

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

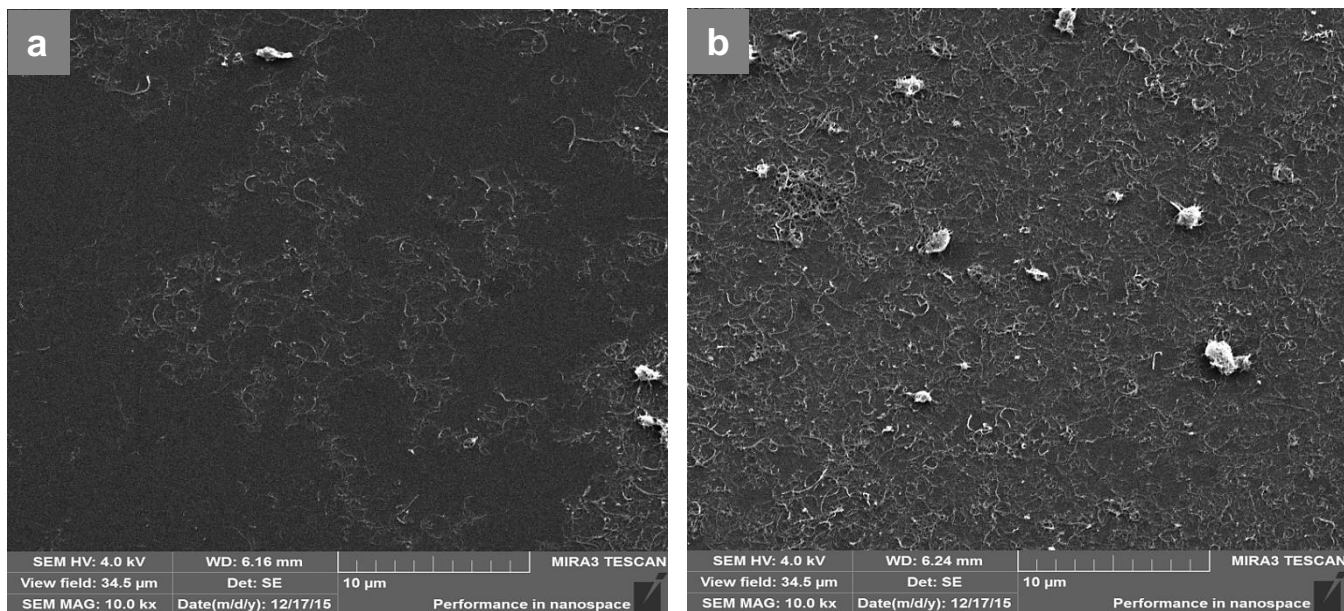
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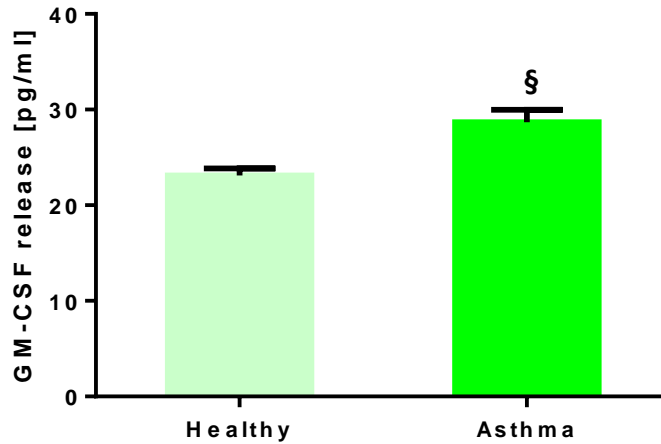
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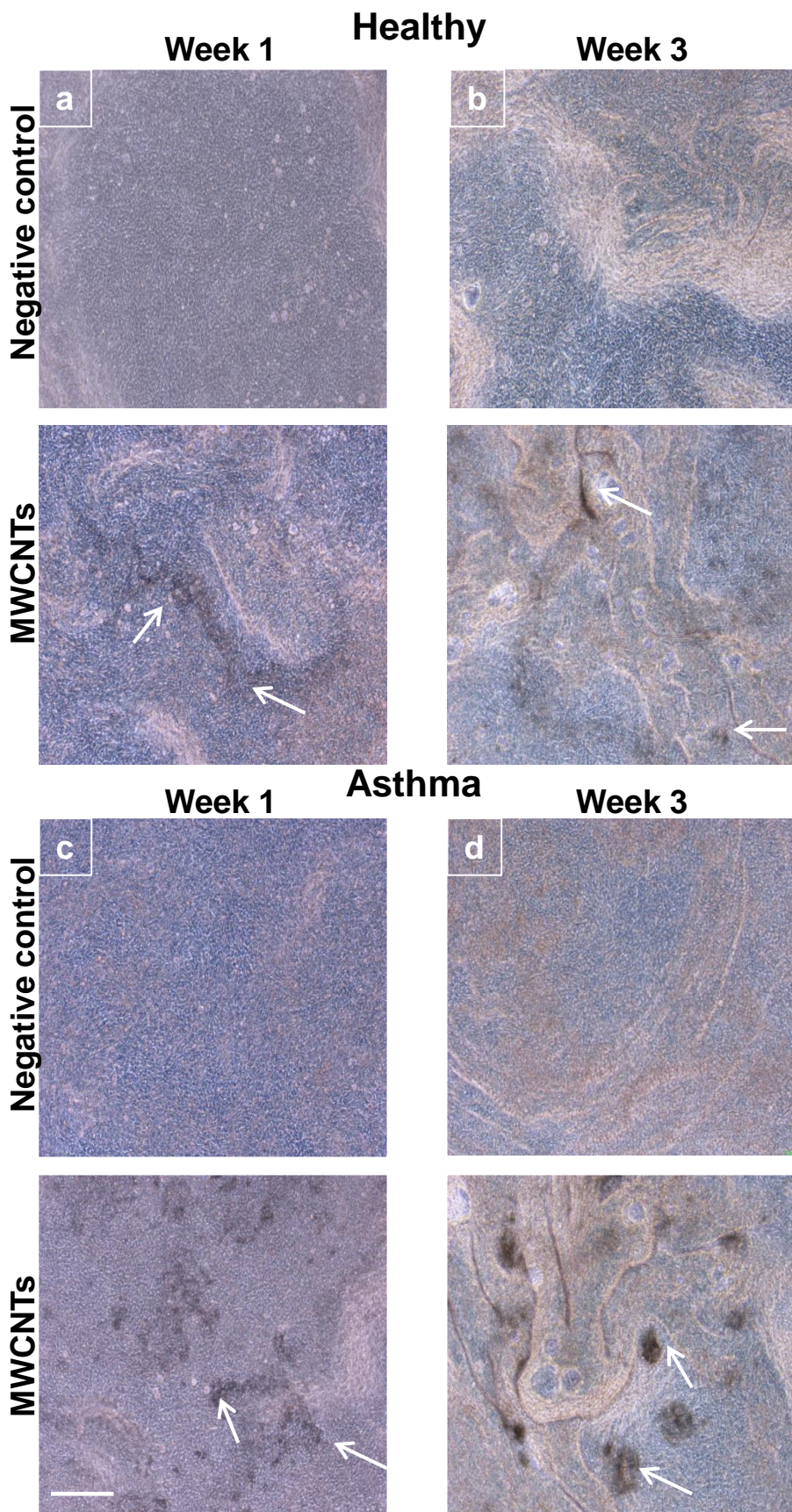
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**Supplementary figure 1: Morphology of deposited MWCNTs upon single and repeated exposures.** SEM images of aerosolized MWCNTs following (a) single (d1) and (b) 5 repeated exposures (w1).

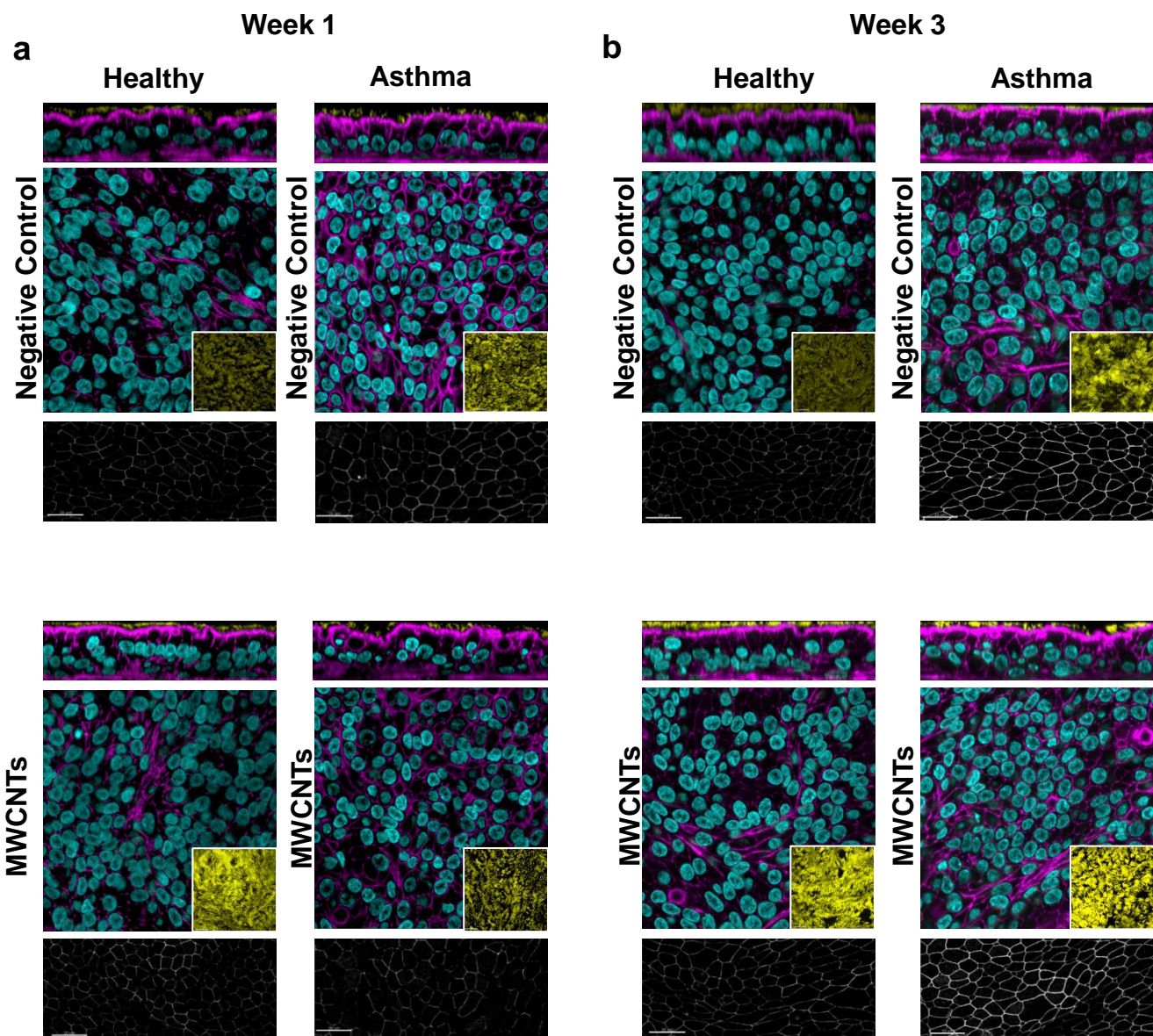


**Supplementary figure 2: CM-CSF activity of asthmatic and nonasthmatic control cultures.** Quantification of GM-CSF release in healthy and asthmatic negative control tissues upon w1. § Shows significant response ( $p < 0.05$ ) of asthma cultures when compared to healthy cultures. Data are presented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

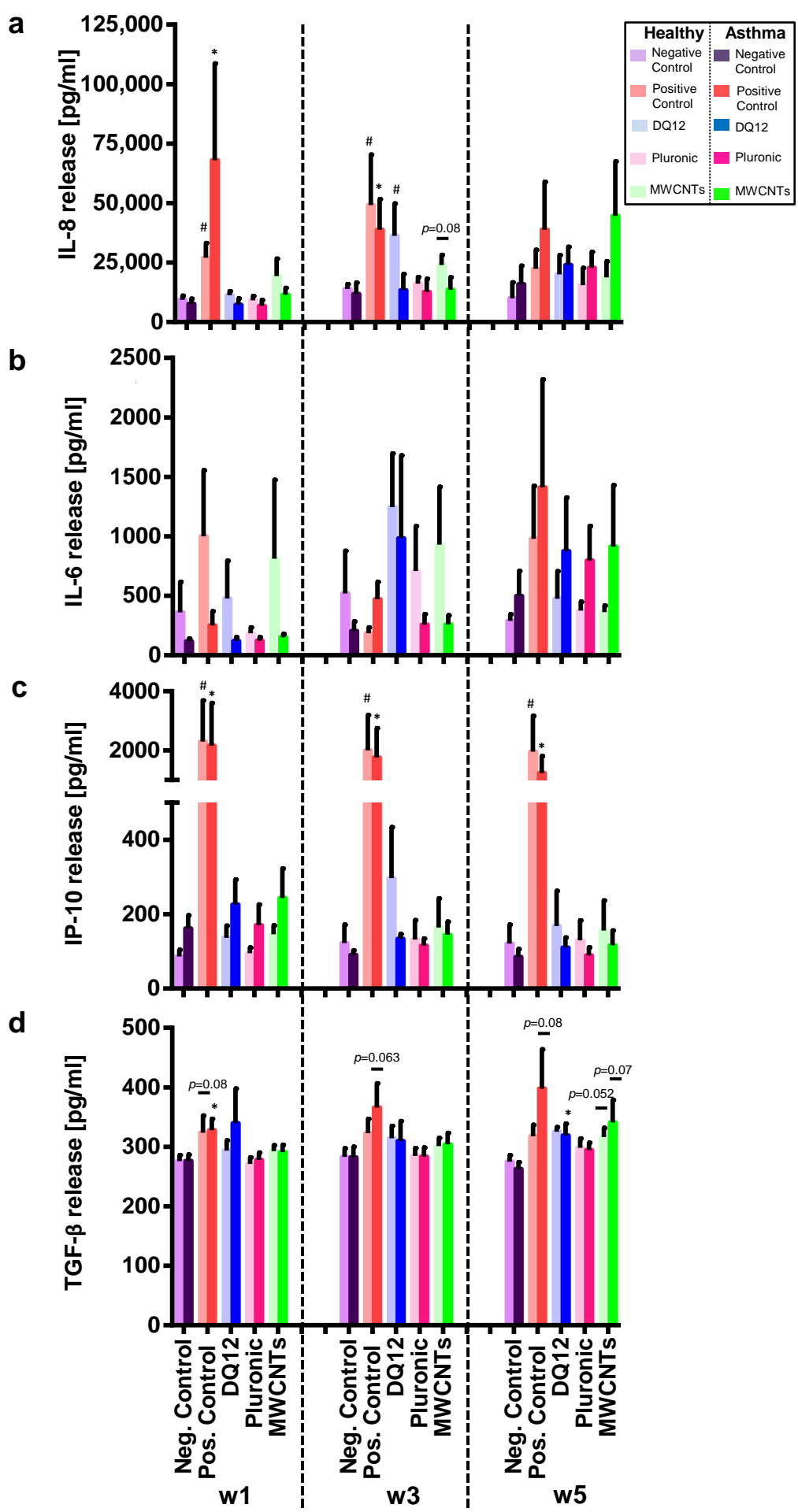


**Supplementary Figure 3: Morphological assessment of healthy and asthma cultures after subchronic MWCNT exposure.** Light microscopy images of healthy cultures at w1 (a) and w3 (b). Illustrated images represent asthma cells at the end of w1 (d) and w3 (e). White arrows indicate MWCNTs accumulated on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



**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- $\beta$  release in the medium (n=4). TNF- $\alpha$  (1 $\mu$ g/ml) was used as positive control for IL-8 and IL-6 induction, while IFN- $\gamma$  (1 $\mu$ g/ml) for IP-10 and TGF- $\beta$  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  $\pm$  standard error of the mean (SEM).

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

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



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

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

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