Supplementary Figures for:

Uncoupling of Oxidative Stress Resistance and Lifespan in Long-lived *isp-1* Mitochondrial Mutants in *Caenorhabditis elegans*

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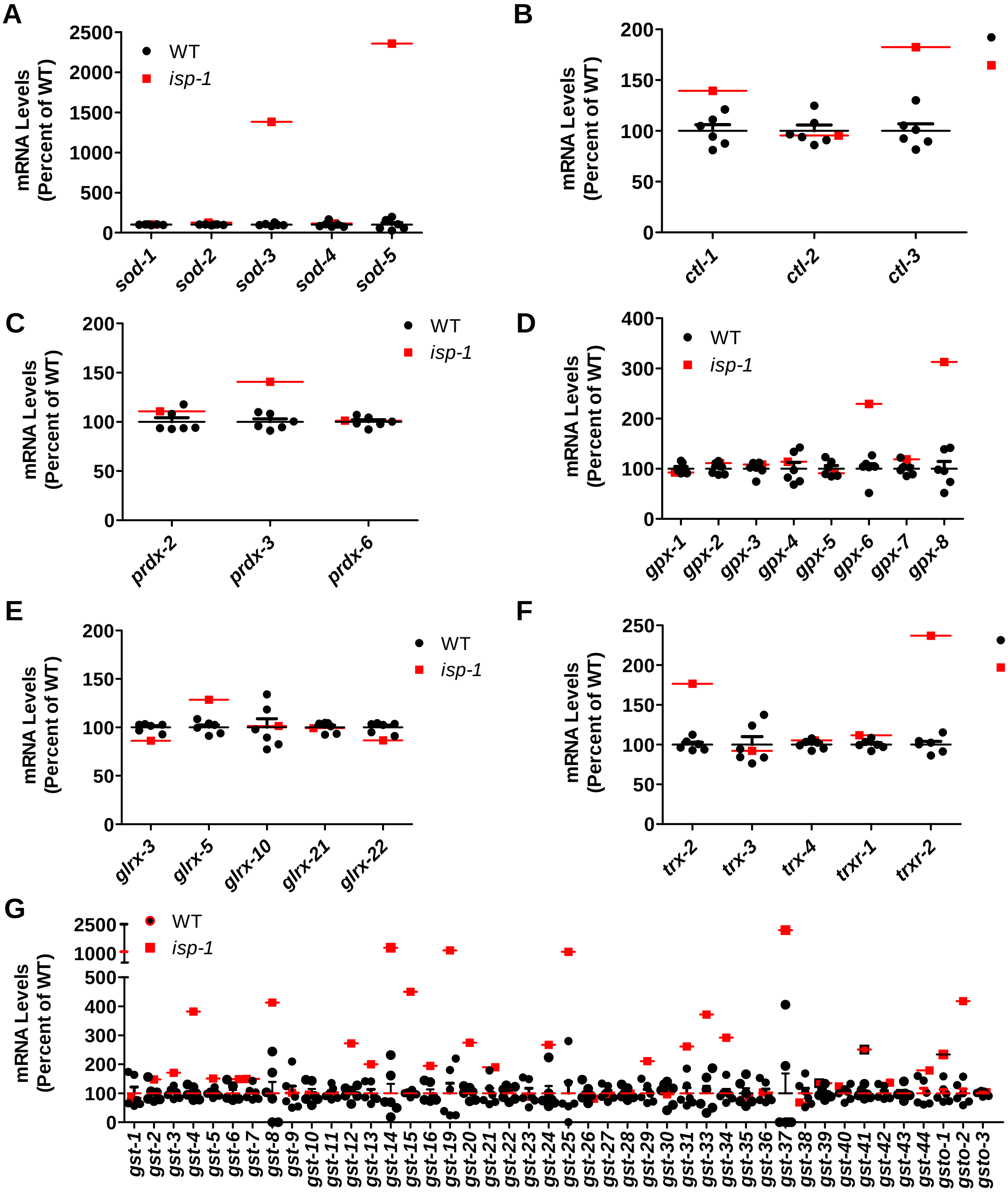
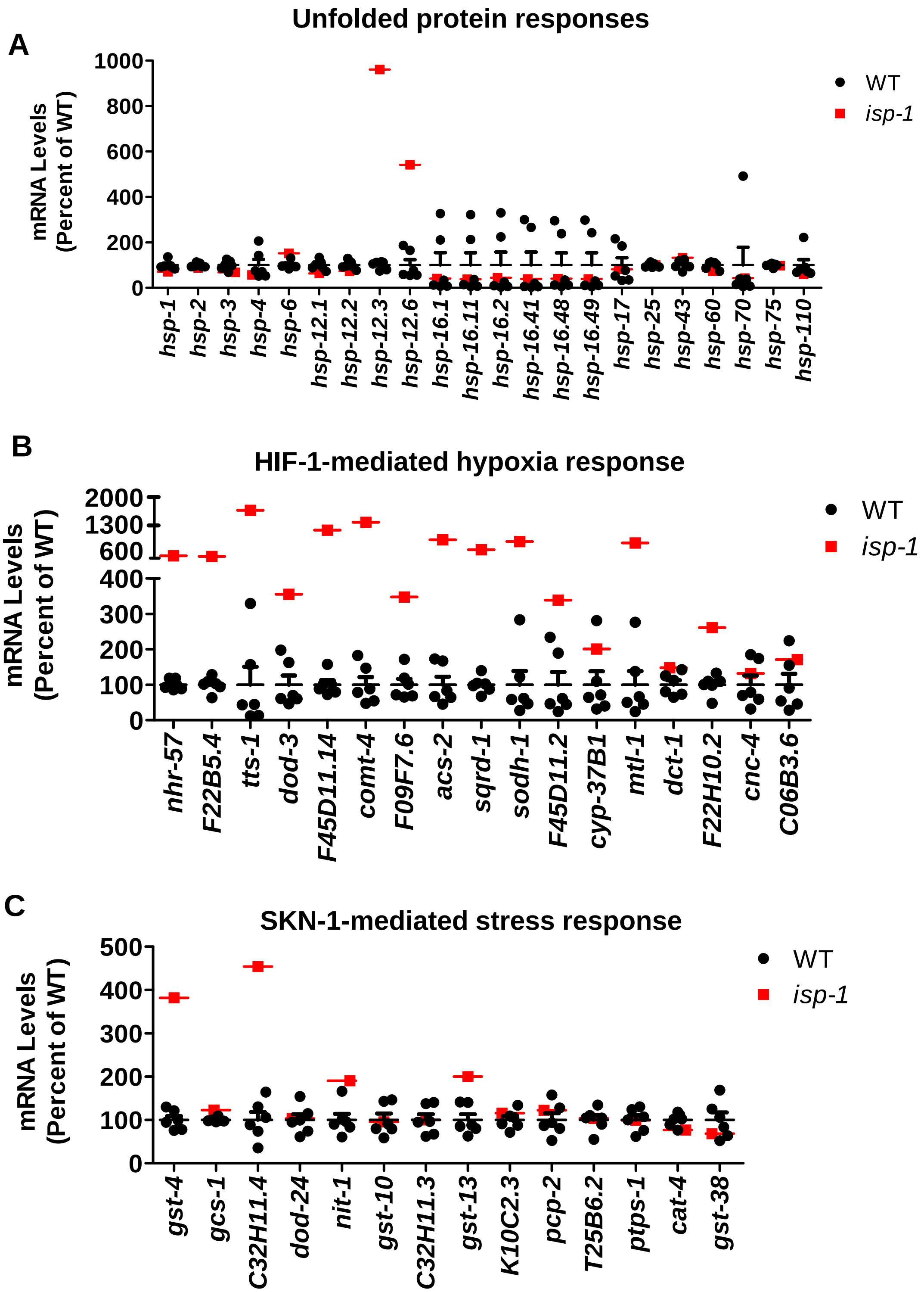
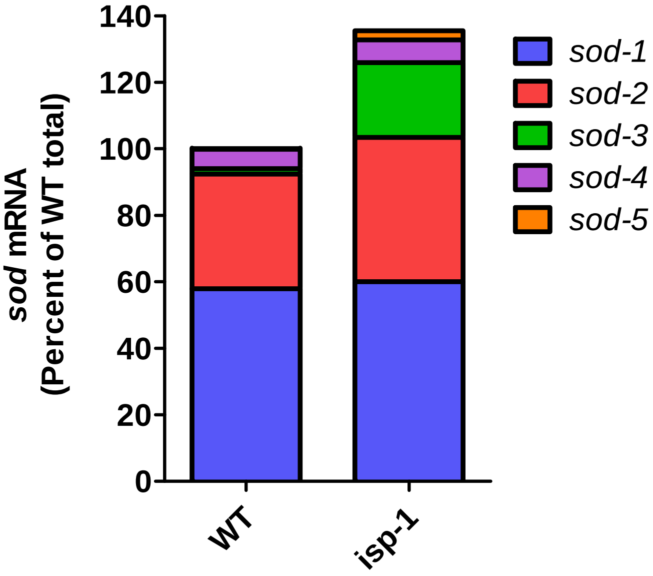


Supplementary Fig. S1. Upregulation of specific antioxidant genes in *isp-1* worms. The expression of antioxidant enzymes was assessed using RNA sequencing. Six independent biological samples were collected for both WT and *isp-1* worms. RNA sequencing for *isp-1* worms was performed on a pooled sample containing equal proportions of the six biological replicates **A.** *sod-3* and *sod-5* were markedly upregulated in *isp-1* worms **B**. Among the catalase genes *ctl-3* expression was increased in *isp-1* worms **C.** None of the peroxireodxin *(prdx*) genes were increased in *isp-1* worms. **D.** Among the glutathione peroxidase genes, *gpx-6* and *gpx-8* were increased in *isp-1* worms. **E.** There was no difference in the expression of glutaredoxin genes. **F.** Thioredoxin 2 (*trx-2*) and thioredoxin reductase 2 (*trxr-2*) were increased in *isp-1* worms. **G.** Multiple glutathione reductase genes showed upregulation in *isp-1* worms. Counts per million (CPM) were normalized to the average CPM of the wild-type samples to obtain percent of WT.

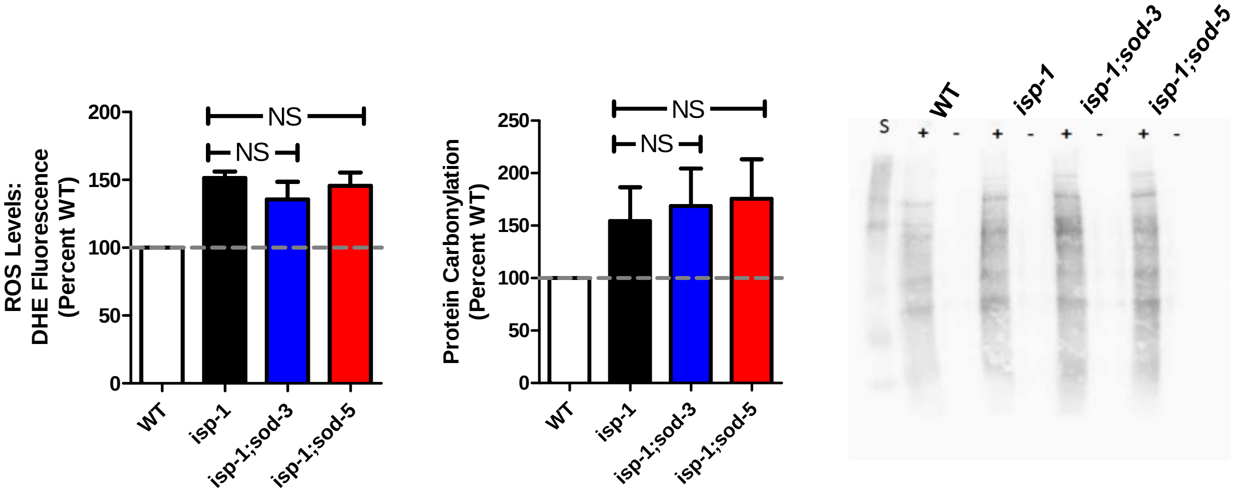


Supplementary Fig. S2. Activation of stress response pathways in *isp-1* worms. The expression of gene targets of different stress response pathways was assessed using RNA sequencing. Six independent biological samples were collected for both WT and *isp-1* worms. RNA sequencing for *isp-1* worms was performed on a pooled sample containing equal proportions of the six biological replicates **A.** *isp-1* worms did not exhibit a marked increase in genes associated with the cytosolic unfolded protein response (*hsp-16.11, hsp-16.2*), the mitochondrial unfolded protein response (*hsp-6,* hsp-60) or the endoplasmic reticulum unfolded protein response (*hsp-4*). i*sp-1* worms did exhibit increased expression of *hsp-12.3* and *hsp-12.6*. **B**. *isp-1* worms exhibited upregulation of many genes associated with the HIF-1-mediated hypoxia response. **C.** Select genes associated with the SKN-1-mediated oxidative stress response were upregulated in *isp-1* worms. Counts per million (CPM) were normalized to the average CPM of the wild-type samples to obtain percent of WT.

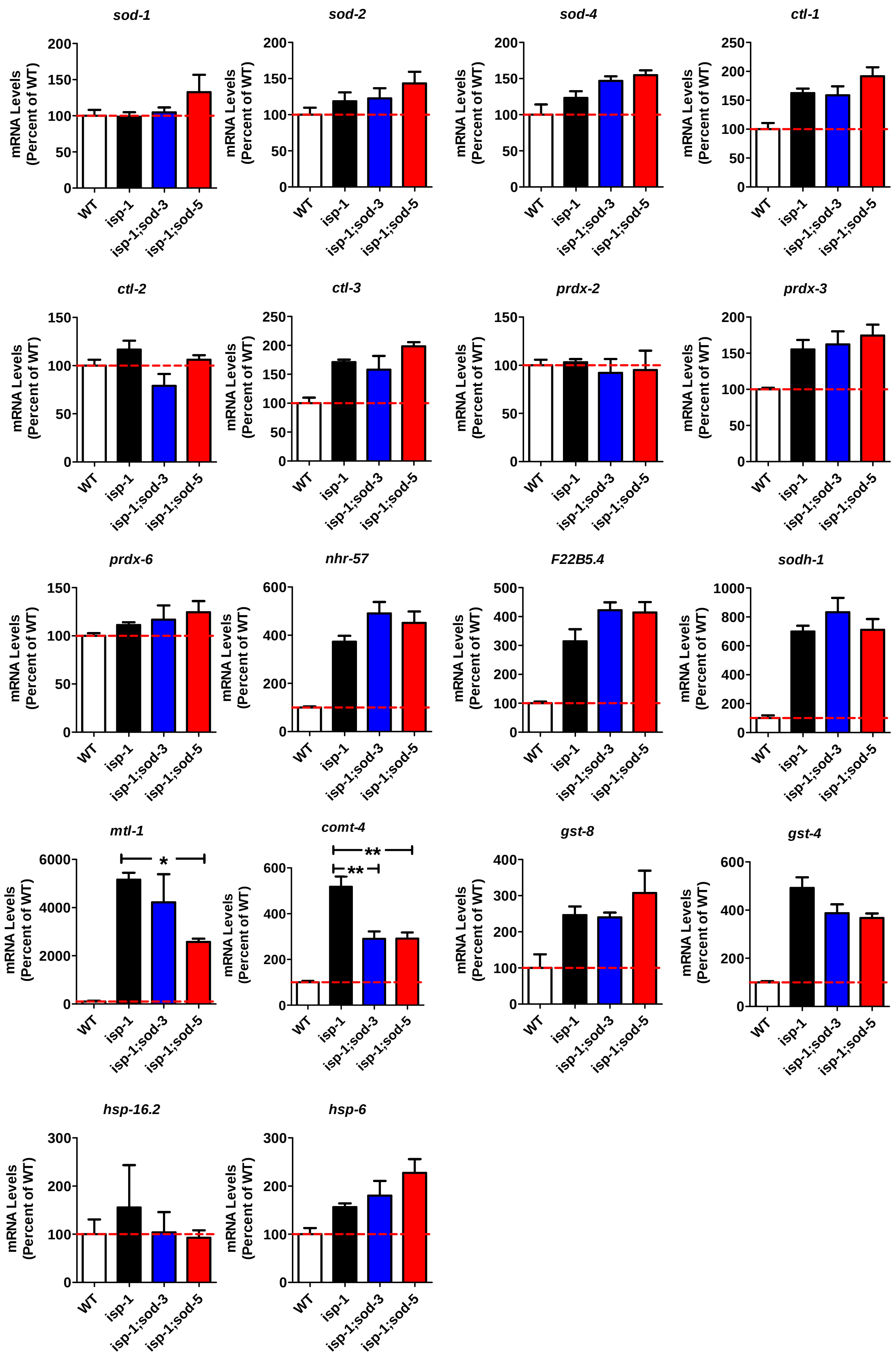


	WT	*isp-1*
sod-1	57.9%	44.3%
sod-2	34.5%	32.1%
sod-3	1.6%	16.6%
sod-4	5.8%	5.0%
sod-5	0.1%	2.1%

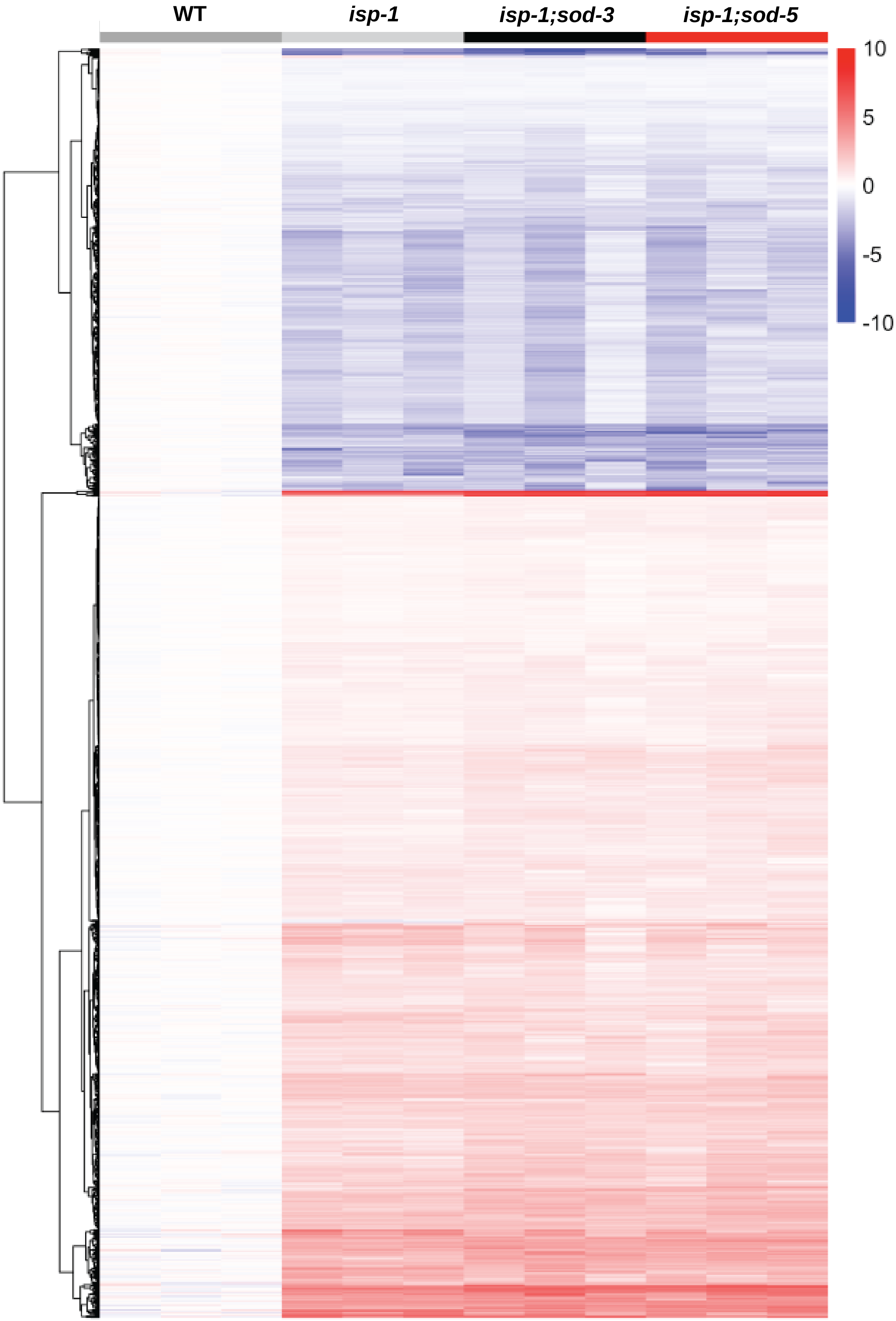
Supplementary Fig. S3. Comparison of *sod* mRNA levels in WT and *isp-1* worms. A. *isp-1* worms exhibit increased expression of *sod-3* and *sod-5* mRNA compared to WT worms leading to an overall increase in *sod* expression. **B.** This table shows the percent contribution of each *sod* gene to the total *sod* mRNA. In *isp-1* worms there is a marked increase in the contribution of *sod-3* and *sod-5.* However, their expression is still less than *sod-2* and *sod-1* in the mitochondria and cytoplasm, respectively.



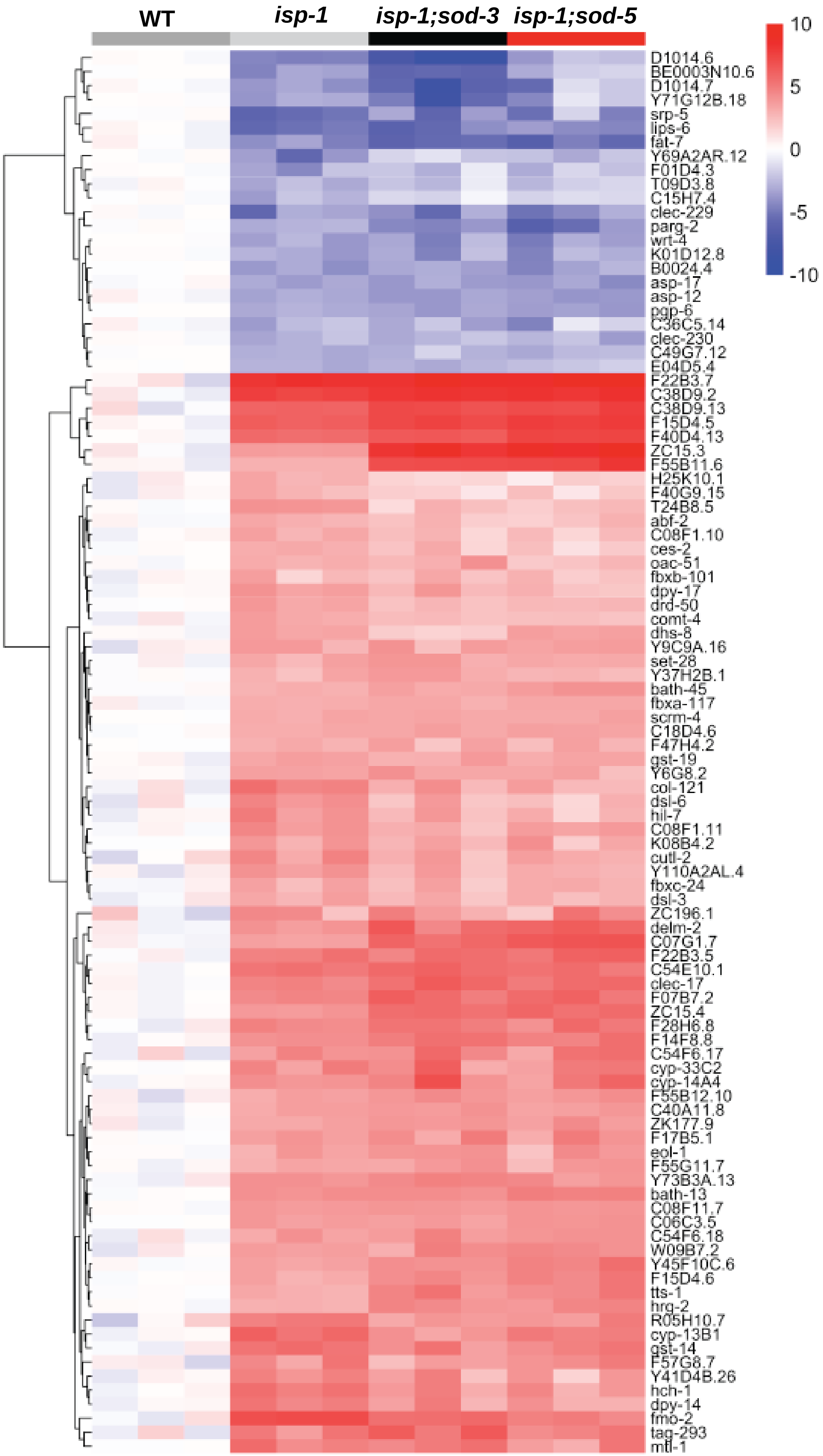
Supplementary Fig. S4. Deletion of inducible *sod* genes does not increase ROS levels or oxidative damage in *isp-1* worms. A. Quantification of ROS levels by staining with the ROS-sensitive dye DHE revealed no difference between *isp-1* worms and *isp-1;sod-3* or *isp-1;sod-5* worms. **B.** Similarly, *isp-1;sod-3* and *isp-1;sod-5* worms showed a similar level of oxidative damage to proteins to *isp-1* worms, as measured by protein carbonylation. **C.** Representative blot for carbonylated proteins. Error bars indicate SEM. Only significant differences between *isp-1* and *isp-1;sod-3* or *isp-1;sod-5* worms are indicated.



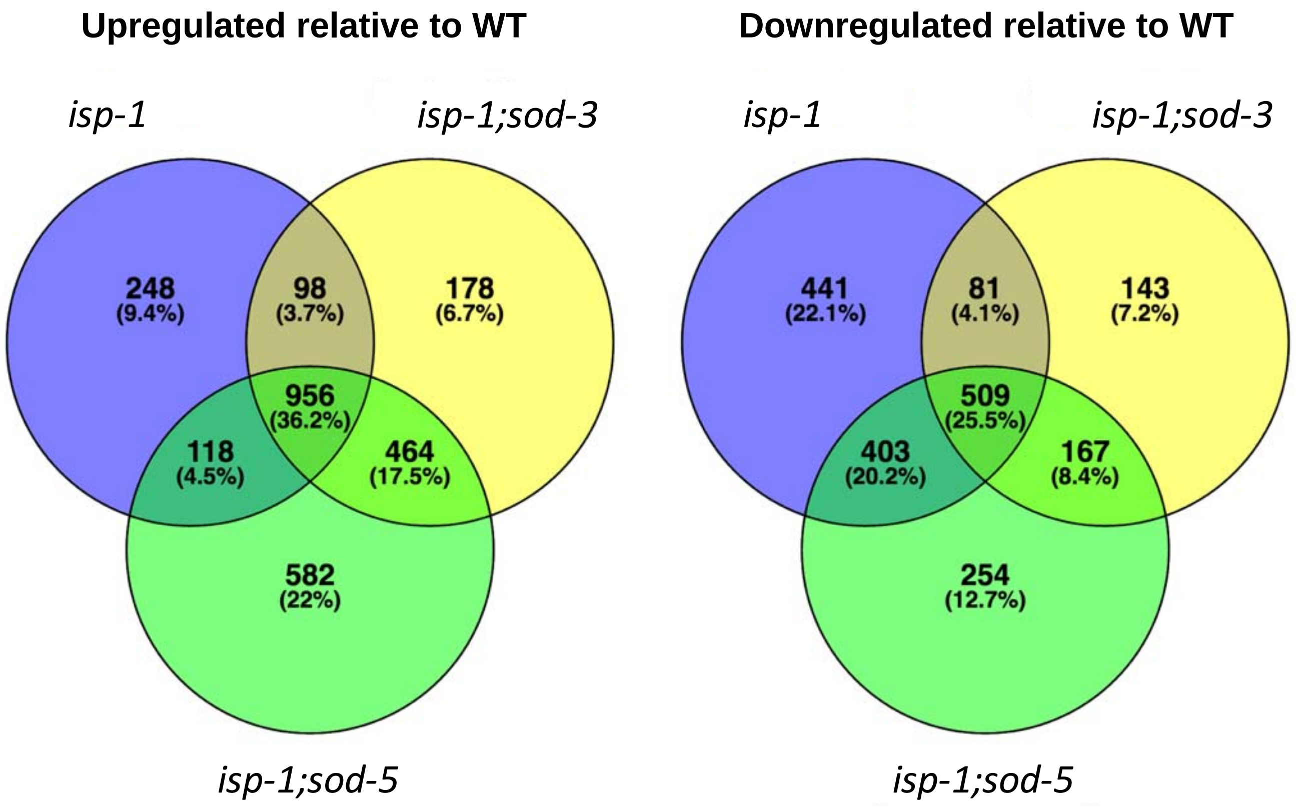
Supplementary Fig. S5. Deletion of inducible *sod* genes has little effect on the expression of select antioxidant and stress response genes in *isp-1* worms. Gene expression was measured by quantitative real-time RT-PCR. Six biological replicates were collected and pooled into three samples for analysis. Error bars indicate SEM. Only significant differences between *isp-1* and *isp-1;sod-3* or *isp-1;sod-5* worms are indicated.



Supplementary Fig. S6. Gene expression changes in *isp-1, isp-1;sod-3* and *isp-1;sod-5* worms compared to WT. RNA sequencing was used to compare gene expression in *isp-1* worms, *isp-1;sod-3* worms and *isp-1;sod-5* worms to WT worms. Overall, *isp-1;sod-3* and *isp-1;sod-5* worms exhibit similar changes in gene expression to *isp-1* worms. Nonetheless, there are multiple genes that show differences in gene expression between *isp-1* worms and the *isp-1;sod* double mutants. Nine biological replicates were collected for each strain and pooled to make three samples for RNA sequencing.

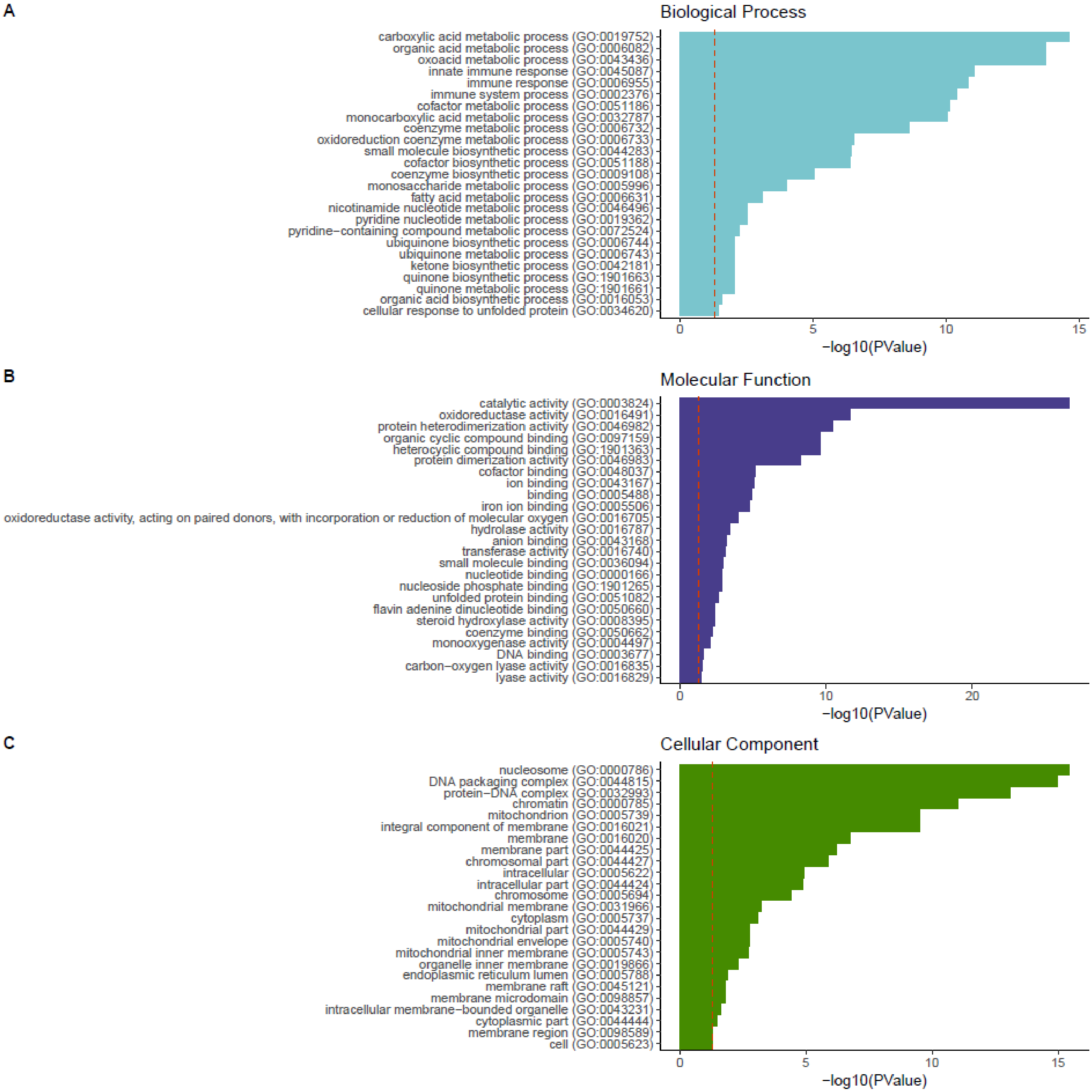


Supplementary Fig. S7. Gene expression changes in *isp-1, isp-1;sod-3* and *isp-1;sod-5* worms compared to WT – top 100 differentially expressed genes. RNA sequencing was used to compare gene expression in *isp-1* worms, *isp-1;sod-3* worms and *isp-1;sod-5* worms to WT worms. Overall, *isp-1;sod-3* and *isp-1;sod-5* worms exhibit similar changes in gene expression to *isp-1* worms. Nonetheless, there are multiple genes that show differences in gene expression between *isp-1* worms and the *isp-1;sod* double mutants. Nine biological replicates were collected for each strain and pooled to make three samples for RNA sequencing.

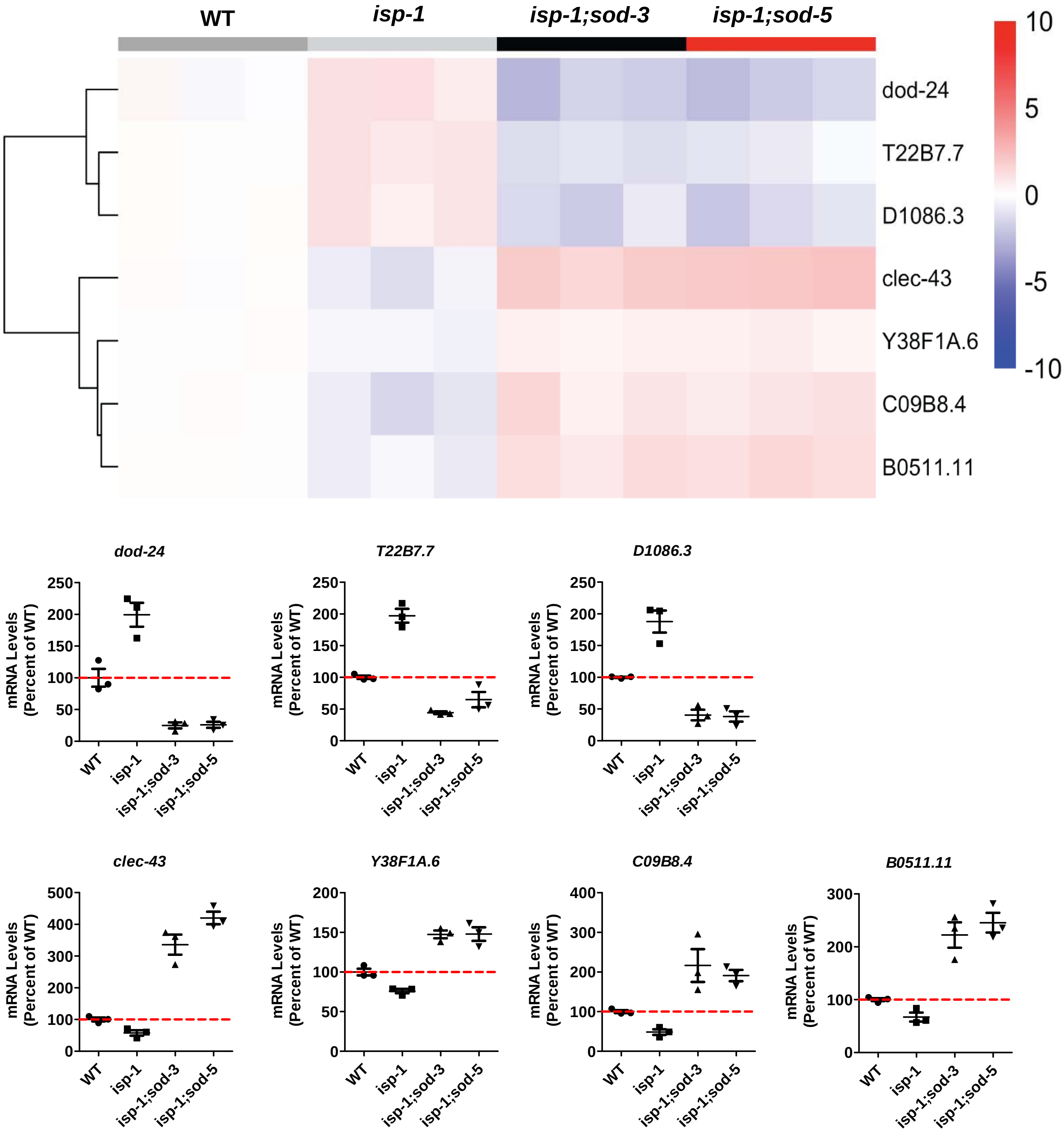

Upregulated relative to WT

Downregulated relative to WT

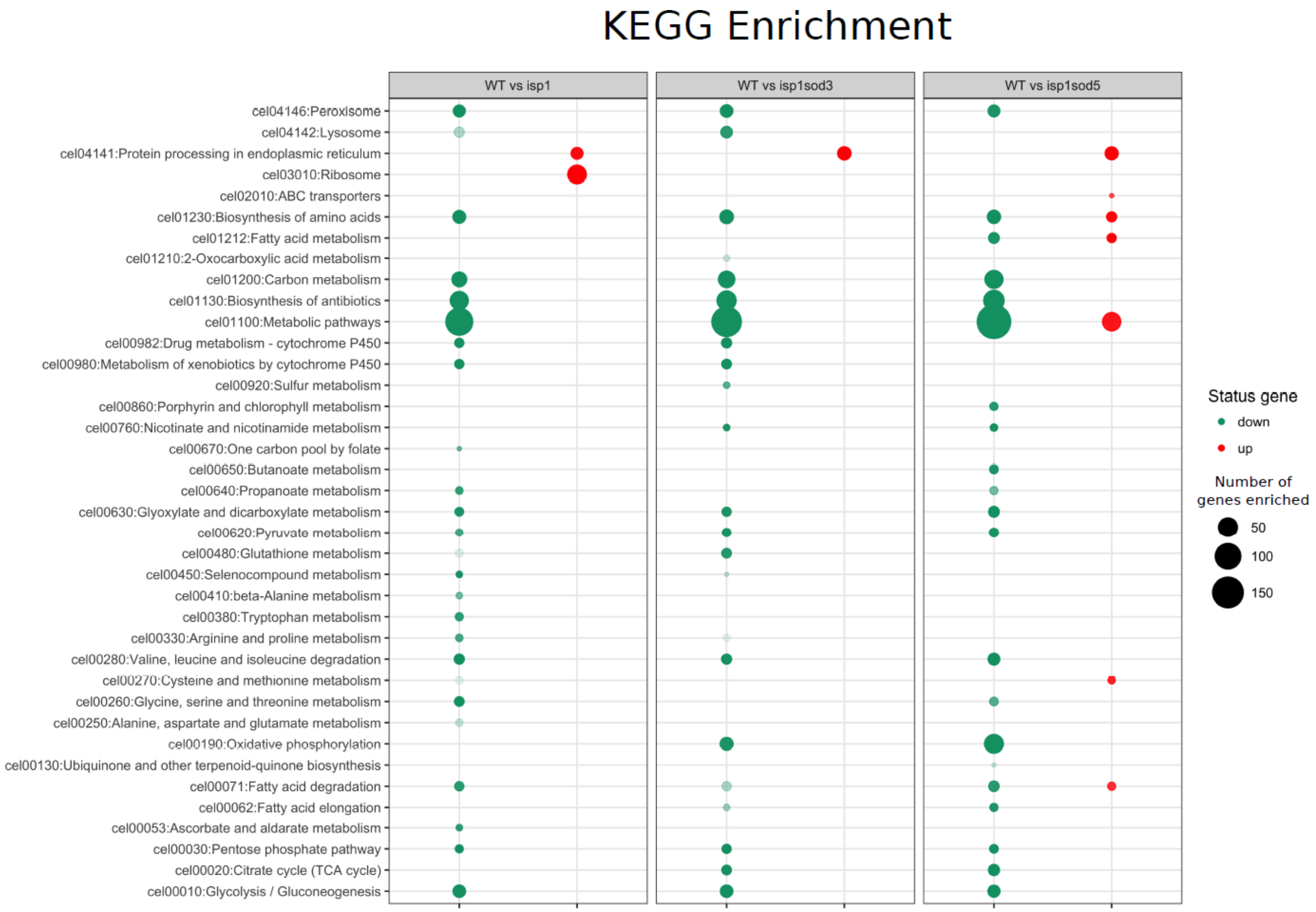
Supplementary Fig. S8. Venn diagrams of differentially expressed genes in *isp-1*, *isp-1;sod-3*, and *isp-1;sod-5* worms relative to WT worms. A large overlap of differentially expressed genes across all *isp-1* mutants exists, suggesting that *isp-1* deficiency drives large transcriptional alterations. Nonetheless, *isp-1;sod-3* and *isp-1;sod-5* mutants exhibit changes in genes expression that are unique from *isp-1* worms that may affect lifespan, stress resistance and physiologic rates. All genes $q < 0.05$.



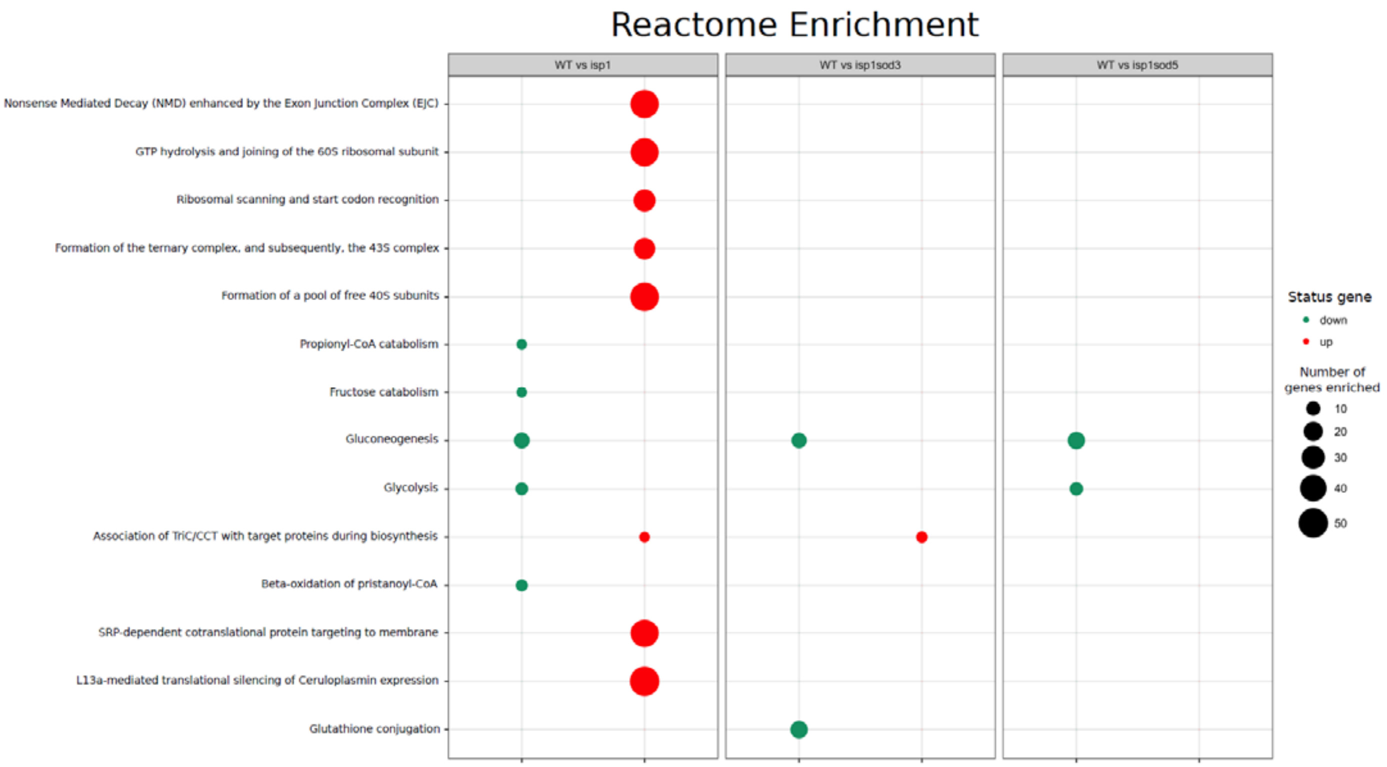
Supplementary Fig. S9. Gene ontology enrichment of gene expression changes in *isp-1, isp-1;sod-3* and *isp-1;sod-5* worms compared to WT. Commonly differentially expressed genes between *isp-1*, *isp-1;sod-3* and *isp-1;sod-5* were analyzed for gene ontology category enrichment in biological processes, molecular function, and cellular components. GO terms can be searched on geneontology.org. Dotted red line indicates P = 0.05.



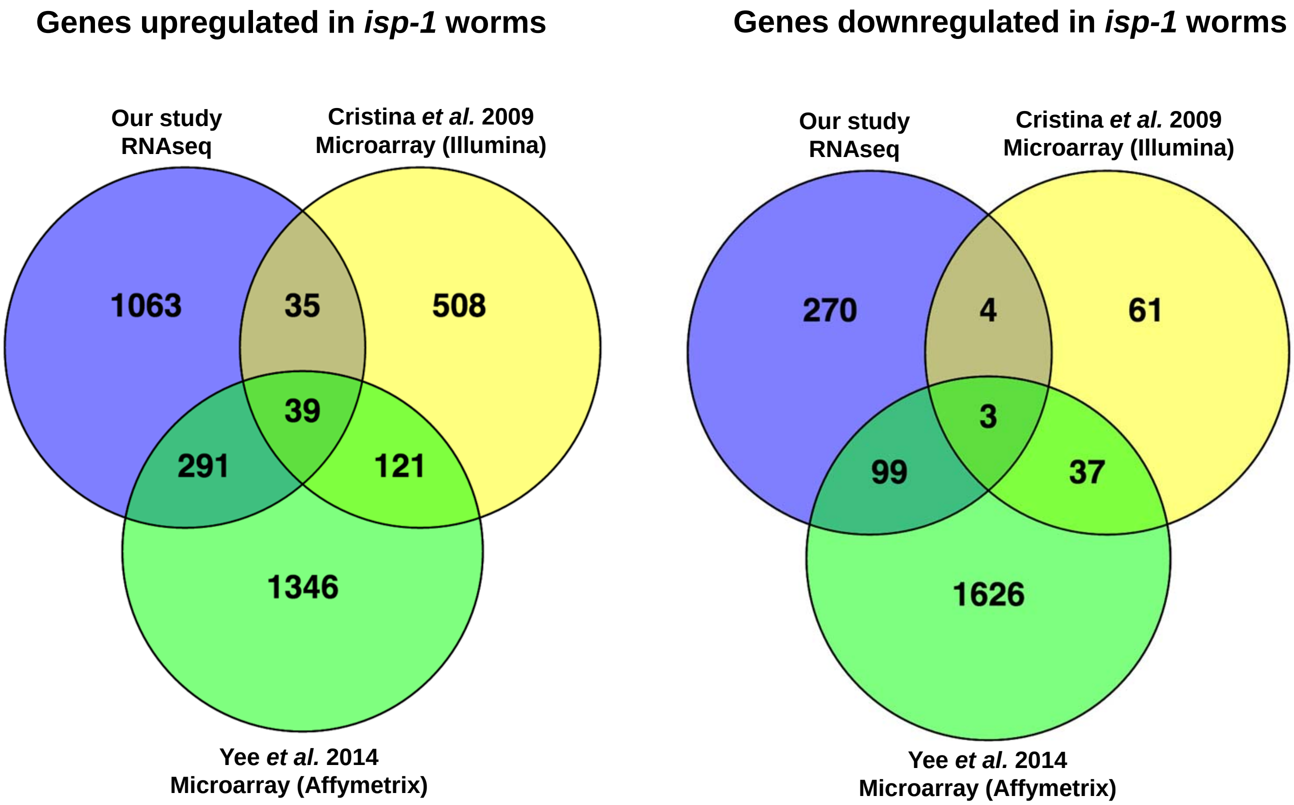
Supplementary Fig. S10. A subset of genes shows opposite expression patterns between *isp-1* worms and *isp-1;sod-3* and *isp-1;sod-5* double mutants. RNA sequencing was used to compare expression in *isp-1* worms, *isp-1;sod-3* worms and *isp-1;sod-5* worms to WT worms. Inversion of expression profiles of differentially expressed genes in *isp-1* mutants reveals genotype specific changes in transcriptional profiles relative to the mean WT expression. Genes that show an altered pattern of expression between *isp-1* worms and *isp-1;sod-3* and *isp-1;sod-5* worms may account for the differences observed in lifespan, stress resistance and physiologic rates.



Supplementary Fig. S11. KEGG enrichment of differentially expressed genes in *isp-1*, *isp-1;sod-3* and *isp-1;sod-5* worms relative to WT worms. Differentially expressed genes in *isp-1* mutants were examined for KEGG pathway enrichment. Enrichment suggests genotype common and unique metabolic changes. The “ribosome” category is upregulated in *isp-1* mutants but not *isp-1;sod-3* or *isp-1;sod-5* double mutants, while “oxidative phosphorylation” and “citric acid cycle” are downregulated in *isp-1;sod-3* and *isp-1;sod-5* mutants but not in *isp-1* mutants. Dot sizes represent the number of differentially expressed genes ($q < 0.05$) that compose that enriched process. Green and red dots are downregulated and upregulated genes, respectively.



Supplementary Fig. S12. Reactome enrichment of differentially expressed genes in *isp-1*, *isp-1;sod-3* and *isp-1;sod-5* worms relative to WT worms. Differentially expressed genes in *isp-1* mutants were examined for reactome pathway enrichment. Enrichment suggests genotype common and unique metabolic changes. Notably, *isp-1* worms exhibit upregulation of seven groups of genes relative to *isp-1;sod-3* and *isp-1;sod-5* worms. Dot sizes represent the number of differentially expressed genes ($q < 0.05$) that compose that enriched process. Green and red dots are downregulated and upregulated genes, respectively.



Genes upregulated in *isp-1* worms

Genes downregulated in *isp-1* worms

Supplementary Fig. S13. Gene expression changes in *isp-1* worms. Venn diagram of overlapping changes in gene expression with previous microarray studies.

Supplementary Table S1. Summary of Lifespan Experiments

	Mean Lifespan	Count	Significance	Max Lifespan	Significance
Combined Results					
isp-1	40.5 ± 12.8	290		63.8 ± 3.2	
isp-1;sod-3	33.0 ± 9.4	244	p < 0.001	54.6 ± 3.0	p < 0.001
isp-1;sod-5	33.8 ± 10.5	272	p < 0.001	58.3 ± 2.6	p < 0.001
Trial 1					
isp-1	42.4 ± 13.7	71		60.6 ± 4.9	
isp-1;sod-3	31.8 ± 12.1	59	p < 0.001	52.2 ± 3.9	p < 0.001
isp-1;sod-5	34.0 ± 11.3	77	p < 0.001	52.3 ± 5.0	p = 0.0015
Trial 2					
isp-1	32.9 ± 9.1	94		49.9 ± 4.7	
isp-1;sod-3	31.1 ± 9.2	59	p = 0.1323	40.4 ± 2.9	p < 0.001
isp-1;sod-5	28.7 ± 6.0	63	p < 0.001	40.1 ± 3.1	p < 0.001
Trial 3					
isp-1	43.3 ± 13.8	55		59.1 ± 1.4	
isp-1;sod-3	35.0 ± 9.4	66	p < 0.001	49.2 ± 3.9	p < 0.001
isp-1;sod-5	37.1 ± 11.5	68	p = 0.008	57.2 ± 1.7	p = 0.015
Trial 4					
isp-1	46.6 ± 10.3	70		61.1 ± 2.6	
isp-1;sod-3	33.9 ± 8.8	60	p < 0.001	47.2 ± 5.0	p < 0.001
isp-1;sod-5	35.1 ± 10.1	64	p < 0.001	51.7 ± 2.7	p < 0.001

Supplementary Table S3. Effect of deleting inducible superoxide dismutase genes on *isp-1* physiologic rates, oxidative stress sensitivity and lifespan.

		isp-1 **(vs. WT)**	***isp-1;sod-3*** **(vs *isp-1*)**	***isp-1;sod-5*** **(vs *isp-1*)**
Physiologic Rates	Post-embryonic development	↑	↑	↑
	Brood size	↓	↓	↓
	Defecation cycle length	↑	↑	↑
	Thrashing Rate	↓	↓	↓
Sensitivity to Oxidative Stress	Development: 0.3 mM Paraquat	↓	↑	↑
	Day 1 Adult: 240 uM Juglone	↑	↑	↑
	Adulthood: 4 mM PQ	↑	↓	↓
Lifespan		↑	↓	↓

Red arrows indicate phenotypic exacerbation or less healthy phenotype, while green arrows represent an improved phenotype.