



Article

Continuous Long-Term Exposure to Low Concentrations of MWCNTs Induces an Epithelial-Mesenchymal Transition in BEAS-2B Cells

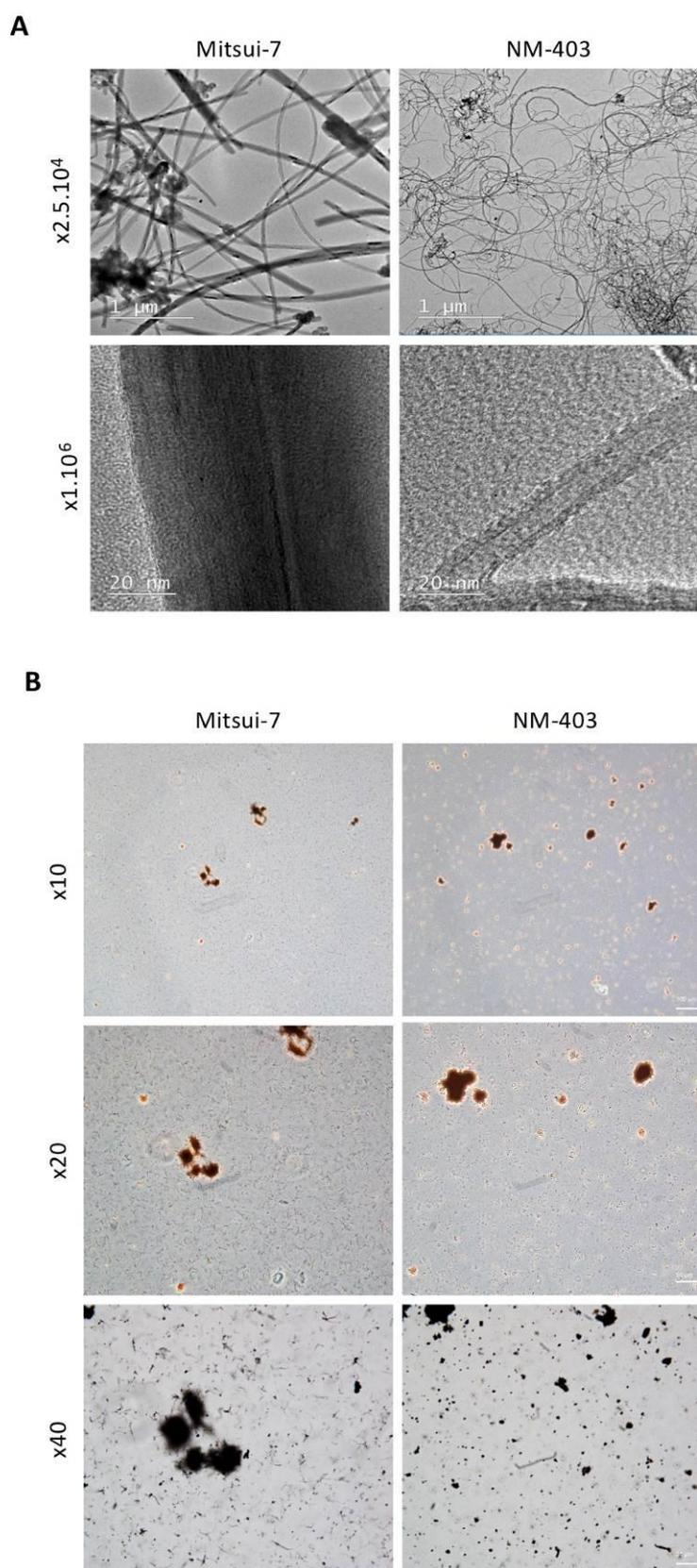
Supplementary data

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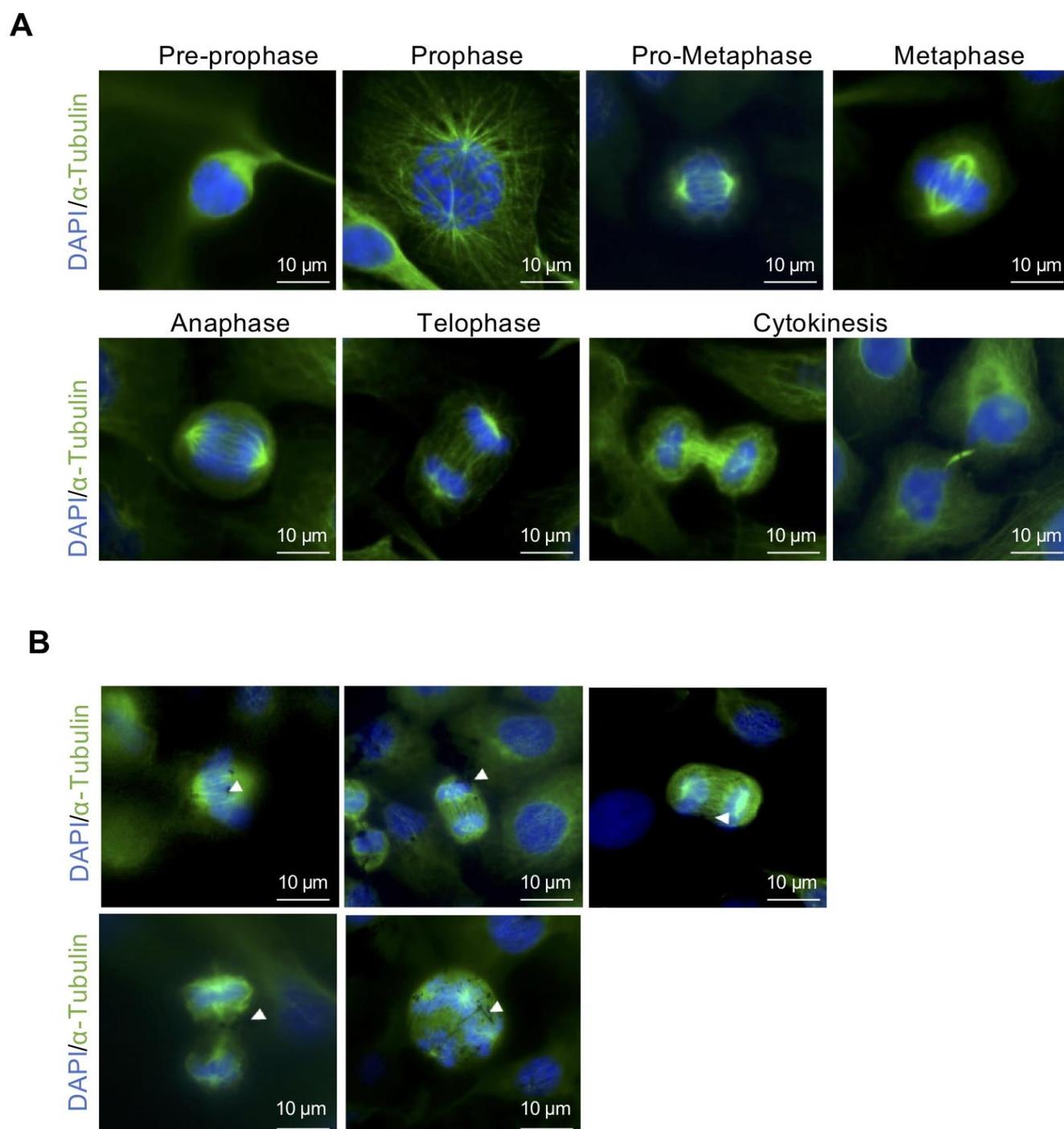
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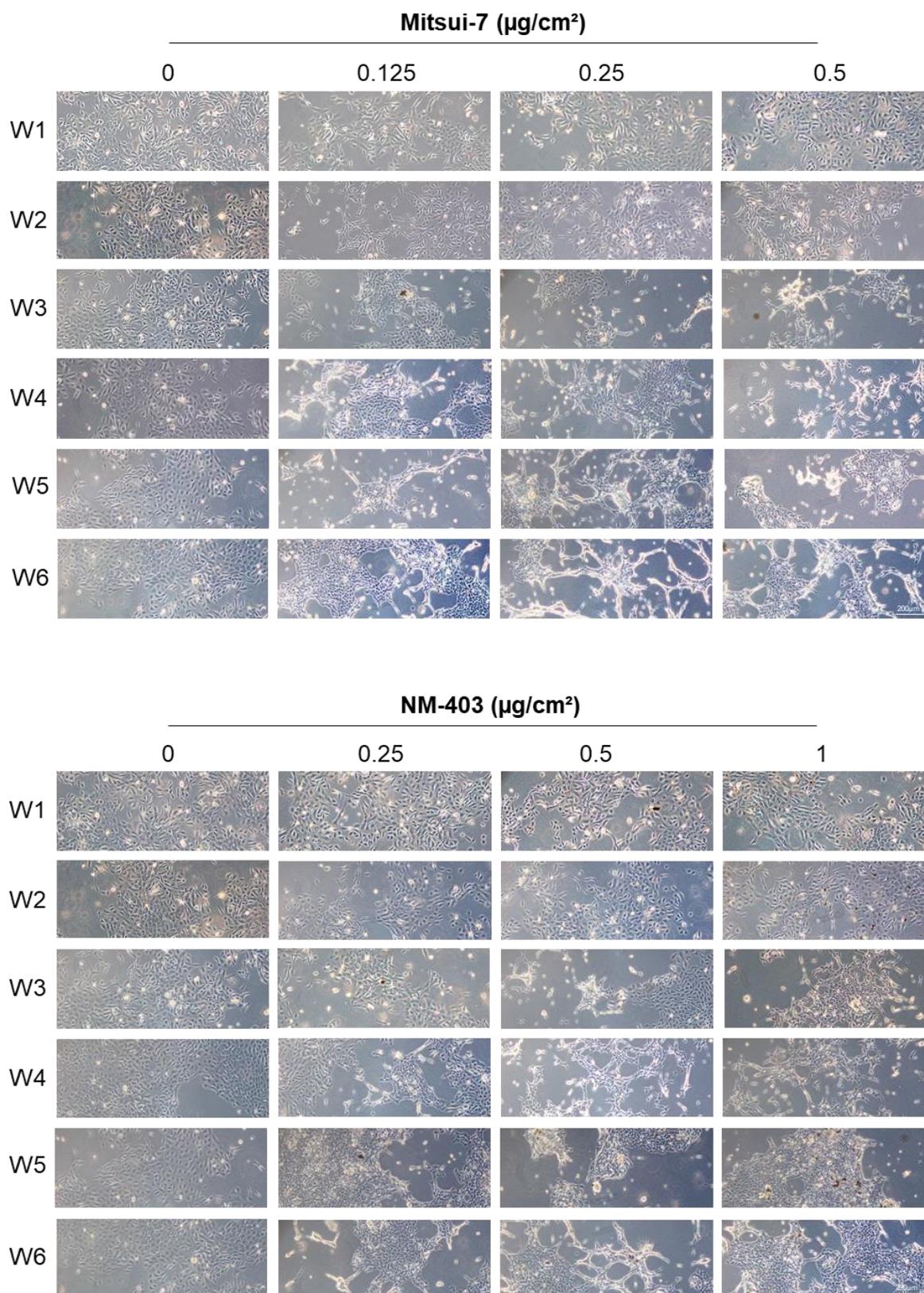
Supplementary figure 1: Images of MWCNTs and suspensions. (A) Transmission electronic microscopy images of Mitsui-7 and NM-403 powder. (B) Phase-contrast images of MWCNT suspensions in BSA 1%-LHC-9 medium after 15 min of sonication.

Supplementary table 1: Concentrations of MWCNTs for BEAS-2B treatments. The cells were cultured in a T75 flask in a final volume of 15 mL.

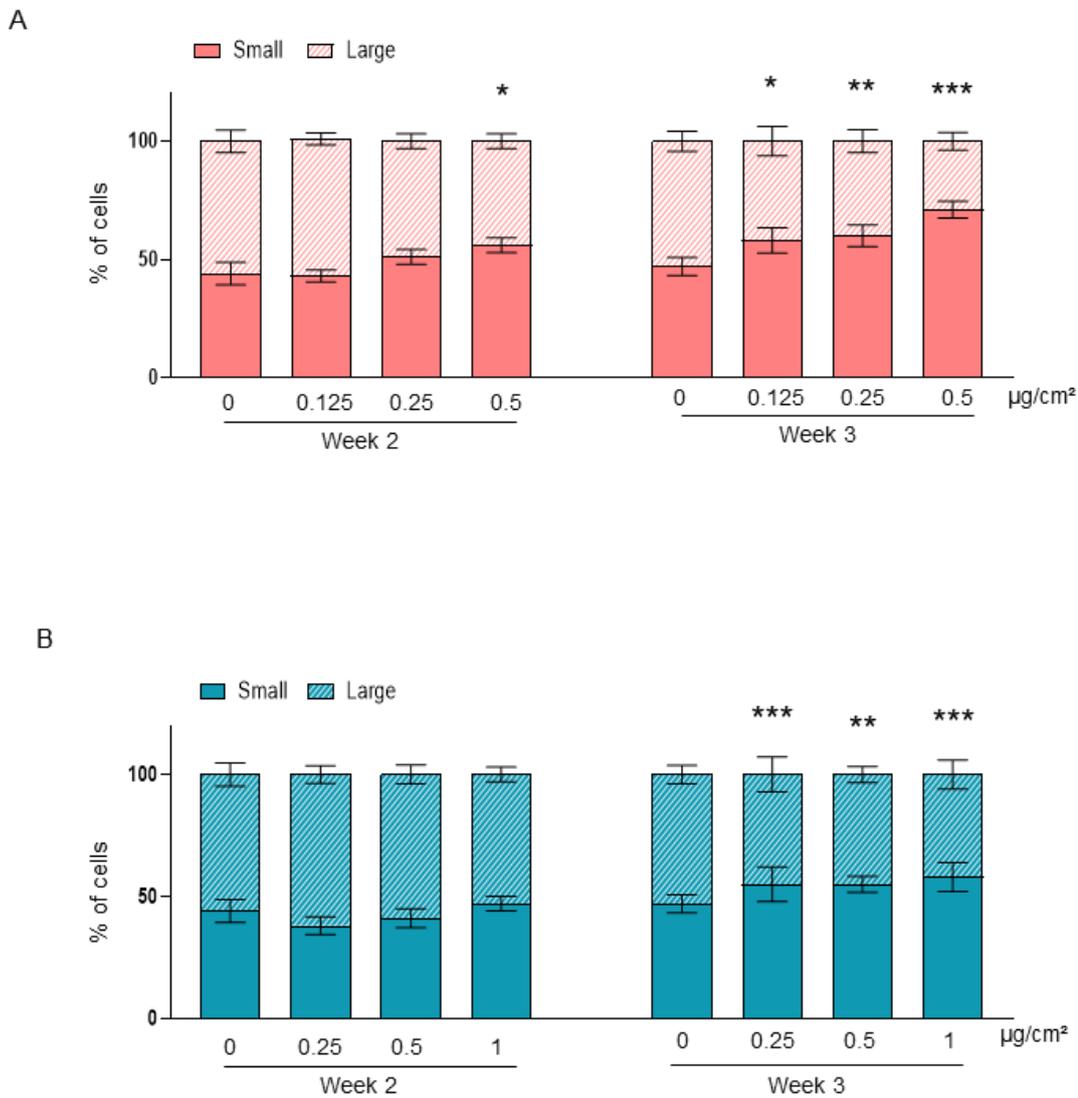
Concentration ($\mu\text{g}/\text{cm}^2$)	Concentration ($\mu\text{g}/\text{mL}$)
0.125	0.625
0.25	1.25
0.5	2.5
1	5



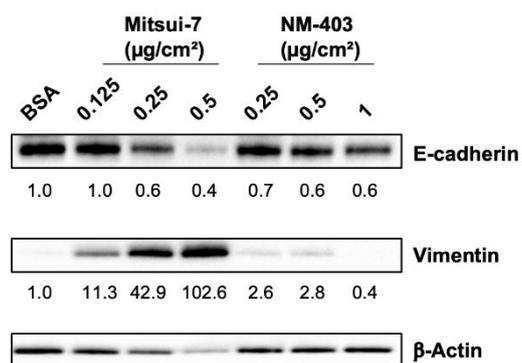
Supplementary figure 2: Normal and abnormal mitoses of BEAS-2B cells. The cells were immunolabelled with α -tubulin-FITC and nuclei stained with DAPI. (A) Images of cells in the different mitosis phases. (B) Following cell treatment by Mitsui-7 or NM-403, carbon nanotubes are visible close to the mitotic spindle and/or nucleus during cell division.



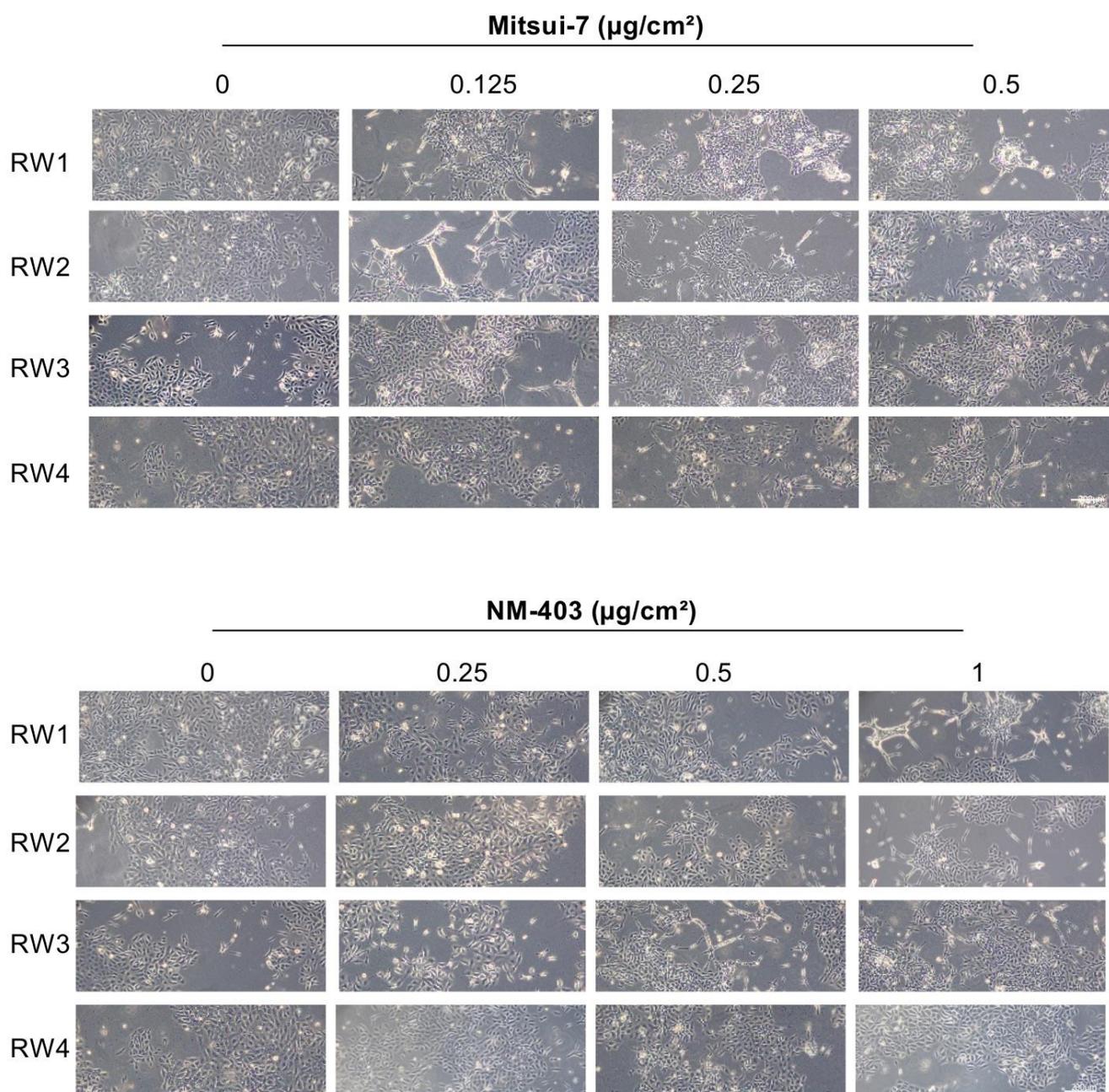
Supplementary figure 3: Mitsui-7 and NM-403 treatment induced changes in BEAS-2B cell morphology. After treatment by the indicated concentration of Mitsui-7 and NM-403, phase-contrast microscopy images were recorded weekly from the 1st (W1) to the 6th (W6) week.



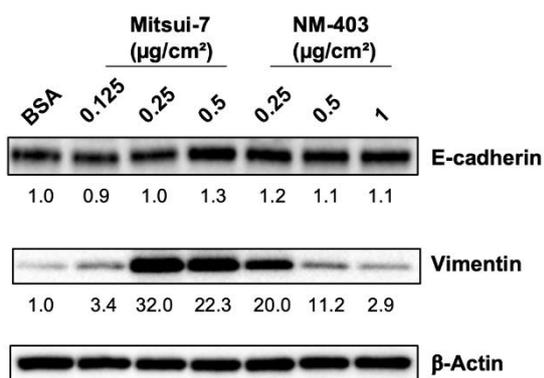
Supplementary figure 4: Cell population after 2 and 3 weeks of treatment with Mitsui-7 and NM-403. The cells were treated with vehicle, (A) Mitsui-7 (0.125, 0.25 and 0.5 µg/cm²) or (B) NM-403 (0.25, 0.5 and 1 µg/cm²) for 2 and 3 weeks and their size and granulometry were analyzed by flow cytometry. Based on SSC:FSC profiles, 2 cell populations were discriminated: with small size and low granulometry (small) or with large size and high granulometry (large). The histograms represents the mean \pm standard error of the mean (SEM) of the quantification of the two cell populations of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significantly different from the control.



Supplementary figure 5: E-cadherin and Vimentin protein level after 6 weeks of treatment with Mitsui-7 and NM-403. Total proteins were extracted from cells treated 6 weeks at the indicated concentrations of Mitsui-7 and NM-403. E-cadherin and Vimentin protein level were analyzed by Western Blot. Quantifications are presented under protein of interest (Protein of interest/Loading control) reported to the vehicle control. Blots are representative of two independent experiments.



Supplementary figure 6: Mitsui-7 and NM-403 treatment induced changes in BEAS-2B cell morphology. Phase-contrast microscopy images were recorded weekly in the recovery period, from the 1st to 4th week (RW1 to RW4).



Supplementary figure 7: E-cadherin and Vimentin protein level after 4 weeks of the recovery period. Total proteins were extracted from cells 4 weeks after the recovery period. E-cadherin and Vimentin protein level were analyzed by Western Blot. Quantifications are presented under protein of interest (Protein of interest/Loading control) reported to the vehicle control. Blots are representative of at least two independent experiments.