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Response to Tonsaker et al.

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Tonsaker et al. state that there are two issues with the results previously published by us in Budovskaya et al. (2008). The first is whether *elt-3* is expressed in the intestine in *C. elegans*. Tonsaker et al. claim that there is no *elt-3* expression and we claimed that there is low level expression in the intestine. Figure 1 shows *elt-3* expression from an elt-3::GFP transcriptional reporter used in Budovskaya et al., 2008 that includes 1999 bp from the upstream region of *elt-3* driving expression of H2B::GFP. We consistently see *elt-3* expression from this transcriptional reporter in the posterior part of the intestine.

We feel that the issue regarding intestinal expression of *elt-3* in the intestine is not relevant to the main points of Budovskaya et al. 2008. We did not state that *elt-3* functions in the intestine to modulate aging, and we feel that Tonsaker et al., have misinterpreted our statements. *elt-3* may be expressed at a low level (our view) or not at all (their view) in the intestine, but in either case this does not address whether *elt-3* functions in the intestine. Genetic mosaic analysis is required to show in which tissue *elt-3* functions, but this analysis has not yet been performed to our knowledge. In principle, expression of *elt-3* anywhere in the worm could affect expression of genes in the intestine via an indirect, cell non-autonomous fashion.

The second issue is whether *elt-3* mutations can suppress the longevity conferred by a *daf-2* mutation. This is one of the points used in Budovskaya et al., 2008 to argue that *elt-3* has a role in worm aging. In Supplemental Table 1 of Budovskaya et al., 2008, we showed four examples of *elt-3* suppression: three times using *elt-3*(RNAi) and one time using the *elt-3(vp1)* null allele performed by K.W. in the lab.

Since 2008, we have replicated our results five times. Figure 2E shows one replicate using the same *daf-2(e1370); elt-3(vp1)* strain (SD1294) used in Budovskaya et al., 2008 performed by YB in the lab. This experiment confirms that *daf-2(e1370); elt-3(vp1)* has a shorter lifespan than *daf-2(e1370)*.

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Next, we repeated this experiment four times in a blinded experiment performed by two different people in the lab (YB and EVN)(Figure 2). These experiments included two new strains constructed using the *elt-3(vp1)* null mutation and one strain using the *elt-3(gk121)* null mutation. Table 1 shows the Mann-Whitney Rank statistics comparing *daf-2* to *daf-2; elt-3* from experiments 2, 4, and 5 in Figure 2. In all four experiments, we saw that mutations in *elt-3* significantly suppress the longer lifespan conferred by *daf-2(e1370)*, confirming results from Budovskaya et al., 2008.

We also analyzed the data to determine whether suppression of the *daf-2* longevity phenotype by *elt-3* was partial or complete. To do this, we compared the lifespan of the *daf-2; elt-3* strains to either N2 or *elt-3*. If the suppression is only partial, then *daf-2; elt-3* will live significantly longer than N2 or *elt-3*. Using Mann-Whitney Rank statistics, we find that suppression is partial rather than complete in every case, indicating that there are likely other genes, besides *elt-3*, acting downstream of *daf-2* to confer longevity (Table 2).

In summary, we have compared the lifespan of *daf-2; elt-3* to *daf-2* a total of nine times and observed partial suppression in every case. These results cannot be due to an artifact from one strain or allele of *elt-3* as we have used two *elt-3* null alleles as well as *elt-3(RNAi)*. These results are not likely to involve differences in genetic background, as we see suppression using *elt-3(RNAi)* which uses the same *daf-2* genetic background. These differences are not likely to be due to artifacts in scoring from one person, as we obtained similar results performed by three people in the lab.

We are not sure why the *elt-3* suppression result from Figure 2 of Tonsaker et al. does not agree with ours, and we are deeply committed to finding out the true answer. We have requested the strains from Dr. McGhee and will perform the appropriate experiments to determine the source of the difference. One possibility is that the *daf-2(e1370)* strain used by Tonsaker et al. (CB1370) has a background mutation that shortens lifespan itself. This background mutation could mask the effects of an *elt-3* mutation on suppressing longevity. Backcross experiments using strains from both labs should reveal the correct result, and we will report this result as soon as possible.

In summary, we disagree with the two issues raised by Tonsaker et al. The first issue on intestinal expression is not relevant to the main results of our paper on the role of *elt-3* in worm aging. We again apologize if other readers were misled into thinking that that our model required that *elt-3* function in the intestine to modulate aging. The second issue on suppression of *daf-2* longevity by mutations in *elt-3* is not resolved. Our lab can clearly replicate the published lifespan results and it will be necessary to exchange strains to get at the source of the difference. Besides *daf-2* suppression by *elt-3*, there are many other results that support our model that GATA transcription factors have a role in worm aging, such as lifespan extension by RNAi treatment of *elt-5* GATA and *elt- 6* GATA, decreasing expression of *elt-3* GATA with age, increasing expression of *elt-5* GATA and *elt-6* GATA with age, decreasing expression of the targets of *elt- 3* GATA with age, etc. Thus, we do not think that the points raised by Tonsaker et al. should cause one to discard our model for modulation of lifespan by *elt-3*. Since 2008, we have continued to study the role of *elt-3* in

aging and our new results support the main points of Budovskaya et al., 2008 and allow us to modify and extend the original model.

References

Budovskaya YV, Wu K, Southworth LK, Jiang M, Tedesco P, Johnson TE, Kim SK. An elt-3/elt-5/elt-6 GATA transcription circuit guides aging in *C. elegans*. Cell. 2008; 134:291–303. [PubMed: 18662544]

Tonsaker T, Pratt RM, McGhee JM. Re-evaluating the Role of ELT-3 in a GATA Transcription Factor Circuit Proposed to Guide Aging in *C. elegans*.

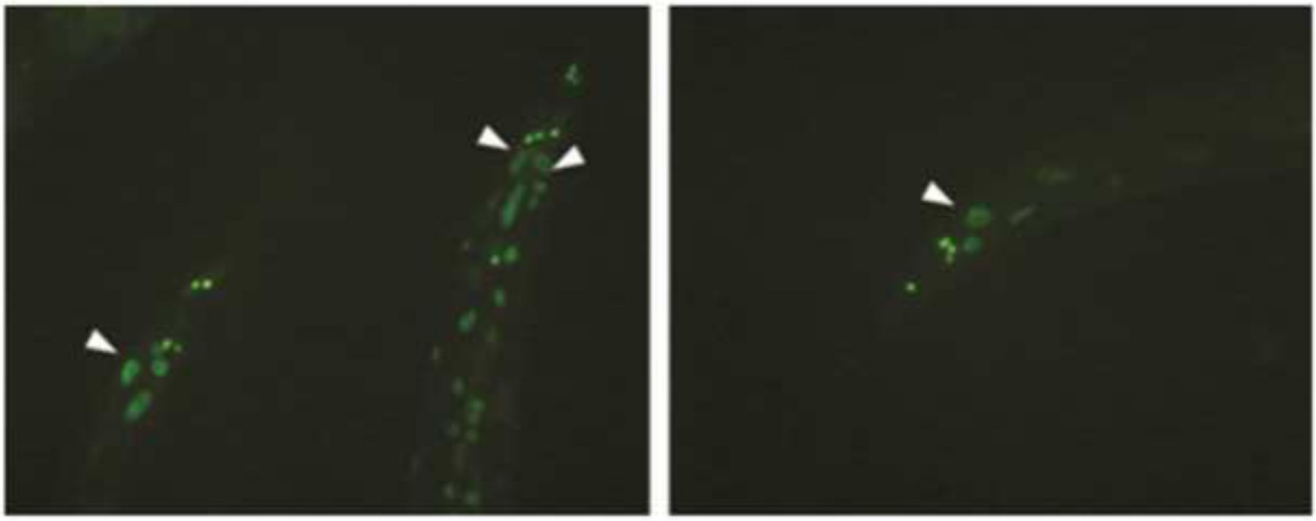

Figure 1.

Expression of elt-3::H2B::GFP (SD1276 (*pha-1(e2123)III*, Ex[*elt-3pro::*GFP::H2B; *pha-1(+)*])). The *elt-3* promoter includes the 2kb upstream of K02B9.4 transcript (genomic location X:13927330..13929329bp). The figure shows worms at day 2 of adulthood, and the arrowheads show GFP expression in the posterior two intestinal cells. Figures are from data used in Budovskaya et al., 2008.

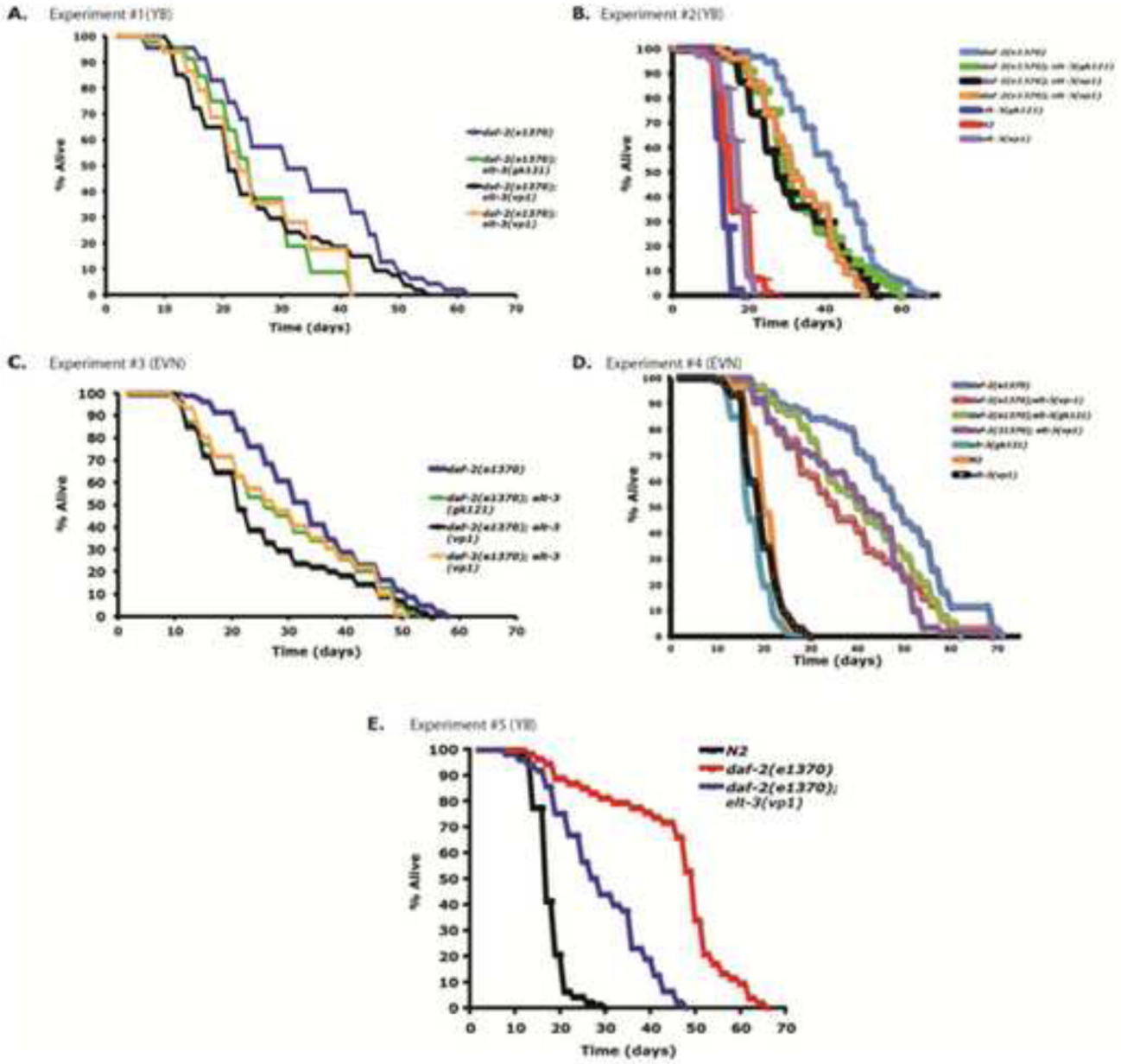

Figure 2.

elt-3(vp1) suppresses longevity conferred by *daf-2(e1370)*. All experiments were performed at 20°C. We used on average 150-200 animals per experiment. L4 larvae stage was counted as day 0 of adulthood. At day 2, all animals were transferred to NGM plates containing FUDR to prevent their progeny from developing. During these experiments, we censored animals that displayed severe bagging, vulva protrusion, and gonad explosion.

Table 1

Suppression of the *daf-2* longevity phenotype by *elt-3*

Experment #2	**p-value**
daf-2(e1370) vs. daf-2(e1370); elt-3(vp1)#1	1.22×10^{-05}
daf-2(e1370) vs. daf-2(e1370); elt-3(vp1)#2	4×10^{-15}
daf-2(e1370) vs. daf-2(e1370); elt-3(gk121)	9.61×10^{-07}
Experment #4	
daf-2(e1370) vs. daf-2(e1370); elt-3(vp1)#1	7.57×10^{-5}
daf-2(e1370) vs. daf-2(e1370); elt-3(gk121)	5.41×10^{-5}
daf-2(e1370) vs. daf-2(e1370); elt-3(vp1)#2	3.1×10^{-4}
Experiment #5	
daf-2(e1370) vs. daf-2(e1370);elt-3(vp1)	5.5×10^{-5}

Table 2

Suppression by *elt-3* of the *daf-2* longevity phenotype is partial.

Experment #2	**p-value**
N2 vs. elt-3(gk121)	0.000154
N2 vs elt-3(vp1)	0.162
N2 vs. daf-2(e1370); elt-3(vp1)#1	1×10^{-27}
N2 vs. daf-2(e1370); elt-3(vp1)#2	1×10^{-27}
N2 vs. daf-2(e1370); elt-3(gk121)	1×10^{-27}
elt-3(gk121) vs. daf-2(e1370); elt-3(vp1)#1	1×10^{-27}
elt-3(gk121) vs. daf-2(e1370); elt-3(vp1)#2	1×10^{-27}
elt-3(gk121) vs. daf-2(e1370); elt-3(gk121)	1×10^{-27}
elt-3(vp1) vs. daf-2(e1370); elt-3(vp1)#1	1×10^{-27}
elt-3(vp1) vs. daf-2(e1370); elt-3(vp1)#2	1×10^{-27}
elt-3(vp1) vs. daf-2(e1370); elt-3(gk121)	1×10^{-27}
Experment #4	
N2 vs. elt-3(gk121)	1.3×10^{-9}
N2 vs elt-3(vp1)	0.0244
N2 vs. daf-2(e1370); elt-3(vp1)#1	1×10^{-27}
N2 vs. daf-2(e1370); elt-3(vp1)#2	1×10^{-27}
N2 vs. daf-2(e1370); elt-3(gk121)	1×10^{-27}
elt-3(gk121) vs. daf-2(e1370); elt-3(vp1)#1	1×10^{-27}
elt-3(gk121) vs. daf-2(e1370); elt-3(vp1)#2	1×10^{-27}
elt-3(gk121) vs. daf-2(e1370); elt-3(gk121)	1×10^{-27}
elt-3(vp1) vs. daf-2(e1370); elt-3(vp1)#1	1×10^{-27}
elt-3(vp1) vs. daf-2(e1370); elt-3(vp1)#2	1×10^{-27}
elt-3(vp1) vs. daf-2(e1370); elt-3(gk121)	1×10^{-27}
Experiment #5	
daf-2(e1370) vs. N2	1×10^{-27}
daf-2(e1370);elt-3(vp1) vs. N2	1.6×10^{-15}