

Supporting Information

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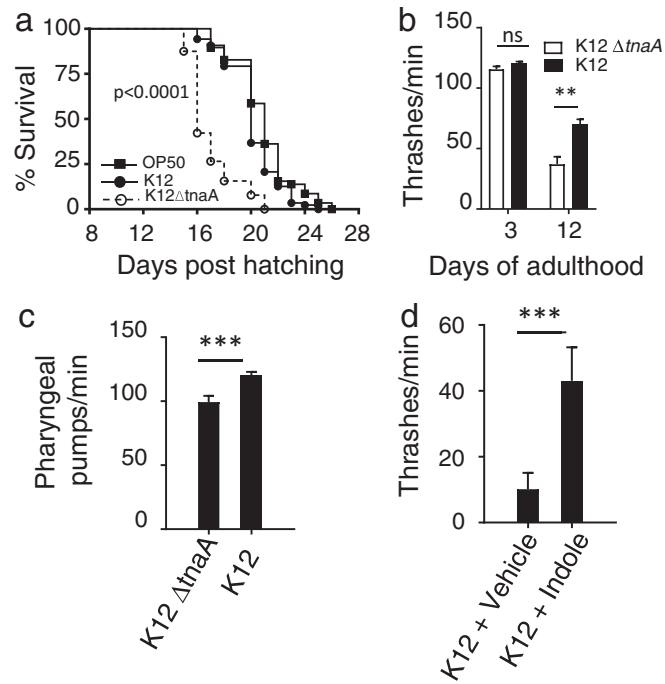


Fig. S1. Effects of indoles on healthspan. (A) Lifespan curve of N2 with K12, K12 Δ tnaA, or OP50 ($n > 60$ worms per condition; $P < 0.0001$ for K12 Δ tnaA vs. other strains; differences between K12 and OP50 were not significant). (B) Thrashing motility in liquid ($n = 8$ –12 worms per condition); (C) pharyngeal pumping rate per minute (day 12 adults, $n = 8$ –12 worms per condition) of N2 adults grown on K12 or K12 Δ tnaA. (D) Thrashing motility of N2 animals grown on K12 animals supplemented with 100 μ M indole or vehicle (day 20 adults, $n = 12$ worms).

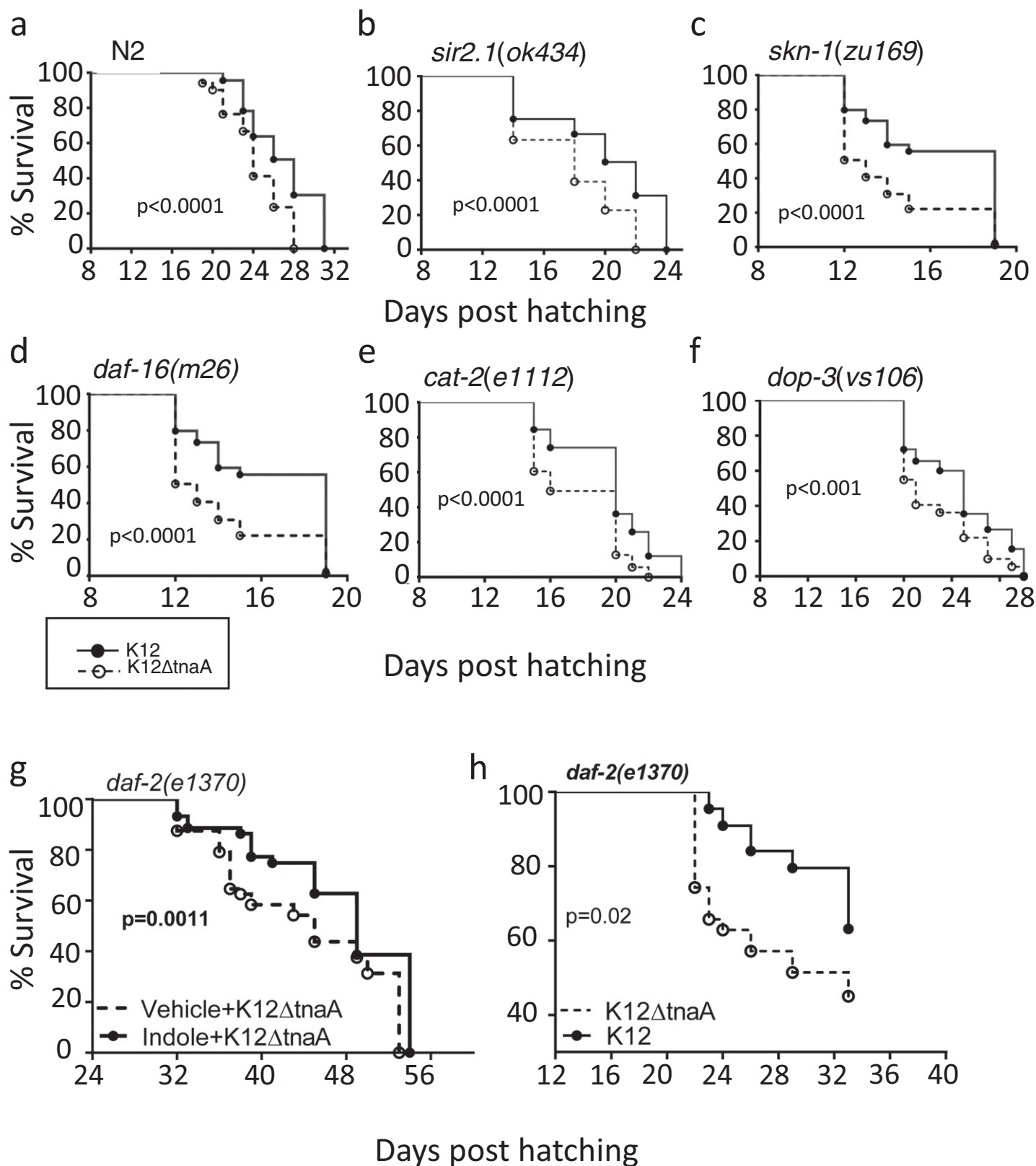


Fig. S2. Genes associated with lifespan and stress response regulation in *C. elegans* do not mediate healthspan effect of indole. (A–F) Kaplan–Meier lifespan curves of wild-type *C. elegans*, N2 (A), *sir2.1(ok434)* (B), *skn-1(zu169)* (C), *daf-16(m26)* (D), *cat-2(e1112)* (E), *dop-3(vs106)* (F), *daf-2(e1370)* (G) on *E. coli* K12 or K12 Δ tnaA mutant; *daf-2(e1370)* on K12 Δ tnaA plus vehicle or K12 Δ tnaA plus indole (H) ($n > 50$ worms/condition). Graphs are representatives of at least two independent experiments. Details of the lifespan experiments are listed in Table S3.

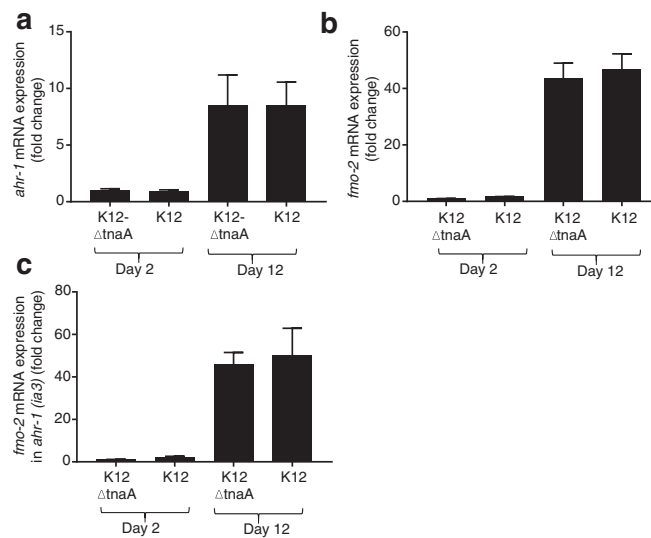


Fig. S3. Expression of *ahr-1* and *fmo-2* increases with age but not with indole. (A and B) Real-time PCR analysis of expression levels of *ahr-1* (A) and *fmo-2* (B) in young and old N2 animals grown in K12 or K12 Δ *tnaA*. (C) *fmo-2* expression level in young and old *ahr-1(ia3)* animals grown in K12 or K12 Δ *tnaA*. *act-1* expression levels were used to normalize the *ahr-1* and *fmo-2* mRNA levels.

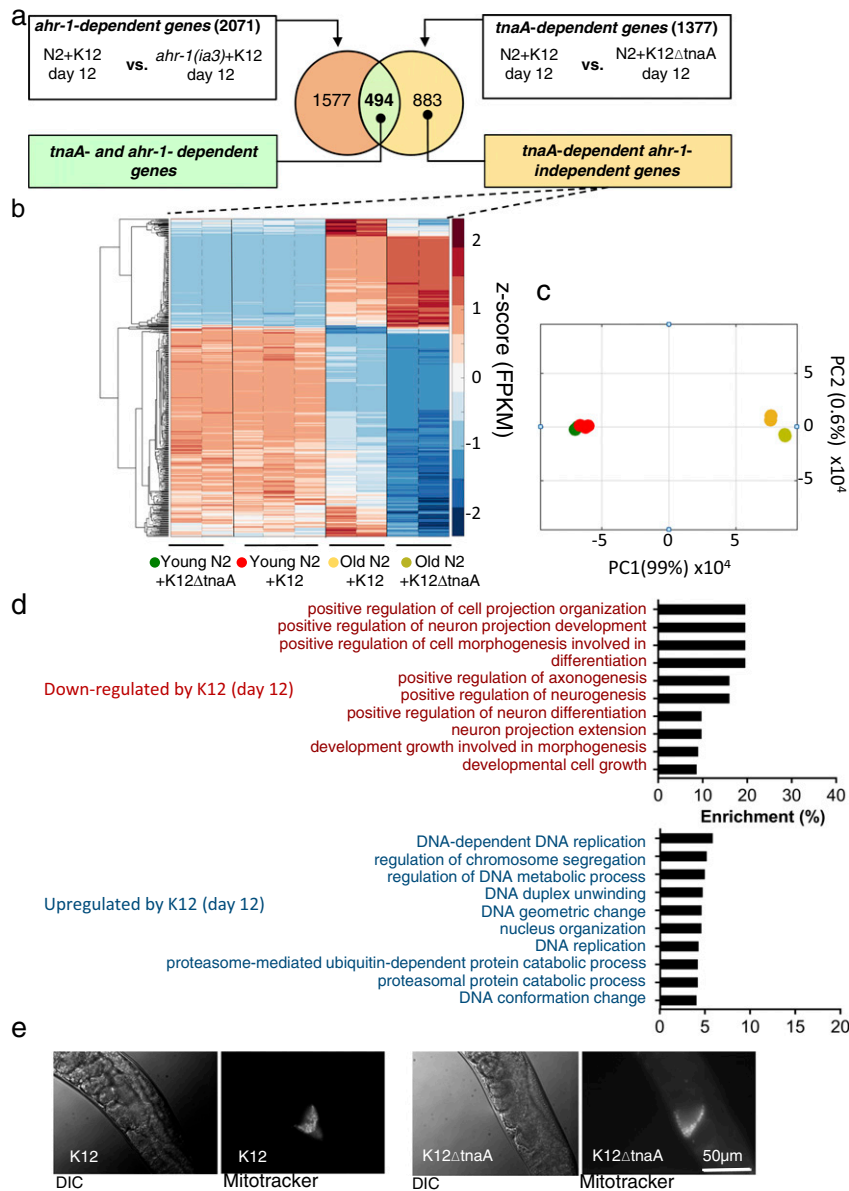


Fig. S4. Characterization of transcriptional responses from *C. elegans*. (A) Schematic for the identification TnaA-dependent and Ahr-independent genes. (B) Expression profile of TnaA-dependent and Ahr-independent genes in young and old animals grown in K12 or K12Δ*tnaA* through hierarchical clustering of genes based on their corresponding z-score values. The replicates in each condition are separated by dotted black line; conditions are demarcated by solid black line. (C) PCA with FPKM values of TnaA-dependent and Ahr-independent genes in young and old animals grown in K12 or K12Δ*tnaA*. (D) GO output with TnaA-dependent and Ahr-independent genes performed using GOAmigo software. (E) Images of spermatheca of day 12 adult N2 hermaphrodites grown in K12 or K12Δ*tnaA*, after mating with males whose sperm had been labeled with MitoTracker. (Scale bar, 50 μ m.)

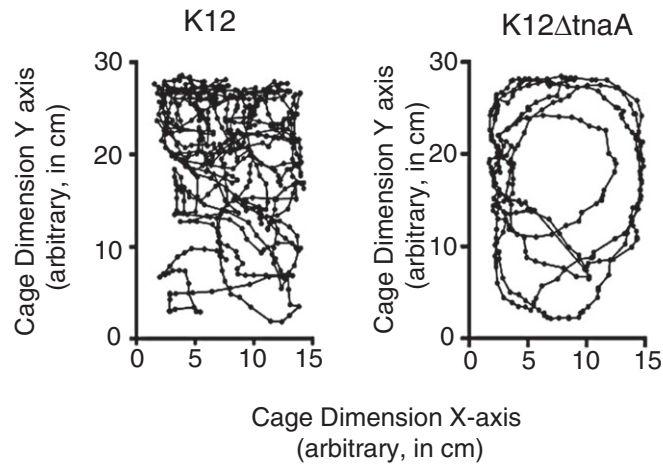


Fig. S5. Mouse motility in geriatric BALB/c animals colonized with K12 or K12 Δ tnaA. Motility tracking of geriatric animals for 2 min. The y axis represents the long side of a cage in centimeters, and the x axis represents the short side of the cage in centimeters. The images show representative tracks from two geriatric animals one colonized with K12 (Left), and the other with K12 Δ tnaA (Right) for 90 d. From these data, the total distance traveled was calculated.

Table S1. FPKM values of *ahr-1* and *fmo-2* in young and old N2 animals grown in K12 or K12 Δ tnaA and FPKM values of *fmo-2* in young and old *ahr-1(ia3)* animals grown in K12 or K12 Δ tnaA

Gene	Conditions	FPKM values
<i>ahr-1</i>	N2-young + K12 Δ tnaA	7.1, 3.2
<i>ahr-1</i>	N2-young + K12	4.7, 14.3
<i>ahr-1</i>	N2-old + K12 Δ tnaA	28.9, 33.9, 23.1
<i>ahr-1</i>	N2-old + K12	47, 31.3
<i>fmo-2</i>	N2-young + K12 Δ tnaA	32.5, 25.8
<i>fmo-2</i>	N2-young + K12	55.65, 48.5
<i>fmo-2</i>	N2-old + K12 Δ tnaA	505.8, 375.4, 518.5
<i>fmo-2</i>	N2-old + K12	413.8, 397.2
<i>fmo-2</i>	<i>ahr-1(ia3)</i> -young + K12 Δ tnaA	83.5, 63.9, 74.5
<i>fmo-2</i>	<i>ahr-1(ia3)</i> -young + K12	54.9, 62.8, 83.1
<i>fmo-2</i>	<i>ahr-1(ia3)</i> + K12 Δ tnaA	1,251.3, 681.5, 756.2
<i>fmo-2</i>	<i>ahr-1(ia3)</i> -old + K12	439.5, 951.5, 357.2

Table S2. Alignment summary of total RNA-Seq reads to *C. elegans* reference genome (ce10)

Sample ID	Read length	No. of reads	% Total aligned	% Unaligned	% Stranded
14_N2tnaA_LATE2	1 × 151	17,077,925	98.53	1.47	99.57
15_N2tnaA_LATE3	1 × 151	11,568,564	91.67	8.33	99.57
16_N2K12_LATE1	1 × 151	17,635,082	98.41	1.59	99.67
18_N2K12_LATE3	1 × 151	16,765,410	97.66	2.34	99.66
2_N2tnaA_EARLY2	1 × 151	17,265,225	98.98	1.02	99.82
37_AHRK12_LATE1	1 × 151	13,349,441	96.24	3.76	99.58
39_AHRK12_LATE3	1 × 151	14,609,013	96.54	3.46	99.65
3_N2tnaA_EARLY3	1 × 151	16,956,473	98.84	1.16	99.81
4_N2K12_EARLY1	1 × 151	16,414,814	98.70	1.30	99.81
5_N2K12_EARLY2	1 × 151	16,813,260	98.88	1.12	99.76
6_N2K12_EARLY3	1 × 151	16,406,106	98.79	1.21	99.80

Table S3. Summary of the lifespan experiments

Figures	Conditions	Maximum lifespan + SEM, d	Median lifespan + SEM, d	N	Censored	P value
Fig. 1A	N2 + K12 Δ <i>tnaA</i>	25.5 \pm 1.0	17.2 \pm 0.8	534	15	<0.0001
	N2 + K12	27.7 \pm 1.1	20.2 \pm 0.7	677	15	
Fig. 1C	N2 + K12 Δ <i>tnaA</i> + vehicle	29.0 \pm 1.7	18.2 \pm 0.5	542	3	<0.0001
	N2 + K12 Δ <i>tnaA</i> + indole	29.6 \pm 1.2	22.4 \pm 1.2	492	5	
Fig. 1D	N2 + K12 + vehicle	32.5 \pm 0.5	22 \pm 1	226	0	<0.0001
	N2 + K12 + indole	36.5 \pm 1.5	25.5 \pm 1.5	243	0	
Fig. 1L	W1118 + K12 Δ <i>tnaA</i>	103.0 \pm 2.0	90.5 \pm 2.5	116	0	<0.0001
	W1118 + K12	110.0 \pm 4.0	100.0 \pm 5.0	60	0	
Fig. 2A	<i>ahr-1(ju145)</i> + K12 Δ <i>tnaA</i>	31.3 \pm 0.6	22.8 \pm 1.5	117	12	0.15
	<i>ahr-1(ju145)</i> + K12	32.0 \pm 0.6	22.3 \pm 1.2	112	5	
Fig. 2B	<i>ahr-1(ia3)</i> + K12 Δ <i>tnaA</i>	33.0 \pm 0.7	24.8 \pm 0.8	95	0	0.065
	<i>ahr-1(ia3)</i> + K12	31.5 \pm 0.5	23.0 \pm 0.9	104	0	
Fig. 2D	<i>ahr-1(ju145)</i> + K12 Δ <i>tnaA</i> + vehicle	28.7 \pm 2.0	24.0 \pm 2.0	98	1	0.051
	<i>ahr-1(ju145)</i> + K12 Δ <i>tnaA</i> + indole	29.0 \pm 1.7	22.7 \pm 2.3	111	1	
Fig. 2E	<i>ahr-1(ia3)</i> + K12 Δ <i>tnaA</i> + vehicle	30.5 \pm 1.5	21.5 \pm 1.5	368	3	0.36
	<i>ahr-1(ia3)</i> + K12 Δ <i>tnaA</i> + indole	30.5 \pm 1.5	20 \pm 2	258	2	
Fig. S2B	<i>sir-2.1(ok434)</i> + K12 Δ <i>tnaA</i>	22.0 \pm 0.0	18.0 \pm 0.0	79	0	<0.0001
	<i>sir-2.1(ok434)</i> + K12	24.0 \pm 0.0	21.0 \pm 1.0	93	0	
Fig. S2C	<i>skn-1(ju169)</i> + K12 Δ <i>tnaA</i>	19.0 \pm 0.0	12.5 \pm 0.5	80	0	<0.0001
	<i>skn-1(ju169)</i> + K12	19.0 \pm 0.0	15.0 \pm 1.0	79	0	
Fig. S2D	<i>daf-16(m26)</i> + K12 Δ <i>tnaA</i>	23.0 \pm 0.0	18.0 \pm 0.0	88	7	<0.0001
	<i>daf-16(m26)</i> + K12	23.0 \pm 0.0	20.3 \pm 0.3	59	5	
Fig. S2E	<i>cat-2(e1112)</i> + K12 Δ <i>tnaA</i>	21.3 \pm 0.7	17.6 \pm 1.55	71	0	<0.0001
	<i>cat-2(e1112)</i> + K12	24.0 \pm 1.0	20.0 \pm 0.0	56	2	
Fig. S2F	<i>dop-3(vs.106)</i> + K12 Δ <i>tnaA</i>	30.0 \pm 0.0	22.0 \pm 1.0	91	0	<0.0001
	<i>dop-3(vs.106)</i> + K12	30.0 \pm 0.0	25.0 \pm 0.0	90	0	

P values were obtained from log-rank (Mantel–Cox) test.