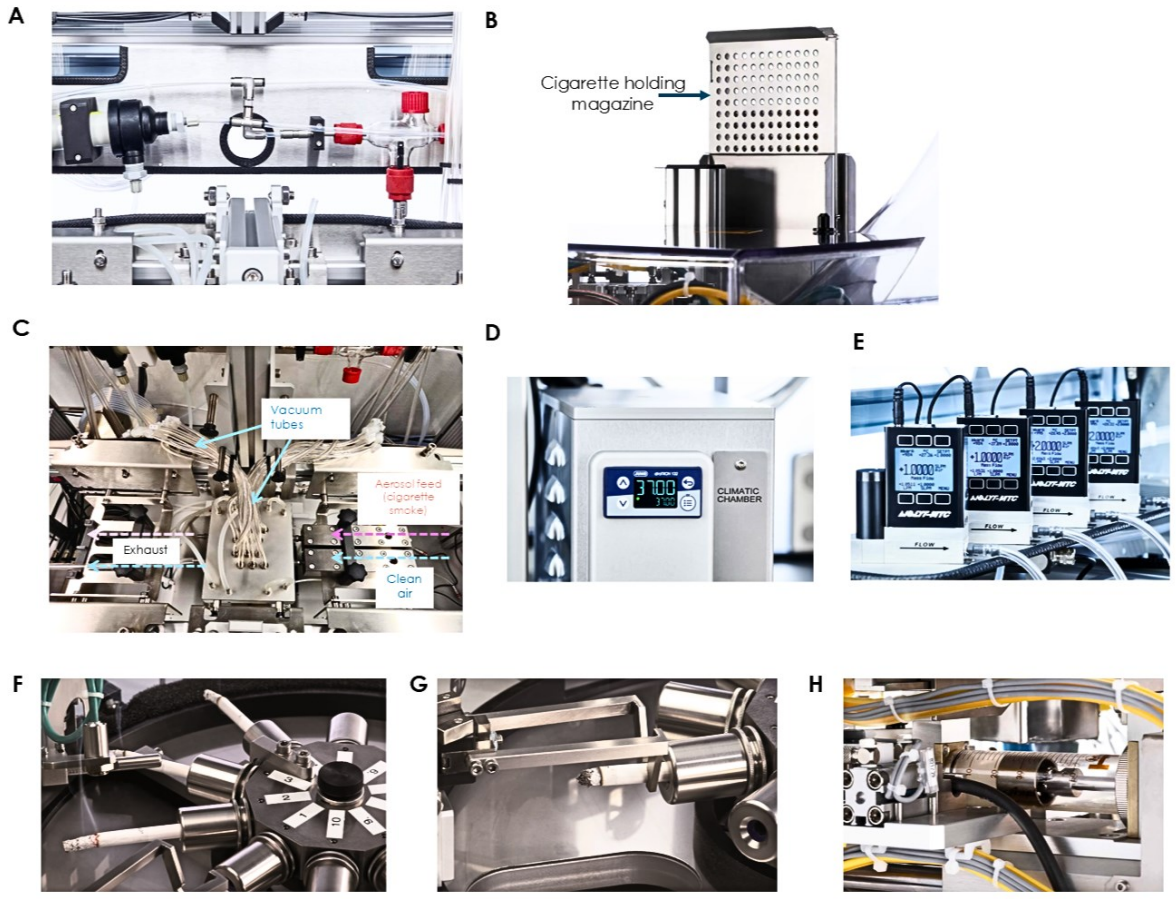
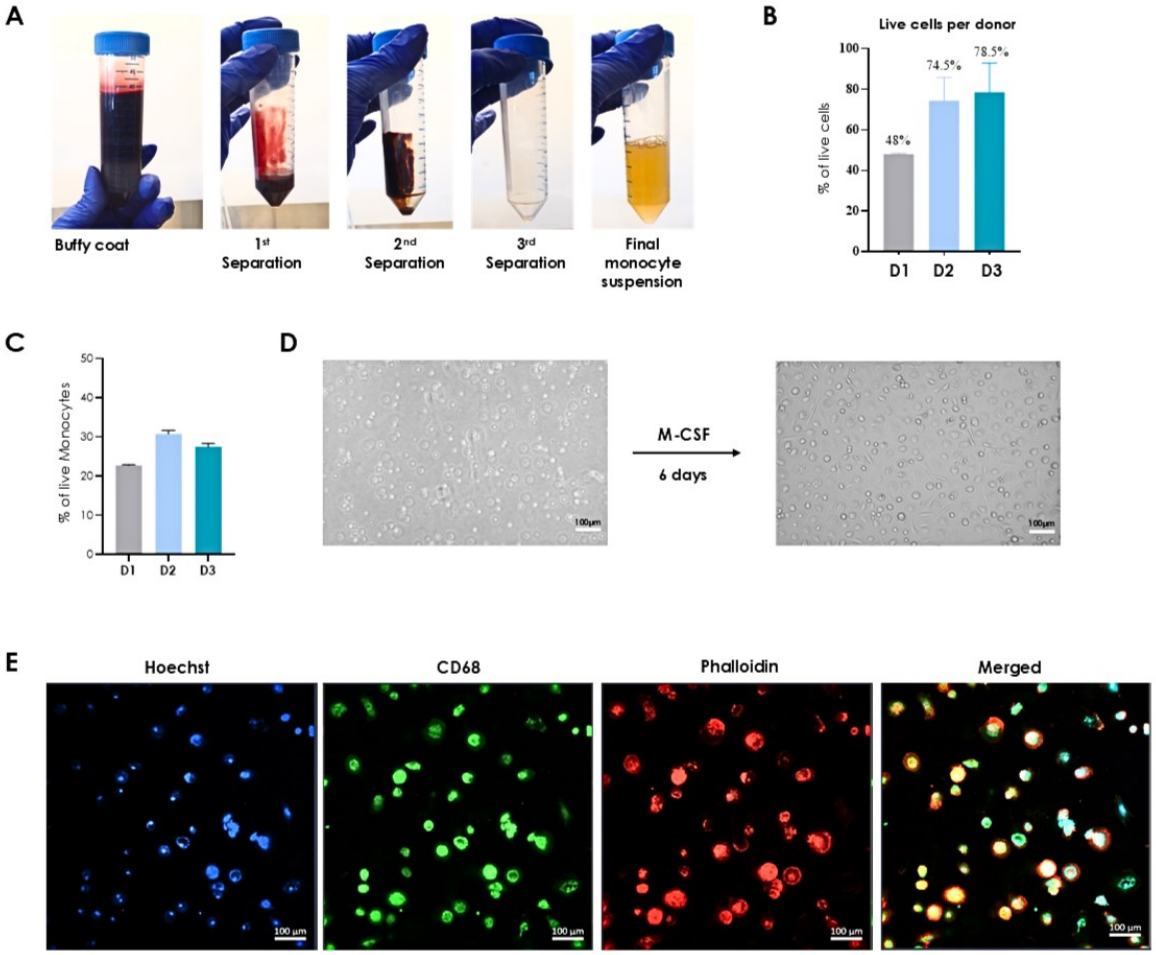
A next-generation system for smoke inhalation integrated with a breathing Lung-on-Chip to model human lung responses to cigarette exposure

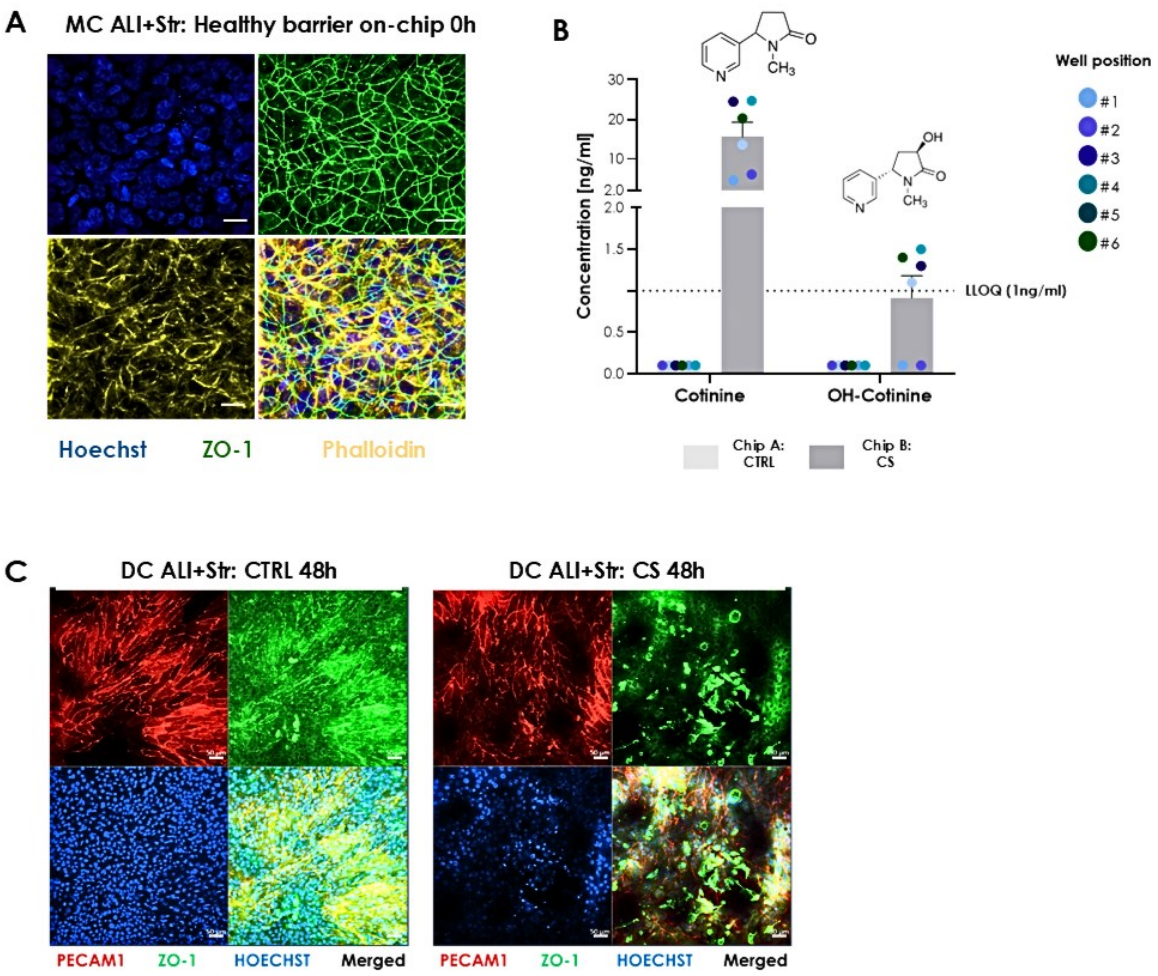
Supplementary Information:



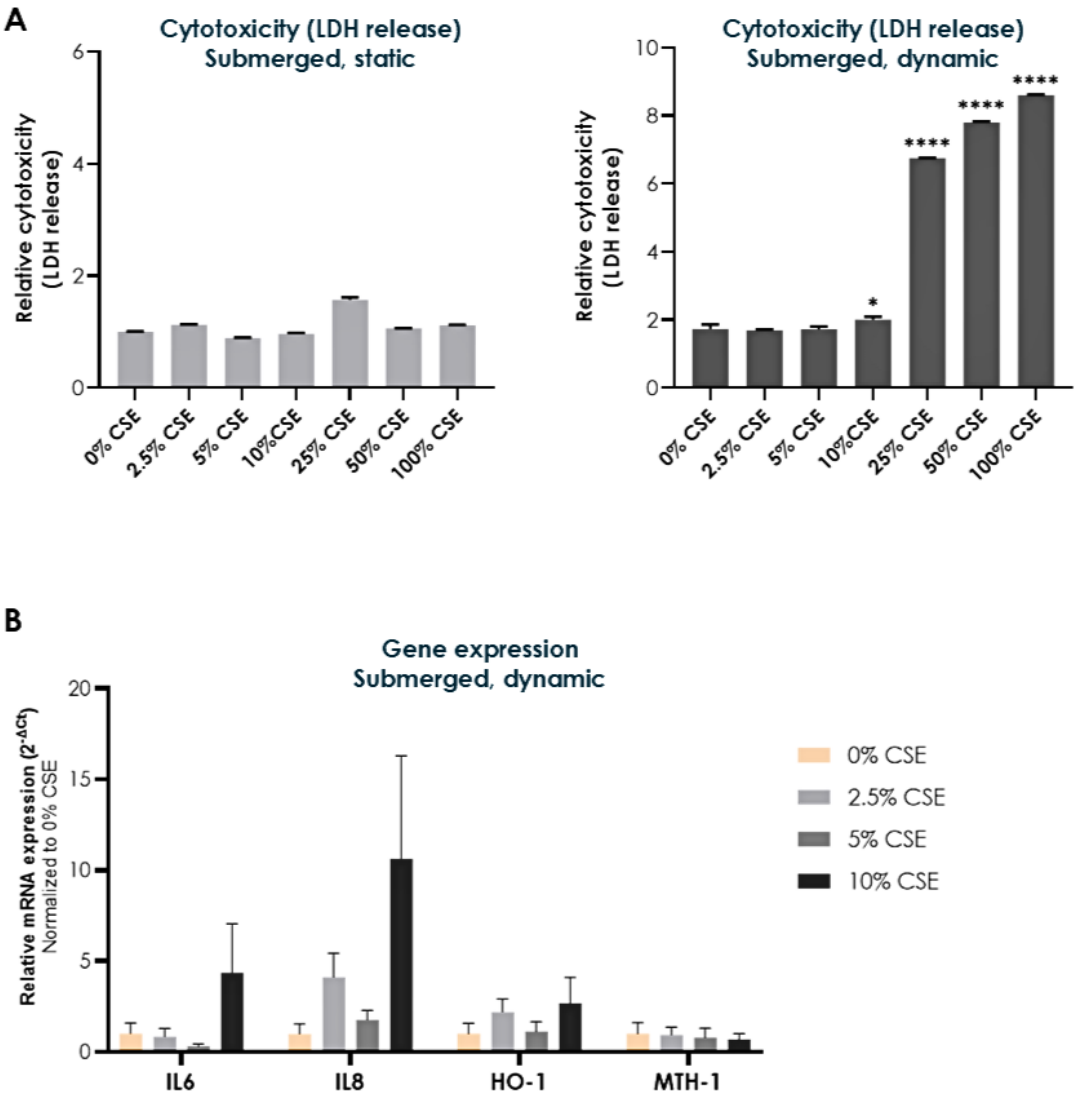
Supplementary Fig 1. Elements within the CFAX12. **(A)** The controlled environment around the exposure module and the AX12 during an experiment is maintained two electrical heaters, using four fans for air circulation, and a warm water bath **(D)** to replicate the humidity and temperature of inhaled air, typically 37°C and 90-95% humidity. **(B)** The cigarette holding magazine takes up to 20 cigarettes. **(C)** VC 10 smoking robot connected to the dilution system and exposure module and the two parallel smoke paths allow to have different exposure conditions on the same AX12. **(E)** Mass flow controllers for the regulation of dilution air. **(F)** The cigarettes are automatically loaded into the cigarette holder taken one by one from the magazine unit. **(G)** The cigarette butt retractor clamp removes the cigarette from the holder when one smoking cycle is finished. **(H)** The syringe pump draws the desired volume of smoke (puff volume) from the cigarette and then pushes the smoke towards the exposure module.



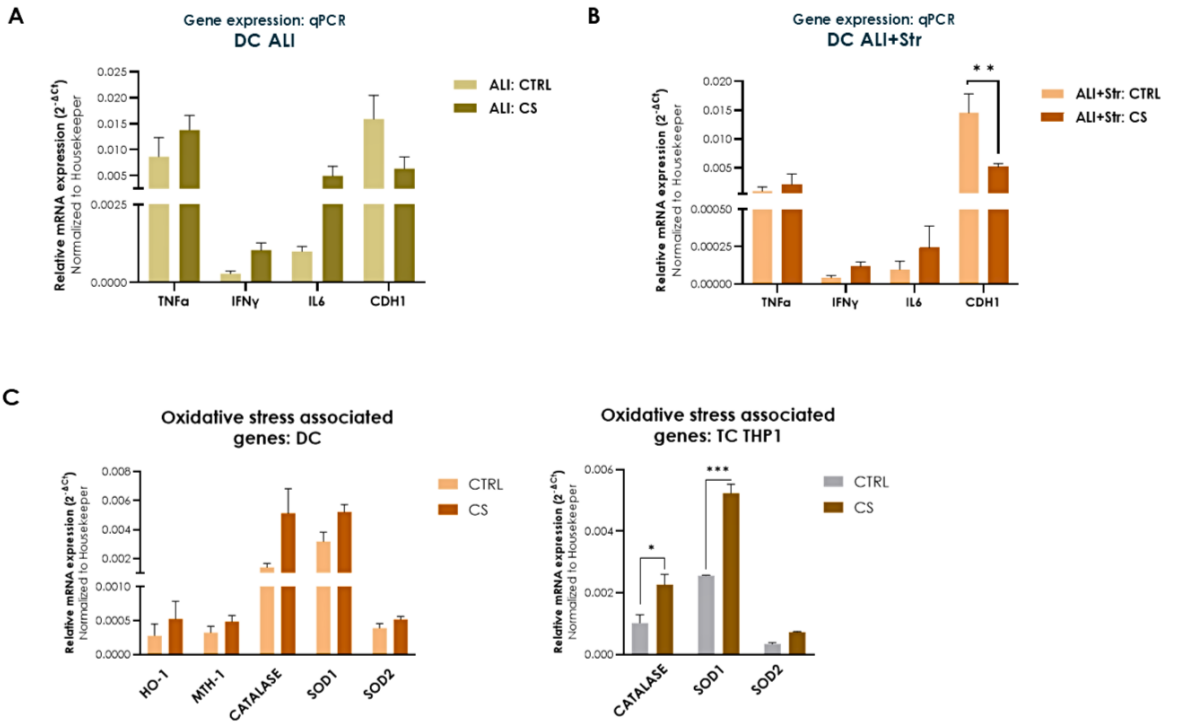
Supplementary Figure 2. Characterization of the primary blood-derived macrophages. **(A)** Illustration of the different steps of the protocol used to isolated monocytes from the buffy coats of doners. **(B)** Flow cytometry analysis revealed an average of 67% of live monocytes after isolation from 3 donors (#D1, #D2 and #D3) and an average of 76.5% live cells from donors #D2 and #D3. **(C)** Among the live cells, ca. 28% of CD14+ monocytes were identified in average from the three donors. **(D)** Representative brightfield pictures of the isolated monocytes, before and after treatment with M-CSF for 6 days. Scale bar = 100 µm. **(E)** From left to right, the cells were stained for nuclei (Hoechst; in blue), CD68 (in green) and F-actin (Phalloidin; in red). Scale bar = 100 µm.



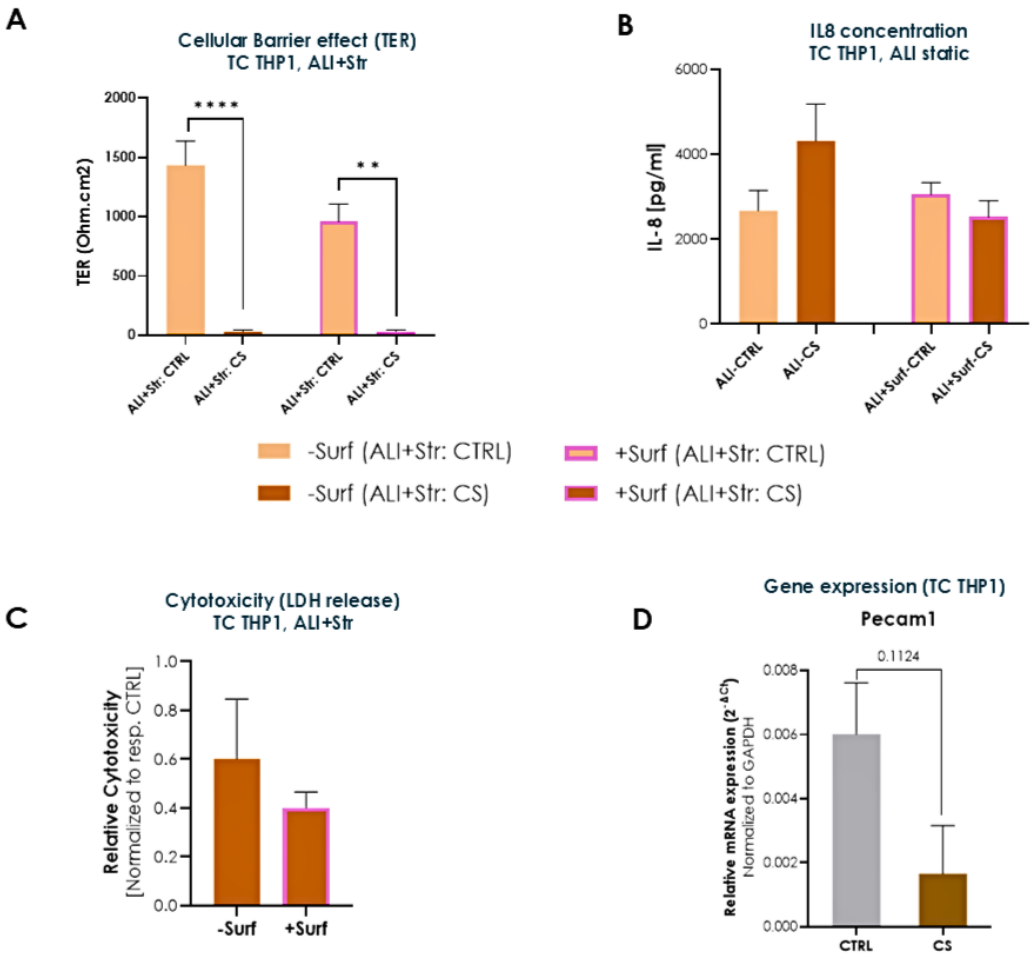
Supplementary Figure 3: (A) Representative immunofluorescence staining of CTRL ALI+Str (^{AX}iAECs) in monoculture on-AX12 (scale bar 20µm) at 0h time-point. Cells were stained for Zonula Occludens 1 (ZO-1, in green), F-actin (Phalloidin; in red) and nuclei (Hoechst, in blue). **(B)** Concentration of cotinine, and OH-cotinine (in ng/ml) within the wells of Chip A and Chip B is depicted. The individual dots represent measurements from specific wells. Lower limit of quantification (LLOQ) for these metabolite concentrations were at 1ng/ml. Data are shown as mean ± SEM. **(C)** CTRL and CS exposed ALI+Str cells in dual-culture; DC conditions at 48h time-point after CS exposure (scale bar 50µm). Cells were stained for Zonula Occludens 1 (ZO-1, in green), PECAM-1 (in red) and nuclei (Hoechst, in blue).



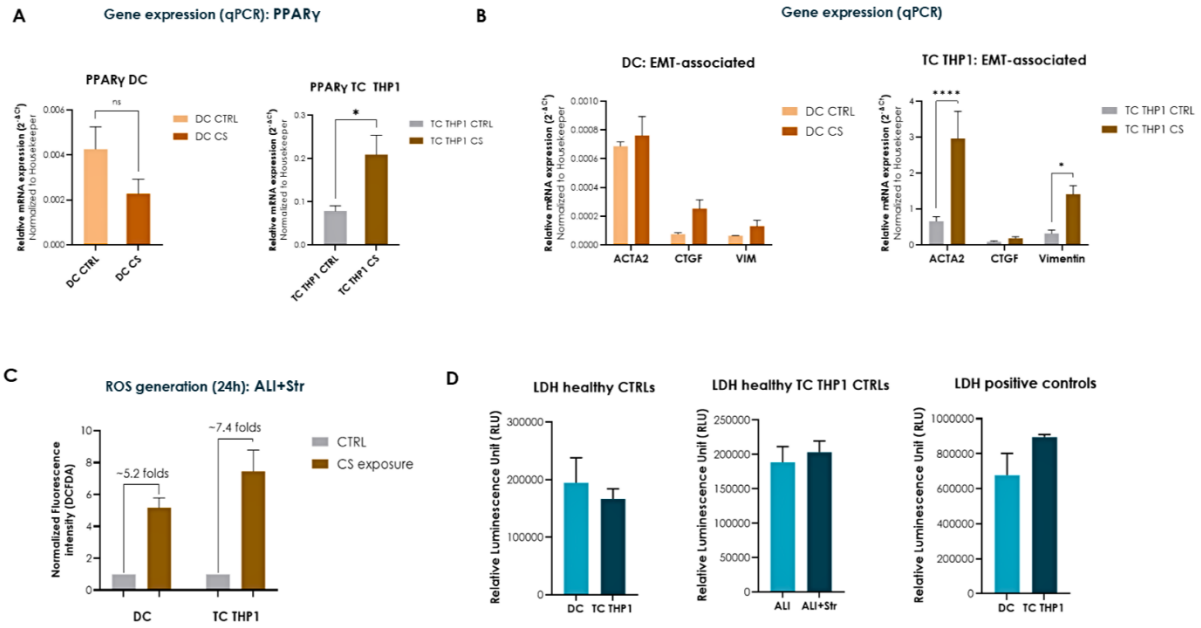
Supplementary Figure 4: Higher concentrations of CSE treatment induce cytotoxic and inflammatory effects. **(A)** Cytotoxicity was calculated from LDH release at 24h after CSE treatment in static and dynamic conditions (N=1; n=4/concentration). Significance for cytotoxicity was calculated for the treated cells (from 2.5% to 100%) relative to the non-treated 0% CSE control cells. **(B)** mRNA was harvested at 24h time-point post-treatment of CSE. Gene expression for inflammation (IL6, Interleukin 6; IL8, Interleukin 8) and oxidative stress-related (HO-1, Heme Oxygenase 1; MTH-1, Human MutT Homolog 1) genes were measured in dynamic cell samples (N = 2, n = 3/concentrations). Data are shown as mean ± SEM.



Supplementary Figure 5: Effect of CS exposure. **(A)** mRNA was harvested at 48h time-point post-exposure to CS under ALI static conditions. Gene expression for inflammation (Interleukin 6, IL6; Tumor Necrosis Factor a, TNFa; Interferonγ, IFNγ) and epithelial-related (CDH1; Cadherin1) genes were measured (N=3, n=3) **(B)** mRNA was harvested at 48h time-point post-exposure to CS under ALI+Str dynamic conditions. Gene expression for inflammation (IL6, TNFa, IFNγ) and epithelial-related (CDH1) markers were measured (N=3, n=3). Data are shown as violin plots, medians are indicated by black dotted lines. **(C)** mRNA was harvested at 48h time-point post-exposure to CS from DC and (E) TC THP1 culture conditions. Gene expression for oxidative stress associated genes (Heme Oxygenase-1; HO-1, mitochondrial oxidative stress marker Human MutT Homolog 1; MTH-1, Catalase, and Superoxide Dismutase 1 & 2; SOD1, SOD2) genes were measured (N=2, n=3). Data are shown as mean ± SEM.



Supplementary Figure 6: Impact of CS exposure on gene expression, oxidative stress, and barrier integrity. **(A)** TER (Ohm.cm²) was measured under ALI+Str conditions using the TC THP1 model at 48h post-CS exposure to assess barrier integrity (N=3, n=3/time-point). **(B)** IL-8 levels (pg/ml) were quantified from supernatants collected 48h post-CS exposure using ELISA (N=2, n=3/condition). **(C)** Cytotoxicity was assessed by LDH release at 48h post-CS with and without surfactant (+Surf or -Surf) exposure (N=2, n=2/time-point). **(D)** Gene expression analysis in the TC THP1 model at 48h post-CS exposure, focusing on PECAM 1expression (N=1, n=3). Data are shown as mean ± SEM.



Supplementary Figure 7: Effect of CS exposure on PPARγ, EMT and ROS generation. (A) mRNA was harvested at 48h post-CS exposure from DC and TC THP1 under ALI+Str conditions. Gene expression analysis for PPARγ performed using qPCR (N=1, n=5). (B) mRNA was harvested at 48h post-CS exposure from DC and TC THP1 under ALI+Str conditions. Gene expression analysis for PPARγ performed using qPCR (N=1, n=5). Gene expression for EMT markers (ACTA2, CTGF and Vimentin) were analyzed (N=2, n=4). (C) ROS generation was measured at 24h post-exposure under ALI+Str conditions in both DC and TC THP1 model using the DCFDA assay (N=1, n=4). (D) Cytotoxicity was calculated from LDH release at 0h time-point before CS exposure from healthy controls from DC and TC THP1 models (N=3; n=6/condition) LDH positive control tested from DC and TC THP1 model at endpoint (N=2; n=3/model). Data are shown as mean ± SEM.

Gene name	Forward (5′-3′)	Reverse (5′-3′)
TNFa	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGTCACTC
IL8	AGCACTCCTTGGCAAAACTG	CGGAAGGAACCATCTCACTG
IL6	AGGAGACTTGCCTGGTGAAA	GTCAGGGGTGGTTATTGCAT
IFNγ	AGCTCTGCATCGTTTTGGGTT	GTTCCATTATCCGCTACATCT
CDH1	CGAGAGCTACACGTTCACGG	CGAGAGCTACACGTTCACGG
HO-1	AGCAACAAAGTGCAAGATTCT	TGTAAGGACCCATCGGAGAAG
MTH-1	CTCAGCGAGTTCTCCTGG	GGAGTGGAAACCAGTAGC TGTC
PPARγ	GAG AGA TCC ACG GAG CTG AT	AGG CCA TTT TGT CAA ACG A
Catalase	TAA GAC TGA CCA GGG CAT C	CAA ACC TTG GTG AGA TCG AA
SOD1	CTG AAG GCC TGC ATG GAT TC	CCA AGT CTC CAA CAT GCC TCT C
SOD2	CAC TGC AAG GAA CAA CAG GC	ACC AGG CTT GAT GCA CAT CTT
GAPDH	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTTC

Supplementary Table 1. Primer sequences used in qRT-PCR. IL6, Interleukin 6; IL8, Interleukin 8; TNFa, Tumor necrosis factor a; IFNγ, Interferon γ; CDH1, Cadherin1; HO-1, Heme Oxygenase 1; MTH-1, Human MutT Homolog 1; PPARγ, Peroxisome proliferator-activated receptor; Catalase, SOD1 and 2, Superoxide dismutase 1 and 2; GAPDH (housekeeper), Glyceraldehyde-3-phosphate dehydrogenase.