Supplemental Material

Modulators of *Enterococcus faecalis* cell envelope integrity and antimicrobial resistance influence stable colonization of the mammalian gastrointestinal tract

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Table S1

Figure S1-S7

Table S1 Bacterial strains and plasmids used in this work

Strain or plasmid	Genotype/description	Source
Strains		
E. coli		
DH5α	*E. coli* host for routine cloning	Laboratory stock
E. faecalis		
OG1	Wild-type reference strain	(1)
OG1RF	Spontaneous Rif^R, Fus^R derivative of OG1 – primary wild type strain	(2)
V583	Vancomycin resistant *E. faecalis* clinical isolate (MDR strain)	(3)
CK119	OG1RF Δ*ireK*	(4)
CK125	OG1RF Δ*ireP* Δ*ireK*	(5)
CK206	V583 Δ*ireK*	This work
23J13	OG1RF *brp/Blh*::MarTN	(6)
28M17	OG1RF *ispA*::MarTN	(6)
35H2	OG1RF *sigV*::MarTN	(6)
SB6	OG1RF Δ*croR croS*	S. Kellogg and C. Kristich, unpublished
Δ*ireK**	CK119 suppressor mutant	This work
IB21	CK119 Δ*271*	This work
IB22	CK119 Δ*272*	This work
IB23	CK119 Δ*271-272*	This work
IB25	CK119 Δ*270*	This work
CK164	OG1RF Δ*ireB*	(7)
CK121	OG1RF Δ*ireP*	(5)
IB18	OG1RF Δ*271*	This work
IB19	OG1RF Δ*272*	This work
IB20	OG1RF Δ*271-272*	This work
BL102	OG1 *ireK K41R*	(8)
IB36	BL102 Δ*271-272*	This work
Plasmids		
pJRG8	Expression vector with constitutive promoter (Erythromycin resistance)	(5)
pJRG9	Expression vector with constitutive promoter (chloramphenicol resistance)	(9)
pJH082	*E. faecalis* allelic exchange vector (chloramphenicol resistance, *LacZ, repA V71G*, *thyA** counterselection)	This work
pJH086	*E. faecalis* allelic exchange vector (chloramphenicol resistance, *LacZ, repA V71G*, *pheS** counterselection)	(10)
pCJK74	*ireK* deletion plasmid	(4)

pCJK160	pJRG8::*ireP-ireK*	(5)
pCJK205	Constitutive expression of *lacZ* (erythromycin resistance)	(11)
pCJK216	pJRG8::*ireP-ireK*	(5)
pIB11	*271* in pJRG9	This work
pIB12	*272* in pJRG9	This work
pIB13	*271-272* in pJRG9	This work
pIB14	*876* in pJRG9	This work
pIB28	*270* in pJRG9	This work
pIB10	Δ*271* deletion allele in pJH082 (*271* Δ*K6-L412*, 97% deletion)	This work
pIB15	Δ*272* deletion allele in pJH082 (*272* Δ*F2-A175*, 92% deletion)	This work
pIB16	Δ*271-272* deletion allele in pJH082 (*271* Δ*K6-272 A175*, 94% deletion)	This work
pIB18	Δ*270* deletion allele in pJH086 (*270* Δ*K2-L343*, 99% deletion)	This work

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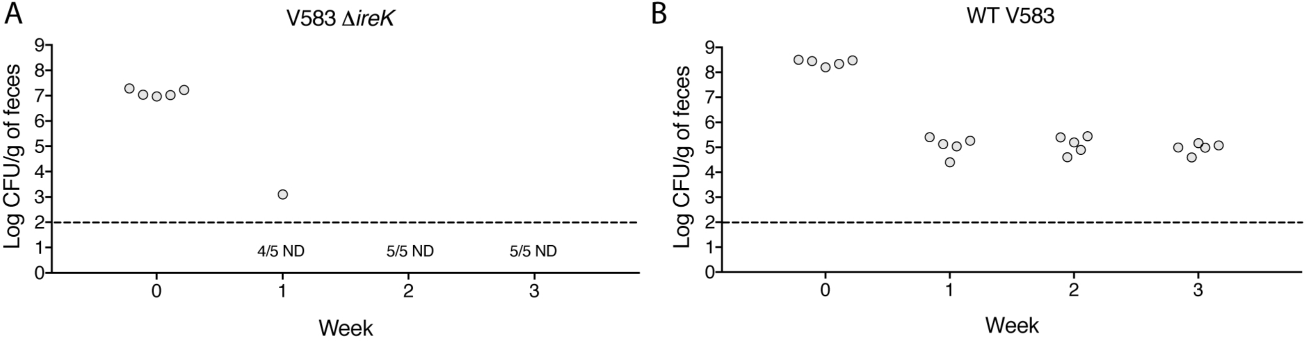
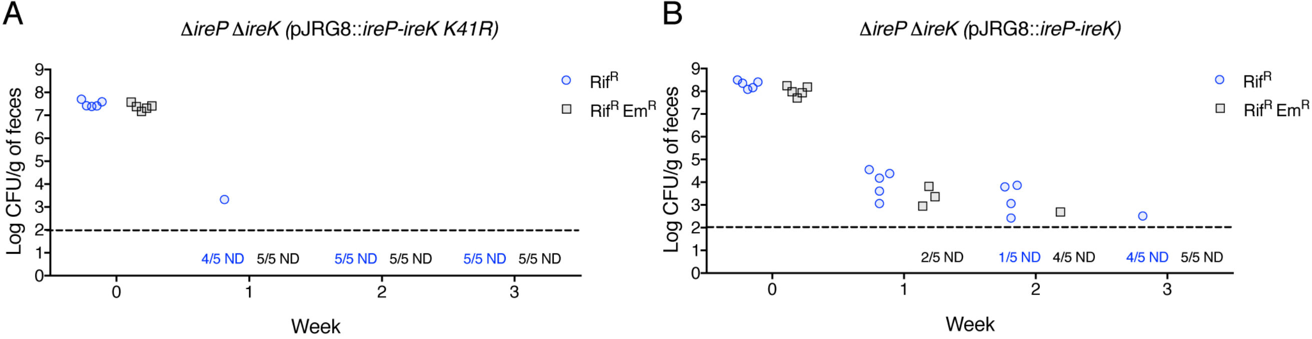
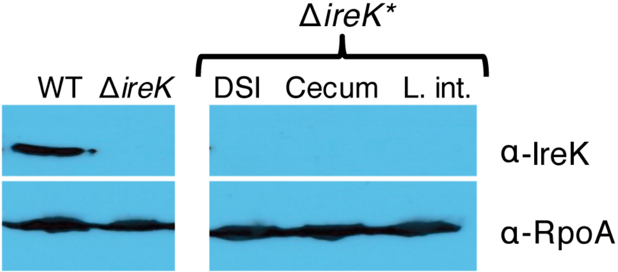


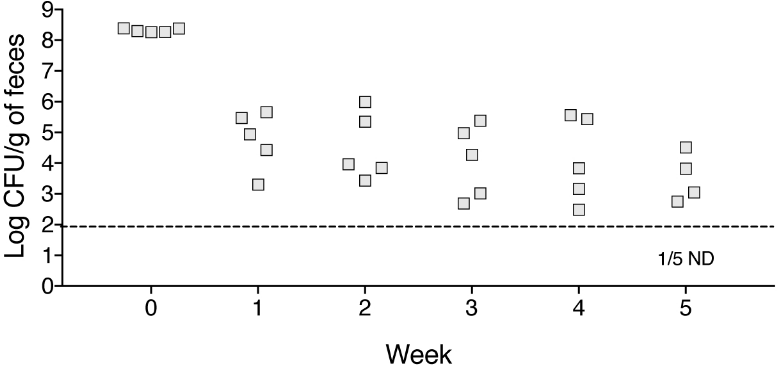
Supplemental figure 1. Deletion of *ireK* in *E. faecalis* V583 impairs intestinal colonization. Intestinal colonizations of MDR *E. faecalis* strain (V583) and corresponding Δ*ireK* mutant (CK206) were assessed. Groups of mice (5 per group) were colonized with *E. faecalis* V583 (panel B) or the corresponding Δ*ireK* mutant (CK206; panel A). Bacterial loads were determined by enumerating the enterococcal strains in feces by culture on kanamycin-supplemented BHI agar. Dotted line represents the limit of detection. Symbols for mice with undetectable colonization levels were omitted, and instead the number of mice for which colonization was not detected (ND) is shown underneath the dotted line.



Supplemental figure 2. Ectopic expression of WT IreK enhances GIT colonization. Mice (5 per group) were fed with Δ*ireP* Δ*ireK* double mutant (CK125) carrying a plasmid encoding either IreP and IreK-K41R (pCJK216) (A) or WT IreP and IreK in tandem (pCJK160) (B). Colonization levels in the feces were determined by enumerating Rif-resistant clones. Plasmid retention was assessed by enumerating Rif and Erythromycin (Em)-resistant clones. Dotted line represents the limit of detection. Symbols for mice with undetectable colonization level were omitted, and instead the number of mice for which colonization was not detected (ND) is shown underneath the dotted line.



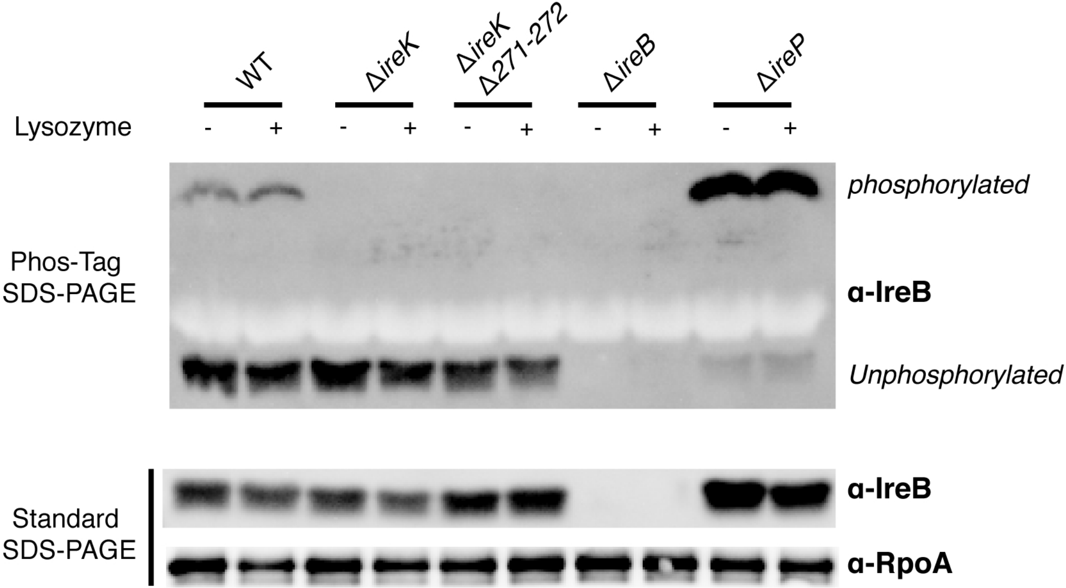
Supplemental figure 3. Δ*ireK clones lack IreK.** Expression of IreK in WT *E. faecalis* (OG1RF), Δ*ireK* (CK119) and Δ*ireK** was assessed by Western blot analysis probing for IreK and RpoA (loading control). Δ*ireK** samples originate from clones obtained from the distal small intestine (DSI), cecum and large intestine (L. int.) of the mouse in Figure 3.



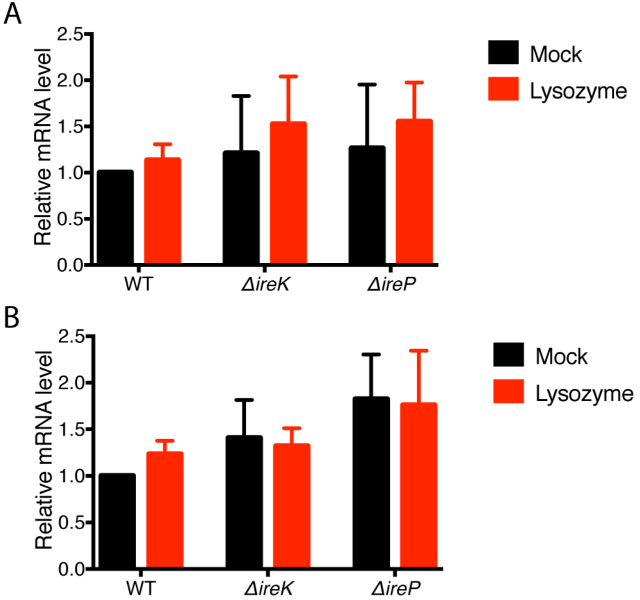
Supplemental figure 4. Deletion of *271* and *272* in Δ*ireK* background restores GIT colonization. 5 mice were colonized with the Δ*ireK* Δ*271-272* (IB23) triple mutant and colonization was assessed by enumerating fecal viable counts on rifampin-supplemented BHI agar. Dotted line represents the limit of detection.

Strain	Cholate MIC (mM)	Lysozyme MIC (mg/ml)
WT (pJRG9)	256	32
Δ*ireK* (pJRG9)	32	4
Δ*ireK* Δ*270* (pJRG9)	256	16
Δ*ireK* Δ*270* (pJRG9::*270*)	256	8
ΔireK Δ270 (pJRG9::*271-272*)	32	4

Supplemental figure 5. *270* does not modulate antimicrobial resistance in *E. faecalis*. Cholate and lysozyme resistance was determined for WT *E. faecalis* (OG1RF), Δ*ireK* (CK119), and Δ*ireK* Δ*270* (IB25) carrying the empty vector pJRG9 or expressing wild-type copies of the indicated genes. Reported MICs represent the median value from three independent biological replicates.



Supplemental figure 6. 271 and 272 do not alter expression and phosphorylation of IreB in Δ*ireK* background. Western blots of Phos-tag SDS-PAGE (top) and standard SDS Page gels (bottom) probing for IreB and RpoA (loading control) in WT (OG1RF), Δ*ireK* (CK119), Δ*ireK Δ271-272 (*IB23), Δ*ireB* (CK164), and Δ*ireP* (CK121). Lysates of exponential phase bacteria that were lysozyme or mock-treated were analyzed.



Supplemental figure 7. IreK does not affect mRNA levels of *271* and *272*. mRNA from lysozyme and mock-treated cells was isolated and levels of *271* (A) and *272* (B) were quantified by qRT-PCR. Relative mRNA expression was determined following the Pfaffl method using *gyrB* as the reference gene. Samples were obtained WT *E. faecalis* (OG1RF), Δ*ireK* (CK119) and Δ*ireP* (CK121) mutants. Samples for each strain were from three independent biological replicates, each independent culture was examined with three technical replicates and error bars represent standard deviations.