1 Supplementary material.

2 SFigure 1



SFig. 1. The rac1 and rhoA proteins co-purified with RSV particles. HEp2 cells were 5 6 RSV-infected using a multiplicity of infection of 0.01 and at 48 hrs post-infection the virus was purified as described previously (Radkakrishnan et a, 2010). (A) The bands containing 7 8 infectious virus particles (band 1) and non-infectious virus particles (band 2) were harvested 9 and (B) the presence of the cdc42, rhoA and rac1 proteins detected by immunoblotting using appropriate antibodies. Proteins are expected are highlighted (black arrow). (C) Cell lysate 10 11 prepared from RSV-infected cells (V) and purified cdc42 protein (cdc42) detected by 12 immunoblotting using anti-cdc42.

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18 SFigure 2. Time course of infection with RSV in HEp2 cells. HEp2 cells were infected 19 with RSV using a multiplicity of infection of 0.1 and at between 2 and 18 hrs post-infection 20 the cells were co-stained with anti-G and anti-P, and visualized using immunofluorescence 21 microscopy (objective x20 magnification). The representative infected cells (*) are shown in

22	the enlarged insets. The inclusion bodies (short arrows) and anti-G stained cells (long arrow)
23	are highlighted.
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41 SFigure 3



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43 SFigure 3. Temporal appearance of the virus filaments in HEp2 cells. HEp2 cells were 44 infected with RSV using a multiplicity of infection of 0.1 and at (A) 12, (B) 14 (C) 16 and 45 (D) 18 hrs post-infection the cells were co-stained with anti-G and anti-P. The co-stained 46 cells were imaged using confocal microscopy at a focal plane that allowed visualization of (i) 47 the virus filaments and (ii) the inclusion bodies. The virus filaments (white arrows), inclusion 48 bodies (*) and punctuate non-filamentous anti-G staining pattern (yellow arrows) are 49 highlighted. Insets are enlarged regions taken from the main plates.

50 SFigure 4





52 SFigure 4. Effect of NSC23766 RSV-infected cells. (A and B) HEp2 cells were either (i) 53 mock-infected or RSV-infected with a multiplicity of 0.1 in (ii) the absence (NT) or (iii) 54 presence of NSC23766 using a multiplicity of infection of 2. At 18 hrs post-infection the cells 55 were co-stained with anti-G and phalloidin-FITC (F-actin) and visualized using

56	immunofluorescence microscopy. (A) (objective x20 magnification). (ii) The anti-G and
57	phalloidin-FITC co-stained filamentous projections in the non-treated cells are highlight (by
58	white arrows). and (objective x100 magnification). (C) RSV infected cells were stained using
59	anti-actin and anti-F and imaged using confocal microscopy. (i) Individual channels (grey
60	scale) and (ii) Merged (two-color) image showing the corresponding actin and F protein
61	distribution. Inset is an enlarged image of the region highlighted (white open-box).
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76 SFigure 5





by fluorescence microscopy (objective x100 magnification).



The cells were image using (A) objective magnification x20 or (B(i)) and (C(i)) objective magnification x100 (oil). The virus filaments are highlighted (black arrow). In each case (ii) is an enlarged image taken from corresponding the anti-filamin1 stained plate in (i). (C(ii)) the filamentous anti-filamin1 staining pattern (black arrow) following RSV infection is highlighted.

142 Table. S1.

RSV-F(f)	5'GCGGTACCATGGAGTTGCTAATCCTC3'
RSV-F(r)	5'GCCTCGAGTTAGTTACTAAATGCAATATTATTATACC3'
RSV-F-FLAG(r)	5'CGCCTCGAGAAT <u>TTTATCGTCATCGTCTTTGTAATC</u> CAAACTAAATGCAATATTATTTATACC3'
RSV-G(f)	5'GCGGTACCATGGCCAAAAACAAGGACC3'
RSV-G(r)	5'GCCTCGAGCTACTGGCGTGGTGTGTT3'
RSV-G-FLAG (r)	5'CGCCTCGAGCTATTTATCGTCATCGTCTTTGTAATCCTGGCGTGGTGTGTGGGGGGAGA 3'

The nucleotide sequence of the forward (f) and reverse (r) PCR primers used in the cloning of the RSV A2 F and G genes. The FLAG sequence is underlined.