Title: Nutritional status and fecundity are synchronised by muscular exopheresis

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Abstract

Organismal functionality and reproduction depend on metabolic rewiring and balanced energy resources. However, the crosstalk between organismal homeostasis and fecundity, and the associated paracrine signaling mechanisms are still poorly understood. Using the *Caenorhabditis elegans* we discovered that secretory vesicles termed exophers, attributed in neurons to the removal of neurotoxic components, are released by body wall muscles to support embryonic growth. We found that exopher formation (exopheresis) is a non-cell autonomous process regulated by egg formation in the uterus. Our data suggest that exophers serve as transporters for muscle-generated yolk proteins used for nourishing and improving the growth rate of the next generation. We propose that the primary role of muscular exopheresis is to stimulate the reproductive capacity, thereby influencing the adaptation of worm populations to the current environmental conditions.

Main

The proper cellular function relies on the removal of unwanted contents by proteolysis or degradation mainly via the ubiquitin-proteasome system (UPS) and autophagy[1]. Recently a complementary mechanism was described in *Caenorhabditis elegans*. Under proteotoxic stress, worms' neurons can remove protein aggregates and damaged mitochondria via large membrane-surrounded vesicles called exophers[2]. Waste excretion by exophers in mouse neuronal HT22 cells under oxidative stress conditions was also reported[3]. This extrusion phenomenon could constitute a significant but still poorly explored metabolic waste management pathway. However, not only neurons can remove cellular content via exophers. Using worms expressing fluorescent reporters in the body wall muscle cells (BWM), we identified muscle-derived exophers; these reporters allow simultaneous tracking of proteasomes, closely associated with proteostasis, and mitochondria, which can be extruded in

neuronal tissue[2]. Muscular exophers are filled with proteasomes (both 19S and 20S subunits) (**Fig. 1a**), and approximately 12% of them contain mitochondria (**Extended Data Fig. 1**). Muscular exophers are generally more abundant and prominent (with diameters ranging from 2 to 15 μm) (**Fig. 1b**) than neuronal exophers[2], and the majority are generated by adult hermaphrodite mid-body muscles (**Fig. 1c**). Exophers are formed in the muscle cell and expelled outside via a pinching off mechanism (**Fig. 1d**). Some exophers that bud off remain connected with the extruding BWM via a thin but elastic tube that permits continued transfer of a large amount of cellular material into the extruded vesicle (**Fig. 1e, Supplementary Video 1**), similar to neuronal exophers[2]. Proteostasis impairment significantly increases neuronal exopher output[2,3]. In contrast, the number of muscular exophers did not change in response to depletion of the central proteostasis transcription factor HSF-1 (via *hsf-1* RNAi) or to heat stress, and the number increased slightly under conditions of oxidative stress (**Fig. 1f-g**). These observations suggest that proteostasis regulation might be not the core function of muscle exopheresis.

Next, we assessed the number of exophers at different time points of the *C. elegans* hermaphrodite life cycle. Reminiscent of neuronal exophers, muscular exophers are not produced during the larval stages, and their maximum level is reached around the second and third days of hermaphrodite adulthood (**Fig. 2a**). Because this time point coincides with the worm's maximum reproductive rate, we wondered if reproduction could influence exopher formation. To examine this possibility, we followed exopheresis in males. For the first three days of adulthood, males did not produce any exophers (**Fig. 2a**). This finding suggests that germ cell maturation in the reproductive system of hermaphrodite worms, the process of oocyte fertilization, or embryonic development might regulate muscle exopheresis.

To test these hypotheses, we took advantage of a thermosensitive *C. elegans fem-1* mutant strain that does not produce viable sperm at the restrictive temperature of 25 °C. At the permissive temperature of 15 °C, some animals can reproduce as well as wild type hermaphrodites, whereas the rest of the population is sterile[4]. The offspring-producing *fem-1* mutant animals grown at 15 °C generated a high number of muscular exophers. Contrarily, animals raised either at 15 °C or 25 °C that were unable to fertilize oocytes did not activate muscular exopheresis (**Fig. 2b-c**), indicating that neither the presence of the female gonad nor the temperature itself were sufficient to trigger exopher release. Notably, hermaphrodites sterilized via fluorodeoxyuridine (FUdR) treatment[5] extruded no exophers or only a few per animal (**Fig. 2d**), suggesting that the occurrence of developing embryos could indeed stimulate muscular exopheresis. We also found that FUdR-treated hermaphrodites often contained exopher-like structures in their BWM (**Fig. 2e**, middle and right panels). Interestingly, we detected objects resembling non-extruded exophers in the BWM of males (**Fig. 2e**, left panel), indicating that males are devoid of mechanisms triggering their expulsion.

The above results suggest that the occurrence of developing embryos could induce muscular exopheresis. Consistently, we observed a positive correlation between the number of exophers released and the number of eggs present in the worm uterus (**Fig. 3a**). To further explore this link, we depleted the mRNA of genes responsible for various processes associated with egg-laying. RNAi depletion of the G-protein signalling gene *goa-1* leads to hyperactive egg-laying behaviour, resulting in the presence of fewer early-stage eggs within the uterus[6]. As expected, *goa-1* knockdown caused a significant drop in the level of exopher release. In contrast, the egg-laying defects induced by *egl-1* and *egl-4* RNAi, which lead to egg retention in the uterus[7], increased exopher formation by muscle cells (**Fig. 3b**). In the absence of food, worms halt egg-laying and retain fertilized eggs in the uterus[8,9]; therefore, we anticipated that worms would generate more exophers when experiencing a food shortage. Indeed, the accumulation of

91 developing eggs in the uterus caused by the transfer of adult worms to food-free plates resulted
92 in a significant increase in muscle exopher secretion (**Fig. 3c**). Next, we tested whether worm
93 embryos could directly induce exopher production. We exposed young adult worms to extract
94 from developing eggs from the wild-type strain (**Fig. 3d**). Intriguingly, worms placed in contact
95 with material derived from lysed embryos increased exopheresis by approximately 20% (**Fig.**
96 **3e**). This observation suggests that molecules that diffuse from embryos *in utero* are
97 responsible for exopheresis induction. Finally, we decided to confirm that even in the face of
98 disturbed proteostasis specifically in the BWM, a signal associated with the developing
99 embryos in the uterus would be the primary regulator of muscular exopheresis. To this end, we
100 knocked down the myosin-directed chaperone UNC-45, which results in severe defects in the
101 organization of thick muscle filaments, as well as embryonic defects in cytokinesis and polarity
102 determination[10-12]. We initiated *unc-45* depletion in L4 larvae, which first leads to a disturbance
103 of proteostasis in BWM, and later, in young gravid adults, to inhibition of embryonic
104 development. Despite the dysfunction of a myosin-chaperone network in muscle cells, which
105 was indicated by complete paralysis of worms, we observed a dramatic inhibition of exopher
106 formation (**Fig. 3f**), highlighting the predominant role of maturating eggs in muscular
107 exopheresis.

108 Production of a single exopher involves the elimination of a substantial amount of the muscle
109 cell content. The high number of exophers produced during the *C. elegans* lifespan by BWM
110 leads to the removal of a relatively significant portion of cellular mass by a single tissue. We
111 hypothesized that this process should have substantial effects on worm muscle functionality
112 and healthspan. To this end, we selected three types of worms from the synchronized
113 population of gravid adults based on the number of extruded exophers, i.e., few (< 2), many (>
114 20), and control (6-8) animals (**Fig. 4a**), and analyzed their locomotory behaviour. In neurons,
115 exophers production is correlated with improved cell functionality; however, worms with

intensified exopheresis did not show enhanced mobility (**Fig. 4b**, **Extended Data Fig. 2**). On the contrary, these animals displayed a reduction in exploratory behaviour (**Fig. 4c-d**). Furthermore, the active use of muscular exopheresis was correlated with prolonged lifespan, revealing a long-term beneficial effect (**Fig. 4e**).

Because, developing embryos stimulate exopher release from the mother BWM, we next wondered whether this process benefited the offspring. Previous work showed that neuronal exopher content can be transported through the worm body to reach distant scavenger cells (coelomocytes)[2]. Moreover, muscle-specific transcriptomic analysis revealed the presence of significant levels of vitellogenin mRNAs (i.e., *vit-2*, *-5* and *-6*)[13]. Therefore, we hypothesized that the components of the yolk from BWM are transported through exophers to be used as a source of raw materials for maturating eggs. To address this possibility, we used RNAi knockdown of *vit-1* (a vitellogenin-coding gene) to reduce the level of the principal yolk protein[14] and investigated whether it led to an increase in exopher biogenesis as a possible compensatory mechanism. Indeed, the number of accumulated muscle-released exophers nearly doubled in response to yolk protein depletion (**Fig. 4f**). Moreover, intensification of exopheresis in the mother increased the amount of vitellogenin in developing eggs (**Fig. 4g**). Next, we followed the localization of vitellogenin-2 (VIT-2) fused to GFP and muscle exopher markers in the hermaphrodite. We detected the presence of VIT-2::GFP in the BWM of day-2 adult worms, as well as a significant accumulation in many muscle exophers (**Fig. 4h, Extended Data Fig. 3**). These results suggest that exophers can mediate the transport of additional portions of muscle-produced vitellogenin, which is ultimately deposited in oocytes from the body cavity[15]. Finally, we followed the growth of the progeny of hermaphrodites exhibiting different levels of exopheresis (**Fig. 4a**). We found that offspring from mothers with a high number of muscle-released exophers (which showed no change in egg-laying behaviour)

grew faster (**Fig. 4i**). This is in line with a previous reports showing that yolk-reach eggs support animals post-embryonic survival and development[14,16,17].

Discussion

We have shown here that muscular exopheresis in *C. elegans* represents a previously uncharacterized nutrient management program associated with nourishing the next generation of worms. The availability of food for mothers affects the number of developing eggs in the uterus and the developmental stage at which the eggs are laid[18]. Activation of exopheresis occurs with the first appearance of developing eggs in the uterus and intensifies in situations of environmental food depletion. Likewise, disturbance of yolk synthesis increases exopher biosynthesis. Consequently, in mothers with highly active exopheresis, the volume of yolk content in eggs increased. Our results show that yolk protein produced in BWM is transferred to exophers and ultimately delivered to oocytes. Therefore, the use of exophers for the transport of vitellogenin represents an elegant mechanism by which remote cells can help to enrich the nourishment of developing embryos. This process leads to the production of larvae that are better prepared to thrive in the current environmental conditions. Thus, muscular exopheresis is likely an adaptive mechanism that affects the dynamics of population growth. The impact of exopheresis on early reproduction may be particularly crucial for wild worms, given the significantly shortened life expectancy observed under more natural conditions[19].

Neuronal exopher formation occurs in higher animals[3]. In addition, yolk protein can be synthesized in the muscles of oviparous animals like zebrafish[20], and is supplemented from the mother to the intraovarian embryo in viviparous animals[21]. Hence, it is tempting to speculate that the role of muscular expheresis in supporting progeny development could be evolutionarily conserved. However, the exact mechanisms by which oocyte fertilization and subsequent embryonic development initiate exopher formation and how this exopheresis is executed at the molecular level require further studies.

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Figures

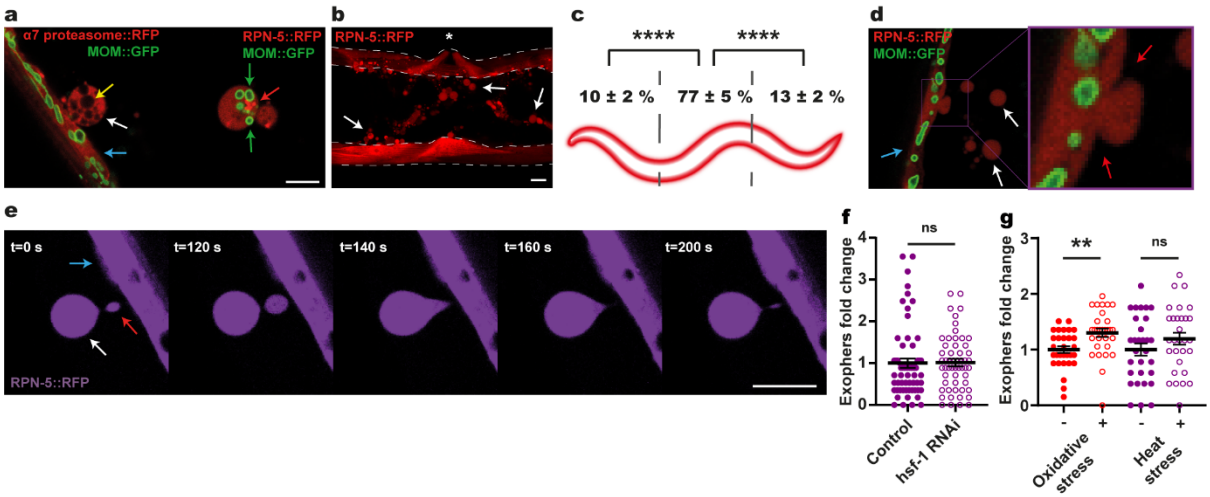


Figure 1. *C. elegans* muscles expel cellular content via exophers. a, Muscular exophers contain organelles and large protein complexes. Arrows: white – exopher, blue – muscle cell, green – mitochondria, red – proteasome foci, yellow – unidentified vesicle. MOM – mitochondrial outer membrane. **b**, BMW actively releases significant amounts of exopher. The image shows the middle part of the worm's body with muscles marked with dashed lines. Arrows indicate representative exophers, and the asterisk indicates the position of the vulva. **c**, Production of muscular exophers is not evenly distributed across all muscle cells. The highest number of exophers is produced by the muscles adjacent to the vulva. n = 46 animals; three biological replicates. **d**, Exophers are formed via a pinching-off mechanism. Arrows: white – exopher, blue – muscle cell, red – distorted muscle cell membrane during exopher formation. **e**, Exophers may remain connected to the sending BWM cells via thin elastic tubes that allow further transfer of cellular material. Arrows: white – exopher, blue – muscle cell, red – cellular material transferred to exopher via elastic tube. **f**, Proteostasis disruption by *hsf-1* knockdown does not increase exopher production. n = 60 and 55 animals, two biological replicates. **g**, Proteostasis induced via oxidative stress but not via heat stress increases exopher production. n = 30 animals; three biological replicates.

Scale bars are 10 μm. Data are shown as mean ± SEM. ns – not significant, ** $P < 0.01$, **** $P < 0.0001$; **c**, **f**, **g**, two-tailed Welch's t-test.

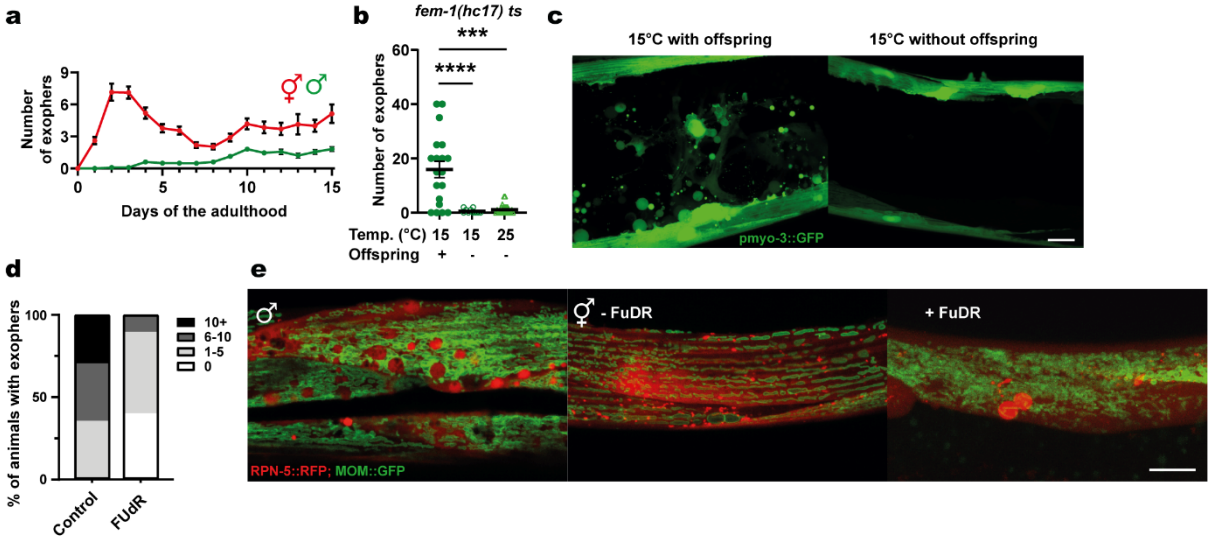


Figure 2. Exopher formation is sex-specific and fertility-dependent. a, The highest number of exophers is produced during the hermaphrodite reproductive period and in aging animals. Males do not produce exophers during the first days of adulthood and begin to generate a small number of exophers later in life. Starting n = 90 hermaphrodites and 150 males; three biological replicates. **b**, Feminized hermaphrodites of thermosensitive a *fem-1* mutant strain do not produce exophers regardless of growth temperature. n = 10 - 26 animals; one biological replicate. **c**, Representative images of the middle part of the worm body in panel b. **d**, Hermaphrodites sterilized via FUdR treatment produce no exophers or only a few per animal. n = 17 and 20 animals; two biological replicates. **e**, Males and sterile hermaphrodites (via FUdR treatment) show the formation of spherical structures in the BWM that resemble mature exophers. MOM – mitochondrial outer membrane.

Scale bars are 10 µm. Data are shown as mean ± SEM; *** $P < 0.001$, **** $P < 0.0001$; **b**, two-tailed Welch's t-test.

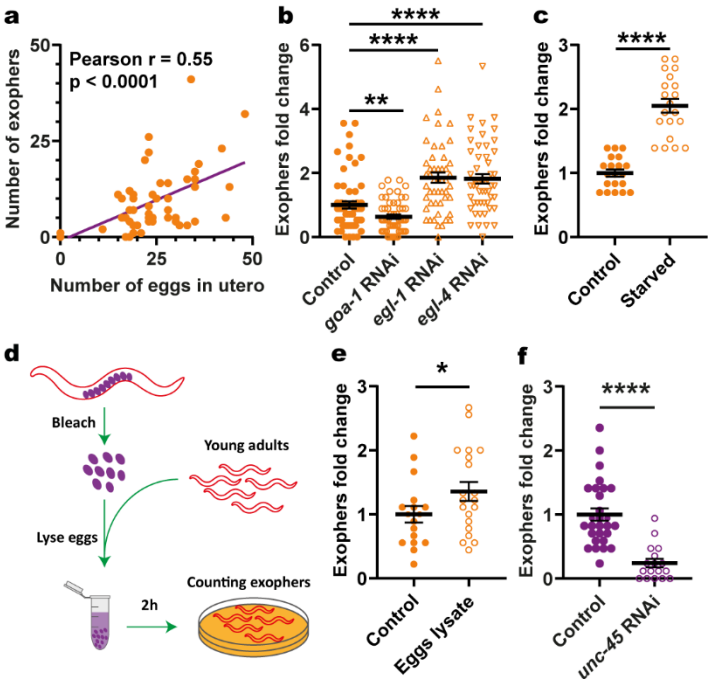


Figure 3. Muscular exopheresis is a non-cell autonomous process regulated by *in utero* developing embryos. a, The number of produced exophers positively correlates with the number of *in utero* embryos. The violet line is a linear regression line, and each orange point represents one animal. All animals were 1 - 3 days old; n = 54 animals; three biological replicates. **b**, RNAi knockdown of genes regulating the egg laying rate and their presence in the uterus influences exopher production. n = 50 - 60 animals; two biological replicates. **c**, Egg retention in the uterus caused by starvation increases exopher production. n = 20 animals; one biological replicate. **d**, Schematic representation of the experimental setup for investigating the influence of egg lysate on exopher production. **e**, Exposure of young-adult worms to embryo-derived substances increases exopher production. n = 17 and 20 animals; one biological replicate. **f**, RNAi knockdown of the essential muscular co-chaperone *unc-45* reduces exopher production. n = 28 and 17 animals; two biological replicates.

Data are shown as mean ± SEM; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$; **b**, **c**, **f**, two-tailed Welch's t-test; **e**, one-tailed Welch's t-test.

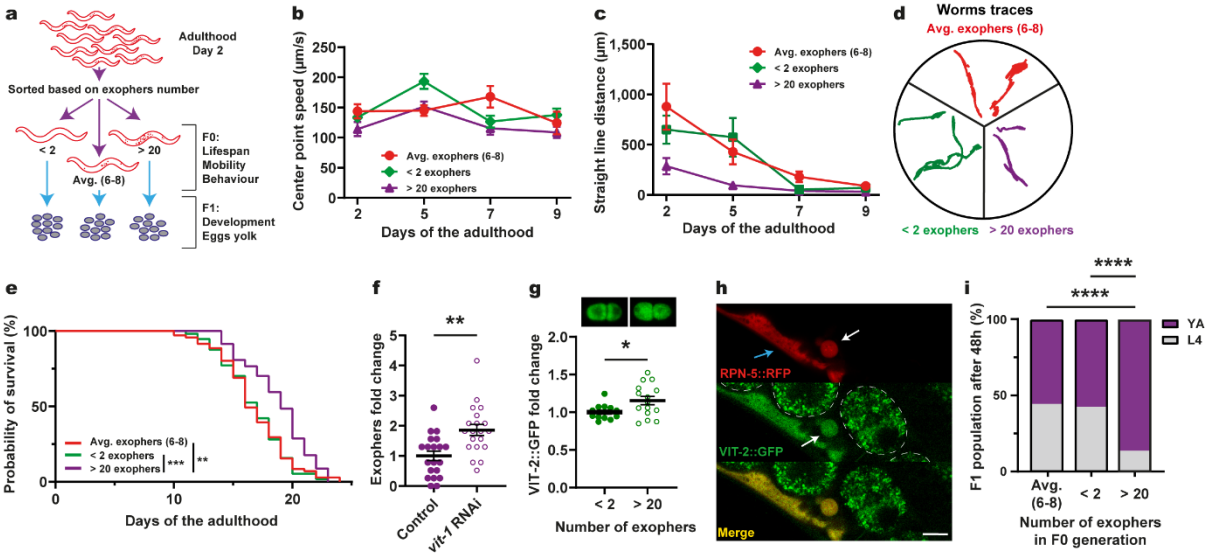
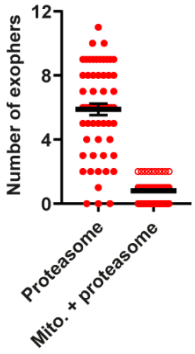
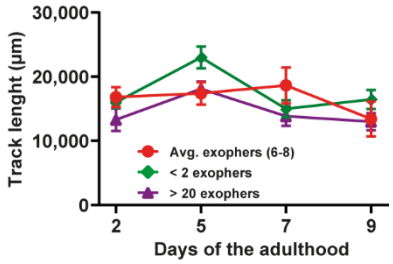


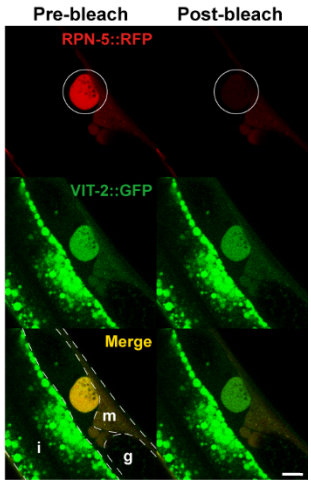
Figure 4. Worms with overactive muscular exopheresis live longer and their offspring develop faster. a, Schematic representation of the experimental setup for investigating the influence of overactive exopheresis on F0 and F1 worm generation. **b**, Exopher production does not improve muscle functionality. n = 11 - 38; three biological replicates. **c-d**, Animals with overactive exopheresis have reduced exploratory behaviour presented as a reduction in straight line distance travelled (c) and by representative worms traces on the plate (d). n = 11 - 34; three biological replicates. **e**, Production of a high number of exophers increases lifespan. n = 63 - 88 animals; three biological replicates. **f**, RNAi knockdown of the egg yolk precursor protein VIT-1 increases the number of muscular exophers. n = 20 animals; two biological replicates. **g**, Embryos from hermaphrodite mothers that produce a high number of exophers contain more egg yolk precursor protein VIT-2. Representative images of embryos with VIT-2::GFP levels from mothers with different exopheresis activity are show above the graph. n = 15 and 14 eggs, five and six biological replicates (animals). **h**, Muscle-produced VIT-2 is released from muscles via exophers. The image shows the midbody of worms expressing the proteasome subunit RPN-5 tagged with RFP in BWM and VIT-2::GFP endogenous expression. Arrows: white – exopher, blue – muscle cell. Dashed lines mark eggs present in the uterus. Scale bar is 10 µm. **i**, Offspring of worms with overactive exopheresis develop faster. YA – young adult stage, L4 – last larval stage. n = 317 – 372 animals; two biological replicates. Data are shown as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; **e**, Log-rank (Mantel-Cox) test; **f**, **g**, two-tailed Welch's t-test; **i**, Fisher's exact test.



Extended Data Figure 1. Small fraction of muscular exophers contain mitochondria. n = 60; six biological replicates. Data are shown as mean ± SEM.



Extended Data Figure 2. Active exophoresis affects worm mobility. Graph shows the track lengths of animals exhibiting different exopheresis activities. n = 11 - 38; three biological replicates. Data are shown as mean ± SEM.



Extended Data Figure 3. Exophers contain muscle-produced vitellogenin. Images show the formation of an exopher filled with the proteasome and vitellogenin. Images were captured before and after RPN-5::RFP photobleaching, confirming that the high signal from VIT-2::GFP in forming exophers is not an imaging artefact. The white circle marks the area that

304 was bleached using a 555 nm laser and the position of developing exopher. Dashed lines mark
305 different tissue borders: m – muscle, i – intestine, g – gonad. Scale bar is 10 μm.

Methods

Data reporting

No statistical methods were used to predetermine the sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment except for lifespan measurement and exploratory behaviour experiments.

Worm maintenance and strains

Worms were maintained on Nematode Growing Medium (NGM) plates seeded with OP50 *Escherichia coli* bacteria at 20 °C unless otherwise stated[1]. A list of all strains used in the study, together with the information in which experiments they were used is provided in Supplementary Table 1.

Generation of plasmids

All constructs were cloned using the SLiCE method[2] and were sequenced for verification. Construct for the expression of GFP on the mitochondrial outer membrane in the worm's body wall muscles was prepared as follows. First, destination vector pMT26 containing pCG150 vector backbone with the *myo-3* promoter and *unc-54* 3'UTR sequences separated by KspAI restriction site was made. Next, codon-optimized sequences[3] for GFP, linker containing attB5 sequence, and the sequence of the first 55 amino acids from TOMM-20 protein[4] (from a plasmid that was synthesized for this study) were PCR amplified and inserted into linearized pMT26 vector. To generate construct for expression of RPN-5 or PAS-7 tagged with RFP in worm's body wall muscles, sequences encoding respective proteins were PCR amplified and inserted into pMT26 linearized destination vector. As a template for the *rpn-5*-coding sequence, we used plasmid bearing codon-optimized *rpn-5* cDNA with 3 artificial introns, which was synthesized for this study. The sequence of *pas-7* gene was directly amplified from

N2 worms genomic DNA, and the sequence of RFP (wrmScarlet) was amplified from pSEM89 plasmid[5].

Transgenic strains generation

Worms transgenic strains were created by microparticle bombardment using *unc-119(ed3)* rescue as a selection marker[6]. After phenotypic confirmation of successful plasmid insertion, transformants were backcrossed two times against N2 strain to remove the *unc-119(ed3)* background.

Scoring exophers and Microscopy

For scoring of exophers, a confocal microscope or a stereomicroscope was used.

When using the confocal microscope, animals were transferred onto 3 % agarose pads prepared in H_2O and formed on a microscope slide. Next, animals were immobilized on the pad using 6 µL of PolySciences 0.05 µm polystyrene microspheres or 25 µM tetramisole and covered with a glass coverslip. Immediately afterward, animals were imaged using an inverted Zeiss LSM800 laser-scanning confocal microscope with 40x or 63x oil immersion objectives. The 488 nm and 561 nm lasers were used for excitation of GFP and RFP fluorescent proteins, respectively. Z-stacks, which covered the whole animal, were taken and the number of exophers released by muscles was counted. Finally, data were normalized to the average number of exophers present in control animals and compared between conditions.

For scoring exophers with the stereomicroscope, we used Leica M165FC stereomicroscope equipped with Leica EL6000 lamp and standard Texas Red and GFP filter sets. Age synchronized, freely moving, day-2 adult animals were directly visualized on culturing NGM plates and the number of visible exophers released by muscles were counted. Finally, data were

normalized to the average number of exophers present in control animals and compared between conditions.

Representative pictures of exophers presented in the manuscript were taken with the usage of an inverted Zeiss 700 laser-scanning confocal microscope equipped with a 40x oil objective. The 488 nm and 555 nm lasers were used for excitation of GFP and RFP fluorescent proteins, respectively. To investigate the presence and distribution of exophers, z-stacks were taken and processed with ZEN software.

RNA interference

RNA interference in *C. elegans* was performed using the standard RNAi feeding method and RNAi clones[7]. For experiments, we used NGM plates supplemented with 1 mM IPTG and 25 µg/µl carbenicillin seeded with HT115 *E. coli* bacteria expressing double-stranded RNA (dsRNA) against the gene of interest or, as a control, with bacteria without a vector. Worms were placed on freshly prepared RNAi plates, either as age-synchronized pretzel-stage eggs, L1 larvae, or L4 larvae. The number of exophers was counted on day 2 adult worms using a confocal microscope or a stereomicroscope.

Stress influence on exophers production

Worms were age-synchronized using alkaline hypochlorite solution (bleaching procedure), as previously described[8]. The harvested eggs were incubated overnight at 16 ˚C for hatching. Approx. 1000 L1 larvae were transferred on NGM plates and incubated at 20 °C till they reached day-2 of adulthood. The worms were further channeled to the respective stress treatments.

Oxidative stress

Approx. 100, day-2 adult worms were washed-off from the NGM plates and rinsed 3 times with M9 buffer. The worms to be stressed were suspended in 1 ml of 5 mM hydrogen peroxide solution prepared in M9 buffer, whereas control worms were suspended in M9 buffer. The tubes were incubated on a shaker at 20 ˚C for 60 minutes.

Heat stress

Similarly, approx. 100, day-2 adult worms were washed-off form NGM plates and rinsed 3 times with M9 buffer. The worms were further suspended in a 1 ml M9 buffer. The worms to be heat stressed were incubated in a shaker at 33 °C for 60 minutes, whereas the control animals were incubated at 20 °C for 60 minutes.

Exophers quantification

From each stress/control treatment, 30 worms were picked onto agarose pad slides individually for the quantification of exophers. Exophers were quantified using a confocal microscope. Obtained data were normalized to the average number of exophers present in control animals and compared between conditions.

Number of exophers on consecutive days

For each biological replicate, 30 L4 larvae hermaphrodites or 50 L4 larvae males were transferred to fresh NGM plates (5 hermaphrodites or 10 males per plate). For the next consecutive 15 days, the number of exophers in each animal was counted using a stereomicroscope. Hermaphrodites were transferred to fresh plates every 2 – 3 days. Males were kept on the same plate until the end of the experiment. All animals that died during the experiment time course were removed from the plate.

Measuring exophers in *fem-1* mutant

Approx. 50 L1 larvae from *fem-1(hc17)ts* mutant strain expressing GFP in BWM were transferred to 2 fresh NGM plates. During the time course of the experiment, one of the plates was kept at 15 °C while the second one was kept at 25 °C. When worms reached L4 larval stage, each worm was transferred to a separate plate and grown at the same temperature as before. After 48 hours at 25 °C or 72 hours at 15 °C, using a stereomicroscope, a number of exophers released from worms muscles was counted and plates were scored for F1 offspring to assign all worms as fertile or infertile.

FUdR assay

Age-synchronized animals were placed on NGM plates seeded with OP50 *E. coli* bacteria as a food source until they reached young adulthood (day 0). Next, animals were selected and moved to test plates containing 25 µM fluorodeoxyuridine (FUdR) to prevent embryonic development and egg hatching[9] or control plates without FUdR. The number of exophers was counted on adult day-2 using confocal microscopy.

Exophers and *in utero* eggs correlation

For correlating the number of exophers with the number of eggs present in worms uterus, 1 to 3-days old animals were used. First, muscular exophers for a single worm were counted using the stereomicroscope. Next, worm was transferred to a 10 µl drop of 1.8 % hypochlorite solution put on a microscope slide. Finally, after approx. 5 minutes when the hermaphrodite mother was fully bleached, the number of eggs released from worm's uterus was counted. Data analysis was performed using GraphPad Prism 8 software.

Starvation assay

To assess the influence of worms starvation on the exophers production, day-2 adult worms were moved to bacteria-free NGM plates. After 24 hours of food deprivation, the number of exophers was counted using a stereomicroscope. As a control, day-3 adult worms grown for the whole time on NGM plates seeded with bacteria were used. Obtained data were normalized to the average number of exophers present in control animals and compared between conditions.

Eggs lysate assay

To obtain eggs, age-synchronized N2 gravid hermaphrodites were bleached using an alkaline hypochlorite solution. Harvested eggs were suspended in appropriate volume of M9 buffer to reach the concentration of approx. 200 eggs per µl. Next, eggs were flash-frozen in liquid nitrogen, thawed on ice, and sonicated 3 times for 10 seconds to obtain eggs lysate. 150 µl of eggs lysate was mixed with 150 µl of concentrated OP50 *E. coli* bacteria and placed in a 0.5 ml Eppendorf tube. Approx. 30 day-1 young adult hermaphrodites were transferred to Eppendorf tube containing a mixture of eggs lysate and bacteria and were placed on the rotator for 2 hours at room temperature. After 2 hours, the content of the tube was placed on the fresh NGM plate seeded with OP50 bacteria. 18 hours later the number of exophers in each worm was counted. For control animals, the whole protocol was the same except that for bleaching step no worms were used.

Worm exploratory behaviour

Age-synchronized, day-2 adult worms which had an average (6-8), few (< 2), or many (> 20) exophers were sorted to separate NGM plates. Approximately 10 worms per replicate were brought onto NGM plates and the worm movement was recorded for 2 minutes using the WormLab system (MBF Bioscience). The frame rate, exposure time, and gain were set to 7.5

frames per second, 0.0031 s, and 1, respectively. The track length, straight-line distance, center point speed, and the overall track pattern of individual worms were analyzed using the WormLab software (MBF Bioscience).

Lifespan assay

For each biological replicate, approx. 300 late L4 larvae were transferred to fresh NGM plate. On day 2 of worms' adulthood, the number of exophers in each animal was counted and worms with less than 2 or more than 20 visible exophers were transferred to new plates (25 – 30 animals). Additional 25 – 30 worms were picked blindly from the same original population, moved to a fresh plate, and served as a control. Lifespan measurements were performed on these three respective populations. During lifespan measurements, worms were scored daily for movement and pharyngeal pumping until their death. All worms were moved to fresh plates every 1 – 2 days during the eggs-laying period and every 3 – 5 days afterward. Animals that crawled off the plate or exhibited baggy phenotype were censored from the experiment. Data analysis was performed using GraphPad Prism 8 software.

Vitellogenin levels in embryos

Vitellogenin levels in embryos were measured based on the GFP signal from fluorescently tagged VIT-2 protein. Approx. 200 late L4 larvae were transferred to fresh NGM plate. On day 2 of worms adulthood, the number of exophers in each animal was counted and worms with less than 2 or more than 20 visible exophers were transferred to new plates. Next, animals from each group were individually transferred to a 10 µl drop of M9 buffer which was placed on a microscope slide. Using a sharp injection needle, worm was cut open to release eggs from the uterus. Fluorescent signal from 2-cell embryo stage was captured using Leica M165FC stereomicroscope equipped with Leica EL6000 lamp, standard GFP filter set, and Leica

DFC365 FX CCD camera. Magnification used for recording pictures was set to 192x. Exposure time and gain were set to 600 ms and 2, respectively. The fluorescent signal was quantified using Leica Las X software and was normalized to the average signal from eggs, which were obtained from animals with less than 2 exophers.

Worms development assay

25 – 30 age-synchronized, day-2 adult worms which had an average (6-8), few (< 2), or many (> 20) exophers were sorted to separate NGM plates. Gravid adults were allowed to lay eggs for 4 hours, afterward removed from the plates, and the development of their offspring was followed. 46 hours later, using the stereomicroscope, developmental stage of each animal was checked, and the proportion between L4 larvae stage worms and young adult worms was calculated.

Statistical analysis

Statistical tests used in this study were two-tailed Welch's t-test, one-tailed Welch's t-test, Log-rank (Mantel-Cox) test, and Fisher's exact test. P-value < 0.05 was considered significant.

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Author contributions

M.T., W.P., M.P., N.S., M.N., and K.K. conducted and designed experiments, M.T. and W.P. conceived the project and supervised the study. W.P., A.C. and M.T. secured the funding. W.P. and M.T. wrote the manuscript with input from A.C. and M.P

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550 **Supplementary Information**

551 Supplementary Video 1

552 The video shows the cellular content being transfer from BWM cell to exopher via elastic tube.

553 Supplementary Table 1

554 The file contains a table with the list of all *C. elegans* strains used in this study.

555 Supplementary Table 2

556 The file contains a table with the statistical summary of lifespan experiments

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