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Supporting Information

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Scalable Multiplexed Drug-Combination Screening Platforms Using 3D Microtumor Model for Precision Medicine

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Supplementary Figures

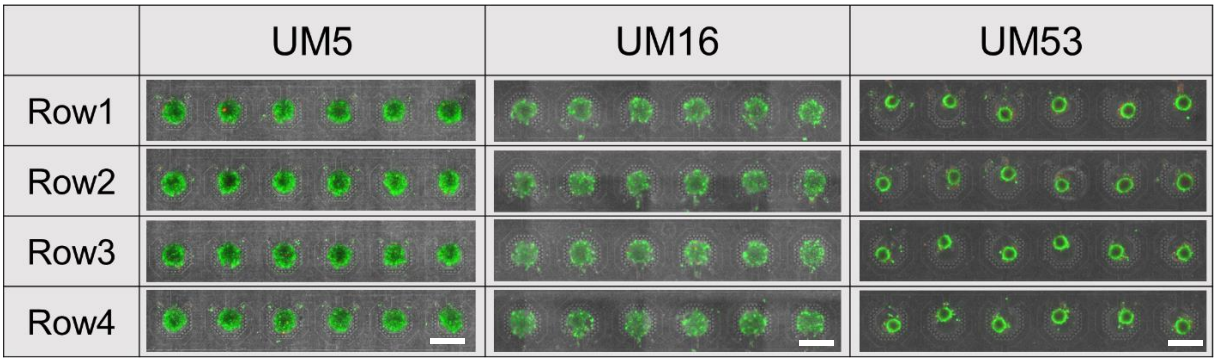

Figure S1. Spheroids of the media control groups in all rows across three pancreatic PDX cell lines, UM5, UM16, and UM53, after 4 days culture, showing uniform viability and spheroid size in each cell line). (Scale bar = 300 μm)

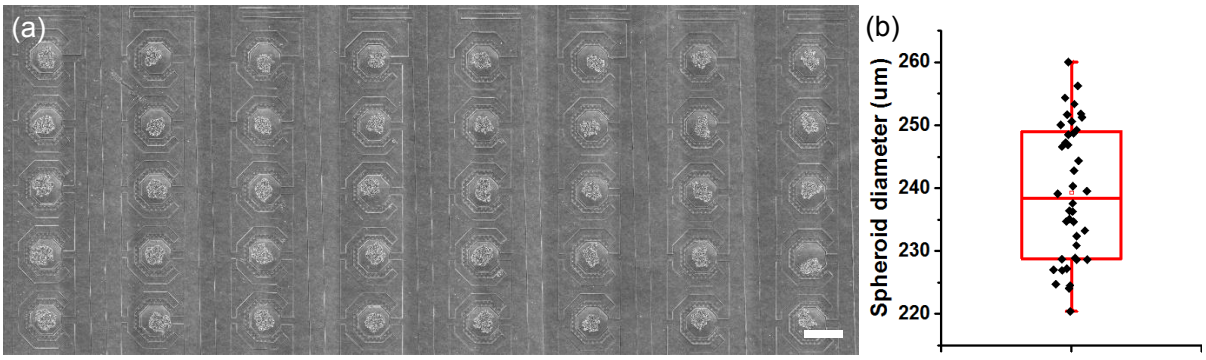

Figure S2. (a) A microscope view containing 40 SUM159 spheroids showing uniform spheroids size. (b) Scattering plot showing a small variation of < 4.4% in the diameter size. (Scale bar = 300 μm)

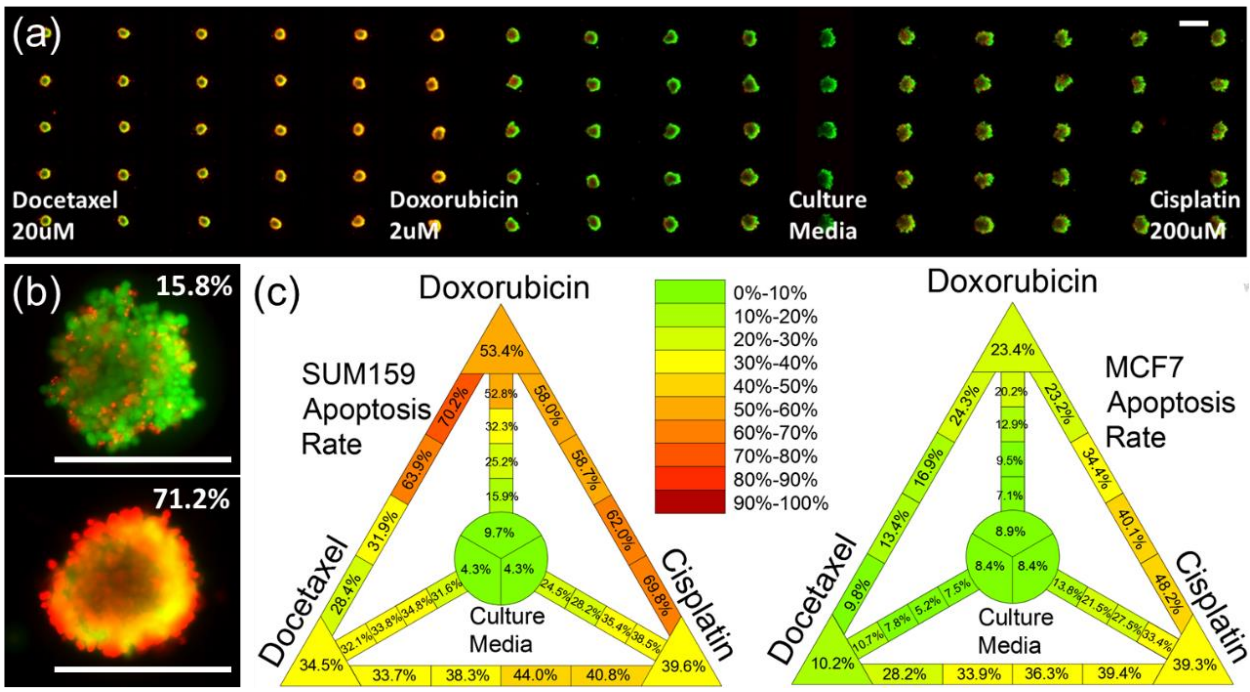

Figure S3. SUM159 and MCF7 drug combination susceptibility test. (a) Using 20 μM docetaxel, 2 μM doxorubicin, culture media, and 200 μM cisplatin. Live (green) / dead (red) staining are used after drug treatment for cell viability readout. (b) Example images of 2 spheroids with death rate of 15.8% and 71.2% measured by custom software, respectively. (c) Heterogeneous drug response between SUM159 and MCF7 breast cancer cell lines. Cell death rate under certain drug treatment condition is quantified using different color. SUM159 is susceptible to doxorubicin + docetaxel and doxorubicin + cisplatin, while MCF7 is only sensitive to doxorubicin + cisplatin. (Scale bar = 300 μm)

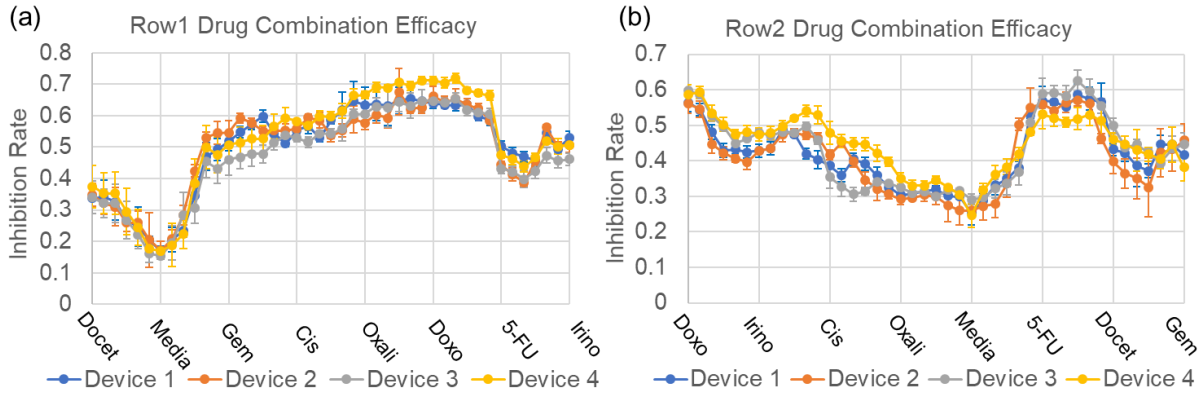

Figure S4. Chip-to-chip variance characterization experiment. We repeated drug combination screening experiments on breast cancer cell line SUM159, using 4 fabricated chips under identical treatment conditions. (a) Drug combination screening result in row 1. The average standard deviation among 4 devices is 3.11%, with maximum standard deviation of 5.26% and minimum of 0.79%. (b) Drug combination screening result in row 2. The average standard deviation among 4 devices is 3.36%, with maximum standard deviation of 5.54% and minimum of 0.70%.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
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25	23	27	21	29	19	31	17	33	15	35	13	37	11	39	9	41	7	43	5	45	3	47	1	49	2	50	4	48	6	46	8	44	10	42	12	40	14	38	16	36	18	34	20	32	22	30	24	28	26

Figure S5. A "sudoku puzzle" with 50 by 50 entries, guaranteeing all the "horizontal adjacent pairs" are non-repeating while covering all the possible pairwise combinations.

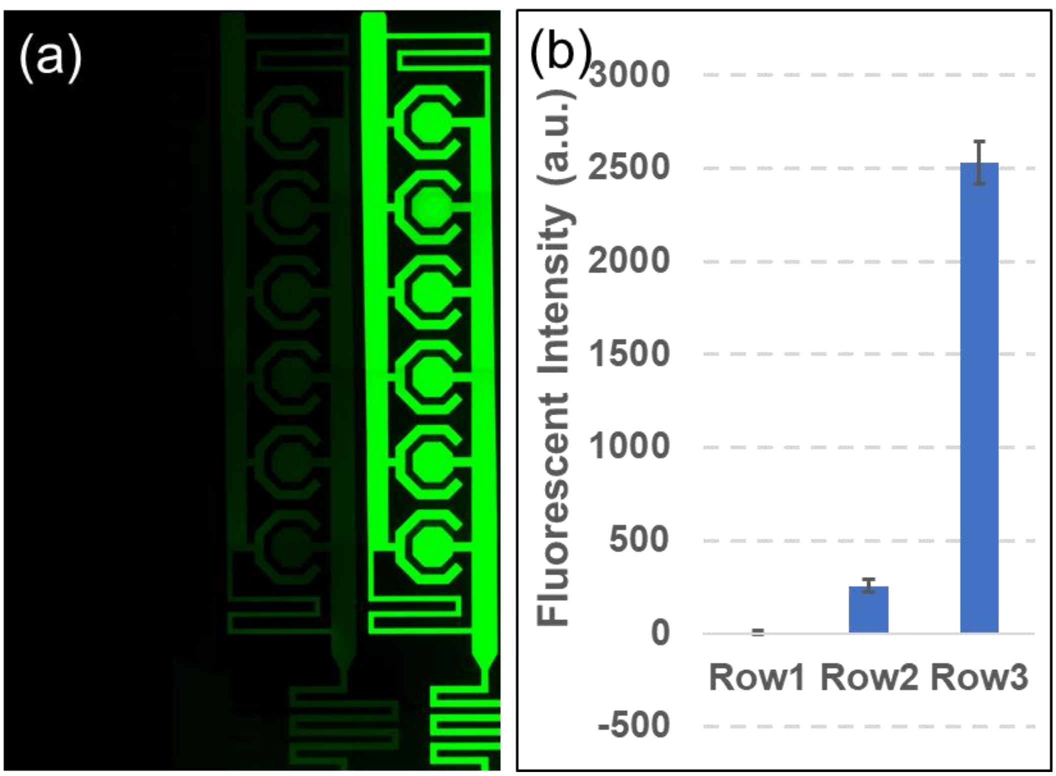

Figure S6. Validation of logarithmic mixing using long fluorescence exposure time of 300ms. (a) Zoom-in microscopy image of the channels with PBS + Fluorescein mixed in 100%:0%, 99%:1%, and 90%:10%. (b) Fluorescent intensity measurement of the PBS + Fluorescein channels with long exposure time, verifying that the fluorescein concentration ratio is 0:1:10

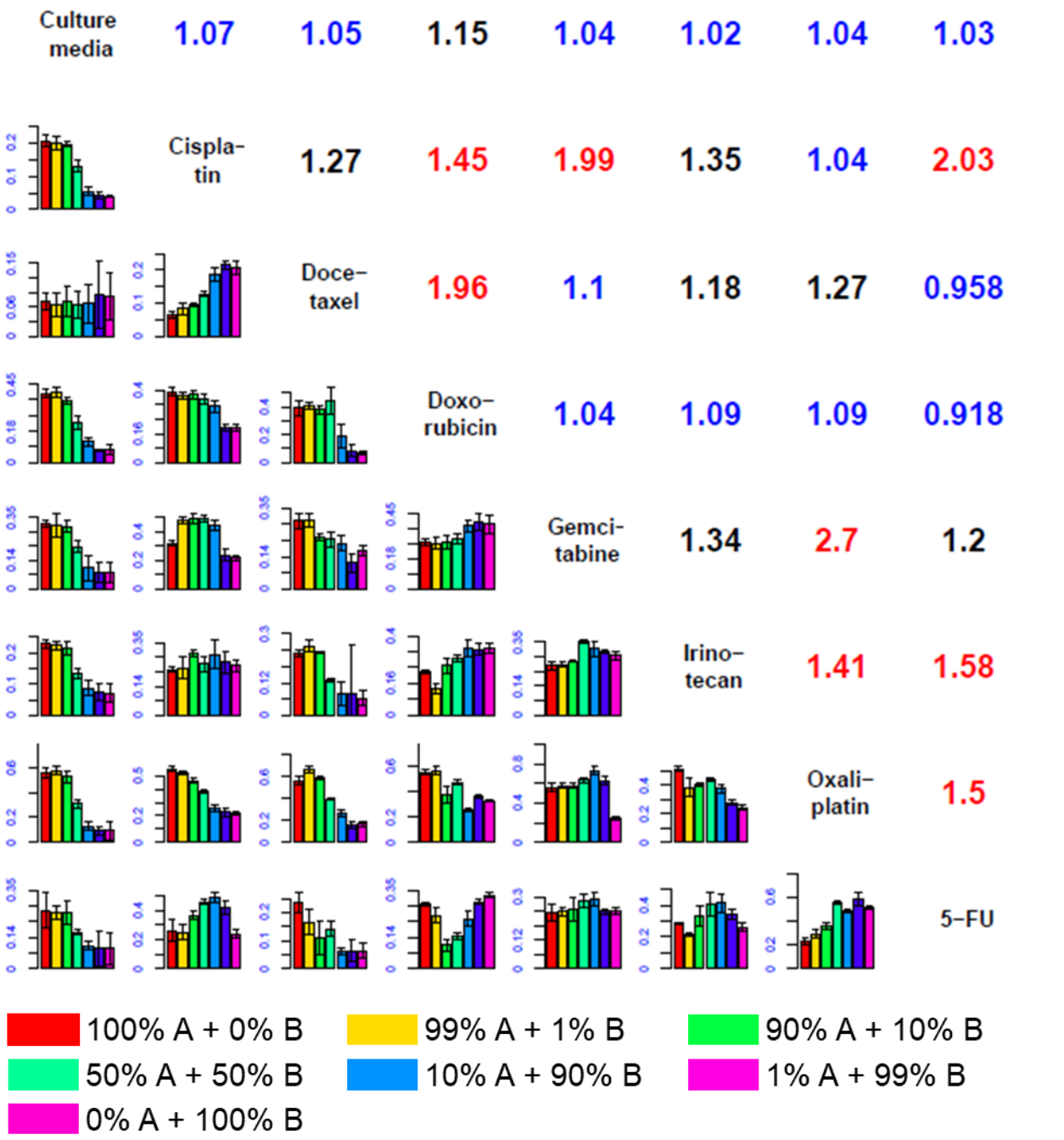

Figure S7. Drug combination screening results of UM5 using 7 commonly used chemo-drugs and control (culture media). With the name of all compounds are denoted at diagonal entries, each subplot illustrates the cell death rate under the combination of drugs at each corresponding row and column. Maximum Synergistic Index (MSI) is denoted at upper triangle table. Highly synergistic pairs are highlighted in red (MSI > 1.4), while non-synergistic ones are highlighted in blue (MSI < 1.1).

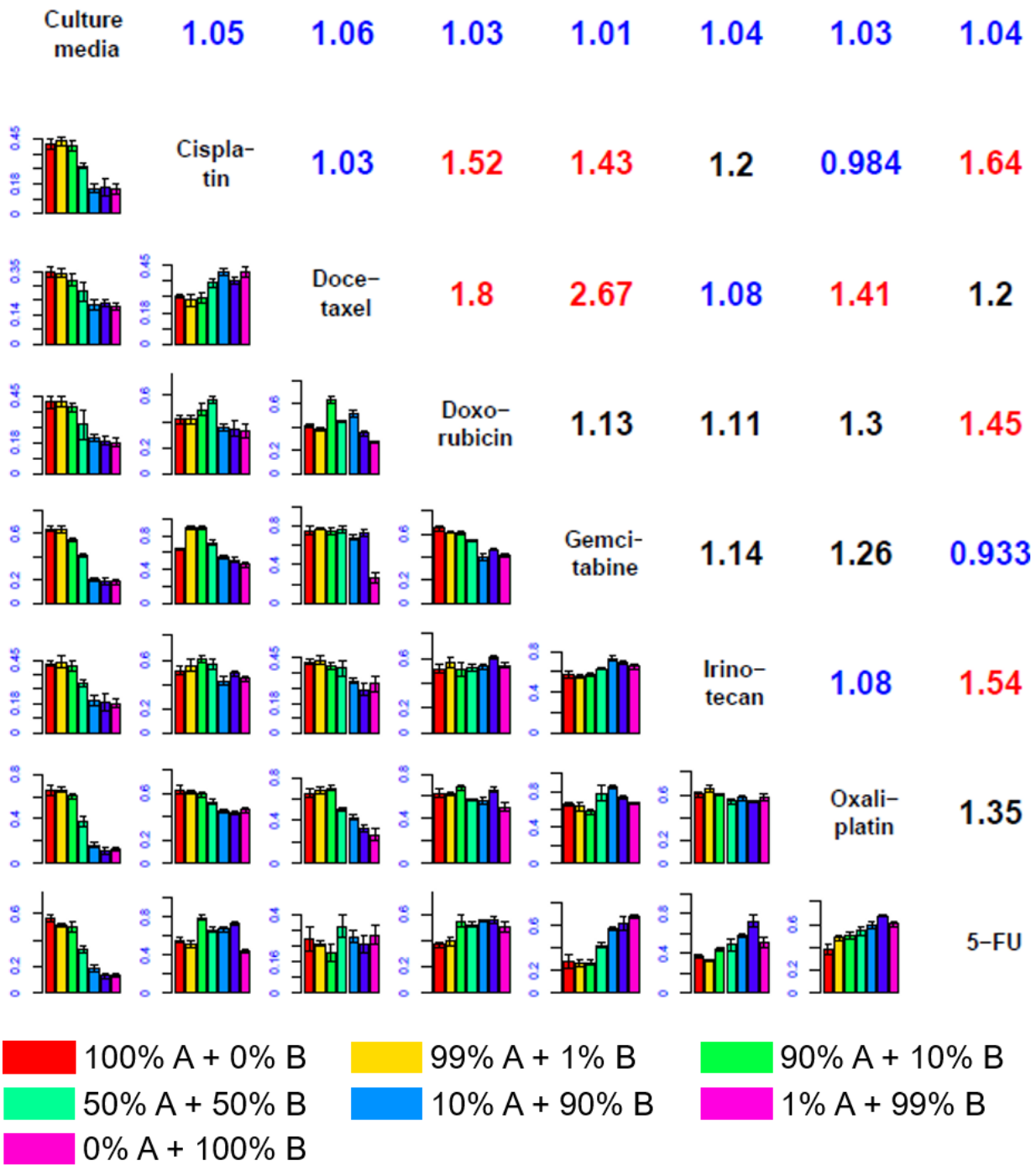

Figure S8. Drug combination screening results of UM16 using 7 commonly used chemo-drugs and control (culture media). With the name of all compounds are denoted at diagonal entries, each subplot illustrates the cell death rate under the combination of drugs at each corresponding row and column. Maximum Synergistic Index (MSI) is denoted at upper triangle table. Highly synergistic pairs are highlighted in red (MSI > 1.4), while non-synergistic ones are highlighted in blue (MSI < 1.1).

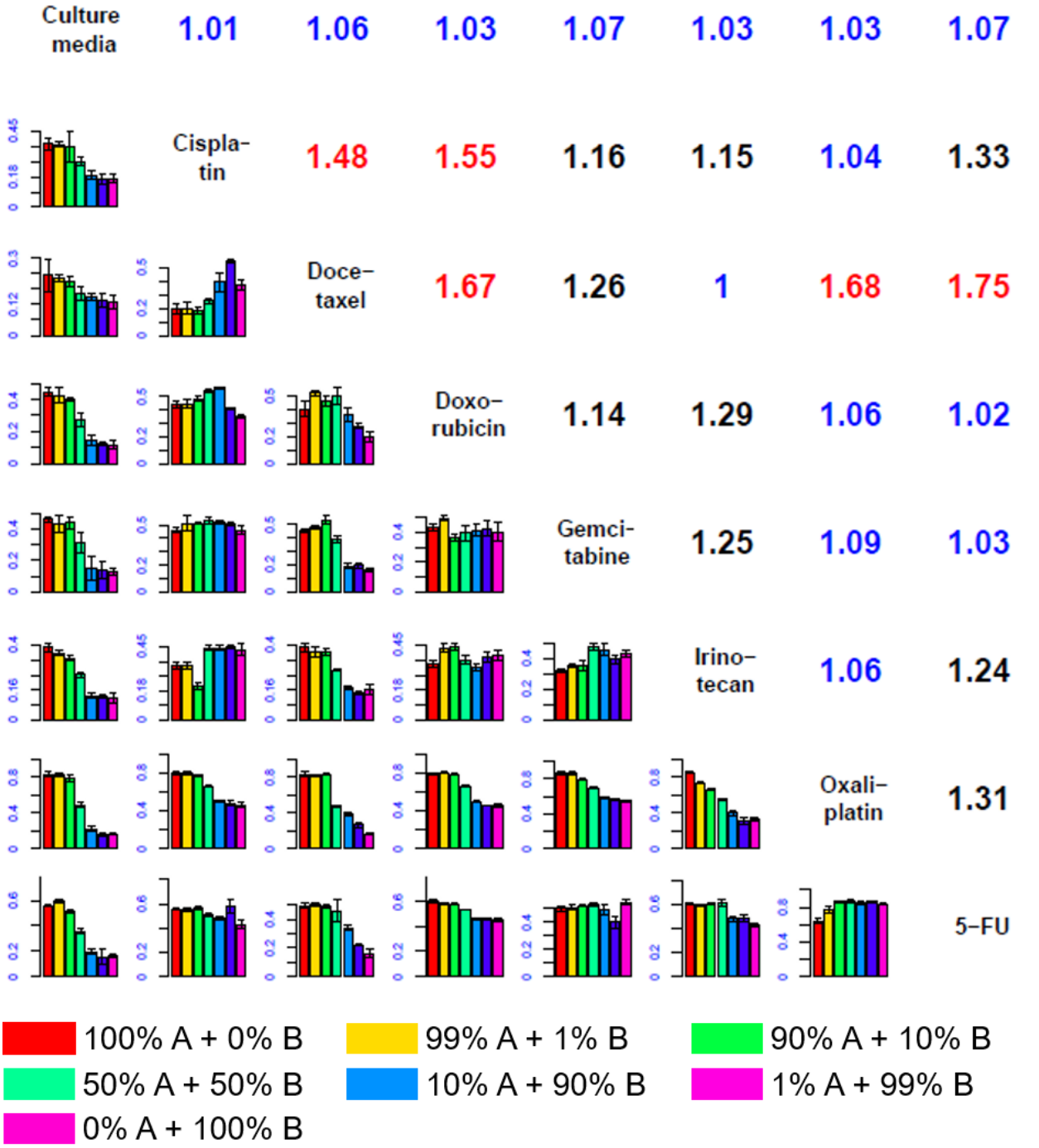

Figure S9. Drug combination screening results of UM53 using 7 commonly used chemo-drugs and control (culture media). With the name of all compounds are denoted at diagonal entries, each subplot illustrates the cell death rate under the combination of drugs at each corresponding row and column. Maximum Synergistic Index (MSI) is denoted at upper triangle table. Highly synergistic pairs are highlighted in red (MSI > 1.4), while non-synergistic ones are highlighted in blue (MSI < 1.1).

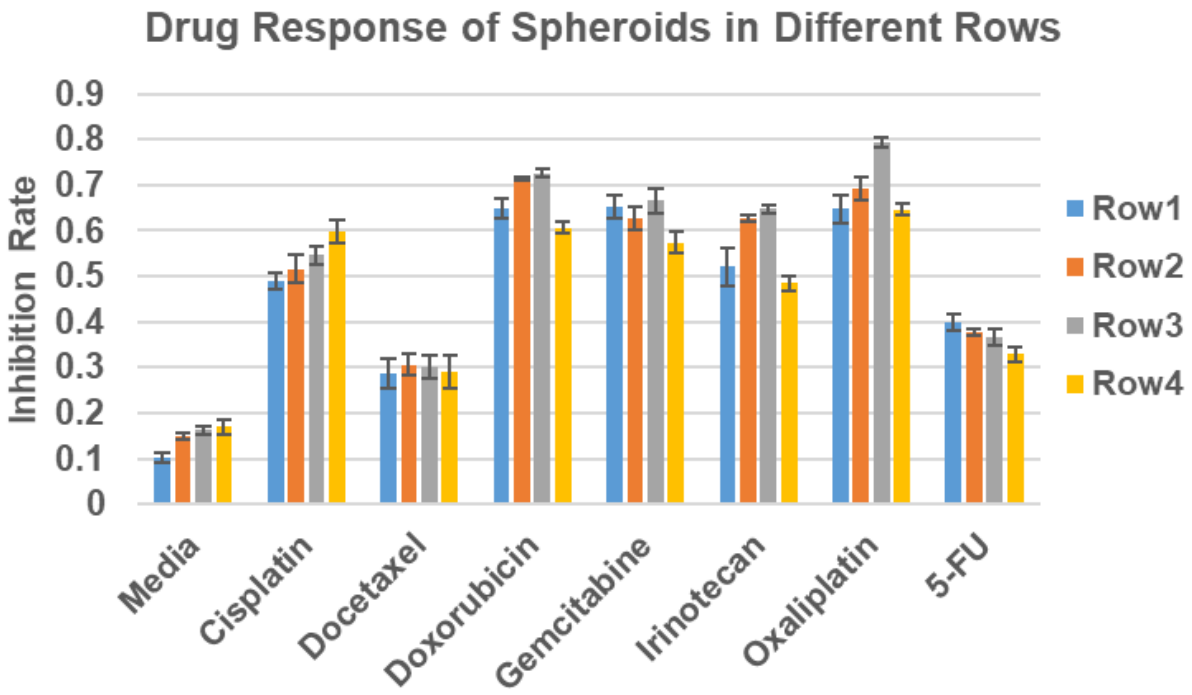

Figure S10. Drug response of MIA PaCa-2 spheroids in different rows. The small difference of drug inhibition rates among different rows indicates that the variation is negligible. (N=6 for each bar)

Table. S1. Mean drug response of spheroids in different rows.

	Row1	**Row2**	**Row3**	**Row4**	**Ave.**	**Std. Dev.**
Media	0.102979	0.148457	0.16246	0.169489	0.145846	0.025881
Cisplatin	0.489244	0.516173	0.546117	0.599366	0.537725	0.040881
Docetaxel	0.288366	0.306008	0.301793	0.289793	0.29649	0.007576
Doxorubicin	0.649611	0.712915	0.725218	0.606656	0.6736	0.048133
Gemcitabine	0.65291	0.626685	0.665292	0.573745	0.629658	0.035162
Irinotecan	0.520501	0.62722	0.647198	0.484306	0.569806	0.068969
Oxaliplatin	0.64777	0.692494	0.792823	0.646332	0.694855	0.059529
5-FU	0.398503	0.377241	0.36568	0.329774	0.3678	0.024911

Supplementary Document 1. Microfluidic tree structure as log-scale concentration gradient generator

In conventional "Christmas tree" concentration gradient generator, solutions containing different compounds are introduced from the top inlets and flowed through the microchannel network. The fluid streams are combined in each branch channel stage, yielding mixture of distinct compositions, and split to next stage. Finally, a concentration gradient is generated across the last stage of branch channel [1]. The splitting ratio of the flow at each stage is determined by the flow resistance, i.e. dimension of the meander channels, which could be understood with an equivalent "electronic circuit" model. Each microfluidic channel in this "Christmas tree" could be regarded as a resistor in circuit, while the fluid flow can be seen as electric current. As is shown in Figure 1, two different solutions, drug A and drug B, are introduced through I_{01} and I_{02}. We ignore the flow resistance of all horizontal channels, since they are designed to be two orders of magnitude smaller than that of the vertical channels. According to Ohm's law, we can derive the current flowing through the middle channel stage,

$$I_{11} = \frac{R_{12}}{R_{11}+R_{12}} \cdot I_{01} \qquad (\#1)$$

$$I_{12} = \frac{R_{11}}{R_{11}+R_{12}} \cdot I_{01} + \frac{R_{13}}{R_{12}+R_{13}} \cdot I_{02}$$

$$I_{13} = \frac{R_{12}}{R_{12}+R_{13}} \cdot I_{02}$$

If we assume the microfluidic structure is symmetric, and we applied the same pressure to drive drug A and drug B. We have $I_{11} = I_{13}$, and $I_{12} = 0.5\, I_{01} + 0.5\, I_{02}$. The concentration flowing through any resistor is given by:

$$C_{mixer} = \frac{\sum_{i=1}^{n} I_i \cdot C_i}{\sum_{i=1}^{n} I_i}$$

We apply the same analysis for the next channel stage,

$$I_{21} = \frac{R_{22}}{R_{21}+R_{22}} \cdot I_{11}$$

$$I_{22} = \frac{R_{21}}{R_{21}+R_{22}} \cdot I_{11} + \frac{R_{23}}{R_{22}+R_{23}} \cdot I_{12} \qquad (\#2)$$

$$I_{23} = \frac{R_{22}}{R_{22}+R_{23}} \cdot I_{12} + \frac{R_{24}}{R_{23}+R_{24}} \cdot I_{13}$$

$$I_{24} = \frac{R_{23}}{R_{23}+R_{24}} \cdot I_{13}$$

If all the microfluidic channels are designed to be of the same size (width, height, length), a linear concentration gradient will be finally established in the last stage. However, in some cases, drug combination therapy could be most effective when the concentration ratio of the two drugs is 1:100, or even smaller. In conventional multi-stage mixer systems, the compound concentration only covers one order of magnitude. Larger concentration ranges are required to obtain a comprehensive drug efficacy screening.

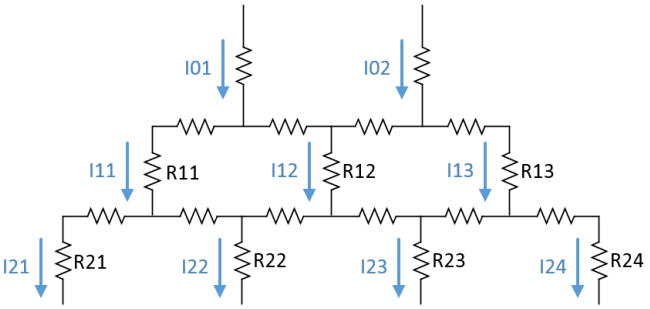

Figure S6. Equivalent electronic circuit schematic for "Christmas tree" concentration gradient generator system. Each resistor stands for a microfluidic channel. Electric current, for example, "I21" stands for the fluid flow through the channel "R21".

We proposed a "biased tree" structure with non-uniform channels sizes to achieve a log-scale mixing ratio gradient between two different compounds. From equation (#2), there are 2 components in I_{22}: I_{11} and I_{12}, among which I_{12} contains 50% drug A and 50% drug B, while I_{11} contains 100% drug A. In order to decrease the concentration of drug B in I_{22}, we need to increase the flow from I_{11} through R_{22}, while decreasing the flow from I_{12} through R_{22}. According to equation (#1), I_{11} is proportional to $\frac{R_{12}}{R_{11}+R_{12}}$. We could increase I_{11} by increasing R_{12} and decreasing R_{11}. The same method applies to I_{22}, that it requires increasing R_{12} and decreasing R_{13}. According to Hagen-Poiseuille equation, hydraulic resistance of a channel is approximately inversely proportional to squared channel width [2]. Therefore, the channels for R_{11} and R_{13} are designed to be wider than that for R_{12}. Similar calculations could also be applied to the following channel stages where more branch channels are incorporated. In general, channels on both sides are designed to be wider than those at the center. Therefore, the flow of certain compounds will be mostly guided to its own side, with only a small portion mixed with the other compound. In this way, various concentration ratio, as large as 1:1E6, could be obtained at mixer outlets.

Supplementary Document 2. Detailed Description of a General Solution to Adjacency Sudoku Problem

Since all the requirements are made on number adjacency relationship, we introduce an "adjacency matrix" to help filling in the table when the number goes up. Take the adjacency matrix for N = 6 as example:

	1	2	3	4	5	6
1	x					
2		x				
3			x			
4				x		
5					x	
6						x

There are $6 \times 6 = 36$ entries in this adjacency matrix. Each entry represents the existence of certain adjacent number pairs in the original Sudoku table. For example, if we put an "x" in the entry located at row2 colomn3, it means that the combination of "2-3" already exist in the original Sudoku table. Similarly, entry in row5 colomn1 means that "5-1" is covered. In this case, if all the entries in this "adjacency matrix" is filled with a "x", its corresponding Sudoku table is a good solution. It is obvious that the diagonal entries don't exist, so we ignore those entries.

To fill in this table, we first assume this n-by-n array should be symmetric. In this way, it is convenient to guarantee “a-b” and “b-a” appear at the same time. Also, it is easy to proof that the first row and first column are arbitrary. To make it simple, we fill in number 1~6 in original Sudoku table, together with its corresponding adjacency matrix as follows:

1	2	3	4	5	6
2					5
3					4
4					3
5					2
6	5	4	3	2	1

	1	2	3	4	5	6
1	x	a				
2	a	x	a			
3		a	x	a		
4			a	x	a	
5				a	x	a
6					a	x

We keep filling in the entries in third line along diagonal direction.

1	2	3	4	5	6
2	4			3	5
3	1			6	4
4	6			1	3
5	3			4	2
6	5	4	3	2	1

	1	2	3	4	5	6
1	x	a	b			
2	a	x	a	b		
3	b	a	x	a	b	
4		b	a	x	a	b
5			b	a	x	a
6				b	a	x

Taking advantage of the symmetry of the table, we could derive:

1	2	3	4	5	6
2	4	1	6	3	5
3	1			6	4
4	6			1	3
5	3	6	1	4	2
6	5	4	3	2	1

	1	2	3	4	5	6
1	x	a	b	c		c
2	a	x	a	b		
3	b	a	x	a	b	c
4	c	b	a	x	a	b
5			b	a	x	a
6	c		c	b	a	x

Finally, we fill in the 5th line along diagonal direction, we could get the full solution to 6 by 6 adjacency Sudoku table as follows:

1	2	3	4	5	6
2	4	1	6	3	5
3	1	5	2	6	4
4	6	2	5	1	3
5	3	6	1	4	2
6	5	4	3	2	1

	1	2	3	4	5	6
1	a	a	b	c	d	c
2	a	x	a	b	e	d
3	b	a	x	a	b	c
4	c	b	a	x	a	b
5	d	e	b	a	x	a
6	c	d	c	b	a	x

The same method using “adjacency matrix” could be applied to get any arbitrary N numbers. As an example, an “sudoku puzzle” with 50 by 50 entries is attached.

Reference:

[1] Chung, Bong Geun, et al. "Human neural stem cell growth and differentiation in a gradient-generating microfluidic device." *Lab on a Chip* 5.4 (2005): 401-406.
[2] Mortensen, Niels Asger, Fridolin Okkels, and Henrik Bruus. "Reexamination of Hagen-Poiseuille flow: Shape dependence of the hydraulic resistance in microchannels." *Physical Review E* 71.5 (2005): 057301.