Supporting Information (SI)

A Spectrum of CodY Activities Drives Metabolic Reorganization and Virulence Gene Expression in *Staphylococcus aureus*

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Table S1. Additional strains and plasmids.

Strain or plasmid	Genotype	Source or Reference[a]
E. coli		
NEB 5α	*fhuA2* Δ*(argF-lacZ)U169 phoA glnV44* Φ80 Δ*(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	New England Biolabs
Plasmids		
pMAD	Gram-positive allelic exchange vector, Ap^R Em^R	(Arnaud *et al.*, 2004)
pCN38	Source of *cat194* cassette	(Charpentier *et al.*, 2004)
pMRSI	sGFP-sDsRed double reporter shuttle vector, Ap^R Cm^R	
pMRSI-nuc	pMRSI with *nuc-gfp* reporter, Ap^R Cm^R	
pSRB44	pMAD with s*ucD::cat194* allele, Ap^R Em^R Cm^R	
pSRB57	pMAD mutated *codY* fragment to generate *codY57*, Ap^R Em^R	
pSRB58	pMAD mutated *codY* fragment to generate *codY58*, Ap^R Em^R	
pSRB65	pMAD with in-frame deletion fragment to generate Δ*ilvE1,* Ap^R Em^R	
pSRB66	pMAD mutated *codY* fragment to generate *codY66*, Ap^R Em^R	
pTET	pTnT with *tet*(M)	(Bose *et al.,* 2013)

[a]Unless otherwise noted, strains and plasmids were constructed during the course of this study.

Table S2. Oligonucleotides used in this study.

Oligo	Sequence (5' → 3')	Purpose	Source or reference[a]
KPPE-TIR-f	GGTACCAGCTGCAGAATTCTGATTAACTTTATAAGGAG GAAAAACATATGC	pMRSI construction	
NNSB-TIR*-r	GCCGGCTAGCACTAGTGGATCCAGATAATCTATAAAAG GAGG	pMRSI construction	
oSRB198	TGG AGG TAG AAT TCA AGC AGG CGG AGT	*sucD* deletion allele	
oSRB199	TTT CCT AGG CAT CTT AGT GCT CCC ATC CTT T	*sucD* deletion allele	
oSRB200	GCA CTA AGA TGC CTA GGA AAA AAC TCG AGT AAA GTT AAA AGA TGA TAT AA	*sucD* deletion allele	
oSRB201	ATA AAG ATG GAT CCA ATA TAC ATT AAC	*sucD* deletion allele	
oSRB233	AGG ATC GAG TCT AAA TGA ATT ATT AAA AAG TCA AGA AAT TAT TCA AAT GT	R61E allele	
oSRB234	ACA TTT GAA TAA TTT CTT GAC TTT TTA AT	R61E allele	
oSRB235	AGG ATC GAG TCT AAA TGA ATT ATT AAA AAG TCA AAA AAT TAT TCA AAT GT	R61K allele	
oSRB236	ACA TTT GAA TAA TTT TTT GAC TTT TTA AT	R61K allele	
oSRB237	AGG ATC GAG TCT AAA TGA ATT ATT AAA AAG TCA ACA TAT TAT TCA AAT GT	R61H allele	
oSRB238	ACA TTT GAA TAA TAT GTT GAC TTT TTA AT	R61H allele	
oSRB248	TTT CAT TCC TAT GGA TCC TGA TTC AAT T	*codY* cloning flank	
oSRB249	ACG AAA GTT GCC ATG GAT TAA ACA ATA TGA A	*codY* cloning flank	
oSRB316	GAA ACA AGG ATC CGT TAT AAT TTA	*ilvE* deletion allele	
oSRB317	CAT GGT GAT TGC CTC CTA ATA ATA	*ilvE* deletion allele	
oSRB318	TAT TAT TAG GAG GCA ATC ACC ATG CCC GGG TAA TAA AAA TTG AAT ATG ATC ATG	*ilvE* deletion allele	
oSRB319	TAA CAC CGT CGA CCC AAT TAA TTT	*ilvE* deletion allele	
oSRB320	TCTATAAAAATATACAAAAGGAGA	*codY* sequencing	
oSRB321	GTTACGACTAGGACATTGAATTAT	*codY* sequencing	
oSRB451	ATC GTG TAC TCG ATC AAG TAC TAA TGT	G129D allele	
oSRB452	ACA TTA GTA CTT GAT CGA GTA CAC GAT	G129D allele	
oSRB453	TAC AAA TAT TGG ATC CTT TAC ACA ATC A	G129D allele	

pCN51-S1-f	CTCACATGTTCTTTCCTGCGTTATCC	pMRSI sequencing	
pCN51-S-r	GTTCTTGTTGCTGTTCCTGTTCTG	pMRSI sequencing	
Pnuc1-r1	GGGCATAACTAACACCTCTTTCTTTTTAGTTAATTTTAA TATTAAACG	pMRSI-nuc construction	
sDsRed-S-r	CTGTTGATGGTTCCCAACCC	pMRSI sequencing	
sGFP-S-r	GTAGCATCACCTTCACCCTCTC	pMRSI sequencing	
SphI-Pnuc-f	CATAGCATGCGTGAATAATAAGATAGAGAAAACTGAAA AACGC	pMRSI-nuc construction	
Nuc substrate	Cy3—CCCCGGATCCACCCC—BHQ2	FRET assay	(Kiedrowski *et al.*, 2011)

Quantitative, real-time RT-PCR oligos

Oligo	Sequence (5' → 3')	Specificity
oDS001	CGA AAG AAC AAT ACG CAA AGA GG	*nuc*
oDS002	TGC ATT TGC TGA GCT ACT TAG A	*nuc*
oNSF1	CAG GTG ACA CAG CGG GTA TT	polC
oNSR1	TGC CGG GTT GTG ATG CTA TT	polC
oNW043	TGA ACA AGA AGC CTG ACA TAA A	*icaA*
oNW044	CGT ATT TGA GTG CAA GAA CAT TAG	*icaA*
oSRB239	GGA TTG GCT TCA CCT GAA AA	*rpoC*
oSRB240	CTT TCA CGA CGT ACT TTA GA	*rpoC*
oSRB241	AAG GAG ATC ACC AAG CAC CA	*ilvD*
oSRB242	CTG CAA GCT CTC TTA AAT GA	*ilvD*
oSRB243	AAC ATA AAT TGG GAG CAG CA	*fnbA*
oSRB244	TTG TCT TTT GTT CTG ATG CT	*fnbA*
oSRB356	GTG AAT TTG TTC ACT GTG TCG ATA A	*hld*
oSRB357	GGA GTG ATT TCA ATG GCA CAA G	*hld*
oSRB454	GGT GTT GAA AGT GTA GGC AAT C	*brnQ2*
oSRB455	AGC ACG TGG AAT ACC GTA AA	*brnQ2*

[a]Unless noted, oligos are from this study.

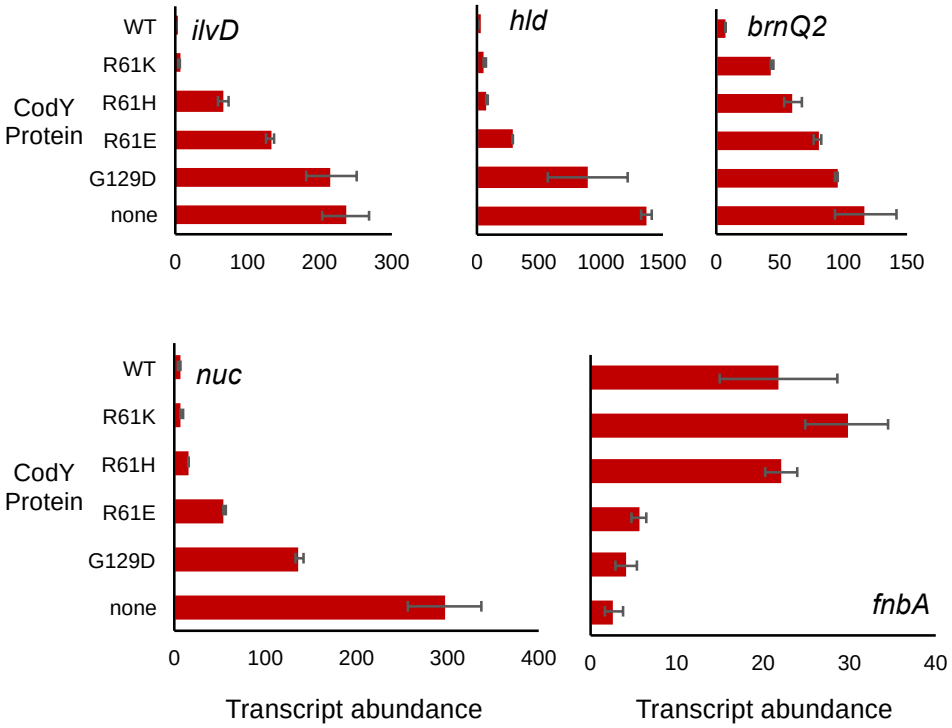

Figure S1: CodY proteins retain varying amounts of residual regulatory activity. Strains producing variant proteins were cultivated in TSB. qRT-PCR was performed on cDNA versions of RNA extracted from cells collected during exponential growth when CodY activity is maximized. In all cases, copies of target transcript were normalized to copies of *rpoC* transcript whose expression is stable (mean transcript abundance) and plotted. Error bars denote standard error of the mean of at least two independent experiments, each performed in technical triplicate.

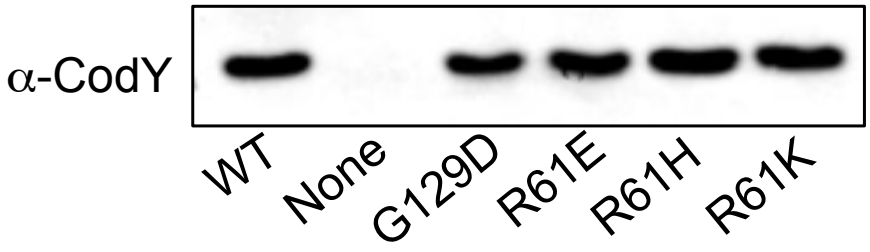

Figure S2: Variant CodY proteins achieve similar abundances during in vitro growth. Soluble protein extracts were prepared from exponentially growing *S. aureus* cells in TSB at 37°C as described in *Experimental Procedures*. An equal amount of each protein sample was separated using SDS-PAGE, transferred to nitrocellulose and analyzed by Western Blot with anti–CodY antibody raised against *Bacillus subtilis* CodY protein.

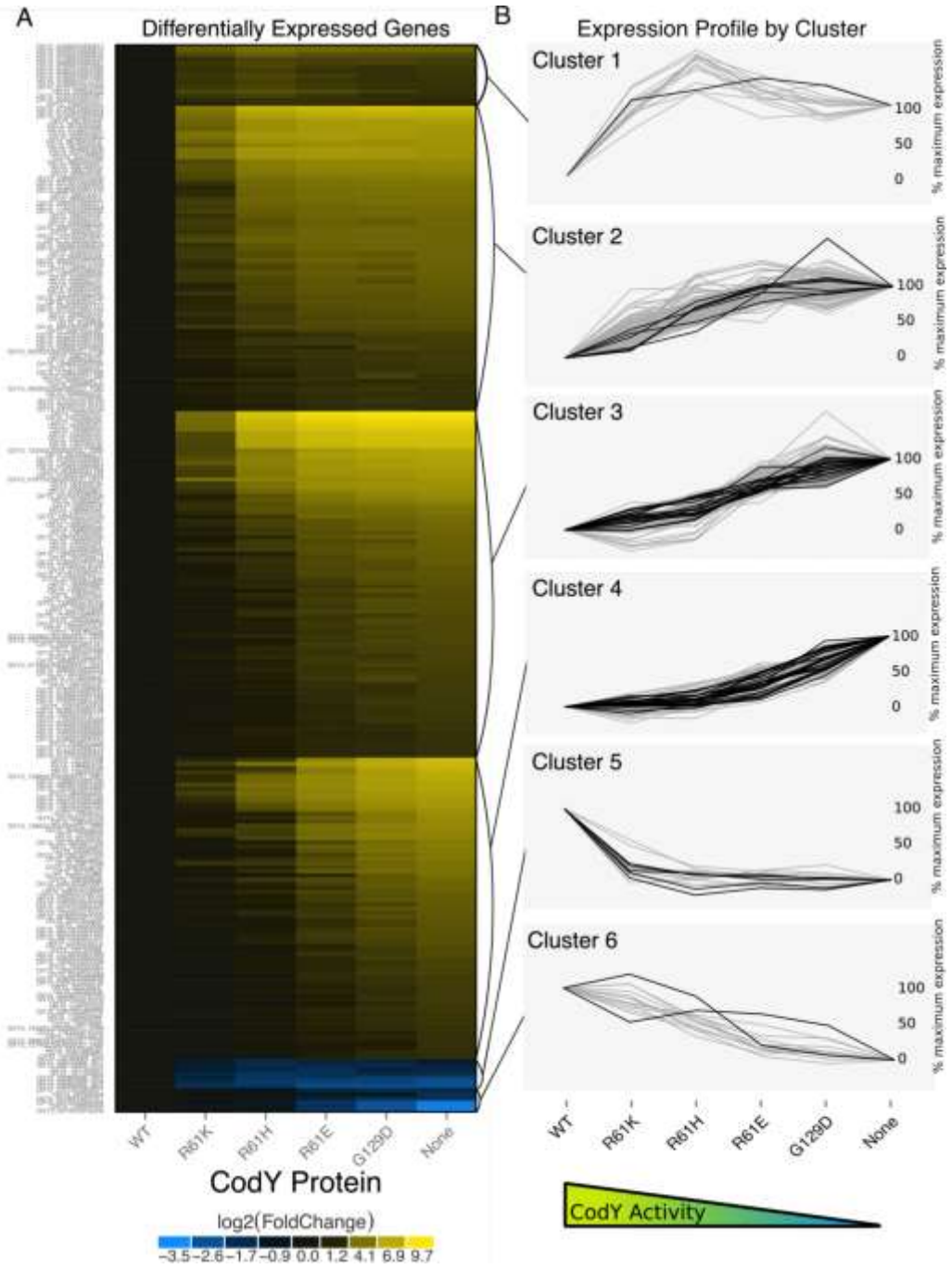

Figure S3: Graded CodY activity turns genes on and off sequentially. A. Heatmap of differentially expressed genes. Fold-changes are color-coded, with yellow indicating an increase in expression and blue indicating a decrease in expression relative to UAMS-1 (wild-type, WT) on a log(2) scale. **B.** *k*-means clustering of genes based on relative expression. Repressed genes were set to 100% in the *codY* null mutant, stimulated genes were set to 100% in UAMS-1 (wild-type, WT). Grey lines denote metabolism genes, black lines denote virulence genes. The plots do not imply a linear relationship between the strains.

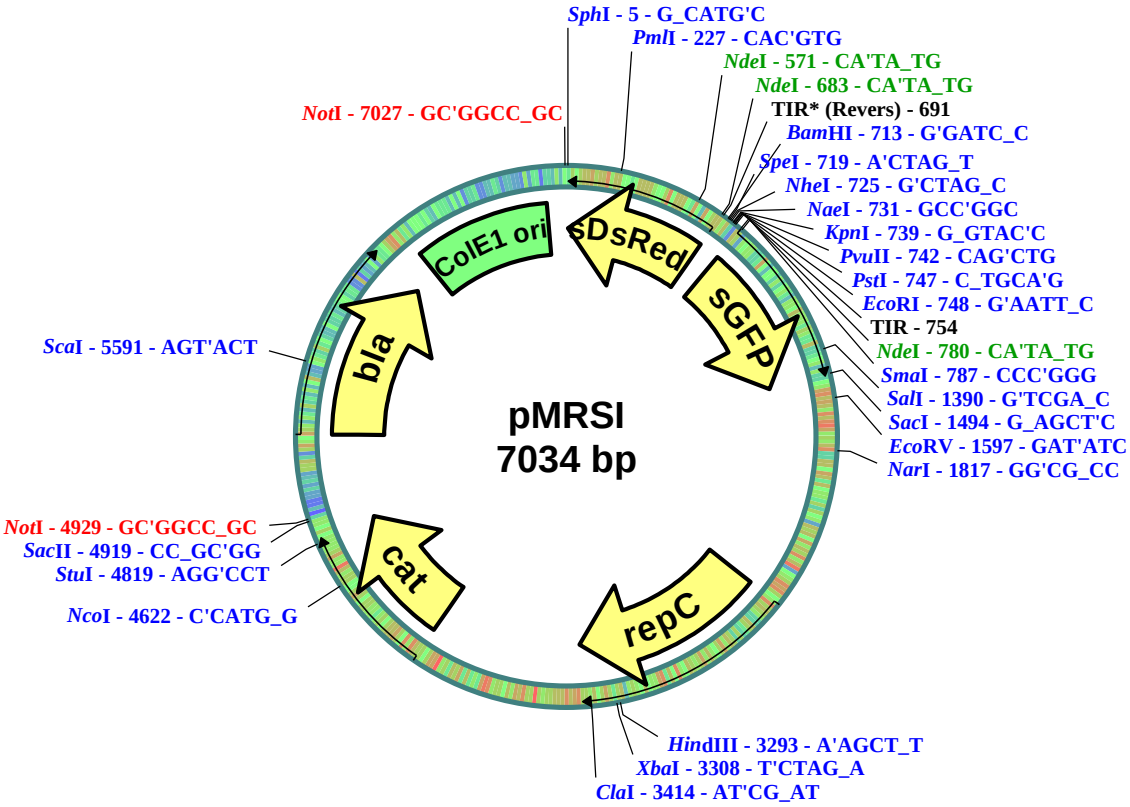

Figure S4: Map of the pMRSI double reporter shuttle vector used in this study. Optimized translation initiation regions (TIR – TGATTAACTTTATAAGGAGGAAAAA and TIR* - AGATAATCTATAAAGGAGGAA) are indicated upstream of the s*GFP* and s*DsRed* genes correspondingly. Unique recognition sequences for the Type II restriction endonucleases are indicated in blue. The pMRSI map was generated using pDRAW32 (AcaClone Software [http://www.acaclone.com]).

References

Arnaud, M., A. Chastanet & M. Débarbouillé, (2004) New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. *Appl. Environ. Microbiol.* **70**: 6887-6891.

Bose, J.L., P.D. Fey & K.W. Bayles, (2013) Genetic tools to enhance the study of gene function and regulation in Staphylococcus aureus. *Appl Environ Microbiol* **79**: 2218-2224.

Charpentier, E., A.I. Anton, P. Barry, B. Alfonso, Y. Fang & R.P. Novick, (2004) Novel cassette-based shuttle vector system for gram-positive bacteria. *Appl Environ Microbiol.* **70**: 6076-6085.

Kiedrowski, M., J. Kavanaugh, C. Malone, J. Mootz, J. Voyich, M. Smeltzer, K. Bayles & H. AR, (2011) Nuclease modulates biofilm formation in community-associated methicillin-resistant *Staphylococcus aureus*. *PLoS One.* **6**: e26714.