Figure S1. A multiple sequence alignment of CpTEII orthologs from represenative species. The CpTEII orthologs with query cover ≥50% and expect value ≤ e-5 were retrieved from NCBI protein databases and multiple sequence alignment was performed by using T-coffee program. All TEII orthologs from *Cryptosporidium* spp. were retained, while the nearly identical sequences from other species were removed from the dataset containing 32 sequences from apicomplexans, algae and bacteria. This alignment shows TEII sequences from 15 representative species. The two conserved motifs are shaded. The three residues representing catalytic triad are boxed. Asterisks (*) indicate residues conserved among all listed sequences.

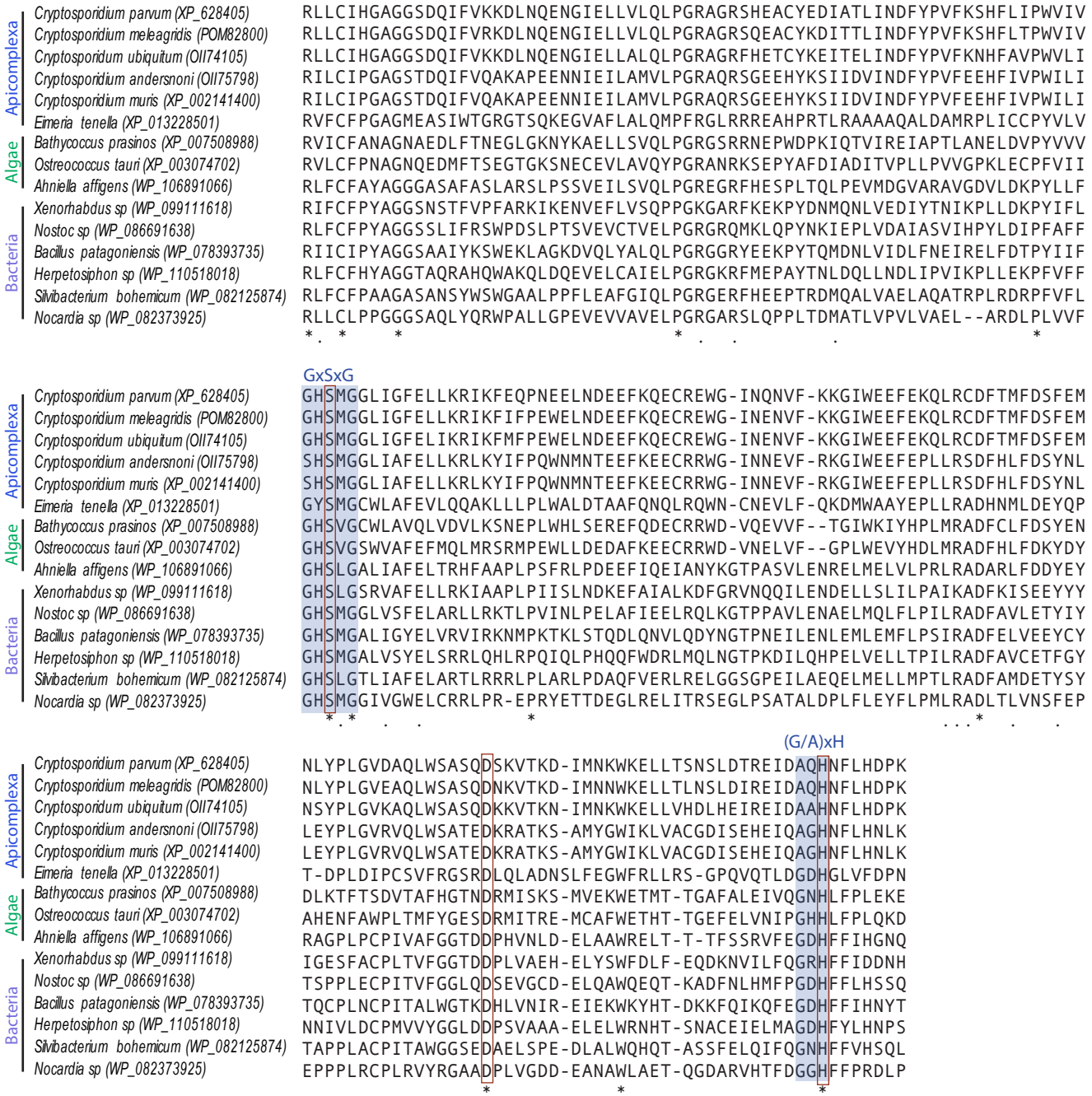

Figure S2. MBP-tag itself had no or little effect on the CpTEII activity assay. The assay was performed in 200 µL of HEPES buffer (0.1 M, pH 7.4) containing 50 mM of KCl, 50 µM of a specified fatty acyl-CoA, 50 uM of DTNB and 10 µg of MBP-CpTEII or MBP-tag only.

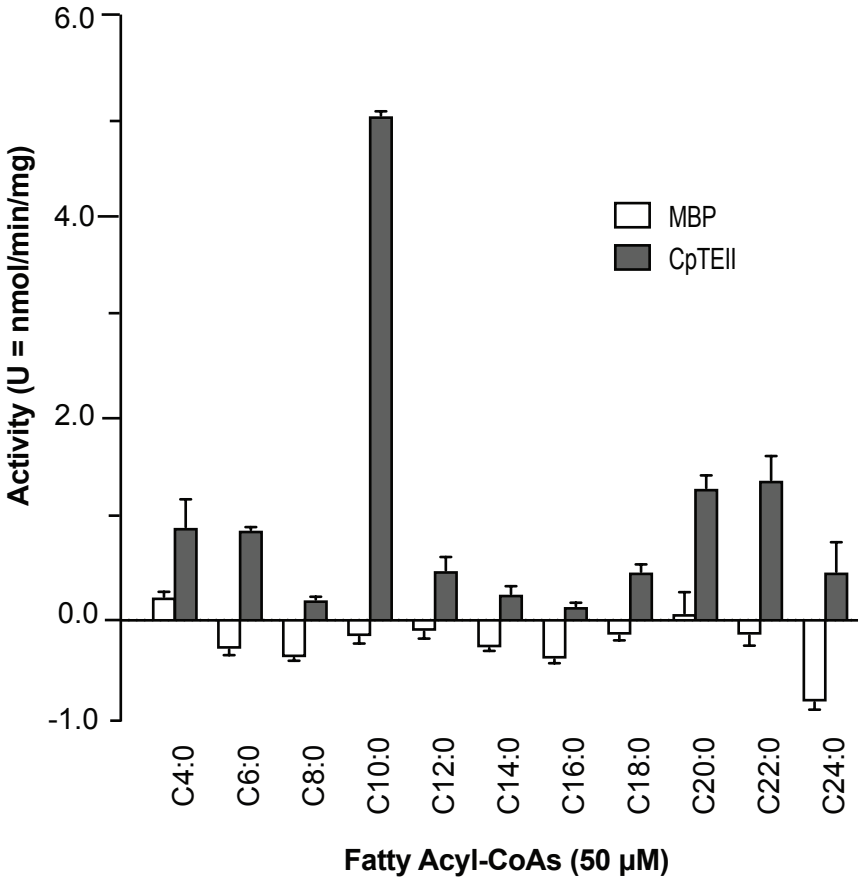

Figure S3. Effect of bovine serum albumin (BSA) on CpTEII activity. Significant improvement of CpTEII activity on behenoyl coenzyme A (C22:0 CoA) was observed. The assay was performed in 200 µL of HEPES buffer (0.1 M, pH 7.4) containing 50 mM of KCl, 50 µM of a specified fatty acyl-CoA and 50 uM of DTNB. The reaction started by adding MBP-CpTEII only or MBP-CpTEII with BSA at molar ration 4.5:1.

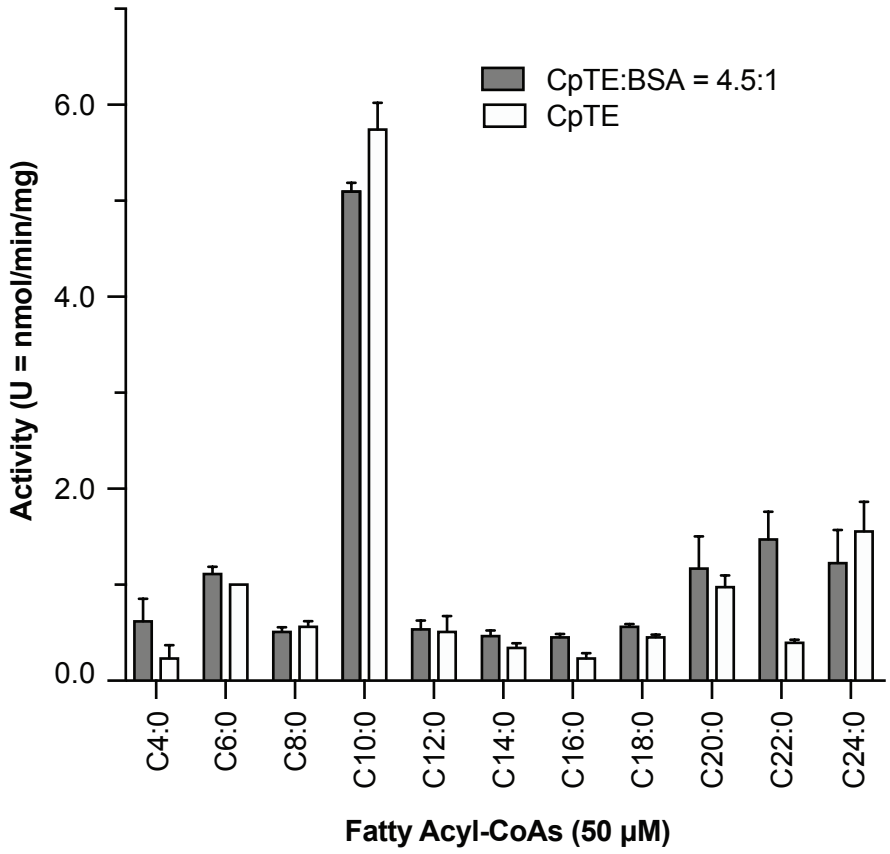

Figure S4. The anti-CpTEII antibodies specificity assay by western blotting. (A) The purified CpTEII (0.1 µg), MBP (0.1 µg), and crude extracts of oocysts (1 x 10^7/lane) and sporozoites (Spz) (~4 x 10^7/lane) were separated by 4-20% SDS-PAGE. (B) The fractionated proteins from the gel (A) were transferred onto nitrocellulose membrane for western blot analysis. The membranes were probed with anti-CpTEII antibody (1:1,000 dilution) (left) or the antibody (1:1,000 dilution) presoaked with MBP and CpTEII proteins immobilized on nitrocellulose membrane, followed by incubation with a goat anti-rabbit secondary antibody conjugated with horseradish peroxidase (HRP; 1:20,000 dilution). The membranes were developed with SuperSignal West Femto maximum sensitivity substrate and imaged using ChemiDoc XRS+ system (Bio-Rad).