ADVANCED HEALTHCARE MATERIALS

# Supporting Information

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Evaluating the Impact of a Biomimetic Mechanical Environment on Cancer Invasion and Matrix Remodeling

*Auxtine Micalet, Judith Pape, Deniz Bakkalci, Yousef Javanmardi, Chloe Hall, Umber Cheema* and Emad Moeendarbary**

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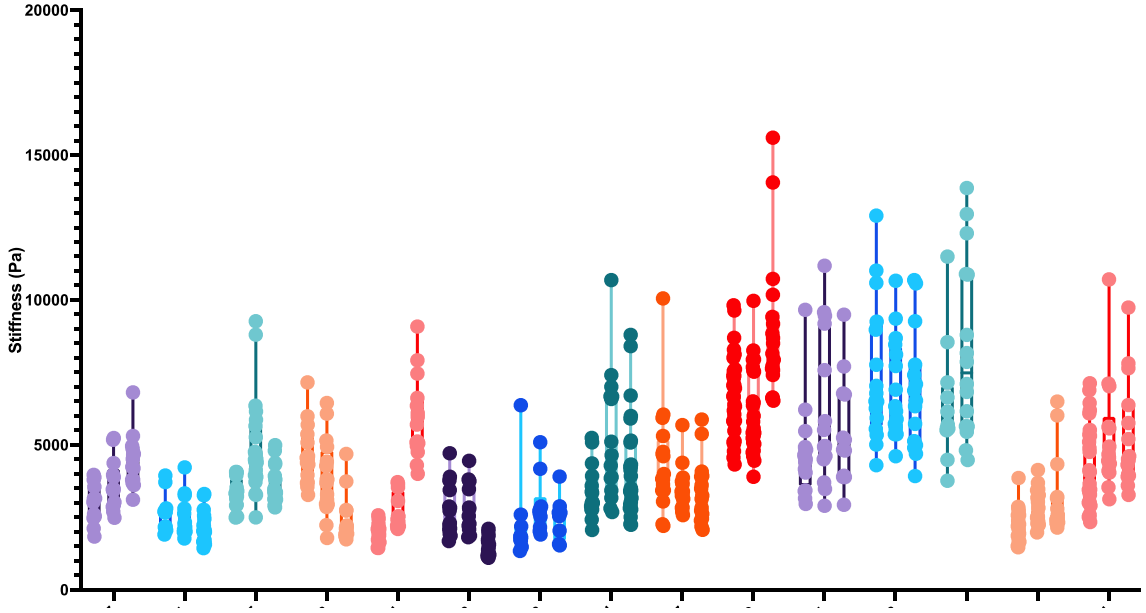


*Figure S1- Stiffness (Pa) of all acellular controls made for this work. Each experiment was set with an n=3 of acellular controls, which are shown here as one colour. The stiffness varies within one sample but also from gel to gel and experiment to experiment due to the experimental process.*

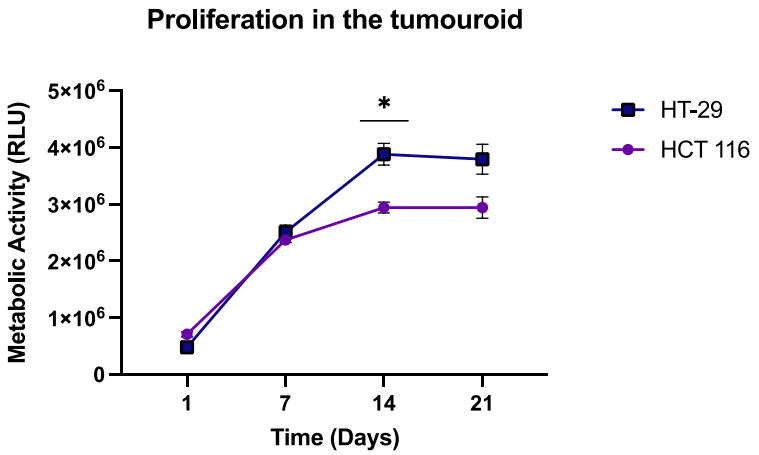


*Figure S2- Metabolic activity of cancer cell lines HT-29 and HCT 116 in compressed collagen gel over 21 days using CellTiter Glo®. The proliferation is uncorrelated with stiffness of the tumouroids. If the 'soft' felt by AFM was due to a higher cell number, we would expect the HCT 116 to have a higher proliferation rate. All n=3, showing (mean ± SEM). Shows unpaired t test significance at each time point. All p-value significance is indicated as: 0.05<*, 0.01<**, 0.001<*** and 0.0001<****.*

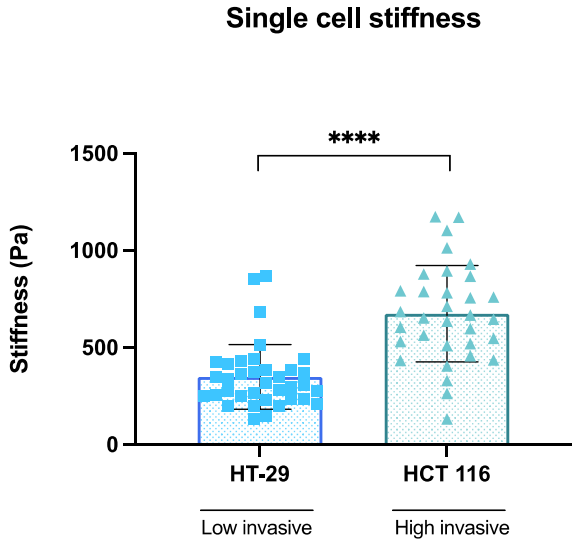


*Figure S3- Stiffness (Pa) of HT-29 and HCT 116 single cells measured by AFM. Cells measured after 24 hours on a glass petri dish, with a cantilever of k ~0.1 N/m, a bead of 10 µm, and a force of 2 nN. All n>34, showing (mean ± SD) and showing Mann-Whitney significance (p<0.0001).*

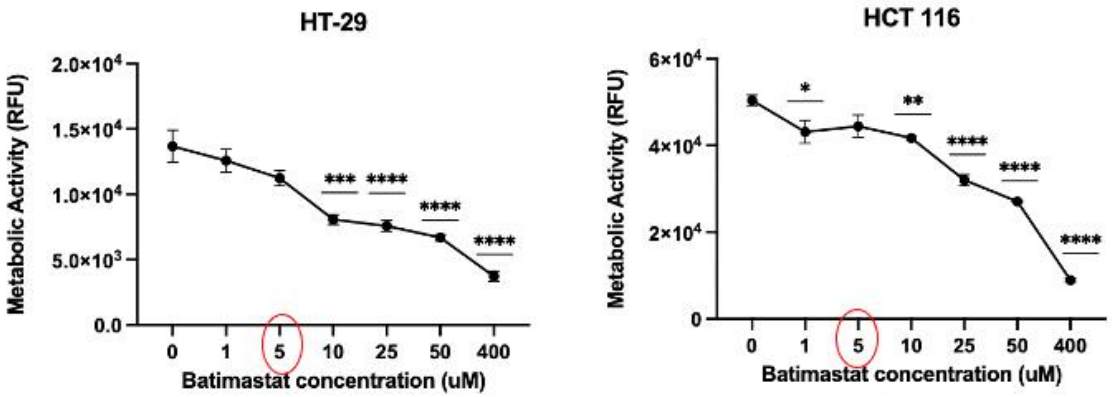


*Figure S4- Cell viability assay in 2D for HT-29 and HCT 116 cells, treated with increasing concentrations of Batimastat in 0.1% DMSO (from 1 µM to 400 µM). 5 µM was chosen as the optimum concentration as it was it highest concentration possible without affecting cell viability.*

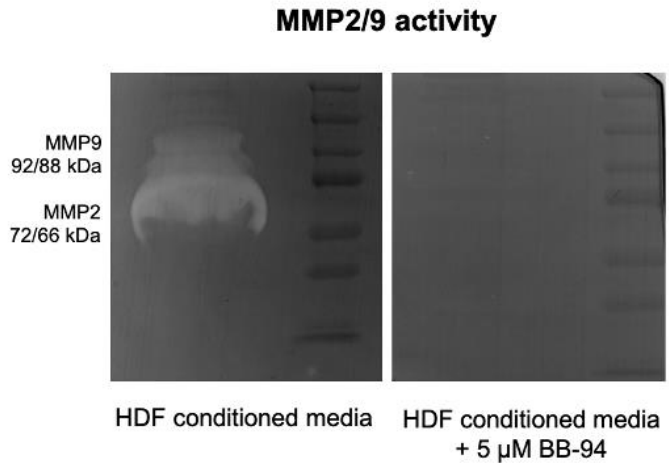


*Figure S5- Zymography using human dermal fibroblast conditioned media. It shows MMP-2 and MMP-9 presence and activity in the untreated condition and no MMP activity when incubated with 5 µM of BB-94*

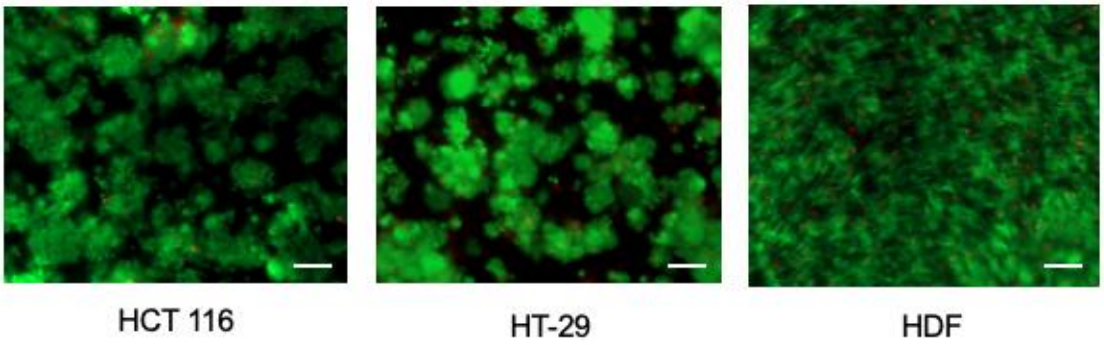


*Figure S6- Live/dead cell viability staining of BB-94 treated tumouroids after 21 days. MMP inhibition via BB-94 treatment, at 5µM every 48 hours. Green = Live, Red = Dead. Scale bars = 100 µm*