Single-cell western blotting after whole-cell imaging to assess cancer chemotherapeutic response

*Chi-Chih Kang†, Jung-Ming G. Lin†,‡, Zhuchen Xu†, Sanjay Kumar†,‡, Amy E. Herr†,‡**

†Department of Bioengineering, UC Berkeley, ‡The UC Berkeley/UCSF Graduate Program in Bioengineering, Berkeley, California 94720, United States

Supplementary Figure 1. Ponceau Staining of a western blot.

Supplementary Figure 2. *sc*Western blots of Alexa Fluor 555-labeled purified bovine serum albumin (BSA) and Alexa Fluor 488-labeled purified trypsin inhibitor (TI) as presented in Figure 2A.

Supplementary Figure 3. *sc*Western blots of GAPDH and β–tubulin under the 4 ^{o}C and 50 ^{o}C lysis conditions as presented in Figure 3A.

Supplementary Figure 4. Time-lapse fluorescence images of Alexa Fluor 555-labeled purified BSA electro-migrating during PAGE at the 4 ^{o}C and 50 ^{o}C conditions.

Supplementary Figure 5. Time-lapse fluorescence intensity plot of GFP in U373-GFP cells after pouring cold (4 ^{o}C) or hot (50 ^{o}C) PBS.

Supplementary Figure 6. *sc*Western blots of GFP under 4 ^{o}C and 50 ^{o}C lysis conditions.

Supplementary Figure 7. *In situ* chemical lysis of cells in microwells at 37 ^{o}C conditions suggests slight improvements in protein diffusional losses offset by sub-optimal protein separation resolution.

Supplementary Figure 8. The schematic process of performing enclosed microwell lysis by application of a lid slide.

Supplementary Figure 9. Toxicity of daunomycin (DNR) for U373 MG cells.

Supplementary Figure 10. *sc*Western blots of probed cleaved caspase 8 and GAPDH in U373 MG control and DNR-treated U373 MG cells as presented in Figure 4A.

Supplementary Figure 11. Correlation assessment of cell imaging and corresponding β-Tubulin *sc*Western blotting signal at open lysis condition suggests the existence of cross-contamination.

Supplementary Figure 12. Two distributions of cleaved caspase 8 signal defined a fluorescence threshold for negative (< 0.2) and positive (> 0.2) cleaved caspase 8 populations.

Supplementary Figure 13. Scatter plot of cleaved caspase 8 and DNR uptake shows cell-to-cell variation in response to DNR.

Supplementary Figure 14. *sc*Western blots of probed P-glycoprotein (P-gp) in DNR-treated U373 MG cells as presented in Figure 4E.

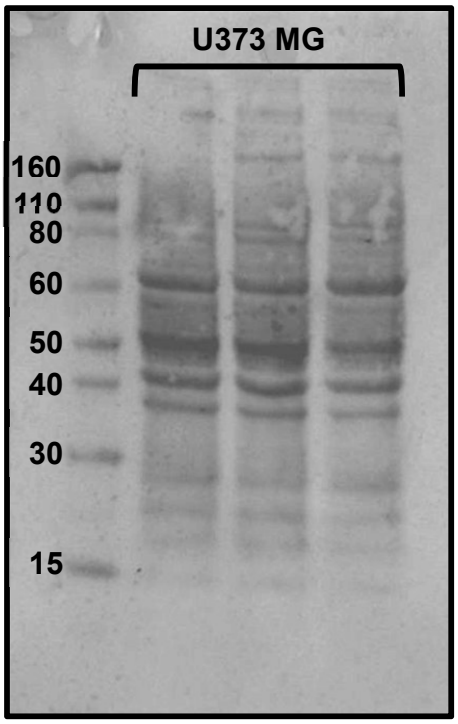
Supplementary Figure 15. Two distributions of P-gp signal defined a fluorescence threshold for low (< 0.1) and high (> 0.1) P-gp expression.

Supplementary Figure 16. *sc*Western blots of probed GAPDH signal before and after 4 month storage as presented in Figure 5B.

Supplementary Figure 17. *sc*Western blots of probed β–tubulin signal in a *sc*Western slide stored for 4 months as presented in Figure 5C.

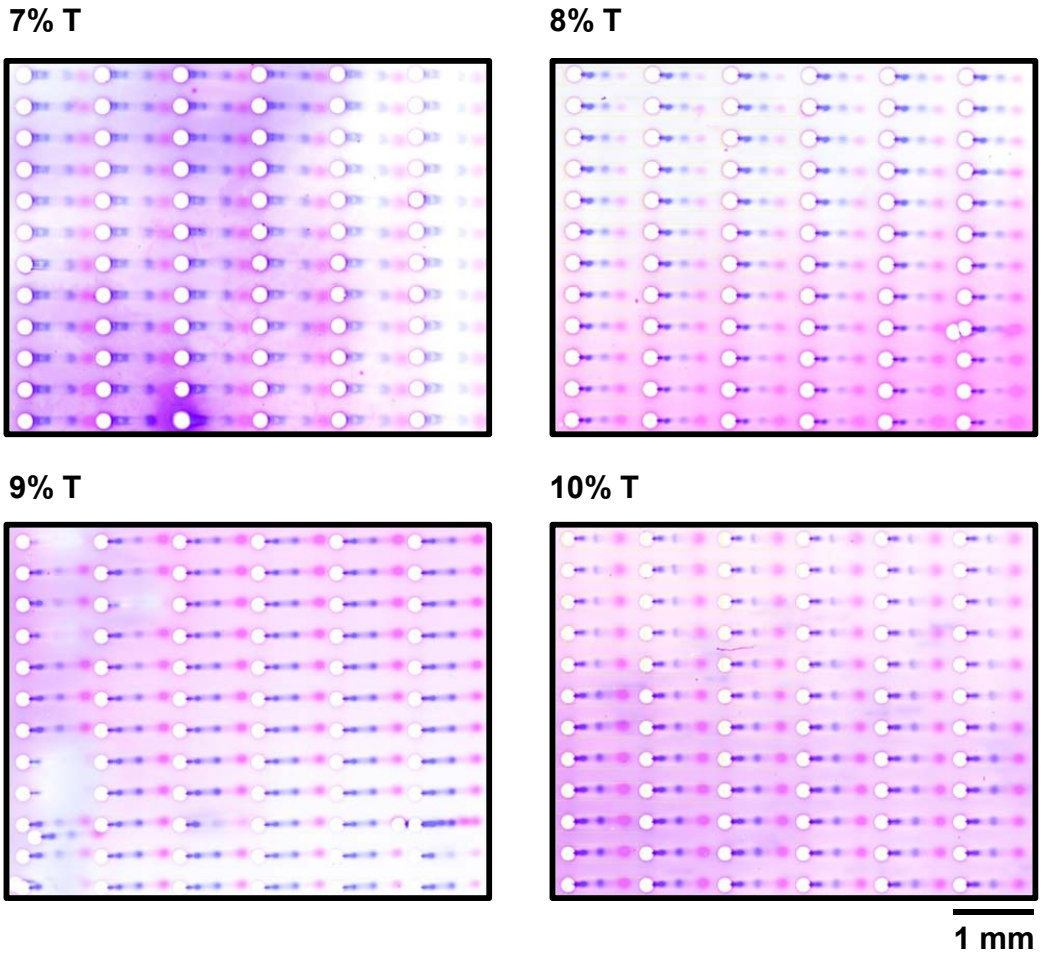
Supplementary Figure 18. *sc*Western blots of probed β-Tubulin and cleaved caspase 8 in DNR-treated U373 MG cells.

Supplementary Figure 1



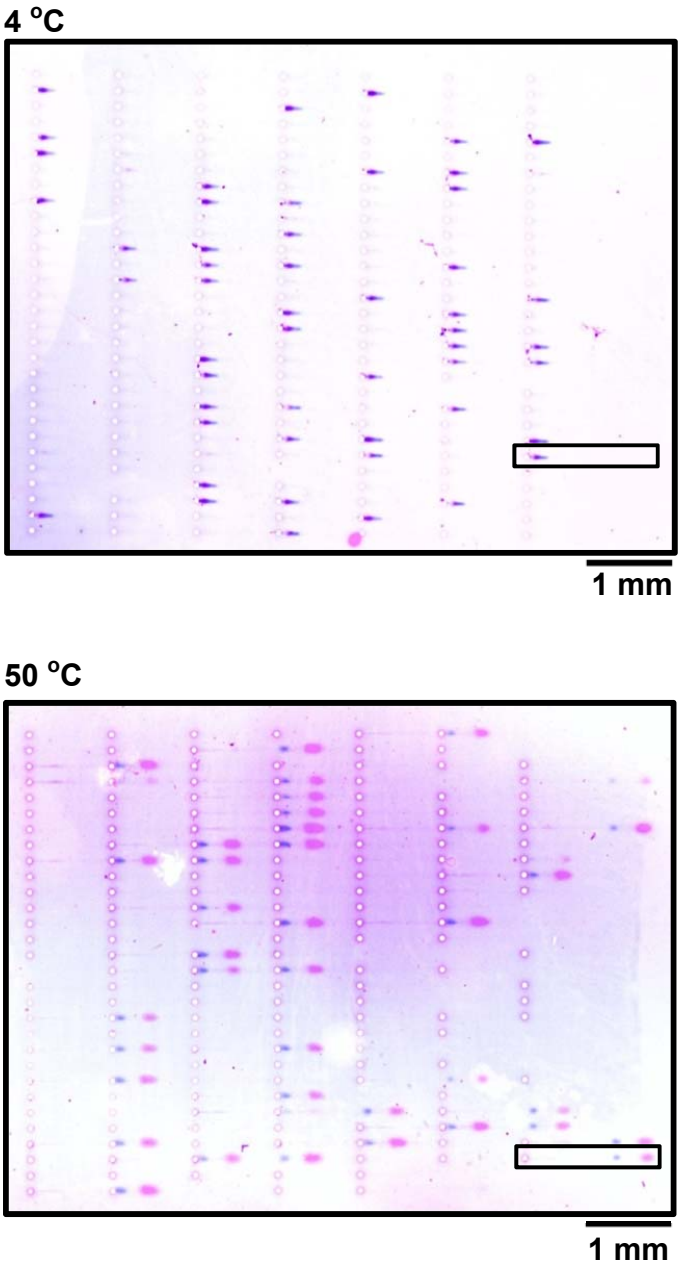
Supplementary Figure 1. Ponceau Staining of a western blot from U373 MG cell lysate showed that the molecular weight of most proteins is located in the range from 20 to 100 kDa.

Supplementary Figure 2



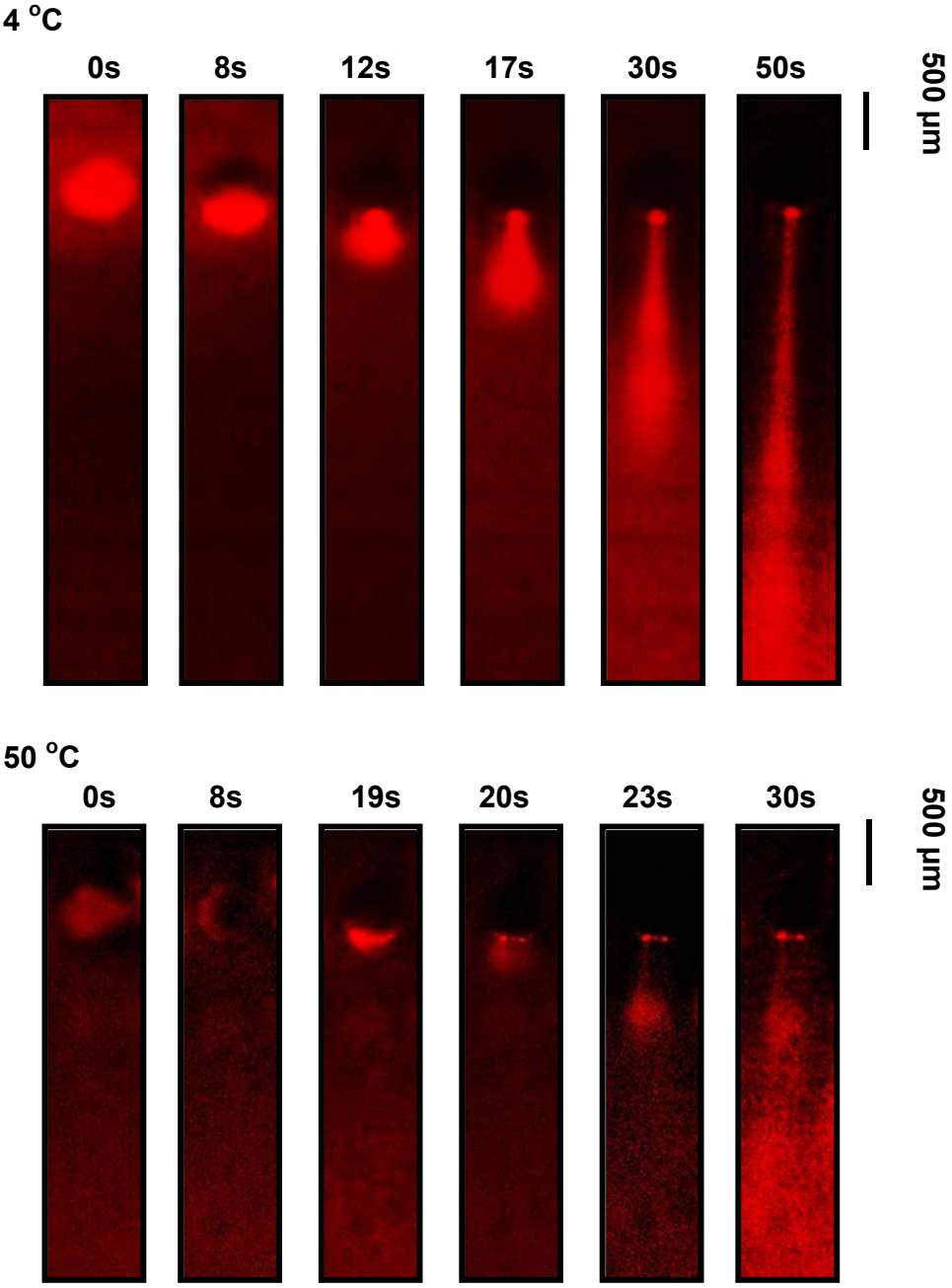
Supplementary Figure 2. *sc*Western blots of Alexa Fluor 555-labeled purified bovine serum albumin (BSA) and Alexa Fluor 488-labeled purified trypsin inhibitor (TI) as presented in Figure 2A. The separation resolution between BSA (blue signal) and TI (magenta signal) is 0.56 ± 0.08 at 7% T (±s.d., n = 57); 0.67 ± 0.05 at 8% T (±s.d., n = 103); 0.89 ± 0.11 at 9% T (±s.d., n = 73); 1.04 ± 0.09 at 10% T (±s.d., n = 103). Protein PAGE time is 21 s (7%T); 20 s (8%T), 23 s (9%T), and 26 s (10%T). Circular features are the microwells. E = 40 V cm^{-1}.

Supplementary Figure 3



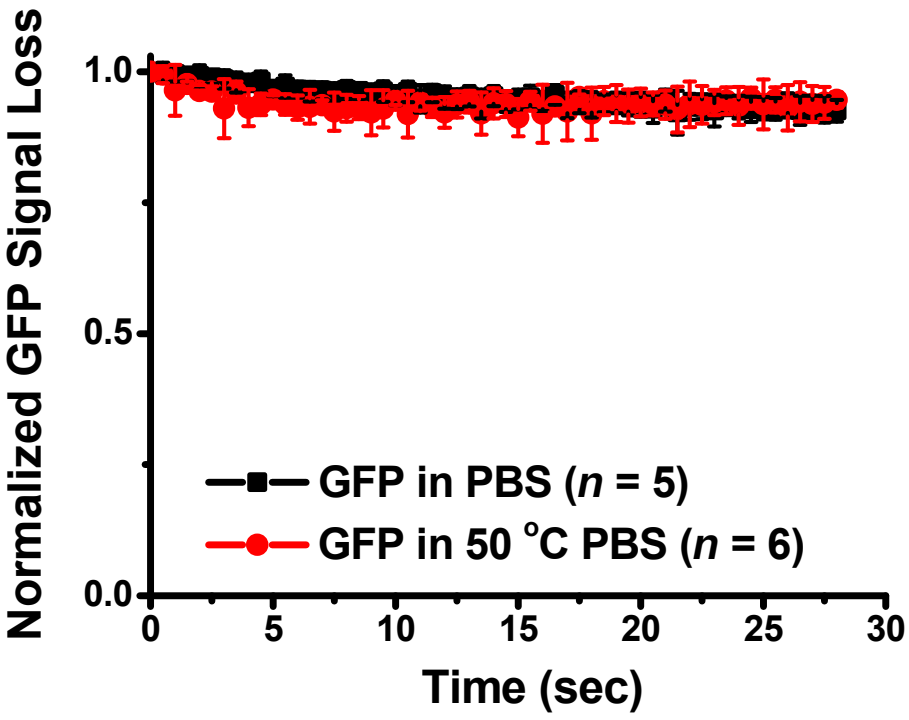
Supplementary Figure 3. *sc*Western blots of GAPDH (magenta signal) and β–tubulin (blue signal) under the 4 °C (top) and 50 °C (bottom) lysis conditions. The black squares in the images highlight the *sc*Westerns presented in Figure 3A. E = 40 V cm^{-1}, lysis time = 20 s, electrophoresis time = 40 s, 10%T PA gel, (GAPDH: Alexa Fluor 555-labeled secondary antibody, β–tubulin: Alexa Fluor 647-labeled secondary antibody).

Supplementary Figure 4



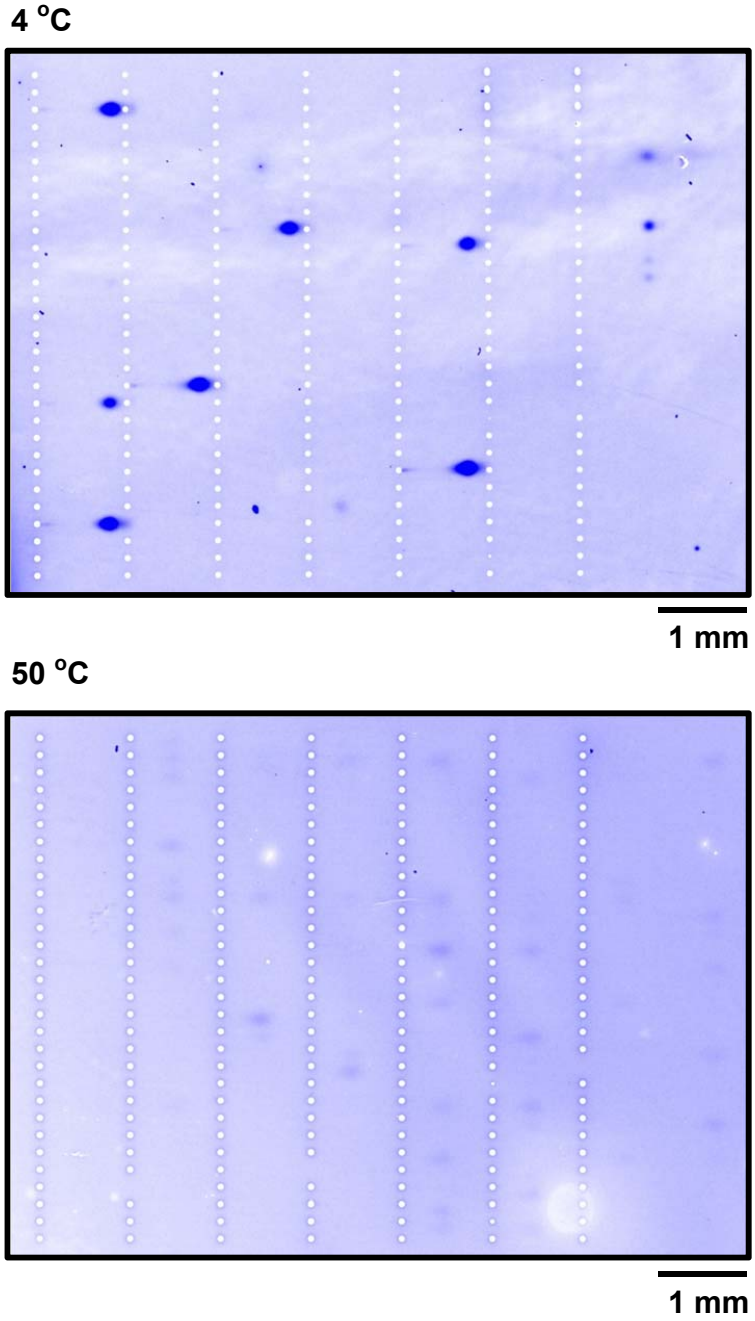
Supplementary Figure 4. Time-lapse fluorescence images of Alexa Fluor 555-labeled purified BSA (red signal) electro-migrating during PAGE at the 4 °C and 50 °C conditions. We observed better protein stacking and less protein dispersion at the 50 °C condition.

Supplementary Figure 5



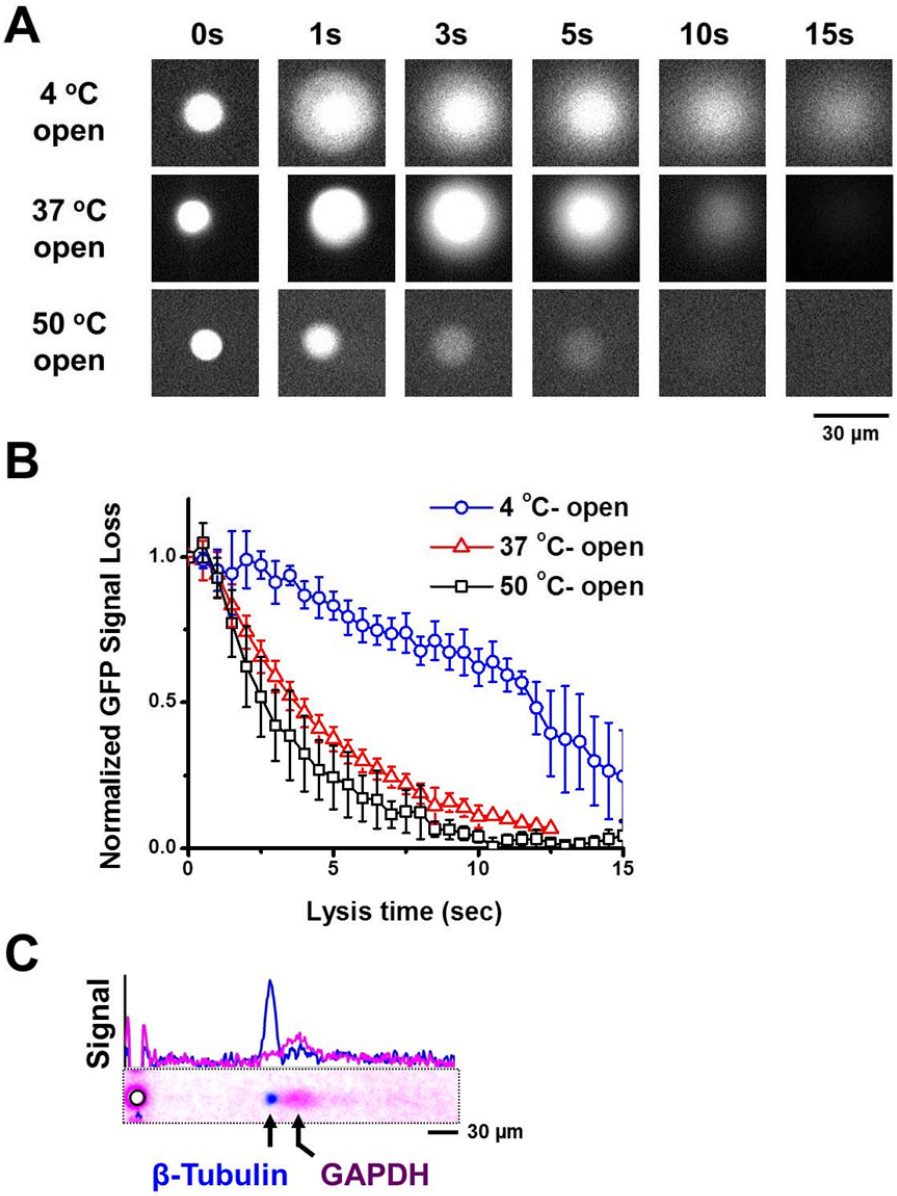
Supplementary Figure 5. Time-lapse fluorescence intensity plot of GFP in U373-GFP cells after pouring cold (4 ^{o}C) or hot (50 ^{o}C) PBS. The images were taken every 0.5 s. The slight loss (<10%) of intensity over time is likely due to the photobleaching of GFP. The similar intensity loss of GFP in 50 ^{o}C PBS suggested the fact that GFP is resistant to heat. The error bars came from 5 or 6 individual cells from three independent experiments.

Supplementary Figure 6



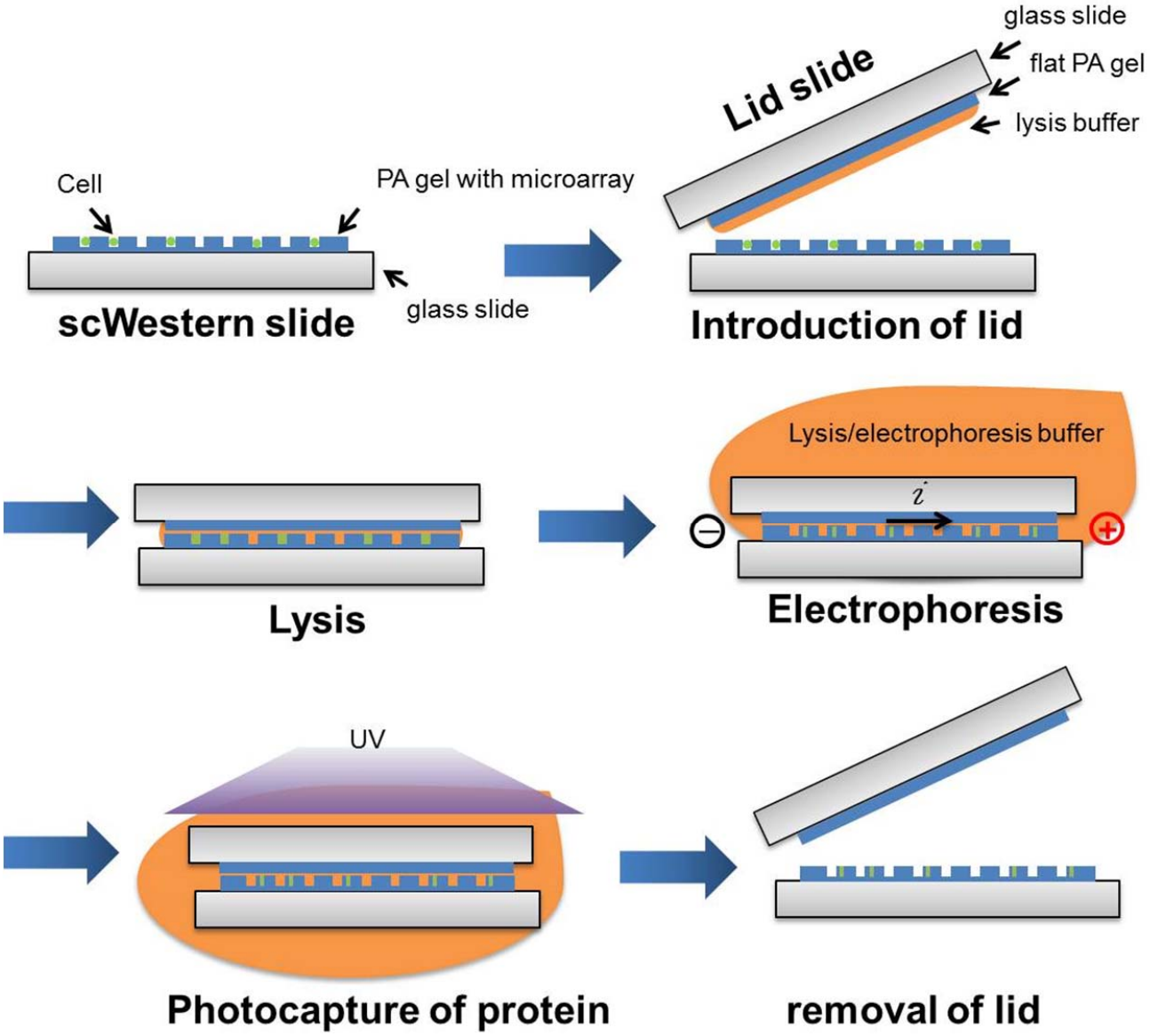
Supplementary Figure 6. *sc*Western blots of GFP (blue signal) under 4 °C and 50 °C lysis conditions. Strong GFP signal was observed under 4 °C lysis conditions. Proteins were extracted by modified RIPA lysis/extraction/electrophoresis buffer for 20 s, and then analyzed by protein PAGE for 30 s (1-mm long separation distance, 10%T PA gel). Circular features are the microwells. E = 40 V cm^{-1}.

Supplementary Figure 7



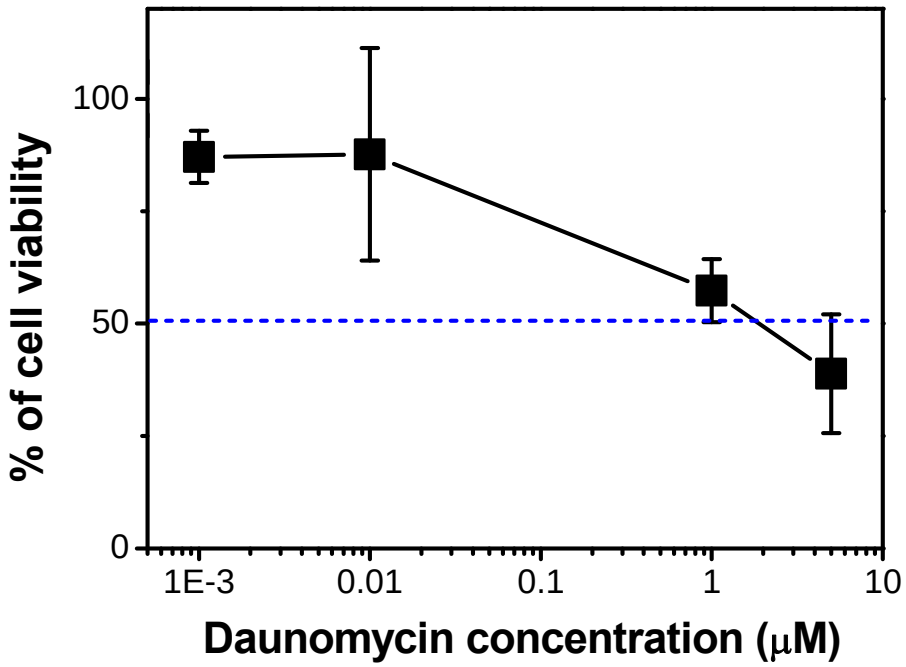
Supplementary Figure 7. *In situ* chemical lysis of cells in microwells at 37 °C conditions suggests slight improvements in protein diffusional losses offset by sub-optimal protein separation resolution. (A) Fluorescence micrographs during in-microwell lysis of U373-GFP cells under 4 °C, 37 °C and 50 °C lysis conditions, suggesting more protein diffusional losses with increasing temperature. (B) Time course of the total integrated GFP fluorescence signal from each microwell in (B). Error bars from 3-5 independent experiments indicate standard deviation. (C) Fluorescence micrographs and intensity profiles show *sc*Westerns at 37 °C lysis buffer conditions, suggesting decrease in protein separation resolution comparing to lysis condition at 50 °C (Figure 3). E = 40 V cm^{-1}, lysis time = 20 s, electrophoresis time = 40 s, 10%T PA gel. (GAPDH: Alexa Fluor 555-labeled secondary antibody, β–tubulin: Alexa Fluor 647-labeled secondary antibody).

Supplementary Figure 8



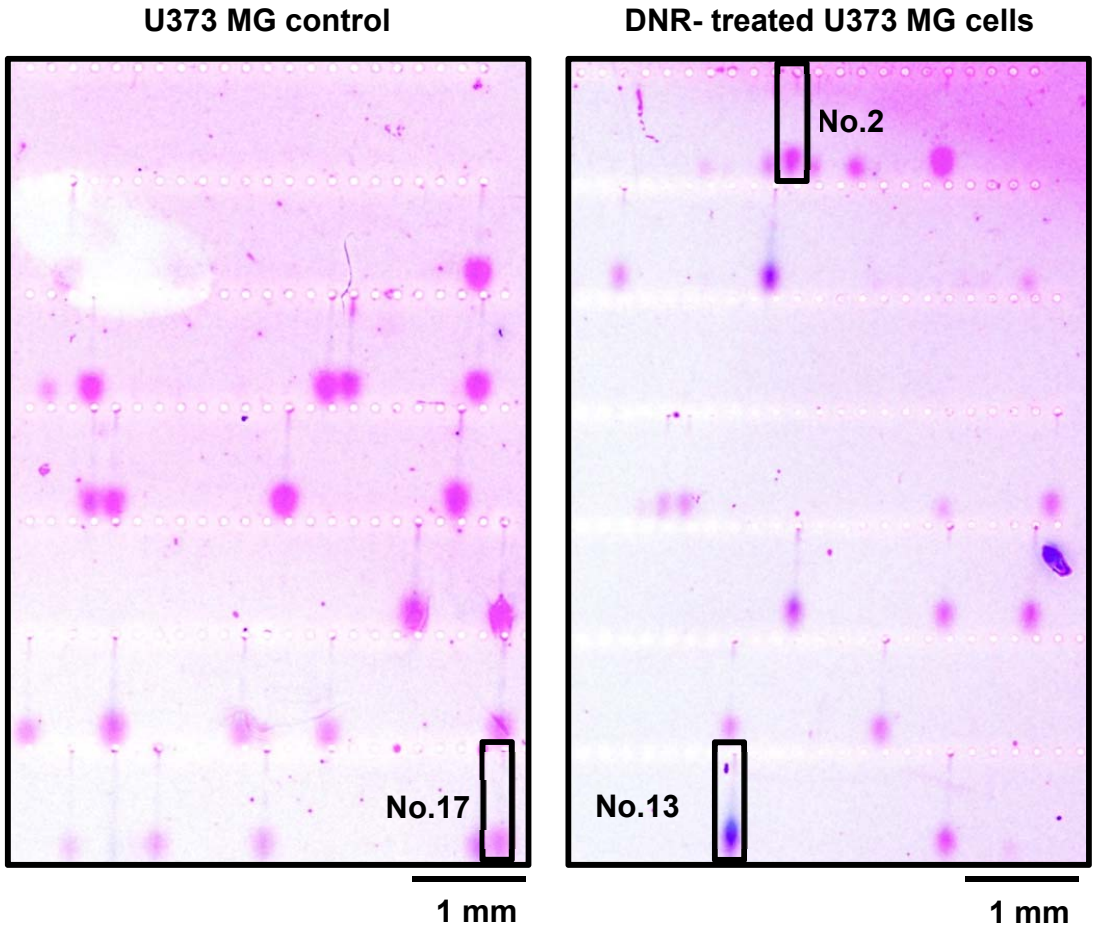
Supplementary Figure 8. The schematic process of performing enclosed microwell lysis by application of a lid slide. After cell settling on the *sc*Western PA gel slide, a plain, flat PA gel lid slide immersed with 50 ^{o}C lysis buffer is placed on top of the *sc*Western slide for performing enclosed microwell-lysis. After 20 s of lysis time, without removal of the lid slide, we pour the lysis/electrophoresis buffer over this sandwich-like lid-*sc*Western slide assembly, apply electrophoresis for 40 s, and photo-immobilize protein by UV directly. After protein immobilization, the lid is removed from the *sc*Western slide, allowing subsequent antibody probing procedures.

Supplementary Figure 9



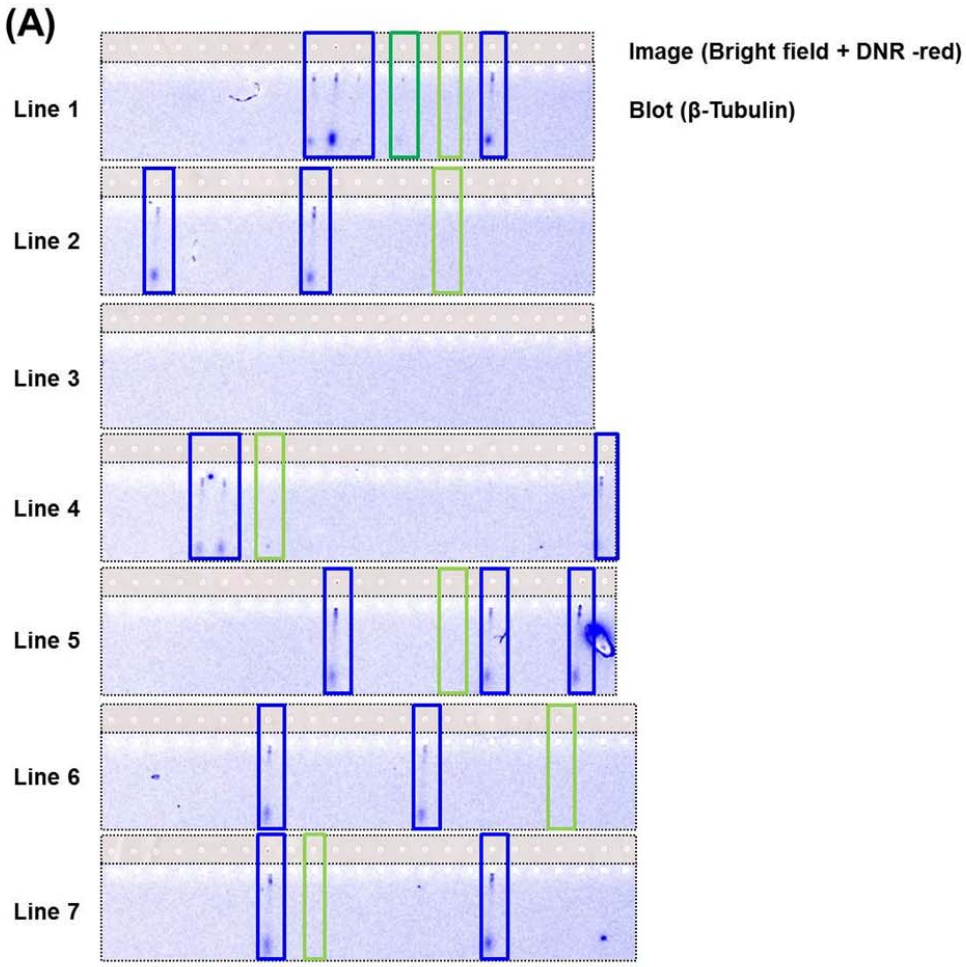
Supplementary Figure 9. Toxicity of daunomycin (DNR) for U373 MG cells. Cell viability was measured by the WST-1 proliferation assay after 24 hours of incubation with DNR at a concentration of 0.001, 0.01, 1, 5 μM. Values are given in mean % of untreated control cells ± standard deviation (SD). The mean and SD came from three independent measurements.

Supplementary Figure 10



Supplementary Figure 10. *sc*Western blots of probed cleaved caspase 8 (blue signal) and GAPDH (magenta signal) in U373 MG control (left) and DNR-treated U373 MG cells (right). The black squares in the images highlight the *sc*Westerns presented in Figure 4A. Proteins were extracted by modified RIPA lysis/extraction/electrophoresis buffer for 20 s, and then analyzed by protein PAGE for 30 s (1-mm long separation distance, 10%T PA gel). Circular features are the microwells. E = 40 V cm^{-1}. (GAPDH: Alexa Fluor 555-labeled secondary antibody, cleaved caspase 8: Alexa Fluor 647-labeled secondary antibody).

Supplementary Figure 11



(B)

		Image	
Blot		With cell (22)	No cell (138)
	With β-Tubulin (17)	16	1
	No β-Tubulin (143)	6	137

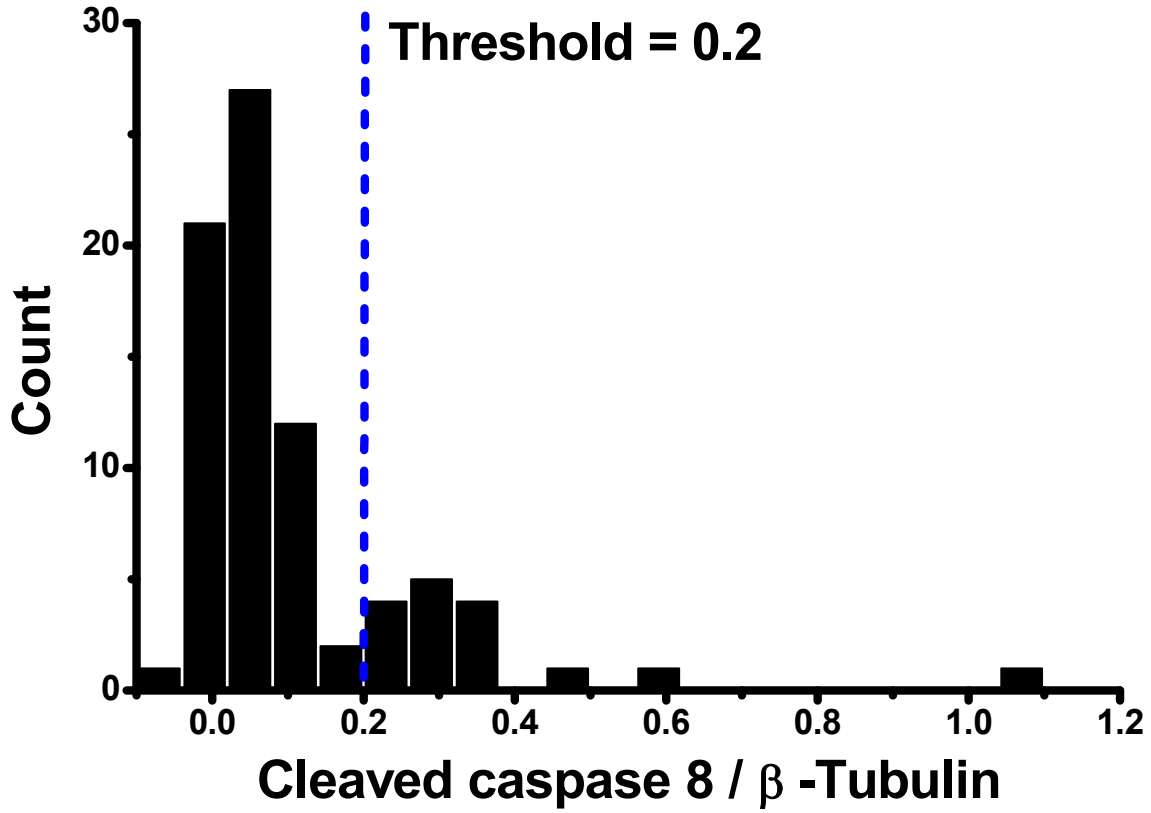
Accuracy = $\frac{16+137}{160}$ = 95.63%

False positive rate = $\frac{1}{138}$ = 0.72%

False negative rate = $\frac{6}{22}$ = 27.27%

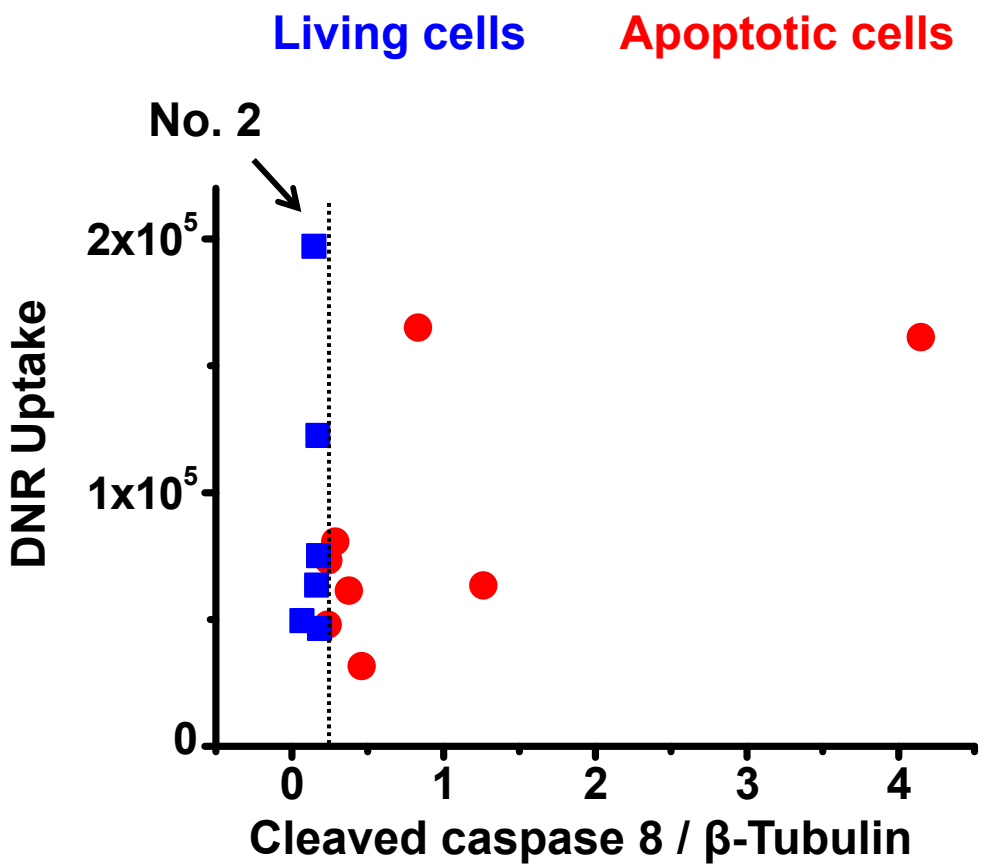
Supplementary Figure 11. Correlation assessment of cell imaging and corresponding β-Tubulin *sc*Western blotting signal at open lysis condition suggests the existence of cross-contamination. (A) A block of merged images (bright field and DNR stain) and their corresponding *sc*Western blots. The blue squares highlight the cell-containing microwells with its *sc*Western blots probing for β-Tubulin. The light green squares highlight the cell-containing microwells yet no results in its corresponding western blots. The single dark green square in Line 1 marks the empty microwells with a positive β-Tubulin signal.

Supplementary Figure 12



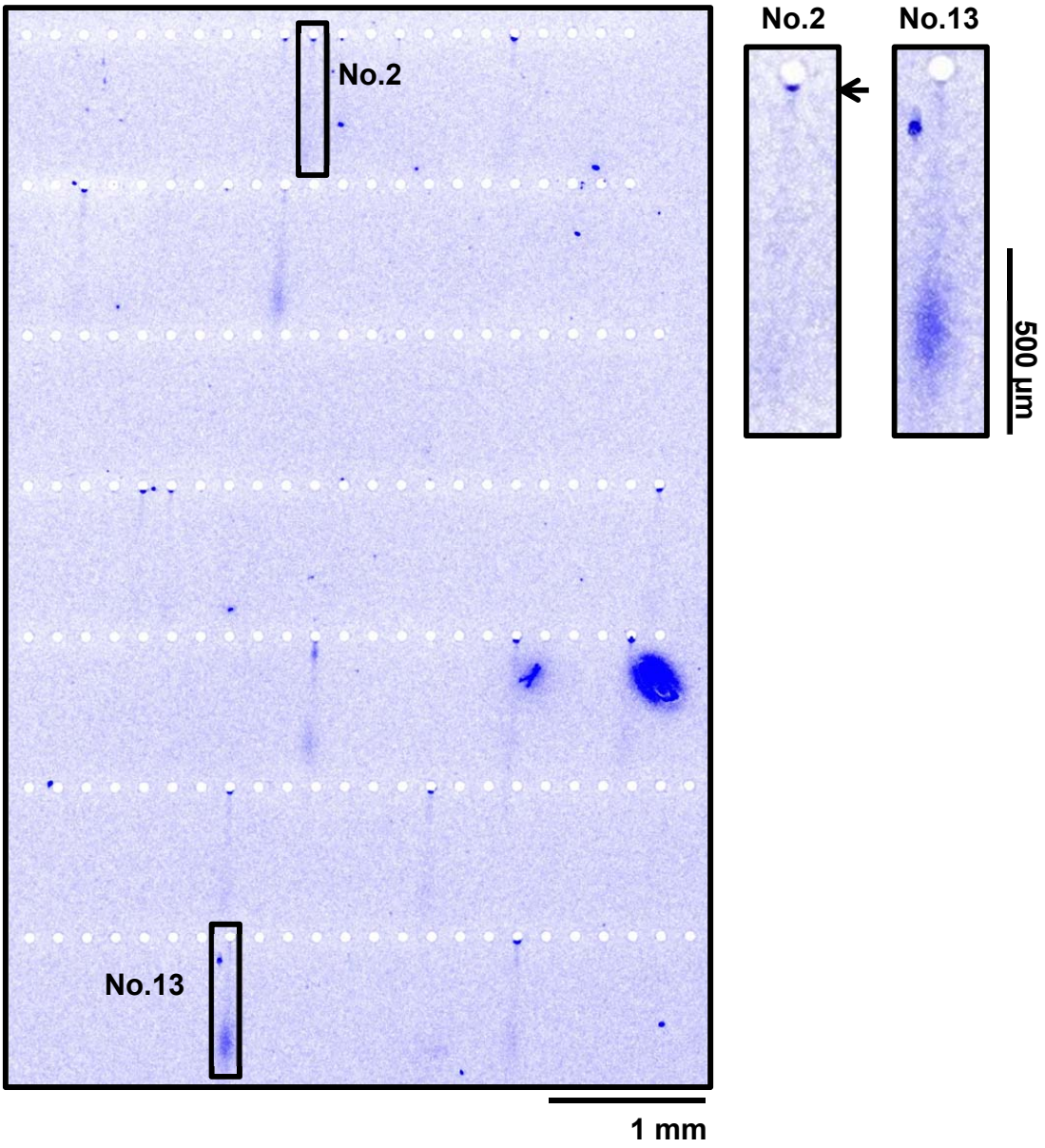
Supplementary Figure 12. Two distributions of cleaved caspase 8 signal defined a fluorescence threshold for negative (< 0.2) and positive (> 0.2) cleaved caspase 8 populations. All the cleaved caspase 8 signals are normalized to their corresponding β-Tubulin signals.

Supplementary Figure 13



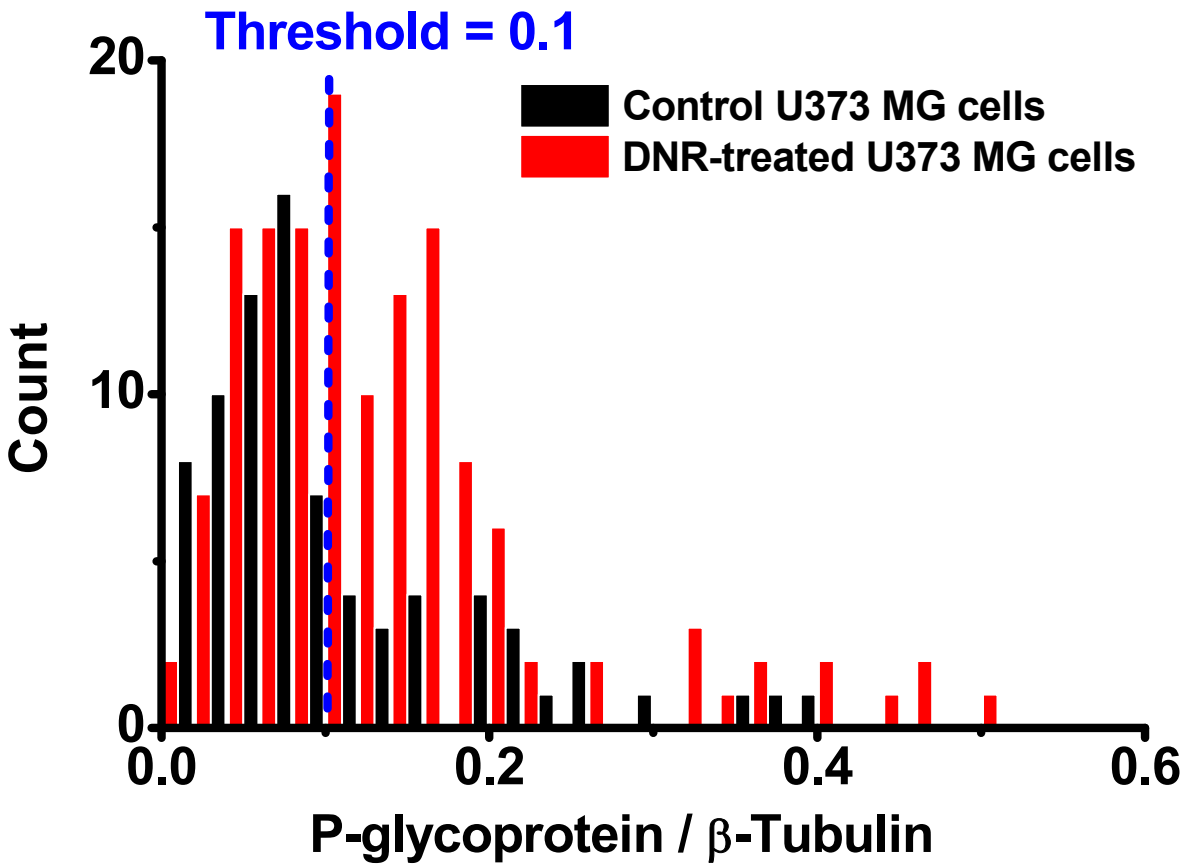
Supplementary Figure 13. Scatter plot of cleaved caspase 8 and DNR uptake shows cell-to-cell variation in response to DNR. Dashed line indicates the 0.2 threshold line defined in Supplementary Figure 12. The cell number 2 as marked by the arrow corresponds to the cell shown in Supplementary Figure 10 and 14. All the cleaved caspase 8 signals are normalized to their corresponding β-Tubulin signals.

Supplementary Figure 14



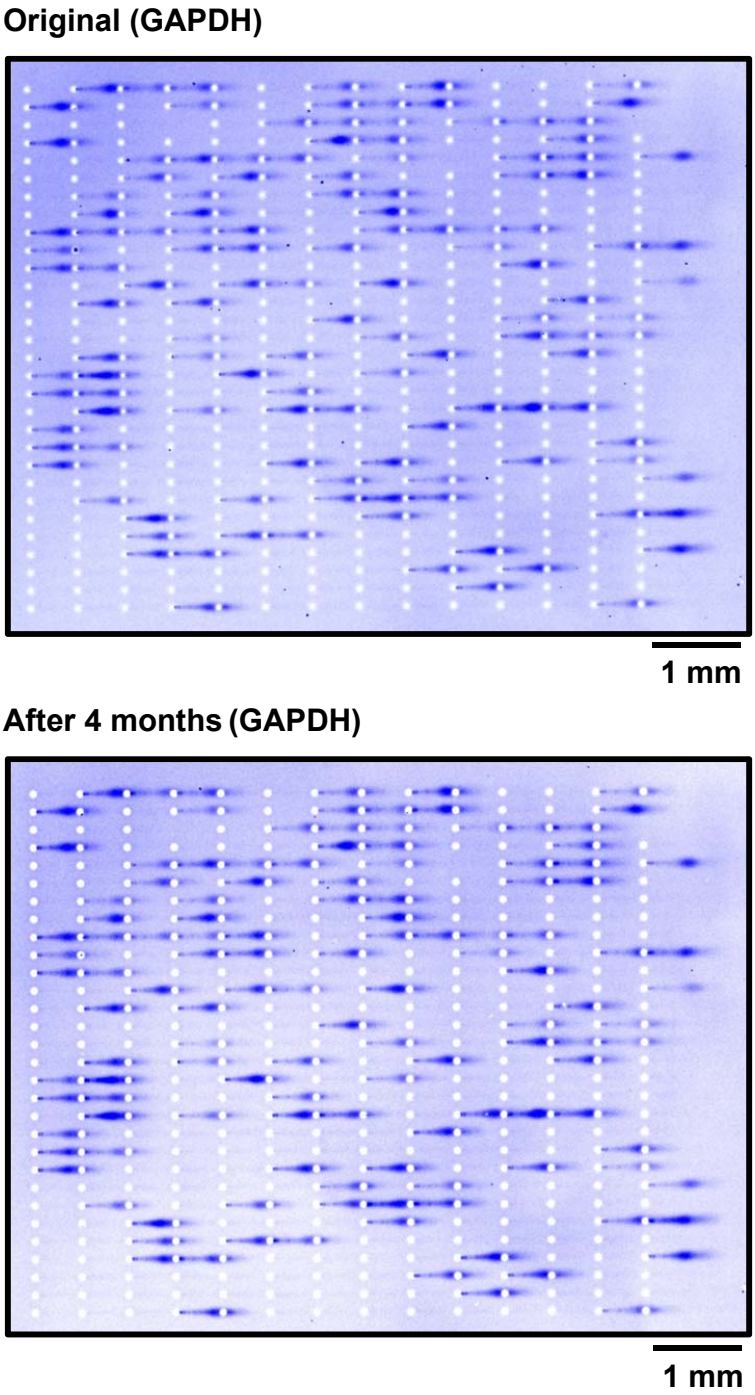
Supplementary Figure 14. *sc*Western blots of probed P-glycoprotein (P-gp, blue signal) in DNR-treated U373 MG cells. The black squares in the images highlight the *sc*Westerns presented in Figure 4E. At 10%T PA gel and protein PAGE for 30 s, P-gp (140kDa) only electro-migrates to the edge of the microwell. The selected fluorescence micrographs are marked in black. It is apparent that cell number 2 has strong P-gp signal (indicated by arrow), while cell number 13 has almost undetectable signal at the same location. Circular features are the microwells. The high motility protein signals are the original probed cleaved caspase 8 signal. E = 40 V cm^{-1}. (P-glycoprotein: Alexa Fluor 647-labeled secondary antibody).

Supplementary Figure 15



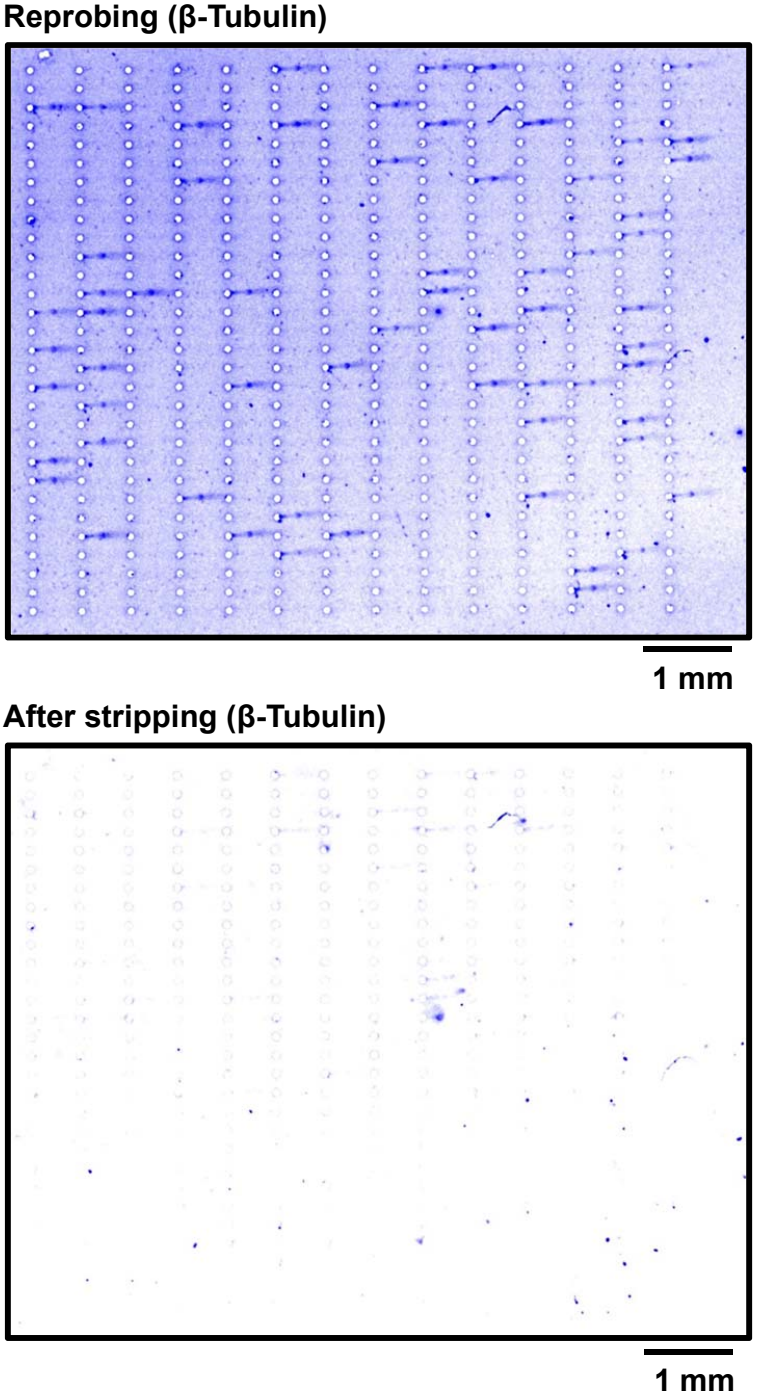
Supplementary Figure 15. Two distributions of P-gp signal defined a fluorescence threshold for low (< 0.1) and high (> 0.1) P-gp expression. All the P-gp signals are normalized to their corresponding β-Tubulin signals.

Supplementary Figure 16.



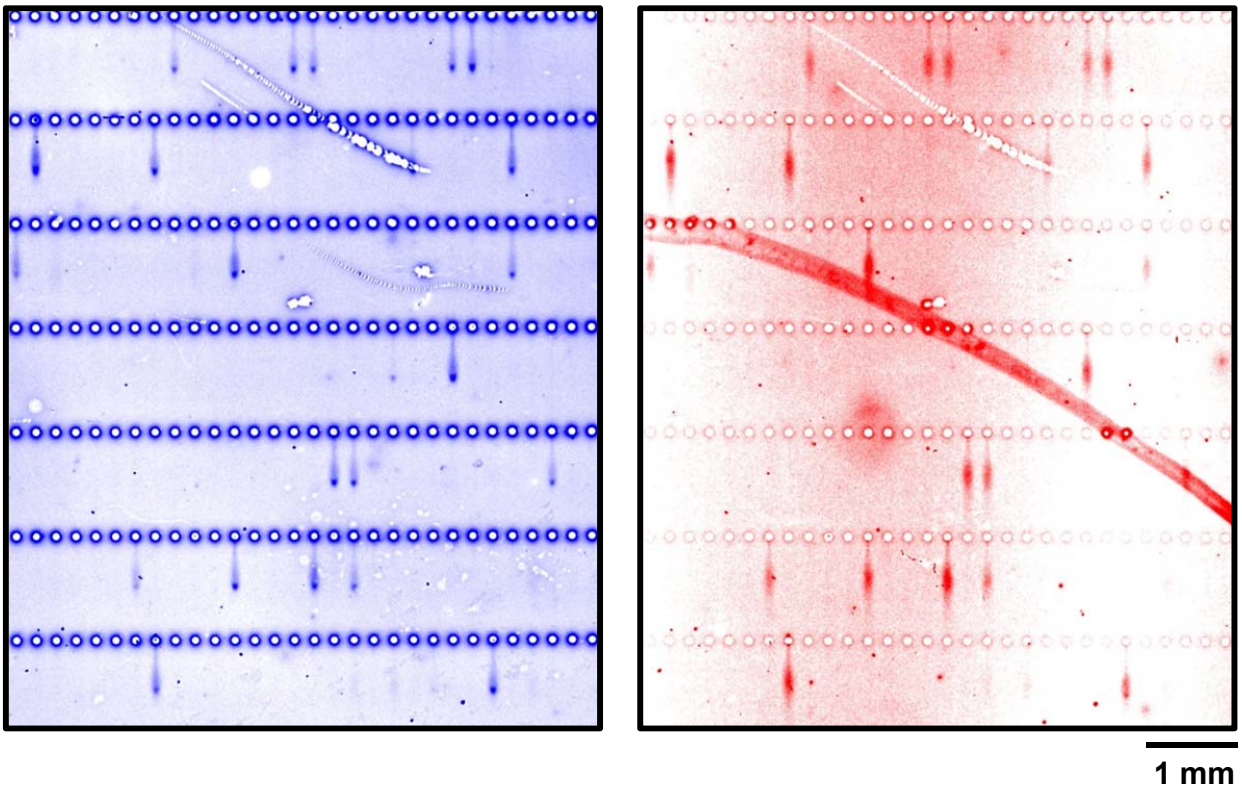
Supplementary Figure 16. *sc*Western blots of probed GAPDH signal (blue signal) before (top) and after 4 months storage (bottom) as presented in Figure 5B. There was no discernible difference in the signal between the original slide and slide stored for 4 months. Proteins were extracted by modified RIPA lysis/extraction/electrophoresis buffer for 25 s, and then analyzed by protein PAGE for 20 s (0.5-mm long separation distance, 12%T PA gel). Circular features are the microwells. E = 40 V cm^{-1}. (GAPDH: Alexa Fluor 647-labeled secondary antibody)

Supplementary Figure 17.



Supplementary Figure 17. *sc*Western blots of probed β–tubulin signal (blue signal) in a *sc*Western slide stored for 4 months as presented in Figure 5C. Both the signal and background were significantly reduced after stripping. Proteins were extracted by modified RIPA lysis/extraction/electrophoresis buffer for 25 s, and then analyzed by protein PAGE for 20 s (0.5-mm long separation distance, 12%T PA gel). Circular features are the microwells. E = 40 V cm^{-1}. (β–tubulin: Alexa Fluor 594-labeled secondary antibody).

Supplementary Figure 18



Supplementary Figure 18. *sc*Western blots of probed β-Tubulin (Left block; blue signal) and cleaved caspase 8 (Right block; red signal) in DNR-treated U373 MG cells. All the cells assayed in this block showed positive cleaved caspase 8 signal. Circular features are the microwells. Proteins were extracted by modified RIPA lysis/extraction/electrophoresis buffer for 20 s, and then analyzed by protein PAGE for 35 s (1-mm long separation distance, 10%T PA gel). E = 40 V cm^{-1}. (β-Tubulin: Alexa Fluor 594-labeled secondary antibody; cleaved caspase 8: Alexa Fluor 594-labeled secondary antibody; β-Tubulin was probed after stripping of cleaved caspase 8) .