## S1. Oligonucleotide table

Oligonu cleotide	Sequence	Descript ion
SC1	CGCGGATCCAGGAACATTAAAAGACCTTTAGCT	graS upstream
SC2	TTAAAATGACAATCTTGTCACCTTAGAAAACCAAAACCATCGAAAATTAT TCAT	frame deletion mutant
SC3	ATGAATAATTTTCGATGGTTTTGGTTTTCTAAGGTGACAAGATTGTCATTT TAA	<i>graS</i> downstrea
SC4	ACGCACGCACGTCGACTCCTACTTGTGAGCCTTCTTT	in-frame deletion mutant
SC5	CGCGGATCCGGATTATACCTTGTACAAAGCGTG	<i>vraG</i> upstream
SC6	AGCTATAATAGCAAATACTGCATATTTGAAGATAATTTCATTAAAGTTCA T	frame deletion mutant
SC7	ATGAACTTTAATGAAATTATCTTCAAATATGCAGTATTTGCTATTATAGCT	<i>vraG</i> downstrea
SC8	ACGTCCCGGGTGTTTCATCGACTGTCTTAAGAT	in-frame deletion mutant
SC9	TTAAAAACAATATCCAAAATATCCAGTTCATACGAGATTTTATAAAAAAAC CAAAACCATC	Swaps intermem
SC10	ATGAACTGGATATTTGGATATTGTTTTTAA	S. <i>_epidermid</i>
SC11	TACACTTTCAACTGAAATCTCATAATCGATTAGACTAATGCCTAACATAA GC	<i>is</i> NIH0514
SC12	GATTATGAGATTTCAGTTGAAAGTGTATTTTATATTGTTTCTTTGAATTTA AGTTTA	75 GraS with S.
SC13	TTTAAAATATGTCAATATAAGAAAAATCATTGT	MW2 GraS
SC14	ACAATGATTTTTCTTATATTGACATATTTTAAAGAAGTCCGTCTATCTA	-
SC15	GCAGCAGCAGCAGCAGCAGCAGCAGCATTTTATATAGTGATATTAAACG TAGGATTG	GraS 9 amino
SC16	TGCTGCTGCTGCTGCTGCTGCTGCTATGTAGGCAACACCTAATAAAA TAATGT	acid extracellu lar loop to alanines

SC17	GCATATGAGATTTCAGTTGAAAGTGTATTTTATATAGTGATATTAAACGT AGGATT	ΔEL::AY EISVESV
SC18	TACACTTTCAACTGAAATCTCATATGCTATGTAGGCAACACCTAATAAAA TAATGT	gras
SC19	AGCTAGGATCCCAGAAGATATAATTACATTACATCTTGTT	ΔEL::DY DFPIDSL
SC20	TAAACTGTCTATTGGAAAATCATAATCTATGTAGGCAACACCTAATAAAA TAATGT	<i>graS</i> , swaps extracellu
SC21	GATTATGATTTTCCAATAGACAGTTTATTTTATATAGTGATATTAAACGTA GGATT	lar loop of <i>S</i> .
SC22	AGCTAGTCGACCACTCCTGTCATACTTCAATCAAAT	epidermid is NIH0514 75 GraS with S. aureus MW2 GraS
SC23	ACGTTAAAGAATCAGAGACAAGCAAATGAAGATATAGAAACAAGAAGT GAAG	ΔEL <i>vraG</i> , deletes
SC24	ATTTGCTTGTCTCTGATTCTTTAACGTAACATCATGTGGTGAACTCAGTAA	residues 334 to 490 of extracellu lar domain
SC25	GGTAGACCGACACTTATTTTAAATGAT	$\Delta GL$ <i>vraG</i> ,
SC26	ATCATTTAAAATAAGTGTCGGTCTACCATTTATCCCTTTTCTCAAACTTAC TTT	-deletes residues 432 to 441 of extracellu lar domain
SC27	GGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTAGACCGACACTTATTTT AAATGAT	$\Delta GL::Gly$ 10 <i>vraG</i> ,
SC28	ACCACCACCACCACCACCACCACCACCATTTATCCCTTTTCTCAAAC TTACTTT	replaces residues 432 to 441 of extracellu lar domain with glycines

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

SC42	AACTTCGAAGCAGCTCCAGCCTACACATATTTAAATTGAAAGTTCATCCA TATTCACCTC	flanked Kanamyci
SC43	ATAACGTGGCTTTTTTTGGTAAGCAGAAAATAAGTCATTAGTGAAAATAT GTCCATATGAATATCCTCCTTAG	In cassette and introduces downstrea $m \lambda$ -red integratio n target sequence
SC44	CAAAAGCGGTCAAATTATACGGTG	Primer set amplifies
SC45	ATAACGTGGCTTTTTTGGTAAGC	fused fragments of $\lambda$ -red integratio n amplicon
SC46	TTCGTTCCACTGAGCGTCA	Generates complete
SC47	AACTCACGTTAAGGGATTTTGGT	fragment for Gibson assembly
SC48	TGACGCTCAGTGGAACGAACACTTGATACTGTATGAGCATACA	<i>recA</i> coding
SC49	ACCAAAATCCCTTAACGTGAGTTGCAAAAGGGCCGCAGATG	and native promoter from <i>E.coli</i> K12 for Gibson assembly
SC50	ACGCGGATCCGACATGGATAGTGCAATTGAAACCAAA	graS upstream
SC51	AGCATAATCTGGAACATCATATGGATAAAATGACAATCTTGTCACCTTAG ACAT	frame C- terminal HA-tag
SC52	TATCCATATGATGTTCCAGATTATGCTTAAACGTGCGTTTTGTTACTTAC	<i>graS</i> downstrea
SC53	ACGCCCCGGGTCCTACTTGTGAGCCTTCTTT	m region, in-frame C- terminal HA-tag
SC54	ACGCGGATCCGCGGCGTTAAGTCGTAGT	<i>vraG</i> upstream

SC55	AGCATAATCTGGAACATCATATGGATAATTAAAGTTCATAATTGATGCCA C	region, in- frame N- terminal HA-tag
SC56	TATCCATATGATGTTCCAGATTATGCTGAAATTATCTTCAAAAACTTTAA ACAA	vraG downstrea m region, in-frame N- terminal
SC57	GGAGGATCCCATTTGCAATCATGATATGCTTAG	Fuses promoter
SC58	TATTTTCTTTAATTAATTCTTCACCCATGTTTTCACTTCGATTCTAATTCAA T	of <i>mprF</i> to coding sequence
SC59	ATTGAATTAGAATCGAAGTGAAAACATGGGTGAAGAATTAATT	of the mRuby
SC60	ACGCACGGTCGACTTTATATAATTCATCCATACCACCACC	t protein

Table S2. Complete strain and plasmid table

Strain or plasmid	<b>Relevant genotype or description</b>	Source
S. epidermidis		
NIH051475		(1)
ALC9220	$\Delta graS$	This Study
ALC9221	ΔgraS 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	<i>graS</i> -IM <sup>MW2</sup> , replaces NIH051475 GraS intramembrane regions with S. aureus MW2's	This Study
ALC9225	ΔEL::Alanine <sub>9</sub> graS	This Study
ALC9226	ΔEL::AYEISVESV graS	This Study
ALC9227	ΔEL::DYDFPIDSL graS, replaces NIH051475 9 residue extracellular loop with S. aureus MW2's	This Study
ALC9228	$\Delta vraG$	This Study
ALC9229	$\Delta v raG$ wild type revertant	This Study
ALC9230	$\Delta EL vraG$	This Study
ALC9231	$\Delta EL vraG$ wild type revertant	This Study

ALC9232	$\Delta GL vraG$	This Study
ALC9233	$\Delta GL vraG$ wild type revertant	This Study
ALC9234	$\Delta GL::Gly_{10}$ vraG	This Study
ALC9235	<i>vraF</i> -G40A, Alanine point mutation in Walker A motif	This Study
ALC9236	vraF-G40A wild type revertant	This Study
ALC9237	NIH051475 with C-terminal HA tagged GraS	This Study
ALC9238	NIH051475 with C-terminal HA tagged EL/DYDFPIDSL 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
E.Coli		
DC10B	mcrA, $\Delta$ (mrr-hsdRMS-mcrBC), $\varphi$ 80lacZ $\Delta$ M15, $\Delta$ lacX74, recA1, araD139, $\Delta$ (ara-leu)7697, galU, galK, rpsL, endA1, nupG, $\Delta$ dcm	(2)
DTH1	F-, cya-854, ilv 691::Tn10, recA1, endA1, gyrA96 (Nalr), thi1, hsdR17, spoT1, rfbD1, glnV44(AS)	(3)
C41(DE3)	F-, 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
Plasmids		
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</i> . <i>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 recA 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 graS	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 graS	This study
pMAD9	pMAD derivative for EL/DYDFPIDSL graS	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 $\Delta graS$ mutant	This study
pUT18-graS	graS ORF cloned into pUT18	This study
pUT18-graS-9	EL/DYDFPIDSL graS ORF cloned into pUT18	This study

pUT18-graS-3	EL/ DYEASVESV graS ORF cloned into pUT18	This study
pUT18-graS-1	EL/ AYEISVESV graS ORF cloned into pUT18	This study
pKT25-vraG	vraG ORF cloned into pKT25	This study
pKT25-vraG-GL	ΔGL <i>vraG</i> ORF cloned into pKT25	This study
pKT25-vraG-EL	ΔEL <i>vraG</i> ORF cloned into pKT25	This study
pKT25-vraG- Gly	GL-Gly <sub>10</sub> vraG 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- $\Delta$ EL mutant is expected to be approximately 55 kDa. The expected size for monomeric GraS is approximately 35 kDa. Solid black line separating  $\Delta$ GL::Gly10 VraG:HA and  $\Delta$ EL VraG:HA indicates removal of empty intervening lane.

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