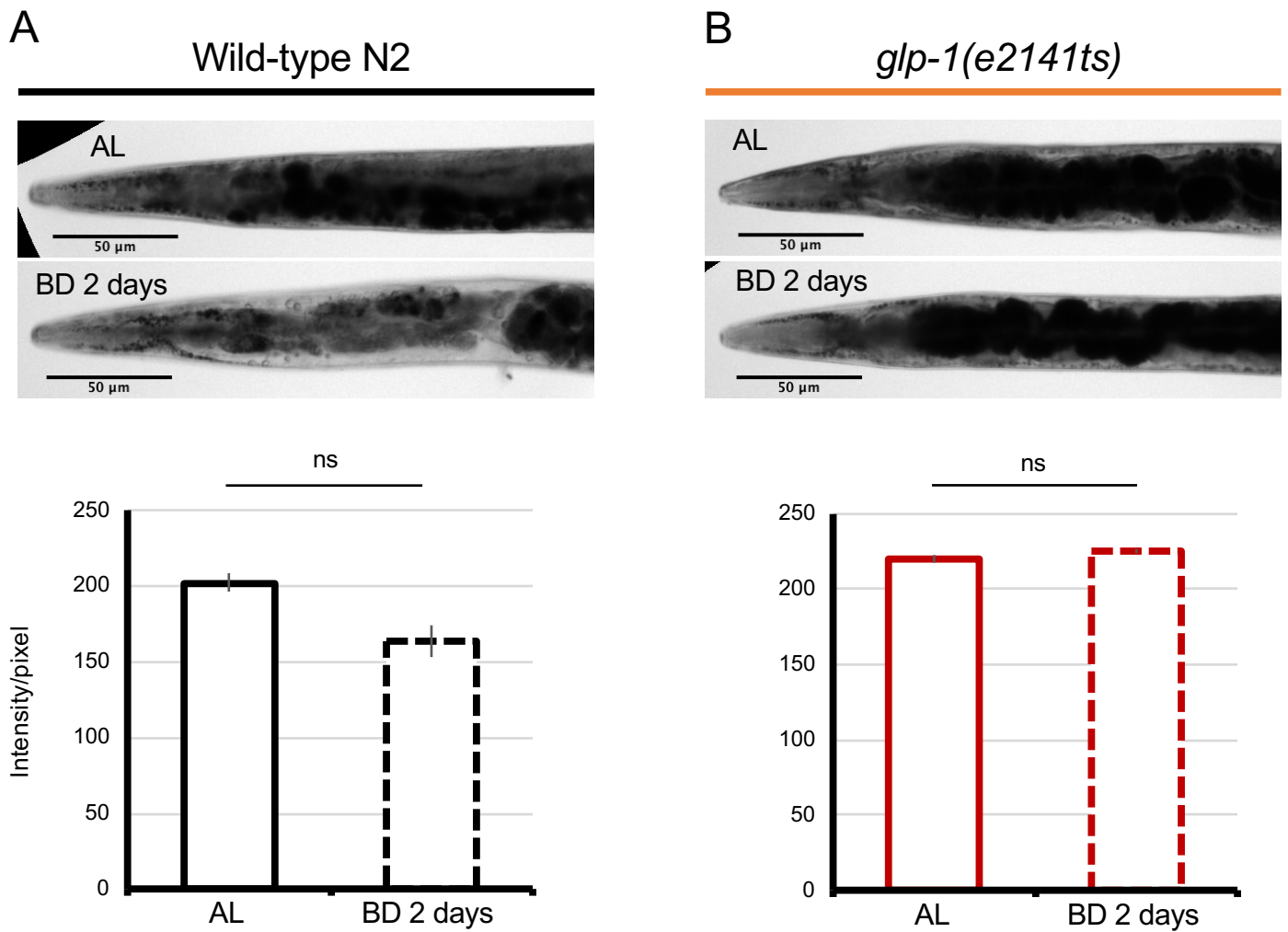


Cell Reports, Volume 28

## Supplemental Information

### **Coelomocytes Regulate Starvation-Induced Fat Catabolism and Lifespan Extension through the Lipase LIPL-5 in *Caenorhabditis elegans***

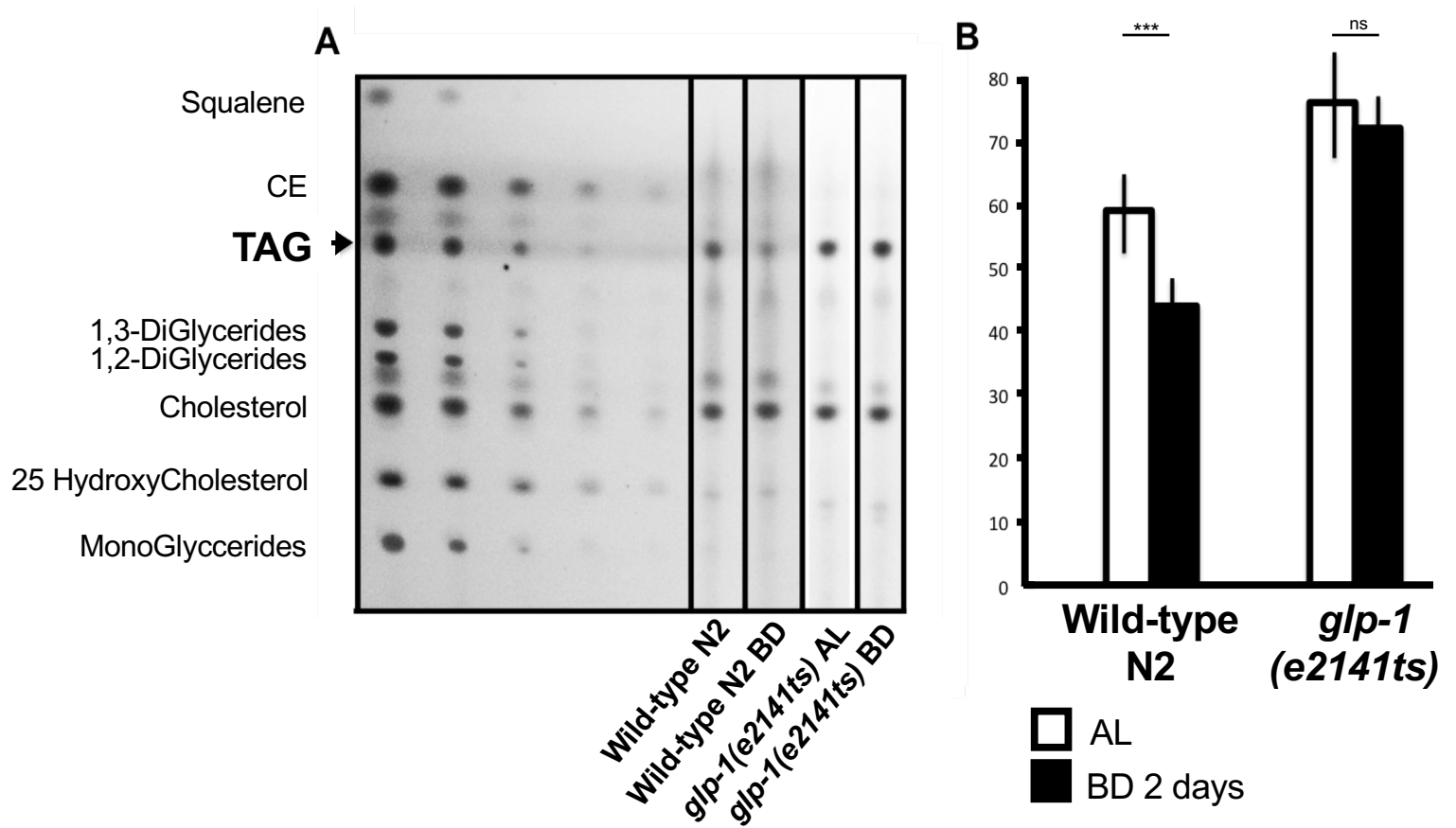
**Alexia Buis, Stéphanie Bellemin, Jérôme Goudeau, Léa Monnier, Nicolas Loiseau, Hervé Guillou, and Hugo Aguilaniu**



**Figure S1. Sudan Black Staining of Lipids in Fed and Starved *C. elegans*.**

**Relates to Figure 1**

(A and B) Sudan Black staining of representative whole animals (upper panels) and densitometric quantification of staining of the two first cells of the intestine after background removal (lower panels) in wild-type *C. elegans* and *glp-1(e2141ts)* mutants fed *ad libitum* (AL) or subjected to bacterial deprivation (BD) for 2 days. Mean ± SD of n 6. ns, not significant by two-tailed Mann-Whitney U test. Representative of 3 biological replicates.



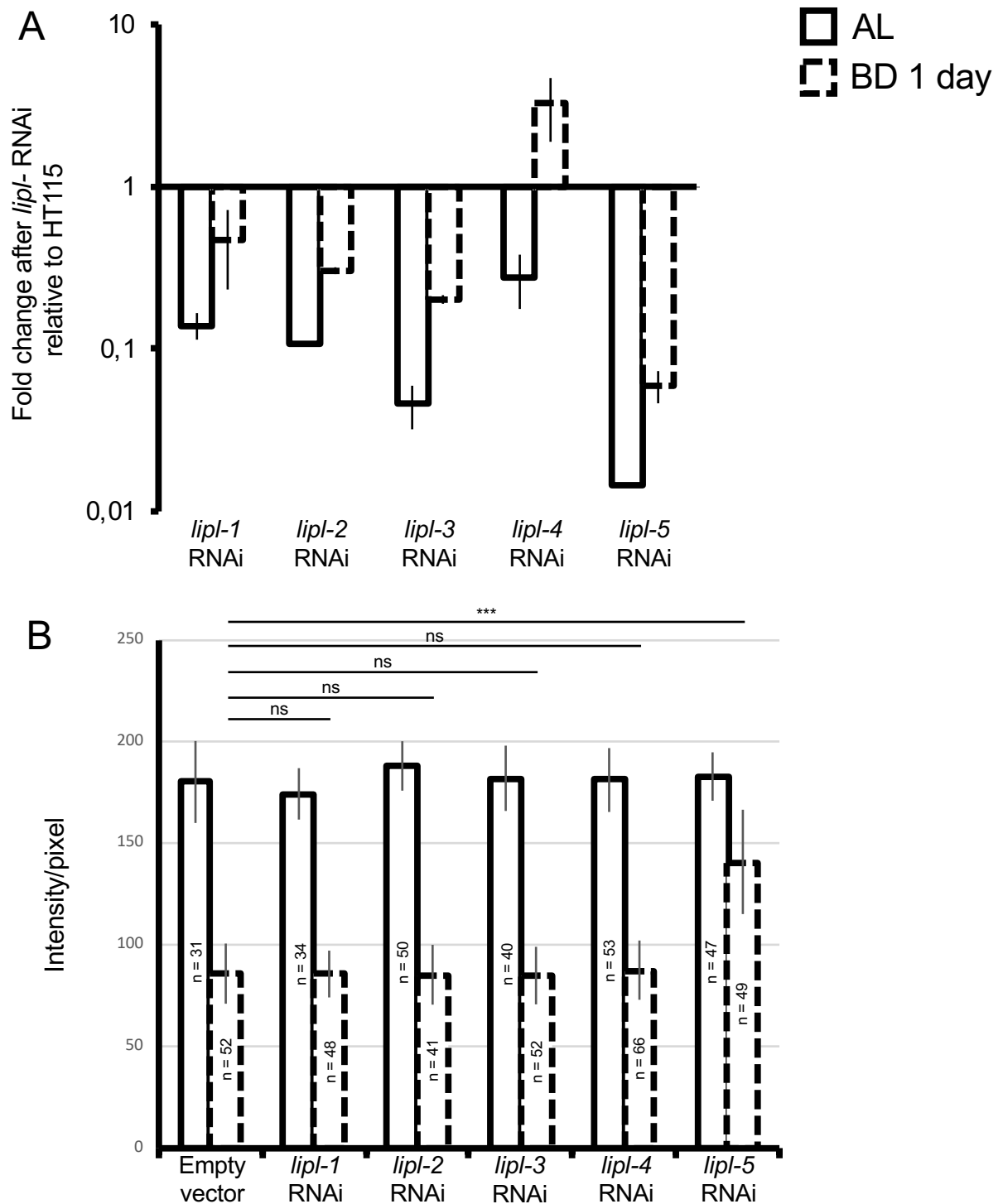
**Figure S2. Thin Layer Chromatography of Lipids in Fed and Starved *C. elegans*.**

**Relates to Figure 1**

(A) Thin layer chromatography of triacylglycerol (TAG) in wild-type N2 fed *ad libitum* (AL) or subjected to bacterial deprivation (BD) for 2

days. (B) Quantification of TG levels. Mean  $\pm$  SD of  $n =$  at least 6. ns, not significant; \*\*\*  $p < 0.001$  by two-tailed Student's t-test.

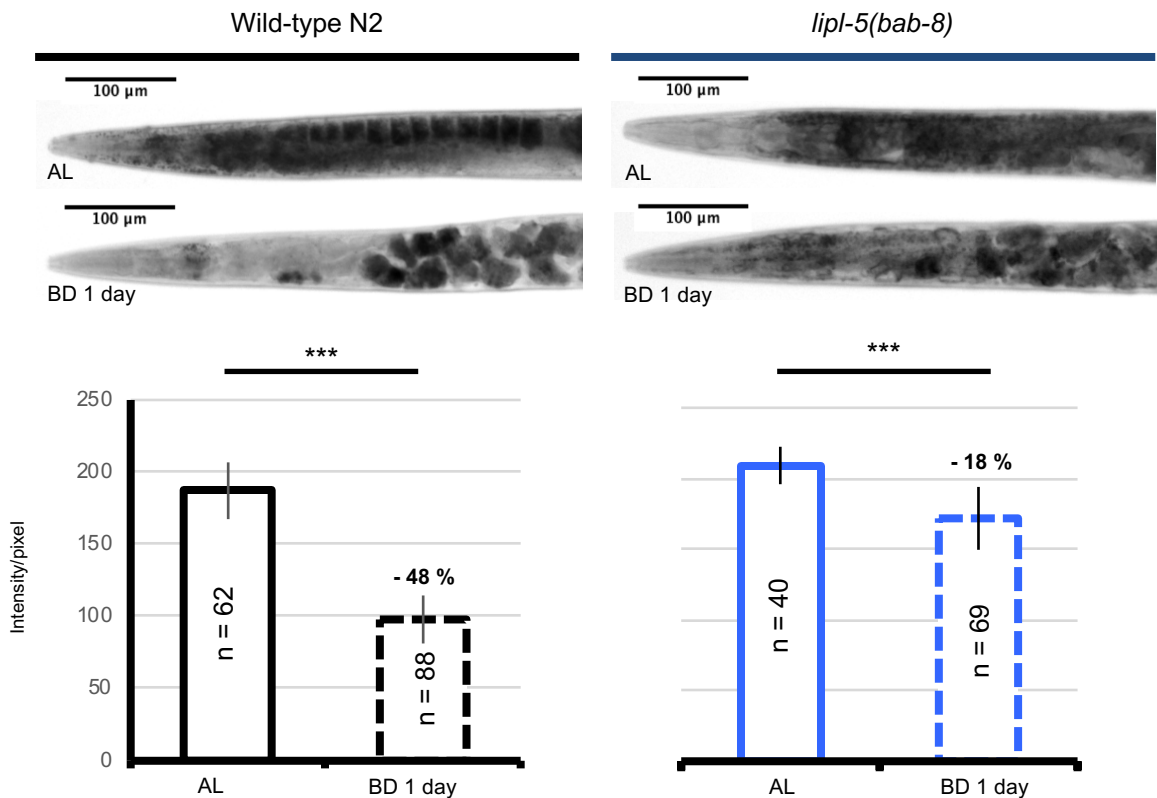
Representative of 2 biological replicates.



**Figure S3. *lip1-5* RNAi Efficiency and Effect on Fat Catabolism in Fed and Starved *C. elegans***

**Relates to Figure 1**

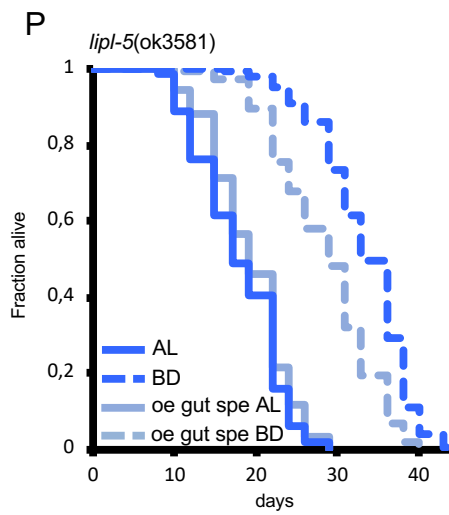
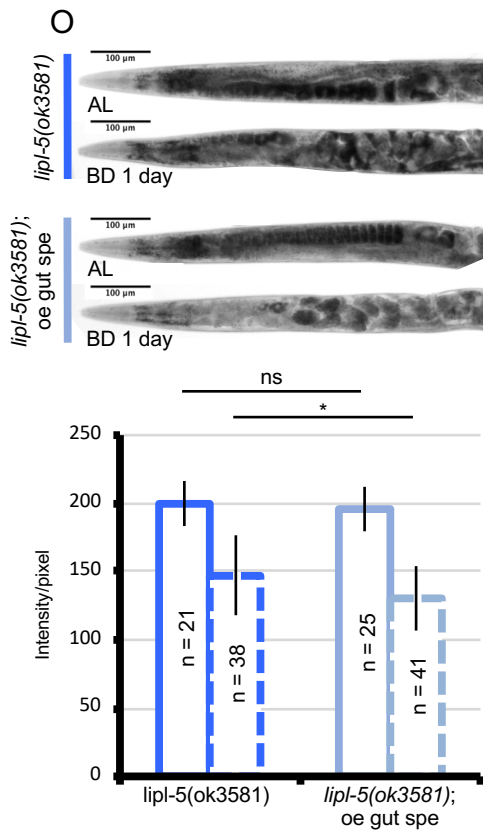
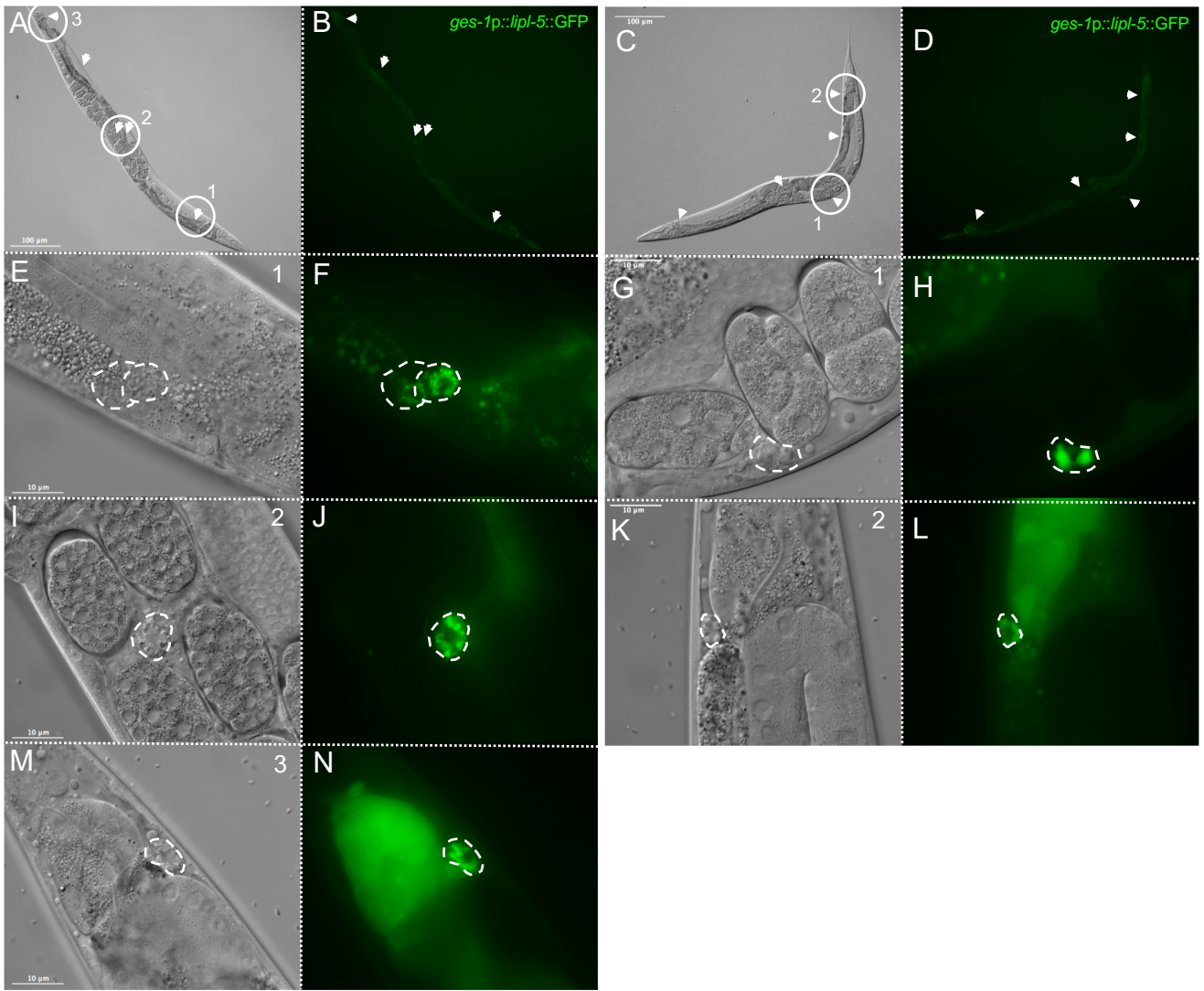
(A) RT-qPCR analysis of *lip1-1*, *lip1-2*, *lip1-3*, *lip1-4*, and *lip1-5* mRNA in wild-type *C. elegans* subjected to RNAi and fed *ad libitum* (AL, solid bars) or subjected to bacterial deprivation (BD, dashed bars) for 1 day. Results are normalized to mRNA levels in animals fed bacteria transfected with empty vector. Representative of 3 biological replicates. The relatively inefficiency of *lip1-4* RNAi after 1 day of BD is likely due to the particularly strong induction of this lipase by BD. Note, however, that this isoform has little effect on TAG levels under these conditions (O'Rourke et al., 2013). (B) Densitometric quantification of Oil Red O staining of fat stores of the two first intestinal cells after background removal in wild-type *C. elegans* subjected to *lip1-1*, -2, -3, -4, or -5 RNAi and fed AL (solid bars) or subjected to BD (dashed bars) for 1 day. Mean  $\pm$  SD of n = at least 20 worms ns, not significant; \*\*\*p < 0.001 by two-tailed Mann-Whitney U test. Representative of 3 biological replicates.



**Figure S4. A Second *lipI-5* Mutation Confirms that Suppression of LIPL-5 Activity Mediates the Reduction in Fat Catabolism and Enhancement in Longevity Induced by Bacterial Deprivation**

**Relates to Figure 2**

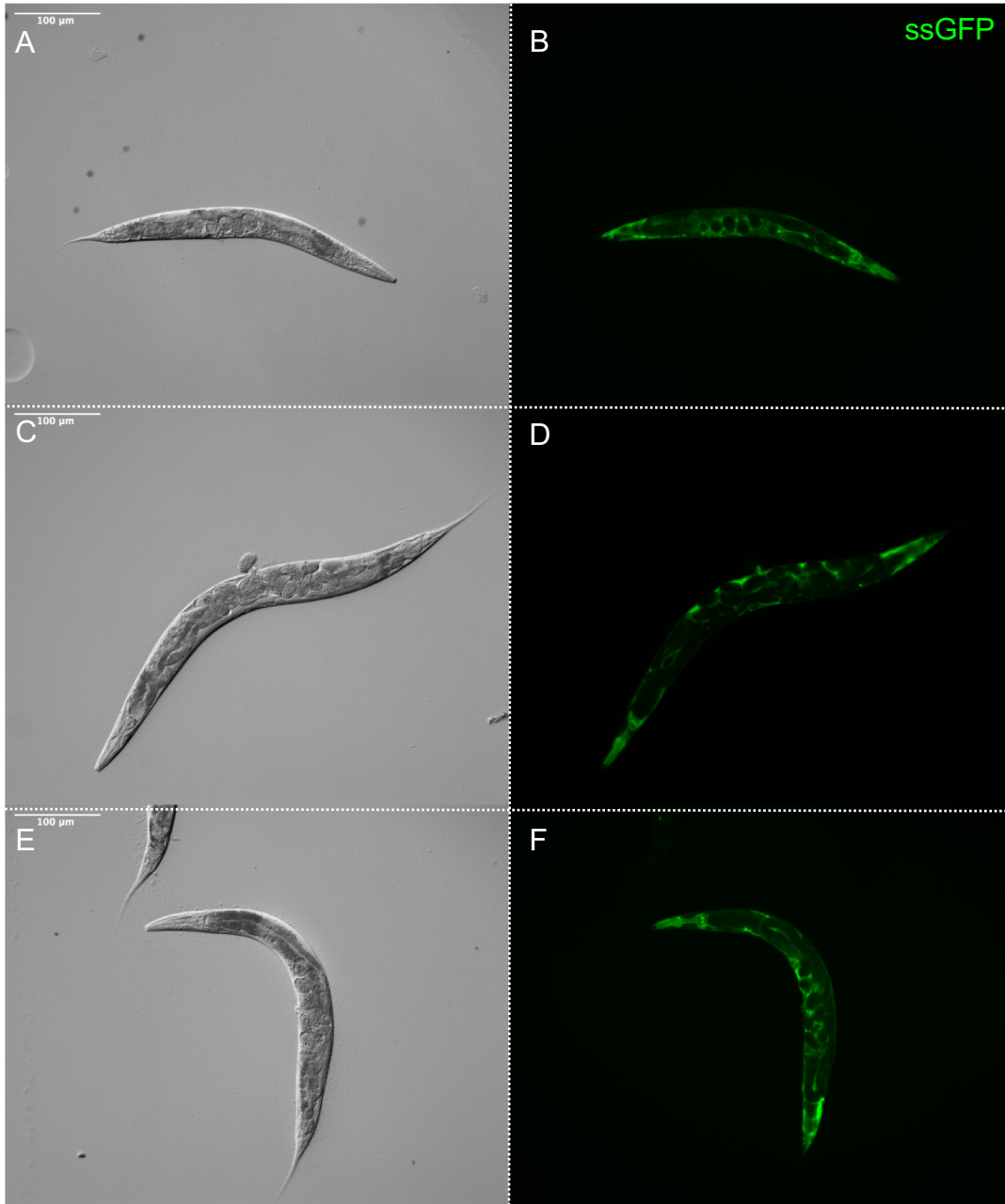
Light micrographs of representative Oil Red O (ORO)-stained whole animals (upper panels) and densitometric quantification of staining of the two first intestinal cells after background removal (lower panels) for wild-type *C. elegans* and *lipI-5(bab-8)* CRISPR-generate mutants fed *ad libitum* (AL) or subjected to bacterial deprivation (BD) for 1 day. Mean  $\pm$  SD of  $n = 20$ . \*\*\* $P < 0.001$  by two-tailed Mann-Whitney U test. Representative of 5 biological replicates.



**Figure S5. Gut-specific *lipl-5* Overexpression in *lipl-5(ok358)* Mutants Partially Reverses the Reduced Fat Catabolism and Enhanced Lifespan Extension Induced by Bacterial Deprivation.**

**Relates to Figure 2**

(A, C, E, G, I, K, M) DIC images and (B, D, F, H, J, L, N) fluorescence images of *lipl-5(ok358)* mutants overexpressing LIPL-5::GFP in the gut (*ges-1* promoter). The A, C, E, G, I, K and M panels and right 6 panels show two adult animals at low magnification (A, B, C and D) and high magnification (E, F, G H, I, J, K, L, M, N); scale bars, 100  $\mu\text{m}$  and 10  $\mu\text{m}$ , respectively). White arrows and dotted lines indicate coelomocytes. Each experiment was replicated at least 3 times (biological replicates). (O) Light micrographs of representative Oil Red O (ORO)-stained whole animals (upper panels) and densitometric quantification of staining of the two first intestinal cells after background removal (lower panel) for *lipl-5(ok358)* mutants with or without gut specific (*ges-1*-driven) *lipl-5* overexpression. Animals were fed ad libitum (AL) or subjected to bacterial deprivation (BD) for 1 day. Mean  $\pm$  SD of n = at least 20 worms ns, not significant; \*\* p < 0.001 by two-tailed Mann-Whitney U test. Representative of 3 biological replicates. (P) Lifespan analyses of *C. elegans* fed AL or subjected to BD. BD extended lifespan by 85% for *lipl-5(ok3581)* mutant (P < 0.0001) and by 48% for *lipl-5(ok3581)* mutants over-expressing *lipl-5* specifically in the intestine (P < 0.0001). When fed ad libitum, the latter strain exhibits a 7% increase when compared to *lipl-5(ok3581)* mutant animals (P < 0.05). In BD, *lipl-5(ok3581)* mutants over-expressing *lipl-5* specifically in the intestine live 14% shorter than simple *lipl-5(ok3581)* mutant animals (P < 0.001). Mantel-Cox log-rank test and the two-tailed Student's t-test. Representative of 3 replicate experiments (See table S2 for number of replicates).



**Figure S6. Coelomocyte-Deficient Animals Exhibit a Cup Phenotype.**

**Relates to Figure 5**

(A, C, E) DIC images and (B, C, F) fluorescence images of adult coelomocyte-deficient animals (coel.-) expressing a soluble form of GFP secreted from body wall muscle cells. In wild-type *C. elegans*, GFP secreted into the pseudocoelom is taken up by coelomocytes; in coel.- animals, worms imaged, GFP accumulates in the pseudocoelom (coelomocyte uptake [cup] phenotype).



Genotype	Origin	Strain number
<i>glp-1(e2141ts)III</i>	Gift from Kenyon Lab	CF1903
<i>daf-2(e1370)III</i>	CGC	CB1370
<i>lipl-5(ok3581)V</i>	CGC	RB2573
<i>lynEx03**</i>	UMS3421	MCP39
<i>lipl-5(ok3581)V;lynEx03</i>	Our lab	HGA2912
<i>unc-119(ed3)III;pwls50***</i>	CGC	RT258
<i>lipl-5::td-tomato</i>	UMS3421	MCP34
<i>lipl-5::td-tomato;pwls50</i>	Our lab	HGA2914
<i>coel.(-)</i>	Gift from Fares Lab	NP717
<i>coel.(-);lipl-5(ok3581)V</i>	Our lab	HGA2915
<i>coel.(-);lynEx03</i>	Our lab	HGA2916
<i>lipl-5(ok3581)V;lynEx04****</i>	Our lab	HGA2917
<i>lipl-5(bab-8)V</i>	UMS3421	MCP12
<i>lipl-5(ok3581)V;lynEx02*****</i>	Our lab	HGA8021

\* CGC = Caenorhabditis Genetics Center

\*\*lynEx03 = [(pAB02(*lipl-5p::lipl-5::td-tomato*) and co-injection marker *myo-2p::mCherry*]

\*\*\*pwls50 = [*lmp-1::GFP* + *Cbr-unc-119(+)*]

\*\*\*\*lynEx04 = [(pAB02(*pcc1::lipl-5::td-tomato*) and co-injection marker *myo-2p::mCherry*]

\*\*\*\*\*bls1 = [*vit-2::GFP* + *rol-6(su1006)*]

\*\*\*\*\*lynEx02 = [(pAB01(*ges-1p::lipl-5::gfp*) and co-injection marker *myo-2p::mCherry*]

Table S1: Strain list, Related to all figures

Genotype	Condition	Mean LS	±	# Worms	% Change	P value
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Figure 1 A	Wild-type N2	AL	16,372	0,411	194/201	62,98	< 0.0001
		BD	26,683	0,558	188/194		
	Wild-type N2	AL	17,703	0,621	132/139	31,78	< 0.0001
		BD	23,329	0,500	146/190		
	<b>Wild-type N2</b>	<b>AL</b>	<b>15,881</b>	<b>0,396</b>	<b>190/198</b>	<b>57,64</b>	<b>&lt; 0.0001</b>
		<b>BD</b>	<b>25,035</b>	<b>0,446</b>	<b>144/176</b>		

Figure 4 A	<b>Wild-type N2</b>	<b>AL</b>	<b>18,495</b>	<b>0,318</b>	<b>233/239</b>	<b>56,42</b>	<b>&lt; 0.0001</b>
		<b>BD</b>	<b>28,930</b>	<b>0,401</b>	<b>228/239</b>		
	Wild-type N2	AL	17,638	0,527	118/126	41,84	< 0.0001
		BD	25,017	0,569	95/116		
	Wild-type N2	AL	20,150	0,600	120/124	47,59	< 0.0001
		BD	29,740	0,640	100/129		
	<i>lipI-5(ok3581)</i>	AL	16,959	0,408	122/123	98,53	< 0.0001
		BD	33,668	0,396	131/142		
	<b><i>lipI-5(ok3581)</i></b>	<b>AL</b>	<b>18,162</b>	<b>0,474</b>	<b>122/124</b>	<b>85,57</b>	<b>&lt; 0.0001</b>
		<b>BD</b>	<b>33,703</b>	<b>0,476</b>	<b>99/146</b>		
	<i>lipI-5(ok3581)</i>	AL	16,929	0,278	267/284	79,10	< 0.0001
		BD	30,320	0,398	157/171		
	Wild-type N2	AL	<b>18,495</b>	<b>0,318</b>	<b>233/239</b>	-1,80	0.515
	<i>lipI-5(ok3581)</i>		<b>18,162</b>	<b>0,474</b>	<b>122/124</b>		
Wild-type N2	BD	<b>28,930</b>	<b>0,401</b>	<b>228/239</b>	16,50	P < 0.001	
<i>lipI-5(ok3581)</i>		<b>33,703</b>	<b>0,476</b>	<b>99/146</b>			
Figure 4 B	<i>lipI-5(ok3581)</i>	AL	18,653	0,492	98/103	85,20	< 0.0001
		BD	34,546	0,428	86/146		
	<i>lipI-5(ok3581)</i>	AL	18,653	0,492	98/103	85,20	< 0.0001
		BD	34,546	0,428	86/146		
	<b><i>lipI-5(ok3581)</i></b>	<b>AL</b>	<b>18,160</b>	<b>0,510</b>	<b>111/141</b>	<b>72,25</b>	<b>&lt; 0.0001</b>
		<b>BD</b>	<b>31,280</b>	<b>0,770</b>	<b>67/109</b>		
	<i>lipI-5(ok3581); oe lipI-5 (endogenous promoter)</i>	AL	18,178	0,361	189/193	64,10	< 0.0001
		BD	29,830	0,455	126/144		
	<b><i>lipI-5(ok3581); oe lipI-5 (endogenous promoter)</i></b>	<b>AL</b>	<b>18,410</b>	<b>0,400</b>	<b>136/141</b>	<b>50,03</b>	<b>&lt; 0.0001</b>
		<b>BD</b>	<b>27,620</b>	<b>0,610</b>	<b>99/116</b>		
	<i>lipI-5(ok3581); oe lipI-5 (endogenous promoter)</i>	AL	18,178	0,361	189/193	64,10	< 0.0001
		BD	29,830	0,455	126/144		
	<i>lipI-5(ok3581)</i>	AL	<b>18,160</b>	<b>0,510</b>	<b>111/141</b>	1,38	0.651
	<i>lipI-5(ok3581); oe lipI-5 (endogenous promoter)</i>		<b>18,410</b>	<b>0,400</b>	<b>136/141</b>		
<i>lipI-5(ok3581)</i>	BD	<b>31,280</b>	<b>0,770</b>	<b>67/109</b>	-11,70	P < 0.001	
<i>lipI-5(ok3581); oe lipI-5 (endogenous promoter)</i>		<b>27,620</b>	<b>0,610</b>	<b>99/116</b>			

Figure 6A	Wild-type N2	AL	17,638	0,527	118/126	41,84	< 0.0001
		BD	25,017	0,569	95/116		
	coel.(-)	AL	14,815	0,440	107/110	89,44	< 0.0001
		BD	28,066	0,646	89/107		
	coel.(-)	AL	14,405	0,383	122/123	109,48	< 0.0001
		BD	30,175	0,706	106/130		
	coel.(-)	AL	13,718	0,422	106/120	108,32	< 0.0001
		BD	28,578	0,780	96/126		
	coel.(-)	AL	16,611	0,564	99/118	78,63	< 0.0001
		BD	29,672	0,670	101/144		
	coel.(-)	AL	16,237	0,568	113/116	90,49	< 0.0001
		BD	30,930	0,749	77/134		
	coel.(-)	AL	17,173	0,341	207/218	91,36	< 0.0001
		BD	32,862	0,399	213/231		
Wild-type N2	AL	17,638	0,527	118/126	-16,01	< 0.001	
coel.(-)		14,815	0,440	107/110			
Wild-type N2		BD	25,017	0,569			95/116
coel.(-)	28,066		0,646	89/107	12,19	< 0.001	

Figure 7 B	coel.(-)	AL	14,815	0,440	107/110	89,44	< 0.0001
		BD	28,066	0,646	89/107		
	coel.(-); <i>lipI-5(ok3581)</i>	AL	12,676	0,449	107/121	154,80	< 0.0001
		BD	32,299	0,528	108/134		
	coel.(-); <i>lipI-5(ok3581)</i>	AL	15,266	0,522	116/123	106,20	< 0.0001
		BD	31,479	0,701	103/138		
	coel.(-); <i>lipI-5(ok3581)</i>	AL	17,335	0,536	119/130	80,65	< 0.0001
		BD	31,315	0,828	90/147		
	coel.(-); <i>lipI-5(ok3581)</i>	AL	17,232	0,387	171/178	87,73	< 0.0001
		BD	32,349	0,480	160/171		
coel.(-)	AL	14,815	0,440	107/110	3,04	0.185	
coel.(-); <i>lipI-5(ok3581)</i>		15,266	0,522	116/123			
coel.(-)	BD	28,066	0,646	89/107	12,16	< 0.05	
coel.(-); <i>lipI-5(ok3581)</i>		31,479	0,701	103/138			
Figure 7 D	coel.(-)	AL	14,815	0,440	107/110	89,44	< 0.0001
		BD	28,066	0,646	89/107		
	coel.(-); <i>oe lipI-5 (endogenous promoter)</i>	AL	14,543	0,666	89/103	92,32	< 0.0001
		BD	27,969	0,564	124/143		
	coel.(-); <i>oe lipI-5 (endogenous promoter)</i>	AL	15,589	0,479	123/131	75,37	< 0.0001
		BD	27,339	0,679	72/115		
	coel.(-); <i>oe lipI-5 (endogenous promoter)</i>	AL	15,436	0,444	122/123	95,45	< 0.0001
		BD	30,170	0,440	101/155		
coel.(-)	AL	14,815	0,440	107/110	-1,84	0.234	
coel.(-); <i>oe lipI-5 (endogenous promoter)</i>		14,543	0,666	89/103			
coel.(-)	BD	28,066	0,646	89/107	-0,35	0.309	
coel.(-); <i>oe lipI-5 (endogenous promoter)</i>		27,969	0,564	124/143			

Figure S5 C	<i>lipl-5(ok3581)</i>	AL	<b>17,987</b>	<b>0,388</b>	<b>172/183</b>	85,45	< 0.0001
		BD	<b>33,357</b>	<b>0,464</b>	<b>143/157</b>		
	<i>lipl-5(ok3581)</i>	AL	23,130	0,480	193/194	32,77	< 0.0001
		BD	30,709	0,429	155/180		
	<i>lipl-5(ok3581)</i>	AL	23,847	0,533	177/179	47,73	< 0.0001
		BD	35,229	0,417	157/184		
	<i>lipl-5(ok3581); oe lipl-5 (pges-1 promoteur)</i>	AL	<b>19,240</b>	<b>0,352</b>	<b>185/192</b>	48,43	< 0.0001
		BD	<b>28,558</b>	<b>0,522</b>	<b>142/167</b>		
	<i>lipl-5(ok3581); oe lipl-5 (pges-1 promoteur)</i>	AL	24,192	0,413	205/210	19,56	< 0.0001
		BD	28,925	0,461	165/176		
	<i>lipl-5(ok3581); oe lipl-5 (pges-1 promoteur)</i>	AL	23,643	0,521	182/183	37,41	< 0.0001
		BD	32,487	0,562	144/189		
	<i>lipl-5(ok3581)</i>	AL	<b>17,987</b>	<b>0,388</b>	<b>172/183</b>	6,97	< 0.05
	<i>lipl-5(ok3581); oe lipl-5 (pges-1 promoteur)</i>		<b>19,240</b>	<b>0,352</b>	<b>185/192</b>		
<i>lipl-5(ok3581)</i>	BD	<b>33,357</b>	<b>0,464</b>	<b>143/157</b>	-14,39	< 0.001	
<i>lipl-5(ok3581); oe lipl-5 (pges-1 promoteur)</i>		<b>28,558</b>	<b>0,522</b>	<b>142/167</b>			

**Table S2: Lifespan data, Related to Figures 1, 4, 6, 7, 8 and S5**

Lifespan data corresponding to lifespan curves shown in the figures appear in bold in the table.

	Forward	Reverse
Plasmid pAB01 construction	Sall/lipl-5 F: 5'-CTGGTCGACATGTGGC GGTTTGCCGTTTTTC-3'	lipl-5/KpnI R: 5'-GTGGTACCTTTCCCAAATAATCGT CAG TGCACAGC-3'
Plasmid pAB02 construction	lipl-5p/lipl-5 gibson F: 5'-CAC AAC GAT GGA TAC GCT AAC AAC TTG GAA ATG AAA TAA GCT TCT AAA ACA ATT ATT TTC AGA CGA TAC TTG GTC-3'	lipl-5p/lipl-5 gibson R: 5'-GAT GAC CTC CTC GCC CTT GCT CAC CAT TAC CGG TAC CGA TTT TCC CAA ATA ATC GTC AGT GC-3'
Plasmid pAB03 construction: primers PCR1	F1 (Plasmid+Pcc1): 5'-CGC TAA CAA CTT GGA AAT GAA ATA AGC TTG TTG ACA CGC AGT TTC CCT- 3'	R1F2 (Pcc1+lipl-5): 5'-AAC GGC AAA CCG CCA CAT ATT GTG AGC CCA ATG AAG TAA AAT TTC-3'
Plasmid pAB03 construction: primers PCR2	R1F2 (Pcc1+lipl-5): 5'-GAA ATT TTA CTT CAT TGG GCT CAC AAT ATG TGG CGG TTT GCC GTT-3'	R2 (lipl-5+Plasmid): 5'-CCC TTG CTC ACC ATT ACC GGT ACC GAT TTT CCC AAA TAA TCG TCA GTG CAC-3'
RT-qPCR primer PMP-3	5'-GTT CCC GTG TTC ATC ACT CAT-3'	5'-ACA CCG TCC AGA AGC TGT AGA-3'
RT-qPCR primer CDC-42	5'-CTG CTG GAC AGG AAG ATT ACG-3'	5'-CTC GGA CAT TCT CGA ATG AAG-3'
RT-qPCR primer ACT-1	5'-GCT GGA CGT GAT CTT ACT GAT TAC C-3'	5'-GTA GCA GAG CTT CTC CTT GAT GTC-3'
RT-qPCR primer LILP-1	5'-CGG TTT GCG CTG GAC TTA-3'	5'-GAA CAC GAG TTG CGT TAA-3'
RT-qPCR primer LIPL-2	5'-TGG ATG CAG ATG GTT CGC AA-3'	5'-GCC CTT GAT GGC TCC GAA AT-3'
RT-qPCR primer LIPL-3	5'-GCT CGT GAT TCT TGC GGT TC-3'	5'-CGG ATA ACC CCA TCG CTC AA-3'
RT-qPCR primer LIPL-4	5'-GAA ACG TTG TTC GCG CAG TT-3'	5'-AAC TTG GCT GGC TGC ATT TG-3'
RT-qPCR primer LIPL-5	5'-ACT GGG GAA CCA AGA CGA AC-3'	5'-TAT CAG CCA ACC AAT CGG CA-3'

**Table S3: Oligonucleotide information, Related to Figures 2, 3, 4, 5, 6, 7, S3 and S7.**