Table S4. Alleles and constructs used in this study.

	Allele/ construct name	Predicted protein product(s)[a]	Method used to generate (template gDNA)	Primers used to generate allele/construct (see Table S3)[c-d]				Plasmids containing allele/construct[e]	
				US forward	US reverse	DS forward	DS reverse	Blunt-end cloning vector	Final plasmid[f]
A. In-frame, chromosomal deletion alleles	Δ*ebpA*	EbpA$^{1\text{-}47:1142\text{-}1143}$	SOE-PCR (OG1RF)	HVN001	HVN002	HVN003	HVN004	pSJH-533 (F)	pSJH-529 (G)
	Δ*ebpB*	EbpB$^{1:458\text{-}476}$	SOE-PCR (OG1RF)	HVN009	HVN010	HVN011	HVN012	pSJH-534 (F)	pSJH-530 (G)
	Δ*ebpC*	EbpC$^{1\text{-}4:624\text{-}625}$	SOE-PCR (OG1X)	HVN015	HVN016	HVN017	HVN018	pSJH-518 (E)	pSJH-523 (G)
	Δ*ebpAB*	EbpA$^{1\text{-}47}$:EbpB$^{458\text{-}476}$	SOE-PCR (OG1RF)	HVN001	HVN005	HVN006	HVN012	pSJH-535 (F)	pSJH-531 (G)
	Δ*ebpBC*	EbpB^{1}:EbpC$^{624\text{-}625}$	SOE-PCR (OG1X)	HVN009	HVN013	HVN014	HVN018	pSJH-536 (F)	pSJH-532 (G)
	Δ*ebpABC*	EbpA$^{1\text{-}47}$:EbpC$^{624\text{-}625}$	SOE-PCR (OG1X)	HVN001	HVN007	HVN008	HVN018	pSJH-520 (E)	pSJH-524 (G)
	Δ*ebpABCsrtC*	EbpA$^{1\text{-}47}$	SOE-PCR (OG1RF)	HVN001	HVN078	HVN079	EF1094e-r3	n/a	pSJH-279 (G)
	Δ*srtC*	None; entire ORF deleted	SOE-PCR (OG1X)	EF1094e-f3	EF1094 sew-r	EF1094 sew-f	EF1094e-r3	n/a	pSJH-189 (G)
B. MIDAS motif mutant allele	*ebpA*^{AWAGA}	EbpA$^{D315A,\ S317A,\ S319A}$	SOE-PCR (OG1RF)	HVN226	HVN229	HVN228	HVN227	n/a	pSJH-509 (H)
C. *E. faecalis* expression constructs	*ebpAp-ebpABC*	EbpA, EbpB, EbpC	PCR (OG1RF)	HVN145	n/a	n/a	HVN150	n/a	pSJH-491 (I)
	ebpAp-ebpAB	EbpA, EbpB	PCR (OG1RF)	HVN145	n/a	n/a	HVN147	n/a	pSJH-492 (I)
	ebpB(internal)-*ebpC-srtC*	EbpA, EbpB, EbpC, SrtC	PCR (OG1RF)	HVN201	n/a	n/a	HVN184	n/a	pSJH-496 (I)
	ebpA^{AWAGA} (internal)	EbpA$^{D315A,\ S317A,\ S319A}$, EbpB, EbpC, SrtC	PCR (EbpA^{AWAGA})	HVN080	n/a	n/a	HVN230	n/a	pSJH-559 (I)
D. *E. coli* expression constructs	EbpA-X	N-term[b]:EbpA$^{634\text{-}1117}$	PCR (OG1RF)	HVN117	n/a	n/a	HVN118	pSJH-542 (F)	pSJH-541 (J)
	EbpB-X	N-term[b]:EbpB$^{27\text{-}488}$	PCR (OG1RF)	HVN107	n/a	n/a	HVN108	n/a	pSJH-547 (J)
	EbpCA-X	N-term[b]:EbpC$^{33\text{-}592}$	PCR (OG1RF)	EF1093A Fw	n/a	n/a	EF1093A Rev	pSJH-516 (E)	pSJH-550 (J)

[a] Protein products predicted to be generated from mutant alleles once incorporated into the *E. faecalis* chromosome (sections A-B) or from final plasmids containing the respective expression constructs (sections C-D). A colon denotes a fusion of non-consecutive amino acids within the same protein or from two different proteins.

[b] The N-terminal amino acid sequence (N-term) added by the pQE-30Xa vector is: **RGSHHHHHH**GSGSGSGIEGRPYNGTG(SA)-. The RGS-6×His tag is bolded, the Factor Xa cleavage recognition site is underlined, and the residues in parentheses are only present in the EbpB-X construct.

[c] For SOE-PCR, PCRs to generate US and DS fragments using the respective forward and reverse primers were performed first. Products were mixed in 1:1 molar ratios and used as template for the SOE-PCR reaction with the US forward and DS reverse primers.

[d] Primers listed in sections A, B, C, and D of this table are described in the same sections of Table S3.

[e] The letter in parentheses refers to the section of Table S2 where the noted plasmid is described.

[f] Final plasmids are derivatives of pJRS233 (section A), pGCP213 (section B), pGCP123 (section C), or pQE-30Xa (section D).