nature research

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Last updated by author(s): Jun 16, 2020

Reporting Summary

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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	Data were acquired using next generation sequencing on HiSeq2500 or on MiSeqNano. WES was performed by Macrogen using a NovaSeq6000. BD FACSDiva Software Version 8.0.1 was used to acquire FACS data. FlowJo 10.4 was used to analyse FACS data.
Data analysis	Analysis of QuantSeq data was performed with an in-house established pipeline: Adapter and polyA sequences are clipped with bbduk (v38.06). Abundant sequences (iGenomes GRCm38) are removed with bbmap (v38.06). Cleaned reads are aligned against the genome (GRCm38, primary_assembly) with STAR (v2.6.0c). Reads are counted towards their corresponding gene (Ensembl 94) with featureCounts (v1.6.2). Differentially expressed genes are detected with DESeq2 (v1.18.1). Coverage tracks are created with deepTools (normalized BPM; v3.0.2). GO-terms were extracted, and data was visualized using pcaExplorer and pheatmap (package version 1.0.12). Upstream mediator analysis was performed using the Ingenuity Pathway Analysis software (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis). Additional heatmaps were generated using Heatmapper. Venn diagrams were generated using Venny 2.1. Bioinformatic analysis of exome sequencing data. Analysis of exome sequencing data was performed using the bcbio (https://github.com/bcbio/bcbio-nextgen) community resource using the following tools: alignment with bwa, SNV variant calling with an ensemble of samtools and varscan retaining only variants passing the filtering in both tools. Common SNVs in all samples were determined using vcftools and excluded from all further analysis. Low-complexity regions (LCRs) were called analogous to Li H., Biofinformatics 2014 (10.1093/bioinformatics/btu356) on the mouse genome using SeqClean mdust (ftp://occams.dfci.harvard.edu/pub/bio/tgi/software/seqclean/) and all SNVs in LCRs were excluded from all further analysis. Variants were annotated using Annovar and the final annotated variant set was analysed and visualized using maftools. The mouse oncoKB list was created based on the OncoKB human cancer gene list (downloaded September 26, 2019) and mapping mouse orthologs using the biomaRt R package. Bioinformatic analysis of CaTCH barcoding data. The reads were processed in Python (v3.6.7) using Biopython (v1.70). The reads were scanned for their barcode using the regular expression CGCCG[ATGC]{7}GCC[ATGC], and the sample and genotype tags were then identified at a fixed offset from the matched barcode, as per the design of the construct. A counter was kept for each distinct barcode

sequence encountered. To prevent artificial inflation of the barcode counts due to random sequencing errors, barcodes with a Hamming distance of 2 or less (i.e. up to 2 substitutions) were subsequently assumed to represent the same barcode and their counts were combined. The barcode counts where then analysed in R (v3.5.1) with help of the data.table (v1.12.2) and tidyr packages. They were visualized using the R packages ggplot2(v3.2.1) and ggrepel(v0.8.1). Numeric IDs were assigned to the barcodes in decreasing order of absolute abundance (count) in the reference sample. The proportional abundance of each barcode in each sample was calculated as the fraction of its count over the library size. Only barcodes with at least 50 reads were considered and barcodes representing more than 1% of the assigned reads were highlighted. These results were visualized using the R packages ggplot2(v3.2.1) and ggrepel(v0.8.1) and pheatmap(v1.0.12).

GraphPad Prism v7 was used for data entry, graph construction and data analysis.

FlowJo v10.4 was used for analysis of FACS data.

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 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

mRNA sequencing data (QuantSeq) have been deposited at the Gene Expression Omnibus (GEO) under accession number GSE139236.

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	No sample size calculations were performed. Sample size was determined based on preliminary experiments that defined the adequate number of samples to consistently identify differences between groups. Sample sizes are indicated in figure legends.
Data exclusions	No data-points were excluded with the exception of mice that had to be euthanized due to necrotic tumors. For mean calculation of tumor growth curves only time points were n>=3 mice were measured are considered (not applicable for start- and endpoints). To deconvolute and increase clarity of certain mean growth curves, selected data points (caliper measurements) were omitted (only when not affecting the slope of the curve).
Replication	For all experiments, the replication experiments were succesful and showed comparable results.
Randomization	Mice were randomized based on tumor burden to achieve equal tumor volume between treatment groups. Mice used were litter-mates and sex-matched.
Blinding	Investigators were not blinded to mouse treatment groups. Data reported for mouse experiments is not subjective but was repeated by independent investigators.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Immunoblotting:
pERK (clone D13.14.4E, CST, Cat: 4370L, Lot: 12), 1:2000
tERK (clone L34F12, CST, Cat: 4696S, Lot: 22), 1:2000
pAKT (clone D9E, CST, Cat: 4060L, Lot:16), 1:2000
tAKT (clone 40D4, CST, Cat: 2920s, Lot: 3), 1:2000
Histone H3 (polyclonal, abcam, Cat: ab1791, Lot: GR3237694-1), 1:1000

Flowcytometry:
CD45 PECy7 (clone 30-F11, BD Pharmingen, Cat: 552848, Lot: 7270660), 1:500
Thy1.1/CD90.1 AF700 (clone OX-7, BioLegend, Cat: 202528, Lot:B185299), 1:350
Thy1.1/CD90.1 PE-Cy7 (clone OX-7, BD Pharmingen, Cat: 561404, Lot:7143537), 1:350

Validation

The antibodies used are commercially available and validated throughout experiments.

https://www.cellsignal.at/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370
https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-l34f12-mouse-mab/4696
https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060
https://www.cellsignal.com/products/primary-antibodies/akt-pan-40d4-mouse-mab/2920
https://www.abcam.com/histone-h3-antibody-nuclear-loading-control-and-chip-grade-ab1791.html

http://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/mouse/pe-cy7-rat-anti-mouse-cd45-30-f11/p/561868
https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-rat-cd90-mouse-cd90-1-thy-1-1-antibody-5883
https://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/mouse/pe-cy7-mouse-anti-rat-cd90mouse-cd901-ox-7-also-known-as-ox7/p/561404

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

A375 melanoma: ATCC
Mouse (BRAFV600E/WT, PTEN-/-, CDNK2a-/-): Markus Bosenberg Laboratory
Lenti X: Clontech
Platinum-E (Plat-E): Cell Biolabs Inc.
K562 and MOLM13: Johannes Zuber Laboratory
NIH/3T3, HEK293 and U937: Alexander Stark Laboratory

Authentication

The cell lines used were not authenticated, but specificity for BrafV600E inhibitors was confirmed in the Braf-mutated cell lines.

Mycoplasma contamination

All cell lines were tested negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

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

Laboratory animals

Laboratory animals – Mus Musculus
6-12-week-old female/male C57BL/6

Wild animals

Did not involve wild animals.

Field-collected samples

Did not involve field-collected samples.

Ethics oversight	All experiments using animals were performed in accordance with our protocol approved by the Austrian Ministry (BMBWF-66.015/0009-V/3b/2019 or GZ: 340118/2017/25).

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

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	For flow cytometry of whole tumours (from subcutaneous injected mouse cell lines), tumours were dissected, cut into small pieces and dissociated for 1.5 hours using Collagenase A (1 mg/ml) in PBS. Single cell suspensions were strained through a 70 µm nylon mesh, washed in FACS buffer (0.5% BSA, 2 mM EDTA) and incubated for 10 min on 4 degree with anti-mouse Fc-Block CD16/CD32 antibody (clone 2.4G2, BD Pharmingen, 1:10). Cells were subsequently stained with a live-dead stain (Fixable viability dye eFluor 780, eBioscience, 1:1000) and a pan mouse immune-cell antibody CD45 PECy7 (clone 30-F11, BD Pharmingen, 1:500) in FACS buffer for 30 min on 4 degree. For flow cytometry of cultured cells, cells were detached using 0.05% Trypsin, inhibited with full medium and stained in FACS buffer (0.5% BSA, 2mM EDTA). Thy1.1/CD90.1 was detected using the following antibodies mouse CD90.1 AF700 (clone OX-7, 1:400 BioLegend) or mouse CD90.1 PE-Cy7 (clone OX-7, 1:400 BD Pharmingen). Cells were washed in PBS and resuspended in FACS buffer (0.5% BSA, 2mM EDTA).
Instrument	BD LSR Fortessa for analysis BD FACS Aria for sorting
Software	DIVA software for acquisition FlowJo V10.4 for analysis
Cell population abundance	All relevant sorted cell lines where after expansion in tissue culture analyzed by FACS for their respective fluorescent expression markers and were only used with a purity of at least 90%. For cell lines sorted from subcutaneous tumors for mRNA sequencing, post-sort analysis was only performed for selected samples for which a purity of ca. 90% could be confirmed.
Gating strategy	For cell lines: A singlet discrimination (FSC-A vs SSC-A > SSC-A vs SSC-H or FSC-A vs FSC-H) was performed for every cell line. Fluorescent signals were then either gated against each other, against FSC-A or an unoccupied channel (i.e. APC-A). Negative and positive signal boundaries were determined by using a negative control of the respective cell line. Gating strategies of the respective experiments: Fig 1 b: mCherry+ > BFP+ > AF700+ (if sgRNA infected) Fig 1 d, Ext. Data Fig 3 a: mCherry+ >BFP+ SgRNA infection rates for samples were checked in parallel and ranged from 75-80% (gating mCherry+ >BFP+ > PE-CY7) Fig 2 c: PE-Cy7+ (if infected) Fig 2 b, Ext. Data Fig 4 a: Singlet discrimination only Ext. Data Fig 1 a: mCherry+ > BFP+ (if rtTA3 infected) or AF700+ (if sgRNA infected) Ext. Data Fig 1 b, c: mCherry+ > BFP+ > AF700+ (if sgRNA infected) Ext. Data Fig 2 b: Fig 1 b: mCherry+ > BFP+ > AF700+ (if sgRNA infected) Ext. Data Fig 3 c: CaTCH approach: BFP+ > PE-CY7+; Alternative approach: PE-Cy7+ Ext. Data Fig 4 c: Singlet discrimination only Ext. Data Fig 8: For cell sorts from subcutaneous tumors: A singlet discrimination and live-dead cell exclusion was performed. To gate for injected CaTCH-isolated tumor cells, we gated on CD45- cells and then on GFP expressing cells.

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