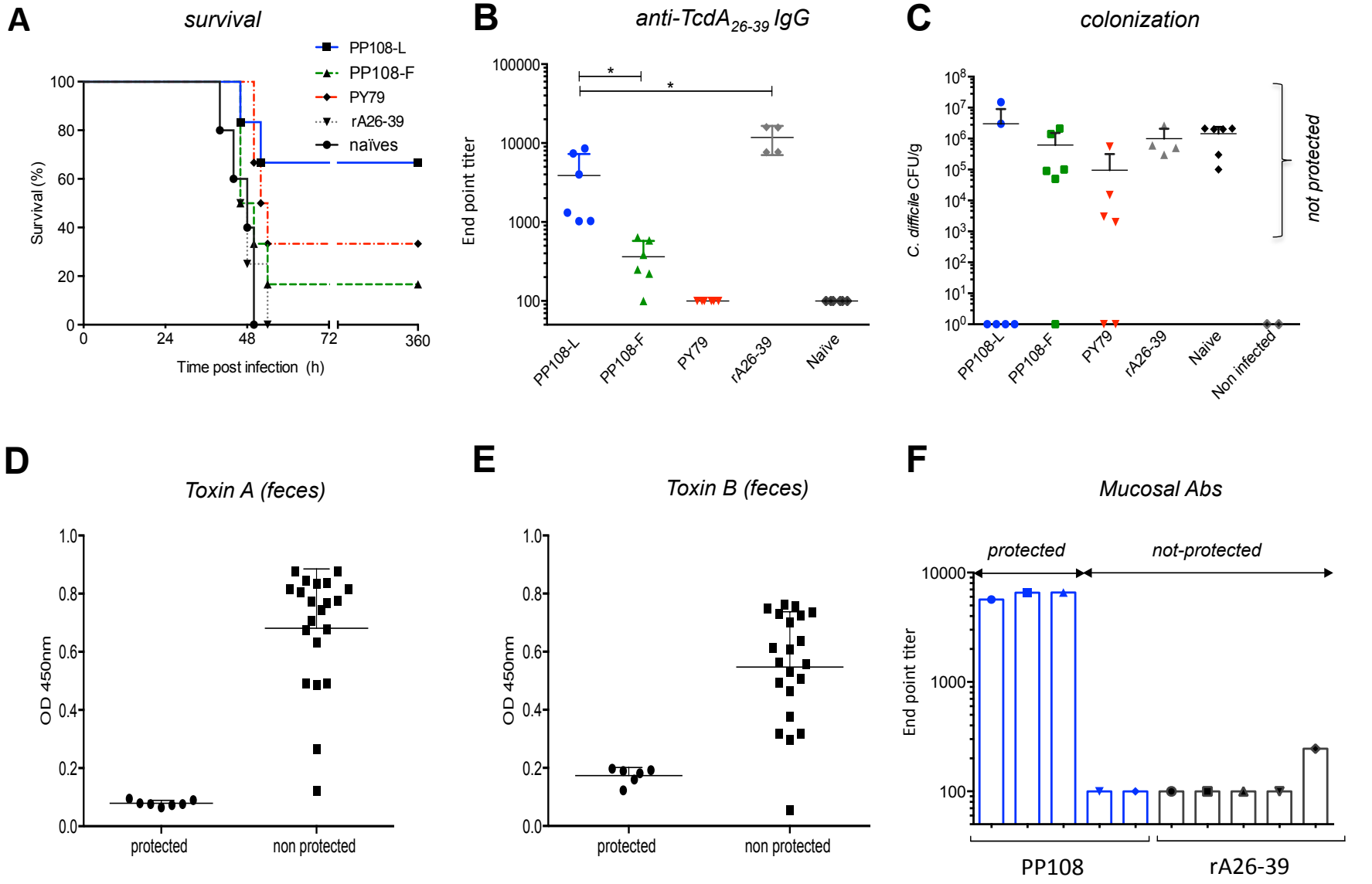

Figure 1.1: Mucosal Vaccination with CDVAX/PP108 Spores. Hamsters (6/group) were immunized using the s.o. route with live (PP108-L) or formalin-inactivated (PP108-F) PP108 spores, live PY79 spores or rTcdA$_{26-39}$ protein by injection (i.m.). *Panel A* shows Kaplan-Meier survival estimates after oral challenge of these animals with 100 spores of *C. difficile* 630. *Panel B* shows anti-TcdA$_{26-39}$ IgG titers in individual animals from each group 2 days before challenge, *, p = <0.05. *Panel C* shows counts of 630 spores in feces 24h post-challenge in individual animals. *Panel D and E* show levels of toxin A (*panel D*) and toxin B (*panel E*) detected in hamster feces protected vs non-protected animals challenged with *C. difficile* strain 630 corresponding to *panel A* and with individual data values shown in **Fig. 1.5**. Capture ELISA was used to determine toxins and the presence of functional toxins also confirmed using a cell cytotoxicity assay to measure toxin A (HT29 cells) and toxin B (Vero cells) (data not shown). *Panel F* shows analysis of fecal IgA obtained from a separate hamster experiment where IgA could be detected using an anti-mouse secondary antibody. In this experiment groups (n =5) were orally dosed with PP108 spores or by injection (i.m.) with rTcdA$_{26-39}$ with 3/5 hamsters protected in the PP108 dosed group.

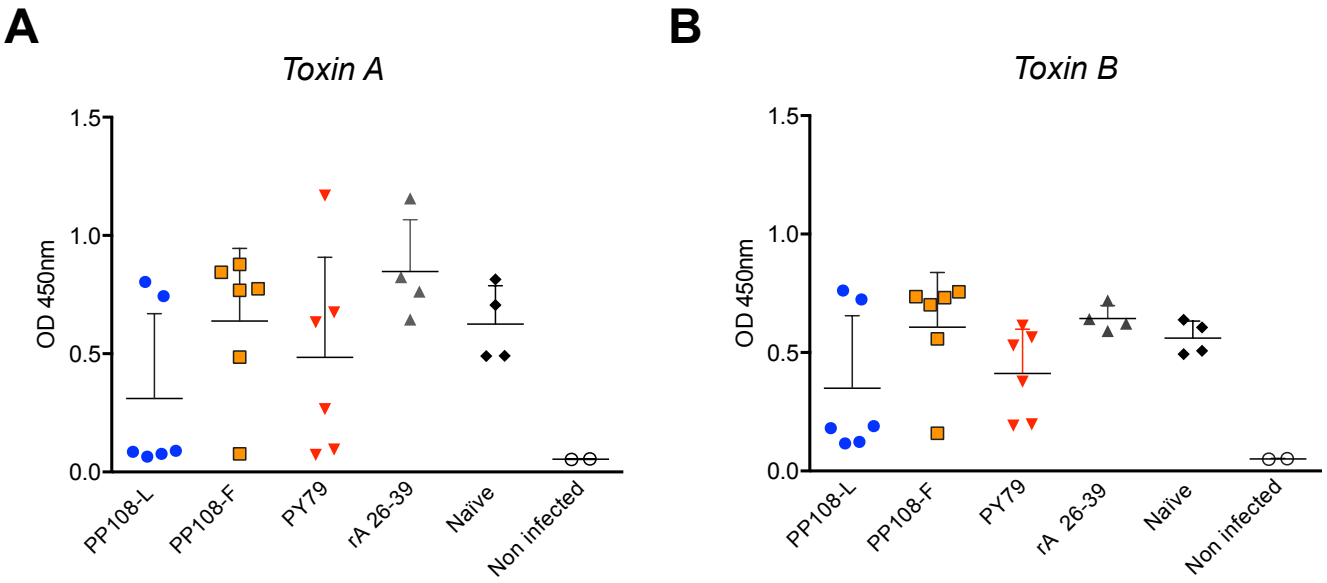

Figure 1.2: Levels of toxin A and toxin B in challenged animals.
Levels of toxin A and toxin B detected in hamster feces in animal groups (immunized or naïve) challenged with *C. difficile* strain 630 corresponding to Fig. 1A. Capture ELISA was used to determine toxins and the presence of functional toxins also confirmed using a cell cytotoxicity assay to measure toxin A (HT29 cells) and toxin B (Vero cells) (data not shown).

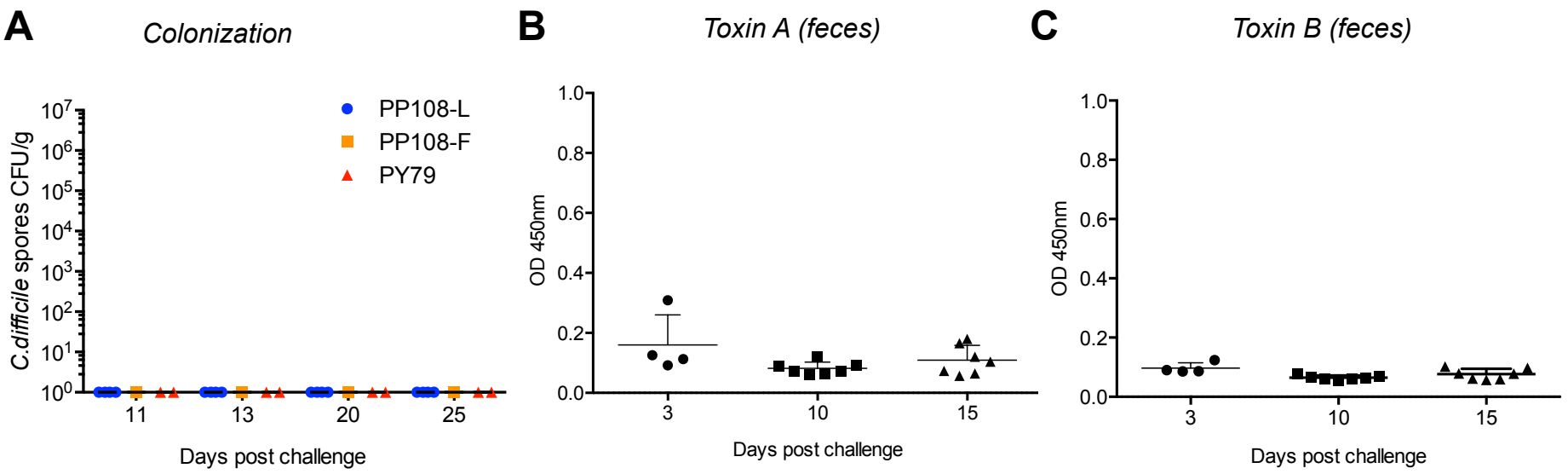

Figure 1.3: Relapse study.
Animals surviving challenge with *C. difficile* 630 were dosed (i.g.) with clindamycin (~7 days after the completion of the primary challenge experiments). The presence of *C. difficile* in feces, indicating colonization, was determined during relapse on days 3, 10 and 15 after induction of recurrence. The day 3 samples were 4 animals only due to sampling error. To confirm the absence of *C. difficile* levels of toxins A and toxin B in feces was determined by ELISA and also using the cell cytotoxicity assay using HT29 and Vero cells (data not shown).

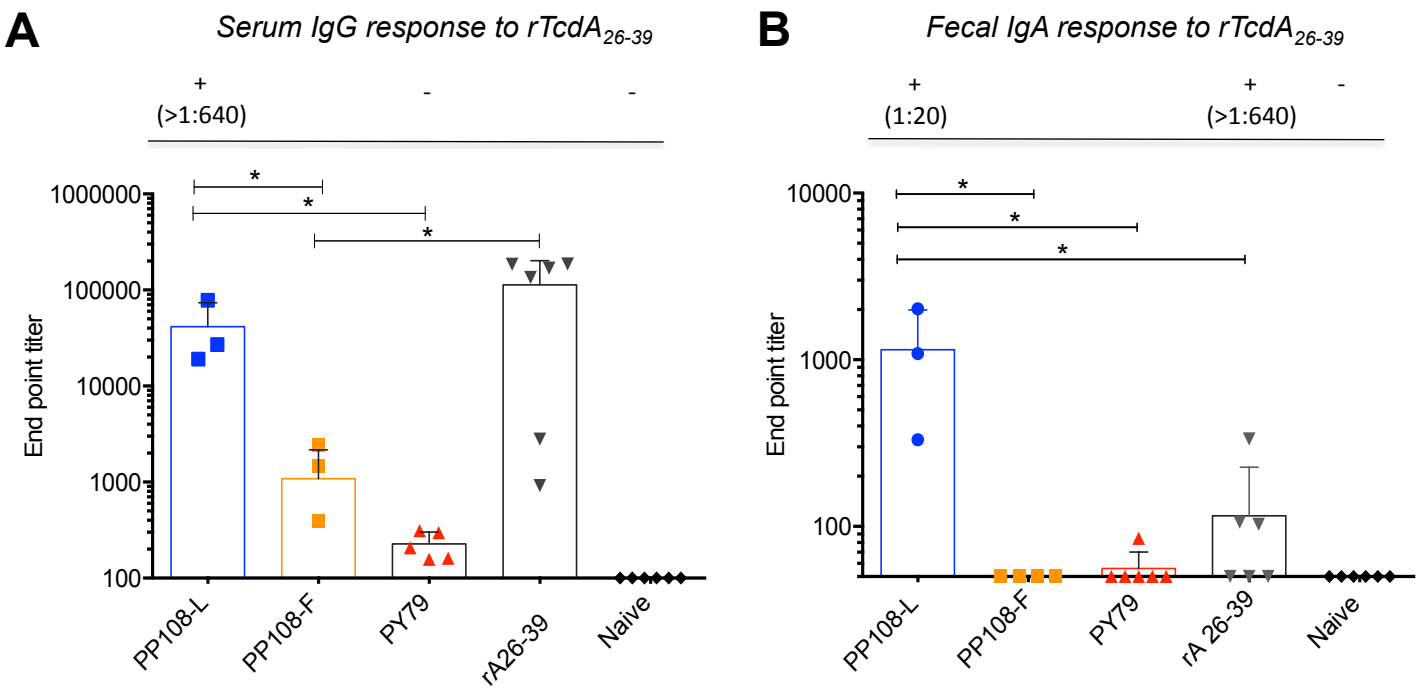

Figure 1.4: Immune responses in CD1 immunized mice. Groups (n=6) of CD-1 mice were immunized (i.g.) with live spores of PP108 (PP108-L), formaldehyde-inactivated PP108 spores (PP108-F), live PY79 (PY79-L) spores or parenterally (i.m.) with $rTcdA_{26\text{-}39}$ (5 mg/dose). Oral dosing used 5 x 10^{10} cfu/dose was on days 0, 1, 2, 14, 15, 16, 28, 29, 30, 55, 56, 57. Parenteral dosing was on days 0, 14, 28. Fecal IgA was collected 10-15 days after the final immunization and serum IgG 21 days after (*panel A*) and anti-$TcdA_{26\text{-}39}$ (*panel B*) titres determined. *, p <0.05. Neutralisation endpoint titers are shown above the bars and were determined as described elsewhere (21).

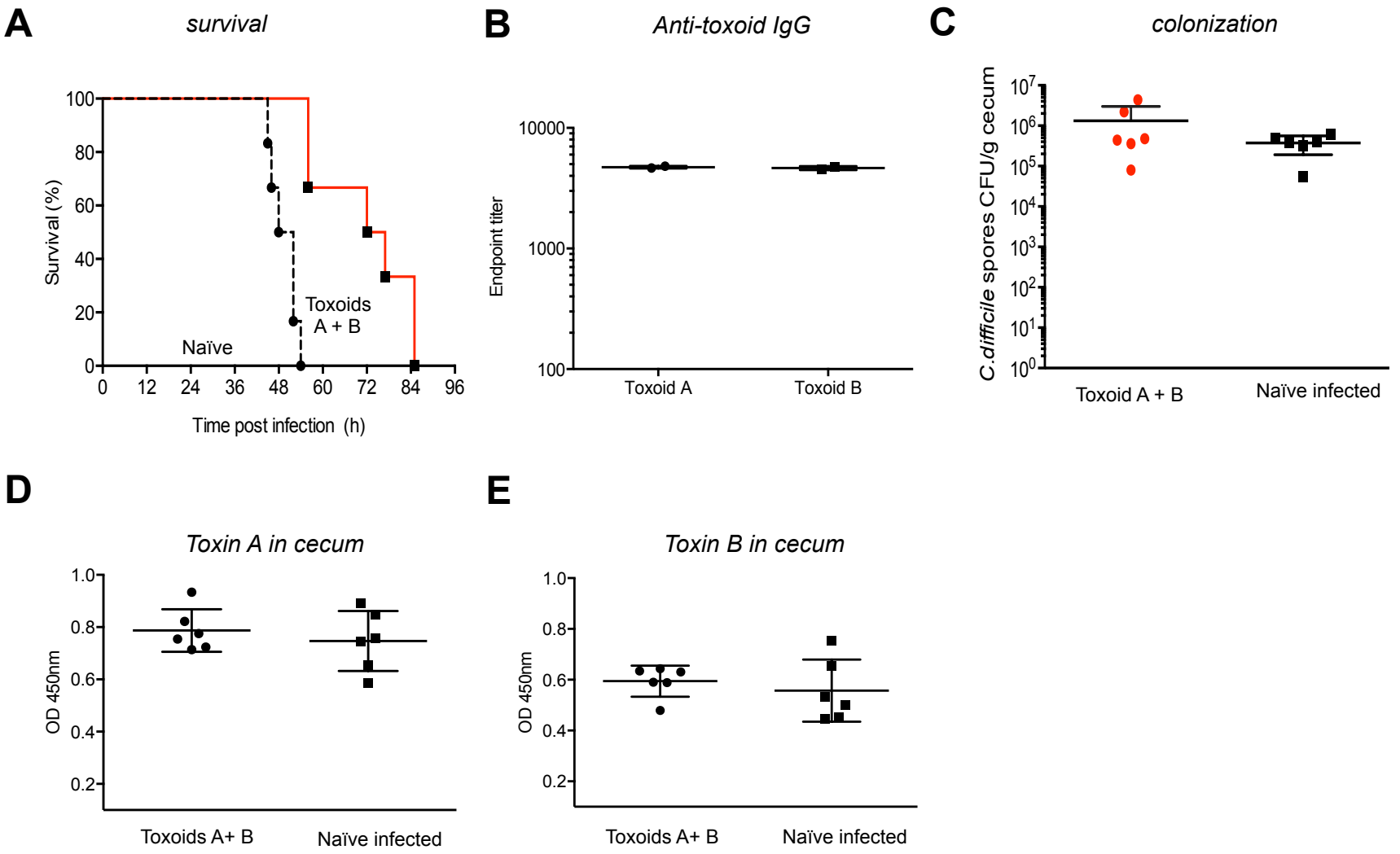

Figure 1.5: Parenteral Vaccination with Toxoids. Hamsters (6/group) were immunized with toxoids A and B using three injections (i.m.) and then challenged with 100 spores of *C. difficile* 630. *Panel A* shows survival of animals compared to the naïve groups. *Panel B* shows anti-toxoid A and anti-toxoid B IgG in immunized groups 2 days before challenge. *Panel C* shows counts of 630 spores in feces 24h post-challenge in immunized and naïve groups. This experiment has been repeated once. *Panels D and E* show levels of toxins A (*panel D*) and B (*panel E*) detectable in cecal samples determined by capture ELISA are shown together with non-immunized animals (naïve infected). Cell cytotoxicity assays were also used to confirm the presence of toxins.

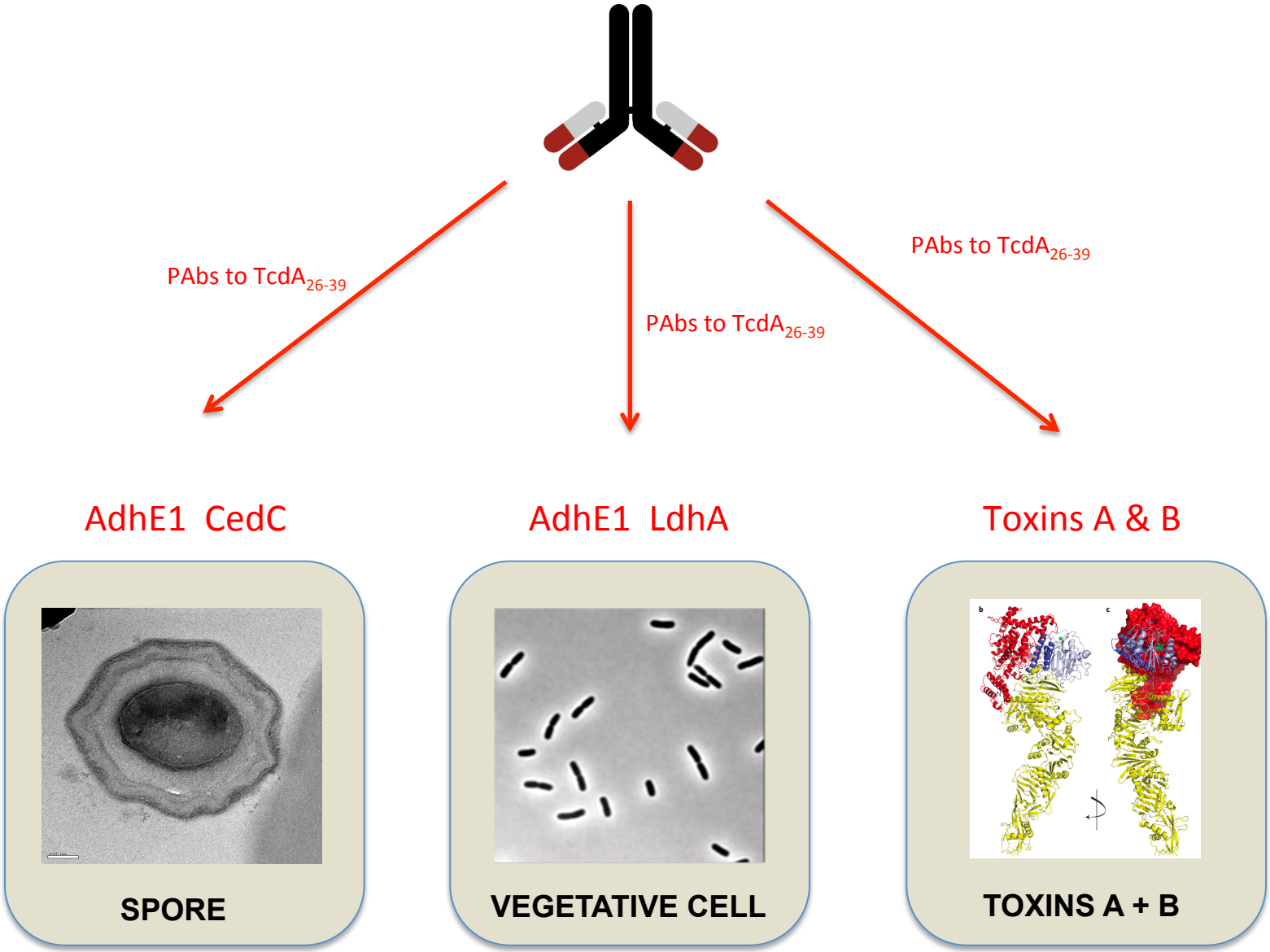

Figure 1.6: Cross-recognition of anti-TcdA26-39 antibodies with proteins present on the spore or vegetative cells of *C. difficle*