Detailed comments to the critique
Response to Editorial Comments.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

We have proofread the manuscript.

2. Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

We have made the required changes.

1) Lines 151-155 need to be rewritten in the imperative voice and made into steps.

We have rewritten these lines.

2) 2.1: Unclear if this is a title or step.

It is a step. The redaction of this step was corrected to make it clear.

3. Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. ***Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps.*** *There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:*
1) 1.1: unclear how the genes are identified, and what we can film here. Please clarify.
2) 1.3.1: Use g for all centrifuge speeds

1.1 The text was changed from "identify" to "select" the genes that are going to be used by the researcher to inactivate expression by RNAi. The genes to be selected depends on the researcher interest.
1.3.1 The rotational speed has been changed to RCF (g).

4. Please add a one-line space after each protocol step.

We have made the required changes.

5. After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

601 Elmwood Avenue • Box 633 • Rochester, NY 14642
585-273-2409 • 585-273-1450 fax • andrew_samuelson@urmc.rochester.edu

In the revised manuscript, the protocol section is 7 pages long and the filmable content is 3 pages long.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

We checked the article to make sure that all steps have the appropriate sub-steps highlighted.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

We have made the required changes.

3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

We have made the required changes.

4) Notes cannot be filmed and should be excluded from highlighting.

We have made the required changes.

6. JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Thank you pointing this out. We have highlighted (as a comment) how each paragraph of the discussion addresses the aforementioned criteria. We apologize for the length of our discussion, but as Reviewer 1 has noted (point 1 below): this protocol is commonly used by *C. elegans* proteostasis aficionados and we felt that it was important to emphasize the continued relevance of this method and highlight future modification and application of this method. While some details could be moved to the main text (e.g. alternatives to the use of FUDR), we were guided in their placement based on out previous JoVE publication (*Cornwell et al. 2018*) and comments from the reviewers.

7. *Please spell out journal names.*

We apologize for the error. We have applied the JoVE-1 style in Endnote X8:
(https://www.jove.com/files/JoVE.ens).

8. If your figures and tables are original and not published previously or you have already obtained

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figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Figures 1 & 2 are original, generated for this manuscript.
Figure 3 was published in PLoS Genetics in 2017. PLoS states (https://www.plos.org/license):

> "PLOS applies the Creative Commons Attribution (CC BY) license to works we publish. Under this license, authors retain ownership of the copyright for their content, but they allow anyone to download, reuse, reprint, modify, distribute and/or copy the content as long as the original authors and source are cited."

In the Figure legend for Figure 3 we state: "This figure is reprinted from reference [31] with permission via a Creative Commons Attribution (CC BY) license." (lines 401-402).

Response to Reviewer #1.

1. This manuscript is somewhat unsatisfying as it does not present a major advancement of a common protocol. The counting of polyQ foci is performed in numerous publications as a very basic tool to access general proteostasis and may not justify an extra protocol. The authors used two published strains by the Morimoto lab and their own contribution was solely the crossing with the hpk-1 oe line (+ RNAi) that was also already published.

We thank the reviewer for raising this concern. While this protocol is commonly used by *C. elegans* investigators in the proteostasis field, we agreed to contribute this protocol to the collection to: further advocate the continued use of these strains, highlight their versatility and relevance, and to lower the barrier for their use by scientists that are new to the field. As the reviewer is aware, the Morimoto laboratory was one of the labs that founded the field of proteostasis. We enthusiastically advocate for the use and continued relevance of these tools in aging and proteostasis research! We tried to strike a neutral tone in the original submission, but should the reviewer desire, we are happy advocate further and cite more relevant references to give additional credit for the development and use of these tools.

We apologize if the reviewer is under the impression that we are claiming undue credit. We do not seek to claim credit for the advent of this method, only to advocate on its value. Our laboratory's use of these tools to contribute to the field of proteostasis is relatively modest: two publications from our laboratory in PLoS Genetics used these strains (*Johnson et al. 2014 PMID: 24699255 and Das et al. 2017 PMID: 29036198*), in which we identified and characterized novel transcriptional regulators of the proteostatic network. Nonetheless, we believe we have demonstrated sufficient expertise to contribute to this collection.

We chose to adhere to the outlines of the initial solicitation from the guest editors:

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> "*We hope that this Methods Collection will be the definitive record of techniques to set the standard for reproducibility within the community. We are aiming to cover standard and advanced experimental approaches to monitor proteostasis at the molecular, cellular and organismal level in C. elegans.*"

We made the decision to limit our method to standard approaches that have gone through peer review. As we mention in the discussion, we have used a modified higher-throughput version of this method to simultaneously quantify age-associated changes in proteostasis for over 100 RNAi clones that produce premature aging phenotypes and have a manuscript in preparation of these findings. We did not add this to the primary methods section in the original submission because our results are unpublished, but believe this article will help to "pave the way" for our forthcoming study and as currently discussed provides an example of "modifications and future applications", which is in line with JoVE's expectations for a discussion.

Finally, we believe our article is worthwhile based on JoVE's FAQ page:

> "JoVE videos are a unique and effective tool to learn and share a particular technique. We are primarily concerned about publishing highly reproducible methodologies regardless of novelty. These methodologies can be previously published protocols or new techniques. Previously published protocols must be properly cited. Please note that results for experiments can be representative."

2. In addition, the presented data would be more comprehensive if the control lines: unc-54::YFP and rgef-1::YFP would have been analyzed in parallel - this should also be pointed out to the readers. Both control strains were generated by the Morimoto lab as well and are available. So the extra work is negligible.

We thank the reviewer for their comment. Our experiments were done in parallel for both reporters (*unc-54::Q35::YFP* and *rgef-1::Q40::YFP*), but the days we used for foci quantification were different because the polyQ aggregates in the nervous system form later in adulthood (after D4) compared to polyQ aggregates in the muscle (after D1).

3. Some spelling mistakes and typos can be found: e.g. line 196: instead of unc-54::G35::YFP it should read: unc-54::Q35::YFP.

We thank the reviewer and have corrected these mistakes.

Response to Reviewer #2.

1. The method include a section on Synchronization of C. elegans that involves hypochlorite treatment of gravid adults, While this method as stated as many advantages in the ease of getting synchronized population of worms, it causes stress to the animals and can impact regulators of

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proteostasis (Karady et al 2013, J. Vis. Exp. (82), e50840), similar to the use of FUdR that is discussed here.

We thank the reviewer for pointing this out to us, and agree that hypochlorite treatment may impact proteostasis. However, we looked at the *Karady et al.* manuscript in depth and while it states that synchronization via hypochlorite causes stress and affects proteostasis, the manuscript does not actually demonstrate the validity of this claim nor does that manuscript provide a reference. To our surprise, after looking through other papers from the Ben-Zvi lab, and conducting a broader literature search, we could not find actual evidence demonstrating that bleaching affects proteostasis. We would welcome any additional insight to identify the specific relevant study. Nevertheless, we agree that this is a potential concern and have included a cautionary note about hypochlorite treatment in the "Synchronization of *C. elegans*" section and referenced the aforementioned study.

2. If deciding to synchronize animals via egg lay, it is best to do it in two steps. When first getting syncronized day 1 adults (by placing gravid adults for example) and then letting these worms lay eggs - older worms retain eggs and so eggs' age can vary and thus using young adults is important.

We thank the reviewer for their insight and have added that synchronized young adult animals should be used for egg-laying. Also, we have included a note about how old animals may release eggs that are in a more advance developmental stage.

3. The concern about the use of FUdR for proteostasis discussed in paper should be noted in section 3 when its use is described.

We thank the reviewer and have made the requested addition (lines 189-190 in the revised manuscript).

4. Proteostasis collapse in C. elegans has some elements that are gradual, however, its regulation was demonstrated to change within hours (Ref 2 in the manuscript; Labbadia and Morimoto 2015 Mol Cell. 2015 59(4):639-50; Shemesh et al., Aging Cell. 2013 12(5):814-22). This should be noted in the discussion.

We thank the reviewer for their insight and have added the following to the discussion (lines 415-417) and added references:

> "In *C. elegans,* the transcriptional inducibility of multiple forms of stress response rapidly decline within a few hours after the onset of reproduction due to the formation of repressive chromatin marks at stress loci".

5. Lines 413-417 discuss a specific work: daf-2 mutant animals showing increased aggregation but improved proteostasis - relating to Cohen et al Science. 2006 Sep 15;313(5793):1604-10. Moreover, ref 40 discussed here show via RNAi screen that aggregation and toxicity are not correlated.

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We thank the reviewer for catching our unintended oversight. We apologize and have added references for the Cohen et al. and Silva et. al. studies to this portion of the discussion.

6. There is no ref to FRAP data on protein aggregation, please add citation to refs: 15 and 31 in line 439.

We thank the reviewer for our oversight. We found several additional instances where FRAP was mentioned, but not properly referenced. In the revised manuscript we reference Morley 2002, Brignull 2006, and Garcia 2007 throughout.

7. Also, there are biochemical methods to asses aggregation that can also be used and should be mentioned - Nollen et al., Proc Natl Acad Sci U S A. 2004 101(17):6403-8

We thank the reviewer and have added a line to the discussion and this reference.

8. In line 485 the a problem with the full name of GABA.

We have made the required changes.

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