Supplementary data

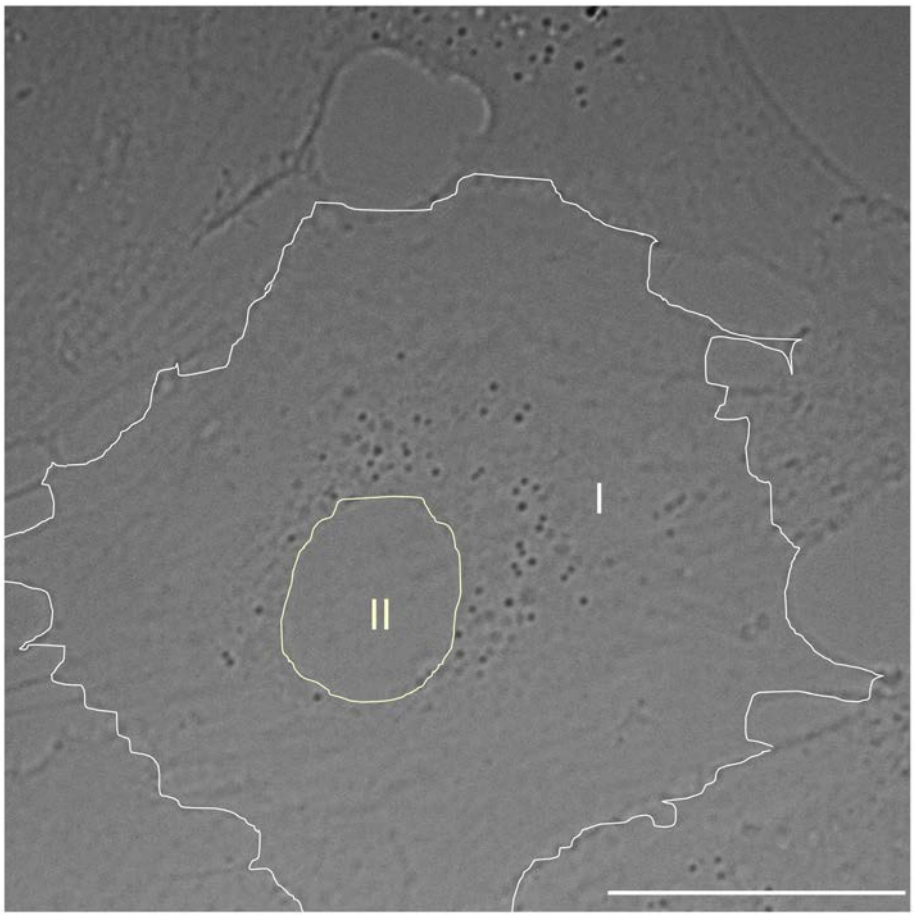

FIG. S1. Representative DIC image of a BS-C-1 cell. In the DIC image the plasma membrane and the nucleus are indicated with a white and off-white line, respectively. The numbers I and II designate cytoplasm and nucleus, respectively. Particles that fused above or in the direct vicinity (< 3 µm) of the nucleus (II) were excluded from tracking behavior analysis. Scale bar: 25 µm.

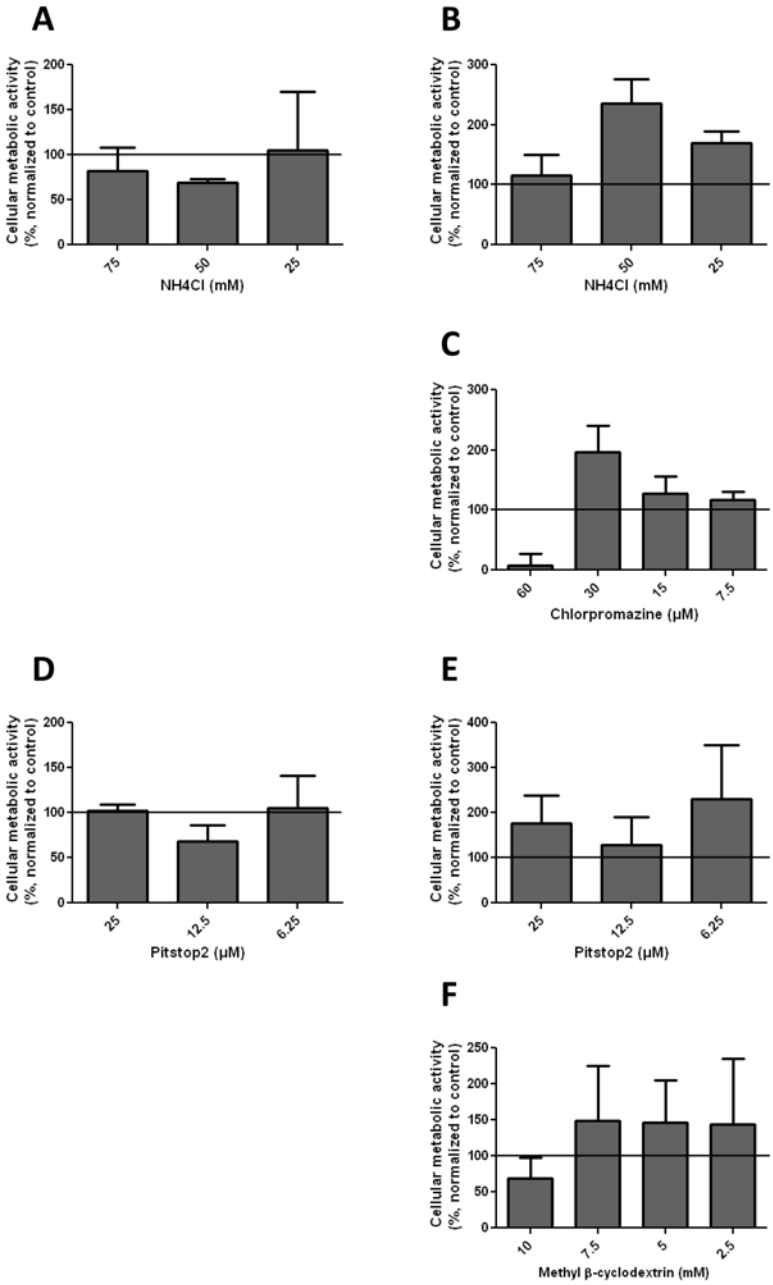

FIG. S2. Cell viability assays. MTT assays of BS-C-1 cells upon treatment with NH4Cl (A, B), Chlorpromazine (C), Pitstop2 (D, E), and methyl-bèta-cyclodextrin (F). In A and D cells were incubated overnight (18 h) in presence of the inhibitor to mimic conditions during overnight infections. In B, C, E and F cells were incubated with the inhibitor for a similar period as for the microscopic cell entry and fusion assay, specifically 1.5 h for NH4Cl; 2.5 h for Chlorpromazine; 45 min for Pitstop2; and 1 h for methyl-bèta-cyclodextrin. For all compounds two individual experiments were performed in triplicate. Error bars represent SD.

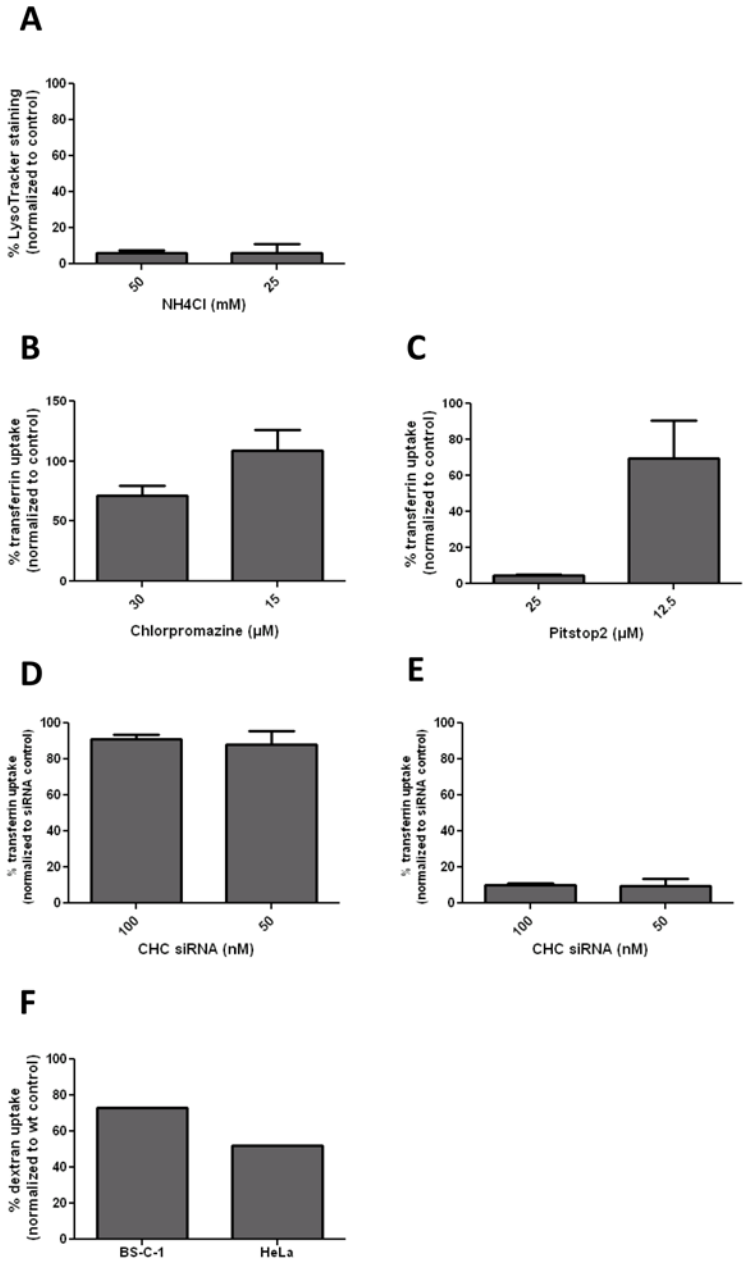

FIG S3. Cargo control assays. LysotrackerGreen, Transferrin-AF633 and Dextran-TexasRed were used as cargo controls for NH4Cl (A); Chlorpromazine (B), Pitstop2 (C) and anti-CHC siRNAs (D,E); and Rab5-S34N (F), respectively. A-D, BS-C-1 cells; E, HeLa cells; F, both BS-C-1 and HeLa cells. (A-E) Cargo controls were analyzed by microscopy. Total fluorescence intensity was analyzed by the 'Particle Analyzer' plugin in ImageJ and normalized to the non-treated control or non-targeting siRNA control. At least three independent experiments were carried out for the cargo control experiments related to NH4Cl, Chlorpromazine, Pitstop2 and siRNAs. Error bars represent SD. (F) Cells were transfected with either wildtype Rab5-GFP or Rab5-S34N-GFP and 2 d post-transfection Dextran was added to the cells. Dextran uptake was analyzed by microscopy and assessed by eye for at least 70 GFP-positive cells per condition. The uptake of Dextran was normalized to the wild type control.

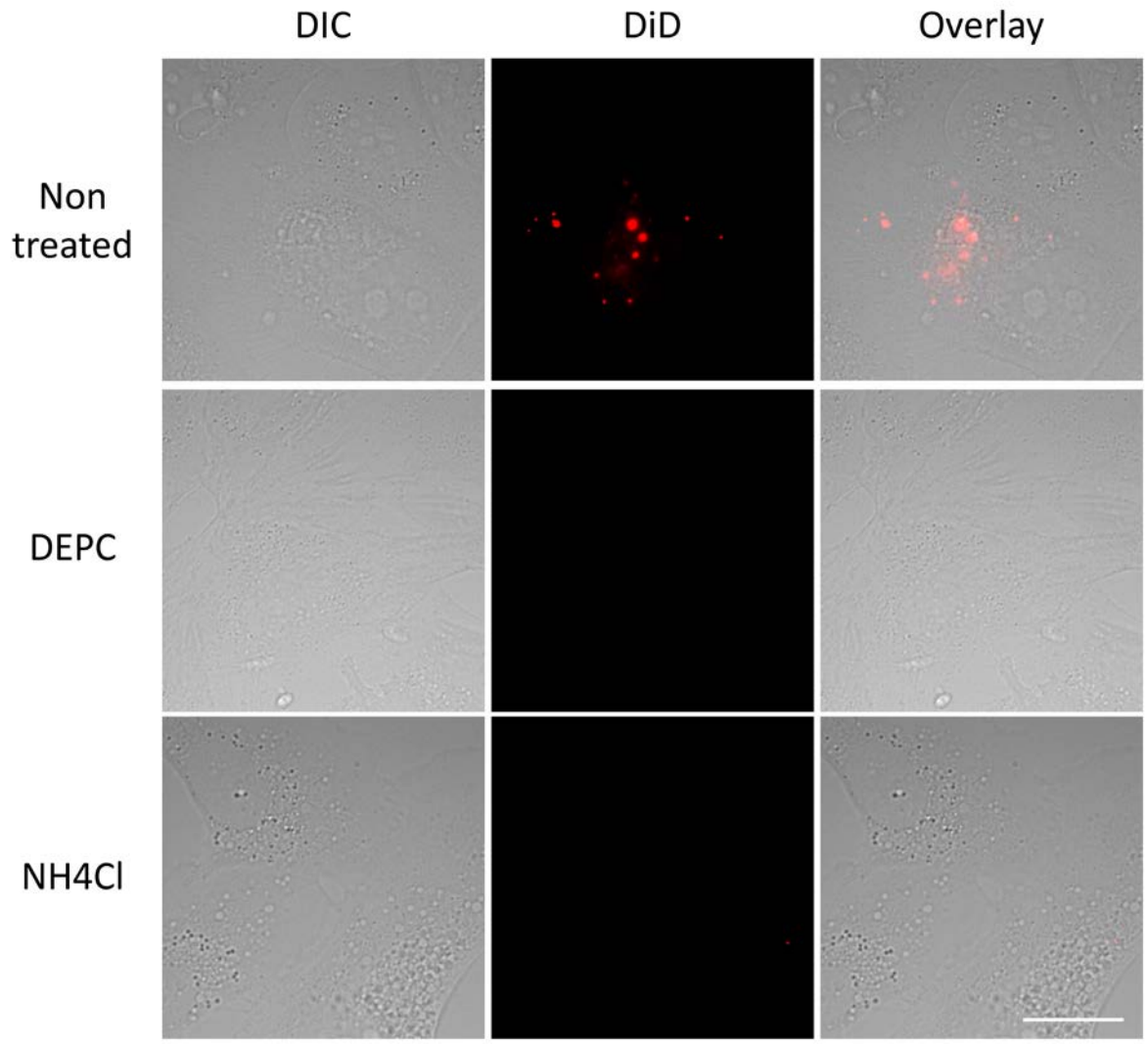

FIG. S4. CHIKV microscopic entry/fusion assay. Representative images of cells infected with DiD-labeled CHIKV. Top, non-treated positive control; middle, DEPC treated DiD-labeled CHIKV (2 mM DEPC for 30 min); bottom, BS-C-1 cells prior treated with 50 mM NH4Cl for 1 h. One representative image per condition (DIC, DiD, overlay) is shown. Intensity is equally enhanced for visual purposes. Fusion events appear as bright red spots in the DiD channel. Scale bar: 25 µm.

Supplementary data
Fig. 5

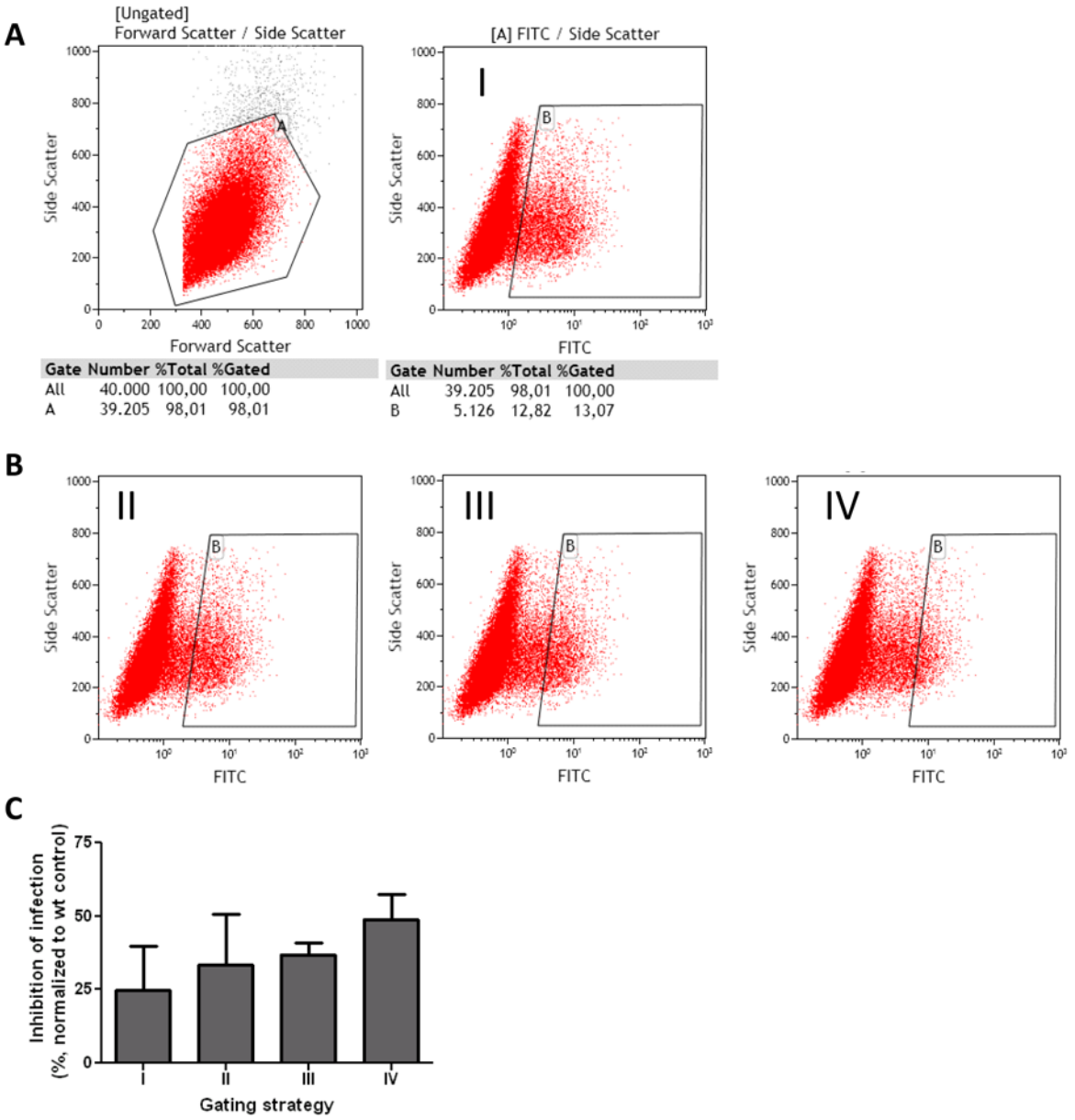

FIG S5. Flow cytometry gating strategy in Rab5-GFP-expressing cells. (A) Standard gating (I) strategy. (B) Step-wise gating as function of GFP expression (II-IV). (C) The percentage inhibition of infection found for Rab5-S34N compared to wild type Rab5 in the different GFP-gating strategies. The experiment is repeated twice in triplicate. Error bars represent SD.

Video S1. CHIKV fusion event. DiD-labeled CHIKV particle (red) resides in the left down corner before travelling towards the right upper corner and fusing. Fusion is seen as a sudden burst in fluorescence intensity. Movie shows the same particle as in Fig. 1A (no treatment). Image recording was performed at 1 frame/s. Virtual time is shown in the right down corner.

Video S2. CHIKV/clathrin colocalization. Movie S2A, S2B, S2D show a DiD-labeled CHIKV particle (red) that first colocalizes with clathrin-YFP, then the clathrin signal disappears and the virus travels through the cell and is visible till the moment of membrane fusion. Clathrin-YFP is green and colocalization is visible as yellow. Membrane fusion occurs in the last seconds of the movie and can be detected as a sudden increase in fluorescence intensity. In Movie S2C and S2E we zoom in on CHIKV-clathrin colocalization of movie S2B and S2D, respectively. Image recording was performed at 1 frame/s. For all movies the virtual time is shown in the right down corner.

Video S3. CHIKV fusion upon Rab5 colocalization. S3A. DiD-labeled CHIKV particle that travels towards a Rab5-positive endosome and fuses within 2 s after colocalization. S3B. DiD-labeled CHIKV particle that travels towards a Rab5-positive endosome and fuses within 21 s after colocalization (virtual time). S3C. DiD-labeled CHIKV particle moves towards an early endosome and fuses approximately 30 seconds after initial colocalization (virtual time). For all movies virtual time is shown in the right down corner.