Electronic Supplementary Information for

Assessing chemotherapeutic effectiveness using a paper-based tumor model

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Experimental

SN-38 dose-response assay

A 5 mM stock solution of SN-38 (Cayman Chemical) was prepared in DMSO (Sigma-Aldrich). A range of SN-38 concentrations were prepared at 2x the working concentration (0, 0.001, 0.002, 0.01, 0.02, 0.1, 1, 10 μM) by dissolving the SN-38 stock solution into McCoy's 5A medium. Each SN-38 solution contained 0.2% (v/v) DMSO.

3D cultures were prepared by pipetting 0.5 μL of cell-laden Matrigel (20,000 cells/μL) into single-zone scaffolds (Fig. S1). Additional scaffolds were prepared with 0.5 μL of cell-free Matrigel to serve as background controls. The single-zone scaffolds were placed into individual wells of a 96-well plate containing 100 μL of McCoy's 5A medium and incubated for 24 h prior to dosing. Scaffolds were dosed with SN-38 by adding 100 μL of the respective concentrations to each well. After dosing for 48 h, scaffolds were transferred to wells containing 100 μL of culture medium.

2D cultures were prepared by adding 5,000 cells in 100 μL of medium to wells of a 96-well plate. Cultures were incubated for 24 h, then dosed with 100 μL of the respective SN-38 concentrations for 48 h. After dosing, 100 μL of medium was removed from each well.

Cellular viability was assessed with CellTiter-Glo™ (CTG, Promega). For the 3D and 2D cultures, 100 μL of CTG reagent was added to each cell-containing well. The plates were agitated on an orbital shaker at 800 rpm at room temperature for 15 min prior to analysis. Chemiluminescence was measured on a SpectraMax M5 (Molecular Devices) spectrophotometric plate reader with an integration time of 500 ms. Emission intensities from wells without cells (2D cultures) or wells with cell-free single-zone scaffolds (3D cultures) were used as background. All background-subtracted intensities were averaged and normalized to the vehicle control.

Fluorescein penetration assay

Sodium fluorescein (Fluka) was dissolved in McCoy's 5A medium to prepare a 0.5 mg/mL solution. PBCs containing 12 scaffolds seeded at a cell density of 84,000 cells/zone were placed in a 6-well plate without additional medium and situated on the stage of an incubated

inverted microscope (Axiovert 40 CFL, Zeiss). The wells of the top metal holder were filled with 100 µL of the fluorescein solution, and fluorescence images were of the bottom of the stack were recorded every minute for 11 h. Images were taken using an Axiovert 40 CFL (Zeiss) inverted microscope equipped with a LED light source (wLS-LG-MB, QImaging), 470±20 nm excitation filter, 540±40 nm emission filter, and monochrome 12-bit camera (QIC-F-12-C, QImaging).

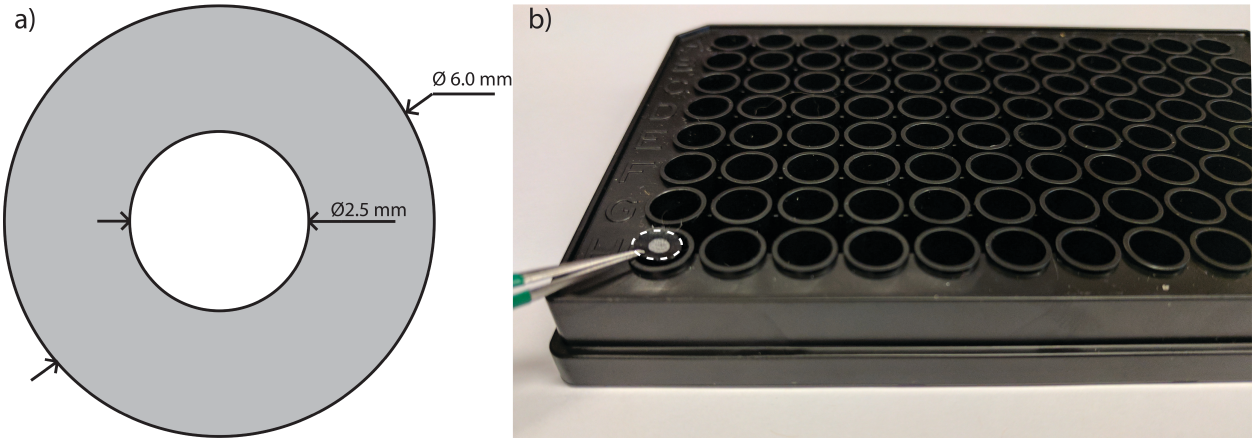

Fig. S1 a) Schematic of single-zone scaffolds, prepared using previously described protocols.[1] Each circular scaffold is 6 mm in diameter with a 2.5 mm diameter zone for seeding cells. The grey color corresponds to the wax-patterned region of the scaffold. b) Photograph of the single-zone scaffold (white outline) being placed into the well of a 96-well plate.

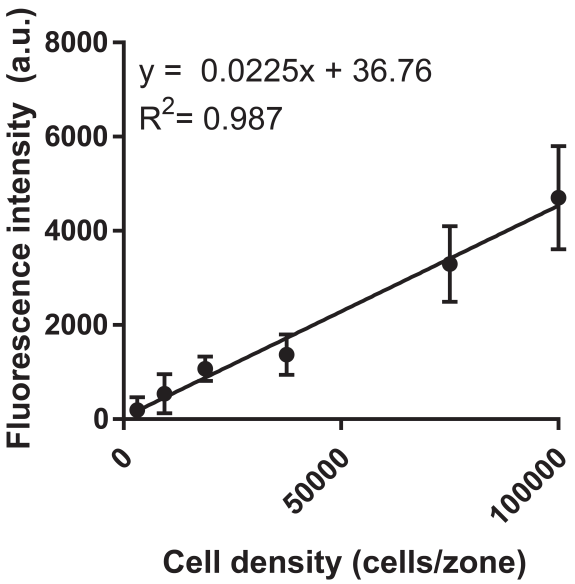

Fig. S2 Calibration curve relating mCHR fluorescence intensity to number of the number of HCT116 mCHR cells in a zone of a paper-based scaffold, imaged with a Typhoon 9400 scanner. These measurements utilized scaffolds patterned with the 9-zone format: five zones contained 0.5 µL of cell-laden Matrigel with increasing densities of HCT 166 mCHR cells, the remaining four zones contained 0.5 µL of cell-free Matrigel. Each point on the curve represents the average and standard deviation of n = 5 zones.

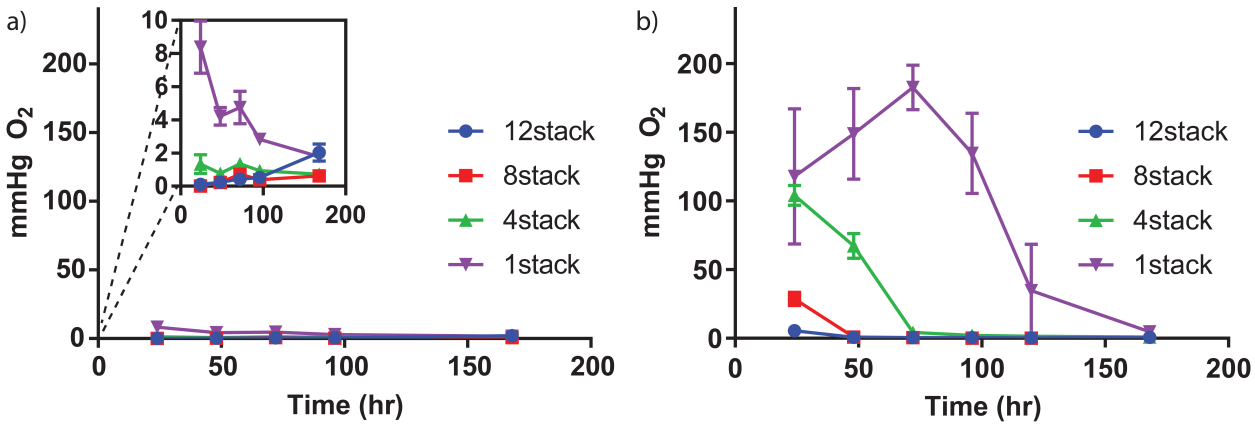

Fig. S3 Oxygen tensions at the bottom of stacked cultures containing 1, 4, 8, or 12 scaffolds. These cultures were seeded at cell densities of either a) 84,000 cells/zone or b) 21,000 cells/zone, and incubated for up to 168 h. The inset of a) is to better visualize differences in oxygen tension for cultures with 84,000 cell/zone. Each point represents the average and standard deviation of n = 5 measurements from the same experimental setup.

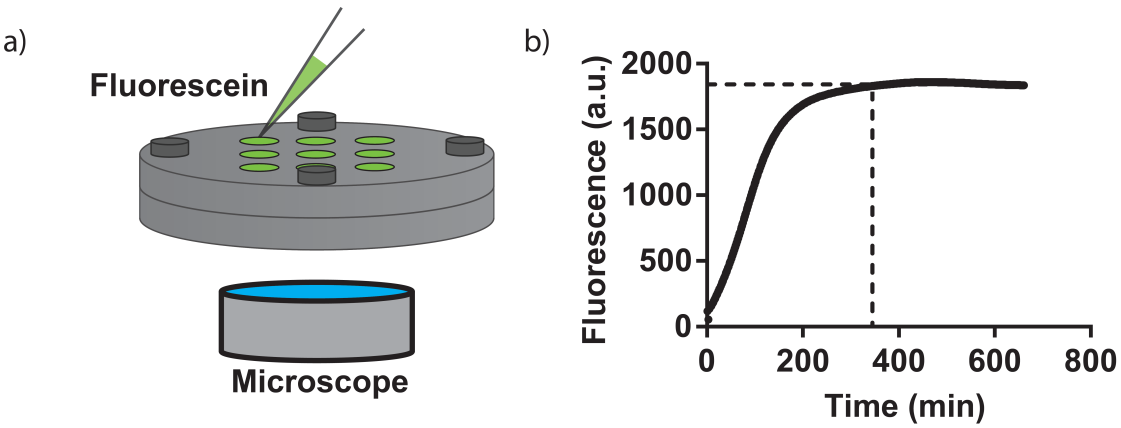

Fig. S4 Measuring the penetration of fluorescein into stacked paper-based cultures (PBCs) using fluorescence microscopy. a) Experimental setup. A 12-stack PBC culture was assembled, 100 µL of a 0.5 mg/mL fluorescein was added to the top of each culture, and images of the bottom collected with an inverted fluorescence microscope. b) Fluorescence intensity at the bottom over an 11 h incubation period at 37 °C. Images were collected in 1-minute increments. The constant fluorescence intensity after ~350 minutes indicates that an equilibrium in fluorescein had been reached.

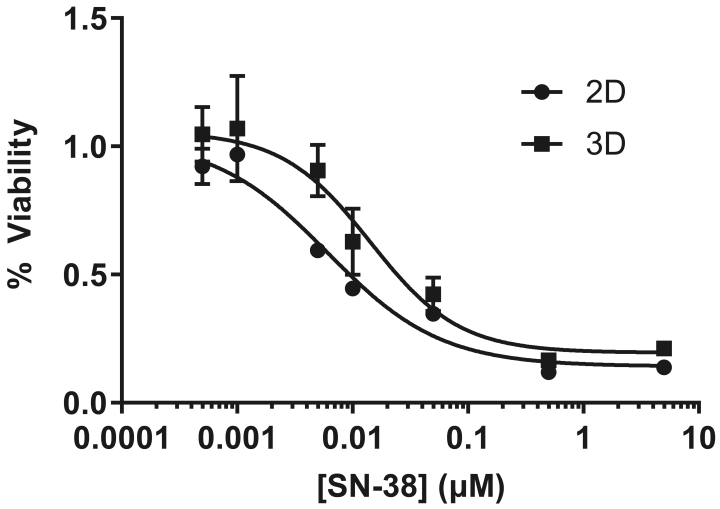

Fig. S5 Dose-response curves for SN-38 treatment of HCT116 mCHR cells in 2D and 3D cultures. Doses of SN-38 were administered at increasing concentrations to adherent (2D; 5,000 cells/well) and Matrigel-suspended (3D; 20,000 cells/zone) HCT116 mCHR cells. The resulting IC_{50} values for 2D and 3D conditions were 0.006±0.001 and 0.014±0.006, respectively. Each point represents the average and standard error of the mean of n = 4 measurements.

Table S1: Statistical comparisons between mCHR fluorescence intensity of cultures seeded at 84,000 cells/zone and dosed with different concentrations of SN-38.[a]

	Vehicle vs.			1.4 μM vs.		0.14 μM vs.
Scaffold[b]	1.4 μM	0.14 μM	0.014 μM	0.14 μM	0.014 μM	0.014 μM
1	****	-	-	****	****	*
2	****	****	****	**	***	-
3	****	****	****	****	*	-
4	****	****	****	-	-	-
5	****	**	**	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	*	-	-	-	-	-
12	-	-	*	-	-	-

[a] The comparisons in this table correspond to the data presented in **Figure 4a** of the main text. (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$)

[b] Scaffold 1 was closest to the source of fresh medium, and scaffold 12 was the furthest from the source of fresh medium.

Table S2: Statistical comparisons between mCHR fluorescence intensity of cultures seeded at 21,000 cells/zone and dosed with different concentrations of SN-38.[a]

	Vehicle vs.		1.4 µM vs.
Scaffold[b]	1.4 µM	0.014 µM	0.014 µM
1	****	****	**
2	****	-	****
3	****	-	*
4	****	-	****
5	**	-	****
6	*	-	****
7	-	-	*
8	**	-	*
9	**	-	**
10	-	-	-
11	***	-	****
12	-	-	-

[a] The comparisons in this table correspond to the data presented in **Figure 4b** of the main text. (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$)

[b] Scaffold 1 was closest to the source of fresh medium, and scaffold 12 was the furthest from the source of fresh medium.

References

1 C. C. Lloyd, M. W. Boyce and M. R. Lockett, *Curr. Protoc. Chem. Biol.*, 2017, **9**, 1–20.