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Corresponding author(s): Colin R Goding

Last updated by author(s): 14/12/2021

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	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	*Provide a description of all commercial, open source and custom code used to analyse the data in this study, specifying the version used OR state that no software was used.*

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

All the ChIP-seq raw, processed and bioinformatics pipelines involved are available at GSE137776

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

Life sciences study design

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

Sample size	SMT fast - Ncells = 28; 24;26; Ndisplacements = 82,252; 102,765; 107,370 for WT MITF, K206R and K206Q mutants respectively SMT slow - Ncells= 43, 43, 45, 30, 35, 36; Nbound molecules = 5141, 6193, 3631, 1185, 2374, 1225, for WT MITF, K206R,K206Q, Dbasic, USF1 and p53 respectively
Data exclusions	*Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Replication	*Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.*
Randomization	*Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.*
Blinding	*Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

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	HA Roche Cat# 11666606001; RRID: AB_514506 HA Sigma-Aldrich Cat# H3663; RRID: AB_262051 HA Cell Signaling Technology Cat# 2367; RRID: AB_2314619 Acetylated-Lysine Cell Signaling Technology Cat# 9441; RRID: AB_331805 MITF Millipore Cat# MAB3747; RRID: AB_570596 ERK 2 Santa Cruz Biotechnology Cat# sc-153; RRID: AB_2141293 ERK 2 Santa Cruz Biotechnology Cat# sc-1647; RRID: AB_627547 Thr202/Tyr204, ppERK Cell Signaling Technology Cat# 4377; RRID: AB_331775 GAPDH Santa Cruz Biotechnology Cat# sc-32233; RRID: AB_627679 GFP Abcam Cat# ab290 (RRID:AB_303395) Donkey Anti-Mouse IgG (H+L) Antibody, Alexa Fluor 488 Conjugated Thermo Fisher Scientific Cat# A-21202 (RRID:AB_141607) Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 Thermo Fisher Scientific Cat# A10040 (RRID:AB_2534016) MITF-K206Ac - In house
Validation	MITF-K206Ac has been validated using peptide array

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	501mel parental cell line obtained from Ruth Halaban, Yale (Zakut et al., 1993) and 501mel engineered to express ectopic-doxycycline-inducible-HA-tagged-murine-Mitf and HIS-tagged-human-MITF were generated in house for this paper
Authentication	501mel cell line has been authenticated using eurofins genomic service

Mycoplasma contamination	All cell lines were routinely check for mycoplasma contamination using the Ludwig Institute routine (monthly) mycoplasma test service
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Animals and other organisms

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

Laboratory animals	Zebrafish AB/TPL lines were bred and maintained as previously described {Westerfield, 2000 #5199}.
Wild animals	N/A
Field-collected samples	N/A
Ethics oversight	All zebrafish experiments are performed in accordance with the Animals (Scientific Procedures) Act 1986, and approved by the University of Edinburgh Animal Welfare and Ethical Review Body. Zebrafish AB/TPL lines were bred, raised and maintained as described {Westerfield, 2000 #5199}.

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

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	GSE137776
Files in database submission	iMitf_K206Q_0ng_R1_1.fastq.gz iMitf_K206Q_0ng_R1_2.fastq.gz iMitf_K206Q_0ng_R2_1.fastq.gz iMitf_K206Q_0ng_R2_2.fastq.gz iMitf_K206Q_20ng_R1_1.fastq.gz iMitf_K206Q_20ng_R1_2.fastq.gz iMitf_K206Q_20ng_R2_1.fastq.gz iMitf_K206Q_20ng_R2_2.fastq.gz iMitf_K206Q_100ng_R1_1.fastq.gz iMitf_K206Q_100ng_R1_2.fastq.gz iMitf_K206Q_100ng_R2_1.fastq.gz iMitf_K206Q_100ng_R2_2.fastq.gz iMitf_K206Q_Input_1.fastq.gz iMitf_K206Q_Input_2.fastq.gz iMitf_K206R_0ng_R1_1.fastq.gz iMitf_K206R_0ng_R1_2.fastq.gz iMitf_K206R_0ng_R2_1.fastq.gz iMitf_K206R_0ng_R2_2.fastq.gz iMitf_K206R_20ng_R1_1.fastq.gz iMitf_K206R_20ng_R1_2.fastq.gz iMitf_K206R_20ng_R2_1.fastq.gz iMitf_K206R_20ng_R2_2.fastq.gz iMitf_K206R_100ng_R1_1.fastq.gz iMitf_K206R_100ng_R1_2.fastq.gz iMitf_K206R_100ng_R2_1.fastq.gz iMitf_K206R_100ng_R2_2.fastq.gz iMitf_K206R_Input_1.fastq.gz iMitf_K206R_Input_2.fastq.gz iMitf_WT_0ng_R1_1.fastq.gz iMitf_WT_0ng_R1_2.fastq.gz iMitf_WT_0ng_R2_1.fastq.gz iMitf_WT_0ng_R2_2.fastq.gz iMitf_WT_20ng_R1_1.fastq.gz iMitf_WT_20ng_R1_2.fastq.gz iMitf_WT_20ng_R2_1.fastq.gz iMitf_WT_20ng_R2_2.fastq.gz iMitf_WT_100ng_R1_1.fastq.gz iMitf_WT_100ng_R1_2.fastq.gz

iMitf_WT_100ng_R2_1.fastq.gz
iMitf_WT_100ng_R2_2.fastq.gz
iMitf_WT_Input_1.fastq.gz
iMitf_WT_Input_2.fastq.gz
GSM4087709_iMitf_K206Q_0ng_R1.ucsc.bedGraph.gz
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GSM4087719_iMitf_K206R_20ng_R2.ucsc.bedGraph.gz
GSM4087720_iMitf_K206R_100ng_R1.ucsc.bedGraph.gz
GSM4087721_iMitf_K206R_100ng_R2.ucsc.bedGraph.gz
GSM4087722_iMitf_K206R_Input.ucsc.bedGraph.gz
GSM4087723_iMitf_WT_0ng_R1.ucsc.bedGraph.gz
GSM4087724_iMitf_WT_0ng_R2.ucsc.bedGraph.gz
GSM4087725_iMitf_WT_20ng_R1.ucsc.bedGraph.gz
GSM4087726_iMitf_WT_20ng_R2.ucsc.bedGraph.gz
GSM4087727_iMitf_WT_100ng_R1.ucsc.bedGraph.gz
GSM4087728_iMitf_WT_100ng_R2.ucsc.bedGraph.gz
GSM4087729_iMitf_WT_Input.ucsc.bedGraph.gz

Genome browser session
(e.g. UCSC)

http://genome-euro.ucsc.edu/s/Pakavarin/iMITF_titration_206_colour_adjusted

Methodology

Replicates

2 biological replicates (each with 30x 15cm dishes) prepared consecutively but all steps post-fixation were carried out in parallel

Sequencing depth

iMit_K206Q_0ng_Dox_Replicate1 Total number of reads=28424720 Uniquely mapped reads=25929609 Av. Input Length=150 paired
iMit_K206Q_0ng_Dox_Replicate2 Total number of reads=30245021 Uniquely mapped reads=27528967 Av. Input Length=150 paired
iMit_K206Q_20ng_Dox_Replicate1 Total number of reads=33702659 Uniquely mapped reads=30779108 Av. Input Length=150 paired
iMit_K206Q_20ng_Dox_Replicate2 Total number of reads=32900533 Uniquely mapped reads=30009364 Av. Input Length=150 paired
iMit_K206Q_100ng_Dox_Replicate1 Total number of reads=27100903 Uniquely mapped reads=24741618 Av. Input Length=150 paired
iMit_K206Q_100ng_Dox_Replicate2 Total number of reads=31639213 Uniquely mapped reads=28845804 Av. Input Length=150 paired
iMit_K206Q_Input_control Total number of reads=38542817 Uniquely mapped reads=35227922 Av. Input Length=150 paired

iMit_K206R_0ng_Dox_Replicate1 Total number of reads=31555438 Uniquely mapped reads=28798671 Av. Input Length=150 paired
iMit_K206R_0ng_Dox_Replicate2 Total number of reads=31352594 Uniquely mapped reads=28684192 Av. Input Length=150 paired
iMit_K206R_20ng_Dox_Replicate1 Total number of reads=33733351 Uniquely mapped reads=30941219 Av. Input Length=150 paired
iMit_K206R_20ng_Dox_Replicate2 Total number of reads=31079871 Uniquely mapped reads=28520630 Av. Input Length=150 paired
iMit_K206R_100ng_Dox_Replicate1 Total number of reads=30973546 Uniquely mapped reads=28344856 Av. Input Length=150 paired
iMit_K206R_100ng_Dox_Replicate2 Total number of reads=33097713 Uniquely mapped reads=30396518 Av. Input Length=150 paired
iMit_K206R_Input_control Total number of reads=31162561 Uniquely mapped reads=28473720 Av. Input Length=150 paired

iMit_WT_0ng_Dox_Replicate1 Total number of reads=23821343 Uniquely mapped reads=21786519 Av. Input Length=150 paired
iMit_WT_0ng_Dox_Replicate2 Total number of reads=27099478 Uniquely mapped reads=24805484 Av. Input Length=150 paired
iMit_WT_20ng_Dox_Replicate1 Total number of reads=29491670 Uniquely mapped reads=26928341 Av. Input Length=150 paired
iMit_WT_20ng_Dox_Replicate2 Total number of reads=25850947 Uniquely mapped reads=23689580 Av. Input Length=150 paired

iMit_WT_100ng_Dox_Replicate1 Total number of reads=34846438 Uniquely mapped reads=32066253 Av. Input Length=150 paired
iMit_WT_100ng_Dox_Replicate2 Total number of reads=36319862 Uniquely mapped reads=33368844 Av. Input Length=150 paired
iMit_WT_Input_control Total number of reads=31351213 Uniquely mapped reads=28567297 Av. Input Length=150 paired

Antibodies

HA Cat# 11666606001; RRID: AB_514506

Peak calling parameters

Mapping
STAR --runThreadN 24 --genomeDir /t1-data/user/plouphra/STAR_Genome_hg38/ --readFilesIn "$1" "$2" --outFileNamePrefix "$3" --readFilesCommand zcat -c --chimSegmentMin 20 --outReadsUnmapped Fastx
"$1" = *_1.fastq.gz
"$2" = *_2.fastq.gz
"$3" = output file name prefix

Peak calling & annotation
makeTagDirectory "$1" -genome /t1-data/user/plouphra/hg38/ "$2"
findPeaks "$1" -style factor -norm 3e7 -i "$3" -o "$1"_peaks.txt
annotatePeaks.pl "$1"/peaks.txt -genome /t1-data/user/plouphra/hg38/ -CpG -gsize 2790000000 > "$1"/annotated_peaks.txt -go "$1"/GO.txt -genomeOntology "$1"/genomeOntology.txt

"$1" = samples name/directory e.g. iMitf_WT_0ng_R1
"$2" = input SAM file output from STAR
"$3" = tag_directory generated from STAR-mapped SAM files of the input control samples

Generating normalised Bedgraph for UCSC
makeUCSCfile "$1" -fsize 1e50 -o auto

Data quality

All peaks were called using the following fixed parameters
FDR rate threshold = 0.001000000
Fold over input = 4.00
Poisson p-value over input = 1.00e-04
Fold over local region = 4.00
Poisson p-value over local region = 1.00e-04
and variable parameters (modelled according to each dataset as these are sequencing depth dependent)
FDR effective poisson threshold
FDR tag threshold

iMit_K206Q_0ng_Dox_Replicate1 # FDR effective poisson threshold = 3.145650e-06 # FDR tag threshold = 17.0 Fragment length=196 Peaks called=78579 Differential peaks over input (filtered)=74467 Local background filtering (filtered)= 2145 Clonal filtering (filtered)=0 ChIP-efficiency=0.24% total peaks (passed) = 1967

iMit_K206Q_0ng_Dox_Replicate2 # FDR effective poisson threshold = 2.514935e-06 # FDR tag threshold = 18.0 Fragment length=210 Peaks called=82617 Differential peaks over input (filtered)=78729 Local background filtering (filtered)= 2288 Clonal filtering (filtered)=0 ChIP-efficiency=0.17% total peaks (passed) = 1600

iMit_K206Q_20ng_Dox_Replicate1 # FDR effective poisson threshold = 3.296687e-06 # FDR tag threshold = 19.0 Fragment length=210 Peaks called=82641 Differential peaks over input (filtered)=75944 Local background filtering (filtered)= 2011 Clonal filtering (filtered)=0 ChIP-efficiency=1.00% total peaks (passed) = 4686

iMit_K206Q_20ng_Dox_Replicate2 # FDR effective poisson threshold = 2.638019e-06 # FDR tag threshold = 19.0 Fragment length= 204 Peaks called= 85601 Differential peaks over input (filtered)= 78454 Local background filtering (filtered)= 2083 Clonal filtering (filtered)= 0 ChIP-efficiency=1.12% total peaks (passed) = 5064

iMit_K206Q_100ng_Dox_Replicate1 # FDR effective poisson threshold = 2.834221e-06 # FDR tag threshold = 17.0 Fragment length= 198 Peaks called=77637 Differential peaks over input (filtered)= 71864 Local background filtering (filtered)= 2199 Clonal filtering (filtered)= 0 ChIP-efficiency= 0.67% total peaks (passed) = 3574

iMit_K206Q1010ng_Dox_Replicate2 # FDR effective poisson threshold = 2.492754e-06 # FDR tag threshold = 18.0 Fragment length= 206 Peaks called= 88142 Differential peaks over input (filtered)= 81656 Local background filtering (filtered)=2589 Clonal filtering (filtered)= 0 ChIP-efficiency=0.72% total peaks (passed) = 3897

iMit_K206R_0ng_Dox_Replicate1 # FDR effective poisson threshold = 7.334091e-06 # FDR tag threshold =17.0 Fragment length= 204 Peaks called= 106808 Differential peaks over input (filtered)= 88369 Local background filtering (filtered)= 10058 Clonal filtering (filtered)= 0 ChIP-efficiency=0.99% total peaks (passed) = 8381

iMit_K206R_0ng_Dox_Replicate2 # FDR effective poisson threshold =5.694232e-06 # FDR tag threshold =17.0 Fragment length= 204 Peaks called= 105830 Differential peaks over input (filtered)=86841 Local background filtering (filtered)= 10386 Clonal filtering (filtered)= 0 ChIP-efficiency=0.99% total peaks (passed) = 8603

iMit_K206R_20ng_Dox_Replicate1 # FDR effective poisson threshold = 1.332323e-05 # FDR tag threshold = 19.0 Fragment length=206 Peaks called=148494 Differential peaks over input (filtered)=84809 Local background filtering (filtered)=9536

Clonal filtering (filtered)= 0 ChIP-efficiency=11.98% total peaks (passed) = 54149

iMit_K206R_20ng_Dox_Replicate2 # FDR effective poisson threshold = 5.147797e-06 # FDR tag threshold =19.0 Fragment length=198 Peaks called=128399 Differential peaks over input (filtered)= 68606 Local background filtering (filtered)= 7919 Clonal filtering (filtered)= 0 ChIP-efficiency=11.87% total peaks (passed) = 51874

iMit_K206R_100ng_Dox_Replicate1 # FDR effective poisson threshold =5.961963e-06 # FDR tag threshold = 19.0 Fragment length=218 Peaks called= 124891 Differential peaks over input (filtered)= 59170 Local background filtering (filtered)=7304 Clonal filtering (filtered)= 0 ChIP-efficiency=14.23% total peaks (passed) = 58417

iMit_WT_0ng_Dox_Replicate1 # FDR effective poisson threshold = 3.651436e-06 # FDR tag threshold =17.0 Fragment length= 208 Peaks called= 73101 Differential peaks over input (filtered)= 55932 Local background filtering (filtered)= 4323 Clonal filtering (filtered)= 0 ChIP-efficiency=2.55% total peaks (passed) = 12845

iMit_WT_0ng_Dox_Replicate2 # FDR effective poisson threshold = 5.008143e-06 # FDR tag threshold = 18.0 Fragment length= 202 Peaks called= 81440 Differential peaks over input (filtered)= 65900 Local background filtering (filtered)=4598 Clonal filtering (filtered)= 0 ChIP-efficiency=2.03% total peaks (passed) = 10942

iMit_WT_20ng_Dox_Replicate1 # FDR effective poisson threshold = 9.104248e-06 # FDR tag threshold = 19.0 Fragment length= 210 Peaks called= 116466 Differential peaks over input (filtered)=65761 Local background filtering (filtered)= 6358 Clonal filtering (filtered)= 0 ChIP-efficiency=9.54% total peaks (passed) = 44347

iMit_WT_20ng_Dox_Replicate2 # FDR effective poisson threshold = 7.311743e-06 # FDR tag threshold = 18.0 Fragment length= 220 Peaks called= 113694 Differential peaks over input (filtered)= 55696 Local background filtering (filtered)= 6124 Clonal filtering (filtered)= 0 ChIP-efficiency=13.17% total peaks (passed) = 51874

iMit_WT_100ng_Dox_Replicate1 # FDR effective poisson threshold = 6.461421e-06 # FDR tag threshold = 21.0 Fragment length= 224 Peaks called= 142484 Differential peaks over input (filtered)=53281 Local background filtering (filtered)=7108 Clonal filtering (filtered)= 0 ChIP-efficiency=22.87% total peaks (passed) = 82095

iMit_WT_100ng_Dox_Replicate2 # FDR effective poisson threshold = 1.289524e-05 # FDR tag threshold = 21.0 Fragment length= 224 Peaks called= 148377 Differential peaks over input (filtered)= 61584 Local background filtering (filtered)= 7356 Clonal filtering (filtered)= 0 ChIP-efficiency=21.78% total peaks (passed) = 79437

Software

STAR v 2.5.1b, Genome GRCh38 v23 (Dobin et al., 2013)

Homer v4.9.1, Genome hg38 v5.10 (Heinz et al., 2010)

ggplot2-v3.0.0 – under R-v3.5.1 https://CRAN.R-project.org/package=ggplot2

TreeView (Saldanha, 2004)

Bowtie 1.1.2 Genome hg19 (Langmead and Salzberg, 2012)

PicardTools version 1.96, http://picard.sourceforge.net

MACS2 v2.1.0 (Zhang et al., 2008)

CisGenome (Ji et al., 2008)

MEME (Machanick and Bailey, 2011)

BedTools (Quinlan and Hall, 2010)