# He et al. Figure S1

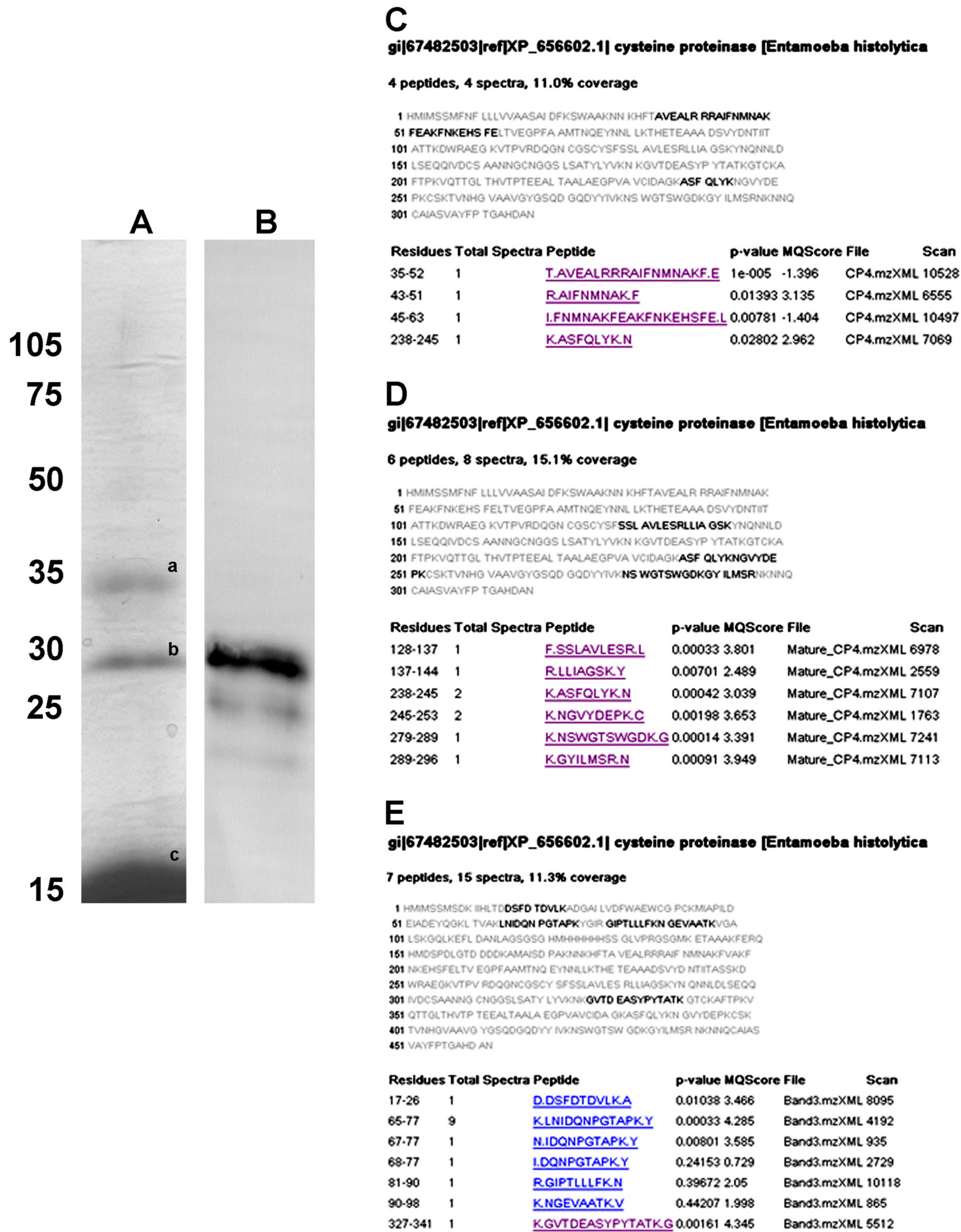


Fig. S1. Mass-spectrometry identified the refolded and activated recombinant EhCP4 protein. *A.* Refolded rEhCP4 that underwent autocatalytic processing was separated in a 12% SDS-polyacrylamide gel. Coomassie staining indicated a band of intermediate auto-processed EhCP4 fragment from the zymogen form (a), a band of mature EhCP4 (b), a band of degraded protein fragments (c). *B.* The same proteinase sample was reacted with DCG04 and blotted with streptavidin-alkaline phosphatase, indicating the mature rEhCP4. *C.* The Mass-spectrometry result of band a, indicating peptides from the pro-domain. *D.* The mass-spectrometry result of band b, indicating peptides from the mature enzyme. *E.* The mass-spectrometry result of band c, indicating peptides from the fusion protein's tag region and degraded fragments belonging to the enzyme proper.

# He et al. Figure S2

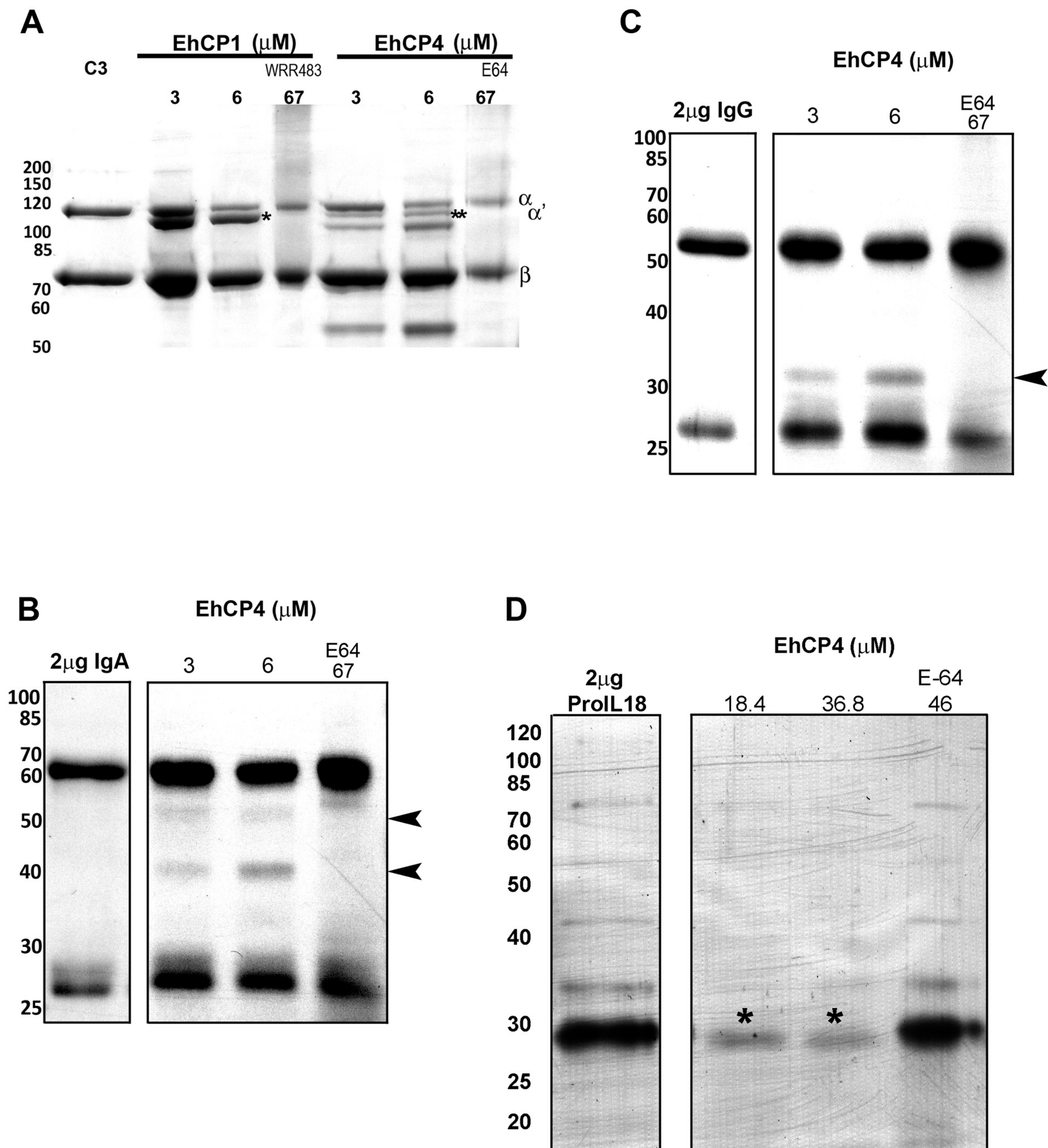


Fig. S2. Cleavage of proteins of the host immune response by rEhCP4. *A*. Cleavage of C3. C3 (2μg) was incubated with EhCP4 or EhCP1 and the fragments were resolved by a 4-50% SDS polyacrylamide gel. Coomassie stained bands resolved from cleavage of the α chain are marked with * (rEhCP1) and ** (rEhCP4). Inhibitors, WRR483 (rEhCP1) or E64 (rEhCP4) blocked the cleavage. *B*. rEhCP4-mediated cleavage of IgA. rEhCP4 mediated cleavage of human IgA (2μg). Preincubation with E64 blocked the cleavage. Arrowheads indicate the fragments of the immunoglobin heavy chain. *C*. rEhCP4 mediated cleavage of human IgG (2μg). Preincubation with E64 blocked the cleavage. Arrowheads indicate the fragments of the immunoglobin heavy chain. *D*.. Cleavage of Pro-IL-18. rEhCP4 and recombinant pro-IL-18 (2μg) were incubated. The decreased amount of pro-IL18 proteins are indicated with *. The degradation of pro-IL-18 was completely inhibited by pre-incubation with E-64. B-D, protein samples were separated with 15% SDS-polyacrylamide gels and stained with Coomassie blue.

# He et al  Figure S3

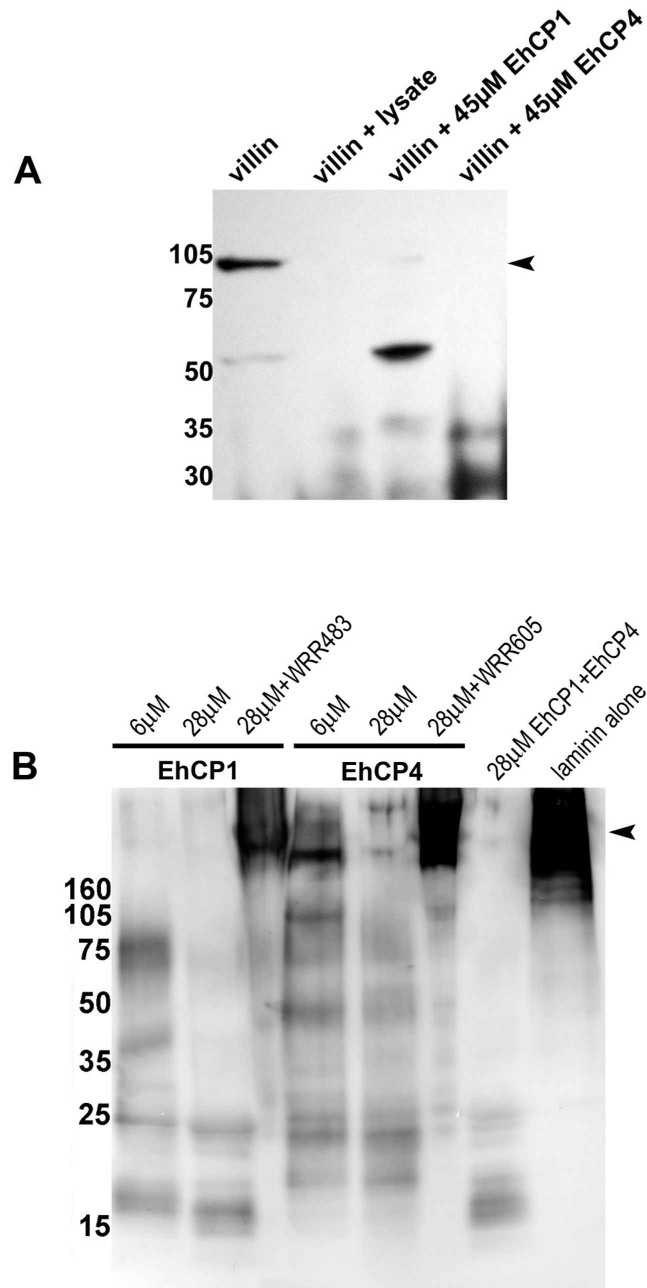


Fig. S3. Cleavage of villin and basement membrane laminin-1 by rEhCP4. A. Cleavage of villin-1. Immunoprecipitated human villin-1 was incubated with amebic lysate, rEhCP1 and rEhCP4. The proteinase activity (based on substrate Z-RR-AMC) of the amebic lysate was equivalent to that of rEhCP1 The resulting immunoblot were probed with villin-1 antibody. *B.* rEhCP4 and rEhCP1 degraded laminin-1. Laminin-1 (4 μg) was incubated with rEhCP1 or rEhCP4 or both. The resulting laminin-1 fragments were analyzed by SDS-PAGE and the immunoblot was probed with anti-L-laminin-1 antibody. The EhCP1 specific inhibitor, WRR483 and the EhCP4 specific inhibitor, WRR605 were added at a concentration of 28 μM. Intact laminin is indicated with an arrowhead.

# He et al. Figure S4

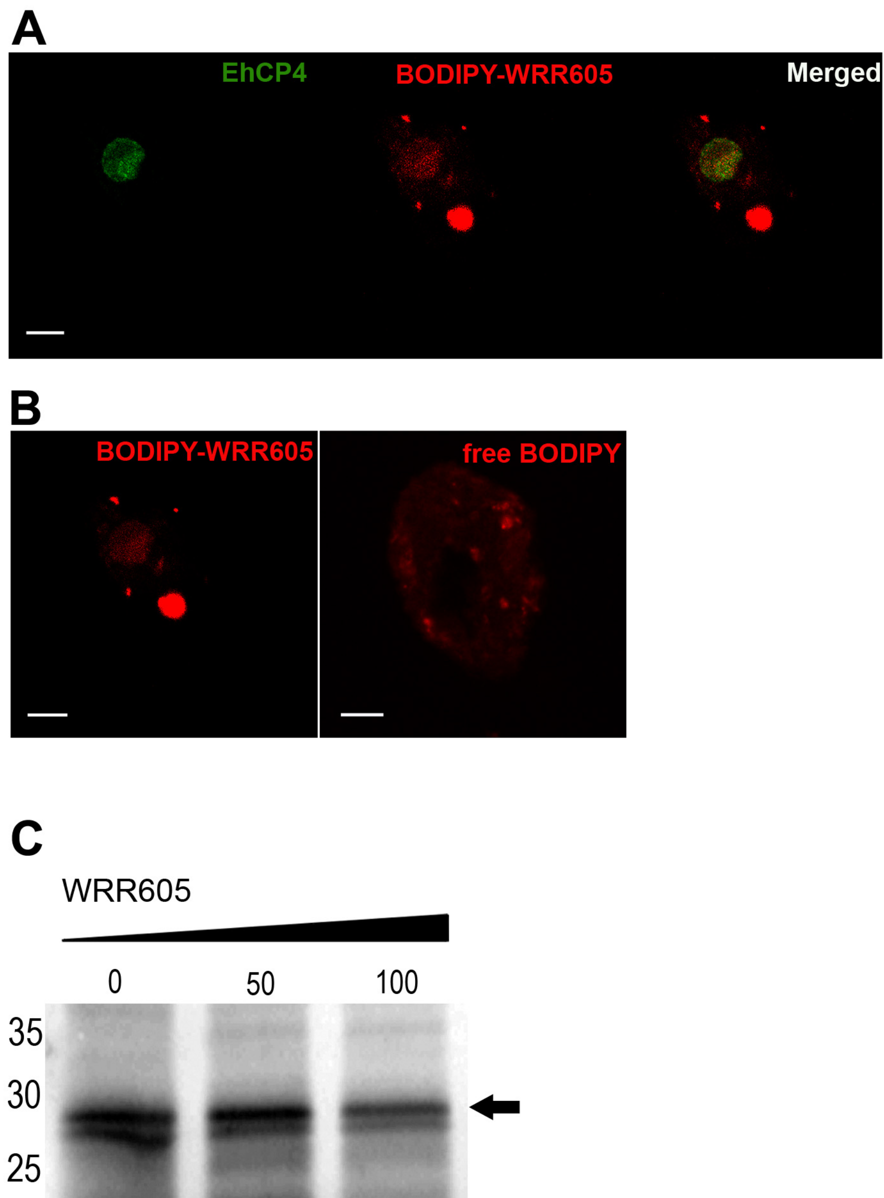


Fig. S4. BODIPY labeled WRR-605 binds rEhCP4 and intracellular EhCP4. *A*. An optical section of a trophozoite shows co-localization of EhCP4 (green) and BODIPY-WRR605 (red) in the nuclear region of a trophozoite *B*. Cellular distribution of BODIPY-WRR605 and Free BODIPY fluorescence dye in trophozoites. Z-stack thickness, 0.5μm; Scale bar, 5.0 μm. *C*. The activated rEhCP4 was treated with BODIPY labeled WRR605 without (0 μM) or with increasing concentrations of unlabeled WRR605, demonstrating competitive binding between WRR605 and BODIPY-WRR605. The arrow indicates the location of mature rEhCP4.