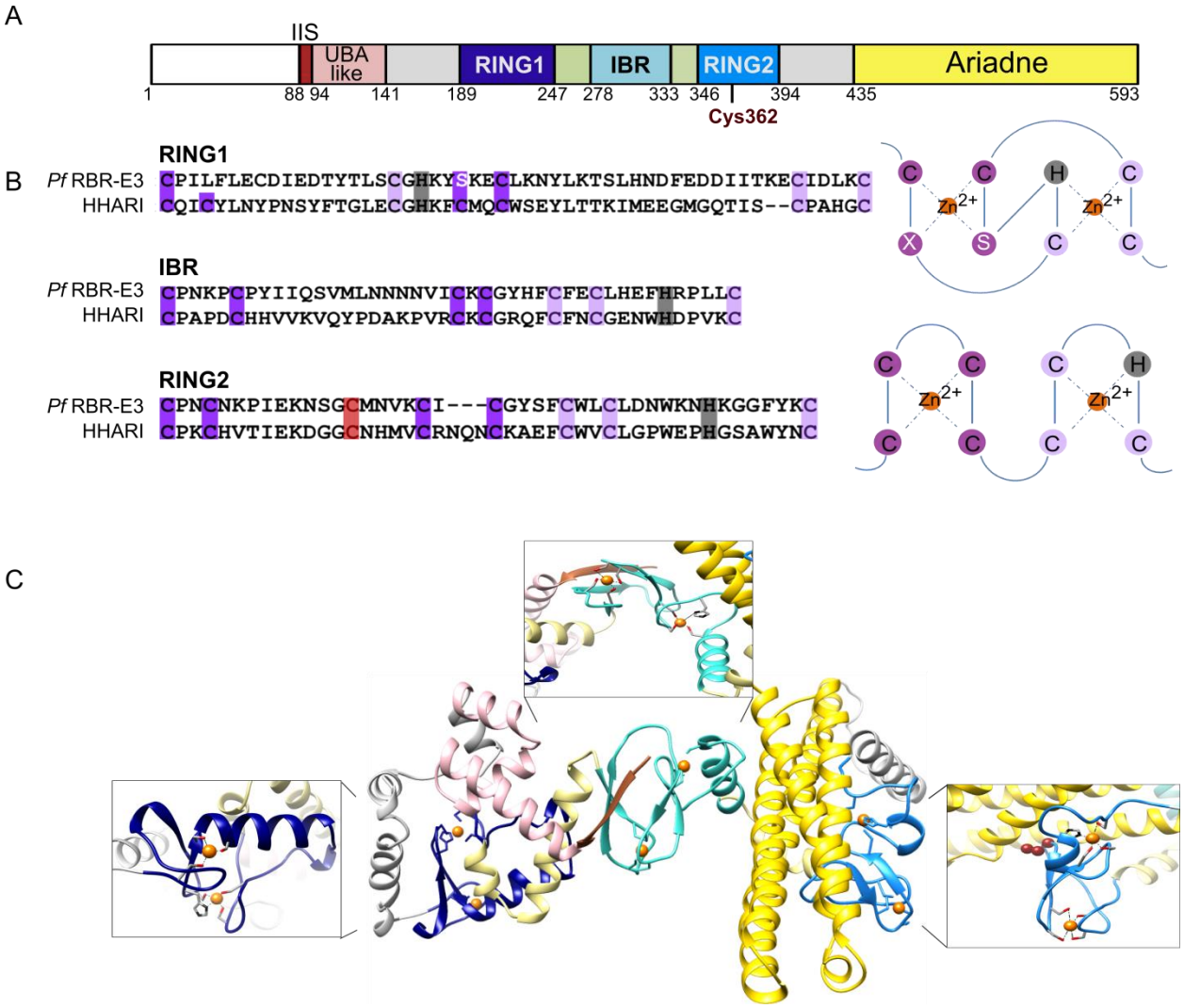

Scheme 1: (A) Schematic representation of domains of *Pf* RBR-E3 ligase. Color code corresponds to IBR interacting strand (IIS; sienna color), UBA-like (pink), RING1 (navy blue), IBR (turquoise), RING2 (dodger blue) and Ariadne (yellow). Linkers connecting IBR domain with RING1 and RING2 domains are shown in light green color and interdomain spacers are shown in gray. Cys362 is the catalytic cysteine (in red). (B) Alignment of *Pf* RBR-E3 RING1, IBR and RING2 domains showing conserved Zn^{2+} coordinating residues with respect to human ortholog, HHARI (Uniprot ID: Q9Y4X5). RING1 domain has cross-braced Zn^{2+} coordination pattern with non-conserved 2^{nd} and 5^{th} Zn^{2+} coordinating residues in parasite. IBR and RING2 domains have Zn^{2+} coordination in sequential pattern. (C) Structure of *Pf* RBR-E3 with close-up view of Zn^{2+} binding domains (RING1, IBR and RING2). Zn^{2+} atom is shown as orange sphere, Zn^{2+} coordinating Cys and His amino acids are shown as sticks and catalytic cysteine is shown in red sphere.

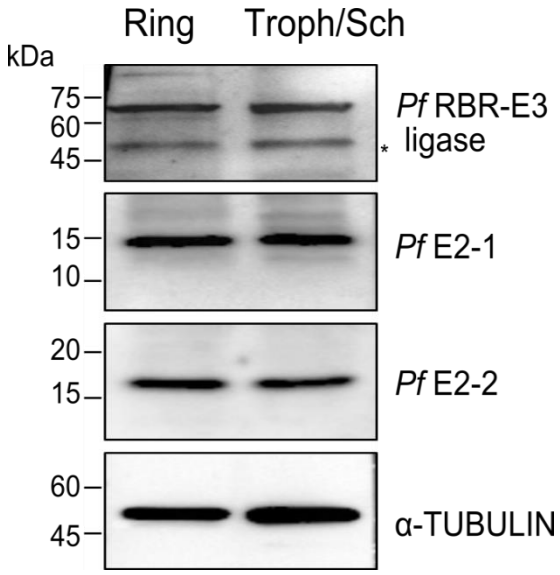

Figure 1: Cellular expression of *Pf* RBR-E3 ligase (PF3D7_0303800) and its probable E2s (*Pf* E2-1: PF3D7_1203900 and *Pf* E2-2: PF3D7_0527100) in ring and trophozoite/schizont stages of blood phase of lifecycle of human malaria parasite (PF3D7 strain). Polyclonal antibodies were raised against parasite proteins and their expression was checked in parasite lysate. (Full length is 72 kDa, * probably is a degradation band at 54 kDa).

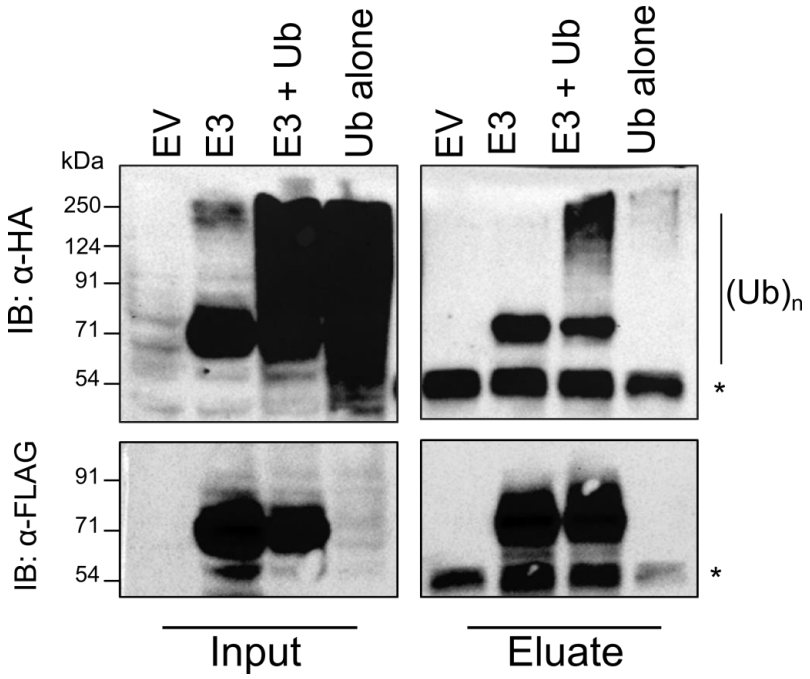

Figure 2: Ubiquitination activity of *Pf* RBR-E3 ligase. Immunoprecipitation and immunoblot analysis of extracts of HEK293T cells transfected with empty vector (EV), HA-Ub alone or co-transfected with *Pf* RBR-E3 and HA-Ub. Samples were immunoprecipitated with anti-FLAG antibody and immunoblotted with both anti-FLAG and anti-HA antibodies. * corresponds to IgG heavy chain (54 kDa).

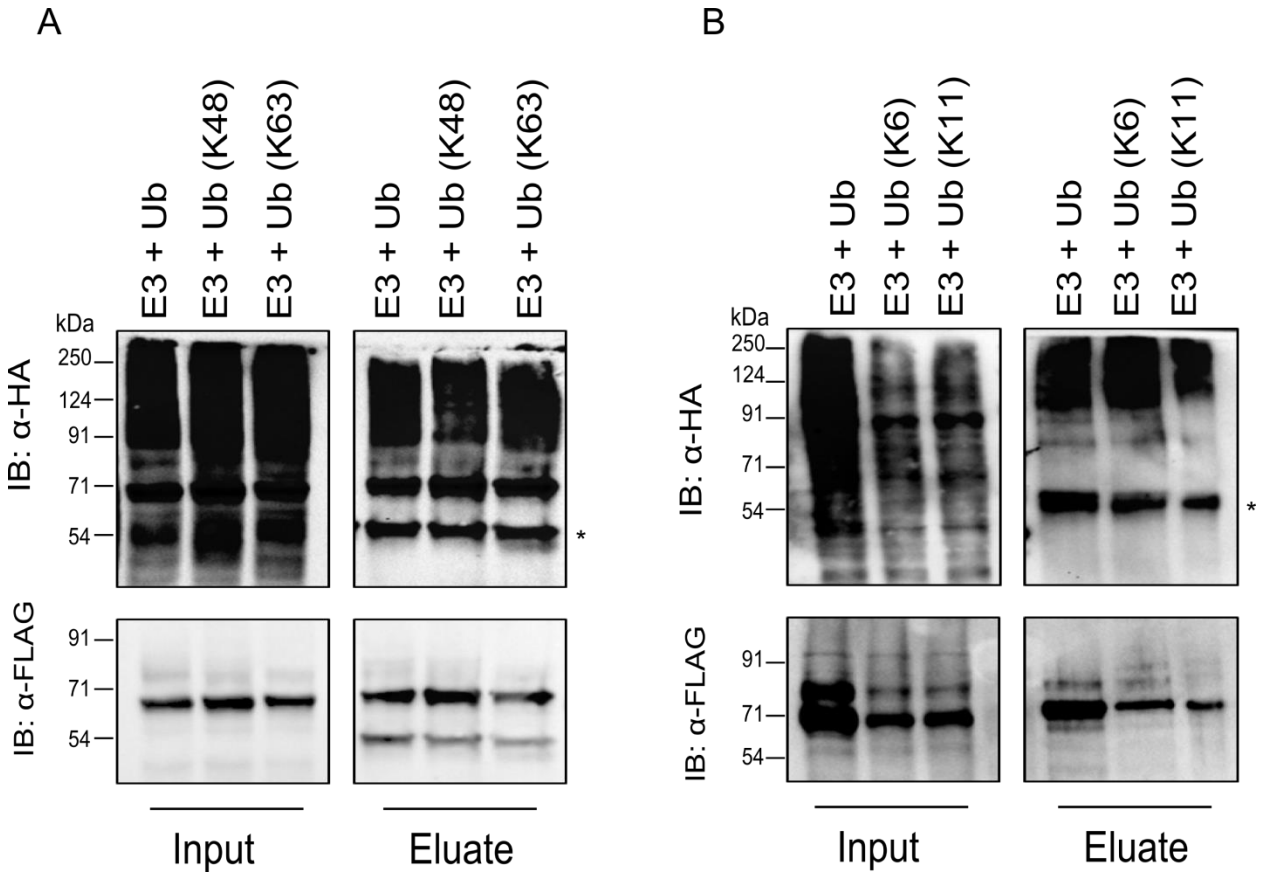

Figure 3: *Pf* RBR-E3 ligase mediate ubiquitination through different lysine linkages. Immunoprecipitation and immunoblot analysis of extracts of HEK293T cells co-transfected with *Pf* RBR-E3 and HA-Ub or HA-Ub mutants with lysine at different positions (K48, K63, K6, K11). Samples were immunoprecipitated with anti-FLAG antibody and immunoblotted with anti-FLAG and anti-HA primary antibody with light chain-specific secondary antibody. * corresponds to IgG heavy chain (54 kDa).

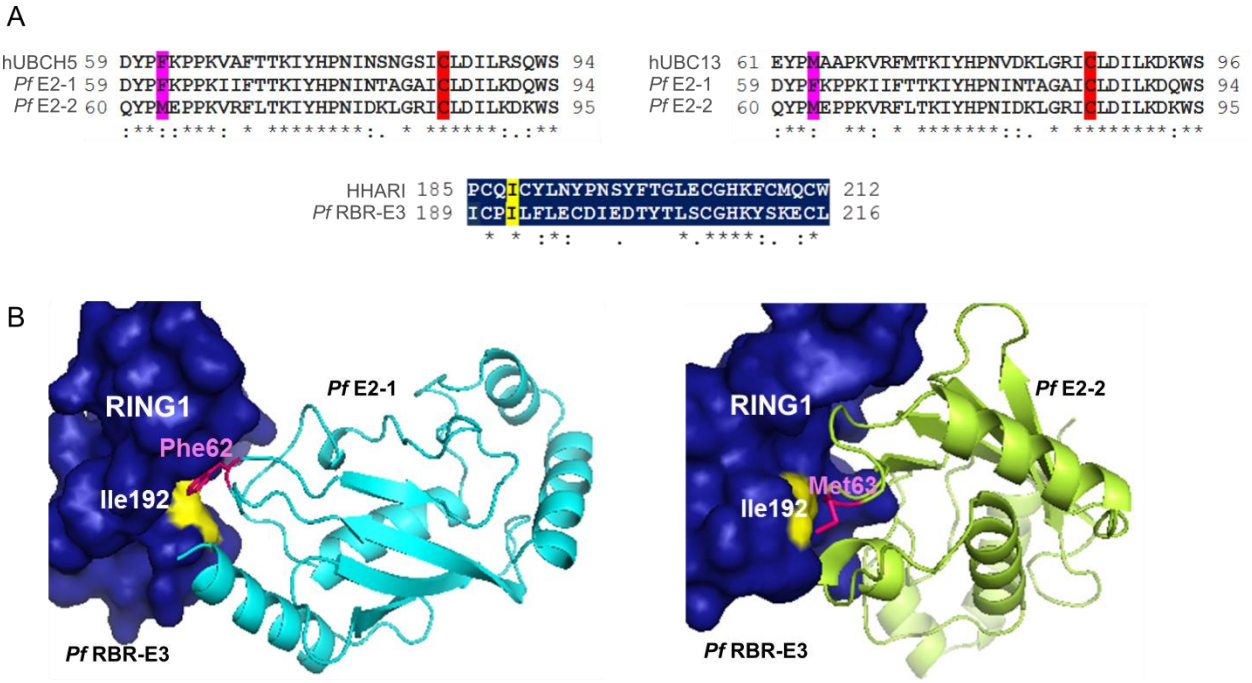

Figure 4: Interaction of RING1 domain of *Pf* RBR-E3 ligase with E2 enzyme. (A) Sequence alignment of human E2s (UBCH5 and UBC13) with their respective *P. falciparum* E2s (PF3D7_1203900 and PF3D7_0527100). The conserved hydrophobic residue and catalytic cysteine are marked in magenta and red, respectively. Sequence alignment of RING1 domain of HHARI and *Pf* RBR-E3 ligase showing conserved isoleucine residue (yellow). (B) Docking showing interaction of hydrophobic residue in *Pf* E2s with isoleucine in RING1 domain of *Pf* RBR-E3 ligase. RING1 is represented as surface model (navy blue) and *Pf* E2-1 (cyan) and *Pf* E2-2 (green) are represented as ribbon.

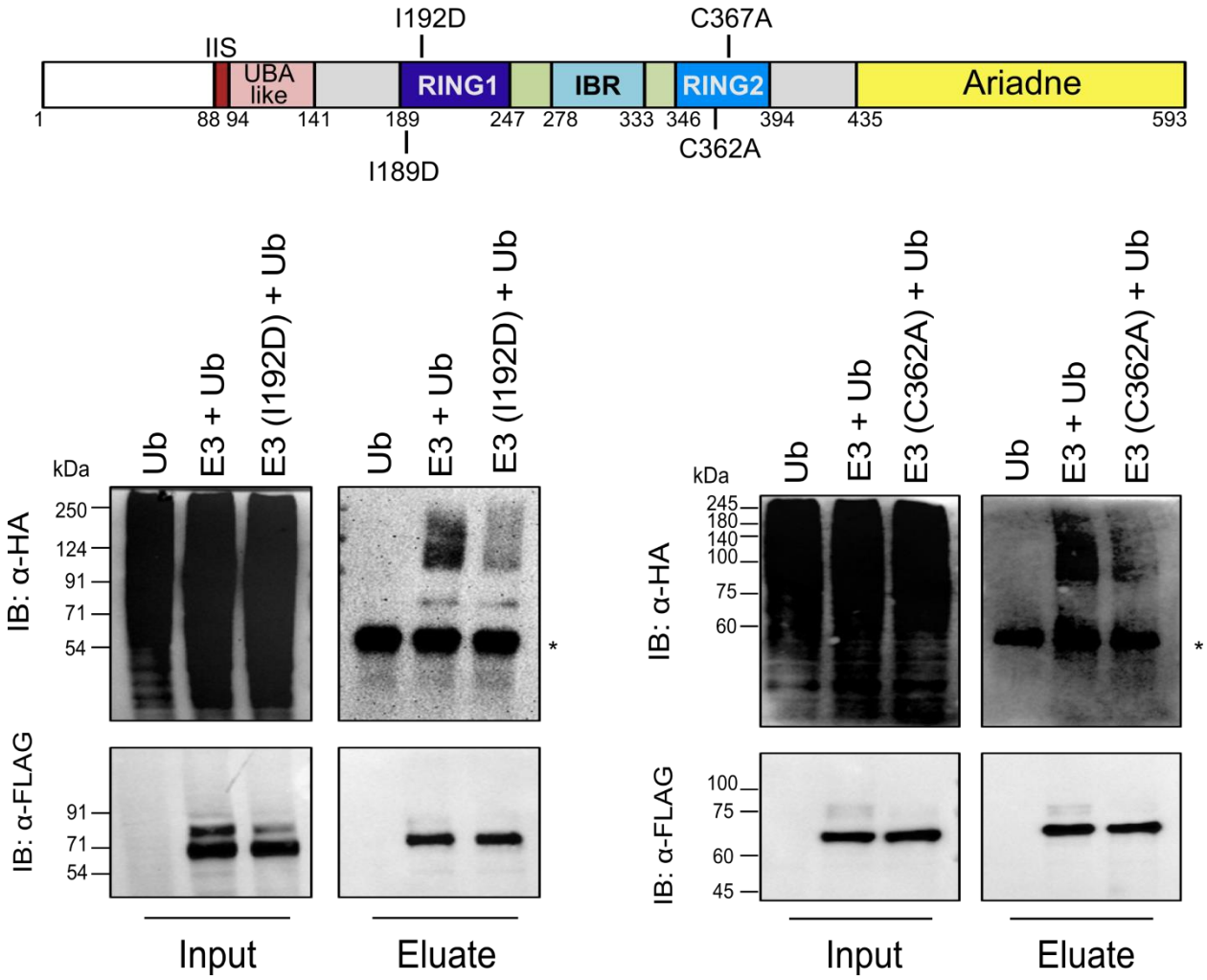

Figure 5: Mutations in RING1 and RING2 domains affect *Pf* RBR-E3 ligase activity. Immunoprecipitation and immunoblot analysis of extracts of HEK293T cells co-transfected with HA-Ub and *Pf* RBR-E3 or its RING1 (I192D) or RING2 mutant (C362A). Samples were immunoprecipitated with anti-FLAG antibody and immunoblotted with both anti-FLAG and anti-HA primary antibody with light chain-specific secondary antibody. * corresponds to IgG heavy chain (54 kDa).

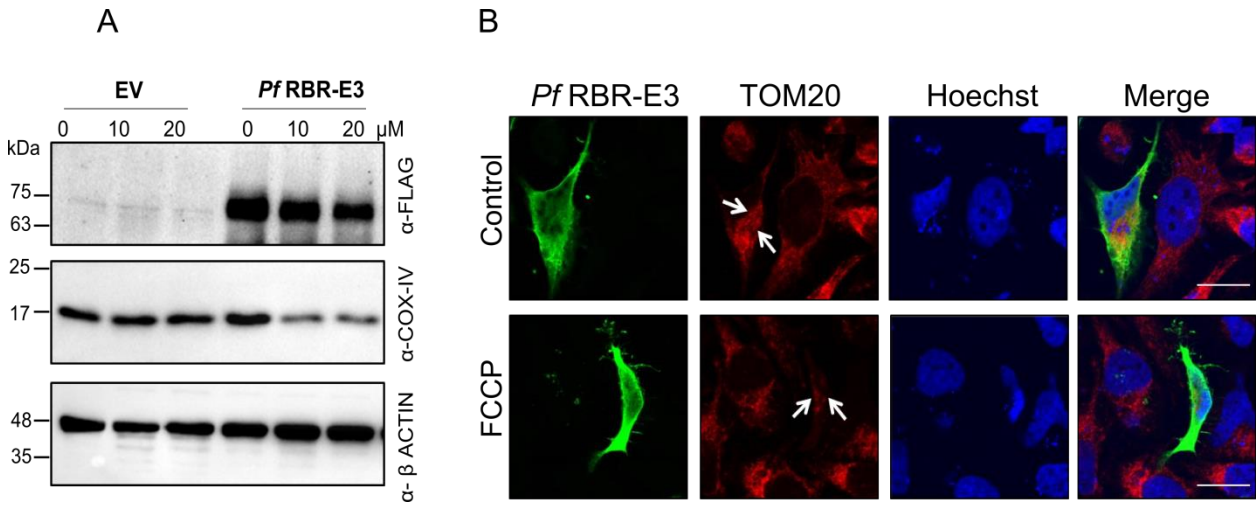

Figure 6: Role of *Pf* RBR-E3 in mitochondrial quality control. HeLa cells transfected with empty vector (pCIneo) or *Pf* RBR-E3 were treated with FCCP (10 or 20μM, 6 h). (A) Immunoblotting was done with FLAG, ACTIN and COX-IV antibodies. (B) Fluorescence microscopy of HeLa cells, ectopically expressing *P. falciparum* RBR-E3 ligase before and after exposure to FCCP (20 μM, 6 h). Hoechst and TOM20 antibody was used for staining nucleus and mitochondria, respectively. Scale bar represents 20 μm.

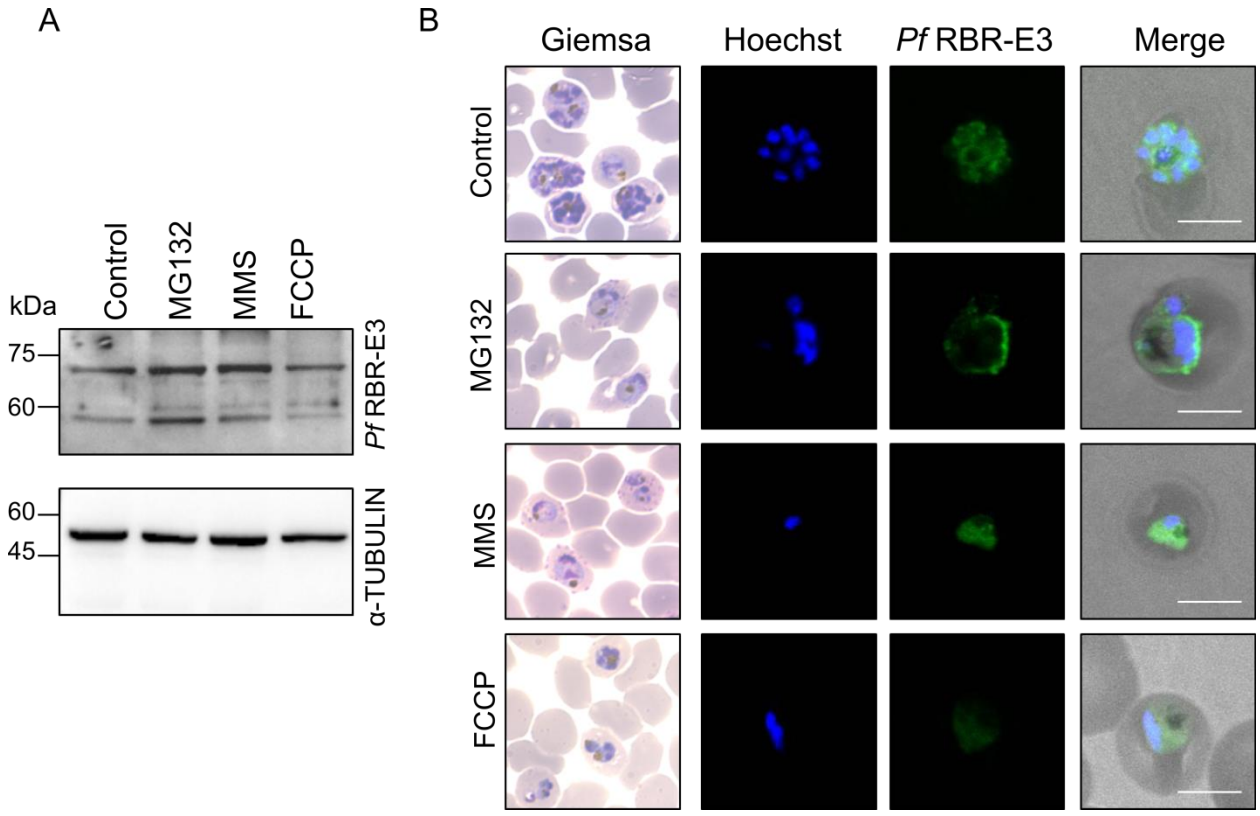

Figure 7: Expression and localization of *Pf* RBR-E3 in parasite under different stress conditions. Infected-RBCs were treated with 2.5 µM MG132, 0.025 % MMS and 7.5 µM FCCP at early trophozoite stage for 6 h. (A) Immunoblot analysis of *Pf* RBR-E3 expression in parasite under different stress conditions. (B) Giemsa and fluorescent microscopic images of infected-RBCs. Immunofluorescence microscopy was done using anti-*Pf* RBR-E3 antibody and parasite nuclei were stained with Hoechst. Scale bar represent 5µm.

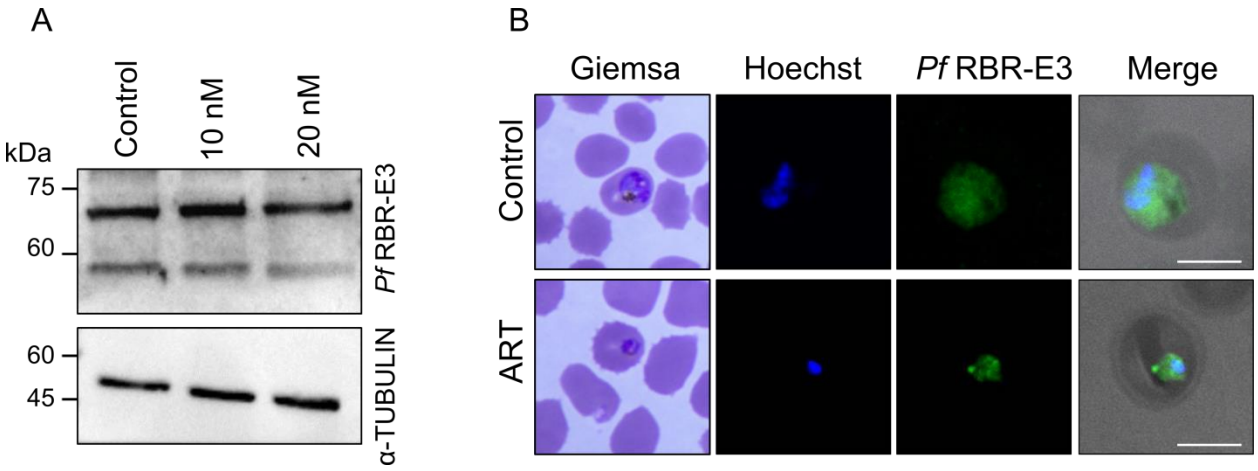

Figure 8: Expression and localization of *Pf* RBR-E3 in presence of α/β-Arteether. Infected-RBCs were treated with Arteether at rings stage for 14 h. (A) Immunoblot analysis of *Pf* RBR-E3 after α/β Arteether (ART) treatment, (B) Giemsa and fluorescent microscopic images of infected-RBCs. Immunofluorescence microscopy was done using anti-*Pf* RBR-E3 antibody and parasite nuclei were stained with Hoechst. Scale bar represent 5µm.