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Reporting Summary

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

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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 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
Software and code

Policy information about <u>availability of computer code</u>

Data collection

For data collection: ZEN LE software 2 (Carl Zeiss®), AxioVision, Rel. 4.9.1 (Carl Zeiss®), EVOS FLc Cell Imaging System®, Versamax microplate reader at 490 nm (Molecular devices®), IncuCyte™ Zoom software

Data analysis

Data analysis was carried out with Microsoft Excel® 2010, Imaris software 8.2.0 (Bitplane®), IncuCyte™ Zoom software, Adobe Photoshop CC® using their standard functions/modules.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about <u>availability of data</u>

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

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. If needed, additional information is available from the corresponding author upon reasonable request.

Field-spe	ecific r	eporting				
Please select the o	ne below tha	it is the best fit for y	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 w	ith all sections, see <u>nature</u>	re.com/documents/nr-reporting-summary-flat.pdf			
Life sciences study design						
All studies must dis	sclose on the	se points even wher	en the disclosure is negative.			
Sample size	The chosen s	sample size are based on the numbers used for previous publications, which is most optimal to generate statistically significant				
Data exclusions	No data wer	re excluded for statistical analyses.				
Replication	All attempts	s at replication were successful.				
Randomization	Randomizati	tion was not relevant in this study as there was no comparison of cohorts.				
Blinding	Blinding was	ding was not relevant to the study because all cells/samples were analyzed in the same way.				
We require informati	on from autho	ors about some types o	naterials, systems and methods of materials, experimental systems and methods used in many studies. Here, indicate whether each material, are not sure if a list item applies to your research, read the appropriate section before selecting a response.			
Materials & experimental systems		l systems	Methods			
n/a Involved in th	•		n/a Involved in the study			
Antibodies			ChIP-seq			
Eukaryotic cell lines			Flow cytometry			
Palaeontology			MRI-based neuroimaging			
Animals and other organisms						
Human research participants						
Clinical dat	ta					
Antibodies						
Antibodies used		anti-RhoC (sc-393090 anti-Myc (sc-764) dilu anti-GST (sc-138) dilu (11814460001) dilute	diluted 1:1000 (Santa Cruz Biotechnology), anti-RhoA (sc-418) diluted 1:100 (Santa Cruz Biotechnology), 0) diluted 1:100 (Santa Cruz Biotechnology), anti-GST (sc-138) diluted 1:100 (Santa Cruz Biotechnology), uted 1:100 (Santa Cruz Biotechnology), anti-RhoC (sc-393090) diluted 1:100 (Santa Cruz Biotechnology), atted 1:100 (Santa Cruz Biotechnology), anti-GFP ed 1:100 (Roche®), anti-Flag (F3165) diluted 1:100 (Sigma®), KillerRed (AB961), diluted 1:1000 (Evrogen®), diluted 1:10000 (Abcam®), anti-mouse (sc-2061) diluted 1:10000 (Santa Cruz Biotechnology), anti-mouse			

(D3V2A) diluted 1:1000 (Cell Signaling Technology®). For Immunofluorescence : anti-RhoA (sc-179) diluted 1:50 (Santa Cruz Biotechnology), anti-Myc (sc-764) diluted 1:50 (Santa Cruz Biotechnology), Alexa Fluor 488 or 546 diluted 1:500 (Molecular Probes_Thermo Fisher Scientific®). For RhoA activity: anti-RhoA diluted 1:50 (Cytoskeleton®), HRP–conjugated secondary antibody diluted 1:250 (Cytoskeleton®)

Validation

Antibody validation was deferred to the manufacturers and was supported by multiple publications.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

ARPE-19 a human retinal pigment epithelial cell line was purchase from ATCC (#CRL2302). The TOV-112D and TOV-1946 ovarian cancer cell lines were respectively derived from a high-grade endometrioid tumor and a high-grade serous carcinoma, and were used to downregulate the expression of Ran and RhoA. Both cell lines are known to express high levels of Ran and have been previously described by our lab as indicated in Methods section.

Authentication

TOV-112D and TOV-1946 ovarian cancer cell lines were used with further authentication: Short Tandem Repeat (STR) analysis

Mycoplasma contamination

Cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines (See <u>ICLAC</u> register)

No commonly misidentified cell lines were used in this study.