Hypotaurine promotes longevity and stress tolerance via the stress response factors DAF-16/FOXO and SKN-1/NRF2 in *Caenorhabditis elegans*

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Supporting information

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Fig. S1

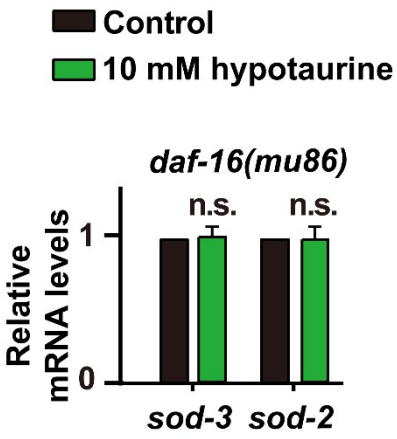

Fig. S1 Upregulation of *sod-2* and *sod-3* induced by hypotaurine depended on *daf-16*. qPCR analysis of mRNA level of *sod-2* and *sod-3* in *daf-16(mu86)* mutant exposed to 10 mM hypotaurine versus control worms (Student's t test; mean ± SD; no significance is abbreviated as n.s.; $n \geq 3$).

Fig. S2

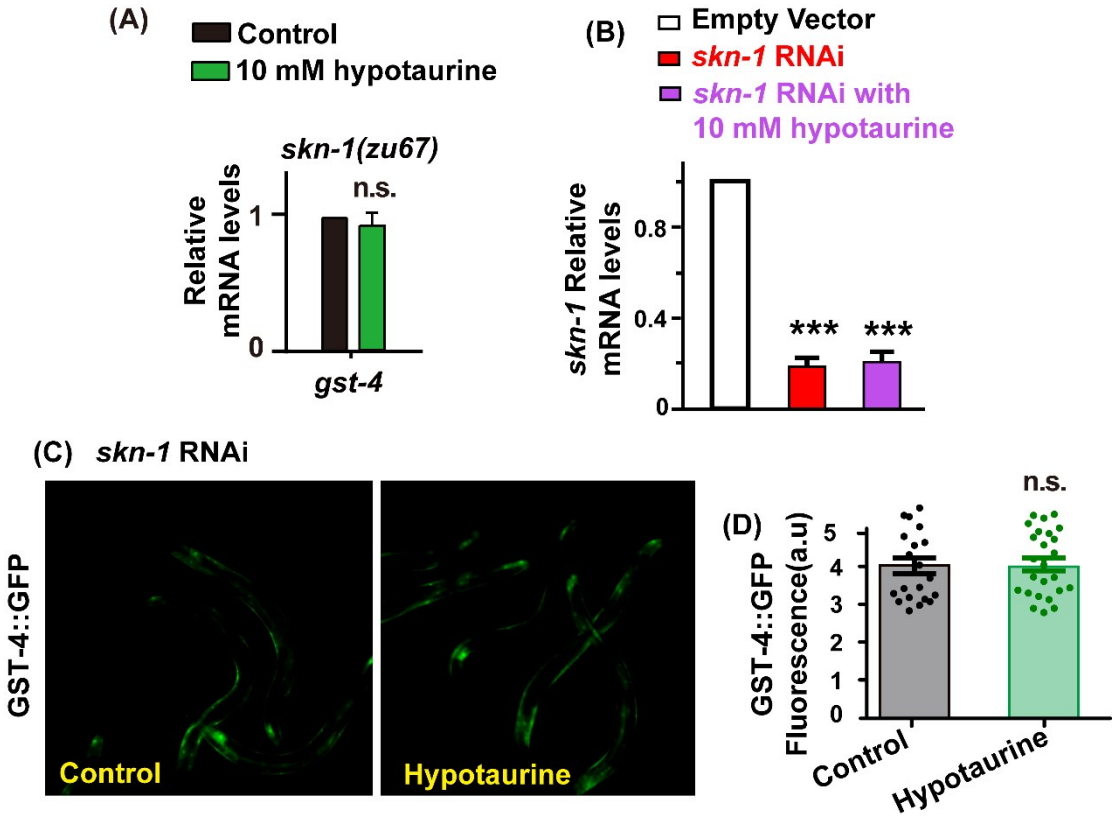

Fig. S2 Hypotaurine-induced *gst-4* upregulation required for SKN-1. (A) qPCR analysis of mRNA level of *gst-4* in *skn-1(zu67)* mutant exposed to 10 mM hypotaurine versus control worms. (B) *skn-1* RNA levels in whole worms after treatment of *C. elegans* with *skn-1* RNAi versus control RNAi. (Student's t test; mean ± SD; no significance is abbreviated as n.s., ***$p < 0.001$, $n \geq 3$). (C) Images and quantification of GFP fluorescence of P_{gst-4}::GFP after animals subjected to *skn-1* RNAi (no significance is abbreviated as n.s.; Student's t test; mean ± SD; $n \geq 30$).

Table S1

Figure	Strains	Treatments	Mean Lifespan ± SEM (days)	*P* value VS control	% Change in mean lifespan	N
	N2 (WT)					
Fig 1A	EXP. 1	20℃ / control	23.380±0.441			71
	EXP. 1	20℃ / 0.02mM Hypotaurine	24.936±0.370	0.025	6.66	76
	EXP. 1	20℃ / 0.1mM Hypotaurine	24.926±0.448	0.031	6.61	81
	EXP. 1	20℃ / 0.5mM Hypotaurine	25.200±0.461	0.006	7.78	80
	EXP. 1	20℃ / 2mM Hypotaurine	25.071±0.573	0.006	7.23	70
	EXP. 1	20℃ / 10mM Hypotaurine	27.132±0.563	<0.0001	16.05	78
	EXP. 2	20℃ / control	24.902±0.535			82
	EXP. 2	20℃ / 0.02mM Hypotaurine	26.656±0.629	0.039	7.0	64
	EXP. 2	20℃ / 0.1mM Hypotaurine	26.672±0.610	0.0085	7.1	61
	EXP. 2	20℃ / 0.5mM Hypotaurine	26.508±0.676	0.052	6.4	61
	EXP. 2	20℃ / 2mM Hypotaurine	25.538±0.777	0.518	#	52
	EXP. 2	20℃ / 10mM Hypotaurine	29.317±0.664	<0.0001	17.73	63
	EXP. 3	20℃ / control	24.626±0.446			91
	EXP. 3	20℃ / 0.02mM Hypotaurine	28.095±0.626	<0.0001	14.086	74
	EXP. 3	20℃ / 0.1mM Hypotaurine	25.756±0.629	0.045	4.58	78
	EXP. 3	20℃ / 0.5mM Hypotaurine	26.222±0.728	0.005	6.48	72
	EXP. 3	20℃ / 2mM Hypotaurine	24.059±0.627	0.678	#	68
	EXP. 3	20℃ / 10mM Hypotaurine	29.809±0.622	<0.0001	21.05	89
	N2 (WT) with 5 mM PQ					
Fig 2B	EXP. 1	20℃ / control	7.576±0.351			59
	EXP. 1	20℃ / 10mM hypotaurine	9.373±0.368	<0.0001	23.720	67
	EXP. 2	20℃ / control	7.526±0.294			76
	EXP. 2	20℃ / 10mM hypotaurine	8.725±0.304	0.002	15.931	91
	EXP. 3	20℃ / control	7.487±0.267			76
	EXP. 3	20℃ / 10mM hypotaurine	9.193±0.312	<0.0001	22.786	83
	daf-16(mu86) Ⅰ					
Fig 2C	EXP. 1	20℃ / control	20.761±0.378			67
	EXP. 1	20℃ / 10mM hypotaurine	21.200±0.312	0.858	#	65
	EXP. 2	20℃ / control	21.160±0.229			100
	EXP. 2	20℃ / 10mM hypotaurine	20.741±0.210	0.21	#	116
	EXP. 3	20℃ / control	21.717±0.223			120
	EXP. 3	20℃ / 10mM hypotaurine	20.741±0.210	0.001	#	116

	skn-1(zu67)IV					
Fig 2D	EXP. 1	20°C / control	20.444±0.433			90
	EXP. 1	20°C / 10mM hypotaurine	20.059±0.429	0.734	#	102
	EXP. 2	20°C / control	21.105±0.392			76
	EXP. 2	20°C / 10mM hypotaurine	20.789±0.415	0.284	#	76
	EXP. 3	20°C / control	20.105±0.392			69
	EXP. 3	20°C / 10mM hypotaurine	19.789±0.415	0.357	#	73
	sir-2.1(ok434)IV					
Fig 3E	EXP. 1	20°C / control	22.078±0.215			256
	EXP. 1	20°C / 10mM hypotaurine	21.264±0.276	0.063	#	230
	EXP. 3	20°C / control	20.463±0.232			177
	EXP. 3	20°C / 10mM hypotaurine	20.127±0.211	0.570	#	260
	EXP. 4	20°C / control	22.130±0.424			115
	EXP. 4	20°C / 10mM hypotaurine	21.697±0.396	0.324	#	81
	EXP. 5	20°C / control	19.053±0.203			225
	EXP. 5	20°C / 10mM hypotaurine	19.623±0.341	0.025	#	130
	eat-2(ad1116) II					
Fig 3D	EXP. 1	20°C / control	24.5±0.249			116
	EXP. 1	20°C / 10mM hypotaurine	24.974±0.215	0.181	#	156
	EXP. 2	20°C / control	24.182±0.255			143
	EXP. 2	20°C / 10mM hypotaurine	23.814±0.251	0.411	#	156
	EXP. 3	20°C / control	24.570±0.220			193
	EXP. 3	20°C / 10mM hypotaurine	23.883±0.200	0.058	#	196
	EXP. 4	20°C / control	26.485±0.356			134
	EXP. 4	20°C / 10mM hypotaurine	26.367±0.264	0.602	#	226
	clk-1(e2519)III					
Fig 4C	EXP. 1	20°C / control	26.545±0.306			191
	EXP. 1	20°C / 10mM hypotaurine	25.498±0.290	0.044	#	203
	EXP. 2	20°C / control	23.573±0.401			124
	EXP. 2	20°C / 10mM hypotaurine	24.294±0.413	0.145	#	136
	EXP. 3	20°C / control	29.429±0.442			133
	EXP. 3	20°C / 10mM hypotaurine	29.698±0.534	0.431	#	86
	isp-1(qm150)IV					
Fig 4A	EXP. 1	20°C / control	29.671±0.706			140
	EXP. 1	20°C / 10mM hypotaurine	30.056±0.619	0.791	#	160
	EXP. 3	20°C / control	29.533±0.649			105
	EXP. 3	20°C / 10mM hypotaurine	29.190±0.520	0.339	#	121
	EXP. 4	20°C / control	28.761±0.438			208
	EXP. 4	20°C / 10mM hypotaurine	28.431±0.439	0.812	#	122

	daf-2(e1370) Ⅲ					
Fig 3A	EXP. 1	20℃ / control	54.641±0.598			145
	EXP. 1	20℃ / 10mM hypotaurine	54.310±0.531	0.159	#	155
	EXP. 2	20℃ / control	52.402±0.661			127
	EXP. 2	20℃ / 10mM hypotaurine	54.920±0.661	0.023	#	150
	EXP. 3	20℃ / control	52.379±0.537			116
	EXP. 3	20℃ / 10mM hypotaurine	52.526±0.487	0.931	#	133
	aak-2(ok524) X					
Fig 3C	EXP. 1	20℃ / control	20.777±0.253			121
	EXP. 1	20℃ / 10mM hypotaurine	20.277±0.230	0.546	#	137
	EXP. 2	20℃ / control	20.357±0.323			112
	EXP. 2	20℃ / 10mM hypotaurine	20.421±0.299	0.822	#	114
	EXP. 3	20℃ / control	19.528±0.147			108
	EXP. 3	20℃ / 10mM hypotaurine	19.943±0.161	0.441	#	105
	glp-1(e2144) III					
Fig 3B	EXP. 1	20℃ / control	34.595±0.450			168
	EXP. 1	20℃ / 10mM hypotaurine	33.798±0.506	0.206	#	124
	EXP. 2	20℃ / control	34.079±0.492			189
	EXP. 2	20℃ / 10mM hypotaurine	35.627±0.576	0.021	#	161
	EXP. 3	20℃ / control	34.414±0.548			186
	EXP. 3	20℃ / 10mM hypotaurine	32.923±0.572	0.094	#	168
	rsks-1(ok1255) III					
Fig 3F	EXP. 1	20℃ / control	32.228±0.040			184
	EXP. 1	20℃ / 10mM hypotaurine	33.413±0.442	0.019	#	167
	EXP. 2	20℃ / control	35.480±0.334			223
	EXP. 2	20℃ / 10mM hypotaurine	35.621±0.407	0.508	#	182
	EXP. 3	20℃ / control	32.687±0.445			198
	EXP. 3	20℃ / 10mM hypotaurine	32.823±0.438	0.989	#	186
	mev-1(kn1) III					
Fig 4B	EXP. 1	20℃ / control	16.392±0.247			143
	EXP. 1	20℃ / 10mM hypotaurine	15.855±0.182	0.082		166
	EXP. 3	20℃ / control	15.563±0.176			199
	EXP. 3	20℃ / 10mM hypotaurine	14.862±0.134	0.003		167
	EXP. 5	20℃ / control	16.608±0.222			74
	EXP. 5	20℃ / 10mM hypotaurine	16.171±0.181	0.106		111
	EXP. 6	20℃ / control	16.559±0.195			143
	EXP. 6	20℃ / 10mM hypotaurine	16.854±0.174	0.126		253

Table S1 Survival analyses are conducted using the Kaplan-Meier method. *P* values were calculated by log-rank test for individual experiments. All statistical were

performed using SPSS package. “N” displayed the number of dead worms. “#”: no calculate (because $p > 0.05$).

Table S2: sequences of PCR primers (qPCR)

Primer sequences used for quantitative PCR (5’→3’):		
Gene	Forward primer sequence	Reverse primer sequence
cdc-42	CTGCTGGACAGGAAGATTACG	CTCGGACATTCTCGAATGAAG
sod-3	AGCATCATGCCACCTACGTGA	AGCATCATGCCACCTACGTGA
skn-1	TGGAGTGTCGTCCATATTCATCT	TGAGGTGTTGGACGATGGTG
ctl-1	GACGTATCCAAAACCCCAAGTG	TTGGCATGAACGACACGCTC
ctl-2	TTCCGATCGAGGACTCCCAG	CTTCACTCCTTGAGTTGGCTTG
ctl-3	CCCACATGGTCAATCTAACGGT	GGAGCTCCATTGGATGTGGT
gst-4	TCCGTCAATTCACTTCTTCCG	AAGAAATCATCACGGGCTGG
sod-2	AGCTTTCGGCATCAACTGTC	AAGTCCAGTTGTTGCCTCAAGT