High-Throughput Force Arrays Reveal Spatial Force Distribution as a Biomarker for Cancer Cell Identification and Drug Resistance Detection

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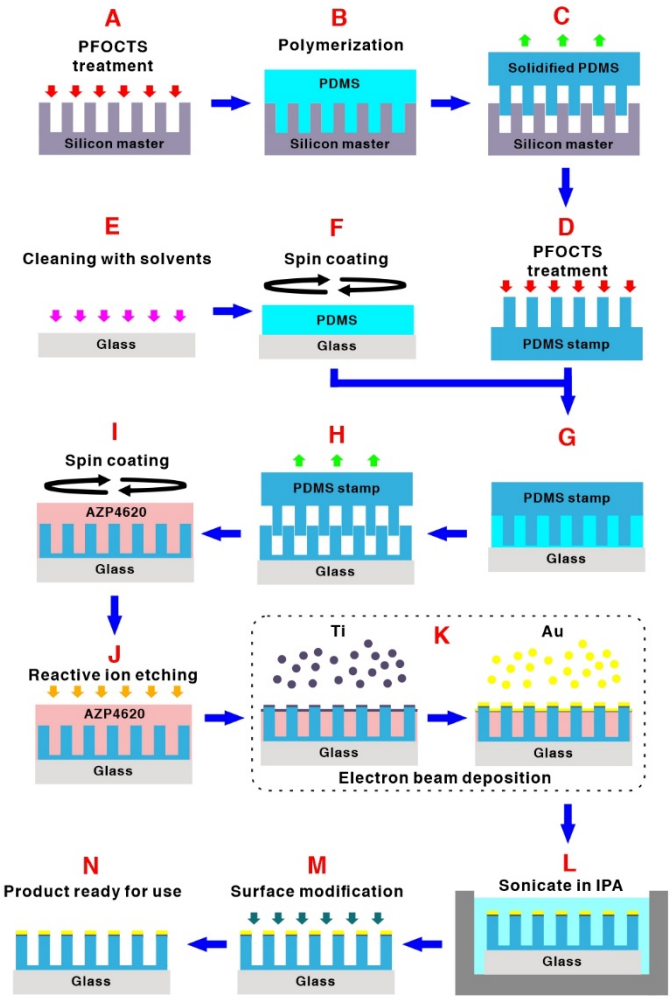


Figure S1. Schematic of the fabrication process of Force Arrays Screening Tool (FAST). (A) Trichloro(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)silane (PFOCTS) treatment of the silicon wafer master. (B)-(C) Generation of negative polydimethylsiloxane (PDMS) hole array replica. (D) PFOCTS treatment of the negative PDMS hole array replica. (E) Glass coverslip cleaning with detergent, ethanol, and water. (F) Spin coating of untreated PDMS onto glass coverslip. (G)-(H) Replica molding of negative PDMS hole array replica onto a glass coverslip. (I) Spin coating of positive photoresist. (J) Reactive ion etching of product. (K) Electron beam deposition of titanium and gold onto micropillar array. (L) Removal of excess positive photoresist via sonication in isopropyl alcohol. (M) Surface treatment of FAST. (N) FAST is ready to be used.

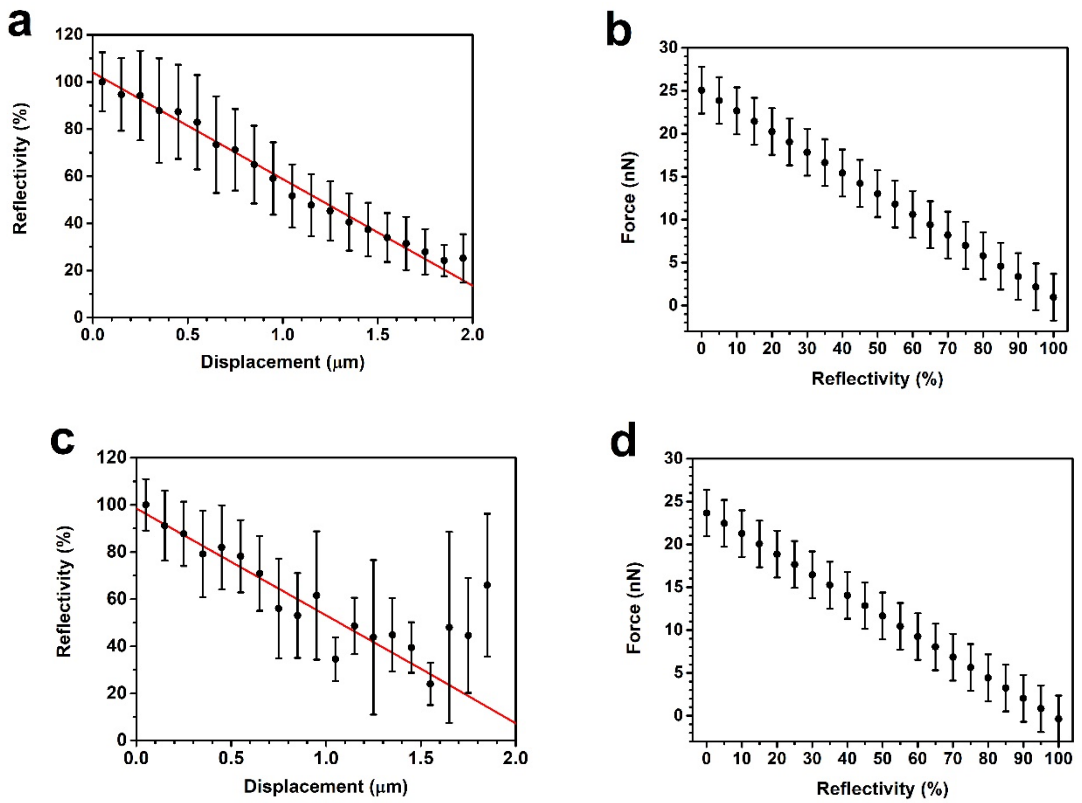


Figure S2. The calibration curve of displacement vs intensity change. We measured the displacements of 120, 000 pillars and their reflected intensity for more than 1000 cells. (a) The displacement and reflectivity of micropillars were measured using 20X. (b) The calibration curve for the reflectivity was measured by the 20 X objective to calculate the traction force using the fitted data in (a). (c) The measured displacement by the 20X objective and reflectivity measured by the 10 X objective (d) Calibration curve for the measured reflectivity using the 10 X objective to calculate traction force using the fitted data in (c).

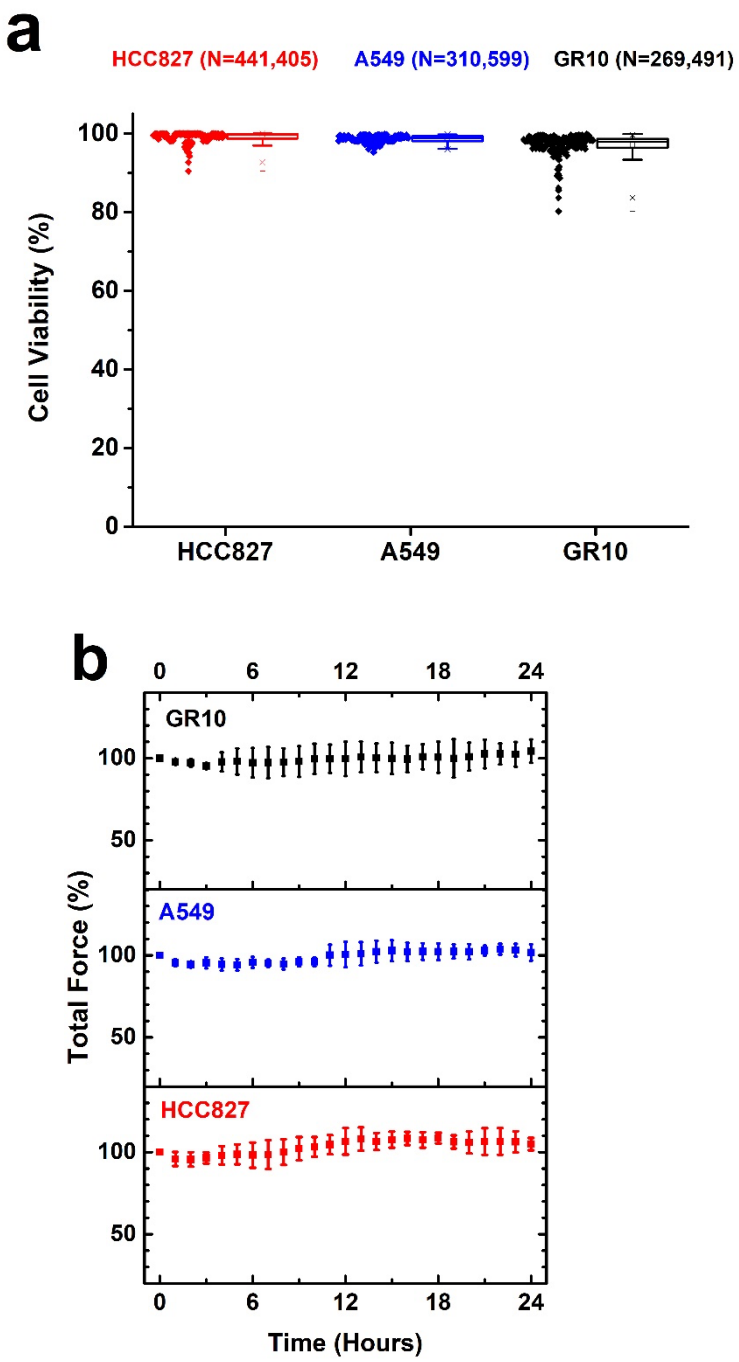


Figure S3 (a) The viability of HCC827, A549, and GR10 cells on the FAST chips after 24 hours of continuous force measurement. N values are the total number of cells on the FAST chips. (b) The time-dependent force per cell for HCC827, A549, and GR10 cells on the FAST chips.

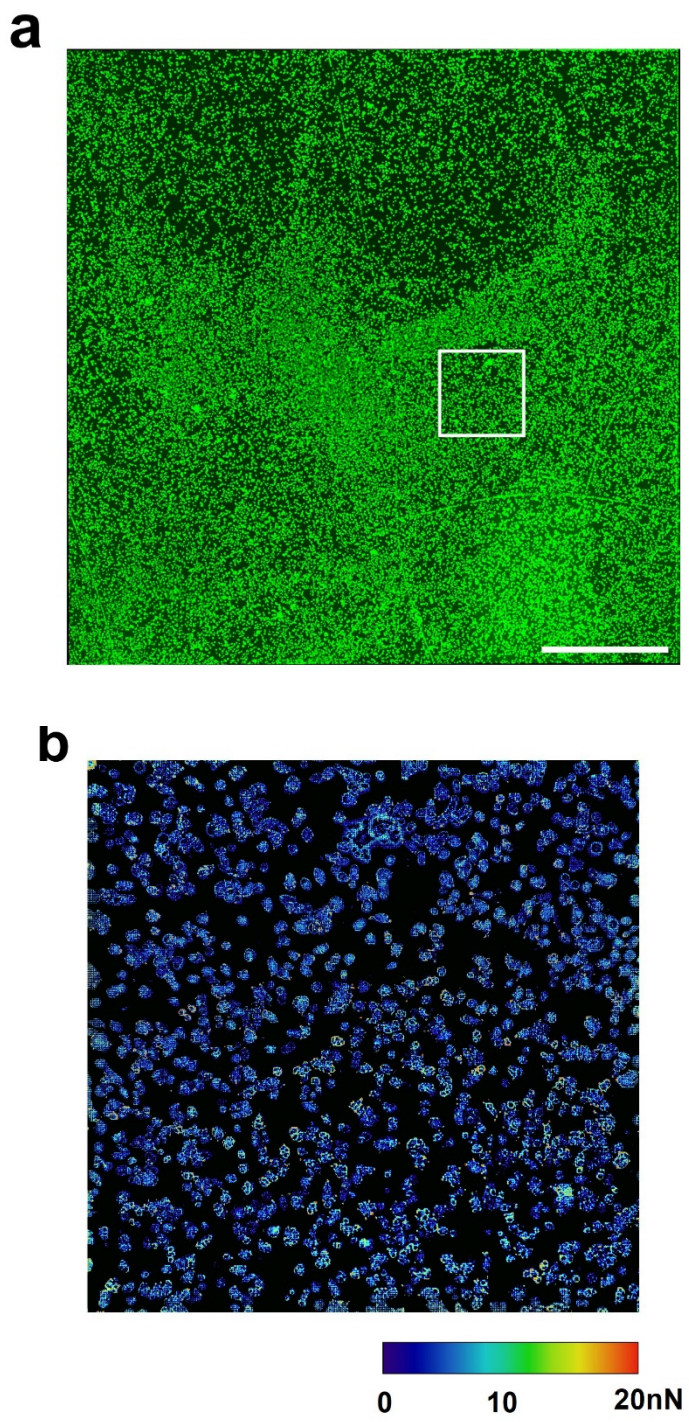


Figure S4 a) The fluorescence image of more than 100,000 cells cultured on the FAST chip. b) The traction force image of the yellow box in (a).

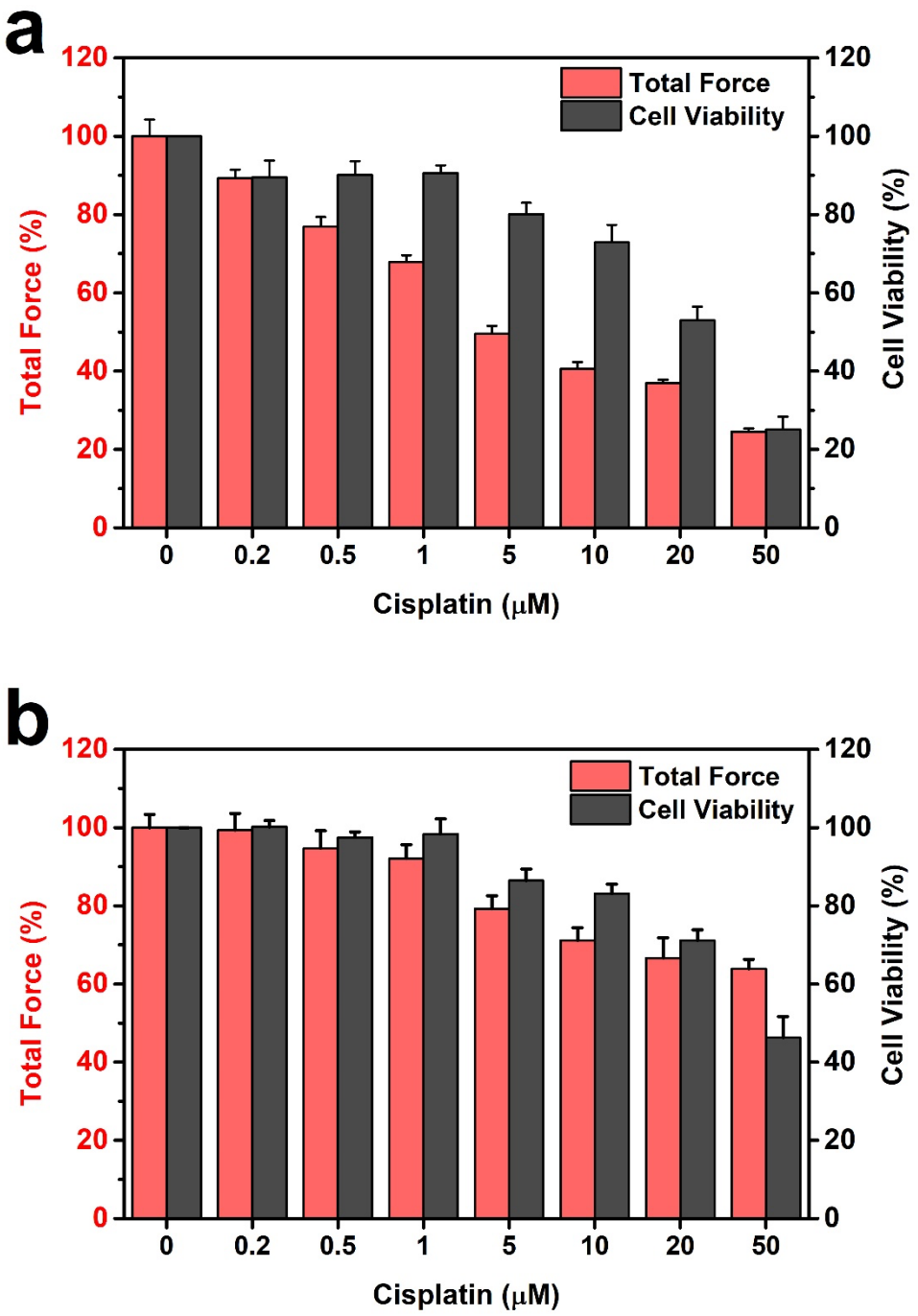


Figure S5 The cytotoxicity and reduction in the average traction force for (a) A549 and (b)HCC827 cells after treatment with various concentrations of cisplatin for 48 hours.

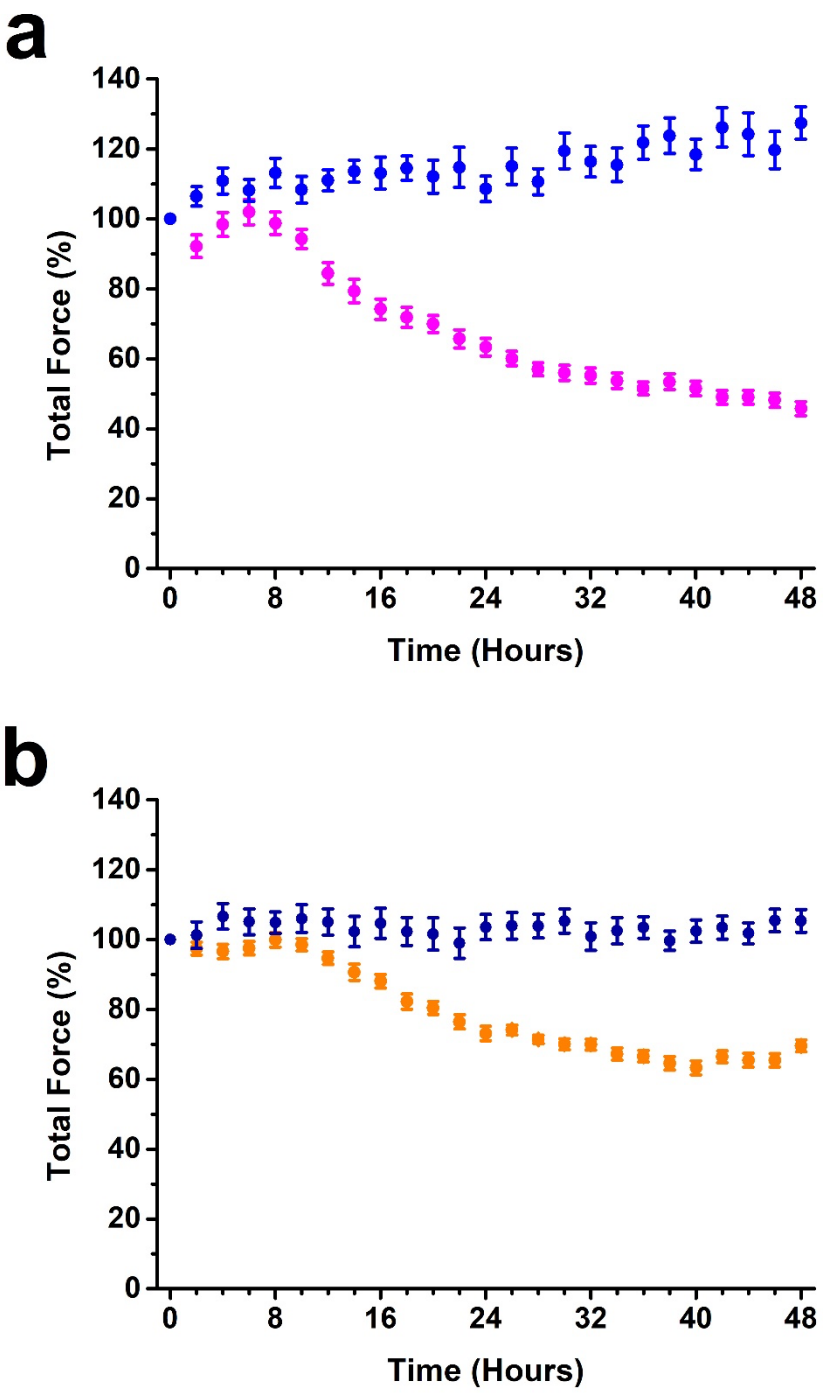


Figure S6 (a) Time-dependent traction force measurement for A549 and (b) HCC827 treated with cisplatin at 50 μM of cisplatin. Blue dots are the total traction force for the control cells, and red dots are the average traction force from the cells treated with cisplatin.

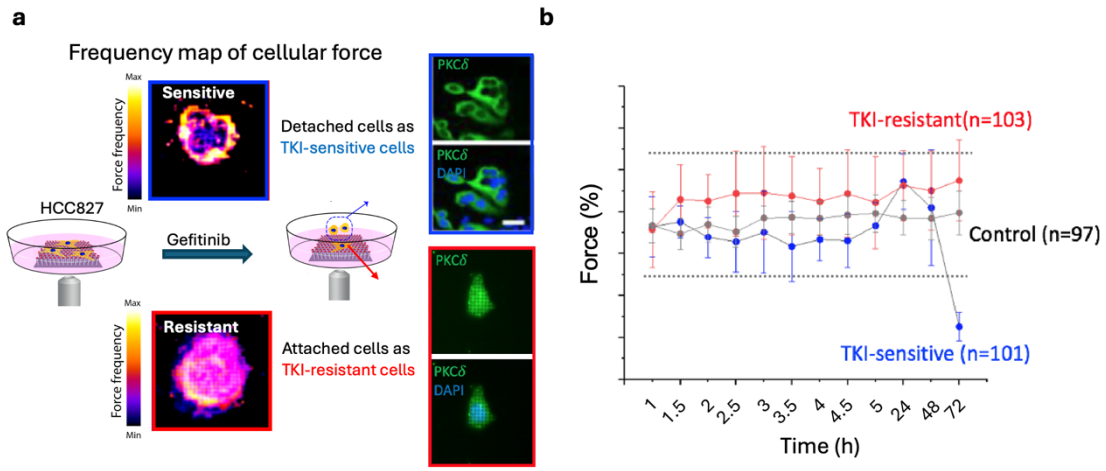


Figure S7. (a) We stained the HCC827 cells with nuclear protein kinase Cδ (PKCδ), which is a common marker for TKI-resistance in lung cancer. In the HCC827 cells that are sensitive to gefitinib, the PKCδ markers are located in the cytosol, whereas the PKCδ markers translocated to the nuclear in the HCC827 cells resistant to gefitinib. (b) Time-dependent changes in cellular traction force upon gefitinib treatment.

The traction force of TKI-sensitive (blue) and TKI-resistant (red) HCC827 cells was monitored over 72 hours following gefitinib treatment. The control group (black) represents untreated cells. TKI-sensitive cells exhibited a significant reduction in traction force after 48 hours, whereas TKI-resistant cells maintained a stable force profile. Error bars indicate standard deviations, and sample sizes are indicated for each group (TKI-resistant: n=103, TKI-sensitive: n=101, Control: n=97).

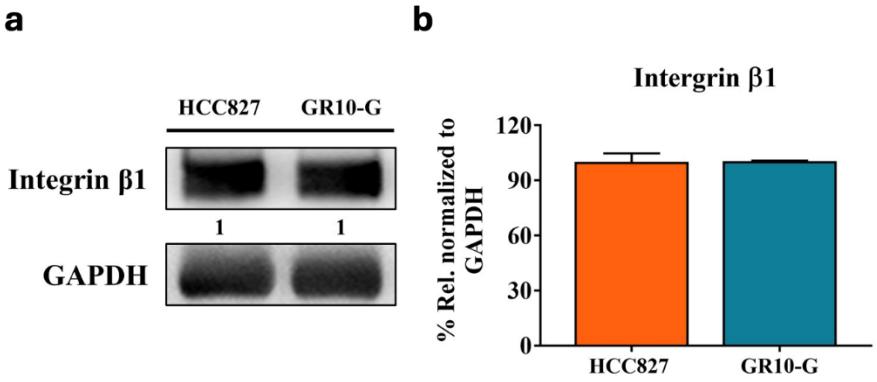


Figure S8 (a) Western blotting of integrin β1in HCC827 and GR10 cells. (b) The relative expression levels of integrin β1in HCC827 and GR10 cells

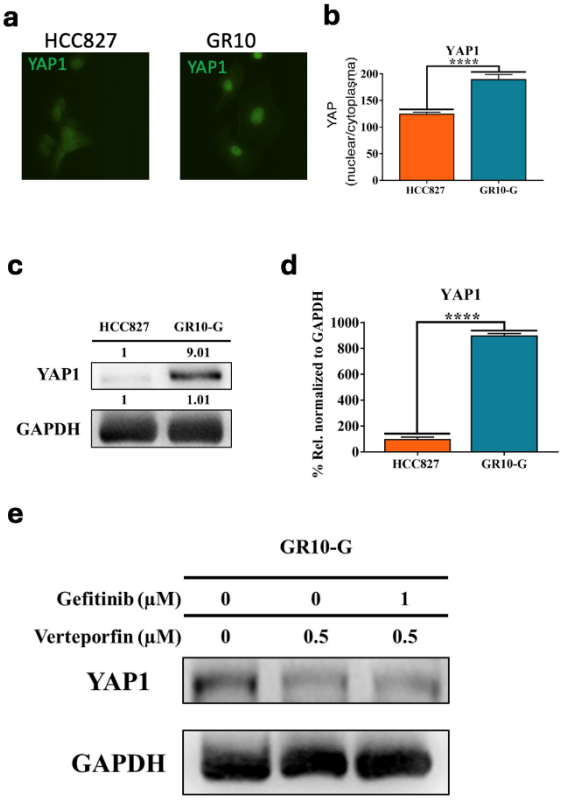


Figure S9 (a) The fluorescence images of HCC827 and GR10 cells stained with YAP1 proteins. (b) The fluorescence intensity ratio of the nuclear region over fluorescence intensity in the cytosol. (c) The Western blot image of YAP1 in HCC827 and GR10 cells. (d) The relative expression level of YAP1 in HCC827 and GR10 cells. (e) The Western blot image of YAP1 in GR10 cells after being treated with YAP inhibitor and gefitinib.

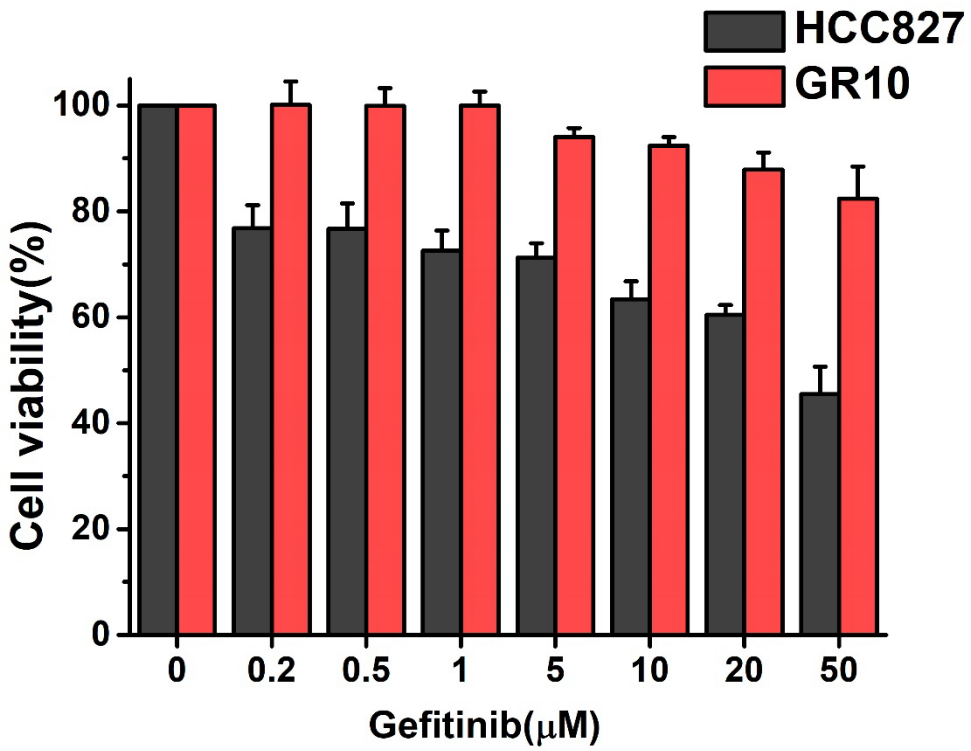
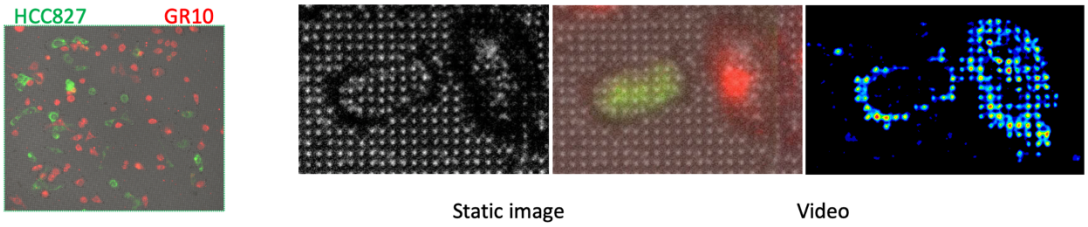


Figure S10 Cell viability for HCC827 and GR10 cells after treatment of various concentrations of Gefitinib for 48 hours.



	Accuracy	Accuracy
HCC827/GR10	91.36%	92.17%
HCC827/GR06	70.64%	91.24%

Figure S11Mixture of HCC827 (green) and GR10 (red) cells in a 1:1 ratio, analyzed using force mapping image processing with the Nyckel platform. The leftmost panel shows the original mixed cell image, followed by force mapping images highlighting traction forces exerted by individual cells. Static image analysis and video-based analysis (5 minutes per frame, 12 frames in total) yielded classification accuracies of 91.36% and 92.17% for HCC827/GR10 cells, and 70.64% and 91.24% for HCC827/GR06 cells, respectively.

Table S1

Comparison of FAST with MTT and CCK8 Assays

MTT and CCK8 assays are widely used, commercially available colorimetric assays for measuring cell proliferation and viability. These methods rely on metabolic activity to indirectly quantify the number of viable cells. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay measures mitochondrial dehydrogenase activity, converting MTT into formazan crystals, which require solubilization before absorbance measurement. CCK8 (Cell Counting Kit-8) assay, based on WST-8, eliminates the solubilization step and enables a more straightforward measurement of cell viability through water-soluble formazan production.

While these assays are widely used and reliable for assessing cell proliferation, they have several limitations:

Feature	FAST (Force Arrays Screening Tool)	MTT/CCK8 Assays
Measurement Type	Direct mechanical force mapping	Metabolic activity assay
Readout Sensitivity	Detects early mechanical responses (pre-cell death)	Detects late-stage viability loss
Single-Cell Resolution	Yes	No (population-based)
Real-Time Measurement	Yes, continuous tracking	No, endpoint measurement
Label-Free	Yes	No (requires reagents)
Throughput	High (100,000+ cells per chip)	Medium (multi-well plates)
Drug Sensitivity Testing	Detects drug response within **3 hours**	Requires **12–24 hours** to show changes
Cost	Low (reusable chip-based platform)	Medium (reagents required for each assay)
Ease of Use	Simple, minimal sample prep	Requires multiple steps and incubation times