Supplementary information for

Scanning ion conductance microscopy revealed cisplatin-induced morphological changes related to apoptosis in single adenocarcinoma cells

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S1 Characterization of nanopipette size

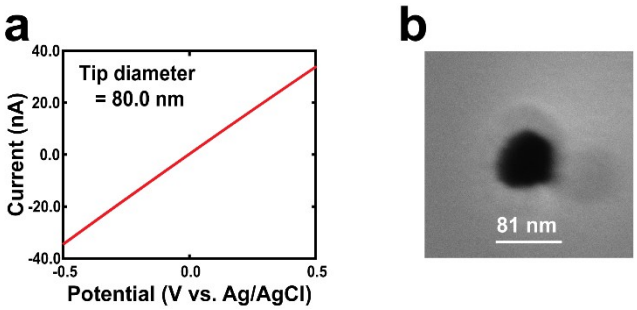

Fig. S1. Nanopipettes were characterized using cyclic voltammetry (CV) and scanning electron microscopy (SEM). (a) A cyclic voltammogram showing the use of ion current flow for measuring the tip diameter of a nanopipette, which was determined to be 80 nm. (b) The SEM image of the nanopipette tip shows a diameter of 81 nm (i.e. in close agreement to the CV result).

S2 Topographical maps of PBS, cisplatin, and untreated single A549 cells after 24 h

Fig. S2a-b serves as a continuation of Fig. 2, while Fig. S2c-d are evidence for the PBS solution having no effect on the cell topography, which confirms the effect of cisplatin on the morphology of cells.

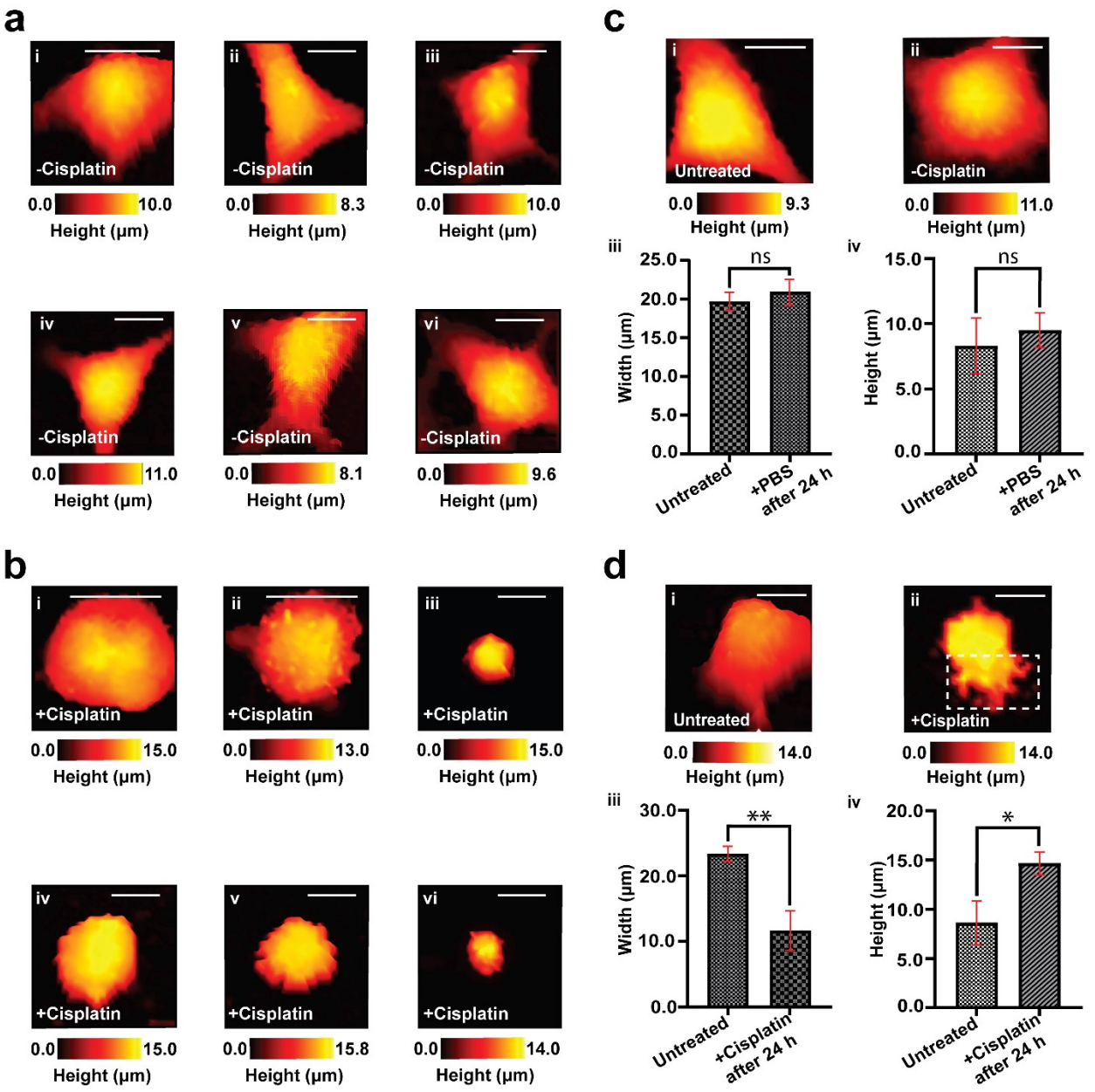

Fig. S2. Topographical maps of A549 cells. (a) Cells treated with PBS after 24 h. (b) Cells treated with cisplatin after 24 h. (c) SICM topography maps of cells i) untreaed, and ii) 24 h after being treated with the vehicle, phosphate buffered saline (PBS) solution. SICM images were analyzed, and a t-test was used for the comparison of iii) width and iv) height of A549 cells, untreated and treated with PBS after 24 h. (d) SICM images of A549 cells i) untreated and ii) 24 h after treatment with cisplatin in PBS solution, and the broken box shows membrane blebbing. T-test showing the comparison of iii) width and iv) height of untreated A549 cells and 24 h after treatment with cisplatin. (n = 3, *P < 0.05, and **P < 0.01). Scale bar: 10 μm.

S3 Topographical map of untreated A549 cell before and after 24 h

As an additional control experiment, we have scanned the topography of the same single A549 cell untreated with neither the drug or vehicle, before and after 24 h. The height does not change as well as the width. Fig. S4 shows the SICM topography of the same single A549 cell before and after 24 h.

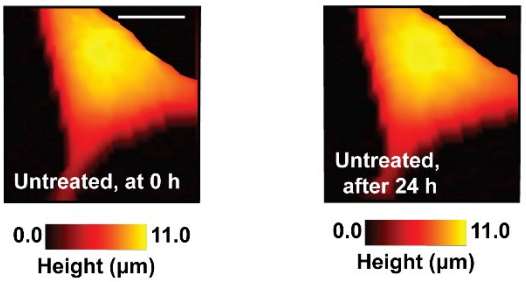

Fig. S3. The topography of the same single A549 cell before and after 24 h treatment. Scale bar: 10 μm.

S4 Topographical maps of the same single A549 cells after 36, 48, 60, and 72 h treatment with cisplatin, corresponding to the continuation of the 72 h studies

The height of A549 cells remained increased after 36 h treatment with cisplatin, but after 48 h treatment the height was observed to decrease. The membrane undergoes extensive fragmentation after 36 to 72 h treatment with cisplatin. This fragmentation is associated with loss of the major part of the cell body as well as the decrease in the height of the cell membrane. The blebbing of the plasma membrane during apoptosis and the formation of apoptotic bodies are regarded as the morphological characteristics of apoptosis, and cell membrane is modified at the terminal stage of apoptosis.[1]

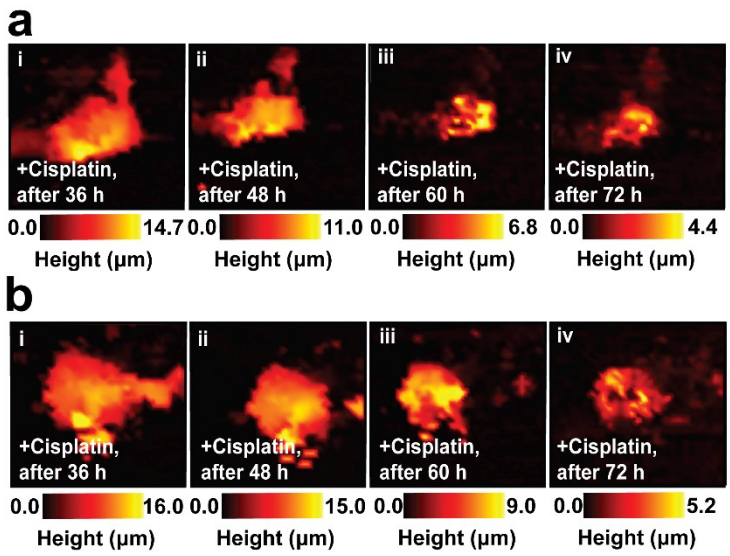

Fig. S4. The continuation of the 3 days longitudinal studies of the same three A549 cells for the studies of the dynamic effect of cisplatin. All images are 30 × 30 µm (in the x and y axes).

S5 Treatment of A549 cells with higher concentration of cisplatin

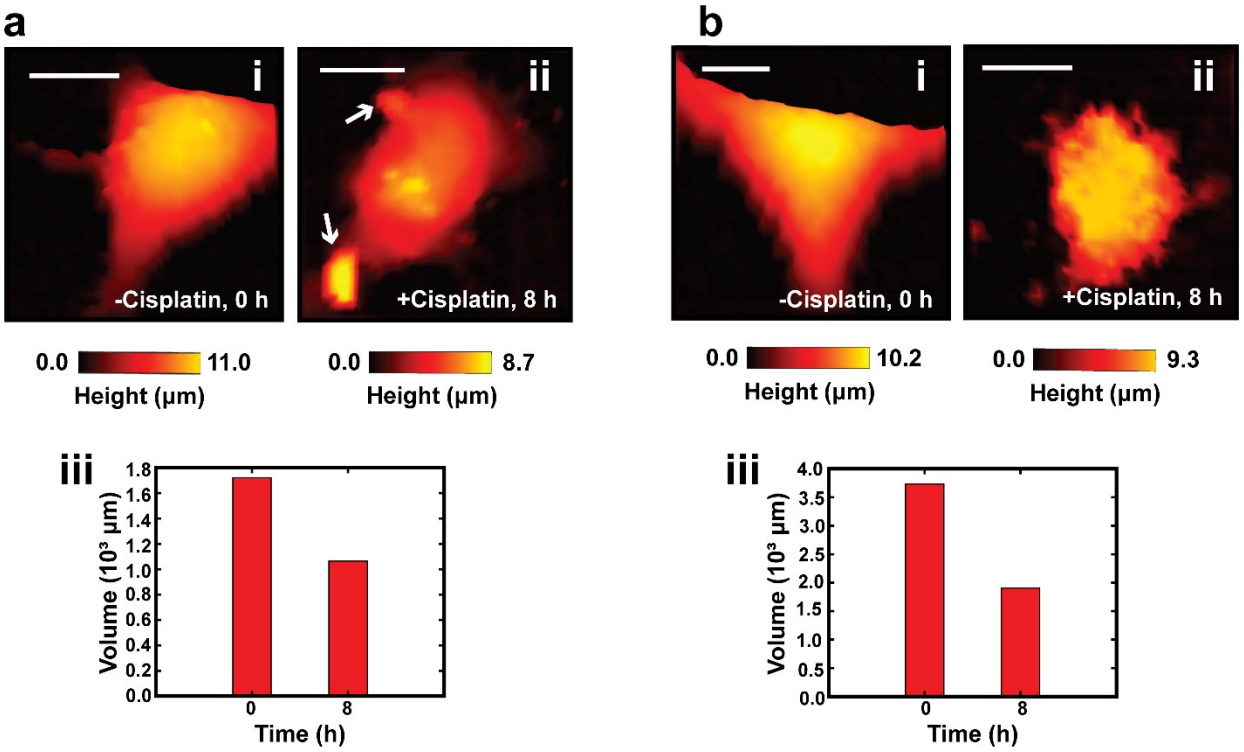

Fig. S5. Apoptosis of A549 cells induced with 100 μM cisplatin. (a-b) A549 cell morphology and volume before and during apoptosis.

S6 Membrane bulges observed from mapping of the small membrane region of A549 cell

During apoptosis, the cytoskeleton of the cell breaks to form outward bulges on the membrane, in a process known as blebbing.[2] A549 cells treated with cisplatin have displayed the presence of circular membrane bulges, an increased height, and irregularities around the membrane, compared to cells that were not treated with cisplatin.

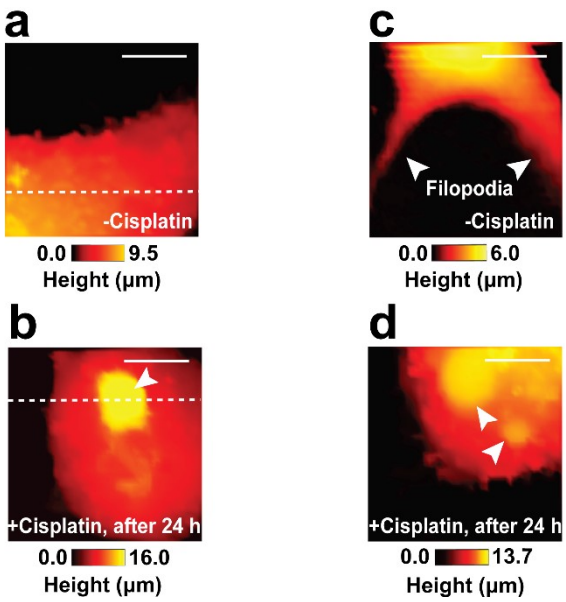

Fig. S6. High resolution SICM topography of single A549 cells. (a) The edge of -cisplatin A549 cell. (b) The edge of +cisplatin A549 cell. (c) -Cisplatin A549 cell showing its filopodia. (d) The edge of the +cisplatin A549 cell. The membrane of cisplatin treated cells displayed an increase in height as well as presence of circular bulges as indicated by the white arrow. Scale bar 5 µm.

S7 Mapping of Petri dish surface used for cell attachment

We have also mapped the topography of a Petri dish surface to determine its roughness to further confirm the surface of the Petri dish is at zero when estimating the volume. Fig. S7 shows the height of the Petri dish surface scanned.

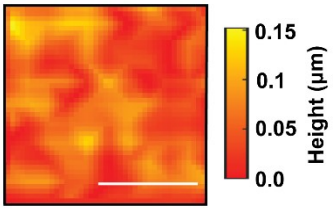

Fig. S7. A topographical map of a Petri dish surface shows roughness, when viewed at the scale of ~100 nm. These features are a combination of actual surface roughness, and the noise associated with imaging. At the fast approach rates used in this study (to enable fast hopping mode imaging), this roughness is expected, and is significantly smoother than the surface of the cell membrane observed in the cell images. Scale bar: 10 µm.

References

1. Zhang, Y., Chen, X., Gueydan, C. & Han, J. Plasma membrane changes during programmed cell deaths. *Cell Res.* **28**, 9–21 (2018).

2. Tinevez, J. Y. *et al.* Role of cortical tension in bleb growth. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 18581–18586 (2009).