Table S4. Strains and plasmids used in this study.

Strain	Genotype	Origin
E. coli		
NEB-10 beta	Δ*(ara-leu) 7697 araD139 fhuA* Δ*lacX74 galK16 galE15 e14- ϕ80*d*lacZ*Δ*M15 recA1 relA1 endA1 nupG rpsL* (Str^R) *rph spoT1* Δ*(mrr-hsdRMS-mcrBC)*	New England Biolabs
NEB-5 alpha	*fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	New England Biolabs
HB101 (RP4)	*supE44 aa14 galK2 lacY1* Δ(*gpt-proA*) 62 *rpsL20 (*Str^R*)xyl-5 mtl-1 recA13* Δ(*mcrC-mrr*) *hsdS*$_B$ (r_B-m_B-) RP4 (Tra^+ IncP Ap^R Km^R Tc^R)	Laboratory stock
BL21-AI	F^-*ompT hsd*S_B (r_B^- m_B^-) *gal dcm ara*B::T7RNAP-*tet*A	Invitrogen
BL21-AI_ΔCRISPR	F^-*ompT hsd*S_B (r_B^- m_B^-) *gal dcm ara*B::T7RNAP-*tet*A ΔCRISPR	Laboratory stock
KD620	BL21-AI_ΔCRISPR carrying CRISPR 12 "mini-array"	This work
KD623	BL21-AI_ΔCRISPR carrying CRISPR 16 "mini-array"	This work
KD626	BL21-AI_ΔCRISPR carrying one-repeat CRISPR "mini-array" lacking spacer	This work
EC0344	KD620 carrying pDIA6349 and pDIA6351 *cas* expressing plasmids	This work
EC0346	KD623 carrying pDIA6349 and pDIA6351 *cas* expressing plasmids	This work
EC0348	KD626 carrying pDIA6349 and pDIA6351 *cas* expressing plasmids	This work
EC0367	KD620 carrying pDIA6351 *cas* expressing plasmids	This work
EC0369	KD623 carrying pDIA6351 *cas* expressing plasmids	This work
EC0371	KD626 carrying pDIA6351 *cas* expressing plasmids	This work
C. difficile		
630	Sequenced reference strain	Laboratory stock
630Δ*erm*	630 Δ*ermB*	Laboratory stock (Hussain et al. 2005)
R20291	PCR-ribotype 027 epidemic strain	Laboratory stock
Plasmid		
pCDF-1b	$P_{T7\ RNAP}$ Str^R expression vector with replication origin derived from CloDF13	Novagen
pRSF-1b	$P_{T7\ RNAP}$ Km^R expression vector with RSF1030 replication origin	Novagen
pDIA6351	pCDF-1b derivative carrying coding regions of *CD2982, CD2981, CD2980, CD2979, CD2978* and *CD2977*	This work
pDIA6349	pRSF-1b derivative carrying coding regions of *CD2976* and *CD2975*	This work

pT7Blue	Ap^R cloning vector	Novagen
pDIA6361	pT7Blue derivative carrying CRISPR 12 spacer 1	This work
pDIA6362	pT7Blue derivative carrying CRISPR 12 spacer 1 with single mismatch	This work
pDIA6363	pT7Blue derivative carrying CRISPR 16 spacer 1	This work
pDIA6364	pT7Blue derivative carrying CRISPR 16 spacer 1 with single mismatch	This work
pMTL84121	Tm^R *Clostridium-Escherichia coli* shuttle vector	(Heap et al. 2009)
pDIA5989	pMTL84121 derivative carrying CCA PAM with CRISPR 12 spacer 1	This work
pDIA5990	pMTL84121 derivative carrying CCT PAM with CRISPR 12 spacer 1	This work
pDIA5991	pMTL84121 derivative carrying GAG with CRISPR 12 spacer 1	This work
pDIA5999	pMTL84121 derivative carrying AAT with CRISPR 12 spacer 1	This work
pRPF185	P_{tet}-*gusA* Tm^R expression and cloning vector	(Fagan and Fairweather 2011)
pDIA6103	pRPF185 Δ*gus* vector derivative	(Soutourina et al. 2013)
pDIA6365	pRPF185 derivative carrying CCA PAM and CRISPR 16 spacer 1	This work
pDIA6367	pRPF185 derivative carrying CCA PAM and CRISPR 16 spacer 1 with single mismatch (G to A substitution at first position)	This work

Plasmid and bacterial strain construction. For inducible expression of *C. difficile cas* genes, we used the pCDF-1b or pRSF-1b expression vector system carrying the T7 RNAP promoter (Novagen). The coding regions of *cas6, cas8, cas7, cas3, cas4* genes (*CD2982, CD2981, CD2980, CD2979, CD2978, CD2977)* and *cas1, cas2* genes (*CD2976*, *CD2975)* were amplified by PCR on *C. difficile* 630 genomic DNA and the resulting 7169 and 1282-bp fragments were cloned into the *Nco*I and *Not*I sites of pCDF-1b and pRSF-1b vectors, respectively, under the control of T7 promoter. For interference assays in the *E. coli*, the protospacer perfectly matching the CRISPR spacer or carrying a single mismatch at first position preceded by CCA PAM was introduced into pT7Blue vector through blunt-end cloning (Novagen). To construct the *E. coli* strains carrying *C. difficile* CRISPR "mini-arrays", the resident CRISPR1 cassette (corresponding to the positions 1002803-1003730 of NC_012947.1) was replaced by the fragment containing T7 RNAP promoter, *lacO*, and T7 terminator in the *E. coli* BL21-AI strain (Invitrogen) lacking endogenous *cas* genes and carrying the T7 RNAP gene under the control of the arabinose-inducible *araBAD* promoter. Then we amplified by PCR on genomic DNA from *C. difficile* 630 three different CRISPR regions. The first fragment contained the *C. difficile* leader region starting from position -65 relative to the major transcriptional start site (TSS), and the first two repeats separated by the first spacer from the CRISPR 12 array (genomic position from 2908123 to 2907796 on the

minus strand). The second fragment carried the CRISPR 16 array sequence identical to the CRISPR 3 array, including the leader region starting from position -28 relative to the TSS and two repeats flanking the first spacer (genomic positions from 3397851 to 3397523 on the minus strand for CRISPR 16 array and from 1124790 to 1125118 for the CRISPR 3 array). Third fragment contained only the CRISPR leader region with the first direct repeat without the adjacent spacer (genomic position from 2908123 to 2907861 on the minus strand). These three arrays were introduced by recombination into the genome of *E. coli* BL21-AI_ΔCRISPR derivative strain downstream from the *lacO* region flanked by T7 RNAP promoter and transcription terminator sequences (resulting strains KD620, KD623 and KD626). *E. coli* genomic modifications were performed using the technique based on the previously described method (Datsenko and Wanner 2000).

For interference assays in *C. difficile,* we introduced a 44 bp fragment composed of a protospacer sequence corresponding to the first spacer of the CRISPR 12 array, preceded by either a tri-nucleotide PAM motif (CCA or CCT) or a different tri-nucleotide GAG or AAT into BamHI and HindIII sites of the pMTL84121 vector (Heap et al. 2009). pRPF185 derivatives were constructed by introducing the CCA PAM motif followed by either a protospacer perfectly matching the first spacer of the CRISPR 16 array or a protospacer with a single mismatch at the first position into the StuI and BamHI sites of the pDIA6103 vector. The resulting derivative plasmids were transformed into the *E. coli* HB101 (RP4) and subsequently mated with *C. difficile* 630Δ*erm*. *C. difficile* transconjugants were selected by sub-culturing on BHI agar containing Tm (15 μg.ml^{-1}) and Cfx (25 μg.ml^{-1}).

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