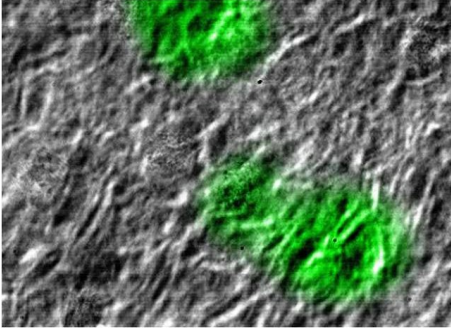
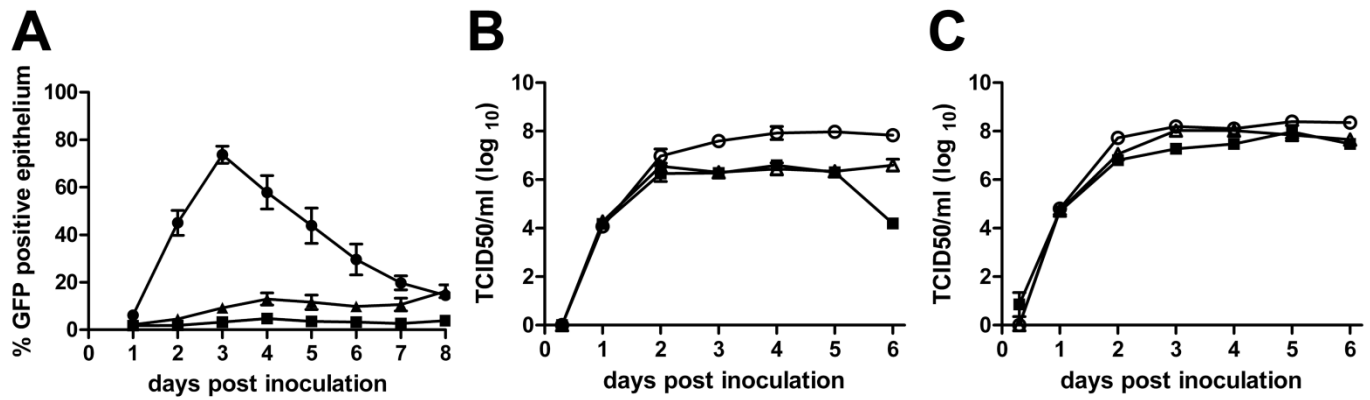


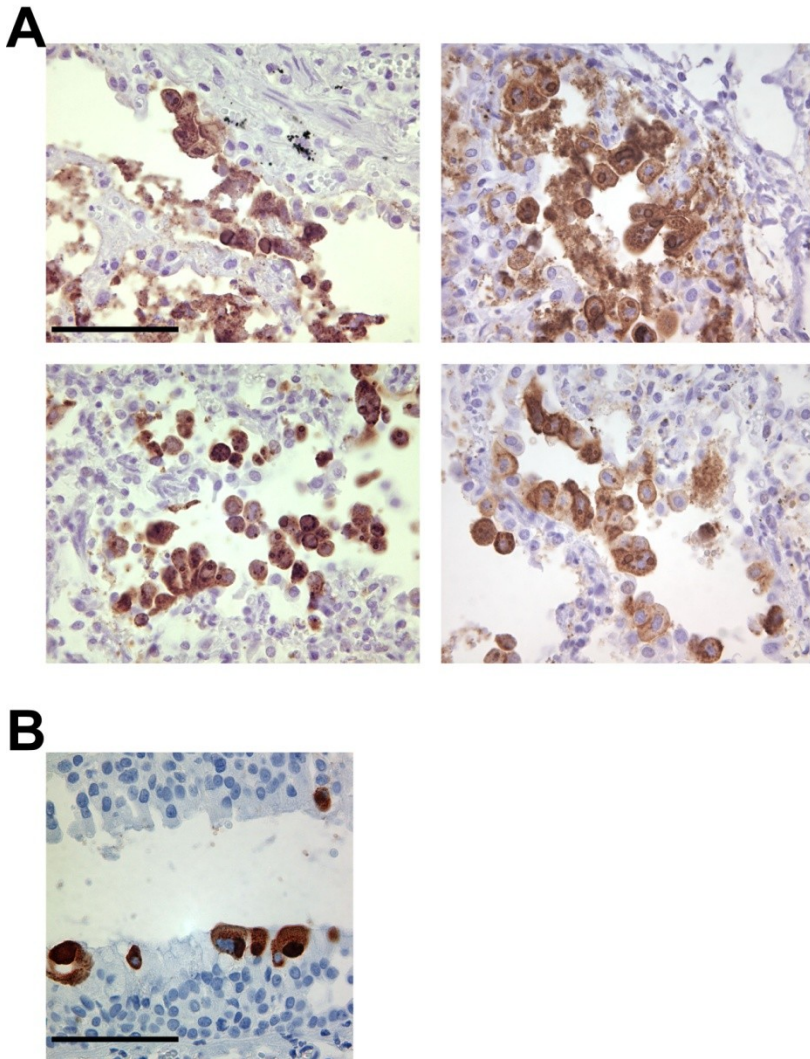
A



Supplemental Figure 1. RSV infected ciliated cells display cilia beat asynchrony. (A) En face image of HAE infected with RSV-GFP (green) showing location of cilia and infected cells. **(B)** Corresponding video of cilia beat on RSV-GFP-infected and uninfected ciliated cells in HAE. Note the asynchrony of cilia beat direction on RSV-infected cells, identified by GFP positivity in (A), compared to uninfected neighboring ciliated cells.



Supplemental Figure 2. Replication of RSV with NS gene deletions and PIV3 viruses in HAE and cell lines. (A) The number of infected cells over time was quantified as the percentage of the epithelium surface area positive for GFP fluorescence in HAE inoculated with RSV (circles), RSVΔNS1 (squares), or RSVΔNS2 (triangles). All viruses express GFP. Data (mean ± SEM) are representative of independent experiments utilizing n = 3-4 cultures per donor obtained from 3 different donors. (B, C) Growth kinetics of recombinant PIV3 (open circles) and PIV3 expressing either RSV NS1 (PIV3-NS1, closed squares) or NS2 (PIV3-NS2, open triangles) in A549 cells (B) and Vero cells (C) inoculated at MOI = 0.1. Data (mean ± SEM) represent n = 4. All viruses express GFP.



Supplemental Figure 3. Infection of the smaller and larger airway regions of RSV-infected patients. (A) Additional example images of histologic cross-sections of human lower airways obtained post-mortem from several patients naturally infected by RSV. Note infected cells exhibit pleomorphic morphology and are often in the process of shedding or fully detached from the basal lamina, resulting in accumulation of shed cells in the airway lumen. (B) Example image of histologic cross-section of the pseudostratified columnar airway, indicative of larger airway regions, obtained post-mortem from a patient naturally infected with RSV. Note infected cells exhibit rounded morphology, although the airway lumen is free of infected cell debris, likely due to effective mechanical clearance of shed cells from the lumen. Sections were stained with an RSV-specific antibody to detect infected cells. Scale bar represents 50 μm .