

Supplementary Table 1: Phage and SaPI titres

Background		pC221		pI258		pGO1	
		Phage titre ^a	SaPI titre ^b	Phage titre	SaPI titre	Phage titre	SaPI titre
80 α		10.02 (\pm 0.21)	-	9.74 (\pm 0.04)	-	9.96 (\pm 0.05)	-
80 α Δ terS		<1 ^c	-	<1	-	ND ^d	-
80 α ::SaPI1cpmAB		<1	-	<1	-	ND	-
80 α	SaPI1	8.40 (\pm 0.21)	7.98 (\pm 0.37)	8.55 (\pm 0.21)	7.86 (\pm 0.30)	8.28 (\pm 0.22)	7.64 (\pm 0.38)
80 α Δ terS	SaPI1	<1	7.99 (\pm 0.51)	<1	8.10 (\pm 0.70)	ND	ND
80 α	SaPI1 Δ terS	8.55 (\pm 0.32)	5.89 (\pm 0.17)	9.06 (\pm 0.46)	5.75 (\pm 0.23)	ND	ND
80 α Δ terS	SaPI1 Δ terS	<1	<1	<1	<1	ND	ND
80 α	SaPI1 Δ cpmAB	9.88 (\pm 0.13)	9.06 (\pm 0.04)	9.66 (\pm 0.12)	8.74 (\pm 0.25)	9.75 (\pm 0.37)	8.83 (\pm 0.31)
80 α ::SaPI1cpmAB	SaPI1	<1	7.01 (\pm 0.21)	<1	6.86 (\pm 0.35)	ND	ND
80 α	SaPIbov2	8.25 (\pm 0.42)	7.79 (\pm 0.18)	8.08 (\pm 0.10)	8.03 (\pm 0.22)	8.58 (\pm 0.18)	7.83 (\pm 0.12)

^aMean log₁₀ Plaque-forming units (Pfu) per ml of lysate in RN4220 recipient, n = 3 (\pm std).

^bMean log₁₀ Transduction units (TrU) per ml of lysate in RN4220 recipient, n = 3 (\pm std).

^cLimit of detection = 1.

^dND, Not determined.

Supplementary Table 2: Transfer abilities of the evolved pGO1 plasmids.

Plasmid	Size (bp)	Potential for conjugative transfer ^a	Median conjugation efficiency (range) ^{b,c}	Potential for transduction (< 45kb)	Mean Log ₁₀ TrU per ml (±SD, n=3) ^d	Median transduction efficiency (range) ^{e,f}
pGO1	54,000	Yes (<i>tra</i> ⁺ , <i>nes</i> ⁺ , <i>oriT</i> ⁺)	4.42 x 10 ⁻⁴ (3.85 x 10 ⁻⁵ to 5.96 x 10 ⁻⁴)	No	<1	< 1.00 x 10 ⁻⁸
pGO1evol	27,574	No (<i>tra</i> ⁻ , <i>nes</i> ⁺ <i>oriT</i> ⁺)	< 7.5 x 10 ⁻⁹	Yes	1.32 (± 0.28)	3.00 x 10 ⁻⁸ (1.00 x 10 ⁻⁸ to 3.00 x 10 ⁻⁸)
pGO1evol _A *	41,026	Yes (<i>tra</i> ⁺ , <i>nes</i> ⁺ <i>oriT</i> ⁺)	3.55 x 10 ⁻³ (1.50 x 10 ⁻³ to 1.55 x 10 ⁻²)	Yes	2.23 (± 0.26)	1.80 x 10 ⁻⁷ (9.00 x 10 ⁻⁸ to 3.00 x 10 ⁻⁷)
pGO1evol _B *	41,026	Yes (<i>tra</i> ⁺ , <i>nes</i> ⁺ <i>oriT</i> ⁺)	ND	Yes	2.42 (± 0.09)	2.80 x 10 ⁻⁷ (2.10 x 10 ⁻⁷ to 3.10 x 10 ⁻⁷)
pGO1evol _C *‡	40,932	No (<i>tra</i> ⁻ , <i>nes</i> ⁻ <i>oriT</i> ⁻)	< 7.5 x 10 ⁻⁹	Yes	5.03 (± 0.13)	1.12 x 10 ⁻³ (7.80 x 10 ⁻⁴ to 1.41 x 10 ⁻³)
pGO1evol _F *	41,737	Yes (<i>tra</i> ⁺ , <i>nes</i> ⁺ <i>oriT</i> ⁺)	2.63 x 10 ⁻³ (5.65 x 10 ⁻⁵ to 4.97 x 10 ⁻³)	Yes	2.91 (± 0.03)	8.20 x 10 ⁻⁷ (7.50 x 10 ⁻⁷ to 8.80 x 10 ⁻⁷)
pGO1evol _J *‡	43,843	No (<i>tra</i> ⁺ , <i>nes</i> ⁻ <i>oriT</i> ⁻)	< 7.5 x 10 ⁻⁹	Yes	7.04 (± 0.13)	9.80 x 10 ⁻² (8.90 x 10 ⁻² to 1.53 x 10 ⁻¹)
pGO1evol _E †	30,899	No (<i>tra</i> ⁻ , <i>nes</i> ⁺ <i>oriT</i> ⁺)	< 7.5 x 10 ⁻⁹	Yes	1.16 (± 0.28)	1.00 x 10 ⁻⁸ (1.00 x 10 ⁻⁸ to 3.00 x 10 ⁻⁸)
pGO1evol _H †	30,899	No (<i>tra</i> ⁻ , <i>nes</i> ⁺ <i>oriT</i> ⁺)	ND	Yes	0.33 (± 0.58)	<1.00 x 10 ⁻⁸ (<1.00 x 10 ⁻⁸ to 1.00 x 10 ⁻⁸)
pGO1evol _I †	30,899	No (<i>tra</i> ⁻ , <i>nes</i> ⁺ <i>oriT</i> ⁺)	< 7.5 x 10 ⁻⁹	Yes	1.80 (± 0.27)	5.00 x 10 ⁻⁸ (4.00 x 10 ⁻⁸ to 1.30 x 10 ⁻⁷)

ND: Not determined; ^a The products of the conjugative transfer cluster genes (*tra*), relaxase (*nes*) and origin of transfer (*oriT*) are necessary for mating pore formation and translocation of plasmid DNA between cells; ^b Gentamicin-resistant transconjugant cells/donor cells, n = 3; ^c No transfer was considered to be < 7.5 x 10⁻⁹; ^d Transduction of 100µl of lysate into 1ml of RN4220 recipient cells; ^e Gentamicin-resistant transductant cells per transducing particle following infection of RN4220 recipient cells with 10⁸ transducing particles (10⁷ transducing particles where SaPIbov2 is present), n = 3; ^f No transfer was considered to be < 1.0 x 10⁻⁸; *SaPIbov2-driven remodelling; †SaPI1Δ*cpm*AB-driven remodelling; ‡pGO1-SaPIbov2 hybrid species.

Supplementary Table 3: Size reduction of pGO1evol forces a trade-off between mechanisms for mobilization.

Mating			Distribution of pGO1 and pGO1evol plasmids in transconjugant populations					
Donor 1	Donor 2	Recipient	Median conjugation efficiency (range) ^a	Neomycin-resistant colonies ^b			Frequency of pGO1 transfer (%)	Frequency of pGO1evol _x transfer (%)
				Exp't 1	Exp't 2	Exp't 3		
SH1000 pGO1	SH1000 pGO1evol	RN4220	2.98×10^{-5} (3.16×10^{-6} to 1.04×10^{-4})	100/100	100/100	100/100	100.0 (± 0.00)	0.00 (± 0.00)
SH1000 pGO1	SH1000 pGO1evol _A	RN4220	9.83×10^{-4} (6.56×10^{-4} to 6.29×10^{-3})	19/100	9/100	33/100	20.3 (± 12.1)	79.7 (± 12.1)
SH1000 pGO1	SH1000 pGO1evol _F	RN4220	8.36×10^{-4} (6.05×10^{-4} to 1.22×10^{-3})	100/100	100/100	100/100	100.0 (± 0.00)	0.00 (± 0.00)
SH1000 pGO1	SH1000 pGO1evol _E	RN4220	2.12×10^{-4} (4.16×10^{-5} to 2.30×10^{-4})	100/100	100/100	100/100	100.0 (± 0.00)	0.00 (± 0.00)
SH1000 pGO1	SH1000 pGO1evol _I	RN4220	1.60×10^{-6} (1.17×10^{-6} to 1.69×10^{-4})	34/34	43/43	100/100	100.0 (± 0.00)	0.00 (± 0.00)

^a Three independent mating experiments were carried out for each donor combination; ^b Neomycin resistance was used to differentiate pGO1 from the derivative plasmids as all pGO1evol_x plasmids are sensitive to neomycin.

Supplementary Table 4: Bacterial strains and plasmids

Strain	Description	Reference
RN450	<i>Staphylococcus aureus</i> NCTC8325 cured of ϕ 11, ϕ 12 and ϕ 13	1
RN4220	Restriction-deficient derivative of RN450	Lab strain
SH1000	<i>rsbU</i> ⁺ derivative of <i>S. aureus</i> NCTC8325-4	Lab strain
RN10359	RN450 80 α	2
SH1000 pGO1	SH1000 pGO1	3
JP20320	SH1000 80 α	This study
JP19979	SH1000 pGO1 80 α	This study
JP19994	JP19979 SaPI1 <i>tst::tetM</i>	This study
JP19996	JP19979 SaPIbov2 <i>bap::tetM</i>	This study
JP21467	RN4220 80 α pGO1evol _A	This study
JP21468	RN4220 80 α pGO1evol _B	This study
JP21469	RN4220 pGO1evol _C ::SaPIbov2 <i>bap::tetM</i>	This study
JP21470	RN4220 pGO1evol _F	This study
JP21471	RN4220 pGO1evol _J ::SaPIbov2 <i>bap::tetM</i>	This study
JP21472	RN4220 pGO1evol _E	This study
JP21473	RN4220 pGO1evol _H	This study
JP21474	RN4220 pGO1evol _I	This study
JP19881	RN4220 80 α pGO1evol	This study
JP20000	SH1000 pGO1evol	This study
JP20050	JP20320 pGO1evol	This study
JP21271	SH1000 pGO1evol _A	This study
JP21272	SH1000 pGO1evol _C ::SaPIbov2 <i>bap::tetM</i>	This study
JP21273	SH1000 pGO1evol _F	This study
JP21274	SH1000 pGO1evol _J ::SaPIbov2 <i>bap::tetM</i>	This study
JP21275	SH1000 pGO1evol _E	This study
JP21276	SH1000 pGO1evol _I	This study
JP14151	RN4220 SAOUHSC 00390:: <i>cadCA</i> ; Cd ^R marker 10 kb downstream SaPI type II <i>attB</i>	This study
JP19998	JP14151, spontaneous rifampicin-resistant mutant	This study
JP20580	JP20320 pGO1evol _A	This study
JP20581	JP20320 pGO1evol _B	This study
JP20582	JP20320 pGO1evol _C ::SaPIbov2 <i>bap::tetM</i>	This study
JP20585	JP20320 pGO1evol _E	This study
JP20583	JP20320 pGO1evol _F	This study
JP20586	JP20320 pGO1evol _H	This study
JP20587	JP20320 pGO1evol _I	This study
JP20584	JP20320 pGO1evol _J ::SaPIbov2 <i>bap::tetM</i>	This study
ST126	RN4220 80 α SaPI1 <i>tst::tetM</i> Δ <i>cpmAB</i>	4

JP19997	JP20320 SaPI1 <i>tst::tetM</i> Δ <i>cpmAB</i>	This study
JP18286	RN4220 pI258	5
JP18292	JP18286 80 α	This study
JP18410	JP18292 SaPI1 <i>tst::tetM</i>	This study
JP18413	JP18292 SaPI _{bov2} <i>bap::tetM</i>	This study
JP12871	RN4220 80 α Δ <i>terS</i>	This study
JP18208	JP12871 pI258	This study
JP18661	JP18208 SaPI1 <i>tst::tetM</i>	This study
JP18693	JP18292 SaPI1 <i>tst::tetM</i> Δ <i>terS</i>	This study
JP18694	JP18208 SaPI1 <i>tst::tetM</i> Δ <i>terS</i>	This study
JP18525	JP18292 SaPI1 <i>tst::tetM</i> Δ <i>cpmAB</i>	This study
ST82	RN4220 80 α ::SaPI1 <i>cpmAB</i>	4
JP18669	ST82 pI258	This study
JP18692	JP18669 SaPI1 <i>tst::tetM</i>	This study
JP18288	RN4220 pC221	6
JP18293	JP18288 80 α	This study
JP18665	JP18293 SaPI1 <i>tst::tetM</i> Δ <i>cpmAB</i>	This study
JP18406	JP18293 SaPI1 <i>tst::tetM</i>	This study
JP18408	JP18293 SaPI _{bov2} <i>bap::tetM</i>	This study
JP18425	JP12871 pC221	This study
JP18865	JP18425 SaPI1 <i>tst::tetM</i>	This study
JP18866	JP18293 SaPI1 <i>tst::tetM</i> Δ <i>terS</i>	This study
JP18867	JP18425 SaPI1 <i>tst::tetM</i> Δ <i>terS</i>	This study
JP19946	ST82 pC221	This study
JP19950	JP19946 SaPI1 <i>tst::tetM</i>	This study
JP1220	<i>Staphylococcus xylosus</i>	Lab strain
JP831	<i>Staphylococcus epidermidis</i> BM96184	Lab strain
JP7422	<i>Listeria monocytogenes</i> SK1351	Lab strain
JP21894	RN4220, spontaneous rifampicin-resistant mutant	This study
JP21480	RN450 pC221	This study
JP21924	JP21480 SaPI1 <i>tst::tetM</i>	This study
JP21481	RN10359 pC221	This study
JP21925	JP21481 SaPI1 <i>tst::tetM</i>	This study

Plasmid	Description	Reference
pGO1	<i>S. aureus</i> conjugative plasmid, 54 kb; confers resistance to bleomycin, gentamicin, kanamycin, neomycin, quaternary ammonium compounds, and trimethoprim	³
pI258	<i>S. aureus</i> plasmid, 29 kb; confers resistance to erythromycin, cadmium, arsenic, and mercury	⁷
pC221	<i>S. aureus</i> mobilizable plasmid, 4.6 kb; confers resistance to chloramphenicol	⁶

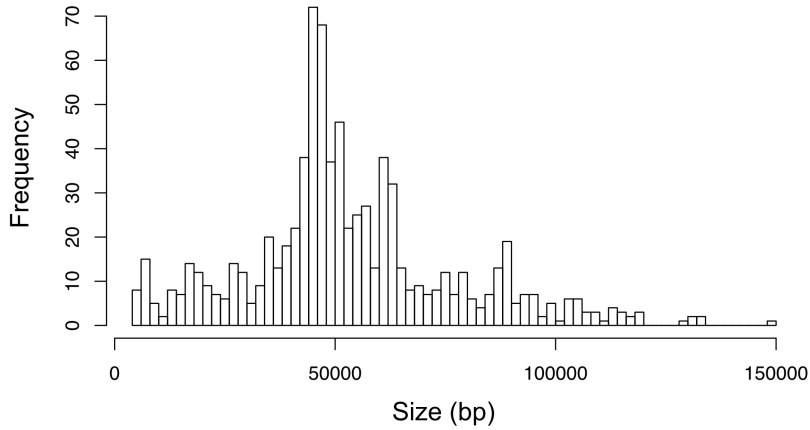
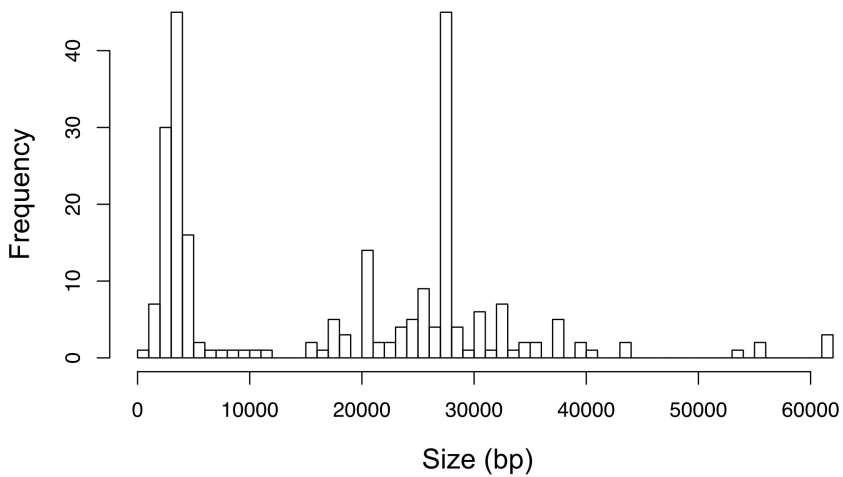
Supplementary Table 5: Oligonucleotides

Primer	Sequence (5'-3')	Description
Differentiation of pGO1 and pGO1evolved plasmids		
pGO1_p01-F	GATAACAACAAAAACGAATAATATAGGAGGGGTT	Region common to both pGO1 and pGO1-derivatives
pGO1_p01-R	GTTACCCACCTCTTTAATACTGTAGGCTA	
pGO1-1F	CACTCAACTATTATGAGAAGAAAAATATGGAAAACA ATCCA	PCR for differentiation of pGO1 and pGO1evol
pGO1_42317-R	GTCTTGATAGTTAGGTAACATTCCTATTGAGTG	
pGO1-Frag21F	TTGCAATATTGAAAGGAACACCTAAAAATATATCAGC AC	PCR for differentiation of pGO1 and pGO1evol _A
pGO1-Frag21R	TTTCTCAGCATAATTAATGGCCCGTGATG	
Southern blot DNA probes		
pGO1_GmR-F	AGAGCCAAATTATTGGAGTAAAGGAATTGG	<i>aacA-aphD</i> (gentamicin-resistance) probe
pGO1_GmR-R	TATCTGTATAATCTAAACCGTGCATTTGTC	
pGO1_nes-F	TTTGTGGAATAAAGTTCATGATGTTGAGAACAGA	<i>nes</i> probe
pGO1_Frag4R	TTCATCTTCAAATTTTCGTGCTTTCCAACC	
tetM-1mS	ACGCGTCGACCAAATATGCTCTTACGTGC	<i>tetM</i> (SaPI _{bov2} <i>bap::tetM</i>) probe
REV tetM mid	CATCAACATGAGCTAAAACCTCCAA	

References

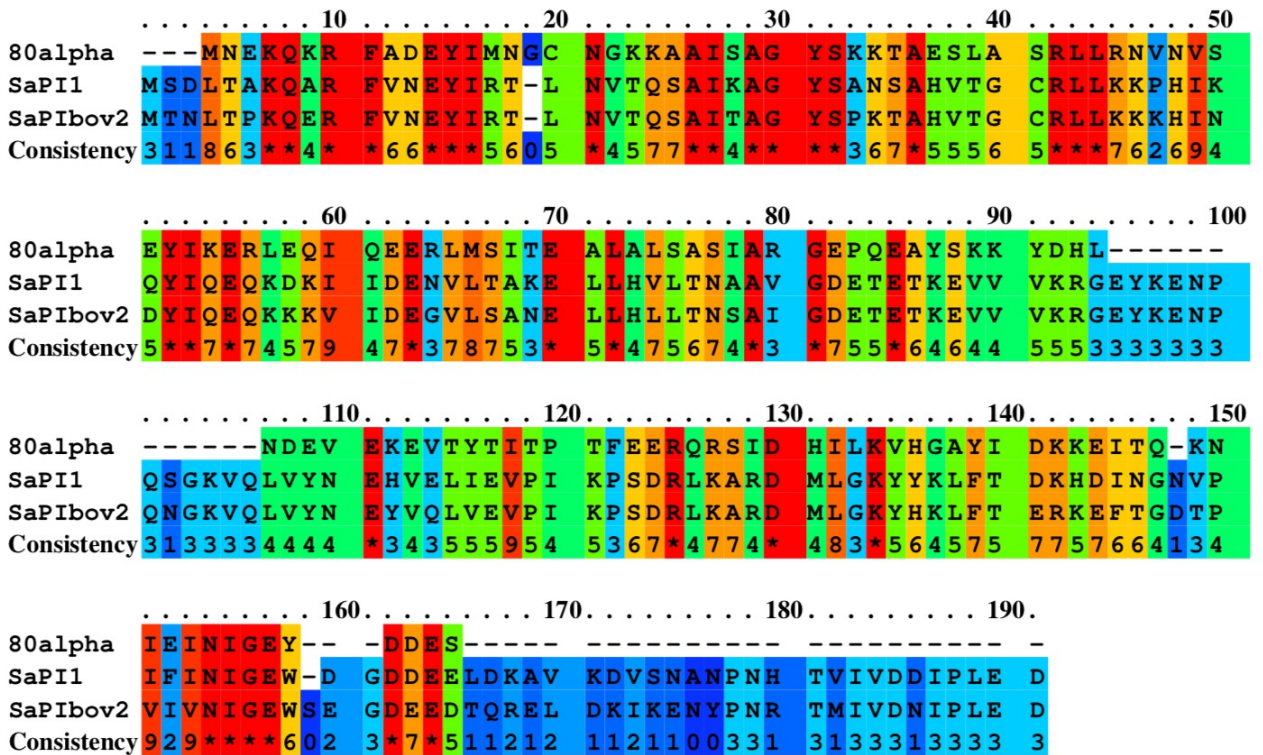
1. Novick, R. Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. *Virology* **33**, 155–166 (1967).
2. Christie, G. E. *et al.* The complete genomes of *Staphylococcus aureus* bacteriophages 80 and 80 α --implications for the specificity of SaPI mobilization. *Virology* **407**, 381–390 (2010).
3. Caryl, J. A. & O'Neill, A. J. Complete nucleotide sequence of pGO1, the prototype conjugative plasmid from the Staphylococci. *Plasmid* **62**, 35–38 (2009).
4. Damle, P. K. *et al.* The roles of SaPI1 proteins gp7 (CpmA) and gp6 (CpmB) in capsid size determination and helper phage interference. *Virology* **432**, 277–282 (2012).
5. Wang, P. Z. & Novick, R. P. Nucleotide sequence and expression of the beta-lactamase gene from *Staphylococcus aureus* plasmid pI258 in *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. *J. Bacteriol.* **169**, 1763–1766 (1987).

6. Projan, S. J. *et al.* Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *Staphylococcus aureus*. *Mol. Gen. Genet.* **199**, 452–464 (1985).
7. Laddaga, R. A., Chu, L., Misra, T. K. & Silver, S. Nucleotide sequence and expression of the mercurial-resistance operon from *Staphylococcus aureus* plasmid p1258. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5106–5110 (1987).

a**b**

Supplementary Figure 1. Staphylococcal plasmids display bimodal size distribution. 295 complete *S. aureus* sequences were downloaded from the NCBI database and screened for the presence of prophages (n=813) and plasmids (n=243). **(a)** Prophage genomes ranked according to size. **(b)** Plasmid sequences ranked according to size. Almost all plasmids were below 45 kb, which is consistent with phage capsid capacity. Prophages display unimodal distribution with a peak at 44 kb, while plasmid sequences reveal a clear bimodal size distribution with peaks at 4 kb and 27 kb.

Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved



Supplementary Figure 2. Comparison of phage 80 α and SaPI small terminase sequences. Small terminase (TerS) amino acid sequences from phage 80 α , SaPI1 and SaPIbov2 aligned using PRALINE software.