



Supporting Figure SF8 (figshare) – Different spheroid formation time does not alter phenotypes in 3D. **A** DLD-1 colon cancer cells were seeded at different cell numbers for spheroid formation (1000, 1500, 2000, 3000) and these spheroids were for different time periods in order to reach the same spheroid size prior to experimentation (e.g. 3000 cells were incubated for 24 hrs, whereas 1000 cells were incubated for 96 hrs to reach the same sphere size). The initial differences of spheroid sizes are shown below in A after 24 hrs. After 24, 48 and 72 hrs of further incubation spheroids formed by 2000, 1500 and 1000 cells, respectively, reached the same size as 3000 cells after 24 hrs. **B** These spheroids of the same size but with different spheroid formation periods were then subjected to cell cycle analysis (EdU incorporation) and DNA staining (7-AAD) and displayed no apparent differences in cell cycle distribution. **C** To generalize these observations the same experimental approach was repeated with HCT116 cells and showed the same results.