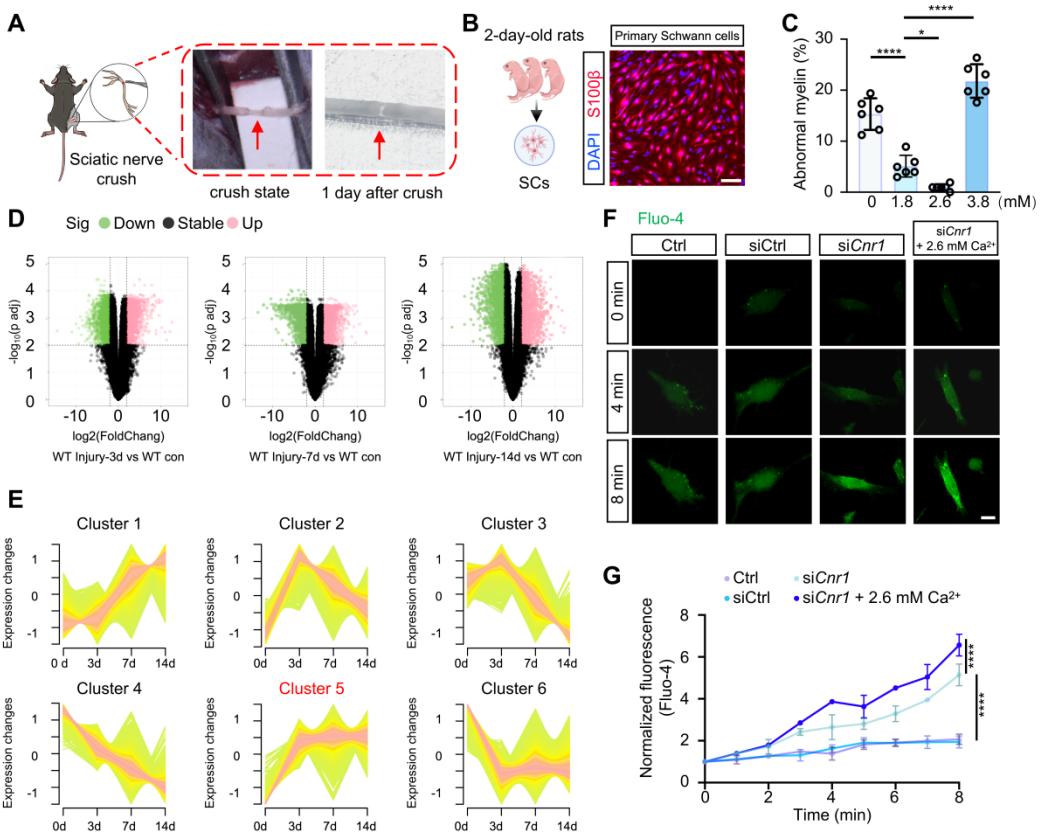
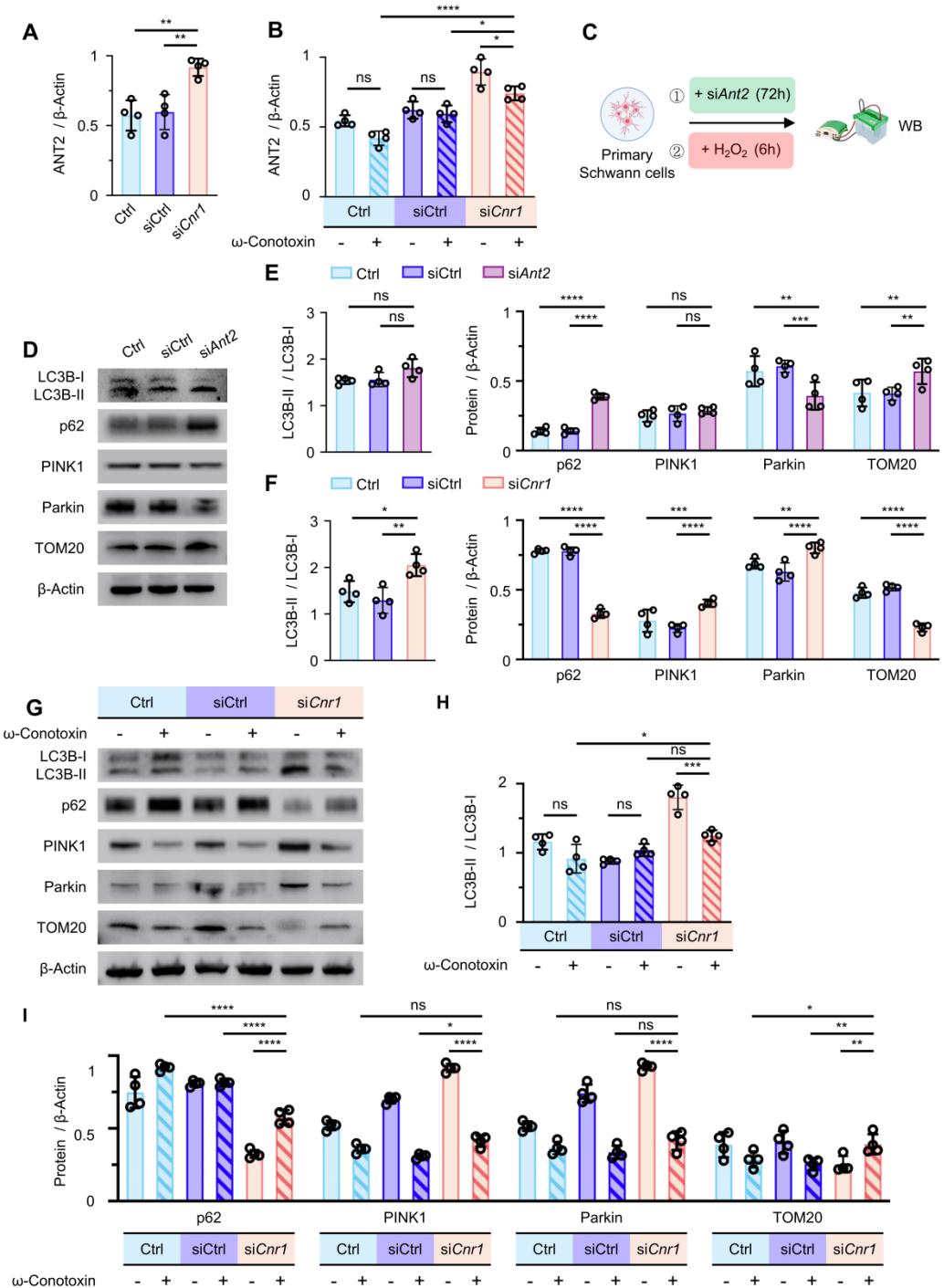


## Supplementary Figures

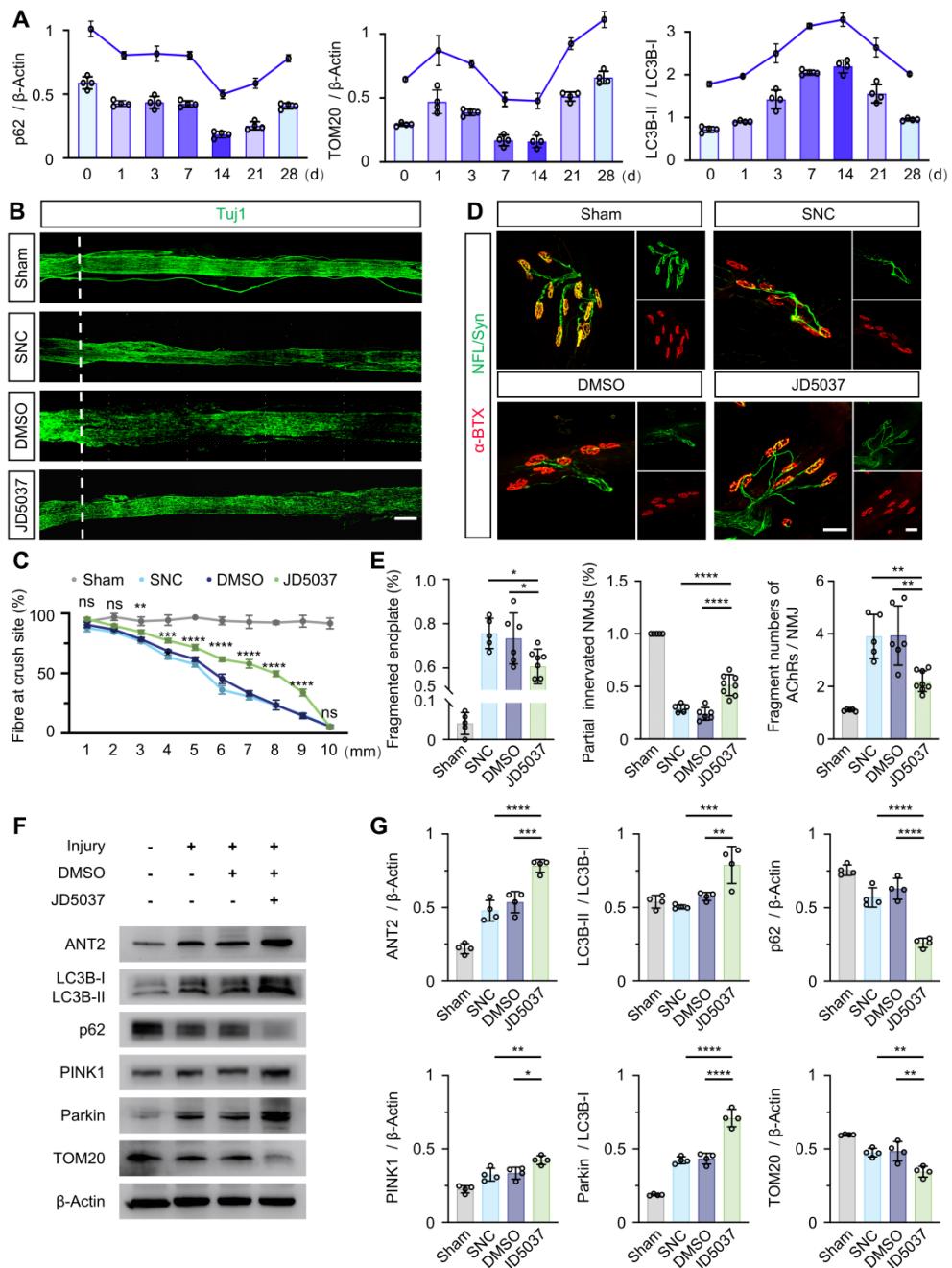


**Figure S1. Establishment of the peripheral nerve injury model, Schwann cell isolation, and transcriptomic analysis.** (A) Schematic diagram illustrating the sciatic nerve crush injury model. The left panel shows the procedure of crush injury, and the right panel shows the injured sciatic nerve at 1 day post-injury. (B) Workflow of primary SC isolation from sciatic nerves of 2-day-old rats. Representative IF image shows SCs stained with S100 $\beta$  (red) and DAPI (blue), confirming high purity. Scale bar = 10  $\mu$ m. (C) Quantification of abnormal myelination in sciatic nerve explants treated with varying extracellular Ca $^{2+}$  concentrations (0 mM, 1.8 mM, 2.6 mM, 3.8 mM). n = 6. (D) Volcano plots showing differentially expressed genes (DEGs) at 3, 7, and 14 days post-injury relative to uninjured control (WT con). Upregulated genes are shown in pink, downregulated genes in green, and non-significant genes in black. (E) Mfuzz clustering of gene expression patterns at 0, 3, 7, and 14 days post-injury. Cluster 5 (highlighted in red) displays strong association with injury-induced calcium changes. (F) Fluo-4 fluorescence imaging of intracellular Ca $^{2+}$  levels in CB1R-knockdown Schwann cells (siCnr1), under conditions including Ctrl, siCtrl, siCnr1, and siCnr1 + 2.6 mM Ca $^{2+}$ , at 0, 4, and 8 min. Scale bar = 10  $\mu$ m. (G) Quantification of Fluo-4 fluorescence intensity indicating intracellular Ca $^{2+}$  dynamics across experimental groups. n = 3. All data are presented as mean  $\pm$  SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test (C), and two-way ANOVA followed by Bonferroni's post hoc test (G). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.



**Figure S2. CB1R regulates ANT2 expression and PINK1/Parkin-mediated mitophagy in Schwann cells. (A, B)**

Quantification of relative expression of ANT2 in Figure 4H and 4I. n = 4. (C) Primary Schwann cells were treated with si*Ant2* for 72 h, followed by H<sub>2</sub>O<sub>2</sub> for 6 h, and then subjected to western blot analysis. (D) Western blot analysis of mitophagy-related proteins (LC3B, p62, PINK1, Parkin, and TOM20) after silencing of *Ant2*. (E) Quantification of relative expression of LC3BII/I, p62, PINK1 and Parkin after silencing of *Ant2*. n = 4. (F) Quantification of relative expression of LC3BII/I, p62, PINK1 and Parkin after silencing of *Cnr1* as shown in Figure 5I. n = 4. (G) Western blot analysis of mitophagy-related proteins after treatment with  $\omega$ -Conotoxin or saline in indicated groups. (H, I) Quantification of relative expression of LC3BII/I, p62, PINK1, Parkin, and TOM20 in (G). n = 4. All data are presented as mean  $\pm$  SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001, \*\*\*\* $p$  < 0.0001; ns, not significant.



**Figure S3. JD5037 promotes axonal regeneration, neuromuscular junction repair, and PINK1/Parkin-mediated mitophagy.** (A) Quantitative analysis of relative expression of p62, TOM20 and LC3BII/I in the distal sciatic nerve stump at 0, 1, 3, 7, 14, 21, and 28 days post-injury, as shown in Figure 6B. (B) IF staining of regenerating axons in the distal sciatic nerve labeled with Tuj1 (green). Scale bar = 1 mm. (C) Quantification of axon regeneration at 3–9 mm distal to the injury site at indicated groups. n = 4. (D) IF staining of NMJs with NFL/Syn (green) marking nerve terminals and  $\alpha$ -BTX (red) labeling postsynaptic acetylcholine receptors (AChRs). Scale bar = 20  $\mu$ m. (E) Quantification of fragmented endplates (left), partially innervated NMJs (middle), and fragmented AChRs per NMJ (right) in each group. n = 4. (F) Western blot analyzed the expression of ANT2 and mitophagy-related proteins (LC3B, p62, PINK1, Parkin, TOM20) in sciatic nerve tissues isolated from mice with or without JD5037 administration. (G) Quantification of relative expression of ANT2, LC3BII/I, p62, PINK1, Parkin and TOM20 in (F). n = 4. All data are presented as mean  $\pm$  SD. Statistical analysis was performed using two-way ANOVA followed by Bonferroni's post hoc test (C), and one-way ANOVA followed by Tukey's post hoc test (A, E, G). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001; ns, not significant.

## Supplementary Tables

**Table S1**

( [Ca<sup>2+</sup>]: Calcium quantification; WB: Western blot )

Category	Patient ID	Sex (M/F)	Age (years)	Sampling Site	Time Since Injury	Assay
Congenital Polydactyly (Con)	P1	M	16	Right digital nerve	–	[Ca <sup>2+</sup> ] & WB
	P2	M	21	Right digital nerve	–	[Ca <sup>2+</sup> ] & WB
	P3	M	18	Left digital nerve	–	[Ca <sup>2+</sup> ] & WB
Severe Peripheral Nerve Injury (PNI)	P4	M	31	Right digital nerve	5.5 h	[Ca <sup>2+</sup> ] & WB
	P5	M	22	Right ulnar nerve	6 h	[Ca <sup>2+</sup> ] & WB
	P6	M	15	Right digital nerve	3 h	[Ca <sup>2+</sup> ] & WB

**Table S2**

Antibodies	Source	Identifier
Mouse Anti-S100β	Sigma	Cat#S2532
Mouse Anti-Tuj1	Abcam	Cat#ab78078
Rabbit Anti-Synapsin-1	Cell Signaling Technology	Cat#5297
Rabbit Anti-NFL	Cell Signaling Technology	Cat#2837
Mouse Anti-β-Actin	Transgen	Cat#HC201
Rabbit Anti-Cannabinoid Receptor 1	Abcam	Cat#ab3558
Rabbit Anti-NeuN	Cell Signaling Technology	Cat#24307
Rabbit Anti-ANT2	Proteintech	Cat#83104-1-RR
Monoclonal Anti-Thy1.1	Sigma	Cat#M7898
Rabbit Anti-PINK1	Proteintech	Cat#23274-1-AP
Rabbit Anti-PARK2/Parkin	Proteintech	Cat#14060-1-AP
Rabbit Anti-TOM20	Proteintech	Cat#11802-1-AP
Mouse Anti-LC3B	Cell Signaling Technology	Cat#83506
Rabbit Anti-p62(SQSTM1)	MBL International	Cat#PM045
Alexa Fluor 488 Donkey Anti-Mouse	Invitrogen	Cat#A32766
Alexa Fluor 488 Donkey Anti-Rabbit	Invitrogen	Cat#A11008
Alexa Fluor 546 Donkey Anti-Mouse	Invitrogen	Cat#A10040
Alexa Fluor 546 Donkey Anti-Rabbit	Invitrogen	Cat#A11035
Alexa Fluor 647 Goat Anti-Mouse	Invitrogen	Cat#A31571
HRP-Donkey-anti-rabbit	Beyotime	Cat#A0208
HRP-Donkey-anti-mouse	Beyotime	Cat#A0216

**Table S3**

<b>siRNA sequences</b>		
CNR1	Sense	5'-GGGAAGAUGAACACAAGCUUAUC-3'
	Anti-sense	5'-UAAGCUUGUUCAUCUUCCGA-3'
ANT2	Sense	5'-CCUUCAAAGAUAAAUCACAAGC-3'
	Anti-sense	5'-UUGUAUUUAUCUUUGAAGGCG-3'
Negative	Sense	5'-UUCUCCGAACGUGUCACGUTT-3'
	Anti-sense	5'-ACGUGACACGUUCGGAGAATT-3'

**Table S4**

<b>Chemicals, peptides, recombinant proteins, and Reagent Resource</b>		
JD5037	MedChemExpress	Cat#HY-18697
Tris HCl	Beyotime	Cat#ST772
BAPTA	MedChemExpress	Cat# HY-100168
DMEM/F12	Bioscience	Cat#3130-0500
CaCl <sub>2</sub>	Sigma	Cat#C5670
Corn Oil	MedChemExpress	Cat#HY-Y1888
Opti-MEM	Gibco	Cat#31985-070
Tamoxifen	MedChemExpress	Cat#HY-13757A
Mdivi-1	MedChemExpress	Cat#HY-15886
Cy3- $\alpha$ -Bungarotoxin	Biotium	Cat#90-1022
$\omega$ -Conotoxin GVIA/MVIIC TFA salt	Aladdin	Cat#274837
Rhod-2 AM	Abcam	Cat#142780
DAPI	Genview	Cat#GD3410
Hochest 3342	Solarbio	Cat#23491
Poly-L-lysine Hydrobromide	Sigma	Cat#P8954
Forskolin	Sigma	Cat#F6886
Recombinant Human NRG1- $\beta$ 1	R&D	Cat#377-HB-050
Collagenase from Clostridium histolyticum	Sigma	Cat#C0130
Complement, Rabbit Serum	Calbiochem	Cat#3386909
Fetal Bovine Serum	Sigma	Cat#F8318
1XPBS	Bioss	Cat#C01-01001
1XTBST	Servicebio	Cat#G2150
NGF	Sino Biological	Cat#11050-HNAC
Protease inhibitor mixture	Sangon Biotech	Cat# C50008
Poly-L-ornithine hydrobromide	Sigma	Cat#P3655
Lipofectamine 3000	Invitrogen	Cat#L3000150

Bovine serum Albumin	Genview	Cat#FA016
Donkey serum	Servicebio	Cat#G1217
Goat serum	Servicebio	Cat#G1208
DMSO	Sigma	Cat#D2650
Penicillin-Streptomycin Solution	Beyotime	Cat#C0222
Multicolor Prestained Protein Ladder	EpiZyme	Cat#WJ103
Tween-20	Ding Guo	Cat#DH358-3
Tween-80	Ding Guo	Cat#DH360-3
PEG-300	Shyuanye	Cat#25322-68-3
TritonX-100	Ding Guo	Cat#DH351-4
Western Blot Antibody Stripping Solution	Epizyme	Cat#PS107

**Table S5**

<b>Critical commercial assays</b>		
Calcium Colorimetric Assay Kit	Beyotime	Cat#S1063S
Fluo-4 Calcium Fluorometric Assay Kit	Elab Science	Cat#E-BC-F100
ROS Assay Kit	Beyotime	Cat#S0033S
Mitochondrial Permeability Transition Pore Assay Kit or MPTP Assay Kit	Beyotime	Cat#C2009S
TB Green™ Premix Ex Taq™	TaKaRa	Cat#RR420A
CCK-8 Assay Kit	DoJinDo	Cat#CK04
JC-1	Beyotime	Cat#C2006
Tissue or Cell Total Protein Extraction Kit	Sangon Biotech	Cat#C510003