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SUPPLEMENTARY MATERIALS

Repetitive exposure to nicotine-free vaping impairs airway defences and delays recovery

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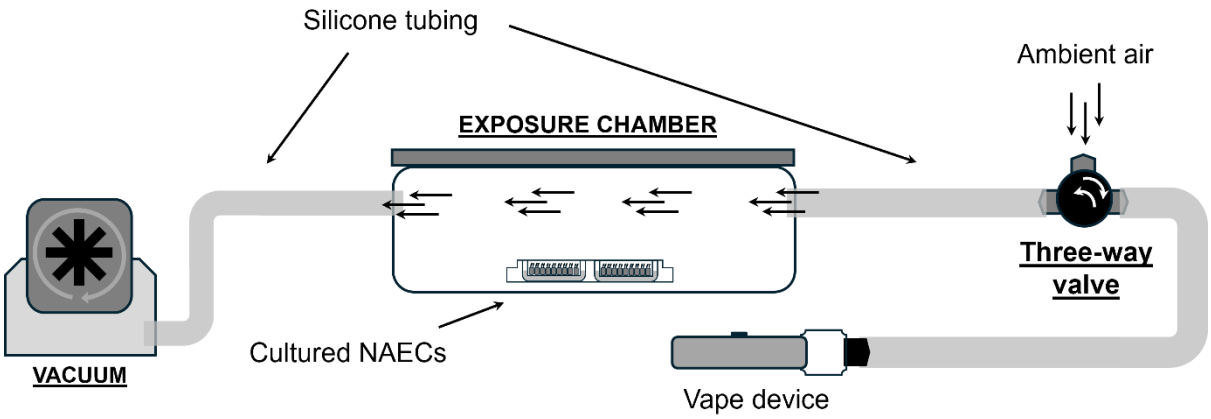
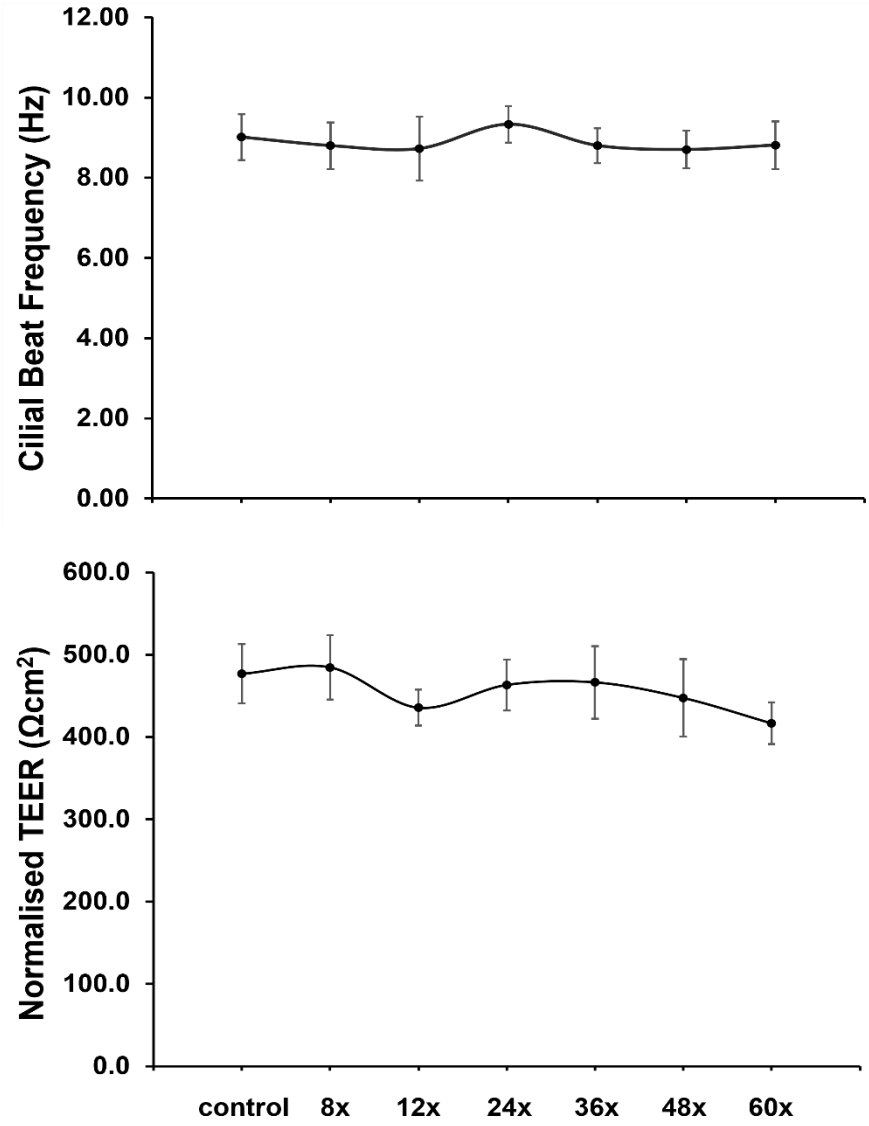
SUPPLEMENTARY FIGURE 1



Supplementary figure 1. Schematic of the e-cigarette exposure apparatus. Cultured NAECs were placed in a 0.9L airtight chamber, which was connected to the vape pen under low vacuum. A three-way valve positioned between the chamber and the pen enabled switching between aerosol and ambient air. Silicone tubing (40 cm on each side of the valve; total distance 85 cm [including the valve]) separated the chamber from the vape pen. Temperature monitoring confirmed that aerosol entering the chamber was cooled to ambient levels (23-25^{0}C). Cells were exposed to e-cigarette aerosols for 3 seconds before recovered with ambient air for 57 seconds. The exposure cycles were repeated 10 times.

SUPPLEMENTARY FIGURE 2



Supplementary figure 2. Ambient air only exposures do not affect epithelial cell function. Cultured NAECs were placed into the exposure chamber and exposed to 8, 12, 24, 36, 48 or 60 cycles of ambient air only under low vacuum. Both CBF (top panel) and TEER (bottom panel) was largely unchanged following repetitive exposures to ambient air. Data are presented as average ± SD.

Supplementary Table 1. Comparison of secreted cytokines from cells exposed to 36 repeated vaping.

Cytokines/ Chemokines/ Growth factors	Amount secreted in the basolateral compartment (pg/mL), Median ± IQR		Fold change	Statistical significance
	Unchallenged control	*36 repetitive vaping*		
IL-1β	**4.63 ± 1.70**	**6.83 ± 0.96**	**1.48**	**<0.01**
IL-1ra	411.38 ± 1,552.49	7,650.40 ± 14,687.79	18.60	NS
IL-2	110.53 ± 53.88	163.72 ± 36.74	1.48	NS
IL-4	**4.56 ± 1.58**	**6.50 ± 1.44**	**1.43**	**<0.05**
IL-5	**114.90 ± 54.73**	**191.15 ± 10.64**	**1.66**	**<0.01**
IL-6	**8.62 ± 4.00**	**36.63 ± 9.64**	**4.25**	**<0.01**
IL-7	43.52 ± 22.41	55.25 ± 5.84	1.27	NS
IL-8	8,934.36 ± 14,345.01	12213.42 ± 12,325.53	1.37	NS
IL-9	**47.13 ± 39.51**	**61.87 ± 20.54**	**1.31**	**<0.05**
IL-10	3.83 ± 4.57	10.12 ± 5.42	2.64	NS
IL-12	**7.22 ± 5.96**	**15.87 ± 1.36**	**2.20**	**<0.05**
IL-13	**1.19 ± 1.26**	**2.04 ± 1.25**	**1.71**	**<0.01**
IL-15	**227.86 ± 175.52**	**355.67 ± 59.04**	**1.56**	**<0.05**
IL-17	**14.31 ± 6.44**	**22.69 ± 4.16**	**1.59**	**<0.01**
Eotaxin	**1.89 ± 0.48**	**2.89 ± 0.22**	**1.53**	**<0.01**
FGF-basic	**1,034.38 ± 512.39**	**3,071.80 ± 1,229.80**	**2.97**	**<0.01**
G-CSF	123.79 ± 531.78	286.03 ± 653.70	2.31	NS
GM-CSF	17.34 ± 16.71	15.76 ± 4.29	0.91	NS
IFN-γ	**5.63 ± 11.79**	**48.30 ± 53.93**	**8.58**	**<0.05**
IP-10	55.93 ± 294.82	131.54 ± 4,590.73	2.35	NS
MCP-1	**14.29 ± 4.93**	**20.69 ± 2.14**	**1.45**	**<0.01**
MIP-1α	**0.82 ± 0.18**	**1.09 ± 0.20**	**1.33**	**<0.01**
MIP-1β	**30.60 ± 19.56**	**36.40 ± 14.89**	**1.19**	**<0.01**
PDGF-β	187.14 ± 108.84	228.03 ± 36.43	1.22	NS
RANTES	**17.80 ± 5.10**	**30.87 ± 8.06**	**1.73**	**<0.01**
TNF-α	52.04 ± 68.79	89.61 ± 40.03	1.72	NS
VEGF	**504.24 ± 371.19**	**2,580.15 ± 562.04**	**5.12**	**<0.01**

Data presented as median concentration (pg/mL) ± IQR. Changes in secreted levels are presented as fold-change. Analytes with ≥3 fold increase in levels following repeated vaping are highlighted.