

Supplemental Materials: IB-ART-12-2014-000281

Vega et al.,

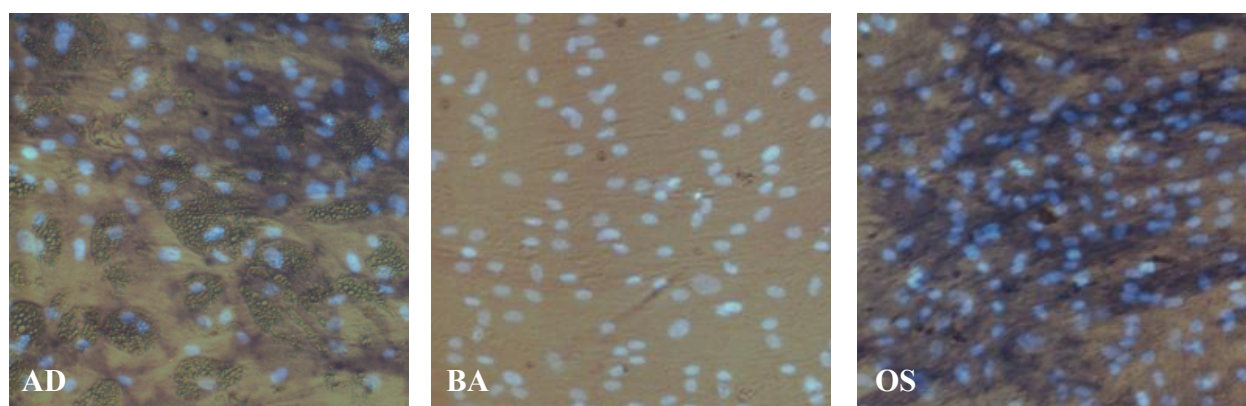
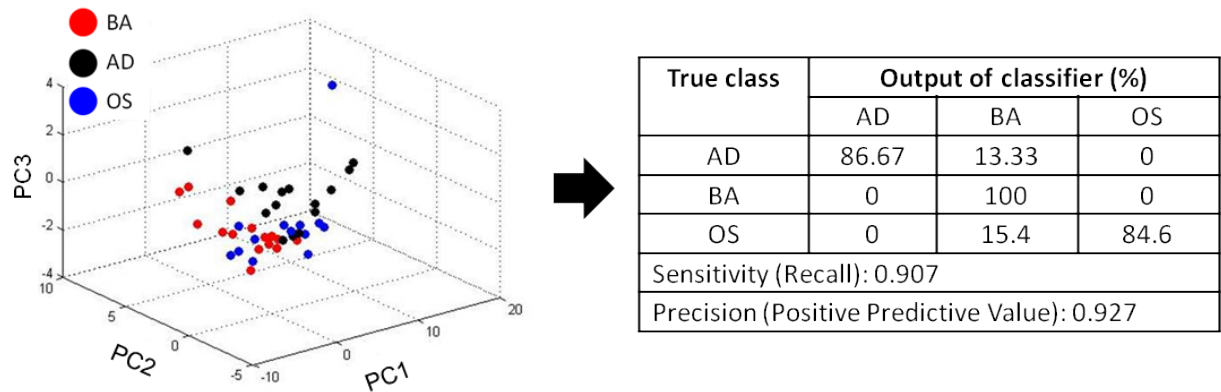


Figure S1. Osteogenic and adipogenic differentiation of hMSCs. . hMSCs were cultured in BA, AD, or OS media for 14 days.) Osteogenic differentiation was analyzed using Fast Blue staining assay (blue) and adipogenic differentiation was analyzed using Adipored assay (green). Images were taken using a fluorescence microscopy at 10x magnification.



C

Figure S2. hMSC phenotype classification using EZH2 organization for cells exposed to different culture media. hMSCs were cultured in BA, AD, or OS medium for 72 hours and EZH2 organization was analyzed by high-resolution imaging. High content analysis of EZH2 organization was subsequently performed and 26 Haralick texture descriptors were evaluated for EZH2. PCA was then performed to dimensionally reduce the Haralick descriptors to principal components that account for > 95% of the variance in data and J48 decision tree analysis was done to classify cells exposed to different culture conditions (BA, AD, or OS). **A)** Depicts cells in 3-D Principal Component space, **B)** shows output of the classifier using decision tree analysis and **C)** lists the principal components

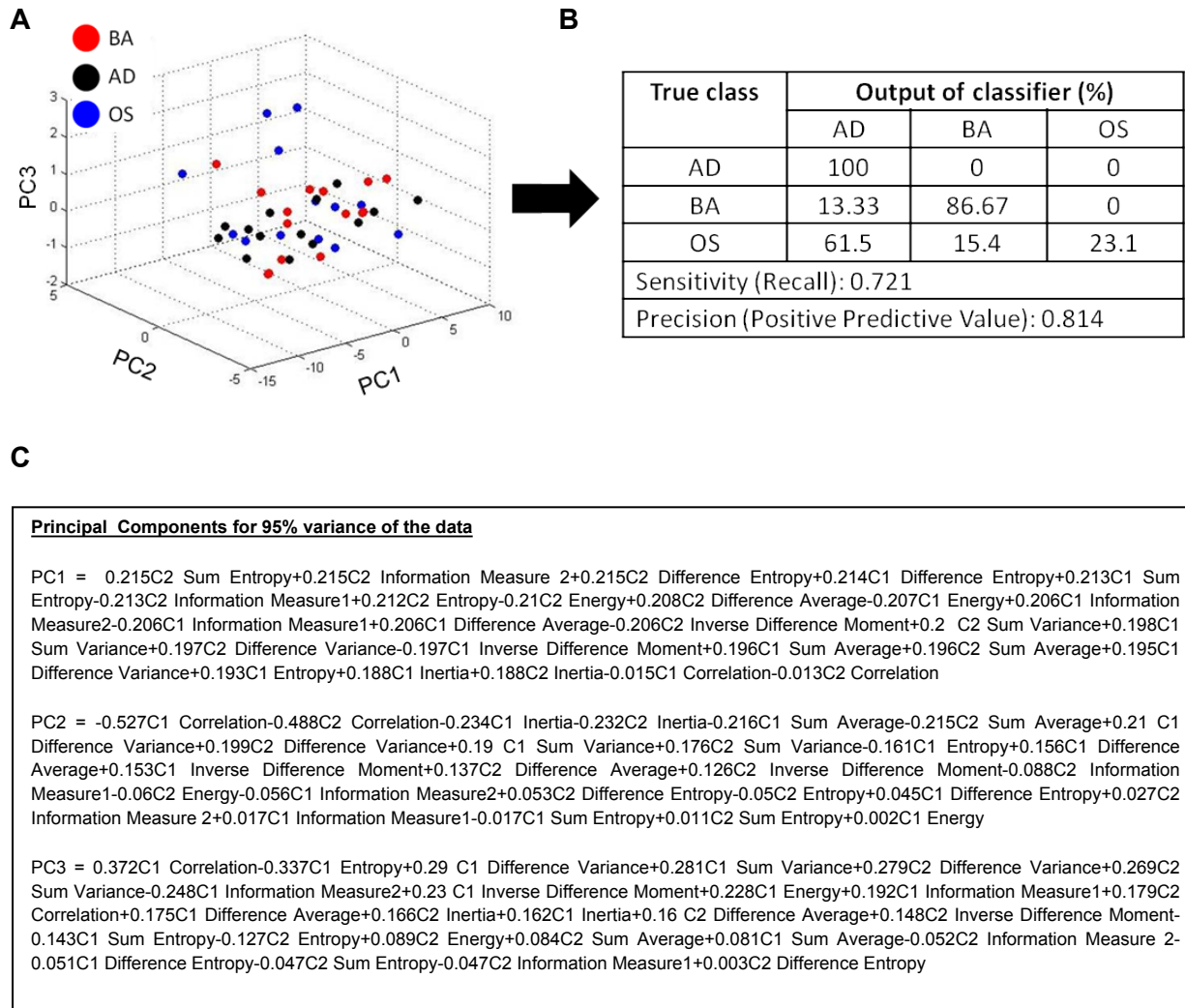


Figure S3. hMSC phenotype classification using RUNX2 organization for cells exposed to different culture media. hMSCs were cultured in BA, AD, or OS inducing conditions for 72 hours and RUNX2 organization was analyzed by high-resolution imaging. High content analysis of RUNX2 organization was subsequently performed and 26 Haralick texture descriptors were evaluated for RUNX2. PCA was then performed to dimensionally reduce the Haralick descriptors to principal components that account for > 95% of the variance in data and J48 decision tree analysis was done to classify cells exposed to different culture conditions (BA, AD, or OS). **(A)** Depicts cells in 3-D Principal Component space, **(B)** shows output of the classifier using decision tree analysis, and **(C)** lists the composition of the principal components.

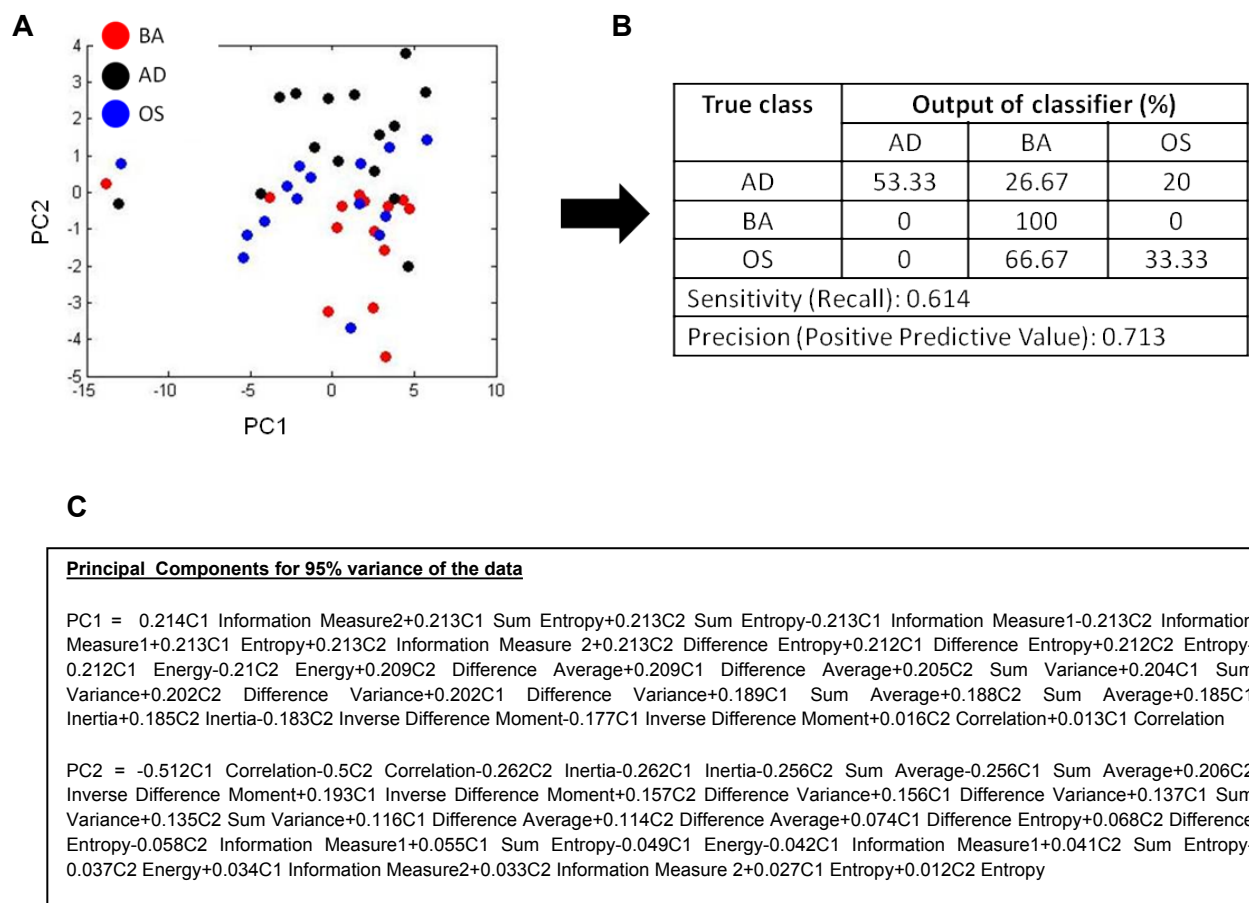


Figure S4. hMSC phenotype classification using lamin organization for cells exposed to different culture media. hMSCs were cultured in BA, AD, or OS inducing conditions for 72 hours and Lamin organization was analyzed by high-resolution imaging. High content analysis of Lamin organization was subsequently performed and 26 Haralick texture descriptors were evaluated for Lamin. PCA was then performed to dimensionally reduce the Haralick descriptors to principal components that account for > 95% of the variance in data and J48 decision tree analysis was done to classify cells exposed to different culture conditions (BA, AD and OS). **A)** depicts cells in the 3-D Principal component space, **B)** shows output of the classifier using decision tree analysis and **C)** lists the principal components

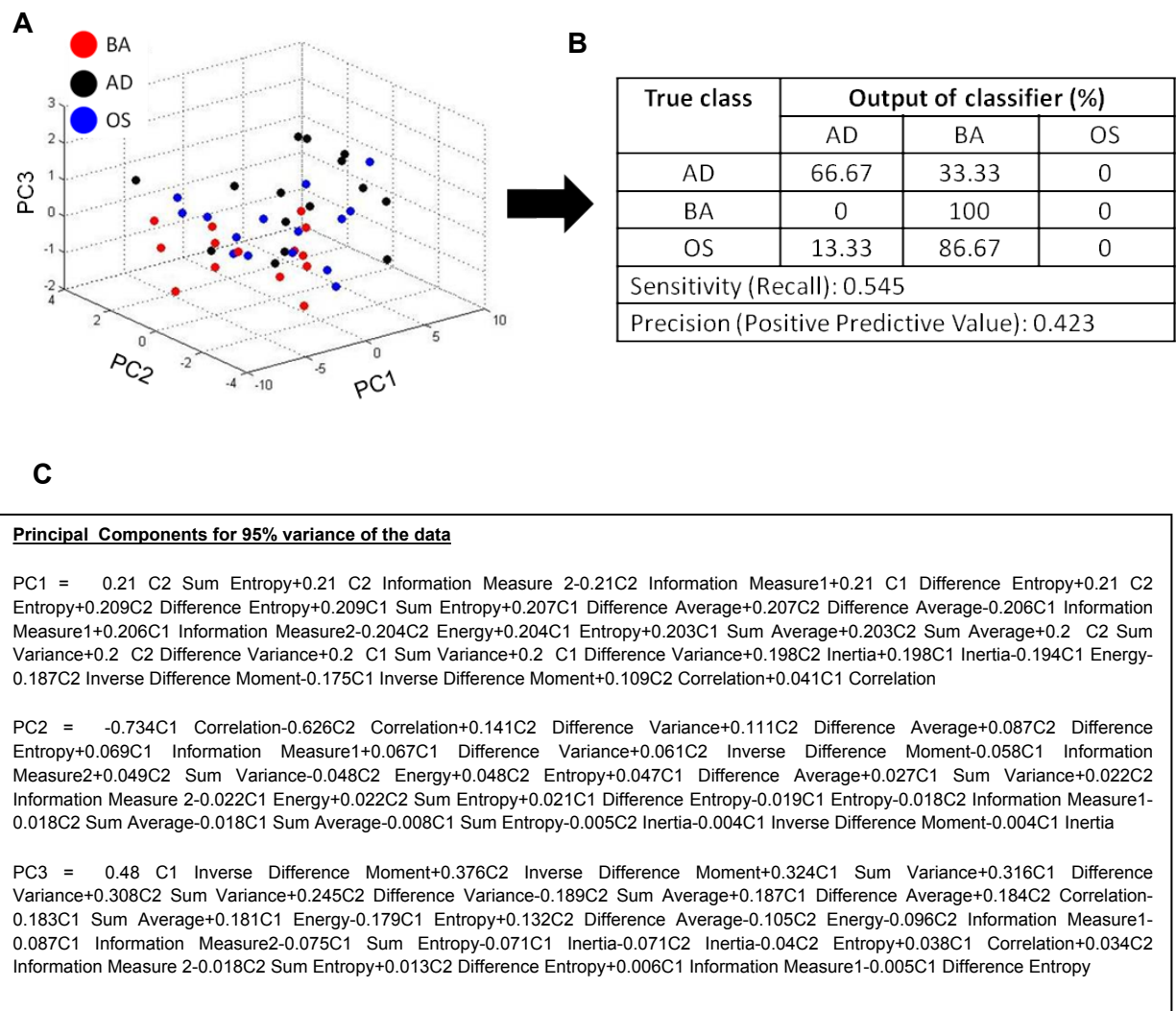
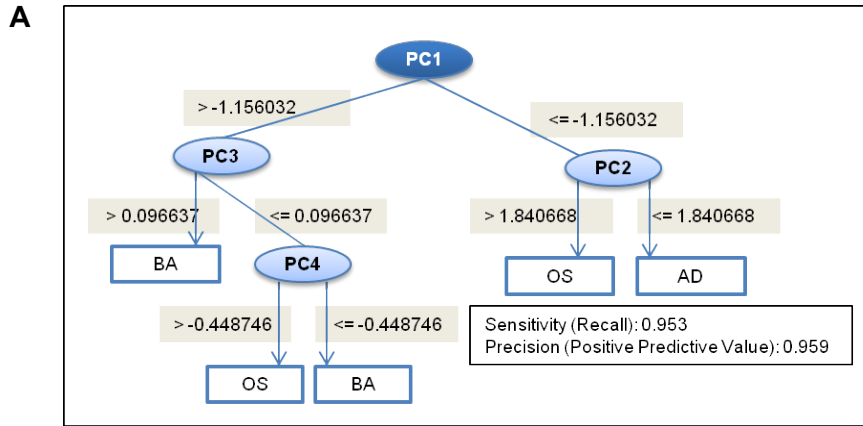


Figure S5. hMSC phenotype classification using H3K4me3 organization for cells exposed to different culture media. hMSCs were cultured in BA, AD, and OS inducing conditions for 72 hours and H3K4me3 organization was analyzed by high-resolution imaging. High content analysis of H3K4me3 organization was subsequently performed and 26 Haralick texture descriptors were evaluated for H3K4me3. PCA was then performed to dimensionally reduce the Haralick descriptors to principal components that account for > 95% of the variance in data and J48 decision tree analysis was done to classify cells exposed to different culture conditions (BA, AD, or OS). **A**) Depicts cells in 3-D Principal Component space, **B**) shows output of the classifier using decision tree analysis, and **C**) lists the composition of the principal components.



B

Principal Components for 95% variance of the data

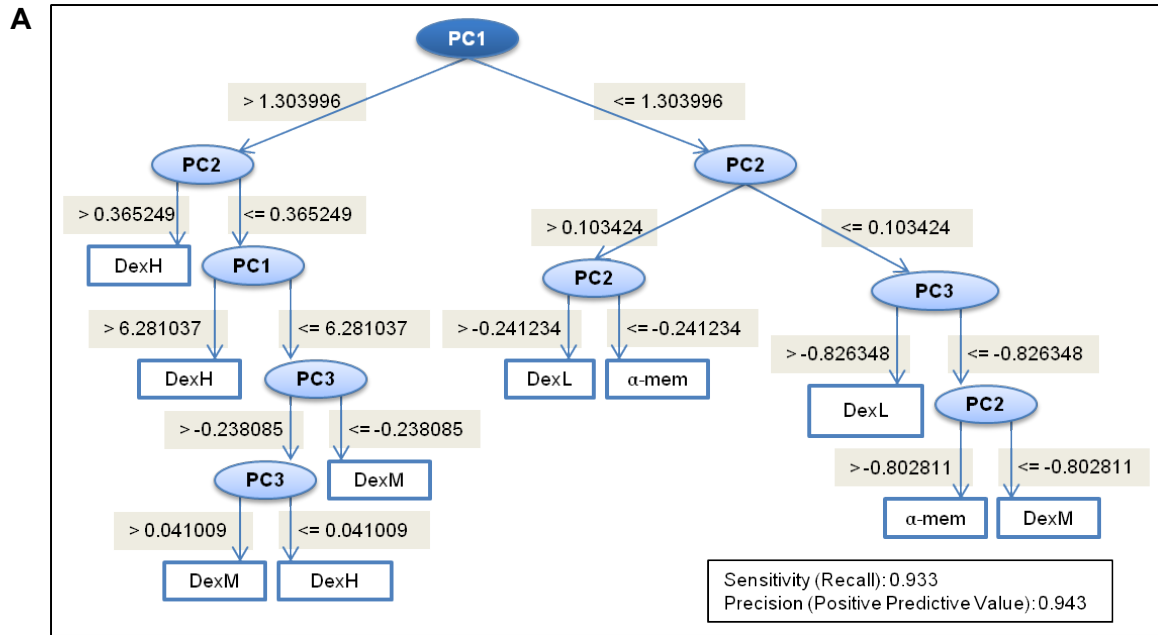
PC1 = -0.234(C2 Information Measure1)+0.233(C2 Information Measure2)+0.233(C2 Difference Average)+0.232(C2 Difference Entropy)+0.23(C2 Sum Entropy)+0.23(C2 Entropy)+0.222(C1 Difference Average)+0.22(C2 Sum Average)+0.219(C1 Sum Average)+0.217(C2 Difference Variance)-0.215(C1 Inverse Difference Moment)+0.213(C1 Difference Entropy)+0.206(C1 Sum Entropy)+0.205(C2 Inertia)+0.205(C1 Inertia)+0.196(C1 Entropy)-0.184(C2 Inverse Difference Moment)-0.182(C1 Energy)+0.174(C1 Information Measure2)-0.172(C2 Correlation)+0.17(C2 Sum Variance)+0.159(C1 Difference Variance)-0.133(C1 Information Measure1)+0.128(C1 Sum Variance)-0.109(C1 Correlation)-0.031(C2 Energy)

PC2 = 0.42(C2 Energy)-0.369(C1 Sum Variance)-0.34(C1 Difference Variance)-0.319(C2 Sum Variance)+0.277(C1 Entropy)+0.266(C1 Information Measure2)+0.229(C1 Sum Entropy)-0.225(C1 information Measure1)-0.187(C2 Difference Variance)+0.174(C1 Difference Entropy)-0.17(C1 Energy)+0.156(C2 Inverse Difference Moment)-0.155(C1 Difference Average)-0.154(C2 Correlation)-0.135(C1 Inverse Difference Moment)-0.105(C2 Inertia)-0.104(C1 Inertia)-0.059(C2 Difference Average)+0.058(C2 Entropy)+0.052(C2 Sum Entropy)+0.041(C2 Difference Entropy)+0.038(C2 Information Measure1)+0.023(C1 Correlation)+0.007(C2 Sum Average)+0.007(C2 (Information Measure2)+0.003(C1 Sum Average)

PC3 = 0.476(C1 Information Measure1)-0.296(C1 Information Measure2)-0.291(C1 Sum Variance)+0.281(C1 Energy)-0.275(C2 Inverse Difference Moment)-0.232(C1 Difference Variance)+0.23(C1 Sum Average)+0.227(C2 Sum Average)-0.208(C2 Sum Variance)+0.1939(C1 Inertia) +0.191(C2 Inertia)+0.145(C2 Energy)-0.142(C1 Inverse Difference Moment)-0.134(C1 Difference Entropy)-0.13(C2 Correlation)-0.127(C1 Sum Entropy)+0.121(C2 Entropy)+0.117(C2 Sum Entropy)-0.115(C1 Entropy)-0.11(C1 Difference Average)+0.106(C2 Mean Difference Entropy)+0.081(C2 Information Measure2)-0.074(C2 Difference Variance)-0.03(C1 Correlation)-0.029(C2 Information Measure1)+0.029(C2 Difference Average)

PC4 = -0.685(C1 Correlation)-0.459(C2 Correlation)-0.298(C2 Energy)-0.243(C2 Inertia)-0.242(C1 Inertia)-0.164(C1 Energy)-0.136(C2 Sum Average)-0.136(C1 Sum Average)-0.109(C1 Sum Variance)-0.093(C2 Inverse Difference Moment) +0.086(C1 Information Measure1)-0.073(C2 Sum Variance)-0.058(C1 Sum Entropy)-0.057(C1 Information Measure2)-0.052(C1 Inverse Difference Moment)-0.05(C1 Difference Entropy)+0.038(C2 Difference Average)+0.037(C2 Difference Variance)-0.033(C1 Entropy)-0.023(C1 Difference Variance)+0.021(C2 Difference Entropy)+0.016(C2 Entropy)-0.013(C1 Difference Average)+0.012(C2 Information Measure1)-0.005(C2 Sum Entropy)+0.004(C2 Information Measure2)

Figure S6. hMSC phenotype classification using SC-35 organization for cells exposed to different culture media. SC-35 organization was assessed in hMSCs exposed to BA, AD, or OS medium for 72 hours by evaluating 26 Haralick texture features. PCA was then performed for the Haralick descriptors to obtain principal components that account for > 95% of the variance between hMSCs exposed to BA, AD, or OS. Cells exposed to different culture conditions were classified using J48 decision tree analysis on the principal components. **A)** Shows the decision tree analysis and **B)** lists the principal components (PC1, PC2, PC3, PC4).



B

Principal Components for 95% variance of the data

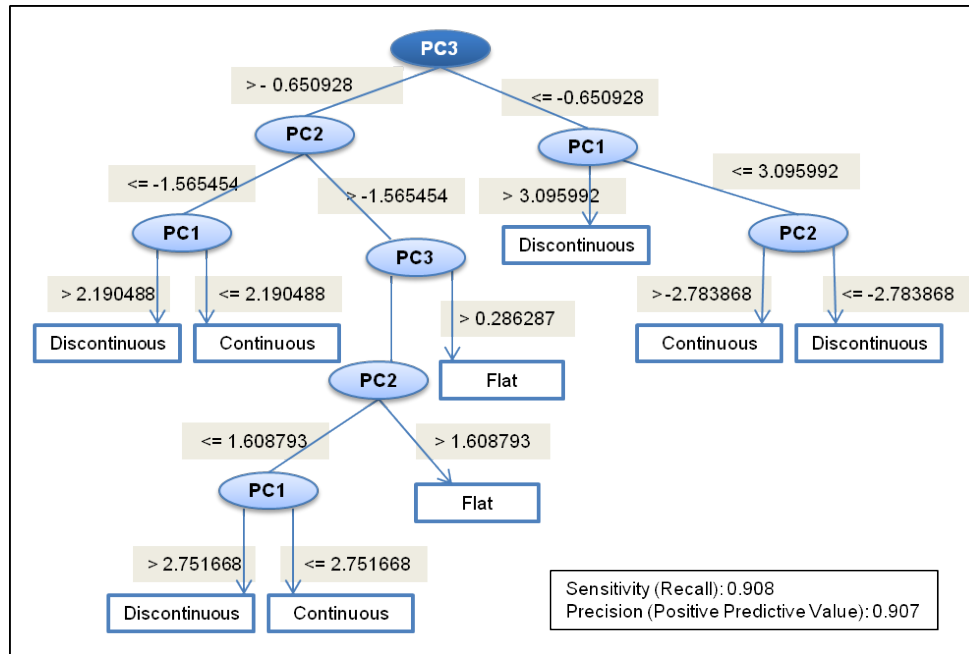
PC1 = -0.217(C2 Information Measure1)+0.217(C2 Information Measure2)-0.217(C1 Information Measure1)+0.216(C2 Difference Entropy)+0.216(C2 Difference Average)+0.215(C1 Difference Entropy)+0.213(C1 Information Measure2)+0.212(C1 Difference Average)+0.211(C2 Sum Entropy)+0.21(C2 Entropy)+0.209(C1 Sum Entropy)+0.208(C2 Difference Variance)+0.206(C1 Sum Average)+0.206(C2 Sum Variance)+0.205(C2 Sum Average)+0.204(C1 Difference Variance)-0.204(C1 Energy)+0.203(C1 Sum Variance)+0.202(C2 Inertia)+0.201(C1 Inertia)+0.192(C1 Entropy)-0.177(C2 Inverse Difference Moment)-0.167(C2 Energy)-0.122(C1 Inverse Difference Moment)-0.074(C1 Correlation)-0.066(C2 Correlation)

PC2 = 0.552(C1 Correlation)+0.482(C2 Correlation)-0.34(C1 Inverse Difference Moment)+0.322(C2 Energy)+0.187(C2 Inverse Difference Moment)+0.183(C1 Entropy)+0.178(C2 Sum Average)+0.171(C1 Sum Average)-0.137(C2 Difference Variance)+0.114(C2 Sum Entropy)+0.105(C1 Sum Entropy)+0.104(C2 Entropy)+0.096(C1 Inertia)+0.095(C2 Inertia)-0.089(C1 Difference Variance)-0.086(C2 Difference Average)-0.086(C1 Difference Average)+0.085(C1 Energy)+0.068(C1 Information Measure2)-0.053(C2 Sum Variance)-0.045(C1 Sum Variance)+0.032(C2 Difference Entropy)+0.029(C2 Information Measure2)+0.025(C1 Difference Entropy)+0.025(C2 Information Measure1)+0.006(C1 Information Measure1)

PC3 = 0.446(C1 Inverse Difference Moment)+0.43(C2 Correlation)+0.282(C1 Correlation)-0.269(C1 Entropy)+0.235(C1 Sum Variance)-0.218(C2 Energy)-0.205(C2 Inverse Difference Moment)+0.205(C2 Inertia)+0.205(C2 Sum Variance)+0.203(C1 Inertia)+0.193(C1 Difference Variance)-0.165(C1 Sum Entropy)+0.159(C1 Energy)-0.153(C2 Entropy)+0.124(C1 Difference Average)-0.124(C2 Sum Entropy)-0.11(C2 Difference Entropy)-0.11(C1 Information Measure2)-0.092(C1 Difference Entropy)+0.084(C2 Difference Variance)+0.06(C1 Sum Average)-0.058(C2 Information Measure2)+0.049(C2 Sum Average)+0.022(C2 Difference Average)-0.015(C1 Information Measure1)-0.008(C2 Information Measure1)

Figure S7. Classification of hMSCs cultured in presence of different dexamethasone (dex) concentrations using SC-35 organization. SC-35 organization was assessed in hMSCs cultured in the presence of no dex (a-mem), low dex (DexL), medium dex (DexM), and high dex (DexH) concentrations by evaluating 26 Haralick texture descriptors. Next, PCA was performed to obtain principal components that account for 95% of the variance and different culture conditions were classified using J48 decision tree analysis. **A)** Shows the decision tree analysis and **B)** lists the principal components (PC1, PC2, PC3).

A



B

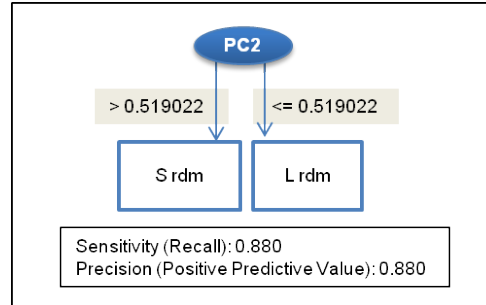
Principal Components for 95% variance of the data

PC1 = -0.219(C2 Information Measure1)-0.218(C1 Information Measure1)+0.216(C2 Sum Entropy)+0.216(C2 Information Measure2)+0.216(C2 Difference Entropy)+0.216(C1 Difference Entropy)+0.214(C1 Sum Entropy)+0.214(C1 Information Measure2)+0.213(C2 Difference Average)+0.21(C2 Entropy)+0.209(C1 Difference Average)+0.206(C1 Sum Average)+0.205(C2 Sum Average)+0.2(C1 Entropy)+0.196(C2 Difference Variance)+0.195(C2 Inertia)-0.194(C2 Inverse Difference Moment)+0.194(C2 Sum Variance)+0.194(C1 Inertia)+0.193(C1 Sum Variance)+0.191(C1 Difference Variance)-0.181(C1 Energy)-0.171(C1 Inverse Difference Moment)-0.16(C2 Energy)+0.112(C2 Correlation)+0.078(C1 Correlation)

PC2 = -0.463(C1 Correlation)-0.428(C2 Correlation)-0.321(C2 Energy)-0.297(C1 Energy)-0.22(C2 Inertia)-0.219(C1 Inertia)-0.214(C1 Sum Variance)-0.189(C1 Difference Variance)-0.188(C2 Sum Variance)+0.17(C2 Entropy)+0.158(C1 Entropy)-0.127(C2 Inverse Difference Moment)+0.125(C2 Difference Entropy)-0.124(C2 Sum Average)-0.122(C1 Sum Average)+0.117(C1 Sum Entropy)+0.115(C2 Information Measure2)+0.114(C1 Difference Entropy)+0.102(C2 Sum Entropy)-0.099(C2 Difference Variance)+0.099(C1 Information Measure2)-0.076(C1 Difference Average)-0.053(C1 Inverse Difference Moment)-0.044(C1 Information Measure1)-0.025(C2 Information Measure1)+0.015(C2 Difference Average)

PC3 = 0.437(C1 Inverse Difference Moment)-0.348(C1 Correlation)+0.321(C2 Difference Variance)+0.298(C1 Difference Variance)+0.272(C2 Sum Variance)+0.259(C1 Sum Variance)-0.251(C2 Correlation)+0.231(C1 Difference Average)-0.214(C2 Energy)-0.2129(C1 Entropy)+0.211(C2 Difference Average)+0.145(C2 Inverse Difference Moment)-0.135(C1 Information Measure2)-0.115(C2 Sum Average)-0.114(C1 Sum Average)-0.1(C2 Entropy)-0.099(C1 Sum Entropy)-0.097(C2 Sum Entropy)-0.056(C2 Information Measure2)+0.048(C1 Energy)+0.046(C1 Information Measure1)+0.03(C2 Inertia)+0.025(C2 Difference Entropy)+0.022(C1 Inertia)+0.014(C1 Difference Entropy)-0.007(C2 Information Measure1)

Figure S8. Cell state classification for hMSCs cultured on patterned surfaces using SC-35 organization. SC-35 organization was assessed in hMSCs cultured on surface topographies of different degrees of continuity (flat, continuous, discontinuous) by evaluating 26 Haralick texture descriptors. Next, PCA was performed to obtain principal components that account for 95% of the variance and different culture conditions were classified using J48 decision tree analysis. **A)** Shows the decision tree analysis and **B)** lists the principal components (PC1, PC2, PC3).

A**B****Principal Components for 95% variance of the data**

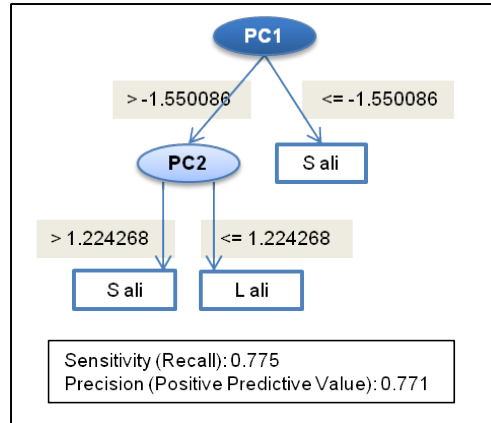
PC1 = -0.221(C2 Information Measure1)+0.219(C2 Information Measure2)+0.217(C2 Difference Average)+0.216(C2 Sum Entropy)+0.216(C2 Difference Entropy)+0.216(C1 Difference Average)+0.214(C2 Entropy)-0.212(C1 Information Measure1)+0.21(C2 Sum Average)+0.21(C1 Sum Average)+0.209(C1 Difference Entropy)+0.203(C1 Information Measure2)+0.202(C1 Sum Entropy)+0.2(C2 Difference Variance)+0.195(C2 Sum Variance)+0.195(C1 Sum Variance)+0.194(C2 Inertia)+0.194(C1 Difference Variance)+0.193(C1 Inertia)+0.19(C2 Correlation)+0.188(C1 Entropy)+0.179(C1 Correlation)-0.164(C1 Inverse Difference Moment)-0.16(C1 Energy)-0.118(C2 Inverse Difference Moment)-0.114(C2 Energy)

PC2 = 0.361(C2 Energy)-0.358(C1 Energy)+0.355(C2 Inverse Difference Moment)-0.353(C1 Inverse Difference Moment)+0.28(C1 Entropy)+0.218(C1 Sum Entropy)+0.207(C1 Information Measure2)-0.204(C2 Inertia)-0.204(C1 Inertia)-0.198(C1 Sum Variance)-0.186(C2 Sum Variance)-0.18(C1 Difference Variance)+0.171(C1 Difference Entropy)-0.145(C2 Difference Variance)-0.124(C1 Information Measure1)+0.111(C2 Entropy)-0.104(C2 Correlation)+0.094(C2 Sum Entropy)-0.094(C1 Sum Average)-0.086(C2 Sum Average)+0.086(C2 Difference Entropy)-0.081(C1 Difference Average)-0.053(C2 Difference Average)+0.045(C2 Information Measure2)-0.006(C1 Correlation)+0.001(C2 Information Measure1)

PC3 = -0.488(C2 Energy)-0.484(C2 Inverse Difference Moment)-0.356(C1 Correlation)-0.28(C2 Correlation)-0.256(C1 Difference Variance)-0.2539(C1 Sum Variance)-0.244(C2 Sum Variance)-0.158(C2 Difference Variance)+0.146(C2 Entropy)+0.112(C2 Information Measure2)+0.103(C2 Sum Entropy)+0.096(C2 Difference Entropy)+0.091(C1 Entropy)+0.088(C1 Information Measure2)-0.087(C1 Difference Average)-0.082(C1 Information Measure1)+0.071(C2 Sum Average)+0.071(C1 Sum Average)+0.059(C1 Sum Entropy)-0.053(C2 Inertia)-0.049(C1 Inertia)+0.045(C1 Difference Entropy)-0.042(C2 Information Measure1)-0.035(C1 Inverse Difference Moment)-0.031(C1 Energy)-0.006(C2 Difference Average)

Figure S9. Cell state classification for hMSCs cultured on randomly oriented fibers using SC-35 organization. hMSCs were cultured on small and large randomly oriented fibers for 72 hours and high content analysis was done for SC-35 organization to evaluate 26 Haralick texture descriptors. The descriptors were dimensionally reduced by PCA to principal components that account for 95% of the variance and small versus large randomly oriented fiber classification was performed using J48 decision tree analysis. **A)** Shows the decision tree analysis and **B)** lists the principal components (PC1, PC2, PC3).

A



B

Principal Components for 95% variance of the data

PC1 = -0.213(C2 Information Measure1)+0.212(C2 Information Measure2)+0.211(C2 Difference Entropy)+0.211(C2 Sum Entropy)+0.21(C2 Entropy)+0.209(C1 Difference Entropy)+0.209(C2 Difference Average)+0.208(C1 Difference Average)-0.206(C1 Information Measure1)+0.205(C2 Sum Average)+0.205(C1 Sum Entropy)+0.205(C1 Sum Average)+0.201(C1 Information Measure2)+0.194(C1 Entropy)+0.193(C2 Difference Variance)+0.193(C2 Sum Variance)+0.193(C1 Sum Variance)-0.192(C2 Energy)+0.191(C2 Inertia)+0.19(C1 Inertia) +0.19(C1 Difference Variance)-0.188(C2 Inverse Difference Moment)-0.18(C1 Energy)-0.169(C1 Inverse Difference Moment)+0.165(C2 Correlation)+0.135(C1 Correlation)

PC2 = -0.347(C1 Inverse Difference Moment)-0.317(C1 Energy)+0.291(C1 Correlation)-0.268(C1 Difference Variance)-0.263(C1 Sum Variance)+0.261(C1 Entropy)-0.26(C2 Sum Variance)-0.249(C2 Difference Variance)-0.237(C2 Inertia)-0.236(C1 Inertia) +0.223(C1 Information Measure2)+0.179(C1 Sum Entropy)+0.159(C2 Correlation)-0.154(C1 Information Measure1)-0.136(C1 Difference Average)+0.121(C2 Energy)-0.12(C2 Difference Average)+0.113(C2 Inverse Difference Moment)+0.111(C1 Difference Entropy)-0.1(C1 Sum Average)-0.098(C2 Sum Average)+0.094(C2 Entropy)+0.089(C2 Sum Entropy)+0.053(C2 Information Measure2)+0.039(C2 Difference Entropy)-0.008(C2 Information Measure1)

PC3 = -0.647(C1 Correlation)-0.576(C2 Correlation)-0.227(C1 Inverse Difference Moment)-0.22(C1 Energy)+0.144(C2 Difference Entropy)+0.134(C1 Entropy)+0.129(C2 Entropy)-0.113(C2 Inertia)-0.11(C1 Inertia)+0.1(C2 Difference Variance)-0.098(C2 Energy)+0.094(C2 Difference Average)+0.087(C1 Sum Entropy)+0.086(C1 Difference Entropy)+0.085(C2 Sum Entropy)-0.059(C2 Sum Average)-0.056(C1 Sum Average)-0.056(C1 Sum Variance)+0.055(C2 Inverse Difference Moment)+0.046(C1 Information Measure1)+0.041(C2 Information Measure2)+0.028(C1 Information Measure2)-0.026(C2 Sum Variance)+0.009(C2 Information Measure1)-0.004(C1 Difference Average)-0.002(C1 Difference Variance)

Figure S10. Cell state classification for hMSCs cultured on aligned fibers using SC-35 organization. hMSCs were cultured on small and large aligned fibers for 72 hours and high content analysis was done for SC-35 organization to evaluate 26 Haralick texture descriptors. The descriptors were dimensionally reduced by PCA to principal components that account for 95% of the variance and small versus large aligned fiber classification was performed using J48 decision tree analysis. **A)** Shows the decision tree analysis and **B)** lists the principal components (PC1, PC2, PC3).

Principal Components for 95% variance of the data

PC1 = -0.215(C2 Information Measure1)+0.215(C2 Information Measure2)+0.215(C1 Difference Entropy)+0.213(C2 Sum Entropy)+0.213(C1 Sum Entropy)+0.212(C2 Difference Average)+0.211(C2 Entropy)+0.211(C2 Difference Entropy)+0.21(C1 Difference Average)+0.202(C1 Information Measure2)+0.201(C2 Sum Average)+0.201(C1 Sum Average)-0.2(C1 Information Measure1)+0.2(C1 Entropy)-0.2(C2 Energy)+0.195(C2 Difference Variance)+0.194(C2 Inertia)+0.193(C1 Inertia)+0.193(C2 Sum Variance)+0.192(C1 Sum Variance)+0.188(C1 Difference Variance)-0.187(C1 Energy)-0.183(C2 Inverse Difference Moment)+0.176(C2 Correlation)+0.124(C1 Correlation)-0.117(C1 Inverse Difference Moment)

PC2 = -0.458(C1 Inverse Difference Moment)-0.376(C1 Difference Variance)-0.347(C1 Sum Variance)-0.345(C2 Sum Variance)-0.32(C2 Difference Variance)+0.261(C1 Correlation)-0.215(C2 Inverse Difference Moment)-0.192(C1 Difference Average)-0.142(C2 Difference Average)+0.137(C1 Sum Average)+0.136(C2 Sum Average)+0.125(C2 Correlation)-0.121(C1 Energy)+0.107(C1 Information Measure2)+0.106(C1 Entropy)+0.103(C2 Entropy)+0.101(C2 Sum Entropy)+0.078(C1 Sum Entropy)-0.071(C1 Information Measure1)+0.061(C2 Information Measure2)-0.06(C2 Inertia)-0.055(C1 Inertia)+0.032(C2 Difference Entropy)+0.03(C2 Energy)-0.009(C2 Information Measure1)+0.008(C1 Difference Entropy)

PC3 = 0.368(C1 Inertia)+0.362(C2 Inertia)+0.359(C1 Energy)+0.324(C1 Sum Average)-0.324(C1 Entropy)+0.319(C2 Sum Average)-0.295(C1 Information Measure2)+0.272(C1 Information Measure1)-0.209(C1 Inverse Difference Moment)-0.188(C1 Sum Entropy)-0.151(C2 Inverse Difference Moment)+0.119(C2 Energy)-0.11(C1 Difference Entropy)+0.029(C1 Sum Variance)+0.026(C2 Sum Variance)+0.024(C2 Difference Variance)-0.022(C2 Information Measure2)+0.021(C1 Difference Variance)-0.018(C2 Correlation)-0.016(C1 Difference Average)+0.015(C2 Information Measure1)+0.013(C2 Difference Entropy)+0.009(C2 Difference Average)+0.006(C1 Correlation)+0.006(C2 Sum Entropy)-0.001(C2 Entropy)

PC4 = -0.632(C1 Correlation)-0.455(C2 Correlation)-0.326(C1 Inverse Difference Moment)-0.219(C2 Inverse Difference Moment)+0.203(C2 Difference Entropy)+0.184(C1 Information Measure1)-0.16(C2 Energy)+0.156(C2 Entropy)-0.138(C1 Energy)-0.128(C1 Inertia)-0.127(C2 Inertia)+0.121(C2 Sum Entropy)+0.11(C2 Difference Variance)-0.106(C1 Information Measure2)+0.099(C2 Difference Average)+0.07(C1 Difference Entropy)-0.061(C1 Sum Variance)+0.039(C2 Information Measure1)-0.036(C2 Sum Variance)+0.027(C2 Information Measure2)-0.024(C2 Sum Average)-0.022(C1 Sum Average)+0.018(C1 Sum Entropy)-0.011(C1 Difference Average)-0.011(C1 Difference Variance)+0.009(C1 Entropy)

Figure S11. Cell state classification for hMSCs cultured on different TopoUnits using SC-35 organization. hMSCs were cultured in BA media on different TopoUnits (termed TopoA-D) for 72 hours and high content analysis was done for SC-35 organization to evaluate 26 Haralick texture descriptors. The descriptors were dimensionally reduced by PCA to principal components that account for 95% of the variance and different culture conditions (TopoUnits) were classified using J48 decision tree analysis. List of principal components (PC1, PC2, PC3, PC4) with corresponding descriptors and linear weights that account for > 95% of the variance are listed.

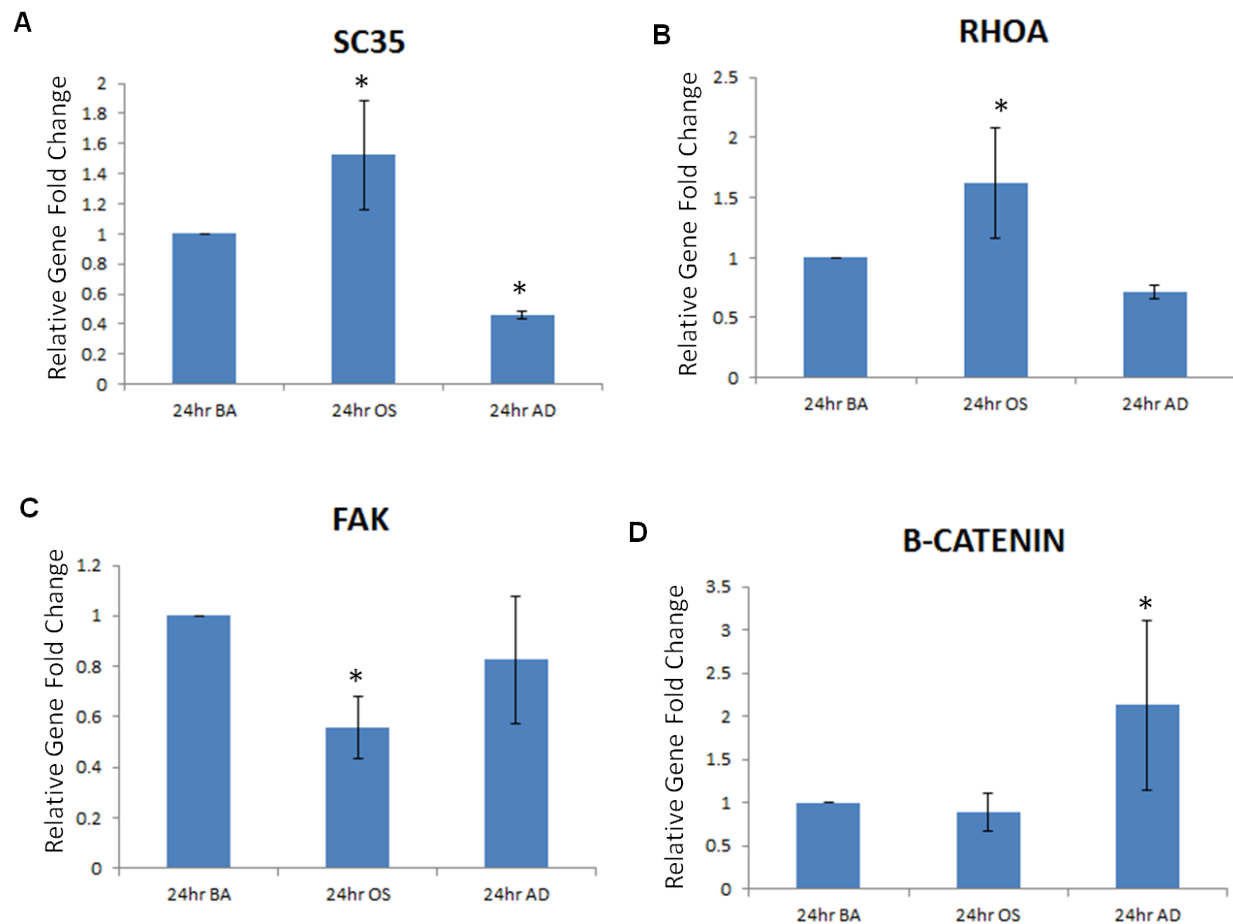


Figure S12. Microarray analysis of RhoA, FAK and β -catenin expression in hMSCs cultured in BA, AD and OS media. hMSCs were cultured in differentiation media for 24 hours, and the relative gene expression for RhoA, FAK, and β -catenin for hMSCs cultured in AD or OS media was analyzed with respect to BA using microarray analysis at 24 hours. Statistical analysis was done using ANNOVA test where the gene expression in cells cultured in AD or OS media was statistically compared to gene expression in BA condition. * indicates statistical significance ($p < 0.01$).

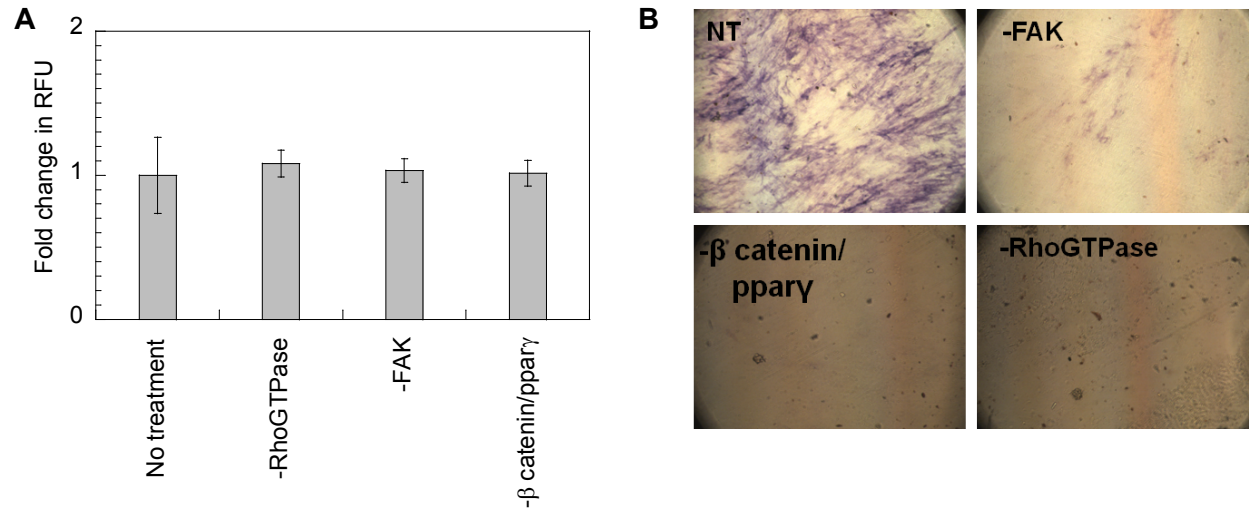


Figure S13. Effect of pharmacological inhibitors on cell viability and osteogenic differentiation. Rho was specifically inhibited 0.5 μ g/ml C3 transferase (-RhoGTPase), FAK was inhibited using 1 μ M FAK inhibitor 14 (-FAK) and β -catenin/ppar- γ were both inhibited 15 μ M FH535. Following pretreatment with the inhibitors, cells were cultured in osteogenic induction media in presence of inhibitor for 72 hours and 14 days for analyzing effect of inhibitors on cell viability and osteogenic differentiation, respectively. **A)** Cell viability was analyzed 72 hours post culturing cell in presence of inhibitor using Alamar Blue assay. Fold change in proliferation was assessed with respect to proliferation of untreated sample, and plotted. **B)** Osteogenic differentiation was analyzed 14 days post culturing cells in osteogenic differentiation media and in presence of inhibitors using Fast Blue staining assay. Fast blue staining was analyzed by fluorescence microscopy at 20x magnification.

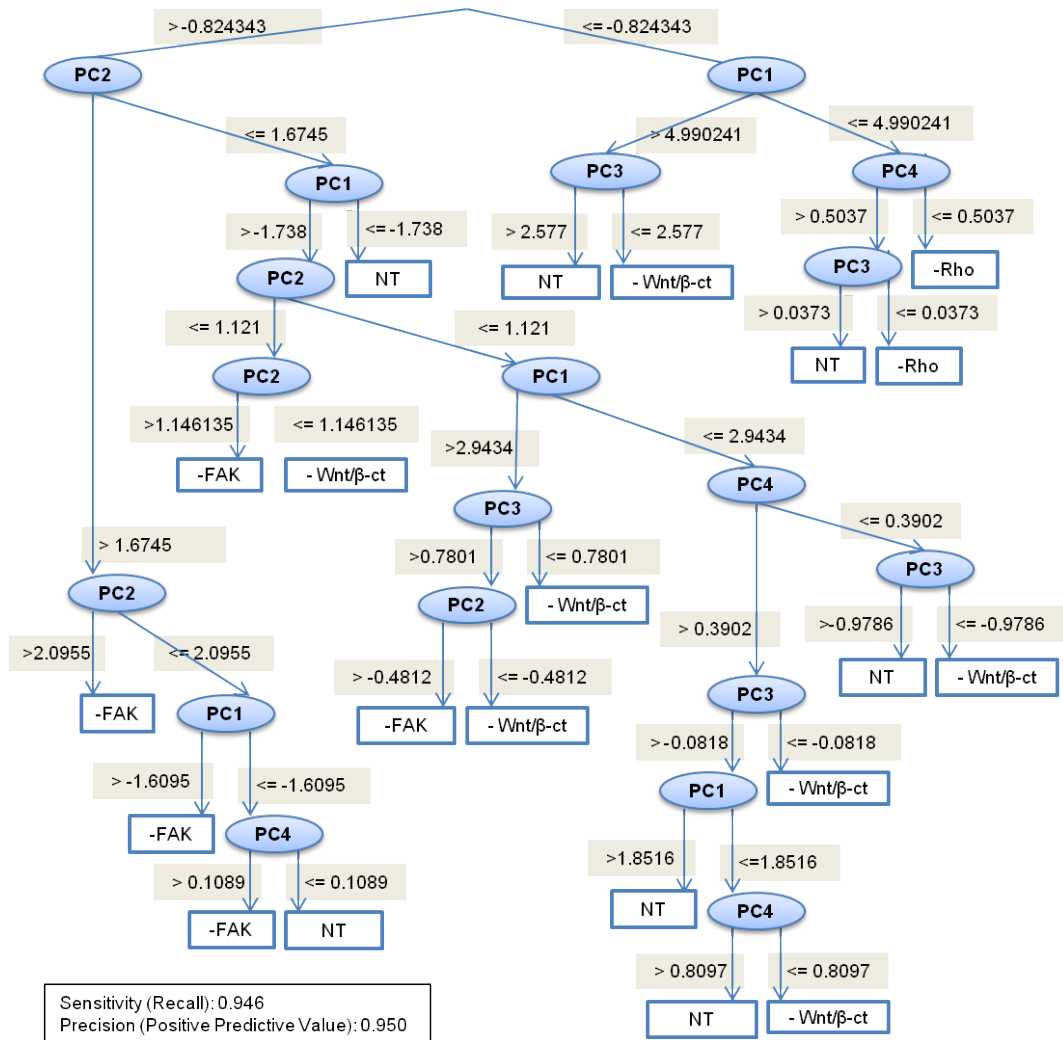


Figure S14. Classification for hMSCs treated with different inhibitors using SC35 organization. Rho was specifically inhibited 0.5 $\mu\text{g/ml}$ C3 transferase (-RhoGTPase), FAK was inhibited using 1 μM FAK inhibitor 14 (-FAK) and β -catenin/ppar- γ were both inhibited 15 μM FH535. Following pretreatment with the inhibitors, cells were cultured in osteogenic induction media in presence of inhibitor for 72 hours. The SC35 organization was then analyzed using high content analysis and 26 Haralick texture descriptors were evaluated. The descriptors were dimensionally reduced by PCA to principal components that account for 95% of the variance and different culture conditions (small vs large randomly oriented fibers) were classified using J48 decision tree analysis. **A)** shows the decision tree analysis.

Principal Components for 95% variance of the data

PC1 = -0.221(C2 Information Measure1)+0.218(C2 Difference Entropy)+0.218(C2 Information Measure2)+0.217(C2 Difference Average)-0.216(C1 Information Measure1)+0.215(C2 Sum Entropy)+0.215(C2 Sum Average)+0.215(C1 Sum Average)+0.212(C1 Difference Average)+0.209(C2 Entropy)+0.208(C1 Difference Entropy)+0.204(C2 Inertia)+0.204(C1 Inertia)+0.202(C2 Difference Variance)+0.2(C2 Sum Variance)+0.198(C1 Sum Variance)+0.197(C2 Correlation)+0.197(C1 Difference Variance)+0.195(C1 Information Measure2)+0.194(C1 Sum Entropy)-0.193(C2 Inverse Difference Moment)+0.177(C1 Correlation)+0.166(C1 Entropy)-0.166(C1 Inverse Difference Moment)-0.101(C2 Energy)-0.045(C1 Energy)

PC2 = 0.428(C2 Energy)+0.39(C1 Entropy)+0.287(C1 Sum Entropy)+0.282(C1 Information Measure2)-0.23(C1 Sum Variance)-0.229(C1 Inverse Difference Moment)-0.211(C2 Correlation)-0.206(C2 Sum Variance)-0.204(C1 Difference Variance)+0.201(C1 Difference Entropy)+0.2(C2 Entropy)-0.162(C1 Correlation)-0.159(C1 Difference Average)-0.155(C2 Difference Variance)+0.152(C2 Sum Entropy)-0.128(C1 Information Measure1)-0.123(C2 Inertia)-0.121(C1 Inertia)+0.105(C2 Difference Entropy)+0.102(C2 Information Measure2)-0.097(C2 Difference Average)+0.084(C1 Mean Energy)+0.073(C2 Inverse Difference Moment)-0.018(C1 Sum Average)-0.015(C2 Sum Average)+0.004(C2 Information Measure1)

PC3 = 0.753(C1 Energy)+0.354(C2 Energy)+0.198(C2 Inverse Difference Moment)+0.185(C1 Inertia)+0.184(C2 Inertia)+0.181(C1 Inverse Difference Moment)+0.181(C2 Difference Variance)+0.173(C1 Difference Variance)+0.132(C2 Sum Variance)+0.127(C1 Sum Variance)-0.114(C2 Correlation)+0.104(C2 Sum Average)+0.104(C1 Sum Average)-0.101(C1 Correlation)+0.076(C1 Information Measure1)-0.075(C2 Information Measure2)+0.063(C2 Information Measure1)-0.054(C2 Entropy)-0.047(C1 Information Measure2)-0.046(C2 Sum Entropy)+0.04(C1 Difference Average)+0.038(C2 Difference Average)-0.009(C1 Difference Entropy)-0.008(C1 Sum Entropy)+0.007(C1 Entropy)-0.005(C2 Difference Entropy)

PC4 = -0.405(C1 Correlation)+0.342(C1 Inverse Difference Moment)+0.325(C2 Inverse Difference Moment)-0.268(C1 Inertia)-0.26(C2 Inertia)-0.24(C1 Sum Average)-0.239(C2 Sum Average)+0.228(C2 Difference Variance)+0.221(C1 Difference Variance)+0.22(C2 Sum Variance)+0.192(C2 Difference Average)+0.175(C1 Difference Average)-0.171(C1 Energy)+0.167(C1 Sum Variance)+0.135(C1 Difference Entropy)+0.132(C2 Difference Entropy)-0.098(C2 Correlation)+0.093(C1 Sum Entropy)+0.081(C1 Entropy)+0.072(C1 Information Measure2)-0.068(C2 Energy)-0.061(C2 Information Measure1)+0.058(C2 Sum Entropy)-0.053(C1 Information Measure1)+0.053(C2 Entropy)+0.045(C2 Information Measure2)

Figure S14 B) lists the principal components (PC1, PC2, PC3, PC4) obtained after doing PCA for descriptors defining SC35 organization in hMSCs treated with specific pharmacological inhibitors.

Table S1. List of the 13 Haralick texture features. Descriptors were calculated using two different pixel groupings (termed C1, C2) resulting in 26 Haralick texture features in total. The features are divided into four groupings: entropy (red), correlation (blue), granularity (purple), and inertia (green), which represent a measure of speckle homogeneity, elongation, size, and distribution with respect to nuclear centroid, respectively. The potential biological relevance for each feature is also listed.

<i>Measure</i>	<i>Biological Relevance</i>
Entropy	Randomness of the intensity distribution; low values correspond to small differences in neighboring pixel intensity (e.g., even intensity of SC-35 domains across nuclear ROI).
Energy	A measure of image uniformity (opposite of entropy); low value when the window is not orderly (e.g., uneven distribution of SC-35 domains across nuclear ROI).
Sum Entropy	Measure of homogeneity of histogram of gray level similarities; low for uniform histograms.
Difference Entropy	Measure of homogeneity of histogram of gray level differences; high for uniform histograms.
Correlation	Image linearity; high value of Correlation if an image contains a considerable amount of linear structure (e.g., elongated SC-35 domains). Low value if intensity within ROI is perfectly uniform.
Information Measure 1	Information measure of the correlation (i.e., numerical uncertainty in correlation measure).
Information Measure 2	Information measure of the correlation (i.e., numerical uncertainty in correlation measure).
Sum Average AKA Granularity	Measure of grain size; high values indicate coarse texture having a grain size equal to or larger than the magnitude of the displacement vector (e.g., large versus small SC-35 domains).
Sum Variance	Standard deviation of Sum Average (e.g., uniform versus broad distribution in size of SC-35).
Difference Average	Measure of grain size; small values indicate coarse texture having a grain size equal to or larger than the magnitude of the displacement vector.
Difference Variance	The dispersion around the mean of combinations of reference and neighbor pixels in the summation histogram of the GLCM.
Inertia	Absolute value of the weighted averages away from the diagonal; Inertia exponentially increases in value according to homogeneity in protein intensity within ROI.
Inverse Difference Moment	Inverse of inertia. Inverse Difference Moment exponentially decreases in value according to homogeneity in protein intensity within ROI.

Table S2. Summary of most significant Haralick features across different growth factor conditions (i.e., adipogenic, osteogenic induction media, and varying levels of dexamethasone). The features are divided into four groupings: entropy (red), correlation (blue), granularity (purple), and inertia (green), which represent a measure of speckle homogeneity, elongation, size, and distribution with respect to nuclear centroid, respectively.

Granularity + Entropy

Media: BA vs. AD vs. OS

Information Gain	Attribute
1.3308	C1 Inverse Difference Moment
1.0881	C2 Sum Average
1.0881	C1 Sum Average
0.834	C1 Entropy
0.834	C2 Entropy
0.834	C2 Sum Entropy
0.7809	C2 Difference Entropy
0.7809	C2 Information Measure 2
0.7266	C1 Sum Entropy
0.721	C2 Information Measure 1
0.7059	C2 Difference Average

Granularity + Entropy

Media: BA vs. dex low vs. dex med vs. dex high

Information Gain	Attribute
1.0761	C1 Difference Average
1.0608	C2 Energy
1.0274	C2 Difference Average
1.026	C2 Difference Variance
0.9619	C2 Inverse Difference Moment
0.9515	C1 Difference Entropy
0.9474	C1 Difference Variance
0.9351	C2 Information Measure 1
0.9314	C1 Sum Variance
0.9105	C2 Sum Variance
0.8366	C1 Information Measure 1

Table S3. Summary of most significant Haralick features across different topographies (i.e., surface texture, fibrous scaffolds, topographical micropillars). The features are divided into four groupings: entropy (red), correlation (blue), granularity (purple), and inertia (green), which represent a measure of speckle homogeneity, elongation, size, and distribution with respect to nuclear centroid, respectively.

Correlation + Granularity + Inertia

Topography: flat vs. cont. vs. discontin.

Information Gain	Haralick Descriptor
0.458	C2 Correlation
0.422	C1 Correlation
0.332	C1 Inverse Difference Moment
0.312	C2 Sum Average
0.312	C1 Sum Average
0.302	C1 Inertia
0.285	C2 Inertia
0.271	C2 Energy
0.22	C1 Difference Variance
0.212	C1 Sum Variance
0.209	C1 Difference Average

Entropy + Correlation

Topography: small ali/rdm vs. large ali/rdm

Information Gain	Haralick Descriptor
0.794	C2 Energy
0.793	C1 Entropy
0.71	C1 Sum Entropy
0.649	C1 Information Measure 2
0.588	C1 Difference Entropy
0.574	C2 Entropy
0.486	C2 Difference Entropy
0.469	C2 Sum Entropy
0.459	C2 Information Measure 2
0.42	C2 Information Measure 1
0.377	C1 Information Measure 1

Entropy + Granularity

Topography: topographical micropillars

Information Gain	Haralick Descriptor
0.309	C1 Mean Energy
0.265	C1 Mean Sum Variance
0.245	C2 Mean Sum Variance
0.236	C1 Mean Difference Variance
0.225	C1 Mean Entropy
0.219	C2 Mean Difference Variance
0.205	C1 Mean Difference Average
0.189	C1 Mean Information Measure 1
0.185	C2 Mean Energy
0.175	C1 Mean Information Measure 2
0.147	C2 Mean Difference Average

Table S4. Summary of most significant textural attributes defining SC-35 organizational metrics modulated by different signaling molecules (RhoGTPase, FAK, Wnt/ β -catenin). The features are divided into four groupings: entropy (red), correlation (blue), granularity (purple), and inertia (green), which represent a measure of speckle homogeneity, elongation, size, and distribution with respect to nuclear centroid, respectively.

Entropy + Correlation

RhoGTPase inhibition versus no treatment

Information gain	Textural attribute
0.591	C2 Energy
0.366	C1 Entropy
0.222	C1 Sum Entropy
0.216	C1 Energy
0.203	C1 Information Measure 2
0.155	C1 Difference Entropy
0.137	C2 Entropy
0.121	C1 Sum Variance
0.117	C2 Inverse Difference Moment
0.117	C2 Correlation

Entropy + Correlation

Wnt/ β -catenin inhibition versus no treatment

Information gain	Textural attribute
0.763	C2 Energy
0.733	C2 Information Measure 2
0.728	C2 Difference Entropy
0.727	C2 Information Measure 1
0.724	C1 Information Measure 1
0.719	C2 Sum Entropy
0.719	C2 Entropy
0.709	C1 Difference Entropy
0.688	C2 Difference Average
0.681	C1 Information Measure 2

Entropy + Inertia

FAK inhibition versus no treatment

Information gain	Textural attribute
0.234	C1 Inverse Difference Moment
0.232	C1 Entropy
0.158	C2 Energy
0.126	C1 Sum Entropy
0.114	C2 Entropy
0.112	C1 Information Measure 2