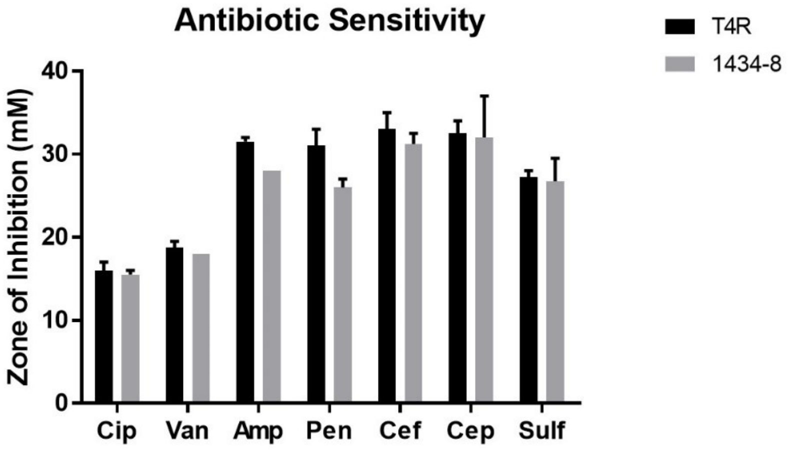
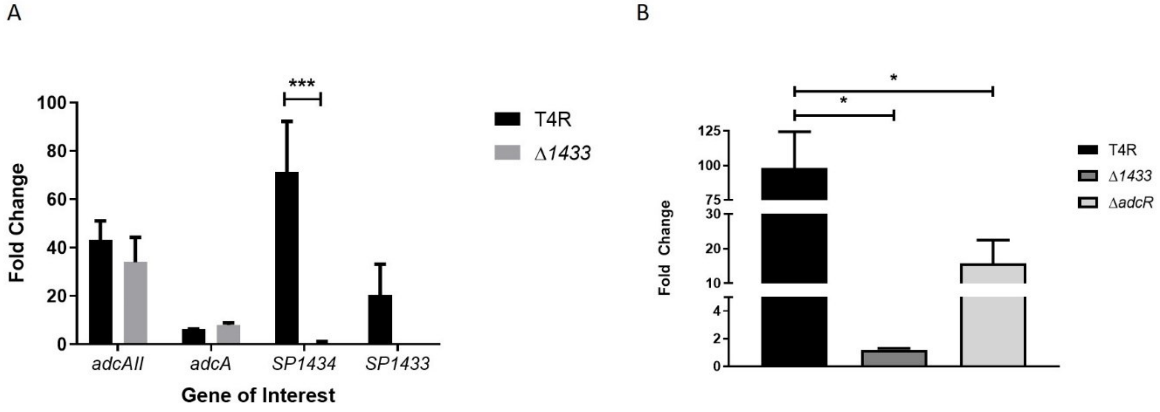
Supplemental Information

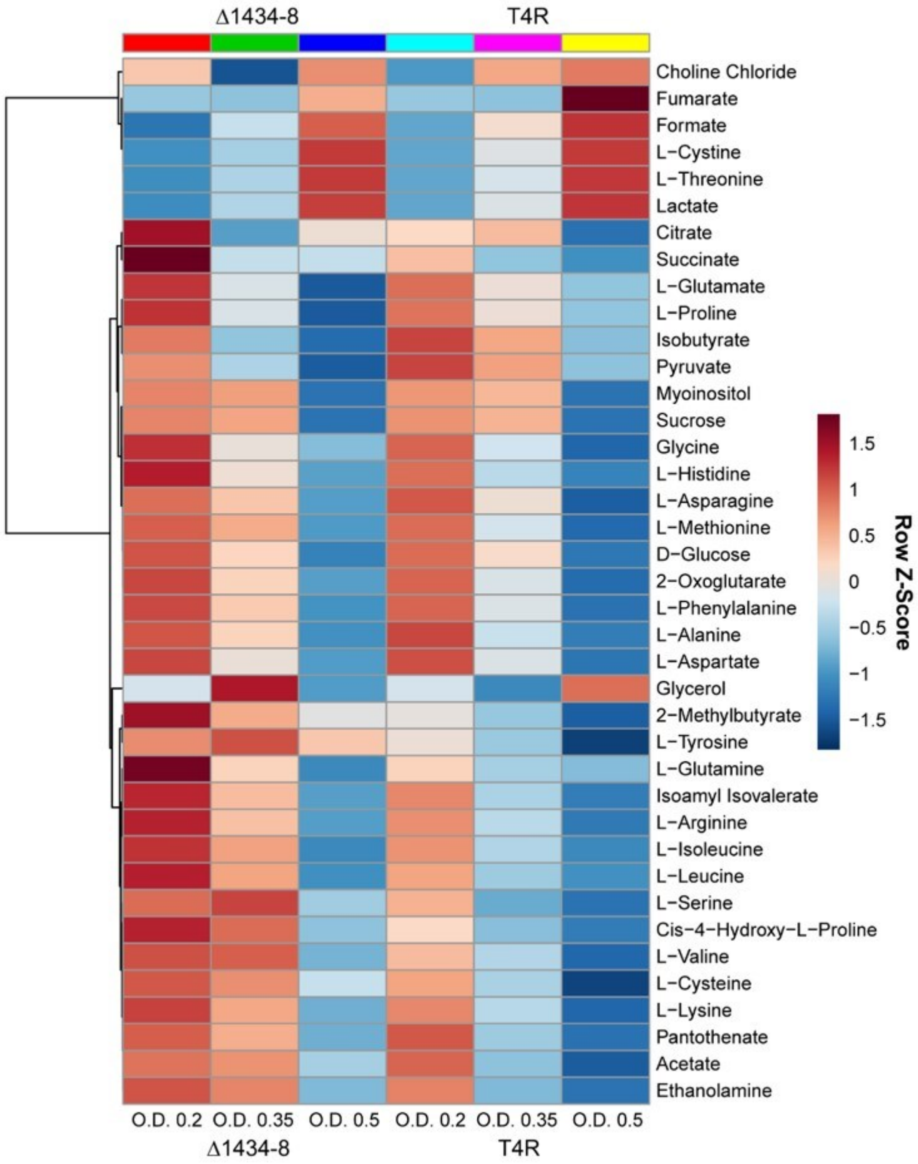
Supplemental Figure 1. Antibiotic sensitivity in T4R and Δ*1434-8*



Supplemental Figure 2. *SP1433* specifically regulates *SP1434-8* and not characterized zinc importers



Supplemental Figure 3. Heatmap of metabolite concentrations across pneumococcal growth phases



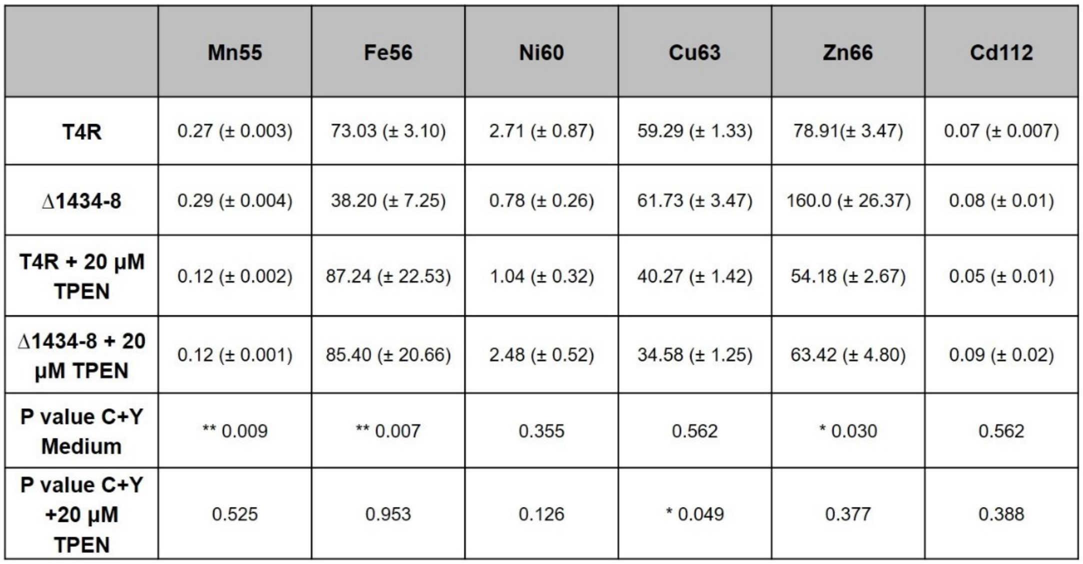
Supplemental Table 1. Microarray data from TIGR4 following TPEN treatment

Gene Name	Predicted Gene Function	Fold Change
SP1435	ABC transporter, ATP-binding protein	4.201
SP1173	conserved hypothetical protein	3.626
SP1436	hypothetical protein	3.382
SP1434	ABC transporter, ATP-binding-permease	3.241
SP0052	hypothetical protein	3.156
SP1002	adhesion lipoprotein (*adcAII*)	3.125
SP1003	conserved hypothetical protein (*phtD*)	2.993
SP2171	zinc ABC transporter, ATP-binding protein (*adcC*)	2.943
SP0641	serine protease, subtilase family	2.523
SP1437	conserved domain protein	2.298
SP1472	oxidoreductase, putative	2.233
SP2172	adc operon repressor (*adcR*)	2.209
SP0053	phosphoribosylaminoimidazole carboxylase	2.111
SP0117	pneumococcal surface protein A (*pspA*)	1.955
SP0327	hypothetical protein, interruption	1.832
SP2206	ribosomal subunit interface protein	1.716
SP1470	thiamine biosynthesis protein (*apbE*)	1.67
SP1471	oxidoreductase, putative	1.66
SP1438	ABC transporter, ATP-binding protein	1.629
SP1690	ABC transporter, substrate-binding protein	1.605
SP1647	Endopeptidase O	1.514
SP1215	Putative FNT family transporter	1.512
SP0534	Hypothetical protein	1.501

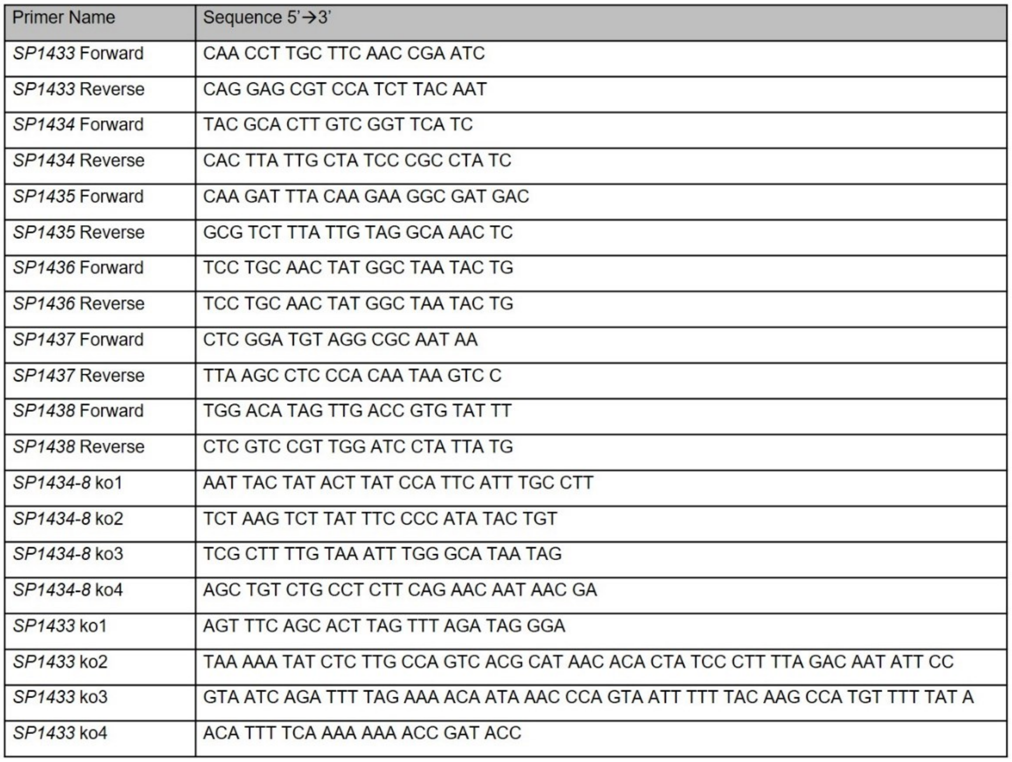
Supplemental Table 2. BLAST analysis of the SP1433-1438 operon

Gene Name	Uniprot ID-Description	Protein length	Protein domains (Position)	Protein COG	Top BLASTp Alignment	Protein length (% Ident)	Query Cover	E value
SP1433	A0A0H2UQW4 Transcriptional regulator, AraC family	330 AA	Helix-turn-helix DNA-binding (219-317)	Transcription	SoxS regulatory protein	330 AA (99.70%)	100%	0.0
SP1434	A0A0H2UQM1 ABC transporter, ATP-binding/permease protein	586 AA	Transmembrane (21-41, 61-86 147-175, 246-276, 274-298) Nucleotide binding (370-377)	Defense mechanisms	IrtA: iron transport ATP-binding/permease protein	584 AA (99.83%)	99%	0.0
SP1435	A0A0H2UQM0 ABC transporter, ATP-binding protein	581 AA	Transmembrane (32-52, 64-81, 145-161, 167-184, 248-274) Nucleotide binding (371-378)	Defense mechanisms	NvdA: β-1,2 glucan export	573 AA (99.65%)	100%	0.0
SP1436	A0A0H2UQL1 Uncharacterized protein	209 AA	Transmembrane (12-33, 39-57, 64-97, 113-133, 163-184, 190-208)	Function unknown	Conserved hypothetical integral membrane protein	194 AA (100.00%)	100%	1e-127
SP1437	A0A0H2UQT8 Conserved domain protein	147 AA	Signal peptide (1-18) Transmembrane (126-146)	Inorganic ion transport & metabolism	Putative HMP/thiamine permease protein	231 AA (96.22%)	88%	1e-118
SP1438	A0A0H2UQX0ABC transporter, ATP-binding protein	374 AA	Nucleotide binding (34-41) ABC transporter (2-239)	Inorganic ion, carbohydrate transport & metabolism	Ribose ABC transporter ATP-binding protein	454 AA (99.73%)	100%	0.0

Supplemental Table 3. Intracellular metal ion concentrations in ppb (µg/L)



	Mn55	**Fe56**	**Ni60**	**Cu63**	**Zn66**	**Cd112**
T4R	0.27 (± 0.003)	73.03 (± 3.10)	2.71 (± 0.87)	59.29 (± 1.33)	78.91(± 3.47)	0.07 (± 0.007)
∆1434-8	0.29 (± 0.004)	38.20 (± 7.25)	0.78 (± 0.26)	61.73 (± 3.47)	160.0 (± 26.37)	0.08 (± 0.01)
T4R + 20 µM TPEN	0.12 (± 0.002)	87.24 (± 22.53)	1.04 (± 0.32)	40.27 (± 1.42)	54.18 (± 2.67)	0.05 (± 0.01)
∆1434-8 + 20 µM TPEN	0.12 (± 0.001)	85.40 (± 20.66)	2.48 (± 0.52)	34.58 (± 1.25)	63.42 (± 4.80)	0.09 (± 0.02)
P value C+Y Medium	** 0.009	** 0.007	0.355	0.562	* 0.030	0.562
P value C+Y +20 µM TPEN	0.525	0.953	0.126	* 0.049	0.377	0.388



Primer Name	Sequence 5'→3'
SP1433 Forward	CAA CCT TGC TTC AAC CGA ATC
SP1433 Reverse	CAG GAG CGT CCA TCT TAC AAT
SP1434 Forward	TAC GCA CTT GTC GGT TCA TC
SP1434 Reverse	CAC TTA TTG CTA TCC CGC CTA TC
SP1435 Forward	CAA GAT TTA CAA GAA GGC GAT GAC
SP1435 Reverse	GCG TCT TTA TTG TAG GCA AAC TC
SP1436 Forward	TCC TGC AAC TAT GGC TAA TAC TG
SP1436 Reverse	TCC TGC AAC TAT GGC TAA TAC TG
SP1437 Forward	CTC GGA TGT AGG CGC AAT AA
SP1437 Reverse	TTA AGC CTC CCA CAA TAA GTC C
SP1438 Forward	TGG ACA TAG TTG ACC GTG TAT TT
SP1438 Reverse	CTC GTC CGT TGG ATC CTA TTA TG
SP1434-8 ko1	AAT TAC TAT ACT TAT CCA TTC ATT TGC CTT
SP1434-8 ko2	TCT AAG TCT TAT TTC CCC ATA TAC TGT
SP1434-8 ko3	TCG CTT TTG TAA ATT TGG GCA TAA TAG
SP1434-8 ko4	AGC TGT CTG CCT CTT CAG AAC AAT AAC GA
SP1433 ko1	AGT TTC AGC ACT TAG TTT AGA TAG GGA
SP1433 ko2	TAA AAA TAT CTC TTG CCA GTC ACG CAT AAC ACA CTA TCC CTT TTA GAC AAT ATT CC
SP1433 ko3	GTA ATC AGA TTT TAG AAA ACA ATA AAC CCA GTA ATT TTT TAC AAG CCA TGT TTT TAT A
SP1433 ko4	ACA TTT TCA AAA AAA ACC GAT ACC

Supplemental Table 4. Primer sequences used in this study