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Corresponding author(s): Ravid Straussman

Last updated by author(s): 2021.07.15

# 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<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

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| Data collection | The Harmony software version 4.1 was used to image and count GFP-positive cells imaged by the Operetta automated imaging system (PerkinElmer). |
| Data analysis | Detailed explanation of data analysis was given in the methods section.<br>In short - TopHat (version 2.0.10) algorithm was used to read alignment of bulk RNA-Seq reads. HTseq (version 0.6.1p1) was used for estimation of mRNA abundance. R package DEseq2 (version 1.6.3) algorithm was used for looking for differential expression. Quantification of western blots were done by ImageJ (version 1.53a). FlowJo version 10.7.1 was used for cell-cycle analysis by FACS. Raw MS files were analyzed by MaxQuant (version 1.5.3.36) with the integrated Andromeda search engine. The label-free quantification (LFQ) algorithm (version 1.5.3.36) was used in the proteome analysis.<br>The code used in the manuscript (Extended Data 10) is available at https://doi.org/10.5281/zenodo.5036166 |

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 mass spectrometry phospho-proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD026824, PXD026842, PXD026844, PXD026857, PXD026805. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD026834. RNA-seq data that support the findings of this study have been deposited in the

Gene Expression Omnibus (GEO) under accession codes GSE178978 and GSE179240.

# 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.

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| Sample size | No calculations were done to determine sample size. In most experiments, three biological repeats (with additional technical repeats) were done to compare between groups (e.g. clones with differential CTP, effect of siRNA/over-expression etc.). To demonstrate the continuous nature of CTP we generated ~400-550 clones from each cell line. This number was determined by our technical ability. First RNA-Seq experiments were done on eight different clones and as no significant results were achieved we increased this number to 194 clones. Here again, the number 194 was determined by the technical feasibility that we had at this time. |
| Data exclusions | In the phospho-proteomics experiments samples with lower coverage than expected (= less than 7000 phospho-sites detected), were excluded from the analysis. |
| Replication | Both technical and biological replications were done. The number of the technical and biological repeats are depicted in the text and in the legend for each figure and sup. figure separately. |
| Randomization | Randomization was used in the MARS-seq library preparation and in the analysis of the phospho-proteomics samples as described in the methods. |
| Blinding | Blinding was used in assessment of viability and pIRS staining by an independent pathologist as described in the methods. |

# 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 |
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| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

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| Antibodies used | pIGF1R (Tyr1165/1166) was purchased from Santa-cruz (#sc-135767) diluted 1:200. Anti pIRS1 ser1101 (LifeSpan BioSciences Inc; #LS-C352384, 1:100 dilution). phospho-ERK (Thr202/Tyr204) rabbit monoclonal Ab diluted 1:1000,pEGFR (Tyr1068) rabbit monoclonal Ab diluted 1:200, GAPDH rabbit monoclonal Ab diluted 1:10000 and HSP90 polyclonal Ab diluted 1:1000 were purchased from Cell Signaling (#4370, #3777, #2118, #4874). β-tubulin mouse monoclonal diluted 1:10000 was purchased from Sigma Aldrich (#SAB4200715). Anti-mouse secondary Ab and anti Rabbit secondary Ab were purchased from Li-Cor (#926-32211, #926-68070) both diluted 1:8000. |
| Validation | All antibodies used are commercially available.<br>pIGF1R validation details can be found at: https://www.scbt.com/p/p-igf-ir-antibody-50-y1165-1166<br>pIRS1 validation details can be found at: https://www.lsbio.com/antibodies/irs1-antibody-phospho-ser1101-ihc-wb-western-ls-c352384/363505<br>pERK validation details can be found at: https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370<br>pEGFR validation details can be found at: https://www.cellsignal.com/products/primary-antibodies/phospho-egf-receptor-tyr1068-d7a5-xp-rabbit-mab/3777 |

## Eukaryotic cell lines

Policy information about cell lines

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| Cell line source(s) | HCC2935 (#CRL-2869), G361 (#CRL-1424) and SKBR3 (#HTB-30) were purchased from the ATCC, EFM192A (#ACC 736) were purchased from DSMZ. The PC9 cell line was a gift from Dr. Channing Yu of the Broad Institute of Harvard and MIT. |
| Authentication | Cell-lines authentication by fingerprinting analysis was performed for SKBR3, PC9 (non clonal population) and two PC9 high CTP clones (Cl.4 and B12) to confirm their identity. HCC2935, G361 and EFM192A were not authenticated. |
| Mycoplasma contamination | All cell lines were tested negative for mycoplasma contamination by PCR. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified lines were used in this study. |

## Animals and other organisms

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

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| Laboratory animals | For the establishment of PDX models 6-week-old NOD/SCID mice were purchased from Harlan.<br>For NSCLC study twenty 8 weeks old male NOD/SCID mice were purchased from the SPF unit, the faculty of medicine, Bar Ilan, Israel.<br>For HNSCC study 7 weeks old female NOD/SCID mice were purchased from the SPF unit, the faculty of medicine, Bar Ilan, Israel. |
| Wild animals | No wild animals were used in the study. |
| Field-collected samples | No field collected samples were used in the study. |
| Ethics oversight | All procedures were performed under sterile conditions at the Faculty of Medicine, Bar Ilan University SPF facility, and carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the ethics committee of animal experiments of Bar Ilan University, Israel. |

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

## Human research participants

Policy information about studies involving human research participants

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| Population characteristics | NSCLC for ex-vivo organ culture (Fig. 5G) was obtained from Sheba medical center. Informed consent was obtained. |
| Recruitment | The patient was recruited by the surgeons involved in the study and was chosen depending only upon the availability of tissue and the willingness of the patients to participate in the study. Age, race, tumor location, presence of metastatsis, were not taken into consideration in choosing patients for the study. |
| Ethics oversight | The study was approved by the Sheba medical center IRB (protocol #SMC-4744) as well as by the Israeli ministry of health ethics committee (protocol #: 057-2007). |

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

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| Sample preparation | Floating and adherent cells were collected and fixed overnight in 100% cold methanol. Cells were rehydrated in PBS for 30 min, followed by staining with Propidium Iodide (25µg/ml)/ RNAse A (50µg/ml). |
| Instrument | BD LSR II |
| Software | FlowJo version 10.7.1 |

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| Cell population abundance | Average of 76,544 single-cells out of total of 100,000 cells were analyzed. |
| Gating strategy | Single cells were gated according to PI-A/PI-H. Cell cycle phases were determined by the cell-cycle module of FlowJo program based on PI fluorescence. |

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