

Multimic comparison of sulfur mustard-exposed human and rat EpiAirway™ tissues

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ABSTRACT

Translating cellular responses to chemical exposures at the *in vitro* level to the *in vivo* level is a challenge within the entire scientific community. This is especially difficult for chemical defense research due to the limited availability of exposure data in humans, as well as the difficulty in accumulating large amount of animal model exposure data. Recent advances in cell culture techniques have presented unique opportunities to evaluate effects of chemical warfare agents (CWAs) on human cells engineered to mimic complex three-dimensional (3D) tissue types *in vitro*, such as the respiratory tract, skin, liver, kidney, digestive tract, and brain. Recently, a respiratory tract model has been developed from rat primary airway epithelial cells (rat EpiAirway™). The development of these types of *in vitro* 3D models using cells from experimental animals allow for a more direct comparison to what is occurring in response to chemical injury *in vivo*. In this study, we compare the cellular responses of human EpiAirway™ and rat EpiAirway™ to sulfur mustard vapor exposure. We evaluate the cellular inflammatory signaling pathways associated with sulfur mustard exposure through both classical techniques (e.g. histopathology) as well as newly developed multimic techniques (e.g. proteomics, metabolomics, lipidomics). These types of comparisons with cells derived from humans and experimental animals are necessary to aid in the translation of cell culture model exposure data to *in vivo* animal model exposure data.

MATERIALS AND METHODS

EpiAirway™ Tissue Models: Human EpiAirway (AIR-100) and rat EpiAirway™ (AIR-100-R) three-dimensional airway models were purchased from MatTek, Corp. (Ashland, MA, USA) and cultured according to the manufacturer's specified instructions (Dhumakupt *et al.*, 2022; Wallace *et al.*, 2023)

Sulfur Mustard (HD) Preparation and Exposure: Due to the acute hazards with HD, all experiments involving this chemical were performed by qualified personnel in certified chemical fume hoods equipped with an advanced filtration system that protects the user and the environment at DEVCOM CBC according to all Federal, State, and International guidelines. The HD used in this study was synthesized and purified (HD: 99.6 ± 0.4 wt. %) by U.S. Army DEVCOM CBC chemists in accordance with international regulations. HD exposures were performed using vapor cups as described with slight modifications (Dalton *et al.*, 2006; Hayden *et al.*, 2009). Briefly, 10 µL neat HD was placed in the cotton plug of a vapor cup lid and then placed on top of the cell vessel for 8 min. At the end of the exposure, the lids containing the HD were removed and the tissues were allowed to off-gas under a chemical fume hood for 10 minutes. Then, the cells are moved to a new cell culture plate and placed in the cell culture incubator (37 °C/5%CO₂), which was located under a chemical fume hood, until processed.

Transepithelial Electrical Resistance (TEER) Measurements: TEER measurements were performed using the EVOM2 volt-ohmmeter (World Precision Instruments; Sarasota, FL) using the STX EVOM Electrode. TEER was calculated and reported as Ω x cm².

Determination of Cellular Viability: The MTT assay (MatTek) was used to determine cellular viability of EpiAirway™ tissues following exposure to HD vapor. Preparation and execution of this assay was performed according to the manufacturer's stated protocol. The processed plates were then read at 650nm for background reading and at 570nm for the assay reading on a SpectraMax plate reader. The results were displayed as a percentage of viability compared to untreated control.

Histological Analysis: The exposed EpiAirway™ tissues were fixed overnight in 10% formalin at room temperature and then washed with PBS. The fixed tissues were then paraffin-embedded using routine histological techniques as described (Dhumakupt *et al.*, 2022). The rehydrated slides were stained with hematoxylin and eosin and then visualized using the EVOS M5000 Imaging System (ThermoFisher Scientific).

Preparation of EpiAirway™ Tissues for Multimic Analysis: At the specified time point, the EpiAirway™ tissues were removed from the inserts, placed in centrifuge tubes (Eppendorf Lo-Bind, 2 mL centrifuge tubes), and then flash frozen in liquid N₂. The tubes were then stored at -80 °C until further processing. Then, 1 mL of ice-cold Fisher Scientific (Waltham, MA) Optima™ methanol with 0.1% Optima™ formic acid added to each tube containing tissue sample. Keeping the tubes on ice, each sample was ground using a Potter tissue homogenizer for 1-2 minutes. The samples were then centrifuged for 10 minutes at 20,000 x g at 4 °C. The supernatant was then removed to a glass vial for lipid/metabolite extraction, and the protein pellet was stored at -80 °C for protein extraction.

Omics Analysis: Proteomic or metabolomics analysis was performed as stated in Dhumakupt *et al.*, 2022.

FIGURE 1

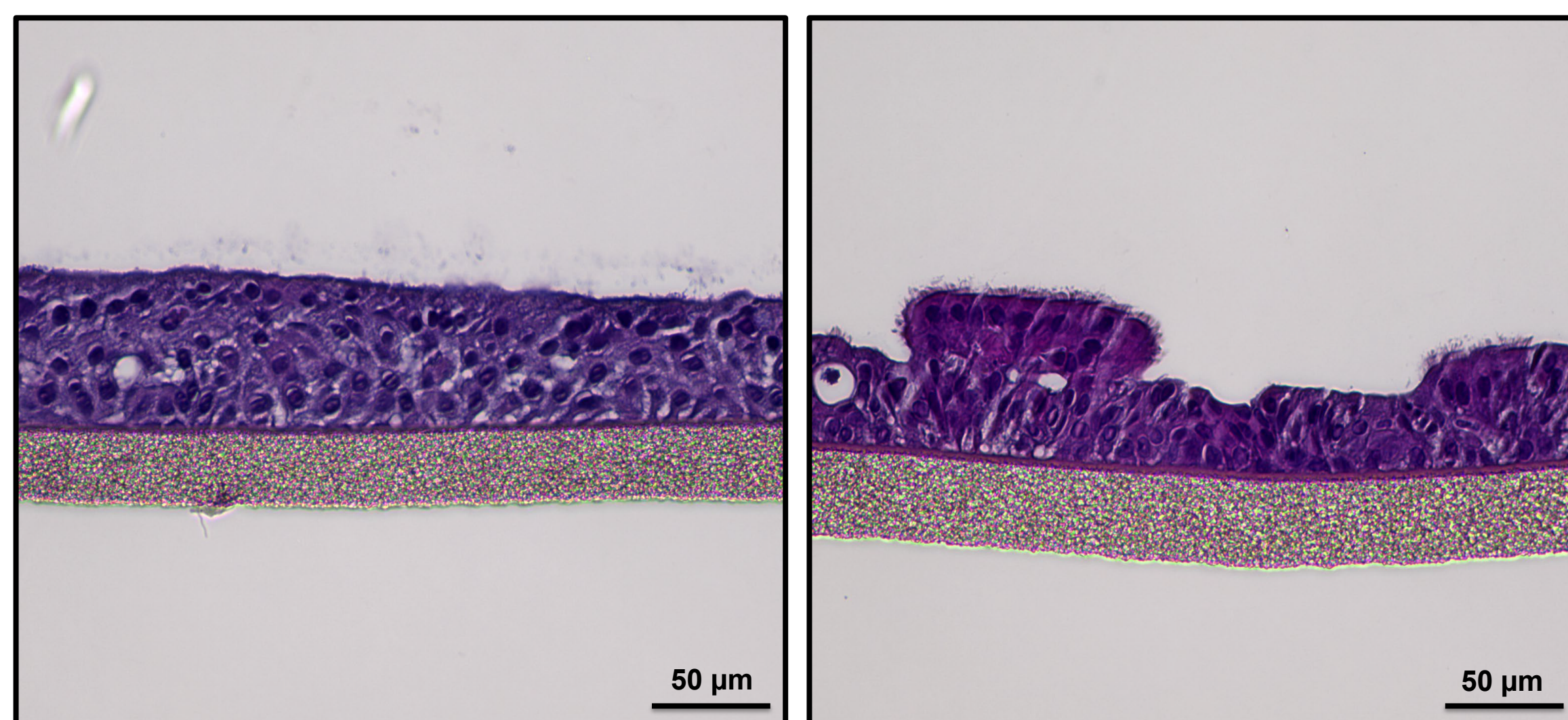


Figure 1: Histological evaluation of human and rat EpiAirway™ tissues. Paraffin-embedded sections from human and rat EpiAirway™ tissues were stained with hematoxylin and eosin. Stained slides were visualized through an EVOS M5000 Imaging System. Scale bars, 50 µm.

FIGURE 2

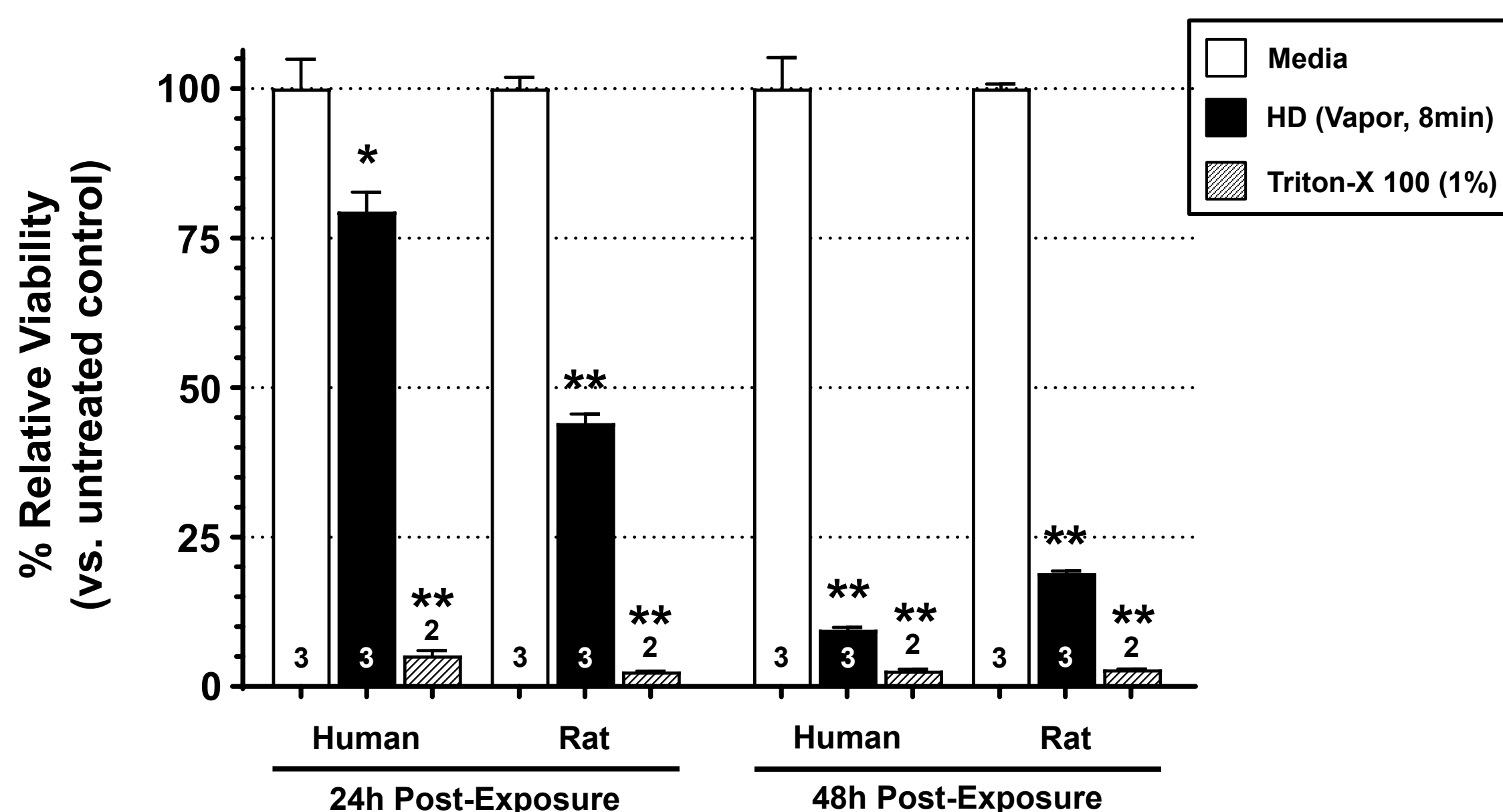


Figure 2: Effect of HD vapor on human and rat EpiAirway™ tissue viability. Cellular viability (MTT assay) was evaluated at 24h and 48h following an initial 8 min HD vapor exposure. Open bars represent mean ± SEM of % relative cellular viability of untreated human and rat EpiAirway™ tissues. Closed bars represent mean ± SEM of % relative cellular viability of human and rat EpiAirway™ tissues exposed to HD vapor. Cross-hatched bars represent mean ± SEM of % relative cellular viability of human and rat EpiAirway™ tissues following exposure to 1% Triton-X 100 (positive control). The experimental n is indicated within each bar. *p < 0.05 versus time-matched media control. **p < 0.01 versus time-matched media control. ***p < 0.001 versus time-matched media control.

FIGURE 3

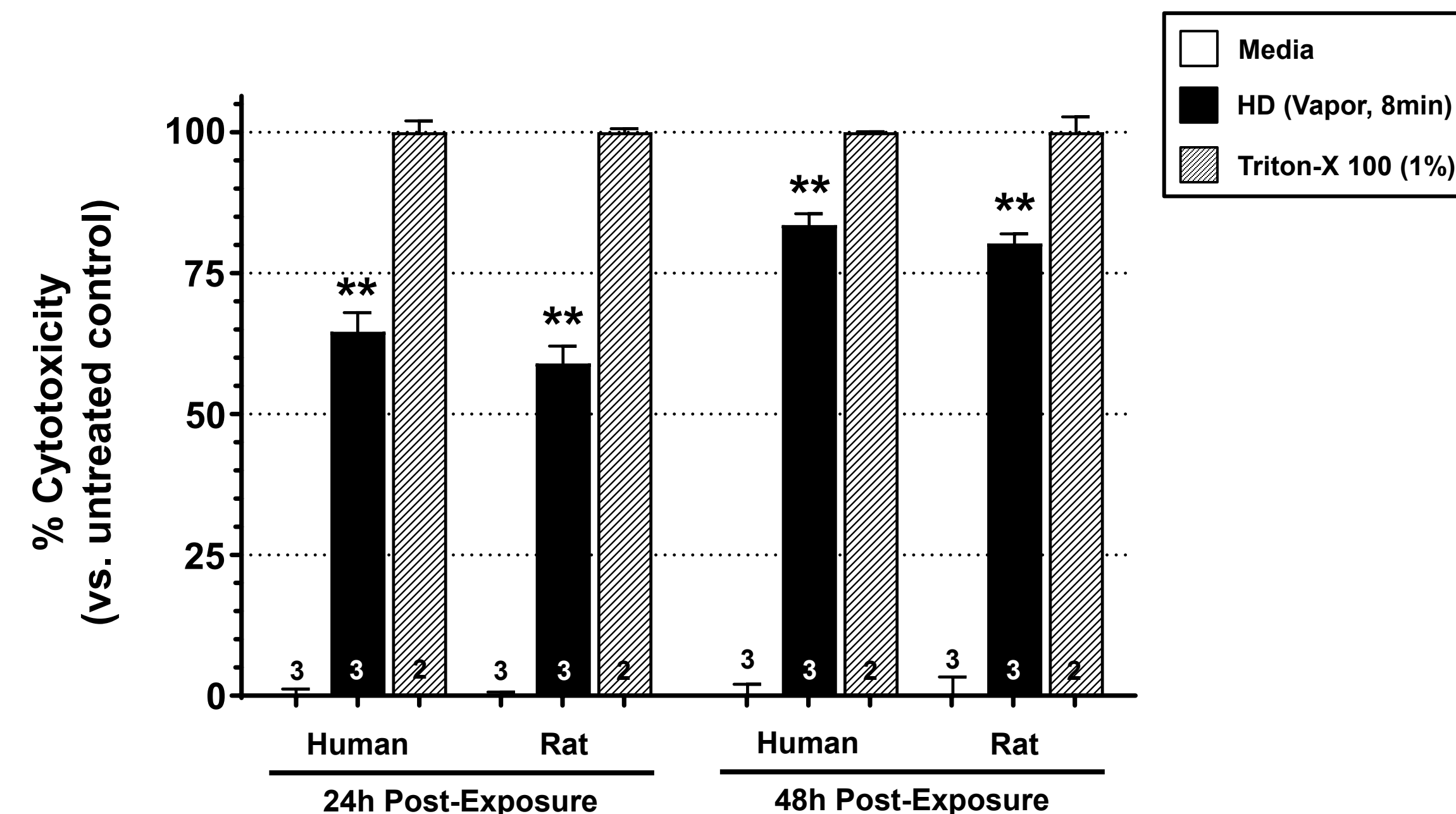


Figure 3: Effect of HD vapor on human and rat EpiAirway™ Cytotoxicity. Cytotoxicity (LDH assay) was evaluated at 24h and 48h following an initial 8 min HD vapor exposure. Open bars represent mean ± SEM of % cytotoxicity of untreated human and rat EpiAirway™ tissues. Closed bars represent mean ± SEM of % cytotoxicity of human and rat EpiAirway™ tissues exposed to HD vapor. Cross-hatched bars represent mean ± SEM of % cytotoxicity of human and rat EpiAirway™ tissues following exposure to 1% Triton-X 100 (positive control). The experimental n is indicated within each bar. **p < 0.001 versus time-matched media control.

FIGURE 4

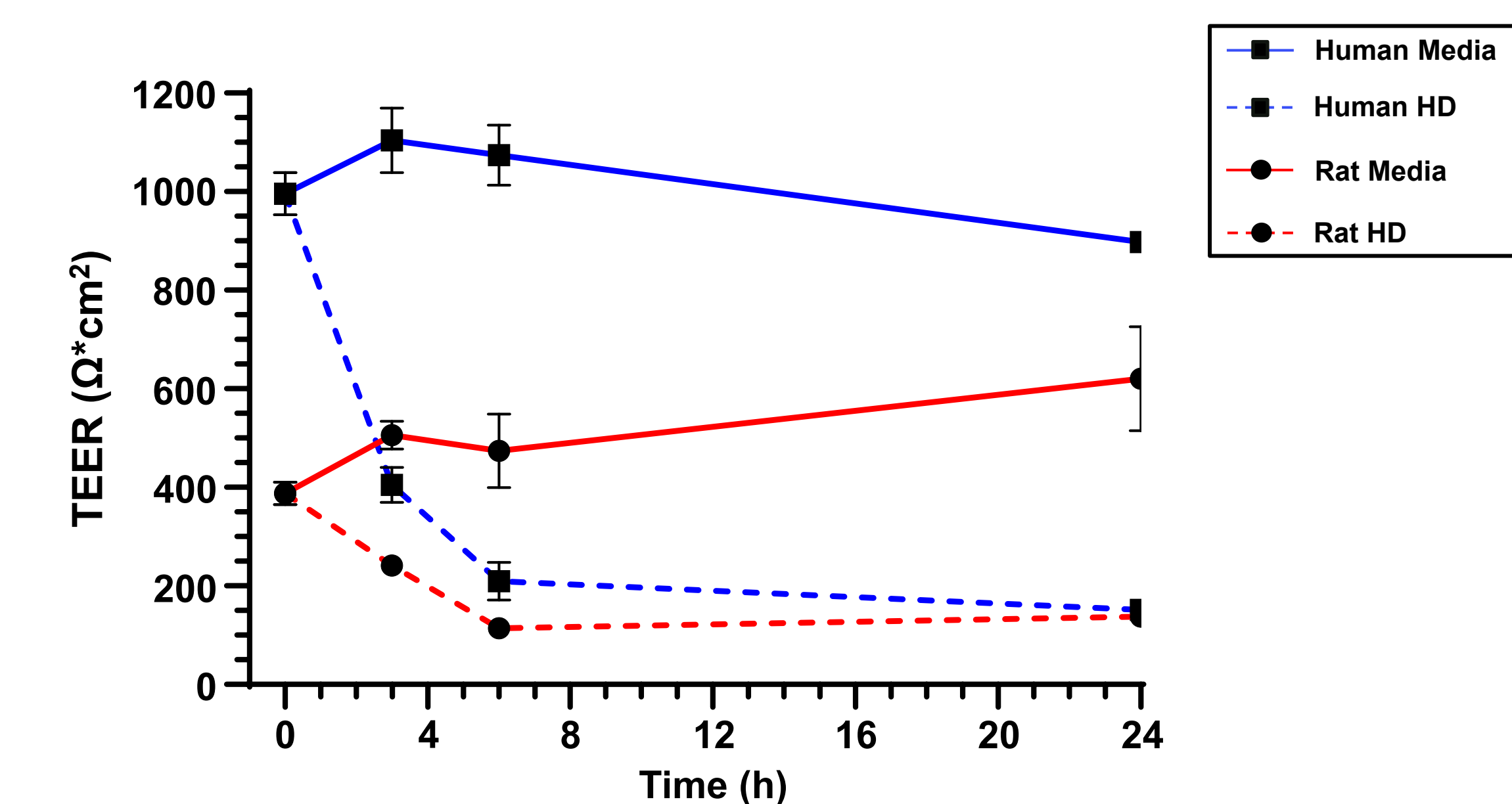


Figure 4: Effect of HD vapor on human and rat EpiAirway™ TEER values. The squares with solid lines (blue) represent mean ± SEM of TEER from media exposed human EpiAirway™ tissue. The squares with dashed lines (blue) represent mean ± SEM of TEER from HD exposed human EpiAirway™ tissue. The circles with solid lines (red) represent mean ± SEM of TEER from media exposed rat EpiAirway™ tissue. The squares with dashed lines (red) represent mean ± SEM of TEER from HD exposed rat EpiAirway™ tissue.

FIGURE 5

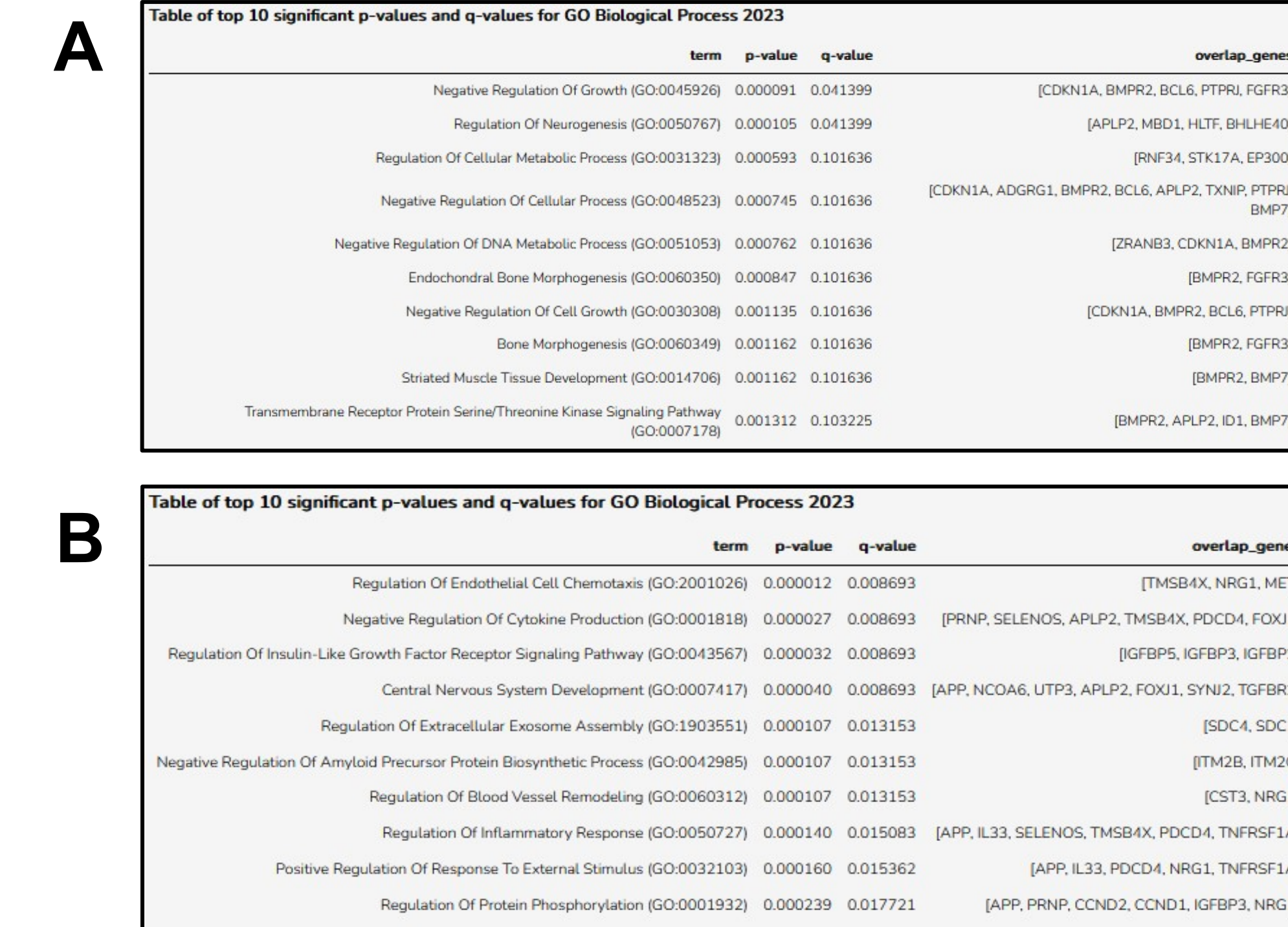


Figure 5: Proteomic evaluation of Human and Rat EpiAirway™ tissues following HD vapor exposure. Proteomic analysis was performed on (A) rat EpiAirway™ tissue and (B) human EpiAirway™ tissue 24h following HD vapor exposure.

CONCLUSIONS

- Both human and rat EpiAirway™ tissues form a well-defined three-dimensional structure.
- HD induces a dose- and time-dependent loss of cellular viability in both the rat and human tissues.
- Loss of viability due to HD exposure appears to be a combination of apoptotic and necrotic cell death.
- Proteins dysregulated following HD exposure include proteins associated with cellular growth, metabolic processes, and inflammation.

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