



Supporting Figure SF9 (figshare) – The presence of methylcellulose does not affect treatment response. DLD-1 colon cancer cells, grown in 2D and 3D for 24h, were treated with DMSO, which served as control, Rapamycin, the pan mTOR inhibitor Torin and the AKT inhibitor MK2206 for further 24h in the presence or absence of 0.3% methylcellulose. Spheroid formation was carried out in ultra low attachment plates (96 well U-shaped, Thermo Scientific) to avoid cell attachment in the absence of methylcellulose. 2D and 3D cultures were harvested and whole protein extracts were subjected to Western blot analysis of different proteins of the PI3K/AKT/mTOR pathway. No difference in Akt and S6 phosphorylation was detected in methylcellulose containing culture conditions versus methylcellulose free incubation in DMSO controls and in Torin1 or MK2206 treated cells.