Supplemental Information

Mesenchymal Stem Cells Exploit Extracellular Matrix as Mechanotransducer

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Inventory of Supplemental Information

Supplemental Figures

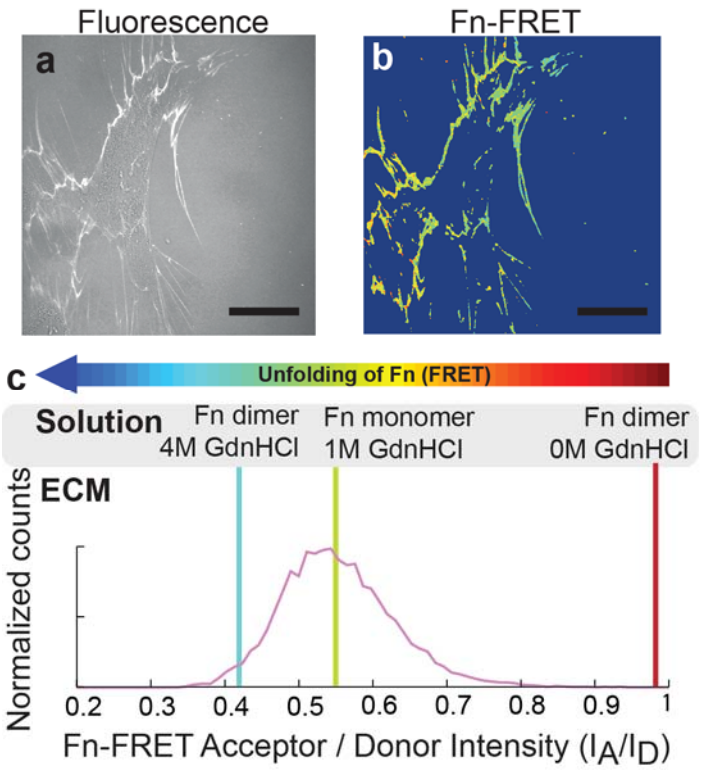

Figure S1: Undifferentiated hMSCs initially harvest plasma Fn and assemble it into early ECM already within the first 24 hours.

(a) Merged images of hMSC-assembled Fn ECM (fluorescence image) with brightfield images of hMSCs cultured for 24 hours on glass coverslips in growth medium supplemented with Fn-FRET at low (1×10^3 cells/cm^2) seeding densities.

(b) FRET false colors of hMSC-assembled Fn ECM at low (1×10^3 cells/cm^2) seeding densities. The FRET false color scheme represents the relative stretching of Fn fibrils with a color range of red to blue indicating folded to completely unfolded states of Fn, respectively.

(c) Histograms of Fn-FRET I_A/I_D ratios of hMSC-assembled Fn ECM in 24 hour cultures.

Scale bars: 50µm.

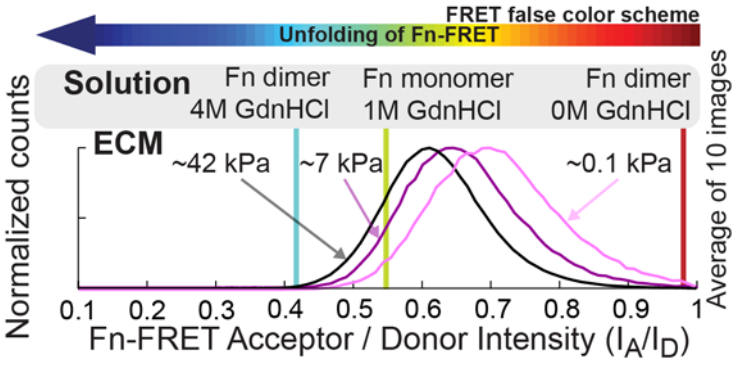

Figure S2: Stretching of Fn matrix increases with higher substrate rigidity. Related to Figure 2.

hMSCs were cultured in mixed induction medium supplemented with Fn-FRET and excess unlabeled Fn at an initial seeding density of $3\times10^3/cm^2$ on Fn-functionalized polyacrylamide substrates of high (~42kPa, black curve), medium (~7kPa, purple curve) and low (~0.1kPa, pink curve) rigidity. Confocal microscopic images of donor and acceptor peak intensities and differential interference contrast images (DIC) were taken 2μm above the glass-cell interface, followed by image analysis using MATLAB. Histograms of average I_A/I_D ratios of Fn matrices (average of 10 images of each rigidity) are shown. Solution denaturation values for dimeric Fn-FRET in 0M GdnHCl, monomeric Fn-FRET in 1M and dimeric Fn-FRET 4M GdnHCl are shown as red, green and blue lines respectively.

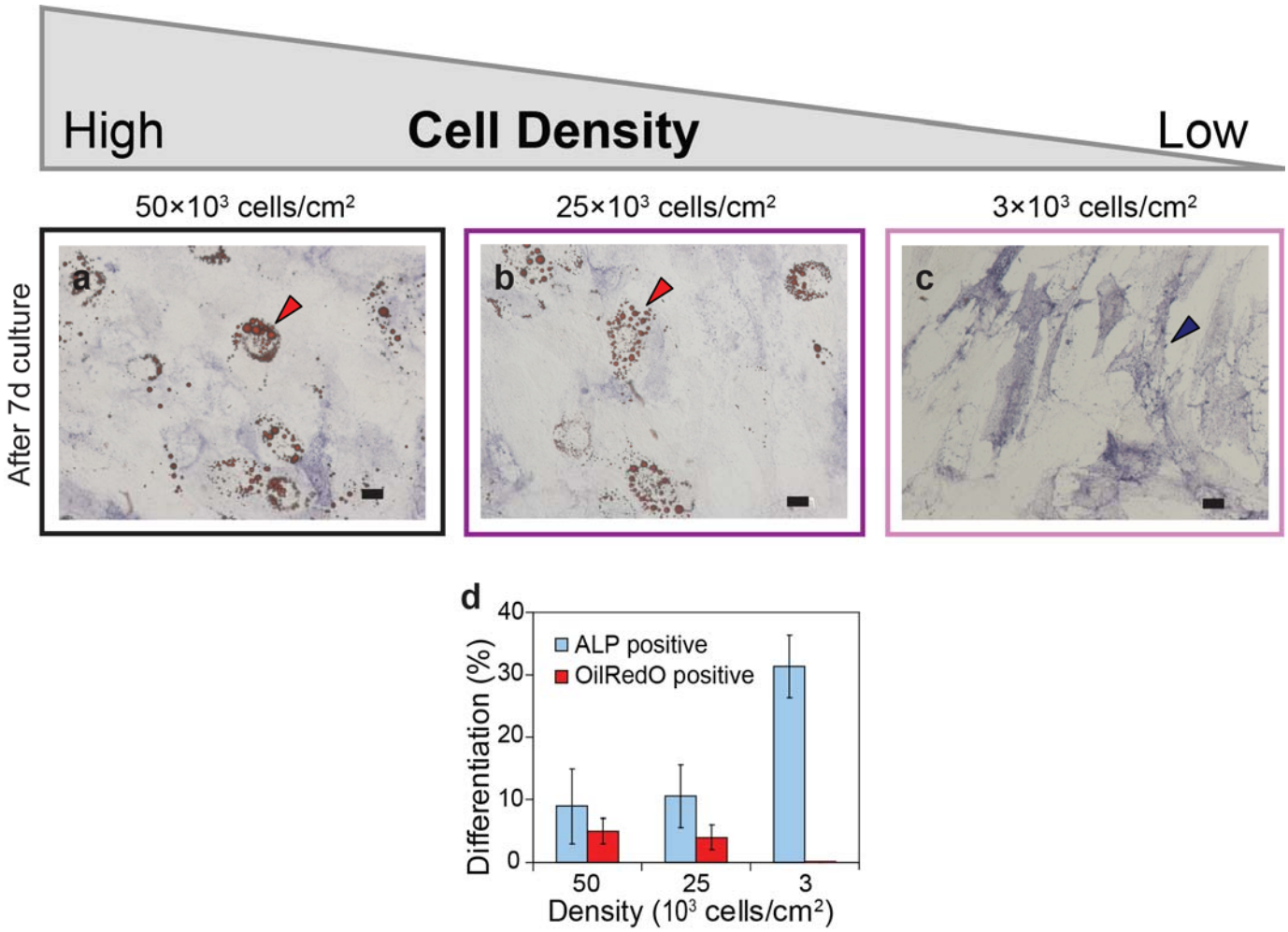

Figure S3: Higher cell density decreases osteogenesis and increases adipogenesis of hMSCs in 2D.

(a-c) Brightfield images of hMSCs cultured at seeding densities of 50×10^3 (a), 25×10^3 (b) or 3×10^3 cells/cm^2 (c) for 7 days on glass coverslips in mixed induction medium supplemented with Fn-FRET (5 µg/ml Fn-FRET, 45 µg/ml unlabeled Fn), with histochemical staining for ALP (blue, blue arrows) and OilRedO (red, red arrows). Scale bars: 50µm.

(d) Percentage of OilRedO and ALP positive hMSCs. OilRedO positive cells were mostly suppressed at a seeding density of 3×10^3 cells/cm^2. Results are shown as the mean ± s.d. (n=3).

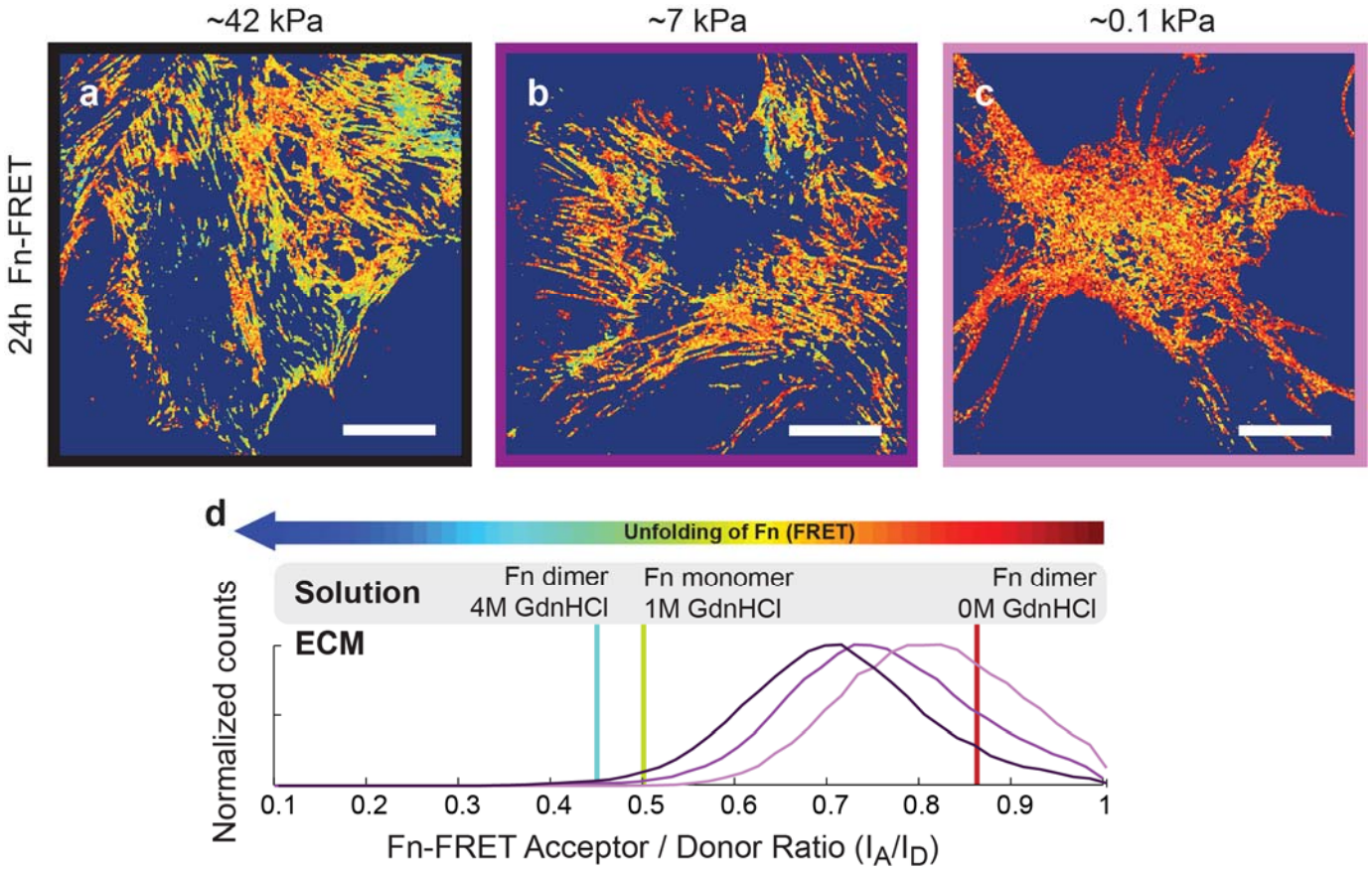

Figure S4: Stretching of early Fn matrix fibrils correlates with the rigidity of collagen I-functionalized polyacrylamide gels.

hMSCs were cultured in mixed induction medium supplemented with Fn-FRET and excess unlabeled Fn at an initial seeding density of $3\times10^3/cm^2$ on collagen I-functionalized polyacrylamide substrates of high (a, black curve), medium (b, purple curve) and low (c, pink curve) rigidity. Histograms of I_A/I_D ratios of Fn matrices are shown in d. Solution denaturation values for dimeric Fn-FRET in 0M GdnHCl, monomeric Fn-FRET in 1M and dimeric Fn-FRET 4M GdnHCl are shown as red, green and blue lines respectively.

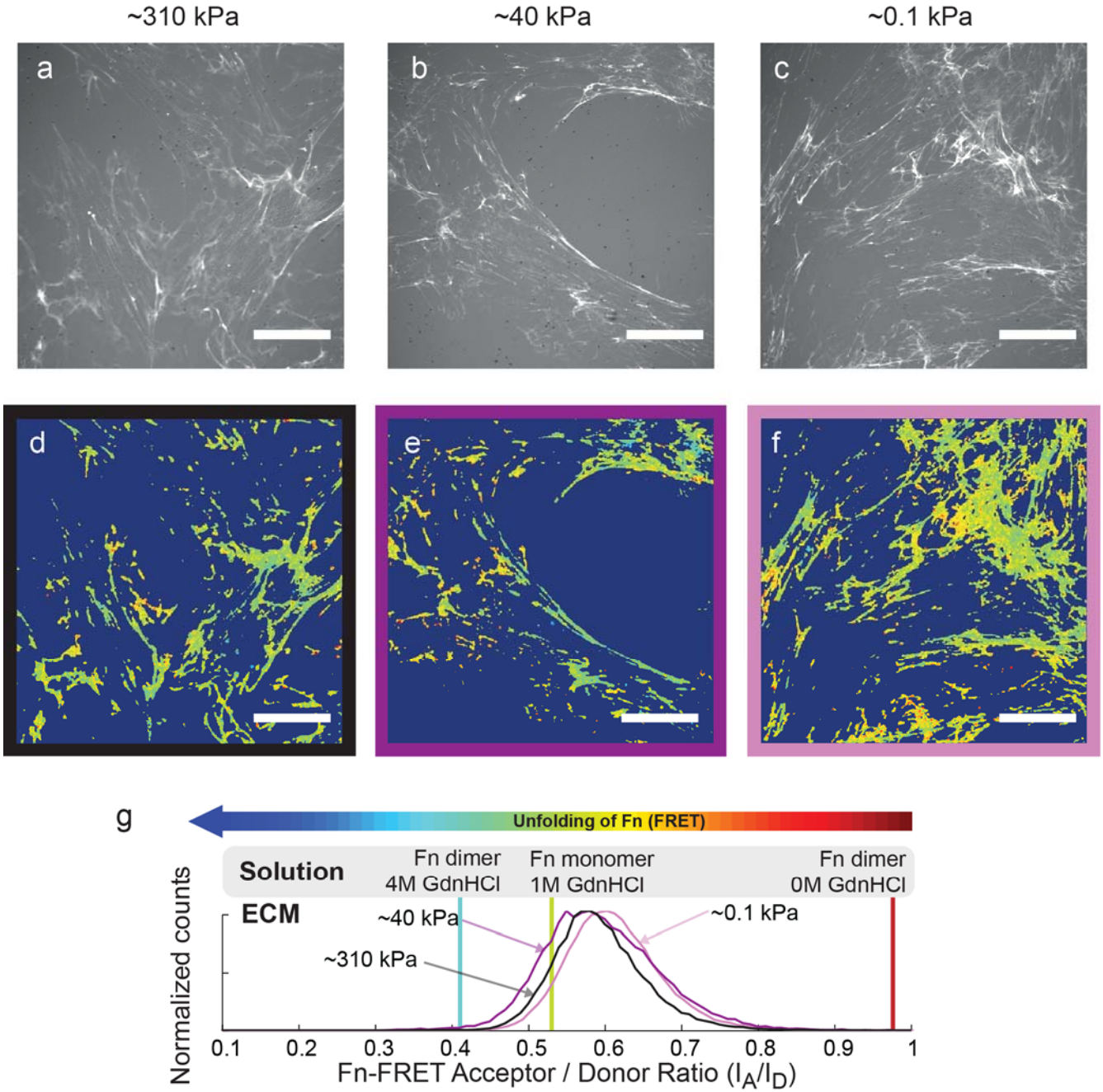

Figure S5: hMSCs cultured on PDMS substrates of varying rigidity which are covalently crosslinked to Fn as previously described [1].

(a-c) Merged images of hMSC-assembled Fn matrices (fluorescence image) with brightfield images of hMSCs cultured on rigid (a, ~310 kPa), medium (b, ~40 kPa) or soft (c, ~0.1 kPa) Fn-functionalized PDMS substrates at a seeding density of 3×10^3 cells/cm^2 for 24 hours in growth medium supplemented with trace amounts of Fn-FRET.

(d-f) FRET false colors of hMSC assembled Fn matrices on rigid (d), medium (e) or soft (f) Fn-functionalized PDMS substrates. Scale bars: 50µm.

(g) Histograms of Fn-FRET I_A/I_D ratios of hMSC assembled 24 hour Fn matrices on rigid (black curve), medium (purple curve) or soft (pink curve) Fn-functionalized PDMS substrates. Solution denaturation values for dimeric Fn-FRET in 0M GdnHCl, monomeric Fn-FRET in 1M and dimeric Fn-FRET 4M GdnHCl are shown as red, green and blue lines respectively.

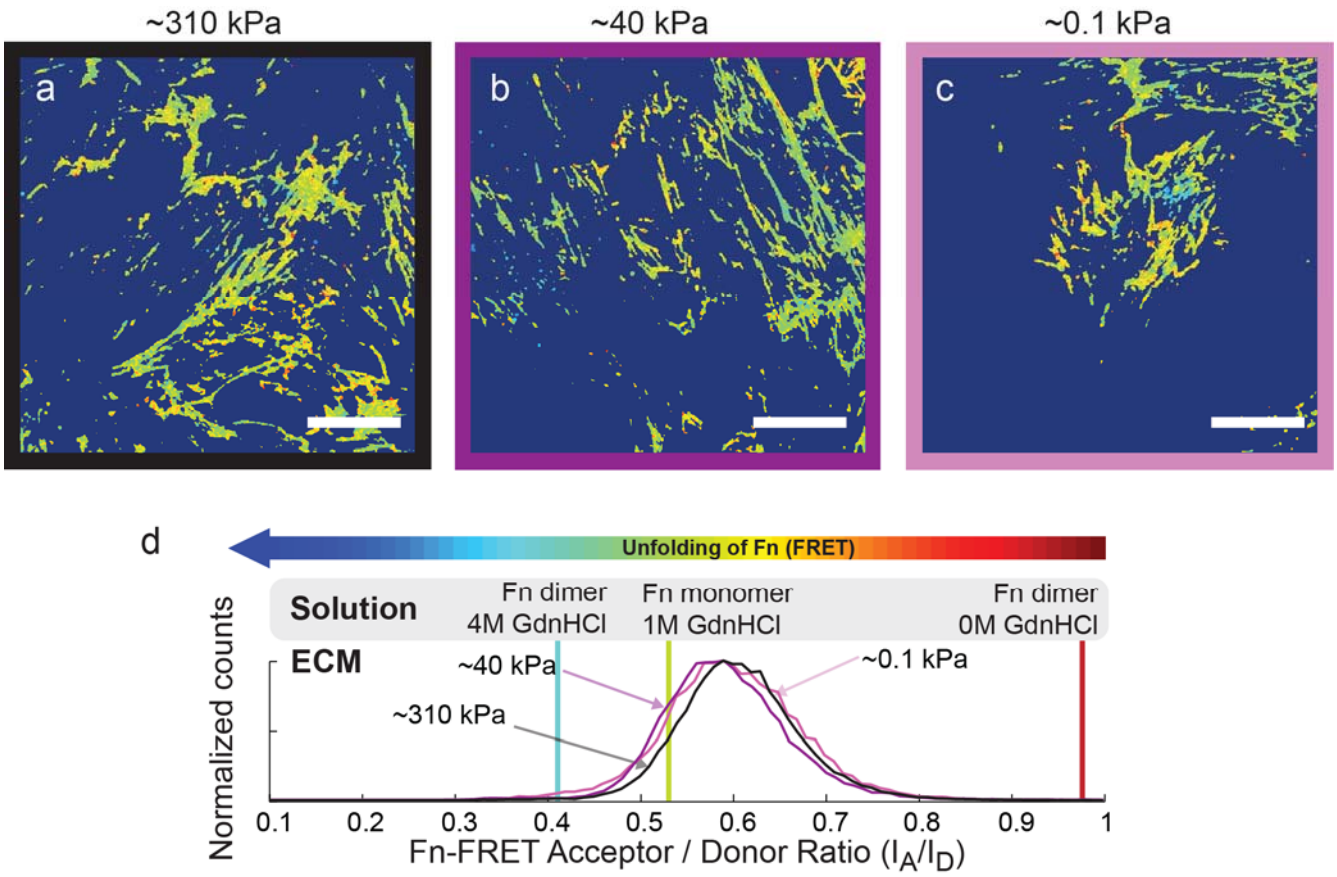

Figure S6: hMSCs cultured on PDMS substrates of varying rigidity which are crosslinked to collagen I.

(a-c) Fn-FRET false colors of hMSC assembled Fn matrices on rigid (a), medium (b) or soft (c) Collagen I-functionalized PDMS substrates. Scale bars: 50µm.

(d) Histograms of Fn-FRET I_A/I_D ratios of hMSC assembled 24 hour Fn matrices on rigid (black curve), medium (purple curve) or soft (pink curve) Collagen I-functionalized PDMS substrates. Solution denaturation values for dimeric Fn-FRET in 0M GdnHCl, monomeric Fn-FRET in 1M and dimeric Fn-FRET 4M GdnHCl are shown as red, green and blue lines respectively.

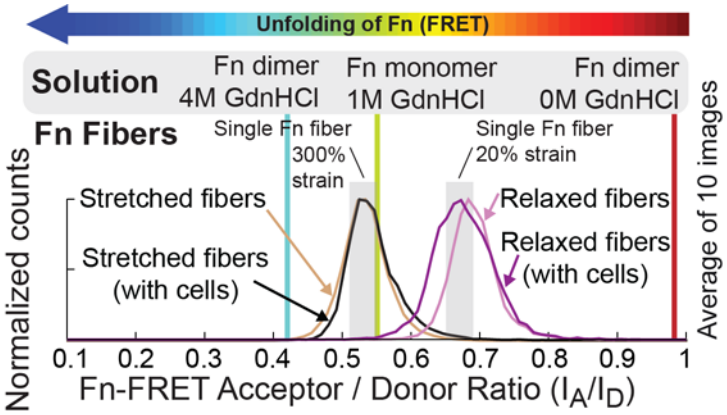

Figure S7: Histograms of the average Fn-FRET I_A/I_D ratios of 10 relaxed fibers before (pink curve) and after cell were cultured for 2 days on these fibers (purple curve), and 10 stretched fibers before (brown curve) and after (black curve). Fiber strains corresponding to 20% and 300% strain are shown as gray bars. Solution denaturation values for dimeric Fn-FRET in 0M, monomeric Fn-FRET in 1M and dimeric Fn-RET in 4M GdnHCl are shown as red, green and blue vertical lines respectively.

Supplemental References

1. Trappmann, B. *et al.* Extracellular-matrix tethering regulates stem-cell fate. *Nat. Mater.* **11**, 642-649 (2012).