Supplementary Figures for:

Mitochondrial thioredoxin system is required for enhanced stress resistance and extended longevity in long-lived mitochondrial mutants

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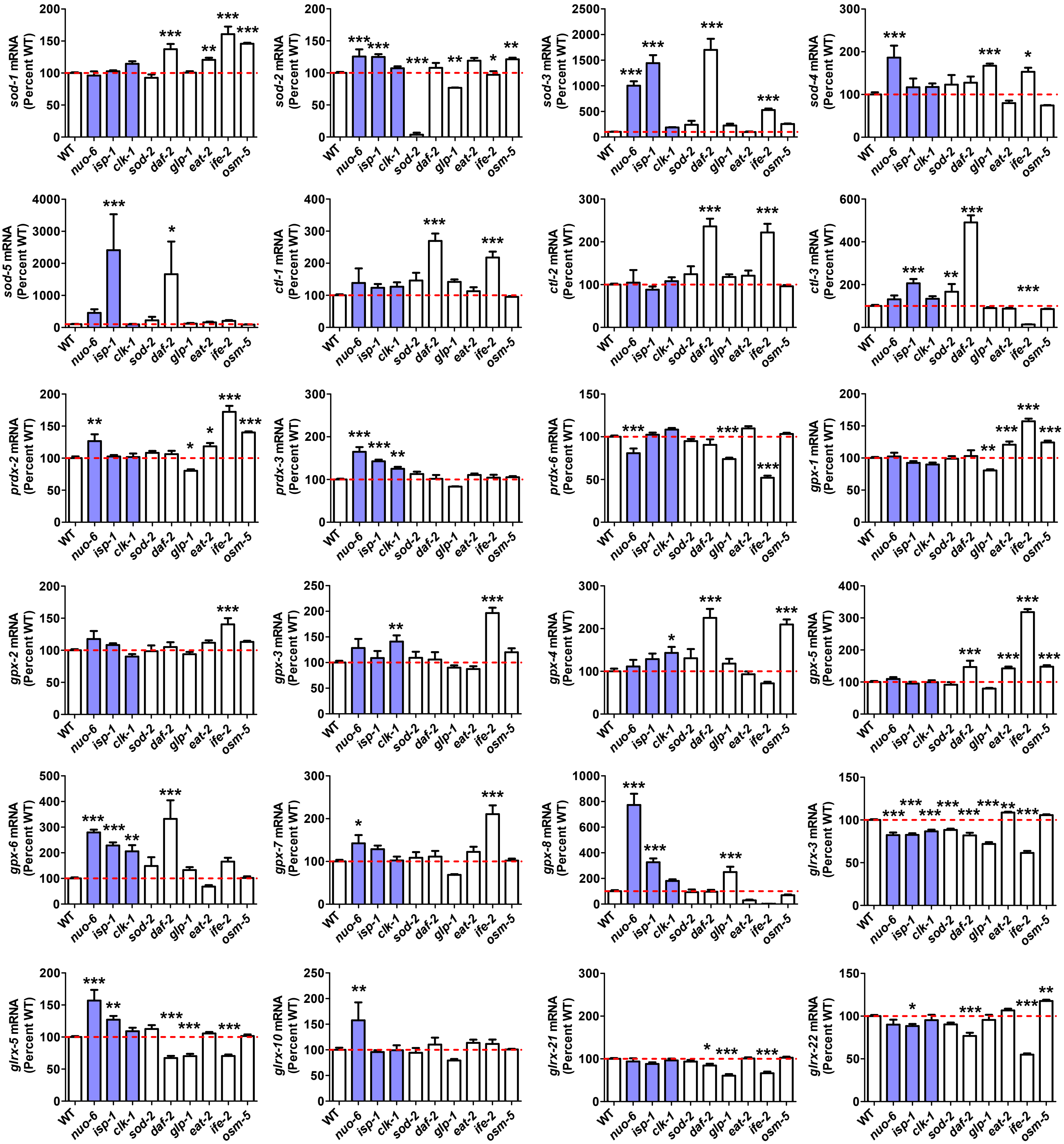

Fig. S1. *prdx-3* is specifically upregulated in long-lived mitochondrial mutants. To determine which antioxidant genes are specifically upregulated in long-lived mitochondrial mutants, we quantified gene expression from RNA sequencing data of six biological replicates per strain. Expression of *prdx-3* was specifically upregulated in the long-lived mitochondrial mutants *nuo-6, isp-1* and *clk-1,* but not in other long-lived strains. Statistical significance was assessed using a one-way ANOVA with Dunnett's multiple comparison test. Error bars indicate SEM. *p<0.05, **p<0.01, ***p<0.001.

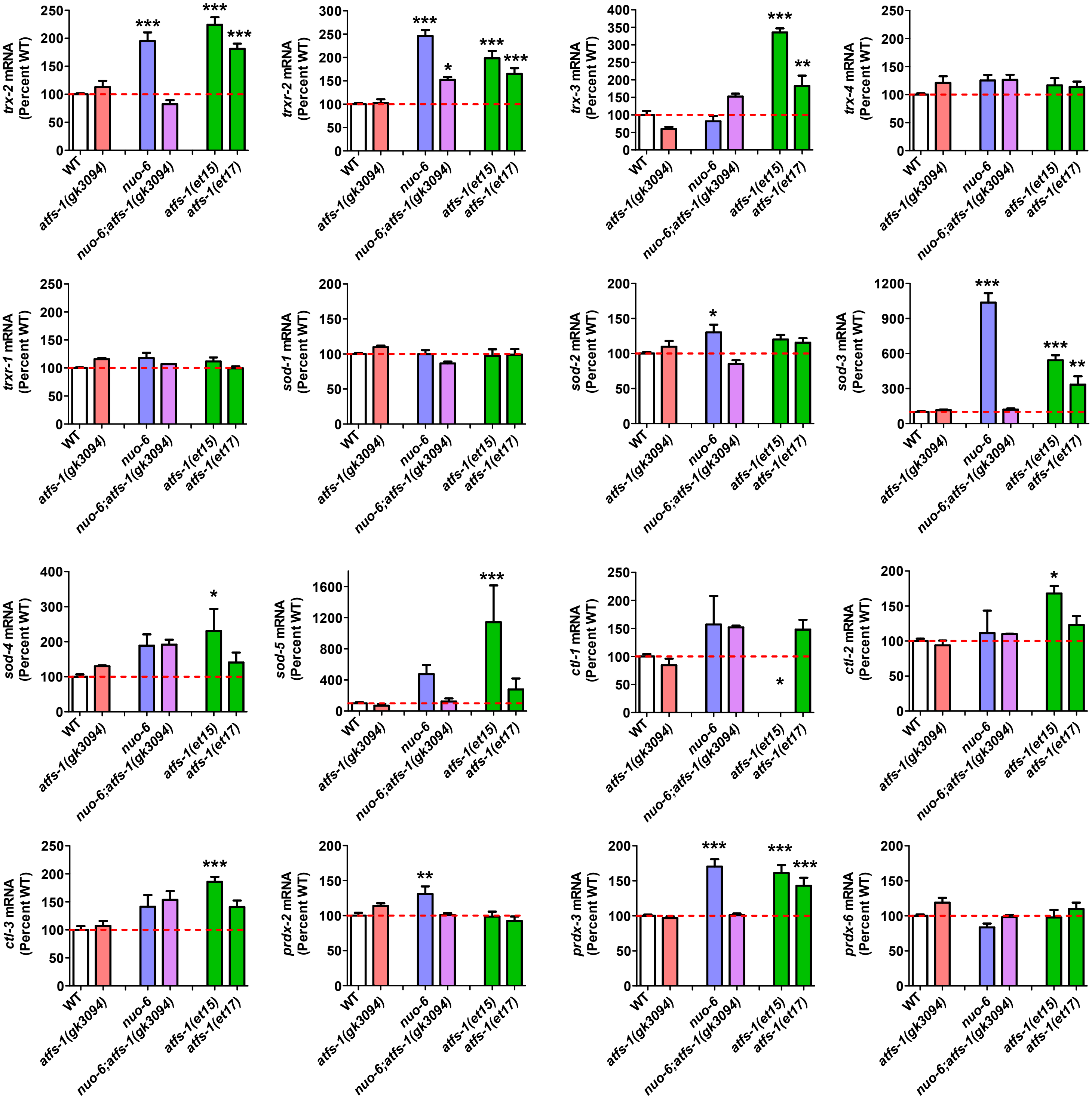

Fig. S2. Activation of mitoUPR results in upregulation of *sod-3* and *prdx-3.* To examine the role of the mitochondrial unfolded protein response (mitoUPR) in the upregulation or expression of antioxidant genes, we examined antioxidant gene expression in wild-type, *atfs-1(gk3094)* deletion mutants, *nuo-6* mutants, *nuo-6;atfs-1* mutants, and two constitutively active *atfs-1* mutants, *et15* and *et17.* Similar to *trx-2* and *trxr-2,* both *sod-3* and *prdx-3* showed ATFS-1-dependent upregulation in *nuo-6* worms and upregulation in both constitutively active *atfs-1* mutants. Gene expression was measured using RNA sequencing with 3-6 biological replicates per strain. Statistical significance was assessed using a one-way ANOVA with Dunnett's multiple comparison test and indicates the difference from wild-type. Error bars indicate SEM. *p<0.05, **p<0.01, ***p<0.001.

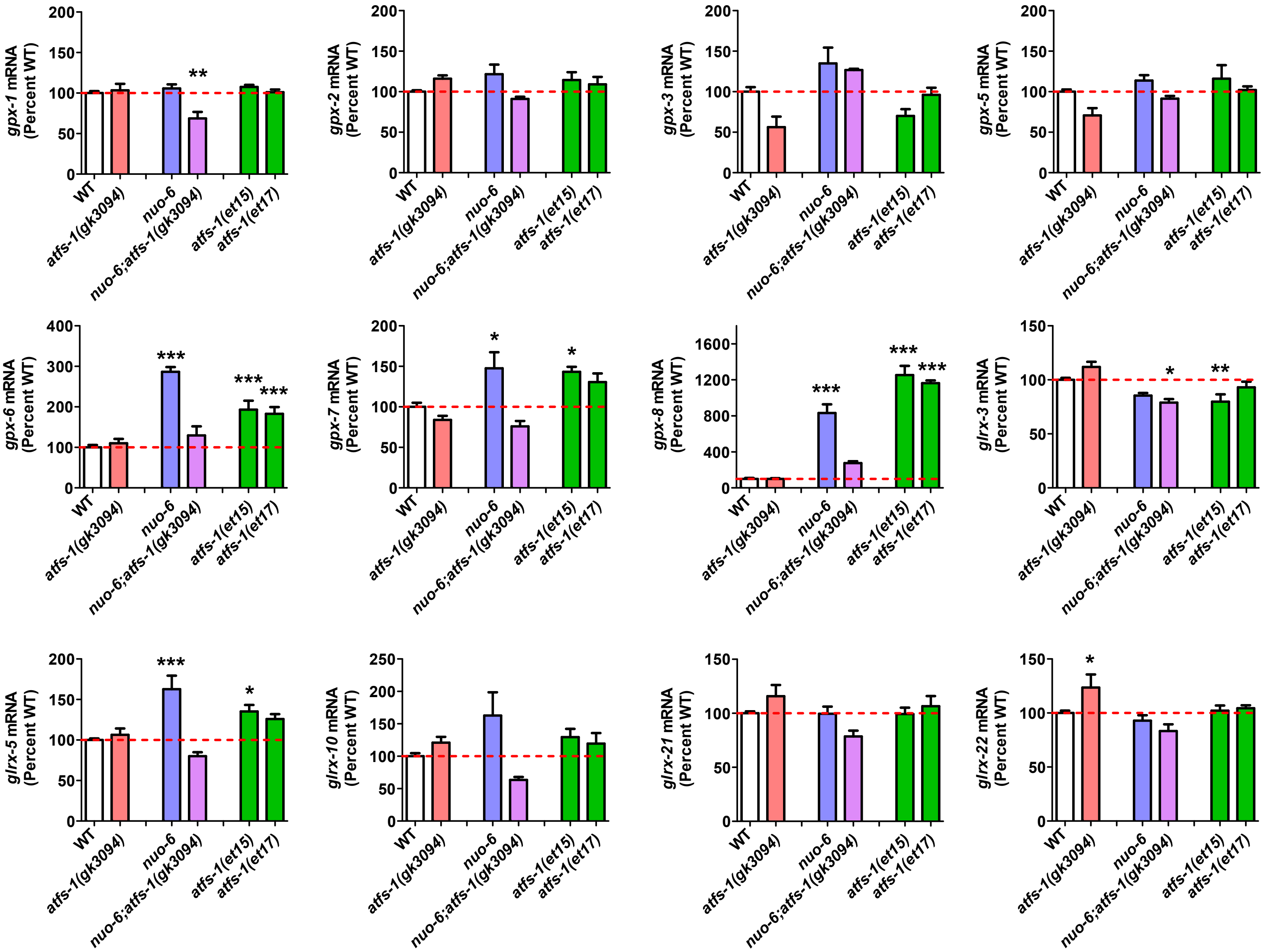

Fig. S3. Activation of mitoUPR results in upregulation of *gpx-6* and *gpx-8*. To examine the role of the mitochondrial unfolded protein response (mitoUPR) in the upregulation or expression of antioxidant genes, we examined antioxidant gene expression in wild-type, *atfs-1(gk3094)* deletion mutants, *nuo-6* mutants, *nuo-6;atfs-1* mutants, and two constitutively active *atfs-1* mutants, *et15* and *et17.* Similar to *trx-2* and *trxr-2,* both *gpx-6* and *gpx-8* showed ATFS-1-dependent upregulation in *nuo-6* worms and upregulation in both constitutively active *atfs-1* mutants. Gene expression was measured using RNA sequencing with 3-6 biological replicates per strain. Statistical significance was assessed using a one-way ANOVA with Dunnett's multiple comparison test and indicates the difference from wild-type. Error bars indicate SEM. *p<0.05, **p<0.01, ***p<0.001.

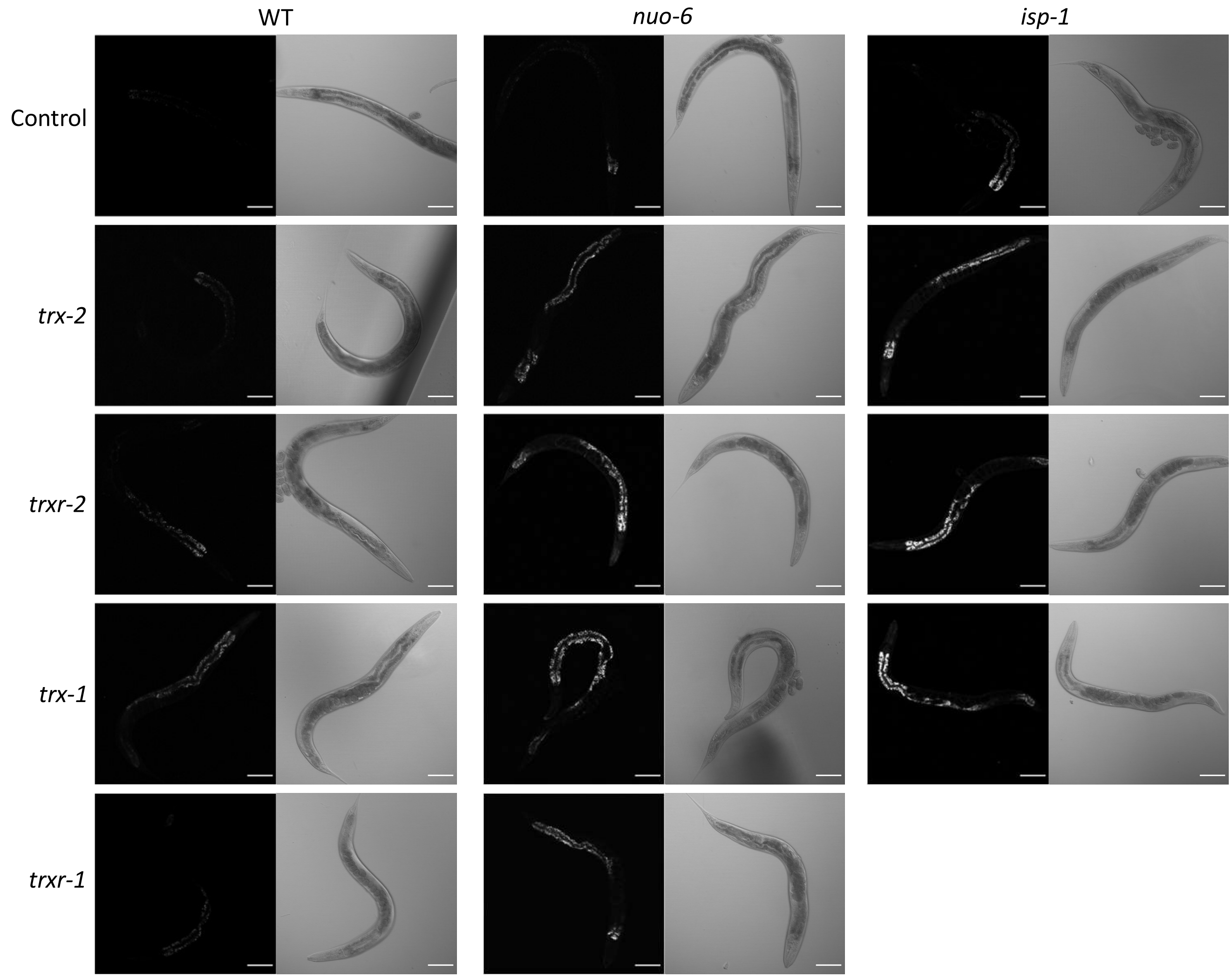

Fig. S4. Disruption of mitochondrial or cytoplasmic thioredoxin systems increases levels of reactive oxygen species. ROS levels were measured by staining whole worms with dihydroethidium (DHE) and quantifying the resulting fluorescence. Representative images of each genotype are shown. Scale bar indicates 100 µM. Quantification of fluorescence can be found in **Figure 3**.

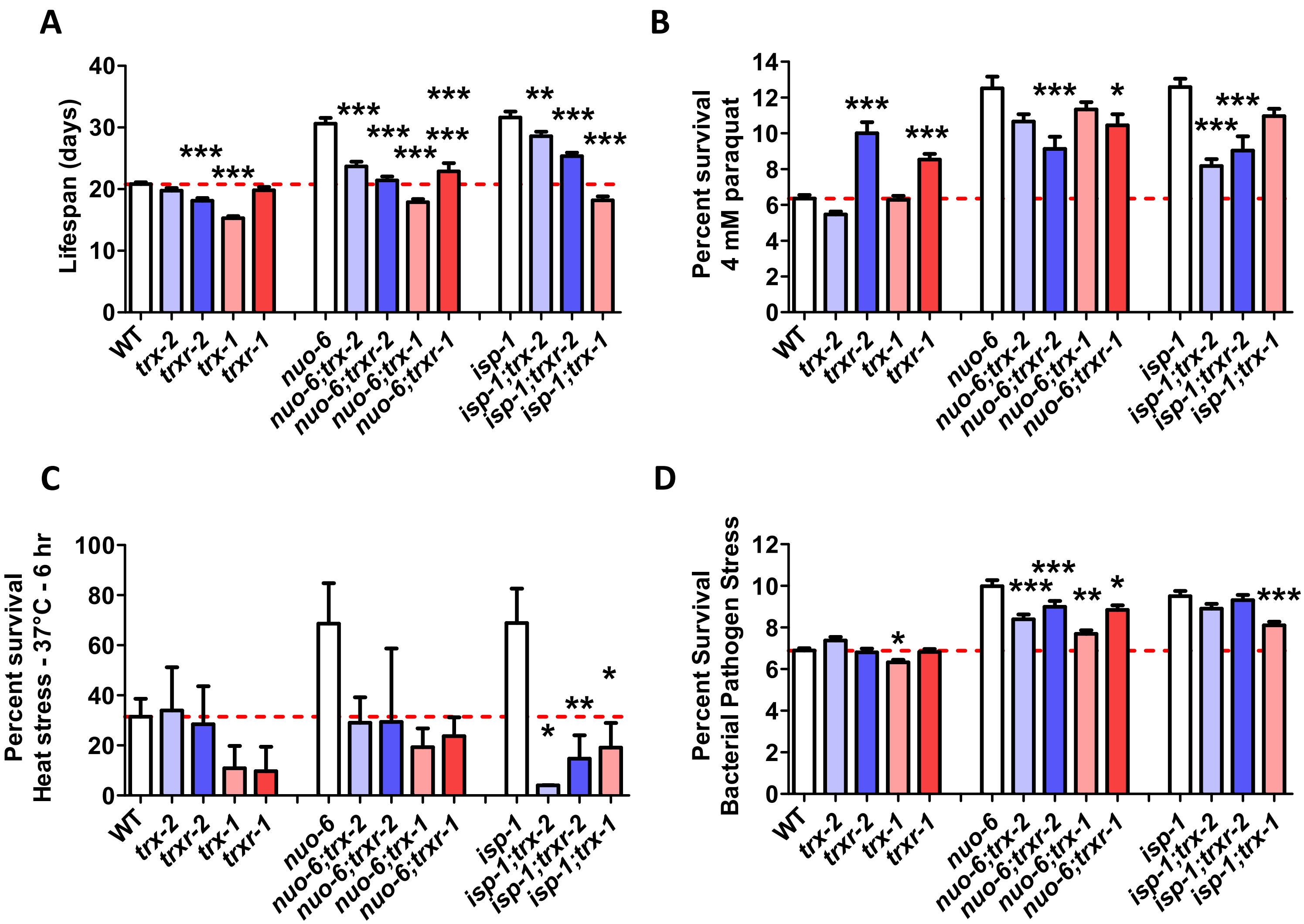

Fig. S5. Disruption of thioredoxin system genes affects lifespan and resistance to stress. Data from figures 4 (**A**; lifespan), figure 5 (**B**; oxidative stress resistance), figure 6 (**C**; heat stress) and figure 8 (**D**; bacterial pathogen resistance) were reformatted into a bar graph to facilitate comparisons across all genotypes. Raw data can be found in **Table S1**. Statistical significance was assessed using a one-way ANOVA with Dunnett's multiple comparison test. Error bars indicates SEM. *p<0.05, **p<0.01, ***p<0.001.

Table S2. Summary of results

"=" indicates no significant difference, one arrow indicates less than 15% change; two arrows indicates 15-30% change; three arrows indicates greater than 30% change

		trx-2	*trxr-2*	*trx-1*	*trxr-1*
Wild-type	ROS levels	↑↑	↑↑↑	↑↑↑	↑↑
	Lifespan	=	↓	↓↓	=
	Oxidative Stress Resistance	↓	↑↑↑	=	↑↑↑
	Heat Stress Resistance	=	=	↓↓↓	↓↓↓
	Bacterial pathogen resistance	↑	=	↓	=
	Osmotic stress resistance	=	=	↓↓↓	=
nuo-6	ROS levels	↑↑↑	↑↑↑	↑↑↑	↑↑↑
	Lifespan	↓↓	↓↓	↓↓↓	↓↓
	Oxidative Stress Resistance	↓	↓↓	↓	↓↓
	Heat Stress Resistance	↓↓↓	↓↓↓	↓↓↓	↓↓↓
	Bacterial pathogen resistance	↓↓	↓	↓↓	↓
	Osmotic stress resistance	=	↓↓↓	↓↓	=
isp-1	ROS levels	↑↑↑	↑↑↑	↑↑↑	NA
	Lifespan	↓	↓↓	↓↓↓	NA
	Oxidative Stress Resistance	↓↓↓	↓↓↓	↓	NA
	Heat Stress Resistance	↓↓↓	↓↓↓	↓↓↓	NA
	Bacterial pathogen resistance	=	=	↓	NA
	Osmotic stress resistance	↓↓↓	↓↓↓	↓↓↓	NA