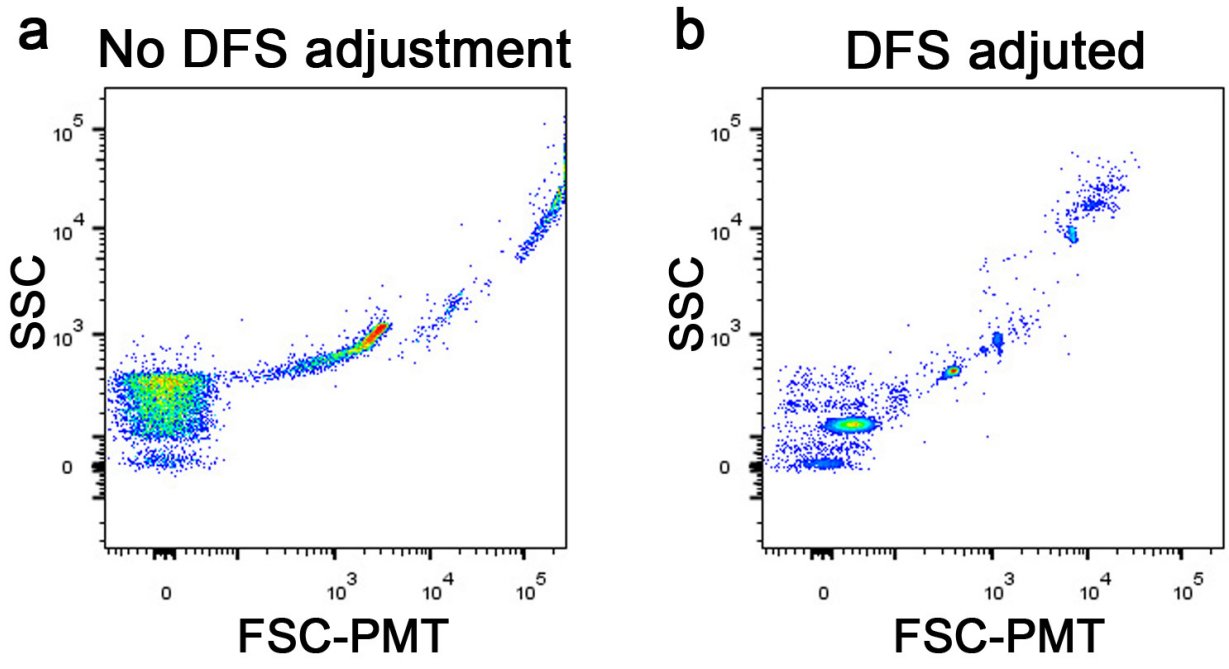
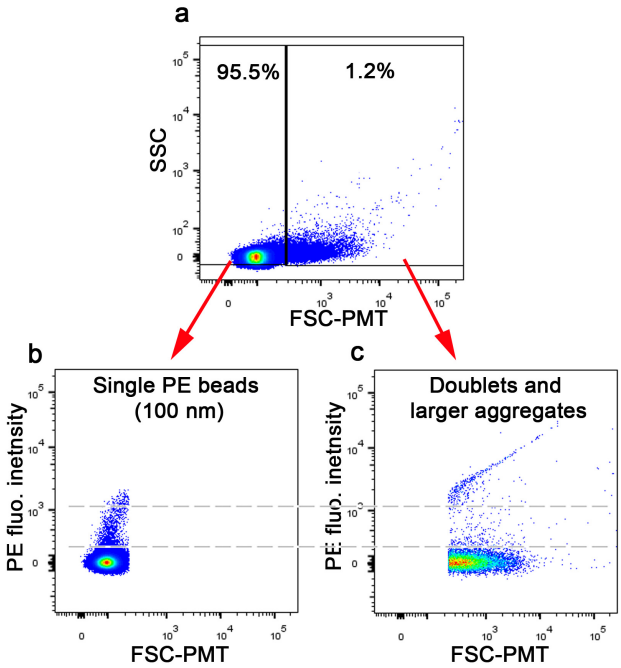
Supplementary Figure 1



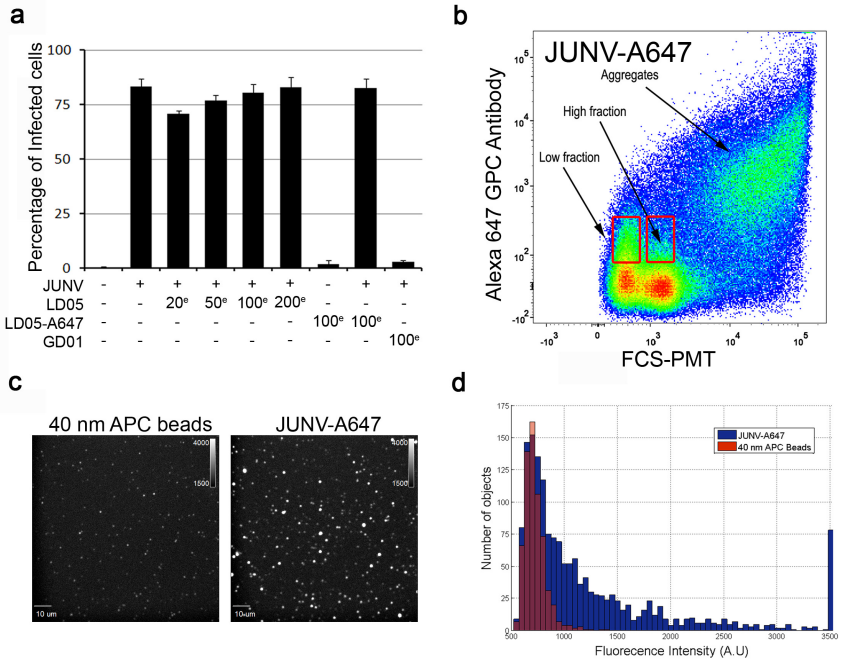
Supplementary Figure 1. Efficiency of the digital focusing system (DFS). Megamix Plus-FSC beads of 100, 300, 500 and 900 nm were run with **(b)** or without **(a)** DFS adjustment. The plots show Side scatter (SSC) in function of FSC-PMT. Sample resolution is strongly improved by DFS correction.

Supplementary Figure 2



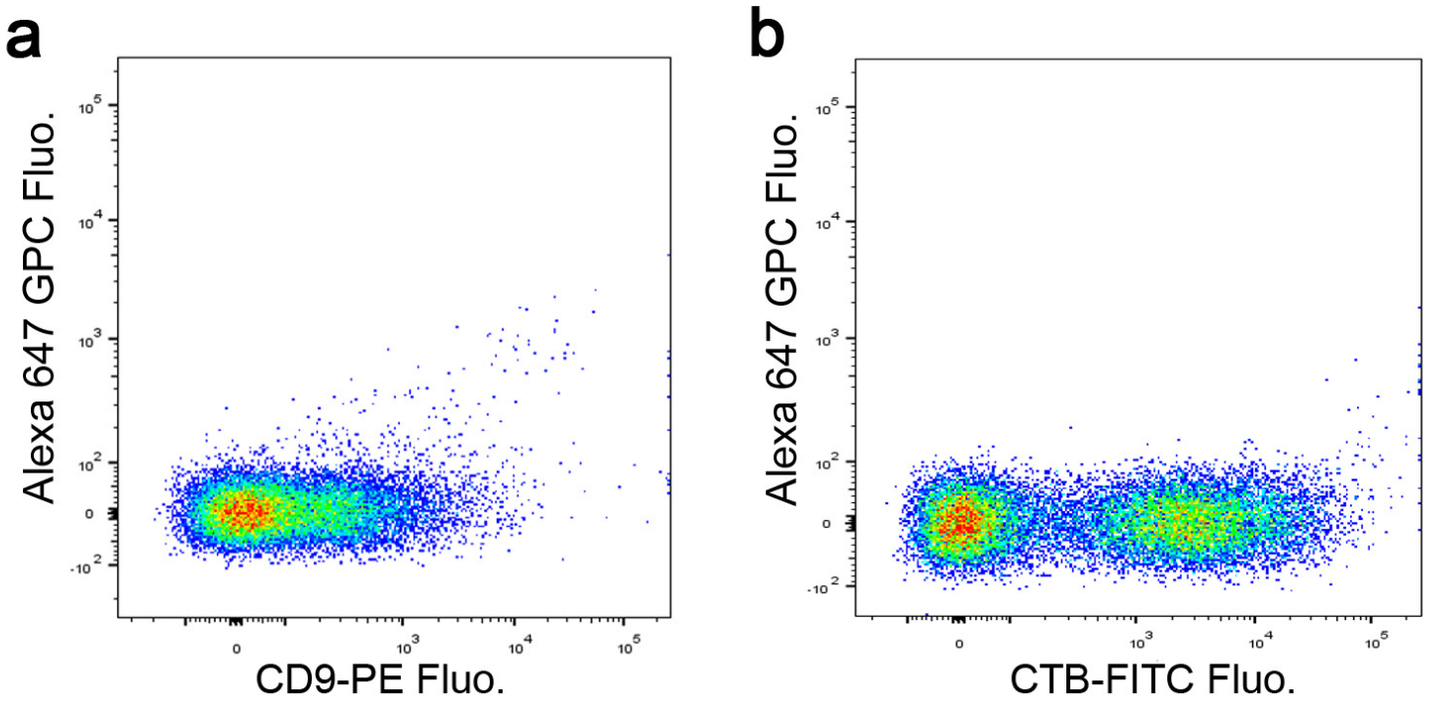
Supplementary Figure 2. Flow cytometry detection of single 100 nm objects. (a-c) 100 nm PE-conjugated latex beads were analyzed on our custom-made flow cytometer. (a) The Plot represents SSC as a function of the FSC-PMT parameter. Two gates were generated. The left one represents events at the level of the background. The right one corresponds to events that could be detected by FSC-PMT alone and therefore of at least 150-200 nm in diameter. From each gates PE fluorescence intensity was analyzed (b,c). From the left gate, events with low but significant PE fluorescence intensity could be detected (b), likely corresponding to single beads (or events smaller than 150 nm). From the right gate, events with higher PE fluorescence intensity could be detected (c), likely corresponding to doublets and larger aggregates. The area between the two gray dashed lines shows PE positives events that are above the noise level but below the intensity of doublets and larger aggregates.

Supplementary Figure 3



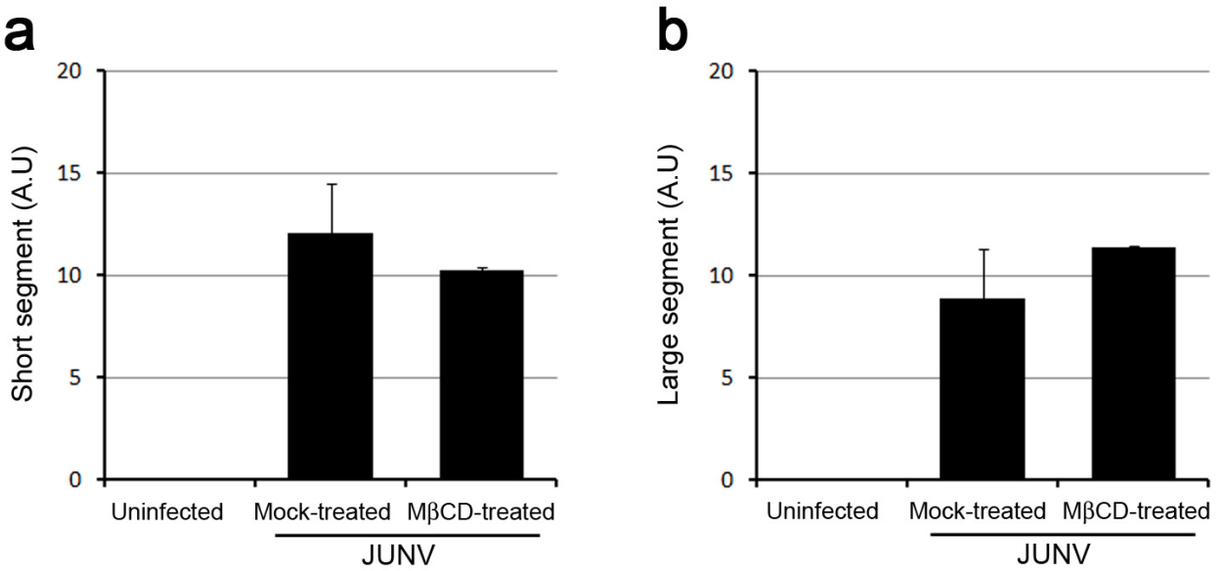
Supplementary Figure 3. Characterization of the JUNV-A647 preparation (a) Vero cells were cooled down at 4°C for 15 min and cold JUNV was added to the cells for 30 min. Then, viruses were washed away and indicated concentrations of the LD05, LD05 coupled to Alexa Fluor 647 or GD01 antibodies, all GPC-specific, were incubated for an additional 30 min at 4°C. Cells were then extensively washed and incubated for 16 hrs at 37°C to allow infection of the bound viruses. Next, cells were harvested, fixed, permeabilized and stained using the anti-Nucleoprotein (NP) antibody SA02 coupled to an Alexa Fluor 647 to detect infected cells by flow cytometry. The LD05 antibody, either coupled to a fluorophore or not, was poorly neutralizing, while the GD01 antibody was very neutralizing. Flow virometry assays were all performed at a dilution of the LD05 antibody of at least 1:200. (b) Plots from figure 1g representing the gates used to sort the low and high fractions. (c) 40 nm APC beads (left panel) or JUNV particles stained with a GPC antibody coupled to an Alexa Fluor 647 (right panel), were adsorbed on glow discharged glass coverslip and mounted on slides. Micrographs were acquired by spinning disk confocal microscopy. The acquisition procedure and contrast parameters (upper right) used were the same for both samples. Bar = 10 µm. (d) The histograms show the intensity distribution of the detected beads (red) or viruses (blue). While beads exhibit low homogeneous intensity levels, JUNV-A647 presents a broader distribution and higher mean intensity.

Supplementary Figure 4



Supplementary Figure 4. Single labeled particles show no crosstalk to other relevant channels. (a,b) Flow virometry assay of JUNV particles stained with CD9-PE only (a) or CTB-FITC only (b). Dot plots represent the Alexa Fluor 647 GPC fluorescence (y axis) as a function of PE or FITC fluorescence (x axis). Fluorophores are excited from 488 nm and 640 nm lasers and fluorescence emission collected through 530/30 (FITC), 575/25 (PE) and 660/20 (Alexa Fluor 647) bandpass filters, allowing minimal crosstalk between fluorophores.

Supplementary Figure 5



Supplementary Figure 5. MβCD treatment does not affect JUNV RNA particle release. (a,b) Vero cells infected for 24 hrs with JUNV were mock-treated or treated with 10 mM Methyl-β-Cyclodextrin (MβCD-treated) for 1 hr at 37°C. Cells were then extensively washed in PBS and incubated for an additional 12 hrs at 37°C in DMEM containing 2% FBS. Subsequently, JUNV-containing supernatant was harvested and pelleted by ultracentrifugation for RNA purification. Relative amounts of RNA contained in 500 µl of mock-treated or MβCD-treated samples were measured by reverse transcriptase RT-qPCR with primers targeting the Short (a) or the Large (b) segment of JUNV. Both segments are expressed in similar amount in mock-treated and MβCD-treated conditions. Error bars are mean +/- SD of RT-qPCR duplicates from an experiment performed in duplicate.