

# Supplementary Material

# Rewiring of Gene Expression in *Pseudomonas aeruginosa* During Diauxic Growth Reveals an Indirect Regulation of the MexGHI-OpmD Efflux Pump by Hfq

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## **1** Supplementary Figures and Tables

### **1.1 Supplementary Tables**

**Supplementary Table 1** Compilation of the T3/T1, T2/T1 and T3/T2 RNA-seq, Ribo-seq and translatome datasets. The numbers of transcripts, ribosome footprints and peptides with a fold-change  $\geq 2$  or  $\leq 2$  and a multiple testing adjusted *p*-value  $\leq 0.05$  are listed in bold, whereas values below these thresholds are shown in pale writing.

### **1.2 Supplementary Figures**



**Supplementary Figure S1** Venn diagrams showing the number of transcripts with increased (left panels) or decreased (right panels) abundance in RNA-seq, Ribo-seq and the MS data obtained after analysis of the following datasets: (A) T3 vs T1 (mannitol uptake vs succinate uptake); (B) T2 vs T1; (C) T3 vs T2; For significance only a FC  $\geq 2$  or  $\leq 2$  and a multiple testing adjusted *p*-value  $\leq 0.05$  are considered for the RNA-seq, Ribo-seq and MS data. The corresponding transcripts, ribosomal footprints and MS data with increased or decreased abundance are listed in **Supplementary Table 1**.





**Supplementary Figure S2** Meta-analysis of the dataset T3 vs T1 of normalized synthesis / expression of differentially abundant proteins resulting from the MS data and transcripts revealed by Ribo-seq and RNA-seq, respectively. The genes are grouped into the corresponding pathways (<u>http://www.kegg.jp/kegg-bin/show\_organism?org=pae</u>). For each group the averaged log<sub>2</sub> fold changes of significantly modulated members are shown. The colour code shown in the scale at the left denotes log<sub>2</sub>-fold changes. Red indicates an overall decrease and green indicates an overall increase in the protein and mRNA levels in a particular pathway.



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		T2 vs T1									
PA	gene	RNA-seq		Ribo-seq		MS					
number	name	FC	p-value	FC	p-value	FC	p-value				
Pentose phosphate pathway											
PA3183	zwf	25.4	0.00E+00	1.91	2.94E-17	4.99	3.38E-04				
Entner–Doudoroff pathway											
PA3181	edaA	82.5	0.00E+00	5.84	6.37E-54						
PA3194	edd	14.0	0.00E+00	2.28	4.86E-31	-1.22	4.56E-01				
PA3182	pgl	85.6	0.00E+00	3.21	3.07E-31	7.09	3.70E-04				
Mannitol metabolism											
PA2344	mtlD	17.2	0.00E+00	1.02	7.69E-01	1.42	5.73E-01				
PA2344	mtlZ	8.14	0.00E+00	1.03	7.06E-01	1.61	5.95E-01				

**Supplementary Figure S3** (A) *Pae* utilizes the pentose phosphate (PP) and the Entner-Doudoroff (ED) pathways for metabolization of different carbohydrates, such as mannitol (Dolan et al., 2020; Park et al., 2020). Key enzymes for these pathways are highlighted in red. (B) RNA-seq, Ribo-seq and MS data for the functions highlighted in red in (A).