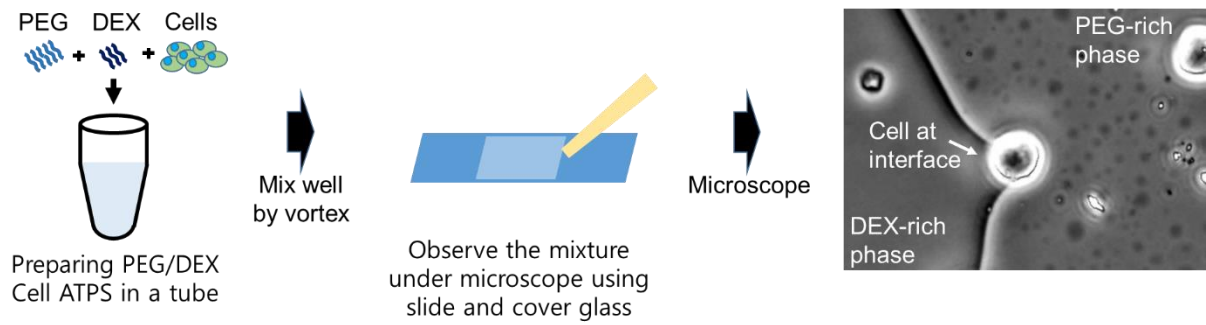


Supplementary Information

Formation and manipulation of cell spheroids using a density adjusted PEG/DEX aqueous two phase system

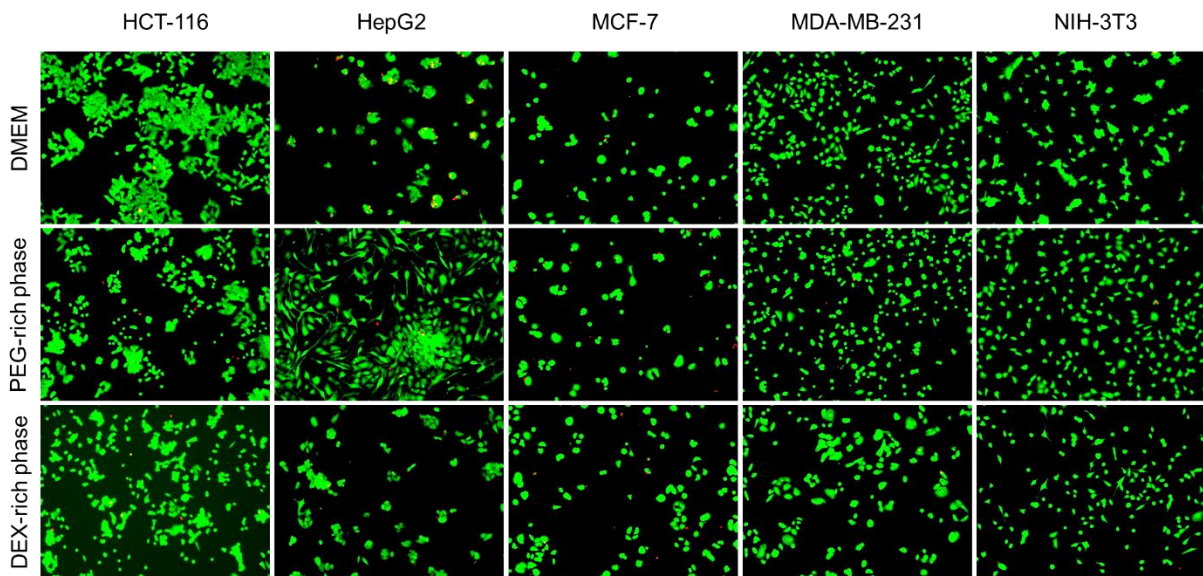
Chungmin Han¹, Shuichi Takayama^{2*} and Jaesung Park^{1,3**}

Figure S1



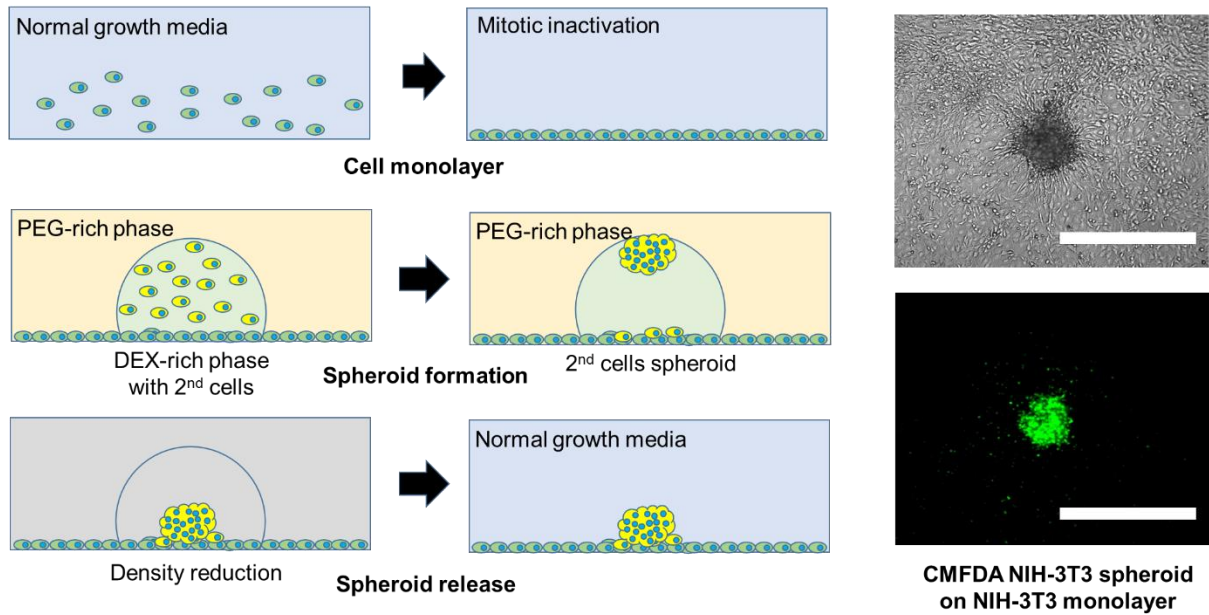
Contact angle measurement. Well-mixed PEG/DEX/cell mixture was observed under phase-contrast microscope. Sample was mount using slide and cover glasses. By chance, a few cells were trapped at PEG/DEX phase interface. Contact angle observation indicated that DEX-cell interactions are more favorable than PEG-cell interactions.

Figure S2



PEG and DEX cytotoxicity determination. HCT-116, HepG2, MCF-7, MDA-MB-231 and NIH-3T3 cells were first cultured with normal growth media, PEG- and DEX-rich phases for 48 hours. After that, cells were stained with calcein AM and ethidium homo-dimer for live and dead analysis. Green (calcein AM) signal indicates live cells and red (ethidium homo-dimer) signal indicates dead cells.

Figure S3



Spheroid-monolayer co-culture system. First, 1st cell types were culture as monolayer in normal growth media. After the culture reaches ~90% confluent, cells were mitotically inactivated by mitomycin c treatment. (left top) Then, 2nd cell types were patterned using DEX-in-PEG ATPS for spheroid formation for 2 days. (left mid) When spheroid was formed, polymer free media was added to the culture to release the spheroid on previously formed 1st cell type monolayer. After 1 day incubation, polymer media was changed to fresh and polymer free media for further analysis. (left bottom) Microscopic picture was taken with CMFDA labeled NIH-3T3 spheroid cultured on non-labeled NIH-3T3 monolayer. (right) Scale bars: 400 μ m

Table 1S. Primers for real time quantitative PCR

Name	Ref. Seq. #	Sequence	Size (bp)	Etc.
Actb (Actin)	NM_007393.3	Fwd- ACGTTGACATCCGTAAAGAC	100	House Keeping
		Rev- GCAGTAATCTCCTTCTGCAT		
Pou5f1 (Oct-3/4)	NM_013633.3	Fwd- GTTGGAGAAGGTGGAACCAA	61	Pluripotency
		Rev- CTCCTTCTGCAGGGCTTTC		
Brachyury	NM_009309.2	Fwd- CAGCCACCTACTGGCTCTA	72	Mesoderm
		Rev- GAGCCTGGGGTGATGGTA		
Hand1	NM_008213.2	Fwd- CAAGCGGAAAAGGGAGTTG	84	Mesoderm
		Rev- GTGCGCCCTTTAATCCTCTT		
Gata4	NM_008092.3	Fwd- GGAAGACACCCCAATCTCG	72	Endoderm
		Rev- CATGGCCCCACAATTGAC		
Sox1	NM_009233.3	Fwd- GTGACATCTGCCCCATC	60	Ectoderm
		Rev- GAGGCCAGTCTGGTGTGTCAG		
Otx2	NM_144841.4	Fwd- AGAATCCAGGGTGCAGGTAT	98	Ectoderm
		Rev- CAGGCCTCACTTTGTTCTGA		