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Supplementary Information for

Swim exercise in *Caenorhabditis elegans* extends neuromuscular and gut healthspan, enhances learning ability, and protects against neurodegeneration

Ricardo Laranjeiro, Girish Harinath, Jennifer E. Hewitt, Jessica H. Hartman, Mary Anne Royal, Joel N. Meyer, Siva A. Vanapalli, and Monica Driscoll

Monica Driscoll
Email: Driscoll@dls.rutgers.edu

This PDF file includes:

Other supplementary materials for this manuscript include the following:

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SI Materials and Methods

Long-term swim exercise protocols

We used two different methods for our long-term swim exercise experiments: the picking method and the washing method.

For the picking method, we synchronized the population by allowing 30-40 gravid hermaphrodites to lay eggs on a 60 mm seeded NGM plate for 3h before removing the parents. After 64-68h, we began the long-term exercise regimens by picking approximately 60 Ad1 animals to a 60 mm unseeded NGM plate (non-exercise control) or to a 35 mm unseeded NGM plate flooded with 1.5 mL of M9 buffer (exercise). Note that we picked just a few animals at a time (1-5 animals) to minimize the transfer of bacteria to control and exercise plates. After 90 min, we picked animals from both conditions to 60 mm seeded NGM plates. We repeated this procedure multiple times over the first four days of adulthood depending, on the exercise regimen specified.

For the washing method, we synchronized the population by allowing 300-400 gravid hermaphrodites to lay eggs in a 100 mm seeded NGM plate for 3h before removing the parents. After 64-68h, we began the long-term exercise regimens by washing the Ad1 animals off the plate with M9 buffer into a 15 mL conical tube and allowing them to gravity settle (washing step performed by a gentle swirl of the plate to minimize bacterial transfer). Then, using a glass Pasteur pipette to minimize nematode loss, we split the pellet between a 100 mm unseeded NGM plate (non-exercise control) and a 100 mm unseeded NGM plate flooded with 8-10 mL of M9 buffer (exercise). After 90 min, animals from both conditions were washed off with M9 buffer into 15 mL conical tubes and allowed to settle under gravity. We transferred animals to 100 mm seeded NGM plates using a glass Pasteur pipette. We repeated this washing method multiple times over the first four days of adulthood depending, on the exercise regimen specified.

We performed swim exercise sessions at the following timings: 1+1+1+1 regimen, 1:00 PM each day; 2+2+2+2 regimen, 7:00 AM and 1:00 PM each day; 3+3+2+2 regimen, 7:00 AM, 1:00 PM, and 7:00 PM on the first two days, 7:00 AM and 7:00 PM on the last two days; 3+3+3+3 regimen, 7:00 AM, 1:00 PM, and 7:00 PM each day; 4+4+4+4 regimen, 7:00 AM, 1:00 PM, 7:00 PM, and 1:00 AM each day; 3+2 regimen, 7:00 AM, 1:00 PM, and 7:00 PM on the first day, 7:00 AM and 7:00 PM on the second day; 3+3 regimen, 7:00 AM, 1:00 PM, and 7:00 PM each day; 2+2+2 regimen, 7:00 AM and 7:00 PM each day.

RNA extraction and quantitative PCR

We performed RNA extraction and qPCR as previously described (1). Briefly, we collected *C. elegans* (20-40 animals per sample) into TRIzol Reagent (Thermo Fisher Scientific) and immediately froze animals in liquid nitrogen. After three freeze-thaw cycles, we extracted total RNA following the manufacturer's instructions (Thermo Fisher Scientific) and synthesized cDNA from 400 ng of total RNA using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific). We carried out qPCR using diluted cDNA, PerfeCTa SYBR Green FastMix (Quantabio), and 0.5 μM of gene-specific primers (SI Appendix, Table S2) in a 7500 Fast Real-Time PCR System (Thermo Fisher Scientific), using a two-step cycling protocol (3 seconds at 95°C, 30 seconds at 60°C) for 40 cycles. We calculated relative expression using the ΔΔCt method with *cdc-42* and Y45F10D.4 as reference genes.

Maximum and mean velocity

We measured maximum and mean velocity as previously described (2), with minor modifications. Briefly, we transferred single animals to a 60 mm unseeded NGM plate and immediately recorded a 30-second video at a rate of 20-25 frames per second with a Prosilica GC 1380 camera (Allied Vision) connected to a Macro Zoom 7000 lens (Navitar) using StreamPix 5 software (NorPix). We used approximately 20 animals per trial. After recording, we returned animals to a 60 mm seeded

NGM plate for re-use in later time points. Maximum and mean velocity were calculated with the ImageJ plugin wrMTrck (3).

Microfluidic assays

We synchronized the population by allowing 30-40 gravid hermaphrodites to lay eggs in a 60 mm seeded NGM plate for 3h before removing the parents. After 64-68h, we began the 3+3+2+2 regimen by inserting approximately 80 Ad1 animals (5-15 animals per chamber) in small chamber height microfluidics (non-exercise control) and in large chamber height microfluidics (exercise), both filled with M9 buffer. After 90 min, we washed animals from both conditions off the microfluidic chambers with M9 buffer and returned them to 60 mm seeded NGM plates. We repeated this procedure for a total of 10 times over the first four days of adulthood at the standard timings for the 3+3+2+2 regimen. We used microfluidic chambers with the following heights: 20 µm for Ad1 control animals, 30 µm for Ad2-4 control animals, and ~300 µm for all exercise animals. We recorded representative videos at a rate of 20 frames per second with a Prosilica GC 1380 camera (Allied Vision) connected to a Macro Zoom 7000 lens (Navitar) using StreamPix 5 software (NorPix).

Burrowing Assay

We prepared a 26% (w/v) Pluronic F-127 (Sigma-Aldrich) solution in water several days before the assay and stored it at 4°C to facilitate complete dissolving. The Pluronic F-127 solution remains liquid at cooler temperatures, but fully gels to a solid at room temperature. At least one hour before the burrowing assay, we placed the Pluronic F-127 solution at 14°C and allowed it to equilibrate before use (at this temperature it remains fully liquid). We carried out the burrowing assay at room temperature (20°C) by adding 30 µL of Pluronic F-127 solution to the center of a well in a 12-well plate. We immediately transferred approximately 40-45 animals to the 30 µL droplet using a nematode pick. Once the droplet was fully gelled, we added ~4 mL of additional Pluronic F-127 solution to form a final gel height of ~0.75 cm. After the top layer of Pluronic F-127 had fully gelled, we added 20 µL of *E. coli* OP50-1 chemoattractant solution (100 mg of *E. coli* OP50-1 per mL of liquid NGM) to the center of the gel surface (this was considered t = 0 min). The number of animals that reached the gel surface was scored at regular intervals up to 3h. For each trial, we performed the burrowing assay in triplicate.

2D chemotaxis assay

For the 2D chemotaxis assay, we divided 100 mm unseeded chemotaxis agar plates (2% agar, 25 mM potassium phosphate, 1 mM calcium chloride, 1 mM magnesium sulfate, and 10 µg/ml nystatin) in two halves and spotted 5 µl of *E. coli* OP50-1 chemoattractant solution (100 mg of *E. coli* OP50-1 per mL of liquid NGM) at the edge of one half and 5 µl of water at the edge of the other half. After moving animals to an unseeded NGM plate to get rid of bacteria, we transferred 40-60 animals to the center of the chemotaxis plate and incubated for 1h at 20°C. We calculated chemotaxis index (CI) as follows: CI = (animal number at OP50-1 – animal number at water) / (total animal number – immobile animal number at origin).

Confocal microscopy

We fixed *C. elegans* in 2% paraformaldehyde/phosphate buffered saline for 30 min at room temperature and then stored them at 4°C in M9 buffer until imaging. We performed confocal imaging with a CSU-X1 spinning disk unit (Yokogawa) mounted to an Axio Imager Z1 microscope (ZEISS) using MetaMorph Premier software (Molecular Devices). For the $P_{myo\text{-}3}$*mitoGFP* animals (SJ4103 strain), we imaged two different regions of the anterior body wall muscle per animal, and scored images blind to exercise condition using Blinder software (4). For the animals with GFP-labeled GABAergic motor neurons in the pro-aggregant Tau background (BR5707 strain), we

used 30-40 animals per trial and quantified the total number of gaps in the ventral and dorsal cords per animal. We performed quantification blind to exercise condition.

Oxygen consumption rates

We measured oxygen consumption rates as previously described (5), with some modifications. We exercised *C. elegans* with the washing method and, on the day of the assay, we washed animals three times with M9 buffer in a 15 mL conical tube to get rid of bacteria. We transferred 20-50 animals to each well of a Seahorse XF24 Cell Culture Microplate (Agilent) with 150 µL of M9 buffer, leaving 2-4 wells for blanks. We added 375 µL of EPA water (a lower-saline solution to ensure solubility of drugs (6)) to each well. We performed oxygen consumption rate measurements in a Seahorse XFe24 Analyzer (Agilent). We measures basal respiration first in every well, followed by injection of Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) in half of the wells to uncouple mitochondria or dicyclohexylcarbodiimide (DCCD) in the other half of the wells to inhibit ATP synthesis. We injected sodium azide at the end of the assay in every well to completely block mitochondrial respiration. All respiration parameters were normalized to the number of animals per well.

Lifespan assay

After a long-term exercise regimen, we started a lifespan assay with approximately 45-55 animals per 60 mm NGM plate. We scored and moved animals to fresh plates daily until Ad8 and every two days after that. Animals were considered dead when they mounted no response to vigorous prodding; we censored animals that bagged or crawled off the plates.

Mid-life survival (from Ad12 to Ad20) of exercised animals increased for the 3+3+2+2 regimen and decreased for the 4+4+4+4 regimen relative to non-exercised controls. During this period, the percentage survival increase for the 3+3+2+2 exercise population ranged from ~4% on Ad12 to ~20% on Ad16, whereas the percentage survival decrease for the 4+4+4+4 exercise population ranged from ~15% on Ad12 to ~7% on Ad20. We calculated the average survival difference for exercised animals using Ad12, Ad14, Ad16, Ad18, and Ad20 values.

Pharyngeal pumping rate

We recorded 30-second videos at a rate of 30 frames per second of *C. elegans* on seeded NGM with a Lt225 camera (Lumenera) connected to an Axiovert 35M microscope (ZEISS) using StreamPix 6 software (NorPix). We used approximately 25 animals per trial.

To quantify pharyngeal pumping in the liquid environment during an exercise session, we recorded 30-second videos (rate of 30 frames per second) of Ad2 WT animals crawling on an unseeded NGM plate (45 min after transfer to unseeded plate) or swimming in M9 buffer (45 min after transfer to liquid) with a Revolve microscope (Echo). We used 6-11 animals per trial. We manually counted the number of pharyngeal pumps during the 30 seconds using ImageJ.

Smurf assay

We performed Smurf assays as previously described (7), with minor modifications. Briefly, we incubated 25-40 animals for 3h at 20°C in 5% (w/v) of blue food dye (Erioglaucine disodium salt, Sigma-Aldrich) in *E. coli* OP50-1 liquid culture grown overnight in lysogeny broth (LB). We then transferred the animals to an unseeded NGM plate and immobilized them with 1M sodium azide before scoring for intestinal leakage in a SZX2 microscope (Olympus). We collected representative images using a 14MP MU1403 microscope digital camera (AmScope); AmScope 3.7 software (AmScope). Animals with blue dye in the germline were not considered Smurf animals unless the dye was also observed in the body cavity.

Associative learning assay

We performed associative learning assays as previously described (8, 9), with some modifications. We exercised *C. elegans* with the washing method and, on the day of the assay, we washed animals off the plate with M9 buffer into a 15 mL conical tube. After three washes with M9 buffer, we used some animals for naïve chemotaxis assay and starved the rest in 4 mL of M9 buffer for 1h. After starvation, animals were conditioned in a 100 mm seeded NGM plate with three 2 µL streaks of 10% butanone (Sigma-Aldrich) in absolute ethanol placed on the inside of the lid. We parafilmed the plate and incubated it for 1h at room temperature. We washed animals off the plate with M9 buffer into a 15 mL conical tube. After two washes with M9 buffer, we used some animals for the 0h post-conditioning chemotaxis assay and split the rest between two 100 mm seeded NGM plates (hold plates). After 0.5h or 1h at room temperature, we washed animals off the hold plates with M9 buffer into a 15 mL conical tube, washed them two additional times with M9 buffer, and used them for the 0.5h and 1h post-conditioning chemotaxis assays.

For the chemotaxis assays, we marked 100 mm unseeded NGM plates with spots on the bottom (origin) and each side of the plate (9). We spotted 1 µL of 10% butanone in absolute ethanol at one side of the plate and 1 µL of 1:1000 isoamyl alcohol (Sigma-Aldrich) in absolute ethanol at the other side of the plate. We also spotted 1 µL of 1M sodium azide at each side of the plate to paralyze animals that reached the odorant spots. We transferred approximately 100-200 animals with a glass Pasteur pipette to the origin spot on each plate, and incubated for 1h at room temperature. We performed two technical replicates for each chemotaxis assay. We took images with a Prosilica GC 1380 camera (Allied Vision) connected to a Macro Zoom 7000 lens (Navitar) using StreamPix 5 software (NorPix) at the start of the assay to count the total number of animals, and at the end of the assay to count the number of animals at each spot. We calculated chemotaxis index (CI) as follows: CI = (animal number at butanone – animal number at isoamyl alcohol) / (total animal number – immobile animal number at origin). Learning index (LI) was calculated as follows: $LI = CI_{Post\text{-}conditioning} - CI_{Naïve}$.

We found it essential to use isoamyl alcohol for the chemotaxis assays because Ad5 naïve animals were strongly attracted to butanone when absolute ethanol was used as the control odorant. Conversely, Ad5 naïve animals were attracted almost exclusively to isoamyl alcohol rather than butanone, which vastly increased the dynamic range over which to test how exercise affected the food-butanone association.

Chemotaxis assay toward benzaldehyde

We maintained the CL2355 strain, which enables temperature-sensitive expression of neuronal $A\beta_{1\text{-}42}$, at 16°C. We raised animals at 23°C (i.e. expression of $A\beta_{1\text{-}42}$ induced) from the egg stage onward for the exercise experiments. For the chemotaxis assay, we divided 60 mm unseeded NGM plates in two halves and spotted 1 µL of 0.1% benzaldehyde (Sigma-Aldrich) in absolute ethanol at the edge of one half and 1 µL of absolute ethanol at the edge of the other half. After moving animals to an unseeded NGM plate to get rid of bacteria, we transferred 20-25 animals to the center of the chemotaxis plate and incubated for 2h at 23°C. We performed two technical replicates for each chemotaxis assay. We calculated chemotaxis index (CI) as follows: CI = (animal number at benzaldehyde half – animal number at ethanol half) / (total animal number – immobile animal number at origin).

Touch sensitivity assay

We performed touch sensitivity assays as previously described (10), with minor modifications. Briefly, animals were scored by a gentle touch with a thin platinum wire (0.025 mm of diameter, Alfa Aesar) in the anterior region followed by a gentle touch in the posterior region a few seconds later. We repeated this procedure for a total of 5 anterior and 5 posterior touches per animal.

Normally, a positive touch response resulted in animals backing away from the touch. We scored animals blind to exercise condition.

Statistical analyses

We used log-rank test to compare the proportion over time of control vs. exercise populations that reached a specific phenotype (burrowing and lifespan assays). We used chi-square test to compare the distribution (actual number of subjects) into multiple categories of control vs. exercise populations (muscle mitochondrial morphology and touch sensitivity assays). For all other assays, we used two-tailed Student's *t* test to determine statistical significance given the comparison of control vs. exercise means for a single time point. We used a paired Student's *t* test when control and exercise values consisted of matched pairs, whereas unpaired Student's *t* test was used when control and exercise values were compiled from multiple trials.

The specific number of data points and the statistical test used for each figure in the manuscript are as follows:

Fig. 1C. Number of independent trials performed: $n_{1+1+1+1} = 4$, $n_{2+2+2+2} = 4$, $n_{3+3+2+2} = 5$, $n_{3+3+3+3} = 4$, $n_{4+4+4+4} = 4$. We used 30-40 animals per sample in each trial and we determined statistical significance by paired two-tailed Student's *t* test.

Fig. 1D. Number of independent trials performed: $n_{\text{Ad5 } 1+1+1+1} = 4$, $n_{\text{Ad8 } 1+1+1+1} = 4$, $n_{\text{Ad5 } 2+2+2+2} = 3$, $n_{\text{Ad8 } 2+2+2+2} = 3$, $n_{\text{Ad5 } 3+3+2+2} = 5$, $n_{\text{Ad8 } 3+3+2+2} = 6$, $n_{\text{Ad5 } 3+3+3+3} = 4$, $n_{\text{Ad8 } 3+3+3+3} = 4$, $n_{\text{Ad5 } 4+4+4+4} = 3$, $n_{\text{Ad8 } 4+4+4+4} = 3$. We used ~20 animals per sample in each trial and determined statistical significance by paired two-tailed Student's *t* test.

Fig. 2B. Number of animals used for analysis: $n_{\text{Control}} = 392$, $n_{\text{Exercise}} = 370$. We compiled data from 3 independent trials and determined statistical significance by a log-rank test comparing control vs. exercise curves.

Fig. 2D. Number of body wall muscle images used for analysis: $n_{\text{Ad5 Control}} = 170$, $n_{\text{Ad5 Exercise}} = 160$, $n_{\text{Ad8 Control}} = 160$, $n_{\text{Ad8 Exercise}} = 162$, $n_{\text{Ad11 Control}} = 160$, $n_{\text{Ad11 Exercise}} = 160$. We compiled data from 4 independent trials and determined statistical significance by chi-square test.

Fig. 3A-G. Number of Seahorse XF24 Microplate wells used for analysis: $n_{\text{Ad5 Control}} = 30$ in B, 14 in C and D, 15 in E, F, and G, $n_{\text{Ad5 Exercise}} = 30$ in B, 15 in C, D, E, F, and G, $n_{\text{Ad8 Control}} = 30$ in B, 13 in C, 14 in D, E, F, and G, $n_{\text{Ad8 Exercise}} = 30$ in B, 13 in C, 14 in D and E, 15 in F and G. We compiled data from 3 independent trials. We used 20-50 animals per well in each trial. We determined statistical significance by unpaired two-tailed Student's *t* test.

Fig. 3H. Number of animals used for analysis: $n_{\text{Control}} = 245$, $n_{\text{Exercise}} = 221$. We compiled data from 3 independent trials and determined statistical significance by a log-rank test comparing control vs. exercise survival curves.

Fig. 4A. Number of animals used for analysis: $n_{\text{Ad5 Control}} = 66$, $n_{\text{Ad5 Exercise}} = 66$, $n_{\text{Ad8 Control}} = 76$, $n_{\text{Ad8 Exercise}} = 77$, $n_{\text{Ad11 Control}} = 85$, $n_{\text{Ad11 Exercise}} = 87$, $n_{\text{Ad15 Control}} = 75$, $n_{\text{Ad15 Exercise}} = 69$. We compiled data from 3 independent trials and determined statistical significance by unpaired two-tailed Student's *t* test.

Fig. 4E. Number of independent trials performed: $n_{\text{Ad8}} = 4$, $n_{\text{Ad11}} = 8$, $n_{\text{Ad15}} = 8$. We used 25-40 animals per sample in each trial and determined statistical significance by paired two-tailed Student's *t* test.

Fig. 5B. $n = 8$ independent trials. We used 200-400 animals per sample in each trial. We determined statistical significance by paired two-tailed Student's *t* test.

Fig. 6A. Number of animals used for analysis: $n_{\text{Ad5 Control}} = 59$, $n_{\text{Ad5 Exercise}} = 60$, $n_{\text{Ad8 Control}} = 60$, $n_{\text{Ad8 Exercise}} = 60$, $n_{\text{Ad11 Control}} = 54$, $n_{\text{Ad11 Exercise}} = 57$. We compiled data from 3 independent trials and determined statistical significance by unpaired two-tailed Student's *t* test.

Fig. 6C. n = 5 independent trials of 30-40 animals per sample in each trial. We determined statistical significance by paired two-tailed Student's t test.

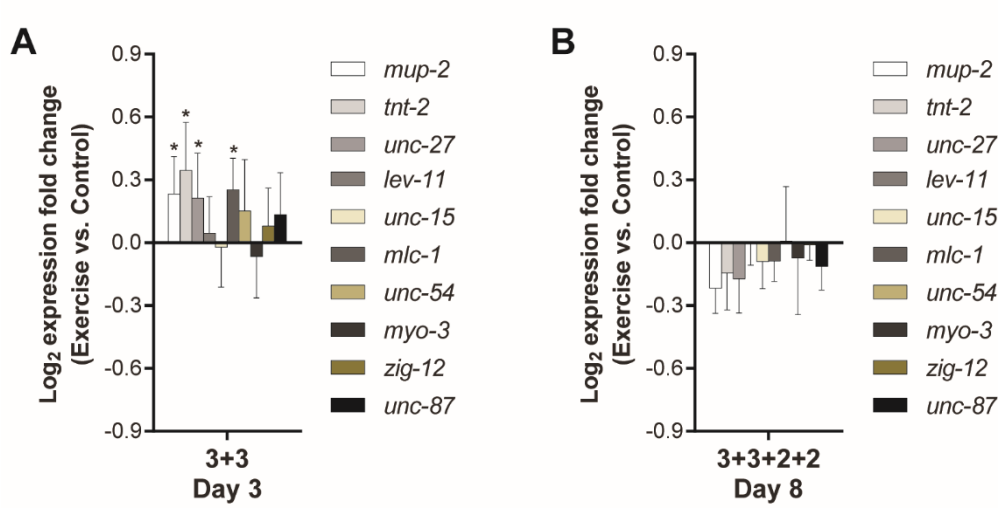
Fig. 6D. n = 5 independent trials of 40-50 animals per sample in each trial. We determined statistical significance by paired two-tailed Student's t test.

Fig. 6E. Number of animals used for analysis: $n_{\text{Ad5 Control}}$ = 248, $n_{\text{Ad5 Exercise}}$ = 233, $n_{\text{Ad8 Control}}$ = 155, $n_{\text{Ad8 Exercise}}$ = 147, $n_{\text{Ad11 Control}}$ = 122, $n_{\text{Ad11 Exercise}}$ = 119. We compiled data from 5 independent trials and determined statistical significance by chi-square test.

Fig. 7B. Number of animals used for analysis: $n_{\text{Ad9 Control}}$ = 60, $n_{\text{Ad9 Exercise}}$ = 60, $n_{\text{Ad12 Control}}$ = 60, $n_{\text{Ad12 Exercise}}$ = 59. We compiled data from 3 independent trials and determined statistical significance by unpaired two-tailed Student's t test.

Fig. 7C. Number of animals used for analysis: $n_{\text{Ad9 Control}}$ = 60, $n_{\text{Ad9 Exercise}}$ = 60, $n_{\text{Ad12 Control}}$ = 59, $n_{\text{Ad12 Exercise}}$ = 60. We compiled data from 3 independent trials and determined statistical significance by unpaired two-tailed Student's t test.

Fig. S1



C

Exercise regimen	Day of adulthood	Maximum velocity (mm/s) (mean ± s.e.m.) Control	Exercise
1+1+1+1	5	0.263 ± 0.006	0.281 ± 0.014
	8	0.218 ± 0.007	0.224 ± 0.015
2+2+2+2	5	0.290 ± 0.012	0.331 ± 0.015
	8	0.230 ± 0.003	0.254 ± 0.015
3+3+2+2	5	0.270 ± 0.007	0.300 ± 0.012
	8	0.200 ± 0.011	0.246 ± 0.007
3+3+3+3	5	0.281 ± 0.016	0.321 ± 0.015
	8	0.219 ± 0.020	0.249 ± 0.017
4+4+4+4	5	0.261 ± 0.027	0.311 ± 0.017
	8	0.188 ± 0.009	0.220 ± 0.015

Figure S1. (**A, B**) qPCR results for 10 genes encoding proteins important for muscle contraction in Ad3 WT animals exposed to the 3+3 regimen (**A**) and in Ad8 WT animals exposed to the 3+3+2+2 regimen (**B**). Expression data are presented as the $\log_2$ fold change of exercise samples relative to control samples. Number of independent trials performed: $n_{\text{Ad3 3+3}} = 4$, $n_{\text{Ad8 3+3+2+2}} = 5$. We used 25-40 animals per sample in each trial and determined statistical significance by paired two-tailed Student's *t* test. (**C**) Maximum velocity values of Ad5 and Ad8 WT animals exposed to the five tested long-term exercise regimens. Note that these values were used to calculate the percentage increase in maximum velocity of exercised animals presented in Fig. 1D. Number of independent trials performed: $n_{\text{Ad5 1+1+1+1}} = 4$, $n_{\text{Ad8 1+1+1+1}} = 4$, $n_{\text{Ad5 2+2+2+2}} = 3$, $n_{\text{Ad8 2+2+2+2}} = 3$, $n_{\text{Ad5 3+3+2+2}} = 5$, $n_{\text{Ad8 3+3+2+2}} = 6$, $n_{\text{Ad5 3+3+3+3}} = 4$, $n_{\text{Ad8 3+3+3+3}} = 4$, $n_{\text{Ad5 4+4+4+4}} = 3$, $n_{\text{Ad8 4+4+4+4}} = 3$. We used ~20 animals per sample in each trial. *$P \leq 0.05$.

Fig. S2

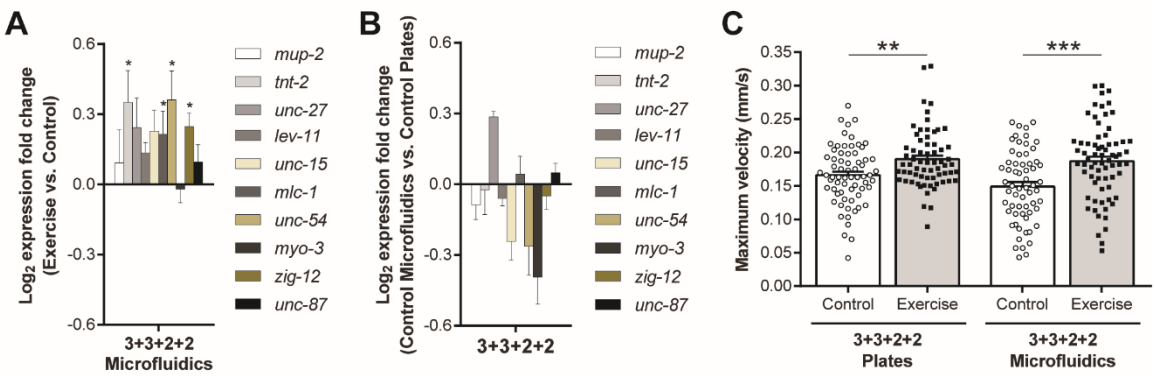


Figure S2. (**A**) qPCR results for 10 genes encoding proteins important for muscle contraction in Ad5 WT animals that executed the 3+3+2+2 regimen in microfluidic devices. Expression data are presented as the log2 fold change of exercise samples (microfluidic device permissive for swimming) relative to control samples (microfluidic device that restricts locomotion to prevent swimming in the liquid environment of the chamber). *n* = 4 independent trials. We used ~20 animals per sample in each trial and we determined statistical significance by paired two-tailed Student's *t* test. (**B**) qPCR results for 10 genes encoding proteins important for muscle contraction in Ad5 WT animals. Expression data are presented as the log2 fold change of control microfluidic samples relative to control plate samples. *n* = 4 independent trials. We used ~20 animals per sample in each trial and we determined statistical significance by paired two-tailed Student's *t* test. (**C**) Crawling maximum velocity of Ad8 WT animals exposed to the 3+3+2+2 regimen on NGM plates (left) and in microfluidic devices (right). Each point represents a single animal. Number of animals used for analysis: $n_{\text{Control Plates}}$ = 67, $n_{\text{Exercise Plates}}$ = 66, $n_{\text{Control Microfluidics}}$ = 62, $n_{\text{Exercise Microfluidics}}$ = 70. We compiled data from 4 independent trials and determined statistical significance by unpaired two-tailed Student's *t* test. **P*≤0.05, ***P*≤0.01, ****P*≤0.001.

Fig. S3

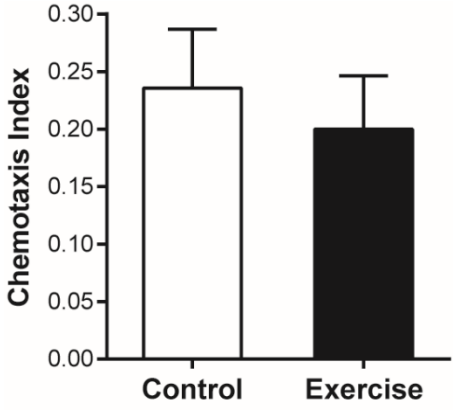


Figure S3. 2D chemotaxis index toward OP50-1 of Ad5 WT animals exposed to the 3+3+2+2 regimen. For the chemotaxis assay, we divided 100 mm unseeded chemotaxis agar plates into two halves and spotted *E. coli* OP50-1 chemoattractant solution (100 mg of *E. coli* OP50-1 per mL of liquid NGM) at the edge of one half and water at the edge of the other half. We calculated the chemotaxis index (CI) as follows: CI = (animal number at OP50-1 – animal number at water) / (total animal number – immobile animal number at origin). *n* = 3 independent trials. We used 40-60 animals per sample in each trial and determined statistical significance by paired two-tailed Student's *t* test.

Fig. S4

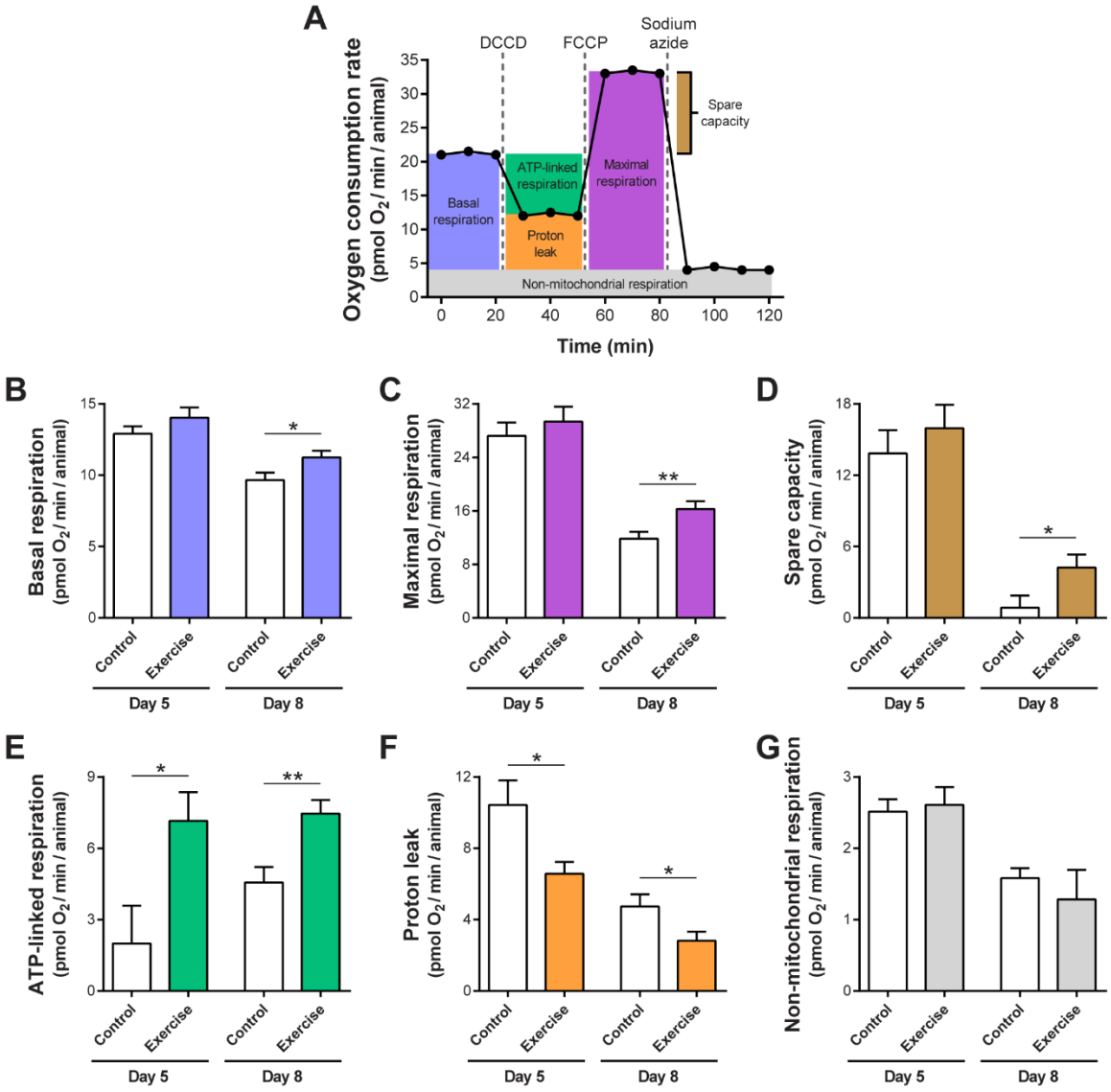
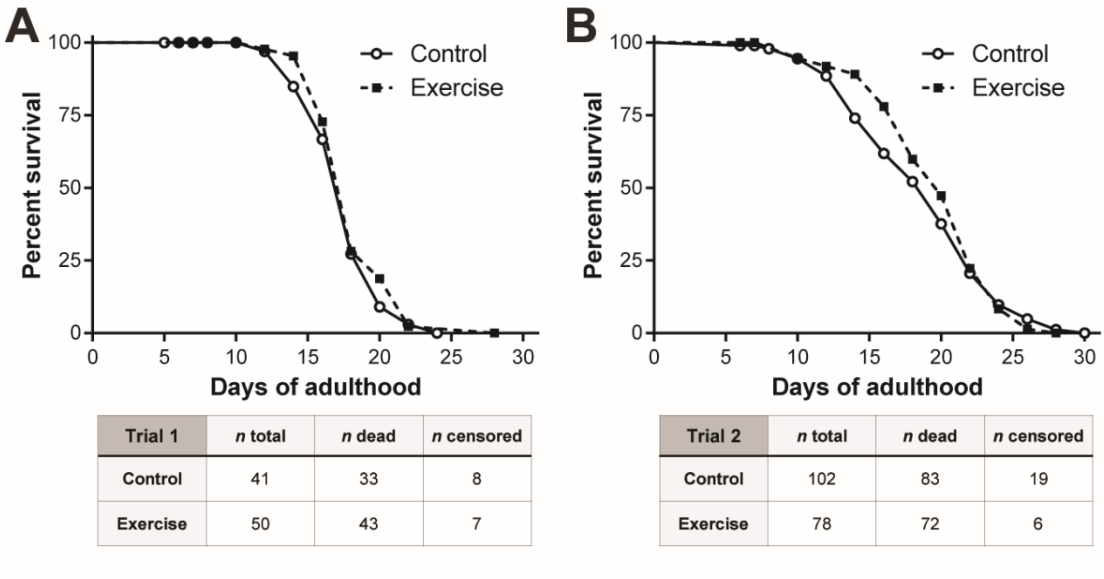


Figure S4. (**A**) Diagram of oxygen consumption rates measured in a Seahorse XFe24 Analyzer consequent to addition of mitochondrial inhibitors. In the Seahorse protocol for measuring oxygen consumption rates, basal respiration is first monitored, followed by addition of DCCD, FCCP, and sodium azide, allowing for the calculation of the six respiration parameters represented by different colors. (**B-G**) basal respiration (**B**), maximal respiration (**C**), spare capacity (**D**), ATP-linked respiration (**E**), proton leak (**F**), and non-mitochondrial respiration (**G**) values of Ad5 and Ad8 WT animals exposed to the 4+4+4+4 regimen. Number of Seahorse XF24 Microplate wells used for analysis: $n_{\text{Ad5 Control}}$ = 36 in **B**, 18 in **C**, **D**, **E**, **F**, and **G**, $n_{\text{Ad5 Exercise}}$ = 44 in **B**, 22 in **C**, **D**, **E**, **F**, and **G**, $n_{\text{Ad8 Control}}$ = 39 in **B**, 20 in **C**, 19 in **D**, **E**, **F**, and **G**, $n_{\text{Ad8 Exercise}}$ = 41 in **B**, 22 in **C** and **D**, 19 in **E**, **F**, and **G**. We compiled data from 4 independent trials of 20-25 animals per well per trial. We determined statistical significance by unpaired two-tailed Student's *t* test. **P*≤0.05, ***P*≤0.01.

Fig. S5



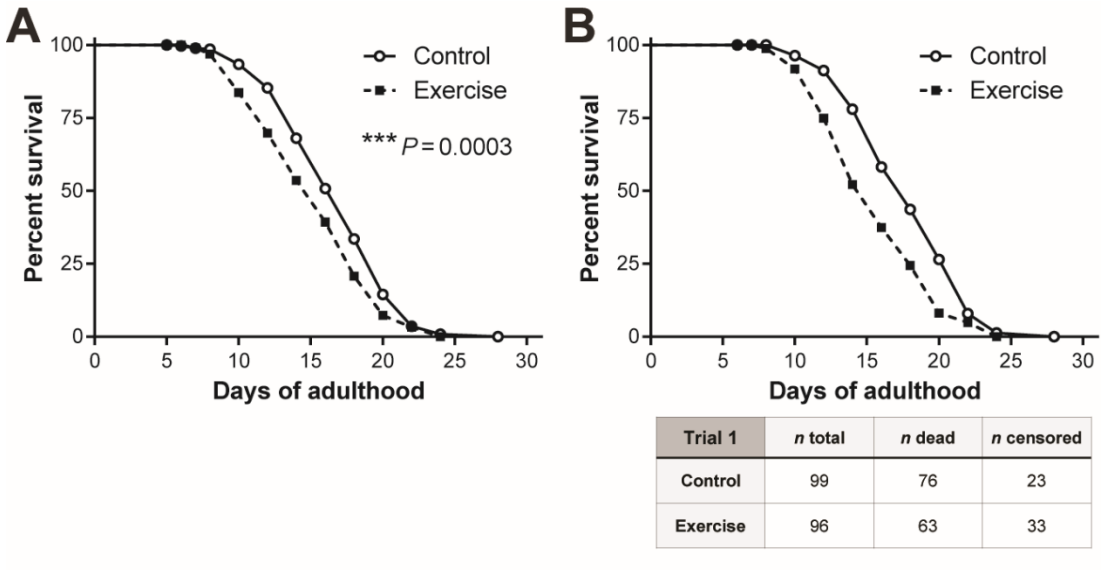
Trial 1	*n* total	*n* dead	*n* censored
Control	41	33	8
Exercise	50	43	7

Trial 2	*n* total	*n* dead	*n* censored
Control	102	83	19
Exercise	78	72	6

Trial 3	*n* total	*n* dead	*n* censored
Control	102	81	21
Exercise	93	71	22

Figure S5. (**A-C**) Survival curves from the three independent trials performed with WT animals exposed to the 3+3+2+2 regimen. The number of animals and their outcome are indicated for each trial.

Fig. S6



Trial 1	*n* total	*n* dead	*n* censored
Control	99	76	23
Exercise	96	63	33

Trial 2	*n* total	*n* dead	*n* censored
Control	95	68	27
Exercise	118	88	30

Trial 3	*n* total	*n* dead	*n* censored
Control	103	78	25
Exercise	113	71	42

Figure S6. (**A**) Survival curve of WT animals exposed to the 4+4+4+4 regimen. Number of animals used for analysis: $n_{Control}$ = 297, $n_{Exercise}$ = 327. We compiled data from 3 independent trials and determined statistical significance by a log-rank test comparing control vs. exercise survival curves (P = 0.0003). (**B-D**) Survival curves from the three independent trials performed with WT animals exposed to the 4+4+4+4 regimen, with number of animals and their outcome indicated for each trial.

Fig. S7

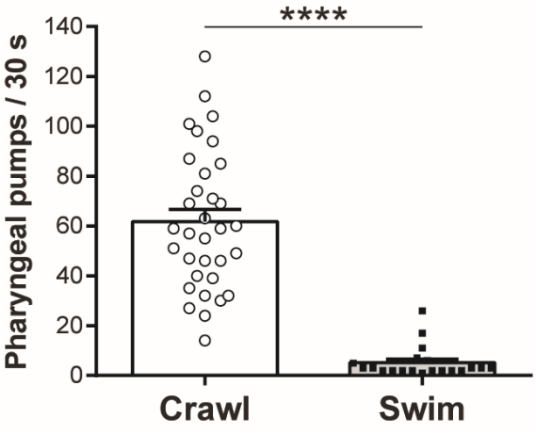


Figure S7. Pharyngeal pumping rate of Ad2 WT animals crawling on an unseeded NGM plate (45 min after transfer to unseeded plate) and swimming in M9 buffer (45 min after transfer to liquid). Note that animals used for this quantification were not exposed to a previous exercise regimen. Each point represents a single animal. Number of animals used for analysis: n_{Crawl} = 33, n_{Swim} = 20. We compiled data from 3 independent trials and determined statistical significance by unpaired two-tailed Student's *t* test. ****$P \leq 0.0001$.

Fig. S8

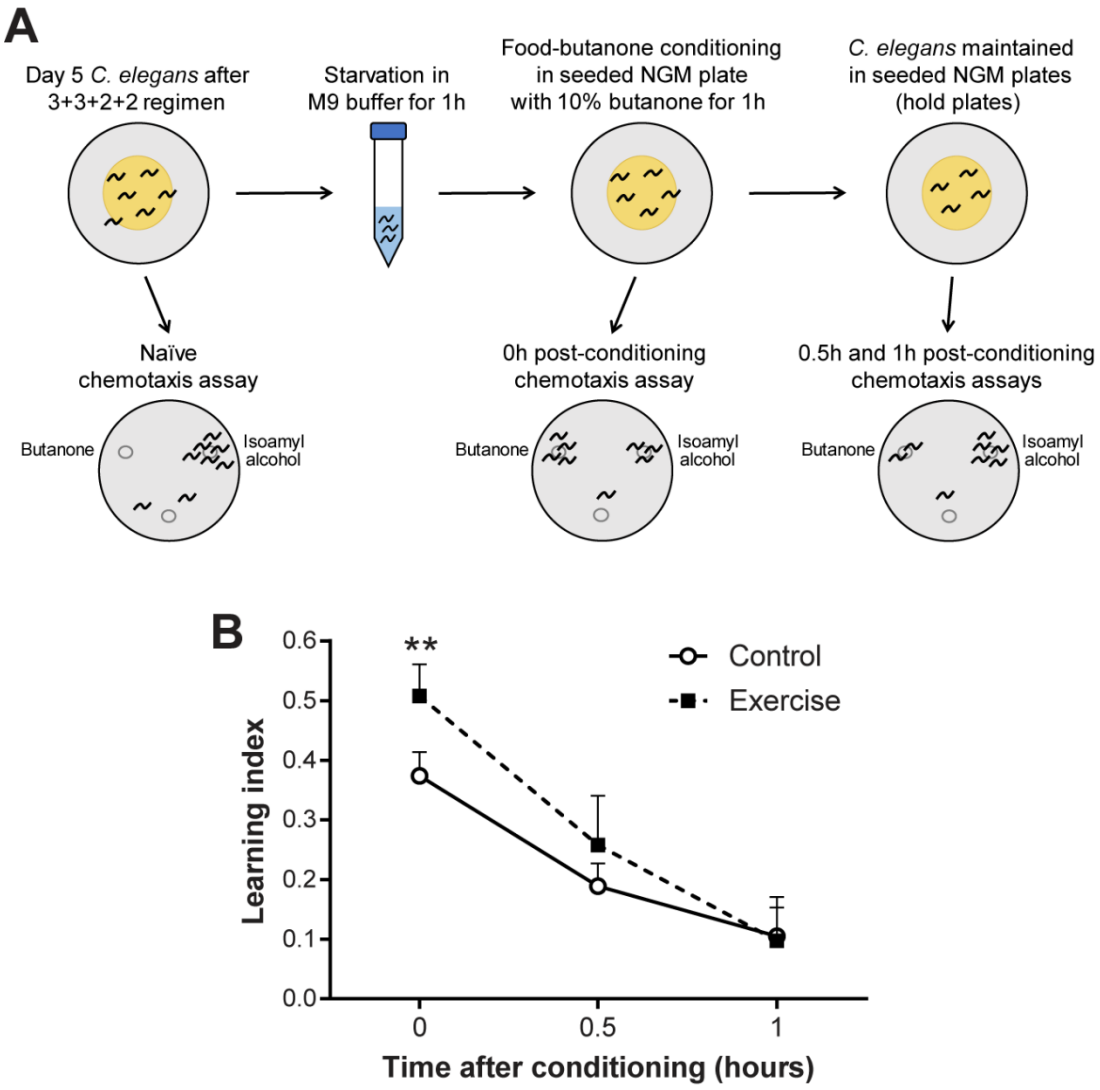


Figure S8. (**A**) Diagram of the associative learning assay. After the 3+3+2+2 regimen, we starved Ad5 animals in M9 buffer for 1h followed by food-butanone conditioning in a seeded NGM plate with 10% butanone solution on the inside of the lid. After conditioning, we maintained animals on seeded NGM plates (hold plates). At different points of the protocol, we used part of the nematode population for naïve and post-conditioning chemotaxis assays. We performed chemotaxis assays by testing animal attraction to 10% butanone vs. 1:1000 isoamyl alcohol. Naïve animals are attracted almost exclusively to isoamyl alcohol whereas a significant proportion of food-butanone conditioned animals choose butanone over isoamyl alcohol. (**B**) Learning index of Ad5 WT animals for up to 1h post-conditioning after exposure to the 3+3+2+2 regimen. Chemotaxis index (CI) = (animal number at butanone – animal number at isoamyl alcohol) / (total animal number – immobile animal number at origin). We calculated learning index by subtraction of naïve CI from post-conditioning CI. Number of independent trials performed: n_{0h} = 8, $n_{0.5h}$ = 4, n_{1h} = 4. We used 200-400 animals per sample in each trial and determined statistical significance by paired two-tailed Student's *t* test. ***P*≤0.01.

Fig. S9

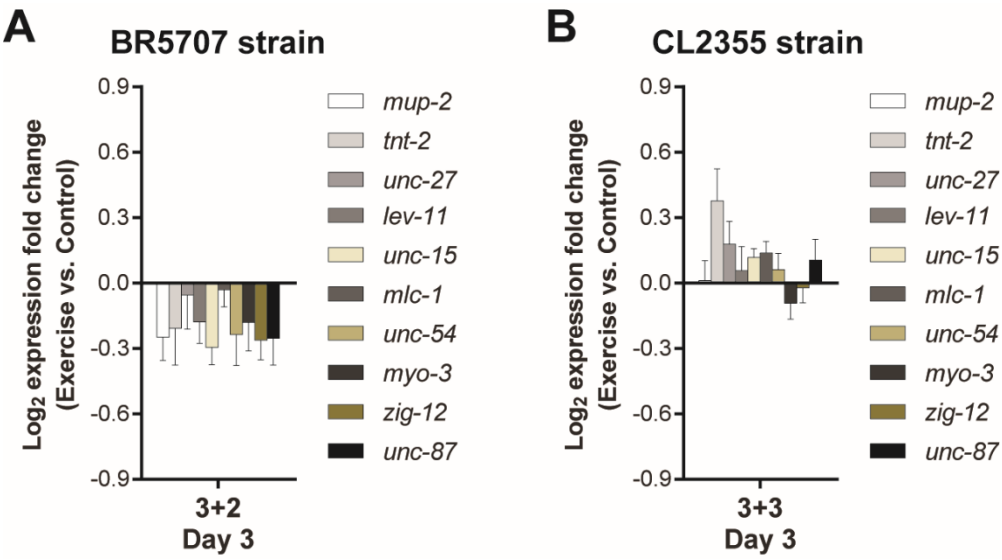


Figure S9. (**A, B**) qPCR results for 10 genes encoding proteins important for muscle contraction in Ad3 animals expressing aggregating Tau in all neurons and GFP in GABAergic motor neurons (BR5707 strain) exposed to the 3+2 regimen (**A**) and in Ad3 animals expressing neuronal $A\beta_{1-42}$ (CL2355 strain) exposed to the 3+3 regimen (**B**). Note that BR5707 animals did not exhibit a standard swimming motion due to a severe uncoordinated phenotype and CL2355 animals crawled and swam slower than WT animals. Expression data are presented as the $\log_2$ fold change of exercise samples relative to control samples. n = 4 independent trials of 25-40 animals per sample per trial. We determined statistical significance by paired two-tailed Student's t test.

Fig. S10

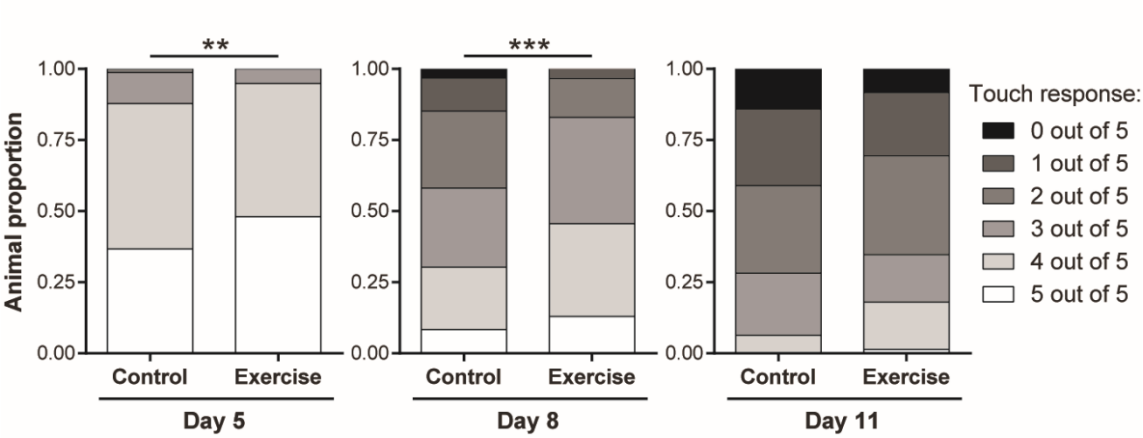


Figure S10. Posterior touch sensitivity of Ad5, Ad8, and Ad11 animals expressing polyQ128 in the touch receptor neurons ($P_{mec\text{-}3}$*htt57Q128*) exposed to the 3+3+2+2 regimen. We scored animals blind to exercise condition. Number of animals used for analysis: $n_{\text{Ad5 Control}}$ = 248, $n_{\text{Ad5 Exercise}}$ = 233, $n_{\text{Ad8 Control}}$ = 155, $n_{\text{Ad8 Exercise}}$ = 147, $n_{\text{Ad11 Control}}$ = 122, $n_{\text{Ad11 Exercise}}$ = 119. We compiled data from 5 independent trials and determined statistical significance by chi-square test. **$P \leq 0.01$, ***$P \leq 0.001$.

Table S1. Genes selected for qPCR analysis.

***C. elegans* genes**	**Mammalian homologs**
mup-2	Troponin T
tnt-2	Troponin T
unc-27	Troponin I
lev-11	Tropomyosin 1
unc-15	Paramyosin
mlc-1	Myosin light chains
unc-54	Myosin heavy chains
myo-3	Myosin heavy chains
zig-12	Titin
unc-87	Calponin-like

Table S2. Primers used for qPCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Amplification efficiency
cdc-42	CTGCTGGACAGGAAGATTACG	CTCGGACATTCTCGAATGAAG	1.03
lev-11	CCGCTGAAGAGAAAGTCCGT	TCGTCTCCGGTCTGAGTCAT	0.93
mlc-1	TGGAGCCTTTGCCATGTTC	CTTGACCTCATCCTCGTCCAAT	0.99
mup-2	AACGCAAGGCTAAGGCTGAT	AGCTCCGGCTTCAACTCTTC	0.92
myo-3	AGGGAGACTTGAAGGTTGCG	AGCGAGCTTAGCATTGGTGT	0.93
tnt-2	ATGGGGACGCAAAGAGAACG	AATTGGGTTGACTGGTGGCT	1.00
unc-15	CGAGGAAGCCAATGGACGTA	AAATCAGCTTGAGCGGTGGA	0.95
unc-27	CGTGGAAAGTTCGTCAAGCC	TCTTGAGGTTGGCACGGAAG	0.98
unc-54	ACTACCAACACGAAGCCGAG	GGCGTTAGCCTTGGAGAGTT	0.94
unc-87	ATGACTGGATTCGGACAGCC	AGCTTGAGAAGCAAAACGGT	0.98
Y45F10D.4	GTCGCTTCAAATCAGTTCAGC	GTTCTTGTCAAGTGATCCGACA	0.94
zig-12	GATCAGAGAACGGGTCGGTG	CTCCTCAAGCTCGTCTGGTC	1.02

Video S1 (separate file). Representative video of Ad2 WT animals in an exercise-permissive microfluidic chamber (large chamber height).

Video S2 (separate file). Representative video of Ad2 WT animals in a control microfluidic chamber (small chamber height non-permissive for swimming).

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