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Direct interaction of β-catenin with nuclear ESM1 supports stemness of metastatic prostate cancer

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EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

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?	We used a simple method, the"resource equation" method, to calculate the N number of animal studies. This method is based on ANOVA, it is applicable to all animal experiments. Any sample size, which keeps E between 10 and 20 should be considered as adequate. E can be measured by the following formula: E = Total number of animals – Total number of groups.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We have calculated the sample size for animal studies using resource equation.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No data exclusion criteria were laid out and have thus not been included in the manuscript.
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 randomized based on weight prior to experiment set up.
For animal studies, include a statement about randomization even if no randomization was used.	We have allocated the mice randomly based on their body weight.
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	Blinding of animal study was not applicable.
5. For every figure, are statistical tests justified as appropriate?	All data were statistically analyzed with GraphPad Prism 5 and SigmaPlot 10.0 software. A two-tailed t test was utilized to analyze the difference between two groups. Data were presented as mean ± SD. All findings were considered significant at a P value threshold of < 0.05. Where results of statistical test are shown, *P < 0.05, **P < 0.01 unless otherwise indicated.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	To assess whether the data can meet our assumptions, Student's t test was used to compare independent groups under the assumptions of normal distribution and standard deviation.
Is there an estimate of variation within each group of data?	Variation within each group of data were calculated by Excel. Standard deviation was used to estimate the variance compared to the average of the numbers in each group.
Is the variance similar between the groups that are being statistically compared?	The variance is similar between the groups. We have assessed the variance by using Student's t test to compare independent groups under the assumptions of normal distribution and standard deviation.

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

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

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

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

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).	The following antibodies were used: ESM1 (ab56914, Abcam) for immunohistochemistry staining, ESM1 (LIA-1001, Lunginnov) for immunoblotting, ESM1 (customized antibody) for immunoprecipitation, mouse anti-HA (Sigma), Flag (8146, Cell signaling), GFP (sc8334, Santa Cruz), β-catenin (sc1496, Santa Cruz), TCF4 (2569, Cell signaling), BCL9 (15096, Cell signaling), RelA/p65 (8242, Cell signaling), NFκB/p50 (GTX100772, Genetex), Axin1 (2087, Cell signaling), APC (2504, Cell signaling), LRP6 (2560, Cell signaling), Dvl3 (3218, Cell signaling), CK1 (2655, Cell signaling), GSK3β (GTX59576, Genetex), βTrCP (4394, Cell signaling), IκBα (4814, Cell signaling), IKKα (2682, Cell signaling), IKKβ (2684, Cell signaling).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Human prostate cancer cell lines PC3-P and PC3-M were purchased from Level Biotechnology Inc. 22Rv1-P and 22Rv1-M were gifts from Dr. Ming-Shyue Lee. All the cell lines have been tested 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.	Animals were housed under pathogen-free conditions, according to protocols approved by the Nation Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee, with the IACUC Approval number: 20170354. Male non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice with aged 6-8 weeks were randomized and placed into groups based on weight. All mice were housed in a specific pathogen-free environment and kept in a room with controlled temperature (~23 ℃) and humidity under 12 h light/dark cycle.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal procedures were using protocols approved by the Nation Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee, with the IACUC Approval number: 20170354.
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.	Male non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice aged 6-8 weeks were anesthetized by exposure to 1–3% isoflurane and orthotopically or subcutaneously injected with 10 to 100 µl of 1 × 106 22Rv1-Luc+ cells suspended in Matrigel or PBS into the prostate gland or hypodermis. Ten days later, tumor imaging will be done by i.p. administration of 150 µg/ml luciferin and by using bioluminescence technology (Xenogen IVIS-100 imaging system). Tumor growth and metastases will be monitored once weekly by bioluminescent imaging and external caliper measurements (tumor size = [length × width × height] × 0.52) for 6 weeks. These experiments will last for 10 weeks. Xenografts weights will be determined at the end(10 weeks after implant) of the study when mice were sacrificed. All animal work will be performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the College of Medicine, National Taiwan University. Anesthesia in animal study：Isofluren (inhalation anesthesia) Endpoint of animal study：All experiments will be terminated after cell injected 6-10 weeks or mice body weight loss is up to 20 % in the experiments or the tumor volume exceeds 10% of the body weight or exceeds 20 mm in diameter, or any animal distress symptom is shown, self-damage, cachexia, etc. Euthanasia of animal： Isoflurane/inhaled administered with Oxygen. Cervical dislocation will be performed secondarily to assure euthanasia.

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	We have uploaded the RNA sequencing data to Gene Expression Omnibus (GEO). The data has been deposited in the Gene Expression Omnibus with accession numbers GSE157496.
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. 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