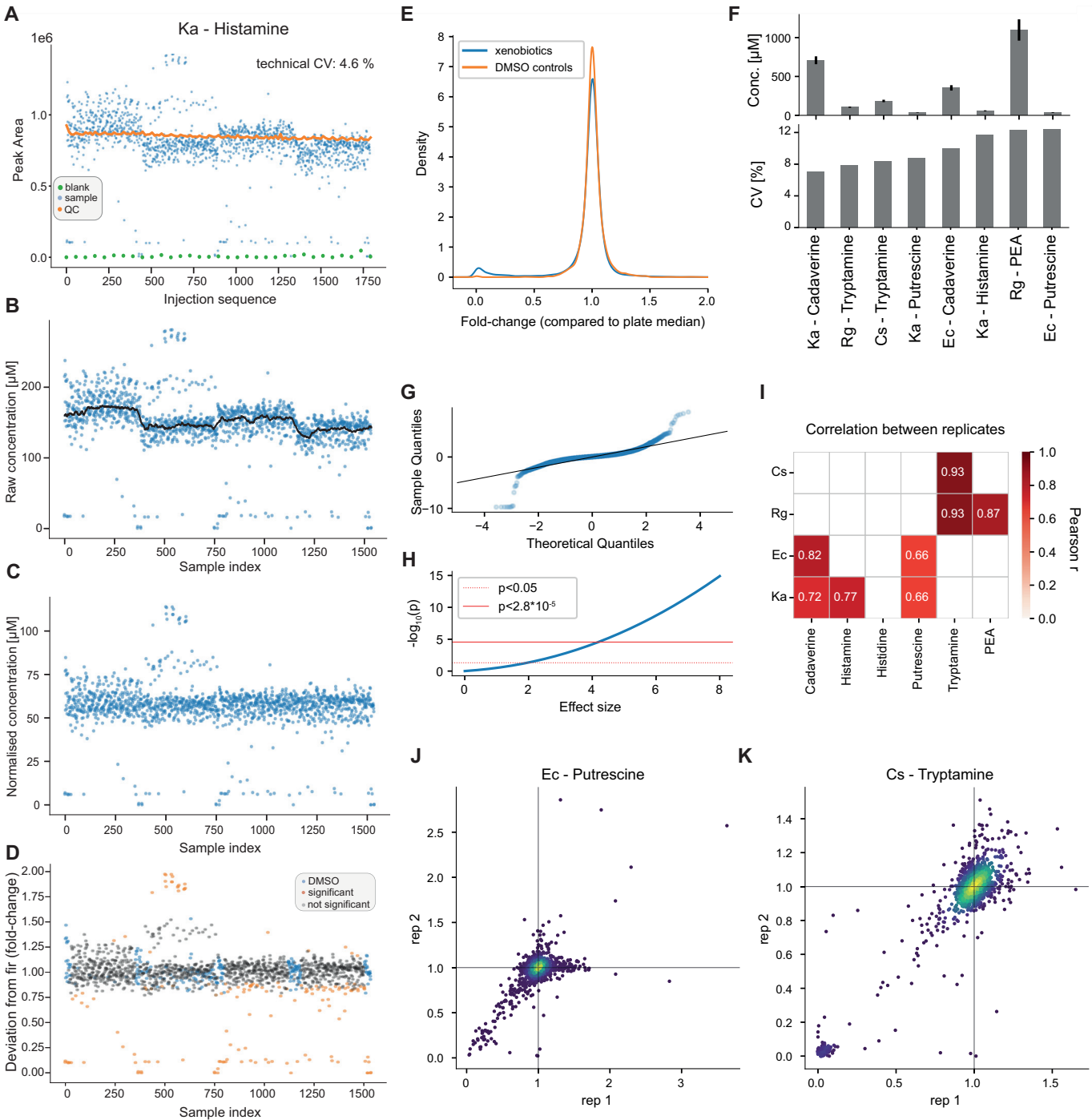
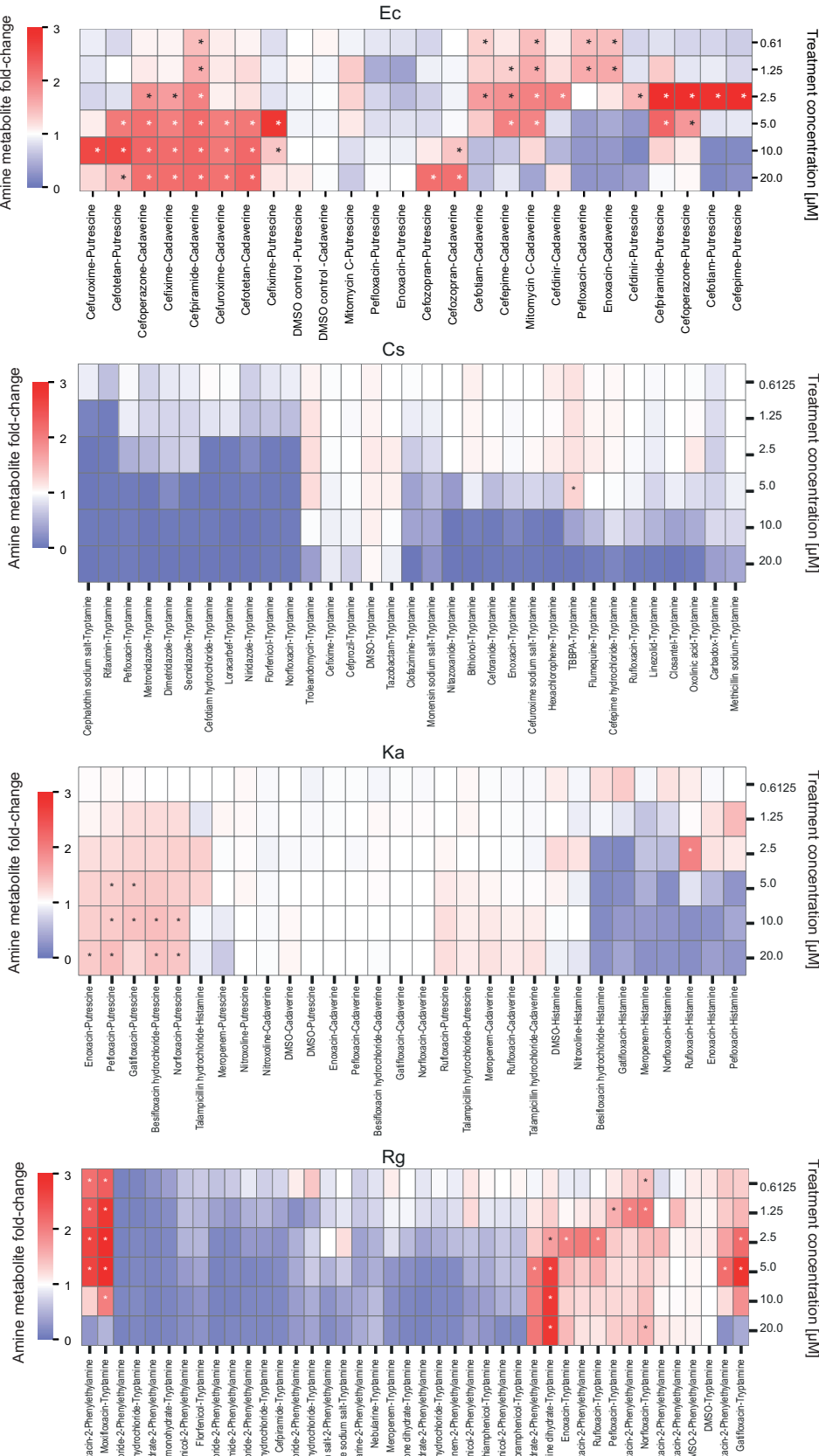


Expanded View Figures

Figure EV1. Quality control indicators.

(A) In each LC-MS run, performance was monitored by regular injection of serially diluted analytical standards, a QC sample (DMSO-control sample from respective bacterial species) and water blanks. All runs were inspected manually and a technical coefficient of variation (CV, indicating the stability of instrument performance) was computed based on the distribution of values obtained for the QC sample. (B) Batch variation between each 384-well sample plate was apparent. This was corrected using the strategy described in Methods. In brief, a smoothed line (black) was fitted to outlier-filtered data and the distance of each datapoint to the line was added to the overall median to produce batch-corrected concentration estimates. (C) Concentration values after normalisation. (D) Hit calling was performed on each sample (ie biological replicate) separately. The deviation of the sample from the fitted line (see B) was divided by the standard deviation of the DMSO controls, for each 96-well culture plate (indicating the degree of combined biological and technical noise in the assay) separately. This z-score was converted to a p value using the normal distribution. (E) Distribution of fold changes (relative to mean of DMSO controls) for DMSO controls and xenobiotic-treated samples. As the vast majority of compounds had no effect on a bacterial species, the distributions are similar, although increased density around 0 is observed in treated samples. (F) Based on DMSO controls, the baseline median concentration (top) and the overall level of biological and technical noise in the assay (bottom) were quantified. CVs fell between 8 and 18%, a typical range observed in biological mass spectrometry. $N > 600$ biological replicates, error bars indicate the standard deviation. (G) Quantile-quantile (Q-Q) plot of fold-change values of DMSO controls across all species and amines. This indicates approximately normal distribution of the values, albeit with slightly heavy tails. (H) Power calculation of the Z-test used to determine likelihood that individual datapoints are compatible with the null distribution derived from DMSO controls. The red lines indicate significance thresholds without multiple testing correction ($P < 0.05$) and with Bonferroni correction (1772 compounds). FDR correction with the Benjamini-Hochberg method was applied to the data. The Bonferroni threshold is shown as this is the initial, strictest threshold applied in this method. (I) Two biological replicates were recorded for each xenobiotic-species pair. The Pearson correlation between these replicates is indicated in the heatmap. (J, K) Representative plots illustrating the correlation between the two biological replicates for different metabolite-species pairs.





**Figure EV2. Dose-response behaviour for selected xenobiotics.**

Heatmaps show amine metabolite fold changes across species (panels), compound-metabolite pairs (rows) and treatment concentrations (columns). Hits (same criteria as main screen: mean $\log_2(\text{fold change}) > 0.32$, both replicates $P_{\text{adj}} < 0.05$ (two-sided FDR-corrected z-test), consistent direction of metabolite change) with increased amine production are marked with '*'. Heatmaps columns were clustered using Euclidean distance and the Average method.

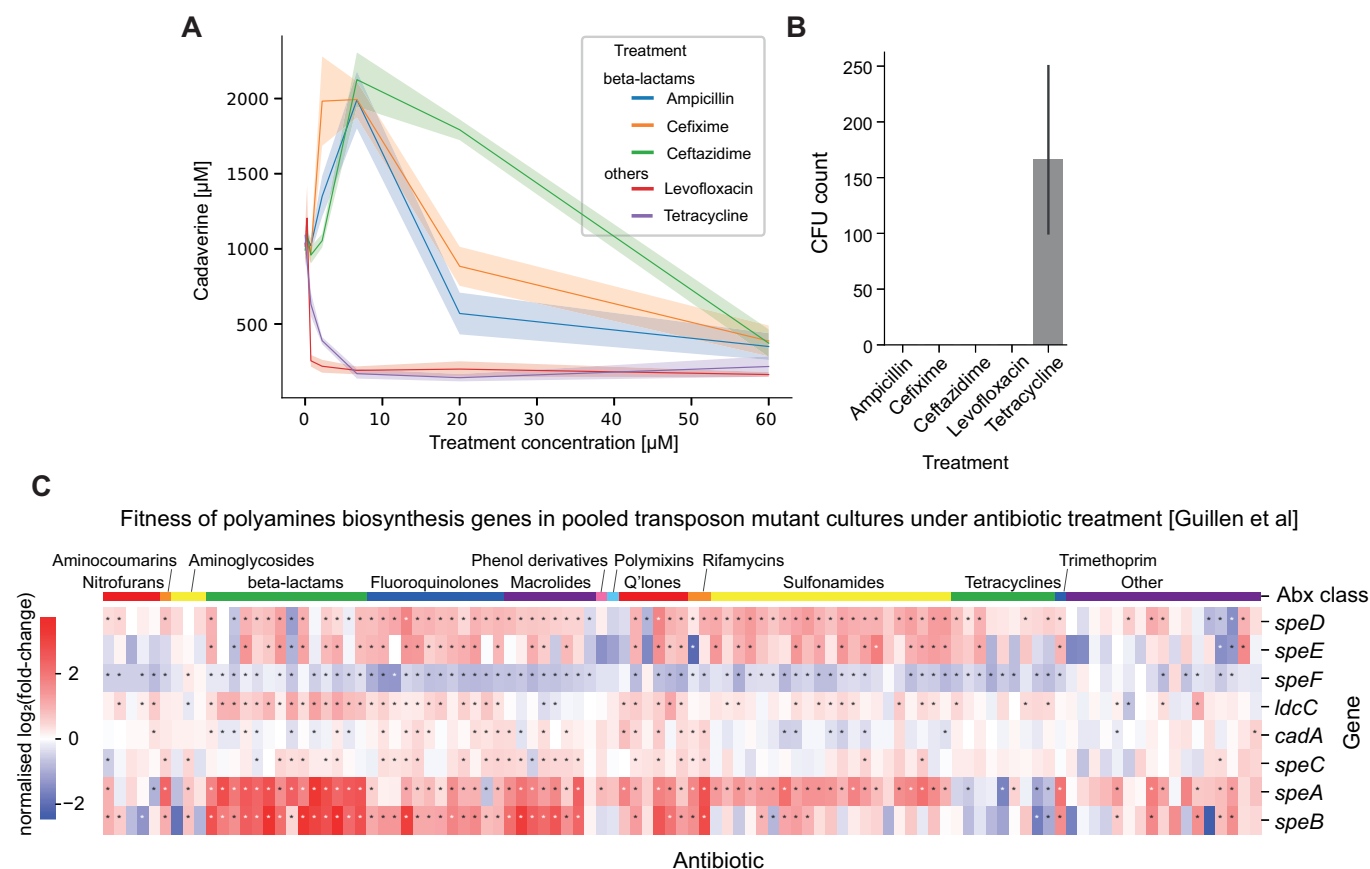


Figure EV3. Additional antibiotics experiments.

(A) Validation of the main screen results using a subset of antibiotics from different classes, prepared and measured independently. Lines indicate the mean and shaded areas the standard deviation of $n = 3$ biological replicates. (B) Number of colony-forming units of *E. coli* after 3.5 h incubation in mGAM medium with 20 μM of each antibiotic. Bar heights indicate the mean and error bars the standard deviation of $n = 3$ biological replicates. (C) Analysis of published dataset by Guillen et al (Noto Guillen et al, 2024) capturing the fitness of pooled, genome-wide knock-out mutants in the presence of various drugs. The analysis is based on Supplementary Table 3 in the above publication, using the normalised $\log_2(\text{fold-change})$ data (sheet 'normLFC') and adjusted P values (sheet ' P_{adj} ', see the source publication for details). We filtered the data for known polyamine biosynthetic enzymes (y axis) and antibiotics (column antibiotic == 1). Significant interactions are marked "*", using the same cut-off of $P_{\text{adj}} < 0.25$ as used by the authors. Rows were clustered using Euclidean distance and the Average method. Columns were sorted by antibiotic class.

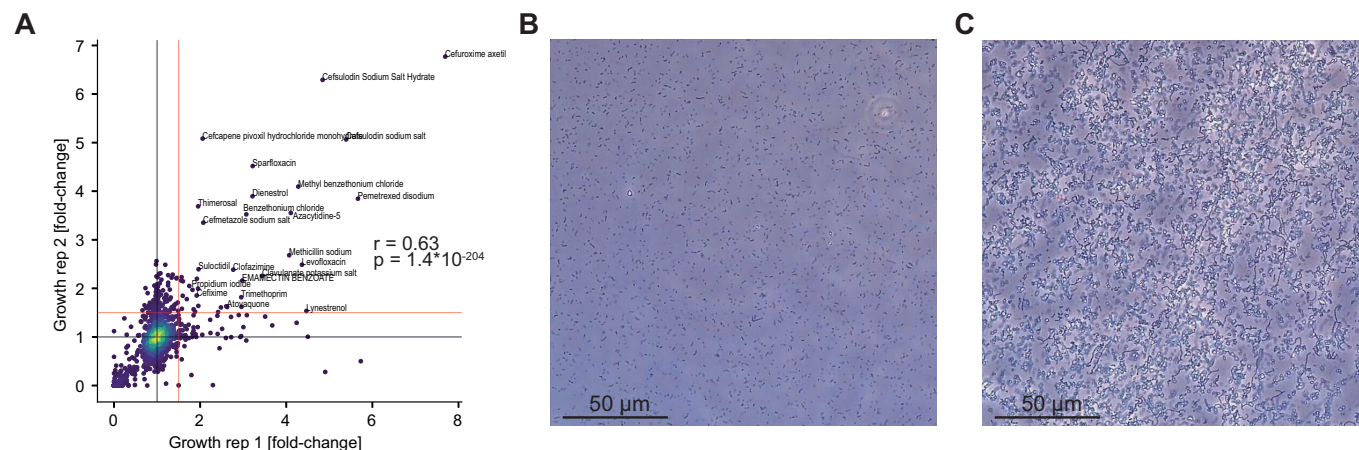


Figure EV4. Cell aggregation in *R. gnavus* confounds OD measurements.

(A) Growth fold changes for *R. gnavus* across the two biological replicates. The Pearson correlation coefficient and associated two-sided *P* value is shown in the plot. Very high values are often consistent across replicates. (B) Phase contrast micrograph of *R. gnavus* cells in control conditions (mGAM 1% DMSO). Images were acquired using a Leica DM1000 LED microscope, a 20x/0.4 PH1 objective and Leica ICC50 W camera. (C) Similar micrograph of *R. gnavus* cells treated with compound causing abnormally high OD readings.