EMBO reports

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Genome-wide screen identifies signaling pathways that regulate autophagy during C. elegans development

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Nonia Pariente

1st Editorial Decision 16 January 2014

Thank you for your patience while your study was peer-reviewed over the Christmas holidays. I have now received reports from the three referees that were asked to assess your study, which you will see below. Although referee 2 is more negative about the general interest of the resource provided by your dataset, both referees 1 and 3 are more positive and consider it will be a valuable contribution. Nevertheless, they raise several issues that would be worth addressing during revision to strengthen the study and thus its general interest.

We agree with the more positive view of the value that your dataset will have and, thus, if the referee concerns can be satisfactorily addressed, we will be happy to consider your study for publication in EMBO reports. It will be important nevertheless to deepen the analysis of the consequences of defective ribosomal synthesis, as referee one requests, and analyze whether the transcriptional response controlling the expression of autophagy genes depends on the Yme6, Sin3, Rpd3 pathway, as referee 3 points out.

In addition, there are comments to the confusing nature of the text and figure organization throughout the reports of referees 1 and 3, which need to be addressed. Please note that you can present up to five figures if necessary for clarity (preferably 4).

The text is 10,000 characters longer than we can accommodate, a more direct, to the point writing will help shorten it and may alleviate some of the concerns raised by referee 1. Please note that the Materials and methods section is rather succinct and cannot be further shortened.

Please also go through your manuscript carefully and ensure that all relevant figure legends include information on the number of independent experiments (or animals) measured, the represented value and type of error bars used, statistical test applied to the data and values considered significant, were applicable. It is more useful to the reader to place this information in (or at the end of) the legends that in the methods section.

Please get in touch with me if I can be of any help. I look forward to receiving the revised version of your study.

REFEREE REPORTS:

Referee #1:

Review for Guo et al., 2013
Genome-wide screen identifies multiple signaling pathways regulating autophagy activity during C. elegans development.

This current study by Guo et al., shows that ribosomal protein RPL-43 activity is required for degradation and removal of p62 homolog SQST-1 in the intestine. In the absence of RPL-43 activity, SQST-1::GFP accumulates in the intestine to form aggregates. SQST-1::GFP aggregate formation in the RPL-43 mutant is suppressed by induction of autophagy. They performed a genome-wide RNAi screen to identify genes that regulate autophagy during larval development. From that screen, they identified 139 genes as a negative regulator of autophagy activity. Further, they show that various signaling pathways regulate autophagy in C. elegans. Though the manuscript lacks clarity due to complex sentences and grammatical error, it is a very interesting study. Findings in this manuscript will be of interest to the field of autophagy. This manuscript is apt for publication in this journal. However, the following comments should be addressed prior to publication.

Major Comments
The authors did not offer explanation on why defects in ribosomal synthesis would lead to defect in clearance of autophagic aggregates. Since translation inhibition in response to rapamycin promotes autophagy, one might have expected an opposite result where the rpl-43 mutant has increased autophagy. For example, Ramirez-Valle et al 2008 shows that eIF4GI depletion leads to induction of autophagy via TOR signaling. The authors need to look at lgg-1::gfp puncta in rpl-43 mutants. Is it possible that ribosomal deficiency leads to ribophagy and the normal SQST-1::GFP clearance is attenuated ? I feel that the authors did not analyze in detail on why ribosomal deficiency would lead to reduced SQST::GFP clearance. The screen identified several genes inactivations that suppress the SQST-1::GFP clearance defect in rpl-43. But the common theme of all the hits from the screen is that increasing autophagy suppresses the SQST-1::GFP clearance defect suggesting an indirect effect rather than mechanistic explanation of why ribosomal deficiency leads to autophagy defect. The authors say that SQST::GFP gets weaker as the worms age; since perturbations in ribosomal translation prolongs the life span of worms (Tavernarakis, Kenyon, and Ruvkun labs) is it possible that the rpl-43 mutant defect in SQST::GFP elimination is due to slowed aging. Did the authors look at old rpl-43 mutants? Since translation is required for synthesis of all proteins, is it possible that the levels of the terminal proteins required for lysosomal clearance of aggregates is lower in rpl-43 mutants? The authors need to look at the protein levels of core autophagic pathway components.

Minor comments
1. Figure S1C does not show expression in the larval stages. It shows expression only in the embryo. Please correct second sentence in page 5.

2. In figure S1E-H, it is hard to see any difference in SQST-1::GFP aggregates in different larval

stages.

3. In all the figure and figure legend, please follow the C. elegans nomenclature to write the genotype. Write the gene name in small letter and allele name in parenthesis (e.g. rpl-43 (bp399)).

4. Figure S1C2 -F2 shows that PGL-1 expression is unaffected in the rpl-43(bp399) mutant but did not show any data for SEPA-1 as mentioned in the text. Please include that data or remove SEPA-1 from the text. Authors need to use another autophagy substrate expressed in the intestine to show that autophagy is not affected and RPL-43 is not a essential component of autophagy pathway.

5. Page 6, second paragraph is confusing. Please rewrite.

6. It is not clear from the text whether genes identified from the secondary screens are excluded from the genes list mentioned in the table 1 and table S1. If those genes identify by secondary screens were not excluded, including that data to the table S1 might be useful.

7. In page 10, a brief introduction about LGG-1 and why it is being tested will help the reader to understand better.

8. In figure 2C, D, G, & H, lin-35 is shown but the text indicates that lin-35, lin-54 and lin-61. Please include the data for lin-54 and lin-64 or rewrite the text accordingly.

9. Please briefly explain in the text what we are supposed to see in figure S3L-Q and why authors concluded that SynMuvB gene repress autophagy independent of its other roles during development.

10. Page 16, "we found that loss of function of hlh-30....." sentence is complex and hard to understand, please rewrite.

11. All the figure including supplementary figures have lots of panels and cited in different places, which makes it harder to follow. It requires reorganization of the figures so as to follow it easily. Split the figure and cite them with the flow of the text.

Referee #2:

In an unbiased screen Guo and colleagues identified a c elegans mutant which had accumulations of SQST-1/p62 aggregates in larval intestine. They mapped this mutation to the ribosomal protein subunit RPL-43. Using this mutant background they performed a screen to look for negative regulators of autophagy, that is genes whose loss promoted removal of the p62 aggregates. The result was the identification of 139 genes whose loss promoted autophagy. This group contained representatives of many signalling pathways, som of which are known regulators of autophagy. Finally the authors validated a number of these hits providing a degree of confidence in the results. However, the results are still largely that of a "screen paper" and in some cases the pathways identified have been implicated in the regulation of autophagy. Therefore in my opinion the paper is not suitable for EMBO reports but for a more speacialized audience.

Referee #3:

Guo et al identify a mutation in the ribosomal protein encoding gene rpl-43 that causes accumulation of SQST-1/p62 in the worm intestine, and SQST-1 foci can be removed by the activation of autophagy. Using this as a tool, the authors utilize a nearly genome-wide RNAi approach to screen for and identify genes that when reduced in function, promote autophagy. I am usually skeptical about such screens, as in theory any gene that induces cell stress could activate autophagy. The reason that I support publication of this study is that the screen is thorough, includes a number of important secondary screens and controls, and also reports a number of surprising findings.

Therefore, this manuscript is certain to be of interest to the readership of EMBO Reports. My concerns are relatively minor, and I encourage the authors to consider the following issues.

1) The authors should provide a clear statement to readers to reduce the concern that the mutations identified (or at least a subset of the mutations) are not simply activating stress that indirectly induce autophagy.

2) One of the most interesting findings in the manuscript is that the new genes identified influence the transcription of autophagy genes, and this presumably influences autophagy. Surprisingly, the TFEB homologue HLH-30 is not required for this autophagy, and this is also interesting. However, the authors fail to consider that this transcriptional response may be controlled by a mechanism identified by the Klionsky lab involving Ume6, Sin3 and Rpd3 (PNAS 2012). Minimally, the authors should reference this paper and potential mechanism. In addition, the authors should provide any evidence from their screen that this may not be the potential mechanism if they have this information.

3) On pp. 5-6 the authors refer to bp399 mutants before they have determined that the mutation is in rpl-43. This is confusing, as they reference an immuno-blot in Fig.1E that is labeled rpl-43 (instead of bp399). This could easily corrected by either changing the labeling of Fig. 1E, or reversing 1E and 1F (and adjusting the text as appropriate).

4) Fig. 3 A,B are labeled as being wild type on the figure but are described as being rpl-43 mutants in the text. This must be corrected.

5) It is difficult to read the labels on the graphs because the font size is extremely small. This is only going to get worse when the figures are reduced, and a solution is needed.

1st Revision - authors' response 14 March 2014

We thank the reviewers for their constructive comments. Using these comments as a guide, we performed additional experiments to further support our conclusions. We believe that the genes identified in this study will provide a "resource" for further investigating autophagy regulation under physiological conditions.

Here are the reviewers' comments with our point-by-point responses:
Referee #1:

1). The authors did not offer explanation on why defects in ribosomal synthesis would lead to defect in clearance of autophagic aggregates.

As in *rpl-43* mutants, we found that RNAi inactivation of genes encoding components of other ribosomal subunits, ribosomal maturation factors and translational initiation factors causes the accumulation of SQST-1::GFP aggregates in the intestine (Table S1 in the revised manuscript). SQST-1::GFP aggregates in *rpl-43* mutants are co-stained by anti-ubiquitin antibody. These results suggest that impairment of translation results in accumulation of misfolded proteins in the intestine, which could be due to a reduction in synthesis of proteins involved in protein folding. Consistent with this idea, we found that RNAi inactivation of genes involved in protein folding (*hsp-110*) causes accumulation of a large number of SQST-1::GFP aggregates in the intestine.

The accumulated SQST-1 aggregates in *rpl-43* mutants cannot be efficiently removed by basal autophagy activity. Upon autophagy induction, those aggregates are removed. (See also our response to #4 below, where we explain that loss of function of *rpl-43* does not impair autophagy activity). In this manuscript, we used the removal of SQST-1 aggregates in *rpl-43* mutants by elevated autophagy activity as a model to identify autophagy regulators.

2). Since translation inhibition in response to rapamycin promotes autophagy, one might have expected an opposite result where the rpl-43 mutant has increased autophagy. For example, Ramirez-Valle et al 2008 shows that eIF4GI depletion leads to induction of autophagy via TOR signaling. The authors need to look at lgg-1::gfp puncta in rpl-43 mutants.

RNAi inactivation of *ifg-1* (encoding the eIF4G1 homolog) did not suppress the SQST-1 aggregate accumulation phenotype in *rpl-43* mutants. However, inactivation of Tor signaling promotes the removal of SQST-1 aggregates in *rpl-43* mutants. We also found that *rpl-43* mutants have a significantly shortened life-span. As suggested by the reviewer, we also examined levels of the *C. elegans* Atg8 homolog LGG-1, which has been widely used to measure autophagy activity. Compared to wild-type animals, levels of both lipidated and non-lipidated forms of LGG-1 remain unchanged in *rpl-43* mutants in an immunoblotting assay. Furthermore, GFP::LGG-1 puncta do not accumulate in the intestine in *rpl-43* mutants. We also measured mRNA levels of *lgg-1*, *atg-7, epg-8* and *bec-1* and found that they remain unaltered in *rpl-43* mutants (Figure S2Q-T in the revised manuscript). Taken together, these results confirm that loss of function of *rpl-43* does not elevate autophagy activity.

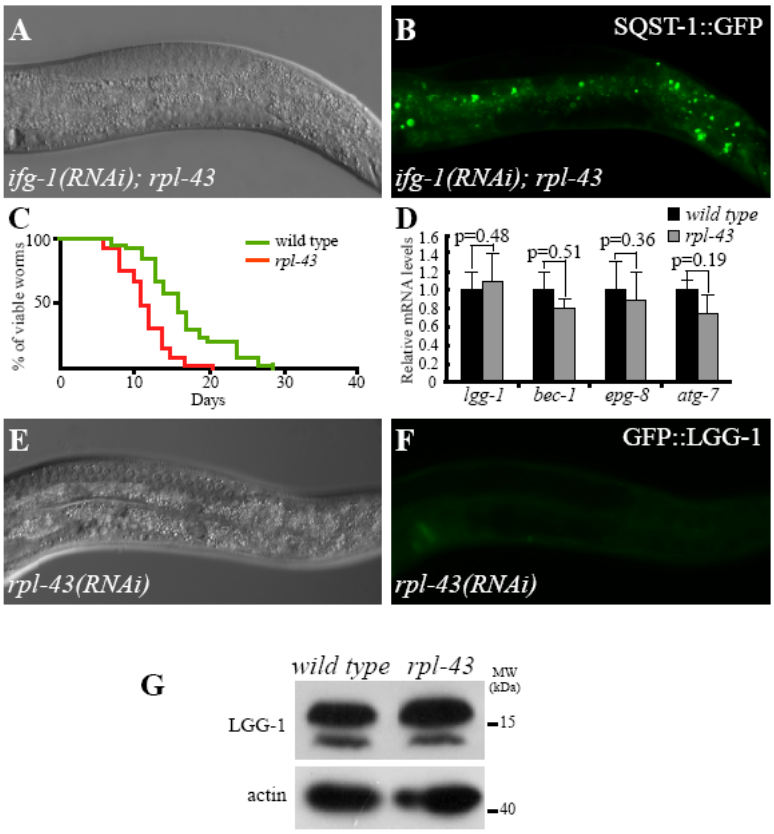


Figure 1. (A-B) Loss of function of *ifg-1* does not suppress the accumulation of SQST-1::GFP in *rpl-43(bp399)* mutants. (C) *rpl-43* mutants show a shortened life-span. (D) mRNA of levels of autophagy genes remained unchanged in *rpl-43(bp399)* mutants. (E-F) *rpl-43(RNAi)* does not cause accumulation of LGG-1 puncta in the intestine. (G) Levels of LGG-1 remained unchanged in *rpl-43* mutants.

3). Is it possible that ribosomal deficiency leads to ribophagy and the normal SQST-1::GFP clearance is attenuated ?

As suggested by the reviewer, we examined the expression of reporters for ribosomal proteins and found that in *rpl-43* mutants, RPS-3::GFP and RPL-43(bp399)::GFP are diffusely localized in the cytoplasm and their levels remain unchanged. Thus, loss of activity of *rpl-43* does not lead to ribophagy. Ribophagy has not been demonstrated in *C. elegans*.

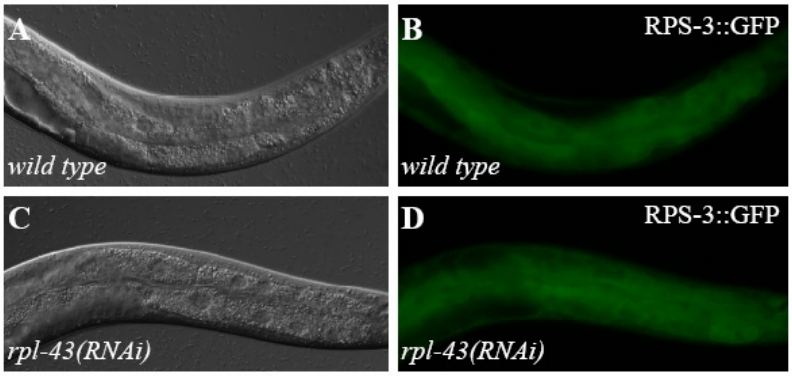
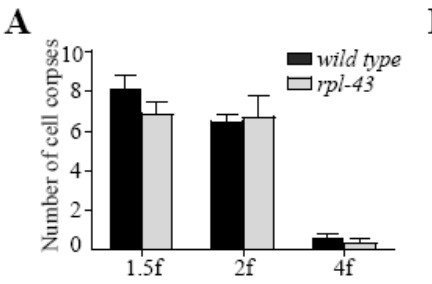


Figure 2. Expression of RPS-3::GFP remains unchanged in *rpl-43* mutants.

4). I feel that the authors did not analyze in detail on why ribosomal deficiency would lead to reduced SQST::GFP clearance. The screen identified several genes inactivations that suppress the SQST-1::GFP clearance defect in rpl-43. But the common theme of all the hits from the screen is that increasing autophagy suppresses the SQST-1::GFP clearance defect suggesting an indirect effect rather than mechanistic explanation of why ribosomal deficiency leads to autophagy defect.

Our results demonstrated that loss of function of *rpl-43* does not impair autophagy activity. First, in *rpl-43* mutants, SQST-1 aggregates accumulate only in larval intestine and are absent in embryos and other larval tissues. In contrast, a large number of SQST-1 aggregates accumulate in most cells in embryos and also in many tissues in larvae in autophagy mutants. Second, upon autophagy induction, the SQST-1 aggregates in *rpl-43* mutants are degraded, but persist in autophagy mutants. This suggests that RPL-43 is not an essential component of the autophagy pathway. Third, degradation of other well-characterized autophagy substrates, including PGL-1, SEPA-1, C35E7.6 and ZK1053.4, is not affected in *rpl-43* mutants (Figure S2A-P in the revised manuscript). Fourth, loss of function of *rpl-43* has no effect on other autophagy-regulated processes, including the removal of apoptotic cell corpses during embryogenesis and also the fate specification of the ASEL neurons in *lsy-6* hypomorphic mutants. Fifth, we examined levels of the *C. elegans* Atg8 homolog LGG-1, which has been widely used to measure autophagy activity. We found that compared to wild-type animals, levels of both lipidated and non-lipidated forms of LGG-1 remain unchanged in *rpl-43* mutants in an immunoblotting assay. Furthermore, GFP::LGG-1 puncta do not accumulate in the intestine in *rpl-43* mutants (Figure S2Q-T in the revised manuscript). Taken together, these results show that the autophagy pathway is not impaired by loss of function of *rpl-43*.



B

Animals expressing *lim6*^{pro}*::gfp* (%) in ASEL

	ASEL	ASER	ASEL ASER	n	*p* value
wild type	0		100	241	
rpl-43	0		100	220	
lsy-6(ot150)	21.2		78.8	288	
rpl-43; lsy-6(ot150)	21.3		78.7	211	=0.97

Figure 3. (A) Loss of function of *rpl-43* has no effect on the number of cell corpses at the 1.5-fold, 2-fold and 4-fold embryonic stages. (B) Loss of function of *rpl-43* has no effect on the specification of ASEL neuron fate in *lsy-6(ot150)* mutants.

5).The authors say that SQST::GFP gets weaker as the worms age; since perturbations in ribosomal translation prolongs the life span of worms (Tavernarakis, Kenyon, and Ruvkun labs) is it possible that the rpl-43 mutant defect in SQST::GFP elimination is due to slowed aging. Did the authors look at old rpl-43 mutants? Since translation is required for synthesis of all proteins, is it possible that the levels of the terminal proteins required for lysosomal clearance of aggregates is lower in rpl-43 mutants? The authors need to look at the protein levels of core autophagic pathway components.

As suggested by the reviewer, we examined the expression of EPG-8::GFP and EPG-1::GFP and found that their expression levels are not evidently altered in *rpl-43* mutants. In our response to #4, we showed that levels of LGG-1 are not affected in *rpl-43* mutants. Therefore, *rpl-43(bp399)* mutants do not exhibit a reduction in synthesis of essential autophagy components. Furthermore, we showed that loss of function of 139 genes promotes autophagy activity in *rpl-43* mutants. Among the identified 139 genes, many of them, are not involved in translation. We also found that *rpl-43(bp399)* mutants have a significantly shortened life-span. Previous studies showed that perturbations in ribosomal translation prolong the life-span of worms. Thus, a reduction of protein synthesis at different levels may results in distinct phenotypes. Perturbation of ribosomal translation at a low level extends life-span, while a severe reduction in translation such as in *rpl-43(bp399)* mutants results accumulation of misfolded proteins. This could be because of a severe reduction in synthesis of factors involved in protein folding in *rpl-43* mutants. Consistent with this hypothesis, we found that RNAi inactivation of genes involved in protein folding (*hsp-110*) causes accumulation of a large number of SQST-1::GFP aggregates in the intestine. The accumulated SQST-1 aggregates in *rpl-43* mutants cannot be efficiently removed by basal autophagy activity. Upon autophagy induction, those aggregates are removed.

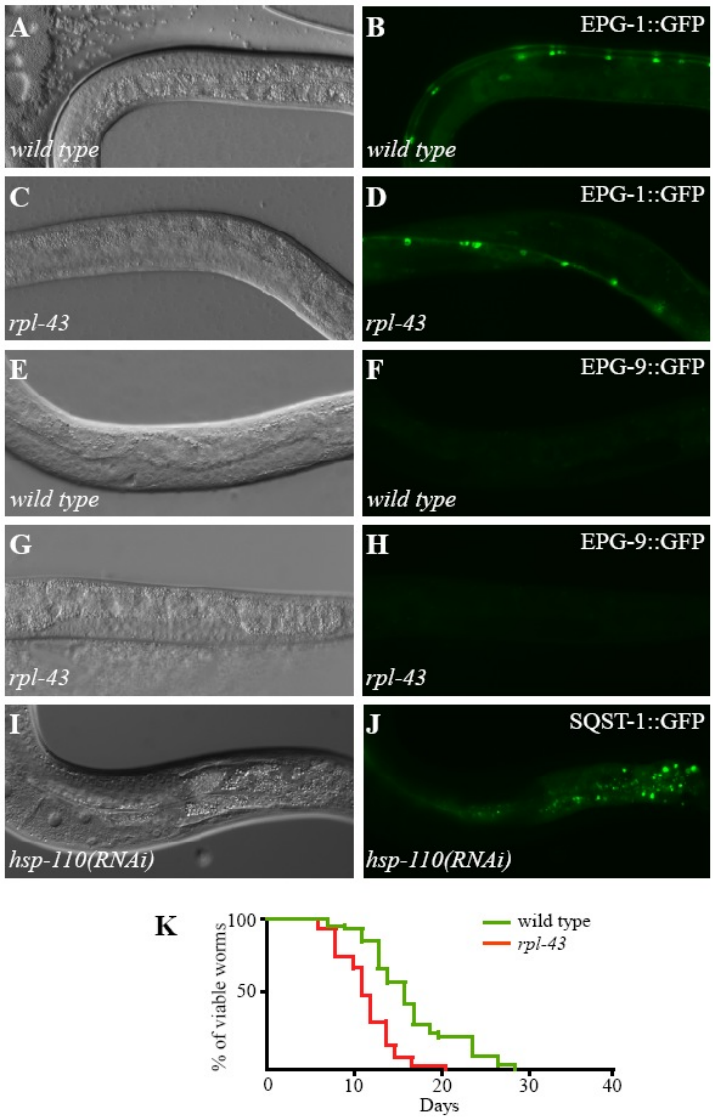


Figure 4. (A-H) Expression of EPG-1::GFP (A-D) and EPG-9::GFP (E-H) remains unchanged in *rpl-43* mutants. (I-J) *hsp-110(RNAi)* animals show accumulation of SQST-1::GFP in the intestine. (K) *rpl-43* mutants have a shortened life-span.

We examined the temporal pattern of SQST-1::GFP in *rpl-43* mutants and autophagy mutants. In autophagy mutants, SQST-1::GFP aggregates strongly accumulated in almost all cells during embryogenesis. At larval stages, a large number of SQST-1::GFP aggregates persist in the intestine throughout the larval stages in strong autophagy mutants. In *rpl-43* mutants, SQST-1::GFP aggregates started to be detected at the L1 stage and then gradually increase in number as development proceeds. The aggregates in *rpl-43* mutants appear to decrease in young adult animals but then increase in older adults, indicating a temporal regulation of autophagy activity during *C. elegans* development. Loss of function of *daf-2*, which slows the aging process, results in elevated autophagy activity and also promotes the removal of SQST-1 aggregates in *rpl-43* mutants. This is consistent with our idea that autophagy activity is tightly controlled by developmental signaling.

6). Figure S1C does not show expression in the larval stages. It shows expression only in the embryo. Please correct second sentence in page 5.

This has been corrected in the revised manuscript (Page 4, line 8).

7). In figure S1E-H, it is hard to see any difference in SQST-1::GFP aggregates in different larval stages.

In the revised manuscript, we included DIC images for each larval stage (Figure S1E-T). In hypomorphic mutants such as *atg-3(bp412)*, SQST-1::GFP aggregates strongly accumulate in early larvae and then decrease as development proceeds. In strong autophagy mutants such as *epg-8(bp251)*, large number of SQST-1::GFP aggregates persist in the intestine. In contrast, intestinal SQST-1::GFP aggregates are not detected in *rpl-43* mutants until the late L1 larval stage and gradually increase as animals develop into late larvae.

Our unpublished data also revealed that not all known *Atg* genes are equally employed in the larvae. Mutants of some essential *Atg* genes show strong accumulation of SQST-1 in embryos and weak defects in late larvae.

8). In all the figure and figure legend, please follow the C. elegans nomenclature to write the genotype. Write the gene name in small letter and allele name in parenthesis (e.g. rpl-43 (bp399)).

This has been corrected in the revised manuscript.

9). Figure S1C2 -F2 shows that PGL-1 expression is unaffected in the rpl-43(bp399) mutant but did not show any data for SEPA-1 as mentioned in the text. Please include that data or remove SEPA-1 from the text. Authors need to use another autophagy substrate expressed in the intestine to show that autophagy is not affected and RPL-43 is not a essential component of autophagy pathway.

Data on SEPA-1 has been included in the revised manuscript (Figure S2E-H). We also demonstrated that in *rpl-43* mutants, the removal of other autophagy substrates, including C35E7.6 and ZK1053.4, are not affected. These results have been included in the revised manuscript (Figure S2I-P). We also demonstrated that loss of function of *rpl-43* has no effect on several other autophagy-regulated processes, including the removal of apoptotic cell corpses during embryogenesis and the fate specification of the ASEL neurons in *lsy-6(ot150)* mutants. Taken together, these results show that RPL-43 is not an essential component of the autophagy pathway.

10). Page 6, second paragraph is confusing. Please rewrite.

This paragraph has been rewritten in the revised manuscript (Page 4, lines 20-22; Page 5, lines 1-4).

11). It is not clear from the text whether genes identified from the secondary screens are excluded from the genes list mentioned in the table 1 and table S1. If those genes identify by secondary screens were not excluded, including that data to the table S1 might be useful.

Genes identified by secondary screens have been excluded from table 1 and table S1. This has been clearly stated in the revised manuscript (Page 6, line 10).

12). In page 10, a brief introduction about LGG-1 and why it is being tested will help the reader to understand better.

As suggested by the reviewer, this has been added in the revised manuscript (Page 5, lines 6-8).

13). In figure 2C, D, G, & H, lin-35 is shown but the text indicates that lin-35, lin-54 and lin-61. Please include the data for lin-54 and lin-64 or rewrite the text accordingly.

Data on *lin-54* and *lin-64* has been included in the revised manuscript (Figure S5A-D).

14). Please briefly explain in the text what we are supposed to see in figure S3L-Q and why authors concluded that SynMuvB gene repress autophagy independent of its other roles during development.

The *lin-35*/Rb synMuvB pathway is involved in specification of vulval cell fate, RNA interference (RNAi) and also soma-germ fate determination. Knockdown of components of *lin-3*/EGF signaling in vulval cell lineage specification (*lin-3*, *let-23* and *let-60*), RNAi pathway (*mut-7* and *dcr-1*), germline specification or P granule genes (*pie-1*, *pgl-1*, *pgl-3*, *glh-1*) did not cause reappearance of SQST-1 aggregates in *lin-35; rpl-43* mutants. These results have been included in the revised manuscript (Figure S5H-M).

The function of the LIN-35 DRM complex in vulval development and soma-germ fate determination is antagonized by chromatin remodeling complexes, including the NuA4 complex, the compass complex and the ISW1 complex. RNAi knockout of genes in these complexes, including *mrg-1*, *gfl-1*, *mes-4* and *isw-1*, did not restore the SQST-1 aggregates in the intestine in *lin-35; rpl-43* mutants. Taken together, these results show that the *lin-35*/Rb synMuvB pathway represses autophagy activity independent of its role in specifying other processes.

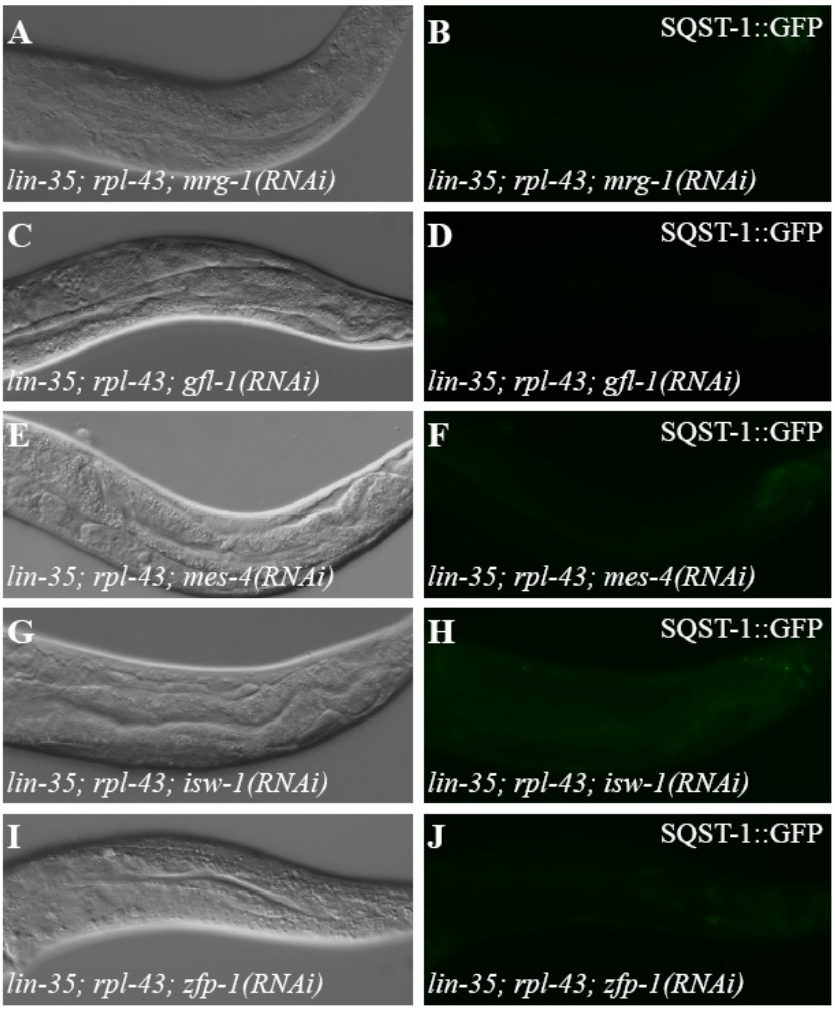


Figure 5. (A-J) Loss of function of components of chromatin remodeling complexes does not cause reappearance of SQST-1::GFP aggregates in *lin-35; rpl-43* double mutants.

15). Page 16, "we found that loss of function of hlh-30....." sentence is complex and hard to understand, please rewrite.

This sentence has been rewritten in the revised manuscript (Page 12, lines 10-13).

16). All the figure including supplementary figures have lots of panels and cited in different places, which makes it harder to follow. It requires reorganization of the figures so as to follow it easily. Split the figure and cite them with the flow of the text.

The figures have been split and cited with the flow of the text in the revised manuscript.

Referee #2:

In an unbiased screen Guo and colleagues identified a c elegans mutant which had accumulations of SQST-1/p62 aggregates in larval intestine. They mapped this mutation to the ribosomal protein subunit RPL-43. Using this mutant background they performed a screen to look for negative regulators of autophagy, that is genes whose loss promoted removal of the p62 aggregates. The result was the identification of 139 genes whose loss promoted autophagy. This group contained representatives of many signalling pathways, some of which are known regulators of autophagy. Finally the authors validated a number of these hits providing a degree of confidence in the results. However, the results are still largely that of a "screen paper" and in some cases the pathways

identified have been implicated in the regulation of autophagy. Therefore in my opinion the paper is not suitable for EMBO reports but for a more speacialized audience.

The regulation of autophagy activity during multicellular organisms is poorly understood. In this study, by using the degradation of SQST-1 aggregates in *rpl-43* mutants as a model, we identified 139 genes which, when inactivated, promote autophagy activity. Autophagy activity is promoted by gene inactivations that induce stress, and by loss of activity of a variety of developmental signaling pathways, including TGF-β, *lin-35*/Rb, *glp-1* and *daf-2*. We also demonstrated that mitochondrial stress induces autophagy activity that is mediated by the transcriptional factor ATFS-1. Our study provides a framework for understanding the role of signaling pathways in regulation of autophagy under physiological conditions.

Referee #3:

1) The authors should provide a clear statement to readers to reduce the concern that the mutations identified (or at least a subset of the mutations) are not simply activating stress that indirectly induce autophagy.

This has been clearly stated in the revised manuscript (Page 13, line 6).

2) One of the most interesting findings in the manuscript is that the new genes identified influence the transcription of autophagy genes, and this presumably influences autophagy. Surprisingly, the TFEB homologue HLH-30 is not required for this autophagy, and this is also interesting. However, the authors fail to consider that this transcriptional response may be controlled by a mechanism identified by the Klionsky lab involving Ume6, Sin3 and Rpd3 (PNAS 2012). Minimally, the authors should reference this paper and potential mechanism. In addition, the authors should provide any evidence from their screen that this may not be the potential mechanism if they have this information.

In yeast, transcription of *ATG8* is repressed under growing conditions by the Ume6-Sin3-Rpd3 complex. Loss of function of *Ume6*, *Sin3* and *Rpd3* results in upregulation of *Atg8* and a concomitant increase in autophagy activity. We showed here that loss of function of synMuvB genes suppresses the accumulation of SQST-1::GFP defects in *rpl-43* mutants. HDA-1/Rpd3 functions along with other components of the NuRD complex, including MEP-1 and LET-418, in the synMuvB pathway in the vulval development. While MEP-1 and LET-418 act in the SUMO-recruited Mec complex, but not in the NuRD complex, for preventing germline gene expression in the soma. *hda-1(RNAi)* worms show no detectable misexpression of germline-specific genes in the soma. In contrast to *hda-1*, loss of function of *sin-3* suppresses the SynMuv phenotype (Cui et al., Plos Genetics, 2006). Thus, *sin-3* and *hda-1* play an opposite role in the synMuv pathway.

As suggested by the reviewer, we investigated the role of *sin-3* and *hda-1/Rpd3* in regulating the autophagy activity in *C. elegans*. A homolog of Ume6 cannot be identified in *C. elegans*. We found that *sin-3(RNAi)* fails to suppress the SQST-1 aggregate accumulation phenotype in *rpl-43* mutants, and does not increase the number of GFP::LGG-1 puncta in the intestine. *sin-3(RNAi)* causes weak accumulation of SQST-1::GFP aggregates in the intestine and greatly enhances the defect in *rpl-43* mutants. RNAi inactivation of *mep-1* and *let-418* suppresses the *rpl-43* defect. *hda-1(RNAi)* causes a lethal defect and the treated animals failed to lay any embryos, preventing us from analyzing its role in suppressing the defect in *rpl-43* mutants.

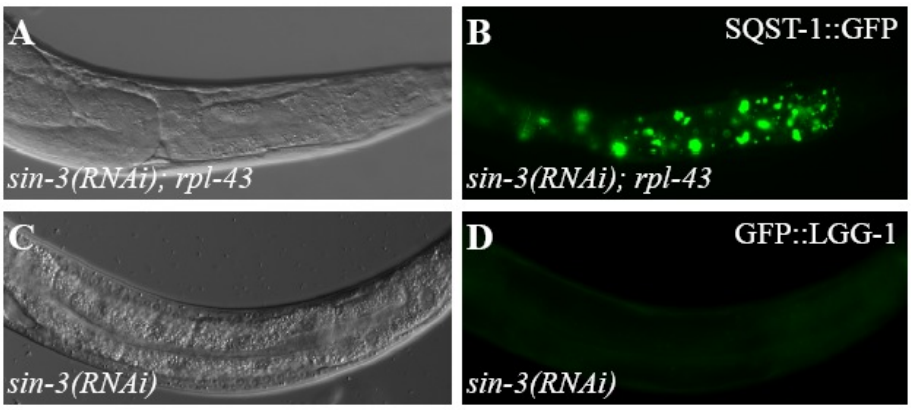


Figure 6. (A-B) Loss of function of *sin-3* enhances the accumulation of SQST-1::GFP in *rpl-43* mutants. (C-D) Loss of function of *sin-3* does not cause formation of GFP::LGG-1 puncta in the intestine.

3) On pp. 5-6 the authors refer to bp399 mutants before they have determined that the mutation is in rpl-43. This is confusing, as they reference an immuno-blot in Fig.1E that is labeled rpl-43 (instead of bp399). This could easily corrected by either changing the labeling of Fig. 1E, or reversing 1E and 1F (and adjusting the text as appropriate).

As suggested by the reviewer, *bp399* was used in Fig. 1A-E in the revised manuscript.

4) Fig. 3 A,B are labeled as being wild type on the figure but are described as being rpl-43 mutants in the text. This must be corrected.

This has been corrected in the revised manuscript.

5) It is difficult to read the labels on the graphs because the font size is extremely small. This is only going to get worse when the figures are reduced, and a solution is needed.

A larger font for the labels on the graphs has been used in the revised manuscript.

2nd Editorial Decision 25 March 2014

Thank you for your patience while we have reviewed your revised manuscript. As I mentioned, referees 1 and 3, who assessed your revised study, now support its publicaiton and have no further comments. I am thus writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once a few minor issues/corrections have been addressed, as follows.

- The materials and methods section is rather succinct. I think it would be helpful for readers if the "Preparation and induction of RNAi bacterial clones" and "Immunofluorescence" sections were moved to the main text.

- The number of animals/samples analyzed is missing in a few figure legends. This needs to be indicated wherever errors and/or statistical information is given. I have noted this on figures 2F,L; 3M; S2T; S6J,K; and S8B2. Please go through all figure legends in detail once more in preparation for publication.

- Lastly, we now encourage the publication of original source data -particularly for electrophoretic gels and blots, but also for graphs- with the aim of making primary data more accessible and transparent to the reader. If you agree, you would need to provide one PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figures and an

Excel sheet or similar with the data behind the graphs. The files should be labeled with the appropriate figure/panel number, and the gels should have molecular weight markers; further annotation could be useful but is not essential. The source files will be published online with the article as supplementary "Source Data" files and should be uploaded when you submit your final version. If you have any questions regarding this please contact me.

After these minor corrections have been attended to, you will receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

Many thanks you for your contribution to EMBO reports.

2nd Revision - authors' response 27 March 2014

1. The materials and methods section is rather succinct. I think it would be helpful for readers if the "Preparation and induction of RNAi bacterial clones" and "Immunofluorescence" sections were moved to the main text.

These two parts have been moved to the main text.

2. The number of animals/samples analyzed is missing in a few figure legends. This need to be indicated wherever errors and/or statistical information are given. I have noted this on figures 2F, L; 3M; S2T; S6J, K; and S8B2. Please go through all figure legends in detail once more in preparation for publication.

The number of animals used for western blotting and RT-PCR analysis and also the statistical information has been included in the revised manuscript.

3rd Editorial Decision 28 March 2014

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Thanks again for your contribution to EMBO reports and congratulations on a successful publication.

