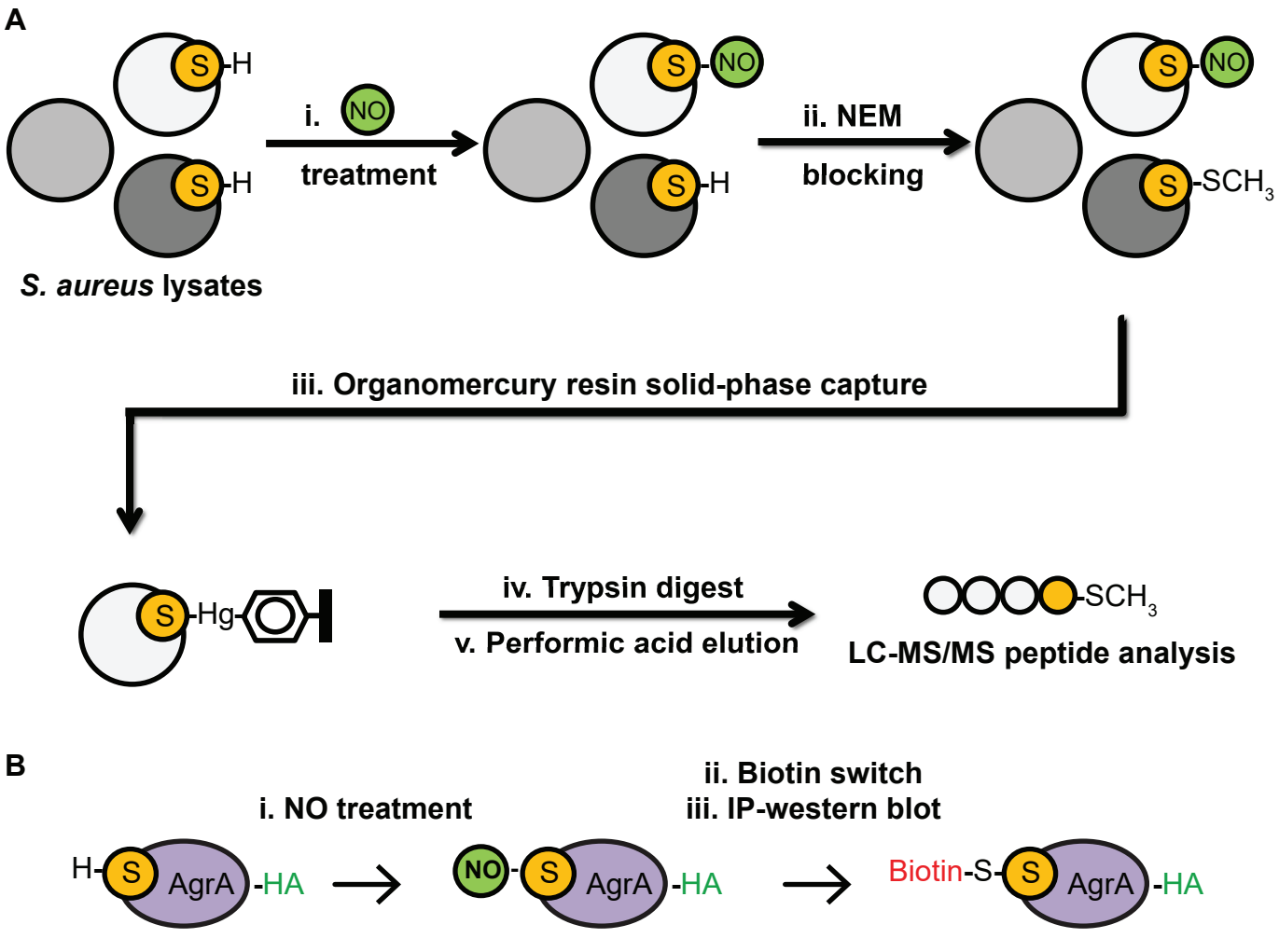


Figure S1 Related to Figure 1. Biochemical Methods to Detect *S*-nitrosylation in *S. aureus* Lysates

(A) Experimental strategy to identify *S*-nitrosylated proteins following NO treatment of bacterial lysates by mercury resin-assisted capture of *S*-nitrosopeptides and mass spectrometry-based identification. NEM, N-ethylmaleimide.

(B) Biotin-switch assay of NO-treated bacterial lysates containing hemagglutinin-tagged AgrA with biotinylation of *S*-nitrosylated sulfhydryl groups and detection by immunoprecipitation-western blot. HA, hemagglutinin; IP, immunoprecipitation.

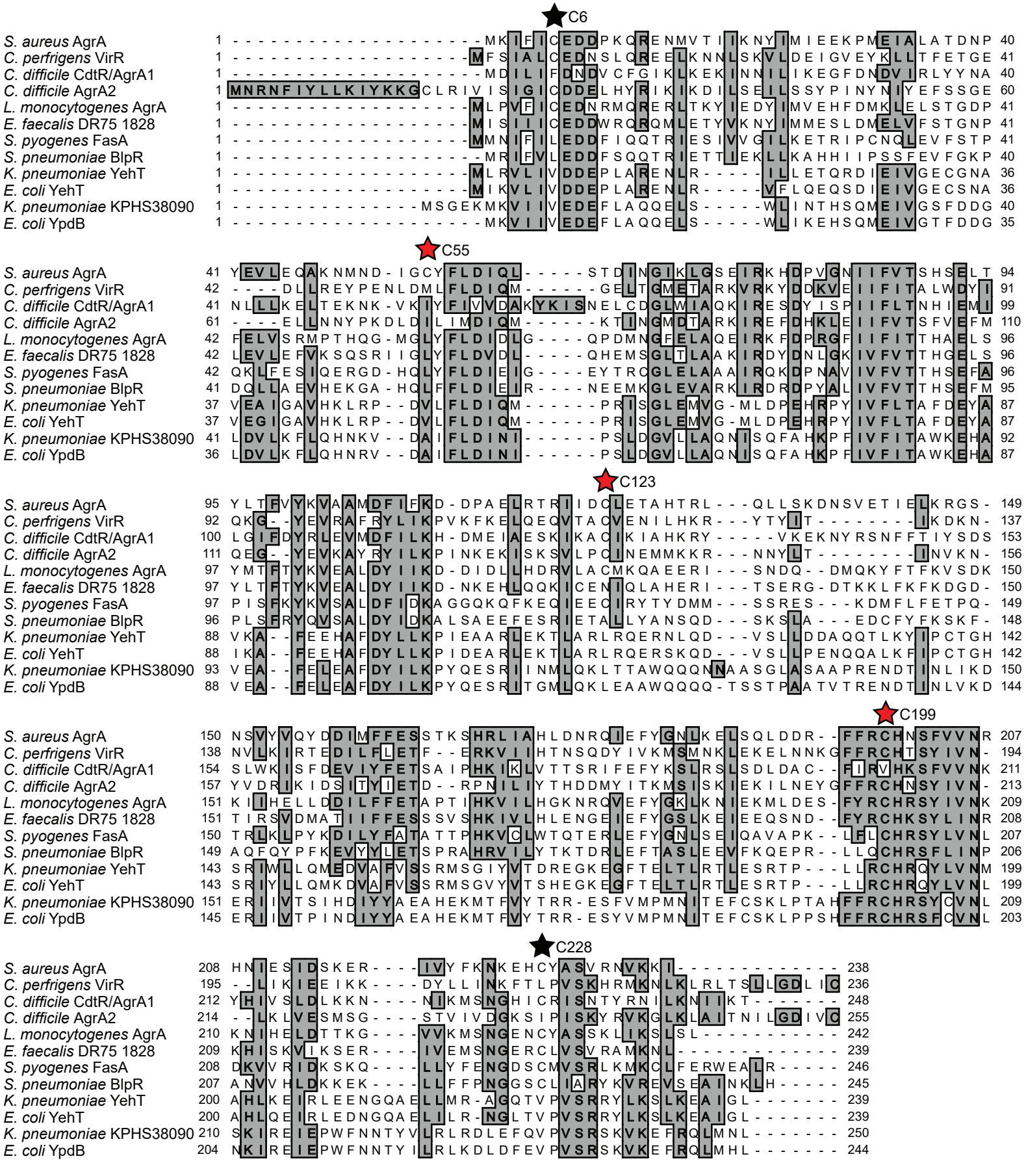

C6
C55
C123
C199
C228
S. aureus AgrA
C. perfrigens VirR
C. difficile CdtR/AgrA1
C. difficile AgrA2
L. monocytogenes AgrA
E. faecalis DR75 1828
S. pyogenes FasA
S. pneumoniae BlpR
K. pneumoniae YehT
E. coli YehT
K. pneumoniae KPHS38090
E. coli YpdB

Figure S2 Related to Figure 3. Amino Acid Sequence Alignment of AgrA with LytTR Transcription Regulators

Amino acid similarities and identities are shaded gray; stars show the position of cysteine residues also present in *S. aureus* AgrA; red stars indicate cysteines shown to mediate NO inhibition in this study.

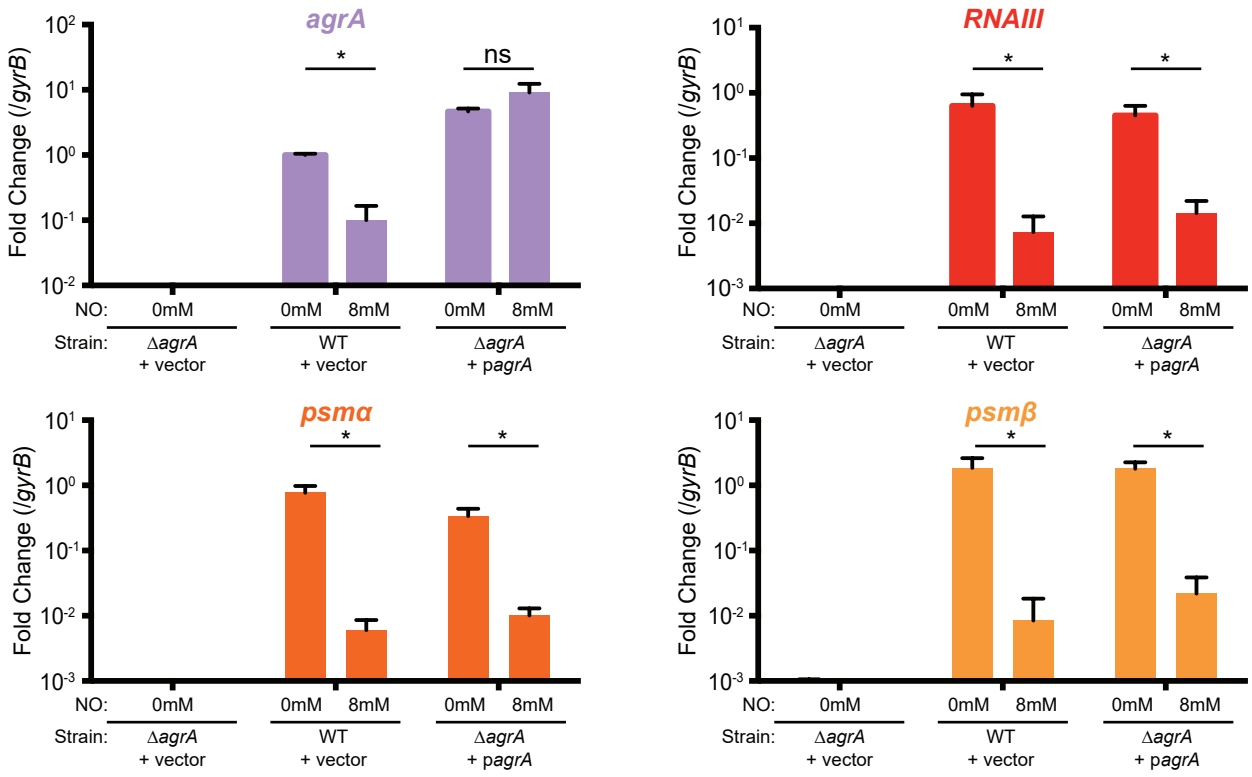
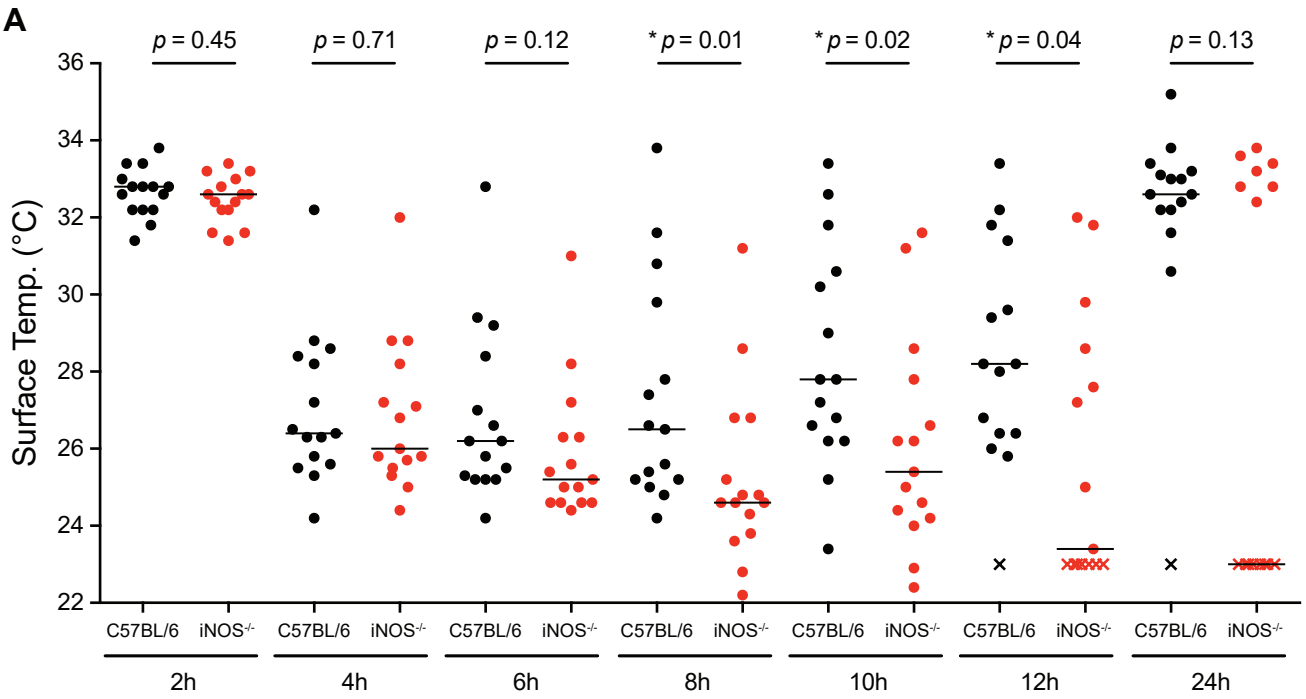


Figure S3 Related to Figure 4. Nitric Oxide-Sensitivity of AgrA-Activitated Genes in *S. aureus* with Constitutive Expression of AgrA from a Non-Native Promoter

RT-qPCR of AgrA-regulated genes after NO-treatment of *S. aureus* Δ*agrA* p*agrA* cultures expressing AgrA from the T5X non-native promoter (n=3). Data are represented as means with error bars showing standard deviation.

Student's t test, *p<0.05; ns, not significant.


A
p = 0.45
p = 0.71
p = 0.12
*p = 0.01
*p = 0.02
*p = 0.04
p = 0.13
Surface Temp. (°C)
36
34
32
30
28
26
24
22
C57BL/6
iNOS-/-
2h
4h
6h
8h
10h
12h
24h

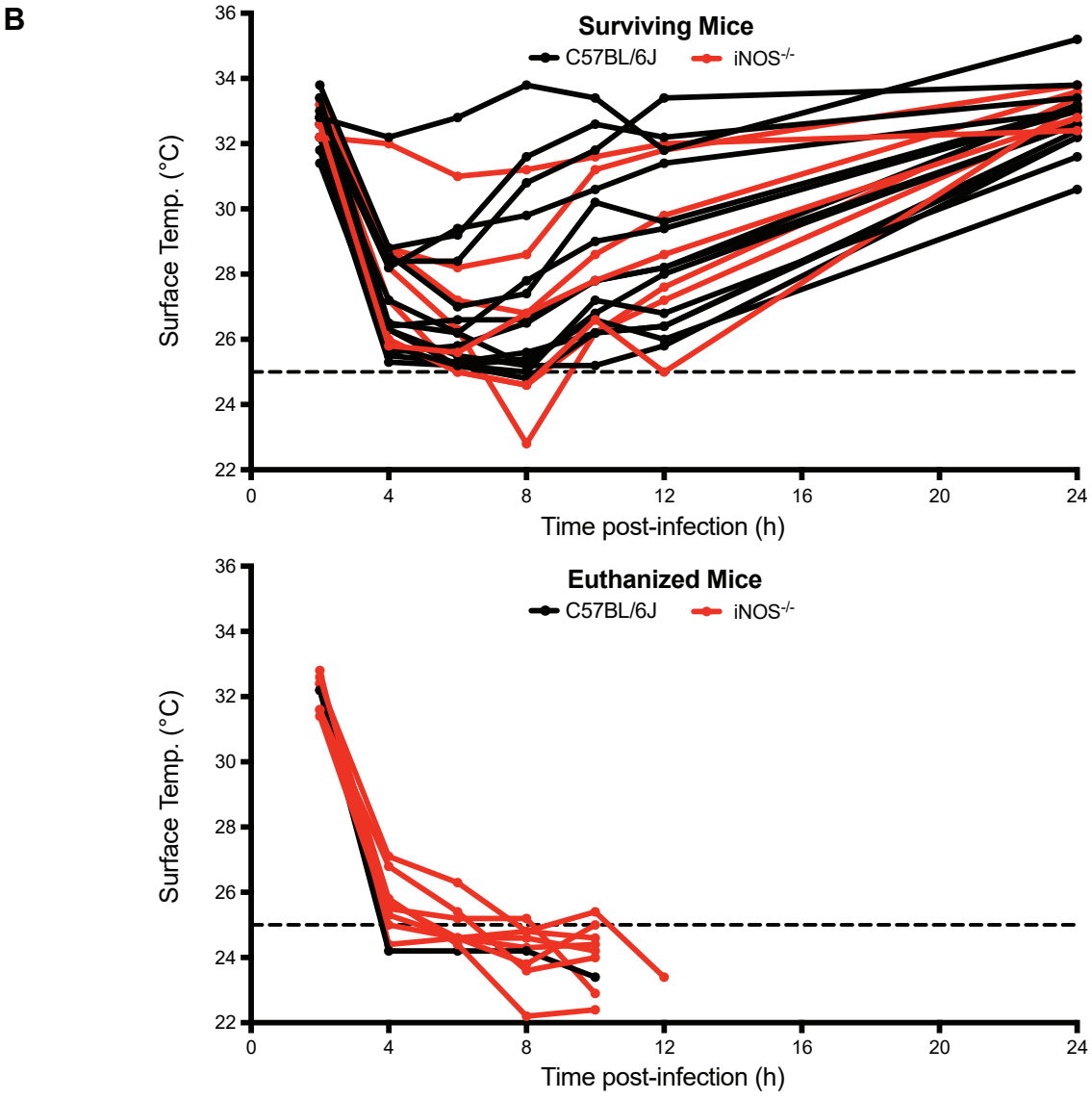

B
Surviving Mice
C57BL/6J
iNOS-/-
Surface Temp. (°C)
Time post-infection (h)
Euthanized Mice
C57BL/6J
iNOS-/-
Surface Temp. (°C)
Time post-infection (h)

Figure S4 Related to Figure 5A. Body Surface Temperature of Mice Infected with *S. aureus*

(A) Surface temperature of C57BL/6 (black circles, n=15) and congenic iNOS$^{-/-}$ (red circles, n=15) mice at indicated time points post-infection with *S. aureus* Newman. Individual values are plotted with horizontal lines showing medians of groups. X= mice euthanized due to a moribund state; Mann-Whitney U test, *$p<0.05$.

(B) Measurements of body surface temperature in surviving (top) and moribund (bottom) mice (C57BL/6, black lines, n=15; iNOS$^{-/-}$, red lines, n=15) at indicated time points post-infection. Dashed line at y=25˚C marks one criterion used for euthanasia. Other euthanasia criteria included low mobility, labored breathing and a moribund state.

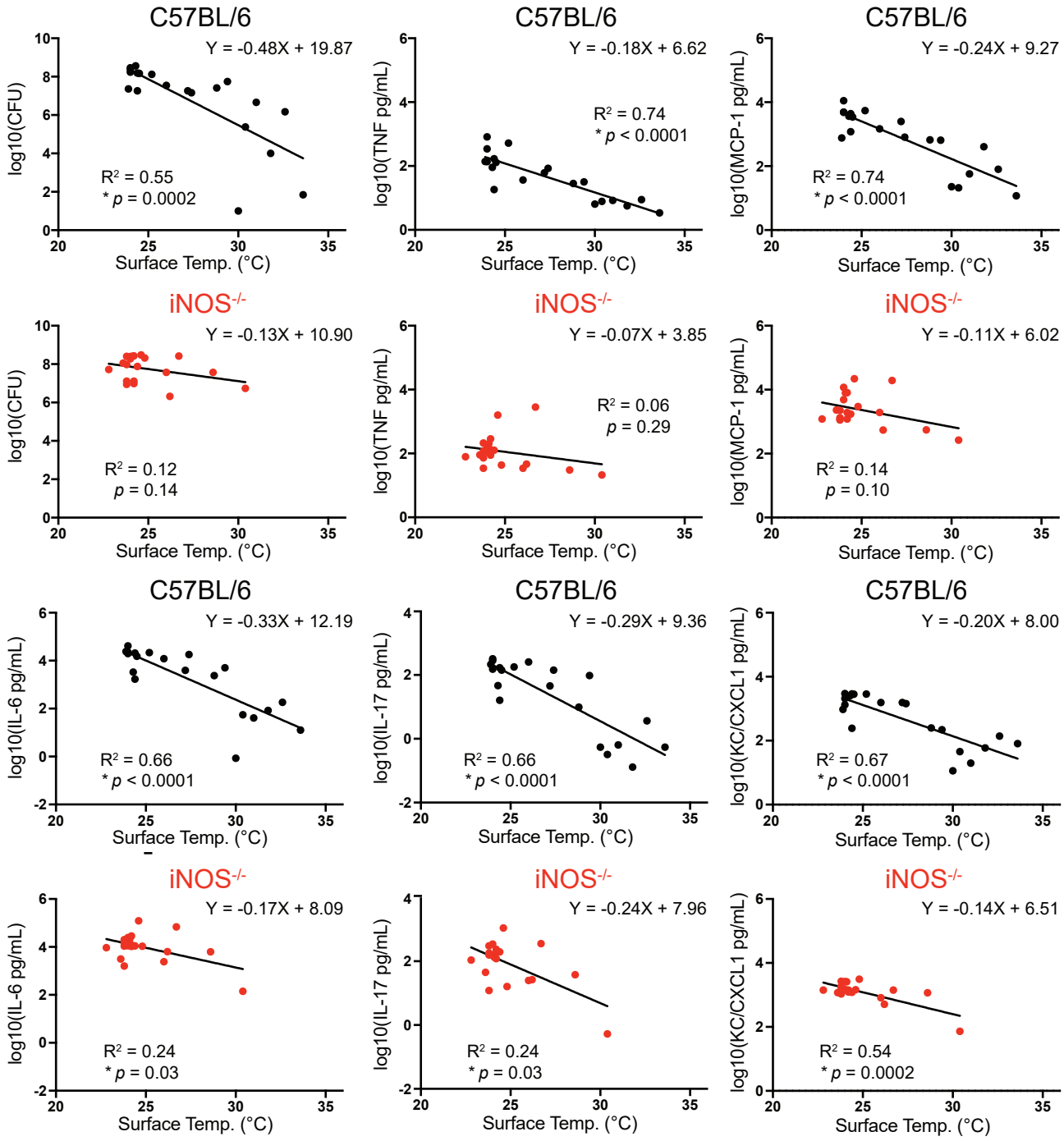


Figure S5 Related to Figure 5B. Linear Regression Analysis of Body Surface Temperature Against Bacterial Burden and Serum Cytokines

Relationship of mouse body surface temperature with log10 transformed bacterial burden (CFU/Lung) and cytokine serum levels (pg/mL) in C57BL/6 (black) and congenic iNOS knockout mice (red).

R^2 indicates goodness of fit; *p<0.05 indicates slope is different from zero, n=20.

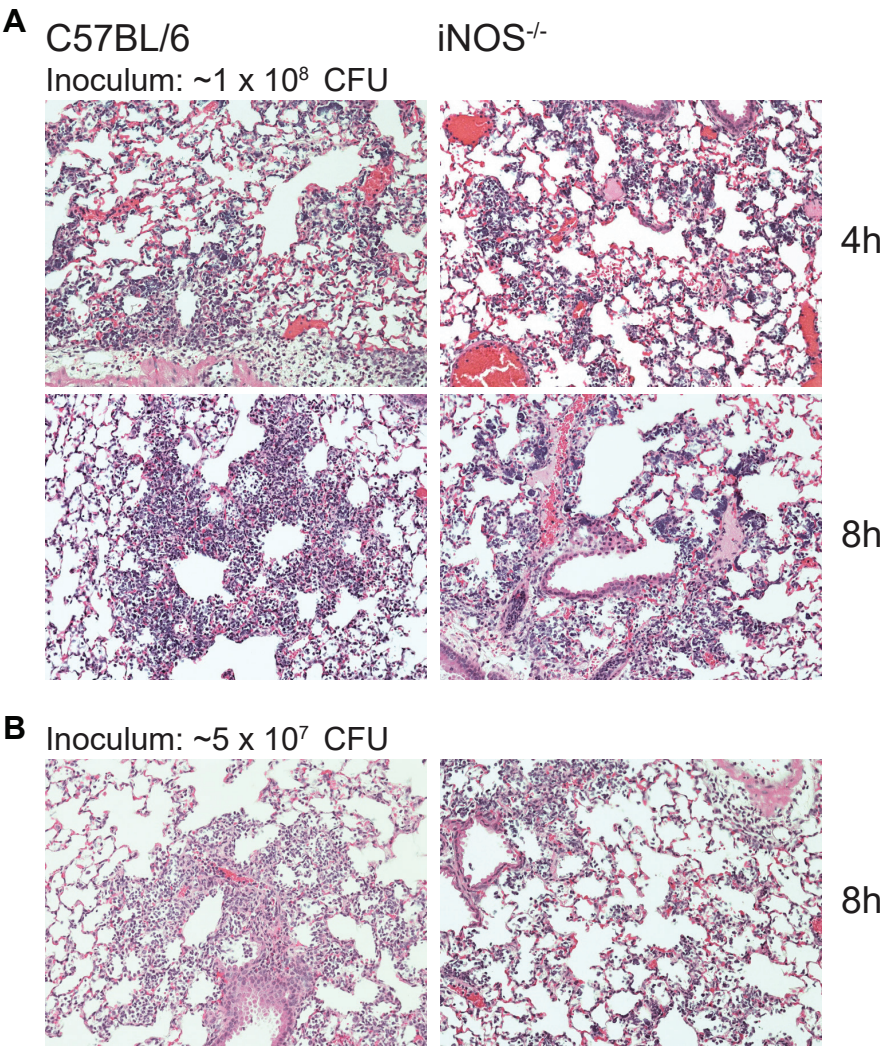
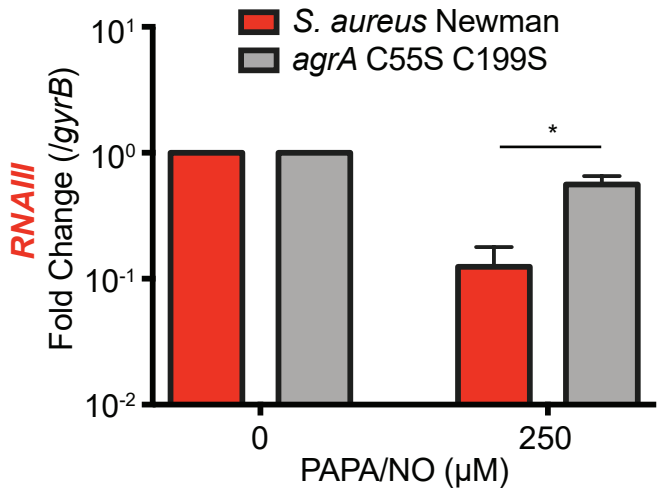


Figure S6 Related to Figure 6. Deficiency in Host Nitrc Oxide Production Alters the Histopathological Pattern of Staphylococcal Pneumonia

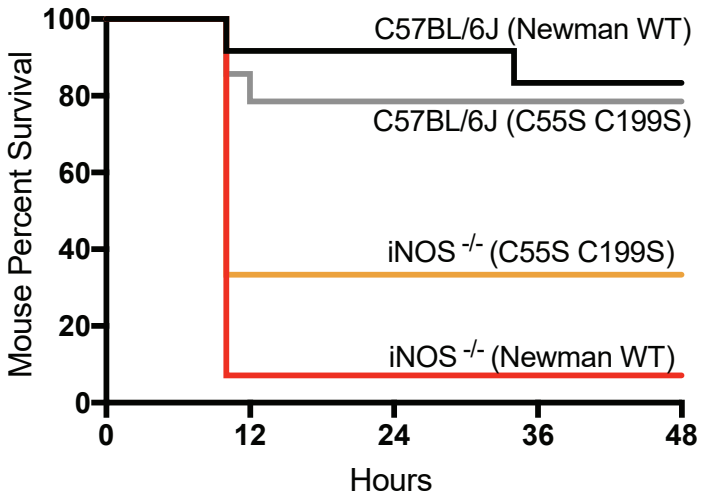
(A) Representative H&E stains of lung sections at 20x magnification from C57BL/6 and iNOS knockout female mice infected with ~1 x 10^8 CFU at 4 h (top panels) and 8 h (bottom panels) post-infection.

(B) Representative H&E stains of lung sections at 20x magnification from C57BL/6 and iNOS knockout female mice infected with ~5 x 10^7 CFU at 8 h post-infection.

A



B



***p* values**

	C57BL/6 (Newman WT)	iNOS$^{-/-}$ (Newman WT)	C57BL/6 (C55S C199S)	iNOS$^{-/-}$ (C55S C199S)
C57BL/6 (Newman WT)		* < 0.0001	0.74	* 0.01
iNOS$^{-/-}$ (Newman WT			* < 0.0001	0.10
C57BL/6 (C55S C199S)				* 0.02
iNOS$^{-/-}$ (C55S C199S)				

Figure S7 Related to Figure 7. AgrA cysteine double mutant is partially resistant to Agr-inhibition by NO *in vitro* but not hypervirulent *in vivo*

(A) RT-qPCR of RNAIII in PAPA/NO-treated *S. aureus* Newman and *agrA* C55S C199S (double cysteine mutant) (n=3). Data are represented as means with error bars showing standard deviation. Student's t test, **p*<0.05.

(B) Mouse percent mortality at indicated time points post-infection. C57BL/6 and congenic iNOS$^{-/-}$ mice were infected with either *S. aureus* Newman or *agrA* C55S C199S (~5 x 10^7 CFU, n > 12). Kaplan-Meier plot (left) and *p* values (right), **p*<0.05.

Table S2 Related to Figure 7. Histology Scores for Lung Sections

Mouse Group (n=mice per group)	**Predominant Lesion Pattern**	**Mean Inflammatory Cell Accumulation (scale: 1-4)**	**Mean Bacterial Load (scale: 1-4)**
4 h post-infection Inoculum: ~1 x 10^8 CFU			
C57BL/6 Males (n=6)	Alveolar	1.2	2.2
C57BL/6 Females (n=5)	Alveolar	1.2	2.4
iNOS$^{-/-}$ Males (n=5)	Mixed	1	2
iNOS$^{-/-}$ Females (n=6)	Mixed	1.3	1.8
8 h post-infection Inoculum: ~1 x 10^8 CFU			
C57BL/6 Males (n=7)	Focal Aggregation	3.9	3
C57BL/6 Females (n=8)	Focal Aggregation	2.4	2.5
iNOS$^{-/-}$ Males (n=6)	Alveolar	1.8	3.7
iNOS$^{-/-}$ Females (n=8)	Alveolar	2.1	2.5
8 h post-infection Inoculum: ~5 x 10^7 CFU			
C57BL/6 Females (n=7)	Mixed	2.1	1.3
iNOS$^{-/-}$ Females (n=5)	Alveolar	1	1.8

Table S3 Related to STAR METHODS. Primers Used in This Paper

Primer	Sequence 5'-3'	Reference
Cloning primers		
RU044	ATATGGTACCCTCGGATGAAGCTAAAGTAATAAG	This paper
RU045B	GACTTTATTATCTTATTACATACATTCACATCCTT A TGGCTAG	This paper
RU048B	GGATGTGAATGTATGTAATAAGATAATAAAGTCA G TTAACGGC	This paper
RU049	ATATGCGGCCGCCGTAAGCCCTCTGCTGATATG	This paper
RU044A	ATATGGTACCCTGCGCAAGTTCCGTCATGATTAT GTC	This paper
RU049AA	ATATGCGGCCGCTCATTTATACGAAGGGAGCAG A TG	This paper
pIMAYΔ*agrC*		
RU076	ATATGGTACCCGTATAATGACAGTGAGGAG	This paper
RU077	CACATCCTTATGGCTACATTTGAGTTAATACGAA T AAAAC	This paper
RU078	CGTATTAACTCAAATGTAGCCATAAGGATGTGAA T GTATG	This paper
RU079	ATATGCGGCCGCTCACCGATGCATAGCAGTG	This paper
pIMAY*agrA-HA*		
RU138	TTAAGCATAATCTGGAACATCATATGGATATATTT T TTTAACGTTTCTCACCGATGCATAGC	This paper
RU139	TATCCATATGATGTTCCAGATTATGCTTAATAAGA T AATAAAGTCAGTTAACGGCG	This paper
p*agrA*		
RU106	ATATGGTACCCATAAGGATGTGAATGTATG	This paper
RU107	ATATGGATCCAACAAGATTTACAATTGAATACGC	This paper
p*agrA-HA*		
RU116	AGCATAATCTGGAACATCATATGGATATATTTTTT TAACGTTTCTCACCGATGCATAGCAGTGTTC	This paper
Site-directed mutagenesis		
p*agrA* C6V		
RU094	GGATGTGAATGTATGAAAATTTTCATTGTCGAAG AC GATCCAAAACAAAGAGAAAACATG	This paper
RU095	CATGTTTTCTCTTTGTTTTGGATCGTCTTCGACAA T GAAAATTTTCATACATTCACATCC	This paper
p*agrA* C55S		
RU070	GCAAGCTAAAAATATGAATGACATAGGCTCTTAC TT TTTAGATATTCAACTTTC	This paper
RU071	GCAAGCTAAAAATATGAATGACATAGGCTCTTAC TT TTTAGATATTCAACTTTC	This paper
p*agrA* C123N		

RU352	GCTGAATTAAGAACTCGAATTATAGACAATTTA GAAACTGCACATACACGCTTAC	This paper
RU353	GTAAGCGTGTATGTGCAGTTTCTAAATTGTCTAT AA TTCGAGTTCTTAATTCAGC	This paper
p*agrA* C199S		
RU050	GAGTCAATTAGATGATCGTTTCTTTAGATCTCAT AA TAGCTTTGTCGTCAATCGC	This paper
RU051	GCGATTGACGACAAAGCTATTATGAGATCTAAAG A AACGATCATCTAATTGACTC	This paper
pIMAY*agrC* C91S C371S		
RU080	GTAACGTTTATTGTTATGAATAAATCCGCTGATG AT ATACCACGCATTCATG	This paper
RU081	CATGAATGCGTGGTATATCATCAGCGGATTTATT CA TAACAATAAACGTTAC	This paper
RU082	GACTTCGCAGATTATTCTATACTCTGCTAACTAC AT GTATATAG	This paper
RU083	CTATATACATGTAGTTAGCAGAGTATAGAATAAT C TGCGAAGTC	This paper
qPCR primers		
agrA_F	GAAGACGATCCAAAACAAAGAG	This paper
agrA_R	GTCATTCATATTTTTAGCTTGCTC	This paper
agrB_F	CCAGTTTGCCACGTATCTTC	This paper
agrB_R	GCACCATGTGCATGTCTTC	This paper
agrIR_F	GAAGATACGTGGCAAACTGGTC	This paper
agrIR_R	TCCTACAGTTAGGCAATATAATG	This paper
$ChIP_c$_F	CAAATGCACTGTATAGCTGGC	This paper
$ChIP_c$_R	CAAAATAAGATTCACGGAGTAGG	This paper
gmk_F	AAGGTGCAAAGCAAGTTAGAA	Hirschhausen et al., 2012
gmk_R	CTTTACGCGCTTCGTTAATAC	Hirschhausen et al., 2012
gyrB_F	TTATGGTGCTGGGCAAATACA	Goerke et al., 2000
gyrB_R	CACCATGTAAACCACCAGATA	Goerke et al., 2000
hmp_F	CGTTTAACGCCAAAAGTTAAATGG	Richardson et al., 2006
hmp_R	TGACTTTAGTGAATTTACACCAGG	Richardson et al., 2006
nrdG_F	CAGTGTTTATGTATCAGGATGTCC	Richardson et al., 2006
nrdG_R	GTTCGCCACCTAATAGACTTAGCC	Richardson et al., 2006
proC_F	GGCAGGTATTCCGATTGA	Theis et al.,

		2007
proC_R	CCAGTAACAGAGTGTCCAAC	Theis et al., 2007
psmα_F	CATCGCTGGCATCATTAAAG	This paper
psmα_R	5-ACCAGTGAATTTCTCAATTAATCC	This paper
psmβ_F	CGCAATTAAAGATACCGTAACTG	This paper
psmβ_R	CGATTGCTTCTGCTAGTCCAG	This paper
RNAIII_F	GAAGGAGTGATTTCAATGG	Goerke et al., 2000
RNAIII_R	TAAGAAAAATACATAGCACTGAG	Goerke et al., 2000
rpoD_F	AACTGAATCCAAGTGATCTTAGTG	Richardson et al., 2006
rpoD_R	TCATCACCTTGTTCAATACGTTTG	Richardson et al., 2006