

Supplemental Material

Title:

Collective cell detachment forms multicellular spheroids in ascites that maintain intratumoral heterogeneity during ovarian cancer metastasis.

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Table S1: Primary antibodies used in this study

Figure S1: Epithelial ovarian cancer cells can spontaneously detach as single cells or clusters in 2D culture.

Figure S2: Epithelial ovarian cancer cells can spontaneously detach as single cells or clusters.

Figure S3: Quantification of the size distribution of multicellular clusters isolated from ascites in mice

Video S1: Collective detachment of OV90 cells.

Video S2: Single cell detachment of OV90 cells.

Video S3: Single cell detachment of OV90 cells with fluorescent caspase marker.

Video S4: Collective detachment of OV90 cells with fluorescent caspase marker.

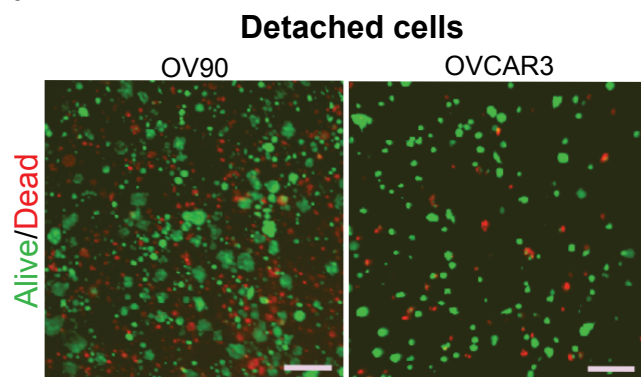
Video S5: Single detachment of OVCAR3 cells with fluorescent caspase marker.

Video S6: Collective detachment of OVCAR3 cells with fluorescent caspase marker.

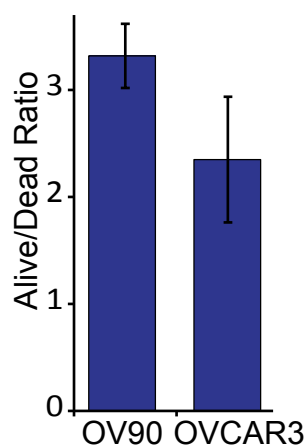
Supplementary Table 1: Primary antibodies used in this study.

Primary antibody	Application	Dilution	Incubation	Company	Catalogue number
Actin	WB	1:500	O/N in 5% milk	Santa Cruz	sc-8432
E-cadherin	WB	1:5000	O/N in 5% milk	CST	3195
ZEB1	WB	1:500	O/N in 5% BSA	CST	3396
Vimentin	WB	1 in 5000	O/N in 5% milk	CST	5741
Pan CK	WB	1:2000	O/N in 5% milk	CST	4545
ZO-1	WB/IF	1:1000	O/N in 5% milk (WB) or 5% GS (IF)	CST	8193
E-cadherin	IF	1:500	O/N in 5% GS	BD transduction	610181
Ki67	IF	1:500	O/N in 5% GS	Abcam	ab15580

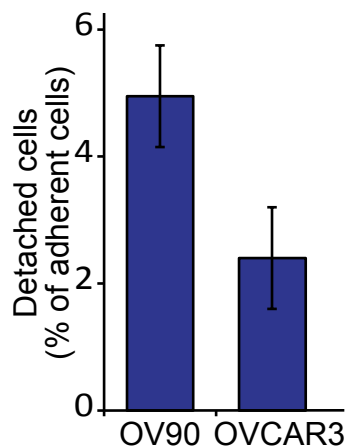
a



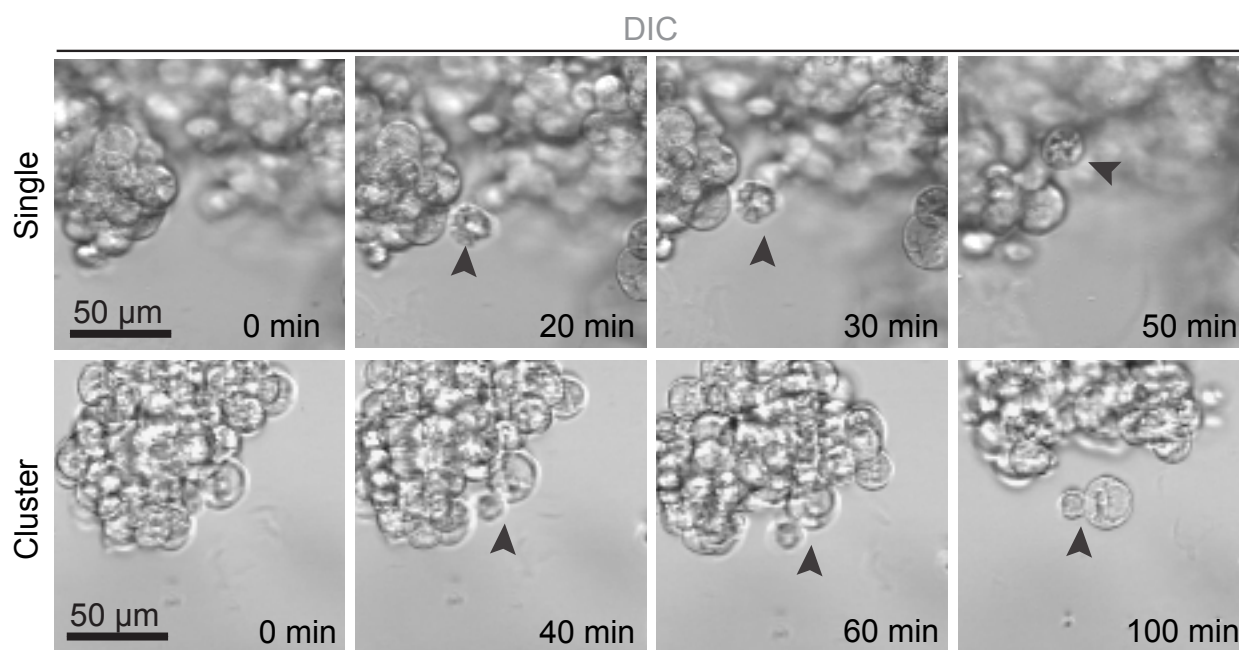
b



c

**Supplementary Figure S1 (related to Figure 1)**

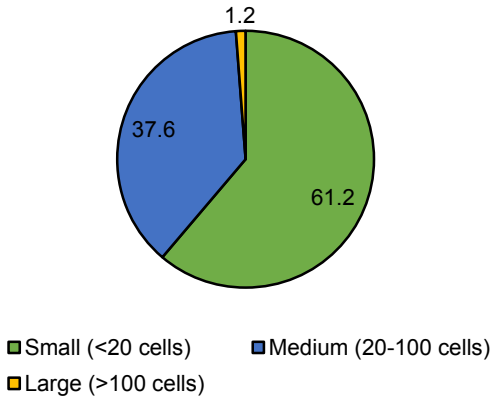
Epithelial ovarian cancer cells can spontaneously detach as single cells or clusters in 2D culture. A, fluorescence images showing spontaneously detached cells from OV90 and OVCAR3 monolayers stained with calcein (green) and ethidium homodimer (red) to identify live and dead cells, respectively. B, quantification of the proportion of attached and detached cells in the indicated cell lines. C, quantification of the live/dead ratios of detached cells (n=3).

**Supplementary Figure S2 (related to Figure 1)**

Epithelial ovarian cancer cells can spontaneously detach as single cells or clusters. DIC images showing a representative time-lapse series of single cell and collective cell detachment events for OVCAR3 cells.

a

% Size distribution of ascites clusters



Supplementary Figure S3 (related to Figure 3)

Quantification of the size distribution of multicellular clusters isolated from ascites in mice.