Supplementary Information (SI) for Lab on a Chip.

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Repeated Exposure of Anticancer Agents to Tumorspheres in Open-Surface Microwell Arrays for Modeling Chemotherapy-Induced Dormancy in Colorectal Cancer

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Supplementary Data and Methods

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Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

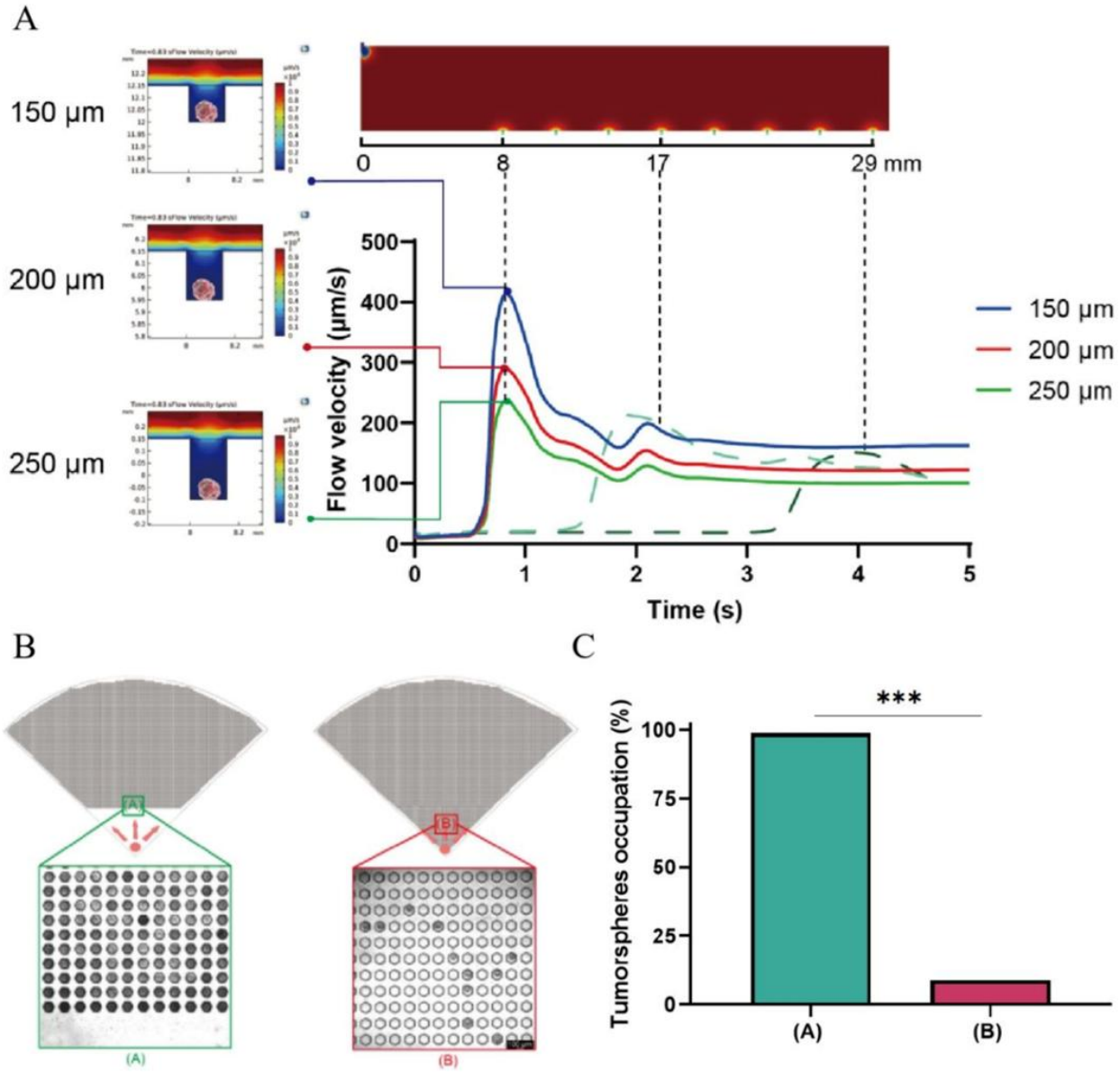

Figure S1. Investigation of fluid disturbance of tumorspheres in microwells. A) Simulation of the disturbance of fluid flow to the liquid within microwells of varying depths. B) Occupation of tumorspheres within the microwell array, both with and without a liquid flow buffer area. C) Occupation rate of tumorspheres in the fluid exchange area following fluid exchange, as observed under an optical microscope(scale bar:100 μm).

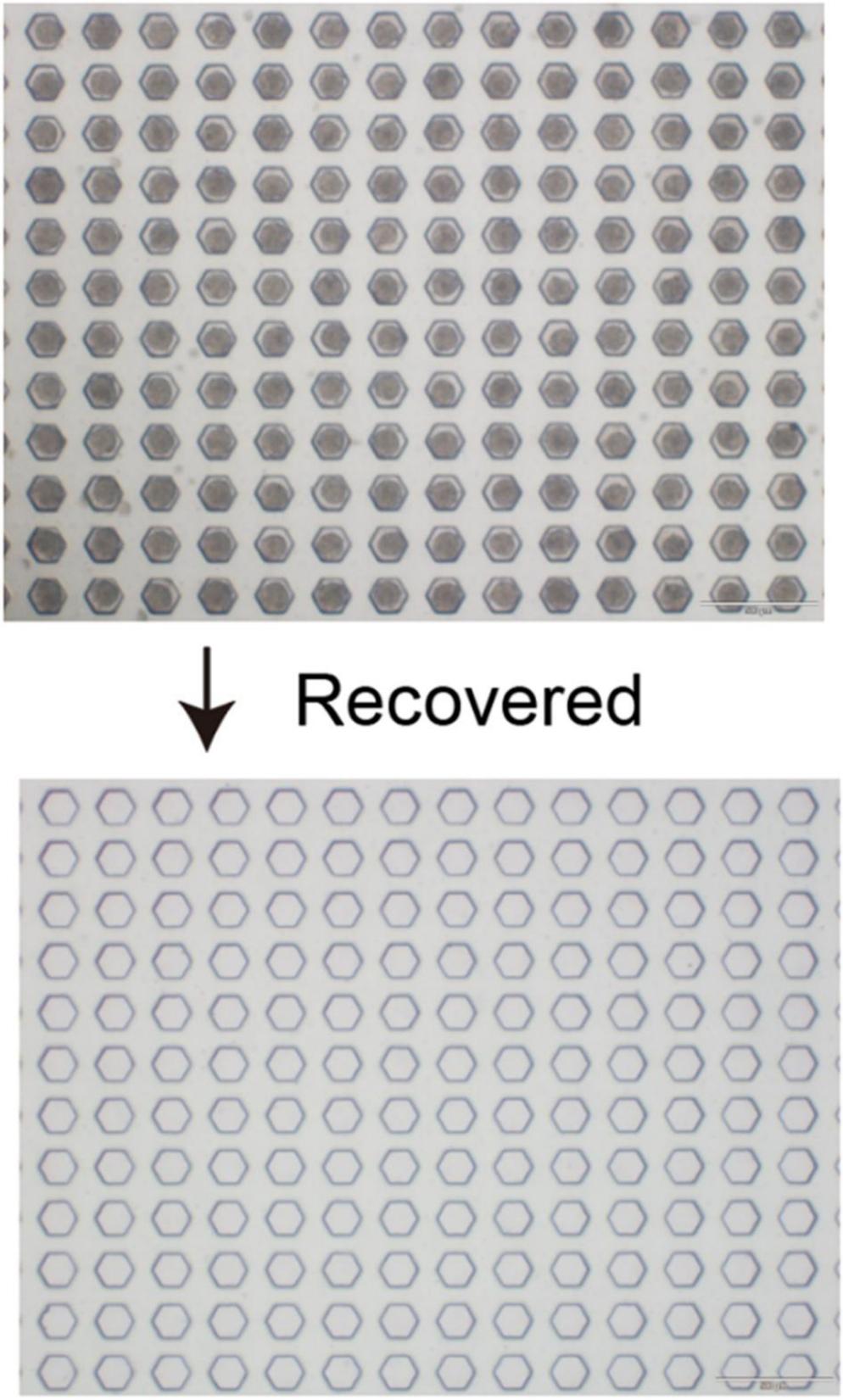

Figure S2. Recovery of tumorspheres from the microwell array (scale bar:200 μm).

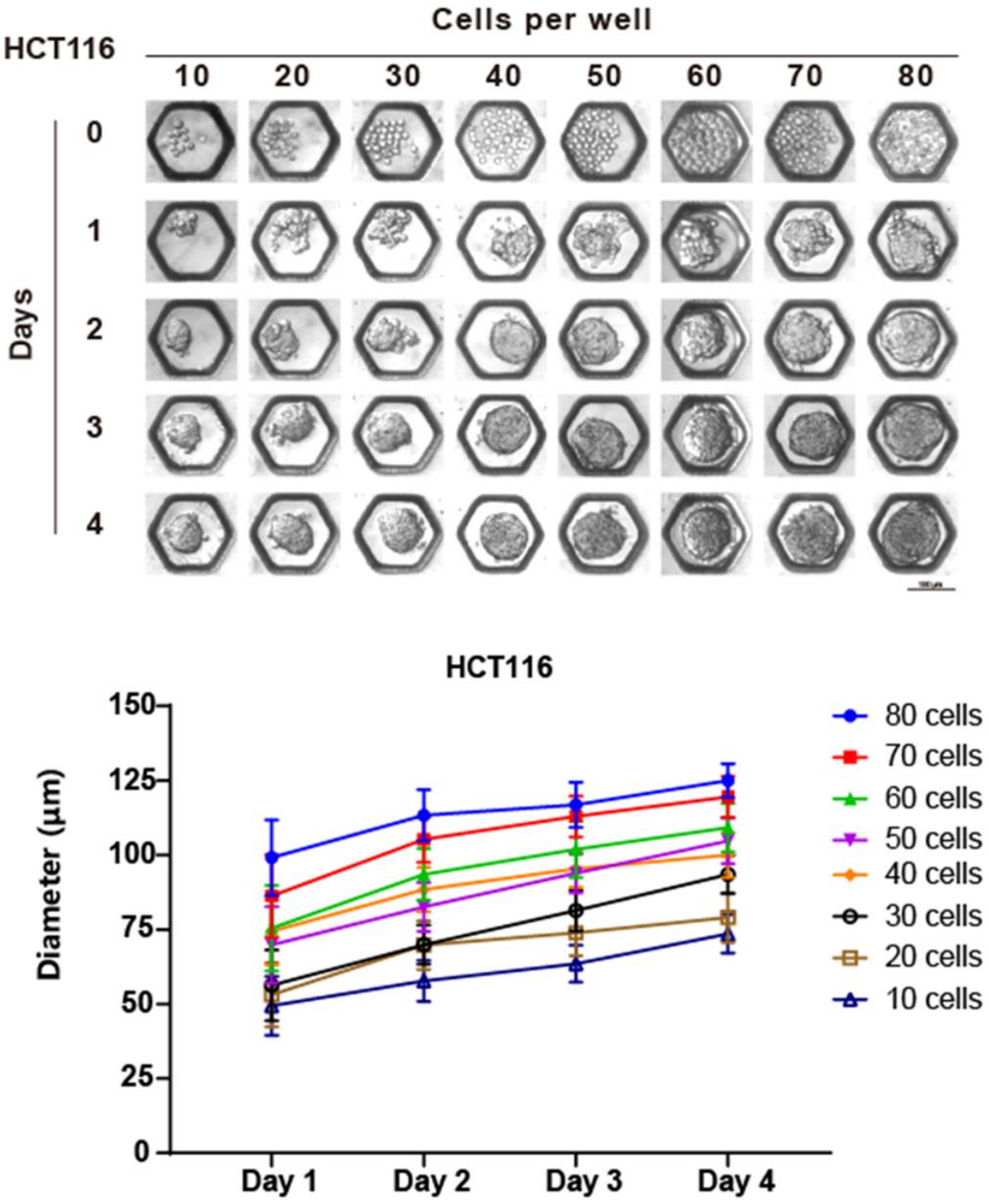

Figure S3. Tumorspheres generated through the microwell array culture of HCT116 cells(scale bar:100 µm).

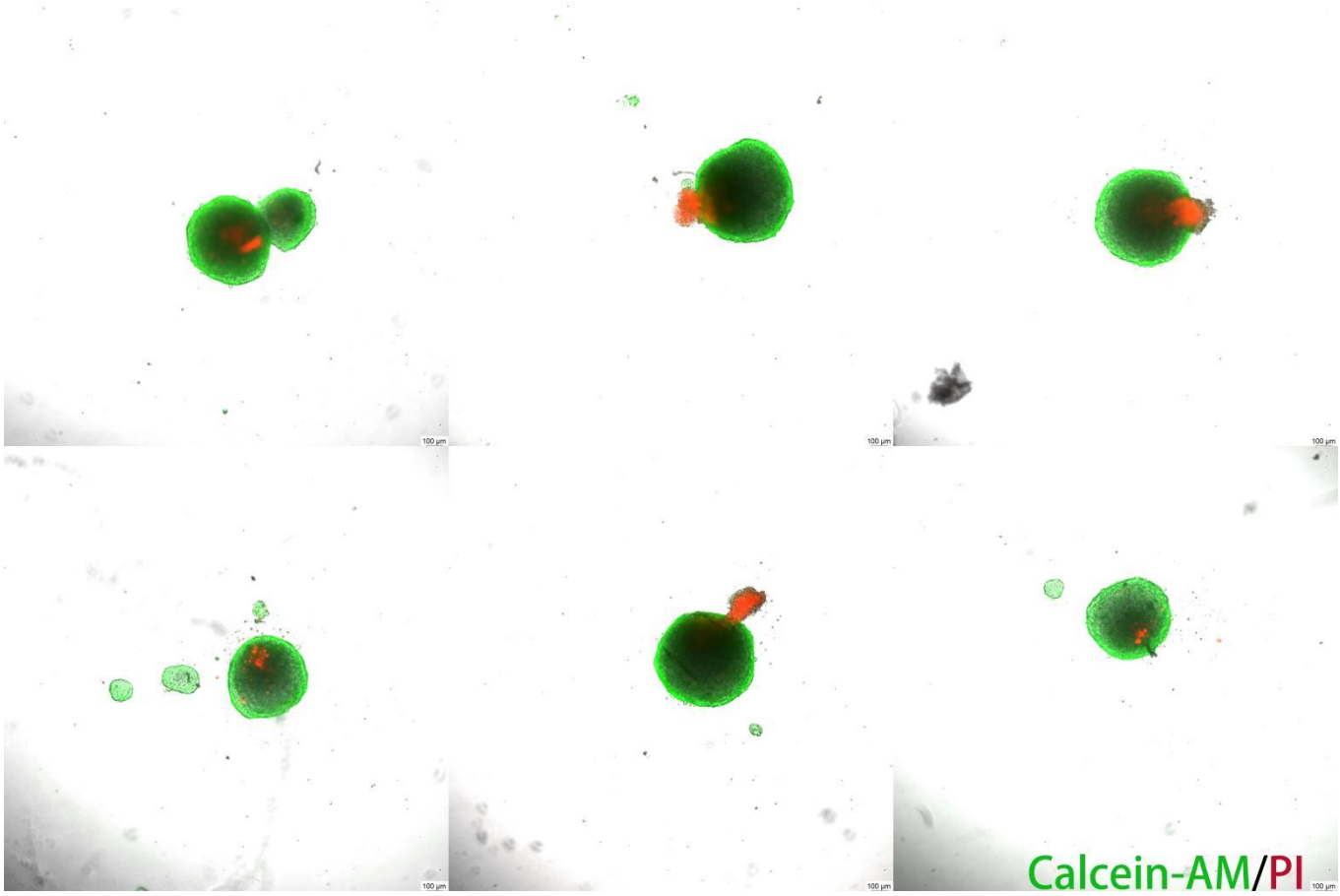

Figure S4. Representative images showing multiple tumorsphere formation in the ULA 96-well plate (scale bar:100 μm).

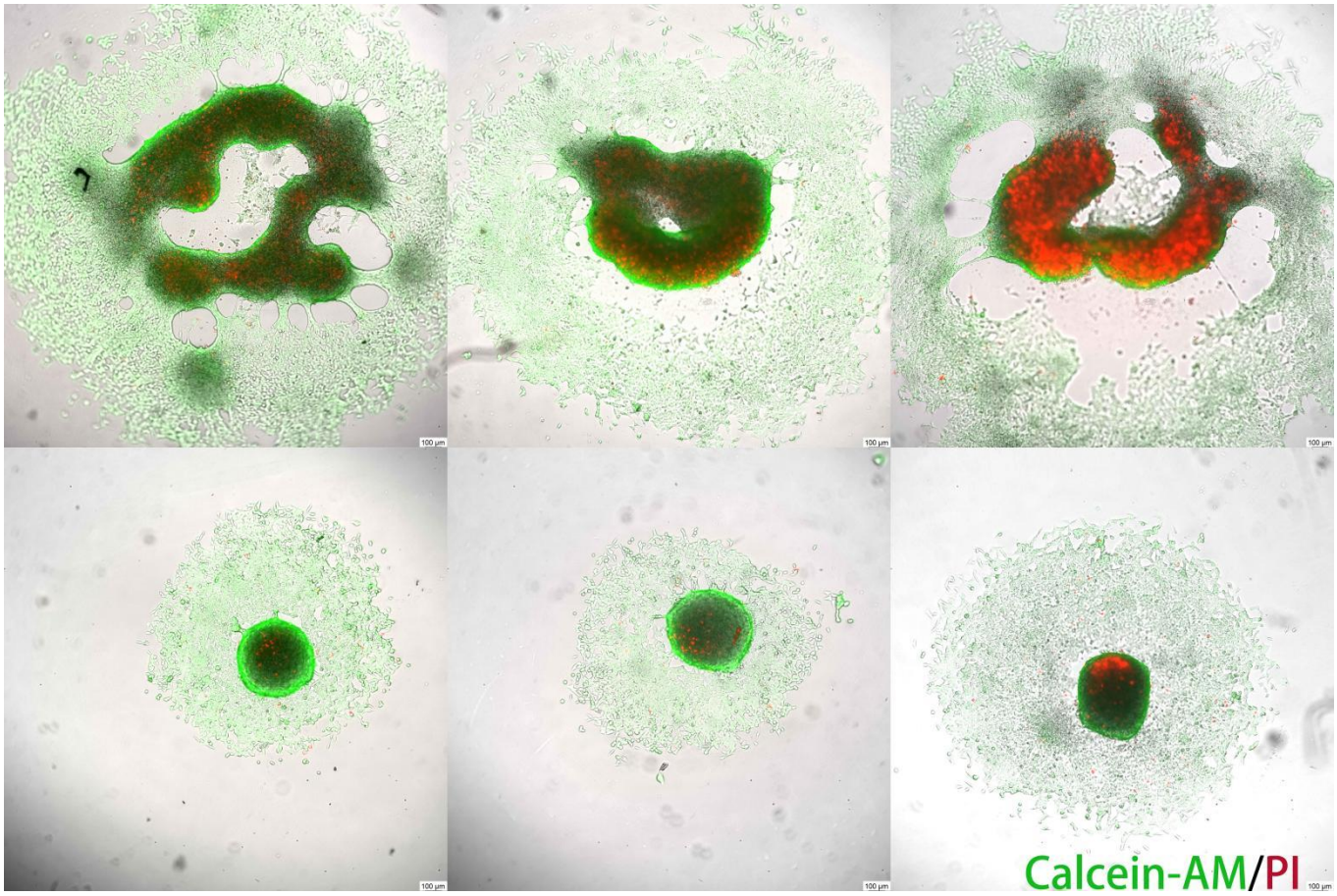

Figure S5. Representative images showing incomplete elimination of cell adhesion in the ULA 96-well plate (scale bar:100 μm).

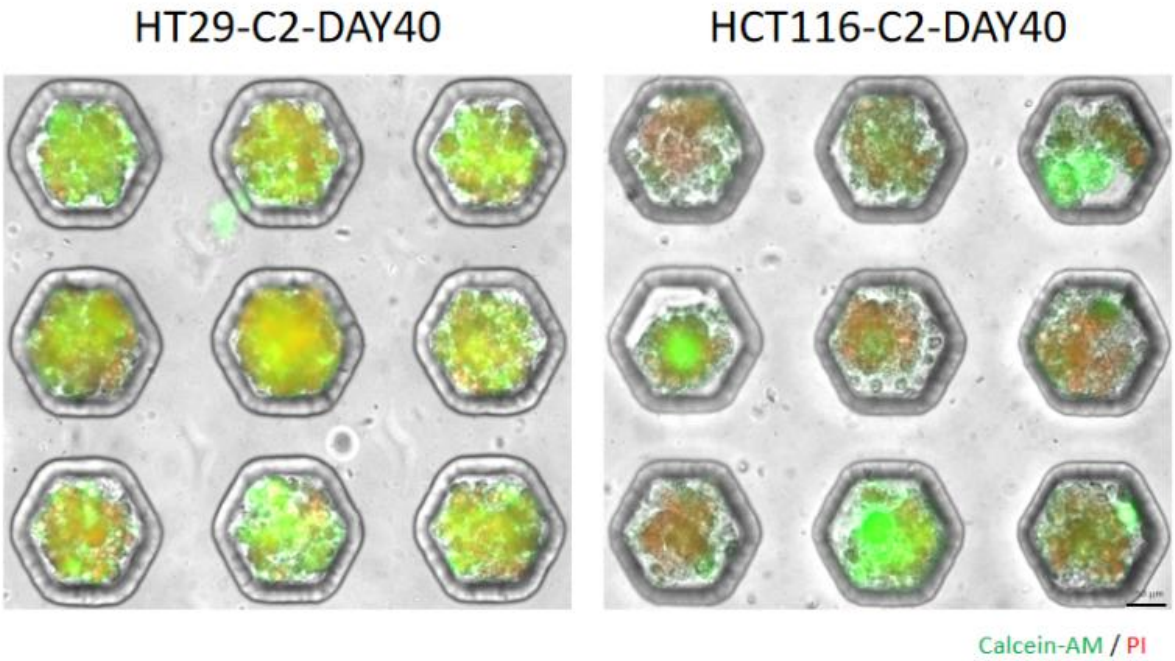

Figure S6. Live/dead staining of cells in C2 tumorspheres after a 40-day drug-free interval(scale bar:100 μm).

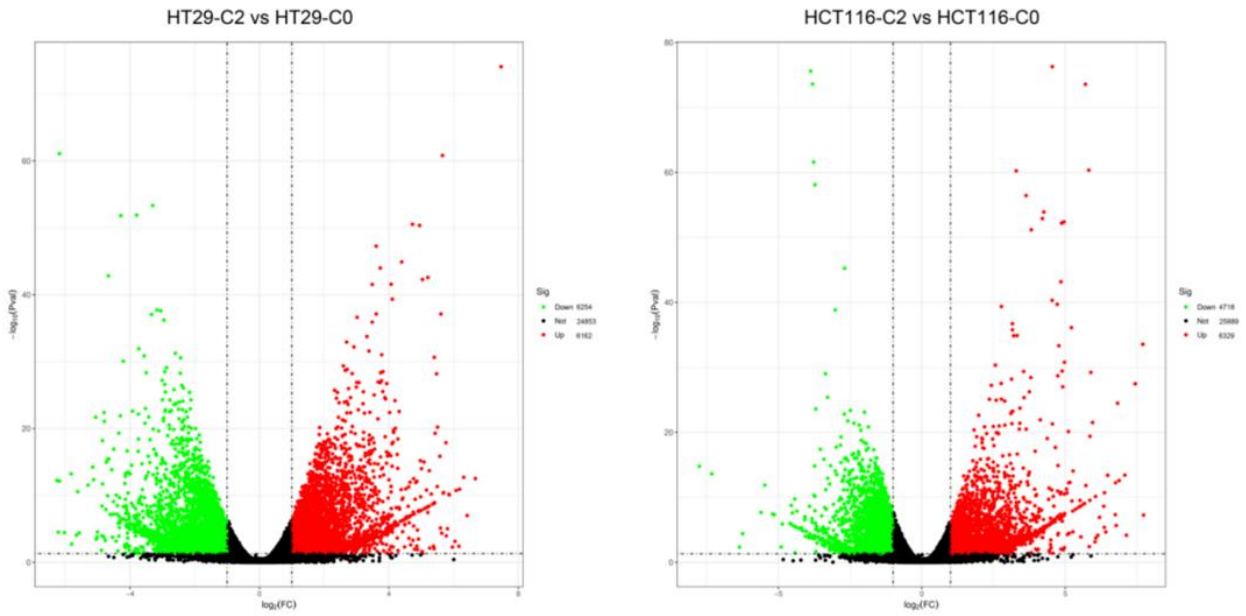

Figure S7. Volcano plots depict the differentially expressed genes between experimental conditions, highlighting changes in tumorosphere cells derived from colorectal cancer subsequent to drug administration.

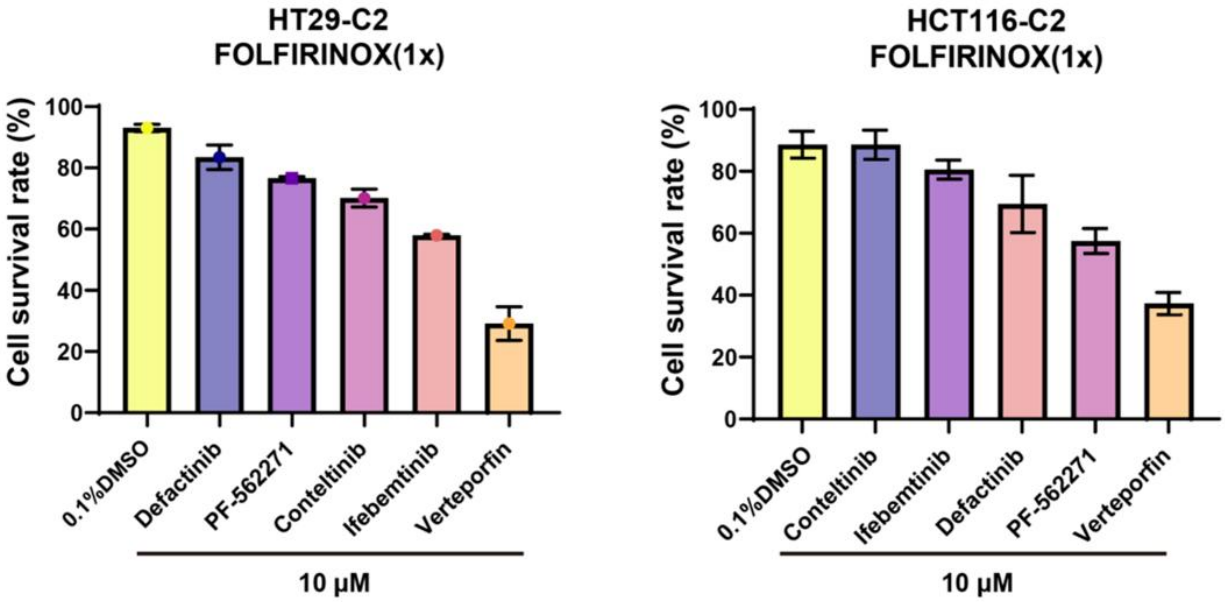

Figure S8. Survival rate of cells within C2 tumorspheres following drug treatments with FOLFIRINOX (1x) in combination with potential dormancy inhibitors.

Fluid Simulation

Two-phase fluid transient simulations were conducted utilizing COMSOL version 6.1 to analyze flow velocity and pressure distributions, thereby facilitating the optimization of the microfluidic chip design (refer to Figure S1). Rectangular geometric arrays with three distinct depths—150 μm, 200 μm, and 250 μm—were modeled to represent microchambers of varying depths. The simulation conditions assumed incompressible flow, ambient pressure fixed at 1 atm, and a temperature of 293.15 K. No-slip boundary conditions were applied, with an inlet flow rate set to 4 μL/s and an outlet pressure maintained at 0 Pa. Surface tension was specified as 1×10^{-5} N/m. The simulations were performed over a duration of 5 seconds, employing a time step of 0.01 seconds, and a channel thickness of 1 mm was used in the model.

Contact Angle Measurement

Two flat, fully cured PDMS pieces were utilized, with one piece being treated with a 2% hydrophilic copolymer solution. A 10 μL droplet of pure water was applied vertically to the surface of the treated PDMS, and the droplet shape was analyzed using a contact angle measuring instrument (Dongguan Dingjing Instrument Co., LTD., SDC-2005). The software subsequently calculated the contact angle.