**Supplemental Material**

*Worm strains and culture conditions*

The following strains were used in this study: WT (N2 Bristol), NL2099/*rrf-3(pk1426)*II, CB4037/*glp-1(e2141)*III, CF1814/*rrf-3(pk1426)*II; *daf-2(e1370)*III, TU3401/*sid-1(pk3321)*V; uIs69[*pCFJ90 (myo-2p::mCherry) + unc-119p::sid-1*]V, PD4251/ccIs4251 [*(pSAK2) myo-3p::GFP::LacZ::NLS + (pSAK4) myo-3p::mitochondrial GFP + dpy-20(+)*] I, VP303/kbIs7 [*nhx-2p::rde-1 + rol-6(su1006)*], NR222/kzIs9 [*(pKK1260) lin-26p::NLS::GFP + (pKK1253) lin-26p::rde-1 + rol-6(su1006)*], WM118/neIs9 [*myo-3::HA::RDE-1 + rol-6(su1006)*], JK4143/qIs57 [*lag-2p::GFP*] II. qIs140 [*lag-2p::rde-1 + (pRF4) rol-6(su1006)*], DP132/edIs6 [*unc-119::gfp + (pRF4) rol-6(su1006)*] IV. All strains were acquired from the *Caenorhabditis* Genetics Centre (CGC) at the University of Minnesota, except the following: QU34/izEx5[*pAy39.1, bec-1p::BEC-1::RFP*] was kindly provided by Alicia Meléndez (Queens College, Flushing, NY), BAT922/*rrf-3(pk1426)* II; adIs2122 [*lgg-1p::GFP::lgg-1* + *rol-6(su1006)*], BAT1007/*rrf-3(pk1426)* II; bpIs151 [*sqst-1p::sqst-1::GFP* + *unc-76(+)*], BAT1032/*daf-16(mgDf47)* I; *daf-2(e1370)* III; *rrf-3 (pk1426)* II, BAT 1004/*rrf-3(pk1426)* II; edIs6 [*unc-119::gfp* + *(pRF4) rol-6(su1006)*] IV. *rrf-3* worms and all BATstrains were outcrossed to WT three times. Standard procedures for *C. elegans* strain maintenance were used (Brenner 1974). All stock animals were maintained on nematode growth medium (NGM) plates and fed with EV expressing HT115 bacteria. Experimental animals were maintained in a liquid culture system as described in the following section.

*Age synchronous liquid culture of C. elegans*

NGM liquid media was prepared with 100 µg/ml Ampicillin and EV HT115 bacteria re-suspended to a final density of 3x109 cells ml-1. Cell culture flasks with breathable filter caps were filled to the following capacities: 75 cm2 to a maximum of 50 ml, 175 cm2 to a maximum of 100 ml and 300 cm2 to a maximum of 200 ml, in order to provide optimal aeration. Capacities were determined by the desired worm load in each flask with a maximum density of 0.5 worms per µl per flask. Stock worms were washed from two 10 cm2 NGM plates, sedimented and placed in a 100 ml cell culture flask. All procedures from this point were carried out under a laminar flow hood to avoid contamination. Worm cultures were placed at 20 °C in a shaking incubator at 40 rpm. Once dense with egg producing adults, they were synchronized with NaOCl treatment followed by an over-night egg hatch in M9 buffer. L1 worms were counted and placed back into culture at the appropriate density. Worms were maintained in this manner for a minimum of three and a maximum of eight generations at optimal conditions. For experimental samples, eggs were harvested from age synchronous adults at no later than day 1 of life. These offspring were allowed to grow until day 0 and were then subject to twice daily sedimentation to remove offspring (Supplemental Fig. S1, A-D). The sedimentation protocol was as follows. Adult worms were allowed to settle in 50 ml Falcon tubes at a 1:1 ratio to the density of the liquid culture. Sedimentation proceeded for 5-10 minutes depending on the age of the worms, adult worms sediment faster than larvae. The supernatant containing L1-L3 worms was removed by aspiration. Worms were re-suspended in NGM containing EV bacteria at a density of 1x109 cell ml-1. This was repeated six times. Worms were then carefully layered onto a 15 ml column of 36% Percoll with NGM in a 50 ml Falcon (Fabian and Johnson 1994). Worms were allowed to settle through the Percoll for 10 minutes and the top two thirds of the Percoll containing: dead worms, bagging worms, and egg clumps was aspirated. Worms were washed a further three times with NGM plus before being transferred into a fresh food culture. Percoll sedimentation was performed twice daily for the first five days and then once daily thereafter. A final sedimentation of in 40% Percoll was performed on days where samples were extracted.

*Lysotracker staining*

Worms were grown as described and transferred to respective RNAi treatments at day 9. On day 15, 30 aged-synchronized worms were picked into M9 containing food and stained with 5 nM LysoTracker® Deep Red (Molecular Probes L12492) for 3 hours at room temperature rotating. Worms were paralyzed by 0.5% NaAz and mounted on a 2% agar pad on a glass microscope slide. 488 nm, 561 nm or 633 nm signals were acquired using a STED CW super-resolution microscope (Leica) and a 63x/1.4 NA Oil immersion objective. At least 10 whole worms were imaged per condition.

*Quantification of LGG-1::GFP foci*

All GFP::LGG-1 foci were quantified using ImageJ according to standard procedures (Palmisano and Meléndez 2016; Alberti et al. 2010). Each image was processed in the same manner. Briefly, the original image is examined and the hypodermis outlined to avoid other regions such as muscle or seam cells in young worms (Supplemental Fig. S10, A and B). Images are converted into a bit map following automatic threshold adjustment using the max entropy calculation to remove background (Supplemental Fig. S10, C and D). The region corresponding to the outlined hypodermis is selected for analysis Particles are analyzed in this selected region with the following criteria: particles were selected for quantification based on a size range of 0.2 - 4 µm2 and with a circularity of 0 – 1 (Supplemental Fig. S10E). The outlines of the identified and counted particles are checked to avoid spurious counts (Supplemental Fig. S10F). Images were not considered for quantification where the hypodermis was not clear or where foci were not visible following thresholding. In total, at least 60 total regions were quantified from 50 different worm samples per condition.

*Proteasome peptidase activity assay*

*In vitro* proteasomal peptidase assay was performed as previously described (Kisselev and Goldberg 2005). Briefly, synchronized worms were grown in liquid culture and transferred to RNAi feeding plates at day 9. Day 16 old animals were homogenized in proteasome activity assay buffer (50 mM Tris-HCl, pH 7.5, 250 mM sucrose, 5 mM MgCl2, 0.5 mM EDTA, 2 mM ATP and 1 mM dithiothreitol), using an ultrasonicator (Bioruptor, Diagenode). The lysate was centrifuged at 10,000 x g for 15 minutes at 4 °C. 25 μg of total protein lysate was transferred to a 96-well black microplate (Greiner) and incubated with fluorogenic substrate. As a control condition, the lysate was treated with the proteasome inhibitor MG-132 (25µM in DMSO) and the other conditions were supplemented with the matching volume of DMSO as a vehicle control. Chymotrypsin-activity was assayed with hydrolysis of the fluorogenic peptide substrates Suc-LLVY-AMC (Enzo Life Sciences). Fluorescence (380 nm excitation, 460 nm emission) was recorded every 5 minutes for 1 hour at 25 °C with a microplate fluorometer (Infinite M1000, Tecan). Assays were performed in three biological replicates, each averaging two technical replicates.

*Bortezomib treatment*

To pharmacologically inhibit the proteasome, animals were treated with Bortezomib (EMD Millipore Calbiochem). Briefly, worms were grown as described in liquid culture until day 14.5 and subsequently incubated with 2 mM Bortezomib or DMSO vehicle control over night (~12 hours). The drug treatment was performed in M9 liquid medium supplemented with EV HT115 bacteria at a density of 3x109 cell ml-1, while shaking at 20 °C. The worms were harvested at day 15, following a 40% Percoll wash. This wash was repeated in the event that the cleaned worms contained less than 95% living worms.

*RNA sequencing*

Worms were grown in liquid culture as described. Worms were harvested at day 3, day 6, day 9, day 12, day 15 and day 18 following sedimentation in 40% Percoll. 200 worms were collected and flash frozen in 400 µl of Trizol. RNA extraction and cDNA synthesis was carried out following the manufacturer’s instruction. 60 NGS libraries were prepared using Illumina's TruSeq stranded Total RNA LT Sample Prep Kit following Illumina’s standard protocol (Part # 15031048 Rev. E). The ribozero steps of the protocol for rRNA depletion were carried out manually, while the following steps of library preparation were performed using the Bravo Automated Liquid Handling Platform (Agilent). Libraries were prepared from a starting amount of 500 ng of total RNA and amplified in 11 PCR cycles. Libraries were profiled in a High Sensitivity DNA chip on a 2100 Bioanalyzer (Agilent technologies) and quantified using the Qubit dsDNA HS Assay Kit, in a Qubit 2.0 Fluorometer (Life technologies). 15 libraries were pooled together in equimolar ratio (60 libraries into 4 pools), and each pool was sequenced on 2 HiSeq 2500 lanes of a High Output run, single-read for 51 cycles plus 7 cycles for the index read. The libraries were sequenced on a HiSeq2500 with a read length of 51 bp single read. Base calling and demultiplexing were performed using bcl2fastq (version 1.8.4). Reads were mapped against *C. elegans* genome assembly WBcel235 (ENSEMBL release 84) using STAR (Dobin et al. 2013) (version 2.5.1b, additional parameters: --outFilterMultimapNmax 10 --outFilterMismatchNmax 2 --alignIntronMin 21 --sjdbOverhang 50). Strand specific read counts per gene were generated using featureCounts (Love et al. 2014) (version 1.4.6-p2 subread package). Differentially expressed genes were determined using DESeq2 (Liao et al. 2014) (version 1.12.3) with an FDR cutoff of 1% and default filtering criteria. Samtools (Quinlan and Hall 2010) (version 1.3), BEDTools (Kent et al. 2010) (version 2.25) and UCSC tools (Li et al. 2009) were used to generate coverage tracks.

*Real-time quantitative PCR*

Worms were grown in liquid culture as described. RNAi treatment commenced at day 0 or day 9. Samples were harvested at day 3, day 12, day 15, or day 18 respectively. Total RNA was isolated from flash frozen samples of at least 100 age synchronised worms using Trizol extraction. cDNA synthesis was performed using First Strand cDNA Synthesis Kit (ThermoFisher) with 800 ng of RNA and oligo-dT primers. Quantitative PCR was performed in a ViiA™ 7 Real-Time PCR System (ThermoFisher) using SYBR® Green PCR Master Mix (Life technologies). Values were normalized to *cdc-42* and *pmp-3* as internal controls. Primers used are listed in supplementary Supplemental Table 13.

**References**

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