Supplementary Information (SI) Methods

Chemicals and reagents

Foetal Bovine Serum (South American origin, Gibco, Life Technologies, 10270-106), Phosphate Buffer Saline (HIMEDIA, M1866), 0.25% Trypsin-EDTA 1X (Gibco, Life Technologies, 25200-056), F12 media (Gibco, Life Technologies, 11765-054), Antibiotic-Antimycotic 100X (Gibco, Life Technologies, 15240-062), MTT (Sigma Aldrich, M2128), DMSO (Merck, SK5S650642), Alexa Fluor 488 anti-rabbit (Invitrogen, A11034), Alexa Fluor 594 anti-mouse (Invitrogen, A11032), Prolong gold Antifade with DAPI(Invitrogen, 8961S), Piperine (Sigma), Dexamethasone (HIMEDIA), Methacholine (TCI, M0073), TPER (Thermo Scientific, 78510), RIPA (Thermo Scientific, 89901), Protease and phosphatase Inhibitor (Thermo Scientific, A32961), Protein Assay Dye Reagent Concentrate (BIO-RAD, 5000006), Trans-Blot Turbo 5X Transfer buffer (BIO-RAD, 10026938), Ponceau S solution (Sigma Aldrich, P7170), Bovine serum albumin (HIMEDIA, MB083), primary antibodies E-cadherin (Abclonal, A18135), N-cadherin (Abclonal, A0433), Vimentin (Abclonal, A2584), α-SMA (Abclonal, A17910), OPA1 (Abclonal, A9833), DRP1 (CST, 8480S), α-tubulin (CST, 8480S), β-actin (Abclonal, AC026). TRIzol reagent (Ambion, Life Technologies, 15596018), cDNA synthesis kit (Takara, 6110A), PowerUp™ SYBER™ Green Master Mix (A25742), primers from Integrated DNA technologies- PINK1 (F- 5’ AGAGAGGTCCCAAGCAACTA 3’, R- 5’ GCAGGGTACAGGGATAGTTC 3’), PARKIN (F- 5’ CCCAGTGACCATGATAGTGTTT 3’, R- 5’ CAGTCCAGTCATTCCTCAGC 3’) and GAPDH (F- 5’ TCATTTCCTGGTATGACAACGA 3’, R- 5’ AGGGGAGATTCAGTGTGGTG 3’). All the analytical grade chemicals were used and were purchased from Sigma and Thermo.

Nicotine Estimation by HPLC

The concentration of nicotine extracted in phosphate buffer saline was estimated by using Dionex Ultimate 3000 UHPLC system equipped with (photodiode array) PDA detector. The chromatographic analysis was performed on Hypersil Gold C8 colulmn (150 X 4.6mm, 5µm) using 0.1% TFA in water and acetonitrile (9:1) as mobile phase with a flow rate of 0.5mL/min. The detection wavelength was 260nm.

Supplementary Figure legends

Figure S1: HPLC chromatogram of nicotine from Cigarette smoke and IQOS extract recorded at 260nm.

Figure S2: (A) Pictorial representation of histogram plots for the DCFDA assay to measure intracellular ROS generation in BEAS-2B cells upon exposure of CS, or IQOS, or CS+IQOS for 24h. (B) Bar graph representing mean fluorescence intensity changes upon DCFDA staining. Values are represented as mean±SEM (n=4). A one-way ANOVA followed by post-hoc Tukey's test was used to define statistical significance. $*p<0.05$, $**p<0.01$, $***p<0.001$ vs unexposed.

Figure S3: (A) Representative images for MitoSOX assay to assess mitochondrial superoxide generation in BEAS-2B cells upon exposure of CS, or IQOS, or CS+IQOS for 24h. (B) Bar graph representing mean fluorescence intensity changes upon MitoSOX staining. Values are represented as mean±SEM (n=3). A one-way ANOVA followed by post-hoc Tukey's test was used to define statistical significance. $*p<0.05$, $**p<0.01$, $***p<0.001$ vs unexposed, $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ vs CS exposed.

Figure S4: (A) Representative images of BEAS-2B cells stained with mitotracker and lysotracker to show mitophagy upon exposure of CS, IQOS, or CS+IQOS for 6h. (B) Bar graph represents

Pearson’s co-localisation coefficient between mitotracker and lysotracker staining. Values are represented as mean±SEM (n=3). A one way ANOVA followed by Tukey’s test was applied to determine the statistical significance. *$p<0.5$, **$p<0.01$, ***$p<0.001$ vs. unexposed.