



Fig. S2. Moving junction formation determined by RON4 staining. Naturally egressed parasites were allowed to invade host-cell monolayer for 5 min and cells were immediately fixed for staining. Extracellular parasites were stained with Alexa-594 conjugated DG52 (anti-SAG1). Subsequently cells were permeabilized with 0.1% saponin and TgRON4 were stained with rabbit anti-RON4 followed by Alexa-488 conjugated goat anti-rabbit IgG and total parasites were detected by DG52 followed by Alexa-350 conjugated goat anti-mouse secondary antibody. Based on the staining pattern, parasites were classified as: “invading” when they were partially extracellular and had a RON4 ring; “invaded” if they were completely negative in the extracellular stain; and “bound but no MJ” when they were fully extracellular with no RON4 staining signal. Images representing parasites from each category were shown. Scale bar = 5 μ m.