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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 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	Incucyte s3 software (Essen) was used for automated microscopy. BD Accurri was used for flow cytometry. Odyssey CLx imaging system (LI-COR) was used for Western blot imaging. NIS-Elements software (Nikon) was used for confocal microscopy imaging. MassHunter Software suite was used for LC/MS data collection and analysis (Agilent).
Data analysis	For screen analysis we used custom Python scripts available at https://bitbucket.org/dmorgens/castle (see methods section, 'Genome-wide CRISPR-Cas9 screens in Ramos cells'). Incucyte s3 software (Essen) was used for automated microscopy analysis. Flowjo 9.9 was used for flow cytometry analyses. ImageJ 1.5i was used for Western quantification (https://imagej.nih.gov/ij/), Agilent MassHunter Software was used for LC/MS analysis (https://www.agilent.com/en/products/software-informatics/masshunter-suite/masshunter/masshunter-software).

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 complete results of all screens in Ramos cells are in Table S1-4. The sequences of sgRNAs used in this work are in Table S6. The uncropped Western blots with size marker indications are summarized in the Supplementary Figure. All data are available from the corresponding author upon reasonable request.

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 the screen, two independent replicate screens were performed, which are sufficient for screening technologies as previously reported. See Methods section, 'Genome-wide CRISPR-Cas9 screens in Ramos cells'.
Data exclusions	No data was excluded.
Replication	Once experiments and procedures were fully optimized, all attempts at replication were successful. Number of replicates for each experiment is indicated in figure legends.
Randomization	Randomization was not performed. Knock out clones were allocated into experimental groups based on their genotype. Negative control cells were grown together with experimental cells as control.
Blinding	Blinding was performed in assessing confocal microscopy images. Other experiments were not blinded.

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	mouse monoclonal anti-EEA1 (1:1000, BD 610457) rabbit monoclonal anti-LAMP1 (1:1000, CST 9091S) rabbit monoclonal anti-EGFR (1:2000, abcam ab52894) rabbit monoclonal anti-CTSD (1:1000, abcam ab75852) mouse monoclonal anti-GAPDH (1:10000, Fisher AM4300) goat polyclonal anti-Human IgG Fcγ fragment specific, Alexa Fluor 488-conjugated (1:500, Jacksons Immuno Research 109-545-098) goat anti-mouse IRDye 680RD (1:10000, Licor 925-68070) anti-Her2 ADC TDM1 (Kadcyla) was a gift from lab of Mark Pegram All other antibodies (Anti-CD22 ADC, anti-CD79b) were made in-house and concentration used is described in main text.
Validation	anti-EEA1 (BD 610457), validation: Hsu, F. et al. Rab5 and Alsin regulate stress-activated cytoprotective signaling on mitochondria. Elife 7, (2018). anti-LAMP1 (CST 9091S), validation: Bento, C. F., Ashkenazi, A., Jimenez-Sanchez, M. & Rubinsztein, D. C. The Parkinson's disease-associated genes ATP13A2 and SYT11 regulate autophagy via a common pathway. Nat. Commun. 7, 11803 (2016). anti-EGFR (abcam ab52894), validation: Xu, J. et al. HER2 overexpression reverses the relative resistance of EGFR-mutant H1975 cell line to gefitinib. Oncol. Lett. 12, 5363–5369 (2016). anti-GAPDH (Fisher AM4300), validation: Liao, G., Wang, R., Rezey, A. C., Gerlach, B. D. & Tang, D. D. MicroRNA miR-509 Regulates ERK1/2, the Vimentin Network, and Focal Adhesions by Targeting Plk1. Sci. Rep. 8, 12635 (2018).

anti-Human IgG (Jacksons Immuno Research 109-545-098), validation: Zhong, G et al. Rational design of aptazyme riboswitches for efficient control of gene expression in mammalian cells. Elife. pii: e18858 (2016).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cell lines used: Ramos (RA 1) (ATCC® CRL-1596™) K562 (ATCC® CCL-243) SKBR3(ATCC® HTB-30™) ZR-75-1(ATCC® CRL-1500™) NCI-N87(ATCC® CRL-5822) All cell lines were obtained from ATCC.
Authentication	Cell lines were authenticated by the vendor (ATCC). No additional authentication has been performed.
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.

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	Cells were collected and washed in cold dPBS, stained on ice for 30 minutes using antibody (anti-CD22-AF488) or fluorescently-labeled lectins (SNA,MAA2, PNA, or ECL). Cells were then washed 3x with cold dPBS and analyzed by flow cytometry.
Instrument	BD Accurri
Software	BD Accuri C6 and FlowJo (v.10.5)
Cell population abundance	All analyzed samples are pure samples that has undergone identical staining procedure.
Gating strategy	Cells were first gated on FSS/SCC for live cells. Unstained cells and cells incubated with free fluorophore was used to determine the boundaries between negative and positive populations.

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