

# Cell based high-throughput screening of cell-cell adhesion inhibitors: approaches to develop a new combination therapy for reversing multicellular resistance.

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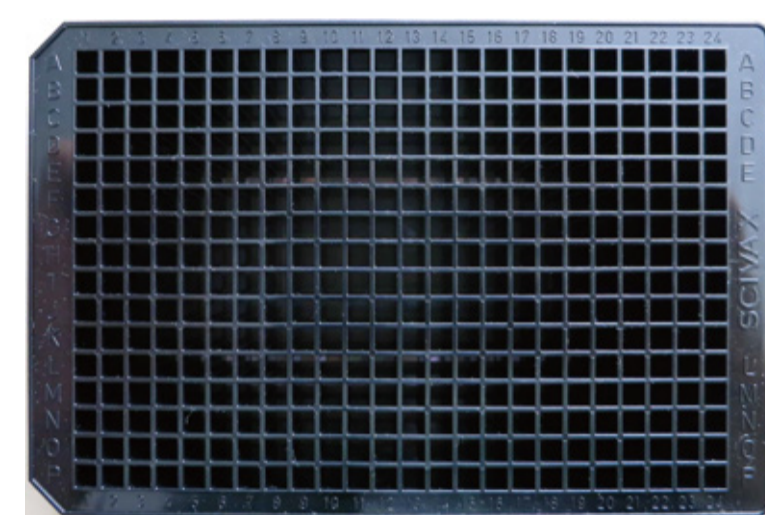


## Abstract

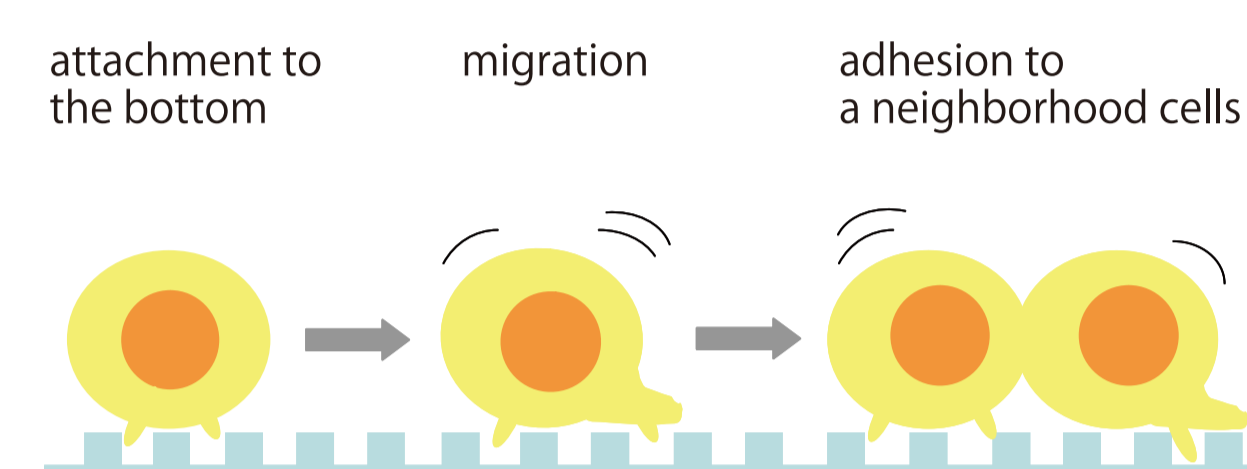
Solid tumors are more resistant to chemo- and radio-therapy than *in vitro* cancer cells under conventional monolayer culture condition. This phenomenon can be explained by "multicellular resistance" (MCR), and recently, therapeutic approaches increasing effect of chemo- and radio-therapy by preventing MCR are proposed. The mechanism of MCR is 1) rising anti-apoptotic ability, which is attributed to cell-cell contacts and cell-matrix contacts, 2) the three-dimensional structure of tissue, conduces to a difficulty of drug penetration and heterogeneous cell populations. In light of this, it is considered that one of approach for keeping cancer cells from acquired MCR is inhibition of cell-cell/cell-matrix adherence. However, as a lot of molecule is involved in cell-cell and cell-matrix adhesion, it is difficult to screen the MCR preventing compounds, which can work effectively *in vivo*, by *in vitro* protein-protein interaction assay. Multicellular spheroids are three dimensional *in vitro* microscale tissue analogs, and also recapture MCR phenomenon. Herein, we tried to develop a new cell based assay, based on spheroid culturing system and monitoring method of degree of cell-cell adherence, for exploring compounds capable of reversing MCR. In this report, we indicate novel high throughput screening method of cell-cell adherence inhibitors, and several hit compounds selected as candidates of chemosensitizing agents.

## Cells migrate, adhere to each other and then form spheroids in NanoCulture® Plate

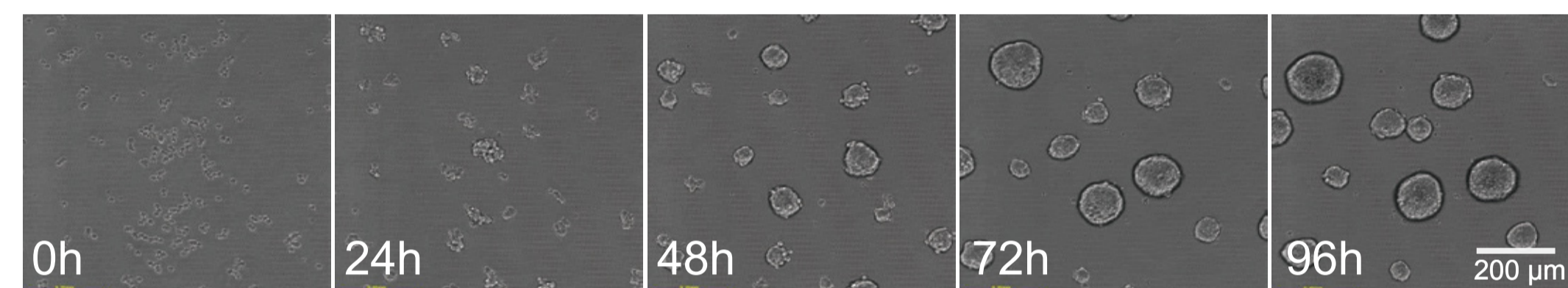
### NanoCulture® Plate (384 well plate)



### Schematic diagram of spheroid formation on NCP

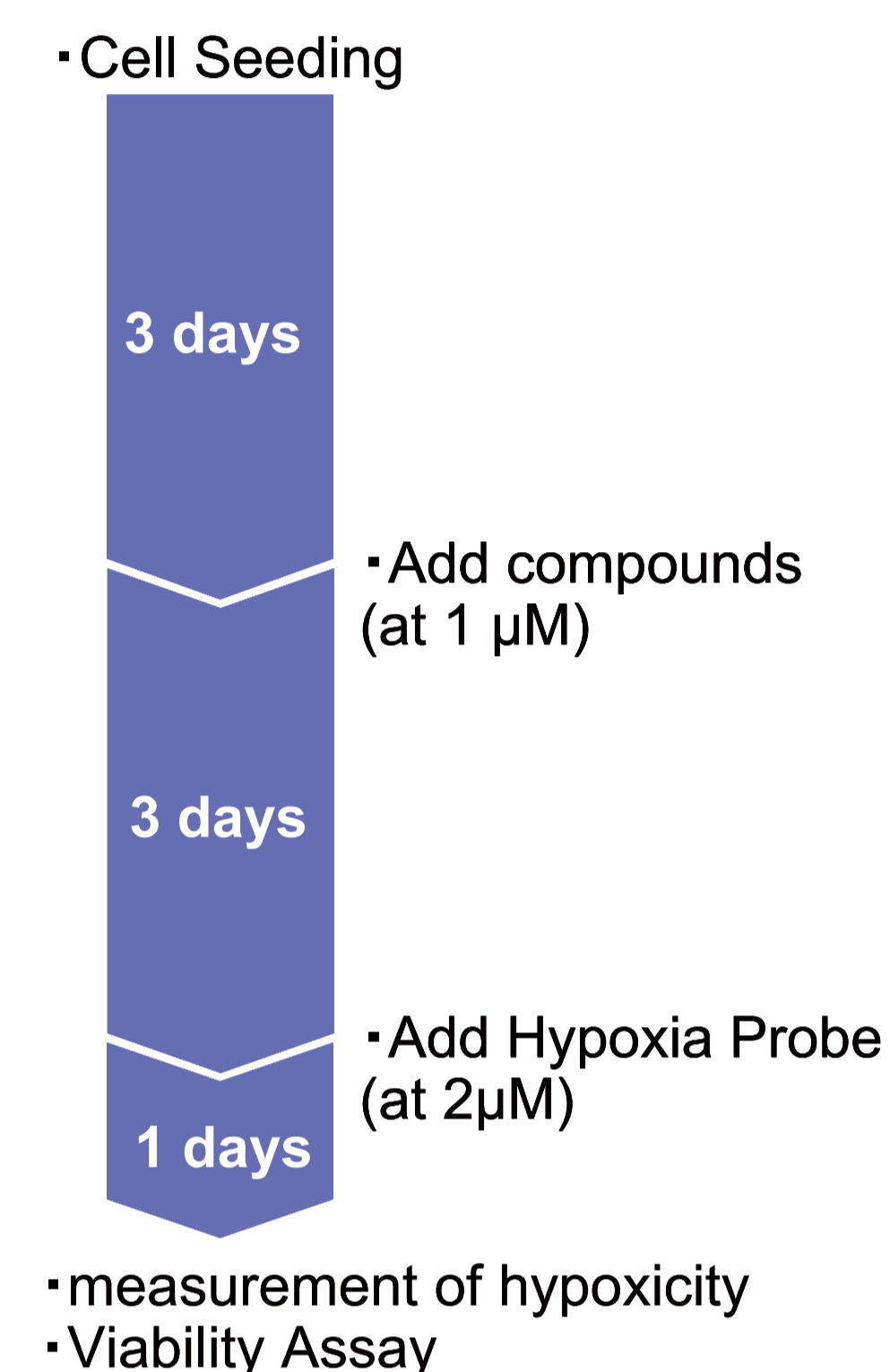


### Time Lapse images of spheroid formation on NCP

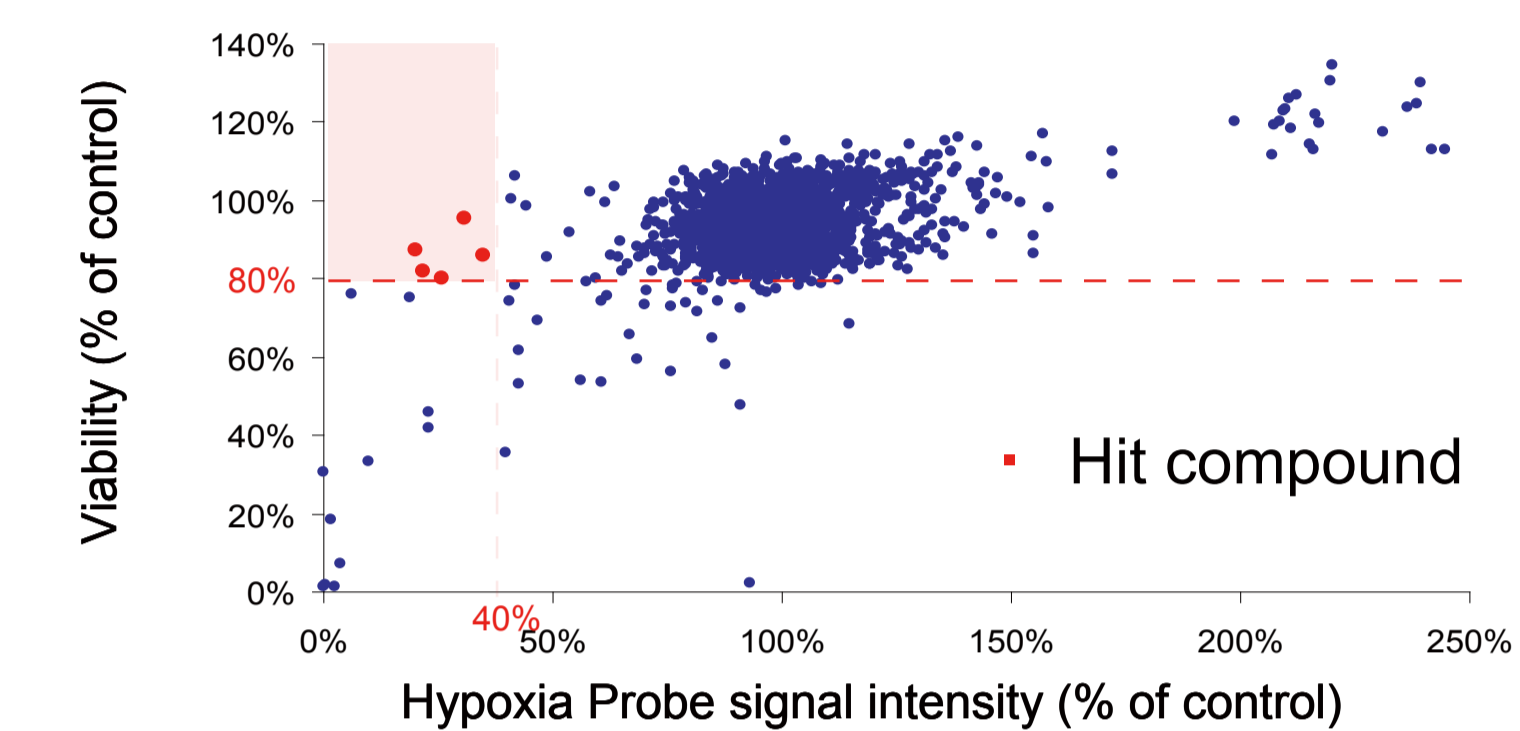


## Development of screening system of cell-cell adhesion inhibitor, and a pilot screening

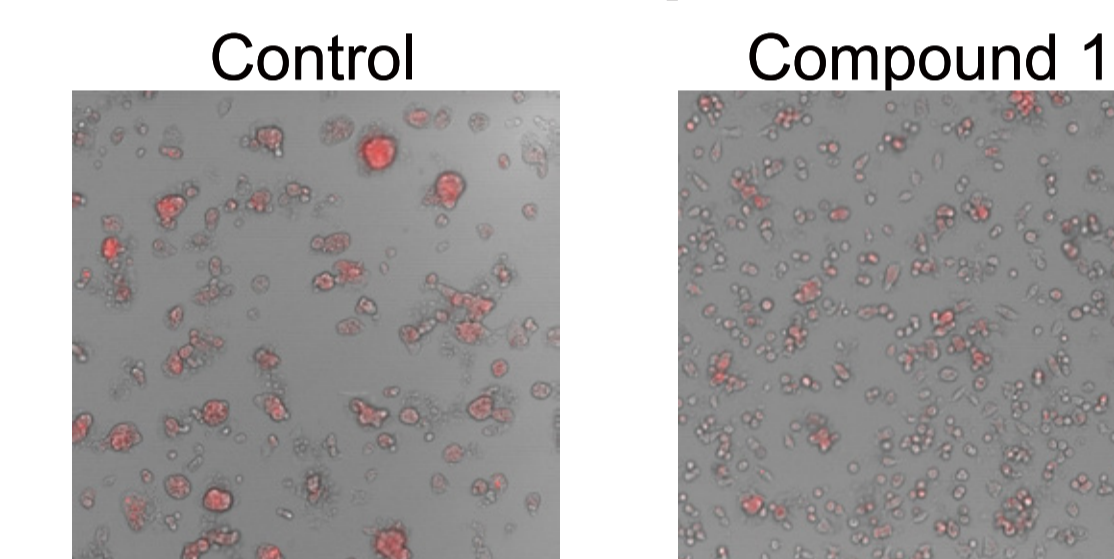
### Screening schedule



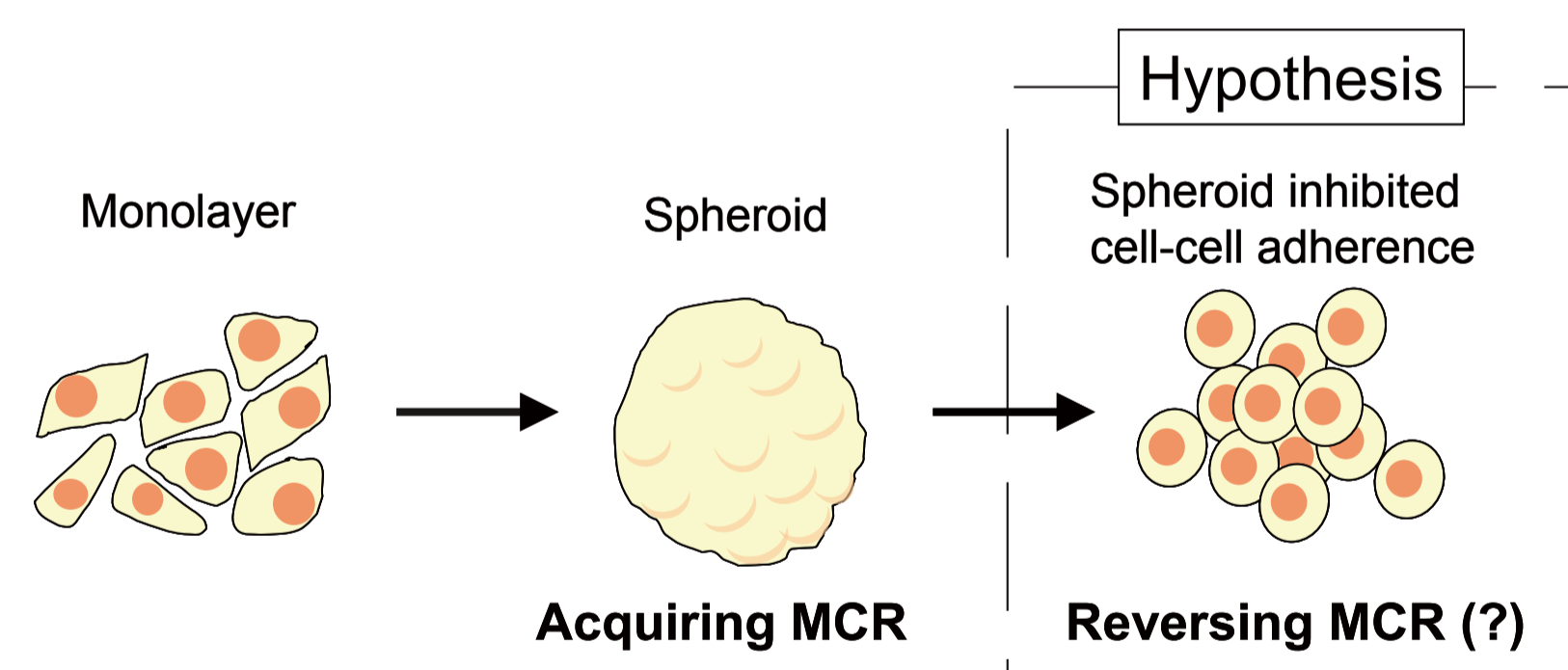
### 5 hits from 1330 chemical compounds



### Phenotype of spheroids which were treated with compound 1



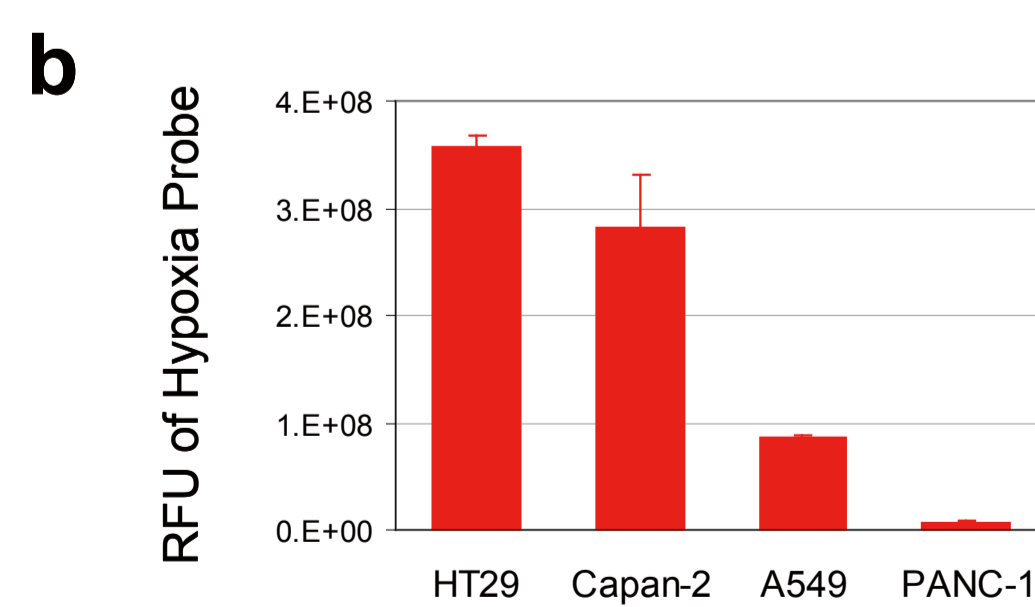
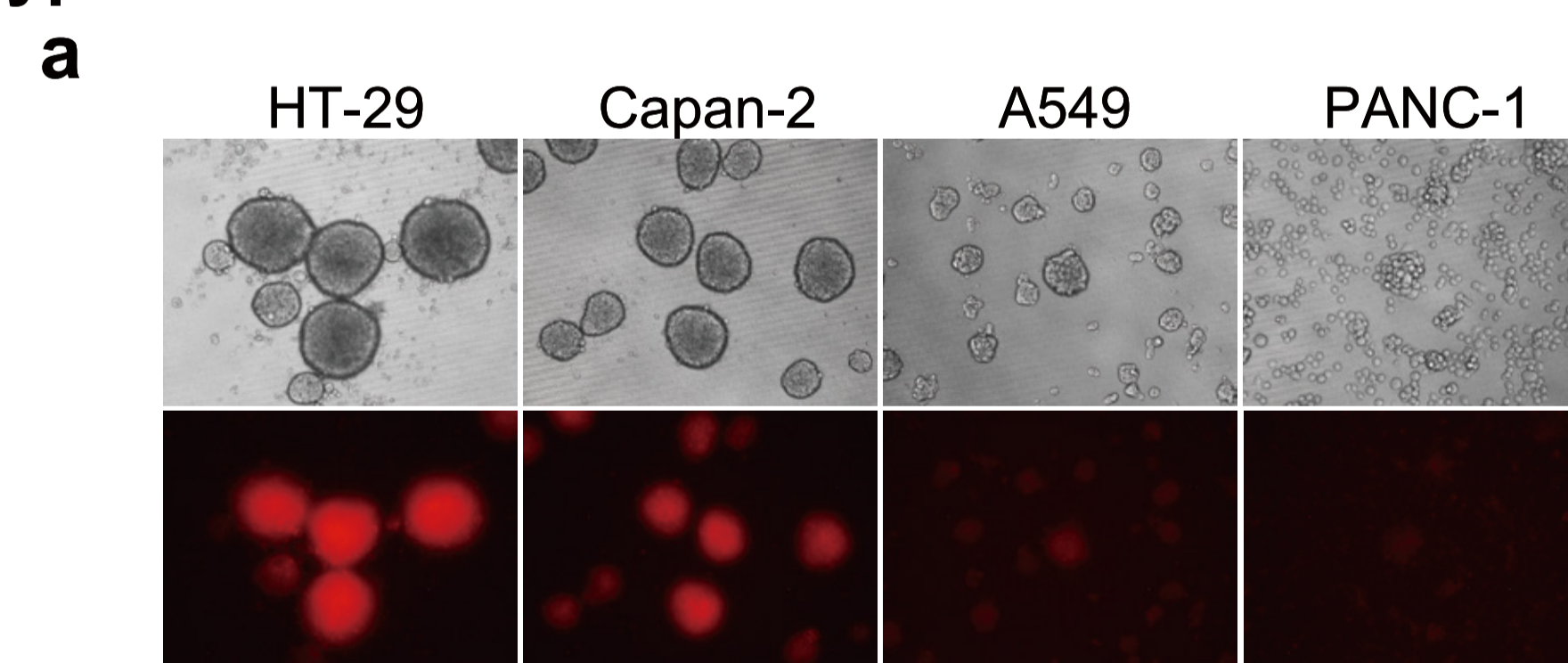
## Hypothesis and Purposes



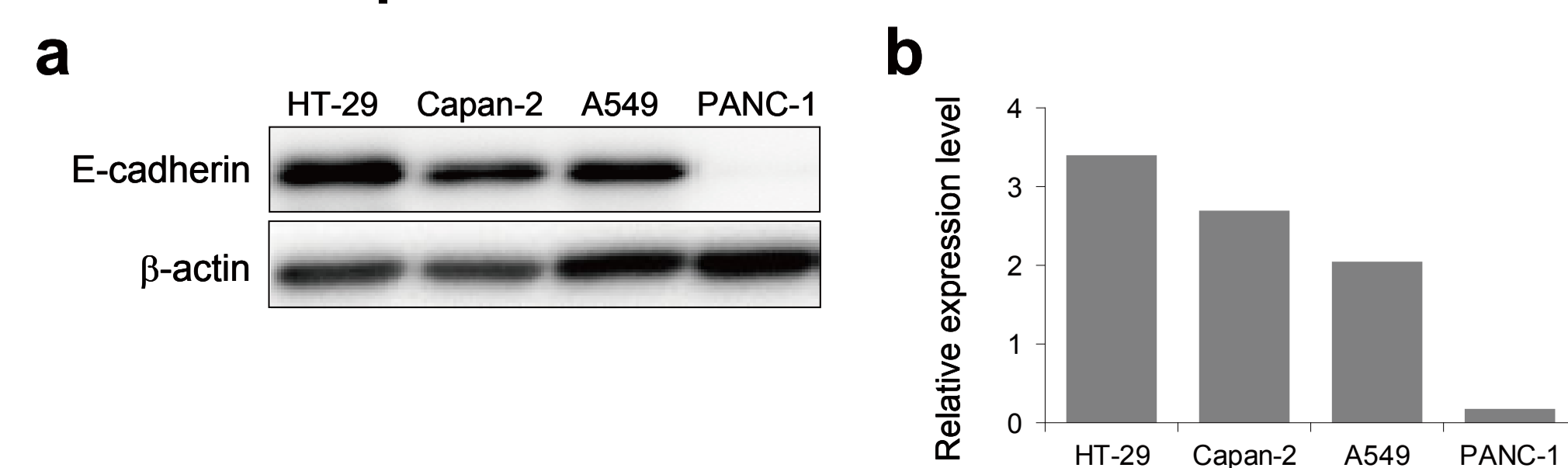
- Purpose**
- Development a screening methods of cell-cell adhesion inhibitors
  - Screening cell-cell adherence inhibitors which candidates of chemosensitizing agents.

## Hypoxia Probe is capable of an indicator of cell-cell adherence of spheroid

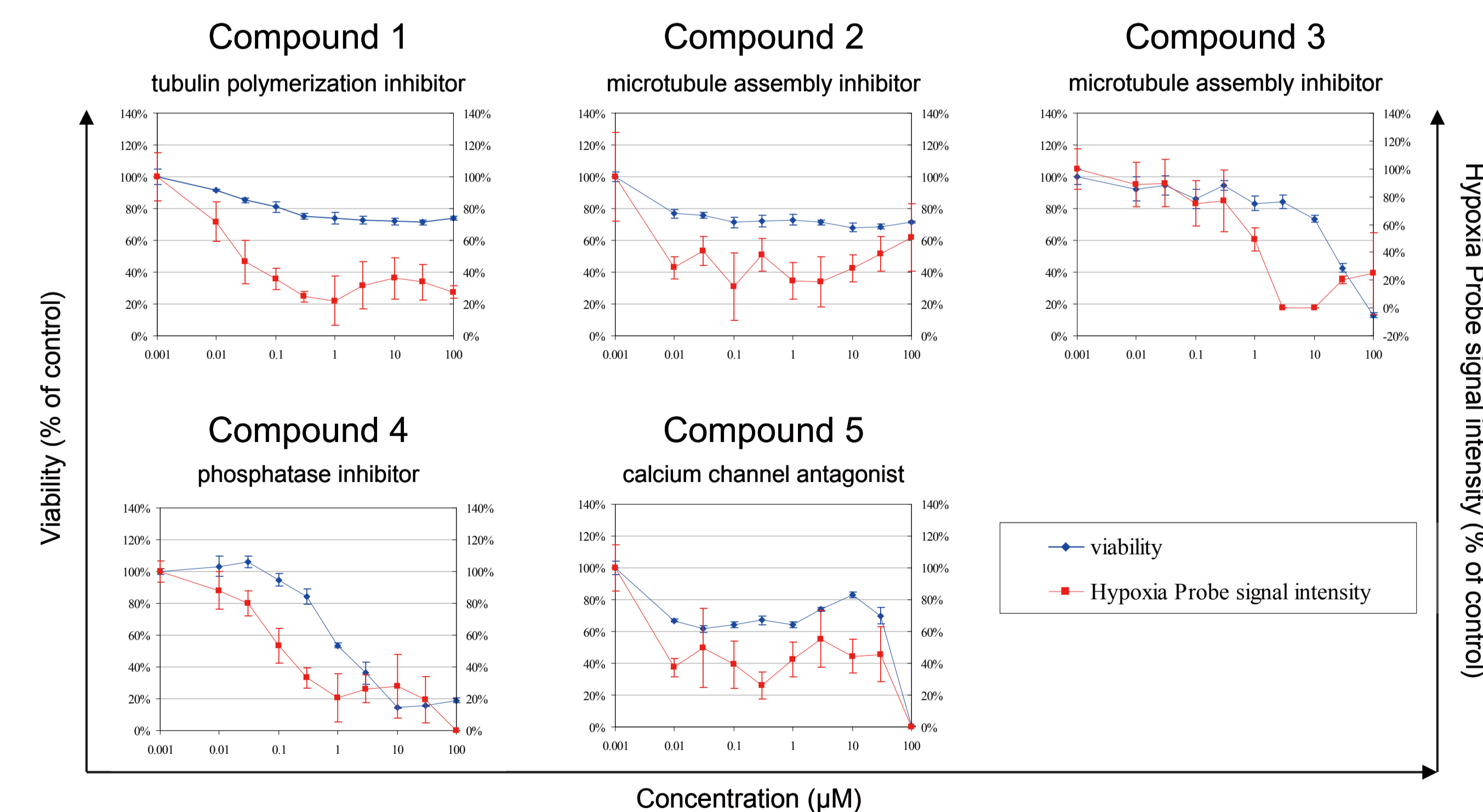
### Spheroids of 4 cancer cell lines and intra-spheroid hypoxia



### E-cadherin expression levels of 4 cancer cell lines



## Validation of 5 hit compounds from pilot screening



## Methods

### 3D cell culture

We adopted 3D cell culture method on NanoCulture® Plate (SCIVAX USA Inc.), a scaffold type spheroid culture plate, as a culture method which can confirm spheroid morphologies.

### Intracellular Hypoxia sensing

The intra-spheroid hypoxia was visualized using Hypoxia Probe (SCIVAX USA Inc.).

### Evaluation of the cell-cell adhesion status

Fluorescence intensity of the Hypoxia Probe of intra-spheroid in whole well was detected using Celigo® Imaging Cytometer (Brooks Life Science Systems), as an indicator for the cell-cell adhesion status.

## conclusions

- We developed a novel high throughput screening method of cell-cell adherence inhibitors.
- Five hit compounds were acquired from pilot screening.
- We are now evaluating these compounds as chemosensitizing agents.