Supplementary materials

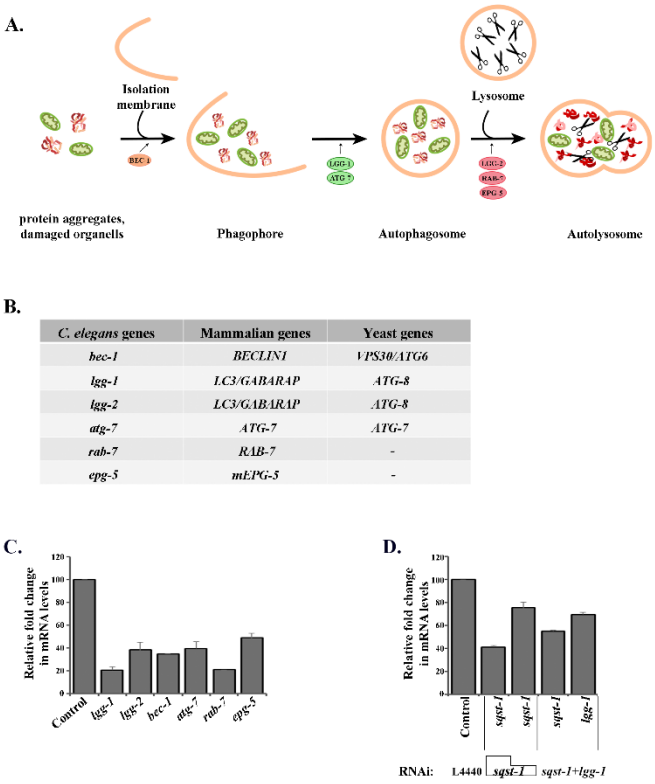

Figure S1. Efficient downregulation of autophagy genes following RNAi (A) Overview of the Autophagy Lysosomal Pathway (ALP). The pathway starts with the formation of an isolation membrane and ends with the fusion of the autophagosome with the lysosome. Autophagy genes investigated in this study are indicated according to their role at the respective steps in the pathway. (B) *C. elegans* genes used in this study and their homologs in human and yeast. (C) and (D) RNAi efficiently decreases mRNA levels of the corresponding genes. (C) Expression of *lgg-1, lgg-2, bec-1, atg-7, rab-7* or *epg-5* mRNA in RNAi-treated *rrf-3(pk1426)* animals was investigated with qPCR. Graph shows the average fold change in mRNA levels compared to control (set as 1). Results are mean of 2-3 independent experiments in triplicate, except for *bec-1* and *rab-7* RNAi with one experiment in triplicate. Error bar, SD. (D) Expression of *sqst-1* or *lgg-1* mRNA levels upon RNAi targeting with either empty vector control (L4440), *sqst-1,* diluted *sqst-1* (RNAi with *sqst-1* and L4440 in 1:1 ratio) or both *sqst-1* and *lgg-1* (RNAi of *sqst-1* and *lgg-1* in 1:1 ratio). Graph shows the average fold

change in mRNA levels compared to control (set as 1). Results are mean of 2-3 independent experiments in triplicate. Error bar, SD.

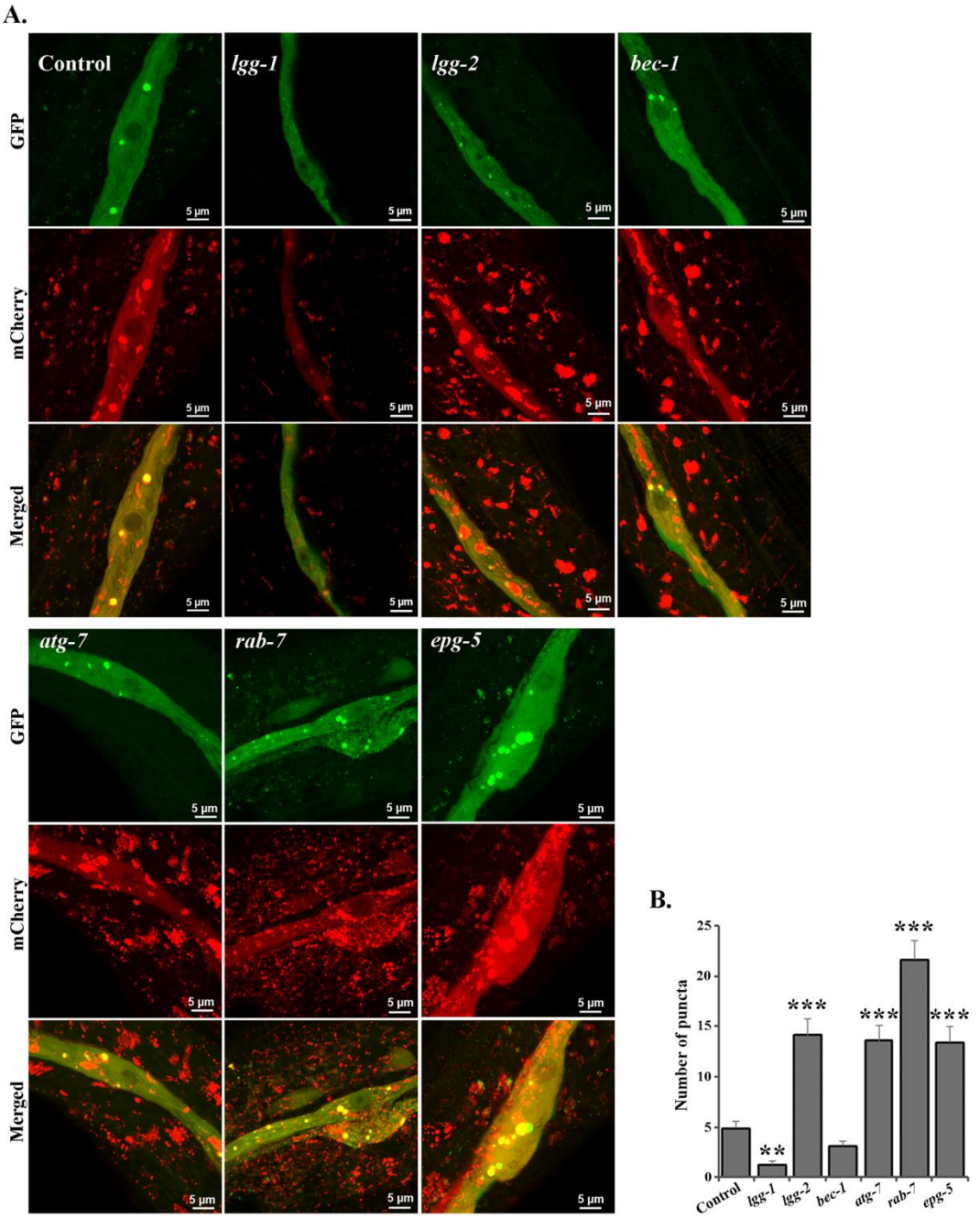

Figure S2. Expression of *mCherry::GFP::LGG-1* reporter in *C. elegans* following RNAi of autophagy genes. (A) Images representing fluorescent micrographs of transgenic N2 (wild-type) animals expressing *mCherry::GFP::LGG-1* after treatment with control, *lgg-1, lgg-2, bec-1, atg-7, rab-7* or *epg-5* RNAi. (B) Quantification of the number of autophagosomes (APs, overlayed GFP and mCherry positive puncta) in hypodermal seam cells. Results are the

mean of quantifications from 3 independent experiments (number of hypodermal cells = 12) (See Table S5). Error bars, SEM, **P<0.01 and ***P<0.001 compared to control.

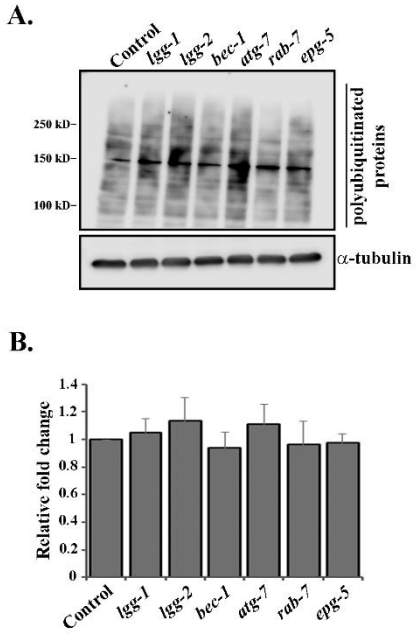

Figure S3. Downregulation of autophagy genes does not affect the amount of polyubiquitinated proteins in whole animal lysates. (A) Lysates of *rrf-3(pk1426)* animals treated with control, *lgg-1, lgg-2, bec-1, atg-7, rab-7* or *epg-5* RNAi were separated on SDS-PAGE and immunoblotted against polyubiquitinated proteins (upper panel) and α-tubulin (lower panel). (B) Quantification of the amount of polyubiquitinated proteins. Graph shows the average fold change in the amount of polyubiquitinated proteins compared to control RNAi (set as 1) and normalized against α-tubulin. Results are the mean of quantifications of 5 independent experiments. Error bars, SEM.

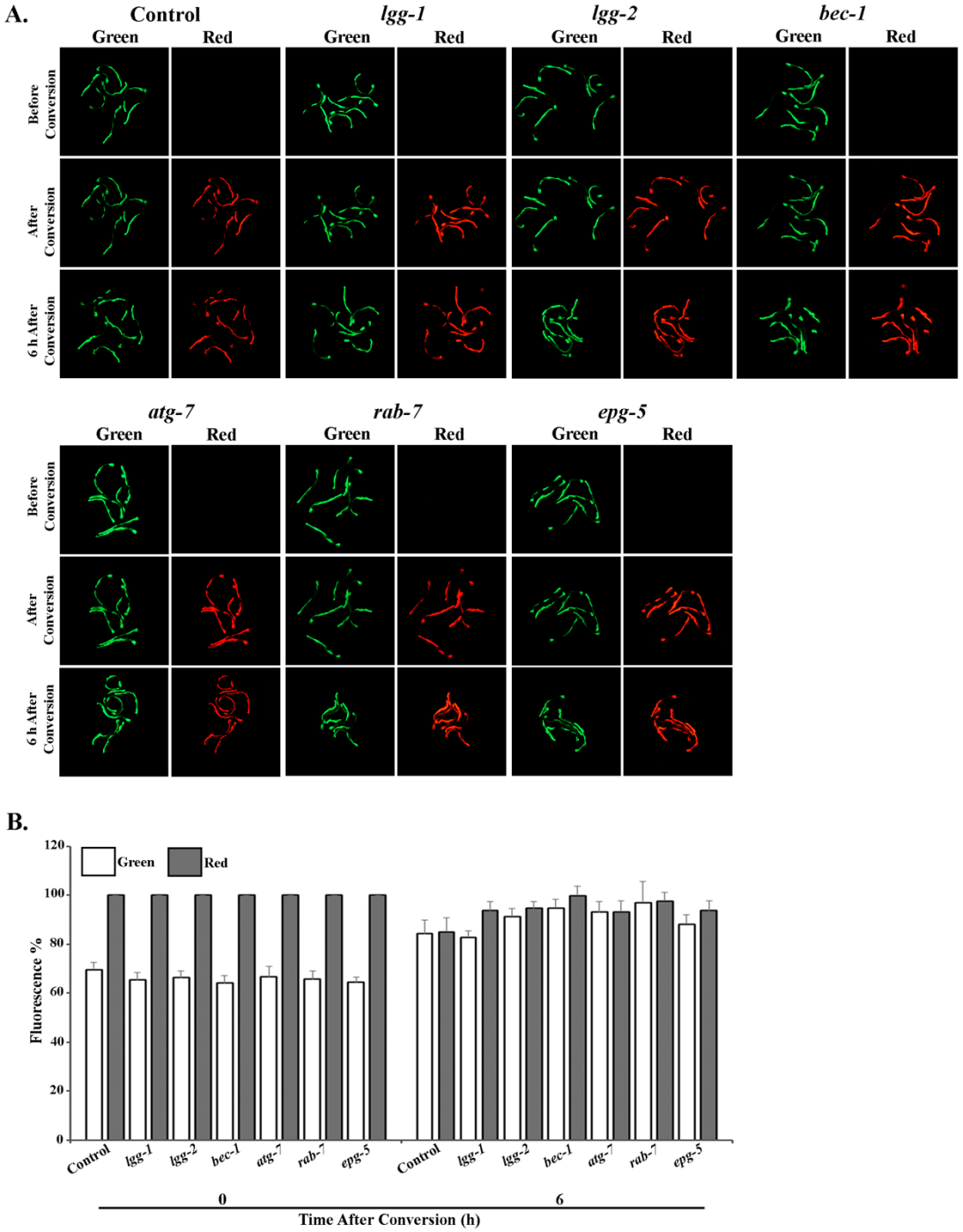

Figure S4. Knockdown of autophagy genes does not affect the stability of Dendra2 reporter in intestinal cells. (A) Fluorescent micrographs of control, *lgg-1, lgg-2, bec-1, atg-7, rab-7* or *epg-5* RNAi treated N2 animals expressing Dendra2 in intestinal cells before, immediately after, and 6 h after photoconversion. (B) Quantification of Dendra2 degradation

in intestinal cells. Graph shows the average percentage of green of red fluorescence relative to initial fluorescent intensity (set as 100%) or intensity at the point of photoconversion (set as 100%), respectively. Results are the mean of quantification from 3 independent experiments (number of animals (n) = 60) (See Table S3). Error bars, SEM.

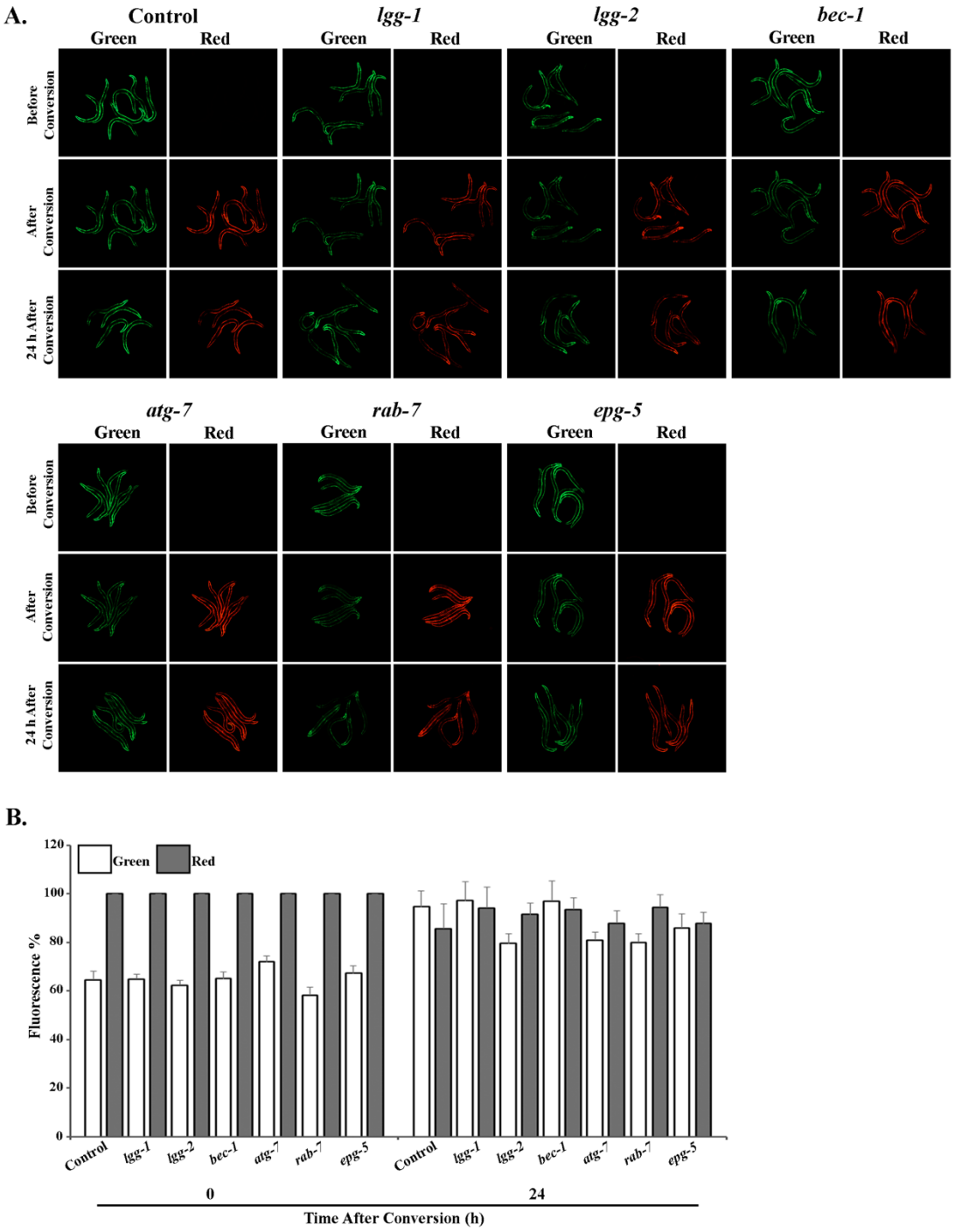

Figure S5. Knockdown of autophagy genes does not affect the stability of Dendra2 reporter in body-wall muscle cells. (A) Fluorescent micrographs of control, *lgg-1, lgg-2, bec-1, atg-7, rab-7* or *epg-5* RNAi treated N2 animals expressing Dendra2 in body-wall muscle cells before, immediately after, and 24 h after photoconversion. (B) Quantification of Dendra2

degradation in body-wall muscle cells. Graph shows the average percentage of green of red fluorescence relative to initial fluorescence intensity (set as 100%) or intensity at the point of photoconversion (set as 100%), respectively. Results are the mean of quantification from 3 independent experiments (number of animals (n) = 60) (See Table S3). Error bars, SEM. (Table S3)

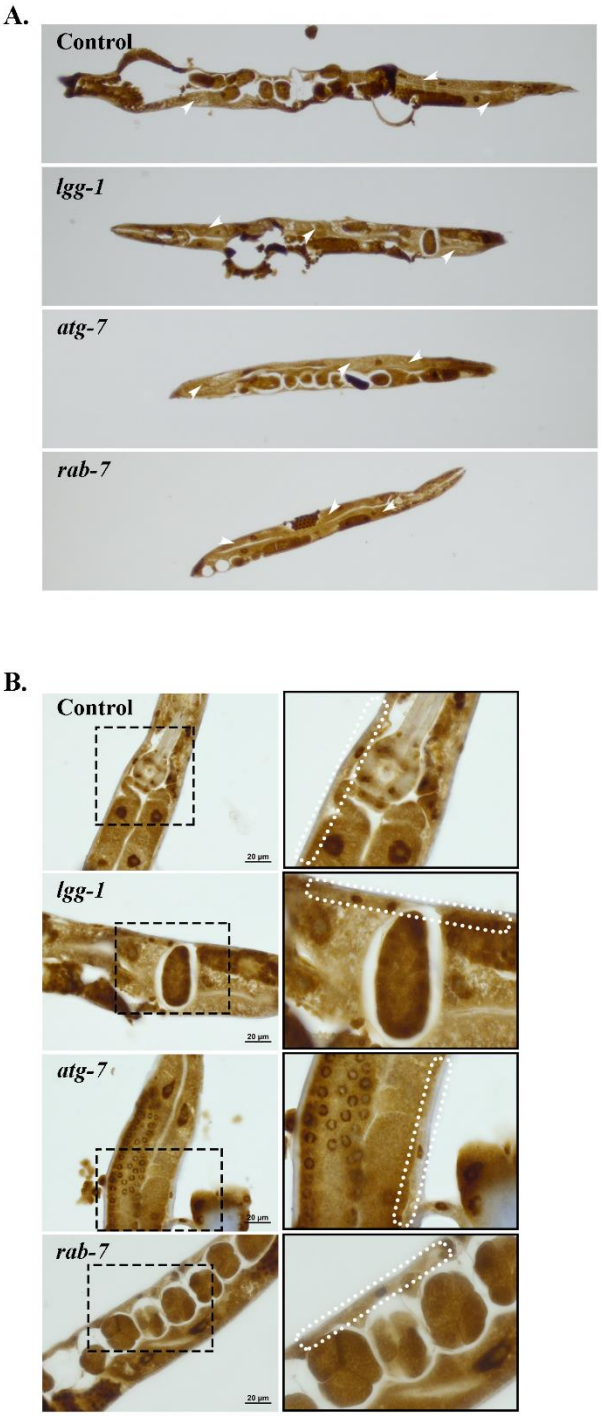

Figure S6. RNAi of *lgg-1, atg-7* and *rab-7* does not affect proteasome tissue expression. (A) Images showing 20S proteasome immunoreactivity of *rrf-3(pk1426)* animals treated with control, *lgg-1, atg-7* or *rab-7* RNAi in the intestinal cells (indicated by white

arrowheads). (B) Higher magnification images showing 20S immunoreactivity in body-wall muscle cells (outlined with white dash line).

Table S1. List of *C. elegans* strains used in this study

Strain	Genotype	Origin
[1]N2		CGC
[2]NL2099	*rrf-3(pk1426)*	CGC
[3]MAH215	*sqIs11[lgg-1p::mCherry::gfp::lgg-1 + rol-6]*	CGC
[4]YD1	*xzEx1[Punc-54::Dendra2]*	[40]
[5]YD3	*xzEx3[Punc-54::UbG76V::Dendra2]*	[40]
[6]YD25	*xzEx25[Pvha-6::Dendra2]*	[35]
[7]YD27	*xzEx27[Pvha-6::UbG76V::Dendra2]*	[35]
[8]YD90	*xzIs1[Pvha-6::UIM2::ZsProSensor]*	[28]
[9]YD116	*rrf-3(pk1426);xzIs2[Punc-54::UIM2::ZsProSensor]*	Generated for this study

[1] Wild-type Bristol strain

[2] RNAi-sensitive strain

[3] Dual fluorescent reporter for autophagy studies

[4] and [6] Dendra2, a photoconvertible green-to-red fluorescent protein expressed in the body-wall muscle and intestinal cells, respectively.

[5] and [7] UbG76V-Dendra2, which consists of the non-hydrolyzable ubiquitin moiety UbG76V fuse to Dendra2. The photoconvertible UbG76V-Dendra2 is degraded by the proteasome and measures UPS-mediated protein degradation independently of translation of reporter proteins, expressed in the body-wall muscle and intestinal cells, respectively.

[8] and [9] Fluorescent polyubiquitin reporter, which binds to endogenous LYS-48-linked polyubiquitinated proteasomal substrates, expressed in the intestinal and muscle cells, respectively.

Table S2. Numbers of imaged polyubiquitin reporter animals

Strain	Imaged tissue	RNAi	Number of animals imaged (number of experiments)	p-value against control RNAi
YD90[xzIs1[Pvha-6::UIM2::ZsProSensor]]	Intestine	*L4440*	90 (6)	
		lgg-1	90 (6)	0,010667
		lgg-2	75 (5)	0,020198
		bec-1	90 (6)	0,013207
		atg-7	75 (5)	0,007871
		rab-7	90 (6)	0,045469
		epg-5	90 (6)	0,833626
		sqst-1	90 (6)	0,141818
		sqst-1+lgg-1	90 (6)	0,034358
YD116[rrf-3(pk1426);xzIs2[Punc-54::UIM2::ZsProSensor]]	Body-wall muscle	*L4440*	90 (6)	
		lgg-1	75 (5)	0,055731
		lgg-2	90 (6)	0,009538
		bec-1	75 (5)	0,000847
		atg-7	60 (4)	0,411909
		rab-7	60 (4)	0,384942
		epg-5	90 (6)	0,000762

		sqst-1	50 (3)	0,211944
		sqst-1+epg-5	50 (3)	0,055069

Table S3. Numbers of imaged UbG76V-Dendra2 and Dendra2 reporter animals

Strain	Imaged tissue	RNAi	Number of animals imaged (number of experiments)	p-value against control RNAi (degradation UbG76V-Dendra2)
YD27[xzEx27[Pvha-6::UbG76V::Dendra2]]	Intestine	*L4440*	115 (5)	
		lgg-1	112 (5)	0,0016458
		lgg-2	105 (5)	0,0004006
		bec-1	112 (5)	0,0002728
		atg-7	105 (5)	0,00497
		rab-7	100 (5)	0,29413
		epg-5	105 (5)	0,1925351
		sqst-1	65 (3)	0,2417188
		sqst-1+lgg-1	65 (3)	0,0202486
YD25[xzEx25[Pvha-6::Dendra2]]	Intestine	*L4440*	65 (3)	
		lgg-1	65 (3)	0,3441761
		lgg-2	63 (3)	0,1544679
		bec-1	65 (3)	0,0657256
		atg-7	60 (3)	0,286508
		rab-7	65 (3)	0,0978616

		epg-5	70 (3)	0,1084852
YD3[xzEx3[Punc-54::UbG76V::Dendra2]]	Body-wall muscle	*L4440*	105 (5)	
		lgg-1	105 (5)	0,917595
		lgg-2	105 (5)	0,145795
		bec-1	105 (5)	0,978631
		atg-7	105 (5)	0,735148
		rab-7	105 (5)	0,217689
		epg-5	105 (5)	0,032891
YD1[xzEx1[Punc-54::Dendra2]]	Body-wall muscle	*L4440*	65 (3)	
		lgg-1	65 (3)	0,44017
		lgg-2	60 (3)	0,62759
		bec-1	65 (3)	0,422
		atg-7	60 (3)	0,95776
		rab-7	65 (3)	0,16752
		epg-5	65 (3)	0,82245

Table S4. Significance values of *in vitro* assays

Approach	RNAi	Number of experiments	p-value compared to control RNAi	
In-gel proteasome activity assay			From whole animal lysates	
	L4440	10		
	lgg-1	10	0,008881	
	lgg-2	10	0,257055	
	bec-1	10	0,720861	
	atg-7	10	0,520055	
	rab-7	10	0,149359	
	epg-5	10	0,000215	
Western Blotting			From whole animal lysates	
	L4440	6		
	lgg-1	6	0,643918	
	lgg-2	6	0,0006	
	bec-1	6	0,003655	
	atg-7	6	0,05713	
	rab-7	6	0,331061	
	epg-5	6	0,655201	
Immunohistochemical analysis			Intestinal cells	Body-wall muscle cells
	L4440	3		
	lgg-1	1	NA	NA
	lgg-2	3	0,697217	0,035077
	bec-1	3	0,015465	0,010164

	atg-7	1	NA	NA
	rab-7	1	NA	NA
	epg-5	3	0,34077	0,198936

Table S5. Numbers of imaged *mCherry::GFP::LGG1* reporter animals

Strain	**Imaged cell**	**RNAi**	**Number of cells imaged (number of experiments)**	**p-value against control RNAi**
MAH215[sqIs11[lgg-1p::mCherry::gfp::lgg-1 + rol-6]]	Hypodermal seam cell	*L4440*	15(3)	
		lgg-1	15(3)	0,000816
		lgg-2	15(3)	0,000199
		bec-1	15(3)	0,062158
		atg-7	15(3)	0,000386
		rab-7	15(3)	4,01E-06
		epg-5	15(3)	0,000131

Table S6. List of qPCR oligonucleotides used in this study

C. elegans	**Forward**	**Reverse**
lgg-1	aggagacaagatccgcagaa	gacgaagttggatgcgtttt
lgg-2	ttagacgacgcctccaactt	ctggatcacgctcttgactg
bec-1	gatcctgttggagcgtatcg	cgaattccaggatcaattcc
atg-7	atcgcttcatcaaaccgaag	agggtaccggacattgaca
rab-7	ttacgaggtttctgccaagg	ggaaatcgttggtttcctga
epg-5	ctccaccaccacgtgtttc	tggtgctaccgctgtagttg
act-1	tcggtatgggacagaaggac	catcccagttggtgacgata
cdc-42	ctgctggacaggaagattacg	ctcggacattctcgaatgaag
pmp-3	gttcccgtgttcatcactcat	acaccgtcgagaagctgtaga