Supplementary Information for

A Polyion Complex Sensor Array for Markerless and Noninvasive Identification of Differentiated Mesenchymal Stem Cells from Human Adipose Tissue

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Table of contents

Section 1: Materials and method

Titration of PEGylated polyamines to enzymes. β-Galactosidase from *Aspergillus oryzae* (GAO), β-galactosidase from *Escherichia coli* (GEC), lipase from *Aspergillus niger* (LAN), 4-methylumbelliferyl-β-D-galactopyranoside (MUG), 4-methylumbelliferyl oleate (MUO), Triton X-100, 3-(*N*-morpholino)propanesulfonic acid (MOPS), and 200 mM L-glutamine solution were obtained from Sigma Chemical Co. Dimethyl sulfoxide (DMSO) was obtained from Wako Pure Chemical Ind. Chemically defined serum-free CDCHO medium was obtained from Invitrogen. Quaternized poly(ethylene glycol)-*block*-poly(*N,N*-dimethylaminoethyl methacrylate) (PEG-*b*-QPAMA) (**P1** and **P2**) were synthesized as reported previously.[1] The degrees of polymerization of **P1** and **P2** were 102 for PEG and 35 for QPAMA.

A solution of 4.3 nM GAO, 1.7 nM GEC, and 86.5 nM LAN in 10 mM MOPS (pH 7.0) was prepared and cryopreserved at –80°C. Frozen enzyme solutions were thawed immediately before the experiments in a 37°C water bath. Various concentrations of PEGylated polyamines were mixed with enzymes in 10 mM MOPS (pH 7.0). PEGylated polyamine/enzyme solution (182 μL) and 10 μL of CDCHO medium supplemented with 8 mM L-glutamine were loaded into each well of 96-well plates (96 Well Black Flat-Bottom Polystyrene NBS™ Microplates; Corning Inc.). After incubation for 30 minutes at 30°C, 8 μL of substrate in DMSO (for LAN, 7.5% Triton X-100 was mixed with stock substrate solution) was added to each well, and the time course of the increase in fluorescence at 460 nm was recorded using a microplate reader (Fluoroskan Ascent; Thermo Labsystems) with excitation at 355 nm. The final concentrations were 0.5 nM GAO and 1.0 mM MUG; 0.2 nM GEC and 1.0 mM MUG; 10 nM LAN, 0.05 mM MUO, and 0.3% Triton X-100. The samples were measured in triplicate.

Concentrations of enzymes were determined from the absorbance at 280 nm using a spectrophotometer (UV-2450; Shimadzu Corporation, Kyoto, Japan) with extinction coefficients of 192075 M^{-1} cm^{-1} (GAO), 1046760 M^{-1} cm^{-1} (GEC), and 48275 M^{-1} cm^{-1} (LAN).[2]

Cell culture. The human lung adenocarcinoma epithelial cell line (A549), human osteosarcoma cell line (MG63), and human hepatoma cell line (HuH7) were obtained from the Japanese Collection of Research Bioresources. Normal human lung fibroblast (NHLF), normal human dermal fibroblasts (NHDF), and human adipose-derived stem cells (ADSCs) were obtained from Lonza. Normal human fetal lung diploid fibroblasts (WI-38) were obtained from Health Science Research Resource Bank. All of the cells were grown in growth medium consisting of DMEM supplemented with L-glutamine and phenol red (Wako Pure Chemical Ind.) and 10% (*v*/*v*) fetal bovine serum (FBS) GOLD (PAA Laboratories GmbH) and 1% Penicillin-Streptomycin-Neomycin antibiotic mixture (Life Technologies)

in a humidified 5% CO_2 incubator at 37°C. The procedure for collecting culture supernatants is shown in Figure S3 (1 – 3). Cancer cells and fibroblasts in the growth medium were seeded in 24-well tissue culture plates (AGC Techno Glass Co.). After 16 hours, cells were washed twice with 200 μL of chemically defined serum-free CDCHO medium supplemented with 8 mM L-glutamine, and then 200 μL of CDCHO medium was added to each well. Following culture in CDCHO for 48 hours, 160 μL of the culture supernatants was collected and centrifuged at 3000 × *g* for 10 minutes to remove cell debris. The supernatants were finally stored at –80°C until use. ADSCs in the growth medium were seeded at a density of 3.0×10^4 cells/cm^2 in 24-well tissue culture plates, and after 24 hours, ADSCs were cultured in growth medium, adipogenic differentiation medium (PromoCell), or osteogenic differentiation medium (PromoCell). The medium was changed every other day. After 21 days of induction, ADSCs were treated as well as normal/cancer cells to collect the culture supernatants. Following supernatant collection, cell viability was evaluated with a Live/Dead Cell Staining Kit (BioVision Inc.), and the results indicated that almost all cells were viable after culture in CDCHO.

Cell staining. Prior to staining of ADSC-derived cells, the cells cultured for 21 days in growth, osteogenic differentiation, or adipogenic differentiation media were washed twice with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 15 minutes. Alizarin Red S staining: The fixed cells were washed three times with distilled water and incubated in chilled methanol for 10 minutes. Finally, the cells were washed once with distilled water and soaked in 30 mM Alizarin Red S (pH ~ 4.2) for 15 minutes at 37°C, washed with distilled water, and then observed under an optical microscope. Oil Red O staining: The fixed cells were washed twice with PBS and incubated in 60% 2-propanol for 2 minutes, and then soaked in 0.4 mM Oil Red O in 60% 2-propanol for 15 minutes at 37°C, washed with PBS, and then observed in PBS under an optical microscope.

Sensing of culture supernatants. The sensing procedure is shown in Figure S3 (4 – 8). Total protein in the culture supernatants was quantified using the Bradford assay with Bradford Reagent (Sigma Chemical Co.) according to the manufacturer's instructions. Each culture supernatant was then diluted to a total protein concentration of 5.0 μg/mL with CDCHO medium supplemented with 8 mM L-glutamine. In enzyme assays, aliquots of 182 μL of solutions containing PEGylated polyamines and enzymes were loaded into each well of 96-well plates. Subsequently, 10 μL of diluted culture supernatant was added. After incubation for 30 minutes at 30°C, 8 μL of substrate in DMSO (for LAN, 7.5% Triton X-100 was mixed with stock substrate solution) was added to each well, and the time course of the increase in fluorescence at 460 nm was recorded using a microplate reader with excitation at 355 nm. The final concentrations were 0.5 nM GAO, 30 nM **P1** or 30 nM **P2**, and 1.0 mM MUG; 0.2

nM GEC, 9 nM **P1** or 9 nM **P2**, and 1.0 mM MUG; 10 nM LAN, 30 nM **P1** or 9 nM **P2**, 0.05 mM MUO, and 0.3% Triton X-100. This process was repeated for the culture supernatants with 6 PICs in six replicates each. This data set matrix was subjected to linear discriminant analysis (LDA). Similar procedures were also performed for a blind test (see below). After the cell identity was recognized by LDA, the cell density at the seeding time was deduced from the nonlinear curve of total protein concentration against the cell density (Figures 4C and S5C).

Linear discriminant analysis (LDA). SYSTAT 13 (Systat Inc.) was used to carry out all LDA analysis. LDA is frequently used supervised pattern recognition method for dimensionality reduction and for classification of multivariate data. LDA develops discriminant functions with the objective of maximizing the between-class variance relative to the within-class variance to describe the relationship between the observed variables and their known classes. The first discriminant function Z_1 is the linear combination of variables that best discriminates among the groups, and the second discriminant function Z_2 is orthogonal to the first one and is the next best combination of variables:

$$Z_k = a_1x_1 + a_2x_2 + \ldots + a_nx_n + C$$

where x_i are discriminating variables (enzyme activities in our case), a_i are discriminant weights, and C is a constant. Discriminant scores are calculated from the discriminant functions, and provides a graphical output to give an insight into clustering of the data by plotting discriminant scores (e.g., Figure 4B).

Jackknife classification (leave-one-out) procedure is used to test the predictability of sensor arrays and also to determine a minimal number of sensor elements. This procedure removes only one sample at a time from the data set and considers it as a "test data". The rest of the data set is used as a "training data". Test data is classified based on its Mahalanobis distances to the centroid of each group, i.e., the closer a case is to one group, the more likely it is to be assigned to that group. The procedure is repeated until all samples have been left out and classified. As the data of unknown identity can be later classified to one of the classes based on the similarity of its responses to the responses of the samples in the training data, the predictive power of a sensor array can be evaluated (e.g., Table S2). After the jackknife procedure is completed, true test data are finally predicted based on the proximity to known group calculated from all data set (e.g., Table S3).

Section 2: Supplementary Figures and Tables

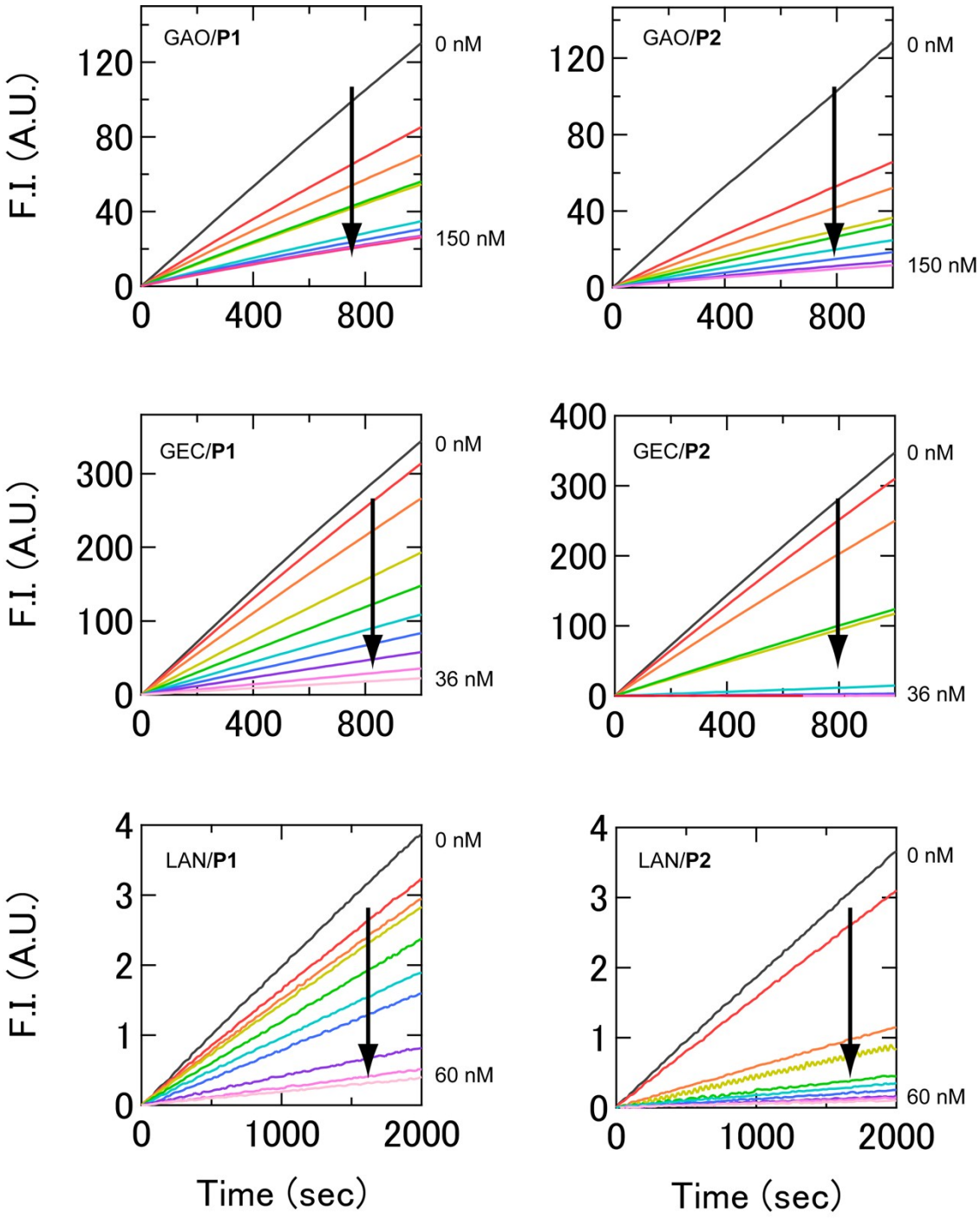


Figure S1. Time courses of increase in fluorescence intensity in the presence of various concentrations of PEGylated polyamines. Titration of PEGylated polyamines to 0.5 nM GAO, 0.2 nM GEC, and 10 nM LAN in 10 mM MOPS (pH 7.0) with 5% chemically defined serum-free CDCHO medium.

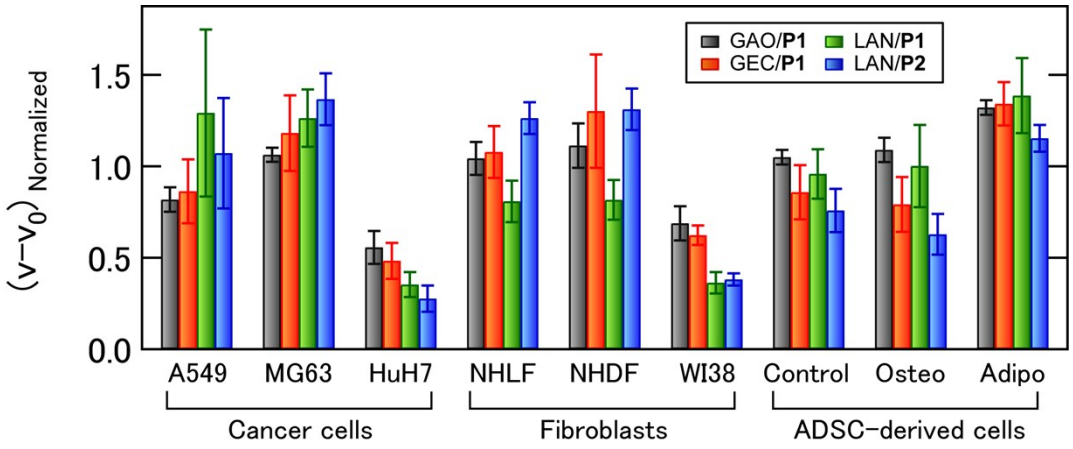


Figure S2. Enzyme activity patterns for culture supernatants collected from cancer cells, fibroblasts, and ADSC-derived cells. Each normalized value represents the average of six parallel measurements with 1 S.D.

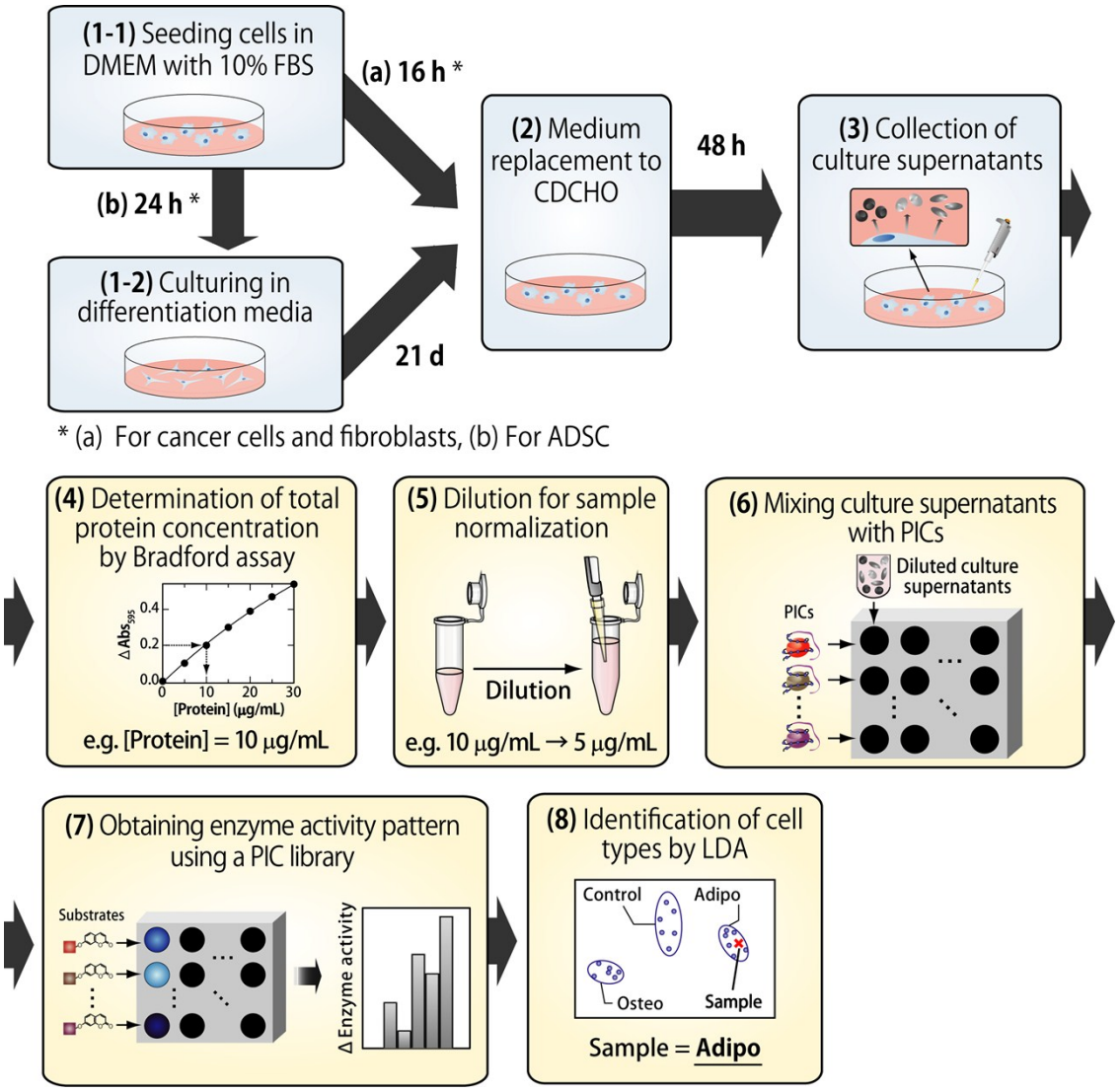


Figure S3. Schematic representation for the sensing procedure of the culture supernatants using a PIC sensor array.

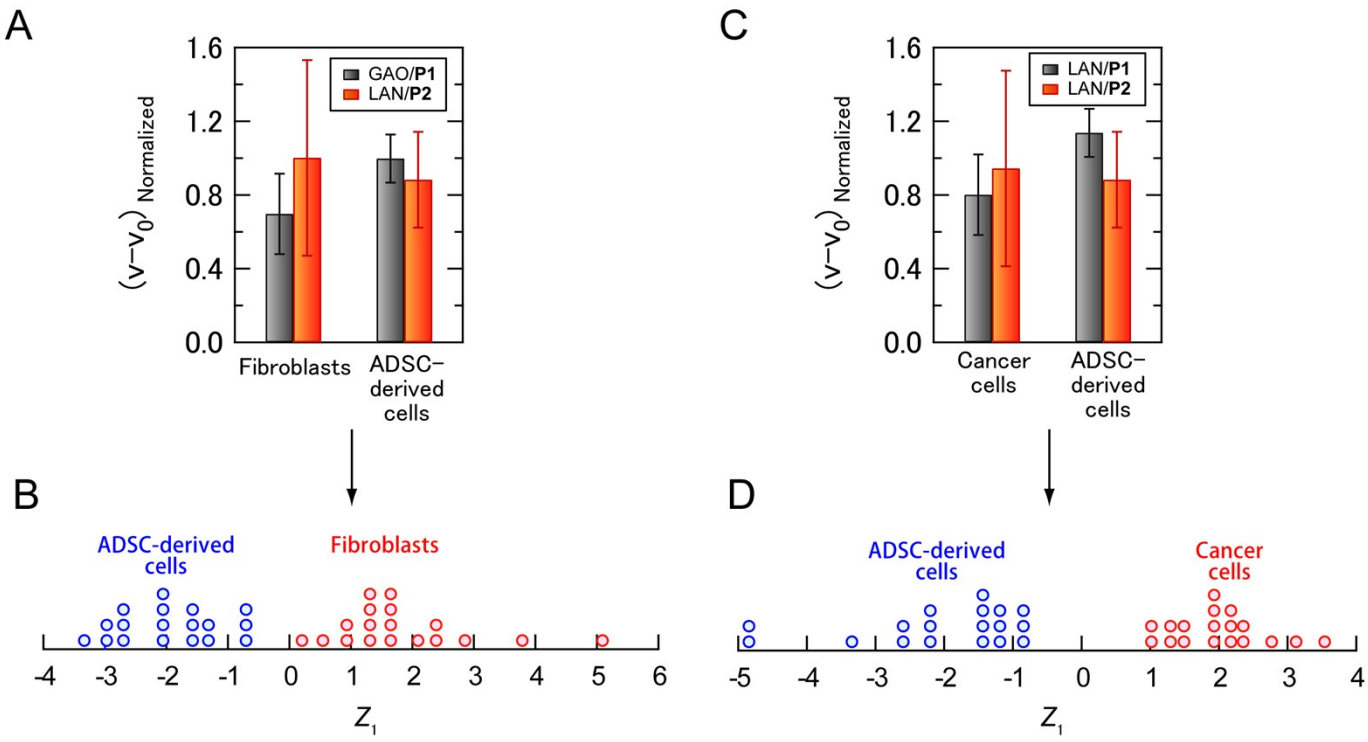


Figure S4. Enzyme activity patterns for culture supernatants collected from (A) fibroblasts and ADSC-derived cells; (C) cancer cells and ADSC-derived cells. Each normalized value represents the average of 18 parallel measurements with 1 S.D. Discriminant score plot for (B) fibroblasts and ADSC-derived cells; (D) cancer cells and ADSC-derived cells.

Table S1. Data set matrix of differences between initial slope of fluorescent intensity before (v_0) and after (v) the addition of individual cell culture supernatants (v-v_0) generated from the sensor array containing 6 PICs. To facilitate a visual comparison between each PIC result, data for each PIC were divided by the root mean square of corresponding PIC data set in Figures 4, 5, and S5 (see Section 3 in Supplementary Information):

$$(v-v_0)_{Normalized\,ij}{}^{(h)} = \frac{(v-v_0)_{ij}{}^{(h)}}{\sqrt{\frac{1}{NM}\sum_{j=1}^{M}\sum_{i=1}^{N}(v-v_0)^2{}_{ij}{}^{(h)}}}$$

where i is the number of samples, j is cell type or lineage, and h is PIC type. For example, in Figure 4A, N = 6, M = 3 (A549, MG63, HuH7), and h = 3 (GAO/**P1**, LAN/**P1**, LAN/**P2**).

Cellular category	Analytes	[Total protein] (μg/mL)	Enzyme activity ($v - v_0$)					
			GAO/**P1** (×10²)	GAO/**P2** (×10²)	GEC/**P1** (×10²)	GEC/**P2** (×10²)	LAN/**P1** (×10⁴)	LAN/**P2** (×10⁴)
Cancer cells	A549	14.25	2.85	2.09	6.78	9.91	3.81	8.16
	A549	16.30	2.61	1.66	5.54	7.55	3.75	6.62
	A549	11.41	3.26	2.71	9.78	11.95	7.16	11.86
	A549	15.81	2.86	2.13	6.88	8.00	4.05	8.52
	A549	11.11	2.91	2.11	6.57	9.54	5.46	11.18
	A549	8.81	2.63	2.54	6.78	11.01	8.26	14.34
	MG63	23.89	3.60	2.64	8.85	13.93	4.99	13.25
	MG63	24.61	3.52	2.34	10.36	12.68	4.57	10.89
	MG63	23.46	3.69	2.34	7.76	8.51	5.06	12.83
	MG63	21.38	3.70	2.94	8.41	14.36	5.46	14.25
	MG63	22.36	3.88	2.94	12.43	15.20	5.20	11.87
	MG63	20.68	3.82	3.32	10.11	15.01	6.51	14.25
	HuH7	26.95	2.17	1.47	3.61	5.62	1.10	2.02
	HuH7	36.93	1.94	1.03	3.43	3.28	1.13	1.74
	HuH7	20.86	1.86	0.89	5.29	7.41	1.65	3.23
	HuH7	23.03	2.36	1.31	3.06	4.38	1.71	2.31
	HuH7	25.51	1.85	0.85	4.48	6.87	1.68	2.95
	HuH7	15.35	1.45	0.99	3.88	6.44	1.64	3.41
Fibroblasts	NHDF	13.01	3.85	2.96	13.67	14.34	3.66	12.91
	NHDF	13.23	3.62	2.73	9.55	12.99	3.52	12.66
	NHDF	13.90	3.72	2.86	11.73	10.95	3.53	13.53
	NHDF	15.94	3.42	2.48	6.24	12.96	2.53	10.66
	NHDF	15.61	4.63	2.55	11.45	12.16	3.83	12.96
	NHDF	14.85	4.02	2.55	11.23	10.03	3.46	11.51
	NHLF	13.89	3.53	2.14	6.79	11.62	2.82	12.54
	NHLF	11.83	3.66	2.54	9.10	11.64	3.57	12.70
	NHLF	12.68	3.54	2.50	9.80	11.33	3.69	12.26
	NHLF	14.29	3.42	2.56	9.80	11.74	3.03	11.05
	NHLF	13.42	4.25	2.41	9.22	8.25	4.08	12.24
	NHLF	14.07	3.43	2.14	8.22	12.92	3.14	10.74
	WI38	16.68	2.38	1.28	5.40	8.09	1.70	4.11
	WI38	18.54	2.18	1.23	4.58	6.73	1.13	3.58
	WI38	19.49	1.97	1.22	5.12	7.55	1.58	3.42
	WI38	17.72	2.33	1.32	4.53	7.57	1.59	3.69

	WI38	16.78	2.70	1.34	5.49	5.36	1.36	3.18
	WI38	16.38	2.84	1.29	5.45	9.18	1.80	3.65
ADSC-derived cells	Control	58.60	3.70	2.63	7.89	7.83	4.92	8.42
	Control	53.17	3.60	2.14	5.66	6.75	4.17	8.08
	Control	58.65	3.65	2.70	6.78	8.05	3.51	6.09
	Control	49.72	3.89	2.64	8.67	6.84	4.37	7.91
	Control	59.86	3.64	2.78	7.41	7.82	3.56	6.61
	Control	57.87	3.46	2.32	5.65	6.53	3.59	5.83
	Osteo	103.52	3.56	3.18	7.43	5.50	6.06	7.55
	Osteo	119.36	3.89	2.36	4.35	6.25	4.10	5.39
	Osteo	100.06	4.14	2.58	7.55	6.89	3.87	6.63
	Osteo	153.74	3.55	1.88	5.87	4.10	3.38	4.52
	Osteo	109.61	3.93	2.39	6.39	7.03	3.78	5.84
	Osteo	123.47	3.71	1.91	7.23	5.73	4.04	5.62
	Adipo	89.28	4.46	3.58	11.89	9.21	6.53	11.97
	Adipo	98.42	4.47	3.55	10.07	12.49	5.77	10.89
	Adipo	95.00	4.75	3.77	11.68	13.35	7.03	10.60
	Adipo	97.92	4.51	3.54	9.85	9.26	4.94	11.14
	Adipo	91.62	4.67	3.46	11.90	11.23	5.79	10.81
	Adipo	102.07	4.76	3.28	10.37	10.86	4.85	9.85

Table S2. Classification accuracy of sensor arrays for discrimination of three cancer cell lines seeded at 2.25×10^4 cells/cm^2.

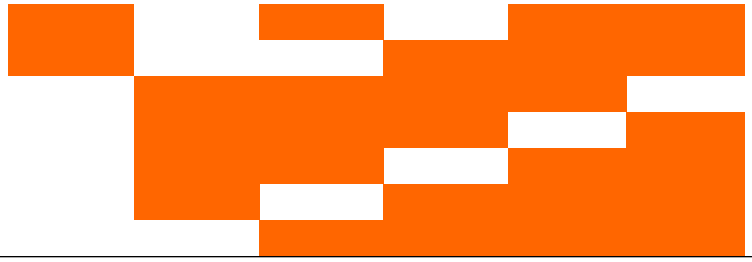
	Selected PICs						%correct			
	GAO/**P1**	GAO/**P2**	GEC/**P1**	GEC/**P2**	LAN/**P1**	LAN/**P2**	A549	HuH7	MG63	Total
1 PIC	■						83	83	100	89
		■					50	100	67	72
			■				67	100	67	78
				■			67	100	83	83
					■		17	100	50	56
						■	50	100	83	78
2 PICs	■	■					83	100	100	94
	■		■				83	83	100	89
	■			■			83	100	83	89
	■				■		83	100	100	94
	■					■	83	100	100	94
		■	■				67	100	83	83
		■		■			50	100	67	72
		■			■		67	100	67	78
		■				■	50	100	67	72
			■	■			67	100	83	83
			■		■		83	100	67	83
			■			■	50	100	83	78
				■	■		83	100	83	89
				■		■	50	100	83	78
					■	■	83	100	100	94
3 PICs	■	■	■				83	100	100	94
	■	■		■			100	100	100	100
	■	■			■		83	100	100	94
	■	■				■	100	100	100	100
	■		■	■			83	100	83	89
	■		■		■		83	100	100	94
	■		■			■	83	100	100	94
	■			■	■		83	100	83	89
	■			■		■	83	100	83	89
	■				■	■	100	100	100	**100**
		■	■	■			67	100	83	83
		■	■		■		67	100	83	83
		■	■			■	50	100	83	78
		■		■	■		67	100	83	83
		■		■		■	50	100	67	72
		■			■	■	83	100	100	94
			■	■	■		67	100	83	83
			■	■		■	50	100	83	78
			■		■	■	83	100	100	94
				■	■	■	83	100	83	89

Table S3. Blind test of 33 culture supernatants collected from the three kinds of cancer cell lines seeded at different cell densities by the PIC sensor array consisting of GAO/**P1**, LAN/**P1** and LAN/**P2**. The average deviation of seeding density was 18%.

Identification	Seeding density ($\times10^4$ cells/cm^2)	[Total protein] (μg/mL)	Enzyme activity (v-v_0)			Verification		
			GAO/**P1** ($\times10^2$)	LAN/**P1** ($\times10^4$)	LAN/**P2** ($\times10^4$)	Cell	Seeding density ($\times10^4$ cells/cm^2)	Deviation
A549	1.50	5.94	3.42	9.09	16.46	A549	1.13	–24.4%
A549	2.25	8.60	3.31	8.21	13.68	A549	1.71	–24.0%
A549	3.00	16.47	2.85	5.17	8.65	A549	3.65	21.8%
A549	1.50	7.44	3.09	6.52	12.47	A549	1.45	–3.0%
A549	2.25	10.37	2.50	6.24	11.80	A549	2.11	–6.0%
A549	3.00	17.48	2.88	4.67	8.36	A549	3.93	31.1%
A549	1.50	8.00	3.13	6.72	12.17	A549	1.58	5.2%
A549	2.25	10.83	2.44	6.03	11.85	A549	2.22	–1.2%
A549	3.00	18.64	3.00	4.90	9.65	A549	4.27	42.2%
MG63	0.75	11.00	3.33	4.86	21.68	MG63	0.92	22.2%
MG63	1.50	18.81	3.14	5.39	24.47	MG63	1.72	14.6%
MG63	2.25	21.94	3.59	6.89	15.59	MG63	2.08	–7.4%
MG63	3.00	34.66	3.75	7.89	12.72	**A549**	**3.96**	**Fail**
MG63	0.75	7.51	4.50	8.25	17.52	MG63	0.60	–20.1%
MG63	1.50	13.92	4.06	7.08	15.72	MG63	1.20	–20.0%
MG63	2.25	19.05	4.23	7.59	16.85	MG63	1.75	–22.4%
MG63	3.00	24.24	4.38	6.40	15.82	MG63	2.37	–21.0%
MG63	0.75	9.31	3.16	5.84	14.06	MG63	0.76	1.4%
MG63	1.50	16.45	4.44	6.71	14.06	MG63	1.46	–2.6%
MG63	2.25	22.27	4.05	7.20	15.35	MG63	2.12	–5.6%
MG63	3.00	29.80	4.05	6.05	13.43	MG63	3.15	4.9%
HuH7	0.75	8.68	2.96	1.61	2.87	**A549**	**0.70**	**Fail**
HuH7	1.50	17.29	2.56	1.20	2.49	HuH7	1.28	–14.7%
HuH7	2.25	25.17	1.83	1.84	3.37	HuH7	1.97	–12.5%
HuH7	3.00	30.33	2.34	1.12	2.24	HuH7	2.46	–18.0%
HuH7	0.75	16.56	1.90	0.94	1.36	HuH7	1.22	62.6%
HuH7	1.50	23.09	1.76	1.09	2.04	HuH7	1.78	18.7%
HuH7	2.25	21.20	1.50	1.67	3.08	HuH7	1.61	–28.3%
HuH7	3.00	38.91	1.88	1.11	2.16	HuH7	3.37	12.3%
HuH7	0.75	9.06	2.30	1.09	2.46	HuH7	0.63	–16.3%
HuH7	1.50	17.18	2.46	1.14	2.40	HuH7	1.27	–15.3%
HuH7	2.25	21.08	2.75	1.37	2.65	HuH7	1.60	–28.8%
HuH7	3.00	28.23	2.67	1.36	2.59	HuH7	2.26	–24.8%

Table S4. Classification accuracy of sensor arrays for discrimination of three ADSC-derived cells.

	Selected PICs						%correct			
	GAO/**P1**	GAO/**P2**	GEC/**P1**	GEC/**P2**	LAN/**P1**	LAN/**P2**	Adipo	Control	Osteo	Total
1 PIC	■						100	83	50	78
		■					100	67	67	78
			■				100	67	50	72
				■			67	83	67	72
					■		67	50	0	39
						■	100	67	67	78
2 PICs	■	■					100	67	67	78
	■		■				100	67	50	72
	■			■			100	83	83	89
	■				■		100	67	50	72
	■					■	100	67	83	83
		■	■				100	67	33	67
		■		■			100	67	50	72
		■			■		100	67	50	72
		■				■	100	50	67	72
			■	■			100	67	50	72
			■		■		100	50	17	56
			■			■	100	50	67	72
				■	■		83	83	67	78
				■		■	100	83	67	83
					■	■	100	67	83	83
3 PICs	■	■	■				100	67	50	72
	■	■		■			100	83	83	89
	■	■			■		100	50	67	72
	■	■				■	100	50	83	78
	■		■	■			100	83	83	89
	■		■		■		100	67	50	72
	■		■			■	100	67	83	83
	■			■	■		100	83	83	89
	■			■		■	100	100	83	94
	■				■	■	100	100	67	89
		■	■	■			100	67	17	61
		■	■		■		100	50	33	61
		■	■			■	100	50	50	67
		■		■	■		100	83	50	78
		■		■		■	100	67	67	78
		■			■	■	100	83	67	83
			■	■	■		100	67	67	78
			■	■		■	100	83	50	78
			■		■	■	100	83	83	89
				■	■	■	100	83	67	83
4 PICs	■	■	■	■			100	83	67	83
	■	■	■		■		100	50	50	67
	■	■	■			■	100	50	67	72
	■	■		■	■		100	67	83	83
	■	■		■		■	100	83	83	89
	■	■			■	■	100	100	67	89
	■		■	■	■		100	67	100	89
	■		■	■		■	100	83	83	89



100	83	67	83
100	100	100	**100**
100	67	33	67
100	50	67	72
100	83	67	83
100	83	67	83
100	83	67	83

Table S5. Blind test of 18 culture supernatants collected from the three kinds of ADSC-derived cells by the PIC sensor array consisting of GAO/**P1**, GEC/**P2,** LAN/**P1** and LAN/**P2**.

Identification	[Total protein] (μg/mL)	Enzyme activity ($v - v_0$)				Verification	Accuracy
		GAO/**P1** ($\times 10^2$)	GEC/**P2** ($\times 10^2$)	LAN/**P1** ($\times 10^4$)	LAN/**P2** ($\times 10^4$)		
Control	56.49	4.02	8.05	3.97	7.03	Control	Yes
Control	58.14	3.56	6.50	3.83	5.90	Control	Yes
Control	57.57	4.31	5.97	3.98	6.70	**Osteo**	**No**
Control	56.68	3.91	7.20	3.33	6.07	Control	Yes
Control	52.8	3.68	7.00	3.77	6.17	Control	Yes
Control	52.15	3.41	7.10	4.22	6.69	Control	Yes
Osteo	116.59	3.57	7.29	3.78	5.25	**Control**	**No**
Osteo	107.96	4.15	5.92	3.63	5.61	Osteo	Yes
Osteo	138.23	4.00	4.73	3.24	4.89	Osteo	Yes
Osteo	154.38	3.68	5.92	3.45	4.45	Osteo	Yes
Osteo	142.3	3.52	5.80	2.88	4.32	Osteo	Yes
Osteo	105.31	3.38	6.91	3.66	5.65	**Control**	**No**
Adipo	93.81	4.60	11.27	6.40	11.43	Adipo	Yes
Adipo	91.31	5.12	9.17	6.01	11.98	Adipo	Yes
Adipo	101.93	4.58	11.27	5.34	10.67	Adipo	Yes
Adipo	97.53	4.99	9.66	5.93	11.34	Adipo	Yes
Adipo	88.6	5.33	11.19	5.70	11.20	Adipo	Yes
Adipo	111.01	4.07	9.78	5.04	9.78	Adipo	Yes

Section 3: Discrimination of human lung-derived cells

To test our strategy in the model case for neoplastic cell transformation and contamination with cancerous cells, normal and cancerous human lung-derived cells were selected: NHLF and WI38 are normal fibroblasts, whereas A549 is a cancerous cell line. The culture supernatants of lung-derived cells generated distinct response patterns (Figure S5A and Table S1). Cells were classified into respective cell types with 100% accuracy using only two PICs (GAO/**P1** and LAN/**P1**) according to LDA with jackknife analysis (Figure S5B and Table S6). In a blind test, human lung-derived cells with different seeding densities were identified with 96% accuracy (26 of 27), and the cell densities at the seeding time were determined within ± 15% (Table S7). Pattern generation was likely independent on seeding density (Figure S5D) as well as cancer cells (Figure 4D)

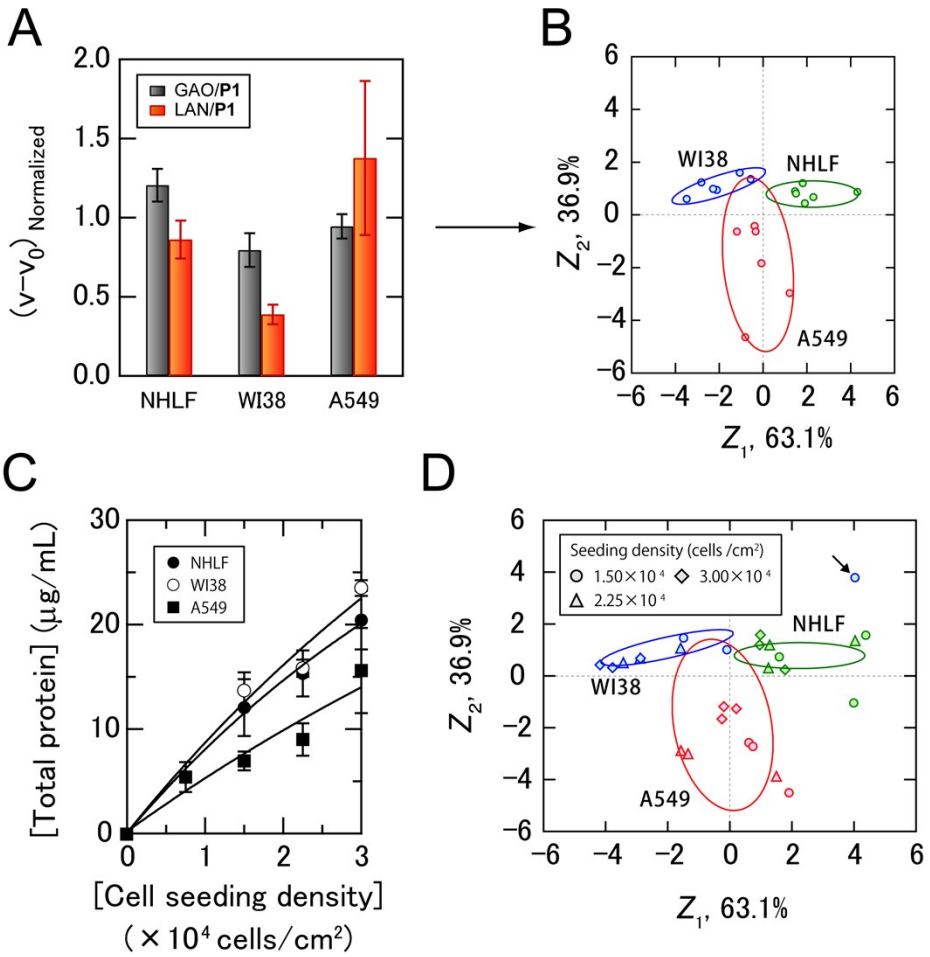


Figure S5. Sensing of normal/cancer cells from human lung. **(A)** Enzyme activity patterns for culture supernatants from three human lung cell types seeded at 2.25×10^4 cells/cm^2. Each normalized value represents the average of six parallel measurements with 1 S.D. **(B)** Discriminant score plot of the first two discriminant functions. Enzyme activity patterns obtained from two PICs were subjected to LDA. The ellipses represent confidence intervals (± 1 S.D.) for the individual cell types from the human lung.

(C) Total protein concentrations of the culture supernatants with different seeding densities determined by Bradford assay. The values are the averages of four parallel measurements with ± 1 S.D. **(D)** Effects of cell seeding density on pattern generation. Discriminant scores of enzyme activity patterns for the three kinds of human lung-derived cells with various seeding densities were calculated using the first two discriminant functions obtained from training data. The ellipses are the same as those shown in (B), and the arrow indicates the misclassified sample.

Table S6. Classification accuracy of sensor arrays for discrimination of three cell types from human lung seeded at 2.25×10^4 cells/cm^2.

	Selected PICs						%correct			
	GAO/**P1**	GAO/**P2**	GEC/**P1**	GEC/**P2**	LAN/**P1**	LAN/**P2**	A549	NHLF	WI38	Total
1 PIC							50	100	67	78
							50	67	100	72
							67	83	100	83
							33	83	83	67
							50	100	100	83
							33	67	100	67
2 PICs							67	100	100	89
							67	100	83	83
							67	100	83	83
							100	100	100	**100**
							67	100	100	89
							67	83	100	83
							50	83	100	78
							83	100	100	94
							33	67	100	67
							67	67	100	78
							67	83	100	83
							50	83	100	78
							67	83	100	83
							50	83	100	78
							83	100	100	94

Table S7. Blind test of 27 culture supernatants collected from the three kinds of cell types from human lung seeded at different cell densities by the PIC sensor array consisting of GAO/**P1** and LAN/**P1**. The average deviation of seeding density was 15%.

Identification	Seeding density ($\times 10^4$ cells/cm^2)	[Total protein] (mg/mL)	Enzyme activity (v-v_0)		Verification		
			GAO/**P1** ($\times 10^2$)	LAN/**P1** ($\times 10^4$)	Cell	Seeding density ($\times 10^4$ cells/cm^2)	Deviation
A549	1.50	5.94	3.42	9.09	A549	1.13	–24.5%
A549	2.25	8.60	3.31	8.21	A549	1.71	–24.0%
A549	3.00	16.47	2.85	5.17	A549	3.66	21.8%
A549	1.50	7.44	3.09	6.52	A549	1.45	–3.1%
A549	2.25	10.37	2.50	6.24	A549	2.11	–6.0%
A549	3.00	17.48	2.88	4.67	A549	3.94	31.2%
A549	1.50	8.00	3.13	6.72	A549	1.58	5.1%
A549	2.25	10.83	2.44	6.03	A549	2.22	–1.2%
A549	3.00	18.64	3.00	4.90	A549	4.27	42.3%
NHLF	1.50	10.19	4.29	3.35	NHLF	1.30	–13.2%
NHLF	2.25	15.50	3.37	2.60	NHLF	2.14	–4.8%
NHLF	3.00	19.15	3.27	2.49	NHLF	2.81	–6.3%
NHLF	1.50	10.03	4.11	6.06	NHLF	1.28	–14.7%
NHLF	2.25	13.16	4.17	3.42	NHLF	1.76	–22.0%
NHLF	3.00	23.74	3.29	2.08	NHLF	3.80	26.6%
NHLF	1.50	12.11	3.45	3.26	NHLF	1.59	6.1%
NHLF	2.25	14.33	3.33	3.56	NHLF	1.95	–13.5%
NHLF	3.00	17.30	3.49	3.83	NHLF	2.46	–17.9%
WI38	1.50	15.86	2.97	2.36	WI38	1.96	30.8%
WI38	2.25	14.72	2.53	1.71	WI38	1.80	–20.0%
WI38	3.00	24.43	1.75	1.49	WI38	3.33	11.0%
WI38	1.50	12.13	2.57	1.36	WI38	1.44	–3.7%
WI38	2.25	16.02	2.14	1.67	WI38	1.98	–11.8%
WI38	3.00	23.65	1.87	1.74	WI38	3.19	6.4%
WI38	1.50	14.35	4.24	0.80	**NHLF**	**1.95**	**Fail**
WI38	2.25	15.86	1.97	1.66	WI38	1.96	–12.8%
WI38	3.00	23.25	2.14	1.68	WI38	3.12	4.1%

Section 4: References

(1) S. Tomita, T. Soejima, K. Shiraki and K. Yoshimoto, *Analyst*, 2014, **139**, 6100.

(2) C. N. Pace, F. Vajdos, L. Fee, G. Grimsley and T. Gray, *Protein Sci.*, 1995, **4**, 2411.