

Supplemental information

**PRMT2-mediated upregulation of miR-323-3p
in sensory neurons promotes trigeminal
neuropathic pain by targeting Kv2.1 channels**

Renfei Qi, Yu Tao, Shoupeng Wang, Yueting Zhou, Yufang Sun, Dongsheng Jiang, Zitong Huang, Gang Chen, Gang Zhao, Yuan Zhang, Yongjun Rui, and Jin Tao

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PRMT2-mediated upregulation of miR-323-3p in sensory neurons promotes trigeminal neuropathic pain by targeting Kv2.1 channels

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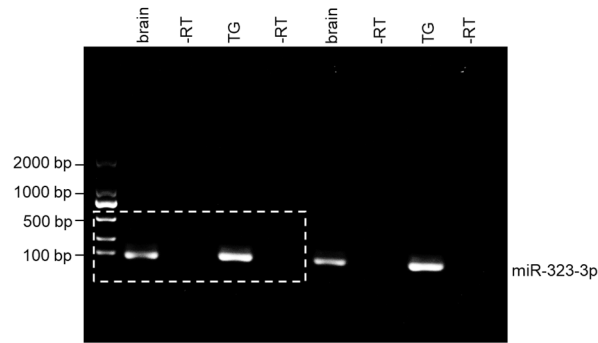


Fig. S1: Expression of miR-323-3p in the brain and TGs. Shown is the full-length gel image for Fig. 1D.

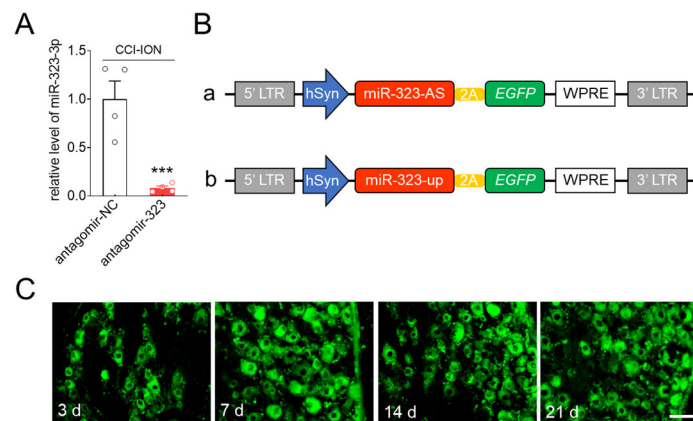


Fig. S2: Supplemental data for Figure 2.

(A) qPCR analysis of miR-323-3p in the TG of CCI-ION rats after administration of antagomir-NC or antagomir-323. $n = 4$ rats/group. *** $p < 0.001$ (vs. antagomir-NC) by unpaired t test.

(B) Schematic of lentiviral system for lenti-hSyn-miR-323-3p-antisense (miR-323-AS, *a*) and lenti-hSyn-miR-323-3p-up (miR-323-up, *b*). LTR = long terminal repeat, hSyn = human synapsin promoter, EGFP = enhanced green fluorescent protein, and WPRE = woodchuck hepatitis virus post-regulatory element.

(C) Lentiviral delivery of lenti-hSyn-miR-323-3p-antisense (miR-323-AS) carrying EGFP.

Scale bar = 50 μ m.

Data are represented as mean $\pm$ SEM.

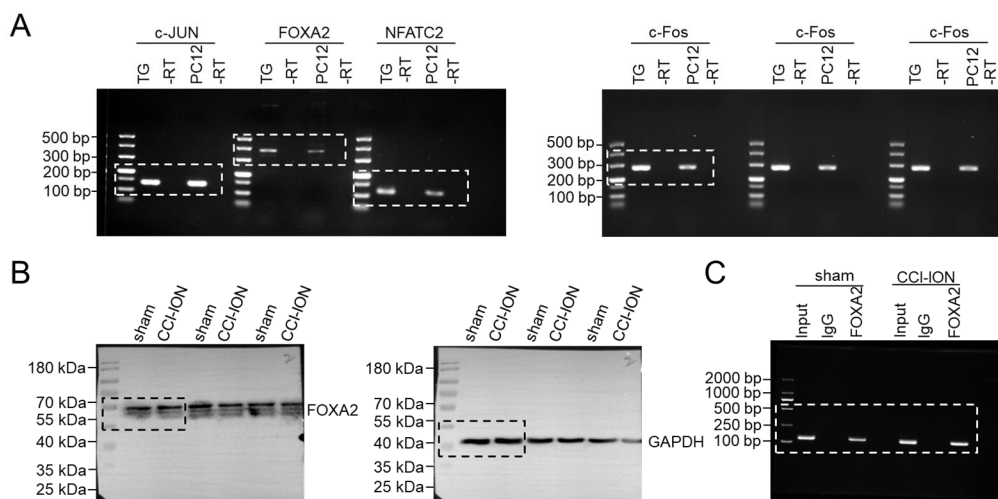


Fig. S3: Full-length gel and blot images for Figure 3.

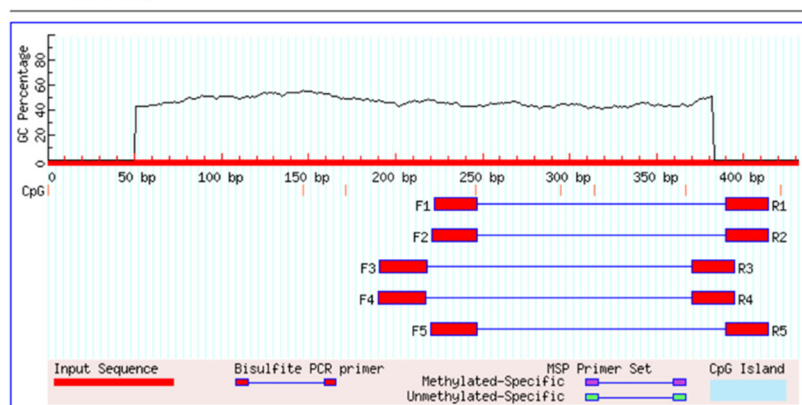
(A) RT-PCR analysis of c-Jun, c-Fos, FOXA2, and NFATC2 in rat TGs and PC12 cells. Full-length gel images for Fig. 3E are shown.

(B) Immunoblot analysis of FOXA2 in the injured TG following CCI-ION or sham surgery. Full-length blot images for Fig. 3H are shown.

(C) ChIP analysis shows that the binding activity of FOXA2 in the *miR-323-3p* gene promoter in the TG after CCI-ION- or sham-operation. Shown is the full-length gel image for Fig. 3K.

MethPrimer result

Please cite MethPrimer: Li LC and Dahiya R. [MethPrimer: designing primers for methylation PCRs](#). PMID: [12424112](#)



Sequence Name:
Sequence Length: 431

CpG island prediction results
(Criteria used: Island size > 100, GC Percent > 50.0, Obs/Exp > 0.6)
No CpG islands were found in your sequence
Primer picking results for bisulfite sequencing (or restriction) PCR

Fig. S4: Analysis of CpG islands using MethPrimer. No CpG islands were identified within the ΔS region of the *miR-323-3p* promoter.

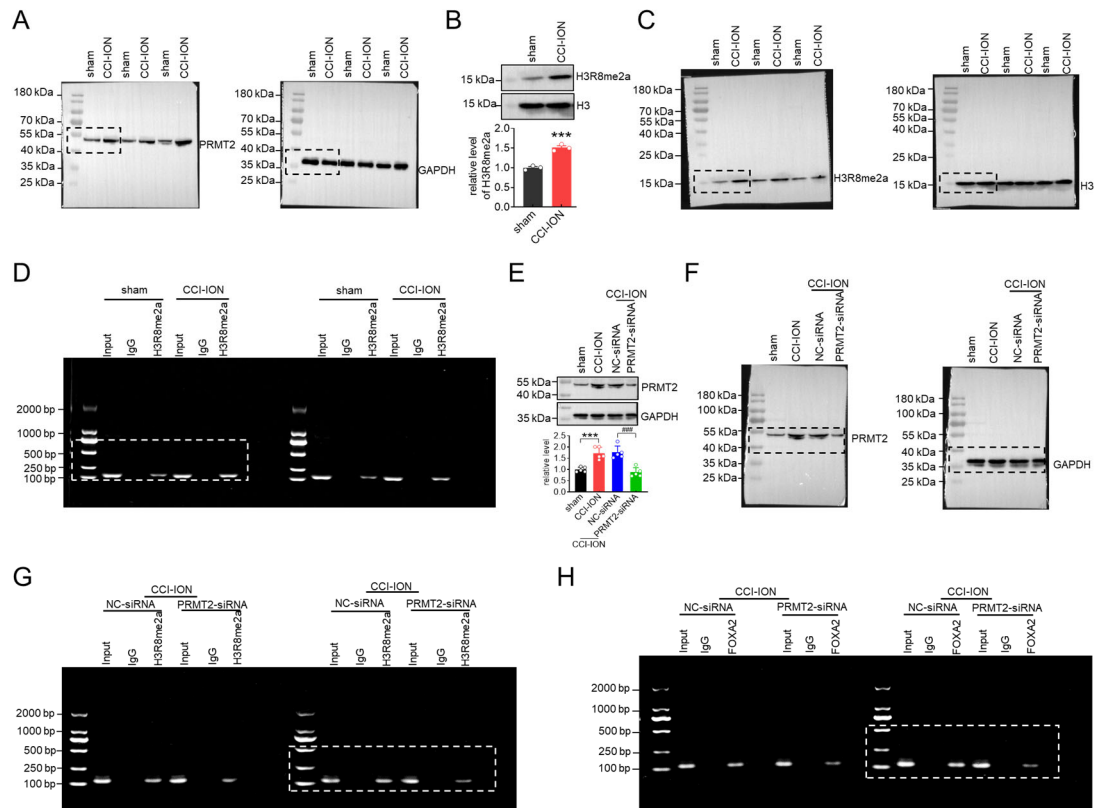


Fig. S5: Supplemental data, full-length gel and blot images for Figure 4.

(A) Immunoblot analysis of PRMT2 in the injured TG following CCI-ION or sham surgery. Full-length blot images for Fig. 4B are shown.

(B) Immunoblot analysis showing the protein expression levels of H3R8me2a in rat TGs after CCI-ION or sham-operation. $n = 3$ rats/group. $***p < 0.001$ (vs. sham) by unpaired t test.

(C) Full-length blot images for Panel B are shown.

(D) ChIP analysis showing the binding of H3R8me2a to the *miR-323-3p* gene promoter. Shown is the full-length gel image for Fig. 4E.

(E) Administration of PRMT2-siRNA attenuated the CCI-ION-induced increase in PRMT2 protein expression. $n = 5$ rats/group. $***p < 0.001$ (vs. sham), $####p < 0.001$ (vs. CCI-ION + NC-siRNA), by one-way ANOVA.

(F) Full-length blot images for Panel E are shown.

(G) Administration of PRMT2-siRNA decreased the binding activity of H3R8me2a to the *miR-323-3p* gene promoter in the injured TG of CCI-ION rats. Shown is the full-length gel image for Fig. 4F.

(H) Administration of PRMT2-siRNA decreased the binding activity of FOXA2 to the *miR-323-3p* gene promoter in the injured TG of CCI-ION rats. Shown is the full-length gel image for Fig. 4G.

Data are represented as mean $\pm$ SEM.

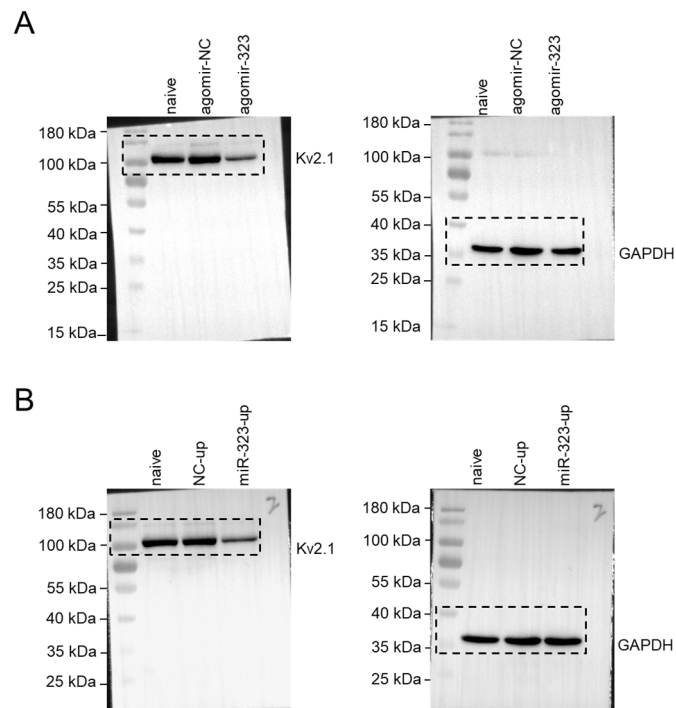


Fig. S6: Full-length blot images for Figure 5.

(A) Administration of agomir-323 decreased the protein abundance of Kv2.1 in naive rats. Full-length blot images for Fig. 5J are shown.

(B) Administration of miR-323-up decreased the protein abundance of Kv2.1 in naive rats. Full-length blot images for Fig. 5M are shown.

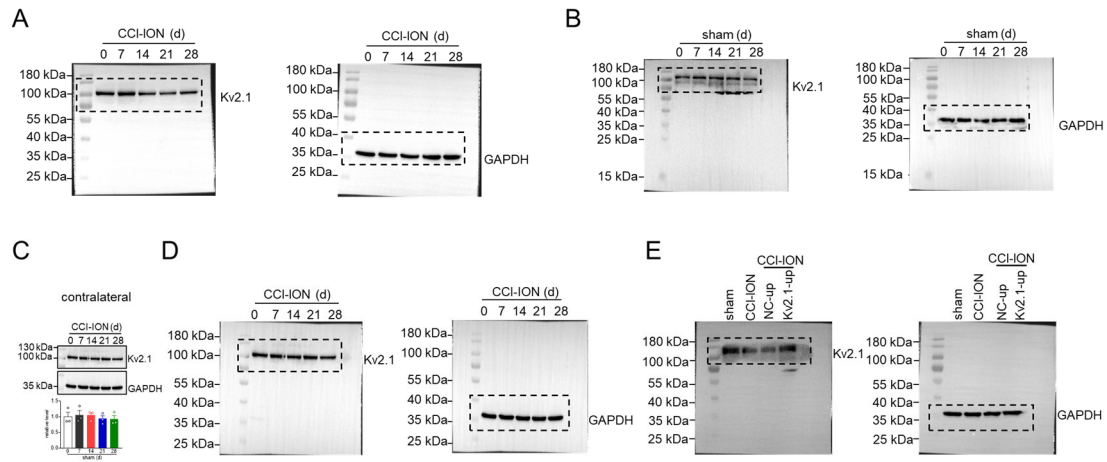


Fig. S7: Supplemental data and full-length blot images for Figure 6.

(A and B) Expression of Kv2.1 in the ipsilateral TGs after CCI-ION-operation (A) or sham surgery (B). Full-length blot images for Figs. 6A and B are shown.

(C) Protein expression of Kv2.1 in the contralateral TG after CCI-ION.

(D) Full-length blot images for Panel C are shown.

(E) Administration of Kv2.1-up reversed the CCI-ION-induced increase in the protein expression of Kv2.1. Full-length blot images for Fig. 6G are shown.

Data are represented as mean $\pm$ SEM.

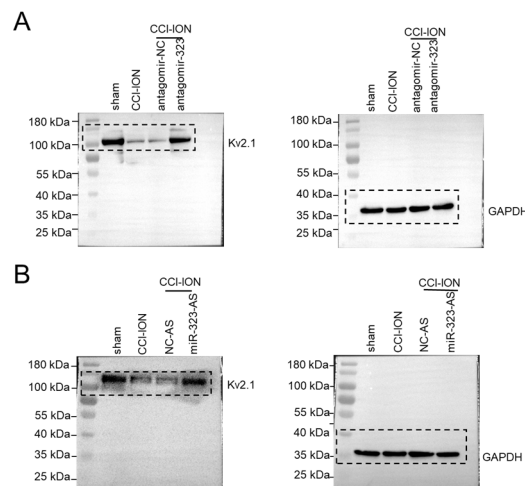


Fig. S8: Full-length blot images for Figure 7.

(A) Administration of antagomir-323 reversed the CCI-ION-induced increase in the protein expression of Kv2.1. Full-length blot images for Fig. 7A are shown.

(B) Administration of miR-323-AS reversed the CCI-ION-induced increase in the protein expression of Kv2.1. Full-length blot images for Fig. 7F are shown.

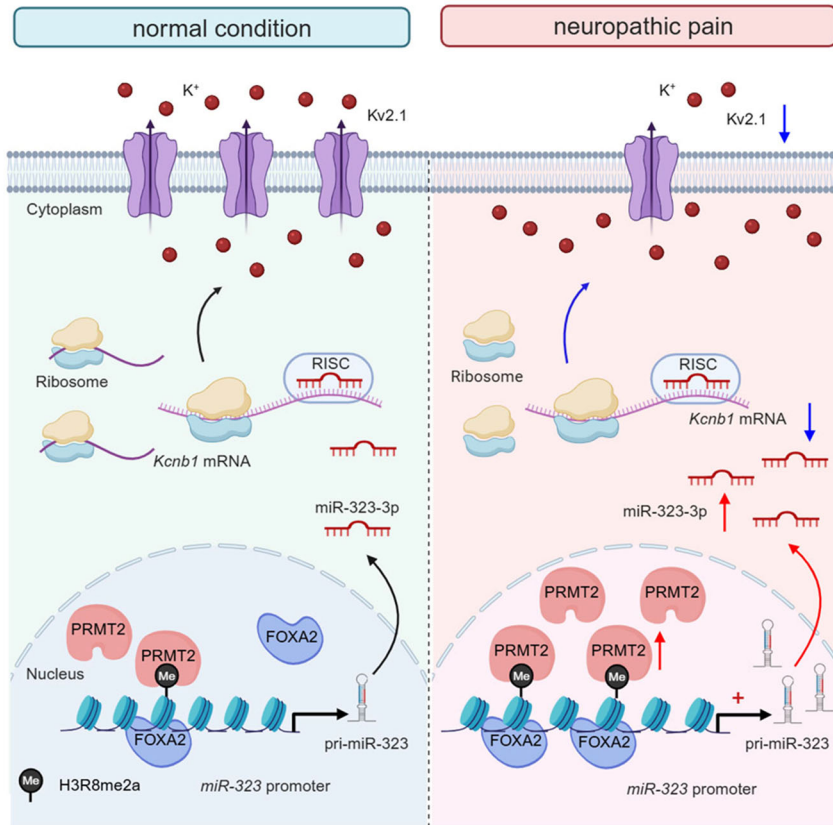


Fig. S9: Schematic diagrams showing the sensory PRMT2/FOXA2/miR-323-3p/Kv2.1 signaling axis in trigeminal-mediated neuropathic pain. *Left panel*, under normal conditions, TG neurons with miR-323-3p promoter hypomethylation have low levels of miR-323-3p. *Right panel*, nerve injury induces the upregulation of miR-323-3p and the reduction in Kv2.1 expression in the CCI-ION-treated rats. First, peripheral nerve injury induces the upregulation of PRMT2, which promotes the asymmetrical dimethylation of H3R8, thereby facilitating binding of the transcription factor FOXA2 to the promoter of *miR-323-3p* and resulting in the upregulation of miR-323-3p expression. Next, mature single-stranded miR-323-3p, guided by the RNA-induced silencing complex (RISC), recognizes and binds to complementary sequences within the Kv2.1 mRNA 3'-UTR, resulting in Kv2.1 mRNA degradation. Consequently, the upregulation of miR-323-3p during nerve injury decreases the protein abundance of Kv2.1, resulting in a decreased Kv2.1 channel current. MiR-323-3p-mediated Kv2.1 downregulation subsequently induces TG neuron hyperexcitability and eventually leads to neuropathic pain symptoms.

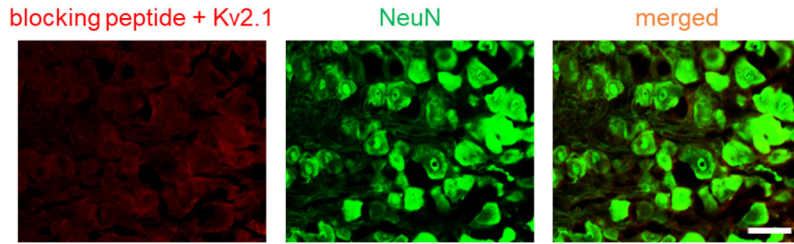


Fig. S10: The specificity of the Kv2.1 antibody was verified using a Kv2.1 blocking peptide. Pre-incubation of the Kv2.1 antibody with a recombinant blocking peptide abolished the detection of Kv2.1 in rat TGs by immunofluorescence assays, ensuring the high specificity of the Kv2.1 antibody. Scale bar, 50 μ m.

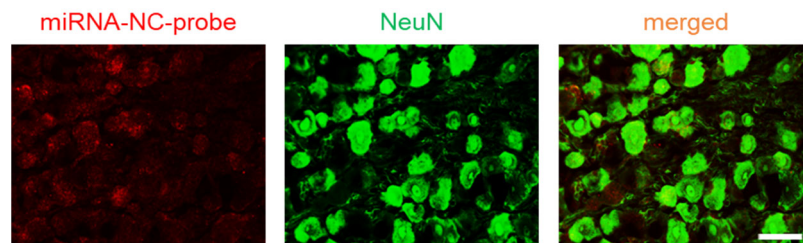


Fig. S11: The specificity of the rno-miR-323-3p FISH probe was verified using miRNA-NC-probe as a negative control. RNA fluorescence in situ hybridization (FISH) with the miRNA-NC-probe combined with NeuN immunofluorescence staining. Scale bar, 50 μ m.

[\[Download table\]](#)

Conserved
No Conserved Found

Context++ score and features that contribute to the context++ score are evaluated as in [Agarwal et al., 2015](#).
Conserved branch lengths and PCT are evaluated as in [Friedman et al., 2008](#), with an expanded 84-species alignment as described in [Agarwal et al., 2015](#).

Poorly conserved							
	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT
Position 46-52 of KCNB2 3' UTR	5' ... UCAUUCUUAUAAAGCAUAA ... 3' UGGGAGUAGUGUAAUCGAAUCU	7mer-A1	-0.18	84	-0.18	0	< 0.1
mo-miR-155-5p	5' ... CGCCCCUAAUACAGUGCAUAA ... 3' ACUGGGAAGGGUCCAGUAA	7mer-A1	-0.24	93	-0.24	2.288	N/A
Position 97-103 of KCNB2 3' UTR	5' ... CGCCCCUAAUACAGUGCAUAA ... 3' GGUUAGGAACGGGCGCAGUAA	7mer-A1	-0.23	92	-0.23	2.288	N/A
mo-miR-500-3p	5' ... AAUACAGUGCAUAA--AAUGUAAU ... 3' UCUUACAGGGGGGCUACAAA	7mer-A1	-0.13	94	-0.13	2.452	N/A
Position 105-111 of KCNB2 3' UTR	5' ... UUUAAUAAAGAGUGAGACGCG ... 3' GAAGAAAGGCUACUAGACU	7mer-m8	-0.35	97	-0.35	0.420	N/A
mo-miR-543-3p	5' ... UGAGACUCCAUUUUAUAAAC ... 3' UCUUAGAGGAAA--AUUGUUC	7mer-A1	-0.18	92	-0.18	0	N/A
Position 163-169 of KCNB2 3' UTR	5' ... AAACAAACCGAGUCCUGAACAG ... 3' UACUACUUGGUGGACGACUUGU	7mer-A1	-0.15	77	-0.15	0	N/A
mo-miR-544-5p	5' ... AAUAAAGUCCUUCUUAAGGAC ... 3' UACCCUCCCCCGUCCUACUCCU	7mer-A1	-0.02	38	-0.01	0	N/A
Position 196-202 of KCNB2 3' UTR	5' ... AUAAAGUCCUUCUAGGAACCA ... 3' UUUAGUGUAGGCGUCCUUGG	8mer	-0.52	98	-0.34	0.227	N/A
mo-miR-743b-5p							
Position 224-230 of KCNB2 3' UTR							
mo-miR-3573-3p							
Position 226-233 of KCNB2 3' UTR							
mo-miR-23b-5p							

Fig. S12: No conserved target sites in the 3' UTR of KCNB2 were identified by miR-323-3p. MiRNAs targeting KCNB2 were predicted using the TargetScan (www.targetscan.org) algorithm.

Table S1: Agomir, antagomir, siRNAs, and probes used in the current study.

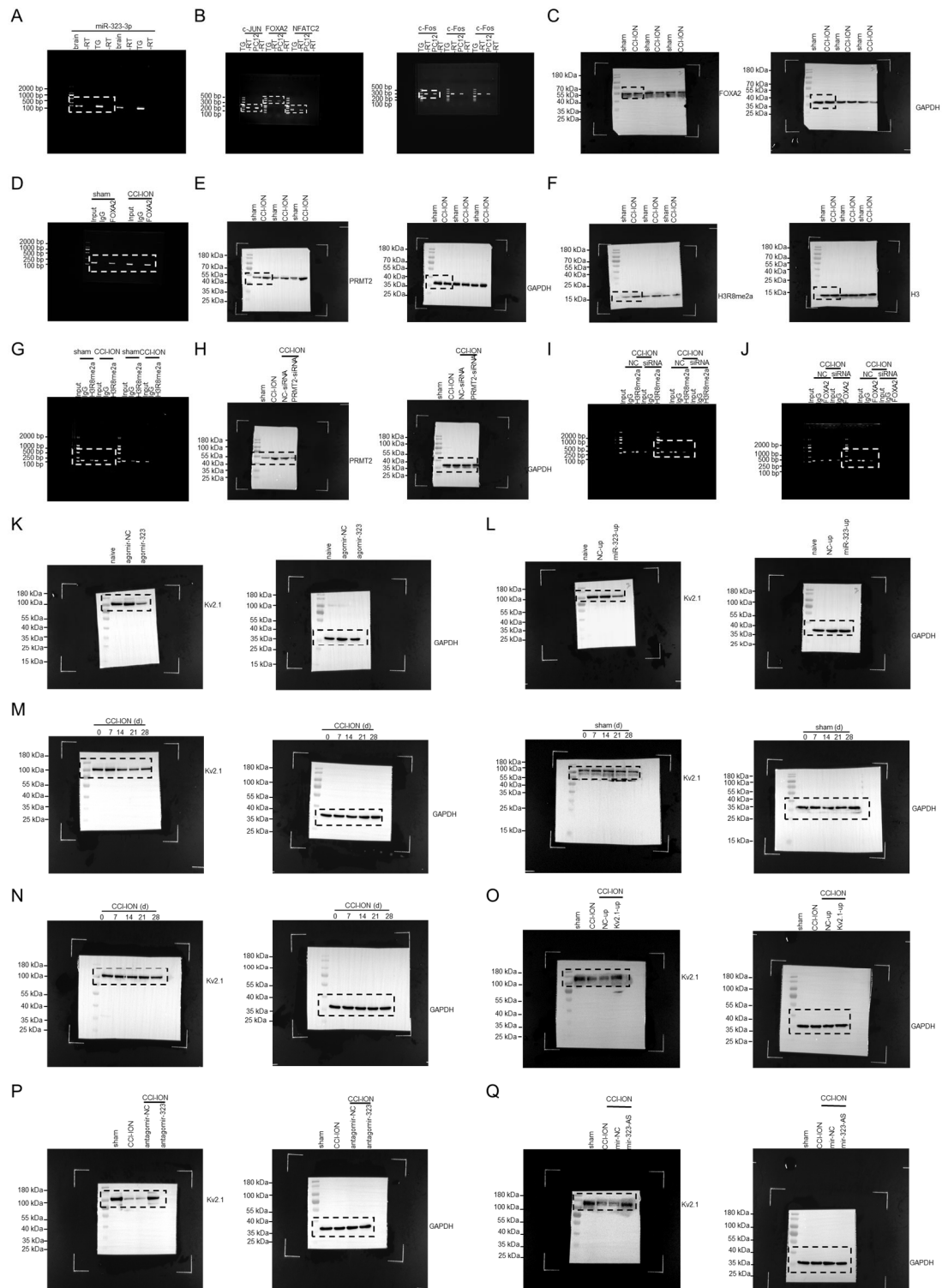
Name	Sense	Antisense
rno-miR-323- 3p agomir	CACAUUACACGGUCGACCUCU	AGGUCGACCGUGUAAUGUGUU
agomir-NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
rno-miR-323- 3p antagomir	AGAGGUCGACCGUGUAAUGUG	
antagomir-NC	CAGUACUUUUGUGUAGUACAA	
rno-miR-323- 3p mimics	CACAUUACACGGUCGACCUCU	AGGUCGACCGUGUAAUGUGUU
mimics NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
PRMT2- siRNA	GCACUCAAGUCGUUAGCAATT	UUGCUAACGACUUGAGUGCTT
NC-siRNA	GGCUCUAGAAAAGCCUAUGCTT	GCAUAGGCUUUUCUAGAGCCTT
rno-miR-323- 3p FISH probe	AGAGGTCGACCGTGTAATGTG	

Table S2: Primers of miRNAs used in the current study.

Name	RT	Forward	Reverse
miR-21-5p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACTCAACAT	GCCGCTAGCTTATC AGACTGA	ATCCAGTGCAG GGTCCGAGG
miR-142-3p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACTCCATAA	TGTCCGCCTGTAGT GTTTCCTAC	
miR-146a-5p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACAACCCAT	CCGCCTGAGAACT GAATTCC	
miR-184	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACACCCTTA	AACCGGTGGACGG AGAACTGA	
miR-221-3p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACGAAACCC	AACGGCAGCTACA TTGTCTGCT	
miR-323-3p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACAGAGGT	ACGCCGCACATTA CACGGTC	
miR-342-3p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACACGGGTG	ACGCTGAATCTCA CACAGAAATCG	
miR-370-3p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACACCAGGT	AACTTGATGCCTG CTGGGGTG	
miR-433-3p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACACACCGA	AACGGCATCATGAT GGGCTCC	
miR-758-3p	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACGGTTAGT	AACGGCTTTGTGA CCTGGTCC	
U6	GTCGTATCCAGTGCAGG GTCCGAGGTATTCGCAC TGGATACGACAAAATA	AGAGAAGATTAGC ATGGCCCCTG	

Table S3: Sequences of nucleotides used in the current study.

Name	Forward	Reverse
GAPDH	GTGCTGAGTATGTCGTGGAGT	CAGTCTTCTGAGTGGCAGTGAT
PRMT2	CGCGGCTGAGACGTGAT	AGCCATAGTCCACAGGGTCT
c-Fos	AGATACGCTCCAAGCGGAGA	GGGCTCAGGGTCATTGAGAA
c-Jun	CGCTTGATGACTCAGCCGGAA	TTCCGGCTGAGTCATCAAGCG
FOXA2	GAGGTAAGCTCTTGCCCTCG	ATTCTTTGCCTGCACCTCTGT
NFATC2	CAGACTTACCTGGATGACGTT	GAAGGGGTCCAAAGTAGAAGG
KCNB1	GAGGGCGAGGAGTTTGACAACAC	CGATGGTGGACAGGACGATGAAC
GREM2	CTGCACAACCTCGCTGTTTC	AGAGCTTCCAGAACATCCTGC
VSNL1	GCACAGCGCTATAACTGCAAAA	AGCCCTTGTACCACTGCTTG
ATP11A	GCAGCCTATTGAGGACGCT	TCCAGAAGGTGTACTTGGAGG
FMR1	AAAGTCCAGAGGGGGATGGT	TCTCTCCAAACGCAACTGGT
ChIP	CAGATTACTCAGTGCTTGGCTAT	ATGCCGCTATGACTTCCAG
S1	CCGCTCGAGGGGAACTCTTGCCAT CT	CCCAAGCTTAGGGAGACCGGGAC ACT
S2		CCCAAGCTTAGTTAGTACGGCTAC CAGA
S3		CCCAAGCTTCCATAACTAGTGCTG TGC
S4		CCCAAGCTTAAGTAAGCCAAGGA ACAAC
S5		CCCAAGCTTCTTAGAGGCTGCATT GA



Data S1: Unedited gel and blot images in this study.

(A) Unedited gels for Figures 1D and S1.

(B) Unedited gels for Figures 3E and S3A.

(C) Unedited blots for Figures 3H and S3B.

(D) Unedited gels for Figures 3K and S3C.

- (E) Unedited blots for Figures 4B and S5A.
- (F) Unedited blots for Figures S5B and S5C.
- (G) Unedited gels for Figures 4E and S5D.
- (H) Unedited blots for Figures S5E and S5F.
- (I) Unedited gels for Figures 4F and S5G.
- (J) Unedited gels for Figures 4G and S5H.
- (K) Unedited blots for Figures 5J and S6A.
- (L) Unedited blots for Figures 5M and S6B.
- (M) Unedited blots for Figures 6A, 6B, S7A and S7B.
- (N) Unedited blots for Figures S7C and S7D.
- (O) Unedited blots for Figures 6G and S7E.
- (P) Unedited blots for Figures 7A and S8A.
- (Q) Unedited blots for Figures 7F and S8B.