nature portfolio

Corresponding author(s): Kenkichi Masutomi

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|---|---|
| ☐ | ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | No software was used. |
| Data analysis | The following software was used to analyse the data:<br>- Mageck algorithm (Li et al. 2014)<br>- Prism version 7.03 (GraphPad Software)<br>- Image J version 1.52a<br>- Trim Galore! (version 0.6.4_dev)<br>- Trimmomatic (version 0.39)<br>- cutadapt (version 2.10)<br>- STAR (version 2.7.9a)<br>- featureCounts (version 2.0.1)<br>- DESeq2 package (version 1.20.0)<br>- MBCluster.Seq package (Si et al. 2014)<br>- clusterProfiler package (Yu et al. 2012) |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All sequencing datasets have been deposited at the Gene Expression Omnibus (GEO) with accession codes GSE226966 and GSE226994. All the data supporting the findings of this study are available within the article and its supplementary information files.

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | Not applicable |
| Population characteristics | Not applicable |
| Recruitment | Not applicable |
| Ethics oversight | Not applicable |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | Sample size was based on experimental feasibility, sample availability, and N necessary to obtain definitive results. |
| Data exclusions | No data were excluded from our study. |
| Replication | All the biological experiments were repeated three times or twice and reproduced. |
| Randomization | Mice were assigned randomly to experimental and control groups for xenotransplantation. For experiments using culture cells, randomization is not relevant as all experiments were performed using the same experimental conditions. |
| Blinding | The investigators were not blinded, since experiments were performed by the same investigator. However, all experiments were independently performed multiple times. All results are based on quantitative and objective measures. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

# Antibodies

Antibodies used

The following commercial antibodies were used:
anti-phospho-SR proteins mouse mAb (1H4, Merck, MABE50, 1:800 for IB),
anti-SF3B1 mAb (clone 16, MBL Co., Ltd., D221-3, 1:1000 for IB)
anti-U2AF1 polyclonal Abs (pAbs) (MBL Co., Ltd., RN085PW, 1:1000 for IB)
anti-histone H2A rabbit pAbs (Cell Signaling Technology, 2578S, 1:1000 for IB)
anti-BRCA2 mouse mAb (Ab-1, Merck, OP95, 1:250 for IB)
anti-dsRNA mouse mAb (J2, SCICONS, 10010200, 1:200 for IB),
anti-RNA:DNA hybrid mouse mAb (S9.6, Merck, MABE1095, 1:5000 for IB and 1:100 for IP),
anti-dsDNA mouse mAb (HYB331-01, Santa Cruz Biotechnology, sc-58749, 1:5000 for IB),
anti-hTERT sheep pAbs (abx120550, Abbexa Ltd., 1:50 for IP),
anti-SC-35 mAb (against SRRM2) (SC-35, Merck, S4045, 1:2000 for IF),
anti-gammmaH2AX rabbit mAb (20E3, Cell Signaling Technology, 9718, 1:500 for IF),
anti-PML rabbit mAb (EPR16792, abcam, ab179466, 1:500 for IF),
anti-TRF2 goat pAbs (Novus Biologicals, NB100-56694, 1:250 for IF),
Alexa Fluor 488 goat anti mouse IgG (Thermo Fisher Scientific Inc., A-11029, 1:1000 for IF)
Alexa Fluor 568 goat anti mouse IgG (Thermo Fisher Scientific Inc., A-11036, 1:1000 for IF)
Mouse TrueBlot ULTRA Anti-Mouse Ig HRP (Rockland, 18-8817-31, 1:4000 for IB)
Anti-Mouse IgG, HRP-Linked Whole Ab Sheep (Jackson Immunoresearch Laboratories, NA931, 1:5000 for IB).

The following antibodies were generated in house and used in this study:
anti-hTERT mouse mAb (clone 10E9-2, 1:100 for IF and IP) (Maida et al. 2014),
anti-hTERT mouse mAb (clone 2E4-2, 1:200 for IB) (Maida et al. 2016),
anti-p-hTERT mouse mAb (clone TpMab-3, 1:200 for IB and 1:100 for IF) (Matsuda et al. 2022),
anti-p-hTERT mouse mAb (clone TpMab-35, 1:200 for IB and 1:100 for IF) (generated in this study).

Validation

All of the commercial antibodies were validated to recognize the relevant human protein, as specified on the relevant catalog pages on the suppliers' websites. Detailed information about usage, validation, and citation is available from the supplier's websites:
anti-phospho-SR proteins mouse mAb: https://www.merckmillipore.com/JP/ja/product/Anti-Phosphoepitope-SR-proteins-Antibody-clone-1H4,MM_NF-MABE50
anti-SF3B1 mAb: https://ruo.mbl.co.jp/bio/dtl/A/index.html?pcd=D221-3
anti-U2AF1 pAbs: https://ruo.mbl.co.jp/bio/dtl/A/?pcd=RN085PW
anti-histone H2A rabbit pAbs: https://www.cellsignal.jp/products/primary-antibodies/histone-h2a-antibody-ii/2578
anti-BRCA2 mouse mAb: https://www.merckmillipore.com/JP/ja/product/Anti-BRCA2-Ab-1-Mouse-mAb-2B,EMD_BIO-OP95
anti-dsRNA mouse mAb (J2): https://search.cosmobio.co.jp/view/p_view.asp?PrimaryKeyValue=25052332&ServerKey=Primary&selPrice=1
anti-RNA:DNA hybrid mouse mAb (S9.6): https://www.merckmillipore.com/JP/ja/product/Anti-DNA-RNA-Hybrid-Antibody-clone-S9.6,MM_NF-MABE1095
anti-dsDNA mouse mAb (HYB331-01): https://www.scbt.com/ja/p/ds-dna-marker-antibody-hyb331-01
anti-hTERT sheep pAbs: https://www.abbexa.com/htert-antibody-1
anti-SC-35 mAb (against SRRM2): https://www.sigmaaldrich.com/JP/ja/product/sigma/sab4200725
anti-gammmaH2AX rabbit mAb: https://www.cellsignal.jp/products/primary-antibodies/phospho-histone-h2a-x-ser139-20e3-rabbit-mab/9718
anti-PML rabbit mAb: https://www.abcam.co.jp/products/primary-antibodies/pml-protein-antibody-epr16792-ab179466.html
anti-TRF2 goat pAbs: https://www.novusbio.com/products/trf-2-antibody_nb100-56694
Alexa Fluor 488 goat anti mouse IgG: https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11029
Alexa Fluor 568 goat anti mouse IgG: https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11036
Mouse TrueBlot ULTRA Anti-Mouse Ig HRP: https://www.rockland.com/categories/trueblot/mouse-trueblot-ultra-anti-mouse-ig-hrp-18-8817-30/
Anti-Mouse IgG, HRP-Linked Whole Ab Sheep: https://cdn.cytivalifesciences.com/api/public/content/digi-11297-pdf?_gl=1*1k8t2p5*_gcl_au*MjAxNzM5NTI5MC4xNzEyNzE1MDY4

Anti-hTERT mAbs (clones 10E9-2 and 2E4-2) and an anti-p-hTERT mouse mAb (clone TpMab-3) have been validated and used in the following studies: Maida, Y. et al. Mol Cell Biol, 2014, Maida, Y. et al. Mol Cell Biol, 2016, and Matsuda, Y et al. J Pathol, 2022.
An anti-p-hTERT mouse mAbs (clone TpMab-35) was validated in this study. Antibody specificity is validated by western blot and immunofluorescence analyses with negative controls in the figures.

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | The following cell lines were purchased:<br>VA-13 (RCB0251, RIKEN BRC),<br>Saos-2 (RCB0428, RIKEN BRC),<br>HeLa (CVCL_0030, ATCC),<br>Huh-7 (JCRB0403, JCRB Cell Bank),<br>TOV-21G (CVCL_3613, ATCC),<br>Capan-1 (HTB-79, ATCC),<br>HEK-293T (293T) (CVCL_0063, ATCC).<br><br>The FANCF transduced human ovarian cancer cell line TOV-21G FANCF and the BRCA2-restored Capan-1 clones (C2-1 and C2-2) were previously generated in house:<br>TOV-21G FANCF (Taniguchi et al. 2003),<br>The BRCA2-restored Capan-1 clones (C2-1 and C2-2) (Sakai et al. 2008). |
| Authentication | The cell lines purchased were verified by the commercial supplier. All cell lines were authenticated in our lab by morphological examination using microscope. |
| Mycoplasma contamination | Cell lines were routinely tested and confirmed to be free of Mycoplasma contamination. |
| Commonly misidentified lines<br>(See ICLAC register) | No commonly misidentified cell lines were used. |

## Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

| | |
|---|---|
| Laboratory animals | Xenotransplantation of human cell lines was performed in NOD/SCID mice. Generation of antibody was performed in BALB/c mice. Experimental mice were typically observed for 3-5 weeks. All mice were closely monitored by investigators and facility technicians every two or three days. |
| Wild animals | The study did not involve wild animals. |
| Reporting on sex | Six-week-old male mice were used as recipients for xenotransplantation. |
| Field-collected samples | The study did not involve samples collected from the field. |
| Ethics oversight | All animal experiments were performed in accordance with the study protocols approved by the Animal Care and Use Committee of Kanazawa University and Tohoku University. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.