

Electronic Supplementary Information

Naringenin prolongs lifespan and delays aging mediated by IIS and MAPK in *Caenorhabditis elegans*

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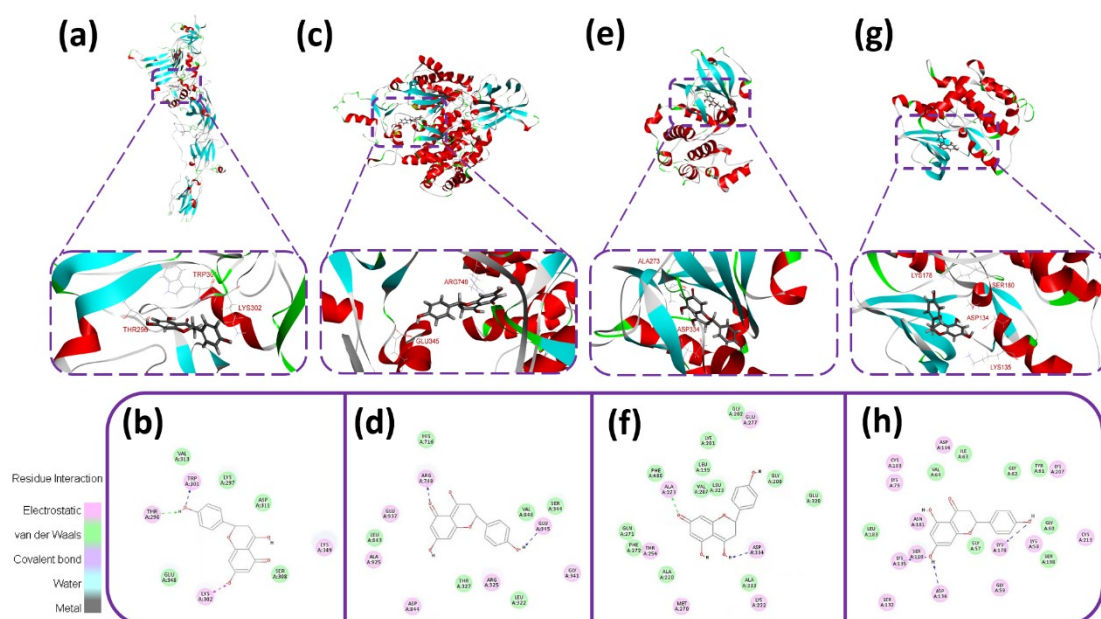


Fig. S1 Molecular docking study of NN with target proteins in *C. elegans*. Figure (a), (c), (e) and (g) represented the interaction of NN with DAF-2, AGE-1, AKT-1 and SEK-1, respectively. Two-dimensional docked figure (b), (d), (f) and (h) represented maximum interactions between NN and DAF-2, AGE-1, AKT-1, SEK-1 in *C. elegans*, respectively.

Table S1 Primers used for measuring mRNA expression

Gene	Gene ID	Primer sequences
<i>act-1</i>	NM_073418.9	Forward primer: GGAATTGCTGATCGTATGC
<i>act-1</i>	NM_073418.9	Reverse primer: CTGTTGGAAGGTGGAGAG
<i>daf-2</i>	NM_065249.7	Forward primer: TCGGACGCTATTCATATTACG
<i>daf-2</i>	NM_065249.7	Reverse primer: GGCTTATCGGCTACAATCG
<i>age-1</i>	NM_064061.6	Forward primer: ATTGTGCTCTCCGAACTC
<i>age-1</i>	NM_064061.6	Reverse primer: GCTGCTCATTCAACTTCTC
<i>akt-1</i>	NM_001028475.5	Forward primer: AGTCAGCTAAAGCATGGGAGA
<i>akt-1</i>	NM_001028475.5	Reverse primer: TCTTTGATTTGACGTTCACTGG
<i>daf-16</i>	NM_001381205.1	Forward primer: TCAAGCCAATGCCACTACC
<i>daf-16</i>	NM_001381205.1	Reverse primer: TGGAAGAGCCGATGAAGAAG
<i>hsf-1</i>	NM_060630.7	Forward primer: CATCATCTTCGGCTCCATCTG
<i>hsf-1</i>	NM_060630.7	Reverse primer: AATAGTCTTGTTGCGGCTGAG
<i>hsp-16.1</i>	NM_072953.5	Forward primer: CACTTTACCACTATTTCCGTCCAG
<i>hsp-16.1</i>	NM_072953.5	Reverse primer: GGCTTGAAGTGCAGACATTG
<i>hsp-16.2</i>	NM_001392482.1	Forward primer: CTCAACGTTCCGTTTTGGT
<i>hsp-16.2</i>	NM_001392482.1	Reverse primer: CGTTGAGATTGATGGCAAAC
<i>skn-1</i>	NM_171345.6	Forward primer: CACTGTCTCCTCTCATCATTGG
<i>skn-1</i>	NM_171345.6	Reverse primer: GAGTGTCTCTGTGAGTGATATGG
<i>sek-1</i>	NM_076921.8	Forward primer: TGGCTCTGTCGGTCATTG
<i>sek-1</i>	NM_076921.8	Reverse primer: TCTCTGGTGGCATGTAAGG
<i>sod-3</i>	NM_078363.9	Forward primer: GGCTAAGGATGGTGGAGAAC
<i>sod-3</i>	NM_078363.9	Reverse primer: ACAGGTGGCGATCTTCAAG
<i>ctl-1</i>	NM_064578.6	Forward primer: GCGTATCTTCTCCTACAC
<i>ctl-1</i>	NM_064578.6	Reverse primer: ACTCCTTCACATCATCAC
<i>ctl-2</i>	NM_001027302.8	Forward primer: TACAGTCGGTGGTGAGAG
<i>ctl-2</i>	NM_001027302.8	Reverse primer: GGTGAGTCTGTGGATTACG
<i>gst-4</i>	NM_069447.8	Forward primer: CTCTTGCTGAGCCAATCCGTATC

<i>gst-4</i>	NM_069447.8	Reverse primer: TGACTGACCGAATTGTTCTCCATC
<i>mtl-1</i>	NM_072295.6	Forward primer: TGGAGACAAGTGTGAGAGAAG
<i>mtl-1</i>	NM_072295.6	Reverse primer: TTAATGAGCCGCAGCAGTTC

Table S2 Effects of NN on the lifespan of N2 and mutant worms for the 1st replicate.
(mean $\pm$ SD)

Genotype	Treatment	Mean lifespan (days)	Maximum lifespan (days)	% change in mean lifespan	p value
N2	0 μ M	15.85 $\pm$ 2.04	24.13 $\pm$ 3.23	/	/
	50 μ M	17.48 $\pm$ 2.16	26.45 $\pm$ 2.86	10.07**	p<0.01
	100 μ M	17.95 $\pm$ 1.83	27.08 $\pm$ 3.53	13.27**	p<0.01
	200 μ M	18.52 $\pm$ 1.97	29.37 $\pm$ 2.17	20.98**	p<0.01
	0 μ M (H ₂ O ₂)	1.92 $\pm$ 0.29	3.29 $\pm$ 0.41	/	/
	200 μ M (H ₂ O ₂)	2.48 $\pm$ 0.23	4.33 $\pm$ 0.60	29.57**	p<0.01
	0 μ M (35 °C)	12.24 $\pm$ 1.73	19.62 $\pm$ 3.03	/	/
	200 μ M (35 °C)	12.03 $\pm$ 2.26	20.14 $\pm$ 2.42	-1.63	n. s.
	<i>daf-16 (mu86)</i> 0 μ M	11.85 $\pm$ 2.11	20.06 $\pm$ 3.32	/	/
	<i>daf-16 (mu86)</i> 200 μ M	12.15 $\pm$ 1.78	18.84 $\pm$ 1.61	2.52	n. s.
	<i>daf-2 (e1370)</i> 0 μ M	23.60 $\pm$ 3.69	36.26 $\pm$ 3.92	/	/
	<i>daf-2 (e1370)</i> 200 μ M	24.03 $\pm$ 2.30	34.19 $\pm$ 4.74	1.83	n. s.

<i>hsf-1 (sy441)</i>	0 μ M	13.30 $\pm$ 2.45	22.52 $\pm$ 3.81	/	/
	200 μ M	15.11 $\pm$ 1.63	25.05 $\pm$ 2.80	13.61**	p<0.01
<i>eat-2 (ad1116)</i>	0 μ M	25.24 $\pm$ 3.27	40.14 $\pm$ 3.77	/	/
	200 μ M	27.93 $\pm$ 2.63	43.22 $\pm$ 3.02	10.65**	p<0.01
<i>skn-1 (ZU67)</i>	0 μ M	11.03 $\pm$ 1.21	18.41 $\pm$ 2.15	/	/
	200 μ M	11.41 $\pm$ 0.93	19.28 $\pm$ 1.48	3.42	n. s.

n.s.: no significance.

Table S3 Effects of NN on the lifespan of N2 and mutant worms for the 2nd replicate.
(mean $\pm$ SD)

Genotype	Treatment	Mean lifespan (days)	Maximum lifespan (days)	% change in mean lifespan	p value
N2	0 μ M	16.43 $\pm$ 1.52	23.52 $\pm$ 2.26	/	/
	50 μ M	18.12 $\pm$ 2.02	26.77 $\pm$ 2.73	10.29**	p<0.01
	100 μ M	18.91 $\pm$ 1.33	27.96 $\pm$ 3.10	15.09**	p<0.01
	200 μ M	19.83 $\pm$ 1.60	28.60 $\pm$ 2.52	20.69**	p<0.01
	0 μ M (H ₂ O ₂)	1.79 $\pm$ 0.17	3.13 $\pm$ 0.34	/	/
	200 μ M (H ₂ O ₂)	2.25 $\pm$ 0.20	4.84 $\pm$ 0.41	25.70**	p<0.01
	0 μ M (35 °C)	12.76 $\pm$ 1.04	21.02 $\pm$ 3.58	/	/
	200 μ M (35 °C)	12.83 $\pm$ 1.27	20.44 $\pm$ 2.74	0.55	n. s.
	<i>daf-16 (mu86)</i> 0 μ M	12.16 $\pm$ 1.43	22.31 $\pm$ 3.19	/	/
	<i>daf-16 (mu86)</i> 200 μ M	11.75 $\pm$ 1.01	21.14 $\pm$ 2.98	-3.37	n. s.
	<i>daf-2 (e1370)</i> 0 μ M	21.92 $\pm$ 2.13	34.42 $\pm$ 3.53	/	/
	<i>daf-2 (e1370)</i> 200 μ M	22.23 $\pm$ 1.42	33.71 $\pm$ 2.81	1.41	n. s.

<i>hsf-1 (sy441)</i>	0 μ M	14.54 $\pm$ 1.33	24.77 $\pm$ 3.25	/	/
	200 μ M	17.02 $\pm$ 1.89	25.84 $\pm$ 2.16	17.06**	p<0.01
<i>eat-2 (ad1116)</i>	0 μ M	26.67 $\pm$ 2.12	42.73 $\pm$ 3.19	/	/
	200 μ M	29.33 $\pm$ 2.51	44.36 $\pm$ 2.47	9.97**	p<0.01
<i>skn-1 (ZU67)</i>	0 μ M	10.16 $\pm$ 0.78	17.97 $\pm$ 2.96	/	/
	200 μ M	10.03 $\pm$ 1.02	17.14 $\pm$ 1.84	-1.28	n. s.

n.s.: no significance.

Table S4 Effects of NN on the lifespan of N2 and mutant worms for the 3rd replicate.
(mean $\pm$ SD)

Genotype	Treatment	Mean lifespan (days)	Maximum lifespan (days)	% change in mean lifespan	p value
N2	0 μ M	14.36 $\pm$ 1.37	22.89 $\pm$ 3.78	/	/
	50 μ M	16.10 $\pm$ 1.55	23.41 $\pm$ 3.30	12.12**	p<0.01
	100 μ M	16.94 $\pm$ 1.34	24.57 $\pm$ 3.12	17.97**	p<0.01
	200 μ M	17.21 $\pm$ 2.42	25.11 $\pm$ 3.47	19.85**	p<0.01
	0 μ M	1.88 $\pm$	3.48 $\pm$	/	/
	(H ₂ O ₂)	0.12	0.22		
	200 μ M	2.31 $\pm$	4.02 $\pm$	22.87**	p<0.01
	(H ₂ O ₂)	0.19	0.41		
	0 μ M (35 °C)	11.56 $\pm$ 1.32	18.21 $\pm$ 1.76	/	/
	200 μ M	11.17 $\pm$	20.05 $\pm$	-3.37	n. s.
	(35 °C)	2.01	1.49		
<i>daf-16 (mu86)</i>	0 μ M	10.78 $\pm$ 1.24	18.34 $\pm$ 2.60	/	/
	200 μ M	11.12 $\pm$ 1.85	19.16 $\pm$ 1.08	3.15	n. s.
<i>daf-2 (e1370)</i>	0 μ M	23.26 $\pm$ 1.97	36.88 $\pm$ 3.03	/	/
	200 μ M	22.74 $\pm$ 1.71	35.34 $\pm$ 3.82	-2.24	n. s.

<i>hsf-1 (sy441)</i>	0 μ M	13.67 $\pm$ 2.04	21.56 $\pm$ 2.79	/	/
	200 μ M	15.22 $\pm$ 2.32	25.16 $\pm$ 1.93	11.34**	p<0.01
<i>eat-2 (ad1116)</i>	0 μ M	23.67 $\pm$ 2.64	38.37 $\pm$ 4.30	/	/
	200 μ M	26.81 $\pm$ 1.44	42.18 $\pm$ 3.26	13.27**	p<0.01
<i>skn-1 (ZU67)</i>	0 μ M	11.87 $\pm$ 1.41	19.12 $\pm$ 1.04	/	/
	200 μ M	11.55 $\pm$ 1.24	17.69 $\pm$ 2.43	-2.70	n. s.

n.s.: no significance.

Table S5 The category of Gene Ontology (GO) projects linked to NN

Category	Term	Project
GOTERM_MF_DIRECT	GO:0005515	protein binding
GOTERM_MF_DIRECT	GO:0042802	identical protein binding
GOTERM_MF_DIRECT	GO:0005524	ATP binding
GOTERM_MF_DIRECT	GO:0042803	protein homodimerization activity
GOTERM_MF_DIRECT	GO:0004674	protein serine/threonine kinase activity
GOTERM_MF_DIRECT	GO:0008144	drug binding
GOTERM_MF_DIRECT	GO:0032403	protein complex binding
GOTERM_MF_DIRECT	GO:0016301	kinase activity
GOTERM_MF_DIRECT	GO:0008134	transcription factor binding
GOTERM_MF_DIRECT	GO:0019899	enzyme binding
GOTERM_MF_DIRECT	GO:0050661	NADP binding
GOTERM_MF_DIRECT	GO:0019902	phosphatase binding
GOTERM_MF_DIRECT	GO:0016740	transferase activity
GOTERM_MF_DIRECT	GO:0002020	protease binding
GOTERM_MF_DIRECT	GO:0001078	transcriptional repressor activity, RNA polymerase II core promoter proximal region sequence-specific binding
GOTERM_MF_DIRECT	GO:0020037	heme binding
GOTERM_MF_DIRECT	GO:0034736	cholesterol O-acyltransferase activity
GOTERM_MF_DIRECT	GO:0031406	carboxylic acid binding
GOTERM_MF_DIRECT	GO:0005041	low-density lipoprotein receptor activity
GOTERM_MF_DIRECT	GO:0008374	O-acyltransferase activity
GOTERM_MF_DIRECT	GO:0001784	phosphotyrosine binding
GOTERM_MF_DIRECT	GO:0004707	MAP kinase activity
GOTERM_MF_DIRECT	GO:0070402	NADPH binding
GOTERM_MF_DIRECT	GO:0005319	lipid transporter activity
GOTERM_MF_DIRECT	GO:0004602	glutathione peroxidase activity
GOTERM_MF_DIRECT	GO:0033613	activating transcription factor binding
GOTERM_CC_DIRECT	GO:0005829	cytosol
GOTERM_CC_DIRECT	GO:0005739	mitochondrion
GOTERM_CC_DIRECT	GO:0070062	extracellular exosome
GOTERM_CC_DIRECT	GO:0005783	endoplasmic reticulum
GOTERM_CC_DIRECT	GO:0005794	Golgi apparatus
GOTERM_CC_DIRECT	GO:0005789	endoplasmic reticulum membrane
GOTERM_CC_DIRECT	GO:0043235	receptor complex
GOTERM_CC_DIRECT	GO:0043231	intracellular membrane-bounded organelle
GOTERM_CC_DIRECT	GO:0005901	caveola
GOTERM_CC_DIRECT	GO:0043234	protein complex
GOTERM_CC_DIRECT	GO:0005764	lysosome

GOTERM_CC_DIRECT	GO:0005769	early endosome
GOTERM_CC_DIRECT	GO:0005777	peroxisome
GOTERM_CC_DIRECT	GO:0016020	membrane
GOTERM_CC_DIRECT	GO:0005770	late endosome
GOTERM_CC_DIRECT	GO:0034362	low-density lipoprotein particle
GOTERM_CC_DIRECT	GO:0015630	microtubule cytoskeleton
GOTERM_CC_DIRECT	GO:0031143	pseudopodium
GOTERM_CC_DIRECT	GO:0032839	dendrite cytoplasm
GOTERM_CC_DIRECT	GO:0005925	focal adhesion
GOTERM_CC_DIRECT	GO:0016323	basolateral plasma membrane
GOTERM_CC_DIRECT	GO:0010008	endosome membrane
GOTERM_BP_DIRECT	GO:0042493	response to drug
GOTERM_BP_DIRECT	GO:0042632	cholesterol homeostasis
GOTERM_BP_DIRECT	GO:0032869	cellular response to insulin stimulus
GOTERM_BP_DIRECT	GO:0007568	aging
GOTERM_BP_DIRECT	GO:0042953	lipoprotein transport
GOTERM_BP_DIRECT	GO:0008203	cholesterol metabolic process
GOTERM_BP_DIRECT	GO:0030855	epithelial cell differentiation
GOTERM_BP_DIRECT	GO:0098869	cellular oxidant detoxification
GOTERM_BP_DIRECT	GO:0007584	response to nutrient
GOTERM_BP_DIRECT	GO:0046326	positive regulation of glucose import
GOTERM_BP_DIRECT	GO:0010742	macrophage derived foam cell differentiation
GOTERM_BP_DIRECT	GO:0045471	response to ethanol
GOTERM_BP_DIRECT	GO:0000187	activation of MAPK activity
GOTERM_BP_DIRECT	GO:0000302	response to reactive oxygen species
GOTERM_BP_DIRECT	GO:0034379	very-low-density lipoprotein particle assembly
GOTERM_BP_DIRECT	GO:0034383	low-density lipoprotein particle clearance
GOTERM_BP_DIRECT	GO:0032872	regulation of stress-activated MAPK cascade
GOTERM_BP_DIRECT	GO:0030299	intestinal cholesterol absorption
GOTERM_BP_DIRECT	GO:0055114	oxidation-reduction process
GOTERM_BP_DIRECT	GO:0006749	glutathione metabolic process
GOTERM_BP_DIRECT	GO:0006629	lipid metabolic process
GOTERM_BP_DIRECT	GO:0010745	negative regulation of macrophage derived foam cell differentiation
GOTERM_BP_DIRECT	GO:0071380	cellular response to prostaglandin E stimulus
GOTERM_BP_DIRECT	GO:0006805	xenobiotic metabolic process
GOTERM_BP_DIRECT	GO:0009636	response to toxic substance
GOTERM_BP_DIRECT	GO:0042593	glucose homeostasis
GOTERM_BP_DIRECT	GO:0033344	cholesterol efflux

GOTERM_BP_DIRECT	GO:0030168	platelet activation
GOTERM_BP_DIRECT	GO:0043200	response to amino acid
GOTERM_BP_DIRECT	GO:0031663	lipopolysaccharide-mediated signaling pathway
GOTERM_BP_DIRECT	GO:0001934	positive regulation of protein phosphorylation
GOTERM_BP_DIRECT	GO:0006641	triglyceride metabolic process
GOTERM_BP_DIRECT	GO:0043407	negative regulation of MAP kinase activity
GOTERM_BP_DIRECT	GO:0042157	lipoprotein metabolic process
GOTERM_BP_DIRECT	GO:0032720	negative regulation of tumor necrosis factor production
GOTERM_BP_DIRECT	GO:1901215	negative regulation of neuron death
GOTERM_BP_DIRECT	GO:0035338	long-chain fatty-acyl-CoA biosynthetic process
GOTERM_BP_DIRECT	GO:0014823	response to activity
GOTERM_BP_DIRECT	GO:0048538	thymus development
GOTERM_BP_DIRECT	GO:0045893	positive regulation of transcription, DNA-templated
GOTERM_BP_DIRECT	GO:0031100	organ regeneration
GOTERM_BP_DIRECT	GO:0009408	response to heat
GOTERM_BP_DIRECT	GO:0042542	response to hydrogen peroxide
GOTERM_BP_DIRECT	GO:0008152	metabolic process
GOTERM_BP_DIRECT	GO:0001666	response to hypoxia
GOTERM_BP_DIRECT	GO:0051260	protein homooligomerization
GOTERM_BP_DIRECT	GO:0010871	negative regulation of receptor biosynthetic process
GOTERM_BP_DIRECT	GO:0061308	cardiac neural crest cell development involved in heart development
GOTERM_BP_DIRECT	GO:0000189	MAPK import into nucleus
GOTERM_BP_DIRECT	GO:0034435	cholesterol esterification
GOTERM_BP_DIRECT	GO:0070301	cellular response to hydrogen peroxide
GOTERM_BP_DIRECT	GO:0001523	retinoid metabolic process
GOTERM_BP_DIRECT	GO:0006954	inflammatory response
GOTERM_BP_DIRECT	GO:0008217	regulation of blood pressure
GOTERM_BP_DIRECT	GO:0051384	response to glucocorticoid
GOTERM_BP_DIRECT	GO:0042159	lipoprotein catabolic process
GOTERM_BP_DIRECT	GO:0006084	acetyl-CoA metabolic process
GOTERM_BP_DIRECT	GO:0090170	regulation of Golgi inheritance
GOTERM_BP_DIRECT	GO:0070849	response to epidermal growth factor
GOTERM_BP_DIRECT	GO:0006006	glucose metabolic process
GOTERM_BP_DIRECT	GO:0032868	response to insulin
GOTERM_BP_DIRECT	GO:0033591	response to L-ascorbic acid

GOTERM_BP_DIRECT	GO:0010891	negative regulation of sequestering of triglyceride
GOTERM_BP_DIRECT	GO:0072584	caveolin-mediated endocytosis
GOTERM_BP_DIRECT	GO:0031325	positive regulation of cellular metabolic process
GOTERM_BP_DIRECT	GO:1900034	regulation of cellular response to heat
GOTERM_BP_DIRECT	GO:0007623	circadian rhythm
GOTERM_BP_DIRECT	GO:0014066	regulation of phosphatidylinositol 3-kinase signaling
GOTERM_BP_DIRECT	GO:0010887	negative regulation of cholesterol storage
GOTERM_BP_DIRECT	GO:0046321	positive regulation of fatty acid oxidation
GOTERM_BP_DIRECT	GO:0060440	trachea formation
GOTERM_BP_DIRECT	GO:0043066	negative regulation of apoptotic process
GOTERM_BP_DIRECT	GO:0006468	protein phosphorylation
GOTERM_BP_DIRECT	GO:0032287	peripheral nervous system myelin maintenance
GOTERM_BP_DIRECT	GO:0045944	positive regulation of transcription from RNA polymerase II promoter
GOTERM_BP_DIRECT	GO:0032355	response to estradiol
GOTERM_BP_DIRECT	GO:2000641	regulation of early endosome to late endosome transport
GOTERM_BP_DIRECT	GO:0002674	negative regulation of acute inflammatory response
GOTERM_BP_DIRECT	GO:0006975	DNA damage induced protein phosphorylation
GOTERM_BP_DIRECT	GO:0035865	cellular response to potassium ion
GOTERM_BP_DIRECT	GO:0097011	cellular response to granulocyte macrophage colony-stimulating factor stimulus
GOTERM_BP_DIRECT	GO:0016310	phosphorylation
GOTERM_BP_DIRECT	GO:0042158	lipoprotein biosynthetic process
GOTERM_BP_DIRECT	GO:0042473	outer ear morphogenesis
GOTERM_BP_DIRECT	GO:0032930	positive regulation of superoxide anion generation
GOTERM_BP_DIRECT	GO:0060020	Bergmann glial cell differentiation
GOTERM_BP_DIRECT	GO:0006853	carnitine shuttle
GOTERM_BP_DIRECT	GO:0032000	positive regulation of fatty acid beta-oxidation
GOTERM_BP_DIRECT	GO:0001649	osteoblast differentiation
GOTERM_BP_DIRECT	GO:0071356	cellular response to tumor necrosis factor

GOTERM_BP_DIRECT	GO:0030278	regulation of ossification
GOTERM_BP_DIRECT	GO:0071222	cellular response to lipopolysaccharide
GOTERM_BP_DIRECT	GO:0045995	regulation of embryonic development
GOTERM_BP_DIRECT	GO:0060425	lung morphogenesis
GOTERM_BP_DIRECT	GO:0018105	peptidyl-serine phosphorylation
GOTERM_BP_DIRECT	GO:0014912	negative regulation of smooth muscle cell migration
GOTERM_BP_DIRECT	GO:1902176	negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathway
GOTERM_BP_DIRECT	GO:0019395	fatty acid oxidation
GOTERM_BP_DIRECT	GO:0010875	positive regulation of cholesterol efflux
GOTERM_BP_DIRECT	GO:0032270	positive regulation of cellular protein metabolic process
GOTERM_BP_DIRECT	GO:0060397	JAK-STAT cascade involved in growth hormone signaling pathway
GOTERM_BP_DIRECT	GO:0010667	negative regulation of cardiac muscle cell apoptotic process
GOTERM_BP_DIRECT	GO:0045725	positive regulation of glycogen biosynthetic process
GOTERM_BP_DIRECT	GO:0097194	execution phase of apoptosis
GOTERM_BP_DIRECT	GO:0010804	negative regulation of tumor necrosis factor-mediated signaling pathway
GOTERM_BP_DIRECT	GO:1904355	positive regulation of telomere capping
GOTERM_BP_DIRECT	GO:0030301	cholesterol transport
GOTERM_BP_DIRECT	GO:0071276	cellular response to cadmium ion
GOTERM_BP_DIRECT	GO:0060324	face development
GOTERM_BP_DIRECT	GO:0033189	response to vitamin A
GOTERM_BP_DIRECT	GO:0051493	regulation of cytoskeleton organization
GOTERM_BP_DIRECT	GO:0043536	positive regulation of blood vessel endothelial cell migration
GOTERM_BP_DIRECT	GO:0060716	labyrinthine layer blood vessel development
GOTERM_BP_DIRECT	GO:0071385	cellular response to glucocorticoid stimulus
GOTERM_BP_DIRECT	GO:1901687	glutathione derivative biosynthetic process
GOTERM_BP_DIRECT	GO:0032496	response to lipopolysaccharide
GOTERM_BP_DIRECT	GO:0071398	cellular response to fatty acid
GOTERM_BP_DIRECT	GO:0070371	ERK1 and ERK2 cascade

Table S6 The enrichment of Gene Ontology (GO) projects

Category	The number of GO projects	Total GO projects	Ratio (%)
GO_MF	26	166	15.66
GO_CC	22		13.25
GO_BP	118		71.09

Table S7 The enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG)

Term	Count	Target
hsa04910 Insulin signaling pathway	7	FASN, INSR, MAPK1, AKT1, PIK3CG, ACACA, MAPK3
hsa04066 HIF-1 signaling pathway	6	INSR, MAPK1, AKT1, RELA, PIK3CG, MAPK3
hsa04010 MAPK signaling pathway	6	CASP3, MAPK1, AKT1, RELA, MAP2K6, MAPK3
hsa04014 Ras signaling pathway	6	INSR, MAPK1, AKT1, RELA, PIK3CG, MAPK3
hsa04015 Rap1 signaling pathway	6	INSR, MAPK1, AKT1, PIK3CG, MAP2K6, MAPK3
hsa04024 cAMP signaling pathway	6	MAPK1, AKT1, PPARA, RELA, PIK3CG, MAPK3
hsa04068 FoxO signaling pathway	6	INSR, CAT, MAPK1, AKT1, PIK3CG, MAPK3
hsa04071 Sphingolipid signaling pathway	6	ABCC1, MAPK1, AKT1, RELA, PIK3CG, MAPK3
hsa04620 Toll-like receptor signaling pathway	6	MAPK1, AKT1, RELA, PIK3CG, MAP2K6, MAPK3
hsa04151 PI3K-Akt signaling pathway	6	INSR, MAPK1, AKT1, RELA, PIK3CG, MAPK3
hsa04920 Adipocytokine signaling pathway	5	CPT1A, ADIPOQ, AKT1, PPARA, RELA
hsa04662 B cell receptor signaling pathway	5	MAPK1, AKT1, RELA, PIK3CG, MAPK3
hsa04664 Fc epsilon RI signaling pathway	5	MAPK1, AKT1, PIK3CG, MAP2K6, MAPK3
hsa05221 Acute myeloid leukemia pathway	5	MAPK1, AKT1, RELA, PIK3CG, MAPK3

Table S8 Effects of NN on paralysis of CL4176 (mean $\pm$ SD, n=3)

Genotype	Treatment	Mean paralyzed	% change in mean	p value
		time (hours)	paralyzed time	
CL4176	0 μ M	39.35 $\pm$ 2.92	/	/
	50 μ M	42.32 $\pm$ 2.27	7.54*	p<0.05
	100 μ M	41.71 $\pm$ 3.32	6.01*	p<0.05
	200 μ M	45.58 $\pm$ 2.48	15.86**	p<0.01

Table S9 Effects of NN on chemotaxis index of CL2122 and CL2355 (mean $\pm$ SD, n=3)

Genotype	Treatment	CI	p value
CL2122	0 μ M	0.36 $\pm$ 0.03	/
	50 μ M	0.38 $\pm$ 0.01	n. s.
	100 μ M	0.35 $\pm$ 0.01	n. s.
	200 μ M	0.34 $\pm$ 0.03	n. s.
CL2355	0 μ M	0.14 $\pm$ 0.02 ^{**}	p<0.01
	50 μ M	0.18 $\pm$ 0.01 [#]	p<0.05
	100 μ M	0.26 $\pm$ 0.03 ^{##}	p<0.01
	200 μ M	0.23 $\pm$ 0.02 ^{##}	p<0.01

n.s.: no significance.

Table S10 Molecular docking between NN with receptor proteins of *C. elegans*.

Ligand name	Receptor name	Interactive residues	Hydrogen bonds involved residues	CDOCKER energy (kJ/mol)
NN	DAF-16	GLY 96, MET 98, ASN 97, LEU 103, PRO 130, VAL 129, TRP 95	GLY 96, MET 98	-26.9271
	SKN-1	ARG 104, THR 133, THR 149, HIS 152, PRO 132, PHE 148, GLY 77, MET 80, TRP 103, THR 23, SER 25, GLY 24, ALA 102, PHE 27, ILE 78, HIS 76, TRP 44, PHE 131, PHE 105, VAL 130	PHE 148, PRO 132, THR 23, TRP 103, HIS 152	-72.7362
	SOD-3	HIS 46, LEU 45, MET 0, LYS 1, GLU 42, ALA 57, ILE 58, GLN 61, GLU 43, VAL 49, HIS 2, LEU 64, VAL 49, LEU 45, GLY 52, LEU 54	MET 0	-21.4896
	GST-4	VAL 334, ALA 333, LYS 372,	ALA 333, ALA 386, LYS 372, ASP 381	-45.1753

	VAL 332, ALA 386, ASP 381, ARG 390, GLN 387, TRP 340, ILE 335, ASN 379, LEU 337, PRO 336, LYS 338, LEU 382, LEU 389		
DAF-2	VAL 313, LYS 297, ASP 311, LYS 349, TRP 301, THR 296, GLU 348, LYS 302, SER 308	TRP 301, THR 296, LYS 302	-73.3912
AGE-1	HIS 716, ARG 749, GLU 937, LEU 843, ALA 925, ASP 844, THR 327, ARG 325, LEU 322, GLY 341, GLU 345, VAL 846, SER 344	ARG 749, GLU 345	-40.7825
AKT-1	GLY 202, GLU 277, LYS 201, LEU 199, VAL 207, LEU 323, PHE 480, ALA	ALA 273, ASP 334	-37.4168

	273, GLN 271,		
	PHE 272, THR		
	254, ALA 220,		
	MET 270, LYS		
	222, ALA 333,		
	ASP 334, GLY		
	200, GLU 320		
SEK-1	LYS 207, TYR 61,	SER 180, LYS 135,	-116.2217
	GLY 62, ILE 63,	ASP 134, LYS 178	
	ASP 194, VAL 64,		
	CYS 193, LYS 79,		
	LEU 183, ASN		
	181, LYS 135,		
	SER 180, SER		
	132, ASP 134,		
	GLY 57, GLY 59,		
	LYS 178, LYS 58,		
	SER 198, GLY 60,		
	CYS 213		
