Supplementary Materials: Receptor Binding Domains of TcdB from *Clostridioides difficile* for Chondroitin Sulfate Proteoglycan-4 and Frizzled Proteins are Functionally Independent and Additive

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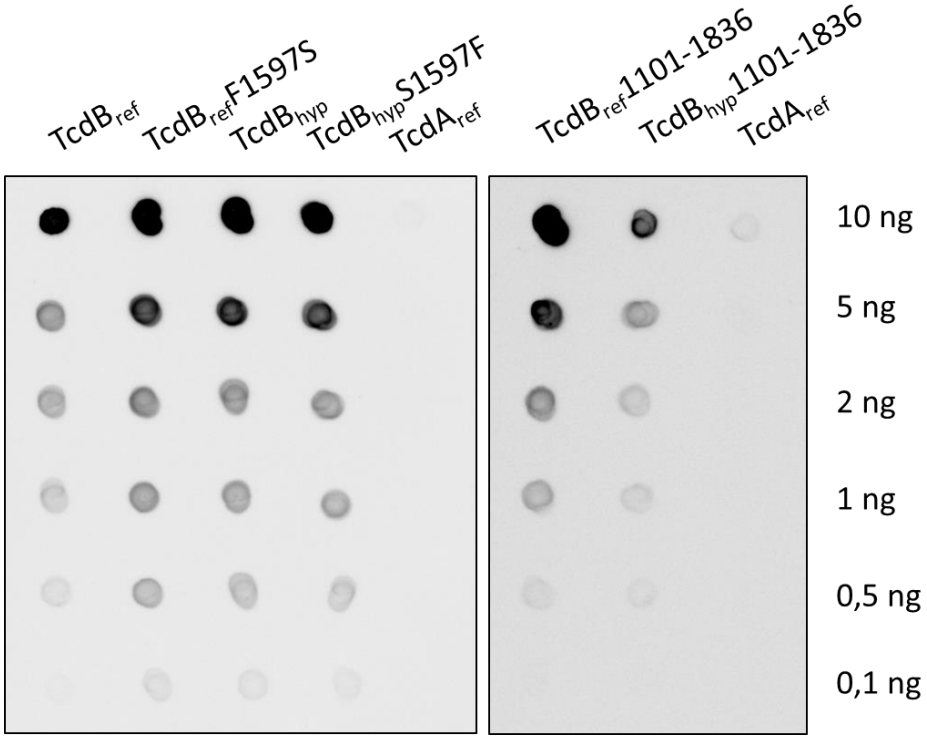

Supplementary figure 1

Figure S1. Recognition of toxins and fragments of different toxinotypes by affinity purified polyclonal antibody raised against full-length TcdB from historical strain VPI10463. **Method**: Indicated amount of toxins or toxin fragments were spotted onto nitrocellulose, air dried, and blocked with 5% skimmed milk powder in TBS containing 0.2% Tween-20 (TBS-T). Incubation with purified polyclonal anti-TcdB IgG (100 ng/mL in TBS-T supplemented with 1% skimmed milk powder) was performed for one hour, followed by three times washing with TBS-T. The nitrocellulose was incubated with secondary antibody (monoclonal anti-rabbit IgG, HRP conjugated) for one hour. After three times washing with TBS-T the nitrocellulose was incubated with Supersignal West Femto enhanced chemiluminescence substrate (Thermo Scientific) and signals were detected by the LAS-3000 Imaging System from Fuji. **Results:** Full-length TcdB$_{R20}$ and TcdB$_{R20}$ S1597F were recognized by anti-TcdB with a comparable specificity and sensitivity as TcdB$_{VPI}$ and TcdB$_{VPI}$ F1597S. In contrast to full-length TcdB the isolated intermediate domain aa 1101–1836 of TcdB$_{R20}$, comprising the FZD-binding domain and part of the CSPG4 binding domain, was recognized three-fold less sensitive than TcdB$_{VPI}$ 1101–1836. A weaker recognition of CROP-depleted TcdB$_{R20}$ compared with CROP-depleted TcdB$_{VPI}$ by monoclonal phage-display derived antibodies was previously observed (Chung et al., (2018) Frontiers in Microbiology, 9:2314, doi: 10.3389/fmicb.2018.02314).