Supplemental Materials Molecular Biology of the Cell

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Supplementary Figure 1

Msp1 expression levels from various plasmids. The strains were grown in glucose-containing medium at 30°C. Whole cell extracts were analyzed for levels of Msp1. Pgk1 was decorated as loading control.

Supplementary Figure 2

UPR^{mt} induction in wild type and an alternative *mspn-1* mutant. Wild type (+/+) and *mspn-1* (tm3831) animals carrying the P_{hsp-6}GFP transcriptional reporter (strains MD4433 and MD4431, respectively) were analyzed by brightfield and fluorescence microscopy. The P_{hsp-6}GFP fluorescence intensities were quantified. Error bars indicate standard deviations. The difference between the two strains is statistically significant by unpaired t test with Welch's correction (p= 0.0002; n=6).

Supplementary Figure 3

- A) Msp1-dependent binding of the proteasome to isolated mitochondria. Mitochondria were isolated from a W303 and $\Delta msp1$ strain and subsequently subjected to sucrose gradient purification. Gradient purified mitochondria were analyzed by SDS-PAGE, Western blot and immuno decoration with an antibody raised against the whole purified proteasome.
- **B)** Rpn10-dependent chase of arrested precursor from isolated mitochondria. Recombinant Cytb2-ΔTM-DHFR was pre-folded in the presence of methotrexate and bound to mitochondria of the indicated strains. After indicated time points mitochondria were re-isolated, washed and analyzed by SDS-PAGE, Western blot and immuno decoration and quantified.







