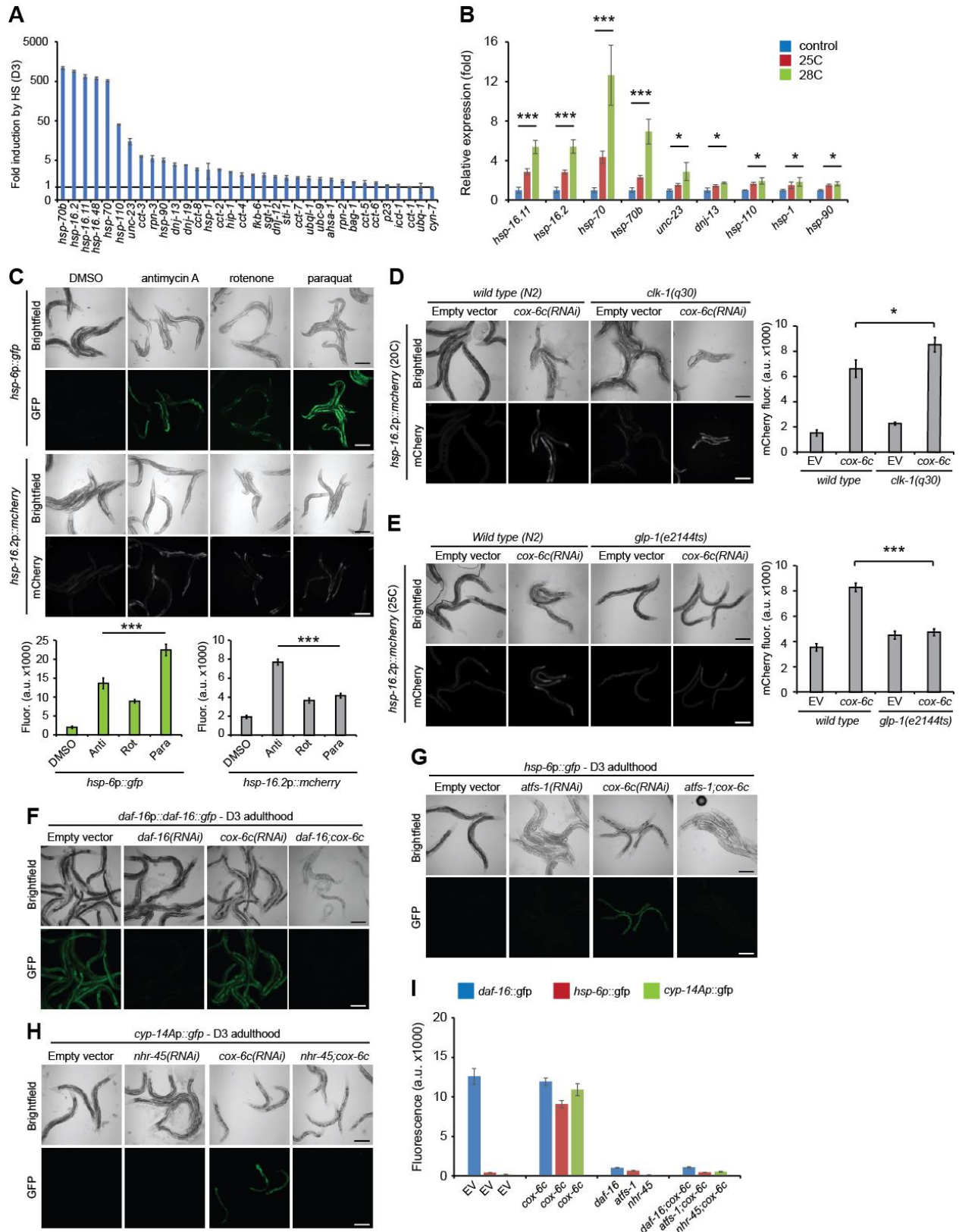**Supplemental Figure 1 (related to main figure 1)**

Supplemental Figure 1: ETC stress does not increase HSF-1 activity through known stress response pathways (related to Figure 1)

A – Relative expression of HSF-1 target genes following heat shock (33°C, 30 min) compared to control treated animals (20°C) on day 3 of adulthood. Values plotted are the mean of 4 biological replicates.

B – Relative expression of HSF-1 target genes on day 3 of adulthood in wild type animals exposed to control conditions (20°C), 25°C for 1 hour or 28°C for 1 hour. Expression was calculated relative to the HK genes *cdc-42, rpb-2* and *pmp-3*. Values plotted are the mean of 4 biological replicates.

C – Representative images and fluorescence quantification of *hsp-6*p::gfp and *hsp-16.2*p::mcherry reporters on day 3 of adulthood following treatment with DMSO, antimycin A, rotenone or paraquat. Values plotted are the mean fluorescence of at least 15 worms per treatment group.

D & E – Representative images and fluorescence quantification of day 3 adult wild type and (**D**) *clk-1(qm30)* or (**E**) *glp-1(e2144ts)* worms expressing *hsp-16.2*p::mcherry following exposure to empty vector (EV) control (L4440) or *cox-6c(RNAi).* Experiments involving *glp-1* and *clk-1* mutants were performed at 25°C and 20°C respectively. Values plotted are the mean fluorescence of at least 15 worms per treatment group.

F-H – Representative images of (**F**) *daf-16*p::*daf-16::*GFP, (**G**) *hsp-6*p::gfp and (**H**) *cyp14A*p::*gfp* worms following growth on empty vector (EV) control, *cox-6c(RNAi)* or EV/*cox-6c(RNAi)* combined with (**F***), daf-16(RNAi),* (**G**) *atfs-1(RNAi)* or (**H**) *nhr-45(RNAi).*

I - Fluorescence quantification of day 3 adult *daf-16*p::*daf-16::*GFP, *hsp-6*p::gfp and *cyp14A*p::*gfp* worms. Values plotted are the mean fluorescence of at least 15 worms per treatment group.

All scale bars = 250 µM. Statistical significance was calculated by: (B and C) one-way ANOVA with Tukey post-analysis comparison of groups and (D and E) two-way ANOVA with post analysis pairwise comparison of groups. All error bars represent SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplemental Figure 2 (related to main figure 2):
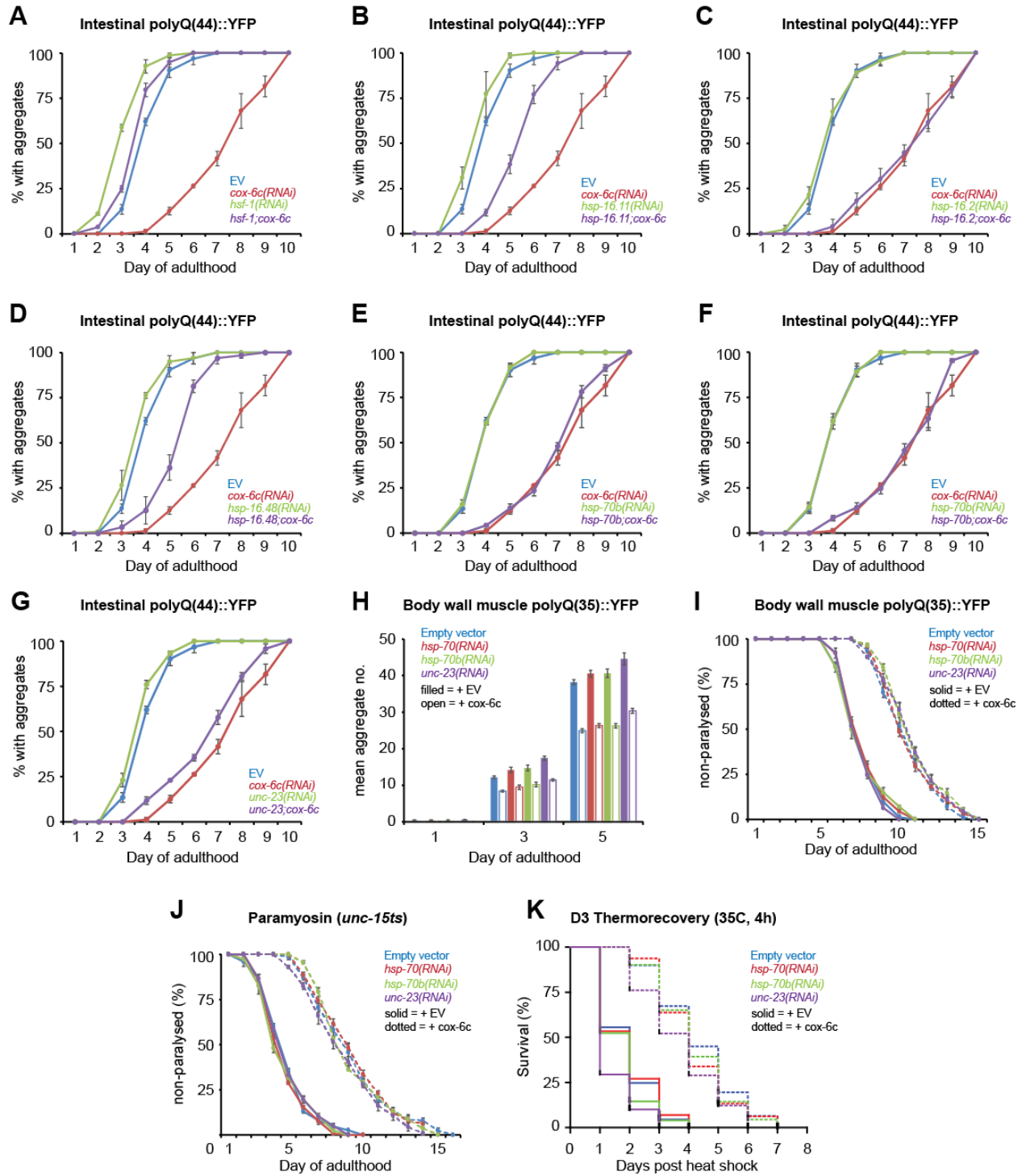
Supplemental Figure 2: Inducible HSP70 and BAG family chaperones are not required for mitochondria to suppress protein aggregation and toxicity (related to main figure 2)

A-G - Quantification of intestinal polyglutamine (Q44)::YFP aggregation with age in worms grown on EV control or *cox-6c(RNAi)* combined with RNAi against *hsf-1, hsp-16.11, hsp-16.2, hsp-16.48, hsp-70, hsp-70b,* or *unc-23*. All experiments were performed in parallel with the same EV and *cox-6c(RNAi)* groups (included on each graph).

H - Quantification of body wall muscle polyglutamine (Q35)::YFP aggregation with age in worms grown on EV control or *cox-6c(RNAi)* combined with RNAi against *hsp-70, hsp-70b,* or *unc-23*.

I - Age-related paralysis of worms expressing polyglutamine (Q35)::YFP in body wall muscle cells and grown on EV control or *cox-6c(RNAi)* combined with RNAi against *hsp-70, hsp-70b,* or *unc-23*.

J - Age-related paralysis of worms expressing metastable paramyosin (*unc-15ts)* and grown on EV control or *cox-6c(RNAi)* combined with RNAi against *hsp-70, hsp-70b,* or *unc-23*.

K - Survival curves for worms following 4 hour heat shock at 35°C on day 3 of adulthood following growth on EV control or *cox-6c(RNAi)* combined with RNAi against *hsp-70, hsp-70b,* or *unc-23*. See supplemental table 1 for survival statistics.

(**A-J**) All values plotted are the mean of 4 biological replicates and error bars represent SEM.

Supplemental Figure 3 (related to main figure 3)
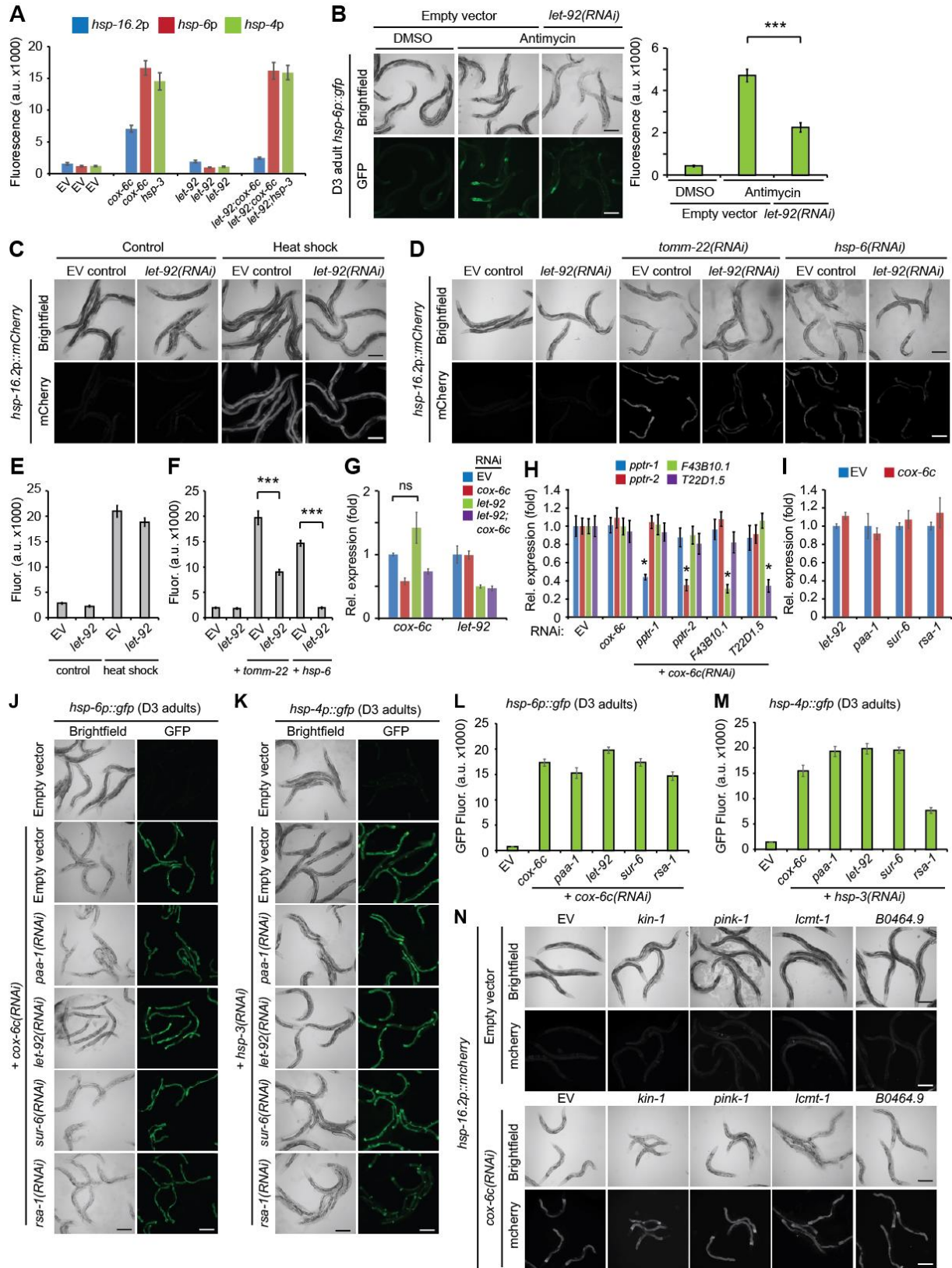
Supplemental Figure 3: *let-92* is necessary for activation of the UPR^{mt} in response to antimycin treatment (related to main figure 3)

A - Quantification of fluorescence intensity in *hsp-16.2*p::mcherry (blue bars), *hsp-6*p::gfp (red bars) and *hsp-4*p::gfp (green bars) reporter worms on day 3 of adulthood following growth on empty vector (EV) control (L4440), *cox-6c(RNAi)* or *hsp-3(RNAi)* combined with RNAi against *let-92*. Values plotted are the mean fluorescence intensity of at least 15 worms per treatment group. Error bars represent SEM.

B – Representative images and quantification of GFP fluorescence intensity of day 3 adult *hsp-6*p::gfp worms exposed to antimycin in the presence or absence of *let-92(RNAi)*. Values plotted are the mean fluorescence intensity of at least 15 worms per treatment group. Scale bar = 250 µM

C – Representative images of *hsp-16.2*p::mcherry worms 24 hours after heat shock (35°C, 1h) on day 3 of adulthood following growth on empty vector control (EV) or *let-92(RNAi)*. Scale bar = 250 µM

D – Representative images of *hsp-16.2*p::mcherry worms on day 3 of adulthood following growth on empty vector control (EV) or *let-92(RNAi)* in the presence or absence of *tomm-22(RNAi)* or *hsp-6(RNAi)*.

E & F – Quantification of fluorescence intensity in *hsp-16.2*p::mcherry reporter worms on day 3 of adulthood following (**E**) heat shock or (**F**) exposure to *tomm-22* or *hsp-6* RNAi. Values plotted are the mean fluorescence intensity of at least15 worms per treatment group. Error bars represent SEM.

G – Relative *cox-6c* and *let-92* mRNA levels on day 3 of adulthood following growth on empty vector (EV) control (L4440) (blue bars) or *cox-6c(RNAi)* (red bars) combined with *let-92(RNAi)* (green and purple bars). Values plotted are the mean of 4 biological replicates and error bars represent SEM.

H - Relative mRNA levels of PP2A subunits *pptr-1* (blue bars), *pptr-2* (red bars), *F43B10.1* (green bars) or *T22D1.5* (purple bars) on day 3 of adulthood following exposure to EV control, *cox-6c(RNAi)*, or *cox-6c(RNAi)* combined with RNAi against *pptr-1*, *pptr-2*, *F43B10.1* or *T22D1.5*. Values plotted are the mean of 4 biological replicates and error bars represent SEM. Expression was normalized to the geometric mean of the housekeeping genes *rpb-2, cdc-42,* and *pmp-3*. Statistical significance was calculated by one-way ANOVA. * = $p < 0.001$

I – Relative mRNA levels of PP2A subunits on day 3 of adulthood following exposure to EV control (blue bars) or *cox-6c(RNAi)* (red bars) conditions. Values plotted are the mean of 4 biological replicates and error bars represent SEM. Expression was normalized to the geometric mean of the housekeeping genes *rpb-2, cdc-42,* and *pmp-3*.

J & K – Representative images of (**I**) *hsp-6p::gfp* and (**J**) *hsp-4p::gfp* reporter worms on day 3 of adulthood following growth on empty vector (EV) control (L4440), *cox-6c(RNAi)*, or *hsp-3(RNAi)* combined with RNAi against the PP2A subunits, *let-92, paa-1, sur-6,* and *rsa-1*. Scale bar = 250 µM

L & M – Fluorescence quantification of (**K**) *hsp-6*p::gfp and (**L**) *hsp-4*p::gfp reporter worms on day 3 of adulthood following growth on empty vector (EV) control (L4440), *cox-6c(RNAi)*, or *hsp-3(RNAi)* combined with RNAi against the PP2A subunits, *let-92, paa-1, sur-6,* and *rsa-1*. Values plotted are the mean fluorescence intensity of at least 15 worms per group. Error bars represent SEM.

N – Representative images of day 3 adult *hsp-16.2p::mcherry* worms after exposure to EV control or *cox-6c(RNAi)* in combination with RNAi against the PP2A regulators *kin-1, pink-1, lcmt-1,* and *B0464.9/PPME1*.
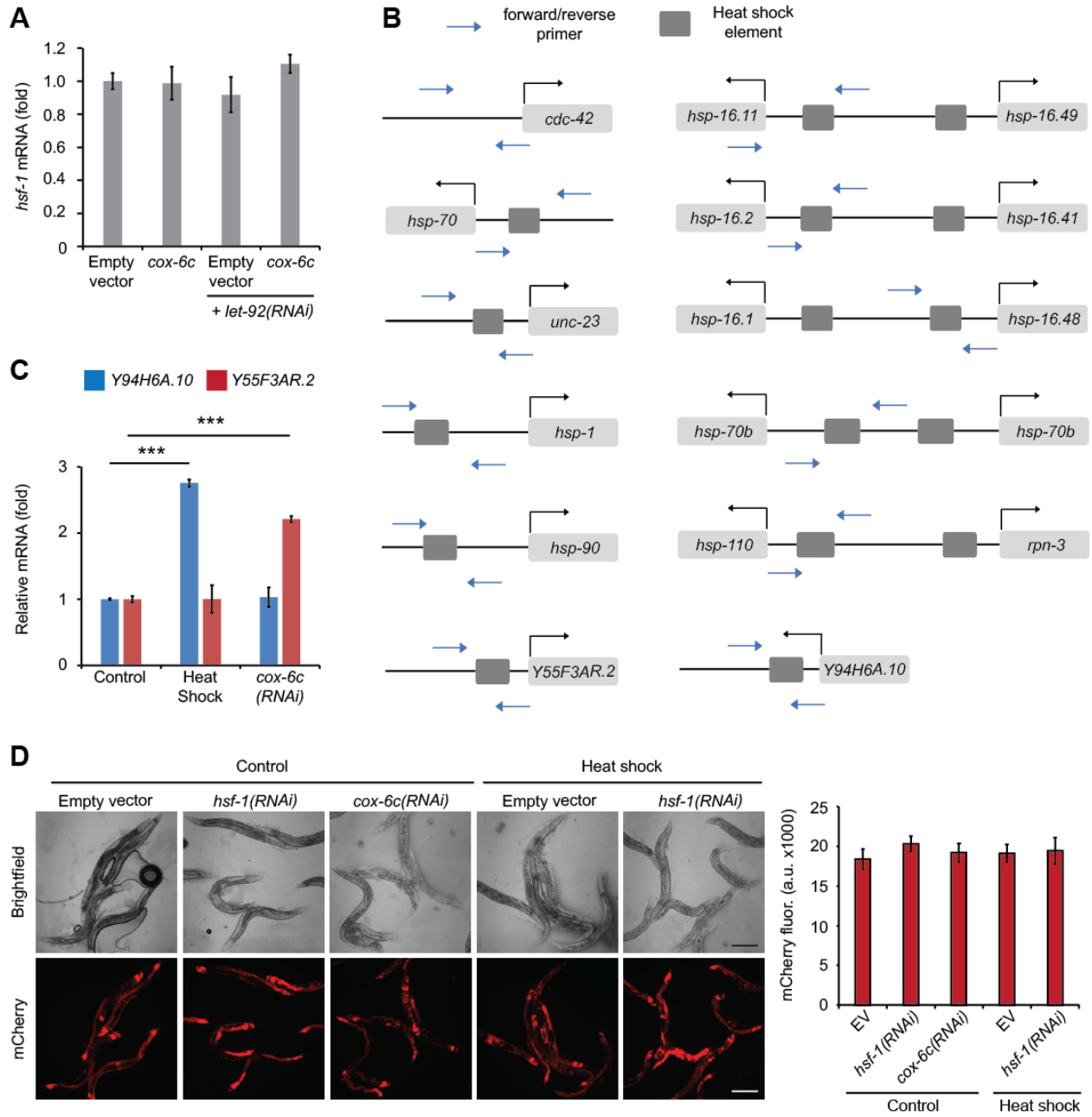
Supplemental Figure 4 (related to main figure 4)

A intestinal polyQ44::YFP (Day 2 adults)

B body wall muscle polyQ35::YFP (Day 1 adults)
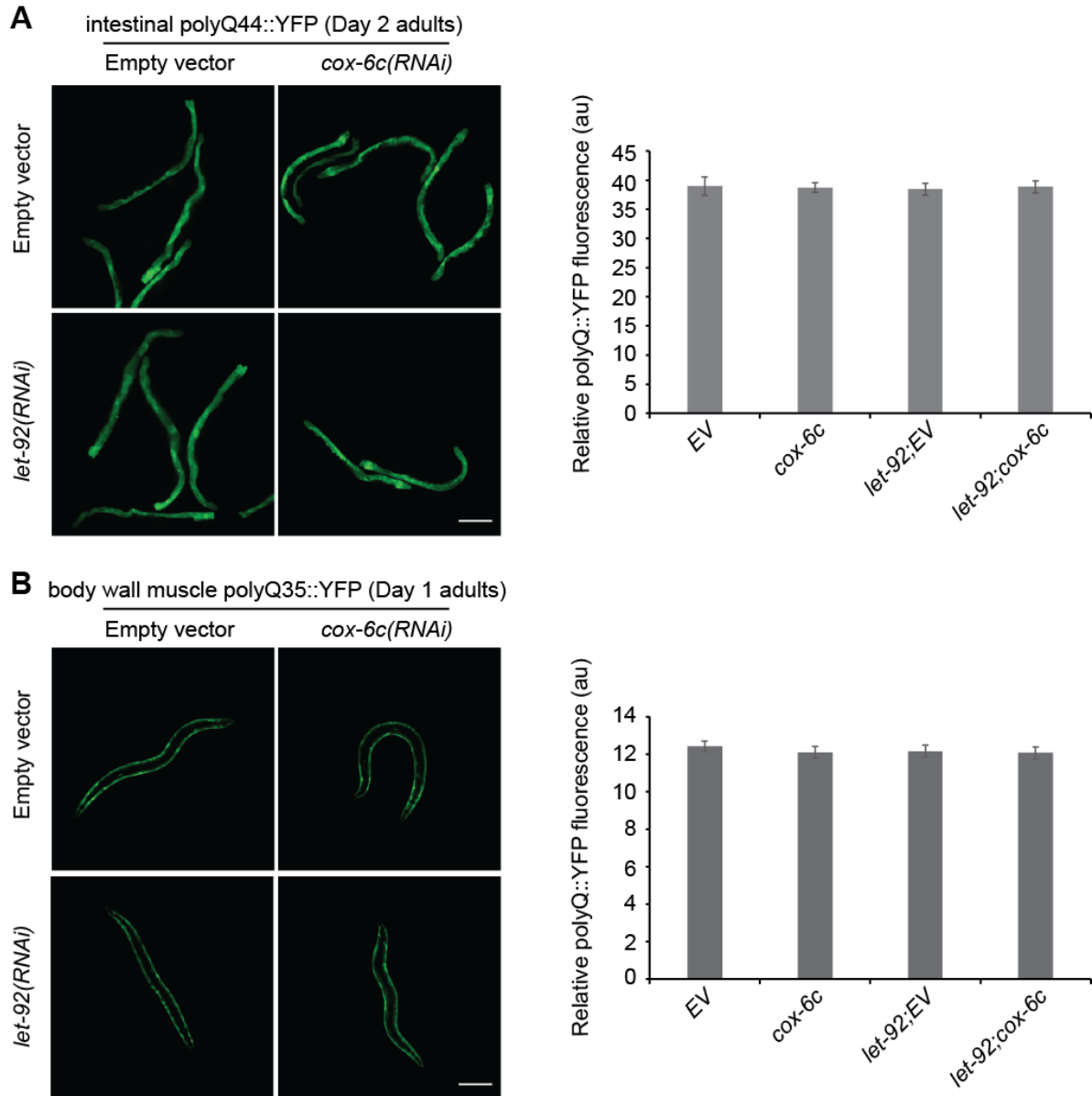
Supplemental Figure 4 (related to main figure 4): *let-92* **knockdown does not affect intestinal or muscle polyglutamine expression**

A – Representative images and quantification of intestinal polyglutamine(Q44)::YFP fluorescence in empty vector (EV) control (L4440), *cox-6c(RNAi), let-92(RNAi);EV* and *let-92(RNAi);cox-6c(RNAi)* worms at day 2 of adulthood. Scale bar = 250 µM

B - Representative images and quantification of body wall muscle polyglutamine(Q35)::YFP fluorescence in empty vector (EV) control (L4440), *cox-6c(RNAi), let-92(RNAi);EV* and *let-92(RNAi);cox-6c(RNAi)* worms at day 1 of adulthood. Scale bar = 250 µM

Supplemental Figure 5 (related to main figure 5)

**Supplemental Figure 5: Knockdown of *let-92* does not alter *hsf-1* mRNA levels (related to main figure 5)**

A – Relative *hsf-1* mRNA levels on day 3 of adulthood following treatment with empty vector (EV) control, *cox-6c(RNAi), let-92(RNAi);EV* or *let-92(RNAi);cox-6c(RNAi)*. Values are the mean of 4 biological replicates and error bars represent SEM.

B – Location of primer pairs used for ChIP-QPCR relative to position of transcription start site and heat shock elements (consensus DNA binding motif for HSF-1)

C – Relative expression of *Y94H6A.10* and *Y55F3AR.2* at day 3 of adulthood in worms exposed to empty vector control, heat shock (35°C, 1h) or *cox-6c(RNAi)*. Values are the mean of 4 biological replicates and error bars represent SEM.

D – Representative images and fluorescence quantification of unc-119::mCherry co-expression marker in day 3 adult HSEp::gfp worms. Unc-119::mcherry was expressed from the same construct as HSE::gfp. Scale bars = 250 µM. Values plotted are the mean fluorescence intensity of at least 15 worms per group. Error bars represent SEM.

Supplemental Figure 6 (related to main figure 6)
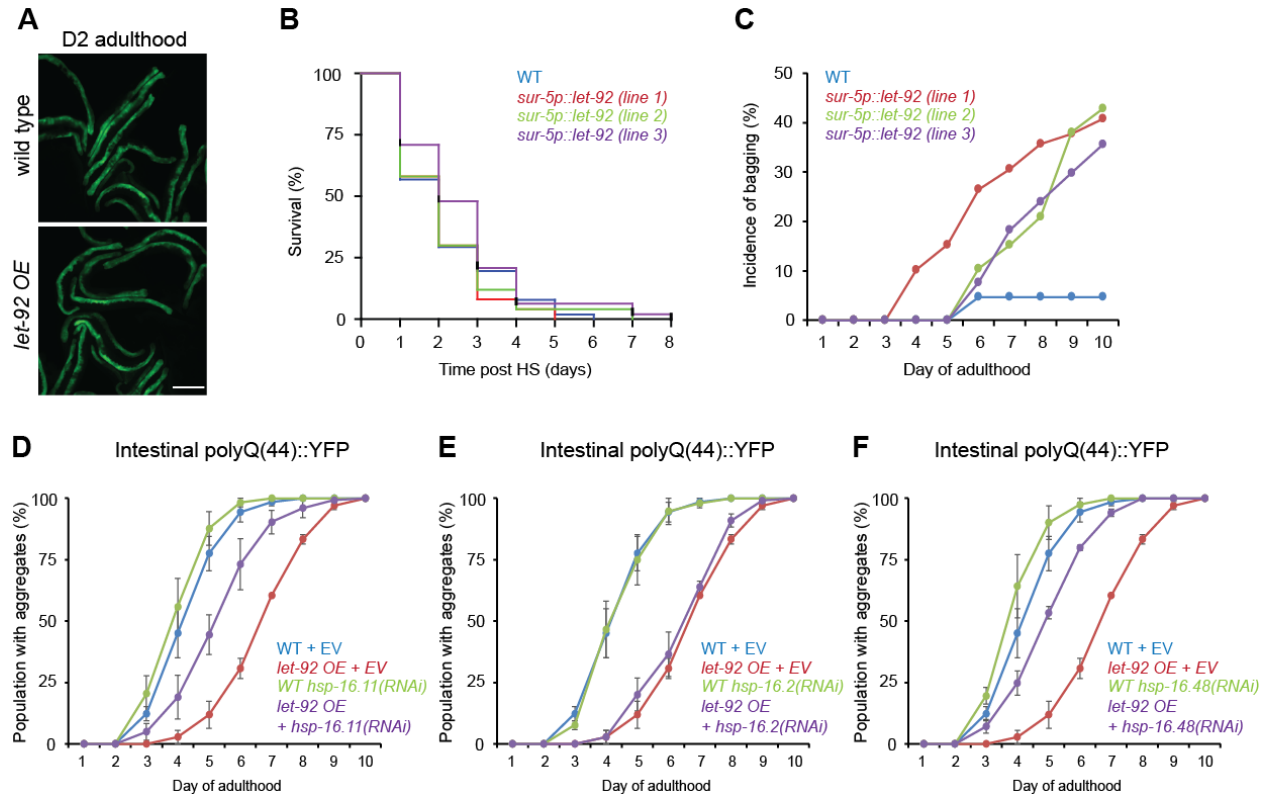
Supplemental figure 6: Overexpression of *let-92* increases bagging but does not alter polyglutamine expression or stress resistance (related to main figure 6)

A – Representative images of day 2 adult wild type or *let-92 OE* worms expressing intestinal polyglutamine (Q44) fused to YFP. Scale bar = 250 µM

B – Survival of wild type or let-92 OE worms following heat shock (35°C, 4 hours) on day 3 of adulthood

C – Incidence of bagging in wild type or *let-92 OE* worms during the first 10 days of adulthood.

D-F – Quantification of intestinal polyglutamine (Q44)::YFP aggregation with age in wild type and *let-92* OE worms grown on EV control or RNAi against *hsp-16.11, hsp-16.2,* or *hsp-16.48*.