Figure S9.

A

```
                                   DNA-binding domain (M1-Y51)
                                          ΔHTH (I10-Y46)
C. difficile RstA                  MEILSLGEKIKKLRKEKNMTLKELAGDRITAAQISHIERDKSHTSYELLEYLGDKLDVSV
C. sordellii ATCC9714_31891        MEILSLGEKIKKLRKEKNLTLKELAGERITAAQISHIERDKSHTSHELLEYLASCLEVSV
C. perfringens CPE1448             MEILSLGEKIKRRRKELNMTLKELAGERITPGQISLVESGRSNPSMDLLEYIAITLNTSV
C. acetobutylicum CA_c0957         MEILSLGEKIKARRKELNMTLKDLAGDRITPGQISLVESGKSNPSMDLLEYLAGALNTSV
                                   ***********  *** *:***:***:*** .*** :* .:*: * :****:.  *:.**

C. difficile RstA                  EYLLETKEMQSKKITDNLILQSEIYIKENELNKAEDQIKEVLDICKRYDLMENYGRCNFL
C. sordellii ATCC9714_31891        DYLLETKEMQAKKLTDNLIIQSEIYIKCKELEKAKEEINKVMSICTEYQLIDNYGKCNLL
C. perfringens CPE1448             EYLMETEKIQAEKICLYYEQIAESAILNDDIKKGEKFIEYAIYYADKYDLIYRKAKNLAL
C. acetobutylicum CA_c0957         EYLMESEETQAEKICVYYENTAEAYMLNDELMEAEQYLEKAMYYAENYKISYRKARTLYL
                                   :**:*::: *::*:        :*  :  .:: :.:. :: .:  . .*.:  . .:   *

C. difficile RstA                  MANINLKKSNYNDAIENFEKALYFFIKNSDSENILKCYLNIGKIYMQEDFYKGAISHFNF
C. sordellii ATCC9714_31891        LATINLRQKDYNAAIINFEKSLYFFIQNDDKENIARCYINIGKIYMEENFHKGALSNFKF
C. perfringens CPE1448             KAEMYLKSEEYVLAQQYFLSANMLFLQINSNIDMVKTYLNIAKISLHSKAYYSANSYLKE
C. acetobutylicum CA_c0957         YGCINMNKNELGVAQQFFLSSNVLFIKLNHYEDIINTFINLGKITLNLKAYNSSCSYFEQ
                                    . : :...:   *   * .:  :*:: .   :: . ::*:.** :. . : .: * ::

C. difficile RstA                  AEEILSENKFEDVNIHKELYSKMAYCYIKLDNPNMSLKYIEKINE-IDNRNDRKEDVDIL
C. sordellii ATCC9714_31891        AEEIIEENKLNDVNIKKDLYSNISFCYTQLNKDDDALEYIEKIKN-LDITINCKEEAGLM
C. perfringens CPE1448             AEKIYINNKLTEDLLLGEIYYLISKTYFFIENIQKAINFAFLAKSEFEKVYNKKAYAKSL
C. acetobutylicum CA_c0957         AERVSRDNDIGNDFLIGEIYYYMSLVYFKMNNSNKTIKYCLMAKEKFAELENKKEYARTL
                                   **.:  :*.: :  :  ::*  ::  *  ::: : ::::    :. :    : *  .  :

C. difficile RstA                  VLKANNMLEIGKYEESRDYFKKALKILESEDNKTGLANVYLTICDVYRKIGETDRVLEYS
C. sordellii ATCC9714_31891        VSKANKLMSVGKYEEAKQQFKTALDLLEKEDNKTGLANVYIRICEVYESIGESEKSLEYS
C. perfringens CPE1448             ELLAEEHAKVGDLESAIAYSKQSLNVYKELEQLDKISSIEDSLGKLFFEFDNIEESFEHY
C. acetobutylicum CA_c0957         LILSKEYSKSGDVDNAIKYSKKALKAFKENDNMFAVADIENELGKLFYEFENIEESFIHL
                                      :::  . *. :.:    * :*.  :. ::   ::.:   : .:: .: : :. : :

C. difficile RstA                  QKVYDIKKNDEDEYMMSGLFKIIEAYIDKEDYDLARKYCK-IALASSIKNKNKFNEYKAL
C. sordellii ATCC9714_31891        KKAYEIKRYDEDECSIDVIIKIIQSYIKLNNFEEARKYSK-TALASAIKNKNKYFEYKAL
C. perfringens CPE1448             NRAIKIREENTDSKLLDTLFNLCENHIKTKNMNKCIEILNEIKNKLNVSDVNRLIEYKIL
C. acetobutylicum CA_c0957         NKAKEIRKKTKGHEFIETLCNICENYIKLKDTENSQKVLNEIKENIDGNNVFDILKYYML
                                   ::. .*:.   .   :. : :: : :*. :: : . :  :        .:     :*  *

C. difficile RstA                  RFYANMYKNQNEVTLAIEYLIKCIKIVSDLGNNRILANLYIDLGQLYSNISKEKE-LEYY
C. sordellii ATCC9714_31891        KYYSEIYKKEDNNKLAIEYLLKCINIIKELNDKKTLADLYIELGKLYSNISKEKE-LEYY
C. perfringens CPE1448             TFRIEVLQ--NNIKEAENILLDTLEIAKRNDMKKKEADISIRLGKFYLELKRDYEAAKYL
C. acetobutylicum CA_c0957         KYRISILN--RSLKEAENILLRALQYAENMELEKEAAEFSVIIGKYYIDNGDDVEAAKYL
                                    :  .: :   . . * : *:  ::  .    ::  *:: : :*: * :   : *  :*

C. difficile RstA                  QKGVFMYKNLEIM---
C. sordellii ATCC9714_31891        QKGVCLYQNLEII---
C. perfringens CPE1448             DKGINNLKNLGVIKS-
C. acetobutylicum CA_c0957         NSGIEKFKKIGVLKDL
                                   :.*:   ::: ::
```

B

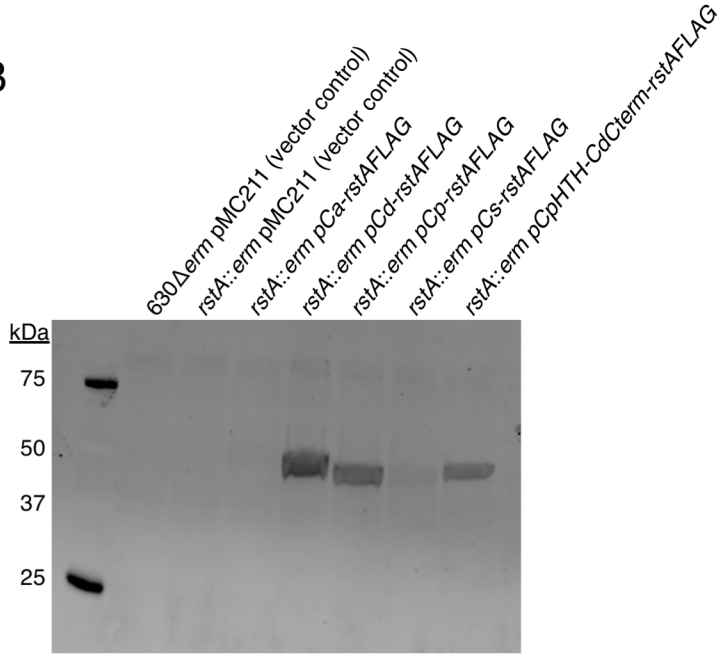

Figure S9. Alignment and stability of RstA orthologs encoded in *C. difficile* 630, *C. sordellii* ATCC 9714, *C. perfringens* S13, *C. acetobutylicum* ATCC 824. (**A**) Multiple sequence alignment performed by EMBL-EMI Clustal Omega tool. The regions denoting the DNA-binding domain (from M1 to Y51), which were used in the RstA *C. perfringens* DNA-binding domain-*C. difficile* C-terminal domains hybrid construct, and the residues deleted in conserved helix-turn-helix motif for the *rstA*ΔHTH-3XFLAG construct are marked. The key is as follows: (*) identical; (:) strongly conserved; and (.) weakly conserved residues. The colors group the amino acid residues based on their physiochemical properties: (red) small, hydrophobic residues; (blue) acidic residues; (magenta) basic residues; and (green) hydroxyl-sulfhydryl- or amine-containing residue. (**B**) Western blot analysis using FLAG M2 antibody to detect recombinant RstA-3XFLAG proteins (~51 kDa) expressed in 630Δ*erm* pMC211 (MC282; vector control), *rstA*::*erm* pMC211 (MC505; vector control), *rstA*::*erm* pMC828 (MC1323; *Clostridium acetobutylicum rstA* [*Ca_C0957*]), *rstA*::*erm* pMC675 (MC1004; *Clostridium difficile rstA*), *rstA*::*erm* pMC829 (MC1324; *Clostridium perfringens rstA [CPE1448*]), *rstA*::*erm* pMC830 (MC1325; *Clostridium sordelii rstA* [*ATCC9714_3891*]) and *rstA*::*erm* pMC798 (MC1257; pP*cprA-rstACpHTHCdCterminal*-3XFLAG) grown in TY medium supplemented with 2 μg/ml thiamphenicol and 1 μg/ml nisin at H_{24}.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7:539.