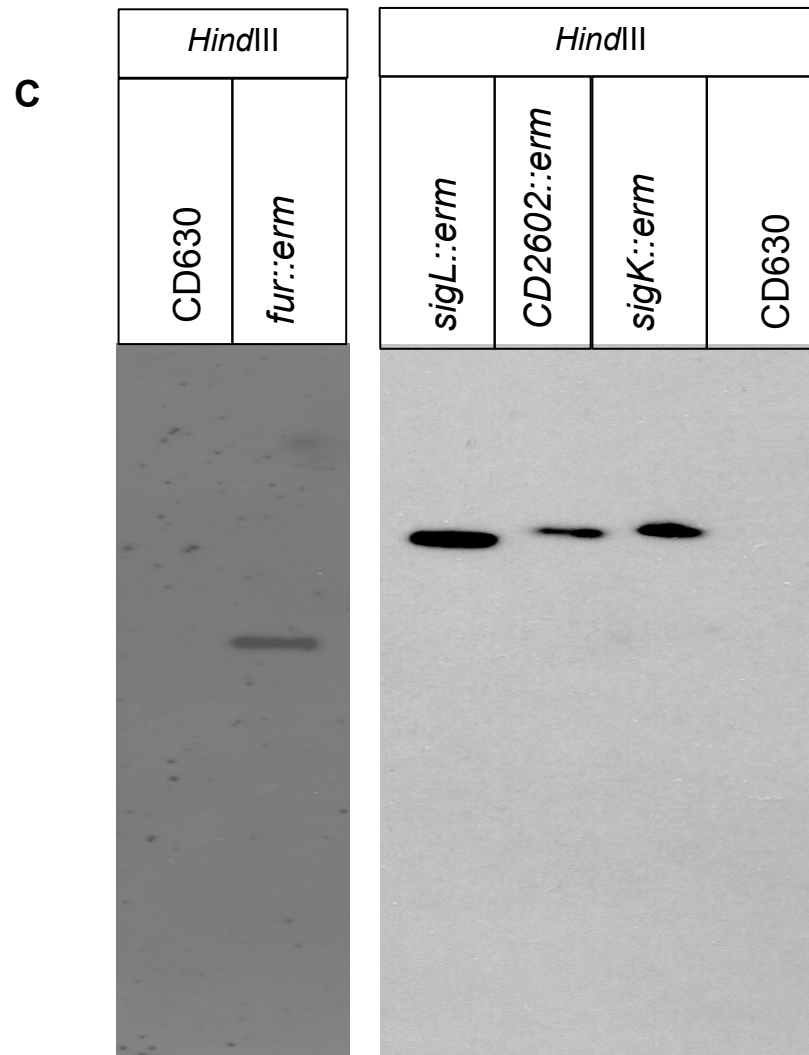**Fig. S1**: **Construction the *C. difficile fur*, *cysK*, *sigL* and *CD2602* mutants using the ClosTron system.**

A Schematic diagram of the ClosTron technology. The ClosTron delivery system encoded on plasmid pMTL007 consists of a group-II intron (black arrow) with an internal Retrotransposition-Activated Marker (RAM) conferring erythromycin resistance (white arrow) that is itself interrupted by a *td* group-I intron (hatched box). The group-II intron is retargeted to the target gene (grey arrow) by altering the sequence of the IBS/EBS region using overlap PCR. Splicing the group-II intron into the target gene disrupts it, and splicing the *td* group-I intron of the *erm* RAM restores the functional *erm* gene, allowing positive selection of the mutants. **B**. Confirmation of the mutant knockout by PCR. PCR using the primer pair Ram-F/Ram-R (lane 1 and 2, black arrows) was performed to confirm the splicing out of the group-I intron in the mutant. To verify the integration of the Ll.LtrB intron in the targeted gene, we performed PCR with two other primer pairs: the intron primer EBSu associated with a primer within the targeted gene (lanes 3 and 4, red and green arrow) and the wo primers flanking the insertion site in the targeted gene (lanes 5 and 6, red and blue arrows). The primer pairs LS182/LS183, OS214/OS215, cysK-verif-F/cysK-verif-R and 2602-verif-F/2602-verif-R2 and the primers LS182, OS215, cysK-verif-R and 2602-verif-R2 in association with EBSu (Tables S1) were used for the target genes *sigL*, *fur*, *cysK* and *CD2602,* respectively. Each PCR was performed on chromosomal DNA of Em^R conjugant strains (lanes 1, 3 and 5) and strain 630Δ*erm* as a negative control (lanes 2, 4 and 6). Smart Ladder (Eurogentec) was used as a molecular-weight marker.

Fig. S1: **Construction the *C. difficile fur, cysK, sigL* and *CD2602* mutants using the ClosTron system.**

C: Southern blot analysis of genomic DNA from *C. difficile* 630Δ*erm,* 630Δ*erm::fur,* 630Δ*erm::sigL*, 630Δ*erm::CD2602* and 630Δ*erm::cysK* strains with an intron probe. Chromosomal DNA (6 µg in each reaction) was digested with *Hind*III. Southern blot analyses were performed using Amersham ECL Direct Nucleic Acid labelling and detection reagents, in accordance with the manufacturer's guidelines and visualised using Super Signal West Femto Maximum Sensitivity Substrate (Thermo Scientific). The probe was produced by PCR using OBD522 and OBD523 primers (Table S1), designed within the group II intron sequence.
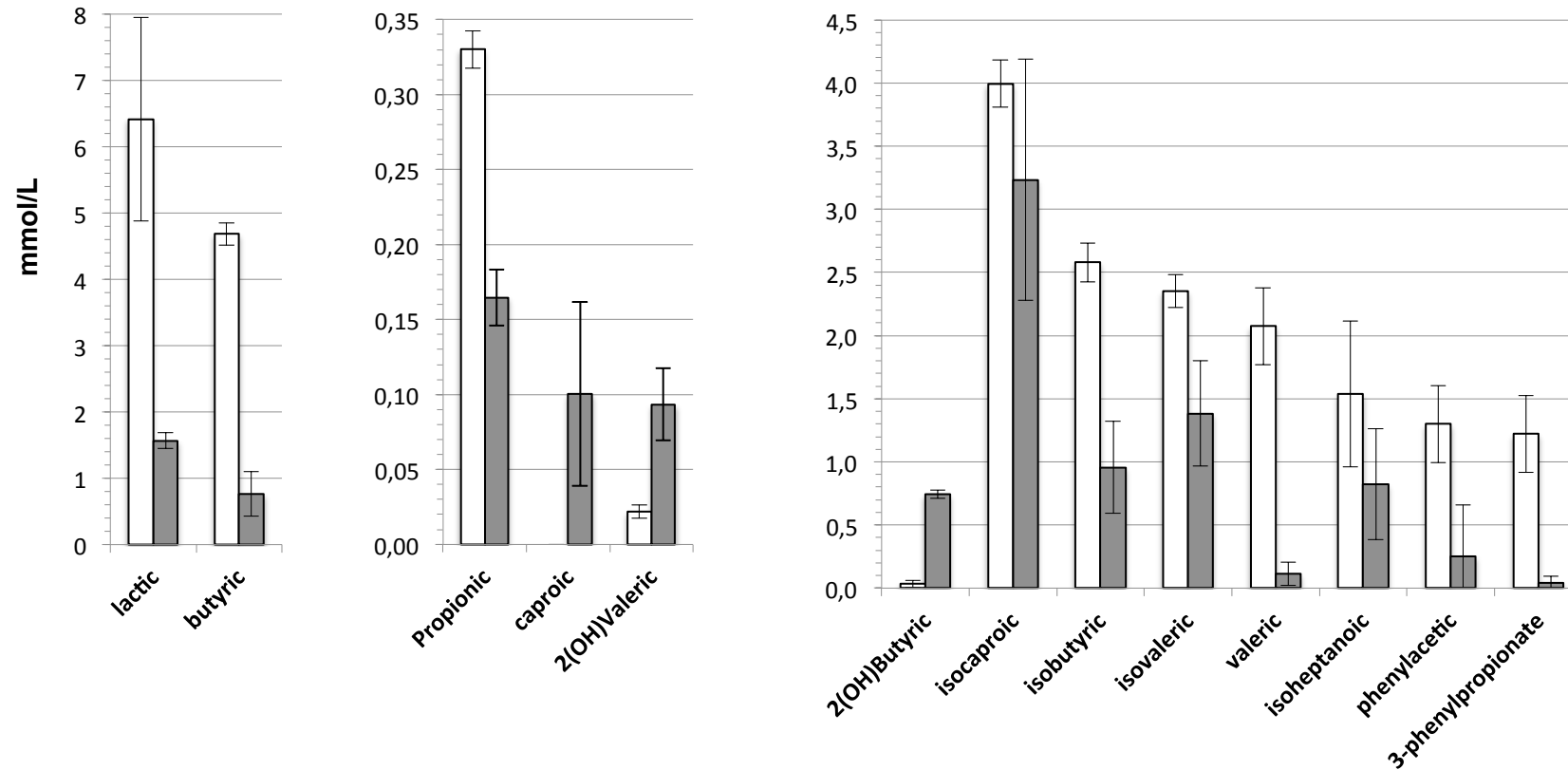
Fig. S2: **Fermentation end products of the strain 630Δ*erm* grown in the presence or absence of 10 mM cysteine .**

Determination of the concentration (mmol L^{-1}) of the short-chain fatty acids (SCFA) was performed by gas-liquid chromatography analysis with strain 630Δ*erm* after 48 h of growth in PY (white boxes) or PYC (grey boxes) medium. For each graph, the results indicated are the means of three independent experiments.
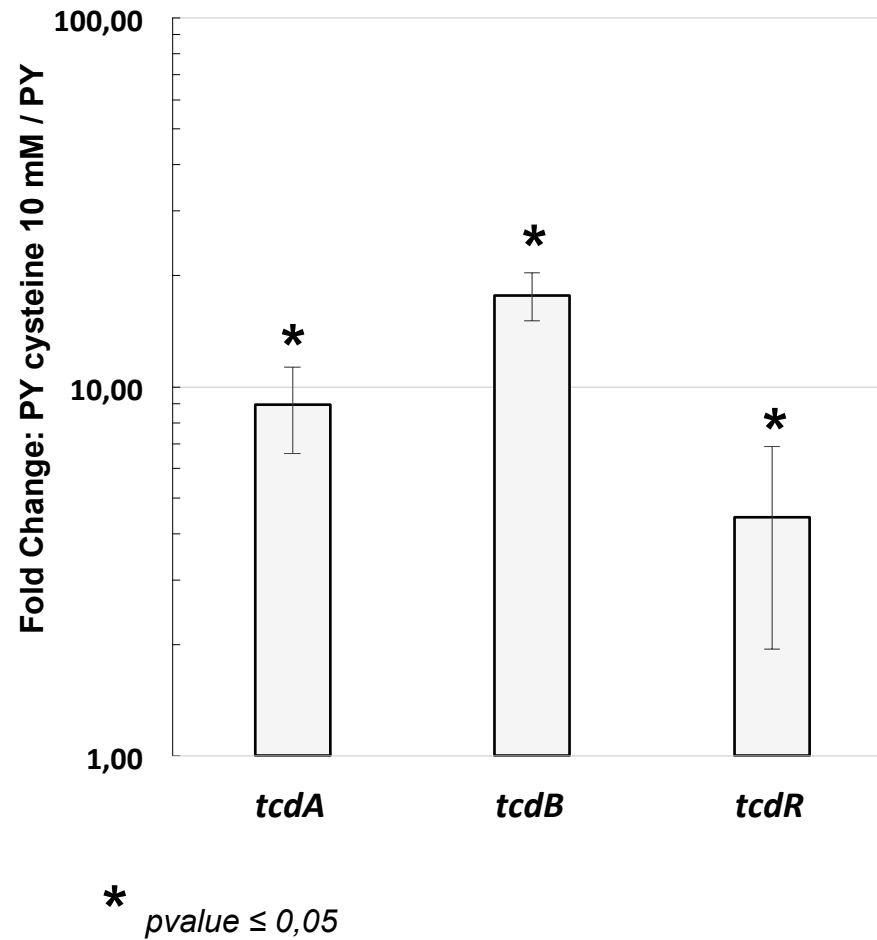
Fig. S3: **Transcript levels of *tcdA*, *tcdB* and *tcdR* genes of strain 630Δ*erm* after 1 h of exposure to cysteine .**

The strain was grown in PY for 9 h, and 10 mM cysteine was then added to the medium; the cells were centrifuged 1 h later. qRT-PCR results are presented as the ratio between the amount of mRNA (arbitrary units) normalized by the DNA *polIII* gene of bacterial cells grown in the presence of cysteine compared to the amount of mRNA in the untreated cells. Data are the averages of at least three independent experiments (error bars are the standard deviations from the mean values). The statistical analysis was performed by using a t-test for all genes. .
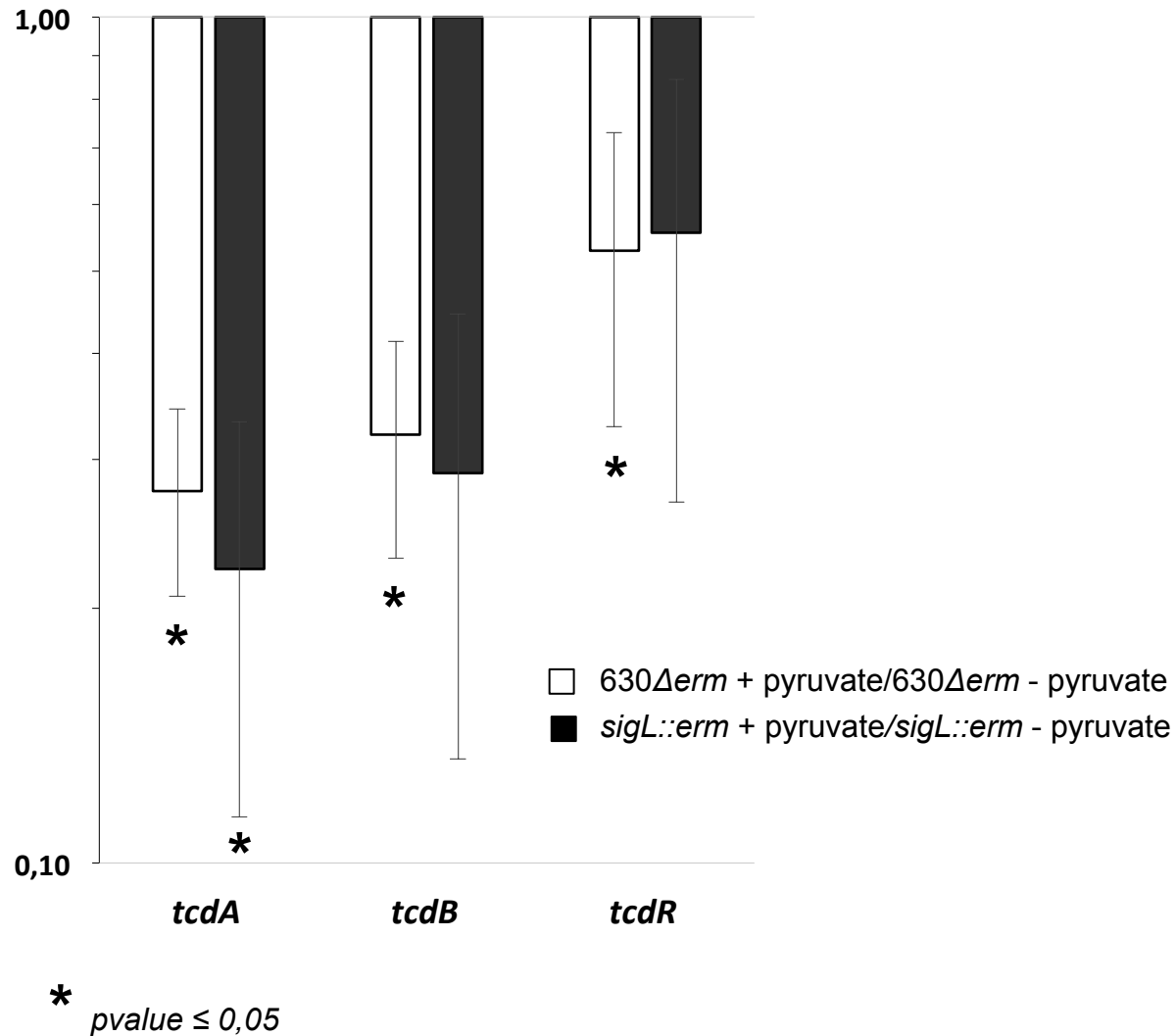
Fig. S4: **Transcript levels of *tcdA*, *tcdB* and *tcdR* genes of the strain 630Δ*erm* (white boxes) and a 630Δ*erm::sigL* (black boxes) after 1 h of exposure to pyruvate..**

Both strains were grown in PY for 9 h, and 50 mM pyruvate was then added to the medium. Cells were centrifuged 1 h later. qRT-PCR results are presented as the ratio between the amount of mRNA (arbitrary units) of each gene normalized by the DNA *polIII* gene from bacterial cells grown in the presence of pyruvate compared to the amount of mRNA in the untreated cells. Data are the averages of at least three independent experiments (error bars are the standard deviations from the mean values). The statistical analysis was performed by using a t-test for all genes, with an exception for *tcdR* and *tcdB* +pyruvate (Mann-Whitney test). .