Expanded View Figures

Figure EV1. Injury-induced *Atf3*:BAC2 Tg mouse and *Rpt3* CKO mouse.

A Time-dependent GFP expression in injured hypoglossal motor neurons of *Atf3*:BAC2 Tg mouse at 20, 36, and 48 h after injury.
B Representative image stained by ATF3 and GFP antibodies at 5 days following hypoglossal nerve injury of *Atf3*:BAC2 Tg mouse.
C The graph showing the percentage of GFP (+) or GFP (−) hypoglossal MNs in ATF3(+) MNs of control and injured sides at 5 days after injury of *Atf3*:BAC2 Tg mouse (n = 3 mice).
D Schematic of the breeding strategy to generate injury-induced *Rpt3* CKO mouse.
E Diagram for *Atf3*:BAC2 transgene and *Rpt3* gene. Arrows indicate the location of the genotyping primers (1)–(5) for *Atf3*:BAC2 Tg and *Rpt3* CKO mouse. The gray triangles show loxP sites.
F Representative genotyping results using primers (1)–(4) to obtain *Rpt3* CKO mouse. PCR products on the gel are amplified using indicated primer pairs.
G Representative genotyping using tail samples of adult *Rpt3* CKO mice from #1 to #4 shows that the floxed *Rpt3* alleles are not deleted.

Data information: Dashed lines outline hypoglossal nucleus in (A and B). XII, hypoglossal nucleus; cc, central canal. Scale bars, 150 μm in (A and B).
Source data are available online for this figure.

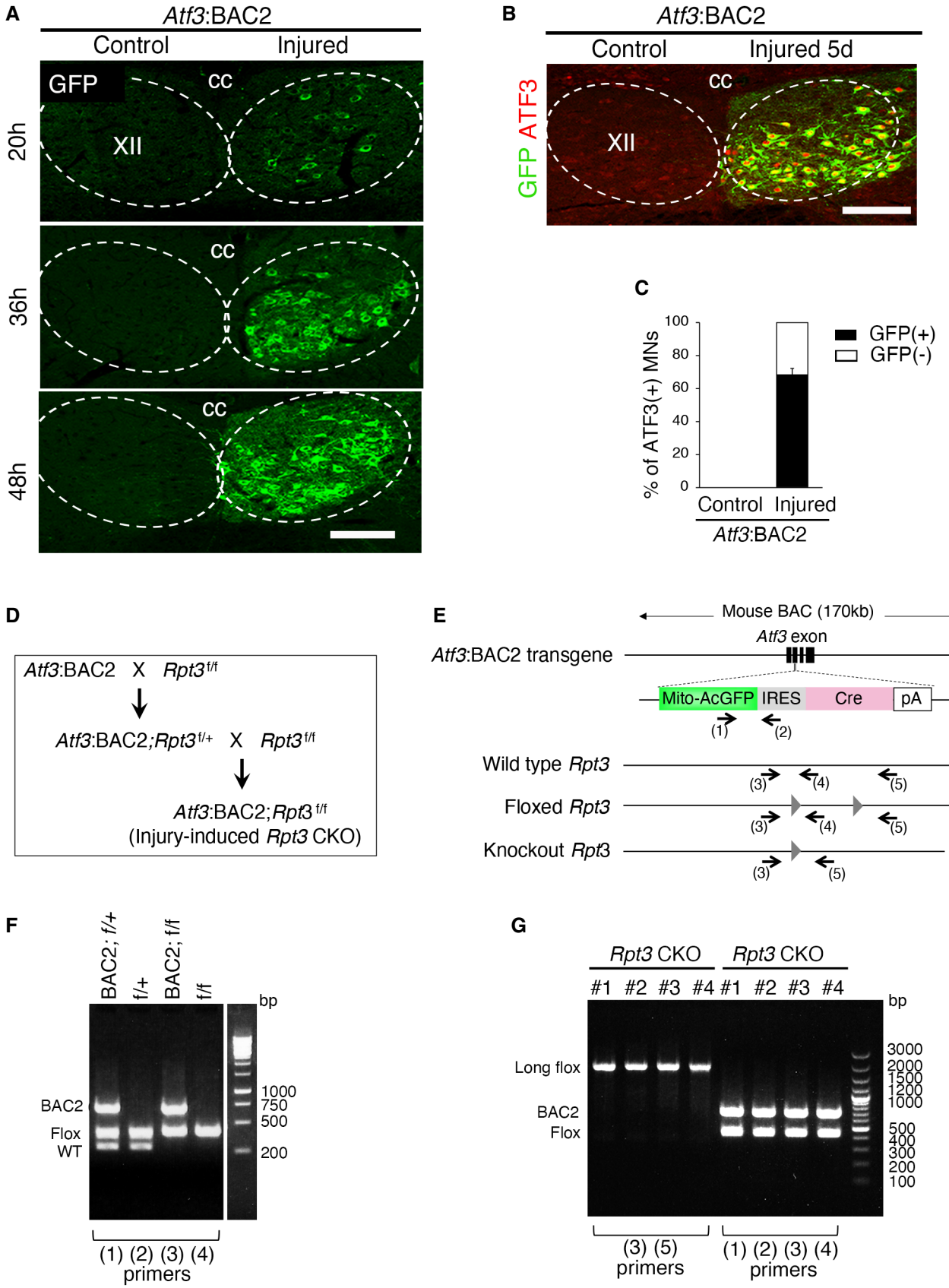

Figure EV1.

Figure EV2. GFP-labeled mitochondria in injured hypoglossal motor neurons of *Atf3*:BAC2 Tg and *Rpt3* CKO mice.

A Whole-mount observation of hypoglossal nerve of *Atf3*:BAC2 Tg mouse without staining.
B Immunostaining of hypoglossal nerve at 5 and 10 days after injury, using anti-GFP, Lamp1, and neurofilament (NF) antibodies. Arrows show Lamp1 positive signals in injured nerve.
C Graph shows mitochondria number in hypoglossal (XII) axon at 5 days and 10 days after injury ($n = 4$ mice).
D Graph shows lysosome number in hypoglossal (XII) axon at 5 days and 10 days after injury ($n = 4$ mice).
E The GFP expression of injured hypoglossal motor neurons at 5 days following injury. XII, hypoglossal nucleus; cc, central canal.
F The percentage of GFP-positive injured motor neurons compared with ChAT-positive uninjured motor neurons ($n = 3$ mice per group).
G Super-resolution images of GFP-stained mitochondria in injured hypoglossal motor neuron. Stacked image of 2 μm thickness.
H Single layer image corresponding to boxed area in (G) and plot profile of the fluorescence intensity corresponding to the line in the image.

Data information: Dashed lines outline axon in (A) and hypoglossal nucleus in (E). Data are shown as the mean ± s.e.m. *$P = 0.0247$, **$P = 0.0011$ in (C), ***$P < 0.0001$ in (D), $P = 0.7854$ in (F), determined by one-way ANOVA followed by *Tukey post hoc* test. Scale bars, 300 μm in (A; left two panels) and 10 μm in (A; right panel), 5 μm in (B), 150 μm in (E), 4 μm in (G), and 2 μm in (H).
Source data are available online for this figure.

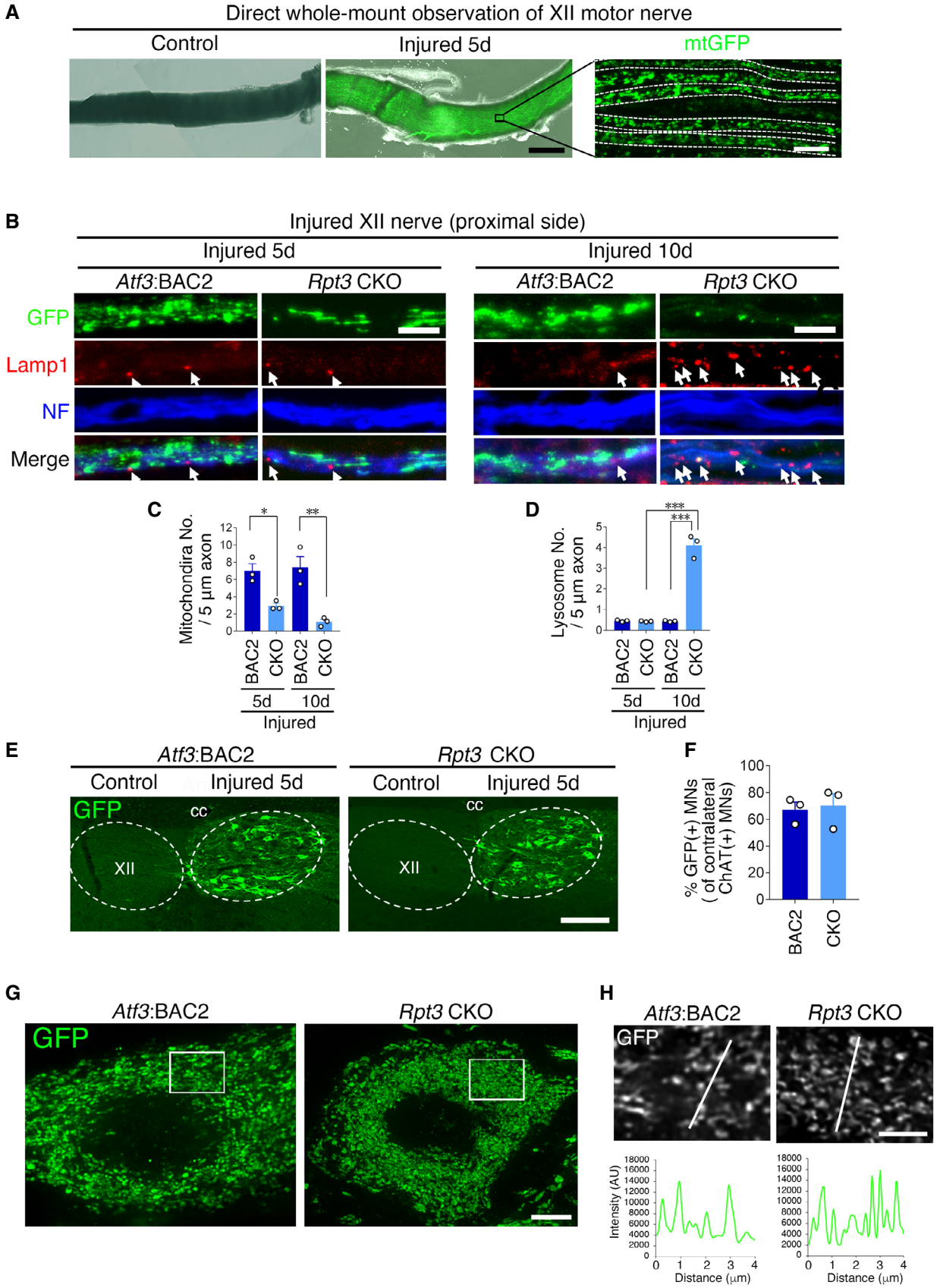

Figure EV2.

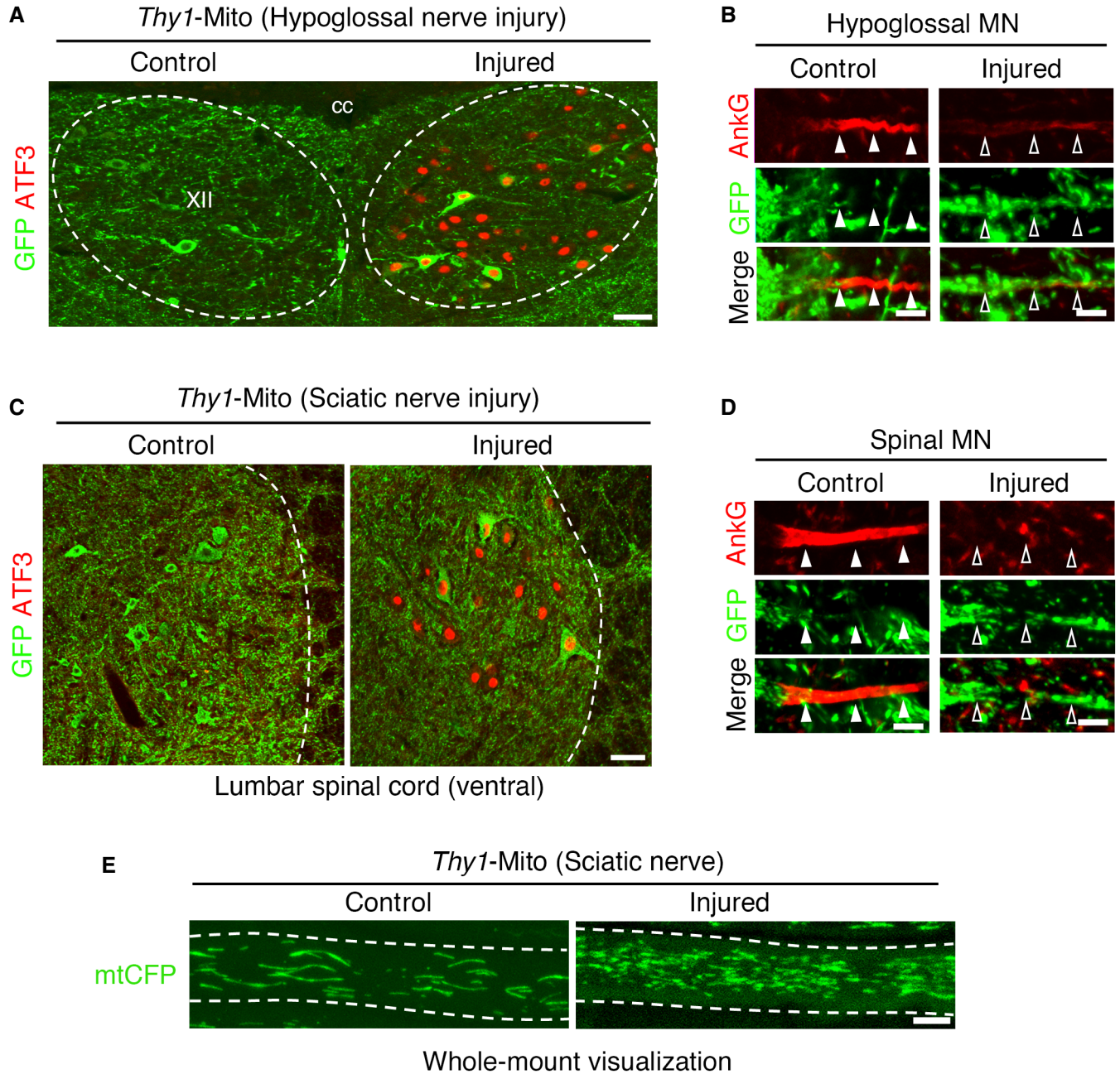

Figure EV3. Expression of CFP-labeled mitochondria in *Thy1*-mito-CFP Tg (*Thy1*-Mito) mouse before and after motor nerve injury.

A Immunostaining of hypoglossal nucleus (XII) of *Thy1*-Mito mouse using ATF3 (red) and GFP (green) antibodies at 5 days following hypoglossal nerve injury. XII, hypoglossal nucleus; cc, central canal.

B The localization of AnkG and CFP-labeled mitochondria in the AIS of uninjured and injured hypoglossal motor neurons of *Thy1*-Mito mouse.

C Immunostaining of the lumbar spinal motor neurons of *Thy1*-Mito mouse using ATF3 (red) and GFP (green) antibodies at 5 days following sciatic nerve injury.

D The localization of AnkG and CFP-labeled mitochondria in the AIS of uninjured and injured spinal motor neurons of *Thy*1-Mito mouse.

E Whole-mount observation of CFP-labeled mitochondria (mtCFP) in sciatic nerve.

Data information: Dashed lines outline hypoglossal nucleus (A), spinal cord (C) and axon (E). Closed arrowheads indicate the AIS, while open arrowheads indicate the region where the AIS is disrupted in (B and D). Scale bars, 50 μm (A and C) and 5 μm (B, D and E).

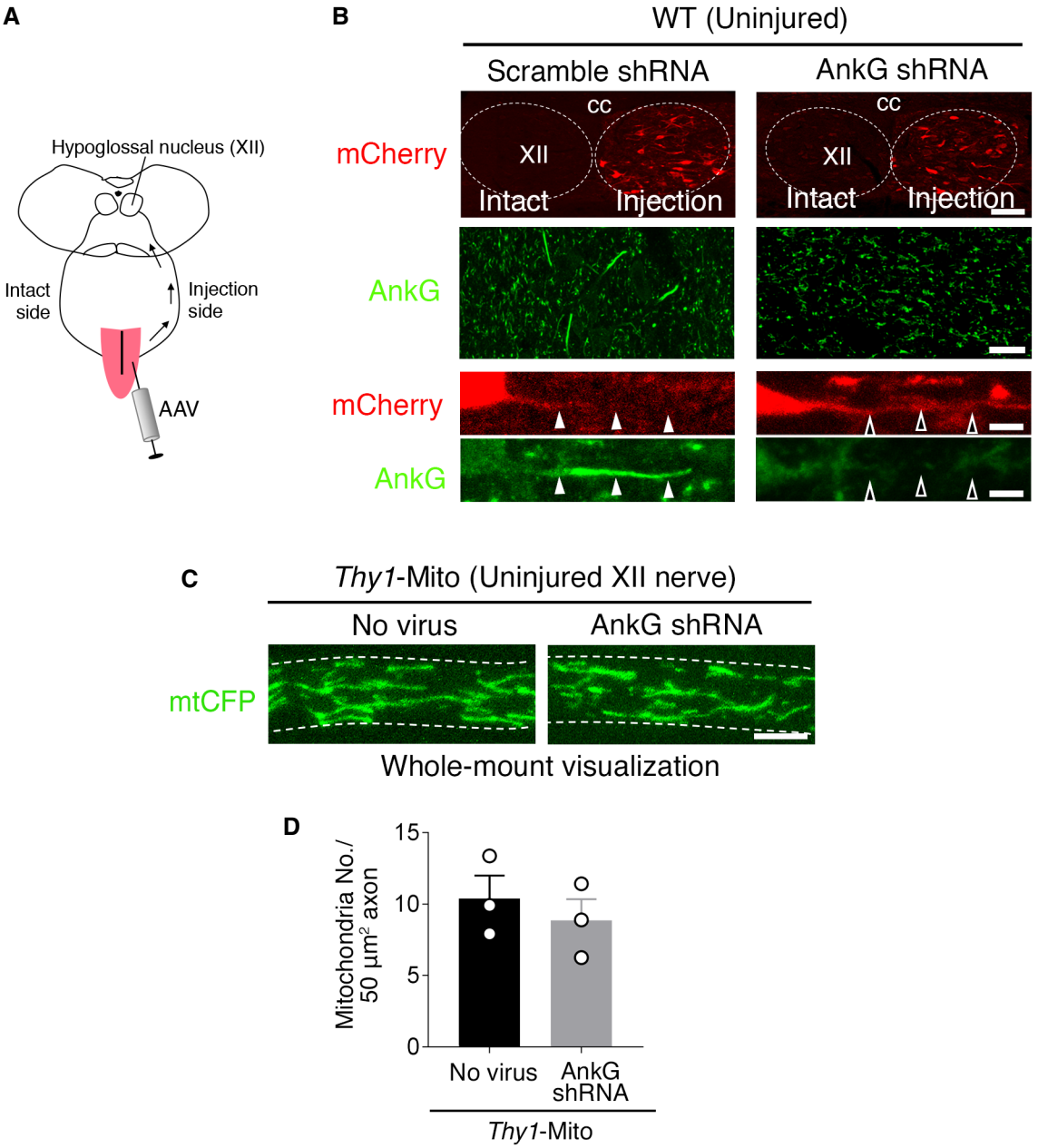

Figure EV4. Suppression of AnkG in uninjured hypoglossal motor neurons.

A Schematic diagram for AAV injection to tongue.

B Hypoglossal motor neurons labeled by mCherry (red) in injection side of AAVs. The decrease of AnkG (green) immunoreactivity is observed after the infection of AAV-AnkG shRNA but not AAV-scramble shRNA. Dashed lines show hypoglossal nucleus (XII). cc, central canal. Closed arrowheads indicate the AIS, while open arrowheads indicate the region where the AIS is disrupted.

C CFP-labeled mitochondria in uninjured hypoglossal nerve of *Thy1*-Mito mouse after the infection of AAV-AnkG shRNA to tongue. Dashed lines show axon.

D Graph shows the number of CFP-labeled mitochondria in uninjured hypoglossal (XII) axon of *Thy1*-Mito mouse before and after AAV infection. Data are shown as the mean ± s.e.m. (no significant difference ($P = 0.519$), determined by two-tailed Student's *t*-test, $n = 3$ mice).

Data information: Scale bars = 100 µm (top), 20 µm (middle), and 5 µm (bottom) in (B) and 5 µm in (C).
Source data are available online for this figure.

Figure EV5. Characterization of *Atf3*:BAC;SOD1^{G93A} (*Atf3*;SOD1) mouse.

A Schematic of the breeding strategy to generate *Atf3*;SOD1 mouse.
B Kaplan–Meier survival analysis (long-rank test, $P = 0.4674$). The *Atf3*:BAC transgene has no significant effect on life span of SOD1^{G93A}.
C Representative image of spinal motor neurons at 45, 70, and 130 days, immunostained with ChAT.
D Quantification of age-dependent ChAT-positive MN in the ventral horn of lumbar spinal cord. Data are shown as the mean ± s.e.m., no significant differences at same age, determined by unpaired *t*-test, $n = 3$ mice per group.
E The expression of GFP and ChAT in spinal motor neurons of *Atf3*;SOD1 at 45, 60, 90 and 130 days.
F The percentage (%) of GFP-positive MNs (black bar) of ChAT-positive MNs in *Atf3*; SOD1 mice. Data are shown as the mean ± s.e.m. $n = 5$ mice at each age.
G Immunostaining of spinal motor neurons in *Atf3*;SOD1 at P65 using GFP, MMP-9, OPN, and ChAT antibodies. Arrows denote GFP-positive motor neurons co-localized with MMP-9 or OPN.

Data information: Scale bar, 100 μm in (C), 50 μm in (E), and 20 μm in (G).
Source data are available online for this figure.

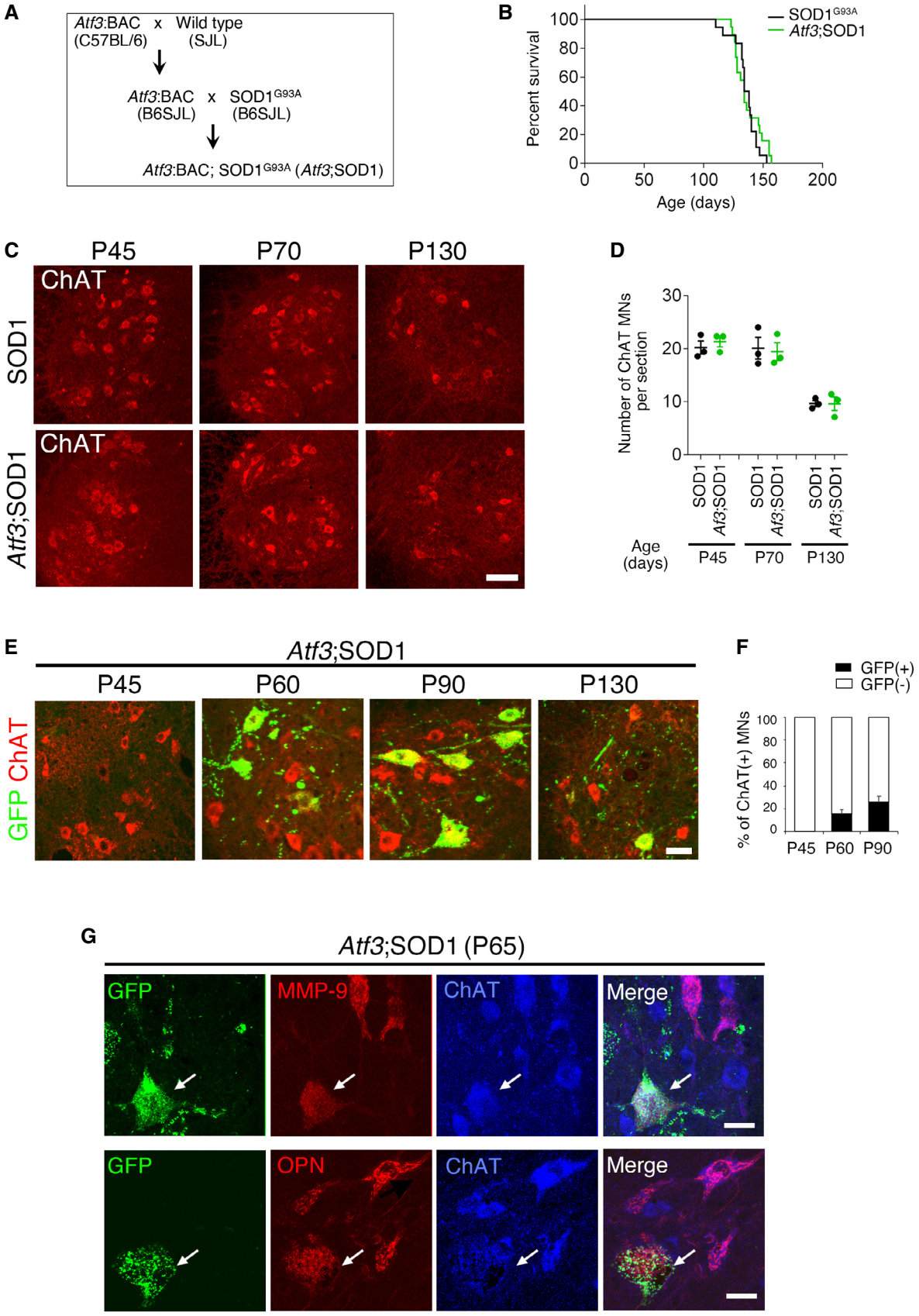

Figure EV5.