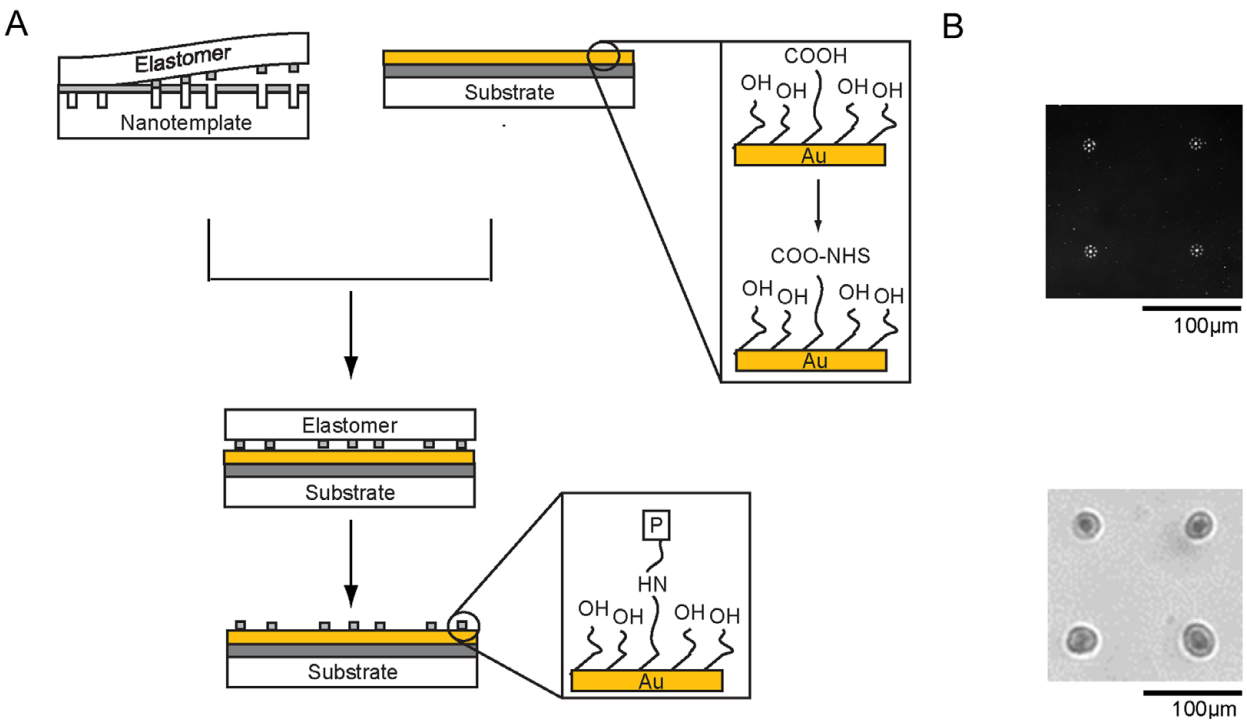


Fig. S1. Cell adhesive substrates with nanopatterned geometries. (**A**) Anchoring groups (COOH) on mixed self-assembled monolayers on gold are activated with EDC/NHS to produce amine-reactive NHS-esters. A flat elastomer stamp uniformly coated with FN is brought into contact with a high-precision nanotemplate to selectively remove FN from the stamp and create the desired FN patterns on the stamp. Pressing the stamp onto the substrates with NHS-esters results in the transfer and tethering of FN molecules onto the substrate. (**B**) NIH3T3 fibroblasts cultured overnight on nanopatterned substrates attach as single cells and remain rounded (top: fluorescence microscopy image of FN nanopatterns, bottom: phase contrast image of cells).

Characteristics of adhesion cluster designs analyzed

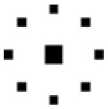
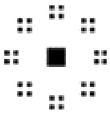
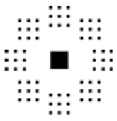
Pattern	Design	Number of Islands per pad	Island size (nm)	Island area (μm^2)	Pad Area (μm^2)	Total Area (μm^2)
1000 nm×1		1	1000	1.00	1.00	12.0
500 nm×4		4	500	0.25	1.00	12.0
333 nm×9		9	333	0.11	1.00	12.0
500 nm×1		1	500	0.25	0.25	6.0
250 nmx2		2	250	0.06	0.13	5.0
250 nm×4		4	250	0.06	0.25	6.0
250 nm×9		9	250	0.06	0.56	8.5
Center only		0	0	0	0	4.0
10 µm Diameter		N/A	N/A	N/A	N/A	78.5

Fig. S2. Characteristics of adhesion cluster designs analyzed.

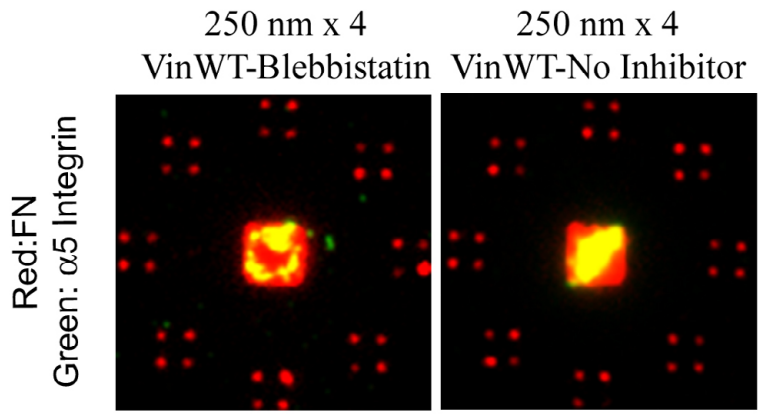


Fig. S3. Blebbistatin treatment (20 μM, 60 min prior to analysis) has no effect on the lack of assembly of integrin-FN clusters on 250 nm islands for cells expressing wild-type vinculin. Fluorescence microscopy images for integrin binding (green) to FN (red) adhesive zones on 250 nm×4 patterns; scale bar: 1 μm.