SUPPLEMENTAL MATERIAL

Type IV pili promote *Clostridium difficile* adherence and persistence in a mouse model of infection

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Table S1. Strains and cell lines used in this study

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Figure S1. Viability of epithelial cell lines under anaerobic conditions.

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Table S1. Strains and plasmids used in this study.

Strain/Plasmid	Description/Purpose	Reference
Plasmids		
pMC123	*E. coli – C. difficile* shuttle vector; Amp^R, Cm^R/Tm^R	[1]
pMC-P_{cpr}	pMC123 with *cpr* promoter in the multiple cloning site	[2]
pDccA	pMC-P_{cpr}::*dccA* (CD630_14200)	[2]
pDccA^{mut}	pMC-P_{cpr}::*dccA*^{mut} (AADEF)	[2]
pSigD	pMC-P_{cpr}::*sigD* (CD630_02660)	[3]
pPilA1	*pilA1* complementation plasmid with native promoter and riboswitch, pMC-P_{pilA1}-RB-*pilA1* (CD630_3513), abbreviated as pPilA1	This study
pPilA1^{mut}	*pilA1* complementation plasmid with native promoter and riboswitch unable to bind c-di-GMP, pMC-P_{pilA1}-RB^{A70G}-*pilA1*, abbreviated as pPilA1^{mut}	This study
***E. coli* strains**		
DH5α	F- φ80*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *recA1 endA1 hsdR*17(rκ -, mκ+) *phoA supE44 thi*-1 *gyrA96 relA1* λ- *tonA*	Invitrogen [4]
HB101	F− *mcrB mrr hsdS20*(rB− mB−)*recA13 leuB6 ara*-14 *proA2 lacY1 galK2 xyl*-5 *mtl*-1 *rpsL20*	[5]
RT1026	HB101(pRK24) pPilA1	This study
RT1027	HB101(pRK24) pPilA1^{mut}	This study
***C. difficile* strains**		
630Δerm	Ribotype 012, erythromycin-sensitive derivative of *C. difficile* 630	[6]
R20291	Ribotype 027, epidemic isolate	[7]
RT761	630Δerm *pilA1*::*ermB*	[3]
RT762	630Δerm pMC-P_{cpr}	[3]
RT763	630Δerm pDccA	[3]
RT764	630Δerm pDccA^{mut}	[3]
RT765	630Δerm *pilB1*::*ermB,* pMC-P_{cpr}	[3]
RT766	630Δerm *pilB1*::*ermB,* pDccA	[3]
RT767	630Δerm *pilB1*::*ermB,* pDccA^{mut}	[3]
RT768	630Δerm *pilA1*::*ermB,* pMC-P_{cpr}	[3]
RT769	630Δerm *pilA1*::*ermB,* pDccA	[3]
RT770	630Δerm *pilA1*::*ermB,* pDccA^{mut}	[3]
RT1075	630Δerm *sigD*::*ermB*	[3]
RT1138	630Δerm *sigD*::*ermB* pSigD	This study
RT1136	630Δerm *sigD*::*ermB* pMC-P_{cpr}	[3]
RT1265	630Δerm *sigD*::*ermB* pDccA	This study
RT1266	630Δerm *sigD*::*ermB* pDccA^{mut}	This study
RT1362	630Δerm *pilA1*::*ermB* pPilA1	This study
RT1366	630Δerm *pilA1*::*ermB* pPilA1^{mut}	This study
Eukaryotic cell lines		
Caco-2	Caco-2 (ATCC® HTB-37) human colorectal adenocarcinoma cells	[8]
HT-29	HT-29 (ATCC® HTB-38) human colorectal adenocarcinoma cells	[9]
MDCK	Madin-Darby Canine Kidney cells (ATCC® CCL-34)	[10]

Table S2. Primers used in this study.

Oligonucleotide	Sequence
R978	GACTGCAGCATTCATAATTGAACATTAAAGAAAATAG
R1183	CCGAATTCAAATTCTGTTTCAATATGTAAAAAGTTG
R1184	CAATTTCATAGCCGGCTGCACCACTAACTCAATA
R1185	TATTGAGTTAGTGGTGCAGCCGGCTATGAAATTG

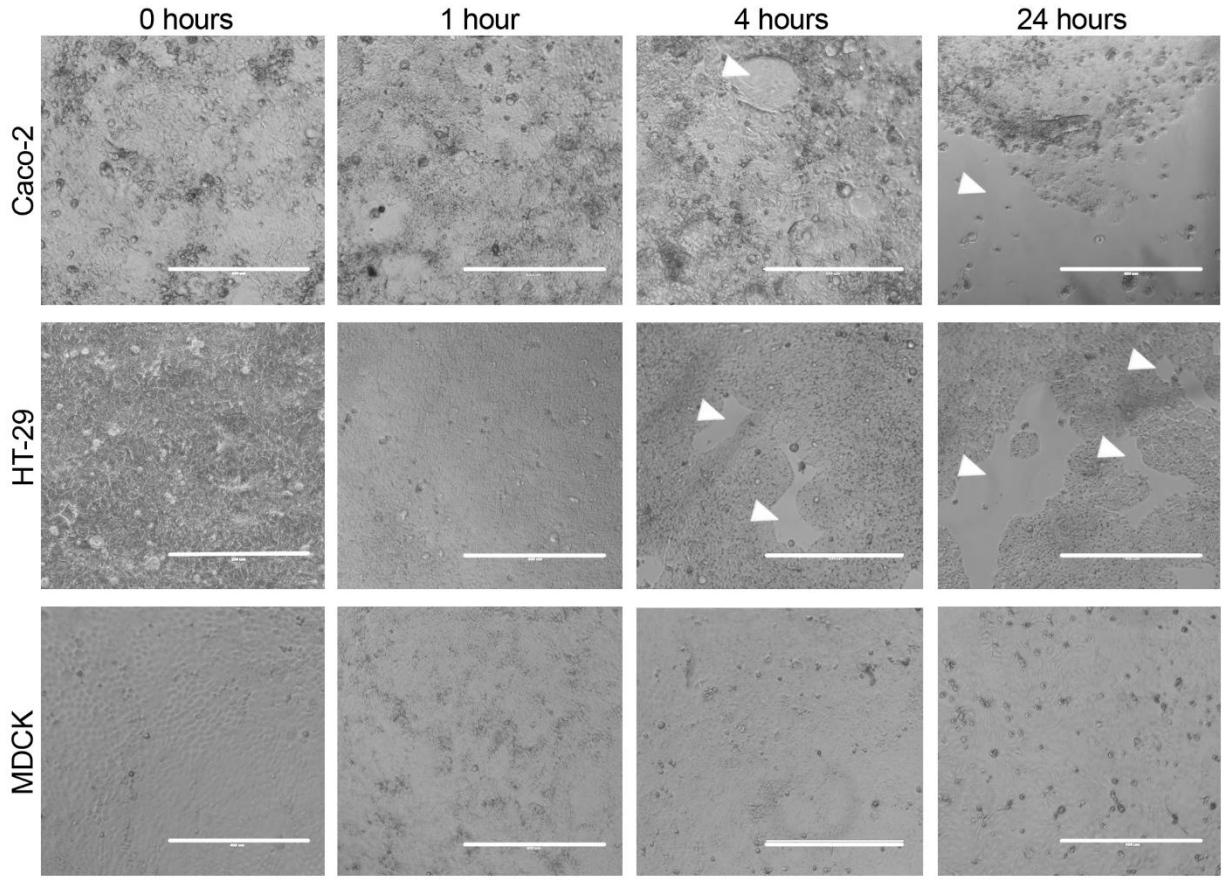

Figure S1. Viability of epithelial cell lines under anaerobic conditions. Monolayers of Caco-2, HT-29, and MDCK cells before and after incubation in anaerobic conditions for 1, 4 and 24 hours. Scale bar = 400 μm. White arrows indicate gaps in monolayers.

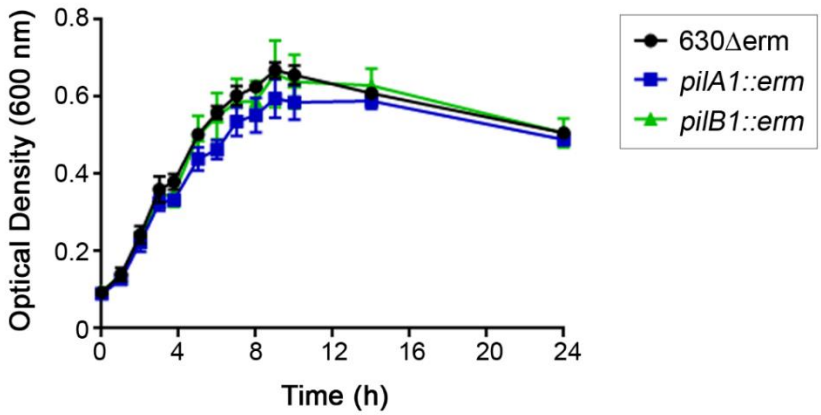

Figure S2. Growth of *C. difficile* strains in DMEM culture medium. 630ΔErm, the *pilA1* mutant, and *pilB1* mutant were grown in DMEM + 10% FBS without phenol red, with OD_{600} measured over the indicated time frame.

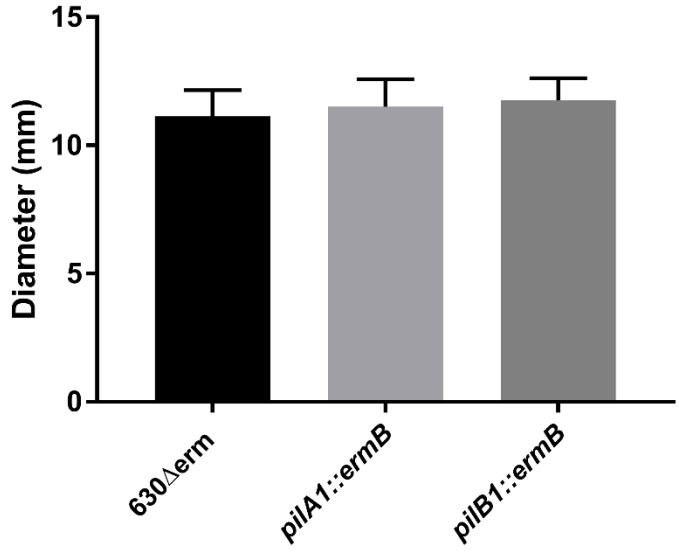

Figure S3. Swimming motility of 630Δerm and TFP mutants. Bacteria from individual colonies were inoculated into soft agar plates containing 0.5X BHIS and 0.3% agar and incubated at 37 ^{o}C under anaerobic conditions for 48 hours. Shown are the means and standard deviations of the diameters of the colony swarms from 4 biological replicates. Data were analyzed by one-way ANOVA; no significant differences were found.

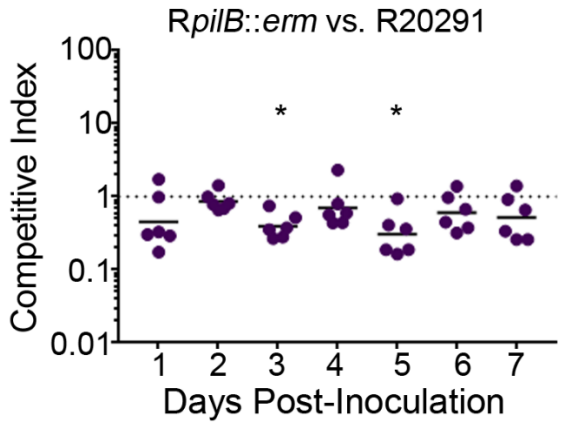

Figure S4. Competition of R20291 and R20291 *pilB1*::*ermB* strains in the mouse model. R20291 *pilB1*::*ermB* versus the R20291 parent strain were co-inoculated into antibiotic-treated C57BL/6 mice. CFU recovered from feces were used as a proxy for bacterial load in the intestine. Fecal samples were collected and homogenized at the indicated times post-inoculation and plated on TCCFA and TCCFA supplemented with lincomycin at 20 µg/ml to enumerate total CFU and mutant CFU, respectively. Competitive indices (CI) were calculated as detailed in the Materials and Methods. Symbols reflect CI from individual mice, bars indicate standard deviation. * $P < 0.05$, Wilcoxon matched-pairs signed rank test.

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