

## Supporting Information

### Table S1

Overexpression strain	Bacteria	Mean lifespan $\pm$ s.e.m. (days)	75% (days)	n	% change	P
<i>drr-2</i> overexpression in wild type (N2) background						
N2	OP 50	18.50 $\pm$ 0.81	22	57/72	-	-
EQ18 ( <i>drr-2</i> )	OP 50	19.26 $\pm$ 1.06	24	42/72	4%	0.57
N2	OP 50	21.75 $\pm$ 0.53	25	81/90	-	-
EQ18 ( <i>drr-2</i> )	OP 50	22.93 $\pm$ 0.69	27	76/90	5%	0.019
N2 *	OP 50	19.30 $\pm$ 0.60	23	59/72	-	-
EQ19 ( <i>drr-2::gfp</i> ) *	OP 50	19.34 $\pm$ 0.86	23	52/72	5%	0.32
N2	OP 50	19.99 $\pm$ 0.53	23	61/72	-	-
EQ19 ( <i>drr-2::gfp</i> )	OP 50	19.15 $\pm$ 0.54	21	50/72	-4%	0.26
N2	OP 50	19.87 $\pm$ 0.58	23	58/72	-	-
EQ19 ( <i>drr-2::gfp</i> )	OP 50	20.58 $\pm$ 0.58	21	62/72	4%	0.31
N2	HT115	16.56 $\pm$ 0.76	21	66/80		-
EQ19 ( <i>drr-2::gfp</i> )	HT115	17.06 $\pm$ 0.74	21	66/80	3%	0.78
<i>drr-2</i> overexpression in <i>eat-2(ad1116)</i> background						
N2 *	OP 50	19.99 $\pm$ 0.53	23	61/72	-	-
<i>eat-2(ad1116)</i> *	OP 50	29.41 $\pm$ 0.68	33	56/72	47%	< 0.0001
EQ191 ( <i>drr-2::gfp</i> ) *	OP 50	22.98 $\pm$ 0.67	26	57/72	15%	< 0.0001 ( < 0.0001 )
EQ192 ( <i>drr-2::gfp</i> ) *	OP 50	21.55 $\pm$ 0.79	26	42/72	8%	0.034 ( < 0.0001 )
N2	OP 50	19.87 $\pm$ 0.58	23	58/72	-	-
<i>eat-2(ad1116)</i>	OP 50	30.18 $\pm$ 1.26	36	47/72	52%	< 0.0001
EQ191 ( <i>drr-2::gfp</i> )	OP 50	25.16 $\pm$ 0.86	30	49/60	27%	< 0.0001 ( < 0.0001 )
EQ192 ( <i>drr-2::gfp</i> )	OP 50	24.37 $\pm$ 0.86	28	47/60	23%	< 0.0001 ( < 0.0001 )
N2	OP 50	20.98 $\pm$ 0.56	25	70/90	-	-
<i>eat-2(ad1116)</i>	OP 50	30.64 $\pm$ 0.67	35	67/90	46%	< 0.0001
EQ49 ( <i>drr-2::gfp</i> )	OP 50	23.41 $\pm$ 0.68	27	70/90	12%	0.0002 ( < 0.0001 )
N2	OP 50	21.40 $\pm$ 0.54	25	79/90	-	-
<i>eat-2(ad1116)</i>	OP 50	29.97 $\pm$ 0.69	35	71/90	40%	< 0.0001
EQ49 ( <i>drr-2::gfp</i> )	OP 50	23.17 $\pm$ 0.55	27	74/88	8%	0.0043 ( < 0.0001 )
N2	OP 50	18.83 $\pm$ 0.72	24	54/60	-	-
<i>eat-2(ad1116)</i>	OP 50	28.04 $\pm$ 1.14	35	52/69	49%	< 0.0001
EQ49 ( <i>drr-2::gfp</i> )	OP 50	23.44 $\pm$ 0.53	26	63/72	24%	< 0.0001 ( < 0.0001 )

**Table S1 Con't**

Overexpression strain	Bacteria	Mean lifespan $\pm$ s.e.m. (days)	75% (days)	n	% change	P
Solid plate dietary restriction						
N2 *	Killed OP 50; 1 x 10 <sup>12</sup>	15.48 $\pm$ 0.47	19	76/80	-11%	0.011
	<b>Killed OP 50; 1 x 10<sup>11</sup></b>	17.34 $\pm$ 0.62	19	75/80	-	-
	Killed OP 50; 1 x 10 <sup>10</sup>	21.27 $\pm$ 0.86	27	69/80	23%	0.0001
	Killed OP 50; 1 x 10 <sup>9</sup>	25.11 $\pm$ 0.82	30	69/80	45%	< 0.0001
	Killed OP 50; 1 x 10 <sup>8</sup>	30.76 $\pm$ 0.94	36	66/80	77%	< 0.0001
	Killed OP 50; 1 x 10 <sup>7</sup>	29.93 $\pm$ 0.95	36	65/80	73%	< 0.0001
<i>daf-16(mu86)</i> *	Killed OP 50; 1 x 10 <sup>12</sup>	12.08 $\pm$ 0.32	13	73/80	-14%	0.0008
	<b>Killed OP 50; 1 x 10<sup>11</sup></b>	14.09 $\pm$ 0.53	15	64/80	-	-
	Killed OP 50; 1 x 10 <sup>10</sup>	18.54 $\pm$ 0.50	23	69/80	32%	< 0.0001
	Killed OP 50; 1 x 10 <sup>9</sup>	21.91 $\pm$ 0.71	27	66/80	56%	< 0.0001
	Killed OP 50; 1 x 10 <sup>8</sup>	28.15 $\pm$ 0.85	34	74/80	100%	< 0.0001
	Killed OP 50; 1 x 10 <sup>7</sup>	27.55 $\pm$ 0.82	32	72/80	96%	< 0.0001
EQ19 ( <i>drr-2::gfp</i> ) *	Killed OP 50; 1 x 10 <sup>12</sup>	15.73 $\pm$ 0.57	19	65/81	-10%	0.033
	<b>Killed OP 50; 1 x 10<sup>11</sup></b>	17.51 $\pm$ 0.64	21	73/80	-	-
	Killed OP 50; 1 x 10 <sup>10</sup>	17.97 $\pm$ 0.74	23	74/80	3%	0.404
	Killed OP 50; 1 x 10 <sup>9</sup>	20.82 $\pm$ 0.81	27	78/80	19%	0.0002
	Killed OP 50; 1 x 10 <sup>8</sup>	23.45 $\pm$ 0.83	29	83/83	34%	< 0.0001
	Killed OP 50; 1 x 10 <sup>7</sup>	22.24 $\pm$ 0.82	29	73/76	27%	< 0.0001
N2	<b>Killed OP 50; 1 x 10<sup>11</sup></b>	17.86 $\pm$ 0.48	19	68/75	-	-
	(with FUDR) Killed OP 50; 1 x 10 <sup>9</sup>	21.06 $\pm$ 0.64	23	72/75	18%	< 0.0001
EQ19 ( <i>drr-2::gfp</i> )	<b>Killed OP 50; 1 x 10<sup>11</sup></b>	11.70 $\pm$ 0.61	14	70/75	-	-
	(with FUDR) Killed OP 50; 1 x 10 <sup>9</sup>	11.67 $\pm$ 0.59	14	70/75	0%	0.985
N2	<b>Killed OP 50; 1 x 10<sup>11</sup></b>	17.25 $\pm$ 0.69	21	65/80	-	-
	(with FUDR) Killed OP 50; 1 x 10 <sup>9</sup>	21.67 $\pm$ 0.75	26	69/80	26%	< 0.0001
EQ19 ( <i>drr-2::gfp</i> )	<b>Killed OP 50; 1 x 10<sup>11</sup></b>	13.84 $\pm$ 0.68	18	70/80	-	-
	(with FUDR) Killed OP 50; 1 x 10 <sup>9</sup>	15.71 $\pm$ 0.71	21	72/80	14%	0.052

**Table S1. Lifespan analysis of the effect of *drr-2* over-expression on DR.** Adult mean lifespan, in days, observed in lifespan analysis. Lifespan experiments were carried out at 20°C, unless noted otherwise. 100  $\mu$ g/ml of FUDR was added to each plate to stop the progeny production, if indicated. ‘75%’ is the mean lifespan of the 75<sup>th</sup> percentile (the

age at which the fraction of animals alive reaches 0.25). 'n' shows number of observed deaths relative to total number of animals started. The difference between these numbers represents the number of animals censored during the experiment, and includes animals that exploded, bagged (i.e. exhibited internal progeny hatching), or crawled off the plates. '% change' is the percentage of lifespan extension of either mutants compared to the N2 control or the *ad libitum* ( $1 \times 10^{11}$ ) control. *P* values were calculated by pair-wise comparisons to the N2 control, each consisting of control and experimental animals examined at the same time. *P* values in parentheses indicate comparisons between EQ49, EQ191, EQ192 and *eat-2(ad1116)*. EQ191 and EQ192 were generated by crossing the pAH11(*drr-2p::drr-2::gfp*) extrachromosomal array into the *eat-2(ad1116)* mutant background, while EQ49 was generated by injecting 30ng/ $\mu$ l pAH11(*drr-2p::drr-2::gfp*) plasmid into *eat-2(ad1116)* mutants. We used Statview 5.01 (SAS) software for statistical analysis and to determine means and percentiles. The logrank (Mantel-Cox) test was used to test the hypothesis that the survival functions among groups were equal. Repetitions of the same experiments are listed in order. '\*' indicates the sets of experiments plotted and shown in Figures.

**Table S2**

Strain	RNAi treatment	Mean lifespan $\pm$ s.e.m. (days)	75% (days)	n	% change	P
<i>drr-2</i> overexpression strain on TOR/ <i>let-363</i> (+Raptor/ <i>daf-15</i> ) RNAi						
<i>Adult only at 25°C</i>						
N2	Vector (control)	13.05 $\pm$ 0.48	16	66/80	-	-
	TOR/ <i>let-363</i> RNAi	15.51 $\pm$ 0.52	18	69/80	19%	0.0007
EQ19 ( <i>drr-2::gfp</i> )	Vector (control)	13.04 $\pm$ 0.44	14	60/72	-	-
	TOR/ <i>let-363</i> RNAi	11.42 $\pm$ 0.43	14	59/72	-12%	0.0134
N2 *	Vector (control)	15.97 $\pm$ 0.51	20	70/88	-	-
	TOR/ <i>let-363</i> +Raptor/ <i>daf-15</i> RNAi	20.90 $\pm$ 0.66	24	62/84	31%	<0.0001
EQ19 ( <i>drr-2::gfp</i> ) *	Vector (control)	16.64 $\pm$ 0.60	20	63/72	-	-
	TOR/ <i>let-363</i> +Raptor/ <i>daf-15</i> RNAi	16.57 $\pm$ 0.61	22	67/73	0%	0.89
N2	Vector (control)	14.04 $\pm$ 0.65	19	60/72	-	-
	TOR/ <i>let-363</i> +Raptor/ <i>daf-15</i> RNAi	21.22 $\pm$ 0.88	28	64/70	51%	<0.0001
EQ19 ( <i>drr-2::gfp</i> )	Vector (control)	14.79 $\pm$ 0.74	20	68/72	-	-
	TOR/ <i>let-363</i> +Raptor/ <i>daf-15</i> RNAi	14.23 $\pm$ 0.66	20	66/72	-4%	0.38
<i>drr-2</i> overexpression strain on S6K/ <i>rsk-1</i> RNAi						
N2 *	Vector (control)	18.56 $\pm$ 0.57	21	65/72	-	-
	S6K/ <i>rsk-1</i> RNAi	25.33 $\pm$ 0.79	30	65/72	36%	<0.0001
EQ19 ( <i>drr-2::gfp</i> ) *	Vector (control)	18.87 $\pm$ 0.54	21	50/72	-	-
	S6K/ <i>rsk-1</i> RNAi	25.15 $\pm$ 0.93	30	51/72	33%	<0.0001
N2	Vector (control)	19.02 $\pm$ 0.75	24	68/72	-	-
	S6K/ <i>rsk-1</i> RNAi	22.77 $\pm$ 1.01	29	65/72	20%	0.0005
EQ19 ( <i>drr-2::gfp</i> )	Vector (control)	20.11 $\pm$ 0.90	26	54/72	-	-
	S6K/ <i>rsk-1</i> RNAi	24.09 $\pm$ 1.05	29	55/72	20%	0.0016
<i>drr-2</i> overexpression strain on <i>rab-10</i> RNAi						
N2 *	Vector (control)	17.22 $\pm$ 0.72	22	68/72	-	-
	<i>rab-10</i> RNAi	23.90 $\pm$ 0.85	29	55/72	39%	<0.0001
EQ19 ( <i>drr-2::gfp</i> ) *	Vector (control)	17.14 $\pm$ 0.68	22	60/72	-	-
	<i>rab-10</i> RNAi	18.39 $\pm$ 0.73	22	60/72	7%	0.14
N2	Vector (control)	16.56 $\pm$ 0.76	21	66/80	-	-
	<i>rab-10</i> RNAi	20.00 $\pm$ 0.84	27	70/80	21%	0.0006
EQ19 ( <i>drr-2::gfp</i> )	Vector (control)	17.06 $\pm$ 0.74	21	66/80	-	-
	<i>rab-10</i> RNAi	18.75 $\pm$ 0.73	26	61/80	10%	0.1
<i>drr-2</i> overexpression strain on <i>sams-1</i> RNAi						
N2 *	Vector (control)	17.22 $\pm$ 0.72	22	68/72	-	-
	<i>sams-1</i> RNAi	24.55 $\pm$ 0.87	29	58/72	43%	<0.0001
EQ19 ( <i>drr-2::gfp</i> ) *	Vector (control)	17.14 $\pm$ 0.68	22	60/72	-	-
	<i>sams-1</i> RNAi	19.08 $\pm$ 0.68	22	65/72	11%	0.054
N2	Vector (control)	16.56 $\pm$ 0.76	21	66/80	-	-
	<i>sams-1</i> RNAi	21.73 $\pm$ 0.97	29	58/80	31%	<0.0001
EQ19 ( <i>drr-2::gfp</i> )	Vector (control)	17.06 $\pm$ 0.74	21	66/80	-	-
	<i>sams-1</i> RNAi	17.51 $\pm$ 0.59	21	67/80	3%	0.8028

**Table S2. Lifespan analysis of *drr-2* over-expressing animals grown on various RNAi bacteria.** Adult mean lifespan, in days, observed in lifespan analysis. Lifespan experiments were carried out at 25°C for TOR/*let-363* RNAi experiments and at 20°C for all other experiments. RNAi treatments were initiated from hatching, except for TOR/*let-363* RNAi experiments, which was initiated at Day 1 of adulthood. ‘75%’ is the mean lifespan of the 75<sup>th</sup> percentile (the age at which the fraction of animals alive reaches 0.25). ‘n’ shows number of observed deaths relative to total number of animals started. The difference between these numbers represents the number of animals censored during the experiment, and includes animals that exploded, bagged (i.e. exhibited internal progeny hatching), or crawled off the plates. ‘% change’ is the percentage of lifespan extension compared to the empty vector (L4440) control. *P* values were calculated by pair-wise comparisons to the vector control, each consisting of control and experimental animals examined at the same time. We used Statview 5.01 (SAS) software for statistical analysis and to determine means and percentiles. The logrank (Mantel-Cox) test was used to test the hypothesis that the survival functions among groups were equal. Repetitions of the same experiments are listed in order. ‘\*’ indicates the sets of experiments plotted and shown in Figures.

**Table S3**

Strain	RNAi treatment	Mean lifespan $\pm$ s.e.m. (days)	75% (days)	n	% change	P
<i>pha-4(zu225)</i> on <i>drr-2</i> RNAi						
N2 *	Vector (control)	16.49 $\pm$ 0.61	22	65/80	-	-
	<i>drr-2</i> RNAi	20.43 $\pm$ 0.65	24	68/80	24%	<0.0001
<i>pha-4(zu225)</i> *	Vector (control)	14.70 $\pm$ 0.59	17	67/72	-	-
	<i>drr-2</i> RNAi	17.68 $\pm$ 0.77	22	61/72	20%	0.0013
N2	Vector (control)	14.04 $\pm$ 0.65	19	60/72	-	-
	<i>drr-2</i> RNAi	17.34 $\pm$ 0.72	22	64/72	24%	0.0006
<i>pha-4(zu225)</i>	Vector (control)	16.58 $\pm$ 0.45	191	88/90	-	-
	<i>drr-2</i> RNAi	19.20 $\pm$ 0.62	22	64/72	16%	0.0003
<i>sams-1</i> & <i>rab-10</i> overexpression strains on <i>drr-2</i> RNAi						
N2 *	Vector (control)	17.41 $\pm$ 0.53	19	65/72	-	-
	<i>drr-2</i> RNAi	21.17 $\pm$ 0.47	23	63/72	22%	<0.0001
EQ2 ( <i>sams-1::gfp</i> ) *	Vector (control)	16.10 $\pm$ 0.63	19	59/72	-	-
	<i>drr-2</i> RNAi	20.58 $\pm$ 0.67	25	64/72	28%	<0.0001
N2	Vector (control)	18.44 $\pm$ 0.58	21	61/72	-	-
	<i>drr-2</i> RNAi	20.44 $\pm$ 0.51	23	65/72	11%	0.034
EQ2 ( <i>sams-1::gfp</i> )	Vector (control)	17.33 $\pm$ 0.82	23	59/72	-	-
	<i>drr-2</i> RNAi	20.33 $\pm$ 0.77	26	62/72	17%	0.0066
N2 *	Vector (control)	18.76 $\pm$ 0.55	21	64/72	-	-
	<i>drr-2</i> RNAi	22.15 $\pm$ 0.54	25	65/72	18%	<0.0001
EQ28 ( <i>rab-10::gfp</i> ) *	Vector (control)	16.59 $\pm$ 0.44	19	65/72	-	-
	<i>drr-2</i> RNAi	20.86 $\pm$ 0.50	23	58/72	26%	<0.0001
N2	Vector (control)	21.68 $\pm$ 0.62	26	68/72	-	-
	<i>drr-2</i> RNAi	25.20 $\pm$ 0.70	28	58/72	16%	0.0003
EQ2 ( <i>sams-1::gfp</i> )	Vector (control)	17.83 $\pm$ 0.87	25	69/72	-	-
	<i>drr-2</i> RNAi	20.80 $\pm$ 0.96	28	66/74	17%	0.016
EQ28 ( <i>rab-10::gfp</i> )	Vector (control)	17.98 $\pm$ 0.46	21	57/72	-	-
	<i>drr-2</i> RNAi	20.37 $\pm$ 0.48	23	68/75	13%	0.0002

**Table S3. Lifespan analysis of various mutants grown on *drr-2* RNAi bacteria.**

Adult mean lifespan, in days, observed in lifespan analysis. Lifespan experiments were carried out at 20°C, unless noted otherwise. ‘75%’ is the mean lifespan of the 75<sup>th</sup> percentile (the age at which the fraction of animals alive reaches 0.25). ‘n’ shows

number of observed deaths relative to total number of animals started. The difference between these numbers represents the number of animals censored during the experiment, and includes animals that exploded, bagged (i.e. exhibited internal progeny hatching), or crawled off the plates. ‘% change’ is the percentage of lifespan extension compared to the empty vector (L4440) control. *P* values were calculated by pair-wise comparisons to the vector control, each consisting of control and experimental animals examined at the same time. We used Statview 5.01 (SAS) software for statistical analysis and to determine means and percentiles. The logrank (Mantel-Cox) test was used to test the hypothesis that the survival functions among groups were equal. Repetitions of the same experiments are listed in order. ‘\*’ indicates the sets of experiments plotted and shown in Figures.