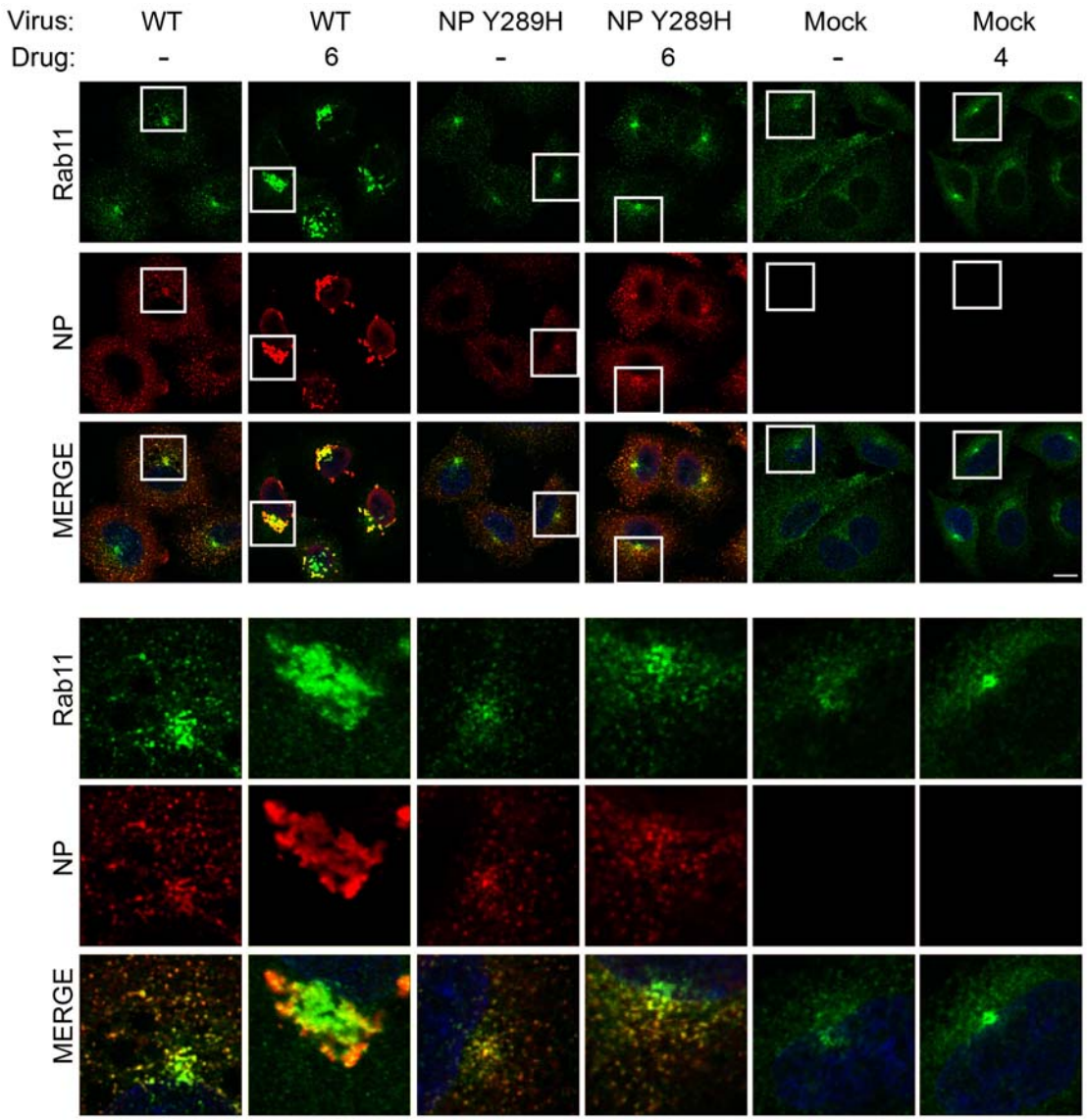
Supplemental Figure Legends:

Fig S1. Cytoplasmic colocalisation of NP and Rab11 with and without nucleozin treatment. A549 cells were infected (or mock infected) with WT virus and either left untreated or treated with 1 μM nucleozin at the indicated times. Samples were fixed at 8hpi and (A) stained for Rab11 and NP. The lower half of the figure shows high magnification images of the inset regions in the upper half.

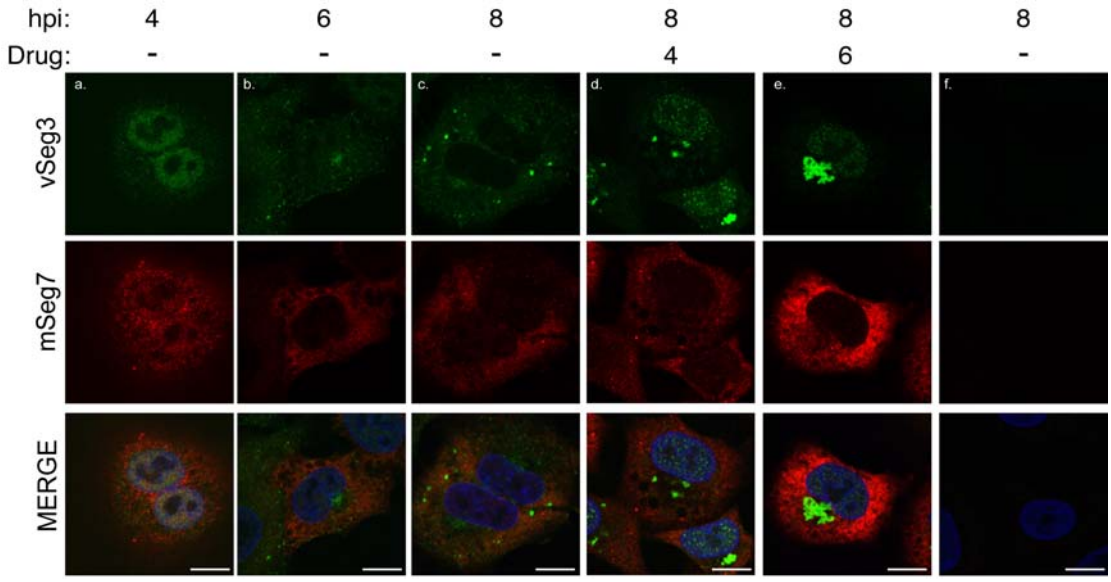
Fig S2. Nucleozin does not induce aggregation of viral mRNA. A549 cells were infected (a.-e.)(or mock infected, f.) with WT PR8 virus, treated with 1 μM nucleozin at the times shown (d. and e.) and fixed at 4 (a.), 6 (b.) and 8h.p.i. (c.-e.). Coverslips were stained for segment 7 viral mRNA and for segment 3 vRNA. Merged images include a DAPI channel shown in blue. Scale bar represents 10 μm. Images were acquired using a SPE confocal microscope and images processed using LAS AF Lite.

Fig S3. Nucleozin overrides ordinary transient interactions between vRNP decorated vesicles. A549 cells were transfected with GFP-NP and 12 h later, infected with WT PR8 before imaging at high magnification under time-lapse conditions (every 1.406 seconds) at 8h p.i. either without (A) or (B) 3 min after nucleozin addition. Selected still images of the whole cell and the inset regionss are shown to illustrate vRNP interactions (white arrowheads). Scale bars represent 7.5 μm. Images were acquired using a SPE confocal microscope and images processed using LAS AF Lite and Image J Fiji. See Supplementary Movies S4-5.

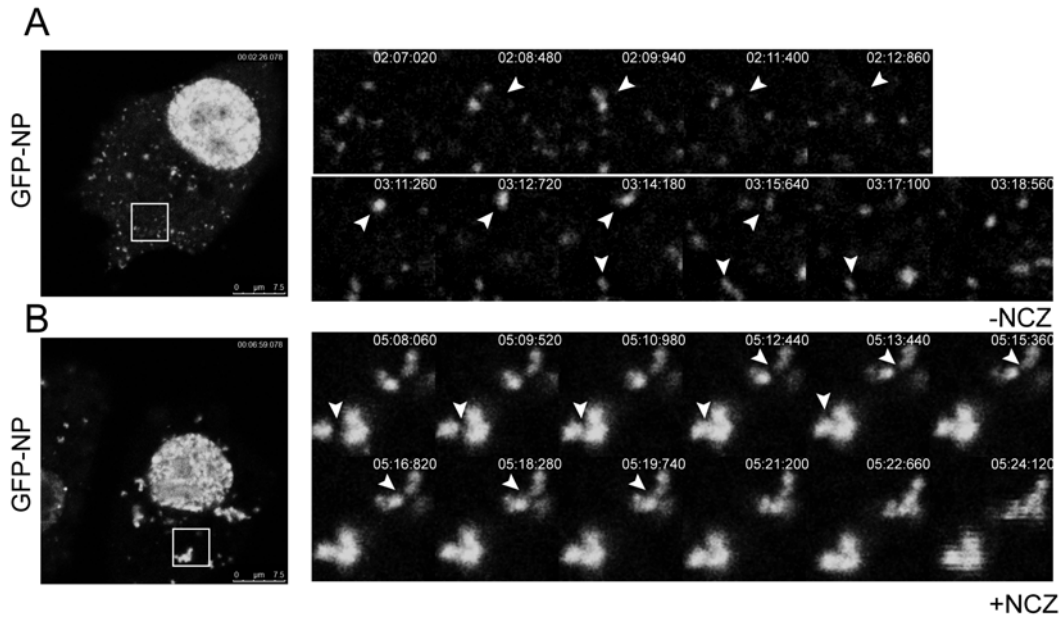
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Movie Legends

Each movie is representative of a minimum of 5 independent experiments, each experiment containing a minimum of 4 movies for each condition.

Movies S1-S3 Time lapse movies of GFP-NP and RFP-Rab11 trafficking in living infected cells. A549 cells were transfected with GFP-NP and RFP-Rab11 plasmids and incubated at 37 ºC for 12h before (S1) a final concentration of 2 μM nucleozin was added (when the screen goes black) and imaging continued, (S2) being infected with PR8 virus at a MOI=3 for 8 h prior to imaging, (S3) infected with PR8 at an MOI=3 for 8 h prior to imaging, a final concentration of 2 μM nucleozin added (when the screen goes black) and imaging continued. All movies were taken on a Leica SPE confocal microscope at a rate of 1 frame/ 4 sec for a period of 30 minutes. Scale bars were added to each movie, and total witdh correspondes to a length of ~40 (Movie S1, S3) or 27.5 μm (Movie S2).

Movies S4-S5 Vesicular trafficking of GFP-NP in living infected cells. A549 cells were transfected with GFP-NP plasmid and incubated at 37 ºC for 12h before (S4) being infected with PR8 virus at a MOI=3 for 8 h prior to imaging for 5 minutes, or (S5) adding a final concentration of 2 μM nucleozin and acquiring images (from 1 min to 20 min after adding nucleozin) on a Leica SPE confocal microscope at a rate of 0.33 frame/sec. The total width represents a length of 5 μm. Movie was saved at 10 fps rate.