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Supporting Information

The novel dipeptide Tyr-Ala (TA) significantly enhances the lifespan and healthspan of *Caenorhabditis elegans*

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The Effect of Bacterial Metabolism on TA activity

E. coli OP50 was killed in a boiling water bath for 15 min. We treated wild-type *C. elegans* that had just reached adulthood with heat-killed

E. coli OP50 bacteria and 10 mM TA for 48 h and then exposed the animals to juglone (500 μM) at 20 °C. The number of dead worms was

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counted and recorded every hour. The stress resistance effect in the inactive bacterial group was not significantly different from that of the group treated with live *E. coli* OP50 and 10 mM TA (Fig. S1). This result indicates that TA does not require bacterial metabolism for its activity.

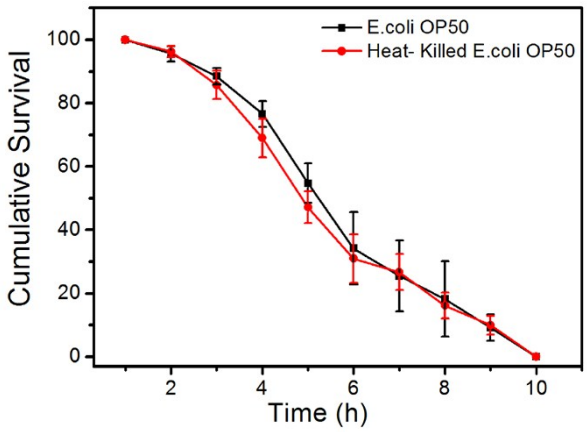

Fig. S1. Cumulative survival of wild-type *C. elegans* N2 that were treated with 10 mM TA and E. *coli* OP50 (black) or Heat-killed *E.coli* OP50 (red) under oxidative stress conditions. All survival curves are presented based on three individual experiments.

Stress resistance assay after treatment with Tyr

As Tyr is practically insoluble in water, we prepared a 100 mM Tyr stock solution by adding NaOH to an aqueous solution until the Tyr dissolved and diluted the solution to 10 mM in *E.coli* OP50. Worms that had just reached adulthood were pre-treated with various concentrations of Tyr (1 mM, 10 mM) for 48 h and then exposed to juglone (500 µM) at 20 °C. Heat-shock assays were performed using 2-day-old adult worms at 35 °C, which was considered to be a heat stressor. The worms were subjected to the same treatment described for the oxidative stress assay for 2 days at 20 °C and then transferred to an incubator at 35 °C. The number of dead worms was counted and recorded every hour. Our findings showed that the supplementation of tyrosine in the culture medium did not extend the lifespan under stress, which means TA works as an active unit in prolonging the lifespan of *C. elegans*.

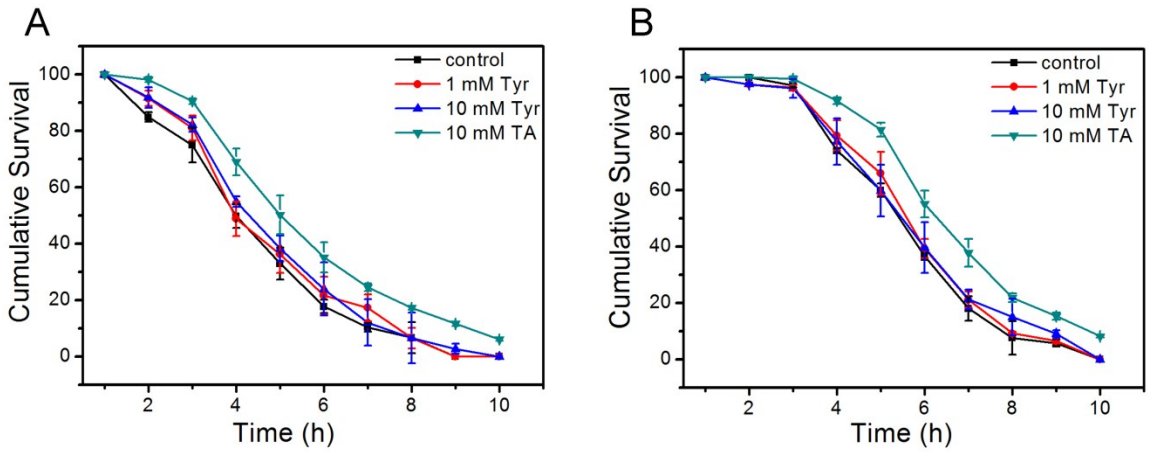

Fig. S2. Protective effects of Tyr on wild-type *C. elegans* N2 during oxidative stress (A) and heat stress (B).