SUPPLEMENTARY INFORMATION

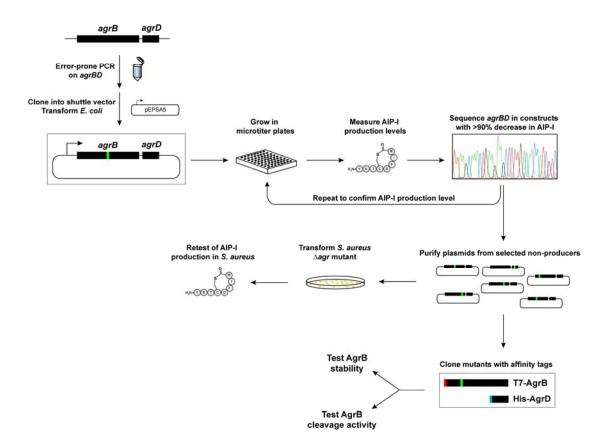


Figure 1. Schematic of the screen for identifying *agrBD* mutants. As outlined in the text, the *agrBD* genes were mutagenized by error-prone PCR and cloned onto *E. coli* – *S. aureus* shuttle vector pEPSA5. Spent media from the *E. coli* transformants was tested for AIP-I production using a *lux*-based *agr* activation bioassay with an *S. aureus* reporter strain. Transformants producing less than 10% bioluminescence were retested in the bioassay, and the mutagenized *agrBD* genes that confirmed in the retest were sequenced. After removing candidates with multiple or nonsense mutations, the pEPSA5-*agrBD* mutant plasmids were transformed into *S. aureus Δagr* strain (AH1292) for testing AIP-I production in the native host. In parallel, T7-AgrB and His6-AgrD constructs were built for testing AgrB cleavage activity.

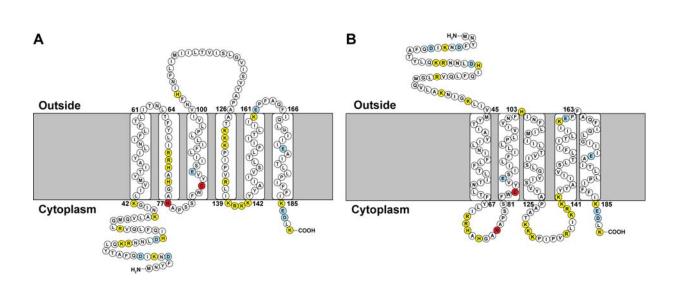


Figure S2. Topology maps of AgrB. **A**, The topology map reported by Zhang, et al. (Zhang et al., 2002) based on alkaline phosphatase fusions. **B**, AgrB topology as predicted by the topology analysis program TOPCONS (Bernsel et al., 2009). Positively charged residues are shaded yellow, negatively charged ones are blue. Red indicates the catalytic histidine and cysteine residues.

		1 10 20 30 40 50 60 70
S. aureus type 1	(1)	LNYFDNKIDQFATYLQKRNNLDHIQ-FLQVRLGMQVLAKNIGKLIVMYTIAYILNIFLFTLITNLTFYLIRRH
S. aureus type 2	(1)	LNYFDNK <mark>I</mark> DQF <mark>A</mark> TY <mark>LQ</mark> KRN <mark>NL</mark> DH <mark>I</mark> Q-F <mark>L</mark> QVRLGMQIIVG <mark>N</mark> FF <mark>K</mark> ILVT <mark>Y</mark> SISIFLSVFLFTLVTHLSYMLI <mark>R</mark> YN
S. aureus type 3		LNYFDNK <mark>I</mark> DQF <mark>A</mark> TY <mark>LQ</mark> KRN <mark>NL</mark> DH <mark>I</mark> Q-F <mark>L</mark> QVRLGMQVLAKNIGKLIVMYTIAYILNI <mark>F</mark> IFTLITNISFYLIRRY
S. aureus type 4		MNYFDNKIDQFATYLQKRNNLDHIQ-FLQVRLGMQVLAVNIGKLIVMYTIAYILNIFLFTLITNLTFYLIRH
S. capitis		MNIIDRKIDEFANYLQRKNNLDHIQ-FLKVRLGMQVVLTNIEKTIIVYGLALIFQTFYYTLLTHLSYFLIRSN MOKVLERKIDAWAQALQKRNNLDRIA-YLKIKLGLEVFFNNLFKTIVVYGLALLFHVFLYTLTVHLSYFAIRHY
S. carnosis S. epidermidis		MQKVLEKKIDAWHQALQKKNNDKIA-ILKIRLGLEVFFNNDFKIIVVIGLALLFHVELIIIIVHLSIFAIRH MKIIDKKIEQFAQYLQRKNNLDHIQ-FLKIRLGMQVLAINIEKSIVVYGLAIIFHTFFYTLLTHLSYFLIRH
S. haemolyticus		MAIDARIEQFALYLQRKNNLDHIQ-FLKVRLGLQVVVSNLAKTIVTYGVALIFHTFLYTLFTHVSYFLVR
S. intermedius		MLLIDNGIEKMALKLQORONLSHIE-FLKVRLGMOVVVINTFKAIVTYGLALLLNIFLYTLIVHLTFLTLRTY
S. lugdunensis		LKAIDKK <mark>I</mark> ERF <mark>A</mark> RY <mark>LQ</mark> RQN <mark>NLDHI</mark> Q-F <mark>L</mark> KIR <mark>LG</mark> IQVALG <mark>N</mark> FF <mark>K</mark> TIVT <mark>Y</mark> GVALLFHT <mark>F</mark> LY <mark>TL</mark> ITHLTYFFV <mark>R</mark> RF
S. warneri		MKIIDTK <mark>I</mark> DQF <mark>A</mark> NYLQRRNNLDRIQ-FLKVRLGMQVVVS <mark>N</mark> IAKFIVT <mark>Y</mark> GLAIIFHI <mark>F</mark> WYTLTMHLAYMILRFY
C. acetobutylicum		MKGKSSVMEKLAEVVSLKLNKHLKMEGIE-LIKLKLGVEIIFINISKLAILFLVSYYFGLIKETIIMLAAFGFLRSN
C. beijerinckii C. botulinum		MEVDLSKKALSEEIALKLVAKINRDNQLDNVN-YKKMKYGLEVIIINLSKTSFILLIAFLLGILQKTLTIMLSFAFIRRY MKLSEKFSIKVTNYIKKTLPNKTEEDLQIIKYGVELLFMNFTKLPIILVVGYILNIFKFTVYAMVIFAFIRRF
C. butyricum		-MKNKIQISFMERLAETIVNNMNSYLHKEGLE-IQKMKLGMEILLINISKLIIIFLVAAAFHLLGRTIFILLIFTIIRRN
C. cellulolyticum		MLEVITKKITNEIVLNVPGITEEKAEQIDYGLYMALADGLKLLAVLIAALLLGQLKYAVVAVIVFSLNKSY
C. difficile 1		MFKRYAEKMTSVLICNNMIDNNE-SKVYSYGFEILIAFIVNITTMLFIGFLFGKFTYVLFFLMCYCPIRQF
C. difficile 2		MFKRLSYKFANILVNNEIVES-EDFEIYRYGFETLIYFIVNISVALFIGIIFDRFIHTVIFLSCYCTLRQF
C. novyi		MKLSEKFSEKVTTYVKNTLPNKTEEDLEIIKYGVELLFMNFTKLPLILIVGYMLNIFKMTVCAMIIFSVIRRF
C. thermocellum L. grayii		MPLLKKLCENITNVIKERVKDIDDEKAEIINYGLYLWIADIIKLAIIFTAACLLRVFKLAVVFVVCFGLLRVF MSNYSPNVPLSARLAEKIITKERWADDEEGYLKVKYGLEIILINAMKFIAVYGVALLLGIVWQTITVHLAYLMIRRY
L. innocua		MSNFTAKVPLSERMADVLISKDRWKDDEEGYLKVKYGLEIILINVMKFAIVYGIALVTGLLLQTVTVHLSYLWLRRY
L. monocytogenes		LSNFTAKVPLSERMADVLISKDRWKDDEEGYLKVKYGLEIILINVMKFALVYGIALVTGLLLQTVTVHLSYLWLRRY
L. seeligeri		MSNFTAKIPLSERMADILISKDRWKDDEEGYLKVKYGLEIILINVMKFAIVYGIALVTGVLLQTMTVHLSYLWLRRY
L. welshimeri	(1)	MSNFTVKVPLSERMADVLISKDRWKDDEEGYLKVKYGLEIILINVMKFAIVYGISLATGLLLQTVTVHMSYLWLRRY
		80 90 100 110 120 130 140
S. aureus type 1	(73)	AHGAHAPSSFWCYVESIILFILLPLVIVNFHINFLIMIILTVISLGVISVYAPAATKKKPIPVRLIKRKKYYAIIV
S. aureus type 2	(73)	A <mark>H</mark> GAHAKSSILCYIQSILT <mark>F</mark> VFV <mark>P</mark> YFLINIDINFTYLLALSIIGLISVVIYAPAA <mark>TKK</mark> QPIPIKLVKRKKYLS <mark>I</mark> IM
S. aureus type 3		A <mark>HGAHA</mark> PSSFWCYIESITL <mark>F</mark> IVL <mark>P</mark> LLVLHFHINETLMMFLALISVGVVIKYAPAA <mark>TKKKPI</mark> PARLVKQKRYFS <mark>I</mark> II
S. aureus type 4		AHGAHAPSSFWCYVESIFLFTLLPLILVNYHINFLIMTIMTVIAIGMIIRYAPAATKKKPIPVRLIKRKRNYAIIV
S. capitis S. carnosis		A <mark>HGAHA</mark> KSSLLCHIQNIIL <mark>F</mark> ILF <mark>P</mark> YLIIKFDVKYLILLFLALIGFIIVIKNAPAATKKQPIPKRLLKRKKILS <mark>I</mark> VL A <mark>HGAHA</mark> KSTFACYIESIIL <mark>F</mark> VIL <mark>F</mark> WILIKVDIPQIFMIVLAAVAFILICLYSPAITRKQPIPNHMRKKKKITA <mark>I</mark> FV
S. epidermidis		ABGAHANSIFACTIESTIEVILETIIKVDIPO-IEMIVEAVATIETUSTATENOVETENNÖKKKKKITÄLEV AHGTHANSSLLCHIONIIFFIIFPYLIIKLDINYFVLLSMALVGLIITILYAPAATKKOPIPRRLVKRKKILSIFL
S. haemolyticus		AHGAHAKSSLLCHVQNLALFVALPWLLVYFQVNLGIMYSVVAIGTVLIIYYAPSATKKOPIPSHLKMKKKLLSIII
S. intermedius	(73)	SHGAHAKTSMLCHVQNIVA <mark>F</mark> VML <mark>P</mark> WLIVQYDISFQFLLILSLLSALIVIKYAPAA <mark>TKK</mark> RP <mark>I</mark> APKKVKGLKIKS <mark>I</mark> IV
S. lugdunensis		A <mark>HGAHARSSLICHIQNLVLFVALP</mark> WSIVHFQVSWTFMILLAFIAFIIIICYAPAA <mark>TKK</mark> QP <mark>I</mark> LPHLRKKKKRNA <mark>I</mark> LI
S. warneri		A <mark>H</mark> GAHAKSSLLCHIQNIII <mark>F</mark> LLM <mark>P</mark> WLIVYLSIGKFEMLIFALIGYLIVIIYAPAA <mark>TKK</mark> QPIPKRLVKKKRILS <mark>I</mark> AV
C. acetobutylicum C. beijerinckii		AFGLHAKNSIVCTVMSLLMFVLGAYLSKYLLFNNYMVLASFIIVNLLLFRYAPGDTEAHPLVGAKLRDKLKKQAVLMG AFGIHAKNSISCTILTSIYFFTGAYIPNFIHADNYMILLMFSTTILLLYLYAPADTDARPLTGRKLRKSLKKKALLSG
C. boljerinckii C. botulinum		AAGIHARNSISUITEISIIFFIGAITENTHADNTHILENFSITTEEETTEETTEETTEETTEETTEETTEETTEETTEET
C. butyricum		TFGLHAKNSFICTLVSLLIFVFGSYLSYYLKFNNYMVFILFTIINILLYKYAPADTENHPLFSADLRNKLKKDSVITG
C. cellulolyticum	(72)	LGGVHAKTQIGCVITHFLFIFGTVYLAQILNIKFLNIVLFAISGIFVFLYAPADLVSKPIVTEKRKRE-LRIKGSIL
C. difficile 1		SGGYHADNYFRCLLTFIFIILSTILIIENINIDLFKNIIMIIASVSWVGICVLCPIEHRSNPISDREKFVYKKTAIFIST
C. difficile 2		TGGYHARNYKECTLTFAVIYLITIFSANNIDINKYKYLLVLLMIISILTIYKLAPLEHRNKPLSESEKKHYRKTVQKILF
C. novyi C. thermocellum		AAGIHARKSYTCLASTMLVIYGSIYLSLNFKLSNILKIIIFCICFIIYLKYSPADTEEKPYLNKNLRKKLKVKSIAVI AGGSHAKTFWGCLLTNSAITFGSVYLSLLLSSIKPIFLFMLVMPFCAVVLYLYAPADHENKPVVSKKQKRKLKIVAYMVL
L. grayii		SFGLHATKSWVCTVVSVSMFDLIPYFAKGLLLNNWIVLGVFIFVLLNVFFFAPADTESLPLIGKDNRKKLKRKAMVCT
L. innocua		SFGLHATKTLNCTLISLTMFVLAPFIFQNIPSNNWIVLGTFAFILLNMFLFAPADTESLPLIGEEHRKKLKRKAMIGT
L. monocytogenes		SFGLHATKTLNCTLISLLMFVLAPFVFQNIPSNNWIVLGTFGFILLNMFLFAPADTESLPLIGEEHRKTLKRKAMIGT
L. seeligeri		SFGLHATKTLNCTLISLTMFVLTPWIFQNVLSNNWIVLGTFAFILLNMFLFAPADTENLPLIGEGHRKKLKRKAMIGT
L. welshimeri	(78)	SFGLHATKTLNGTLISLAMFVLAPFVFQNIPSNNWIVLGTFAFILLNMFLFAPADTESLPLIGEKHRKTLKRKAMIGT
		150 160 170 180
S. aureus type 1		SLTLFIITLIIKEPFAQFIQL <mark>G</mark> IIIEA-ITL <mark>LPI</mark> FFIKEDLK(189)
S. aureus type 2		YLLVLILSLIIHPFYAQFMLL <mark>G</mark> ILVES-ITL <mark>L</mark> P <mark>I</mark> FFPKED(187)
S. aureus type 3 S. aureus type 4		STILFIITLFVKEPYTQFIQL <mark>G</mark> IIIQA-ITLLP <mark>I</mark> YYSKED(187)
S. aureus type 4 S. capitis		SLIFFIITLIIKEPFAQFMQL <mark>G</mark> IIIEA-ITL <mark>PI</mark> FFVRRT(187) YCFILVVSFVTFEPVNKLILF <mark>G</mark> EFLES-LTL <mark>SI</mark> FFPKEDT(188)
S. carnosis		AGILLIISFFIKQPFNELVQLGIVLIG-AAQLPIFFPKQTKEG(191)
S. epidermidis	(149)	YCTIVVISLVTKEPVNKLILF <mark>G</mark> VILES-LTL <mark>L</mark> P <mark>I</mark> FFPKEDINHGKHF(194)
S. haemolyticus		TMVLLIISFLAPEPFKQLILL <mark>G</mark> ITLES-ITL <mark>LPI</mark> FFPREDN(188)
S. intermedius		FVLLMTIACIVPPPYNRFVVY <mark>G</mark> VLLQS-FTLLP <mark>I</mark> FSIKEEV(188)
5. lugdunensis S. warneri		SICFLVLSLLVSEPYMQLIAL <mark>G</mark> MCIEA-ITL <mark>L</mark> PIFFSKEET(188) YMLLVIISFIIKEPYSQLILF <mark>G</mark> IIVES-ITL <mark>LPI</mark> FFPKED(187)
		MLLMAITLIIPDELIKTCISLSSYFEI-ISILPITYKVLGRRYKNYYEFERTIKOS-(209)
C. beijerinckii		SILMTLTFFINDFSLKFFISYGALCEA-LTIIPIIYKLFNRYKNYEYYQRNID(210)
C. botulinum	(152)	TLYFLLSLIFNENIFISNILIHFIWIEGVLILPLTYKIFNRRYNNYENYEETI(204)
		LILMIFTLLIPNNAIKTLVSLAAISAV-TLILPITYNLLKRSYKNYENYEKDFI(209)
C. cellulolyticum		LVICFTVTLIVPNIYSNIISVITIISS-VNITPIVYRLTKNKRGGIIT(194)
C. difficile 1 C. difficile 2		VVLLITILSLSLSIFVDYFTYSAFAMFWIFVMLVLGKLKAKV(192) VIICLIILCKILNIFQQYVIYAIISIYWIAILIYIGMKVNNDQ(193)
		ILYFLLSLVFNKNMFISNILIHFIWIEGILIPLTYKIFNRYNNYENYE(201)
		IAEYLISLSITQNTFSNVIILSTLFVC-LGMLPVTYKIMGARHGNGL(199)
L. grayii	(156)	LLLTGIALLVPIAEMKVLIMLGALYQV-VSINPLVYKILNRRYHNYESYE(204)
L. innocua		LILTGIALLIPFAEMKTLIMVGSLFQV-ISINPLTYKLLKRRYRNYEKYE(204)
		LILTGIALLIPFAEMKTLIMVGSLFQV-ISINPLTYKLLKRRYRNYEKYE(204) LILTGITLLIPFAEMKTLIMIGSLFQV-VSINPLTYKLLRRRYRNYEKYE(204)
L. seeligeri L. welshimeri		LILTGITLLIPFAEMKTLIMIGSLFQV-VSINPLTYKLLKKKYKNYEKYE(204) LILTGIALLIPFAEMKTLIMVGSLFQV-ISINPLSYKLLKRRYRNYEKYE(204)
	1200/	(not)

Figure S3. Sequence alignments of AgrB. All known AgrB sequences from *Staphylococcus, Clostridium* and *Listeria species* were aligned using Vector NTI Advance 11.0 AlignX software (Invitrogen). Residues that are completely conserved in all species are boxed in gray and residues conserved in *Staphylococcus species* are boxed in yellow. Residues where mutations were found to decrease AIP production are bolded in red.