Nanoscale Molecular Quantification of Stem Cell-Hydrogel Interactions

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Supplementary Information

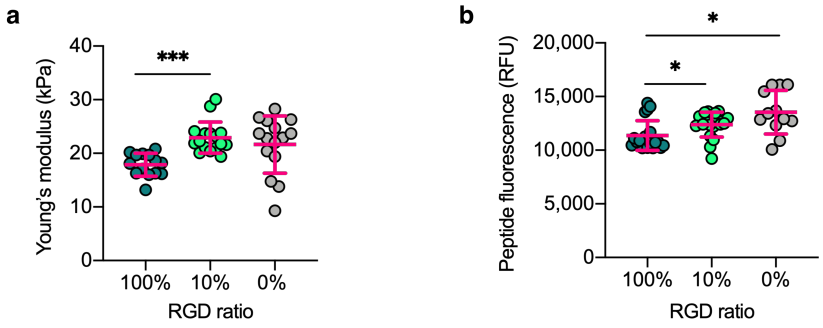

Figure S1. Characterization of RGD peptide-functionalized hydrogels. (a) Young's modulus of hydrogels was measured using AFM. N = 14-16 gels for each condition. Mean ± SD for each condition are 18 ± 2 kPa, 23 ± 3 kPa, and 22 ± 5 kPa, for 100%, 10%, and 0% RGD, respectively. (b) Fluoraldehyde assay of total peptide content in different hydrogel conditions. N = 4 measurements each from 4-5 gels per condition. Brown-Forsythe and Welch unequal variances unpaired one-way ANOVA, Dunnett's T3 multiple comparison test. Dot plots represent mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

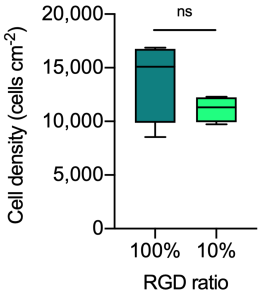

Figure S2. Analysis of number of cells bound to hydrogels. Total number of nuclei per cm^2, as measured from images taken at 10x magnification within a 1.2 x 1.2 mm region of hydrogel. N = 4 images per condition. Parametric unpaired two-tailed t-test. Box plots represent median ± IQR, whiskers represent minimum and maximum. ns = not significant.

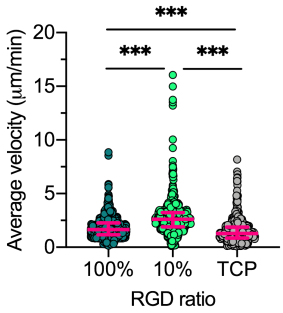

Figure S3. Average migration velocity of hMSCs. Average velocity of hMSC tracks on hydrogels of 100% and 10% RGD concentration, compared to TCP controls. N = 613-1203 tracks per condition. Non-parametric Kruskal-Wallis ANOVA with Dunn's multiple comparison test. Dot plots represent median ± IQR. ***$p < 0.001$.

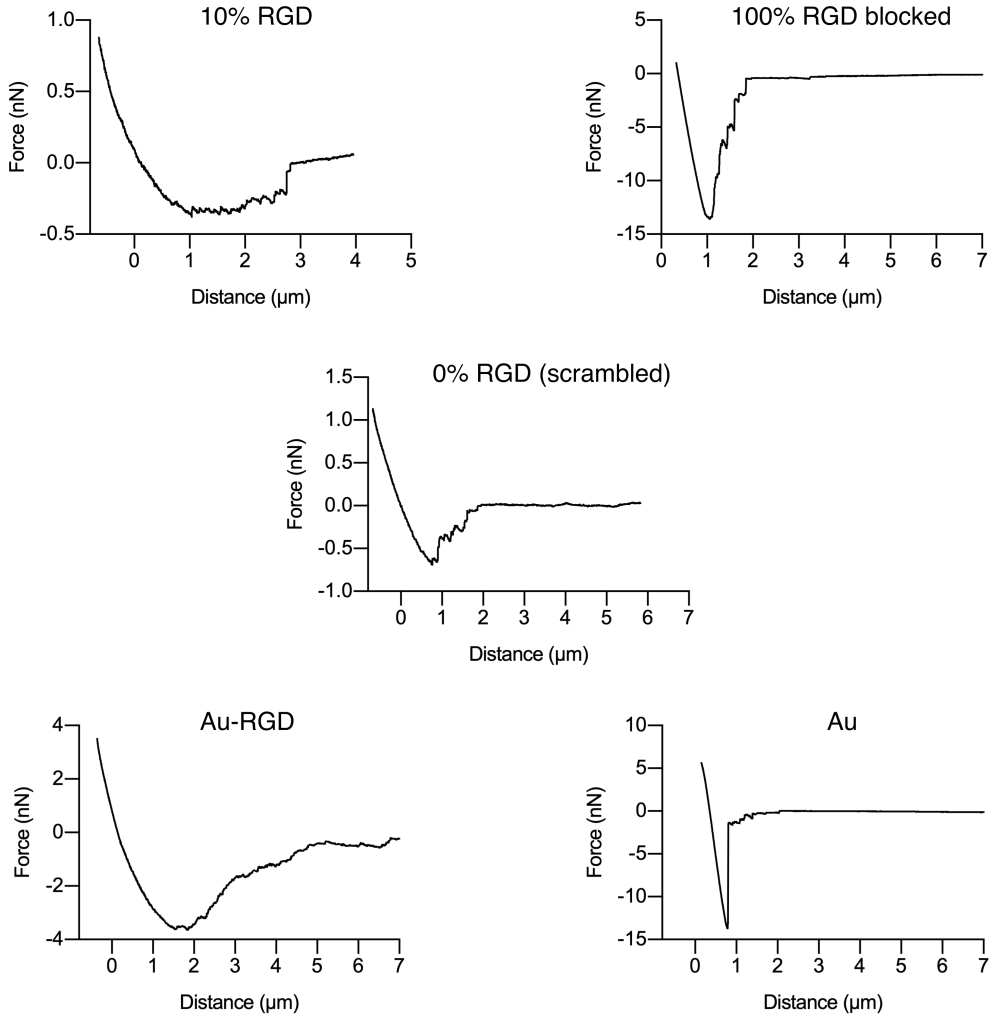

Figure S4. AFM force curve controls. Example force-distance curves of hMSC unbinding from 10% RGD hydrogel, 100% RGD hydrogel following blocking of the hMSC in RGD, 0% RGD (scrambled) hydrogel, RGD functionalized gold surface (Au-RGD), and unfunctionalized gold surface (Au).

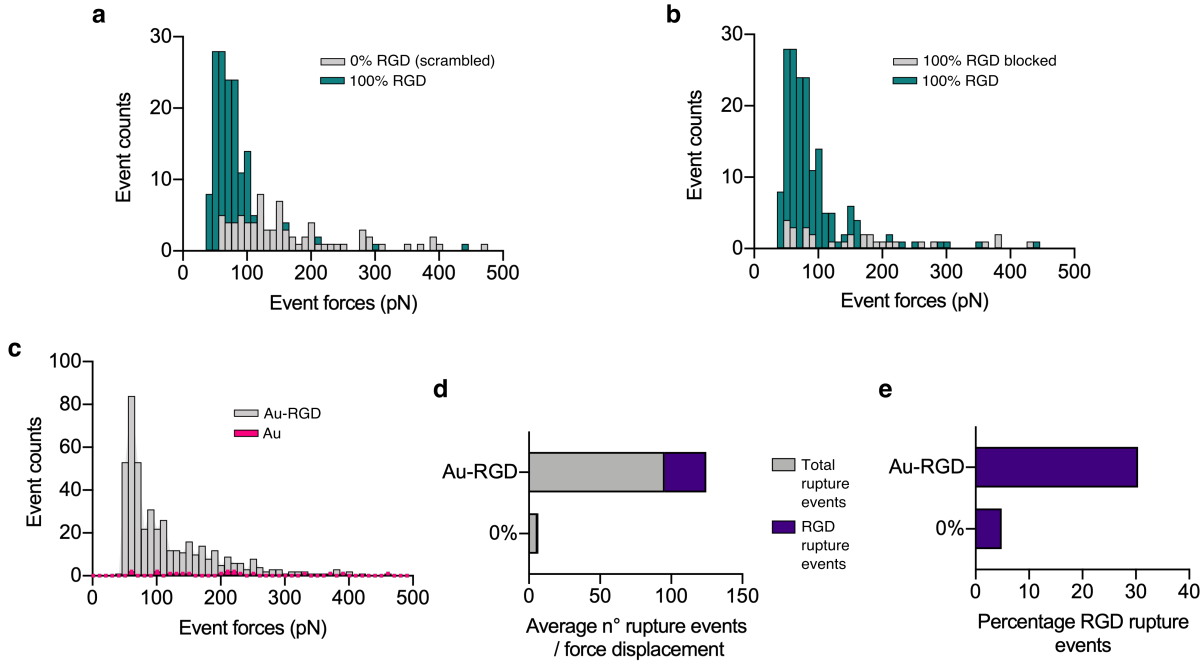

Figure S5. AFM event forces for controls. Comparison of total event forces when hMSCs were interfaced with (a) 100% RGD hydrogel and 0% RGD (scrambled) hydrogel, (b) 100% RGD hydrogel and 100% RGD hydrogel following RGD blocking of hMSC, (c) Au and RGD-functionalized Au. (d) Average total and RGD-specific rupture events on 0% RGD hydrogel and RGD functionalized Au substrates. (e) Percentage of RGD rupture events per force displacement curve on 0% RGD hydrogel and RGD functionalized Au substrates. N = 3, n = 5 per condition.

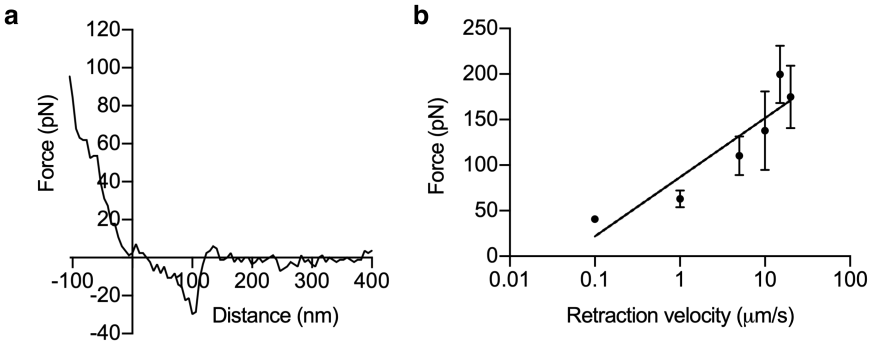

Figure S6. AFM loading rate. (a) Representative force-distance curve showing a single molecule unbinding event, between hMSC and 100% RGD hydrogel with a retraction velocity of 0.1 μm/s. (b) Dependence of the average rupture force measured for single molecule unbinding events on the 100% RGD hydrogel with loading rate. N = 3, n = 40 per retraction velocity condition.

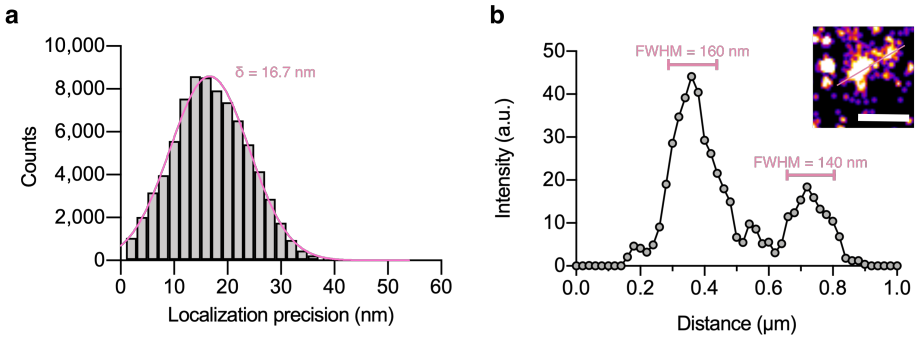

Figure S7. dSTORM localization precision. (a) Localization precision of single AlexaFluor647 detections was measured, median = 16.7 nm. (b) Intensity profile of two clusters (line profile inset) gives a full width half maximum (FWHM) of 140-160 nm. Scale bar = 500 nm.

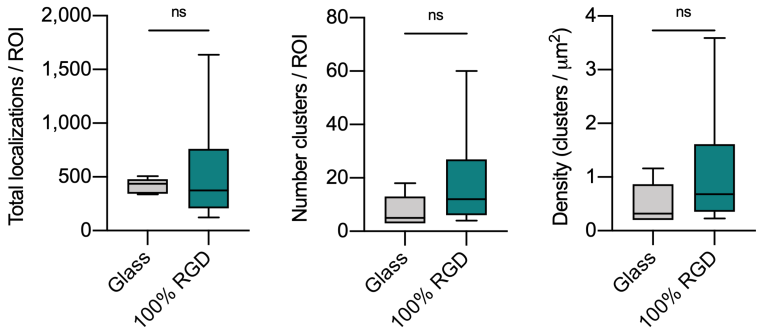

Figure S8. dSTORM cluster analysis for control. Analysis of total number of surface localizations, number of clusters and density of clusters of integrin α5β1 per ROI for hMSCs in contact with untreated glass control and 100% RGD hydrogels. N = 5-15. Welch's unequal variances unpaired two-tailed t-test. Box plots represent median ± IQR. ns = not significant.