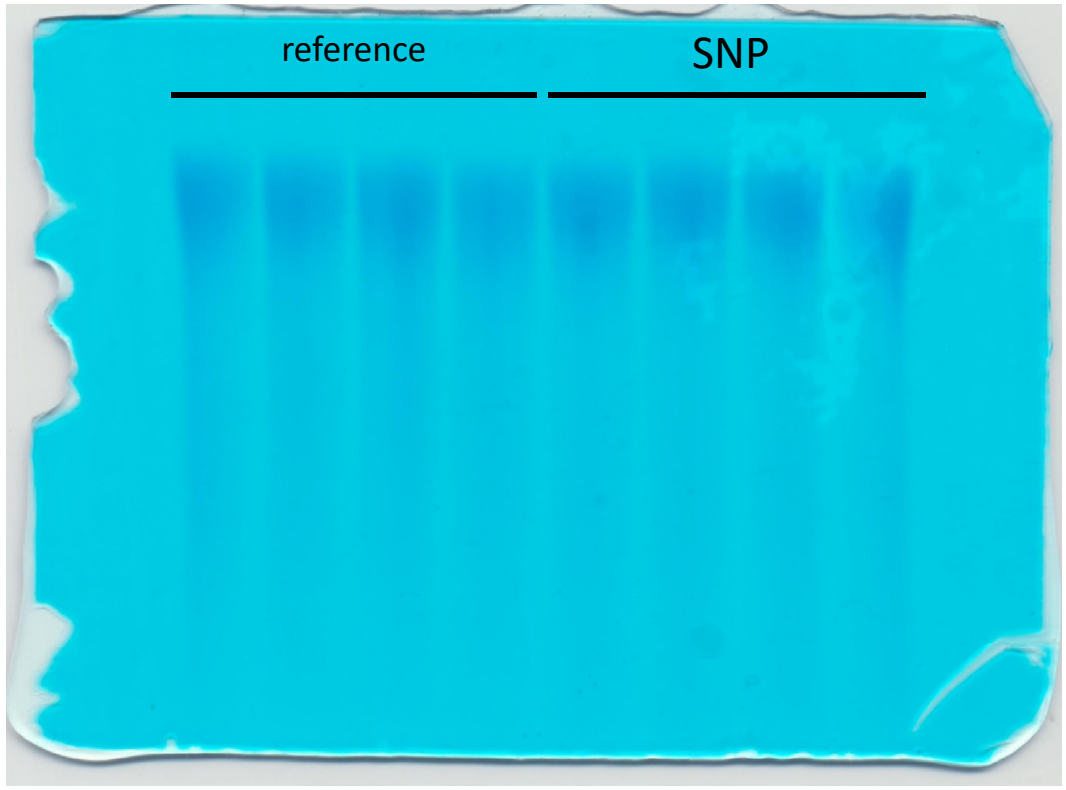
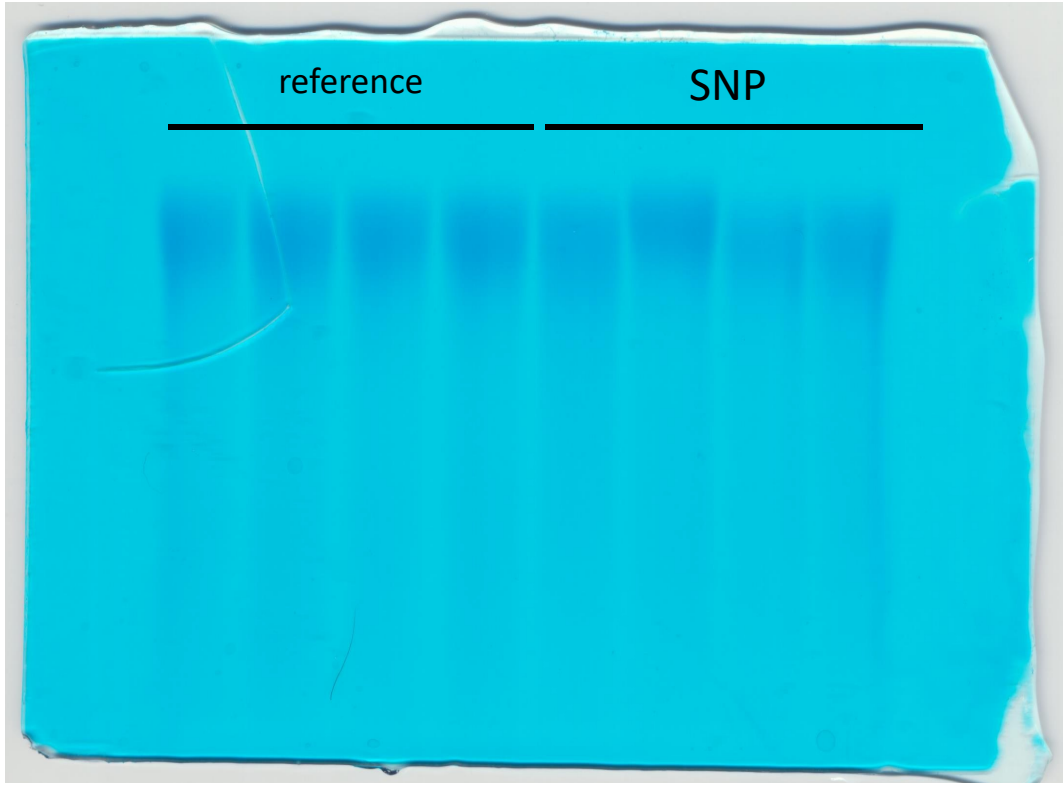
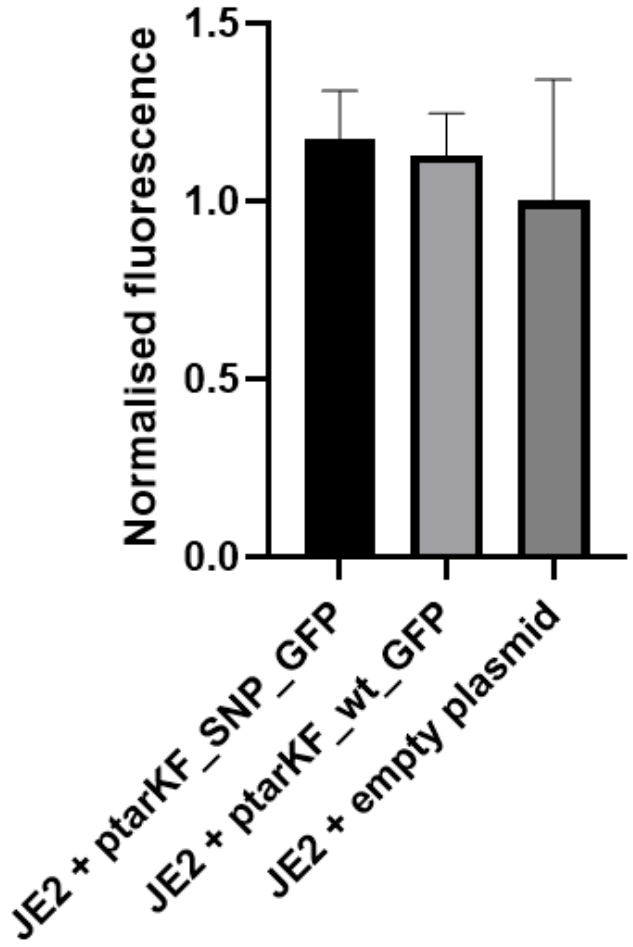
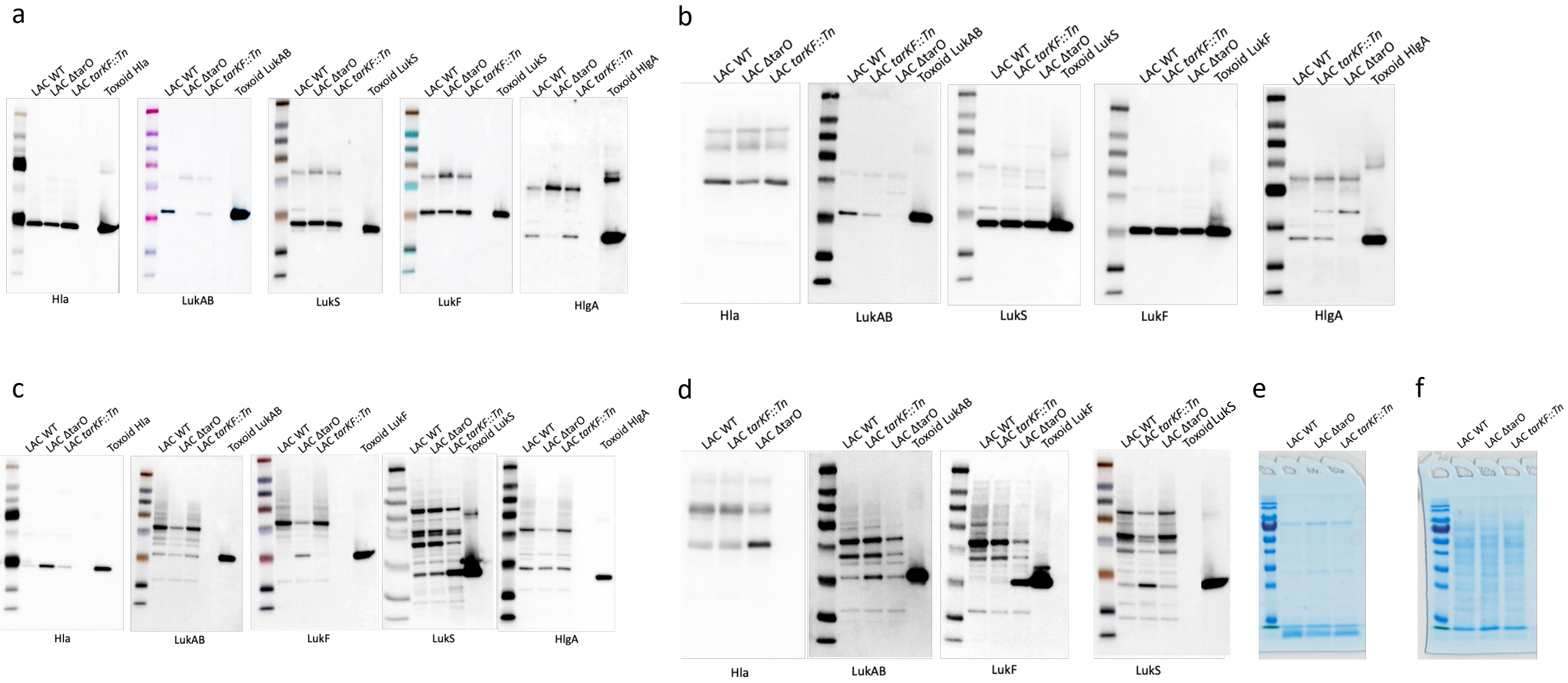




Supp. Fig. 1: The presence of the intergenic SNP does not affect WTA length/polymerisation. WTA was extracted from 16 clinical isolates, eight with the reference (wild type) *tar* intergenic sequence, and eight with the intergenic SNP. No difference in the length or polymerisation of the WTA was evident.



Supp. Fig. 2: Transcription from the *tarF* promoter is undetectable by promoter GFP fusion. The graph shows the fluorescence detected from overnight growths of JE2 transformed with pOS1 plasmid containing the GFP reporter fusion with the wild type (wt) intergenic region, pOS1 plasmid containing the GFP reporter fusion with the SNP intergenic region and empty pOS1 plasmid. Fluorescence values were normalised to the empty plasmid control.



Supp Fig. 3: Toxins abundance in whole cell lysates and supernatants replicates. Western replicates (a) supernatant, replicate 1. (b) supernatant, replicate 2. (c) whole cell lysate, replicate 1. (d) whole cell lysate, replicate 2. Since HlgA was not detectable in the first replicate, the western was not repeated. Coomassie gels replicates (e) supernatants, replicate 2. (f) whole cell lysate, replicate 2.

Accession	Description	Score	Coverage	Area	# AAs	MW [kDa]
Q2FWM8	Delta-hemolysin	26.65	96.15	3.782×10^{7}	26	3.0
Q2FZA4	Phenol soluble modulin beta 1	13.75	70.45	8.575×10^{6}	44	4.5
P0C7Y1	Phenol-soluble modulin alpha 1 peptide	4.68	85.71	9.601×10^{7}	21	2.3
P0C818	Phenol-soluble modulin alpha 4 peptide	4.32	85.00	1.680×10^{8}	20	2.2
Q2FZA3	Phenol soluble modulin beta 2	4.17	40.91	4.538×10^{5}	44	4.5

Supp. Table 1. PSMs band identification. The table shows the proteins identified in the 3kDa band on SDS page gel from JE2 supernatants. Coverage: percentage of the protein sequences covered by identified peptides. Areas: distribution of areas calculated by the Peptide and Protein Quantifier node over the sample groups. # Aas: the theoretical length of the protein sequence. MW [kDa]: theoretical molecular weight of the protein.

Toxin	isoelectric point
LukG	8.42
LukH	9.30
Hla	7.94
LukS	8.90
LukF	8.96
HlgA	9.53
Hld	9.03
Psmα1	9.70
Psmα2	10.00
Psmα3	9.52
Psmα4	9.70
Psmβ1	4.78
Psmβ2	5.36

Supp. Table 2. Isoelectric points of the toxins whose secretion is impaired in a WTA mutant. The isoelectric points were calculated on the amino acid sequence, excluding the signal peptide, using the online tool Compute pI/Mw (https://web.expasy.org/compute_pi/)**.**

Supp. Table 2. Clinical strains used in this study. The strain names for 90 isolates of *S. aureus* of the ST239 lineage are listed. Whether they contain the *tarF* promoter SNP is indicated, as is their toxicity as measured by vesicle lysis.

Strain ID	Tarf SNP?	Toxicity (fluor.units)	Strain ID	Tarf SNP?	Toxicity (fluor. units)	Strain ID	Tarf SNP?	Toxicity (fluor.units)
IU12	SNP	86313	IU19		25421	DEU12		15424
IU9	SNP	85826	DEU6		25366	DEU49		15328
IU4	SNP	85146	DEU25		25121	HU19		15119
IU17	SNP	85090	DEU8		23850	DEU5		14901
IU20	SNP	83862	IU6		22072	IU11	SNP	14864
HU13	SNP	83420	DEU15		20765	DEU22		14845
HU7	SNP	82231	DEU23		20679	HU21		14826
HU8	SNP	82125	MU1		20127	DEU35		14607
DEU29		81384	DEU41		19862	DEU36		14555
HU17	SNP	80774	MU19		19774	DEU46		14341
MU11	SNP	79495	IU16		19587	HU14		13637
IU10	SNP	72244	IU7		19443	MU2		13452
DEU20		62940	HU9		18330	DEU47		13390
DEU17		62740	DEU19		17354	MU6		13264
DEU37		62085	DEU11		17264	IU8		12317
DEU9		60435	DEU39		17119	DEU10		11988
HU16		59307	MU20		16907	DEU2		11827
HU11		58291	DEU16		16780	HU18		11402
HU5		52212	DEU28		16752	HU2		11383
HU6		46616	IU13		16622	HU15		11299
HU4		40677	IU3	SNP	16590	MU4	SNP	10580
DEU42		34291	DEU38		16316	DEU43		10115
DEU3		33352	DEU14		16179	MU10		9426
DEU40		33022	HU12		16062	HU10		9426
DEU1		31815	IU18		16053	MU7		8989
DEU27		28779	DEU50		16017	MU5		8829
DEU30		27803	MU3		15943	HU23		7859
DEU4		27328	IU14		15893	MU9		7567
IU1		27103	IU2	SNP	15832	HU26		5049
DEU7		25698	IU15		15793	HU24		4693