

## **Supporting Information (SI)**

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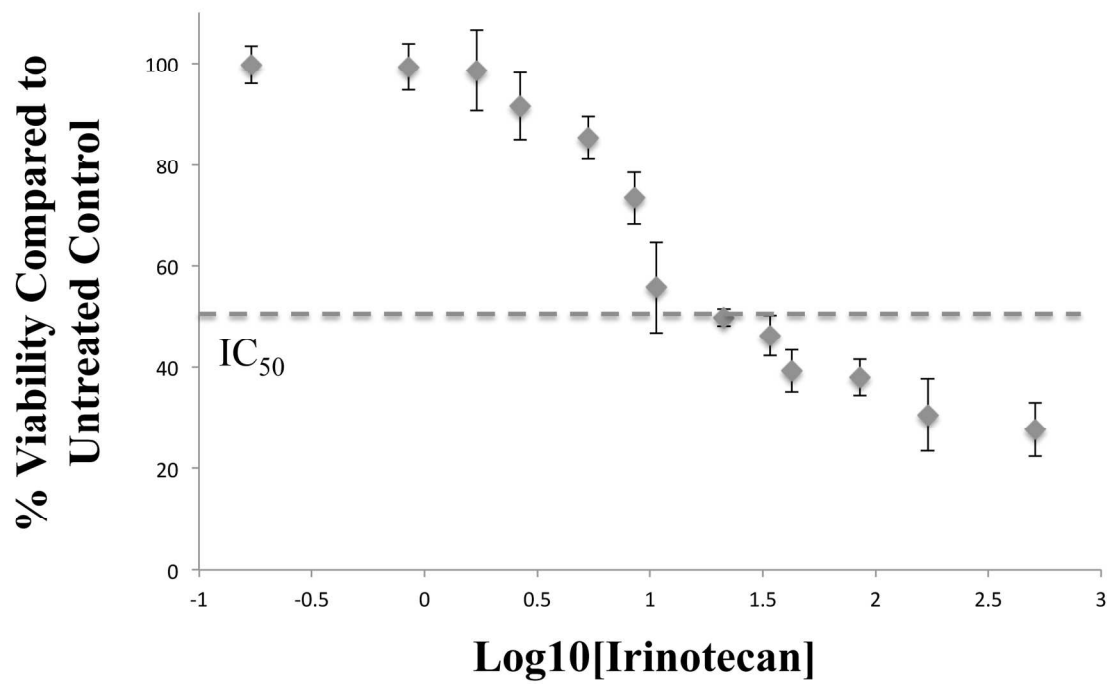
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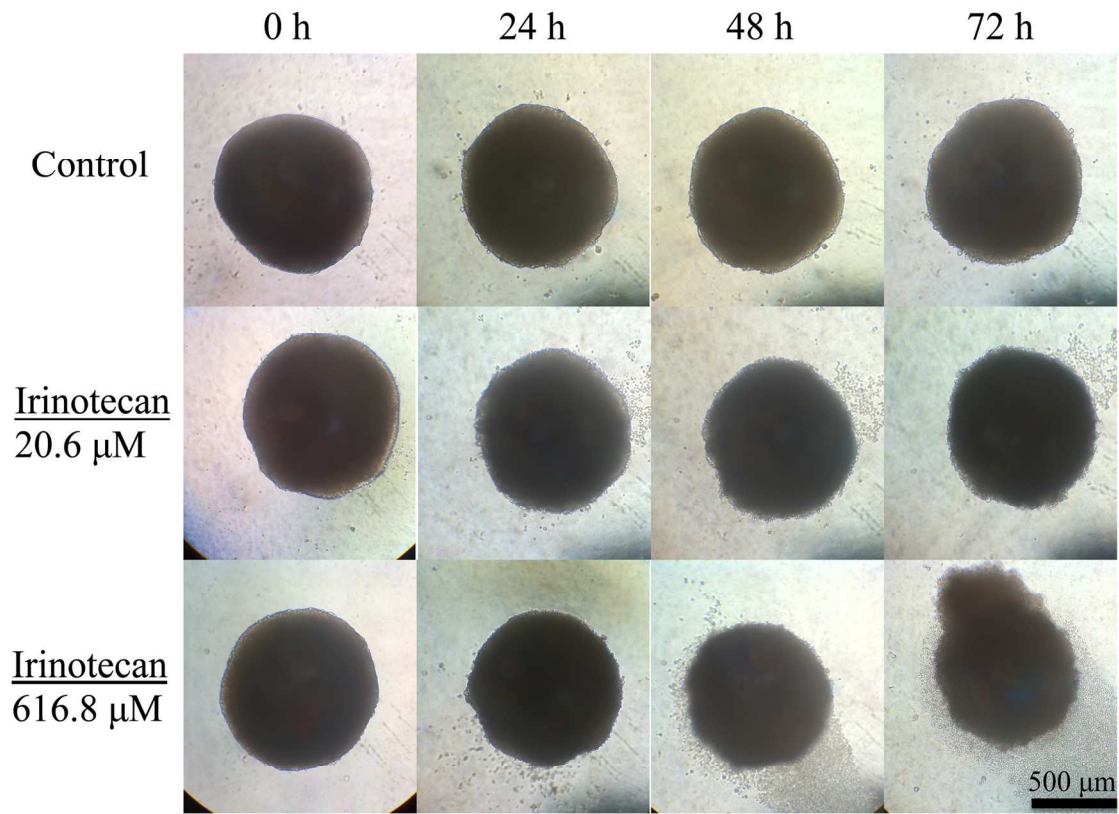
### **Title: Evaluation of Therapeutics in Three-Dimensional Cell Culture Systems by MALDI Imaging Mass Spectrometry**

**Abstract:** The supplemental information includes additional information on the IC<sub>50</sub> result, spheroids morphological integrity studies, division of regions on spheroid section for statistical analysis, Ion chromatograms and MS/MS spectra for metabolites of irinotecan, and spectra from untreated spheroid section. Supplemental Figure 1 shows the dose response curve of HCT 116 spheroids to irinotecan. Spheroids integrity after drug treatment is illustrated by Supplemental Figure 2. Supplemental Figure 3 gives the definition of three regions of interest on treated spheroid section for comparison of drug signal intensity. Ion chromatograms and HCD MS/MS spectra for irinotecan and its metabolites are shown in Figure 4. Supplemental Figure 5 provides the MALDI IMS spectra from a control spheroid section.

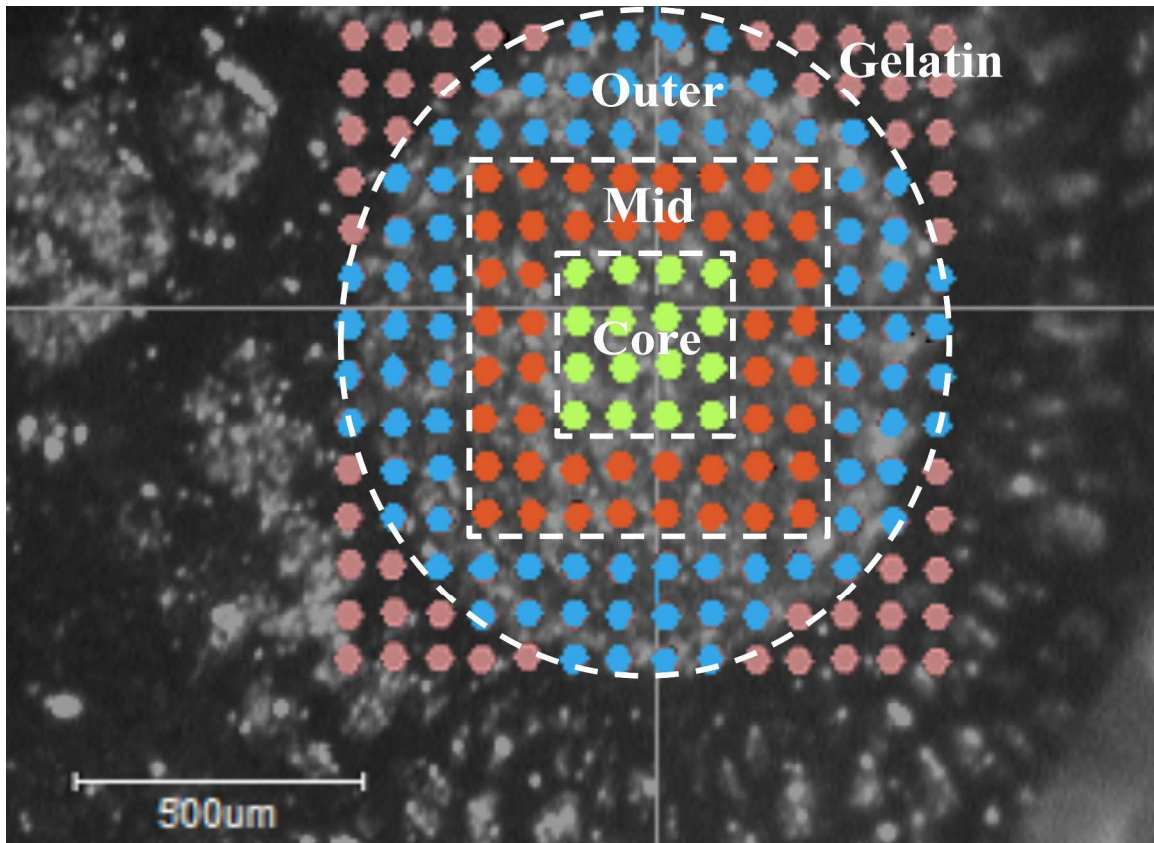


**Supplemental Figure 1.** HCT 116 spheroids dose response curve to irinotecan treatment.

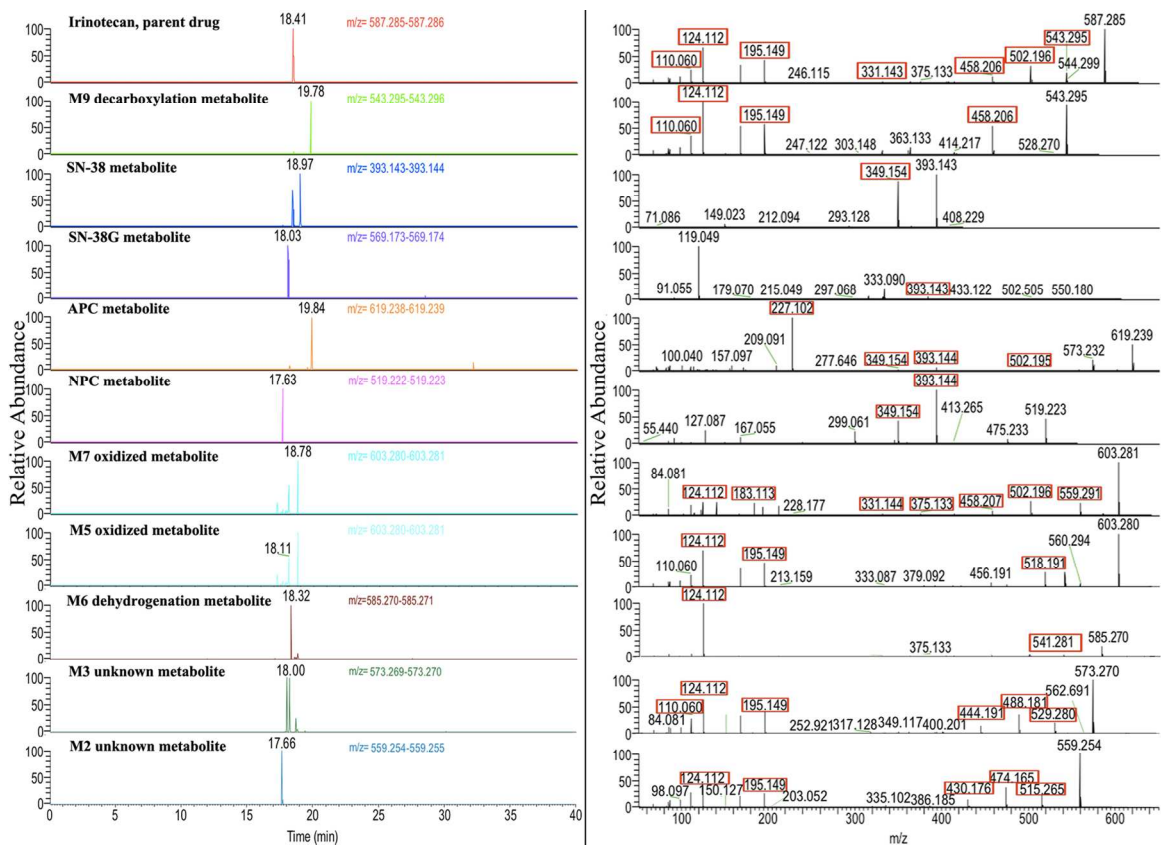
IC<sub>50</sub> for the drug was determined to be 20.6 μM. The Five measurements were collected for each concentration of irinotecan by using Cell Titer-Blue Viability assay. Error bars represent the median of five measurements.



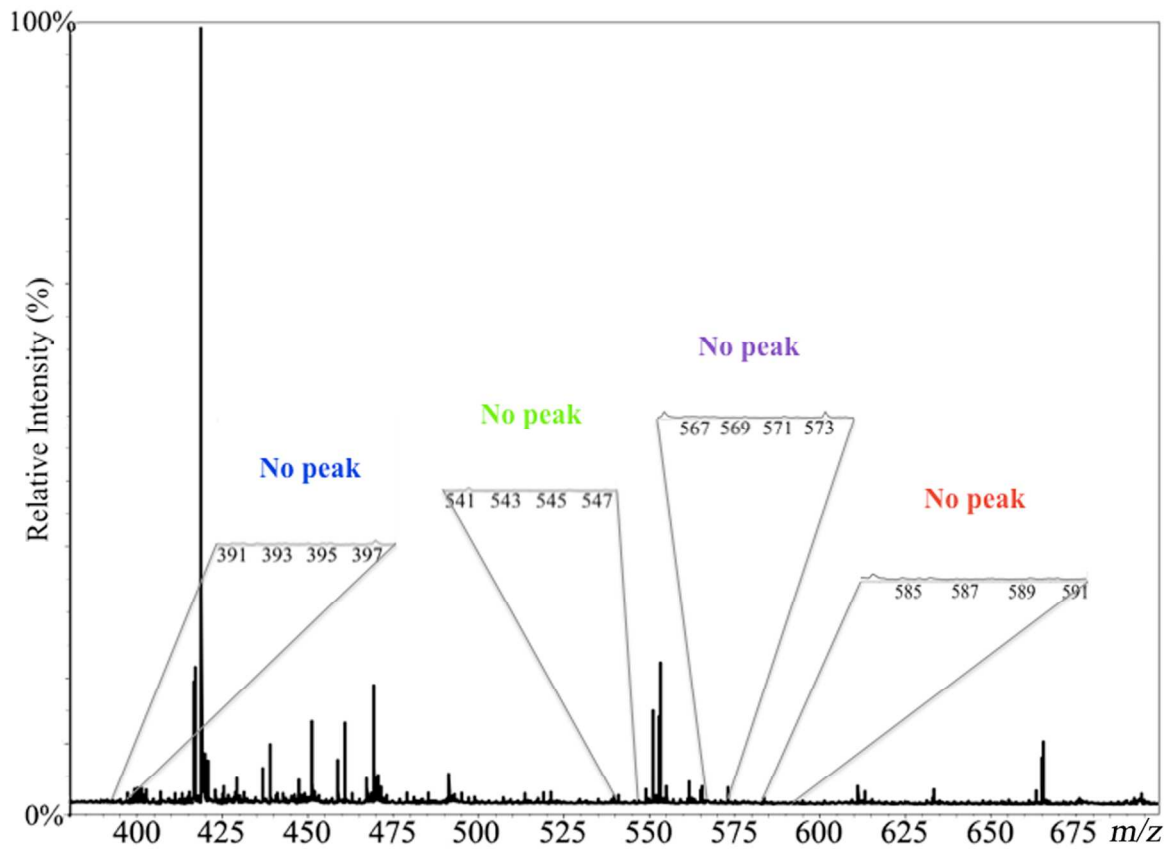
**Supplemental Figure 2.** Spheroid integrity following treatment with Irinotecan. Optical images of HCT 116 tumor spheroids at the onset of treatment and after 24h, 48h, and 72h treatment interval with 0  $\mu$ M, 20.6  $\mu$ M, 616.8  $\mu$ M Irinotecan; magnification 10 $\times$  objective.



**Supplemental Figure 3.** Division of three regions of interest based on the optical image of a dosed spheroid section. Color dots represent the laser spot positions on the sample surface. Pixels on gelatin were removed by setting a suitable threshold above the image noise. The region of the core (green spots) is defined as 4\*4 pixels in the center; the mid region (red spots) is defined as 8\*8 pixels minus those from the core; the outer region contains the remaining pixels except the pixels on gelatin.



**Supplemental Figure 4.** Ion chromatograms (left) illustrating the irinotecan and ten metabolites detected and confirmed by MS<sup>2</sup> from irinotecan treated spheroids extract. MS<sup>2</sup> spectra are shown on the right with corresponding fragment ions shown in red boxes.



**Supplemental Figure 5.** MALDI IMS spectra from a drug-free spheroid as a blank control.