SUPPORTING INFORMATION

Heavy metal exposure led to rapid changes in cellular biophysical properties

Peiran Zhu^{1,2,3#}, Jamar Hawkins^{1#}, Will Hamilton Linthicum⁴, Menglin Wang^{2,5}, Ningwei Li¹, Nanjia Zhou^{2,3}, Qi Wen⁴, Alicia Timme-Laragy⁶, Xiaofei Song^{7*}, Yubing Sun^{1,8*}

¹Department of Mechanical and Industrial Engineering, University of Massachusetts, Amherst, Massachusetts 01003, USA.

²Key Laboratory of 3D Micro/Nano Fabrication and Characterization of Zhejiang Province, School of Engineering, Westlake University, 18 Shilongshan Road, Hangzhou 310024, Zhejiang Province, China.

³Institute of Advanced Technology, Westlake Institute for Advanced Study, 18 Shilongshan Road, Hangzhou 310024, Zhejiang Province, China.

⁴Department of Physics, Worcester Polytechnic Institute, Worcester, Massachusetts 01609, USA.

⁵Department of Chemistry and Chemical Engineering, Anhui University, Hefei 230601, Anhui Province, China.

⁶Department of Environmental Health Sciences, University of Massachusetts, Amherst, Massachusetts 01003, USA.

⁷School of Environment and Energy, South China University of Technology, Guangzhou 510006, China.

⁸Department of Chemical Engineering, University of Massachusetts, Amherst, Massachusetts 01003, USA.

[#]These authors contribute equally to this work.

*Correspondence should be addressed to X. Song (<u>songxf@scut.edu.cn</u>) and Y. Sun (<u>ybsun@umass.edu</u>)

Supporting Information:

Supporting Figures and Captions



Figure S1. Schematic diagram showing the fabrication of PDMS micropost arrays (PMAs) and cell culture on PMAs.



Figure S2. The LDH assay for characterizing MDCK cells viability. Bar plot presenting the cell viability after lead treatment tested by LDH assay. MDCK cells were exposed to Lead Nitrate with 0, 0.01, 0.1, 1 mM for 24 hours. Data represents mean and standard deviation from at least two independent experiments. ***, P < 0.001, n.s., P > 0.05.



Figure S3. Lead-induced changes in both cellular and nuclear shape. Representative fluorescence images showing the MDCK cells exposed to the increasing concentration of lead. MDCK cells were exposed to Lead Nitrate with 0, 0.01, 0.1, 1 mM for 24 hours. Cells were fixed and stained for F-actin (Phalloidin, pink), Nuclei (DAPI, blue). Scale bar, 25 µm.



Figure S4. Lead-induced monolayer permeability changes. Bar plots depicting normalized TEER value of MDCK monolayers treated with indicated concentration of lead. Data is normalized to the initial TEER value of the respective transwell. MDCK cells were exposed to Lead Nitrate with 0, 0.01, 0.1, 1 mM for 0, 2, 4, and 24 hours. Data represents mean and standard deviation from at least three independent experiments. *, P < 0.05, **, P < 0.01, n.s. n > 9 monolayers in each condition.



Figure S5. The Live/Dead assay and MTT assay for characterizing cadmium cytotoxicity. (a) Bar plot presenting the cell viability after cadmium treatment tested by MTT assay. MDCK cells were exposed to Cadmium Nitrate with 0, 0.005, 0.05, 0.5 mM for 12, and 24 hours. Data represents mean and standard deviation from at least two independent experiments. ***, P < 0.001, n.s., P > 0.05. (b) Plots showing the cell number as a function of time. Gray lines show the results from each independent measurement.



Figure S6. LDH assay for characterizing REF cells viability after lead exposure. Bar plots showing the cell viability tested by LDH assay after 2 (a), 4 (b), and 24hrs (c). REF cells were exposed to Lead Nitrate with 0, 0.01, 0.1, 1 mM for 2, 4, and 24 hours. Data represents mean \pm standard deviation from two independent experiments. ***, P < 0.001, n.s., P > 0.05.



Figure S7. REF cell migration speed and distance declined rapidly after lead exposure. (a) Bar plot showing the total cell migration distance from 0 to 8 hours. n = 25 cells. (b) Bar plot depicting the average migration speed over 8 hours (c) Bar plot presenting the cell migration average speed per 2 hrs from 0 to 8 hrs. REF cells were exposed to Lead Nitrate with 0, 0.01, 0.1, 1 mM for 2, 4, 6, and 8 hours. The migration velocity was analyzed at 2, 4, 6, and 8 hours, respectively. Data represents mean \pm standard deviation from at least three independent experiments. *, P < 0.05, **, P < 0.01, ***, P < 0.001, n.s., P > 0.05.



Figure S8. Lead-induced reduction of cell traction forces of REF cells. Bar plots comparing the TTFs (a) and TFAs (b) after 24hrs. REF cells were exposed to Lead Nitrate with 0, 0.01, 0.1, 1 mM for 24 hours. Data represents mean and standard deviation from at least three independent experiments. *, P < 0.05, **, P < 0.01, ***, P < 0.001, n.s., P > 0.05. n > 30 cells in each condition.