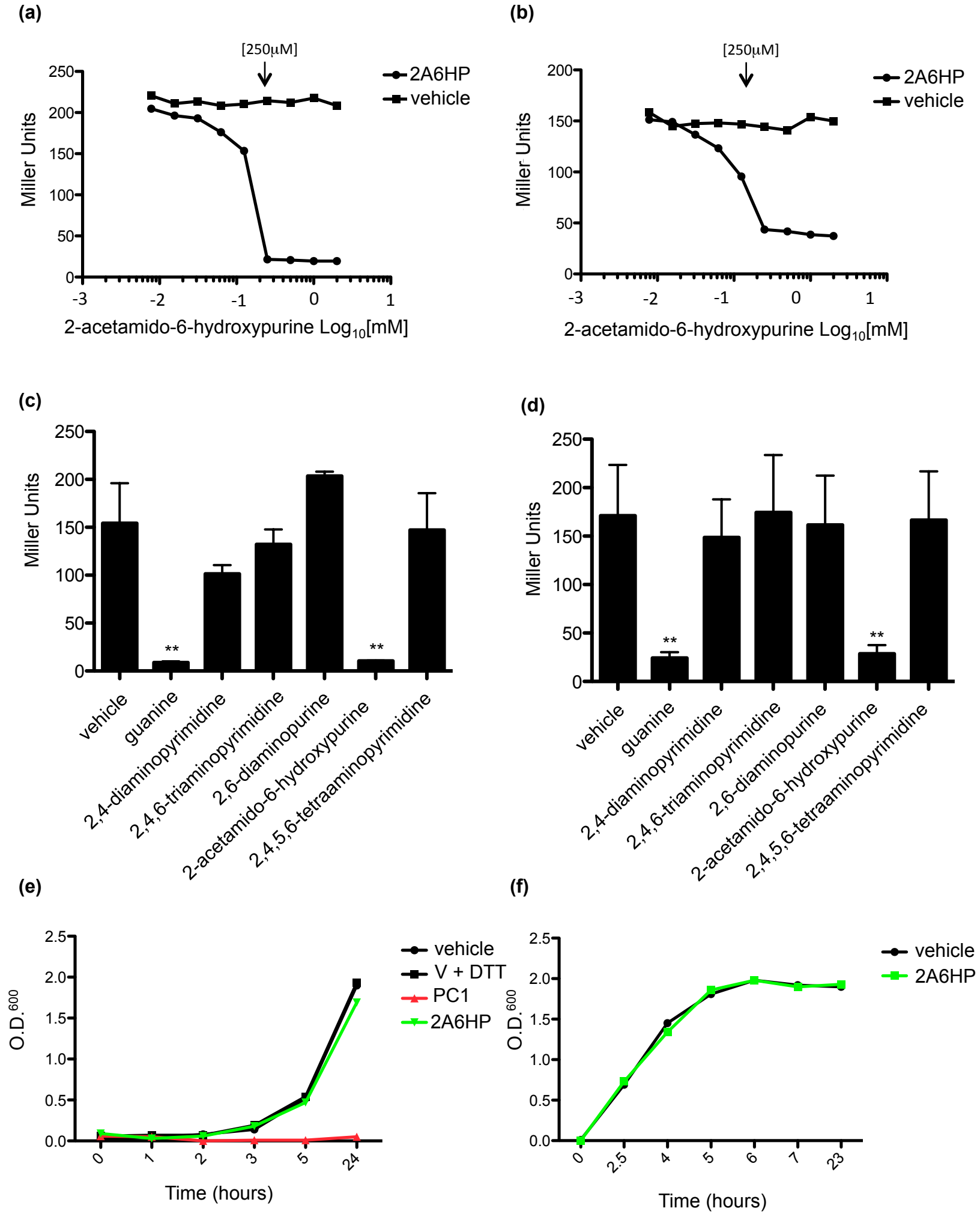
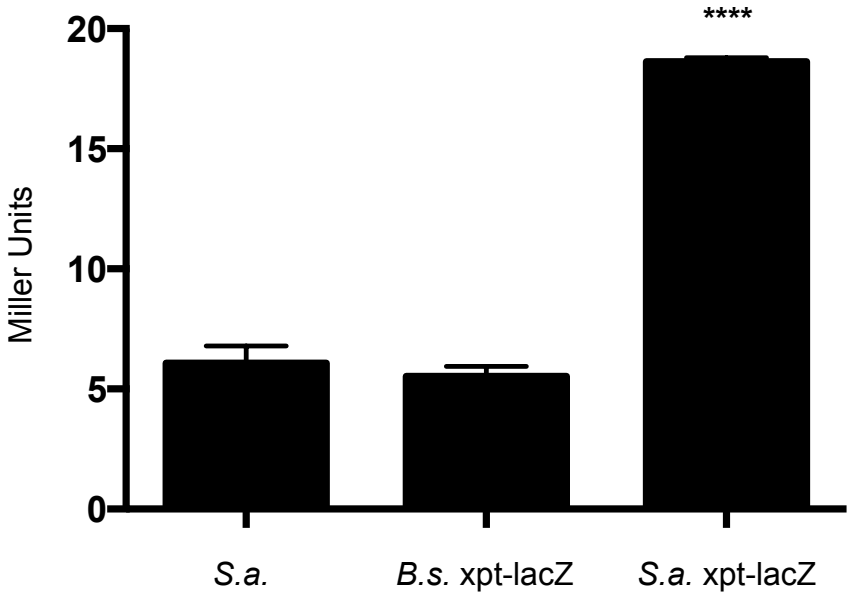
Supplementary Figure 1



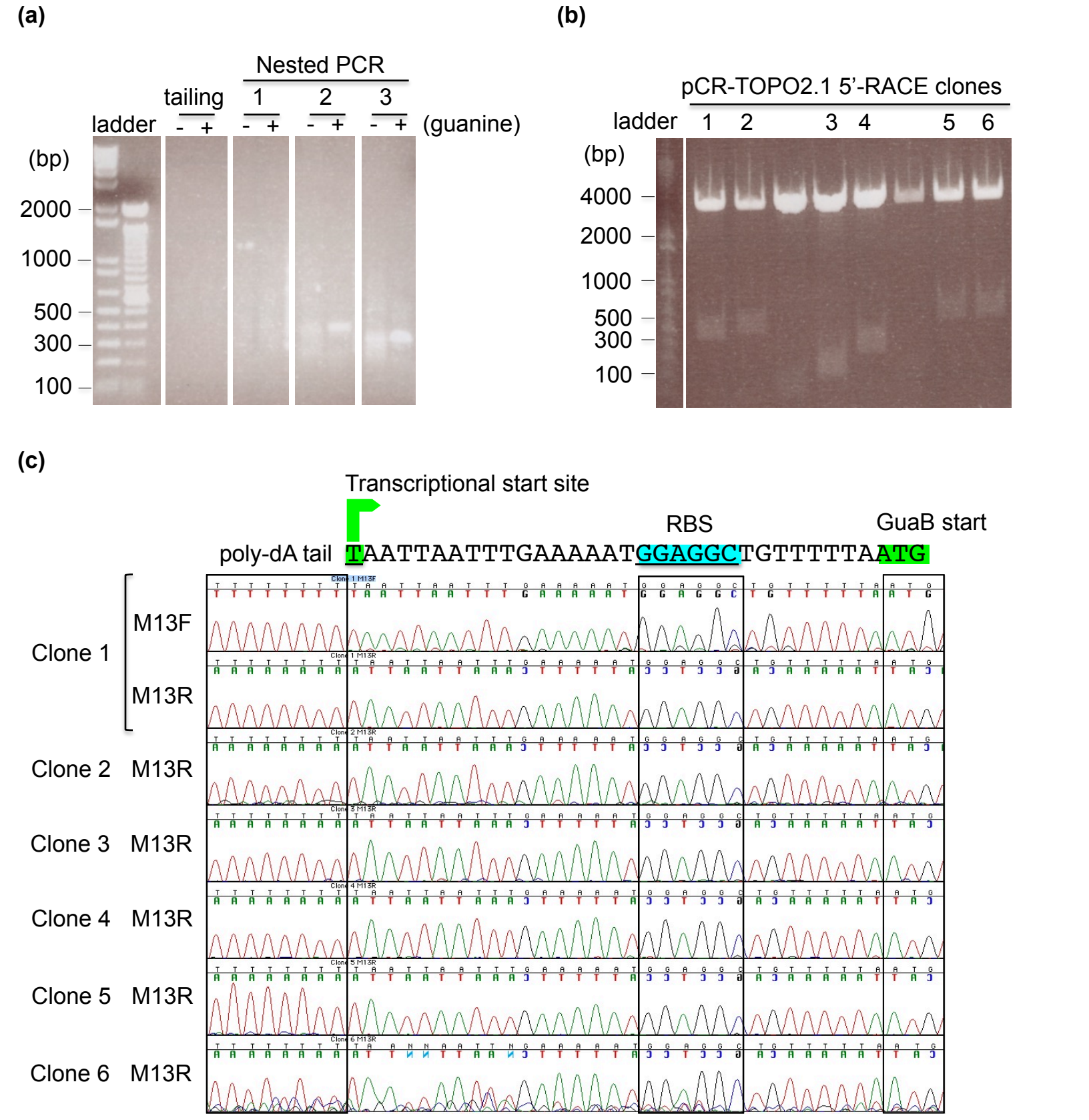
Supplementary Figure 1. Riboswitch reporters respond to guanine analog 2-acetamido-6-hydroxypurine. (a) EK1 was grown in Bacillus media to log phase in varying concentrations of 2-acetamido-6-hydroxypurine (2A6HP) or vehicle alone and reporter activity was measured by Miller assay. **(b)** EK2 was grown to log phase in CDM as described in **(a)**. **(c)** EK1 was grown to log phase in Bacillus media containing vehicle, or 1mM exogenous guanine, 2,4-diaminopyrimidine, 2,4,6-triaminopyrimidine, 2,6-diaminopyrimidine, 2A6HP, or 2,4,5,6-tetraaminopyrimidine and reporter activity was measured by Miller assay. **(d)** EK2 was grown in CDM as described in **(b)**. **(e)** Growth of WT *S. aureus* was monitored by optical density over 24 hours in CDM containing vehicle (0.002M NaOH) alone, vehicle + 1mM DTT, 1mM PC1, or 1mM 2A6HP. **(f)** Growth of WT *S. aureus* in TSB containing vehicle, or 1mM 2A6HP was monitored by optical density over 24 hours. Data are presented as mean+/- S.D. (n=3) (Student's t-test; $p < 0.05$*, $p < 0.01$**, $p<0.001$***).

Supplementary Figure 2



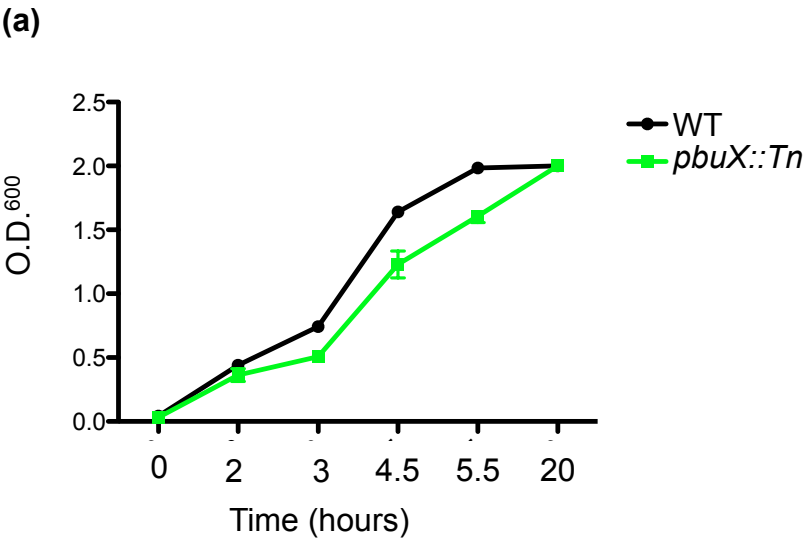
Supplementary Figure 2. Activity of *S. aureus* and *B. subtilis* xpt-lacZ transcriptional reporters stably integrated in *S. aureus* NRS384. Transcriptional reporter activity was measured in the parental *S. aureus NRS384ΔhsdRΔsauUSI* (*S.a.*) strain, and reporter strains *S. aureus NRS384ΔhsdRΔsauUSIΦ85att::pIMC85-B.s. xpt*(-305)*lacZ* (EK17; *B.s.* xpt-lacZ) and *S. aureus NRS384ΔhsdRΔsauUSIΦ85att::pIMC85-S.a. xpt*(-452)*lacZ* (EK3; *S.a.* xpt-lacZ) and grown to log phase in CDM by Miller assay. Comparison of *B.s.* xpt-lacZ (EK17) and the parental *S. aureus NRS384ΔhsdRΔsauUSI* (*S.a.*) strain shows that the *B. subtilis* xpt-reporter is not active in the heterologous host *S. aureus* (P = 0.3274), in contrast to S.a. xpt-lacZ (P<0.0001****). Data are presented as mean +/- S.D. of biological triplicates. Statistical significance determined using student's t-test.

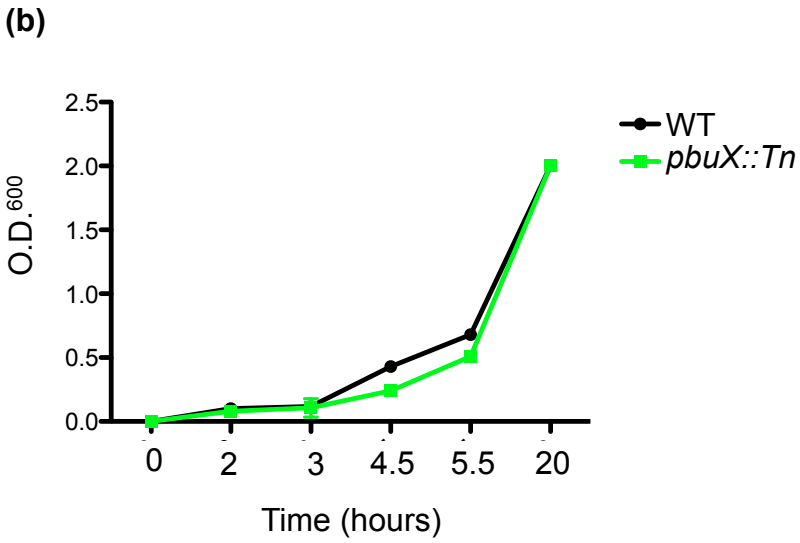
Supplementary Figure 3



Supplementary Figure 3. Identification of the guaB transcriptional start site by 5'-rapid amplification of cDNA ends (5'-RACE). (a) Total RNA was harvested from *S. aureus* grown to log phase in tryptic soy broth with (+), or without (-) 0.25mM exogenous guanine followed by first-strand cDNA synthesis, poly-A tailing with terminal deoxynucleotidyl transferase, and sequential nested PCR. The first PCR contained gene-specific primer 1(GSP1) combined with bridging primer QT that contains a poly-dT 3'-tail, followed by GSP2/QI, and GSP3/QI. PCR products were separated in a 0.8% agarose gel and stained with ethidium bromide and identified a single ~300bp product. **(b)** 5'-RACE products were TA cloned into pCR2.1-TOPO and screened for white colonies indicating an insert that disrupted lacZ expression. Plasmid DNA isolated from these clones were digested with NotI/BamHI to verify insertion of products, and sequenced **(c)** using M13F and M13R primers to identify the transcriptional start site of *guaB*. Sequence analysis was performed using Sequencher software. Agarose gel panels were from a single gel, but images were cropped to remove lanes unrelated to the *guaB* 5'-RACE.

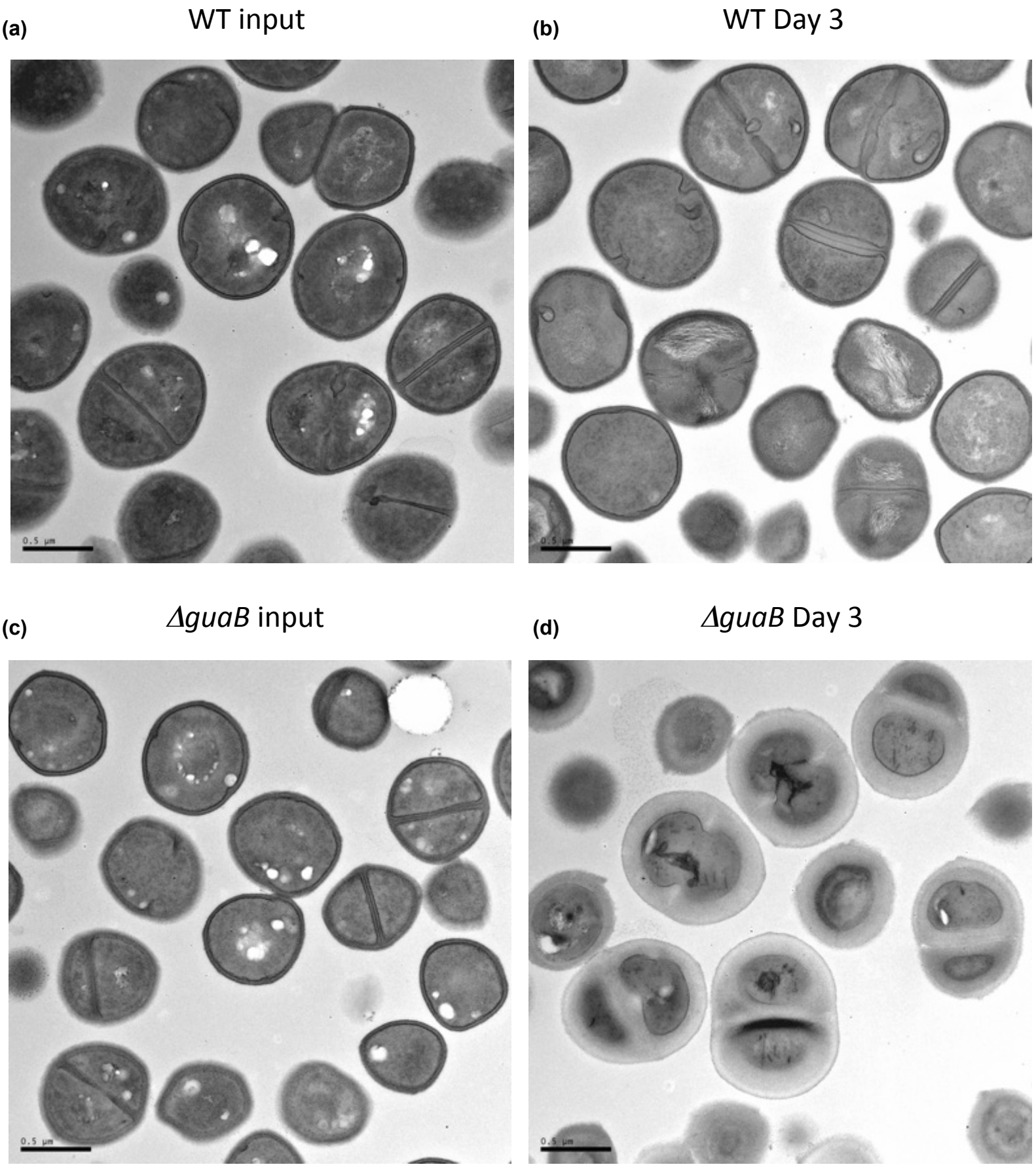
Supplementary Figure 4





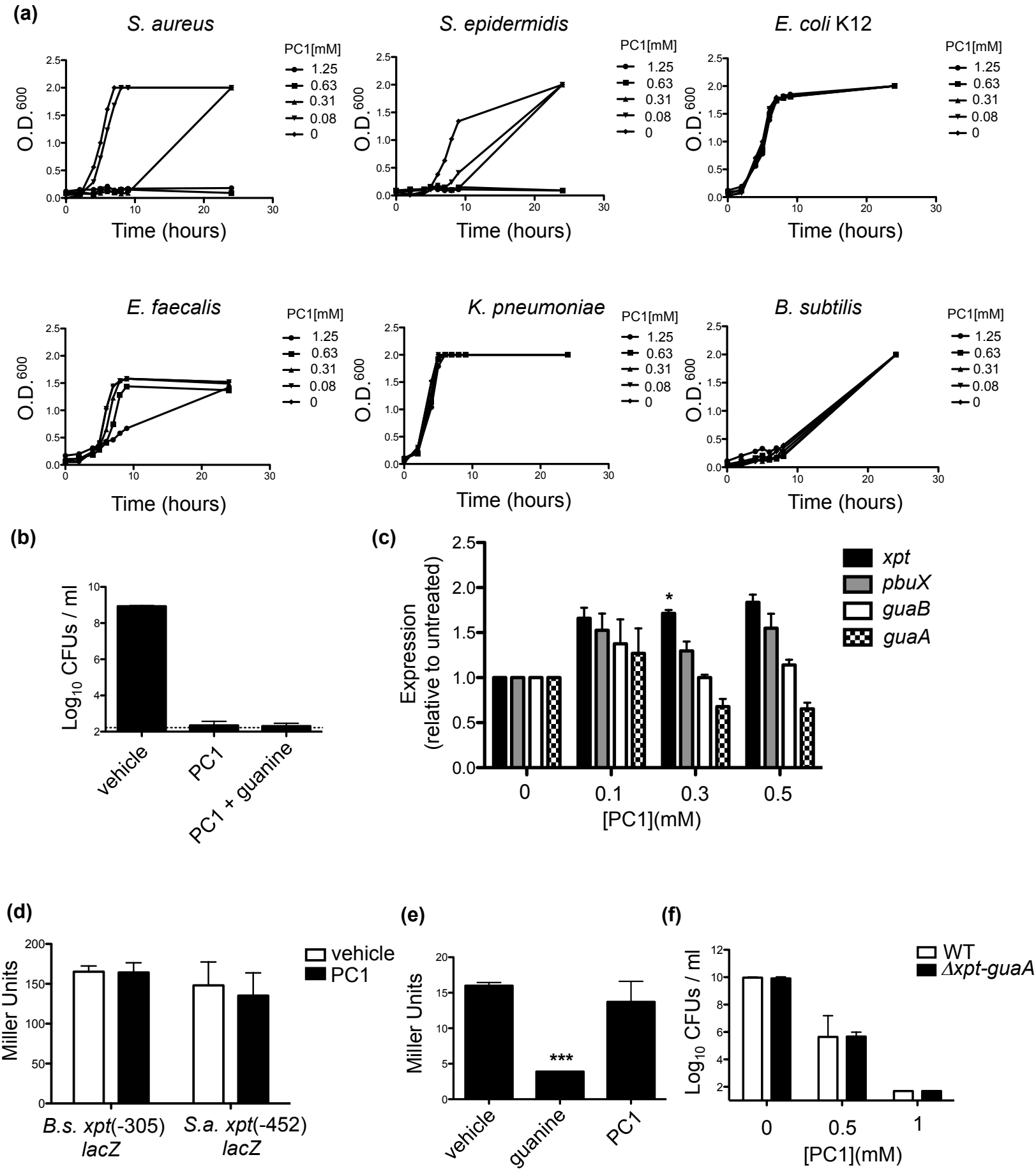
Supplementary Figure 4. Growth curves for *pbuX::Tn* grown in TSB and CDM. (a) Growth of WT and *pbuX::Tn* were monitored by optical density at 600nm over 20 hours in TSB. **(b)** Growth of WT and *pbuX::Tn* in CDM as in (a).

Supplementary Figure 5



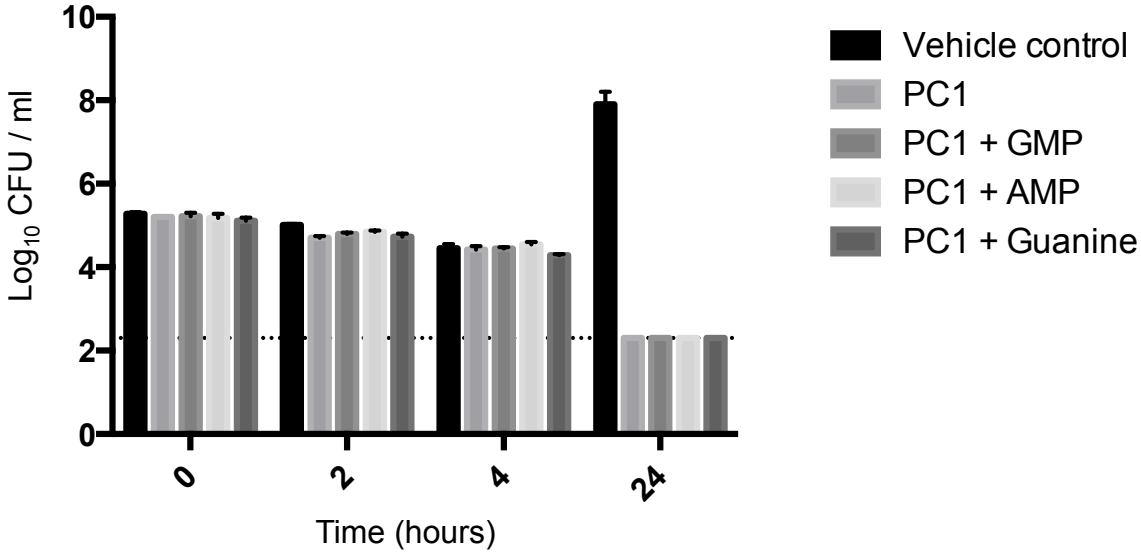
Supplementary Figure 5. Transmission electron microscopy of wild-type and *ΔguaB* input and after three days of guanine withdrawal. (a) TEM of WT input. **(b)** TEM of WT day 3. **(c)** TEM of Δ*guaB* input. **(d)** TEM of Δ*guaB* day 3. All fields are 6000x.

Supplementary Figure 6



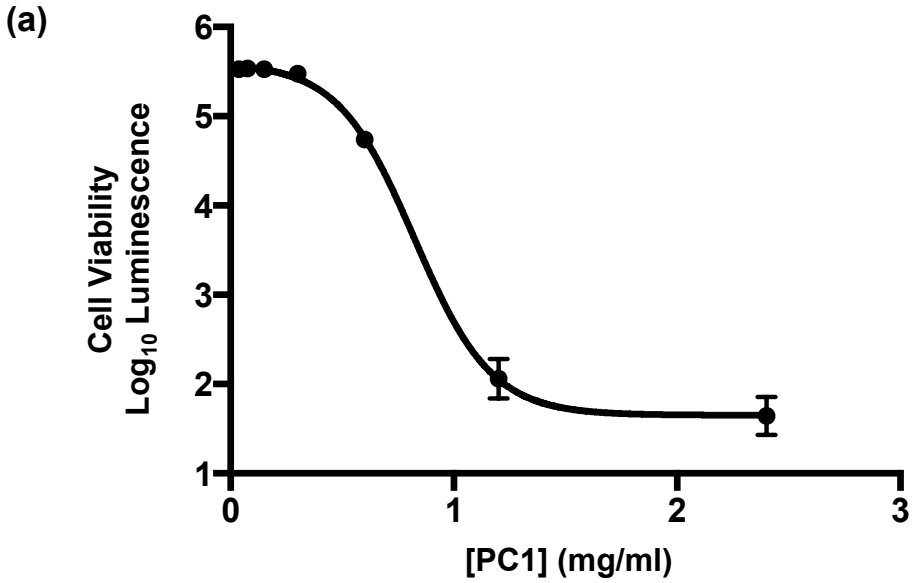
Supplementary Figure 6. Bactericidal guanine analog PC1 acts independent from the *xpt*-riboswitch and the guanine nucleotide biosynthetic pathway in *S. aureus*. (a) Growth of *S. aureus, S. epidermidis, E. coli, E. faecalis, B. subtilis*, and *K. pneumonia* was measured by reading optical density at 600nm over time for 24 hours of culture in TSB containing 0, 0.08, 0.31, 0.63, and 1.25 mM PC1. **(b)** WT *S. aureus* was grown in TSB containing vehicle (0.002M NaOH, 1mM DTT), 1mM PC1, or 1mM PC1 combined with 1mM guanine and CFUs enumerated 24 hours later. **(c)** RNA from log phase WT *S. aureus* grown in TSB alone, or supplemented with 0.1, 0.3, and 0.5 mM PC1 was isolated and TaqMan qPCR was performed to quantify transcripts of *xpt, pbuX, guaB,* and *guaA* relative to the untreated controls. **(d)** Transcriptional reporter activity was measured in *B. subtilis* for *B.s. xpt*(-305)*lacZ* and *S.a. xpt*(-452)*lacZ* grown to log phase in Bacillus media with or without 1mM PC1 by Miller assay. **(e)** *S. aureus* carrying the *S.a. xpt*(-452)*lacZ* reporter was grown to log phase in TSB containing vehicle, 1mM of guanine, or 1mM PC1 and assayed for reporter activity. **(f)** WT and Δ*xpt-guaA* were grown in Muller-Hinton broth containing 0, 0.5, and 1mM PC1 followed by enumeration of CFUs 24 hours later. All data are presented as mean +/- S.D. (n=3). Statistical analyses performed using Student's t-test; $p < 0.05$*, $p < 0.01$**, $p<0.001$***.

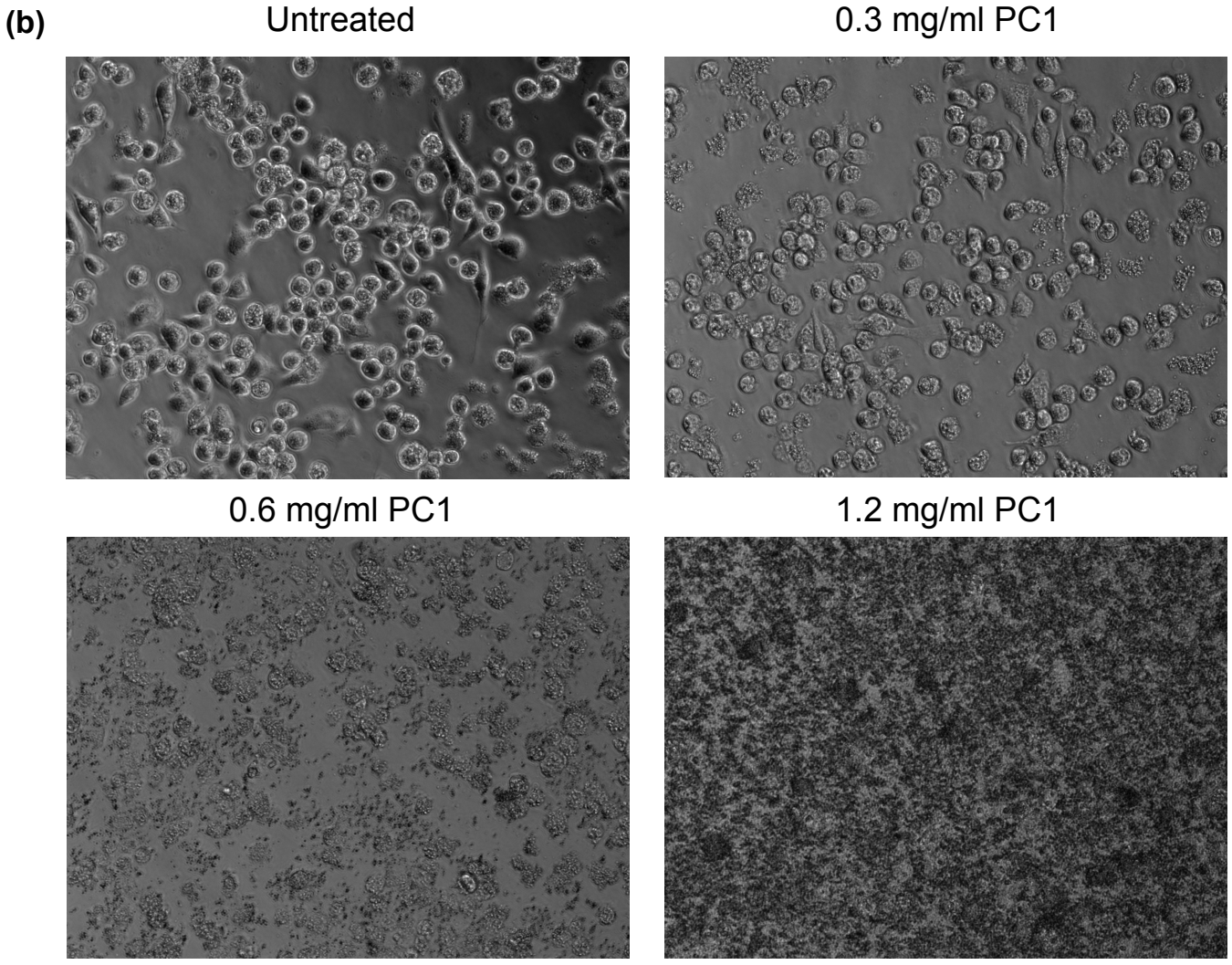
Supplementary Figure 7



Supplementary Figure 7. Guanosine 5'-monophosphate (GMP), adenosine 5'-monophosphate (AMP), and guanine fail to prevent death of *S. aureus* in response to PC1. *S. aureus* NRS384*ΔhsdRΔsauUSI* was grown to log phase in cation-adjusted Mueller-Hinton Broth II (MHBII) and diluted to 1x10^5 CFU/ml in fresh MHBII broth containing vehicle alone, 600μg/ml PC1, 600μg/ml PC1 + 100μM GMP, 600μg/ml PC1 + 100μM AMP, and 600μg/ml PC1 + 100μM guanine and CFU/ml was determined by serial dilution at 0, 2, 4, and 24 hours. MHBII was supplemented with 0.002%Triton X-100 and PC1 was solubilized using a vehicle (9.2mM NaOH + 450μM DTT) to prevent oxidative self-condensation (Muhlbacher et al., 2010 & Ster et al., 2013). Bacteria were incubated stationary at 37C in 1ml total volume in 96-well polypropylene blocks, and 20μl was removed for serial dilutions at each time-point. CFUs were enumerated by spotting 5μl of serial dilutions on MHBII / 5% sheep-blood agar plates in biological triplicate. The limit of detection in this experiment was 200 CFU/ml indicated by the dotted line.

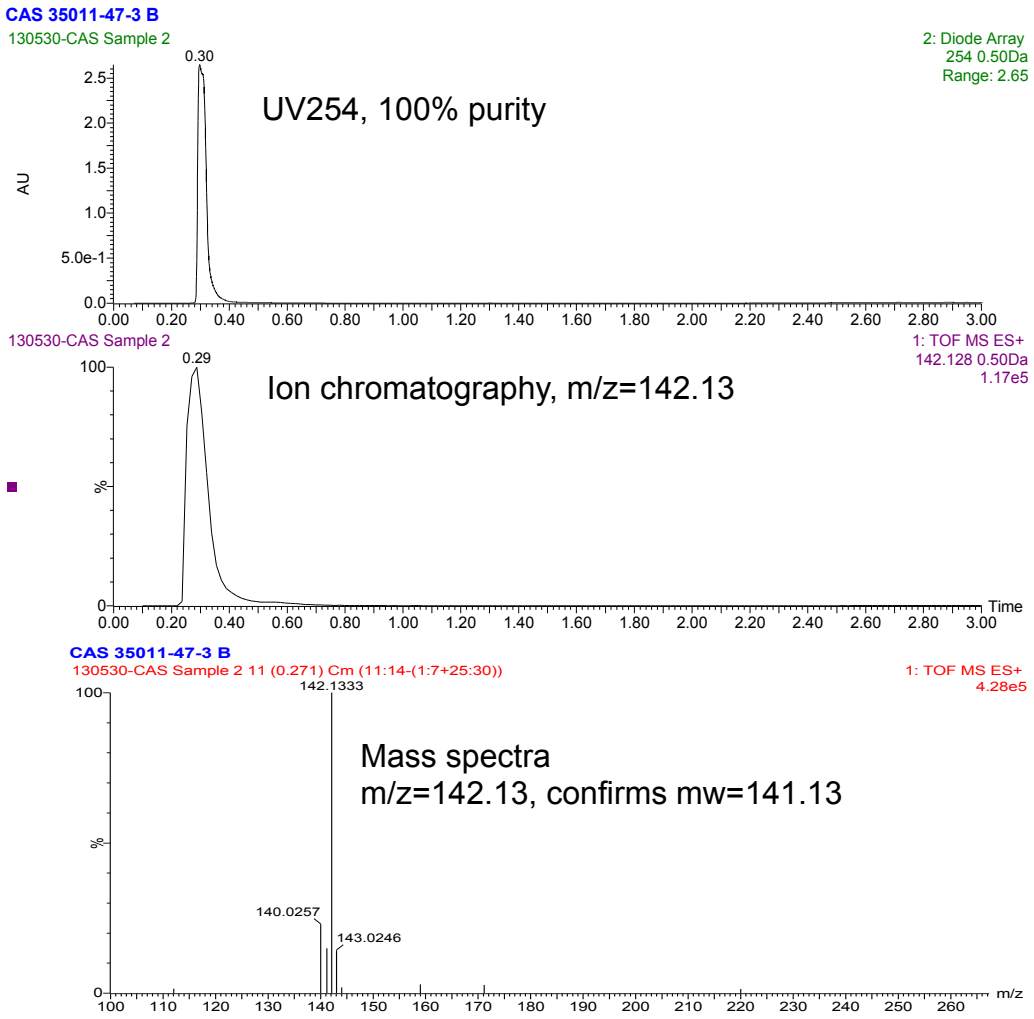
Supplementary Figure 8





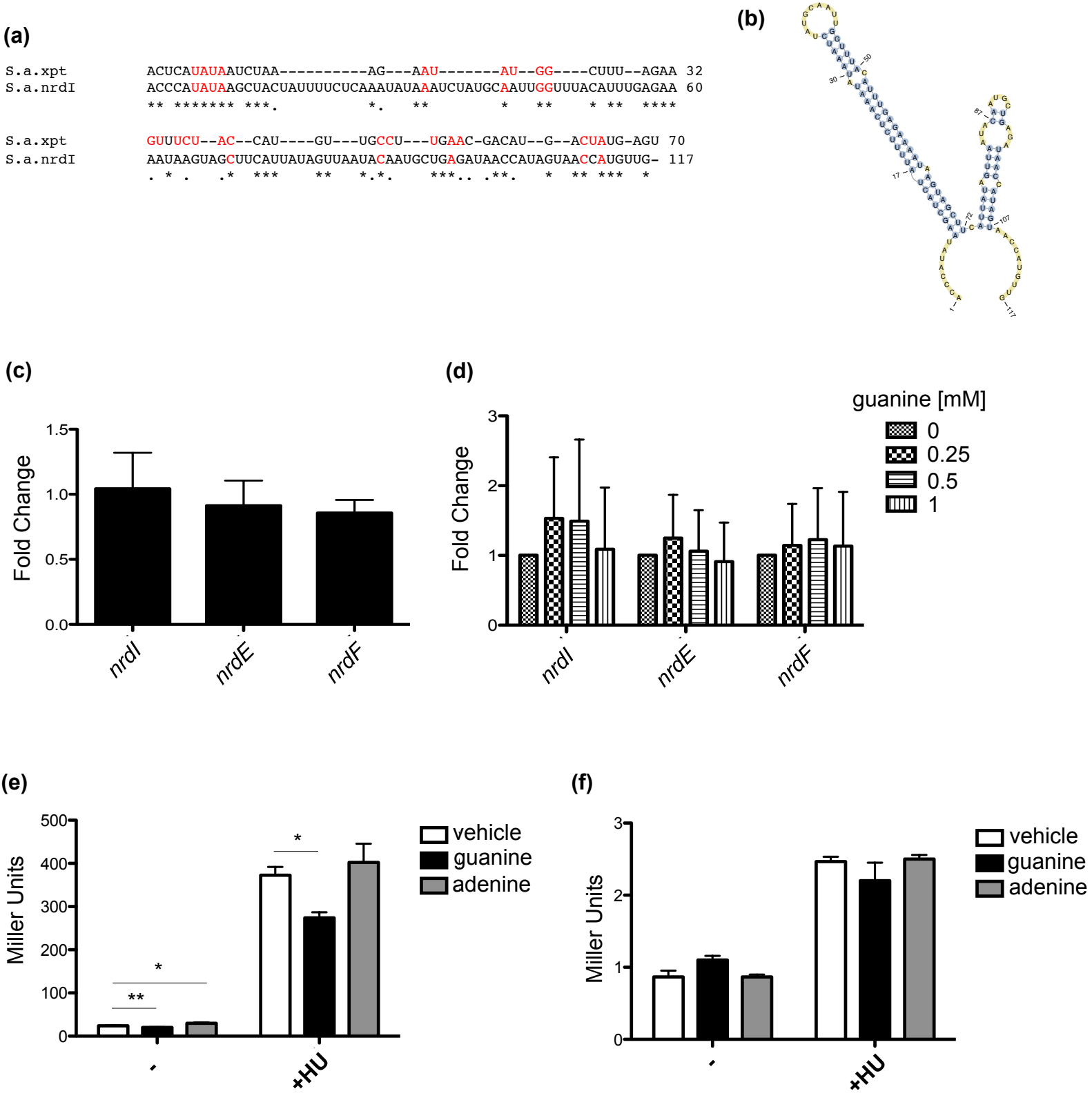
Supplementary Figure 8. Balb/c peritoneal macrophage viability in response to PC1. (a) Thioglycollate-elicited Balb/c peritoneal macrophages were treated with varying concentrations of PC1 and cell viability measured using CellTitre Glow (Promega) at 8 hours. **(b)** Differential interference contrast images of peritoneal macrophages treated with varying concentrations of PC1. Data in **(a)** are presented as mean +/- S.E.M of biological triplicates.

Supplementary Figure 9



Supplementary Figure 9. LCMS data of compound PC1 (CAS 35011-47-3). The purity was 100% by UV254nm at a retention time of 0.23min. The observed ion $(M+H)^+$=142.25 that confirmed the identity of the compound (calculated mw=141.13). LCMS system: Waters Acquity UPLC and LCT (Time of Flight) mass spectrometer. Mobile phase A: water+0.05%TFA; Mobile phase B: acetonitrile; LC gradient: 0-98%B over 3min; LC column: Waters Acquity UPLC BEH C18, 1.7um, 2.1×50mm; MS method: positive ESI with 100-800amu/sec scan. CAD-Charged Aerosol Detector. MS-Mass Spectrometry. (Sigma Cat. No. 17376; 4-Hydroxy-2,5,6-triaminopyrimidine sulfate salt; CAS 35011-47-3).

Supplementary Figure 10



Supplementary Figure 10. 5'-UTR of the *S. aureus nrdI-nrdE-nrdF* operon does not contain a guanine riboswitch. (a) Pairwise sequence alignment of the minimal aptamer domain of *S. aureus xpt*-riboswitch and the putative *S. aureus nrd*-riboswitch predicted computationally (12) to be located upstream of *nrdI* in *S. aureus* N315 highlighting (in red) conserved nucleobases that define the guanine-binding riboswitch (4). **(b)** Predicted secondary structure of the putative *nrd*-riboswitch aptamer of *S. aureus* deviates from the characteristic three-junction stem-loop structure of the purine riboswitch (56). **(c)** RNA from WT *S. aureus* grown overnight in CDM alone or with 1mM exogenous guanine was isolated and TaqMan qPCR performed to quantify the transcripts of *nrdI*, *nrdE*, and *nrdF* compared to untreated controls. **(d)** RNA from WT *S. aureus* grown to log phase in TSB containing 0, 0.25, 0.5, and 1 mM exogenous guanine was isolated and TaqMan qPCR performed to quantify transcripts of *nrdI*, *nrdE*, and *nrdF* compared to untreated controls. **(e)** *B. subtilis* carrying a *S. aureus nrd*(-218)*lacZ* reporter was grown in Bacillus media to log phase in the presence of vehicle or 1mM exogenous guanine or adenine, with or without 50mM hydroxyurea and reporter activity was analyzed by Miller assay. **(f)** *S. aureus* carrying the *S. aureus nrd*(-218)*lacZ* reporter was grown in CDM as described in **(e)**. All data are presented as mean +/- S.D. (n=3). Statistical analyses performed using Student's t-test; $p < 0.05$*, $p < 0.01$**, $p<0.001$***.

Supplementary Table 1. Primers and plasmids used in this study.

Plasmids & Primers used in this study	Purpose	Source
pIMAY	Allelic replacement in *S. aureus* NRS384*ΔhsdRΔsauUSI*	(Monk et al., 2012)
pIMC85	Stable integration into the into Φ85 att site of *S. aureus* NRS384*ΔhsdRΔsauUSI*	This work
pCR2.1-TOPO	Linearized vector with single T overhangs for TA cloning 5'-RACE products	Invitrogen
pDG1661	Stable integration of *lacZ* transcriptional fusions into *amyE* locus of *B. subtilis*	Bacillus Genetics Stock Center

Quantitative Real-time PCR primers	Purpose	Source
TGATGTGCCATGTTTATTTGCGAAA	Forward TaqMan qPCR for *xpt*	This work*
CAATGACCGTACTTGTTTTATTTTTAGTAAATGAATG	Reverse TaqMan qPCR for *xpt*	This work*
FAM-CCATCCGTCAAAGTGC-BHQ1	Probe TaqMan qPCR for *xpt*	This work*
GGCTTACTTGGGATGGTCGATATTA	Forward TaqMan qPCR for *pbuX*	This work*
CGGCACTGGGATGCCTAA	Reverse TaqMan qPCR for *pbuX*	This work*
FAM-CCAACCGGCATGATTG-BHQ1	Probe TaqMan qPCR for *pbuX*	This work*
AGCAAAAGATGAACATGGTCGTCTA	Forward TaqMan qPCR for *guaB*	This work*
GCTTCGACTAATTTTTGAGCACGAATA	Reverse TaqMan qPCR for *guaB*	This work*
FAM-TAGCCGCAGCAATTG-BHQ1	Probe TaqMan qPCR for *guaB*	This work*
TGAAGGCGACATGGTTATGGA	Forward TaqMan qPCR for *guaA*	This work*
GCGATCTTTCGCATTAACACGAATA	Reverse TaqMan qPCR for *guaA*	This work*
FAM-CCTTCACCGAATTGC-BHQ1	Probe TaqMan qPCR for *guaA*	This work*
GCCCCTTAGTGCTGCAGCTA	Forward TaqMan qPCR for *rrsA*	This work*
AGTTTCAACCTTGCGGTCGTA	Reverse TaqMan qPCR for *rrsA*	This work*
FAM-CGCATTAAGCACTCCGCCTGGG-BHQ1	Probe TaqMan qPCR for *rrsA*	This work*

Allelic Replacement Primers	Purpose	Source
ATATGGTACCTTTAAAAATTTGTTTATTTAATC	*Δxpt-pbuX-guaB-guaA* A / KpnI	This work
TCTTAAAACCTCCTCAGATTTGTGTGAAAC	*Δxpt-pbuX-guaB-guaA* B / KpnI	This work
GTTTCACACAAATCTGAGGAGGTTTTAAGATTATATATAATAAA ACCACCTGTTTGCACG	*Δxpt-pbuX-guaB-guaA* C / KpnI	This work
ATATGAATTCACACACTCGTTCAATAGGCAACCTAAAACA	*Δ*xpt-pbuX-guaB-guaA D / KpnI	This work
TACAGTAATATCTGCCATAGTTGTCGCCCC	*Δxpt-pbuX-guaB-guaA* OUT F / KpnI	This work
AAATAATTCCGATTACACAAAAGGGACGAT	*Δxpt-pbuX-guaB-guaA* OUT R / KpnI	This work
TACATGTCAAGAATAAACTGCCAAAGC	pIMAY MCS forward sequencing primer	(Monk et al., 2012)
AATACCTGTGACGGAAGATCACTTCG	pIMAY MCS reverse sequencing primer	(Monk et al., 2012)
GTTTCACACAAATCTGAGGAGGTTTTAAGATACGCAGGTGCTA TCTTAGTTCCAATCATT	*Δxpt-pbuX* C	This work
ATATGAATTCCCAAGTAAGCCAGCACCTATCGTTCCTAA	*Δxpt-pbuX* D / EcoRI	This work
TCTAAACGGCACTGGGATGCCTAACCAACC	*Δxpt-pbuX* OUT R	This work
GTAAATTTGCAAAAGAATCATAGTTAACGTTTGATGATGTGTT	B1xSTOP F (stop codon engineered in complementation vector)	This work
AACACATCATCAAACGTTAACTATGATTCTTTTGCAAATTTAC	B1xSTOP R (stop codon engineered in complementation vector)	This work
CAAAAGAACAAGAGTTAATCTAGCTTGTCTTAGACTTTGGTAG	A1xSTOP F (stop codon engineered in complementation vector)	This work
CTACCAAAGTCTAAGACAAGCTAGATTAACTCTTGTTCTTTTG	A1xSTOP R (stop codon engineered in complementation vector)	This work

Supplementary Table 1 (continued)

Transcriptional Reporter Primers	Purpose	Source
AAAAGAATTCAACCCTTACATTATTAAGTT	Forward *S. aureus xpt*-5'-UTR riboswitch reporter for *lacZ* fusion in pDG1661/EcoRI	This work
AAAAGGATCCTAGTAACTCCACTCTTAAAAC	Reverse *S. aureus xpt*-5'-UTR riboswitch reporter for *lacZ* fusion in pDG1661/BamHI	This work
AATAGAATTCAGACTCTTTTATATCGA	Forward *B. subtilis xpt*-5'-UTR riboswitch reporter for *lacZ* fusion in pDG1661/EcoRI	This work
TTTTGGATCCTTTCAGTGCTTCCATCCTGTC	Reverse *B. subtilis xpt*-5'-UTR riboswitch reporter for *lacZ* fusion in pDG1661/BamHI	This work
AAAAGAATTCTAGGACAAAAAGTAAAGGAAGACGGCGTTG	*S. aureus* reporter *pbuX* (-568)F /EcoRI	This work
AAAAGGATCCTAAATTTTTCATTATTCTTCTCCCACCAAT	*S. aureus* reporter *pbuX* (-568)R / BamHI	This work
AAAAGAATTCTCGCAAAATGTAGGACTTGTTTCTTTATCC	*S. aureus* reporter *guaB* (-454)F / EcoRI	This work
AAAAGGATCCTCTTTCGGTAAAATATCAGATTGTGCTGGA	*S. aureus* reporter *guaB* (-454)R / BamHI	This work
AAAAGAATTCATGGCTCGTCAAGGTGGTTTAGGTGTTATT	*S. aureus* reporter *guaA* (-1305)F / EcoRI	This work
AAAAGAATTCGGTGTGGATGTCTTAGTTATCGATACAGCA	*S. aureus* reporter *guaA* (-764)F / BamHI	This work
AAAAGAATTCGGTTATGTTAGGTAGCTTATTAGCAGGTAC	*S. aureus* reporter *guaA* (-414)F / EcoRI	This work
AAAAGGATCCTCCATATTTGTCGTTCTCCTTTATCTTAAT	S. aureus reporter *guaA* (-X)R / BamHI	This work
AAAAGAATTCAATTCAGTATCAAGCTAA	*S. aureus* reporter *nrd* (-218)F/ EcoRI	This work
AAAAGGATCCTATTATTTTCATTGGATC	*S. aureus* reporter *nrd* (-218)R / BamHI	This work

lacZ sequencing Primers	Purpose	Source
CGTCTGAATTTGACCTGAGC	*lacZ* sequence verification 1	This work
GAAAATGGTCTGCTGCTGCTGAAC	*lacZ* sequence verification 2	This work
CCGATATTATTTGCCCGATG	*lacZ* sequence verification 3	This work
ATGTCGCTCCACAAGGTAAACAG	*lacZ* sequence verification 4	This work
ATGCGGTGCTGATTACGACC	*lacZ* sequence verification 5	This work

Northern blot probes	Purpose	Source
TACTGAAGAAAGCCCAGGCG	PCR amplification of *guaB* for radioactive probe (F)	This work
AAACCAGCAGGACCCATACG	PCR amplification of *guaB* for radioactive probe (R)	This work
ACGCGATTTTACGCCAAGTG	PCR amplification of *guaA* for radioactive probe (F)	This work
TAGACTACGCGGTTGACGTG	PCR amplification of *guaA* for radioactive probe (R)	This work
TCAACCGTGGAGGGTCATTG	PCR amplification of *rrsA* for radioactive probe (F)	This work
TGCACCACCTGTCACTTTGT	PCR amplification of *rrsA* for radioactive probe (R)	This work

Supplementary Table 1 (continued)

Riboswitch aptamer and *in-vitro* transcription primers	Purpose	Source
AAAAGCGGCCGCTAATACGACTCACTATAGGGCGAAGCAGT TATTGAAAAAATGCCG	*S. aureus xpt*-ribswitch aptamer with T7 TSS & cloning into pIMC85/NotI	This work
TATAGGATCCCACTCTTAAAACCTCCTCAGATTTGTGTGAAAC	*S. aureus xpt*-riboswitch aptamer with T7 TSS & cloning into pIMC85/BamHI	This work
AAAAGCGGCCGCTAATACGACTCACTATAGGGCGAATATAA TAGGAACACTC	*B. subtilis xpt*-riboswitch aptamer with T7 TSS & cloning into pIMC85/NotI	This work
TATAGGATCCCATCCTGTCTACCTCCGTTATGAGAATAA	*B. subtilis xpt*-riboswitch aptamer with T7 TSS & cloning into pIMC85/BamHI	This work
TAATACGACTCACTATAGGGCGAATAATTTACATAAACTC	*S. aureus* 93 xpt aptamer (F)	This work
GTTGTTACTCATAGTCATGTC	*S. aureus* 93 xpt aptamer (R)	This work
TAATACGACTCACTATAGGGCGAATATAATAGGAACACTC	*B. subtilis* 93 xpt aptamer (F)	This work
GTTCCATTGCTCACCCATAG	*B. subtilis* 93 xpt aptamer (R)	This work

5'-RACE primers	Purpose	Source
CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTT TTTTTTTTTT	QT	Invitrogen
CCAGTGAGCAGAGTGACG	Q0	Invitrogen
GAGGACTCGAGCTCAAGC	QI	Invitrogen
TTCTGCTTCATAAACGCTTT	Gene specific primer 1 (*guaB*)	This work
CTTCTGGCGTTAAGAAAAAT	Gene specific primer 2 (*guaB*)	This work
CGCTTGTTCTTCAACGCCCA	Gene specific primer 3 (*guaB*)	This work

*TaqMan Primer/Probe sets engineered by Life Technologies™ Custom Design
(F) and (R) designate forward and reverse primers respectively

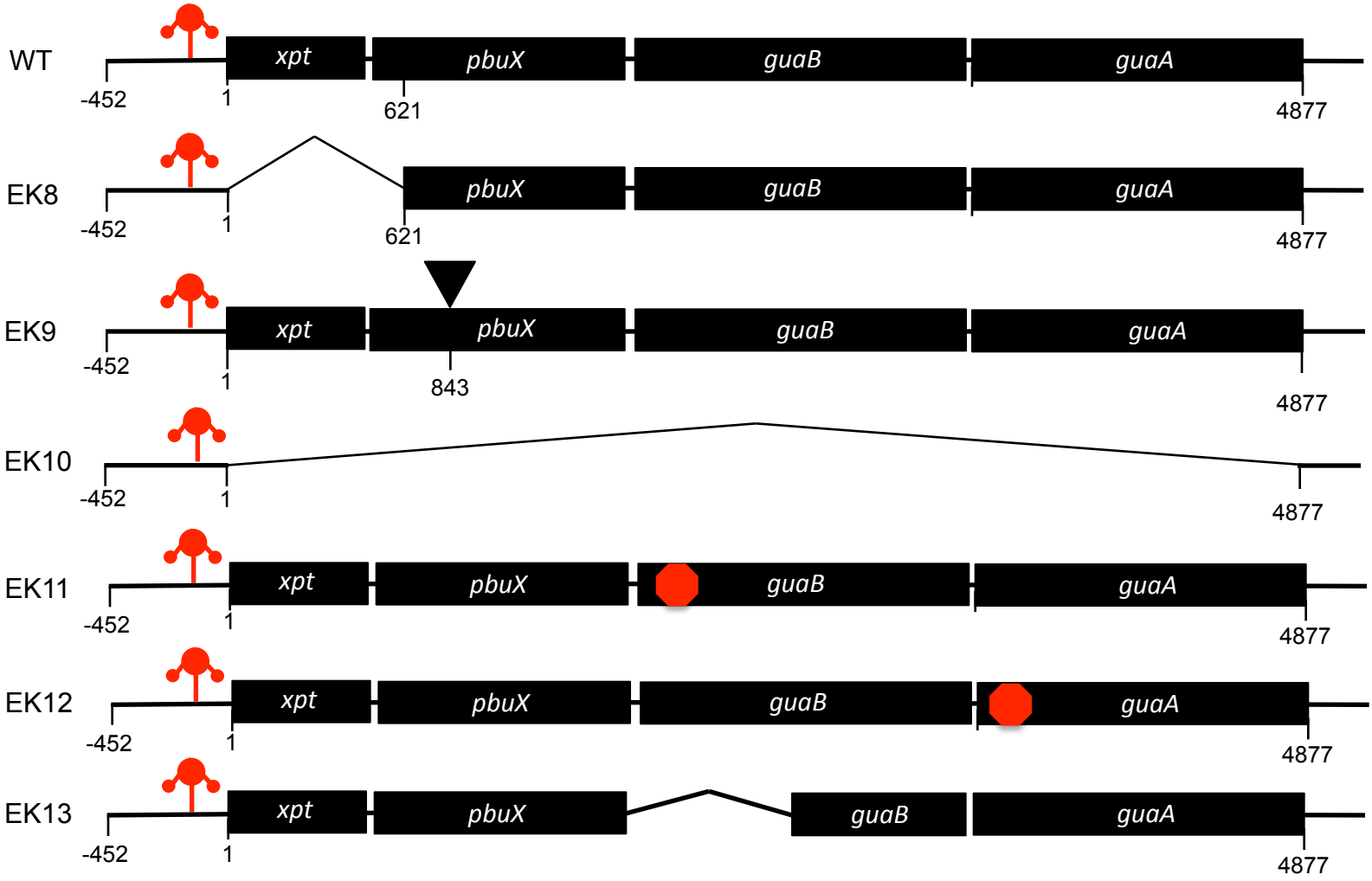
Supplementary Table 1 (continued)

pIMC85 construction primers	Name / Purpose	Source
ATAT*AGATCT*AT*GCATGC*CTTTGGCAGTTTATTCTTGACATGT AGTGAGGGGGCTGGTATAATCACATAAGGAGGATATATATG AACAAAAATATAAAATATTCTC	IM202 Pcp25-*ermB* F to amplify *ermB* (BglII/SphI)	This work
*CTGCAG*AT*CCATGG*ATATCCTCCTTTATTTCCTCCCGTTAAAT AATAG	IM203 *ermB* R to amplify *ermB* (PstI/NcoI)	This work
AGGAGGATAT*CCATGG*AT*CTGCAG*CGATTTTTTATTAAAACG TCTCAAAATCG	IM204 *repB* F to amplify *repB* (RBS/PstI/NcoI)	This work
ATAT*GAATTCAAGCTT*AATAGTCAAAAGCCTCCGGTCGGAGG CTTTTGACTTTATGCTTTCGATTCTGAAATCACCATTT	*repB* R to amplify *repB* (EcoRI/HindIII)	This work
ATAT*CCATGG*AAGTAGCAATTTATACTAGAGTGAG	IM287 *int* 85 F to amplify *int* (NcoI)	This work
ATAT*CTGCAG*TTAATAAAACTCTATACCCGTAATC	IM288 *int* 85 R to amplify *int* (PstI)	This work
ATAT*AGATCT*TCGTTGAGTAAAGACATAGACTTAGC	312 attP 85 F to amplify *attP* (BglII)	This work
ATAT*GCATGC*TAATTGGAAGTTCGGAATAACTATGC	313 attP 85 R to amplify *attP* (SphI)	This work
ATAT*GCATGC*TATGCTTTGGCAGTTTATTCTTGAC	291 Phelp-cat AF to amplify *cat* (SphI)	This work
CCA**A**GGAATAATAGAAAGAGAAAAAGC	308 cat BR to amplify *cat*	This work
TTTCTCTTTCTATTATTCC**T**TGGACTTCATTTACTGGGTTTAAC	309 cat	This work
ATAT*CCATGG*ATATCCTCCTTTATAAAAGCCAGTCATTAGG	292 cat DR (NcoI/RBS)	This work
ACTGTGGTCGAATACAAATTATCACACC	289 85 Con F	This work
ATTAATGGCATTATTAGCTTTAAGTAG	290 85 Con R	This work

Underlined: Gram positive ribosome binding site. Underlined/Itallics: Restriction site. Bold: NcoI point mutation.

Supplementary Table 2. Schematic of strain-specific modifications of the *xpt-guaA* operon in *S. aureus*.

(a) Wild-type, clean-deletion, and knockout strains in *S. aureus*



(b) Transcriptional reporter constructs (integrated in *S. aureus*)