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Reporting Summary

Nature Portfolio 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 Portfolio 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	OD600 growth curves were acquired on a BioTek Epoch2 microplate reader using BioTek Gen5 acquisition software (readings taken through the 20 h endpoint). Barcode fitness measurements used 150-nucleotide paired-end Illumina sequencing on a NovaSeq X Plus (300-cycle); base-calling and demultiplexing used the instrument's standard onboard Illumina software. No custom code was used to collect data.
Data analysis	Raw reads were processed to per-barcode counts with cutadapt 5.0, bowtie2 2.5.4, and minimap2 2.26. All downstream analysis was performed in Python 3.12.12: Polars 1.38.1, NumPy 2.4.2, SciPy 1.17.1, and pandas 3.0.1 (data handling and statistics); LightGBM 4.6.0, scikit-learn 1.8.0, SHAP 0.49.1, and PyTorch 2.10.0 (regression, classification, and model interpretation); matplotlib 3.10.8, Altair 6.0.0, logomaker, and joypy (figures). Figure 6 evolutionary statistics used py-mfdca (mean-field direct-coupling analysis; pinned commit 24bb3adf) together with pyhmmer/HMMER for family back-mapping. The Latent Generative Landscape used for Figure 6D–F is the LGL-VAE model at https://github.com/morcoslab/LGL-VAE. All custom analysis code is public (GitHub + Zenodo, below); the complete environment is pinned in pyproject.toml + uv.lock and reproducible with uv sync.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Processed fitness and epistasis tables, per-genotype AUC and barcode read counts, the Pfam PF13354 alignment underlying Figure 6, and the analysis code are available in the public repository https://github.com/msadikyildiz/Gaszek_Yildiz_Meng_2025, archived at Zenodo (concept DOI 10.5281/zenodo.21442350, which resolves to the latest version). Raw barcode-sequencing reads are deposited in the NCBI Sequence Read Archive under BioProject PRJNA1370879 and will be released upon publication. The catalogue of clinically observed TEM-1 substitutions was compiled from the NCBI beta-lactamase resource (https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/). Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Not applicable — no human participants.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable — no human participants.
Population characteristics	Not applicable — no human participants.
Recruitment	Not applicable — no human participants.
Ethics oversight	Not applicable — no human participants.

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

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	The combinatorial mutant library (TEM-1^CML) was designed to be combinatorially complete across the 18 clinical substitutions at 13 residues, yielding exactly 55,296 genotypes; no statistical sample-size calculation was used because the library is a full enumeration of the chosen mutational subspace rather than a random sample. Every genotype was assayed in three independent biological replicates per drug × concentration × timepoint (n = 3). The monoculture IC50/MIC validation panel comprised n = 13 variants for ampicillin and n = 18 variants for aztreonam, chosen to span the observed fitness range.
Data exclusions	Genotypes that did not match the intended combinatorial design were excluded before analysis (pre-established criterion). Low-read genotypes — flagged by a pre-established majority_low filter (≥2 of 3 replicates with a summed read count <10 at a given concentration) — were retained in the primary analysis and drawn as open markers in Supplementary Figure S17; their fraction per condition is disclosed. A library-invariance analysis confirmed that the main-text conclusions are preserved when the low-read tail is removed (pairwise-epistasis matrix r = 1.00; order-resolved R^2 change ≤ 0.05; SHAP-ranking Spearman ρ = 0.87).
Replication	Three independent biological replicates were performed per drug × concentration × timepoint (n = 3), each initiated from a separate culture of the same TEM-1^CML stock, grown in parallel under identical antibiotic regimens and sampled at 3 h intervals over 12 h. Replicate agreement is quantified in Supplementary Figure S16: pooled-replicate Pearson r ranges 0.69–0.85 at informative concentrations (declining at sub-MIC and supra-MIC extremes, as expected from compressed dynamic range and extinction-driven undersampling). Independent monoculture IC50/MIC measurements reproduce the pooled fitness ranking (Pearson r = 0.88 for AMP, 0.80 for AZT; Supplementary Figures S7–S8). All replication attempts were successful.
Randomization	Randomization is not applicable to this pooled-library design: all 55,296 genotypes are measured simultaneously in a single competition culture per condition, so there is no allocation of samples to experimental groups. Drug and concentration are the only imposed variables and are applied to aliquots of the same starting pool.
Blinding	Blinding is not relevant. The pooled fitness readout is a fully automated sequencing-and-counting pipeline with no subjective scoring. The monoculture IC50/MIC validation was quantified from OD600 readings via an automated pipeline (validation module), so no investigator judgement enters data acquisition or scoring.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.