

Supplemental Figure 3. Quantification of infection in FTE cells by flow cytometry is comparable to fluorescent microscopy. HeLa cells and non-polarized FTE cells were cultured on coverslips in 24-well plate and 12 well plate. Cells were either mock-infected with media alone or infected with CTE3024-mCherry (10 MOI). HeLa cells were cultured with cycloheximide (CHX)-containing medium (500 ng/mL) to prevent over expansion. Cells on coverslips were fixed for microscopy and cells in 12-well plates were harvested 36 hours post-infection to evaluate infection rates. A: Representative immunofluorescent microscopy images of cultures stained with DAPI (blue) and anti-RFP (red) with drawings of DAPI and mCherry outlined particles quantified by ImageJ software. B: Percentage of infected cells in HeLa and non-polarized FTE weas determined by flow cytometry. Dot plots of mock-infected or infected HeLa (N=1) and non-polarized FTE (concatenated, N=3 donors) measuring mCherry expression. Percentages in gate are  $\pm$  SD. C: Bar graph of percent of infected cells as determined by fluorescence microscopy from **A** and flow cytometry from **B**. Statistical analysis of non-polarized FTE cells was performed using non-parametric T test.