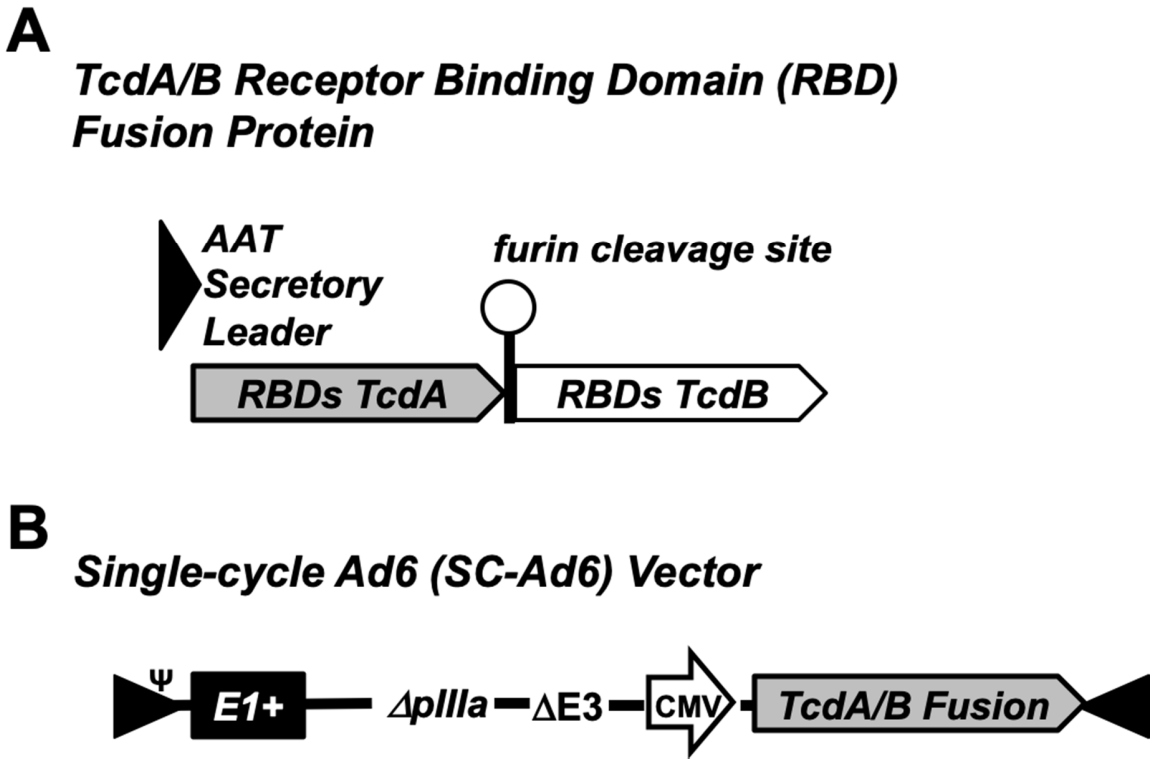
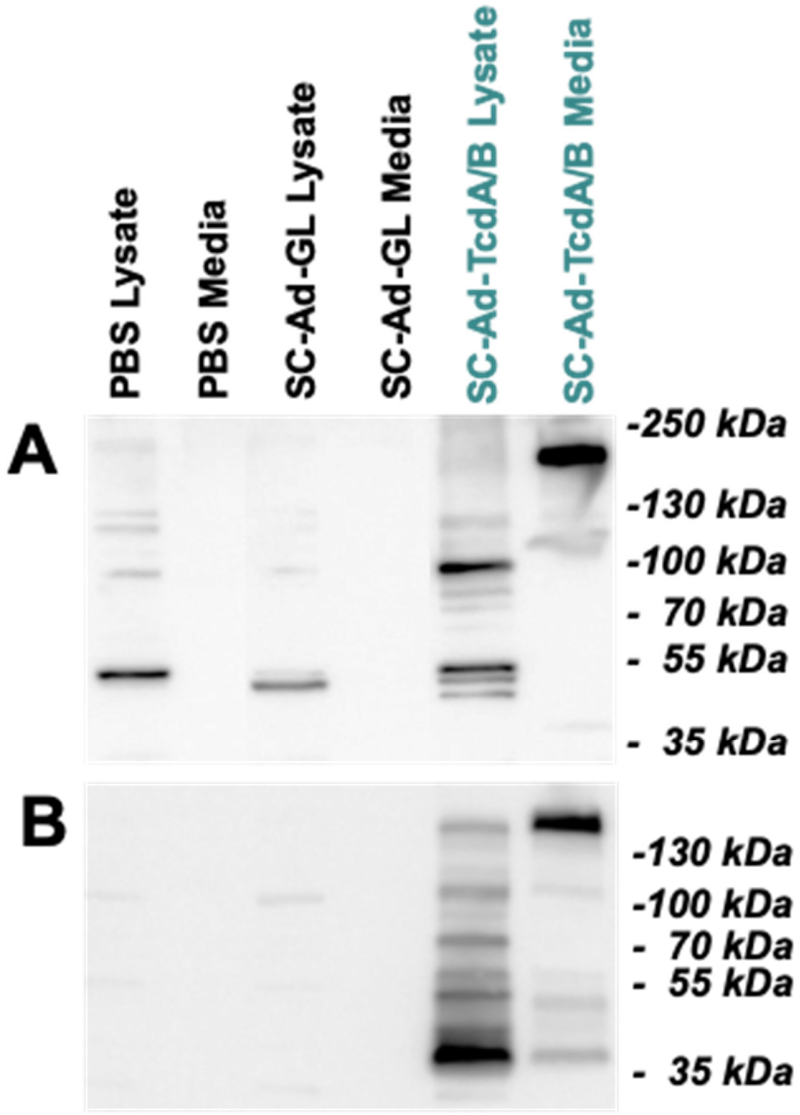
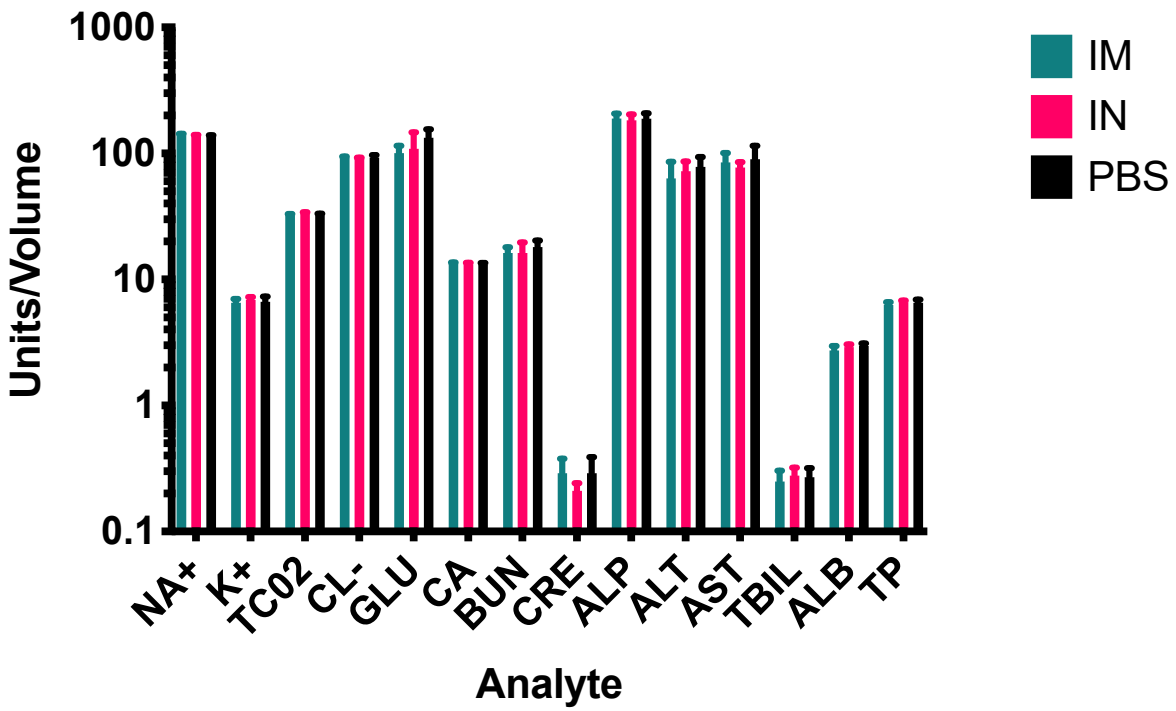


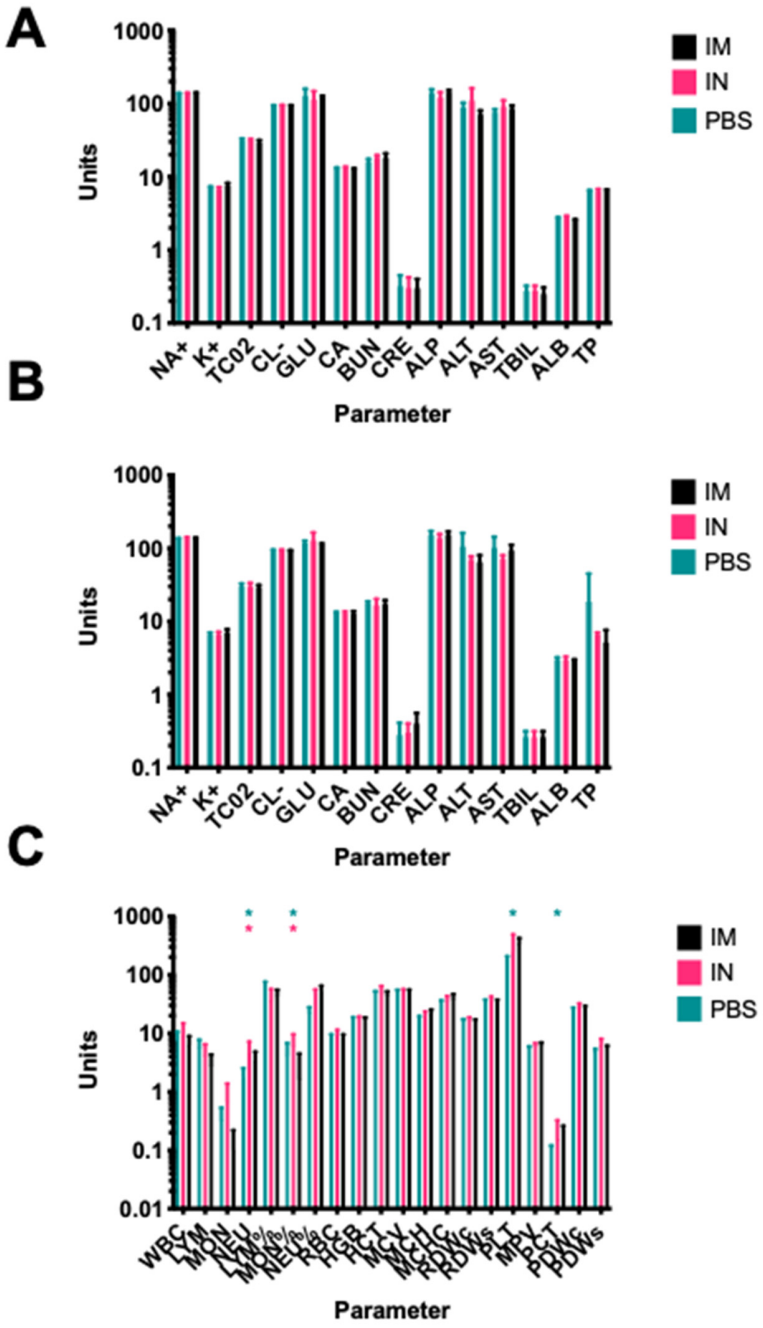

**Supplemental Figure S1. Schematic of TcdA/B fusion protein and SC-Ad6 plasmids expressing fusion protein (A)** TcdA/B fusion protein consists of a human *alpha* 1-antitrypsin (AAT) secretory leader sequence and the RBDs of TcdA and TcdB that are separated by two furin cleavage sites. **(B)** SC-Ad6, which is E1 expressing (E1+), protein IIIa deleted (ΔpIIIa), and E3 deleted (ΔE3) expressing the TcdA/B fusion protien using the CMV promoter.




**Supplemental Figure S2. Single-cycle adenovirus expression of *C. difficile* TcdA/B fusion protein.** Western blot detecting expression of **(A)** TcdA and **(B)** TcdB fragments in the A549 cell lysates and media taken from uninfected cells, cells infected with an Ad control (SC-Ad6-GL), and cells infected with SC-Ad6-TcdA/B. The fusion protein (~MW 150 kDA), individual TcdA (~100 kDa) and TcdB (~50 kDa) fragments can be seen in SC-Ad6-TcdA/B infected cells. Non-specific binding to proteins the cells, and Ad control can be seen in both blots.




**Supplemental Figure S3. Blood chemistry of SC-Ad6-TcdA/B vaccinated animals.** Groups of 10 Syrian hamsters were immunized a single time with $10^{11}$ virus particles of SC-Ad6-TcdA/B by the i.n. or i.m. route. Control animals received i.n. PBS. Blood was collected 3 days after immunization for clinical chemistry. Abbreviations shown in Table S1




**Supplemental Figure S4. Blood chemistry and CBC of SC-Ad6-TcdA/B vaccinated animals.** Groups of 10 Syrian hamsters were immunized a single time with $10^{11}$virus particles of SC-Ad6-TcdA/B by the i.n. or i.m. route. Control animals received i.n. PBS. Blood was collected ½ of the cohort (n=5 per group) **(A)** 3 and the other ½ of the cohort (n=5 per group) **(B)** 4 days after immunization for clinical chemistry. **(C)** Blood was collected on day 4 from ½ of the hamsters (n=5 per group) for CBC Abbreviations for panel A and B in Table S1 and in Table S2 for panel C.

**Supplemental Table S1.** Veterinary Hematology (CBC) Assay on the Abaxis VetScan HM5 Analyzer Results Table.

| **Parameter** | **Abbreviation** | **Units** |
|---|---|---|
| Sodium | NA+ | mmol/L |
| Potassium | K+ | mmol/L |
| Total Carbon Dioxide | tCO2 | mmol/L |
| Chloride | CL- | mmol/L |
| Glucose | GLU | mg/dL |
| Calcium | CA | mg/dL |
| Blood Urea Nitrogen | BUN | mg/dL |
| Creatinine | CRE | mg/dL |
| Alkaline Phosphatase | ALP | U/L |
| Alanine Aminotransferase | ALT | U/L |
| Aspartate Aminotransferase | AST | U/L |
| Total Bilirubin | TBIL | mg/dL |
| Albumin | ALB | g/dL |
| Total Protein | TP | g/dL |

**Supplemental Table S2.** Blood Chemistry Assay on the Piccolo Xpress Chemistry Analyzer Results Table.

| **Parameter** | **Abbreviation** | **Units** |
|---|---|---|
| Total White Blood Cell count | WBC | 10^9L |
| Lymphocyte count | LYM | 10^9L |
| Monocyte count | MON | 10^9L |
| Neutrophil count | NEU | 10^9L |
| Eosinophil count | EOS | 10^9L |
| Basophil count | BAS | 10^9L |
| Lymphocyte percentage | LYM% | % |
| Monocyte percentage | MON% | % |
| Neutrophil percentage | NEU% | % |
| Eosinophil percentage | EOS% | % |
| Basophil percentage | BAS% | % |
| Red Blood Cell count | RBC | 10^12L |
| Hemoglobin | HGB | g/dL |
| Hematocrit percentage | HCT | % |
| Mean Corpuscular Volume | MCV | fL |
| Mean Corpuscular Hemoglobin | MCH | pg |
| Mean Corpuscular Hemoglobin Concentration | MCHC | g/dl |
| Red Cell Distribution Width, coefficient of variation % | RDWc | % |
| Red Cell Distribution Width | RDWs | fL |
| Platelet count | PLT | 10^9L |
| Mean Platelet Volume | MPV | fL |
| Platelet crit % | PCT | % |
| Platelet Distribution Width, coefficient of variation % | PDWc | % |
| Platelet Distribution Width | PDWs | fL |