nature research

Corresponding author(s): Walid T. Khaled & Ewan. St. John Smith

Last updated by author(s): Jan 13, 2020

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	FASTQ files were aligned to the reference genome of either Mus Musculus (GRCm38 with GRC38.91 annotation from ENSEMBL) or to the HetGla2.0 reference genome with the GCF_000247695.1 annotation from NCBI. The mapping was performed using the STAR aligner (2.7.1a) with default settings and –quantMode GeneCounts to derive gene level counts. The counts were then normalized with the trimmed mean of M-values (TMM) method to account for differences in sequencing depth and library composition19. The principle component analysis (Supplementary Figure 8) for mouse and naked mole rat cell lines was performed on log-transformed CPM (counts-per-million) values for a set of 16054 published orthologous genes16. Genes with high or low loading for PC2 (>0.02 or < -0.02) were characterized by gene set enrichment analysis based on gene-ontology (GO) terms using topGO with default settings (Alexa, A. & Rahnenfuhrer, J. TopGO: Enrichment Analysis for Gene Ontology. R package version 2.28.0 (2016)). All differential expression tests were performed using edgeR20 by fitting a negative binomial generalised log-linear model with the cell line as covariate. Genes that have a higher log fold change than 1 at an FDR of 0.01 were identified using the 'glmTreat' function.
Data analysis	FASTQ files were aligned to the reference genome of either Mus Musculus (GRCm38 with GRC38.91 annotation from ENSEMBL) or to the HetGla2.0 reference genome with the GCF_000247695.1 annotation from NCBI. The mapping was performed using the STAR aligner (2.7.1a) with default settings and –quantMode GeneCounts to derive gene level counts. The counts were then normalized with the trimmed mean of M-values (TMM) method to account for differences in sequencing depth and library composition19. The principle component analysis (Supplementary Figure 8) for mouse and naked mole rat cell lines was performed on log-transformed CPM (counts-per-million) values for a set of 16054 published orthologous genes16. Genes with high or low loading for PC2 (>0.02 or < -0.02) were characterized by gene set enrichment analysis based on gene-ontology (GO) terms using topGO with default settings (Alexa, A. & Rahnenfuhrer, J. TopGO: Enrichment Analysis for Gene Ontology. R package version 2.28.0 (2016)). All differential expression tests were performed using edgeR20 by fitting a negative binomial generalised log-linear model with the cell line as covariate. Genes that have a higher log fold change than 1 at an FDR of 0.01 were identified using the 'glmTreat' function.

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

The RNAseq data will be deposited into publicly available data repositories and accession numbers provided in the manuscript. All other other supporting raw data will be available from the authors upon request.

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	The number of naked mole-rats analysed in this study was dependent on age and sex. For the majority of the naked mole-rats we attempted to generate as many cell lines from as many tissues as possible using different vectors and transfection. This led to the generation of 106 cell lines. Full details of all the lines generated are available in Supplementary Table 1.
Data exclusions	NA
Replication	All experiments in this study were repeated multiple times (number of experimental repeats is noted for each figure and in figure legends)
Randomization	Cell lines used in the xenograft assays were assigned random names by one experimenter and handed over to a second experimenter who then injected them into mice. Each cell line was injected into four mice and equal number of mice of both genders were used for each cell line. In some cases, animals from different groups were housed in the same cage. The animal technician who measured the tumour masses were unaware of the grouping and cell line identity.
Blinding	Cell lines used in the xenograft assays were assigned random names by one experimenter and handed over to a second experimenter who then injected them into mice. Each cell line was injected into four mice and equal number of mice of both genders were used for each cell line. In some cases, animals from different groups were housed in the same cage. The animal technician who measured the tumour masses were unaware of the grouping and cell line identity.

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	The following antibodies were used: SV40LT (sc-148, Santa Cruz #sc-58665), RasG12V (D2H12, Cell Signalling #14412), Tubulin (DM1A, Abcam #ab7291). For details of dilutions of antibodies used please refer to the 'Protein Extraction, Antibodies and Immunoblotting' section of the Material & Methods

Validation	Protein extract from primary cell lines (not expressing any of the transgenes) were used to validate the antibodies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	106 cell lines were generated from 5 tissues (skin, pancreas, lungs, kidneys and intestines) of 11 different Naked Mole-Rats (Supplementary Table 1). Details of how the cell lines were generated are in the methods section of the manuscript
Authentication	NA
Mycoplasma contamination	All cell lines used in this study were tested and negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	NA

Animals and other organisms

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

Laboratory animals	Cells were derived from 11 (2 females, 9 males) adult naked mole-rats (age range: 37 - 244 weeks). Mice used for xenograft assay were of the NSG strain. Mouse skin cell lines were generated from 23 weeks old C57BL/6 female mice.
Wild animals	This study did not involve the use of wild animals
Field-collected samples	NMRs used in the study were housed in custom-made caging system with conventional mouse/rat cages connected by different lengths of tunnel. Bedding and nesting material were provided along with a running wheel. The room was warmed to 28 °C, with a heat cable to provide extra warmth running under 2-3 cages, and red lighting (08:00 - 16:00) was used.
Ethics oversight	All animal experiments were carried out under Home Office licenses P7EBFC1B1 and P1958D980.

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