S1 Text. Supplemental Materials and Methods

Plasmid construction

pTP016 [allelic replacement plasmid] was constructed by inserting annealed oligos oTP048 and oTP049 into pTP009 between BamHI and NcoI. pTP009 was constructed by isothermal assembly from three PCR products. Product 1 was derived from pMAD [1] (amplified with oTP023 and oTP024) and contained the pBR322 origin, *bla*, and *erm*. Product 2 was derived from pBursa [2] (amplified with oTP025 and oTP026) and contained the temperature-sensitive origin pE194ts. Product 3 was derived from pMAD (amplified with oTP027 and oTP028) and contained *lacZ*.

pTP078 [Δ*noc*::*spec* insertion-deletion plasmid] was constructed by isothermal assembly using three PCR products and the allelic replacement vector pTP016 cut with EcoRI and EagI. Product 1 was derived from pWX466 (amplified with oTP046 and oTP047) and contained the *spec* gene. Product 2 is the 1 kb region of DNA upstream of the *noc* gene (amplified from HG003 gDNA using oTP120 and oTP200). Product 3 is the 1 kb region of DNA downstream of the *noc* gene (amplified from HG003 gDNA using oTP201 and oTP123). pWX466 is a plasmid harboring the *spec* gene flanked by *loxP* sites (XW and DZR, unpublished).

pTP083 [Δ*parB*::*kan* insertion-deletion plasmid] was constructed by isothermal assembly using three PCR products and the allelic replacement vector pTP016 cut with EcoRI and EagI. Product 1 was derived from pWX470 (amplified with oTP046 and oTP047) and contained the *kan* gene. Product 2 is the 1 kb region of DNA upstream of the *parB* gene (amplified from HG003 gDNA using oTP226 and oTP227). Product 3 is the 1 kb region of DNA downstream of the *parB* gene (amplified from HG003 gDNA using oTP228 and oTP229). pWX470 is a plasmid harboring the *kan* gene flanked by *loxP* sites (XW and DZR, unpublished).

pTP088 [Δ*rbd*::*kan* insertion-deletion plasmid] was constructed by isothermal assembly using three PCR products and the allelic replacement vector pTP016 cut with EcoRI and EagI. Product 1 was derived from pWX470 (amplified with oTP046 and oTP047) and contained the *kan* gene. Product 2 is the 1 kb region of DNA upstream of the *rbd* gene (amplified from HG003 gDNA using oTP244 and oTP245). Product 3 is the 1 kb region of DNA downstream of the *rbd* gene (amplified from HG003 gDNA using oTP246 and oTP247).

pTP095 [Δ*comEB*:*kan* insertion-deletion plasmid] was constructed by isothermal assembly using three PCR products and the allelic replacement vector pTP016 cut with EcoRI and EagI. Product 1 was derived from pWX470 (amplified with oTP046 and oTP047) and contained the *kan* gene. Product 2 is the 1 kb region of DNA upstream of the *comEB* gene (amplified from HG003 gDNA using oTP265 and oTP266).). Product 3 is the 1 kb region of DNA downstream of the *comEB* gene (amplified from HG003 gDNA using oTP267 and oTP268).

pTP077 [transposon containing plasmid with NotI sites] was constructed by isothermal assembly from two PCR products. Product 1 was amplified from pTM402 [3] using oTP196 and 197. Both oligonucleotide primers containined NotI sites. Product 2 was amplified from pTM402 using oTP198 and oTP199.

pTP044 [L54a integrase expression plasmid] was constructed by isothermal assembly from two DNA fragments. Fragment 1 was a PCR product derived from pFA545 [2] (amplified using oTP096 and oTP097) that contained the *repC* and *tetR* region. Fragment 2 was derived from pYL112d19 [4] (digested with StuI and ApaI) and contained the *bla* and L54a *int* gene.

pTP069 [P_{tet}-^{Sa}*noc S. aureus* integration plasmid] was generated in a 2-way ligation with a PCR product containing the *S. aureus noc* gene with its native RBS (amplified from HG003 gDNA using oTP126 and oTP127) and plasmid pTP063 digested with HpaI and EcoRI. pTP063 (P_{tet} integration vector) was generated by replacing the P_{spank} promoter and *lacI* gene from pTP041 with the $P_{xyl/tet}$ promoter, *tetR*, and *lacZ* region from pRAB14-*lacZ* (both plasmids were digested with EcoRI and HindIII). pTP041 (P_{spank} integration vector) was generated by isothermal assembly from four PCR products. Product 1 was derived from pDR110 (amplified using oTP078 and 090) contained the P_{spank} promoter and *lacI*. Product 2 was derived from pWX465 (amplified with oTP088 oTP089) contained the *cat* gene. Product 3 derived from pLL29 (amplified using oTP091 and oTP083) contained *attP*(L54a). Product 4 derived from pML6 (amplified with oTP084 and oTP085) contained the pACYC origin and *bla*. pRAB14-*lacZ* is a *S. aureus* expression vector containing *tetR*-$P_{xyl/tet}$ [5]. pLL29 is an integration vector containing *attP*(L54a) [6]. pDR110 contains the P_{spank} promoter (DZR unpublished). pWX465 is a plasmid harboring the *cat* gene flanked by *loxP* sites (XW and DZR unpublished). pML6 is a vector containing the pACYC origin and *bla gene* (PML and DZR unpublished).

pTP167 [P_{tet}-(optimized RBS) ^{Bs}*noc S. aureus* integration plasmid] was constructed by site-directed mutagenesis using oligos oTP441 and oTP442 (containing a consensus RBS) and plasmid pTP144. pTP144 [P_{tet}-^{Bs}*noc*] was constructed by isothermal assembly of a PCR product containing the *B. subtilis noc* gene (amplified from PY79 gDNA using oTP414 and oTP415) and pTP063 digested by HpaI and EcoRI.

pTP170 [P_{tet}-^{Sa}noc_{his} *S. aureus* integration plasmid] was constructed by isothermal assembly of a single PCR product from plasmid pTP069 using oTP445 and oTP310 (containing a His6 tag). The PCR product was treated with DpnI to degrade the template DNA prior to isothermal assembly.

pTP171 [P_{tet}-(optimized RBS) ^{Bs}noc_{his} *S. aureus* integration plasmid] was constructed by isothermal assembly of a single PCR product from plasmid pTP167 using oTP446 and oTP310 (containing a His6 tag). The PCR product was treated with DpnI to degrade the template DNA prior to isothermal assembly.

pTP137 [*ycgO*::P_{spank}-^{Bs}*noc spec*] was constructed in a 2-way ligation with a PCR product containing the *B. subtilis noc* gene with its native RBS (amplified from PY79 gDNA using oTP389 and oTP402) and pER107 digested with NheI and XmaI. pER107 is an ectopic integration vector containing the P_{spank} promoter for insertions in the nonessential *ycgO* gene (ER and DZR, unpublished).

pTP169 [*ycgO*::P_{spank}-(optimized RBS) ^{Sa}*noc spec*] was constructed by site-directed mutagenesis using oTP439 and oTP440 (containing a consensus RBS) and pTP136. pTP136

was constructed in a 2-way with a PCR product containing the *S. aureus noc* gene with its native RBS (amplified from HG003 gDNA using oTP387 and oTP401) and pER107 digested with NheI and XmaI.

pTP173 [*ycgO*::P_{spank}-$^{Bs}noc_{his}$ *spec*] was constructed by isothermal assembly of a single PCR product from plasmid pTP137 using oTP446 and oTP447. The PCR product was treated with DpnI to degrade the template DNA prior to isothermal assembly.

pTP174 [*ycgO*::P_{spank}-(optimized RBS) $^{Sa}noc_{his}$ *spec*] was constructed by isothermal assembly of a single PCR product from plasmid pTP169 using oTP445 and oTP447. The PCR product was treated with DpnI to degrade the template DNA prior to isothermal assembly.

pTP200 [*amyE*::P_{xylA}-(optimized RBS) ^{Sa}*noc-yfp* spec] was constructed by isothermal assembly using the ectopic integration vector pDR150 digested with BamHI and EcoRI and a PCR product amplified from an isothermal assembly reaction using oTP 501 and oTP511. The isothermal assembly reaction contained three PCR products. Product 1 derived from pDR150 (amplified with oTP501 and oTP510) contained the P_{xyl} and *xylR*. Product 2 containing *yfp* was amplified from gDNA extracted from strain bKM1585 using oTP502 and oTP511. Product 3 containing ^{Sa}*noc* with an optimized RBS was amplified from pTP174 with oTP505 and oTP508. pDR150 is an ectopic integration vector containing the P_{xyl} promoter for insertions in the nonessential *amyE* gene (DZR, unpublished).

S. aureus strain construction

Construction of the knock-out mutants

S. aureus knock-out mutants were generated by allelic replacement. pMAD-based plasmids (pTP078, 083, 088, 095) were transformed into strain RN4220 (for pTP083, 088, 095) or TM18 (for pTP078), followed by selection on TSB plates containing erythromycin (10 µg/ml) at 30˚C. After incubation for 2 days, transformants were streaked on TSB plates containing erythromycin (10 µg/ml) and X-gal (250 µg/ml). One blue colony was inoculated in liquid TSB medium supplemented with erythromycin (10 µg/ml), and incubated with shaking for 3 h at 30˚C followed by 6 h at 37˚C. 100ul of a 20-fold dilution was then plated onto TSB plate containing erythromycin (10 µg/ml) and X-gal (250 µg/ml) and incubated overnight at 37˚C to generate single-crossover integrants. A single blue colony was then inoculated into TSB medium without antibiotics and grown at 30˚C. After the culture became turbid, cells were diluted 30-fold into the same medium and grown again at 30˚C. After the culture became turbid, cells were 30-fold diluted and grown at 30˚C for 3 h, followed by 37˚C for 6 h. Serial dilutions of this culture were then plated on TSB supplemented with X-Gal and the antibiotic corresponding to the antibiotic resistance cassette used to replace the target gene (kanamycin/neomycin for Δ*comEB,* Δ*rbd,* Δ*parB*, and spectinomycin for Δ*noc*). White colonies were then tested for erythromycin sensitivity. The insertion-deletions were then confirmed by colony PCR using primers amplifying a region 1 kb upstream and downstream of the target gene. The insertion-deletions were transduced into HG003 using phage 80alpha.

Construction of complementation strains
HG003 (Δ*noc*::*spec*, *geh*::pTP069) was constructed by phage transduction using *S. aureus* phage 80alpha infected RN4220 (*geh*::pTP069) as donor, and HG003 (Δ*noc*::*spec*) as recipient. RN4220 (*geh*::pTP069) was constructed by electroporating plasmid pTP069 into strain RN4220 (pTP044), and selection on TSB plate containing chloramphenicol (5 µg/ml). In the presence of L54a integrase, expressed from pTP044, pTP069 integrates into the *attB*(L54a) site within the *geh* gene of *S. aureus*. RN4220(Δ*noc*::*spec*, *geh*::pTP069) was constructed in the same way, except that RN4220(Δ*noc*::*spec*) was used as recipient.

HG003 (Δ*noc*::*spec*, *geh*::pTP170), and HG003 (Δ*noc*::*spec*, *geh*::pTP171) were constructed in the same way as HG003 (Δ*noc::spec*, *geh*::pTP69).

HG003 (Δ*noc*::*spec*, Δ*rbd*::*kan*, *geh*::pTP069) and HG003 (Δ*noc*::*spec*, Δ*comEB*::*kan*, *geh*::pTP69) were constructed by phage transduction using phage 80alpha infected RN4220 (Δ*rbd*::*kan*) or RN4220 (Δ*comEB*::*kan*) as donor, and HG003 (Δ*noc*::*spec*, *geh*::pTP69) as recipient.

HG003 (Δ*noc*::*spec*, Δ*rbd*::*kan*) and HG003 (Δ*noc*::*spec*, Δ*comEB*::*kan*) were constructed by phage transduction using phage 80alpha infected RN4220 (Δ*rbd*::*kan*) or RN4220 (Δ*comEB*::*kan*) as donor, and HG003 (Δ*noc*::*spec*) as recipient. Transductants of HG003 (Δ*noc*::*spec*, Δ*rbd*::*kan*) were selected on agar plates with 0.5X LB lacking NaCl supplemented with kanamycin/neomycin (25 µg/ml each), and incubated at 30°C for 2 days. Transductants of HG003 (Δ*noc*::*spec*, Δ*comEB*::*kan*) were selected on agar plates with LB lacking NaCl supplemented with kanamycin/neomycin, and incubated at 37°C overnight.

Reconstruction of suppressor mutants
HG003 (Δ*noc*::*spec dnaA*^{sup1}), HG003 (Δ*noc*::*spec dnaA*^{sup2}), and RN4220 (Δ*noc*::*spec dnaA*^{sup1}) were constructed by phage transduction using phage 80alpha. The originally suppressor strains aTP512 HG003 (Δ*noc*::*spec* Δ*rbd*::*kan dnaA*^{sup1}) and aTP522 HG003 (Δ*noc*::*spec* Δ*rbd*::*kan dnaA*^{sup2}) were used as donors and HG003 or RN4220 as recipient. Transductants were selected on TSB plates supplemented with spectinomycin, and screened for kanamycin sensitivity. To screen for transductants that contained the linked *dnaA* suppressor mutation, colony PCR was performed on the transductants using oTP342 and oTP343 and the products were purified and sequenced using oTP342.

Reconstructed suppressor strains aTP774 HG003 (Δ*noc*::*spec* Δ*rbd*::*kan dnaA*^{sup1}) and aTP776 HG003 (Δ*noc*::*spec* Δ*rbd*::*kan dnaA*^{sup2}) were constructed in the same way as HG003 (Δ*noc*::*spec dnaA*^{sup1}), except that HG003 (Δ*rbd*::*kan*) was used as recipient, and that transductants were selected on agar plates with 0.5X LB lacking NaCl supplemented with spectinomycin at 30°C.

Construction of strains for immunoblot and ChIP-seq
Strains used for immunoblot analsyis and ChIP-seq contained a transposon insertion in the *spa* gene encoding Surface Protein A. The donor strain was NE286, a USA300 strain harboring a mariner transposon insertion in *spa* [7]. The transducing phage used was phage 80alpha. Transductants were selected on TSB plates supplemented with 5 ug/ml erythromycin.

Construction of strains for Tn-Seq validation
Transposon insertions from the Nebraska Transposon Mutant Library [7] were first transduced into HG003 and then into the Noc depletion strain (aTP359) using phage 80alpha selecting for erythromycin resistance.

Construction of *Bacillus subtilis* strains
bTP039 [Δ*noc*::*tet*, *ycgO*::P_{spank}-$^{Bs}noc_{his}$ *spec*] and bTP041 [Δ*noc*::*tet*, *ycgO*::P_{spank}-$^{Sa}noc_{his}$ *spec*] were constructed by transforming gDNA from bRB73 (Δnoc::*tet*) into strains bTP035 (*ycgO*::P_{spank}-$^{Bs}noc_{his}$ *spec*) and bTP037 ([Δ*noc*::*tet*, *ycgO*::P_{spank}-$^{Sa}noc_{his}$ *spec*). bTP035 and bTP037 were constructed by transforming SacII-digested plasmid pTP173 and pTP174 into (*ycgO*::*cat*) selecting for Spec(R) and screening for Cm(S).

bTP043 [Δ*noc*::*tet* Δ*minD*::*kan ycgO*::P_{spank}-$^{Bs}noc_{his}$ *spec*] and bTP045 [Δ*noc*::*tet* Δ*minD*::*kan ycgO*::P_{spank}-$^{Sa}noc_{his}$ *spec*] were constructed by transforming gDNA from bML712 (Δ*noc*::*tet* Δ*minD*::*kan*) into strains bTP039 and bTP041 selecting for Kan(R) and screening for Tet(R) and Spec(R).

bTP061 [Δ*noc*::*tet amyE*::P_{xyl}-^{Sa}*noc-yfp spec*] was constructed by transforming the SacII-digested plasmid pTP200 into PY79 Δ*noc*::*tet* selecting for Spec(R) and screening for the inability to degrade starch.

Supplemental References

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S1 Table Candidate *sln* genes identified by Tn-seq

Gene name	Annotation	p-value	Fold-change	Validation
comEB	deoxycytidylate deaminase	0.06	17.5	+++
mprF	lysyl-phosphotidylglycerol synthase	0.007	9.3	-
SAOUHSC_01496	cytidylate kinase	0.001	7.5	N/A
SAOUHSC_01270	putative uncharacterized protein	0.35	7.5	-
yabA	regulator of DNA replication initiation	0.49	7.4	-
ecsB	putative ABC transporter	0.028	6.8	+
SAOUHSC_01154	SepF cell division protein	0.021	6.8	N/A
SAOUHSC_02337	MurA peptidoglycan precursor biosynthesis	0.046	6.7	-
rbd	rhomboid protease, putative	0.07	6.3	+++
SAOUHSC_01497	L-asparaginase, putative	0.10	6.1	-
tagA	techoic acid biosynthesis	0.010	5.6	N/A
SAOUHSC_01050	putative uncharacterized protein	0.013	3.8	+
fmtA	Affects methicillin resistance level	0.038	3.6	N/A

S2 Table Quantification of abnormal septa and lysis in cells lacking Noc and Rbd

	total cells counted	percent with abnormal septa	percent lysed cells
WT	724	1.1%	0
Δ*noc*	310	4.2%	4.5%
Δ*rbd*	507	5.9%	0.6%
Δ*noc* Δ*rbd*	353	27.2%	23.8%

S3 Table Δ*noc* Δ*rbd* and Δ*noc* Δ*comEB* suppressors

strain	mapped reads	loci	aa change	confirmed
Δ*noc*Δ*rbd*	664,476			
#2	935,976	Deletion in 5' UTR of *dnaA*		Yes
#3	1,877,640	SAOUHSC_01907 Aldo-keto reductase (AKRs) superfamily Point mutation upstream of SAOUHSC_R0005	N10S N/A	
#4	257,669	No mutation detected		
#5	1,260,537	SAOUHSC_00018: DnaC helicase	A352V	Yes
#6	787,308	upstream of SAOUHSC_01866	N/A	
#7	2,723,259	*dnaA*	V141L	Yes
#8	1,846,980	*pheS*: phenylalanyl-tRNA synthetase subunit alpha	M48K	Yes
#9	3,067,565	rRNA: Sa5SA, Gene: SAOUHSC_R00011	N/A	
#10	2,172,921	*aroA*: 3-phosphoshikimate 1-carboxyvinyltransferase	T291frameshift	
Δ*noc*Δ*comEB*				
#22	996,316	*dnaA*	R254Q	Yes
#23	978,230	SAOUHSC_01679: MiaB 2-methylthioadenine synthetase	Q150*	Yes
#24	1,234,711	*trmB*: tRNA (guanine-N(7)-)-methyltransferase trmB SAOUHSC 01866: Phosphotransferase enzyme family	deletion: 1772372-1772564bp	
#25	3,175,065	SAOUHSC_02963: clfB clumping factor B	D617G	
#26	1,658,211	SAOUHSC_01866: Phosphotransferase enzyme family	E157G	Yes
#27	2,465,322	SAOUHSC_01679: MiaB 2-methylthioadenine synthetase SAOUHSC_01866: Phosphotransferase enzyme family	Q150* L185R	
#28	1,249,282	SAOUHSC_00018: DnaC helicase	A280V	Yes
#29	2,025,633	SAOUHSC_01866: Phosphotransferase enzyme family	D147G	

S4 Table Strains used in this study

Strain	Relevant Genotype and Features	Source
S. aureus		
RN4220	*S. aureus* subsp. aureus NCTC8325 derivative; MSSA; r−m+;	Nair 2011
HG003	*S. aureus* subsp. aureus NCTC8325 with *rsbU* and *tcaR* repaired	Herbert 2010
TM17	RN4220 *geh*::pTM304; replication of pT181-ori containing plasmids in trans	Wang 2011
TM18	RN4220 ΔattBφ11::Orf5 (non-lysogenic and represses bacteriophage φ11 replication)	Wang 2011
TM51	TM18 (pTM378), transposon recipient strain	Wang 2011
aTP310	TM17 (pTP077), transposon donor strain	this work
aTP310	TM17 (pTP077), transposon donor strain	this work
aTP315	TM18 Δ*noc*::*spec*	this work
aTP317	TM18 Δ*noc*::*spec*, (pTM378) transposon recipient strain	this work
aTP341	HG003 Δ*noc*::*spec*	this work
aTP977	RN4220 Δ*noc*::*spec*	this work
aTP411	RN4220 Δ*rbd*::*kan*	this work
aTP428	HG003 Δ*rbd*::*kan*	this work
aTP477	RN4220 Δ*comEB*::*kan*	this work
aTP506	HG003 Δ*comEB*::*kan*	this work
aTP730	RN4220 Δ*parB*::*kan*	this work
aTP742	HG003 Δ*parB*::*kan*	this work
aTP983	RN4220 Δ*noc*::*spec geh*::pTP069	this work
aTP359	HG003 Δ*noc*::*spec geh*::pTP069	this work
aTP431	HG003 Δ*noc*::*spec* Δ*rbd*::*kan geh*::pTP069 (^{Sa}*noc*)	this work
aTP508	HG003 Δ*noc*::*spec* Δ*comEB*::*kan geh*::pTP069 (^{Sa}*noc*)	this work
aTP510	HG003 Δ*noc*::*spec* Δ*rbd*::*kan*	this work
aTP550	HG003 Δ*noc*::*spec* Δ*comEB*::*kan*	this work
aTP979	RN4220 Δ*noc*::*spec dnaA*^{sup1}	this work
aTP768	HG003 Δ*noc*::*spec dnaA*^{sup1}	this work
aTP770	HG003 Δ*noc*::*spec dnaA*^{sup2}	this work
aTP774/512	HG003 Δ*noc*::*spec* Δ*rbd*::*kan dnaA*^{sup1}	this work
aTP776/522	HG003 Δ*noc*::*spec* Δ*rbd*::*kan dnaA*^{sup2}	this work
aTP557	HG003 Δ*noc*::*spec* Δ*comEB*::*kan dnaA*^{sup3}	this work
aTP518	HG003 Δ*noc*::*spec* Δ*rbd*::*kan dnaC*$_{A352V}$	this work
aTP780	HG003 Δ*noc*::*spec dnaA*^{sup1} *geh*::pTP069 (^{Sa}*noc*)	this work
aTP782	HG003 Δ*noc*::*spec dnaA*^{sup2} *geh*::pTP069 (^{Sa}*noc*)	this work
aTP811	HG003 Δ*noc*::*spec geh*::pTP170 (^{Sa}*noc*$_{his}$)	this work
aTP813	HG003 Δ*noc*::*spec geh*::pTP171 (^{Bs}*noc*$_{his}$)	this work

NE286	USA300 *spa*::Tn	Fey 2013
aTP394	HG003 *spa*::Tn	this work
aTP403	HG003 Δ*noc*::*spec spa*::Tn	this work
aTP851	HG003 Δ*noc*::*spec dnaA*^{sup1} *spa*::Tn	this work
aTP853	HG003 Δ*noc*::*spec dnaA*^{sup2} *spa*::Tn	this work
aTP959	HG003 Δ*noc*::*spec geh*::pTP170 ($^{Sa}noc_{his}$) *spa*::Tn	this work
aTP961	HG003 Δ*noc*::*spec geh*::pTP171 ($^{Bs}noc_{his}$) *spa*::Tn	this work
aTP821	HG003 pLOW-FtsZ-GFP	this work
aTP845	HG003 Δ*noc*::*spec*, pLOW-Zgfp	this work
aTP823	HG003 Δ*noc*::*spec dnaA*^{sup1}, pLOW-Zgfp	this work
aTP825	HG003 Δ*noc*::*spec dnaA*^{sup2}, pLOW-Zgfp	this work
aTP919	HG003 pNDX2	this work
aTP921	HG003 pNDX2-*dnaA*	this work
aTP923	HG003 pNDX2-*dnaA*^{R318H}	this work
aTP925	HG003 *spa*::Tn, pNDX2	this work
aTP927	HG003 *spa*::Tn, pNDX2-*dnaA*	this work
aTP929	HG003 *spa*::Tn, pNDX2-*dnaA*^{R318H}	this work
aTP949	HG003 Δ*noc*::*spec,* pNDX2	this work
aTP951	HG003 Δ*noc*::*spec,* pNDX2-*dnaA*	this work
B. subtilis		
PY79	wild type	Youngman 1983
bRB73	PY79 Δ*noc*::*tet*	Lab stock
bDR3019	PY79 Δ*soj(parA)*	Lab stock
bDR2292	PY79 Δ*spo0J(parB)*::*spec*	Lab stock
bML712	PY79 Δ*noc*::*tet* Δ*minD*::*kan*	Lab stock
bTP039	PY79 Δ*noc*::*tet ycgO*::P_{spank}-$^{Bs}noc_{his}$ *(spec)*	this work
bTP041	PY79 Δ*noc*::*tet ycgO*::P_{spank}-$^{Sa}noc_{his}$ *(spec)*	this work
bTP043	PY79 Δ*noc*::*tet* Δ*minD*::*kan ycgO*::P_{spank}-$^{Bs}noc_{his}$ *(spec)*	this work
bTP045	PY79 Δ*noc*::*tet* Δ*minD*::*kan ycgO*::P_{spank}-$^{Sa}noc_{his}$ *(spec)*	this work
bTP061	PY79 Δ*noc*::*tet amyE*::P_{xyl}-^{Sa}*noc-yfp (spec)*	this work
4702	168 *trpC2* Δ*noc*::*tet amyE*::P_{xyl}-^{Bs}*noc-yfp (spec)*	Wu 2009
bRB467	PY79 Δ*noc*::*tet amyE*::P_{xyl}-^{Bs}*noc-yfp (spec)*	Lab stock

S5 Table Plasmids used in this study

Plasmid	Relevant features	Source
pLOW-FtsZ-GFP	*S. aureus* FtsZ-GFP expression plasmid	Liew 2011
pMAD	Vector for allelic replacement	Arnaud 2004
pNDX2	*S. aureus* vector containing aTc-inducible promoter	Kurokawa 2009
pNDX2-dnaA	*S. aureus* aTc-inducible *dnaA* expression plasmid	Kurokawa 2009
pNDX2-R318H	*S. aureus* aTc-inducible $dnaA_{R318H}$ expression plasmid	Kurokawa 2009
pTM304	pCL25-P_{Pen} repC11 TT	Wang 2011
pTM378	$pWV01^{Ts}$ *ori aphA-3* $Gram^{+}$ RBS HMAR1 C9 transposase	Wang 2011
pTP016	pMAD derivative	this work
pTP044	L54a integrase expression plasmid	this work
pTP069	P_{tet}-^{Sa}*noc S. aureus* integration plasmid	this work
pTP077	Transposon containing plasmid with NotI sites	this work
pTP078	Δ*noc*::*spec* insertion-deletion plasmid	this work
pTP083	Δ*parB*::*kan* insertion-deletion plasmid	this work
pTP088	Δ*rbd*::*kan* insertion-deletion plasmid	this work
pTP095	Δ*comEB*:*kan* insertion-deletion plasmid	this work
pTP137	*ycgO*::P_{spank}-^{Bs}*noc spec*	this work
pTP167	P_{tet}-(optimized RBS) ^{Bs}*noc S. aureus* integration plasmid	this work
pTP169	*ycgO*::P_{spank}-(optimized RBS) ^{Sa}*noc spec*	this work
pTP170	P_{tet}-$^{Sa}noc_{his}$ *S. aureus* integration plasmid	this work
pTP171	P_{tet}-(optimized RBS) $^{Bs}noc_{his}$ *S. aureus* integration plasmid	this work
pTP173	*ycgO*::P_{spank}-$^{Bs}noc_{his}$ *spec*	this work
pTP174	*ycgO*::P_{spank}-(optimized RBS) $^{Sa}noc_{his}$ *spec*	this work
pTP200	*amyE*::P_{xyl}-(optimized RBS) ^{Sa}*noc-yfp spec*	this work

S6 Table Oligonucleotides primers used in this study

Used for plasmid construction	
oTP023	GTTACGTTACACATTAACTAGACCATGGTATGGATCCATCACCGATACGCGAGCG
oTP024	CGTGTGATGCGCTGCGTCCGCTACACCTCCGGATAATAAATATAT
oTP025	ATATATTTATTATCCGGAGGTGTAGCGGACGCAGCGCATCACACG
oTP026	CAACATAATATGCTAAAGGCCTCGGTCTTTTGCGCAGTCGGC
oTP027	GCCGACTGCGCAAAAGACCGAGGCCTTTAGCATATTATGTTG
oTP028	CGCTCGCGTATCGGTGATGGATCCATACCATGGTCTAGTTAATGTGTAACGTAAC
oTP046	CGGCTCGAGTTCTGCTCCCTCGCTCAG
oTP047	CGGGTCGACATCAGGGAGCACTGGTCAAC
oTP048	CATGGTATGAATTCATAGTCGACTTACTCGAGTATGCTAGCAATCGGCCGATTG
oTP049	GATCCAATCGGCCGATTGCTAGCATACTCGAGTAAGTCGACTATGAATTCATAC
oTP078	CCCCGAAAAGTGCCACCTGGAATTCGACTCTCTAGCTTGAG
oTP083	GTCAATTGTCTGATTCGTTACCTCCCCACACAACCAACAAAAC
oTP084	GTTTTGTTGGTTGTGTGGGGAGGTAACGAATCAGACAATTGAC
oTP085	CTCAAGCTAGAGAGTCGAATTCCAGGTGGCACTTTTCGGGG
oTP088	GCAATTAATGTGAGTTAGGAAGCTTCATACGGCAATAGTTACCC
oTP089	GCCCCGCAAAAGACATAATGGAGCTGTAATATAAAAACCTTC
oTP090	GGGTAACTATTGCCGTATGAAGCTTCCTAACTCACATTAATTGC
oTP091	GAAGGTTTTTATATTACAGCTCCATTATGTCTTTTGCGGGGC
oTP096	TTATAAAAGCCAGTCATTAGGGCTAGCCACTCATAGTTCTAAAC
oTP097	ATCATCTCAATATCCGAATAGGAAATTCAGAGAAGCCTTTGAG
oTP120	CATTAACTAGACCATGGTATGCCAGAAGACTTAGATTATAGTAAG
oTP123	CGTATCGGTGATGGATCCAATCGCCGGAAACGTTGCAAATATAC
oTP126	ATAGTTAACAAGGAGCGAATGGATAATGAAAAAAC
oTP127	AATGAATTCCTACTACTAACGTTTATATATTCGAA
oTP196	ATGGCATGCCGAATTGGGCCGGCGGCCGCTCTAGTAACAGGTTGGCTG
oTP197	AAAGGATCCAGACTGGACGGGCGGCCGCTCTAGTAACAGGTTGGCTG
oTP198	CCGTCCAGTCTGGATCC
oTP199	GGCCCAATTCGGCATGC
oTP200	GAGGGAGCAGAACTCGAGCCGCTTCAATATGTCCAATGATGTCATC
oTP201	GTGCTCCCTGATGTCGACCCGCGTTAGTAGTAGGATGTCGTATAC
oTP226	CATTAACTAGACCATGGTATGCTGTAAAATCAACGTACTTAAATTTG
oTP227	GAGGGAGCAGAACTCGAGCCGTTTTGACAATTCACTCACATCAC
oTP228	GTGCTCCCTGATGTCGACCCGCGTAGGTATGGTAAATAGTTACAC
oTP229	CGTATCGGTGATGGATCCAATCGGTTGAGGTGTTTTAATACCTTC
oTP244	CATTAACTAGACCATGGTATGGCACCTTTGTGTTTCTCCC
oTP245	GTGCTCCCTGATGTCGACCCGGGCTATTACTATTAAGTGAATCG
oTP246	GAGGGAGCAGAACTCGAGCCGGTCTATGTTCATTTAGTCCTCC

oTP247	CGTATCGGTGATGGATCCAATCGACGATTGTGTAGCGCATGG
oTP265	CATTAACTAGACCATGGTATGGACATATTTGTCTTGGTTAGC
oTP266	GAGGGAGCAGAACTCGAGCCGCATAAAATATTCTTCCCATTTG
oTP267	GTGCTCCCTGATGTCGACCCGCTGACTAAAGGTTAATGTTTTGC
oTP268	CGTATCGGTGATGGATCCAATCCGATAAATGAAGCTAATTGTGC
oTP310	CTTGAGGGTAGCGGACAAG
oTP387	ATACCCGGGAAGGAGCGAATGGATAATGAAAAAAC
oTP389	ATACCCGGGAAGGTGGTGTAGGTACATG
oTP401	CGAGCTAGCCTACTACTAACGTTTATATATTCGAA
oTP402	CGAGCTAGCCTATTTTGGTATGCGAATCGTT
oTP414	GTATGATGGTACCGTTAACAAGGTGGTGTAGGTACATG
oTP415	GAAAAGTGCCACCTGGAATTCCTATTTTGGTATGCGAATCGTT
oTP439	GTCCCGGGAAGGAGGAACTACTATGAAAAAACCTTTTTCAAAATTATTTGG
oTP440	GAAAAAGGTTTTTTCATAGTAGTTCCTCCTTCCCGGGACAAG
oTP441	GTACCGTTAACAAGGAGGAATAAAAAATGAAGCATTCATTCTCTCG
oTP442	GAATGAATGCTTCATTTTTTATTCCTCCTTGTTAACGGTACC
oTP445	GGTGGTGGTGGTGGTGCCCGGGACGTTTATATATTCGAATTTTTATTTCATAATAATC
oTP446	GGTGGTGGTGGTGGTGCCCGGGTTTTGGTATGCGAATCGTTAATTG
oTP447	GGGCACCACCACCACCACCATTAGGCTAGCTCGCATGCAAGC
oTP501	AGTAGTTCCTCCTTAAGCTTGCATGCCTGCAGG
oTP502	CTCGAGGGTTCCGGAGTGAG
oTP505	AAGCTTAAGGAGGAACTACTATGAAAAAACC
oTP508	CTCACTCCGGAACCCTCGAGACGTTTATATATTCGAATTTTTATTTCATAA
oTP510	CGCCATTCGCCAGGGCTG
oTP511	TGGTAGCGACCGGCGCTCAGGATCCTTATTTGTATAGTTCATCCATGCCATG
Used for marker frequency analysis	
oTP478	CAGAAACACGCCCAGTACTAA
oTP479	CTGCAACTTTCTTACAGCCAAG
oTP480	GAGTATGGTGGACGTGGTATG
oTP481	TTTGTACTGGCGTAGGCTTT
oTP482	GGGTAGTAGGCCAGCAATTTA
oTP483	CCTTGATAAGTACATGGCGATTTG
oTP484	CCTCATGTACAAGACCGGTAAG
oTP485	GTTCCTCCATGTTCAGCTACA
Confirmation of *dnaA* suppressor mutants	
oTP342	CGTCCAACTCATGATTTTATAAG
oTP343	GATTACATTTCCCAAAGTTTCC
Confirmation of insertion into *ycgO* site	
oKM011	CAATTCCCGGATTCCTGG
oKM012	CCACTGGCTTTTGCAGTTC

S7 Table Peaks enriched by ChIP-seq in *S. aureus* using anti-^{Bs}Noc antibody

Peak #	Peak position (bp)	FIMO identified motif position (bp)	Score[1]	*p*-value[2]	q-value[3]	Matched sequence
1	67149 - 67300	67228 - 67241	15.95	2.03E-06	0.26	ATTTCTCG GGCAAT
2	83280 - 83445	83342 - 83355	6.058	8.85E-05	1	ATTTCCTG GGTTAC
3	104230 - 104382	104303 - 104316	15.95	2.03E-06	0.26	ATTTCCCA GGCAAT
4	128017 - 128170	128098 - 128111	11.01	1.53E-05	0.861	ATAACCCG GAAAAT
5	133029 - 133188	133107 - 133120	15.95	2.03E-06	0.26	ATTGCCCA GGAAAT
6	143823 - 143988	143895 - 143908	16.96	4.98E-07	0.128	ATTTCCCG GGGCAT
7	153267 - 153427	153339 - 153352	21.91	5.34E-08	0.0301	ATGTCCCG GGAAAT
8	169562 - 169711	169633 - 169646	21.91	5.34E-08	0.0301	ATTTCCCG GGAATT
9	200985 - 201140	201061 - 201074	15.95	2.03E-06	0.26	ATATCCTG GGAAAT
10	266582 - 266747					
11	307853 - 308009	307912 - 307925	10	3.47E-05	0.981	ATTTCCTA GGAAAA
12	331029 - 331175	331107 - 331120	10	3.47E-05	0.981	ATTTCCTA GGGAAT
13	345989 - 346139	346071 - 346084	14.95	3.26E-06	0.288	ATTTCCTCG GAAAT
14	415983 - 416134	416059 - 416072	15.95	2.03E-06	0.26	ATTGCCTG GGAAAT
15	429234 - 429391	429295 - 429308	12.01	4.64E-06	0.385	ATTTCCCG GGTGGT
16	477582 - 477741	477659 - 477672	11.01	1.53E-05	0.861	ATTTCCCA GGTTAT
17	567954 - 568098	568017 - 568030	14.95	3.26E-06	0.288	ATTTCTCA GGAAAT
18	578431 - 578600	578495 - 578508	11.01	1.53E-05	0.861	ATTGCCCA GGAAAA
19	691103 - 691282	691185 - 691198	20.90	1.50E-07	0.053	ATTTCCTG GGAAAT
20	877608 - 877773	877680 - 877693	15.95	2.03E-06	0.26	ATTTCCTG GGTAAT
21	2261104 - 2261269	2261177 - 2261190	20.90	1.50E-07	0.053	ATTTCCCC GGAAAT

22	2272079 - 2272224	2272134 - 2272147	14.95	3.26E-06	0.288	ATTTCCCA GTAAAT
23	2324836 - 2324988	2324889 - 2324902	10	3.47E-05	0.981	ATGTCCTA GGAAAT
24	2371742 - 2371901	2371807 - 2371820	21.91	5.34E-08	0.0301	ATTTCCCG GGAAAA
25	2405033 - 2405205	2405114 - 2405127	16.96	4.98E-07	0.128	ATCACCCG GGAAAT
26	2429616 - 2429779	2429687 - 2429700	14.95	3.26E-06	0.288	ATTTCCTA GGAAAT
27	2436417 - 2436578	2436491 - 2436504	15.95	2.03E-06	0.26	ATTTCCCA GGCAAT
28	2508131 - 2508295	2508218 - 2508231	14.95	3.26E-06	0.288	ATTTCCTG GTAAAT
29	2511766 - 2511931	2511842 - 2511855	14.95	3.26E-06	0.288	ATTTCCCG AAAAAT
30	2566488 - 2566633	2566560 - 2566573	10	3.47E-05	0.981	ATTGCTCA GGAAAT
31	2577721 - 2577893	2577796 - 2577809	14.95	3.26E-06	0.288	ATTTCTTGG GAAAT
32	2587242 - 2587401	2587325 - 2587338	14.95	3.26E-06	0.288	ATTTCCTTG GAAAT
33	2619613 - 2619751	2619677 - 2619690	14.95	3.26E-06	0.288	ATTTACTG GGAAAT
34	2635932 - 2636083	2636010 - 2636023	15.95	2.03E-06	0.26	ATTTCCCTG GAAAA
35	2685378 - 2685536	2685433 - 2685446	20.90	1.50E-07	0.053	ATTTACCG GGAAAT
36	2725979 - 2726137	2726054 - 2726067	21.91	5.34E-08	0.0301	GTTTCCCG GGAAAT
37	2749873 - 2750038	2749938 - 2749951	21.91	5.34E-08	0.0301	ATTTCCCG GGACAT
38	2754594 - 2754806	2754679 - 2754692	15.95	2.03E-06	0.26	ATTTCCTG GGACAT
39	2771924 - 2772081	2771996 - 2772009	16.96	4.98E-07	0.128	CTTGCCCG GGAAAT
40	2791904 - 2792075	2791983 - 2791996	12.01	4.64E-06	0.385	ATTTCCCG GGTGAA
41	2808311 - 2808476					

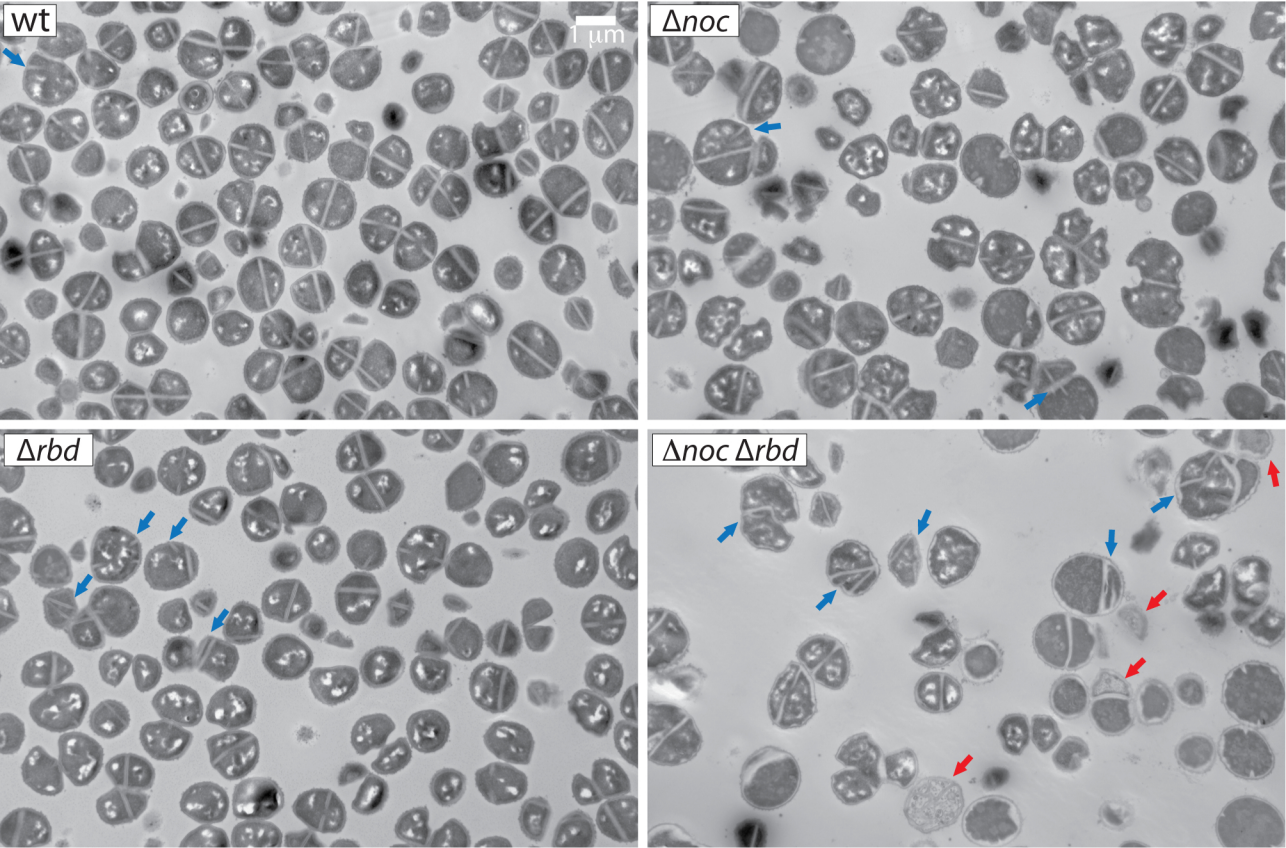
1. The score is for the strength of the match to the consensus motif.

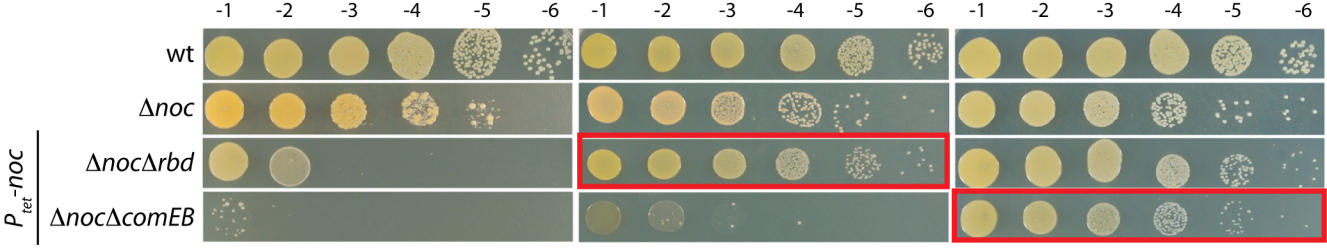
2. The *p*-value is defined as the probability of a random sequence of the same length as the motif matching that position of the sequence with as good or better a score.

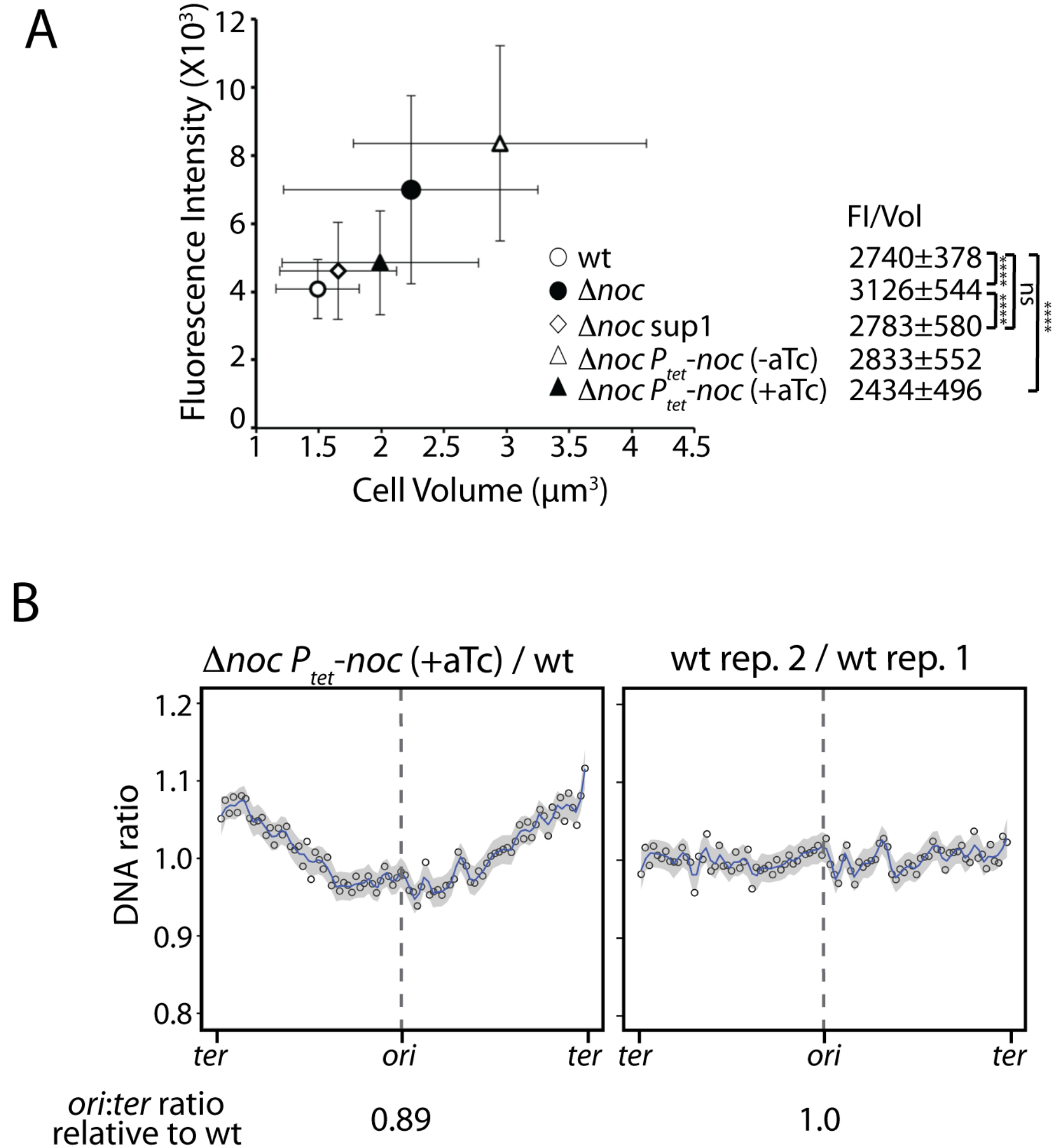
3. The q-value is defined as the false discovery rate if the occurrence is accepted as significant.

Peaks #10 and #41 did not contain a potential motif that matches the consensus motif.

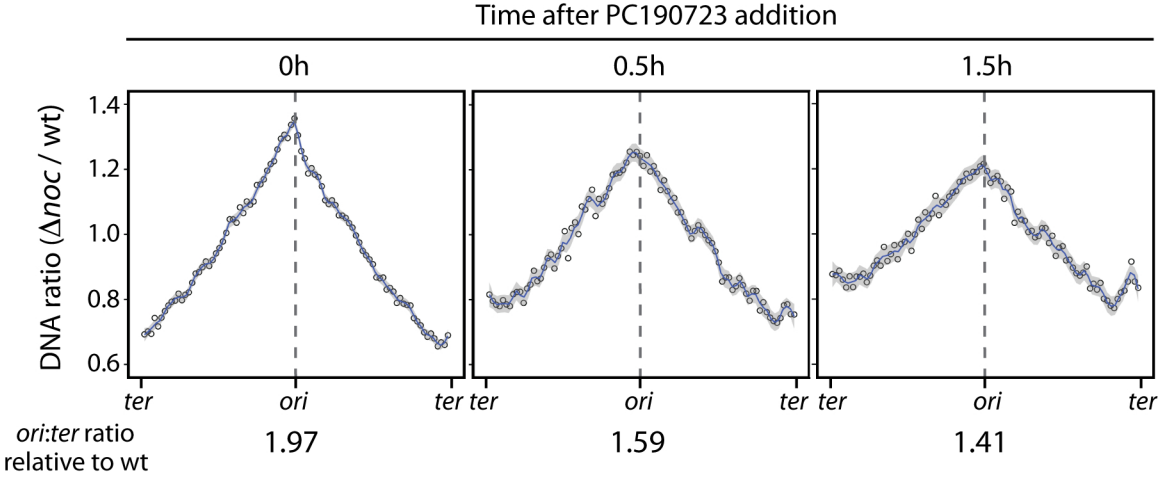
Peak #9, #40, #41 were more modestly enriched and peaks #2, #4, #10, #16, #18, #23 were not enriched in the ChIP-seq experiment in which in $^{Bs}Noc_{his}$ was expressed in *S. aureus*.


wt
1 µm
Δnoc
Δrbd
Δnoc Δrbd

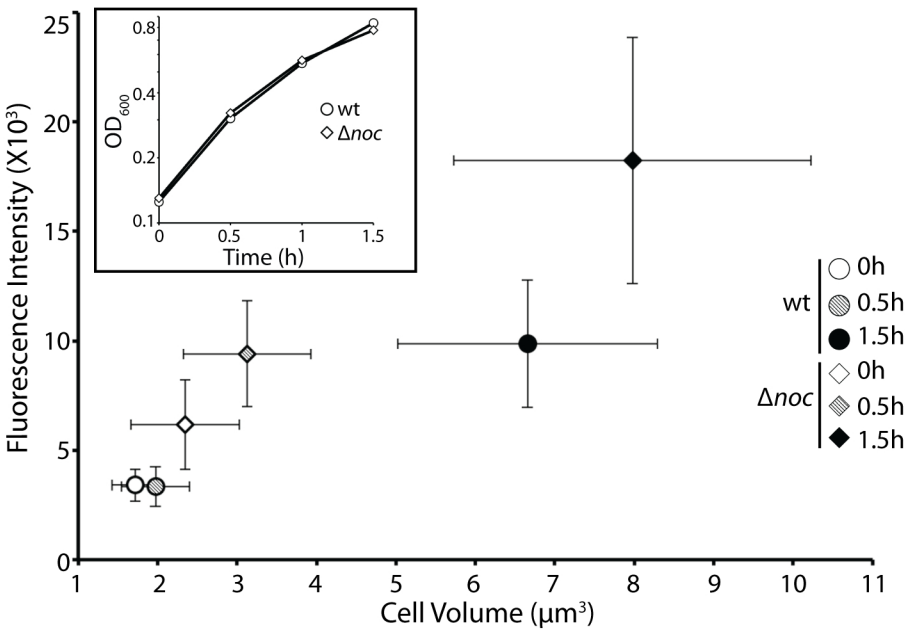

-1 -2 -3 -4 -5 -6
-1 -2 -3 -4 -5 -6
-1 -2 -3 -4 -5 -6
wt
Δnoc
ΔnocΔrbd
ΔnocΔcomEB
P tet -noc
LB 0.5% NaCl, 37 ˚C
0.5X LB no NaCl, 30 ˚C
LB no NaCl, 37 ˚C

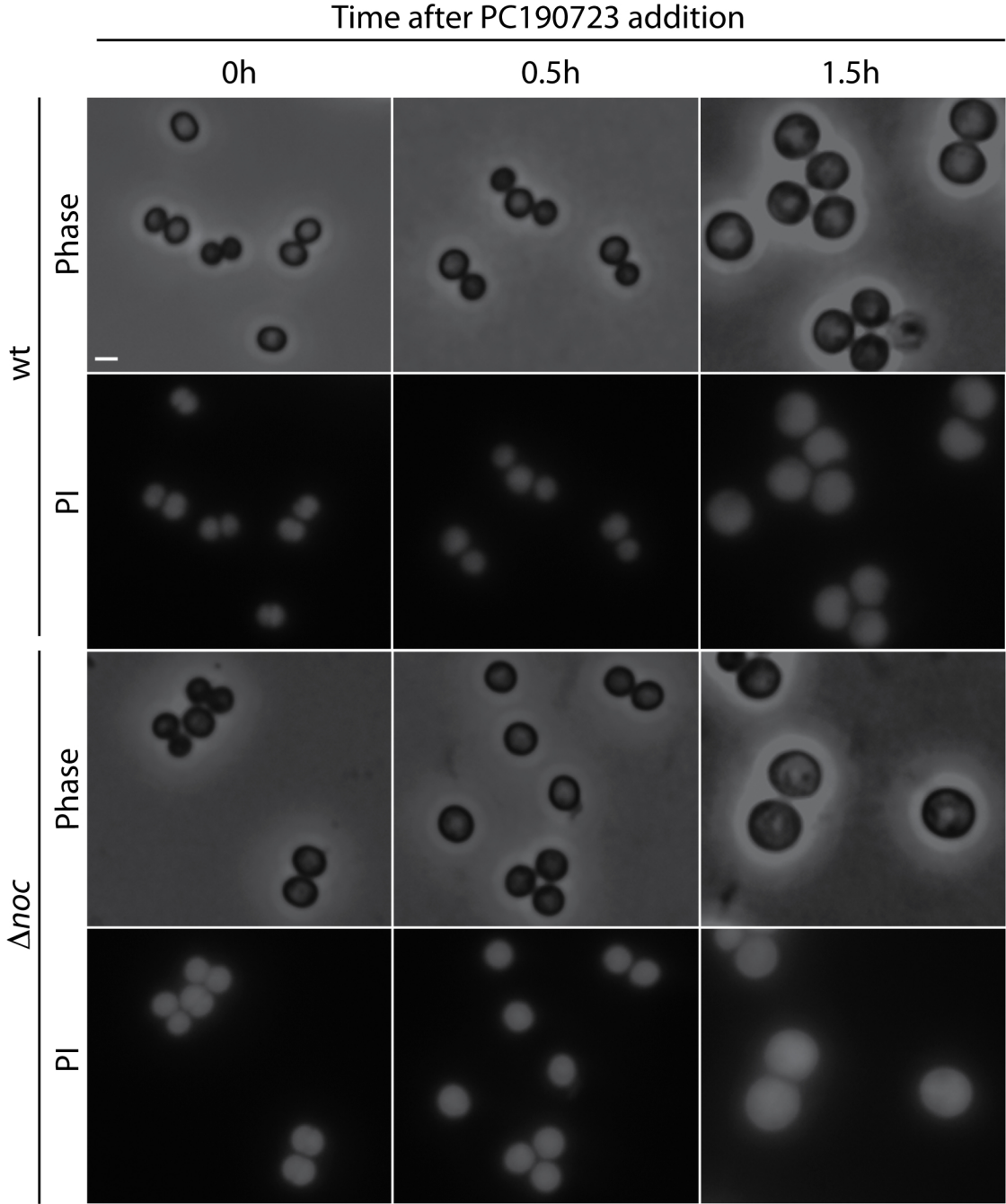

A
Fluorescence Intensity (X10³)
Cell Volume (µm³)
wt
Δnoc
Δnoc sup1
Δnoc P_tet-noc (-aTc)
Δnoc P_tet-noc (+aTc)
FI/Vol
2740±378
3126±544
2783±580
2833±552
2434±496
****
****
ns
****
B
Δnoc P_tet-noc (+aTc) / wt
wt rep. 2 / wt rep. 1
DNA ratio
ter
ori
ter
ori:ter ratio relative to wt
0.89
1.0

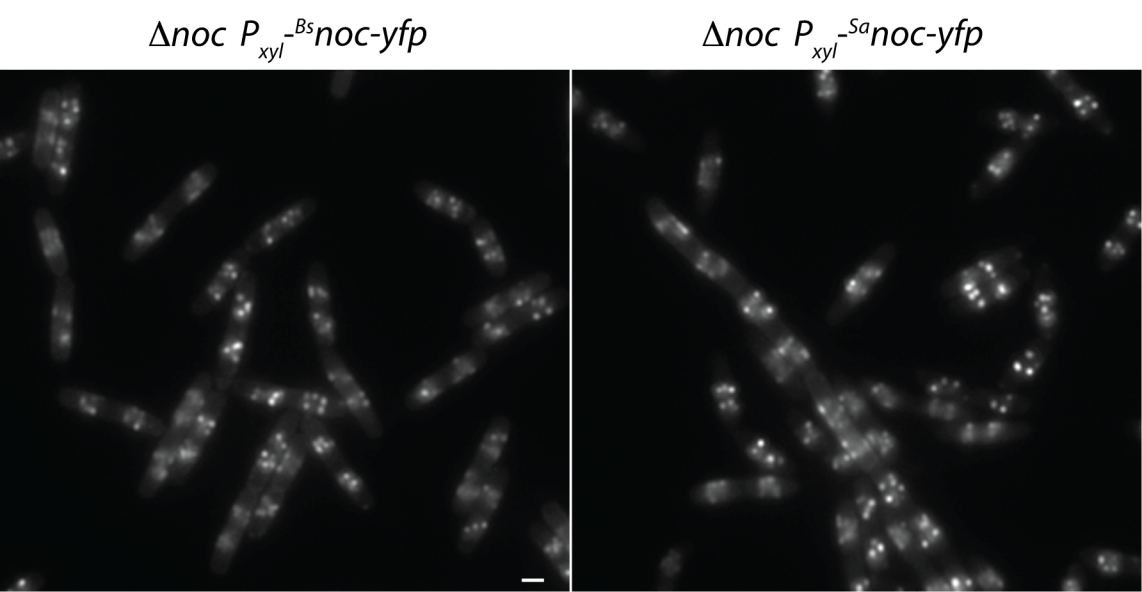
A

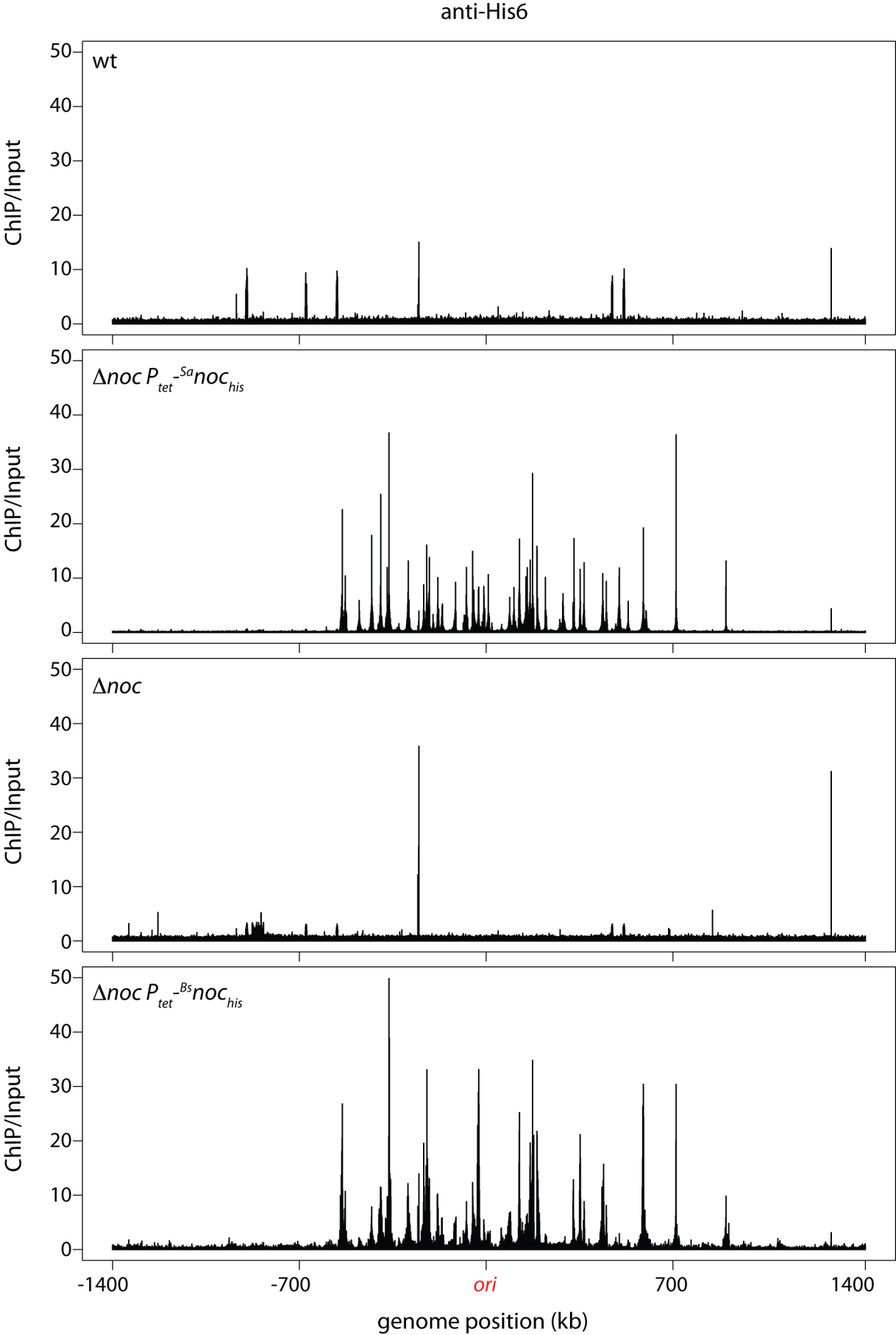

Time after PC190723 addition
0h
0.5h
1.5h
DNA ratio (Δnoc / wt)
1.4
1.2
1.0
0.8
0.6
ter
ori
ter
ori:ter ratio relative to wt
1.97
1.59
1.41

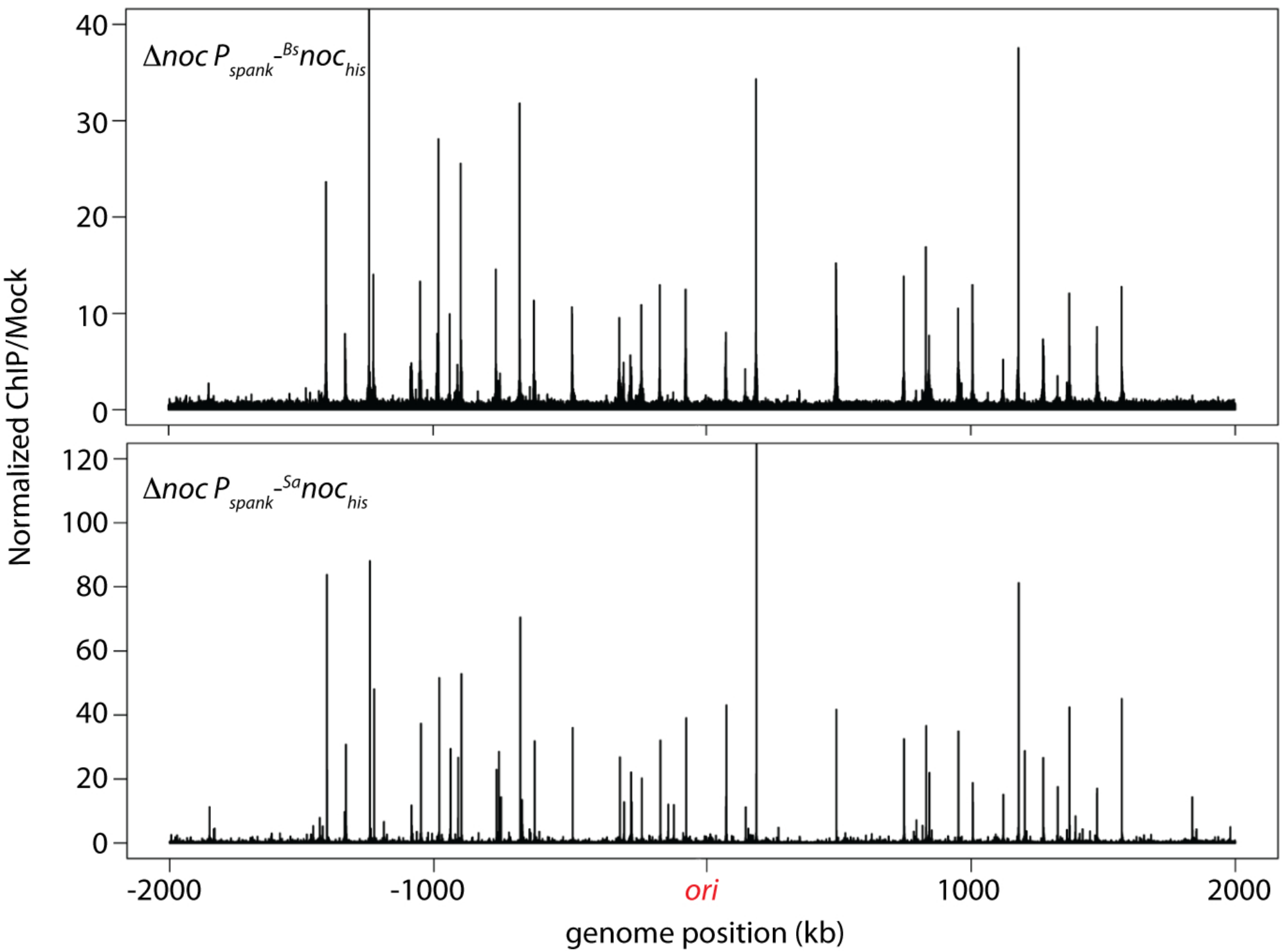
B

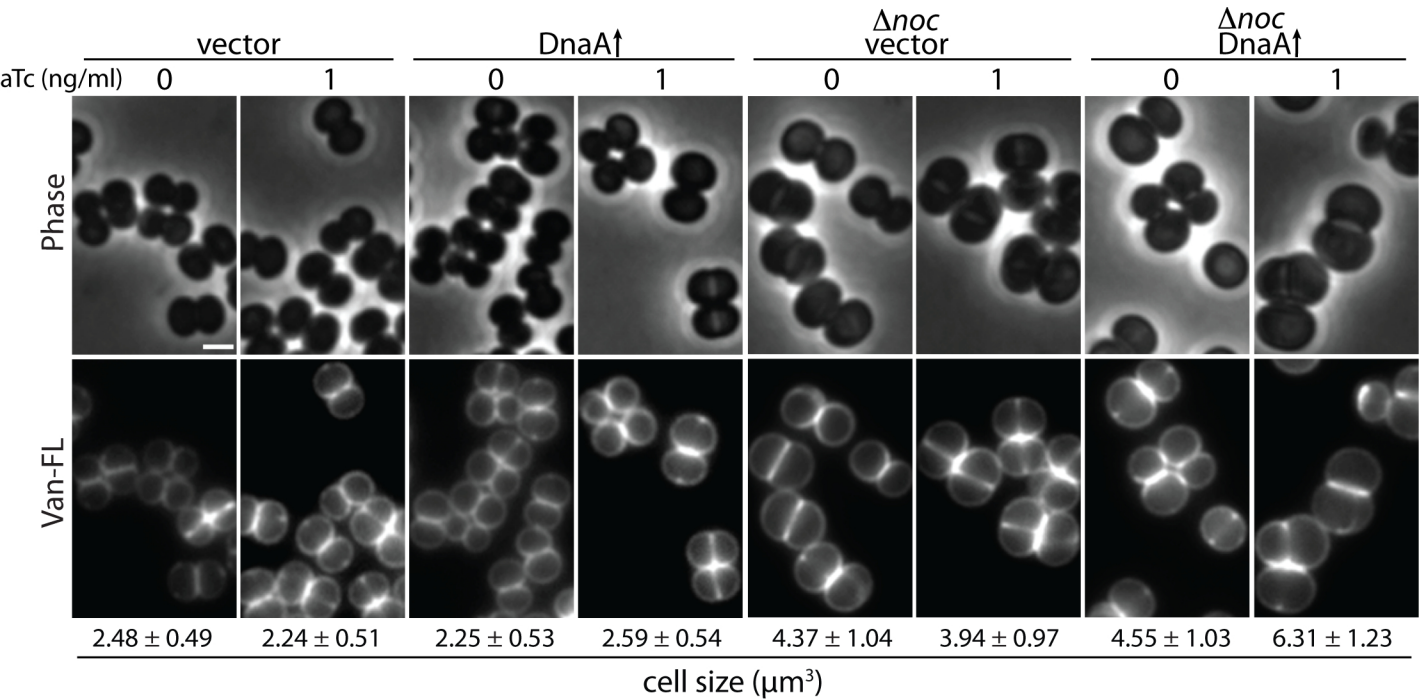

Fluorescence Intensity (X10³)
Cell Volume (μm³)
OD600
Time (h)
wt
Δnoc
0h
0.5h
1.5h

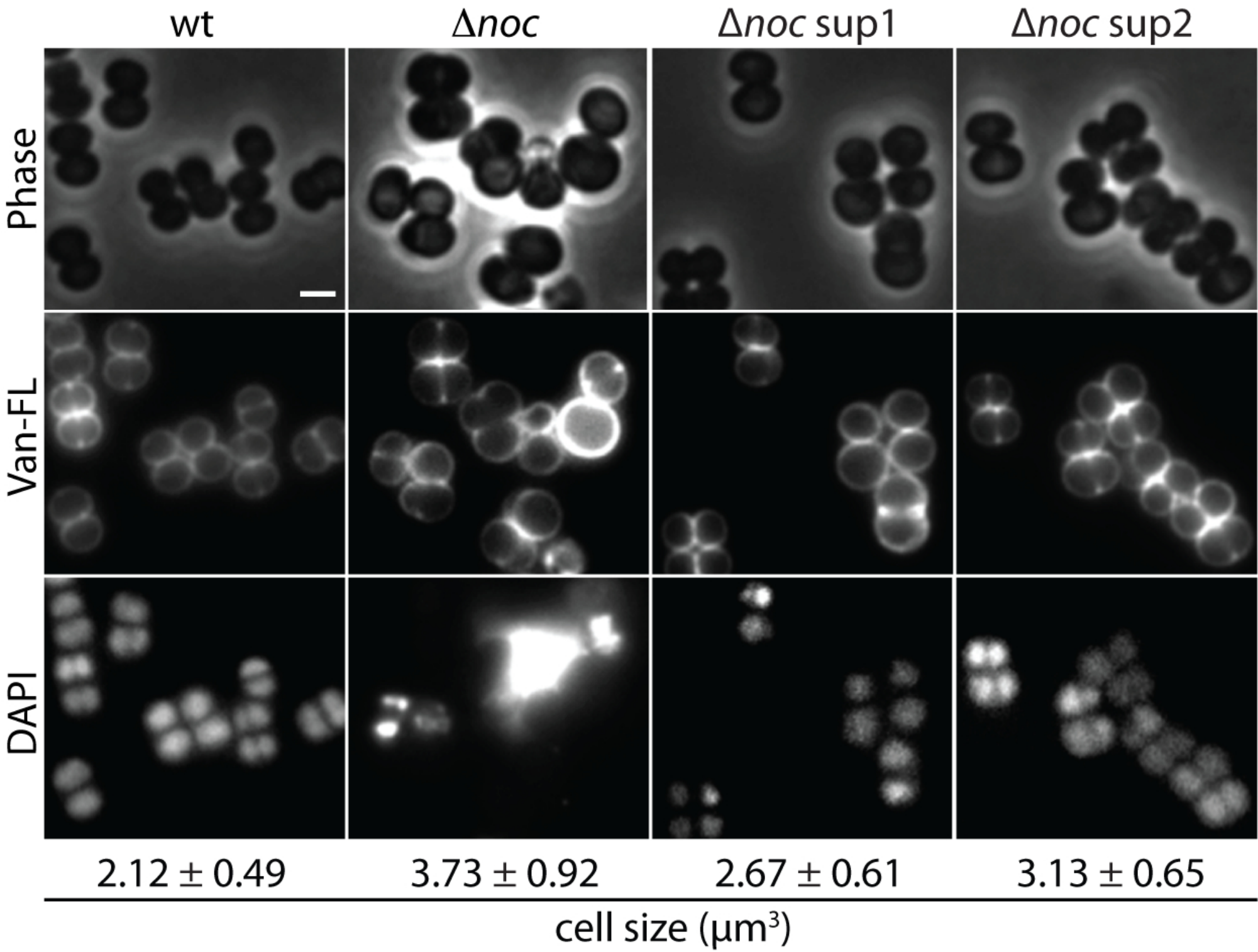

Time after PC190723 addition
0h
0.5h
1.5h
wt
Phase
PI
Δnoc
Phase
PI

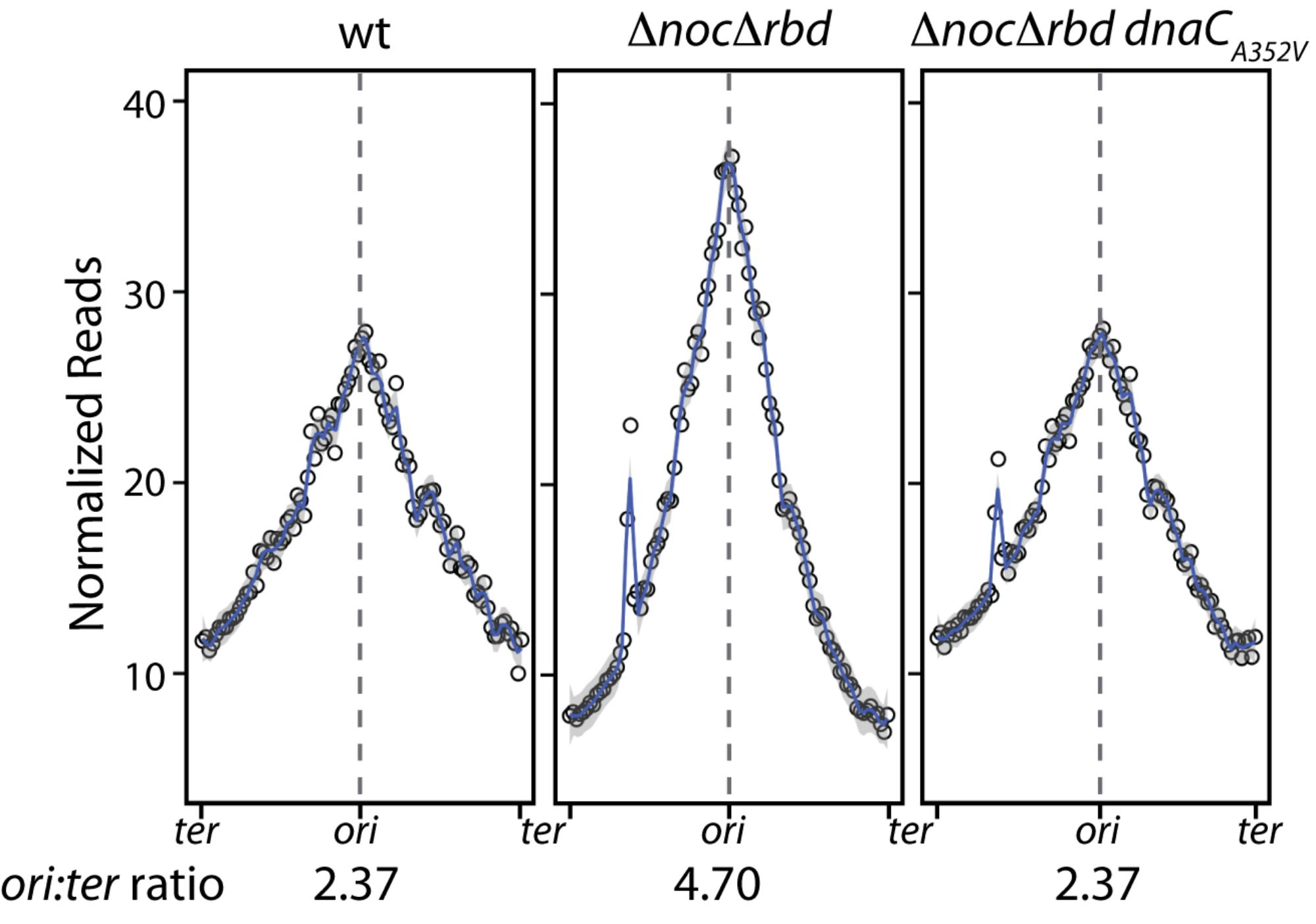

Δnoc P_{xyl}-^{Bs}noc-yfp
Δnoc P_{xyl}-^{Sa}noc-yfp


anti-His6
wt
Δnoc P tet -Sa noc his
Δnoc
Δnoc P tet -Bs noc his
ChIP/Input
0
10
20
30
40
50
-1400
-700
ori
700
1400
genome position (kb)


Δnoc P spank -Bs noc his
Δnoc P spank -Sa noc his
Normalized ChIP/Mock
0
10
20
30
40
0
20
40
60
80
100
120
-2000
-1000
ori
1000
2000
genome position (kb)


vector
DnaA↑
Δnoc vector
Δnoc DnaA↑
aTc (ng/ml)
0
1
0
1
0
1
0
1
Phase
Van-FL
2.48 ± 0.49
2.24 ± 0.51
2.25 ± 0.53
2.59 ± 0.54
4.37 ± 1.04
3.94 ± 0.97
4.55 ± 1.03
6.31 ± 1.23
cell size (μm³)


wt
Δnoc
Δnoc sup1
Δnoc sup2
Phase
Van-FL
DAPI
2.12 ± 0.49
3.73 ± 0.92
2.67 ± 0.61
3.13 ± 0.65
cell size (μm³)


wt
ΔnocΔrbd
ΔnocΔrbd dnaC A352V
Normalized Reads
40
30
20
10
ter
ori
ter
ter
ori
ter
ter
ori
ter
ori:ter ratio
2.37
4.70
2.37