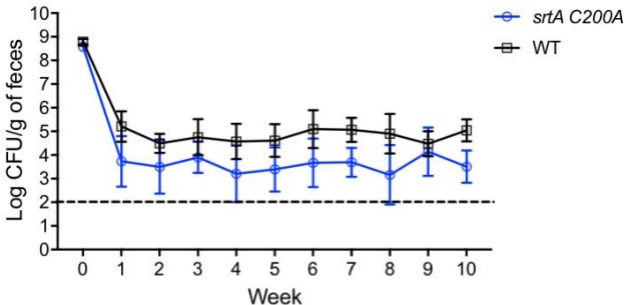
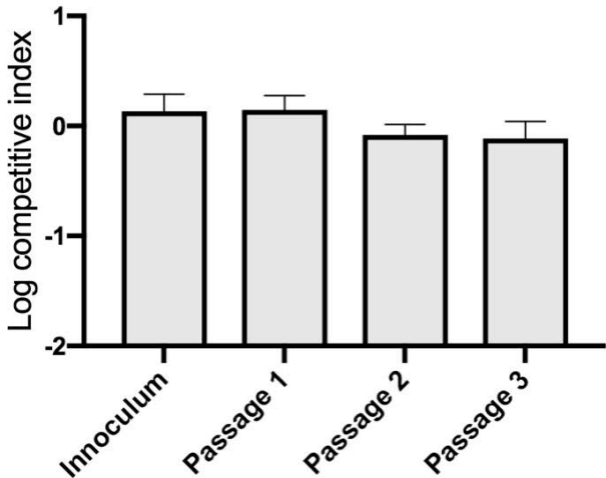


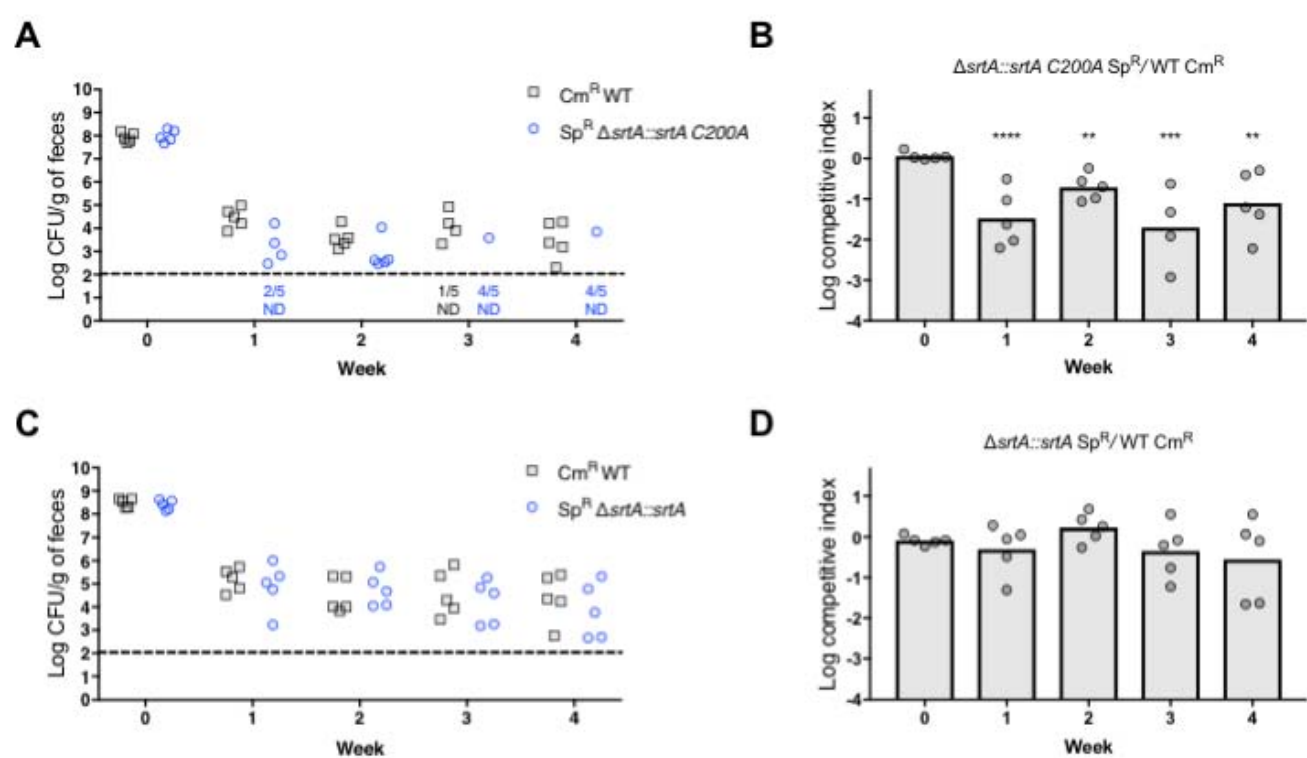
Supplemental figure 1: GIT Colonization by *E. faecium* Δ *srtA* mutants. Groups of mice (n=5) were colonized with either the Δ *srtA* strain (JL622) or its parental wild type (JL619). Colonization was assessed by enumerating viable colonies from fecal samples on rifampin-supplemented BHI agar, geometric standard deviations and line connecting geometric means for each group of mice are shown. Dotted line represents the limit of detection.



Supplemental figure 2: GIT Colonization by an *E. faecalis* mutant carrying catalytically inactive sortase encoded by the *C200A* allele. Groups of mice (n=5) were colonized with either the *E. faecalis srtA C200A* strain (IB37) or its isogenic wild type parent (OG1RF). Colonization was assessed by enumerating viable colonies from fecal samples on rifampin-supplemented BHI agar, geometric means and standard deviations for each group of mice are shown. The dotted line represents the limit of detection.

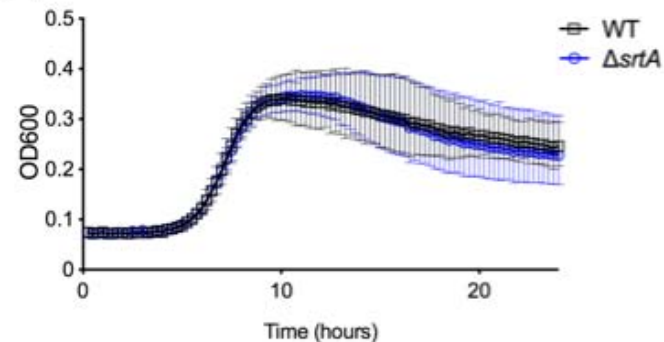


Supplemental figure 3: *in vitro* competitive fitness of $\Delta srtA$ mutants. mixed cultures containing equal quantities of Sp-resistant WT *E. faecalis* (IB38) and Cm-resistant *ace::MarTn* were grown at 37°C and passaged 3 times. The abundance of each strain was determined by dilution plating on Sp or Cm containing BHI agar and the competitive index was determined. Three independent mixed culture were set up, average competitive indices and standard deviations are shown.

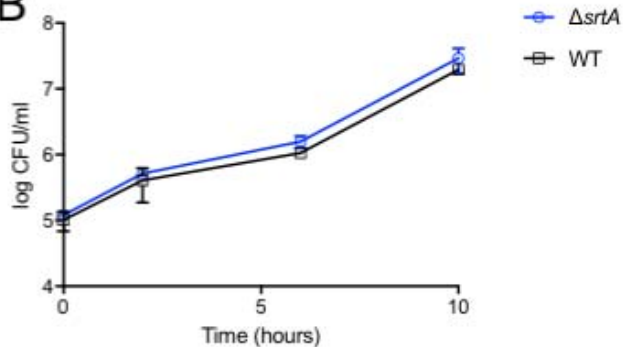


Supplemental Figure 4. Ectopic expression of WT SrtA enhances competitive fitness of the Δ srtA mutant. (A) Differentially marked *E. faecalis* strains SK11 (Cm resistant WT) and IB50 (Sp resistant Δ srtA complemented with *srtA* C200A allele at an ectopic chromosomal location) were co-fed to a group of 5 mice for two weeks after which colonization was assessed by enumerating viable colonies from fecal samples on rifampin-supplemented BHI agar containing Cm or Sp. (C) Differentially marked strains SK11 (Cm resistant WT) and IB49 (Sp resistant Δ srtA complemented with a WT *srtA* allele at an ectopic chromosomal location) were fed in equal quantities to a group of 5 mice and colonization was assessed by enumerating viable colonies from fecal samples on rifampin-supplemented BHI agar containing Cm or Sp. (B and D) competitive indices of IB50 and IB49 relative to SK11 counterparts were calculated for the co-administration colonization experiments in panels A and C respectively. Geometric means are shown as bar graph; individual data points denote competitive indices for individual mice. Statistical significance was evaluated by one sample t-test comparing means to a value of 1. *, $P < 0.01$, **, $P < 0.001$, ***, $P < 0.0001$. Dotted line represents the limit of detection, symbols for mice with undetectable colonization level were omitted, instead the number of mice for which colonization was not detected (ND) is shown underneath the dotted line.

A



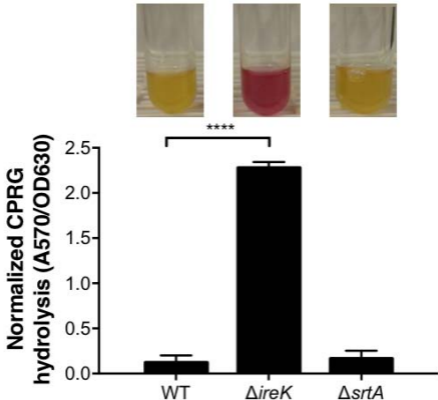
B



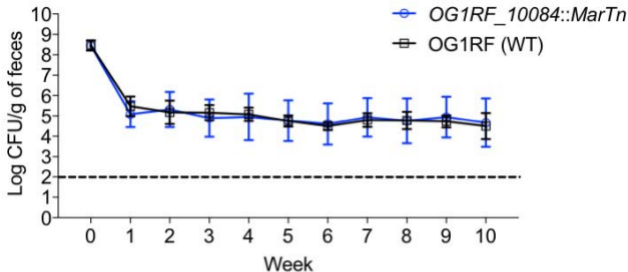
Supplemental Figure 5: Growth curves of WT *E. faecalis* and $\Delta srtA$. (A) cultures of WT *E. faecalis* (OG1RF) and the $\Delta srtA$ mutant (IB10) were started in MH broth and growth was tracked by measuring OD600 using an automated plate reader. Datapoints shown represent the average of three independent cultures, error bars representing standard deviations are shown. (B) cultures of OG1RF and IB10 mutant were started in fecal suspensions and growth was tracked by plating and determining viable counts. Datapoints shown represent the average of three independent cultures, error bars representing standard deviations are shown.

Antimicrobial	$\Delta srtA$ MIC	WT MIC
Lysozyme	8mg/ml	8mg/ml
Polymixin B	>1024 μ g/ml	>1024 μ g/ml
Colistin	>1024 μ g/ml	>1024 μ g/ml
Cholate	>256mM	>256mM
Ceftriaxone	16 μ g/ml	16 μ g/ml
Human Defensin 5	8 μ g/ml	8 μ g/ml

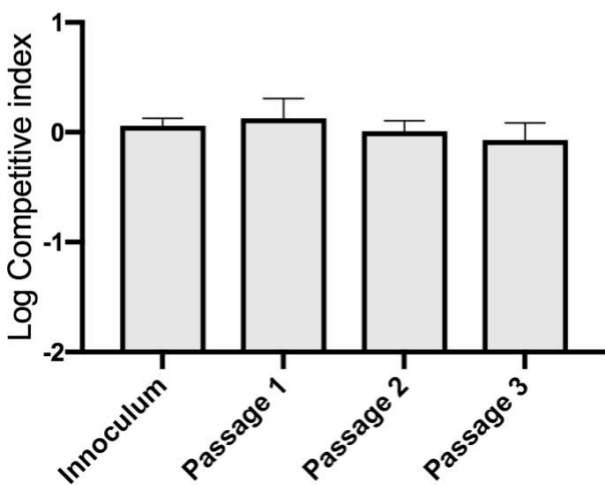
Supplemental Figure 6. Resistance to various antimicrobials was determined for WT *E. faecalis* (OG1RF) and the $\Delta srtA$ mutant (IB10). Minimal inhibitory concentration (MIC) reported represents the median value from three independent biological replicates.



Supplemental Figure 7. Cell envelope permeability. CPRG hydrolysis was measured for WT *E. faecalis*, the $\Delta ireK$ mutant and the $\Delta srtA$ mutant. CPRG hydrolysis was quantified by measuring the absorbance at 570 nm of cell-free supernatants and normalizing to the optical density at 630 nm. Measurements represent averages from three independent cultures and error bars represent standard deviations. Statistical significance was evaluated by student's t test. ****, $P < 0.0001$ versus WT.



Supplemental Figure 8: GIT Colonization by the *E. faecalis* OG1RF_10084 SDP mutant. Groups of mice (n=5) were colonized with either the *E. faecalis* OG1RF_10084::MarTn mutant or the isogenic wild type parent (OG1RF). Colonization was assessed by enumerating viable colonies from fecal samples on rifampin-supplemented BHI agar, and geometric means and standard deviations for each group of mice are shown. Dotted line represents the limit of detection.



Supplemental Figure 9: *in vitro* competitive fitness of *ace::MarTn* mutants. mixed cultures containing equal quantities of Sp-resistant WT *E. faecalis* (IB38) and Cm-resistant *ace::MarTn* were grown at 37°C and passaged 3 times. The abundance of each strain was determined by dilution plating on Sp or Cm containing BHI agar and the competitive index was determined. Three independent mixed culture were set up, average competitive indices and standard deviations are shown.

Table S1. Bacterial strains and plasmids

Strain or plasmid	Genotype/description	Source
Strains		
<i>E. coli</i>		
DH5α	<i>E. coli</i> host for routine cloning	Laboratory stock
<i>E. faecalis</i>		
OG1RF	Spontaneous Rf ^R , Fus ^R wild type strain	(1)
IB10	OG1RF Δ <i>srtA</i>	This work
SK11	OG1RF (<i>OG1RF_10894-OG1RF_10895</i> :: <i>MarTN</i> – Cm ^R)	This work
IB38	OG1RF (<i>OG1RF_10894-OG1RF_10895</i> :: <i>ada9</i> – Sp ^R)	This work
IB40	IB10 (<i>OG1RF_10894-OG1RF_10895</i> :: <i>MarTN</i> – Cm ^R)	This work
IB39	IB10 (<i>OG1RF_10894-OG1RF_10895</i> :: <i>ada9</i> – Sp ^R)	This work
JL619	Spontaneous Rf ^R <i>E. faecium</i> 1141733	This work
JL622	JL619 Δ <i>srtA</i>	This work
IB37	OG1RF <i>srtA C200A</i>	This work
IB49	IB10 (<i>OG1RF_10894- OG1RF_10895</i> :: <i>srtA-aad9</i>)	This work
IB50	IB10 (<i>OG1RF_10894- OG1RF_10895</i> :: <i>srtA C200A-aad9</i>)	This work
CK119	OG1RF Δ <i>ireK</i>	(2)
7B3	<i>OG1RF_10056</i> :: <i>MarTN</i>	(3)
13N10	<i>OG1RF_10084</i> :: <i>MarTN</i>	(3)
12N2	<i>OG1RF_10088</i> :: <i>MarTN</i>	(3) (3)
15F18	<i>OG1RF_10508</i> :: <i>MarTN</i>	(3)
32M18	<i>OG1RF_10811</i> :: <i>MarTN</i>	(3)
5P24	<i>OG1RF_10878</i> :: <i>MarTN</i>	(3)
1D18	<i>OG1RF_11531</i> :: <i>MarTN</i>	(3)
5O10	<i>OG1RF_11764</i> :: <i>MarTN</i>	(3)
3K10	<i>OG1RF_11924</i> :: <i>MarTN</i>	(3)
9B6	<i>OG1RF_12054</i> :: <i>MarTN</i>	(3)
18P6	<i>OG1RF_12303</i> :: <i>MarTN</i>	(3)
34B14	<i>OG1RF_12506</i> :: <i>MarTN</i>	(3)
AP1	OG1RF Δ <i>OG1RF_10786</i>	This work
AP2	OG1RF Δ <i>OG1RF_12251</i>	This work
IB15	OG1RF Δ <i>OG1RF_10485</i> Δ <i>OG1RF_11037</i> Δ <i>OG1RF_12346</i> Δ <i>OG1RF_12558</i>	This work
TX5608	OG1RF Δ <i>ebpA</i> Δ <i>ebpB</i> Δ <i>ebpC</i>	(4)
IB44	TX5608(<i>OG1RF_10894-OG1RF_10895</i> :: <i>MarTN</i>)	This work
Plasmids		

pJRG8	Expression vector with constitutive promoter (Erythromycin resistance)	(5)
pDV75-2	Vector for ectopic integration of genes at the <i>OG1RF_10894-OG1RF_10895</i> locus	(6)
pJH082	<i>E. faecalis</i> allelic exchange vector (chloramphenicol resistance, <i>LacZ</i> , <i>repA</i> <u>V71G</u> , <i>thyA</i> * counterselection)	(7)
pIB53	pJRG8:: <i>srtA</i>	This work
pIB6	Δ <i>srtA</i> deletion allele in pJH082 (<i>srtA</i> Δ P3-M228, 93% deletion)	This work
pSK37	pDV75-2:: <i>MarTN</i>	This work
pIB51	pDV75-2:: <i>aad9</i>	This work
pIB67	pDV75-2:: <i>srtA-aad9</i>	This work
pIB68	pDV75-2:: <i>srtA C200A-aad9</i>	This work
pIB7	<i>srtA C200A</i> allele in pJH082	This work
pIB43	Δ <i>OG1RF_10786</i> deletion allele in pJH082 (<i>OG1RF_10786</i> Δ L6-V456, 98% deletion)	This work
pIB44	Δ <i>OG1RF_12251</i> deletion allele in pJH082 (<i>OG1RF_12251</i> Δ S6-K128, 92% deletion)	This work
pIB8	Δ <i>OG1RF_11037</i> deletion allele in pJH082 (<i>OG1RF_11037</i> Δ K3-S614, 97% deletion)	This work
pIB6A	Δ <i>OG1RF_12258</i> deletion allele in pJH082 (<i>OG1RF_12558</i> Δ I2-I1647, 99% deletion)	This work
pIB5	Δ <i>OG1RF_12346</i> deletion allele in pJH082 (<i>OG1RF_12346</i> Δ K2-G108, 87% deletion)	This work
pIB4	Δ <i>OG1RF_10485</i> deletion allele in pJH082 (<i>OG1RF_10485</i> Δ L24-V107, 66% deletion)	This work

Supplemental References

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