**Supporting Information for**

# Human Asthmatic Bronchial Cells Are More Susceptible to Sub-Chronic Repeated Exposures of Aerosolized Carbon Nanotubes at Occupationally-Relevant Doses than Healthy Cells

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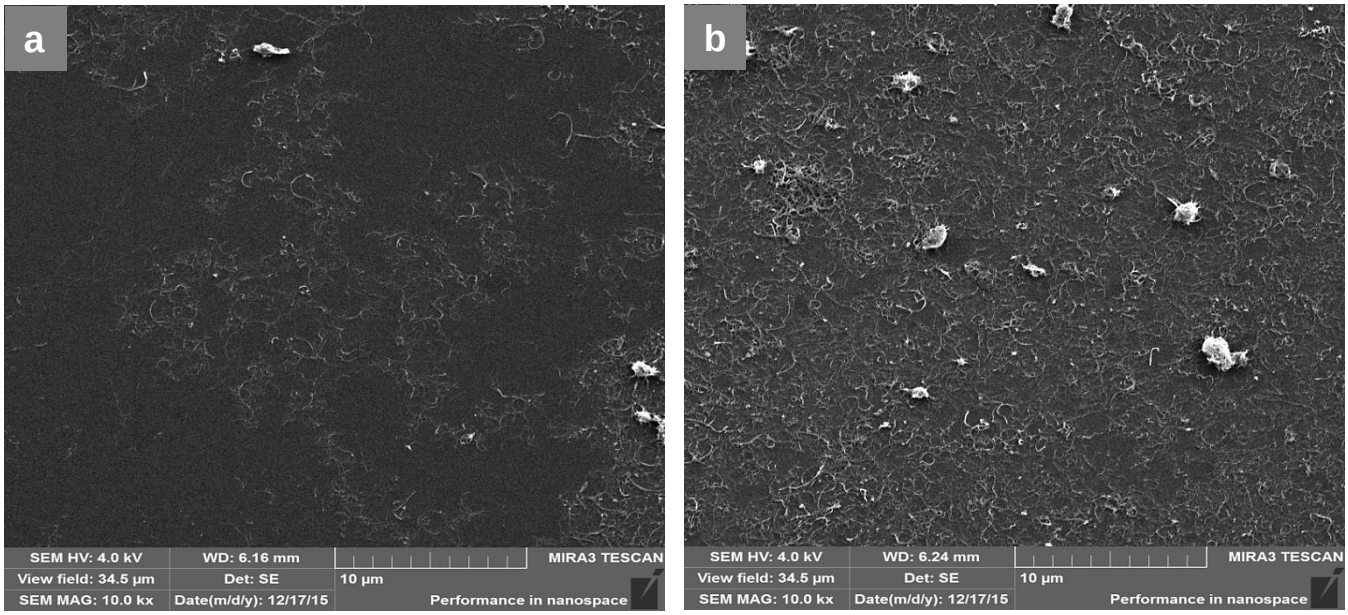
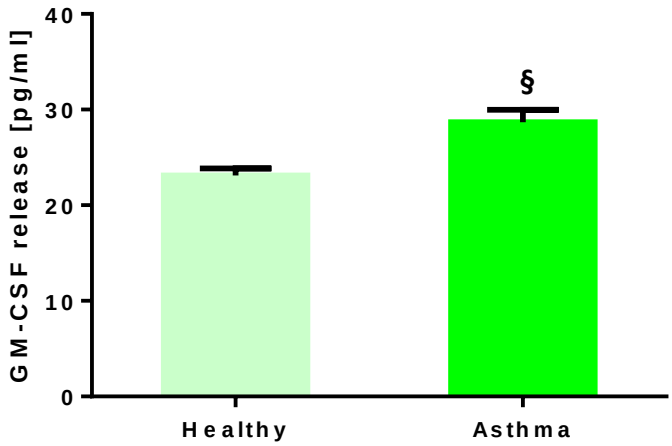
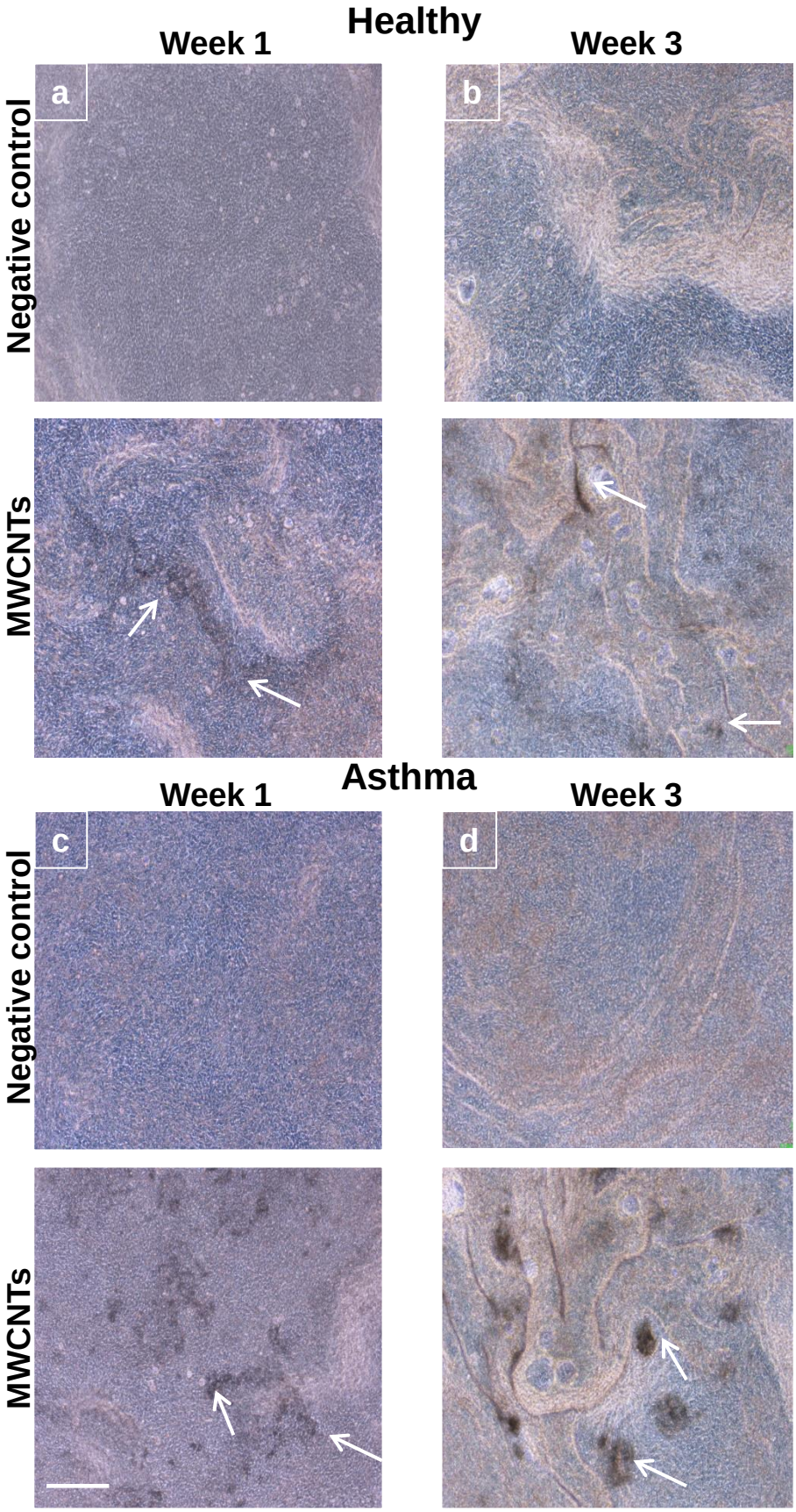


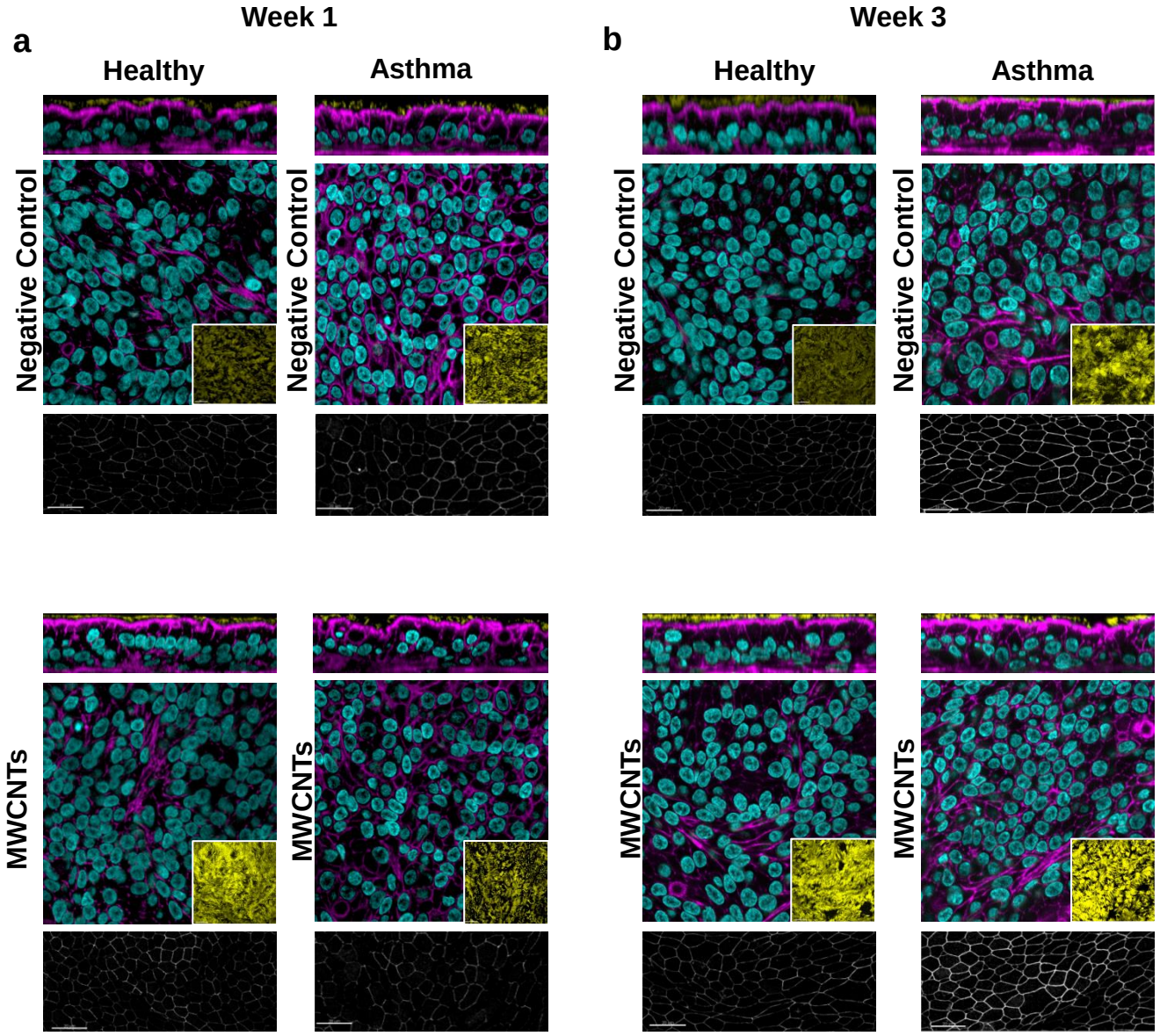

**Supplementary figure 1: Morphology of deposited MWCNTs upon single and repeated exposures.** SEM images of aerosolized MWCNTs following (**a**) single (d1) and (**b**) 5 repeated exposures (w1).



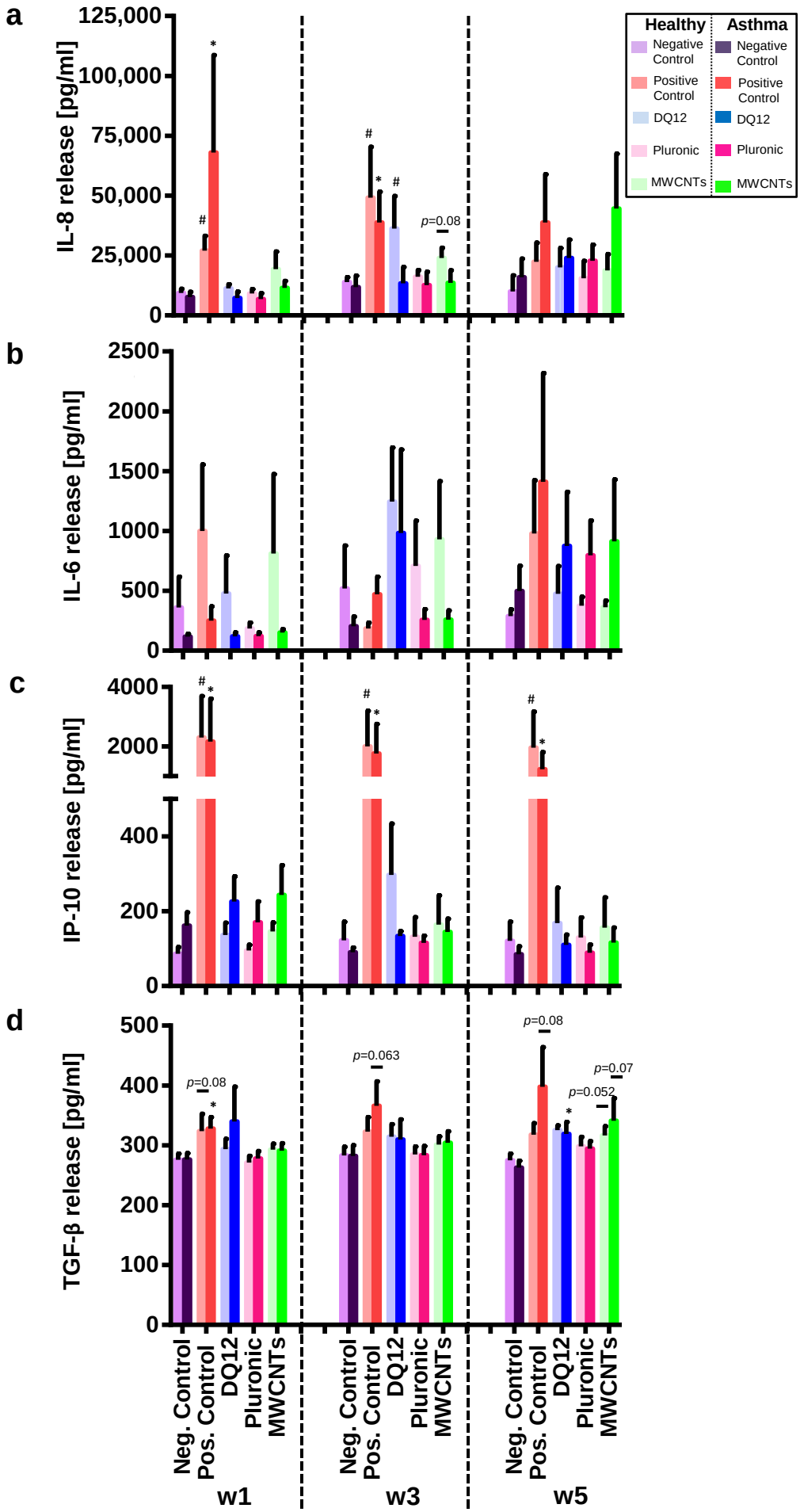

**Supplementary figure 2: CM-CSF activity of asthmatic and nonasthmatic control cultures.** Quantification of GM-CSF release in healthy and asthmatic negative control tissues upon w1. § Shows significant response ($p < 0.05$) of asthma cultures when compared to healthy cultures. Data are presented as the mean ± standard error of the mean (SEM).




**Supplementary Figure 3: Morphological assessment of healthy and asthma cultures after subchronic MWCNT exposure.** Light microscopy images of healthy cultures at w1 (**a**) and w3 (**b**). Illustrated images represent asthma cells at the end of w1 (**d**) and w3 (**e**). White arrows indicate MWCNTs accumulated on the cells surface (scale bars: 100µm).




**Supplementary figure 4: Morphology of healthy and asthmatic human bronchial epithelial cells after long term exposure to aerosolized MWCNTs.** Illustrated LSM images represent cells exposed repeatedly to MWCNTS for either 1 week (**a**) or 3 weeks (**b**). Magenta color shows F-actin (cytoskeleton), blue color shows DNA (cell nuclei), yellow represents cilia and white the tight junctions (scale bars: 20 μm).


a
b
c
d
IL-8 release [pg/ml]
IL-6 release [pg/ml]
IP-10 release [pg/ml]
TGF-β release [pg/ml]
Healthy
Asthma
Negative Control
Positive Control
DQ12
Pluronic
MWCNTs
p=0.08
p=0.063
p=0.07
p=0.052
Neg. Control
Pos. Control
DQ12
Pluronic
MWCNTs
w1
w3
w5

**Supplementary figure 5: (Pro-)inflammatory response in cells exposed repeatedly to MWCNTs for up to 5 weeks.** (**a**) IL-8, (**b**) IL-6 (**c**) IP-10 and (**d**) TGF-β release in the medium (n=4). TNF-α (1μg/ml) was used as positive control for IL-8 and IL-6 induction, while IFN-γ (1μg/ml) for IP-10 and TGF-β respectively. # Represents a significant increase ($p < 0.05$) in healthy cultures compared to the negative control.* Indicates statistical significance in asthmatic cells compared to the negative control ($p < 0.05$). § Shows significant response ($p < 0.05$) of asthma cultures when compared to healthy cultures upon w5. Data are presented as the mean ± standard error of the mean (SEM).

| MWCNTs | Before aerosolisation | After aerosolisation |
|---|---|---|
| **Length (µm)** | 1-10[27] | 3.4 ± 2.2[25] |
| **Width (nm)** | 5-30[27] | 30 ± 10.3[25] |
| Other MWCNT characteristics: | | |
| **Shape (Form)** | Fibrous (bundled)[27, 28] | |
| **Elemental Contaminants (%wt)** | Fe(0.05), Mg(0.01), Ni(0.12), Co(<0.001)[27, 28] | |
| **Endotoxin Content** | ND[27, 28] | |

**Supplementary table 1: Physicochemical characteristics of MWCNTs.** Summary of key physicochemical MWCNTs characteristics before and after aerosolisation process as previously reported by [25,27,28].

| Genes | Direction | Primer Sequence |
|---|---|---|
| *GADPH* | forward | AAC AGC CTC AAG ATC ATC AGC |
| | reverse | GGA TGA TGT TCT GGA GAG CC |
| *HMOX-1* | forward | TTC TCC GAT GGG TCC TTA CAC T |
| | reverse | GGC ATA AAG CCC TAC AGC AAC T |
| *SOD-2* | forward | CTG CTG GGG ATT GAT GTG TGG |
| | reverse | TGC AAG CCA TGT ATC TTT CAG T |
| *IL-8* | forward | CTG GCC GTG GCT CTC TTG |
| | reverse | CCT TGG CAA AAC TGC ACC TT |
| *IL-6* | forward | CCA GGA GCC CAG CTA TGA AC |
| | reverse | CCC AGG GAG AAG GCA ACT G |
| *IP-10* | forward | CCA TTC TGA TTT GCT GCC TTA TC |
| | reverse | GCA GGT ACA GCG TAC AGT TCT |
| *TGF-β* | forward | CCC TAC ATT TGG AGC CTG GAC ACG |
| | reverse | CGG GTT ATG CTG GTT GTA CAG GGC |

**Supplementary table 2: List of analysed genes and primer sequences used for real-time PCR.**

| MWCNTs Sub-chronic effects | w1 | | w3 | | w5 | |
|---|---|---|---|---|---|---|
| | Healthy | Asthma | Healthy | Asthma | Healthy | Asthma |
| MCC | N.T | N.T | N.T | N.T | 22.7 ±3.9 | 26.7 ±3.2 |
| CBF | N.T | N.T | N.T | N.T | 9.37 ±0.05 | 9.86 ±0.13 |
| HMOX-1 (expression) | 3.95 ±3.0 | 18.1 ±10.9 | 5.3 ±4 | 6.9 ±4 | 6.57 ±2.9 | 22.6 ±10.8 |
| SOD-2 (expression) | 1.18 ±0.13 | 4.95 ±2.8 | 3.73 ±2.2 | 10.55 ±8.4 | 79.2 ±45.1 | 104.7 ±74.6 |
| IL-8 (expression) | 19.6 ±11.6 | 8.8 ±3.8 | 9.48 ±6.6 | 10.84 ±10.5 | 9.2 ±1.99 | 26.4 ±7.9 |
| (secretion) | 19360 ±7306 | 11465 ±2923 | 23938 ±4310 | 13582 ±5181 | 18792 ±6819 | 44637 ±22882 |
| IL-6 (expression) | 7.49 ±3.4 | 6.1 ±2.6 | 41.4 ±4.5 | 2.4 ±1.1 | 120.9 ±62.3 | 196.8 ±118.2 |
| (secretion) | 812 ±662.1 | 151.4 ±27.9 | 931.0 ±483.6 | 259.5 ±75.5 | 363.75 ±53.5 | 914.4 ±514.5 |
| IP-10 (expression) | 5.6 ±3.7 | 0.17 ±0.1 | 2.3 ±0.6 | 13.7 ±8.6 | 92.9 ±34.6 | 29.8 ±12.4 |
| (secretion) | 145.8 ±23.7 | 243.6 ±79.2 | 164.28 ±77.84 | 144.79 ±35.16 | 156.1 ±80.6 | 116.1 ±39.8 |
| TGF-β (expression) | 5.9 ±4.5 | 5.2 ±2.8 | 5.2 ±4 | 3.1 ±1.6 | 84.7 ±69.3 | 144.8 ±94.1 |
| (secretion) | 292.9 ±9.7 | 291.2 ±11.9 | 301.02 ±13.63 | 304.3 ±18.79 | 316.46 ±15.29 | 341.02 ±37.55 |

**Supplementary table 3: Summary of the measured biological response induced by long-term MWCNT exposure in bronchial epithelial cells *in vitro*.** Values are presented as the mean ± SEM. Red values represent statistical significant increase. Orange values show notable increase, while green values a weak increase. Black values indicate no change compared to control. N.T: Not tested.