Supplementary information

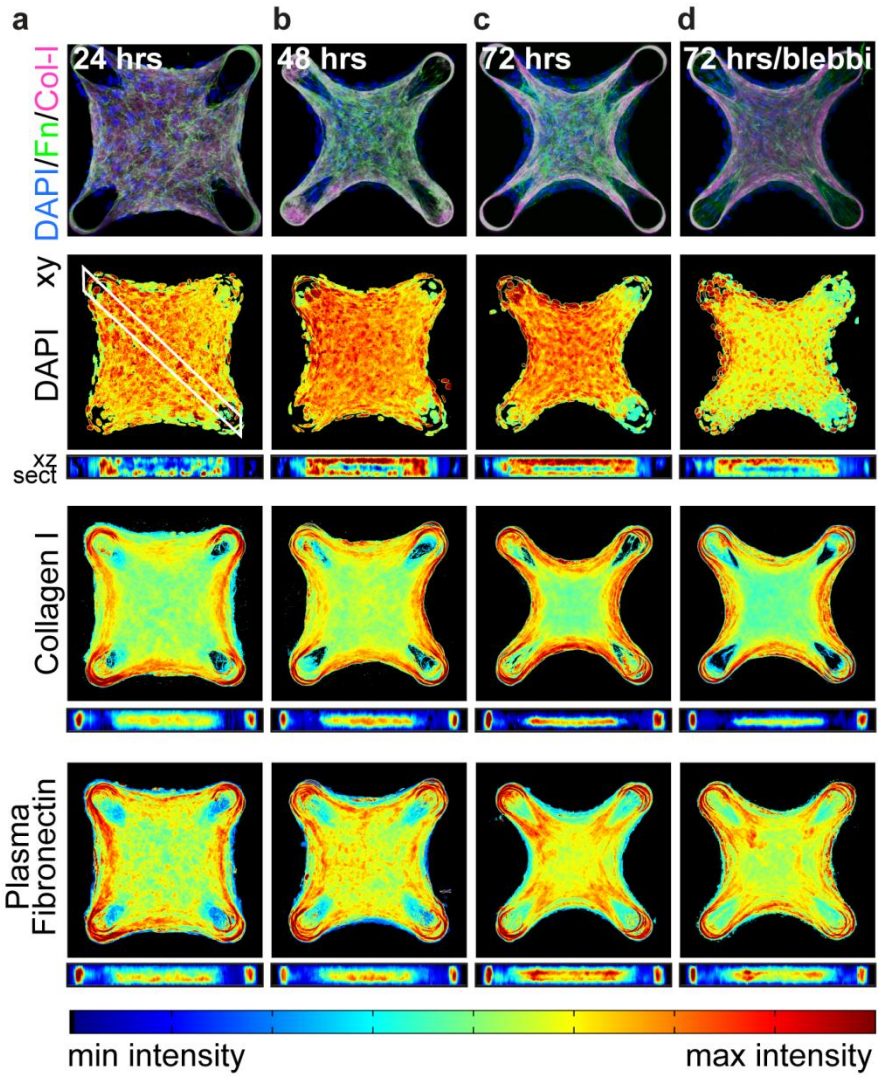

Figure S1: 3D averaged density maps of ECM protein in microtissues (normalized within each time point). **a-d)** Immunofluorescent images showing DAPI, collagen I and Fn within microtissues fixed after 24, 48 and 72 hours of remodeling, or after 72 hours of remodeling with acute (2 hours prior to fixing) incubation with 50 µM blebbistatin. Density maps represent the DNA or protein density at a given location averaged over the axis orthogonal to the image plane. In order to register images between tissues of different thickness, cross-sections (xz sect) are the plotted from the uppermost to the lower most surface of the tissues (ie normalized by tissue thickness). All density maps are the average of 10 individual microtissues from each condition. To better show the distributions of proteins within each condition, color scales are normalized to depict the min and max values within each time point. Thus, the intensities displayed at different time points for the same stain are not directly comparable.

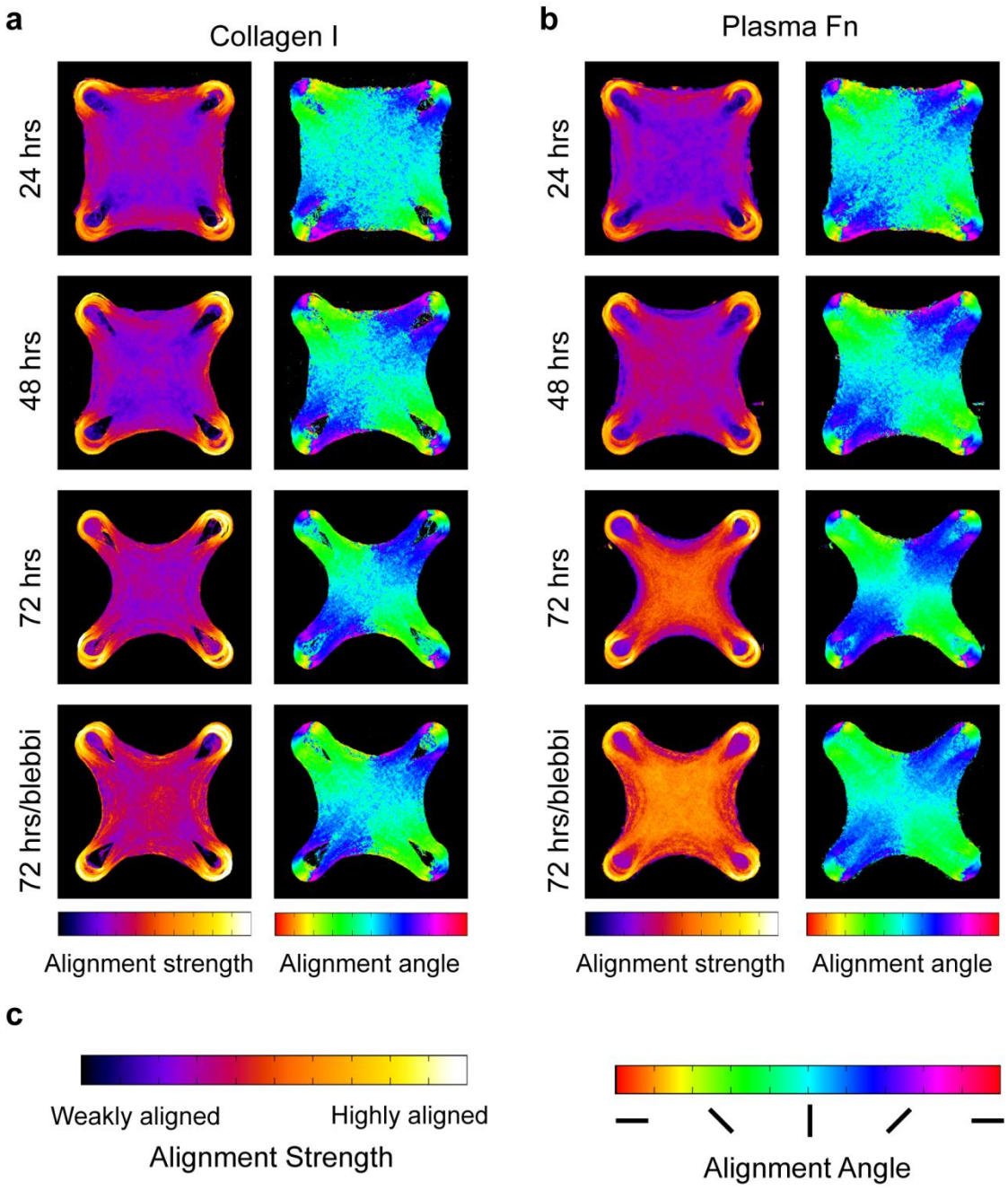

Figure S2: Average protein alignment maps. **a, b)** Heatmaps of alignment strength and angle of collagen I and Fn within microtissues fixed after 24, 48 and 72 hours of remodeling, or after 72 hours of remodeling with acute (2 hours prior to fixing) incubation with 50 μM blebbistatin. Using the confocal image data, principal component analysis (PCA) of the image gradient vector computes a new orthogonal basis such that the variance of the projection on one axis is maximal while the variance of the projection on the orthogonal axis is minimal. The principal eigenvector corresponds to the direction of fiber alignment. Alignment strength maps are computed from ratio of the minimal and maximal eigenvalues as described in Methods. High strength areas (str ~ 1) represent fibrillar regions with uniformly aligned fibers. Low strength areas (str ~ 0) represent either fibrillar regions with heterogeneous orientations, or non-fibrillar (diffuse) staining. Heatmaps of alignment angle represent the eigenvectors of the above decomposition, and correspond to the long axis of the protein fibrils. **c)** Detailed explanations for the color bars shown in (a and b).

Figure S3

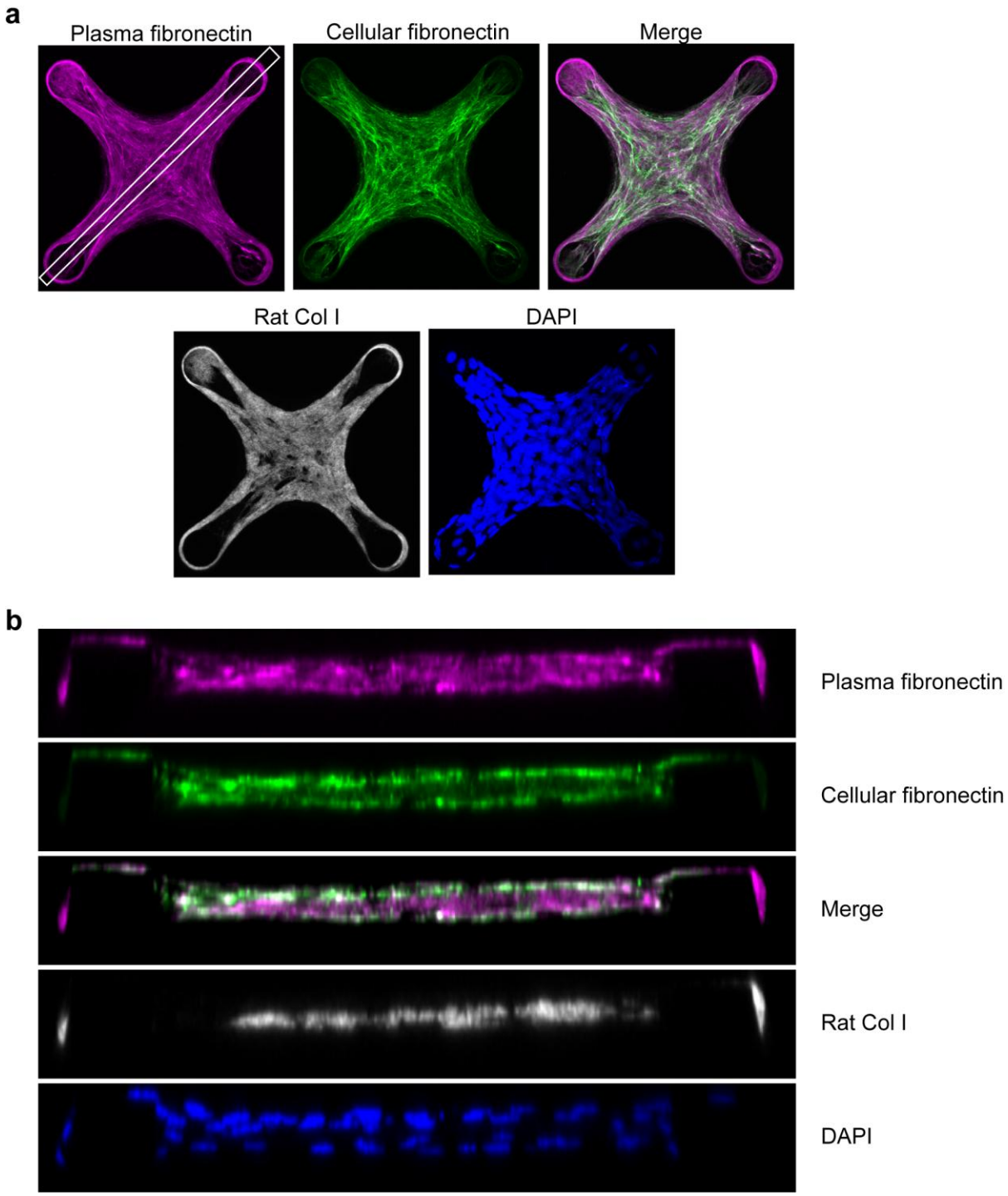

Figure S3: Localization and accumulation of cellular and plasma derived Fn. **a, b)** Maximum intensity projections for top down and cross-section (as outlined in the white box in (a) for microtissues fixed after 72 hours of culture. Microtissues were dual labeled with exogenous plasma Fn (pink), the EDA splice variant specific to cell-derived Fn as distinguished by a EDA-specific antibody (green), collagen I (white), and DAPI (blue). Cell-derived Fn accumulated at the periphery of the microtissue where it was highly colocalized with plasma Fn. In contrast, only plasma Fn was highly colocalized with the compacted collagen scaffold.

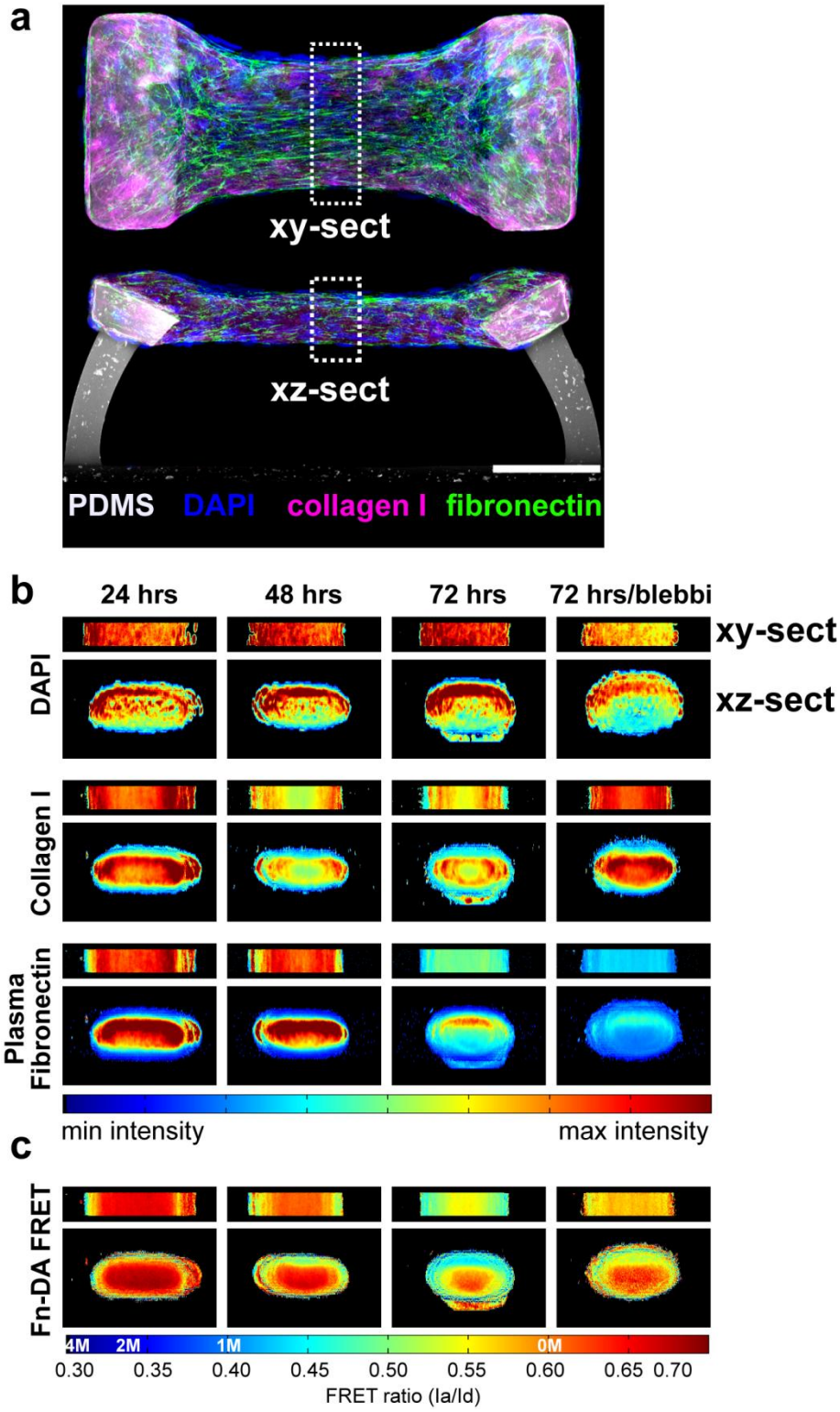

Figure S4: 3D averaged density maps of ECM protein in microtissues tethered to 2 flexible cantilevers. **a)** Representative top down and cross-section views for microtissues showing plasma-Fn (green), collagen (pink) and DAPI (blue) after 48 hours of remodeling. Dashed boxes indicate regions for calculation of xy and xz protein density maps and Fn-DA FRET maps. Scale bar = 100 µm. **b)** Protein density maps showing DAPI, collagen I and Fn within microtissues fixed after 24, 48 and 72 hours of remodeling, or after 72 hours of remodeling with acute (2 hours prior to fixing) incubation with 50 µM blebbistatin. Density maps represent the average protein density at a given location. All

density maps are the average of 25 individual microtissues from each condition. Color scales are normalized to depict the min and max values for each stain. Thus, the intensities measured at different time points for the same stain are comparable, but the intensities of different protein stains are not.

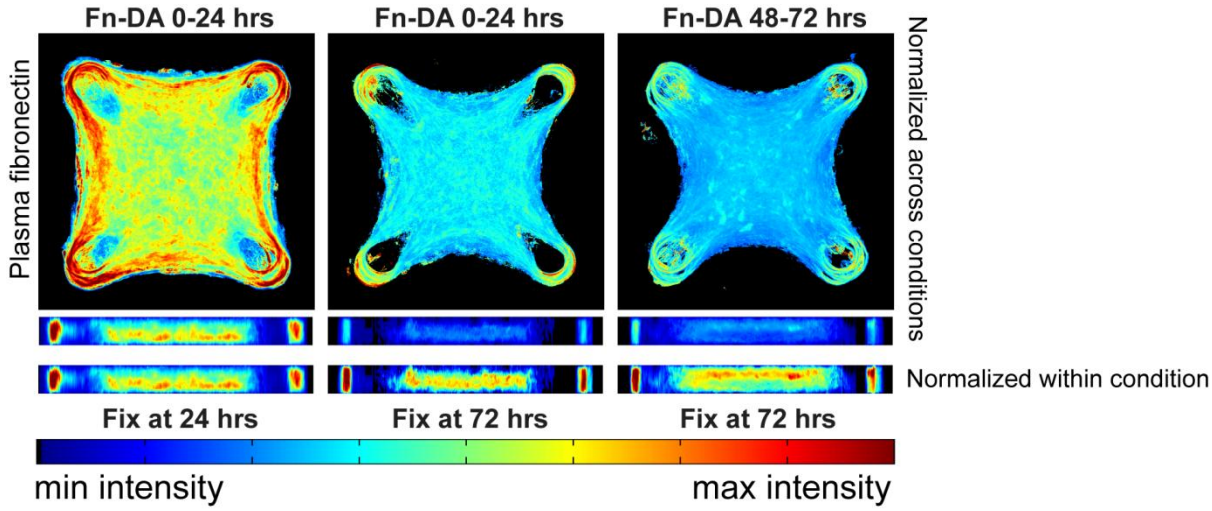

Figure S5: Fluorescence intensity of plasma Fn assembled during specific windows of remodeling. Protein density maps showing microtissues incubated with Fn-DA from 0-24 hours of remodeling and then replaced with unlabeled Fn (fixed after either 24 or 72 hours), or incubated with unlabeled Fn from 0-48 hours, replaced with Fn-DA from 48-72 hours of remodeling and fixed after 72 hours. Density maps represent the protein density at a given location averaged over the axis orthogonal to the image plane. Cross-section views are of the same regions as those shown in **Fig. 2** and **Fig S1**. Color scales for the upper panel are normalized to depict the min and max values across all conditions. Thus, the intensities measured at different time points are comparable. The lower panel depicts internally normalized cross-section views. Fn-DA in microtissues fixed after 24 hours was brighter than Fn-DA present in microtissues from 0-24 hours and then fixed after 72 hours, indicating that proteolytic degradation of plasma Fn was occurring during tissue remodeling. Fn-DA in microtissues fixed after 24 hours was also brighter than Fn-DA in microtissues incorporated from 48-72 hours and fixed after 72 hours. Because these two populations of Fn-DA were remodeled for the same duration of time, the differences in brightness likely represent a dilution of plasma Fn-DA with endogenous cell-derived Fn assembled between 48 and 72 hours.

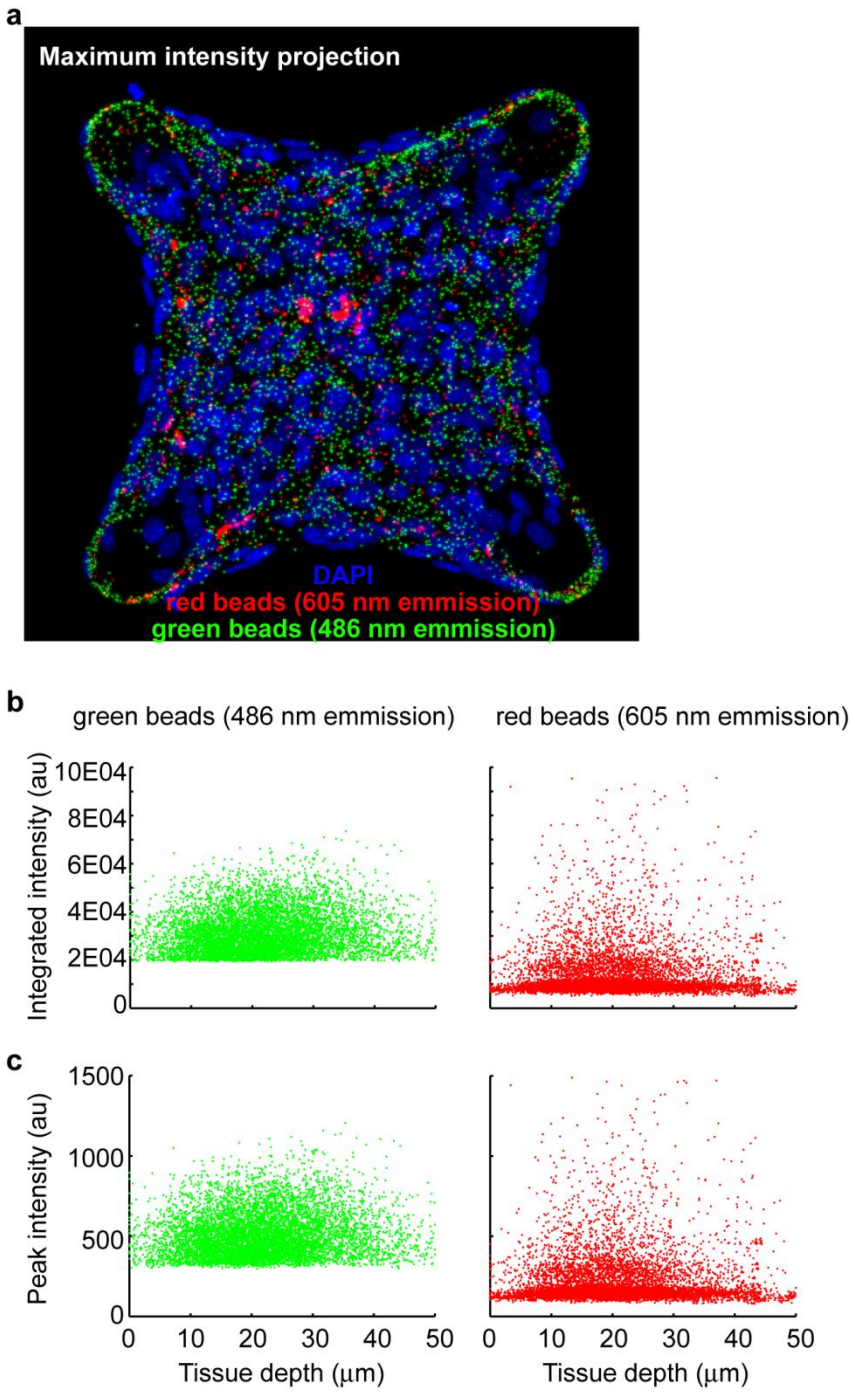

Figure S6: Fluorescent bead intensities as a function of imaging depth within microtissues. **a)** Maximum intensity projection of a microtissue fixed after 24 hours with green (486 nm emission) and red (605nm emission) 0.5 µm beads embedded within the collagen matrix. **b)** Scatter plot of the integrated intensity of fluorescent beads plotted throughout the depth of the tissue. **c)** Scatter plot of the peak intensity of fluorescent beads plotted throughout the depth of the tissue. Neither the integrated nor the peak intensity of the red or green beads show an influence of the imaging depth. This indicates that imaging through the collagen based microtissue will not confound the FRET results.

Table S1

Figure 4b: Total Fn-DA				
	24 hrs	48 hrs	72 hrs	72 hrs/blebbi
24 hrs	-	ns	**	**
48 hrs	ns	-	**	ns
72 hrs	**	**	-	ns
72 hrs/blebbi	**	ns	ns	-

Figure 4d: Collagen colocalized Fn-DA				
	24 hrs	48 hrs	72 hrs	72 hrs/blebbi
24 hrs	-	ns	**	**
48 hrs	ns	-	**	ns
72 hrs	**	**	-	ns
72 hrs/blebbi	**	ns	ns	-

Figure 4d: Non-Collagen colocalized Fn-DA				
	24 hrs	48 hrs	72 hrs	72 hrs/blebbi
24 hrs	-	ns	**	**
48 hrs	ns	-	**	ns
72 hrs	**	**	-	ns
72 hrs/blebbi	**	ns	ns	-

Table S1: Statistical analysis of FRET signal from 4-post tethered microtissues. Statistical comparisons were performed using a Kruskal–Wallis one-way analysis of variance and Tukey's honestly significant difference (HSD) tests. Data are from n = 10 independent samples for each condition. ns – not significant, * alpha = 0.05, ** alpha = 0.01.

Table S2

Figure 5d: Tissue Tension				
	24 hrs	48 hrs	72 hrs	72 hrs/blebbi
24 hrs	ns	ns	*	**
48 hrs	ns	ns	*	**
72 hrs	*	*	ns	**
72 hrs/blebbi	**	**	**	ns

Figure 5e: Cross-section area				
	24 hrs	48 hrs	72 hrs	72 hrs/blebbi
24 hrs	-	**	**	ns
48 hrs	**	-	ns	**
72 hrs	**	ns	-	**
72 hrs/blebbi	ns	**	**	-

Figure 5f: Cross-section stress				
	24 hrs	48 hrs	72 hrs	72 hrs/blebbi
24 hrs	-	*	**	**
48 hrs	*	-	ns	**
72 hrs	**	ns	-	**
72 hrs/blebbi	**	**	**	-

Figure 5f: Fn-DA FRET				
	24 hrs	48 hrs	72 hrs	72 hrs/blebbi
24 hrs	-	*	**	**
48 hrs	*	-	**	*
72 hrs	**	**	-	*
72 hrs/blebbi	**	*	*	-

Table S2: Statistical analysis of 2-post tethered microtissues. Statistical comparisons were performed using a Kruskal–Wallis one-way analysis of variance and Tukey's honestly significant difference (HSD) tests. Data are from n = 25 independent samples for each condition. ns – not significant, * alpha = 0.05, ** alpha = 0.01.

Table S3

Figure 6a: Total Fn-DA				
	0-24 hrs	24-48 hrs	48-72 hrs	0-24 Fix at 24 hrs
0-24 hrs	-	ns	**	ns
24-48 hrs	ns	-	ns	*
48-72 hrs	**	ns	-	**
0-24 Fix at 24 hrs	ns	*	**	-

Figure 6b: Collagen colocalized Fn-DA				
	0-24 hrs	24-48 hrs	48-72 hrs	0-24 Fix at 24 hrs
0-24 hrs	-	ns	**	ns
24-48 hrs	ns	-	ns	**
48-72 hrs	**	ns	-	**
0-24 Fix at 24 hrs	ns	**	**	-

Figure 6b: Non-Collagen colocalized Fn-DA				
	0-24 hrs	24-48 hrs	48-72 hrs	0-24 Fix at 24 hrs
0-24 hrs	-	ns	**	ns
24-48 hrs	ns	-	**	ns
48-72 hrs	**	**	-	**
0-24 Fix at 24 hrs	ns	ns	**	-

Table S3: Statistical analysis of FRET signal from 4-post tethered microtissues under Fn-DA pulses. Statistical comparisons were performed using a Kruskal–Wallis one-way analysis of variance and Tukey's honestly significant difference (HSD) tests. Data are from n = 10 independent samples for each condition. ns – not significant, * alpha = 0.05, ** alpha = 0.01.