Expanded View Figures

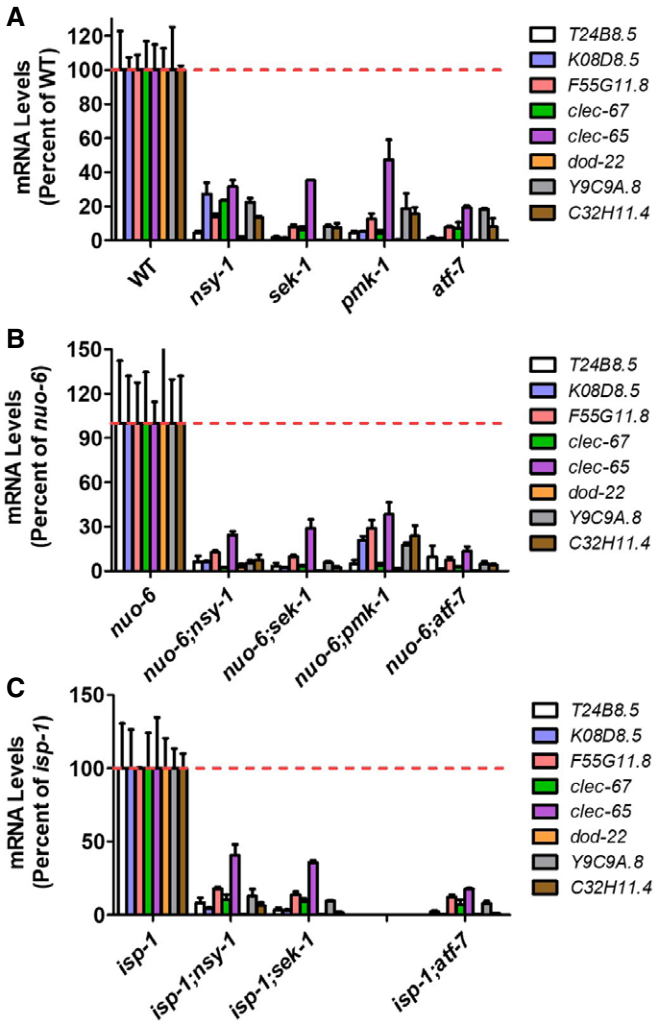

Figure EV1. Upregulation of innate immunity genes in long-lived mitochondrial mutants requires the p38-mediated innate immune signaling pathway.

A–C Mutation of genes involved in the p38-mediated innate immune signaling pathway (*nsy-1*, *sek-1*, *pmk-1*, *atf-7(gof)*) decreases the expression of genes involved in innate immunity in wild-type (A), *nuo-6* (B), and *isp-1* (C) worms. Gene expression was determined by quantitative real-time RT–PCR on three biological replicates of pre-fertile young adult worms.

Data information: Error bars indicate SEM. All differences from control are significant $P < 0.05$. Statistical significance was assessed for each gene independently using a one-way ANOVA with Dunnett's multiple comparisons test.

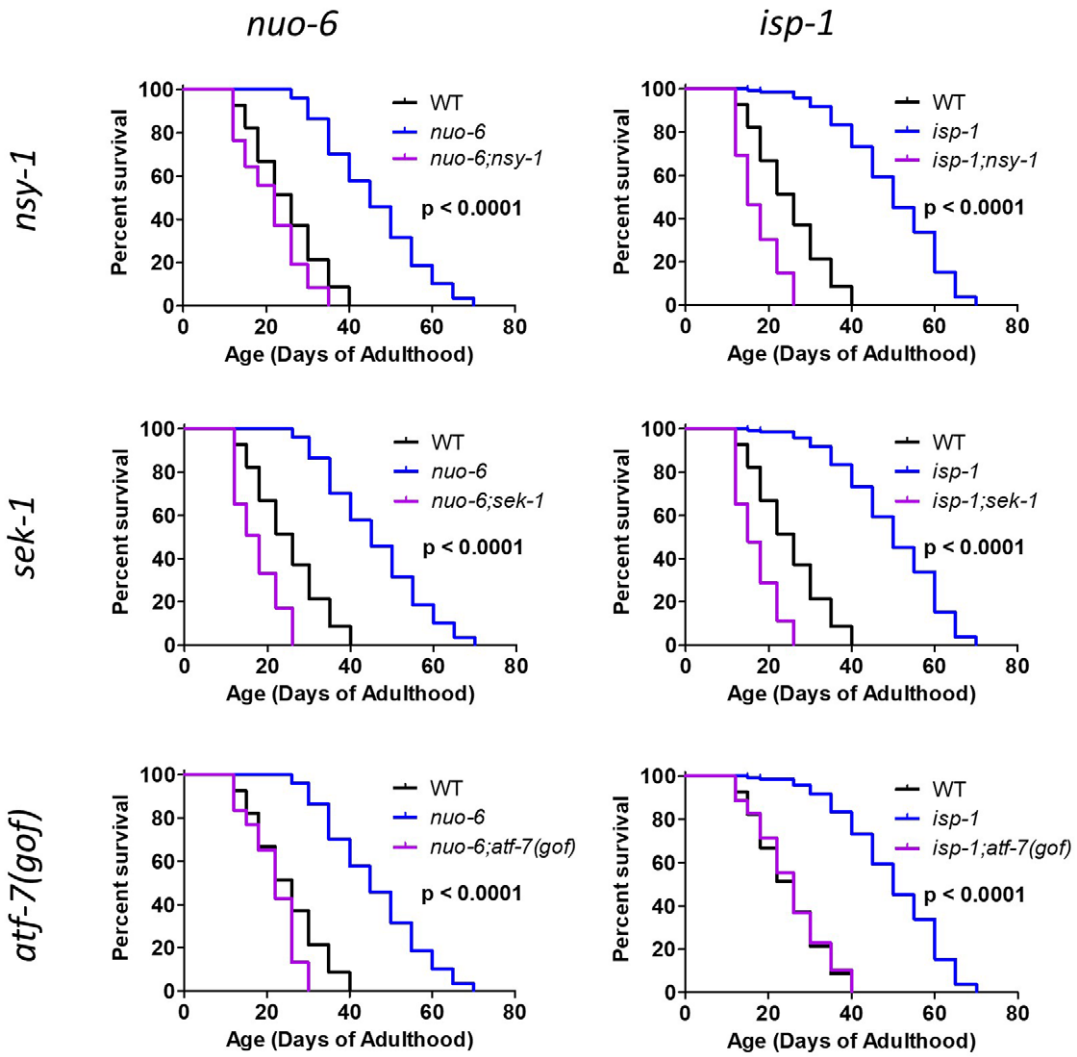

Figure EV2. Disruption of genes involved in the p38-mediated innate immune signaling pathway abolishes the extended longevity of long-lived mitochondrial mutants independently of bacterial proliferation.

Quantification of *nuo-6* and *isp-1* lifespan on non-proliferating bacteria revealed that their long lifespan is independent of bacterial proliferation. Similarly, lifespan extension in *nuo-6* and *isp-1* mutants is completely dependent on having a function p38-mediated innate immune signaling pathways as deletion of *nsy-1*, *sek-1*, or *atf-7(gof)* completely prevented the increase in lifespan in long-lived *nuo-6* and *isp-1* mutants. Lifespans were performed in liquid culture with worms fed *ad libitum*. Bacteria proliferation was prevented through treatment with cold and antibiotics. All strains were tested in a single parallel experiment. Two biological replicates per strain were measured with a total of at least 274 animals per strain.Data information: Statistical significance for survival plots was determined with the log-rank test. *P*-value indicates significance of difference between blue and purple lines. Control strains are shown in multiple panels for direct comparison.

Source data are available online for this figure.

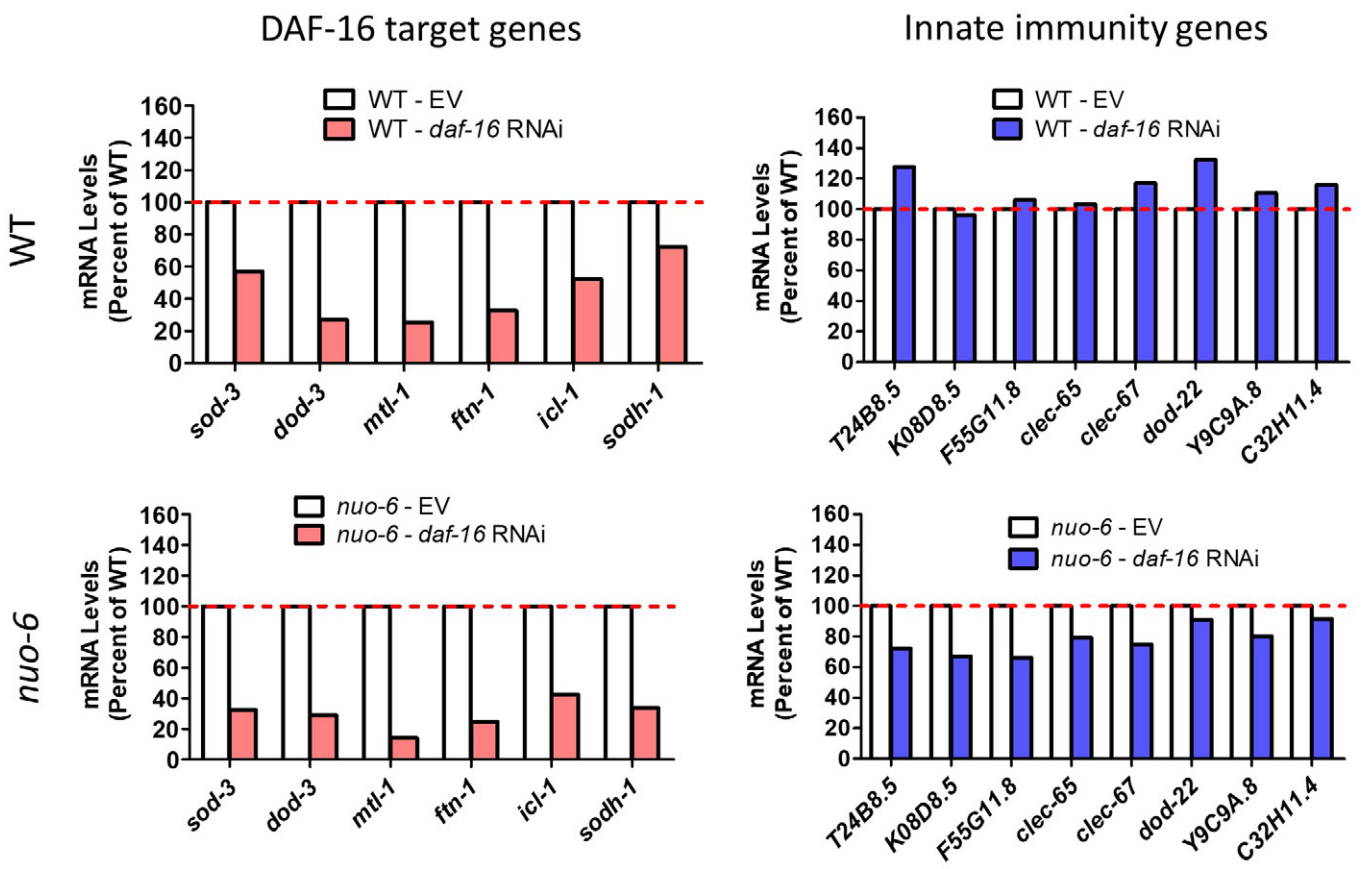

Figure EV3. DAF-16 is not required for expression of innate immune signaling pathway target genes.

daf-16 expression was knocked down using RNAi beginning at the L4 stage of the parental generation. While *daf-16* RNAi effectively decreases the expression of DAF-16 target genes (**Left;** red bars; *sod-3*, *dod-3*, *mtl-1*, *ftn-1*, *icl-1*, *sodh-1*) in both wild-type and *nuo-6* mutants, it does not markedly affect the expression of any of the innate immunity genes (**Right;** blue bars; *T24B8.5*, *K08D8.5*, *F55G11.8*, *clec-65*, *clec-67*, *dod-22*, *Y9C9A.8*, and *C32H11.4*). This suggests that DAF-16 is not required for expression of innate immune signaling pathway target genes in wild-type worms and *nuo-6* mutants. RNA was isolated from six biological replicates at the young adult stage of the experimental generation. RNA from the six biological replicates was pooled for RNA sequencing.

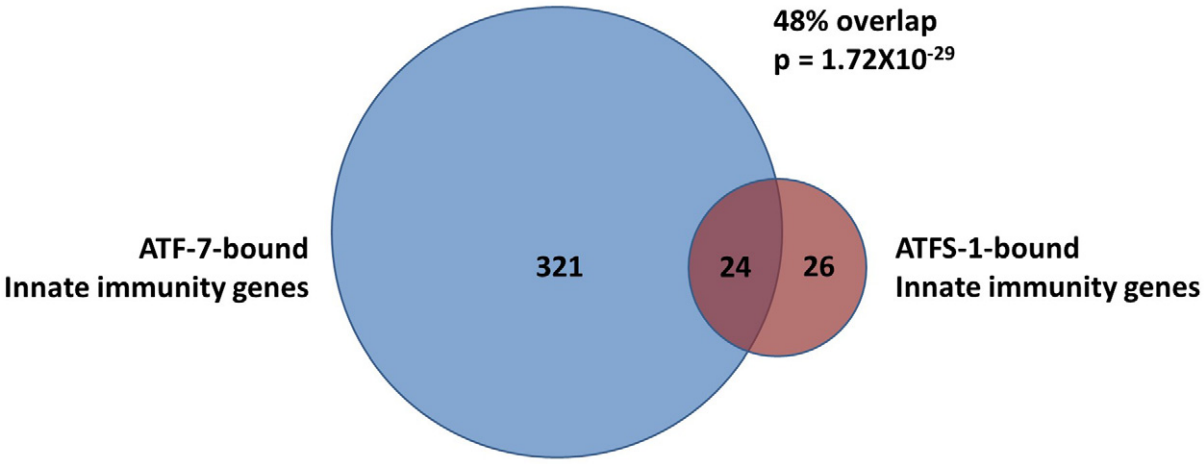

Figure EV4. ATFS-1 and ATF-7 bind to the same genes involved in innate immunity.

Innate immunity genes are defined as genes that are upregulated by a 4-h exposure to PA14. This list was obtained from Fletcher *et al* (2019b). ATF-7-bound genes are genes bound by ATF-7 after exposure to PA14 from a CHIP-seq experiment conducted by Fletcher *et al* (2019b). ATFS-1-bound genes are genes bound by ATFS-1 after mitochondrial stress resulting from RNAi against *spg-7* as determined by a CHIP-seq experiment conducted by Nargund *et al* (2015b). ATF-7 was found to be bound to 345 innate immunity genes after exposure to PA14. ATFS-1 was found to be bound to 50 innate immunity genes after exposure to mitochondrial stress. Of these 50 genes, 24 were found to be in common with ATF-7-bound innate immunity genes. This highly significant overlap ($P = 1.72 \times 10^{-29}$) clearly demonstrates that ATF-7 and ATFS-1 can bind to and regulate the same innate immunity genes. See Dataset EV2 for the complete gene lists.

Data information: The *P*-value indicates the significance of the difference between the observed number of overlapping genes between the two gene sets and the expected number of overlapping genes if the genes were picked at random.

Figure EV5. ATF-7 and ATFS-1 can bind to the promoter region of the same innate immunity genes.

To better understand how ATF-7 and ATFS-1 act to modulate the same innate immunity genes, we mapped out the regions identified by ChIP-seq studies as well as the location of the consensus binding sites. Ten of the 24 genes that show binding of both ATF-7 and ATFS-1 demonstrated binding of ATF-7 and ATFS-1 in close proximity in the promoter region of innate immunity target genes. Diagrams of seven of those genes are shown here, while diagrams of the other three genes can be found in Appendix Fig S15. The binding regions identified for ATF-7 and ATFS-1 by ChIP-seq experiments are shown by red and blue bars, respectively. The consensus binding sites for ATF-7 and ATFS-1 are indicated by yellow triangles and orange triangles, respectively. The distance between dotted lines is 1 kb. Exons are indicated by dark blue bars. Untranslated regions are indicated by gray bars, and introns are indicated by black lines joining together two exons. Note that not all transcripts for each gene are illustrated, only those with differing promoter regions and or markedly different structures.

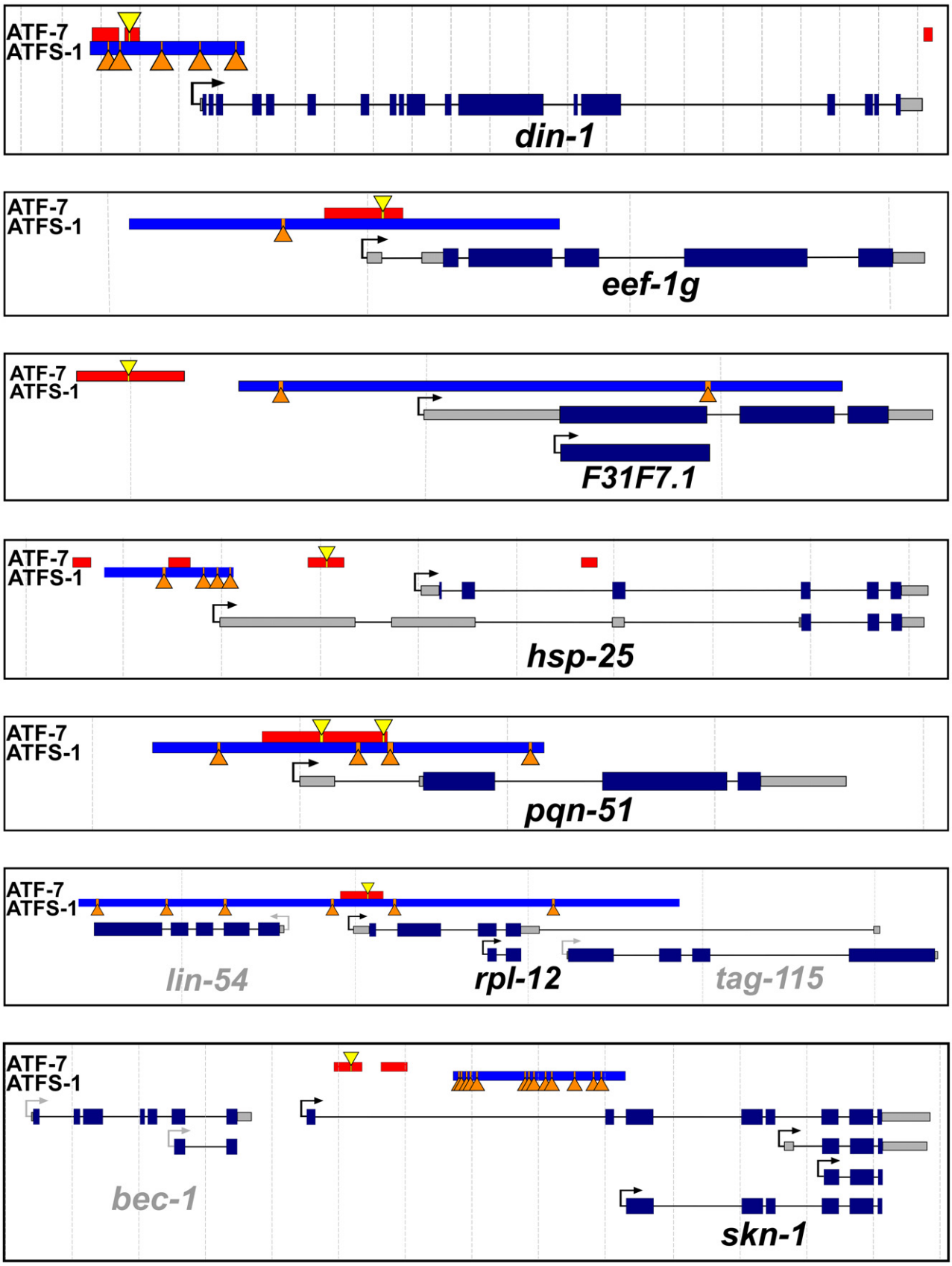

Figure EV5.

