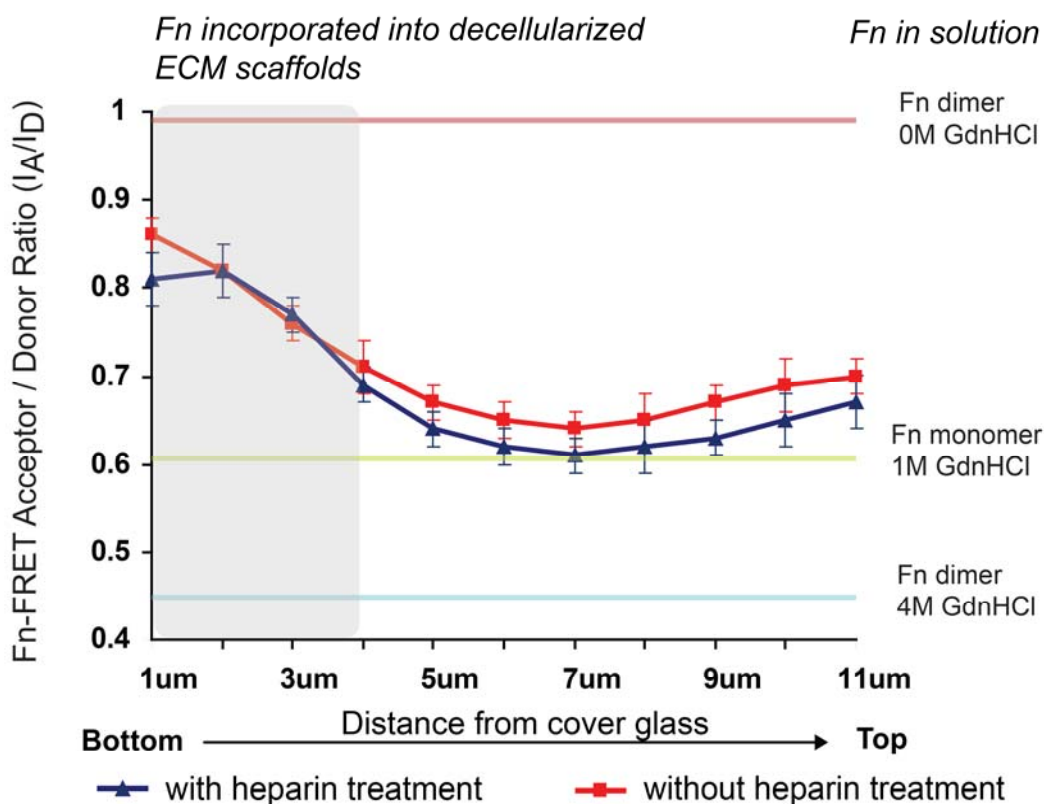


## Heparin-induced conformational changes of fibronectin within the extracellular matrix promote hMSC osteogenic differentiation

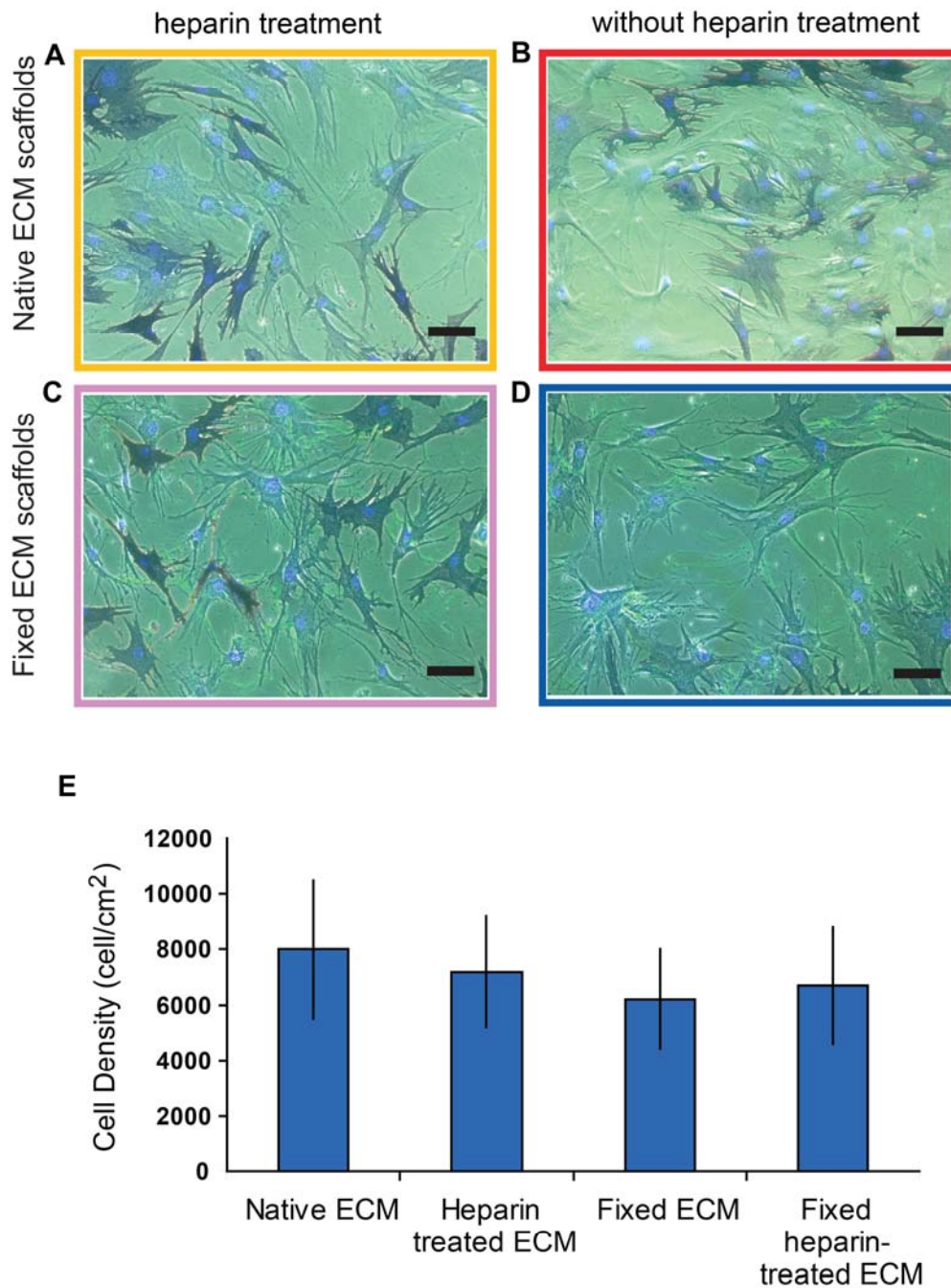
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### Inventory of Supplemental Information

#### Supplemental Figures



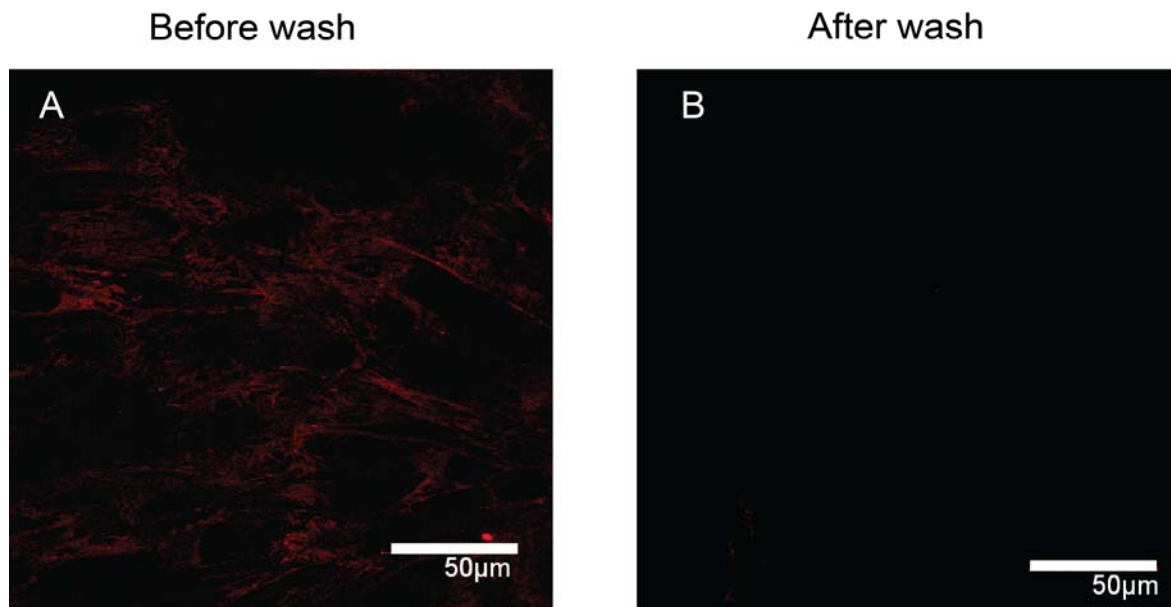
**Figure S1: Heparin treatment decreases FRET ratios of Fn within 3D ECM scaffolds throughout the Z-volume images.** Fn-FRET was incorporated into 3D ECM as described. Confocal Z-stack with a step size of 1µm were acquired for both donor (Alexa 488) and acceptor (Alexa 546) channels and the corresponding FRET  $I_A/I_D$  ratios were calculated for each Z-slice. In the graph, average  $I_A/I_D$  ratios (average of 10 images) are plotted as a function of the distance from the coverglass for 3D ECM scaffolds with (blue) or without (red) heparin treatment. The results show slightly lower FRET values for the heparin-treated scaffolds throughout the Z-volume images, except at the distances less than 3 µm from the coverglass. It is possible that the FRET ratios at distances close to the glass surface may be affected by the autofluorescence of glutaraldehyde that has been used to functionalize the glass for the production of the scaffolds. The FRET values for Fn in solution, native or GdnHCl denatured, are indicated by the horizontal lines.



**Figure S2: Cell densities on different ECM scaffolds are similar after 7 days in cell culture.**

(A, B, C and D) Merged brightfield and fluorescence images of hMSCs cultured for 7 days in mixed induction medium on heparin-treated (A), native (B), fixed heparin-treated (C) and fixed native ECM scaffolds (D). Decellularized ECM scaffolds were labeled with Fn-FRET (green); cell nuclei were stained with DAPI (blue dot) and histochemical staining was performed for ALP (dark blue). Scale bars: 100 μm.

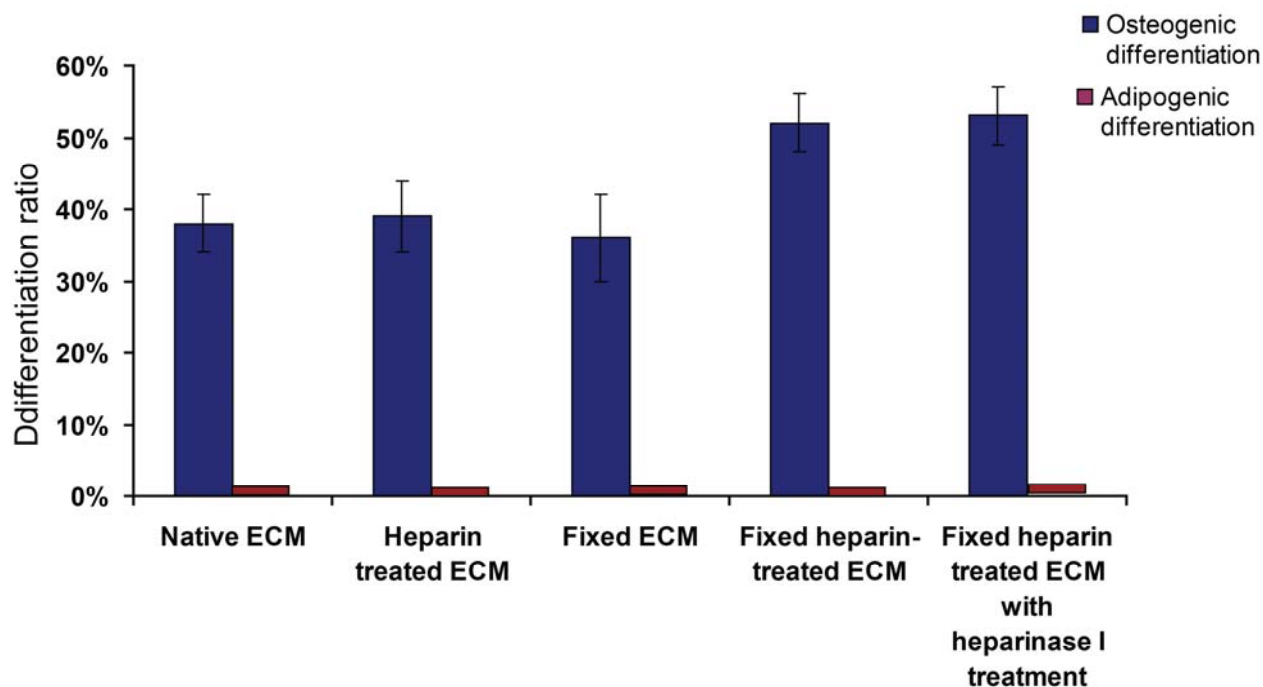
(E) Cell densities on heparin-treated or native ECM with or without fixation. Data are shown for cell densities and represent a mean ± s.d. (n=3).



**Figure S3: Heparin does not remain bound to ECM scaffolds after washing with PBS.**

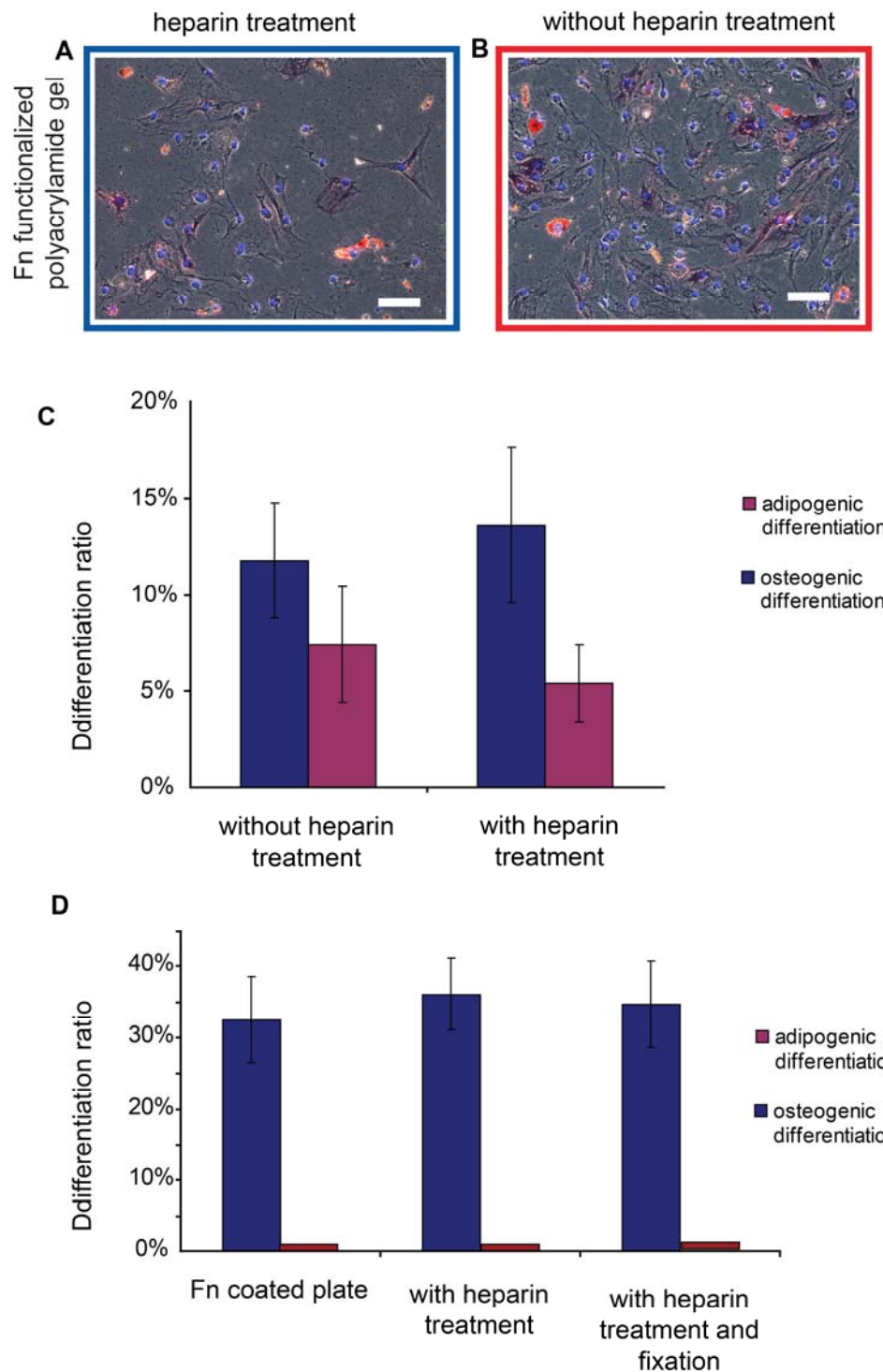
Alexa 633 labeled heparin was used to treat ECM scaffolds for 12 h at 4 °C. Following the incubation, the solution was removed and the scaffolds were imaged by confocal fluorescence microscope (A). Subsequently, the scaffolds were washed 3 times with PBS and imaged again (B). The heparin that remains associated with the ECM scaffolds is removed by washing with a buffer of physiological ionic strength (PBS) as indicated by the loss of fluorescence.

Scale bars: 50µm.



**Figure S4: Removal of remaining heparin from fixed heparin-treated ECM by active degradation with heparinase I did not significantly impact hMSC osteogenic differentiation.**

hMSCs were cultured for 7 days in mixed induction medium on native ECM, heparin treated ECM, fixed native ECM, fixed heparin treated ECM and fixed heparin treated ECM that was treated with heparinase I. Percentage of OilRedO and ALP positive hMSCs were shown as the mean  $\pm$  s.d. (n=3).

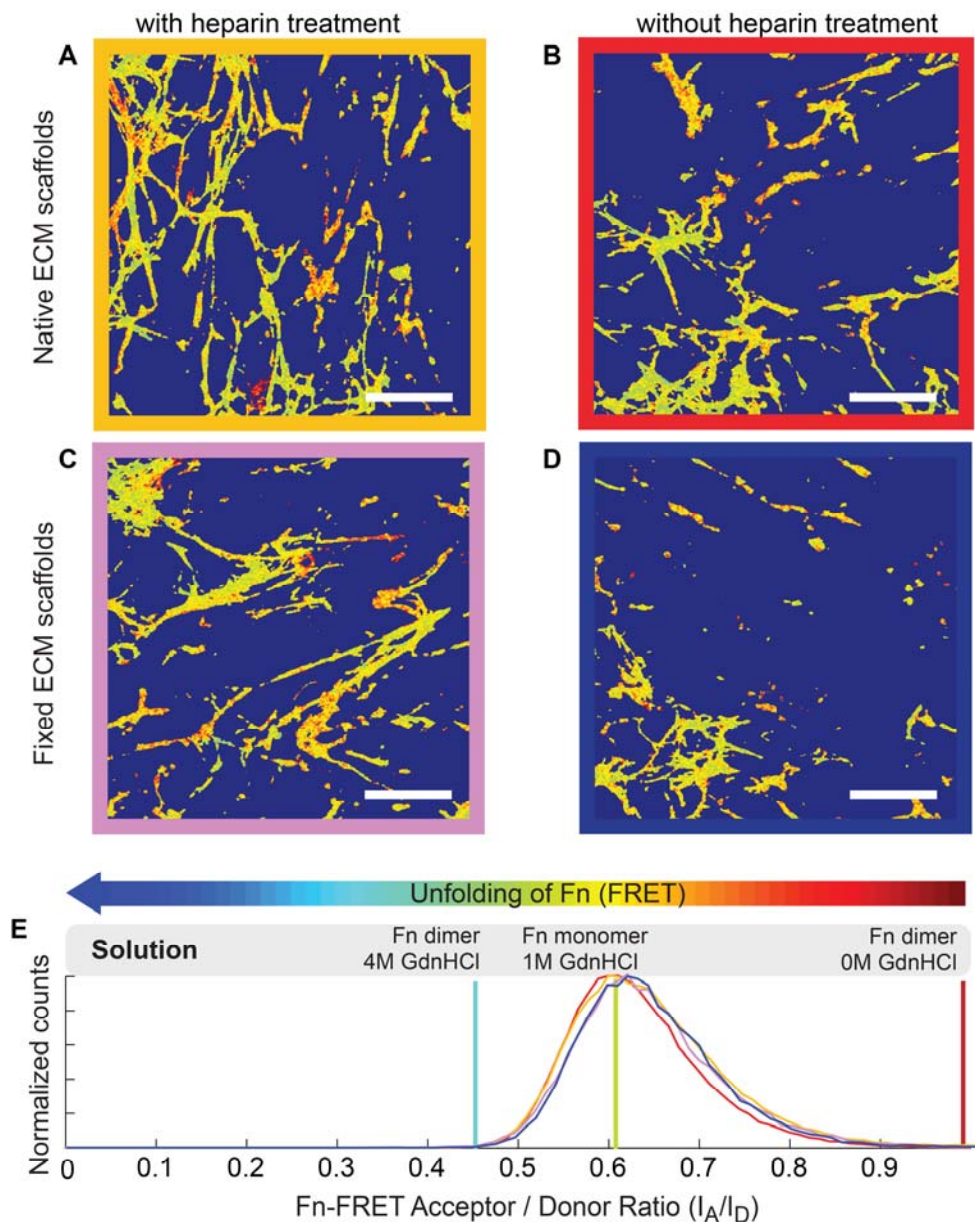


**Figure S5: Heparin treatment of either Fn functionalized polyacrylamide gels or Fn coated glass coverslips had no effect on hMSC differentiation.**

(A, B) Brightfield images of hMSCs cultured on soft (0.1kPa) Fn-Functionalized polyacrylamide gels with (A) or without (B) heparin treatment. hMSCs were seeded at a  $5 \times 10^3$  cells/cm<sup>2</sup> density and were cultured for 7 days in mixed induction medium, with histochemical staining for ALP (blue) and OilRedO (red). Scale bars: 50 $\mu$ m

(C) Percentage of OilRedO and ALP positive hMSCs on Fn functionalized soft polyacrylamide gels with or without heparin treatment.

(D) Percentage of OilRedO and ALP positive hMSCs on Fn coated (not crosslinked) glass with or without heparin treatment. Results are shown as the mean  $\pm$  s.d. (n=3).



**Figure S6: The Fn conformations in ECM newly assembled by hMSCs are similar.**

It has been reported that hMSCs could assemble ECM within 24 hours after reseeding onto decellularized ECM scaffolds, and the Fn conformations in new assembled ECM could influence hMSC differentiation. In order to assess the effect of hMSC assembled ECM on hMSC differentiation, the Fn conformations in new assembled ECM were observed using FRET after 24 hours of reseeding of hMSCs ( $3 \times 10^3$  cells/cm<sup>2</sup>) on decellularized HFF derived ECM scaffolds.

(A, B, C and D) FRET false color images of hMSC assembled ECM on heparin-treated (A), native (B), fixed heparin-treated (C) or fixed native ECM scaffolds (D). The FRET false color scheme represents the relative conformations of Fn fibrils with a color range of red to blue indicating compact to completely unfolded states of Fn respectively. Scale bars: 50 μm.

(E) Histograms of Fn-FRET  $I_A/I_D$  ratios of hMSCs assembled Fn ECM following hMSCs attachment. hMSCs cultured on native (red curve, B), heparin-treated (yellow curve, A), fixed heparin-treated (purple curve, C) and fixed native ECM (blue curve, D). Solution denaturation values for dimeric Fn-FRET in 0M GdnHCl, monomeric Fn-FRET in 1M and dimeric Fn-FRET in 4M GdnHCl are shown as red, green and blue lines respectively.