

Supplementary Information_2

Elucidation of independently modulated genes in *Streptococcus pyogenes* reveals carbon sources that control its expression of hemolytic toxins

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1. Supplementary Table

Table S1: Primers used in this study

Primer	Sequence (5'-3')	Purpose
MalR2KOupF	CCCAAGCTTTCCTTTTGGAAAATGGTTTATGA	Construction of pHY304- <i>malR2</i> KO
MalR2KOupR	CCAGTGATTTTTTCTCCATCATTTTGGTTATATCCGAATTC	Construction of pHY304- <i>malR2</i> KO
MalR2KOdownF	GAGTGGCAGGGCGGGGCGTAACGCCTGAGAATTAACCTATC	Construction of pHY304- <i>malR2</i> KO
MalR2KOdownR	ATAGTTTAGCGGCCGCTCCCTTGAACGGATTGTAA	Construction of pHY304- <i>malR2</i> KO
MalR2KOcatF	ATGGAGAAAAAATCACTGGATATACC	Construction of pHY304- <i>malR2</i> KO
MalR2KOcatR	TTACGCCCCGCCCTGCCACTCATCGCA	Construction of pHY304- <i>malR2</i> KO
MalR2KOconfirmF	GCGAAGGAACTGCTTACGTC	Confirmation of <i>malR2</i> deletion
MalR2KOconfirmR	GCCGAGCTCAGTTTACTTGG	Confirmation of <i>malR2</i> deletion

2. Supplementary Figures

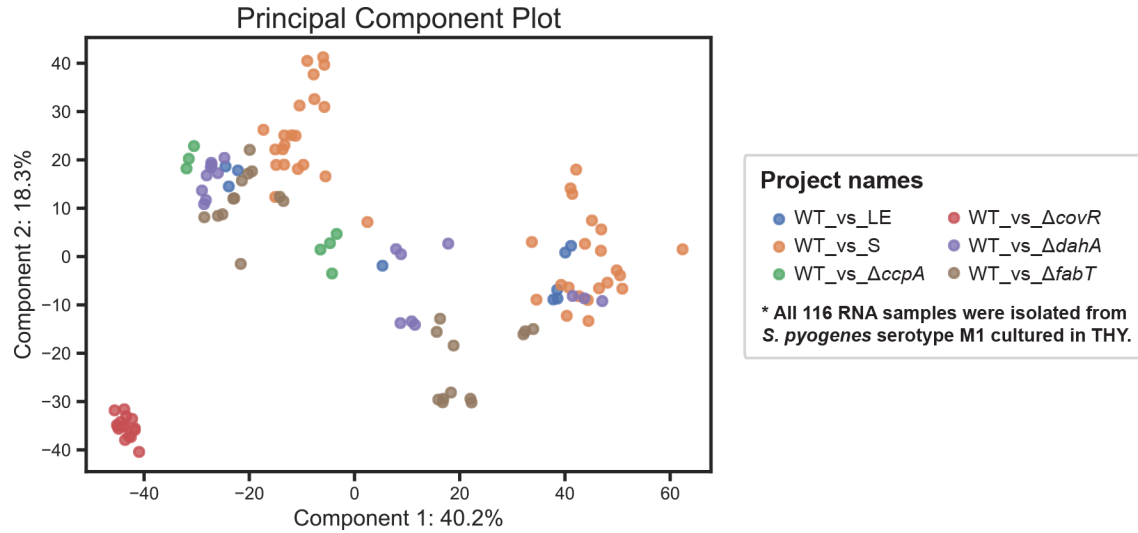


Figure S1. Diversity of 116 RNA-seq data sets used for ICA decomposition. Loadings of the first two principal components (PC). The variation in locations across 116 RNA-seq samples demonstrates the diversity of the compendium.

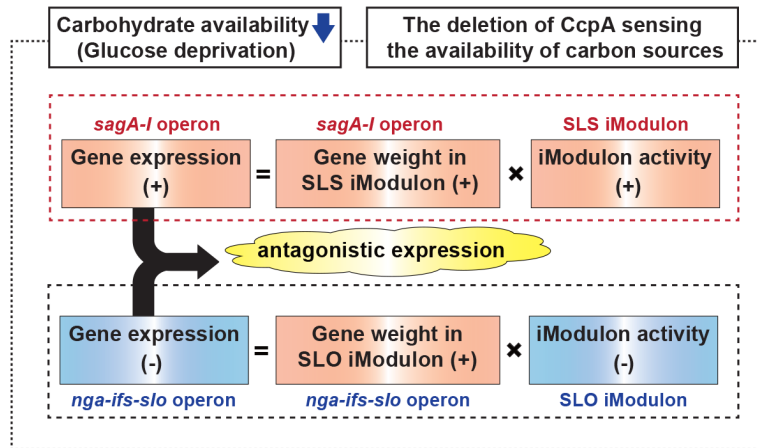


Figure S2. Conditions that *S. pyogenes* may show the antagonistic expression of *sag A-I* and *nga-ifs-slo* operons.

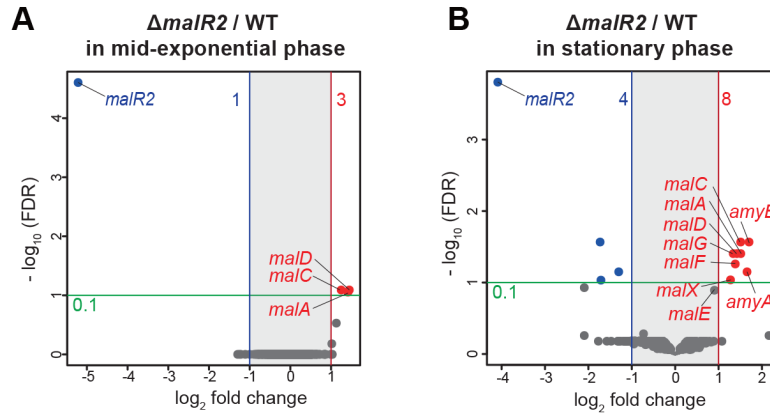


Figure S3. The actual MalR2 regulon. Differentially expressed genes (DEGs) from comparisons of the $\Delta malR2$ mutant and WT strains at (A) mid-exponential, and (B) stationary growth phases in THY broth. Colored circles indicate significantly upregulated (red) and downregulated (blue) genes (absolute $\log_2$ fold change, > 1 ; adjusted $P < 0.1$).

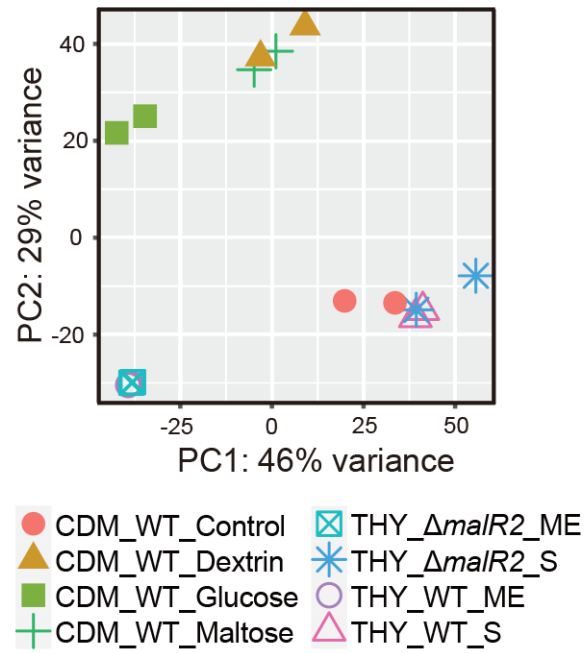


Figure S4. Principal component analysis (PCA) plot of TPM data from 16 RNA-seq data set in this study. ME, Mid-Exponential growth phase. S, Stationary growth phase.