

Supplementary Information

Natural transformation allows transfer of SCC mec -mediated methicillin resistance in *Staphylococcus aureus* biofilms

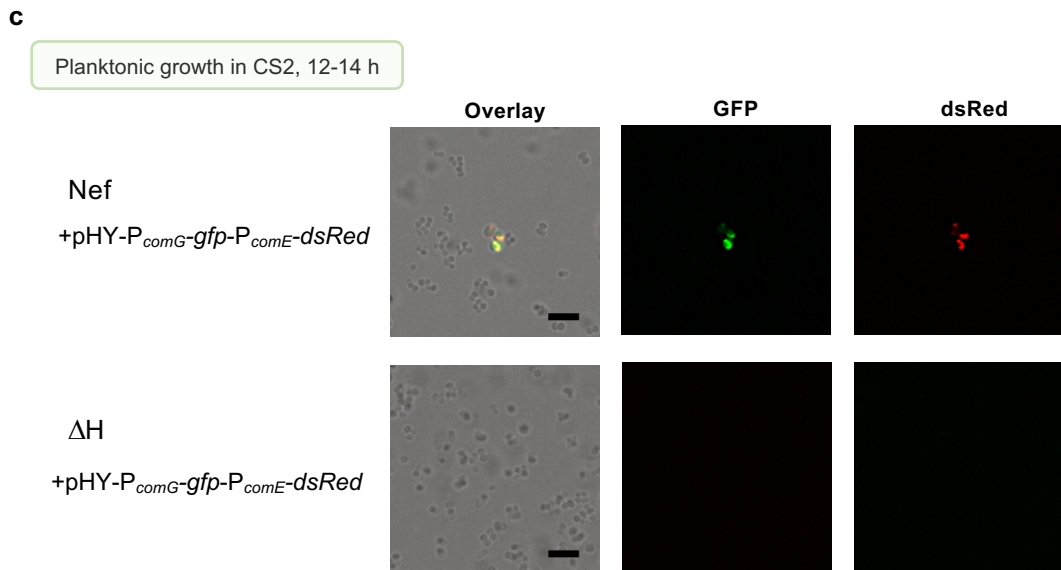
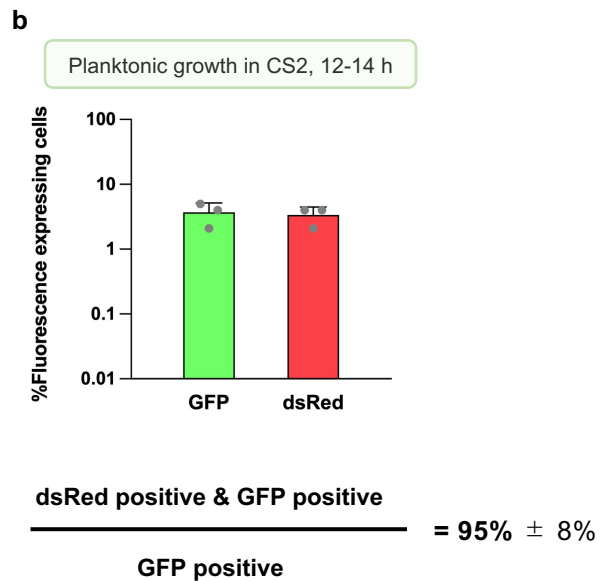
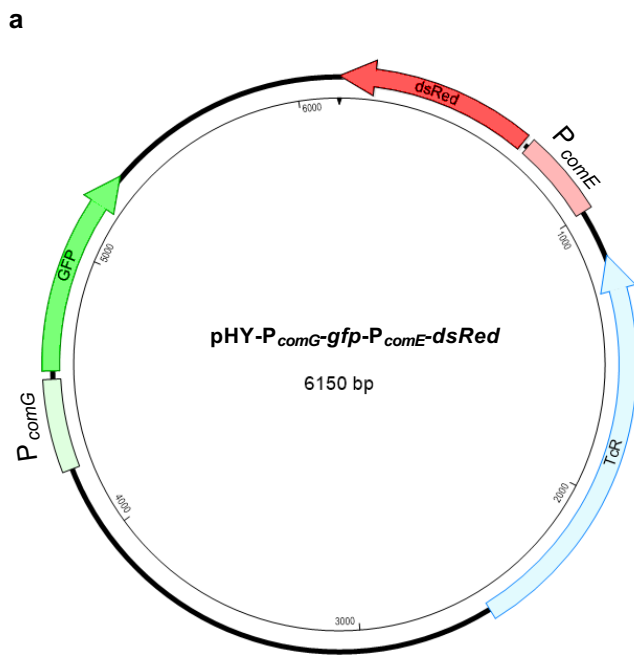
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Supplementary References



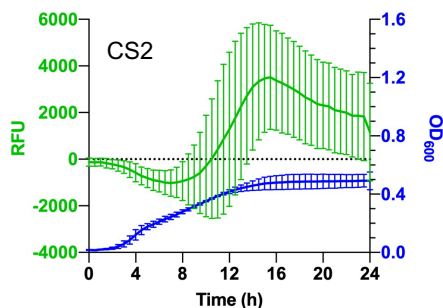
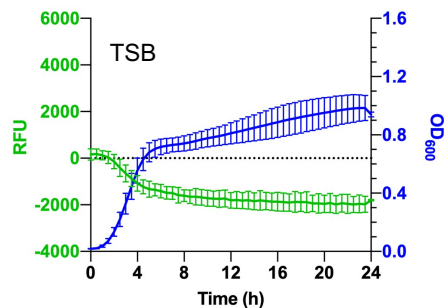
Supplementary Figure 1. P_{comG} activity is a proper indicator of competence genes expression.

a, Schematic map of the dual reporter plasmid *pHY-P_{comG}-gfp-P_{comE}-dsRed*.

b, Nef carrying *pHY-P_{comG}-gfp-P_{comE}-dsRed* was grown in CS2 medium with shaking. The population percentage expressing the reporter was determined after 12-14 h of growth by fluorescent microscopy. At least 100 cells were counted in each independent experiment. The mean of $n = 3$ independent experiments is shown with SD. 95% of GFP positive cells were also positive in dsRed, indicating that P_{comG} activity is a suitable indicator for competence gene expression.

c, Fluorescence microscopy images of Nef and ΔH carrying *pHY-P_{comG}-gfp-P_{comE}-dsRed*. No signals were detected from ΔH carrying *pHY-P_{comG}-gfp-P_{comE}-dsRed*. Scale bars, 5 μm.

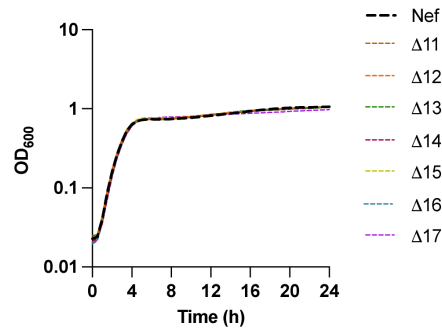
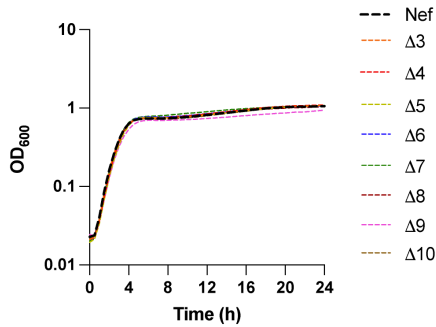
The experiment was repeated at least three times independently with similar results. Source data are provided as a Source Data file.

a**b****Supplementary Figure 2. Reporter assay for *comG* promoter activity.**

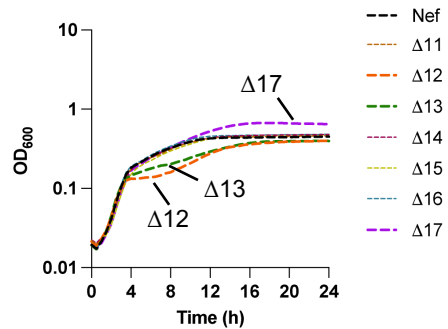
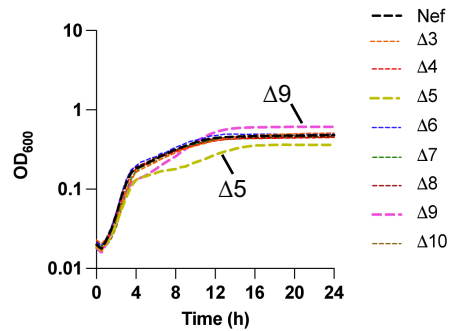
Nef carrying the P_{comG} -*gfp* reporter was grown in either CS2 medium (a) or TSB (b) with shaking. Fluorescence intensity (green line) and OD₆₀₀ (blue line) were measured every 30 min. The mean of $n = 3$ independent experiments is shown with SD. Source data are provided as a Source Data file.

a

Planktonic growth in TSB

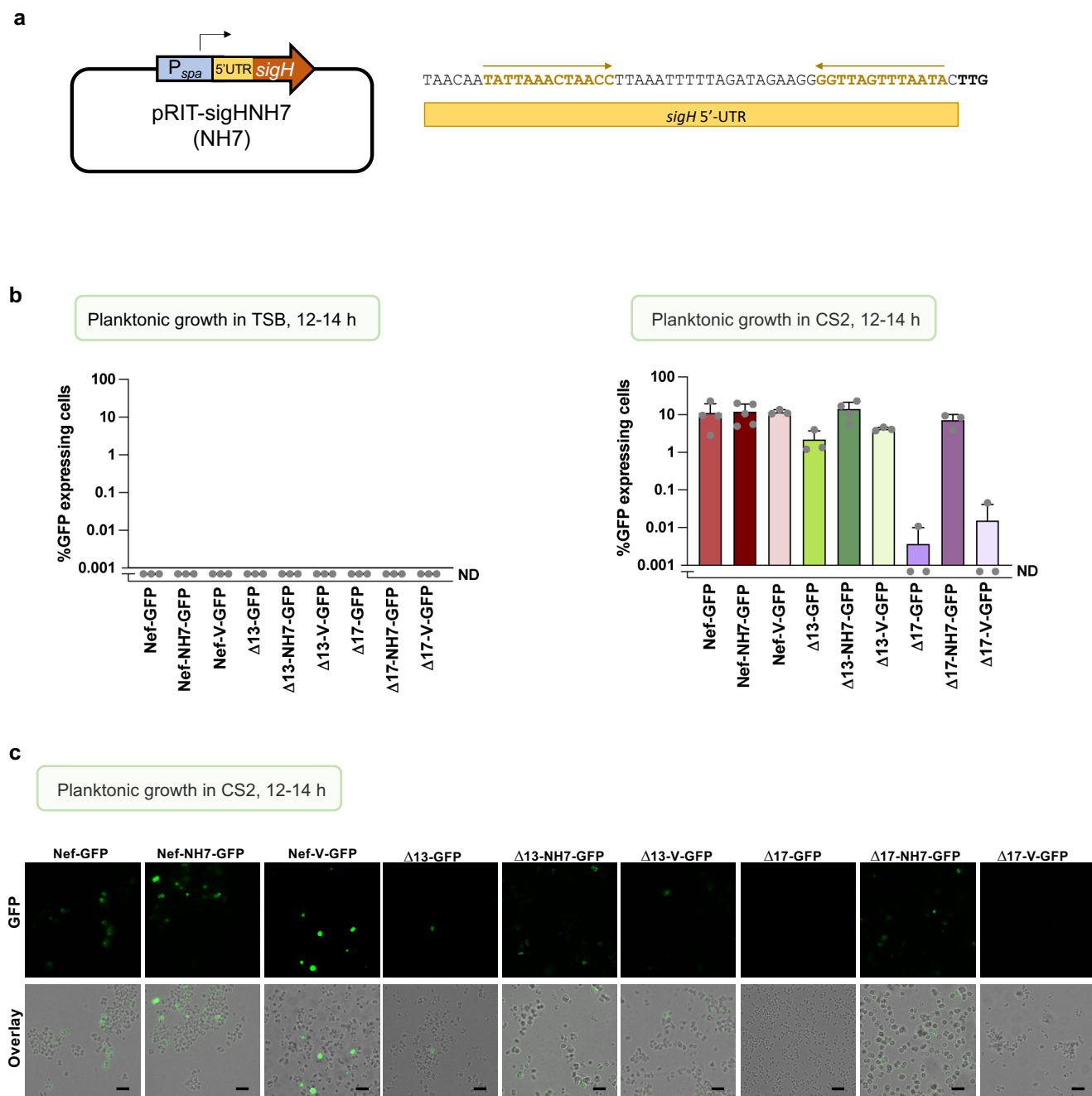
**b**

Planktonic growth in CS2



Supplementary Figure 3. Growth curves of Nef and its derivative Δ TCS.

Cells were grown in either TSB (a) or CS2 medium (b) with shaking. OD₆₀₀ was measured every 30 min. The mean of $n = 3$ independent experiments is shown. Error bars are omitted for clarity, but all standard deviations did not exceed ± 0.144 . Source data are provided as a Source Data file.

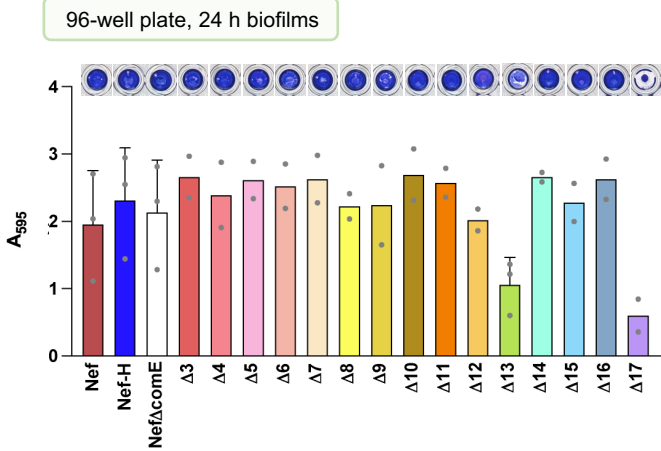
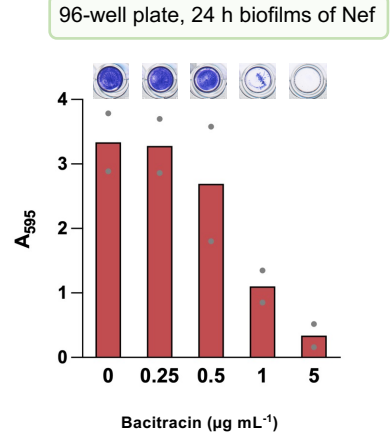
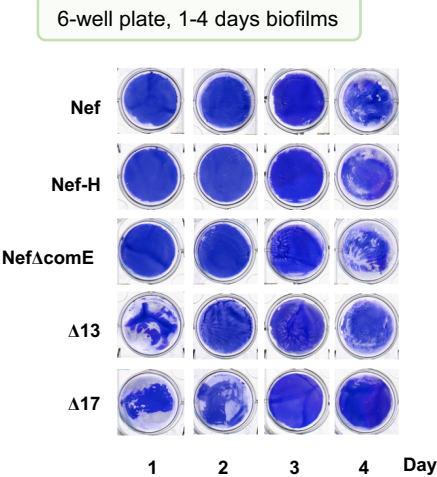


Supplementary Figure 4. Absence of TCS13 and TCS17 can be compensated for by *sigH* expression in P_{comG} expression.

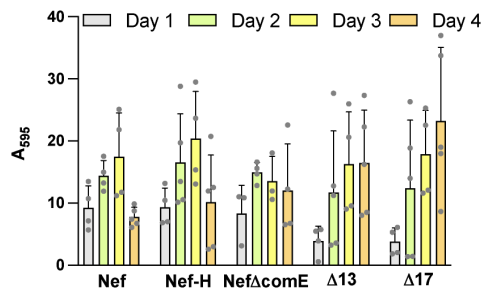
a, Schematic map of pRIT-sigHNH7 plasmid (NH7). The *sigH* mRNA including its 5'-UTR region is constitutively expressed under the *spa* promoter (P_{spa}). The *sigH* 5'-UTR includes inverted repeat sequences that suppress SigH translation.

b, The population percentage of Nef and its derivatives expressing P_{comG} -*gfp* reporter with or without NH7 plasmid was determined after 12-14 h of growth by fluorescent microscopy. The vector control strains are shown by "-V-" within the strain name, e.g., Nef-V-GFP. Cells were grown in TSB or CS2 medium with shaking. At least 200 cells were counted in each experiment. The mean of at least $n = 3$ independent experiments is shown with SD. ND: none detected.

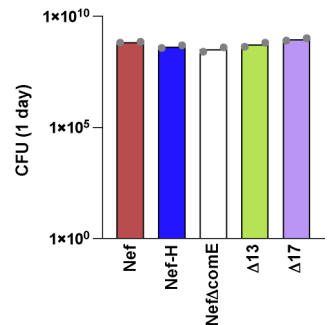
c, Fluorescence microscopy images of the cells grown in CS2 for 12-14 h. Scale bars, 5 μ m. The experiment was repeated at least three times independently with similar results. Source data are provided as a Source Data file.

a**c****b**

6-well plate, 1-4 days biofilms



6-well plate, CFU of day 1 biofilm



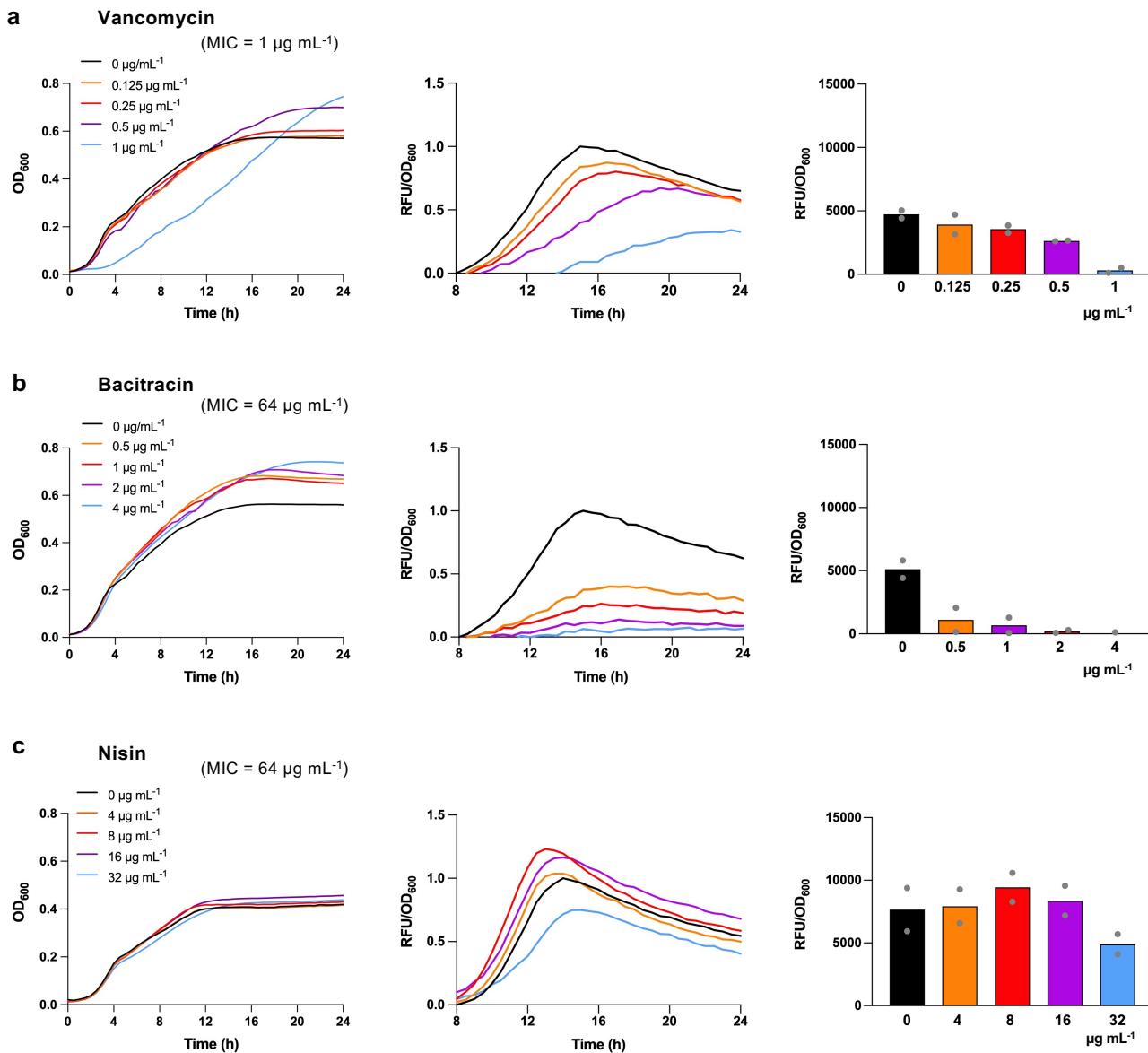
Supplementary Figure 5. Biofilm formation is impaired in the TCS13 and TCS17 mutants.

a, Nef and its derivatives were statically grown in CS2 medium in a 96-well plate for 24 h. The biofilm was quantified as described in Methods. The mean of at least $n = 2$ independent experiments is shown. Error bars represent SD. **b**, left and middle, Time-course of biofilm quantity in Nef and its derivatives. The cells were statically grown in CS2 medium in a 6-well plate for up to 4 days. The mean of at least $n = 3$ independent experiments is shown with SD.

b, right. CFU of the whole cells after 1 day (without washing non-biofilm cells).

c, Low concentration of bacitracin prohibits Nef biofilm formation in CS2 medium. The mean of $n = 2$ independent experiments is shown. Source data are provided as a Source Data file.

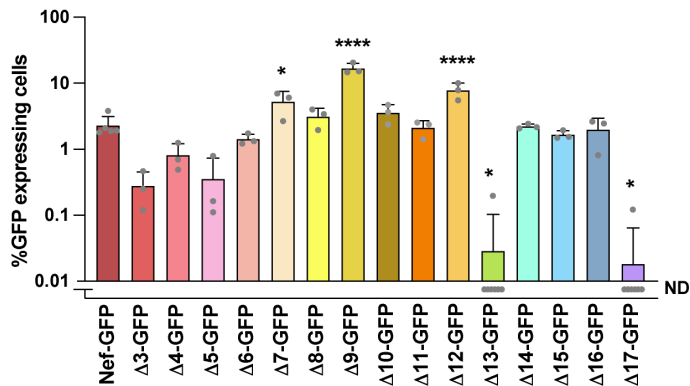
Planktonic growth in CS2



Supplementary Figure 6. *comG* promoter activity is affected by cell wall-targeting antibiotics.

Nef-GFP was grown with vancomycin (a), bacitracin (b), or nisin (c) in CS2 medium with shaking for 24 h. Fluorescence (RFU) and OD₆₀₀ were measured every 30 min. Data shown are OD₆₀₀ values (left panels), RFU/OD₆₀₀ values (middle panels) or increases in RFU/OD₆₀₀ values during 8-24 h of growth (right panels). The mean of $n = 2$ independent experiments is shown. Source data are provided as a Source Data file.

3-day biofilm in CS2



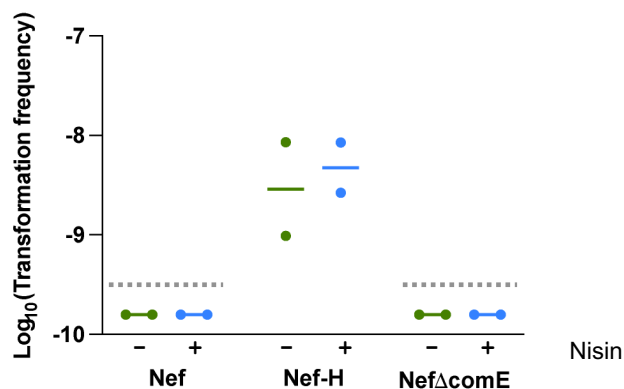
Supplementary Figure 7. *comG* promoter activity is affected by multiple TCSs in biofilm.

Nef and its derivative Δ TCSs carrying the P_{comG} -*gfp* reporter were statically grown in CS2 medium for 3 days. The percentage of GFP-expressing cells was calculated as observed by fluorescence microscopy. Bars represent the mean of at least $n = 3$ independent experiments with error bars indicating SD. ND: none detected. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparison test. Nef-GFP vs Δ 7-GFP * $P = 0.0107$, Nef-GFP vs Δ 13-GFP * $P = 0.0159$, Nef-GFP vs Δ 17-GFP * $P = 0.0152$, **** $P < 0.0001$. Source data are provided as a Source Data file.

Donor: purified plasmid pHY300PLK (tet^R)

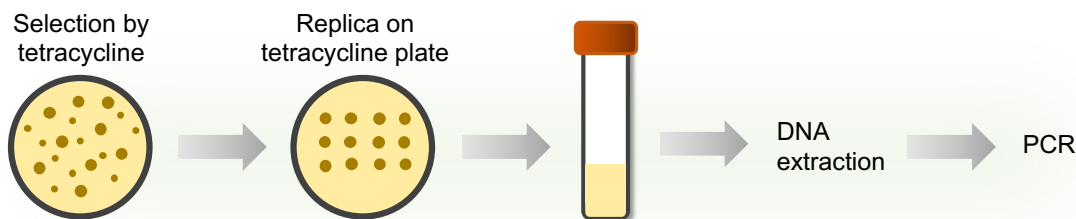
Planktonic in CS2

Selection: tetracycline



Supplementary Figure 8. Nisin has no detectable effect on natural transformation.

Nef and its derivatives were grown in CS2 medium with or without nisin (8 $\mu\text{g mL}^{-1}$). Transformation frequencies were determined after 10 h of planktonic growth. The transformants were selected by tetracycline. The dotted line represents the detection limit. The mean of $n = 2$ independent experiments is shown. Transformation remained undetectable, regardless of the nisin treatment, and there were no significant effects in the SigH-overexpressing strain (Nef-H). Source data are provided as a Source Data file.

a

Donor: Heat-killed *NefΔcls2-tet^R* cells

Recipient: *Nef-pRIT5H*

biofilm in CS2

Selection: tetracycline

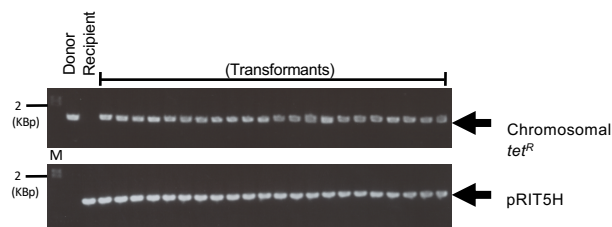
Donor: Heat-killed *Nef* (no tetracycline resistance gene) cells

Recipient: *Nef-pRIT5H*

biofilm in CS2

Selection: tetracycline

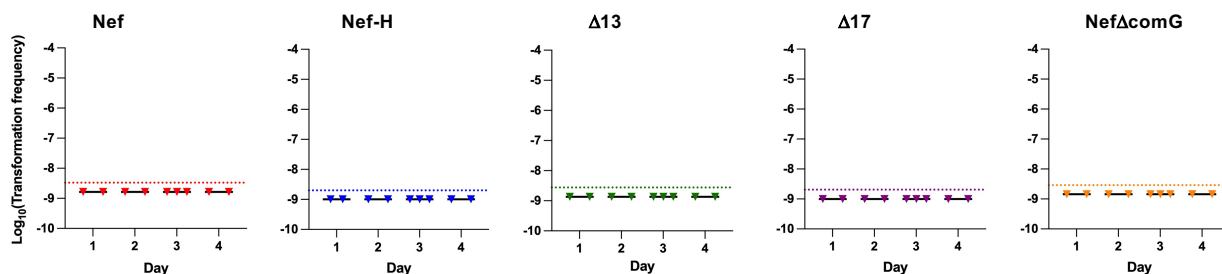
PCR check

**b**

Donor: Heat-killed *Nef* (no tetracycline resistance gene) cells

biofilm in CS2

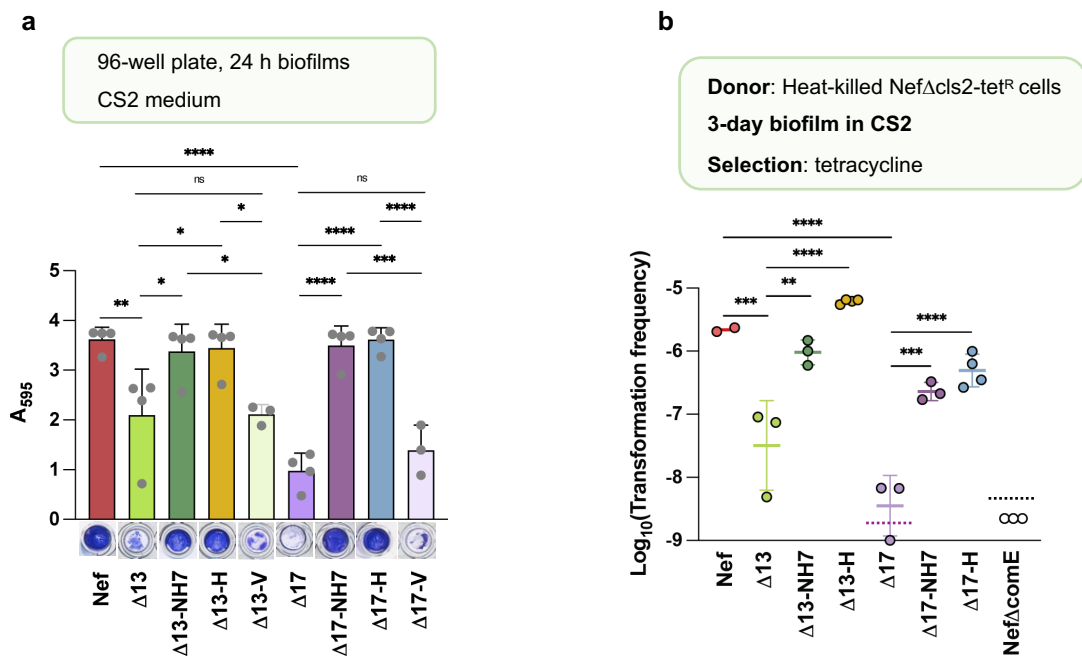
Selection: tetracycline



Supplementary Figure 9. Spontaneous tetracycline resistant colonies do not emerge.

a, Transformants were selected by tetracycline. Emergent colonies were replicated on fresh tetracycline plates. These replicate colonies were then inoculated in liquid cultures followed by DNA extraction for PCR. A representative image of the colonies following the initial tetracycline selection is shown. In the absence of tetracycline resistance gene in donor (*Nef*), no colony emerges. The tetracycline resistance gene can be detected by PCR in the transformants as well as in the donor (*NefΔcls2-tet^R*). The pRIT5H plasmid in the recipient (*Nef-pRIT5H*) was detected in the transformants. M: DNA size marker, λ HindIII.

b, When a heat-killed *Nef* donor (with no tetracycline resistance gene) is used, no colonies emerge in any recipient. *Nef* and its derivatives were statically grown in CS2 medium. Transformation frequencies were determined every 24 h. Dotted lines represent the detection limit. Data points represent independent experiments. Source data are provided as a Source Data file.

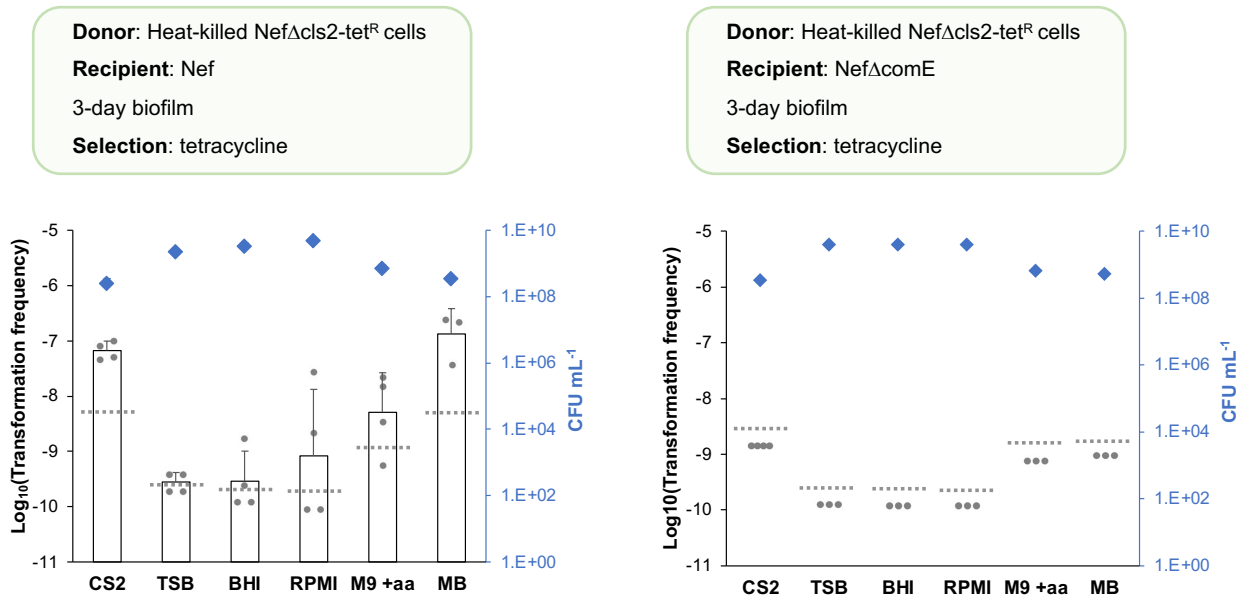
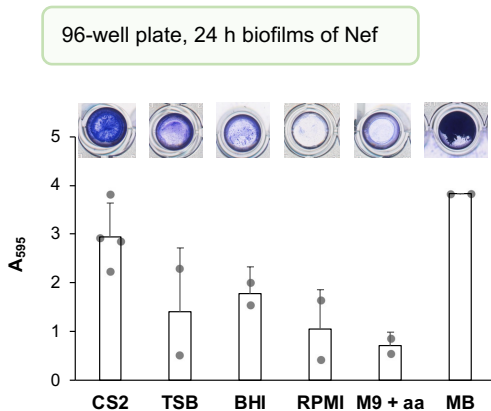


Supplementary Figure 10. Absence of TCS13 and 17 can be compensated for by expression of *sigH* in biofilm formation and transformation.

a, Quantification of 24 h biofilms of Nef and its derivatives. The *sigH* was overexpressed by pRIT-sigH^{NH7} (shown by “-NH7” in strain names) or SigH by pRIT-sigH (“-H”): see Supplementary Figure 4 regarding these plasmids. Vector controls are shown by “-V”. The mean of at least $n = 3$ independent experiments is shown with SD. The *sigH*/SigH overexpression restored the biofilm formation in both Δ13 and Δ17, while vector control did not. Statistical significance was determined by one-way ANOVA with Tukey’s multiple comparison test. Nef vs. Δ13 $^{**}P=0.0044$, Δ13 vs. Δ13-NH7 $^{*}P=0.0243$, Δ13 vs. Δ13-H $^{*}P=0.0156$, Δ13-V vs. Δ13NH7 $^{*}P=0.0497$, Δ13-V vs. Δ13-H $^{*}P=0.0337$, Δ17-V vs. Δ17-NH7 $^{***}P=0.0002$, $^{****}P<0.0001$, ns $P>0.9$.

b, Transformation frequencies of Nef and its derivatives were determined at day 3 in the biofilm growth condition. Heat-killed NefΔcls2-tet^R donor was used. Transformants were selected by tetracycline. The dotted lines represent the detection limit. The mean of at least $n = 2$ independent experiments is shown. Error bars represent SD. ND: None detected, $<10^{-9}$. The *sigH*/SigH overexpression restored the transformation frequencies in both Δ13 and Δ17. NefΔcome served as a negative control for the transformation assay. Statistical significance was determined by one-way ANOVA with Tukey’s multiple comparison test. Nef vs. Δ13 $^{***}P=0.0002$, Δ13 vs. Δ13-NH7 $^{***}P=0.0007$, $^{****}P<0.0001$.

Source data are provided as a Source Data file.

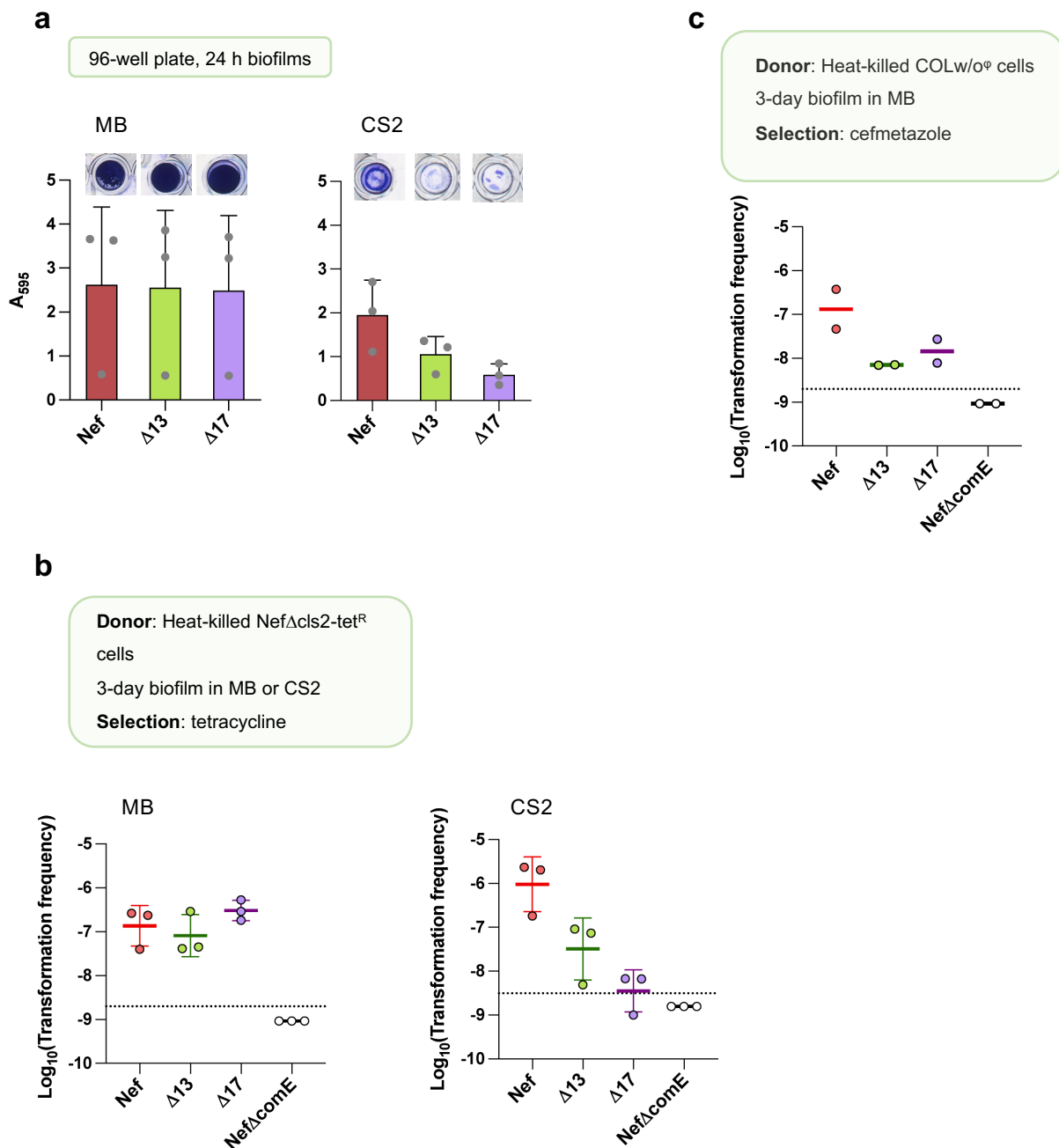
a**b**

Supplementary Figure 11. Biofilm formation and natural genetic transformation in distinct growth media.

a, Nef (left) and NefΔcomE (right) were statically grown in different growth media for 3 days. Transformation frequencies (bars) and CFU mL⁻¹ (blue) were determined after 3 days. A heat-killed NefΔcls2-tet^R donor was used. Transformants were selected by tetracycline. The dotted lines represent the detection limit. The mean of at least $n = 3$ independent experiments is shown with SD.

b, Biofilm formation of Nef was assessed in 96-well plates after static growth for 24 h in different growth media. The mean of at least $n = 2$ independent experiments is shown.

Source data are provided as a Source Data file.



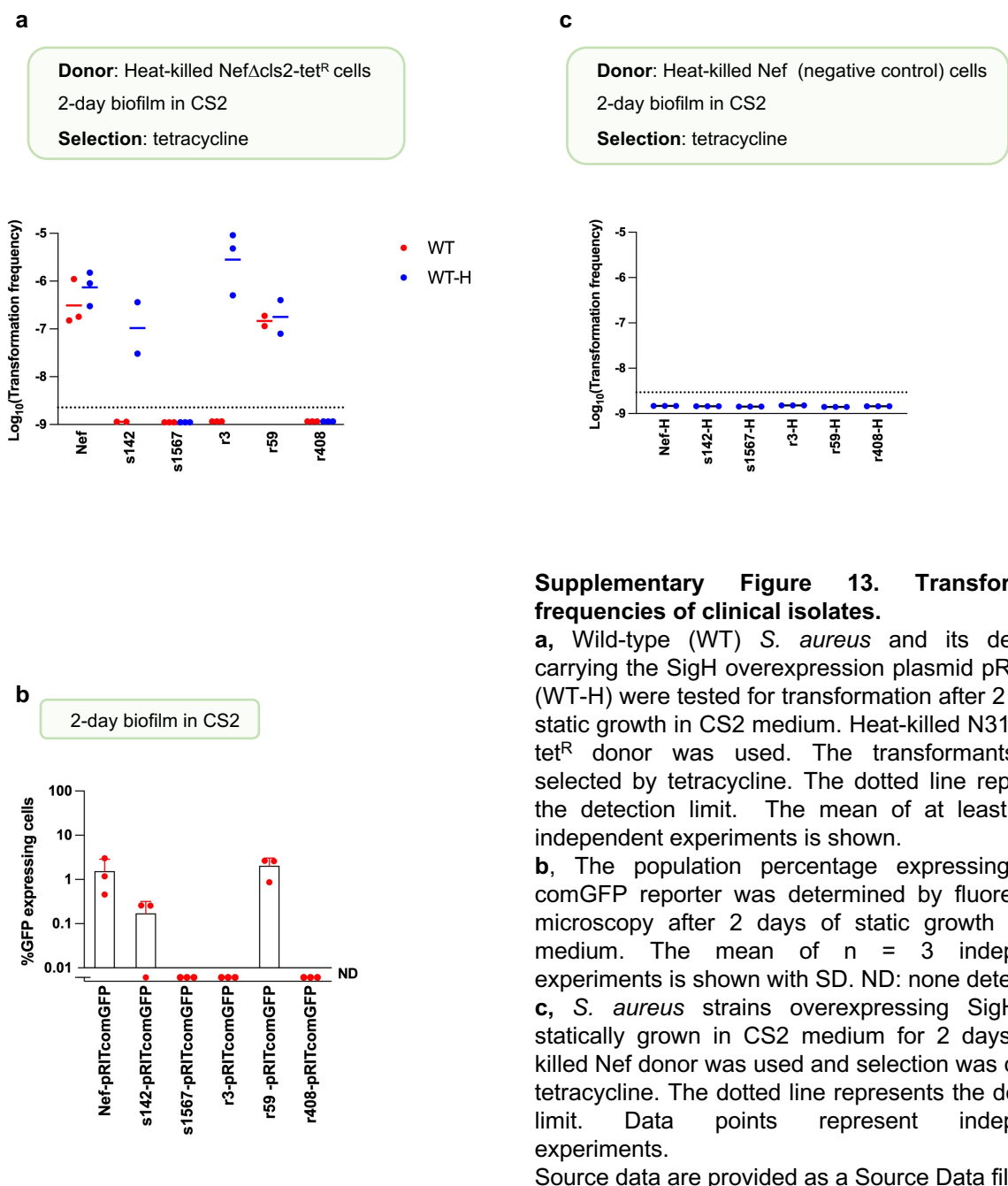
Supplementary Figure 12. TCS13 and TCS17 are dispensable for transformation in MB.

a-b, Cells were statically grown in MB (whole milk & BHI medium [1:1]) or CS2. **a**, Biofilm formation was assessed in 96-well plates after 24 h. The mean of $n = 3$ independent experiments is shown with SD.

b, Transformation frequencies were determined after 3 days. A heat-killed *NefΔcls2-tet^R* donor was used. Transformants were selected by tetracycline. Dotted lines represent the detection limit. The mean of $n = 3$ independent experiments is shown with SD.

c, Cells were statically grown in MB. Transformation frequencies were determined after 3 days. A heat-killed COLw/oφ donor was used. Transformants were selected by cefmetazole. Dotted lines represent the detection limit. The mean of $n = 2$ independent experiments is shown.

Source data are provided as a Source Data file.



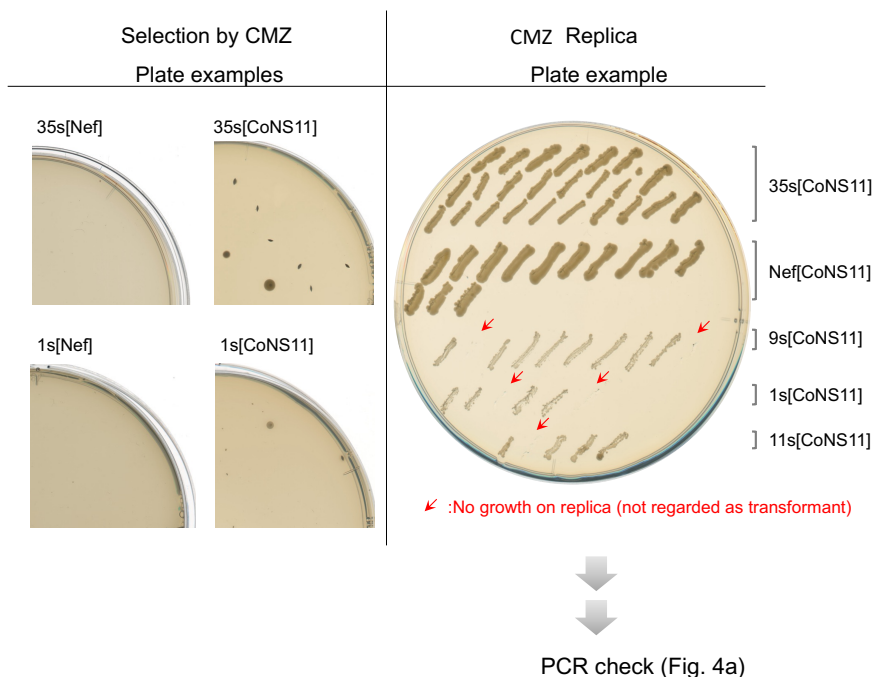
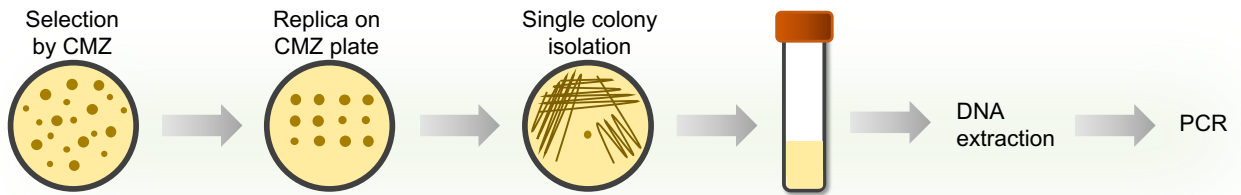
Supplementary Figure 13. Transformation frequencies of clinical isolates.

a, Wild-type (WT) *S. aureus* and its derivative carrying the SigH overexpression plasmid pRIT-sigH (WT-H) were tested for transformation after 2 days of static growth in CS2 medium. Heat-killed N315 Δ cls2-tet^R donor was used. The transformants were selected by tetracycline. The dotted line represents the detection limit. The mean of at least $n = 2$ independent experiments is shown.

b, The population percentage expressing pRIT-comGFP reporter was determined by fluorescence microscopy after 2 days of static growth in CS2 medium. The mean of $n = 3$ independent experiments is shown with SD. ND: none detected.

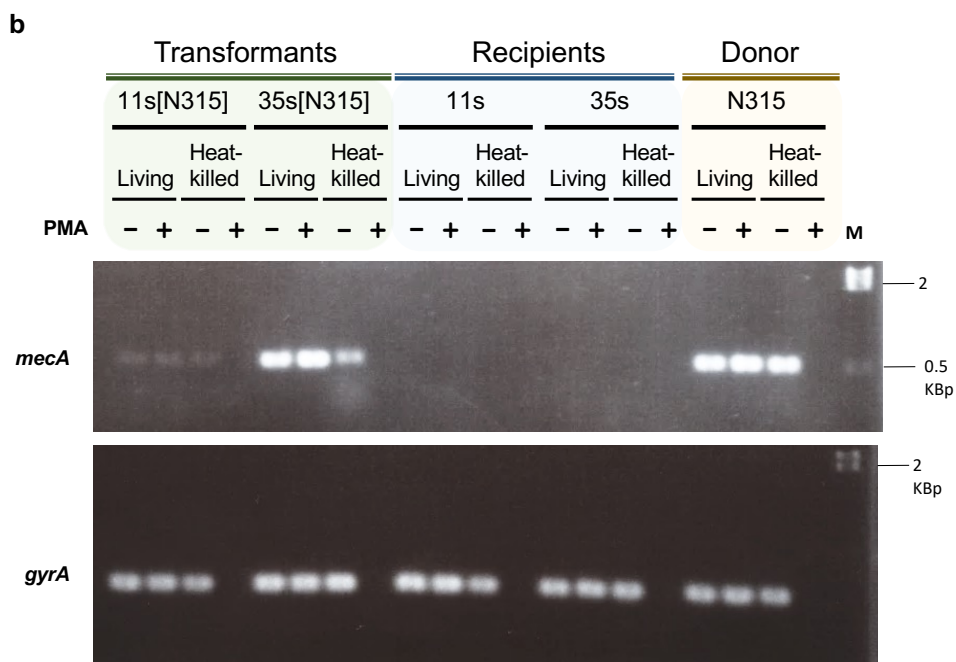
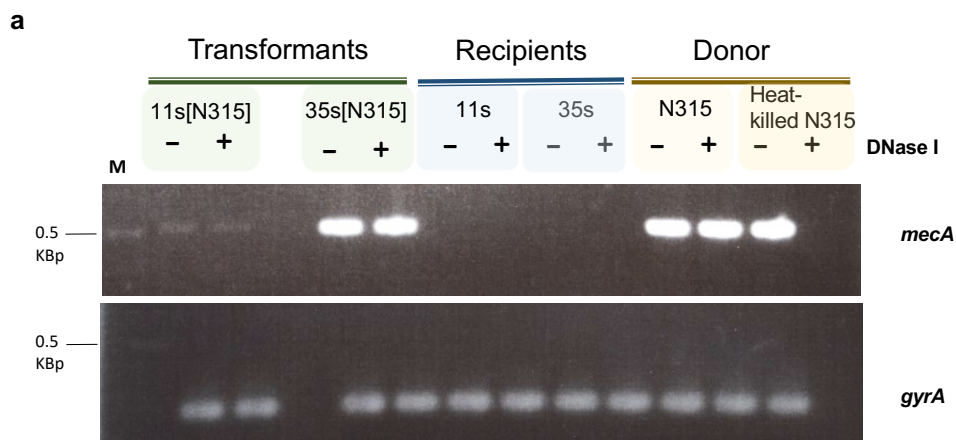
c, *S. aureus* strains overexpressing SigH were statically grown in CS2 medium for 2 days. Heat-killed Nef donor was used and selection was done by tetracycline. The dotted line represents the detection limit. Data points represent independent experiments.

Source data are provided as a Source Data file.



Supplementary Figure 14. The *mecA* transformant selection by cefmetazole (CMZ).

After transformation and selection by cefmetazole, generated colonies are replicated onto fresh agar plates containing cefmetazole. In order to prepare a PCR template, cells capable of growth on replica plates undergo single colony isolation and liquid culture to avoid any contamination of donor DNA. Images show representative plates following initial selection by cefmetazole (left) and replication (right). Tiny colonies tend not to grow on replica plates and are not counted as transformants (red arrows). Stable transformants make rigid colonies on replica plates (e.g. 35s[CoNS11]) but unstable ones also grow on replica plates (e.g. 9s[CoNS11]).



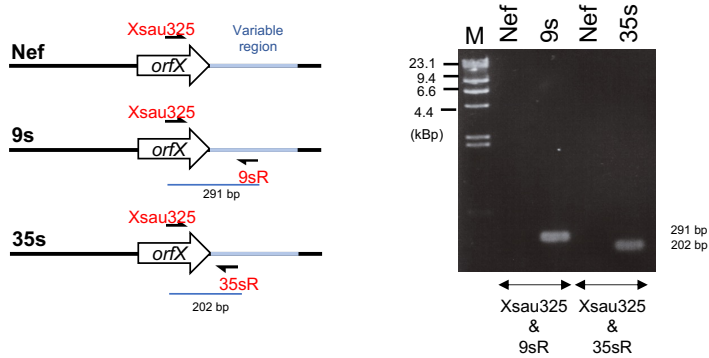
Supplementary Figure 15. Confirmation of *mecA* incorporation into cells.

Living and heat-killed cells were treated with 50 $\mu\text{g mL}^{-1}$ DNase I for 3 hours at 37 °C (a) or with 50 μM PMA (Propidium monoazide, Biotium, CA) (b), followed by genome extraction. PCR for *mecA* and *gyrA* is shown. M: DNA size marker, λ HindIII.

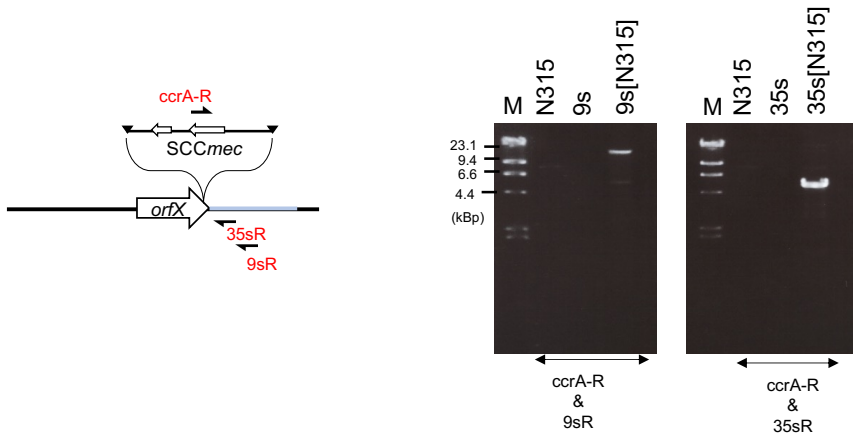
DNase I and PMA treatment abolished PCR amplification in heat-killed cells but were unable to abolish *mecA* PCR signals from unstable transformants, such as 11s[N315], confirming that *mecA* is incorporated into cells. Each experiment was repeated at least twice independently with similar results.

Source data are provided as a Source Data file.

a



b



Supplementary Figure 16. Confirmation of site-specific integration of *SCCmec* in transformants.

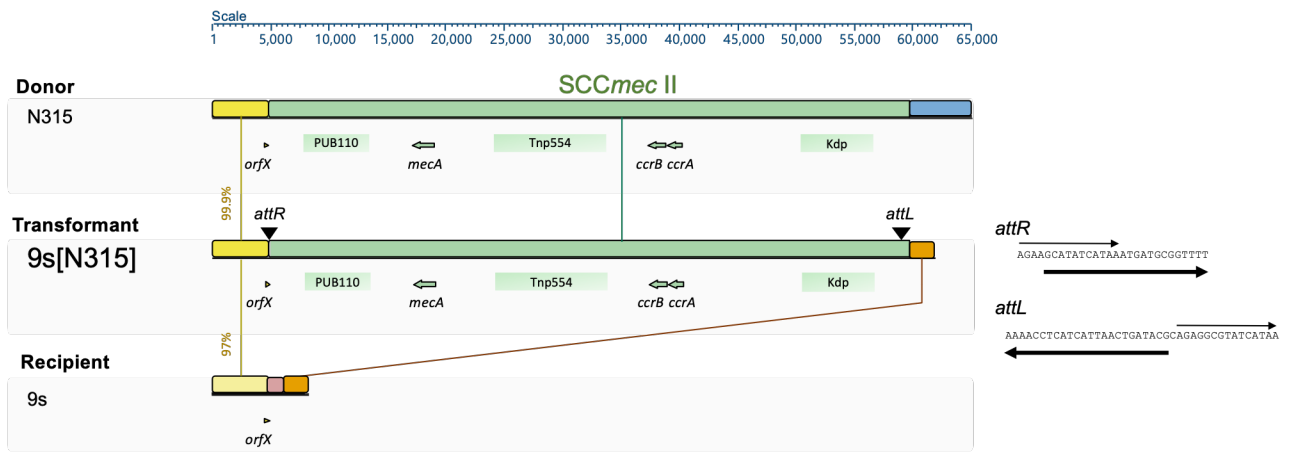
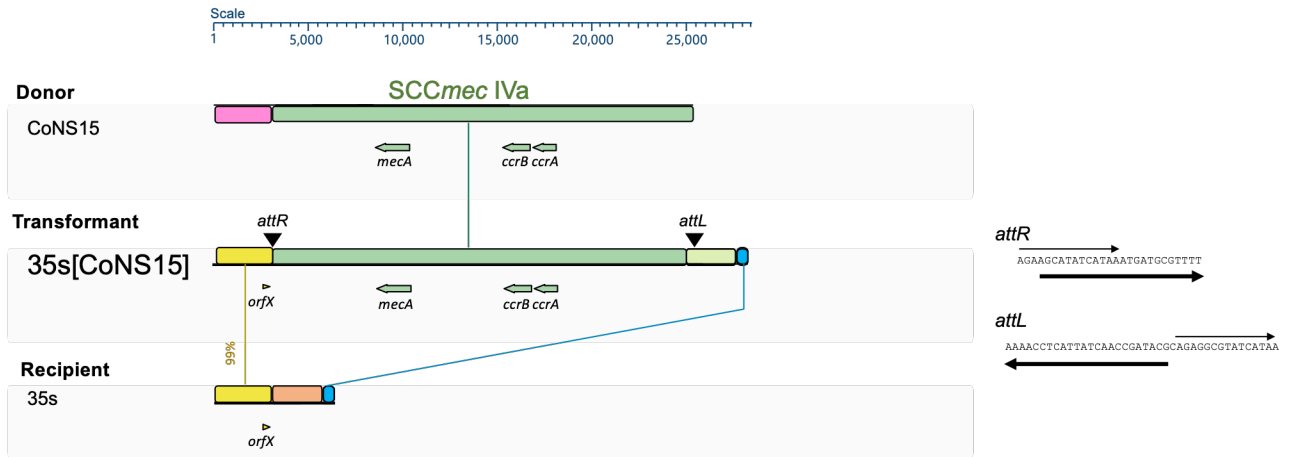
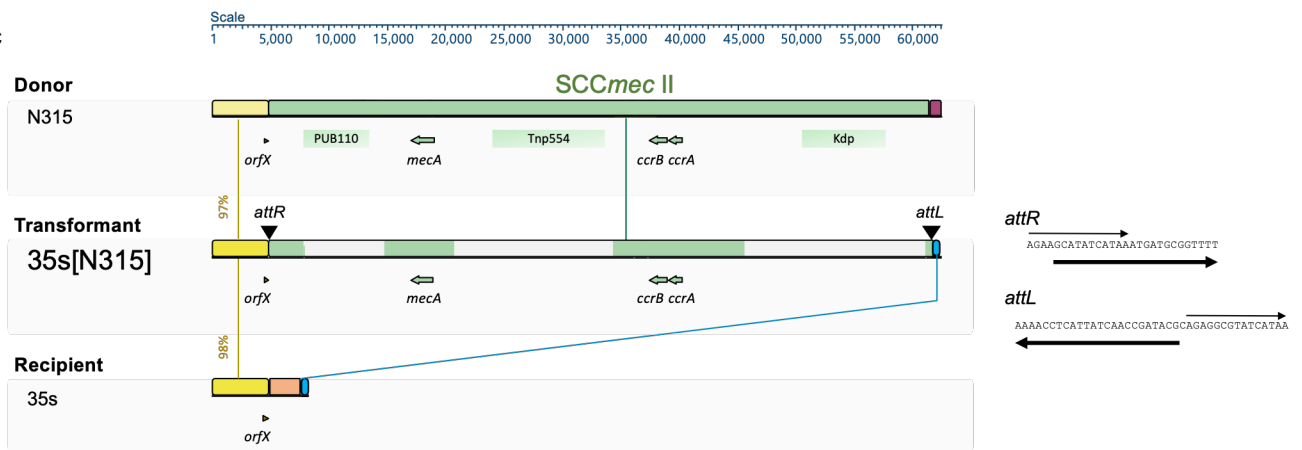
a, Primers 9sR and 35sR are specific for strains 9s and 35s, respectively, and Nef does not have these sequences. The primer Xsau325 is compatible for Nef (as shown in Figure 4) as well as for 9s and 35s.

b, The primer *ccrA-R* is designed for the *ccrA* region in *SCCmec* type II in N315. M: DNA size marker, λ HindIII.

The experiment was repeated at least twice independently with similar results.

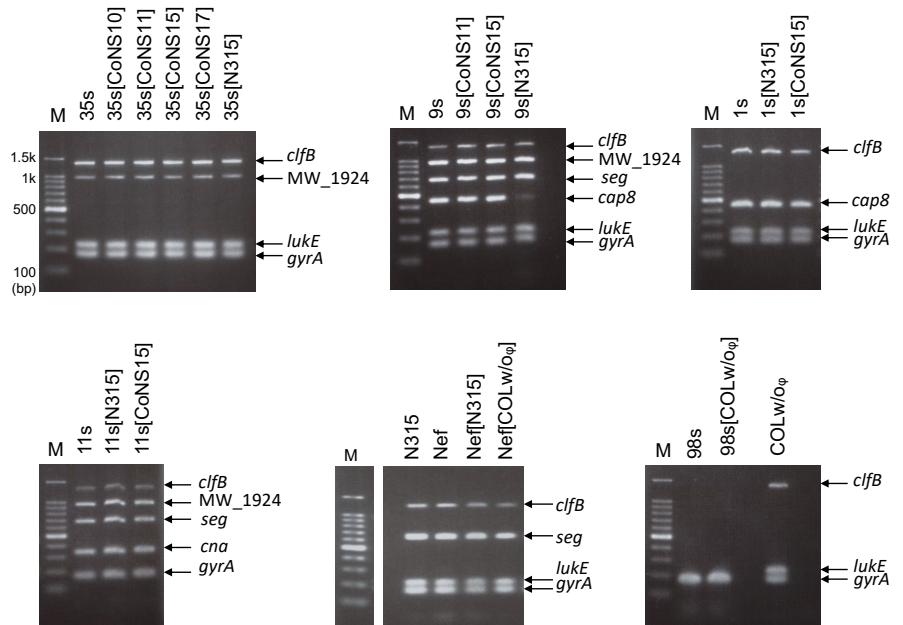
The PCR with 9sR or 35sR primer, together with *ccrA-R*, confirmed the site-specific integration of *SCCmec* in the chromosome of transformants.

Source data are provided as a Source Data file.

a**b****c**

Supplementary Fig. 17. Confirmation of SCC integration into chromosome by genome sequencing.

Sequenced genome contigs of transformants (a, 9s[N315], b, 35s[CoNS15], c, 35s[N315]) were aligned with known sequences or contigs of donors, and recipients. *SCCmec* region is shown by green. Homologous regions are shown by similar colored blocks and are connected by lines. Upstream regions of transformants 35s[CoNS15] and 35s[N315] were identical to the original recipient sequences, but the upstream region of 9s[N315] was more similar to the donor, implicating additional homologous recombination. The percent identity of the aligned upstream regions (yellow blocks) is shown. In all cases, downstream regions in transformants were identical to the original recipient sequences. The *att* sequences detected in transformants are shown on the right. Although insertion boundaries were sometimes rearranged (e.g. 35s[CoNS15]; pale green region), chromosomal backbones of transformants were identical to the recipients, which was also confirmed by multiplex PCR (Supplementary Fig. 18).

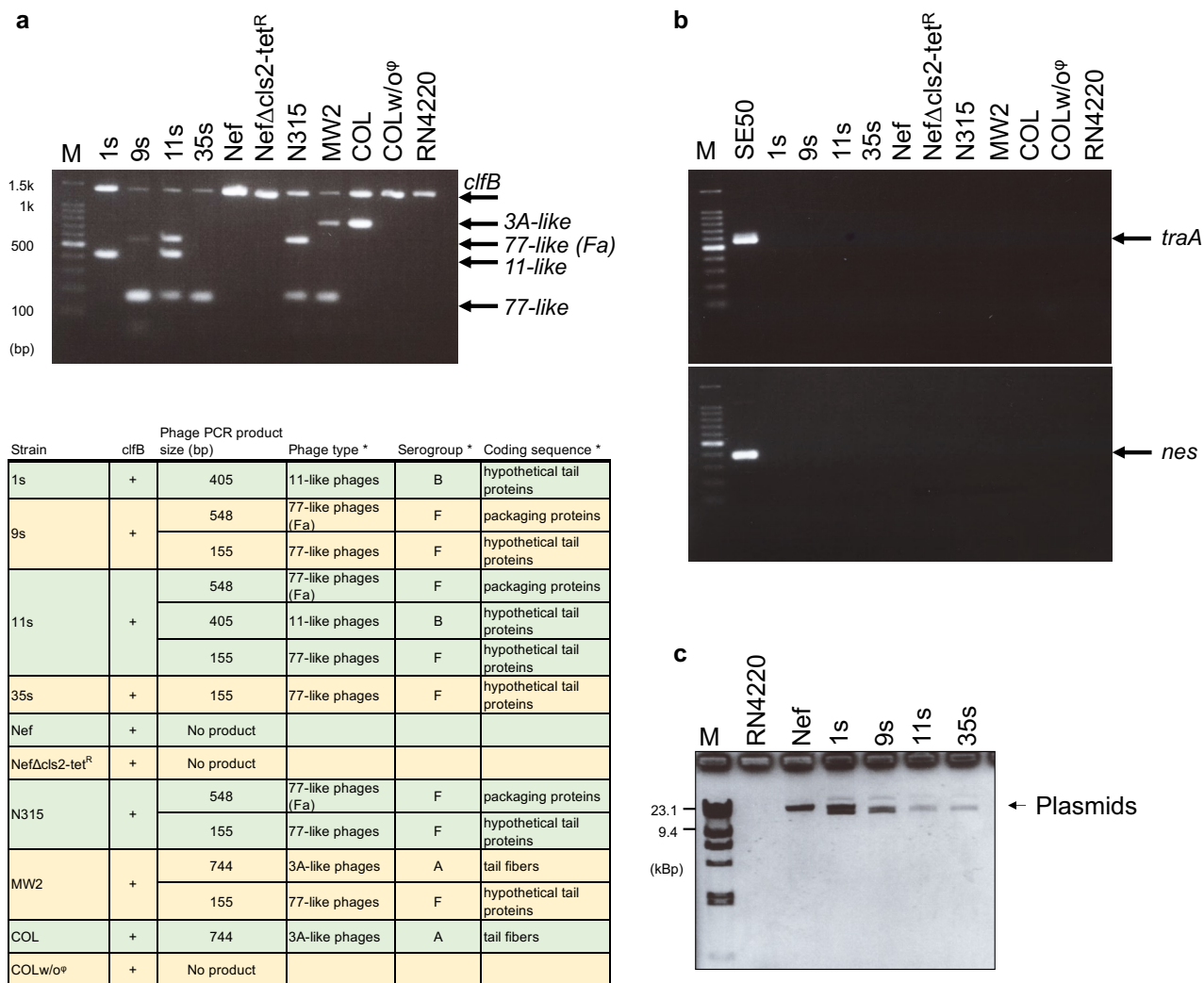
a**b**

Supplementary Figure 18. Validation of the SCCmec transformants.

a, 35s transformants and CoNS donors were replicated onto Mannitol-Salt agar. Mannitol utilization is represented by the yellow color of the medium. Transformants were positive for mannitol utilization while CoNS donors were negative, confirming that these transformants are *S. aureus*.

b, Transformants share the same genetic background as the recipient as validated by multiplex PCR: The amplification patterns are same between transformants and each original recipient. M: 100 bp DNA ladder (Takara). **a**, **b**, The experiments were repeated at least twice independently with similar results.

Source data are provided as a Source Data file.



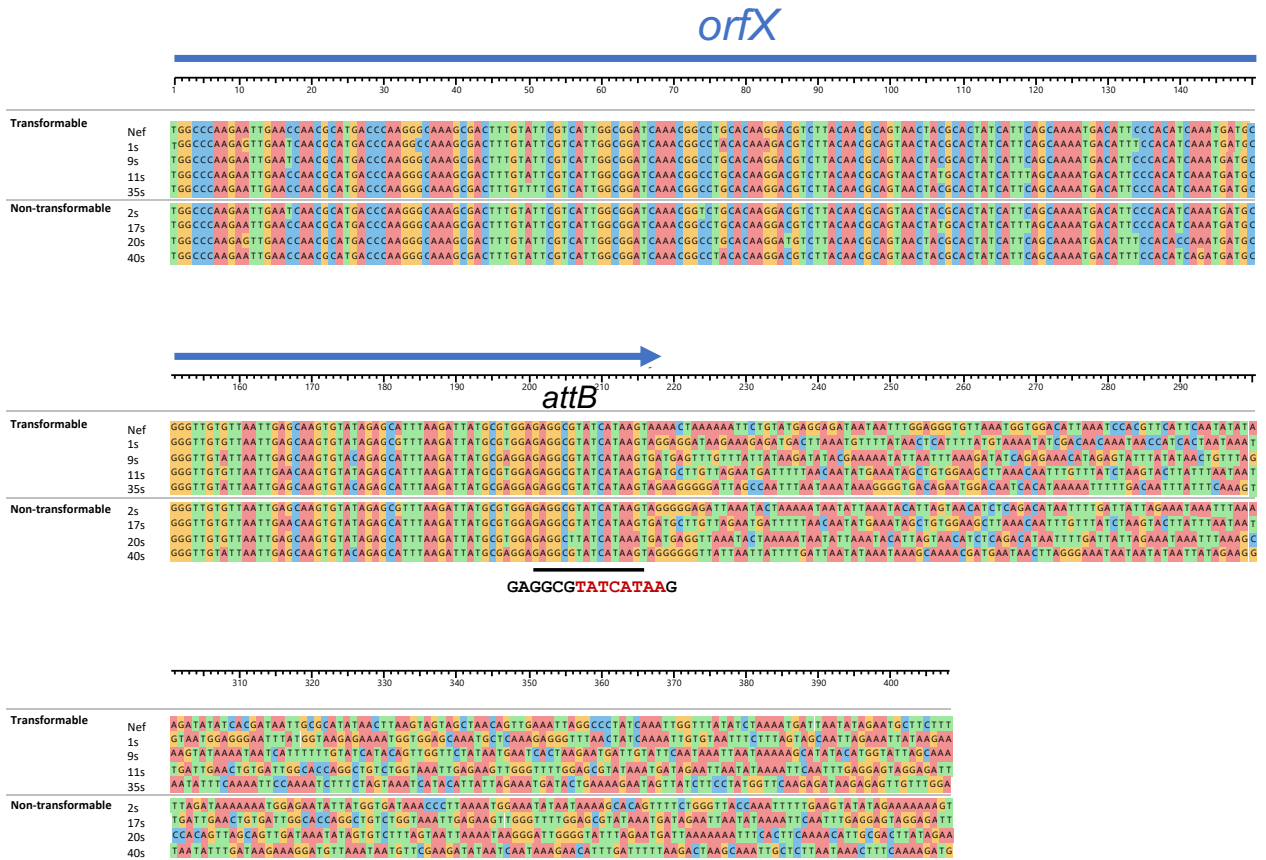
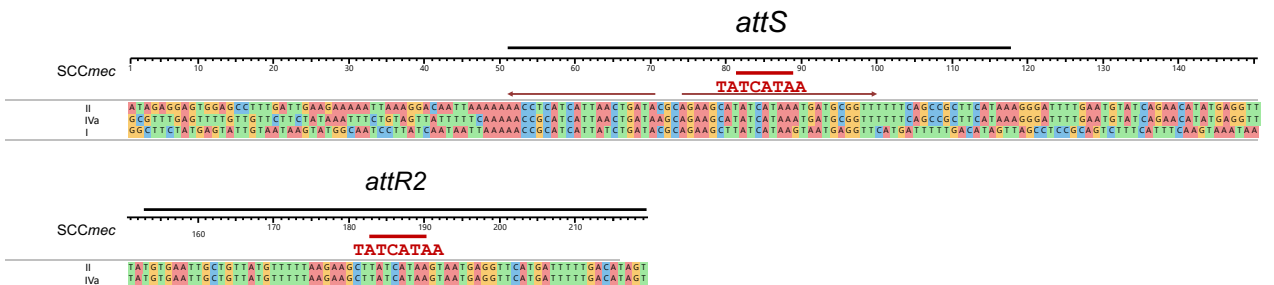
Supplementary Figure 19. Presence of phages, conjugative elements, and plasmids.

Chromosomal DNA of donors and recipients were tested for presence of phage (a) or conjugative elements (b). RN4220 was used as negative control. SE50 was used as positive control for presence of conjugative elements (*traA* and *nes*). M: 100bp marker (Takara). c, Plasmid DNA was purified from log-phase cells. Transformable MSSA isolates (Nef, 1s, 9s, 11s, 35s) have plasmids. RN4220 has no plasmid and was used as a negative control. M: DNA size marker, λ HindIII.

To confirm the absence of conjugative elements (including *traA* and *nes*), PCR using the method and primers described by Cafini et al., 2016 was conducted¹.

*Phages were detected using the multiplex PCR method described by Pantůček et al., 2004².

Source data are provided as a Source Data file.

a**b****Supplementary Figure 20. att sequences in recipients and donors.**

a, Sequences encompassing *attB* site in MSSA strains. The 15 bp core sequence of the *attB* attachment site is shown below the alignment.

b, The *attS* and *attR2* sequences of SCCmec³. Type II (N315), Type IVa (MW2), and Type I (COL) share conserved *attS* core sequence (TATCATAA), which is also found in the other *att* sites. The *attR2* in SCCmec II and IVa can be used for SCC excision but it can also affect the insertion frequency³.

Supplementary Table 1. MIC ($\mu\text{g mL}^{-1}$) of cell wall-targeting antibiotics in CS2, TSB, or MH.

	Vancomycin			Bacitracin		Nisin	
	CS2	TSB	MH	CS2	TSB	CS2	TSB
Nef	1	2	1	64	> 256	64	128
$\Delta 12$	0.25	0.5	0.25	-	-	-	-
$\Delta 12(\text{pHY-12})$	1	1	1	-	-	-	-
$\Delta 12(\text{pHY})$	0.25	0.5	0.5	-	-	-	-
$\Delta 17$	-	-	-	4	8	8	16
$\Delta 17(\text{pHY-17})$	-	-	-	32	256	32	64
$\Delta 17(\text{pHY})$	-	-	-	4	8	8	16

Supplementary Table 2. MSSA clinical isolates screened for *mecA*-transformability.

Screened by using MR-CoNS8 as donor					Screened by using MR-CoNS3 as donor					Screened by using COLw/oφ as donor**								
isolate	CC	<i>blaZ</i>	<i>blaI</i>	Result *	isolate	CC	<i>blaZ</i>	<i>blaI</i>	Result *	isolate	CC	Result*	isolate	CC	Result*	isolate	CC	Result*
1s	133	+	+	+	21s	8	+	+	-	41s	1	-	61s	untypable	Δ/-/Δ	81s	1	Δ/-/-
2s	1	-	-	-	22s	5	+	+	-	42s	8	-	62s	1	Δ/-/-	82s	45	Δ/-/Δ
3s	1	-	-	-	23s	45	-	-	-	43s	133	-	63s	30	Δ/-/Δ	83s	8	-
4s	59	+	+	-	24s	5	+	+	-	44s	1	-	64s	1	Δ/-/-	84s	8	+
5s	133	-	-	-	25s	1	-	-	-	45s	45	-	65s	1	Δ/-/Δ	85s	133	-
6s	untypable	-	-	-	26s	untypable	-	-	-	46s	8	-	66s	1	-	86s	133	-
7s	1	-	-	-	27s	1	+	+	-	47s	30	-/Δ/-	67s	5	Δ/-/-	87s	1	-
8s	8	+	+	-	28s	133	+	+	-	48s	1	-	68s	30	Δ/-/-	88s	133	Δ/Δ/Δ
9s	untypable	+	+	+	29s	133	+	+	-	49s	45	-/Δ/-	69s	30	-	89s	5	-
10s	5	+	+	-	30s	5	+	+	-	50s	untypable	Δ/Δ/-	70s	untypable	Δ/-/Δ	90s	133	-
11s	45	+	+	+	31s	1/30	-	-	-	51s	untypable	-	71s	untypable	Δ/Δ/Δ	91s	8	-
12s	1	-	-	-	32s	5	+	+	Δ	52s	8	Δ/Δ/-	72s	8	-	92s	8	-
13s	untypable	-	-	-	33s	1/30	+	+	-	53s	133	-	73s	8	+	93s	8	-
14s	1	-	-	-	34s	133	-	-	-	54s	untypable	-	74s	133	Δ/Δ/Δ	94s	8	-
15s	8	-	-	-	35s	8	+	+	+	55s	133	-	75s	untypable	-	95s	30	-
16s	30	+	+	-	36s	8	-	-	-	56s	45	Δ/-/-	76s	133	Δ/Δ/-	96s	133	-
17s	45	-	-	-	37s	45	-	-	-	57s	30	Δ/Δ/-	77s	45	Δ/Δ/-	97s	8	-
18s	59	-	-	-	38s	8	+	+	-	58s	untypable	Δ/-/-	78s	45	Δ/Δ/Δ	98s	untypable	+
19s	30	+	+	-	39s	1	-	-	Δ	59s	5	-	79s	1	Δ/-/Δ	99s	133	-
20s	untypable	-	-	-	40s	1	+	+	-	60s	30	-	80s	8	Δ/-/-			

* +: Transformation confirmed by *mecA* PCR after replica (including unstable transformation)

-: No colony emerged in the cefmetazole selection, or emerged colony could not grow on replica.

Δ: Colonies emerged in the selection by cefmetazole and could grow on replica, but not further tested by PCR.

** Three independent transformation experiments were done. e.g. Δ/-/-: colony emerged in 1st screening, but none in 2nd and 3rd.

Low reproducibility is probably because of the low transformation efficiency close to the detection limit.

20 MSSA (1s~20s) were tested by using MR-CoNS8 as a donor. 20 MSSA (21s~40s) and 59 MSSA (41s~99s) were by MR-CoNS3 and COLw/oφ, respectively. In each experiment, NefΔ_{comE} was used as a negative control. CC: clonal complex. The presence of *blaZ* and *blaI* that has a role in the regulation of *mecA* expression (see Discussion) was tested by PCR. Strains selected for detail analysis are marked (1s, 9s, 11s, 35s, and 98s).

Supplementary Table 3. Intra and interspecies transformation of distinct SCCmec elements in biofilm growth conditions. (Number of transformants obtained).

3-day biofilm in CS2

Selection: cefmetazole

Heat-killed donor		SCC type	Recipient								
			Nef	NefΔcomE	NefattB*	1s	9s	9sΔcomE	11s	35s	98s
S. aureus	COL	I	7 (n = 1), ND (n = 1)	ND (n = 2)		3 (n = 2)	9.5 (n = 2)		8 (n = 2)	4 (n = 2)	
	COLw/oφ	I	3 (n = 2)	ND (n = 4)		3.5 (n = 2)	3 (n = 2)		2.5 (n = 2)	4 (n = 2)	4 ± 2.6 (n = 3)
	N315	II	21 ± 16 (n = 5)	ND (n = 4)	ND (n = 6)	11 ± 9.9 (n = 5)	5.3 ± 5.3 (n = 6), ND (n = 2)	ND (n = 4)	4.6 ± 3.6 (n = 5)	10 ± 5.9 (n = 7)	
	N315ΔccrAB	II (ΔccrAB)	ND (n = 4)	ND (n = 6)	ND (n = 3)		ND (n = 3)	ND (n = 3)			
	Nef	none		ND (n = 2)	ND (n = 4)	ND (n = 3)	ND (n = 3)		ND (n = 3)	ND (n = 3)	
	35s[CoNS17]	IVa	3 (n = 1), ND (n = 1)	ND (n = 2)		14 (n = 2)	5.5 (n = 2)		1 (n = 1), ND (n = 1)	3.5 (n = 2)	
MR-CoNS	MW2	IVa	4.5 (n = 2)	ND (n = 2)		11 (n = 2)	21 (n = 2)		2 (n = 2)	10 (n = 2)	
	CoNS16	I	4 (n = 1), ND (n = 1)	ND (n = 5)	ND (n = 3)	8 (n = 2)	19 (n = 1), ND (n = 1)		5 (n = 2)	19 ± 29.5 (n = 3)	
	CoNS9	III	26 (n = 2)	ND (n = 2)		23 (n = 1)	20 ± 5.5 (n = 3)		29 (n = 1)	11 (n = 2)	
	CoNS10	IVa	82 (n = 2)	ND (n = 5)	ND (n = 3)	13 (n = 1)	27 (n = 1)		12 ± 9.1 (n = 3)	10 (n = 2)	
	CoNS11	IVa	47 ± 65.6 (n = 3)	ND (n = 5)	ND (n = 4)	20 (n = 2)	7 ± 1.2 (n = 3)	ND (n = 2)	16 (n = 2)	14 (n = 2)	
	CoNS15	IVa	5 (n = 2)	ND (n = 5)	ND (n = 3)	9 (n = 2)	10 (n = 2)		6 (n = 2)	11 (n = 2)	
CoNS17	IVa	9 (n = 2)	ND (n = 5)	ND (n = 3)	13 ± 7.9 (n = 5)	13 ± 3.6 (n = 4)		14 (n = 1)	8 (n = 2)		

The mean of **number of transformants** obtained from single well is shown with ± SD.

n: number of independent experiments.

ND: none detected (0)

Supplementary Table 4. MIC ($\mu\text{g mL}^{-1}$) of cefmetazole and ceftiofur in TSB.

	strain	Cefmetazole	Ceftiofur
MSSA recipients	Nef	4	4
	1s	4	4
	9s	4	4
	11s	4	4
	35s	4	4
MRSA donors	N315	8	8
	MW2	16	16
MR-CoNS donors	CoNS10	16	16
	CoNS11	64	64
	CoNS15	64	64
	CoNS17	32	32
Transformants (Recipient[Donor])	35s[N315]	64	64
	35s[CoNS10]	64	64
	35s[CoNS11]	64	64
	35s[CoNS15]	64	64
	35s[CoNS17]	64	64
	1s[CoNS11]	8	8
	1s[CoNS15]	8	8
	9s[CoNS11]	8	8
	9s[CoNS15]	8	8
	11s[CoNS11]	8	8
	11s[CoNS15]	8	8

Supplementary Table 5. Bacterial strains and plasmids used in this study.

Strain or plasmid	Description	Source
Strains		
RN4220	Derivative of 8325-4, restriction minus, modification plus	4
N315	Pre-MRSA, SCCmec II, Km ^R , Erm ^R , clonal complex 5, carrying <i>blaI</i> and <i>blaZ</i> in plasmid	5
N315ex	N315 cured of SCCmec, Km ^S , Erm ^R	6
N315ΔccrAB	N315 lacking <i>ccrAB</i> locus	This study
N315Δcls2-tet ^R	N315Δcls2 mutant, Tet ^R	7
Nef	N315ex w/oφ (N315ex cured of the φN315 prophage)	8
Nef-GFP	Nef carrying pMK3-com-gfp	This study
Nef-NH7-GFP	Nef carrying pRIT-sigHH7 and pMK3-com-gfp	This study
Nef-pRIT5H	Nef carrying pRIT5H	8
Nef-pRITcomGFP	Nef carrying pRIT-com-gfp	This study
Nef-pT181	Nef carrying pT181	This study
NefattB*	Nef with mutated <i>attB</i> site	This study
Nef-H	Nef carrying pRIT-sigH	8
Nef-H-GFP	Nef-H carrying pMK3-com-gfp	This study
Nef-V-GFP	Nef carrying pRIT5H and pMK3-com-gfp	This study
NefΔcls2-tet ^R	NefΔcls2 mutant, Tet ^R (transduction of Tet ^R from N315Δcls2)	This study
NefΔcomE	Nef lacking <i>comE</i> operon	8
NefΔcomG	Nef lacking <i>comG</i> operon	8
ΔH	Nef lacking <i>sigH</i>	This study
ΔH-GFP	ΔH carrying pMK3-com-gfp	This study
COLw/oφ	COL strain cured of the φL54a prophage, clonal complex 8	8
1s	MSSA clinical isolate, clonal complex 133	This study
9s	MSSA clinical isolate, clonal complex untypable	This study
9sΔcomE	9s mutant lacking <i>comE</i> operon	This study
11s	MSSA clinical isolate, clonal complex 45	This study
35s	MSSA clinical isolate, clonal complex 8	This study
98s	MSSA clinical isolate, clonal complex untypable	This study
MR-CoNS3	Methicillin resistant <i>S. epidermidis</i> , SCCmec nontypeable	This study
MR-CoNS8	Methicillin resistant <i>S. lugdunensis</i> , SCCmec nontypeable	This study
MR-CoNS9	Methicillin resistant <i>S. epidermidis</i> , SCCmec III	This study
MR-CoNS10	Methicillin resistant <i>S. epidermidis</i> , SCCmec IVa	This study
MR-CoNS11	Methicillin resistant <i>S. epidermidis</i> , SCCmec IVa / I	This study
MR-CoNS15	Methicillin resistant <i>S. epidermidis</i> , SCCmec IVa	This study
MR-CoNS16	Methicillin resistant <i>S. caprae</i> , SCCmec I	This study
MR-CoNS17	Methicillin resistant <i>S. epidermidis</i> , SCCmec IVa	This study
r3	MRSA clinical isolate, SCCmecII, clonal complex 5	9
r3-H	r3 carrying pRIT-sigH	This study
r3-pRITcomGFP	r3 carrying pRIT-com-gfp	This study
r408	MRSA clinical isolate, SCCmecII, clonal complex 5	9
r408-H	r408 carrying pRIT-sigH	This study
r408-pRITcomGFP	r408 carrying pRITcomGFP	This study
r59	MRSA clinical isolate, SCCmecII, clonal complex 5	9
r59-H	r59 carrying pRIT-sigH	This study
r59-pRITcomGFP	r59 carrying pRIT-com-gfp	This study
s142	MSSA clinical isolate	9
s142-H	s142 carrying pRIT-sigH	This study
s142-pRITcomGFP	s142 carrying pRIT-com-gfp	This study
s1567	MSSA clinical isolate, clonal complex 5	9
s1567-H	s1567 carrying pRIT-sigH	This study
s1567-pRITcomGFP	s1567 carrying pRIT-com-gfp	This study
Δ3	Nef lacking <i>TCS3</i>	This study
Δ3-GFP	Δ3 carrying pMK3-com-gfp	This study
Δ4	Nef lacking <i>TCS4</i>	This study
Δ4-GFP	Δ4 carrying pMK3-com-gfp	This study
Δ5	Nef lacking <i>TCS5</i>	This study
Δ5-GFP	Δ5 carrying pMK3-com-gfp	This study
Δ6	Nef lacking <i>TCS6</i>	This study
Δ6-GFP	Δ6 carrying pMK3-com-gfp	This study
Δ7	Nef lacking <i>TCS7</i>	This study
Δ7-GFP	Δ7 carrying pMK3-com-gfp	This study

Supplementary Table 5. Bacterial strains and plasmids used in this study (continued).

Strain or plasmid	Description	Source
Strains		
Δ8	Nef lacking <i>TCS8</i>	This study
Δ8-GFP	Δ8 carrying pMK3-com-gfp	This study
Δ9	Nef lacking <i>TCS9</i>	This study
Δ9-GFP	Δ9 carrying pMK3-com-gfp	This study
Δ10	Nef lacking <i>TCS10</i>	This study
Δ10-GFP	Δ10 carrying pMK3-com-gfp	This study
Δ11	Nef lacking <i>TCS11</i>	This study
Δ11-GFP	Δ11 carrying pMK3-com-gfp	This study
Δ12	Nef lacking <i>TCS12</i>	This study
Δ12-GFP	Δ12 carrying pMK3-com-gfp	This study
Δ12(pHY-12)-GFP	Δ12 carrying pMK3-com-gfp and pHY-12 (<i>TCS12</i> complementary strain)	This study
Δ12(pHY)-GFP	Δ12 carrying pMK3-com-gfp and pHY300PLK (empty vector)	This study
Δ13	Nef lacking <i>TCS13</i>	This study
Δ13-GFP	Δ13 carrying pMK3-com-gfp	This study
Δ13(pHY-13)-GFP	Δ13 carrying pMK3-com-gfp and pHY-13 (<i>TCS13</i> complementary strain)	This study
Δ13(pHY)-GFP	Δ13 carrying pMK3-com-gfp and pHY300PLK (empty vector)	This study
Δ13(<i>TCS13</i>)	Δ13 with chromosomal <i>TCS13</i> complementation	This study
Δ13(<i>TCS13</i>)-GFP	Δ13 with chromosomal <i>TCS13</i> complementation carrying pMK3-com-gfp	This study
Δ13-NH7	Δ13 carrying pRIT-sigHNNH7	This study
Δ13-NH7-GFP	Δ13 carrying pRIT-sigHNNH7 and pMK3-com-gfp	This study
Δ13H	Δ13 carrying pRIT-sigH	This study
Δ13-V	Δ13 carrying pRIT5H	This study
Δ13-V-GFP	Δ13 carrying pRIT5H and pMK3-com-gfp	This study
Δ14	Nef lacking <i>TCS14</i>	This study
Δ14-GFP	Δ14 carrying pMK3-com-gfp	This study
Δ15	Nef lacking <i>TCS15</i>	This study
Δ15-GFP	Δ15 carrying pMK3-com-gfp	This study
Δ16	Nef lacking <i>TCS16</i>	This study
Δ16-GFP	Δ16 carrying pMK3-com-gfp	This study
Δ17	Nef lacking <i>TCS17</i>	This study
Δ17(<i>TCS17</i>)	Δ17 with chromosomal <i>TCS17</i> complementation	This study
Δ17(<i>TCS17</i>)-GFP	Δ17 with chromosomal <i>TCS17</i> complementation carrying pMK3-com-gfp	This study
Δ17-GFP	Δ17 carrying pMK3-com-gfp	This study
Δ17(pHY-17)-GFP	Δ17 carrying pMK3-com-gfp and pHY-17 (<i>TCS17</i> complementary strain)	This study
Δ17(pHY)-GFP	Δ17 carrying pMK3-com-gfp and pHY300PLK (empty vector)	This study
Δ17-NH7	Δ17 carrying pRIT-sigHNNH7	This study
Δ17-NH7-GFP	Δ17 carrying pRIT-sigHNNH7 and pMK3-com-gfp	This study
Δ17-H	Δ17 carrying pRIT-sigH	This study
Δ17-V	Δ17 carrying pRIT5H	This study
Δ17-V-GFP	Δ17 carrying pRIT5H and pMK3-com-gfp	This study
Nef-pHY- <i>P_{comG}-gfp-P_{comE}-dsRed</i>	Nef carrying pHY- <i>P_{comG}Gfp-P_{comE}dsRed</i>	This study
ΔH-pHY- <i>P_{comG}-gfp-P_{comE}-dsRed</i>	ΔH carrying pHY- <i>P_{comG}Gfp-P_{comE}dsRed</i>	This study
SE50	<i>S. epidermidis</i> , carries <i>cfr</i> pSCFS7-like plasmid and conjugative genes	1
DH5α	<i>E. coli</i> plasmid cloning host	Toyobo Ltd., Japan
Plasmids		
pMADtet	pMAD derivative, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	8
pMADtet-Δ3	Vector for deletion of <i>TCS3</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ4	Vector for deletion of <i>TCS4</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ5	Vector for deletion of <i>TCS5</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ6	Vector for deletion of <i>TCS6</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ7	Vector for deletion of <i>TCS7</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ8	Vector for deletion of <i>TCS8</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ9	Vector for deletion of <i>TCS9</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ10	Vector for deletion of <i>TCS10</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ11	Vector for deletion of <i>TCS11</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ12	Vector for deletion of <i>TCS12</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ13	Vector for deletion of <i>TCS13</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ14	Vector for deletion of <i>TCS14</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ15	Vector for deletion of <i>TCS15</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ16	Vector for deletion of <i>TCS16</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-Δ17	Vector for deletion of <i>TCS17</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-ΔH	Vector for deletion of <i>sigH</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-ΔccrAB	Vector for deletion of <i>ccrAB</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtetcomEII	Vector for deletion of <i>comE</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	8
pMADtet- <i>TCS13</i>	Vector for complementation of <i>TCS13</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet- <i>TCS17</i>	Vector for complementation of <i>TCS17</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pMADtet-attB*	Vector for mutational substitution of <i>attB</i> locus, Amp ^R (<i>E. coli</i>), Erm ^R , Tet ^R (<i>S. aureus</i>)	This study
pHY- <i>P_{comG}-gfp-P_{comE}-dsRed</i>	<i>P_{comG}-gfp</i> transcriptional fusion and <i>P_{comE}-dsRed</i> transcriptional fusion in pHY300PLK, Tet ^R (<i>E. coli</i> , <i>S. aureus</i>)	This study
pMK3-com-gfp	<i>P_{comG}-gfp</i> transcriptional fusion in pMK3, Amp ^R (<i>E. coli</i>), Tet ^R (<i>S. aureus</i>)	8
pRIT5H	Shuttle vector, ori-pC194, Amp ^R (<i>E. coli</i>), Cm ^R (<i>S. aureus</i>), <i>P_{spa}</i> -MCS	9
pRIT-sigHNNH7	pRIT5H carrying <i>sigH</i> gene in MCS, <i>sigH</i> mRNA overexpressing plasmid	8
pRIT-sigH	pRIT5H carrying <i>sigH</i> gene with <i>SigA</i> -type translation initiation sequences, allowing constitutive expression of <i>SigH</i>	8
pRIT-com-gfp	<i>P_{comG}-gfp</i> transcriptional fusion in pRIT	8
pHY300PLK	Shuttle vector, ori-pAMα1, Amp ^R (<i>E. coli</i>), Tet ^R (<i>S. aureus</i>)	Takara, Japan
pHY-12	pHY300PLK- <i>TCS12</i> , <i>TCS12</i> complementation plasmid	This study
pHY-13	pHY300PLK- <i>TCS13</i> , <i>TCS12</i> complementation plasmid	This study
pHY-17	pHY300PLK- <i>TCS17</i> , <i>TCS17</i> complementation plasmid	This study

Supplementary Table 6. List of primers used in this study.

Primer	Sequence	Source
ATCS construction (pMADtet targetting vector construction)		
TCS3-A	GGAGGATCCAGAAGATTTTGTTCCCTATC	This study
TCS3-B	CATTGAATCATCTCCAAAAATTTATGATG	This study
TCS3-C	TTTTTGGAGATGATTCAATGAAATAGATTAGCACATAACTAATGATTATG	This study
TCS3-D	GGAGTCGACGTGTATCATTGACTGGTTTTG	This study
TCS4-A	GGAGGATCCGGCATGTTAGAGCATATGC	This study
TCS4-B	CACGATAGCACCTCAGTGAATT	This study
TCS4-C	GAGGTGCTATCGTGCTTTAACAGTAATCCTTTTTTTATGCATTTTAC	This study
TCS4-D	GGAGTCGACACAAATAGCGGTSCGAATAAG	This study
TCS5-A	GGAGGATCCGAAACAATTGAACGCCTAGG	This study
TCS5-B	CATCCATATCACCCAATATCATTTAG	This study
TCS5-C	GATATTGGGTGATATGGATGTAACATGCGTTTTTGTACTTAGAATTG	This study
TCS5-D	GGAGTCGACTTTGTCCACCAGACAACTTCAG	This study
TCS6-A	GGAGGATCCAGCTCAAACACTTCTGTGTT	This study
TCS6-B	CATCTATTTTTTACCTCTGTTCTTAGG	This study
TCS6-C	GAGGTGAAAAATAGATGTCAATCCGATTTATTATAAAAATAAAATGC	This study
TCS6-D	GGAGTCGACTTTTAAGCCAAAGAGCTG	This study
TCS7-A	GGAGGATCCATTGCCGGAAGATGTTAAACC	This study
TCS7-B	CATATTTTATTCGCCCTTTTAAAAATGAC	This study
TCS7-C	GGGCGGAATAAAATATGATCTAAATACAAACAAAAAGTATTGAGTG	This study
TCS7-D	GGAGTCGACGCAATAAATGTATCACCATCC	This study
TCS8-A	GGAGGATCCCGCATGATTACGCTATTTTAG	This study
TCS8-B	CATTTGTACACCTCATATTACGACTTTTTTC	This study
TCS8-C	GTAATATGAGGTGTACAAATGTTTTAATCATGTCTGAGACGTCAATC	This study
TCS8-D	GGAGTCGACACCTTCCAAAGTCTGTTGATC	This study
TCS9-A	GGAGGATCCGATGATAGAGGACGTACAG	This study
TCS9-B	CAGGTCATACCTCCACAC	This study
TCS9-C	GAGGTATGACCTGTATGGAATAAAACTGAATATAGTTATTTAAGAACGC	This study
TCS9-D	GGAGTCGACGTCACTACTGCGCATTGATC	This study
TCS10-A	GGAGGATCCGCAACACATGGTACAGCTC	This study
TCS10-B	CATGGTATGCCCTCCCTAACTTATAA	This study
TCS10-C	GGAGGCATACCATGGAATAAAAAATTAAAGTGTAACAGCGC	This study
TCS10-D	GGAGTCGACCATTTGTAATCATCTGTGATG	This study
TCS11-A	GGAGGATCCGCTTCTTAACATGCGCATCAAG	This study
TCS11-B	CATCAAATCGCTCCAATTCATTTTTAC	This study
TCS11-C	AATTGGAGCGATTGTGATGATTAGAATGAGCTTTTAAATATTTGTGC	This study
TCS11-D	GGAGTCGACTGTACCCATTACGAGTCTC	This study
TCS12-A	GGAGGATCCCTGTTCTTATTATTGGAGTGTG	This study
TCS12-B	CATCGATAAATCACCTCTACG	This study
TCS12-C	GAGGTGATTTATCGATGCAATAGTTCGTATCGAATTAAAGAAAAG	This study
TCS12-D	GGAGTCGACACTTGGATTGTGACGAACAAG	This study
TCS13-A	GGAGGATCCGCCACGTATCTTCAAAAGAG	This study
TCS13-B	CAATACGGCTCTACTTCCATAG	This study
TCS13-C	GAAGTAGAGCCGTATTGATATAATAAGATAATAAAGTCAGTTAACGGC	This study
TCS13-D	GGAGTCGACATGGTGCTGCCGTATATTTG	This study
TCS14-A	GGAGGATCCCTGATTACCCGTTATAGTGTG	This study
TCS14-B	CATAACCTTCACCTCGATAGC	This study
TCS14-C	CGAGGTGAAGGTTATGAAATAATTAAATAAAAAAGATCGCTGCC	This study
TCS14-D	GGAGTCGACGTCTGTGAGTAAAGGTGTATG	This study
TCS15-A	GGAGGATCCATGCGCATACATTGTGTCG	This study
TCS15-B	CATAGCTATAAACTCCCTTATCTTTTTTC	This study
TCS15-C	GGGAGTTTATAGCTATGCATTAATCTCTACCTCTGAAAAAAC	This study
TCS15-D	GGAGTCGACTTTCATATTGATAAGCGCTCC	This study
TCS16-A	GGAGGATCCATTGTAGAGTGGTGTGCC	This study
TCS16-B	CATGACTTACACCCTAATTCAATC	This study
TCS16-C	TAGGGTGTAAAGTCATGTTTAGAGTTTGAAATTAATATAATTCAATATAA	This study
TCS16-D	GGAGTCGACATGGTCATACGAAAGCATATC	This study
TCS17-A	GGAGGATCCAAATGATGGACCATGCC	This study
TCS17-B	CATCTATAATCTTCTTCTTCAATTG	This study
TCS17-C	GGAAGAAGATTATAGATGGAATAAAACTTTCAATATTGTAAGTATACTA	This study
TCS17-D	GGAGTCGACAAGTGCACCTGTAGGTTT	This study

Supplementary Table 6. List of primers used in this study (continued).

Primer	Sequence	Source
Confirmation of target deletion		
TCS3-E	GTGTGTTATCAGAAACAATTGATC	This study
TCS3-F	GGTGCGTTAATCTTGGTCC	This study
TCS4-E	GTCGAGTTAAAGAAACATATGATAC	This study
TCS4-F	GGCCACACCAATACCATAC	This study
TCS5-E	CATACCTGGGAGTCGTTATG	This study
TCS5-F	CTCTGCAGCAGATGTTTCG	This study
TCS6-E	GTTTGTTTAGCTTAAGCAACCC	This study
TCS6-F	CAATTTGATGATGGTGTGGTG	This study
TCS7-E	TTATGATACTAAGTTACTTGAAAATCG	This study
TCS7-F	TATCAACCCCTATAAGCCTAAC	This study
TCS8-E	GTGAGAATCATGTCAATTAGAAAC	This study
TCS8-F	TGATCTGAAACAATTCCTGCTG	This study
TCS9-E	CCAACCTCAAGTGATAACAAGTG	This study
TCS9-F	CCATCATACTCATATCATCACC	This study
TCS10-E	CATTAAATTAAGAAACAGGTCATGC	This study
TCS10-F	CCATTAAAGTGATGCAATCCTAC	This study
TCS11-E	TGCCTTAACATTGCTTTGTATATC	This study
TCS11-F	CGAAAATTGGTTGGTTATCTGG	This study
TCS12-E	ATGACACACAAATATATATCAACGC	This study
TCS12-F	CTGTAATTAGTCATTTCCCTATTTCG	This study
TCS13-E	TGAGGAGAGTGGTGTAATTTG	This study
TCS13-F	TGTAGTCATTATACGAAGGGAG	This study
TCS14-E	ACCTGATGCACTAGATGTAAAC	This study
TCS14-F	GATCTAAGGTTATGTAATTGGCC	This study
TCS15-E	CGTTATCGTTCAATAGCACAAATG	This study
TCS15-F	AGTGAAAGGGACAAACCAATG	This study
TCS16-E	ATTTATGTGTTAAACCCAGATGCGTC	This study
TCS16-F	ATTTTAAACAAGACACTACAGTCAC	This study
TCS17-E	GGTCATTTCTTTGGCATGCG	This study
TCS17-F	TAACATCATCAATGCCTTTGACG	This study
in <i>trans</i> complementation (pHY-12, 13, 17 construction)		
TCS12CF	ATTC ~~CCGGG~~ AAAGAACAACTTAGCAAGTT	This study
TCS12CR	GTA ~~GGATCCT~~ TTTCTTAATTCGATACGAA	This study
TCS13CPrF	TACAAGCTTCAGTTAAGTATTATTTCCT	This study
TCS13CR	AAAG ~~GGATCCC~~ CAAGATTTACAATTGAATAC	This study
TCS17CF	GTGGAATTC ~~CAAAATATGAATC~~ AAAGCAGT	This study
TCS17CR	TCAG ~~GATCCT~~ GTTAGTTCATATTTAAAC	This study
Chromosomal complementation (pMADtet-TCS13, TCS17 construction)		
TCS13-G	TAT ~~GGATCCC~~ AAGTGATTTTTGTTTACCT	This study
TCS13-H	TAGGTC ~~GACT~~ TAAGGTAAACCTGTTGATA	This study
TCS17-G	ATG ~~GGATCCT~~ TTGTCCCTTTTAAATATGAA	This study
TCS17-H	AAT ~~GTCGACG~~ ACGTTTTTGAATCAAGTG	This study
ΔH mutant construction		
H-A	GGAG ~~GATCC~~ GAAATGCAATTAATAGATGCAATTGC	This study
H-B	CAAGTATTAACTAACCCCTTCTATC	This study
H-C	GATAGAAGGGGTTAGTTTAATACTTGATTTGAAAAGCGCC	This study
H-D	GGAGTC ~~GACT~~ GTCTTCACTCCTCTAACCC	This study
ΔH mutant check		
H-E	CGCCATTGCAAAAGAGAATTTAGAAG	This study
H-F	TATGCACTGCATACCAACGCTTTG	This study
Construction of dual reporter plasmid		
clpBterF	CCG ~~GAATTC~~ TAAAATTGAACCAAGAATG	This study
clpBterR	GTCATATCATGATAAACACATTAAATCATTTAAAAAGT	This study
PcomEF	AATGTGTTTATCATGATATGAC	This study
PcomER	GGATCCAAATCTTTATAGCGTAAT	This study
dsRedF	CGCTATAAAGATTTGGATCCGAGGAGGTTTATT	This study
dsRedR	ATTGTC ~~GAC~~ CTGCAGTTACAGGAACAG	This study
pHYF	GAAGAT ~~CTC~~ TGTTCAGACCAAGTTTACTC	This study
pHYR	CGGGGTACCGACATTTCCCGAAAAGTGC	This study
PcomGF	CGGGG ~~TACCT~~ ATGTGTTTCGATGAATTC	This study
gfpR	GAAGAT ~~CTC~~ TATTTGTATAGTTTCATCCATGCCATGTG	This study

Supplementary Table 6. List of primers used in this study (continued).

Primer	Sequence	Source
SCCmec amplification		
mecAF	GTAGTTGTCGGGTTTGGT	8
mecAR	GGTATCATCTGTACCCA	8
3.0-R	CTCAGACAGCAATTTCCTCG	8
ccrA-F	ACGTCAAAGTACGATGAACAAC	8
ccrA-R	CTGACTTGTCTCCAATGTTATCTG	8
Xsau325	GGATCAACAGCCTGCACA	8
attL-F	ACTTATGATACGCCTCTGCTT	This study
attR-R	AGAAGCTTATCATAAGTAATGAGG	This study
ccrA_F	TGAATGCTTCACGCTTTGTCT	This study
ccrA_R	TTGGGTTTGTCTCTGAACG	This study
plsR	TACTCAAGGGAATGGCCAAG	This study
plsF	TCACACCATCTGCACCATTT	This study
ccrA1R	GATTGCGATGAAGTCGGTTT	This study
ccrA1F	ATCGAGGCATTAGCCAAAA	This study
9sR	CACTAAGTAATATCTTGGTTGACATAC	This study
35sR	CAGTAAGACAGTGGGGAAA	This study
attB* mutant construction		
attB-A	CGCGGATCCCCAGCAGCGATGTTGTAT	This study
attB-B	TTTAGTTTACTTGTGGTAAGCTTCTCCACGCATAATCTTA	This study
attB-C	CGGTGGAGAGCTTACCACAAGTAAAACTAAAAAATTCGT	This study
attB-D	AATGTCGACCATTAAGCAGTTCATAATTTGTCATCA	This study
attB* mutant checck		
attB-E	AAAAATTTGGTATAATAAGAGG	This study
attB-F	AATGTCGACGCAAAATCAATTCCGAAGT	This study
Amplification of attB flanking regions		
orfXfor	GAGAAATATTGGAAGCAAGCC	10
unirev	GCACAGTGGGAATTAATCGAAGC	10
ΔccrAB mutant construction		
ccrAB-A	ATTGCGGGATCCGCAACACAGGCAATCGTATG	This study
ccrAB-B	GATAGCCTGTTCTGTGCAAGAGA	This study
ccrAB-C	CAGCACAGAAACAGGCTATCTAAGGGATTTCGAGATTGCAAGA	This study
ccrAB-D	TTACGCGTCGACATGCAGGTGTTCTTGTTTCATG	This study
ΔccrAB mutant check		
ccrAB-E	AAAAGTTGGCACAAGGCATC	This study
ccrAB-F	GTGGAGCTCAGTCGATTCT	This study
blaZ, blaI check		
blaZF	CAAGATGATATAGTTGCTTATTCTCC	11
blaZR	TGCTTGACCACTTTATCAGC	11
blaIF	ATGACCAATAAGCAAGTTGAAA	12
blaIR	CATATCCCTCCATACAGT	12
CC typing by multiplex PCR		
clfBF	AACAGAGCCAGCTTCAACAAATGA	13
clfBR	GTCTTTCGGATTACTGTGAATC	13
MW_1924F	TCACGAAGTCGAACGAAGAA	13
MW_1924R	GCTGAACGCTCTCTGCTTC	13
segF	AGAATCAACAACCTTTATTTATCTCCG	13
segR	TATGTGAATGCTCAACCCGA	13
arcDF	TGCCATTGATGGATTAGCAA	13
arcDR	GTTTTTCAAAGTGCTTGGGGA	13
cap8F	GGAGGAAATGACGATGAGGA	13
cap8R	TGTCACCCTGCTAGCATCAA	13
cnaF	TGCTGTCCACCTTGAATCTG	13
cnaR	GTATTACGCCAGACGGAGC	13
lukEF	GCATTATGCTTTCTTTCTGGG	13
lukER	AATGGTCCAACAGGTTACGC	13
gyrAF	AAGGTGTTCGCTTAATTCGC	14
gyrAR	ATTGCATTTCCGTGGTGTTC	14
Phage typing by multiplex PCR		
SGA1	TATCAGCGAGAATTAAAGG	2
SGA2	CTTTGACATGACATCCGTTGAC	2
SGB1	ACTTATCCAGTGGYGTATTG	2
SGB2	TGTATTTAATTTCGCCGTTAGTG	2
SGF1	CGATGGACGGCTACACAGA	2
SGF2	TTGTTTCAGAACTTCCCAACCTG	2
SGFa1	TACGGGAAAAATATTCGGAAG	2
SGFa2	ATAATCCGCACCTCATTCCT	2
SGFb1	AGACACATTAAAGTCGCACGATAG	2
SGFb2	TCTTCTCTGGCAGGCTCTCTT	2
SGL1	GCTTAAAAACAGTAACGGTGACAGTG	2
SGL2	TGCTACATCATCAAGAACACCTGG	2
SGD1	TGGGCTTCATTCTACGGTGA	2
SGD2	GTAATTTAATGAATCCACGAGAT	2
Conjugation genes check		
traAF	AATGGCCGAAGATAGAGAGGA	1
traAR	TTGCTATTTGTCCCGAGGCG	1
nesF	ATCATTTGAATCTAGCCAGCA	1
nesR	GGCACAGAAAACACGCTTGA	1
Transformants check		
pRIT5H1	GGAAGATCTGCGGCCGCGTCGACGGATCCCCGGGAAT	This study
pRIT5H2	TGCCACCTGACGTCTAAGAA	This study
TetR(EcoRI)	GGAATTCCTGTTATAAAAAAGGATCAAT	8
TetF(sall)	CATATTGTCGACTAAGTGATGAAATACTG	8

Underlined, restriction site included in the oligonucleotide.
Bold, nucleotidic changes to cause silent mutations in *orfX*.

Supplementary Table 7. Composition of CS2 medium

	stock conc.	final conc.	1000 ml
Sterile MilliQ water			710ml
Solution A			100ml
Na ₂ HPO ₄	71.4 g/L		
KH ₂ PO ₄	30 g/L		
(NH ₄) ₂ SO ₄	20 g/L		
Solution B-1			10 ml
MgSO ₄ • 7H ₂ O	5 g/L	50 mg/L	
MnSO ₄ • 5H ₂ O	0.5418 g/L	5.4 mg/L	
Solution B-2			10 ml
FeSO ₄ • 7H ₂ O	0.28 g/L	2.8 mg/L	
Solution C			25 ml
40% Glucose	40%(w/v)	1%	
Solution D			10 ml
Biotin	10 mg/L	0.1 mg/L	
Nicotinic acid	200 mg/L	2 mg/L	
D-Panthenic acid	200 mg/L	2 mg/L	
Pyridoxine hydrochloride	400 mg/L	4 mg/L	
Riboflavin	200 mg/L	2 mg/L	
Thiamine hydrochloride	200 mg/L	2 mg/L	
Adenine Solution			50 ml
Adenine • 1/2H ₂ SO ₄	300 mg/L	15mg/L	
Guanine Solution*			50 ml
Guanine	592 mg/L	30mg/L	
Trace elements Solution			10 ml
CaCl ₂	109.94 mg/L	**	
1M ZnSO ₄	105.2 ul	0.17mg/L	
100mM CuSO ₄	504 ul	0.08mg/L	
CoCl ₂ • 6H ₂ O	12 mg/L	0.12mg/L	
Na ₂ MoO ₄ • 2H ₂ O	12 mg/L	0.12mg/L	
Amino Acids Solution ***			20 ml
Glutamine Solution			10 ml
L-Glutamine	29.2 g/L	0.29 g/L	
Solution Ca			0.14
CaCl ₂	55.4987 g/L	8.9mg/L**	

* in 0.05N NaOH

** total

*** RPMI1640 amino acids solution (50X) (Sigma, R7131)

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