

Inventory of Supplementary Tables and Figures for

Energy-translation coupling limits anaerobic yeast growth

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Supplementary Table 1. Concentrations of substances, yields, and carbon balances at different specific growth rates under carbon (C), nitrogen (N), and phosphorus (P) limitations. For each substance category, the first row represents C-limited conditions, the second row corresponds to N-limited conditions, and the third row reflects P-limited conditions. Concentrations are in g/kg, and yields are in Cmol/Cmol glucose. Data represent mean $\pm$ SD from three biological replicates.

Specific growth rate [h ⁻¹]	0.05	0.10	0.15	0.20	0.25
C limitation					
Ethanol	9.23 $\pm$ 0.02	10.88 $\pm$ 0.73	9.39 $\pm$ 0.11	8.64 $\pm$ 0.20	8.27 $\pm$ 0.17
Glycerol	1.99 $\pm$ 2E-3	1.89 $\pm$ 0.14	1.88 $\pm$ 0.05	1.65 $\pm$ 0.09	1.77 $\pm$ 0.34
Glucose	0.00	0.05 $\pm$ 0.05	0.004 $\pm$ 6E-3	0.83 $\pm$ 0.48	3.30 $\pm$ 0.74
Biomass	2.15 $\pm$ 1.5E-3	2.27 $\pm$ 7E-3	2.23 $\pm$ 0.02	2.17 $\pm$ 0.02	2.04 $\pm$ 0.17
Y _{CO₂/s}	0.37 $\pm$ 0.008	0.31 $\pm$ 0.0169	0.30 $\pm$ 0.0393	0.35 $\pm$ 0.0140	0.34 $\pm$ 0.0025
Y _{Ethanol/s}	0.48 $\pm$ 0.0008	0.50 $\pm$ 0.0221	0.49 $\pm$ 0.0106	0.46 $\pm$ 0.0122	0.50 $\pm$ 0.0037
Y _{Glycerol/s}	0.082 $\pm$ 7E-5	0.067 $\pm$ 0.0318	0.078 $\pm$ 0.0022	0.070 $\pm$ 0.0037	0.084 $\pm$ 0.0006
Y _{Biomass/s}	0.092 $\pm$ 5E-5	0.097 $\pm$ 5E-4	0.095 $\pm$ 0.0040	0.096 $\pm$ 0.0024	0.104 $\pm$ 0.0025
C balance	1.03 $\pm$ 0.0077	0.99 $\pm$ 0.0372	0.97 $\pm$ 0.0393	0.99 $\pm$ 0.0248	1.03 $\pm$ 0.0130
N limitation					
Ethanol	23.97 $\pm$ 0.74	21.94 $\pm$ 0.51	15.83 $\pm$ 0.17	13.13 $\pm$ 0.85	
Glycerol	2.21 $\pm$ 0.13	2.15 $\pm$ 0.07	1.98 $\pm$ 0.01	1.89 $\pm$ 0.09	
Glucose	0.10 $\pm$ 0.07	7.63 $\pm$ 1.06	23.18 $\pm$ 1.49	27.03 $\pm$ 0.30	
Biomass	3.51 $\pm$ 0.11	3.28 $\pm$ 0.12	2.70 $\pm$ 0.07	2.41 $\pm$ 0.08	
Y _{CO₂/s}	0.30 $\pm$ 0.0058	0.28 $\pm$ 0.0067	0.25 $\pm$ 0.0089	0.23 $\pm$ 0.0045	
Y _{Ethanol/s}	0.56 $\pm$ 0.0208	0.58 $\pm$ 0.0064	0.62 $\pm$ 0.0203	0.59 $\pm$ 0.0348	
Y _{Glycerol/s}	0.041 $\pm$ 0.0024	0.046 $\pm$ 0.0022	0.063 $\pm$ 0.0028	0.067 $\pm$ 0.0029	
Y _{Biomass/s}	0.067 $\pm$ 0.0020	0.071 $\pm$ 0.0034	0.087 $\pm$ 0.0018	0.089 $\pm$ 0.0026	
C balance	0.97 $\pm$ 0.0286	0.99 $\pm$ 0.0152	1.02 $\pm$ 0.0257	0.98 $\pm$ 0.0379	
P limitation					
Ethanol	26.35 $\pm$ 0.42	23.93 $\pm$ 0.74	18.16 $\pm$ 0.52	13.67 $\pm$ 0.92	
Glycerol	3.10 $\pm$ 0.01	3.18 $\pm$ 0.10	2.49 $\pm$ 0.06	2.15 $\pm$ 0.09	
Glucose	0.65 $\pm$ 0.21	7.56 $\pm$ 0.71	28.27 $\pm$ 1.43	37.65 $\pm$ 1.10	
Biomass	3.77 $\pm$ 0.28	3.18 $\pm$ 0.05	2.76 $\pm$ 0.13	2.31 $\pm$ 0.09	
Y _{CO₂/s}	0.254 $\pm$ 0.0079	0.24 $\pm$ 0.0028	0.24 $\pm$ 0.0023	0.24 $\pm$ 0.0033	
Y _{Ethanol/s}	0.52 $\pm$ 0.0101	0.53 $\pm$ 0.0116	0.62 $\pm$ 0.0244	0.63 $\pm$ 0.0186	
Y _{Glycerol/s}	0.049 $\pm$ 0.0001	0.056 $\pm$ 0.0013	0.068 $\pm$ 0.0044	0.079 $\pm$ 0.0026	
Y _{Biomass/s}	0.061 $\pm$ 0.0044	0.058 $\pm$ 0.0012	0.073 $\pm$ 0.0075	0.088 $\pm$ 0.0057	
C balance	0.89 $\pm$ 0.0130	0.89 $\pm$ 0.0103	1.02 $\pm$ 0.0294	1.02 $\pm$ 0.0217	

Supplementary Table 2. Enzyme activity regulation through protein phosphorylation under carbon (C) limitation, with activation or inhibition observed.

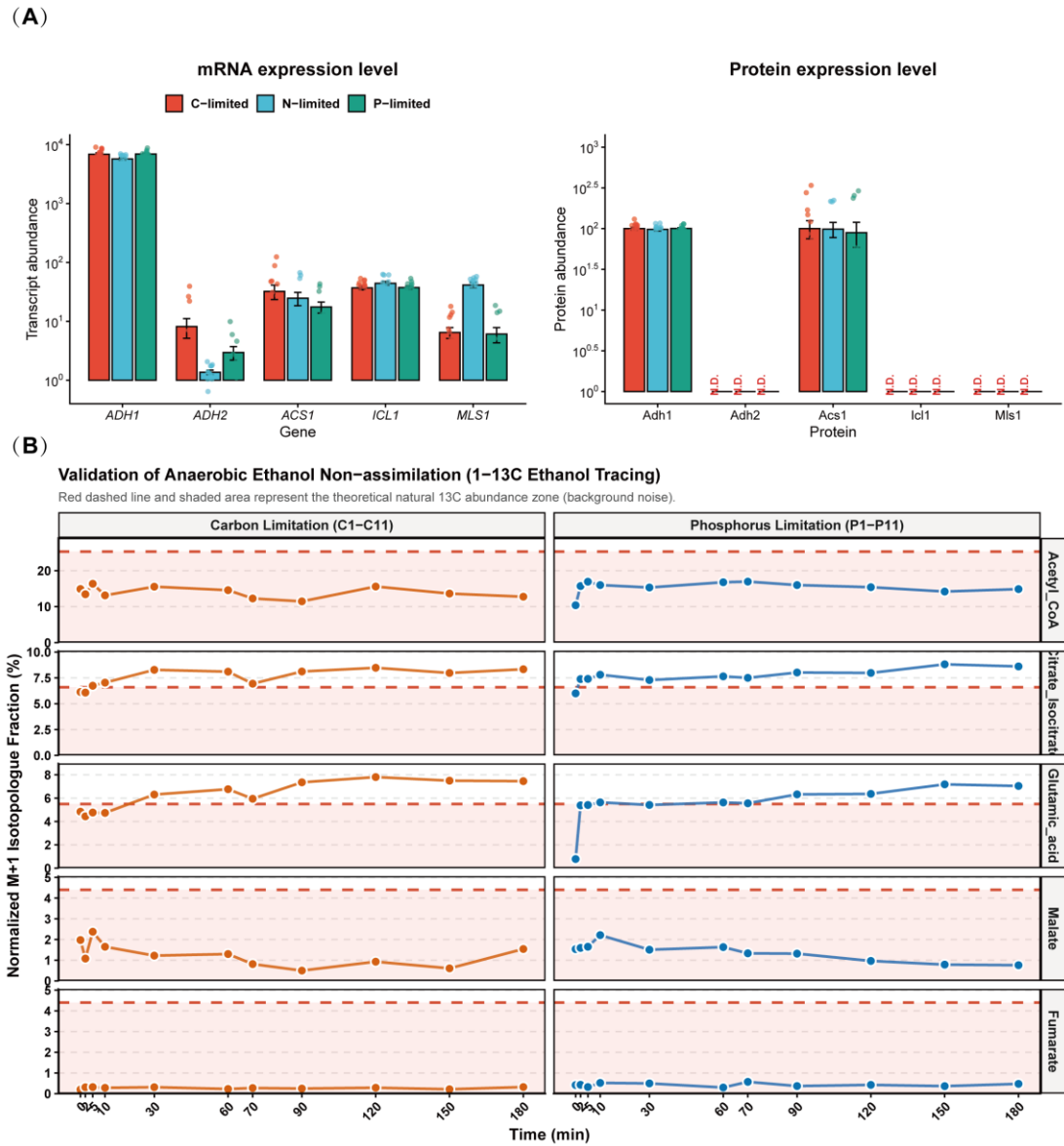
Gene symbol	C limitation			
	0.05→0.1	0.1→0.15	0.15→0.2	0.2→0.25
hxx1-15				Activation (A)
hxx1-293				
HXX1_419		Activation (A)		
TDH2-207				
TDH2-209			Inhibition(I)	
TDH2-235				
TDH2-239				
PGK1-114				
PGK1-392		Activation (A)		
PGK1-86		Activation (A)		Activation (A)
GPM1-116				Activation (A)
ENO1-188	Inhibition(I)	Activation (A)		Activation (A)
ENO1-404		Activation (A)		
ENO2-324	Activation (A)			
CDC19-9		Inhibition(I)		
ADH1-2			Activation (A)	Activation (A)
GPD1_24			Activation (A)	
GPD1-27			Activation (A)	
GPD2-72				Activation (A)

Supplementary Table 3. Enzyme activity regulation through protein phosphorylation under nitrogen (N) limitation, with activation or inhibition observed.

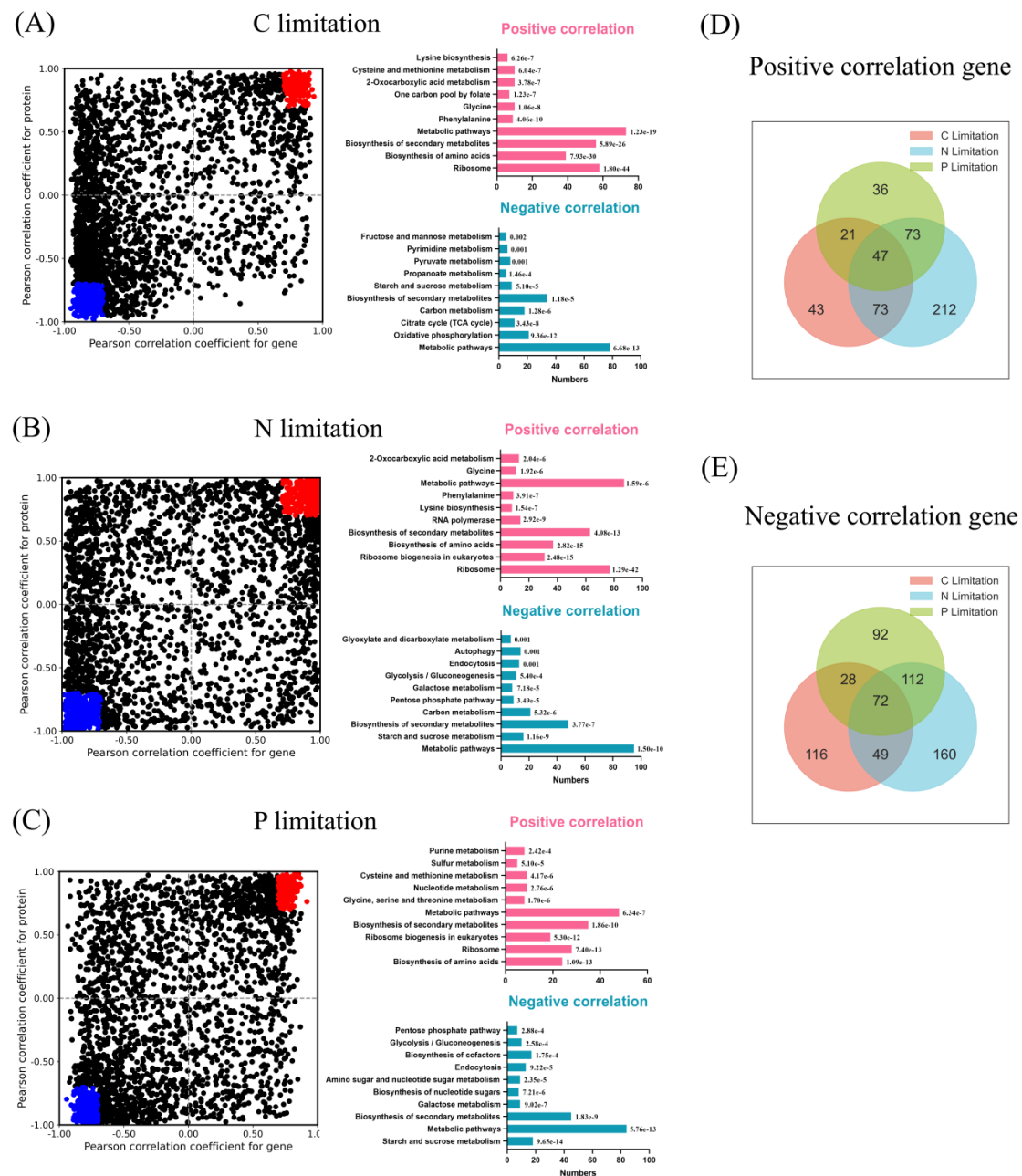
Gene symbol	N limitation		
	0.05→0.1	0.1→0.15	0.15→0.2
hxx1-15	Activation (A)	Activation (A)	Activation (A)
hxx1-249	Activation (A)	Activation (A)	Activation (A)
HXX2_419			
GLK1-2			Activation (A)
PGI1-2			
PGI1-27	Activation (A)	Activation (A)	
PGI1-53			
FBA1-313		Inhibition(I)	
THD2-49			
TDH2-59		Activation (A)	
TDH2-105	Inhibition(I)		
TDH2-185			
TDH2-190			
TDH2-201			
TDH2-207	Inhibition(I)	Inhibition(I)	
TDH2-208	Inhibition(I)		
TDH2-209			
TDH2-227			
GPM1-116			
GPM1-12	Inhibition(I)		Activation (A)
GPM1-127		Inhibition(I)	Activation (A)
GPM1-197	Activation (A)		
ENO1-205			Activation (A)
ENO1-251	Activation (A)		
ENO1-376	Activation (A)	Activation (A)	
ENO1-404	Activation (A)		
ADH1-2			
ADH1-288	Inhibition(I)		

Supplementary Table 4. Enzyme activity regulation through protein phosphorylation under phosphorus (P) limitation, with activation or inhibition observed.

Gene symbol	P limitation		
	0.05→0.1	0.1→0.15	0.15→0.2
hvk1-15	Activation (A)		Activation (A)
hvk1-293			
PFK1-179		Inhibition(I)	
PFK1-895			
GPM1-116		Activation (A)	
GPM1-197			
ENO1-188			Activation (A)
ADH1-2		Inhibition(I)	Activation (A)
ADH1-290	Activation (A)		Activation (A)



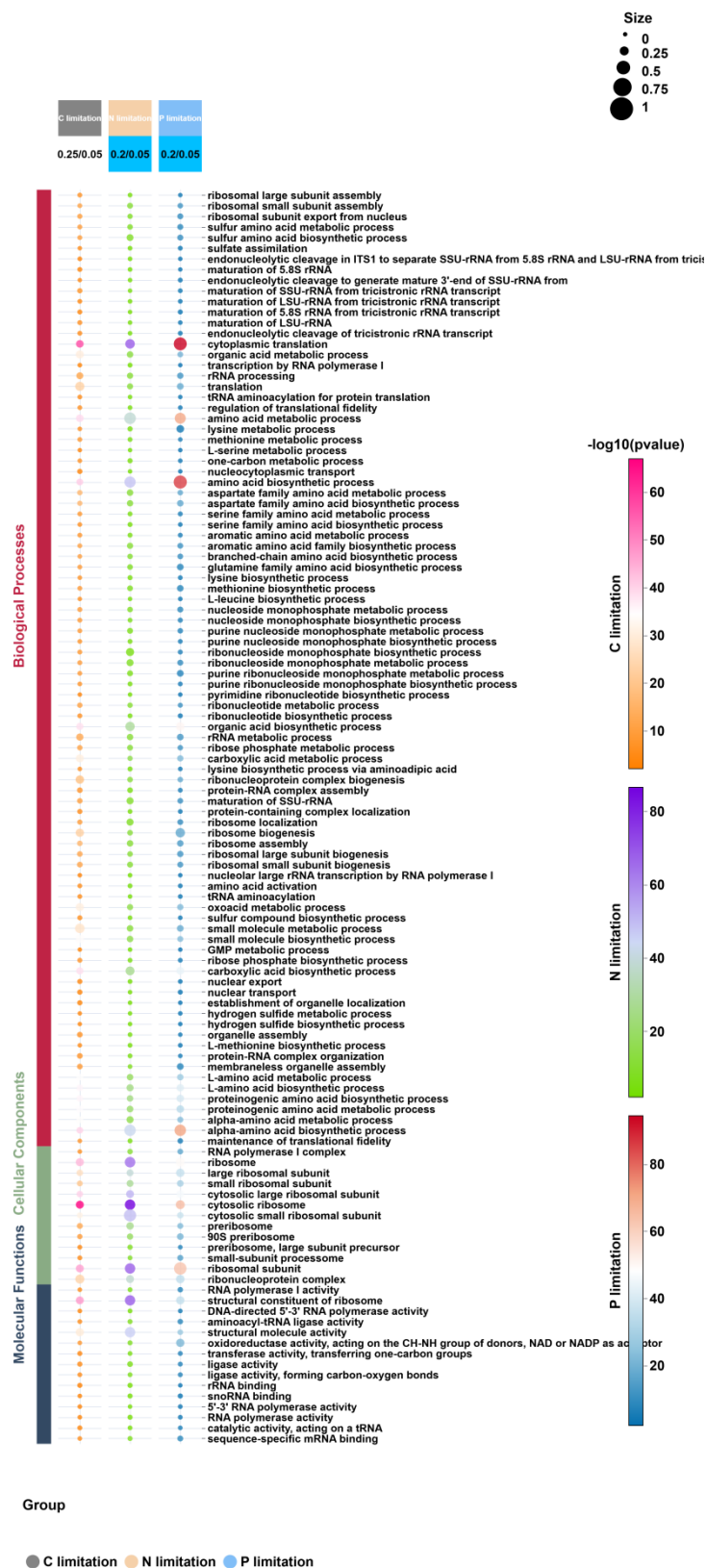
Supplementary Figure 1. Validation of unidirectional ethanol production under strict anaerobiosis. (A) Transcriptomic and proteomic repression of the ethanol assimilation pathway. **(B)** Dynamic 1-¹³C-ethanol isotopic tracing confirms zero metabolic re-assimilation. Data represent mean ± SD from three biological replicates.



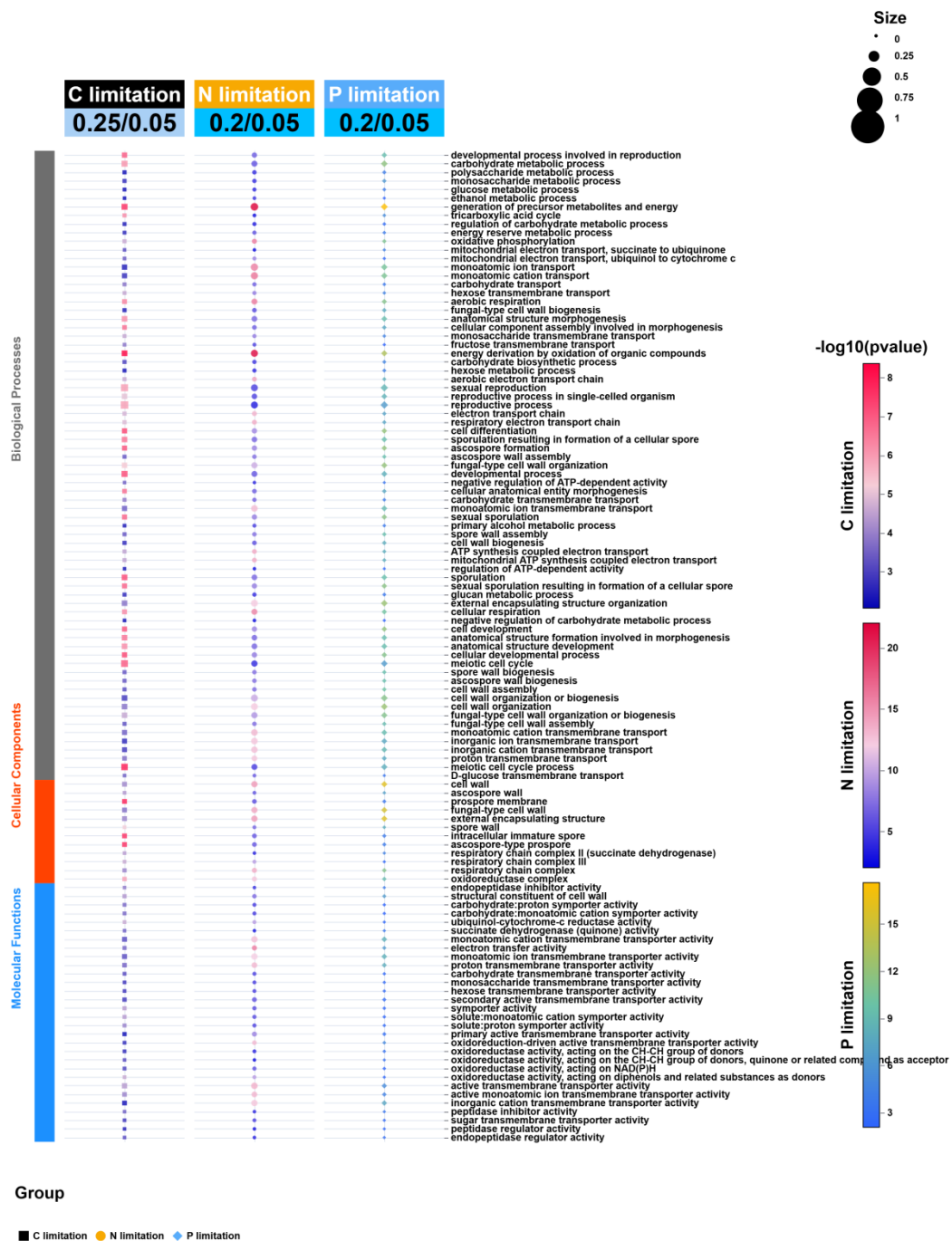
Supplementary Figure 2. Hierarchical regulation of transcript and protein abundance under nutrient limitations. (A-C) Correlations of transcript levels and protein abundances with specific growth rate (μ) in chemostat cultures limited by C, N, or P. Scatter plots depict the Pearson correlation coefficients between transcript level and μ (x-axis) and between protein abundance and μ (y-axis). Genes showing strong positive ($R > 0.7$) or negative ($R < -0.7$) correlations are highlighted in red and blue, respectively. Bar charts indicate KEGG-enriched pathways associated with

positively (red) and negatively (blue) correlated genes. **(D)** Venn diagram showing overlap of positively correlated genes across all three nutrient limitations. **(E)** Venn diagram showing overlap of negatively correlated gene.

Up-regulation

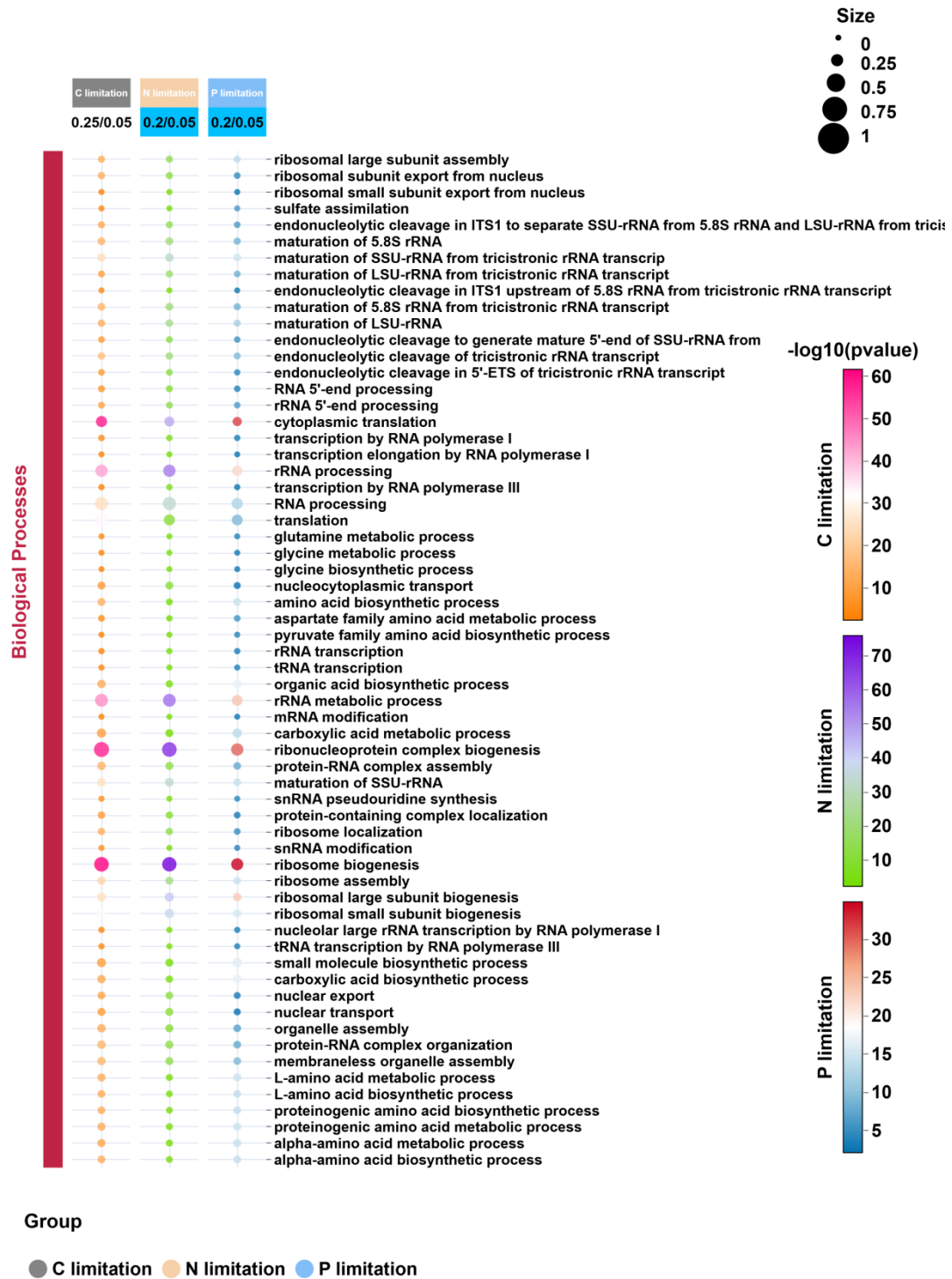


Down-regulation



Supplementary Figure 3. Comparison of gene expression levels under maximum and minimum specific growth rates under elemental limitation conditions. Genes are categorized into two groups: significantly up-regulated genes and significantly down-regulated genes. For the up-regulated genes, those consistently up-regulated across all three elemental limitations were selected for functional enrichment analysis. Similarly, for the down-regulated genes, those consistently down-regulated across three elemental limitations were analyzed for functional enrichment analysis.

Up-regulation



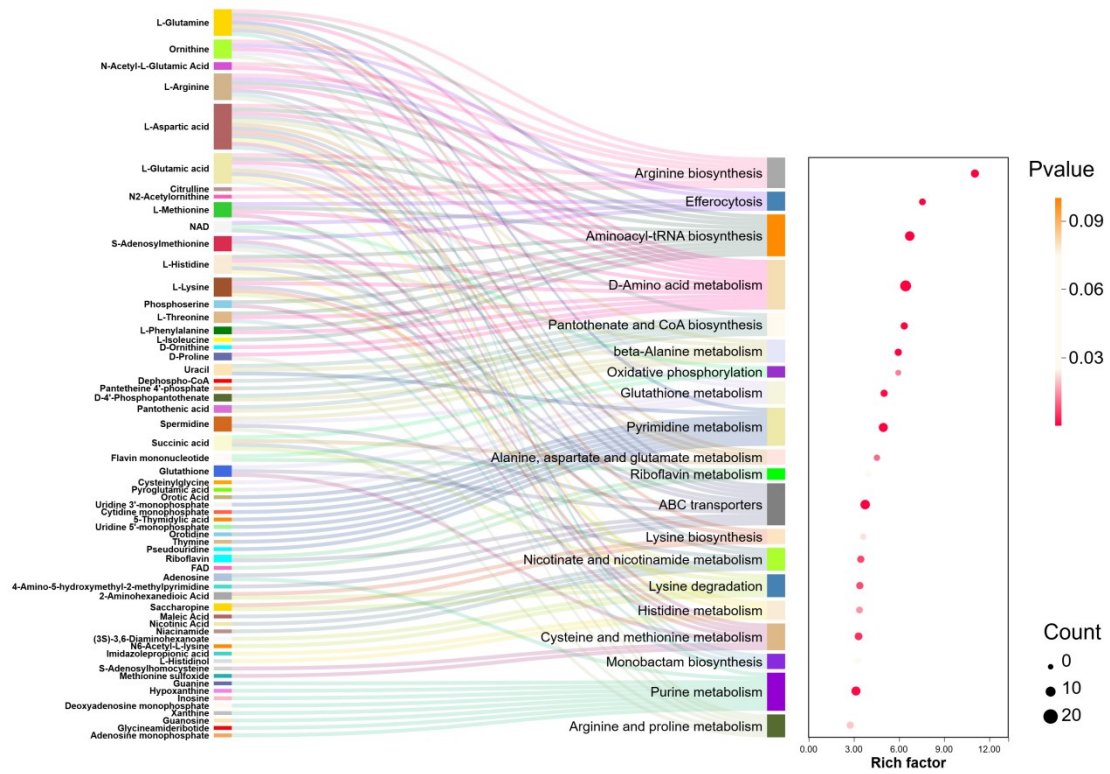
Down-regulation



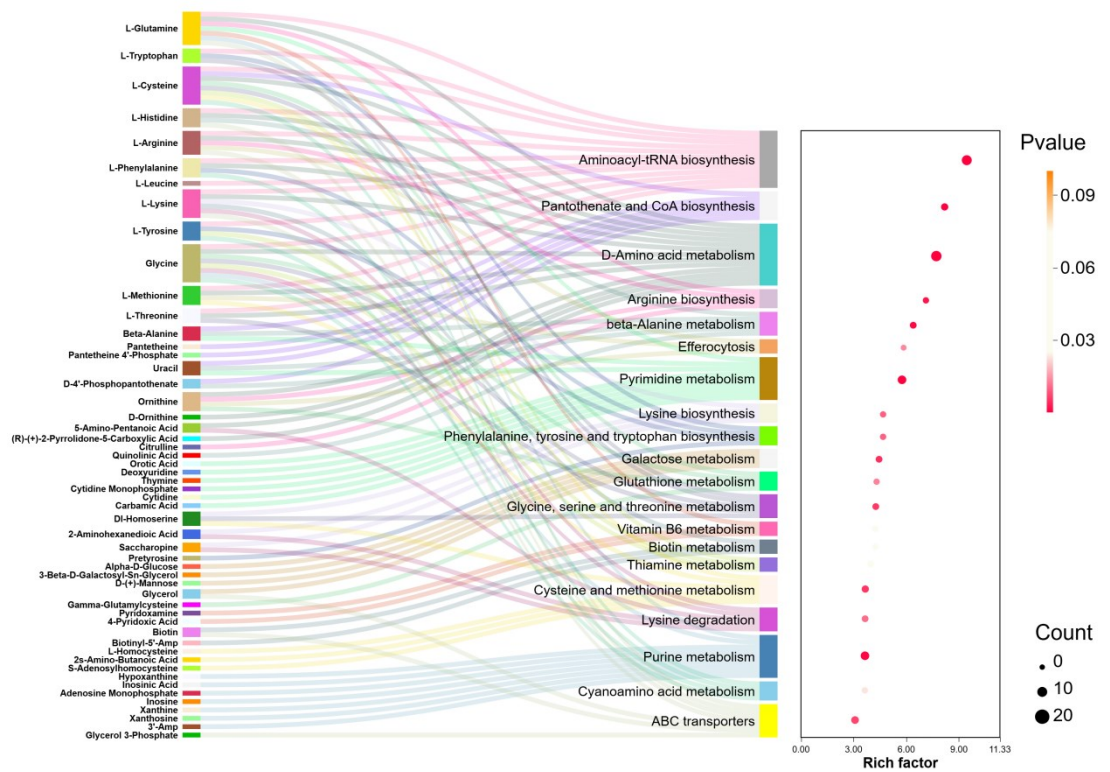
Supplementary Figure 4. Comparison of protein abundance under maximum and minimum specific growth rates under conditions of elemental limitation. Proteins are categorized into two groups: significantly upregulated and significantly

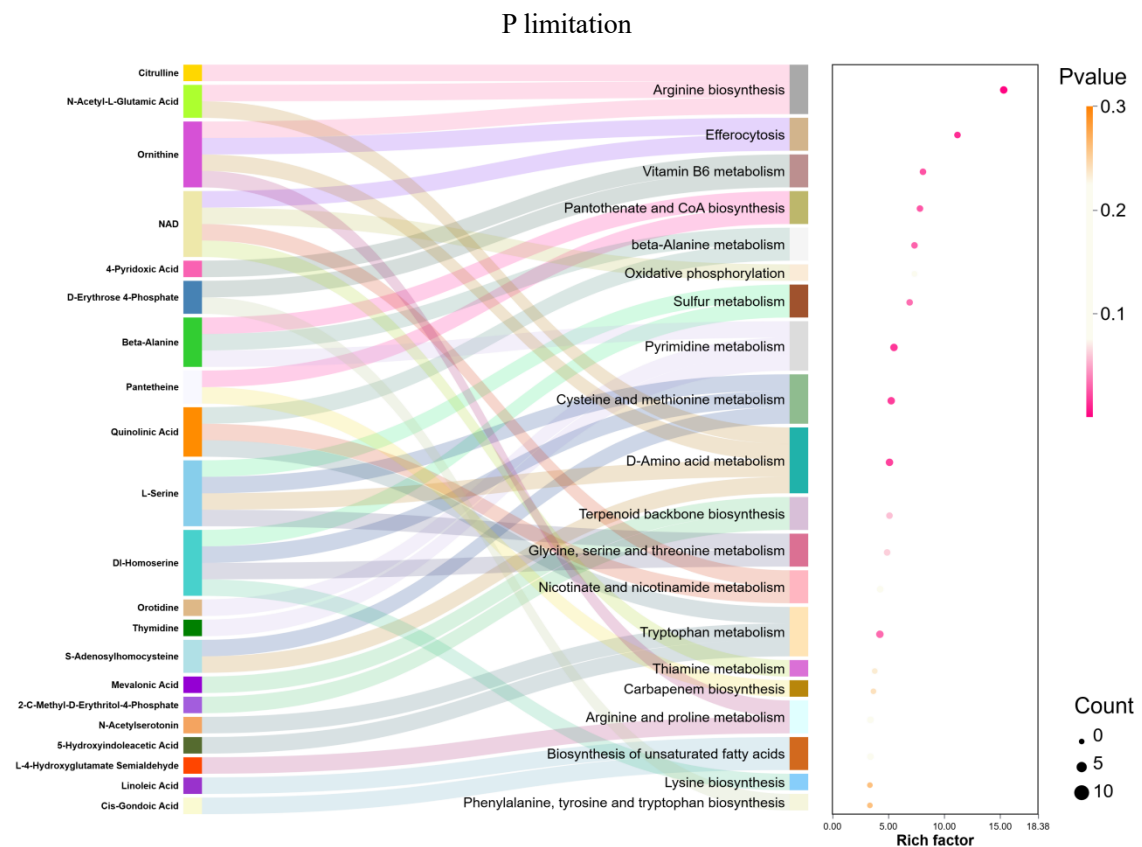
downregulated. For the upregulated proteins, those consistently up-regulated across all three elemental limitations were selected for functional enrichment analysis. Similarly, for the downregulated proteins, those consistently down-regulated across the three elemental limitations were subjected to functional enrichment analysis.

C limitation



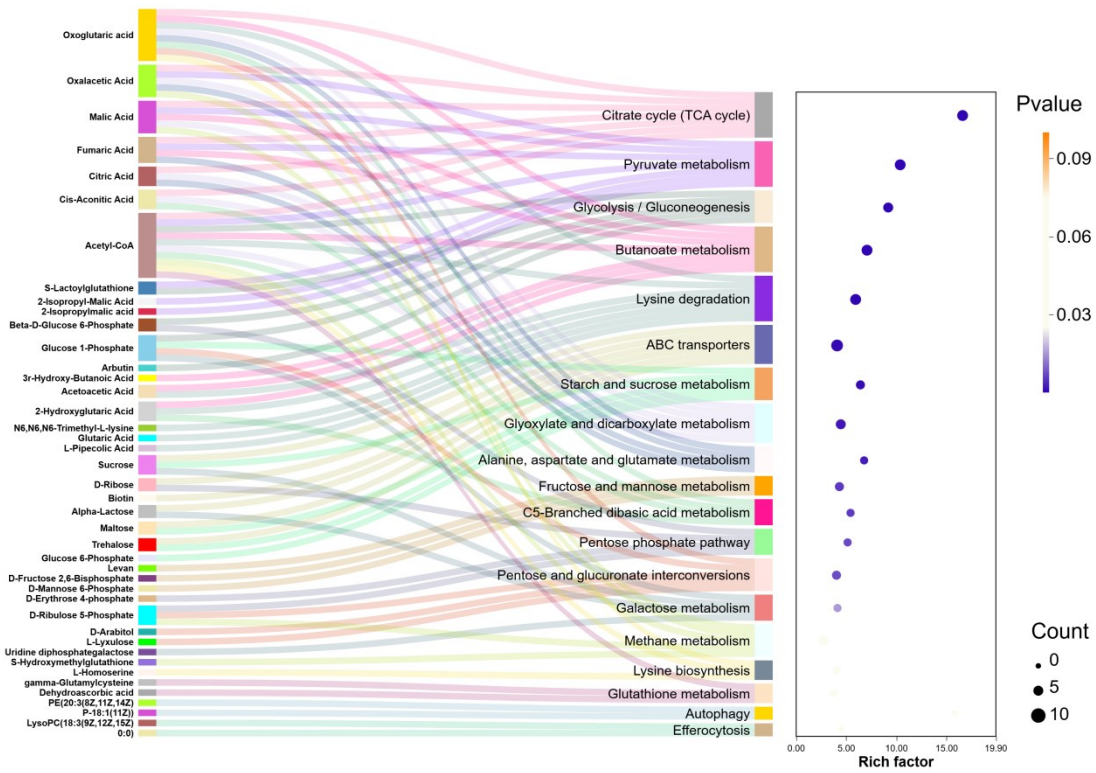
N limitation



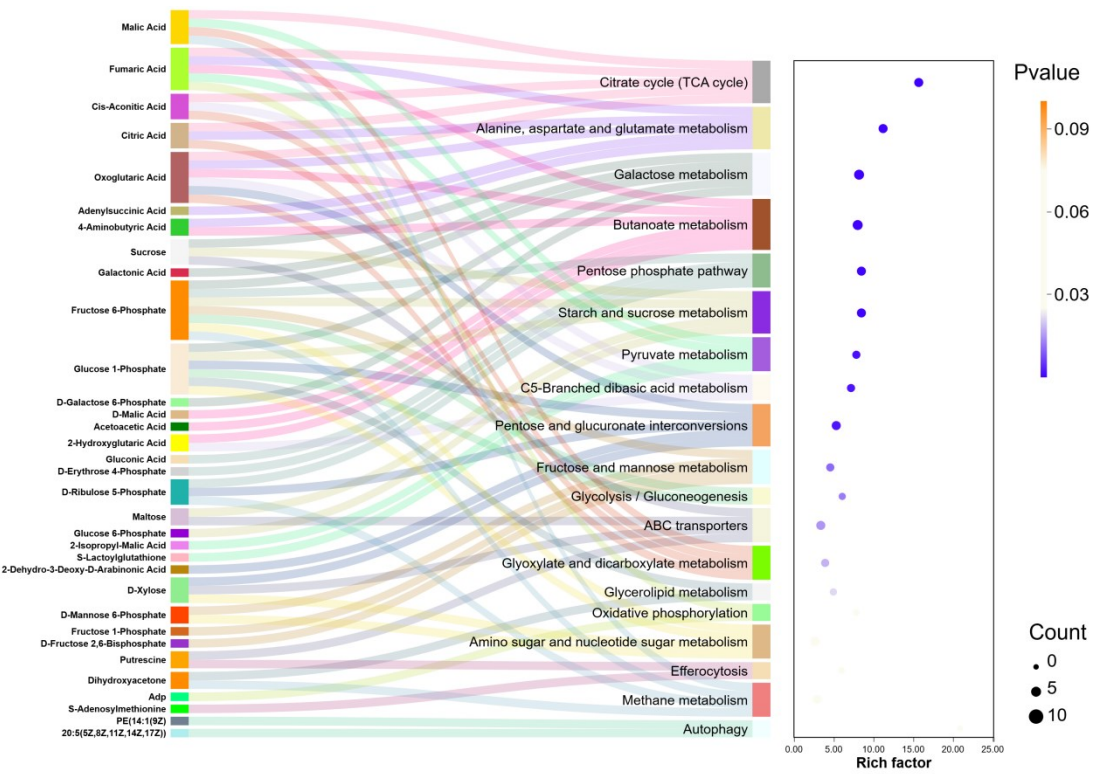


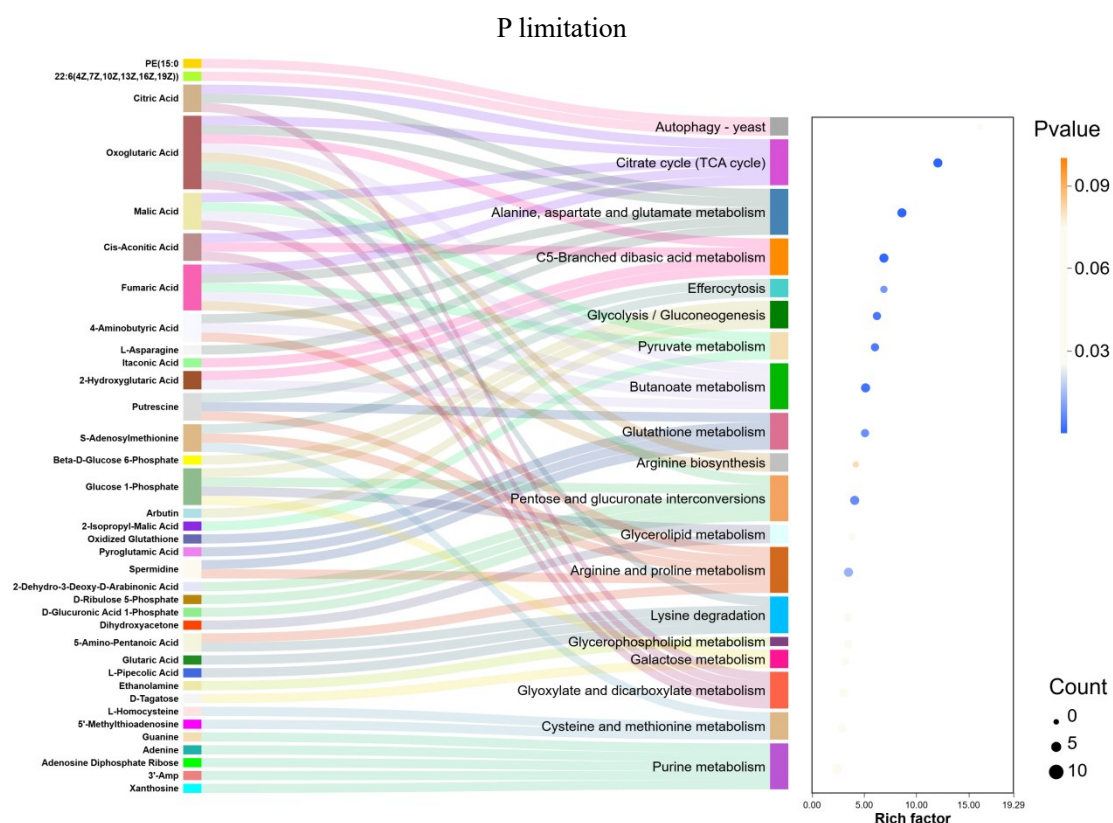
Supplementary Figure 5. Comparison of metabolite concentrations at maximum and minimum specific growth rates under conditions of elemental limitation, highlighting significantly upregulated metabolites. A functional enrichment analysis of the genes associated with these upregulated metabolites was performed. Significantly changed were identified by two-tailed t-test with Benjamini-Hochberg correction ($q < 0.05$). Data represent mean $\pm$ SD from three biological replicates. Functional enrichment of associated genes was performed using KEGG overrepresentation analysis ($q < 0.05$).

C limitation

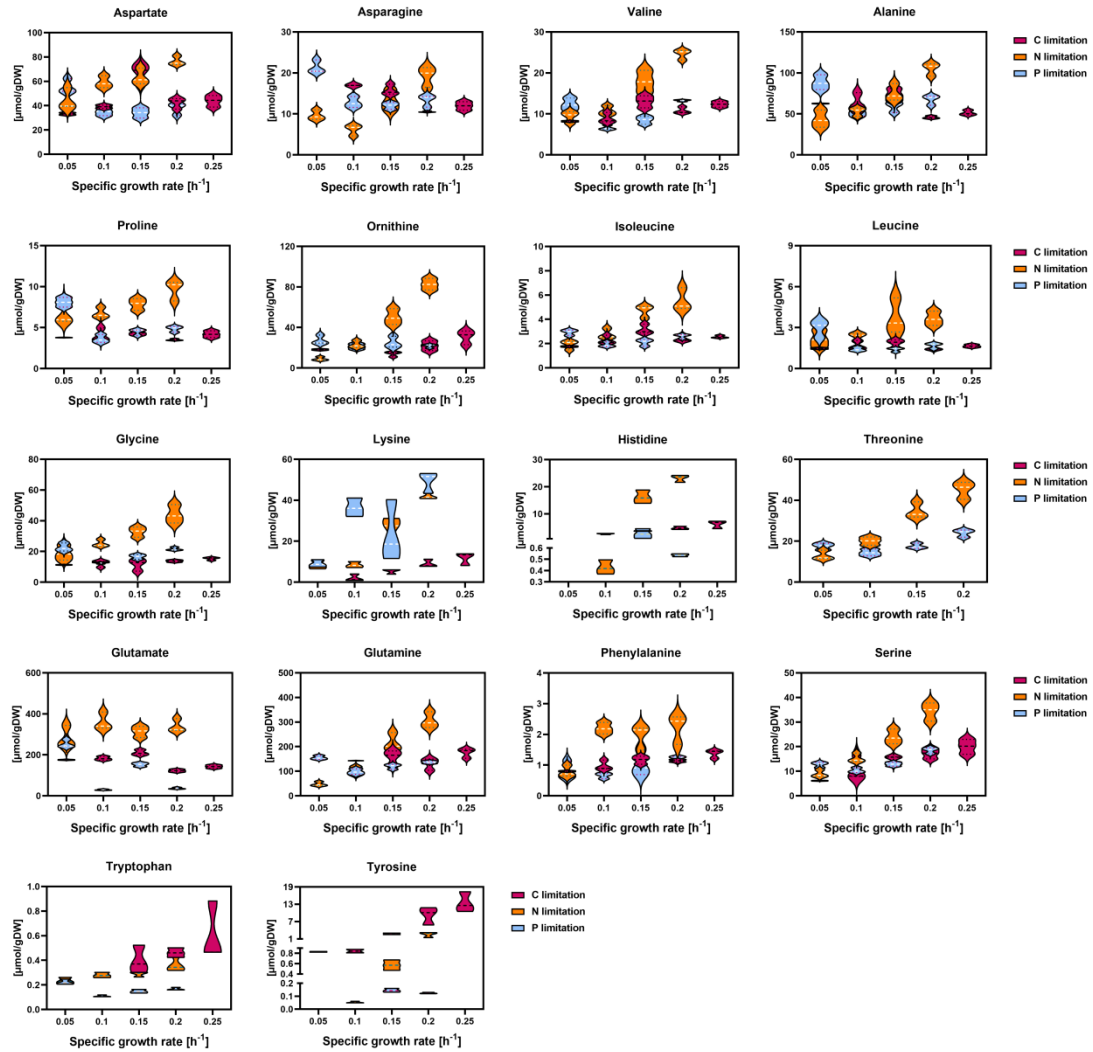


N limitation



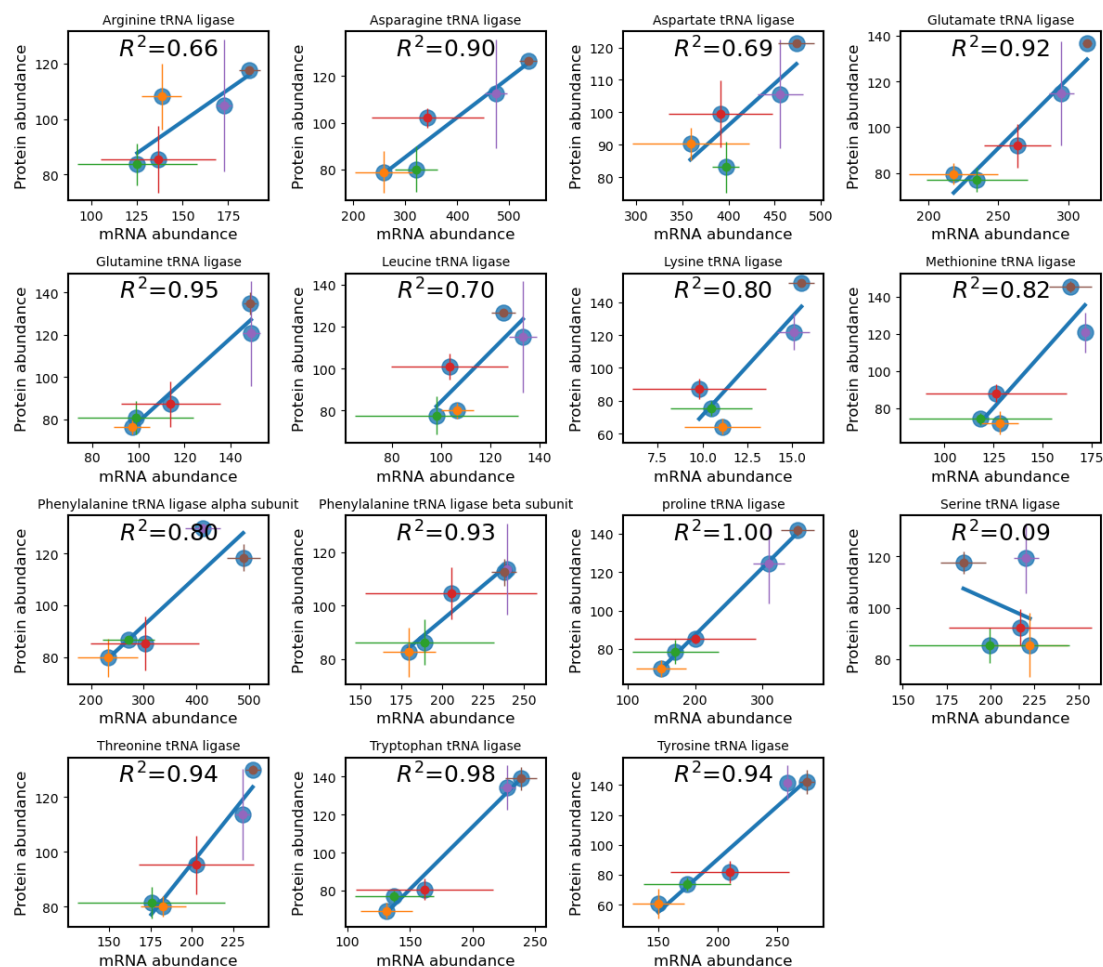


Supplementary Figure 6. Comparison of metabolite concentrations at maximum and minimum specific growth rates under conditions of elemental limitation, highlighting significantly downregulated metabolites. A functional enrichment analysis of the genes associated with these significantly downregulated metabolites was performed. Significantly changed were identified by two-tailed t-test with Benjamini-Hochberg correction ($q < 0.05$). Data represent mean $\pm$ SD from three biological replicates. Functional enrichment of associated genes was performed using KEGG overrepresentation analysis ($q < 0.05$).



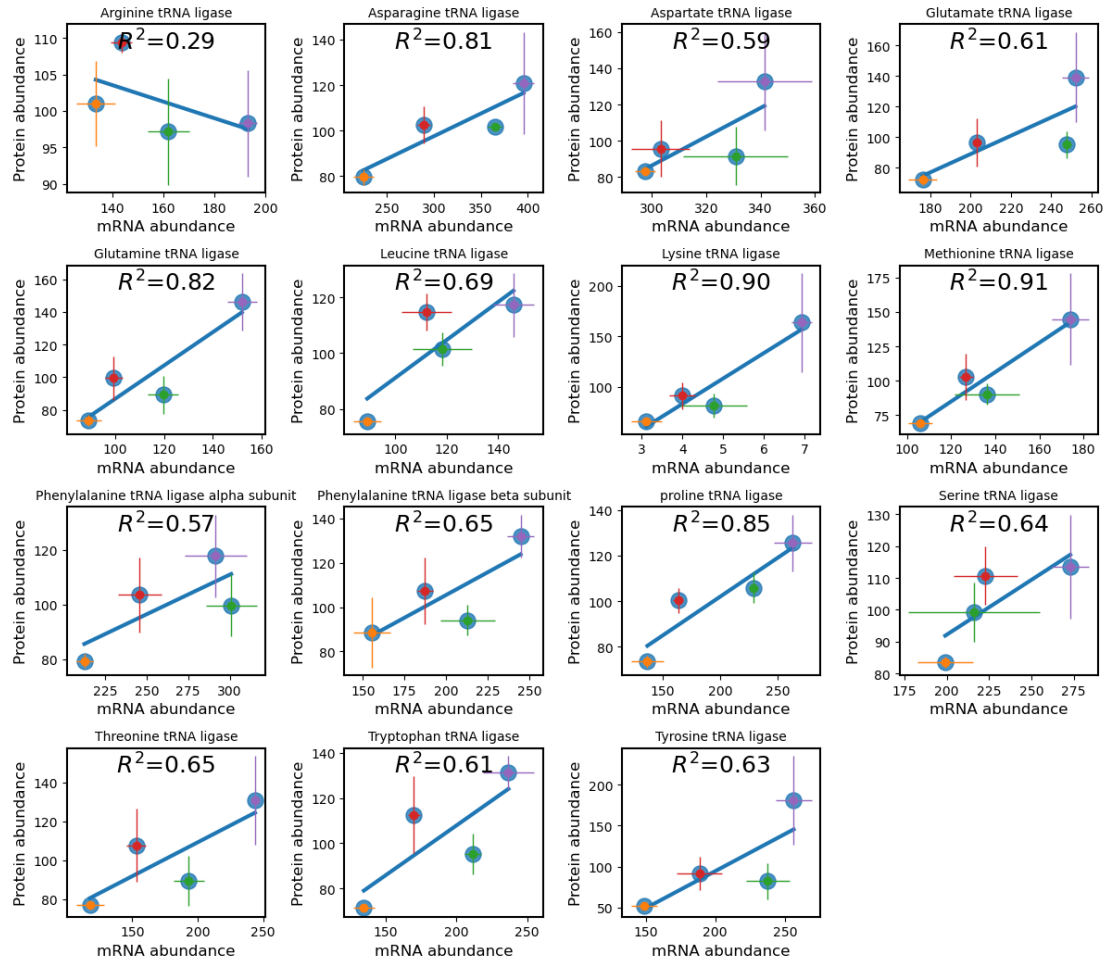
Supplementary Figure 7. Intracellular amino acid amounts under C, N, or P limitation at different specific growth rates, expressed in $\mu\text{mol/gDW}$. Data represent mean $\pm$ SD from three biological replicates.

Correlation between mRNA and protein of tRNA ligase under C-limited condition



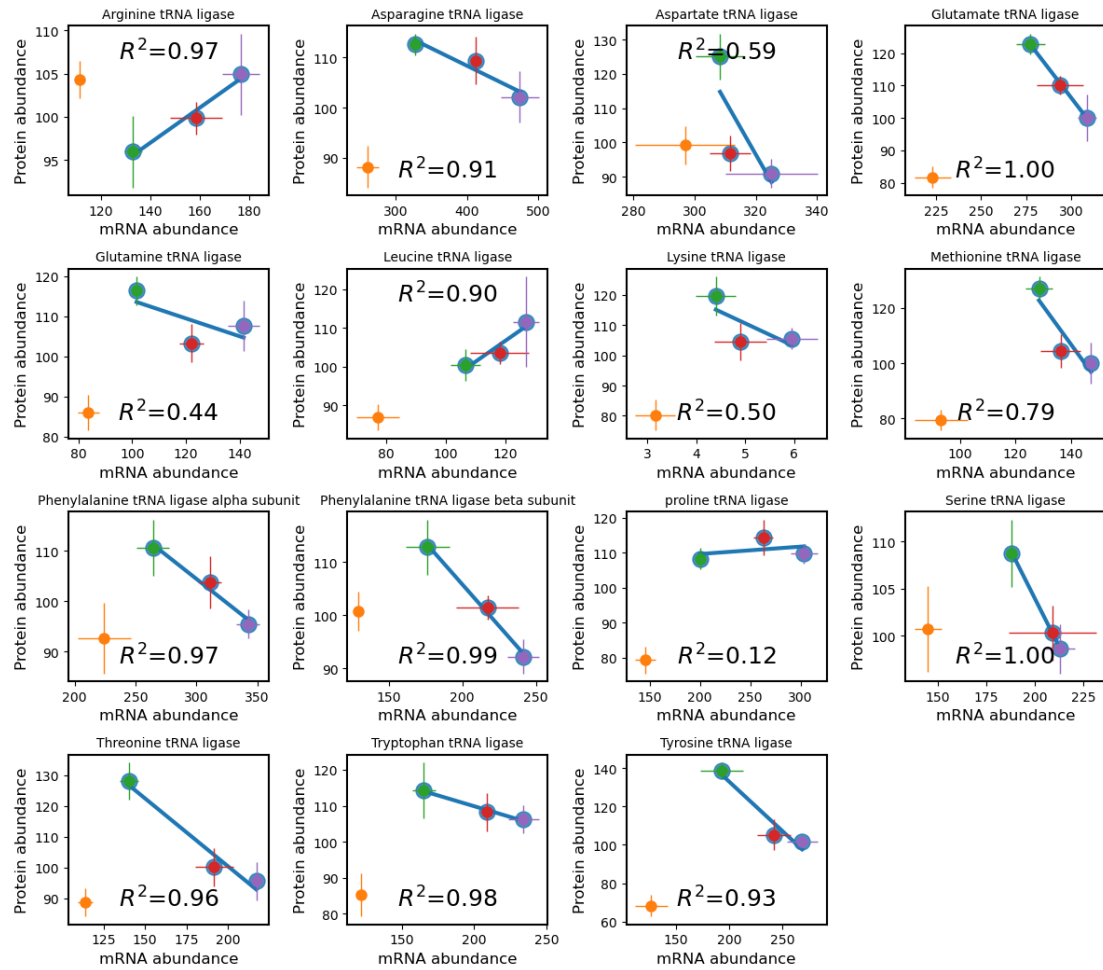
Supplementary Figure 8. Correlation analysis of gene and protein abundances of aminoacyl-tRNA ligases under C-limited culture conditions. Gene expression levels of aminoacyl-tRNA ligases increased with the specific growth rate. However, the protein abundances of serine, phenylalanine, and tryptophan tRNA ligases decreased after a growth rate of 0.2 h^{-1} , while the protein abundance of other tRNA ligases showed an upward trend. Data represent mean $\pm$ SD from three biological replicates.

Correlation between mRNA and protein of tRNA ligase under P-limited condition

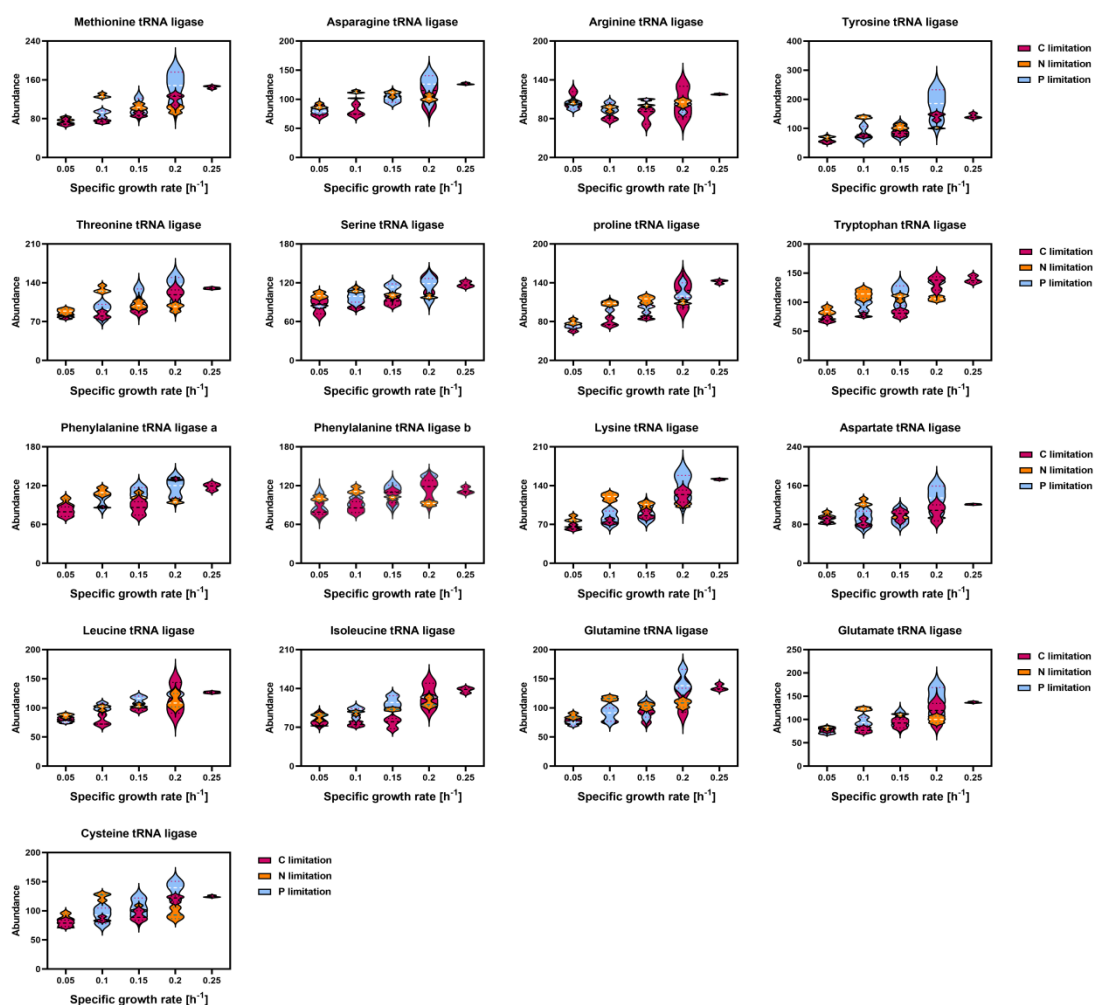


Supplementary Figure 9. Correlation analysis of gene and protein abundances of aminoacyl-tRNA ligases under P-limited culture conditions. Gene expression levels of aminoacyl-tRNA ligases increased with the specific growth rate. The protein levels of tRNA ligases for arginine and phenylalanine decreased, while the protein abundances of other tRNA ligases showed an upward trend. Data represent mean $\pm$ SD from three biological replicates.

Correlation between mRNA and protein of tRNA ligase under N-limited condition

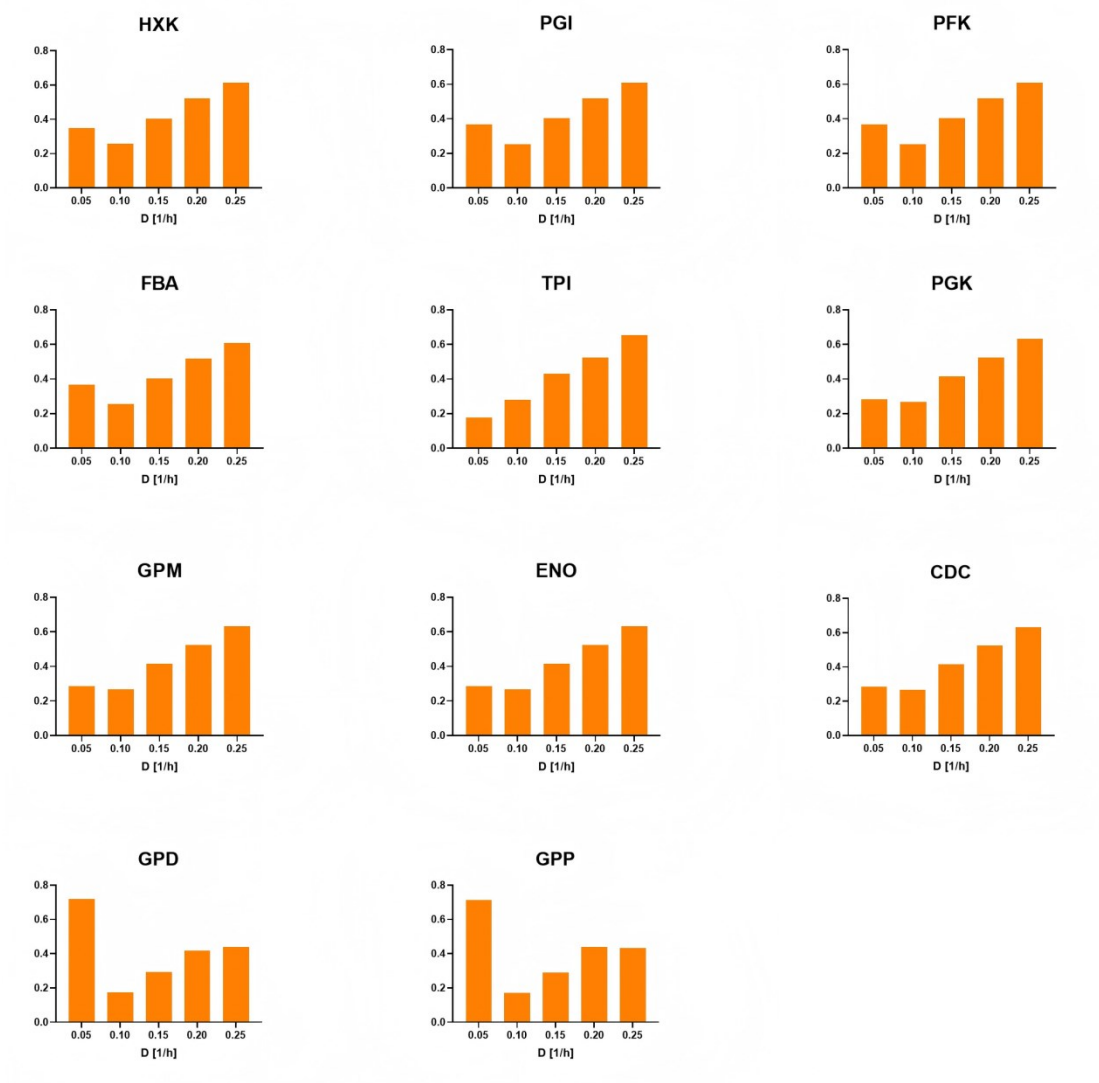


Supplementary Figure 10. Correlation between gene and protein abundances of aminoacyl-tRNA ligases under N-limited culture conditions. Gene expression levels of aminoacyl-tRNA ligases increased with the specific growth rate. However, their protein abundances exhibited two distinct trends after the specific growth rate exceeded 0.1 h^{-1} . Specifically, the protein abundances of tRNA ligases for arginine, leucine, and proline increased, while the protein abundances of tRNA ligases for other amino acids decreased. A strong negative correlation was observed between the protein abundances and gene expression levels of these aminoacyl-tRNA ligases. The results suggest that *S. cerevisiae* regulates the expression of aminoacyl-tRNA ligases at the genetic level, but at the protein level, regulation is constrained by protein resources, preventing the upregulation of aminoacyl-tRNA ligase proteins. Data represent mean $\pm$ SD from three biological replicates.



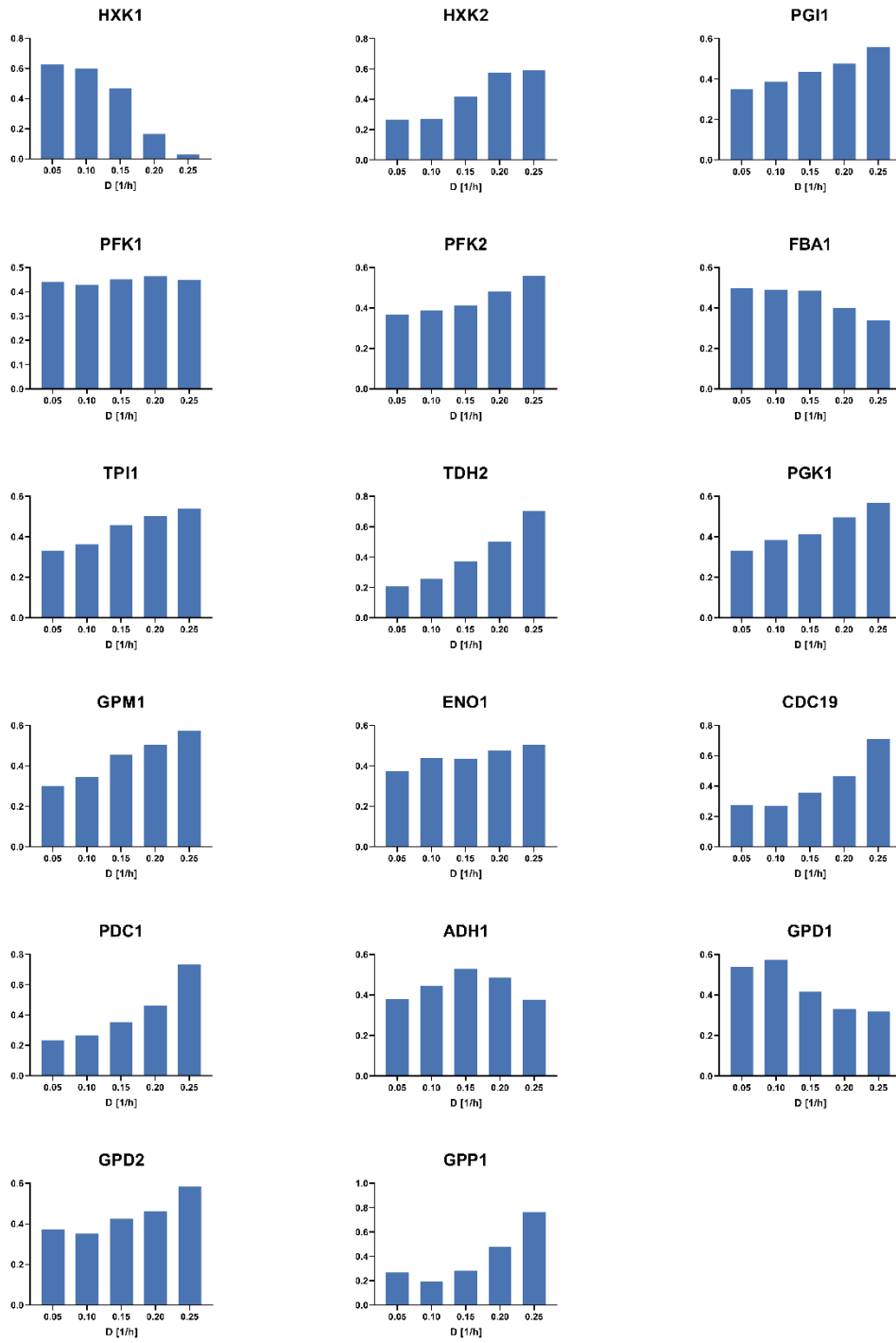
Supplementary Figure 11. Protein abundances of aminoacyl-tRNA ligases at different specific growth rates under C, N, or P limitation. Data represent mean $\pm$ SD from three biological replicates.

Reaction fluxes at different specific growth rates



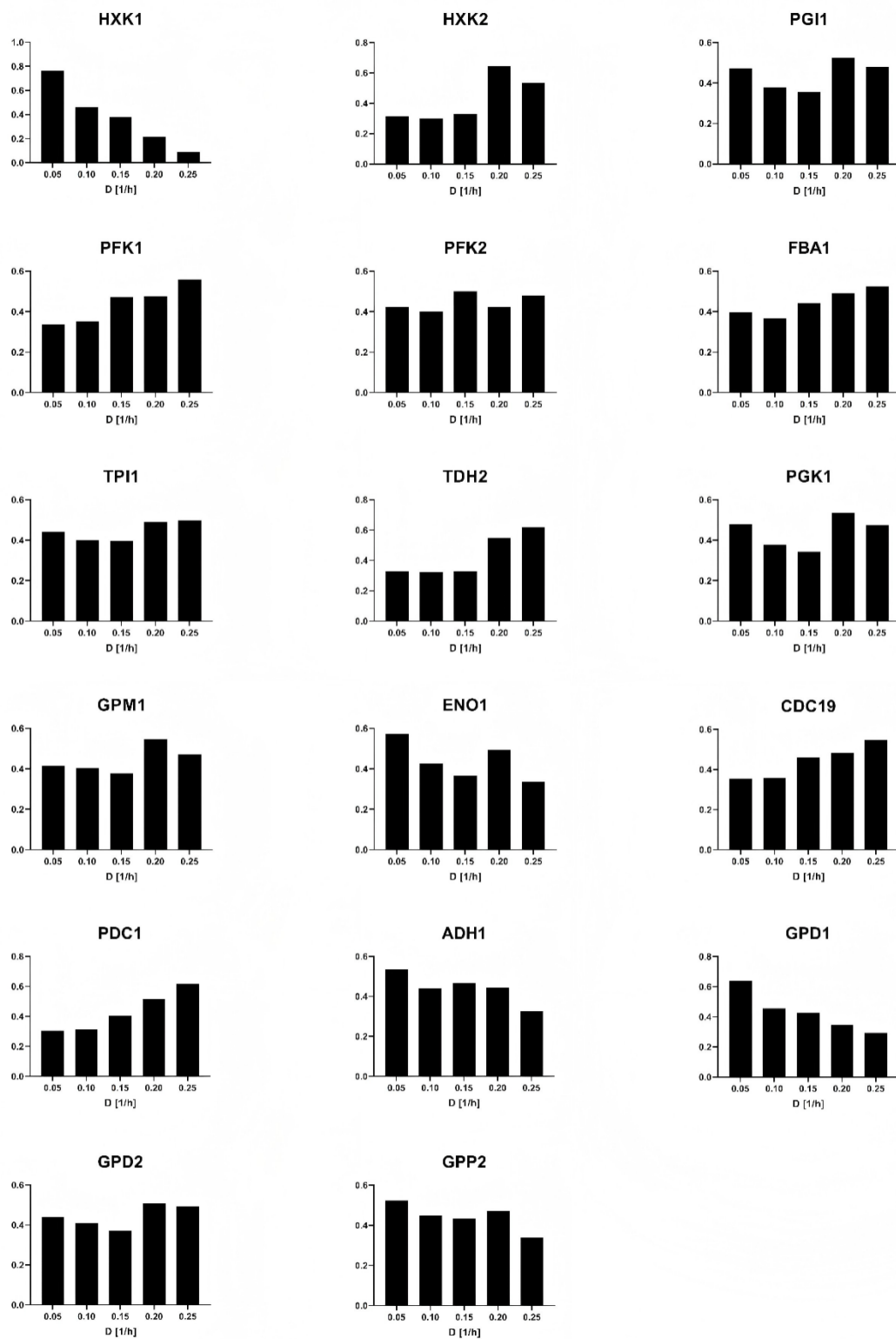
(A)

mRNA levels at different specific growth rates



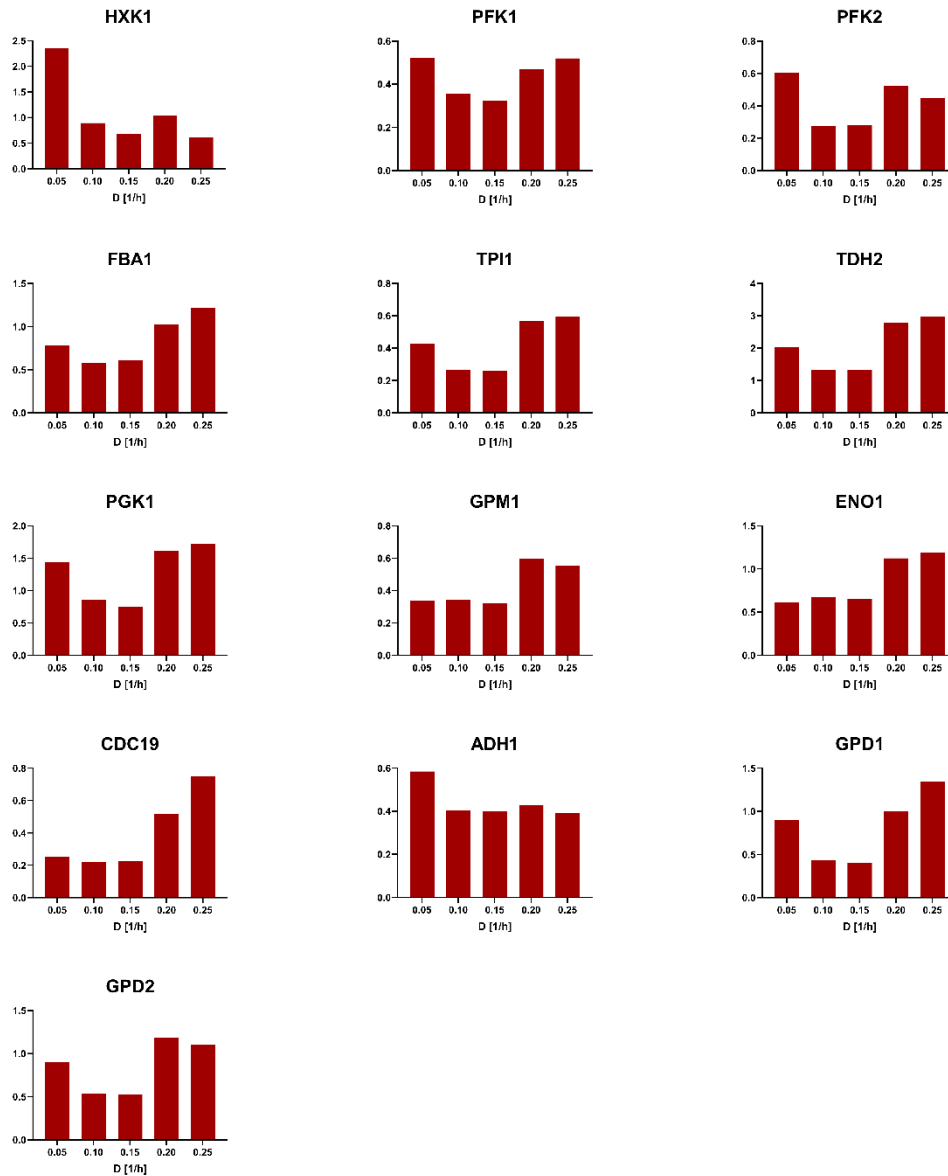
(B)

Protein levels at different specific growth rates



(C)

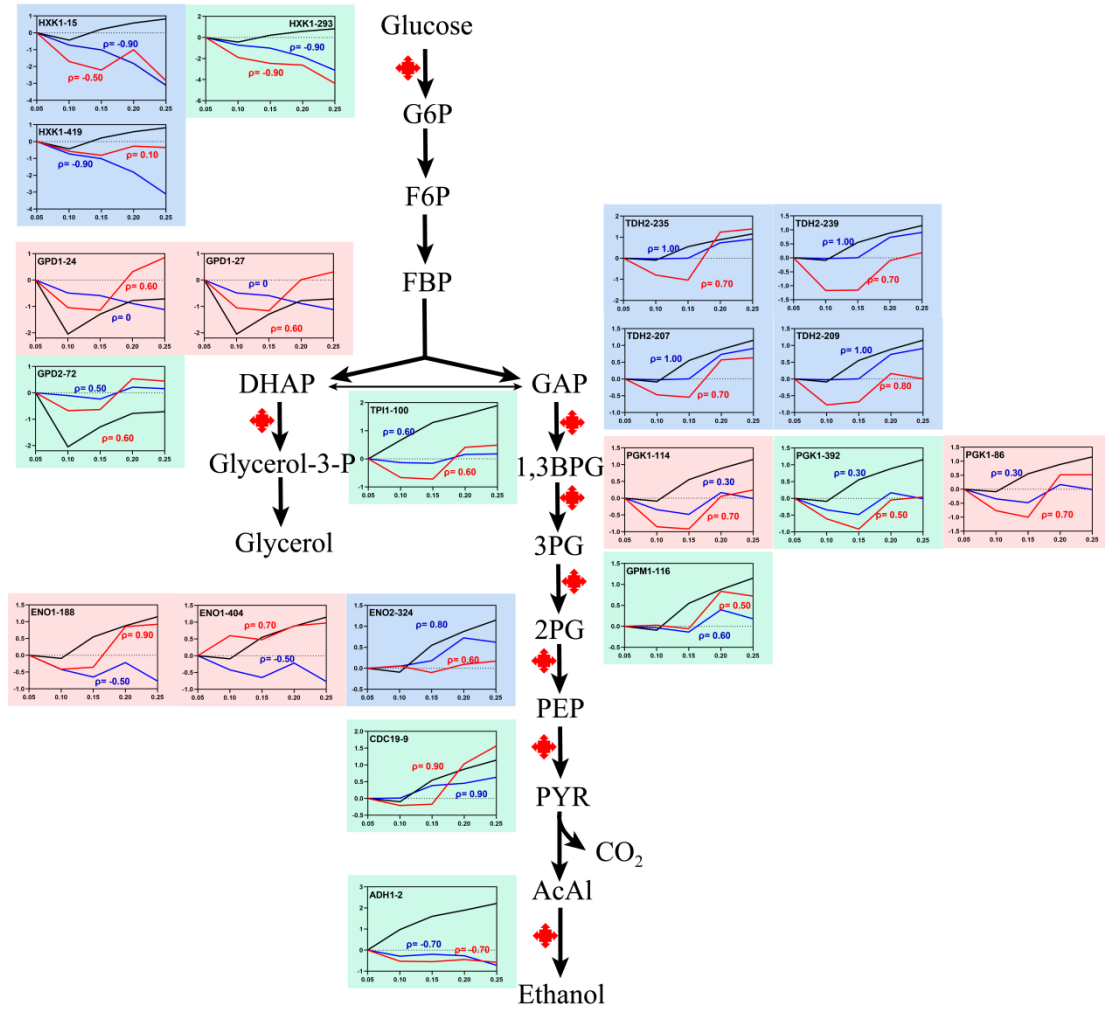
Protein phosphorylation levels at different specific growth rates



(D)

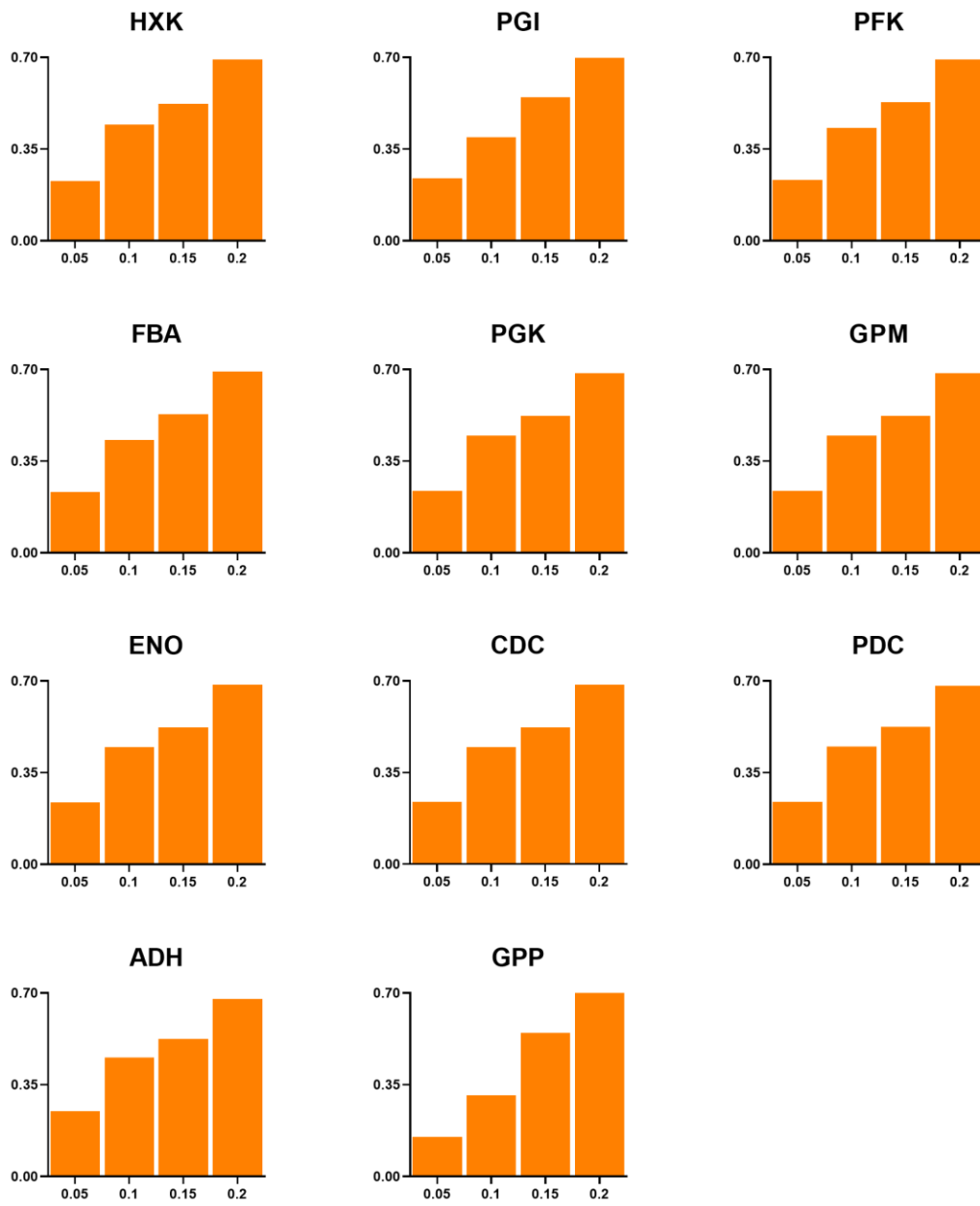
Supplementary Figure 12. Relative abundance of catalytic enzymes involved in glycolysis and ethanol and glycerol metabolism pathways under C-limited culture conditions at the gene, protein, protein phosphorylation, and reaction flux levels. A: Reaction fluxes of glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. B: mRNA abundance in glycolysis and ethanol and glycerol metabolism pathways under five dilution rates. C: A detailed map of the proteins of glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. D: Protein phosphorylation levels in glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. Data represent mean $\pm$ SD from three biological replicates.

C limitation



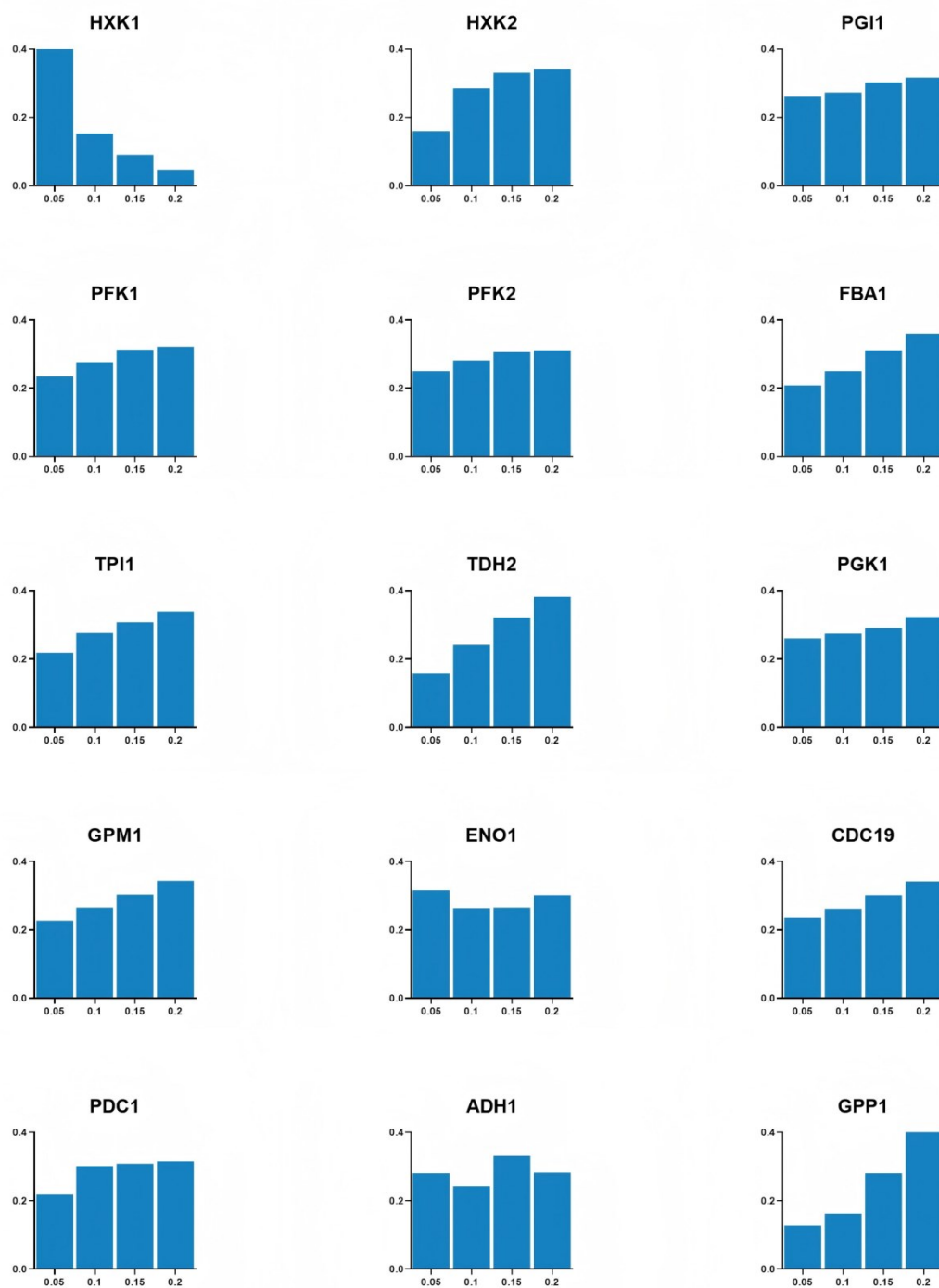
Supplementary Figure 13. Spearman correlations between protein abundance and protein phosphorylation levels of catalytic enzymes in the EMP metabolic pathway and metabolic reaction fluxes under C-limited chemostat culture conditions. The black solid line represents reaction flux, the blue solid line represents enzyme protein abundance, and the red solid line represents enzyme protein phosphorylation level. The blue ρ denotes the correlation coefficient between protein abundance and flux, while the red ρ denotes the correlation coefficient between phosphorylation levels and flux. The blue background indicates a relationship between catalytic enzyme activity and protein abundance, the red background indicates a relationship between catalytic enzyme activity and phosphorylation levels, and the green background indicates a relationship between catalytic enzyme activity, protein abundance and phosphorylation levels. Data represent mean $\pm$ SD from three biological replicates. Spearman's ρ and two-sided p-value are shown in each panel. Blue/red/green backgrounds indicate dominant regulatory mode. All reported ρ are statistically significant at $p < 0.05$ except where noted.

Reaction flux levels at different specific growth rates



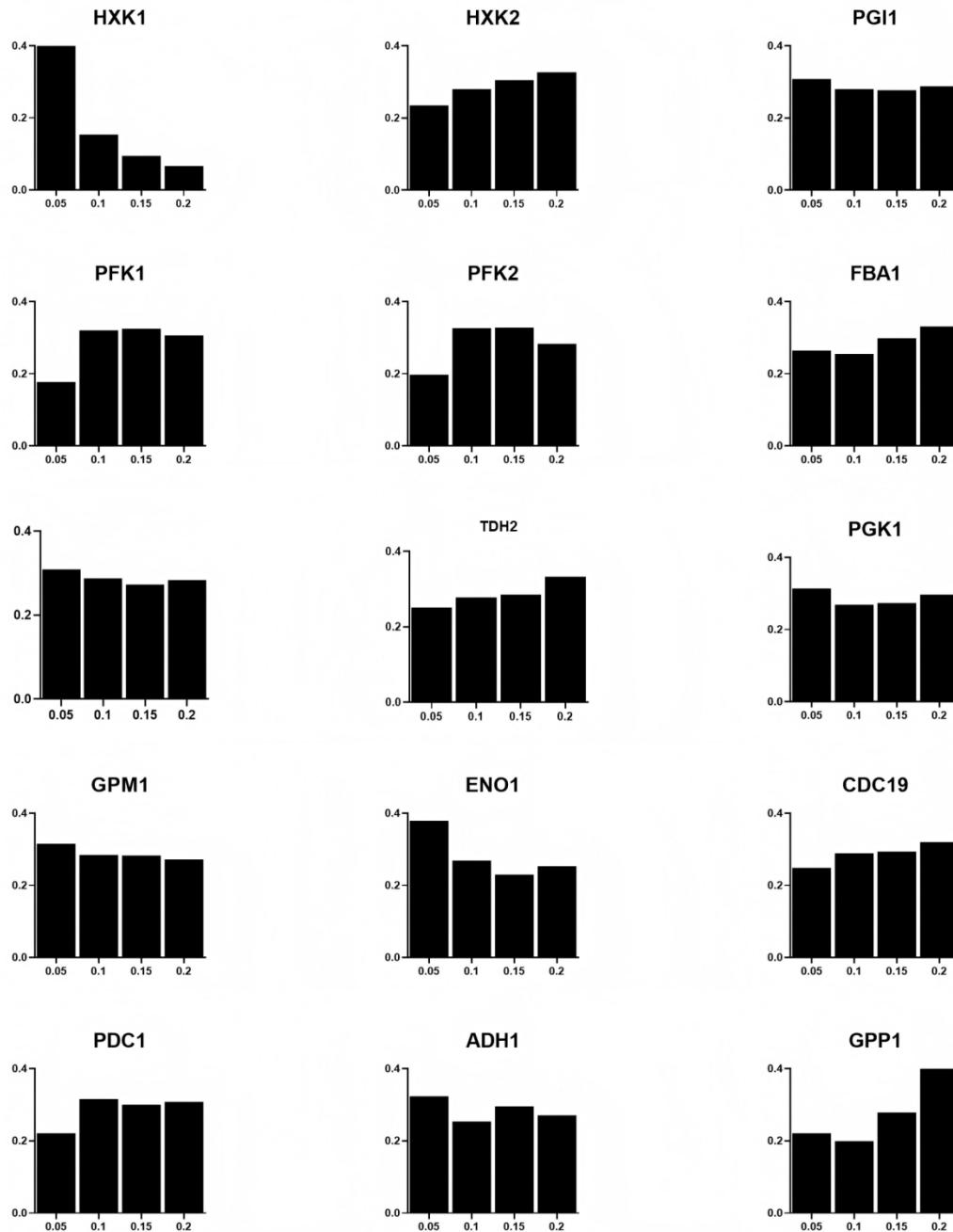
(A)

mRNA levels at different specific growth rates

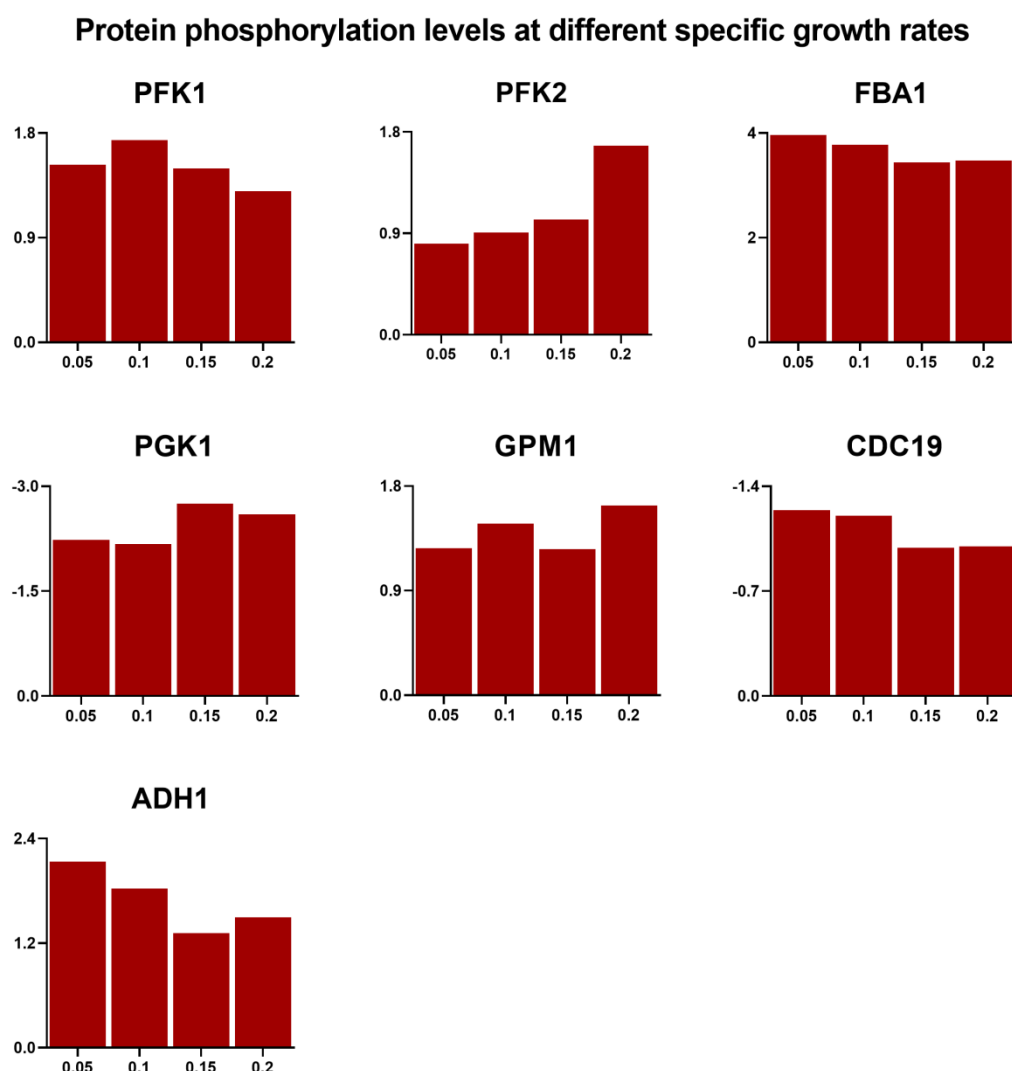


(B)

Protein levels at different specific growth rates



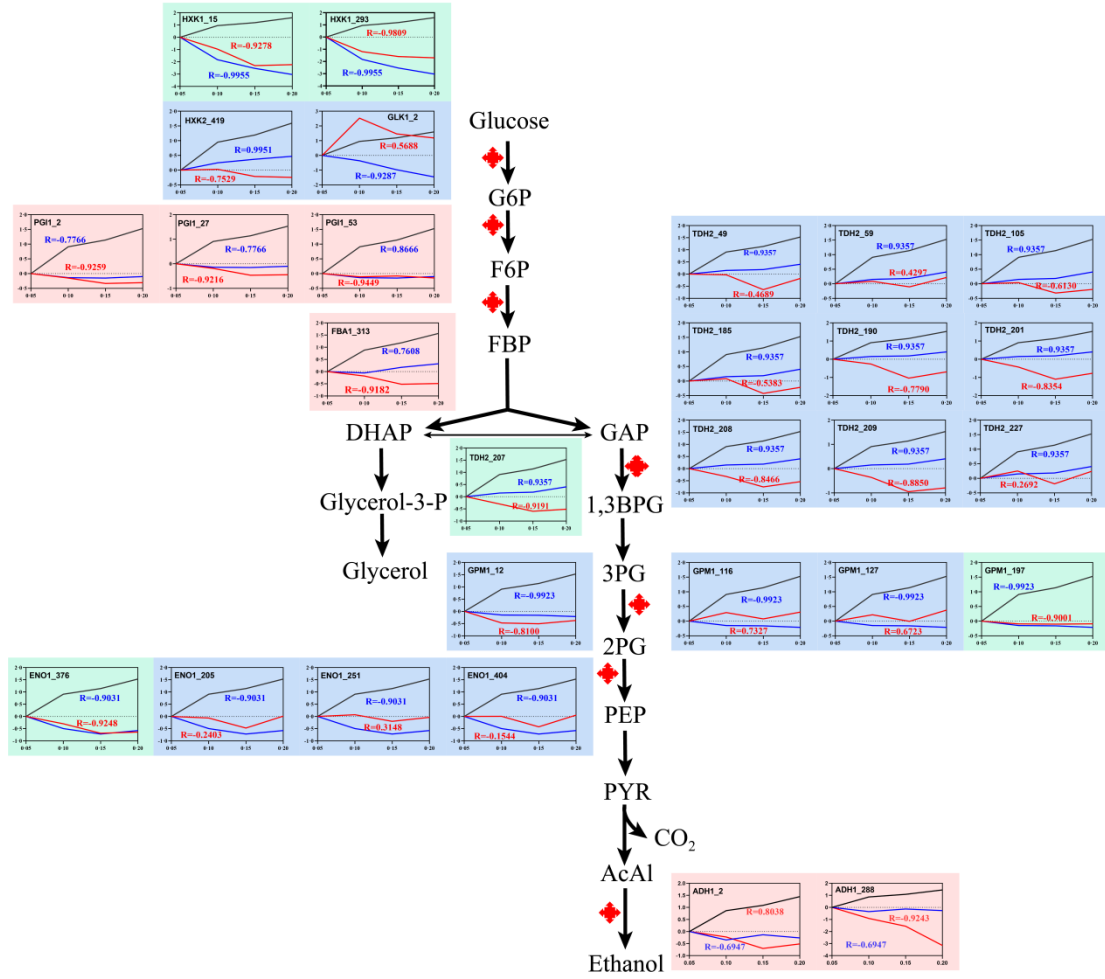
(C)



(D)

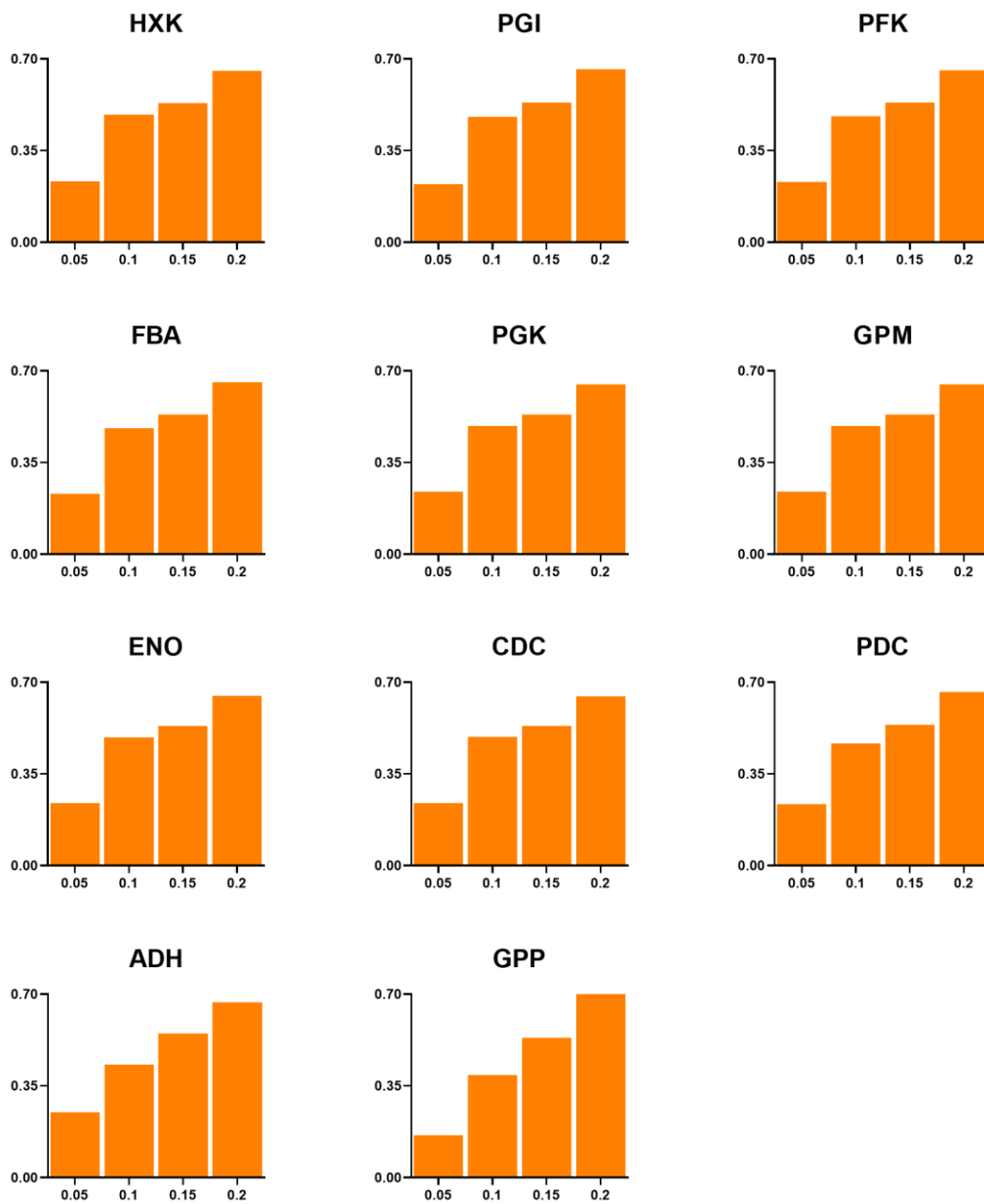
Supplementary Figure 14. Relative abundance of catalytic enzymes involved in glycolysis and ethanol and glycerol metabolism pathways under N-limited culture conditions at gene, protein abundance, protein phosphorylation, and reaction flux level. A: Reaction fluxes of glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. B: mRNA abundance of glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. C: Protein abundance in glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. D: Protein phosphorylation levels in glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. Data represent mean $\pm$ SD from three biological replicates.

N limitation



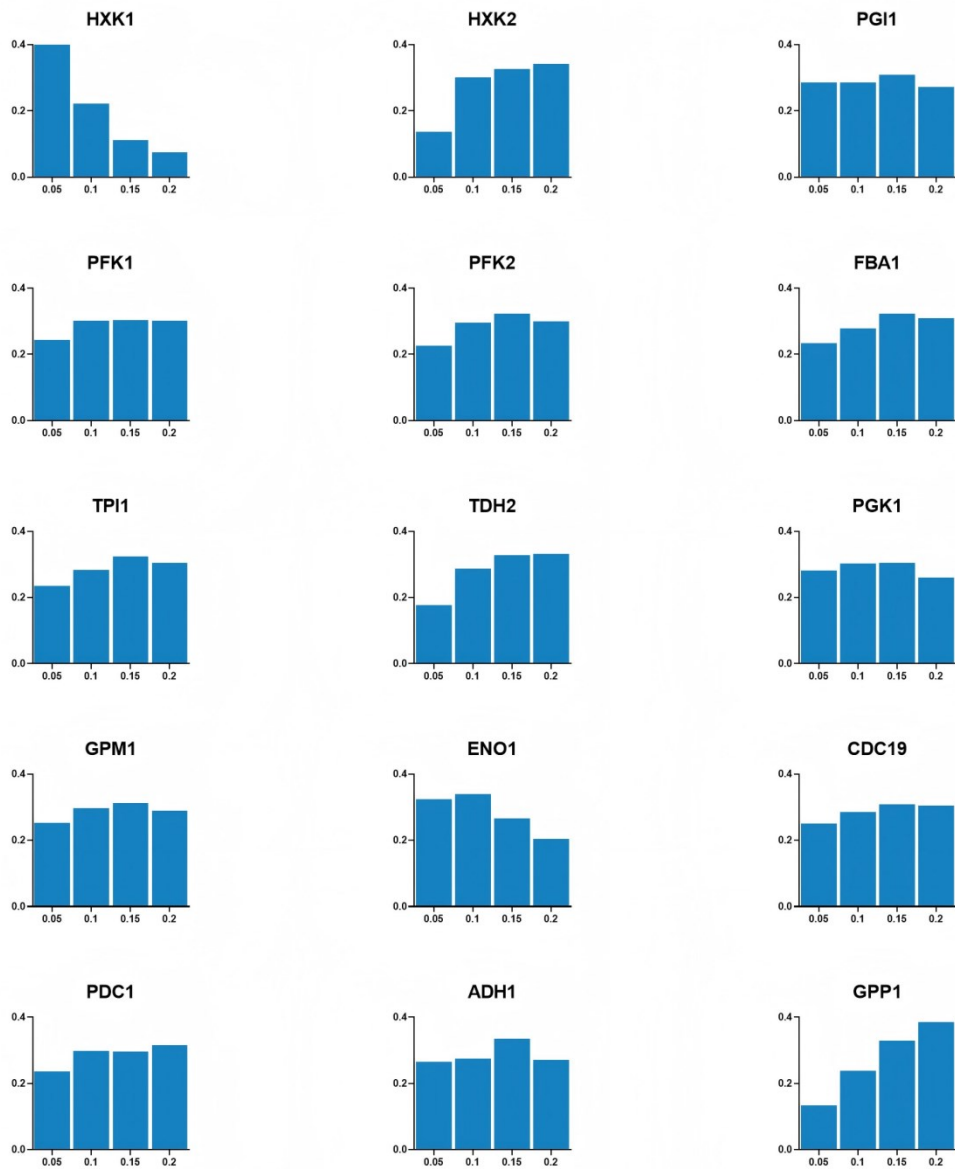
Supplementary Figure 15. Spearman correlations between protein abundance and protein phosphorylation levels of catalytic enzymes in the EMP metabolic pathway and metabolic reaction fluxes under N-limited chemostat culture conditions. The black solid line represents reaction flux, the blue solid line represents enzyme protein abundance, and the red solid line represents enzyme protein phosphorylation level. The blue ρ denotes the correlation coefficient between protein abundance and flux, while the red ρ denotes the correlation coefficient between phosphorylation levels and flux. The blue background indicates a relationship between catalytic enzyme activity and protein abundance, the red background indicates a relationship between catalytic enzyme activity and phosphorylation levels, and the green background indicates a relationship between catalytic enzyme activity, protein abundance and phosphorylation levels. Data represent mean $\pm$ SD from three biological replicates. Spearman's ρ and two-sided p-value are shown in each panel. Blue/red/green backgrounds indicate dominant regulatory mode. All reported ρ are statistically significant at $p < 0.05$ except where noted.

Reaction flux levels at different specific growth rates



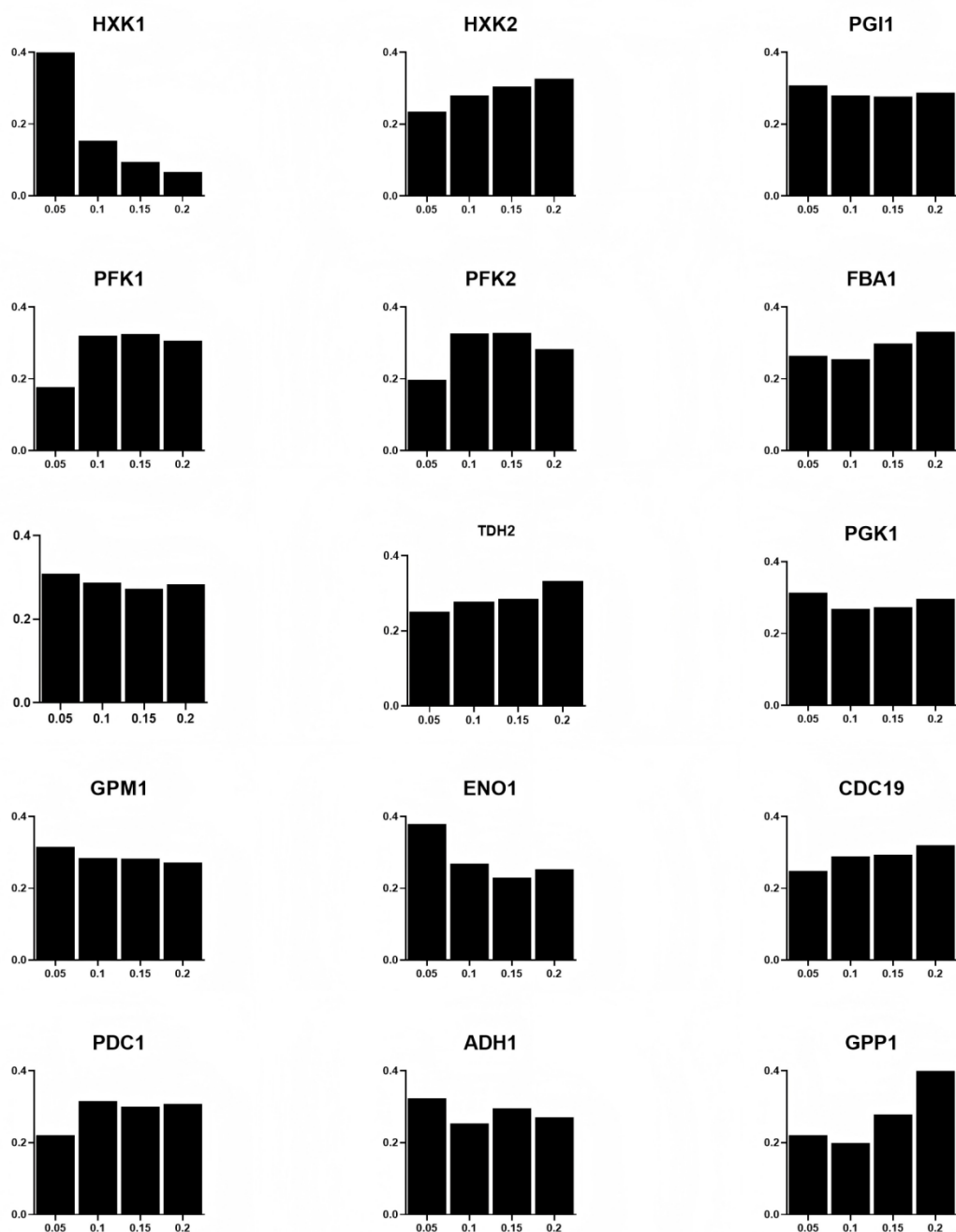
(A)

mRNA levels at different specific growth rates



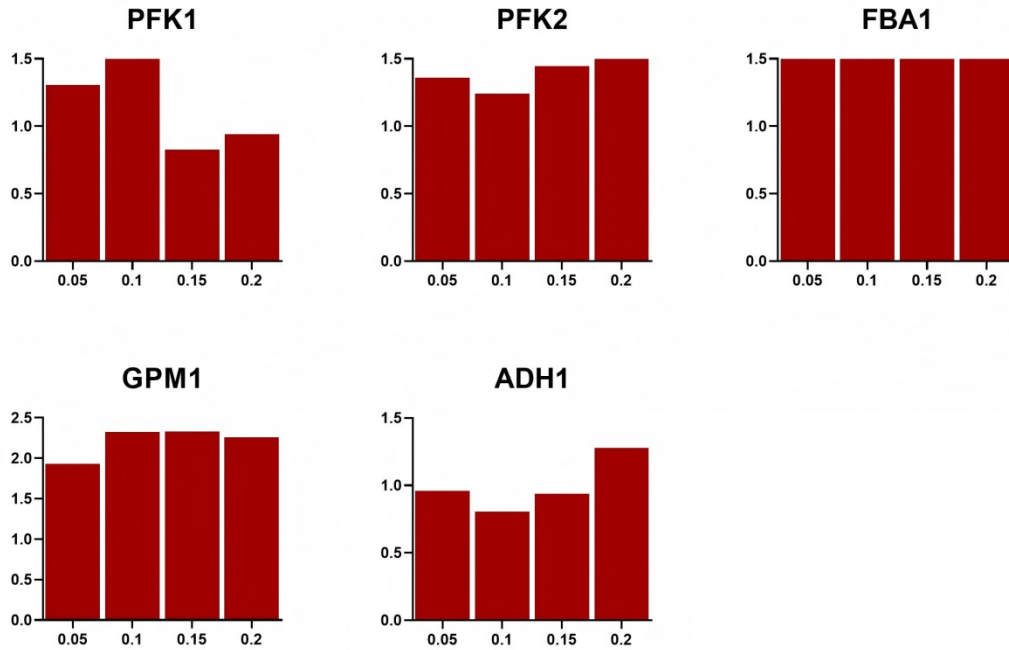
(B)

Protein levels at different specific growth rates



(C)

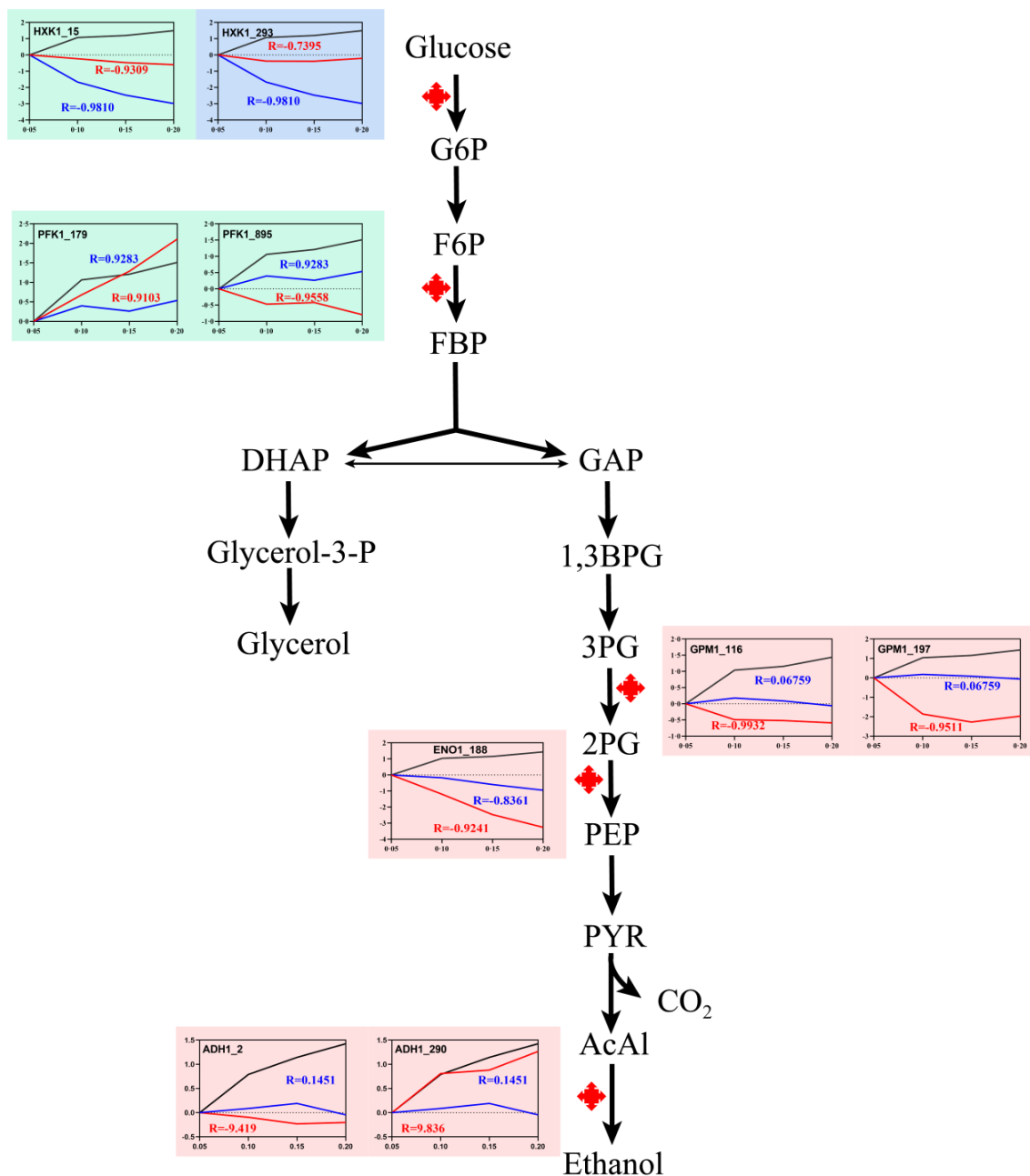
Protein phosphorylation levels at different specific growth rates



(D)

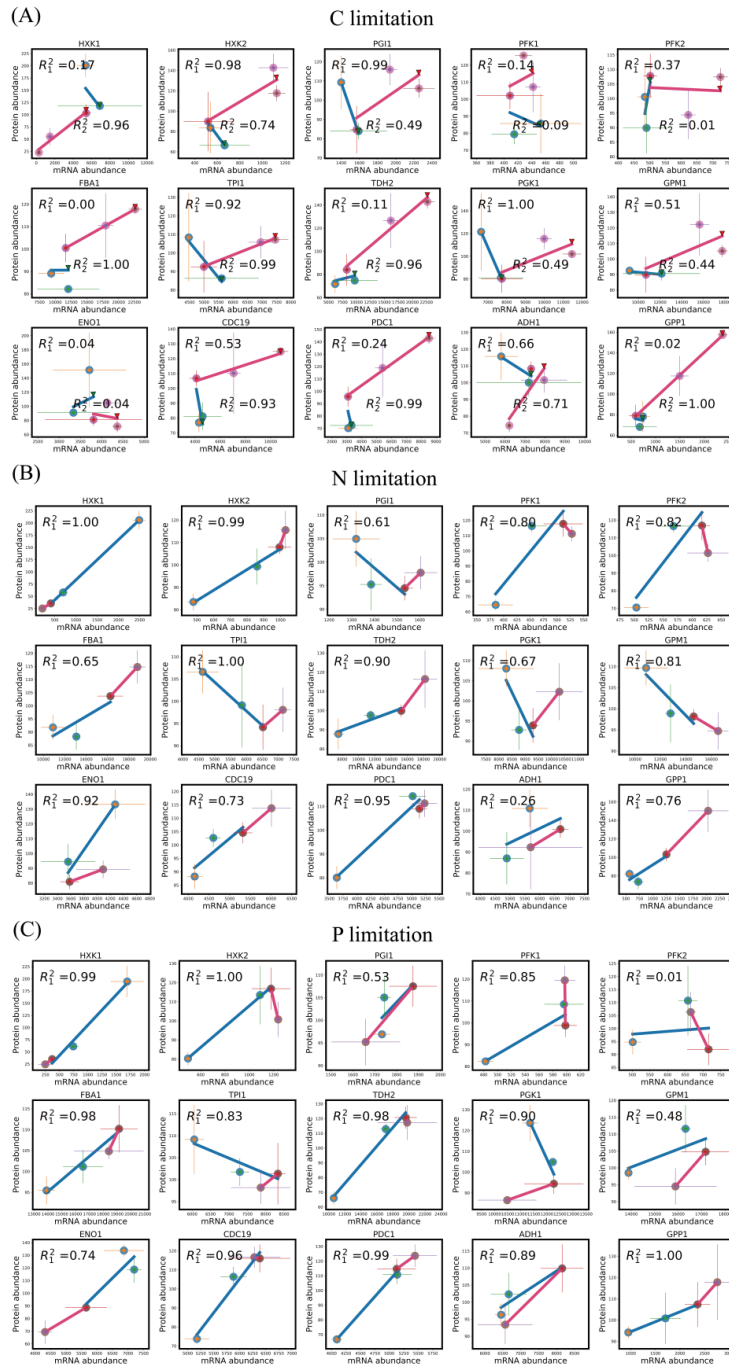
Supplementary Figure 16. Relative abundance of catalytic enzymes involved in glycolysis and ethanol and glycerol metabolism pathways under P-limited culture conditions at gene, protein abundance, protein phosphorylation, and reaction flux level. A: Reaction fluxes of glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. B: mRNA abundance of glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. C: Protein abundance in glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. D: Protein phosphorylation levels in glycolysis and ethanol and glycerol metabolism pathways at five different dilution rates. Data represent mean $\pm$ SD from three biological replicates.

P limitation



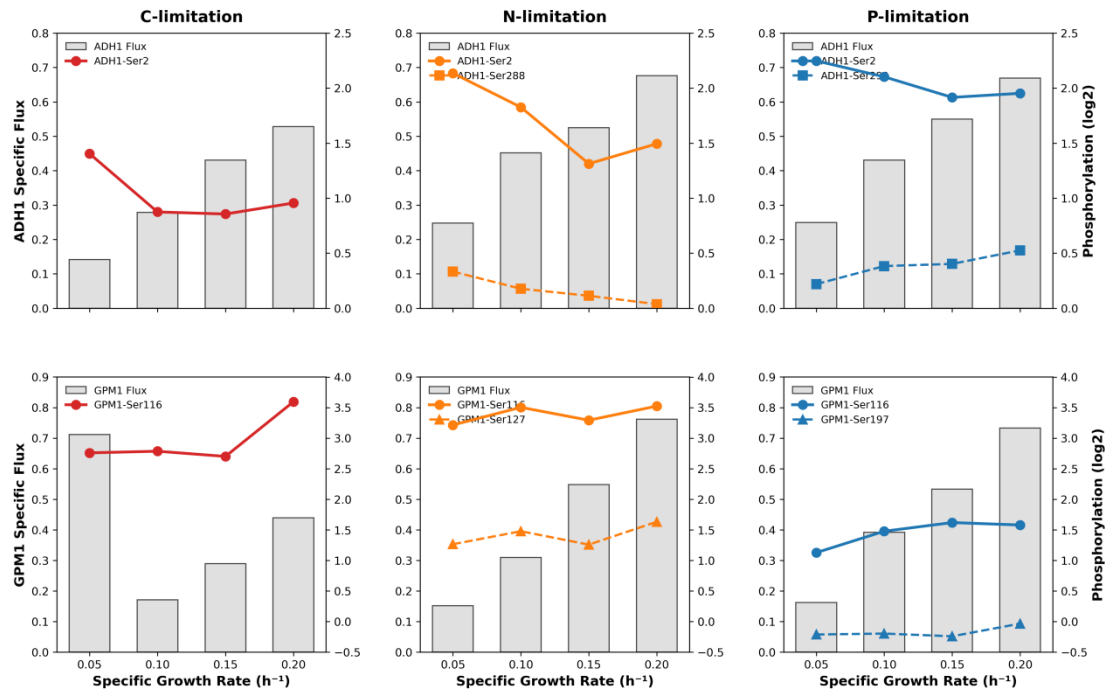
Supplementary Figure 17. Spearman correlations between protein abundance and protein phosphorylation levels of catalytic enzymes in the EMP metabolic pathway and metabolic reaction fluxes under P-limited chemostat culture conditions. The black solid line represents reaction flux, the blue solid line represents enzyme protein abundance, and the red solid line represents enzyme protein phosphorylation level. The blue ρ denotes the correlation coefficient between protein abundance and flux, while the red ρ denotes the correlation coefficient between phosphorylation levels and flux. The blue background indicates a relationship between catalytic enzyme activity and protein abundance, the red background indicates a relationship between catalytic enzyme activity and phosphorylation levels,

and the green background indicates a relationship between catalytic enzyme activity, protein abundance and phosphorylation levels. Data represent mean $\pm$ SD from three biological replicates. Spearman's ρ and two-sided p-value are shown in each panel. Blue/red/green backgrounds indicate dominant regulatory mode. All reported ρ are statistically significant at $p < 0.05$ except where noted.

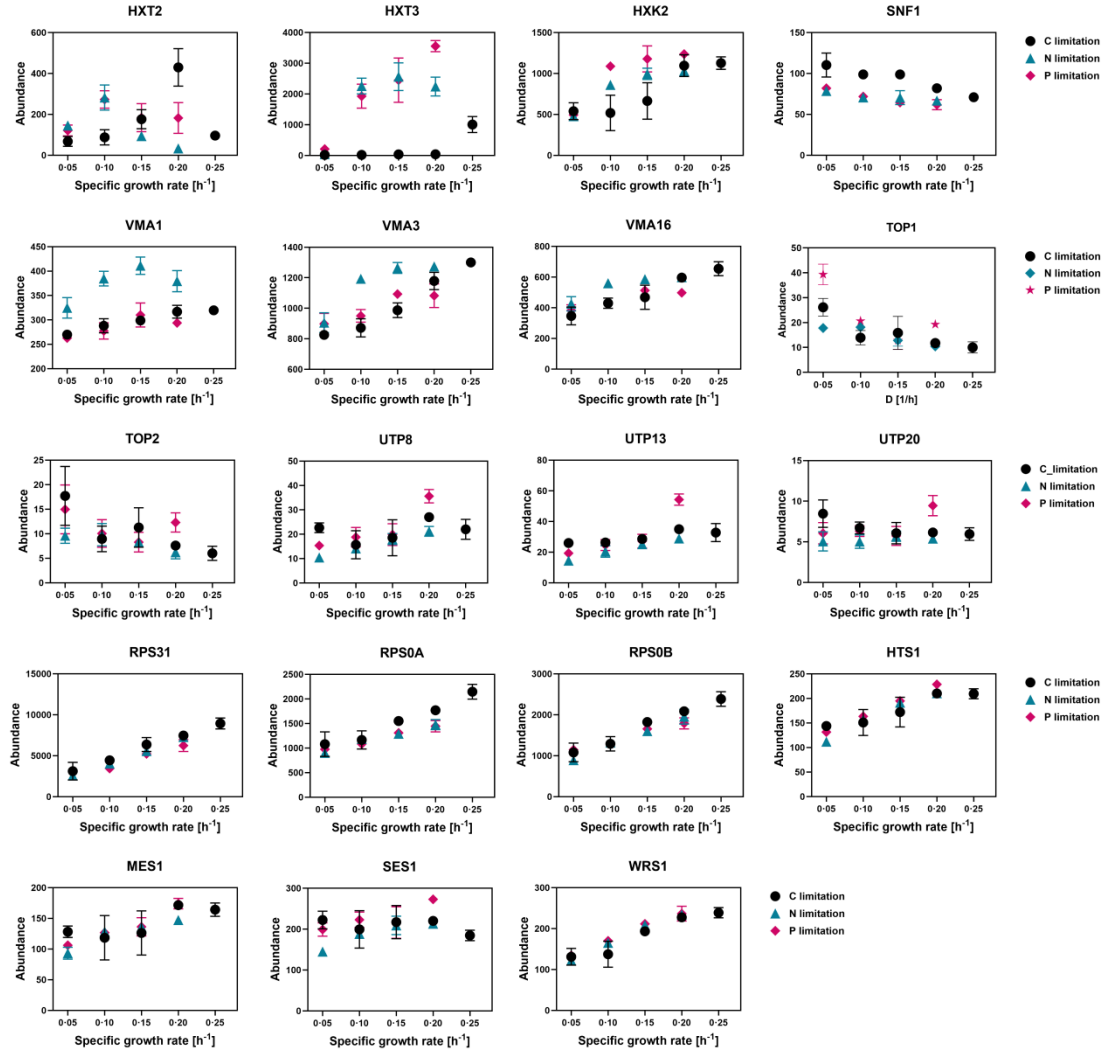


Supplementary Figure 18. Correlation between mRNA and proteins of catalytic enzymes involved in glycolysis and ethanol and glycerol metabolism pathways. A: C-limited conditions. B: N-limited conditions. C: P-limited conditions. In all three conditions, the data were piecewise fitted. For C-limited conditions, the breakpoint was set at $\mu=0.15 \text{ h}^{-1}$, while for N- and P-limited conditions, the breakpoint was set at $\mu=0.2 \text{ h}^{-1}$. The blue line represents the first segment, and the red line represents the second segment. Data represent mean $\pm$ SD from three biological replicates.

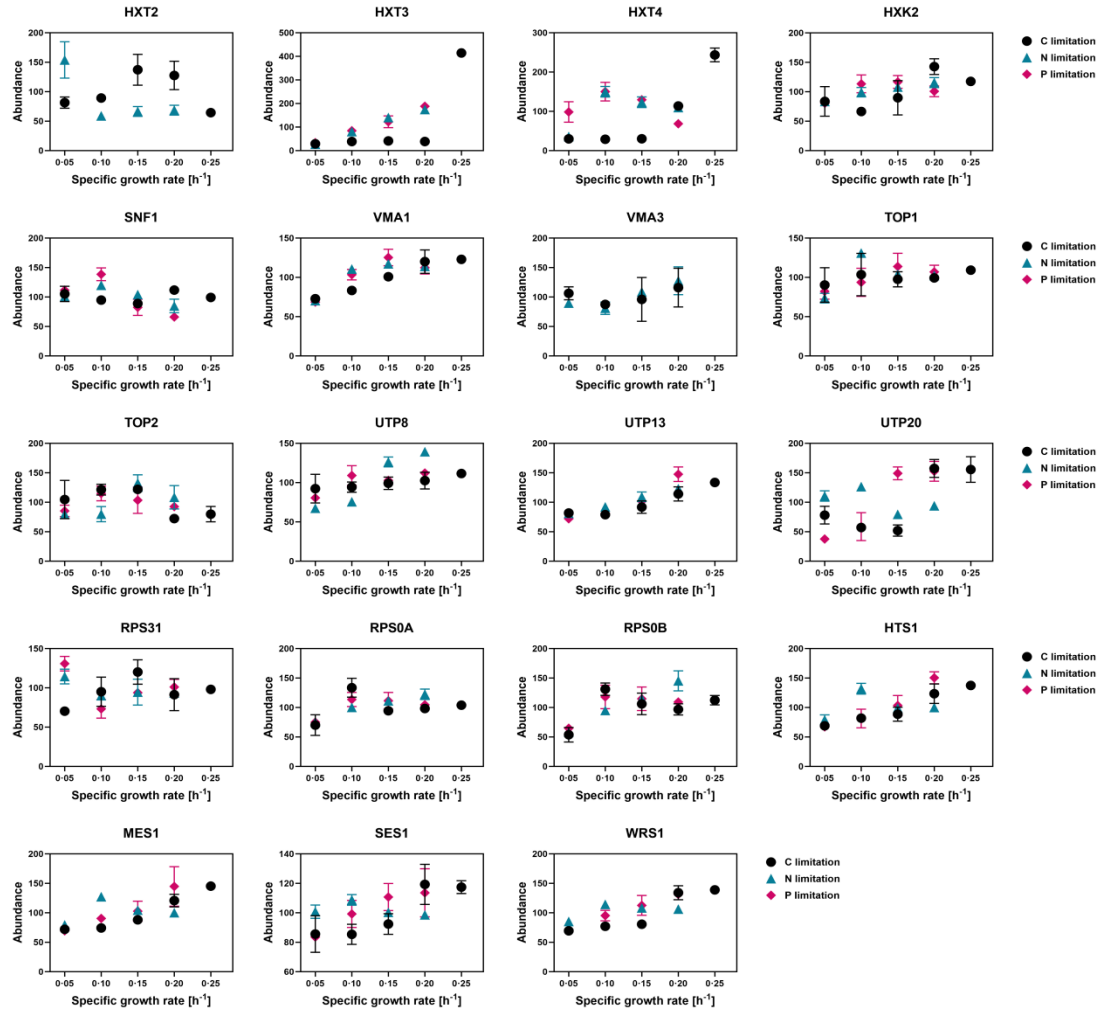
Systems-Level Shift to Activity-Based Control across Multiple Enzymes



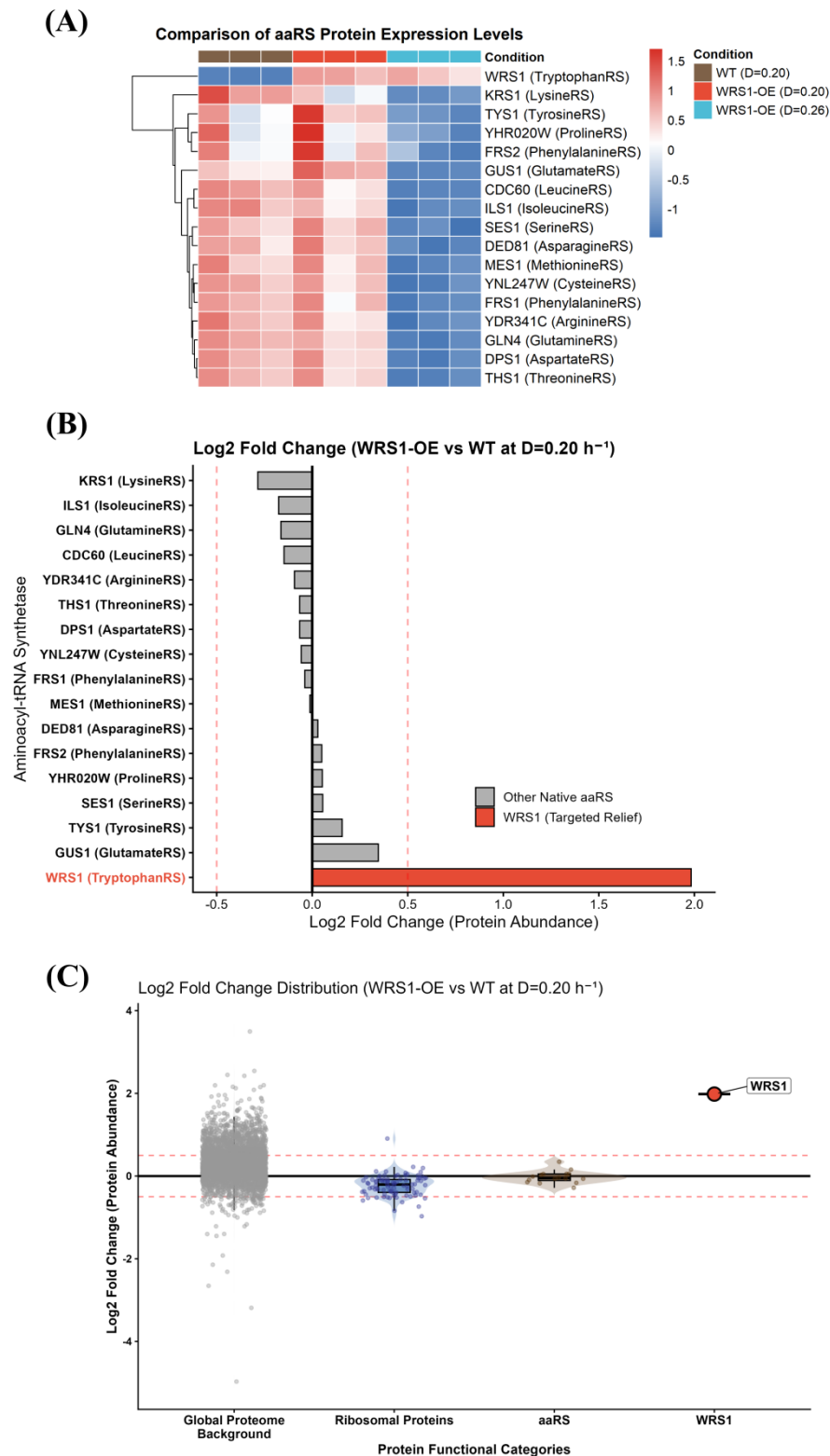
Supplementary Figure 19. Information on changes in protein phosphorylation levels. The macroscopic specific metabolic flux (grey bars) is plotted against relative site-specific phosphorylation levels (colored lines) across varying specific growth rates (μ). Under C-limitation, cells rely predominantly on abundance-based control, maintaining stable or slightly decreasing baseline phosphorylation. In stark contrast, under P-limitation, where severe ATP depletion thermodynamically restricts bulk protein translation (abundance-based control fails), the cell forcefully upregulates the phosphorylation of bottleneck enzymes e.g., ADH1 at Ser2/290 to sustain the essential glycolytic flux. Data represent mean $\pm$ SD from three biological replicates.



Supplementary Figure 20. Variation in mRNA expression levels of molecular validation of gene loci under three elemental limitations as a function of with the specific growth rate. Data represent mean $\pm$ SD from three biological replicates.



Supplementary Figure 21. Variation in protein abundance of molecular validation gene loci under three elemental limitations as a function of the specific growth rate. Data represent mean $\pm$ SD from three biological replicates.



Supplementary Figure 22. Validation of *WRS1* overexpression. (A) aaRS Heatmap. Displays the specific hyper-accumulation of WRS1 in the OE strain; other native aaRSs remain completely stable. (B) Confirms the massive, precise upregulation of WRS1 Log2FC=1.98, $P < 0.01$ without pleiotropic effects on other aaRSs (average Log2FC ≈ 0). (C) Changes in the abundance of translation-related proteins. Data represent mean $\pm$ SD from three biological replicates.