nature research

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Last updated by author(s): Nov 7, 2019

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.

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	CCLE RNA-seq data can be found through link https://portals.broadinstitute.org/ccle. TFAM microarray data are available in NCBI GEO database with accession number GSE63767. The code used to analyze the data was deposited in github (http://github.com/kepbod/CCLE_analysis)
Data analysis	Prism8 and R (v.3.4.1) were used to generate graphs and perform statistical analyses. FlowJo was used for flow cytometry data analysis. ImageJ was used for imaging data analysis. NIS-Elements (version 4.3) using the focinator plug-in (code provided in the reference, Oeck et al. 2017) was used for foci quantification.

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

CCLE RNA-seq data can be found through link https://portals.broadinstitute.org/ccle. TFAM microarray data are available in NCBI GEO database with accession number GSE63767. qPCR biological replicates are provided as an additional supplementary file. All the other data will be provided by the corresponding author upon reasonable request as stated 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	We determined the sample sizes for our study based on prior knowledge from previously published studies on identical analyses.
Data exclusions	The only pre-established exclusion criteria were for replicates that were determined to be technically flawed or determined by statistical tests to contain a legitimate outlier data point. When either of these occurred the entire replicate was omitted and the entire experiment repeated as opposed to reanalyzing the data in the absence of an outlying data point. The number of final replicates used is described in the next section.
Replication	After taking into consideration the above exclusion criterion, all attempts to replicate experiments were successful based on at least 2 biological replicates analyzed. There was some variability in the magnitude of the fold change in the RT-PCR analyses of ISGs between biological replicates, thus the data shown are from one representative biological replicate with the statistics shown for the associated technical replicates. This is indicated clearly in the corresponding figure legends and the other biological replicates are provided in the supplemental materials.
Randomization	The mice used in the tumour studies were randomized prior to emplantation
Blinding	None of our experiments were blinded, because the results were inherently objective and subject to binary interpretation based in statistical tests.

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

TFAM antibody was originally made in David Clayton's lab at Stanford University.
STAT1 (Cell Signaling Technology, 9172S), (1:1000)
p-STAT1 (Tyr 701) (D4A7) (Cell Signaling Technology, 7649), (1:500)
STAT2 (Cell Signaling Technology, 4597S), (1:1000)
NF-κB, p-NF-κB, IKKβ , IκB antibody kit (Cell Signaling Technology, 9936), (1:1000)
HSP60 (Cell Signaling Technology, 12165T), (1:1000 for both IF and Western blot)
cGAS (D3O8O) (Cell Signaling Technology, 31659S), (1:1000)
STING (D2P2F) (Cell Signaling Technology, 13647S), (1:1000)
TBK1 (D1B4) (Cell Signaling Technology, 3504), (1:1000)
MAVS (Cell Signaling Technology, 4983S), (1:1000)
anti-DNA (Millipore CBL186), (1:150)
γH2A.X (Ser139, EMD Millipore 05-636), (1:1000 for western blot, 1:400 for IF)
Histone H3 (Abcam ab1791), (1:1000)
GAPDH (Ambion, AM4300), (1:3000)
VDAC (abcam, ab15895), (1:1000)
actin (Santa Cruz, sc47778), (1:2000)
IRF9 (ProteinTech, 14167-1-AP), (1:500)
p-53BP1 (Cell Signaling Technology, 2675S), (1:300)

Vinculin (Sigma, V9131), (1:10,000)
OXPHOS antibody Cocktail (abcam, ab110413). (1:500)

Validation

TFAM antibody reactivity: mouse. It was validated in our TFAM deficient and knockdown cells and also used in our previous publication (West et al, Nature, 2015).
STAT1 antibody reactivity: mouse, human, rat and monkey. It was validated in our STAT1 knockout cells and by the company. https://www.cellsignal.com/products/primary-antibodies/stat1-antibody/9172?site-search-type=Products. Product citations: 357.
P-STAT1 antibody reactivity: human, mouse and rat. It was validated in our STAT1 knockout cells and by the company. https://www.cellsignal.com/products/primary-antibodies/phospho-stat1-tyr701-d4a7-rabbit-mab/7649?site-search-type=Products. Product citations: 114
STAT2 antibody reactivity: mouse. It was validated in our STAT2 knockdown cells and by the company. https://www.cellsignal.com/products/primary-antibodies/stat2-antibody-mouse-specific/4597. product citations: 11
NF-κB antibody reactivity: human, rat, mouse, monkey, hamster and dog.
p-NF-κB antibody reactivity: human, rat, mouse, monkey, hamster and pig.
IKKβ antibody reactivity: human, rat, mouse, and monkey.
IκBa antibody reactivity: human, rat, mouse, monkey, pig and bovine.
NF-κB, p-NF-κB, IKKβ , IκBa antibodies are from NF-κB pathway sampler kit and are validated by the company. https://www.cellsignal.com/products/primary-antibodies/nf-kb-pathway-sampler-kit/9936
HSP60 antibody reactivity: human, rat, hamster, mouse, monkey, xenopus, pig, zebrafish and bovine. It applies for both western blot and Immunofluorescence and was validated by the company. https://www.cellsignal.com/products/primary-antibodies/hsp60-d6f1-xp-rabbit-mab/12165 Product citations: 33
cGAS antibody reactivity: mouse. It was validated in our cGAS CRISPR knockout cell line pools and by the company. https://www.cellsignal.com/products/primary-antibodies/cgas-d3o8o-rabbit-mab-mouse-specific/31659 Product citations: 7
STING antibody reactivity: mouse and human. It was validated in our STING CRISPR knockout cell line pools and by the company. https://www.cellsignal.com/products/primary-antibodies/sting-d2p2f-rabbit-mab/13647?site-search-type=Products Product citations: 59
TBK1 antibody reactivity: mouse, human, rat and monkey. It was validated in our TBK1 CRISPR knockout cell line pools and by the company. https://www.cellsignal.com/products/primary-antibodies/tbk1-nak-d1b4-rabbit-mab/3504?site-search-type=Products Product citations: 101
MAVS antibody reactivity: mouse and rat. It was validated in our MAVS CRISPR knockout cell line pools and by the company. https://www.cellsignal.com/products/primary-antibodies/mavs-antibody-rodent-specific/4983?site-search-type=Products&N=4294956287&Ntt=4983s&fromPage=plp&_requestid=2262477 Product citations: 16
anti-DNA antibody reactivity: All. This antibody reacts with native denatured DNA as well as synthetic DNA. http://www.emdmillipore.com/US/en/product/Anti-DNA-Antibody-clone-AC-30-10,MM_NF-CBL186 The antibody was used previously in West, et al. Nature 2015.
γH2A.X antibody reactivity: vertebrates. It was a well-established antibody to recognize phospho-H2AX (Ser139) and used in more than 1000 papers. http://www.emdmillipore.com/US/en/product/Anti-phospho-Histone-H2A.X-Ser139-Antibody-clone-JBW301,MM_NF-05-636# Product citations: 1605
Histone H3 antibody reactivity: Mouse, Rat, Chicken, Dog, Human, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Ferret, Indian muntjac, Schizosaccharomyces pombe, Zebrafish, Silk worm, Dictyostelium discoideum, Rainbow trout, Trypanosoma cruzi, Neurospora crassa, Toxoplasma gondii, Rice, Schistosoma mansoni, Candida albicans, Cyanidioschyzon merolae. It was validated by the company and has 2745 references. https://www.abcam.com/histone-h3-antibody-nuclear-loading-control-and-chip-grade-ab1791.html
GAPDH antibody reactivity: amphibian, dog, chicken, fish, human, mouse, Non-human primate, rabbit, rat. It was used for western blotting in 312 publications. https://www.thermofisher.com/antibody/product/GAPDH-Antibody-clone-6C5-Monoclonal/AM4300
VDAC antibody reactivity: mouse, rat, chicken, dog, human, zebrafish, Chinese hamster. It was validated by the company and has 199 references. https://www.abcam.com/vdac1-porin-antibody-mitochondrial-loading-control-ab15895.html
Actin antibody reactivity: mouse, rat, human, avian, bovine, canine, porcine, rabbit, Dictyostelium discoideum and Physarum. It was validated by the company. https://www.scbt.com/p/beta-actin-antibody-c4?requestFrom=search Product citations: 7190
p-53BP1 antibody reactivity: human and monkey. It was validated by the company. https://www.cellsignal.com/products/primary-antibodies/phospho-53bp1-ser1778-antibody/2675?_=1572905550114&Ntt=,%202675S&tahead=true product citations: 20
IRF9 reactivity: mouse, rat and human. It was validated in our siIRF9 cells and by the company. https://www.ptglab.com/Products/IRF9-Antibody-14167-1-AP.htm Product citations: 7
Vinculin antibody reactivity: bovine, canine, mouse, rat, turkey, human, chicken, frog. It was validated by the company and used in 628 peer-reviewed papers. https://www.sigmaaldrich.com/catalog/product/sigma/v9131?lang=en&region=US
OXPHOS antibody Cocktail reactivity: mouse, rat, cow, human, cynomolgus monkey. I was validated by the company and has 441 references. https://www.abcam.com/total-oxphos-rodent-wb-antibody-cocktail-ab110413.html

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The MC38 cell line was obtained from Dr. Peter Glazer's lab at Yale University, and was purchased originally from ATCC. The LMTK- cell line was from David Clayton's lab at Stanford University (where it was generated) and has been propagated in Dr. Shadel's lab for many years. YUMMER cells were generated in Dr. Marcus Bosenberg's lab at Yale University and are maintained in Dr. Shadel's and Dr. Kaech's labs at the Salk Institute.

Authentication

LMTK cells were originally verified to contain no thymidine kinase 1 activity by labeling with BrdU and were assayed for the presence or absence of mtDNA (the key attribute for this study) by qPCR. MC38 cells were purchased from ATCC, but not

	verified further. The YUMMER melanoma cell lines was verified to have PTEN knocked out and to express activated BRAF allele, as well as routinely assayed in vivo for their increased immunogenic potential compared to their parent cell line YUMM. (Wang et al, 2017). CRISPR knockout cell line pools were authenticated by western blotting.
Mycoplasma contamination	Cell cultures were tested regularly and determined to be free of mycoplasma
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other organisms

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

Laboratory animals	TFAM mice: C57BL/6; STAT1 mice: C57BL/6J 2-12 months old female mice were used to make MEFs. Littermate MEFs were used for the study. For the IR experiment, male and female littermate mice were 8 months old. 7-weeks old female athymic nu/nu mice (Hsd:Athymic Nude-Foxn1nu) were used for the tumour xenograft experiment. All housing and experiments were done using IACUC-approved protocols in AAALAC-certified facilities.
Wild animals	No wild animals were used
Field-collected samples	No field-collected samples were used.
Ethics oversight	Salk Institute and Yale University Animal Care and use committee, who confirm use of IACUC-approved methods and approaches.

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 cell viability analysis, WT and Tfam+/- littermate MEFs were seeded into 6 well plates. The next day, the media was replenished with or without 1µM doxorubicin. All cells including floating and adherent cells were pelleted and washed with PBS. Then cells were re-suspended in 60-100µL Annexin V binding buffer (Biolegend, cat#42220) with Pacific Blue Annexin V (Biolegend, cat#640918, 1:20) and 1µg/mL propidium iodide (PI) (MP Biomedicals cat#195458) and incubated for 15-20 minutes prior to flow cytometry analysis. For mitochondrial mass, membrane potential and ROS analysis, cells were treated with or without 500nM doxorubicin for 24 hours and then stained with MitoTraker Green (Thermo Fisher, cat#M7514, 50nM) and Deep Red (Thermo Fisher, cat#M22426, 20nM) or MitoSOX (Invitrogen, cat#M36008, 5µM), for 30min. Cells were washed with PBS and pelleted at room temperature. Cell pellets were resuspended in PBS with 2% FBS and then subjected to flow cytometry. Data were analyzed using FlowJo Software.
Instrument	Stratedigm (STD-13 and STD-13-L)
Software	FlowJo
Cell population abundance	At least 5000 cells were collected for each experiment.
Gating strategy	For cell viability analyses, all cells were analyzed. For mitochondrial mass, membrane potential and mitochondrial ROS analysis, FSC and SSC were used to gate out the cell debris and cell clumps. FSC-W and FSC-A were used for singlets gating.

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