

# Supporting Information for

## Recapitulation and Modulation of the Cellular Architecture of a User-Chosen Cell of Interest Using Cell-Derived, Biomimetic Patterning

*John H. Slater, James C. Culver, Byron L. Long, Chenyue W. Hu, Jingzhe Hu, Taylor F. Birk, Amina A. Qutub, Mary E. Dickinson, and Jennifer L. West*

**Supplemental Table 1:** Metrics Used to Define Cellular Architecture

Cell Metrics		
Metric	Description	Mathematical Representation
Morphology Metrics		
Cell Size	Cell spread area.	$A_C$
Circularity	Shape factor.	$\frac{4\pi \cdot A_C}{P_C^2}$ , where $P_C$ = Cell Perimeter
Elongation	Shape factor.	$\frac{P_C}{A_C}$
Adhesion Site Metrics		
Number of Adhesion Sites	Number of adhesions per cell.	$N_A$
Adhesion Total Area	Sum of the area of all adhesions.	$\sum N_A$
Polarity	Distance between the center of mass of the vinculin stain and the centroid of the cell.	$\sqrt{(\chi_{C,x} - \Omega_{V,x})^2 + (\chi_{C,y} - \Omega_{V,y})^2}$ , where $\chi_C$ = centroid of the cell $C$ $\Omega_V$ = center of mass of the vinculin stain $V$
Maximum Intensity	The maximum of the average vinculin stain intensities of all 10 bands*	$\max(\{I_{B_1} \cdots I_{B_{10}}\})$
Maximum Location	The index of the band* with the highest average vinculin stain intensity	The least $n$ such that $I_{B_n} = \max(\{I_{B_1} \cdots I_{B_{10}}\})$

Above Average Adjusted Distribution	Adjusted, weighted sum of average vinculin stain intensities greater than the mean intensity.	$\frac{\sum_{k=1}^{10} s(I_{B_k}) \cdot I_{B_k} \cdot k}{\max(\{I_{B_1} \cdots I_{B_{10}}\}) \cdot \sum_{k=1}^{10} s(I_{B_k})}$
Slope	Slope of average vinculin stain intensity between bands* $B_8$ and $B_2$ .	$\text{Where } s(I) = \begin{cases} 1 & \text{if } I \geq \text{mean}(I_{B_1} \cdots I_{B_{10}}) \\ 0 & \text{otherwise} \end{cases}$ $\frac{I_{B_8} - I_{B_2}}{8 - 2}$
Comparison to COI	The sum of the Euclidean distance between nearest neighbors of the COI (cell-of-interest) and a second cell (patterned cell) using the COI adhesion sites as reference plus the patterned cells as reference; the shorter the distance, the closer the match; COI compared to COI is an exact match.	$\sum_{NN=1}^{N_A} \sqrt{(\chi_{A,COI,x}^{NN} - \chi_{A,C,x}^{NN})^2 + (\chi_{A,COI,y}^{NN} - \chi_{A,C,y}^{NN})^2}$ <p>using both for the COI and the patterned cell as the initial cell of reference  <math>NN</math> = nearest neighbor adhesion site  <math>\chi_A</math> = centroid of the adhesion site A</p>

### Actin Cytoskeleton Metrics

Polarity	Distance between the center of mass of actin and the centroid of the cell.	$\sqrt{(\chi_{C,x} - \Omega_{A,x})^2 + (\chi_{C,y} - \Omega_{A,y})^2}$ <p>where <math>\Omega_A</math> = center of mass of actin</p>
Uniformity	Sum of squared elements in the histogram counts of the image for pixel intensities. Analogous to energy or sum of squared elements in the grayscale co-occurrence matrix.	$\sum p^2$
Entropy From Histogram	Measure of randomness of the image where $p$ is the histogram counts of the image for pixel intensities, with 256 possible bins for a grayscale image.	$-\sum p * \log_2(p)$
Entropy From GLCM	Measure of randomness of the image where $g$ is the histogram counts of the gray level co-occurrence matrix of the image.	$-\sum g * \log_2(g)$
Correlation	A measure of Pearson's correlation of each pixel to its neighborhood over the whole image. For a perfectly linearly and positively correlated set of pixels, correlation = 1.	$\sum_{i,j} \frac{(i - \mu_i)(j - \mu_j)p(i,j)}{\sigma_i \sigma_j}$
Energy	Sum of squared elements in the grayscale co-occurrence matrix.	$\sum_{i,j} p(i,j)^2$
Homogeneity	Measure of the closeness of the distribution of elements in the grayscale co-occurrence matrix to the diagonal of the matrix. For a diagonal matrix, homogeneity = 1.	$\sum_{i,j} \frac{p(i,j)}{1 +  i - j }$
Maximum Location	The index of the band* with the highest average actin stain intensity	Similar to Adhesion Site maximum location
Above Average Adjusted Distribution	Weighted average location (as a band* index) of the actin stain bands	Similar to Adhesion Site mean location above average

with intensity greater than the mean intensity.

Fiber Alignment Match

Sum of squares difference between histograms of COI and pattern cell actin fiber orientation

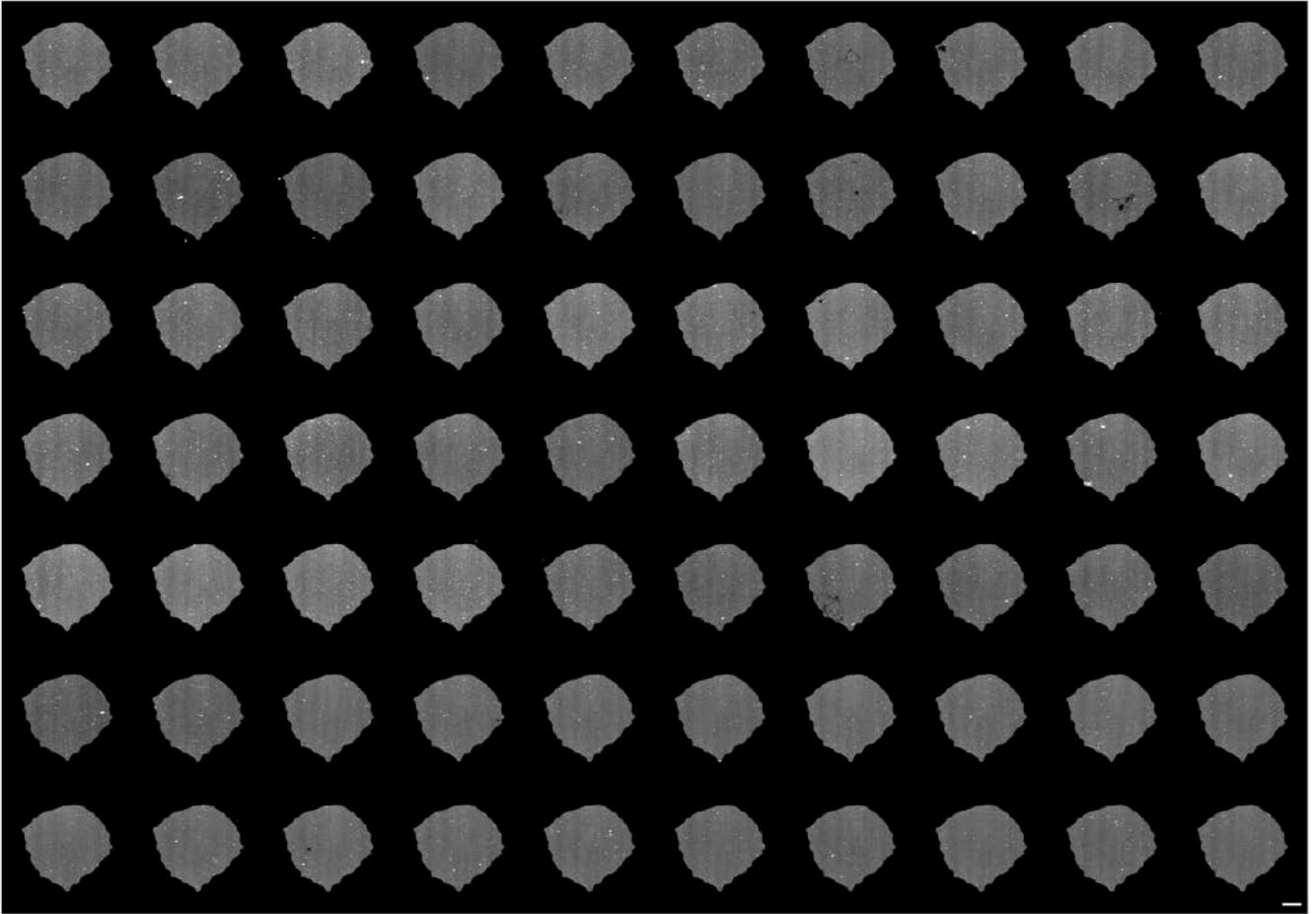
$$\sum_{i=1}^N (h_{COI,i} - h_{pattern,i})^2$$

$N$  = number of histogram bins  
 $h_i$  = size of  $i$ -th bin of histogram

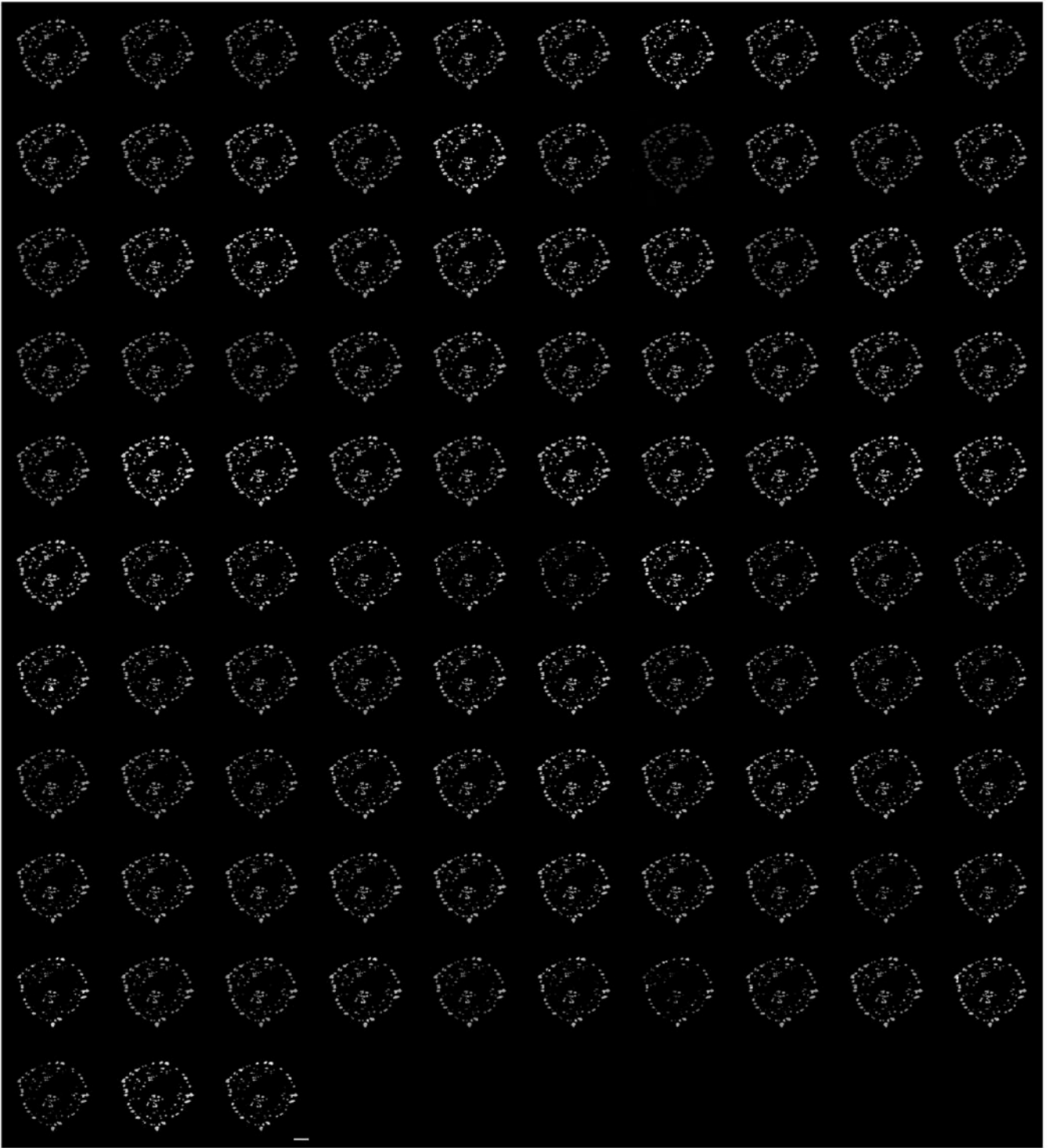
### Nucleus Metrics

Major Axis Length	Length of major axis.	$l_{N,Major}$
Minor Axis Length	Length of minor axis.	$l_{N,Minor}$
Major Minor Axis Ratio	Axis length ratio	$\frac{l_{N,Major}}{l_{N,Minor}}$
Polarity	Distance between the center of mass of DAPI and the centroid of the cell.	$\sqrt{(\chi_{C,x} - \Omega_{D,x})^2 + (\chi_{C,y} - \Omega_{D,y})^2}$ where $\Omega_D$ = center of mass of DAPI

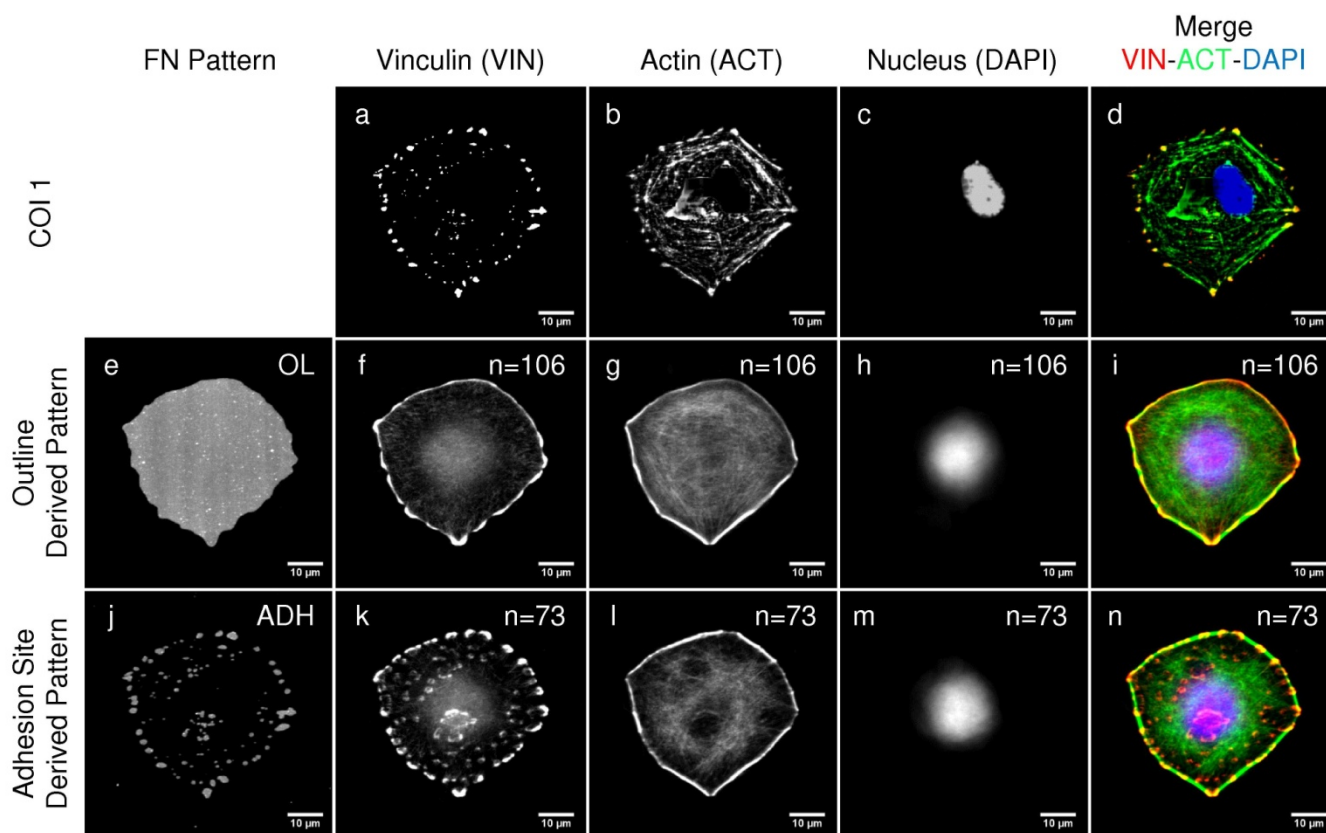
\*A band is a locus of points within the cell cytoplasm at a relative distance from the nucleus. Let  $r$  range over segments of lines radiating from the centroid of a cell nucleus starting at the nucleus perimeter and ending at the cell perimeter. Let  $r_n(1 \leq n \leq 10)$  denote the  $n$ -th of 10 equi-length, separate, and consecutive subsegments of  $r$ . For a given cell, band  $B_n(1 \leq n \leq 10)$  is the locus of all  $r_n$ .



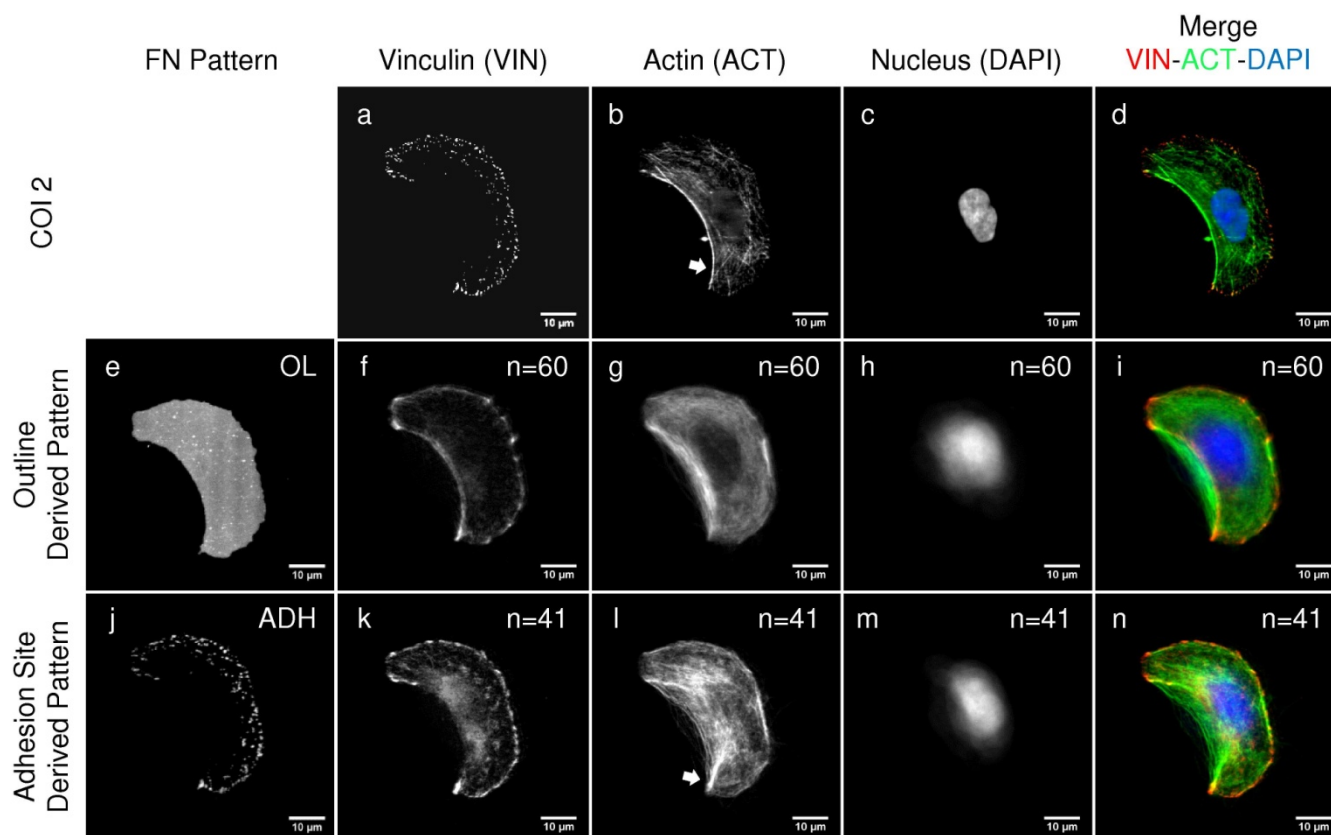
**Supplemental Figure 1:** Montage of images of the morphology pattern derived from COI 1. An outline of COI 1 was used to define this fibronectin pattern configuration. Arrays of the FN pattern were created with IG-LSL, fluorescently immunolabeled, imaged, and compiled into a montage. SB=10  $\mu$ m.



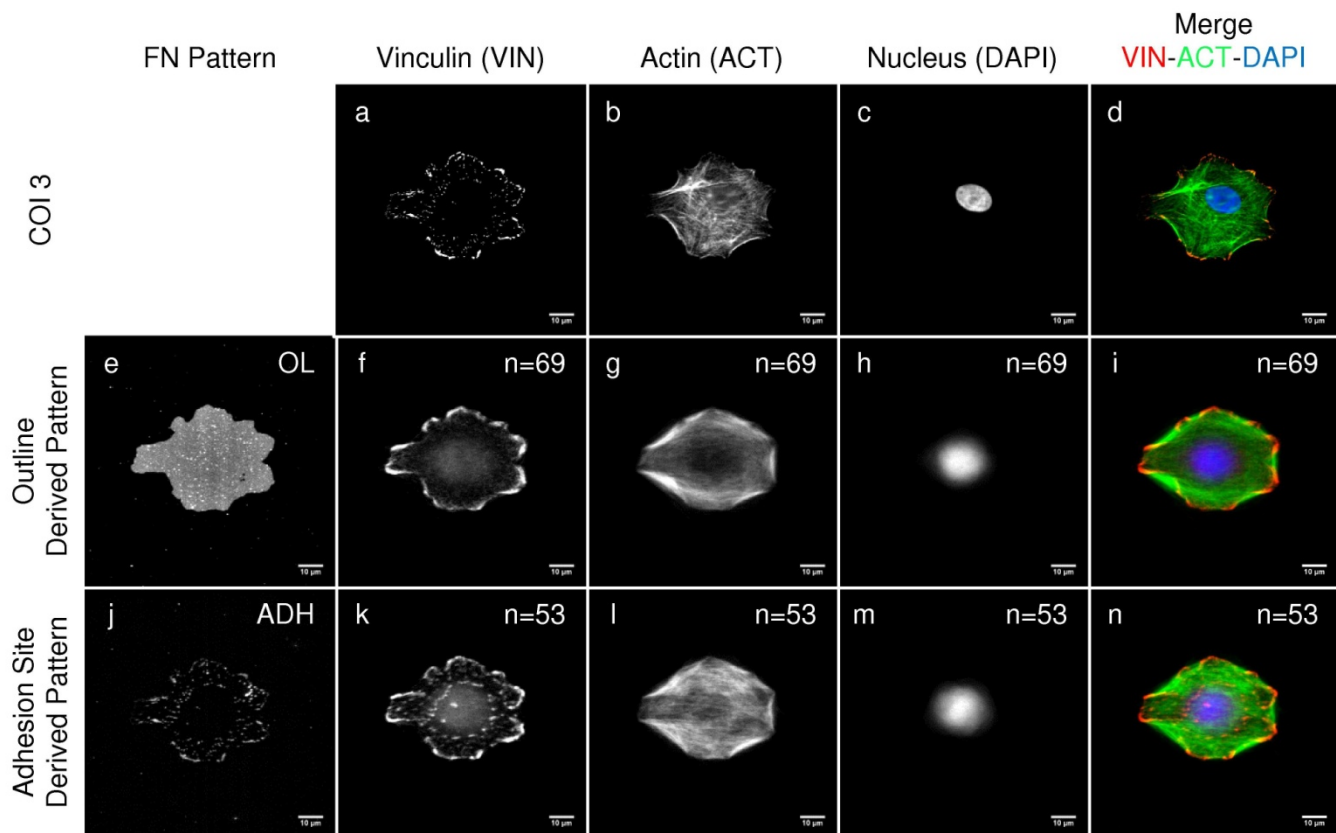
**Supplemental Figure 2:** Montage of images of the adhesion pattern derived From COI 1. An image of the vinculin-containing adhesion sites of COI 1 was used to define this fibronectin pattern configuration. Arrays of the FN pattern were created with IG-LSL, fluorescently immunolabeled, imaged, and compiled into a montage. SB=10  $\mu$ m.



**Supplemental Figure 3:** ‘Average’ cells cultured on patterns derived from COI 1. (a-d) Fluorescent images of the vinculin-containing adhesions, actin cytoskeleton, and nucleus of COI 1 that the (e,j) FN pattern configurations were derived from. HUVECs were cultured on arrays of the (e) morphology (OL) or (j) adhesion site (ADH) derived pattern configuration, fixed, fluorescently immunolabeled for vinculin, actin, counterstained with DAPI, and imaged. (f-h, k-m) The individual cell images,  $n = 106$  and  $73$  for the number of cells cultured on the (f-h) morphology and (k-m) adhesion site derived pattern configurations respectively, for each channel were aligned, overlaid, and intensity averaged. SB=10  $\mu\text{m}$ .

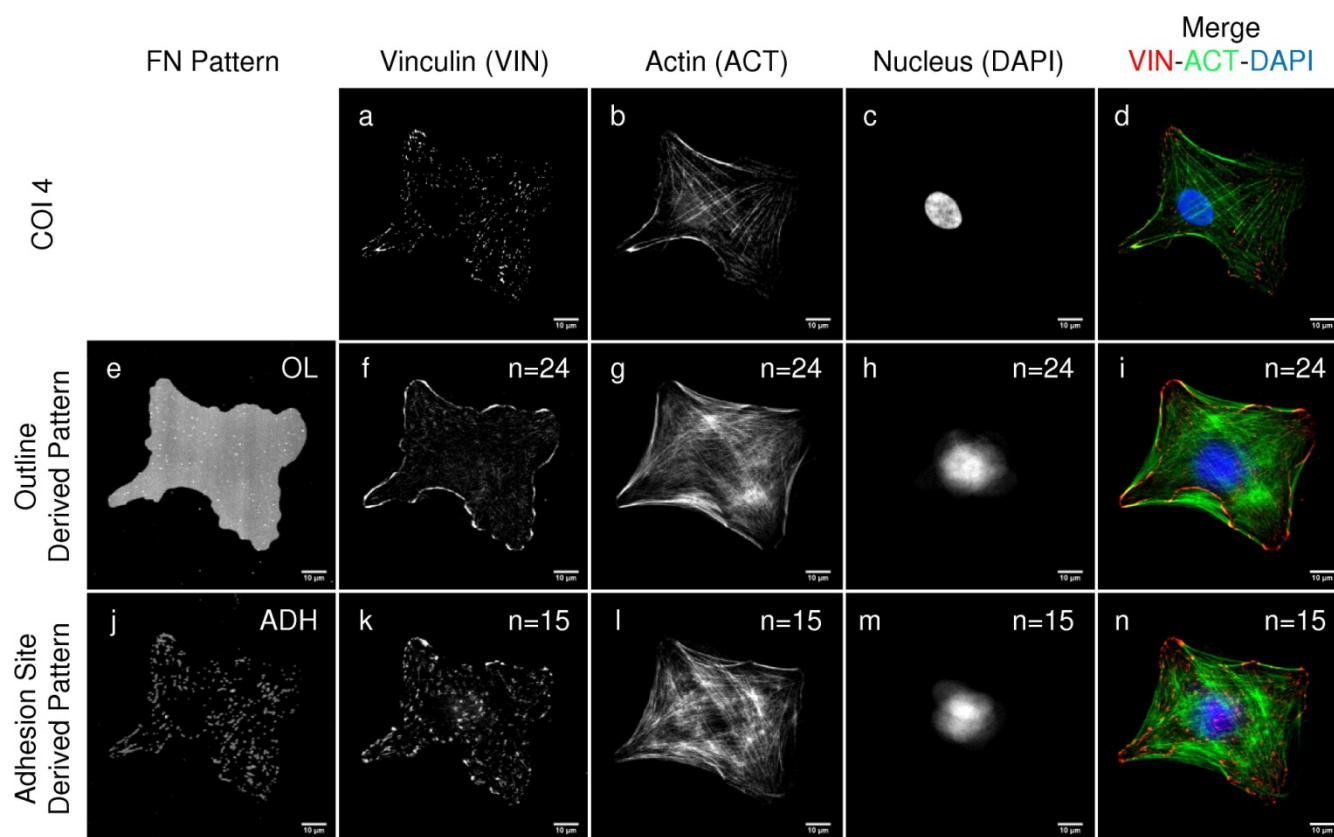


**Supplemental Figure 4:** ‘Average’ cells cultured on patterns derived from COI 2. (a-d) Fluorescent images of the vinculin-containing adhesions, actin cytoskeleton, and nucleus of COI 2 that the (e,j) FN pattern configurations were derived from. HUVECs were cultured on arrays of the (e) morphology (OL) or (j) adhesion site (ADH) derived pattern configuration, fixed, fluorescently immunolabeled for vinculin, actin, counterstained with DAPI, and imaged. (f-h, k-m) The individual cell images,  $n = 60$  and  $41$  for the number of cells cultured on the (f-h) morphology and (k-m) adhesion site derived pattern configurations respectively, for each channel were aligned, overlaid, and intensity averaged. SB=10  $\mu\text{m}$ .



**Supplemental Figure 5:** ‘Average’ cells cultured on patterns derived from COI 3. (a-d) Fluorescent images of the vinculin-containing adhesions, actin cytoskeleton, and nucleus of COI 3 that the (e,j) FN pattern configurations were derived from. HUVECs were cultured on arrays of the (e) morphology (OL) or (j) adhesion site (ADH) derived pattern configuration, fixed, fluorescently immunolabeled for vinculin, actin, counterstained with DAPI, and imaged. (f-h, k-m) The individual cell images,  $n = 69$  and  $53$  for the number of cells cultured on the (F-H) morphology and (k-m) adhesion site derived pattern configurations respectively, for each channel were aligned, overlaid, and intensity averaged. SB=10  $\mu\text{m}$ .





**Supplemental Figure 6:** ‘Average’ cells cultured on patterns derived from COI 4. (a-d) Fluorescent images of the vinculin-containing adhesions, actin cytoskeleton, and nucleus of COI 4 that the (e, j) FN pattern configurations were derived from. HUVECs were cultured on arrays of the (e) morphology (OL) or (j) adhesion site (ADH) derived pattern configuration, fixed, fluorescently immunolabeled for vinculin, actin, counterstained with DAPI, and imaged. (f-h, k-m) The individual cell images,  $n = 24$  and  $15$  for the number of cells cultured on the (f-h) morphology and (k-m) adhesion site derived pattern configurations respectively, for each channel were aligned, overlaid, and intensity averaged. SB=10  $\mu\text{m}$ .