EMBO reports

eIF5A is required for autophagy by mediating ATG3 translation

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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.)

1st Editorial Decision 21 March 2018

Thank you for the transfer of your manuscript from The EMBO Journal to EMBO reports. Given the timeliness and interest of your findings for the autophagy community but in particular from the translation side, we offered publication of a revised manuscript in EMBO reports. Referee 1 had raised concerns that the data do not support a role for eIF5A as an active regulator of autophagy but rather indicate that the identified target ATG3 is hyper-sensitive to the loss of the general translation elongation/termination factor eIF5A due to the amino acid motif DDG. This view was supported by referee 2.

You have now modified the text according to the suggestions of referee 1. Thank you also for providing source data for all Western blots. These will be published together with the manuscript.

I apologize again for the delay in handling your manuscript, but I have now gone through the revised manuscript and all related source data files and I am writing now with an "Accept-in-principle" decision, which means that I will be happy to accept your manuscript once a few remaining editorial issues have been resolved as follows:

- Since the general conclusion has been changed from eIF5A being a "translational regulator" to "translational effector" you might want to review the phrasing in line 235-236 (comment 13, referee 1) and line 237 (comment 14 of referee 1). Moreover, it might also be more accurate the change the phrasing in the paragraph headers since these still emphasize the concept of a more active regulation.

- Regarding comment 28 from referee 1: Is there evidence that the DDG motif is conserved in C. elegans? You might want to comment on this in the discussion.

- Tables EV1 - 3 represent rather complex tables. Please resubmit these as Dataset EV1 to Dataset EV3 and change the nomenclature accordingly in the text and the files.

- Please submit the manuscript as editable Word file.

- Please change the header of the "Competing financial interest" statement to "Conflict of interest"

- The scale bars in Fig. 2A and Fig 4A appear rather thin and might not be well visible at final print size. Please make them a bit thicker.

- Figures 2B, 3B, 3C, 5A, EV2C, EV3C, EV3D: you show the quantification and statistical evaluation of one representative experiment (technical replicates). Please note that it is not accurate to apply statistics to technical replicates since this provides information on technical variability rather than the biological variation. Effectively, n=1 for these experiments. Please provide a quantification of all three independent experiments or alternatively, display data from one experiment as scatter blot showing the individual data points. Since you anyway have data from independent experiments, the first option is preferable.

- Fig. 2E and EV2G: please indicate the number of independent experiments in the respective figure legend.

- Figure EV2H: Please define the nature of the bars and error bars and the number of independent experiments in the figure legend. In general, the number of experiments, the nature of the bars and error bars and the test used to generate p-values must be specified in the figure legends.

- Please define the arrowheads in Fig. 2F in the legend.

If all remaining corrections have been attended to, you will then 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.

Thank you for your contribution to EMBO reports.

1st Revision - authors' response 26 March 2018

Please receive the revised version of our manuscript EMBOR-2018-46072V1. We have now addressed your remaining points as follows:

1. Since the general conclusion has been changed from eIF5A being a "translational regulator" to "translational effector" you might want to review the phrasing in line 235-236 (comment 13, referee 1) and line 237 (comment 14 of referee 1). Moreover, it might also be more accurate the change the phrasing in the paragraph headers since these still emphasize the concept of a more active regulation.
The relevant sentences and paragraph headers have been rephrased accordingly.

2. Regarding comment 28 from referee 1: Is there evidence that the DDG motif is conserved in C. elegans? You might want to comment on this in the discussion.
We have now commented on this point in the discussion (line 425-427).

3. Tables EV1 - 3 represent rather complex tables. Please resubmit these as Dataset EV1 to Dataset EV3 and change the nomenclature accordingly in the text and the files.
The nomenclature in the text and files has been changed accordingly.
In addition, Table EV4 has now been changed to Table EV1.

4. Please submit the manuscript as editable Word file.
The manuscript is now submitted as an editable word file.

5. Please change the header of the "Competing financial interest" statement to "Conflict of interest"

The header has been modified accordingly.
6. The scale bars in Fig. 2A and Fig 4A appear rather thin and might not be well visible at final print size. Please make them a bit thicker.
The scale bars in Figures 2A and 4A have been thickened.

7. Figures 2B, 3B, 3C, 5A, EV2C, EV3C, EV3D: you show the quantification and statistical evaluation of one representative experiment (technical replicates). Please note that it is not accurate to apply statistics to technical replicates since this provides information on technical variability rather than the biological variation. Effectively, n=1 for these experiments. Please provide a quantification of all three independent experiments or alternatively, display data from one experiment as scatter blot showing the individual data points. Since you anyway have data from independent experiments, the first option is preferable.
For figures 5A, EV2C, EV3C, EV3D we now show a quantification of all independent experiments together and we have adjusted the legends accordingly.

For Figures 2B, 3B, 3C we display data from one representative of three experiments, now as scatter blots with individual data points. The reason that we do not combine three independent experiments for these figures specifically, is that the automated quantification of LC3B puncta has varying baseline values between independent biological replicates.

8. Fig. 2E and EV2G: please indicate the number of independent experiments in the respective figure legend.
This is now indicated in the legends.

9. Figure EV2H: Please define the nature of the bars and error bars and the number of independent experiments in the figure legend. In general, the number of experiments, the nature of the bars and error bars and the test used to generate p-values must be specified in the figure legends.
This has now been defined in the legend.

10. Please define the arrowheads in Fig. 2F in the legend.
Arrowheads are now defined in the legend.

2nd Editorial Decision 6 April 2018

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Lisa B. Frankel
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2017-98515

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The data meet the assumptions of the employed statistical tests
Is there an estimate of variation within each group of data?	yes

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
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http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

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http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	LC3 nanotools (0231-100), LC3 CST (2775), GABARAP Abgent (AP1821a), GATE-16 MBL (PM038), ATG3 Sigma (A3231), Vinculin Sigma (V 9131), GAPDH Santacruz (25778), eIF5A Santacruz (sc-390202), Lamin A1 Santacruz (sc-20680), Histone H3 Abcam (ab1791), p62 MBL (PM045), RFP/Cherry Rockland (600-401-379), p-mTOR Cell signalling (2971S), mTOR Cell Signaling (2983), RPL23A Abcam (ab157110), RPS6 CST (2217), RPL10A Santacruz (sc-100827), Hypusine Merck Millipore (ABS1064)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	MCF-7 (Source: ATCC), MCF-7 GFP-LC3 (Source: Marja Jäättelä lab), HEK 293 (Source: ATCC), HeLa (Source: ATCC), BJ (Source: Kristian Helin), MEF (Source: generated in house), MCF-7 GFP-LC3 eIF5A WT (Source: this study), MCF-7 GFP-LC3 eIF5A K50A (Source: this study), MCF-7 GFP-LC3 ATG3 (Source: this study), MCF-7 GFP-eIF5A (Source: this study), MCF-7 GFP-3xFLAG (Source: this study), ATG5 WT and crispr KO Hela (Source: this study). All cell lines are routinely tested for mycoplasma in the lab.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Raw data for the RNA seq are deposited to GEO (GSE104604). Raw data for the proteomics experiments are deposited to PRIDE ProteomeXchange (PXD008874).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Proteomics and RNA seq data are deposited (see above) and additionally available in expanded view section
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA