PAIN REGULATION

miR-183 cluster scales mechanical pain sensitivity by regulating basal and neuropathic pain genes

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Nociception is protective and prevents tissue damage but can also facilitate chronic pain. Whether a general principle governs these two types of pain is unknown. Here, we show that both basal mechanical and neuropathic pain are controlled by the microRNA-183 (miR-183) cluster in mice. This single cluster controls more than 80% of neuropathic pain–regulated genes and scales basal mechanical sensitivity and mechanical allodynia by regulating auxiliary voltage-gated calcium channel subunits α2δ-1 and α2δ-2. Basal sensitivity is controlled in nociceptors, and allodynia involves TrkB$^+$ light-touch mechanoreceptors. These light-touch–sensitive neurons, which normally do not elicit pain, produce pain during neuropathy that is reversed by gabapentin. Thus, a single microRNA cluster continuously scales acute noxious mechanical sensitivity in nociceptive neurons and suppresses neuropathic pain transduction in a specific, light-touch–sensitive neuronal type recruited during mechanical allodynia.

Nociceptive (basal) pain serves as a protective mechanism to bring awareness of injured tissue. Pain is relayed by nociceptors in the dorsal root ganglia (DRGs) that link skin or deep tissues to the spinal cord dorsal horn. Variation in an individual's responses to nociceptive pain (*1*) is partially explained by genetic variation (*2*). Mechanical allodynia during neuropathic pain—which involves hypersensitivity of nociceptive neurons (*3*) and recruitment of touch-activated low-threshold mechanoreceptive (LTMR) neurons into the nociceptive network (*4*)—is governed by changes in gene expression (*3*) susceptible to influence by microRNAs (miRNAs) (*5*, *6*). Down-regulation of microRNA-183 (miR-183, part of miR-183/96/182 or the miR-183 cluster) in sensory neurons characterizes chronic pain (*7*, *8*), whereas overexpression attenuates some forms of allodynia (*8*, *9*). Here we ask how and in which cell types the miR-183 cluster contributes to basal and neuropathic pain.

We observed miR-183/96/182 expressed in most DRG neurons by embryonic day (E) 10.5 and also in the adult, with miR-96 present at lower levels (fig. S1, A to F). To determine the physiological role of the miR-183 cluster for nociception, we generated a conditional targeted allele in mice ($miR^{fl/fl}$ mice) (fig. S2, A and B) that, when bred to *Wnt1-Cre* strain (*10*) (*Wnt1-Cre;*$miR^{fl/fl}$ mice), confirmed a complete elimination of DRG expression by in situ hybridization (fig. S2C). To determine its function in different types of sensory neurons, $miR^{fl/fl}$ mice were crossed to *Wnt1-Cre*, *TH-Cre* (*11*), and $TrkB^{CreERT2/+}$ mice. We generated $TrkB^{CreERT2/+}$ mice by inserting an *IRES-CreERT2* cassette into the 3′ untranslated region of the gene (fig. S2, D to F). The TrkB^{high} neurons are lightly myelinated LTMRs (*12*) of the NF1 type of sensory neurons (*13*). To examine the efficacy of cell type–specific recombination, the driver strains were first crossed onto a $ROSA26^{Tomato}$ reporter strain. *Wnt1-Cre* recombined all neurons of the DRG (fig. S3A), in $TrkB^{CreERT2/+}$;$ROSA26^{Tomato}$ mice 96.0 ± 1.9% of recombined neurons were NECAB2$^+$ NF1 class, which is TrkB^{high} (*13*) (fig. S3B). In *TH-Cre;*$ROSA26^{Tomato}$ mice 98.0 ± 0.6% recombined neurons belonged to the small size, mostly unmyelinated nociceptive neurons, as determined by costaining for TrkA, which is expressed in most or all of these neurons at birth (fig. S3C). Hence, these results show that the three activator strains can be used to address the role of the miR-183 cluster in all sensory neurons (*Wnt1-Cre;*$miR^{fl/fl}$), in the TrkB$^+$ NF1 class of LTMR neurons ($TrkB^{CreERT2/+}$;$miR^{fl/fl}$), and in nociceptors (*TH-Cre;*$miR^{fl/fl}$).

Animals with conditional deletion of the miR-183 cluster in all neurons or only in nociceptors responded normally to light touch, cold, heat, or pinprick but were hypersensitive to mechanical stimuli (Fig. 1, A and C, and fig. S4, A and C). $TrkB^{CreErT2/+}$;$miR^{fl/fl}$ mice were normal in all behavioral tests (Fig. 1B and fig. S4B). Thus, loss of function of the miR-183 cluster results in mechanical hypersensitivity and further tests confirmed also nocifensive-like hyperalgesia (Fig. 1D). Membrane properties were altered, with the action potential (AP) threshold lowered from −10.7 ± 1.6 to −19.9 ± 6.7 mV in *Wnt1-Cre;*$miR^{fl/fl}$ sensory neurons (Fig. 1E), without apparent difference in cell body size or input resistance (fig. S4, D and E). Quantifying the amount of Fos$^+$ spinal cord cells (*14*) following intense innocuous mechanical stimuli (running 10 min, 30 rpm on a rotarod) showed that the miR-183 cluster scales excitability down, because miR-183–deficient mice showed increased numbers in all superficial lamina of the spinal cord (Fig. 1F and fig. S5). Thus, these results show that the miR-183 cluster sets the basal sensitivity to nocifensive mechanical stimuli in nociceptors.

To identify the targets for the miR-183 cluster involved in preventing pain sensitization, we isolated adult L4 to L6 DRGs from *Wnt1-Cre;*$miR^{fl/fl}$ mice and control *Wnt1-Cre* mice for RNA sequencing (table S1). Eighteen up-regulated genes [11 of which were predicted as direct miR-183 cluster targets by TargetScan (*15*)] and 15 down-regulated genes were identified (Fig. 2A; fig. S6, A and B; and table S2). Of the up-regulated genes, *Cacna2d2* could affect pain sensitivity, as *Cacna2d* family members encode voltage-gated calcium channel auxiliary subunits α2δ, which affect nociceptor excitability (*16*–*18*). mRNAs of *Cacna2d1* and *Cacna2d2* expressed in both nociceptors (94 ± 3.5% and 51 ± 8.8%, respectively) and TrkB$^+$ neurons (98 ± 6% and 98 ± 5%, respectively) (Fig. 2B and fig. S6C) were both significantly increased in *Wnt1-Cre;*$miR^{fl/fl}$ DRG (Fig. 2C). Consistently, protein levels in both nociceptors and TrkB$^+$ neurons were elevated (Fig. 2D and fig. S6D). The anticonvulsant gabapentin [1-(aminomethyl) cyclohexane acetic acid], which acts through interacting with α2δ subunits (*19*) and is widely used to treat neuropathic pain (*20*), did not lead to any behavioral changes between the genotypes in cold, heat, or pinprick sensitivity but reversed the mechanical hyperalgesia in *Wnt1-Cre;*$miR^{fl/fl}$ mice (Fig. 2E and fig. S6E).

To analyze whether the miR-183 cluster continuously determines basal sensitivity in the adult, we generated $Rosa26^{CreERT2/+}$;$miR^{fl/fl}$ mice and induced depletion of the miR-183 cluster acutely by tamoxifen administration (100 mg/kg). Mechanical threshold measurements in the same animals before and 6 days after tamoxifen administration revealed sensitization similar to that observed in *Wnt1-Cre;*$miR^{fl/fl}$ mice (Fig. 2F). Tamoxifen administration reduced expression of the miR-183 cluster by about half within 6 days and consequently induced increased expression of *Cacna2d1* and *Cacna2d2* (Fig. 2, G and H). Thus, loss of miR-183 expression in adult rapidly promotes excess *Cacna2d1/2* expression through de-repression. Combined, our results show that the miR-183 cluster continuously suppresses *Cacna2d1/2* expression controlling basal mechanical sensitivity in nociceptors without apparent effects on LTMRs.

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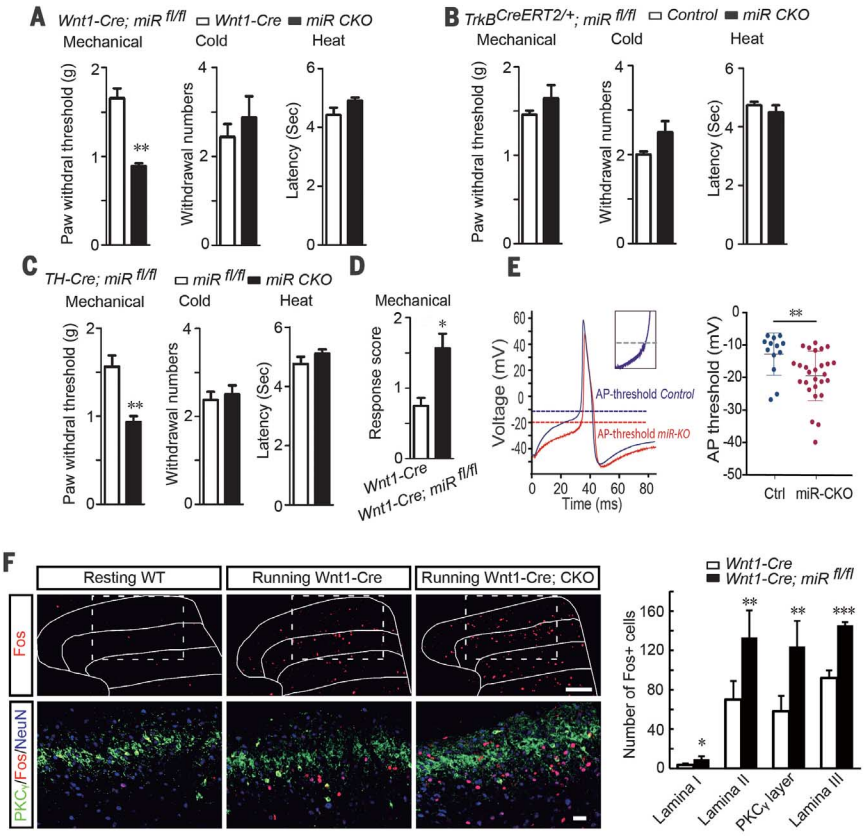

Fig. 1. Increased basal mechanical sensitivity of nociceptors in miR-183–depleted sensory neurons. (**A**) Deficiency of the miR-183 cluster in *Wnt1-Cre;miR$^{fl/fl}$* mice [miR conditional knock out (CKO)] leads to increased acute nocifensive response to mechanical, but not other, stimuli (n = 8 per genotype; $^{**}P < 0.01$, Mann-Whitney test). (**B**) Deficiency of the miR-183 cluster in *TrkB$^{CreERT2/+}$;miR$^{fl/fl}$* mice (miR CKO) leads to no changes in acute pain sensitivity (n = 7 per genotype). (**C**) Deficiency of the miR-183 cluster in *TH-Cre;miR$^{fl/fl}$* mice (miR CKO) leads to increased acute nocifensive response to mechanical, but not other, stimuli (control, n = 8; *TH-Cre;miR$^{fl/fl}$*, n = 9; $^{**}P < 0.01$, Mann-Whitney test). (**D**) Increased nocifensive response of *Wnt1-Cre;miR$^{fl/fl}$* compared with *Wnt1-Cre mice* in response to 1.4 g Von Frey stimuli (n = 7 or 6, respectively, $^{*}P < 0.05$, Mann-Whitney test). (**E**) Action potential (AP) threshold reduction in the absence of the miR-183 cluster. Current protocol is to evoke the first AP in a representative trace. *Wnt1-Cre;miR$^{fl/fl}$* (n = 27) or WT control (n = 13) DRG neurons; P = 0.0016, t test. (**F**) Increases of Fos$^+$ neurons (NeuN$^+$) in the spinal cords of miR-183 cluster–deficient mice (80 min after 10 min of running on a rotarod). The gamma isoform of protein kinase C (PKC$_\gamma$) indicates lamina IIi. Hatched boxes outline the enlarged images below (n = 4 per group; means ± SEM; $^{**}P < 0.01$, $^{***}P < 0.001$, t test; scale bar, 50 μM).

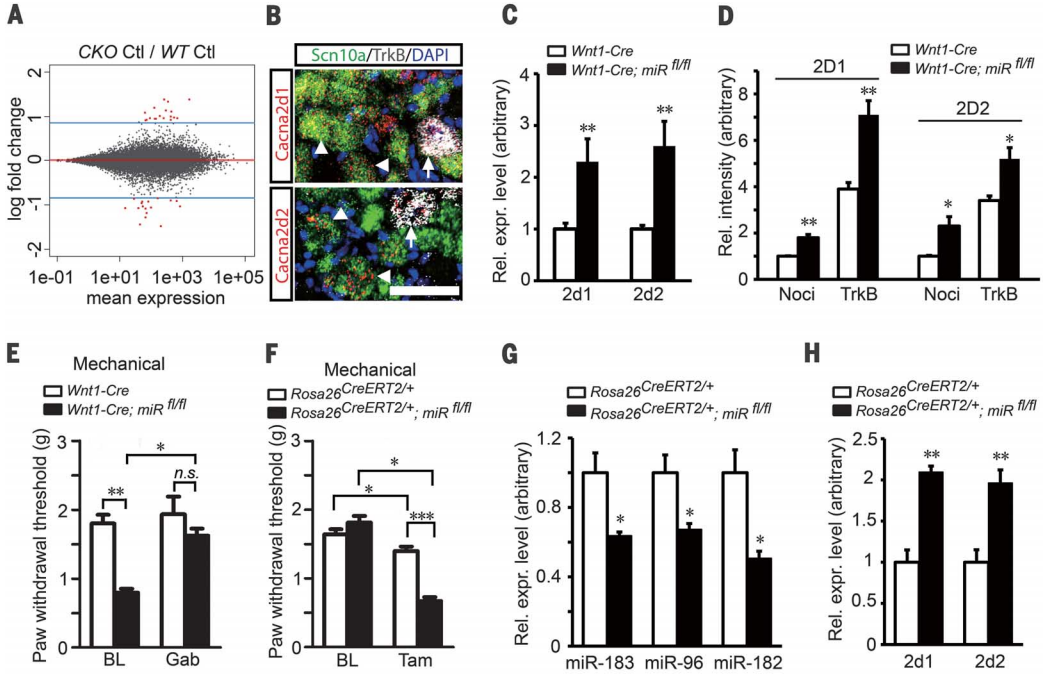

Fig. 2. Control of acute nociceptive mechanical sensitivity through a continuous miR-183-96-182–dependent tuning of auxiliary VGCC expression. (**A**) MA plot of M (log ratio) and A (mean average) scales to show the log$_2$-fold changes. Red dots indicate genes up- or down-regulated more than 1.8-fold (P < 0.01) in DRGs of *Wnt1-Cre;miR$^{fl/fl}$* mice (CKO versus WT, n = 4 or 5, respectively). (**B**) *Cacna2d1* and *Cacna2d2* expression in both nociceptors (Scn10a, arrowheads) and TrkB$^+$ neurons (arrows, triple in situ hybridization), 4′,6-diamidino-2-phenylindole (DAPI) counterstained. Scale bar, 50 μM. (**C**) Quantitative polymerase chain reaction (PCR) of *Cacna2d1* and *Cacna2d2* expression in DRGs of control and *Wnt1-Cre;miR$^{fl/fl}$* mice (n = 6 or 6; means ± SEM; $^{**}P < 0.01$, t test). (**D**) CACNA2D1 and CACNA2D2 immunofluorescence protein intensity levels in nociceptive and TrkB$^+$ neurons of control and *Wnt1-Cre;miR$^{fl/fl}$* mice (n = 3 or 3; means ± SEM; $^{*}P < 0.05$; $^{**}P < 0.01$, t test). (**E**) Relative to *Wnt1-Cre* mice, gabapentin reverses mechanical hypersensitivity of *Wnt1-Cre;miR$^{fl/fl}$* mice (*Wnt1-Cre/Wnt1-Cre;miR$^{fl/fl}$*, (n = 6 or 8, $^{*}P < 0.05$, Wilcoxon test; $^{**}P < 0.01$, Mann-Whitney test). (**F**) Acute depletion of the miR-183 cluster induces mechanical hypersensitivity. Before (BL) and 6 days after tamoxifen (Tam) (*Rosa26$^{CreER/+}$* littermate controls versus *Rosa26$^{CreER/+}$;miR$^{fl/fl}$* mice; n = 10 or 8; $^{*}P < 0.05$, Wilcoxon test; $^{***}P < 0.001$, Mann-Whitney test). (**G**) miR-183/96/182 expression in DRGs 6 days after tamoxifen administration. Quantitative PCR, control mice set to 1 (n = 4 or 3; means ± SEM; $^{*}P < 0.05$, t test). (**H**) *Cacna2d1* and *Cacna2d2* de-repression in DRG 6 days after tamoxifen administration. Quantitative PCR, control mice set to 1 (n = 4 or 3; means ± SEM; $^{**}P < 0.01$, t test).

Human *CACNA2D1* and *CACNA2D2* genes are predicted targets of the miR-183 cluster (fig. S7A). Profiling DRG expression in a human cohort (n = 214) revealed a significant inverse correlation between all three miRNAs and *CACNA2D1* and *CACNA2D2* expression (fig. S7B). The negative correlation for both *CACNA2D1/2* by the miR-183 cluster in both mice and humans suggest a role of the miR-183 cluster in pain mechanisms across mammalian species.

We asked whether the miR-183 cluster also affects neuropathic pain through gene regulation. Wild-type (WT) mice in the spared-nerve injury (SNI) neuropathy model (*21*) at 14 days (table S1) revealed 70 up-regulated and 2 down-regulated genes compared with unlesioned controls (Fig. 3A and table S2). In *Wnt1-Cre;miR$^{fl/fl}$* mice, more genes showed changes in expression (189 up-regulated and 35 down-regulated) (Fig. 3B and table S2). We used the STRING database to analyze functional protein association networks (*22*) (fig. S8, A to F). Several pain-inducing and pain-preventing genes [known from the Pain Gene Database (*23*) and manual interrogation] showed regulatory changes in both WT and *Wnt1-Cre;miR$^{fl/fl}$* mice. Various up-regulated genes were candidate direct targets of miR-183 cluster regulation (genes circled with a green ring in fig. S8, A to F).

Many of the regulated genes attributed to cell signaling, cell adhesion, ubiquitination, neuropeptide modulation, and Ca^{2+} signaling were centered around transcription factors Atf3 and Jun in WT mice with neuropathy. In *Wnt1-Cre;miR$^{fl/fl}$* mice with neuropathy, a larger transcriptional network—including Atf3, Jun, Csrnp1, Nfil3, Ets2, Sox11, and Cited2—passed threshold criteria. Indeed, expression of these transcription factors was also increased for WT mice with neuropathy. Furthermore, Atf3, Csrnp1, Ets2, Sox11, and Cited2 are potential direct miR-183 cluster targets (Fig. 3, C and D). Thus, in addition to directly targeting genes, the miR-183 cluster may affect the global neuropathic pain gene–regulatory network indirectly through targeting core neuropathy-induced transcription factors.

More genes become up-regulated in miR-183 cluster–deficient mice with neuropathy than in WT mice with neuropathy (189 versus 70), whereas only 18 up-regulated genes are controlled by the miR-183 cluster in normal mice. These findings suggest that neuropathy-induced genes in WT mice might be further de-repressed in *Wnt1-Cre;miR$^{fl/fl}$* mice with neuropathy. If the miR-183 cluster regulates genes that are unrelated to genes increased during neuropathic pain in WT mice, the proportion of shared genes between the two genotypes should decrease with increasing relaxation of the fold-change criteria. Maintaining P < 0.01 but lowering fold-change criteria from 2.0-fold to 1.8-fold or 1.6-fold resulted in a disproportionate increase of genes shared between *Wnt1-Cre;miR$^{fl/fl}$* and WT mice with neuropathy and maintenance of close to 80% shared genes regardless of fold-change threshold (Fig. 3, E and F). Consistently, we observed up-regulation of individual known pain genes (fig. S8, G and H). Thus, the miR-183 cluster suppresses expression changes of the neuropathy-regulated genes. Absent the miR183 cluster, gene expression induced during neuropathy is further elevated.

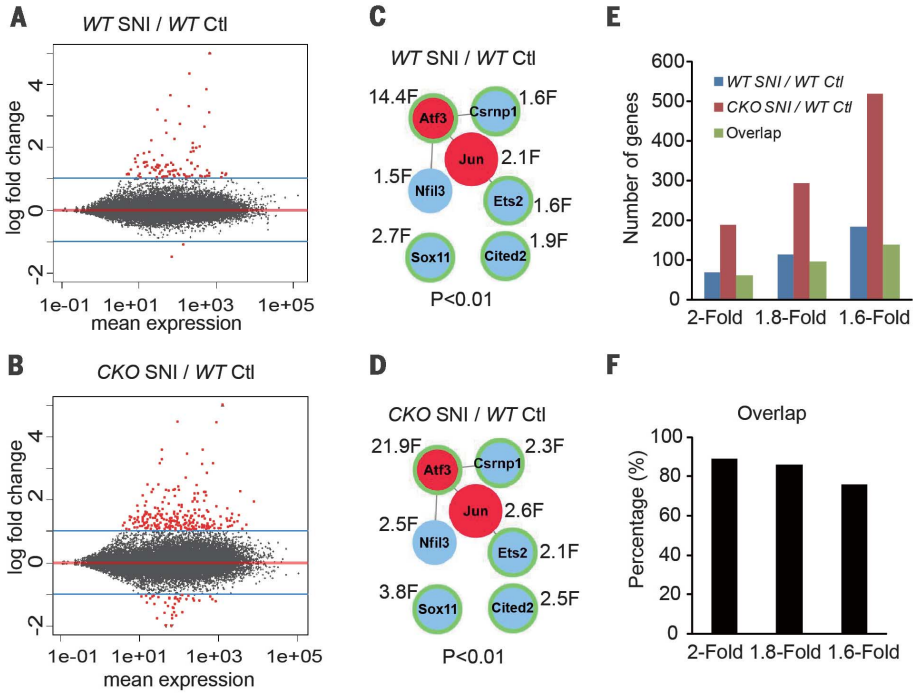

Fig. 3. The global neuropathic pain gene–regulatory network is controlled by the miR-183 cluster. (**A**) MA plot of $\log_2$-fold changes in DRGs of neuropathic WT mice. Red dots indicate >2-fold with P < 0.01. Neuropathic WT (WT SNI, n = 3) compared with unlesioned controls (WT Ctl, n = 5). (**B**) MA plot of $\log_2$-fold changes in DRGs of neuropathic miR-183 cluster–deficient mice. Red dots indicate >2-fold with P < 0.01. Neuropathic *Wnt1-Cre;miR$^{fl/fl}$* mice (CKO SNI, n = 3) compared with WT control mice (WT Ctl, n = 5). (**C** and **D**) Neuropathic pain core transcriptional network with STRING associations and fold change (F). Neuropathic WT and *Wnt1-Cre;miR$^{fl/fl}$* mice compared with unlesioned WT Ctl (P < 0.01). Green circles mark genes predicted as direct targets of the miR-183 cluster. (**E**) Shared regulated genes in DRGs increase between neuropathic WT and *Wnt1-Cre;miR$^{fl/fl}$* mice with relaxed inclusion criterion. (**F**) Maintained percent of shared regulated genes between neuropathic WT and *Wnt1-Cre;miR$^{fl/fl}$* mice with relaxed inclusion criterion.

With neuropathy, the miR-183/96/182 cluster was rapidly down-regulated (fig. S9A), consistent with pain gene overexpression. Mice with neuropathy that were deficient in the miR-183 cluster in all neurons or deficient only in TrkB$^+$ neurons, but not in mice deficient in nociceptors, showed enhanced mechanical sensitization (Fig. 4, A to C, and fig. S9, B to D). Thus, this enhancement of neuropathic pain was mediated through loss of miR-183 activity in TrkB$^+$ NF1 neurons. The NF1 class of TrkB$^+$ neurons are Aδ LTMRs conveying touch sensation (*12*). We asked if TrkB$^+$ neurons can transduce pain. Optogenetic excitation of TrkB$^+$ neurons in *TrkB$^{CreErT2/+}$; ChR2$^{+/-}$* mice (fig. S9E) did not result in a nocifensive response (Fig. 4D and fig. S10A). However, light activation of TrkB$^+$ neurons reduced mechanical threshold (Fig. 4E and fig. S10B). In contrast to naïve animals, light-activation of TrkB$^+$ neurons in animals with neuropathy produced a nocifensive response (Fig. 4F). *Cacna2d1* but not *Cacna2d2*, increased in WT mice during neuropathy; however, the miR-183 cluster suppressed both *Cacna2d1* and *Cacna2d2* expression (Fig. 4G and fig. S10C), and gabapentin completely reversed nocifensive behavior in both neuropathic WT and *Wnt1-Cre;miR$^{fl/fl}$* mice (Fig. 4H and fig. S10, D and E). Painlike behavior initiated by TrkB$^+$ neurons during neuropathy requires auxiliary aδ2 subunits, because gabapentin completely prevented nocifensive responses of optogenetically excited TrkB$^+$ neurons in mice with neuropathy (Fig. 4I and fig. S10F). Thus, during neuropathic pain, the miR-183 cluster targets the majority of pain-regulated genes and attenuates sensitization in the most sensitive hairy skin mechanoreceptors, the TrkB$^+$ neurons (*12*), which contribute to mechanical allodynia during painful neuropathies. However, although *Cacna2d1/2* de-repression is critical once TrkB$^+$ neurons are recruited into the nociceptive network in mice with neuropathy, sensitization was not observed after *Cacna2d1/2* de-repression in TrkB$^+$ neurons of uninjured miR-183 cluster–deficient animals. *Cacna2d1/2* de-repression in these neurons is therefore, by itself, insufficient to cause mechanical hypersensitivity.

Our results show that the miR-183 cluster continuously prevents elevation of basal mechanical sensitivity in nociceptors and enhancement of mechanical allodynia in TrkB$^+$ LTMRs.

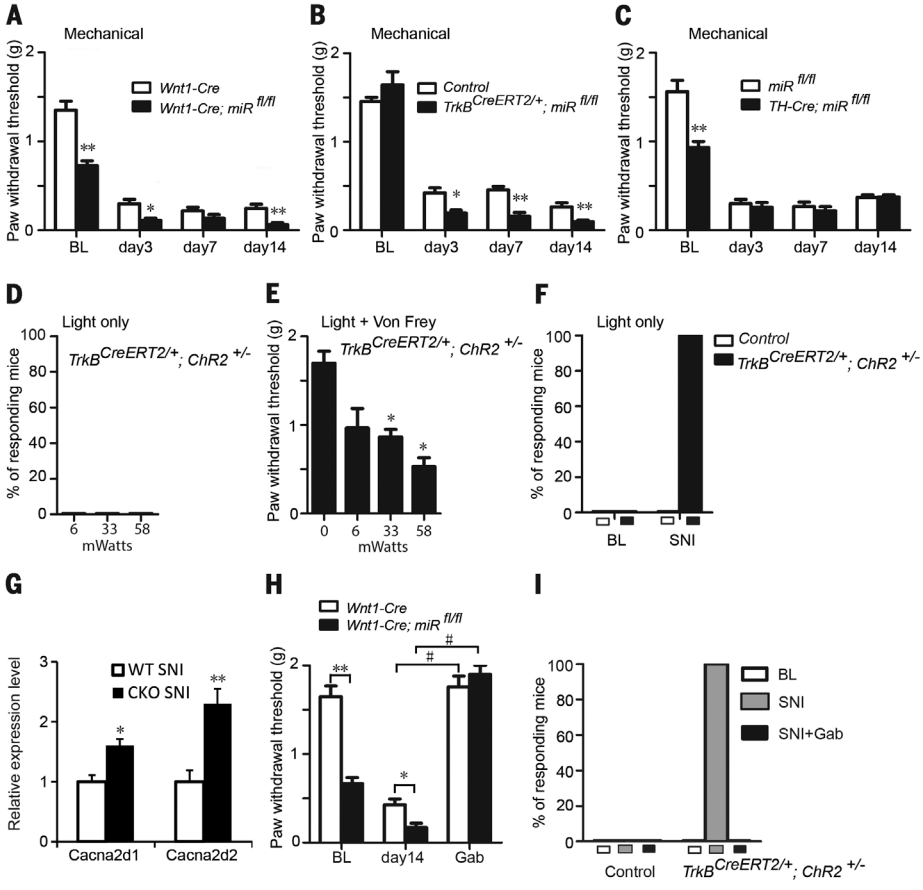

Fig. 4. Pain signaling through TrkB$^+$ LTMRs recruited by neuropathy is suppressed by the miR-183 cluster. (**A**) Enhanced SNI-induced mechanical allodynia in *Wnt1-Cre;miR$^{fl/fl}$* (n = 8 or 8; BL, base line; *P < 0.05, **P < 0.01, Mann-Whitney test). (**B**) Enhanced SNI-induced mechanical allodynia in *TrkB$^{CreERT2/+}$;miR$^{fl/fl}$* mice (n = 7 or 7; *P < 0.05; **P < 0.01, Mann-Whitney test). (**C**) No effect on SNI-induced mechanical allodynia in *TH-Cre;miR$^{fl/fl}$* mice (n = 8 or 9; **P < 0.01, Mann-Whitney test). (**D**) No response by optogenetic excitation of TrkB$^+$ LTMRs (light only) (n = 6). (**E**) Optogenetic excitation of TrkB$^+$ LTMRs increases sensitivity to mechanical stimuli (light + von Frey) (n = 6; one stimuli train; *P < 0.05, Wilcoxon test). (**F**) Excitation of TrkB$^+$ neurons produces pain after SNI. Optogenetic activation (58 mW) of TrkB$^+$ LTMRs before (BL) and after SNI in littermate controls and *TrkB$^{CreERT2/+}$;ChR2$^{+/-}$* mice (n = 6 or 6). (**G**) Quantitative PCR of *Cacna2d1* and *Cacna2d2* expression in wild-type and *Wnt1-Cre;miR$^{fl/fl}$* mice after SNI (n = 3 or 3; means ± SEM; *P < 0.05; **P < 0.01, t test). (**H**) Reversal of neuropathic allodynia by gabapentin in both *Wnt1-Cre;miR$^{fl/fl}$* and *Wnt1-Cre* mice (n = 6 or 6, respectively, *P < 0.05 **P < 0.01, Mann-Whitney test; #P < 0.05, Wilcoxon test). (**I**) Reversal by gabapentin of nocifensive pain-like behavior through optogenetic excitation (58 mW) of TrkB$^+$ neurons (*TrkB$^{CreERT2/+}$;ChR2$^{+/-}$* and littermate controls, n = 6 or 6).

Ongoing activity of TrkB$^+$ neurons also potentiates mechanical transduction from nociceptors in normal mice. Nociceptors have been a focus for development of analgesic drugs for painful neuropathies, TrkB$^+$ neurons may be equally or more important. We propose a unifying concept for scaling mechanical sensitivity through expression of the miR-183 cluster. Thus, somatic mechanosensitivity is a dynamic system able to respond and adapt to various intensities of stimulation.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6343/1168/suppl/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 and S2
References (*24*–*31*)

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MicroRNAs in functional and dysfunctional pain

Pain serves the useful purpose of alerting us to danger. Chronic pain, however, can arise from dysfunctional responses. Peng *et al.* found that a cluster of microRNAs regulates the gene networks behind both physiological and dysfunctional pain (see the Perspective by Cassels and Barde). The recruitment of genes that regulate a subset of the light-touch mechanoreceptors found in hairy skin was critical to the generation of dysfunctional pain.

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