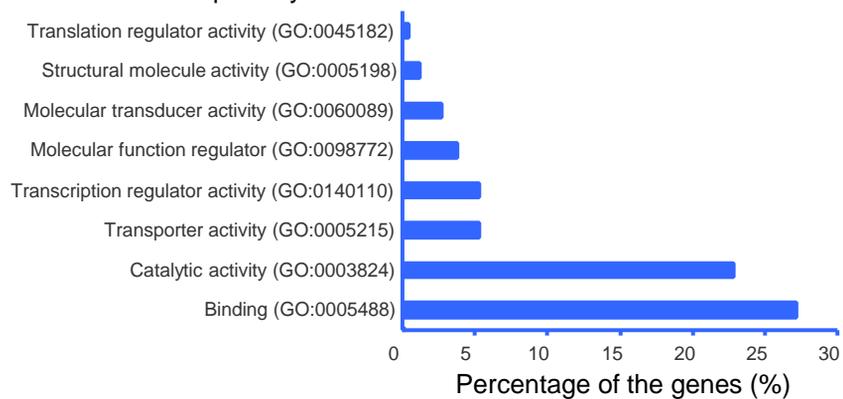
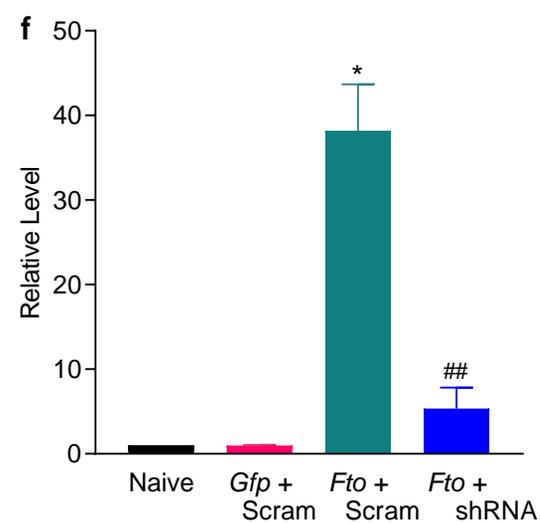
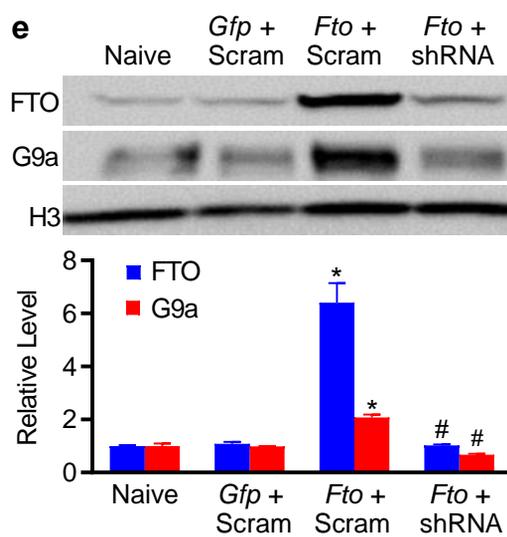
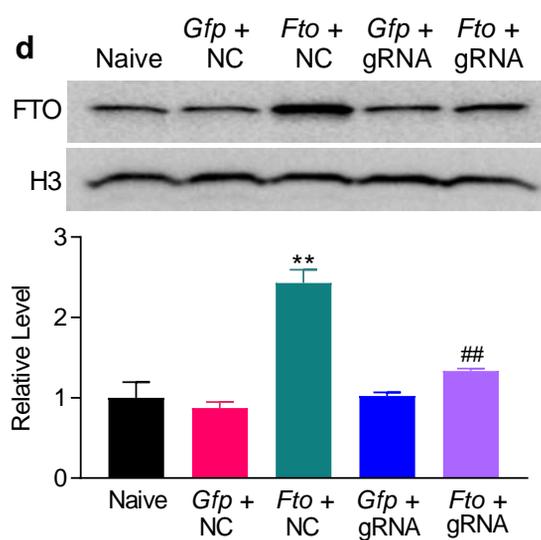
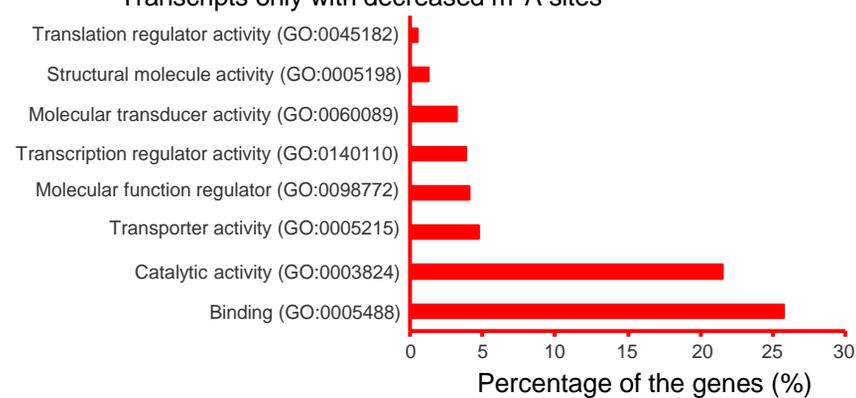
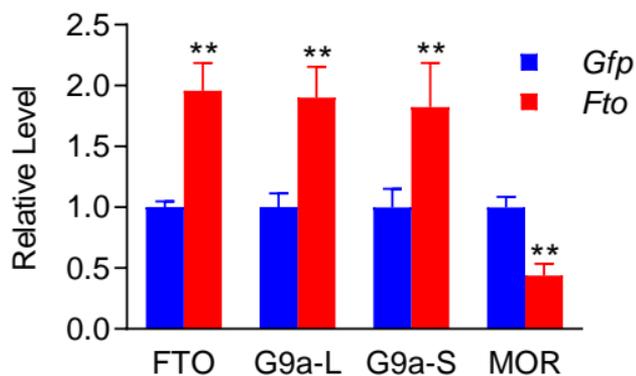
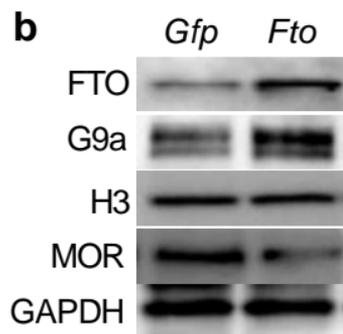
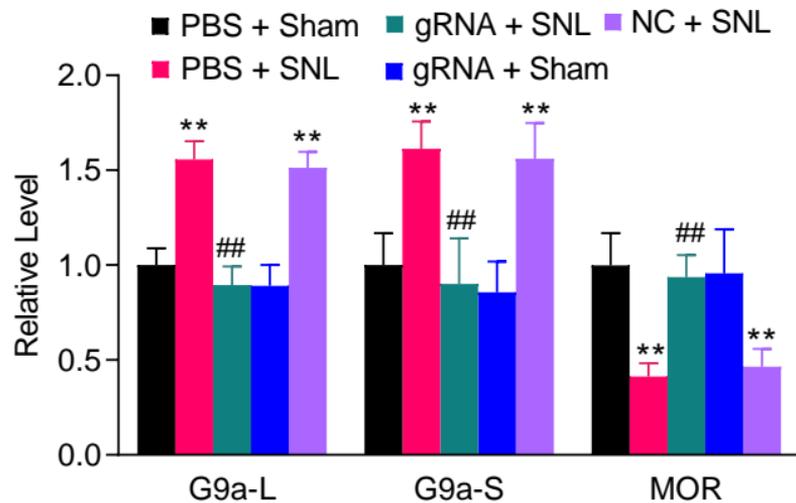
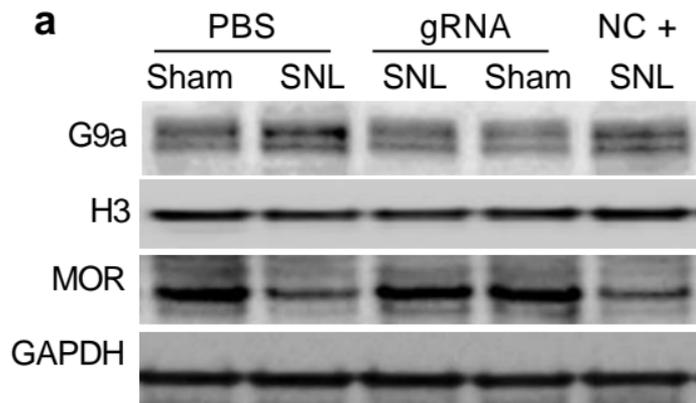


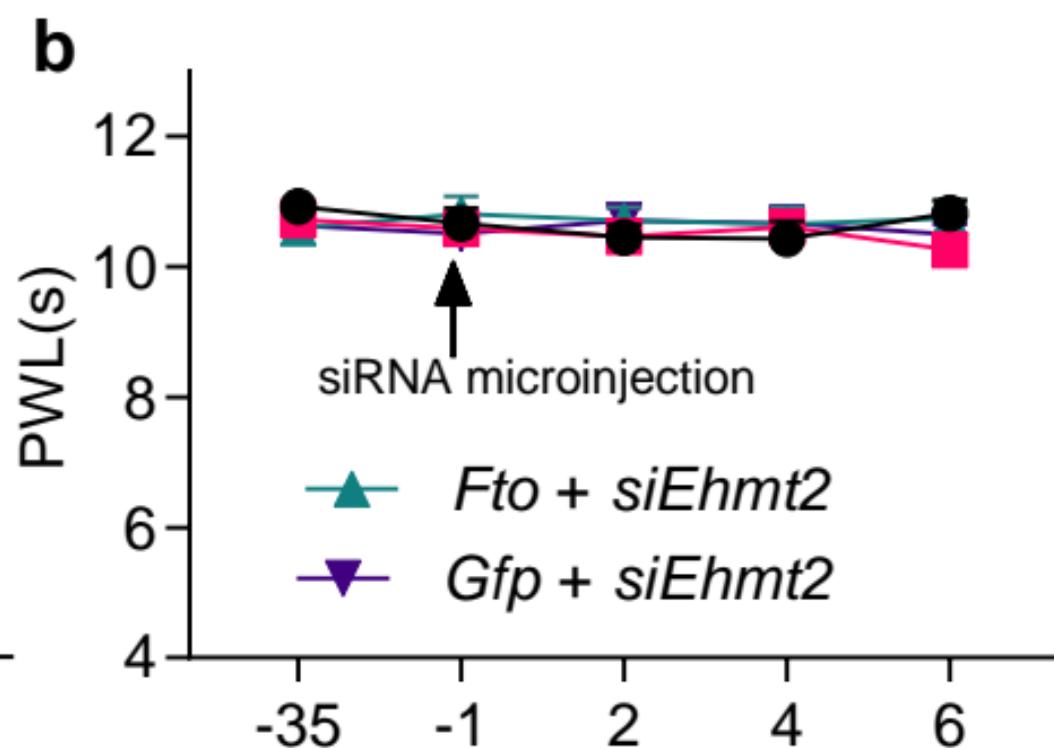
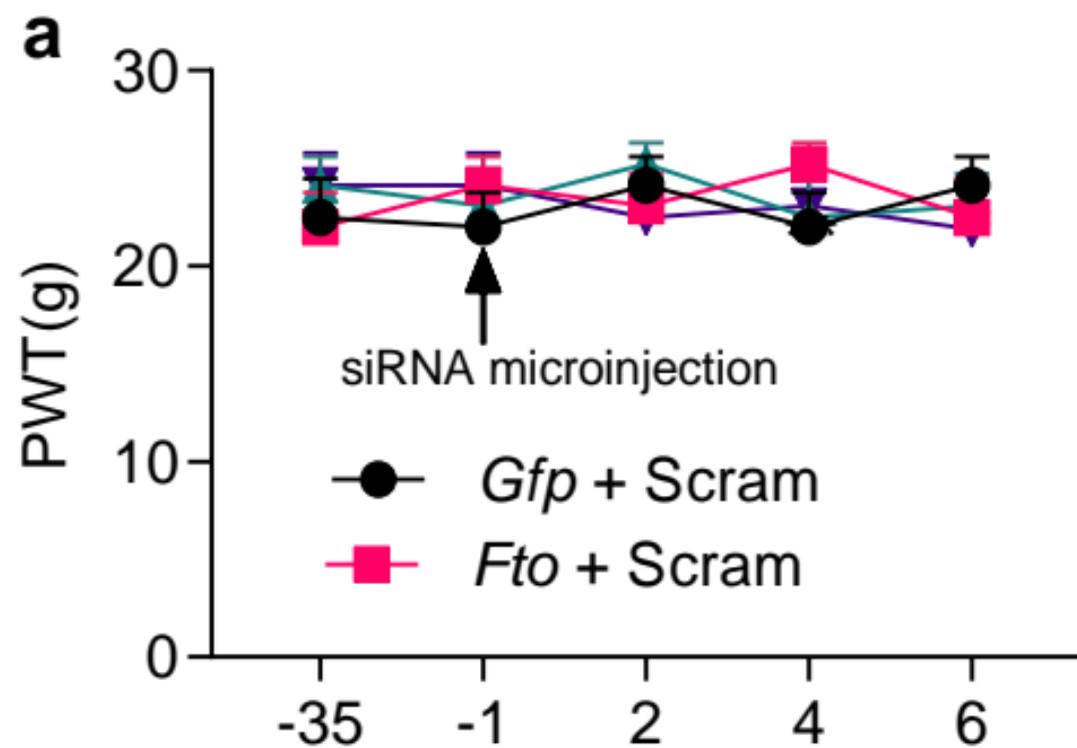
b Transcripts only with increased m⁶A sites



c Transcripts only with decreased m⁶A sites







1 **Supplementary Figure 1 (a)** Expression of FTO in the ipsilateral (Ipsi) L4 DRG and
2 contralateral (Con) L5 DRG after SNL. n = 6 rats/time point. One-way ANOVA with repeated
3 measures followed by *post hoc* Tukey test. **(b)** Expression of FTO in the ipsilateral L4/5 DRGs
4 after injection of complete Freund's adjuvant (CFA) into unilateral hind paw. n = 6 rats/time
5 point. One-way ANOVA with repeated measures followed by *post hoc* Tukey test.

6 **Supplementary Figure 2** Effect of pre-microinjection of Runx1 siRNA or scrambled siRNA
7 (Scr) into the ipsilateral L5 DRG of the rats on the development of SNL-induced mechanical
8 allodynia **(a)**, heat hyperalgesia **(b)** and cold allodynia **(c)** on days 3 and 5 post-surgery. **(a,d)**
9 Paw withdrawal thresholds (PWT) in response mechanical stimulation on the ipsilateral **(a)** and
10 contralateral **(d)** sides. **(b,c,e)** Paw withdrawal latencies in response to heat stimulation in the
11 ipsilateral **(b)** and contralateral **(e)** and to cold stimulation on the ipsilateral side **(c)**. n = 8
12 rats/group. **P* < 0.05, ***P* < 0.01 versus the Scr plus SNL group at the corresponding time
13 points by two-way ANOVA with repeated measures followed by *post hoc* Tukey test.

14 **Supplementary Figure 3** Effect of microinjection of AAV5-*Runx1* or AAV5-*Gfp* into unilateral
15 L4/5 DRGs of the rats on paw withdrawal thresholds (PWT) in response to mechanical
16 stimulation **(a)** and on paw withdrawal latencies (PWL) in response to heat **(b)** and cold **(c)**
17 stimuli on the ipsilateral (Ipsi) and contralateral (Con) sides at time points as shown. BL:
18 baseline. n = 10 rats/group. **P* < 0.05, ***P* < 0.01 versus the AAV5-*Runx1*-treated group on the
19 ipsilateral side at the corresponding time points by two-way ANOVA with repeated measures
20 followed by *post hoc* Tukey test.

21 **Supplementary Figure 4 (a)** Effect of pre-microinjection of *Fto* siRNA (siFTO), scrambled
22 siRNA (Scr) or PBS into the ipsilateral L5 DRG of the rats on the expression of FTO, DNMT1,
23 DNMT3a and Kv1.4 in the ipsilateral L5 DRG on day 7 after SNL or sham surgery. n = 6

1 rats/group. One-way ANOVA with repeated measures followed by *post hoc* Tukey test. $**P <$
2 0.01 versus the corresponding naive group. $##P < 0.01$ versus the corresponding PBS-treated
3 SNL group. **(b,c)** Effect of pre-microinjection of *Fto* siRNA (siFTO), scrambled siRNA (Scr) or
4 PBS into the ipsilateral L5 DRG of the rats on basal paw withdrawal responses to mechanical **(b)**
5 and heat **(c)** stimuli on the contralateral side at days indicated post-SNL or sham surgery. $n = 6$
6 rats/group. Two-way ANOVA with repeated measures followed by *post hoc* Tukey test. **(d,e)**
7 Effect of post-microinjection of *Fto* siRNA (siFTO), scrambled siRNA (Scr) or PBS into
8 ipsilateral L5 DRG of the rats on basal paw withdrawal responses to mechanical **(d)** and heat **(e)**
9 stimuli on the contralateral side at days indicated post-SNL or sham surgery. $n = 6$ rats/group.
10 Two-way ANOVA with repeated measures followed by *post hoc* Tukey test.

11 **Supplementary Figure 5 (a,b)** Levels of FTO protein **(a)** and mRNA **(b)** in PC12 cells
12 transfected with the *Fto* guide RNA (gRNA) or negative control sequence (NC). $n = 3$ biological
13 repeats $*P < 0.05$, $**P < 0.01$ versus the NC-treated group by two-tailed unpaired Student's t-
14 test. **(c,d)** Levels of FTO protein **(c)** and mRNA **(d)** in the cultured rat DRG neurons transduced
15 with AAV5-*Fto* guide RNA (gRNA) or AAV5-negative control sequence (NC). $n = 3$ biological
16 repeats $*P < 0.05$, $**P < 0.01$ versus the AAV5-negative control sequence-treated group by two-
17 tailed unpaired Student's t-test. **(e)** Expression of FTO protein in the ipsilateral L5 DRG on day 7
18 post-SNL or sham surgery from the rats microinjected with AAV5-*Fto* guide RNA (gRNA), PBS
19 or AAV5-negative control sequence (NC). $n = 3$ biological repeats (6 rats)/group. Two-way
20 ANOVA with repeated measures followed by *post hoc* Tukey test. $**P < 0.01$ versus the PBS-
21 treated sham group. $##P < 0.01$ versus the PBS-treated SNL group. **(f)** Levels of p-ERK1/2,
22 ERK1/2 and GFAP in the ipsilateral L5 dorsal horn 7 days after SNL or sham surgery from the
23 rats microinjected with AAV5-*Fto* guide RNA (gRNA), PBS or AAV5-negative control

1 sequence (NC). n = 3 biological repeats (3 rats)/group. Two-way ANOVA with repeated
2 measures followed by post hoc Tukey test. $**P < 0.01$ versus the corresponding PBS-treated
3 sham group. $##P < 0.01$ versus the corresponding PBS-treated SNL group. **(g,h)** Effect of pre-
4 microinjection of AAV5-*Fto* guide RNA (gRNA), PBS or AAV5-negative control sequence
5 (NC) into ipsilateral L5 DRG on basal paw withdrawal responses to mechanical **(g)** and heat **(h)**
6 stimuli on the contralateral side at days indicated post-SNL or sham surgery. PWT: paw
7 withdrawal threshold. PWL: paw withdrawal latency. Two-way ANOVA with repeated measures
8 followed by post hoc turkey test. n = 5 rats/group. **(i-l)** Effect of pre-microinjection of AAV5-*Fto*
9 guide RNA (gRNA), PBS or AAV5-negative control sequence (NC) into ipsilateral L4/5 DRGs
10 on paw withdrawal thresholds (PWT) in response to mechanical stimulation **(i, j)** and paw
11 withdrawal latencies (PWL) in response to heat stimulation **(k, l)** stimuli on the ipsilateral **(i, k)**
12 and contralateral **(j, l)** sides at days indicated post-CCI or sham surgery. Two-way ANOVA with
13 repeated measures followed by post hoc turkey test. n = 6 rats/group. $**P < 0.01$ versus the
14 corresponding PBS-treated sham group. $##P < 0.01$ versus the corresponding PBS-treated CCI
15 group.

16 **Supplementary Figure 6 (a)** FTO protein expression in the ipsilateral L4 DRG, spinal cord
17 (SC) and brain 7 weeks after microinjection of AAV5-*Gfp* (*Gfp*) or AAV5-*Cre* (*Cre*) into
18 unilateral L4 DRG from the *Fto*^{fl/fl} mice. n = 3 biological repeats (6 mice). $**P < 0.01$ versus the
19 corresponding AAV5-*Gfp*-treated group by two-tailed unpaired Student's t-test. **(b)** FTO protein
20 expression in the ipsilateral L4 DRG on day 14 post-SNL or sham surgery from the *Fto*^{fl/fl} mice
21 microinjected with AAV5-*Cre* or AAV5-*Gfp*. n = 3 biological repeats (12 mice)/group. One-way
22 ANOVA followed by post hoc Tukey test. $**P < 0.01$ versus the PBS-treated sham group. $##P <$
23 0.01 versus the AAV5-*Gfp*-treated SNL group. **(c, d)** Effect of pre-microinjection of AAV5-*Gfp*

1 (*Gfp*) or AAV5-*Cre* (*Cre*) into ipsilateral L4 DRG of the *Fto*^{fl/fl} mice on basal paw withdrawal
2 responses to mechanical (**c**) and heat (**d**) stimuli on the contralateral side at days indicated post-
3 SNL or sham surgery. PWF: paw withdrawal frequency. PWL: paw withdrawal latency. Two-
4 way ANOVA with repeated measures followed by post hoc turkey test. n = 10 mice/group.

5 **Supplementary Figure 7** (a) FTO protein expression in the DRGs, spinal cord (SC) and cortex
6 from the *Fto*^{fl/fl} mice (fl/fl) and the conditional *Fto* knockout mice (KO). (b) Level of FTO
7 protein in the ipsilateral L4 DRG on day 14 post-SNL or sham surgery from the *Fto*^{fl/fl} mice
8 (fl/fl) and the conditional *Fto* knockout mice (KO). n = 3 biological repeats (12 mice)/group.
9 One-way ANOVA with repeated measures followed by post hoc Tukey test. **P* < 0.05, ***P* <
10 0.01 versus the sham *Fto*^{fl/fl} mice. ##*P* < 0.01 versus the SNL conditional *Fto* knockout mice. (c-
11 i) Paw withdrawal frequency (PWF) to low (0.07 g; **c** and **g**) and medium (0.4 g; **d** and **h**) force
12 von Frey filament stimuli and paw withdrawal latency (PWL) to heat (**e** and **i**) and cold (**f**)
13 stimuli on the ipsilateral (**c-f**) and contralateral (**g-i**) sides from *Fto*^{fl/fl} mice (fl/fl) or conditional
14 *Fto* knockout mice (KO) after SNL or sham surgery. n = 10 mice/group. ***P* < 0.01 versus the
15 SNL *Fto*^{fl/fl} mice at the corresponding time points by two-way ANOVA with repeated measures
16 followed by post hoc Tukey test.

17 **Supplementary Figure 8** (a) FTO protein expression in the cultured rat DRG neurons
18 transduced with AAV5-*Fto* (*Fto*) or AAV5-*Gfp* (*Gfp*). n = 3 biological repeats/group. ***P* < 0.01
19 versus the AAV5-*Gfp*-treated group by two-tailed unpaired Student's t-test. (b) FTO protein
20 expression in the ipsilateral L4/5 DRGs 8 weeks after microinjection of AAV5-*Fto* (*Fto*) or
21 AAV5-*Gfp* (*Gfp*) into unilateral L4/5 DRGs of the rats. n = 3 biological repeats (6 rats)/group.
22 ***P* < 0.01 versus the AAV5-*Gfp*-treated group by two-tailed unpaired Student's t-test. (c, d)
23 Basal paw withdrawal response to mechanical (**c**) and heat (**d**) stimuli on the contralateral side

1 on weeks as indicated after microinjection of AAV5-*Fto* (*Fto*) or AAV5-*Gfp* (*Gfp*) into
2 unilateral L4/5 DRGs of the rats. BL: baseline. PWT: paw withdrawal threshold. PWL: paw
3 withdrawal latency. n = 12 rats/group. Two-way ANOVA with repeated measures followed by
4 post hoc Tukey test.

5 **Supplementary Figure 9** (a) Dynamic changes of m⁶A sites across transcripts from the
6 ipsilateral L5 DRG on day 7 after SNL or sham surgery. **This chart is based on clustering relative**
7 **tag numbers of peak-associated genes. The intragenic regions of peak-associated genes are**
8 **classified into four categories: intron, CDS (coding sequence region), 5'-UTR and 3'-UTR.**
9 **Peaks are categorized into each of the intragenic regions. Thus, each gene has four values**
10 **according to the relative tag numbers in the four regions. The relative tag numbers are log₂_ratio**
11 **of different tag numbers ((tag number in the SNL group) – (tag number in the sham group)) in**
12 **peaks in each region. If no peaks were detected in one gene locus in both sham and SNL groups,**
13 **this gene was discarded for analysis. (b,c)** GO enrichment analyses of the transcripts with
14 increased (b) and decreased (c) m⁶A sites from the ipsilateral L5 DRG on day 7 after SNL or
15 sham surgery. (d) FTO protein expression in cultured rat DRG neurons transduced with the
16 viruses as indicated. *Gfp*: AAV5-*Gfp*. *Fto*: AAV5-*Fto*. gRNA: AAV5-*Fto* guide RNA. NC:
17 AAV5-control negative sequence. n = 3 biological repeats/group. One-way ANOVA with
18 repeated measures followed by post hoc Tukey test. ***P* < 0.01 versus the *Gfp* plus NC-treated
19 group. ##*P* < 0.01 versus the *Fto* plus NC-treated group. (e,f) Levels of FTO and G9a proteins
20 (e) and *Fto* mRNA (f) in PC12 cells transduced by the vectors as shown. *Gfp*: vector expressing
21 *Gfp*. *Fto*: vector expressing full-length *Fto*. Scr: vector expressing scrambled shRNA. shRNA:
22 vector expressing *Fto* shRNA. n = 3 biological repeats. One-way ANOVA with repeated

1 measures followed by *post hoc* Tukey test. * $P < 0.05$ versus corresponding naive group. # $P <$
2 0.05, ## $P < 0.01$ versus the corresponding *Fto* plus Scr-treated group.

3 **Supplementary Figure 10 (a)** Levels of G9a and MOR protein in the ipsilateral L5 DRG on day
4 7 post-SNL or sham surgery from the rats microinjected with AAV5-*Fto* guide RNA (gRNA),
5 PBS or AAV5-negative control sequence (NC). $n = 3$ biological repeats (6 rats)/group. One-way
6 ANOVA with repeated measures followed by post hoc Tukey test. ** $P < 0.01$ versus the
7 corresponding PBS-treated sham group. ## $P < 0.01$ versus the corresponding PBS-treated SNL
8 group. **(b)** Levels of FTO, G9a and MOR proteins in the cultured rat DRG neurons transduced
9 with AAV5-*Fto* (*Fto*) or AAV5-*Gfp* (*Gfp*). $n = 3$ biological repeats. ** $P < 0.01$ versus the
10 corresponding AAV5-*Gfp*-treated group by two-tailed unpaired Student's t-test.

11 **Supplementary Figure 11** Effect of microinjection of *Ehmt2* siRNA (*siEhmt2*) or scrambled
12 siRNA (Scram) into ipsilateral L4/5 DRGs on contralateral paw withdrawal responses to
13 mechanical **(a)** and heat **(b)** stimuli at time points as shown in the rats pre-microinjected with
14 AAV5-*Fto* (*Fto*) or AAV5-*Gfp* (*Gfp*) into unilateral L4/5 DRGs. PWT: paw withdrawal
15 threshold. PWL: paw withdrawal latency. $n = 10$ rats/group. Two-way ANOVA with repeated
16 measures followed by *post hoc* Tukey test.

17

Supplementary Table 1: Locomotor functions

Treatments/genotyping	Functional test		
	Placing	Grasping	Righting
<i>Fto</i> siRNA + Sham	5 (0)	5 (0)	5 (0)
<i>Fto</i> siRNA + SNL	5 (0)	5 (0)	5 (0)
<i>Fto</i> scramble + SNL	5 (0)	5 (0)	5 (0)
PBS + SNL	5 (0)	5 (0)	5 (0)
PBS + Sham	5 (0)	5 (0)	5 (0)
sg3+Sham	5 (0)	5 (0)	5 (0)
sg3+ SNL	5 (0)	5 (0)	5 (0)
NC + Sham	5 (0)	5 (0)	5 (0)
NC + SNL	5 (0)	5 (0)	5 (0)
<i>Fto</i> ^{fl/fl} + AAV5-Cre + Sham	5 (0)	5 (0)	5 (0)
<i>Fto</i> ^{fl/fl} + AAV5-Cre + SNL	5 (0)	5 (0)	5 (0)
<i>Fto</i> ^{fl/fl} + AAV5-GFP + Sham	5 (0)	5 (0)	5 (0)
<i>Fto</i> ^{fl/fl} + AAV5-GFP + SNL	5 (0)	5 (0)	5 (0)
AAV5-GFP	5 (0)	5 (0)	5 (0)
AAV5-FTO	5 (0)	5 (0)	5 (0)
<i>Fto</i> ^{fl/fl} + Sham	5 (0)	5 (0)	5 (0)
<i>Fto</i> ^{fl/fl} + SNL	5 (0)	5 (0)	5 (0)
<i>Adv</i> ^{cre} <i>Fto</i> ^{fl/fl} + Sham	5 (0)	5 (0)	5 (0)
<i>Adv</i> ^{cre} <i>Fto</i> ^{fl/fl} + SNL	5 (0)	5 (0)	5 (0)

Scores for placing, grasping and righting reflexes were based on counts of each normal reflex exhibited in five trials. All values are Mean (SEM). n = 10 rats or mice/group.

Supplementary Table 2. All primers and other sequences used.

PCR or Real-RT-PCR
<i>Fto</i> -F 5'- TGGATCCGTGCATCTGTAAAGCTCAGG -3'
<i>Fto</i> -R 5'- CCTGACAAATAACGACAATCGAGATG -3'
<i>Ehmt2</i> -F 5'-TGCCTATGTGGTCAGCTCAG-3'
<i>Ehmt2</i> -R 5'-GGTTCTTGCAGCTTCTCCAG-3'
<i>Cre</i> -F 5'-TGC CAC GAC CAA GTG ACA GCA ATG-3'
<i>Cre</i> -R 5'-ACC AGA GAC GGA AAT CCA TCG CTC-3'
Rat- <i>Fto</i> -F 5'- TCTTACAACGCTGCCAGTTG -3'
Rat- <i>Fto</i> -R 5'- GAAACCAGAACTGCCTCAGC -3'
Mouse- <i>Fto</i> -F 5'- GCGGGAAGCTAAGAAACTGA -3'
Mouse- <i>Fto</i> -R 5'- ATGCAGCTCCTCTGGTATGC -3'
Rat- <i>Runx1</i> -F 5'- CGAAGACATCGGCAGAAACT -3'
Rat- <i>Runx1</i> -R 5'- GCTGAGGGTTAAAGGCAGTG -3'
Rat- <i>Ehmt2</i> -F 5'- GATCATCTGCCGGGATGTAG -3'
Rat- <i>Ehmt2</i> -R 5'- AGTGCTGCAGGTGAGTGATG -3'
Mouse- <i>Ehmt2</i> -F 5'- AAATTGGGAACTTGAAATGG-3'
Mouse- <i>Ehmt2</i> -R 5'- CACTACCCGTGAAGGAGGC-3'
<i>Gapdh</i> -F 5'-TCG GTG TGA ACG GAT TTG GC-3'
<i>Gapdh</i> -R 5'-TCC CAT TCT CGG CCT TGA CT-3'
Rat- <i>Oprm1</i> -F 5'- TTCCTGGTCATGTATGTGATTGT-3'
Rat- <i>Oprm1</i> -R 5'- GGGCAGTGTACTGGTCGCTAA-3'
Rat- <i>Oprk1</i> -F 5'- TTTGTGGTGGGCTTAGTGGG -3'
Rat- <i>Oprk1</i> -R 5'- CTCTGGAAGGGCATAGTGGT -3'
<i>Kcna2</i> -F 5'- CTGCAAGGGCAACGTCACAC -3'
<i>Kcna2</i> -R 5'-GGGACAGTGAGATGCTTGGC-3'
ChIP-PCR
<i>Fto</i> -ChIP-F 5'- GAGTGGCACCACACCCTACT -3'
<i>Fto</i> -ChIP-R 5'- GACAGGGTAGGGGGACGTAT-3'
RIP-PCR
Rat <i>Ehmt2</i> -RIP-F 5'- GATCATCTGCCGGGATGTAG-3'
Rat <i>Ehmt2</i> -RIP-R 5'-AGTGCTGCAGGTGAGTGATG-3'
<i>Fto</i> Guide RNA
GATGAGGATGCGAGACACCGGG (PAM: GTGAG, targeting exon 3)
<i>Fto</i> shRNAs
<i>Fto</i> siRNA: ACGUGACUUUGC UAAACUUTT
<i>Fto</i> siRNA: AAGUUUAGCAAAGUCACGUTG
Scramble siRNA: AAGGCUCUAUGAAGAGGCUTG
Cloning
FTO shRNA sense: GATCCGATGAAGTGGACCTTAAGAGAAGCTTGTCTTAAGGTCCACTTCATCTTT TTTT
FTO shRNA antisense: CTAGAAAAAAGATGAAGTGGACCTTAAGACAAGCTTCTCTTAAGGTCCACTTC

ATCG

Runx1 shRNA sense:

GATCCGCAGGCTCCTACCAATTCTGAAGCTTGAGAATTGGTAGGAGCCTGCTTT
TTTT

Runx1 shRNA antisense:

CTAGAAAAAAGCAGGCTCCTACCAATTCTCAAGCTTCAGAATTGGTAGGAGC
CTGCG

Scramble shRNA sense:

GATCCGGTTCAGATGTGCGGCGAGTGAAGCTTGACTCGCCGCACATCTGAACCT
TTTTTT

Scramble shRNA antisense:

CTAGAAAAAAGGTTTCAGATGTGCGGCGAGTCAAGCTTCACTCGCCGCACATCT
GAACCG

Runx1 RT F: TAACCCTGCCTGGGTGTAAG

Runx1 RT R: AATAACGACCACCCAGATGC

Runx1 nested F: CGCTCTAGAGCCACCATGCGTATCCCCGTAGA

Runx1 nested R: ATATAGCGGCCGCTCAGTAGGGCCGCCAGACA

Fto RT F: ATGAAGCGCGTCCAGACCGC

Fto RT R: GGGAGAAAAGCCAAGGACAT

Fto nested F: ATATCCGGAGCCACCATGAAGCGCGTCCAGACC

Fto nested R: GCTAGCGGCCGCTAGGATCTTGCTTCCAG

FTO-luc-F: ATAGGTACCTAAGGGAAGCCTATGCAAGC

FTO-luc-R: ATCAAGCTTACTCAGGCCTGCATCACAG

m⁶A-eCLIP-seq

3'-RNA adaptor 1: ATTGCTT AGATCGGAAGAGCACACGTCT

3'-RNA adaptor 2: ACAAGCCAGATCGGAAGAGCACACGTCT

3'-RNA adaptor 3: AACTTGT AGATCGGAAGAGCACACGTCT

nested specific primer: AGACGTGTGCTCTTCCGA

RT: reverse transcription. F: forward. R: reverse.