

Supplementary Materials: Rational Design of Toxoid Vaccine Candidates for *Staphylococcus aureus* Leukocidin AB (LukAB)

Shweta Kailasan, Thomas Kort, Ipsita Mukherjee, Grant C. Liao, Tulasikumari Kanipakala, Nils Williston, Nader Ganjbaksh, Arundhathi Venkatasubramaniam, Frederick W. Holtsberg, Hatice Karauzum, Rajan P. Adhikari and M. Javad Aman

Table S1. Comparison of expression using pET Duet versus pET24a (+) dual plasmid system.

Vector	IPTG Concentration (mM)	Growth/ Induction Temperature (°C)	Total Yield (mg/L)	EC50 from PMN-Based Toxicity Assay (nM)
pET Duet	0.3	37/25	19.84	0.1
pET24a(+)	0.3	37/25	20.5	0.09

Abbreviations: IPTG (Isopropyl β -D-1-thiogalactopyranoside); PMN (Polymononuclear neutrophils)

Table S2. Bacterial Strains.

Strain	Genotype/Description	Source
JE2	USA300 WT from Nebraska mutant library	NARSA repository
MRSA252, MN8, NCTC 8325, MRSACOL, USA200, USA100, MNHOCH	Standard SA strain	NARSA repository
MRSA ST-80	Standard SA strain	From Dr. Jean Lee, BWH, Harvard University
USA300 (NRS384)	Community MRSA strain	NARSA repository
USA1000, SF8300 and isogenic LukAB mutant	Community MRSA strain and it's isogenic LukAB mutant	From Dr. Binh Diep, UCSF

Abbreviations: SA (*Staphylococcus aureus*); MRSA (multi-drug resistant SA)

LukA_{wt}		LukB_{wt}	
<i>MKNKKRVLIA SLSLCAILL SAATTQANSA</i>		<i>MIKQLYKNIT ICTLALSTTF TVLPATSEAK</i>	1
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<i>HKDSQDQNK EHVDSQQKD KRNVTNKDKN</i>	33	<i>INSEIKQVSE KNLDGDTMY TTATTSDSQ</i>	31
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<i>STAPDDIGKN GKITKRTETV YDEKTNILQN</i>	63	<i>KNITQSLQFN FLTEPNYDKE TVFIKAKGTI</i>	61
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<i>LQDFDIDDPT YDKNVLLVKK QGSIHSNLKF</i>	93	<i>GSGLRILDPN GYWNSTLRWP GSYSVSIQNV</i>	91
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<i>ESHKEEKNSN WLKYPSEYHV DFQVKRNRKT</i>	118	<i>DDNNNTNVD FAPKNQDESR EVKYTYGYKT</i>	121
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<i>EILDQLPKNK ISTAKVDSTF SYSSGGKFD</i>	153	<i>GGDESINRGG LIGNITKESN YSETISYQQP</i>	151
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<i>TKGIGRTSSN SYSKTISYNQ QNYDTIASGK</i>	183	<i>SYRTLLDQST SHKGVGWKVE AHLINMGHD</i>	181
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<i>NNNWHVHWSV IANDLKYGGE VKNRNDLELF</i>	213	<i>HTRQLTNSD NRTKSEIFSL TENGLWAKD</i>	211
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<i>YRNTRIATVE NPELSFASKY RYPALVRSGF</i>	243	<i>NFTPKDKMPV TVSEGFNPEF LAVMSHDKD</i>	241
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<i>NPEFLTYLSN EKSNEKTQFE VTYTRNQDIL</i>	273	<i>KGKSQFVVHY KRSMEFKID WNRHGFWGYW</i>	271
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<i>KNRPGIHYAP PILEKNKDGQ RLIVTYEVDW</i>	303	<i>SGENHVDKKE EKLSALYEVD WKTHNVKFKV</i>	301
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<i>KNKTVKVVDK YSDDNKPYKE G</i>	324	<i>VLNDNEKK</i>	331
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Figure S1. Key residue mutations within LukAB. LukA_{WT} and LukB_{WT} sequences from USA300 strain are shown along with specific mutated residues highlighted in yellow. For LukB, residues crossed out indicate deletion mutations and those colored in blue were substituted with the corresponding residues in HlgB (AEGKITPVSVKKVDDKVTLTKTTATADSD). The leader or signal sequence is shown in bold & italics. Additionally, conservation of residues within LukA_{WT} and LukB_{WT} variants derived from different strains for which annotated genome data is available is shown below the residues as calculated from a multiple sequence alignment using ClustalOmega (Ref). The symbols indicate conservation of: a single residue (*), between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix (:), and between groups of weakly similar properties - scoring ≤ 0.5 in the Gonnet PAM 250 matrix (.).

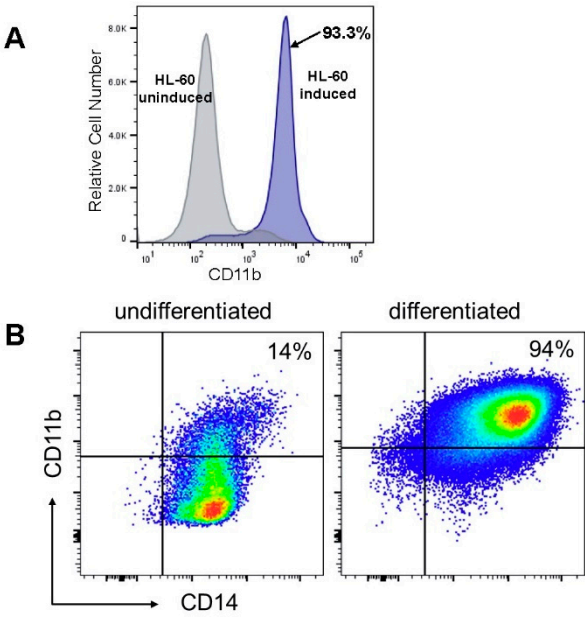


Figure S2. Expression of CD11b and/or CD14 on (A) PMN and (B) THP-1 cells.