

Supplementary data

Table S1 List of gene primers used in qRT-PCR.

| Primer | Sequences (5' to 3') |
|------------------------|--------------------------|
| <i>pmp-3</i> forward | GTTCCCGTGTTTCATCACTCAT |
| <i>pmp-3</i> reverse | ACACCGTCGAGAAGCTGTAGA |
| <i>daf-2</i> forward | TCAAATGAACGAGGAGCCG |
| <i>daf-2</i> reverse | TGGAACACCGTAGGAAGAGC |
| <i>age-1</i> forward | CGGAAAGACCAAACCTGGGATC |
| <i>age-1</i> reverse | CGTAGGCTTCGACGCATAACG |
| <i>akt-1</i> forward | CAAAGCCTAAGGAAGGACAACC |
| <i>akt-1</i> reverse | CATGAATCCAACGCTGACGAAC |
| <i>akt-2</i> forward | ACATTCAGCGAAGCACGAACA |
| <i>akt-2</i> reverse | TACATGACCACTCCGACTCCC |
| <i>daf-16</i> forward | TCGTCGTCTCGTGTTTCTCCA |
| <i>daf-16</i> reverse | TTCCATAGGCACCCGGTAGTG |
| <i>pha-4</i> forward | GAACCACGCAAGCACAGATG |
| <i>pha-4</i> reverse | CGGGTTGGTGGAGCTGTAAA |
| <i>ifg-1</i> forward | AGCAAAGAGATCGTATGCAC |
| <i>ifg-1</i> reverse | ACAAACTTCAATAACCGCAG |
| <i>let-363</i> forward | CACAGGCGAACTAATACCGT |
| <i>let-363</i> reverse | TTTCCGAGTTGCTTGATGAT |
| <i>rsk-1</i> forward | GTTTGTGGGATTCACC |
| <i>rsk-1</i> reverse | TGGCTTTCTCGGGCTCTT |
| <i>lgg-1</i> forward | AACAACCTTGAGAAGCGTCGTGCC |
| <i>lgg-1</i> reverse | ATCTTCTGGACGAAGTTGGATGCG |
| <i>ncr-1</i> forward | AGGCCATGGAAGTTCAGAGA |
| <i>ncr-1</i> reverse | GCTGGCGTCCAGTAAAGAAG |
| <i>asm-1</i> forward | ACGATTCTGGCCAACATGGT |
| <i>asm-1</i> reverse | AAGTCCATCCATAACTTGTG |
| <i>atg-5</i> forward | CTGGCGGAACTCACGGAG |
| <i>atg-5</i> reverse | CGCTTGATCGTAGATCAC |
| <i>vps-34</i> forward | GTCGATTCTGCCTACCGGAT |

| | |
|-----------------------|-----------------------|
| <i>vps-34</i> reverse | GGAGGCGTTGTTCGATCTTG |
| <i>lmp-1</i> forward | ATCCGCCACCGCTTCGCATT |
| <i>lmp-1</i> reverse | TCGAGCTCCCCTCTTTGGCG |
| <i>skn-1</i> forward | TTTTGCCTCCTCTCTTCTGGC |
| <i>skn-1</i> reverse | GGCGTATGGATGTTGGTGATG |

Table S2 Statistical analyses of the lifespan of *C. elegans* treated with astaxanthin

| Conc. (μg/mL) | Number | Mean lifespan (d) | Max. lifespan (d) |
|---------------|--------|-------------------|-------------------|
| 0 | 223 | 15.2 ± 0.2 | 23.3 ± 1.7 |
| 0.5 | 224 | 15.3 ± 0.3 | 24.3 ± 2.1 |
| 1 | 209 | 15.4 ± 0.3 | 25.7 ± 1.5 |
| 5 | 227 | 16.0 ± 0.3* | 25.7 ± 0.8 |
| 10 | 210 | 16.1 ± 0.3* | 26.0 ± 1.0 |
| 20 | 225 | 17.0 ± 0.3* | 27.3 ± 0.7* |

Worms from three independent experiments were combined for the analyses. The mean lifespans were analyzed by the Kaplan–Meir survival analysis of SPSS 22.0 and expressed as mean ± SEM. The maximum lifespans were the average maximum lifespan of three independent experiments.

*Significantly different from the control-treated worms ($p < 0.05$).

Table S3 Effects of astaxanthin on lifespan in *skn-1* RNAi worms

| Group | Number | Mean lifespan (d) | Max. lifespan (d) |
|---------------------------------|--------|-------------------------|-------------------------|
| L4440 + Control | 194 | 16.1 ± 0.3 ^b | 25.3 ± 0.7 ^b |
| L4440 + Astaxanthin | 204 | 17.7 ± 0.2 ^a | 28.3 ± 0.3 ^a |
| <i>skn-1</i> RNAi + Control | 219 | 13.7 ± 0.1 ^c | 19.0 ± 1.0 ^c |
| <i>skn-1</i> RNAi + Astaxanthin | 217 | 13.8 ± 0.2 ^c | 19.3 ± 0.3 ^c |

Worms from three independent experiments were combined for the analyses. The mean lifespans were analyzed by the Kaplan–Meir survival analysis of SPSS 22.0 and expressed as mean ± SEM. The maximum lifespans were the average maximum lifespan of three independent experiments.

Different letters denote mean values that are statistically different at $p < 0.05$.

Table S4 Lifespan of *C. elegans* treated with astaxanthin in the presence of chloroquine

| Group | Number | Mean lifespan (d) | Max. lifespan (d) |
|---------------------------|--------|-------------------------|--------------------------|
| Control | 240 | 14.2 ± 0.1 ^c | 20.0 ± 1.0 ^b |
| Astaxanthin | 226 | 16.0 ± 0.2 ^a | 23.7 ± 0.3 ^a |
| Chloroquine | 239 | 14.9 ± 0.2 ^b | 21.7 ± 0.7 ^{ab} |
| Astaxanthin + Chloroquine | 234 | 14.3 ± 0.2 ^c | 22.7 ± 1.2 ^{ab} |

Worms from three independent experiments were combined for the analyses. The mean lifespans

were analyzed by the Kaplan–Meir survival analysis of SPSS 22.0 and expressed as mean \pm SEM. The maximum lifespans were the average maximum lifespan of three independent experiments. Different letters denote mean values that are statistically different at $p < 0.05$.

Table S5 Effects of astaxanthin on lifespan in *pha-4* RNAi worms

| Group | Number | Mean lifespan (d) | Max. lifespan (d) |
|---------------------------------|--------|-----------------------------|------------------------------|
| L4440 + Control | 246 | 16.5 \pm 0.2 ^b | 25.7 \pm 0.3 ^b |
| L4440 + Astaxanthin | 260 | 17.8 \pm 0.3 ^a | 29.7 \pm 0.3 ^a |
| <i>pha-4</i> RNAi + Control | 254 | 15.5 \pm 0.2 ^c | 23.0 \pm 1.0 ^c |
| <i>pha-4</i> RNAi + Astaxanthin | 255 | 15.6 \pm 0.2 ^c | 25.0 \pm 0.6 ^{bc} |

Worms from three independent experiments were combined for the analyses. The mean lifespans were analyzed by the Kaplan–Meir survival analysis of SPSS 22.0 and expressed as mean \pm SEM. The maximum lifespans were the average maximum lifespan of three independent experiments. Different letters denote mean values that are statistically different at $p < 0.05$.

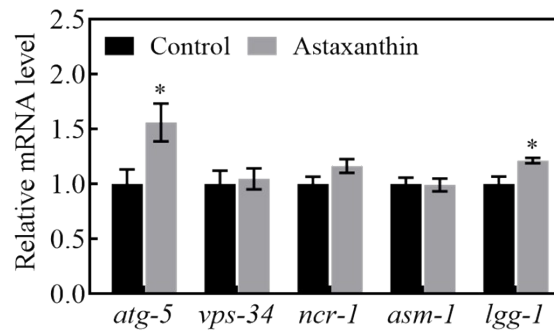


Fig. S1. Effects of astaxanthin (20 μ g/mL) on the expression of ALP-related genes in *skn-1* RNAi worms. The *skn-1* RNAi worms were treated with astaxanthin for 6 d before RNA extraction. The mRNA levels were determined by qRT-PCR and normalized to the expression of *pmp-3*.