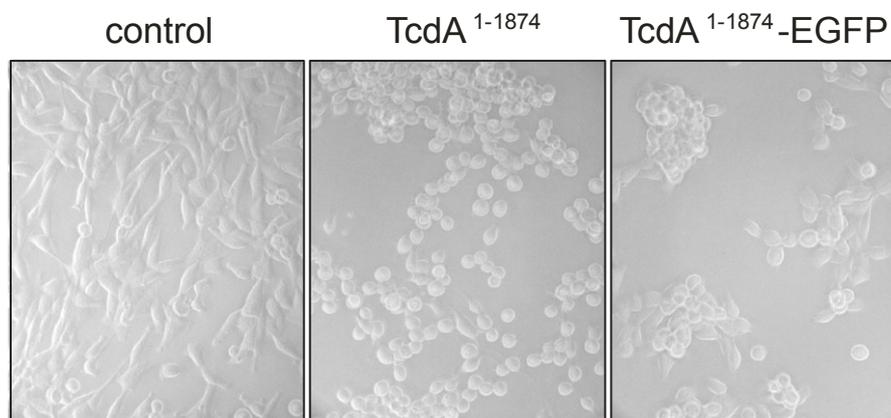


## Supplementary Information

**Figure S1.** HT-29 cells were incubated with TcdA<sup>1-1874</sup> and the fusion protein TcdA1-1874-EGFP for one hour. Both toxins induced comparable cell rounding, indicating that the C-terminal EGFP-Tag does not affect biological function of TcdA<sup>1-1874</sup>.



**Table S1.** Summary of constructs used for this study. Shown are the amino acid sequences of proteins resulting from the cloning strategy.

Construct	Proteins, Tags, Linker	Vector
TcdA <sup>1-2710</sup>	Val-Gln-Thr-Ser-Gly-Ser-[TcdA <sup>1-2710</sup> ]-Gly-Ser-[His] <sub>6</sub>	pWH1520
TcdA <sup>1-542 D285/287N</sup>	Val-Gln-Thr-Ser-[TcdA <sup>1-542</sup> ]-Gly-Ser-[His] <sub>6</sub>	pHis1522
TcdA <sup>1-1065</sup>	Gly-Ser-[TcdA <sup>1-1065</sup> ]-Gly-Ser-[His] <sub>6</sub>	pWH1520
TcdA <sup>1-1874</sup>	Val-Gln-Thr-Ser-[TcdA <sup>1-2710</sup> ]-Thr-Ser-[His] <sub>6</sub>	pWH1520
TcdA <sup>1875-2710</sup> (CROPs)	Thr-Ser-[TcdA <sup>1875-2710</sup> ]-Gly-Ser-[His] <sub>6</sub>	pWH1520
GST-TcdA <sup>1875-2710</sup> (CROPs)	[GST]-[Gly] <sub>5</sub> -Ser-[TcdA <sup>1875-2710</sup> ]-Asp-Pro-Arg-Glu-Phe-Ile-Val-Thr-Asp	pGEX2TGL
GST-TcdB <sup>1848-2366</sup> (CROPs)	[GST]-[Gly] <sub>5</sub> -Ser-[TcdB <sup>1848-2366</sup> ]-Glu-Phe-Ile-Val-Thr-Asp	pGEX2TGL
EGFP-TcdA <sup>1102-1847</sup>	[His] <sub>6</sub> -Gly-Ser-[EGFP]-Ser-[TcdA <sup>1102-1847</sup> ]	pQE30
EGFP-TcdA <sup>1875-2710</sup> (CROPs)	Gly-Ser-[EGFP]-Ser-[TcdA <sup>1102-1847</sup> ]-Gly-Ser-[His] <sub>6</sub>	pWH1520
TcdA <sup>1-1874</sup> -EGFP	Val-Gln-Thr-Ser-[TcdA <sup>1-1874</sup> ]-Thr-Ser-[EGFP]-Gly-Ser-[His] <sub>6</sub>	pHis1522
TcdA <sup>1-542</sup> -EGFP	Gly-Ser-[TcdA <sup>1-542</sup> ]-Thr-Ser-[EGFP]-Gly-Ser-[His] <sub>6</sub>	pHis1522
TcdA <sup>1-1874</sup> -TcdB <sup>1848-2366</sup>	[TcdA <sup>1-1874</sup> ][TcdB <sup>1848-2366</sup> ]-Thr-Ser-[His] <sub>6</sub>	pHis1522
TcdB <sup>1-1852</sup> -TcdA <sup>1875-2710</sup>	[TcdB <sup>1-1852</sup> ][TcdA <sup>1875-2710</sup> ]-Thr-Ser-[His] <sub>6</sub>	pHis1522
TcdB <sup>1848-2366</sup> (CROPs)	[TcdB <sup>1848-2366</sup> ]-Gly-Ser-[His] <sub>6</sub>	pHis1522
TcdB <sup>1-1852</sup>	[TcdB <sup>1-1852</sup> ]-Thr-Ser-[His] <sub>6</sub>	pHis1522
TcdB <sup>1-543</sup>	[TcdB <sup>1-543</sup> ]-Gly-Ser-[His] <sub>6</sub>	pHis1522
TcdB <sup>1-2366</sup>	[TcdB <sup>1-2366</sup> ]-Gly-Ser-[His] <sub>6</sub>	pHis1522

**Figure S2. (A)** Glutathion-S-Transferase (GST)-Pull down assays showing binding of TcdA<sup>1-542</sup> (lane 1, “input” and “TcdA-CROPs”) or TcdA<sup>543-1874</sup> (lane 2, “input” and “TcdA-CROPs”) and the combination of TcdA<sup>1-542</sup> and TcdA<sup>543-1874</sup> (lane 3, “input” and “TcdA-CROPs”) to immobilised TcdA-CROPs. Binding of TcdA<sup>1-542</sup> and TcdA<sup>543-1874</sup> was compared with positive control TcdA<sup>1-1874</sup> (lane 4, “input” and “TcdA-CROPs”). Unspecific binding to GST-beads was checked by pull down of TcdA<sup>1-542</sup> and TcdA<sup>543-1874</sup> in combination (lane 3, “GST”) and of TcdA<sup>1-1874</sup> (lane 4, “GST”). Combination of TcdA<sup>1-542</sup> and TcdA<sup>543-1874</sup> did not increase binding of either domain to TcdA-CROPs; **(B)** GST-Pull down assays showing binding of TcdA<sup>1-1101</sup> (lane 1, “input” and “CROPs”) or TcdA<sup>1102-1847</sup> (lane 2, “input” and “CROPs”) and the combination of TcdA<sup>1-1101</sup> and TcdA<sup>1102-1874</sup> (lane 3, “input” and “CROPs”) to immobilised TcdA-CROPs. Binding of TcdA<sup>1-1101</sup> and TcdA<sup>1102-1874</sup> was compared with positive control TcdA<sup>1-1874</sup> (lane 4, “input” and “CROPs”). Unspecific binding to GST-beads was checked by pull down of TcdA<sup>1-1101</sup> and TcdA<sup>1102-1874</sup> in combination (lane 3, “GST”) and of TcdA<sup>1-1874</sup> (lane 4, “GST”). TcdA<sup>1-1101</sup> did not bind to TcdA-CROPs (lane 1, “CROPs”) and did not increase binding of TcdA<sup>1102-1874</sup> (lane 2, “CROPs”) when added in combination of both (lane 3, “CROPs”).

