

Supplementary Information

Matrix stiffness and cluster size collectively regulate dormancy versus proliferation in brain metastatic breast cancer cell clusters

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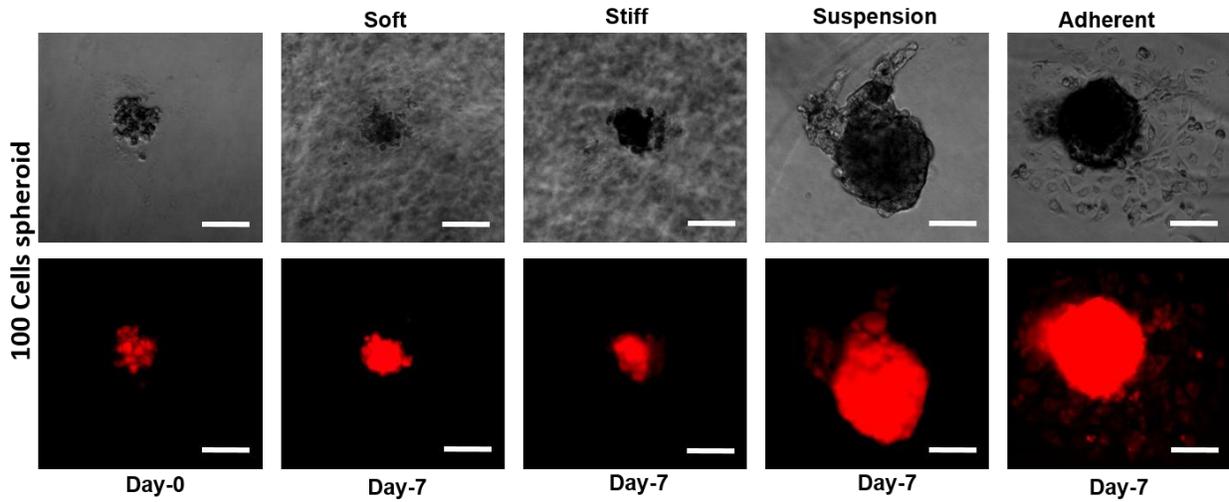


Figure S1: Representative bright field and fluorescent images of 100 cells spheroid at day 7 in various culture conditions. Scale bar = 100 μm .

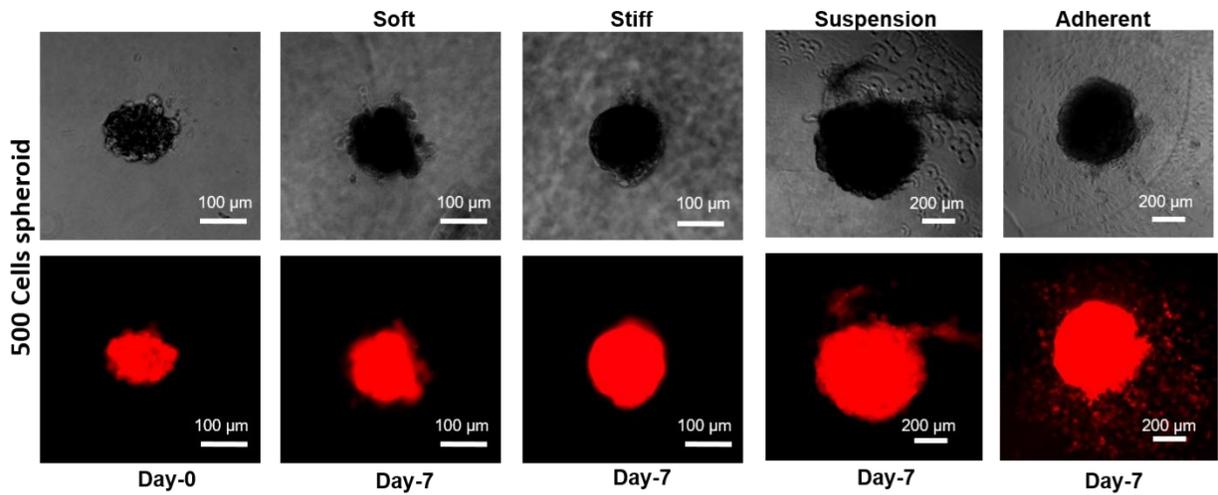


Figure S2: Representative bright field and fluorescent images of 500 cells spheroid at day 7 in various culture conditions.

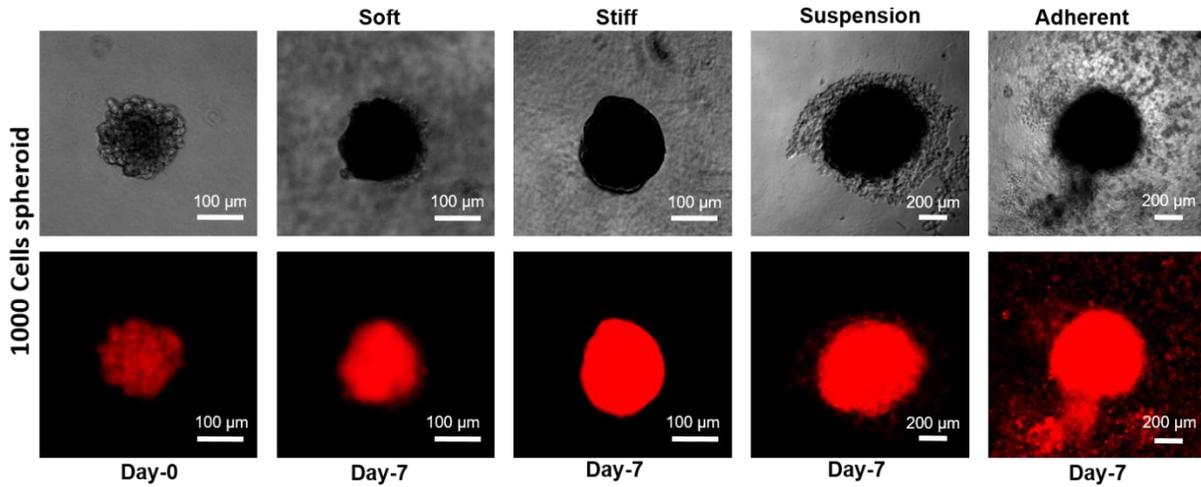


Figure S3: Representative bright field and fluorescent images of 1k cells spheroid at day 7 in various culture conditions.

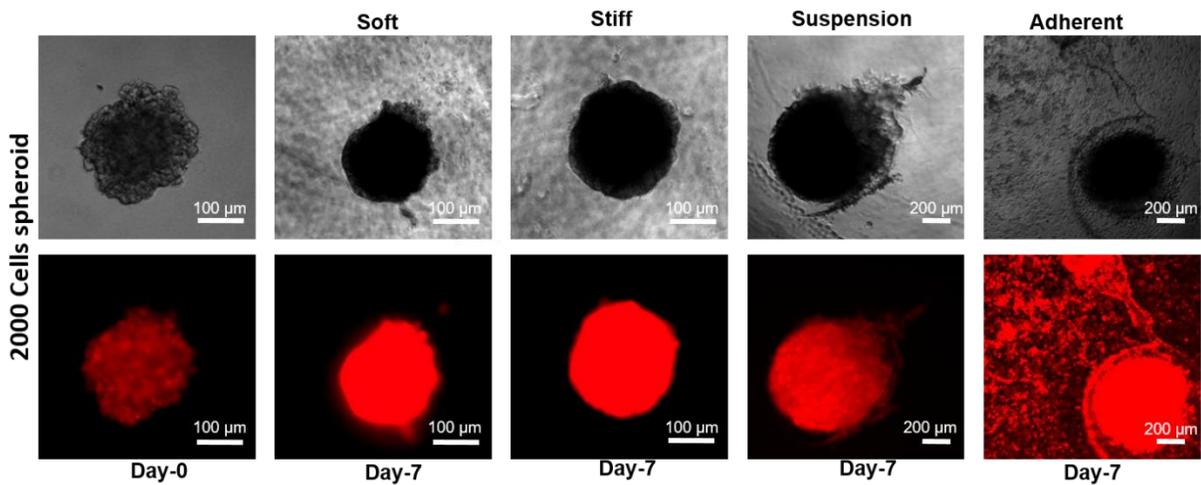


Figure S4: Representative bright field and fluorescent images of 2k cells spheroid at day 7 in various culture conditions.

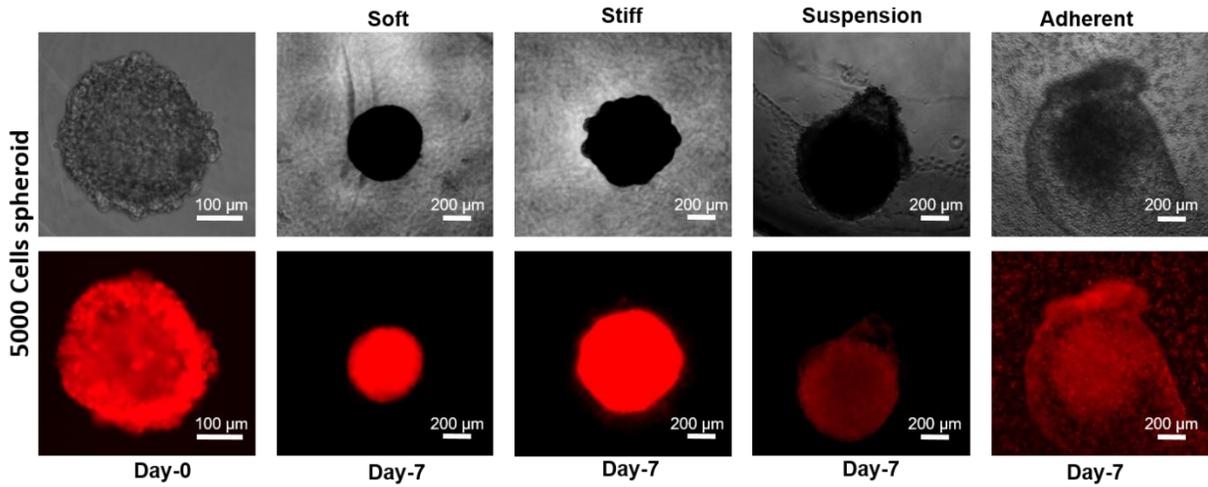


Figure S5: Representative bright field and fluorescent images of 5k cells spheroid at day 7 in various culture conditions.

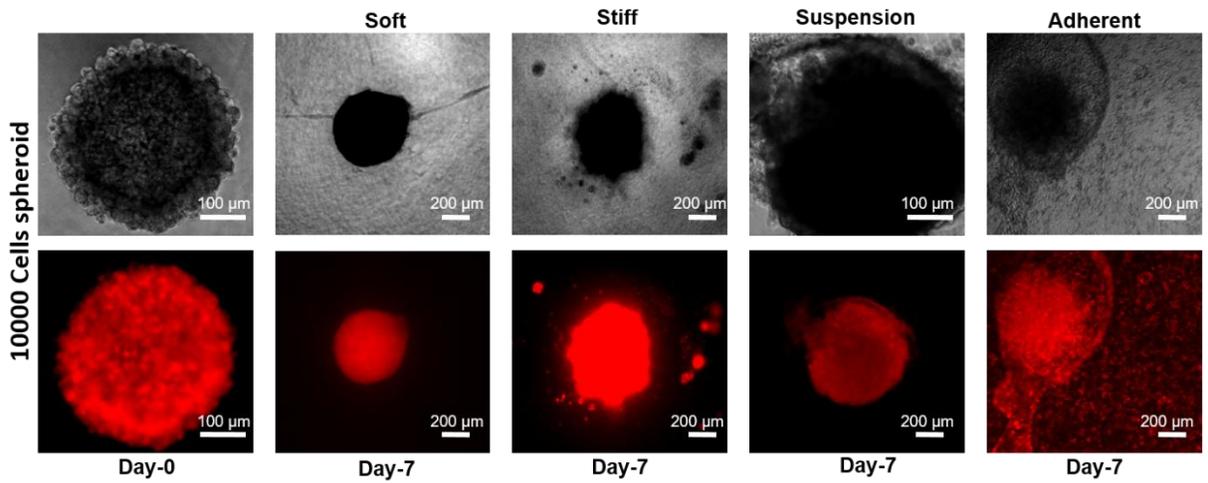


Figure S6: Representative bright field and fluorescent images of 10k cells spheroid at day 7 in various culture conditions.

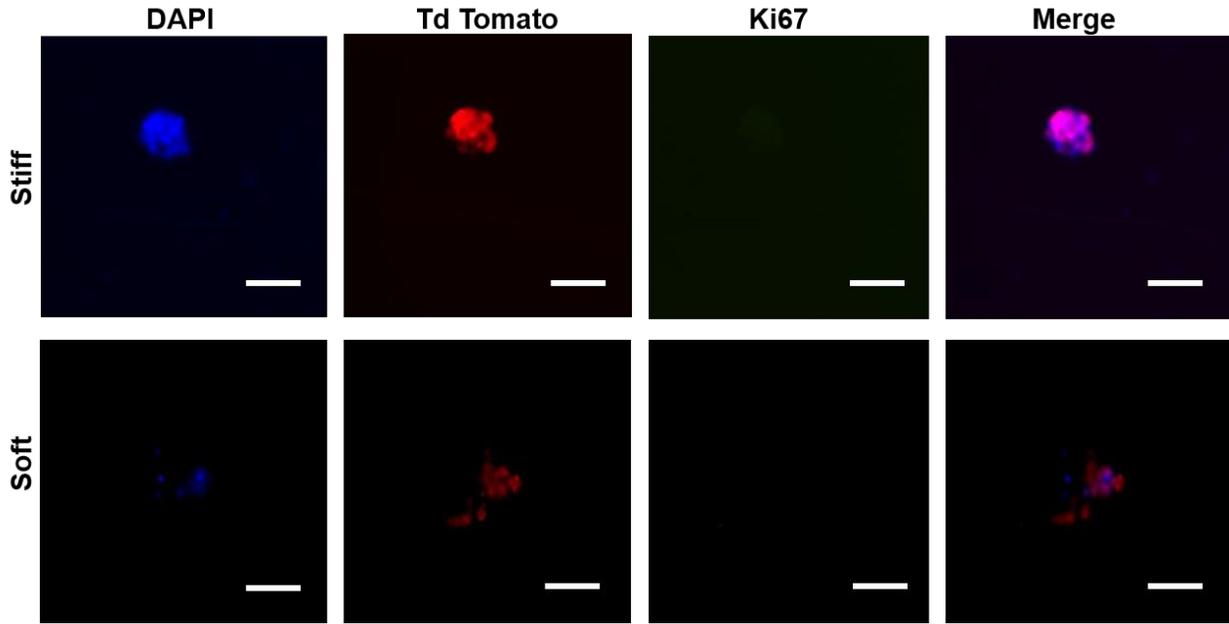


Figure S7: Representative fluorescent images of Ki67 staining for 100 cells spheroid cultured on soft (0.4 kPa) or stiff (4.5 kPa) HA hydrogel. Blue - DAPI; Red - Td tomato, Green - Ki67. Scale bar = 100 μ m.

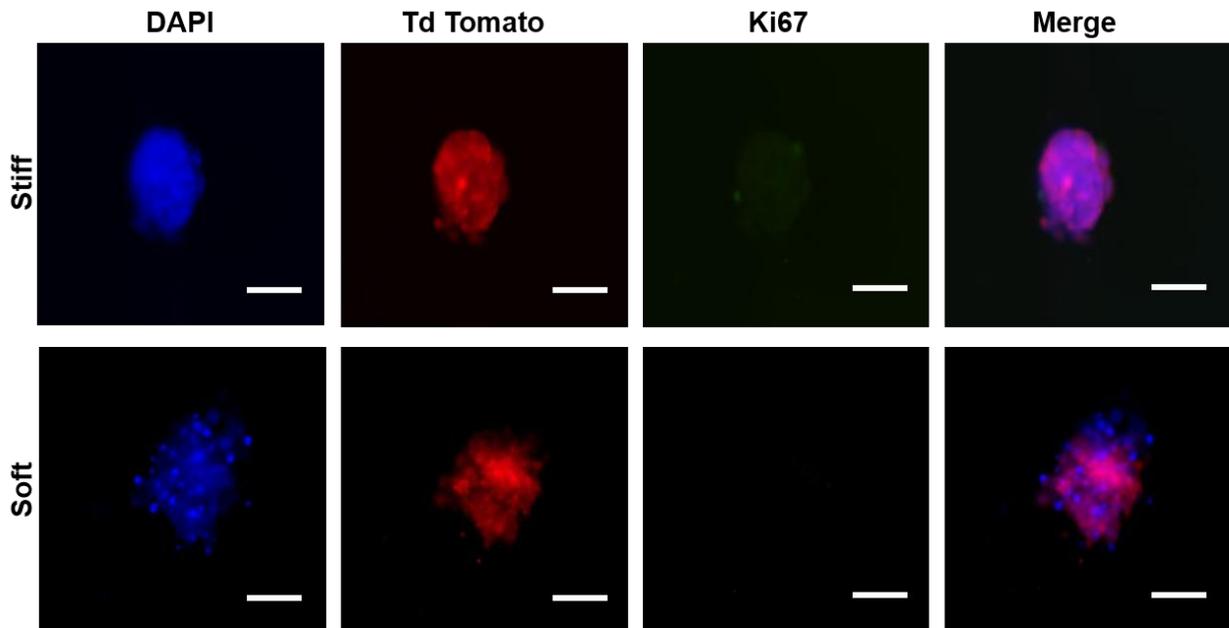


Figure S8: Representative fluorescent images of Ki67 staining for 500 cells spheroid cultured on soft (0.4 kPa) or stiff (4.5 kPa) HA hydrogel. Blue - DAPI; Red - Td tomato, Green - Ki67. Scale bar = 100 μ m.

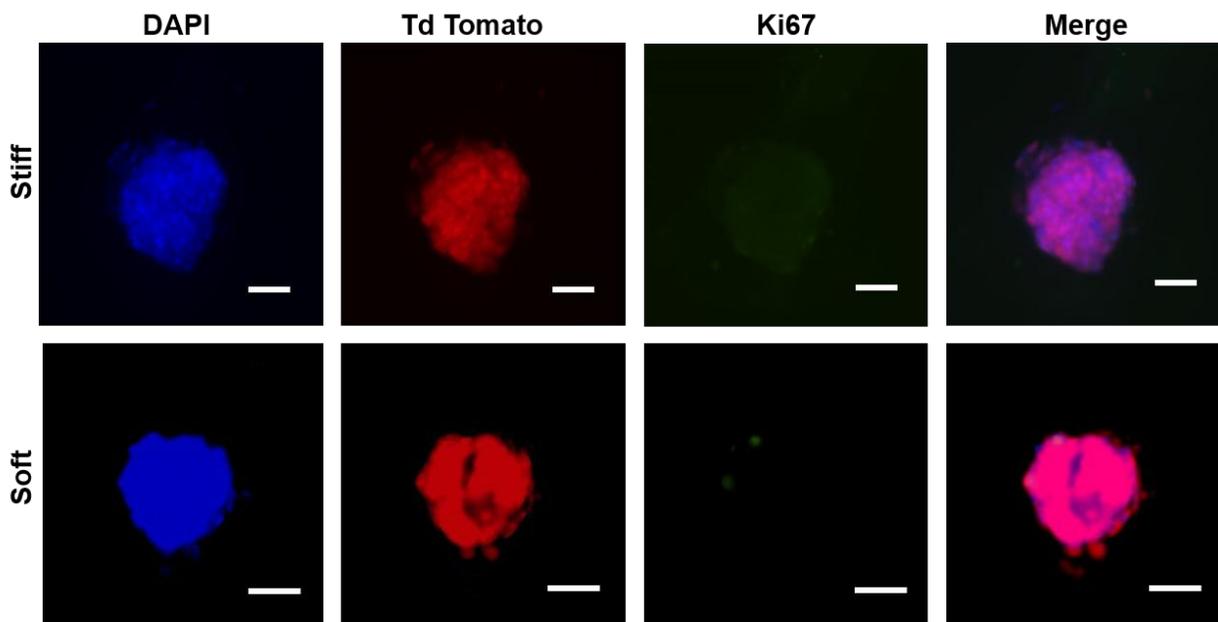


Figure S9: Representative florescent images of Ki67 staining for 1k cells spheroid cultured on soft (0.4 kPa) or stiff (4.5 kPa) HA hydrogel. Blue - DAPI: Red - Td tomato, Green - Ki67. Scale bar = 100 μ m.

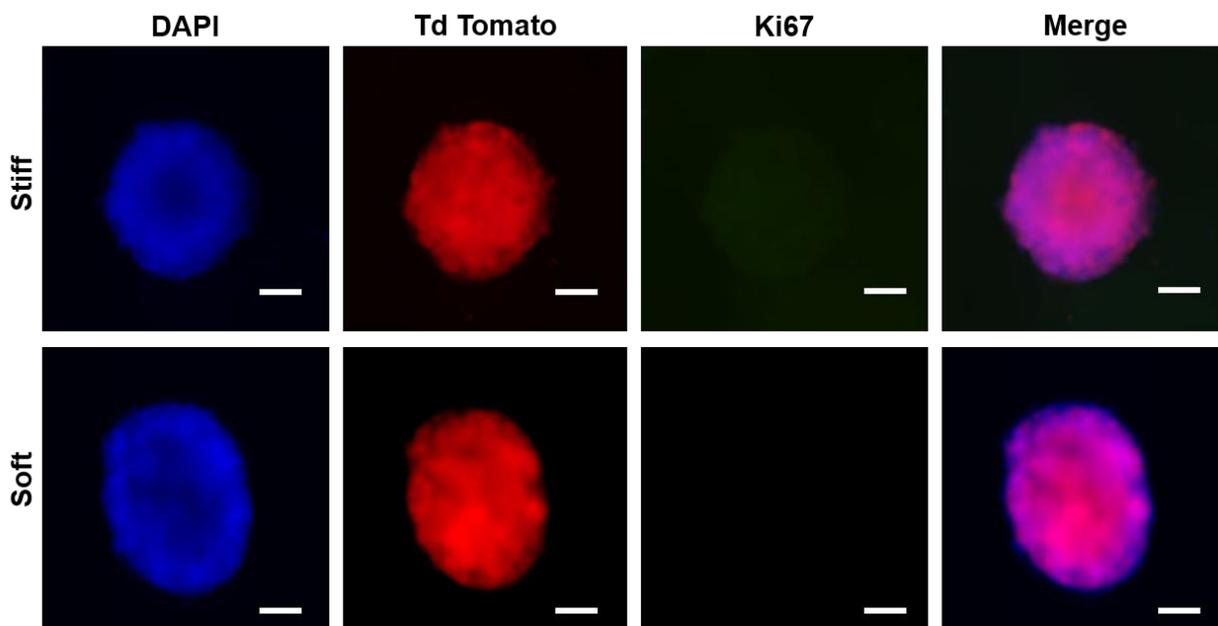


Figure S10: Representative florescent images of Ki67 staining for 2k cells spheroid cultured on soft (0.4 kPa) or stiff (4.5 kPa) HA hydrogel. Blue - DAPI: Red - Td tomato, Green - Ki67. Scale bar = 100 μ m.

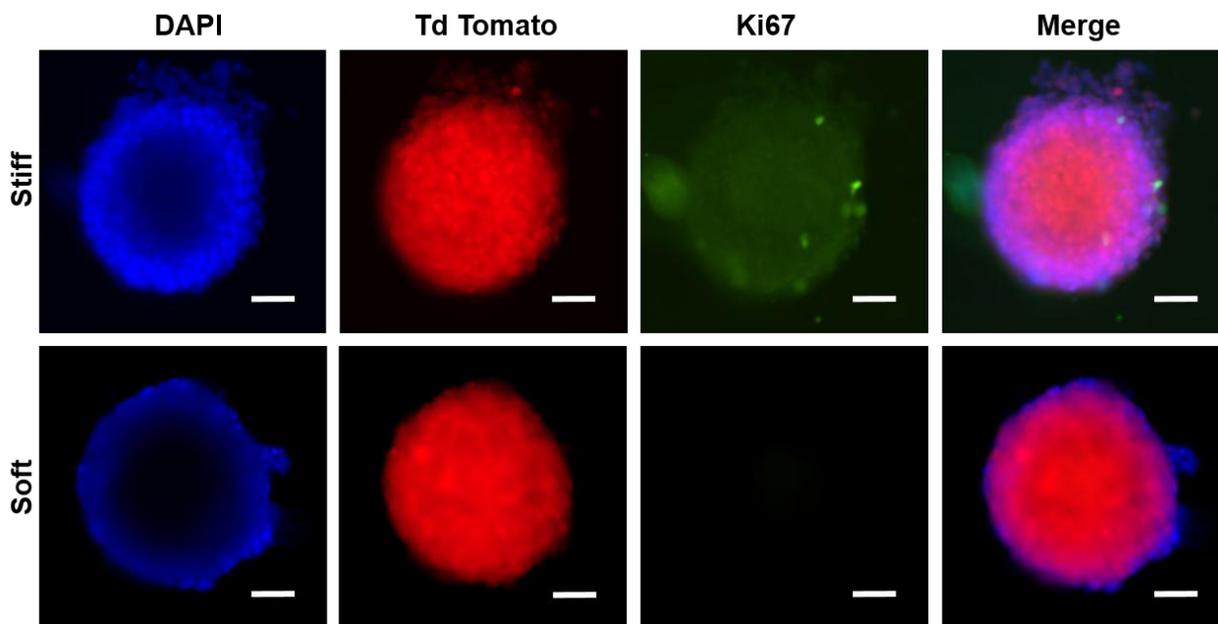


Figure S11: Representative florescent images of Ki67 staining for 5k cells spheroid cultured on soft (0.4 kPa) or stiff (4.5 kPa) HA hydrogel. Blue - DAPI: Red - Td tomato, Green - Ki67. Scale bar = 100 μm .

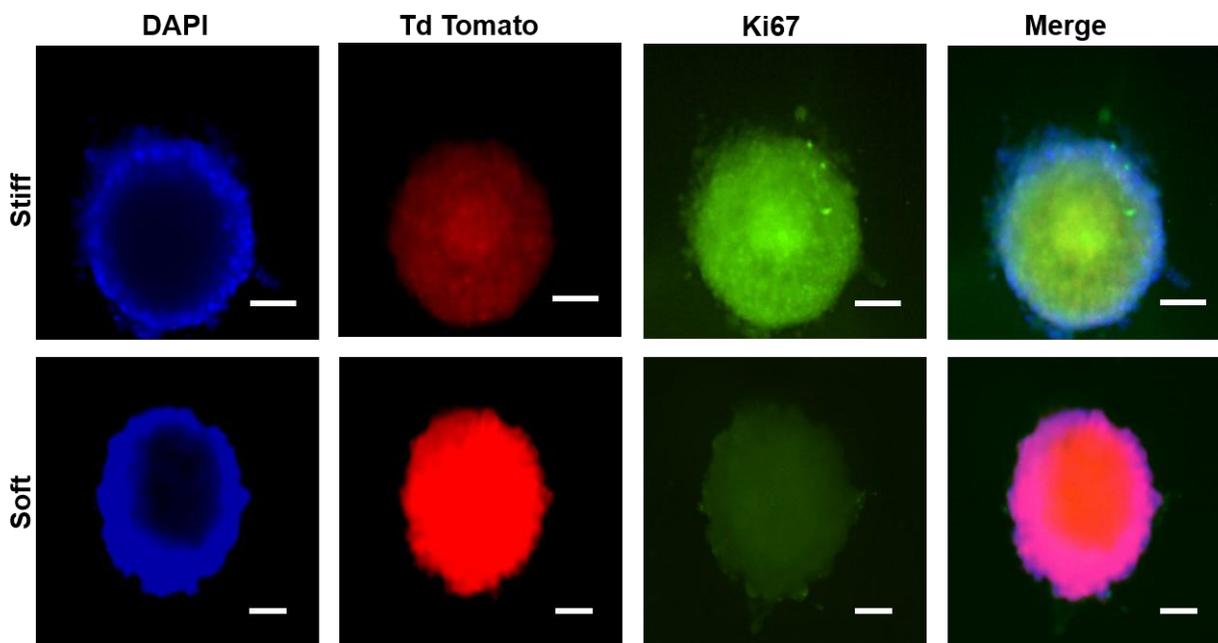


Figure S12: Representative florescent images of Ki67 staining for 10k cells spheroid cultured on soft (0.4 kPa) or stiff (4.5 kPa) HA hydrogel. Blue - DAPI: Red - Td tomato, Green - Ki67. Scale bar = 200 μm .

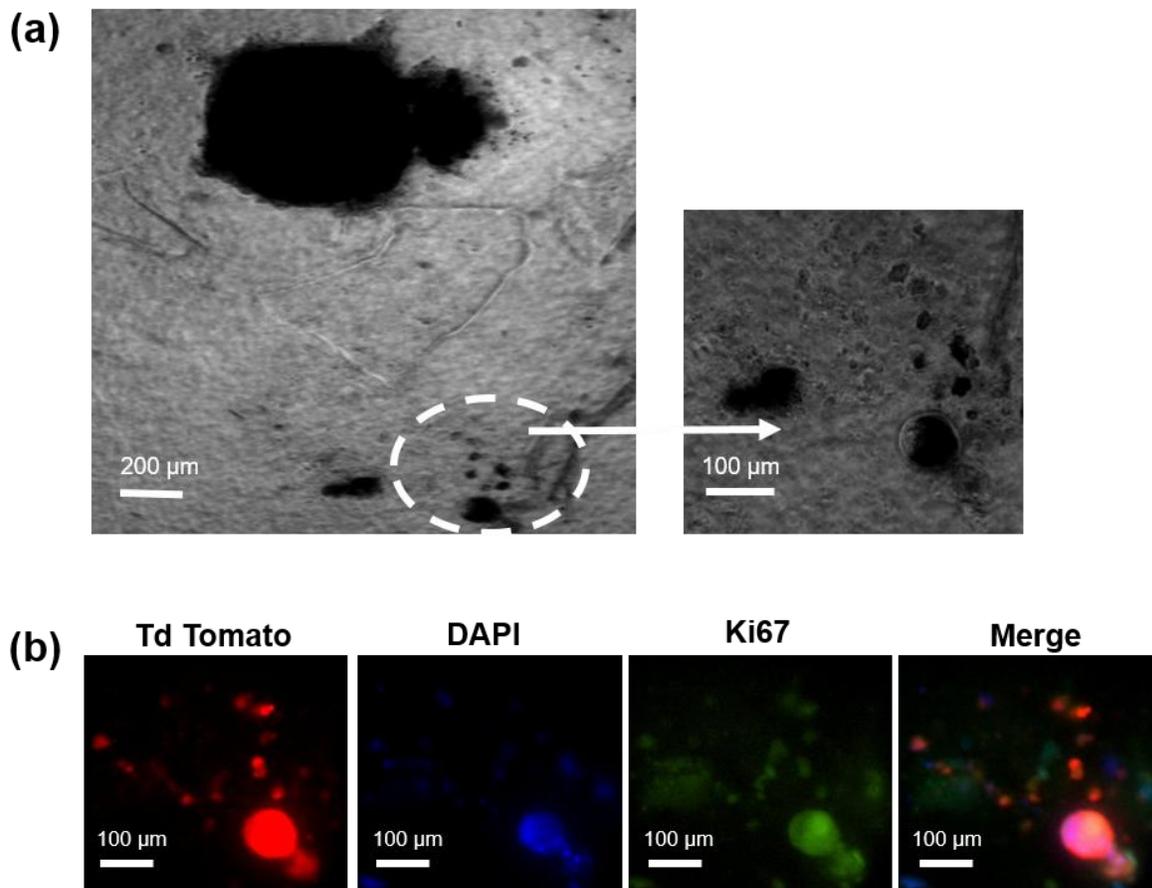


Figure S13: Micro-colonies formed on stiff HA hydrogel (4.5 kPa) for 10k cells spheroid stained positive for Ki67 (a) Bright field image of 10k cells spheroid cultured on stiff HA hydrogel showing the presence of micro-colonies (white dotted circle). (b) Representative fluorescent images for Ki67 staining of micro-colonies. Blue -DAPI, Red-Tdtomato, Green-Ki67.

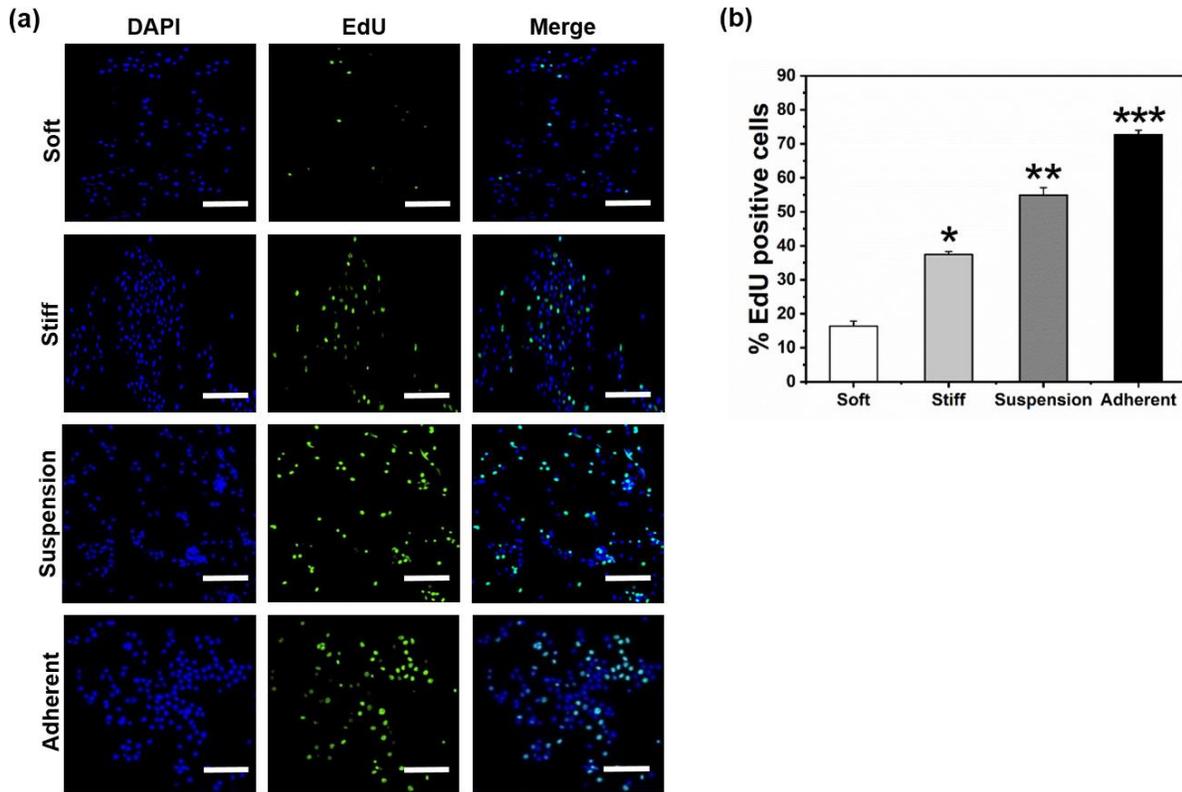


Figure S14: Brain metastatic breast cancer cells in 10k spheroid cultured on soft HA hydrogel (0.4 kPa) were EdU negative and displayed a dormant phenotype compared to stiff HA hydrogel (4.5 kPa). (a) Representative fluorescent images of EdU staining for MDA-MB-231Br cells obtained from 10k spheroid cultured in various culture conditions. Blue – DAPI (nucleus), Green – EdU, Scale bar =100 μ m. (b) Quantification of EdU positive cells in 10k spheroid cultured on soft or stiff HA hydrogel, suspension, and adherent cultures respectively. $N \geq 4$ replicates per condition. ***indicates statistically significant difference ($p < 0.05$) compared to soft and stiff HA hydrogel and suspension culture. ** indicates statistically significant difference ($p < 0.05$) compared to soft and stiff HA hydrogel. * indicates statistically significant difference ($p < 0.05$) compared to soft HA hydrogel. Error bar indicates standard error.

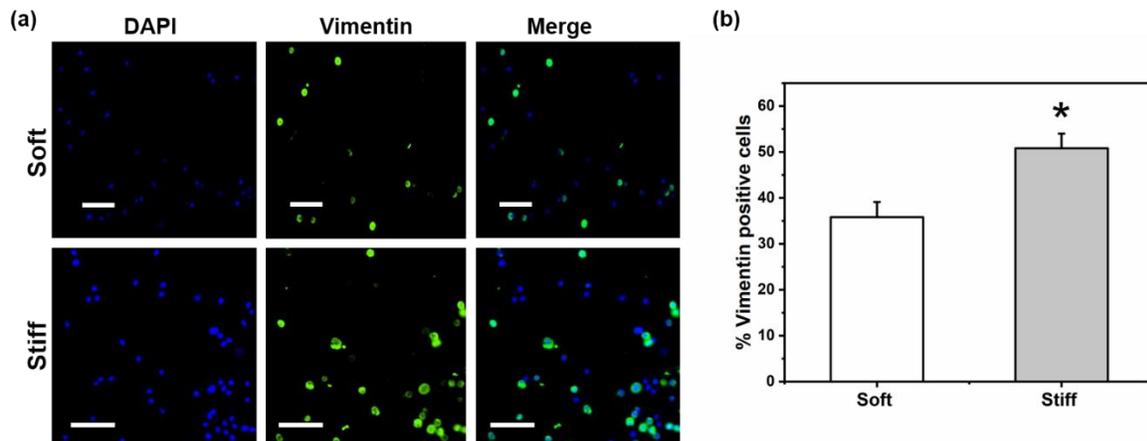


Figure S15: Percentage of Vimentin positive cells in 10k cells spheroid were significantly higher on stiff HA hydrogel (4.5 kPa) compared to soft HA hydrogel (4.5 kPa). (a) Representative fluorescent images of cells obtained on day 7 from 10k cells spheroid cultured on soft or stiff HA hydrogel. Blue – DAPI, Green – Vimentin, Scale bar =100 μ m. (b) Quantification of Vimentin positive cells present in 10k cells spheroid cultured on soft or stiff HA hydrogel. Error bar represents standard error. N = 3 replicates per condition. * indicates statistically significant difference ($p < 0.05$) compared to the soft condition.

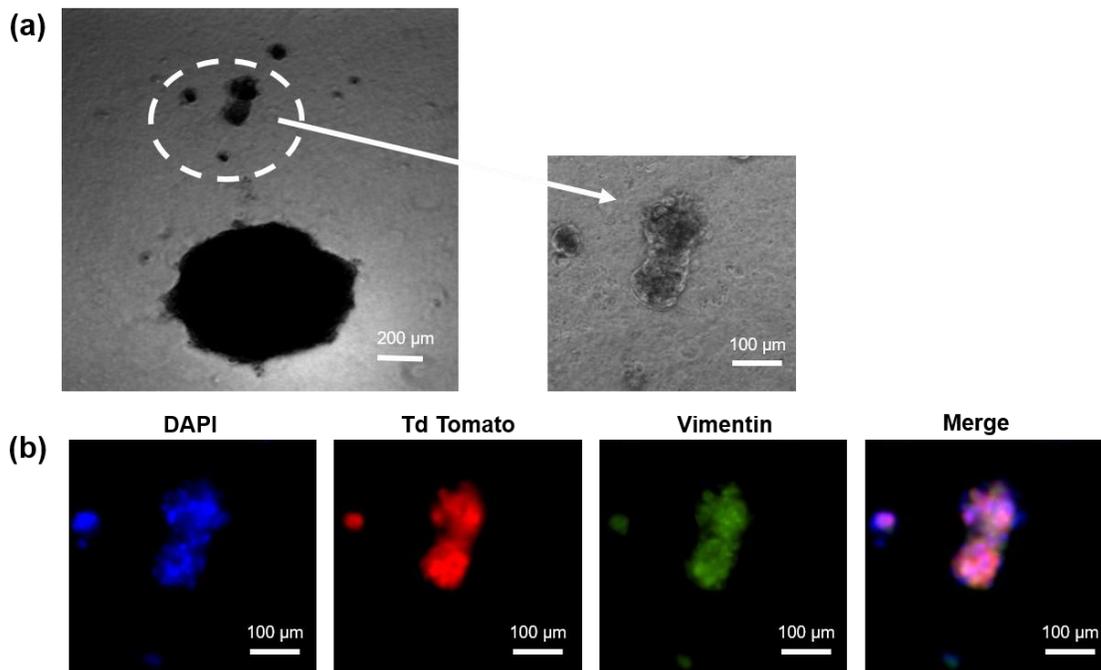


Figure S16: Micro-colonies formed on stiff HA hydrogel (4.5 kPa) for 10k cells spheroid stained positive for Vimentin. (a) Bright field image of 10k cells spheroid cultured on stiff HA hydrogel showing the presence of micro-colonies (white dotted circle). (b) Representative fluorescent images for Vimentin staining of micro-colonies. Blue -DAPI, Red-Tdtomato, Green-Vimentin.

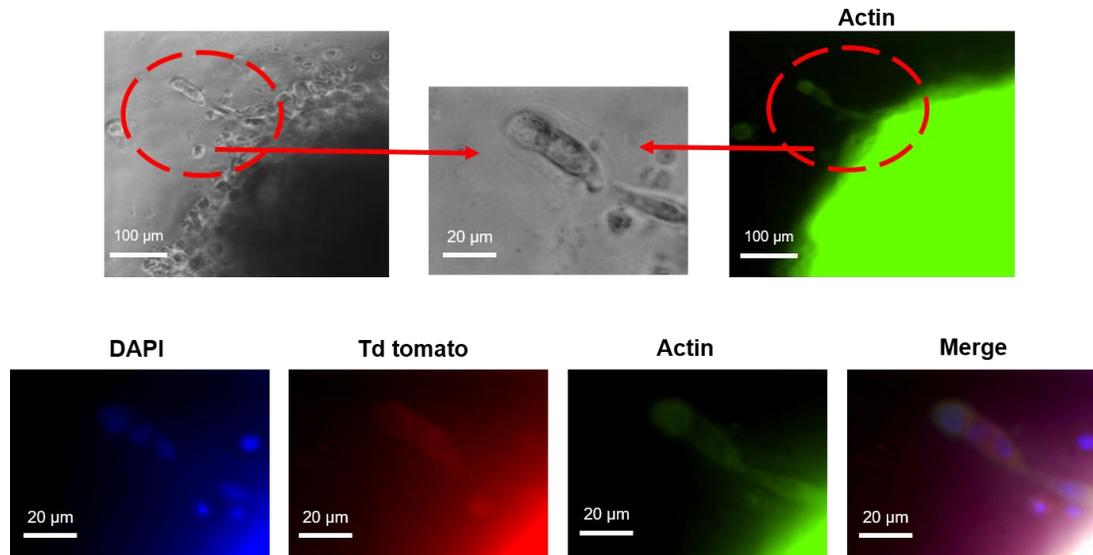


Figure S17: F-actin staining of 10k cells spheroid on stiff HA hydrogel (4.5 kPa) shows the presence of cells with a developed actin cytoskeleton at the spheroid periphery. Blue - DAPI; Red - Td tomato, Green – Actin.

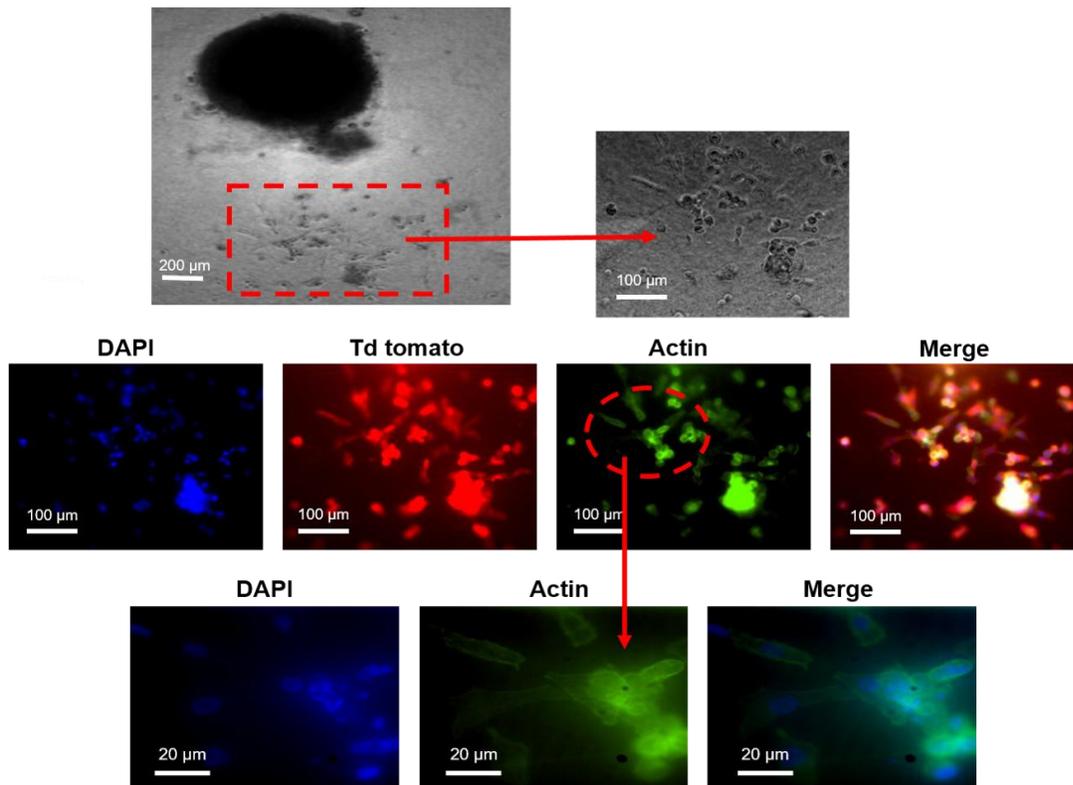


Figure S18: F-actin staining of micro-colonies formed on stiff HA hydrogel (4.5 kPa) for 10k cells spheroid shows the presence of cells with a developed actin cytoskeleton. Blue - DAPI; Red - Td tomato, Green – Actin.

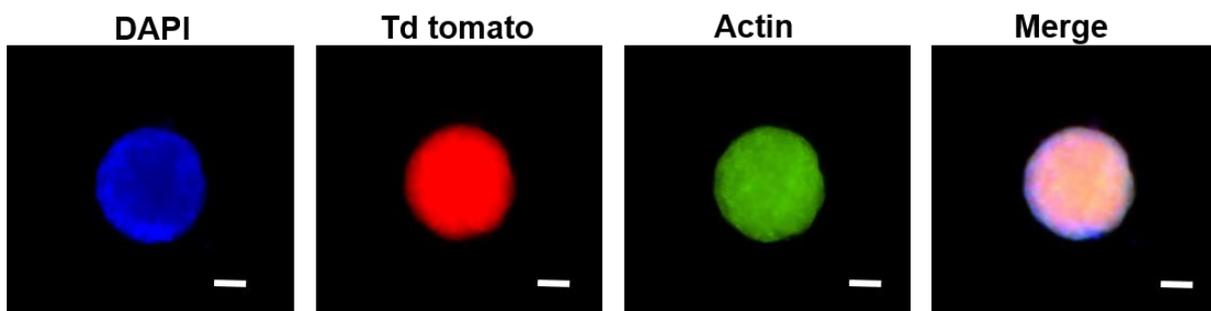


Figure S19: Representative florescent images of F-actin staining for 10k cells spheroid cultured on soft HA hydrogel (0.4 kPa). Blue - DAPI; Red - Td tomato, Green – Actin. Scale bar = 200 μm .

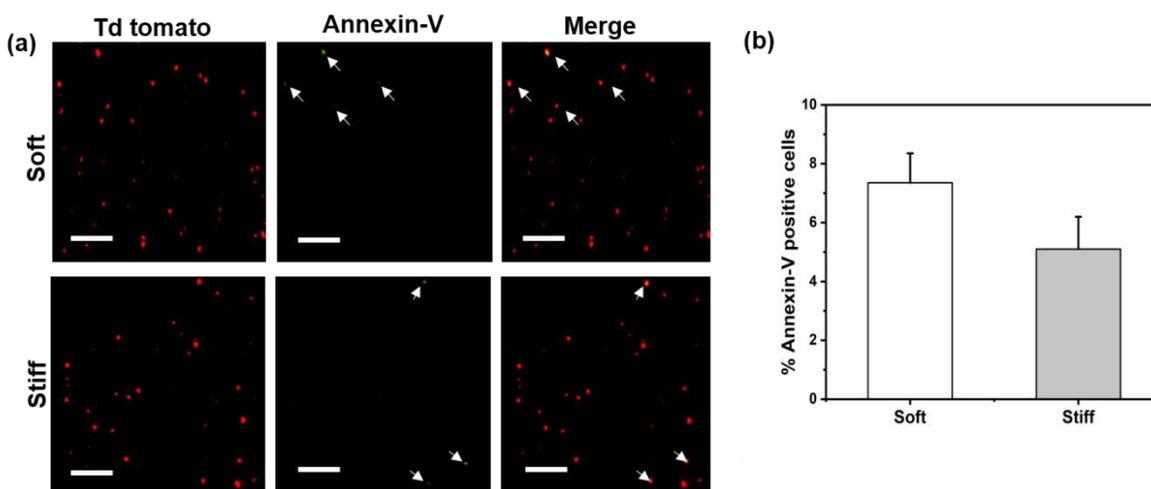


Figure S20: Percentage of Annexin-V positive cells present in 10k cells spheroid on soft (0.4 kPa) or stiff HA hydrogel (4.5kPa) were similar. (a) Representative fluorescent images of cells obtained on day 7 from 10k cells spheroid cultured on soft or stiff HA hydrogel. Red – Tdtomato; Green - Annexin V; Scale bar =100 μm . (b) Quantification of Annexin-V positive cells present in 10k cells spheroid cultured on soft or stiff HA hydrogel. Error bar represents standard error. N = 4 replicates per condition.

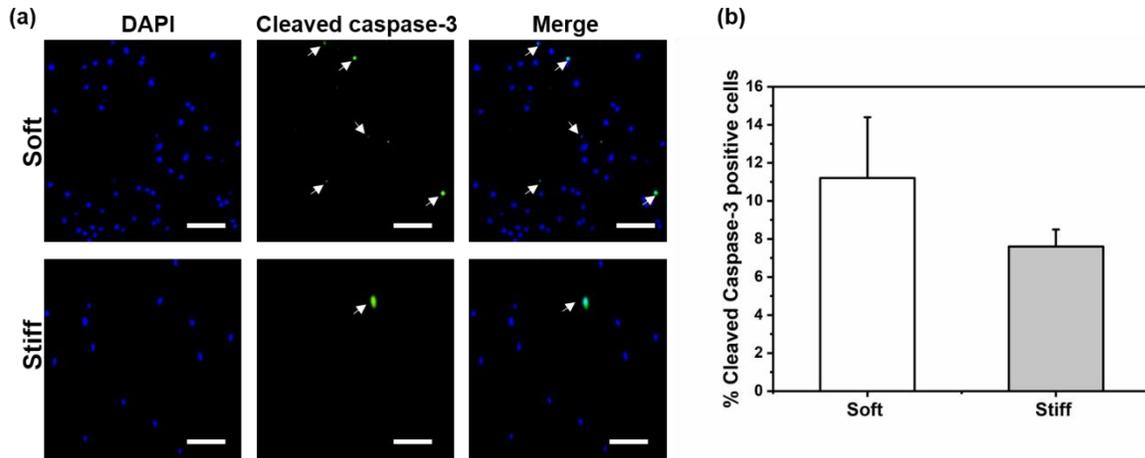


Figure S21: Percentage of cleaved caspase-3 positive cells present in 10k cells spheroid on soft (0.4 kPa) or stiff HA hydrogel (4.5kPa) were similar. (a) Representative fluorescent images of cells obtained on day 7 from 10k cells spheroid cultured on soft or stiff HA hydrogel. Blue – DAPI; Green – Cleaved caspase-3; Scale bar =100 μ m. (b) Quantification of cleaved caspase-3 positive cells present in 10k cells spheroid cultured on soft or stiff HA hydrogel. Error bar represents standard error. N = 3 replicates per condition.

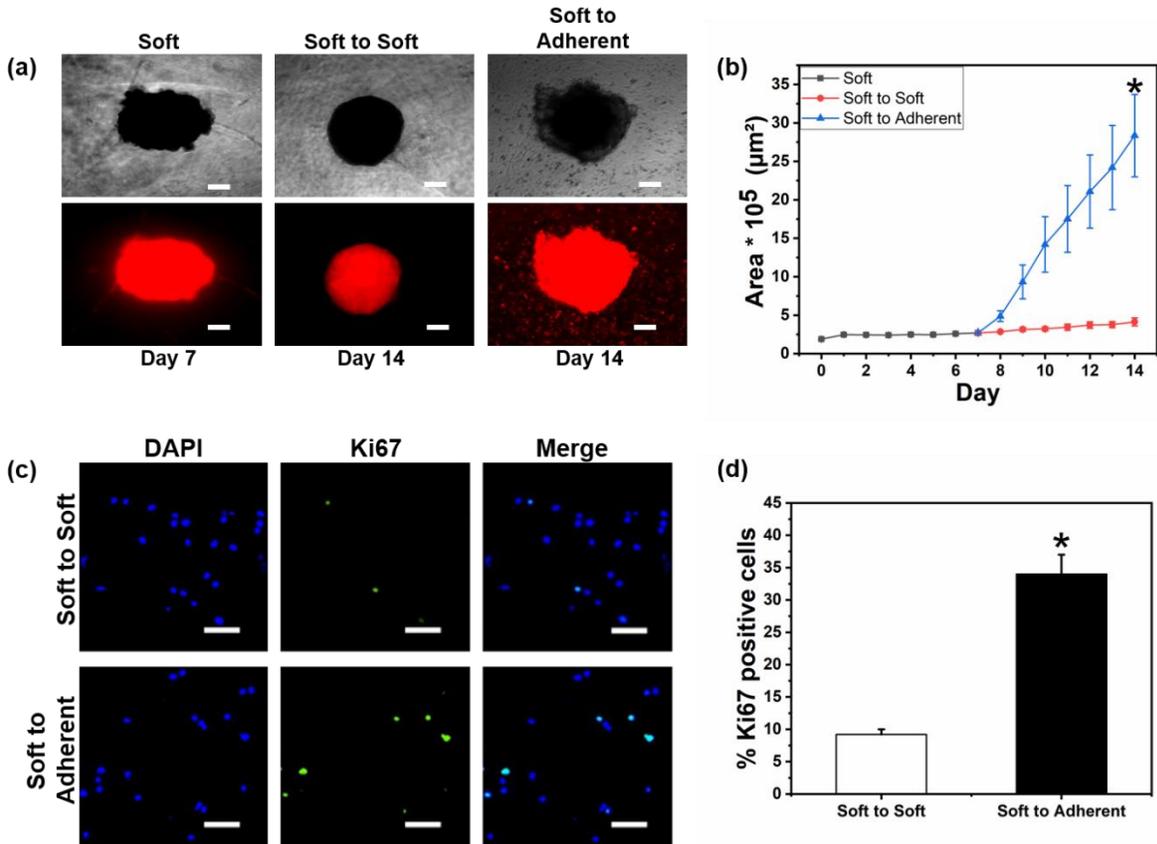


Figure S22: Stiffness induced dormant phenotype observed on the soft HA hydrogel (0.4 kPa) for 10k cells spheroid was reversible. (a) Day-7 bright field and fluorescent images of 10k cells spheroid cultured on soft HA hydrogel and Day-14 bright field and fluorescent images of 10k cells spheroid transferred from soft HA hydrogel on day-7 to soft HA hydrogel or adherent 2D TCPS and cultured for an additional 7 days. Scale bar = 200 μm . (b) Area of 10k cells spheroid measured over a period of 14 days. (c) Representative fluorescent images of Ki67 staining for MDA-MB-231Br cells in 10k cells spheroid cultured on soft HA hydrogel for 7 days and transferred to soft HA hydrogel or adherent 2D TCPS and cultured for an additional 7 days, Blue – DAPI (nucleus), Green – Ki67, Scale bar = 100 μm (d) Quantification of Ki67 positive cells present in 10k cells spheroid on soft HA hydrogel or adherent 2D TCPS post transfer. N = 4 replicates per condition. * indicates statistically significant difference ($p < 0.05$) compared to the ‘soft to soft’ condition. Error bar indicates standard error.