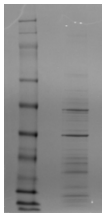
A

C. difficile culture

Culture medium (filtered and concentrated)



In-gel digestion and LC-ion trap MS/MS analysis (115 *C. diff* proteins identified, Table S1 and S2)

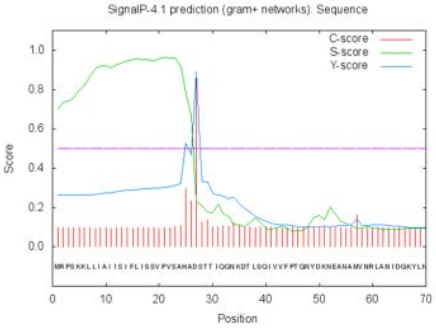
39 signal peptide containing proteins (Table 1)

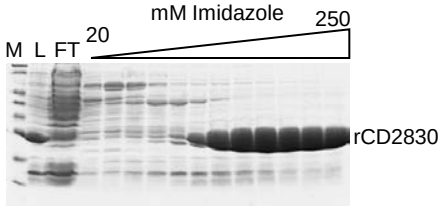
Putative genuinely secreted

CD2830
CD2408
CD3366
CD3145

10 unique peptides
47% sequence coverage
Uncharacterized protein

B





> CD2830

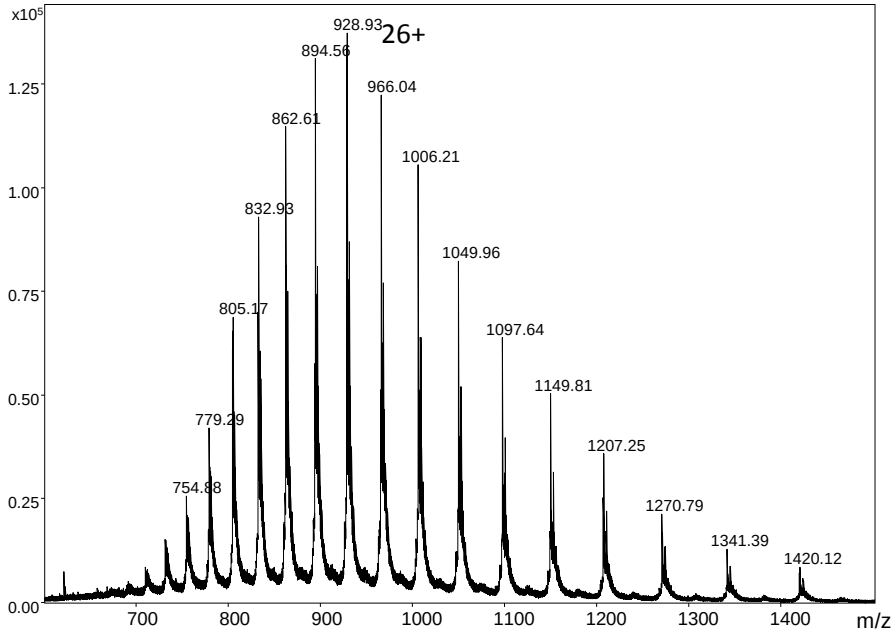
Signal sequence

CD2830: MRPSKKLLIAIISIFLISSVPVSAHADSTTIQQNKDTLSQIVVFPTGNYDKNEANAMVNRLANIDGKYLN

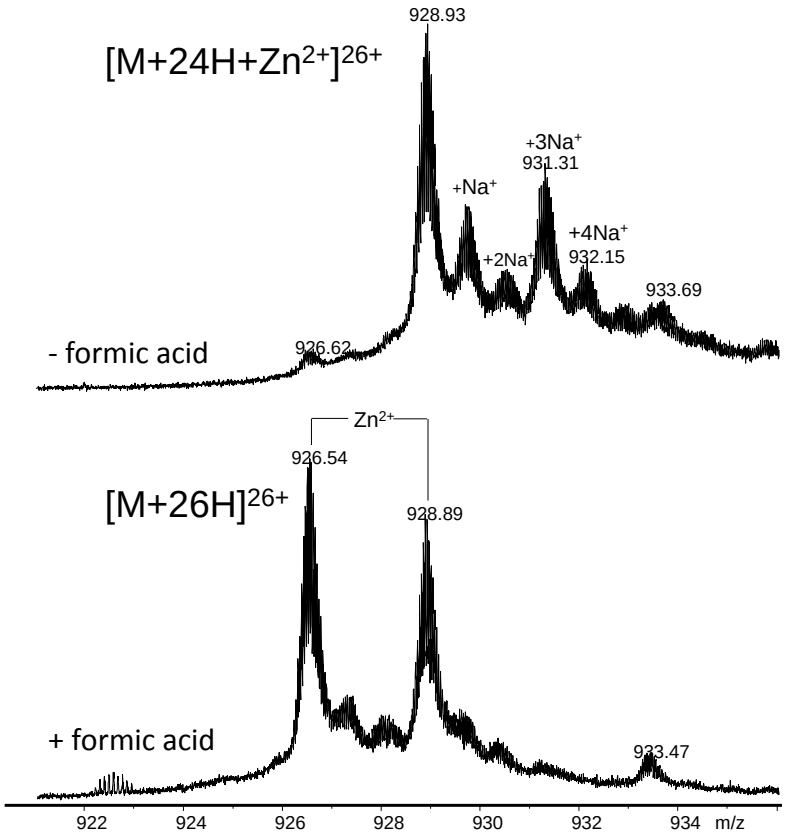
Expression construct: MGHHHHHHHHHHSSGHIEGRHMDSTTIQQNKDTLSQIVVFPTGNYDKNEANAMVNRLANIDGKYLN

Purification tag

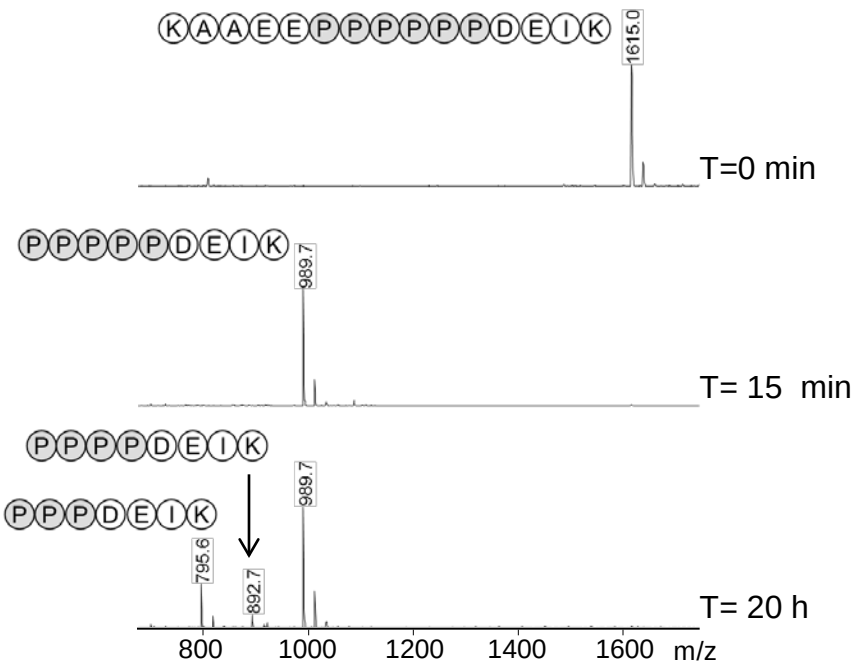
C



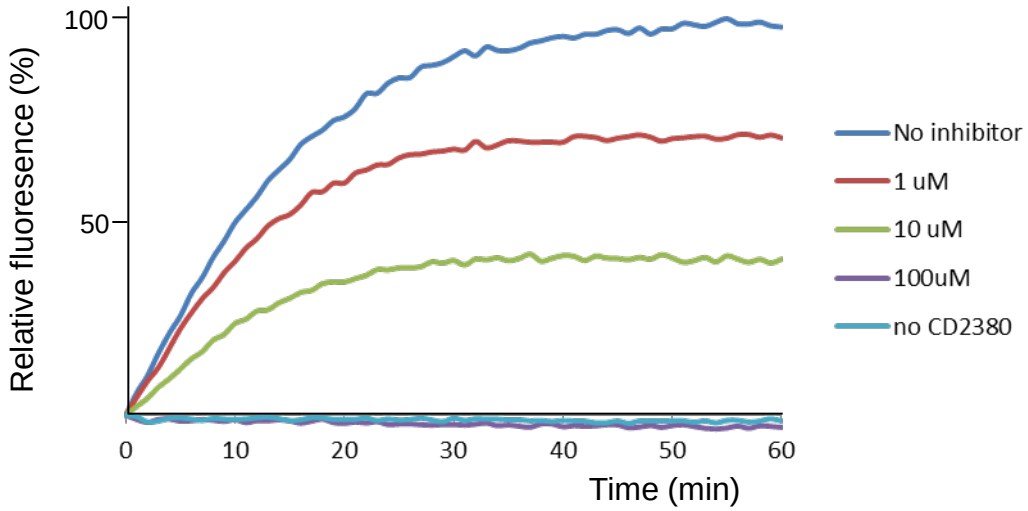
D



Supplemental Fig. 1

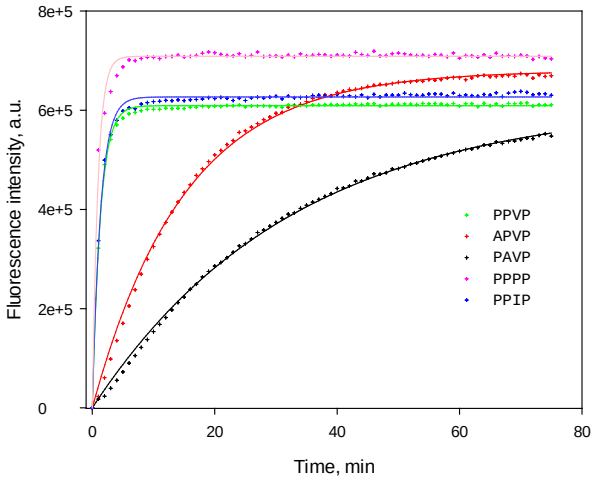


Supplemental Fig. 2



Supplemental Fig. 3

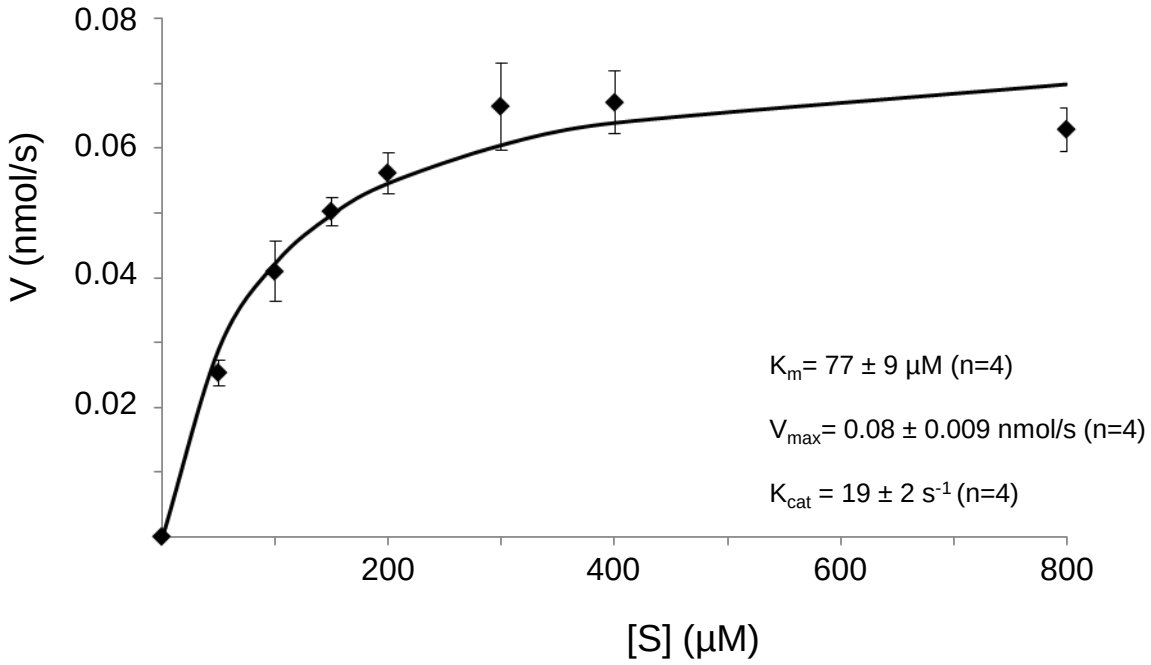
A



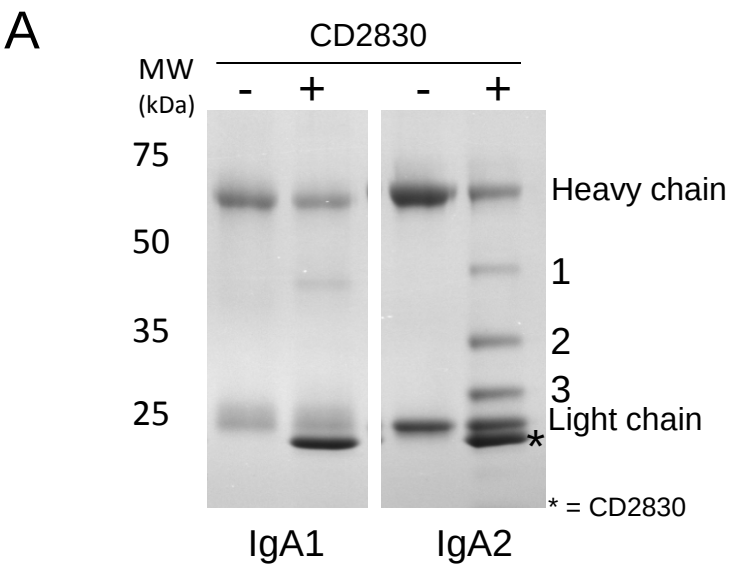
$$[P] = [P_\infty]\,(1 - e^{-kt})$$

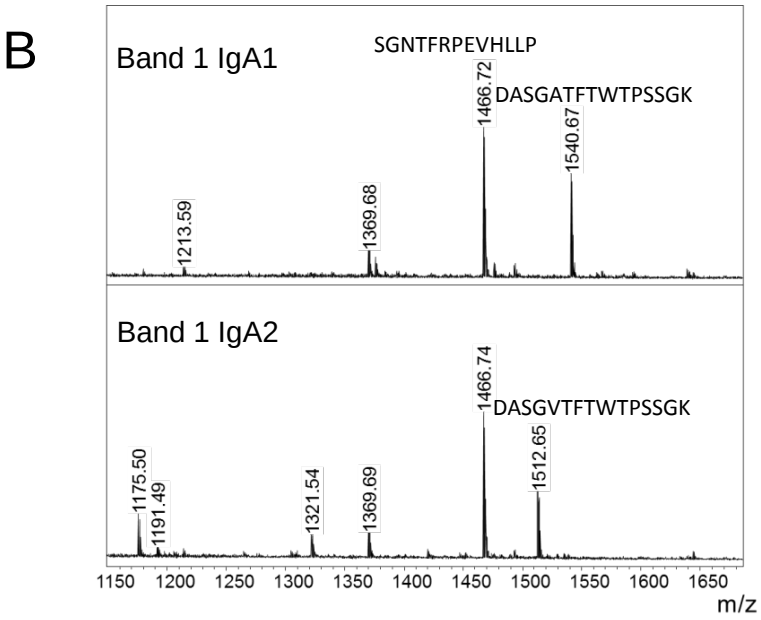
Peptide sequence	$[P_\infty]$	k
EVNPAVPD	6.2E+05 ± 3E+03	3.1E-02 ± 3E-04
EVNAPVPD	6.8E+05 ± 2E+03	6.7E-02 ± 6E-04
EVNPPVPD	6.1E+05 ± 5E+02	7.6E-01 ± 1E-02
EVNPPIPD	6.3E+05 ± 8E+02	7.5E-01 ± 1E-02
EVNPPPPD	7.1E+05 ± 1E+03	1.1E+00 ± 4E-02

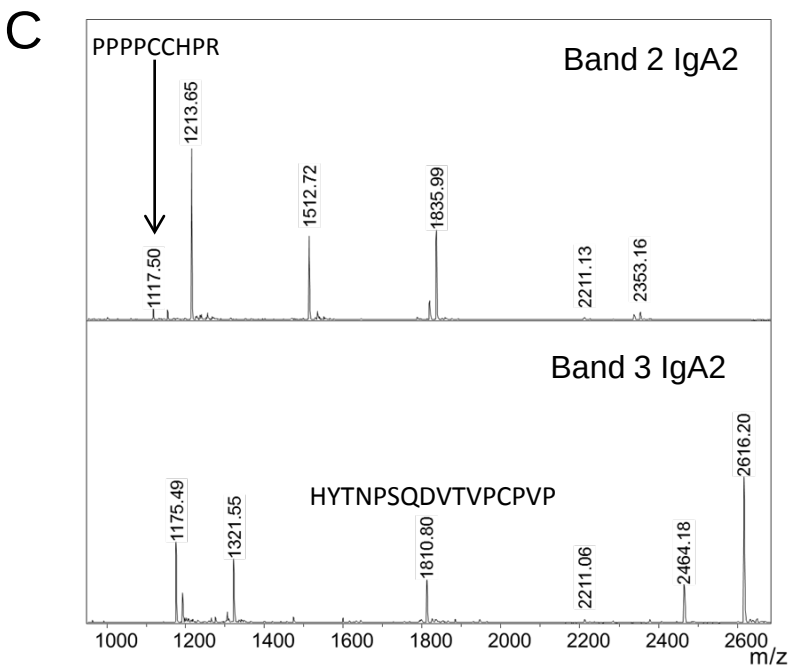
B

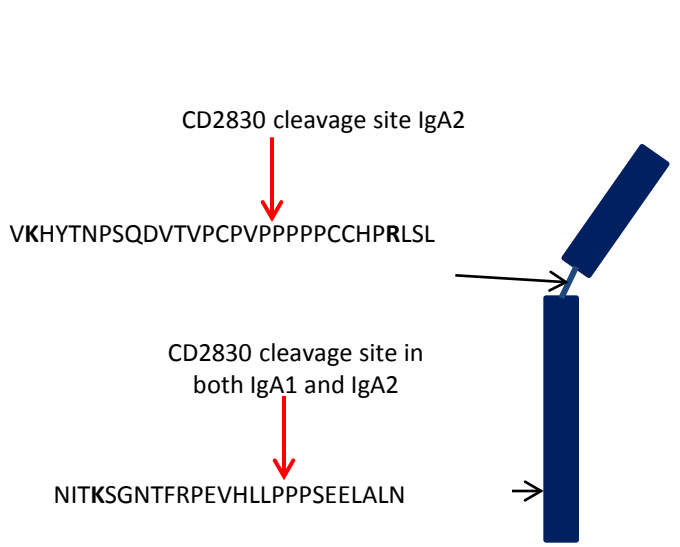


Supplemental Fig. 4









Supplemental Fig. 5

Figure S1. Identification, recombinant production and analysis of CD2830

A) *Clostridium difficile* cells were grown in minimal medium till the beginning of the stationary phase. Conditioned medium was collected, filtered, concentrated and analyzed with SDS-PAGE. Following in-gel digestion with trypsin, proteins were identified by LC iontrap MS/MS and database searching (Table S1 and S2). After selecting only signal peptide containing proteins without a cell surface binding motif (Table 1), the hypothetical protein CD2830 was identified as the major genuinely secreted protein. MW: Molecular weight protein standards

B) Signal peptide cleavage prediction of CD2830 (SignalP, upper left). Coomassie blue-stained SDS-PAGE gel (upper right) displaying protein patterns of selected elution fractions taken during the purification of 10xHis-CD2830 (lower panel) on a His-trap HP column (GE healthcare). M= Marker, prestained pageruler (pierce); L=load ; FT= flow-through. Linear gradient of 20- 250mM imidazole shows elution of a ~25 kDa protein corresponding to the estimated molecular mass of 10xHisCD2830. Correct protein production was confirmed using mass spectrometry after an in-gel tryptic digestion.

C) Mass determination of rCD2830 by ESI-Q-ToF MS. Recombinant CD2830 (250 ug) was desalted on a reversed phase C4 cartridge (Vydac Bio-Select) and eluted with 50% acetonitrille. The mass of rCD2830 was determined by ESI-Q-ToF-MS (maXis, Bruker Daltonics) with direct infusion (3 ul/min).

D) Acidification of rCD2830 results in loss of Zn^{2+}. To remove bound metals rCD2830 was incubated with 5% formic acid for 10 min at room temperature prior to desalting and ESI-Q-ToF-MS measurement.

Figure S2. CD2830-mediated cleavage of a peptide with six consecutive prolines

A synthetic peptide containing six consecutive prolines within a core synthetic peptide was incubated with rCD2830 and analyzed by MALDI-ToF MS. After 15 min of incubation this peptide was fully cleaved and the cleavage site was identified between the first two prolines. After 20 hr of incubation, additional products were observed corresponding to cleavages between other proline residues.

Figure S3. CD2830 is inhibited by o-phenanthroline

Cleavage of a synthetic FRET-peptide (50 µM, $Dabcyl_{Lys}$-EVNPPPPD-$Edans_{Glu}$) by rCD2830 (20 ng) was followed by fluorescence detection in the presence of a range of concentrations of the metalloprotease inhibitor o-phenanthroline.

Figure S4. Enzymatic kinetics of CD2830-mediated cleavage

A) Within a core synthetic FRET-peptide ($Dabcyl_{Lys}$-EVNPPVPD-$Edans_{Glu}$), permutations were introduced at the P1, P1' and P2' (PPV) position. All peptides (50 µM) were incubated with rCD2830 and the formation of cleavage products was followed in time using fluorescence detection (see Experimental procedures for details).The experimental data were approximated using a model of the first order kinetics increase of a product . The parameters of the model were calculated using SigmaPlot 12 curve fitting.

B) Steady state kinetics. A range of concentrations of a preferred synthetic FRET-peptide ($Dabcyl_{Lys}$-EVNPPPPD-$Edans_{Glu}$) were incubated with 100 ng of rCD2830. The CD2830-mediated cleavage was determined at 30 seconds time intervals during the first two minutes of the reaction by fluorescence detection at an excitation wavelength of 355 nm and an emission wavelength of 485 nm in a 96-well plate reader. A calibration curve plotting the fluorescence intensity versus cleaved peptide concentration after complete cleavage of the peptide was used to convert the initial velocity from fluorescence units/s to nmols/s. Data was then fitted to the Michaelis-Menten equation (shown from a representative example) from which the K_m,V_{max} and K_{cat} values were derived.

Figure S5. CD2830 cleaves the IgA2 hinge region.

A) SDS-PAGE analysis of human IgA1 and IgA2 before (-) and after (+) incubation with rCD2830.

B) MALDI-ToF MS spectrum of tryptic digests of the CD2830-cleavage product which was observed with both IgA1 and IgA2 (band 1, Fig. A). The semi-tryptic peptide SGNTFRPEVHLLP (see also D) demonstrated CD2830 cleavage between two prolines in the C-terminal region of the IgA heavy chain. The other indicated peptides are fully tryptic peptides unique for either IgA1 or IgA2.

C) MALDI-ToF MS spectrum of tryptic digests of the IgA2 specific CD2830-cleavage products, demonstrating cleavage between two prolines within the hinge region of IgA2 (see also D).

D) Overview of the CD2830 cleavage sites observed in the heavy chains of IgA1 and IgA2.