Supplemental Table 1: Bacterial strains used in this study

V583	Parental strain	Clinical isolate (1)
IH06	V583Δ*ef0114*	(2)
AH03	V583Δ*ef0362-61*	(2)
AH01	V583Δ*ef2863*	(2)
AH09	V583Δ*ef0114*Δ*ef0362-61*Δ*ef2863* (ΔTM)	(2)
EK49	V583Δ*ef0114*::*ef0114*	This study
EK50	V583Δ*ef0114*Δ*ef0362-61*Δ*ef2863*::*ef0114*	This study
AH03R	V583Ω*ef0362-61*	(2)
EH01	V583Δ*ccpA*	(3)
VI70	V583Δ*ccpA*Δ*ef0114*	(2)
AH05	V583Δ*ccpA*Δ*ef0362-61*	(2)
AH10	V583Δ*ccpA*Δ*ef2863*	(2)
IH09	V583Δ*ccpA*Δ*ef0114*Δ*ef0362-61*	(2)
IH12	V583Δ*ccpA*Δ*ef0362-61*Δ*ef2863*	(2)
IH08	V583Δ*ccpA*Δ*ef0114*Δ*ef2863*	(2)
IH13	V583Δ*ccpA*Δ*ef0114*Δ*ef0362-61*Δ*ef2863*	(2)
EK40	V583Δ*ccpA*Δ*ef0114*::*ef0114*	This study
IH10	V583Δ*mptBACD*	(3)
EK02	V583Δ*ef1516*	(3)
IH10R	V583Ω*mptBACD*	(3)
EK48	V583Δ*mptBACD*Δ*ef1516*	This study
EK54	V583Δ*mptBACD*Δ*ef1516*::*mptBACD*	This study
EK55	V583Δ*mptBACD*Δ*ef1516*::*ef1516*	This study

SP27	V583Δ*ef1317*	This study
EK47	V583Δ*ef1317*::*ef1317*	This study
ZA05	V583Δ*ef0466*	This study
EK46	V583Δ*ef0466*::*ef0466*	This study

Supplementary References

1. Sahm DF, Kissinger J, Gilmore MS, Murray PR, Mulder R, Solliday J, Clarke B. 1989. In vitro susceptibility studies of vancomycin-resistant Enterococcus faecalis. Antimicrob Agents Chemother 33:1588-91.
2. Keffeler EC, Iyer VS, Henderson AJ, Huck IL, Schwarting N, Cortez A, Hancock LE. 2021. Activity of CcpA-regulated GH18 family glycosyl hydrolases that contribute to nutrient acquisition and fitness in Enterococcus faecalis Infection and Immunity (*in press*).
3. Keffeler EC, Iyer VS, Parthasarathy S, Ramsey MM, Gorman MJ, Barke TL, Varahan S, Olson S, Gilmore MS, Abdullahi ZH, Hancock EN, Hancock LE. 2021. Influence of the Alternative Sigma Factor RpoN on Global Gene Expression and Carbon Catabolism in Enterococcus faecalis V583. mBio 12.

Supplemental Table 2: Oligonucleotides used in this study

Primer	Sequence (5'-3')
EF0114P1	GAGAGAATTCCGTGAAGTTACACTGAACAAG
EF0114P4	GAGACTGCAGCTTGATTGACAGCTTCCAGC
EF0114Up	GATGGGAAGCCAGTAATAAGAG
EF0114Down	CGTGCTGGCATATAATTGACC
MptBACDP1	GAGAGAATTCCGAAGACTATGAAATTATTGCG
MptBACDP4	GAGACTGCAGCAGTCCATTCCATGATGTTGT
MptBACDUp	TAGACTGGCATTTAGAAGTGA
MptBACDDown	GCAATTCTTTCATACGATTACG
EF1516P1	GAGAGAATTCCAATGTGGTGTACAATGGTTAC
EF1516P4	CTCTGCATGCATCAGTTACATCCCTTCTAGT
EF1516Up	CGAGCACATATAAGTATGCCT
EF1516Down	GAAGGCCTAACTCTTTCTGCTT
EF1317P1	GAGAGAATTCCTTTCACCTATAAAGCCGATAAG
EF1317P2	TCTCGGATCCATG TTTTCATCTTCATCCTTCTTTC
EF1317P3	GAGAGGATCCGCATAAGACACAAGAAAAAGCTT
EF1317P4	TCTCCTGCAGCATCTTAGAAGCCAAAAGCCTT
EF1317Up	AGATACGGACTGGCAAAAATAT
EF1317Down	CTGTCAACACTCCTTATATGACG
EF0466P1	GAGAGAATTCCGTTGGAAATGACACACAAGAC
EF0466P2	GAGACTGCAGTGCATTTGCTACACGGATAATTTG
EF0466P3	GAGACTGCAGAACCATCAAGATGTTGTTGTA
EF0466P4	GAGAGCATGCCTTACTTATCACCTTTCTTTCTAAC

EF0466Up	AGGTCGAACAGAACAAGGTC
EF0466Down	GTGACCATTCTGCTTCGACTT

Underlined sequences depict restriction sites used for cloning purposes

Supplemental Table 3: Plasmids used in this study

Plasmid	Description	Reference
pLT06	Markerless exchange vector; chloramphenicol resistance	(1)
pEK09	pLT06 + engineered *ef0114* complement	This study
pEK04	pLT06 + engineered *ef1516* deletion	(2)
pEK68	pLT06 + engineered *mptBACD* complement	This study
pEK61	pLT06 + engineered *ef1516* complement	This study
pSP20	pLT06 + engineered *ef1317* deletion	This study
pEK60	pLT06 + engineered *ef1317* complement	This study
pZA12	pLT06 + engineered *ef0466* deletion	This study
pEK62	pLT06 + engineered *ef0466* complement	This study
pEK33	pET21b + EF0114 (full length) + His6 overexpression	(3)
pEK34	pET21b + EF0114 (α) + His6 overexpression	(3)
pEK36	pET21b + EF0114 (γ) + His6 overexpression	(3)
pEK37	pET21b + EF0114 (α+β) + His 6 overexpression	(3)
pEK38	pET21b + EF0114 (β+γ) + His6 overexpression	(3)
pEK47	pET21b + EF0114 E186Q + His6 overexpression	(3)
pEK48	pET21b + EF0114 E662Q + His6 overexpression	(3)

Supplementary References

1. Thurlow LR, Thomas VC, Fleming SD, Hancock LE. 2009. *Enterococcus faecalis* capsular polysaccharide serotypes C and D and their contributions to host innate immune evasion. Infection and Immunity 77.
2. Keffeler EC, Iyer VS, Parthasarathy S, Ramsey MM, Gorman MJ, Barke TL, Varahan S, Olson S, Gilmore MS, Abdullahi ZH, Hancock EN, Hancock LE. 2021. Influence of the Alternative Sigma Factor RpoN on Global Gene Expression and Carbon Catabolism in Enterococcus faecalis V583. mBio 12.

3. Keffeler EC, Iyer VS, Henderson AJ, Huck IL, Schwarting N, Cortez A, Hancock LE. 2021. Activity of CcpA-regulated GH18 family glycosyl hydrolases that contribute to nutrient acquisition and fitness in Enterococcus faecalis Infection and Immunity (*in press*).