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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	Luminex xPONENT version 4.2 was used to collect data.
Data analysis	Data analysis was performed in R version 3.5.1. using custom or publicly-available R packages. Individual packages are explicitly cited in the manuscript and listed below. The custom code is available upon request and from GitHub (https://github.com/broadinstitute/repurposing). Graphpad PRISM version 8 was used for curve fitting and dose response plots. The following software packages were used for data analysis: DESeq2 v3.10 Limma v3.38.3 Atlantis (cancerdatasci/atlantis SHA: 167fc0a338fc2686a27463eb55546f6e316d91b0 on github) MAGeCK v0.5 sva 3.30.1

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

Raw and processed PRISM viability data are available from the Cancer Dependency Map portal (https://depmap.org/repurposing) and archived via Figshare

(doi:10.6084/m9.figshare.9393293). An interactive version of Figure 2a and Figure 4 (with accompanying raw data) is also available on the Cancer Dependency Map portal; scatter plot source data is also deposited in Figshare (doi:10.6084/m9.figshare.9393293). The cell line features used for biomarker analysis are listed in Supplementary Table 10 and also archived via Figshare (doi:10.6084/m9.figshare.10277810). RNA sequencing data were deposited in the Gene Expression Omnibus (GEO, accession number GSE133299). All other data supporting the findings of this study are available from the corresponding author on 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	No sample size calculation was performed. PRISM cell lines were selected based on ability to be transduced with lentivirus and adapt to shared culture conditions. In the primary screen, data was collected for 578 cell lines. 489 cell lines were included in the secondary screen based, in part, on primary screen QC performance. The 578 cell line panel spans a range of multiple cancer types, patient age (pediatric and adult cancer lines), and gender.
Data exclusions	A standardized QC process was employed for the processing of PRISM data. The rationale for filtering data was to remove low quality data points due to technical failures. Replicate-level outliers were filtered using the approach fully described in Methods and Extended Data Fig. 2-4. All filtered data is still included in the raw data files.
Replication	The PRISM screen was performed with 3 independently treated well replicates. Multiple independent cell treatments were performed for confirmatory studies as indicated in the Methods. Results shown are indicative of multiple independent experiments as indicated in the figure legends.
Randomization	All cell lines were tested with active treatments and a vehicle control. No individual experimental groups were used that would necessitate a randomized design.
Blinding	PRISM data was generated without the investigators' knowledge of compound and cell line identities during screening. Investigators were not blind to compound and cell line identities during analysis in order to interpret results within the context of known drug activities.

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	The following antibodies were used: polyclonal rabbit anti-PDE3A from Bethyl Laboratory (A302-740A, 1:1000 dilution), monoclonal mouse anti-V5 from Life Technologies (R960-25, 1:5000 dilution), monoclonal rabbit anti-ABCB1 (D3H1Q) from Cell Signaling Technology (12683, 1:1000 dilution), monoclonal mouse anti-β-Actin (8H10D10) from Cell Signaling Technology (3700, 1:1000 dilution) , and monoclonal rabbit anti-SLC26A2 Antibody (3F6) from Novus Biologicals (H00001836-M04, 1:1000 dilution).
Validation	We validated the ABCB1 and SLC26A2 antibodies by performing gene knockout by CRISPR/Cas9 as reported in this manuscript. For the anti-V5 antibody, the manufacturer provides western blot validation by expressing a V5-tagged transgene. For the PDE3A antibody, IP validation is available from the manufacturer and in the literature (PMID26656089). The actin antibody has validation western blot data available from the manufacturer and is widely cited in the literature.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Parental cell lines were obtained from the Broad-Novartis Cancer Cell Line Encyclopedia (CCLE) project prior to PRISM barcoding (see https://portals.broadinstitute.org/ccle for original sources). For follow-up studies, LS1034, HeLa, HEK293T cells were purchased from the American Type Culture Collection. REC1, SF295, OVISE, COLO320, BEN, HT29 and SNU449 were provided by CCLE. Wildtype and ABCB1 overexpressing (pLX_317 vector) Kuramochi cell lines were gifts from Elizabeth Stover (PMID31462500). HeLa PDE3A CRISPR KO cells (PDE3A-/- cells) were previously described (PMID26656089). LS1034, SF295, A2058, COLO320, SNU449 and OVISE cell lines derived with Cas9 were provided by the Broad Cancer Dependency Map.
Authentication	STR fingerprinting was performed by Genetica using the PowerPlex® 16 HS system (Promega). STR profiles were compared with STR profiles reported by vendors and in literature. Misidentified cell lines or other STR conflicts are listed in Supplementary Table 9. These cell lines are flagged in the data files and are not shown by default in the website interface.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	KPL-1/MCF-7 and NCI-2077/NCI-H1581 have been listed in ICLAC as misidentified or derivative lines.