Electronic Supplementary Material (ESI) for Food & Function.

Supplementary information for

In silico **and** ***in vivo*** **discovery of antioxidant sea cucumber peptides with antineurodegenerative properties**

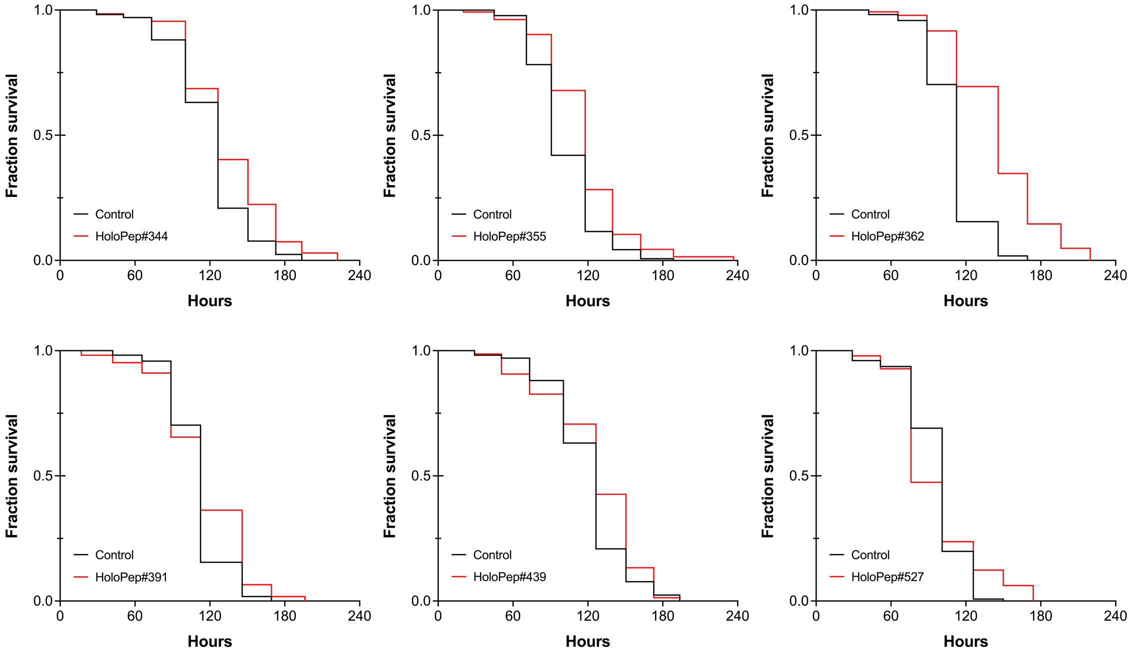
Qiangqiang Wang,[a] Ming Liang,[a,b] Yue Xiao,[a] Zhenhua Li,[c] Xiaohe Chen,[d] Peng Cheng,[c] Bing Qi,[c] Yi Yu,[b] Tao Lei*[d] and Zebo Huang*[a,c]

[a] *Institute for Food Nutrition and Human Health, School of Food Science and Engineering, South China University of Technology, Guangzhou 510641, China. E-mail: huangzebo@scut.edu.cn (Z. Huang)*

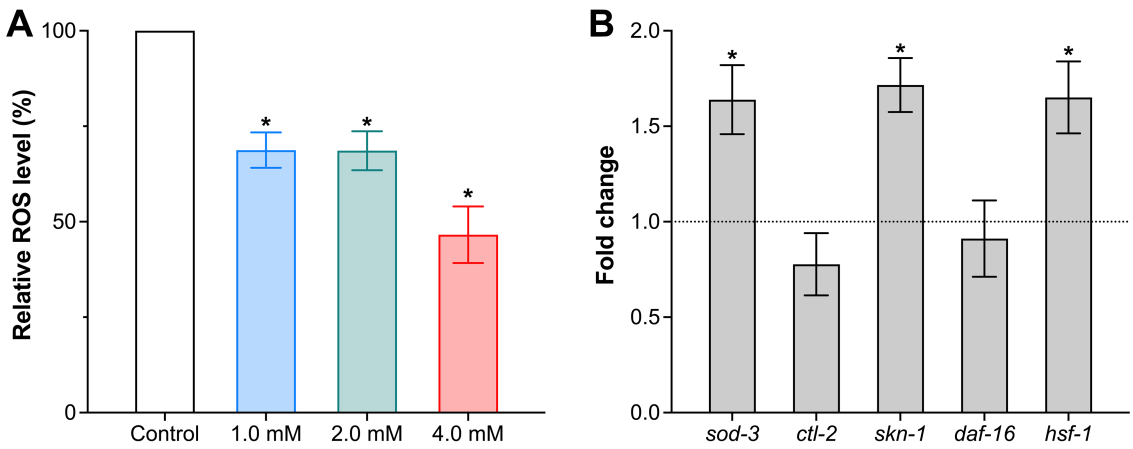
[b] *Research and Development Center, Infinitus (China) Company Ltd, Guangzhou 510405, China*

[c] *School of Biosciences and Biopharmaceutics, Guangdong Pharmaceutical University, Guangzhou 510006, China*

[d] *Rehabilitation Department, Wuhan Children's Hospital, Wuhan Maternal and Child Healthcare Center, Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430016, China. E-mail: rehabtaolei@163.com (T. Lei)*



Supplementary Fig. S1. Effect of selected sea cucumber peptides on *C. elegans* survival under increased oxidative stress. Shown is a graphical presentation of survival data for paraquat-exposed nematodes treated with selected sea cucumber peptides. After pretreatment with 2.0 mM of the peptides, wild-type nematodes were exposed to 50 mM paraquat for survival assay as in Fig. 3C. Representative results are shown as Kaplan–Meier survival curves.



Supplementary Fig. S2. Effect of sea cucumber peptide HoloPep#362 on ROS level and gene expression of *C. elegans* under increased oxidative stress. (A) Relative ROS level of paraquat-exposed *C. elegans*. Wild-type nematodes were pretreated with the indicated concentrations of HoloPep#362 and then exposed to 2 mM paraquat for DCF fluorescence determination as in Fig. 3D. Data are mean ± SEM of relative DCF fluorescence intensity after 120 min reaction. (B) Gene expression level of paraquat-exposed *C. elegans*. Wild-type nematodes were treated as in (A) using 2.0 mM HoloPep#362. Stress-related genes were analyzed by quantitative real-time PCR. Data are presented as mean ± SEM from three independent experiments. * $p < 0.05$ versus nematodes exposed to paraquat alone.

Supplementary Table S1

List of primers used for quantitative real-time PCR analysis.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Reference
sod-3	AGCATCATGCCACCTACGTGA	CACCACCATTGAATTTCAGCG	[1]
ctl-2	CTGGGAGAAGGTGTTGGAT	GGATGAACCTTTGAAAAGTGAT	[2]
skn-1	AGTGTCGGCGTTCCAGATTTC	GTCGACGAATCTTGCGAATCA	[1]
daf-16	GCGGAGCCAAGAAGAGGATA	GGAGAAACACGAGACGACGAT	[3]
hsf-1	TCAGCCGCAACAAGACTA	AGGTGGAAGTCGTTGGAT	[4]
cdc-42	CTGCTGGACAGGAAGATTACG	CTCGGACATTCTCGAATGAAG	[5]

References Supplementary Table S1:

[1] L. Zhang, G. Jie, J. Zhang and B. Zhao, Significant longevity-extending effects of EGCG on *Caenorhabditis elegans* under stress, *Free Radic. Biol. Med.*, 2009, **46**, 414–421.

[2] T. Sugawara, D. Saraprug and K. Sakamoto, Soy sauce increased the oxidative stress tolerance of nematode via p38 MAPK pathway, *Biosci. Biotechnol. Biochem.*, 2019, **83**, 709–716.

[3] Y. Luan, Y. Jiang, R. Huang, X. Wang, X. He, Y. Liu and P. Tan, Polygonati Rhizoma polysaccharide prolongs lifespan and healthspan in *Caenorhabditis elegans*, *Molecules*, 2023, **28**, 2235.

[4] X. Cui, B. Zhang, Z. Li, C. Li and J. Li, Zhuyeqing liquor promotes longevity through enhancing stress resistance via regulation of SKN-1 and HSF-1 transcription factors in *Caenorhabditis elegans*, *Exp. Gerontol.*, 2023, **174**, 112131.

[5] S. Weimer, J. Priebs, D. Kuhlow, M. Groth, S. Priebe, J. Mansfeld, T. L. Merry, S. Dubuis, B. Laube, A. F. Pfeiffer, T. J. Schulz, R. Guthke, M. Platzer, N. Zamboni, K. Zarse and M. Ristow, D-Glucosamine supplementation extends life span of nematodes and of ageing mice, *Nat. Commun.*, 2014, **5**, 3563.

Supplementary Table S2

In vivo antioxidant capacity and *in silico* BBB permeability and toxicity information of selected sea cucumber peptides.

Peptide ID [a]	Sequence	Number of amino acids	MW (Da)	ΔAUC% [b]	BBB [c]		Toxicity [d]
					Permeability	Probability	
HoloPep#344	AGLQFPVGR	9	944.10	12.6%	+	0.92	Non-toxin
HoloPep#355	EAIIKPSTF	9	1005.18	17.4%	−	0.70	Non-toxin
HoloPep#362	FETLMPLWGNK	11	1335.58	36.6%	+	0.70	Non-toxin
HoloPep#391	LLQPIMM	7	845.13	4.7%	+	0.62	Non-toxin
HoloPep#439	WNKFGQDTK	9	1123.23	5.5%	−	0.79	Non-toxin
HoloPep#527	PPPMLR	6	709.90	0.3%	+	0.60	Non-toxin

[a] Selected peptides as in Supplementary Fig. S1.

[b] Relative total survival gain (ΔAUC%), which is the change in the area under the survival curve of nematodes between treatments and control.

[c] The blood-brain barrier (BBB) permeability of peptides was analyzed using the ADME@NCATS program (https://opendata.ncats.nih.gov/adme/predictions). The symbols "+" and "−" indicate moderate-to-high and low permeabilities, respectively. The prediction also provides a corresponding probability score between 0 and 1.

[d] Potential toxicity of peptides was predicted using the ToxinPred online tool (https://webs.iiitd.edu.in/raghava/toxinpred/).

Supplementary Table S3

Differentially expressed proteins between HoloPep#362-treated and control polyglutamine *C. elegans* AM141.

UniProt ID	Protein name	Gene symbol	Treated *vs* untreated	
			p-value	log_2FC [a]
Q86NE0	Peptidase A1 domain-containing protein	*asp-2*	5.76e-06	2.30
G5EEI4	Aspartic protease 1	*asp-1*	6.98e-08	2.29
O01530	Aspartic protease 6	*asp-6*	1.59e-06	1.81
G3MU38	MIF-like protein mif-2	*mif-2*	4.32e-07	1.53
O01532	Peptidase A1 domain-containing protein	*asp-5*	3.65e-06	1.53
Q9N5S7	ThioredoXin domain containing protein homolog	*txdc-12.2*	1.53e-06	1.46
Q20950	Pept_C1 domain-containing protein	*cpr-9*	3.60e-05	1.39
P34340	Putative cuticle collagen 90	*col-90*	1.17e-05	1.33
H2KZG6	Acyl CoA DeHydrogenase	*acdh-1*	1.36e-06	1.33
Q09975	Lysozyme	*lys-8*	6.91e-04	1.33
P34528	Putative serine protease K12H4.7	*K12H4.7*	3.91e-05	1.30
Q23447	Uncharacterized protein	*CELE_ZK180.6*	3.28e-06	1.27
Q9XW83	Ground-like domain-containing protein	*grl-15*	2.11e-06	1.27
H8W3Y1	NOmpA Homolog	*noah-1*	4.12e-05	1.23
Q9N3V3	Ground-like domain-containing protein	*grl-5*	3.62e-05	1.23
Q22972	Peptidase A1 domain-containing protein	*asp-13*	9.59e-06	1.16
Q20603	SCP domain-containing protein	*scl-2*	1.55e-04	1.08
P55956	Aspartic protease 3	*asp-3*	1.73e-05	1.05
Q10008	Uncharacterized protein T19C3.2	*T19C3.2*	3.10e-05	1.05
O62415	Lysozyme-like protein 1	*lys-1*	3.71e-06	1.03
Q86FL8	Saposin B-type domain-containing protein	*spp-5*	2.12e-05	1.02
G5EDV0	Uncharacterized protein	*CELE_E01G4.6*	6.42e-06	1.01
Q067X2	Uterine Lumin Expressed/locailized	*ule-2*	6.15e-06	-1.01
P18835	Cuticle collagen 19	*col-19*	8.57e-05	-1.02
Q9XWU9	Uncharacterized protein	*CELE_Y37D8A.19*	2.03e-04	-1.04
Q19813	Col_cuticle_N domain-containing protein	*col-140*	3.13e-06	-1.09
Q03206	Ras-related protein ced-10	*rac-1*	1.76e-03	-1.11
P90889	Uncharacterized protein	*CELE_F55H12.4*	2.17e-05	-1.14
O17641	Col_cuticle_N domain-containing protein	*col-178*	7.87e-07	-1.20

Q7YTR9	Uncharacterized protein	*C27B7.9*	2.29e-06	-1.22
G5EFS5	Uncharacterized protein	*CELE_F45D11.15*	3.48e-05	-1.23
O44145	PERMeable eggshell	*perm-2*	2.33e-04	-1.32
O17891	Uncharacterized protein	*CELE_F55B11.2*	1.68e-06	-1.36
Q18529	Uncharacterized protein	*C39D10.7*	5.88e-05	-1.38
Q9XWT3	Uterine Lumin Expressed/locailized	*ule-5*	5.90e-04	-1.42
O44144	PERMeable eggshell	*perm-4*	4.69e-05	-1.49
Q9N4J2	Vitellogenin-3	*vit-3*	6.64e-06	-1.51
P55155	Vitellogenin-1	*vit-1*	3.47e-06	-1.66
Q18947	Uterine Lumin Expressed/locailized	*ule-3*	6.04e-08	-1.70
P06125	Vitellogenin-5	*vit-5*	1.52e-05	-2.00
Q18943	Uncharacterized protein	*CELE_D1054.10*	1.96e-05	-2.19
O17635	TransThyretin-Related family domain	*ttr-9*	3.46e-09	-2.50
P18948	Vitellogenin-6	*vit-6*	1.09e-09	-3.05
P05690	Vitellogenin-2	*vit-2*	3.22e-08	-3.69

[a] FC, fold change.