Cooperation between different RNA virus genomes produces a new phenotype

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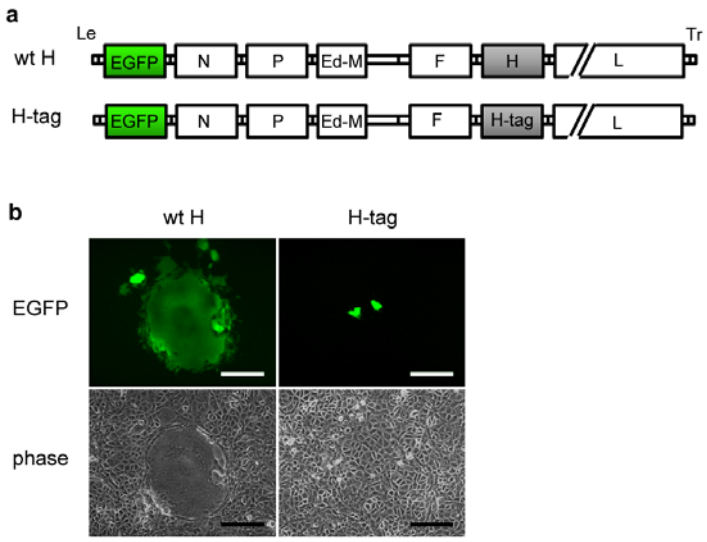
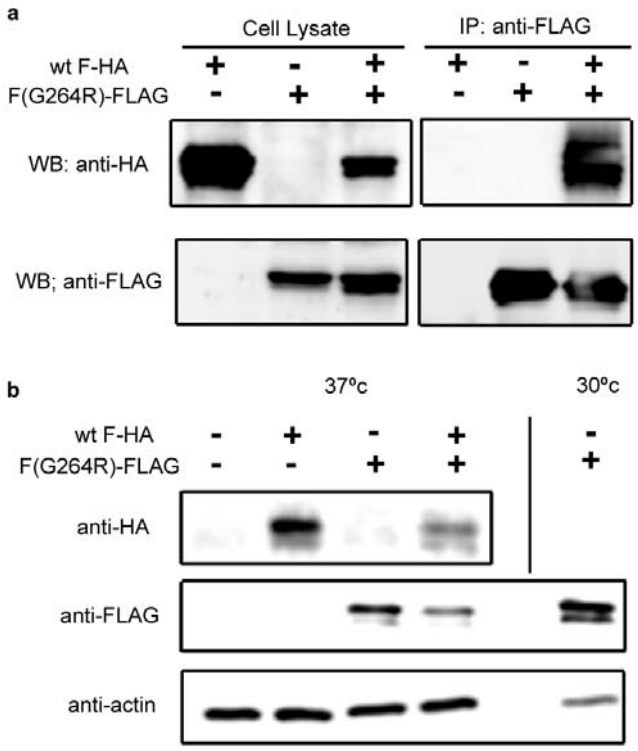
Supplementary Figures: S1-S7

Supplementary Figure S1



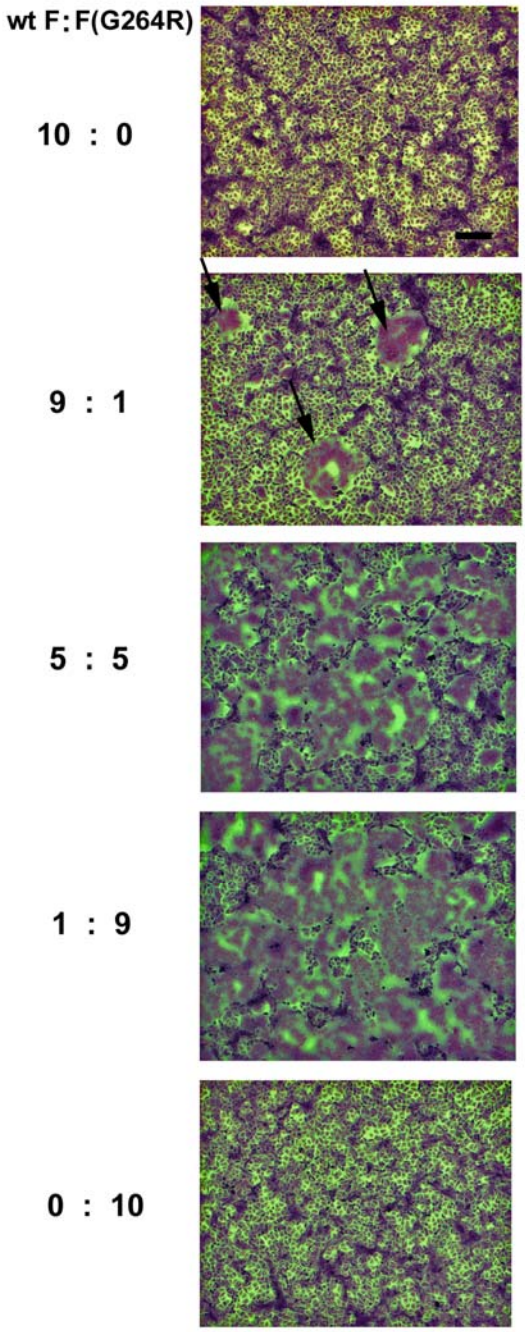
Supplementary Figure S1: Fusion-interfering effect of the FLAG-TEV-MYC tag fused to the MV H protein. (a) The genomes of two recombinant MVs. Although each viral genome is based on the wild-type IC-B strain, the M gene was replaced by the Edmonston strain M gene (Ed-M) to facilitate virus particle formation. The H-tag gene encodes the H protein fused to the FLAG-TEV-MYC tandem tag at its C-terminus. Le, leader. N, nucleocapsid. P, phospho. L, large. Tr, trailer. (**b)** Vero/hSLAM cells were infected with each recombinant virus. At 2 days postinfection, the cells were observed under a fluorescence or a phase-contrast microscope. Scale bars, 200 μm.

Supplementary Figure S2



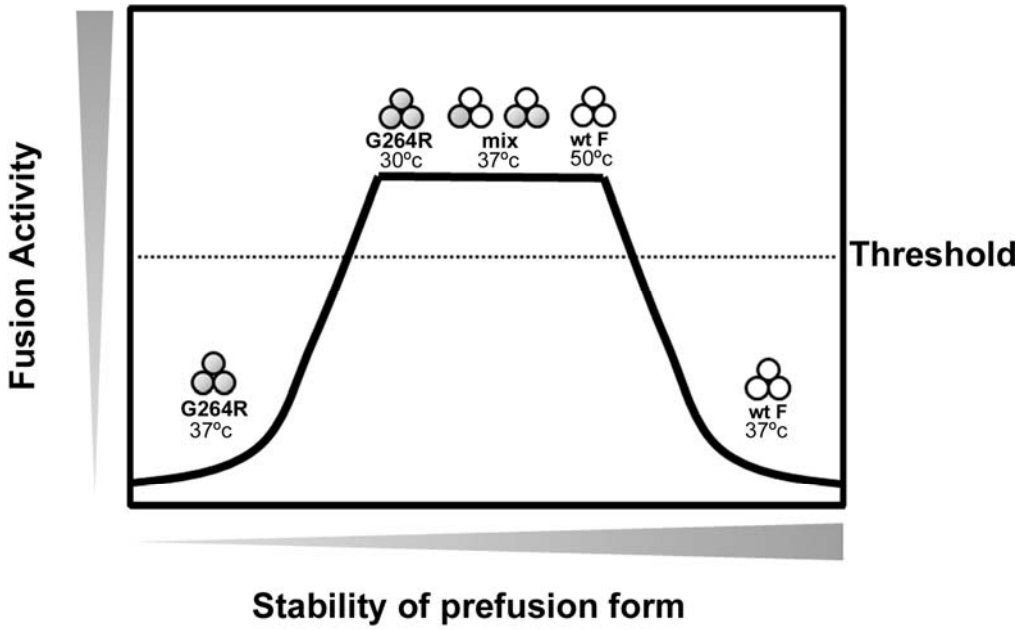
Supplementary Figure S2: Hetero-oligomer formation and protein expression levels of wt F and F(G264R) proteins. (a) Co-immunoprecipitation of wt F and F(G264R) proteins was performed as described in Methods. (b) Expression levels of wt F and F(G264R) proteins were examined under several conditions. Plasmids encoding HA-tagged wt F (2 μg) and/or FLAG-tagged F(G264R) proteins (2 μg) were transfected into 293T cells at 37 °C or 30 °C. The total amount of transfected plasmids was adjusted to 4 μg using empty vector pCA7. Cells were lysed 24 h after transfection and analysed by SDS-PAGE and Western blot analysis.

Supplementary Figure S3



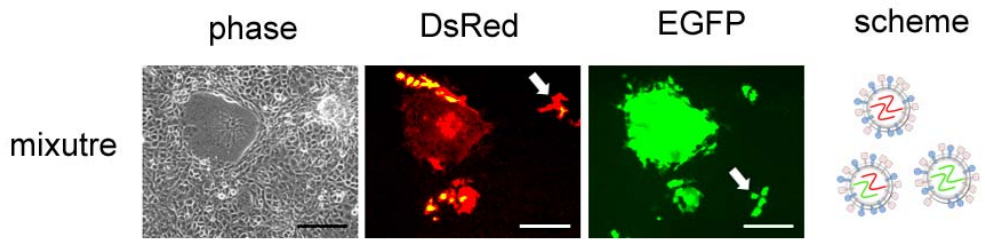
Supplementary Figure S3: Fusion assay by using various ratios of the plasmid encoding wt F to that encoding F(G264R). Vero/hSLAM cells were transfected with plasmids encoding H-tag, and wt F and/or F(G264R) proteins. The ratios of the plasmid encoding the wt F protein to that encoding the F(G264R) protein were changed from 10:0 to 0:10. At 24 h posttransfection, the cells were subjected to Giemsa staining and observed under a light microscope. Scale bars, 200 μm.

Supplementary Figure S4



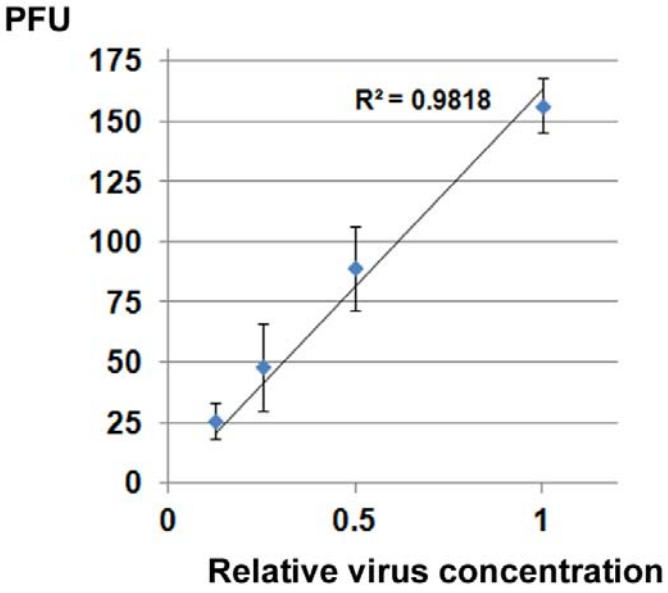
Supplementary Figure S4: A model for fusion triggering. The figure depicts the possible relationship between the stability of the F protein prefusion form and its fusion activity. Protein stability may not be proportional to fusion activity, but there appear to be appropriate levels of stability to induce membrane fusion. When stability is too high or too low, fusion may not occur.

Supplementary Figure S5



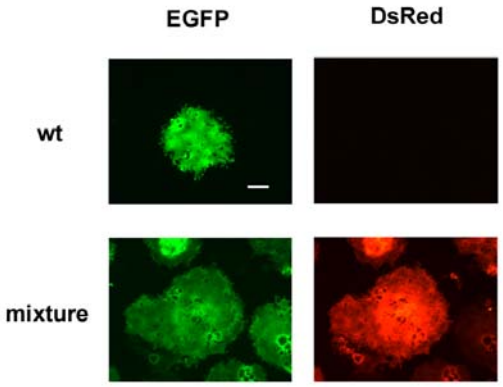
Supplementary Figure S5: Cells infected with recombinant viruses. Viruses were prepared with both the full length genome plasmid carrying the wt F gene and one carrying the mutant F(G264R) gene. The genomes of resulting viruses are schematically shown. Infected cells expressing both DsRed and EGFP formed syncytia, while those expressing either Ds-Red or EGFP did not (white arrow). Scale bars, 200 μm.

Supplementary Figure S6



Supplementary Figure S6: Linear relationship between the numbers of plaques and dilution rates. The same stock of the mixed genome virus was serially diluted, and monolayers of Vero/hSLAM cells on 12-well cluster plates were infected with the diluted samples. After being incubated for 1 h at 37 °C, the cells were overlaid with Dulbecco's modified Eagle's medium containing 7.5% fetal bovine serum and 1% methylcellulose. At 4 days p.i., the numbers of plaques (expressed as plaque-forming units (PFU)) were counted under a fluorescence microscope. R squared (R^2) value of the data is shown. Error bars, standard deviations.

Supplementary Figure S7



Supplementary Figure S7: Viruses recovered from the brains infected with wt and mixed genome viruses. Seven-day-old hamsters were inoculated intracerebrally with the wt or mixed genome (mixture) virus (depicted in Fig. 4). Six days after inoculation, their brains were collected and viruses were recovered in Vero/SLAM cells. The cells were observed under a fluorescence microscope. Scale bars, 200 μm.