Complementation of *crp*-deficient G1278 *E. coli* cells (assay protocol and results)

Competent *crp*-deficient G1278 *E. coli* cells were transformed with TP0262- or *E. coli crp*- encoding constructs as described in the manuscript. Transformants were plated onto McConkey agar plates (Becton-Dickinson, Franklin Lakes, NJ) supplemented with 1% lactose and 1 mg/L crystal violet and grown overnight. In MacConkey plates, wild-type *E. coli* grows as deep red colonies with a halo of precipitated red bile salts, resulting from the pH reduction induced by lactose fermentation; non lactose-fermenting bacilli (as the *crp*-deficient G1278 strain) grow as colorless colonies.

TP0262 was unable to restore the lactose fermenting phenotype in this *E. coli* mutant, in that G1278 *E. coli* cells expressing the TP0262 gene grew as colorless colonies on MacConkey-lactose agar plates (Fig.S1), indicating absence of transcription of the *lac* operon even in presence of lactose as principal carbon source. On the contrary, wild type K12 *E. coli* cells, as well as G1278 cells transformed with a plasmid encoding for the *E. coli crp* gene (p*Eccrp*), grew as deep red colonies surrounded by a red halo of precipitated bile salts (Fig.S1), indicative of local pH decrease induced by lactose fermentation.

FIG. S1: Lack of complementation of the *crp*-deficient G1278 *E. coli* strain by TP0262. Wild type phenotype was not restored by expression of TP0262, as G1278 cells transformed with the pGlow-TOPO vector encoding for TP0262 (pTP0262) were unable to ferment lactose in MacConkey-agar plates. Wild type K12 *E. coli* as well as G1278 cells transformed with a pGlow-TOPO vector encoding for the *E. coli* CRP protein (p*Eccrp*) grew, as expected, as deep red colonies surrounded by a red precipitate. For

graphic purposes, strains were plated in an antibiotic-free MacConkey agar plate. Identical results were obtained with plates supplemented with the proper antibiotics (chloramphenicol for the G1278 strain; chloramphenicol and ampicillin for the G1278 strain + p*Eccrp* or pTP0262 vectors).

Figure S1