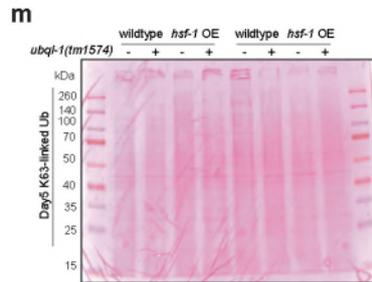
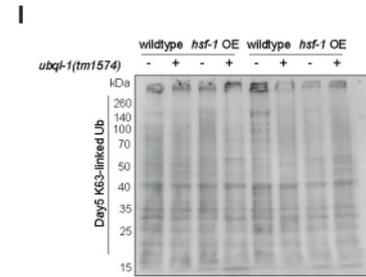
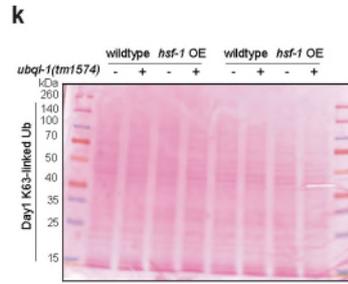
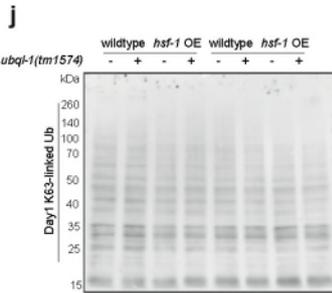
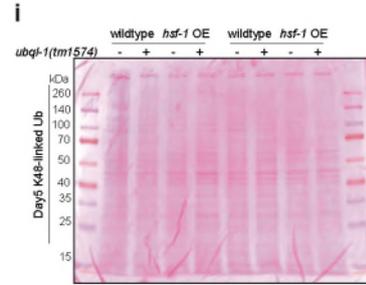
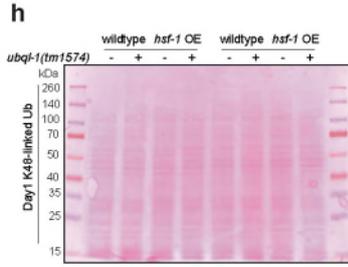
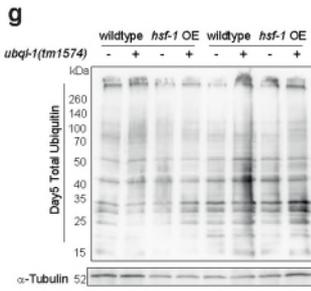
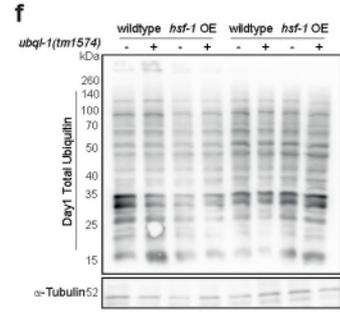
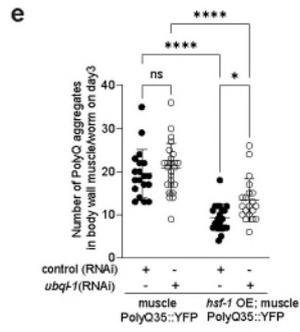
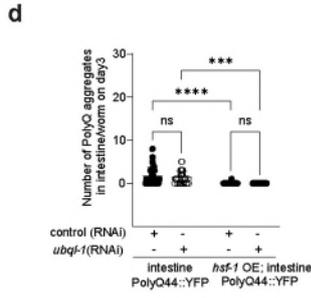
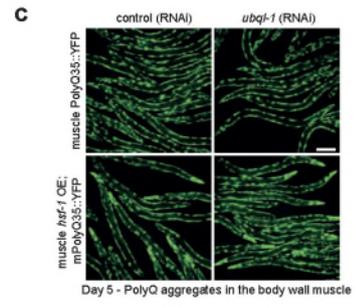
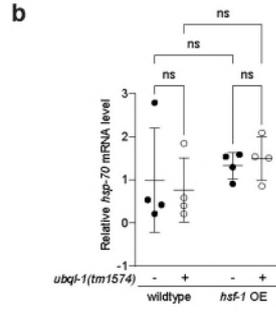
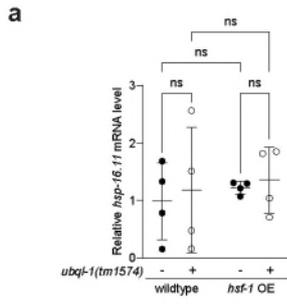


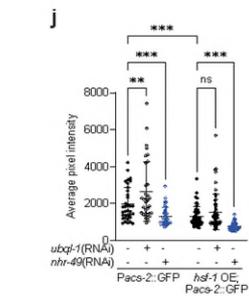
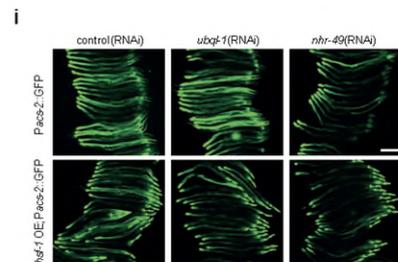
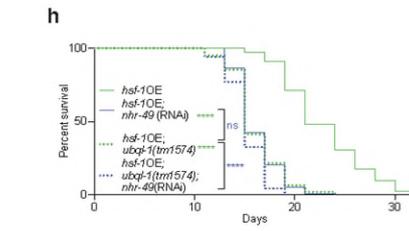
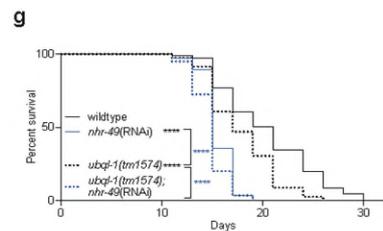
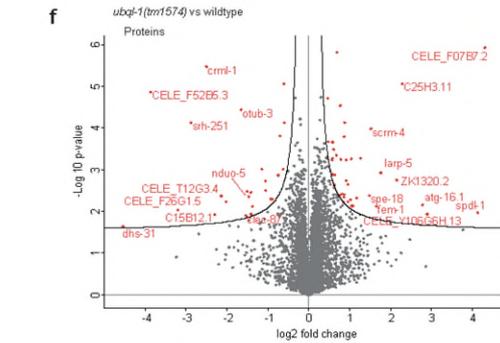
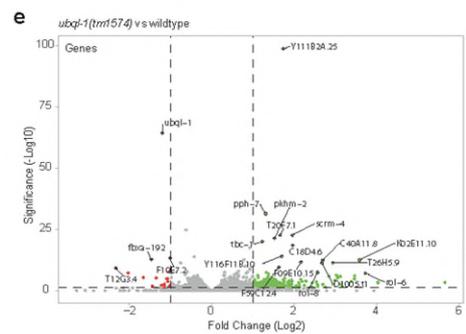
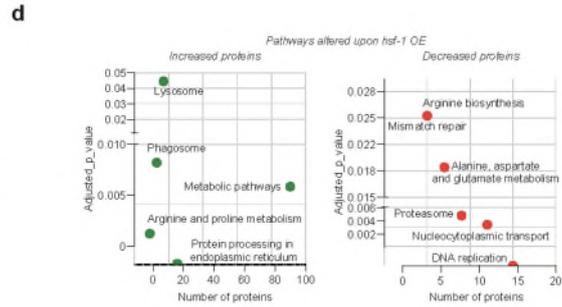
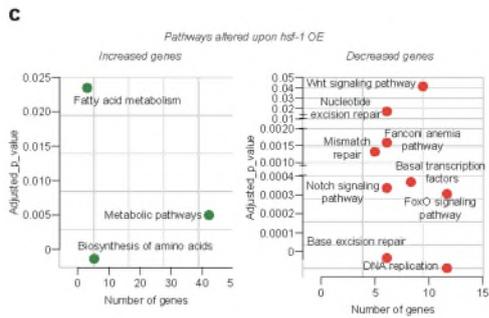
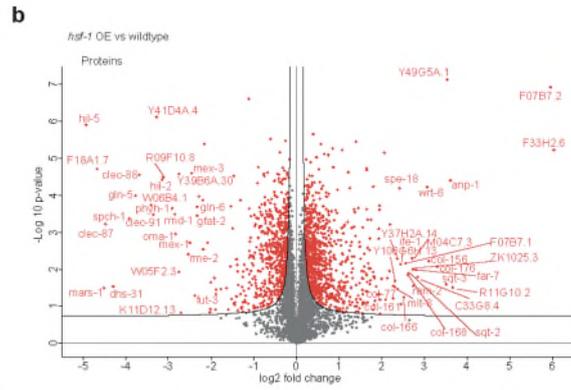
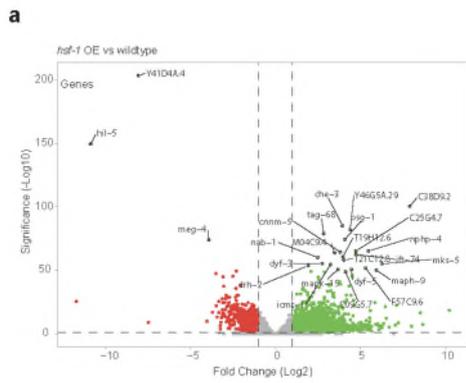
**Supplementary Figure 1: *ubql-1* is bound by HSF-1 and broadly expressed**

(a) Schematic representation of RNAi screen to find modifiers of *hsf-1* OE extended survival. (b) UCSC genome browser view of *ubql-1* genomic region from HSF-1::GFP ChIP-seq data<sup>16</sup>. (c) Schematic diagram of *C. elegans ubql-1* gene and corresponding protein structures. Arrows to the left above exon 3 indicate set of primers used in Supplementary Fig.1f and arrows to the far right above exon 12 indicate primers used in Fig. 1d. (d) Lifespan of wildtype and *hsf-1* OE (AM583) animals on empty vector and *ubql-1*(RNAi) (wildtype vs *ubql-1(tm1574)*,  $p=0.0017$ ; wildtype vs *hsf-1*OE,  $p<0.0001$ ; *hsf-1*OE vs *hsf-1*OE;*ubql-1(tm1574)*,  $p<0.0001$ ). (e) Heatmap depicting tissue/cell-specific expression of *ubql-1*. (f) Relative expression of *ubql-1* mRNA on day 1 of adulthood in wildtype, *ubql-1(tm1574)*, *hsf-1* OE, and *hsf-1* OE;*ubql-1(tm1574)* animals grown on OP50 (wildtype vs *ubql-1(tm1574)*,  $p<0.0001$ ; *hsf-1*OE vs *hsf-1*OE;*ubql-1(tm1574)*,  $p<0.0001$ ; wildtype vs *hsf-1*OE,  $p=0.0015$ ; *ubql-1(tm1574)* vs *hsf-1*OE;*ubql-1(tm1574)*,  $p>0.9999$ ). Data plotted are the mean  $\pm$  SD of 4 biological replicates. All error bars denote SD. Statistical significance was calculated using (f) two-way ANOVA with Fishers LSD test or (d) Mantel-Cox log rank test. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Full statistics for lifespan trials (including n values) can be found in Supplementary Data 2. Source data are provided as a Source Data file.



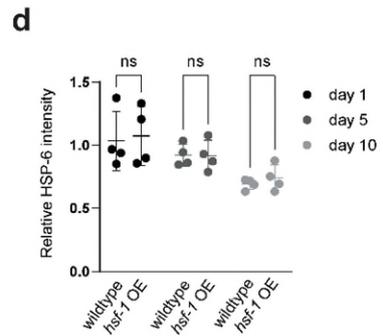
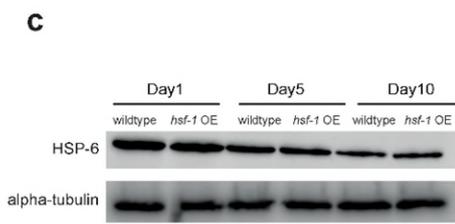
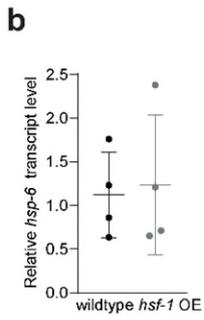
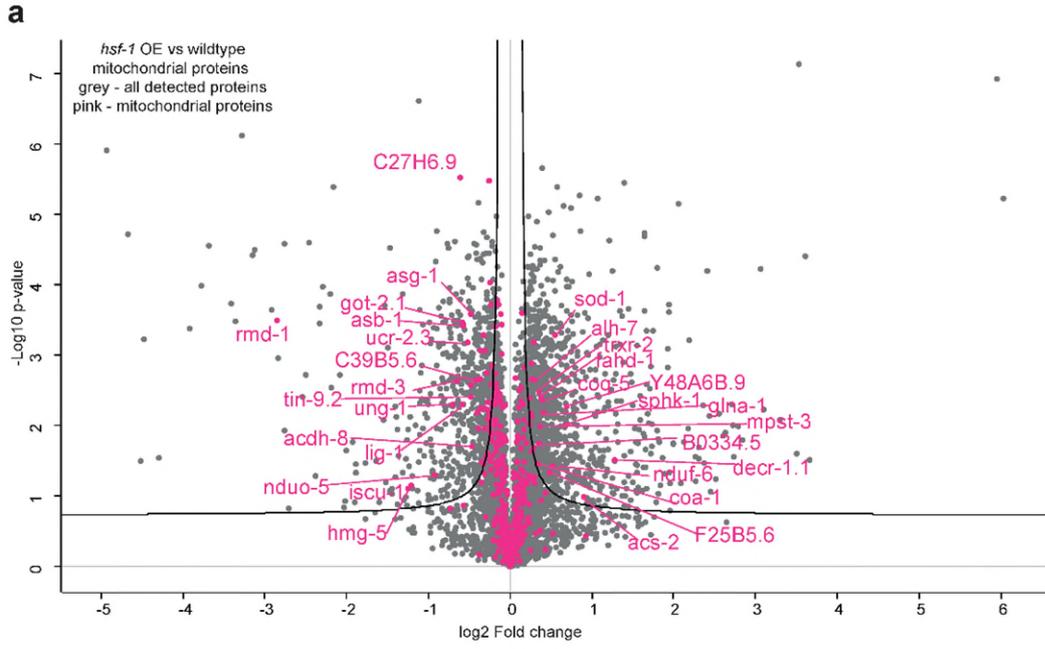
**Supplementary Figure 2: Loss of *ubq1-1* or overexpression of *hsf-1* does not alter the accumulation of polyubiquitylated or K63-linked ubiquitylated proteins with age**

**(a and b)** Relative expression of *hsp-16.11* and *hsp-70* mRNA on day 1 of adulthood in wildtype, *ubq1-1(tm1574)*, *hsf-1* OE or *hsf-1* OE;*ubq1-1(tm1574)* animals grown on OP50. Data plotted are the mean  $\pm$  SD of 4 biological replicates. Statistical comparisons are **(a)** *hsp-16.11* (wildtype vs *ubq1-1(tm1574)*,  $p=0.7087$ ; *hsf-1*OE vs *hsf-1*OE;*ubq1-1(tm1574)*,  $p=0.7936$ ; wildtype vs *hsf-1*OE,  $p=0.6471$ ; *ubq1-1(tm1574)* vs *hsf-1*OE;*ubq1-1(tm1574)*,  $p=0.7293$ ) and **(b)** *hsp-70* (wildtype vs *ubq1-1(tm1574)*,  $p=0.6806$ ; *hsf-1*OE vs *hsf-1*OE;*ubq1-1(tm1574)*,  $p=0.7648$ ; wildtype vs *hsf-1*OE,  $p=0.5395$ ; *ubq1-1(tm1574)* vs *hsf-1*OE;*ubq1-1(tm1574)*,  $p=0.1990$ ). **(c)** Representative images of wildtype and *hsf-1* OE worms expressing body wall muscle polyQ35::YFP grown on empty vector (EV) or *ubq1-1*(RNAi) at day 5 of adulthood. Scale bar, 200  $\mu$ m. **(d and e)** Number of polyglutamine::YFP aggregates present in the **(d)** intestine (Q44::YFP) and **(e)** body wall muscle (Q35::YFP) on day 3 of adulthood in wildtype and *hsf-1* OE animals grown on empty vector and *ubq1-1*(RNAi). One of three independent experiments has been shown for intestinal and muscle PolyQ sensors. Statistical comparisons are **(d)** iPolyQ EV (n=30) vs iPolyQ;*ubq1-1*(RNAi) (n=30),  $p=0.2170$ ; *hsf-1*OE;iPolyQ EV (n=32) vs *hsf-1*OE;iPolyQ;*ubq1-1*(RNAi) (n=26),  $p=0.9246$ ; iPolyQ EV vs *hsf-1*OE;iPolyQ EV,  $p<0.0001$ ; iPolyQ;*ubq1-1*(RNAi) vs *hsf-1*OE;iPolyQ;*ubq1-1*(RNAi),  $p=0.0002$  and **(e)** mPolyQ EV (n=19) vs mPolyQ;*ubq1-1*(RNAi) (n=25),  $p=0.4250$ ; *hsf-1*OE;mPolyQ EV (n=21) vs *hsf-1*OE;mPolyQ;*ubq1-1*(RNAi) (n=20),  $p=0.0110$ ; mPolyQ EV vs *hsf-1*OE;mPolyQ EV,  $p<0.0001$ ; mPolyQ;*ubq1-1*(RNAi) vs *hsf-1*OE;mPolyQ;*ubq1-1*(RNAi),  $p<0.0001$ . **(f and g)** SDS-PAGE followed by western blotting and immunodetection for polyubiquitylated proteins or tubulin in wildtype, *ubq1-1(tm1574)*, *hsf-1* OE or *hsf-1* OE;*ubq1-1(tm1574)* animals on **(f)** day 1 or **(g)** day 5 of adulthood. **(h and i)** Ponceau S staining of western blots used to detect K48-linked ubiquitin on **(h)** day 1 or **(i)** day 5 of adulthood (these panels are an accompaniment to Fig. 2h and i). **(j-m)** SDS-PAGE and western blotting followed by **(j and l)** immunodetection for K63-linked ubiquitylated proteins or **(k and m)** pre-staining of blots with Ponceau S on **(j and k)** day 1 or **(l and m)** day 5 of adulthood. All blots are representative of 4 independent experiments. All error bars denote SD. Statistical significance was calculated using two-way ANOVA with Fishers LSD test. ns, not significant ( $p>0.05$ ), \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , \*\*\*\* $p<0.0001$ . Source data are provided as a Source Data file.



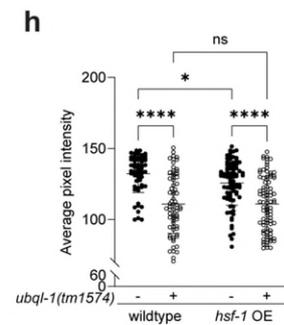
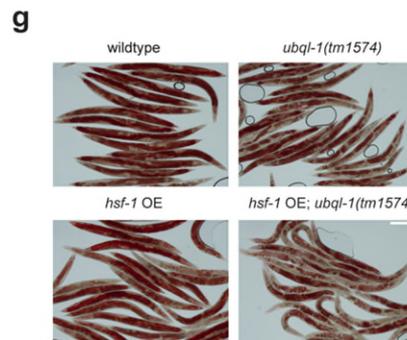
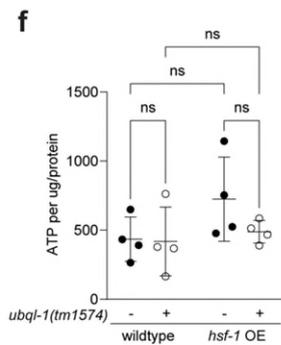
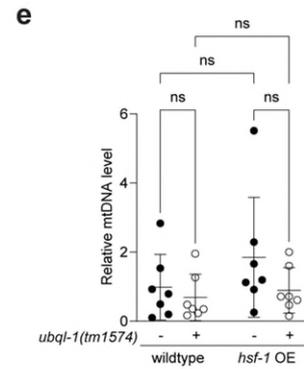
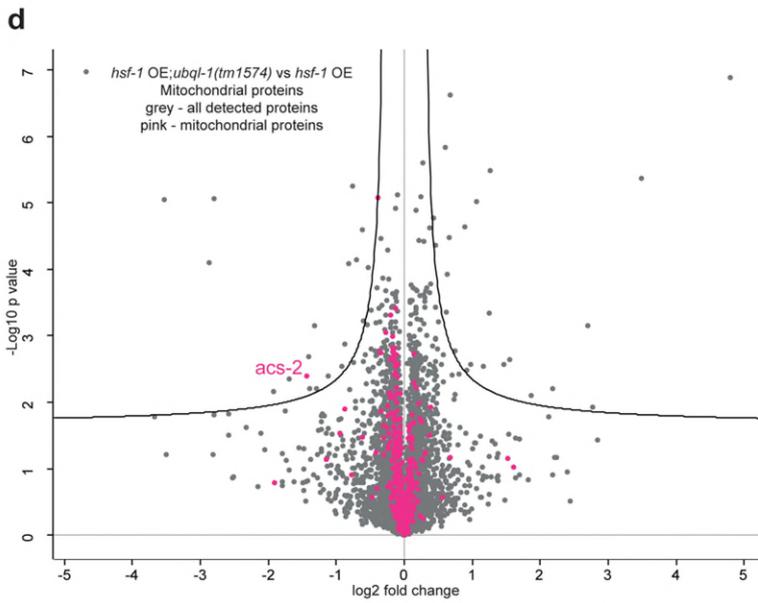
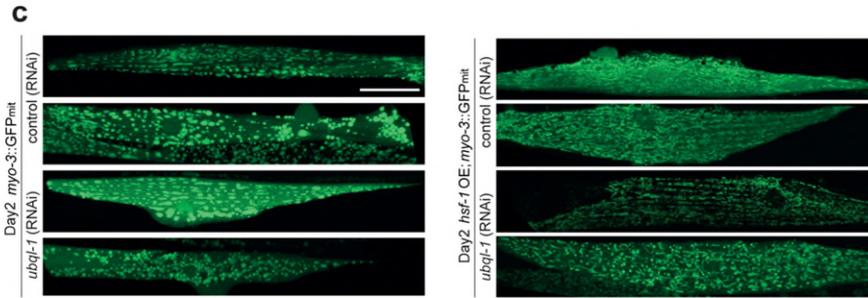
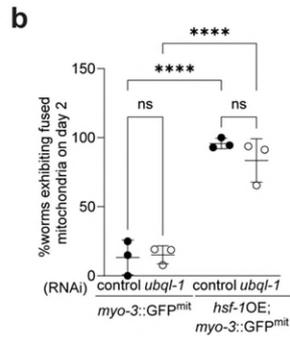
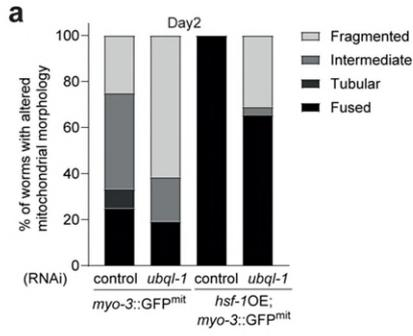
### Supplementary Figure 3: *hsf-1* overexpression leads to changes in genes and proteins associated with metabolic homeostasis

**(a and b)** Volcano plots showing  $\log_2$  (fold change, FC) against  $-\log_{10}$  (p-value) of **(a)** genes identified from RNASeq analysis or **(b)** proteins identified by label-free quantitative mass-spectrometry of *hsf-1* OE vs wildtype worms. **(c and d)** KEGG pathway enrichment analysis of significantly up- and downregulated (right) **(c)** genes and **(d)** proteins upon *hsf-1* overexpression. **(e and f)** Volcano plots showing  $\log_2$  (fold change, FC) against  $-\log_{10}$  (q-value) of **(e)** genes identified from RNASeq analysis or **(f)** proteins identified by label-free quantitative mass-spectrometry of *ubql-1(tm1574)* vs wildtype worms, **(g and h)** Lifespan analysis of **(g)** wildtype and *ubql-1(tm1574)* mutants subjected to *nhr-49* RNAi (wildtype vs *ubql-1(tm1574)*,  $p < 0.0001$ ; wildtype vs wildtype;*nhr-49*(RNAi),  $p < 0.0001$ ; *ubql-1(tm1574)* vs *ubql-1(tm1574);nhr-49*(RNAi),  $p < 0.0001$ ; wildtype;*nhr-49*(RNAi) vs *ubql-1(tm1574)*,  $p < 0.0001$ ) and **(h)** *hsf-1* OE and *hsf-1* OE;*ubql-1(tm1574)* animals subjected to *nhr-49* RNAi (*hsf-1*OE vs *hsf-1*OE;*ubql-1(tm1574)*,  $p < 0.0001$ ; *hsf-1*OE vs *hsf-1*OE;*nhr-49*(RNAi),  $p < 0.0001$ ; *hsf-1*OE;*ubql-1(tm1574)* vs *hsf-1*OE;*ubql-1(tm1574);nhr-49*(RNAi),  $p = 0.0010$ ; *hsf-1*OE;*nhr-49*(RNAi) vs *hsf-1*OE;*ubql-1(tm1574)*,  $p = 0.9438$ ). **(i)** Representative fluorescence images of wildtype, or *hsf-1* OE, NHR-49 reporter animals (*acs-2p::GFP*) on day 1 of adulthood following exposure to empty vector (EV) control, *ubql-1*(RNAi) or *nhr-49*(RNAi). Scale bar, 200  $\mu\text{m}$ . **(j)** Quantification of *acs-2p::GFP* fluorescence on day 1 of adulthood in wildtype, or *hsf-1* OE worms grown on empty vector control, *ubql-1*(RNAi) or *nhr-49*(RNAi). Data are represented as mean  $\pm$  SD (*acs-2p::GFP* EV (n=35) vs *acs-2p::GFP;ubql-1*(RNAi) (n=33),  $p = 0.0011$ ; *acs-2p::GFP* EV vs *acs-2p::GFP;nhr-49*(RNAi) (n=38),  $p = 0.0009$ ; *acs-2p::GFP* EV vs *hsf-1*OE;*acs-2p::GFP* EV (n=50),  $p = 0.0006$ ; *hsf-1*OE;*acs-2p::GFP* EV vs *hsf-1*OE;*acs-2p::GFP;ubql-1*(RNAi) (n=55),  $p = 0.1854$ ; *hsf-1*OE;*acs-2p::GFP* EV vs *hsf-1*OE;*acs-2p::GFP;nhr-49*(RNAi) (n=54),  $p = 0.0009$ ). Statistical significance was calculated by **(g and h)** Mantel-Cox log rank test and **(j)** two-way ANOVA followed by Fishers LSD test. ns, not significant ( $p > 0.05$ ), \*\*\*\* $p < 0.0001$ . Full statistics for lifespan trials (including n values) can be found in Supplementary Data 2. Source data are provided as a Source Data file.



**Supplementary Figure 4: *hsf-1* overexpression alters the abundance of a sub-set of the mitochondrial proteome but does not activate the UPR<sup>mt</sup>**

**(a)** Volcano plot showing log<sub>2</sub> (fold change, FC) against -log<sub>10</sub> (p-value) of mitochondrial proteins detected in *hsf-1* OE and wildtype worms by mass-spectrometry. Pink dots mark all detected mitochondrial proteins, with significantly altered mitochondrial proteins outside the black curve (FDR < 0.05). **(b)** Relative expression of the canonical UPR<sup>mt</sup> gene *hsp-6* in wildtype and *hsf-1*OE worms. Data plotted are the mean +/- SD of 4 biological replicates and values were normalized to the geometric mean of the housekeeping genes *rpb-2*, *pmp-3* and *cdc-42* (unpaired, two-tailed Student's t-test, p=0.8120) **(c)** Representative western blot following immunodetection for HSP-6 or alpha-tubulin in wildtype and *hsf-1* OE protein extracts on day 1, 5, and 10 of adulthood. Blots presented are representative of 4 experiments. **(d)** Quantification of HSP-6 levels relative to tubulin in wildtype and *hsf-1* OE worms on day 1, 5, and 10 of adulthood. Data plotted are the mean +/- SD of four biological replicates. (day 1 wildtype vs *hsf-1*OE, p =0.7158; day5 wildtype vs *hsf-1*OE, p=0.9673; day10 wildtype vs *hsf-1*OE, p=0.6669). Statistical significance was calculated by two-way ANOVA followed by Fishers LSD test. ns, not significant (p>0.05). Source data are provided as a Source Data file.



### Supplementary Figure 5: *Ubql-1* regulates total fat levels

**(a)** Proportion of mitochondrial morphologies observed in  $p_{myo-3}::GFP(mit)$  worms and *hsf-1* OE;  $p_{myo-3}::GFP(mit)$  worms grown on empty vector control or *ubql-1*(RNAi) on day 2 adulthood (*myo-3::GFP(mit)*, n=12; *myo-3::GFP(mit);ubql-1*(RNAi), n=26; *hsf-1*OE; *myo-3::GFP(mit)*, n= 20; *hsf-1*OE; *myo-3::GFP(mit);ubql-1*(RNAi), n=29). **(b)** Prevalence of fused mitochondria in muscle tissues of wildtype and *hsf-1* OE worms, +/- *ubql-1* (RNAi), on day 2 of adulthood. Values are the mean +/-SD of three independent experiments (*myo-3::GFP(mit)* vs *myo-3::GFP(mit);ubql-1*(RNAi), p=0.8344; *hsf-1*OE; *myo-3::GFP(mit)* vs *hsf-1*OE; *myo-3::GFP(mit);ubql-1*(RNAi), p=0.1994; *myo-3::GFP(mit)* vs *hsf-1*OE; *myo-3::GFP(mit)*, p<0.0001; *myo-3::GFP(mit);ubql-1*(RNAi) vs *hsf-1*OE; *myo-3::GFP(mit);ubql-1*(RNAi), p<0.0001). **(c)** Representative confocal microscope images of *myo-3p::GFPmit* within muscle tissues of wildtype or *hsf-1* OE worms +/- *ubql-1*(RNAi) on day 2 of adulthood. Scale bar, 20  $\mu$ m. **(d)** Volcano plot showing log<sub>2</sub> (fold change, FC) against -log<sub>10</sub> (q-value) of mitochondrial proteins detected in *hsf-1* OE and *hsf-1* OE;*ubql-1(tm1574)* worms by mass-spectrometry. Pink dots mark all detected mitochondrial proteins, with significantly altered proteins outside the black curve (FDR < 0.05). **(e)** Levels of mtDNA (*ND1*) relative to genomic DNA (*cdc-42*) as detected by real-time quantitative PCR in wildtype, *ubql-1(tm1574)*, *hsf-1* OE and *hsf-1* OE; *ubql-1(tm1574)* worms on day 1 of adulthood following growth on OP50 bacteria. Data plotted are the mean +/- SD of 7 biological replicates (wildtype vs *ubql-1(tm1574)*, p=0.6171; *hsf-1*OE vs *hsf-1*OE;*ubql-1(tm1574)*, p=0.1148; wildtype vs *hsf-1*OE, p=0.1509; *ubql-1(tm1574)* vs *hsf-1*OE;*ubql-1(tm1574)*, p=0.7265). **(f)** ATP levels in wildtype, *ubql-1(tm1574)*, *hsf-1* OE and *hsf-1* OE; *ubql-1(tm1574)* animals at day 1 of adulthood. Data are the mean +/- SD of 4 biological replicates (wildtype vs *ubql-1(tm1574)*, p=0.9171; *hsf-1*OE vs *hsf-1*OE;*ubql-1(tm1574)*, p=0.1482; wildtype vs *hsf-1*OE, p=0.0821; *ubql-1(tm1574)* vs *hsf-1*OE;*ubql-1(tm1574)*, p=0.6550). **(g and h)** Triglyceride levels in day 5 wildtype, *ubql-1(tm1574)*, *hsf-1* OE and *hsf-1* OE;*ubql-1(tm1574)* adult animals. **(g)** Representative images and **(h)** quantification representing the relative lipid content in whole body of worms. (wildtype (n=58) vs *ubql-1(tm1574)* (n=71), p<0.0001; *hsf-1*OE (n=86) vs *hsf-1*OE;*ubql-1(tm1574)* (n=87), p<0.0001; wildtype vs *hsf-1*OE, p=0.0307; *ubql-1(tm1574)* vs *hsf-1*OE;*ubql-1(tm1574)*, p=0.9626). One of three independent experiments has been shown. All error bars denote SD. Scale bar, 500 $\mu$ m. Statistical significance was calculated by **(b, e, f, and h)** two-Way ANOVA with Fishers LSD test ns, not significant (p>0.05), \*p<0.05, \*\*p < 0.01, \*\*\*\*p<0.0001. Source data are provided as a Source Data file.

Target Gene	Forward sequence	Reverse sequence
ubql-1 (Exon 3)	TCTCACACAGCACAAAATCGC	CCTCCCATTGTTGGTGCAGA
ubql-1 (Exon 12)	GGGAGGAGGAAGACCCTCAT	TTCTGGCACGATCCGAGAAG
hsp-16.11	TGGCTCAGATGGAACGTCAA	TGGCTTGAAGTGCAGACAT
hsp-70 (C12C8.1)	CTACATGCAAAGCGATTGGA	GGCGTAGTCTTGTTCCCTTC
hsp-6	GTTATCGAGAACGCAGAAGGAG	CATCCTTAGTAGCTTGACGCTG
pmp-3	GTTCCCGTGTTCACTCAT	ACACCGTCGAGAAGCTGTAGA
rpb-2	AACTGGTATTGTGGATCAGGTG	TTTGACCGTGTCGAGATGC
cdc-42	TCGACAATTACGCCGTCAACA	GAAACACGTCGGTCTGTGGA
F44E5.4	GTTGAGATCCTCGCCAACTC	GCTGCATCTCCAACCAATCT
nd-1 (genomic)	AGCGTCATTTATTGGGAAGAAGAC	AAGCTTGTGCTAATCCATAAATGT
cdc-42 (genomic)	ATGGTAAAGAAACGCTCGTG	TGAAAAATACGGATGAGTCACA

**Supplementary Table 1.** List of primers used in this study.