SUPPLEMENTAL MATERIALS for Haynes *et. al*: **"*HAF-1 and the mitochondrial UPR*"**

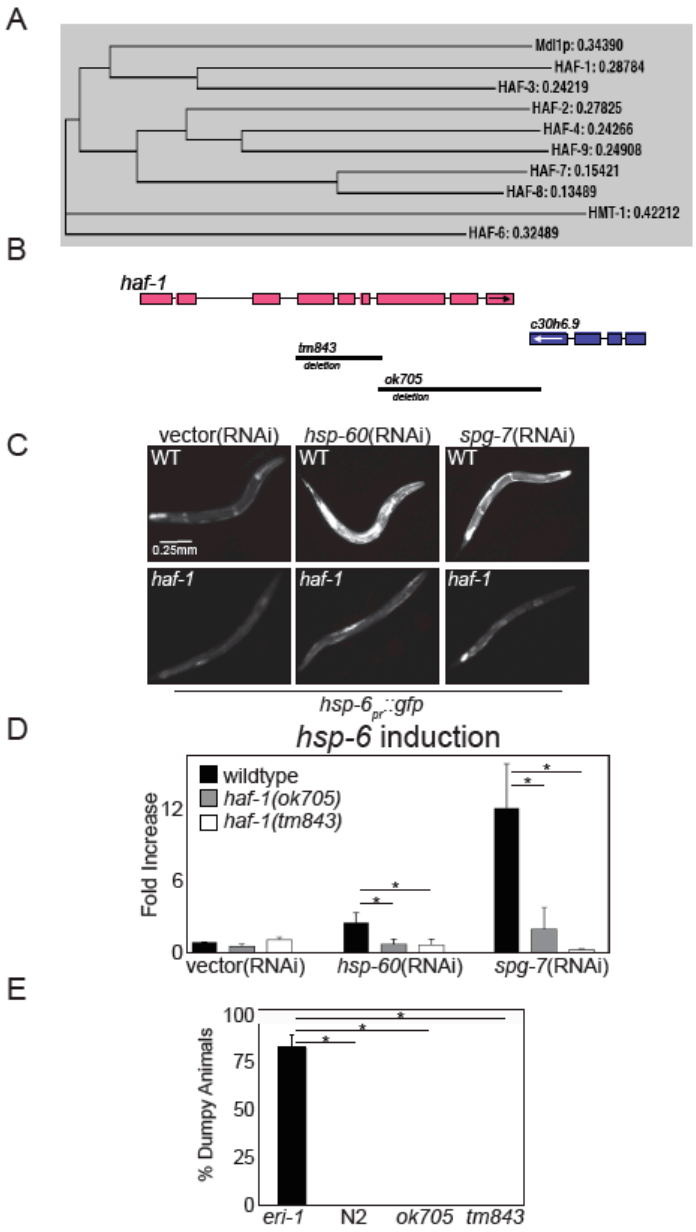
Inventory of Supplemental Information

Supplementary Figure 1:
S1 is associated with Figure 1. S1A serves as an introduction to the findings of Figure 1 and S1B introduces the genetic deletions used throughout the paper, starting with Figure 1A. S1C, S1D and S1E are additional items that would not fit into Figure 1 due to space limits but support the main conclusion of Figure 1.

Supplementary Figure 2:
S2 is associated with Figure 2. S2A-C are additional items that would not fit into Figure 2 due to space limits. These data further support the main point of Figure 2, which is *haf-1* is required for mitochondrial function during conditions that cause mitochondrial unfolded protein stress.

Supplementary Figure 3:
S3 is associated with Figure 6. S3A and S3B indicate that *haf-1* is required for signaling *ubl-5* induction in response to stress and S3C indicates the transcription factor ZC376.7 is required for upregulation of the mitochondrial chaperone *hsp-6*.

Figure S1:

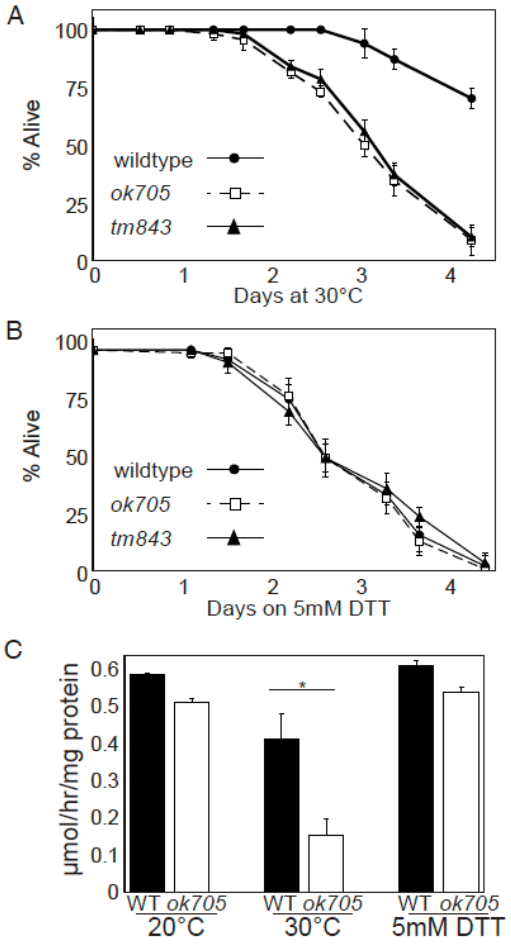


The *C. elegans* orthologue of the yeast ABC transporter, Mdl1p is required for mitochondrial chaperone gene induction.

(A) The nine *C.elegans* proteins most homologous to yeast Mdl1p were aligned using ClustalW2. The cladogram is displayed along with the evolutionary distance from Mdl1p for each *C. elegans* protein. Percent identify to the yeast ABC transporter Mdl1p: HAF-1 35.0%, HAF-2 34.6%, HAF-3 39.2%, HAF-4 33.1%, HAF-5/HMT-1 31.2%, HAF-6 35.1%, HAF-7 32.8%, HAF-8 33.7% and HAF-9 33.2%.
(B) Schematic diagram of the HAF-1 locus with deletion alleles *tm843* and *ok705* highlighted.

(C) Fluorescent photomicrographs of wildtype or *haf-1(ok705)* mutant worms with a UPR^{mt} reporter transgene (*hsp-6*$_{pr}$*::gfp*). Mitochondrial unfolded protein stress was induced by RNAi inactivation of *hsp-60* or *spg-7*, as indicated.
(D) Quantitative analysis (by QRT-PCR) of endogenous *hsp-6* mRNA in wildtype or two different *haf-1* mutant animals (*ok705* and *tm843*) subjected to mitochondrial unfolded protein stress by *hsp-60*(RNAi) or *spg-7*(RNAi). Displayed is the mean +/- SEM, n=3, *p<0.05.
(E) Percent of dumpy animals recovered in wildtype (N2), *eri-1(mg366)* mutant worms (known to be hypersensitive to RNAi) and *haf-1(ok705)* or *haf-1(tm843)* mutant worms raised on *dpy-13*(RNAi).

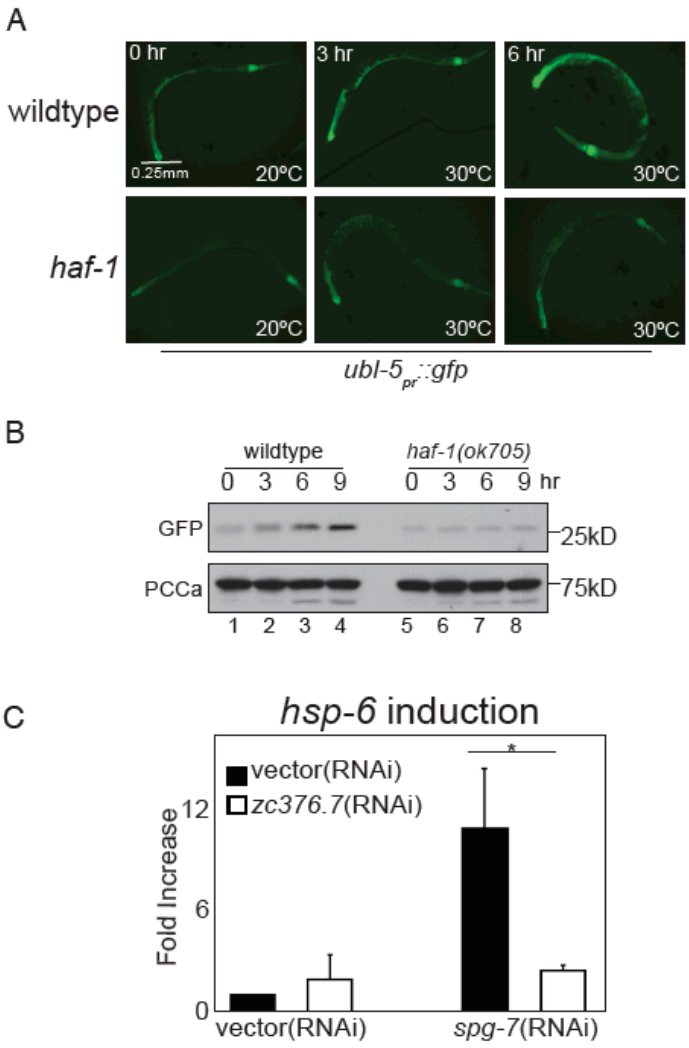
Figure S2:



***haf-1* is required for survival during mitochondrial stress.**

(A) Survival of wildtype, *haf-1(ok705)* and *haf-1(tm843)* L4 worms following shift from 20˚C to 30˚C. Shown is the mean ± S.E.M of fraction of survivors assessed daily (n=3).
(B) Survival of wildtype, *haf-1(ok705)* and *haf-1(tm843)* L4 worms following transfer to plates containing 5mM DTT. Shown is the mean ± S.E.M of fraction of survivors assessed daily (n=3).
(C) Oxygen consumption of wildtype and *haf-1(ok705)* at the indicated temperature or after exposure to 5mM DTT for 24 hours. Shown is the mean ± S.E.M oxygen consumption normalized to protein content (n=3 *p<0.05).

Figure S3:



***haf-1* is required for *ubl-5$_{pr}$::gfp* expression in response to stress.**

(A) Representative fluorescent photomicrographs of *ubl-5$_{pr}$::gfp* transgenic worms in a wildtype or *haf-1* mutant background exposed to 30°C for the indicated time.
(B) A corresponding immunoblot of GFP expressed by *ubl-5$_{pr}$::gfp* transgenic worms in whom mitochondrial unfolded protein stress was induced. The genotypes and treatment are identical to (A). PCCa, detected using avidin-HRP (lower panel) serves as a loading control.
(C) Quantitative analysis (by QRT-PCR) of endogenous *hsp-6* mRNA in vector or *zc376.7*(RNAi) fed N2 worms subjected to mitochondrial unfolded protein stress by *spg-7*(RNAi). Displayed is the mean +/- SEM, n=3, *p<0.05.