nature portfolio

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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 *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 *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	FACS data was obtained on FACS LSR II Fortessa (BD Bioscience). Confocal images were obtained on point laser scanning Zeiss AxioObserverZ1 LSM800 microcope. NGS libraries were sequenced on a Illumina NextSeq 2000 machine.
Data analysis	FACS data analysis: Flowjo (v10.9.0), IF image analysis: ZEN Black (v.2.3) or Zen Blue(v2.3 SP1) Data processing, statistical analysis and visualization: RStudio with R(v4.2.2) CRISPR-Cas9 screens analysis: read processing was done with samtools (v1.10), fastx-toolkit(v0.0.14), bowtie(v2.3.5.1); analysis and visualization was done using Mageck (v0.5.9) and R(v4.2.2).

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

Data availability: All primary and processed data of the genome-wide CRISPR-Cas9 screen are uploaded to Gene Expression Omnibus (GEO) database under the series GSE262309. As the screen was performed in two batches. Batch 1 is available under GSE262307 and Batch 2 under GSE262308. Additionally, source data are provided with this paper. Mutation profiles from all available human melanoma studies are accecible via cBioportal https://www.cbioportal.org/.
Code availability: Code used for analyzing the genome-wide screens in this study are available at Githhub.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	This study does not involve human participants.
Reporting on race, ethnicity, or other socially relevant groupings	This study does not involve human participants.
Population characteristics	This study does not involve human participants.
Recruitment	This study does not involve human participants.
Ethics oversight	This study does not involve human participants.

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	Complexity of the in vitro CRISPR-Cas9 screen was kept above 500 cells per sgRNAs during all the stages of the experiment. Number of mouse cohorts were decided by the known mean of engrafted cells per mouse, multiplied by the number of sgRNAs in the library. In vitro cell numbers were decided by the number of mice that would have to be injected, multiplied by the number of cells to inject and additional the number of cells needed for the in vitro screen. Always taken into account cell loss and therefor 10% extra.
Data exclusions	No data excluded.
Replication	Replicate were generated where indicated. Screens are performed in individual mice, when mice died prior to screening endpoint they were not included in the analysis. Every screen was performed in triplicate. A total of 143 mice for the genome-wide screen were used. TripleColour-StAR experiments are performed in triplicate, where all attempts were successful.
Randomization	Internal controls made randomization unneccessary as the process itself is random.
Blinding	No blinding, however mice were randomly allocated to replicate groups, where after blinded data collection was not performed. But automated analysis using scripts avoided for any bias towards any group.

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Cells were stained with primary antibodies (TagBFP: NanoTag Biotechnologies, N0502-CUSTOM-FluoTag-X2 anti-TagBFP Dylight 405, EGFP: Abcam, A13970, dTomato: Biorbyt, ORB11618) and secondary antibodies (Invitrogen, A78948, A11057). Nuclear staining was performed with To-Pro-3 (Invitrogen, T3605).

Validation

Antibodies used are from commercial sources, which provided validation data on their website and these antibodies were previously used in published studies.
TagBFP: https://nano-tag.com/product/fluotag-x2-anti-tagfp/
EGFP: https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab13970#tab=datasheet
dTomato: https://www.biorbyt.com/mcherry-antibody-orb11618.html
Dilution of antibodies were determined in an antibody titration series to fit the experimental goals.
Cells not expressing antigen were used as a negative control to assess antibody specificity and background fluorescence.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Mouse embryonic stem cells (mESC, AN3-12)
Yumm1.7 450R, Yumm, A375R and A375 melanoma cells were kindly gifted by Anna Obenauf, originally purchased from ATCC, and made resistant.
Lenti-X, Plat-A, 4T1, 4T07, B16F0, B16F1, B16F10, Hepa1.6, EMT6, EO771 and CT26 cells, in house available, originally purchased from ATCC.

Authentication

Cell lines were authenticated visually and phenotypically (e.g. inhibitor resistance).

Mycoplasma contamination

All cell lines were tested every 2 weeks and no Mycoplasma contamination was detected during the period of the study.

Commonly misidentified lines
(See ICLAC register)

Not used in this study

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

B6(Cg)-Rag2tm1.1Cgn/J or B6.SJL-Rag2tm1FwaPtprca mice (Rag2−/−), C57BL/6J and, BALB/cJ. 5- to 16-week-old male/female

Wild animals

Not used in this study

Reporting on sex

In the earlier experiments sex was taken into account, but results did not differ between male and female. Therefore for this study no distincton was made.

Field-collected samples

Not used in this study

Ethics oversight

lAl animal experiments were carried out according to Austrian Veterinary Authorities approved project licenses (IMBA license holders).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Cells were trypsinized, and single cell suspension for analysis was prepared in tubes. Tumors were harvested, dissected into small pieces, and incubated with Collagenase IV (2mg/mL, Worthington, LS004188) and DNaseI (0.2mg/mL, Sigma, 4536282001) for 1hr at 37°C. Cells were then filtered (70um), cultured overnight to isolate tumour cells, and subsequently analysed via FACS.
Instrument	FACS LSR II Fortessa (BD Bioscience)
Software	FACS data analysis: Flowjo (v10.9.0)
Cell population abundance	in vitro samples: total 100,000 cells counted, live cells consisted of 65-80% of whole population, gated TagBFP and dTomato positive populations were more than 12,000-30,000 cells (12-30% of whole population) per each population in control samples. in vivo samples: 180,000-620,000 cells were counted, 13-72% cells were considered live population, gated TagBFP and dTomato positive populations were more than 6,000-26,000 cells (3-8% of whole population) per each population in control samples.
Gating strategy	Live cells were gated using FSC-Area/SSC-Area. Single cells were gated SSC-Area and SSC-hight. miRFP703-/+ cells were gated using 640nm laser and 730/45nm filter, TagBFP+ cells were gated using 405nm laser and 450/50nm filter, dTomato+ cells were gated using 561nm laser and 582/15nm filter, and EGFP-/+ cells were gated using 488nm laser and 530/30nm filter.

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