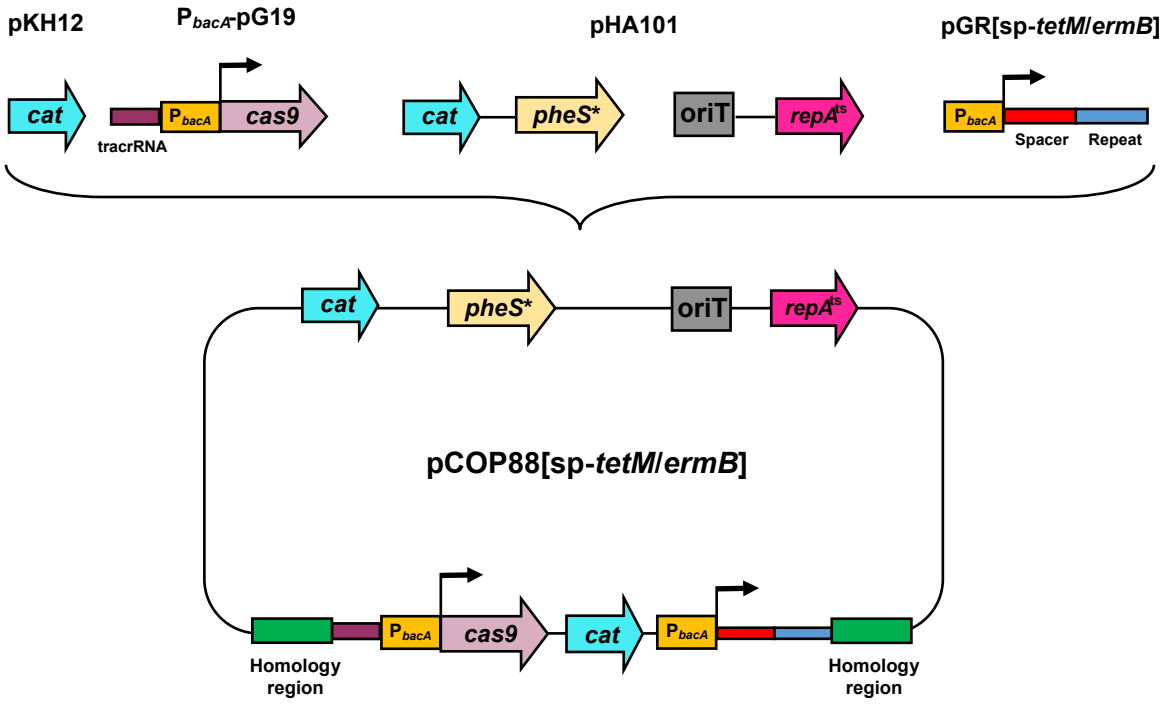
pKH12
P bacA-pG19
pHA101
pGR[sp-tetM/ermB]
cat
P bacA
cas9
tracrRNA
cat
pheS*
oriT
repA ts
P bacA
Spacer
Repeat
cat
pheS*
oriT
repA ts
pCOP88[sp-tetM/ermB]
P bacA
cas9
cat
P bacA
Homology region
Homology region

Figure S1

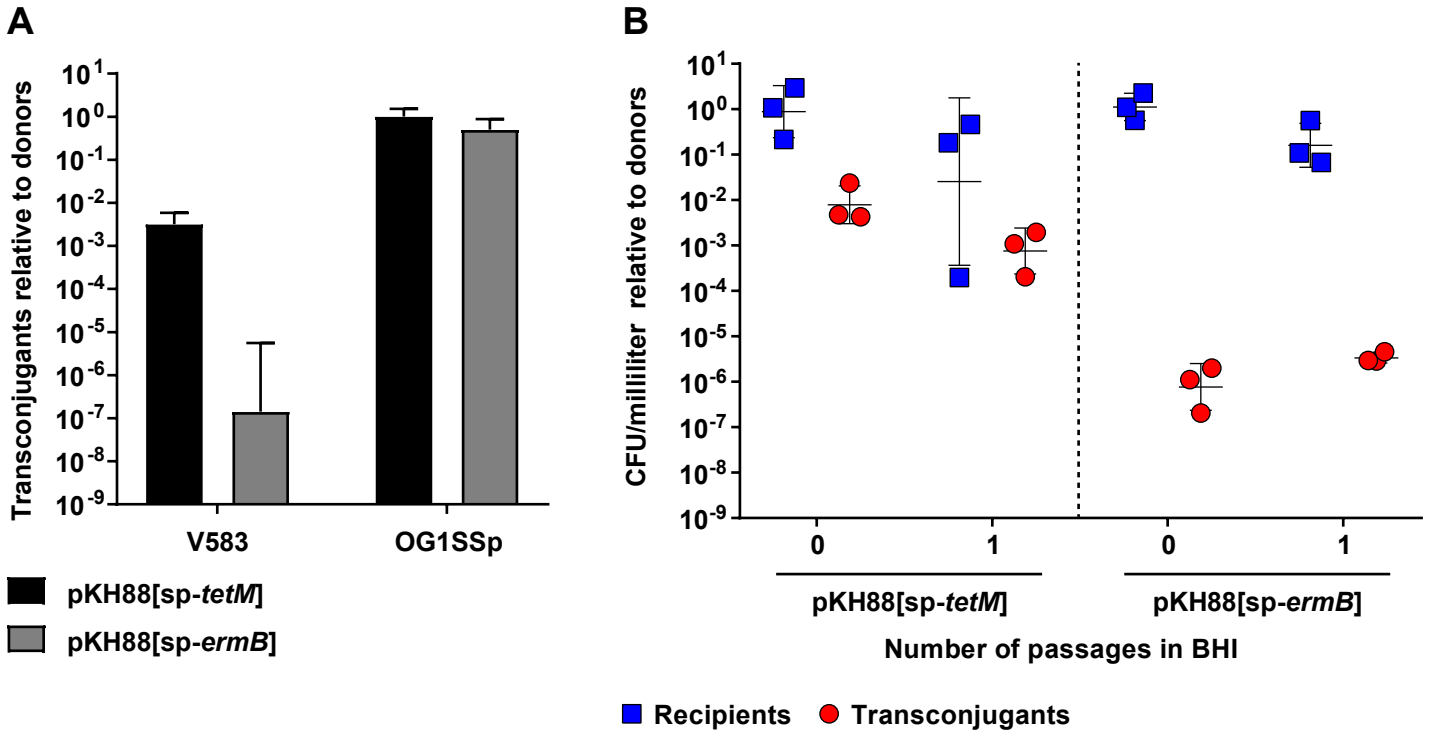
A
Transconjugants relative to donors
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
V583
OG1SSp
pKH88[sp-*tetM*]
pKH88[sp-*ermB*]
B
CFU/milliliter relative to donors
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
0
1
0
1
pKH88[sp-*tetM*]
pKH88[sp-*ermB*]
Number of passages in BHI
Recipients
Transconjugants

Figure S2

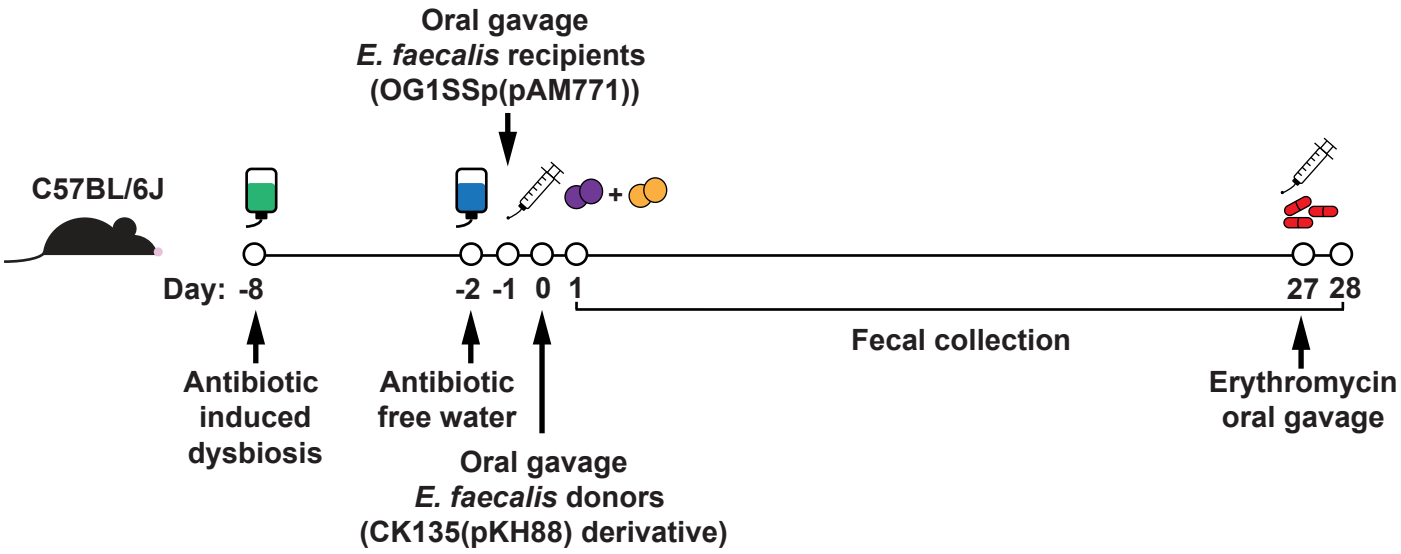
Oral gavage
E. faecalis recipients
(OG1SSp(pAM771))
C57BL/6J
Day: -8
-2 -1 0 1
27 28
Fecal collection
Antibiotic induced dysbiosis
Antibiotic free water
Oral gavage
E. faecalis donors
(CK135(pKH88) derivative)
Erythromycin oral gavage

Figure S3

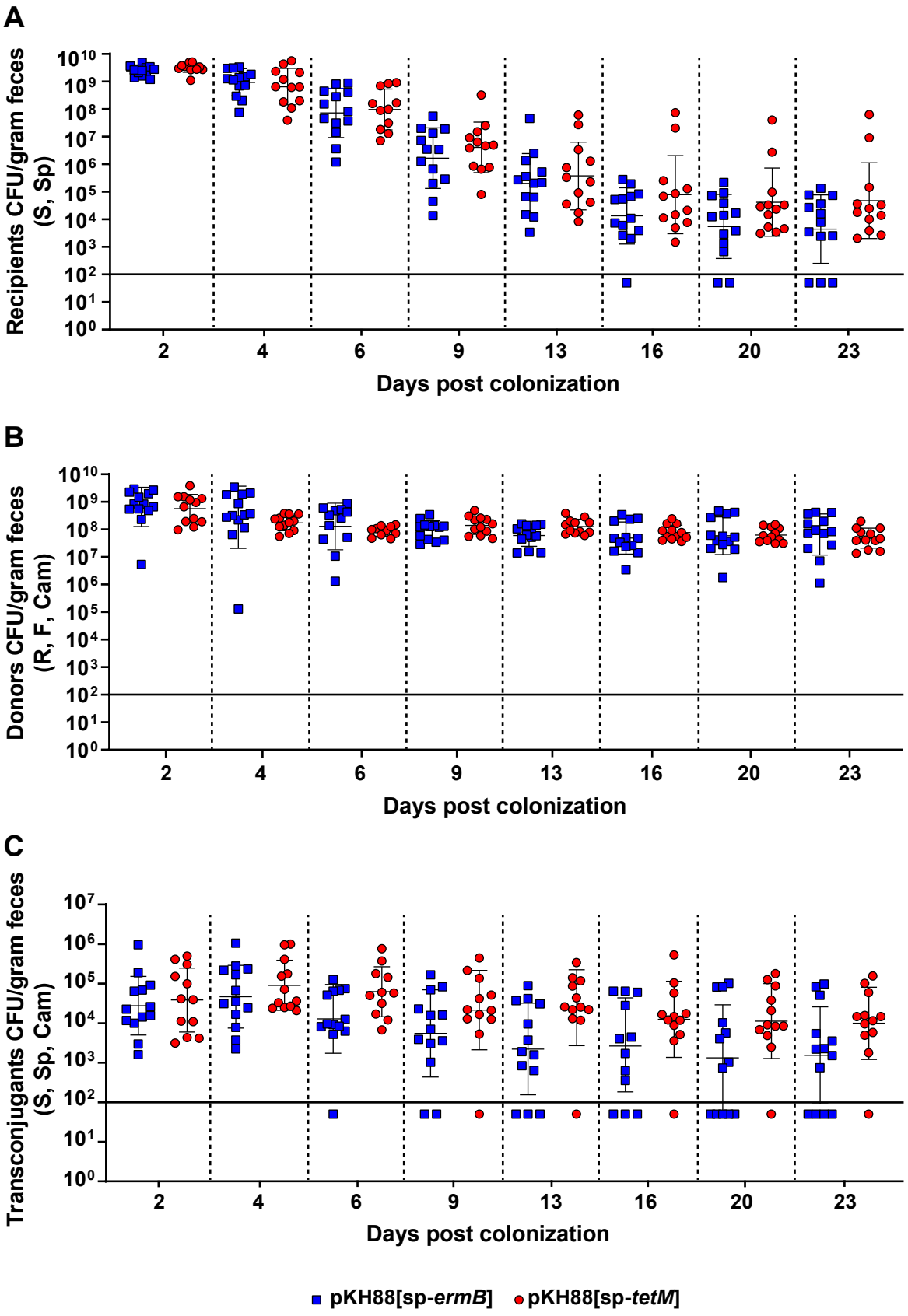
A
Recipients CFU/gram feces (S, Sp)
Days post colonization
B
Donors CFU/gram feces (R, F, Cam)
Days post colonization
C
Transconjugants CFU/gram feces (S, Sp, Cam)
Days post colonization
pKH88[sp-ermB]
pKH88[sp-tetM]

Figure S4

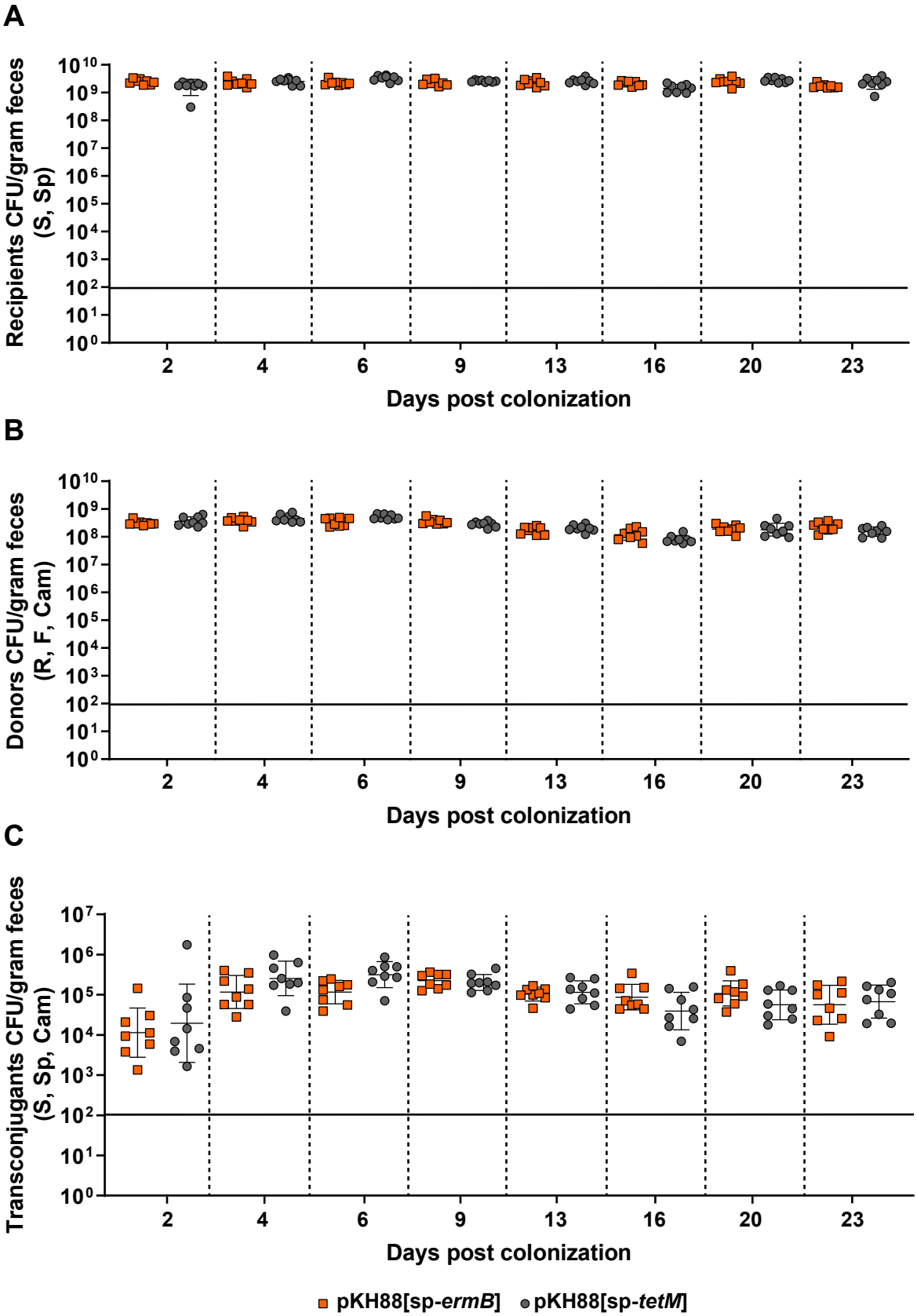
A
Recipients CFU/gram feces (S, Sp)
Days post colonization
B
Donors CFU/gram feces (R, F, Cam)
Days post colonization
C
Transconjugants CFU/gram feces (S, Sp, Cam)
Days post colonization
pKH88[sp-ermB]
pKH88[sp-tetM]

Figure S5

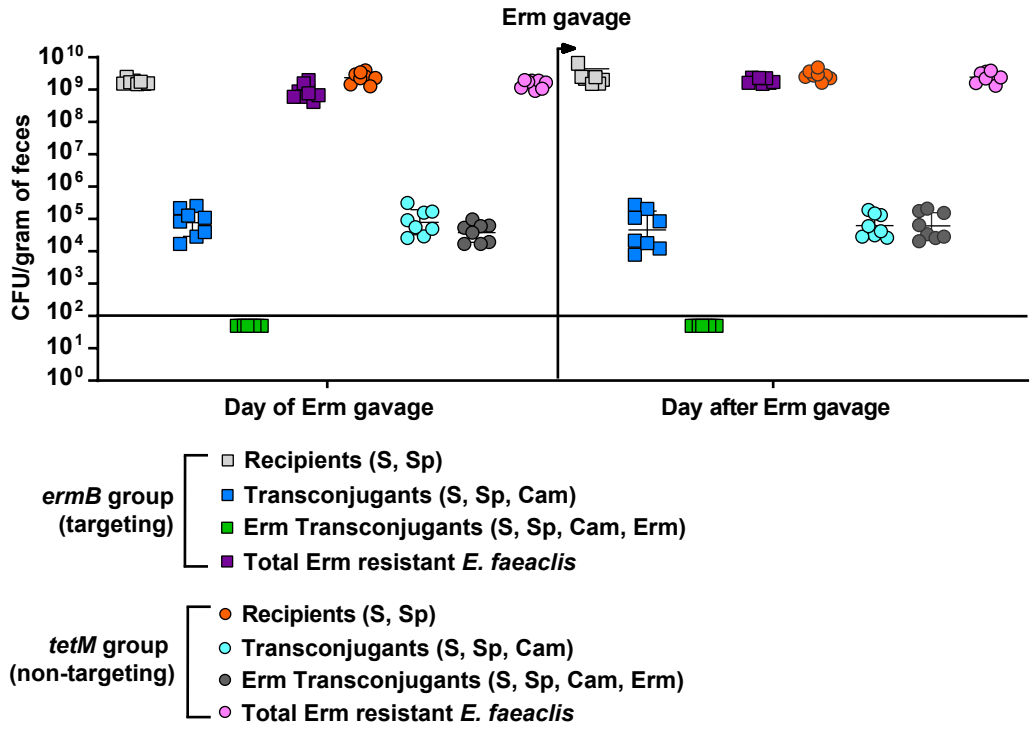
Erm gavage
CFU/gram of feces
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Day of Erm gavage
Day after Erm gavage
ermB group (targeting)
Recipients (S, Sp)
Transconjugants (S, Sp, Cam)
Erm Transconjugants (S, Sp, Cam, Erm)
Total Erm resistant E. faeaclis
tetM group (non-targeting)
Recipients (S, Sp)
Transconjugants (S, Sp, Cam)
Erm Transconjugants (S, Sp, Cam, Erm)
Total Erm resistant E. faeaclis

Figure S6

Supplementary Figure Legends

Figure S1. The allelic exchange vector pCOP88 integrates the P$_{bacA}$-*cas9*-*cat*-guide RNA module into pPD1. pCOP88 derivatives contain elements from the allelic exchange vector pLT06 (1). These include a temperature sensitive *repA* (*repA*^{ts}) allele that facilitates forced integration of pCOP88 derivatives into target DNA sequences under non-permissive temperature (42°C) and a P-*pheS* cassette that allows for the cellular utilization of *p*-chloro-phenylalanine as a counter-selectable marker. The P*bacA*-*cas9*-*cat*-guide RNA module is flanked by 1-kb pPD1 homology regions used for the homologous recombination of the module into native pPD1.

Figure S2. *E. faecalis* V583 restricts conjugation of pKH88 derivatives originating from *E. faecalis* CK135. (A) The ratio of *E. faecalis* V583 and OG1SSp transconjugants following *in vitro* co-culture with *E. faecalis* CK135(pKH88) derivative donors. **(B)** Comparison of normalized *E. faecalis* V583 transconjugant and *E. faecalis* V583 recipient numbers following *in vitro* co-culture with *E. faecalis* CK135(pKH88) derivative donors.

Figure S3. Schematic cartoon of the antibiotic dysbiosis mouse model.

Figure S4. Intestinal colonization of *E. faecalis* populations following co-colonization with OG1SSp(pAM771) and CK135(pKH88) donors in antibiotic-treated mice. (A) Recipients. **(B)** Donors. **(C)** Transconjugants. Markers; S – streptomycin, Sp – spectinomycin, R – rifampicin, F – fusidic acid, Cam – chloramphenicol.

Figure S5. Intestinal colonization of *E. faecalis* populations following co-colonization with OG1SSp(pAM771) and CK135(pKH88) donors in gnotobiotic mice. (A) Recipients. **(B)** Donors. **(C)** Transconjugants. The solid horizontal line indicates the limit of detection. Markers; S – streptomycin, Sp – spectinomycin, R – rifampicin, F – fusidic acid, Cam – chloramphenicol.

Figure S6. Non-targeted *E. faecalis* intestinal transconjugant populations do not bloom following oral erythromycin treatment of gnotobiotic mice. 27 days post co-colonization with *E. faecalis* OG1SSp(pAM771) and CK135(pKH88[sp-*ermB*]) (targeting group) or with *E. faecalis* OG1SSp(pAM771) and CK135(pKH88[sp-

tetM]) (non-targeting group), mice received a single 40 µg dose of oral erythromycin. The number of recipients, transconjugants and erythromycin resistant transconjugants were enumerated from fecal pellets before and after oral erythromycin treatment. The solid horizontal line indicates the limit of detection. Markers; Erm – erythromycin, S – streptomycin, Sp – spectinomycin, Cam – chloramphenicol.

Table S1. Strains used in this study

Organism	Strain Name	Description	Ref
E. coli	EC1000	*E. coli* cloning host, providing *repA in trans.*F- , *araD139 (ara ABC-leu)7679, galU, galK, lacX74, rspL, thi, repA* of pWV01 in *glgB, km*	(2)
E. faecalis	V583	MDR bloodstream isolate, resistant to vancomycin, gentamicin, and erythromycin	(3)
	OG1SSp	Spectinomycin-streptomycin-resistant derivative of OG1	(4)
	CK135	OG1 $rpoB_{H486Y}$ (spontaneous Rif^r derivative)	(5)
	CK135RF	Spontaneous fusidic acid-resistant derivative of CK135	This study
	OG1RF ΔEfaRFI	OG1RF EfaRFI (OG1RF_11622-11621) deletion mutant	(6)

Table S2. Plasmids used in this study

Plasmid	Description	Ref
pLT06	Encodes temperature-sensitive *repA* and *pheS** counter-selection	(1)
pHA101	pLT06 + *oriT*	(7)
pCOP88[sp-*tetM*]	pLT06 derivative used to knock in CRISPR-targeting construct with a spacer targeting *tetM*	This study
pCOP88[sp-*ermB*]	pLT06 derivative used to knock in CRISPR-targeting construct with a spacer targeting *ermB*	This study
pKH88[sp-*ermB*]	pPD1 derivative with CRISPR-targeting cassette for *ermB*; also encodes *cat*	This study
pKH88[sp-*tetM*]	pPD1 derivative with CRISPR-targeting cassette for *tetM*; also encodes *cat*	This study
pAM771	Non-cytolytic derivative of the PRP pAD1 mutagenized with Tn*917,* encodes erythromycin resistance via *ermB*	(8)
pCF10	PRP; encodes tetracycline resistance via *tetM*	(9)
pPD1	PRP; encodes Bac-21 bacteriocin	(10)

Table S3. Primers used in this study

Primer Name	Sequence (5'-3')	Use
pCOP88 Ori for	TGCAGCGTTTCTTTGAATAG	Create pCOP88
pCOP88 Ori rev	GCTTTGCAAAGTCTGAAAAC	Create pCOP88
pCOP88 PheS cat for	TGCCACCTTCGTTTTCAGACTTTGCAAAGCCAAGTTAAGGGATGCAGTTTAAAAATG	Create pCOP88
pCOP88 PheS cat rev	GGCATGATGGTTGCCGGTCGATAAACCCAGCGAAC	Create pCOP88
pCOP88 cas9 for	AAACATTACTCTATAGCAAACACAGTTAACCACG	Create pCOP88
pCOP88 cas9-cat-PSRT rev	CAATATCAGAATCAATCCACTCCTGAATCCCATTC	Create pCOP88
pCOP88 Arm 1 for	CTGGGTTTATCGACCGGCAACCATCATGCCTAAATTTTTATC	Create pCOP88
pCOP88 Arm 1 rev	GTTAACTGTGTTTGCTATAGAGTAATGTTTTAATTTTTTTCTCTTTTTCAG	Create pCOP88
pCOP88 Arm 2 for	GGATTCAGGAGTGGATTGATTCTGATATTGCCAATC	Create pCOP88
pCOP88 Arm 2 rev	CTAAAACGTCCTATTCAAAGAAACGCTGCAAGTCAACTAGAATCTGCTG	Create pCOP88
cas9 rev	TTTATTAAAGTTCATCTAGTCGACAACTTTACGGCGTGTTTC	Create pCOP88

cat for	AAAGTTGTCGACTAGATGAACTTTAATAAAATTG ATTTAGACAAT	Create pCOP88
cat rev	TCAACAAACTGGCCCGTTTGTTGAACTACTTTAT AAAAGCCAGTCATTAGGC	Create pCOP88
PSRT for	AGTAGTTCAACAAACGGGCC	Create pCOP88

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