EMBO reports

Nuclear envelope rupture and NET formation is driven by PKCα-mediated lamin B disassembly

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EMBO PRESS

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

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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?	Sample size was estimated based on pilot experiments which showed the trends of effects. We also chose the sample size of animal experiments by referring the publications of similar studies in the field.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The primary goal of the animal study was to examine our hypothesis for the NET formation in vivo. For the whole animal studies, 5 mice per group of lmnb transgenic mice vs wildtype littermate controls were used for UVB exposure or not. And 5 mice per group were used for skin tisssue collection and IHC staining.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No data were excluded in the study.
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.	Mice were randomly assigned at the time of purchase, weaning, and caging to minimize any potential bias based on the same gender, age, and genotype.
For animal studies, include a statement about randomization even if no randomization was used.	Mice were randomly assigned to the groups for UVB exposure or not (control). This is described in our Materials and Methods section
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.	To avoid the subjective bias, we used the different approches to confirm the findings. i.e. NETosis assessments were conducted both by fluorometric microplate reader and by flurorescent iamging quantification analysis. For the imaging analysis of cultured cells or IHC analysis, the images of samples were taken by one individual, and image quantificantion analyses were conducted by other individuals.
4.b. For animal studies, include a statement about blinding even if no blinding was done	All the lmnb-TG mice and their littermate wild type controls have healthy skin appearance. The animals were randomly caged according to the gender. The animal caregiver provide the genotyping, age, and gender information, and other investigator randomly pick the mice according to the genotype, age and gender from the record to form the groups that were exposed to UVB or
5. For every figure, are statistical tests justified as appropriate?	We have confirmed that statistical tests were as appropriate for every figure.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, the statistical analysis softwares (GraphPad Prism 6) were used for assumption and nomal distribution analysis, and other statistical analysis
Is there an estimate of variation within each group of data?	Yes, we estimated the variation within each group both for in vitro and in vivo studies.

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

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http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes, the variance between groups is similar.

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).	rabbit anti-PKC alpha (#ab32376, abcam), rabbit anti-PKC alpha (phospho S657) (#ab180848, abcam), goat anti Lamin B(C-20) (#sc-6216, santa cruz), rabbit anti Lamin B1 (#NBP2-48966, novusbio), mouse anti Lamin B1 (#66095-1-Ig, proteintech), mouse anti-Vinculin (#66305-1-Ig, proteintech), rabbit anti-phosphoserine/phosphothreonine/phosphotyrosine (#JA10-49, novusbio), rabbit anti-phosphoserine (#NB100-1953, novusbio), rabbit anti-caspase 3 (#9662, CST), rabbit anti-beta actin (#4967, CST), rabbit anti-Histone H3 (#4499, CST). The antibodies are all from commercial sourses and have been validated by their manufacturers. Antibodies were validated based on the size of band in immuno blotting (molecular weight) in our figures.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Primary human neutrophils were isolated from blood of healthy volunteer donors, by dextran sedimentation followed by Ficoll-Paque PLUS H-1077 as previously described (Denny et al., 2010). Primary mouse peritoneal and bone marrow neutrophils were isolated using neutrophil isolation kit (Miltenyl #130-092-332). Human HL-60 cell line and RAW264.7 cells were purchased from ATCC. All cells tested negative for mycoplasma contamination.

* 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.	Lamin B1 transgenic C57BL/6J-Tg (Lmnb1TG) 1Yfu/J mice (JAX stock #023083) and PKCα deficient mice (B6;129-Prkca tm1Jmk/J mice, JAX stock #009068) were purchased from the Jackson Laboratory. All the mice were housed in a pathogen-free environment, and given food and water ad libitum. Female mice aged 8 weeks were used for UVB exposure experiments. Littermate controls with same sex and same age were used.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All the animal experiments were approved by the Animal Care and Use Committee of Philadelphia VA Medical Center.
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.	We confirmed that we did all the animal experiments accroding to the ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Our group has university approved IRB for conducting human studies
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.	The current study only used healthy volunteers for drawing blood for isolation of human blood neutrophils
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	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).	We confirm to follow this recommendation.
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.	No, our study is not under dual use research restrictions.