Published in final edited form as:
Curr Biol. 2007 October 9; 17(19): 1635–1645. doi:10.1016/j.cub.2007.08.058.

The *C. elegans* TGF-β Dauer Pathway Regulates Longevity via Insulin Signaling

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Summary

Background—Previous genetic evidence suggested that the *C. elegans* TGF-β Dauer pathway is responsible solely for regulation of dauer formation, with no role in longevity regulation, while the Insulin/IGF-1 signaling (IIS) pathway regulates both dauer formation and longevity.

Results—We have uncovered a significant longevity-regulating activity by the TGF-β Dauer pathway that is masked by an egg-laying (Egl) phenotype; mutants in the pathway display up to two-fold increases in lifespan. The expression profiles of adult TGF-β mutants overlap significantly with IIS pathway profiles: adult TGF-β mutants regulate the transcription of many DAF-16-regulated genes, including genes that regulate lifespan; the two pathways share enriched Gene Ontology categories; and a motif previously associated with DAF-16-regulated transcription (the DAE, or DAF-16-Associated Element) is highly overrepresented in the promoters of TGF-β regulated genes. The TGF-β Dauer pathway's regulation of longevity appears to be mediated at least in part through insulin interactions with the IIS pathway and the regulation of DAF-16 localization.

Conclusions—Together, our results suggest there are TGF-β-specific downstream targets and functions, but that the TGF-β and IIS pathways may be more tightly linked in the regulation of longevity than has been previously appreciated.

Introduction

In times of environmental stress, crowded conditions, and limited food, juvenile *C. elegans* develop into an alternative larval state called dauer that is highly stress-resistant and long-lived [1]. The decision to enter the dauer state is made during the first larval stage, and is regulated by a complex branched pathway of more than 30 *daf* (dauer formation) genes [1]. Genetic epistasis tests suggested that components of one of the two canonical TGF-β signaling pathways (the TGF-β-like ligand DAF-7, the Type 1 and 2 receptors DAF-1 and DAF-4, and the downstream DAF-3 Smad and DAF-5 Sno/Ski) make up one branch of the dauer regulation pathway, while Insulin/IGF-1 Signaling (IIS) pathway genes, including the Insulin receptor DAF-2 and the FOXO transcription factor DAF-16, occupy a separate downstream branch regulating both dauer formation and longevity [1–3]. The TGF-β dauer pathway has not been previously implicated in the regulation of longevity.

Transcriptional analyses have been carried out on wild-type dauer larvae [4], TGF-β mutant dauer larvae [5], and adult IIS pathway mutants [6–8]. However, the transcriptional profiles of TGF-β Dauer pathway mutant adults have not yet been examined; this is important in order to temporally separate developmental and adult targets. We hypothesized that the transcriptional targets of these pathways likely share dauer-specific targets [5, 8], but should

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diverge significantly in adults if longevity determination is a primary output solely of the IIS pathway.

Here, we have examined the transcriptional output from the adult TGF-β pathway and compared it to the profiles of TGF-β dauer animals and adult IIS pathway mutants. Surprisingly, the adult TGF-β profiles correlated well with the IIS pathway profile, suggesting that we should re-examine the longevity phenotype of the TGF-β mutants and the relationship between the TGF-β and Insulin/IGF-1 Signaling pathways.

Results

The Adult TGF-β Dauer pathway shares transcriptional outputs and Gene Ontology categories with the Insulin-IGF-1 Receptor/FOXO pathway

To identify the downstream targets of the TGF-β pathway in adulthood, we compared the dauer-constitutive mutants *daf-7(e1372)*, *daf-7(m62)*, and *daf-1(m40)* with dauer-defective mutants *daf-3(mgDf90)*, *daf-5(e1386)*, and *daf-7(e1372);daf-3(mgDf90)* double mutants at the permissive temperature, 20°C, on the first day of adulthood (see Fig. 1 and Exp. Proc. for hybridization details). Because *daf-3* and *daf-5* are epistatic to *daf-7* and *daf-1*, these comparisons should identify targets that act downstream of this linear pathway. We compared these profiles with expression data from Liu et al [5] of dauer-stage *daf-7(e1372)*, *daf-8(e1393)*, and *daf-14(m77)* mutants vs wild type L2/L3. Surprisingly, the Pearson correlation between the transcriptional outputs of the TGF-β dauer and adult pathways is low (0.004; Fig. 1a), suggesting that the downstream targets of this pathway vary significantly from dauers to adults.

We used SAM [9], which provides an estimate of false-discovery rate for multiple testing, to identify genes consistently and significantly changed in TGF-β adults (Supp. Table 2, Supp. Fig. 2) and compared them with the Liu, *et al*. dauer data [5]. As the Pearson correlation suggested, very few genes are commonly regulated (Supp. Table 1); in fact, only 14% of the upregulated and 37% of the down-regulated genes are shared between dauer and adult TGF-β pathway mutants. By contrast, the IIS pathway and the adult TGF-β pathway share significant similarity in transcriptional output, as demonstrated by a Pearson correlation of 0.35, almost 90-fold higher than the adult/dauer correlation (Fig. 1b, Supp. Fig. 1). We compared the SAM-determined significantly-regulated genes in the Insulin-IGF-1 Signaling (IIS) pathway [6], and found that 55% of the genes significantly up-regulated by the IIS pathway and 66% of the down-regulated genes are also regulated by the adult TGF-β pathway in the same direction. The transcriptional profiles of adult TGF-β and IIS pathway mutants show that these pathways share many targets in adulthood (Supp. Fig. 3).

To compare the biological roles that the genes regulated by each of these pathways might play, we submitted the lists of significantly up- and down-regulated genes from both the adult TGF-β SAM analysis and from the TGF-β dauer arrays [5] to DAVID [10] for Gene Ontology (GO) analysis. The TGF-β adults and TGF-β dauers' most-enriched GO categories were then compared (Fig. 2; note that because GO terms are not independent, a statistical assessment of the degree of overlap is not possible, thus, we have graphically compared sets of enriched GO terms). Some categories are shared between the dauer and adult upregulated (Fig. 2a, 9 of 37) and downregulated (Fig. 2b, 14 of 47) sets. However, the majority of the top-enriched categories from the up-(28/37) and downregulated (33/47) gene sets are specific for either the adult or the dauer-stage TGF-β worms.

Comparison of the GO categories most highly enriched by the IIS and adult TGF-β pathways reveals that several of the highest-scoring GO categories in both the up- and down-regulated sets overlap: 16 of the 41 upregulated categories (Fig. 2a) and 16 of the 38

downregulated categories (Fig. 2b) are shared between the TGF-β adult and IIS profiles, which exceeds the overlap between TGF-β adult and dauer profiles. Thus, by Pearson correlation of whole transcriptome, percentage of significantly-regulated genes, and GO categories, the adult TGF-β pathway appears to have much more in common with the adult IIS pathway than might have been expected. Many of the GO categories that are known to function in longevity regulation (Fig. 2a, asterisks) [6] were enriched in the TGF-β mutant adults.

Strikingly, many of the "*dod*" genes (downstream of DAF-16) that are responsible for *daf-2* insulin receptor mutants' long life [6] are highly regulated by the TGF-β pathway, in the same direction as in the IIS pathway [6]. Specifically, *sod-3, mtl-1, dod-3, acdh-1/dod-12, sodh-1/dod-11, lys-7, dao-3, gei-7, dod-4/aqp-1, dod-9/acs-17, ges-1, dod-7/asah-1, gcp-1, dod-6, ins-18, fat-7, mdl-1, spp-1, dod-24, dod-22*, and *dod-18*, which had all been previously identified as lifespan-regulating *dod* genes [6], were notable because of their strong expression in TGF-β adults (Supp. Fig. 3b).

The DAF-16 Associated Element (DAE) is enriched in the promoters of Adult TGF-β Dauer pathway targets

We then looked for clusters [11] of genes that are up- or down-regulated and shared or distinct across the strains (Fig. 3, Supp. Table 1), similar to the approach previously used to find longevity genes regulated by *daf-2* and *daf-16* [6]. These comparisons allowed us to identify TGF-β-regulated genes that are specific to and shared in dauer and adulthood. We submitted the promoter sequences from the cluster gene lists to two complementary motif-finding algorithms, BioProspector [12] and Weeder [13], to identify overrepresented promoter sequences (Fig. 3). The yeast-one-hybrid-identified DAF-3 binding sequence, GTCTG, which may direct pharyngeal gene expression during larval development [14] was not apparent in any of the high-scoring motifs. By contrast, the DAE (DAF-16 associated element, CTTATCA), a GATA-like motif that was first identified as an overrepresented sequence in the promoters of genes regulated by DAF-16 [6], and variations of the DAE were the most common sequences in the promoters of these genes. While the presence of the DAE in the promoters of genes regulated by both the IIS and adult TGF-β pathway could be explained by the presence of the IIS genes, the DAE was also strongly associated with Cluster 1 (Fig. 3), which is regulated specifically by the TGF-β pathway. Several novel motifs were also associated with specific clusters.

TGF-β Dauer Pathway Mutants Regulate Longevity

The striking regulation of insulin transcriptional targets previously demonstrated to regulate lifespan [6] by adult TGF-β mutants (Supp. Fig. 3b) suggested that the TGF-β pathway might also regulate lifespan, despite previously-published longevity measurements [2, 3]. To test this hypothesis, we measured the lifespans of ten different TGF-β Dauer pathway mutant alleles. Because these mutants are known to have severe egg-laying (Egl) defects [3, 15], we were concerned that matricide (progeny hatching within the mother, or "bagging"), might cause premature death. In fact, we found that *daf-7* animals display high rates of bagging very early in adulthood compared with wild type (Fig. 4a). Therefore, we used the chemical 5-fluorodeoxyuridine (FUdR), an inhibitor of DNA synthesis, to prevent progeny development [16]. FUdR treatment itself has minimal effects on wild type lifespan [16] (Table 1, Supp. Tables 4–6).

Surprisingly, most mutants in the Dauer pathway extended lifespan substantially and consistently: we observed a significant increase in lifespan in *daf-7, daf-1, daf-4, daf-8*, and *daf-14* mutants relative to wild type worms under the same conditions (Fig. 4b–d; Table 1). In fact, *daf-4(e1364)* mutants lived more than twice as long as wild type in one trial (Fig. 4c,

Table 1). Meanwhile, negative regulators of the dauer pathway (*bra-1*, *daf-5*, and *daf-3*) displayed significant shortening of lifespan relative to wild type (Fig. 4d; Table 1). The long lifespan of *daf-7* mutants was suppressed by *daf-3* mutations (Fig. 4e, Supp. Table 4), similar to *daf-3's* suppression of *daf-7*-mediated dauer formation.

Regulation of Lifespan by the TGF-β Dauer pathway requires DAF-16 activity

Previous studies suggested that the TGF-β and IIS pathways function independently [2, 3], although *daf-16* mutations have been reported to partially suppress dauer formation of TGF-β mutants [3]. To investigate the interaction between the two pathways in longevity regulation, we measured the lifespan of the *daf-16(mu86);daf-7(e1372)* double mutant. We found that loss of *daf-16* activity completely suppressed the longevity phenotype of *daf-7(−)* mutants or RNAi (Fig. 4f, Supp. Table 5). Furthermore, loss of *daf-16* suppressed the slight thermotolerance displayed by *daf-7* (Fig. 4h). By contrast, loss of *daf-3* was not able to suppress the lifespan extension caused by *daf-2* mutations or RNAi (Fig. 4g, Supp. Table 6), suggesting unidirectional communication from the TGF-β Dauer pathway to the IIS/FOXO pathway in longevity regulation.

The TGF-β Dauer pathway acts in adulthood to regulate longevity

Reduction of Insulin/IGF-1 (IIS) pathway signaling during adulthood is sufficient to increase longevity [17], but the dauer decision is made in the L1 larval stage [1]. To determine when TGF-β pathway activity affects lifespan, we carried out a series of temporal temperature-shift and double-stranded RNA interference experiments. When we raised *daf-7(e1372)ts* or *daf-7(m62)ts* animals at 20°C and then shifted them to 15°C as young adults, no lifespan extension was observed (Fig. 5b). By contrast, when these worms were raised at 15°C and then shifted to 20°C as L4/ young adults, lifespan was increased significantly ($p < 0.0001$) (Fig. 5c). Furthermore, when wild type worms were treated with *daf-7* RNAi only in adulthood, lifespan was also increased ($p < 0.0001$) (Fig. 5d). Thus, like the insulin pathway, the TGF-β pathway acts during adulthood rather than in larval stages to regulate longevity, suggesting that this activity is separable from the dauer decision- and formation-requiring *daf-7* activity in larval stages.

The TGF-β Dauer pathway regulates DAF-16 localization and DAF-16 target gene transcription

The suppression of *daf-7* mutants' extended lifespan by *daf-16* mutations suggests that the TGF-β Dauer pathway regulates IIS pathway activity in adults. When *daf-2* insulin receptor signaling is abrogated, DAF-16/FOXO becomes nuclearly localized [18, 19], and mutations in *daf-18*, the PTEN phosphatase that opposes insulin/PI-3-kinase signaling, promote cytoplasmic retention of DAF-16 [18]. *daf-7(m62)* dauer animals were previously shown to nuclearly localize DAF-16 during the L2d pre-dauer state [20]. We found that DAF-16::GFP was excluded from nuclei in many adult *daf-3* mutants (Fig. 6a–c) and with *daf-3* RNAi, similar to *daf-18* mutants [18]. *daf-7(e1372);daf-16::gfp* adults were also heterogeneous, but generally shifted DAF-16::GFP localization from diffuse to nuclearly localized (Fig. 6a–c). These results suggest that the TGF-β Dauer pathway may act through an insulin-signaling-like regulation of DAF-16 localization.

sod-3 (superoxide dismutase) is a direct transcriptional target of DAF-16 [21, 22], and the expression of a *Psod-3::gfp* reporter is increased broadly in *daf-2* mutants [23]. Our microarray results suggested that like the IIS pathway, TGF-β Dauer mutants regulate the transcription of *sod-3* (Fig. 3c). We examined *daf-7(e1372);Psod-3::gfp* mutants and found that fluorescence was increased in intestines, hypodermis, and cuticle, although this pattern was weaker and more heterogeneous than in *daf-2* mutants. *Psod-3::gfp* expression was not noticeably altered below wild type levels in *daf-3(mgDf90)* mutants (Fig. 6d), where

expression is already low and restricted to the pharynx and tail. *daf-3* RNAi did not have a notable effect on the high *Psod-3::gfp* expression in *daf-2* mutants, correlating with its lack of *daf-2* longevity suppression. Together these results suggest that the *daf-2/daf-16* pathway acts downstream of *daf-7* and *daf-3*.

Discussion

The TGF-β pathway regulates longevity through Insulin/IGF-1 Signaling

Our transcriptional analyses of adult TGF-β mutants have identified a number of adult transcriptional targets, functional categories, and regulatory motifs [5][6] that are shared between the TGF-β and IIS pathways, including many *daf-2/daf-16* target genes demonstrated to regulate longevity [6]. These transcriptional results directly suggested the possibility that TGF-β mutants might live long. Supportive of this, we found that TGF-β mutants are indeed long-lived, that the TGF-β pathway acts during adulthood to regulate lifespan, and that this regulation depends on the FOXO transcription factor DAF-16. Together, our results suggest that the TGF-β and Insulin/IGF-1 pathways use similar transcriptionally-regulated mechanisms to survive, and that these pathways are more closely linked than previously appreciated.

One interesting difference between dauer and longevity regulation is that loss of *daf-16* is only able to partially suppress *daf-7* dauer formation [3], but completely suppresses the lifespan extension of *daf-7*, which is weaker than *daf-2*'s longevity effect. This may indicate a greater role by DAF-3 in dauer formation and a more specific role for DAF-16 in longevity regulation. Nevertheless, DAF-16 may play at least a partial role in the regulation of *daf-7* dauer transcriptional targets and the partial suppression of dauer formation.

Lee, *et al.* also noted *daf-7* regulation of DAF-16::GFP in L2d animals [20], and suggested that an insulin might mediate the interaction between the TGF-β and IIS/FOXO pathways, while Liu, *et al.* found several insulins regulated by TGF-β mutants in dauer [5]. The regulation of an insulin-like peptide gene or genes by DAF-3 in adulthood (Fig. 6e, Supp. Fig. 3) would provide a powerful mechanism for the activation of the IIS pathway and the coordination of the two pathways, similar to the proposed coordination of the IIS pathway in the whole animal through *ins-7* signaling [6]. An insulin-based mechanism might allow the coordination of the TGF-β and IIS pathways throughout the animal, reinforcing the dauer decision during larval stages and longevity regulation in adults.

Matricide may significantly mask longevity phenotypes of TGF-β Dauer mutants

Our finding that the TGF-β dauer pathway can regulate lifespan through its interaction with the Insulin/IGF-1 pathway suggests that dauer survival and longevity regulation may be even more closely linked than earlier genetic studies had indicated. While it is surprising that such significant lifespan increases had not been previously reported for any of the mutants in this pathway, it should be noted that some studies had not prevented progeny production to reduce matricide. In other studies, *fer* mutations were used to confer partial sterility (due to the loss of sperm progeny production); however, we have observed that *fer* mutations may also have a slightly deleterious effect on lifespan in TGF-β mutant backgrounds (Supp. Fig. 4). Previous studies also measured lifespan at 25.5°C, which may have caused more severe deleterious effects, either from TGF-β dauer mutant or *fer* pleiotropies, than the temperature we have used here (20°C). Finally, complete deletion of bagged animals from the entire lifespan, rather than standard survival analysis censoring (in which the animal contributes to the "live" and total population until the point at which it is removed), was used in the calculation of lifespan in one study [2], and the second did not censor for bagging events after day 7 [3], when we continue to observe matricide (Fig. 4a).

It is also not surprising that RNAi longevity screens did not pick up these genes, despite the use of sterile strains [24–27], because the *daf-7* RNAi clone appears to act more weakly than does the mutant allele and might not have met the maximum longevity requirement of these screens (W. Shaw and C. T. Murphy, unpublished). Interestingly, another study did identify *daf-7* and *daf-1* in tests of L1 starvation-induced increased longevity [28], and noted their increased thermotolerance as well, but discounted them for further adult longevity tests based on previously-published lifespan reports. In general, it is possible that matricide contributes to deaths more frequently than is currently appreciated, and the suppression of matricide may reveal additional longevity regulators.

Implications for higher organisms

The conservation of TGF-β and insulin signaling pathways between *C. elegans* and mammals is significant. It is possible that more interaction between these pathways than has been previously suspected may also exist in higher organisms, and may affect the survival of specific tissues and ultimately, longevity of these animals.

Experimental Procedures

C. elegans genetics

All strains were cultured at 20°C unless otherwise noted using standard methods [29]. Strain and allele information listed in Supp. Data.

RNA preparation and Microarray hybridization

Standard RNA purification, cRNA generation, labeling, and hybridization on Agilent 4 × 44K *C. elegans* arrays were performed (Supp. Data). 15 replicates of TGF-β adults were hybridized (4 × *daf-1 v daf-5* (2 dye-flipped), 10 × *daf-7(e1372) v daf-3(mgDf90)* or *daf-7(e1372);daf-3(mgDf90)* (4 dye-flipped), 1 × *daf-7(m62) v daf-7(e1372);daf-39(mgDf90)*. Nine replicates of *daf-2(e1370) v daf-16(mu86);daf-2(e1370)* (4 dye-flips) were hybridized.

Microarray Analysis

Data was loaded onto the Princeton University MicroArray database (PUMA, puma.princeton.edu), filtered for array and spot quality, and replicate spots were collapsed to an average value. One-class analysis in SAM [9] was performed with no fold-expression cutoff. Hierarchical clustering by gene and arrays of $\log_2$ expression rations was done after filtering for genes that were present in 80% of the arrays (uncentered correlation, average linking) in Cluster and displayed in TreeView [11].

Gene Ontology analysis

Up-and down-regulated genes identified in the SAM analyses were converted to SwissProt ID and TrEMBLE ID's in wormbase and submitted to DAVID for conversion to DAVID ID's for functional annotation clustering (>70% converted). Molecular/biological functional annotation was assigned to the upregulated and downregulated genes using the default *C. elegans* Gene Ontology criteria in DAVID (http://david.abcc.ncifcrf.gov/), and Enrichment Score (the −log of the Fisher Exact test p-value) was determined [10]. All categories with an enrichment score >1 or the top 30 GO categories are shown (Fig. 2).

Motif analysis

1.5 kb upstream sequences (WormBase Release WS170) of were submitted to two complementary algorithms, BioProspector [12] (a Gibbs sampling-based algorithm) and Weeder [13] (a consensus-based algorithm), to identify overrepresented sequences. p-values

for BioProspector motifs were generated by performing thirty-five Monte Carlo simulations to estimate the null motif score distribution [12]; Weeder's p-value calculator was used for motif significance. Motifs were displayed in WebLogo [30].

Survival analysis

Standard Kaplan-Meier survival analysis (1st day of adulthood as t=0, 20°C unless otherwise indicated, n>60, 50 μM FUdR) was performed (Supp Data).

RNAi

Bacterial feeding RNAi experiments were carried out as described previously [32][17] after verification by PCR and sequencing, on 0.1 M IPTG.

Thermotolerance assay

n> 60 Day 1 adult worms (treated with FUdR from L4) were shifted from 20°C to 35°C at t=0, and time points were taken every 2 hours after 3 hours of incubation at 35°C; standard Kaplan-Meier survival analysis was performed.

DAF-16::GFP localization assay

Day 1 adult worms were scored (3 assays) for nuclear, cytoplasmic, or diffuse GFP localization, as in [33], and plotted as a percentage of the total, with SEP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Center for *C. elegans* Genetics for strains, John Matese and Laurie Kramer for assistance with PUMA, Hani Goodarzi, Max Jan and Kieran Dilks for technical assistance, Zemer Gitai for comments on the manuscript, and Chad Myers for discussion. WMS and CTM designed the experiments, WMS, SL, JA, and CTM carried out the experiments, JL performed motif analyses, and WMS, SL, JL, and CTM wrote the manuscript. WMS is a fellow of the New Jersey Commission on Cancer Research. CTM is an Alfred P. Sloan Fellow and a Pew Scholar in the Biomedical Sciences.

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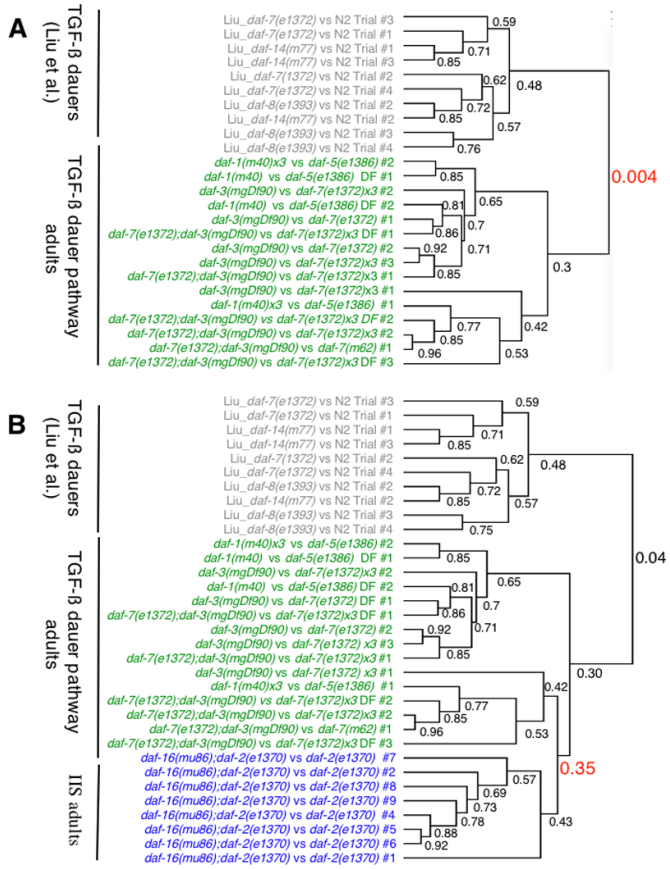

Fig. 1. Hierarchical clustering and Correlations between the TGF-β dauer and Insulin/IGF-1 Signaling pathways

(A) TGF-β dauer stage (Liu, *et al.*) and TGF-β adult profiles (Pearson correlation = 0.004); (B) TGF-β adults and IIS adults (8 IIS arrays), Pearson corr. = 0.35. Note: TGF-β adults and IIS adults Pearson correlations remained high with six IIS arrays (0.42; as in Supp. Fig. 1), when only the adult profiles for the two pathways were compared (0.348), and when all of the TGF-β arrays are forced into a single clade and compared with the IIS clade (0.32).

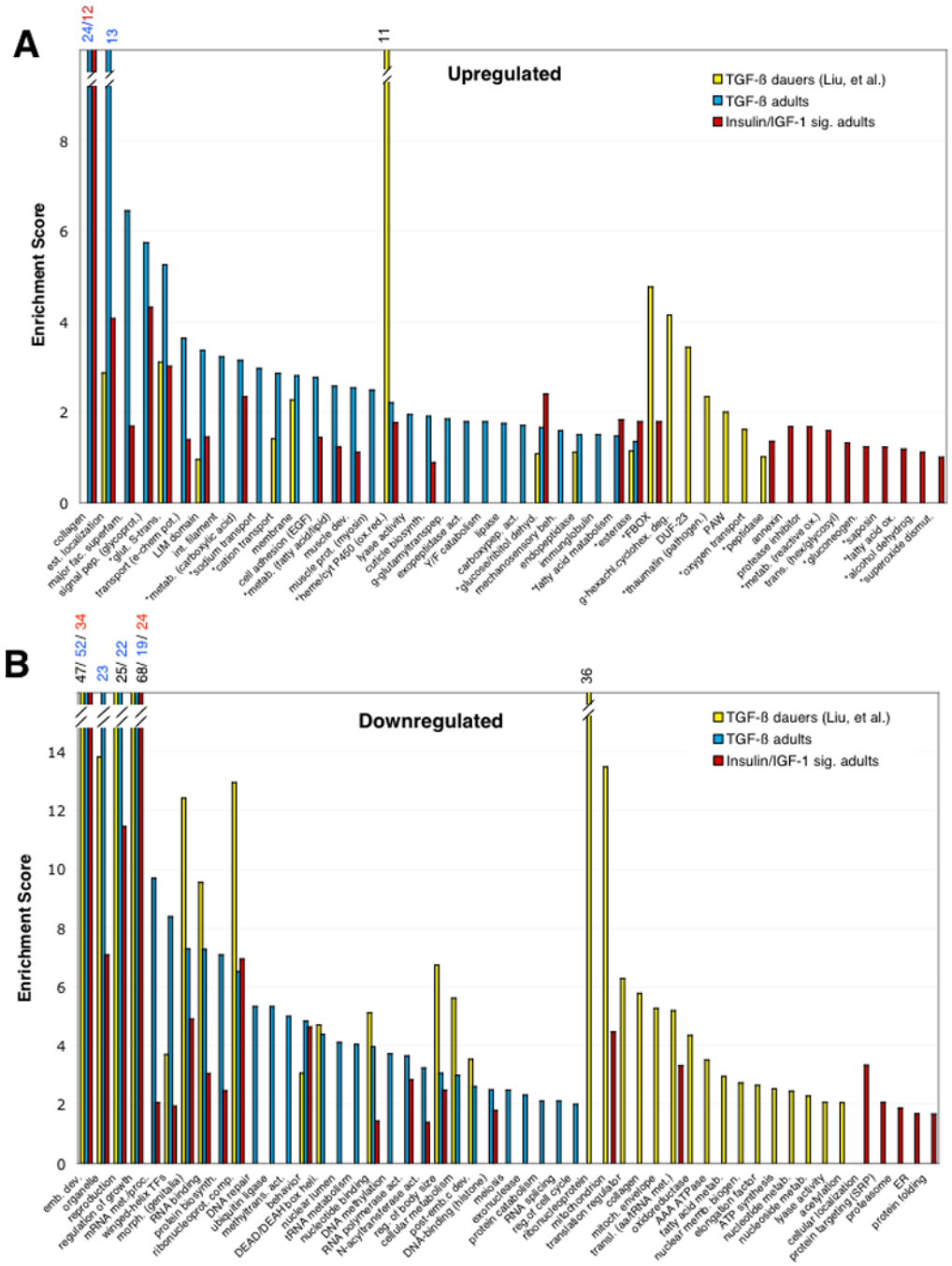

Fig. 2. Gene Ontology analysis of TGF-β targets in dauer and adults

SAM-determined significantly-changed genes in TGF-β dauers (Liu, *et al.*), TGF-β dauer pathway adults (Supp. Table. 2), and IIS mutants were submitted for GO analysis. (A) GO categories of genes upregulated by TGF-β dauers (1381), TGF-β adults (2181 genes), and IIS mutants (1390); (B) GO categories of genes downregulated by TGF-β dauers (2725), TGF-β adults (top 3000 genes), and IIS mutants (1054). Asterisks indicate GO categories that are known to function in longevity regulation.

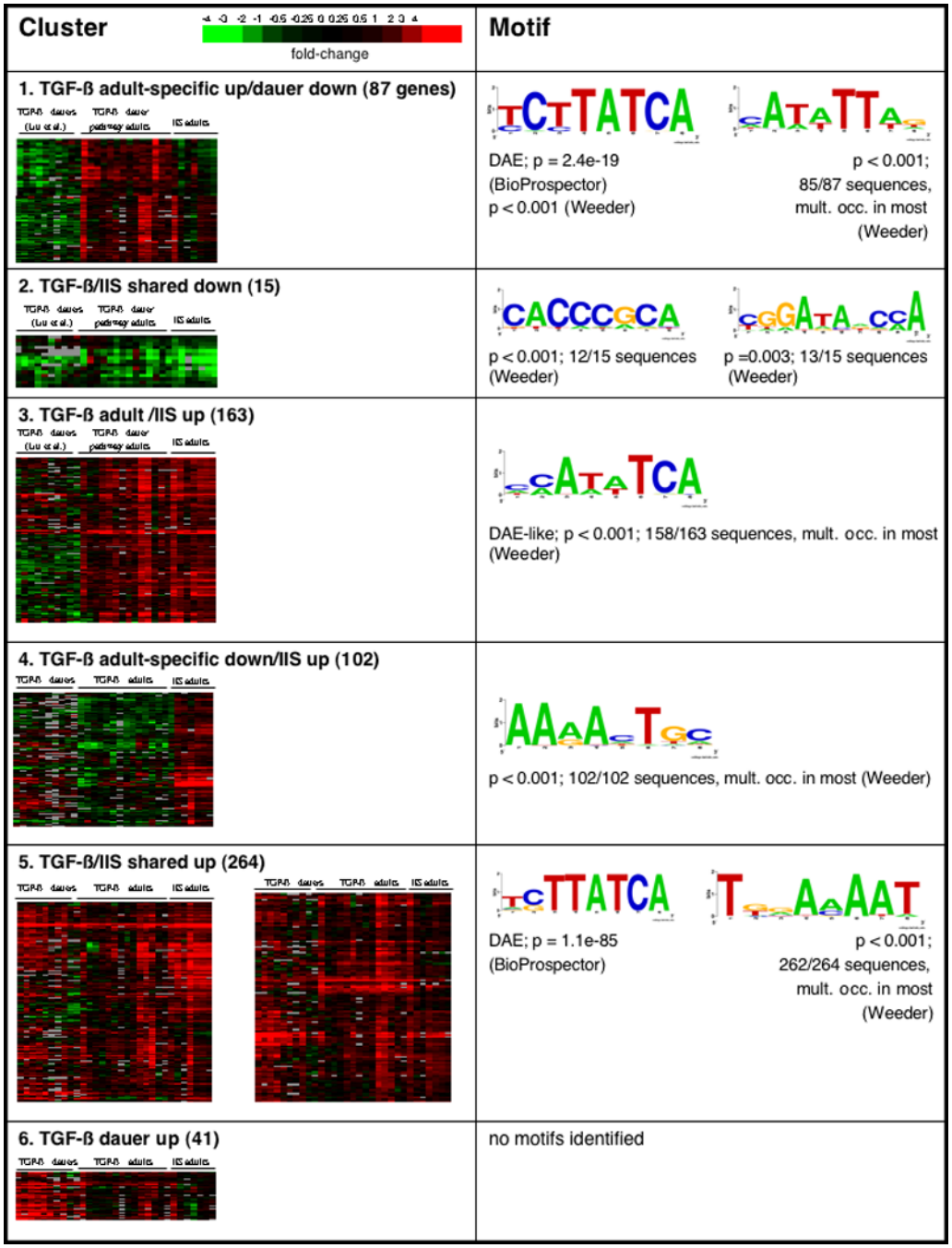

Fig. 3. Transcriptional targets of TGF-β dauer stage, TGF-β adults, and IIS adults with WebLogos of associated motifs

1) TGF-β adult-specific up/dauer downregulated targets; 2) TGF-β/IIS shared downregulated targets; 3) TGF-β adult/IIS shared upregulated; 4) TGF-β adult-specific down/IIS upregulated; 5) TGF-β/IIS shared upregulated; and 6) TGF-β dauer stage upregulated targets. Arrays deposited into Princeton University MicroArray database (PUMA, puma.princeton.edu). Arrays are log2 expression ratios as indicated on scale bar. 1.5 kb of promoter sequences from genes in each cluster were submitted to BioProspector [12] and Weeder [12] to identify overrepresented motifs; high-scoring motifs are depicted by WebLogo [31].

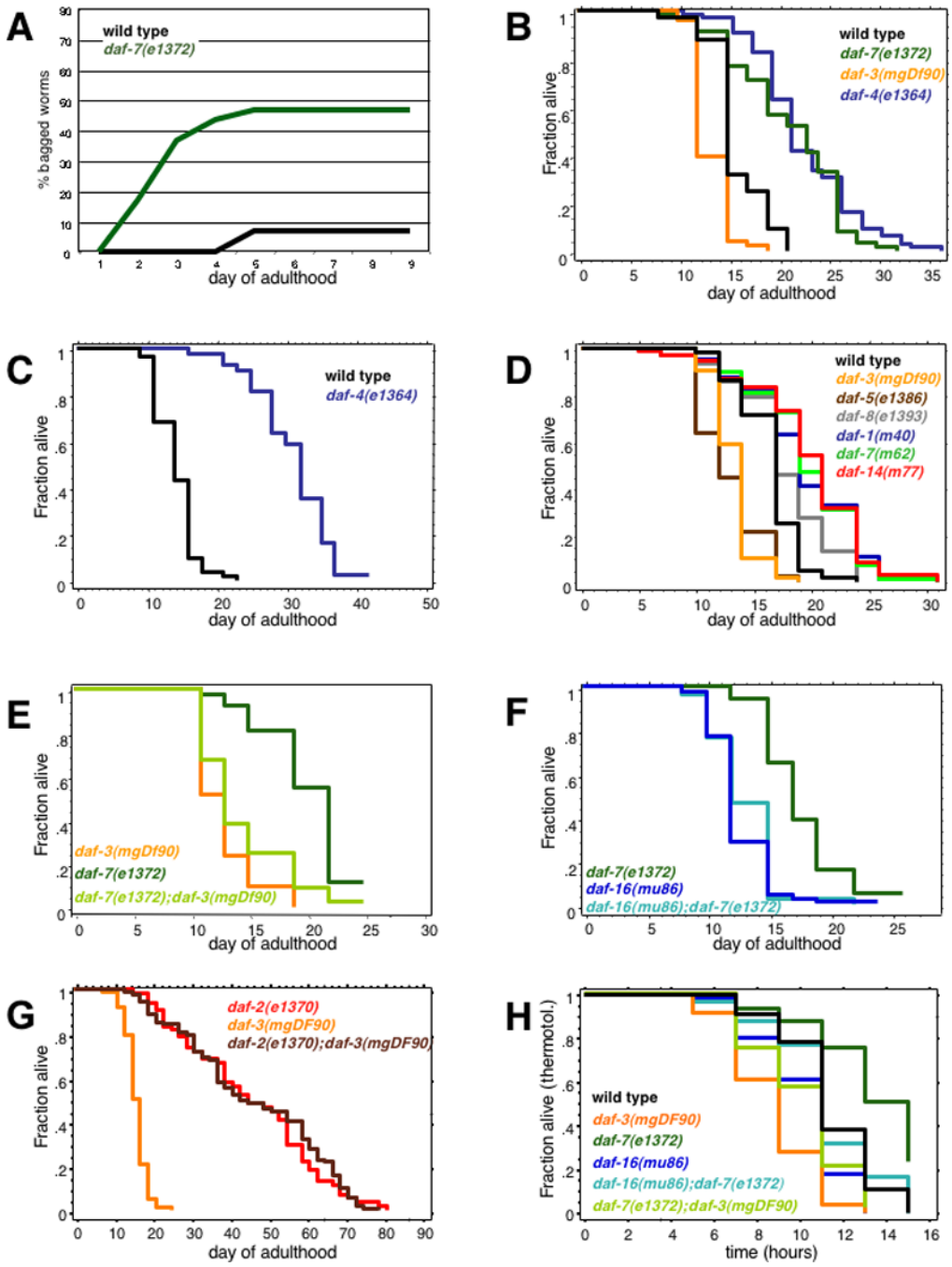

Fig. 4. Longevity and thermotolerance are regulated by TGF-β Dauer pathway signaling in a DAF-3- and DAF-16-dependent manner (see Supp. Data for expanded legend including lifespan data)

(A) *daf-7(e1372)* matricide rates (20°C, no FUdR). (B–G) Lifespans of hermaphrodites treated with 50 μM FUdR during early adulthood to prevent progeny development. (B, C, D) *Daf-c* mutants (*daf-7*, *daf-4*, *daf-8*, *daf-1*, and *daf-14*) are long-lived, while *Daf-d* mutants (*daf-3* and *daf-5*) are short-lived. (E, F) *daf-7(e1372)* mutant lifespan extension is dependent on both *daf-3* (E, 8 replicates, Supp. Table 4) and *daf-16* activity (F, 6 replicates, Supp. Table 5). (G) *daf-2* longevity is independent of *daf-3* (7 replicates, Supp Table 6). (H)

TGF-β mutants are thermotolerant (35°C) in a *daf-3*-, *daf-16*-dependent manner. (*daf-2(e1370)* was very thermotolerant in this assay, with a mean of 21.2 hours.)

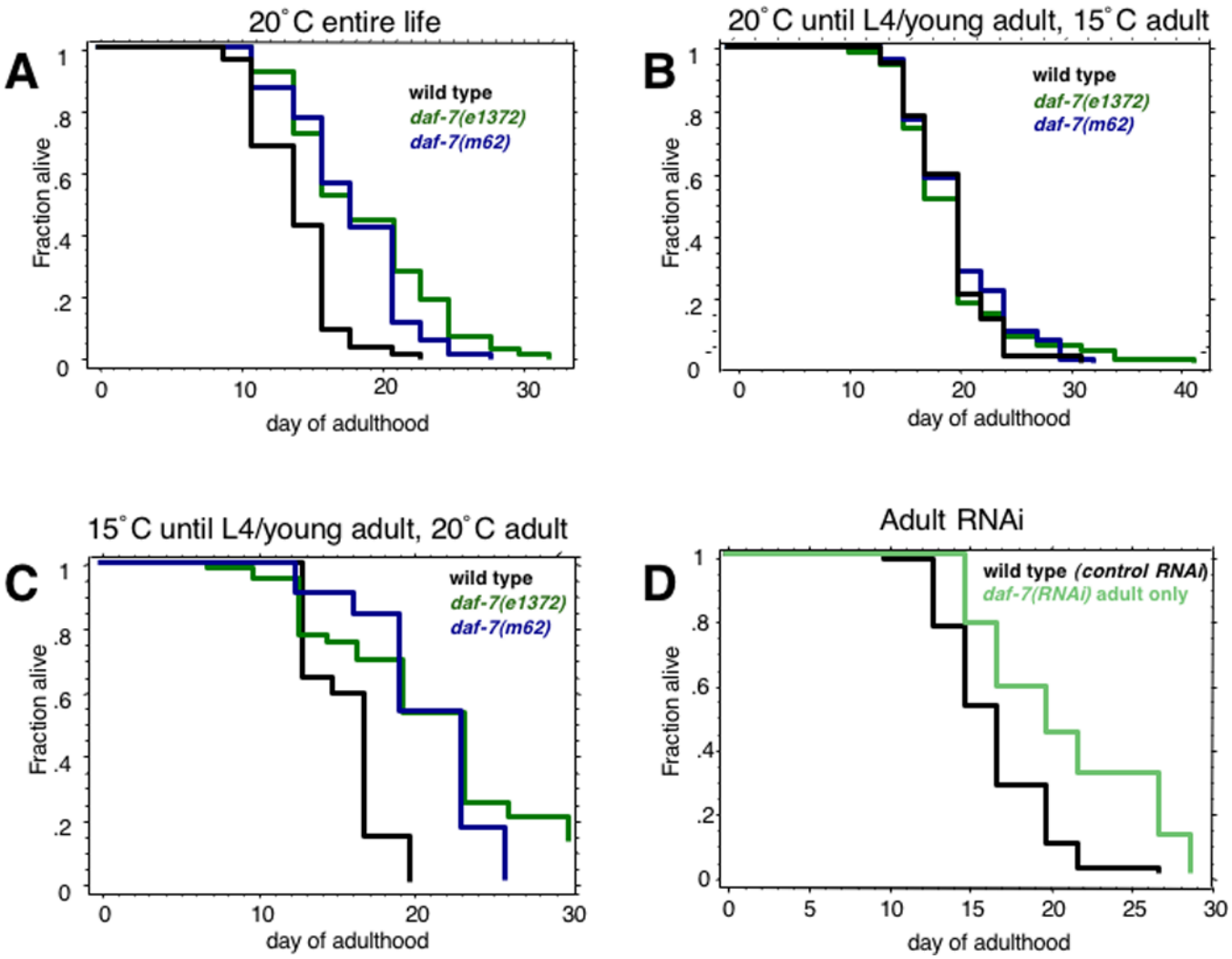

Fig. 5. Temporal Analysis of *daf-7* Longevity Regulation

(A) Lifespan of wild type (14.2 ± 0.4), *daf-7(e1372)* (18.9 ± 0.7, p<0.0001), and *daf-7(m62)* (18 ± 0.5, p<0.0001) worms at 20°C their entire life. (B) Lifespan of wild type (20.1 ± 0.5), *daf-7(e1372)* (20.3 ± 0.6, p=0.9), and *daf-7(m62)* (20.8±0.5, p=0.9) worms raised at 20°C until L4/young adulthood then shifted to 15°C. (C) Lifespan of wild type (15.9 ± 0.5), *daf-7(e1372)* (21.6 ± 0.9, p<0.0001), and *daf-7(m62)* (20.8 ± 0.5, p<0.0001) worms raised at 15°C until L4/young adulthood, then shifted to 20°C. (D) Lifespan of wild type worms treated with vector control (16.7 ± 0.4), or *daf-7* RNAi during adulthood only (21.1 ± 1.1, p<0.0001).

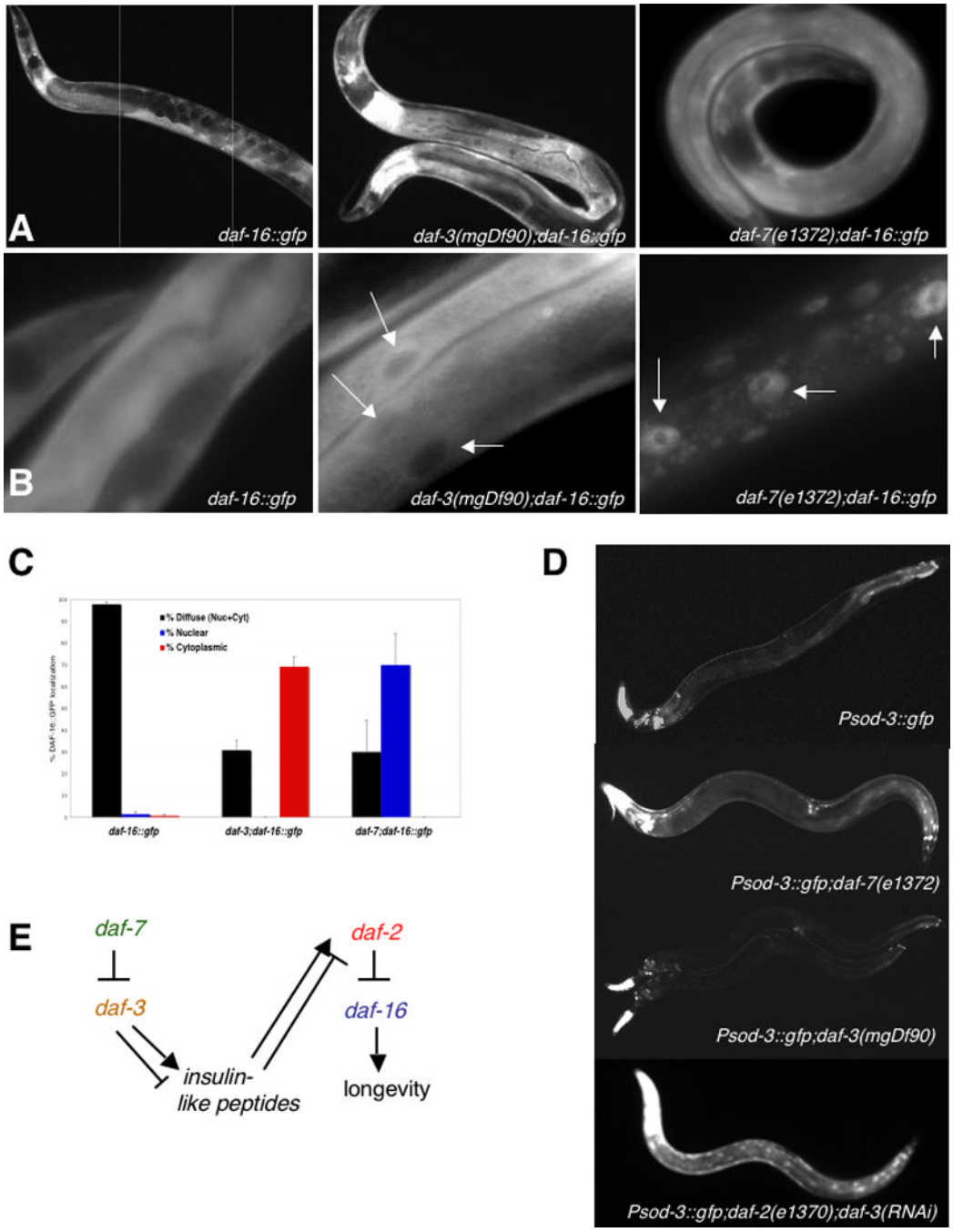

Fig. 6. The TGF-β Dauer pathway regulates DAF-16 localization and *sod-3* transcription

(A) 100× and (B) 400× images of DAF-16::GFP animals. DAF-16::GFP remains diffuse in a wild-type background, but is excluded from nuclei in many *daf-3* animals, and is partially nuclearly localized in many *daf-7* mutants. (C) Ratios of animals with diffuse (black), cytoplasmic (red), and nuclearly-localized (blue) DAF-16::GFP in wild type and *daf-7(e1372)* and *daf-3(mgDf90)* mutant backgrounds. (D) *Psod-3::gfp*, *Psod-3::gfp;daf-7(e1372)*, *Psod-3::gfp;daf-3(mgDf90)*, and *daf-2(e1370);Psod-3::gfp* animal treated with *daf-3* RNAi. (E) Model of TGF-β Dauer and IIS pathway interactions regulating longevity.

Table 1
Lifespans of TGF-β Dauer pathway mutants

Lifespan experiments were carried out at 20°C on 50 μM FUdR; n > 60 for each sample.

Genotype	Mean LS	Std error	P-value	% change
wild type	15.8	0.4	--	--
daf-7(e1372)	21.1	0.7	<0.0001	+33%
daf-4(e1364)	22.1	0.6	<0.0001	+40%
daf-3(mgDf90)	13.2	0.3	<0.0001	−16%
wild type	15.7	0.4	--	-
daf-7(e1372)	17.8	0.8	0.15	+13%
daf-3(mgDf90)	13.2	0.3	<0.0001	−16%
wild-type	14.4	0.3	--	--
daf-1(m40)	21.0	0.6	<0.0001	+46%
bra-1(nk1)	10.8	0.2	<0.0001	−25%
daf-3(e1376)	14.4	0.2	0.76	0%
wild type	13.5	0.3	--	--
daf-7(e1372)	18.8	0.8	<0.0001	+39%
daf-7(m62)	16.3	0.5	<0.0001	+21%
daf-8(e1393)	17.7	0.5	<0.0001	+31%
daf-14(m77)	19.4	0.5	<0.0001	+44%
daf-3(mgDf90)	12.0	0.2	<0.0001	−11%
wild type	14.2	0.4	--	--
daf-7(e1372)	18.9	0.7	<0.0001	+33%
daf-7(m62)	18.0	0.5	<0.0001	+27%
daf-4(e1364)	31.0	0.8	<0.0001	+120%
wild type	16.4	0.2	--	--
daf-7(e1372)	16.5	0.4	0.45	0%
daf-7(m62)	19.7	0.5	<0.0001	+20%
daf-1(m40)	19.5	0.5	<0.0001	+19%
daf-8(e1393)	17.8	0.4	0.3	+9%
daf-14(m77)	19.9	0.6	<0.0001	+21%
bra-1(nk1)	15.4	0.3	0.2	−6%
daf-5(e1386)	13.3	0.4	<0.0001	−19%
daf-3(e1376)	15.5	0.4	0.03	−6%
daf-3(mgDf90)	13.3	0.2	<0.0001	−19%
wild type	17	0.4	--	--
daf-7(e1372) × 3	21	1.7	<0.0001	+24%
daf-7(m62) × 3	22	0.6	<0.0001	+29%
daf-14(m77) × 3	21	0.4	<0.0001	+24%

Genotype	Mean LS	Std error	P-value	% change
daf-1(m40) × 3	20	0.40	<0.0001	+18%