

Mitochondrial entry into damaged axons is restricted by impaired disassembly of the axon initial segment

Sumiko Kiryu-Seo, Reika Matsushita, Yoshitaka Tashiro, Takeshi Yoshimura, Yohei Iguchi, Masahisa Katsuno, Ryosuke Takahashi, Hiroshi Kiyama

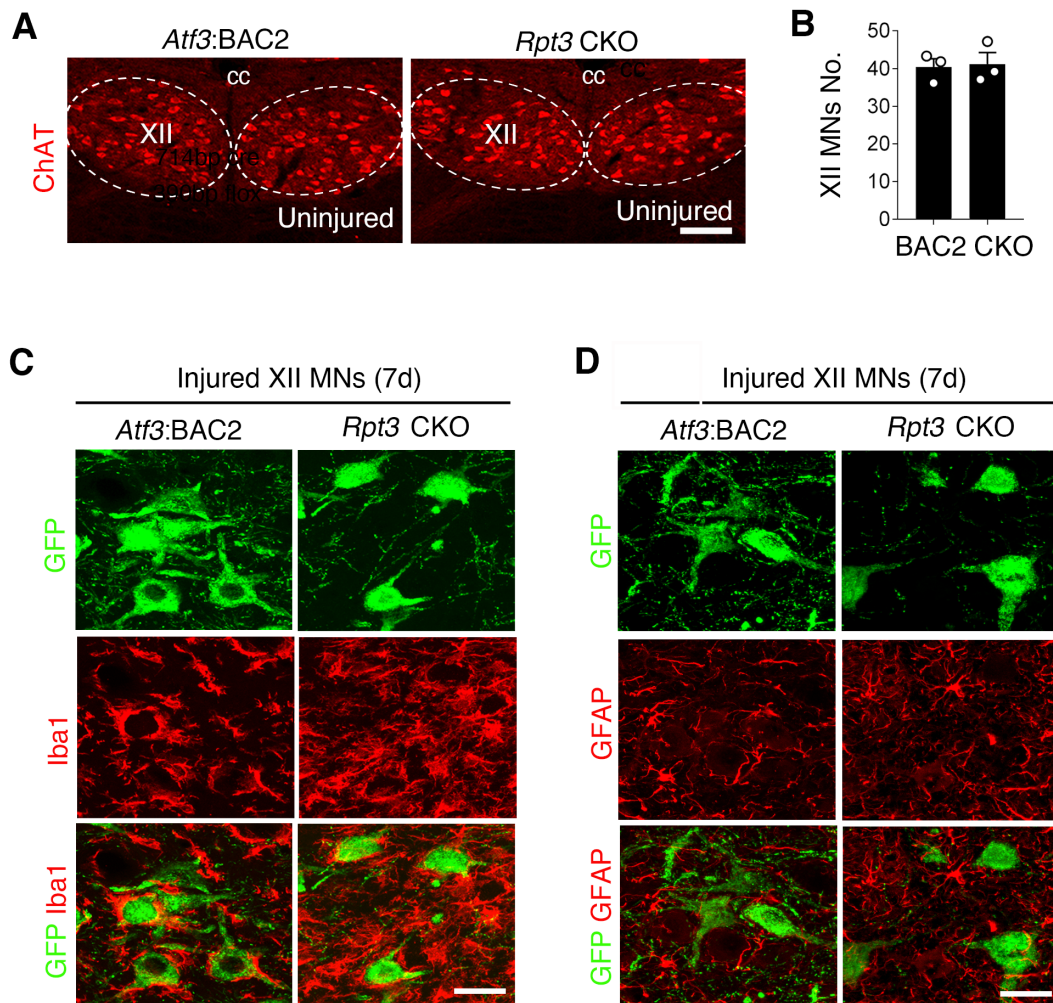
Table of contents

Appendix Figure S1: Hypoglossal motor neurons and glial cells of *Atf3*:BAC2 Tg and *Rpt3* CKO mice before and after nerve injury

Appendix Figure S2: The expression of GFP and AnkG in spinal motor neurons of *Atf3*:BAC2 Tg and *Rpt3* CKO mice after sciatic nerve injury

Appendix Figure S3: The GFP-labeled hypoglossal motor neurons in *Atf3*:BAC;SOD1 mouse

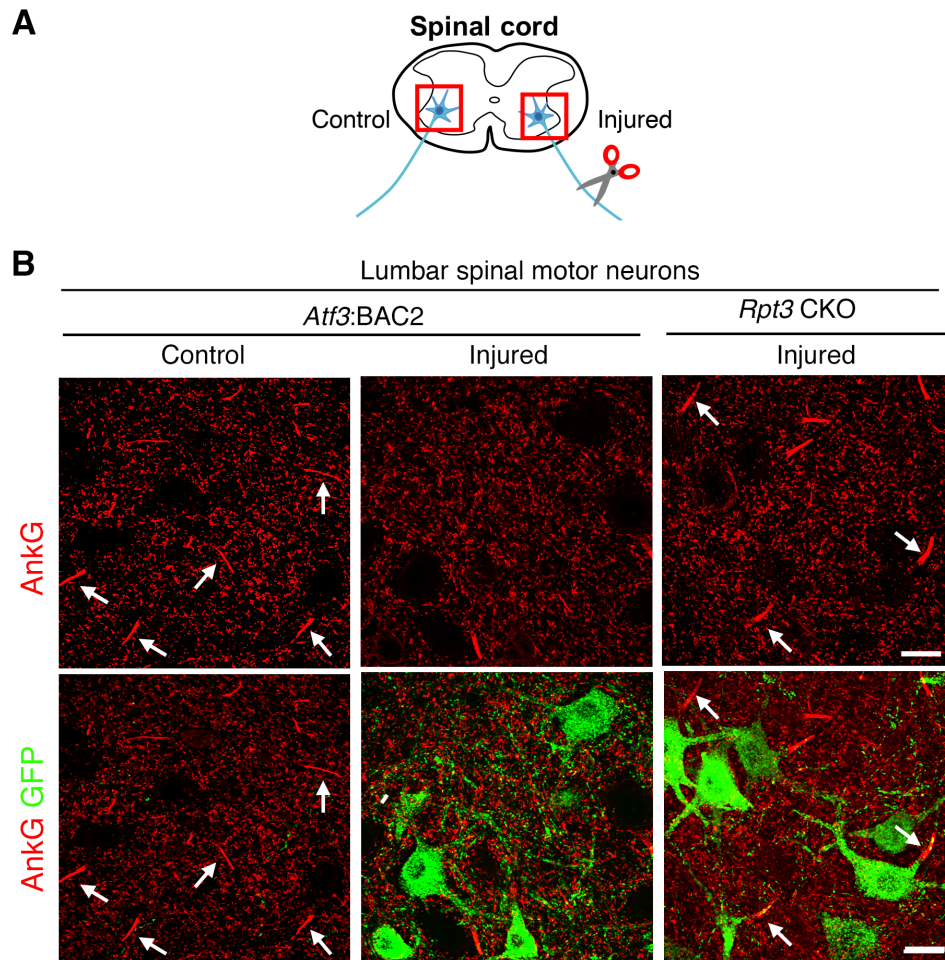
Appendix Table S1 : Primer list



Appendix Figure S1: Hypoglossal motor neurons and glial cells of *Atf3*:BAC2 Tg and *Rpt3* CKO mice before and after nerve injury.

- A.** Uninjured hypoglossal nucleus immunostained by anti-ChAT antibody. XII, hypoglossal nucleus; cc, central canal; MNs, motor neurons. Dashed lines outline hypoglossal nucleus
- B.** The graph showing the number of motor neurons in unilateral hypoglossal nucleus per section. Data are shown as the mean \pm s.e.m. and no significance, determined by Student's *t*-test ($n=3$ mice per group).
- C.** Injured hypoglossal nucleus at 7 days after injury, immunostained by anti-GFP and -Iba1 antibodies. Microglial cells are more activated around injured hypoglossal motor neurons (MNs) of *Rpt3* CKO mouse.
- D.** Injured hypoglossal nucleus at 7 days after injury, immunostained by anti-GFP and -GFAP antibodies.

Scale bars, 150 μ m in (A) and 30 μ m in (C and D).

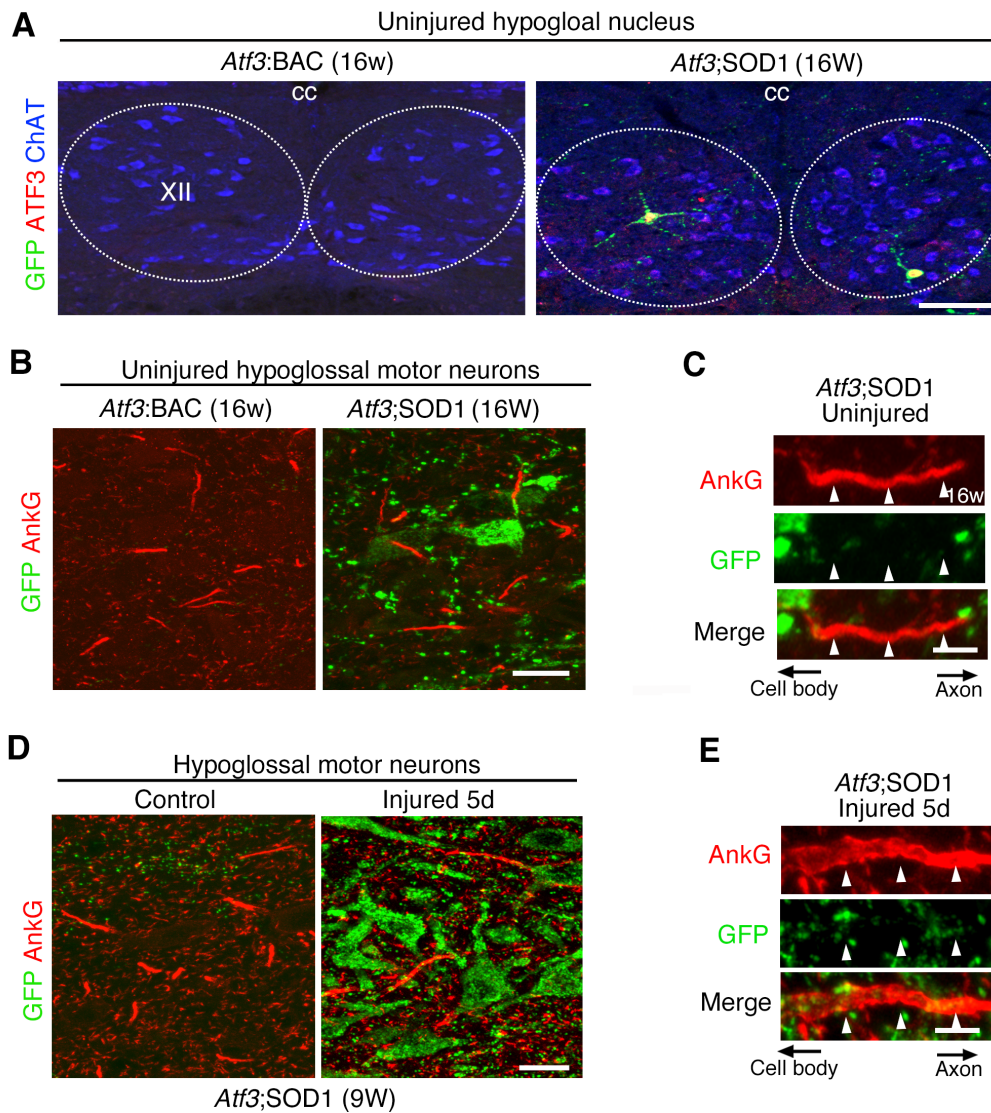


Appendix Figure S2: The expression of GFP and AnkG in spinal motor neurons of *Atf3*:BAC2 Tg and *Rpt3* CKO mice after sciatic nerve injury.

A. Schematic diagram of sciatic nerve injury model. The area surrounded by red box is observed in (B).

B. Spinal motor neurons of *Atf3*:BAC2 Tg and *Rpt3* CKO mice before and at 5 days after injury, immunostained for AnkG and GFP. Arrows indicate the AIS stained by AnkG.

Scale bar, 30 μ m.



Appendix Figure S3: The GFP-labeled hypoglossal motor neurons in *Atf3*;SOD1 mouse.

- A.** Uninjured hypoglossal motor neurons of *Atf3*;SOD1 mouse at 16 weeks old, immunostained by GFP, ATF3 and ChAT. Dashed lines outline hypoglossal nucleus (XII). cc, central canal.
- B.** Uninjured hypoglossal motor neurons of *Atf3*;SOD1 mouse at 16 weeks old, immunostained by AnkG- and GFP- antibodies.
- C.** The localization of the AIS and mitochondria in uninjured GFP-positive motor neurons of *Atf3*;SOD1 mouse at 16 weeks old.
- D.** Hypoglossal motor neurons of *Atf3*;SOD1 mouse (9 weeks old) at 5 days after injury, immunostained by AnkG- and GFP- antibodies.
- E.** The localization of AnkG and GFP-labeled mitochondria in the AIS of uninjured and injured hypoglossal motor neurons of *Atf3*;SOD1 mouse at 9 weeks old. Arrowheads indicate the AIS.
- Scale bars, 100 μ m in (A), 20 μ m in (B and D), 5 μ m in (C and E).

Appendix Table S1: Primer list

Oligonucleotides	
Sprr1a-S	TTGTGCCCCCAAACCAAG
Sprr1a-A	GGCTCTGGTGCCTTAGGTTG
Atf3-S	ATGTCAGTCACCAAGTCTGAGGC
Atf3-A	TGGATAAAGAGGTTCTCTCGTCTTCC
Gadd45a-S	CTGCTGCTACTGGAGAACGAC
Gadd45a-A	CGACTTTCCCGGCAAAAACAAA
Cd44-S	CACCATTGCCTCAACTGTGC
Cd44-A	TTGTGGGCTCCTGAGTCTGA
Fos-S	CCATGATGTTCTCGGGTTTC
Fos-A	TGTCACCGTGGGGATAAAGT
Gap43-S	TGGTGTCAAGCCGGAAGATAA
Gap43-A	GCTGGTGCATCACCCCTTCT
Sox11-S	CGACGACCTCATGTTTCGACC
Sox11-A	GACAGGGATAGGTTCCCCG
Smad1-S	GCTTCGTGAAGGGTTGGGG
Smad1-A	CGGATGAAATAGGATTGTGGGG
Dine-S	GTCTCTGAACTACGGGGGTATTGGCAC
Dine-A	GTAGGCCAGCTTGAGGCCTCCCATGTC
Rbfox3-S	ATCGTAGAGGGACGGAAAATTGA
Rbfox3-A	GTTCCCAGGCTTCTTATTGGTC
Gapdh-S	GGTGAAGGTCGGTGTGAACG
Gapdh-A	CGTGAGTGGAGTCATACTGGA
Pan AnkG-S	CTAGAGTCCCCAGCGCAAGCTCG
Pan AnkG-A	GCCTTTGCTTCTGGAATGACTTCCG
Giant AnkG-S	CGAGTTCACATCTAAGACACCA
Giant AnkG-A	TCACGATCTGTTTCCTTCTCC
Chat-S	GACCAGCTAAGGTTTGCAGC
Chat-A	CAGGAAGCCGGTATGATGAGA
Genotyping primers	
primer (1)	CAATAAGATGGAGTACAACACTACAACGC
primer (2)	GACTCTTTCCACAACACTATCCAACCTCAC
primer (3)	TGAGCTGTGTATCAAGGTCC
primer (4)	TAGAAGCTGCCTAAGGCACA
primer (5)	TGCAATCCCTTGTGAGGAGA