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Swimming exercise promotes post-injury axon regeneration and functional restoration through AMPK

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Keywords: *C. elegans*, Axotomy, PLM neuron, Swimming Exercise, Axon Regeneration, AAK-2

Abstract

Restoration of lost function following a nervous system injury is limited in adulthood as the regenerative capacity of nervous system declines with age. Pharmacological approaches have not been very successful in alleviating the consequences of nervous system injury. On the contrary, physical activity and rehabilitation interventions are often beneficial to improve the health conditions in the patients with neuronal injuries. Using touch neuron circuit of *Caenorhabditis elegans*, we investigated the role of physical exercise in the improvement of functional restoration after axotomy. We found that a swimming session of 90 minutes following the axotomy of Posterior Lateral Microtubule (PLM) neuron can improve functional recovery in larval and adult stage animals. In older age, multiple exercise sessions were required to enhance the functional recovery. Genetic analysis of axon regeneration mutants showed that exercise-mediated enhancement of functional recovery depends on the ability of axon to regenerate. Exercise promotes early initiation of regrowth, self-fusion of proximal and distal ends, as well as post-regrowth enhancement of function. We further found that the swimming exercise promotes axon regeneration through the activity of cellular energy sensor AAK-2/AMPK in both muscle and neuron. Our study established a paradigm where systemic effects of exercise on functional regeneration could be addressed at the single neuron level.

82

83 **Significance Statement**

84 Accelerating axonal regeneration and subsequent functional restoration is a major
85 challenge to the people with nervous system injury. Research on rodents and humans
86 suggests that rehabilitation therapy helps regain the lost function after neuronal injury.
87 The nematode *C. elegans* provides an advantage to investigate the role of exercise in
88 facilitating the axonal regeneration at the level of single neuron. Our study shows that
89 swimming exercise promotes functional restoration via structural and functional changes
90 in injured mechanosensory neuron. The benefit of exercise in regeneration depends on
91 the metabolic energy sensor AAK-2/AMPK. This study provides a molecular perspective
92 to exercise-mediated enhancement of axon regeneration.

93

94

Introduction

Neuronal injuries are accompanied by physical disruption of the axons, which leads to the loss of sensory or motor function (Johnson et al., 2013; Yang et al., 2015). Central nervous system has limited capacity to regenerate due to intrinsic failure and several inhibitory factors in the environment (Huebner and Strittmatter, 2009; He and Jin, 2016; van Niekerk et al., 2016). In case of peripheral nervous system, regeneration from the injured proximal stump and subsequent growth towards the target tissues can lead to functional restoration (He and Jin, 2016; Laha et al., 2017). However, this capability declines with age, resulting in partial or no functional restoration (Verdú et al., 2000; Geoffroy et al., 2016; Abay et al., 2017; Basu et al., 2017). Indeed, intrinsic capacity of axonal regrowth declines with age (Verdú et al., 2000) and external microenvironment poses various challenges in long distance axon regrowth (Brosius Lutz and Barres, 2014).

Although there is comprehensive understanding of axon regeneration pathways (He and Jin, 2016; Mahar and Cavalli, 2018; Richardson and Shen, 2019), protective measures against aging-related loss of regenerative potential are rather lacking. On the contrary, accumulating evidences suggest a beneficial role of rehabilitation therapies and physical exercise in promoting functional recovery following spinal cord and other injuries in human (van Hedel and Dietz, 2010; Formento et al., 2018). Physical exercise enhances functional restoration after nervous system injury in primates and rat models as well (Capogrosso et al., 2016; Fu et al., 2016). Electrical stimulation and modulation of central pattern generator leads to dramatic increase in locomotor activity in humans and rats after spinal cord lesion (Wagner et al., 2018). The benefit of exercise is

accompanied by remodelling of various parts of brain (Karssemeijer et al., 2017; Horowitz et al., 2020). Although many evidence suggest that exercise promotes regrowth of injured axon in peripheral system (Park and Höke, 2014; Gordon and English, 2016; Chen et al., 2017b), it is not completely clear whether functional improvement is the outcome of rewiring of injured axon or remodelling of spared circuitry. Also, cellular and molecular mechanisms involving exercise-mediated functional improvement after nerve injury are not clear.

C. elegans is an excellent model to investigate the cellular and molecular mechanism of axon regeneration following laser-assisted injury (Hammarlund et al., 2009; He and Jin, 2016; Richardson and Shen, 2019). Axonal injury leads to calcium influx and activates the conserved p38-MAPK pathway involving Dual Leucine Zipper Kinase (DLK)-1, which initiates the transcription of regeneration associated genes (Hammarlund et al., 2009; Ghosh-Roy et al., 2010; Nix et al., 2011). Several molecular pathways controlling axon regrowth potential are identified using mechanosensory and motor neurons as model systems (Byrne and Hammarlund, 2017; Hisamoto and Matsumoto, 2017). The two posterior lateral microtubule (PLM) neurons and the DA9 motor neurons allowed correlating functional restoration with axon regrowth at the single neuron level (Abay et al., 2017; Basu et al., 2017; Ding and Hammarlund, 2018). However, it has not been tested whether physical exercise would promote axon regeneration and functional recovery. In *C. elegans*, a single or multiple swimming sessions show exercise-like features (Laranjeiro et al., 2017; Laranjeiro et al., 2019). Swimming sessions extend neuromuscular and gut health span, enhance learning ability, and protects against neurodegeneration (Laranjeiro et al., 2019). Exercise in an

electrotactic flow chamber ameliorates the age-related degeneration (Chuang et al., 2016).

In this study, we have investigated the role of swimming exercise in improving functional restoration after axotomy of the PLM neurons. We found that a single swim session of 90 minutes could improve functional restoration through axon regeneration process irrespective of age. This exercise regimen can also improve the age-related decline in touch response function. To understand how swimming exercise promotes functional restoration, we imaged and correlated the anatomical pattern of regrowth to the recovery index of function. This revealed that exercise promotes regrowth, self-fusion events, and post-regrowth functional recovery. Improvement in functional restoration upon swimming depends on the function of the cellular energy sensor AMP Kinase-2/AAK-2 in both neurons and muscle.

Materials and Methods

C. elegans strains

All the strains were grown and maintained at 20^0 C in nematode growth media (NGM) under standard conditions (Brenner, 1974). We used the following strains: Bristol N2, *aak-2(ok524) X*, *mlk-1(ok2471) V*, *dlk-1(tm4024)* I, *ebp-1(tm1357) V,* and *unc-54(r293) I*. The extra-chromosomal DNA-containing strains used were *aak-2 (ok524)*; *shrEx362* (P*mec-4::aak-2*), *aak-2(ok524); shrEx364* (P*myo-3::aak-2*) and *aak-2(ok524); shrEX420* (P*dpy-7::aak-2*). First, the extra-chromosomal arrays were obtained in *Pmec-7::GFP (muIs32)* background by injecting the rescue transgenes at 10 ng/µl. Then the

transgenes were introduced into the *aak-2* mutant backgrounds by crossing. Homozygosity for all mutations was confirmed by either PCR or sequencing. All loss of function mutations are denoted as *(0)*. We used the following transgenes: *Pmec-7::GFP (muIs32), Pmec-4::GFP (zdIs5)* (Basu et al., 2017), and *Pmec-4::mcherry::RAB-3 (tbIs227)* (Sood et al., 2018).

Age synchronisation of worms

50 gravid adults were transferred to fresh NGM plates seeded with OP50 for egg-laying and kept at 20 ^{0}C for 2-3 hours. Worms were removed from the plates after they had laid eggs. The eggs were allowed to hatch and after 2 days, 40-50 L4 worms were transferred to a fresh NGM plate containing 50 µM 5-Fluoro deoxyuridine (FUDR) (Sigma; Catalog No.F0503) (Basu et al., 2017). The worms at different life stages were used for experiments.

Swimming exercise paradigm

A single swimming session is considered as acute exercise paradigm in *C. elegans* (Laranjeiro et al., 2017; Laranjeiro et al., 2019). In our study, we have adopted mostly the single swim-session paradigm for exercise after axotomy of PLM neurons (Figure 1A, Video 1). The worms were subjected to swimming in M9 buffer in 96 well plate for the duration ranging from 15 to 120 minutes. Single animals were kept in wells containing 200 µl of M9 buffer (1 worm/well). After the desired duration of swimming, the

184 animals were recovered on NGM plate. In the control group, worms were kept in a plate
185 without OP50 food for the same duration and then returned to plates containing food.
186 We found that the ATP level is significantly dropped as reported before (Chaudhari and
187 Kipreos, 2017) after a 90 minutes swim session and therefore we used this 90 minutes
188 session for most of the experiments.

189

190 **Measurement of ATP level after swim session**

191 To measure the change in the ATP level following swim-exercise, we used an ATP
192 bioluminescence assay kit CLSII (Roche Diagnostics, catalog number: 11699695001)
193 (Palikaras and Tavernarakis, 2016). Briefly, 60 A3 worms from 'non-swimming' as well
194 as the 'swimming' group were collected in 50 µl of M9 buffer in a 1.5 ml tube. The
195 samples were then frozen in liquid nitrogen. The frozen tubes were kept in boiling water
196 for 15 minutes. The samples were centrifuged at 14,800 g for 10 minutes at 4 ^{0}C. The
197 supernatants were transferred to a fresh tube and diluted tenfold by adding water before
198 measurement. The ATP levels were determined using Glomax luminometer (Promega).
199 Before measurement, 100 µl of sample or ATP standard was added to 100 µl of
200 luciferase in the well and incubated for 10 seconds at room temperature and then the
201 luminescence was measured. The ATP level in the worm sample was derived from the
202 ATP standard curve. Finally, the ATP levels were normalized with respect to the total
203 protein (mg) measured through BCA protein estimation method.

204

205 **Femtosecond laser, axotomy and imaging**

For axotomy of PLM neurons, the animals were immobilized using 0.1 µm polystyrene beads on a 5% agarose pad under a coverslip. For all the experiments, only one PLM axon corresponding to either left or right side of the animal was axotomized at a distance of 50-60 µm from the cell body, as described before (Basu et al., 2017). The side corresponding to the axotomized PLM neuron is called 'cut side' and other called the 'control side'. Simultaneous two-photon imaging and axotomy was performed according to the previous published protocol (Basu et al., 2017). The PLM axon was imaged and axotomized with 920 nm and 720 nm lasers respectively under a 60X (Olympus) water-immersion objective of 1.1NA on a two-photon microscope (Basu et al., 2017). This system has 2 tunable (wavelength range 690-1040 nm), automated depression compensated femtosecond lasers from Spectra Physics (Mai Tai with Deepsee). The imaging was done with a 6 mm galvanometer scanning system and axotomy was performed with a 3 mm galvanometer system.

Gentle Touch Assay

Each worm was recovered after axotomy on NGM plate and then gentle touch assay was performed. Gentle Touch Response (Chalfie and Sulston, 1981; Chalfie et al., 1985; Basu et al., 2017) was assayed from both right and left sides of the worm. Following the touch assay in one side, the worm was flipped and kept for 20 minutes before touching the other side (Basu et al., 2017). 10 alternative anterior and posterior touches were given with the eyelash tip. The anterior touch was given to a forward-moving animal, which in response started moving backward. When a backward moving

228 animal was given a posterior touch it started moving forward in response. A positive
229 response was denoted as 1, and no response as 0. Then the PTRI or 'Posterior Touch
230 Response Index' was measured as the ratio of total number of positive response to total
231 number of touch stimuli applied as described before (Basu et al., 2017).
232

233 **Correlation of functional recovery with axon regeneration events at the level of a**
234 **single worm**

235 At 3 h after axotomy, PTRIs of both axotomized and control (uncut) side, were denoted
236 as the PTRI postaxotomy. Each worm was labeled based on the side of axotomy and
237 kept in single plate at 20 °C. After 24 h, PTRI values from both the sides were
238 measured and compared with the corresponding values at the postaxotomy stages. For
239 a given side, the Recovery Index was obtained by the following formula: Recovery Index
240 = $PTRI_{24\ h}/PTRI_{3\ h}$ (Extended data Figure 1-2). After the behavioral test at 24 h, the
241 regrowth pattern of the PLM axon was scored either using a Leica DM5000 fluorescent
242 microscope at 40X magnification or a Spinning disc confocal microscope. Specifically, it
243 was noted whether it was a fusion event (Figure 5C) or non-fusion event (Figure 5F).
244 For scoring successful fusion events, we carefully evaluated whether the proximal end
245 has just touched the distal counterpart or it has successfully joined and fused with the
246 distal end (Figure 5C). In case of casual touching, the distal end eventually undergo
247 degeneration and these events are referred as reconnection (Figure 5C) (Neumann and
248 Hilliard, 2019).
249

Imaging of axon regrowth events

At 24 h postaxotomy, for imaging of the regeneration events, the animals were immobilized using 10 mM levamisole hydrochloride. Axonal regrowth was imaged using a Zeiss 864 Axio-Observer Z1 microscope equipped with Yokogawa CSU-XA1 spinning-disk confocal scan-head and a Photometrics Evolve EMCCD camera, the images were taken using 63X oil objective of 1.46 NA. The P*mec-7*::GFP labelled PLM axon was imaged with 30% input power of 480 nm laser. The images were acquired with the exposure time of 300 ms with the 70% of camera gain settings. The images were then exported as czi files and analyzed using ImageJ software. For regrowth length measurement, simple neurite tracer plugin of ImageJ was used. *Pmec-4::mcherry::RAB-3 (tbIs227)* reporter was used to image the formation synapse-like structures during axon regeneration. Imaging was done using a Nikon A1 plus (Nikon corporation) confocal microscope. PLM neurons expressing *muIs32 and tbIs227* reporter were simultaneously imaged after 24 h of axotomy. Imaging was done under a 60x oil objective (NA=1.4) at 1 µm slice interval. Excitation power was 0.8 and 1 for 488 nm and 561 nm laser, respectively. The PMT power for 488 nm channel was 100 and 104 for 561 channel with offset value of 20.

Each PLM neuron has a ventral branch (arrowhead, Figure 1A), which makes synapse onto the postsynaptic interneuron (Chalfie and Sulston, 1981; Chalfie et al., 1985). This allowed us to set the dorsal-ventral axis of the worm (Figure 5F) while analyzing the direction of axon regrowth. The axons that regrew up to 35-45 µm depth in ventral direction and extended along the ventral nerve cord were characterized as the 'ventral targeting' event (Figure 5Fc). An accumulation of the pre-synaptic reporter

mCherry::RAB-3 along the ventral cord (yellow arrowheads, Figure 5Fc) was also noticed in these events. This accumulation pattern resembled the original chemical synapses of PLM neurons. When the distal end intensity was prominently less and showed beaded appearance, it was categorized as 'distal degeneration' (Figure 5Fc) otherwise categorized as 'distal intact' (Figure 5Fa-b).

Paralysis of the worms in swimming well

At 3 h after axotomy, the animals were treated with 5 mM levamisole hydrochloride for 15 seconds in order to paralyze them. After this brief treatment with levamisole, they were placed in swimming wells. We found that this brief treatment is sufficient to perturb the swimming (Video 2, Figure 1E) during the 90 minutes swim session. To verify whether this brief treatment of levamisole has any effect in the gentle touch response of the animal, we performed similar paralysis without performing axotomy and then measured the touch response after 24 hours. We found that the PTRI value was not affected by this brief treatment (Extended data Figure 1-1B). Therefore, we used this experimental design to test the effect of blocking the swimming on enhancement of functional regeneration (Figure 1D-F).

Measurement of thrashing frequency during swimming

For the measurement of thrashing frequency, digital videos of worm movements were acquired during the swimming sessions, using a Leica MC 120 HD camera. The videos were recorded for 30 seconds at 15 frames per second using LAS V4.4 software in a Leica stereo microscope M165 FC at 1.25X magnification. The thrashing frequency were measured as body bends per second using wrMTrck plugin of imageJ software (http://www.phage.dk/plugins/wrmtrck.html). The change in the direction of bending at the mid-body was defined as one body bend (Truong et al., 2015). A mutant for muscle myosin *unc-54* (Pulak and Anderson, 1988) showed drastically reduced thrashing frequency using this plugin. Thrashing frequency in *unc-54* mutant was 18.27±13.04 as opposed to 130.8±28.97 for wild type. This suggests that our analysis is sensitive enough to show the difference in swimming ability due to various experimental conditions.

Metformin treatment on paralyzed worms

At 3 h postaxotomy, worms were first treated with 5 mM levamisole hydrochloride solution for 15 seconds in order to cause the muscle paralysis. The experimental swimming well had 50 mM Metformin hydrochloride (Sigma-Aldrich; Catalog no. PHR1084). Therefore, the paralyzed worms were treated with Metformin during the 90 minute swim-session. Concentration of Metformin were chosen to activate AMPK/AAK-2 based on the previous report in *C. elegans* (Chen et al., 2017a).

Molecular Biology and Transgenes.

For touch neuron, muscle and epidermal specific expression of *aak-2*, first a gateway (Thermo Fisher Sciectific) entry clone of *aak-2* [pCR8::*aak-2* (pNBRGWY115)] was constructed by PCR with the primers 5'-ATGTTTTCTCATCAAGATCGAGA-3' and 5'-TCTCGATCTTGATGAGAAAACAT-3'. Then the entry clone was recombined with pCZGY553 (P*mec-4* destination vector), pCZGWY925 (P*myo-3* destination vector) and pCZGWY44 (P*dpy-7* destination vector) to generate P*mec-4*::*aak-2* (pNBRGWY116), *Pmyo-3*::*aak-2* (pNBRGWY117) and P*dpy-7*::*aak-2* (pNBRGWY149), respectively.

Statistics

All the statistical analyses were performed using GraphPad Prism software version 9.0.2. For two-way comparisons, an unpaired t-test with Welch's correction was used. The median values were compared with the Mann Whitney U-test. Fisher's exact test was used for proportions. Three or more samples were compared with ANOVA (nonparametric) with a Post-hoc Tukey's multiple comparisons test. The sample numbers (n) presented on each bar are the total sample value accumulated over the total number of biological replicates (N) in a given experiment.

Results

A single swim-session after axotomy of PLM neurons promotes functional recovery

Previous studies have indicated that physical exercise following nervous system injury promotes axon regeneration and functional recovery in various model systems (Doyle and Roberts, 2006; Asensio-Pinilla et al., 2009; Sachdeva et al., 2016; Kuwabara et al., 2018). To address whether physical exercise can enhance functional restoration in *Caenorhabditis elegans*, we designed a swimming exercise paradigm in conjunction with PLM axon regeneration (Figure 1A, Video 1). A single session of swimming in *C. elegans* mimics the features of mammalian exercise (Laranjeiro et al., 2017). As previously reported that there is a sharp drop in functional recovery of gentle touch behaviour after the axotomy of Posterior Lateral Microtubule (PLM) neuron at day 3 adult worms (A3) (Basu et al., 2017) (Extended data Figure 1-1A), we tested whether a swim-session after axotomy would enhance recovery. We measured the Posterior Touch Response Index (PTRI) at 3 and 24 hours postaxotomy (Figure 1A). The extent of functional recovery was represented as the normalized PTRI at 24 h with respect to that measured at 3 h postaxotomy, which we called 'recovery index' (Figure 1B, Extended data Figure 1-2). A recovery index value, higher than 1 is an indication of improvement of touch response over the time after axotomy. A swimming session of 90 minutes or above significantly enhanced the recovery index at 24 hours as compared to the non-swimming control (Figure 1B, Extended data Figure1-2). It also correlated with the significant drop in ATP level after the swimming session measured fluorometrically from the worm lysate (Figure 1C). After 90 minutes, the worms did episodic swimming rather than continuous swimming (Ghosh and Emmons, 2008). Therefore, we chose 90-minute exercise window for our further experiments. To confirm that the swimming-induced improvement in functional recovery is not due to the stress in liquid

environment, rather due to exercise, we paralyzed the worm during swimming using levamisole that causes muscle hypercontraction (Culetto et al., 2004) (Figure 1D-E). The change in swimming ability was measured as thrashing frequency (Buckingham and Sattelle, 2009) (Figure 1E). We observed that the recovery index was significantly reduced when swimming was perturbed (Video 2) (Figure 1F). Levamisole treatment in the swimming well *per se* did not affect the touch response index measured after the withdrawal of levamisole (Extended data Figure1-1B).

To determine the time duration required for seeing the benefit of swimming, we varied the time window between the swim-session and evaluation of touch response (PTRI) (Figure 1G). Essentially, the swim-session was gradually shifted towards the time of post-regeneration PTRI measurement (Figure 1G). When the window was lower than 12 hours, in this case 6 hours, the functional improvement was non-significant (Figure 1H). But when we increased the window, by shifting the time of post-regeneration PTRI measurement further by 18 hours, the functional improvement was significant (Figure 1I). Therefore, a critical time of 12 hours is needed for proper manifestation of positive effect of swimming in regeneration.

Multiple swim sessions are needed in older ages for the improvement in functional restoration.

Next, we expanded our single swim-session paradigm across various ages and found that this exercise regimen can improve the recovery index significantly when axotomy was performed at L4, A1, A3 and A4 stages (Figure 2A, Extended data Figure 2-1).

379 However, this improvement was non-significant at day 5 (A5) (Figure 2A, Extended data
380 Figure 2-1). We noticed that the thrashing frequency during swimming was significantly
381 reduced at A5 stage (Figure 2B), which might have reduced the beneficial effect of
382 swimming in functional restoration. We wanted to test whether multiple swimming
383 sessions would be required at A5 stage for significant improvement in functional
384 recovery. To test this hypothesis, we increased the number of swim sessions after the
385 axotomy at A5 stage, one at 4 h and the other at 12 h postaxotomy (Figure 2C).
386 Increasing the number of swim-session raised the recovery index value significantly
387 (Figure 2D). Overall, our data suggest that swimming-related exercise promotes
388 functional restoration irrespective of age, although in older age multiple exercise
389 sessions are important.

390

391 **Swim exercise also prevents the age-related decline in touch neuron function**

392 It might be possible that swim-exercise improves the touch neuron function in general,
393 especially in older age when function is known to decline (Basu et al., 2017). As
394 reported previously, we found that PTRI value is significantly dropped at A5 and
395 subsequent life stages (Figure 3B). A single swim-session of 90 minutes, one day prior
396 to the PTRI measurement (Figure 3A) improved the PTRI value significantly at A5 and
397 A6 stages (Figure 3B-C). However, in A8 stage the improvement in PTRI value due to
398 single session swimming exercise was poor (Figure 3B-C). No improvement at A8 stage
399 was also noticed in the *zdIs5* (P*mec-4::*GFP) transgenic reporter background (Figure
400 3C). We asked whether a longer gap between swim-session and functional assessment
401 would be sufficient to enhance the PTRI value significantly at A8 stage. However, no

402 further improvement in PTRI value was noticed when the exercise session was
403 anticipated by one day (Figure 3C). To test whether multiple swimming sessions across
404 multiple days are helpful, we subjected the animals to 4 swimming sessions starting
405 from A1 with one day interval between two sessions and then measured touch response
406 at A8 stage (Figure 3D). Multiple swimming sessions significantly elevated the PTRI
407 value at A8 stage as compared to that obtained from non-swimming control (Figure 3D-
408 E). Our data highlight that exercise improves both age-dependent decline in post-
409 axotomy functional recovery, as well as prevents the age-related decline in touch
410 neuron function. This raises the possibility that the exercise might only improve the
411 neuronal function. Therefore, it needs to be resolved whether our exercise regimen
412 would also enhance axon regrowth potential after axotomy.

413

414 **Swim-session mediated improvement in post-axotomy functional restoration**
415 **involves initiation of axonal regeneration**

416 Physical exercise mediated improvement in functional recovery after neuronal injury
417 often involves remodelling of spared neuronal circuits (van den Brand et al., 2015).
418 Therefore, we wanted to test whether the benefit of swimming is due to compensatory
419 mechanisms or regrowth of the axotomized PLM neuron. The p38 MAP kinase pathway
420 involving dual leucine zipper kinase-1/DLK-1/MLK-1 is required in the early stages of
421 axon regeneration in a cell-autonomous manner (Figure 4A) (Hammarlund et al., 2009;
422 Yan et al., 2009; Ghosh-Roy et al., 2010). In the absence of this signalling cascade,
423 injured axon cannot initiate the growth cone formation after axotomy. Therefore, the
424 mutants affecting the DLK-1 cascade serve as a tool to block the regeneration from the

injured proximal stump after axotomy (red arrows. Figure 4D). A swim-session neither in *dlk-1(0)* nor in *mlk-1(0)* could promote functional restoration in A3 (Figure 4B). Similar observation was made in *dlk-1(0),* when the experiment was conducted at L4 stage (Figure 4C). Similarly, an upregulation of microtubule dynamics through microtubule plus-end binding protein-1 EBP-1 is critical for the efficient axon regrowth (Chen et al., 2011; Ghosh-Roy et al., 2012). We found that, the benefit of exercise was not seen in the absence of *ebp-1* (Figure 4B-C). Consistently, swimming could not promote the axon regrowth from the cut stump in these mutants (Red arrows, Figure 4D-E). To rule out the possibility of reduced swimming ability in these mutants, we analysed the movies of their swimming. We found that thrashing frequency in these mutants are comparable to that in wild type control (Figure 4F).

These results confirm that exercise mediated increase in functional recovery involves regrowth from the injured proximal stump of the PLM neuron and subsequent rewiring into the functional circuit.

Swim-session promotes axon regrowth and post-regrowth functional recovery

Although swimming induced improvement in functional restoration involves initiation of axon regrowth after axotomy, it is not clear whether this exercise paradigm would enhance anatomical features of axon regrowth or it could simply enhance the functional aspect after axon regrows. The fact that swimming can enhance touch sensation behaviour in older age, leaves the second possibility open. To test this, we performed confocal imaging of the regrowth events after assaying for the functional restoration

(Figure 5A), and correlated the behavioural recovery with the anatomical patterns of regeneration. We found that swimming exercise in A3 worms accelerated the initiation of the regrowth as revealed by the increased number of filopodia-like extension at the cut stump at 6 hour postaxotomy (arrowheads, Figure 5B). The median value for the number of filopodia is increased from 1 to 2 due to the exercise session (*P=0.01, Mann Whitney U test). At 24 h postaxotomy, there was an enhancement in axonal regrowth as compared to the control condition (Figure 5B). Regrowth value in the swimming group becomes 114.0 ± 63.81 µm as compared to the value 83.46 ± 41.62 µm obtained in non-swimming group (*P=0.04, unpaired t test). We also noticed that the percentage of self-fusion events with respect to the reconnection events (Ghosh-Roy et al., 2010; Neumann et al., 2011; Neumann et al., 2015; Basu et al., 2017; Neumann and Hilliard, 2019) between proximal and distal ends are significantly increased due to the swimming session (Figure 5C-D).

We further investigated whether the improvement in functional restoration seen due to swim-exercise correlates with the ‘fusion’ or ‘non-fusion’ events, or both the categories of regrowth. We found that there is a significant increase in the recovery index for both the fusion event and non-fusion events (Figure 5E). Upon correlating the regrowth pattern of the non-fusion events, we found that the regrowing axon, which goes towards the ventral cord and shows an enrichment of the pre-synaptic reporter mCherry::RAB-3 at the ventral cord (arrowheads, Figure 5Fc) corresponds to successful recovery in the ‘swimming’ group as the value of the recovery index of this class is 2.66$\pm$0.73 (Figure 5G). We called this category of as ‘ventral targeting’ events. The recovery index corresponding to the ‘ventral targeting’ events was significantly higher as compared to

the index obtained in the other classes such as 'straight regrowth' (Figure 5Fa) and 'multi-branch regrowth' (Figure 5Fb). The percentage of 'ventral targeting' events got increased upon swim-exercise (Figure 5H). Since the distal end often persisted after injury (Figure 5Fa-b), we asked whether the 'intact distal end' could be contributing to the functional recovery. However, the recovery indices in the 'distal intact' and 'distal degenerated' categories were comparable in both swimming and non-swimming groups (Figure 5I). Therefore, the enhancement of functional restoration due to swim-exercise corresponds to successful rewiring process.

Exercise-mediated improvement in touch neuron function requires metabolic energy sensor AAK-2

During exercise, there is a consumption of energy in the form of ATP, which results in an increase in an AMP: ATP ratio (Chen et al., 2003). The increase in this ratio is sensed by the metabolic energy sensor kinase known as AMPK (Hardie, 2011). The physical exercise in mammalian system leads to activation of AMPK (Chen et al., 2003; Gibala et al., 2009). As in mammals, the two catalytic subunits of AMPK in *C. elegans* are encoded by two genes *aak-1* and *aak-2* (Apfeld et al., 2004). We hypothesized that the decrease in ATP levels after the 90-min swimming session might be sensed by AAK-2 in neurons or muscle to regulate regeneration. To test this possibility, we gave the *aak-2* mutant a 90-minutes swim-session at various life stages starting from A1 to A7 and measured the posterior touch response (PTRI) after 24 hours (Figure 6A). We found that there is drop in touch response index value in *aak-2* mutant starting from A5 stage similar to the wild type (Figure 6B). However, the swim session couldn't elevate

493 the PTRI in any of the life stages (Figure 6B). We wondered, whether the abrogation of
494 the exercise-induced elevation in PTRI value in *aak-2(0)* is due the reduced swimming
495 ability of the mutant. However, the thrashing frequency in this mutant at L4, A3 and A5
496 stages were comparable to the same values in wild type control (Figure 6C). This
497 indicated that AAK-2 might be specifically required for the exercise-induced changes in
498 neuronal regeneration and function (Figure 6D). To understand the tissue specific
499 requirement of *aak-2* in this phenomenon, we have expressed *aak-2* in muscle,
500 epidermal cells and touch neuron exclusively. We found that both touch neuron and
501 muscle specific expression of *aak-2* can rescue the swim-session induced phenomenon
502 significantly (Figure 6E). However, no rescue was observed when *aak-2* was expressed
503 in epithelial cells situated just next to the PLM axon (Figure 6E). Next, we tested
504 whether the AMPK activator metformin (Foretz et al., 2014; Rena et al., 2017) can
505 mimic the benefits of swimming in touch neuron function (Figure 6F). To address this
506 question, we treated the worms with 50 mM metformin while they were kept paralyzed
507 in the swimming well for 90 minutes (Figure 6F). Metformin treatment was sufficient to
508 enhance the PTRI value in day 5 (A5) worms (Figure 6G). This effect of metformin was
509 absent in *aak-2* mutant (Figure 6G) indicating that effect of metformin in our assays is
510 specific to AAK-2 function. These observations suggest that neuron- and muscle-
511 specific activity of AAK-2 is important for swim-exercise induced enhancement of touch
512 neuron function.

513

514 **Activation of the energy sensor AAK-2 after axotomy is sufficient to promote**
515 **axon regrowth and functional restoration**

To address the role of AMPK/AAK-2 during swimming, we treated the 3-day old (A3) worms at 3 h postaxotomy with 50 mM metformin only during the 90 minute swim-session while these animals were paralyzed with levamisole in the swimming well (Figure 7A). Previously we have shown that when worms are paralyzed during the swim session, enhancement of functional restoration is blocked (Figure 1F). We found that metformin treatment significantly enhanced the recovery index as compared to the non-treated control (Figure 7B). Total regrowth from the proximal stump (red arrows, Figure 7C) at 24 h postaxotomy was also increased significantly in the metformin treated group (Figure 7C-D). The regrowth value becomes 91.77±36.19 µm in metformin treated group as compared to 62.87±24.36 µm in untreated group. We noticed that there is an increase in the recovery index for both 'non-fusion' and 'fusion' related events at A3 stage (Figure 7E). These observations suggest that AMPK activation is sufficient to mimic the effect of swimming exercise in axonal regeneration as well as functional recovery.

The *aak-2* mutant shows reduced axon regrowth (Hubert et al., 2014) (Extended data Figure 7-1A-B), which resulted in a loss of swim-exercise mediated enhancement of post-axotomy recovery index at A3 stage (Figure 7F). We found that the expression of *aak-2* either in touch neurons or in muscle rescues the axon regrowth defect in *aak-2* mutant at L4 stage (Extended data Figure 7-1A-B). However, the post-injury recovery index was only rescued significantly by the touch neuron-specific expression of *aak-2* at L4 stage (Extended data Figure 7-1C). These transgenes also rescued the loss in benefit of swim-session in functional recovery in *aak-2* mutant at A3 stage (Figure 7F). Expression of *aak-2* in epithelial cells however did not rescue these phenotypes in *aak-*

539 *2* mutant (Figure 7F, Extended data Figure 7-1A-C). These results indicate that AAK-2
540 acts both cell-autonomously in neuron and non-cell-autonomously in muscle for
541 transducing the effect of swim-exercise in effective axon regeneration.

542

543 **Discussion**

544 In this study, using a well-established swimming paradigm we have studied the effect of
545 physical exercise in functional recovery after the axonal injury of mechanosensory
546 neuron. A swim-session following axotomy promotes functional recovery irrespective of
547 age. This exercise regimen can also help overcome age related decline in touch neuron
548 function. By correlating anatomical features of axon regrowth with the behavioural
549 index, we found that the swimming exercise enhances both post-injury axon regrowth
550 as well as functional recovery. We further showed that the activity of the metabolic
551 energy sensor AMPK in muscle as well as in injured neuron is critical in converting the
552 energy spent during exercise into the positive effect in axon regeneration (Figure 8).

553 In *C. elegans,* it is shown that a single swim-session of 90 minutes presents exercise-
554 like features such as muscle fatigue, a reduction of fat level in muscle, decrease in
555 carbohydrate metabolism and enhanced mitochondrial oxidation (Laranjeiro et al.,
556 2017). Many of these phenomena share commonalities with the physical exercise in
557 vertebrate models (Thompson et al., 2001; Rivera-Brown and Frontera, 2012; Vina et
558 al., 2012). In continuation to this finding, multiple and regular exercise sessions increase
559 life-span, health quality, and protect against neurodegeneration in models of tauopathy,
560 Alzheimer's disease, and Huntington's disease (Laranjeiro et al., 2019). It also

increases overall learning ability as seen in other models and humans (Kobilo et al., 2014; Moon et al., 2016). These findings made it very relevant to address the role of physical exercise in promoting the repair of the injured neuronal circuitry using a worm model. We found that a 90 minutes swim session after the axotomy is sufficient to overcome age-related decline in axon regeneration and functional restoration. Intriguingly, this exercise paradigm is also sufficient to improve the functional recovery in early stages of life. Previous literature has demonstrated the benefit of physical exercise in peripheral nerve regeneration (Park and Höke, 2014; Gordon and English, 2016; Chen et al., 2017b). Since our behavioural assay is specific to single neuron responsible for touch sensation, we provided a direct evidence that enhanced axon regrowth due to physical exercise drives functional recovery.

An immediate effect of exercise is a reduction in the ratio of ATP/AMP (Chen et al., 2003), which is sensed by AMP Kinase (Hardie, 2011). Following the swimming session, we recorded a significant drop in ATP level. Therefore, we speculated a role of AMPK/ AAK-2 in exercise-mediated improvement in touch neurons function. Consistent with this hypothesis, the loss of *aak-2* significantly abolished the improvement in touch neurons regeneration and function due to swimming exercise. Conversely, activation of AMPK using metformin was sufficient to promote axon regeneration. Tissue specific rescue experiments suggested that AAK-2 acts both in neuron and muscle for enhanced axon regeneration and functional recovery. Few studies indicated that activation of AMPK by metformin has positive effect after spinal cord injury (Zhang et al., 2017; Guo et al., 2018). Pharmacological activation of AMPK can promote muscle fibre regeneration in a mouse myopathy model (Peralta et al., 2016). The AMPK agonist

584 AICAR enhances spatial memory in wild type animals, but this improvement was lacking
585 in muscle-specific mutant of AMPK pointing towards the link between muscles and
586 nervous system (Kobilo et al., 2014).

587 The question is how activated AAK-2 could be enhancing axon regeneration. It has
588 been observed that post-exercise activation of AMPK leads to the ATP synthesis via
589 various metabolic pathways (Winder and Hardie, 1996; Hutber et al., 1997; Hardie,
590 2004). It also leads to mitochondrial biogenesis through proliferator–activated receptor
591 gamma coactivator–1α (PGC-1α) (Zong et al., 2002; Kukidome et al., 2006). After a
592 neuronal injury, the growth cone formation and subsequent regrowth is driven by
593 various enzymes and motors. This requires high level of energy (Bradke et al., 2012;
594 Zhou et al., 2016). Therefore, AMPK-driven ATP production can boost up the initiation
595 of axon regeneration process as seen in our analysis. Apart from restoring the cellular
596 energy levels, AMPK also regulates autophagy which allows the cell to survive the
597 metabolic stress (Zhao and Klionsky, 2011). Autophagy induction has been linked with
598 the stabilization of microtubules and enhancement of axonal regeneration after neuronal
599 injury (He et al., 2016; Ko et al., 2020). Other possibility through which the AMPK can
600 enhance axon regeneration is through DAF-16/FOXO1 activation. The activated AMPK
601 can directly phosphorylate and regulate DAF-16 (Greer et al., 2007), which is known to
602 regulate axon regeneration (Byrne et al., 2014). It would be an interesting direction to
603 unravel how AMPK signalling couples muscle and neuron in neuronal regeneration.

604

605

606 **Legends**

607 **Figure 1: A single swim-session after axotomy of PLM neurons enhances**
608 **functional restoration in adult worm.** (A) Experimental paradigm of swim session
609 after the axotomy of a PLM neuron in day-3 adult (A3) worms expressing P*mec-7::*GFP
610 (*muIs32*) reporter. Following axotomy, the gentle touch response assay was performed
611 to measure the reduction in posterior touch response index (PTRI) (Extended data
612 Figure 1-1 A). Then the animals were subjected to a swimming session for a duration
613 ranging from 30 minutes to 2 hours and recovered in NGM plate for further analysis.
614 Another touch response assay was performed at 24h postaxotomy to assess the
615 functional recovery. Arrowhead denotes developmental synapses of PLM neurons. (B)
616 Quantification of the recovery index expressed as PTRI at 24 h postaxotomy / PTRI at 3
617 h postaxotomy for swim session of varying duration. N=4-5 independent replicates,
618 n=20-25 number of animals tested. The raw data used for calculating recovery index
619 values are given in Extended data Figure 1-2. (C) The bar graph represents the ATP
620 levels measured from the total extract prepared from 60 A3-stage animals after the
621 swim-session of varying duration, N=4 independent replicates. (D) Schematics for
622 paralyzing the animals during the swim session. In order to prevent the worms from
623 swimming in the well, they were treated with 5 mM levamisole for 15 seconds prior to
624 the swimming session. The brief exposure to levamisole did not affect basal level
625 posterior touch response index (Extended data Figure 1-1B). (E) The bar graph
626 represents the thrashing frequencies measured from the time-lapse imaging of the
627 worms in the swimming well. N=3-4, n= 38-86. (F) Recovery indices obtained at 24 h
628 post-axotomy for control and paralyzed worms during the swim-session, N=3-4, n=22.

(G) Scheme for determining the critical time required for seeing the beneficial effect of the swim session. In this experiment, the duration between swim session and post-regeneration PTRI measurement was varied. (H-I) Bar graphs showing the recovery indices measured according to the experimental paradigm described in G at 24 h postaxotomy and 36 h postaxotomy for H and I respectively, N=3-5, n= 20-27. Statistics, for B, C, F and H, *p<0.05, **p<0.01, ***p<0.001 ANOVA with Tukey's multiple comparison test. For E and I, **p<0.01, ***p<0.001; unpaired t-test. Error bars represent SD. ns, not significant.

Extended data Figure 1-1: (A) Bar graph with scatter plot showing the posterior touch response index (PTRI) before axotomy and at 3 h and 24 h postaxotomy. This analysis is presented for L4 and A3 stage animals expressing *Pmec-7*::GFP (*muIs32*) reporter. N=4 independent replicates, n=22-31 number of worms tested. (B) Bar graph showing the effect of levamisole treatment on PTRI at A3 stage animals expressing P*mec-7::*GFP (*muIs32*) reporter. The worms were treated with different concentrations of levamisole for 15 seconds and then kept in the swimming well for 90 minutes. The PTRI was measured at 24 h post-levamisole treatment, N=3, n = 14-17. Statistics, for A & B ***p<0.001; ANOVA with Tukey's multiple comparison test. Error bars represent SD. ns, not significant.

649 **Extended data Figure1-2:** The Posterior Touch Response Index (PTRI) values at 3 h
650 and 24 h postaxotomy at A3 stage. The recovery index values were obtained by
651 normalizing the PTRI values at 24 h with respect to that at 3 h. The data is presented
652 from groups, which underwent swimming session of varying duration (30 min-120 min).

653

654 **Figure 2: Multiple swimming-sessions after axotomy are critical for functional**
655 **recovery in older ages.** (A) The scatter plot with bars showing the effect of swimming
656 exercise on functional recovery in L4 to A5 stage. The experiment was done in the
657 transgenic background P*mec-7::*GFP (*muIs32*), N=4-5 independent replicates, n= 20-25
658 number of animals tested. The raw data used for calculating recovery index values are
659 given in Extended data figure 2-1. (B) Thrashing frequency of worms during the swim-
660 session at different life-stages N=3, n =37-78. (C) Scheme for determining the effect of
661 multiple swim-sessions on functional recovery at A5-stage. Worms were allowed to
662 swim for two sessions of 90 minutes each. (D) Recovery Indices in worms at 24 h
663 postaxotomy, which underwent multiple swimming sessions after axotomy at A5 stage
664 as shown in C-panel, N=3, n= 21. Statistics, For A & B, *p<0.05, **p<0.01, ***p<0.001,
665 ANOVA with Tukey's multiple comparison test. For D, **p<0.01, unpaired t-test. Error
666 bars represent SD. ns, not significant.

667

668 **Extended data Figure 2-1:** The Posterior Touch Response Index (PTRI) values at 3 h
669 and 24 h postaxotomy in wild type worms at different life stages (L4-A5). The recovery

index values were obtained by normalizing the PTRI values at 24 h with respect to that at 3 h. The experimental group underwent a swimming session of 90 min duration.

Figure 3: Swimming exercise prevents age-related decline in touch neuron function. (A) A paradigm to study the effect of swimming exercise of 90 minutes duration on age-dependent decline of posterior gentle touch response. Touch response assay was performed at 24 h post-swimming session. (B-C) Bar plots show the posterior touch response indices (PTRIs) measured at 24 h post-swimming session at various life stages in two different reporter backgrounds, P*mec-7::*GFP (*muIs32*) (B) and P*mec-4*::GFP (*zdIs5*) (C). In *zdIs5* background, an additional single swim session was performed at 48 h before PTRI measurement at A8 stage; N=3-5 independent replicates, n=20-72 number of worms tested. (D) Scheme for multiple swimming sessions to enhance touch response at A8 stage. (E) The effect of multiple swim sessions on PTRI values at A8 stage, N=3, n=24-25. Statistics, For B and C, *p<0.05, **p<0.01, ***p<0.001 ANOVA with Tukey's multiple comparison test. For E, ***p<0.001, unpaired t-test. Error bars represent SD. ns, not significant.

Figure 4: Swimming session-related improvement in functional recovery is dependent on axon regeneration. (A) Pathway diagram of two parallel p38 MAP kinase pathways involving DLK-1 and MLK-1 in axon regeneration. (B) The quantification showing the effect of swimming exercise on the recovery index measured

691 at 24 h postaxotomy in the *dlk-1(0)*, *mlk-1(0)* and *ebp-1(0)* at A3 stage. N=3-4
692 independent replicates, n=19-42 number of worms tested. (C) The recovery indices
693 measured at 24 h postaxotomy in the *dlk-1(0)* and *ebp-1(0)* at L4 stage with and without
694 swimming conditions N=3-5, n=22-30. (D-E) Representative confocal images of PLM
695 axons at 24 h postaxotomy in the wild type control and mutants affecting axon
696 regeneration pathways (D) and axon regrowth values (E) measured from the proximal
697 cut tips (Arrows in D), N=3-4, n=19-25. Red arrowheads represent the filipodia-like
698 structure at the regrowing tips, and arrows indicate the position of axotomy. (F)
699 Thrashing frequencies in the mutants at A3 stage N=3-4, n=24-85. Statistics, For B, C,
700 E and F, **p<0.01, ***p<0.001; ANOVA with Tukey's multiple comparison test. Error
701 bars represent SD. ns, not significant.

702

703 **Figure 5: Swimming exercise promotes both axon regrowth and the functional**
704 **recovery.** (A) Experimental design to correlate functional recovery with the anatomical
705 features of axon regrowth in worms expressing P*mec-7*::GFP (*muIs32*) at A3 stage. The
706 confocal imaging was done after the measurement of PTRI values at the 24 h
707 postaxotomy. (B) Representative images and illustration of PLM axons from control and
708 swimming group at 6 h and 24 h postaxotomy. Arrows and arrowheads indicate the
709 position of axotomy and filopodia-like structures, respectively. (C) Confocal images of
710 'fusion' and 'reconnection' events. Arrows indicate the position of axotomy. (D) Bar
711 graph representing the % of different types of regeneration events in swimming and
712 non-swimming groups, N=3-5 independent replicates, n =123-145 neurons. (E) Bar

graph with scatter plots presenting changes in the recovery index corresponding to the fusion and non-fusion events due to swim-session. N=3-5, n= 52-91 worms. (F) Confocal images representing different types of 'non-fusion' regeneration events. The worms are expressing the presynaptic marker P*mec-4*::mCherry-RAB3 (*tbIs227*) and P*mec-7::*GFP (*muIs32*). Yellow dashed box showing the enrichment of RAB-3 at the ventral cord region in the 'ventral targeting' event. Arrowheads indicate enriched RAB-3 puncta; Red arrows indicate the position of axotomy, 'Cm' denotes coelomocyte cell expressing the co-injection marker. (G) Bar graph with scatter plots showing the changes in the recovery index due to swim exercise in different classes of non-fusion events, N=3-5, n=21-39. (H) The change in the % of ventral targeting events due to swimming exercise. (I) A comparison of recovery indices between the 'distal axon intact' and 'distal axon degenerated' events with respect to swimming exercise, N=3-5, n=31-60. Statistics, For E, G and I, **p<0.01, ***p<0.001 ANOVA with Tukey's multiple comparison test; For D and H **p<0.01 Chi-square test. Error bars represent SD. ns, not significant.

Figure 6: Swimming mediated improvement in touch response in older age is dependent on AAK-2. (A) Schematic to study the effect of single swimming session on age- related decline in touch neurons function in *aak-2* mutant. Touch response assay was performed 24 h post-swimming session. (B) The posterior touch response indices (PTRIs) measured at 24 h post-swimming session in *aak-2(0)* at different life stages, N=4-6 independent replicates, n=23-68 number of animals. (C) The thrashing

735 frequencies (body bends/min) in wild type and *aak-2(0)* at larval (L4) and adult stages,
736 N=3-4, n=33-90. (D) A pathway diagram explaining how a swim session might enhance
737 touch neuron function through AMPK. (E) The effect of swimming exercise on the
738 decline in touch response at A5-stage in *aak-2(0)* expressing *Pmec-4::aak-2*
739 *(shrEx362), Pmyo-3:: aak-2 (shrEx364),* and *Pdpy-7::aak-2(shrEx420)* transgenes, N=3,
740 n=25-41. (F) Schematics of metformin treatment to the paralyzed worms in the
741 swimming well to study the effect of AMPK activation in touch neuron function at A5
742 stage. The wild type and *aak-2(0)* worms were pre-treated with 5 mM levamisole for 15
743 seconds prior to the swimming session. (G) Changes in PTRI values at at 24 h post-
744 treatment with 50 mM metformin as shown in F, N=5, n=28-51. Statistics, For B and C,
745 ***p<0.001; ANOVA with Tukey's multiple comparison test. For E and G, ***p<0.001;
746 unpaired t-test. Error bars represent SD. ns, not significant.

747

748 **Figure 7: Swimming-mediated improvement in functional recovery after axon**
749 **regeneration involves the energy sensor AAK-2.** (A) Strategy for the AMPK
750 activation during the swim-session by treating with metformin. Wild type worms were
751 pre-treated with 5 mM levamisole for 15 seconds prior to the swimming session in order
752 to paralyze them. 50 mM metformin was applied to the paralyzed worms in the
753 swimming well. (B) The effect of metformin treatment on recovery index at 24 h
754 postaxotomy at A3 stage N=5 independent replicates, n= 47-48 number of worms. (C-
755 D) Representative confocal images (C) and quantification (D) of PLM axon regrowth at
756 24 h post-injury in wild type worms treated with or without metformin after axotomy at

A3 stage. N=5, n=27-32. Arrows indicate the position of axotomy. (E) Bar graph represents the comparison of recovery index in control and metformin treated worms at A3 stage, N=3, n=48-49. (F) The effect of touch neuron, muscle and epidermal cell specific expression of *aak-2* in *aak-2* mutant on swimming induced enhancement in recovery index. The rescue transgenes P*mec-4::aak-2* (*shrEx362*), P*myo-3::aak-2* (*shrEx364*), and P*dpy-7::aak-2* (*shrEx420*) were used, N=3, n=20-37. The regrowth length and basal level of recovery index values in *aak-2(0)* mutant with or without the transgenes of *aak-2* at L4 stage is presented in Extended data Figure 7-1. Statistics, For, B, D, E and G, *p<0.05, **p<0.01, ***p<0.001 unpaired t-test. Error bars represent SD. ns, not significant.

Extended data Figure 7-1: (A) Confocal images of a PLM neurons labelled with *Pmec-7::GFP (muIs32)* in *aak-2(0) and* tissue specific rescue background at 24 h postaxotomy at the L4 stage. Red arrows indicate the position of axotomy. (B) Rescue of axon regrowth values in *aak-2(0)* at 24 h postaxotomy by the touch neuron, muscle and epidermal cell specific expression of *aak-2* cDNA. N=3-4, n=17-30 animals. (C) The rescue of recovery index at 24 h postaxotomy in *aak-2(0)* by the touch neuron, muscle and epidermal cell specific expression of *aak-2* cDNA. Rescue transgenes P*mec-4*::*aak-2* (*shrEx362*), P*myo-3*::*aak-2* (*shrEx364*) and P*dpy-7*::*aak-2* (*shrEx420*) were used, N=2-4, n=21-26. Statistics, for A, C and D *p<0.05, **p<0.01, ***p<0.001; unpaired t test. n = number of worms tested. Error bars represent SD. ns, not significant.

Figure 8: Proposed model illustrating how swimming exercise promotes axon regeneration. Exercise session after axonal injury leads to the consumption of cellular ATP resulting in activation of AMPK. An activated form of AMPK promotes axon regeneration and functional restoration.

Video 1: After the measurement of post-axotomy touch response index (PTRI), worms were transferred to wells and allowed to swim for 90 minutes. The videos were recorded at 15 frames per second (fps) and represented at 50 fps.

Video 2: Swimming of control and paralyzed worms. In order to paralyze the worms, the worms were treated with 5 mM Levamisole and transferred to the swimming well. Movies were acquired at 15 fps and represented at 50 fps.

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Figure 1

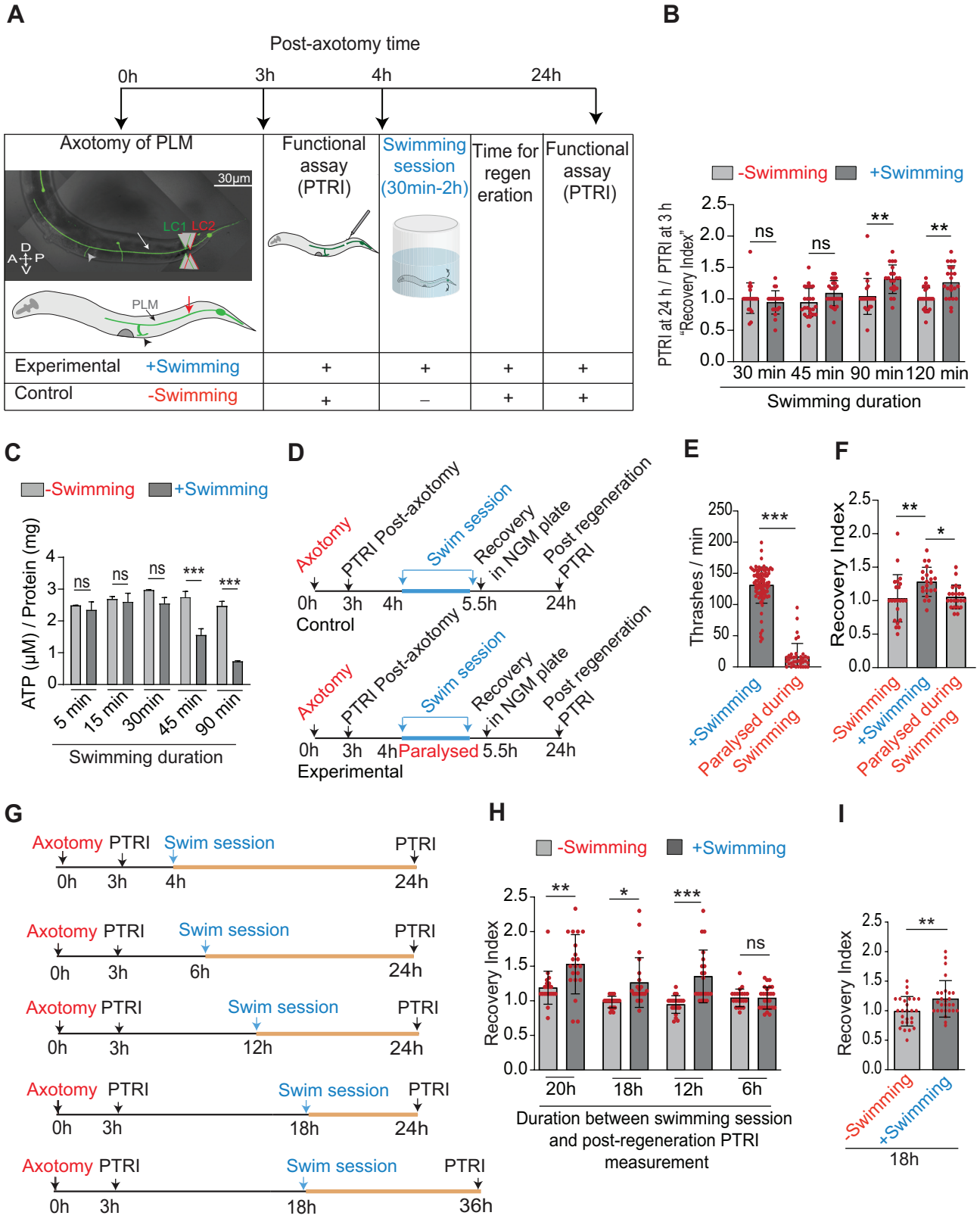
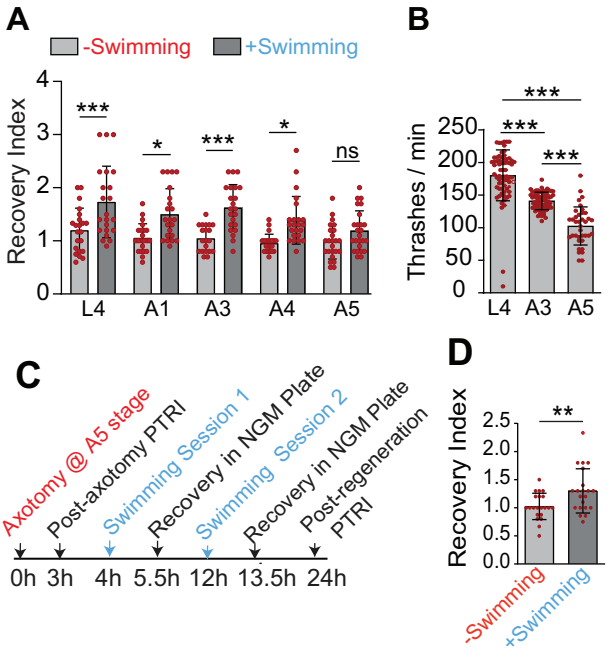


Figure 2



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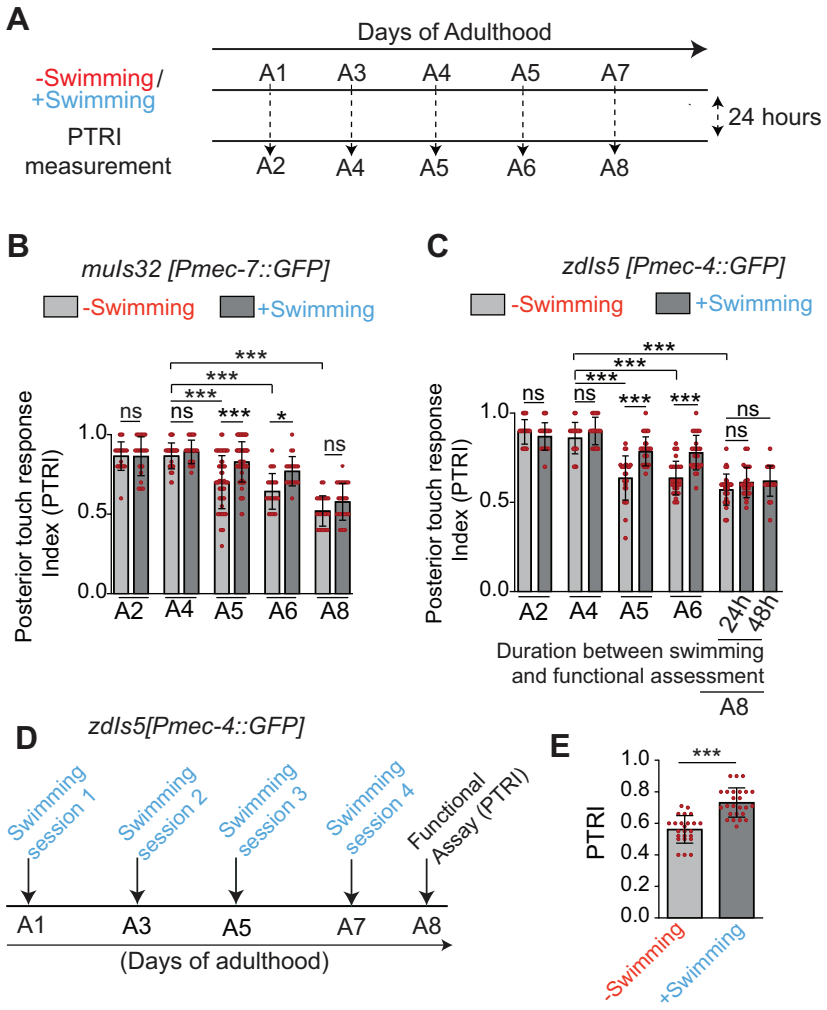

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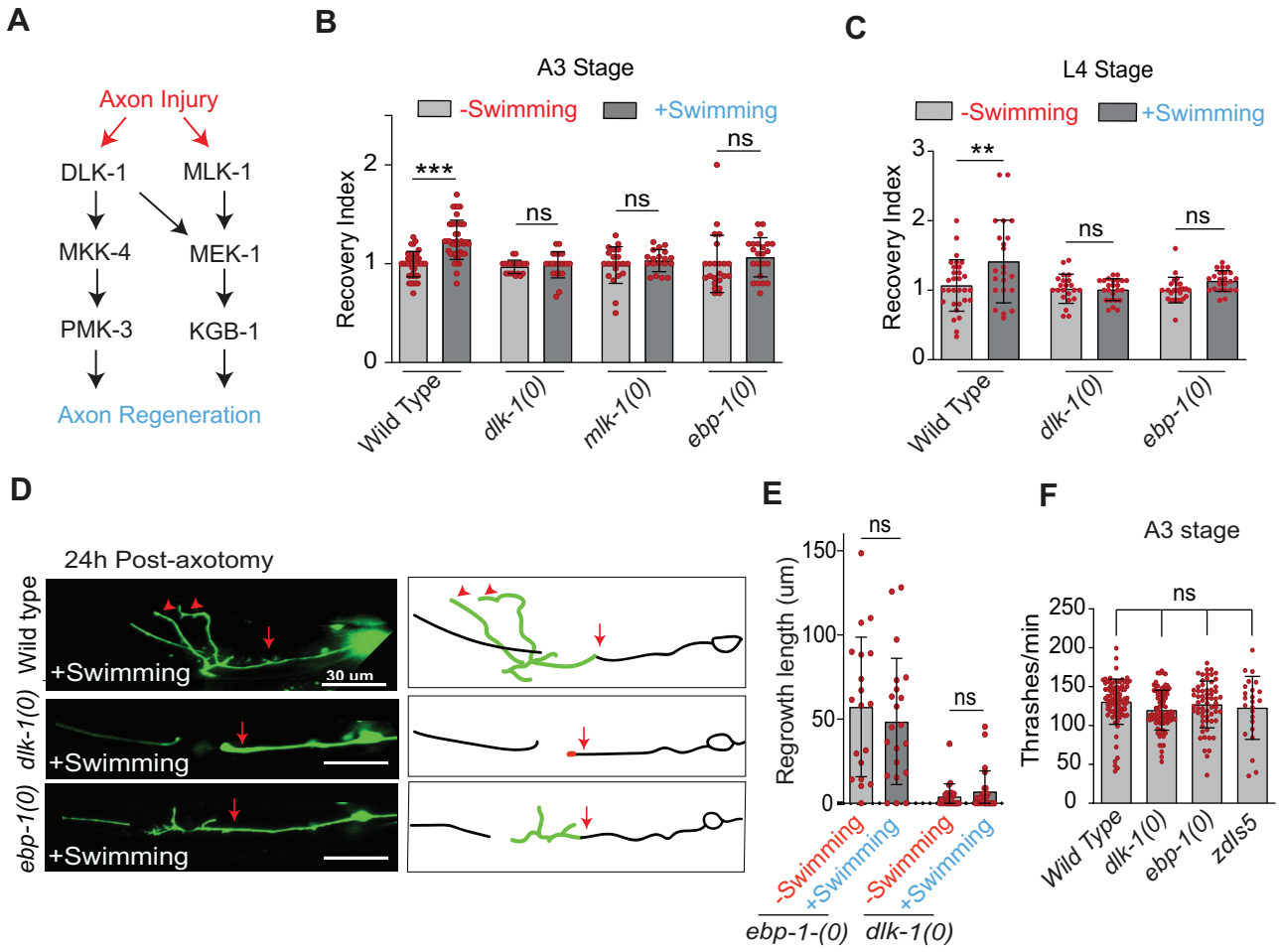

Figure 5

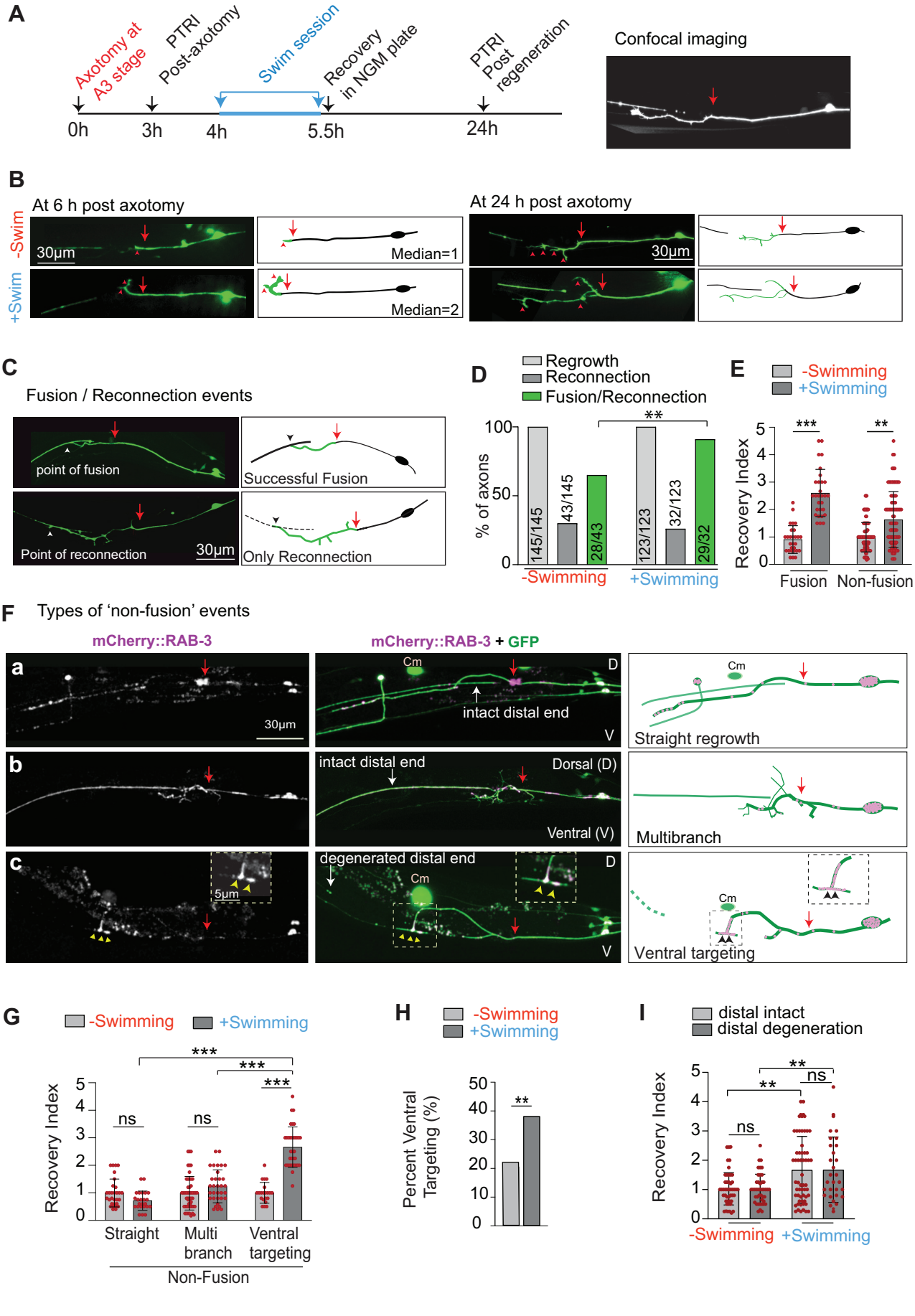

Figure 6

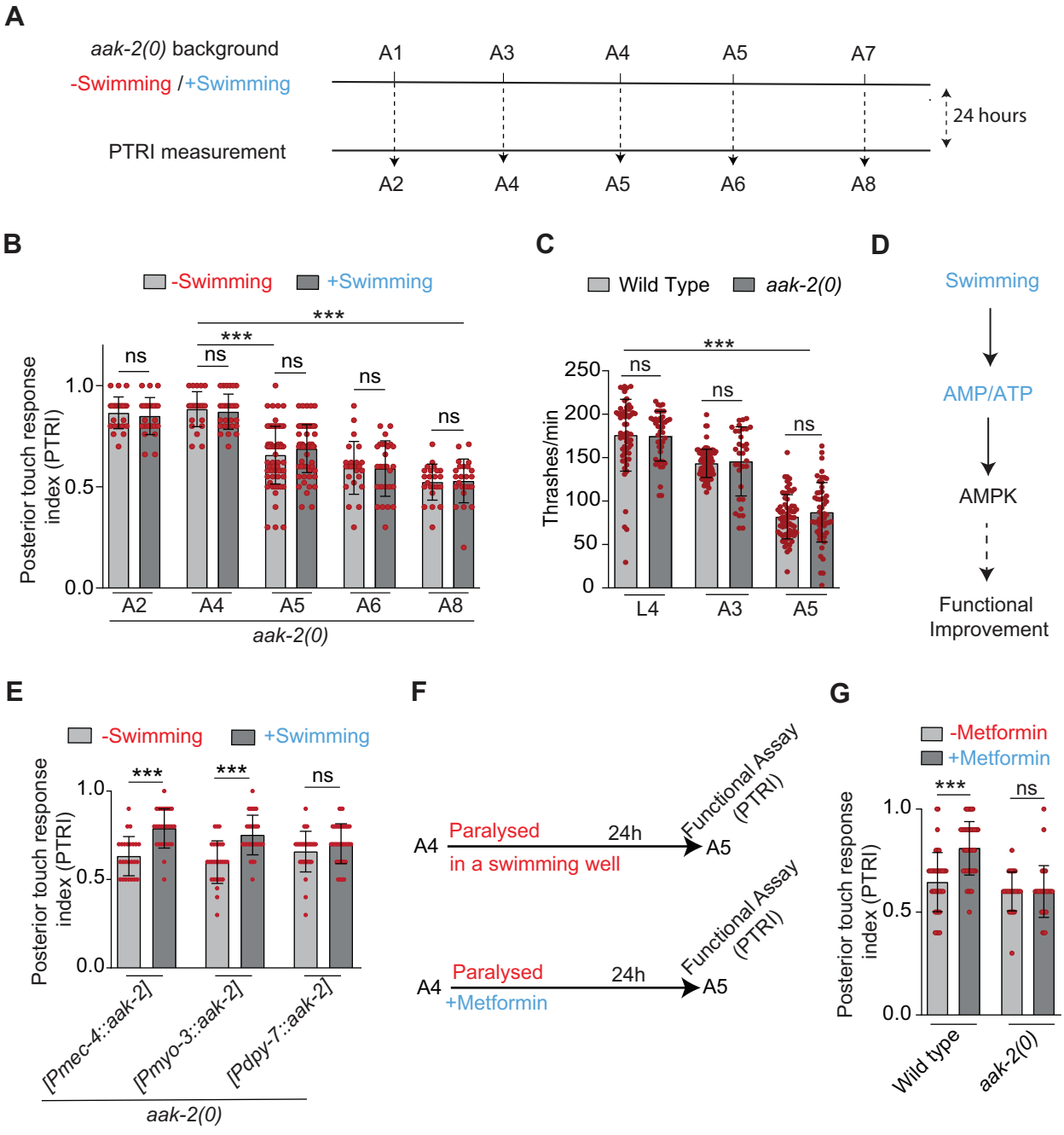

Figure 7

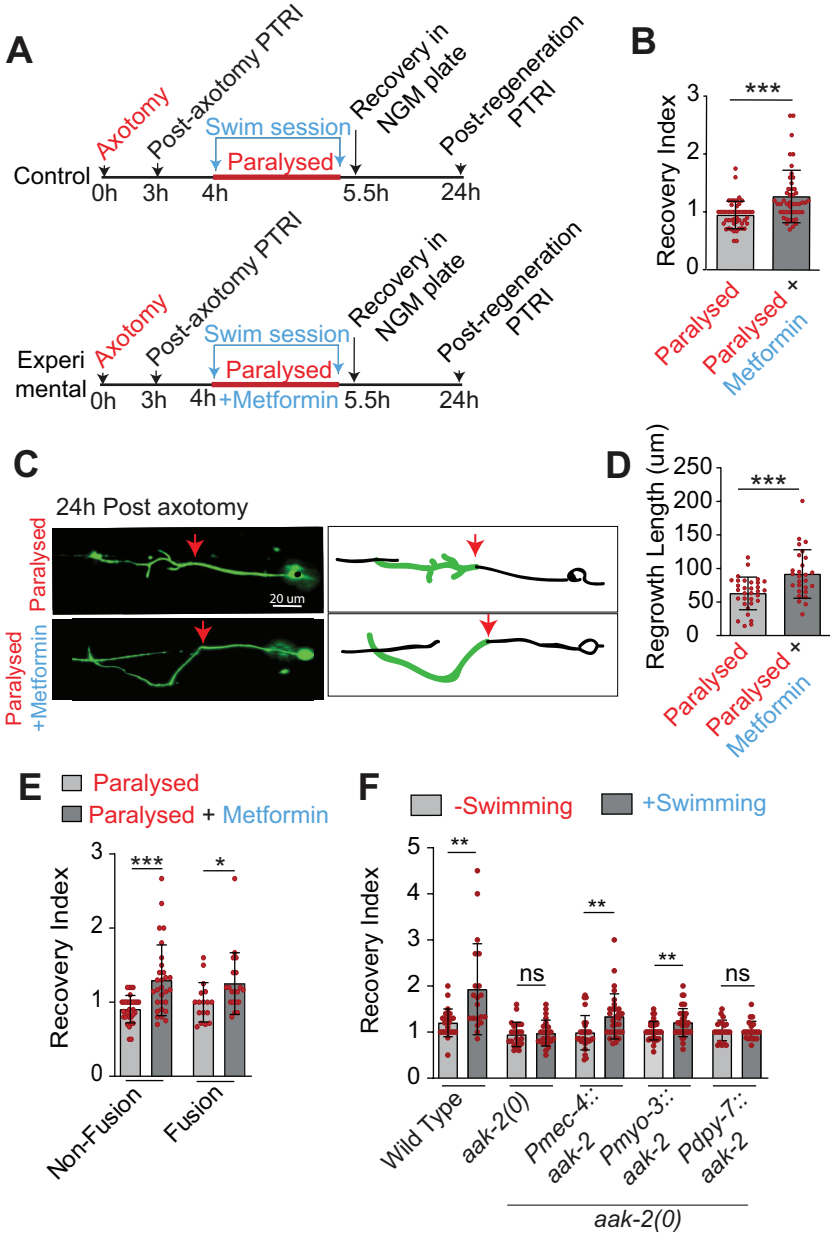

Figure 8