Supplementary Methods

Removing BHT from EVA

Poly(ethylene-co-vinyl acetate) (EVA) (Sigma) (40% vinyl acetate by weight) was first washed to remove butylhydroxytoluene (BHT) according to a protocol adapted from Langer et al. (44). Briefly, polymer pellets were washed ten times each, first in water and then in ethanol at 37°C with stirring. After each successive wash, the absorbance of the ethanol was analyzed spectrophotometrically at 230 nm to quantify the relative level of BHT until its absorbance was less than twenty times the original reading. Following washing, pellets were dried in a desiccator at room temperature.

Drug Release and Loading Efficiency

To test the effect of over-spotting on release of drugs, 5% EVA in cyclohexanol was loaded with 10% (v/v) 7-Diethylamino-4-methycoumarin (Acros Organics, Morris Plains, NJ) dissolved in DMSO and 5% diH_2O (v/v) then printed onto glass coverslips in an arrayed fashion as described above. Following printing, arrays were placed in 35 mm petri dishes with 3 ml PBS containing 1% Tween® 20, and 20 µl samples were taken at defined intervals and analyzed on a Wallac 1420 Multilabel Counter (PerkinElmer, Waltham, MA). For over-spotted samples, unloaded EVA was immediately printed over the dye-loaded islands before being incubated in PBS. Sampling and analysis thereafter were identical.

Loading efficiency is defined as the amount of drug that was partitioned into the polymer as a percentage of the total amount of drug that was initially loaded. To determine the loading efficiency of factors, polymer formulations were made as described above. 100 µl EVA loaded with factors was then pipetted on glass coverslips and allowed to dry overnight under vacuum. Films were removed from the glass substrates and weighed. Films were washed briefly with PBS before being placed in 1 ml toluene to dissolve the polymer. Absorbance was then analyzed using a Nanodrop-ND-1000 spectrophotometer. Standard curves were generated for each factor, and loading efficiency was calculated as the percent of drug embedded in the polymer compared to the theoretical drug concentration.

For drug release studies, polymer formulations were again made as described above. Glass coverslips were weighed prior to printing. Arrays consisting of 900 drug-loaded polymer islands were manufactured and allowed to dry overnight. Samples were weighed and placed in 3 ml of PBS containing 1% Tween® 20 at 37 ºC with gentle agitation. Samples were taken at the specified times and analyzed. To calculate flux, EVA films were loaded with nutlin-3a and camptothecin for a range of concentrations as described above. Samples were collected at 24 h and analyzed. We quantified flux for different loading amounts and analyzed the result in the manner of Higuchi, et. al. (44), which describes a linear relationship between the flux of drug released (in units of $\mu g/cm^2/h^{1/2}$) and the loading dose (in units of $(mg/cm^3)^{1/2}$). We calculated the flux for nutlin-3a and camptothecin from EVA films over a range of concentrations at 24 h.

Array Fabrication

Arrays with PEG-based non-fouling backgrounds and amine-terminated silane adhesion islands were manufactured as reported (30) (Fig. 1a). Briefly, glass coverslips were cleaned in an oxygen plasma etcher (Terra Universal, Fullerton, CA). Arrays of (3-Aminopropyl) trimethoxysilane (NH2-terminated silane)

(Sigma-Aldrich, St. Louis, MO) were printed on clean coverslips using a Calligrapher Miniarrayer printer (Bio-Rad, Hercules, CA) with 1500 µm center to center distances and a pin diameter of 400 µm. The silane printed coverslips were then coated with 175 Å of titanium (Ti; 99.995% pure) and 225 Å of gold (Au; 99.999% pure) (Williams Advanced Materials, Buffalo, NY). Following coating, gold-coated arrays were sonicated to remove gold from the amine spots, exposing NH2-terminated silane islands. The coverslips were incubated with 0.1 M, methyl-terminated alkanethiol ($CH_3(CH_2)_{11}SH$) (Sigma) for 30 min. Substrates were incubated in 10% Pluronic® F-127 (BASF Corporation, USA) for 3 h and 1% heat-denatured BSA for 30 min to create a non-fouling surface around the adhesive amine islands (Fig. 1b). Three layers of the drug-loaded EVA films were printed over the amine islands and placed in a desiccator between each successive layer. EVA is a biocompatible polymer commonly used in drug delivery applications, and when formulated as an oil/water emulsion both hydrophilic and hydrophobic molecules can be loaded (45). Poly-d-lysine (0.1%) was over-spotted onto the EVA films to promote cell attachment. The arrays were placed in 35 mm petri dishes containing PBS with 2% penicillin and 2% streptomycin for 30 minutes to rehydrate the non-fouling PEG background and as a non-caustic sterilization step.

Human subjects

Tissues from colon cancer patients were retrieved under pathologic supervision with Institutional Review Board approvals at the University of Michigan and the University of Florida as previously described (40).

Cells and Microarray Seeding

HCT116 (p53+/+, ATCC, Manassas, VA) human colon cancer cells were maintained in McCoy's 5a Medium supplemented with 10% fetal bovine serum (Thermo Scientific, Waltham, MA), 1% penicillin G and 1% streptomycin (Thermo Scientific). The cells were cultured at 37ºC in a humidified incubator containing 5% CO_2. Following microarray fabrication, 100,000 HCT116 cells were seeded over each array in 3 ml serum-free media and allowed to incubate on a rocking plate at room temperature until cell attachment to the EVA islands occurred, with minimal attachment to background, typically 10-15 min. Microarrays were gently washed in PBS, placed in a 35 mm petri dish with complete media, and placed in an incubator for 24-72 h.

To establish microarray fidelity, microarrays were manufactured as described above. HCT116 cells were then seeded over microarrays and incubated for 24 or 72 h. Microarrays were fixed in 4% paraformaldehyde and stained with Hoechst 34580 dye.

Genomic Analysis

For p53 mutational analysis, genomic DNA was isolated using a DNeasy Tissue kit (Qiagen GmbH, Hilden, Germany); exons 4-9 were amplified with a Taq polymerase Master Mix (Promega, Madison, WI) using a Touchdown PCR program (45 cycles; 60˚C to 50˚C; 0.5˚C decrease per cycle) and previously described primers (46). The resulting PCR products were fractionated by agarose gel electrophoresis; excised and isolated using a QIAquick extraction kit (Qiagen GmbH, Hilden, Germany) and sequenced using an ABI 3130xI Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Sequences were analyzed using Sequencher v. 5.0 (Gene Codes, Ann Arbor, MI).

Calculating comparison to microtiter plates

Dimensions for a typical microtiter plate were obtained from Corning®. The length x width is 127.8 mm x 85.6 mm. Based on our island spacing of 1.5 mm, 85 islands (i.e., (127.8)/(1.5)) can fit along the length axis, and 57 (i.e., (85.6)/(1.5)) islands can fit along the width of a traditional plate. Thus (85 x 57) yields 4845 total islands that fit within the footprint of a standard microtiter plate. We assumed 10,000 cells per well as a typical seeding density in a 96 well plate. Performing experiments in triplicate with 16 unique drug combinations therefore requires 480,000 cells total (i.e., (10,000)*(16)*(3)). On the drug-eluting microarray, approximately 180 cells were seeded per island. For 16 unique conditions with n=3 conditions per array and n=3 arrays per experiment, the total cell requirement (180*16*3*3) equals 25,920 cells or 5.4% of 480,000.

Statistical Analyses

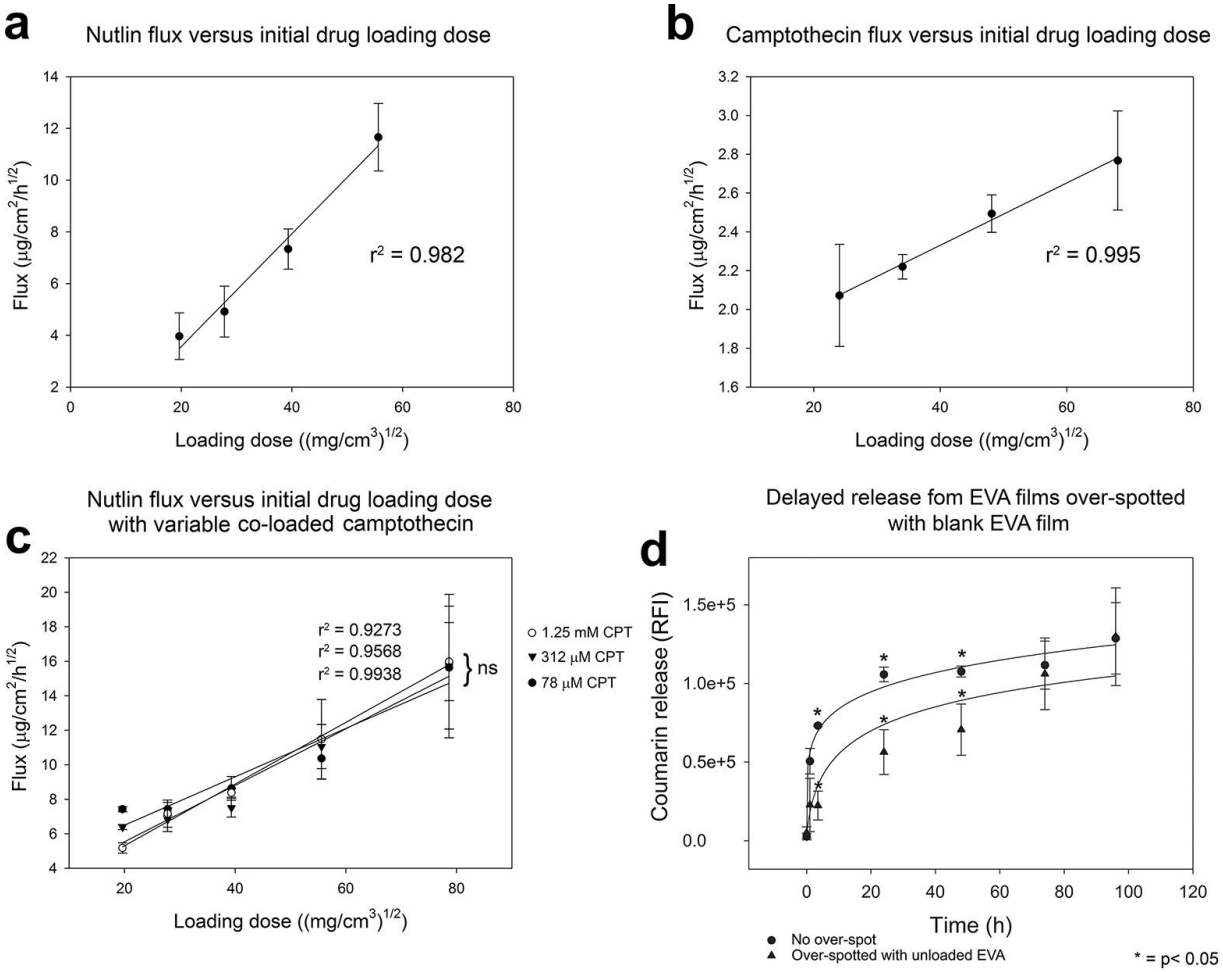
Proliferation values were normalized to control (0 μM drug). Values for concentration-response curves were transformed to non-proliferation by subtracting the normalized value from 100%. Modeling was performed for each concentration interval using the equation $\mathbf{E = E_0 + (E_{max} \times C)/(C + D_{50})}$, and E_{max} and D_{50} values were obtained where E is the effect (either non-proliferation or apoptosis), E_0 is the initial value, E_{max} is the maximum effect, C is the drug concentration, and D_{50} is the dose at which a 50% maximum effect (E_{max}) is observed (36). Drug sensitivity values were obtained by taking the inverse of the D_{50} and multiplying by 100. Those values marked with the # sign indicate that the r^2 value of the curve-fit was below 0.65. N/A values are present where negative parameters were obtained.

Supplementary Figures

S1. Loading efficiencies of small molecules in microarrayed EVA films.

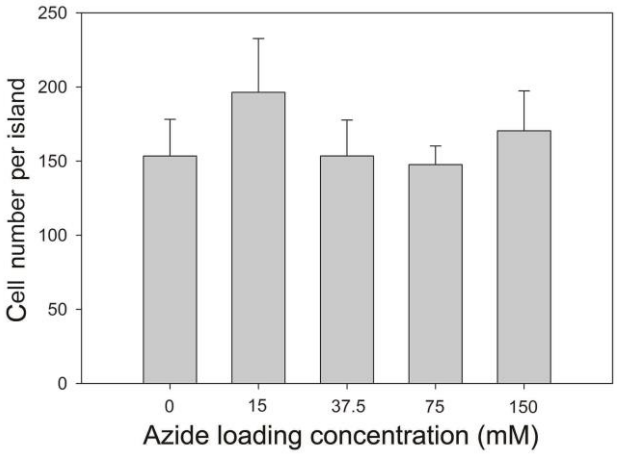
COMPOUND	LOADING EFFICIENCY (%)
AZIDE	73 +/- 8
CAMPTOTHECIN	92 +/- 3
NUTLIN-3A	81 +/- 7

Loading efficiency of drugs was analyzed by measuring the absorbance of EVA films dissolved in toluene and analyzed using a Nano-Drop 1000 spectrophotometer. Azide, a metabolic toxin, had a loading efficiency of 73%. Camptothecin, a topoisomerase inhibitor, was loaded at 92% efficiency, while for nutlin-3a, a p53 activating agent, loading efficiency was 81%.

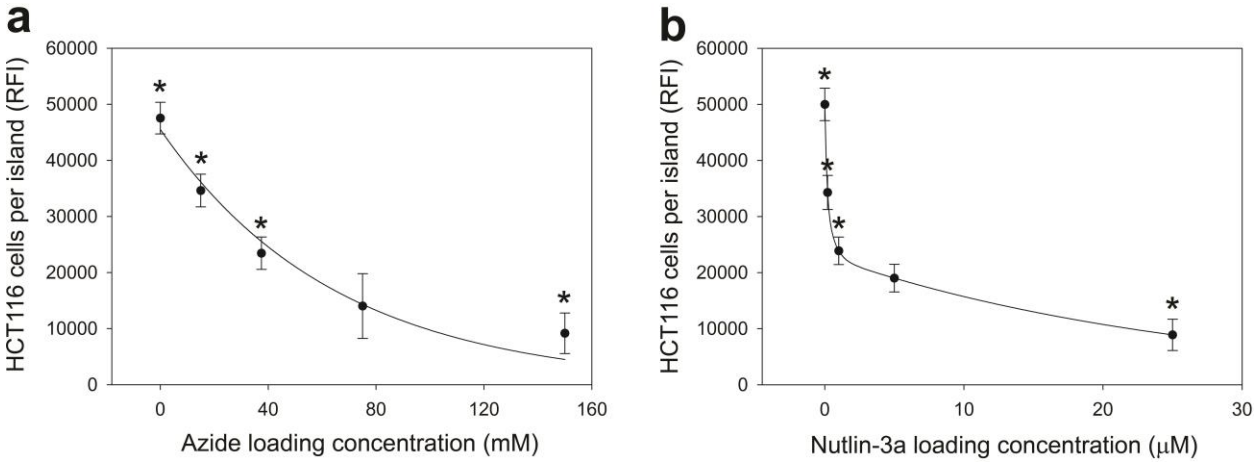


S2. Flux and release kinetics of small molecules from EVA films. Linear relationships were observed for the flux of (**a**) nutlin-3a and (**b**) camptothecin in relationship to the loading concentration at 24 h. (**c**) Quantification of flux of nutlin-3a from EVA films that were co-loaded with variable concentrations of camptothecin. For each group, the slope and y-intercept were determined. Student's t-test between these

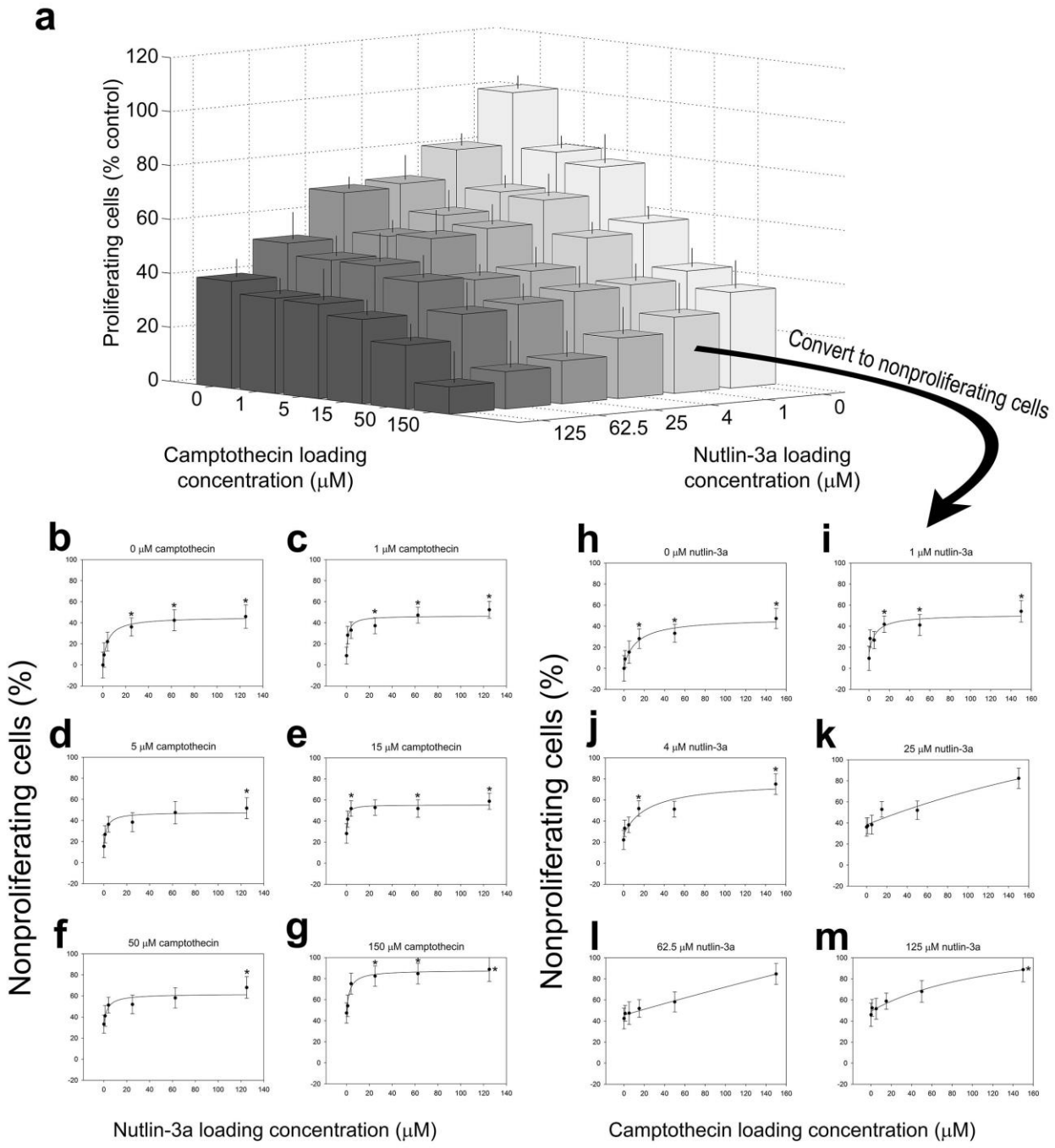
values revealed no significant change among groups ($p = 0.721$ for slope; $p = 0.429$ for y-intercept), indicating that loading the two drugs combinatorially had no effect on release kinetics. (**d**) Overspotting of unloaded EVA over coumarin-loaded EVA mitigates bolus release. Statistical differences were determined by student's t-test (*: $p < 0.05$).



S3. Cell number of HCT116 colorectal cancer cells on microarray loaded with azide. Microarrays were fixed with 4% paraformaldehyde and stained with Hoechst 34580 nuclear dye 1 h after seeding to quantify initial cell attachment. No statistical difference was found by ANOVA ($p = .490$).



S4. HCT116 cell numbers exhibit dose dependent responses to drug loading concentration. Cell numbers decreased with increasing loading concentrations of **(a)** azide after 24 h and **(b)** nutlin-3a after 72 h. Cells were fixed then stained with Hoechst following incubation and quantified using Axiovision software. Significant differences were determined by ANOVA, followed by Tukey's post-hoc analysis. (*: $p < 0.05$ compared to all other concentrations).



S5. HCT116 cell proliferation and dose-response curves from combinatorial drug-eluting microarrays. **(a)** Increasing concentrations of combination treatments increased overall antiproliferative activity. Following 24 h incubation on arrays with both nutlin-3a, {$F(5,590) = 15.481$, $p < 0.05$}, and camptothecin, {$F(5,590) = 21.696$, $p < 0.05$}, proliferation of HCT116 cells significantly decreased as revealed by two-way ANOVA. Additionally, a sub-additive effect was observed for the combination treatments as evidenced by the decrease in proliferation compared to either drug alone. Cell number for each condition did not significantly change following 24 h incubation ($p = 0.272$). Error bars represent standard error of the mean. (**b-g**) Dose response curves for fixed camptothecin concentrations of 0, 1, 5, 15, 50, and 150 μM, with

nutlin-3a varying over a concentration range. E_{max}, the maximum value of non-proliferation, was significantly increased by combination drug treatments by 83.2% with the addition of 150 μM compared to 0 μM camptothecin (86.5 vs 47.2, $p < 0.05$; Fig. S6). Further, the addition of 5 μM camptothecin increased sensitivity to nutlin-3a >5-fold (106 vs. 19.9, $p < 0.05$) and 50 μM camptothecin increased sensitivity >9-fold (189 vs. 19.9, $p < 0.1$) compared to nutlin-3a alone (see Fig. S6). (*: $p < 0.05$). (**h-m**) Dose response curves for fixed nutlin-3a concentrations of 0, 1, 4, 25, 62.5, and 125 μM, with variable camptothecin over a range of concentrations. The sensitivity to camptothecin significantly increased with the addition of all concentrations of nutlin-3a from >2-fold for 1 μM up to >18-fold for 125 μM nutlin-3a (see Fig. S6), greatly lowering the concentration required to induce non-proliferation when these drugs are used in concert. Proliferation data were transformed to non-proliferation data by subtracting the former from 100%. (*: $p <$ 0.05 compared to 0 μM drug).

S6. E_{max} and Sensitivity ($1/D_{50}$) values generated from combinatorial drug-eluting microarrays quantifying percent non-proliferative HCT116 cells.

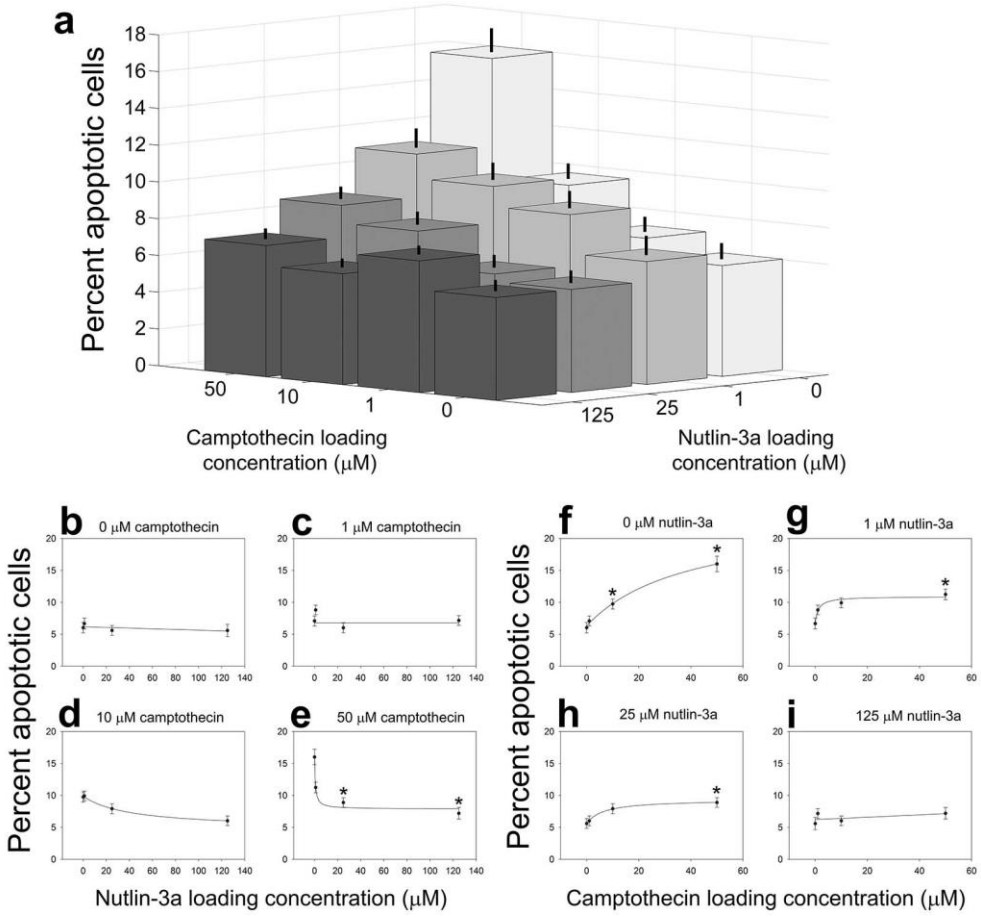
HCT116 Proliferation											
Hyperbolic Fit Parameters of Nutlin Dose Response Upon Addition of Camptothecin						Hyperbolic Fit Parameters of Camptothecin Dose Response Upon Addition of Nutlin					
2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data	2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data
CPT	0	0.0 +/- 1.0	47.2 +/- 0.8	19.9 +/- 23 *	Fig S5b	NUT	0	2.80 +/- 3.2	47.1 +/- 5.5	9.55 +/- 3.1 *	Fig S5h
	1	10.6 +/- 5.8	46.0 +/- 8.3	111 +/- 18	Fig S5c		1	15.0 +/- 6.5	52.0 +/- 13	21.4 +/- 4.5 *	Fig S5i
	5	16.6 +/- 3.9	46.8 +/- 6.6	106 +/- 15 $	Fig S5d		4	27.4 +/- 5.4	61.0 +/- 16	64.5 +/- 6.9**	Fig S5j
	15	28.1 +/- 2.9	55.1 +/- 6.8	#	Fig S5e		25	35.1 +/- 1.9	63.4 +/- 6.8	75.6 +/- 5.8**	Fig S5k
	50	34.4 +/- 4.8	60.0 +/- 12	189 +/- 15 $	Fig S5f		62.5	44.5 +/- 1.3	64.1 +/- 18	188 +/- 11***	Fig S5l
	150	45.9 +/- 3.1	86.5 +/- 7.5 *	160 +/- 14	Fig S5g		125	49.0 +/- 1.7	70.7 +/- 20	193 +/- 12***	Fig S5m

Statistical analysis of combination dose responses was performed by curve-fitting the equation $\mathbf{E = E_0 + (E_{max} \times C)/(C + D_{50})}$ to the concentration response curves of one drug in combination with a second drug. Note that higher ($1/D_{50}$) values equate to greater drug sensitivity. **Left Table.** Curve fit parameter values from the response curves to nutlin-3a dosing in the presence of five different camptothecin concentrations plus an unloaded control. E_{max} values increased with the addition of camptothecin and a significant increase of 83.2% was observed with the addition of 150 μM camptothecin compared to 0 μM (86.5 vs 47.2) (*: $p < 0.05$). Further, there was an increase in sensitivity to nutlin-3a with the addition of camptothecin. Specifically, the addition of 5 μM camptothecin increased sensitivity >5-fold (106 vs. 19.9, $p < 0.05$) and 50 μM camptothecin increased sensitivity >9-fold (189 vs. 19.9, $p < 0.1$) compared to nutlin-3a alone. Hyperbolic curve fits yielding an R^2 value less than 0.7 are indicated with "#". **Right Table.** Curve fit parameter values from the combined response curves to camptothecin dosing in the presence of five different nutlin-3a concentrations plus an unloaded control. Again, E_{max} values increased in response to camptothecin with the addition of nutlin-3a. Further, sensitivity to camptothecin significantly increased with the addition of all concentrations of nutlin-3a. These results indicate an increase in antiproliferative activity with combinations of the two drugs compared to either drug alone. (*: $p < 0.05$, $: $p < 0.1$, **: $p <$ 0.05 compared to * and ***, ***: $p < 0.05$ compared to * and **).

S7. Coefficient of variation values for microarrays from HCT116 proliferation experiments

CPT concentration	Nut concentration	CV between arrays (%)	CV within arrays (%)
0 μM	0 μM	1	1
0 μM	1 μM	2	24
0 μM	4 μM	17	13
0 μM	25 μM	15	21
0 μM	62.5 μM	17	14
0 μM	125 μM	1	13
1 μM	0 μM	5	16
1 μM	1 μM	8	5
1 μM	4 μM	12	16
1 μM	25 μM	25	16
1 μM	62.5 μM	20	16
1 μM	125 μM	8	12
5 μM	0 μM	12	6
5 μM	1 μM	26	10
5 μM	4 μM	21	9
5 μM	25 μM	15	13
5 μM	62.5 μM	15	4
5 μM	125 μM	3	9
15 μM	0 μM	9	1
15 μM	1 μM	8	10
15 μM	4 μM	11	17
15 μM	25 μM	16	13
15 μM	62.5 μM	5	9
15 μM	125 μM	8	7
50 μM	0 μM	12	21
50 μM	1 μM	20	16
50 μM	4 μM	18	11
50 μM	25 μM	11	12
50 μM	62.5 μM	11	10
50 μM	125 μM	12	13
150 μM	0 μM	12	6
150 μM	1 μM	20	39
150 μM	4 μM	18	11
150 μM	25 μM	11	8
150 μM	62.5 μM	11	8

Coefficient of variation was calculated for each condition on the microarrays from HCT116 proliferation experiments. Coefficient of variation was found for both between separate microarrays, and for the replicates within a single microarray.



S8. HCT116 cell apoptosis and dose-response curves from combinatorial drug-eluting microarrays. **(a)** Nutlin-3a and camptothecin demonstrate differing effects on inducing apoptosis as revealed by two-way ANOVA, where camptothecin was pro-apoptotic, {$F(3,77) = 15.475$, $p < 0.05$}, nutlin-3a was not, {$F(3,80) = 0.695$, $p > 0.05$}, and with a significant interaction effect revealed {$F(9,342) = 3.371$, $p < 0.05$}. Error bars depict standard error of the mean. (**b-e**) Dose response curves for fixed camptothecin concentrations of 0, 1, 10, 50 μM, with nutlin-3a varying over a concentration range. (**b**) Nutlin-3a showed no effect on the proportion of HCT116 cells that underwent apoptosis when administered alone (6.0% for 0 μM vs 5.6% for 125 μM, $p > 0.05$; $E_{max} = 0.0$, Fig. S9). (**c-e**) Increasing the nutlin-3a concentrations conferred protection from the apoptotic response to camptothecin as revealed by a decreasing trend in E_{max} values in response to nutlin-3a ($p > 0.05$). This antagonistic effect was particularly evident at the 10 μM and 50 μM camptothecin concentrations which yielded negative apoptotic responses (E_{max} = -5.9 and -4.3 for 10 μM and 50 μM respectively). (**f-i**) Dose response curves of fixed nutlin-3a concentrations of 0, 1, 25, 125 μM, over a range of camptothecin concentrations. (**f**) Camptothecin, administered alone, promoted apoptosis in a dose dependent manner. (**g-i**) As shown by others (37, 47), addition of nutlin-3a attenuated the apoptotic dose dependent response to camptothecin, and blocked it altogether at the 125 μM nutlin-3a concentration. The maximum effect to camptothecin was decreased 87% by the addition of 125 μM nutlin-3a compared to 0 μM nutlin-3a (2.0 vs. 15.2, see S9). (*: $p < 0.05$ compared to 0 μM)

S9. E_{max} and Sensitivity ($1/D_{50}$) values generated from combinatorial drug-eluting microarrays of HCT116 cells undergoing apoptosis.

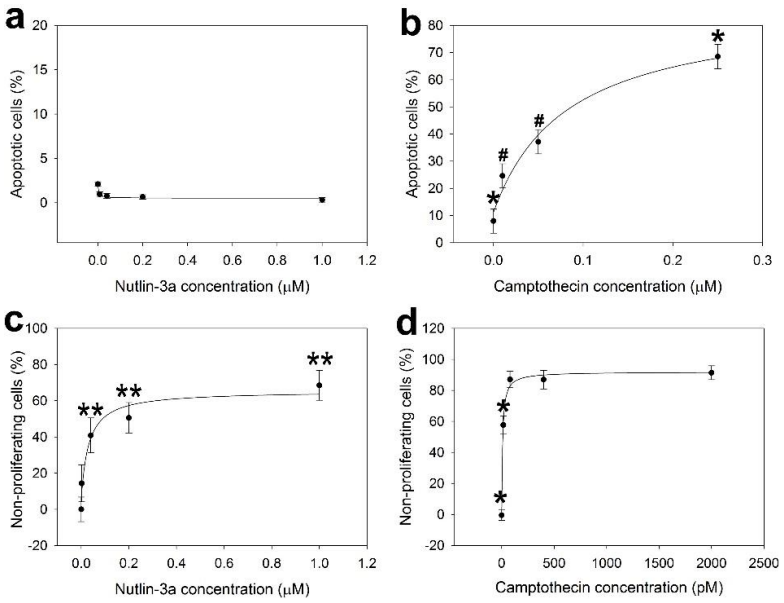
HCT116 Apoptosis											
Hyperbolic Fit Parameters of Nutlin Dose Response Upon Addition of Camptothecin						Hyperbolic Fit Parameters of Camptothecin Dose Response Upon Addition of Nutlin					
2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data	2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data
CPT	0	5.6 +/- 1	0.0 +/- 1	#	Fig S8b	NUT	0	5.8 +/- 1	15.2 +/- 3 *	2.53 +/- 1.1	Fig S8f
	1	6.7 +/- 2	0.0 +/- 2	#	Fig S8c		1	6.6 +/- 1	4.1 +/- 3	83.3 +/- 76	Fig S8g
	10	9.1 +/- 1	-5.9 +/- 1	2.48 +/- 0.83	Fig S8d		25	5.5 +/- 1	3.8 +/- 3	15.9 +/- 2.8	Fig S8h
	50	13.9 +/- 2	-4.3 +/- 2	143 +/- 98	Fig S8e		125	5.5 +/- 1	2.0 +/- 3 *	N/A	Fig S8i

Statistical analysis of combination dose responses was performed by curve-fitting the equation $\mathbf{E = E_0 + (E_{max} \times C)/(C + D_{50})}$ to the concentration response curves of one drug in combination with a second drug. Note that higher ($1/D_{50}$) values, equate to greater drug sensitivity. **Left Table.** Curve fit parameter values from the response curves to nutlin-3a dosing in the presence of four different camptothecin concentrations. The addition of camptothecin revealed a decreasing trend in E_{max} values in response to nutlin-3a ($p > 0.05$). At low camptothecin concentrations, there was minimal response to ranges of nutlin-3a concentrations ($E_{max} = 0$) and increasing the fixed camptothecin concentration yielded a negative apoptotic response ($E_{max} = -5.9$ and -4.3 for 10 μM and 50 μM, respectively). Curve fits yielding an R^2 value less than 0.7 were replaced with "#". **Right Table.** Curve fit parameter values from the combined response curves to camptothecin dosing in the presence of four different nutlin-3a concentrations. There was a significant decrease (of 87%) in the E_{max} values at 125 μM nutlin-3a compared to 0 μM nutlin-3a (2.0 vs. 15.2) indicating a decrease in apoptotic activity. No statistical differences in sensitivity were found. (*: $p < 0.05$). "N/A" is listed when negative parameters were obtained. The poor curve-fit for these parameters are attributed to the antagonistic effect of the drug combinations creating non-standard dose response curves.

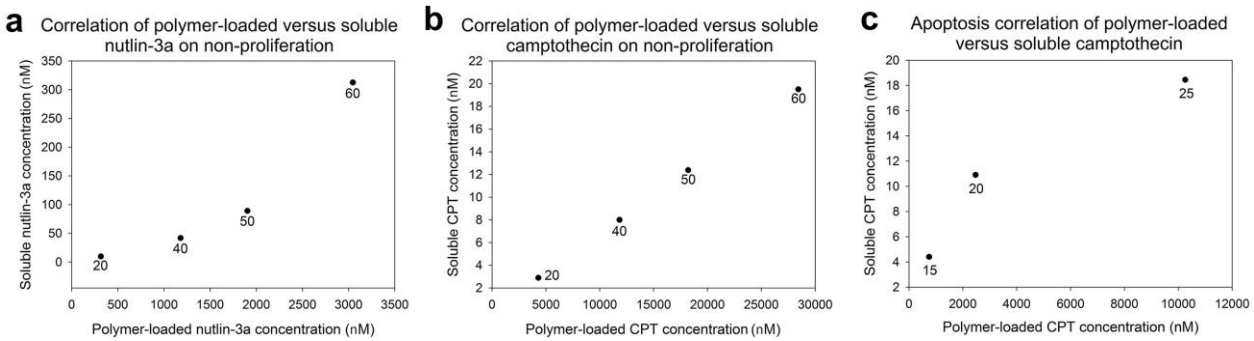
S10. Coefficient of variation values for microarrays from HCT116 apoptosis experiments

CPT concentration	Nut concentration	CV between arrays (%)	CV within arrays (%)
0 μM	0 μM	5	6
0 μM	1 μM	19	13
0 μM	25 μM	7	8
0 μM	125 μM	19	11
1 μM	0 μM	9	4
1 μM	1 μM	9	6
1 μM	25 μM	20	8
1 μM	125 μM	4	9
10 μM	0 μM	24	10
10 μM	1 μM	31	8
10 μM	25 μM	28	8
10 μM	125 μM	19	10
50 μM	0 μM	4	13
50 μM	1 μM	19	11
50 μM	25 μM	21	4
50 μM	125 μM	8	8

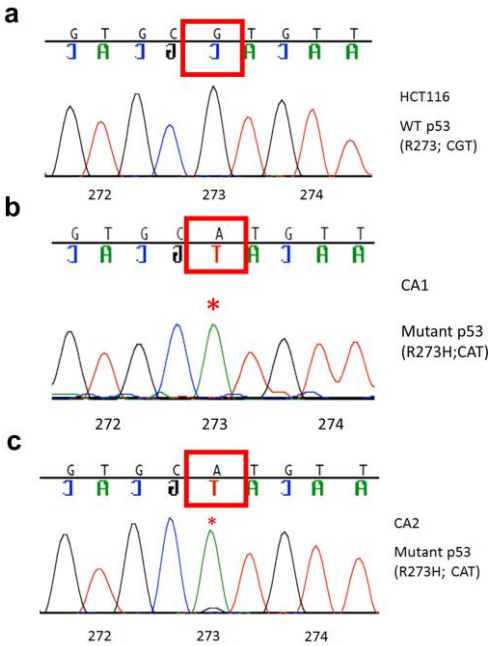
Coefficient of variation was calculated for each condition on the microarrays from HCT116 apoptosis experiments. Coefficient of variation was found for both between separate microarrays, and for the replicates within a single microarray.



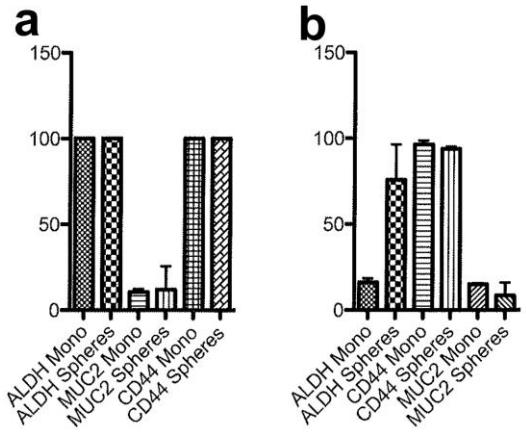
S11. Apoptosis and proliferation dose-response curves from HCT116 cells incubated with soluble drugs in standard 96-well plates demonstrate similar response curves as those from drug-eluting microarrays. **(a)** Percent apoptotic HCT116 cells incubated 24 h with soluble nutlin-3a. Results showed no effect on inducing apoptosis of HCT116 cells, as was observed with the microarray. (**b**) Percent of apoptotic HCT116 cells incubated 24 h with soluble camptothecin. Results displayed a dose-dependent effect on inducing apoptosis of HCT116 cells. Significant differences were determined by ANOVA, followed by Tukey's post-hoc analysis. (**c**) Percent of non-proliferating HCT116 cells incubated 24 h with soluble nutlin-3a. Nutlin-3a showed a dose-dependent effect on reducing proliferation of HCT116 cells. Significant differences were determined by ANOVA, followed by Tukey's post-hoc analysis. (**d**) Percent of non-proliferating HCT116 cells incubated 24 h with soluble camptothecin. Camptothecin had a dose-dependent effect on reducing proliferation of HCT116 cells. Significant differences were determined by ANOVA, followed by Tukey's post-hoc analysis (n=3). (*: $p < 0.05$ compared to all other conditions, #: $p < 0.05$ from all conditions marked *, **: $p < 0.05$ from 0 μM).



S12. Correlation between soluble drug and polymer-loaded drug amounts for identical cell responses allows estimation of effective local drug concentrations released from drug-loaded polymer spots on the microarray. (**a**) Cellular response to soluble nutlin-3a compared to nutlin-3a loaded into EVA films based on non-proliferation data. The numbers adjacent to the data points represent the percentage of non-proliferation of HCT116 cells associated with the corresponding concentration (i.e., 50% non-proliferation was achieved with ~1800 μM nutlin-3a loaded into polymer or ~100 μM soluble nutlin-3a). (**b**) Cellular response to soluble camptothecin compared to camptothecin loaded into EVA films based on non-proliferation data. (**c**) Cellular response to soluble camptothecin compared to camptothecin loaded into EVA films based on apoptosis.



S13. Genomic DNA from HCT116, CA1 and CA2 patient-derived cells. Mutational analysis of p53 was carried out at codons 4-9, which are known to contain the majority of reported mutations for p53. **(a)** No mutations were detected for HCT116. **(b)** CA1 and (**c**) CA2 cells contained a single base pair transition (G to A) at amino acid 273 located in exon 8. The resulting missense mutations, denoted by an asterisk, cause an amino acid change from an arginine (R) to a histidine (H).



S14. Cell surface marker expression in patient-derived colorectal cancer stem-like cells (CCSCs) comparing monolayer to spheroid cultures. Markers investigated include aldehyde dehydrogenase (ALDH, marker for CCSCs(25)), mucin 2 (MUC2, a marker for goblet cell lineage), and cluster of differentiation 44 (CD44, a marker, in combination with other markers, for stem cells in solid tumors, notably in breast cancer(41)). **(a)** In CA1 cells, ALDH, MUC2, and CD44 expression were unchanged when cultured as monolayers or spheres. **(b)** CA2 cells showed decreased expression of ALDH when cultured as monolayers compared to spheroids and showed slightly higher MUC2 expression in monolayers versus spheroid culture. While the decrease in ALDH expression may suggest decreased tumor initiating potential, CD44 expression was unchanged.

S15. E_{max} and Sensitivity ($1/D_{50}$) values generated from combinatorial drug-eluting microarrays quantifying percent non-proliferative CA1 CCSCs.

CA1											
Hyperbolic Fit Parameters of Nutlin Dose Response Upon Addition of Camptothecin						Hyperbolic Fit Parameters of Camptothecin Dose Response Upon Addition of Nutlin					
2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data	2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data
CPT	0	1.0 +/- 20	37.8 +/- 20	28.6 +/- 4.7*	Fig 3b	NUT	0	1.4 +/- 18	33.6 +/- 6	36.3 +/- 14	Fig 3f
	1	19.1 +/- 20	18.6 +/- 24	N/A	Fig 3c		1	9.9 +/- 18	38.8 +/- 6	6.1 +/- 14	Fig 3g
	10	34.8 +/- 20	19.6 +/- 24	50.0 +/- 1.3*	Fig 3d		25	30.6 +/- 18	51.6 +/- 6	6.5 +/- 11	Fig 3h
	50	54.2 +/- 20	27.0 +/- 20	70.6 +/- 29	Fig 3e		125	35.3 +/- 18	43.3. +/- 9	2.6 +/- 11	Fig 3i

Statistical analysis of combination dose responses was performed by curve-fitting the equation $\mathbf{E = E_0 + (E_{max} \times C)/(C + D_{50})}$ to the concentration response curves in response to one drug in combination with a second drug. Note that higher ($1/D_{50}$) values, equate to greater drug sensitivity. **Left Table.** Curve fit parameter values from the response curves to nutlin-3a dosing in the presence of four different camptothecin concentrations. There was no significant change in E_{max} values of the nutlin-3a response with the presence of camptothecin. However, there was a significant increase (by 75%) in the sensitivity to nutlin-3a when combined with 10 μM camptothecin compared to nutlin-3a alone (28.6 vs. 50.0), indicative of an increase in antiproliferative activity (*: $p < 0.05$). **Right Table.** Observing the response curves to camptothecin in the presence of fixed amounts of nutlin-3a revealed an increasing trend in E_{max} values, though this finding wasn't significant ($p > 0.05$). Differences in sensitivity could not be discerned due to high error. "N/A" is listed when negative parameters were obtained.

S16. Coefficient of variation values for microarrays from CA1 experiments

CPT concentration	Nut concentration	CV between arrays (%)	CV within arrays (%)
0 μM	0 μM	9	13
0 μM	1 μM	8	14
0 μM	25 μM	14	13
0 μM	125 μM	10	15
1 μM	0 μM	10	7
1 μM	1 μM	15	18
1 μM	25 μM	9	9
1 μM	125 μM	9	9
10 μM	0 μM	15	14
10 μM	1 μM	12	14
10 μM	25 μM	16	10
10 μM	125 μM	11	18
50 μM	0 μM	5	12
50 μM	1 μM	16	25
50 μM	25 μM	38	24
50 μM	125 μM	9	28

Coefficient of variation was calculated for each condition on the microarrays from CA1 experiments. Coefficient of variation was found for both between separate microarrays, and for the replicates within a single microarray.

S17. E_{max} and Sensitivity ($1/D_{50}$) values generated from combinatorial drug-eluting microarrays quantifying percent non-proliferative CA2 CCSCs.

CA2											
Hyperbolic Fit Parameters of Nutlin Dose Response Upon Addition of Camptothecin						Hyperbolic Fit Parameters of Camptothecin Dose Response Upon Addition of Nutlin					
2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data	2nd Drug	Conc (μM)	E_0	E_{max}	Sensitivity ($1/D_{50}$x100)	Source Data
CPT	0	2.9 +/- 9	59.0 +/- 13*$	171 +/- 49	Fig 3k	NUT	0	5.3 +/- 11	43.7 +/- 21	133 +/- 5.3 *	Fig 3o
	1	25.0 +/- 9	20.5 +/- 13	189 +/- 328	Fig 3l		1	59.5 +/- 14	2.9 +/- 21	1.60 +/- 7.9*	Fig 3p
	10	49.1 +/- 9	5.7 +/- 13 $	N/A	Fig 3m		25	40.0 +/- 14	8.3 +/- 37	N/A	Fig 3q
	50	42.3 +/- 9	-8.3 +/- 16 *	303 +/- 260	Fig 3n		125	59.2 +/- 11	-1.7 +/- 37	#	Fig 3r

Statistical analysis of combination dose responses was performed by curve-fitting the equation $\mathbf{E = E_0 + (E_{max} x C)/(C + D_{50})}$ to the concentration response curves in response to one drug in combination with a second drug. Note that higher ($1/D_{50}$) values, equate to greater drug sensitivity. **Left Table.** Curve fit parameter values from the response curves to nutlin-3a dosing in the presence of four different camptothecin concentrations. For CA2 CCSCs, there was a decrease of 90% in the E_{max} values of the response to nutlin-3a in the presence of 10 μM camptothecin compared to 0 μM camptothecin (5.7 vs. 59.0), and a decrease of 114% in the E_{max} values with the addition of 50 μM (-8.3 vs 59.0), indicating a decrease in antiproliferative activity. Differences in sensitivity could not be discerned due to high error. (*: $p < 0.05$, $: $p < 0.1$). "N/A" values are shown where negative values were obtained. **Right Table.** Observing the response curves to ranges of camptothecin in the presence of fixed amounts of nutlin-3a revealed a decreasing trend in E_{max} values, though this finding wasn't significant ($p > 0.05$). Sensitivity significantly decreased by 100-fold due to the addition of 1 μM of nutlin-3a compared to camptothecin

alone. “N/A” values are shown where negative values for sensitivity were obtained. At high nutlin-3a concentrations, there was minimal response to ranges of camptothecin concentrations. Therefore, hyperbolic curve fits yielded parameters with extremely high variation and values were replaced with “#”.

S18. Coefficient of variation values for microarrays from CA2 experiments

CPT concentration	Nut concentration	CV between arrays (%)	CV within arrays (%)
0 μM	0 μM	1	10
0 μM	1 μM	14	15
0 μM	25 μM	10	22
0 μM	125 μM	23	25
1 μM	0 μM	8	17
1 μM	1 μM	7	19
1 μM	25 μM	33	11
1 μM	125 μM	34	16
10 μM	0 μM	15	16
10 μM	1 μM	17	9
10 μM	25 μM	22	12
10 μM	125 μM	39	15
50 μM	0 μM	13	20
50 μM	1 μM	9	18
50 μM	25 μM	16	21
50 μM	125 μM	7	18

Coefficient of variation was calculated for each condition on the microarrays from CA2 experiments. Coefficient of variation was found for both between separate microarrays, and for the replicates within a single microarray.