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Supplemental Information

TOR Signaling and Rapamycin Influence Longevity by Regulating SKN-1/Nrf and DAF-16/FoxO

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Supplemental Experimental Procedures

C. elegans

Worms were cultured at 20°C on NGM plates, unless otherwise indicated. The streptomycin-resistant *E. coli* strain OP50-1 (Caenorhabditis Genetics Center) was used as the standard food. The following *C. elegans* strains were used in this study, with strain ENH196 generated by crossing:

Number	Genetic background	Transgene	Array number	Reference
LD001	N2	Is[*SKN-1B/C::GFP*]	007	(An and Blackwell, 2003)
LD1008	N2	Ex[*SKN-1op::GFP*]	009	(Tullet et al., 2008)
LD1171	N2	Is[*gcs-1p::GFP*]	003	(Wang et al., 2010)
LD1271	N2	Ex[*pRF4 rol-6*]		(Tullet et al., 2008)
TJ356	N2	Is[DAF-16a::GFP]		(Henderson et al., 2006)
	N2	Ex[DAF-16::GFP]		(Lin et al., 2001)
	N2	[*gst-4p::GFP*]		(Link and Johnson, 2002)
CF1553	N2	[*sod-3p::GFP*]		(Libina et al., 2003)
DA2123	N2	adIs2122[*lgg-1::GFP*]		(Melendez et al., 2003)
LD1175	*skn-1(zu67)*	Is[*gcs-1p::GFP*]	003	(Wang et al., 2010)
CF1874	*daf-16(mu86)*	muIs84[*sod-3p::GFP]*		(Yamawaki et al., 2008)
VP288	*rde-1(ne219)*	Ex[*pnhx-2::rde-1;rol-6*]		(Qadota et al., 2007)
ENH196	*rict-1(mg451)*	Is[*skn-1B/C::GFP*]	007	This Study
EU1	*skn-1(zu67)*	-	-	(Bowerman et al., 1992)
	daf-16(mgDf50)	[*daf-16a::RFP*]		(Kwon et al., 2010)
	daf-16(mgDf50)	[*daf-16f::GFP*]		(Kwon et al., 2010)
EU31	*skn-1(zu135)*	-	-	(Bowerman et al., 1992)
RB1202	*kri-1(ok1251)*	-	-	(Berman and Kenyon, 2006)
DG2389	*glp-1(bn18)*	-	-	(Dorsett et al., 2009)
CF1038	*daf-16(mu86)*	-	-	(Lin et al., 1997)
LD1263	*daf-16(mgDf47);skn-1(zu67)*	-	-	(Wang et al., 2010)
	daf-16(mgDf47)	-	-	(Ogg et al., 1997)
WM27	*rde-1(ne219)*	-	-	(Tabara et al., 1999)

Stress resistance assays

To assay TBHP resistance, late L4 stage worms were fed with rapamycin, RNAi or control bacteria for three days at 20°C, then transferred to NGM plates that contained 15.4

mM TBHP (Sigma-Aldrich) and were seeded with OP50, and had been prepared two hours previously. For heat resistance, worms that had been treated as above were transferred to seeded NGM plates and placed at 35°C. In each TBHP or heat experiment, three plates of 20 worms each were analyzed. For all stress experiments, worms were scored as dead when they did not respond to repeated gentle prodding with a platinum wire.

Transgenic reporter scoring

Unless otherwise indicated, expression or nuclear accumulation of transgenic GFP proteins was scored as “Low, Medium, or High” essentially as published (An and Blackwell, 2003; Tullet et al., 2008; Wang et al., 2010). For the *gcs-1* or *gst-4* transcriptional reporters, ‘‘High’’ indicates GFP detection at high levels throughout most of the intestine, while ‘‘Medium’’ refers to animals with robust GFP signal present only anteriorly or posteriorly. For SKN-1::GFP or DAF-16::GFP reporters, “High” indicates that a strong SKN-1::GFP signal was present in all intestinal nuclei, and “Medium” that nuclear SKN-1::GFP was present at high levels anteriorly, posteriorly or both, but barely visible midway through the intestine, or that a weak signal was observed in all intestinal nuclei. In most RNAi assays, early day 1 adults were placed on feeding RNAi plates 3 days before scoring.

ChIP analysis

ChIP was performed as described (Glover-Cutter et al., 2008), with the following modifications. 2 ml packed N2 worms were collected from plates, washed with M9 three times, washed with crosslinking buffer (PBS, 1% Formaldehyde) twice, resuspended in 6ml crosslinking buffer and frozen dropwise in liquid N_2. Pea-size frozen pellets were crushed in a dry-ice-chilled mortar and pestle, and ground into powder until worm carcasses were disturbed. After thawing, formaldehyde crosslinking was performed at room temperature for 20 minutes. These conditions would capture interactions at one angstrom (Glover-Cutter et al., 2008). The reaction was quenched with 125 mM glycine at room temperature for 5 minutes, pelleted at 4500rpm, washed 3 times with PBS, and resuspended in 6 ml complete RIPA. Sonication (Branson Sonifer 4900) was performed at 4^{o}C at 30% amplitude for 32 cycles of 15 seconds followed by 59 seconds of rest. After protein concentration by Bradford analysis, 1mg/ml samples were and frozen at -80^{o}C in 1ml aliquots. IPs were performed as described (Glover-Cutter et al., 2008), using antibodies for phosphorylated CTD Ser2 (polyclonal) and Ser5 (H14 monoclonal) (Glover-Cutter et al., 2008), and SKN-1 (monoclonal FC4) (Bowerman et al., 1993).

The SKN-1 target genes that were analyzed by ChIP all have SKN-1 binding sites located nearby in their predicted regulatory regions, as described in Oliveira, et al. (2009). For ChIP analysis, qPCR primers were designed according to the best "rank", "score" , and "delta G" from the primer design program LightCycler Probe Design Software 2.0. The resolution of the technique is approximately 250-500 bp (Glover-Cutter et al., 2008).

Autophagy analysis

Puncta of transgenic LGG-1::GFP, an autophagosome marker (Melendez et al., 2003), were scored as an indicator of autophagy. Animals were treated with RNAi beginning at the L4 stage, and then analyzed at day 3 of adulthood. GFP positive puncta were counted in 2-10 seam (lateral epidermal) cells of approximately 20-30 animals in 3 independent experiments.

Translation assay

Translation was measured as ^{35}S incorporation, using an adapted version of a published protocol (Hansen et al., 2007). OP50-1 bacteria were cultured overnight in LB containing 10µCi/mL ^{35}S-methionine (Perkin Elmer NEG709A), then concentrated 10 fold in Eppendorf tubes. Day 1 adults (~2000/sample) were treated with RNAi or 100µM Rapamycin for 3 days on plates containing FUdR. Worms were washed off the plates with S-basal, washed three times, then aliquotted into 4 tubes containing 100µl of radiolabeled OP50. 3 of these tubes were incubated at 22°C for 3 hours with constant rotation. The 4th tube, used to determine the level of contamination by unincorporated ^{35}S-methionine, was incubated for 1 minute. After incubation, worms were washed 3 times with S-basal, incubated with 100µl of unlabeled OP50 for 30 minutes. Worms were washed 3 times with S-basal and the pellet was flash frozen in liquid nitrogen. Samples were thawed and resuspended in 100µl of 1% SDS and boiled for 15 minutes with vortexing, then centrifuged for 20 minutes at 16,000 x g. Supernatants were precipitated with 10% trichloroacetic acid (TCA) for 1 hour on ice. TCA precipitates were pelleted and washed 2 times with ice-cold ethanol. After air drying, the pellet was resuspended in 100µl 1% SDS, 0.1M Tris-HCl pH8.0 and boiled for 30 minutes. Protein concentrations were measured with a BCA protein assay kit (Thermo Scientific prod # 23225) and ^{35}S radioactivity was measured by liquid scintillation (Beckman LS6500) after application to glass microfibers (Whatman).

Mice and treatments

Male C57BL/6 mice were obtained from Taconic at approximately 8 weeks of age. Chronic rapamycin treatment was performed by injecting 8 to 10 week old mice intraperitoneally once daily with either 2 mg/kg rapamycin suspended in 0.9% NaCl and 2% ethanol at a concentration of 0.5 mg/mL (547 µM), or vehicle only for 14-28 days. Following an overnight, 16-hour fast, mice were re-fed (or not) for 45 minutes and then euthanized with CO2 prior to organ harvest. All experiments were carried out with approval from the Committee for Animal Care at MIT and under supervision of the Department of Comparative Medicine at MIT.

RNAi, RNA, isolation, and quantitative (q)RT-PCR

Feeding RNAi was performed with sequenced clones as described, using HT115 and pL4440 as control (Wang et al., 2010). For *C. elegans* RNA sample preparation, early day-1 adults were fed RNAi or control bacteria or rapamycin for 3 days. After transfer to clean plates, total RNA was extracted from 200 worms using Trizol (Sigma-Aldrich). Total RNA was extracted from frozen mouse liver using a Qiagen RNeasy Mini Kit according to manufacturer's instructions. The concentration and purity of RNA were determined by absorbance at 260/280 nm. RNA from some mouse samples was previously analyzed (for different genes) in Lamming, et al. (2012). cDNA was synthesized using the Superscript RT Kit (Life Technologies). qRT-PCR was performed on an ABI 7900 using the SYBER Green kit (Life Technologies).

Statistical Analysis

All stress, lifespan, and healthspan data were analyzed using JMP, with p values representing log rank. P values for transgenic reporter scoring were obtained by the chi^2 method. Autophagy data were analyzed by an unpaired two-tailed t-test, with error bars representing ± SEM. ^{35}S incorporation levels were calculated by normalizing ^{35}S counts per min., corrected for nonspecific background, to total numbers of worms. Statistical analysis

was done with a one-sided paired Student's *t*-test with error bars representing ± SEM. The relative levels of ^{35}S incorporation were calculated by normalizing the ^{35}S incorporation levels of RNAi- or rapamycin-treated worms to pL4440 empty vector control, which was set to 100. Quantitative (q)RT-PCR data were analyzed by the standard curve method (Glover-Cutter et al., 2008), with normalization to either the number of worms or an internal standard. All qRT-PCR and ChIP p-values in this study were calculated by one- or two-sided Student's *t*-test, as appropriate, with error bars representing the SEM.

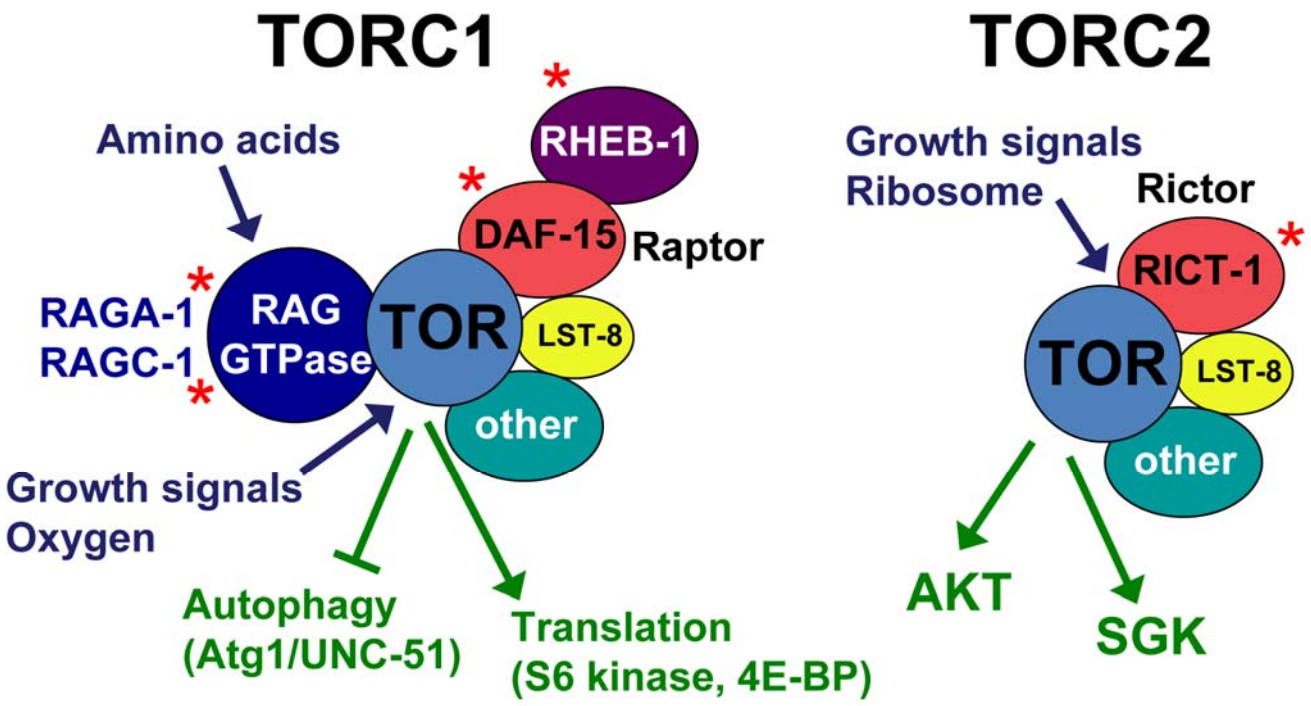

Figure S1. Schematic Illustrating the TORC1 and TORC2 Complexes, Related to Figure 1

Not all subunits, inputs, or activities attributed to these kinase complexes are shown (Cybulski and Hall, 2009; Kapahi et al., 2010; Ma and Blenis, 2009; Zoncu et al., 2011). TORC1 is defined by its unique component Raptor (*C. elegans* DAF-15). Through the heterodimeric Rag GTPases (*C. elegans* RAGA-1 and RAGC-1), TORC1 receives a signal that indicates amino acid availability. When amino acids are plentiful, these GTPases direct TORC1 to be recruited to the lysosomal surface, where TORC1 is activated through its interaction with Rheb (*C. elegans* RHEB-1). TORC1 inhibits autophagy by phosphorylating the kinase Atg1(*C. elegans* UNC-51), and promotes mRNA translation through inhibitory phosphorylation of 4E-BP, and activating phosphorylation of the p70/S6 kinase (*C. elegans* RSKS-1) and other substrates. The functions of the TORC2 complex, which is defined by presence of Rictor (*C. elegans* RICT-1) are not as well understood. In mammals TORC2 phosphorylates and activates several AGC-family kinases, including AKT and SGK, and its activity is stimulated by growth factor signaling and binding to the ribosome (Cybulski and Hall, 2009; Oh et al., 2010; Zinzalla et al., 2011). Proteins that were analyzed functionally by RNAi knockdown in this study are indicated by red asterisks.

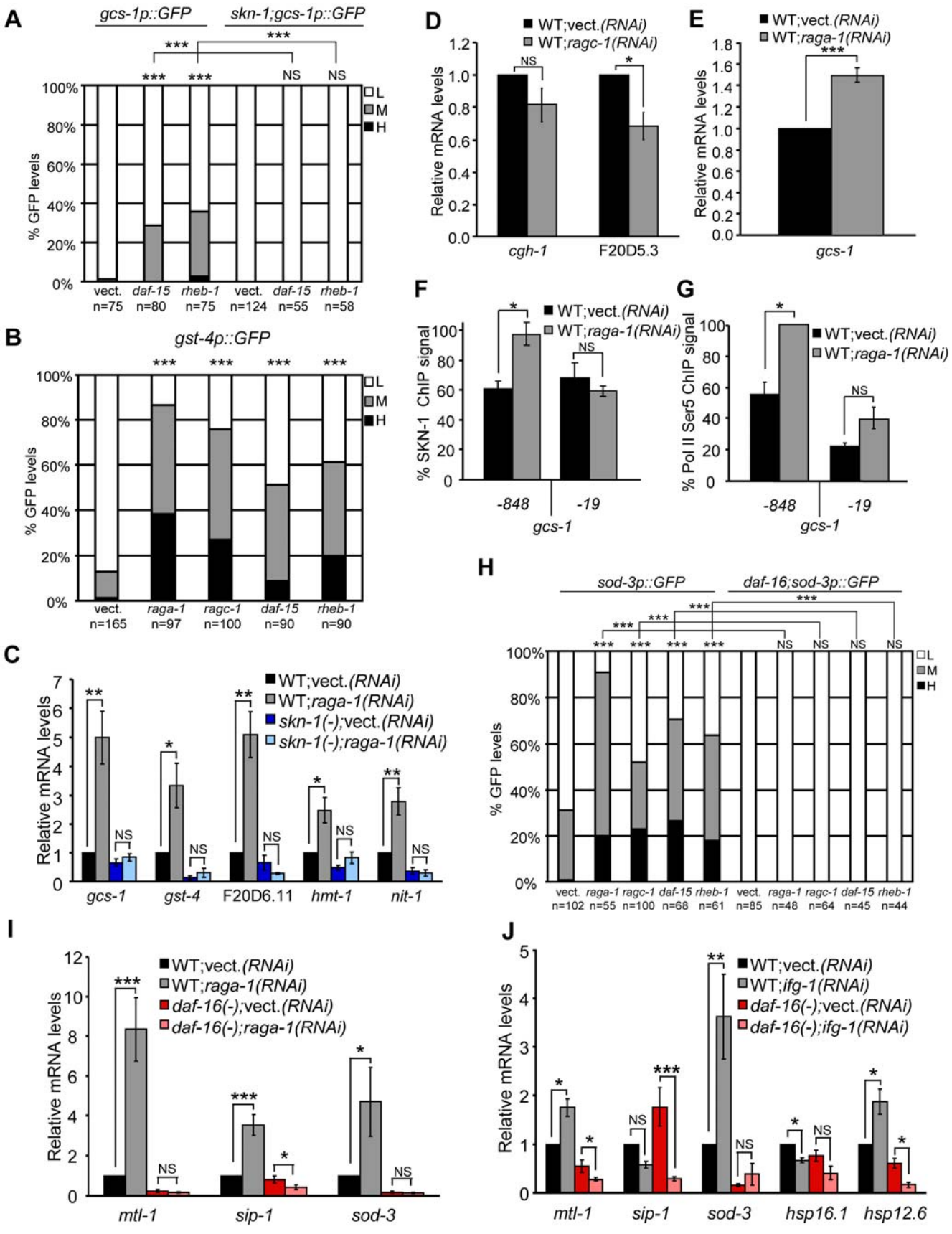
A
gcs-1p::GFP
skn-1;gcs-1p::GFP
% GFP levels
L
M
H
vect. n=75
daf-15 n=80
rheb-1 n=75
vect. n=124
daf-15 n=55
rheb-1 n=58
B
gst-4p::GFP
% GFP levels
vect. n=165
raga-1 n=97
ragc-1 n=100
daf-15 n=90
rheb-1 n=90
C
Relative mRNA levels
WT;vect.(RNAi)
WT;raga-1(RNAi)
skn-1(-);vect.(RNAi)
skn-1(-);raga-1(RNAi)
gcs-1
gst-4
F20D6.11
hmt-1
nit-1
D
WT;vect.(RNAi)
WT;ragc-1(RNAi)
Relative mRNA levels
cgh-1
F20D5.3
E
WT;vect.(RNAi)
WT;raga-1(RNAi)
Relative mRNA levels
gcs-1
F
% SKN-1 ChIP signal
WT;vect.(RNAi)
WT;raga-1(RNAi)
-848
-19
gcs-1
G
% Pol II Ser5 ChIP signal
WT;vect.(RNAi)
WT;raga-1(RNAi)
-848
-19
gcs-1
H
sod-3p::GFP
daf-16;sod-3p::GFP
% GFP levels
vect. n=102
raga-1 n=55
ragc-1 n=100
daf-15 n=68
rheb-1 n=61
vect. n=85
raga-1 n=48
ragc-1 n=64
daf-15 n=45
rheb-1 n=44
I
Relative mRNA levels
WT;vect.(RNAi)
WT;raga-1(RNAi)
daf-16(-);vect.(RNAi)
daf-16(-);raga-1(RNAi)
mtl-1
sip-1
sod-3
J
Relative mRNA levels
WT;vect.(RNAi)
WT;ifg-1(RNAi)
daf-16(-);vect.(RNAi)
daf-16(-);ifg-1(RNAi)
mtl-1
sip-1
sod-3
hsp16.1
hsp12.6

Figure S2. Induction of SKN-1- and DAF-16-Mediated Transcriptional Responses by Genetic TORC1 Inhibition, Related to Figure 3

(A) *skn-1*-dependent upregulation of the *gcs-1p::GFP* reporter in response to knockdown of TORC1 pathway genes, analyzed and graphed as in Figure 3A. *gcs-1* encodes a rate-limiting enzyme in glutathione synthesis (gamma-glutamyl cysteine synthase), and is a conserved target of SKN-1/Nrf proteins (An and Blackwell, 2003). L=Low, M=Medium, H=High. ***P< 0.0001, N.S.=not significant, chi^2 method. (B) Upregulation of the *skn-1*-dependent *gst-4p::GFP* reporter in response to genetic TORC1 inhibition, analyzed and graphed as in (A). ***P< 0.0001. (C) Activation of endogenous SKN-1 target genes by *raga-1* RNAi, measured by quantitative (q)-RT-PCR as in Figure 3B. Parallel data obtained from *skn-1* mutants are shown. Fold induction relative to WT vector control is shown for all qRT-PCR data. (D) Representative negative controls for qRT-PCR analyses. Note that these genes, including the SKN-1 target F20D5.3 (Oliveira et al., 2009), were not upregulated by Rag GTPase gene RNAi. (E-G) Transcriptional activation of the SKN-1 target *gcs-1* by *raga-1* RNAi, detected by qRT-PCR (E) in lysates that were analyzed by ChIP in (F, G). The relative ChIP signal is shown for SKN-1 (F) and a marker of transcription initiation (Serine 5-phosphorylated Pol II CTD; Ser5)(Bentley, 2005) (G) along the gene. Positions indicated below the bar graphs correspond to the middle of each qPCR amplicon relative to the predicted transcription start site. Both SKN-1 recruitment and Ser5 phosphorylation were enhanced after genetic TORC1 inhibition, with signals of 30% and 10% representing thresholds for specific presence of SKN-1 and P-Ser5, respectively (As indicated by parallel analyses of intergenic regions and genes that are not regulated by SKN-1). *gcs-1* is located within an operon and is transcribed from both upstream operon and immediately 5' promoters, which accounts for the high Ser5 signal distal to the start site (-848) (Garrido-Lecca and Blumenthal, 2010). (H) Knockdown of TORC1 pathway genes results in *daf-16*-dependent induction of the DAF-16 target gene reporter *sod-3::GFP* (Antebi, 2007) in the intestine. Analysis and scoring were performed as in Figure 3A. ***P< 0.0001, NS= not significant, chi^2 method. L=Low, M=Medium, H= High. (I) Activation of endogenous DAF-16 target genes by *raga-1* RNAi. (J) Activation of endogenous DAF-16 target genes by knockdown of the translation initiation factor *ifg-1* (eIF4G). ***P<0.001, **P<0.01, *P<0.05, NS=not significant. All qRT-PCR and ChIP p-values in this study were calculated by one- or two-sided Student's *t*-test, as appropriate. In all plots, error bars represent ± SEM

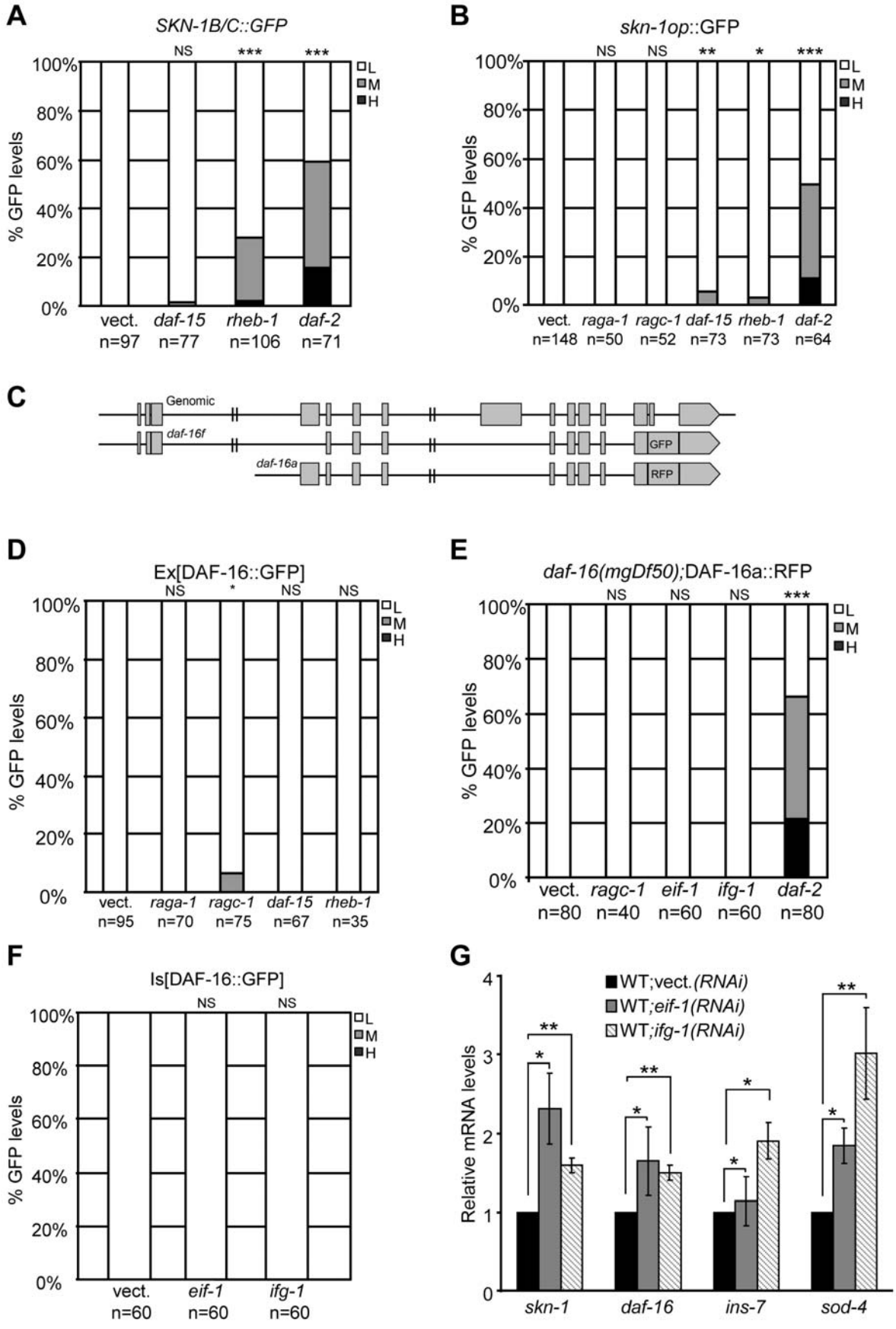
A
SKN-1B/C::GFP
NS
***
***
100%
80%
60%
40%
20%
0%
% GFP levels
L
M
H
vect.
daf-15
rheb-1
daf-2
n=97
n=77
n=106
n=71
B
skn-1op::GFP
NS
NS
**
*
***
vect.
raga-1
ragc-1
daf-15
rheb-1
daf-2
n=148
n=50
n=52
n=73
n=73
n=64
C
Genomic
daf-16f
daf-16a
GFP
RFP
D
Ex[DAF-16::GFP]
NS
*
NS
NS
vect.
raga-1
ragc-1
daf-15
rheb-1
n=95
n=70
n=75
n=67
n=35
E
daf-16(mgDf50);DAF-16a::RFP
NS
NS
NS
***
vect.
ragc-1
eif-1
ifg-1
daf-2
n=80
n=40
n=60
n=60
n=80
F
Is[DAF-16::GFP]
NS
NS
vect.
eif-1
ifg-1
n=60
n=60
n=60
G
WT;vect.(RNAi)
WT;eif-1(RNAi)
WT;ifg-1(RNAi)
Relative mRNA levels
skn-1
daf-16
ins-7
sod-4

Figure S3. Distinct Transcriptional Responses to Genetic Inhibition of TORC1 and IIS, Related to Figure 4

(A, B) Genetic TORC1 inhibition generally does not increase SKN-1 occupancy in intestinal nuclei, except for a modest effect observed after *rheb-1* RNAi. The *SKN-1B/C::GFP* and *SKN-1op::GFP* transgenes encode two and three of the three SKN-1 isoforms, respectively, as full-length proteins fused to GFP (An and Blackwell, 2003; Tullet et al., 2008). A *daf-2* RNAi control (reduced IIS) is shown. (C) Schematic illustrating DAF-16 isoforms, and transgenes described in Kwon, et al. (2010), not to scale. (D) Analysis of DAF-16 nuclear occupancy in the intestine, analyzed as in (A, B). This transgene encodes DAF-16 isoform a (Lin et al., 2001). (E) Transgenic DAF-16a expressed from a different transgene (Kwon et al., 2010) does not localize to nuclei after inhibition of TORC1 or translation initiation. (F) DAF-16a expressed from a third transgene (Henderson et al., 2006) fails to accumulate in intestinal nuclei after inhibition of translation initiation. ***P< 0.0001, **P< 0.004, *P< 0.05, N.S.= not significant, chi^2 method. L=Low, M=Medium, H=High. GFP reporters were scored as described in Experimental Procedures. (G) Transcription induction of *skn-1*, *daf-16*, and other genes (see text) by inhibition of translation initiation, detected by qRT-PCR. **P<0.01, *P<0.05 *t*-test. Error bars represent ± SEM

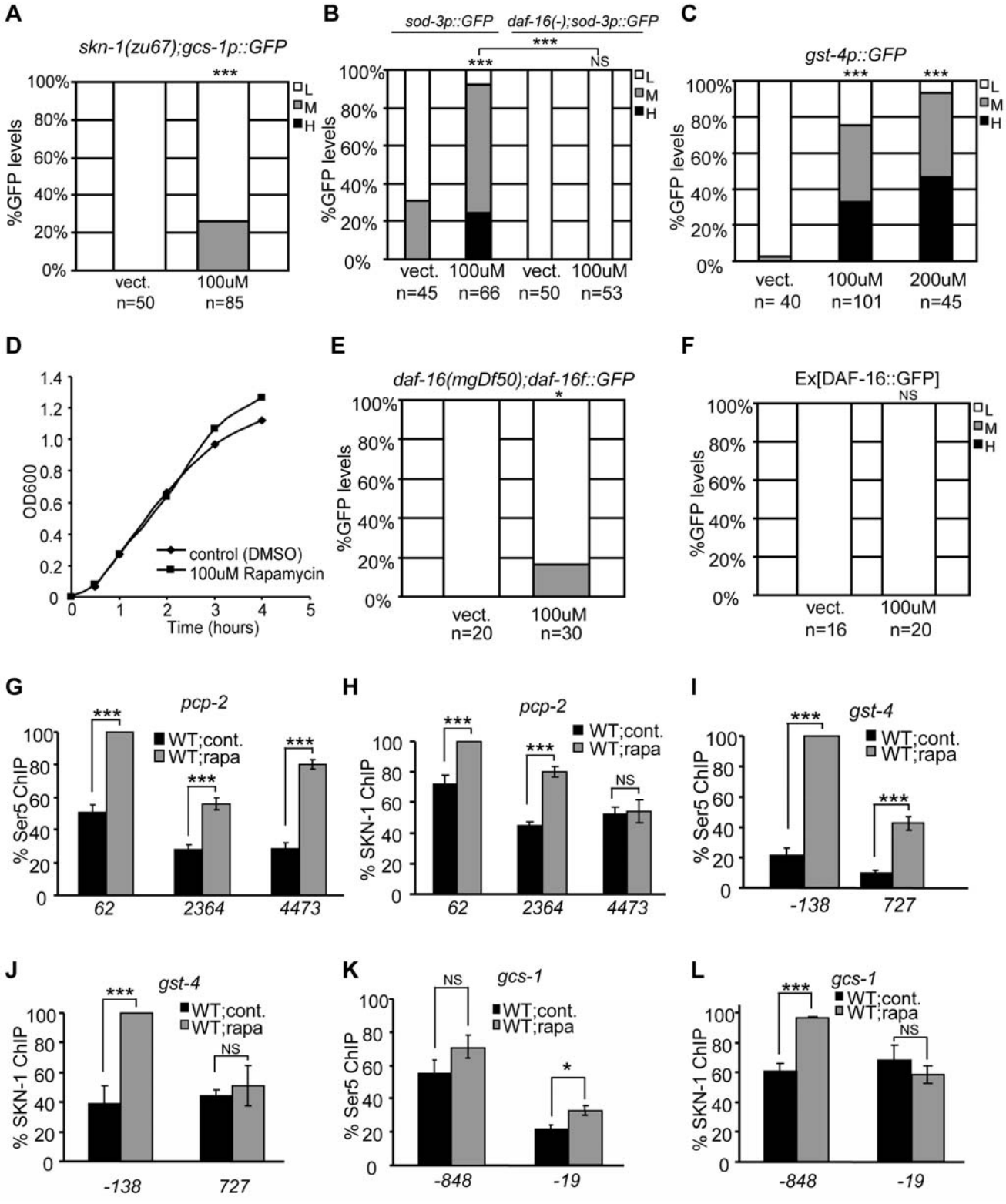

Figure S4. Regulation of SKN-1- and DAF-16-Mediated Transcription by Rapamycin, Related to Figure 5

(A) SKN-1-dependence of *gcs-1::GFP* induction. Compare with the robust reporter gene activation shown in Figure 5A, which occurred in parallel. (B) *daf-16*-dependent activation of intestinal *sod-3::GFP* by rapamycin. (C) Upregulation of the SKN-1 target *gst-4::GFP* in the

intestine by rapamycin. (D) Rapamycin does not inhibit bacterial growth. Proliferation of a liquid culture of OP50 was monitored. (E) Modest nuclear accumulation of DAF-16f (Kwon et al., 2010) induced by rapamycin. (F) Nuclear accumulation of DAF-16a (Henderson et al., 2006) is not increased by rapamycin. ***P< 0.0001, **P< 0.054, N.S.= not significant, chi^2 method. L=Low, M=Medium, H=High. (G-L) Rapamycin-induced accumulation of endogenous SKN-1, and Serine 5-phosphorylated Pol II CTD (Ser5) at the endogenous SKN-1 target genes *pcp-2*, *gst-4*, and *gcs-1* (Oliveira et al., 2009), detected by ChIP as in Figures S2E-S2G. ***P<0.001, **P<0.01, *P<0.05, *t*-test. In all plots, error bars represent ± SEM

Table S1. Individual TBHP Survival Trials, Performed and Analyzed as in Figure 1C, Related to Figure 1

Strain (+TBHP)	RNAi/ Rapamycin treatment	Mean treatment survival (hours ± SEM)	75th %ile (hours)	No. treatment animals	Mean Control Survival (hours ± SEM)	75th %ile (hours)	No. Control animals	% Mean survival extension	*P* value vs. Control	Assay #
WT	*raga-1*	16.28 ± 0.2	17	50/50	8.98 ± 0.2	10	49/49	81	<.0001	1
	raga-1	15.92 ± 0.3	16	26/26	7.30 ± 0.2	8	55/55	118	<.0001	2
	raga-1	14.66 ± 0.2	16	59/59	9.07 ± 0.3	9	57/57	62	<.0001	3
	raga-1	16.87 ± 0.2	18	61/62	9.67 ± 0.2	11	64/64	74	<.0001	4
	ragc-1	16.44 ± 0.3	18	59/59	10.26 ± 0.2	11	57/57	60	<.0001	5
	ragc-1	18.21 ± 0.3	19	57/57	10.23 ± 0.3	11	46/46	78	<.0001	6
	ragc-1	15.04 ± 0.2	16	53/55	9.27 ± 0.2	10	49/49	62	<.0001	8
	daf-15	16.55 ± 0.2	18	51/56	9.27 ± 0.2	10	49/49	79	<.0001	8
	daf-15	17.59 ± 0.1	18	44/51	8.71 ± 0.3	10	51/53	102	<.0001	9
	daf-15	16.70 ± 0.1	17	43/43	9.25 ± 0.3	11	55/55	81	<.0001	10
	rheb-1	16.12 ± 0.2	17	55/55	9.27 ± 0.2	10	49/49	74	<.0001	8
	rheb-1	17.25 ± 0.2	18	40/40	8.71 ± 0.3	10	51/53	98	<.0001	9
	rheb-1	16.20 ± 0.2	17	49/49	9.25 ± 0.3	11	55/55	75	<.0001	10
	100uM	16.69 ± 0.3	17	36/36	7.71 ± 0.3	9	44/44	116	<.0001	1
	100uM	15.84 ± 0.1	16	44/47	7.49 ± 0.2	9	39/39	111	<.0001	7
skn-1(zu67)	*raga-1*	7.96 ± 0.2	9	48/48	7.81 ± 0.2	9	57/57	2	.3623	1
	raga-1	5.91 ± 0.4	8	22/22	5.66 ± 0.3	7	32/32	4	.0087	2
	raga-1	6.92 ± 0.2	8	49/49	7.39 ± 0.2	9	57/57	-6	.0311	3
	ragc-1	8.15 ± 0.2	9	47/47	9.32 ± 0.1	10	56/56	-13	<.0001	5
	ragc-1	7.29 ± 0.2	8	48/48	7.77 ± 0.2	9	39/39	-7	.0128	6
	ragc-1	6.61 ± 0.1	7	62/62	7.02 ± 0.2	8	49/49	-6	.0220	8
	daf-15	6.46 ± 0.1	7	46/46	7.02 ± 0.2	8	49/49	-8	.0039	8
	daf-15	7.79 ± 0.2	9	52/52	7.80 ± 0.3	8	54/54	0	.3003	10
	rheb-1	7.07 ± 0.2	8	43/43	7.02 ± 0.2	8	49/49	0	.9886	8
	rheb-1	8.12 ± 0.3	8	33/33	7.80 ± 0.3	8	54/54	4	.9996	10
	100uM	6.46 ± 0.2	8	57/57	6.77 ± 0.3	8	35/35	-5	.3204	1
	100uM	6.79 ± 0.1	8	53/53	7.04 ± 0.1	8	51/51	-4	.1897	7
	400uM	7.57 ± 0.3	9	35/35	6.77 ± 0.3	8	35/35	12	.1179	1
daf-16(mgDf47)	*raga-1*	15.89 ± 0.2	17	54/54	7.11 ± 0.2	8	47/47	123	<.0001	1
	raga-1	15.37 ± 0.2	17	60/60	8.76 ± 0.2	9	67/67	75	<.0001	3
	raga-1	17.58 ± 0.3	20	50/50	9.87 ± 0.2	11	60/60	78	<.0001	4
	ragc-1	19.13 ± 0.3	22	70/70	11.23 ± 0.3	13	69/70	70	<.0001	5
	ragc-1	16.92 ± 0.3	19	59/59	10.79 ± 0.3	12	52/52	60	<.0001	6
	ragc-1	17.03 ± 0.2	18	59/59	9.85 ± 0.3	11	39/39	73	<.0001	8
	daf-15	17.40 ± 0.3	18	52/52	9.85 ± 0.3	11	39/39	77	<.0001	8
	daf-15	16.13 ± 0.1	17	52/52	10.17 ± 0.3	12	59/59	59	<.0001	10
	rheb-1	16.42 ± 0.1	17	55/55	9.85 ± 0.3	11	39/39	67	<.0001	8
	rheb-1	15.73 ± 0.1	16	41/41	10.17 ± 0.3	12	59/59	55	<.0001	10
	100uM	15.85 ± 0.1	16	53/53	8.27 ± 0.2	9	55/55	92	<.0001	7
	400uM	15.06 ± 0.2	16	18/18	6.93 ± 0.4	9	28/28	117	<.0001	1
daf-16(mgDf47); skn-1(zu67)	*raga-1*	8.56 ± 0.2	9	50/50	7.67 ± 0.1	8	51/51	12	<.0001	1
	raga-1	6.79 ± 0.2	8	63/63	6.73 ± 0.2	8	55/55	0	.9094	2
	raga-1	7.05 ± 0.2	8	44/44	7.00 ± 0.1	8	60/60	0	.8782	4
	ragc-1	7.86 ± 0.3	9	57/57	8.48 ± 0.2	9	56/56	-7	.1678	5
	100uM	6.68 ± 0.1	7	62/62	6.87 ± 0.1	8	54/54	-3	.1043	7
	400uM	7.38 ± 0.2	8	48/48	7.21 ± 0.3	8	38/38	2	.7637	1

Assay numbers indicate experiments that were performed in parallel. 100µM and 400µM indicate concentrations of rapamycin that were used. All treatments were performed during adulthood, with DMSO and pL4440 empty vector plates used for rapamycin and RNAi controls, respectively. *P* values were obtained by the log rank.

Table S2. Individual Heat Stress (35°) Survival Trials, Performed and Analyzed as in Figure 1D, Related to Figure 1

Strain (+Heat)	RNAi treatment	Mean RNAi Survival (hours ± SEM)	75th %ile (hours)	No. RNAi animals	Mean Control Survival (hours ± SEM)	75th %ile (hours)	No. Control animals	% Mean survival extension	*P* value vs. Control	Assay #
WT	*ragc-1*	24.38 ± 0.5	27	48/48	22.22 ± 0.4	25	49/51	10	<.0001	1
	ragc-1	21.29 ± 0.2	22	38/49	18.94 ± 0.1	20	53/53	12	<.0001	2
skn-1(zu67)	*ragc-1*	19.85 ± 0.4	23	46/46	17.92 ± 0.2	19	50/50	11	<.0001	1
	ragc-1	16.92 ± 0.1	18	49/49	15.50 ± 0.1	16	46/46	9	<.0001	2
daf-16(mgDf47)	*ragc-1*	24.16 ± 0.4	26	45/57	20.76 ± 0.2	22	62/62	16	<.0001	2
	ragc-1	21.00 ± 0.2	22	52/52	18.52 ± 0.1	19	52/52	13	<.0001	2
daf-16(mgDf47); skn-1(zu67)	*ragc-1*	17.76 ± 0.2	19	42/42	17.89 ± 0.2	19	46/46	0	.6960	1
	ragc-1	17.37 ± 0.2	18	57/57	16.89 ± 0.1	17	45/45	3	.0118	2

Assay numbers indicate experiments that were performed in parallel. All treatments were performed during adulthood, with pL4440 empty vector plates used for RNAi controls. *P* values were obtained by the log rank.

Table S3. Effects of TORC1 Pathway and Translation Factor Gene RNAi on *C. elegans* Lifespan, Related to Figure 2

Strain	Mean RNAi Lifespan (days ± SEM)	Median Lifespan (days)	75th %ile (days)	*P* value (log-rank) vs. Control	% Mean Lifespan extension	N	No. of Exp.	Figure
WT;vect.(*RNAi)*	23.08 ± 0.3	24	25	-	-	110/113	2	2A
WT;*ragc-1(RNAi)*	29.09 ± 0.4	29	32	<.0001	26	110/116	2	
skn-1(zu67); vect.*(RNAi)*	20.48 ± 0.4	21	21	-	-	95/102	2	
skn-1(zu67);ragc-1(RNAi)	20.48 ± 0.3	20	21	.9815	0	108/116	2	
WT;vect.(*RNAi)*	23.39 ± 0.2	24	25	-	-	151/174	3	2B
WT;*ragc-1(RNAi)*	29.01 ± 0.4	30	32	<.0001	24	139/173	3	
*daf-16(mgDf47);*vect.*(RNAi)*	20.67 ± 0.2	21	23	-	-	150/178	3	
daf-16(mgDf47); ragc-1(RNAi)	20.79 ± 0.2	21	23	.5184	0	158/181	3	
WT;vect.(*RNAi)*	22.93 ± 0.2	24	25	-	-	153/157	3	data
WT;*raga-1(RNAi)*	28.91 ± 0.5	29	33	<.0001	26	206/210	3	not
*skn-1(zu67);*vect.*(RNAi)*	20.13 ± 0.2	20	22	-	-	157/169	3	graphed
skn-1(zu67);raga-1(RNAi)	21.46 ± 0.3	21	24	<.0001	7	177/184	3	
*daf-16(mgDf47);*vect.*(RNAi)*	18.09 ± 0.2	18	20	-	-	186/190	3	
daf-16(mgDf47);raga-1(RNAi)	18.51 ± 0.3	18	20	.0382	2	167/183	3	
*daf-16(mgDf47);skn-1(zu67);*vect.*(RNAi)*	18.65 ± 0.3	18	20	-	-	98/104	2	
daf-16(mgDf47);skn-1(zu67);raga-1(RNAi)	18.64 ± 0.3	18	20	.9215	0	120/125	2	
WT;vect.(*RNAi)*	23.09 ± 0.3	24	25	-	-	69/73	1*	data
WT;*ragc-1(RNAi)*	28.94 ± 0.7	29	33	<.0001	25	35/35	1*	not
WT;*raga-1(RNAi)*	28.98 ± 0.7	29	34	<.0001	26	43/43	1*	graphed
*skn-1(zu67);*vect.*(RNAi)*	19.49 ± 0.5	20	22	-	-	45/56	1*	
skn-1(zu67);ragc-1(RNAi)	19.89 ± 0.3	20	21	.3411	2	37/49	1*	
skn-1(zu67);raga-1(RNAi)	20.13 ± 0.3	20	22	.6802	3	47/53	1*	
*daf-16(mgDf47);*vect.*(RNAi)*	21.62 ± 0.3	22	23	-	-	52/55	1*	
daf-16(mgDf47);ragc-1(RNAi)	21.83 ± 0.3	22	23	.6962	0	53/54	1*	
daf-16(mgDf47);raga-1(RNAi)	23.07 ± 0.2	23	24	.0009	6	43/47	1*	
WT;vect.(*RNAi)*	23.67 ± 0.2	24	25	-	-	144/169	3	data
WT;*rheb-1(RNAi)*	28.51 ± 0.3	30	31	<.0001	20	160/181	3	not
*skn-1(zu67);*vect.*(RNAi)*	18.96 ± 0.4	20	21	-	-	70/106	2	graphed
skn-1(zu67);rheb-1(RNAi)	19.82 ± 0.4	20	22	.1068	5	70/109	2	
*daf-16(mgDf47);*vect.*(RNAi)*	20.61 ± 0.2	21	23	-	-	152/186	3	
daf-16(mgDf47);rheb-1(RNAi)	21.01 ± 0.4	20	25	.0028	2	98/125	3	
WT;vect.(*RNAi)*	23.67 ± 0.2	24	25	-	-	144/169	3	data
WT;*daf-15(RNAi)*	26.43 ± 0.4	27	30	<.0001	12	120/181	3	not
*skn-1(zu67);*vect.*(RNAi)*	18.96 ± 0.4	20	21	-	-	70/106	2	graphed
skn-1(zu67);daf-15(RNAi)	19.45 ± 0.3	20	21	.7740	3	60/105	2	
*daf-16(mgDf47);*vect.*(RNAi)*	20.61 ± 0.2	21	23	-	-	152/186	3	
daf-16(mgDf47);daf-15(RNAi)	19.84 ± 0.3	20	22	.0451	-4	121/175	3	
WT;vect.*(RNAi)*	22.56 ± 0.2	23	25	-	-	145/162	3	2F
WT;*raga-1(RNAi)*	28.65 ± 0.3	29	31	<.0001	27	127/161	3	
*glp-1(bn18);*vect.*(RNAi)*	28.54 ± 0.3	29	31	-	-	152/185	3	
glp-1(bn18);raga-1(RNAi)	33.04 ± 0.4	35	37	<.0001	16	129/192	3	
WT;vect.*(RNAi)*	19.46 ± 0.7	21	23	-	-	45/46	1	data
WT;*ifg-1(RNAi)*	28.95 ± 0.5	28	31	<.0001	49	40/41	1	not
*glp-1(bn18);*vect.*(RNAi)*	26.46 ± 0.4	27	27	-	-	48/63	1	graphed
glp-1(bn18);ifg-1(RNAi)	30.65 ± 0.5	31	33	<.0001	16	66/68	1	
WT;vect.*(RNAi)*	20.98 ± 0.3	21	24	-	-	152/159	3	data
WT;*eif-1(RNAi)*	27.98 ± 0.4	29	31	<.0001	33	134/143	3	not
*glp-1(bn18);*vect.*(RNAi)*	28.73 ± 0.3	28	31	-	-	150/185	3	graphed
glp-1(bn18);eif-1(RNAi)	31.68 ± 0.4	32	35	<.0001	10	178/208	3	
WT;vect.*(RNAi)*	23.96 ± 0.2	24	25	-	-	117/142	2	2G
WT;*raga-1(RNAi)*	30.48 ± 0.5	33	34	<.0001	27	92/139	2	
*kri-1(ok1251);*vect.*(RNAi)*	23.13 ± 0.4	24	26	-	-	102/118	2	
kri-1(ok1251);raga-1(RNAi)	31.21 ± 0.4	33	31	<.0001	35	111/138	2	

WT;*eif-1(RNAi)*	31.65 ± 0.4	33	35	<.0001	32	109/132	2	not
kri-1(ok1251);eif-1(RNAi)	28.94 ± 0.5	29	32	<.0001	25	93/139	2	graphed
rde-1(ne219);vect.(*RNAi)*	24.24 ± 0.3	25	26	-	-	101/117	2	2H
rde-1(ne219);ragc-1(RNAi)	23.69 ± 0.3	24	25	.0282	-2	85/107	2	
VP288;vect.(*RNAi)*	25.29 ± 0.3	25	28	-	-	96/108	2	
VP288;*ragc-1(RNAi)*	31.09 ± 0.5	33	35	<.0001	23	96/105	2	
WT;vect.*(RNAi)*	22.10 ± 0.3	24	25	-	-	90/110	2	controls
WT;*ragc-1(RNAi)*	29.17 ± 0.5	31	33	<.0001	32	100/127	2	for
WT;rol-6; vect.(*RNAi)*	22.83 ± 0.3	24	25	-	-	90/102	2	2H
WT;rol-6; *ragc-1(RNAi)*	29.63 ± 0.4	31	32	<.0001	31	81/117	2	

Assays were performed and analyzed as in Figure 2. Lifespan from hatching is indicated, but RNAi treatments were performed only during adulthood. In *glp-1* and parallel control experiments animals were placed at 25° from the L2 stage until adulthood. In the VP288 strain, the RNAi-defective mutant *rde-1(ne219)* has been rescued by transgenic expression specifically in the intestine (*rde-1[Expnhx-1::RDE-1;rol-6]*)(Qadota et al., 2007). pL4440 empty vector plates were used for the RNAi control, and all experiments included FUdR except for the one indicated by an asterisk. Composites of multiple individual experiments are shown unless otherwise indicated, with the corresponding individual trials tabulated in Table S4.

Table S4. Individual RNAi Analyses of *C. elegans* Lifespan, Related to Figure 2

Strain	RNAi treatment	Mean RNAi Lifespan (days ± SEM)	75th %ile (days)	No. of RNAi animals	Mean Control Lifespan (days ± SEM)	75th %ile (days)	No. of Control animals	% Mean Lifespan extension	*P* value (log- rank) vs. Control	Assay #
WT	*eif-1*	23.97 ± 0.9	28	29/29	19.46 ± 0.6	23	45/46	23	<.0001	37
	eif-1	28.76 ± 0.5	30	48/49	21.36 ± 0.5	24	49/51	35	<.0001	47
	eif-1	29.33 ± 0.6	31	57/65	21.83 ± 0.4	25	58/62	34	<.0001	50
	eif-1	31.88 ± 0.6	34	41/57	23.95 ± 0.4	26	44/65	33	<.0001	58
	eif-1	31.51 ± 0.5	35	68/75	23.96 ± 0.4	25	73/77	32	<.0001	65
	raga-1	24.02 ± 0.4	27	61/63	22.09 ± 0.2	23	47/48	9	<.0001	39
	raga-1	32.90 ± 0.7	38	86/87	23.15 ± 0.4	25	47/50	23	<.0001	41
	raga-1	28.15 ± 0.6	32	59/60	23.44 ± 0.3	25	59/59	20	<.0001	48
	raga-1	26.12 ± 0.6	29	42/55	21.83 ± 0.4	25	58/62	20	<.0001	50
	raga-1	29.94 ± 0.6	34	32/50	22.26 ± 0.4	24	42/52	35	<.0001	57
	raga-1	29.87 ± 0.3	32	53/56	23.78 ± 0.2	25	45/48	26	<.0001	60
	raga-1	30.13 ± 1.0	34	30/61	23.95 ± 0.4	26	44/64	26	<.0001	58
	raga-1	30.65 ± 0.6	34	62/78	23.96 ± 0.3	25	73/77	28	<.0001	65
	ragc-1	28.17 ± 0.7	32	48/56	22.67 ± 0.4	25	51/53	24	<.0001	46
	ragc-1	29.94 ± 0.4	32	52/60	23.44 ± 0.3	25	59/59	28	<.0001	48
	ragc-1	28.31 ± 0.8	33	48/62	21.56 ± 0.5	25	41/51	31	<.0001	55
	ragc-1	29.96 ± 0.6	33	52/65	22.55 ± 0.4	25	49/59	33	<.0001	59
	ragc-1	26.70 ± 0.7	31	43/49	23.58 ± 0.3	25	43/50	13	<.0001	61
	ragc-1	30.18 ± 0.7	34	44/64	23.16 ± 0.3	25	49/65	30	<.0001	62
	daf-15	28.00 ± 0.6	30	32/55	23.08 ± 0.4	25	43/58	21	<.0001	56
	daf-15	24.38 ± 0.6	27	38/58	22.87 ± 0.4	25	45/50	7	.0001	63
	daf-15	26.97 ± 0.6	30	50/68	24.77 ± 0.3	26	56/61	9	<.0001	67
	rheb-1	27.22 ± 0.5	31	54/58	23.08 ± 0.4	25	43/58	18	<.0001	56
	rheb-1	28.62 ± 0.5	31	44/59	22.87 ± 0.4	25	43/58	25	<.0001	63
	rheb-1	29.60 ± 0.5	32	62/64	24.77 ± 0.3	26	56/61	19	<.0001	67
	cco-1	27.87 ± 0.5	29	54/61	22.93 ± 0.4	25	46/53	22	<.0001	69*
	cco-1	29.23 ± 0.4	32	56/56	22.17 ± 0.4	24	41/49	32	<.001	70
	cyc-1	24.34 ± 0.8	29	53/57	22.57 ± 0.5	25	51/56	8	<.0001	52
	cyc-1	27.77 ± 0.6	29	52/66	22.93 ± 0.4	25	46/53	21	<.0001	69*
	cyc-1	28.35 ± 0.5	31	46/47	22.17 ± 0.4	24	41/49	28	<.0001	70
skn-1(zu67)	*raga-1*	21.37 ± 0.5	24	49/50	20.67 ± 0.4	21	54/56	3	.0178	39
	raga-1	22.60 ± 0.7	26	58/60	19.37 ± 0.4	20	49/57	17	.0004	41
	raga-1	20.59 ± 0.3	21	70/74	20.28 ± 0.3	21	54/56	2	.2106	48
	ragc-1	21.61 ± 0.6	25	41/49	20.76 ± 0.4	20	51/46	5	.1040	46
	ragc-1	19.78 ± 0.2	21	67/67	20.28 ± 0.3	21	54/56	-2	.0828	48
	daf-15	18.37 ± 0.5	20	19/55	17.71 ± 0.5	20	33/55	4	.4203	56
	daf-15	19.95 ± 0.3	21	419/50	20.11 ± 0.4	21	37/51	0	.5251	67
	rheb-1	20.21 ± 0.6	23	26/54	17.71 ± 0.5	20	33/55	14	.0012	56
	rheb-1	19.56 ± 0.5	21	44/55	20.11 ± 0.4	21	37/51	-3	.6365	67
daf-16(mgDf47)	*raga-1*	14.95 ± 0.3	14	44/53	15.57 ± 0.3	16	61/64	-4	.0271	39
	raga-1	19.81 ± 0.5	22	72/72	18.63 ± 0.4	20	63/63	-6	.0043	41
	raga-1	19.75 ± 0.2	21	51/58	20.00 ± 0.2	21	62/63	-1	.8084	48
	ragc-1	19.48 ± 0.2	21	67/70	20.00 ± 0.2	21	62/63	-3	.0859	48
	ragc-1	21.65 ± 0.4	24	48/51	20.49 ± 0.5	23	41/49	6	.0698	61
	ragc-1	21.86 ± 0.4	24	43/60	21.70 ± 0.4	24	47/66	0	.7960	62
	daf-15	18.00 ± 0.4	20	34/58	19.15 ± 0.4	21	48/62	-6	.0219	56
	daf-15	19.84 ± 0.4	22	38/56	19.70 ± 0.5	22	35/52	0	.9530	63
	daf-15	21.12 ± 0.4	23	49/61	22.09 ± 0.3	24	69/72	-4	.2593	67
	rheb-1	20.14 ± 0.6	24	43/60	19.15 ± 0.4	21	48/62	5	.0192	56
	rheb-1	20.09 ± 0.5	23	44/65	19.70 ± 0.5	22	35/52	2	.2419	63
	rheb-1	21.69 ± 0.5	25	55/65	22.09 ± 0.3	24	69/72	-2	.1381	67
daf-16(mgDf47);skn-1(zu67)	*raga-1*	18.78 ± 0.5	19	49/50	18.27 ± 0.4	19	51/52	3	.2631	39
	raga-1	18.55 ± 0.4	22	71/75	19.05 ± 0.5	20	47/52	-3	.4417	41
	cco-1	24.60 ± 0.5	27	35/45	20.09 ± 0.4	22	46/53	22	<.0001	69*
	cco-1	27.31 ± 0.8	31	32/34	20.50 ± 0.5	23	48/55	33	<.0001	70
	cyc-1	25.15 ± 0.6	28	41/44	20.09 ± 0.4	22	46/53	25	<.0001	69*
	cyc-1	23.49 ± 0.6	25	30/36	20.50 ± 0.5	23	48/55	15	<.0001	70
WT; *rol-6*	*ragc-1*	29.45 ± 0.6	33	47/62	23.31 ± 0.5	26	45/51	26	<.0001	55
	ragc-1	29.88 ± 0.6	32	34/55	22.36 ± 0.4	24	45/51	34	<.0001	59
rde-1(ne219)	*ragc-1*	21.39 ± 0.5	24	26/39	23.72 ± 0.4	26	52/57	-10	<.0001	55

	ragc-1	24.71 ± 0.2	26	59/68	24.80 ± 0.3	26	49/60	0	.6813	69
VP288	*ragc-1*	30.72 ± 0.9	35	43/45	25.64 ± 0.5	28	45/50	20	<.0001	55
	ragc-1	31.40 ± 0.6	34	53/60	24.98 ± 0.5	28	51/58	26	<.0001	59
glp-1(bn18)	*eif-1*	29.13 ± 0.6	31	61/64	26.46 ± 0.4	27	48/63	10	.0002	37
	eif-1	32.45 ± 0.5	35	53/72	29.35 ± 0.6	33	48/63	11	<.0001	47
	eif-1	33.45 ± 0.5	36	64/72	30.20 ± 0.5	32	54/60	11	<.0001	50
	raga-1	33.07 ± 0.7	36	46/62	30.20 ± 0.5	32	54/60	10	<.0001	50
	raga-1	34.32 ± 0.8	38	47/73	27.39 ± 0.5	29	54/60	25	<.0001	57
	raga-1	31.33 ± 0.4	34	36/57	27.93 ± 0.4	30	42/54	12	<.0001	60
kri-1(ok1251)	*eif-1*	31.52 ± 0.8	35	37/69	22.43 ± 0.7	25	49/61	41	<.0001	58
	eif-1	27.29 ± 0.4	30	56/70	23.74 ± 0.4	26	53/57	15	<.0001	65
	raga-1	31.64 ± 0.6	34	51/67	22.43 ± 0.7	25	49/61	41	<.0001	58
	raga-1	30.83 ± 0.6	34	60/71	23.74 ± 0.4	26	53/57	30	<.0001	65

Many of these experiments correspond to the composites shown in Table S3. Assays were performed and analyzed as in Figure 2. Lifespan from hatching is indicated, but RNAi treatments were performed only during adulthood, except for mitochondrial genes which were initiated at the L3 stage. pL4440 empty vector plates were used for the RNAi control, and all experiments included FUdR except for those indicated by an asterisk. Assay numbers indicate trials that were performed in parallel.

Table S5. Effects of Rapamycin on *C. elegans* Lifespan, Related to Figure 6

Strain	Mean Lifespan (days ± SEM)	Median Lifespan (days)	75th %ile (days)	*P* value (log-rank) vs. Control	% Mean Lifespan extension	N	Assay #	Figure
WT;control	21.89 ± 0.5	24	25	-	-	54/54	51	6A
WT;rapamycin	25.94 ± 0.9	28	31	<.0001	19	33/40	51	
skn-1(zu67); control	17.47 ± 0.3	18	18	-	-	47/50	51	
skn-1(zu67); rapamycin	16.36 ± 0.5	15	18	.4261	-6	42/42	51	
daf-16(mgDf47); control	18.28 ± 0.3	19	20	-	-	54/55	51	
daf-16(mgDf47); rapamycin	21.52 ± 0.4	23	24	<.0001	18	56/56	51	
daf-16(mgDf47);skn-1(zu67); control	17.02 ± 0.3	17	19	-	-	49/53	51	not
daf-16(mgDf47);skn-1(zu67); rapamycin	16.98 ± 0.3	15	18	.0014	-12	38/40	51	graphed
WT;vector *(RNAi)*†	24.27 ± 0.3	25	26	-	-	51/60	53	
WT;rapamycin *(RNAi)*†	31.35 ± 0.5	31	34	<.0001	29	57/57	53	
WT;control	23.36 ± 0.3	24	25	-	-	47/52	53	data
WT;rapamycin	26.33 ± 0.7	26	29	<.0001	13	54/56	53	not
WT;*raga-1 (RNAi)*†	31.56 ± 0.6	32	35	<.0001	-	55/55	53	graphed
WT;*raga-1*;rapamycin *(RNAi)*†	32.24 ± 0.7	33	37	<.0001	2	54/54	53	
daf-16(mgDf47); vector (*RNAi)*†	19.15 ± 0.4	20	20	-	-	48/48	53	
daf-16(mgDf47); rapamycin *(RNAi)*†	22.98 ± 0.6	24	26	<.0001	20	48/48	53	
daf-16(mgDf47); control	20.27 ± 0.4	20	23	-	-	52/54	53	
daf-16(mgDf47); rapamycin	24.27 ± 0.8	23	26	<.0001	21	44/44	53	
daf-16(mgDf47); raga-1 (RNAi)†	19.57 ± 0.5	20	22	.1474	-	44/44	53	
daf-16(mgDf47); raga-1; rapamycin *(RNAi)*†	23.59 ± 0.7	24	29	<.0001	21	49/49	53	
WT;control	23.55 ± 0.6	25	26	-	-	37/44	68*	data
WT;rapamycin	28.89 ± 0.7	30	33	<.0001	23	45/47	68*	not
skn-1(zu67); control	18.25 ± 0.3	18	20	-	-	48/53	68*	graphed
skn-1(zu67); rapamycin	17.89 ± 0.2	18	19	.0685	-2	66/67	68*	
daf-16(mgDf47); control	21.22 ± 0.3	23	23	-	-	55/56	68*	
daf-16(mgDf47); rapamycin	25.04 ± 0.6	26	30	<.0001	18	51/51	68*	
WT;control	23.20 ± 0.6	24	25	-	-	44/44	71	data
WT; rapamycin	29.91 ± 0.6	31	33	<.0001	29	44/45	71	not
daf-16(mu86); control	22.68 ± 0.3	23	24	-	-	62/62	71	graphed
daf-16(mu86); rapamycin	28.61 ± 0.5	30	31	<.0001	26	46/47	71	
WT;vect.*(RNAi)*†	24.05 ± 0.3	24	25	-	-	55/56	79	data
WT;rapamycin*(RNAi)*†	32.66 ± 0.6	35	36	<.0001	36	61/62	79	not
WT;*skn-1(RNAi)*†	21.40 ± 0.5	23	25	-	-	55/55	79	graphed
WT;*skn-1(RNAi)*;rapamycin†	22.70 ± 0.3	24	25	.4511	6	50/51	79	

Lifespan from hatching is indicated, but rapamycin treatment was performed only during adulthood. DMSO plates were used for controls, and all experiments included FUdR except for those indicated by an asterisk. Trials that are grouped together were performed in parallel. † indicates experiments performed on HT115.

Table S6. TORC2 Effects on *C. elegans* Lifespan, Related to Figure 6

Strain	Mean RNAi Lifespan (days ± SEM)	Median Lifespan (days)	75th %ile (days)	*P* value (log-rank) vs. Control	% Mean Lifespan extension	N	No. of Exp.	Figure
WT;vect.*(RNAi)*	22.11 ± 0.3	23	24	-	-	115/118	3	6B
WT;*rict-1(RNAi)*	27.52 ± 0.4	28	31	<.0001	24	145/145	3	
skn-1(zu67);vect.(*RNAi)*	19.00 ± 0.3	20	21	-	-	156/159	3	
skn-1(zu67);rict-1(RNAi)	19.38 ± 0.3	20	24	.0096	2	150/160	3	
WT;vect.(*RNAi)*	23.36 ± 0.2	24	25	-	-	92/115	2	6C
WT;*rict-1(RNAi)*	28.85 ± 0.5	30	33	<.0001	24	97/113	2	
daf-16(mgDf47);vect.*(RNAi)*	21.14 ± 0.3	22	23	-	-	88/115	2	
daf-16(mgDf47);rict-1(RNAi)	26.86 ± 0.5	28	30	<.0001	27	96/115	2	
WT;vect.*(RNAi)*	23.36 ± 0.2	24	25	-	-	92/115	2	Data
WT;*ragc-1;rict-1(RNAi)*	27.32 ± 0.6	30	32	<.0001	17	119/125	2	not
daf-16(mgDf47);vect.*(RNAi)*	21.14 ± 0.3	22	23	-	-	88/115	2	graphed
daf-16(mgDf47);ragc-1;rict-1(RNAi)	27.55 ± 0.4	28	30	<.0001	30	83/101	2	
rde-1(ne219);vect.*(RNAi)*	26.08 ± 0.3	26	28	-	-	118/119	2	6D
rde-1(ne219);rict-1(RNAi)	27.19 ± 0.4	27	29	.0020	4	118/120	2	
VP288;vect.*(RNAi)*	26.79 ± 0.4	27	31	-	-	108/108	2	
VP288;*rict-1(RNAi)*	30.65 ± 0.3	31	33	<.0001	14	117/117	2	
WT;vect.*(RNAi)*	22.21 ± 0.4	24	24	-	-	56/61	1	Controls
WT;*rict-1(RNAi)*	31.46 ± 0.7	31	37	<.0001	42	51/54	1	for
WT;rol-6; vect.(*RNAi)*	23.64 ± 0.3	24	25	-	-	44/49	1	6D
WT;rol-6; *rict-1(RNAi)*	29.98 ± 0.6	31	33	<.0001	27	41/42	1	

Assays were performed and analyzed as in Figure 6. Lifespan from hatching is indicated, but RNAi treatment was performed only during adulthood. pL4440 empty vector plates were used for the RNAi controls and all experiments included FUdR. Composites correspond to individual RNAi experiments that are shown in Table S7. Trials that are grouped together were performed in parallel.

Table S7. Individual RNAi Analyses of TORC2 Effects on *C. elegans* Lifespan, Related to Figure 6

Strain	RNAi treatment	Mean RNAi Lifespan (days ± SEM)	75th %ile (days)	No. of RNAi animals	Mean Control Lifespan (days ± SEM)	75th %ile (days)	No. of Control animals	% Mean Lifespan extension	*P* value (log-rank) vs. Control	Assay #
WT	*rict-1*	28.91 ± 0.5	32	46/52	23.58 ± 0.3	25	43/50	23	<.0001	61
	rict-1	28.78 ± 0.8	33	51/61	23.16 ± 0.3	25	49/65	24	<.0001	62
	rict-1	29.11 ± 0.5	31	47/50	24.22 ± 0.3	26	41/51	20	<.0001	71
	rict-1	29.45 ± 0.3	31	53/53	22.16 ± 0.5	25	37/38	33	<.0001	87
	rict-1	27.91 ± 0.6	31	55/55	22.63 ± 0.5	24	35/37	23	<.0001	91
	rict-1	24.27 ± 1.0	27	37/37	21.65 ± 0.5	24	43/43	12	.0040	92
	ragc-1;rict-1	29.19 ± 0.5	32	52/53	23.58 ± 0.3	25	43/50	24	<.0001	61
	ragc-1;rict-1	25.93 ± 0.9	33	67/72	23.16 ± 0.3	25	49/65	12	<.0001	62
skn-1(zu67)	*rict-1*	21.63 ± 0.4	24	54/55	19.73 ± 0.3	21	55/55	10	<.0001	87
	rict-1	17.93 ± 0.6	20	53/62	18.88 ± 0.5	21	55/58	-5	.8491	91
	rict-1	18.30 ± 0.6	19	43/43	18.28 ± 0.5	19	46/46	0	.9798	92
daf-16(mgDf47)	*rict-1*	27.40 ± 0.6	32	52/57	20.49 ± 0.5	23	41/49	34	<.0001	61
	rict-1	26.21 ± 0.6	29	44/58	21.70 ± 0.4	24	47/66	21	<.0001	62
	ragc-1;rict-1	27.44 ± 0.6	30	46/49	20.49 ± 0.5	23	41/49	34	<.0001	61
	ragc-1;rict-1	27.70 ± 0.5	30	37/52	21.70 ± 0.4	23	47/66	28	<.0001	62
daf-16(mu86)	*rict-1*	29.35 ± 0.4	31	58/58	23.37 ± 0.3	25	60/63	26	<.0001	71
rde-1(ne219)	*rict-1*	28.04 ± 0.8	34	51/53	26.69 ± 0.6	31	58/59	5	.0015	78
	rict-1	26.55 ± 0.3	28	67/67	25.48 ± 0.3	27	60/60	4	.0005	86
VP288	*rict-1*	30.96 ± 0.7	35	48/48	27.57 ± 0.9	31	47/47	12	.0008	78
	rict-1	30.43 ± 0.3	33	69/69	26.16 ± 0.2	27	61/61	16	<.0001	86

Many of these experiments correspond to composites shown in Table S6. Assays were performed as in Figure 6. Lifespan from hatching is indicated, but RNAi treatments were performed during adulthood. pL4440 empty vector plates were used for the RNAi control, and all experiments included FUdR. Assay numbers indicate trials that were performed in parallel.

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