Supporting Information for:

## Generation of Co-Culture Cell Micropattern Model to Simulate Lung Cancer Bone Metastasis for Anti-Cancer Drug Evaluation

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Fig. S1. Mechanical transducer setup for measuring minimum pressure applied to induce cell lysis.



**Fig. S2.** Representative light microscope images of hMSCs on TCP after pressed for 10 s at the pressure of 49.0 kPa (weight of 200 g). Two random locations were selected. The scale bars are 100  $\mu$ m.



**Fig. S3.** Live/dead assay to evaluate cell viability of hMSCs on TCP after cell micropatterning process. HMSCs were pressed for 10 s under the pressure of 49.0 kPa (a weight of 200 g was used). The scale bars are 100  $\mu$ m.



**Fig. S4.** Representative fluorescent microscope images of hMSCs on casein/chitosan multilayer films before micropatterning process (A) and after micropatterning process (B). The scale bars are 100 µm.



**Fig. S5.** Relative cell viability of A549 and OB after cultured in medium with various concentrations of DOX for 24 h. A549, OB cells were first seeded at a density of  $4 \times 10^5$ ,  $8 \times 10^4$  cells/ml, respectively. After growing for one day, normal medium was exchanged with medium containing DOX and cells were incubated for another 24 h. Then MTT assay was performed to quantify the relative cell viability. Data = mean ± SD; n = 3.



**Fig. S6.** Representative light and fluorescent microscope images of A549, A549/OB and OB cell micropatterns after incubated with DOX at a concentration of 100  $\mu$ g/ml for 0, 6 and 24 h. In each type of cell micropatterns, fluorescence intensity in cells increased with incubation time. For A549/OB co-culture micropattern, it appeared that after cultured in medium with DOX for 6 h, fluorescence intensity in OB cells was stronger than that in A549 cells. The scale bars are 100  $\mu$ m.



**Fig. S7.** Representative light microscope images of (A) A549 cells, (B) A549 cell micropattern, (C) and (D) A549/OB co-culture micropattern, (E) and (F) OB cells after ALP staining. Cell micropatterns of one type of cells and two types of cells were first fabricated using the  $\mu$ -eraser strategy and cultured in normal medium for 4 days. Then ALP staining was conducted to evaluate the expression of ALP. The scale bars are 100 µm.