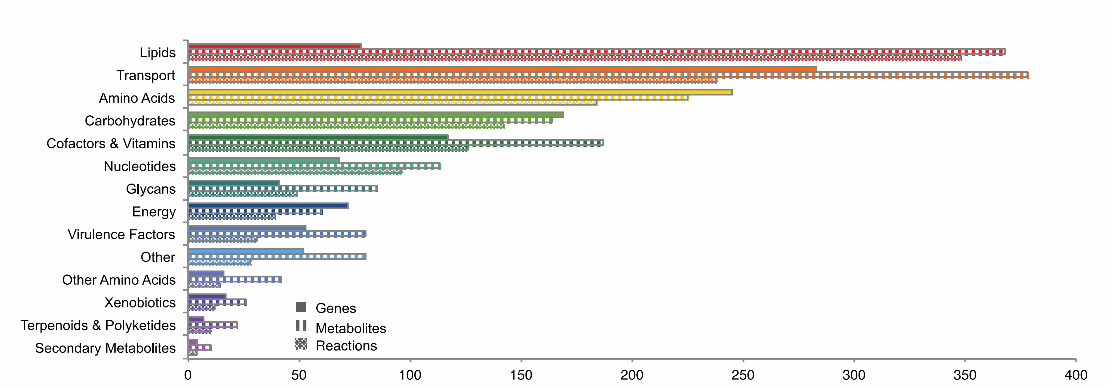
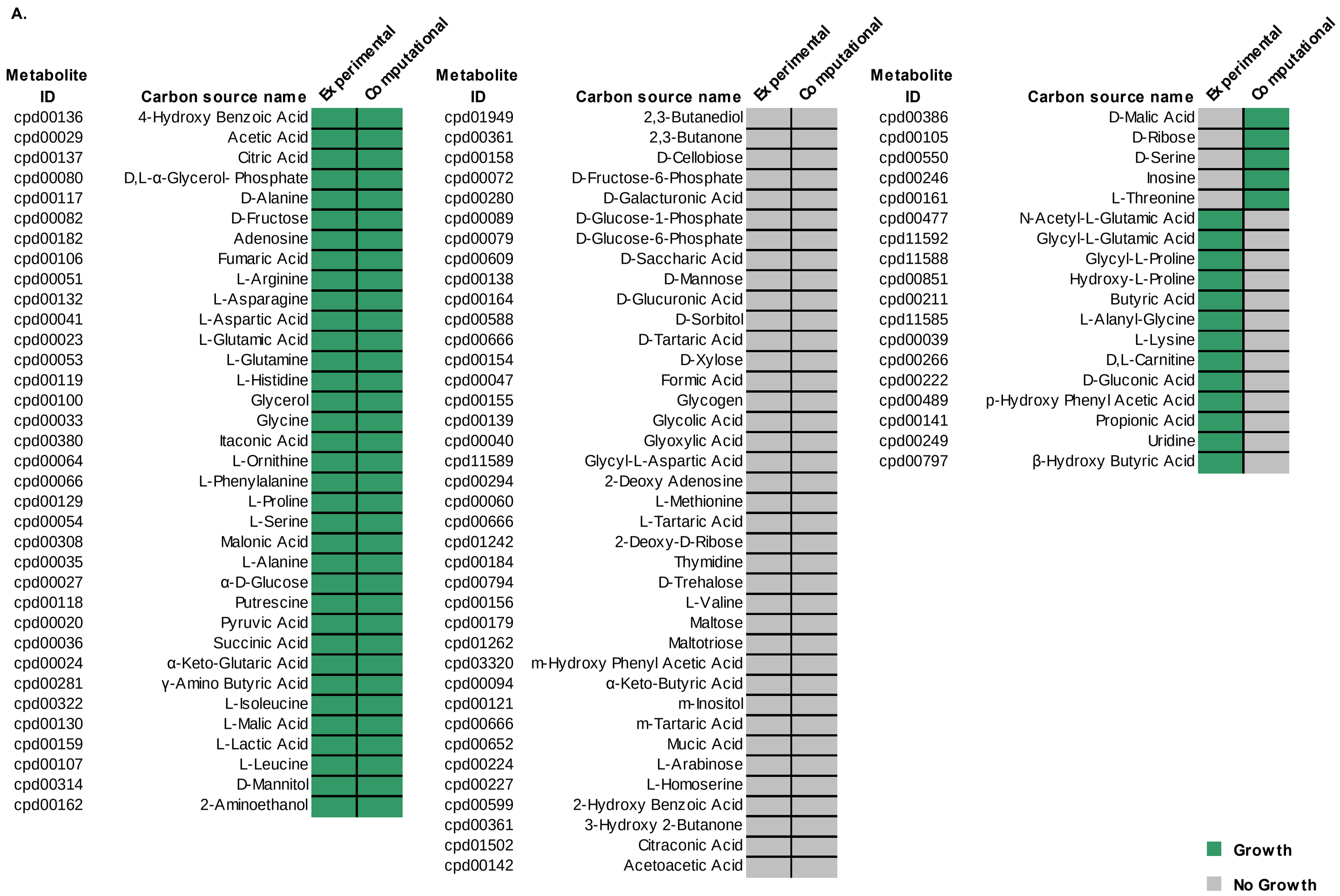
Supplementary Information



Supplementary Figure 1. Network component distribution of mPAO1. The distribution of the genes, metabolites and reactions in mPAO1 across KEGG functional categories.



A.

Metabolite ID	Carbon source name	Experimental	Computational
cpd00136	4-Hydroxy Benzoic Acid	🟩	🟩
cpd00029	Acetic Acid	🟩	🟩
cpd00137	Citric Acid	🟩	🟩
cpd00080	D,L-α-Glycerol- Phosphate	🟩	🟩
cpd00117	D-Alanine	🟩	🟩
cpd00082	D-Fructose	🟩	🟩
cpd00182	Adenosine	🟩	🟩
cpd00106	Fumaric Acid	🟩	🟩
cpd00051	L-Arginine	🟩	🟩
cpd00132	L-Asparagine	🟩	🟩
cpd00041	L-Aspartic Acid	🟩	🟩
cpd00023	L-Glutamic Acid	🟩	🟩
cpd00053	L-Glutamine	🟩	🟩
cpd00119	L-Histidine	🟩	🟩
cpd00100	Glycerol	🟩	🟩
cpd00033	Glycine	🟩	🟩
cpd00380	Itaconic Acid	🟩	🟩
cpd00064	L-Ornithine	🟩	🟩
cpd00066	L-Phenylalanine	🟩	🟩
cpd00129	L-Proline	🟩	🟩
cpd00054	L-Serine	🟩	🟩
cpd00308	Malonic Acid	🟩	🟩
cpd00035	L-Alanine	🟩	🟩
cpd00027	α-D-Glucose	🟩	🟩
cpd00118	Putrescine	🟩	🟩
cpd00020	Pyruvic Acid	🟩	🟩
cpd00036	Succinic Acid	🟩	🟩
cpd00024	α-Keto-Glutaric Acid	🟩	🟩
cpd00281	γ-Amino Butyric Acid	🟩	🟩
cpd00322	L-Isoleucine	🟩	🟩
cpd00130	L-Malic Acid	🟩	🟩
cpd00159	L-Lactic Acid	🟩	🟩
cpd00107	L-Leucine	🟩	🟩
cpd00314	D-Mannitol	🟩	🟩
cpd00162	2-Aminoethanol	🟩	🟩

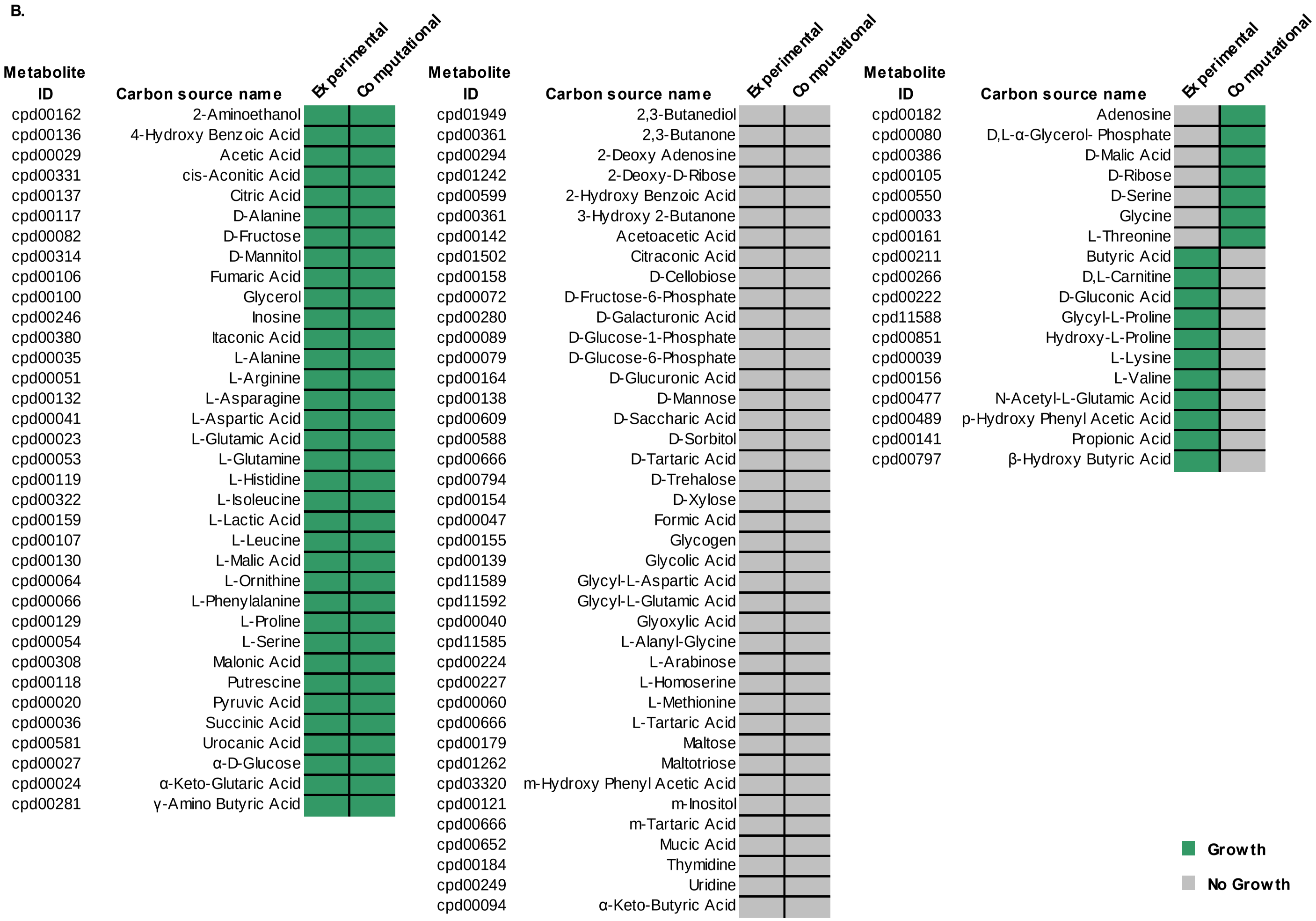
Metabolite ID	Carbon source name	Experimental	Computational
cpd01949	2,3-Butanediol	⬜	⬜
cpd00361	2,3-Butanone	⬜	⬜
cpd00158	D-Cellobiose	⬜	⬜
cpd00072	D-Fructose-6-Phosphate	⬜	⬜
cpd00280	D-Galacturonic Acid	⬜	⬜
cpd00089	D-Glucose-1-Phosphate	⬜	⬜
cpd00079	D-Glucose-6-Phosphate	⬜	⬜
cpd00609	D-Saccharic Acid	⬜	⬜
cpd00138	D-Mannose	⬜	⬜
cpd00164	D-Glucuronic Acid	⬜	⬜
cpd00588	D-Sorbitol	⬜	⬜
cpd00666	D-Tartaric Acid	⬜	⬜
cpd00154	D-Xylose	⬜	⬜
cpd00047	Formic Acid	⬜	⬜
cpd00155	Glycogen	⬜	⬜
cpd00139	Glycolic Acid	⬜	⬜
cpd00040	Glyoxylic Acid	⬜	⬜
cpd11589	Glycyl-L-Aspartic Acid	⬜	⬜
cpd00294	2-Deoxy Adenosine	⬜	⬜
cpd00060	L-Methionine	⬜	⬜
cpd00666	L-Tartaric Acid	⬜	⬜
cpd01242	2-Deoxy-D-Ribose	⬜	⬜
cpd00184	Thymidine	⬜	⬜
cpd00794	D-Trehalose	⬜	⬜
cpd00156	L-Valine	⬜	⬜
cpd00179	Maltose	⬜	⬜
cpd01262	Maltotriose	⬜	⬜
cpd03320	m-Hydroxy Phenyl Acetic Acid	⬜	⬜
cpd00094	α-Keto-Butyric Acid	⬜	⬜
cpd00121	m-Inositol	⬜	⬜
cpd00666	m-Tartaric Acid	⬜	⬜
cpd00652	Mucic Acid	⬜	⬜
cpd00224	L-Arabinose	⬜	⬜
cpd00227	L-Homoserine	⬜	⬜
cpd00599	2-Hydroxy Benzoic Acid	⬜	⬜
cpd00361	3-Hydroxy 2-Butanone	⬜	⬜
cpd01502	Citraconic Acid	⬜	⬜
cpd00142	Acetoacetic Acid	⬜	⬜

Metabolite ID	Carbon source name	Experimental	Computational
cpd00386	D-Malic Acid	⬜	🟩
cpd00105	D-Ribose	⬜	🟩
cpd00550	D-Serine	⬜	🟩
cpd00246	Inosine	⬜	🟩
cpd00161	L-Threonine	⬜	🟩
cpd00477	N-Acetyl-L-Glutamic Acid	🟩	⬜
cpd11592	Glycyl-L-Glutamic Acid	🟩	⬜
cpd11588	Glycyl-L-Proline	🟩	⬜
cpd00851	Hydroxy-L-Proline	🟩	⬜
cpd00211	Butyric Acid	🟩	⬜
cpd11585	L-Alanyl-Glycine	🟩	⬜
cpd00039	L-Lysine	🟩	⬜
cpd00266	D,L-Carnitine	🟩	⬜
cpd00222	D-Gluconic Acid	🟩	⬜
cpd00489	p-Hydroxy Phenyl Acetic Acid	🟩	⬜
cpd00141	Propionic Acid	🟩	⬜
cpd00249	Uridine	🟩	⬜
cpd00797	β-Hydroxy Butyric Acid	🟩	⬜

🟩 Growth

⬜ No Growth

Supplementary Figure 2. A) Comparison of experimental and computational single substrate source utilization for *P. aeruginosa* PA14. B) Comparison of experimental and computational single substrate source utilization for *P. aeruginosa* PAO1.



B.

Metabolite ID	Carbon source name	Experimental	Computational
cpd00162	2-Aminoethanol	🟩	🟩
cpd00136	4-Hydroxy Benzoic Acid	🟩	🟩
cpd00029	Acetic Acid	🟩	🟩
cpd00331	cis-Aconitic Acid	🟩	🟩
cpd00137	Citric Acid	🟩	🟩
cpd00117	D-Alanine	🟩	🟩
cpd00082	D-Fructose	🟩	🟩
cpd00314	D-Mannitol	🟩	🟩
cpd00106	Fumaric Acid	🟩	🟩
cpd00100	Glycerol	🟩	🟩
cpd00246	Inosine	🟩	🟩
cpd00380	Itaconic Acid	🟩	🟩
cpd00035	L-Alanine	🟩	🟩
cpd00051	L-Arginine	🟩	🟩
cpd00132	L-Asparagine	🟩	🟩
cpd00041	L-Aspartic Acid	🟩	🟩
cpd00023	L-Glutamic Acid	🟩	🟩
cpd00053	L-Glutamine	🟩	🟩
cpd00119	L-Histidine	🟩	🟩
cpd00322	L-Isoleucine	🟩	🟩
cpd00159	L-Lactic Acid	🟩	🟩
cpd00107	L-Leucine	🟩	🟩
cpd00130	L-Malic Acid	🟩	🟩
cpd00064	L-Ornithine	🟩	🟩
cpd00066	L-Phenylalanine	🟩	🟩
cpd00129	L-Proline	🟩	🟩
cpd00054	L-Serine	🟩	🟩
cpd00308	Malonic Acid	🟩	🟩
cpd00118	Putrescine	🟩	🟩
cpd00020	Pyruvic Acid	🟩	🟩
cpd00036	Succinic Acid	🟩	🟩
cpd00581	Urocanic Acid	🟩	🟩
cpd00027	α-D-Glucose	🟩	🟩
cpd00024	α-Keto-Glutaric Acid	🟩	🟩
cpd00281	γ-Amino Butyric Acid	🟩	🟩

Metabolite ID	Carbon source name	Experimental	Computational
cpd01949	2,3-Butanediol	⬜	⬜
cpd00361	2,3-Butanone	⬜	⬜
cpd00294	2-Deoxy Adenosine	⬜	⬜
cpd01242	2-Deoxy-D-Ribose	⬜	⬜
cpd00599	2-Hydroxy Benzoic Acid	⬜	⬜
cpd00361	3-Hydroxy 2-Butanone	⬜	⬜
cpd00142	Acetoacetic Acid	⬜	⬜
cpd01502	Citraconic Acid	⬜	⬜
cpd00158	D-Cellobiose	⬜	⬜
cpd00072	D-Fructose-6-Phosphate	⬜	⬜
cpd00280	D-Galacturonic Acid	⬜	⬜
cpd00089	D-Glucose-1-Phosphate	⬜	⬜
cpd00079	D-Glucose-6-Phosphate	⬜	⬜
cpd00164	D-Glucuronic Acid	⬜	⬜
cpd00138	D-Mannose	⬜	⬜
cpd00609	D-Saccharic Acid	⬜	⬜
cpd00588	D-Sorbitol	⬜	⬜
cpd00666	D-Tartaric Acid	⬜	⬜
cpd00794	D-Trehalose	⬜	⬜
cpd00154	D-Xylose	⬜	⬜
cpd00047	Formic Acid	⬜	⬜
cpd00155	Glycogen	⬜	⬜
cpd00139	Glycolic Acid	⬜	⬜
cpd11589	Glycyl-L-Aspartic Acid	⬜	⬜
cpd11592	Glycyl-L-Glutamic Acid	⬜	⬜
cpd00040	Glyoxylic Acid	⬜	⬜
cpd11585	L-Alanyl-Glycine	⬜	⬜
cpd00224	L-Arabinose	⬜	⬜
cpd00227	L-Homoserine	⬜	⬜
cpd00060	L-Methionine	⬜	⬜
cpd00666	L-Tartaric Acid	⬜	⬜
cpd00179	Maltose	⬜	⬜
cpd01262	Maltotriose	⬜	⬜
cpd03320	m-Hydroxy Phenyl Acetic Acid	⬜	⬜
cpd00121	m-Inositol	⬜	⬜
cpd00666	m-Tartaric Acid	⬜	⬜
cpd00652	Mucic Acid	⬜	⬜
cpd00184	Thymidine	⬜	⬜
cpd00249	Uridine	⬜	⬜
cpd00094	α-Keto-Butyric Acid	⬜	⬜

Metabolite ID	Carbon source name	Experimental	Computational
cpd00182	Adenosine	⬜	🟩
cpd00080	D,L-α-Glycerol- Phosphate	⬜	🟩
cpd00386	D-Malic Acid	⬜	🟩
cpd00105	D-Ribose	⬜	🟩
cpd00550	D-Serine	⬜	🟩
cpd00033	Glycine	⬜	🟩
cpd00161	L-Threonine	⬜	🟩
cpd00211	Butyric Acid	🟩	⬜
cpd00266	D,L-Carnitine	🟩	⬜
cpd00222	D-Gluconic Acid	🟩	⬜
cpd11588	Glycyl-L-Proline	🟩	⬜
cpd00851	Hydroxy-L-Proline	🟩	⬜
cpd00039	L-Lysine	🟩	⬜
cpd00156	L-Valine	🟩	⬜
cpd00477	N-Acetyl-L-Glutamic Acid	🟩	⬜
cpd00489	p-Hydroxy Phenyl Acetic Acid	🟩	⬜
cpd00141	Propionic Acid	🟩	⬜
cpd00797	β-Hydroxy Butyric Acid	🟩	⬜

🟩 Growth
⬜ No Growth

Supplementary Figure 2. A) Comparison of experimental and computational single substrate source utilization for *P. aeruginosa* PA14. B) Comparison of experimental and computational single substrate source utilization for *P. aeruginosa* PAO1.

A.

			in vitro		
			+	-	
			95	1037	
in silico	+	110	True Positive 54	False Positive 56	Positive Predictive Value 49.09%
	-	1022	False Negative 41	True Negative 981	Negative Predictive Value 95.99%
			Sensitivity 56.84%	Specificity 94.60%	Accuracy 91.43%

B.

			in vitro		
			+	-	
			96	1052	
in silico	+	110	True Positive 54	False Positive 56	Positive Predictive Value 49.09%
	-	1038	False Negative 42	True Negative 996	Negative Predictive Value 95.95%
			Sensitivity 56.25%	Specificity 94.68%	Accuracy 91.46%

Supplementary Figure 3. Analysis of essential gene predictions compared to *in vitro* essentiality. Overlap of essential genes derived from the PAO1 single transposon mutant library and PA14 single transposon mutant library were used. Both mPA14 (Panel A) and mPAO1 (Panel B) predict gene essentiality with an accuracy of 91%.