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Corresponding author(s): Jordan L. Meier

# 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.

## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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 |
| ☐ | ☒ | An indication of 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 statistics 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 |
| ☐ | ☒ | Clearly defined error bars<br>*State explicitly what error bars represent (e.g. SD, SE, CI)* |

*Our web collection on statistics for biologists may be useful.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Mass spectrometry was performed using a Thermo LTQ Orbitrap Discovery mass spectrometer coupled to an Agilent 1200 series HPLC. Chemiluminescence signal for the western blotting images were obtained using an ImageQuant Las4010 Digitial Imaging System (GE Healthcare). Fluorimetric analysis was performed on Photon Technology International QuantMaster fluorimeter or Biotek Synergy MX plate reader. HPLC analysis was performed on Agilent Technologies 1260 Infinity HPLC equipped with a Phenomenex Kinetex C18 column. |
| Data analysis | The tandem MS data, generated from the 5 MudPIT runs, was analyzed by the SEQUEST algorithm. MS2 spectra matches were assembled into protein identifications and filtered using DTASelect2.0. Quantification of L/H ratios were calculated using the cimage quantification package. All the graphs reported in the manuscript were generated using GraphPad Prism. Softwares and methods used for bioinformatics analyses is listed under relevant section of online methods. |

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 upon request. 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

We have provided datasets S1-S5 for our complete proteomic data. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the identifiers PXD009378 (Datasets S1-S4) and PXD009202 (Dataset S5).

# Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

# Life sciences study design

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

| | |
|---|---|
| Sample size | Sample size calculation based on statistics was not performed for the chemoproteomic or other analyses presented in this work. Widely used sample sizes of 3-4 were employed for chemoproteomics studies. Sample sizes for the bioinformatic analyses performed is reported under the relevant section of online methods. Sample sizes of 2-4 was kinetic, immunoblotting and cell-based experiments as widely adapted in the field of chemical biology. |
| Data exclusions | No data was excluded. |
| Replication | Chemoproteomic analysis of FH-regulated cysteines using IA-alkyne and whole proteome MudPIT were performed in three independent experiments with one LC-MS/MS run each. All gel-based and immunoblotting experiments were performed as two independent experiments with similar results and representative images are shown in figures. |
| Randomization | Randomization was not performed because in each chemoproteomic experiment, only two groups of samples, under the indicated conditions were compared. |
| Blinding | We used isotopic labeling of the samples for quantitative chemoproteomic studies to compare cysteine reactivity as a function of FH mutation status in parallel. Thus, it was not necessary to design a blinding group allocation. |

# Reporting for specific materials, systems and methods

### Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Unique biological materials |
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |

### Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | SMARCC1 (11956), SNF5 (8745), BRG1 (3508), PKM1 (7067), Myc-Tag (2278), FLAG-Tag (14793) and HA-Tag (3724) antibodies were purchased from Cell Signaling Technologies. OAT (A305-355A), HNRNP-L (A303-895A), CBX5 (A300-877A), EEF2 (A301-688A) and MAP2K4 (A302-658A) antibodies were purchased from Bethyl Laboratories, Inc. IP-grade antibodies for SMARCC1 (sc-32763) and BRG1 (sc-17796) were obtained from Santa Cruz Biotechnology. All antibodies were used at 1:1000 dilution unless noted. S-succinated-Cys antibody was obtained from Dr. Norma Frizell and used at 1:3000 dilution. |

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| Validation | All the commercial antibodies were validated by the manufacturers for species and application using recombinant proteins and/or expressing cell types as positive control; these validation studies are reported for each antibody on the product website. S-succinated-Cys antibody was validated by Dr. Norma Frizzell (Nagai et al. 2007, Journal of Biological Chemistry, 282, 34219-34228). |

## Eukaryotic cell lines

Policy information about cell lines

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|---|---|
| Cell line source(s) | HEK-293 cells were obtained from the NCI tumor cell repository. UOK262 (FH-/-), UOK262WT (FH +/+ rescue), UOK268 (FH-/-) and UOK268WT (FH +/+ rescue) cells were obtained from Linehan lab. |
| Authentication | HEK-293 cells were authenticated by ATCC by checking cellular morphology, karyotyping and short tandem repeat profiling. All UOK cell lines were authenticated by Linehan lab by checking cellular morphology, FH gene expression by RT-PCR, western blot analysis and biochemical FH activity assay. |
| Mycoplasma contamination | All cell lines used were tested and verified as free of mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | HEK293 cells were used as a model for nontransformed kidney cells. |