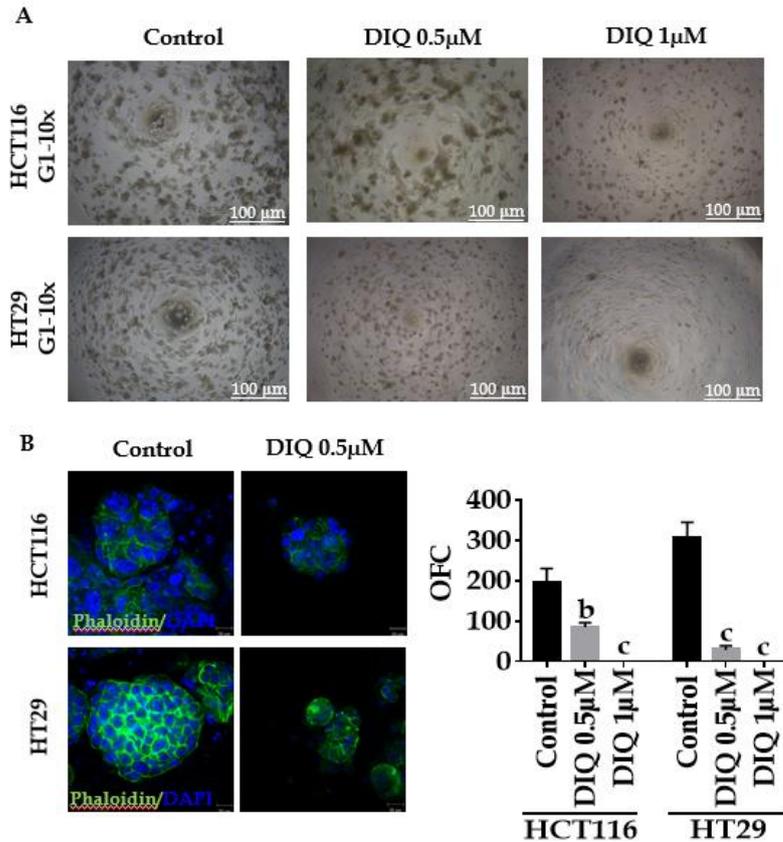


Supplementary Figure 1 Diiminoquinone not targeting non-tumorigenic FHS74Int cells. A: The anticancer effect of 4 μM Diiminoquinone (DIQ) on the proliferation of FHS74Int cells using MTT assay was determined in triplicates at 24, 48 and 72 h. Results are expressed as percentage of proliferation of the treated group compared to control at every time point. The anticancer effect of 4 μM of DIQ on the viability of FHS74Int cells using Trypan blue exclusion assay was determined in triplicates at 24, 48 and 72 h. Results are expressed as percentage of viable cells of the treated group compared to control at every time point. B: FHS74Int cells were seeded at a density of 2000 single cells/well in Matrigel™ for 8 d with and without 1μM DIQ treatment at G1. Spheres were propagated for five generations (G1-G5) in duplicates for each condition. Media or treatment was replenished every 2 d. Spheres were counted at day 8-12 of sphere culture. Results are expressed as SFU which is calculated according

to the following formula: SFU = (number of spheres counted number of input cells) \times 100. Quantification of the average size of G1 to G5 colon cancer spheres with or without treatment conditions. Spheres sizes were measured by Carl Zeiss Zen 2012 image software. Data represent an average diameter (μm) of 50 measured spheres. Representative bright field images of FHS74Int spheres in MatrigelTM taken by the Axiovert inverted microscope are shown. Data represents an average of three independent experiments and is reported as mean \pm SEM (^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001). DIQ: Diiminoquinone; G: generation; OFC: organoid formation count.



Supplementary Figure 2 Diiminoquinone reduces the growth of the cell-derived organoids from HCT116 and HT29 cells. A: Representative bright field images of G1 organoids derived from the CRC HCT116 and HT29 cell lines grown with or without Diiminoquinone (DIQ). Scale bar, 100 µm. B: Immunofluorescent images of organoids derived from HCT116 and HT29 cell lines at G1 in the presence and absence of DIQ treatment (0.5 and 1µM) stained with phalloidin. The nuclei were stained with anti-fade Fluorogel II with DAPI. Representative confocal microscopy images were acquired using a Zeiss LSM 710 laser scanning confocal microscope. Scale bar 20 µm. OFC was calculated in duplicate wells per condition. All mean values were reported as mean ± SEM (^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$). CRC: Colorectal; DIQ: Diiminoquinone; G: generation; OFC: organoid formation count.