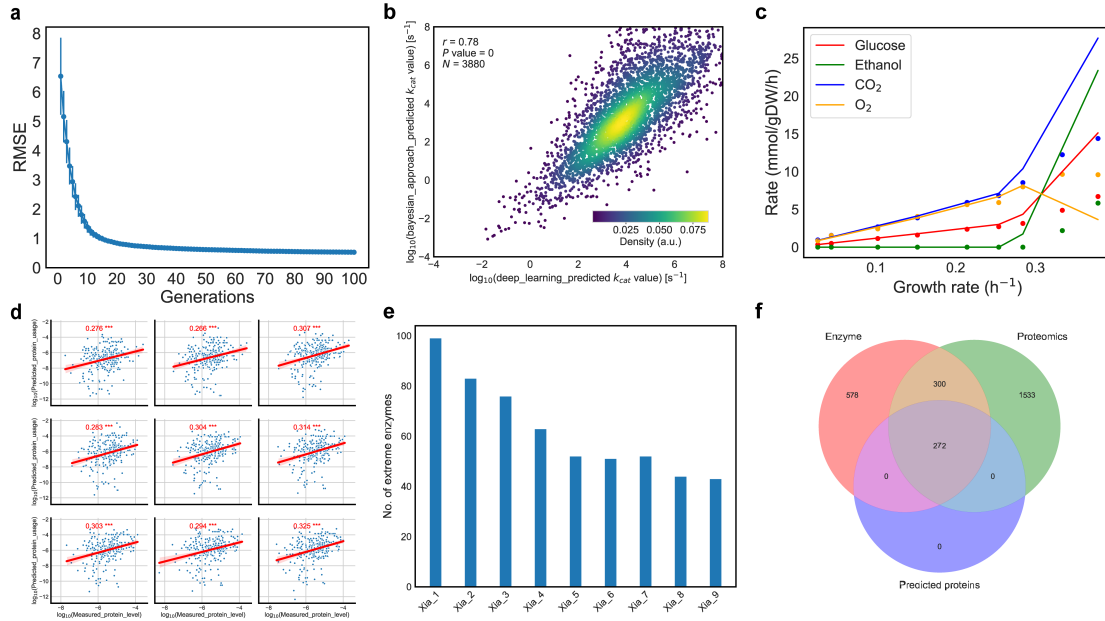


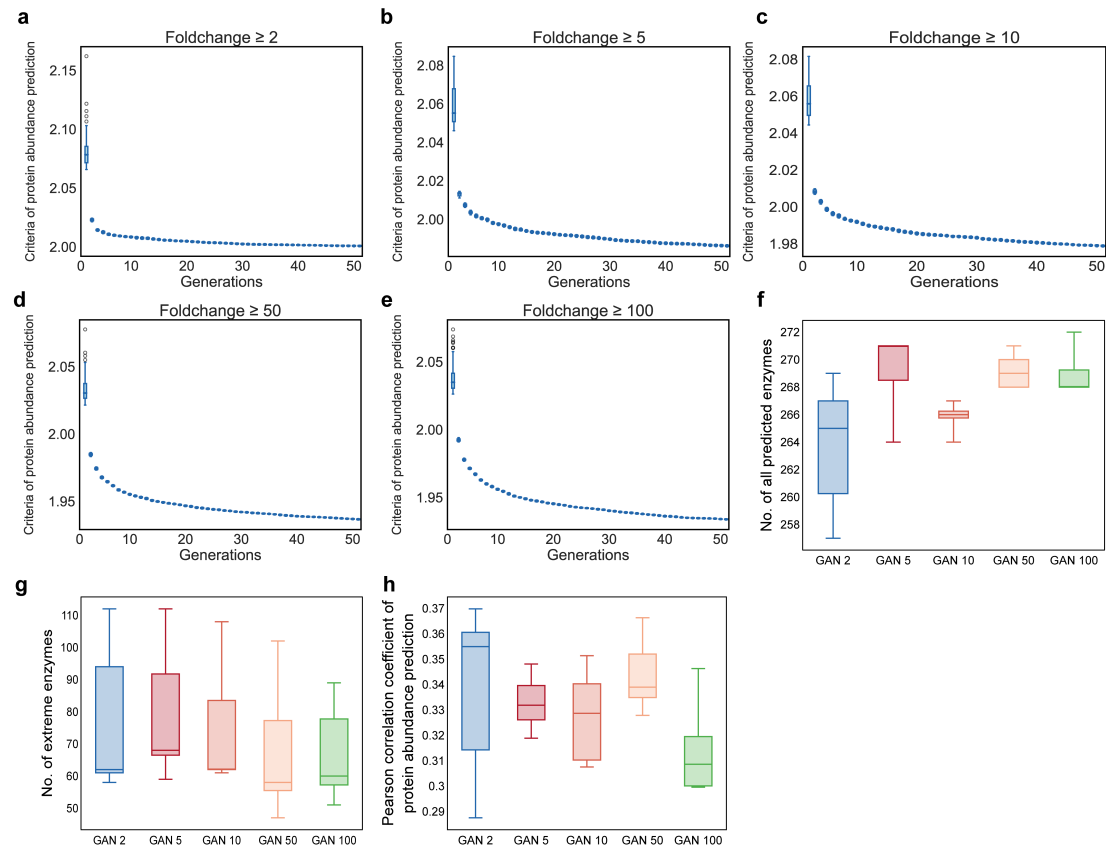
1	Supplementary Figures for	
2	EnzymeTuning Improves Enzyme-constrained Metabolic Modeling and	
3	Proteome Abundance Prediction Through Deep Learning	
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Supplementary Fig. 1 Baseline performance of ecGEM prior to k_{cat} optimization.

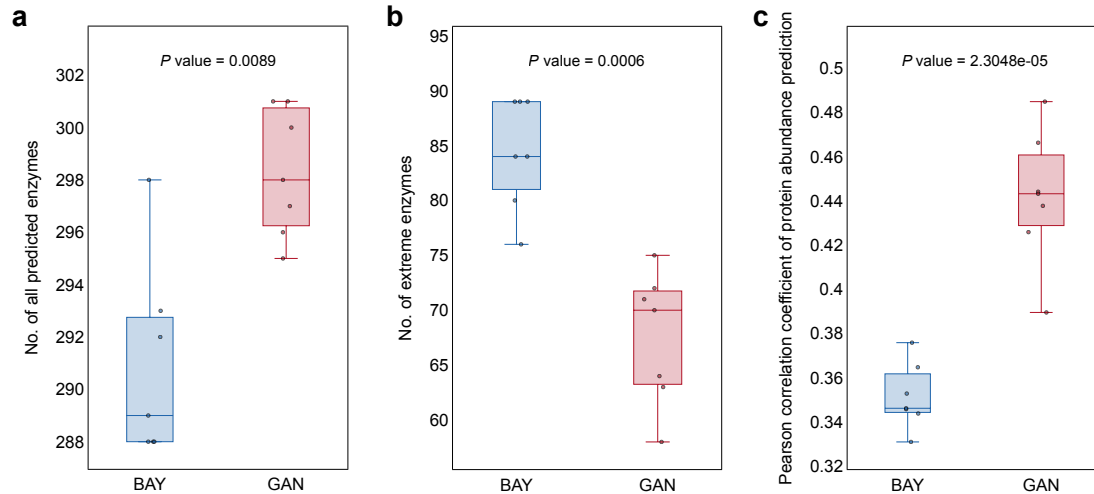
a The RMSE of phenotype measurements and predictions in ecGEM. Data are presented as median $\pm$ SD based on generated k_{cat} parameter sets ($n=100$). **b** Pearson correlation coefficients between k_{cat} values inferred by deep learning-based method and those optimized via the Bayesian approach. The significance of the correlation was assessed using t-test. **c** Predicted exchange rates for the Crabtree effect based on the Bayesian-posterior-parameterized k_{cat} values (lines) compared with experimental data (dots). Raw values are provided in the source data. **d** Performance of ecGEM with Bayesian-posterior-parameterized k_{cat} values in predicting quantitative proteome. Statistical significance was determined using a two-sided t-test. ns: not significant, *: P value < 0.05 , **: P value < 0.01 , ***: P value < 0.005 . **e** The number of extreme enzymes in the protein abundances prediction. **f** Venn diagram representing the overlap among measured proteins, predicted proteins, and enzymes included in the ecGEM.



Supplementary Fig. 2 Comparison of optimization performance for extreme enzymes under different definitions.

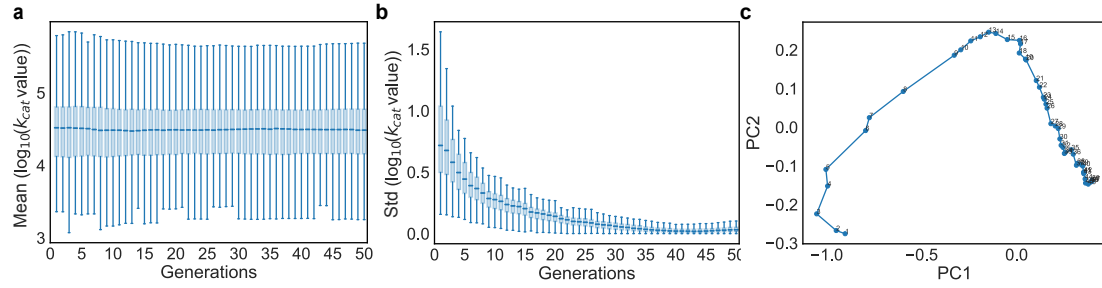
a-e Performance of k_{cat} optimization using the GAN method under different definitions of extreme enzymes, defined by fold change thresholds: more than 2 or less than 0.5 (a), more than 5 or less than 0.2 (b), more than 10 or less than 0.1 (c), more than 50 or less than 0.02 (d), and more than 100 or less than 0.01 (e). The training performance was represented by the absolute value of $\log_2(\text{fold change})$. Data are presented as median $\pm$ SD based on generated k_{cat} parameter sets (n=100). **f** Total number of predictable enzymes after optimization for each training process of the five definitions of extreme enzymes. **g** The number of extreme enzymes identified after GAN-based optimization under each definition. **h** Pearson correlation coefficients of protein

59 abundance prediction after optimization, under each definition of extreme enzymes.
60 The statistics are derived from $n=9$ independent experiments, each performed with three
61 biological replicates. In each box plot, the central band represents the median, the box
62 limits represent the upper and lower quartiles, and the whiskers extend to 1.5 times the
63 interquartile range beyond the box range. Raw values are provided in the source data.



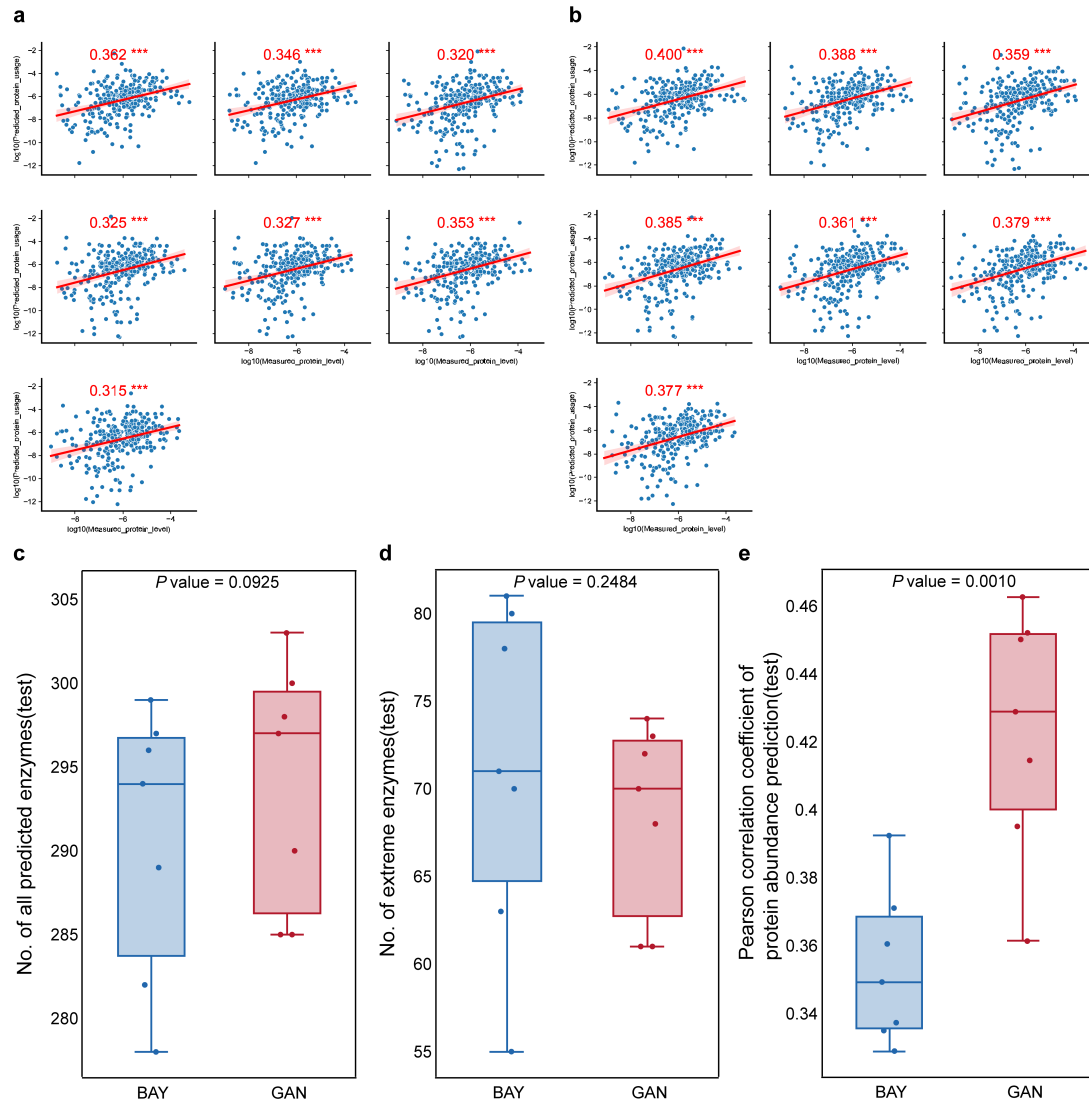
Supplementary Fig. 3 Performance of the EnzymeTuning for protein abundance prediction in *Saccharomyces cerevisiae*.

a Comparison of the total number of predictable enzymes using the GAN method versus the Bayesian approach. **b** Comparison of the number of extreme enzymes using the GAN method and the Bayesian approach. **c** Comparison of Pearson correlation coefficients of protein abundance predictions between the GAN method and the Bayesian approach. The statistics are derived from $n=9$ independent experiments, each performed with three biological replicates. Statistical significance was determined using a two-sided t-test. In each box plot, the central band represents the median, the box limits represent the upper and lower quartiles, and the whiskers extend to 1.5 times the interquartile range beyond the box range. Raw values are provided in the source data.



Supplementary Fig. 4 Convergence and stability of EnzymeTuning-optimized k_{cat} parameters across training generations.

a Distribution of the mean log-transformed k_{cat} values across all k_{cat} variables at each training generation. **b** Distribution of the dispersion (standard deviations, std) of k_{cat} parameters across training generations. **c** Principal component analysis (PCA) of iteration-wise mean k_{cat} vectors. Data are presented as median $\pm$ SD based on generated k_{cat} parameter sets (n=100). In each box plot, the central band represents the median, the box limits represent the upper and lower quartiles, and the whiskers extend to 1.5 times the interquartile range beyond the box range.



Supplementary Fig. 5 Performance of EnzymeTuning for protein abundance prediction under different nitrogen sources with the initial discriminator score.

a Pearson correlation coefficients and corresponding P values for protein abundance predictions based on Bayesian training. ns: not significant, *: P value < 0.05, **: P value < 0.01, ***: P value < 0.005. **b** Pearson correlation coefficients and corresponding P values for protein abundance predictions based on GAN training. ns: not significant, *: P value < 0.05, **: P value < 0.01, ***: P value < 0.005. **c** Comparison of the total number of predictable enzymes under chemostat cultivation

96 with different nitrogen sources using the GAN method versus the Bayesian approach.

97 **d** Comparison of the number of extreme enzymes under chemostat cultivation with

98 different nitrogen sources using the GAN method and the Bayesian approach. **e**

99 Comparison of Pearson correlation coefficients of protein abundance predictions under

100 chemostat cultivation with different nitrogen sources between the GAN method and the

101 Bayesian approach. The statistics are derived from $n=7$ independent experiments, each

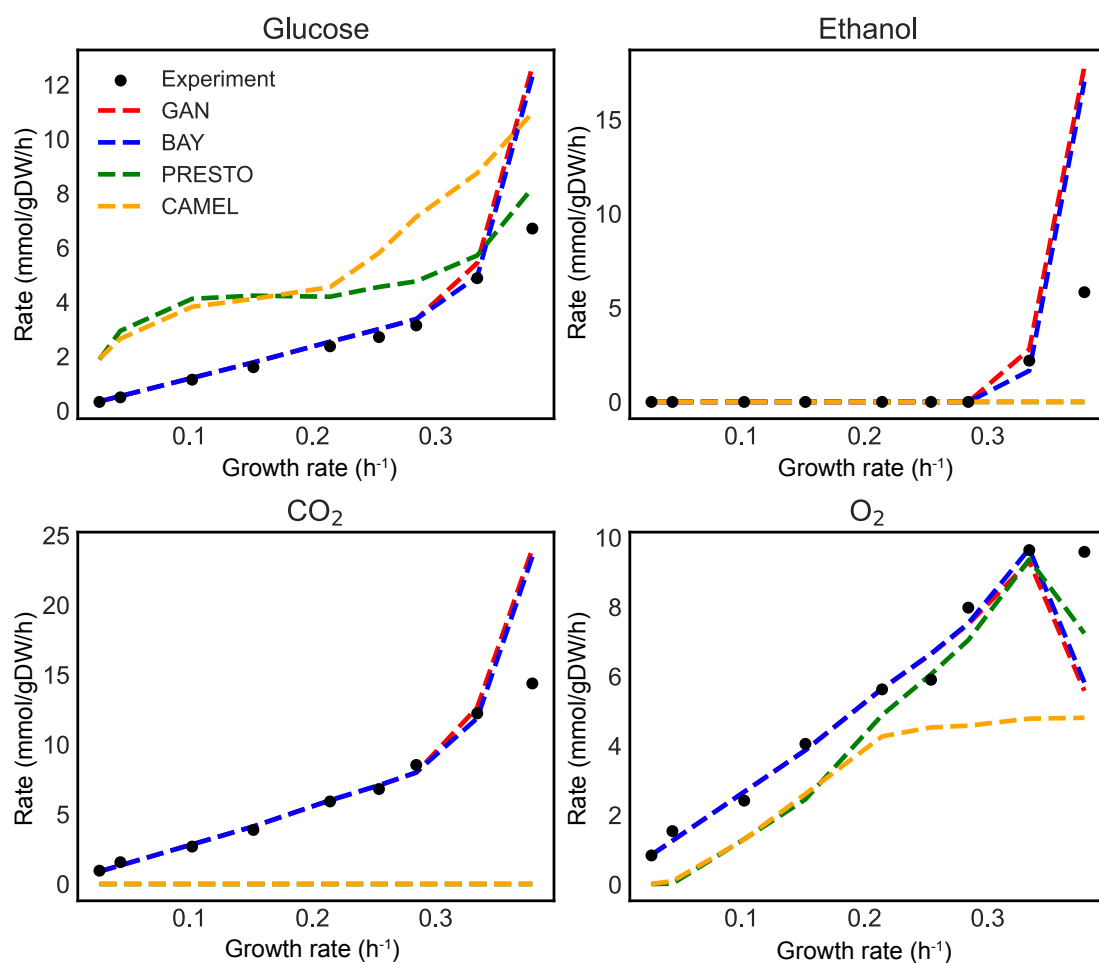
102 performed with three biological replicates. Statistical significance was determined

103 using a two-sided t-test. In each box plot, the central band represents the median, the

104 box limits represent the upper and lower quartiles, and the whiskers extend to 1.5 times

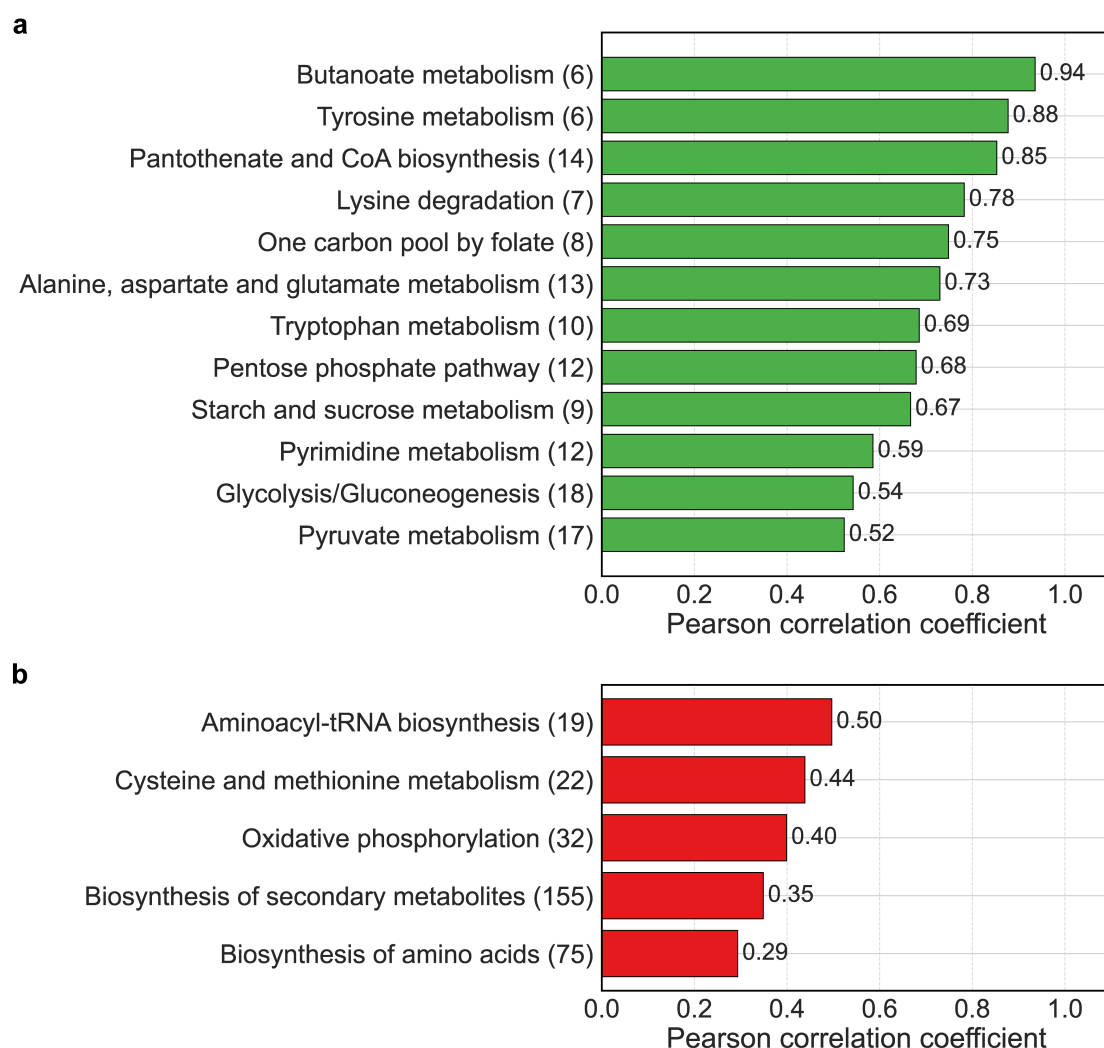
105 the interquartile range beyond the box range. Raw values are provided in the source

106 data.



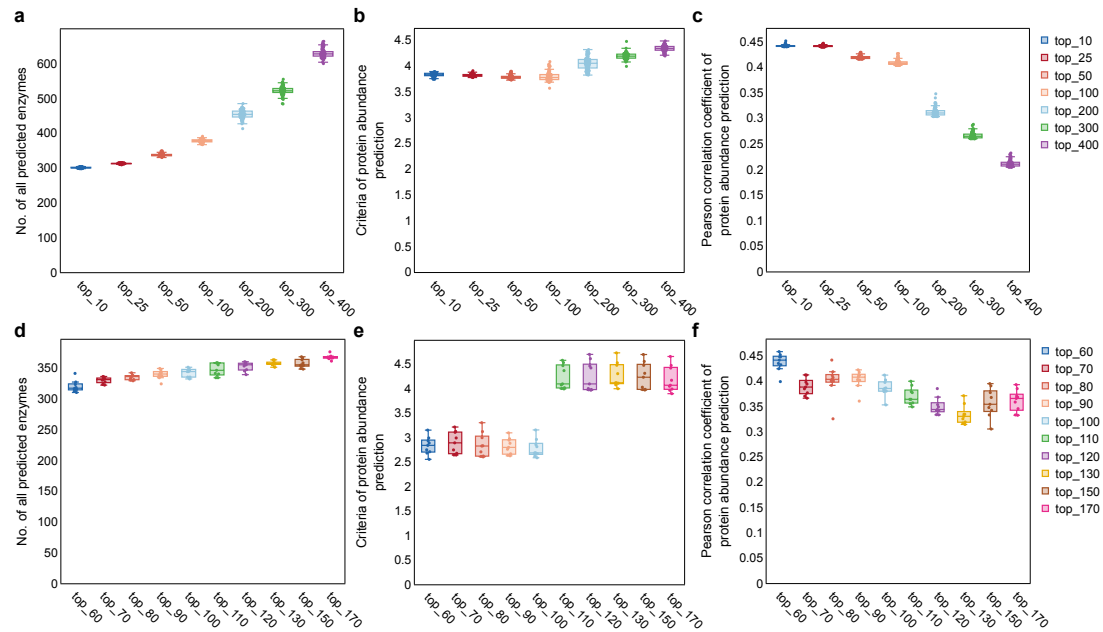
Supplementary Fig. 6 Performance comparison of different k_{cat} parameterization strategies in predicting exchange rates under carbon-limited chemostat conditions.

Experimentally measured and predicted exchange rates of glucose, ethanol, CO₂, and O₂ are plotted against growth rate for ecGEMs parameterized using EnzymeTuning (GAN) (red), Bayesian optimization (BAY) (blue), PRESTO (green), and CAMEL (orange). Black dots represent experimental measurements, and dashed lines represent model predictions.



Supplementary Fig. 7 KEGG pathway-level evaluation of protein abundance prediction accuracy using the GAN method.

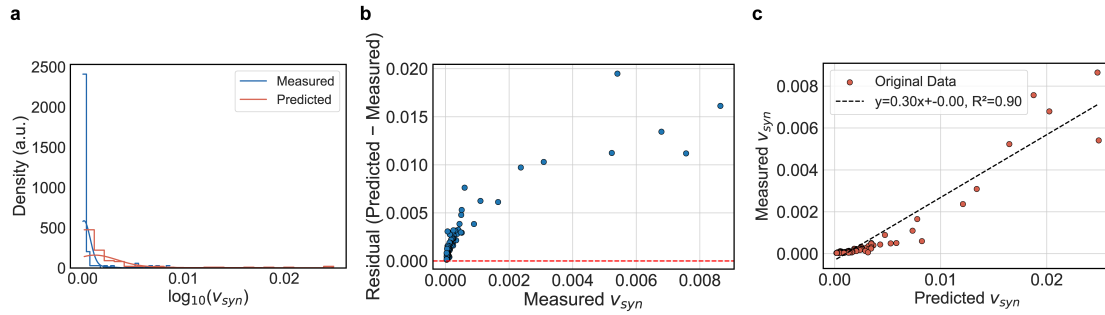
a and **b** KEGG pathways associated with proteins that were predicted with relatively high accuracy (**a**) and relatively low accuracy (**b**) using the GAN method. Bars indicate the Pearson correlation coefficient between predicted and experimentally measured protein abundances for proteins mapped to each pathway. Numbers in parentheses indicate the number of proteins in each pathway.



Supplementary Fig. 8 Comparison of EnzymeTuning in predicting protein synthesis rates at different scales.

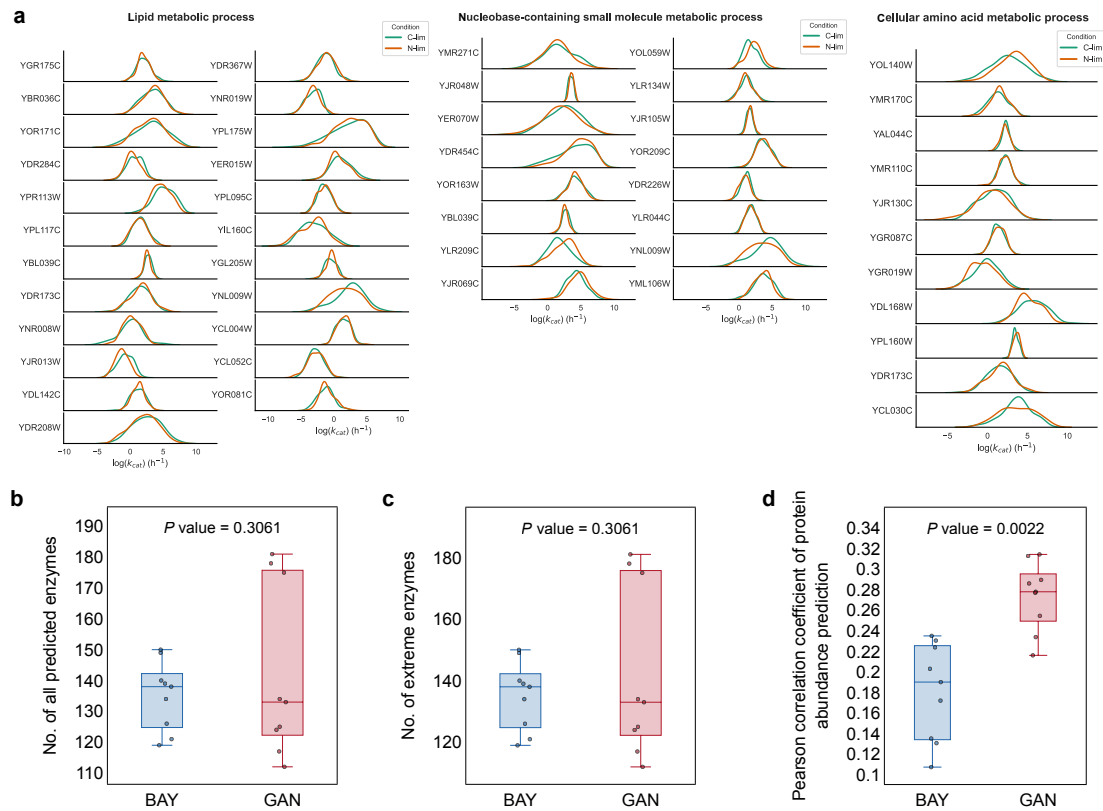
a Comparison of the total number of predictable enzymes cross a wide range of protein synthesis rate scaling sizes with the GAN method. **b** Comparison of the criteria values calculated via Eq. 1 across a wide range of protein synthesis rate scaling sizes with the GAN method. **c** Comparison of Pearson correlation coefficients for protein abundance predictions cross a wide range of protein synthesis rate scaling sizes with the GAN method. **d** Comparison of the total number of predictable enzymes cross an intermediate range of protein synthesis rate scaling sizes with the GAN method. **e** Comparison of the criteria values calculated via Eq. 1 across an intermediate range of protein synthesis rate scaling sizes with the GAN method. **f** Comparison of Pearson correlation coefficients for protein abundance predictions cross an intermediate range of protein synthesis rate scaling sizes with the GAN method. The statistics are derived from $n=9$ independent experiments, each performed with three biological replicates. In each box

138 plot, the central band represents the median, the box limits represent the upper and
139 lower quartiles, and the whiskers extend to 1.5 times the interquartile range beyond the
140 box range. Raw values are provided in the source data.



Supplementary Fig. 9 Evaluation of model performance for protein synthesis rate (v_{syn}) prediction.

a Density distribution of $\log_{10}(v_{syn})$ for predicted values (red) and measured values (blue). The close overlap of the two distributions indicates that the model captures the global distribution and dynamic range of v_{syn} (n=100) across proteins. **b** Residual analysis of v_{syn} predictions, shown as the difference between predicted and measured values (Predicted - Measured) plotted against measured v_{syn} . Residuals are centered around zero for most proteins, indicating the absence of systematic bias, with a modest increase in variance at higher synthesis rates. **c** Scatter plot comparing predicted and measured v_{syn} values. The dashed line denotes the linear regression fit. The high coefficient of determination ($R^2 = 0.90$) demonstrates strong agreement between model predictions and experimental measurements. Raw values are provided in the source data.



Supplementary Fig. 10 The k_{cat} distributions under various C/N conditions and performance of EnzymeTuning in improving protein abundance prediction under anaerobic conditions.

a Ridge plots showing the distributions of log-transformed k_{cat} values (h^{-1}) for representative enzymes enriched in lipid, nucleobase, and amino acid metabolic processes. The distributions were derived from the top 100 GAN-optimized parameter sets ($n=100$). Curves were generated using Kernel Density Estimation (KDE) to visualize the probability density of k_{cat} values under carbon-limited (C-lim, green) and nitrogen-limited (N-lim, orange) chemostat conditions. The y-axis indicated specific enzymes, and the x-axis represented the $\log(k_{cat})$ magnitude. **b-d** Comparison of the total number of predictable enzymes (**b**), the number of extreme enzymes (**c**), and Pearson correlation coefficients of protein abundance predictions (**d**) using the GAN

167 method versus the Bayesian approach under anaerobic conditions. The prediction was
168 performed across three specific growth rates (0.05, 0.15, and 0.25 h⁻¹). The statistics are
169 derived from n=3 independent experiments, each performed with three biological
170 replicates. Statistical significance was determined using a two-sided t-test. In each box
171 plot, the central band represents the median, the box limits represent the upper and
172 lower quartiles, and the whiskers extend to 1.5 times the interquartile range beyond the
173 box range. Raw values are provided in the source data.