

Figure S1. Replication kinetics and morphologic analysis of RSV infected individual pediatric HNO-ALIs and adult HNO-ALIS A) RT-PCR for apical RSV/A/ON for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 days post-infection (dpi). Each HNO-ALI is assigned a specific color, and two replicates of each line are shown on the graph, with composite mean values plotted in a line. B) Corresponding plaque data for RSV/A/ON pediatric HNO-ALIs. C) RT PCR for apical RSV/B/BA for 4 pediatric HNOs at 1, 2, 5, and 8 dpi. D) Corresponding plaque data for RSV/B/BA pediatric HNO-ALIs. E) RT-PCR for apical RSV/A/ON for 4 adult HNO-ALIs at 1, 2, 5, and 8 or dpi. Each HNO-ALI is assigned a specific color, and two replicates of each line are shown on the graph, with composite mean values plotted in a line. F) Corresponding plaque data for RSV/A/ON adult HNO-ALIs. G) RT PCR for apical RSV/B/BA for 4 adult HNO-ALIs at 1, 2, 5, and 8 dpi. H) Corresponding plaque data for RSV/B/BA adult HNO-ALIs. I) Area of the epithelium of 4 adult HNO-ALIs after infection with RSV/A/ON, RSV/B/BA, or mock infection at 1, 2, 5, and 8 dpi. J) Area of the epithelium of 4 pediatric HNO-ALIs after infection with RSV/A/ON, RSV/B/BA, or mock infection at 1, 2, 5, and 8 dpi.



Figure S2. Heat map of cytokine expression for all 4 pediatric HNO-ALI lines. Expression over time for RSV/A/ON apical, RSV/A/ON basolateral, RSV/B/BA apical, and RSV/B/BA basolateral. Each value is normalized to the corresponding mock value at that time point.



Figure S3. Heat map of cytokine expression for all 4 adult HNO-ALI lines.

Expression over time for RSV/A/ON apical, RSV/A/ON basolateral, RSV/B/BA apical, and RSV/B/BA basolateral. Each value is normalized to the corresponding mock value at that time point.



Figure S4. Apical LDH and Caspase levels during RSV infection in individual pediatric and adult HNO-ALIS A) Levels of apical LDH for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 days post-infection (dpi) with RSV/A/ON. Each HNO-ALI is assigned a specific color, and two replicates of each line are shown on the graph, with composite mean values plotted in a line. B) Levels of apical caspase for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of apical LDH and D) Levels of apical caspase for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of apical LDH and **D**) Levels of apical caspase for 3 adult HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. G) Levels of apical LDH and **H**) level of apical caspase for 3 adult HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. G) Levels of apical LDH and **H**) level of apical caspase for 3 adult HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of apical LDH and **H**) level of apical caspase for 3 adult HNO-ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of apical LDH and **H**) level of apical caspase for 3 adult HNO-ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of apical LDH and **H**) level of apical caspase for 3 adult HNO-ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of apical LDH and **H**) level of apical caspase for 3 adult HNO-ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of apical LDH and **H**) level of apical caspase for 3 adult HNO-ALIS at 1, 2, 5, and 8 dpi with RSV/B/BA.



Figure S5. Basolateral LDH and Caspase levels during RSV infection in individual pediatric and adult HNO-ALIs A) Levels of basolateral LDH for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 days post-infection (dpi) with RSV/A/ON. Each HNO-ALI is assigned a specific color, and two replicates of each line are shown on the graph, with composite mean values plotted in a line. B) Levels of basolateral caspase for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of basolateral LDH and D) Levels of basolateral caspase for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of basolateral LDH and D) Levels of basolateral caspase for 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/B/BA. E) Levels of basolateral LDH and F) and levels of basolateral caspase for 3 adult HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. G) Levels of basolateral LDH and H) level of basolateral caspase for 3 adult HNO-ALIs at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. C) Levels of basolateral LDH and H) level of basolateral caspase for 3 adult HNO-ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. ALIS at 1, 2, 5, and 8 dpi with RSV/A/ON. B) Levels of basolateral LDH and H) level of basolateral caspase for 3 adult HNO-ALIS at 1, 2, 5, and 8 dpi with RSV/B/BA.



Figure S6. Cellular and morphological changes in pediatric and adult HNO-ALIs A) Amount of apical ciliated area after infection with RSV in 4 adult HNO-ALIs and **B**) 4 pediatric HNO-ALIs at 1, 2, 5, and 8 days post-infection (dpi). with RSV/A/ON, RSV/B/BA, or mock infection. **C**) Area of mucous quantified by Muc5AC+ after infection with RSV in 4 adult HNO-ALIs and **D**) 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi. **E**) Percentage of CC10+ club cells over / number of DAPI+ cells after infection with RSV in 4 adult HNO-ALIs and **F**) 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi. **G**) Percentage of Krt5+ basal cells /total number of DAPI+ cells after infection with RSV in 4 adult HNO-ALIs and **H**) and 4 pediatric HNO-ALIs at 1, 2, 5, and 8 dpi.