Supplementary Data

A 3D human airway model enables prediction of respiratory toxicity of inhaled drugs in vitro

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**Fig. S1.** Reproducibility and accuracy of respiratory toxicity predictions at different time points in the MucilAir™ 3D human airway in vitro model. (A) Reproducibility of TEER measurement for AZ1 and budesonide between different experiments (mean ± SEM). (B) Accuracy of TEER to predict respiratory toxicity over time (400 µM) as determined by area under the curve (AUC) by receiver operation characteristics (ROC) analysis for toxic (n=8) and non-toxic (n=7) compounds. Significance was determined by two-tailed Student’s *t*-test, * signifies *p* < 0.05, ** *p* <0.01.
Fig. S2. Morphological assessment of whole mount preparations of MucilAir™ epithelium as determined by confocal microscopy against Tubulin B (green), MUC5AC (red) and DAPI (blue). (A) Representative cell images of vehicle treated cells. Apical surface view is shown in the upper and middle panel and cross-sectional view at the bottom. (B) Apical surface view of compound treated cells with different cell coverage grades. Scale bar represents 30 µm.
Fig. S3. Impact of compounds with and without respiratory toxicity on release of LDH and IL-8 in a 3D human airway in vitro model. (A) Effect on cytotoxicity as measured by LDH release over time. Dotted line represents the method detection limit (MDL) of significant change compared to vehicle treated cells. Only compounds with effect levels above MDL at any time point are shown. Compounds with respiratory toxicity are shown in red. Triton X100 (black) represent a positive toxicity control. (B) Time course of compounds with significant change of IL-8 release compared to vehicle. All other tested compounds in experimental set-ups described in A and B were non-significantly changed over MDL or compared to vehicle, respectively, including all 7 non-toxicity controls.
**Fig. S4.** Effect of compounds over time in a 3D human airway *in vitro* model on ciliary beating frequencies (CBF, blue triangles) and (TEER, black circles). Compounds with (red headings) or without (green headings) respiratory toxicity are shown in top and bottom rows, respectively. Data are shown as percent change compared to vehicle controls (mean ± SEM).