Electronic Supplementary Information

# Damage-free evaluation of cultured cells based on multivariate analysis with a single-polymer probe

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## 1. Experimental procedures

### *Materials and instruments*

Human hepatocellular carcinoma cells (HepG2 and HuH7), human lung carcinoma cells (A549), and human normal fetal lung diploid fibroblasts (TIG1) were obtained from the Japanese Collection of Research Bioresources (Osaka, Japan). Human epidermoid carcinoma cells (A431), human cervix carcinoma cells (HeLa), and human breast carcinoma cells (MCF7 and MDA-MB-453) were obtained from RIKEN BioResource Center (Ibaraki, Japan). Dulbecco's modified eagle medium (DMEM) and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). The penicillin–streptomycin–neomycin antibiotic mixture and chemically defined Chinese hamster ovary (CD CHO) medium were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Fetal bovine serum (FBS) was purchased from GE Healthcare UK Ltd. (Buckinghamshire, UK). Tamoxifen citrate (TAM) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 96-Well clear-bottom black plates were purchased from Greiner Bio-One GmbH (Frickenhausen, Germany). 2-Morpholinoethanesulfonic acid (MES), L-glutamine, and Bradford reagent were purchased from Sigma-Aldrich, Co., LLC (St. Louis, MO, USA). Cell Counting Kit-8 and 3-[4-(2-hydroxyethyl)-1-piperazinyl]-propanesulfonic acid (EPPS) were purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). The Apo-ONE Homogeneous Caspase-3/7 Assay Kit was purchased from Promega Corp. (Madison, WI, USA). The PLL-Dnc polymer was synthesized according to a method from a previous study.[1] 384-Well non-binding surface microplates and 96-well half-volume plates were purchased from Corning, Inc. (Corning, NY, USA). The dispensing of solutions was performed using a PIPETMAX liquid handling system (Gilson, Inc., Middleton, WI, USA). Fluorescence spectra and intensities were recorded on a Cytation5 Imaging Reader (BioTek Instruments, Inc., Winooski, VT, USA).

### *Preparation of the cells*

The cells were prepared according to a method from one of our previous studies.[2] For the cell cultures, we used DMEM supplemented with 10% FBS, 0.5 mg/mL penicillin, 0.5 mg/mL streptomycin, and 1.0 mg/mL neomycin (DMEM++) or a serum-free CD CHO medium supplemented with 8 mM L-glutamine (CDCHO+). Unless otherwise mentioned, all incubations were conducted at 37 °C in humidified air with 5% $CO_2$. The culture media for the eight cell lines were prepared as follows: cells ($1.0 \times 10^3$ cells/well − $2.5 \times 10^4$ cells/well) in DMEM++ were seeded on a 96-well clear-bottom black plate and incubated for 24 h. After washing with DPBS (100 μL), the cells were incubated with CDCHO+ (100 μL) for 48 h. Culture media for the TAM-treated HepG2 cells were prepared as follows: HepG2 cells ($5.0 \times 10^4$ cells/well) in DMEM++ were seeded on a 96-well clear-bottom black plate and incubated for 24 h. After washing with DPBS (100 μL), the cells were incubated with 70 μM TAM in CDCHO+ containing 0.1% DMSO (100 μL) for 0.5–12 h. The obtained cells or culture media were used for analyses.

### *Measurement of fluorescence spectra*

In each well of a 384-well microplate, we prepared 60 μL of a mixture of 2 μg/mL PLL-Dnc, 10–50% of cell-culture medium or CDCHO+ alone, and 18 mM buffer solution [MES (pH = 5.5) or EPPS (pH = 8.5)]. After incubation at 35 °C for 10 min, the fluorescence spectra were recorded for two channels [$\lambda_{ex}$ (nm)/$\lambda_{em}$ (nm): 340/385–700 and 320/385–700].

***Measurement of fluorescence responses and statistical analysis of the pattern data***

To each well of a 384-well microplate, we added the following solutions: 20 μg/mL PLL-Dnc (6 μL), water (34.5, 22.5, or 10.5 μL), and 80 mM buffer solution [MES (pH = 5.5) or EPPS (pH = 8.5); 13.5 μL]. After incubation at 35 °C for 10 min, the fluorescence intensities were measured on two channels [$\lambda_{ex}$ (nm)/$\lambda_{em}$ (nm): 340/480 (Ch1) and 320/520 (Ch2)]. Then, cell-culture medium (6, 18, or 30 μL) was added to each well to give a final volume of 60 μL. The final contents were as follows: 2.0 μg/mL PLL-Dnc, 18 mM buffer solution, and 10%, 30%, or 50% cell-culture medium. After incubation at 35 °C for 10 min, the fluorescence intensities were measured again. The fluorescence responses are presented as $F - F_0$, where $F_0$ and $F$ refer to the fluorescence intensities before and after addition of the cell-culture medium, respectively. This process was repeated five or six times to generate a dataset. The dataset was processed via classical linear discriminant analysis (LDA) and hierarchical clustering analysis (HCA) using the SYSTAT software package (version 13; Systat Inc., San Jose, CA, USA).

***Quantification of the total protein concentration***

In a well of a 96-well half-volume plate, cell culture medium (15 μL) or standard solution (15 μL) were mixed with the working reagent (1:1 solution of Bradford reagent and water; 170 μL) and incubated at 5 min under shaded conditions. The total protein concentration was determined based on the absorbance at 595 nm using a calibration curve prepared using a bovine serum albumin standard solution.

***Determination of the cell viability***

The cells were washed with DPBS (100 μL) and then fresh CDCHO+ (50 μL) and Cell Counting Kit-8 working reagent (10 μL) were added. After incubation at 37 °C in humidified air with 5% $CO_2$ for 3 h, 10 mg/mL sodium dodecyl sulfate (10 μL) was added to stop the assay. The cell viability was determined based on the absorbance at 450 nm, and the cell viability of the TAM-untreated control cells was considered to be 100%.

***Apoptotic cell detection***

The cells were washed with DPBS (100 μL) before fresh CDCHO+ (50 μL) and Apo-ONE Homogeneous Caspase-3/7 Assay Kit (50 μL) working reagent were added. After incubation at room temperature for 3 h, the fluorescence intensities of the mixture were recorded at excitation and emission wavelengths of 499 and 521 nm, respectively.

## 2. Supporting tables and figures

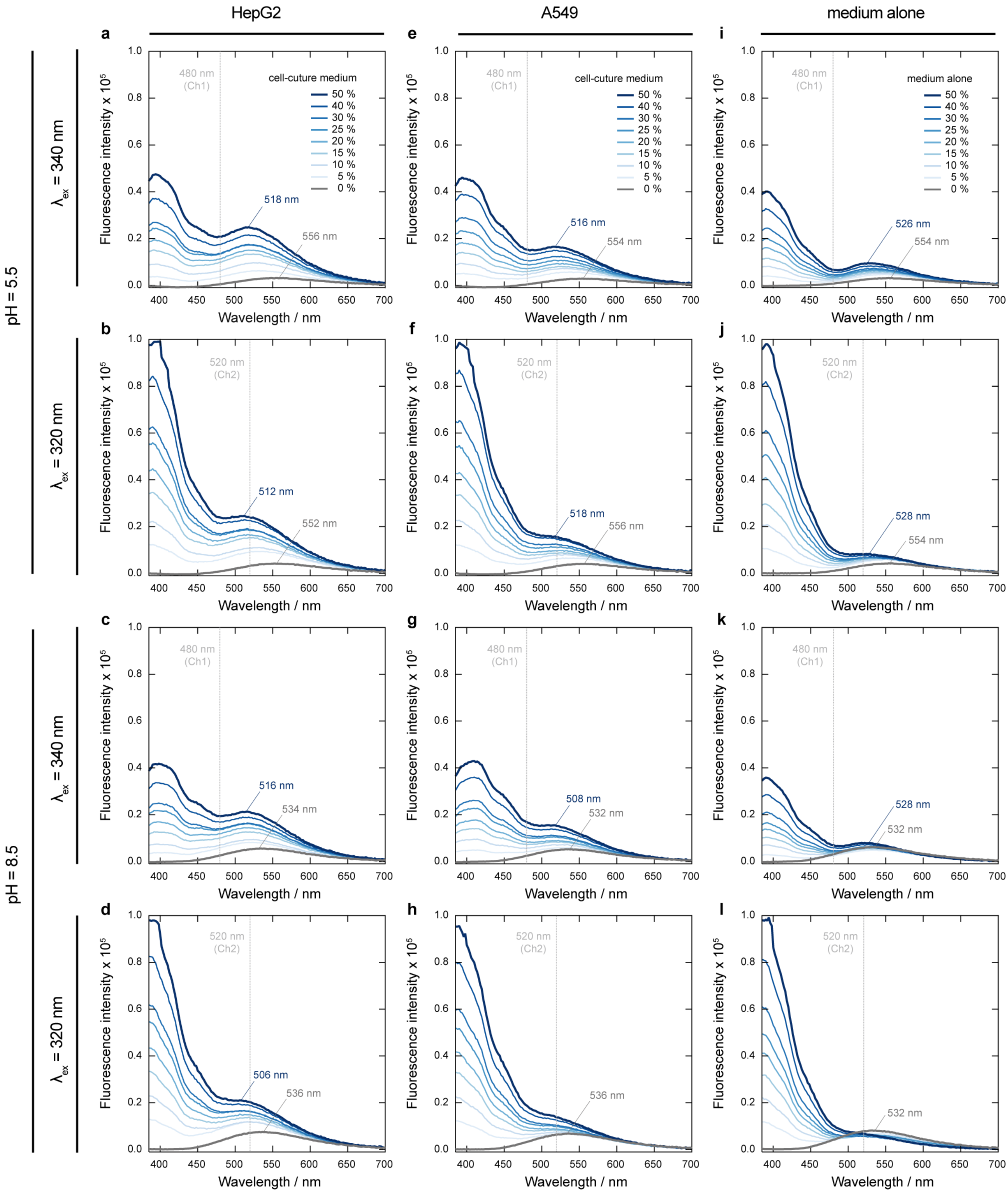


**Fig. S1** Fluorescence spectra of the mixture of PLL-Dnc (2.0 μg/mL), 0–50% cell-culture media of HepG2 (**a**–**d**), A549 (**f**–**h**), or CDCHO+ alone (**i**–**l**) in 18 mM MES (pH = 5.5; **a**, **b**, **e**, **f**, **i**, **j**) or 18 mM EPPS (pH = 8.5; **c**, **d**, **g**, **h**, **k**, **l**). $\lambda_{ex}$ (nm)/$\lambda_{em}$ (nm): 340/385–700 (**a**, **c**, **e**, **g**, **i**, **k**) and 320/385–700 (**b**, **d**, **f**, **h**, **j**, **l**). All spectra represent the average of three scans. The fluorescence peaks at ~400 and 500–560 nm are derived from autofluorescence of CDCHO+ medium and dansyl moiety,[3] respectively.

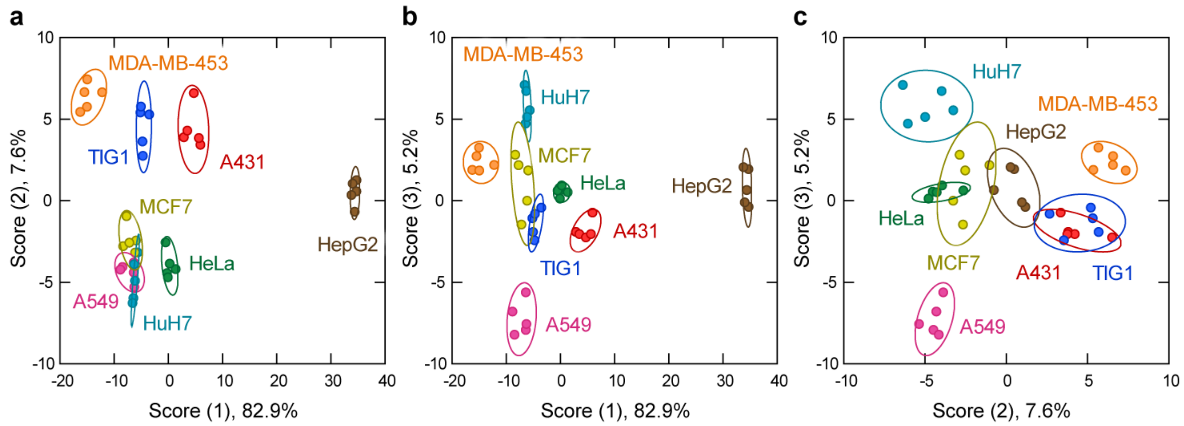


**Fig. S2** 2D discriminant score plots. (**a**) Score (1) vs. score (2). (**b**) Score (1) vs. score (3). (**c**) Score (2) vs. score (3). Ellipses represent confidence intervals ± 1 SD for the individual analytes.

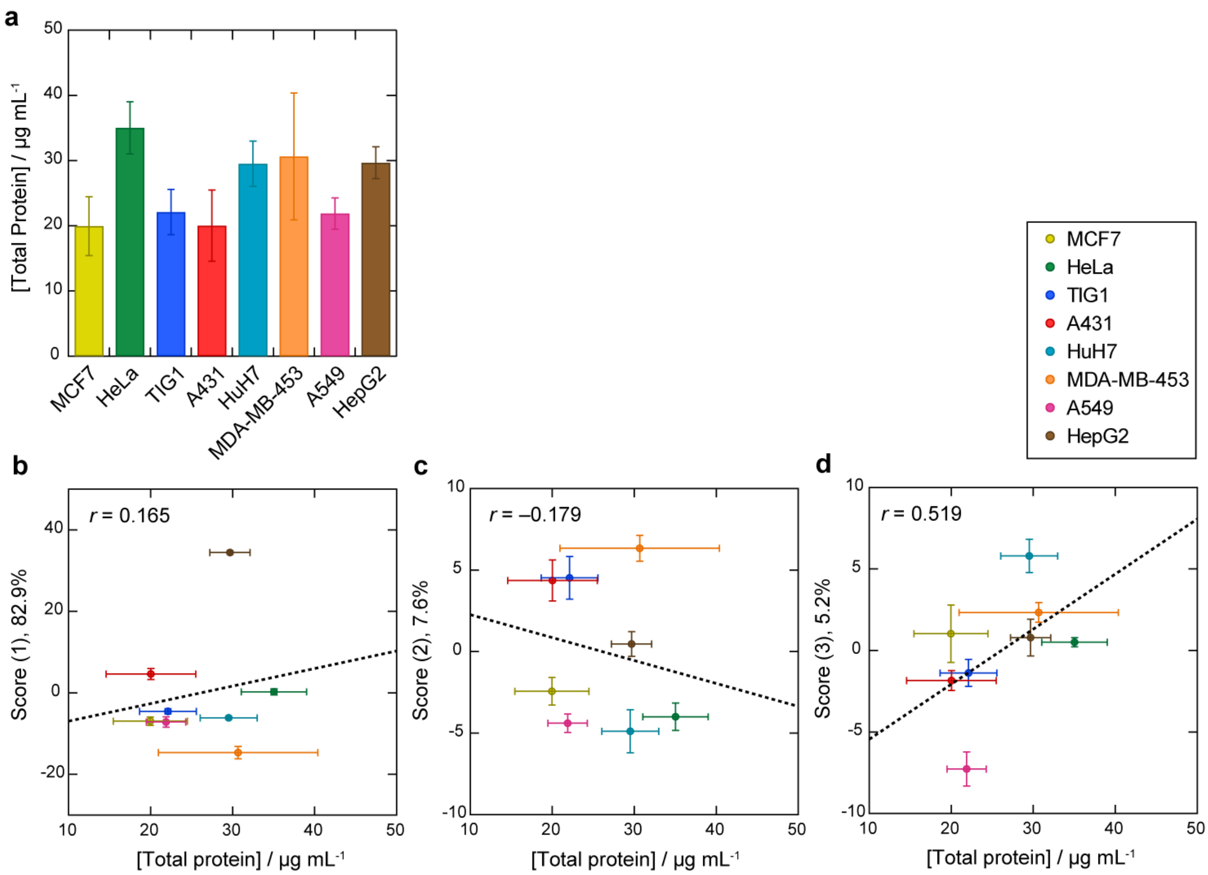


**Fig. S3** (**a**) Total protein concentrations in cell-culture media for each cell line. Values shown are mean values ± SD (*n* = 6). Plots of discriminant scores [(**b**) score (1), (**c**) score (2), and (**d**) score (3)] as a function of total protein concentration.

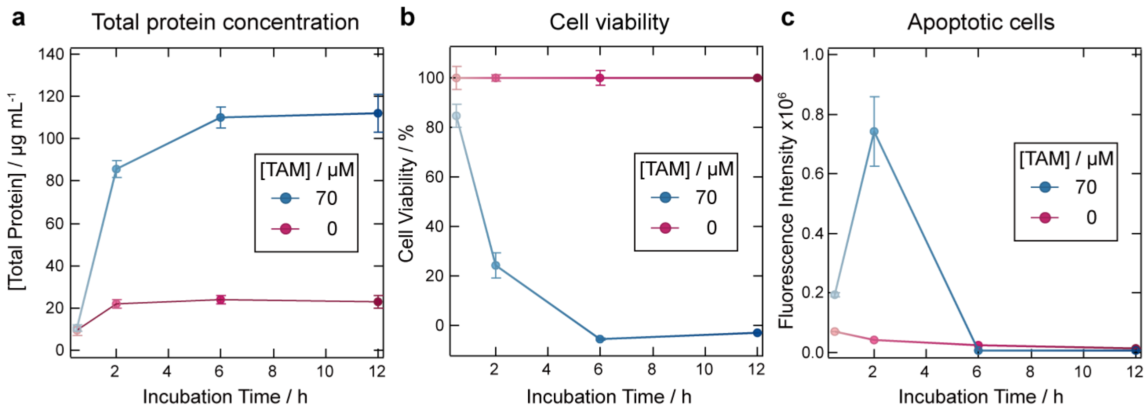


**Fig. S4** Cell-based assays of HepG2 cells both exposed and not exposed to 70 µM TAM for 0.5–12 h. (**a**) The total protein concentration in cell-culture medium, (**b**) cell viability, and (**c**) apoptotic cells. In the caspase-3/7 assay, the observed fluorescence intensities are proportional to the quantity of apoptotic cells. Values shown refer to mean values ± SD [$n = 6$ for (a); $n = 3$ for (b) and (c)].

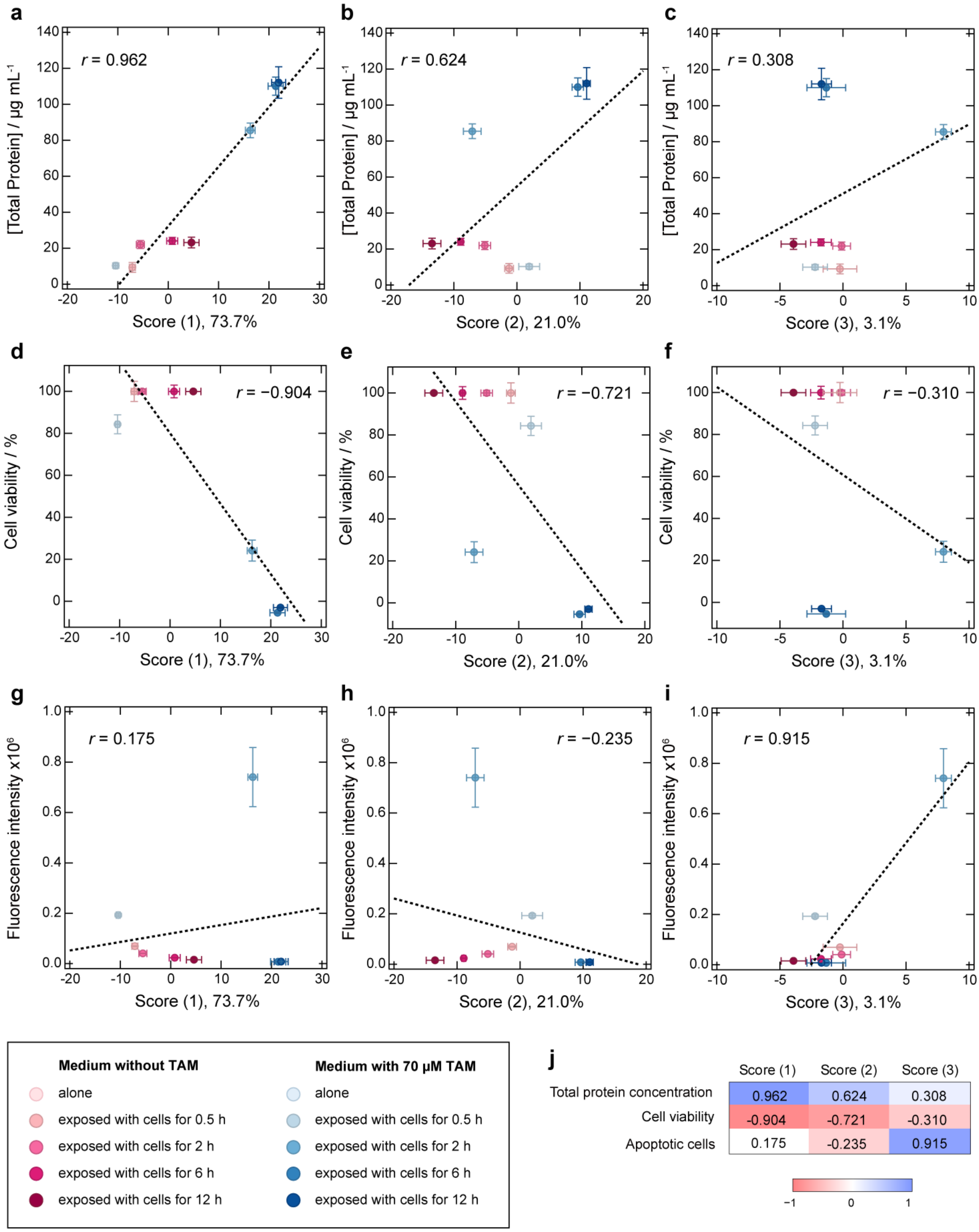


**Fig. S5** Plots of discriminant scores [(**a**, **d**, **g**) score (1), (**b**, **e**, **h**) score (2), and (**c**, **f**, **i**) score (3)] as a function of (**a**–**c**) total protein concentration, (**d**–**f**) cell viability, or (**g**–**i**) fluorescence intensity that is proportional to the quantity of apoptotic cells. (**j**) Summary of correlation coefficients between discriminant scores and total protein concentration, cell viability, or apoptotic cells. The values indicate correlation coefficients.

**Table S1** Dataset of the fluorescence response patterns obtained from the sensing of eight cell-culture media.

| Analyte | $F - F_0$ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **10% cell-culture medium** | | | | **30% cell-culture medium** | | | | **50% cell-culture medium** | | | |
| | **pH = 5.5** | | **pH = 8.5** | | **pH = 5.5** | | **pH = 8.5** | | **pH = 5.5** | | **pH = 8.5** | |
| | **Ch1** | **Ch2** | **Ch1** | **Ch2** | **Ch1** | **Ch2** | **Ch1** | **Ch2** | **Ch1** | **Ch2** | **Ch1** | **Ch2** |
| MCF7 | 1432.0 | 1012.4 | 1094.8 | 243.2 | 3642.8 | 2493.4 | 3160.0 | 1347.2 | 5433.2 | 3672.2 | 4978.4 | 2428.6 |
| MCF7 | 1297.8 | 823.8 | 893.2 | -14.8 | 3455.6 | 2234.0 | 2973.4 | 1110.6 | 5213.6 | 3353.6 | 4783.0 | 2311.8 |
| MCF7 | 1243.4 | 842.6 | 797.0 | -80.6 | 3297.2 | 2230.6 | 2852.2 | 1092.8 | 4951.8 | 3261.8 | 4564.4 | 2145.4 |
| MCF7 | 1203.0 | 804.4 | 661.4 | -170.6 | 3195.4 | 2073.0 | 2675.2 | 892.8 | 4805.8 | 3102.2 | 4444.2 | 1978.2 |
| MCF7 | 1316.8 | 881.6 | 1023.2 | 141.2 | 3301.2 | 2173.0 | 2942.8 | 1258.8 | 5057.0 | 3310.8 | 4567.2 | 2070.6 |
| HeLa | 1974.2 | 1436.4 | 1645.2 | 577.0 | 4458.6 | 3400.0 | 3984.6 | 2008.4 | 6454.0 | 4746.2 | 6238.4 | 3394.8 |
| HeLa | 1827.8 | 1396.2 | 1473.0 | 383.8 | 4276.6 | 3154.6 | 3707.8 | 1743.2 | 6021.8 | 4434.6 | 5693.2 | 3064.4 |
| HeLa | 1739.4 | 1280.2 | 1406.8 | 343.0 | 4061.6 | 3143.0 | 3620.4 | 1770.2 | 5862.0 | 4298.4 | 5603.4 | 3121.2 |
| HeLa | 1736.0 | 1338.0 | 1203.8 | 265.8 | 4094.4 | 3015.0 | 3437.6 | 1574.6 | 5803.4 | 4210.2 | 5431.6 | 2906.6 |
| HeLa | 1842.0 | 1411.2 | 1365.6 | 431.4 | 3985.0 | 3143.2 | 3611.2 | 1768.4 | 5895.2 | 4346.6 | 5569.0 | 2991.6 |
| TIG1 | 977.6 | 595.0 | 992.0 | 214.0 | 2918.4 | 1893.6 | 2494.6 | 909.6 | 4445.2 | 2844.6 | 4018.0 | 1776.6 |
| TIG1 | 1205.6 | 735.0 | 866.0 | 145.0 | 2824.6 | 1832.4 | 2343.8 | 788.2 | 4218.0 | 2666.8 | 3775.4 | 1654.8 |
| TIG1 | 1115.6 | 689.4 | 840.8 | 11.0 | 2649.6 | 1749.4 | 2290.4 | 723.2 | 4096.2 | 2609.0 | 3582.8 | 1509.0 |
| TIG1 | 1146.6 | 757.2 | 812.8 | -13.8 | 2711.0 | 1791.6 | 2152.8 | 659.4 | 4189.8 | 2630.8 | 3682.8 | 1667.4 |
| TIG1 | 1157.6 | 777.8 | 943.0 | 194.0 | 2550.4 | 1727.4 | 2369.6 | 932.0 | 4041.0 | 2648.4 | 3649.4 | 1656.2 |
| A431 | 1375.4 | 990.4 | 1182.2 | 380.4 | 3531.0 | 2524.8 | 2861.8 | 1184.6 | 5287.0 | 3807.4 | 4597.8 | 2396.6 |
| A431 | 1313.8 | 897.6 | 1070.8 | 187.6 | 3098.8 | 2186.6 | 2585.8 | 934.0 | 4707.8 | 3301.6 | 4237.6 | 2206.6 |
| A431 | 1204.4 | 851.6 | 990.0 | 203.6 | 2855.6 | 1959.6 | 2442.8 | 828.2 | 4640.2 | 3278.8 | 3948.0 | 1923.8 |
| A431 | 1262.2 | 902.6 | 928.4 | 137.8 | 3005.8 | 2105.8 | 2327.4 | 779.6 | 4566.8 | 3189.8 | 3869.0 | 1869.4 |
| A431 | 1368.6 | 952.2 | 1003.0 | 185.0 | 3072.2 | 2245.8 | 2588.0 | 1029.6 | 4668.8 | 3368.6 | 4092.0 | 2093.2 |
| HuH7 | 1447.8 | 1037.2 | 1445.4 | 596.8 | 3564.8 | 2488.4 | 3412.6 | 1769.8 | 5348.6 | 3682.8 | 5179.0 | 2822.8 |
| HuH7 | 1378.4 | 985.8 | 1333.0 | 537.8 | 3408.0 | 2312.6 | 3252.0 | 1674.0 | 5102.2 | 3457.8 | 4880.4 | 2744.0 |
| HuH7 | 1417.0 | 974.2 | 1434.4 | 634.2 | 3112.8 | 2122.4 | 3021.8 | 1514.6 | 5013.2 | 3363.4 | 4794.4 | 2640.2 |
| HuH7 | 1331.2 | 946.0 | 1245.0 | 521.6 | 3255.4 | 2244.2 | 2984.6 | 1521.0 | 4942.2 | 3247.0 | 4632.2 | 2520.4 |
| HuH7 | 1464.6 | 1019.6 | 1403.6 | 667.8 | 3227.4 | 2283.6 | 3195.6 | 1669.6 | 4868.0 | 3338.2 | 4778.8 | 2705.0 |
| MDA-MB-453 | 1381.6 | 824.6 | 768.2 | -222.4 | 3118.8 | 1798.8 | 2358.2 | 558.2 | 4625.4 | 2573.6 | 4259.8 | 1675.6 |
| MDA-MB-453 | 1363.6 | 882.4 | 1120.0 | 239.4 | 3016.4 | 1822.6 | 2559.8 | 829.0 | 4506.2 | 2766.6 | 4054.4 | 1763.6 |
| MDA-MB-453 | 1139.8 | 666.0 | 972.8 | 98.4 | 2839.8 | 1607.2 | 2346.0 | 776.8 | 4116.0 | 2283.6 | 3748.6 | 1478.6 |
| MDA-MB-453 | 1193.8 | 722.2 | 839.0 | 103.2 | 2864.4 | 1687.4 | 2308.2 | 606.8 | 4146.2 | 2343.8 | 3643.4 | 1416.0 |
| MDA-MB-453 | 1312.2 | 885.0 | 1170.6 | 310.2 | 3125.6 | 1922.2 | 2647.2 | 1061.8 | 4367.6 | 2605.6 | 4018.6 | 1697.2 |
| A549 | 1019.0 | 499.6 | 684.6 | -315.0 | 2700.2 | 1716.4 | 2601.6 | 800.2 | 4329.4 | 2807.0 | 4468.0 | 2016.8 |
| A549 | 1003.2 | 568.4 | 705.2 | -284.8 | 2744.2 | 1692.6 | 2564.0 | 726.0 | 4208.6 | 2718.4 | 4410.6 | 1989.2 |
| A549 | 1049.2 | 631.2 | 790.6 | -169.4 | 2855.6 | 1942.4 | 2781.8 | 1023.0 | 4454.4 | 2992.2 | 4526.4 | 2047.0 |
| A549 | 1111.4 | 667.8 | 900.6 | 20.4 | 2843.2 | 1988.4 | 2809.8 | 1048.2 | 4443.6 | 2921.0 | 4548.6 | 2076.6 |
| A549 | 1011.8 | 600.6 | 738.8 | -123.6 | 2806.6 | 1835.0 | 2575.2 | 853.6 | 4274.4 | 2776.6 | 4318.2 | 2040.2 |
| HepG2 | 2164.8 | 2036.8 | 2025.2 | 1330.4 | 4587.8 | 4193.0 | 3654.2 | 2556.0 | 6115.6 | 5476.2 | 5405.2 | 3878.8 |
| HepG2 | 1857.6 | 1684.0 | 1760.4 | 1068.2 | 3966.6 | 3578.8 | 3287.0 | 2119.0 | 5713.4 | 5084.8 | 5014.2 | 3662.0 |
| HepG2 | 1823.0 | 1699.8 | 1779.0 | 1090.8 | 4108.8 | 3754.0 | 3385.8 | 2211.2 | 5800.0 | 5195.8 | 4906.0 | 3450.0 |
| HepG2 | 1851.4 | 1691.8 | 1787.4 | 1197.4 | 3904.4 | 3554.8 | 3407.0 | 2222.6 | 5864.0 | 5239.8 | 4816.4 | 3396.6 |
| HepG2 | 1624.2 | 1465.4 | 1741.6 | 1069.8 | 3968.8 | 3572.4 | 3334.2 | 2231.8 | 5571.4 | 4947.6 | 4725.8 | 3334.4 |

**Table S2** Identification accuracies determined by a leave-one-out cross-validation (LOOCV) test in the sensing of eight cell-culture media.

| Sensor elements* | 10% cell-culture medium | | | | 30% cell-culture medium | | | | 50% cell-culture medium | | | | Accuracy % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH = 5.5 | | pH = 8.5 | | pH = 5.5 | | pH = 8.5 | | pH = 5.5 | | pH = 8.5 | | |
| | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | |
| 12 sensor elements | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 100 |
| 8 sensor elements | | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 98 |
| | ■ | ■ | ■ | ■ | | | | | ■ | ■ | ■ | ■ | 98 |
| | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | | | 98 |
| 6 sensor elements | ■ | ■ | | | ■ | ■ | | | ■ | ■ | | | 90 |
| | | ■ | | ■ | | ■ | | ■ | | ■ | | ■ | 88 |
| | ■ | | ■ | | ■ | | ■ | | ■ | | ■ | | 88 |
| | | | ■ | ■ | | | ■ | ■ | | | ■ | ■ | 88 |
| 4 sensor elements | | | | | | | | | ■ | ■ | ■ | ■ | 98 |
| | | | | | ■ | ■ | ■ | ■ | | | | | 98 |
| | ■ | ■ | | | | | | | ■ | ■ | | | 93 |
| | | | | | ■ | | ■ | | ■ | | ■ | | 88 |
| | | | | | | | ■ | ■ | | | ■ | ■ | 88 |
| | ■ | ■ | | | ■ | ■ | | | | | | | 88 |
| | | | | | ■ | ■ | | | ■ | ■ | | | 85 |
| | | ■ | | ■ | | | | | | ■ | | ■ | 85 |
| | | | | | | ■ | | ■ | | ■ | | ■ | 83 |
| | ■ | | ■ | | | | | | ■ | | ■ | | 83 |
| | | | ■ | ■ | | | | | | | ■ | ■ | 83 |
| | | ■ | | ■ | | ■ | | ■ | | | | | 83 |
| | ■ | | ■ | | ■ | | ■ | | | | | | 75 |
| | | | ■ | ■ | | | ■ | ■ | | | | | 73 |
| | ■ | ■ | ■ | ■ | | | | | | | | | 70 |
| 3 sensor elements | | | | ■ | | | | ■ | | | | ■ | 68 |
| | | ■ | | | | ■ | | | | ■ | | | 68 |
| | ■ | | | | ■ | | | | ■ | | | | 65 |
| | | | ■ | | | | ■ | | | | ■ | | 63 |
| 2 sensor elements | | | | | | | | | | ■ | | ■ | 88 |
| | | | | | | | | | | | ■ | ■ | 88 |
| | | | | | | | | | ■ | ■ | | | 88 |
| | | | | | | | | | ■ | | ■ | | 85 |
| | | ■ | | | | | | | | ■ | | | 73 |
| | | | | | | | ■ | ■ | | | | | 73 |
| | | ■ | | ■ | | | | | | | | | 73 |
| | | | ■ | | | | | | | | ■ | | 70 |
| | ■ | | | | | | | | ■ | | | | 68 |
| | | | | | ■ | ■ | | | | | | | 68 |
| | | | ■ | | | | ■ | | | | | | 65 |
| | | ■ | | | | ■ | | | | | | | 65 |
| | | | | | ■ | | ■ | | | | | | 65 |
| | | | | | | ■ | | | | ■ | | | 63 |
| | | | | ■ | | | | ■ | | | | | 63 |
| | ■ | | ■ | | | | | | | | | | 63 |
| | | | ■ | ■ | | | | | | | | | 63 |
| | ■ | ■ | | | | | | | | | | | 63 |
| | | | | ■ | | | | | | | | ■ | 60 |
| | | | | | | ■ | | ■ | | | | | 58 |
| | | | | | | | ■ | | | | ■ | | 53 |
| | | | | | | | | ■ | | | | ■ | 50 |
| | ■ | | | | ■ | | | | | | | | 50 |
| | | | | | ■ | | | | ■ | | | | 45 |

*We examined all combinations of sensor elements consisting of 3 cell-culture-medium-content values, 2 pH values, and 2 channels, i.e., $\{(\sum_{i=1}^{3} {}_{3}C_{i})\cdot(\sum_{j=1}^{2} {}_{2}C_{j})\cdot(\sum_{k=1}^{2} {}_{2}C_{k})\} - ({}_{3}C_{1}\cdot{}_{2}C_{1}\cdot{}_{2}C_{1}) = 51$ combinations. We didn't examine combinations consisting of only one sensor element.

**Table S3** Dataset of the fluorescence response patterns obtained from the sensing of culture media of HepG2 cells both exposed and not exposed to 70 μM TAM.

| Analyte** | $F - F_0$ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10% cell-culture medium | | | | 30% cell-culture medium | | | | 50% cell-culture medium | | | |
| | pH = 5.5 | | pH = 8.5 | | pH = 5.5 | | pH = 8.5 | | pH = 5.5 | | pH = 8.5 | |
| | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 |
| medium alone | 419.6 | 6.8 | 39.4 | -606.6 | 1201.8 | 513.0 | 869.4 | -327.2 | 1985.0 | 980.0 | 1903.6 | 484.6 |
| medium alone | 406.4 | 97.8 | 110.6 | -502.0 | 1171.4 | 546.8 | 888.4 | -321.6 | 1980.6 | 981.0 | 1862.0 | 460.2 |
| medium alone | 412.4 | 80.6 | -38.2 | -727.4 | 1137.2 | 407.8 | 776.4 | -383.6 | 1881.2 | 891.6 | 1815.2 | 371.8 |
| medium alone | 429.6 | 84.8 | -109.4 | -762.4 | 1171.4 | 483.4 | 850.0 | -282.4 | 1912.8 | 969.0 | 1843.4 | 401.0 |
| medium alone | 409.0 | 71.4 | 120.6 | -482.6 | 1187.2 | 494.4 | 706.0 | -497.6 | 1887.8 | 999.0 | 1766.0 | 339.0 |
| medium alone | 405.8 | 116.4 | 61.6 | -585.6 | 1184.0 | 488.4 | 860.8 | -219.8 | 1891.2 | 923.2 | 1803.2 | 377.8 |
| −/0.5 h | 973.0 | 750.4 | 696.8 | 114.0 | 2147.0 | 1686.6 | 1450.6 | 451.6 | 3198.0 | 2469.0 | 2384.6 | 1133.6 |
| −/0.5 h | 1072.6 | 892.8 | 678.6 | 49.4 | 2320.4 | 1886.2 | 1466.6 | 424.2 | 3366.6 | 2666.8 | 2364.2 | 1096.4 |
| −/0.5 h | 989.8 | 758.6 | 381.4 | -239.2 | 2221.8 | 1688.8 | 1174.4 | -4.2 | 3272.2 | 2545.2 | 2172.0 | 907.8 |
| −/0.5 h | 1025.8 | 757.8 | 768.6 | 191.6 | 2317.2 | 1757.2 | 1513.6 | 519.0 | 3304.8 | 2602.6 | 2238.8 | 945.8 |
| −/0.5 h | 1001.6 | 809.2 | 652.6 | 95.6 | 2257.6 | 1747.2 | 1442.6 | 390.4 | 3228.2 | 2495.6 | 2237.2 | 924.8 |
| −/0.5 h | 1014.6 | 829.4 | 723.6 | 221.6 | 2244.4 | 1785.0 | 1482.2 | 407.0 | 3218.0 | 2502.6 | 2235.8 | 967.8 |
| −/2 h | 992.0 | 714.6 | 548.6 | -181.4 | 2410.4 | 1824.8 | 1587.4 | 529.2 | 3563.2 | 2821.6 | 2416.0 | 1138.4 |
| −/2 h | 1005.0 | 720.6 | 467.4 | -166.4 | 2306.6 | 1708.8 | 1396.8 | 257.8 | 3420.0 | 2628.8 | 2521.0 | 1142.2 |
| −/2 h | 972.6 | 792.2 | 530.2 | -104.0 | 2299.8 | 1865.6 | 1503.4 | 444.2 | 3461.0 | 2862.4 | 2421.2 | 1143.4 |
| −/2 h | 979.6 | 795.6 | 534.6 | -46.4 | 2258.8 | 1843.0 | 1488.0 | 536.2 | 3380.4 | 2756.4 | 2361.8 | 1133.2 |
| −/2 h | 1006.8 | 836.6 | 558.6 | -27.8 | 2317.6 | 1962.8 | 1498.8 | 562.6 | 3419.6 | 2902.0 | 2352.6 | 1199.2 |
| −/2 h | 1004.8 | 838.4 | 509.2 | 10.0 | 2317.6 | 1948.8 | 1381.0 | 442.0 | 3473.2 | 2839.4 | 2384.2 | 1198.0 |
| −/6 h | 1278.2 | 1010.8 | 643.4 | 21.2 | 2653.6 | 2195.6 | 1910.6 | 848.2 | 4079.4 | 3484.4 | 2853.8 | 1722.8 |
| −/6 h | 1171.0 | 895.8 | 692.0 | 27.8 | 2692.6 | 2204.4 | 1855.6 | 744.6 | 3970.2 | 3341.2 | 2886.0 | 1653.8 |
| −/6 h | 1130.4 | 941.6 | 778.2 | 140.8 | 2719.6 | 2304.4 | 1859.0 | 894.0 | 3961.4 | 3442.6 | 2734.4 | 1588.0 |
| −/6 h | 1229.0 | 1073.6 | 731.6 | 189.4 | 2800.2 | 2426.0 | 1885.2 | 988.2 | 3985.6 | 3564.8 | 2797.0 | 1784.6 |
| −/6 h | 1043.4 | 817.8 | 690.6 | 115.8 | 2085.2 | 1999.2 | 1774.8 | 822.2 | 3757.2 | 3245.8 | 2723.6 | 1721.0 |
| −/6 h | 1127.2 | 1005.4 | 806.6 | 211.8 | 2666.4 | 2320.0 | 1858.8 | 906.0 | 3966.4 | 3464.4 | 2699.8 | 1750.8 |
| −/12 h | 1364.0 | 1239.6 | 1018.0 | 537.4 | 2970.8 | 2711.6 | 2230.2 | 1279.0 | 4245.8 | 3979.4 | 3308.0 | 2277.4 |
| −/12 h | 1318.4 | 1148.8 | 1063.6 | 457.4 | 4634.4 | 2896.8 | 2289.6 | 1247.0 | 4545.8 | 4099.4 | 3434.2 | 2299.2 |
| −/12 h | 1017.4 | 887.0 | 1052.2 | 465.2 | 3021.4 | 2631.0 | 2131.8 | 1181.0 | 4394.6 | 3822.4 | 3255.0 | 2143.4 |
| −/12 h | 1167.2 | 1021.2 | 944.4 | 339.8 | 2796.8 | 2518.8 | 2033.2 | 1093.6 | 4201.4 | 3768.8 | 3195.4 | 2160.0 |
| −/12 h | 1345.8 | 1203.6 | 1107.6 | 537.4 | 2967.0 | 2733.6 | 2253.4 | 1393.0 | 4450.2 | 4133.0 | 3385.2 | 2384.6 |
| −/12 h | 1344.4 | 1192.2 | 1038.4 | 565.2 | 3064.8 | 2727.2 | 2245.4 | 1395.0 | 4492.8 | 4074.2 | 3272.4 | 2271.2 |
| medium with TAM | 414.0 | -25.4 | 202.6 | -492.2 | 1214.6 | 321.2 | 868.4 | -331.4 | 2016.4 | 797.2 | 1980.6 | 528.8 |
| medium with TAM | 420.4 | 55.0 | -23.2 | -762.2 | 1148.4 | 327.6 | 733.0 | -558.8 | 1962.2 | 819.4 | 1695.0 | 243.0 |
| medium with TAM | 439.2 | 23.0 | 71.6 | -650.6 | 1157.4 | 279.0 | 740.2 | -538.6 | 1886.8 | 759.0 | 1703.2 | 221.2 |
| medium with TAM | 434.4 | 24.4 | 60.8 | -630.4 | 1173.6 | 303.4 | 748.8 | -475.4 | 1924.2 | 822.6 | 1782.0 | 324.4 |
| medium with TAM | 453.8 | 51.6 | 127.2 | -507.6 | 1187.4 | 353.6 | 849.0 | -292.8 | 1906.0 | 847.6 | 1739.4 | 329.6 |
| medium with TAM | 431.2 | 90.8 | 97.8 | -574.4 | 1155.8 | 301.4 | 703.4 | -525.4 | 1913.0 | 854.8 | 1700.4 | 273.4 |
| +/0.5 h | 890.0 | 553.6 | 309.8 | -337.4 | 1988.6 | 1252.4 | 1390.2 | 325.2 | 2915.2 | 1920.4 | 2240.8 | 893.8 |
| +/0.5 h | 942.8 | 595.8 | 324.4 | -289.8 | 2087.8 | 1387.2 | 1265.4 | 132.6 | 3072.2 | 2103.4 | 2406.6 | 1064.8 |
| +/0.5 h | 911.0 | 632.8 | 176.4 | -468.4 | 2006.6 | 1359.0 | 1460.8 | 449.2 | 2955.0 | 2093.4 | 2205.6 | 866.0 |
| +/0.5 h | 815.8 | 557.6 | 417.8 | -154.4 | 1832.2 | 1271.0 | 1443.8 | 385.6 | 2849.8 | 1987.2 | 2232.2 | 940.8 |
| +/0.5 h | 925.0 | 668.2 | 488.8 | -142.0 | 1994.8 | 1353.8 | 1444.6 | 393.2 | 2807.6 | 1986.2 | 2272.8 | 1037.4 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| +/0.5 h | 914.4 | 675.4 | 330.6 | -281.4 | 1954.0 | 1362.8 | 1422.8 | 311.4 | 2844.6 | 2015.2 | 2185.8 | 939.8 |
| +/2 h | 1912.8 | 1792.0 | 1396.0 | 809.2 | 3810.8 | 3633.8 | 2123.2 | 1331.0 | 5153.0 | 5173.2 | 3782.6 | 3218.6 |
| +/2 h | 1853.4 | 1721.0 | 1427.8 | 720.0 | 3736.2 | 3575.8 | 2126.4 | 1405.2 | 5083.0 | 5162.0 | 3647.0 | 3016.4 |
| +/2 h | 1897.6 | 1864.2 | 1541.6 | 906.2 | 3771.6 | 3796.2 | 2285.8 | 1634.6 | 5104.4 | 5447.4 | 3733.6 | 3273.0 |
| +/2 h | 1886.0 | 1913.6 | 1566.6 | 957.2 | 3991.8 | 4177.8 | 2366.8 | 1812.8 | 5012.8 | 5450.8 | 3792.8 | 3509.8 |
| +/2 h | 1847.8 | 1888.4 | 1506.6 | 976.6 | 3806.8 | 3932.8 | 2232.8 | 1666.0 | 4946.4 | 5219.6 | 3743.0 | 3465.2 |
| +/2 h | 1769.8 | 1751.6 | 1208.8 | 694.8 | 3207.6 | 3111.6 | 2024.2 | 1332.0 | 4661.4 | 4926.2 | 3288.8 | 2940.2 |
| +/2 h | 2504.2 | 2613.8 | 1901.2 | 1622.8 | 4403.6 | 4853.6 | 3181.0 | 3246.2 | 5020.2 | 5409.8 | 4299.8 | 4285.6 |
| +/6 h | 2643.2 | 2685.8 | 2018.4 | 1459.2 | 4753.6 | 5192.8 | 3208.6 | 2979.0 | 5392.2 | 5739.4 | 4537.8 | 4356.4 |
| +/6 h | 2469.6 | 2553.4 | 1917.6 | 1360.4 | 4458.2 | 4964.6 | 3177.6 | 2957.4 | 5063.6 | 5410.2 | 4194.8 | 4005.2 |
| +/6 h | 2608.8 | 2622.0 | 1784.0 | 1439.4 | 4452.2 | 4952.4 | 3079.0 | 2866.8 | 5026.8 | 5250.2 | 3969.2 | 3739.8 |
| +/6 h | 2708.6 | 2777.4 | 1912.6 | 1490.8 | 4676.2 | 5301.4 | 3447.0 | 3408.0 | 5228.6 | 5570.0 | 4451.8 | 4274.6 |
| +/6 h | 2620.2 | 2694.8 | 1986.8 | 1580.6 | 4695.0 | 5289.6 | 3327.2 | 3290.6 | 5314.4 | 5696.2 | 4463.2 | 4420.6 |
| +/12 h | 2584.0 | 2657.8 | 2151.0 | 1745.4 | 4510.2 | 5092.0 | 3435.2 | 3395.8 | 5144.8 | 5461.8 | 4390.6 | 4314.2 |
| +/12 h | 2716.2 | 2681.6 | 2061.4 | 1462.6 | 4742.4 | 5198.0 | 3469.4 | 3336.8 | 5345.8 | 5634.6 | 4380.4 | 4138.6 |
| +/12 h | 2585.6 | 2572.4 | 1982.2 | 1451.0 | 4416.0 | 4755.6 | 3229.8 | 3037.4 | 5126.2 | 5374.2 | 4268.8 | 4051.0 |
| +/12 h | 2470.0 | 2411.0 | 1953.8 | 1479.2 | 4453.6 | 4836.8 | 3269.8 | 3065.8 | 4996.6 | 5257.8 | 4359.8 | 4220.6 |
| +/12 h | 2672.6 | 2680.4 | 2274.0 | 1812.2 | 4729.4 | 5195.4 | 3263.2 | 2832.2 | 5325.6 | 5613.8 | 4469.2 | 4300.0 |
| +/12 h | 2712.0 | 2684.6 | 2166.6 | 1695.4 | 4717.8 | 5205.0 | 3476.8 | 3345.4 | 5325.2 | 5641.2 | 4484.0 | 4409.2 |

**− or +/*t* h refers to the culture medium obtained from HepG2 cells exposed with medium alone or medium with 70 µM TAM for *t* h.

## 3. Effect of the cell-seeding density

We investigated the sensitivity of the sensor array to understand its basic performance. Using a PLL-Dnc sensor array, a dataset of fluorescence responses for cell-culture media of HepG2 and A549 cells with different cell-seeding density (i.e., $2.5 \times 10^4$, $5.0 \times 10^3$, and $1.0 \times 10^3$ cells/well) was generated [12 sensor elements (3 cell-culture medium contents × 2 pH values × 2 channels) × 6 analytes × 10 replicates; Table S4]. The LDA of the response patterns resulted in a 2D discriminant score plot (Fig. S6a). The confusion matrix of the leave-one-out cross-validation (LOOCV) test (Fig. S6b) showed that misclassification occurred in both HepG2 and A549 with a cell-seeding density of $1.0 \times 10^3$ and $5.0 \times 10^3$ cells/well. Therefore, the detection limit of this sensor array is estimated to be near $5.0 \times 10^3$ cells/well.

The identification accuracy was quantitatively evaluated using two methods, i.e., LOOCV test and holdout test; in both methods, the entire dataset was separated into 'training data' for model building and 'test data' for validation. In the LOOCV test, one pattern data was removed from the original dataset ($n = 10$) and treated as a test data, while residue was treated as a training data. In the holdout test, the training data ($n = 6$) and test data ($n = 4$) were fixed. In both tests, the test data were assigned to the closest class generated from the training data according to the Mahalanobis distance. The identification accuracy was determined based on whether the class of test data was assigned to the correct class of training data.

We confirmed that the identification accuracy determined by LOOCV test (52/60 samples = 86.7%; Fig. S6b) and holdout test (20/24 samples = 83.3%; Table S4) are almost identical; thus, we decided to carry out only the LOOCV for other experiments in the manuscript (Fig. 3 and Fig. 4).

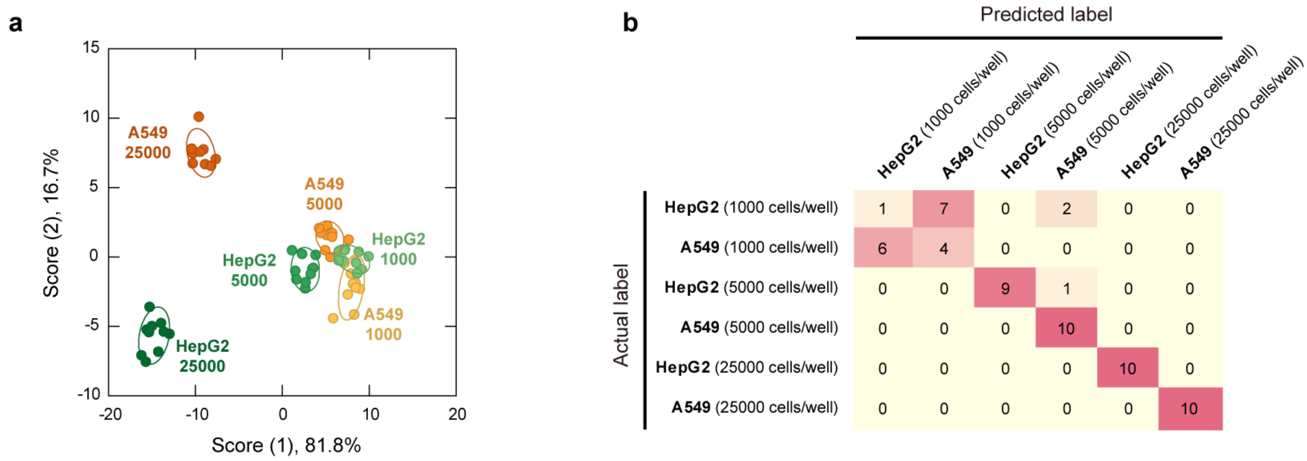


**Fig. S6** Pattern-recognition-based sensing of culture media of HepG2 and A549 cells prepared from different cell-seeding density using a PLL-Dnc array. (**a**) 2D discriminant score plot, wherein the ellipsoids represent the confidence intervals (±1 SD) for the individual analytes. For each analyte, ten independent experimental values are shown. The number in analyte name indicates the cell-seeding density (cells/well). (**b**) Confusion matrix of the LOOCV test.

**Table S4** Dataset of the fluorescence response patterns obtained from the sensing of culture media of HepG2 and A549 cells prepared from different cell-seeding density.

| Analyte*** | $F - F_0$ | | | | | | | | | | | | Holdout test**** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10% cell-culture medium | | | | 30% cell-culture medium | | | | 50% cell-culture medium | | | | | |
| | pH = 5.5 | | pH = 8.5 | | pH = 5.5 | | pH = 8.5 | | pH = 5.5 | | pH = 8.5 | | Verification | Accuracy |
| | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | Ch1 | Ch2 | | |
| HepG2_25000 | 953.2 | 729.4 | 918.2 | 424 | 2116 | 1696.8 | 2117.6 | 1380.2 | 3155.6 | 2798.6 | 3319.6 | 2305.2 | – | – |
| HepG2_25000 | 1055.4 | 887 | 959.2 | 489.2 | 2198.6 | 1784.8 | 2065 | 1338 | 3029.8 | 2855.6 | 3286.6 | 2188.8 | – | – |
| HepG2_25000 | 946.2 | 680.6 | 855.2 | 390.4 | 2094 | 1599.2 | 1921.2 | 1137 | 3001.8 | 2609.4 | 3185 | 2023.2 | – | – |
| HepG2_25000 | 879 | 704.2 | 813.6 | 293.2 | 2104 | 1713.4 | 1901.8 | 1129.2 | 3049 | 2567 | 3139.2 | 2076.4 | – | – |
| HepG2_25000 | 890 | 671 | 943.6 | 500.6 | 2025.4 | 1615.6 | 1950.8 | 1244.4 | 2944.8 | 2644.4 | 3139.2 | 2070.6 | – | – |
| HepG2_25000 | 1015.6 | 787.8 | 981 | 504.2 | 2231.8 | 1833.8 | 2008.6 | 1236.8 | 2964.8 | 2878.8 | 3423.2 | 2047.4 | – | – |
| HepG2_25000 | 995 | 790.6 | 839.8 | 396.4 | 2142.8 | 1711.4 | 1979.6 | 1224 | 3032 | 2836.6 | 3300.2 | 2095 | HepG2_25000 | Yes |
| HepG2_25000 | 885.8 | 730.2 | 747.6 | 304 | 2046.8 | 1554 | 1868.4 | 1102.4 | 2850.8 | 2503 | 2987.2 | 1950.6 | HepG2_25000 | Yes |
| HepG2_25000 | 937.8 | 696.8 | 817.4 | 295 | 2044.8 | 1594.6 | 1840.6 | 1039.2 | 2962.6 | 2662.4 | 3189 | 2095.8 | HepG2_25000 | Yes |
| HepG2_25000 | 926.8 | 718.4 | 839.2 | 405.4 | 2128.2 | 1635.4 | 1812.8 | 1035.4 | 2861.6 | 2609.4 | 3151 | 1924.8 | HepG2_25000 | Yes |
| HepG2_5000 | 528.6 | 361.8 | 429.4 | 17 | 1388 | 984.8 | 1100.8 | 275 | 1918.4 | 1589.8 | 2131 | 1072 | – | – |
| HepG2_5000 | 492 | 304.2 | 416 | 47.4 | 1306.4 | 902.4 | 957.6 | 130.8 | 1813.6 | 1439 | 2037.6 | 968.4 | – | – |
| HepG2_5000 | 554.8 | 355.6 | 380.6 | -49.6 | 1431.8 | 969.6 | 971.6 | 158.6 | 1841.4 | 1601 | 2187 | 879.8 | – | – |
| HepG2_5000 | 505.8 | 305.2 | 419.6 | 43.8 | 1243.8 | 892.4 | 1059.4 | 342.8 | 1844.8 | 1524 | 2158.2 | 892.2 | – | – |
| HepG2_5000 | 468.2 | 252 | 322 | -29.6 | 1273.4 | 820.8 | 871.4 | 128.6 | 1857.6 | 1328.4 | 1892.6 | 940.6 | – | – |
| HepG2_5000 | 563.4 | 320 | 460.2 | 99.2 | 1405.6 | 955.4 | 1105.6 | 351.2 | 1929 | 1559.6 | 2125.2 | 1039 | – | – |
| HepG2_5000 | 542.6 | 333.6 | 435.2 | 63.2 | 1373 | 954.6 | 979 | 176.4 | 1929.8 | 1527.8 | 2027 | 1106 | HepG2_5000 | Yes |
| HepG2_5000 | 516.8 | 318.2 | 382.6 | 43.4 | 1375.6 | 901.6 | 916.4 | 188.8 | 1792 | 1559.4 | 2096.2 | 910.2 | HepG2_5000 | Yes |
| HepG2_5000 | 531.2 | 344.8 | 360.4 | -61.6 | 1302.4 | 897.4 | 888.6 | 128.8 | 1844 | 1461.4 | 2052 | 975.6 | HepG2_5000 | Yes |
| HepG2_5000 | 561 | 325.6 | 405 | 41.6 | 1371 | 982.8 | 905.2 | 103 | 1756.2 | 1592.2 | 2118.2 | 842.8 | HepG2_5000 | Yes |
| HepG2_1000 | 398.8 | 222 | 268.2 | -157.4 | 1078.4 | 612.6 | 655 | -127.2 | 1525.6 | 1082 | 1688 | 637.8 | – | – |
| HepG2_1000 | 384.6 | 174 | 256.4 | -67 | 1011.6 | 605.4 | 581.6 | -86.2 | 1491 | 1039.4 | 1656.4 | 652 | – | – |
| HepG2_1000 | 432.4 | 207.4 | 324 | 51.2 | 1096.2 | 669.2 | 845.2 | 112.2 | 1670.2 | 1104 | 1703.8 | 824.4 | – | – |
| HepG2_1000 | 384.6 | 185 | 286 | -82 | 1096.2 | 640.6 | 760 | -21 | 1520.8 | 1071 | 1689.4 | 652.4 | – | – |
| HepG2_1000 | 391.8 | 190.4 | 205.6 | -157.8 | 988.6 | 588 | 764.6 | 10.4 | 1457.4 | 1036.8 | 1565 | 645.4 | – | – |
| HepG2_1000 | 419.6 | 196.2 | 279.8 | -32.8 | 1113.8 | 701.4 | 766.6 | 54.4 | 1437.6 | 1144 | 1692 | 558.4 | – | – |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HepG2_1000 | 408.4 | 183.2 | 281.6 | -68.2 | 1075 | 633.8 | 717 | 34.2 | 1509.2 | 1126.8 | 1713 | 584.2 | HepG2_1000 | Yes |
| HepG2_1000 | 433.2 | 202.2 | 252.2 | -7.4 | 1116.4 | 678.2 | 881 | 202.4 | 1562.8 | 1135.6 | 1701.4 | 724.2 | HepG2_1000 | Yes |
| HepG2_1000 | 420.8 | 254.4 | 296.8 | -66.2 | 1083 | 651 | 830 | 120.2 | 1506.8 | 1200.2 | 1711.4 | 611.4 | A549_5000 | No |
| HepG2_1000 | 450.6 | 265.8 | 286.2 | -19 | 1128.4 | 718 | 719.4 | 11.2 | 1478.2 | 1217.2 | 1781.2 | 673.6 | HepG2_1000 | Yes |
| A549_25000 | 637 | 346.6 | 583.6 | 71.6 | 1614.2 | 956.4 | 1401 | 345.6 | 2418.6 | 1570.8 | 2484 | 1164.8 | – | – |
| A549_25000 | 601.8 | 350.4 | 556.2 | -9.6 | 1527 | 980.2 | 1327.2 | 327.6 | 2174 | 1605 | 2375.8 | 912.4 | – | – |
| A549_25000 | 635.8 | 349 | 538.2 | 79.8 | 1590.8 | 917.2 | 1323.8 | 315 | 2282 | 1582 | 2425.2 | 996.2 | – | – |
| A549_25000 | 580 | 340.2 | 563.6 | -23.6 | 1476.6 | 895.6 | 1152.8 | 136.4 | 2265.8 | 1502.2 | 2284 | 983 | – | – |
| A549_25000 | 565.4 | 298.6 | 481 | -37.6 | 1469.4 | 873 | 1407 | 429.4 | 2307 | 1576.4 | 2316 | 1049 | – | – |
| A549_25000 | 628.2 | 320.2 | 563.2 | 79.8 | 1620.6 | 1037.4 | 1499 | 485 | 2527.8 | 1703.8 | 2480.8 | 1246.4 | – | – |
| A549_25000 | 685.4 | 380.8 | 596.4 | 50.8 | 1620.4 | 964 | 1418.8 | 412.2 | 2400.2 | 1606.6 | 2548 | 1156.8 | A549_25000 | Yes |
| A549_25000 | 603.6 | 346.8 | 527.8 | -19.8 | 1575.6 | 992.2 | 1337.4 | 322.6 | 2366.8 | 1677.8 | 2428 | 998.2 | A549_25000 | Yes |
| A549_25000 | 575 | 287.6 | 500.2 | -29.2 | 1452.6 | 802.4 | 1493.8 | 482.8 | 2376.6 | 1493.6 | 2259.6 | 1094.4 | A549_25000 | Yes |
| A549_25000 | 627.8 | 341 | 517.6 | -12.2 | 1517.8 | 924.8 | 1315.8 | 192.6 | 2380.6 | 1529 | 2356.2 | 1011 | A549_25000 | Yes |
| A549_5000 | 421.4 | 195.6 | 324.4 | -135.4 | 1163.8 | 716.2 | 781.6 | 9.6 | 1616 | 1264.2 | 1847.4 | 695.6 | – | – |
| A549_5000 | 477 | 266.6 | 314.6 | -48.6 | 1208.6 | 789.8 | 765.8 | 44.6 | 1649.4 | 1318 | 1925.4 | 739 | – | – |
| A549_5000 | 466.6 | 264 | 301 | -93 | 1200.8 | 752.8 | 798 | 4.4 | 1558 | 1271.8 | 1906.8 | 586.2 | – | – |
| A549_5000 | 493.8 | 274.8 | 320.2 | -65 | 1223.6 | 748.8 | 724.6 | -80.2 | 1507 | 1321.6 | 1927.4 | 571.8 | – | – |
| A549_5000 | 404.2 | 196.4 | 259 | -112.2 | 1013.2 | 563.8 | 908 | 169.4 | 1638.8 | 1178.8 | 1730.8 | 669 | – | – |
| A549_5000 | 440 | 195.8 | 362.4 | -54 | 1188.6 | 722.2 | 951.4 | 151.8 | 1597.2 | 1309.4 | 1912.6 | 658.4 | – | – |
| A549_5000 | 476 | 229.4 | 291.2 | -167.2 | 1195 | 731 | 746.6 | 18.2 | 1502.6 | 1281 | 1893.6 | 537.8 | A549_5000 | Yes |
| A549_5000 | 379 | 163.6 | 334.4 | -36.2 | 1090.8 | 613 | 950.8 | 176.8 | 1665.6 | 1077.4 | 1776.6 | 703 | A549_5000 | Yes |
| A549_5000 | 414.8 | 208.8 | 157.2 | -244.6 | 1114 | 615.2 | 878 | 143.4 | 1560.2 | 1301.8 | 1880.4 | 611 | A549_5000 | Yes |
| A549_5000 | 424.8 | 215.4 | 260 | -123.4 | 1140 | 712.8 | 647.2 | -45 | 1384.2 | 1217 | 1832.8 | 429.8 | A549_5000 | Yes |
| A549_1000 | 444.8 | 280.6 | 229.6 | -149.8 | 1201.4 | 754.6 | 649.4 | -88.2 | 1577.6 | 1303 | 1802 | 801.6 | – | – |
| A549_1000 | 450.4 | 272.8 | 161.2 | -205.8 | 1127 | 738.4 | 660 | -91.4 | 1697 | 1237.4 | 1698.6 | 787 | – | – |
| A549_1000 | 1207.2 | 1218.2 | 271.6 | -127 | 2013.6 | 1856 | 763.6 | 109.4 | 1447.4 | 2215.8 | 2497 | 585.6 | – | – |
| A549_1000 | 421.6 | 226.2 | 303.6 | -55.4 | 1103.2 | 700.8 | 720 | 18.4 | 1518.6 | 1200.4 | 1684.8 | 710 | – | – |
| A549_1000 | 558.6 | 390.6 | 290 | 11.4 | 1291.8 | 1022.4 | 934.8 | 288.6 | 1684.6 | 1640.6 | 2005.2 | 937.2 | – | – |
| A549_1000 | 784 | 631.8 | 345.4 | -4.8 | 1627.8 | 1344.2 | 869.8 | 244.2 | 1730.4 | 1983.6 | 2319.2 | 932 | – | – |
| A549_1000 | 384.6 | 179.8 | 288.8 | -68.6 | 1072.2 | 623.8 | 685 | -25.2 | 1355.2 | 1150.6 | 1644.8 | 483.4 | A549_1000 | Yes |
| A549_1000 | 389.4 | 188.4 | 283 | -47.8 | 952.4 | 630.2 | 833.8 | 154 | 1652.4 | 1124 | 1649.6 | 891.6 | HepG2_1000 | No |
| A549_1000 | 392.8 | 198.6 | 304.8 | -89 | 1007.2 | 607.8 | 865 | 121.2 | 1579.6 | 1072 | 1623 | 800 | HepG2_1000 | No |
| A549_1000 | 438.6 | 262.2 | 250 | -76.6 | 1144.6 | 717 | 638.8 | -81.2 | 1510.2 | 1268.4 | 1768.4 | 724 | HepG2_1000 | No |

***The number in analyte name indicates the cell-seeding density (cells/well). ****The far-right column shows the training data (denoted by "–") and the test data as well as the verification result from the holdout test.

## 4. Hierarchical clustering analysis

To understand which sensor elements are important for generating the diverse pattern data obtained from the PLL-Dnc array, the response patterns of the 12 sensor elements were subjected to HCA, where the Euclidean distances between the elements correspond to similarities in the response patterns (Fig. S7).[4] In the HCA dendrogram, the fluorescence responses obtained from the 30% and 50% cell-culture-medium contents were initially clustered with the same channel, indicating that changing the channel rather than the solution conditions contributed to the generation of the various responses. The difference in detection wavelengths (Ch1 and Ch2 corresponding to peak tail and peak top of the dansyl fluorophore, respectively), probably contributes to the efficient generation of diverse fluorescence responses as the emission enhancement of the PLL-Dnc probe is accompanied by a sample-dependent hypsochromic shift (Fig. S1). However, the responses obtained from the 10% cell-culture-medium contents were clustered individually for each pH value, indicating that better differentiated responses were produced at this medium content. Varying pH values are effective to produce the differential fluorescence responses since the pH value directly affects the contribution of electrostatic interactions between compounds in the cell-culture medium and the PLL-Dnc probe. This HCA dendrogram, where the sensor elements are clustered in an unexpected manner, demonstrates the importance of using a combination of all three sensor elements (i.e., channels, pH values, and contents of the cell-culture medium) to construct an effective PLL-Dnc array for cell-culture-medium analysis.

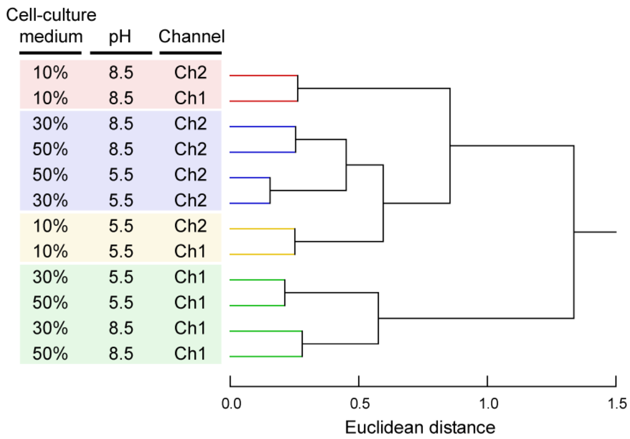


**Fig. S7** HCA of sensor elements in the pattern-recognition-based sensing of eight cell lines using a PLL-Dnc array. The HCA dendrogram was created based on Euclidean distances using the Ward method. The dataset was standardized prior to analysis based on the following equation: $z = (x - \mu)/\sigma$, where $z$ is the standardized score, $x$ is the raw fluorescence response $F - F_0$, $\mu$ is the mean value of the population, and $\sigma$ is the standard deviation of the population.

## 5. References


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