Supplementary material

***Title*:** Reversal of Anchorage-Independent Multicellular Spheroid into a Monolayer Mimics a Metastatic Model

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Methods

Phenotypic Microarray (PMM) analysis

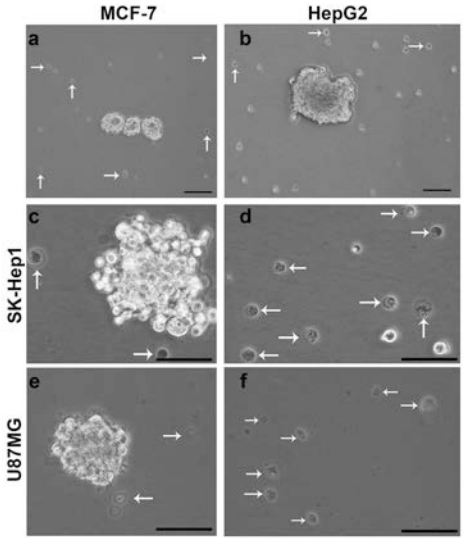
Luc-Huh7 cells of parental population or *rs*-monolayer were used for the PMM array analysis. In brief, 96-well plates, pre-coated with anticancer agents at four-different increasing concentrations (1X, 2X, 3X and 4X) were used to test the chemosensitivity of these two phenotypes (monolayer and reversed-spheroids). After a period of 36 hours of incubation in PMM plates the chemosensitivity of parental and *rs*-monolayer population was assessed based on the bioluminescence signal generated by luciferase which is an indicator of intracellular ATP level and cell viability.

Results

Supplementary Fig. S1.

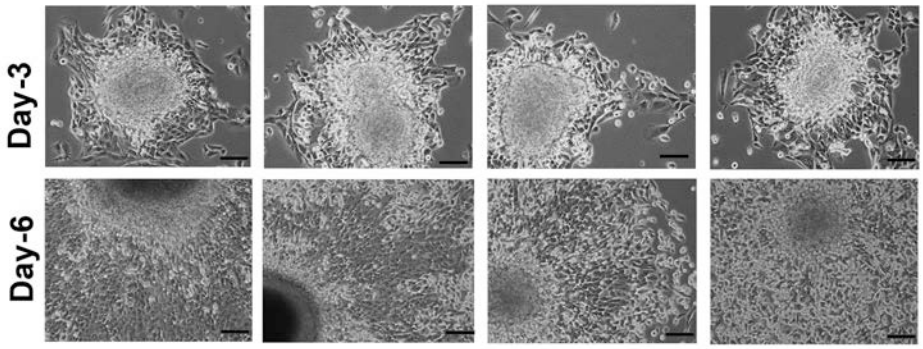
Development of multicellular aggregates or spheroids by anchorage-independence.

Culture of cancer cell lines under ultra-low attachment condition generated multicellular aggregates or spheroids. Arrows indicate cells that did not survive the selection by ultra-low attachment. Scale bar represents 0.5mm



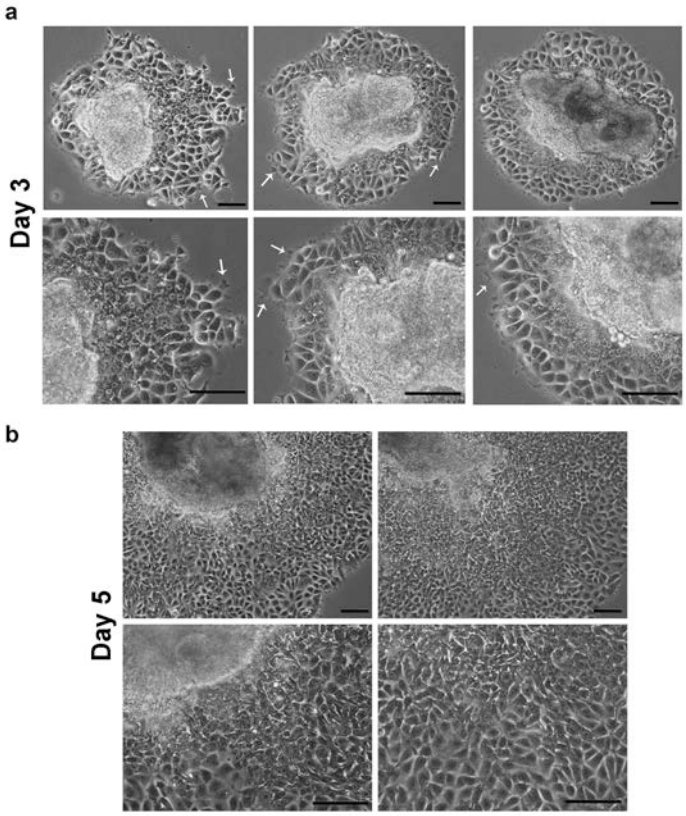
Supplementary Fig. S2.

Induction of reversal of Huh7 multicellular spheroid into monolayer. Huh7 multicellular spheroids aseptically transferred to normal culture condition (adhesive base) resulted in the induction of migration and reversal of spheroid into monolayer. Scale bar represents 0.5mm



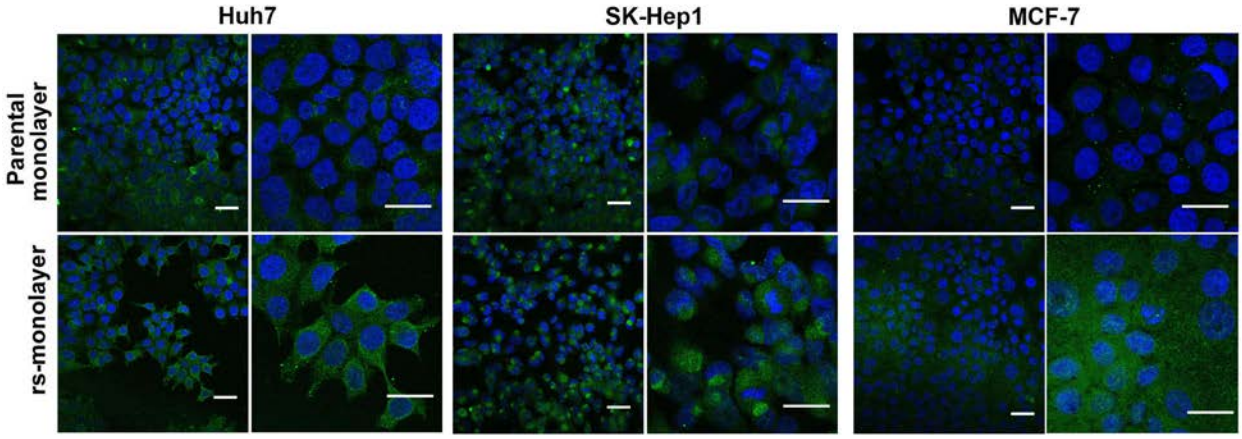
Supplementray Fig. S3.

Induction of reversal in MCF-7 cells. The reversal of spheroid followed a consistent pattern with the migration of cells at the periphery followed by cells from the middle or central region. Note: Microscopic fillopodia-like or lamellipodia-like structures (in panel **a**) are prominent in cells at the leading edge (indicated by arrows at the periphery). Scale bar represents 0.5mm



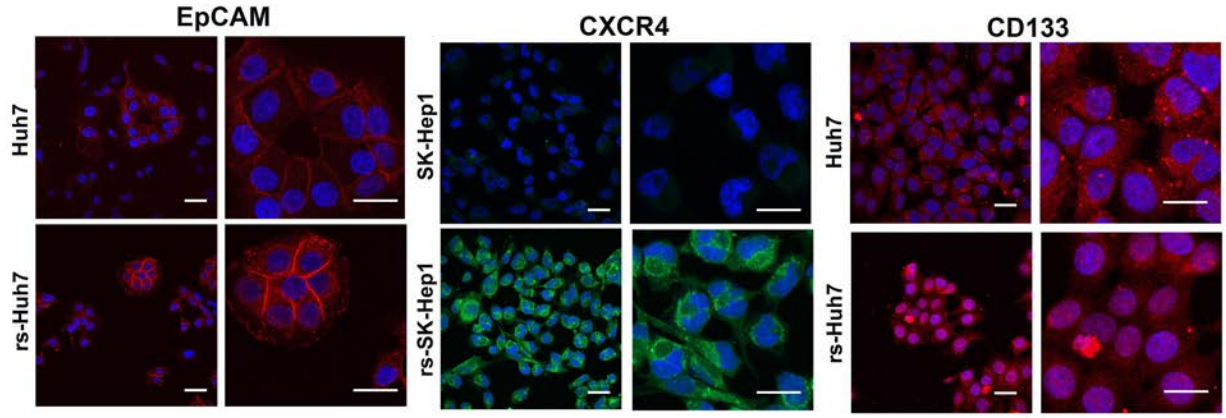
Supplementary Fig. S4.

Transgelin expression is upregulated in the *rs*-monolayer. Multiple cancer cell lines demonstrate that transgelin expression is increased in *rs*-monolayer cells compared to the parental cell line. Scale-bar represents 20μm.



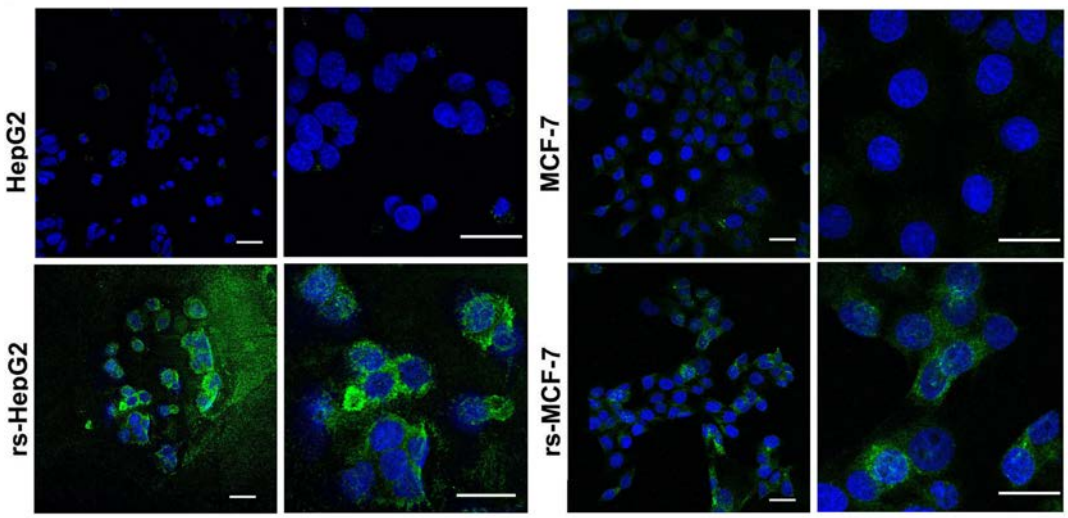
Supplementary Fig. S5.

Cancer stem cell markers are upregulated in the *rs*-monolayer. Multiple cancer cell lines demonstrate that cancer stem cell markers such as EpCAM, CXCR4 and CD133 expression are increased in *rs*-monolayer cells compared to the parental cell line. Scale-bar represents 20μm.

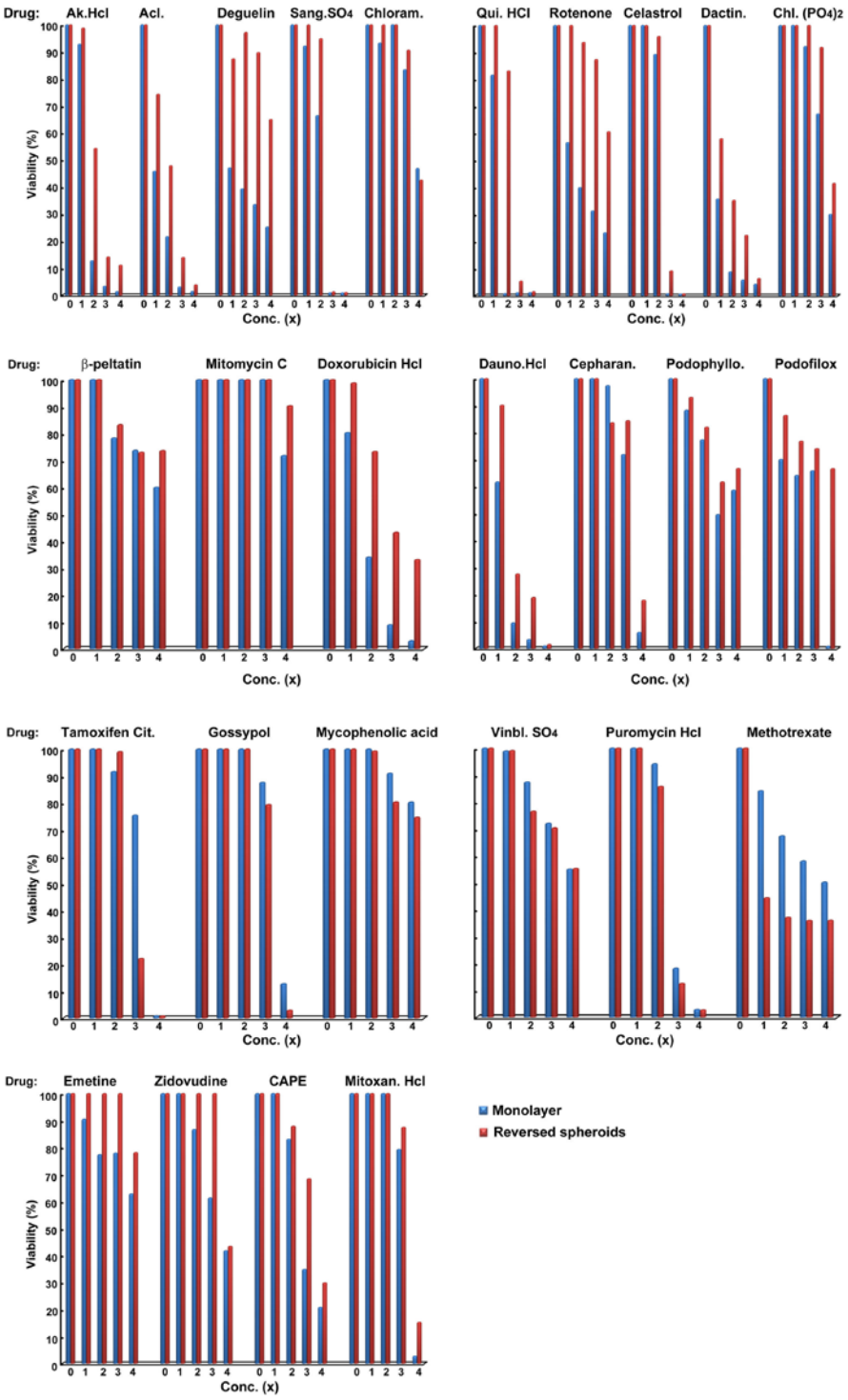


Supplementary Fig. S6.

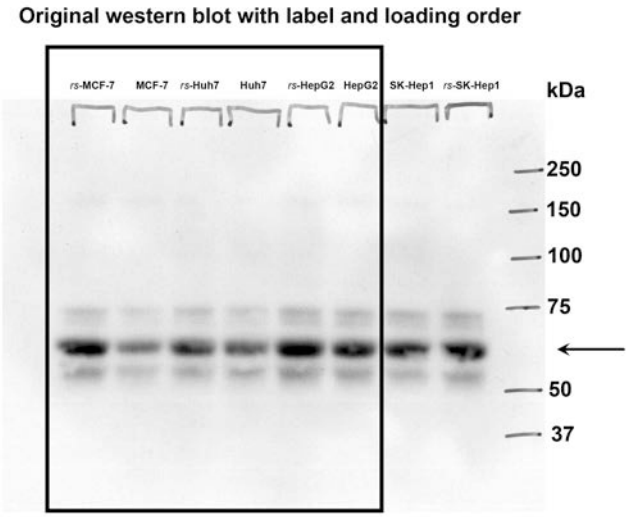
ABCG2 expression is upregulated in the *rs*-monolayer. Multiple cancer cell lines demonstrate that the drug resistance –related gene, ABCG2 expression is increased in *rs*-monolayer cells compared to the parental cell line. Scale-bar represents 20μm.



Supplementary Fig. S7. Differential chemosensitivity of *rs*-monolayer cells. A comparative analysis of chemosensitivity of *rs*-monolayer and parental population of Huh7 cells.



Supplementary Fig. S8. The immunoblot showing the regions that were cropped and used for figure 5c. The boxed area is the part of immunoblot that was cropped and shown in Fig. 5c. The arrow indicates MMP-2 signal. The weak signals above and below the specific (MMP-2) signal could be other forms of MMPs (e.g. MMP-7, MMP-9) which can have slight cross reactivity with the specific antibody. Although we have data from four cell lines and their corresponding reversed spheroids (*rs*), the SK-Hep1 related data were not used due to lack of clarity resulting from smudged signal.



Supplementary Fig. S9. The zymogram gel showing the regions that were cropped and used for figure 5c. The coomassie stained zymogram gel showing MMP-2 signal (indicated by arrow). Similar to the immunoblot, the white box area shows the area of the gel that was cropped and shown in Fig.5c. The signal observed above the MMP-2 could be other forms of MMPs. The identity of the MMP-2 (indicated by arrow) was also confirmed by immunoblotting the zymogram gel with specific antibody (not shown). The lanes of SK-Hep1 were excluded in Fig. 5C zymogram gel, as the corresponding immunoblot in the same figure doesn't have SK-Hep1 data. SK-SK Hep1, G2-HepG2, H7- Huh7, M7-MCF-7, *rs*- reverse spheroid.