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Corresponding author(s): Cédric Blanpain

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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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 *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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Microscope (Axio Imager M2) images were generated using Axiovision 4.8.2 software (Carl Zeiss) The confocal (LSM 780) images were generated using the ZEN 2 software (Carl Zeiss). Flow cytometry data analysis and cell sorting was performed on a FACSAria sorter using the FACS DiVa software (BD Biosciences).
Data analysis	Tumor burden was calculated using AxioVision 4.8.2 software (Carl Zeiss). The confocal imaging data-sets generated were analysed with the ZEN 2 software (Carl Zeiss). Flow cytometry data analysis was performed on a FACSAria sorter using the FACS DiVa software (BD Biosciences). Quantitative PCR analysis was performed using Light Cycler 96 and Light Cycler 96 SW 1.1 software (Roche). Mouse Smo gene was sequenced using ABI 3730XL sequencer and SnapGene version 4.1.3 was used for the analysis. t-test were performed using Excel and Prism (version 7). p-value calculation for Venn diagrams was calculated using R software. The GSEA program was downloaded from the BROAD institute website (http://www.broadinstitute.org/gsea/).We used the GSEA

preranked option with standard parameters of weighted enrichment score calculation to run the GSEA against a user-supplied fold-change-ranked list of genes.Results of the enrichment analysis were plotted using R software.

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 upon request. 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 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data associated with this study have been deposited in the NCBI Gene Expression Omnibus under accession number GSE117458 (microarray analysis).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No statistical methods were used to predetermine sample size. All experiments were repeated at least three times with similar results, except for microarray for which experiments were repeated twice.
Data exclusions	No animals were excluded from the analysis.
Replication	All experiments were repeated at least three times (3 biological independent experiments/mice) showing similar results, except for microarray analysis for which experiments were repeated twice (2 biological independent experiments/mice). All attempts at replication were successfull and are shown, n is described in legends.
Randomization	The experiments were not randomized. The mice included in this study were selected according to their correct genotype. The mice received tamoxifen injection/s when they were 1.5 months, and 8 weeks after they all presented basal cell carcinomas (similar tumor burden). Sex-specific differences were minimized by including similar number of male and female animals.
Blinding	The investigators were not blinded to allocation during experiments and outcome assessment. Blinding was not possible as the same investigator processed the animals and analysed the data.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Vismodegib/GDC0449 (Genentech,San Francisco, US) and LGK-974 (Novartis,Bâle, Switzerland).

Antibodies

Antibodies used

For immunofluorescence and ISH the following primary antibodies were used:
anti-B4-integrin (Rat, 1:200, BD, clone346-11A, ref.553745, lot.5239648), anti-GFP (chiken ,1:3000,Abcam,ref.ab13970, lot.236651-23), anti-Active Caspase-3 (rabbit , 1/600, R&D, ref.AF835, lot.CF23517031), anti-Ki67 (rabbit, 1/1000, Abcam, ref.ab15580, lot.GR3198193-1), anti-Lrig1 (goat, 1/500, R&D, ref.AF3688, lot.ZPH0217111), anti-Lef1 (rabbit, 1/100, Cell Signaling, ref.2230), anti-Lhx2 (goat, 1/500, Santa Cruz, sc-19344, lot.K1615), anti- Cux1 (rabbit,1/6000, Santa Cruz), anti-Tbx1 (rabbit, 1/100, Invitrogen) anti-Alcam (goat, 1/1000, Novus, ref.FAB1172F, lot.AASW0111121), anti-Keratin10(rabbit, 1/3000,Covance,ref.PRB-159P-0100), anti-Keratin1 (rabbit, 1/3000, Covance, ref.PRB-165P-0100),anti-Keratin-14 (rabbit, 1/3000, Thermofisher), anti-scd1(goat, 1/500, Santa Cruz, ref.sc14719,lot.H2610), anti-adipophilin (guinea pig,1/5000, Fitzgerald, ref.20R-AP002, lot.P17030911), anti-BrdU(mouse, 1/200, BD, clone 3D4,ref. 560209, lot.4293550)anti-MKL1 (rabbit,1/200, Sigma, ref.HPA030782,lot.C106712) andanti-ARL13b (rabbit, 1:2000,ref.17711-1-AP, Proteintech, lot.49885). The following secondary antibodies were used: anti-rabbit, anti-rat, anti-goat, antiguinea pig, anti-chicken, conjugated to AlexaFluor488 (Molecular Probes) and to rhodamine Red-X and Cy5 (JacksonImmunoResearch).

Validation

As positive control tissues with known expression of the marker were used. As negative control staining omitting the primary was performed.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

K14-CreER transgenic mice were kindly provided by E. Fuchs, Rockefeller University, USA. Ptch1fl/fl mice and Rosa/SmoM2-YFP 3 mice were obtained from the JAX repository. Lgr5-DTR-GFP mice were kindly provided by Genentech (San Francisco, USA).Tp53fl/fl mice were obtained from the National Cancer Institute at Frederick. Mice included in this study were from mixed genetic background.
Female and male animals have been used for all experiments and equal animal gender ratios have been respected in the majority of the analysis. Mice were included in the study when they were 1.5 months old when the tamoxifen was administred.
Mice colonies were maintained in a certified animal facility in accordance with European guidelines. The experiments were approved by the local ethical committee (CEBEA) under protocols #483 and #632.The study is compliant with all relevant ethical regulations regarding animal research. Mice used in this study were of mixed background.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics

The study includes analysis of skin biopsies from 4 patients :
Patient 1 (57 years, man, superficial BCC, 9 months of treatment, relapse following treatment discontinuation)
Patient 2 (82 years, man, nodular and infiltrative BCCs, 7 months of treatment, relapse following treatment discontinuation)
Patient 3 (84 years, woman, infiltrative BCC, 9 months of treatment, absence of relapse following treatment discontinuation)
Patient 4 (80 years, men, multiple BCCs, 13 months of treatment, relapse following treatment discontinuation and currently under treatment).

Recruitment

Patients presenting locally advanced Basal Cell Carcinoma that received vismodegib treatment.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Sample preparation was performed as described in Jensen et al, Nature Protocols 2010. Briefly, control Lgr5-DTR mice and K14-CreER/Ptch1fl/fl/Lgr5-DTR bearing BCCs and vismodegib-persistent lesions were sacrificed. Back skin was placed in a petri dish and a sterile scalpel was used to remove the adipose tissue and muscle. The epidermal tissue was incubated in thermolysin (Sigma) for 1h at 37oC and after a scalpel was used to separate epidermis from the dermis. The epidermal tissue was chopped in little pieces and resuspended in PBS supplemented with 5% chelated fetal calf serum and filtered in 70um and 40um cell strainers (BD).

	Cells were stained using the anti-Lrig1 (goat polyclonal, R&D) followed by the secondary donkey anti-Goat-Alexa 647 (Invitrogen).
Instrument	FACSAria III (BD Bioscience)
Software	FACSDiva Software 8.0.1 (BD Bioscience)
Cell population abundance	The proportion of the relevant cell populations is depicted in Extended Data Figure 2.m-n.
Gating strategy	Living cells were selected by forward scatter, side scatter, doublets discrimination and by Hoechst dye exclusion. Tumor cell subpopulations were selected based on the expression of Lgr5-GFP and Lrig1-Alexa647.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.