



**SUPPLEMENTARY FIG. S2.** Testing of 5-FU in 2D SW480 monocultures and in 3D in mono- and coculture settings. SW480 cells are not sensitive to treatment with 5-FU in 2D (**A**). In 2D, coculture of the same amount of SW480 cells and fibroblasts leads to a fibroblast monolayer with isolated tumor cells placed on top (**B**, **b1**: SW480 monoculture, **b2**: fibroblast monoculture, PCK (*green*)/vimentin (*red*)). In contrast to 3D coculture, no tumor cell aggregates are built in 2D coculture (**B**). Under static 3D culture conditions, monocultures of tumor cells and fibroblasts (Fib) were not influenced by 5-FU, whereas in coculture, tumor aggregates were smaller and tumor cell numbers were reduced after 5-FU treatment (**C**). Ctrl=control. Scale bar in **B**: 25 μm, Scale bar in **C**: 100 μm. PCK, Pan-cytokeratin.