

# The *E. coli* molecular phenotype under different growth conditions

## Supplementary materials

Mehmet U. Caglar\*, John R. Houser, Craig S. Barnhart,  
Daniel R. Boutz, Sean M. Carroll, Aurko Dasgupta, Walter F. Lenoir,  
Bartram L. Smith, Viswanadham Sridhara, Dariya K. Sydykova,  
Drew Vander Wood, Christopher J. Marx,  
Edward M. Marcotte\*, Jeffrey E. Barrick\*, Claus O. Wilke\*

January 24, 2017

## List of Figures

S1	Significantly differentially expressed molecular functions . . . . .	4
S2	Significantly differentially expressed KEGG pathways for mRNA samples in exponential phase tested for glycerol against glucose . . . . .	5
S3	Significantly differentially expressed KEGG pathways for mRNA samples in exponential phase tested for gluconate against glucose . . . . .	6
S4	Significantly differentially expressed KEGG pathways for mRNA samples in exponential phase tested for lactate against glucose . . . . .	7
S5	Significantly differentially expressed KEGG pathway for protein samples in exponential phase tested for gluconate against glucose . . . . .	8
S6	Significantly differentially expressed KEGG pathways for protein samples in exponential phase tested for lactate against glucose . . . . .	9
S7	Significantly differentially expressed KEGG pathway for protein samples in stationary phase tested for glycerol against glucose . . . . .	10
S8	Significantly differentially expressed KEGG pathway for protein samples in stationary phase tested for gluconate against glucose . . . . .	11
S9	Significantly differentially expressed KEGG pathways for protein samples in stationary phase tested for lactate against glucose . . . . .	12
S10	Significantly differentially expressed KEGG pathways for mRNA samples in exponential phase tested for low $Mg^{2+}$ levels against base $Mg^{2+}$ . . . . .	13
S11	Significantly differentially expressed KEGG pathways for mRNA samples in exponential phase tested for high $Mg^{2+}$ against base $Mg^{2+}$ . . . . .	14
S12	Significantly differentially expressed KEGG pathways for protein samples in exponential phase tested for high $Mg^{2+}$ against base $Mg^{2+}$ . . . . .	15
S13	Significantly differentially expressed KEGG pathway for mRNA samples in stationary phase tested for high $Mg^{2+}$ against base $Mg^{2+}$ . . . . .	16
S14	Significantly differentially expressed KEGG pathway for mRNA samples in exponential phase tested for high $Na^+$ against base $Na^+$ . . . . .	17
S15	Significantly differentially expressed KEGG pathways for protein samples in exponential phase tested for high $Na^+$ against base $Na^+$ . . . . .	18
S16	Significantly differentially expressed KEGG pathways for mRNA samples in stationary phase tested for high $Na^+$ against base $Na^+$ . . . . .	19
S17	Significantly differentially expressed KEGG pathways for protein samples in stationary phase tested for high $Na^+$ against base $Na^+$ . . . . .	20
S18	Significantly differentially expressed GO annotations associated with molecular functions for mRNA samples in exponential phase tested for glycerol against glucose . . . . .	21
S19	Significantly differentially expressed GO annotations associated with molecular functions for mRNA samples in exponential phase tested for gluconate against glucose . . . . .	21
S20	Significantly differentially expressed GO annotations associated with molecular functions for mRNA samples in exponential phase tested for lactate against glucose . . . . .	22
S21	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in exponential phase tested for glycerol against glucose . . . . .	22
S22	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in exponential phase tested for lactate against glucose . . . . .	23
S23	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in stationary phase tested for glycerol against glucose . . . . .	23
S24	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in stationary phase tested for gluconate against glucose . . . . .	24
S25	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in stationary phase tested for lactate against glucose . . . . .	24
S26	Significantly differentially expressed GO annotations associated with molecular functions for mRNA samples in exponential phase tested for low $Mg^{2+}$ levels against base $Mg^{2+}$ levels . . . . .	25
S27	Significantly differentially expressed GO annotations associated with molecular functions for mRNA samples in exponential phase tested for high $Mg^{2+}$ levels against base $Mg^{2+}$ levels . . . . .	25

S28	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in exponential phase tested for low $\text{Mg}^{2+}$ levels against base $\text{Mg}^{2+}$ levels	26
S29	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in exponential phase tested for high $\text{Mg}^{2+}$ levels against base $\text{Mg}^{2+}$ levels . . . . .	26
S30	Significantly differentially expressed GO annotations associated with molecular functions for mRNA samples in stationary phase tested for low $\text{Mg}^{2+}$ levels against base $\text{Mg}^{2+}$ levels	27
S31	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in exponential phase tested for high $\text{Na}^+$ levels against base $\text{Na}^+$ levels	27
S32	Significantly differentially expressed GO annotations associated with molecular functions for mRNA samples in stationary phase tested for high $\text{Na}^+$ levels against base $\text{Na}^+$ levels	28
S33	Significantly differentially expressed GO annotations associated with molecular functions for protein samples in stationary phase tested for high $\text{Na}^+$ levels against base $\text{Na}^+$ levels	28
S34	Change in differentially expressed genes between two controls: "batch" vs "batch + doubling time" . . . . .	29
S35	Flux ratios versus ion concentrations . . . . .	30

## Supplementary Figures

A	mRNA	Protein	
	1.structural constituent of ribosome ▼▼▼ 2.rRNA binding ▼▼▼ 3.structural molecule activity ▼▼▼ 4.RNA binding ▼▼▼ 5.RNA-dependent ATPase activity ▼▼▼	1.L-lactate dehydrogenase activity ▼▼▼ 2.lactate dehydrogenase activity ▼▼▼	lowMg
	1.energy transducer activity ▲ 2.alkanesulfonate transporter activity ▲ 3.oxidoreductase activity ▲▼	1.L-lactate dehydrogenase activity ▼▼▼ 2.lactate dehydrogenase activity ▼▼▼	highMg
		1.binding ▼ 2.protein binding ▼ 3.structural constituent of ribosome ▼▼▼ 4.small molecule binding ▼ 5.nucleotide binding ▼	highNa
	1.structural constituent of ribosome ▼▼▼ 2.structural molecule activity ▼▼▼ 3.rRNA binding ▼▼▼ 4.RNA binding ▼▼▼ 5.tRNA binding ▼▼▼	1.ATP binding ▼▼ 2.adenyl ribonucleotide binding ▼▼ 3.adenyl nucleotide binding ▼▼ 4.molecular transducer activity ▼▼▼ 5.oxidoreductase activity, acting on the CH-OH group of donors, quinon	glycerol
	1.gluconate transmembrane transporter activity ▲ 2.aldonate transmembrane transporter activity ▲		gluconate
	1.structural constituent of ribosome ▲▲ 2.structural molecule activity ▲▲ 3.rRNA binding ▲▲ 4.RNA binding ▲▲ 5.lactate dehydrogenase activity ▲▲	1.catalytic activity ▲▲ 2.lactate dehydrogenase activity ▲▲ 3.hydrolase activity ▲▲ 4.L-lactate dehydrogenase activity ▲▲	lactate
B	mRNA	Protein	
	1.ATPase activity ▼▼▼ 2.hydrolase activity, acting on acid anhydrides, catalyzing transmembr 3.oligopeptide-transporting ATPase activity ▼▼▼ 4.peptide-transporting ATPase activity ▼▼▼ 5.ATPase activity, coupled ▼▼▼		lowMg
			highMg
	1.carbohydrate transmembrane transporter activity ▼▼ 2.carbohydrate transporter activity ▼▼ 3.polyol transmembrane transporter activity ▼▼ 4.alcohol transmembrane transporter activity ▼▼ 5.organic hydroxy compound transmembrane transporter activity ▼▼	1.protein binding ▼ 2.binding ▼ 3.structural constituent of ribosome ▼▼▼ 4.ion binding ▼ 5.structural molecule activity ▼▼▼	highNa
		1.oxidoreductase activity ▼▼ 2.oxidoreductase activity, acting on the aldehyde or oxo group of donors 3.coenzyme binding ▼ 4.oxidoreductase activity, acting on the CH-OH group of donors, quinon 5.cofactor binding ▼	glycerol
		1.siderophore transmembrane transporter activity ▼▼▼ 2.2,3-dihydroxybenzoate-serine ligase activity ▼▼▼ 3.siderophore transporter activity ▼▼▼ 4.iron chelate transmembrane transporter activity ▼▼▼	gluconate
		1.lactate dehydrogenase activity ▲▲ 2.oxidoreductase activity, acting on CH-OH group of donors ▲▲ 3.binding ▲▲ 4.L-lactate dehydrogenase activity ▲▲ 5.cofactor binding ▲▲	lactate

Figure S1: **Significantly differentially expressed molecular functions, as determined by GO annotations.** For each condition, we show the top-5 differentially expressed molecular functions according to either mRNA or protein abundances. Empty boxes indicate that no differentially expressed pathways were found. The arrows next to pathway names indicate the proportion of up- and down-regulated genes among the significantly differentially expressed genes in this pathway. One up arrow indicates that 60% or more of the genes are up-regulated, two arrows correspond to 80% or more genes, and three arrows correspond to 95% or more genes being up-regulated. Similarly, down arrows indicate the proportion of down-regulated genes. (A) Exponential phase. (B) Stationary phase.

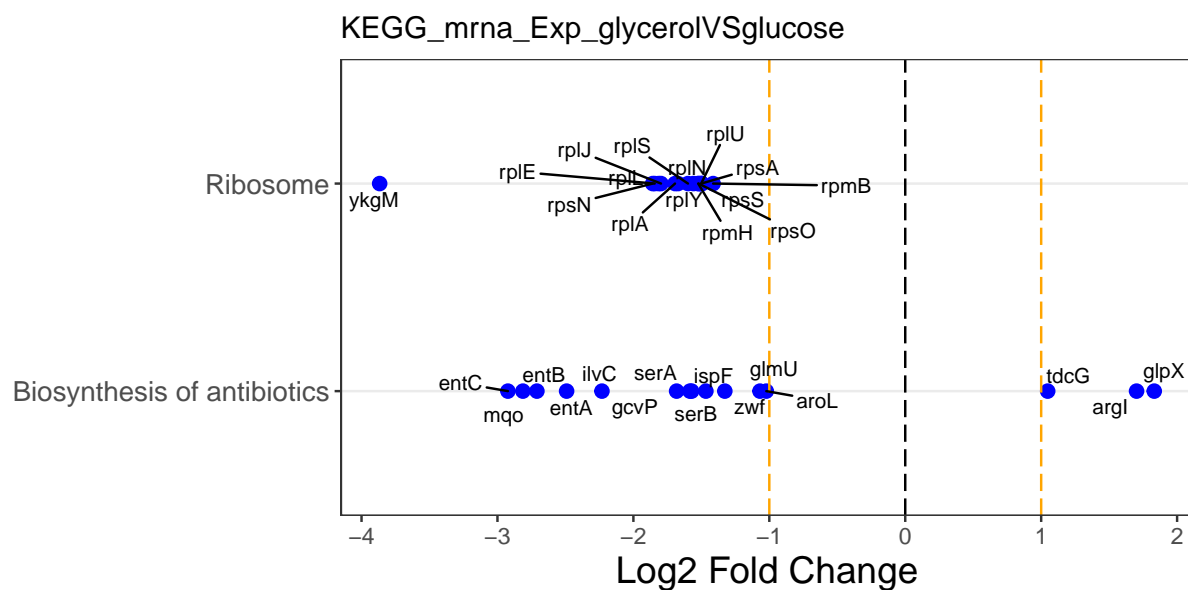


Figure S2: **Significantly differentially expressed KEGG pathways and associated genes with glycerol as carbon source, as determined by mRNA abundances in exponential phase.** The top 2 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway we show up to 15 of the most significantly changing genes.

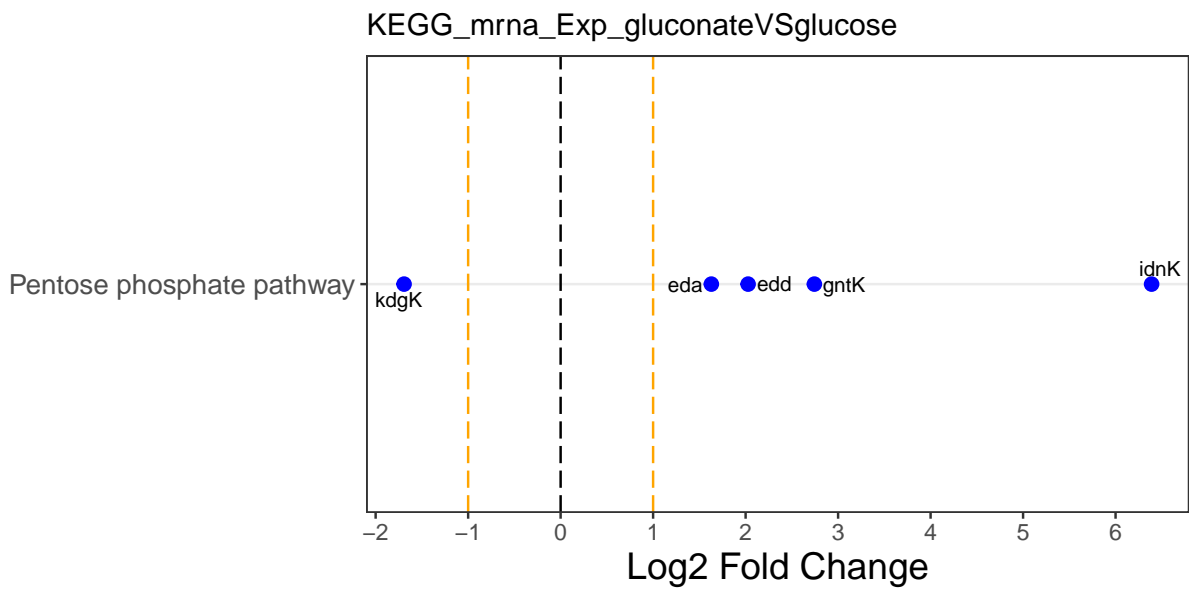


Figure S3: **Significantly differentially expressed KEGG pathway and associated genes with gluconate as carbon source, as determined by mRNA abundances in exponential phase.** The top differentially expressed KEGG pathway is shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway we show up to 15 of the most significantly changing genes.

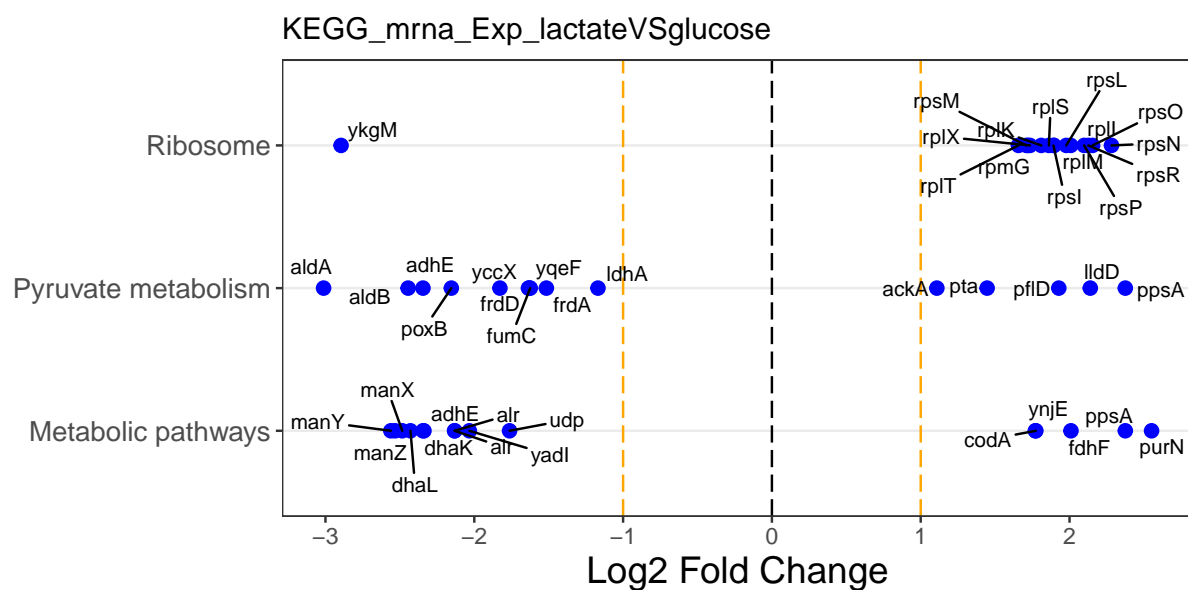


Figure S4: **Significantly differentially expressed KEGG pathways and associated genes with lactate as carbon source, as determined by mRNA abundances in exponential phase.** The top 3 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway we show up to 15 of the most significantly changing genes.

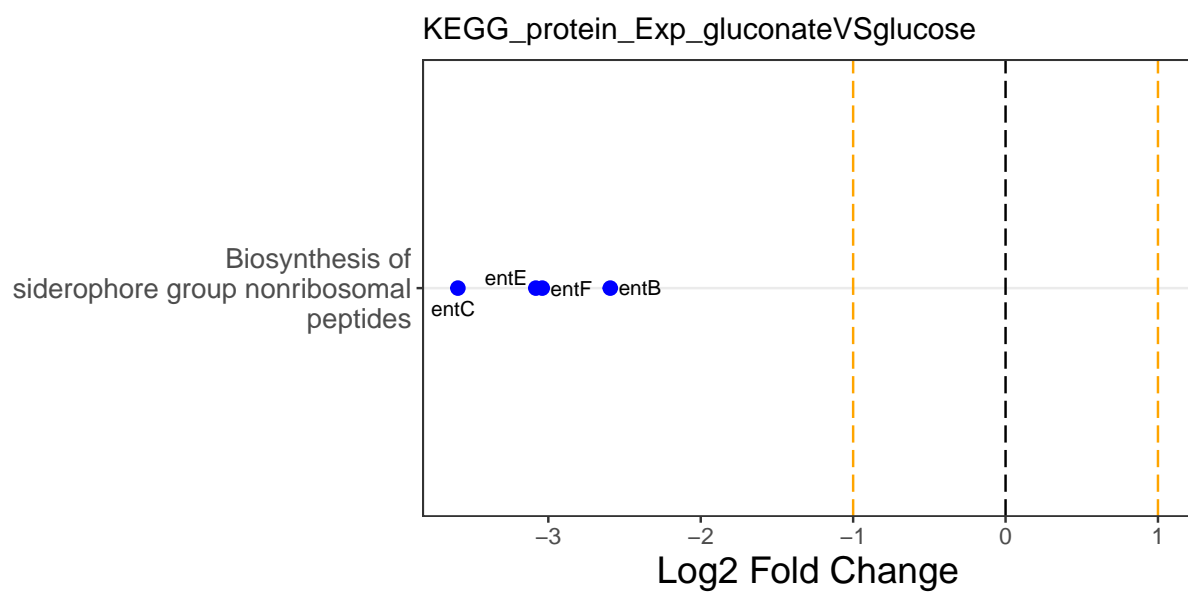


Figure S5: **Significantly differentially expressed KEGG pathway and associated genes with gluconate as carbon source, as determined by protein abundances in exponential phase.** The top differentially expressed KEGG pathway is shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway we show up to 15 of the most significantly changing genes.



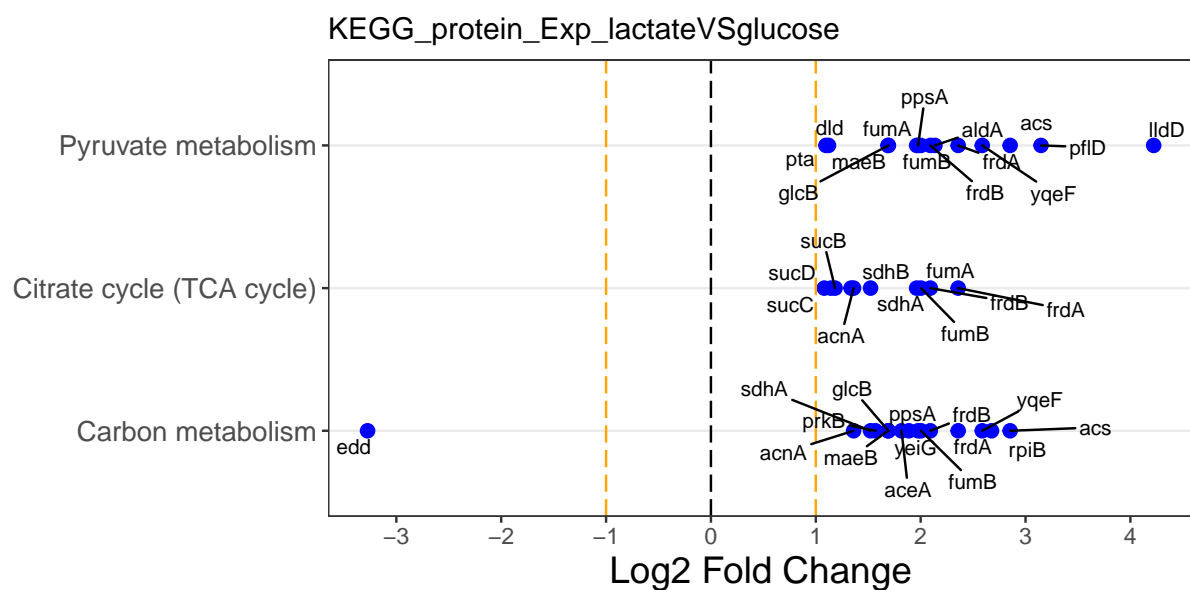


Figure S6: **Significantly differentially expressed KEGG pathways and associated genes with lactate as carbon source, as determined by protein abundances in exponential phase.** The top 3 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway we show up to 15 of the most significantly changing genes.

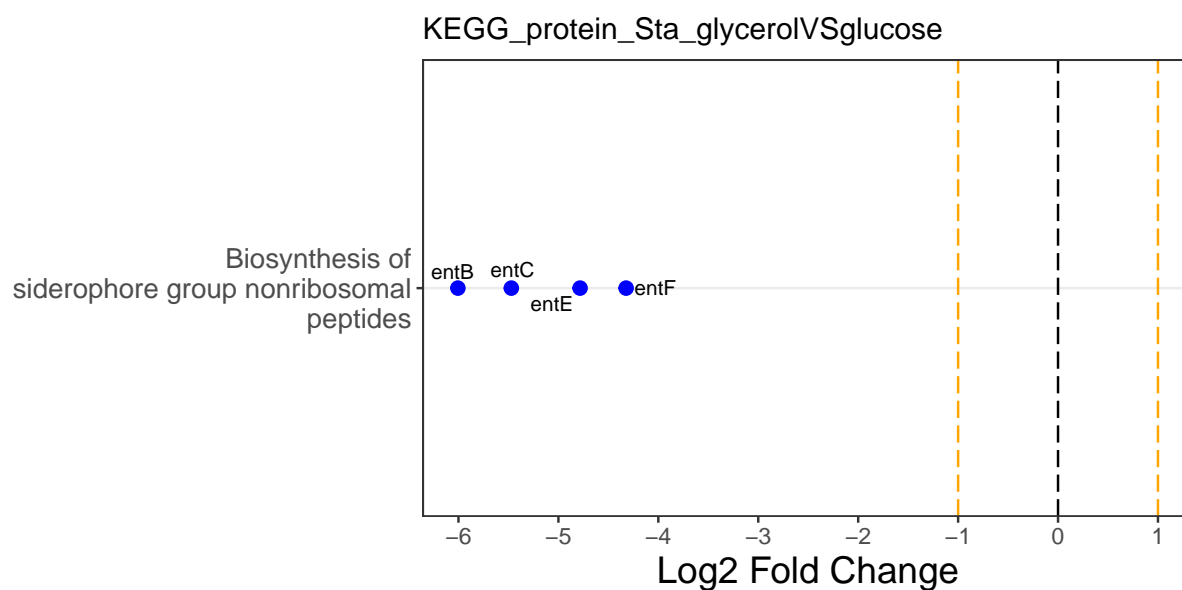


Figure S7: **Significantly differentially expressed KEGG pathway and associated genes with glycerol as carbon source, as determined by protein abundances in stationary phase.** The top differentially expressed KEGG pathway is shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

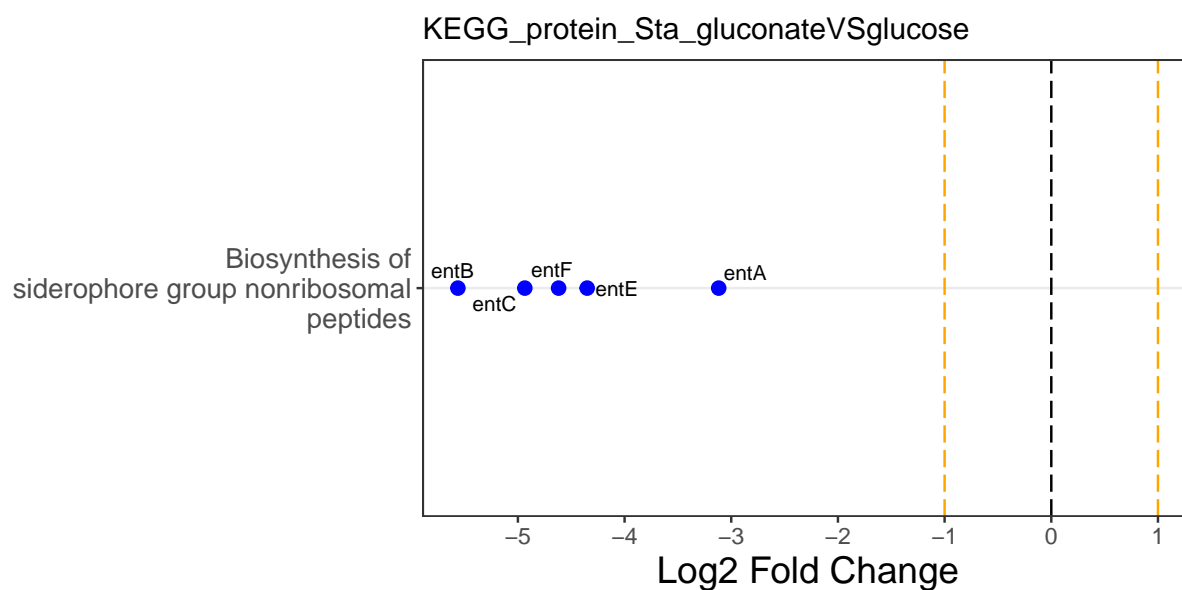


Figure S8: **Significantly differentially expressed KEGG pathway and associated genes with gluconate as carbon source, as determined by protein abundances in stationary phase.** The top differentially expressed KEGG pathway is shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

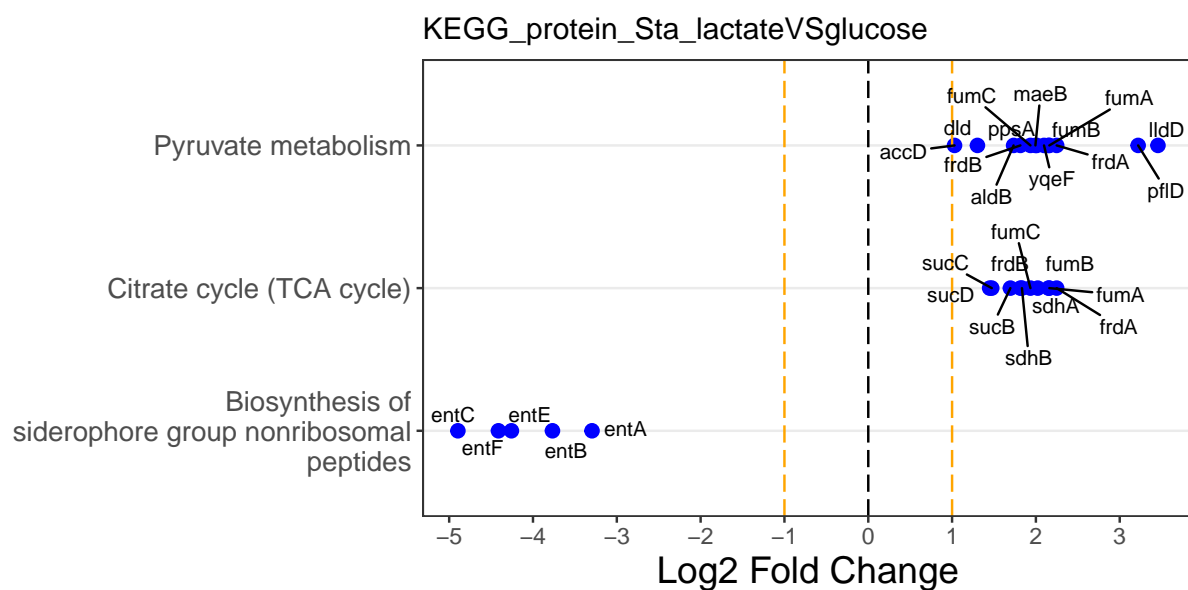


Figure S9: **Significantly differentially expressed KEGG pathways and associated genes with lactate as carbon source, as determined by protein abundances in stationary phase.** The top 3 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

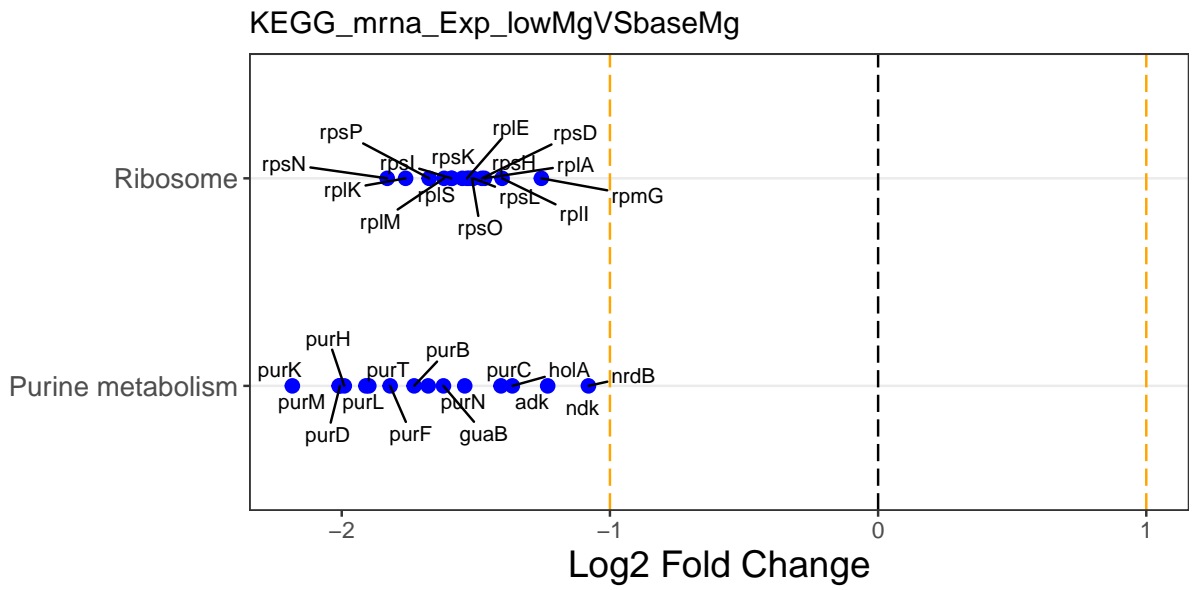


Figure S10: **Significantly differentially expressed KEGG pathways and associated genes with low  $Mg^{2+}$  levels, as determined by mRNA abundances in exponential phase.** The top 2 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

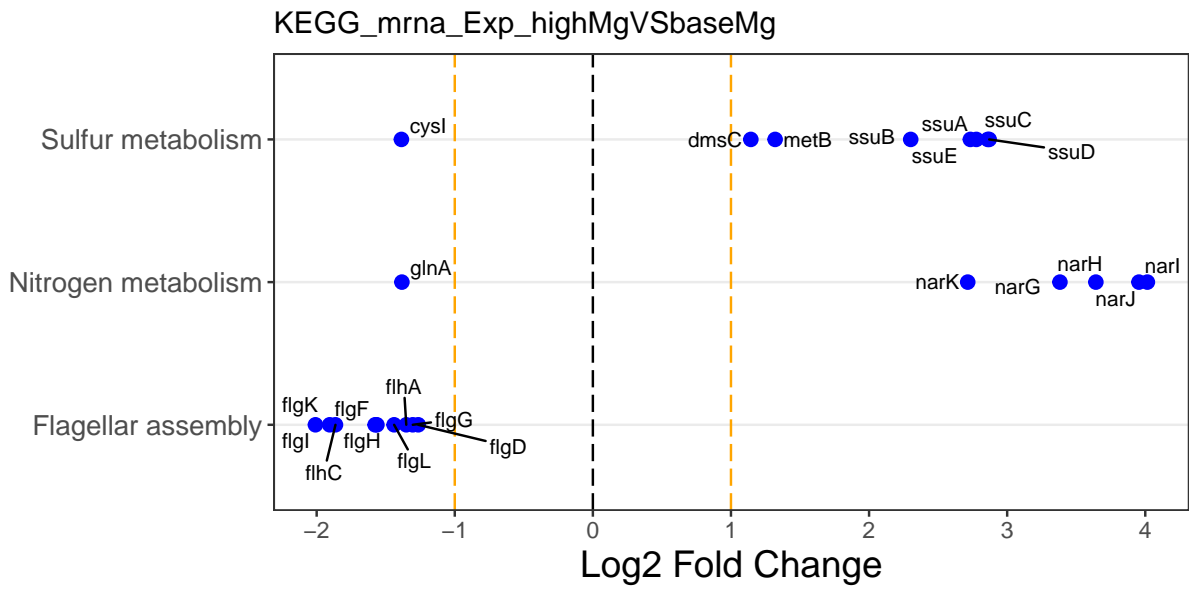


Figure S11: **Significantly differentially expressed KEGG pathways and associated genes with high  $\text{Mg}^{2+}$  levels, as determined by mRNA abundances in exponential phase.** The top 3 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

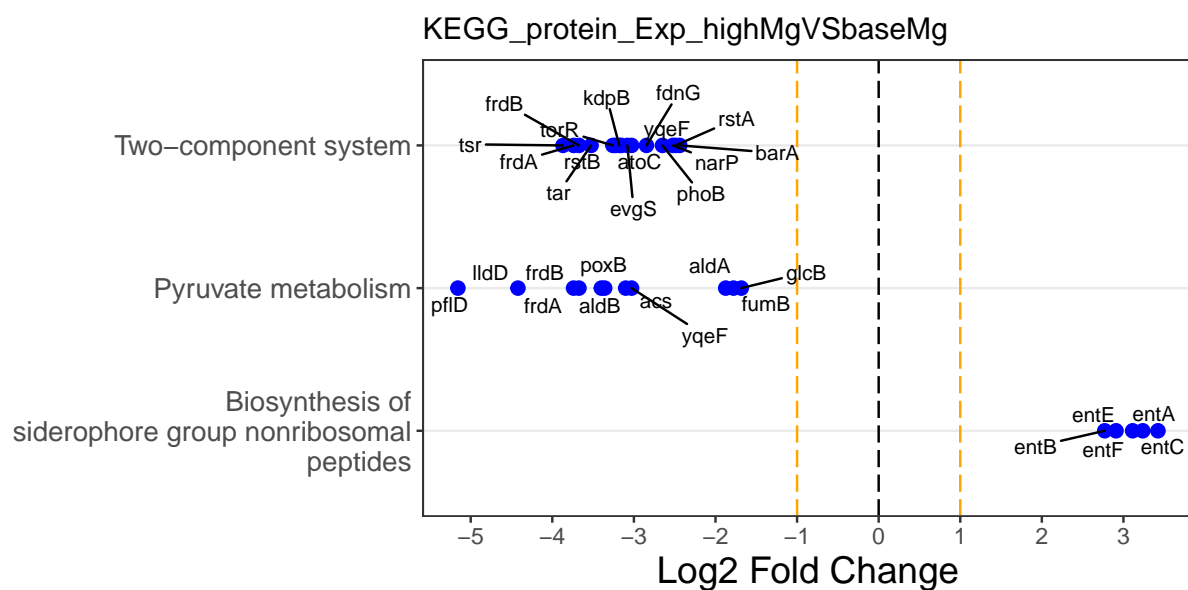


Figure S12: **Significantly differentially expressed KEGG pathways and associated genes with high  $\text{Mg}^{2+}$  levels, as determined by protein abundances in exponential phase.** The top 3 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

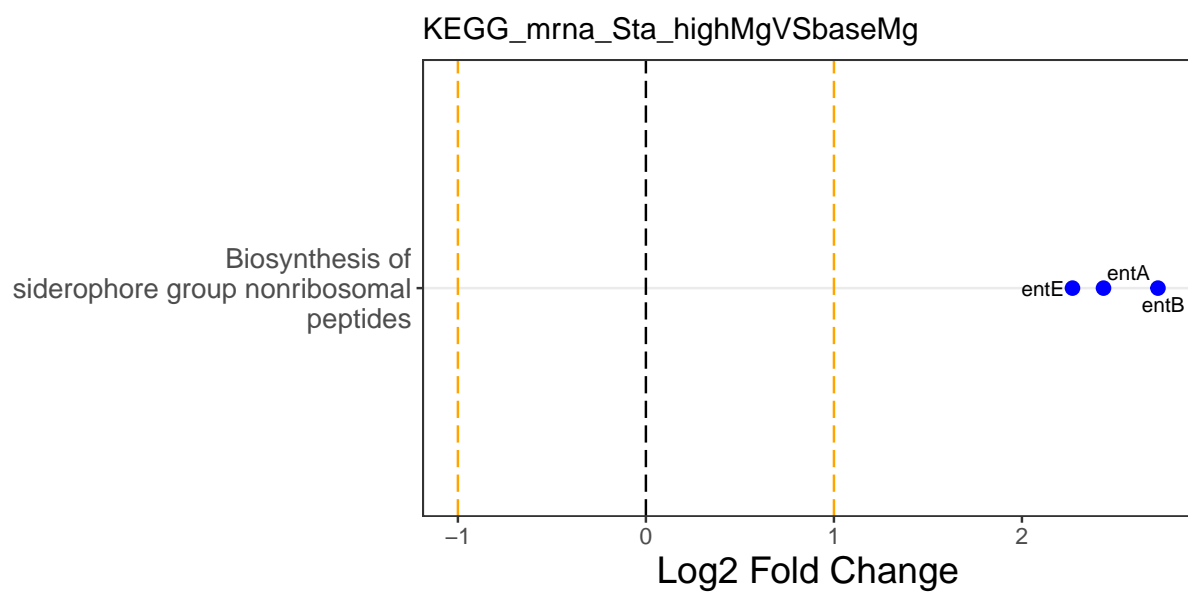


Figure S13: **Significantly differentially expressed KEGG pathway and associated genes with high  $\text{Mg}^{2+}$  levels, as determined by mRNA abundances in stationary phase.** The top differentially expressed KEGG pathway is shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.



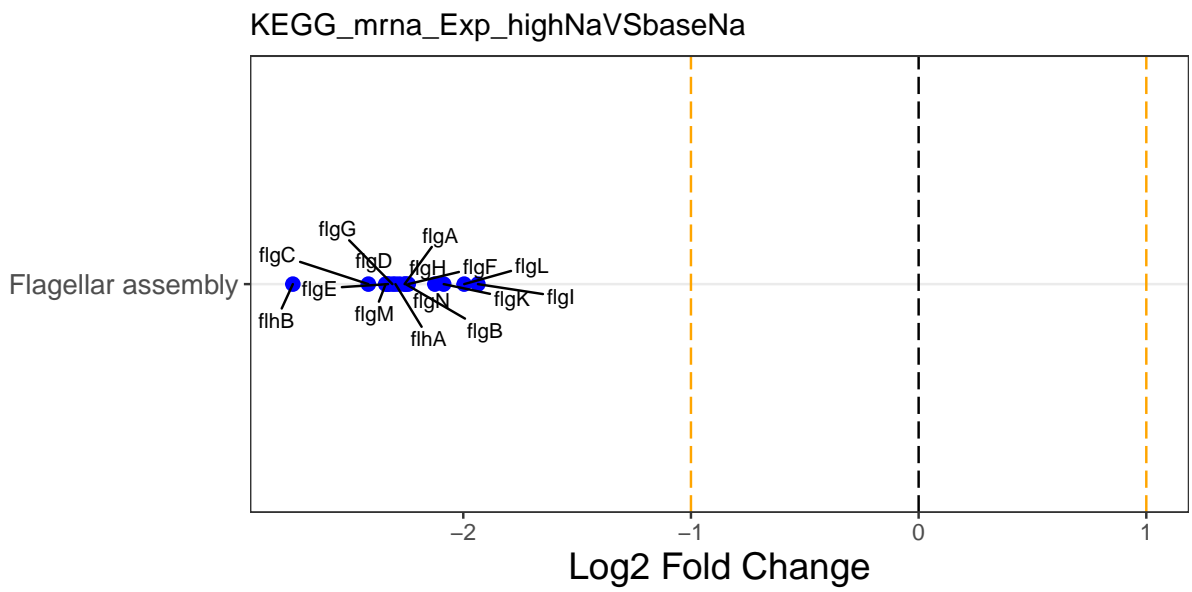


Figure S14: **Significantly differentially expressed KEGG pathway and associated genes with high  $\text{Na}^+$  levels, as determined by mRNA abundances in exponential phase.** The top differentially expressed KEGG pathway is shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

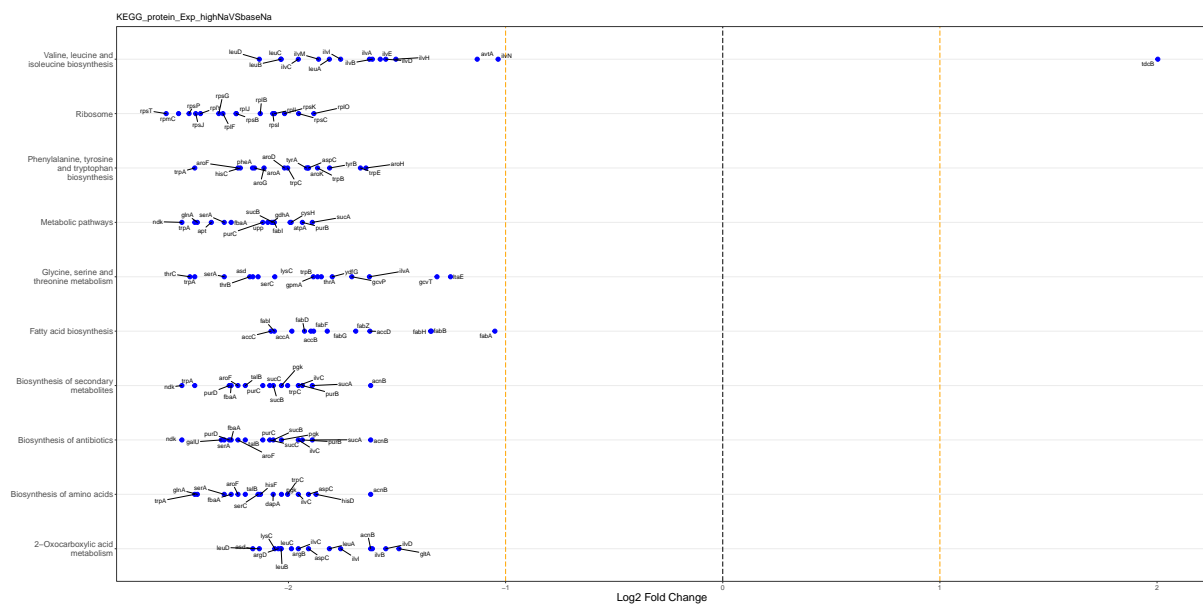


Figure S15: **Significantly differentially expressed KEGG pathways and associated genes with high  $\text{Na}^+$  levels, as determined by protein abundances in exponential phase.** The top 10 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.

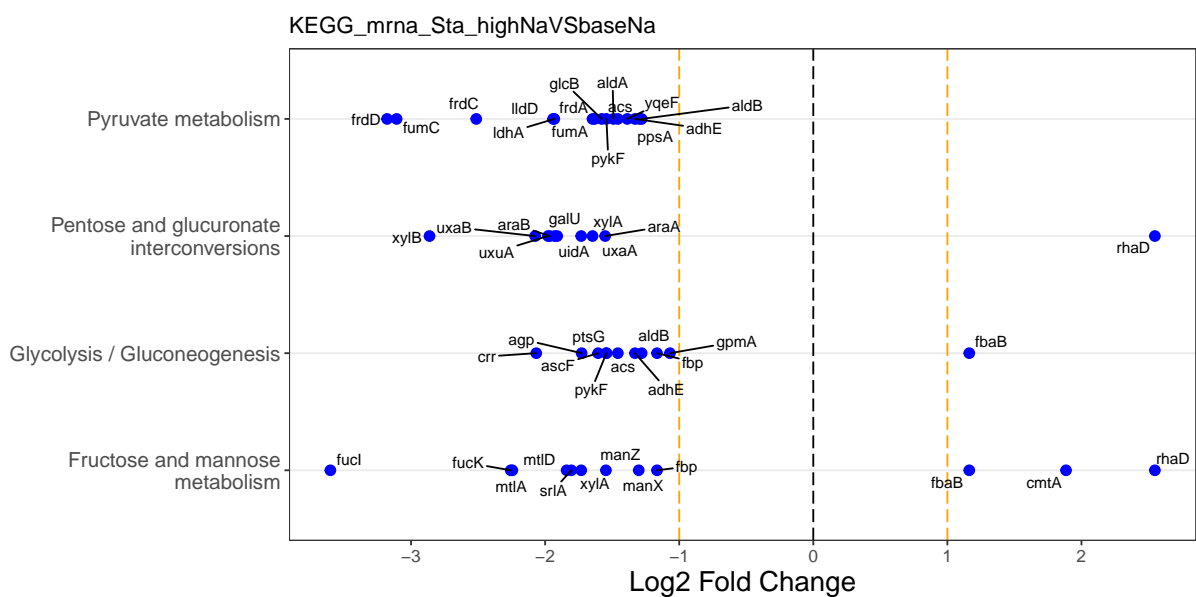
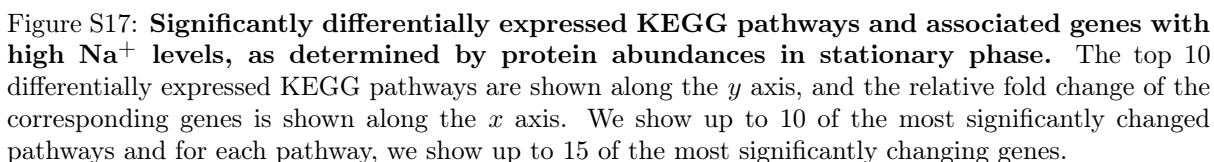


Figure S16: **Significantly differentially expressed KEGG pathways and associated genes with high  $\text{Na}^+$  levels, as determined by mRNA abundances in stationary phase.** The top 4 differentially expressed KEGG pathways are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed pathways and for each pathway, we show up to 15 of the most significantly changing genes.



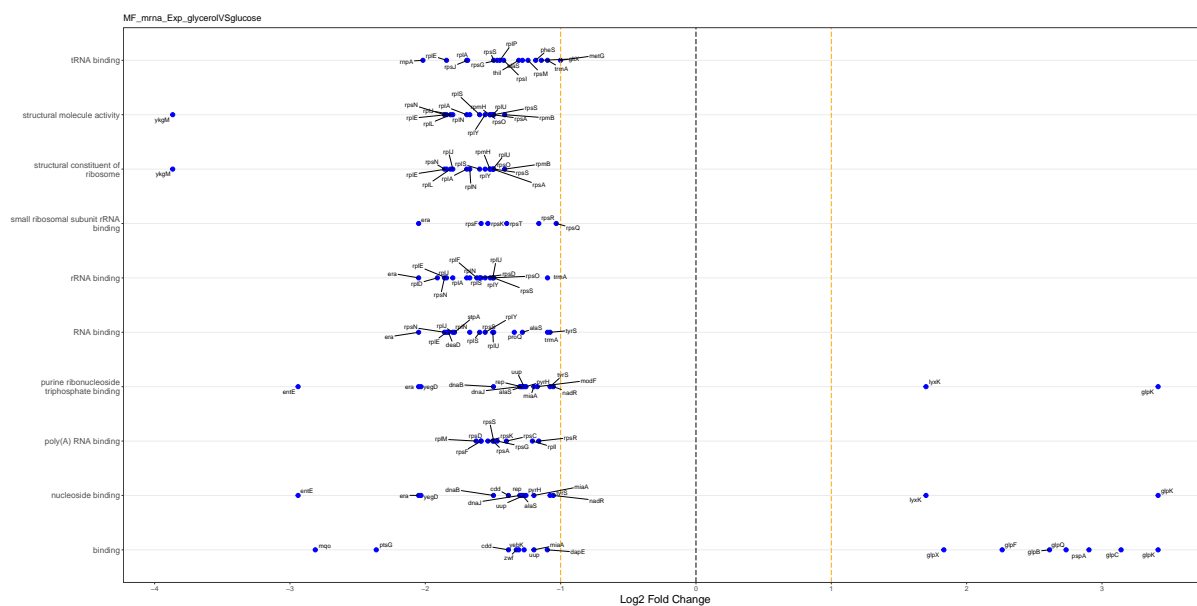


Figure S18: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with glycerol as carbon source, as determined by mRNA abundances in exponential phase.** The top 10 differentially expressed molecular functions are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

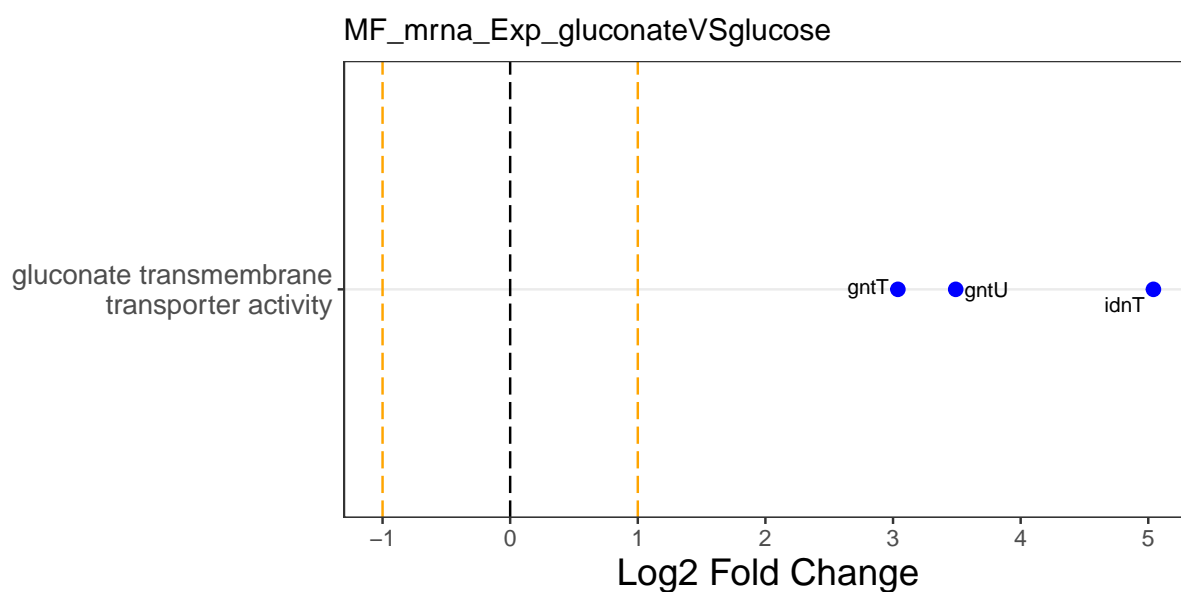


Figure S19: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with gluconate as carbon source, as determined by mRNA abundances in exponential phase.** The top 2 differentially expressed molecular functions are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.



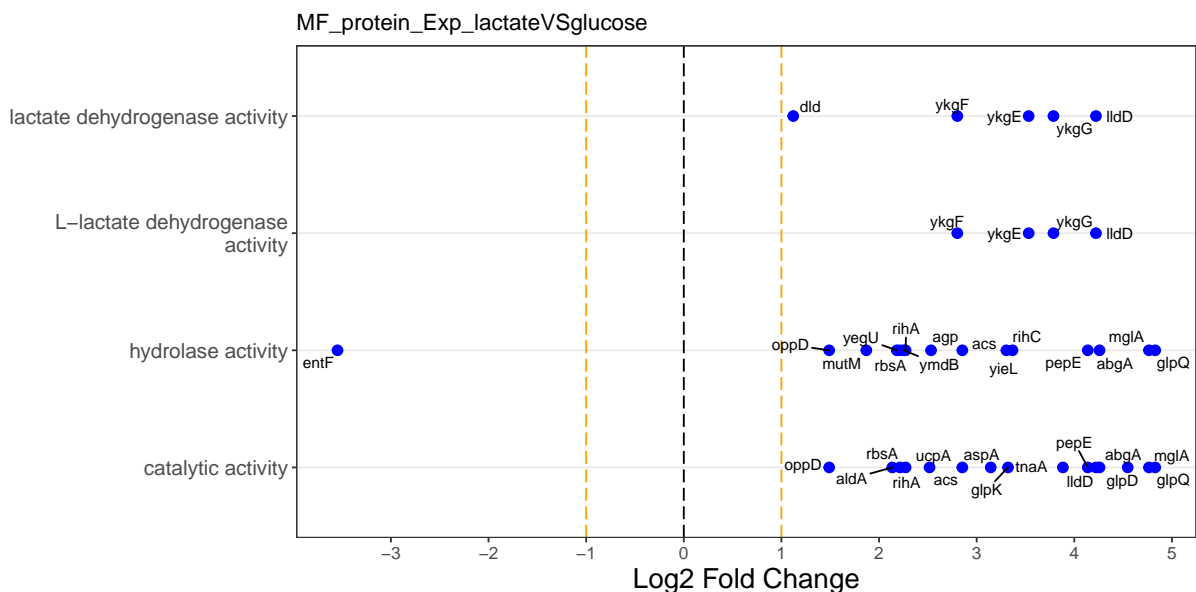


Figure S22: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with lactate as carbon source, as determined by protein abundances in exponential phase.** The top 4 differentially expressed molecular functions are shown along the *y* axis, and the relative fold change of the corresponding genes is shown along the *x* axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

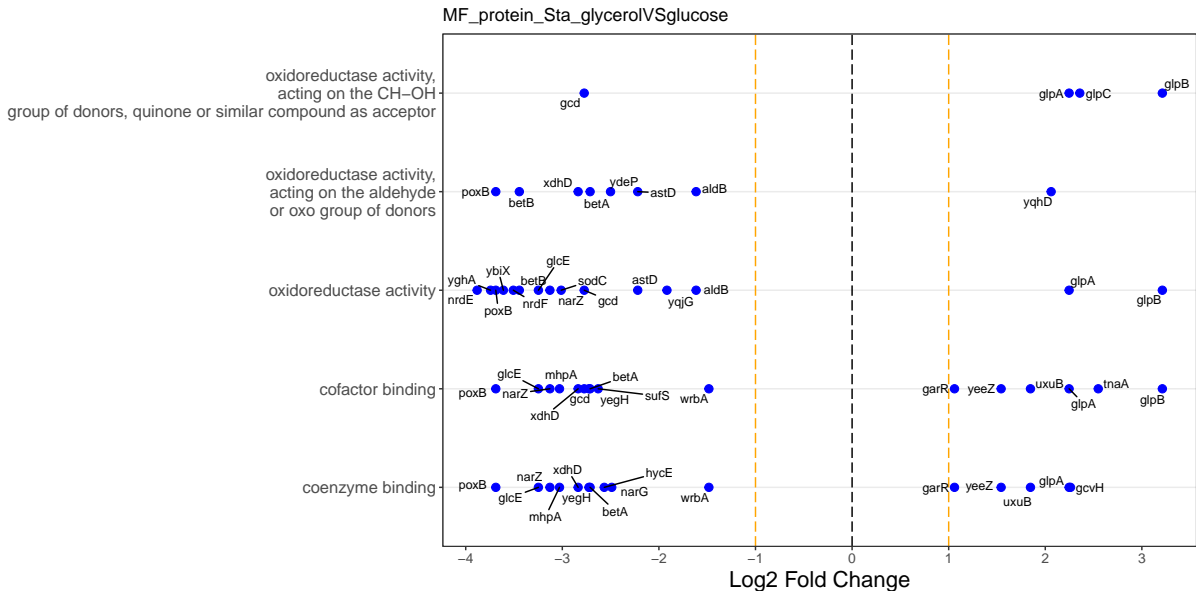


Figure S23: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with glycerol as carbon source, as determined by protein abundances in stationary phase.** The top 5 differentially expressed molecular functions are shown along the *y* axis, and the relative fold change of the corresponding genes is shown along the *x* axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

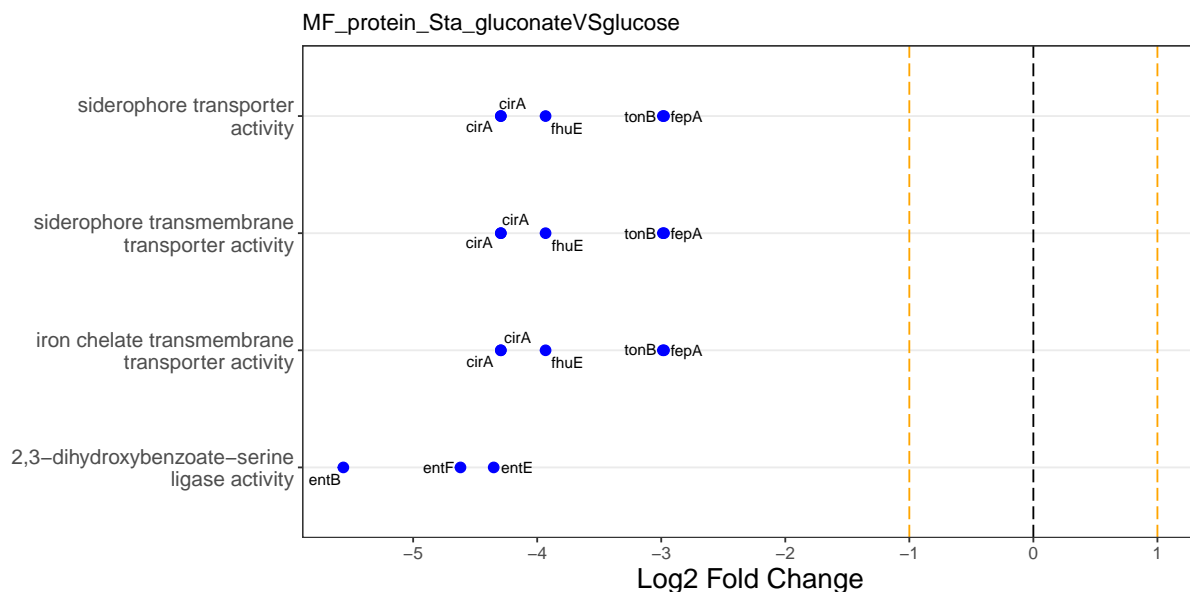


Figure S24: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with gluconate as carbon source, as determined by protein abundances in stationary phase.** The top 4 differentially expressed molecular functions are shown along the *y* axis, and the relative fold change of the corresponding genes is shown along the *x* axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

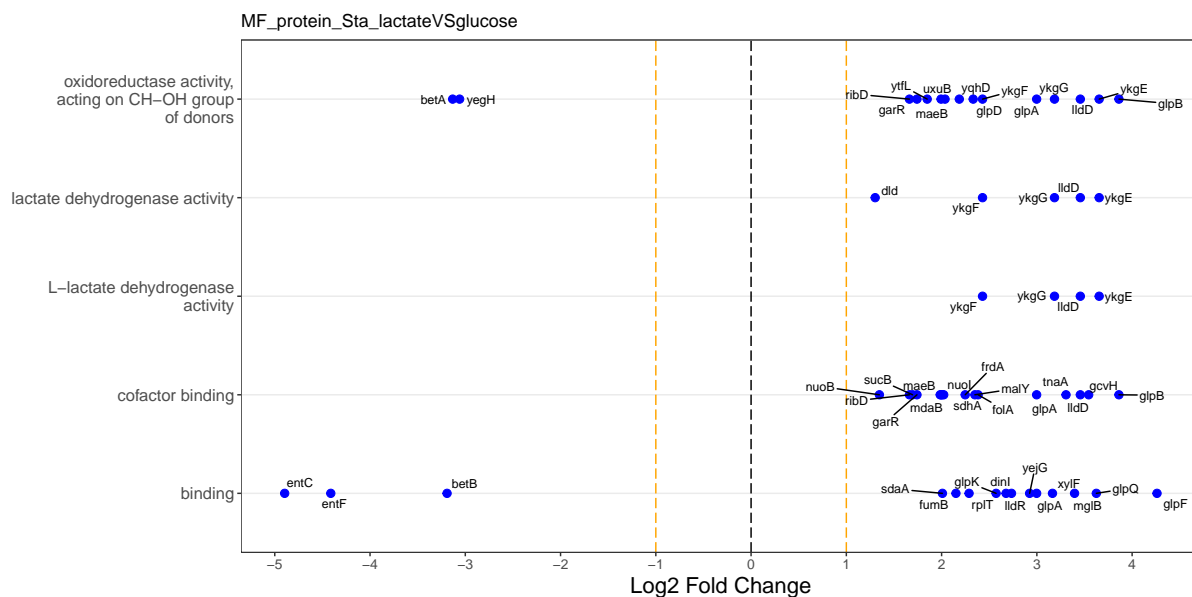
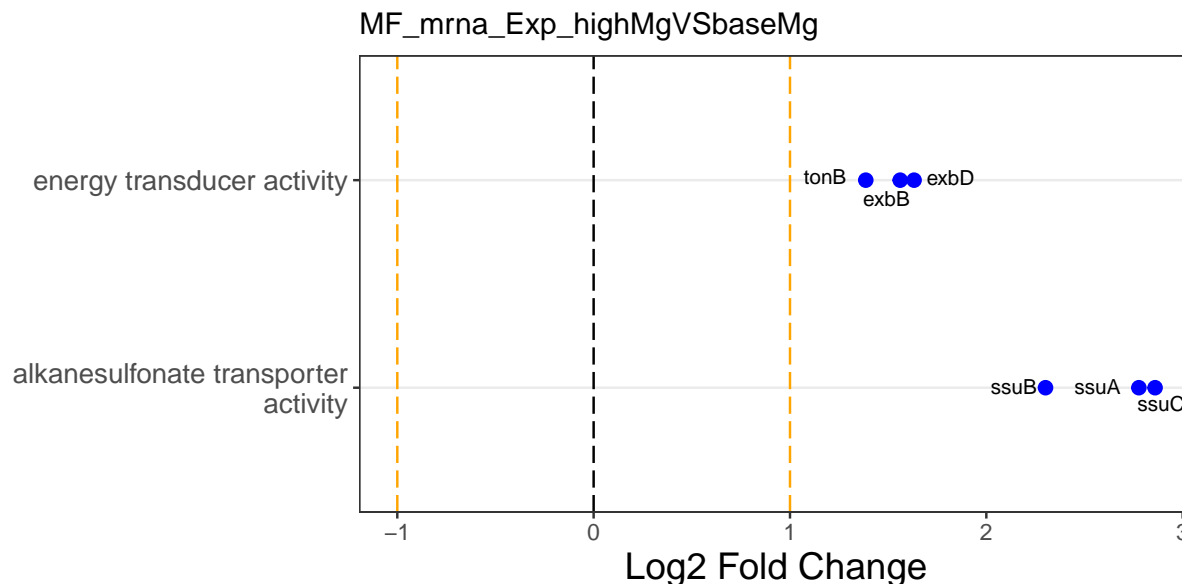
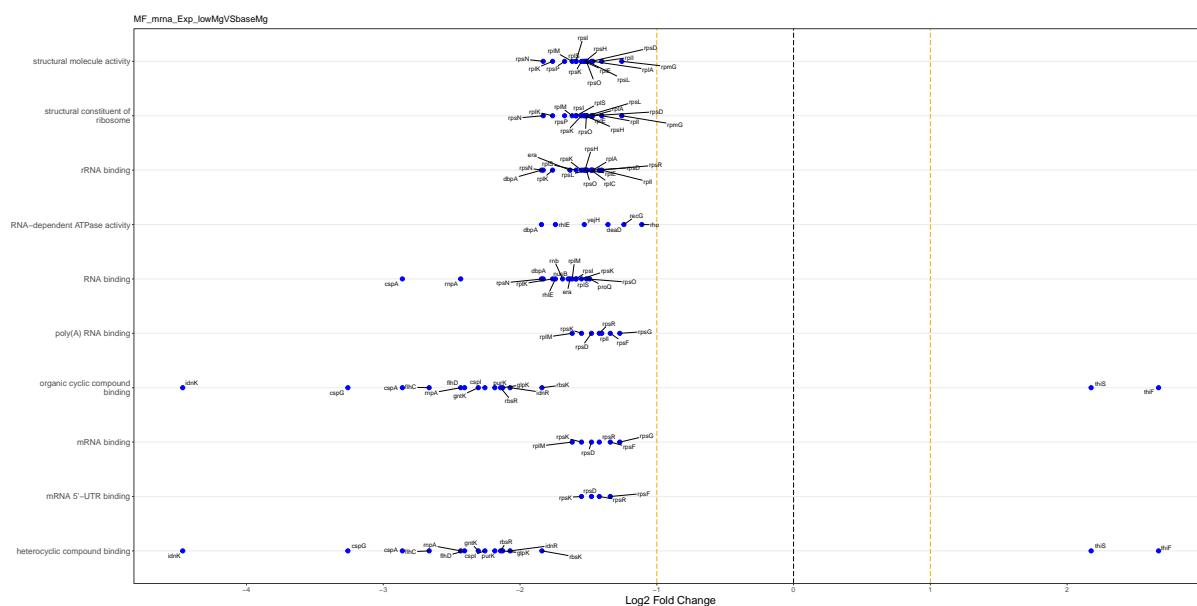


Figure S25: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with lactate as carbon source, as determined by protein abundances in stationary phase.** The top 5 differentially expressed molecular functions are shown along the *y* axis, and the relative fold change of the corresponding genes is shown along the *x* axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.







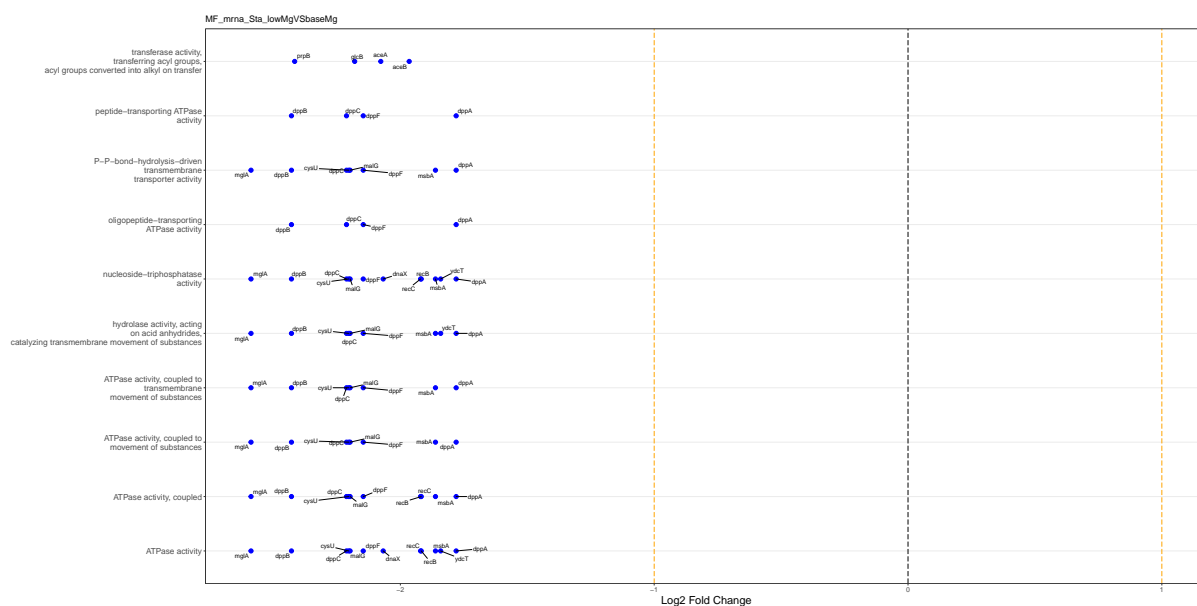


Figure S30: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with low  $Mg^{2+}$  levels, as determined by mRNA abundances in stationary phase.** The top 10 differentially expressed molecular functions are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

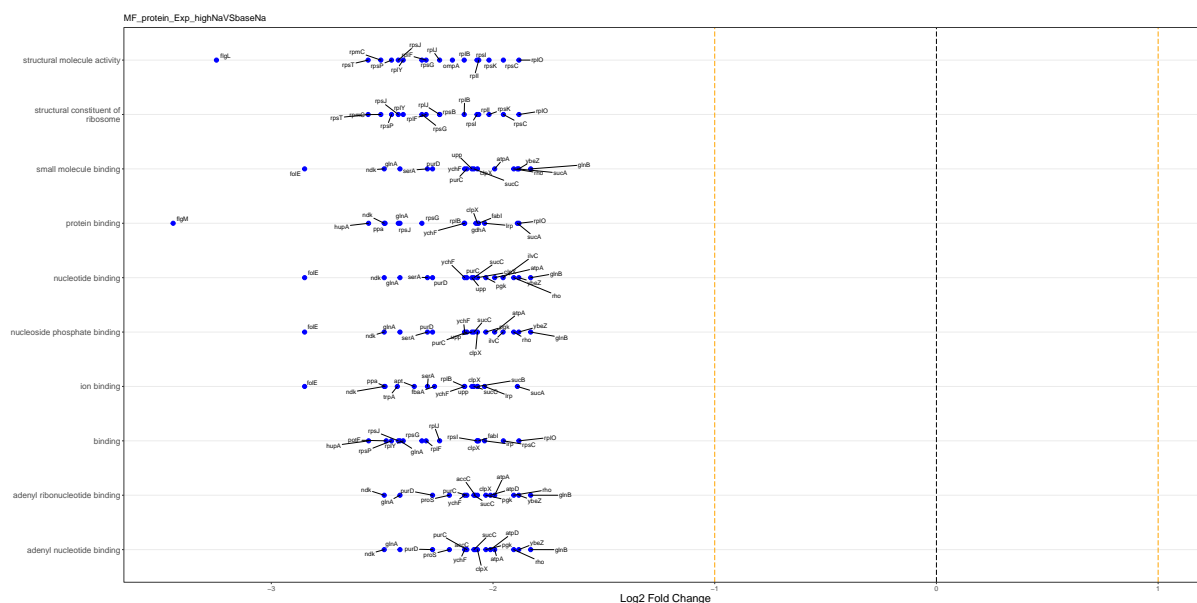


Figure S31: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with high  $Na^{+}$  levels, as determined by protein abundances in exponential phase.** The top differentially expressed molecular functions are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

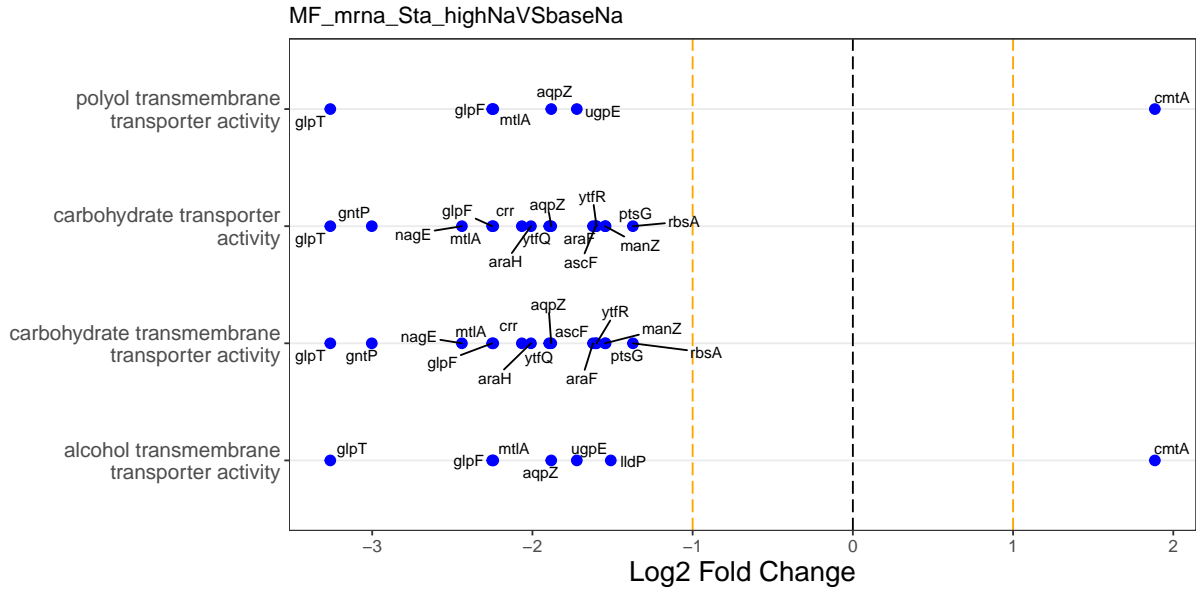


Figure S32: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with high  $\text{Na}^+$  levels, as determined by mRNA abundances in stationary phase.** The top 5 differentially expressed molecular functions are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

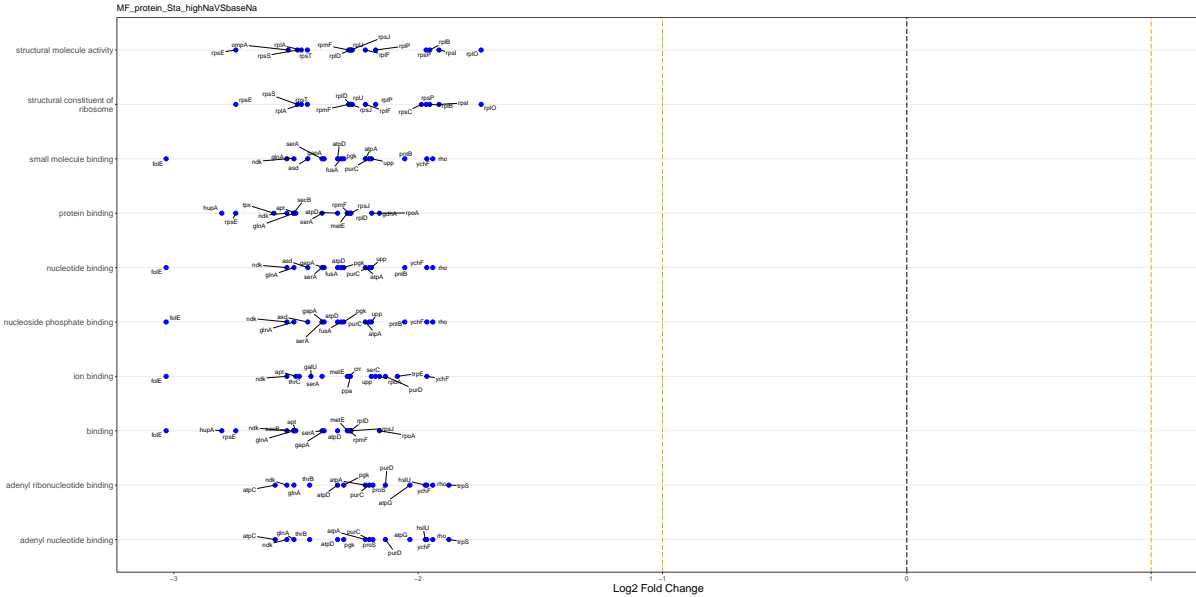


Figure S33: **Significantly differentially expressed GO annotations related with molecular functions and associated genes with high  $\text{Na}^+$  levels, as determined by protein abundances in stationary phase.** The top 10 differentially expressed molecular functions are shown along the  $y$  axis, and the relative fold change of the corresponding genes is shown along the  $x$  axis. We show up to 10 of the most significantly changed molecular functions and for each molecular function, we show up to 15 of the most significantly changing genes.

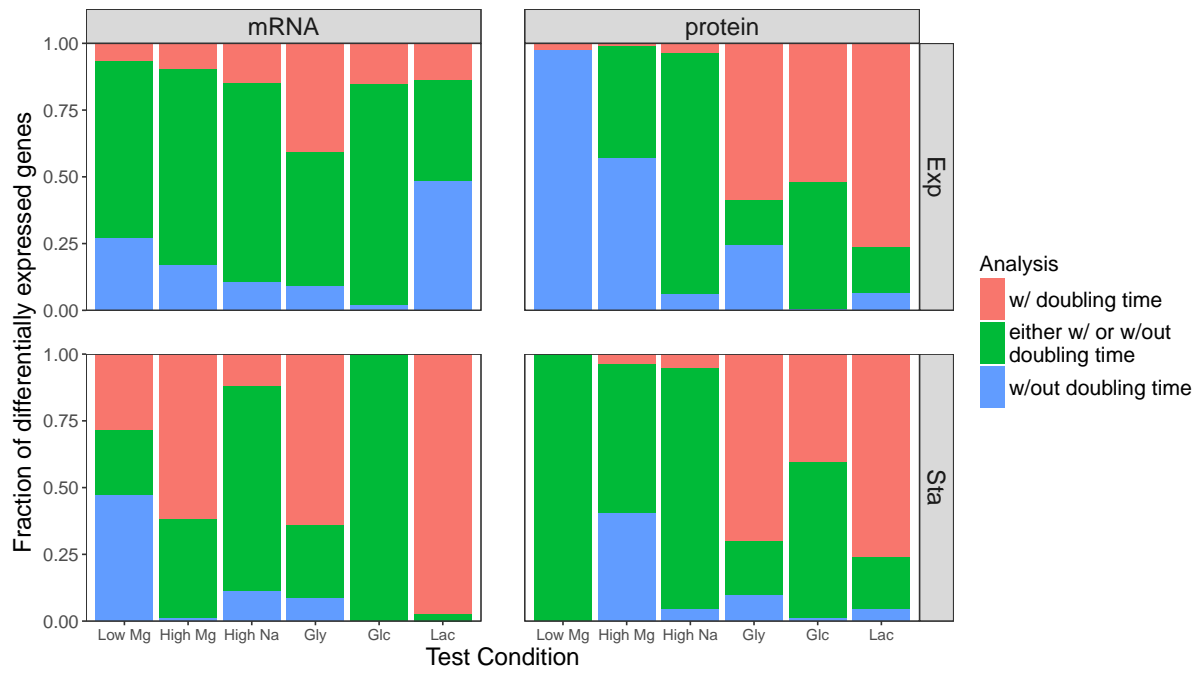


Figure S34: **Change in differentially expressed genes between two controls: "batch" vs "batch + growth rate"**. Red represent the fraction of genes that significantly changed only under control of "batch + doubling time". Green represent the fraction of genes appear in both controlling for "batch + doubling time" and for "batch". Blue represents the fraction of genes appear only under control of "batch". As can be seen from figure 5, the full block of red for lactate, mRNA in stationary phase is because of few to none significantly changing genes under control of "batch". Big blocks of red for high  $Mg^{2+}$  and glycerol for again mRNA data in stationary phase appeared because of the same reason. On the other hand red blocks for protein data associated with carbon sources for both exponential and stationary phases are meaningful and indicates genes that do not change with growth rate, while the cell is under treated with different carbon sources.

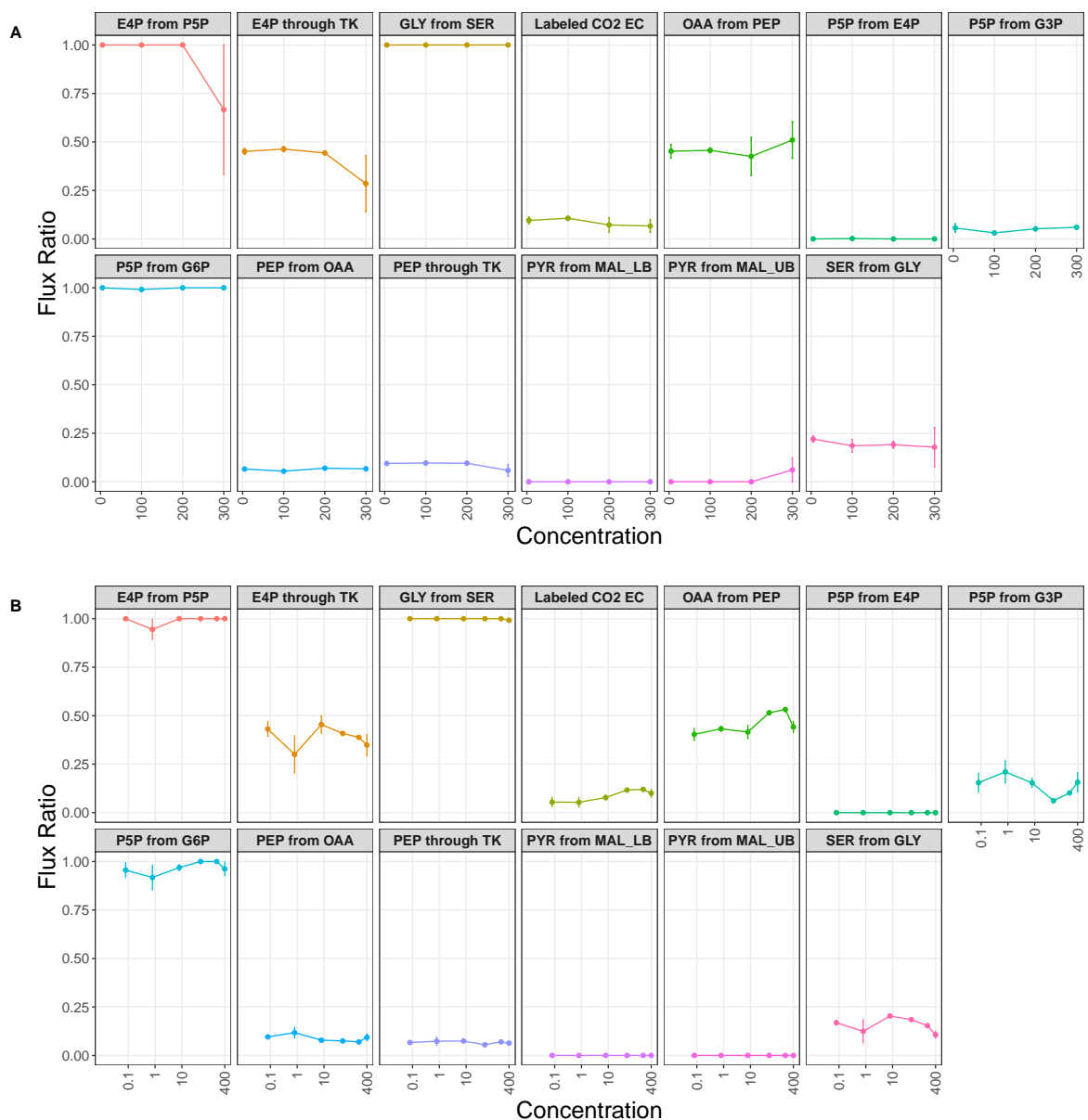


Figure S35: **Flux ratios versus ion concentrations.** 13 different flux ratios were measured with respect to four different Na<sup>+</sup> and five different Mg<sup>2+</sup> concentrations. (A) Concentrations with respect to changing Na<sup>+</sup> concentrations. (B) Concentrations with respect to changing Mg<sup>2+</sup> concentrations. There was no significant trend of increase or decrease in flux ratios with respect to either Na<sup>+</sup> or Mg<sup>2+</sup> concentrations (Supplementary Table 12).