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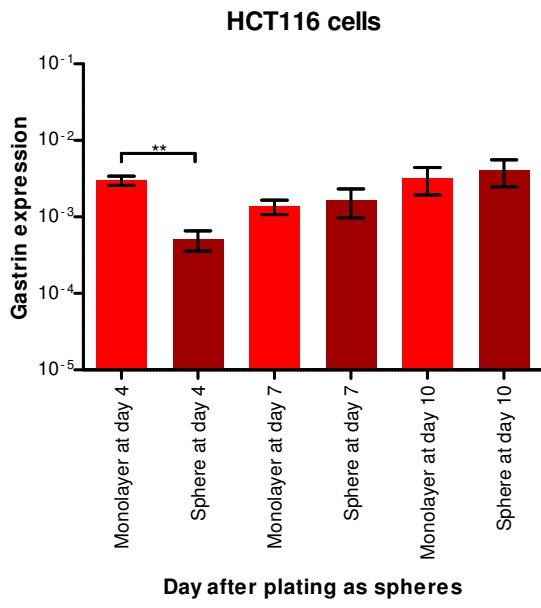
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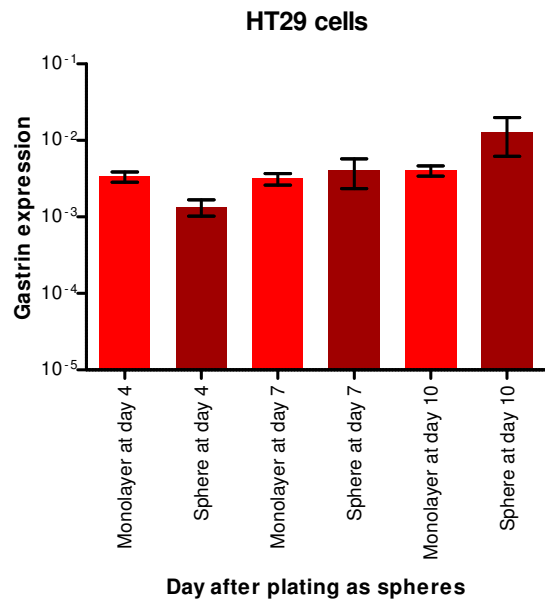
Figure A: The sequence of each of the CCK-2R promoter constructs, compared to that recorded on Ensembl

Each difference between the sequences has been highlighted in green.

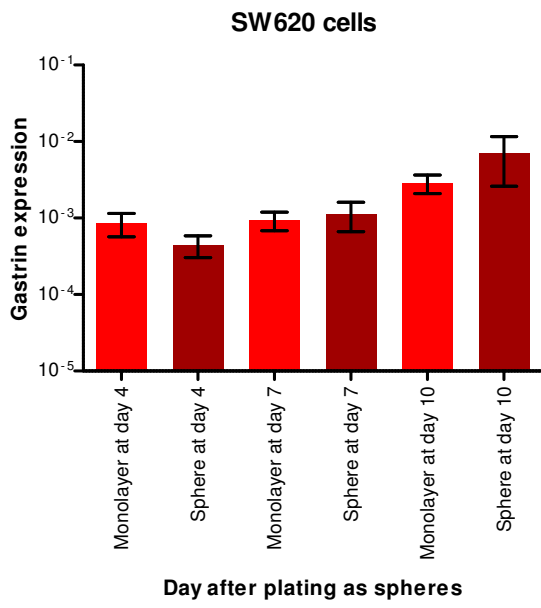
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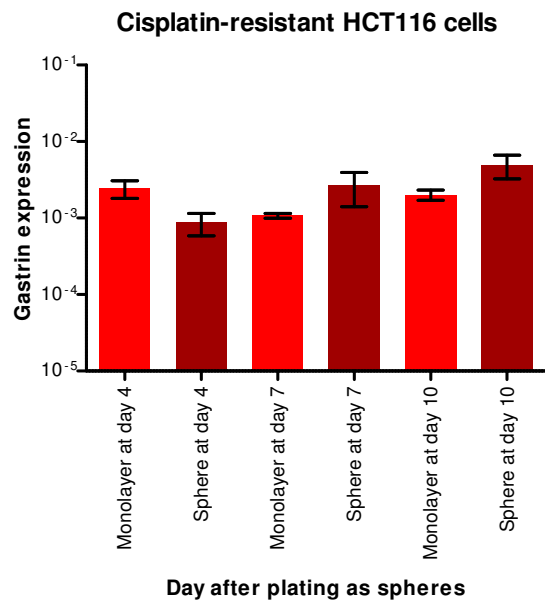
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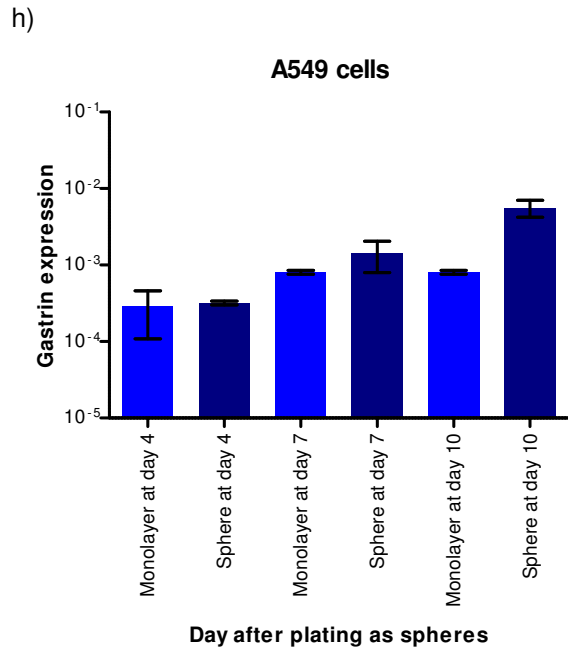
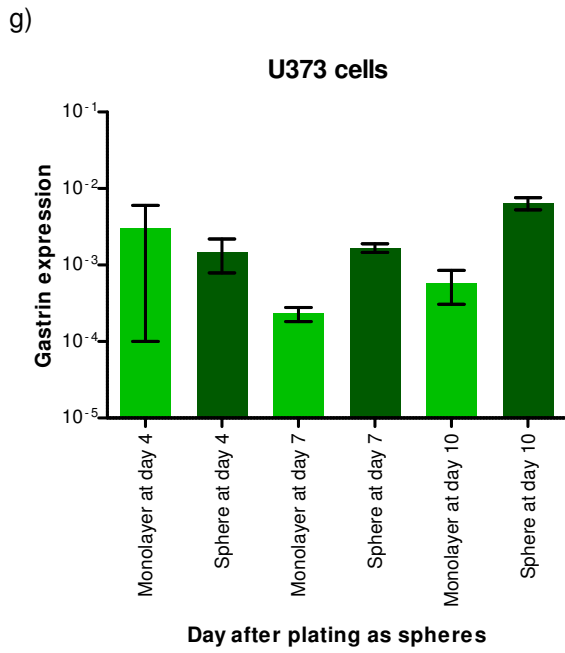
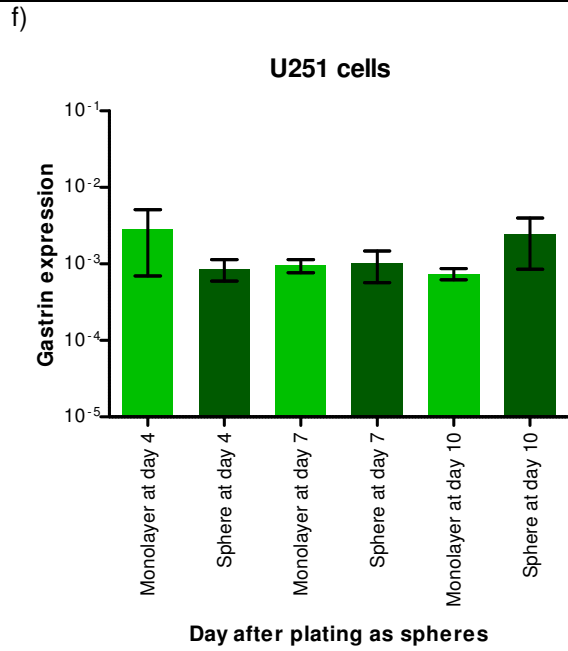
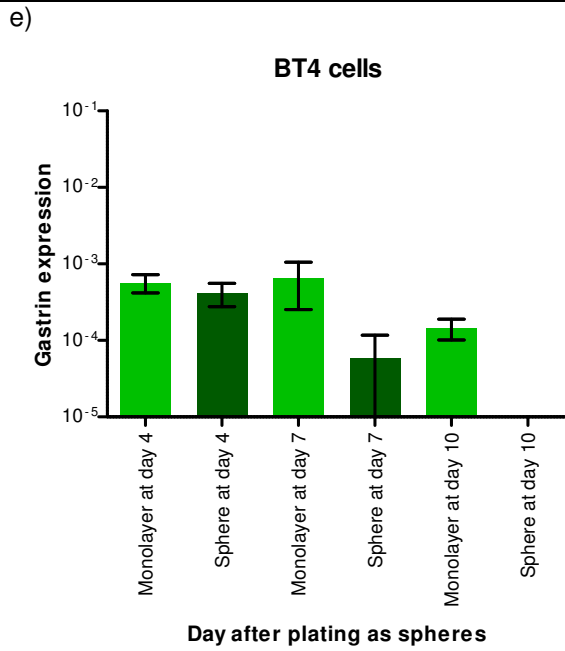


c)



d)





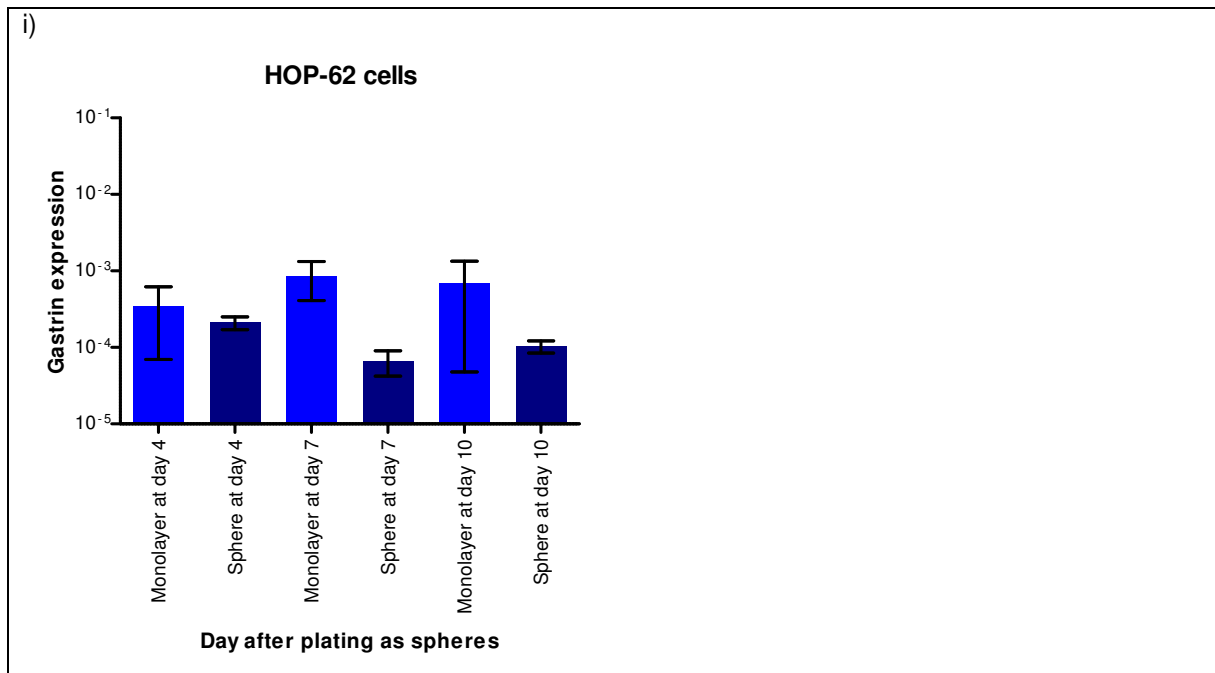
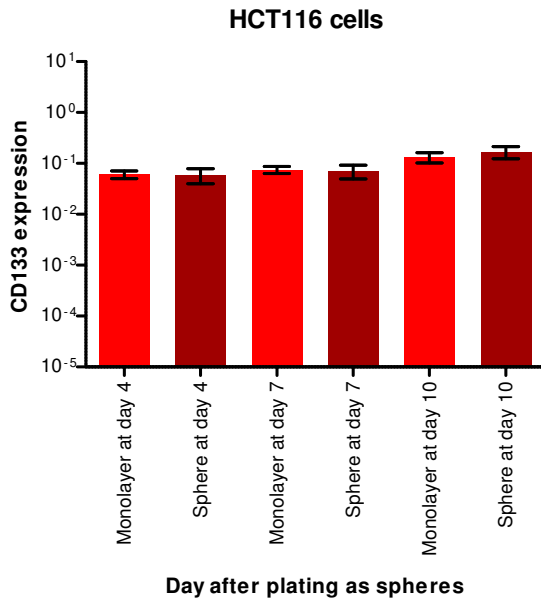


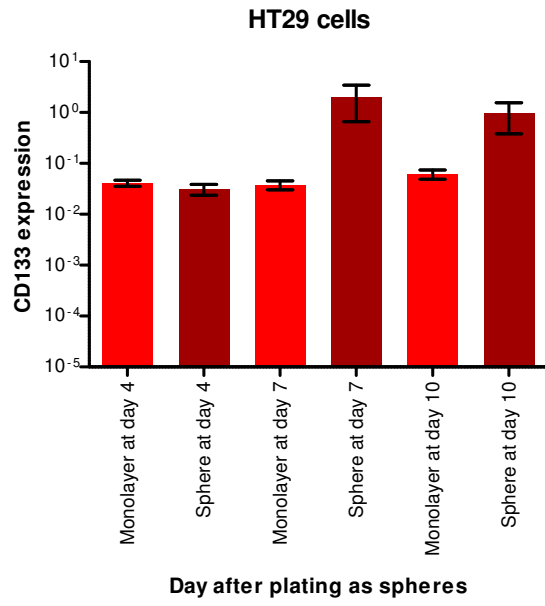
Figure B: Gastrin expression in sphere-forming cells compared to those grown as a monolayer

Logarithmic graphs showing gastrin gene expression in a) HCT116, b) HT29, c) SW620, d) HCT116 cisplatin-resistant, e) BT4, f) U251, g) U373, h) A549 and i) HOP-62 cells. The method of detection for the Gastrin is Sybr Green qRT-PCR and the error bars represent the SEM from at least three replicates (** indicates $p < 0.01$).

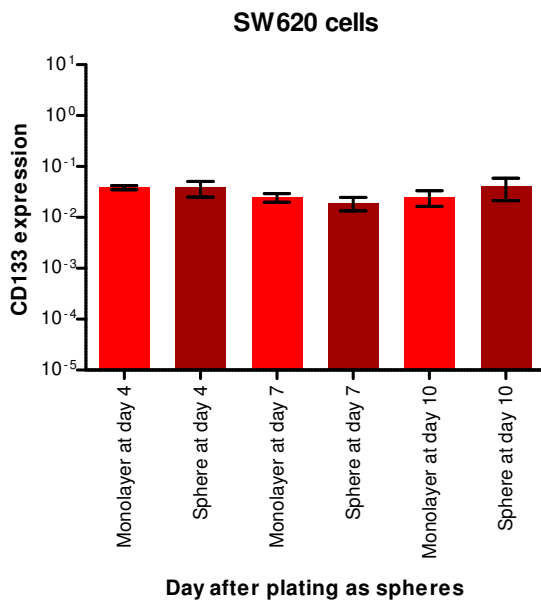
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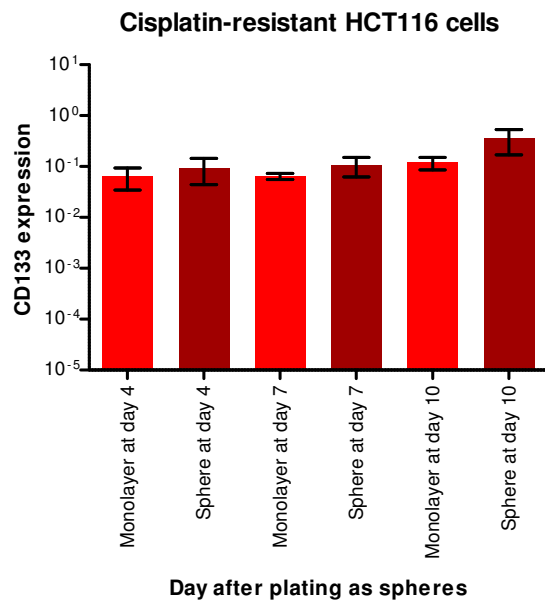
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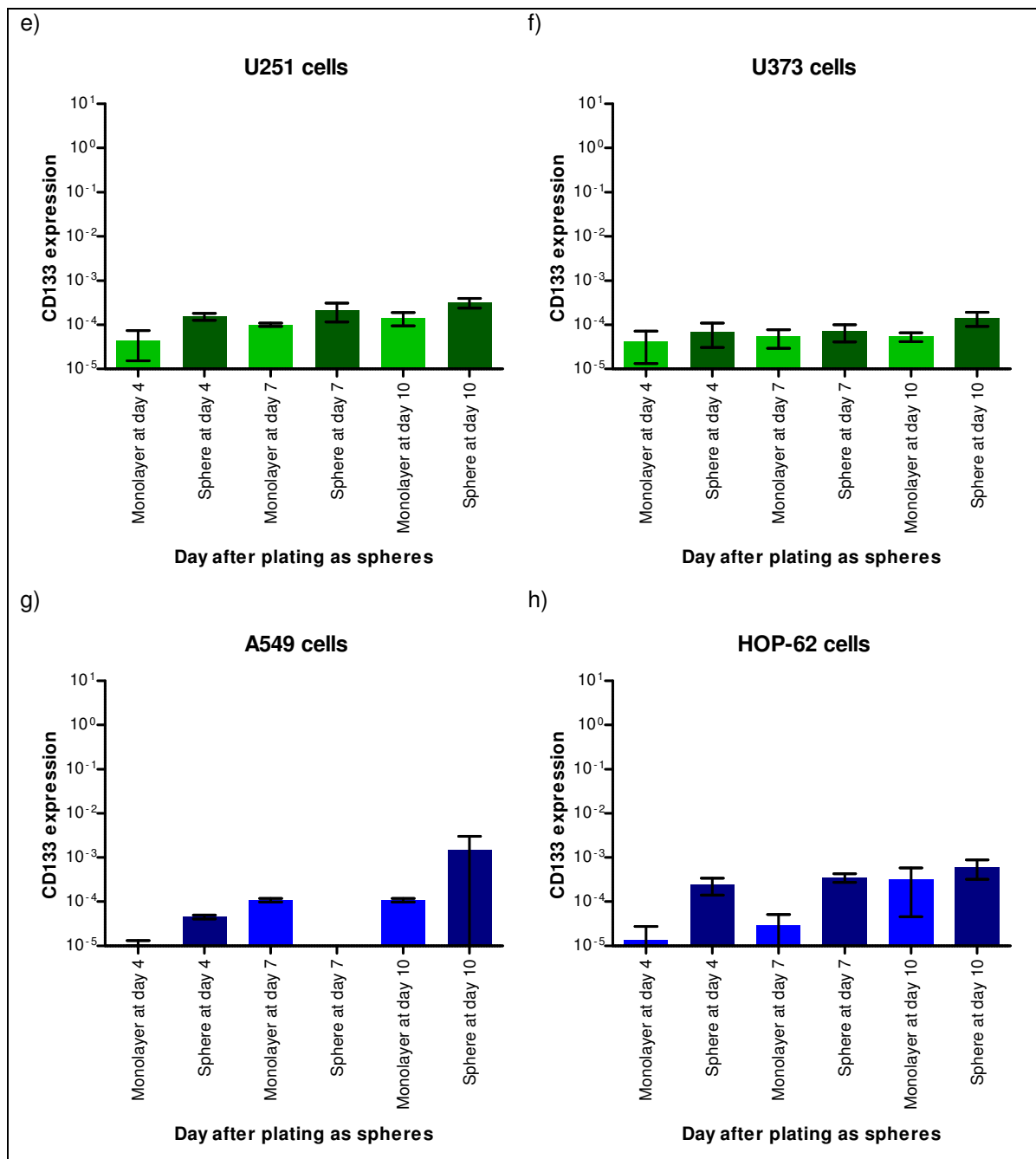


Figure C: CD133 expression in sphere-forming cells compared to those grown as a monolayer

Logarithmic graphs showing CD133 gene expression in a) HCT116, b) HT29, c) SW620, d) HCT116 cisplatin-resistant, e) U251, f) U373, g) A549 and h) HOP-62 cells. The method of detection for the CD133 is Sybr Green qRT-PCR and the error bars represent the SEM from at least three replicates.

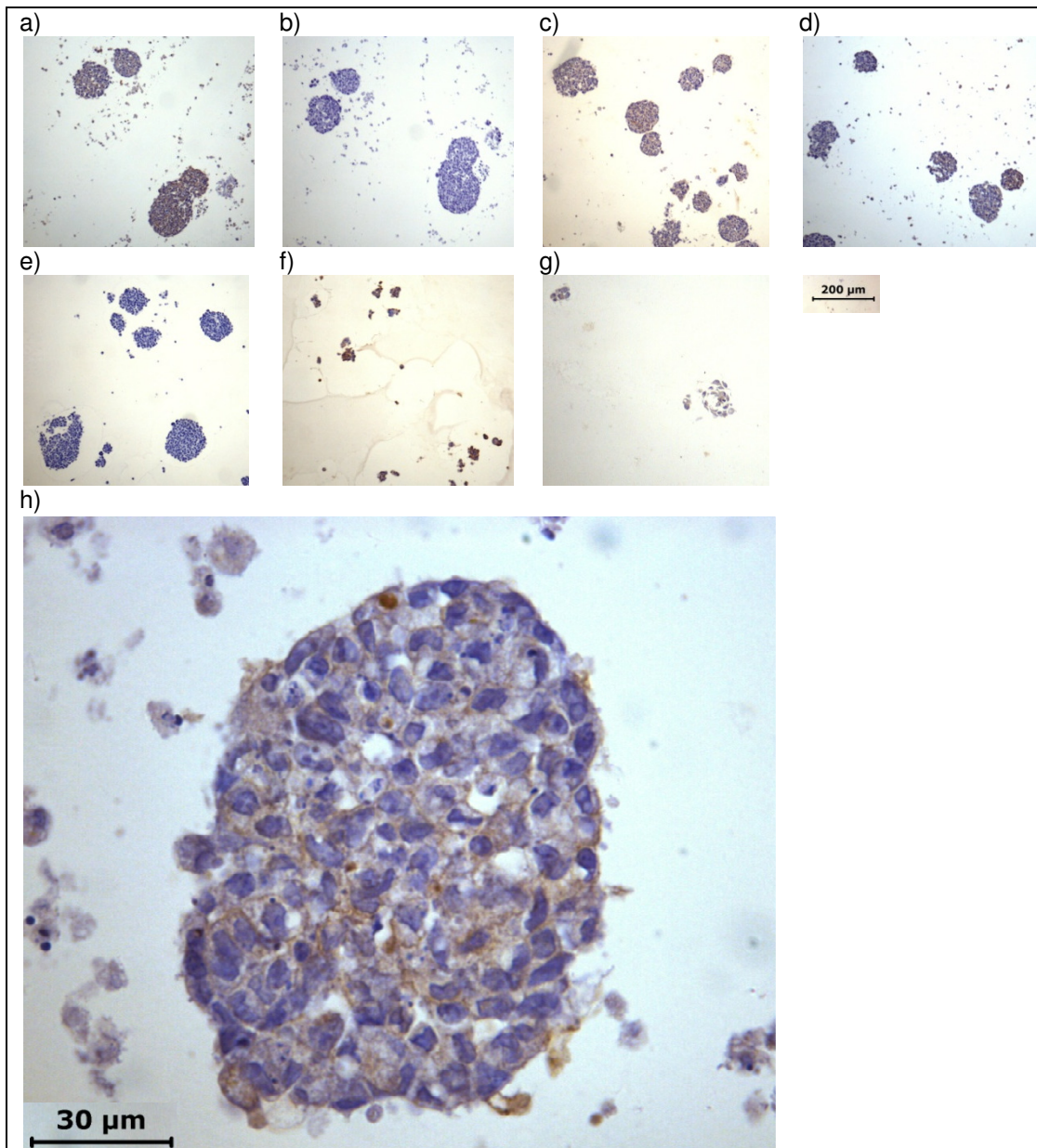
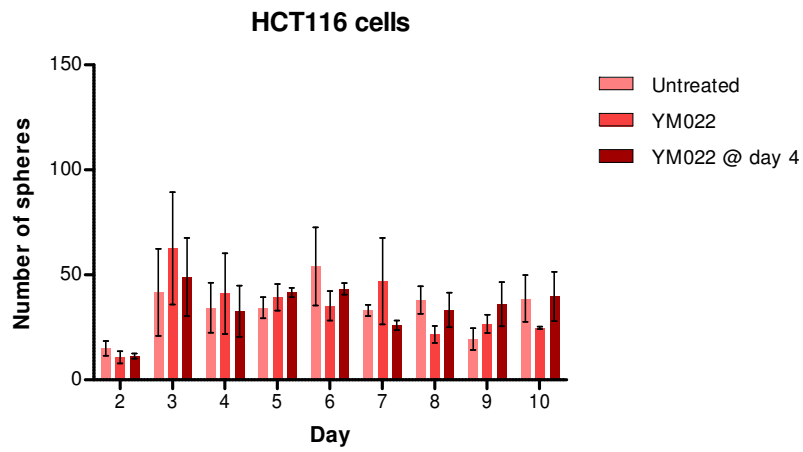


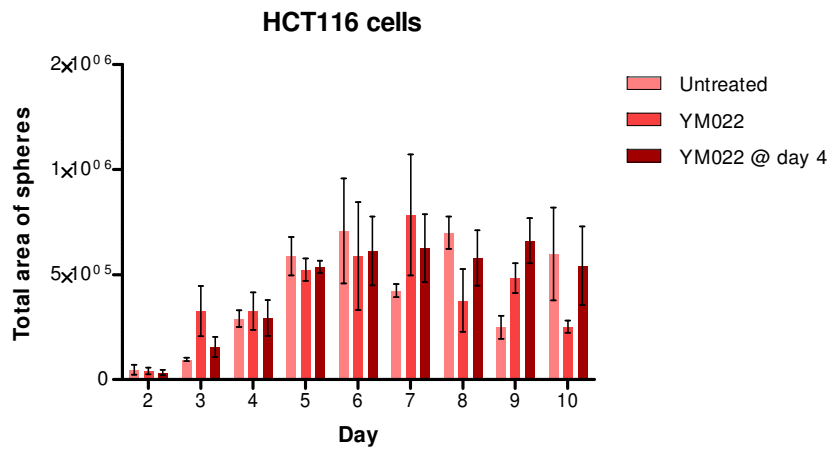
Figure D: Paraffin staining for β -catenin

Sphere structures were formed from cells and stained with a β -catenin antibody (or the negative control with rabbit serum); a) HCT116, b) HCT116 negative, c) HT29, d) cisplatin-resistant HCT116, e) BT4, f) U251, g) HOP-62. The scale of these photographs is shown by the marker for 200 μ m. The photo in h) is a larger scale photo of a HCT116 sphere stained for β -catenin; showing only cytoplasmic localisation.

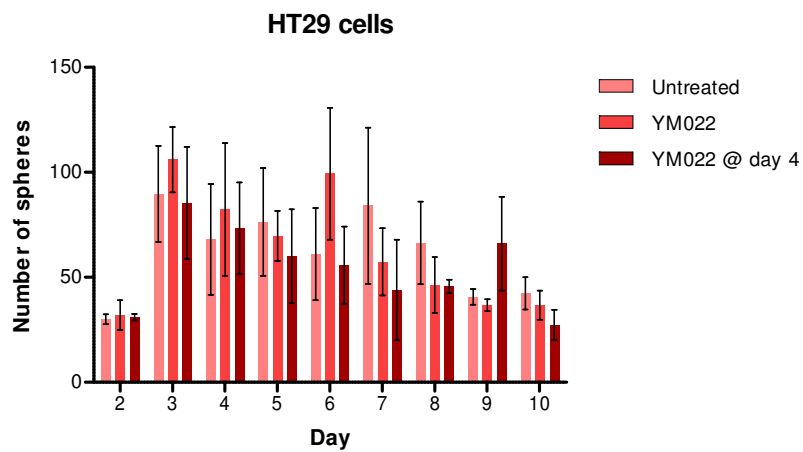
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a) ii)



b) i)



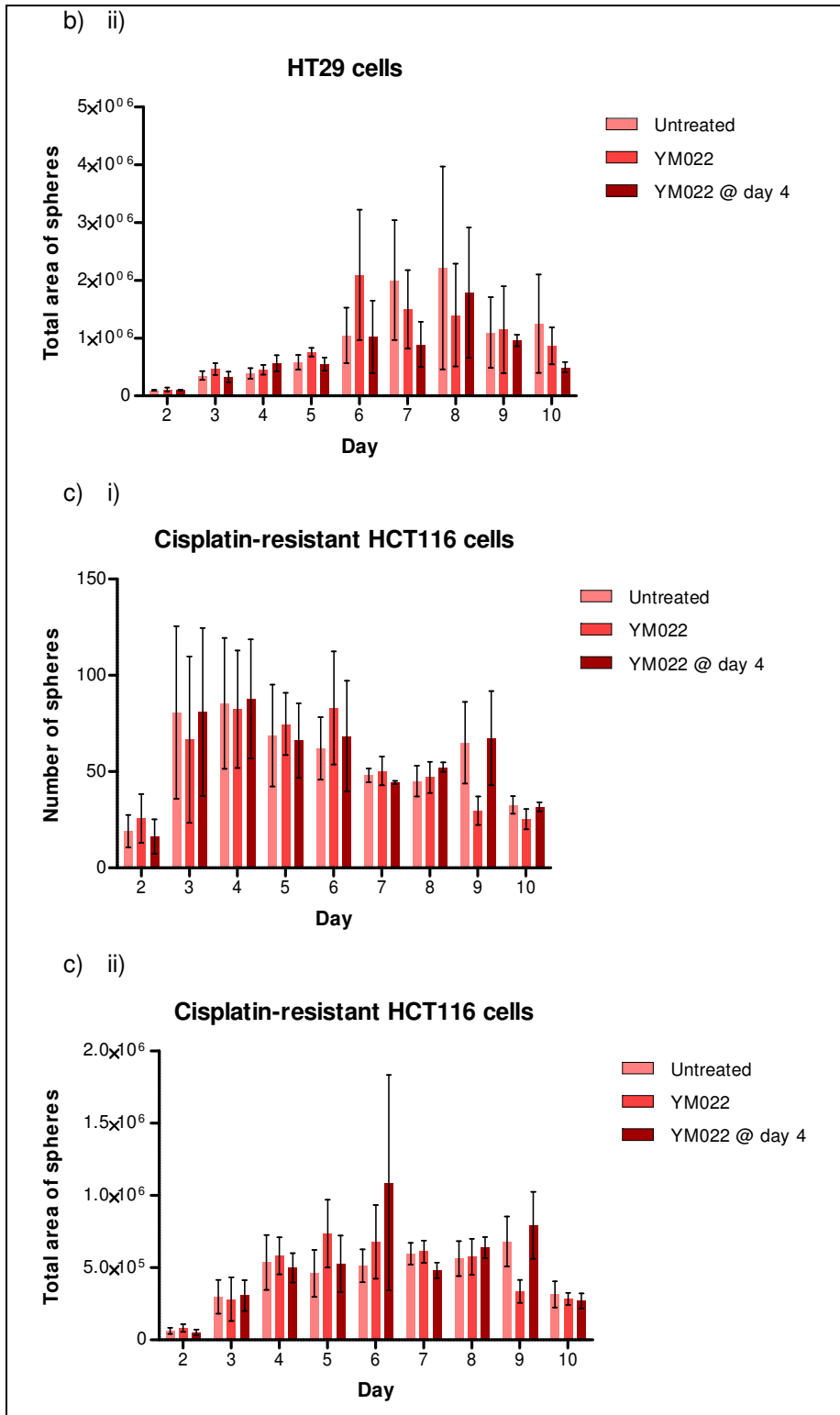


Figure E: Total number and area of spheres formed each day in colorectal cell-lines

The cells were a) HCT116, b) HT29 and c) HCT116 cisplatin-resistant cells, with i) showing total number of spheres and ii) showing total area of spheres (note the different y-axis maximums). Data acquired from 10 photographs taken at 10x magnification for each condition on each day. These were also compared to their monolayer equivalents on each day. The bars show the mean of three experiments and the black error bars represent the SEM.