High throughput profiling drug response and apoptosis of single polar cells

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Supporting Information

Supplementary materials and methods

Figure S13. The SEM photographs of single trapped cells on microwell arrays after treated with drugs.

Figure S14. The fluorescent images of single trapped cells on microwell arrays after treated with DOX drug from 10 μM to 30 μM for 12h.

Figure S15. The fluorescent images of single trapped cells on microwell arrays after treated with PAC drug from 10 μM to 30 μM for 12h.

Figure S16. The drug response of non-polar cells with different spreading areas.

Table S2. The Gini index of cell heterogeneity.

Materials and Chemicals

The photomask for photolithography was obtained from Suzhou Research Materials Microtech Co., Ltd (China). Polydimethylsiloxane (PDMS) precursor -184 for soft-lithography was gained from DOW chemistry. Solvents such as chloroform and alcohol were supplied by Sinopharm Chemical Reagent Co., Ltd (China). Polystyrene (PS, average Mw 250,000 g/mol) was purchased from Acros (China). Hela and MCF-7 cells used here were originated from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. Cell culture medium including DMEM, fetal bovine serum (FBS) were provided by Gibco (USA). Drugs (doxorubicin hydrochloride and paclitaxel) and Caspase Activity Assay Kit and other materials for cell maintaining and staining were obtained from Beyotime Biotechnology (China).

Characterizations

Surficial and cross-sectional morphologies of the microwells on substrates were observed by a scanning electron microscope FlexSEM 1000 (Hitachi, Japan). Water contact angles of the patterned substrates were recorded using an OCA 10 (Dataphysics, Germany). The fluorescent images of the cells were obtained using inverted fluorescence microscopy (DMi 8; Leica, Germany) and laser confocal fluorescent microscopy (Zeiss LSM880; Germany). The concentrations of caspase 1 and caspase 3 were recorded by a Multiskan FC microplate reader (Thermo-Fisher, USA) under the light absorption mode.

Photolithography

The photolithography was performed according to a standard process of CRC8300. The commercial photoresist system is famous for the high contrast (4μm), environmentally-friendly, high adhesion to substrate, and strong mechanical stability. In detail, the positive polyimide photoresist with diazonaphthoquinone (PI and DNQ) was spin-coated onto silicon wafer with a thickness of 20 μm. The photoresist-coated wafer was then prebaked to drive off excess photoresist solvent at 90 °C for 60 s on a hotplate. After prebaking, photomask was placed over positive photoresist on silicon wafer and submitted to lithography machine (URE-2000/35). After exposure to UV light for 10s at a power of 200 mJ/cm^2, the positive photoresist was then developed using tetramethylammonium hydroxide developing solution (2.38 %wt, TMAH). Following by hard-backing at 150°C, the microwell arrays were prepared. To fix the morphologies, the substrates were submitted to thermal treatment at 450°C. The final thickness of profile was about 15 μm.

The resultant PI topologies were then used as the template of soft lithography. As presented in Figure S1, pre-centrifuged PDMS precursor was casted onto the patterned silicon surface and removed gas under vacuum. PDMS was cured in an oven at 80 ℃ for 12h and obtained patterned PDMS template. For easier demold, the surface of the PDMS template was fluorinated using Trichloro (1H,1H,2H,2H-heptadecafluorodecyl) silane. Polystyrene chloroform solution (0.1 g/L) was then cast on the PDMS template. After solvent evaporation, the pattern was transformed to PS substrates, and served as platform for cell culture and analysis.

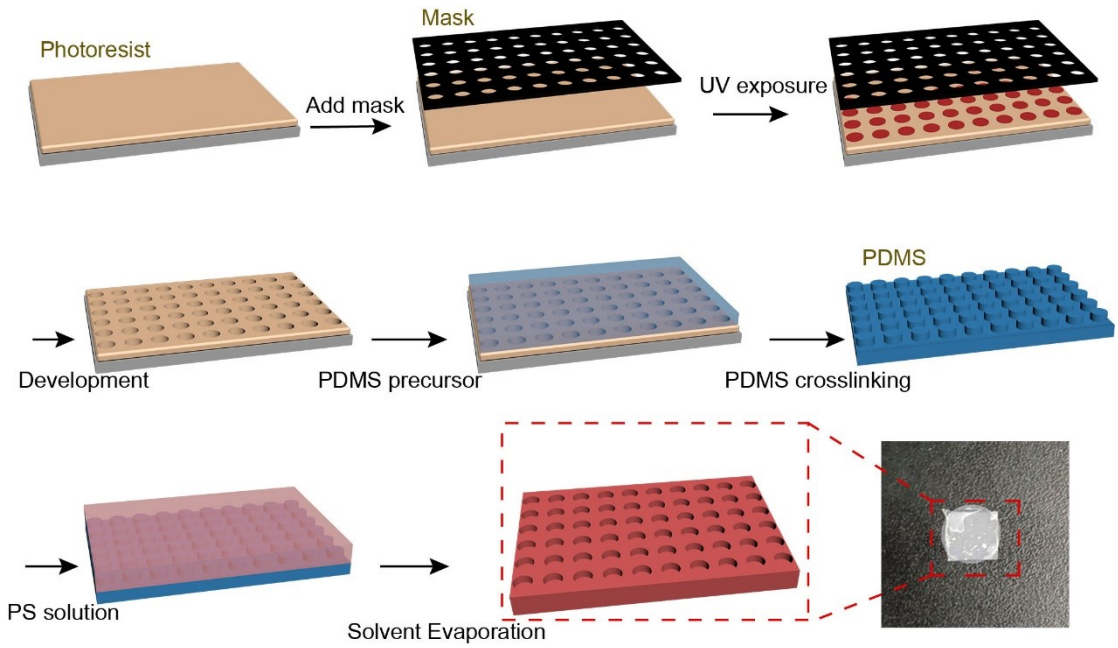

Figure S1. Schematic figure of microwell fabrication, including photolithography and soft lithography. Inserted figure resultant PS microwell film.

Table S1. The density of microwells in each array(well/cm^2).

C1.0	E1.5	E2.0	T1.0
1400	868	710	727

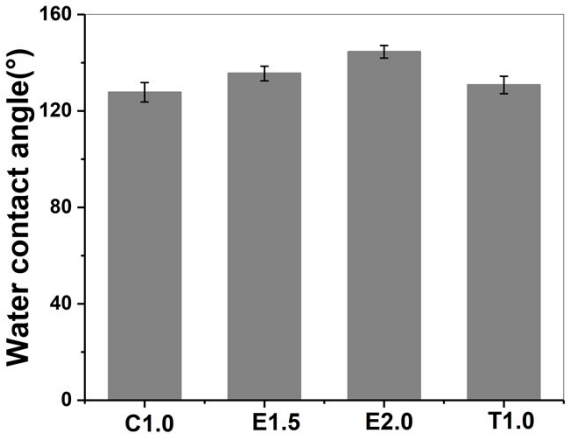

Figure S2. Water contact angles of the microwell arrays.

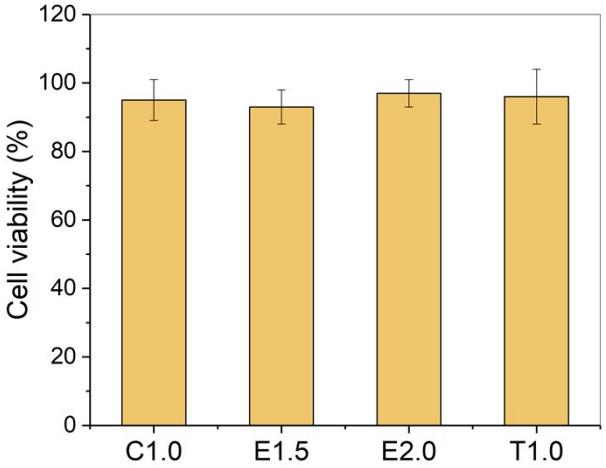

Figure S3. The cell viability after cell trapped into microwells for 48h.

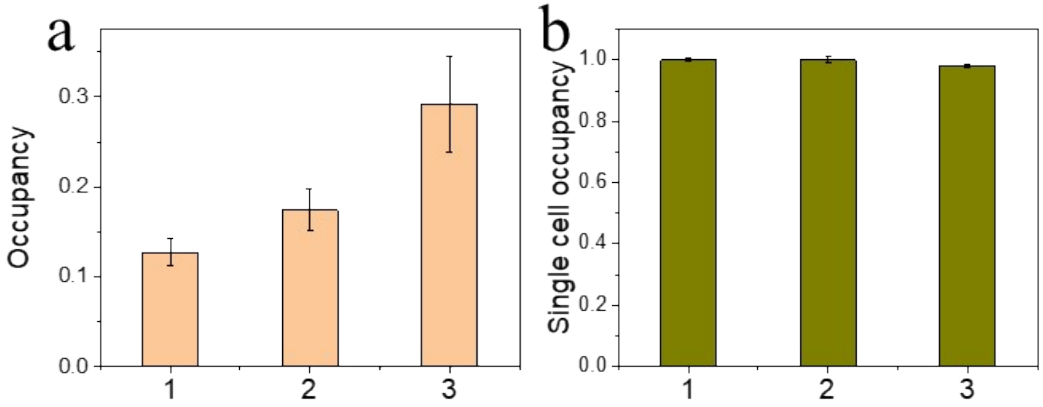

Figure S4. Cell occupancy(a) and single cell occupancy(b) after seeding for three times.

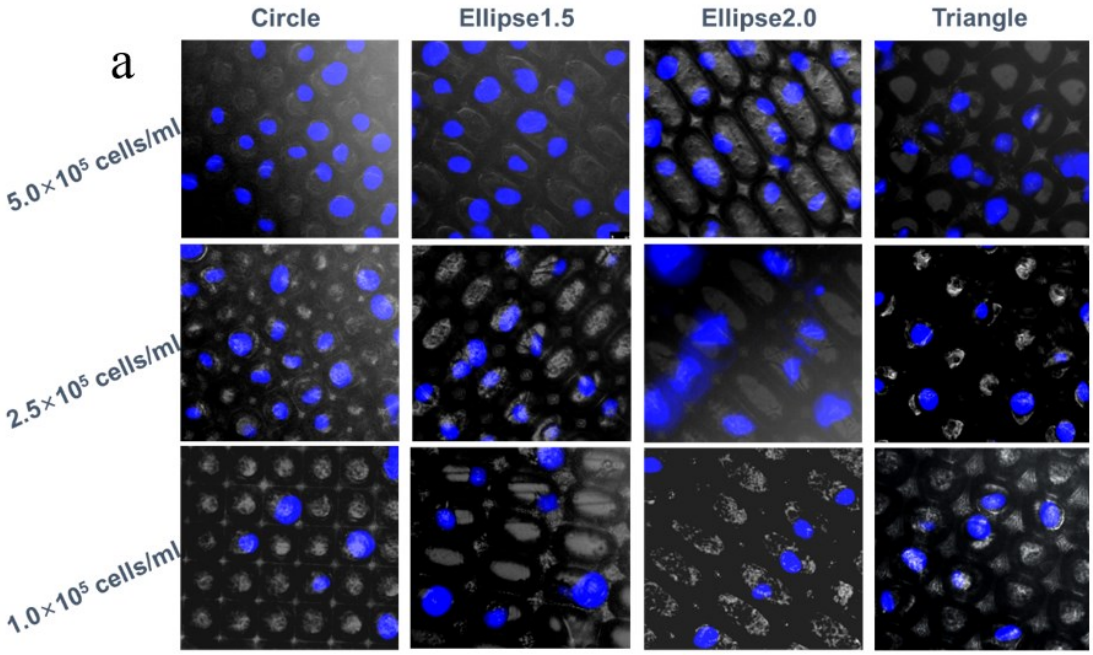

Figure S5. (a)The fluorescent images of cell trapping efficiency using series of concentrations (1×10^5, 2.5×10^5 and 5×10^5).

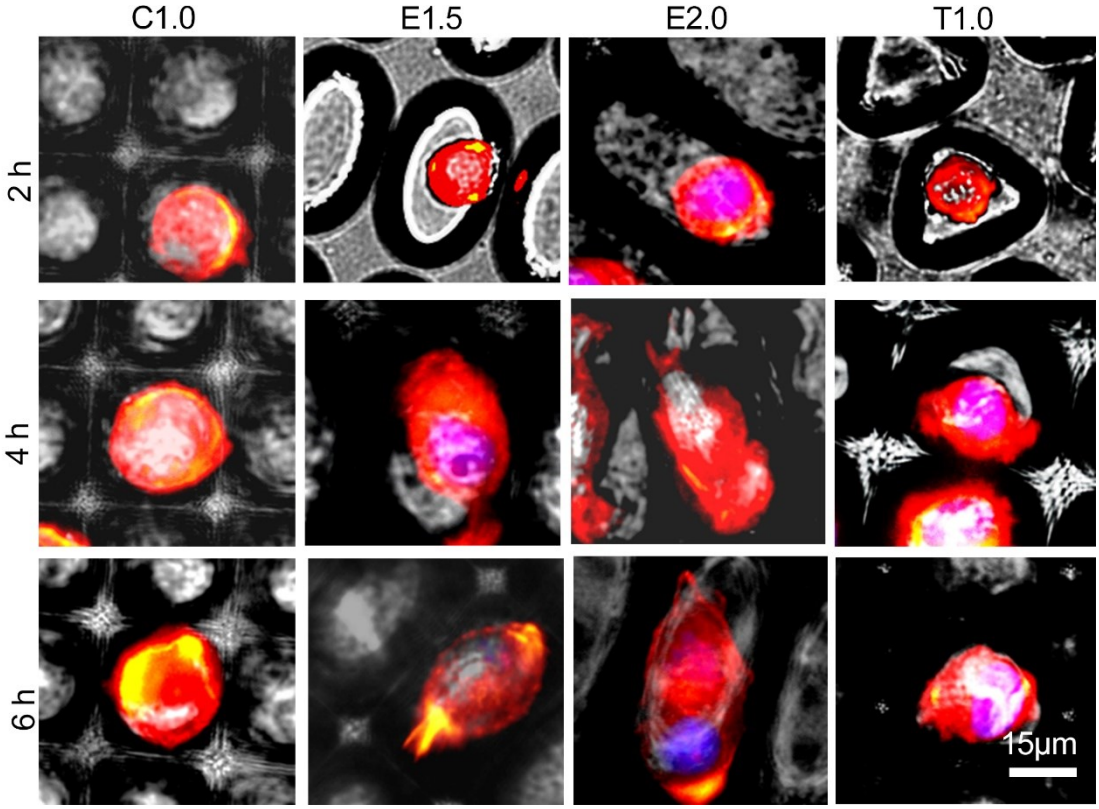

Figure S6. The photographs of single trapped cells on microwell arrays at 2h, 4h and 6h.

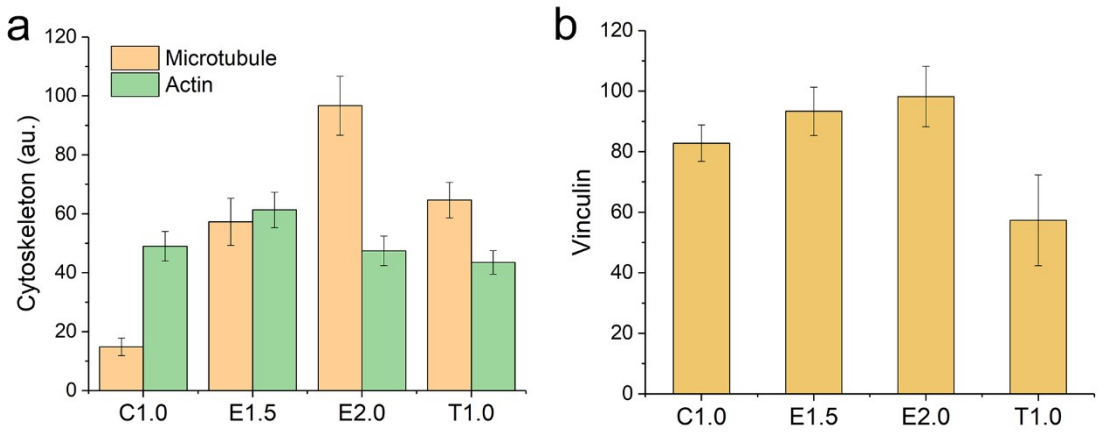

Figure S7. The qualifications of cytoskeleton and vinculin expression after cell trapped in microwell for 8h.

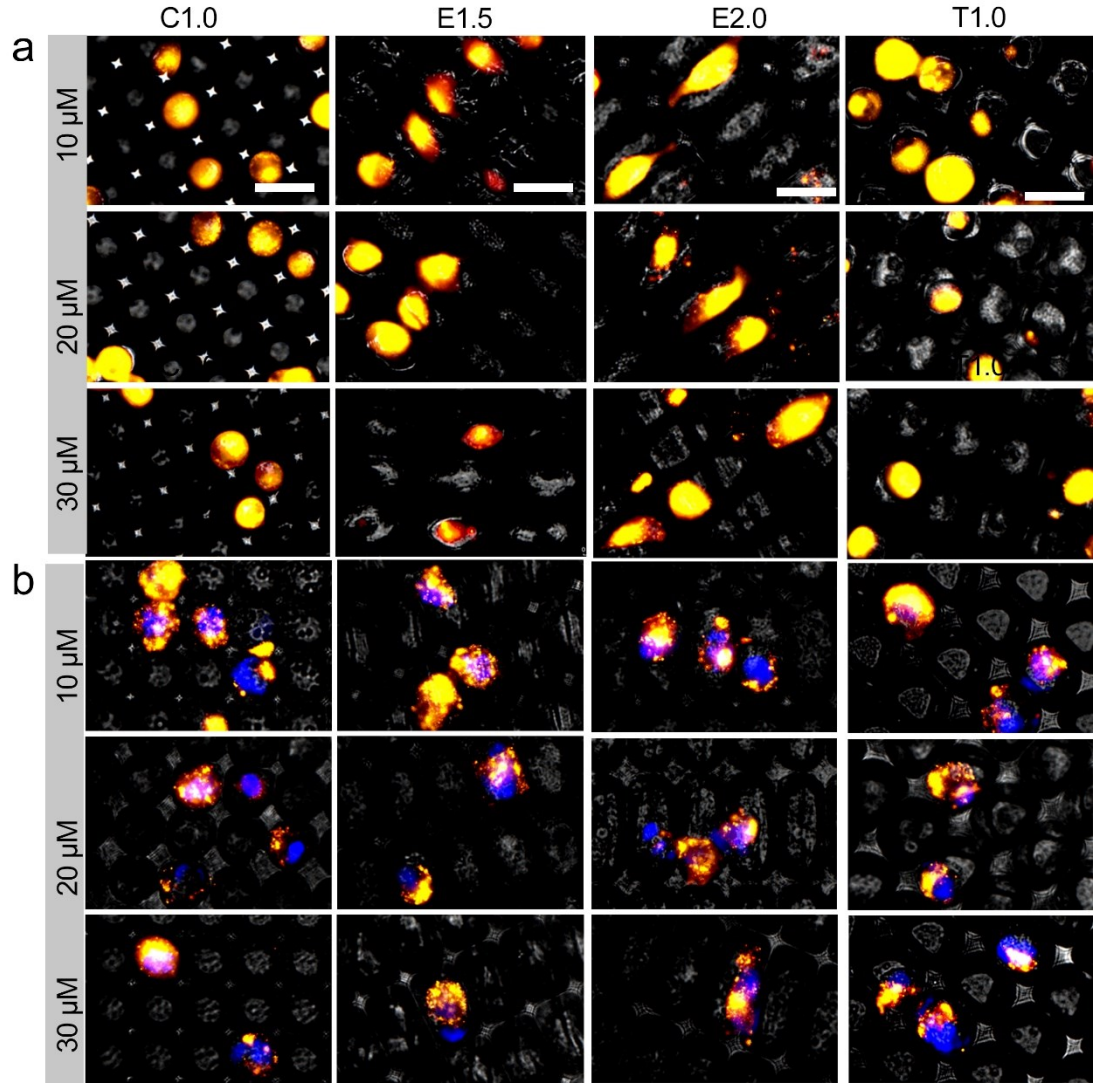

Figure S8. The photographs of single trapped cells on microwell arrays after treated with DOX drug for 4h (a) and 24h (b). The scale bar=30 μm.

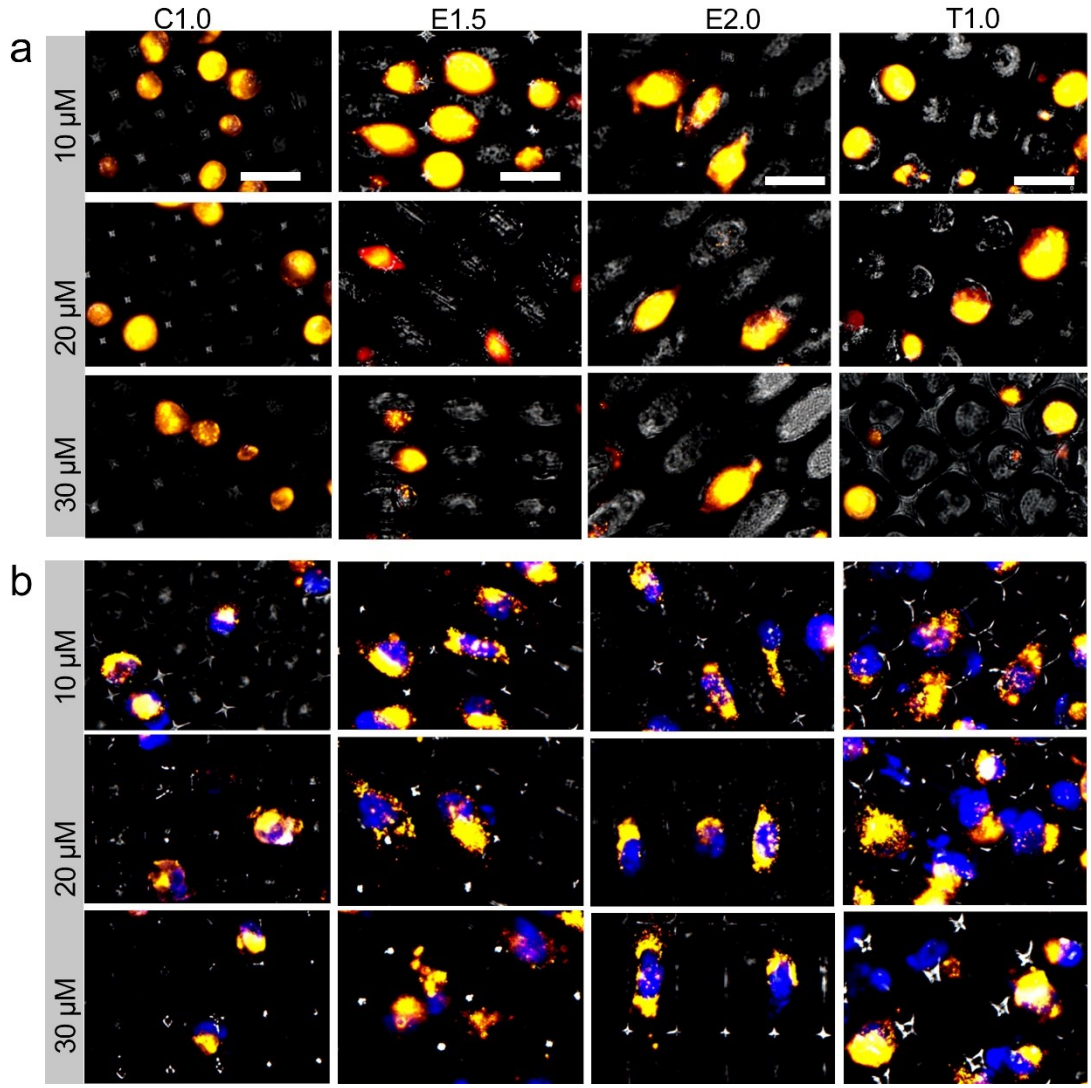

Figure S9. The photographs of single trapped cells on microwell arrays after treated with PAC drug for 4h (a) and 24h (b). The scale bar=30 μm.

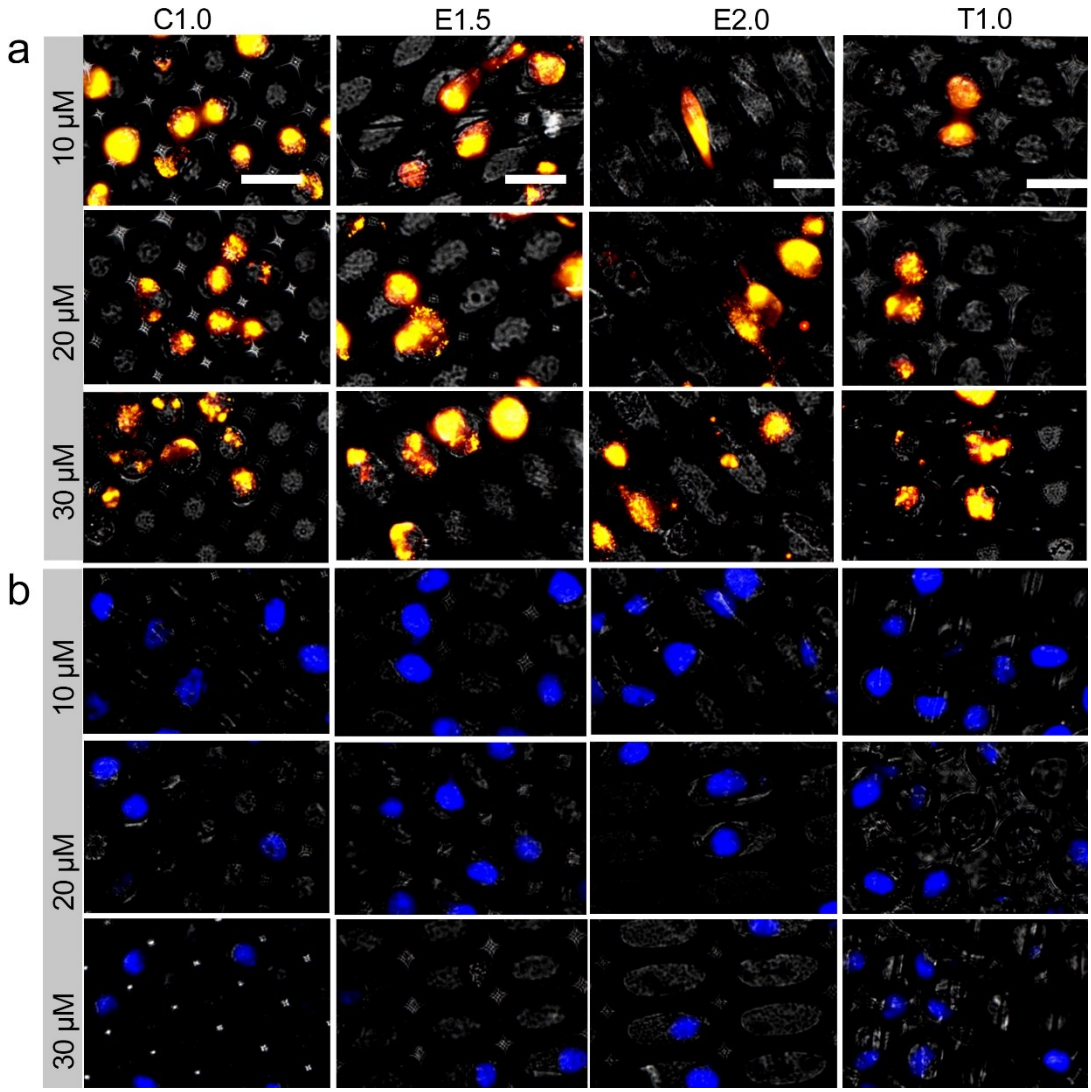

Figure S10. The photographs of single trapped cells on microwell arrays after treated with DOX drug for 12h. (a) is the cell membrane stained by Dil-AM. (b) is nuclei stained by DAPI. Scale bar=30 μm.

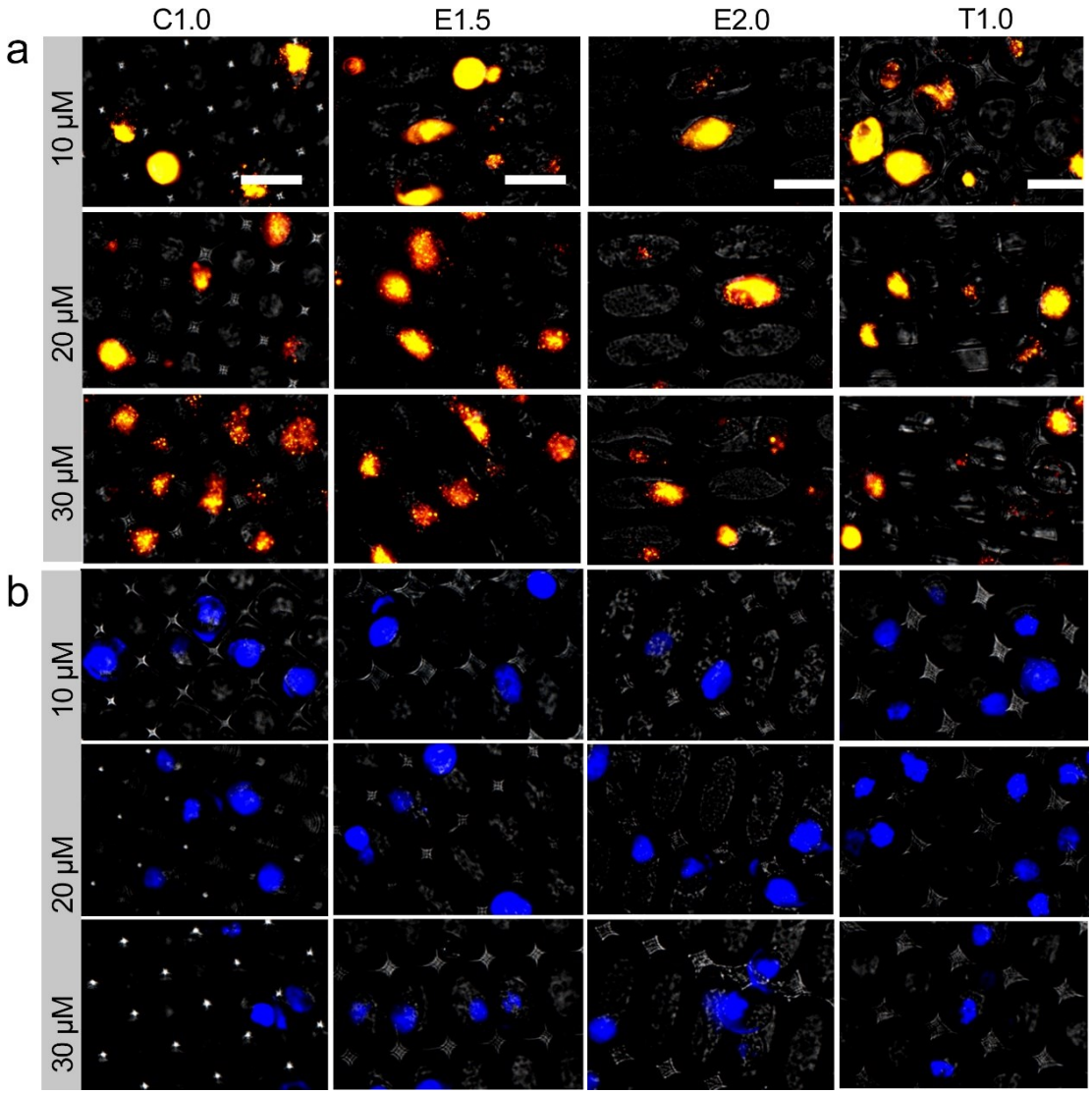

Figure S11. The photographs of single trapped cells on microwell arrays after treated with PAC drug for 12h. (a) is the cell membrane stained by Dil-AM. (b) is nuclei stained by DAPI. Scale bar=30 μm.

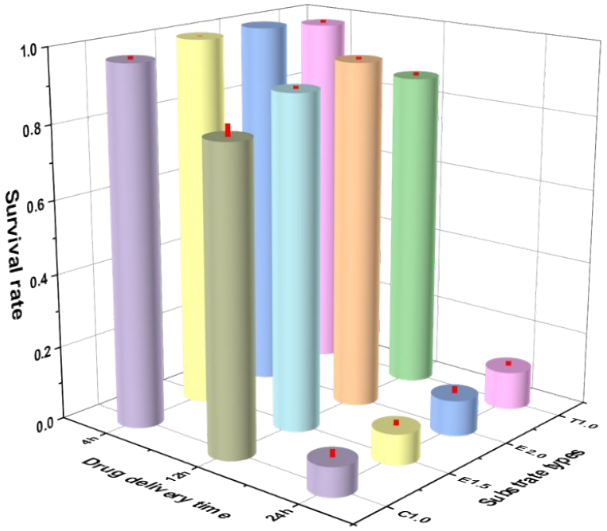

Figure S12. Cell viability in different incubation times (4h, 12h, 24h) with DOX (20μM) treatment.

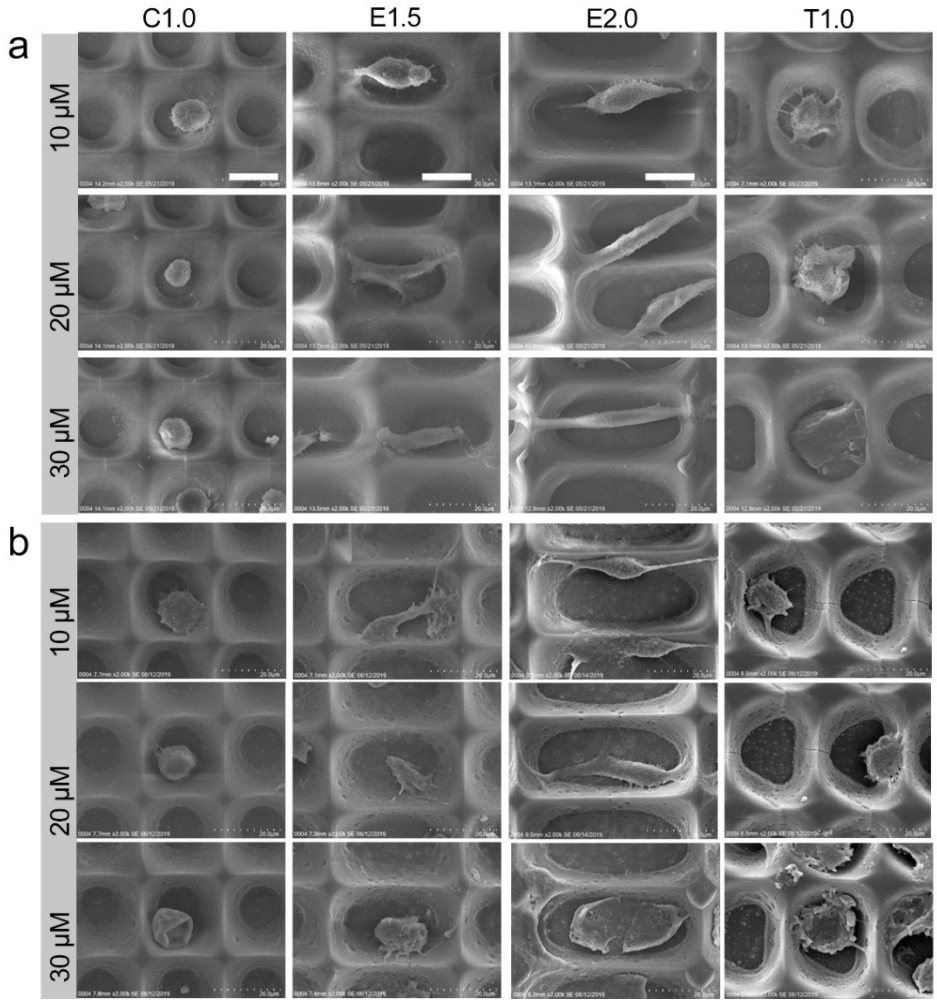

Figure S13. The SEM photographs of single trapped cells on microwell arrays after treated with DOX drug (a) and PAC drug (b) for 12h. Scale bar =10 μm.

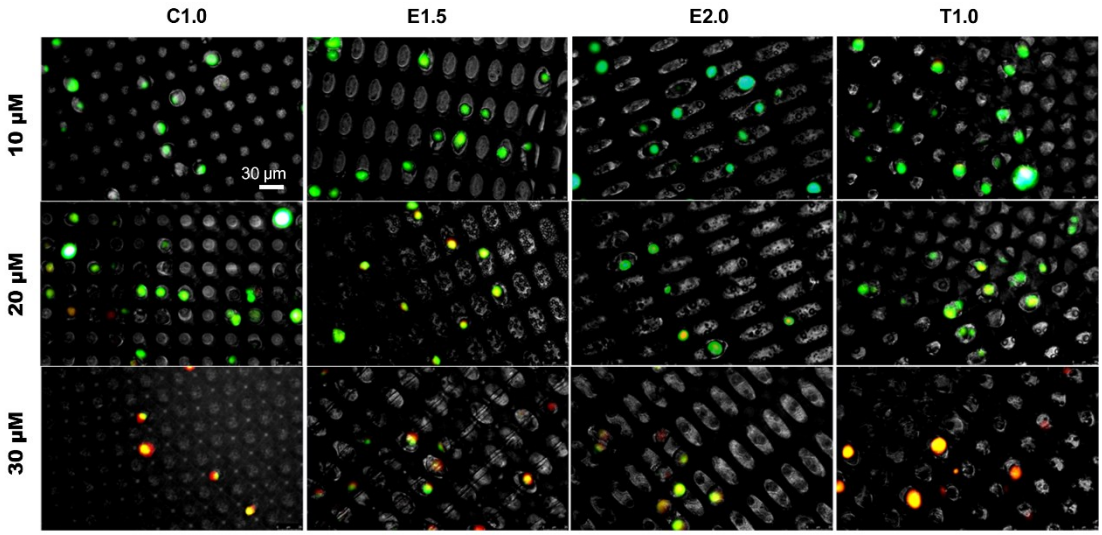

Figure S14. The fluorescent images of single trapped cells on microwell arrays after treated with DOX drug from 10 μM to 30 μM for 12h.

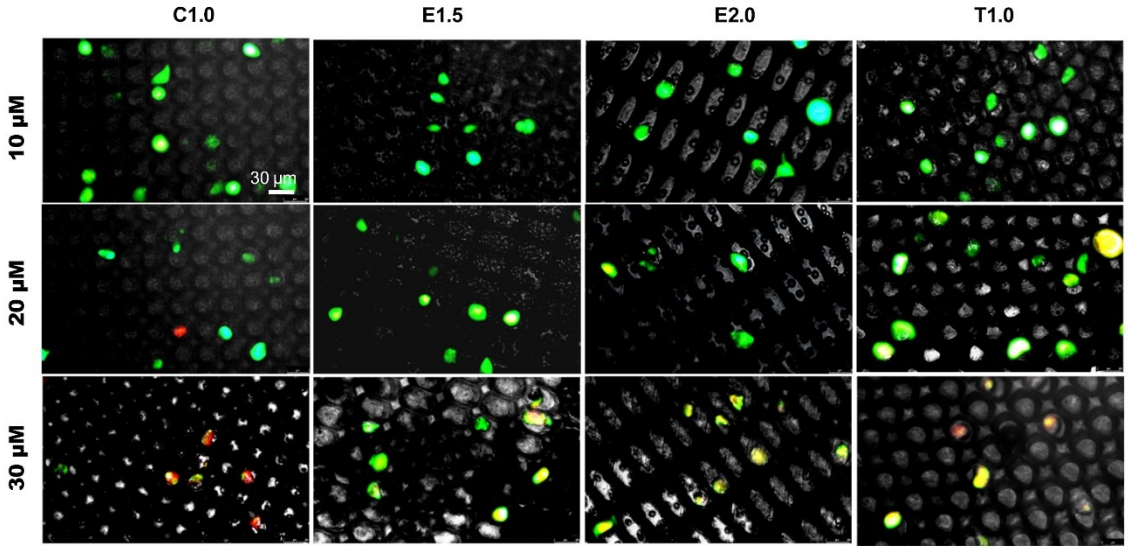

Figure S15. The fluorescent images of single trapped cells on microwell arrays after treated with PAC drug from 10 μM to 30 μM for 12h.

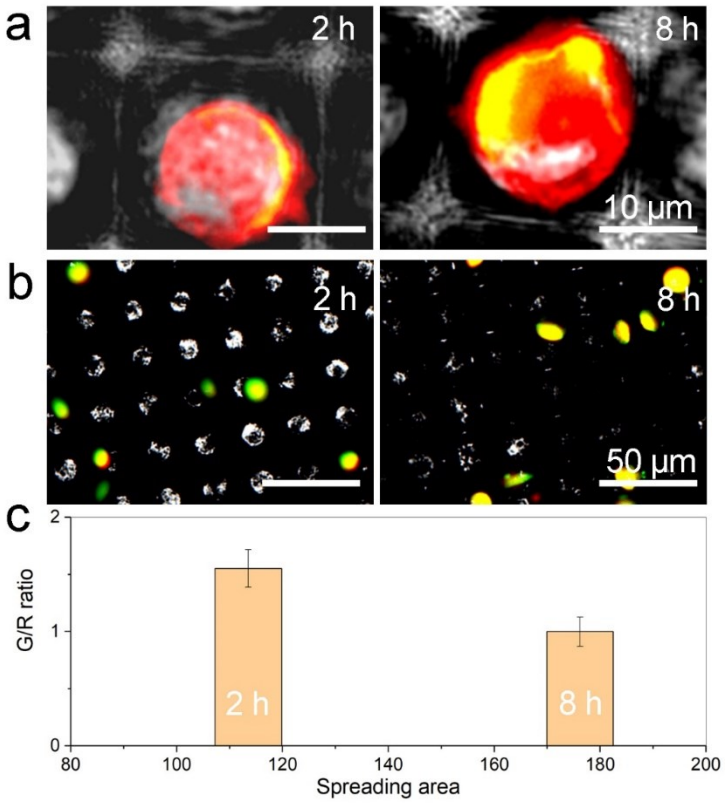

Figure S16. The drug response of non-polar cells with different spreading areas. (a) The cell in C1.0 with different spreading area. (b) AO/EB stained cells in C1.0 after treated by 30 μM for 2h. (c) The Live/Dead (G/R) ratio of non-polar cells with different spreading areas.

Table S2. The Gini index of cell heterogeneity.

C1.0	E1.5	E2.0	T1.0
0.369	0.318	0.261	0.381