SUPPLEMENTARY INFORMATION

HLH-30/TFEB is a conserved regulator of autophagy and modulates longevity in *C. elegans*

Louis R. Lapierre, C. Daniel De Magalhaes Filho, Philip R. McQuary, Chu-Chiao Chu, Orane Visvikis, Jessica T. Chang, Sara Gelino, Binnan Ong, Andrew E. Davis, Javier E. Irazoqui, Andrew Dillin, and Malene Hansen

STRAIN LIST

Published strains used in this study:

N2	Wild-type, WT (Hansen lab (MAH) – originated from Kenyon lab (CF))
CF1041	*daf-2(e1370) III*
CF1903	*glp-1(e2141) III*
CF1908	*eat-2(ad1116) II*
CF2172	*isp-1(qm150) IV*
DA2123	*adIs2122[lgg-1p::gfp::lgg-1 + rol-6(su1006)]* (Avery lab)
HZ859	*him-5(e1490) V; bpIs151[sqst-1/T12G3.1::GFP + unc-76]* (Zhang lab)
IU223	*clk-1(e2519) III* (Lee lab)
MAH14	*daf-2(e1370) III; adIs2122[lgg-1p::gfp::lgg-1 + rol-6(su1006)]*
MAH43	*glp-1(e2141ts) III; adIs2122[lgg-1p::gfp::lgg-1 + rol-6(su1006)]*
RT258	*unc-119(ed3); pwIs50[LMP-1::GFP, Cb-unc-119(+)]* (Grant lab)
VB633	*rsks-1(sv31) III* (Tuck lab)

New strains created for this study:

JIN1375	*hlh-30(tm1978) IV,* outcrossed 6 times to Irazoqui lab (JIN) N2
JIN1679	*jinEx10[hlh-30p::hlh-30::gfp, rol-6(su1006)]*
MAH53	*rsks-1(sv31) III; adIs2122[lgg-1p::gfp::lgg-1 + rol-6(su1006)]*
MAH61	*clk-1(e2519) III; adIs2122[lgg-1p::gfp::lgg-1 + rol-6(su1006)]*
MAH71	*isp-1(qm150) IV; adIs2122[lgg-1p::gfp::lgg-1 + rol-6(su1006)]*
MAH150	*glp-1(e2141ts) III; pwIs50[LMP-1::GFP, Cb-unc-119(+)]*
MAH200	*jinEx10[hlh-30p::hlh-30::gfp + rol-6(su10060]; izEx5[lgg-1p::gfp::lgg-1 + odr-1p::rfp]*
MAH202	*glp-1(e2141) III; jinEx10[hlh-30p::hlh-30::gfp, rol-6(su1006)]*
MAH203	*glp-1(e2141ts) III; him-5(e1490) V?; bpIs151[sqst-1/T12G3.1::GFP+ unc-76]*
MAH204	*daf-2(e1370) III; jinEx10[hlh-30p::hlh-30::gfp, rol-6(su1006)]*
MAH206	*glp-1(e2141ts) III; hlh-30(tm1978) IV*
MAH235	*sqIs19[hlh-30p::hlh-30::gfp, rol-6(su1006)]* outcrossed 4 times to N2 (OE #1)
MAH236	*sqIs13[lgg-1p::gfp::lgg-1p + odr-1p::rfp]* outcrossed 4 times to N2
MAH240	*sqIs17[hlh-30p::hlh-30::gfp, rol-6(su1006)]* outcrossed 4 times to N2 (OE #2)
MAH253	*eat-2(ad1116) II; jinEx10[hlh-30p::hlh-30::gfp, rol-6(su1006)]*
MAH266	*rsks-1(sv31) III; jinEx10[hlh-30p::hlh-30::gfp, rol-6(su1006)]*
MAH267	*clk-1(e2519) III; jinEx10[hlh-30p::hlh-30::gfp, rol-6(su1006)]*

SUPPLEMENTARY METHODS

Quantification of GFP::LGG-1 punctae by confocal microscopy

Day 1 adult wild-type or *rsks-1(sv31)* animals expressing GFP::LGG-1 fed OP50 *E. coli* bacteria and grown at 20°C were mounted on a 2% agarose pad containing 0.1% 1.5 M NaN_3. Worms were imaged using a LSM Zeiss 710 scanning confocal microscope. Z stacks were taken at 0.6 μm slices. GFP excitation/emission was limited to 493/512 nm to eliminate background autofluorescence from the worms. Four experiments with 10-15 L3 worms or day 1 adults were imaged for each experiment. The number of GFP::LGG-1 positive punctae were counted in the seam cells, intestinal cells, and muscle on one 0.6 μm slice. The Z-position of the seam cells and intestinal cells was chosen at a position where the nucleus could be clearly seen. Statistical analysis (ANOVA) was performed with GraphPad Prism software.

TABLE S1. Distribution of CLEAR elements in autophagy-related and lysosomal genes in *C. elegans*

GENE	COSMID	MAMMALIAN ORTHOLOG	CLEAR ELEMENT GTCACGTGAC	POSITION	OVERLAP /10 bp
AUTOPHAGY GENES					
lgg-1	C32D5.9	*lc3*	GTCTCGTTTC	-930	7
			GTCACATGAT	-904	8
atg-18	F4E6.13	*wipi*	GTCAAGTTAG	-815	7
			CTCACTTGAC	-710	8
			ATCAGCTGAC	-211	7
atg-9	T22H9.2	*atg-9*	ATCATGTGAT	-667	7
			GGCACGCGGC	-595	7
			GACAGGTGGC	-402	7
sqst-1	T12G3.1	*sqstm1*	GTGTCGAGAC	-621	7
			TTCGTGTGAC	47	7
LYSOSOMAL GENES					
vps-11	R06F6.2	*vps-11*			
vps-18	W06B4.3	*vps-18*	GTCAGGTCAC	-138	8
lmp-1	C03B1.12	*lamp-1*	GCTACGACAC	-949	7
			GTCACATGTC	-687	8
			ATCAGGTGAC	-513	8
			ATGAGGTGAC	-296	7
vha-15	T14F9.1	*atp6v1h*	GTCTCGCAAC	-249	7
vha-16	C30F8.2	*atp6v0d2*			
vha-17	F49C12.13.1	*atp6v0e1*	GCCGCGAGAC	-378	7
sul-1	K09C4.8	*arsa*	TCCACATGAC	-279	7
			TTGACGAGAC	-237	7
			TTCACTTGTC	-120	7
sul-2	D1014.1	*arsa*	TTTACGTGAA	-683	7

GENE	COSMID	MAMMALIAN ORTHOLOG	CLEAR ELEMENT GTCACGTGAC	POSITION	OVERLAP /10 bp
sul-3	C54D2.4	*arsb*	CTCACTTGAA	-802	7
			GTCAAGTGCC	-662	8
			ATCACTTGGC	-589	7
			ATCACTTGTC	-557	7
			GACAAGTGCC	-512	7
*ctsa**	C08H9.1	*ctsa*	GTTACGTGGA	-540	7
			GTCGTTTGAC	-244	7
cpr-1	C52E4.1	*ctsb*	TGTACGTGAC	-733	7
			GTCATGTGAA	-478	8
			AGCACGTGAG	-154	7
asp-1	Y39B6A.20	*ctsd*			
hlh-30	W02C12.3a	*tfeb*	TTCACGGCAC	-85	7

Table S1: Analysis of nematode orthologs of TFEB autophagy-related and lysosomal target genes reveals the presence of CLEAR (Coordinated Lysosomal Expression And Regulation) elements within 1000 base pairs (bp) upstream and 200 bp downstream of the start codon in the majority of targets. Motifs were determined using Gene Runner 3.0 software. CLEAR elements fully overlap with E-box motifs, a 6-base DNA sequence recognized by HLH-30 (Grove et al., 2009). The positions of CLEAR elements (Sardiello et al., 2009) in the promoters of nematode orthologs of mammalian TFEB autophagy-related and lysosomal target genes were generally located within 1000 bp upstream of the open reading frame (ORF) of *C. elegans* genes. In contrast to their position in the promoters of nematode genes, CLEAR elements in mammalian TFEB target gene promoters were more prevalent within 200 bp of the initiation site of the ORFs (Sardiello et al., 2009). The cutoff for sequence motifs was 7 bp overlap, which limited the numbers of sites found. Notably, despite containing no sequences with 7+ bp overlap, promoter regions of *vha-16* and *asp-1* included several motifs with 6 bp overlap (data not shown).

TABLE S2. Lifespan analysis of *C. elegans* animals with reduced *hlh-30* levels.

STRAINS	*hlh-30* RNAi avg lifespan (days)	Number of RNAi animals	Control RNAi avg lifespan (days)	Number of RNAi animals	Δ avg (%)	P value vs control	
WT	16.5	42/100	16.1	62/100	3	0.99	#
Fig. 3a	17.8	63/100	17.9	69/100	-1	0.67	
	16.3	53/100	16.8	57/100	-3	0.51	
	18.5	76/100	17.1	73/100	8	0.037	
	17.1	68/100	16.7	77/100	2	0.52	
	18.1	59/100	16.8	54/100	8	0.10	
glp-1	15.6	65/100	22.1	92/100	-29	<0.0001	#
Fig. 3b	17.6	89/100	20.5	60/100	-14	0.0012	
	18.0	71/100	20.8	83/100	-14	0.0026	
	16.6	106/125	20.4	95/125	-19	<0.0001	
	16.1	90/100	19.4	80/100	-17	<0.0001	
	17.7	51/100	24.8	64/100	-29	<0.0001	
daf-2	33.5	62/100	48.4	68/100	-31	<0.0001	#
Fig. 3c	31.4	34/100	39.8	41/100	-21	0.0005	
	36.2	68/100	46.5	71/100	-22	<0.0001	
eat-2	18.2	65/100	21.0	75/100	-13	0.0004	#
Fig. 3d	19.1	55/100	21.3	58/100	-10	0.011	
	19.7	69/100	23.0	67/100	-14	0.0001	
clk-1	17.8	44/100	25.0	76/100	-29	<0.0001	#
Fig. 3e	19.5	65/100	21.8	71/100	-11	0.015	
	16.6	75/100	20.0	70/100	-17	0.0002	
rsks-1	17.0	74/100	25.8	77/100	-34	<0.0001	#
Fig. 3f	15.0	79/100	22.3	72/100	-33	<0.0001	
	17.2	75/100	21.6	61/100	-20	<0.0001	
STRAINS	***hlh-30* mutant avg lifespan (days)**	**Number of animals**	**avg lifespan (days)**	**Number of RNAi animals**	**Δ avg (%)**	**P value vs control**	
WT	16.0	68/100	16.0	60/100	0	0.40	#
Fig. S3a	20.5	31/100	19.2	42/100	7	0.28	
	18.8	66/100	17.6	67/100	7	0.050	
glp-1	16.7	93/100	19.8	69/100	-16	<0.0001	#
Fig. S3a	17.6	63/100	34.7	79/100	-49	<0.0001	
	16.5	74/100	23.8	70/100	-31	<0.0001	

Table S2: (**Top**) Results of lifespan analyses of wild-type (WT, N2), *glp-1(e2141), daf-2(e1370), eat-2(ad1116), clk-1(e2519)*, and *rsks-1(sv31)* animals fed bacteria expressing empty vector or *hlh-30* dsRNA during adulthood (from L4 stage for *clk-1(e2519)*). WT and *glp-1(e2141)* were raised at the non-permissive temperature (25°C) and fed standard OP50 *E. coli* during development. *daf-2(e1370)*, *eat-2(ad1116), clk-1(e2519),* and *rsks-1(sv31)* mutants were raised at 20°C and fed OP50 bacteria during development.

(**Bottom**) Results of lifespan analyses of WT, *glp-1(e2141), hlh-30(tm1978),* and *glp-1(e2141); hlh-30(tm1978)* animals raised at the non-permissive temperature (25°C), transferred to 20°C on day 1 of adulthood, and fed OP50 bacteria throughout their life. Lifespan experiments including WT and *glp-1* animals were carried out in parallel and were organized in the same order.

Data show the mean lifespan (avg lifespan), observed events (number of dead animals/total number of animals analyzed), % mean change in lifespan vs control (Δ avg), and *P* value vs control calculated with the Mantel-Cox log-rank test. #, representative lifespan curves displayed in the indicated figures.

TABLE S3. Lifespan analysis of *C. elegans* subjected to *vha-16* or *lmp-1* RNAi.

STRAINS	Adult-only treatment	Target RNAi avg lifespan (days)	Number of RNAi animals	Control RNAi avg lifespan (days)	Number of RNAi animals	Δ avg (%)	P value vs control	
WT	*vha-16*	15.6	72/100	17.5	63/100	-11	0.0015	#
Fig. S3b		14.1	64/100	16.8	54/100	-16	0.0001	
		14.6	68/100	16.1	62/100	-9	0.011	
Fig. S3c	*lmp-1*	16.8	66/100	16.7	77/100	1	0.79	#
		15.8	78/100	17.9	69/100	-12	0.0059	
		17.8	74/100	15.8	64/100	13	0.021	
glp-1	*vha-16*	13.8	65/100	25.9	64/100	-47	<0.0001	#
Fig. S3b		14.8	80/100	24.8	64/100	-42	<0.0001	
		15.0	94/100	22.0	92/100	-32	<0.0001	
Fig. S3c	*lmp-1*	17.2	91/100	19.4	80/100	-11	0.069	#
		16.6	78/100	17.3	77/100	-4	0.58	
		18.3	91/100	20.5	60/100	-11	0.041	

Table S3: Results of lifespan analyses of wild-type (WT, N2) and *glp-1(e2141)* animals fed bacteria expressing control dsRNA or dsRNA against the proton pump subunit *vha-16* or the lysosomal protein *lmp-1* during adulthood. Animals were raised at the non-permissive temperature (25°C) and fed standard OP50 *E. coli* bacteria during development. Lifespan experiments with WT and *glp-1* animals were carried out in parallel and were organized in the same order. *lmp-1* RNAi was confirmed to have a visible effect on an LMP-1::GFP reporter strain (see Supplementary Fig. S3d).

Data show the mean lifespan (avg lifespan), observed events (number of dead animals/total number of animals analyzed), % mean change in lifespan vs control (Δ avg), and *P* value vs control calculated with the Mantel-Cox log-rank test. #, representative lifespan curves displayed in the indicated figures.

TABLE S4. Lifespan analysis of wild-type animals and *hlh-30* mutants subjected to *tor* RNAi.

STRAINS	*tor* RNAi avg lifespan (days)	Number of RNAi animals	Control RNAi avg lifespan (days)	Number of RNAi animals	Δ avg (%)	P value vs control	
WT	21.8	70/100	17.1	60/100	28	<0.0001	#
Fig. 2d	24.8	58/100	18.7	46/100	33	<0.0001	
	26.0	69/100	21.6	49/100	20	<0.0001	
hlh-30	17.8	80/100	17.1	61/100	4	0.18	#
Fig. 2d	17.0	70/100	16.7	51/100	2	0.98	
	19.3	67/100	19.5	60/100	1	0.54	

Table S4: Results of lifespan analyses of wild-type (WT, N2) and *hlh-30(tm1978*) animals fed bacteria expressing control or *tor* dsRNA during adulthood. Animals were raised at 20°C and fed standard OP50 *E. coli* bacteria during development. Lifespan experiments with WT and *hlh-30* animals were carried out in parallel and were organized in the same order.

Data show the mean lifespan (avg lifespan), observed events (number of dead animals/total number of animals analyzed), % mean change in lifespan vs control (Δ avg), and *P* value vs control calculated with the Mantel-Cox log-rank test. #, representative lifespan curves displayed in the indicated figures.

TABLE S5. Lifespan analysis of mitochondrial mutants subjected to autophagy gene RNAi during late larval development.

STRAINS	RNAi treatment from L3/L4 stage	RNAi avg lifespan (days)	Number of RNAi animals	Δ avg (%)	P value vs control
***clk-1* (L4)**	Control	24.9	76/115		
	bec-1	21.4	94/119	-14	<0.0001
	vps-34	20.4	84/116	-18	<0.0001
***clk-1* (L4)**	Control	22.2	71/116		
	bec-1	16.9	79/112	-24	<0.0001
	vps-34	18.0	76/113	-19	<0.0001
***clk-1* (L4)**	Control	25.0	76/100		
	atg-18	12.7	67/100	-49	<0.0001
***clk-1* (L4)**	Control	21.8	71/100		
	atg-18	13.9	61/100	-36	<0.0001
***isp-1* (L3)**	Control	28.3	93/249		
	bec-1	24.5	130/251	-13	<0.0001
	vps-34	23.8	62/248	-16	0.0022
***isp-1* (L4)**	Control	21.4	99/228		
	bec-1	19.8	167/235	-7	0.052
	vps-34	19.7	72/221	-8	0.022
WT (L4)	Control	15.3	70/89		
	bec-1	15.6	74/95	2	0.89
	vps-34	16.4	48/93	7	0.33
***isp-1* (L4)**	Control	21.7	26/72		
	bec-1	16.8	27/73	-23	<0.0001
	vps-34	17.7	26/65	-18	<0.0001
WT (L4)	Control	19.9	83/96		
	bec-1	19.0	65/100	-5	0.25
	vps-34	18.9	73/111	-5	0.22
	lgg-1	19.0	59/69	-5	0.12
***isp-1* (L4)**	Control	22.8	23/121		
	bec-1	15.6	31/121	-32	<0.0001
	vps-34	15.7	24/122	-31	<0.0001
	lgg-1	14.8	22/120	-35	<0.0001

Table S5: Results of lifespan analyses of wild-type (WT, N2), *clk-1(e2519),* and *isp-1(qm150)* animals. Animals were incubated at 20°C and fed control bacteria or bacteria expressing dsRNA against the autophagy genes *bec-1*, *vps-34, lgg-1,* or *atg-18* starting at the indicated larval stages (L3-L4), since inhibition of mitochondrial respiration during this development window is necessary for lifespan extension (Rea et al., 2007). Before RNAi treatments started, the animals were fed standard OP50 *E. coli* bacteria.

Experiments separated by dashed lines were performed in parallel, and showed that RNAi of *bec-1* and *vps-34* from the L4 stage (and L3, data not shown) does not significantly shorten the lifespan of WT animals. Moreover, inhibition of autophagy genes in *clk-1* and *isp-1* mitochondrial mutants starting during L3-L4 larval stages selectively shortened the lifespan of mitochondrial mutants but had no effect on WT animals. This is consistent with previous studies investigating reduced mitochondrial respiration in short-lived autophagy mutants following whole-life administration of *atp-3* RNAi (Toth et al., 2008). We further note that *clk-1(e2519)* L3 larvae stably expressing GFP::LGG-1 (MAH61) displayed a significant increase in GFP::LGG-1–positive foci in seam cells compared to wild-type animals (DA2123) (data not shown), consistent with previous studies of other mitochondrial mutants (Hansen et al., 2008; Yang and Hekimi, 2010).

Data show the mean lifespan (avg lifespan), observed events (number of dead RNAi-treated animals/total number of animals analyzed), % mean change in lifespan vs control (Δ avg), and *P* value vs control calculated with the Mantel-Cox log-rank test.

TABLE S6. Lifespan analysis of S6K mutants subjected to autophagy gene *atg-18* RNAi.

STRAINS	Adult-only treatment	Target RNAi avg lifespan (days)	Number of RNAi animals	Control RNAi avg lifespan (days)	Number of RNAi animals	Δ avg (%)	P value vs control	
WT	*atg-18*	16.8	73/110	17.9	91/110	-6	0.12	#
Fig. S5b		14.4	80/103	17.3	91/101	-17	<0.0001	
		13.0	70/100	15.4	79/100	-16	<0.0001	
rsks-1	*atg-18*	18.4	65/110	21.5	79/110	-14	<0.0001	#
Fig. S5b		16.5	71/102	21.4	65/108	-23	<0.0001	
		16.0	71/100	20.8	65/100	-23	<0.0001	

Table S6: Results of lifespan analyses of wild-type (WT, N2) and *rsks-1(sv31)* animals. *rsks-1* animals were incubated at 20°C and fed control bacteria or bacteria expressing dsRNA against the autophagy gene *atg-18* during adulthood. Animals were raised at 20°C and fed standard OP50 *E. coli* bacteria during development. *atg-18* RNAi also reduced the lifespan of other long-lived animals, including *glp-1(e2141)* (Lapierre et al., 2011) and *clk-1(e2519)* animals (**Table S5**). Lifespan experiments with WT and *rsks-1* animals were carried out in parallel and were organized in the same order.

Data show the mean lifespan (avg lifespan), observed events (number of dead RNAi-treated animals/total number of animals analyzed), % mean change in lifespan vs control (Δ avg), and *P* value vs control calculated with the Mantel-Cox log-rank test. #, representative lifespan curves displayed in the indicated figures.

TABLE S7. Lifespan analysis of *C. elegans* overexpressing HLH-30::GFP.

STRAINS	Bacteria	HLH-30 OE avg lifespan (days)	Number of animals	WT avg lifespan (days)	Number of animals	Δ avg (%)	P value vs control	
HLH-30 OE	OP50	18.2	72/100	15.5	72/100	17	0.0045	
#1		20.2	69/100	17.0	63/100	19	0.0030	
Fig. 4c		18.4	49/100	15.1	64/100	22	0.0001	#
		19.0	60/100	16.4	32/100	16	0.017	
HLH-30 OE	OP50	17.7	77/100	15.5	72/100	14	0.027	
#2		20.0	71/100	17.0	63/100	18	0.0047	
Fig. 4c		18.8	75/100	15.1	64/100	25	<0.0001	#
		19.8	29/100	16.4	32/100	20	0.021	

Table S7: Results of lifespan analysis of animals stably expressing transgenic HLH-30::GFP (referred to as HLH-30 OE) and wild-type (WT, N2) animals incubated at 20°C and fed standard OP50 *E. coli* bacteria. To generate the HLH-30 OE strain, we integrated the strain expressing HLH-30::GFP from an extrachromosomal array used for our nuclear localization studies (JIN1679), and outcrossed two separate integrants (#1 and #2) four times to our WT strain (see Supplementary Methods). These integrated strains were slightly developmentally delayed, and integrant #2 had mildly reduced progeny production (data not shown).

Data show mean lifespan (avg lifespan), observed events (number of dead animals/total number of animals analyzed), % mean change in lifespan vs control (Δ avg), and *P* value vs control calculated with the Mantel-Cox log-rank test. #, representative lifespan curves displayed in the indicated figures.

TABLE S8. Lifespan analysis of HLH-30–overexpressing *C. elegans* subjected to *atg-18* RNAi.

STRAINS	RNAi treatment	RNAi avg lifespan (days)	Number of RNAi animals	Δ avg (%)	P value vs control
WT	Control	16.1	73/100		
	atg-18	15.9	60/100	-1	0.41
HLH-30 OE #1	Control	21.0	68/100		
	atg-18	18.4	69/100	-12	0.0004
HLH-30 OE #2	Control	18.7	67/100		
	atg-18	15.6	79/100	-17	0.0002
HLH-30 OE #1	Control	17.2	70/88		
	atg-18	13.6	70/94	-21	<0.0001
HLH-30 OE #2	Control	18.1	102/102		
	atg-18	16.3	103/123	-10	0.0024

Table S8: Results of lifespan analysis of animals stably expressing transgenic HLH-30::GFP (HLH-30 OE#1 and #2 are two stable and outcrossed integrants described in Table S7) and wild-type (WT) animals incubated at 20°C and fed control bacteria or bacteria expressing dsRNA against *atg-18* during adulthood. Animals were raised at 20°C and fed standard OP50 *E. coli* bacteria during development. Lifespan experiments carried out in parallel were boxed with double line.

Data show mean lifespan (avg lifespan), observed events (number of dead animals/total number of animals analyzed), % mean change in lifespan vs control (Δ avg), and *P* value vs control calculated with the Mantel-Cox log-rank test.

TABLE S9. List of primers used for QPCR

GENE	ANNEALING TEMP. (°C)	DIRECTION	PRIMER SEQUENCE
C. elegans			
*act-1**	58	Forward	CTACGAACTTCCTGACGGACAAG
		Reverse	CCGGCGGACTCCATACC
*cyn-1**	58	Forward	GTGTCACCATGGAGTTGTTC
		Reverse	TCCGTAGATTGATTCACCAC
*cdc-42**	58	Forward	CTGCTGGACAGGAAGATTACG
		Reverse	CTCGGACATTCTCGAATGAAG
*pmp-3**	58	Forward	GTTCCCGTGTTCATCACTCAT
		Reverse	ACACCGTCGAGAAGCTGTAGA
hlh-30	58	Forward	CTCATCGGCCGGCGCTCATC
		Reverse	AGAACGCGATGCGTGGTGGG
atg-18	58	Forward	AAATGGACATCGGCTCTTTG
		Reverse	TGATAGCATCGAACCATCCA
lgg-1	58	Forward	ACCCAGACCGTATTCCAGTG
		Reverse	ACGAAGTTGGATGCGTTTTC
atg-9	64	Forward	GGCCGCCATCCACTCATCGG
		Reverse	TTGACGTCGTGCCGCCGTAG
p62	64	Forward	TGGCTGCTGCATCATCCGCT
		Reverse	TCAATCGTGCCGAGACCGGG
vps-11	58	Forward	TCCGCTTGTCGTCCTGGAGC
		Reverse	TCACACGCCGAGCACTTGGT
vps-18	58	Forward	CGAGCCGGCGCCAGTTGTAA
		Reverse	TCCATCCGGCGAAAGCCACG
lmp-1	58	Forward	ATCCGCCACCGCTTCGCATT
		Reverse	TCGAGCTCCCACTCTTTGGCG
vha-15	64	Forward	CGAGGTTCGTTCCGGACGTCTT
		Reverse	CCTCGGCAGTCAGGAGACGC
vha-16	64	Forward	AGGCGCTGACTCGCGGACTT
		Reverse	TGGTCTCTGGTGAAGAGTTCCGGTG
vha-17	64	Forward	TCGGTTTCCGCCTTCTGGGC
		Reverse	ACATCCAGCAGCAAACAGCAGTCA
sul-1	64	Forward	CAGGATGGGATGAGTGGCACG
		Reverse	GGGTCTTCTGGTCCATGCGGC
sul-2	64	Forward	ATGGCAGCAGAAGGCACCCG
		Reverse	GCCATTTTCCAACCATGCCAGTTGC
sul-3	64	Forward	ACATGGAGCCTGCCGGTGTG
		Reverse	TGGTACGCTTTTTCCGCTGCCA
ctsa	64	Forward	TTCTCCTCGAGGCGCGGGAT
		Reverse	TCCAACGCCAATTGGGGACTC
ctsb	64	Forward	CGCCAAGGACAAGCACTTCGGA
		Reverse	ACCTTGGCCTTTCCGGCGAC
ctsd	64	Forward	GGTGCCAACGCACAAGACCG
		Reverse	AGCCTGGGTCTTGCATGCGG
Mice			
*Rpl23**	60	Forward	TGTCGAATTACCACTGCTGG
		Reverse	CTGTGAAGGGAATCAAGGA
*Arbp**	60	Forward	AGATGCAGCAGATCCGCAT
		Reverse	GTTCTTGCCCATCAGCACC
Tfeb	60	Forward	CCTGCCGACCTGACTCAGA
		Reverse	CTCAATTAGGTTGTGATTGTCTTTCTTC

**: Housekeeping genes*

SUPPLEMENTARY REFERENCES

Grove, C.A., De Masi, F., Barrasa, M.I., Newburger, D.E., Alkema, M.J., Bulyk, M.L., and Walhout, A.J. (2009). A multiparameter network reveals extensive divergence between C. elegans bHLH transcription factors. Cell 138, 314-327.

Hansen, M., Chandra, A., Mitic, L.L., Onken, B., Driscoll, M., and Kenyon, C. (2008). A Role for Autophagy in the Extension of Lifespan by Dietary Restriction in C. elegans. PLoS Genet 4, e24.

Kang, C., You, Y.J., and Avery, L. (2007). Dual roles of autophagy in the survival of Caenorhabditis elegans during starvation. Genes Dev 21, 2161-2171.

Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., Hamazaki, J., Nishito, Y., Iemura, S., Natsume, T., Yanagawa, T., Uwayama, J., Warabi, E., Yoshida, H., Ishii, T., Kobayashi, A., Yamamoto, M., Yue, Z., Uchiyama, Y., Kominami, E., and Tanaka, K. (2007). Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 131, 1149-1163.

Lapierre, L.R., Gelino, S.R., Melendez, A., and Hansen, M. (2011). Autophagy and lipid metabolism coordinately modulate aging in germline-less *C. elegans*. Curr Biol 21, 1507-1514.

Long, X., Spycher, C., Han, Z.S., Rose, A.M., Muller, F., and Avruch, J. (2002). TOR deficiency in C. elegans causes developmental arrest and intestinal atrophy by inhibition of mRNA translation. Curr Biol 12, 1448-1461.

Pankiv, S., Clausen, T.H., Lamark, T., Brech, A., Bruun, J.A., Outzen, H., Overvatn, A., Bjorkoy, G., and Johansen, T. (2007). p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 282, 24131-24145.

Rea, S.L., Ventura, N., and Johnson, T.E. (2007). Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in Caenorhabditis elegans. PLoS Biol 5, e259.

Robida-Stubbs, S., Glover-Cutter, K., Lamming, D.W., Mizunuma, M., Narasimhan, S.D., Neumann-Haefelin, E., Sabatini, D.M., and Blackwell, T.K. (2012). TOR Signaling and Rapamycin Influence Longevity by Regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab 15, 713-724.

Sardiello, M., Palmieri, M., di Ronza, A., Medina, D.L., Valenza, M., Gennarino, V.A., Di Malta, C., Donaudy, F., Embrione, V., Polishchuk, R.S., Banfi, S., Parenti, G., Cattaneo, E., and Ballabio, A. (2009). A gene network regulating lysosomal biogenesis and function. Science 325, 473-477.

Toth, M.L., Sigmond, T., Borsos, E., Barna, J., Erdelyi, P., Takacs-Vellai, K., Orosz, L., Kovacs, A.L., Csikos, G., Sass, M., and Vellai, T. (2008). Longevity pathways converge on autophagy genes to regulate life span in Caenorhabditis elegans. Autophagy 4, 330-338.

Yang, W., and Hekimi, S. (2010). Two modes of mitochondrial dysfunction lead independently to lifespan extension in Caenorhabditis elegans. Aging Cell 9, 433-447.

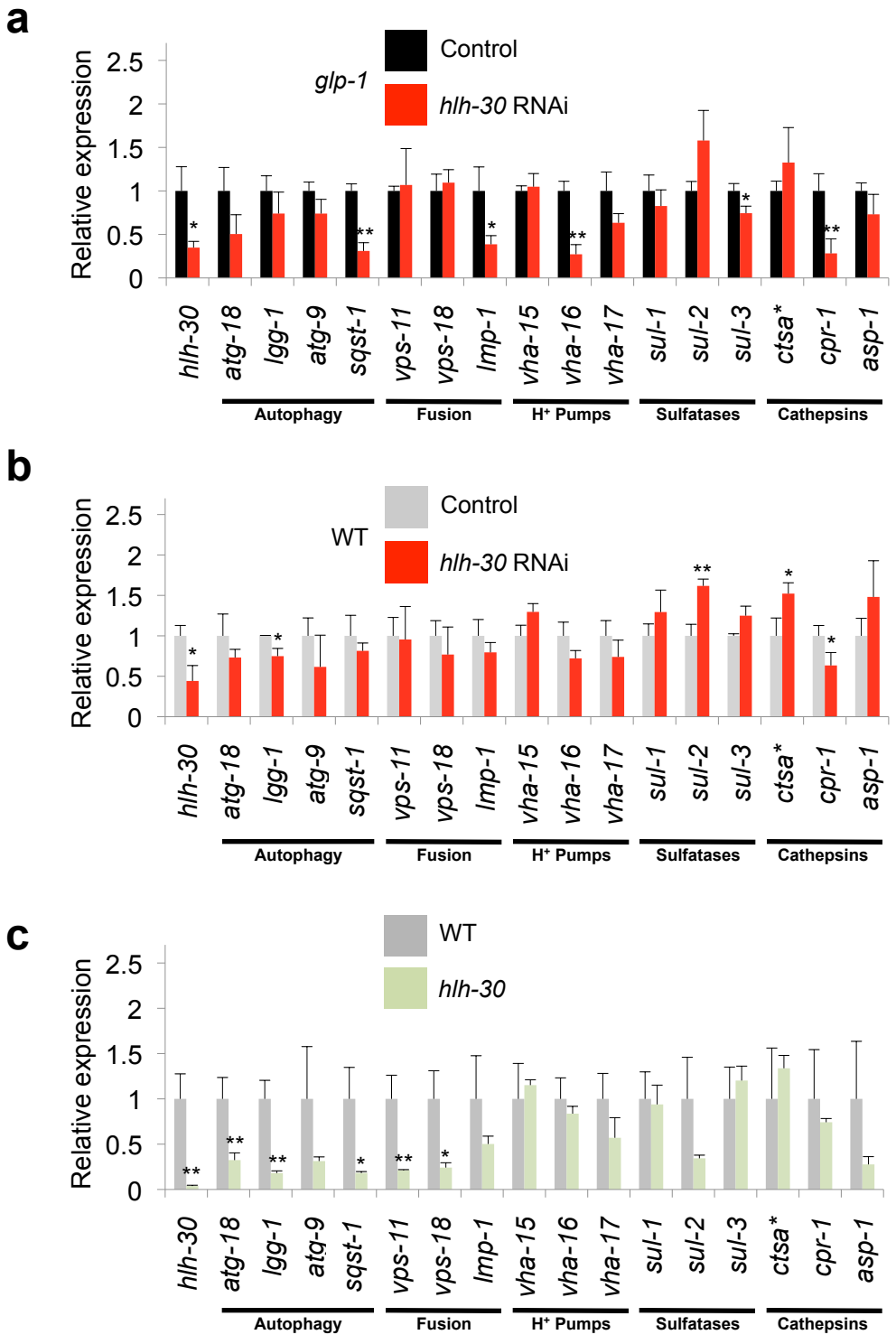

Fig. S1: Analysis of the expression of autophagy-related and lysosomal gene expression in *hlh-30* mutants, and in *hlh-30*–depleted wild-type animals and *glp-1* mutants.
QPCR analysis of autophagy-related and lysosomal gene expression in day 3 (**a**) *glp-1(e2141)* and (**b**) Wild-type (WT, N2) animals raised at the non-permissive temperature (25°C) and fed control bacteria or bacteria expressing dsRNA against *hlh-30* from day 1 of adulthood (at 20°C), or in (**c**) day 1 wild-type (WT, N2) and *hlh-30(tm1978)* mutants fed OP50 *E. coli* and raised at 20°C. Data are mean ± SD of biological triplicates. **P* < 0.05, ***P* < 0.01; Student's *t*-test.

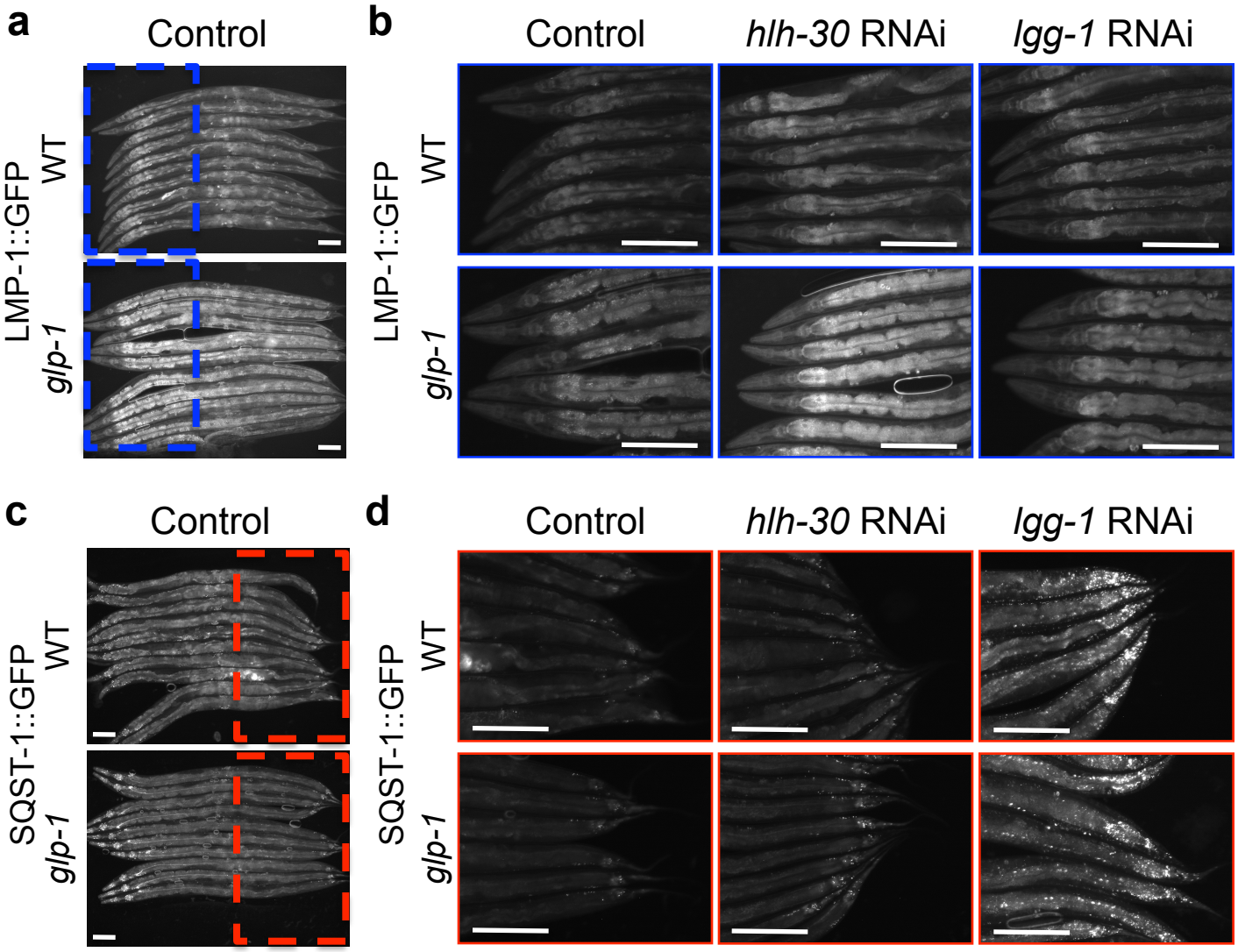

Fig. S2: Expression of LMP-1::GFP and SQST-1::GFP reporters in wild-type animals and *glp-1* mutants.

(**a-b**) Representative micrographs of day 1 wild-type (WT, N2) and *glp-1(e2141)* mutants expressing LMP-1::GFP and raised at the non-permissive temperature (25°C). Animals were fed control bacteria or bacteria expressing dsRNA against *hlh-30* or *lgg-1*/LC3 from hatching. (**c-d**) Representative micrographs of day 1 WT and *glp-1(e2141)* mutants expressing SQST-1/T12G3.1::GFP and raised at the non-permissive temperature (25°C). Animals were fed control bacteria or bacteria expressing dsRNA against *hlh-30* or *lgg-1*/LC3 from hatching. *lgg-1* RNAi is a positive control for inhibition of turnover because mammalian p62/SQSTM1 is targeted for degradation via autophagy by binding directly to LC3 (Komatsu et al., 2007; Pankiv et al., 2007). *hlh-30* RNAi caused an increase in SQST-1::GFP aggregates in the posterior intestine and an increase in LMP-1::GFP levels in the intestine of *glp-1* animals. Higher magnification pictures of these animals are shown in Fig. 1f and 1g. Exposure times were 2 sec for magnifications of 100x (a and c) and 200x (b and d), scale bar 100 μm. Head region is highlighted in blue in (a) and tail region is highlighted in red in (c).

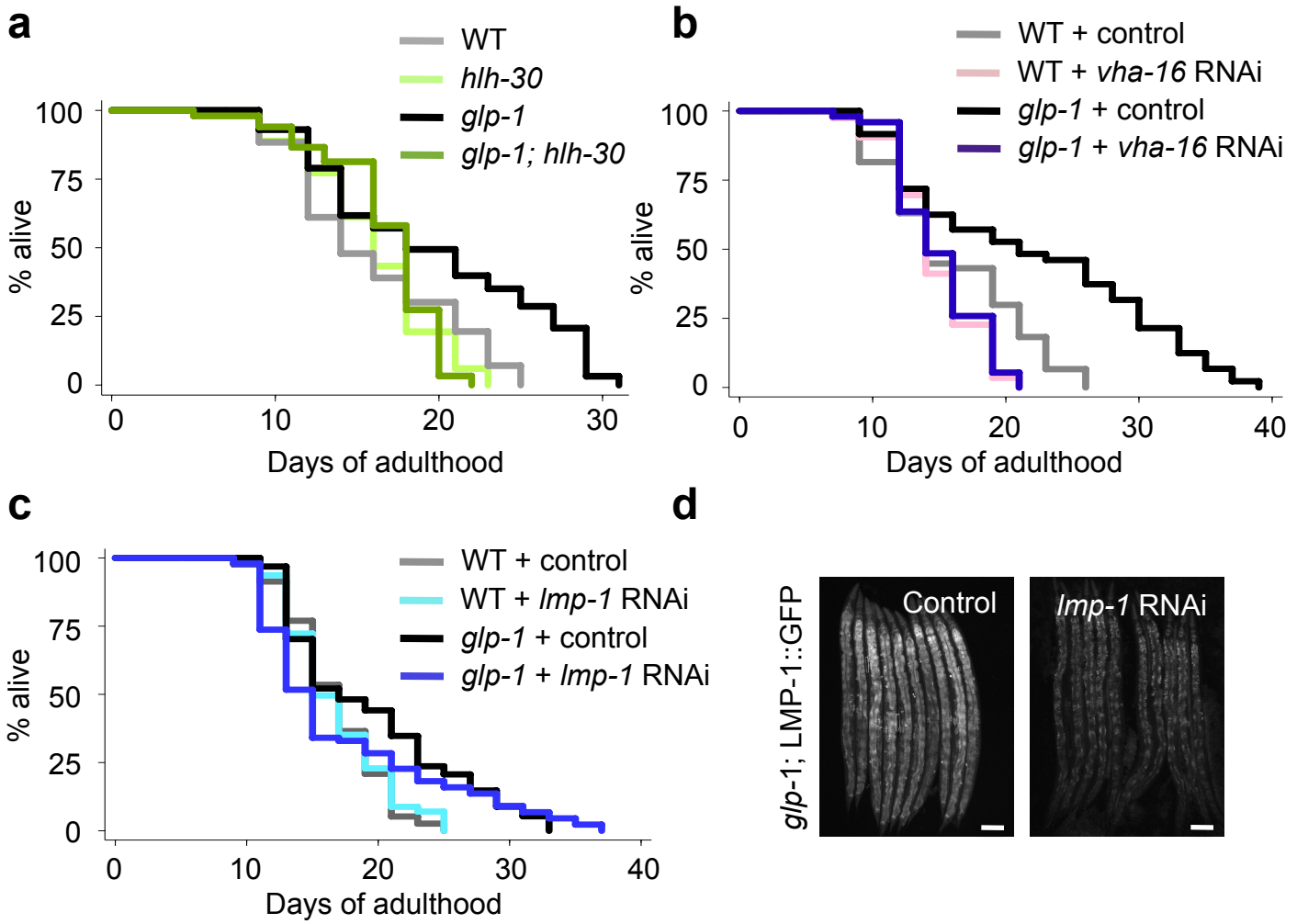

Fig. S3: Role of HLH-30 and select HLH-30–regulated genes in the long lifespan observed in *glp-1* animals.
(**a**) Lifespan analysis of wild-type (WT, N2), *hlh-30(tm1978), glp-1(e2141),* and *glp-1(e2141); hlh-30(tm1978)* animals raised at the non-permissive temperature (25°C), transferred to 20°C on day 1 of adulthood, and fed OP50 *E. coli* (see Table S2 for details and replicate experiments). (**b-c)** Lifespan analysis of WT and *glp-1(e2141)* animals fed control bacteria or bacteria expressing dsRNA against (**b**) *vha-16* or (**c**) *lmp-1* during adulthood (see Tables S2 and S3 for details and replicate experiments). (**d**) Silencing of *lmp-1* by *lmp-1* RNAi was confirmed by evaluating the level of LMP-1::GFP in *glp-1(e2141)* animals fed control bacteria or bacteria expressing dsRNA against *lmp-1* (magnification, 100x; scale bar 100 μm; representative of two independent observations).

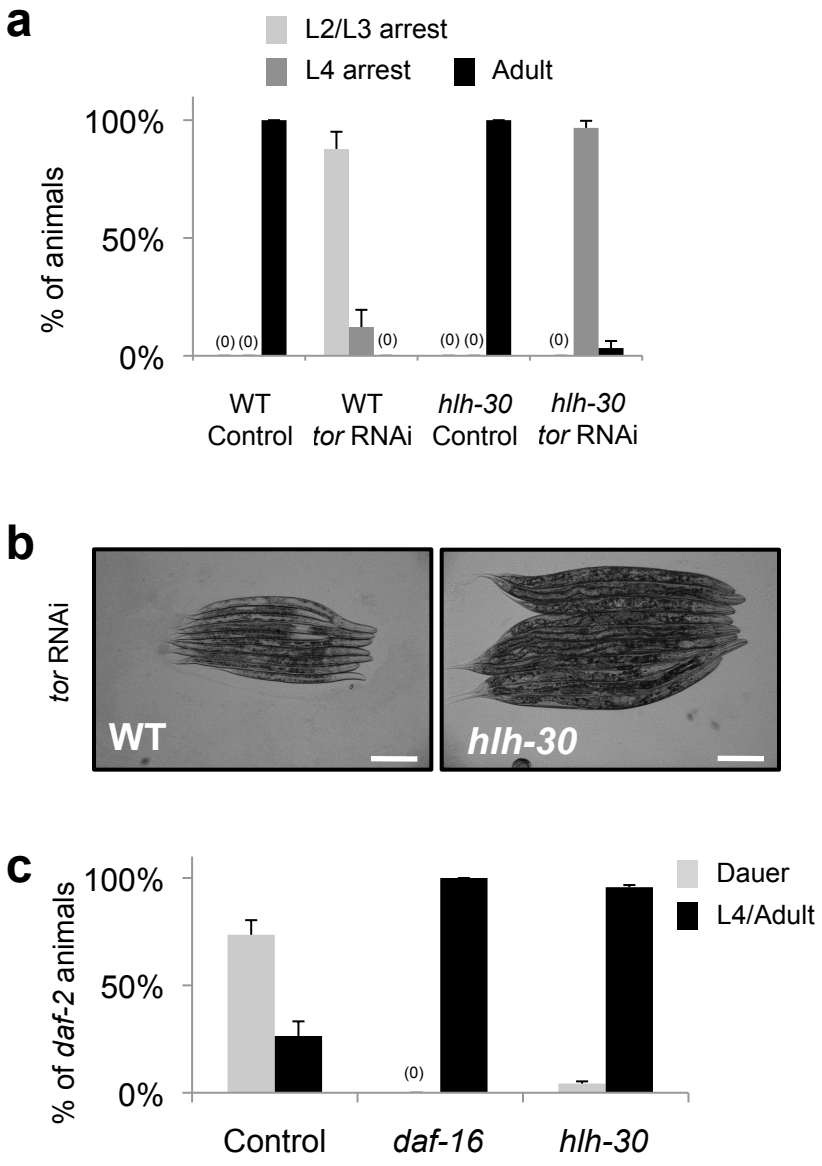

Fig. S4: *hlh*-30 inhibition impairs larval arrest mediated by TOR inhibition and prevents dauer formation in *daf-2* animals.

(**a**) Quantification of larval development of wild-type (N2, WT) and *hlh-30(tm1978)* mutant animals fed bacteria expressing control or *tor* dsRNA for two generations (F1, 20ºC). All animals fed control bacteria reached reproductive adulthood. As previously reported (Long et al., 2002), we observed that progeny of WT animals with reduced *tor* levels arrested almost exclusively at the L2/L3 stage. In contrast, most *tor* RNAi-treated *hlh-30* mutants arrested at the L4 stage, and ~5% of animals bypassed developmental arrest completely and reached reproductive adulthood (n~10-20 animals per plate, 8 plates per treatment, mean ± SD, repeated twice with similar results). (**b**) Micrographs of WT animals and *hlh-30* mutants subjected to *tor* inhibition and arrested at L2/L3 and L4 stages, respectively (magnification, 100x; scale bar 100 μm). (**c**) Quantification of dauer formation in *daf-2(e1370)* mutants fed bacteria expressing control, *daf-16*/FOXO, or *hlh-30 ds*RNA for one generation at ~21°C. The number of dauers or L4 larva/adults were counted after 4 days of incubation (mean ± SD of three independent experiments).

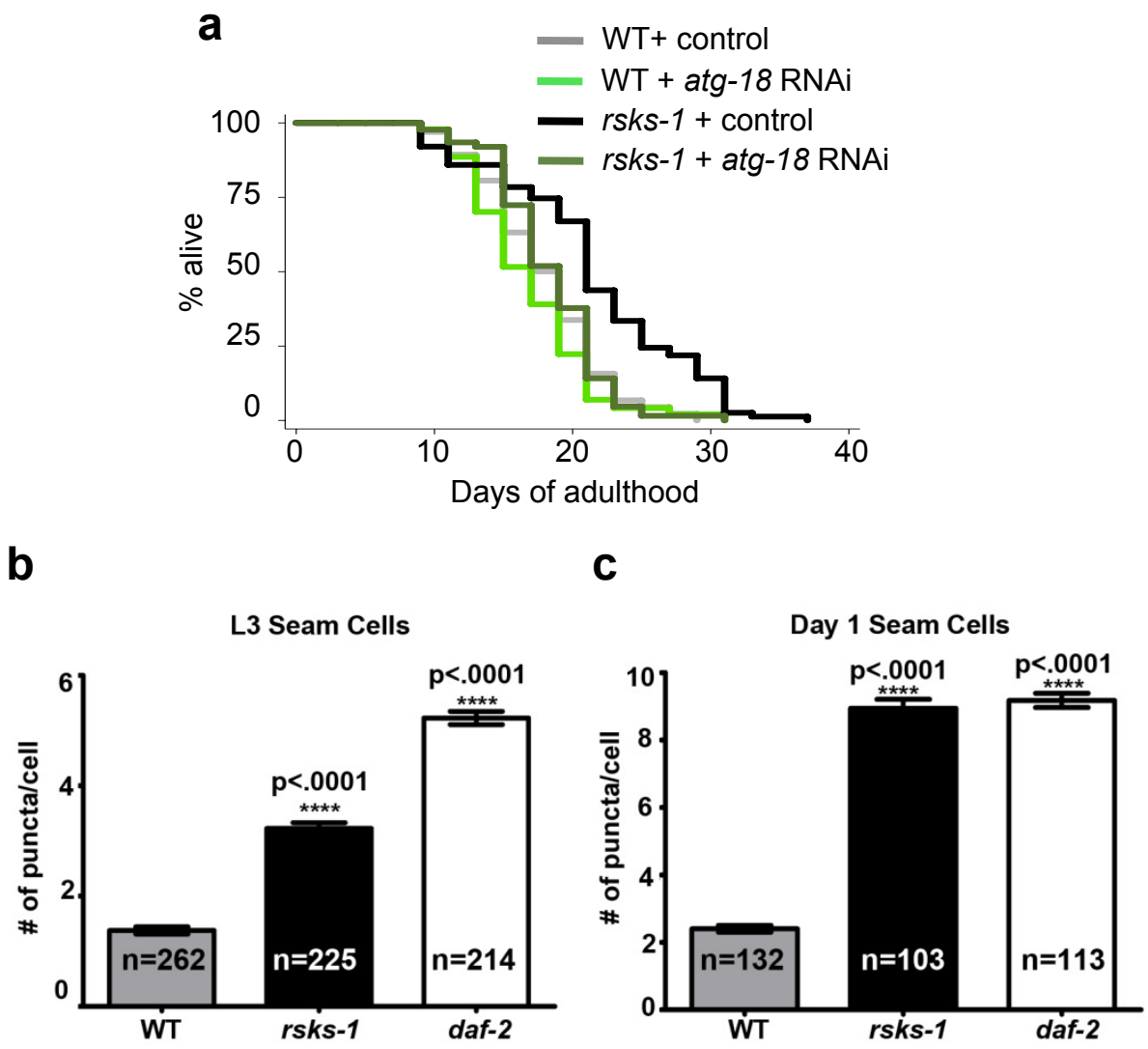

Fig. S5: Autophagy may play a role in the long lifespan of *rsks-1* animals.
(**a**) Lifespan analysis of wild-type (WT, N2) and *rsks-1(sv31)* animals raised at 20°C fed control bacteria or bacteria expressing dsRNA against *atg-18* (see Table S7 for details and replicate experiments). (**b-c**) Quantification of GFP::LGG-1 punctae in WT, *rsks-1(sv31),* and *daf-2 (e1370)* animals (included as a positive control) in seam cells from L3 larvae (**b**) and day 1 adults (**c**). Punctae in 10-15 animals were quantified using confocal microscopy (see Supplemental Methods). Data are mean ± SEM, P values by ANOVA. The analysis was repeated twice with similar results. We previously counted LGG-1::GFP-positive puncta in seam cells of WT and *rsks-1(sv31)* L3 larva using an extrachromosomal array, and found no statistically significant difference (Hansen et al., 2008). The difference between these and our recent results could be due to the use of integrated arrays (Kang *et al*., 2007). A reduction in counts has been observed in day 3 *rsks-1(sv31)* adults compared to WT animals (Robida-Stubbs S. et al., 2012) using the same integrated array we used here, suggesting a very dynamic regulation of LGG-1-positive punctae in rsks-1 animals.

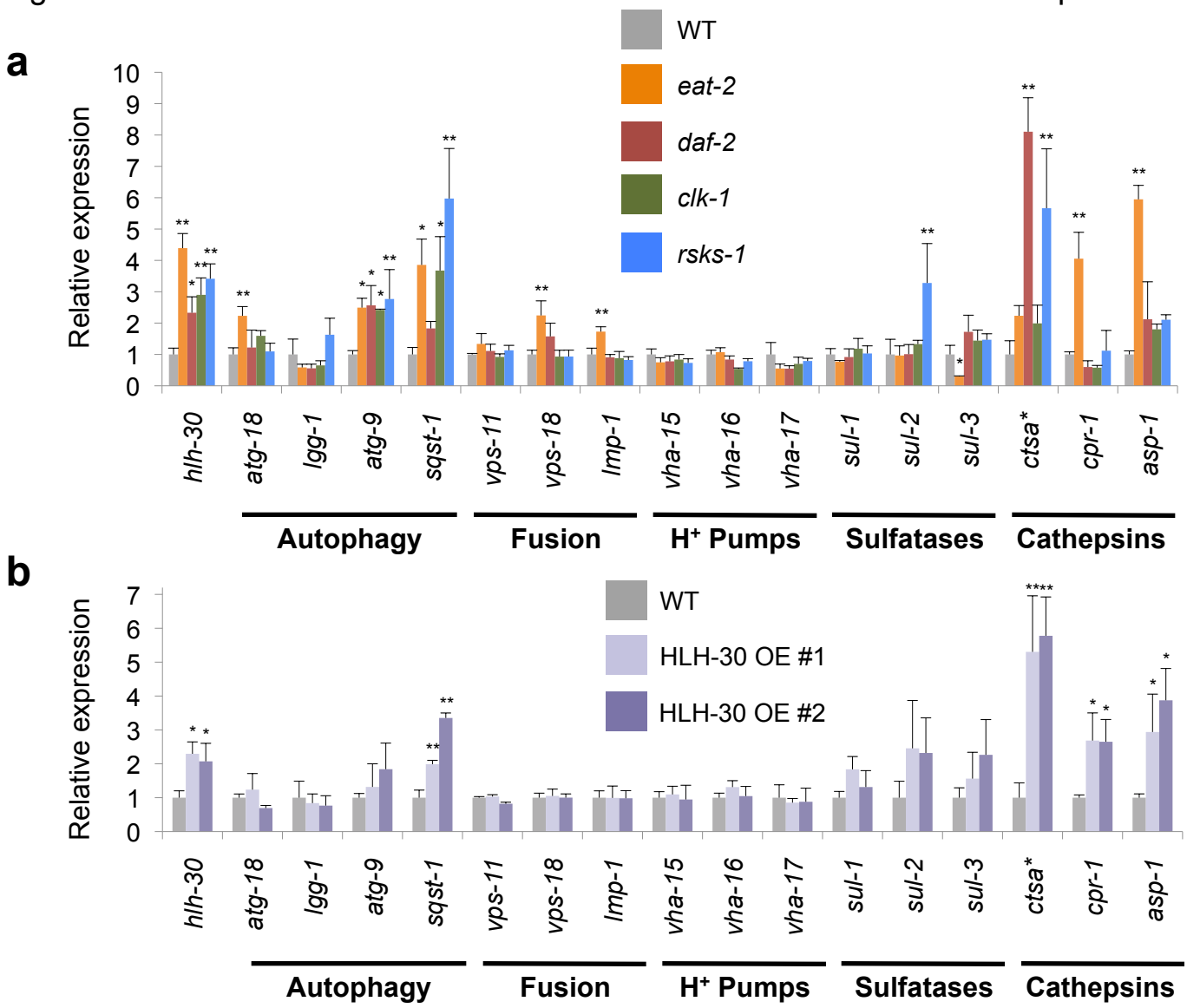

Fig. S6: HLH-30/TFEB target genes are induced in *eat-2*, *daf-2*, *clk-1,* and *rsks-1* mutants and in transgenic HLH-30–overexpressing animals.
(**a**) QPCR analysis of autophagy-related and lysosomal gene expression in day 1 wild-type (WT, N2), *eat-2(ad1116)*, *daf-2(e1370)*, *clk-1(e2519)*, and *rsks-1(sv31)* animals raised at 20ºC. (**b**) QPCR analysis of two different transgenic strains overexpressing HLH-30 (HLH-30 OE #1, #2) raised at 20ºC and collected on day 1. Data are mean ± SD of biological triplicates. **P* < 0.05, ***P* < 0.01; ANOVA.

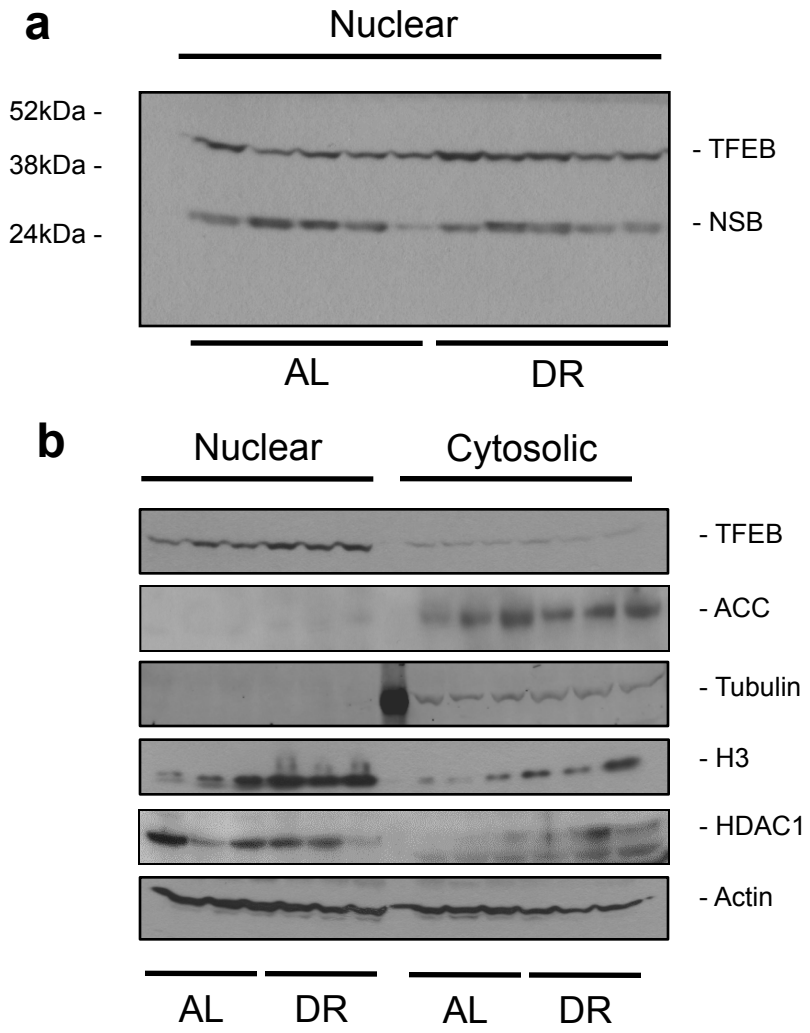

Fig. S7: Dietary restriction increases murine hepatic nuclear TFEB protein levels.
(**a**) Entire membrane shown in Fig. 4f blotted for proteins below 60 kDa with anti-TFEB antibody. NSB: Non-specific band. (**b**) Immunoblot of nuclear and cytosolic fractions of livers from 5 ad libitum (AL) and 5 dietary-restricted (DR) mice (see Methods for details). Membranes were blotted with antibodies against TFEB, the cytosolic proteins Acyl-CoA carboxylase (ACC) and tubulin, the nuclear proteins histone H3 and histone deacetlyase HDAC1, and actin. Each lane was loaded with 40 µg total protein. Note that the anti-tubulin antibody cross-reacted with a marker protein (lane 7, center). Nuclear protein levels were highly variable, but loading equivalence was confirmed by blotting for actin.

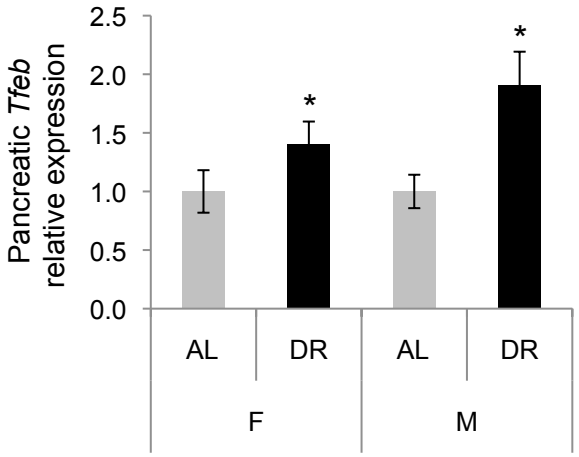

Fig. S8: Dietary restriction increases murine pancreatic TFEB mRNA levels.
Tfeb expression was measured by QPCR in the pancreas of 4.5-month-old female (F) and male (M) mice fed *ad libitum* (AL) or subjected to dietary restriction (DR) for 5.5 weeks starting at 3 months of age (mean ± SEM of ~20 mice per group, **P* < 0.05, Student's *t*-test).