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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 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 *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	NISElements (v4.4, Nikon) was used to acquire confocal IF microscopy images (Figure 5). Incucyte ZOOM/S3 control softwares (v2018a, ESSEN) were used to acquire microscopy images for the transferrin uptake assay (Figure 5).
Data analysis	Code to generate co-essential gene pairs, co-essential modules, modules with cancer type-specific dependencies, and the two-dimensional layout is available at https://github.com/kundajelab/coessentiality and run using Python (version 3.7.4). ClusterOne (version 1.2) was used to generate co-essential modules. Diffusion maps were implemented using NumPy (version 1.16.4) and SciPy (version 1.3.2) packages in Python. GLS was run using the Statsmodels package (Version 0.10.1) in Python. Student's t-tests were performed using Microsoft Excel 2016 or GraphPad Prism (version 9). Lipid molecular species were quantified with the Lipidyzer Workflow Manager using 54 deuterated IS developed with Avanti Polar Lipids covering 10 lipid classes (SCIEX) (Figure 4). Incucyte ZOOM or S3 software (v2018a, ESSEN) was used for automated microscopy analysis (Figure 5). Raw proteomics data was processed using Thermo Proteome Discoverer software version 2.2 (Figure 5, Extended Data Figure 9b).

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 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 dataset used to determine co-essential interactions consists of the 485 genome-wide CRISPR screens from the Achilles project 18Q3 release. Specifically, 17,634 genes were screened in 485 cell lines from 27 distinct lineages using the Avana CRISPR library, and gene-level effects were quantified using the CERES algorithm to account for variability in guide effectiveness and copy number across lines, resulting in a 17,634 x 485 matrix of normalized gene-level effects. Intuitively, gene-level effects represent the number of times fewer cells with the knockout doubled during the screen, compared to control cells. This dataset is publicly available at https://ndownloader.figshare.com/files/12704099, or at https://depmap.org/portal/download/all/ under release "DepMap Public 18Q3" and file "gene_effect.csv". The HUGO Gene Nomenclature Committee Database is accessible at https://www.genenames.org/. The STRING database is accessible at https://string-db.org/. The CORUM database is accessible at https://mips.helmholtz-muenchen.de/corum/. The hu.MAP database is accessible at http://proteincomplexes.org/. The DoRothEA database is accessible at https://saezlab.github.io/dorothea/. The COXPRESdb database is accessible at https://coxpresdb.jp/. Other data in support of this study, such as primer and plasmid sequence data and raw imaging data, are available on reasonable request. Lipidomics raw data, acquisition methods, and quantitative results are available as Supplementary Data 5-7. The raw mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (http://www.ebi.ac.uk/pride) with the dataset identifier PXD023558.

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	For in vitro transferrin uptake assays, lipidomics and proteomics analysis, sample size is indicated in the figure legend or in the description in the methods. The sample size was determined based on previous experience for each experiment to detect specific effects and it was not predetermined with any statistical methods. For the pHrodo-transferrin uptake assay, experiments were performed in triplicate (similar to the pHrodo-EGFR uptake assays in Tsui, C.K., Barfield, R.M., Fischer, C.R. et al. CRISPR-Cas9 screens identify regulators of antibody–drug conjugate toxicity. Nat Chem Biol 15, 949–958 (2019). For lipidomics, samples were prepared in quadruplicate as is frequently used for lipidomic studies (e.g. Nowinski, Sara M., Ashley Solmonson, Scott F. Rusin, J. Alan Maschek, Claire L. Bensard, Sarah Fogarty, Mi-Young Jeong et al. "Mitochondrial fatty acid synthesis coordinates oxidative metabolism in mammalian mitochondria." Elife 9 (2020): e58041.). Samples for proteomics were prepared in duplicate, as is sometimes done for IP-MS studies (e.g. Nobre, Luis V., Katie Nightingale, Benjamin J. Ravenhill, Robin Antrobus, Lior Soday, Jenna Nichols, James A. Davies et al. "Human cytomegalovirus interactome analysis identifies degradation hubs, domain associations and viral protein functions." Elife 8 (2019): e49894.)
Data exclusions	No data were excluded.
Replication	Once experiments and procedures were fully optimized, all attempts at replication were successful.
Randomization	HeLa cell knockout pools were allocated into experimental groups based on their genotype (Figures 4, 5).
Blinding	No blinding was performed in the lipidomics, proteomics experiments because the automated nature of data collection and analysis removed the potential for bias. Additionally, samples for proteomics were labeled with TMT reagents and analyzed in a pooled format, making blinding impossible during data acquisition. No blinding was performed in the transferrin uptake assays as all measurements and analysis were automated and quantitative and thus prevented any bias. Additionally blinding was not feasible in this assay because the uptake defect was visually obvious. Blinding is not commonly performed for similar pHrodo-based assays performed using the Incucyte automated imaging system because the automated analysis prevents user bias (e.g. McQuade, A., Kang, Y.J., Hasselmann, J. et al. Gene expression and functional deficits underlie TREM2-knockout microglia responses in human models of Alzheimer's disease. Nat Commun 11, 5370 (2020); Tsui, C.K., Barfield, R.M., Fischer, C.R. et al. CRISPR-Cas9 screens identify regulators of antibody–drug conjugate toxicity. Nat Chem Biol 15, 949–958 (2019).

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

The following antibodies were used: Rabbit polyclonal anti-TMEM189 (HPA059549, Sigma, 1:250), mouse monoclonal anti-GAPDH (AM4300, Fisher), rabbit polyclonal anti-mCherry (ab167453, Abcam), mouse monoclonal anti-GFP (A-11120, Thermo Fisher) and rabbit polyclonal anti-beta actin (ab8227, Abcam).

Validation

GFP and mCherry antibodies were validated by western blot (Figure 5e) on HeLa cells expressing GFP, mCherry, or control constructs.

TMEM189 antibody (Figure 4) was validated by western blot on TMEM189 knockout HeLa cells.

See the following "Manufacturers Statement" for the other antibodies :

GAPDH mouse mAb (Fisher) recognizes endogenous levels of total human GAPDH protein. Monoclonal antibody is produced by immunizing animals with purified rabbit muscle GAPDH and is confirmed to be reactive towards human GAPDH. Reactivity has been confirmed on western blots with human SH-SY5Y and PC-3 cell lysates, and identifies the target band at ~37 kDa. Product Citations: Chalmers, Fiona, et al. "Inhibition of IRE1α-mediated XBP1 mRNA cleavage by XBP1 reveals a novel regulatory process during the unfolded protein response." Wellcome open research 2 (2017). Crummy, Ellen, et al. "The priming factor CAPS1 regulates dense-core vesicle acidification by interacting with rabconnectin3β/WDR7 in neuroendocrine cells." Journal of Biological Chemistry 294.24 (2019): 9402-9415.

Beta actin rabbit polyclonal antibody (Abcam) recognizes endogenous levels of total human GAPDH protein. Polyclonal antibody is produced by immunizing animals with synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human beta Actin aa 1-100. Reactivity has been confirmed on western blots with human HeLa and HEK293T cell lysates, and identifies the target band at ~42 kDa. Product Citations: Li Y et al. LncRNA-LET relieves hypoxia-induced injury in H9c2 cells through regulation of miR-138. J Cell Biochem 121:259-268 (2020). Xi X et al. MicroRNA-204-3p represses colon cancer cells proliferation, migration, and invasion by targeting HMGA2. J Cell Physiol 235:1330-1338 (2020). Jin Z et al. miR-330-3p suppresses liver cancer cell migration by targeting MAP2K1. Oncol Lett 18:314-320 (2019).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HeLa cells are from ATCC.
Authentication	HeLa cells were authenticated by the vendor (ATCC) using short tandem repeat (STR) profiling to distinguish between individual human cell lines and rule out intra-species contamination.
Mycoplasma contamination	Cell cultures were routinely tested and found negative for mycoplasma infection (MycoAlert, Lonza).
Commonly misidentified lines (See ICLAC register)	None of the cell lines used in this study are in the database of commonly misidentified cell lines.