Supplemental Materials

Table S1: Insertion sites of IS*1181*, IS*1272* and IS*431mec*

Table S2: IS*256* insertions

A) IS*256*-insertions detected in all three strains

B) IS*256*-Insertions present only in strain SA1450/94

C) IS*256* insertions present in SA137/93A and SA137/93G

D) IS*256* insertions present only in SA137/93A

E) IS*256* insertions present only in SA137/93G

Figure S1: Genomic rearrangements caused by recombinations of IS*256* elements

Figure S2: Growth of the *guaA* mutant *S. aureus* HG001 *guaA*::IS*256-ermB* in RPMI medium compared to *S. aureus* HG001.

Figure S3: Supplemetation of the *hemY* mutant *S. aureus* HG001 *hemY*::IS256-*spc* with hemin.

Table S1: Insertion sites of IS*1181*, IS*1272* and IS*431mec*

No.	**Position of direct repeat with regard to the genome of *S. aureus* COL**	**Sequence of the direct repeat next to IR_L**	**Orientation in the genome**	**Additional information**
IS*1181*				
1	553224-31	TATTAAAA	rev	*rplY-pth*
2	711879-86	AAAATAAA	rev	SACOL0687-SACOL0688
3*	1263174-81	GTTTTTAA	rev	*ffh-rpsP*
4	1972872-1974435	GTTAAACA / AAAAGAAA	for	as in SACOL1918,
5	2226431-2227943	TAAAAAAA	for	as in SACOL2155; *tnpA* is truncated
6	2564814-21	TAAATTAA	for	*sarU/galU*
7*	2680737-46	GATTTTTT	rev	*fdaB/mqo2*
IS*1272* and IS*1272*-related				
1	1452885-1453277		rev	IS*1272* related, truncated, 393 bp
2	1779634-1781285		rev	IS*1272* related, 1652 bp
3	1887817-1889426		for	IS*1272* related, 1610 bp
4	2253786-2255410		for	IS*1272* related, 1625 bp
5	42958-44481		rev	IS*1272*, SCC*mec*, 1524 bp
6	1436659-1436955		for	IS*1272* related, truncated, 297 bp
7	2414971-2415327		for	IS*1272* related, truncated, 357 bp
IS*431mec* in SA1450/94				
1ND	36286-93 to 37084-91	GAAAATAA / CATAATAC	rev	SCC*mec*, deletion of 792 bp (HMG-CoA-synthase + hyp. protein)
IS*431mec* in SA137/93A				
1A	36286-93	GAAAATAA / GATTAGAT	rev	SCC*mec,* integrated pUB110
2	38121-28	GATTAGAT / AGTTTTTC	rev	SCCmec, intergrated pUB110
3	22251-58/ 21455-62 [a]	AAAATTCT / AGAATTAA	rev	plasmid
4	29346-53/ 28548-55 [a]	CTATAAAA / CCTAAAAT	rev	plasmid
IS*431mec* in SA137/93G				
1G	36286-93 bis 137832-39	GAAAATAA / ATAAATTT	rev	large deletion (105,767 bp)

Legend: a) Insertion site with regard to plasmid SAP101A, * not present in *S. aureus* SA1450/94.

Table S2: IS*256* insertions

A) IS*256*-insertions detected in all three strains

Locus tag in *S. aureus* COL	**Gene**	**Function**	**Position of direct repeat with regard to the genome of *S. aureus* COL**	**Sequence of the direct repeat next to IR_L**	**Orientation in the genome**
gene disruption					
<u>metabolism</u>					
SACOL2504		permease	2557780-87	GAATTATG	rev
<u>processing of genetic information</u>					
SACOL2012		acetyltransferase (GNAT-Familie)	2073624-31	AAAATCAC	for
<u>regulation</u>					
SACOL2507	*sarU*	transcriptional regulator (HTH-type)	2564338-45	AAAGAATT	for
<u>hypothetical proteins</u>					
SAR1561 [b]		membrane protein	1635824-31	GAATTCGT	rev
SACOL0133		pseudo	149050-57	ATTTTTTG	for
SACOL1507			1548748-55	AAAAATAT	for
intergenic insertions					
SACOL1309/1310			1327221-28	CATTTATA	rev
SACOL0759/0761	*fruA/nagA*		782512-19	AACGGTAG	for
SACOL1691/1692	*recJ/secF*		1723638-45	TAAAAATG	for
SACOL1722/1723	*tig/*	deleted IS*256* (see Fig. S1C)	1754695-702	ATTATTTT	for/rev
Tn*4001* right		= SATW20_20720			
Tn*4001* left		= SATW20_20690			
SACOL1332-1334		deletion of SACOL1333 after recombination of two IS256 (see Fig.S1B)	1350352-59 1349560-67	CACAAATC TAAAAGGC	rev

Legend: b) Insertion site with regard to *S. aureus* MRSA252.

B) IS*256*-Insertions present only in strain SA1450/94

Locus tag in *S. aureus* COL	Gene	Function	Position of direct repeat with regard to the genome of *S. aureus* COL	Sequence of the direct repeat next to IR_L	Orientation in the genome
gene disruption					
<u>metabolism</u>					
SACOL0136	*cap5A*	capsule biosynthesis	153448-55	GTTTTTTT	rev
<u>processing of genetic information</u>					
SACOL1315	*mutS*	DNA repair protein	1331528-35	GTATTTCA	rev
NWMN_1925 [c]	*int*	phage integrase	2131415-22	TAATGACG	rev
SACOL2349		transcriptional regulator, TetR-family	2409142-49	TTAAACTT	for
<u>hypothetical proteins</u>					
SACOL0045			52399-52406	CCAAAATT	rev
SACOL0207			243752-59	CGGTAGAG	rev
SACOL0304			338736-43	CAAATTTA	rev
SACOL0644			675898-905	AGATTATC	rev
SACOL0652			682208-15	CACTTTAA	for
SACOL1858			1908720-27	TTTAGAAT	for
SACOL1859			1910233-40	GGAAAAAG	rev
SACOL2723			2795476-83	ACTAAAAA	for
intergenic insertions					
SACOL0026/0027			35929-36	TAATAAAG	for
SACOL0702/0703			728388-95	AGTAAATC	for
SACOL1099/1100	*/def1*	HP/polypeptide-deformylase	1110104-11	AGATAAAC	rev
NWMN_1876/ 1877 [c]	*scn/chp*	inhibitor of the complement system/ chemotaxis inhibitory protein	2089846-53	AATAATTA	rev
SACOL2202/2203		HP/ClpA-related protein	2284782-89	AAAATATA	for
SACOL2205/2206	*/rpsI*	pseudo HP/ribosomal protein S9	2288400-407	GTATTCTG	rev
SACOL2339/2340	*/gltS*	DNA-3-methyladenine-glycosylase /sodium glutamate symporter	2399578-85	TTGAACTT	for

Legend: c) Insertion site with regard to *S. aureus* Newman, HP hypothetical protein.

C) IS*256* insertions present in SA137/93A and SA137/93G

Insertion site with regard to the genome of *S. aureus* COL	**Gene**	**Function**	**Position of direct repeat with regard to the genome of *S. aureus* COL**	**Sequence of the direct repeat next to IR$_L$**	**Orientation in the genome**
gene disruption					
processing of genetic information					
SACOL1600	*comGB*	competence protein	1632664-71	TAATATCA	rev
SACOL1859		putative uncharacterized protein, deletion (see Fig.S1A)	1909725-32 1912134-41	CGAATATT TAATTCAA	for
metabolism					
SACOL2031	*nrgA*	ammonium transporter	2090132-39	TCATGACG	rev
hypothetical proteins					
hlb-phage [d]		prophage integrated into *hlb*	2108815-22	TAAAAGTT	rev
SACOL0425		membrane protein	428606-13	GAAACCGA	for
SACOL0648			678911-18	TCAAAAAT	for
SACOL2190			2271278-85	ATAATGAA	rev
SACOL2244			2312087-94	TCATTTGC	rev
intergenic insertions					
SACOL2377/2378		/transcriptional regulator (AraC-family)	2436565-72	AGTGTGAA	for
SACOL0282/0283			323864-71	GACTAAAC	rev
SACOL0722/0723		phosphate transporter / LysM related protein	747615-22	ACAATTTA	rev
SACOL2325/2326	*lysR/ fosB*	transcriptional regulator (LysR-family)/ fosfomycin resistance protein	2388839-46	GAATTTTT	rev
SACOL1171/1172			1178745-52	TGTTGACT	for
SACOL1002/1003	*spxA/ mecA2*	transcriptional regulator / adaptor protein	1011042-52	TAATGCTTTAA	for
SACOL2660/2661	*isaB/*	immuno dominant antigen B	2723394-401	ATTTTTAT	rev
SACOL2188/2189	*lacR/*	lactose phosphotransferase system repressor/NAD-dependent deacetylase	2270092-99	GTAAAAAC	for
SACOL1169/1170		fibronectin binding protein precursor/	1177755-62	ATTTTTTG	rev
SACOL1282/1283	*proS/ polC*	prolyl-tRNA synthetase/ DNA-polymerase III	1293133-40	AAAATGAC	for
SACOL0485/0486		/staphylococcal tandem lipoprotein	487494-501	GTTAGTTT	for
SACOL1812/1813	*rot/*	repressor of toxins	1869153-60	AAATATAT	rev
SACOL0441/0442		integrase/enterotoxin	445712-19	GTGATGCG	rev

Legend: d) Insertion site with regard to *S. aureus* TW20.

D) IS*256* insertions present only in SA137/93A

Insertion site with regard to the genome of *S. aureus* COL	**Gene**	**Function**	**Position of direct repeat with regard to the genome of *S. aureus* COL**	**Sequence of the direct repeat next to IR_L**	**Orientation in the genome**
gene disruption					
metabolism					
SACOL1384	*opuD*	BCCT family osmoprotectant transporter	1389315-22	TAAAGCTG	rev
hypothetical proteins					
SACOL0131			147539-46	TCATCTAG	for
SACOL1929		ABC transporter ATP-binding protein	1994146-53	TAATTACT	for
intergenic insertions					
tRNA/ SACOL0019	tRNA-Asp/*yycF*	tRNA/WalR response regulator	24894-901	ATAAAGAA	for
SACOL0078/ 0079	*plc/*	1-phosphatidylinositol phosphodiesterase/ hypothetical protein	89340-47	TAAATATA	for
SACOL0720/ 0721	*vraG/*	ABC-transporter (permease)	745231-38	AAAATAAA	for
SACOL0772/ 0773	*/pabA*	ExsB protein/para-aminobenzoate synthase, glutamine amidotransferase, component II	793549-56	TAAAAAAA	for
SACOL2013/ 2014		/ phage terminase	2074224-31	TGATATAG	for
SACOL0316/ 0317	*/geh*	/ lipase precursor	352616-23	TTAGTATC	for
SAP101A_018/ 020 [e]		/ antiseptic resistance protein	8865-72	CAATTTCT	rev

Legend: e) Insertion site with regard to the plasmid SAP101A.

E) IS*256* insertions present only in SA137/93G

Insertion site with regard to the genome of *S. aureus* COL	**Gene**	**Function**	**Position of direct repeat with regard to the genome of *S. aureus* COL**	**Sequence of the direct repeat next to IR_L**	**Orientation in the genome**
gene disruption					
<u>regulation</u>					
SACOL2352	*tcaA*	teicoplanin resistance associated protein	2412311-18	ATTCACCT	rev
<u>hypothetical proteins</u>					
SACOL1071			1080849-56	AAATTAAA	rev
intergenic insertions					
SACOL1278/ 1279	*frr/uppS*	ribosome recycling factor/ UDP pyrophosphate synthase	1288082-89	CATTTCTG	for
SACOL1733/ 1734	*ndrR /gapA2*	transcriptional regulator NrdR/ glyceraldehyde 3-phosphate dehydrogenase 2	1764961-68	ATGTTTTT	rev

Fig. S1

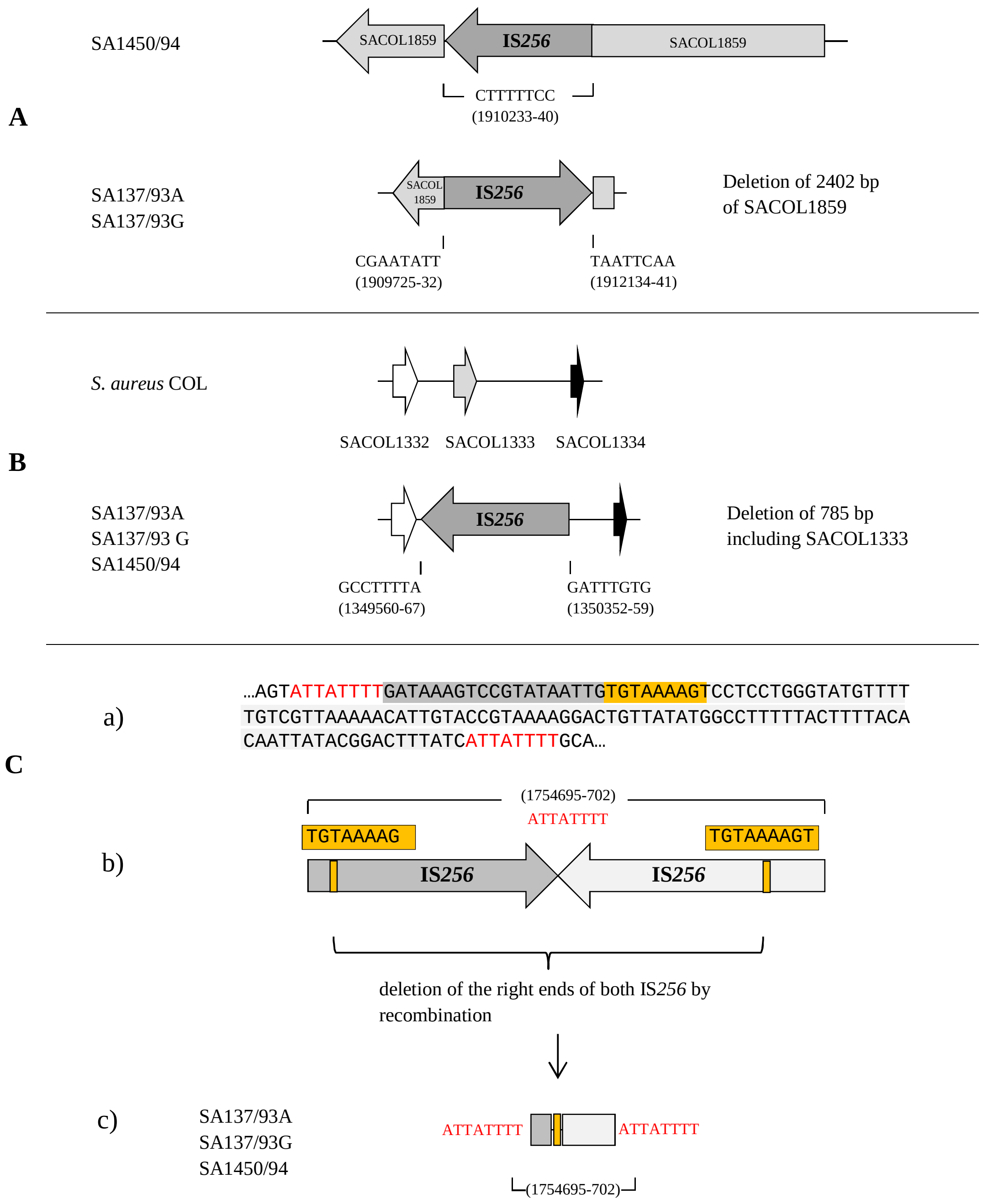

Fig. S1: Genomic rearrangements caused by recombinations of IS*256* elements. All numbers give the insertion sites corresponding to *S. aureus* COL.

A) Schematic view of the insertions in SACOL1859. Strain SA1450/94 contains a reverse insertion that is flanked by the direct repeat shown below. In contrast, in strain SA137/93A and strain SA137/93G, IS*256* is flanked by different sequences, indicating that a homologous recombination between two IS*256* copies might have caused the 2402 bp deletion within SACOL1859.

B) Schematic view of SACOL1333 in *S. aureus* COL and the deletion of SACOL1333 which is present in all three ST247 isolates. Here the indirect repeats IR_L and IR_R are flanked by different sequences, indicating that deletion of SACOL1333 and the neighbouring 785 bp may have been caused by homologous recombination of two IS*256* elements.

C) The insertion between SACOL1722/1723 consists of two truncated IS*256* elements (in each case a part of IR_L is present) which are joined by a TGTAAAAGT sequence (yellow box) ((a), a schematic view is shown in (c)). The TGTAAAAGT sequence forms a direct repeat when two IS*256* are inserted in a head-to-head manner as shown in (b). Therefore, most probably an illegitimate recombination between the two IS elements inserted as shown in (b) yielded the sequence shown in (c). The remnants of the IS elements are flanked by a direct repeat and the recombination did not cause a genomic deletion compared to *S. aureus* COL. This indicates that the two copies of IS*256* must have been inserted directly next to each other.

Fig. S2

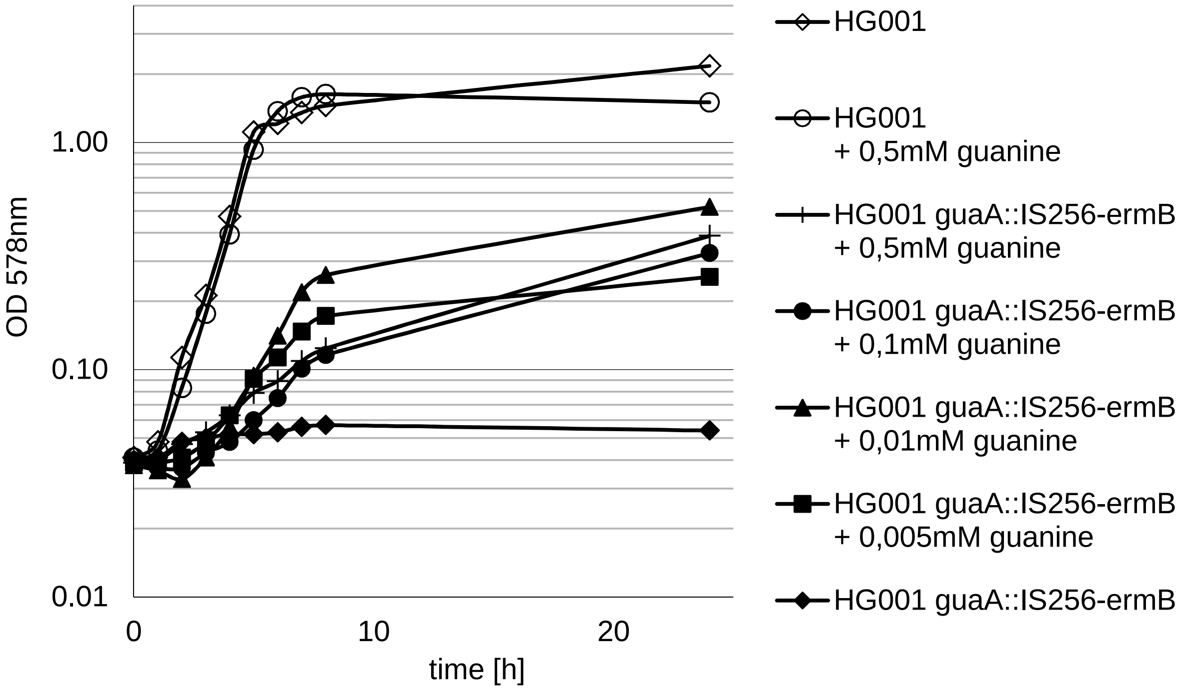

Fig. S2: Growth of the *guaA* mutant *S. aureus* HG001 *guaA*::IS*256-ermB* in RPMI medium compared to *S. aureus* HG001. The cultures were inoculated with a fresh overnight colony that had been resuspended in RPMI supplemented with different concentrations of guanine.

Fig. S3

Fig. S3: Colonies of *S. aureus* HG001 *hemY*::IS256-*spc* on TSA containing 150 µg/ml spectinomycin around a filter disk containing hemin (Oxoid, Basingstoke, UK), after 16 h at 37°C.