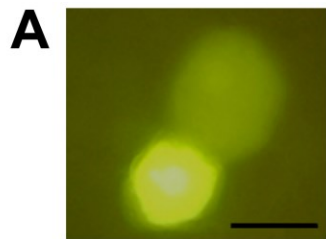
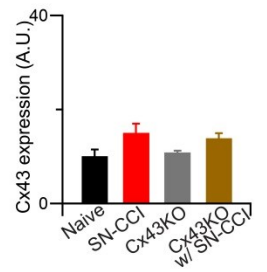
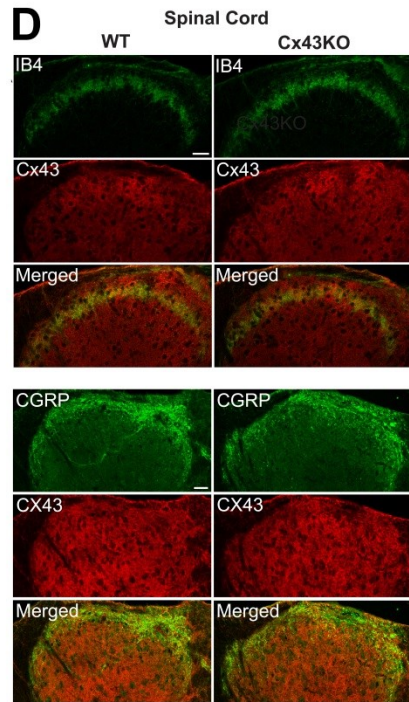
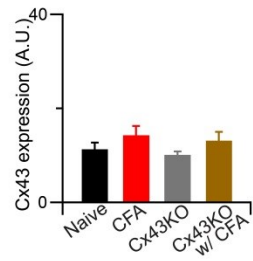
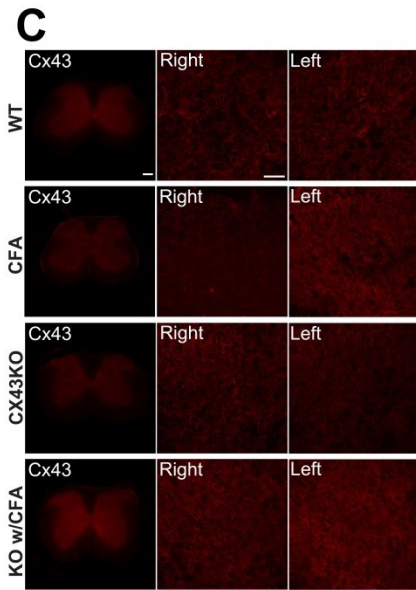
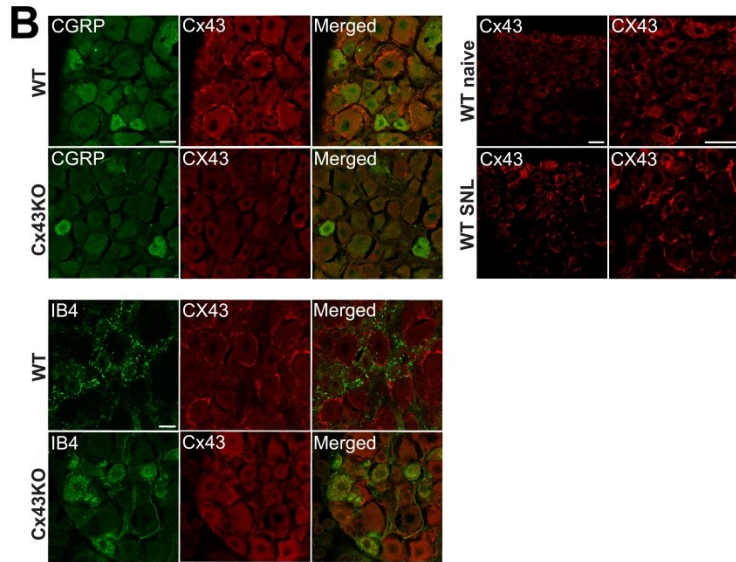
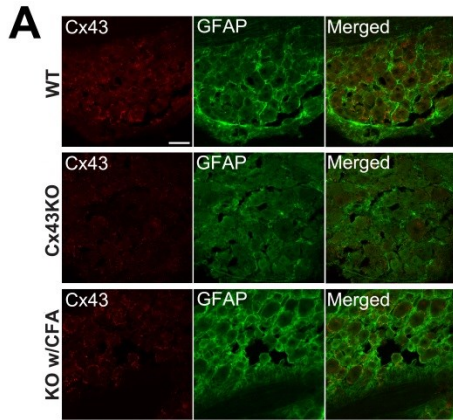


Figure S2, related to Figure 6. Characterization of Cx43 expression in DRG and spinal cord

(A) Cx43 is expressed at low levels in DRG of naïve, Cx43CKO and Cx43CKO after CFA injection. Representative confocal images of Cx43 and GFAP immunostaining from naïve, Cx43CKO, and Cx43CKO with CFA in DRG neurons. Scale bar: 50 μ m. (B) Characterization of Cx43 positive DRG neurons. Double immunostaining of Cx43 (red) and CGRP (green) or IB4 (green) in DRG neurons shows the expression pattern of Cx43 in different types of DRG neurons. After the spinal nerve ligation (SNL) animal model, Cx43 expression is dramatically enhanced surrounding DRG neurons. Scale bars: 20 μ m. (C) Expression level of Cx43 protein in spinal cord is not significantly changed after inflammation. Representative confocal images of Cx43 and expression level of Cx43 protein in spinal cord after inflammation shows that there is no significant change between naïve, CFA, Cx43CKO, or Cx43CKO with CFA (n=7). The quantification is on the right. Left panels, Scale bar: 200 μ m. Middle and right panels, Scale bar: 50 μ m. (D) Expression level of Cx43 protein in spinal cord is not significantly changed after nerve injury. Molecular characterization of Cx43 protein in spinal cord. Double immunostaining of Cx43 (red) and CGRP (green), IB4 (green) in spinal cord shows the expression pattern of Cx43 protein. Representative confocal images of Cx43 and expression level of Cx43 protein in spinal cord is not significantly changed after nerve injury, even with conditional Cx43CKO there is no significant change compared with naïve, SN-CCI, Cx43CKO and Cx43CKO with SN-CCI (n=5). The quantification is on the right. Scale bar: 100 μ m.



Supplemental Movies

Movie S1, related to Figure 2. Representative imaging of *In vivo* whole L4 DRG neurons from naïve animal. Mild press stimulus was indicated in all of the movies. Note that the resolution of the movies was reduced after conversion from Lif (Leica) software to movie format avi.

Movie S2, related to Figure 3. Representative imaging of *In vivo* whole L4 DRG neurons from CFA animal.

Movie S3, related to Figure 4,5. Representative coupled activation event imaging of *In vivo* whole L4 DRG neurons from CFA animal.

Movie S4, related to Figure 5. Representative imaging of decoupled event after systemic gap junction blocker, carbenoxolone (CBX) application of *in vivo* L4 DRG neurons from CFA animal. Compare with Movie S3 for before CBX treatment.

Movie S5, related to Figure 5. Representative imaging of coupled activation event before local CBX application into DRG in *in vivo* whole L4 DRG neurons from CFA animal.

Movie S6, related to Figure 5. Representative imaging of decoupled event after local CBX application into DRG in *in vivo* whole L4 DRG neurons from CFA animal.