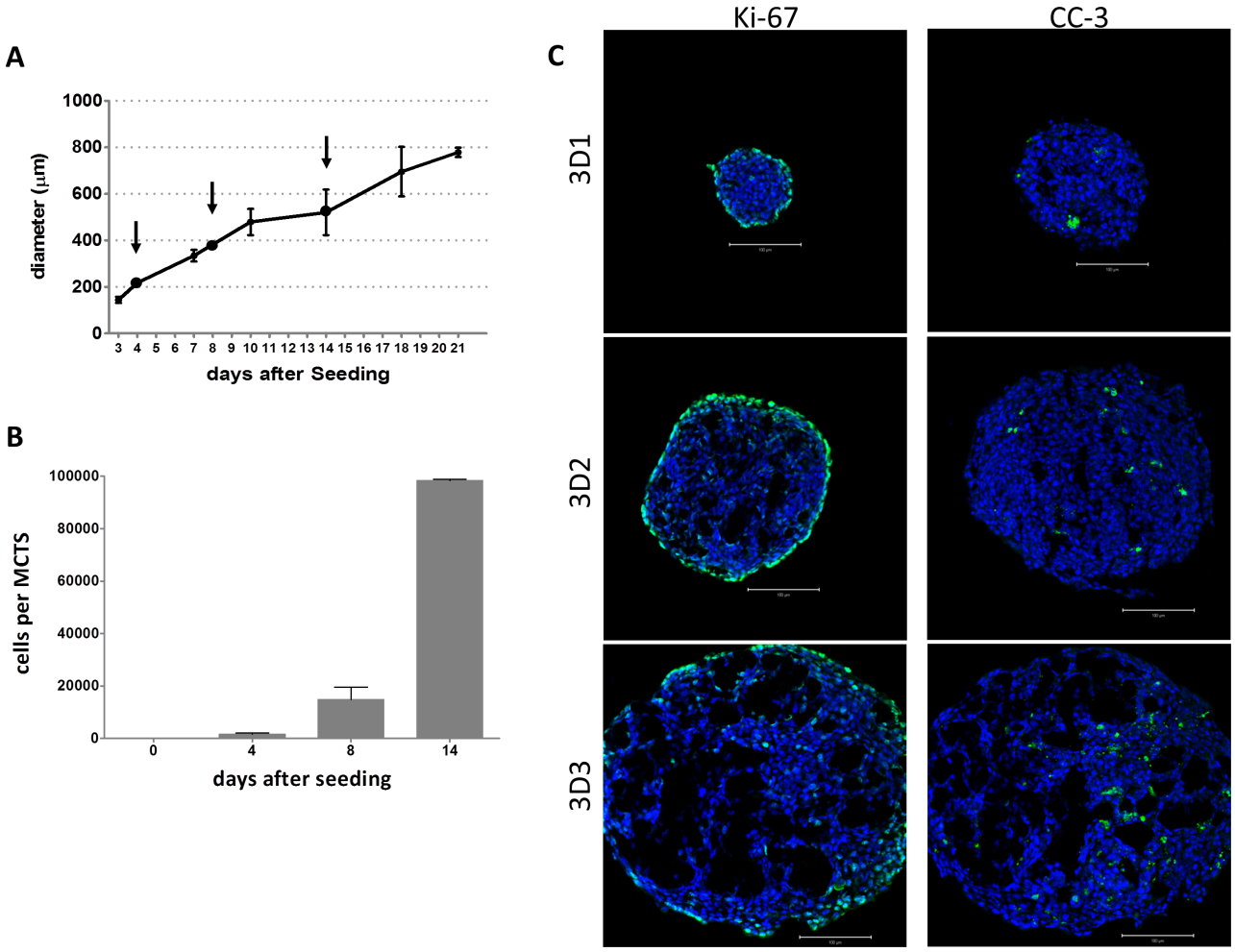
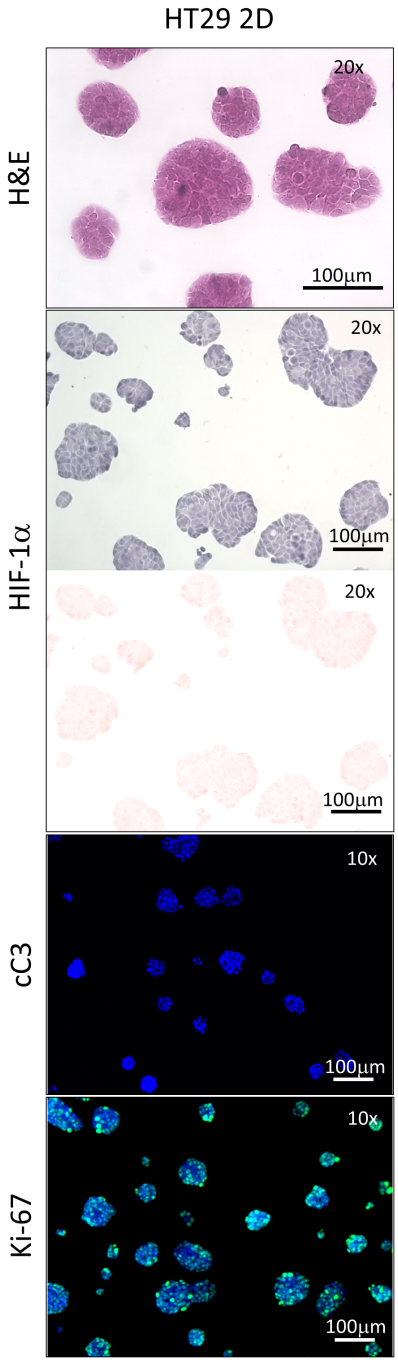
Induction of hypoxia and necrosis in multicellular tumor spheroids is associated with resistance to chemotherapy treatment

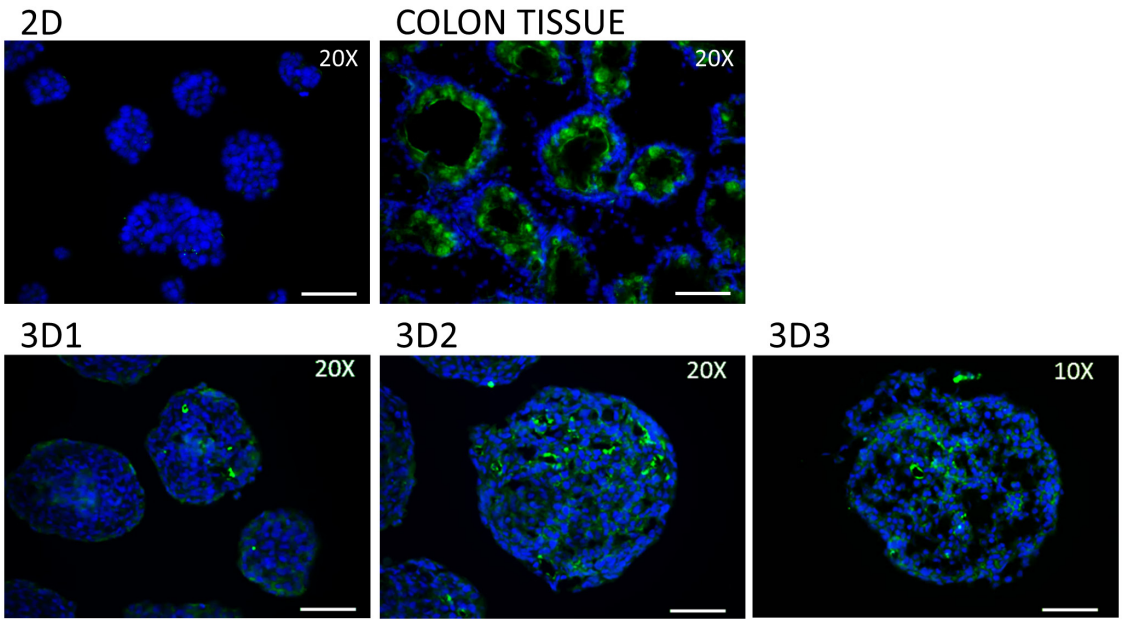
SUPPLEMENTARY FIGURES



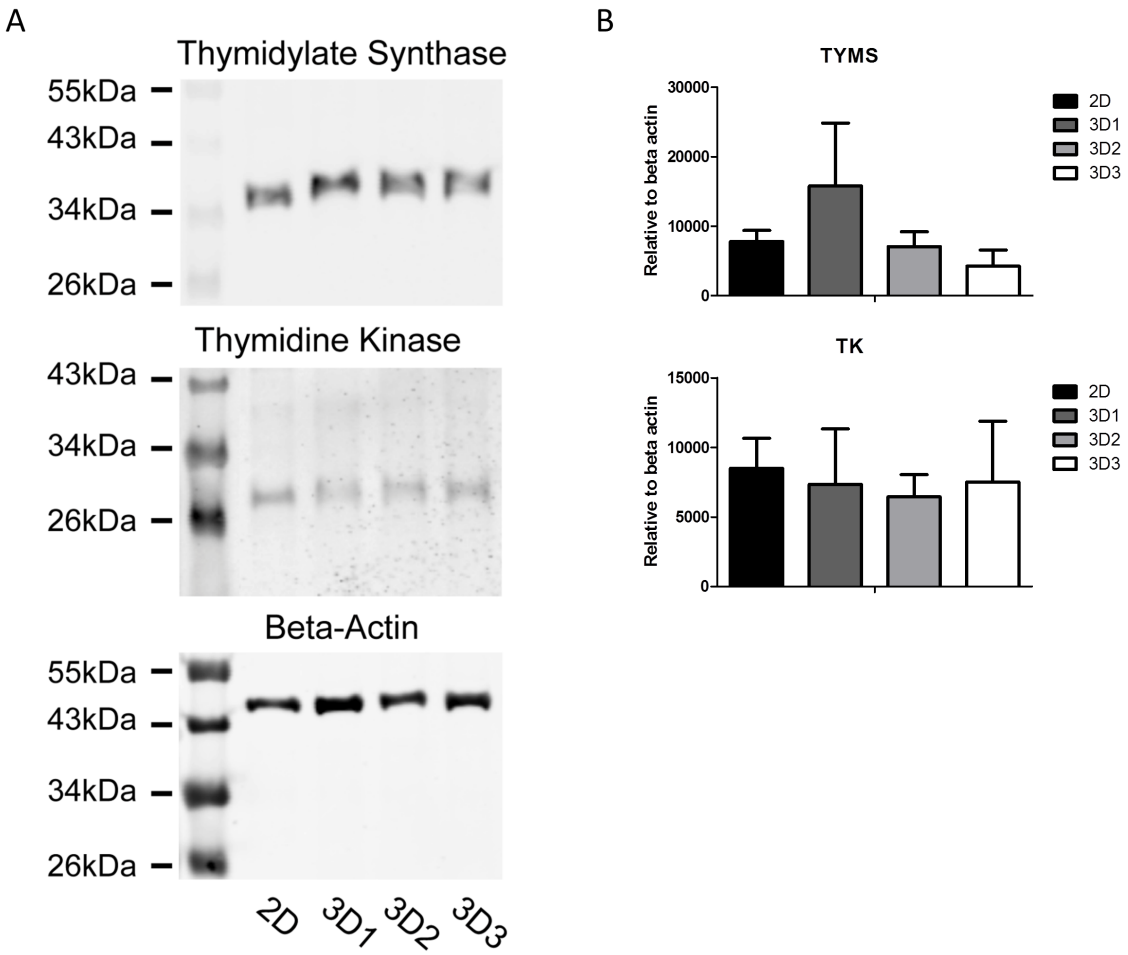
Supplementary Figure S1: Establishment of HCT116 CRC cell MCTS at different stages. HCT116 cells were seeded at 100 cells per hanging drop and cultured up to 21 days. MCTS sizes were measured **A.** and cell numbers were counted **B.** at specific time points, as indicated. Ki-67 and CC3 specific staining was performed on sections derived from MCTS at different stages **C.** Magnification 20x; scale bar 100µm.



Supplementary Figure S2: Histological characterization of HT29 cells cultured in 2D. HT29 cells cultured in 2D were stained for H&E or with anti-HIF1α (upper panel HIF-1a and counterstaining with Hematoxylin, lower panel color deconvolution to show the HIF-1α positivity only), anti-cleaved Caspase 3 (cC3, green) or anti-Ki-67 antibodies (green). In the cC3 and Ki-67 stainings the nuclei were counterstained with DAPI (blue). Magnification 10x and 20x as indicated in the images. Scale bar 100µm as indicated.



Supplementary Figure S3: EBP50 specific staining of HT29 CRC cells in different culture conditions. Cells from healthy colon tissue, HT29 monolayers and HT29 MCTS at different stages were stained with an anti-EBP50 specific antibody. Magnifications 10x and 20x as indicated in the images; scale bar 50μm (3D3 image) and 100μm.



Supplementary Figure S4: Expression of Thymidylate Synthase and Thymidine Kinase in HT29 cells. HT29 cells were cultured in monolayers or MCTS of different sizes. Thymidylate synthase (TYMS) and Thymidine kinase (TK) protein expressions were assessed by Western blot analysis using whole-cell lysates panel **A.** Quantifications of TYMS and TK protein amounts relative to Beta-actin are showed as mean and SD from three independent experiments panel **B.**

Supplementary File S1:

See Supplementary File 1