Table 1 Specific primer sequences

Gene	Primer	Sequence
DNMT1	Forward	5’- GAG GCG GAA ATC AAA GGA GGA -3’
	Reverse	5’- GGG AGT CTC TGG AGC TAC CT -3’
DNMT3a	Forward	5’- GAT GAG CCT GAG TAT GAG GAT GG -3’
	Reverse	5’- CAA GAC ACA ATT CGG CCT GG -3’
DNMT3b	Forward	5’- CTG TCC GAA CCC GAC ATA GC -3’
	Reverse	5’- CCG GAA ACT CCA CAG GGT A -3’
β-actin	Forward	5’-AGA GGG AAA TCG TGC GTG AC-3’
	Reverse	5’-CAA TAG TGA TGA CCT GGC CGT-3’
miR-152-3p	Forward	5’-GAG CGC GTC AGT GCA TGA CA-3’
	Reverse	5’-ATC CAG TGC AGG GTC CGA GG-3’
	Loop primer	5’-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC CAA GT-3’
miR-148a-3p	Forward	5’-AAC ACG TGT CAG TGC ACT ACA GA-3’
	Reverse	5’-ATC CAG TGC AGG GTC CGA GG-3’
	Loop primer	5’-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACA CAA AG-3’
miR-148b-3p	Forward	5’-ATG TGC GTC AGT GCA TCA CAG A-3’
	Reverse	5’-ATC CAG TGC AGG GTC CGA GG-3’
	Loop primer	5’-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACA CAA AG-3’
U6	Forward	5’-CGC TTC TGC CAC ACA GG-3’
	Reverse	5’-ACA CTC GAG ATT ACG TG-3’

Materials and methods

Animals

C57BL/6J mice (6-8 weeks) were obtained from the Experimental Animal Center of Xuzhou Medical University. All animal experiments followed the guidelines approved by the Animal Ethics Committee of Xuzhou Medical University (20110A383).

Type 2 diabetes mellitus (T2DM) mouse models

Mice designated for the diabetic model group were fed a high-sugar and high-fat diet for 8 weeks, followed by intraperitoneal injections of streptozotocin (100 mg/kg) for three consecutive days. Control mice received equivalent volumes of citrate buffer. On the fifth day after the STZ injections, fasting blood glucose (FBG) was measured, confirming the diabetic model with FBG levels above 11.1 mmol/L.

Nociceptive behavioral tests

Mechanical allodynia and thermal hypersensitivity were evaluated using von Frey filaments and Hargreaves tests, as we described previously [17]. Briefly, for the von Frey filaments test, The 50% mechanical withdrawal threshold (MWT) was calculated using the up-down method. For the Hargreaves test, a high-intensity removable radiant heat source was centrally placed on the paw surface. The delay in paw response to the radiant heat source was recorded as the thermal withdrawal delay (TWL). To prevent tissue damage, an automatic cutoff time of 25 seconds was used.

Bioinformatic analysis

Potential miRNAs interacting with DNMT1 were predicted using miRDB, miRWalk, and TargetScan databases. The top three miRNAs most likely associated with DNMT1 were selected.

Adeno-associated virus (AAV) vectors and viral injections

The rAAV2/9-hSyn-mCherry-5'miR-30a-shRNA5(Dnmt1)-3'miR-30a-WPREs (AAV-DNMT1shRNA, 6.45×10^{12} vg/ml) and the control vector rAAV2/9-hSyn-mCherry-5'miR-30a-shRNA5(scramble)-3'miR-30a-WPREs (AAV-mCherry, 5.57×10^{12} vg/ml), the pAAV-CMV-pri-has-mir-152(mut5p)-EF1α-EGFP-3xFLAG-WPRE (AAV-miR-152-3p) and the control vector pAAV-CMV-MCS -EF1α-EGFP-3xFLAG-WPRE (AAV-EGFP), and pAAV-U6-TuD(rno-miR-152-3p)-CMV-EGFP-

WPRE (AAV-miR-152-3p TuD) and the control vector pAAV-U6-shRNA(NC2)-CMV-EGFP-WPRE-spolyA (AAV-EGFP) were obtained from BrainVTA Co., Ltd. (Wuhan, China). Intrathecal injections were performed as we described previously [18]. Briefly, a Hamilton microsyringe was inserted between L5 and L6 vertebrae. Each mouse was injected with a volume of 8 μL at a speed of 1 μL/4 s. The syringe was retained for 1 min after finishing delivery.

Western blotting analysis

Spinal cord tissues from L4 to L6 were taken and incubated overnight at 4°C with the following primary antibodies: anti-DNMT1 (1:1000, Novus Biologicals, Centennial, CO, USA), anti-DNMT3a (1:1000, Abcam, Cambridge, UK), anti-DNMT3b (1:1000, Abcam, Cambridge, UK) and anti-β-actin (Bioworld, Louis Park, USA), followed by incubation with the IRDye 800CW second antibody (Li- Cor, Lincoln, Nebraska, USA). An infrared imaging system (Gene Ltd., Hong Kong, China) was used to detect the immunoreactive bands and ImageJ software was used for analysis.

Real-time quantitative PCR (RT-qPCR)

Total RNA of L4 to L6 spinal cord tissues was extracted by a TRIzol Reagent Kit (Invitrogen, Carlsbad, California, USA) and transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA). RT-qPCR analysis was carried out on the Roche 480 LightCycler detection system. Each sample was analyzed in triplicate. The mRNA level of the target genes was determined using the 2-ΔΔCT method with normalization to β-actin expression. Primer sequences used were shown in Supplementary Table 1.

Immunofluorescence staining

After deep anesthesia, mice were transcardially perfused with 4% paraformaldehyde in PBS. L4 to L6 spinal tissues were removed and postfixed in 4% paraformaldehyde for 6-8 hours and then transferred to 30% sucrose solution until the tissues sank. Coronal slices (30 μm) were obtained with a cryostat (Leica, Wetzler, Germany) at -20°C. After being blocked with 10% normal goat serum in PBS and 0.3% Triton X-100, the sections were incubated with anti-DNMT1(1:100, Novus Biologicals, Centennial, CO, USA), and then with the following primary antibodies: anti-NeuN

(1:100, Abcam, Cambridge, UK), anti-GFAP (1:200, Cell Signaling Technology), and anti-Iba-1 (1:200, Abcam, Cambridge, UK), respectively. After washing, the sections were incubated with suitable secondary antibodies: Alexa Fluor 594 goat anti-mouse IgG or Alexa Fluor 488 goat anti-rabbit IgG (Thermo Fisher Scientific, Waltham, MA, USA) for 2 h. Stained sections were observed with a laser scanning confocal microscope (Leica STELLARIS 5, Germany).

Statistical analysis

Data are expressed as mean ± SEM. An unpaired Student's t-test was used to compare differences between two groups, and one-way ANOVA followed by a Bonferroni test was used for multiple group comparisons. Statistical significance was set at $P<0.05$.