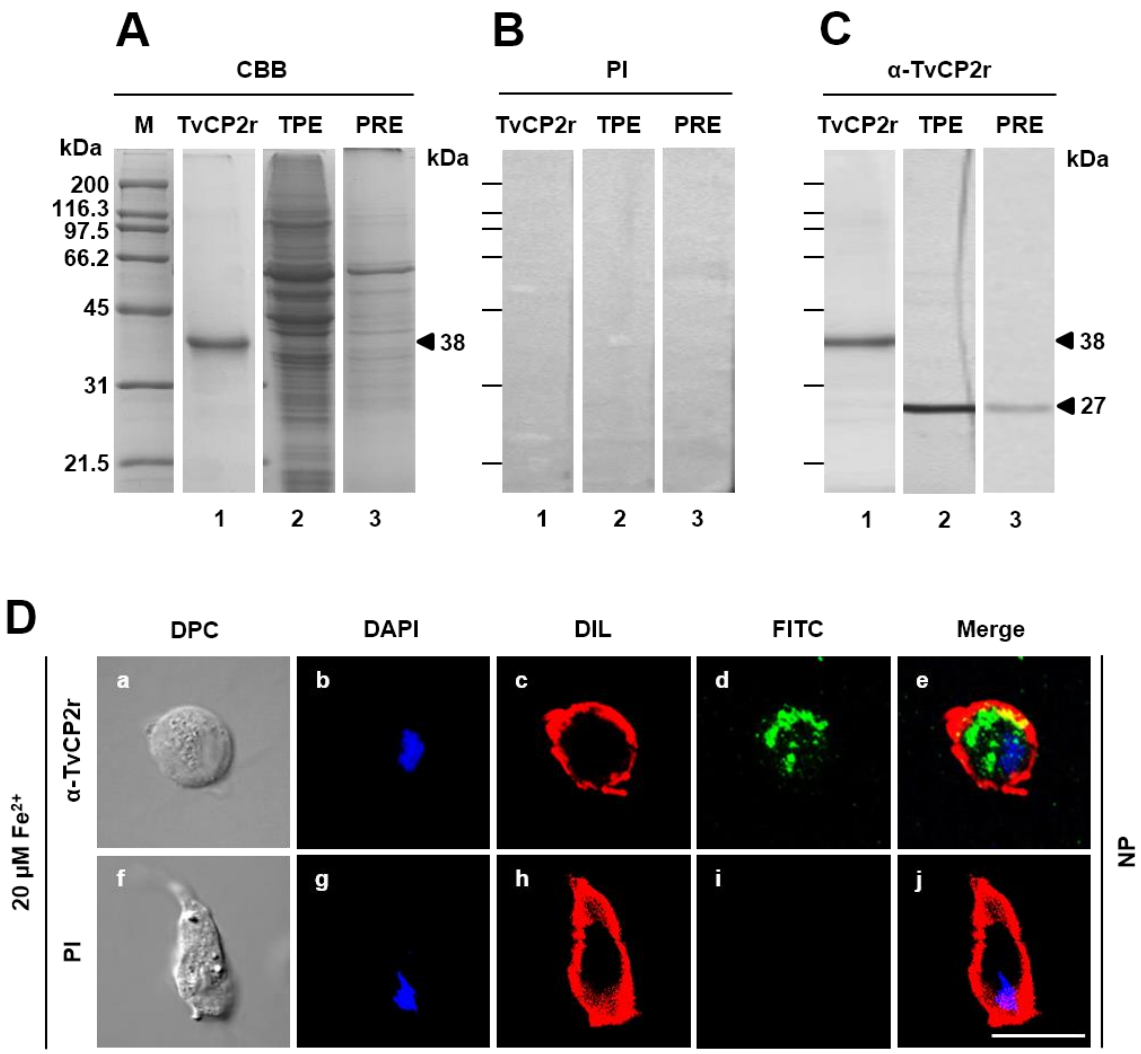

Supplementary Fig. S2. Production of recombinant TvCP2 protein (TvCP2r) and anti-TvCP2r antibody as biological tools to test samples from trichomoniasis patients and TvCP2 localization. (A) Cloning of *tvcp2*, expression of the recombinant TvCP2 protein, and anti-TvCP2r polyclonal antibody production. Coomassie Brilliant Blue-stained (CBB) SDS-PAGE on a 12% gel containing TvCP2r purified by nickel affinity chromatography (lane 1); total protein extract (TPE; lane 2), and protease-resistant extract (PRE; lane 3). M, broad range molecular weight markers (Bio-Rad)

(B) WB assay from a duplicate CBB gel incubated with PI serum at a 1:2000 dilution used as a negative control. (C) WB assay from a duplicate CBB gel using a polyclonal α-TvCP2r antibody at a 1:2000 dilution. Arrowheads show the molecular mass of recombinant (38-kDa) and mature (27-kDa) TvCP2 protein bands in kilodaltons (kDa). (D) IFA of non-permeabilized (NP) parasites incubated with the anti-TvCP2r antibody or preimmune (PI) serum followed by FITC-conjugated goat anti-rabbit IgG (in green). (a and f) Differential phase contrast (DPC) image of parasites grown under normal iron concentrations (NI; 20 μM [Fe^{2+}]); nuclei stained with DAPI (in blue; b and g); parasites labelled with DIL (in red; c and h) as a membrane marker; TvCP2 (labelled with FITC; in green; d) staining of parasites incubated with the anti-TvCP2r antibody (1:100 dilution; panel d), or with a PI serum (1:100 dilution; panel l) used as a negative control, and a FITC-conjugated secondary antibody (1:100 dilution); merge showing the co-localization of TvCP2 with the membrane marker (in yellow; e and j). The slides were observed at 63x magnification by confocal microscopy (Zeiss) 3D maximum projection. Scale bar = 10 μm.