

Supplementary material

**Flavonoids' Effects on *Caenorhabditis elegans*' Longevity, Fat Accumulation,
Stress Resistance and Gene Modulation Involve mTOR, SKN-1 and DAF-16**

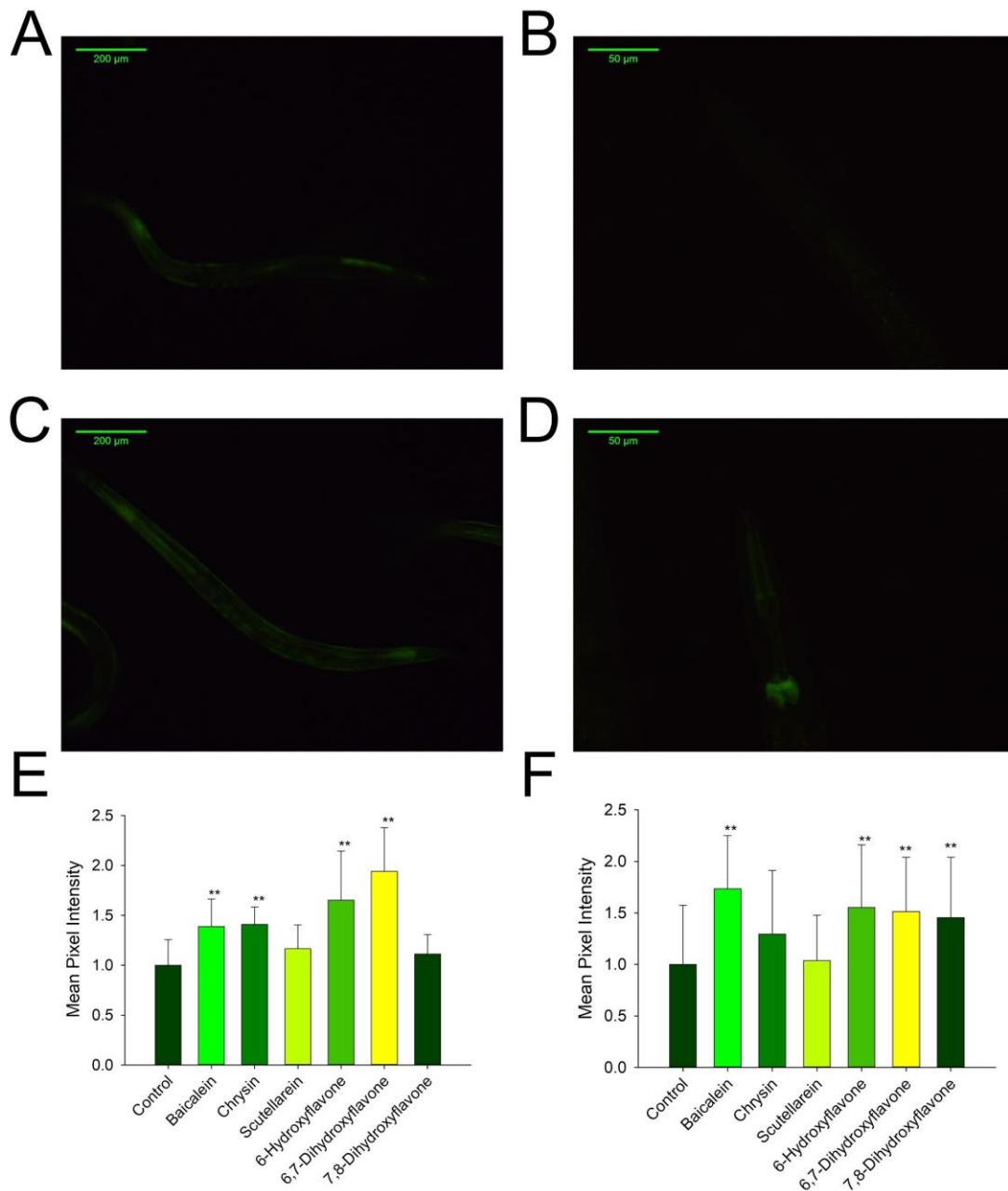


Figure S1. Flavones effect on GST-4 and GSC-1 expression. (a-d) Representative image of: (a) Control *C. elegans* of the CL2166 (GST-4 reporter) strain. (b) Control animal of the LD1171 (GSC-1 reporter) strain. (c) CL2166 worm treated with 6,7-dihydroxyflavone (100 μ M). (d) *C. elegans* LD1171 treated with baicalein (100 μ M). Scale bar for (a) and (c) 200 μ m, magnification 10x, and scale bar for (b) and (d) 50 μ m, magnification 40x. (e) Quantification of the flavones effect on the expression of GST-4. (f) Quantification of the flavones effect on the expression of GSC-1. Data is represented as mean \pm S.D, n > 10, two independent experiments were performed, **significantly at $p \leq 0.05$ by ANOVA test.

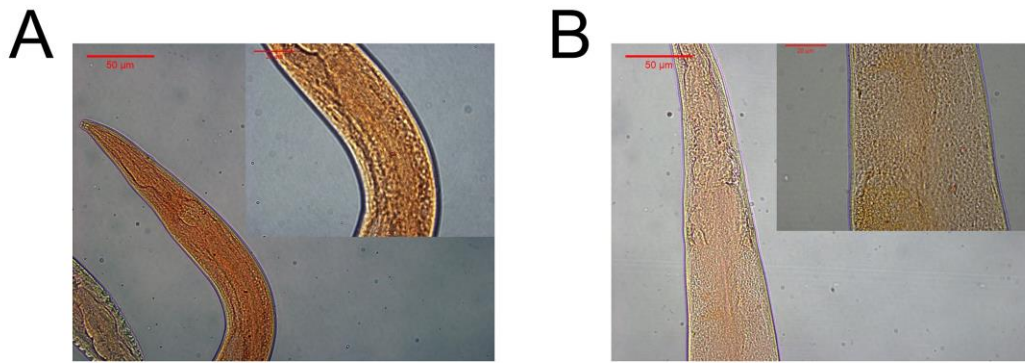


Figure S2. Effect of flavonoids stained with Red O (ORO). **(a)** DMSO control worm magnification 40x, inset magnification 100x. **(b)** Worm treated with 100 μ M of chrysin magnification 40x, inset magnification 100x. Scale bar: 50 μ m, scale bar of the insets 20 μ m.

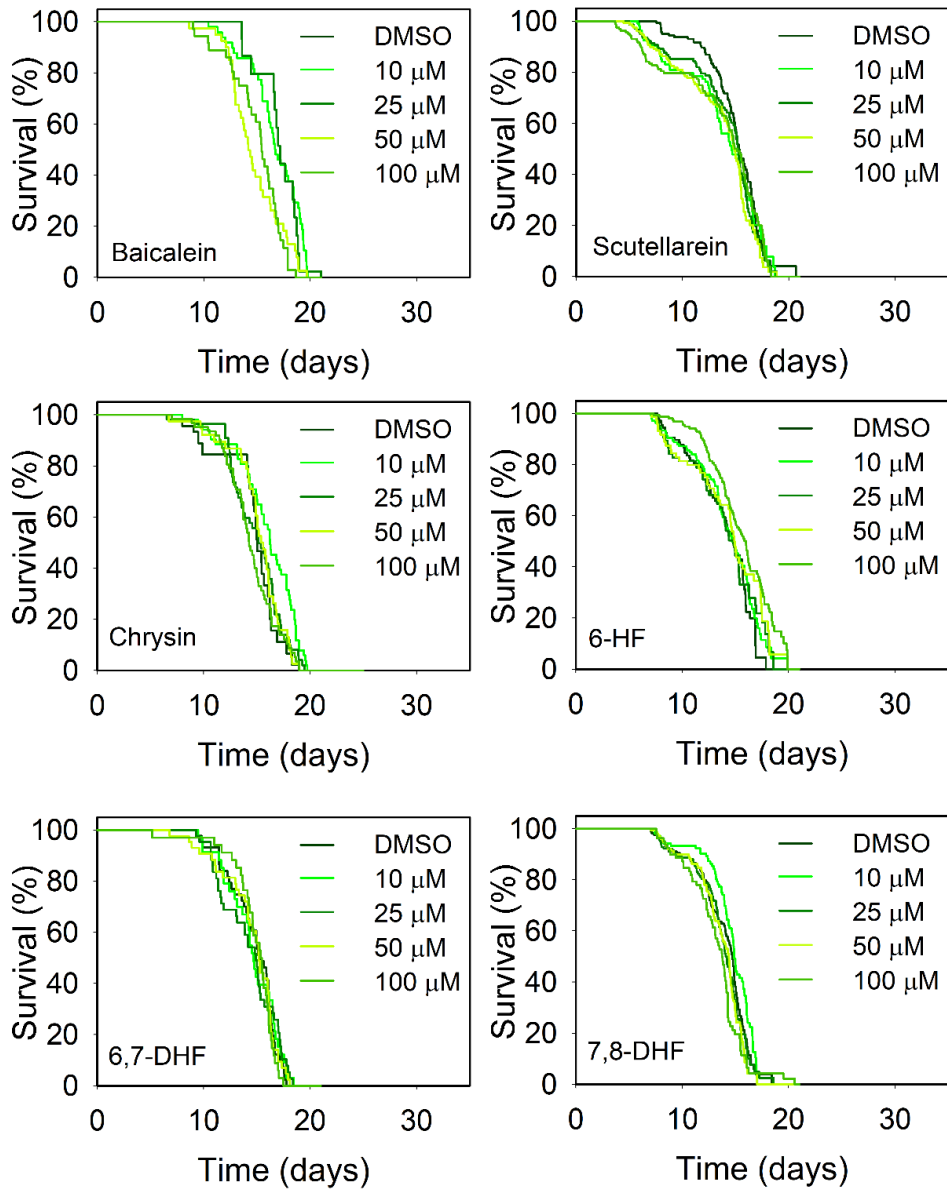


Figure S3. Lifespan curves adjusted to Kaplan-Meier estimator for the *C. elegans* strain N2 treated with individual flavonoids.

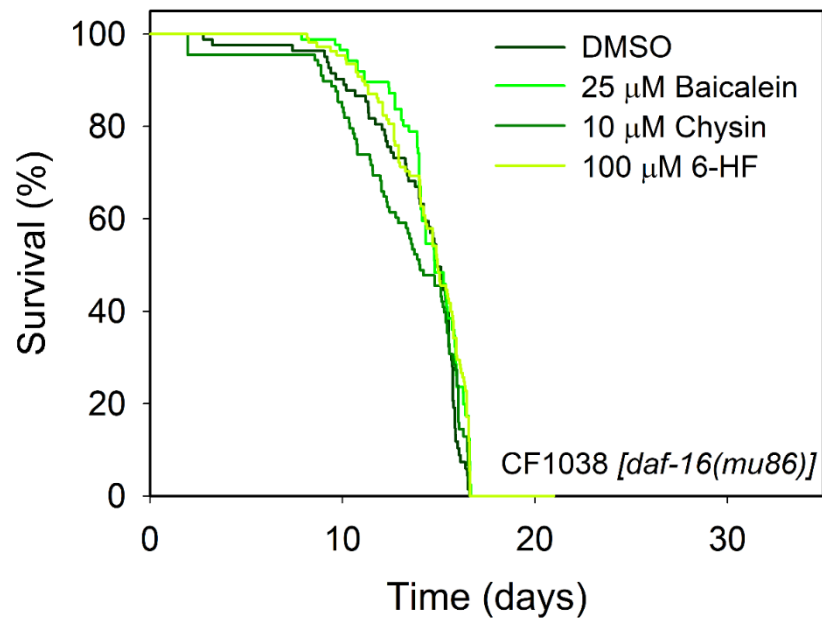


Figure S4. Lifespan curves adjusted to Kaplan-Meier estimator for the *C. elegans* strain CF1038 treated with individual flavonoids baicalein, chrysin and 6-hydroxyflavone.

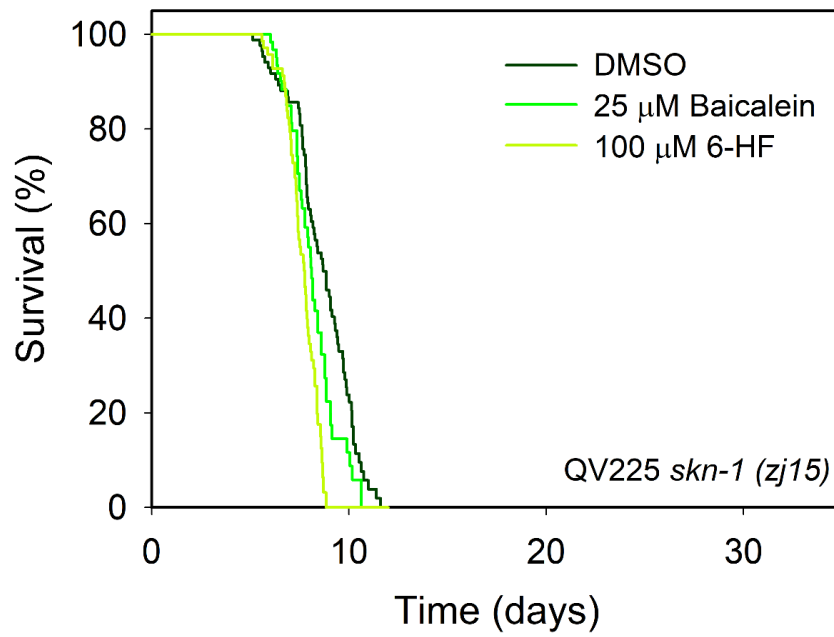


Figure S5. Lifespan curves adjusted to Kaplan-Meier estimator for the *C. elegans* strain QV225 treated with baicalein and 6-hydroxyflavone.

Supplemental Table 1. Survival data for the *C. elegans* treated with flavonoids

<i>C. elegans</i> Strain	Flavonoid	Concentration (μ M)	n	Mean Lifespan (days)	S. E (\pm day)	Lifespan Change (%)	<i>p</i> -value vs Control
N2 Wild-type	Baicalein	0	166	14.33	0.24		
		10	98	16.7	0.25	16.54	0**
		25	95	16.98	0.27	18.49	0**
		50	80	14.72	0.28	2.72	0.0576
		100	72	14.96	0.29	4.40	0.0077**
	Chrysin	0	90	14.66	0.28		
		10	104	15.9	0.28	8.46	0.000006**
		25	110	14.92	0.25	1.77	0.1914
		50	76	15.1	0.29	3.00	0.226
		100	124	14.38	0.23	-1.91	0.4426
	Scutellarein	0	163	14.58	0.29		
		10	109	13.79	0.4	-5.42	0.3076
		25	105	14.08	0.31	-3.43	0.3288
		50	136	13.54	0.32	-7.13	0.0457**
		100	123	13.59	0.36	-6.79	0.3814
	7,8- Dihydroxyflavone	0	175	13.89	0.21		
		10	105	14.58	0.23	4.97	0.0263**
		25	160	13.71	0.22	-1.30	0.4389
		50	156	13.59	0.2	-2.16	0.1045
		100	83	13.24	0.31	-4.68	0.0177
6,7- Dihydroxyflavone	0	88	14.71	0.24			
	10	70	14.55	0.3	-1.09	0.4585	
	25	86	14.29	0.29	-2.86	0.5925	
	50	86	14.52	0.28	-1.29	0.8729	
	100	88	14.77	0.27	0.41	0.2216	
6-Hydroxyflavone	0	111	13.96	0.29			
	10	140	14.29	0.3	2.36	0.3262	
	25	88	14.04	0.4	0.57	0.5242	

		50	74	14.41	0.45	3.22	0.1312
		100	159	15.61	0.25	11.82	0.0001**
		0	82	13.87	0.31		
	Baicalein	25	86	14.56	0.21	4.97	0.0168**
<i>(skn-1(zj15))</i>	Chrysin	10	89	13.15	0.37	-5.19	0.4943
	6-Hydroxyflavone	100	108	14.38	0.21	3.68	0.0079**
		0	85	8.68	0.17		
CF1038 (<i>daf-16(mu86)</i>)	Baicalein	25	61	8.19	0.16	-5.65	0.0172**
	6-Hydroxyflavone	100	70	7.65	0.1	-11.87	7.8e-8**

Supplemental Table 2. Pathways modulated by baicalein treatment. Data

obtained from the RNA microarray results vs DMSO control worms.

Pathway Name	DMSO			Baicalein		
	Enrichment	Enrichment	%	Enrichment	Enrichment	%
	Score	<i>p</i> -value	genes	Score	<i>p</i> -value	genes
RNA transport	0.00	0.00	0.00	13.31	1.66E-06	60.00
Aminoacyl-tRNA biosynthesis	32.42	8.30E-15	52.94	12.05	5.84E-06	70.59
DNA replication	0.00	0.00	0.00	11.64	8.78E-06	78.26
Ribosome biogenesis in eukaryotes	0.00	0.00	0.00	11.49	1.03E-05	76.00
Base excision repair	0.00	0.00	0.00	9.16	1.05E-04	90.91
Oxidative phosphorylation	0.00	0.00	0.00	8.31	2.45E-04	56.60
2-Oxocarboxylic acid metabolism	0.00	0.00	0.00	8.12	2.96E-04	90.00
Fanconi anemia pathway	0.00	0.00	0.00	8.10	3.03E-04	71.43
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	0.00	0.00	0.00	7.84	3.93E-04	140.00
Phenylalanine metabolism	0.00	0.00	0.00	7.83	3.97E-04	100.00
Homologous recombination	0.00	0.00	0.00	6.52	1.48E-03	73.33
Nucleotide excision repair	0.00	0.00	0.00	6.09	2.28E-03	61.54
Biosynthesis of amino acids	0.00	0.00	0.00	5.92	2.69E-03	53.19
Mismatch repair	0.00	0.00	0.00	5.69	3.39E-03	71.43
Basal transcription factors	0.00	0.00	0.00	5.09	6.13E-03	63.16
Spliceosome	0.00	0.00	0.00	4.52	1.09E-02	45.95
Cysteine and methionine metabolism	0.00	0.00	0.00	4.45	1.16E-02	55.56
Tyrosine metabolism	0.00	0.00	0.00	3.74	2.37E-02	58.82
mRNA surveillance pathway	0.00	0.00	0.00	3.56	2.86E-02	46.15
Citrate cycle (TCA cycle)	0.00	0.00	0.00	3.47	3.11E-02	50.00
Longevity regulating pathway - multiple species	0.00	0.00	0.00	3.31	3.64E-02	48.57
Arginine biosynthesis	0.00	0.00	0.00	3.25	3.87E-02	66.67
Carbon metabolism	0.00	0.00	0.00	3.01	4.91E-02	42.47
Lysosome	10.01	4.49E-05	18.67	0.00	0.00	0.00
ABC transporters	4.48	0.01	20.00	0.00	0.00	0.00