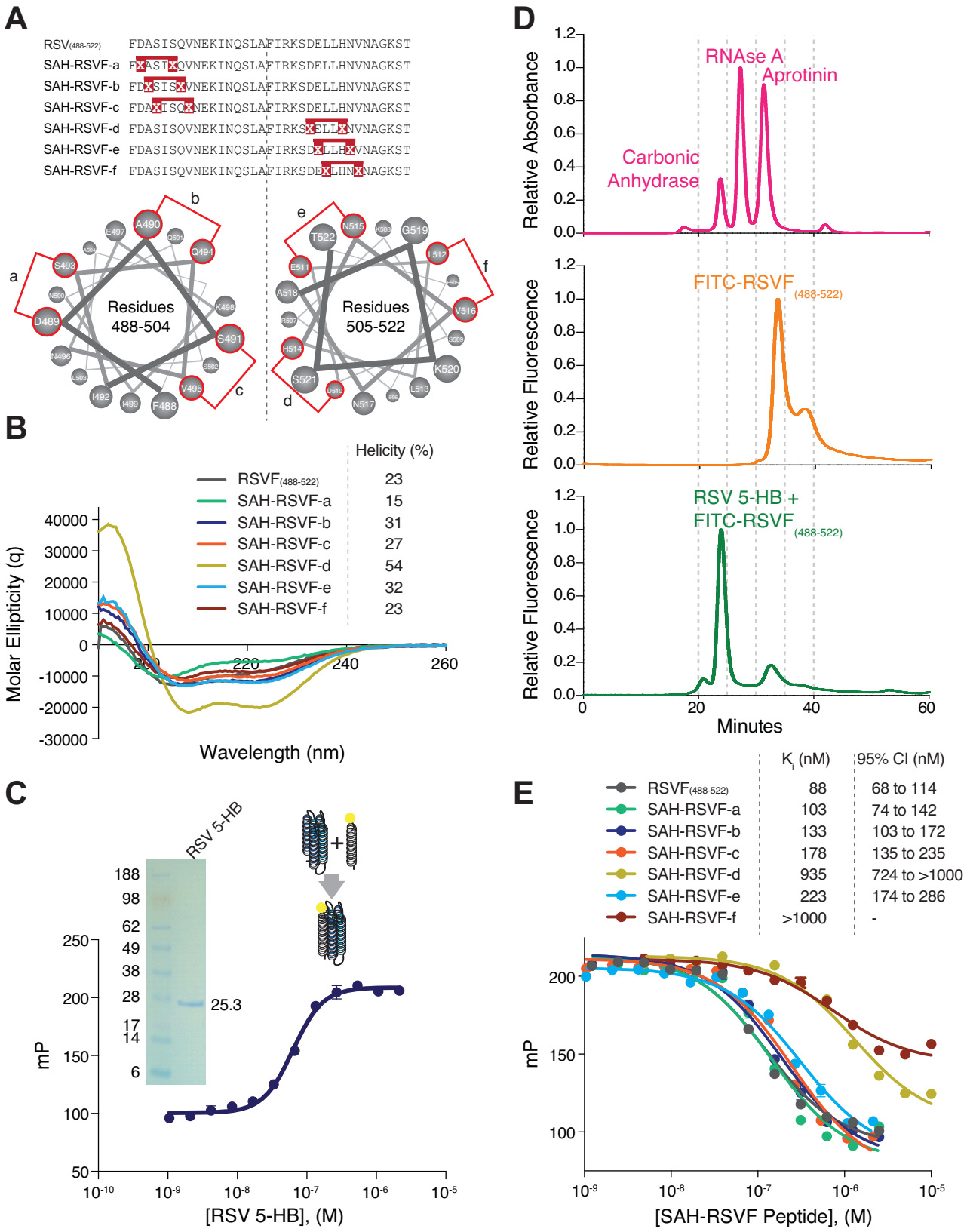
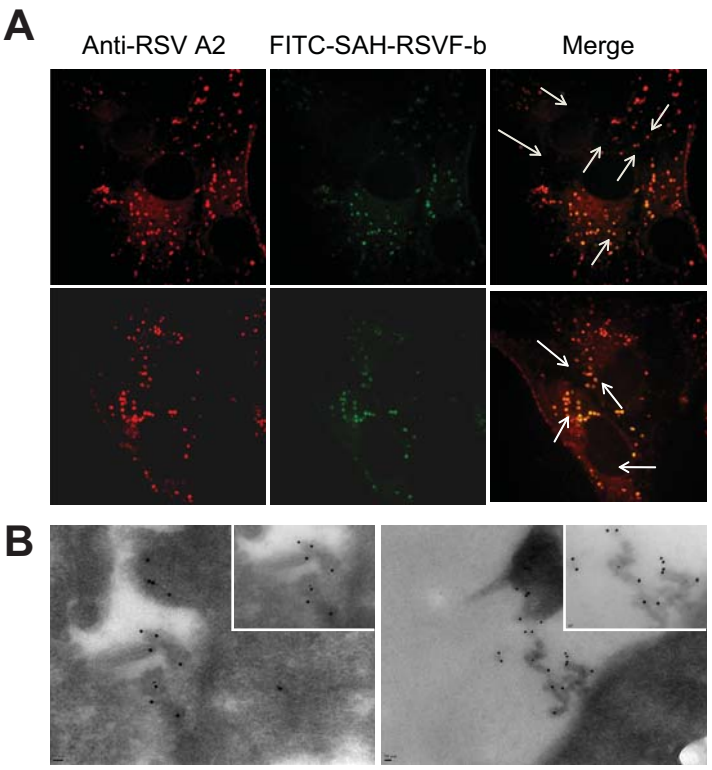
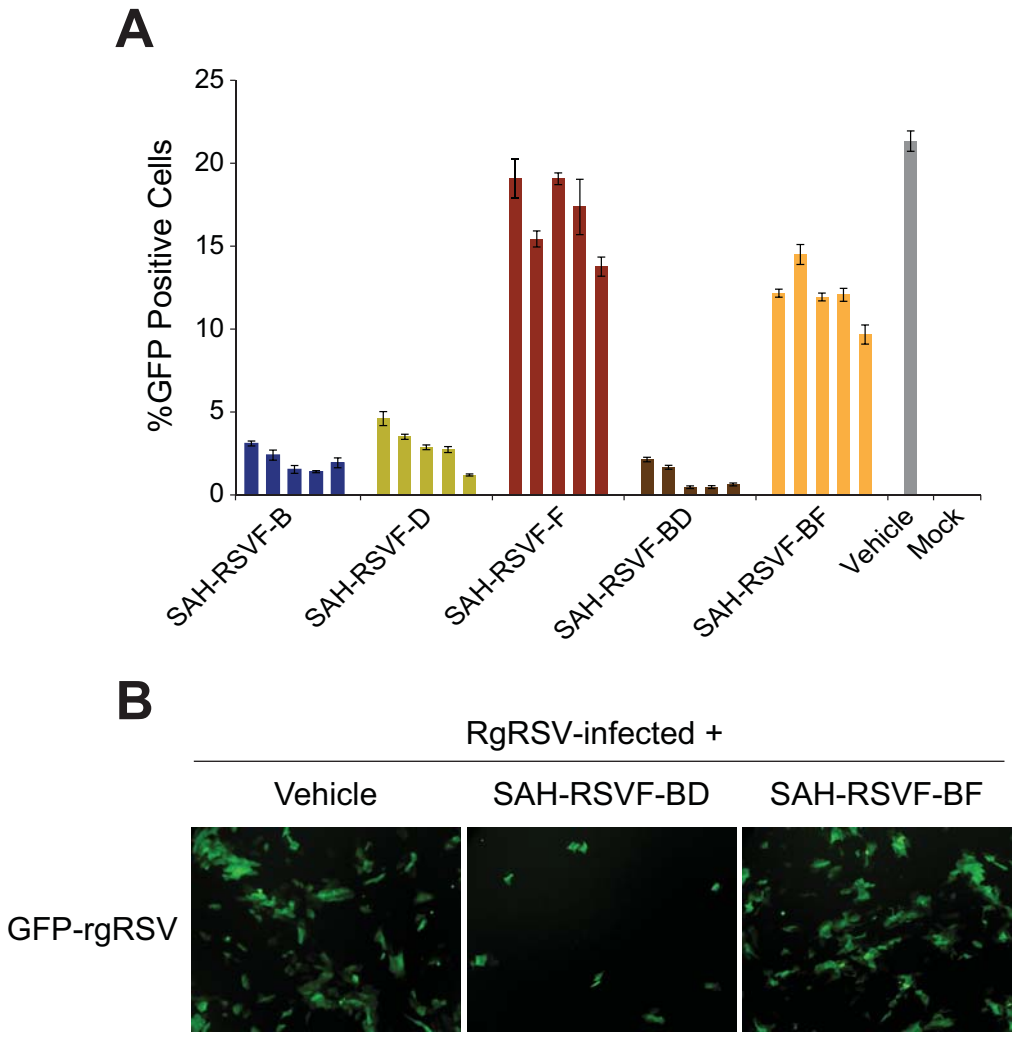


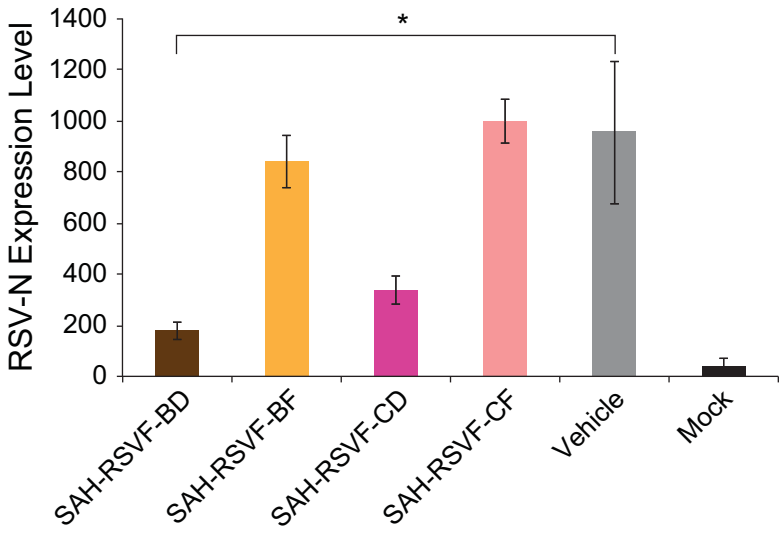
Supplementary Figure 1: Design, synthesis, and 5-HB binding activity of *i*,*i*+4 single stapled SAH-RSVF peptides. (**A**) Sequence compositions and staple placement of *i*,*i*+4 stapled SAH-RSVF peptides. (**B**) Circular dichroism analysis of *i*,*i*+4 stapled SAH-RSVF peptides demonstrating the observed range of α-helical stabilization by hydrocarbon stapling. (**C**) Development of a fluorescence polarization binding assay to measure affinity to the RSV 5-helix bundle (5-HB). Coomassie stain of the purified recombinant 5-HB is shown. FITC-RSVF bound to 5-HB with a K_D of 63 nM. Data represent mean ± s.e.m. for experiments performed in triplicate. (**D**) Fluorescent size-exclusion chromatography demonstrated that FITC-RSVF elutes as a monomeric species (~4 kD). When mixed with excess 5-HB, the fluorescence FITC-RSVF peak shifts to the expected weight of the peptide-protein complex (~29 kD), confirming formation of a 6-HB species. Molecular weight standards: carbonic anhydrase (29 kDa), RNAse A (13.7 kD) and aprotinin (6.5 kD). (**E**) Competitive binding activity of *i*,*i*+4 stapled SAH-RSVF peptides against the FITC-RSVF/5-HB interaction. Data represent mean ± s.e.m. for experiments performed in triplicate.



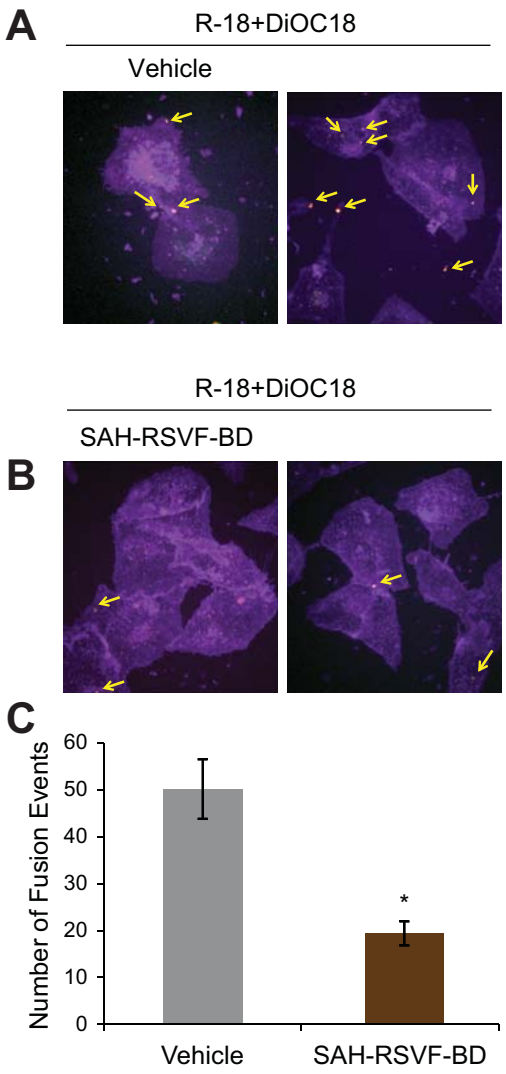
Supplementary Figure 2: Colocalization of RSV A2 virus and SAH-RSVF$_b$ at the plasma membrane of A549 cells. (**A**) Live confocal microscopy documented a striking colocalization of wild-type RSV A2 virus (red) with FITC-labeled SAH-RSVF$_b$ (green) in discrete punctae, seen as yellow spots in the overlay (marked by white arrows). (**B**) Immunoelectron microscopy demonstrated the localization of FITC-SAH-RSVF$_b$ (anti-FITC, 15 nm gold) and RSV A2 (anti-His, 5 nm gold) on the plasma membranes of virus-exposed A549 cells.



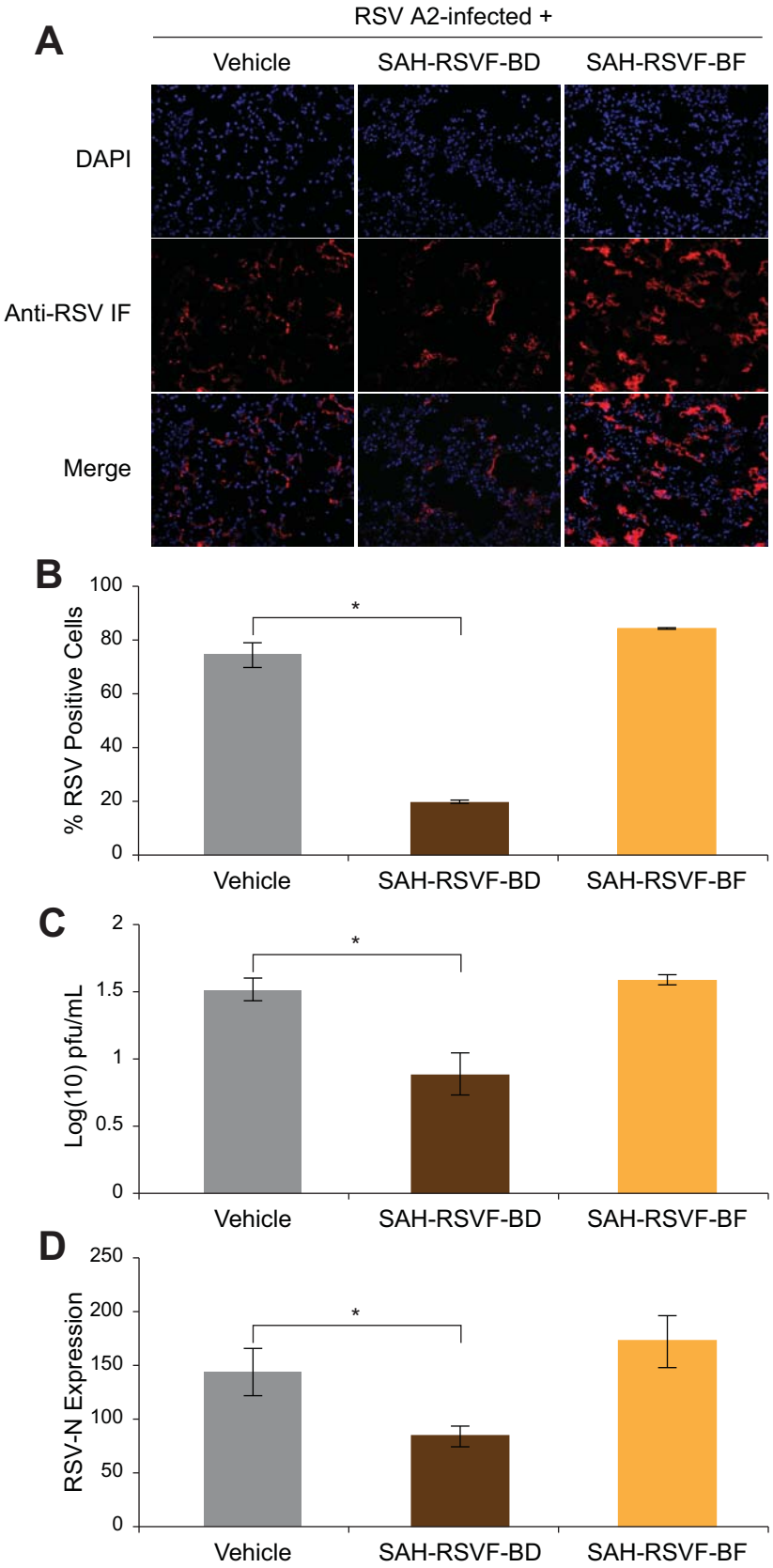
Supplementary Figure 3: Inhibition of RSV infection by SAH-RSVF peptides. (**A**) SAH-RSVF constructs containing *i,i*+7 staples B, D, and BD demonstrated robust viral inhibitory activity over a 1.25 to 15 μM dose range, whereas control peptides F and BF were markedly impaired even at 15 μM dosing. Data represent mean ± s.e.m. for experiments performed in triplicate. (**B**) SAH-RSVF$_{BD}$ suppressed infection of A549 cells by GFP-labeled rgRSV, as evidenced by decreased cellular fluorescence at 5 μM dosing. Cells treated with the negative control peptide, SAH-RSVF$_{BF}$, demonstrated fluorescence equivalent to vehicle control-treated cells.



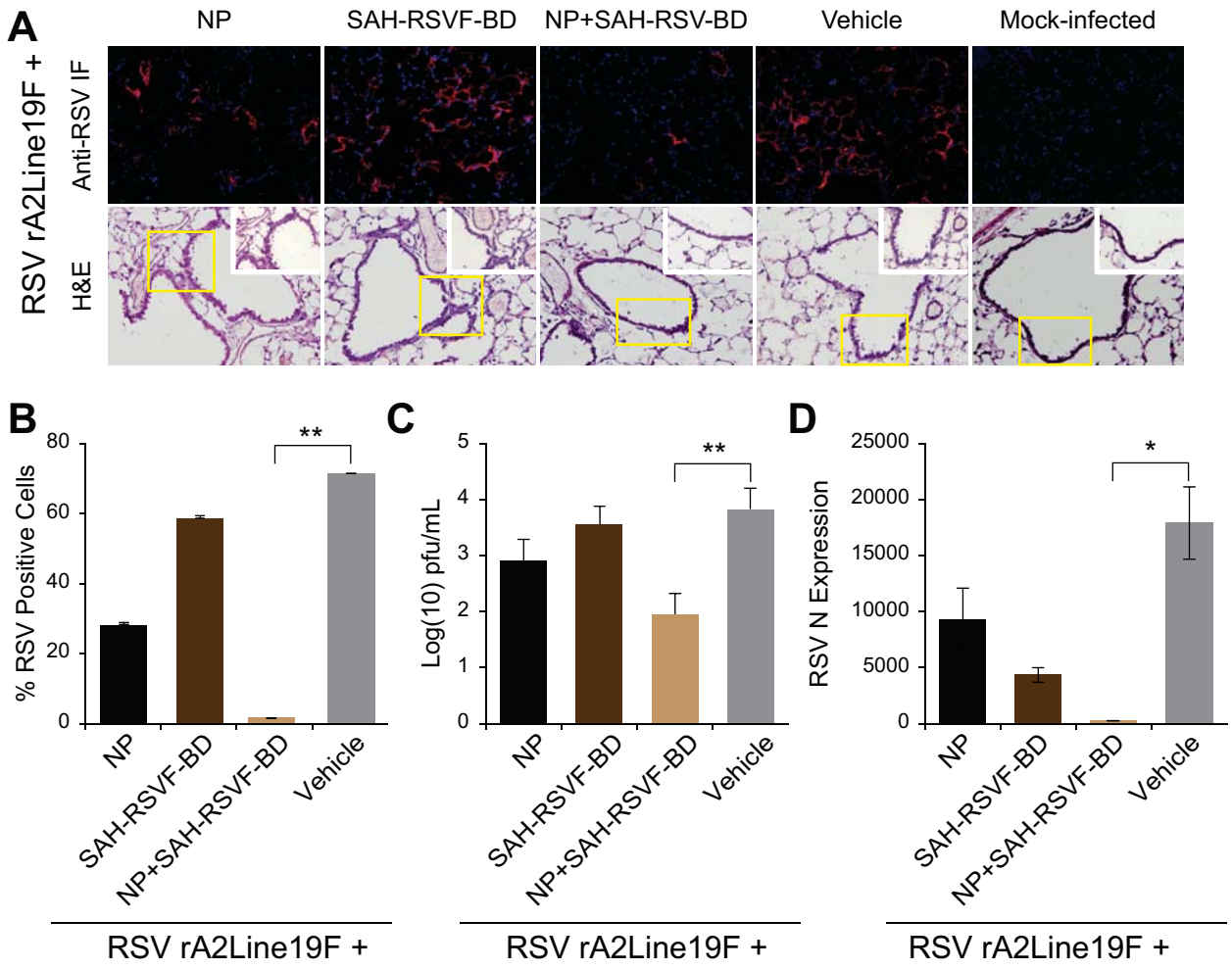
Supplementary Figure 4: Effect of SAH-RSVF treatments on RSV-N expression in RSV A2-infected A549 cells. Whereas SAH-RSVF$_{BF}$ and SAH-RSVF$_{CF}$ had no suppressive effect on RSV-N mRNA levels compared to the untreated control, SAH-RSVF$_{BD}$ and SAH-RSVF$_{CD}$ peptides significantly reduced RSV-N transcript levels, with SAH-RSVF$_{BD}$ manifesting the most potent inhibitory activity. Data are mean ± s.e.m. for experiments performed in triplicate. *, p<0.001



Supplementary Figure 5: SAH-RSVF$_{BD}$ inhibits viral fusion. R18 and DiOC18 double-labeled RSV A2 virus was applied to A549 cells in the presence of SAH-RSVF$_{BD}$ (10 μM) or double-labeled RSV A2 alone for a 30 min attachment period and then fusion monitored by confocal microscopy. (**A**, **B**) Exemplary images demonstrate more fusion events in vehicle-treated cells (**A**) compared to SAH-RSVF$_{BD}$-treated cells (**B**). The yellow-orange spots that reflect fusion events (yellow arrows), whereas red spots represent unfused virus. (**C**) Quantitation of yellow-orange spots documented a 60% reduction in fusion events by SAH-RSVF$_{BD}$ treatment compared to vehicle. Data represent mean ± s.e.m. for experiments performed in triplicate. *, p<0.0001



Supplementary Figure 6: Intranasal prophylaxis with SAH-RSVF$_{BD}$ inhibits spread of RSV infection to the lung. (**A**) Anti-RSV immunofluorescence microscopy of lung sections from 10 week-old BALB/c mice treated with vehicle and SAH-RSVF peptides (50 µL of 125 µM solution in 1.2% DMSO) followed by (1) intranasal RSV A2 inoculation (1x10^6 pfu/mouse) 1 hour later, (2) retreatment and reinfection 16 hours after initial infection, and (3) sacrifice 4 days after the second intranasal infection. Original magnification, 20x. (**B**) Quantitation of average RSV-positivity (red) by Image J analysis of eight images (4 sections) per mouse. Data represent mean percent RSV-positive of total DAPI-positive cells ± s.e.m. (**C**) Viral titers (log10 pfu/mL) were also measured in the supernatants of lung homogenates by HEp-2 plaque assay. Data are mean ± s.e.m. (**D**) Total RNA was isolated from the lungs (right caudal lobe) of treated mice and RSV-N expression quantitated by RT-PCR. Data are mean + s.e.m. *, p<0.0001



Supplementary Figure 7: Intratracheal SAH-RSVF$_{BD}$ as a nanoparticle preparation inhibits pulmonary RSV infection. (**A**) Ten week old BALB/c mice were treated intratracheally with 50 µL of vehicle with NP, SAH-RSVF$_{BD}$ alone (250 µM peptide in 1.2% DMSO), SAH-RSVF$_{BD}$ in combination with NP (1:2.5, peptide:NP), or vehicle alone. Seventy-two hours after treatment, the mice were inoculated intranasally with a single dose of recombinant RSV rA2Line19F at 1x10^6 pfu/mouse. A control group of mice received 50 µL vehicle intratracheally followed by mock inoculation 72 hours later. Mice were sacrificed four days post-infection and 5 µm sections from the left lobes of 1% paraformaldehyde perfused and fixed lungs were subjected to anti-RSV immunofluorescence (IF) analysis (red signal indicates RSV virus) and H&E staining. Comparison of H&E-stained lung sections demonstrated decreased inflammatory cell infiltrates in lung tissue from mice treated with the SAH-RSVF$_{BD}$/NP formulation. Yellow squares mark the regions magnified for each inset. Original magnification 20x, insets 40x. (**B**) Quantitation of average RSV-positivity (red) by Image J analysis of eight images (4 sections) per mouse. Data represent mean percent GFP-positive of total DAPI-positive cells ± s.e.m. (**C**) Viral titers (log10 pfu/mL) were measured in the supernatants of lung homogenates by HEp-2 plaque assay. Data are mean ± s.e.m. (**D**) Total RNA was isolated from the lungs (right caudal lobe) of treated mice and RSV-N expression quantitated by RT-PCR. Data are mean ± s.e.m. *, p<0.001; **, p<0.0001