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Supplementary Information for

Dietary restriction improves proteostasis and increases life span through Endoplasmic Reticulum hormesis

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Supplementary Information Text

Detailed experimental procedures

C. elegans strain maintenance

Unless otherwise mentioned, all the *C. elegans* strains were maintained and propagated at 20°C on *E. coli* OP50 using standard procedures (1). The strains used in this study were: N2 Bristol (wild-type), *eat-2(ad1116)II, Phsp-4:gfp(zcIs4), rrf-3(pk1426)II, ire-1(v33)II, atf-6(ok551)X, pek-1(ok275)X, ire-1(ok799)II, xbp-1(zc12)III, xbp-1(tm2457)III, xbp-1(tm2482)III, hsp-4(gk514)II, Phsp-16.2;gfp(dvIS70), Phsp-6;gfp(zcIs9), ire-1(zc14)II;Phsp-4:gfp, rrf-3(pk1426)II, Punc-54:Q40:yfp(rmIs133).* The above-mentioned strains were obtained from *Caenorhabditis* Genetics Centre, University of Minnesota, USA. The other strains including *eat-2(ad1116)II;Phsp-4:gfp(zcIs4), eat-2(ad1116)II;rrf-3(pk1426)II, pek-1(ok275)X;Phsp-4:gfp(zcIs4), ire-1(v33)II;Punc-54:Q40:yfp(rmIs133)* and *eat-2(ad1116)II;Punc-54:Q40:yfp(rmIs133)* were generated in-house using standard mating techniques.

Preparation of RNAi plates

RNAi plates were poured using autoclaved (NGM) nematode growth medium supplemented with 100 µg/ml ampicillin and 2mM IPTG. Plates were dried at room temperature for 1-2 days. Bacterial culture harbouring RNAi construct was grown in Luria Bertani (LB) media supplemented with 100µg/ml ampicillin and 12.5µg/ml tetracycline, overnight at 37°C in a shaker incubator. Saturated cultures were re-inoculated the next day in fresh LB media containing 100µg/ml ampicillin by using $1/100^{th}$ volume of the primary inoculum and grown in 37°C shaker until OD_{600} reached 0.5-0.6. The bacterial cells were pelleted down by centrifuging the culture at 5000 r.p.m for 10 minutes at 4°C and resuspended in $1/10^{th}$ volume of M9 buffer containing 100µg/ml ampicillin and 1mM IPTG. Around 350µl of this resuspension was seeded onto RNAi plates and left at room temperature for 2 days for drying, followed by storage at 4°C till further use.

Hypochlorite treatment to obtain eggs and synchronizing worm population

Gravid adult worms, initially grown on *E. coli* OP50 bacteria were collected using M9 buffer in a 15 ml falcon tube. Worms were washed thrice by first centrifuging at 1800 r.p.m for 60 seconds followed by resuspension of the worm pellet in 1X M9 buffer. After the third wash, worm pellet was resuspended in 3.5 ml of 1X M9 buffer and 0.5 ml 5N NaOH and 1ml of Sodium hypochlorite solution were added. The mixture was vortexed for 7-10 minutes until the entire worm bodies dissolved, leaving behind the eggs. The eggs were washed 5-6 times, by first centrifuging at 2500 r.p.m, decanting the 1 X M9, followed by resuspension in 1X M9 buffer to remove traces of bleach and alkali. After the final wash, eggs were resuspended in approximately 100-200 µl of M9 and added to different RNAi plates

RNAi life span

Gravid adult worms, initially grown on *E. coli* OP50 were bleached and eggs were hatched on different RNAi plates. On reaching adulthood, 50-60 young adult worms were transferred to the similar RNAi plates in triplicates and overlaid with Fluoro-deoxyuridine (FudR) to final concentration of 0.1 mg/ml of agar (2). At the 7th Day of adulthood, sick, sluggish and slow dwelling worms were removed from the life span population and the remaining were considered as the number of subjects 'N'. Following this, number of dead worms were scored every alternate day and plotted as % survival against the number of days. Statistical analysis for survival was conducted using Mantel–Cox Log Rank test using Oasis software available at (**http://sbi.postech.ac.kr/oasis**). Average life span was also determined using the same method and represented as Mean life span ± Standard Error Mean (S.E.M).

Measurement of Basal UPR^{ER} during larval development

Transgenic worms expressing GFP under *hsp-4* (mammalian ortholog GRP78/Bip) promoter [*Phsp-4:gfp*(*zcIs4*) and *eat-2(ad1116);Phsp-4:gfp(zcIs4*] were bleached and eggs were hatched on control RNAi. Fifty L3, L4, young adult or Day 1 gravid adult worms were immobilized on glass slides coated with 2% agarose using 20 mM sodium azide and visualized under Axio-imager M2 epifluorescent microscope (Carl Zeiss, Germany) equipped with a monochromatic camera lens (MRm) and GFP filter set.

Fluorescence of ≥20 worms at different time points was quantified using NIH Image J software and represented as arbitrary units (AU). Basal UPR^{ER} in other strains like *ire-1(zc14);Phsp-4:gfp(zcIs4)* and on different RNAi like *ire-1* or *drl-1* was measured similarly.

Measurement of induced UPR^{ER} efficiency with age

Transgenic *Phsp-4:gfp*(*zcIs4*) and *eat-2(ad1116);Phsp-4:gfp(zcIs4)* were bleached and eggs were hatched on control RNAi. Worms were allowed to grow till adulthood and then transferred onto plates overlaid with FuDR to a final concentration of 0.1mg/ml. At each successive day (day 1 till day 4 of adulthood), approximately 100 worms were transferred to plates supplemented with 5 or 10 µg/ml tunicamycin (Tm) and incubated for 6 hours at 20°C. Since Tm is dissolved in 0.05% DMSO, it was used as vehicle control. After 6 hours, 50 worms from each treatment were mounted onto 2% agarose slides and visualized using Axio-Imager M2 epifluorescent microscope with a GFP filter set (Carl Zeiss, Germany). Fluorescence of ≥20 Tm-treated and untreated worms was quantified using NIH ImageJ software. Average fluorescence of treated worms was normalized to untreated worms and plotted as normalized GFP fold induction at different days of adulthood. Similar procedure was followed to compare $iUPR^{ER}$ efficiency of *Phsp-4:gfp(zcIs4)* on control and *drl-1* RNAi on different days of adulthood.

Tunicamycin hormesis treatment

Preparation of feed:

Overnight grown bacterial culture expressing Control or test RNAi were re-inoculated in 200 ml fresh LB media and grown at 37°C until OD_{600} reached 0.5-0.6. The culture was divided in two parts (A and B) in a ratio of 1:3 and cells are pelleted separately using a centrifuge (4810R, Eppendorf, Germany) at 5000 r.p.m for 10 minutes at 4°C. Pellet from part B (150 ml) of the culture was resuspended in 1X M9 buffer containing 100µg/ml ampicillin and 1mM IPTG to $1/10^{th}$ of its initial culture volume (for 150 ml culture, 15 ml of resuspension buffer was used) and 350µl was seeded onto each RNAi plate and left for drying at room temperature for 24 hours.

Pellet from Part A was resuspended in 1X M9 buffer containing 100µg/ml ampicillin and 1mM IPTG to ½ of its volume (25ml, if the initial culture volume of Part A was 50ml). This resuspended bacterial culture was used as feed during the Tm treatment.

Preparation of worms

Gravid adult worms initially grown on *E. coli* OP50 bacteria were bleached and eggs were re-suspended in 100-200ul of 1X M9 buffer and standardized for the number of eggs present in a particular volume.

Preparation of the cocktail

An intermediate stock solution of Tunicamycin (10µg/ml) was prepared in MQ using a stock of 25mg/ml (Sigma Aldrich, USA). In the final reaction mixture, this was diluted so as to treat the eggs with a variety of Tm concentrations ranging from 0.062 to 1 µg/ml. Entire composition of cocktail (total volume 1ml) is mentioned as follows:-

Tm Concentration (µg/ml)	**Feed Volume (µl)**	**Tm Volume (10 µg/ml stock conc.) (µl)**	**Eggs volume (µl)**	**M9 (µl)**
0.125	875	12.5	25	87.5
0.25	875	25	25	75
0.5	875	50	25	50
1	875	100	25	0

Eggs were left on rotation in this cocktail at a slow speed for 24 hrs. Following this, the hatched L1 larvae were washed 3-4 times with 1X M9 buffer to remove traces of tunicamycin and added onto seeded control RNAi or test RNAi plates containing no drug. They were allowed to grow till adulthood when 100 worms belonging to each treatment regime were transferred onto FuDR containing RNAi plates and life span was scored as mentioned above. iUPR^{ER} efficiency was also measured on Day 2 of adulthood, as mentioned previously.

RNA isolation

Worms grown on control or test RNAi were collected using 1XM9 buffer and washed thrice to remove bacterial contamination. To 50µl of the worm pellet, 200 µl of Trizol reagent (Invitrogen, USA) was added and subjected to three freeze-thaw cycles in liquid nitrogen. Intermittent vortexing was done to break open worm bodies (at this point, samples were stored in -80 °C). Later, 100 µl of Trizol was again added to the worm pellet and the sample was vortexed vigorously. To this, 150 µl of chloroform was added and tube was gently inverted several times followed by incubation at room temperature for 3 minutes. After incubation, sample was centrifuged at 12000g for 15 minutes at 4°C. The upper aqueous phase was gently removed into a fresh tube without disturbing the bottom layer and interphase. To this aqueous solution, an equal volume of isopropanol was added and the reaction was allowed to sit at room temperature for 10 minutes followed by centrifugation at 12000g for 10 minutes at 4°C. The supernatant was carefully discarded without disturbing the pellet that contained RNA. The pellet was later washed using 1 ml 70% ethanol solution followed by centrifugation at 12000g for 5 minutes at 4°C. RNA pellet was further dried at room temperature and later dissolved in nuclease free water, followed by incubation at 65°C for 10 minutes with intermittent tapping. Concentration of RNA was determined by measuring absorbance at 260 nm using Nano Drop UV spectrophotometer (Thermo Scientific, USA) and quality checked using denaturing formaldehyde gel.

Gene expression analysis using quantitative real time PCR (QRT-PCR)

First strand cDNA synthesis was carried out following the manufacturer's guidelines (Invitrogen, Carlsbad, CA). Briefly, 2.5-5µg of RNA was mixed with 12-18 mer oligo dT primers and dNTPs and reaction was heated at 65 °C for 5 minutes followed by cooling at 4 °C for 1 min. To this mixture, Dithiothreitol (DTT), RNaseOUT, 5X reverse transcriptase buffer and 1 µl/reaction of Superscript Reverse transcriptase III enzyme were added in the required concentrations. The reaction was incubated at 42 °C for 50 minutes and later terminated by incubation at 70 °C for 15 minutes. The prepared cDNA can be stored at -20°C for long term usage.

Gene expression levels were determined using the DyNAmo Flash SYBR Green mastermix (Thermo Scientific, USA) and Eppendorf Realplex PCR system (Eppendorf, USA) according to manufacturer's guidelines. Relative expression of each gene was determined by normalising the data to actin expression levels.

Cycloheximide Chase experiment

A western blot-based approach was taken to monitor the HSP-4 degradation rate *in-vivo*. Day1 adult WT and *eat-2(ad1116)* worms grown on control RNAi were washed from plates to remove adhered bacteria and added to 1X S-basal buffer containing 2mg/ml cycloheximide drug (Sigma Aldrich, USA). The experiment was performed in 24 well plates with each well containing 500 µl of drug-buffer solution with gentle agitation. At each time point, including at 0 hrs, worms were washed thrice with 1XM9 buffer to remove traces of cycloheximide and resuspended in 100 µl of 5% TritonX-100 solution containing 1X-EDTA free protease inhibitor cocktail (Sigma Aldrich, USA). Cell lysates, prepared from these samples were run on a denaturing SDS-PAGE and HSP-4 levels were detected at each time point by western blot analysis.

Western Blotting analysis

Worms resuspended, prepared as above, were sonicated using Bioruptor (Diagenode, USA) at maximum intensity for 15 cycles (30 sec on and 30 sec off pulse). The sonicated sample was centrifuged at 5000g for 5 minutes at 4 °C and supernatant was removed to a fresh tube. Bradford protein assay (Biorad, USA) was used for estimation of protein concentration. Thirty µg of each protein lysate, prepared from untreated and cycloheximide treated samples, was mixed with Laemmli buffer containing β-mercaptoethanol to make the final concentration of the dye to 1X containing 5% β-ME. The lysate was then denatured by heating at 95 °C for 10 minutes followed by brief centrifugation to settle the debris present. The supernatant was run on a 12% polyacrylamide gel. Proteins resolved by SDS page were transferred to PVDF membrane (Millipore, Billerica, MA) at 100 V for 1.5 hours at 4 °C in pre-chilled transfer buffer. Following transfer, the membranes were incubated in primary antibody solutions (Anti GRP78 antibody, Catalogue number 06274, Novus Biologicals, USA) (made in blocking

buffer or 1X PBST) overnight at 4 °C on a rocker shaker. Unbound primary antibody was removed by washing the membrane thrice with 0.1% Tween containing 1XPBST, each for 10 minutes on a rocker shaker. Subsequently, membranes were incubated in secondary anti-rabbit IgG antibody solution prepared in blocking buffer or 1XPBST, for 1 hour at RT. Unbound secondary antibody was washed off as mentioned above. Specific antibody binding onto both the membranes was detected using an ECL reagent (Millipore, USA). For detecting Poly! Using western analysis, anti-GFP antibody (Catalog number sc9996, Santa Cruz, USA).

Ubiquitination assay

Day1 adult WT (Bristol N2) or *eat-2(ad1116)* worms grown on control RNAi were washed to remove adherent bacteria and added to 1X S-basal buffer with or without 100µM of MG-132, a proteasomal inhibitor. The worms were incubated in these solutions in 24-well plates for 6 hours at 20 °C with gentle shaking. They were later washed thrice to remove traces of MG-132. The worm pellets were then resuspended in 100µl of 5% TritonX-100 solution containing 1X EDTA-free protease inhibitor cocktail (Sigma Aldrich, USA). Cell lysates, prepared from these samples were run on denaturing SDS-PAGE and blotted using a poly-Ubiquitin antibody (Catalog number BML-PW8810; Enzo Lifesciences, USA).

Poly-Q aggregation assay

The experiments were performed using an integrated transgenic line containing a stretch of 40 polyglutamine residues coupled to yellow fluorescent protein, expressed in *C. elegans* muscles (using *unc-54* gene promoter) (3-5). WT reporter strain was crossed with *ire-1(v33)* and *eat-2(ad1116)* mutants to assess the polyQ aggregation profile in these mutants as compared to WT. All the strains were grown on control and test-RNAi till adulthood and then transferred onto RNAi plates overlaid with FuDR to a final concentration of 0.1mg/ml. At each successive day from day 1 till day 3 of adulthood, 25 worms of each strain (grown on a particular RNAi) were mounted onto 2% agarose slides and visualized using Axio-Imager M2 Epifluorescent microscope using the YFP filter set. Multiple images were required to cover the entire worm body using a 10X objective.

Total no of bright spots (Puncta or aggregates) on the worm body wall were counted and average number of aggregates for >20 worms was plotted.

HD150Q cell is a stable and inducible mouse neuroblastoma cell line, expressing green fluorescent protein tagged mutant N-terminal huntingtin protein with 150 glutamine residues. Cells were routinely cultured in DMEM with 10% fetal bovine serum and antibiotics containing 0.4 mg/mL Zeocin and 0.4 mg/mL G418 (Geneticin). During experiments, cells were plated onto 6-well tissue culture plates at sub-confluent density and on the following day, they were treated with different concentrations of tunicamycin (0.125 and 0.250 µg/ml) for 5 hrs, washed and then treated with ponasterone A (1µM) to induce the expression of mutant huntingtin. After 36 hours of ponasterone A treatment, cells were observed under fluorescence microscope followed by collection and processing of the cells for immunoblotting experiments. Aggregate formation was manually counted under a fluorescence microscope (approximately 500 cells in each group) and the cell containing more than one aggregate were considered to have a single aggregate.

Cytotoxicity Study Using HD150Q Cell Line

HD150Q cells were cultured in a tissue culture plate with DMEM. Then, the cells were sub-cultured in a 24-well culture plate containing DMEM (0.5 mL each well) and incubated overnight for cells to adhere on to the culture plate. After 24 h, cells were left untreated or treated with 0.125 or 0.25 µg/ml of tunicamycin for 5 h, washed and then induced with ponasterone A (1 µM) in serum free MEM. After 5 days, cell viability was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Typically, each well containing cells was treated with freshly prepared MTT solution and incubated for 4 h. Then, the supernatant was carefully removed, leaving violet formazan on the plate. Formazan was then dispersed in DMF/water containing SDS, and its absorbance was measured at 570 nm with a microplate reader. Cell viability was correlated with the absorbance value, assuming 100% viability for the cells without any treatment.

DAVID analysis

The RNA-seq data for synchronised Day1 adult worms [WT and *eat-2(ad1116)*] grown on *E. coli* OP50 (submitted to NCBI with the BioProject ID PRJNA342407) was analyzed using DAVID functional annotation tool. The listed pathways have $p<0.05$ and FDR<10 (Bonferroni Corrected *p*- value-0.0011).

ChIP-seq data analysis

The ChIP-seq data analysis was performed using parameters mentioned in our previous publication (6). PHA-4 ChIP-seq data (SRA-NCBI GSE50301) was downloaded from modENCODE (http://www.modencode.org/) in .sra format. Downloaded data was converted into fastq format using NCBI-recommended SRA toolkit (version 2.2.2a). Converted fastq of replicates were merged and used for further analysis. Reads were aligned to the *C. elegans* genome (WS230) using Bowtie (v0.12.7) (7) with the following parameters: -q -m 1 --best --strata. Mapped reads were used for peak calling and calculation of read density. Enriched peaks were identified using the peak calling algorithm MACS (v1.4.2)(8) using following parameters: --mfold=5,30 --bw=175 -w. Statistically significant peaks ($P < 1 \text{ X } 10^{-5}$) were used for further analysis. To find target genes, PeakAnalyzer (v1.4)(9) program was used and all genes having peaks within 2 kb of the promoter region were considered for further analysis. UCSC genome browser (10) was used for visualizing aligned data as wig files.

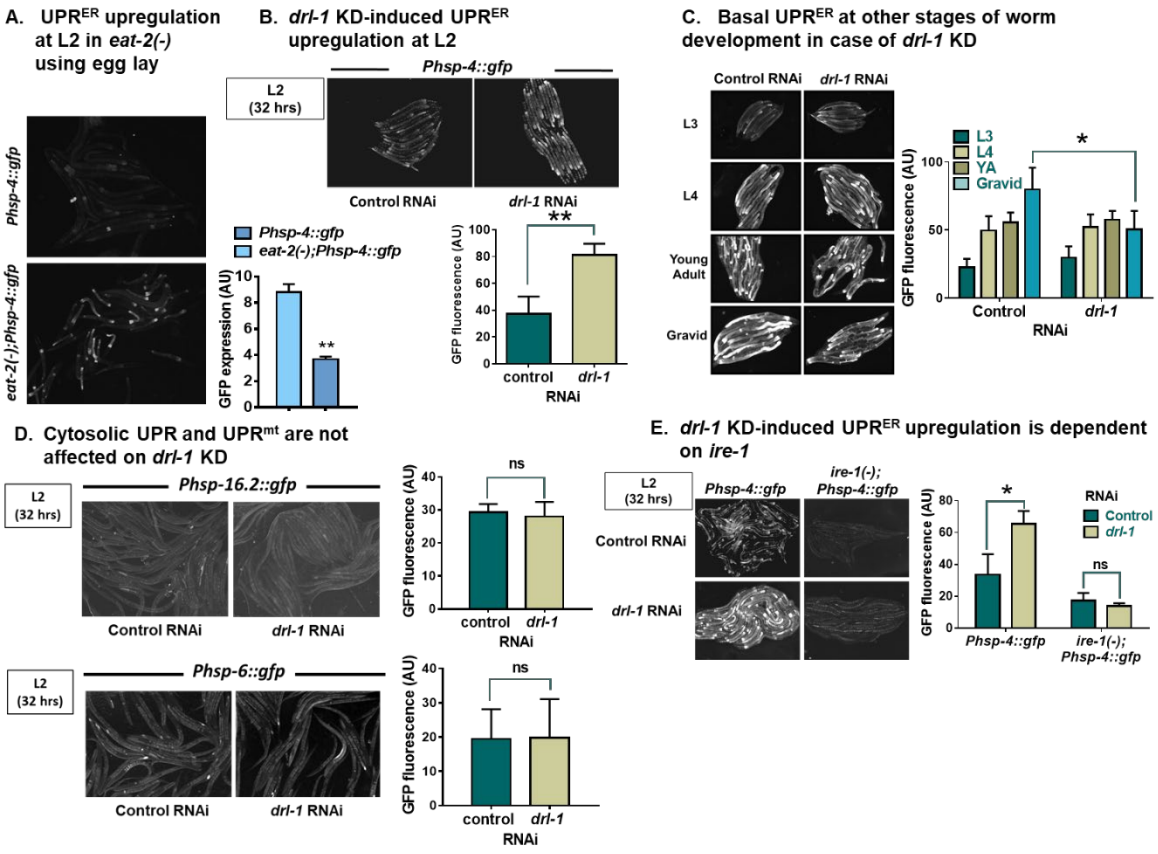

Fig S1: (A) Representative images of *Phsp-4:gfp(zcIs4)* and *eat-2(ad1116);Phsp-4:gfp(zcIs4)* worm at 32 hours after synchronized egg laying. Densitometric quantification averaged over 2 biological repeats, shows the transient upregulation of basal UPR^{ER} in *eat-2(-)*. Error bars-Std. Dev. **(B)** Representative images of *Phsp-4:gfp(zcIs4)* worms, grown on control or *drl-1* RNAi, at L2 stage (32 hours after egg synchronization). Densitometric quantification averaged over 2 biological repeats, shows the transient upregulation of basal UPR^{ER} in *drl-1* RNAi worms. Error bars-Std. dev. **(C)** Representative images of *Phsp-4:gfp(zcIs4)* worms growing on control or *drl-1* RNAi at indicated larval stages and in gravid adults. Densitometric quantification showing *drl-1* knockdown worms having no increase in basal UPR^{ER} response as compared to control RNAi in these stages. Basal UPR^{ER} is lower in *drl-1* RNAi worms at gravid adult stage. Average of 2 biological replicates. Error bars are Std. dev. YA- young adult. **(D)** *Drl-1* knockdown does not affect cytosolic heat shock response (HSR; upper panel). Representative images of *Phsp-16.2:gfp(dvIs70)* worms, representing HSR, grown on control or *drl-1* RNAi at L2 stage. *Drl-1* knockdown does not affect mitochondrial stress

response (lower panel). Representative images of *Phsp-6:gfp(zcIs9)* worms, representing UPR^{mt}, grown on control or *drl-1* RNAi at L2 stage. Densitometric quantifications averaged over 2 biological repeats do not show any difference between *drl-1* knockdown and control RNAi. Error bars- Std. dev. * $p<0.05$, ** $p<0.01$, ns-not significant by Student's *t* test. **(E)** UPR^{ER} up-regulation in L2 larval stage on *drl-1* knockdown is dependent on *ire-1*. Representative images of *Phsp-4:gfp(zcIs4) and ire-1(zc14);Phsp-4:gfp* worms growing on control or *drl-1* RNAi at L2 (32 hours post-egg synchronization). Densitometric quantification of GFP fluorescence averaged over 2 biological repeats is shown on the right. Error bars- SEM. * $p<0.05$ as determined by Two-way Annova.

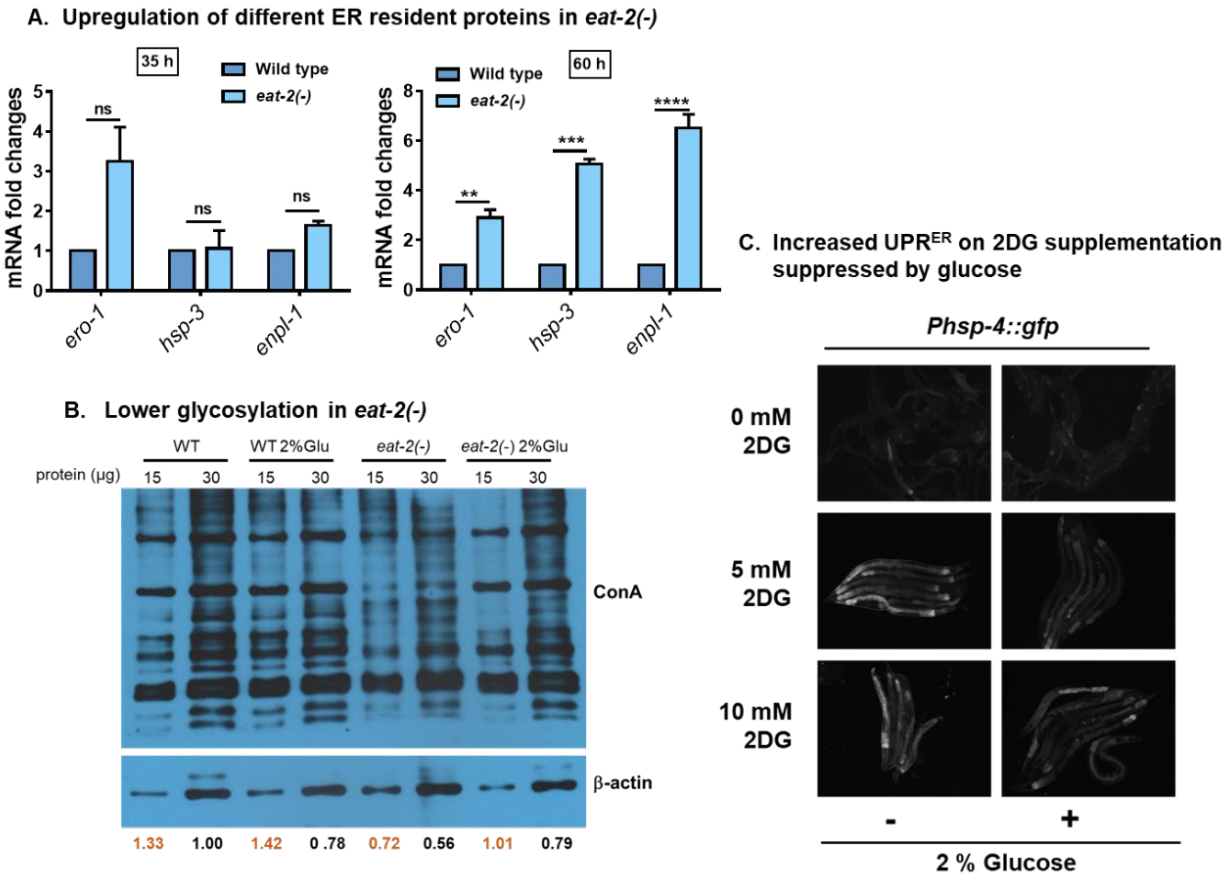

Fig S2: (A) The expression of ER resident proteins is upregulated in *eat-2(-)* as compared to WT during different stages of post-embryonic development, determined by QRT-PCR analysis. Average of 3 biological replicates. Error bars- SEM, ** p<0.01, *** p<0.001, **** p<0.0001, Student's t test. **(B)** Concavalin A western blot analysis was performed after loading different concentrations of WT or *eat-2(-)* (with or without exposure to 2% glucose) proteins. The bands were quantified after normalizing with β-actin. Supplementary data of Figure 1E. **(C)** *Phsp-4:gfp(zcIs4)* worms were exposed to different concentrations of 2-Deoxyglucose (2DG) with or without 2% glucose. Glucose supplementation reduced upregulation of *hsp-4::gfp* at L2 stage in worms treated with 2DG.

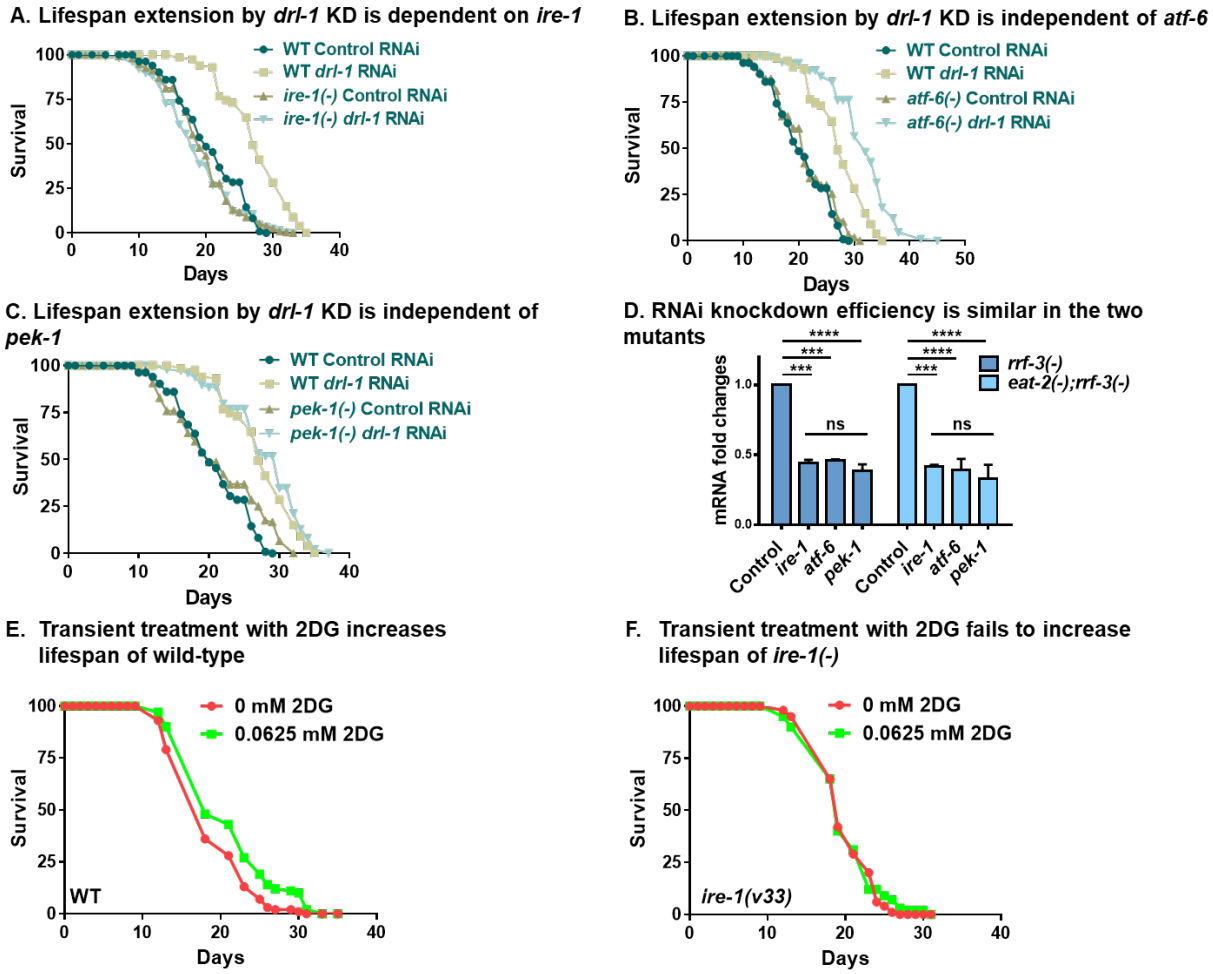

Fig S3: (A) In an *ire-1* mutant, *drl-1* knockdown failed to extend lifespan. **(B-C)** Knocking down *drl-1* can increase the lifespan of *atf-6* **(B)** and *pek-1* mutants **(C)**. **(D)** Efficiency of RNAi knockdown in *rrf-3(-)* and *eat-2(-);rrf-3(-)* as measured by quantitative real time-PCR (QRT-PCR). Average of three biological replicates. Error bars are SEM. *** $p<0.001$, **** $p<0.0001$, ns- non significant by Student's *t-test.* **(E)** Life span of WT is increased on transient supplementation with 2-Deoxyglucose (2DG). WT worms were bleached and eggs were added to M9 buffer containing bacterial feed and supplemented with 0.0625 mM 2DG for the first 24 hours. The hatchlings were washed, transferred to MGM agar plates and later scored for adult lifespan. As a control, worms were treated with water (2DG = 0 mM). **(F)** Transient 2DG treatment failed to increase life span in *ire-1(v33)***.** Complete life span data is present in Table S1.

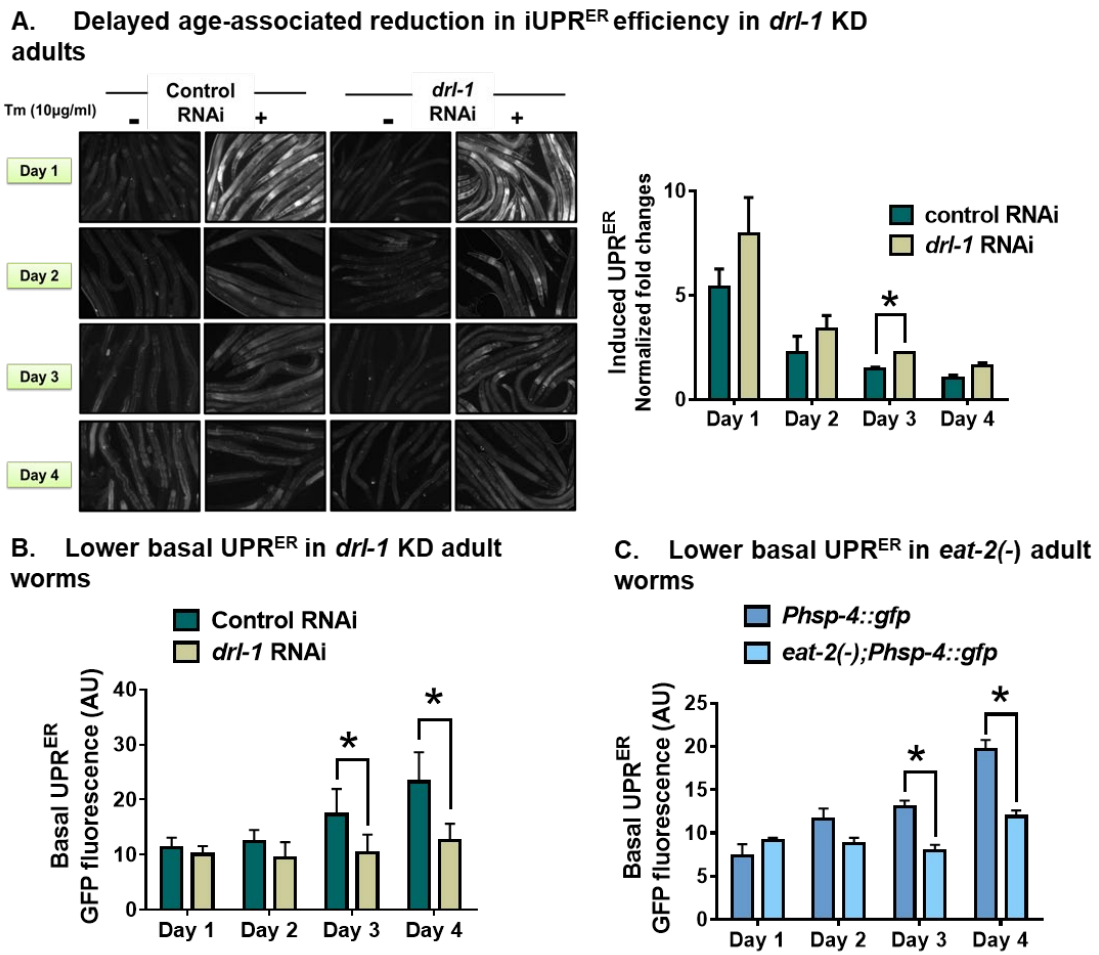

Fig S4: (A) *Drl-1* knockdown delays age-associated decline in iUPR^{ER} efficiency. Representative images of *Phsp-4:gfp(zcIs4)* worms grown on control or *drl-1* RNAi with/without acute Tm treatment (10 µg/ml for 6 hours) on different days of adulthood. Graph represents normalized fold induction in GFP fluorescence after 6 hours of Tm treatment on different days of adulthood. [Normalization was performed with the basal GFP fluorescence (basal UPR^{ER}) for individual experiment]. Error bars- SEM, average of 2 biological replicates. **(B)** Densitometric quantification of GFP fluorescence in control RNAi- or *drl-1* RNAi-treated worms without any treatment (Basal UPR^{ER}) on indicated days of adulthood. Error bars- SEM, 2 biological replicates. (**C**) Densitometric quantification of GFP fluorescence in WT and *eat-2* mutant background without any treatment (Basal UPR^{ER}) on indicated days. Error bars are SEM, 2 biological replicates, * represents $p<0.05$, as determined by Student's *t* test.

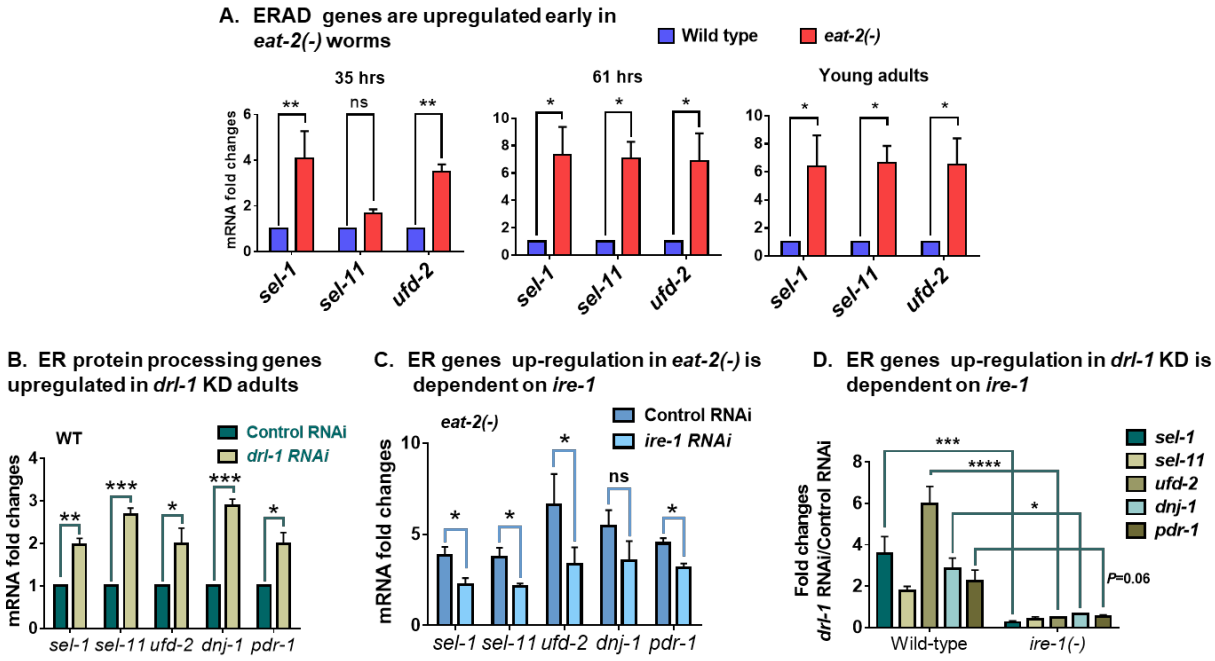

Fig S5: (A) QRT-PCR analysis was used to compare ER protein processing genes in *eat-2(ad1116)* compared to WT at different time points during post-embryonic development. **(B)** QRT-PCR analysis to detect the mRNA levels of ER protein processing genes show significant up-regulation in Day 1 WT adults grown on *drl-1* RNAi as compared to control RNAi. **(C)** QRT-PCR analysis was used to compare ER protein processing genes between *eat-2(ad1116)* and WT adults grown on Control or *ire-1* RNAi. **(D)** QRT-PCR analysis to detect the mRNA levels of ER protein processing genes in Day 1 WT and *ire-1(v33)* adults grown on *drl-1* RNAi as compared to control RNAi. Average of 3 or more biological replicates. Error bars indicate SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ as determined by the Student's *t* test.

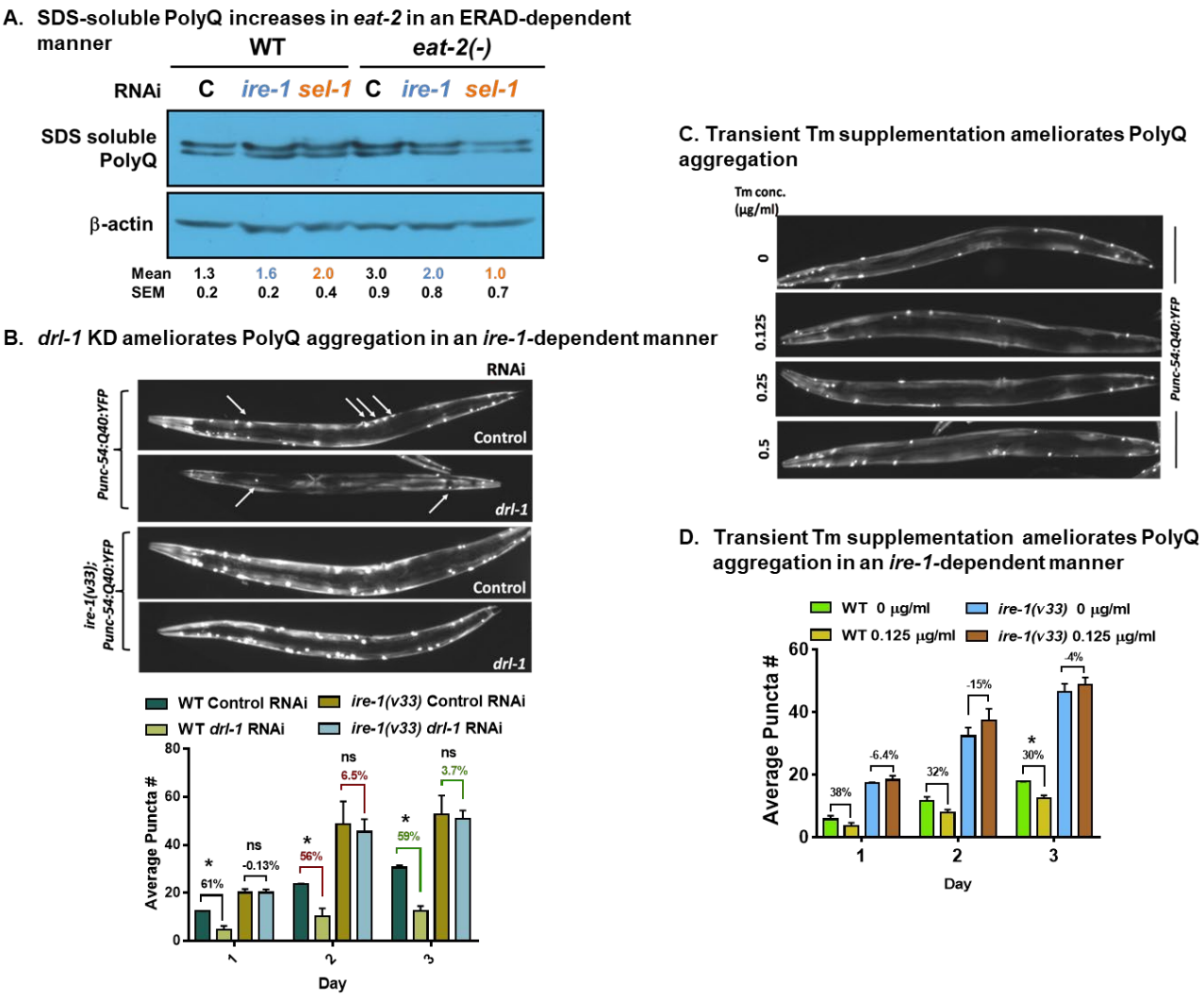

Fig S6: (A) The *Punc-54:polyQ40:yfp* or *eat-2(ad1116;Punc-54:polyQ40:yfp* worms were grown on control, *ire-1* or *sel-1* RNAi and western blot performed for SDS soluble PolyQ aggregates using anti-GFP antibody. β-actin was used to show equal loading of protein. **(B)** Representative images showing polyQ aggregates in *Punc-54:polyQ40:yfp and ire-1(v33);Punc-54:polyQ40:yfp* worms on control or *drl-1* RNAi at day 2 of adulthood. Suppression of polyQ aggregation (indicated in %) on *drl-1* knockdown is abrogated in *ire-1* mutants. Average of three biological replicates is plotted. Error bars- Std. dev. **(C)** Representative images showing polyQ aggregates for *Punc-54:polyQ40:yfp* that has been treated transiently with Tm. Quantification is presented in Figure 6C. **(D)** Graph represents reduction in the number of aggregates for *Punc-54:polyQ40:yfp* and *ire-1(v33);Punc-54:polyQ40:yfp* worms transiently exposed to 0.125 µg/ml Tm during hatching, as compared to untreated worms (Tm=0 µg/ml). Average of two biological

replicates is plotted. Error bars- Std. dev. * represents $p<0.05$ as determined by Student's *t* test, ns=non-significant.

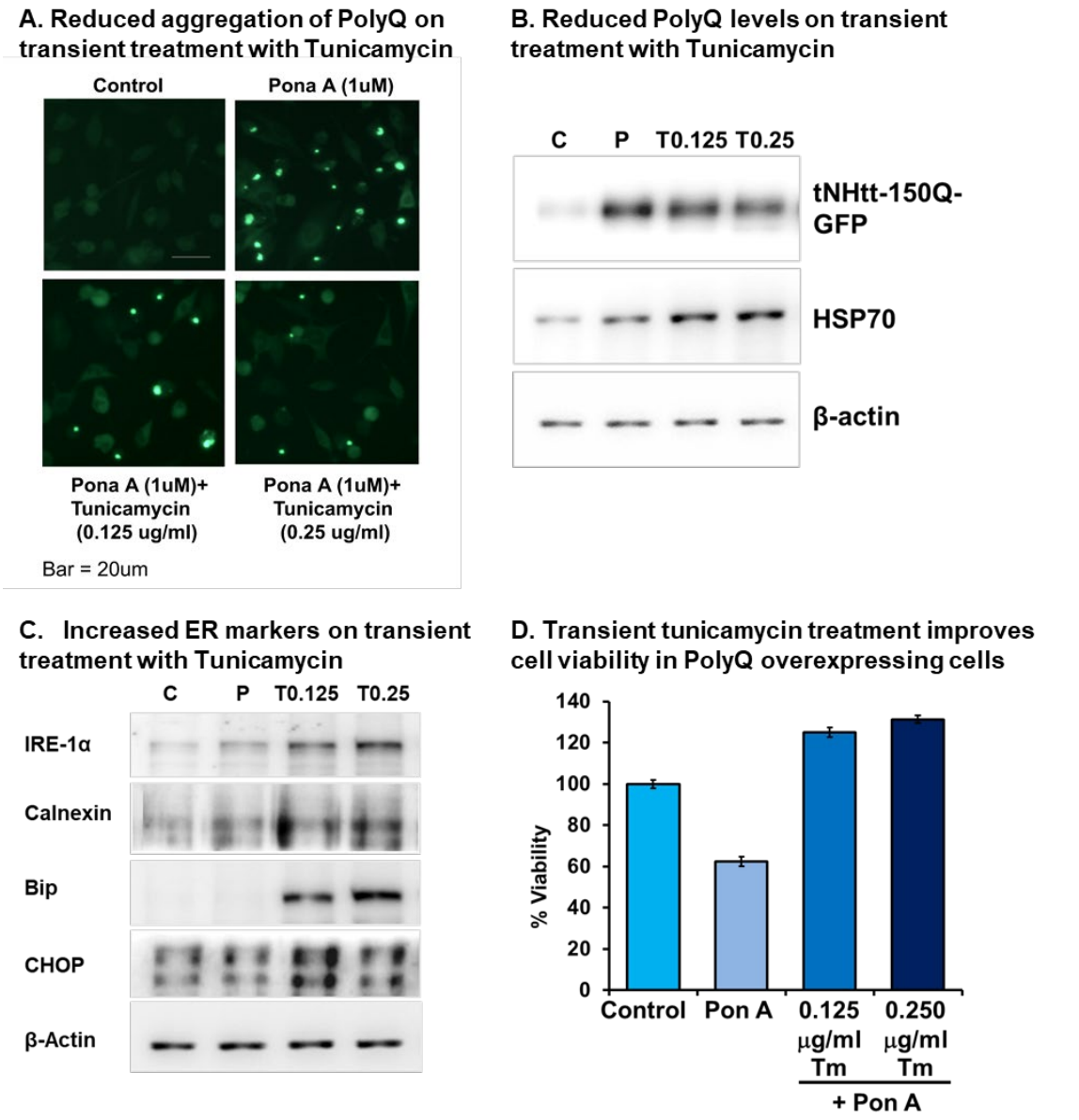

Fig S7: (A) Representative images of PolyQ150 aggregation data presented in Figure 6D. **(B-C)** HD150Q cells were pre-treated with tunicamycin for 5 h, washed and then incubated with ponasterone A (Pon A) for 24 h (which was then washed off) to induce the expression of mutant huntingtin-GFP. At 36 h post-induction, cells were processed for immunoblot analysis of mutant huntingtin-GFP using GFP antibody (B) or for various ER-stress markers (B-C). **(D)** Cell viability of PolyQ cells were determined by MTT assay, five days after transient Tm treatment.

Table S1. Summary of life span analysis

Figure 2A-C

Genetic Background	RNAi	N	Mean ± SEM (days)	% Change	*P*-value	No of experiments
rrf-3(pk1426)	control	256	16.41 ± 0.21			4
eat-2(ad1116):rrf-3(pk1426)	control	526	27.31 ± 0.15	66.42	>0.0001	4
rrf-3(pk1426)	*ire-1*	248	16.69 ± 0.21			4
eat-2(ad1116):rrf-3(pk1426)	*ire-1*	329	21.67 ± 0.16	29.84	>0.0001	4
rrf-3(pk1426)	*pek-1*	191	16.6 **±** 0.24			4
eat-2(ad1116):rrf-3(pk1426)	*pek-1*	299	26.57 **±** 0.21	60.06	>0.0001	4
rrf-3(pk1426)	*atf-6*	187	16.78 **±** 0.27			4
eat-2(ad1116):rrf-3(pk1426)	*atf-6*	319	25.77 ± 0.19	53.58	>0.0001	4

Figure S3A-C

Genetic Background	RNAi	N	Mean ± SEM (days)	% Change	*P*-value	No of experiments
Wild-type	Control	350	20.51 ± 0.27			4
	drl-1	201	27.54 ± 0.33	34.27	>0.0001	4
ire-1(v33)	Control	413	19.45 ± 0.25			4
	drl-1	475	18.60 ± 0.26	-4.37	0.193	4
pek-1(ok275)	Control	228	21.21 ± 0.44			3
	drl-1	147	27.96 ± 0.44	31.80	>0.0001	3
atf-6(ok551)	Control	212	21.22 ± 0.36			3
	drl-1	200	31.55 ± 0.40	48.60	>0.0001	3

Figure 2D-F

Genetic Background	Tm Conc (µg/ml)	N	Mean ± SEM (days)	% Change	*P*-value	No of experiments
Wild-type	0	212	18.45 ± 0.23			3
	0.125	167	21.73 ± 0.28	17.78	>0.0001	3
Wild type (for *ire-1* hormesis expt)	0	275	18.53 ± 0.22			3
	0.125	252	22.02 ± 0.30	18.83	>0.0001	3

ire-1(v33)	0	310	18.80 ± 0.21			3
	0.125	266	18.64 ± 0.21	-0.85	0.163	3
Wild type (for *eat-2* hormesis expt)	0	258	18.63 ± 0.24			2
	0.125	190	21.58 ± 0.36	15.83	>0.0001	2
eat-2 (ad1116)	0	260	27.97 ± 0.36			2
	0.125	168	25.32 ± 0.45	-10.47	>0.0001	2

Figure S2E-F

Genetic Background	2-DG Conc (mM)	N	Mean ± SEM (days)	% Change	*P*-value	No of experiments
Wild-type	0	158	18.99 ± 0.35			3
	0.0625	203	21.21 ± 0.37	11.69	>0.0001	3
ire-1(v33)	0	264	20.20 ± 0.19			3
	0.0625	230	20.07 ± 0.26	-0.64	0.4714	3

Figure 4G

Genetic Background	RNAi	N	Mean ± SEM (days)	% Change	*P*-value	No of experiments
rrf-3(pk1426)	control	256	16.41 ± 0.21			4
eat-2(ad1116):rrf-3(pk1426)	control	526	27.31 ± 0.15	66.42	>0.0001	4
rrf-3(pk1426)	*ufd-2*	177	17.12 ± 0.25			4
eat-2(ad1116):rrf-3(pk1426)	*ufd-2*	342	24.12 ± 0.15	40.89	>0.0001	4
rrf-3(pk1426)	control	260	16.00 ± 0.20			3
eat-2(ad1116):rrf-3(pk1426)	control	409	26.95 ± 0.18	68.44	>0.0001	3
rrf-3(pk1426)	*sel-1*	184	16.55 ± 0.24			3
eat-2(ad1116):rrf-3(pk1426)	*sel-1*	280	22.10 ± 0.20	33.53	>0.0001	3

Figure 5E

Genetic Background	RNAi	Tm Conc (µg/ml)	N	Mean ± SEM (days)	% Change within RNAi	*P*-value	No of experiments
Wild-type	Control	0	118	18.81 ± .41			2
		0.125	134	21.07 ± .41	12.01	0.0001	2
	ire-1	0	138	18.40 ± .27			2
		0.125	104	18.29 ± .34	-0.60	0.7652	2
	pha	0	91	17.38 ± .28			2
		0.125	99	17.66 ± 30	1.61	0.2977	2

Table S2: List of ER protein processing genes up-regulated in *eat-2(ad1116)* mutant as compared to WT at Day 1 of adulthood.

No.	Gene name	Fold up-regulation	*P*-value	Function
1	*skr-5*	2.67	0.050	SKp1 related(ubiquitin ligase component
2	*dnj-29*	2.68	>0.0001	J domain protein
3	*skr-8*	2.01	>0.0001	SKp1 related(ubiquitin ligase component
4	*dnj-7*	3.16	0.002	J domain protein
5	*ZK1307.8*	5.17	>0.0001	predicted to have calcium ion binding activity
6	*skr-13*	3.58	>0.0001	SKp1 related(ubiquitin ligase component
7	*skr-3*	2.03	>0.0001	SKp1 related(ubiquitin ligase component
8	*skr-9*	2.53	0.006	SKp1 related(ubiquitin ligase component
9	*sec-12*	2.59	>0.0001	Prolactin regulatory element binding
10	*T24H7.2*	4.7	>0.0001	Encodes an ortholog of hypoxia-upregulated vertebrate proteins, chaperone
11	*skr-10*	2.87	>0.0001	SKp1 related(ubiquitin ligase component
12	*atf-6*	3.89	0.040	proximal sensor required for theUPR, with a bZIP transcription factor domain
13	*T14G8.3*	5.41	>0.0001	encodes an ortholog of hypoxia-upregulated vertebrate proteins, chaperone
14	*sec-24.1*	2.57	>0.0001	a member of the Sec24-Sec23 subunit of the COPII coat complex
15	*C14B9.2*	3.48	>0.0001	encodes a protein disulfide isomerase
16	*sec-24.2*	3.05	>0.0001	a member of the Sec24-Sec23 subunit of the COPII coat complex
17	*sel-1*	3.5	>0.0001	member of the HRD complex that degrades malfolded ER proteins
18	*F44E5.4*	6.71	0.005	hsp-70 family member
19	*dnj-14*	2.04	0.001	J domain protein
20	*ero-1*	2.72	>0.0001	Oxidoreductase in ER
21	*ubxn-6*	2.05	>0.0001	ubiquitin regulatory X domain-containing protein
22	*dnj-12*	2.4	>0.0001	J domain protein

23	*F44E5.5*	7.64	>0.0001	hsp-70 family member
24	*cdc-48.3*	3.5	0.001	Final step in ubiquitin mediated proteolysis
25	*F10C2.5*	9.16	>0.0001	ortholog of human EDEM2
26	*uggt-1*	4.63	>0.0001	encodes UDP-Glc:glycoprotein glucosyltransferase (UGGT), an ER enzyme involved in quality control
27	*Y105E8A.2*	3.73	>0.0001	endoplasmic reticulum lectin 1
28	*C47E12.3*	2.69	0.002	ortholog of human EDEM1 (ER degradation enhancer, mannosidase alpha-like 1
29	*pdr-1*	2.44	>0.0001	parkin protein
30	*ubc-15*	2.23	0.003	E2 ubiquitin-conjugating enzyme
31	*ufd-2*	4.8	>0.0001	E4 ubiquitin conjugation factor, in complex with cdc48
32	*ile-1*	2.27	>0.0001	intracellular lectin
33	*pek-1*	5.52	>0.0001	UPR sensor, orthologous to human eukaryotic translation initiation factor 2-alpha kinase 3
34	*hsp-70*	3.05	0.002	hsp-70 family member
35	*F54F2.9*	7.47	>0.0001	hsp-70 family member
36	*ZC506.1*	6.07	0.002	ortholog of human EDEM3
37	*unc-23*	4.79	>0.0001	negatively regulate proteosomal degradation by the E3 ubiquitin ligase
38	*cul-1*	2.97	>0.0001	cell cycle protein
39	*ire-1*	3.75	>0.0001	proximal sensor, with an endoribonuclease activity
40	*dnj-1*	4.76	>0.0001	J domain protein
41	*cnx-1*	3.99	>0.0001	Calcium Binding chaperone , for glycoprotein folding

Genes highlighted in **ORANGE** are ERAD pathway genes, in **BLUE** are hsp-70 proteins and in **GREEN** are DnaJ proteins

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