

Evaluation of a Human 3D Airway Tissue Culture Model for the Study of Respiratory Syncytial Virus Infection and the Development of Antiviral Drugs

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BACKGROUND

- Despite respiratory syncytial virus (RSV) being a leading cause of acute respiratory tract infections in children, the immunocompromised, and the elderly, there are limited therapeutic options.
- Animal models do not completely reproduce human RSV infection pathogenesis. As such, there is a need for preclinical models to study RSV and evaluate novel antiviral agents.
- 2D cell culture models are widely used in drug discovery, but antiviral activity may be influenced by factors not relevant to an *in vivo* environment, such as surface stiffness, forced polarity, and uncontrolled cell spreading.
- 3D models provide a unique advantage for respiratory virus studies by recapitulating the different cell types in the airway at an air-liquid interface.
- EpiAirway standard AIR-100 culture tissue by MatTek Corporation (Ashland, MA) is composed of primary human airway tracheal and bronchial epithelial cells grown at an air-liquid interface (pHAEC-ALI). This includes basal cells, mucus-producing goblet cells, tight junctions, and functioning cilia, which are the target cells of RSV.
- EDP-323, a novel non-nucleoside RSV L-inhibitor, and EDP-938, a non-fusion RSV replication inhibitor, were evaluated against RSV-A and -B in the EpiAirway 3D model to determine antiviral activity.

METHODS

- All tissues used in this study are from a 23-year-old Caucasian male donor.
- Tissues were kept in a 12-well transwell plate, with 5 mL basal medium (MatTek) being replaced every 2-3 days. The apical surface was washed every 7 days with transepithelial/transendothelial electrical resistance (TEER) buffer (MatTek).
- RSV growth dynamics were evaluated utilizing plaque-forming units (PFUs) ranging from 3.9×10^3 to 2.5×10^5 . Apical infection was performed for 1 hour at 37 °C with 350 μ L.
- Compounds were added to the basal medium at the time of infection. Antiviral activity was also assessed in the human larynx carcinoma HEP-2 cell line (established via HeLa cell contamination). For both assays, half-maximal effective concentrations (EC_{50} s) were assessed by RT-qPCR at 5 days post-infection.
- Viral growth from the tissue and apical washes over time were quantified by RT-qPCR and plaque assay, respectively.
- For immunofluorescence experiments, tissues were fixed with 10% formalin and immunostained with monoclonal antibodies (mAbs) against the RSV N and F proteins. A mAb against FoxJ1 was used to detect ciliated cells. All immunofluorescence was performed by Visikol Inc. (Hampton, NJ).



RESULTS

RSV RNA viral load and secreted live virus load increases exponentially over 10 days in EpiAirway tissue

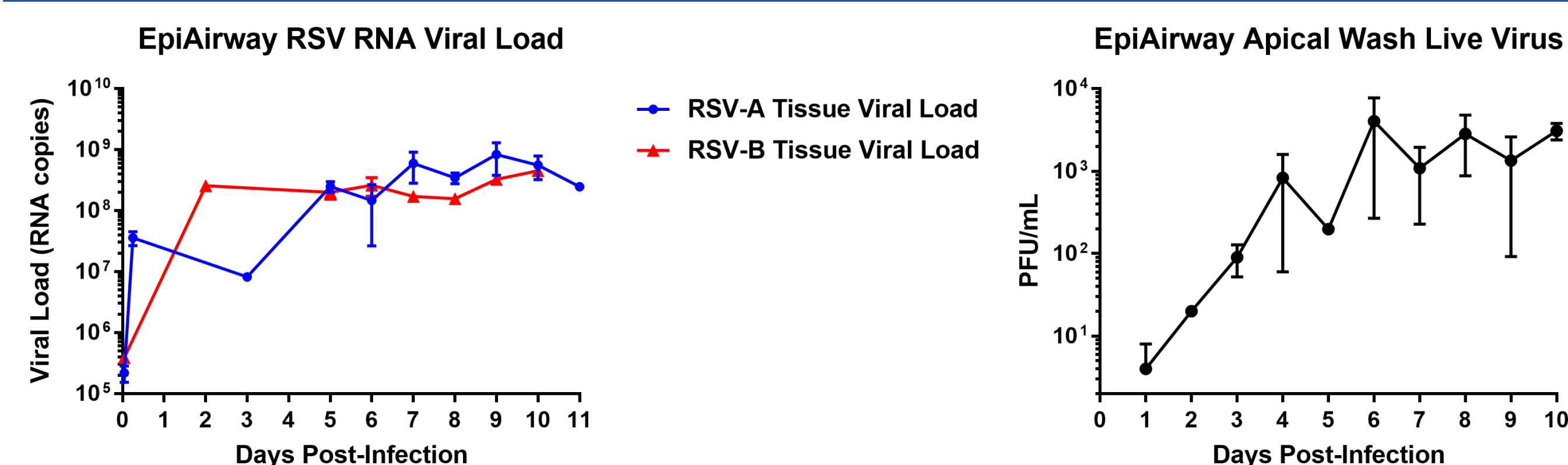


Figure 2. EpiAirway tissues were apically infected with RSV-A Long and RSV-B VR-955 with 3.1×10^4 PFU. Tissue viral RNA load was measured by RT-qPCR. Live virus in apical washes was measured by plaque assay.

Viral inoculum has minimal impact on viral load at 5 days post-infection

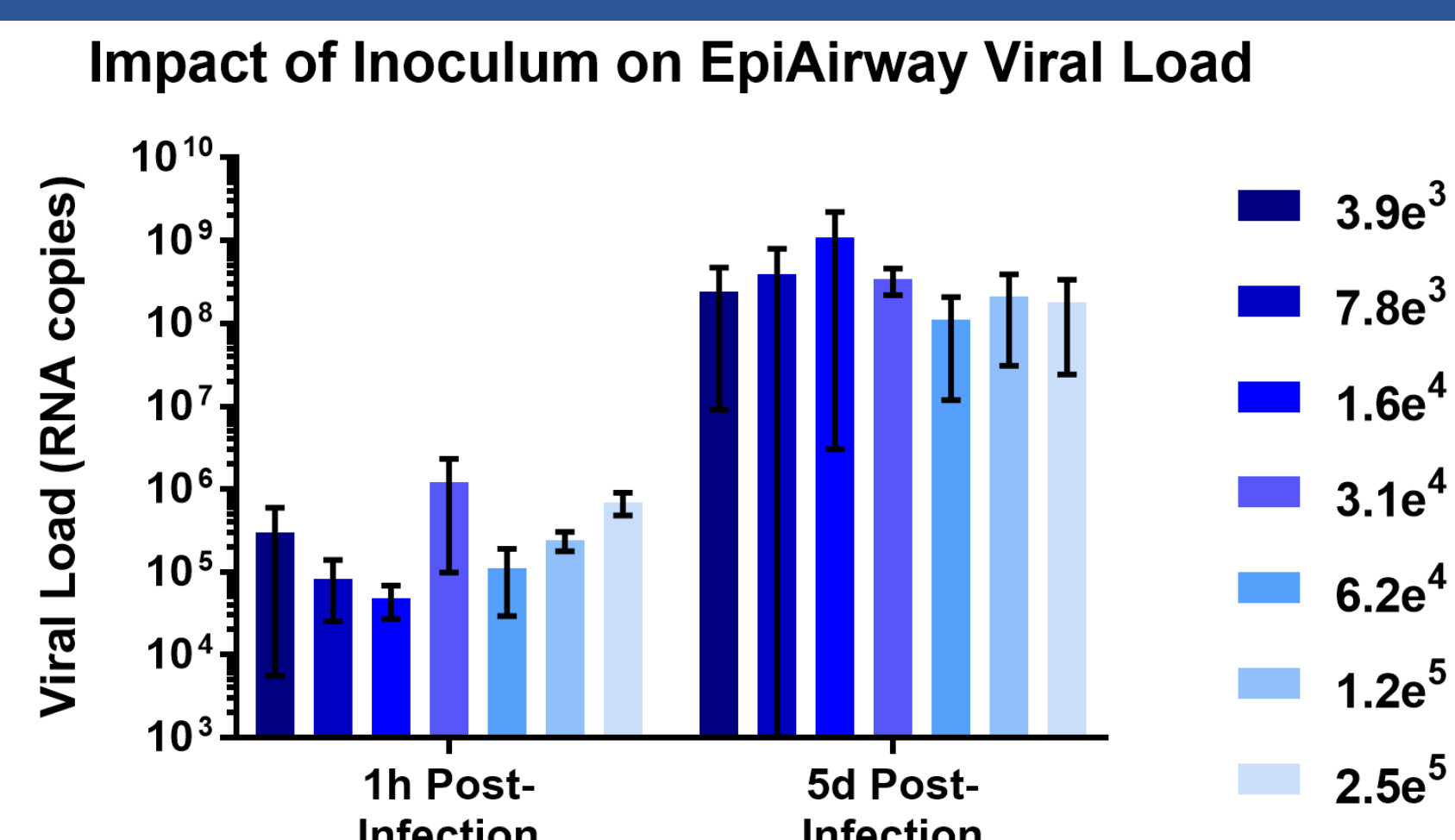


Figure 3. Tissues were apically infected with the indicated PFU. RT-qPCR was used to measure the amount of viral RNA copies in the tissue at 1-hour post-infection and at 5 days post-infection.

RESULTS

EDP-938 and EDP-323 inhibit viral RNA in the EpiAirway 3D system and 2D culture

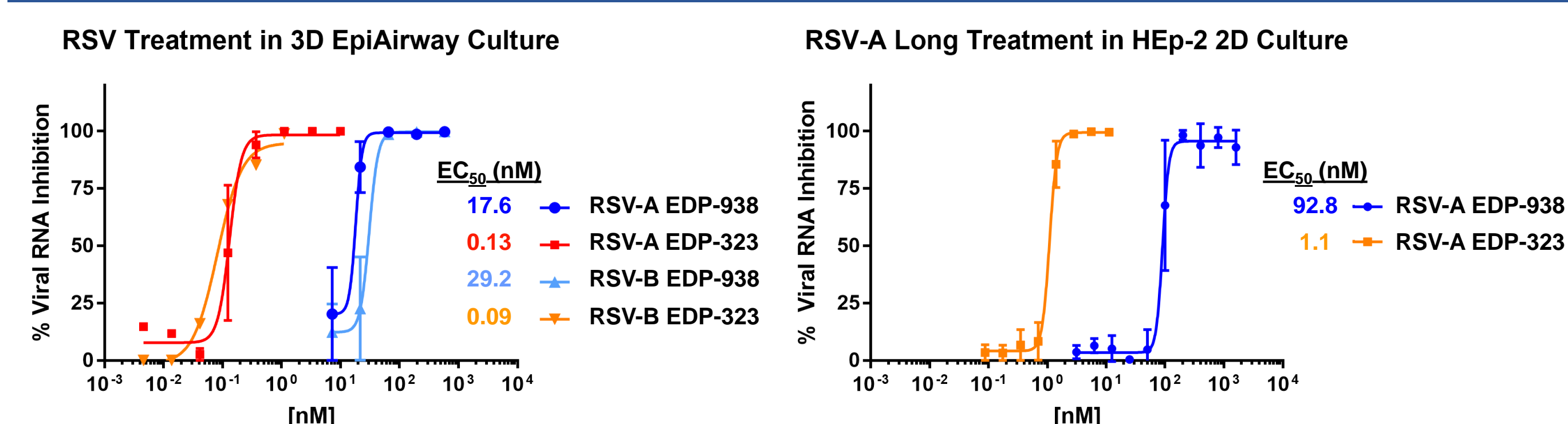


Figure 4. EpiAirway tissues were apically infected with RSV-A Long and RSV-B VR-955 with 3.1×10^4 PFU. HEP-2 cells were infected at a multiplicity of infection (MOI) of 0.1. EpiAirway tissues and HEP-2 cells were treated with EDP-938 and EDP-323 and after 5 days, EC_{50} s were determined by RT-qPCR.

Cell markers visualize ciliated cells in EpiAirway tissue

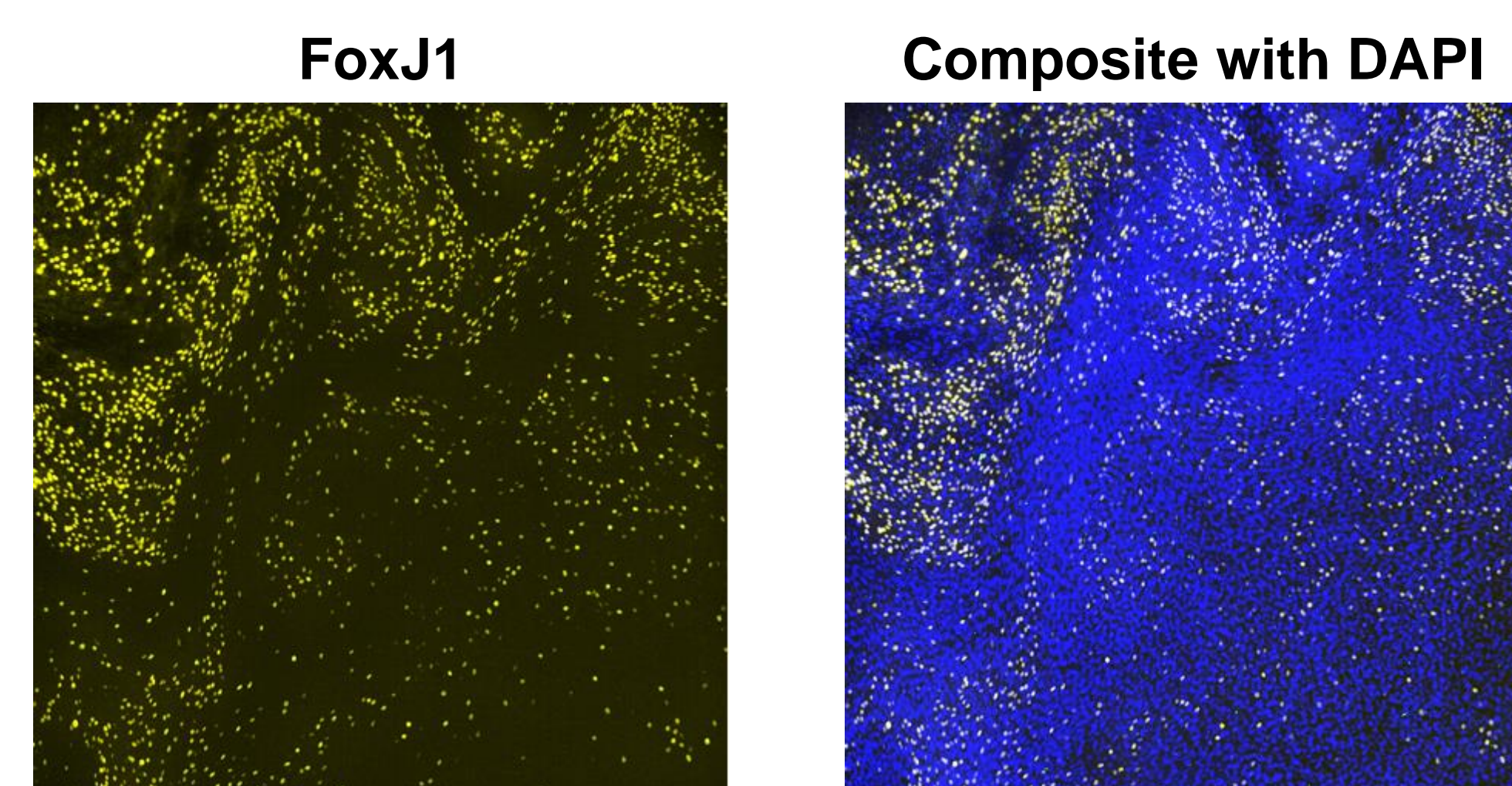


Figure 5. EpiAirway tissues were infected with RSV-A Long with 7.8×10^3 PFU. Tissues were fixed and stained for FoxJ1, shown in yellow, to indicate ciliated cells.

RSV markers are reduced in EDP-323-treated EpiAirway tissue

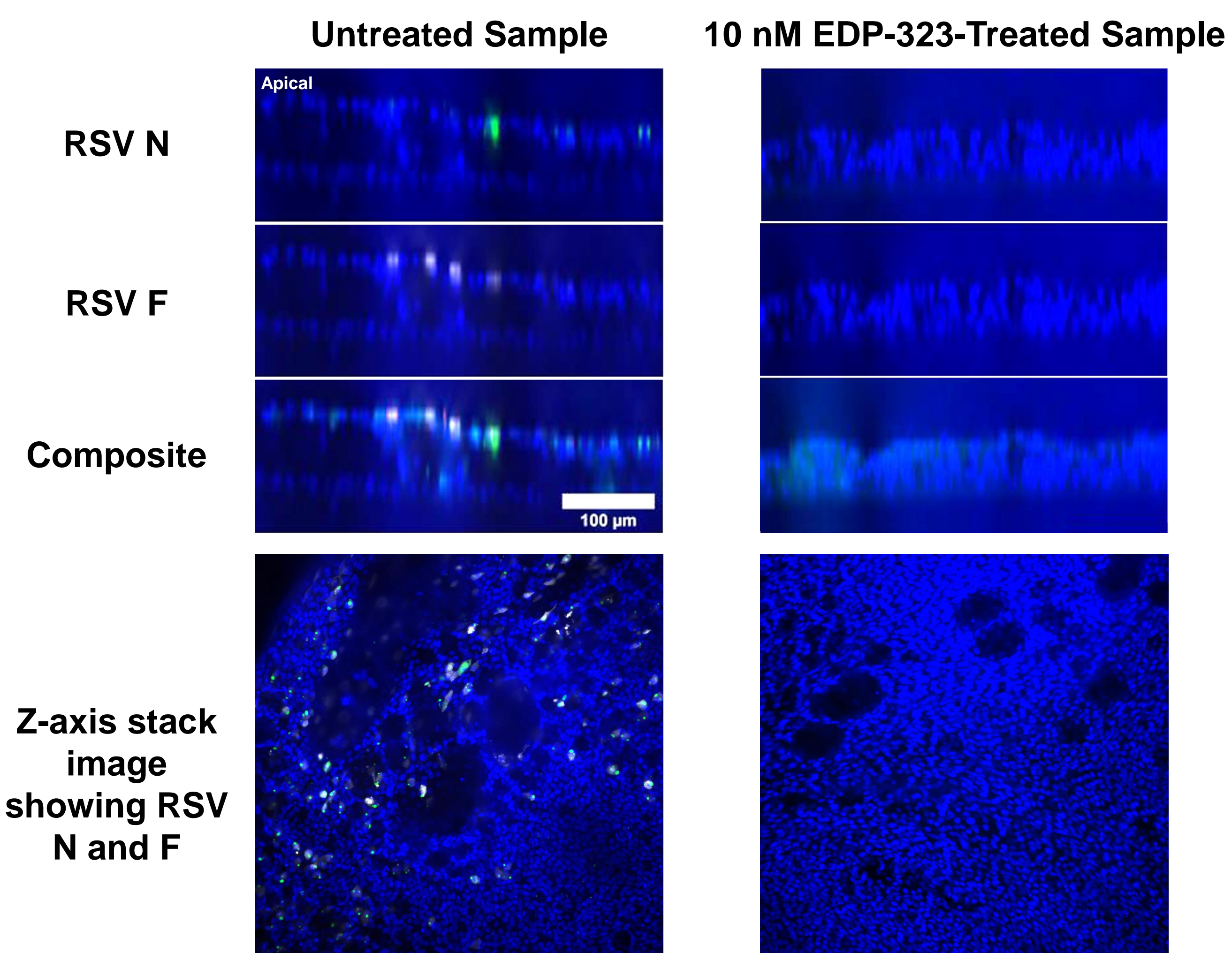


Figure 6. EpiAirway tissues were infected with RSV-A Long with 3.1×10^4 PFU and treated with 10 nM EDP-323 or vehicle control. Five days post-infection, tissues were fixed and sent to Visikol Inc. to stain for RSV N (green) and RSV F (white).

CONCLUSIONS AND ACKNOWLEDGEMENTS

- The EpiAirway model can be successfully used to study the dynamics of RSV infection and evaluate the antiviral activity of novel therapeutics.
- The EpiAirway model generates reproducible viral replication kinetics and consistent compound-induced viral inhibition while surpassing the preclinical testing limits of traditional 2D culture models.
- The EpiAirway model can be utilized for visualization of *in vivo* cell markers as well as reduction of RSV markers with antiviral treatment.
- EDP-323 is expected to begin Ph1 clinical trials in Q4 2022

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DISCLOSURE: All authors are either current or former employees of Enanta Pharmaceuticals, Inc. and received salary and stock compensation.