Supporting information

Size- and shape-dependent pleural translocation, deposition and fibrogenesis by multi-walled carbon nanotubes

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The supporting information contains 5 figures.

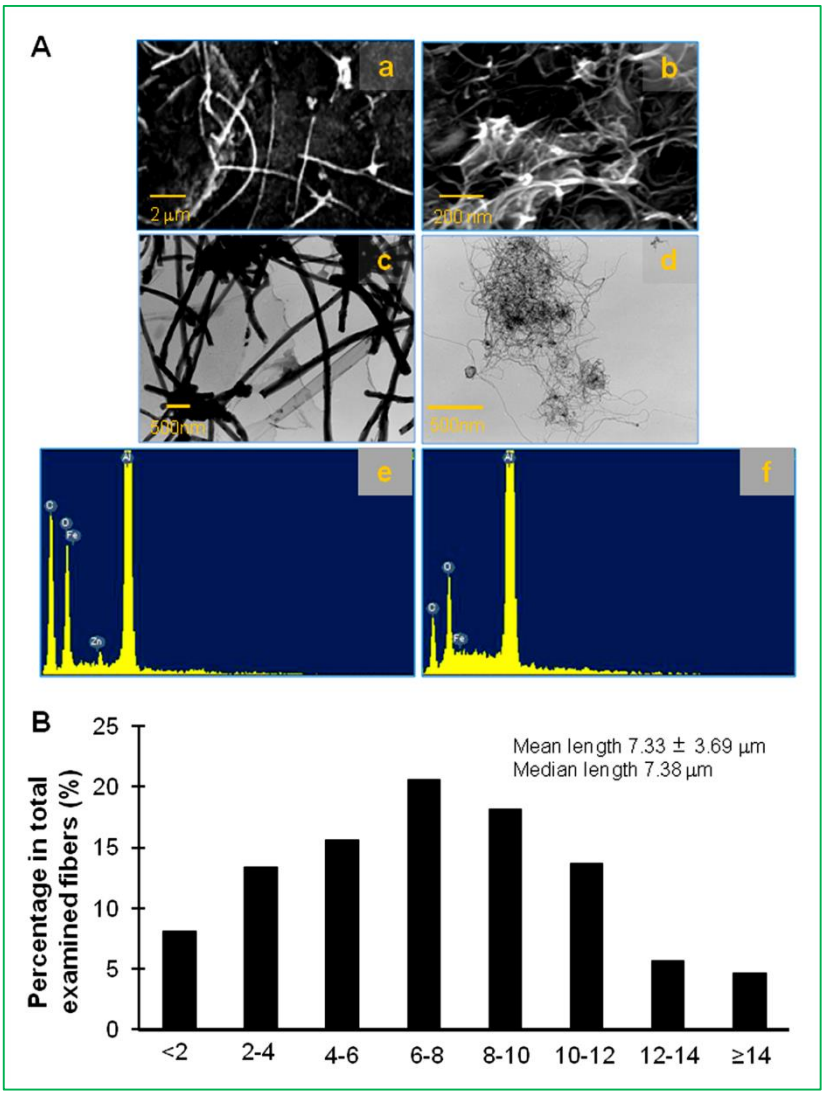

Fig S1 Characterization of MWCNT

A: SEM and TEM observation. Aliquots of diluted MWCNT-L and MWCNT-S suspensions were loaded on aluminum plates and subjected to SEM and TEM observation and elemental analysis. **a, c** and **e**, SEM image, TEM image and elemental analysis of MWCNT-L; **b, d** and **f**, SEM image, TEM image and elemental analysis of MWCNT-S. **B**: Length distribution of MWCNT-L fibers in the suspensions.

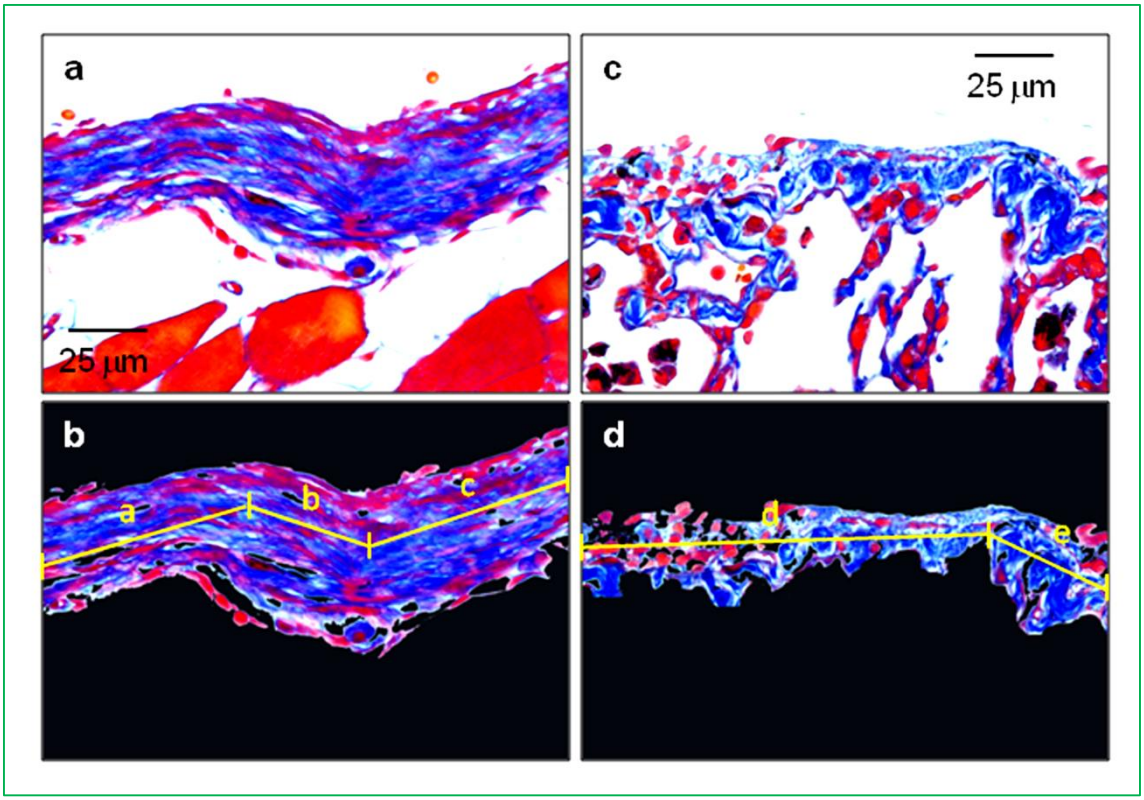

Fig S2 Determination of the thickness of the pleura

Determination of the thickness of the parietal and visceral pleura was based on sections with Azan-Mallory staining. Total area and the length of the parietal pleural region were determined using an image analyzer system (IPAP, Sumika Technos Corp., Osaka, Japan), and the mean thickness of the pleural region was calculated: the thickness of the parietal pleura=total area/(a+b+c) as shown in **a** and **b**, and the thickness of the visceral pleura=total area/(d+e) as shown in **c** and **d**.

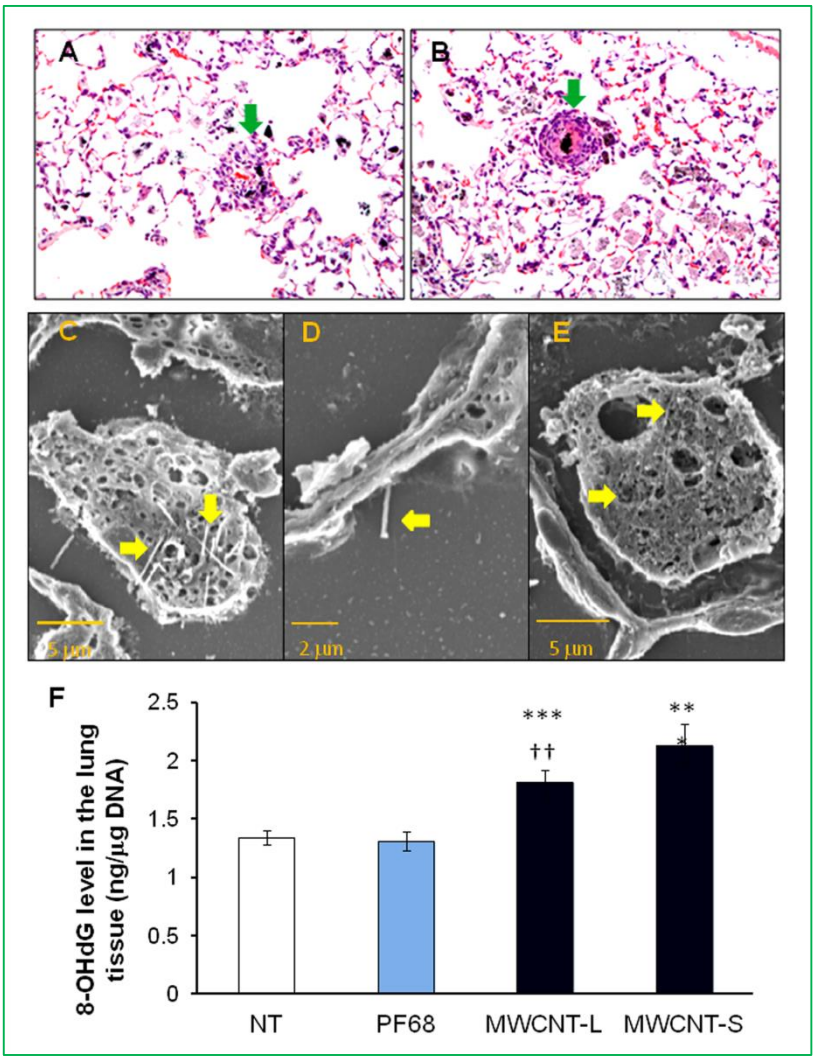

Fig S3 Granuloma formation, alveolar macrophage infiltration and 8-OHdG induction in the lung

Representative images of the lungs of rats treated with MWCNT-L (**A**) or MWCNT-S (**B**) show granulomas (green arrows) and alveolar macrophage induction. Needle-like MWCNT-L fibers phagocytosed by alveolar macrophages (**C**) and penetrating the alveolar epithelium (**D**) were observed. MWCNT-S fibers were found in alveolar macrophages (**E**) by SEM. Yellow arrows indicate MWCNT-L or MWCNT-S fibers. Treatment of MWCNT-L or MWCNT-S induced the production of 8-OHdG (**F**). ***, $p<0.001$, versus PF68; and ††, $p<0.01$, versus MWCNT-S.

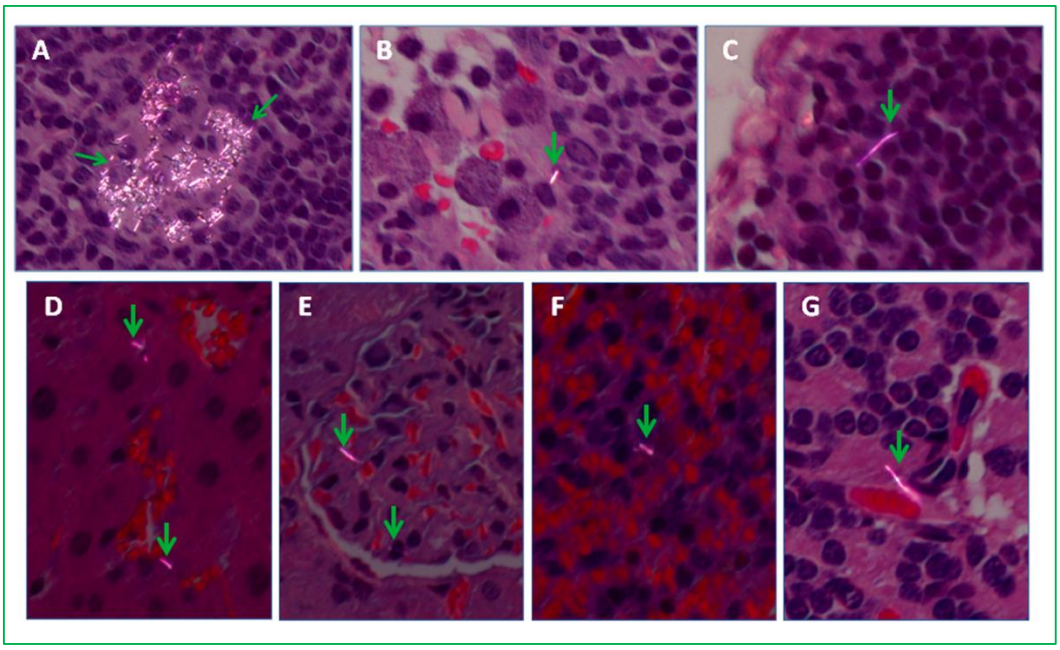

Fig S4 Transportation of MWCNT-L to extra-pulmonary organs

MWCNT-L was observed in the mediastinal (A), submandibular (B) and mesentery (C) lymph nodes, as well as in the liver (D), kidney (E), spleen (F) and brain (G) by polarized light microscopy (PLM).

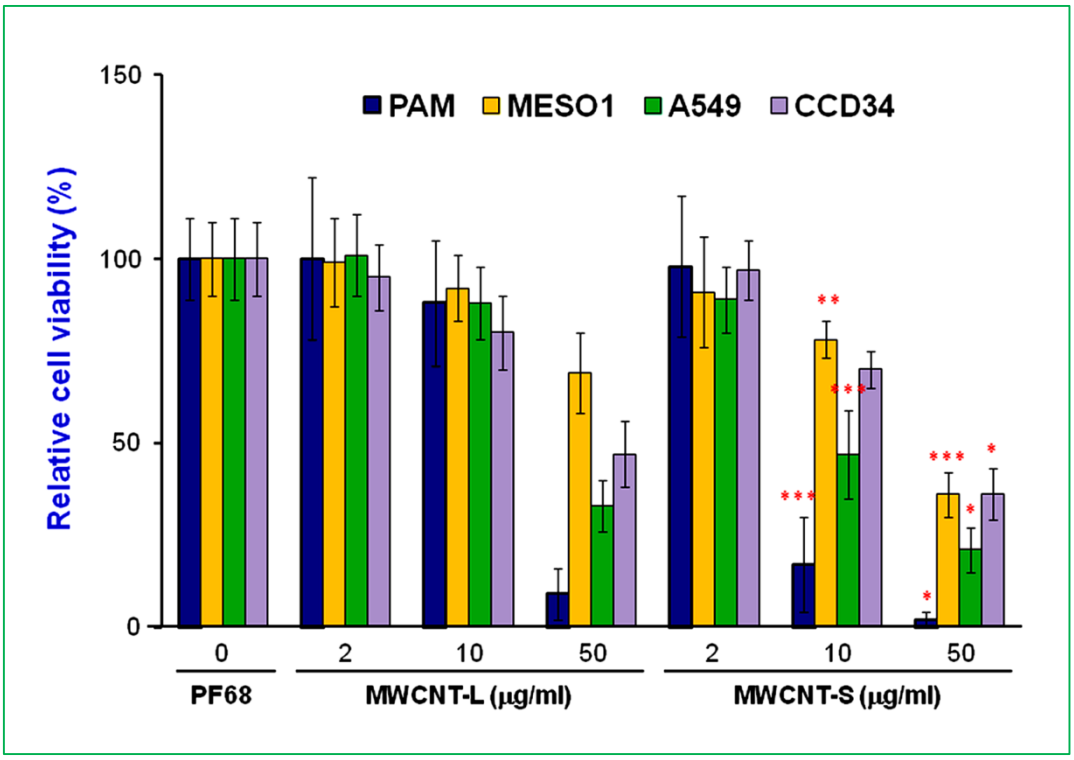

Fig S5 Cytotoxicity of MWCNT-L and MWCNT-S *in vitro*

Rat primary alveolar macrophages (PAM) were isolated as previously described [1]. MESO1, A549 and CCD34 are human cell lines of mesothelioma, lung adenocarcinoma and lung fibroblasts. MESO1, A549 and CCD34 cells were seeded into 96 well culture plates at 2×10^3 cells per well, and PAMs were seeded at 5×10^3 cells per well. After overnight incubation, MWCNT-L or MWCNT-S suspensions were added to the cells to final concentrations of 0, 2, 10 or 50 μg/ml, and the cells were incubated for an additional 72 hours. Cell viability was then determined using Cell Counting Kit-8 (Dojindo Molecular Technologies, Rockville, MD). n=6; *, ** and ***: $p<0.05$, 0.01 and 0.001, respectively, versus the same concentrations of MWCNT-L.

1. Xu, J., et al., Involvement of macrophage inflammatory protein 1alpha (MIP1alpha) in promotion of rat lung and mammary carcinogenic activity of nanoscale titanium dioxide particles administered by intra-pulmonary spraying. Carcinogenesis, 2010. **31**(5): p. 927-35.