# microRNA let-7b enhances spinal cord nociceptive synaptic transmission and induces acute and persistent pain via neuronal and microglial signaling

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Supplementary Tables (2)

Supplementary Figures (7)

**miRNA and RNA sequences used in this study**

| miRNA name | Sequence (From 5' to 3') | Accession No. |
|---|---|---|
| mmu-let-7b | UGAGGUAGUAGGUUGUGUGGUU | MIMAT0000522 |
| mutant Oligo | UGAGGUAGAAGGAUAUAAGGAU | |
| normal let-7b antagomir | AACCACACAACCUACUACCUCA | |
| scrambled antagomir | CCCAAUCUCCAGAUCUCCCUAU | |
| modified let-7b antagomir | [mA[mA][mC][mC][mA][mC][mA][mC][mA][mA][mC][mC][mU][mA][mC][mU][mA][mC][mC][mU][mC][mA] (mN= 2′Ome residue) | |

**Table S1.** Sequences of miRNA and RNAs used in this study. mN: 2′Ome residue.

**Sequence of outer and inner primers for single-cell PCR**

| Target gene (Product length) | Outer primers | Inner primers | Genbank No. |
|---|---|---|---|
| TRPA1 (273 bp, 203 bp) | GGCTTTTGGCCTCAGCTTTTAT<br>ACACGATGGTGGACCTCTGATC | ATGCCTTCAGCACCCCATT<br>TGCGTAAGTACCAGAGTGGCAG | NM_177781.4 |
| GAPDH (367 bp, 313 bp) | AGCCTCGTCCCGTAGACAAAA<br>TTTTGGCTCCACCCCTTCA | TGAAGGTCGGTGTGAACGAATT<br>GCTTTCTCCATGGTGGTGAAGA | XM_001473623.1 |
| TLR7 (421 bp, 359 bp) | AGCCTCGTCCCGTAGACAAAA<br>TTTTGGCTCCACCCCTTCA | TTCTCCAACAACCGGCTTGAT<br>TCAGGAGGCAAGGAATTCAGG | NM_133211.3 |

**Table S2:** Sequences of out primers and inner primers used for single cell PCR.

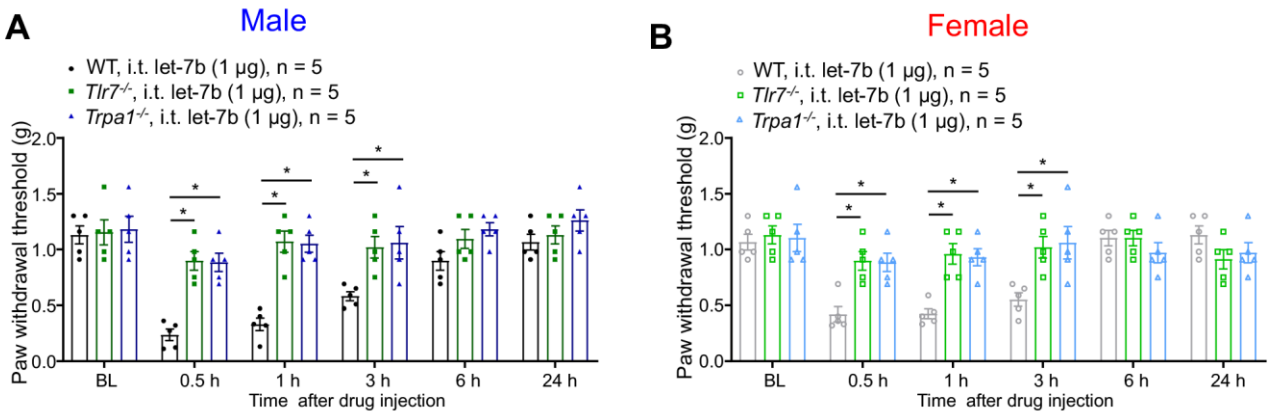


**Fig. S1.** Intrathecal injection of low dose of let-7b (1 μg) induces transient mechanical allodynia in both male mice (A) and female mice (B) via TLR7 and TRPA1. Data are shown as mean ± SEM. $^{*}P < 0.05$. Two-way repeated measures (RM) ANOVA with Bonferroni's post hoc test. (A) $F_{(2, 12)} = 30.53$, $P < 0.0001$; (B) $F_{(2, 12)} = 5.673$, $P = 0.0184$. n = 5 mice per sex per group.

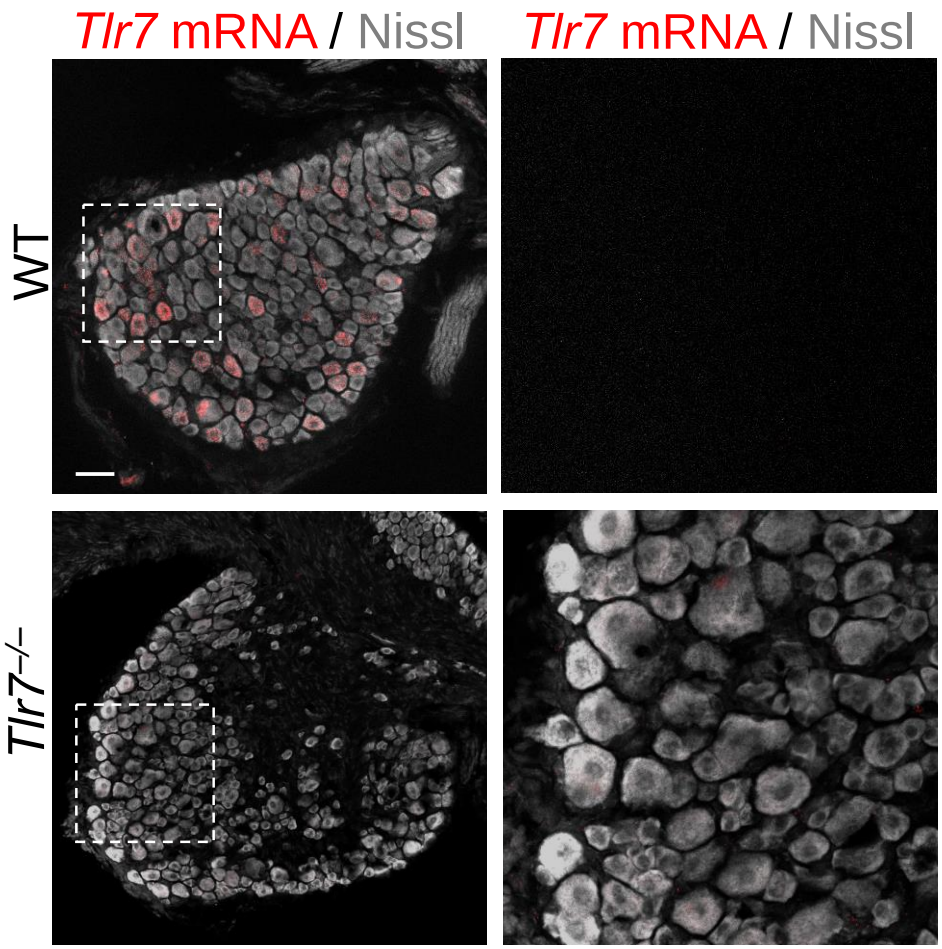


**Fig. S2.** RNAscope fluorescence ISH images showing *Tlr7* expression in WT mice (top) and *Tlr7*$^{-/-}$ mice (bottom). Scale, 50 µm. Small boxes in the left column are enlarged in the right column. Note *Tlr7* mRNA expression in punctate dots in small and medium sized neurons and this expression is lost in *Tlr7*$^{-/-}$ mice.

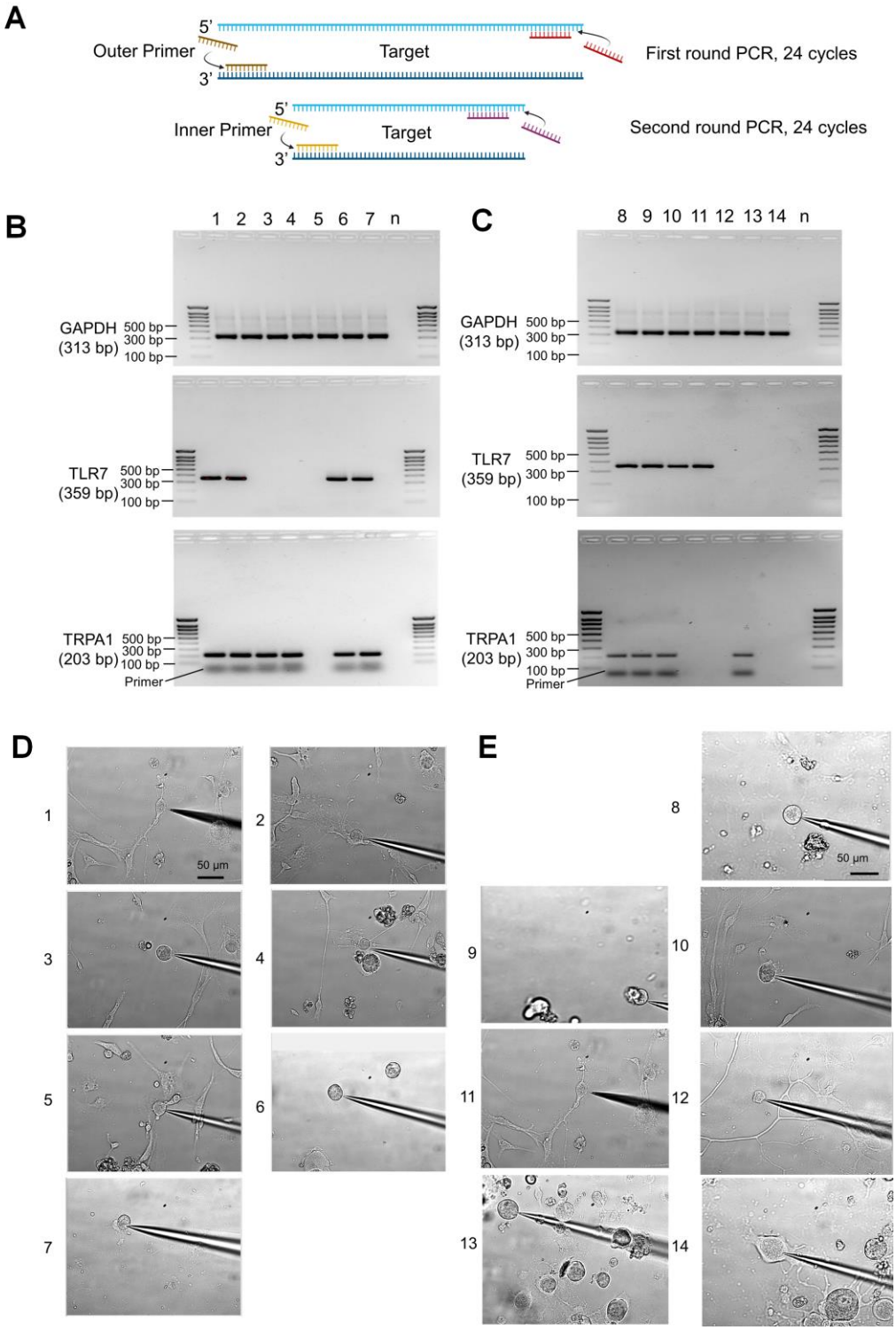


**Fig. S3.** Single-cell PCR showing *Tlr7* and *Trpa1* expression in dissociated DRG neurons. (A) Schematic of two rounds of single cell PCR in mouse DRG neurons using different sets of primers (outer primers and inner primers). See Table S2 for the primer sequences. (B-C) Full gels of single cell PCR showing the expression of *Tlr7*, *Trpa1*, and *Gapdh* transcripts in Experiment 1 (B, cell #1-7) and Experiment 2 (C, cell #8-14). Note all neurons express *Gapdh* as positive control. Also note that the majority of *Tlr7*+ neurons (7 out of 8) express *Trpa1*. n, negative control with glass pipettes but without cells in reaction tubes. (D-E) Images of #1-7 neurons (D) and #8-14 neurons (E) used for single cell PCR in B and C. Note these are small and medium sized neurons with diameters of 20-40 μm.

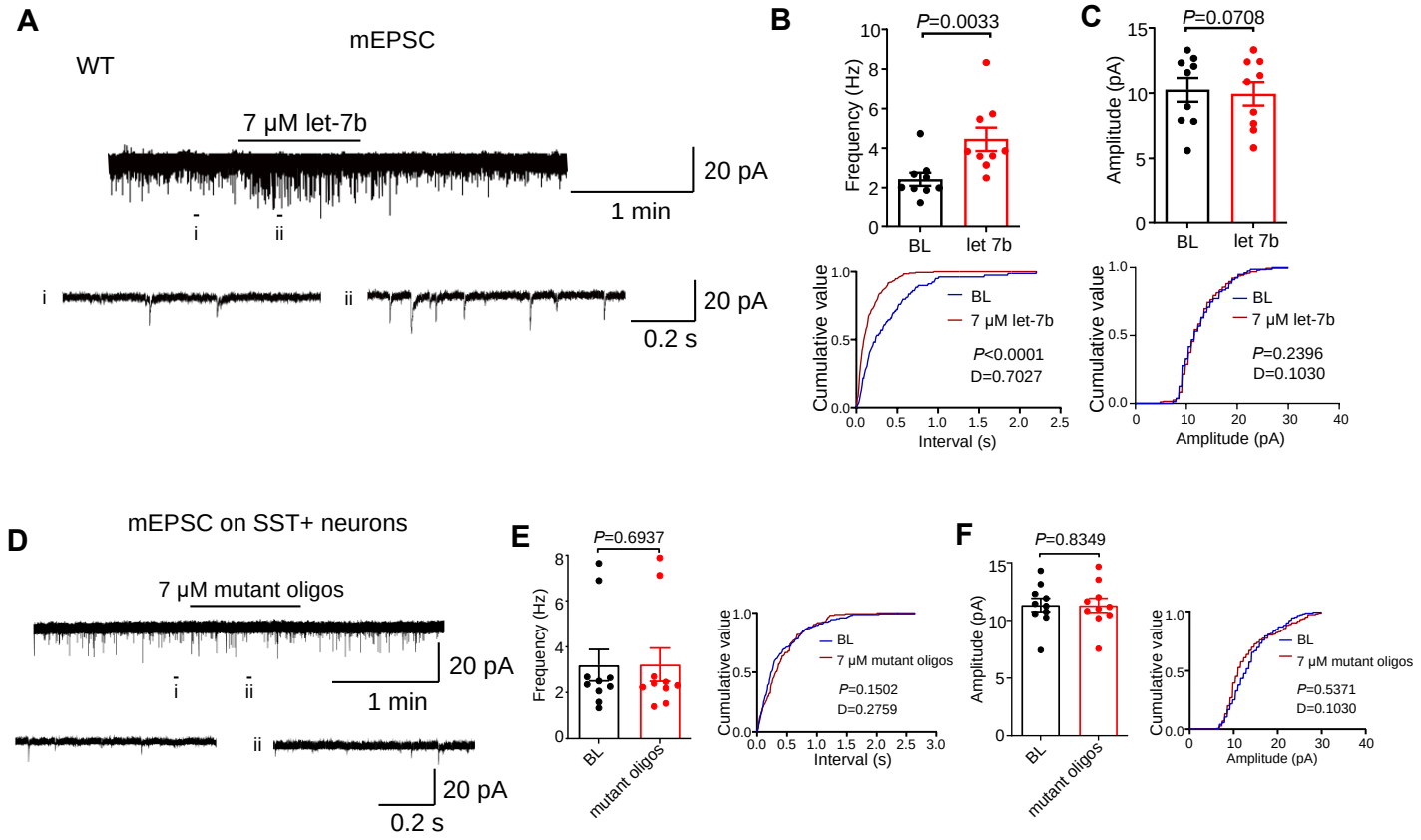


**Fig. S4.** Additional characterization of let-7b induced miniature EPSCs (mEPSCs) in lamina IIo neurons of spinal cord slices. A and D, mEPCS traces showing the effects of 1-min perfusion of 7 μM of let-7b (A) and mutant oligos (D). The traces of (i) and (ii) are enlarged in bottom traces in short time scale. (B, C, E, F), quantification of mEPCS frequency before and after drug perfusion. Paired Student's t-test (B-left, C-left, E-top, F-top). Kolmogorov-Smirnov test (B-bottom, C-bottom, E-right, F-right).
Sample sizes: (B, C) 7 μM let-7b: n = 9 from 7 mice; (E, F) 7 μM mutant oligos: n = 10 neurons from 6 mice; Data are shown as mean ± SEM.

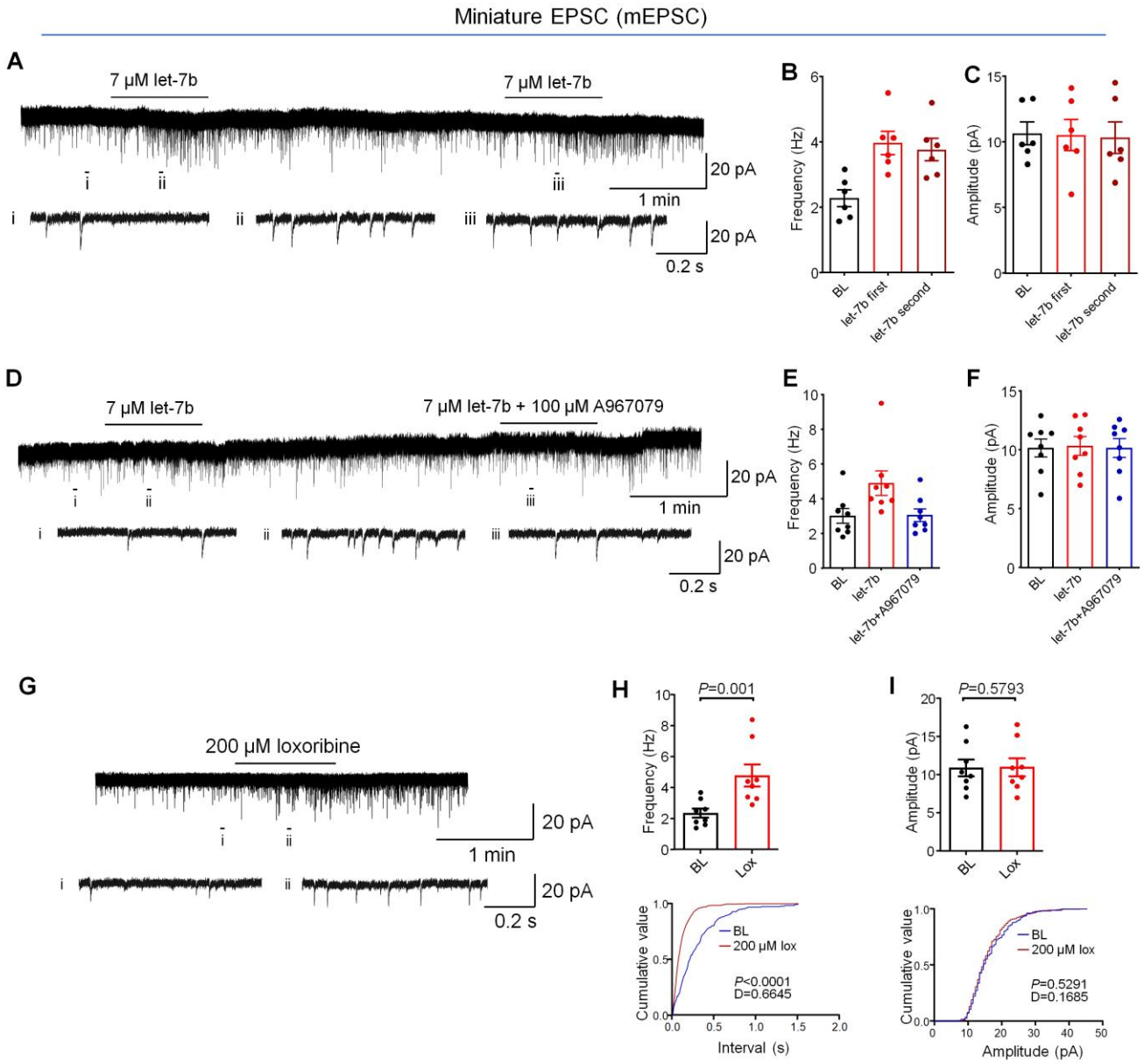


**Fig. S5.** (A-I) Patch clamp recordings of miniature EPSCs (mEPSCs) in lamina IIo neurons of spinal cord slices from *Sst*$^{Cre}$; *Ai9* mice. Left, mEPCS traces showing the effects of 1-min treatment of double perfusion of 7 μM let-7b, TRPA1 antagonist, and loxoribine (A, D, G). The traces of (i) and (ii) are enlarged in bottom traces in short time scale. Right, quantification of mEPCS frequency and amplitude before and after drug perfusion. Repeat perfusion of 7 μM let-7b both significantly increase frequency without affecting amplitude of mEPSC (A, B, C). Co-perfusion of TRPA1 antagonist A967079 and let-7b blocks let-7b effect (D, E, F). Perfusion of 200 μM loxoribine significantly increase frequency without affecting amplitude of mEPSC (G, H, I). Cumulative value of interval and amplitude reveals same finding (H, I-bottom).
Sample sizes: 7 μM let-7b: n = 6 from 5 mice; 7 μM let-7b + 100 μM A967079: n = 8 from 5 mice; 200 μM loxoribine: n = 8 from 5 mice. Data are shown as mean ± SEM. Paired Student's t-test (H, I-top). One-Way ANOVA followed by post-hoc Bonferroni test (B, C, E, F). (B), $F_{(1.553, 7.766)} = 50.41$, $P < 0.0001$. (E), $F_{(1.051, 8.404)} = 27.70$, $P = 0.0006$. Unpaired Kolmogorov-Smirnov test (H, I-bottom).

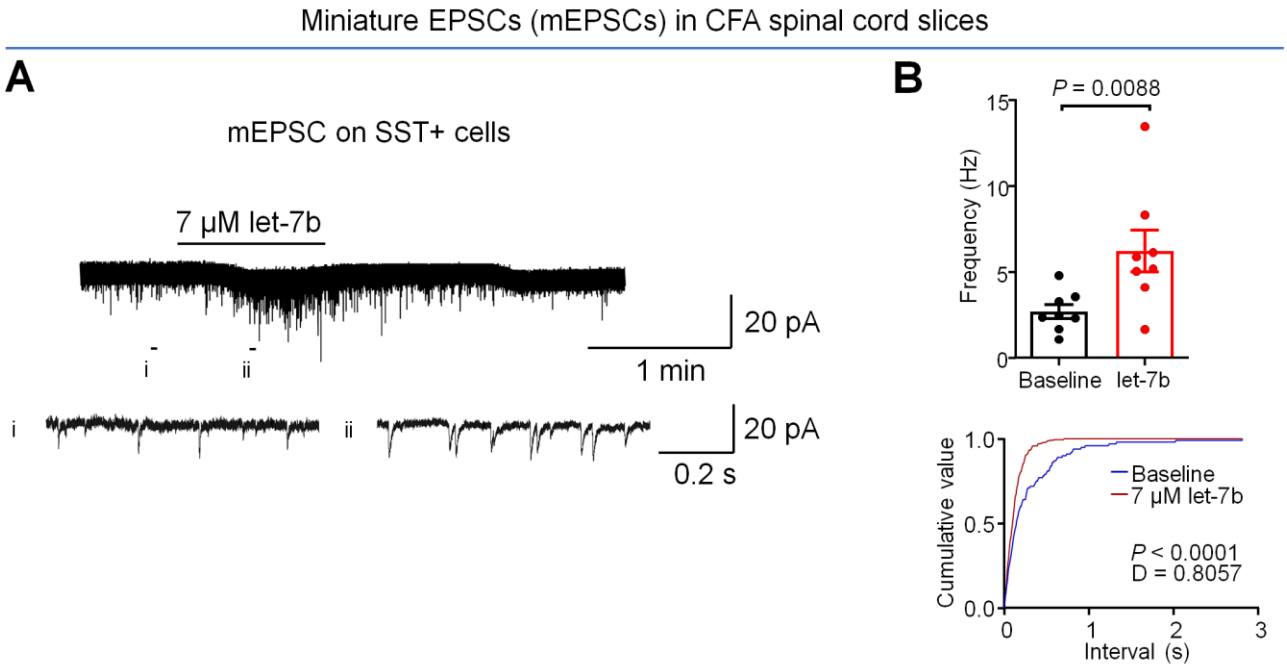


**Fig. S6.** (A, B) Patch clamp recordings of miniature EPSCs (mEPSCs) in lamina IIo neurons of spinal cord slices from CFA mice. (A) mEPSC trace showing the effect of 7 μM let-7b in lamina II SST+ interneurons of spinal cord slices prepared from CFA-treated *Sst*$^{Cre}$; *Ai9* mice. Bottom: mEPSC traces in short time scale. (B) mEPSC frequency before (baseline) and after 1-min application of 7 μM let-7b. Perfusion of 7 μM let-7b significantly increase frequency of mEPSC. Cumulative value of interval reveals same finding. Data are shown as mean ± SEM. Paired Student's t-test (B, top panel). Kolmogorov-Smirnov test (B, bottom panel).

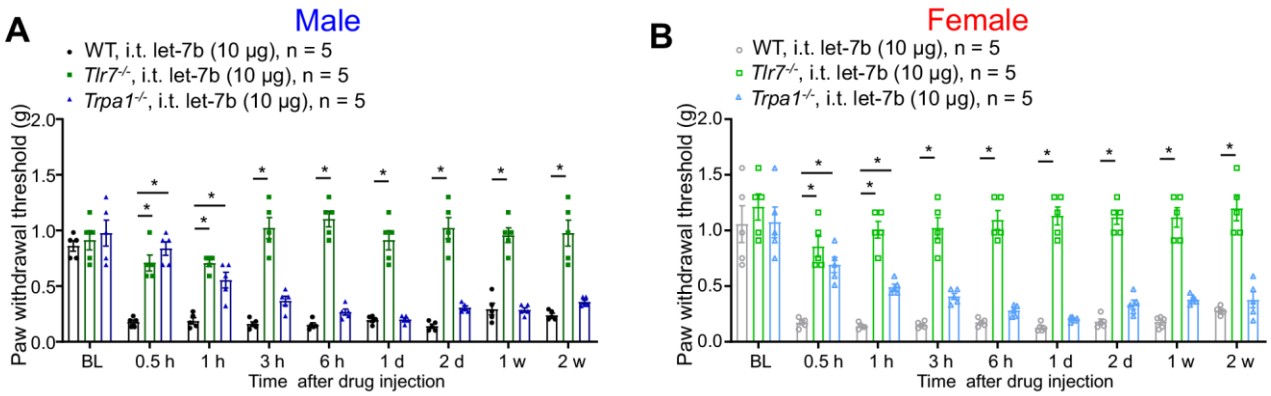


**Fig. S7.** (A, B) Intrathecal injection of high dose of let-7b (10 μg) induces prolonged mechanical allodynia in male mice (A) and female mice (B). Data are shown as mean ± SEM. $^{*}P < 0.05$. Two-way repeated measures (RM) ANOVA with Bonferroni's post hoc test. (A) $F_{(2, 12)} = 203.1$, $P < 0.0001$; (B) $F_{(2, 12)} = 309.7$, $P < 0.0001$.