





SC29	GcACCTTCTGGTTCAGGAAAGACAACCTTTATTAATGTACTCAGTTCGA	<i>vraF</i> -G40A,
SC30	TGTCTTTCCTGAACCAGAAGGTGCCATGATTGATATAAACTCACCTTCAT	introduces point mutation in the Walker A motif to abrogate ATP binding
SC31	CGCGGATCCGAAGTCCGTCTATCTAAACATTTT	
SC32	ACGCCCCGGGGACCTCTTTATAATTATAGTAATGTTC	
SC33	GGAGGATCCTAATAATTTTCGATGGTTTTGGTTT	<i>graS</i> coding sequence,
SC34	GAAGAATCCCAAATGACAATCTTGTCACCTTAGAC	GraS-T18 fusion
SC35	GGAGGATCCtAACTTTAATGAAATTATCTTCAAA	<i>vraG</i> coding sequence,
SC36	GAAGAATTCTTATATCGAATGTTTAATTGTTCTTTT	T25-VraG fusion
SC37	CAAAAAGCGGTCAAATTATACGGTGCGCCCCGTGATTTCAAACAATAA GTATCATAAAAAATTTATTTGCTTTCAGGAAAATTTTCTGTATAAT	Generates upstream $\lambda$ -red integration target sequence and introduces
SC38	CTAGAACTCCTCTCTCAAATTTATGAATCTATTATACAGAAAAATTTTCCT GAAAGCAAA	pN25 promoter upstream of NIH0514 75 <i>hsdMS</i> coding sequence
SC39	GATTCATAAATTTGAGAGAGGAGTTCTAGATGGCAACAATTGGATTTGAA GAAAA	NIH0514 75 <i>hsdMS</i> coding sequence and
SC40	GAAGCAGCTCCAGCCTACACATATTTAAATTGAAAGTTCATCCATATTCA CC	overlap for FRT-flanked Kanamycin cassette
SC41	GAGGTGAATATGGATGAACTTTCAATTTAA ATATGTGTAGGCTGGAGCTGCTTCGAAGTT	Generates FRT-

SC42	AACTTCGAAGCAGCTCCAGCCTACACATATTTAAATTGAAAGTTCATCCA TATTCACCTC	flanked Kanamycin cassette and introduces downstream $\lambda$ -red integration target sequence
SC43	ATAACGTGGCTTTTTTTGGTAAGCAGAAAATAAGTCATTAGTGAAAATAT GTCCATATGAATATCCTCCTTAG	Primer set amplifies fused fragments of $\lambda$ -red integration amplicon
SC44	CAAAAAGCGGTCAAATTATACGGTG	Generates complete pKD46 fragment for Gibson assembly
SC45	ATAACGTGGCTTTTTTTGGTAAGC	<i>recA</i> coding sequence and native promoter from <i>E.coli</i> K12 for Gibson assembly
SC46	TTCGTTCCACTGAGCGTCA	<i>graS</i> upstream region, in- frame C- terminal HA-tag
SC47	AACTCACGTTAAGGGATTTTGGT	<i>graS</i> downstream region, in-frame C- terminal HA-tag
SC48	TGACGCTCAGTGGAACGAACACTTGATACTGTATGAGCATACA	<i>vraG</i> upstream
SC49	ACCAAAATCCCTTAACGTGAGTTGCAAAGGGCCGCAGATG	
SC50	ACGCGGATCCGACATGGATAGTGCAATTGAAACCAAA	
SC51	AGCATAATCTGGAACATCATATGGATAAAATGACAATCTTGTCACCTTAG ACAT	
SC52	TATCCATATGATGTTCCAGATTATGCTTAAACGTGCGTTTTGTTACTTACT A	
SC53	ACGCCCCGGGTCCTACTTGTGAGCCTTCTTT	
SC54	ACGCGGATCCGCGGCGTTAAGTCGTAGT	

SC55	AGCATAATCTGGAACATCATATGGATAATTAAGTTCATAATTGATGCCA C	region, in-frame N-terminal HA-tag
SC56	TATCCATATGATGTTCCAGATTATGCTGAAATTATCTTCAAAAACCTTAA ACAA	<i>vraG</i> downstream region, in-frame N-terminal HA-tag
SC57	GGAGGATCCCATTTGCAATCATGATATGCTTAG	Fuses promoter of <i>mprF</i> to coding sequence
SC58	TATTTTCTTTAATTAATTCTTCACCCATGTTTTCACTTCGATTCTAATTCAA T	of the mRuby fluorescent protein
SC59	ATTGAATTAGAATCGAAGTGAAAACATGGGTGAAGAATTAATTAAGAA AATA	
SC60	ACGCACGGTCGACTTTATATAATTCATCCATACCACCACC	

Table S2. Complete strain and plasmid table

Strain or plasmid	Relevant genotype or description	Source
<i>S. epidermidis</i>		
NIH051475		(1)
ALC9220	$\Delta$ <i>graS</i>	This Study
ALC9221	$\Delta$ <i>graS</i> WTR	This Study

ALC9222	$\Delta graS/pEPSA5::vraG$ , VraG is ectopically overexpressed in a $\Delta graS$ background by the inducible plasmid pEPSA5	This Study
ALC9223	$\Delta graS/pEPSA5::vraFG$ , VraFG is ectopically overexpressed in a $\Delta graS$ background by the inducible plasmid pEPSA5	This Study
ALC9224	$graS-IM^{MW2}$ , replaces NIH051475 GraS intramembrane regions with <i>S. aureus</i> MW2's	This Study
ALC9225	$\Delta EL::Alanine_9 graS$	This Study
ALC9226	$\Delta EL::AYEISVESV graS$	This Study
ALC9227	$\Delta EL::DYDFPIDSL graS$ , replaces NIH051475 9 residue extracellular loop with <i>S. aureus</i> MW2's	This Study
ALC9228	$\Delta vraG$	This Study
ALC9229	$\Delta vraG$ wild type revertant	This Study
ALC9230	$\Delta EL vraG$	This Study
ALC9231	$\Delta EL vraG$ wild type revertant	This Study

ALC9232	$\Delta$ GL <i>vraG</i>	This Study
ALC9233	$\Delta$ GL <i>vraG</i> wild type revertant	This Study
ALC9234	$\Delta$ GL:: <i>Gly</i> <sub>10</sub> <i>vraG</i>	This Study
ALC9235	<i>vraF</i> -G40A, Alanine point mutation in Walker A motif	This Study
ALC9236	<i>vraF</i> -G40A wild type revertant	This Study
ALC9237	NIH051475 with C-terminal HA tagged GraS	This Study
ALC9238	NIH051475 with C-terminal HA tagged EL/DYDFPIDSLS GraS	This Study
ALC9239	NIH051475 with C-terminal HA tagged EL/ Alanine GraS	This Study
ALC9240	NIH051475 with N-terminal HA tagged VraG	This Study
ALC9241	NIH051475 with N-terminal HA tagged $\Delta$ EL VraG	This Study
ALC9242	NIH051475 with N-terminal HA tagged $\Delta$ GL VraG	This Study

ALC9243	NIH051475 with N-terminal HA tagged GL-Gly <sub>10</sub> VraG	This Study
<b><i>E. Coli</i></b>		
DC10B	mcrA, Δ(mrr-hsdRMS-mcrBC), φ80lacZΔM15, ΔlacX74, recA1, araD139, Δ(ara-leu)7697, galU, galK, rpsL, endA1, nupG, Δdcm	(2)
DTH1	F <sup>-</sup> , cya-854, ilv 691::Tn10, recA1, endA1, gyrA96 (Nal <sup>r</sup> ), thi1, hsdR17, spoT1, rfbD1, glnV44(AS)	(3)
C41(DE3)	F <sup>-</sup> , ompT, gal, dcm, hsdSB(rB- mB-)(DE3)	Lucigen
SKC-N05	DC10BΩP <sub>N25</sub> -hsdMS of NIH051475 integrated between the atpI and gidB genes	This study
<b>Plasmids</b>		
pSK236	Shuttle plasmid; ColE1 <i>E. coli</i> origin and Amp <sup>r</sup> selection, pC194 staphylococci origin and Cm <sup>r</sup> selection	(4)
pEPSA5	Xylose inducible complementation vector; p15A <i>E. coli</i> origin and Amp <sup>r</sup> selection, pC194 staphylococci origin and Cm <sup>r</sup> selection	(5)
pMAD	Allelic exchange vector; pBR322 <i>E. coli</i> origin with Amp <sup>r</sup> selection, pE194ts staphylococci origin with Erm <sup>r</sup> selection	(6)
pKD46	λ-red recombineering vector for targeted genetic alterations in <i>E. coli</i> ; repA101ts origin, Amp <sup>r</sup>	(7)



pKD4	Template plasmid for FRT flanked kanamycin cassette; Amp <sup>r</sup> and Kan <sup>r</sup>	(7)
pKD46'	pKD46 harboring <i>E. coli</i> K12 <i>recA</i> coding sequence under native promoter	This study
pKT25	BacTH vector for N-terminal T25 fusion; p15A origin, Kan <sup>r</sup>	Euromedex
pUT18	BacTH vector for C-terminal T18 fusion; ColE1 origin, Amp <sup>r</sup>	Euromedex
pKT25-zip	Contains T25 fused leucine zipper of GCN4	Euromedex
pUT18C-zip	Contains T18 fused leucine zipper of GCN4	Euromedex
pEPSA5-vraG	pEPSA5 harboring the coding sequence of <i>vraG</i> for overexpression	This study
pEPSA5-vraFG	pEPSA5 harboring the coding sequence of <i>vraFG</i> for overexpression	This study
pMADgraS	pMAD derivative for in-frame deletion of <i>graS</i>	This study
pMADvraG	pMAD derivative for in-frame deletion of <i>vraG</i>	This study
pMADgraS-MW2 IM	pMAD derivative for replacement of NIH051475 GraS intramembrane segments with <i>S. aureus</i> MW2's	This study

pMAD-SAla	pMAD derivative for GraS EL replacement with Alanines	This study
pMAD1	pMAD derivative for EL/ AYEISVESV <i>graS</i>	This study
pMAD9	pMAD derivative for EL/DYDFPIDSL <i>graS</i>	This study
pMADvraG-EL	pMAD derivative for in-frame deletion of VraG extracellular loop	This study
pMADvraG-GL	pMAD derivative for in-frame deletion of VraG putative guard loop	This study
pMADGGly	pMAD derivative for VraG putative GL replacement with Glycines	This study
pMADG40A	pMAD derivative for introduction of alanine at residue 40 of VraF	This study
pMADgraS-WTR	pMAD derivative to generate wild type revertants in <i>vraF</i> and <i>vraG</i> mutants	This study
pMADvraFG-WTR	pMAD derivative to generate wild type revertants in the $\Delta$ <i>graS</i> mutant	This study
pUT18- <i>graS</i>	<i>graS</i> ORF cloned into pUT18	This study
pUT18- <i>graS</i> -9	EL/DYDFPIDSL <i>graS</i> ORF cloned into pUT18	This study

pUT18-graS-3	EL/ DYEASVESV <i>graS</i> ORF cloned into pUT18	This study
pUT18-graS-1	EL/ AYEISVESV <i>graS</i> ORF cloned into pUT18	This study
pKT25-vraG	<i>vraG</i> ORF cloned into pKT25	This study
pKT25-vraG-GL	$\Delta$ GL <i>vraG</i> ORF cloned into pKT25	This study
pKT25-vraG-EL	$\Delta$ EL <i>vraG</i> ORF cloned into pKT25	This study
pKT25-vraG-Gly	GL-Gly <sub>10</sub> <i>vraG</i> ORF cloned into pKT25	This study



**Supplementary figure S1. Western blot validation of mutant GraS and VraG expression.**

HA-tagged alleles of indicated mutant VraG (N-terminal) or GraS (C-terminal) were chromosomally integrated via allelic exchange in the NIH051475 background. The predicted size for most VraG mutants is approximately 70 kDa while the *vraG*-ΔEL mutant is expected to be approximately 55 kDa. The expected size for monomeric GraS is approximately 35 kDa. Solid black line separating ΔGL::Gly<sub>10</sub> VraG:HA and ΔEL VraG:HA indicates removal of empty intervening lane.

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