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Supplemental Information

Mitochondrial SKN-1/Nrf Mediates a Conserved Starvation Response

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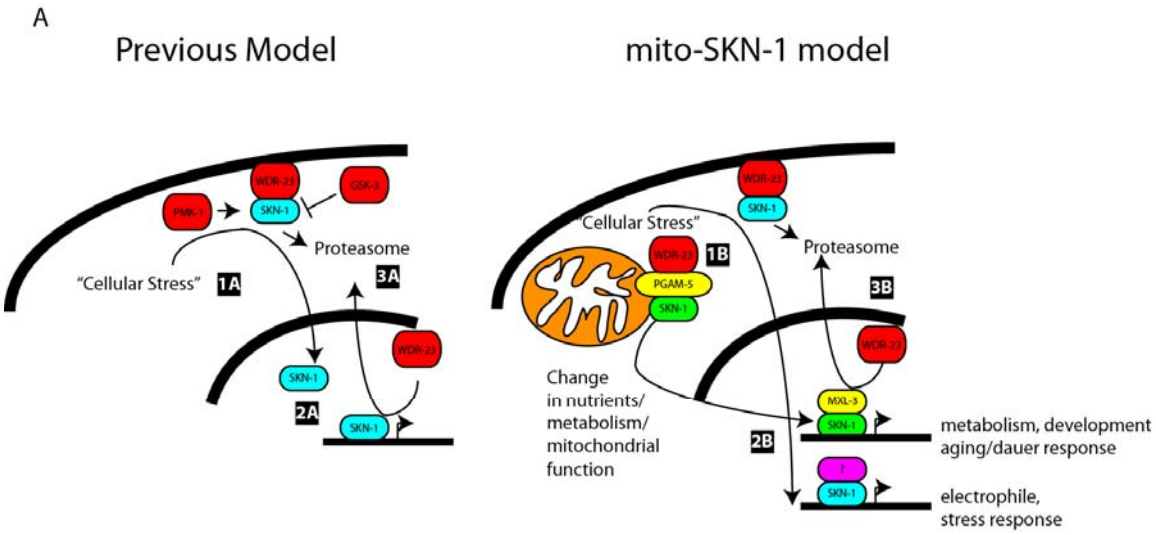

1A - Various forms of stress facilitate the translocation of SKN-1 to the nucleus
1B - Specific changes to the cellular environment activate distinct pools of SKN-1

2A - Once in the nucleus SKN-1 searches for consensus binding sites
2B - With the help of transcriptional reponse elements, a specific and appropriate response is initiated

3A/B - Once the transcriptional response is completed, WDR-23 facilitates SKN-1 nuclear export and delivery to the proteasome for destruction.

Figure S1: Model of mito-SKN-1. SKN-1 is activated by multiple signaling pathways including GSK-3, PMK-1 and WDR-23, however the mechanism by which specific SKN-1 targets are selected remains unclear. mito-SKN-1 associates with the mitochondria and interacts with PGAM-5. Upon nutrient depletion mito-SKN-1 dissociated from the mitochondria, binds to the bZip transcription factor MXL-3 and specifies metabolism, development and aging dauer response genes. WDR-23 facilitates the degradation of SKN-1 following its transcriptional activity.

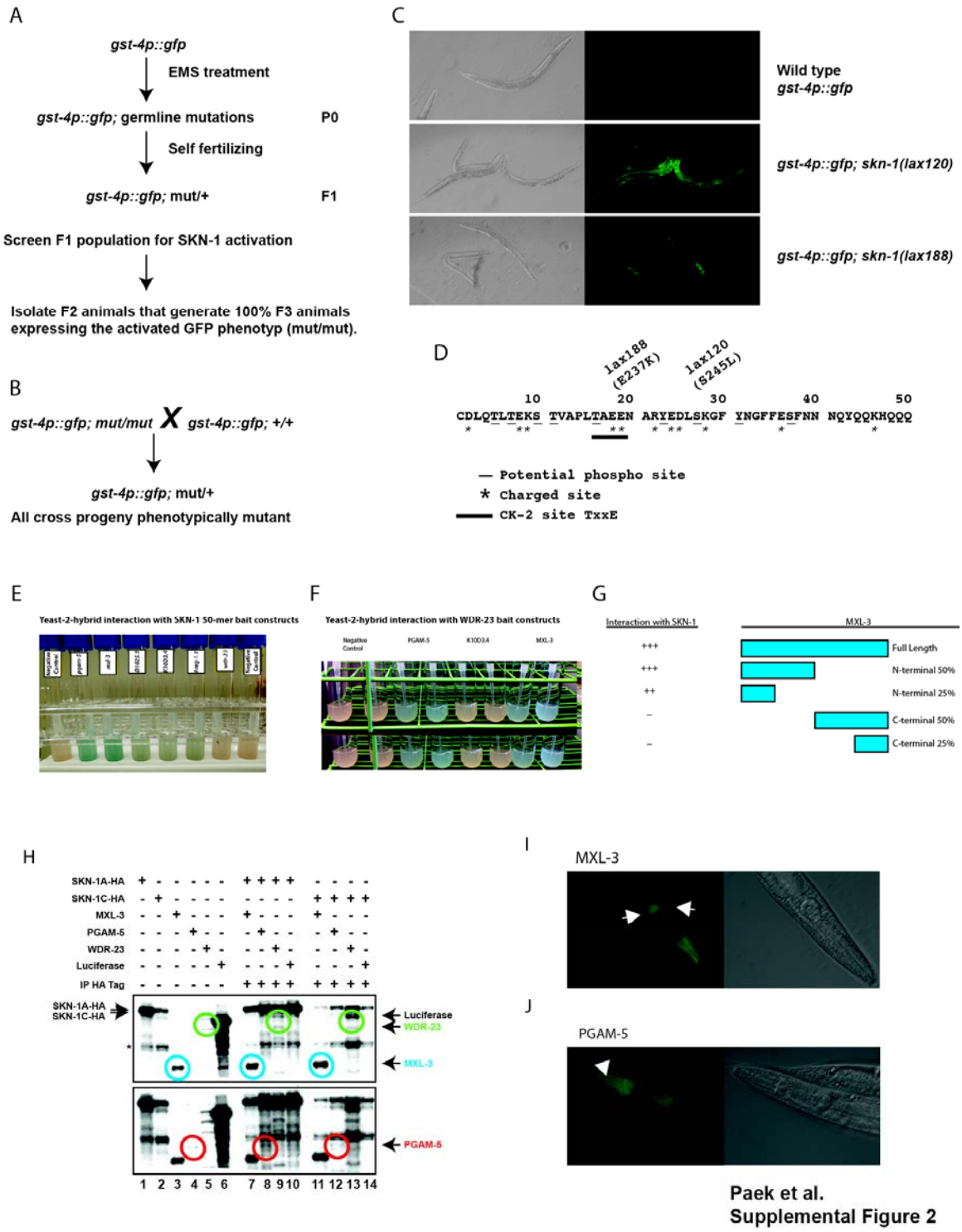

Figure S2: Identification of new protein interactors with SKN-1/Nrf. A. Dominant mutations that activate SKN-1 were isolated in an EMS mutagenesis screen of a *C. elegans* strain harboring the SKN-1 transcriptional reporter *gst-4p::gfp.* F1 generation worms with GFP expression were isolated for further characterization. B. F2 generation animals were singled and F3 progeny were analyzed for 100% penetrance of the activated GFP phenotype. These

homozygous animals were then mated back to the unmutagenized parental strain. Successful matings that yielded 100% F1 progeny expressing the activated GFP phenotype were confirmed as dominant mutants. C. Two dominant mutations activate the *gst-4p::gfp* reporter of SKN-1 activity in *C. elegans* intestinal and hypodermal tissues. D. Bioinformatic (Expasy Prosite) identification of potential phosphorylation sites (light line), Casein kinase 2 recognition site (dark line), and potential phosphorylation sites (*). E. yeast-2-hybrid screen identifies PGAM-5, MXL-3, D1025.1, K10D3.4, HMG-1.1 as protein-protein interactors with the new SKN-1 50-mer domain. F. SKN-1 interacting proteins PGAM-5, K10D3.4, and MXL-3 were tested for binding, using WDR-23 as bait. G. Truncated versions of MXL-3 were tested for binding with SKN-1. H. 35S-methionine labeled SKN-1A-HAtag (lane 1), SKN-1B-HAtag (lane 2), MXL-3 (lane 3), PGAM-5 (lane 4), positive control WDR-23 (lane 5), and negative control Luciferase (Lane 6) were expressed using a Promega TNT coupled *in vitro* transcription and translation System. MXL-3 (green circles), PGAM-5 (red circles) and WDR-23 (blue circles) could bind to SKN-1A:HA (lane 7, 8, and 9) and SKN-1B:HA (lane 11, 12, and 13). Neither SKN-1A:HA or SKN-1B:HA could bind to luciferase (lane 10 and 14). Top panel exposure time 6 hours, bottom panel exposure time 24 hours. Worms harboring extrachromosomal arrays of *mxl-3::gfp* (I) or *pgam-5::gfp* (J) were mounted on agar pads and imaged for GFP fluorescence. Arrows indicated expression in unidentified neuronal and pharyngeal cells.

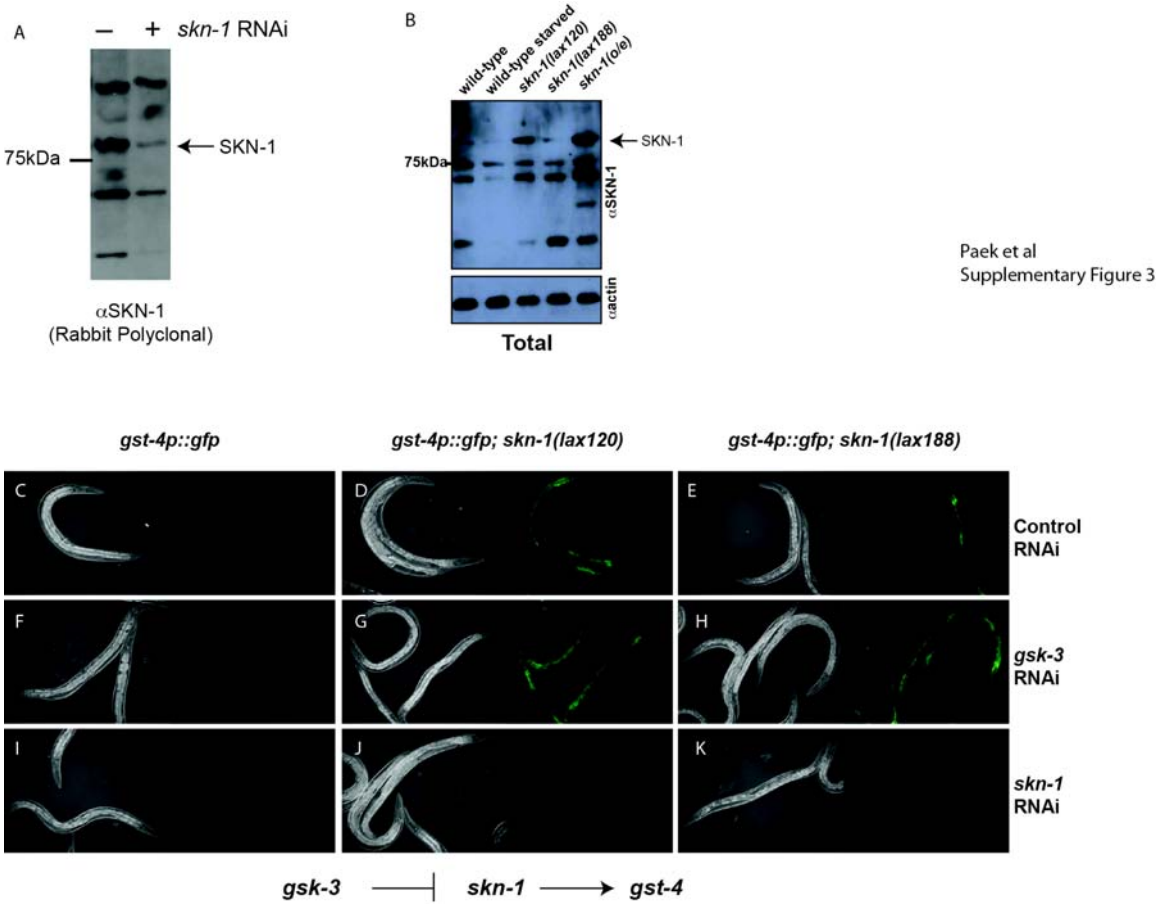

Figure S3: Mitochondrial SKN-1 functions outside of *gsk-3* pathway. A. Identification of SKN-1 specific band from polyclonal antisera. Arrow indicates band that is reduced following treatment with *skn-1* RNAi. B. SKN-1 protein levels in fed and starved wild type animals, *skn-1(lax120), skn-1(lax188),* and *skn-1* overexpression strains. Blots were probed with a monoclonal actin antisera to measure equal loading. C-K, *gsk-3* pathways epistasis**.** Worms of the indicated genotype were treated with control RNAi (C-E), *gsk-3 RNAi* (F-H), or *skn-1 RNAi* (I-K) and imaged for GFP intensity indicative of SKN-1 activation.

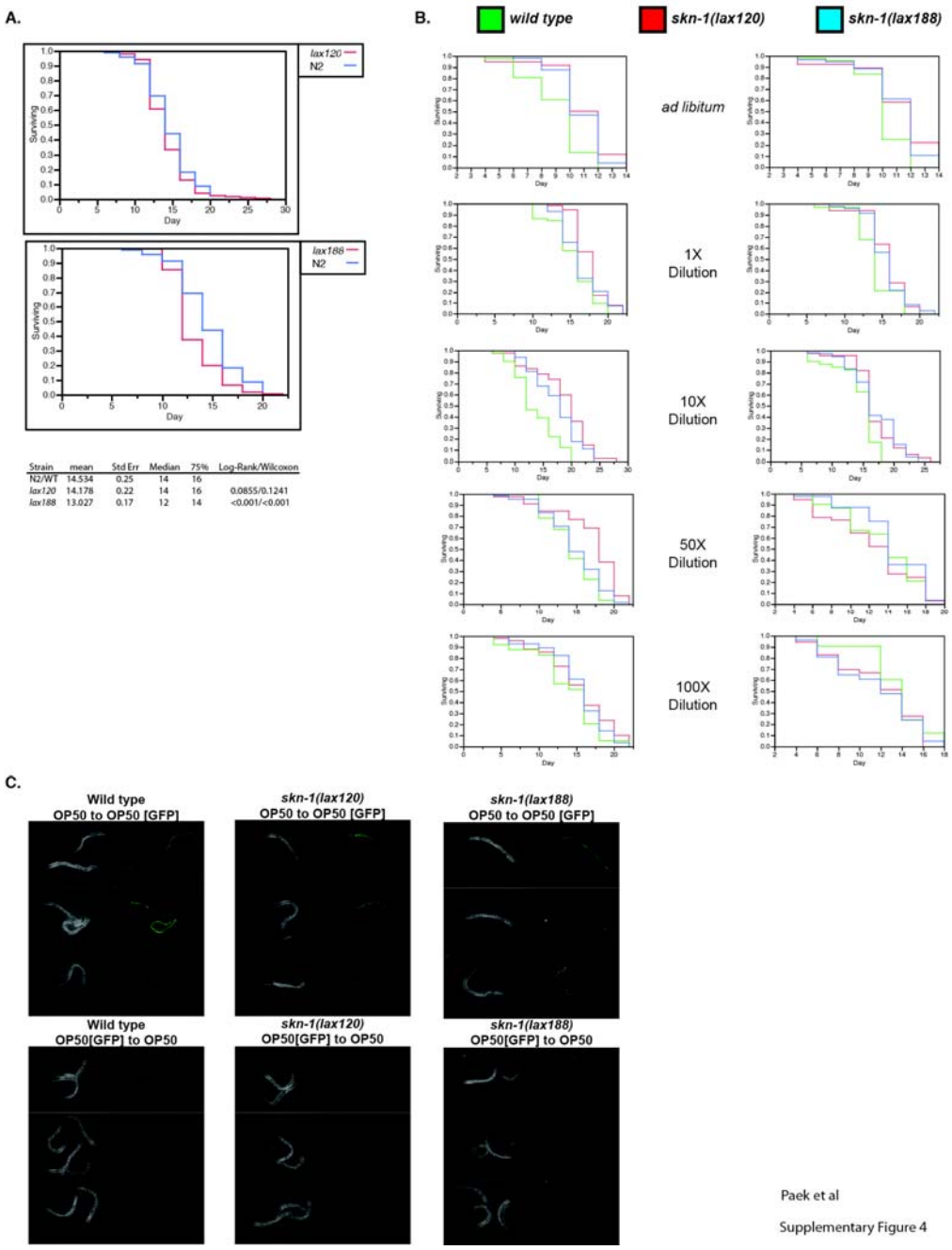

Figure S4: A. DR Lifespan analysis and feeding rates of *skn-1(gf)* mutants. Survival curves of populations of animals of the indicated genotype fed an OP50 diet. Mean is the average lifespan of the population. Std Err is the standard error of the mean. Median lifespan in days. 75% is the day at which 75% of the population has dies. Statistical analysis was performed with JMP software package. B. Lifespan analysis bDR. Survival curves of populations of animals of the indicated genotypes on diluted concentration of OP50. Graphs were generated using the JMP statistical software. C. Feeding and bacterial clearance assays. Worms of the indicated genotype were fed an OP50 diet and then switched to an OP50 strain harboring a GFP plasmid. Feeding rates were

established by the amount of time needed to fill the intestinal lumen with GFP. Bacterial clearance was assed by feeding the indicated strains with OP50-GFP bacteria and then switching to regular OP50 and measuring the time required to clear the GFP fluorescence from the intestinal lumen.

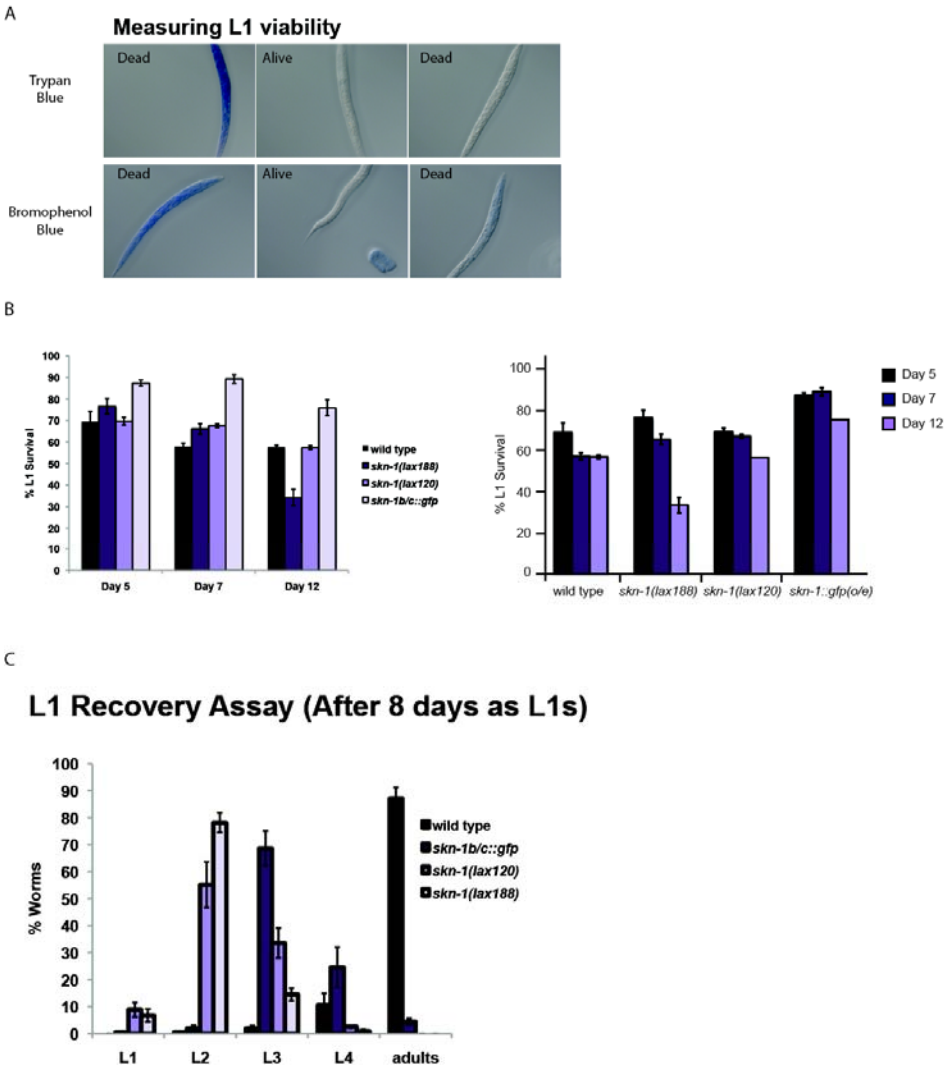

Figure S5: L1 arrest, survival, and recovery assays. A. L1 larvae viability was assessed by staining with Trypan Blue or Bromophenol Blue. B. L1 survival at days 5, 7, and 12 was assessed as described in A for the indicated genotypes. C. Worms of the indicated genotypes were arrested as L1 larvae in the absence of food for 8 days and then allowed to recover on OP50 bacteria. Bars indicate the percentage of worms at each developmental stage following recovery.

Table S1: Raw Affymetrix data worms
Table S2: *wdr-23* RNAi, E.coli B versus E.coli K12 qPCR data
Table S3: *mxl-3* epistasis qPCR data
Table S4: Pharyngeal pumping assays
Table S5: L1 survival and recovery data
Table S6: Raw Affymetrix data keap1 KO mice
Table S7: Go-terms summary

Supplemental Experimental Procedures

Pharyngeal pumping assay. Eggs were isolated from each genotype by hypochlorite/NaOH treatment and allowed to hatch in the absence of food to generate synchronous L1 larvae populations. These animals were placed on NGM plates seeded with OP50 and maintained at 20 °C. L4 larval stage animals were analyzed for, the number of pharyngeal pumps in a 10-second period in the presence of food OP50. Each individual worm was evaluated at least three times. A minimum of 10 worms of each genotype were analyzed. This entire process was performed for at least two biological replicates.

Feeding and bacterial clearance assay. Eggs were isolated from each genotype by hypochlorite/NaOH treatment and allowed to hatch in the presence of the food OP50 or OP50-GFP. These animals were grown at 15 °C until they matured to adulthood and images were taken of their pharynx and intestinal lumen. The animals grown on OP50 were then transferred to OP50-GFP and the worms on OP50-GFP to OP50. They were allowed to feed for 1 hour before they were imaged again.

Quantitative RT-PCR

Single reactions were prepared for each cDNA using the iQ SYBR Green Supermix (Bio-Rad). Each PCR reaction also included a *snb-1* control. Each reaction consisted of 10µl containing 4ng of cDNA and 1pmol of each primer. The real time qPCR was run on Realplex (Eppendorf). The cycling conditions were 1 cycle of denaturation at 95 °C/3 min and 40 three-segment cycles of amplification (95 °C/10 s, 58 °C/20 s, 72 °C/30s), where the fluorescence was automatically measured during PCR. This was followed by one cycle at 72 °C/15 s, a 20 min melting curve to verify the presence of one gene-specific peak. The baseline adjustment method of the Realplex (Eppendorf) software was used to determine the Ct in each reaction.

Mitochondria Enrichment

About 75ul of packed worms were washed in M9(2-3X) and then lysed in RIPA(+ inhibitors) Sonicated 3 x 20 seconds, 25% amplitude. Spun at 1000xg for 15minutes at 4C. Spun sup again at 12,000xg for 20 minutes. The sup was the cyto and the pellet was the mito. Washed pellet 3X and resuspened in appropriate vol (~100ul PBS). Evaluated concentration with BCA method.

Western analysis

A mitochondria enriched fraction from whole worm lysates was isolated by differential centrifugation. Fractions were solubilized, the protein components separated by gel electrophoresis and transferred to PVDF membranes. Blots were then probed with anti-PDHE1 (Abcam cat # 110334) at 1:1000 in 1% milk/TBST, anti-GAPDH (Ambion cat # AM4300) at 1:2000 in 1% milk/TBST and polyclonal anti-SKN-1 at 1:200 in 1%BSA/PBS.

Co-Immunoprecipitation studies

35S-Methionine labeled SKN-1A, SKN-1B, MXL-3, PGAM-5, WDR-23, and Luciferase were translated *in vitro* with Promega TNT coupled transcription translation systems. 50µL of the translation reaction was diluted to 200µL in TBS and was added to 15µL of agarose resin and incubated for 1 hour at 4°C as a non-specific pre-clearing step. 10% of the sample was removed as a loading control. The remaining cleared translation reactions were added to 50µL of anti-HA resin and incubated overnight at 4°C with end-over-end rocking. The resin was washed with 3 x 500µL of TBS-T. SKN-1HA protein complexes were eluted with the addition of 30µL of 2X non-reducing sample buffer and boiled at 95C. B-mercaptoethanol was added to the samples before separation by SDS-PAGE. Gels were fixed with 10% TCA, neutralized in Tris Buffer, and soaked in 1M salicylate. The gels were then dried onto whatman paper and exposed to film for analysis.