SUPPLEMENTAL INFORMATION

Functional Defects in *Clostridium difficile* TcdB Toxin Uptake Identify CSPG4 Receptor Binding Determinants

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Supplementary figure 1. Domain structures of TcdA and TcdB showing short repeats (green) and long repeats (yellow) in the CROP domains.

Supplementary figure 2. Defective TcdB mutants are folded properly. a & b, Autoprocessing activity of recombinant toxins. Recombinant TcdB variants were treated with 100 μM InsP6 (+) or PBS (−) for 3 h and cleavage was visualized by coomassie staining. Bands were quantified by ImageJ software. Vertical lines denote splicing of the gel to exclude lanes between samples. c, GTD activity of recombinant toxins. GST-Rac1 was treated with recombinant toxins, and the level of glucosylation was determined by Western blot analysis using Mab102 that recognizes unglucosyated Rac1 (top) and an anti-Rac1 antibody to determine total Rac1 (bottom) .
d, pH-induced unfolding by TNS fluorescence. Defective mutants were all folded at neutral pH and began unfolding at pH<5. Both mutants (Y1824K and N1839K) show a similar unfolding profile to WT TcdB.

Supplementary Figure 3. Alignment of short and long repeats within the CROP domain of TcdB.

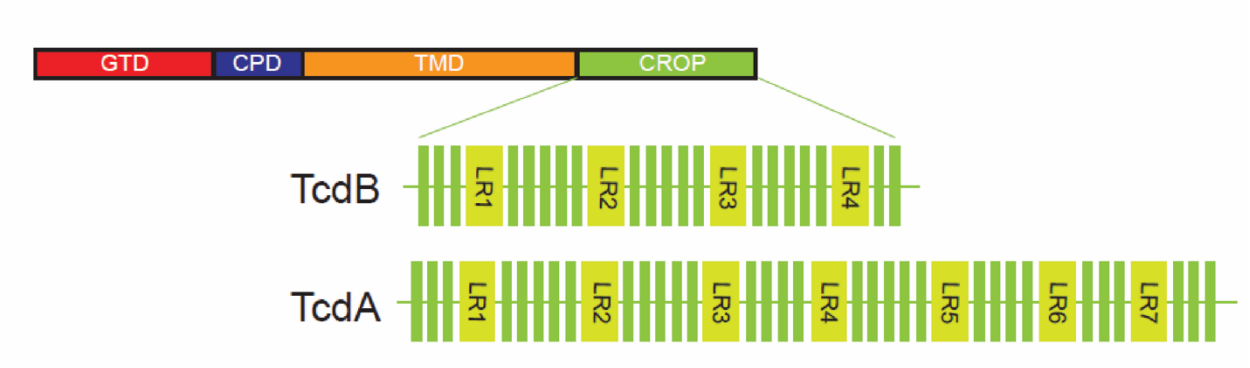
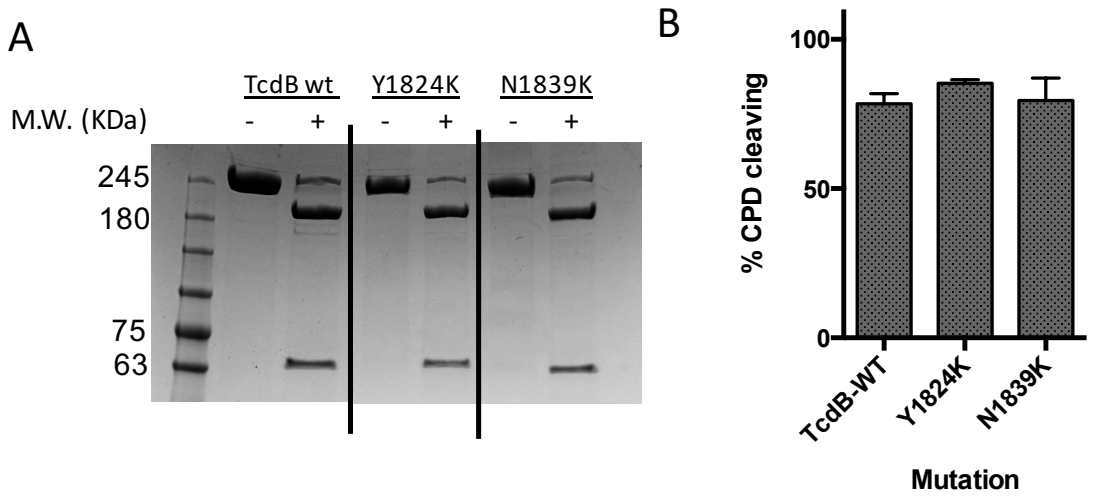
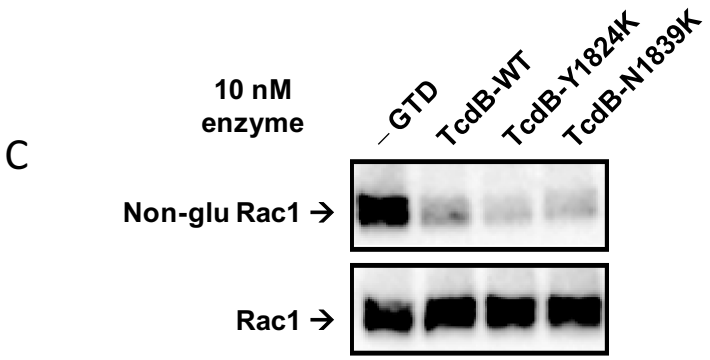

GTD
CPD
TMD
CROP
TcdB
LR1
LR2
LR3
LR4
TcdA
LR1
LR2
LR3
LR4
LR5
LR6
LR7

Fig. 1


A
TcdB wt
Y1824K
N1839K
M.W. (KDa)
- + - + - +
245
180
75
63
B
100
50
0
% CPD cleaving
TcdB-WT
Y1824K
N1839K
Mutation


C
10 nM enzyme
GTD
TcdB-WT
TcdB-Y1824K
TcdB-N1839K
Non-glu Rac1 →
Rac1 →

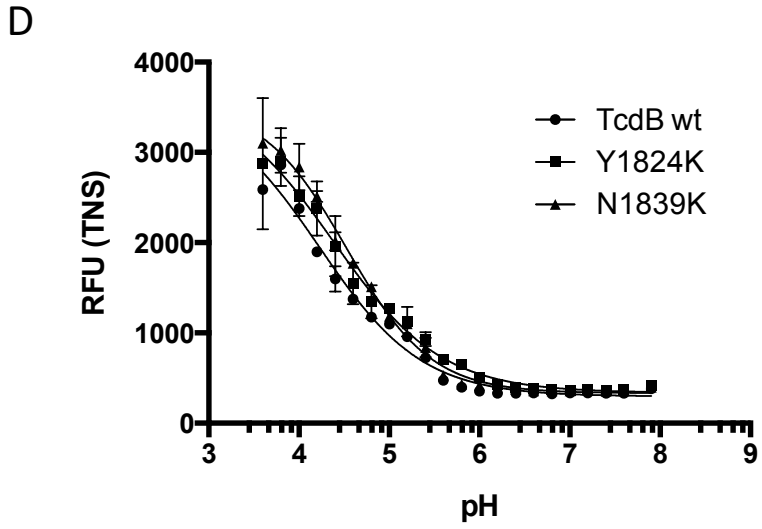

D
4000
3000
2000
1000
0
RFU (TNS)
3
4
5
6
7
8
9
pH
TcdB wt
Y1824K
N1839K

Fig. 2

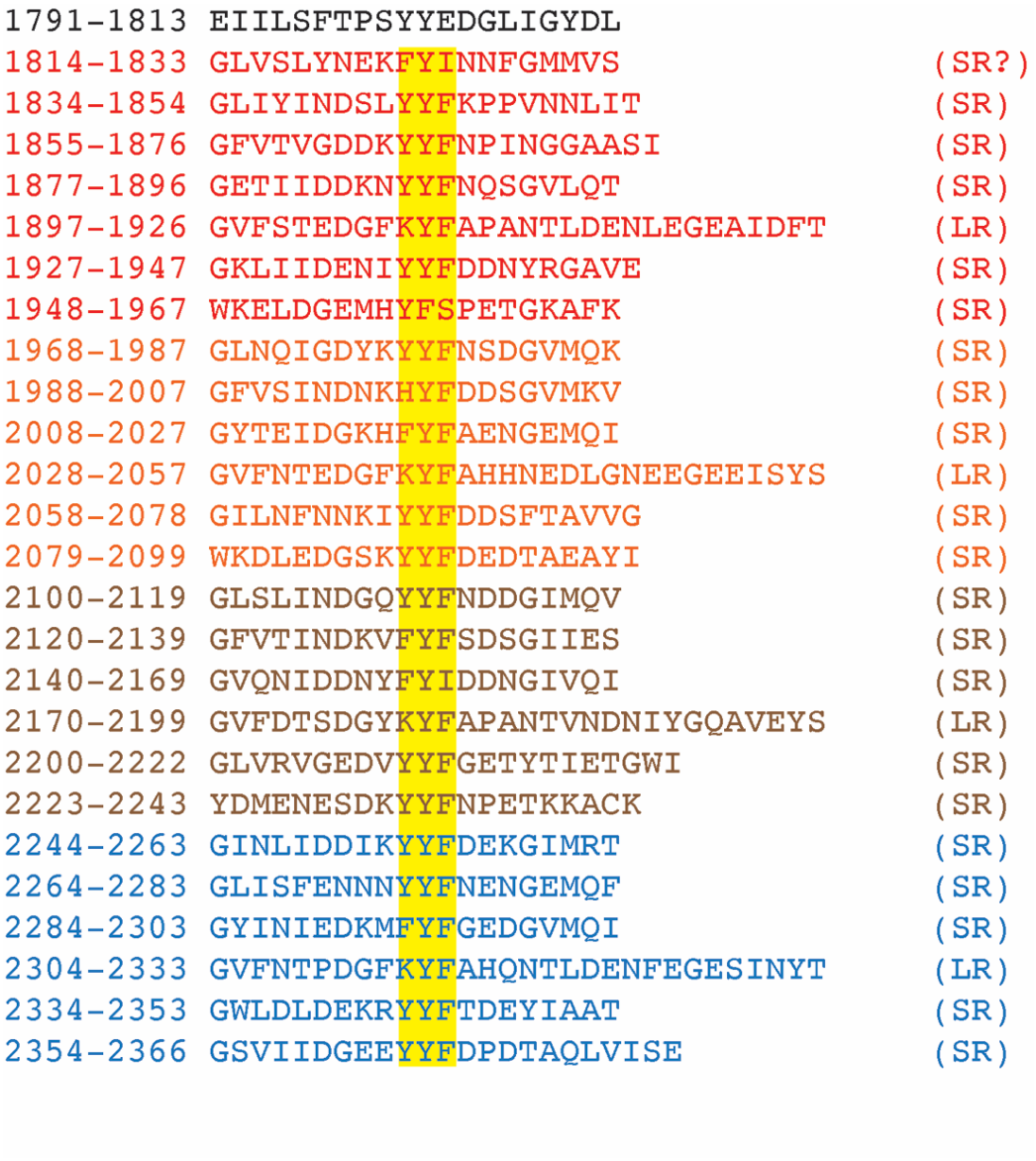

1791-1813 EIILSFTPSYYEDGLIGYDL
1814-1833 GLVSLYNEKFYINNFGMMVS (SR?)
1834-1854 GLIYINDSLYYFKPPVNNLIT (SR)
1855-1876 GFVTVGDDKYYFNPINGGAASI (SR)
1877-1896 GETIIDDKNYYFNQSGVLQT (SR)
1897-1926 GVFSTEDGFKYFAPANTLDENLEGEAIDFT (LR)
1927-1947 GKLIIDENIYYFDDNYRGAVE (SR)
1948-1967 WKELDGEMHYFSPETGKAFK (SR)
1968-1987 GLNQIGDYKYYFNSDGVMQK (SR)
1988-2007 GFVSINDNKHYFDDSGVMKV (SR)
2008-2027 GYTEIDGKHFYFAENGEMQI (SR)
2028-2057 GVFNTEDGFKYFAHHNEDLGNEEGEEISYS (LR)
2058-2078 GILNFNNKIYYFDDSFTAVVG (SR)
2079-2099 WKDLEDGSKYYFDEDTAEAYI (SR)
2100-2119 GLSLINDGQYYFNDDGIMQV (SR)
2120-2139 GFVTINDKVFYFSDSGIIES (SR)
2140-2169 GVQNIDDNYFYIDDNGIVQI (SR)
2170-2199 GVFDTSDGYKYFAPANTVNDNIYGQAVEYS (LR)
2200-2222 GLVRVGEDVYYFGETYTIETGWI (SR)
2223-2243 YDMENESDKYYFNPETKKACK (SR)
2244-2263 GINLIDDIKYYFDEKGIMRT (SR)
2264-2283 GLISFENNNYYFNENGEMQF (SR)
2284-2303 GYINIEDKMFYFGEDGVMQI (SR)
2304-2333 GVFNTPDGFKYFAHQNTLDENFEGESINYT (LR)
2334-2353 GWLDLDEKRYYFTDEYIAAT (SR)
2354-2366 GSVIIDGEEYYFDPDTAQLVISE (SR)

Fig. 3