nature research

Corresponding author(s): Typas

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 *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 *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 *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	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	Data analysis was performed in RStudio Version 1.0.136, using R version 2.15.1. Scripts deposited at https://github.com/AnaRitaBrochado/DrugInteractionsPipeline. Iris (doi:10.1038/nmicrobiol.2017.14) was used to process images of the E. coli gene knockout collection.

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

All data generated during this study are included in this published article and its Supplementary Information files.

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	No prior assumptions were made regarding effect sizes.
Data exclusions	Replicates with inconsistent growth behavior in the screen and validation dataset were excluded from our analysis. For follow up experiments, data exclusion was minimized to cases in which clear outliers due to technical mistakes were spotted. For noisy experiments, we increased the number of replicates.
Replication	We have at least 4 replicates for each drug combination for the screen and present the reproducibility of the data in ED Fig. 3. We have at least 3 (in most times more) replicates for all follow up experiments, and data are always quantified and measurement error is presented. We benchmark our screen against an independently generated validation dataset. For higher resolution checkerboards used for validation experiments and for testing MDR strains, two independent biological replicates were done, and data were kept only if the two agreed. We also performed a sensitivity analysis for the most important statistical parameters of our interaction score, ensuring that the main findings are robust to small parameter changes.
Randomization	Duplicates of the same drugs were randomly distributed in the plates, instead of placed just next to each other, to avoid biases due to any plate spatial effect.
Blinding	in vitro experiments/no blinding required

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	The anti-ArcA polyclonal primary antibody (custom made, Neoystems France) was provided by K.M. Pos (Goethe University, Frankfurt), and used in this study in 1:200,000 dilution in combination with an alkaline-phosphatase-conjugated anti-rabbit secondary antibody (A0545 Sigma, 1:5,000 dilution).
Validation	The anti-AcrA antibody was validated for specificity using the knockout strain.