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Apoptosis Inhibitor 5 is an endogenous inhibitor of Caspase-2

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Transaction Report: Please note that this manuscript was transferred from another journal, where it was initially reviewed. Since the original reviews are not subject to EMBO's transparent review process policy, those reports and the subsequent author response/s cannot be published here.

(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 05 January 2017

Thank you for the submission of your research manuscript to our journal and for your patience while we were assessing it. I have now discussed your manuscript and the transferred referee reports with my colleagues here at EMBO reports and with an additional advisor, who is an expert in the cell death field.

Based on these discussions, I am happy to tell you that we would like to offer publication of the study in EMBO reports. The advisor confirmed that the identification and characterization of a novel negative regulator of caspase-2 represents an interesting and significant finding and considers further experiments that address the biology of this regulation as suggested by reviewer 1 not necessary at this stage and for publication in EMBO reports. Moreover, the expert advisor confirmed that the study is sufficiently controlled and the results are clear and the conclusions supported by the data. S/he agrees thus with reviewer 2 who supported publication of the manuscript in its current form and considered the evidence sufficient.

The advisor however suggests reconfiguring the panels and figures in such a way that their message is conveyed in a more intuitive and clearer way. Moreover, s/he points out that molecular weight markers are lacking for many Western blots. Moreover, reviewer 1 was concerned that Figure S5 lacks a positive control and I agree that such a control should be provided.

1st Revision - authors' response 30 January 2017

The authors made the requested changes and submitted the following letter with their revised manuscript:

Thanks again for quickly reviewing our manuscript and for offering publication in EMBO reports.

We have revised the manuscript as suggested and a new passage on Page 8 has been included: “As CARD domains are also present in other caspases like caspase-9 and caspase-1, we tested if API5 can bind to them. Interestingly, we failed to detect any interaction between API5 in caspase-9 or Caspase-1 (Supplementary Fig. S5a and S5b). Consistently, loss of API5 failed to sensitize cells to Caspase-9 dependent cell death (Fig. 3).”

Please also find attached the author check list and the original blots files.

The Summary and highlights are now embedded in the main text file.

2nd Editorial Decision 07 February 2017

Thank you for the revision of your manuscript and also for the submission of source data, which is very much appreciated. Please accept again my apologies for my delayed response but I have meanwhile gone through your manuscript and noticed several issues that need to be resolved before we can proceed with the official acceptance of your study.

Most of the issues concern the labeling of the figures. In several illustrations the labels are not aligned well and appear to have shifted upwards or sidewards (e.g. 4a, 4d, EV3a etc). This might have happened when you exported your figures to the pdf format? I list some of the cases below, but please review your figures again and correct where necessary.

- Fig. 1c, the labeling of the right part of the blot is not well aligned (API5 monomer, Caspase-2 monomer, Input). Moreover, the label indicating "Control" and "toxin treatment" might not be well visible in the final print size of the figure. The font size is smaller than the font size of the MW marker. What about a 90 degree rotation counter-clockwise of the label and reduce it to 'Control' and ' -toxin'. The concentration and duration of toxin treatment is anyway indicated in the figure legend.

- Fig. 4d: the label of the x-axis appears shifted. It extends into the bars. Moreover, the label that extends currently to the right indicating the identity of the bars could be cut and moved inside the graph (at the upper corner just right of the y-axis). This would enhance the visible separation of this illustration from 4c.

- Fig. 5 looks quite crowded at the moment, which makes it difficult to extract the relevant information. I have taken the liberty to suggest how a slight reshuffling and resizing of the panels could enhance the perceivability of the figure (file attached).

- Please complete the information on data quantification and statistics in the figure legends. The bars and error bars (e.g. SD, SEM) and the test used to calculate the p-values must be provided in the figure legends.

- Thank you for the addition of scale bars to the microscopy image. Please also specify their size in the respective figure legend. Moreover, I noticed that some of the scale bars might not be well visible in final print size, e.g. those in Fig. 4c, because they are rather thin and their appearence seems blurred and does not provide a good contrast to the image.

I am looking forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have any questions or comments.

2nd Revision - authors' response 09 February 2017

The authors made the requested changes and resubmitted their revised manuscript.

3rd Editorial Decision 14 February 2017

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.

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:	Prof. Dr. Krishnaraj Rajalingam
Journal Submitted to:	EMBO Reports
Manuscript Number:	EMBOR-2016-43744V1

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

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 χ2 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.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

USEFUL LINKS FOR COMPLETING THIS FORM

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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?	randomly depending on the experiments
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.	experiments/data were randomly selected without bias
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.	Most of the cases T test/MannWhitney test was performed.A detailed method is provided in the txt and in legends
Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	NA

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).	Antibodies were validated by employing siRNAS
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Routinely Tested for mycoplasma. Cell lines were authenticated by Eurofins by PCR. Data can be shared upon request

* 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 accession codes for deposited data. See author guidelines, under '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	NA
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).	NA
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. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	NA
22. 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

23. 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