Supplementary File S1: Vector Construction

pMC199: The group II intron of pCE240 was retargeted to *oppB* at nucleotide 178s by splicing PCR using primers oMC371, oMC372, oMC373 and EBSu as outlined in the Targetron users manual (Sigma-Aldrich). The primers used for intron retargeting were obtained by using the algorithm provided by J.P. van Pijkeren and Rob Britton of Michigan State University. This region was cloned using *Bsr*GI and *Hind*III sites into pBL100.

pMC211: A 400 bp fragment containing the upstream region of the *cprA* operon was amplified from *C. difficile* 630E genomic DNA using primers oMC215 and oMC346. This region was cloned using *Bam*HI and *Eco*RI into pMC123.

pMC213: A 5.2 kb fragment containing the *oppBCAD* (CD0853-0856) predicted coding sequences and upstream region was amplified from *C. difficile* strain 630Δ*erm* genomic DNA using primers oMC440 and oMC441. This region was cloned using *Bam*HI and *Eco*RI into pMC123.

pMC217: A 517 bp fragment containing the coding region of CD0852 (a putative *scoC* ortholog) was amplified from *C. difficile* strain 630Δ*erm* using primers oMC506 and oMC507. This region was cloned using *Bam*HI and *Pst*I sites into pMC211.

pMC220: The group II intron of pCE240 was retargeted to *appA* at nucleotide 439s by splicing PCR using primers oMC544, oMC545, oMC546 and EBSu as outlined in the Targetron users manual (Sigma-Aldrich). The primers used for intron retargeting were obtained by using the algorithm provided at ClosTron.com. This region was TA cloned into pCR2.1 (Invitrogen).

pMC226: The group II *appA*--targeted intron from pMC220 was subcloned using *Bsr*GI and *Hind*III sites into pCE240.

pMC230: 5.48 kb *Sfo*I/*Sph*I fragment from pMC226 was cloned as *Sph*I/*Sna*BI into pMC123.

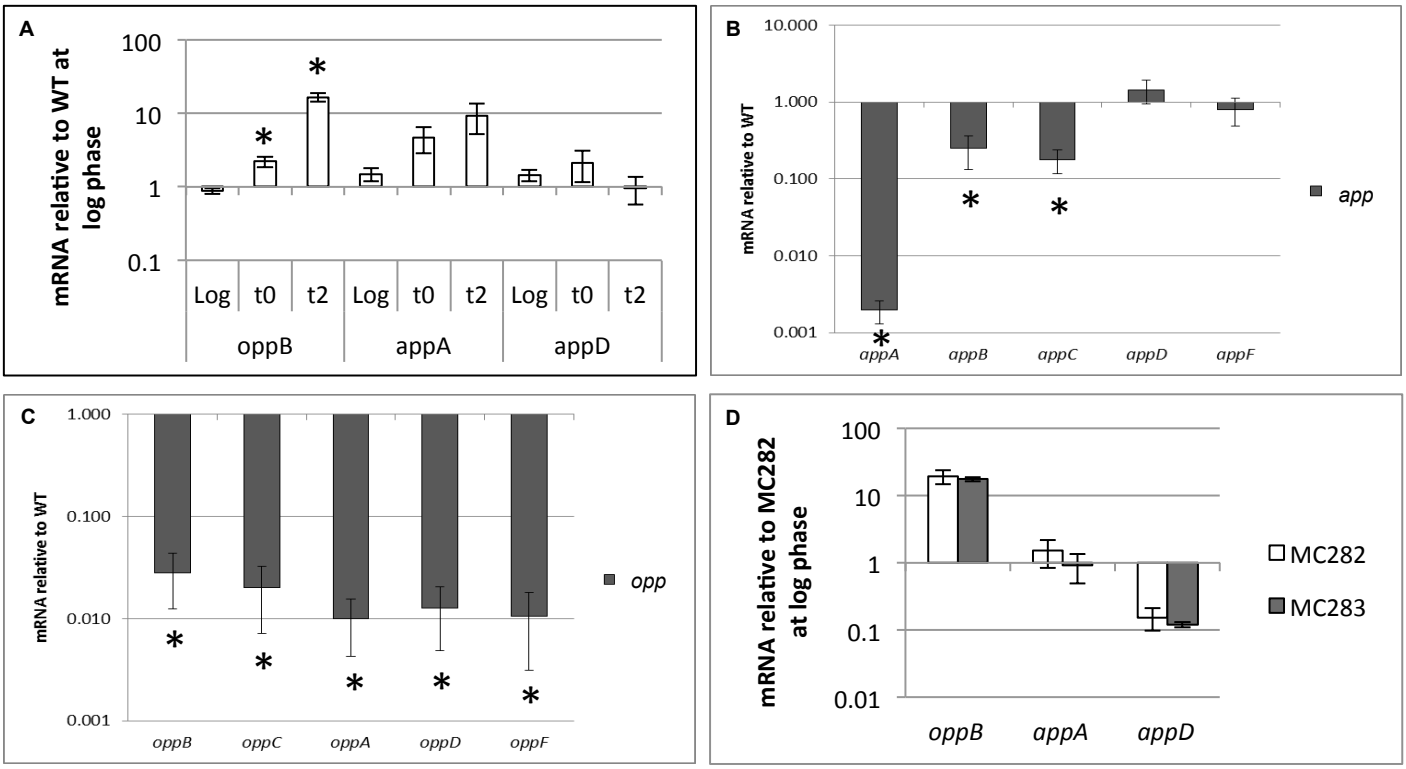
pMC237: A 5.6 kb fragment containing the *appFDABC* coding sequences and promoter regions was amplified from *C. difficile* strain 630Δ*erm* genomic DNA using primers oMC587 and oMC588. This region was TA cloned into pCR2.1 (Invitrogen).

pMC238: The *appFDABC* region from pMC237 was cloned into the *Bam*HI and *Eco*RI sites in pMC123.

pMC239: A 6.1 kb fragment containing the *oppBCADF* predicted coding sequences and upstream region was amplified from *C. difficile* strain R20291 genomic DNA using primers oMC440 and oMC654. This region was TA cloned into pCR2.1 (Invitrogen).

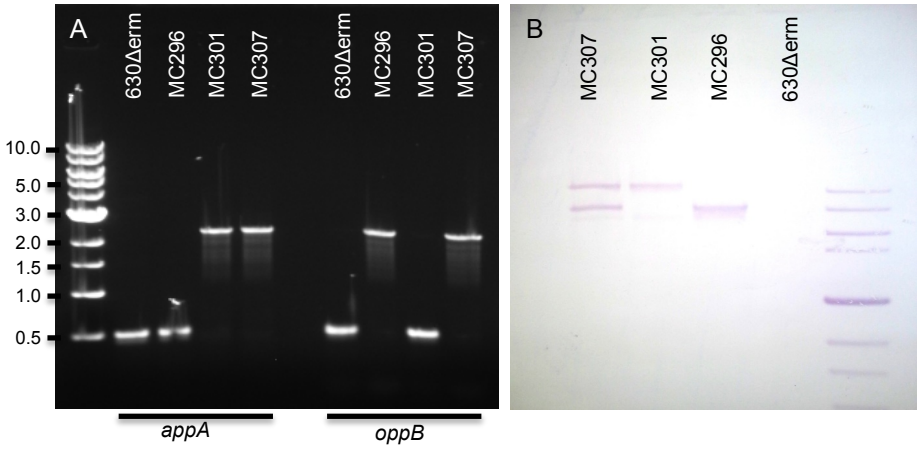
pMC240: The *oppBCADF* region from pMC239 cloned into the *Bam*HI and *Pst*I into pMC123.

Fig. S2



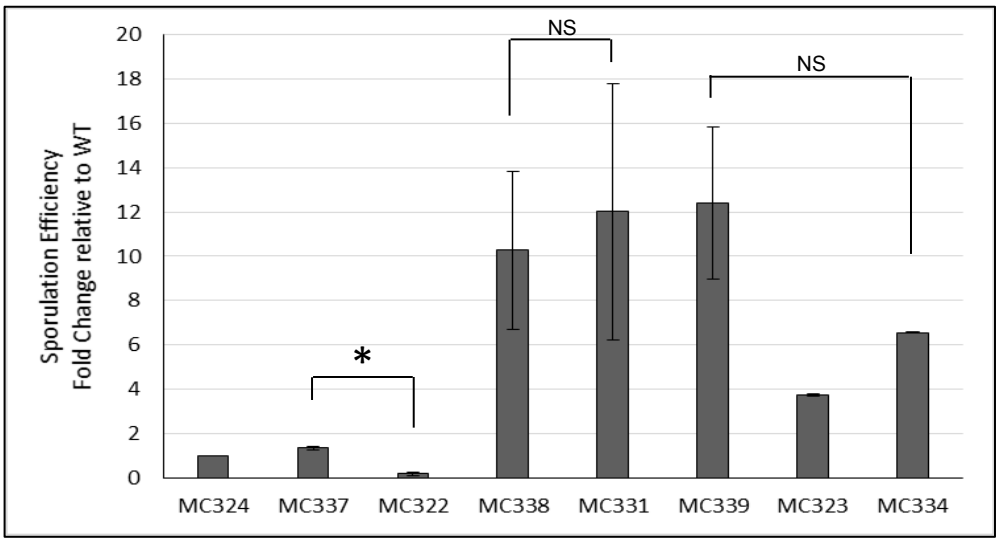
Supplementary Figure S2: Transcriptional analysis of the *app* and *opp* operons in wild-type, *appA* and *oppB* null mutants and in a CD0852 (*scoC*) overexpression strain.. (A) qRT-PCR analysis of *oppB*, *appA* and *appD* expression in 630Δ*erm* at an OD_{600} of 0.5, 1.0 (T_0) and two hours after T_0 (T_2) in 70:30 sporulation medium. (B) qRT-PCR analysis of *appA*, *appB*, *appC*, *appD* and *appF* expression in 630Δ*erm* and MC301 (*app*) grown in 70:30 sporulation medium at T_2. (C) qRT-PCR analysis of *oppB*, *oppC*, *oppA*, *oppD* and *oppF* expression in 630Δ*erm* and MC296 (*opp*) grown in 70:30 sporulation medium at T_2. (D) qRT-PCR analysis of *oppB*, *appA* and *appD* expression in the wild-type strain MC282 (630Δ*erm* pMC211) and the CD0852 (*scoC*) overexpression strain MC283 (630Δ*erm* pMC211::*CD0852*) grown in 70:30 sporulation medium at T_2. Fold changes are normalized to MC282 gene expression at log phase (data not shown). The means and standard error of the means at least three biological replicates are shown (*, $P \leq 0.05$ by a two-tailed Student's *t* test).

Fig. S3



Supplementary Figure S3: Confirmation of targeted disruption of oligopeptide transporter genes. A) PCR amplification of the *oppB* gene (primers oMC350/oMC439) and *appA* gene (primers oMC429/oMC553) from *C. difficile* strains, demonstrating insertion of the Targetron-based intron in mutants. B) Southern blot analysis of genomic DNA from *C. difficile* 630Δ*erm*, MC296 (*oppB*), MC301 (*appA*), and (*oppB appA*) strains using an intron-specific probe as described in Materials and Methods.

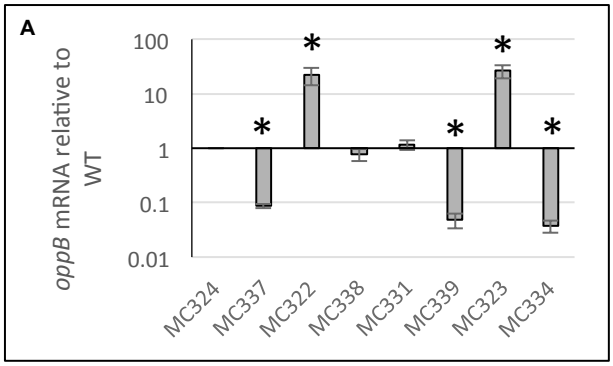
Fig. S4

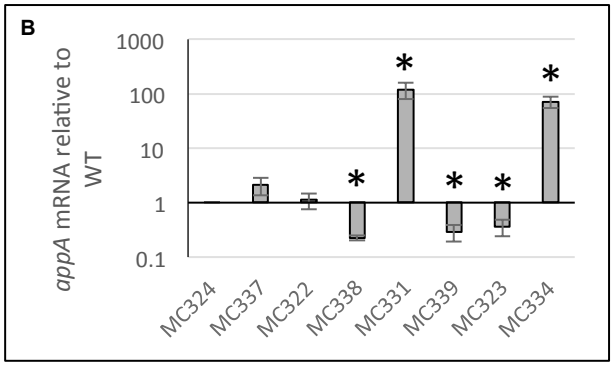


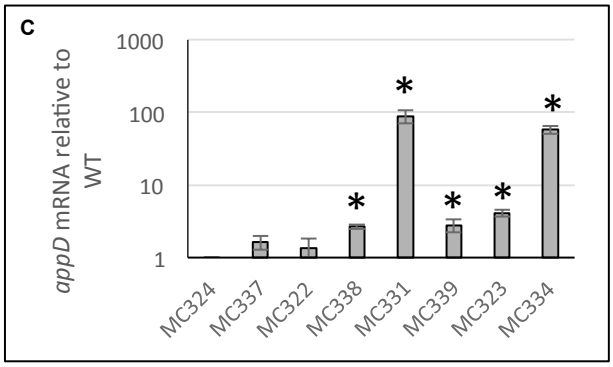
MC324	630Δ*erm* pMC123
MC337	*opp* pMC123
MC322	*opp* p*oppBCAD*
MC338	*app* pMC123
MC331	*app* p*appDFABC*
MC339	*opp app* pMC123
MC323	*opp app* p*oppBCAD*
MC334	*opp app* p*appDFABC*

Supplementary File S4: Complementation of oligopeptide transporter mutants. Sporulation frequency (CFU ml^{-1}) of indicated strains grown in 70:30 sporulation medium at T_{24}. The means and standard error of the means of three biological replicates are shown (*, $P \leq 0.05$ by a two-tailed Student's *t* test).

Fig. S5



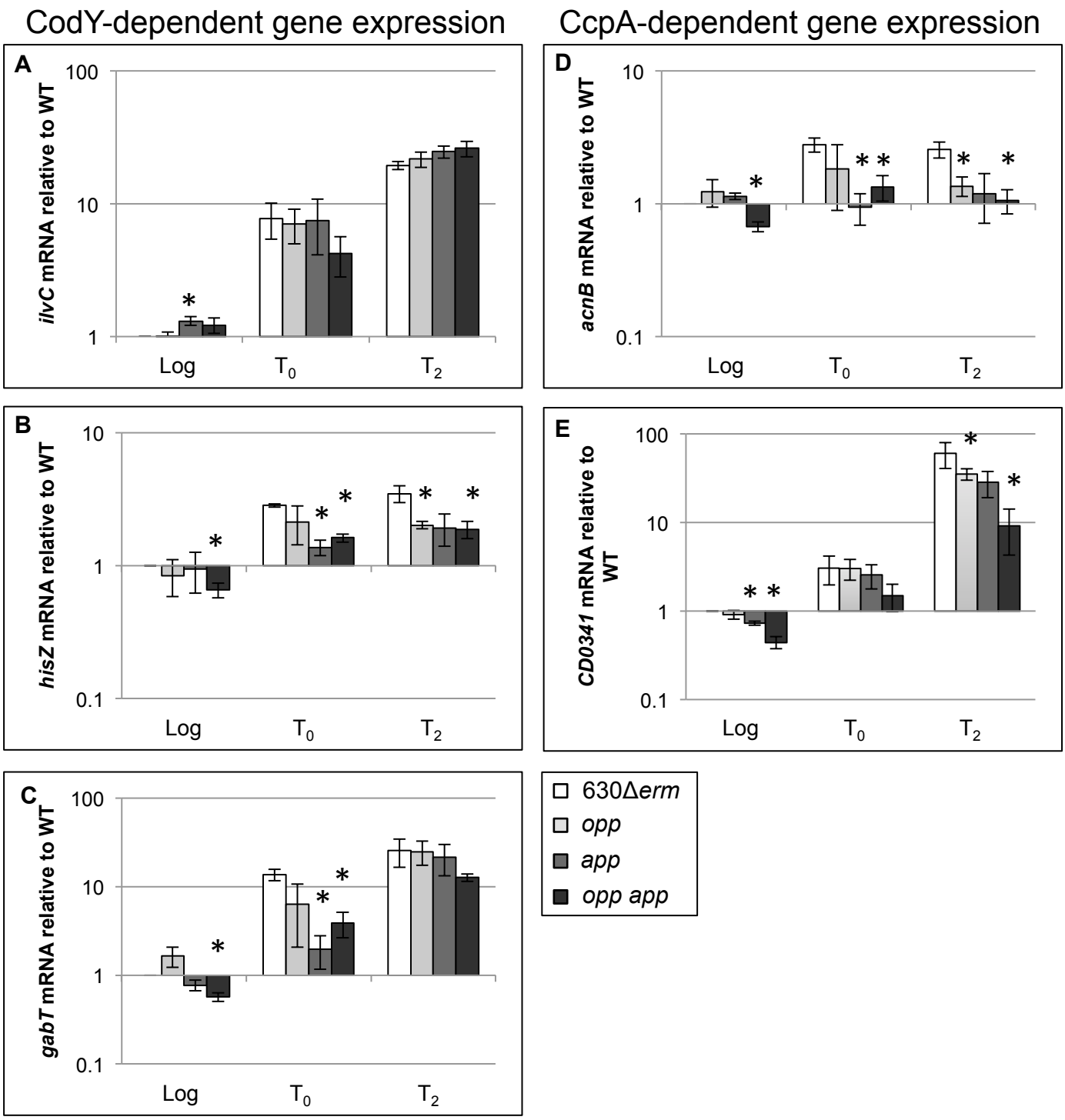




MC324	630Δ*erm* pMC123
MC337	*opp* pMC123
MC322	*opp* p*oppBCAD*
MC338	*app* pMC123
MC331	*app* p*appDFABC*
MC339	*opp app* pMC123
MC323	*opp app* p*oppBCAD*
MC334	*opp app* p*appDFABC*

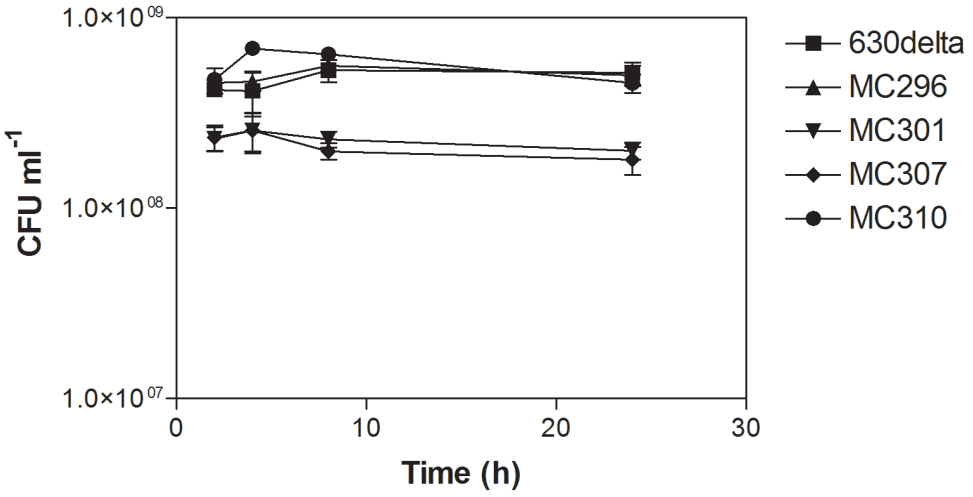
Supplementary File S5: Transcriptional analysis of *opp* and *app* expression in complementation studies. qRT-PCR analysis of *oppB* (A), *appA* (B) and *appD* (C) expression in MC324 (630Δ*erm* pMC123), MC337 (*opp* pMC123), MC322 (*opp* p*oppBCADF*), MC338 (*app* pMC123), MC331 (*app* p*appDFABC*), MC339 (*opp app* pMC123), MC323 (*opp app* p*oppBCADF*), MC334 (*opp app* p*appDFABC*) grown in 70:30 sporulation medium to an OD_{600} of 0.5. The means and standard error of the means of three biological replicates are shown (*, $P \leq 0.05$ by a two-tailed Student's *t* test).

Fig. S6



Supplementary File S6: Effects of oligopeptide transporters on regulation of CodY- and CcpA-dependent gene expression. qRT-PCR analysis of expression of the CodY-dependent genes, *ilvC* (A), *hisZ* (B) and *gabT* (C) and the CcpA-dependent genes *CD0341* (D) and *acnB* (F) in 630Δ*erm*, MC296 (*opp*), MC301 (*app*) and MC307 (*opp app*) grown in 70:30 sporulation medium to an OD_{600} of 0.5, 1.0 (T_0) and two hours after T_0 (T_2) as described in the Materials and Methods. The means and standard error of the means of at least three biological replicates are shown (*, $P \leq 0.05$ by a two-tailed Student's *t* test).

Fig. S7



Supplementary File S7: Total number of all viable cells (vegetative cells and spores) throughout stationary phase growth. 630Δ*erm*, MC296 (*opp*), MC301 (*app*) and MC307 (*opp app*) were grown in 70:30 sporulation medium to an OD_{600} of 1.0 (T_0), and dilutions were plated onto BHIS + 0.1% taurocholate medium at two, four, eight and 24 hours after T_0 (T_2,T_4,T_8 and T_{24}). Cells were enumerated after 24 h of growth. The means and standard deviations of two biological replicates are shown.