

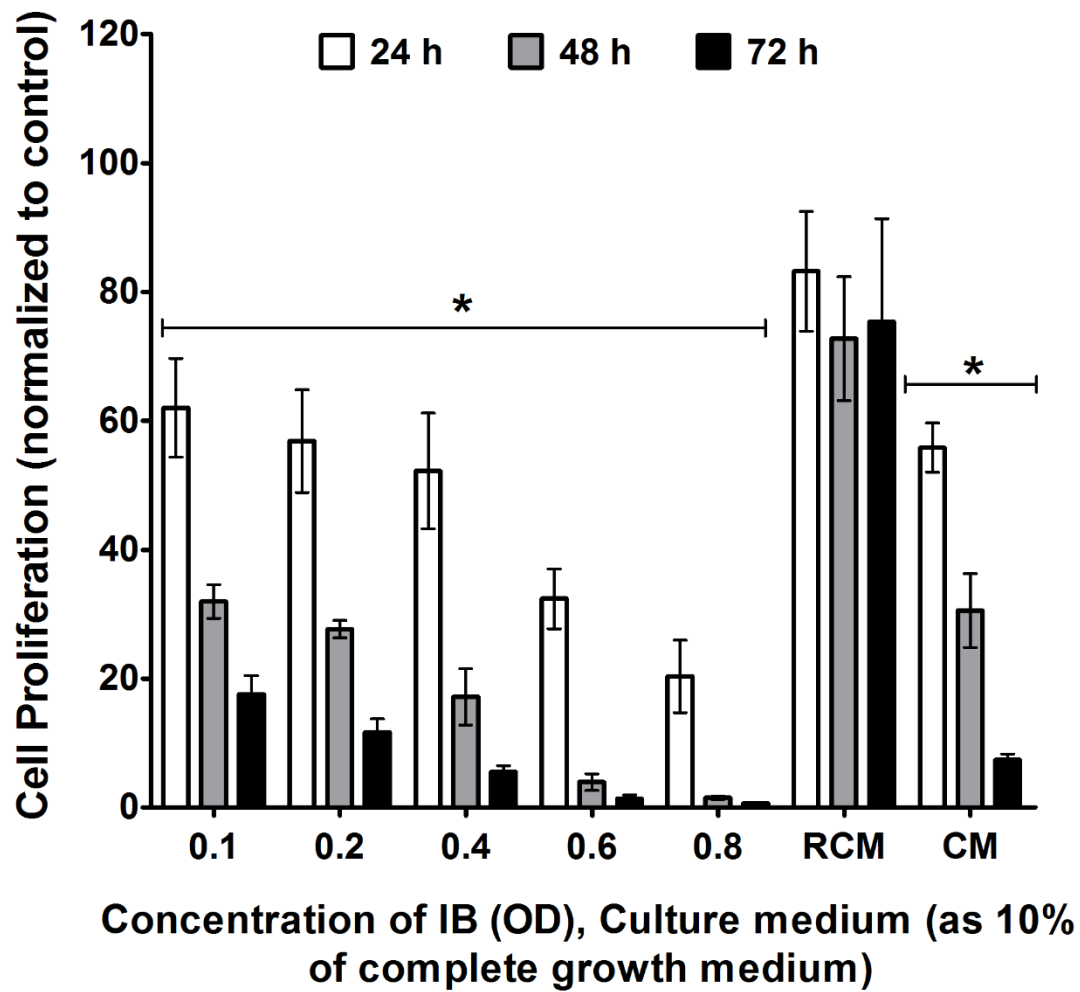
Supplementary Information

Effect of Heat-Inactivated *Clostridium sporogenes* and Its Conditioned Media on 3-Dimensional Colorectal Cancer Cell Models.

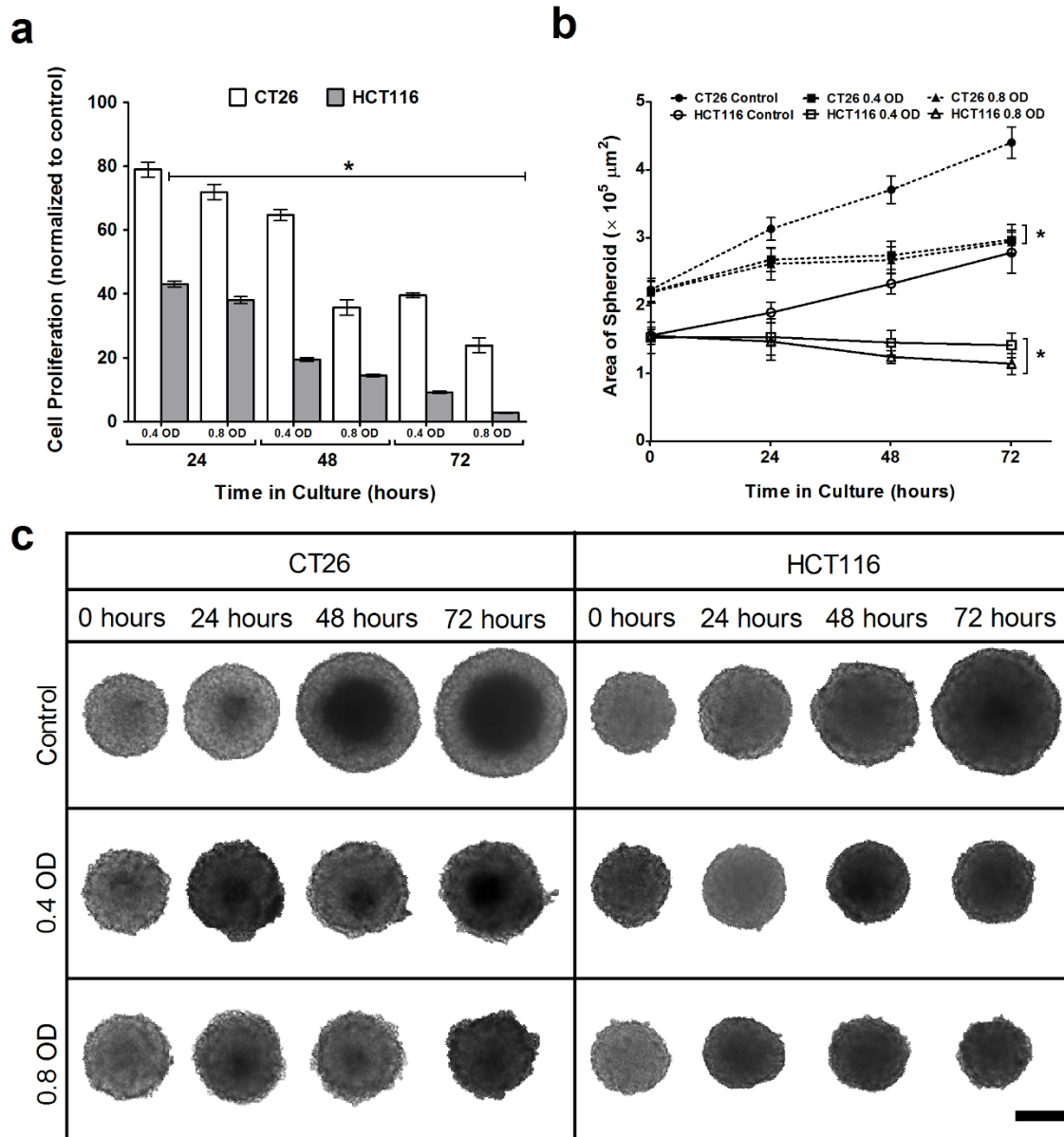
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Supplementary Figure S1. Effect of IB and CM on 2D culture of HCT116 cells. Cell proliferation with WST-1 assay of 2D cell culture exposed to varying concentrations of inactivated bacteria, 10% of Conditioned Media (CM) of *C. sporogenes* and 10% Reinforced Clostridium Media (RCM). 0.1 OD = 2.4×10^6 bacterial cells/ml. *t*-test is a comparison with control. (* $p < 0.005$)



Supplementary Figure S2. Effect of 0.4 OD and 0.8 OD IB on 3D spheroids. (a) Cell proliferation with WST-1 assay of CT26 and HCT116 spheroids exposed to 0.4 OD and 0.8 OD concentration of inactivated bacteria over 72 hours. $0.1 \text{ OD} = 2.4 \times 10^6$ bacterial cells/ml. *t*-test is a comparison with control. (* $p < 0.005$) (b) Area of 3D spheroids after incubation with 0.4 OD and 0.8 OD of inactivated bacteria, at the 0, 24, 48 and 72 hour time points. The area (μm^2) of the spheroids in the images was measured using ImageJ software. *t*-test is a comparison with control (* $p < 0.005$, at all time points). (c) Effect of 0.4 OD and 0.8 OD of inactivated *C. sporogenes* on 3D spheroids, compared with Control. Scale bar represents 1000 μm .