

Supplementary Material

Elevated Trehalose Levels in *C. elegans* *daf-2* Mutants Increase Stress Resistance, Not Lifespan

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Figure S1.

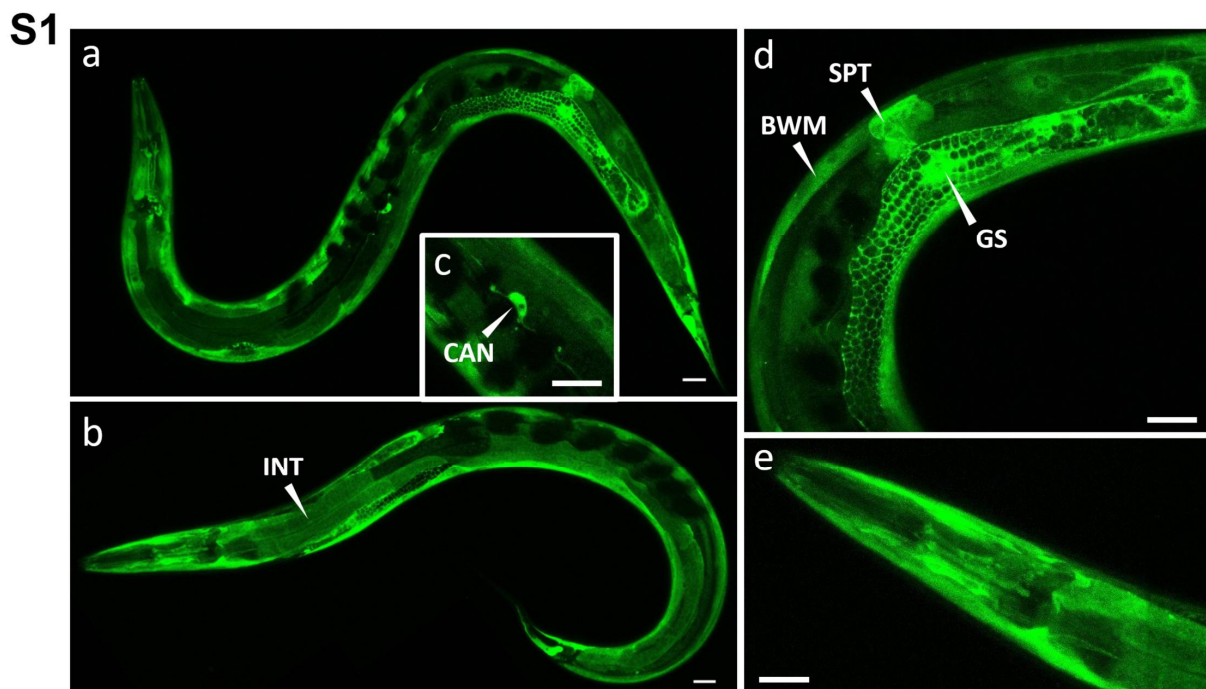


Figure S1. Translational *tps-1::egfp* reporter strain fed with *E. coli* HT115 expressing empty vector (a-c) and *daf-2* RNAi (d,e). CAN, Canal-associated neuron; BWM, body wall muscle; SPT, spermatheca; GS, gonadal sheath; INT, intestine. Scale bars are 25 μm.

Figure S2.

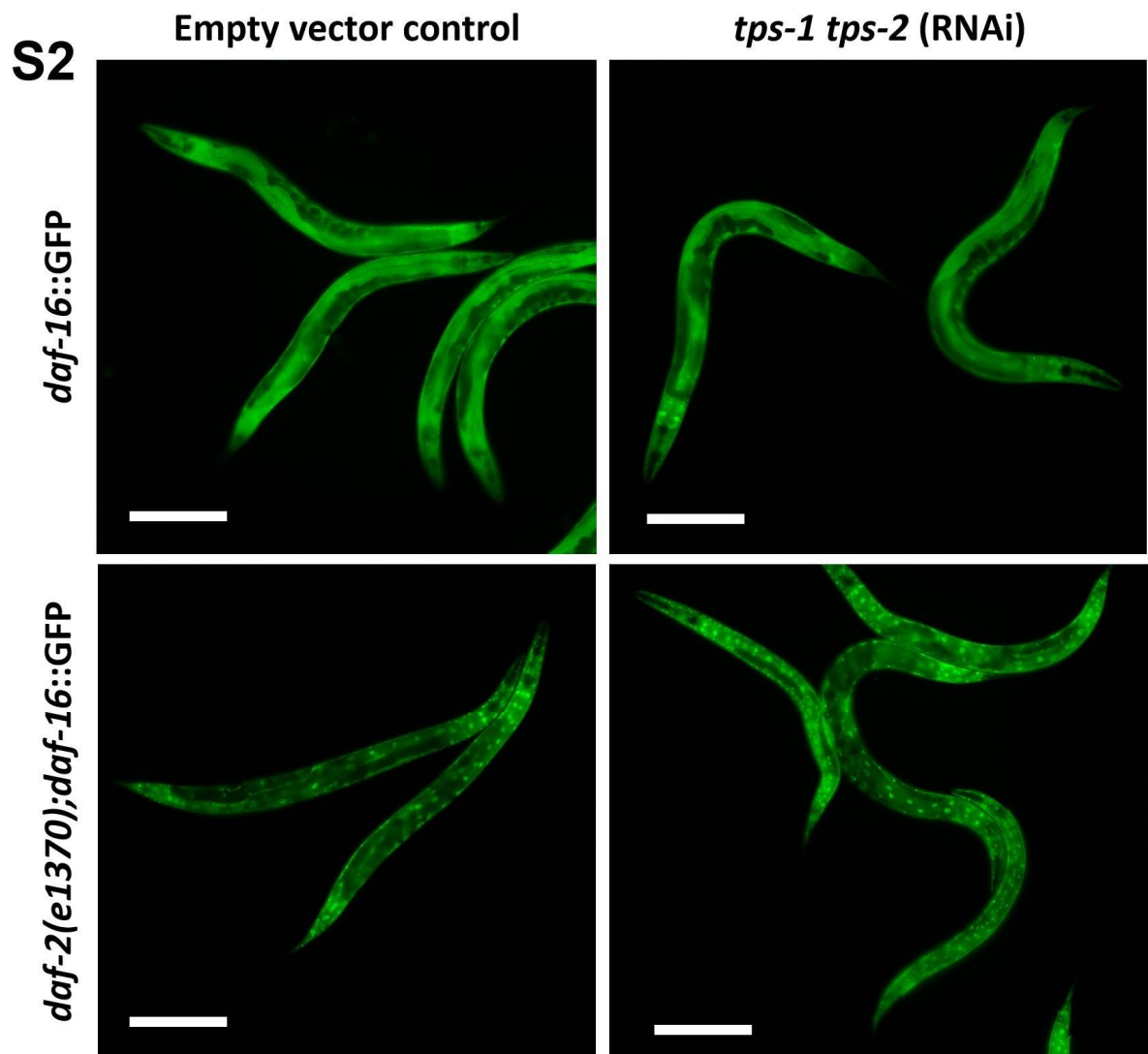


Figure S2. DAF-16::GFP localization in worms with or without *tps* knockdown in wild-type or *daf-2* mutant background. Scale bars are 250 μ m.

Table S1. Strains used in this study.

Strain name	Genotype	RNAi-sensitive tissue	Source
N2	wild type	All	Caenorhabditis Genetics Center
CB1370	<i>daf-2(e1370ts)III</i>	All	
GR1895	<i>daf-2(e1370ts)III; mgl-67 [daf-16p::daf-16::GFP + rol-6(su1006)]</i>	All	
BC14885	<i>dpy-5(e907)I; sEX14885[rCes tps-1::gfp + pCeh361]</i>	All	
BC14876	<i>dpy-5(e907)I; sEX14876[rCes tps-2::gfp + pCeh361]</i>	All	
NR222	<i>rde-1(ne219)V; kzl-9[pKK1260(lin-26p::nls::gfp) + pKK1253(lin-26p::rde-1) + pRF6(rol-6(su1006))]</i>	Hypodermis	
RB798	<i>rrf-1(ok589)I</i>	Germline	A kind gift from T. Johnson
TJ356	<i>zls-356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)]IV</i>	All	
MGH167	<i>sid-1(qt9)V; alx-6[vha-6p::sid-1::sl2::gfp]</i>	Intestine	a kind gift from M. Hansen
MAH410	<i>daf-2(e1370ts)III; sid-1(qt9)V; alx-6[vha-6p::sid-1::sl2::gfp]</i>	Intestine	
AGD855	<i>sid-1(qt9)V; uth-1.37(myo-3p::tomato+myo-3p::sid-1)</i>	Muscles	
MAH411	<i>daf-2(e1370ts)III; sid-1(qt9)V; uth-1.37(myo-3p::tomato+myo-3p::sid-1)</i>	Muscles	
JV34	<i>daf-2(e1370ts)III; rde-1(ne219)V; kzl-9[pKK1260(lin-26p::nls::gfp) + pKK1253(lin-26p::rde-1) + pRF6(rol-6(su1006))]</i>	Hypodermis	This study
JV31	<i>rrf-1(ok589)I; daf-2(e1370ts)III</i>	Germline	
	<i>tps-1p::tps-1::egfp</i>	All	a kind gift from T. Kurzchalia
	<i>daf-2(e1370ts)III; tps-1p::tps-1::egfp</i>	All	
	<i>daf-2(e1370); tps-1(ok373); tps-2(ok526)</i>	All	

Table S2. Mean lifespan of all tested strains.

Mutant/Strain (RNAi active tissue, background)	Treatment (OP50 / RNAi)	Mean lifespan $\pm$ SEM (days)	Sample size/censored
N2 (systemic)	L4440	20.53 $\pm$ 0.61	101/6
	<i>tps-1</i>	21.13 $\pm$ 0.62	100/10
	<i>tps-2</i>	23.87 $\pm$ 0.56	100/4
	<i>tps-1/2</i>	21.11 $\pm$ 0.55	126/4
<i>daf-2</i> (systemic)	L4440	63.92 $\pm$ 1.63	100/3
	<i>tps-1</i>	63.34 $\pm$ 1.75	101/6
	<i>tps-2</i>	63.86 $\pm$ 1.50	100/2
	<i>tps-1/2</i>	62.81 $\pm$ 1.56	101/10
MGH167 (intestine, WT)	L4440	18.41 $\pm$ 0.50	100/16
	<i>tps-1</i>	18.79 $\pm$ 0.57	100/15
	<i>tps-2</i>	18.92 $\pm$ 0.53	100/11
	<i>tps-1/2</i>	18.69 $\pm$ 0.50	100/13
MAH410 (intestine, <i>daf-2</i>)	L4440	63.70 $\pm$ 1.55	100/5
	<i>tps-1</i>	63.18 $\pm$ 1.44	100/1
	<i>tps-2</i>	67.40 $\pm$ 1.23	100/3
	<i>tps-1/2</i>	61.19 $\pm$ 1.35	101/4
AGD855 (muscle, WT)	L4440	21.63 $\pm$ 0.60	101/16
	<i>tps-1</i>	20.59 $\pm$ 0.46	100/10
	<i>tps-2</i>	21.08 $\pm$ 0.54	100/17
	<i>tps-1/2</i>	22.51 $\pm$ 0.55	102/9
MAH411 (muscle, <i>daf-2</i>)	L4440	62.83 $\pm$ 1.21	70/2
	<i>tps-1</i>	58.08 $\pm$ 1.03	86/1
	<i>tps-2</i>	59.42 $\pm$ 1.13	73/0
	<i>tps-1/2</i>	57.64 $\pm$ 1.35	59/0
NR222 (hypodermis, WT)	L4440	24.51 $\pm$ 0.70	125/7
	<i>tps-1</i>	26.41 $\pm$ 0.78	125/6
	<i>tps-2</i>	25.85 $\pm$ 0.73	124/14
	<i>tps-1/2</i>	24.88 $\pm$ 0.66	124/5
JV34 (hypodermis, <i>daf-2</i>)	L4440	66.10 $\pm$ 1.19	115/0
	<i>tps-1</i>	68.42 $\pm$ 1.33	111/10
	<i>tps-2</i>	62.33 $\pm$ 1.49	106/19
	<i>tps-1/2</i>	62.07 $\pm$ 1.61	70/3
RB798 (germline, WT)	L4440	18.43 $\pm$ 0.22	108/15
	<i>tps-1</i>	18.93 $\pm$ 0.44	110/17
	<i>tps-2</i>	18.58 $\pm$ 0.32	106/11
	<i>tps-1/2</i>	19.23 $\pm$ 0.44	109/9
JV31 (germline, <i>daf-2</i>)	L4440	59.90 $\pm$ 1.83	101/17
	<i>tps-1</i>	60.34 $\pm$ 1.14	112/4
	<i>tps-2</i>	62.35 $\pm$ 1.48	100/2
	<i>tps-1/2</i>	62.92 $\pm$ 1.45	97/8

N2	OP50	21,82±0,48	123/8
<i>tps-1;tps-2</i>	OP50	23,50±0,39	120/3
<i>daf-2</i>	OP50	70,38±1,19	126/8
<i>daf-2;tps-1;tps-2</i>	OP50	65,79±0,87	122/5

Table S3. Carbohydrate analysis.

Strain	RNAi treatment	Trehalose (µg/mg protein)	Maltose (µg/mg protein)	Glucose (µg/mg protein)	Glycogen (µg/mg protein)
N2	EV	23.18	4.39	10.21	36.44
		11.77	3.40	5.42	23.00
		14.69	1.83	4.90	60.72
	<i>tps-1</i>	16.14	7.69	11.72	49.64
		5.78	3.04	5.76	33.15
		7.45	1.52	3.82	52.67
	<i>tps-2</i>	4.22	5.78	8.91	65.76
		5.71	1.35	4.31	17.48
		3.78	2.39	4.62	21.82
	<i>tps-1/tps-2</i>	0.81	5.53	7.22	38.34
		1.03	2.51	3.78	17.12
		2.05	1.09	2.80	19.52
<i>daf-2</i>	EV	94.14	12.70	18.37	192.47
		93.31	7.24	14.11	184.98
		75.72	4.36	10.95	113.90
	<i>tps-1</i>	77.94	11.95	21.14	156.42
		83.76	5.10	8.53	148.41
		63.15	6.80	12.94	189.13
	<i>tps-2</i>	13.87	28.81	26.15	208.06
		41.99	7.43	8.72	145.87
		36.51	7.67	12.44	211.61
	<i>tps-1/tps-2</i>	2.74	12.90	12.34	121.35
		10.27	5.10	8.91	133.21
		16.32	4.14	8.76	97.55
<i>daf-2</i>	N/A	61.11	12.17	17.25	307.60
		84.23	14.31	22.73	333.78
		53.05	11.17	13.77	
<i>daf-2;tps-1;tps-2</i>	N/A	-0.06	10.41	15.66	432.81
		-9.37	9.88	15.19	292.36
		0.06	11.29	13.56	

Table S4. Stress survival.

Osmotic stress assay (500 mM NaCl)			
Strain	Biological replicate	Mean survival ± SE (days)	Sample size/censored
N2	1	1.92±0.06	124/0
	2	2.17±0.08	131/0
	3	2.15±0.06	128/0
<i>tps-1;tps-2</i>	1	1.55±0.05	121/0
	2	2±0.06	124/0
	3	1.91±0.04	136/0
<i>daf-2</i>	1	13.06 ±0.64	129/0
	2	18.89±0.63	124/0
	3	17.29±0.58	139/0
<i>daf-2; tps-1;tps-2</i>	1	4.78±0.15	132/0
	2	5.27±0.2	128/0
	3	5.24±0.17	157/0
Oxidative stress assay (0.28% tBOOH)			
Strain	Biological replicate	Median time of death ± SE (min)	
N2	1	198.02±13.10	
	2	176.05±5.76	
	3	207.36±15.41	
<i>tps-1;tps-2</i>	1	215.46±6.63	
	2	183.24±6.45	
	3	214.12±5.24	
<i>daf-2</i>	1	339.40±20.38	
	2	319.08±13.74	
	3	351.59±22.64	
<i>daf-2; tps-1;tps-2</i>	1	314.12±11.51	
	2	360.74±5.73	
	3	345.31±9.33	
Heat stress assay (40°C)			
Strain	Biological replicate	Median time of death ± SE (min)	
N2	1	103.18±2.34	
	2	94.14±5.09	
	3	116.65±5.90	
<i>tps-1;tps-2</i>	1	96.3±4.36	
	2	87.07±3.44	
	3	87.50±4.29	
<i>daf-2</i>	1	170.69±8.01	
	2	178.29±4.60	
	3	195.45±21.69	
<i>daf-2; tps-1;tps-2</i>	1	129.69±5.74	
	2	122.39±5.66	
	3	140.36±5.78	

Table S5. Lifespan experiment setup of three independent studies.

	Honda et al. 2010	Seo et al. 2018	This study	
<i>tps</i> knockdown	Systemic RNAi	Systemic RNAi	Systemic RNAi	Mutants
<i>daf-2</i> allele	<i>e1370</i>	<i>e1370</i>	<i>e1370</i>	<i>e1370</i>
Temperature regimen	20 °C	20 °C	16 °C → 20 °C	16 °C → 20 °C
Food	HT115	HT115	HT115	OP50
Agar	NGM	NGM	NGM	NGM
FUDR	40 µM	200 µM	100 µM	100 µM
Transfer	Every 3 days	Not mentioned	Weekly	Weekly
Lifespan effect of <i>tps</i>	43% ¹	11% ²	3% ¹	9% ¹

¹ Calculated as the *tps*-dependent decrease (%) of the *daf-2* lifespan extension.

² Calculated as the *tps*-dependent decrease (%) of the total *daf-2* lifespan (parallel N2 data are not available in this study). Using N2 of a non-parallel experiment in this study, we estimated the *tps*-dependent decrease of *daf-2* lifespan extension to be around 20%. While N2 and *daf-2* lifespans in our study and Honda's et al study were very similar, those of the Seo et al., study were much shorter.