Supplementary Information

Crocin extends lifespan by mitigating oxidative stress and regulating lipid metabolism through the DAF-16/FOXO pathway

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This PDF file includes:

Gene	Prime forward (5' to 3')	Prime reverse (5' to 3')
actin-1	ggaaatcaccgctcttgccc	cttgcggtgaacgatggatgg
daf-2	cggtgcgaagagaggatatt	tacagaggtcgccgttactg
daf-16	cgatctagtcccgaggaggc	gtacgccgtggattccttcc
skn-1	agtgtcggcgttccagatttc	gtcgacgaatcttgcgaatca
hsp16.2	ctgcagaatctctccatctgagtc	agattggaagcaactgcacc
gst-4	cccattttacaagtcgatgg	cttcctctgcagtttttcca
sod-3	agcatcatgccacctacg	caccaccattgaatttcagcg
fat-1	atgtcgatgatgtcgctgagg	gaagtgatgggcaacgtgtc
fat-2	ctgtgcttacattgcctttgtc	ggagttgcctccaacagatg
fat-4	tcacatccaggtggtagtgc	ggttcttgtgtagggcactc
fat-5	gttggatgggtattcctcctgc	ggaggtagttcttcctctggaac
fat-6	ccacataacaccactcgtgg	catccgaaatagtgagcagcg
fat-7	ctcaggattcggtatcacagc	gggaaatagtgctttctctgg
elo-2	ctctcaaccttgccgtccac	cgatcgatagcacattccagc
atgl-1	caaatgtggattggctccatg	cgaatggtttcttcatgatacttg
lipl-4	ggcactctcactatgtttgc	cggacccatgtttctttacg
lipi-3	tgactacgccccaaatcattg	cgaggccttcaagtttttgtgc
acs-2	accgagacatcccctctagtc	gacgttctcaccacccttcac

Table S1 Primers used in RT-qPCR experiments

Table S2 Primers used in RNAi experiments

Prime	Sequence
L4440-*daf-16*-F	attcgatatcaagcttGAAGAACTCGATCCGTCAC
L4440-*daf-16*-R	cggtatcgataagcttTTACAAATCAAAATGAATATGCTGCC

Table S3 Statistical analysis of the lifespan

Strains	Treatment	Mean lifespan ± SEM	Number	*p*-value
N2 (Wild type)	Control	16.669±0.24	117	
	CR 50 μM	18.431±0.292	121	<0.0001
	CR 100 μM	18.081±0.263	111	<0.0001
	CR 200 μM	19.247±0.253	115	<0.0001
	RES 200 μM	19.532±0.33	110	<0.0001
Mutant strains				
N2 (Wild type)	Control	17.736±0.341	148	
daf-16 (mu86)	Control	16.013±0.331	150	<0.0001 [a]
	CR 200 μM	15.979±0.384	140	0.4256
daf-2 (e1370)	Control	21.347±0.38	147	<0.0001 [a]
	CR 200 μM	22.664±0.451	143	0.0049 [b]

[a] *p*-value indicates significant differences compared to the N2 (wild-type) control group; [b] indicates significant differences compared to the control group of common strain, as determined by the Log-rank test. *p* <0.05 was considered statistically significant. CR: crocin; RES: resveratrol.

47 **Table S4 The 42 hub targets information of PPI network**

Gene Name	Degree	Betweenness	Closeness
MAPK3	19	614.65	40.66667
IL6	18	870.22444	41
MAPK1	18	467.97488	40.33333
STAT3	17	540.44581	39.66667
BCL2	17	669.97778	39
TNF	15	453.34777	39.33333
IL1B	13	180.42712	36.25
RELA	12	333.93453	35
MYC	11	286.40603	34.83333
TGFB1	11	240.94366	32.75
MMP9	11	524.83021	32.58333
CASP3	10	516.04231	35.08333
CREB1	9	119.35705	34.75
ESR1	9	70.67062	32.83333
PTPN11	9	108.22147	31.16667
CASP8	8	151.88508	32.33333
TLR4	8	70.74014	31.91667
IL10	8	17.65963	31.66667
HIF1A	7	16.03675	32.58333
AKT2	7	276.20684	31
CCND1	6	13.70804	30.91667
PTGS2	6	260.8381	30.66667
HRAS	6	32.02895	30
KEAP1	6	211.24232	29.75
BECN1	6	63.28642	29.41667
MAP2K1	6	18.29744	28.91667
GSK3B	6	55.04251	28.9
SQSTM1	6	59.66937	28.11667
NFE2L2	6	96.08782	27.56667
SMAD2	5	99.548	30.08333
ALB	5	351.21183	29.5
JAK2	5	77.07528	29.5
CYCS	5	82.12315	28.91667
CCL5	5	1.36905	28.83333
MYD88	5	24.46984	28.75
MAPK14	5	6.90543	28.41667
MMP2	4	61.75394	28.56667
APP	4	275.2	25.78333
IGF1	3	8.85288	28
NOS2	3	144.0381	24.81667
ELANE	3	23.35098	23.2
SERPINA1	2	18.67149	21.86667

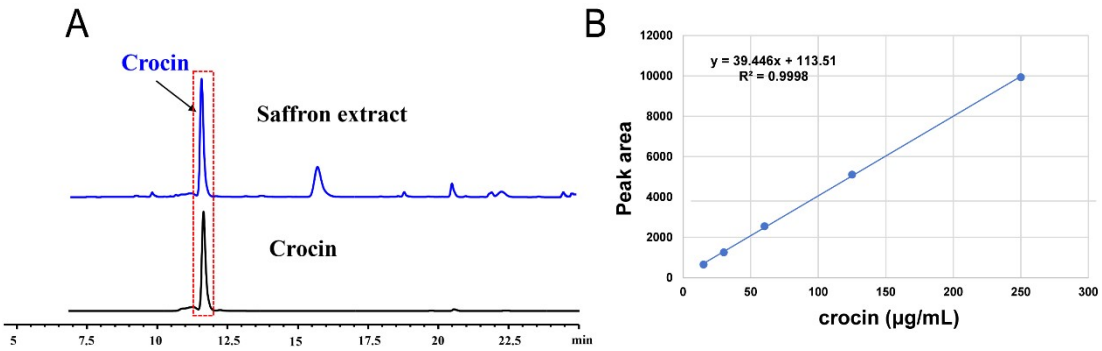

Figure S1 High performance liquid chromatography (HPLC) analysis of saffron extract. (A) HPLC of saffron extract and crocin detected at 440 nm. (B) Standard curve of crocin (15.0625-250 μg/mL) with the linear regression equation (y = 39.446x + 113.51; R^2 = 0.9998) used for quantification.

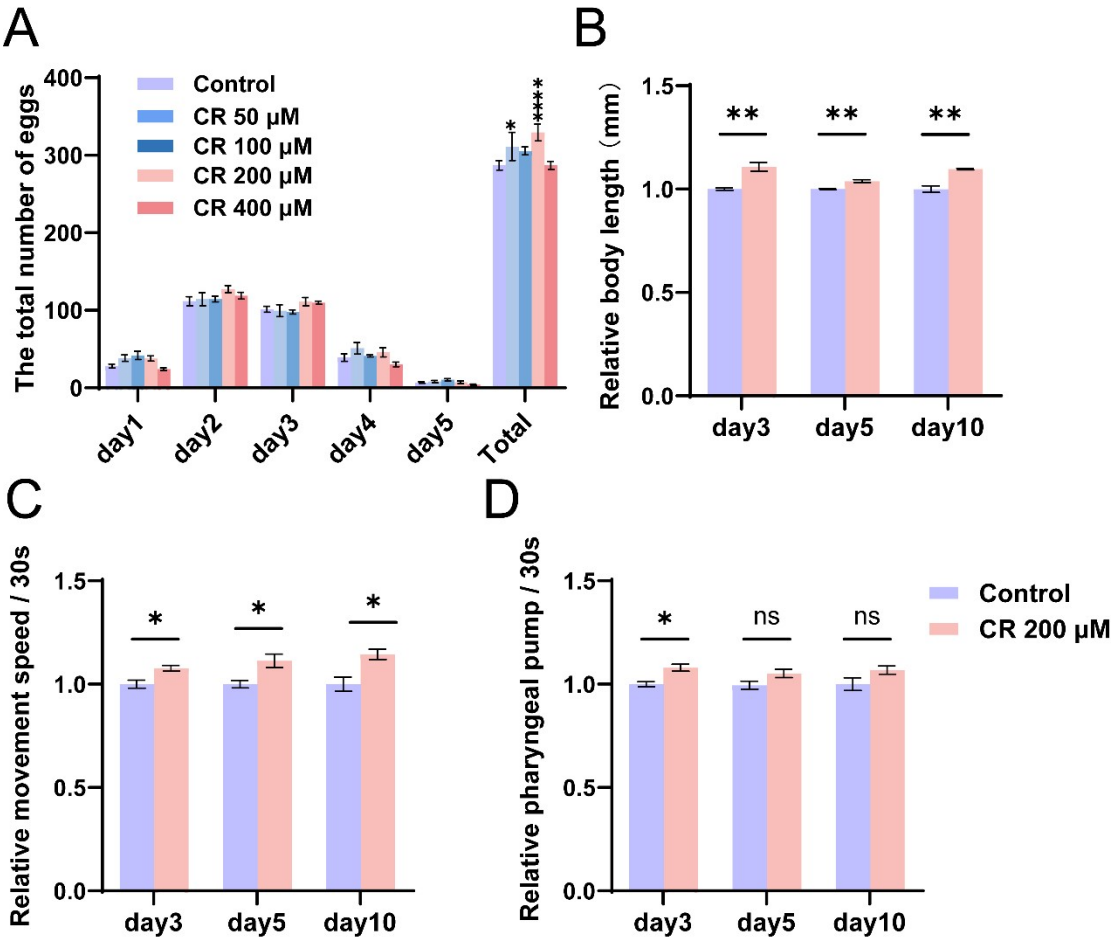

Figure S2 Crocin had no side effect in growth and development of *C. elegans*. (A) Reproductive ability of *C. elegans* treated with crocin (50–400 μM). Data presented as the average total eggs per nematode from ten replicates. (B-D) The indictors of growth

and development in *C. elegans* in different ages, including relative body length(B), relative movement speed in 30 seconds (C) and relative pharyngeal pump in 30 seconds (D). All data represented as mean ± SEM with three biological replicates (n = 20 per replicate). $p < 0.05$ was considered as statistically significant * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

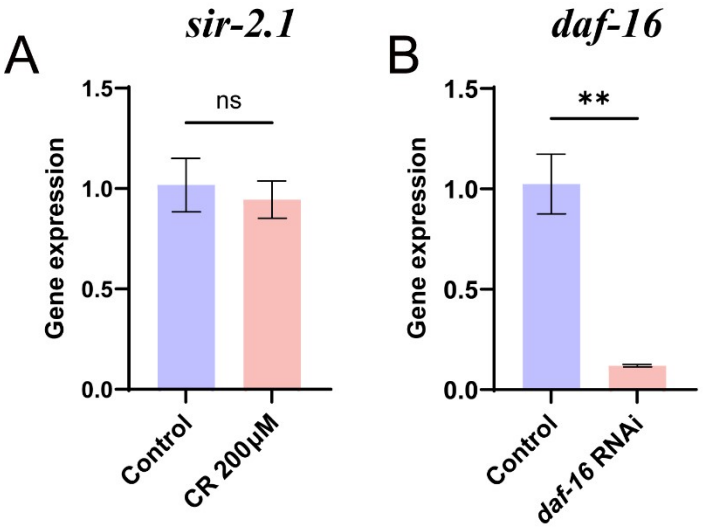

Figure S3 Gene expression of *sir-2.1* and interference efficiency of *daf-16/FOXO*. (A) The expression of *sir-2.1* in crocin-treated aged nematodes. (B) The expression of *daf-16*/*FOXO* gene significantly decreased in *daf-16* RANi nematodes. $p < 0.05$ was considered as statistically significant; ** $p < 0.01$.

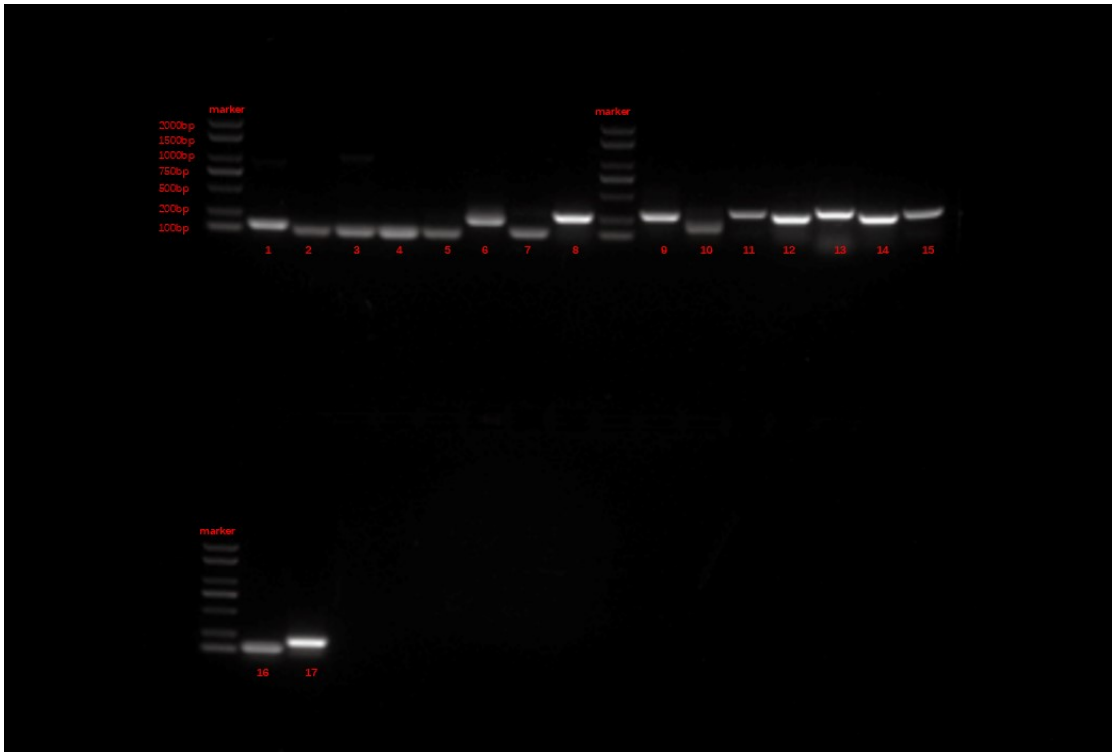

Figure S4 Electrophoresis bands of the genes used in this study. Line 1-17 indicated genes *actin-1*, *daf-16, daf-2, skn-1, gst-4, hsp-16.2, sod-3, atgl-1, lipl-3, lipl-4, acs-2, fat-2, fat-5, fat-6, fat-7, fat-4, elo-2,* respectively.

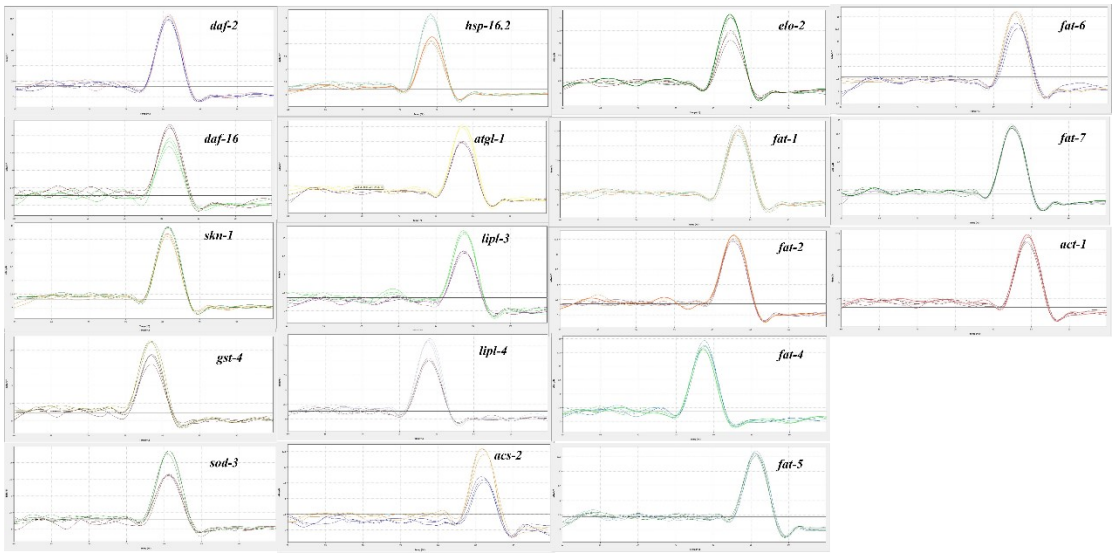

Figure S5 The melting curves of the primers used in RT-qPCR study.

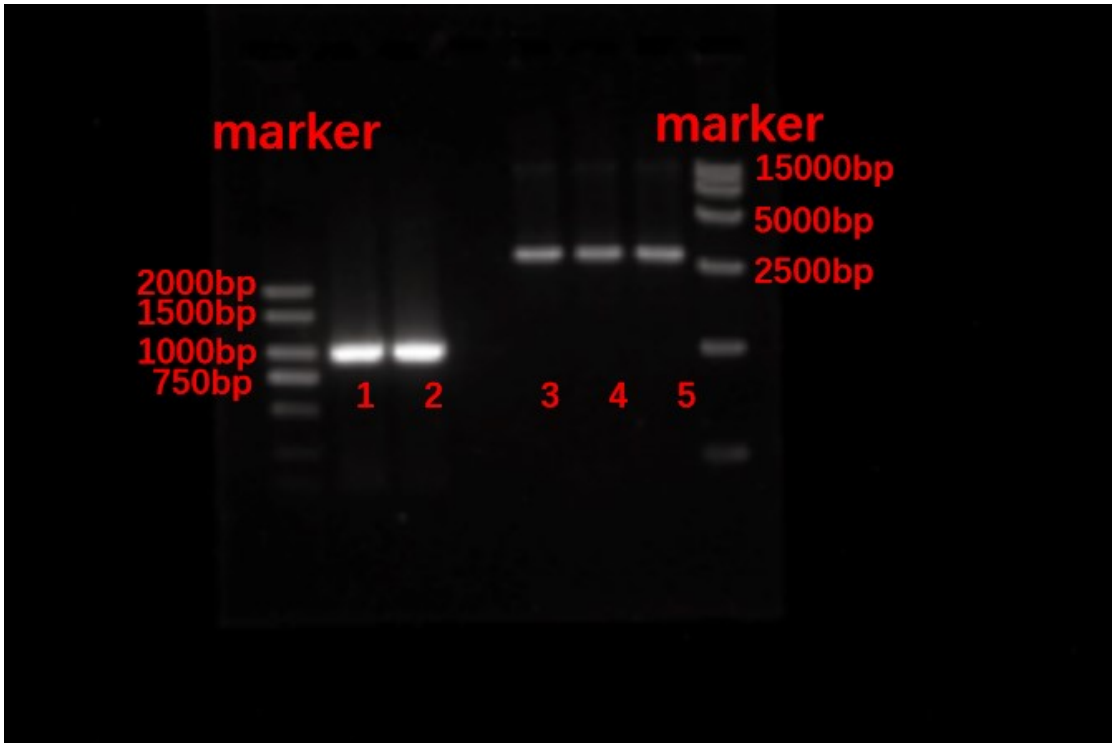

Figure S6 Gel electrophoresis analysis was performed on both the *daf-16* RNAi fragment and the enzyme-digested L4440 plasmid. Lines 1 and 2 were *daf-16* RNAi fragment and lines 3-5 were enzyme-digested plasmid L4440.

Figure S7 Electrophoresis bands of the *daf-16* RNAi fragments in the recombinant plasmid L4440-*daf-16*. Lines 1-6 were the *daf-16* RNAi fragment inserted into the L4440 plasmid using L4440-*daf-16* primers.