nature portfolio

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Last updated by author(s): Jul 7, 2025

# 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<br>*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<br>*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 | FACSCanto (BD Biosciences), Illumina NovaSeq 6000 sequencing platform, Applied Biosystems 7500 Fast Real-Time PCR System, AxioScan (ZEISS), ZEISS Axio Observer |
| Data analysis | FIJI software, FlowJo V10, Graphpad Prism 8, QuPath v0.5.1, R code (https://github.com/MMdR-lab/Feiyang-triple-therapy.git) |

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

All data associated with this study are present in the paper or the Supplementary Information. Source data are provided with this paper. The RNA sequencing data used in this study are available in the GSE301844 (reviewer token gzsdcicuvfevpgb).

## 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 | There is no human participant involved. |
| Reporting on race, ethnicity, or other socially relevant groupings | There is no human participant involved. |
| Population characteristics | There is no human participant involved. |
| Recruitment | There is no human participant involved. |
| Ethics oversight | There is no human participant involved. |

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 | Sample size used determined based on previous experience and similar assays (PMID: 39270021, 37616051). No statistical test was used to pre-determine the sample sizes. |
| Data exclusions | No data was excluded. |
| Replication | Each biological experiments were performed in independent replicates.<br>All experiments were repeated at least 3 times, to ensure reproducibility of the results.<br>There were no experiments that we could not replicate independently in this study. |
| Randomization | In all experiments, mice were randomly assigned to each group and the order in which analysis procedures were performed were done at random. |
| Blinding | Blinding was not applied to most experiments because data collection and analysis were performed by the same 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 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 | Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb Cell Signaling Technology #4370 WB, IHC |

| | |
|---|---|
| Antibodies used | ERK 2 Antibody (C-14) (Santa Cruz, sc-154, WB)<br>Phospho-S6 Ribosomal Protein (Ser240/244) (D68F8) XP® Rabbit mAb (Cell Signaling Technology #5364, WB, IHC)<br>S6 Ribosomal Protein (54D2) Mouse mAb (Cell Signaling Technology #2317, WB)<br>Anti-human cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (Cell Signaling Technology #5625, WB)<br>Anti-mouse cleaved PARP (Asp214) Antibody (Cell Signaling Technology #9544, WB)<br>Anti-human ATF-4 (D4B8) Rabbit mAb (Cell Signaling Technology #11815, WB, IHC)<br>Anti-mouse ATF-4 antibody (Abcam ab216839, WB, IHC)<br>Phospho-eIF2α (Ser51) (D9G8) XP® Rabbit mAb (Cell Signaling Technology #3398, WB)<br>eIF2α (L57A5) Mouse mAb (Cell Signaling Technology #2103, WB)<br>MTHFD1 Polyclonal antibody (Proteintech 10794-1-AP, WB)<br>MTHFD2 Polyclonal antibody (Proteintech 12270-1-AP WB, IHC)<br>Phospho-Histone H2A.X (Ser139) Antibody (Cell Signaling Technology #2577, WB)<br>Ki-67 (8D5) Mouse mAb (Cell Signaling Technology #9449, IHC)<br>Anti-α-actinin Antibody (H-2) (Santa Cruz, sc-17829, WB)<br>Monoclonal Anti-β-Actin antibody (clone AC-15) (Sigma-Aldrich #A5441, WB)<br>GAPDH (D16H11) XP® Rabbit mAb (Cell Signaling Technology #5174, WB)<br>Amersham ECL Mouse IgG, HRP-linked whole Ab (from sheep) (Cytiva NA931V, WB)<br>Amersham ECL Rabbit IgG, HRP-linked whole Ab (from donkey) (Cytiva NA934V, WB)<br>EnVision+ Single Reagent (HRP. Mouse) (Agilent Dako K4001, IHC)<br>EnVision+ Single Reagent (HRP. Rabbit) (Agilent Dako K4003, IHC) |
| Validation | All antibodies used in this study have been previously validated by the manufacturer, as stated on their associated product websites, and by our own lab in previous experiments. |

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | BLM (Gifted by Dr. Ian R Watson, MUHC Research Institute)<br>WM3406 (Gifted by Dr. April Rose, Lady Davis Institute)<br>MeWo (Gifted by Dr. Ian R Watson, MUHC Research Institute)<br>HBL (Gifted by Dr. Ghanem Ghanem, Institut Jules Bordet)<br>YUGOE (Gifted by Dr. April Rose, Lady Davis Institute)<br>WM3623 (Gifted by Dr. April Rose, Lady Davis Institute)<br>MaNRAS1007 (Gifted by Dr. Lionel Larue, INSERM, First described in PMID: 31251472) |
| Authentication | All of the cell lines used were authenticated by short tandem repeat profiling. |
| Mycoplasma contamination | The cell lines were tested as Mycoplasma negative. |
| Commonly misidentified lines (See ICLAC register) | We did not use commonly misidentified lines. |

## 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 | C57BL/6N mice (6-8 weeks old); NOD/SCID mice (6-10 weeks old). Mice were housed under 12 hour light/dark cycle at 20-24°C and 45-65% humidity |
| Wild animals | No wild animals were used in the study. |
| Reporting on sex | We used male C57BL/6N mice and female NOD/SCID mice since MaNRAS1007 cell is from male mice and WM3406 cell is from female patient. |
| Field-collected samples | No field-collected samples were used in the study. |
| Ethics oversight | Animal experiments were conducted according to the regulations established by the Canadian Council of Animal Care, and protocols approved by McGill University Animal Care and Use Committee (#2015-7672). |

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

## Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| | |
|---|---|
| Clinical trial registration | No clinical data is collected in this study. |
| Study protocol | No clinical data is collected in this study. |

| | |
|---|---|
| Data collection | No clinical data is collected in this study. |
| Outcomes | No clinical data is collected in this study. |

## Plants

| | |
|---|---|
| Seed stocks | No plant is used in this study. |
| Novel plant genotypes | No plant is used in this study. |
| Authentication | No plant is used in this study. |

## 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 | Melanoma cells were seeded into 6-well plates at 100,000 cells per well. Following indicated treatments, cells were trypsinized, centrifuged at 240g for 5 min, and washed twice in PBS. For apoptosis detection of non-fixed cells, Alexa Fluor™ 647-Annexin V (Invitrogen™, A23204) and Propidium Iodide (PI) Staining Solution (BD Biosciences, 556463) were diluted in 1×binding buffer (BD Biosciences, 556454), and subsequently mixed with cells following the manufacturer's instructions. |
| Instrument | FACSCanto (BD Biosciences) |
| Software | FlowJo V10 |
| Cell population abundance | Cell population abundance was dependent on the apoptosis level. |
| Gating strategy | For all gating strategy in this article, we gated with debris exclusion (appropriate FSC-A/SSC-A) and doublets exclusion (FSC-A/FSC-H) |

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