An approach to minimize tumour proliferation by reducing the formation of components for cell membrane

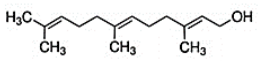
Inmaculada de Dios-Pérez, Álvaro González-Garcinuño

and Eva María Martín del Valle*

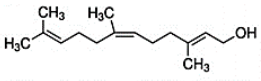
Supplementary Material

Supplementary material S1

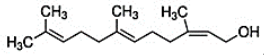
Chemical characterization of FOH isomers and cyclodextrin structure



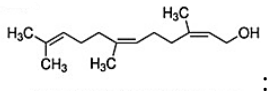
: trans, trans – farnesol (CAS 106-28-5)



: 2-trans, 6-cis – farnesol (CAS 3879-60-5)

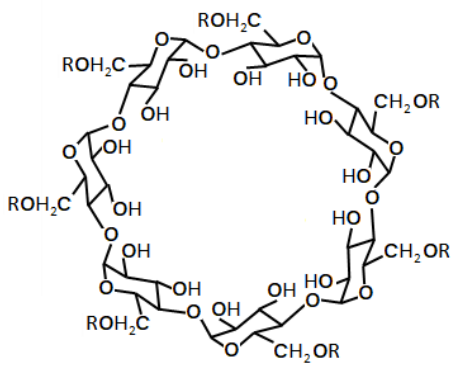


: 2-cis, 6-trans – farnesol (CAS 3790-71-4)



: cis, cis – farnesol (CAS 16106-95-9)

Figure S1. Structural representation of the different geometric isomers of farnesol.



R = -H or sulfobutyl (-CH_2)$_4$-SO_3Na)
Empirical Formula: $C_{42}H_{70\text{-}6.4}O_{35} \cdot (C_4H_8SO_3Na)_{6.4}$

Figure S2. Structural representation of SBE-βCD.

$$[\,]_{FOH} = \frac{\frac{\delta_{FOH}}{MW_{FOH}}}{\frac{\delta_{SBE-\beta-CD}}{MW_{SBE-\beta-CD}}} \cdot [\,]_{SBE-\beta-CD} = \frac{\frac{23{,}4}{10\ H}}{\frac{60{,}0}{6{,}4 \cdot 2\ H}} \cdot 100\ mM = \frac{2{,}34\ mol\ FOH}{4{,}69\ mol\ CD} \cdot 100\ mM \approx 50\ mM$$

$$[\,]_{E,E-FOH} = \frac{\frac{\delta_{E,E-FOH}}{MW_{E,E-FOH}}}{\frac{\delta_{SBE-\beta-CD}}{MW_{SBE-\beta-CD}}} \cdot [\,]_{SBE-\beta-CD} = \frac{\frac{22{,}5}{10\ H}}{\frac{60{,}0}{6{,}4 \cdot 2\ H}} \cdot 100\ mM = \frac{2{,}25\ mol\ FOH}{4{,}69\ mol\ CD} \cdot 100\ mM \approx 50\ mM$$

Equation S3. Calculation of proportion ratio FOH-CD for isomers mix (first line) and E,E-FOH (second line).

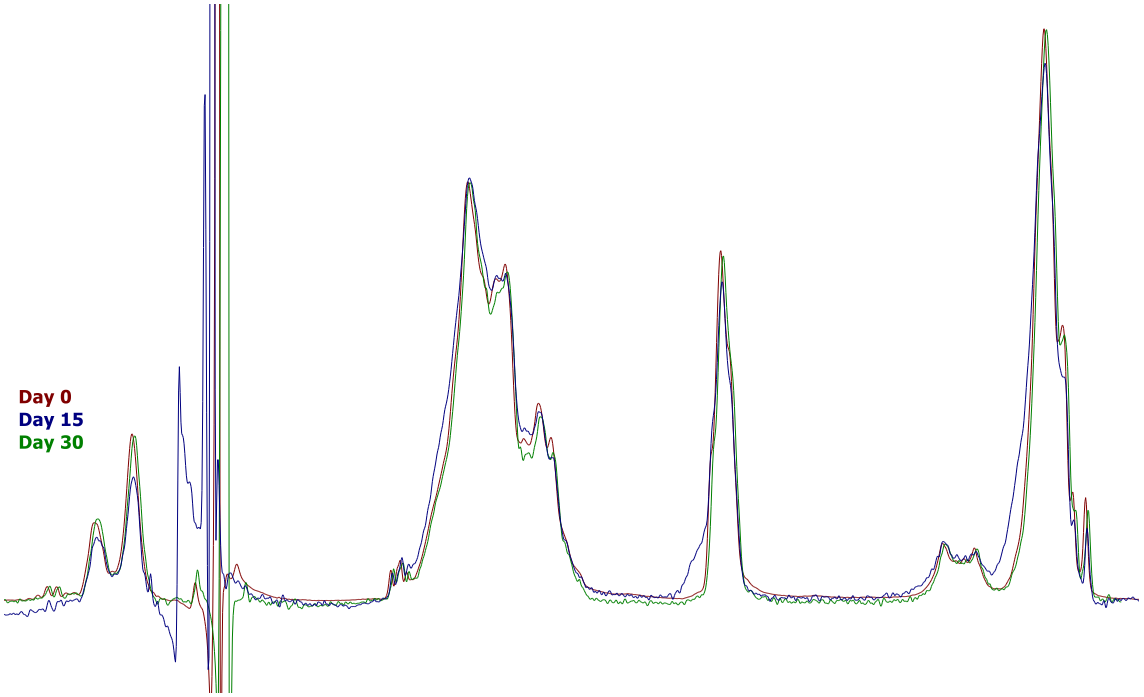


Figure S4: A: NMR-spectra for the complex CD-FOH of the samples collected at different times for one month.

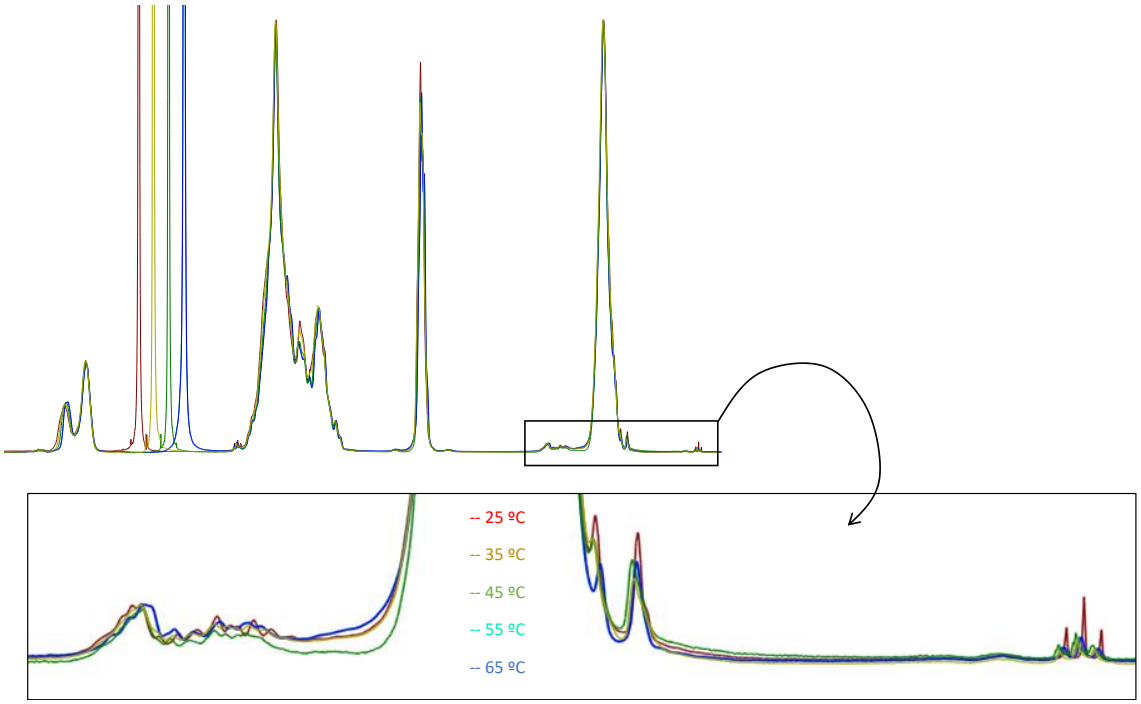


Figure S5: A: NMR-spectra for the complex CD-FOH working with different temperatures.

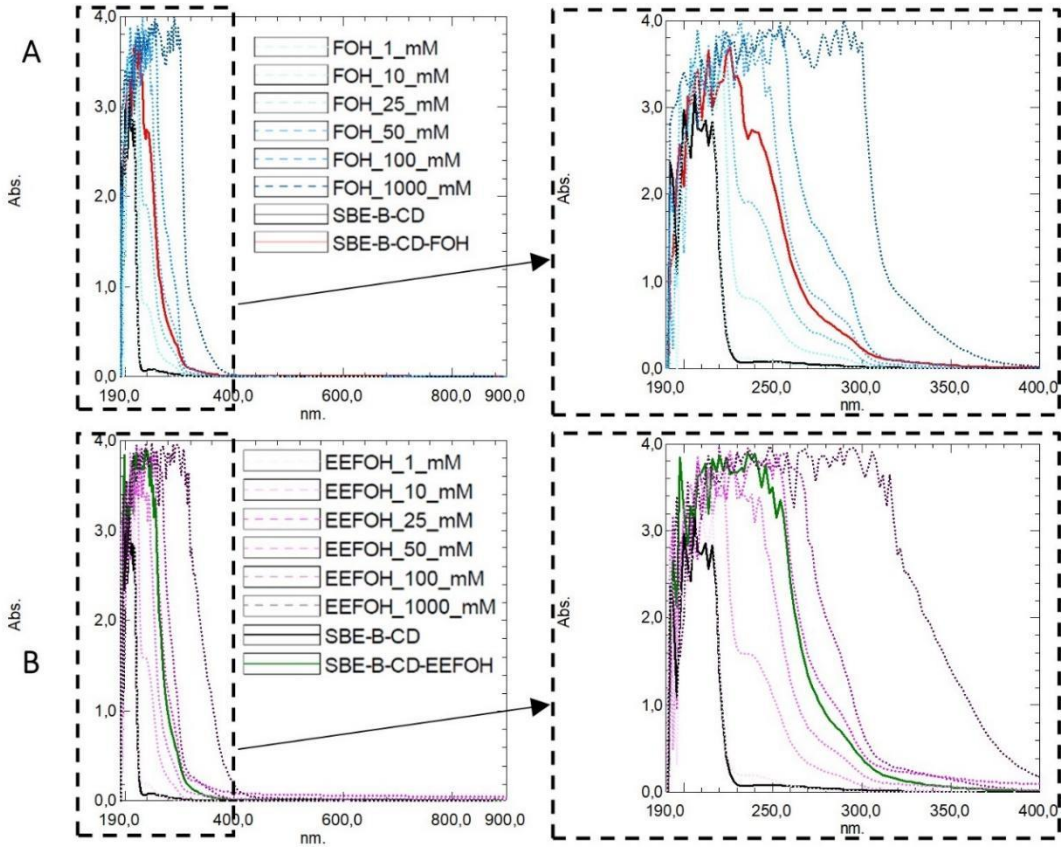


Figure S6: A) UV-Vis spectra for free FOH at different concentrations in ethanol, SBE-β-CD and inclusion complexes (with FOH). B) UV-spectra for free E,E-FOH, SBE-β-CD and inclusion complexes (with E,E-FOH).

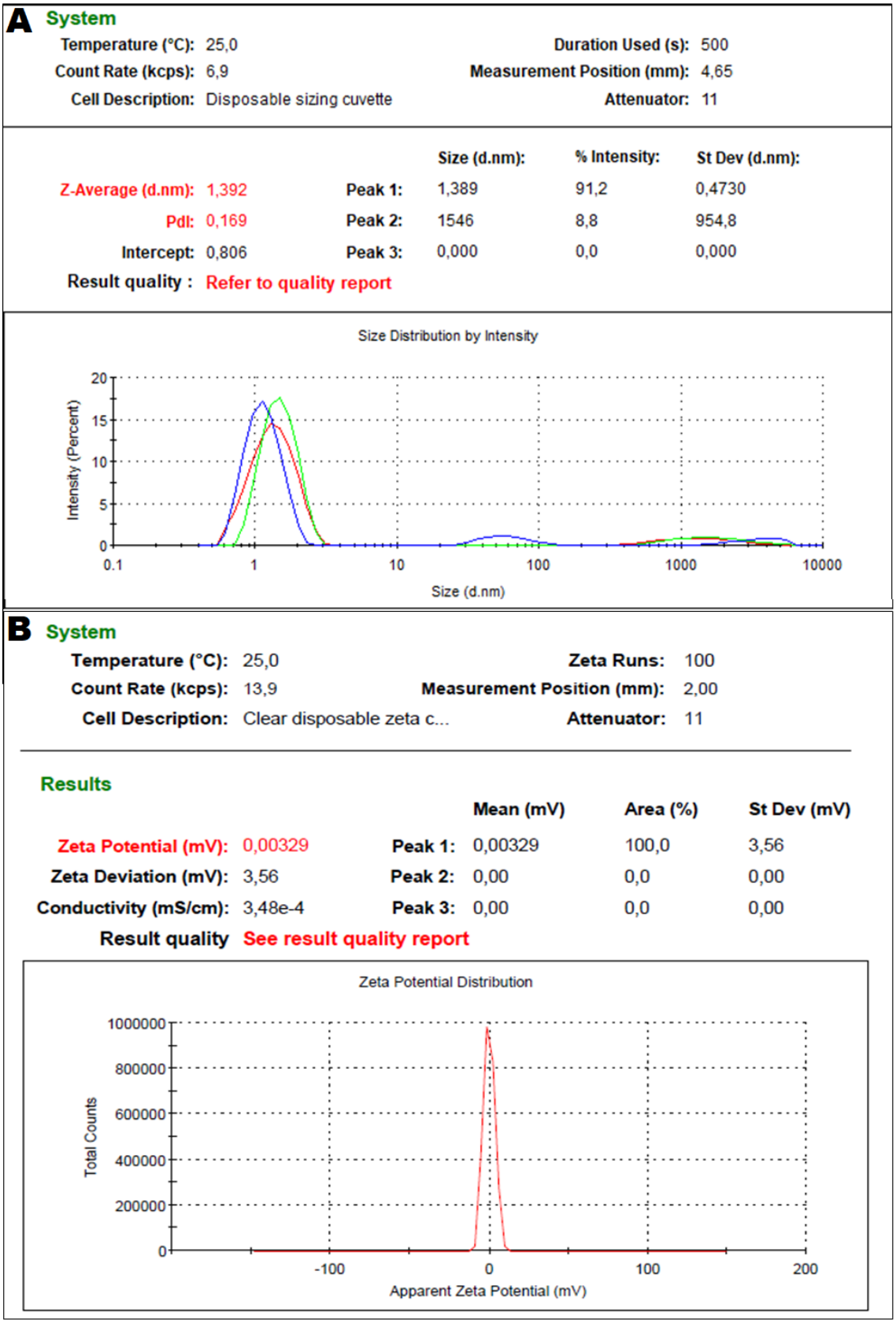


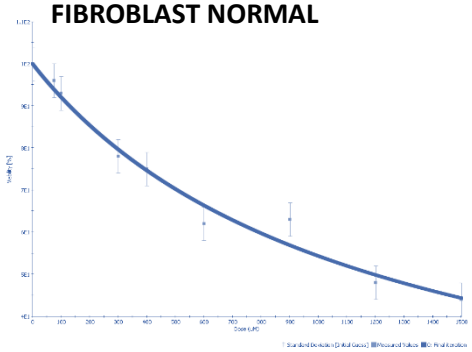
Figure S7: Results size (A) and zeta potential (B) of SBE-β-CD - FOH by DLS.

Supplementary material S2

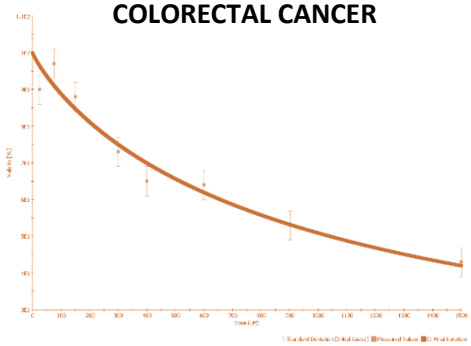
Curves of dose response modelling

CYCLODEXTRIN ESTIMATION

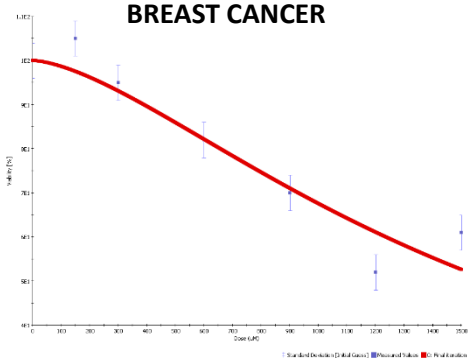
FIBROBLAST NORMAL



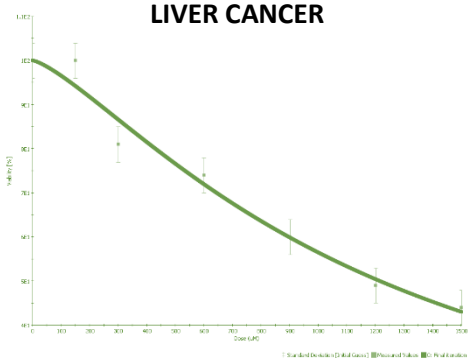
COLORECTAL CANCER



BREAST CANCER

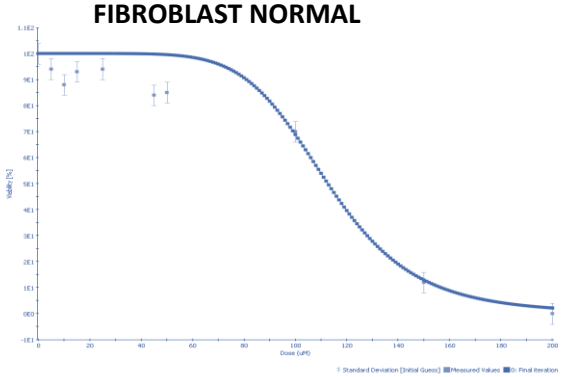


LIVER CANCER

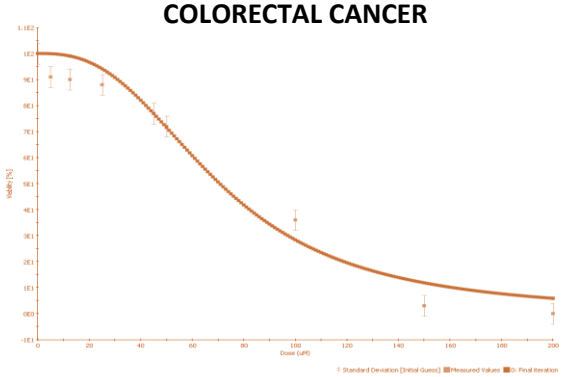


FARNESOL ESTIMATION

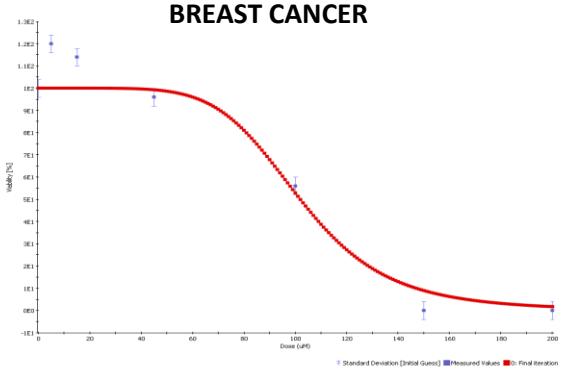
FIBROBLAST NORMAL



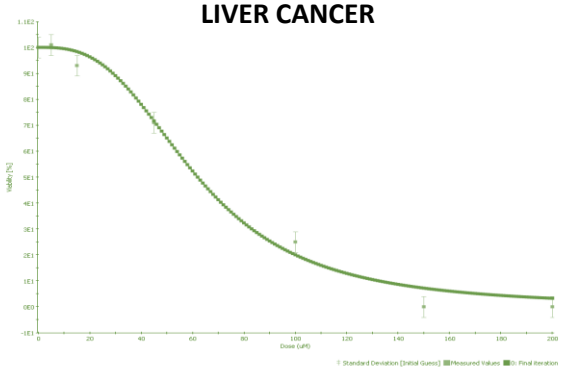
COLORECTAL CANCER



BREAST CANCER



LIVER CANCER



COMPLEX ESTIMATION

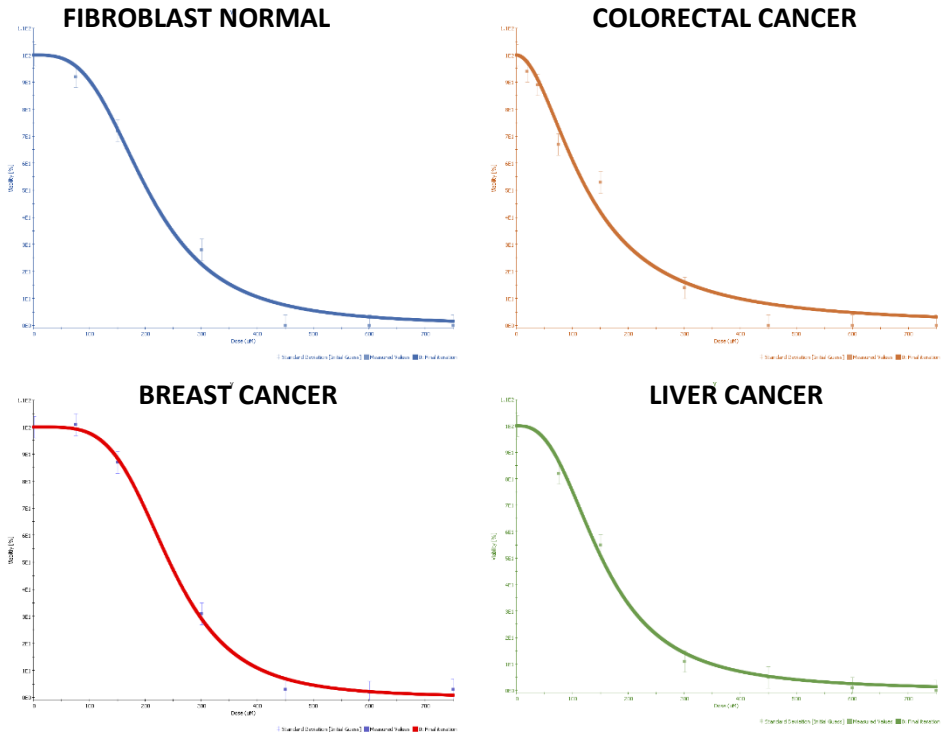


Figure S8: Curves of dose response modelling.

Supplementary material S3

Addition of external PC: in vitro results

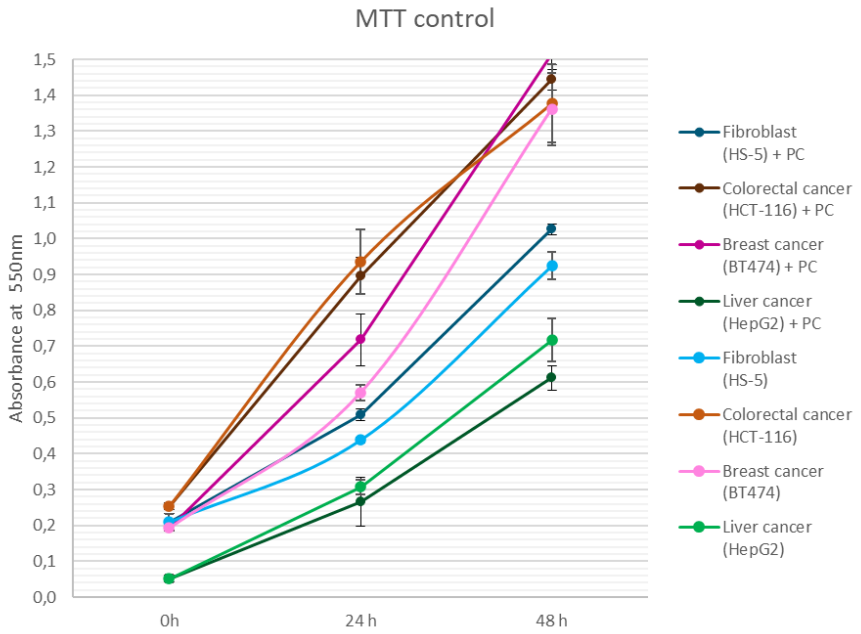


Figure S9: Effect of external addition of PC. Absorbance raw data for four cell lines (with or without addition of PC).

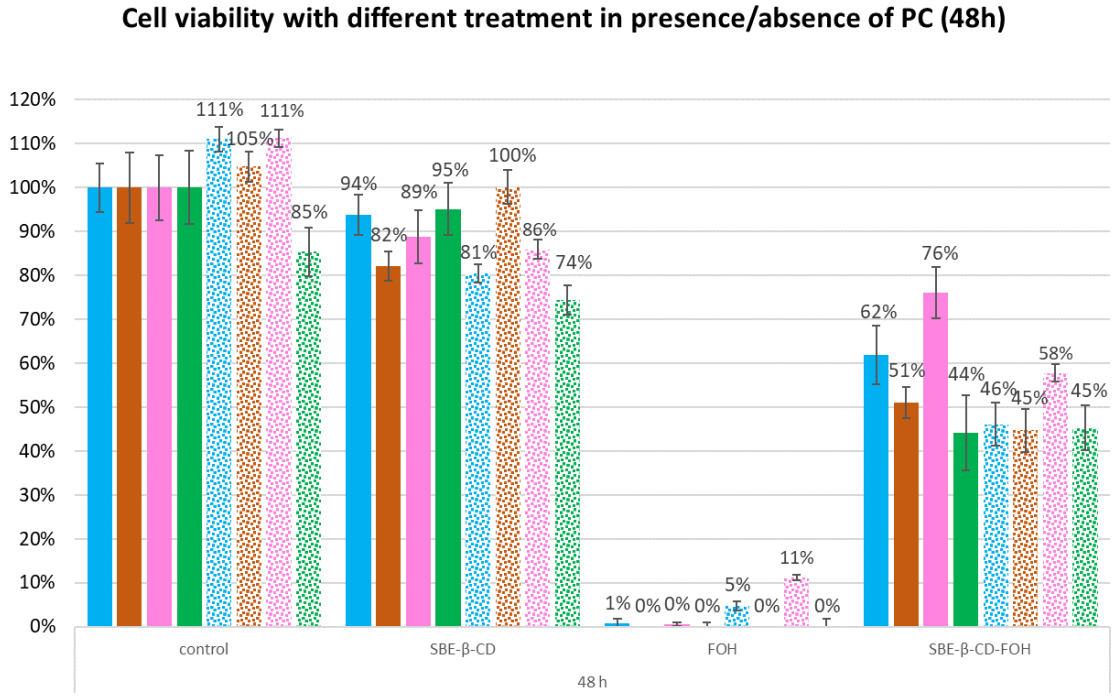


Figure S10: Effect of external addition of PC. Cell viability results calculated from Figure S8.

Supplementary material S4

Statistical analysis: ANOVA tables

ANOVA for colorectal cancer: HTC-116

Source	*Sum of squares*	*FD*	*Medium squared*	*F-value*	*P-value*
Between sample means	36,5616	29	1,26074	354,66	0,0000
Within samples	0,568768	160	0,0035548		
Total	37,1303	189			

ANOVA for fibroblasts: HS-5

Source	*Sum of squares*	*FD*	*Medium squared*	*F-value*	*P-value*
Between sample means	15,0786	29	0,51995	437,91	0,0000
Within samples	0,191164	161	0,00118735		
Total	15,2697	190			

ANOVA for liver cancer: HepG2

Source	*Sum of squares*	*FD*	*Medium squared*	*F-value*	*P-value*
Between sample means	8,04467	29	0,277403	316,06	0,0000
Within samples	0,140431	160	0,000877694		
Total	8,18511	189			

ANOVA for breast cancer: BT474

Source	*Sum of squares*	*FD*	*Medium squared*	*F-value*	*P-value*
Between sample means	35,6583	29	1,2296	426,95	0,0000
Within samples	0,463674	161	0,00287996		
Total	36,122	190			

Supplementary material S5

Cell cycle phase analysis

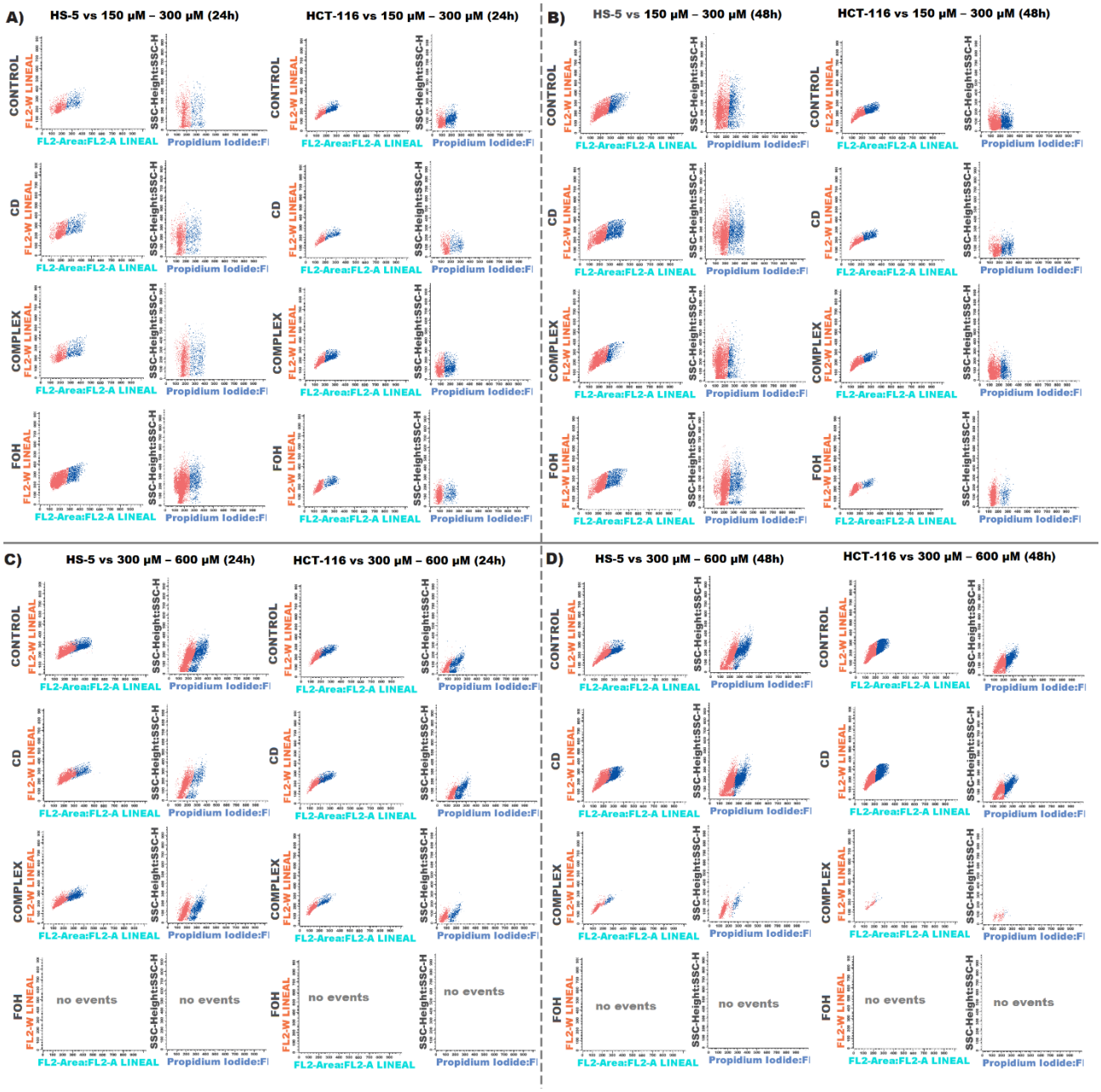


Figure S12: Graphics of cells in G0/G1 (red) or M/G2 (blue) phase after treatment FOH 150 µM and SBE-β-CD 300 µM of fibroblast and colorectal cancer cells for 24 (A) and 48 hours (B), or after treatment FOH 300 µM and SBE-β-CD 600 µM cells for 24 h (A) and 48 h (B).