Table S3. Primers used in this study.

	Primer name	Primer sequence (5' → 3')[c-f]	Type[g-i]	Restriction site(s)	Reference
A. Primers used to generate chromosomal deletion alleles	EF1094 sew-f[a1]	CTGTCCCAGGCTcTCATGCTTTATTTTTAAGGAGGAAGCA/ TAGTATGACGAAAAGGCTAAACATACTAAAAAAAAGAG	DS-f		(1)
	EF1094 sew-r[a1]	CTCTTTTTTTTAGTATGTTTAGCCTTTTCGTCATACTA/ TGCTTCCTCCTTAAAAATAAAGCATGAgAGCCTGGGACAG	US-r		(1)
	EF1094e-f3[a2]	gc**tctaga**ATAACGTGACTTCTGGAGAGTATGCtTATGC	US-f	**XbaI**	(1)
	EF1094e-r3	gc**tctaga**CGCGGAACAAAGGAGCTAAAAACTTAATAGAATA	DS-r	**XbaI**	(1)
	HNV001	tcc***cccgggg*tctaga**ACGTTGTTGAAATCCTTGATG	US-f	**XbaI**, ***SmaI***	This study
	HVN002	TGCGTTTTTCATTGATTACACACT/ATCATTCTCATCTGTTATCATTTGAG	US-r		This study
	HVN003	CTCAAATGATAACAGATGAGAATGAT/AGTGTGTAATCAATGAAAAACGCA	DS-f		This study
	HVN004	gcgt**gtcgac**gcgtTACCATCCCAATGATTAGCAGGCC	DS-r	**SalI**	This study
	HVN005	CCAAATGACTACCATCCCAAT/ATCATTCTCATCTGTTATCATTTGAGCGG	US-r		This study
	HVN006	CCGCTCAAATGATAACAGATGAGAATGAT/ATTGGGATGGTAGTCATTTGG	DS-f		This study
	HVN007	CGGTGATGCTAGAAATTAAGCATT/ATCATTCTCATCTGTTATCATTTGAG	US-r		This study
	HVN008	CTCAAATGATAACAGATGAGAATGAT/AATGCTTAATTTCTAGCATCACCG	DS-f		This study
	HVN009	tcc***cccgggg*tctaga**AATGTTCAAACAACATTAGTGG	US-f	**XbaI**, ***SmaI***	This study
	HVN010	CCAAATGACTACCATCCCAAT/CATTGATTACACACTCCCTTCTGG	US-r		This study
	HVN011	CCAGAAGGGAGTGTGTAATCAATG/ATTGGGATGGTAGTCATTTGG	DS-f		This study
	HVN012	gcgt**gtcgac**gcgtAATAAGTAGCAAGACTGCGCCAC	DS-r	**SalI**	This study
	HVN013	CGGTGATGCTAGAAATTAAGCATT/CATTGATTACACACTCCCTTCTGG	US-r		This study
	HVN014	CCAGAAGGGAGTGTGTAATCAATG/AATGCTTAATTTCTAGCATCACCG	DS-f		This study
	HVN015[a3]	tcc***cccgggg*tctaga**AAAGCTAGcAACCTAGTAGTG	US-f	**XbaI**, ***SmaI***	This study
	HVN016	CTTCGGTGATGCTAGAAATTAAGCATTT/AATTGCTTCATTTTCTCTCT	US-r		This study
	HVN017	AGAGAGAAAATGAAGCAATT/AAATGCTTAATTTCTAGCATCACCGAAG	DS-f		This study
	HVN018	gcgt**gtcgac**gcgtTCGATTGATTGGCCCTTTGTCGC	DS-r	**SalI**	This study
	HVN078	CTCTTTTTTTTAGTATGTTTAGCC/ATCATTCTCATCTGTTATCATTTGAG	US-r		This study
	HVN079	CTCAAATGATAACAGATGAGAATGAT/GGCTAAACATACTAAAAAAAAGAG	DS-f		This study
B. Primers used to generate chromosomal MIDAS motif mutant allele	HVN226	gc**tctaga**CAGAGCAGCCATTACAACGA	US-f	**XbaI**	This study
	HVN227	ccg***ctcgag***CCTGAACAGGACCCACACTT	DS-R	***XhoI***	This study
	HVN228[b]	GGATTTGGTCTTAGTCGTT(g)GcCTGGgCCGGAgcTATGAATGAAAACAATCGGATT	DS-f		This study
	HVN229[b]	AATCCGATTGTTTTCATTCATAgcTCCGGcCCAGgC(c)AACGACTAAGACCAAATCC	US-r		This study
	HVN241	GGATTTGGTCTTAGTCGTTGcCTGGgCCG	SDM (sense)		This study
	HVN242	CGGcCCAGgCAACGACTAAGACCAAATCC	SDM (antisense)		This study

	Primer name	Primer sequence (5' → 3')[c-f]	Type[g-i]	Restriction site(s)	Reference
C. Primers used to generate *E. faecalis* expression constructs	HVN080	gcg***ggatcc***ATAACAGTAGAGGATTCTGCTAAA	f	***BamHI***	This study
	HVN145	ccgccg***ctcgag***ATAAACTCATGGAGCAATTGG	f; US-f	***XhoI***	This study
	HVN147	tccc***cccggg***TCATTTTCTCTCTCCTCTTTTTG	r	***XmaI***	This study
	HVN150	tccc***cccggg***TTAAGCATTTTCTTTTCTACG	r	***XmaI***	This study
	HVN184	tccc***cccggg***CTACTTTGGTTTTCTGGTCGTC	r	***XmaI***	This study
	HVN201	aaa**AGGCCT**GCTAATCATTGGGATGGTAGTC	f	**StuI**	This study
	HVN230	agcttt**CCTGCAGG**TGCTTTTACTTCAGTTAATGTATAGCG	r	**SbfI**	This study
D. Primers used to generate *E. coli* expression constructs	EF1093A Fw	gcg***ggatcc***GAAGAAAATGGGGAGAGCGC	f	***BamHI***	(2)
	EF1093A Rev	gcg**gagctc**ttaGGTACCTTTGTGTTTGTTTGG	r	**SacI**	(2)
	HVN107	cc***gcatgc***GCATCGCAAGCAAGCGTTCAAG	f	***SphI***	This study
	HVN108	cc**ctgcag**ttaAATAGAACGTTCTTCATTTG	r	**PstI**	This study
	HVN114	gcg***ggatcc***ATGAATGGTCGGACAACGTTTCAGCC	f	***BamHI***	This study
	HVN117	cc**gtcgac**ttaCAAGCGTCCTATGCCACCAGTTTCAGG	r	**SalI**	This study
E. Primers used in pGCP123 construction	GP133	gct***gcatgc***tcgatttttattaaaacgtctc		***SphI***	This study
	GP134	gct**agatct**tccgtcgatactatgttatacg		**BglII**	This study
	GP135	gct**agatct**gtaaaacgacggccag		**BglII**	This study
	GP136	gct***gcatgc***caggaaacagctatgac		***SphI***	This study
F. Primers used in pGCP213 construction	oJoy80	***gat***atgattacgccaagcttggtaccgagctcggatcc	5'-P	***ClaI***	This study
	oJoy81	***gat***ggtcatagctgtttcctgtgtgaaattgttatccg	5'-P	***ClaI***	This study
	GP240	ctgcagatatccatcacac**aggcct**tggcggccgctcgagcatgc	SDM (sense)	**StuI**	This study
	GP241	gcatgctcgagcggccgcca**aggcct**gtgtgatggatatctgcag	SDM (antisense)	**StuI**	This study
	GP224	gct***acgcgt***cccgtagaaaagatcaaagg		***MluI***	This study
	GP225	gct**agatct**ggcaagtgtagcggtcac		**BglII**	This study
	GP226	gct***acgcgt***tccctcccttaacttacttattaaat		***MluI***	This study
	GP227	gct**agatct**ctattaatcgcaacatcaaacc		**BglII**	This study

[a1-a3] Primer was designed to anneal to *E. faecalis* V583 sequence, resulting in the following DNA mutations in OG1RF strains: 1) C to T mutation in the *ebpC-srtC* intergenic region in SrtC$^-$, 2) silent mutation in A386 of EbpC in SrtC$^-$, and 3) silent mutation in S155 and a M159V transmutation in EbpB in EbpC$^-$.
[b] An aberrantly added base in HVN228/HVN229 is in parentheses and was removed using SDM with primers HVN241/HVN242 to yield pSJH-509.
[c] Uppercase sequence anneals to the OG1RF chromosome.
[d] A forward slash is inserted in primers where chromosomal sequence was deleted.
[e] XbaI, SalI, AatII, SbfI, BglII, and PstI restriction sites are underlined and bolded; SmaI, XhoI, XmaI, BamHI, and SphI restriction sites are shown in bolded, italics type.
[f] MIDAS motif sequence is underlined but not bolded.
[g] Primers are categorized as forward (f) or reverse (r) (or sense or antisense for SDM) relative to the direction of *ebp* transcription.
[h] US (upstream) or DS (downstream) refers to the relevant splice segment for SOE-PCR primers.
[i] 5'-P denotes 5' phosphorylation of the primer.

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