Supplementary Materials

Supplementary Figures and Figure Legends

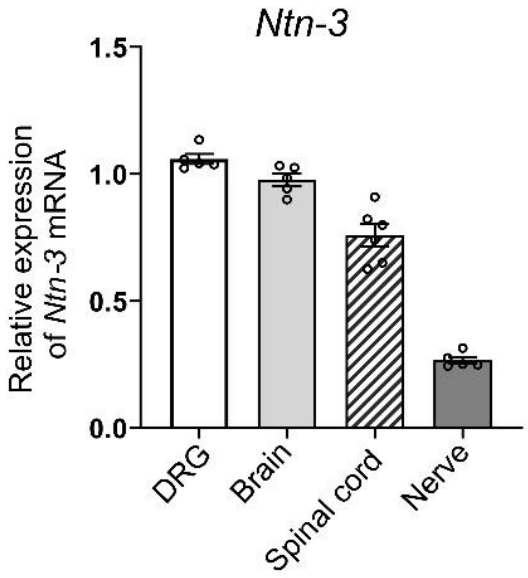

Fig. S1 *Ntn-3* expression pattern in adult mice. qPCR analysis show that *Ntn-3* is expressed in multiple neural tissues of the adult mouse. n = 5–6 mice.

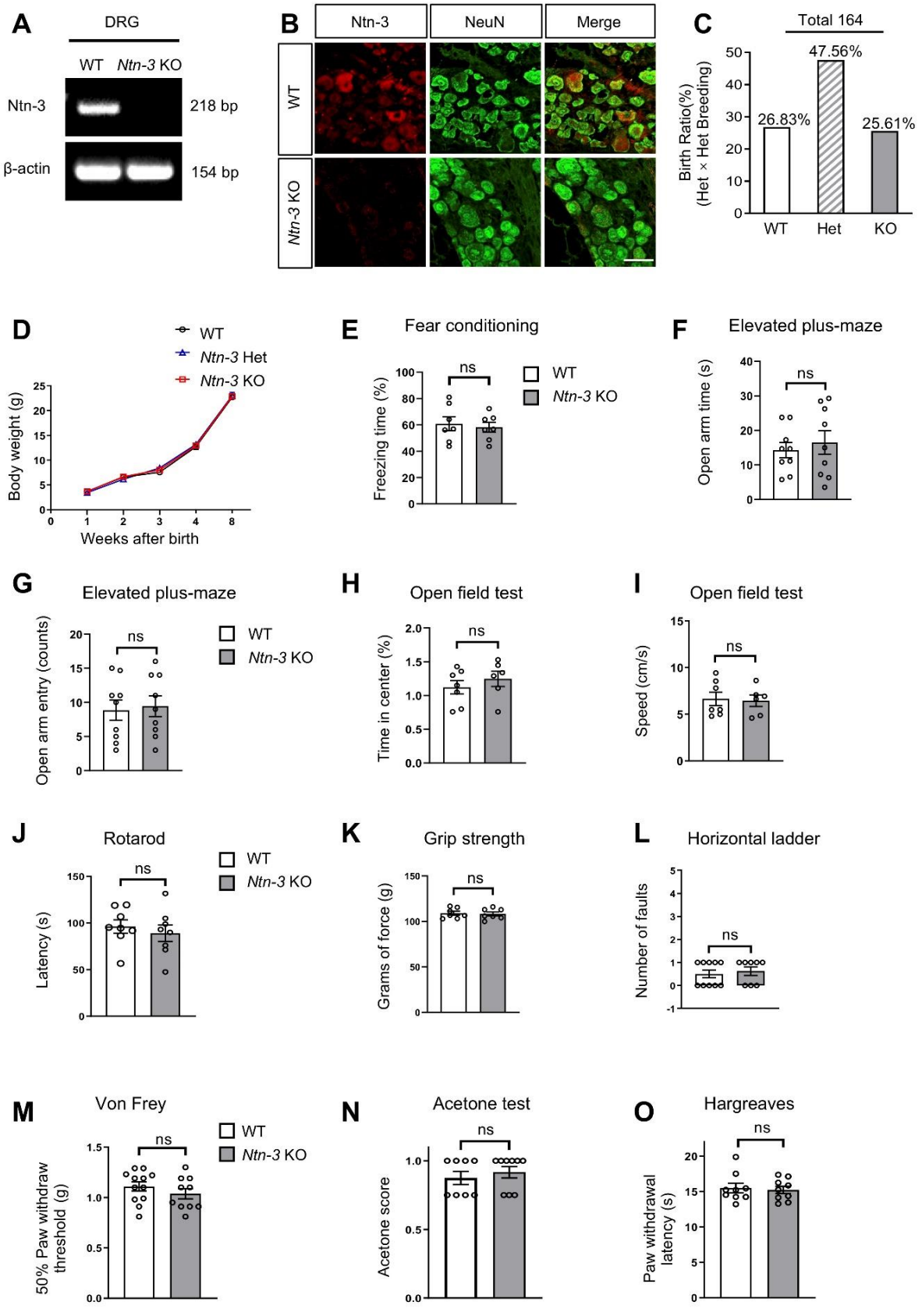
A
DRG
WT
Ntn-3 KO
Ntn-3
218 bp
β-actin
154 bp
B
Ntn-3
NeuN
Merge
WT
Ntn-3 KO
C
Total 164
47.56%
26.83%
25.61%
Birth Ratio(%)
(Het × Het Breeding)
WT
Het
KO
D
WT
Ntn-3 Het
Ntn-3 KO
Body weight (g)
Weeks after birth
E
Fear conditioning
Freezing time (%)
ns
WT
Ntn-3 KO
F
Elevated plus-maze
Open arm time (s)
ns
G
Elevated plus-maze
Open arm entry (counts)
ns
WT
Ntn-3 KO
H
Open field test
Time in center (%)
ns
I
Open field test
Speed (cm/s)
ns
J
Rotarod
Latency (s)
ns
WT
Ntn-3 KO
K
Grip strength
Grams of force (g)
ns
L
Horizontal ladder
Number of faults
ns
M
Von Frey
50% Paw withdraw threshold (g)
ns
WT
Ntn-3 KO
N
Acetone test
Acetone score
ns
O
Hargreaves
Paw withdrawal latency (s)
ns

Fig. S2 *Ntn-3* KO mice show no developmental and behavioral defects. **A, B** Ntn-3 mRNA (**A**) and protein (**B**) are absent in *Ntn-3* KO DRGs. Scale bar, 50 μm. **C** Genotype analysis of 164 offspring of *Ntn-3* heterozygotic (Het) breeding pairs. The birth ratio of each genotype is consistent with Mendel's law. **D** No significant difference in body weights occurs among the different genotypes, $n = 8$ mice per group. **E–O** The behavioral performance of *Ntn-3* KO mice is similar to that of WT littermates. Contextual fear conditioning presented as the percentage of time spent freezing during the 5-min contextual fear conditioning test (**E**); anxiety-related emotional behaviors in the elevated plus maze (**F, G**) and open field maze tests (**H**); locomotor ability indicated by movement speed in the open field maze (**I**); coordination indicated by latency to fall during the accelerating rotarod test (**J**); muscle strength indicated by forelimb grip strength (**K**); and proprioceptive function, as tested *via* the horizontal ladder (**L**). *Ntn-3* KO mice perform normally for sensory sensitivity in the mechanical allodynia (**M**), cold allodynia (**N**), and thermal hyperalgesia (**O**) tests. ns, no significant difference by Student's *t*-test. $n = 6$–17 per group (**E**–**O**).

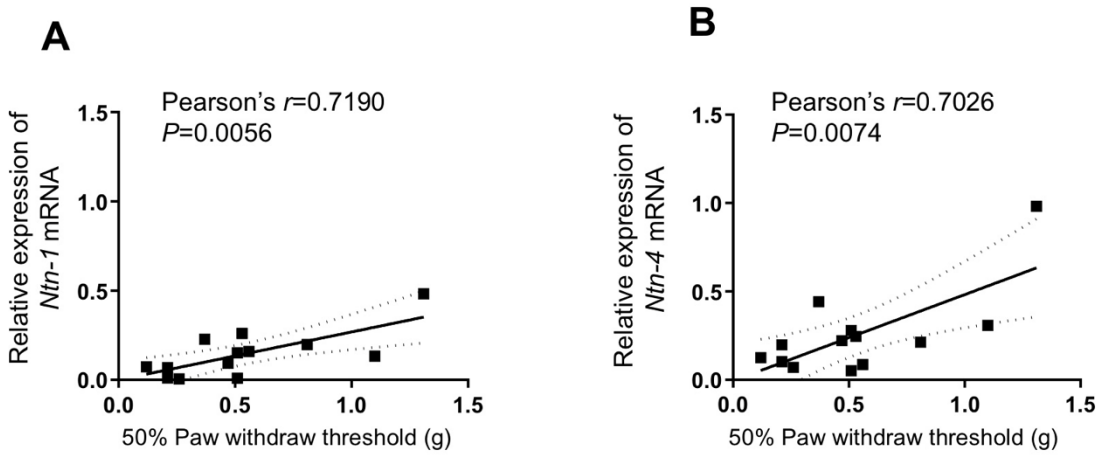

Fig. S3 mRNA expression of *Ntn-1* and *Ntn-4* in diabetic mice. **A, B** Correlation of *Ntn-1* (**A**) and *Ntn-4* (**B**) mRNA levels with mechanical allodynia threshold in diabetic mice. *n* = 13 mice.

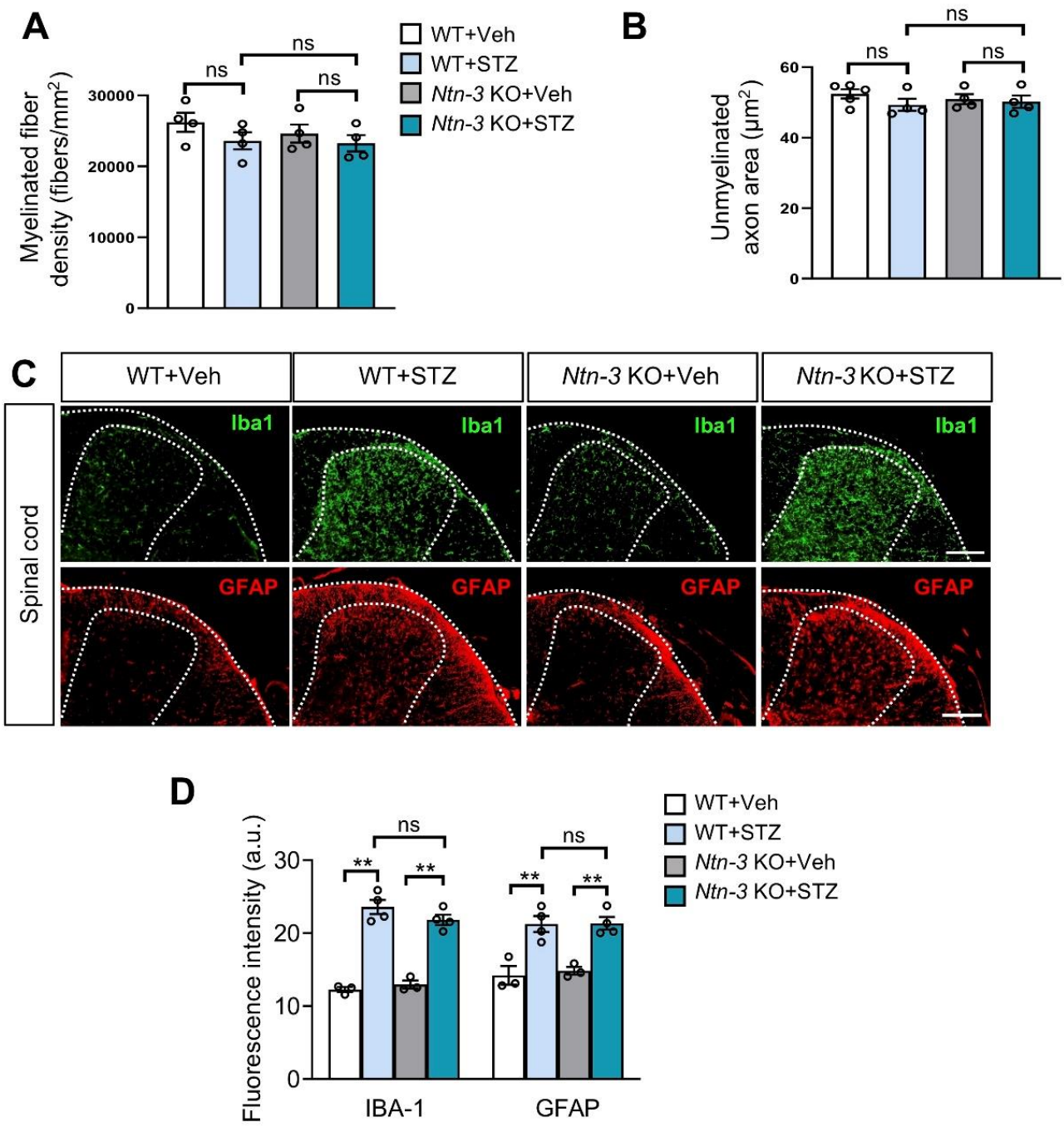

Fig. S4 Ntn-3 deficiency has no effect on DNP-associated peripheral axon loss and spinal cord gliosis. **A, B** Myelinated fiber density (**A**) and unmyelinated axon-covered areas (**B**) indicate no significant axon loss in either diabetic *Ntn-3* KO or WT groups. ns, no significant difference by two-way ANOVA. $n = 4$ sections from 2–3 mice per group. **C** Immunostaining of Iba-1 and GFAP in the spinal cords of WT and

Ntn-3 KO mice 4 weeks after STZ administration. Scale bars, 200 μm. **D** Activation of glial cells in the dorsal horn of the mouse spinal cord quantified by ImageJ and presented as fluorescence intensity (arbitrary units, a.u.). No significant difference in microglia and astrocyte activation is found between diabetic WT and *Ntn-3* KO mice. ***P* <0.01; ns, no significant difference by two-way ANOVA. *n* = 3–4 mice per group.

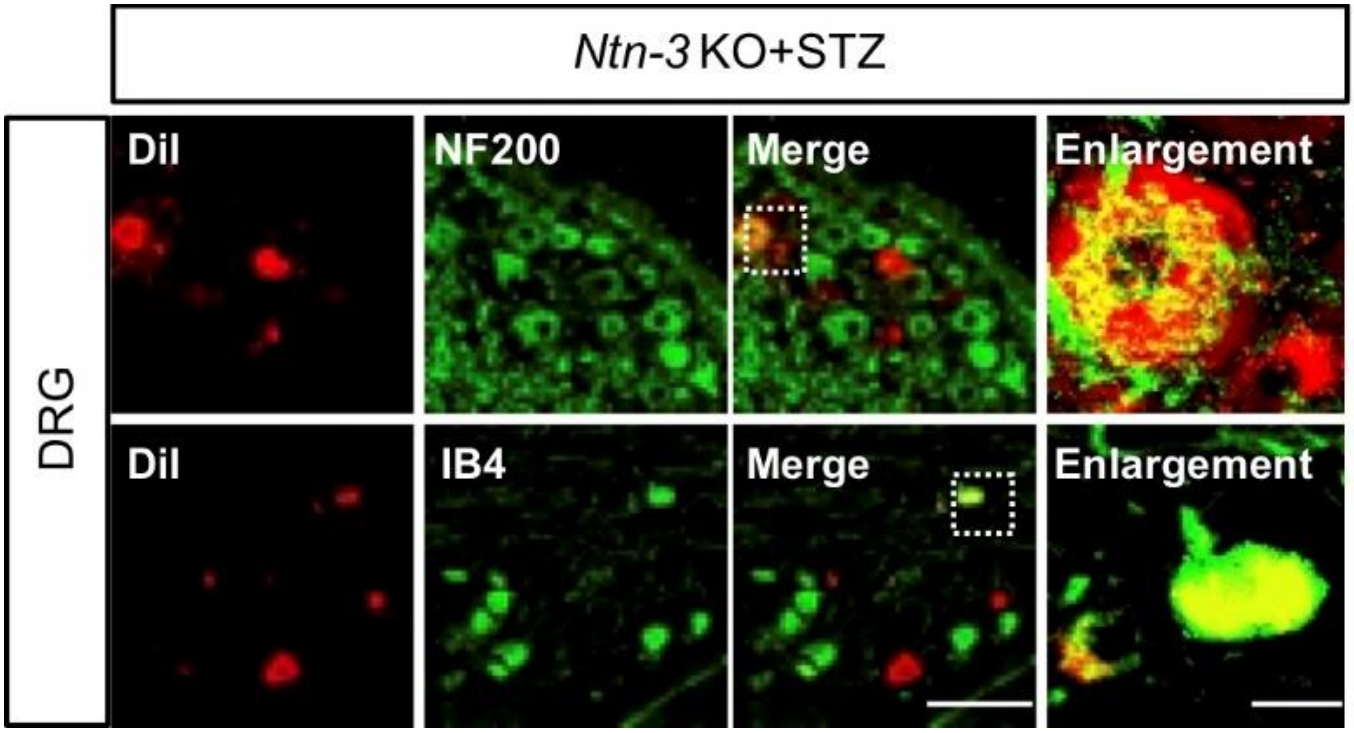

Fig. S5 $NF200^+$ and $IB4^+$ sensory neurons in DRGs retrogradely labeled by Dil. Representative images of NF200, IB4, and Dil labeling in DRG neurons of diabetic *Ntn-3* KO mice. Scale bar, 100 μm. Boxed regions of the merged images are enlarged on the right. Scale bar, 15 μm.

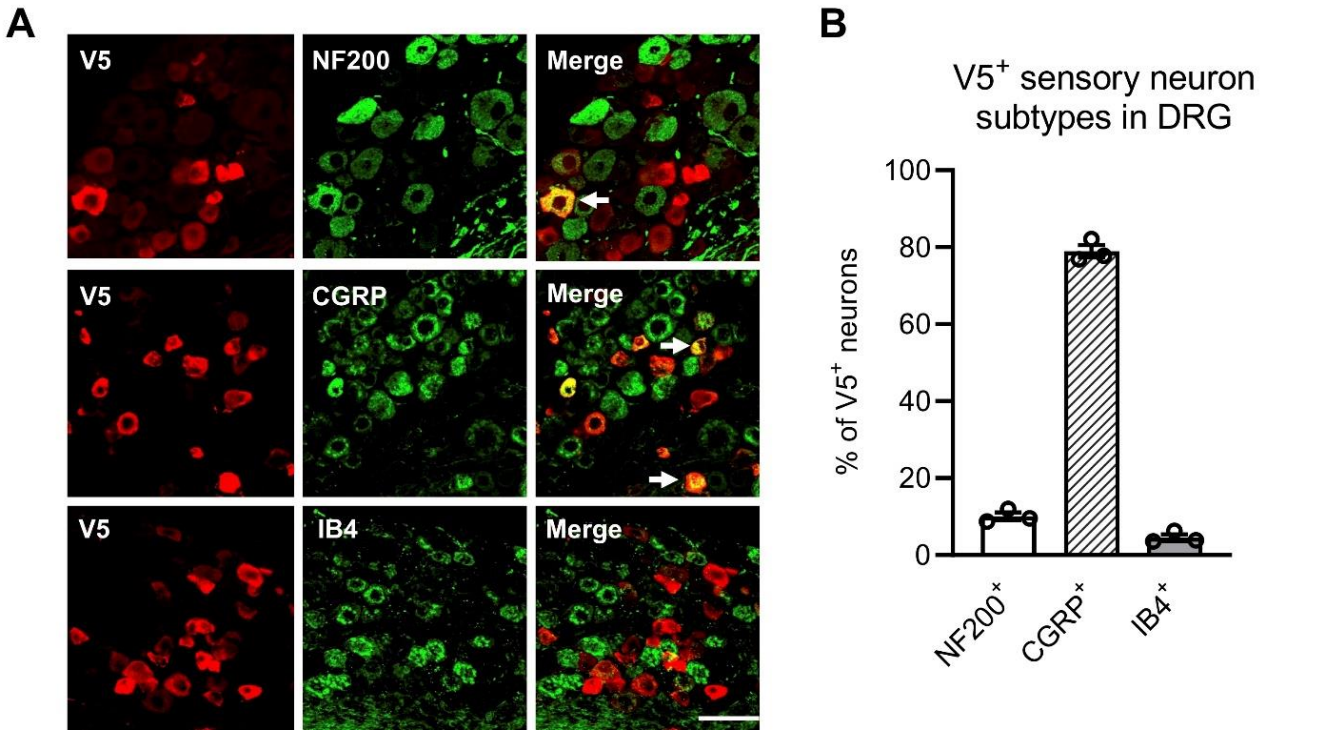

Fig. S6 AAV-induced Ntn-3 overexpression predominantly in CGRP$^+$ neurons. **A** Co-immunostaining of V5 and neuronal subtype markers in mouse DRGs. V5-tagged exogenous Ntn-3 is predominantly expressed in CGRP$^+$ neurons (arrows). Scale bar, 50 μm. **B** Percentages of total V5$^+$ neurons expressing NF200, CGRP, and IB4. *n* = 3 mice.

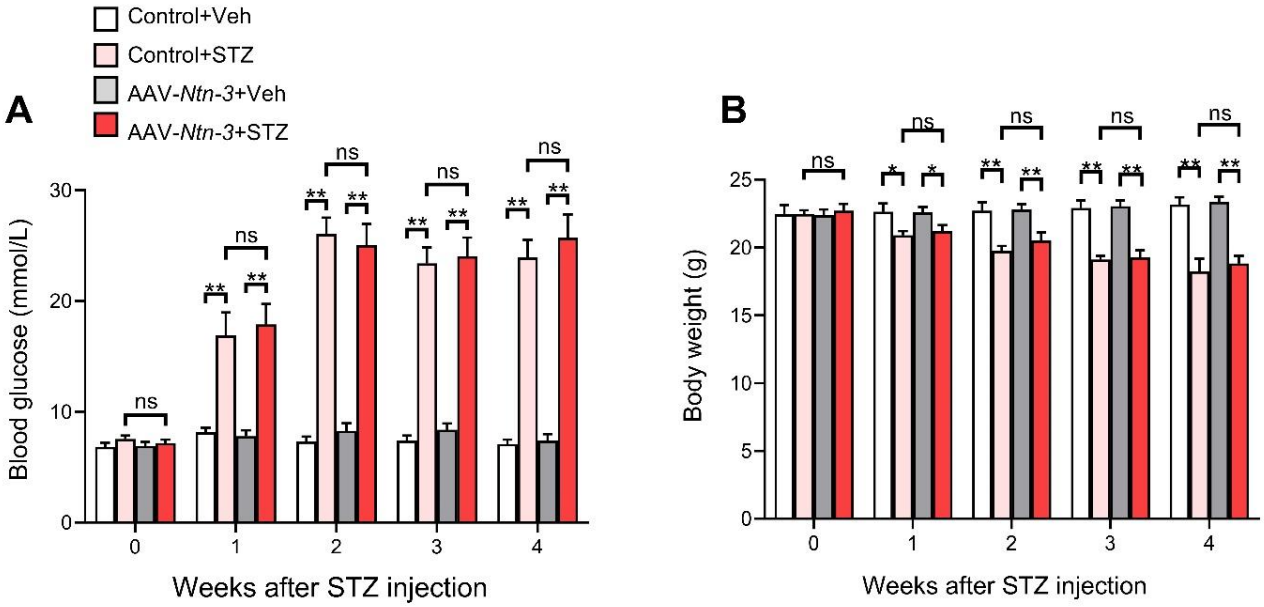

Fig. S7 Elevation of Ntn-3 expression in mouse DRGs has no effect on STZ-induced diabetic progression. **A, B** Weekly blood glucose tests (**A**) and body weight measurements (**B**) show that elevation of Ntn-3 expression by AAV-*Ntn-3* has no effect on the progression of hyperglycemia. **P* <0.05; ***P* <0.01. ns, no significant difference by two-way ANOVA. *n* = 7–13 per group.

Table S1 Primers used for qPCR

Ntn-3-F	CAAGCCCTTCTACTGCGACA
Ntn-3-R	GCAGTGACGACCAGCTGTAT
Ntn-1-F	CTCTATAAGCTATCAGGGCG
Ntn-1-R	TGGTTTGATTGCAGGTCTT
Ntn-4-F	GCAGGCTTGAATGGAGTAGC
Ntn-4-R	GCAGCGTTGCATTTATCACAC
IL-1-F	ACAACTGCACTACAGGCTCC
IL-1-R	TGGGTGTGCCGTCTTTCATT
TGF-β-F	ATGGCGCAAAACAGTCCACA
TGF-β-R	TGTAACATGCACTGGGATACCA
NGF-F	CCAGTGAAATTAGGCTCCCTG
NGF-R	CCTTGGCAAAACCTTTATTGGG
GAPDH-F	AGGTCGGTGTGAACGGATTTG
GAPDH-R	GGGGTCGTTGATGGCAACA