Supplementary Information

EsxA and EsxB do not form a complex

We used recombinant EsxA fused to Glutathione S-transferase (GST-EsxA) and asked whether untagged EsxA or EsxB would engage in complex formation. Size exclusion chromatography indicated that GST-EsxA eluted at a distinct exclusion volume as compared to EsxA or EsxB, albeit that all three proteins eluted in volume fractions consistent with formation of homo-oligomers (Suppl. Figure 1S). When GST-EsxA was incubated with EsxA, a new peak containing both proteins was observed. Co-elution was not observed with EsxB (Suppl. Figure 1S). Conversely, GST-EsxB and EsxA did not co-elute and the elution profile of untagged EsxB was not significantly changed upon incubation with GST-EsxB (Suppl. Figure 1S).

Figure S1

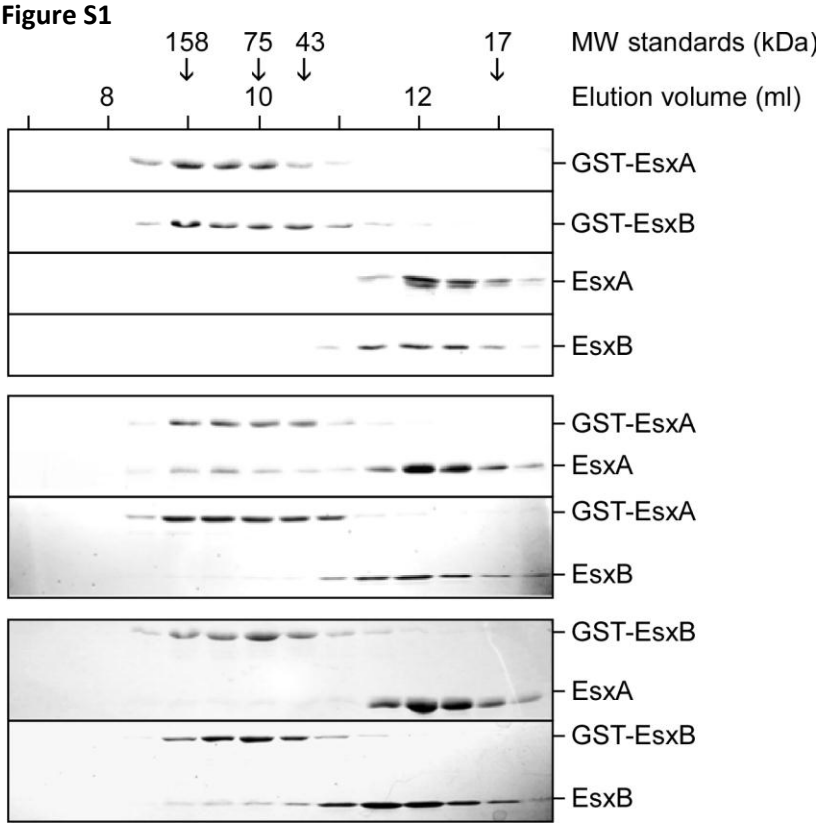

Figure S1. Gel filtration analysis of purified proteins, EsxA, EsxB, GST-EsxA and GST-EsxB, alone or incubated as pairs. The native molecular masses of EsxA and EsxB proteins fused or not to Gluthatione S-

transferase (GST) were estimated by gel filtration following separation on a Superdex^{TM} 75 10/300 GL column. For the top four panels, each protein was loaded individually, for the lower four panels, two proteins were pre-incubated for 1 h at room temperature prior to loading on the column. In all cases, aliquots of eluted fractions were subjected to SDS-PAGE and stained by Coomassie Brilliant Blue. Images of the stained gels are shown. Numbers on top indicate elution volumes in ml. The peak positions of molecular weight (MW) standards in kDa are also indicated: Aldolase (158 kDa), Conalbumin (75 kDa), Ovalbumin (43 kDa), and Myoglobin (17 kDa).

Table S1: *S. aureus* strains used in this study.

Strains	Description	Source
RN4220	*S. aureus sau1 hsdR* laboratory strain used for passaging plasmid DNA	(Kreiswirth et al, 1983; Nair et al, 2011)
Newman	Methicillin sensitive *S. aureus*	(Baba et al, 2008)
USA300	Community-acquired methicillin resistant *S. aureus*	NARSA repository (Diep et al, 2006)
esxA	USA300 carrying an internal deletion of *esxA*	(Anderson et al, 2011)
esxB	USA300 carrying an internal deletion of *esxB*	This study
esxC	USA300 carrying an internal deletion of *esxC*	This study
esxD	USA300 carrying an internal deletion of *esxD*	This study
essA	USA300 carrying an internal deletion of *essA*	This study
essB	USA300 carrying an internal deletion of *essB*	(Chen et al, 2012)
essC	USA300 carrying an internal deletion of *essC*	This study
essD	USA300 carrying a *bursa aurealis* insertion in *essD*	(Anderson et al, 2011)
saeR (Newman)	Newman carrying a *bursa aurealis* insertion in *saeR*	(Bae et al, 2004)
saeS (Newman)	Newman carrying a *bursa aurealis* insertion in *saeS*	(Bae et al, 2004)
saeR (USA300)	USA300 carrying a *bursa aurealis* insertion in *saeR*	This study
saeS (USA300)	USA300 carrying a *bursa aurealis* insertion in *saeS*	This study

Table S2: Sequence and use of oligonucleotides designed for this study.

Name	Nucleotide Sequence	Usage
essA-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTTTTG CTGAAGAGCCACAAGAACC	Cloning of the *essA* deletion mutant in pKOR1 for allelic replacement
essA-15codons-R	AAAGATCTAGATGCTGTTAAAAAAGTTAAAGC	Same as above
essA-15codons-F	AAAGATCTGGGAGACGAACGAAAAATGAATCAG	Same as above
essA-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTGCGCTC ATTATGCTTCATTACTG	Same as above
essC-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTGTTGAT CCATTACCTGTGTCAGAAG	Cloning of the *essC* deletion mutant in pKOR1 for allelic replacement
essC-15codons-R	AAAGATCTGAGCATCTTCAATTGTTTGTTATAT	Same as above
essC-15codons-F	AAAGATCTGAACTTGGCTTGAACATTTTATTTG	Same as above
essC-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTATTC GCTTAACATCTTTCATC	Same as above
esxB-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGAG AGAAATTGCGCATATTGCTG	Cloning of the *esxB* deletion mutant in pKOR1 for allelic replacement
esxB-15codons-R	AAAGATCTATCAACCTTGCCACCATCTGCTTTAA	Same as above
esxB-15codons-F	AAAGATCTGATACATTATCAATTAAGCAAGGGC	Same as above
esxB-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTAATG ACTGTTGCAGCACTACTATAG	Same as above
esxC-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCT GCAATGTTTTTATATGATAAATCAG	Cloning of the *esxC* deletion mutant in pKOR1 for allelic replacement
esxC-15codons-R	AAAGATCTTTTAAATTTCGACTTAACCATTGTTTC	Same as above
esxC-15codons-F	AAAGATCTGCTGGCGAAAAGGCAAGTGAATATTTT	Same as above
esxC-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCGTA TAAATGTATTAATGACACC	Same as above
esxD-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGCAA GGTTGATCAAGCGAAAC	Cloning of the *esxD* deletion mutant in pKOR1 for allelic replacement
esxD-15codons-R	AAAGATCT TGCAATCGTTTCAGCTTTAACAC	Same as above
esxD-15codons-F	AAAGATCTTGGGGTACGGAATTTGCCAAGC	Same as above
esxD-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTGTGAAA TACGACTAACTGCTTG	Same as above
*esxA-Nde*I-F	AAACATATGGCAATGATTAAGATGAGTCCAGAGGA A	For purification of his-EsxA in *E. coli* using pET15b
*esxA-Xho*I-R	AAACTCGAGTTATTGCAAACCGAAATTATTAGAAA GTTG	Same as above
*esxA-BamH*I-F	AAAGGATCCATGGCAATGATTAAGATGAGTCCAGA GGAA	For purification of GST-EsxA in *E. coli* using pGEX-2TK
*esxA-EcoR*I-R	AAAGAATTCTTATTGCAAACCGAAATTATTAGAAAG TTG	Same as above
*esxB-Nde*I-F	AAACATATGGGTGGATATAAAGGTATTAAAGCAGA T	For purification of his-EsxB in *E.coli* using pET15b
*esxB-Xho*I-R	AAACTCGAGTCATGGGTTCACCCTATCAAGCCCTTG CTT	Same as above
*esxB-BamH*I-F	AAAGGATCCATGGGTGGATATAAAGGTATTAAAGC AGAT	For purification of GST-EsxB in *E.coli*

		using pGEX-2TK
*esxB-EcoR*I-R	AAAGAATTCTCATGGGTTCACCCTATCAAGCCCTTGCTT	Same as above
esxD-NdeI-F	AACATATGACGTTGAGTGGAAAAATTAG	For purification of his-EsxD in *E.coli* using pET15b and complementation.
esxD-BamHI-R	AAGGATCCCTATCCCTCAATATTATAG	Same as above
EsxD-6xHis-BamHI-R	AAGGATCCTTAGTGATGGTGATGGTGATGTCCCTCAATATTATAGTAAAGC	His complementation
esxA-FBTH-5’	CGCAGTCTAGACATGGCAATGATTAAGATGAGTCCAGAG	Bacterial 2-Hybrid cloning EsxA
esxA-RBTH-3’	CGCAGGGTACCTTATTGCAAACCGAAATTATTAGAAAGT	Same as above
esxB-FBTH-5’	CGCAGTCTAGACATGGGTGGATATAAAGGTATTAAAGCA	Bacterial 2-Hybrid cloning EsxB
esxB-RBTH-3’	CGCAGGGTACCTCATGGGTTCACCCTATCAAGCCCTTGC	Same as above
esxC-FBTH-5’	CGCAGTCTAGACATGAATTTTAATGATATTGAAACAATG	Bacterial 2-Hybrid cloning EsxC
esxC-RBTH-3’	CGCAGGAATTCTTAATTCATTGCTTTATTAAAATATTCA	Same as above
esxD-FBTH-5’	CGCAGTCTAGACATGACGTTGAGTGGAAAAATTAGTGTT	Bacterial 2-Hybrid cloning EsxD
esxD-RBTH-3’	CGCAGGGTACCCTATCCCTCAATATTATAGTAAAGCTTG	Same as above
EsxD1-99BamHI-R	AAAAGGATCCCTAAAGCTTGGCAAATTCCGTACCCC	EsxD YxxxE motif mutation
EsxD-Y100A-BamHI-R	AAAAGGATCCCTATCCCTCAATATTATAGGCAAGCTTGGCAAATTCCG	Same as above
EsxD-E104A-BamHI-R	AAAAGGATCCCTATCCCGCAATATTATAGTAAAGC	Same as above
EsxD-Y100A-E104A-BHIR	AAAAGGATCCCTATCCCGCAATATTATAGGCAAGCTTGGCAAATTC	Same as above

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