Supplemental information

Pulmonary damage and cytokine release following SWCNT exposure.

To assess pulmonary damage following SWCNT exposure, the activity of LDH and the total amount of protein were assayed spectrophotometrically in BAL specimens. The obtained results revealed that SWCNT induced marked cell/tissue damage in the lung of exposed mice on both days 1 and 7 post exposure showing a significant dose-dependent release of LDH (Figure S1A). Protein levels in BAL fluid were also increased in exposed animals on day 1 and remained elevated on day 7 post exposure (*i.e.* 0.81±0.02 mg/ml in 120 μg/mouse SWCNT group vs 0.45±0.01 mg/ml in control, $p<0.05$). A lower dose of SWCNT (40 μg/mouse) induced only a subtle increase of protein level in BAL fluid found on day 1 post exposure (Figure S3B).

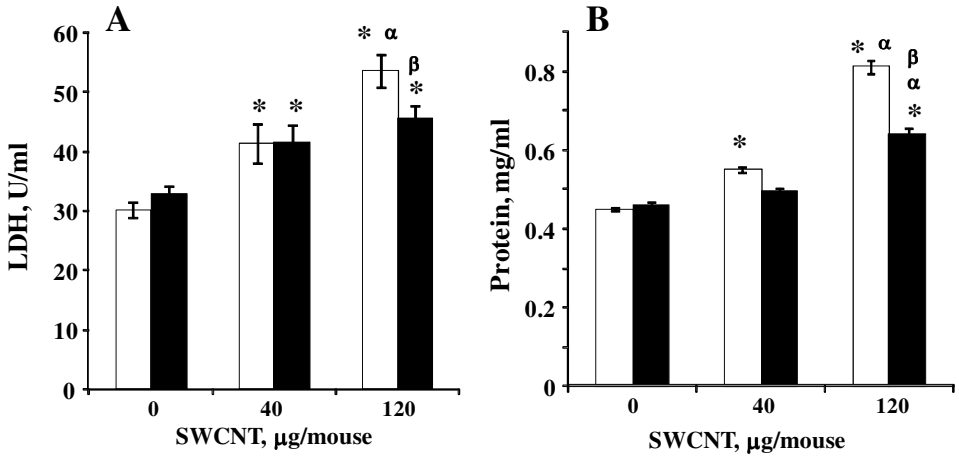

Figure S3. Pulmonary damage after pharyngeal aspiration of SWCNT: A – LDH; B – protein level. Open bars – 1 day post exposure; closed bars – 7 days post exposure. Data are shown as means ± SEM (3 experiments, 6 animals/group); *$p<0.05$ *vs*. control. $^{\alpha}p<0.05$ *vs*. 40 μg/mouse SWCNT exposure, $^{\beta}p<0.05$ *vs*. 1d post exposure.

To further characterize pulmonary inflammation, the levels of cytokines were assayed in the BAL fluid of mice exposed to SWCNT. The concentrations of TNF-α, IL-6, IFN-γ, IL-12p70, IL-10 and MCP-1 were determined using the BD™ Cytometric Bead Array, (Mouse Inflammation kit, BD Biosciences, San Diego, CA) on days 1 and 7 post exposure. A pro-inflammatory pattern of pulmonary cytokine release was found on day 1 post exposure (Figure S2). The TNFα increase (*i.e.* 270.1±74.8 pg/ml in 120 μg/mouse SWCNT group vs 8.47±1.7 pg/ml in control on day 1, $p<0.05$) correlated with PMN influx (peaked at day 1 and strikingly returned toward normal by day 7). MCP-1 (chemotactic signal for monocyte recruitment) was increased more than 60-fold on day 1, and remained significantly elevated but at a lower level on day 7 (*i.e.* day 1: 193.9±40.1 pg/ml; day 7: 13.5±4.2 pg/ml in 120 μg/mouse SWCNT group vs. 3.1±0.7 pg/ml in control, $p<0.05$). IL-6 peaked at day 1 (412.1±96.9 pg/ml in 120 μg/mouse SWCNT group) and returned toward normal by day 7 (3.4±0.4 pg/ml vs. 2.1±0.6 pg/ml in control, $p<0.05$) after SWCNT exposure. IFN-γ was found to be elevated in the BAL fluid only in mice exposed to 120 μg of SWCNT on day 7 post exposure (up to 3.12±1.1 pg/ml, $p<0.05$ vs. control) and was below the detection level in control group. IL-10 and IL-12p70 levels were not significantly altered following SWCNT exposure.

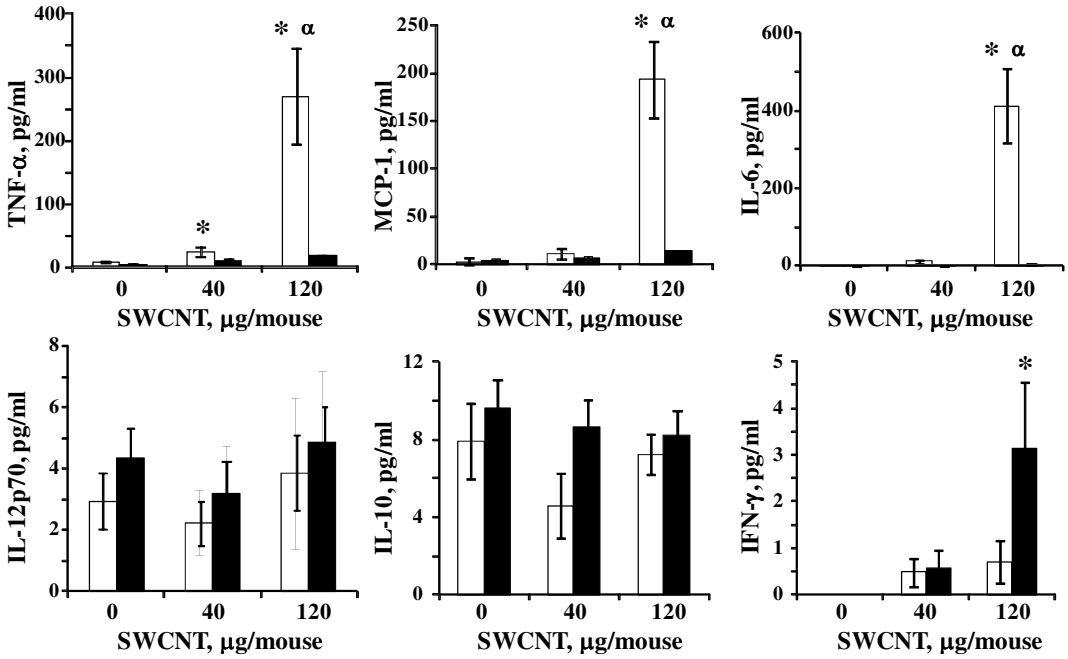

Figure S2. Cytokine profiles in the BAL fluid of mice following aspiration of SWCNT. Open bars – 1 day post exposure; closed bars – 7 days post exposure. Data are shown as means ± SEM (3 experiments, 6 animals/group); *p<0.05 vs. control, $^{\alpha}$p<0.05 vs. 40 μg/mouse SWCNT exposure.

Methods

BAL cytokine analysis

Levels of cytokines were assayed in the acellular BAL fluid following SWCNT exposures. The concentrations of TNF-α, IL-6, IFN-g, IL-12p70, IL-10 and MCP-1 were determined using the BD™ Cytometric Bead Array, Mouse Inflammation kit (BD Biosciences, San Diego, CA).

Total protein and lactate dehydrogenase (LDH) activity in the BAL fluids

Measurement of total protein in the BAL fluid was performed by a modified Bradford assay according to the manufacturer's instructions (BioRad, Hercules, CA) with bovine serum albumin as a standard. The activity of LDH was assayed spectrophotometrically by monitoring the reduction of NAD+ at 340 nm in the presence of lactate (Pointe Scientific, Inc., Lincoln Park, MI) in BAL.