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Title	Host ESCRT factors are recruited during chikungunya virus infection and are required for the intracellular viral replication cycle
Author(s)	Torii, Shiho; Orba, Yasuko; Sasaki, Michihito et al.
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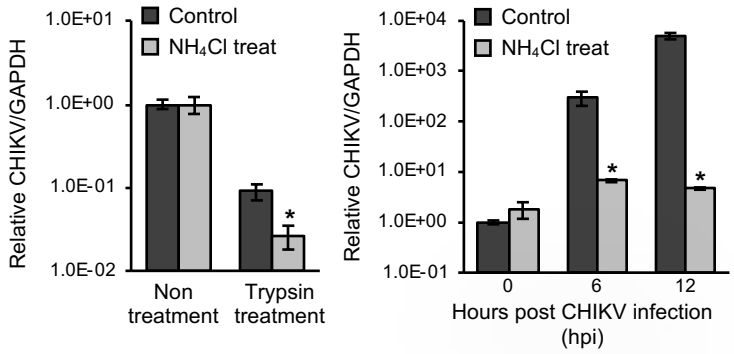


Figure S1. Intracellular CHIKV RNA levels under trypsin and NH_4Cl treatments

Left bar graph showed the intracellular viral RNA levels of control and NH_4Cl-treated cells at 3 hpi of CHIKV infection (MOI=0.1) with or without trypsin treatment before RNA extraction. Right bar graph showed the intracellular viral RNA levels of control and NH_4Cl-treated cells at 0, 6 and 12 hpi of CHIKV infection (MOI=0.1). All of the cells were treated with trypsin before RNA extraction. The data were normalized against control cells and represented as means $\pm$ S.D., collected in triplicate and independently at least 2 times. Statistical significance was assessed using Student's *t*-test and indicated by asterisks (*, $p<0.05$ compared between control cells).

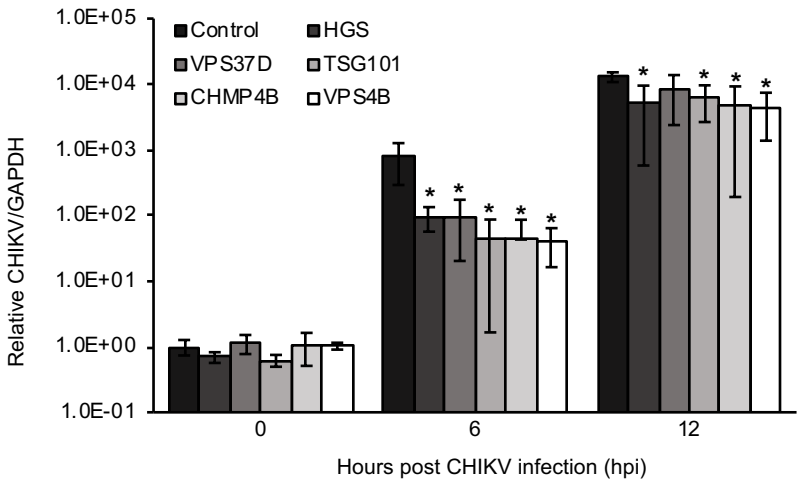


Figure S2. Time course of CHIKV genome expression levels in siRNA-treated HEK293T cells
HEK293T cells were infected with CHIKV (MOI=0.1) and intracellular viral RNA levels were measured at 0, 6 and 12 hpi. Viral RNA levels were determined by qRT-PCR using total RNAs extracted from trypsinized CHIKV-infected cells and primers targeting the CHIKV nsP4 region. All of the cells were treated with trypsin before RNA extraction. The data were normalized against control cells and represented as means ± S.D., collected in triplicate and independently at least 2 times. Statistical significance was assessed using one-way ANOVA with Dunnett's test and indicated by asterisks (*, $p<0.05$ and **, $p<0.001$ compared between control siRNA-treated cells).

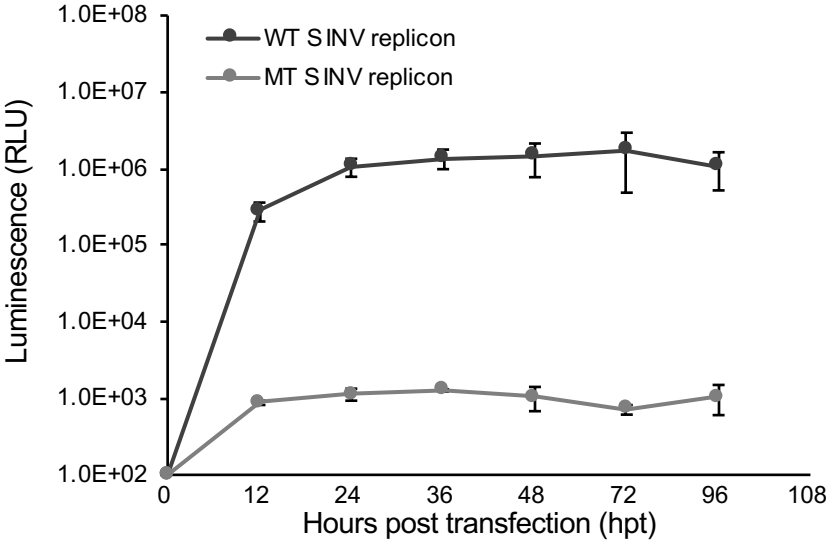


Figure S3. Construction and characterization of Sindbis virus (SINV) replicon
Wild type (WT) SINV replicon- or RNA-dependent RNA polymerase-inactivated mutant (MT) replicon-expressing plasmids were constructed, encoding NanoLuc luciferase. HEK293T cells were transfected with either WT or MT SINV replicon-expressing plasmids and the luciferase activities were measured for 96 hours. The detailed information of SINV and DENV replicon-expressing plasmids was described in Materials and Methods.

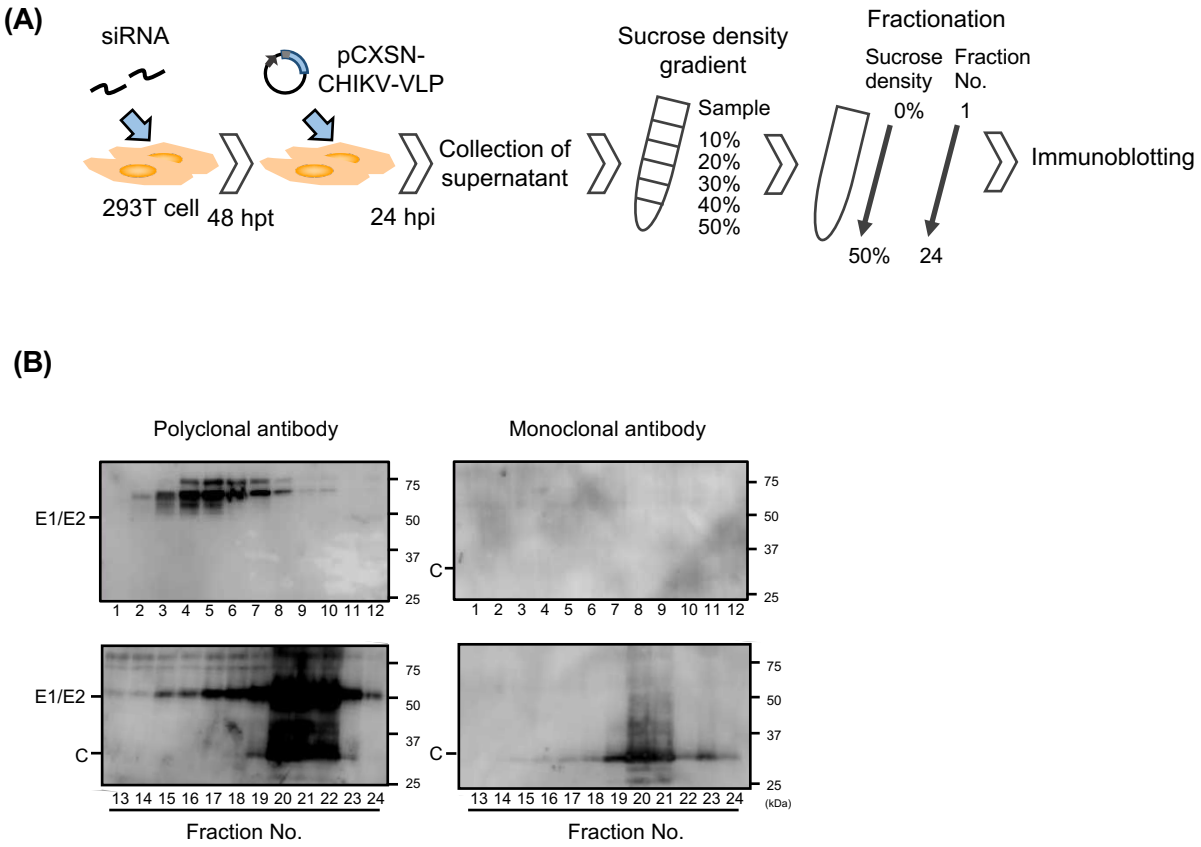


Figure S4. Construction and characterization of chikungunya virus-like particle (CHIKV-VLP)

(A) Scheme of the workflow of the density gradient sedimentation analysis. The cells were transfected with the pCXSN-CHIKV-VLP plasmid and the supernatants were collected at 96 hpt, following the sucrose density gradient sedimentation. (B) The supernatants were fractionated through sucrose gradients (0-50%) into 24 fractions and lysed with TNE buffers. The expression of structural protein in each fraction was confirmed by immunoblotting for structural proteins using anti-CHIKV pAb or mAb 5.5G9, respectively.

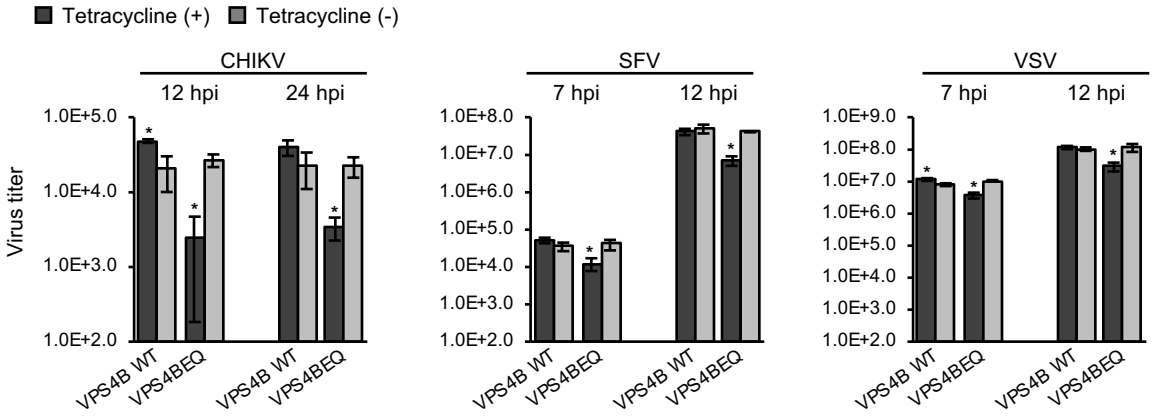


Figure S5. The expression of dominant-negative VPS4B inhibited infection of some alphaviruses
The expression of VPS4B WT or VPS4BEQ were induced with doxycycline for 24 hours, following infection of CHIKV, SFV or VSV (MOI=0.1). Virus titers in the supernatants at the indicated time points were determined by plaque assay. All data were represented as means ± S.D., collected in triplicate and independently at least 2 times. Statistical significance was assessed using Student's *t*-test. and indicated by asterisks (*, $p<0.05$ compared between control cells).