Supplemental Methods

Lifespan assays

For lifespan assays, worms grown at 20°C were picked as L4 and allowed to grow at 20°C until the next day, when 10 young adults were placed on five 3 cm plates. Lifespan assays were performed with OP50 spotted on NGM plates at 25°C. Worms were classified as alive, dead (no movement in response to touch with a wire), or censored (lost or bagged worms) once a day for lifespan assays.

Quantitative PCR

L1 animals from each of the indicated genotypes were synchronized using the hypochlorite method. Animals were plated on OP50 plates and allowed to grow at 20°C for ~48 hours. 500 young adult animals were sorted into 1.5 ml tubes using a COPAS Biosort. 3 biological replicates were performed for each genotype. Purified total RNA was converted to cDNA and quantitative PCR against GFP was carried out on an ABI7500 qPCR system (Applied Biosystems, Fisher Scientific, Foster City, CA) using the SYBR green method. Samples were normalized against expression for the actin gene *act-2*. Each sample was then normalized against the expression of the GFP only control. Raw data for qPCR are included as Table S2.

COPAS fluorescent quantification

Day 1 adult animals expressing each DPR-GFP transgene under the control of the *myo-3* promoter were washed off plates and analyzed using a COPAS Biosort. Fluorescent detection settings were identical for all samples. Only animals with time-of-flight measurements from 400-600 (young adults) were used for the analysis of fluorescence.

Table S1. Raw brood size data for integrated *myo-3p::DPR* strains[1]

GFP[2]	**GFP [3]**	**(PR)$_{50}$-GFP[2]**	**(PR)$_{50}$-GFP[3]**	**(GR)$_{50}$-GFP[2]**	**(GR)$_{50}$-GFP[3]**	**(GA)$_{50}$-GFP[2]**	**(GA)$_{50}$-GFP[3]**	**(PA)$_{50}$-GFP[2]**	**(PA)$_{50}$-GFP[3]**
243	272	119	0	59	0	126	45	37	10
173	218	28	0	63	0	133	70	30	28
160	292	14	0	51	0	98	33	6	37
235	290	24	0	46	0	97	104	46	41
174	237	72	0	62	0	186	98	32	39
188	289	40	0	27	0		157	10	46
151	258	156	0	45	0		61	30	66
159	269	42	0	72	0		112		38
173	267		0		0		128		36
	223		0		0				

[1] – Each box indicates the total brood size for a single animal at 25°C

[2] – *gfp(RNAi)* bacteria

[3]- OP50 bacteria

Table S2. Raw quantitative PCR data

	GFP		**act-2**							
Sample	**gene of interest #1**	**Std.**	**endogenous control**	**Std.**	**ΔC_T**	**Std.**	**$\Delta\Delta C_T$**	**Std.**	**gene of interest #1**	**Fold Change**
	Avg. C_T	**Dev.**	**Avg. C_T**	**Dev.**	**est - endoge**	**Dev.**	**ΔCT - ΔCT,**	**Dev.**	**Relative to Calibrator**	**Relative to Calibrator**
drIs33-1 (myo-3p::GFP)	15.2035		14.0941							
	15.2943		14.16							
	15.3458		14.1878							
Average	15.28	0.07	14.15	0.05	1.13	0.09	0.00	0.09	1.00	1.00
	15.3977		14.2403							
drIs33-2 (myo-3p::GFP)	15.401		14.2208							
	15.34		14.1908							
Average	15.38	0.03	14.22	0.02	1.16	0.04	0.03	0.04	0.98	-1.02
drIs33-3 (myo-3p::GFP)	16.02		14.6771							
	16.0261		14.6468							
	16.0062		14.6507							
Average	16.02	0.01	14.66	0.02	1.36	0.02	0.23	0.02	0.86	-1.17
Sample Mean	**15.56**	**0.04**	**14.34**	**0.03**	**0.91**	**0.04**	**0.00**	**0.05**	**1.00**	**1.00**
drIs35-1 (myo-3p::GA50-GFP)	17.3966		14.7113							
	17.4633		14.7465							
	censored		14.7624							
Average	17.43	0.05	14.74	0.03	2.69	0.05	1.56	0.05	0.340097	-2.94
drIs35-2 (myo-3p::GA50-GFP)	17.1109		14.5717							
	17.2729		14.65							
	17.1916		14.6568							
Average	17.19	0.08	14.63	0.05	2.57	0.09	1.43	0.09	0.370685	-2.70
drIs35-3 (myo-3p::GA50-GFP)	19.5042		15.8019							
	19.4074		15.8645							
	19.3234		censored							
Average	19.41	0.09	15.83	0.04	3.58	0.10	2.44	0.10	0.183701	-5.44
Sample Mean	**18.01**	**0.07**	**15.07**	**0.04**	**2.94**	**0.08**	**2.03**	**0.08**	**0.24**	**-4.09**
drIs37-1 (myo-3p::PA50-GFP)	19.6007		16.5291							
	19.7229		16.5755							
	19.7259		16.5172							
Average	19.68	0.07	16.54	0.03	3.14	0.08	2.01	0.08	0.248503	-4.02
drIs37-2(myo-3p::PA50-GFP)	17.2594		15.2841							
	17.6077		15.2089							
	17.4612		15.2128							
Average	17.44	0.17	15.24	0.04	2.21	0.18	1.07	0.18	0.475132	-2.10
drIs37-3(myo-3p::PA50-GFP)	17.9511		15.5245							
	18.0802		15.569							
	18.1808		15.6256							
Average	18.07	0.12	15.57	0.05	2.50	0.13	1.36	0.13	0.388566	-2.57
Sample Mean	**18.40**	**0.12**	**15.78**	**0.04**	**2.62**	**0.13**	**1.70**	**0.13**	**0.31**	**-3.25**
drIs34-1 (myo-3p::PR50-GFP)	15.8579		14.2747							
	15.8677		14.2701							
	15.7411		14.235							
Average	15.82	0.07	14.26	0.02	1.56	0.07	0.43	0.07	0.743085	-1.35
drIs34-2 (myo-3p::PR50-GFP)	16.1955		14.3932							
	16.0605		14.3669							
	16.0641		14.3544							
Average	16.11	0.08	14.37	0.02	1.74	0.08	0.60	0.08	0.659160	-1.52
drIs34-3 (myo-3p::PR50-GFP)	15.5868		14.1291							
	15.6687		14.1844							
	15.7666		14.1921							
Average	15.67	0.09	14.17	0.03	1.51	0.10	0.37	0.10	0.772925	-1.29
Sample Mean	**15.87**	**0.08**	**14.27**	**0.03**	**1.60**	**0.08**	**0.69**	**0.08**	**0.62**	**-1.61**
drIs28-1 (myo-3p::GR50-GFP)	17.4236		14.5822							
	17.5541		14.4986							
	censored		14.7204							
Average	17.49	0.09	14.60	0.11	2.89	0.15	1.75	0.15	0.296366	-3.37
drIs28-2 (myo-3p::GR50-GFP)	17.2437		14.509							
	17.0185		14.4762							
	censored		14.452							
Average	17.13	0.16	14.48	0.03	2.65	0.16	1.52	0.16	0.349137	-2.86
drIs28-3 (myo-3p::GR50-GFP)	17.0882		14.5592							
	17.0607		14.7095							
	17.0394		14.6388							
Average	17.06	0.02	14.64	0.08	2.43	0.08	1.29	0.08	0.408092	-2.45
Sample Mean	**17.23**	**0.09**	**14.57**	**0.07**	**2.66**	**0.13**	**1.74**	**0.12**	**0.30**	**-3.34**
N2-1 (No dipeptide)	27.2311		14.1427							
	27.2934		14.1264							
	censored		14.1143							
Average	27.26	0.04	14.13	0.01	13.13	0.05	12.00	0.05	0.000244	-4097.56
N2-2 (No dipeptide)	28.9943		14.178							
	28.946		14.1793							
	censored		14.1613							
Average	28.97	0.03	14.17	0.01	14.80	0.04	13.66	0.04	0.000077	-12974.43
N2-3 (No dipeptide)	28.6223		14.1151							
	28.5189		14.1201							
	censored		14.1567							
Average	28.57	0.07	14.13	0.02	14.44	0.08	13.31	0.08	0.000099	-10128.03
Sample Mean	**28.27**	**0.05**	**14.14**	**0.02**	**14.12**	**0.05**	**13.21**	**0.05**	**0.00**	**-9475.92**

Table S3. Nucleotide sequences of codon-varied dipeptide sequences used in this study

(PA)$_{50}$
aagcttgggccggccccggctccggcacctgcgcccgctccagcaccggcgcctgctcccgcaccagctcctgcacccgcgccagcacccgctccagcgcctgctcctgcaccggctcccgcgccagcacctgctccggcgcccgcaccagctcctgccccggccccggctccggcacctgcgcccgctccagcaccggcgcctgctcccgcaccagctcctgcacccgcgccagcacccgctccagcgcctgctcctgcaccggctcccgcgccagcacctgctccggcgcccgcaccagctcctgccccgggttggtaccgagctc**ggatcc**
(GA)$_{50}$
aagcttcccggggccggtgccggagcaggcgctggtgcgggcgccggagctggtgcaggcgcgggagccggtgctggcgcaggagcgggtgcaggcgcgggtgccggagctggcgcaggagcgggtgctggcgccggagcaggtgcgggcgctggggccggggccggtgccggagcaggcgctggtgcgggcgccggagctggtgcaggcgcgggagccggtgctggcgcaggagcgggtgcaggcgcgggtgccggagctggcgcaggagcgggtgctggcgccggagcaggtgcgggcgctggggccggctggtaccgagctc**ggatcc**
(PR)$_{50}$
aagcttggccggccccggccgcgtcctcgcccacgaccgagacctcgtccacgcccgcgacctagaccacggccgcgccctcgaccaagacctcgcccgagaccacgacctcgtccgcgcccaagacctcgaccgcgtccacgccctagaccgcgaccccggccccggccgcgtcctcgcccacgaccgagacctcgtccacgcccgcgacctagaccacggccgcgccctcgaccaagacctcgcccgagaccacgacctcgtccgcgcccaagacctcgaccgcgtccacgccctagaccgcgaccccgggggtaccgagctc**ggatcc**
(PR)$_{25}$
aagcttccccggccgcgtcctcgcccacgaccgagacctcgtccacgcccgcgacctagaccacggccgcgccctcgaccaagacctcgcccgagaccacgacctcgtccgcgcccaagacctcgaccgcgtccacgccctagaccgcgaccccgggggtaccgagctc**ggatcc**
(PR)$_{15}$
aagcttccgcgccctcgaccaagacctcgcccgagaccacgacctcgtccgcgcccaagacctcgaccgcgtccacgccctagaccgcgaccccgggggtaccgagctc**ggatcc**
(PR)$_{5}$
aagcttccgcgtccacgccctagaccgcgaccccgggggtaccgagctc**ggatcc**
(GR)$_{50}$
aagcttgcccggggccggggccgtggtcgcggacgaggcagaggtcgtggacgcggccgaggtagaggacgtggccgcggtcgaggaagaggtcgcggcagaggacgaggtcgtggccgcggaagaggtcgaggccgtggacgcggtagaggccgaggccggggccggggccgtggtcgcggacgaggcagaggtcgtggacgcggccgaggtagaggacgtggccgcggtcgaggaagaggtcgcggcagaggacgaggtcgtggccgcggaagaggtcgaggccgtggacgcggtagaggccgaggccggcggtaccgagctc**ggatcc**

Red – *HindIII* and *BamHI* cloning sites

Table S4. Nucleotide sequences of promoters and targeting sequences used in this study

Myo-3 promoter - FLAG
aagcttgtttgatgaaaaccaatgaaacaagtgattatagtctctgttttcgttaattttgaattttgcttgataaggctgcaacaaagatcaggttgacatatattttcagtaatttattttaacctgtactctatcactgccggctataataagttcttgaataaaataattttcccgacaaaacatgagtatttctttcgaaaataaaagtgcaggctaattagagattattctgtaattaactgcataatttgtcacgtgccatagttttacattccactacgtcatagttcttaaaatactaatctcctgaaaatagaagtaggtgaagaaagtttaattatcagttctaaaatgacaattgatctttggaatatgttctgaaactaccgatcattgaacagatgctatttgaatgatatagaattgtatatttgcaatttctgaaacgcgttcttaaaggcacacagattaattcaaaagggtctggccgcaaaaaggtttatggtggccgattttgagttttgtgtgtgattgctttttcacaatcagtgttttcaggattatgtgatgaactagatcttcaagtttcgttacatttcatatgttttcggaactcacgaagtacatattgggtattgtgctcaaaaaattcagcaatcagcttcgctccgctgactttagaacccaaaaaaatagtatggccaaactgactgtgttacgatcatttcaatttttcaatacatatttaagatttctaagagtaagaaggtcaaaaactgttctggaatacatatatatttttcaggttacaattagtcaaaaagtgcactgaaatatacgttttaatttcacgaataacccaattagttcaatgtatttttggtcaaccaacgttaaagtttggcttccaaccaattatcatttctgatcaaccacaatgtttttctttatctgcaagttaatttttatttttatccagatgtttggcatatttttcaattcttcactagcgcccacttcttgcacttccggcgccctgaatctaatgcatctgttgcaagaattgaaagaccaatcaacacattgttttcttcacgagatactgaagaaaatgaataaaaacagagaaaaagagccatgtgattagtgacaactgttgctaacagataccatagcttggacttggtacgtgatggcaacgtatgggtcaacaaaaatgattgcagagggggtgcaaaacagtcaagtcgagaaaatatgaaaaacagaaaacaaagaacagaaaaatgggtttgagagtcagtataatttataaaagaaaaattgtacatagaaattaaccatttttgtagaagaagttatttttcaagcatcgttaaaaattattcaaagcaccttatttcatatttaattttaaacatggttaaatgaacaacacggtgcgcaatcaggaaaacttgaaatctgaaactgttgttgtgatcttcttcgcaactgttcagatagcactagtgtaatgttaagagtgcgcgaatataatggaatataatggatcacacctcctgccatcaggtaaacgtctctgttatcacatatttccaactattaaatttttaccttttacagttttacatttttttgaaaaaagtaactttttgtcttcaaaatccctgacgaaaatatcaaatattttaatcgagactgcagaggaaccgattgatgatttggaaaatccagctttacctgtgtaagaactgaaaagtttcataaccctagggtattcccagttacattccccactggctaacaatagcacccagtttttcatcacctttcttcaaatttctcggcgatttgttaaaaacaaaatttgtgtcccttctctgatatctctatgtctctaaacacaagttcatcggaaaacgaaggagggtaggtgttggttgggctcccgaagtgaaaatagaagagcaagaatagaatattagagagagagtgcagagagggcgggatagctcccgggattccgttttcttcttctttatcttcaacgatgatgtgtgtgcgtgttgtatagattctgttgctcccccacaactcgctccgaaggctcaatacaattcaattgatattggaggagagcctaccggagtgggaggataagaagaaacataagaagaagaagaagaagcatgcttctggtttttgatgctatgaaaacggcacaaaaagatgattgaggtcccttttcaataccttctctcatctttcaaatcccattgaaacctaaaacttctcaccacgctttaccattgttctccaaaaacttatagcaatgtctataacttttttatctctgaaaagcagtgttccatttttctttttcctattttatttcaattgtttctcacatttcgtttggattctttgcttgtcaaccagcttcttcttccacttttaccgtctaattttcagggcagggagccatcaaacccacgaccactagatccatctagaaATGgattacaaggatgacgacgataag**aagctt**
Myo-3p-signal sequence-transmembrane-FLAG
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Myo-3p-GFP-FLAG-his-58

aagcttgtttgatgaaaaccaatgaaacaagtgattatagtctctgttttcgttaattttgaattttgcttgataaggctgcaacaaagat
caggttgacatatattttcagtaatttattttaacctgtactctatcactgccggctataataagttcttgaataaaataattttcccgacaa
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Unc-47p-FLAG

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Hsp-16.2p-FLAG

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attacaaggatgacgacgataag**aagctt**

Red – HindIII cloning sites

Underline – FLAG sequence

ATG – start codon

Green – GFP sequence

Bold italics – Signal sequence-transmembrane domain

Bold – *his-58* sequence