Supporting Material

Movie S1

Movie S1 : Formation of actin bridges during spreading of a fibroblast on an adhesive pattern

Timelapse sequence of a sub-cutaneous fibroblast transfected with LifeAct EGFP spreading on a circular adhesive pattern (diameter: 71µm) including 8 rectangular non adhesive gaps (width: 8µm). Acquisition rate is 1 image per minute, frame rate is 5 fps. The adhesive pattern was tagged fluorescently using Alexa647 conjugated fibrinogen and imaged before the first time point. Its contour is shown in yellow overlay. The white frame on the first image indicates the cropped area shown in **Figure 5A**. Scale bar is 10µm.

Table S1

Adhesive density of micropatterns

number of non-adhesive branches	4	8	4	8	4	8	4	8
Gap width (µm) \ Total area (µm²)	1000		2000		3000		4000	
2	0.93	0.86	0.95	0.90	0.95	0.90	0.95	0.91
4	0.86	0.72	0.90	0.80	0.90	0.81	0.91	0.82
6	0.79	0.58	0.85	0.71	0.86	0.71	0.86	0.73
8	0.72	0.43	0.80	0.61	0.81	0.62	0.82	0.64
10	0.65	0.33	0.76	0.51	0.76	0.52	0.77	0.55

This table gives the adhesive density for the different patterns, computed as (adhesive area)/(total area). The total area is taken to be that of the outer circle. The diameters of these circles are 36 µm, 50 µm, 62 µm, 71 µm respectively for the 1000 µm², 2000 µm², 3000 µm² and 4000 µm² patterns. All patterns have a smaller circle in the center with 26 µm in diameter, except for the smallest patterns (1000 µm²) where the inner circle is only 18 µm in

diameter. The non-adhesive gaps go from the inner to the outer circle. The shaded boxes are configurations that were not used in this study.

Table S2

Results of Kruskal-Wallis tests

Gap width (µm)	2	4	6	8	10
3T3	0.88	0.48	0.57		
SCF	0.91	0.71	0.97	0.05	0.36

Grouped data	
3T3 (2/4 vs 6/8/10µm)	$9.2 \cdot 10^{-11}$
SCF (2 vs 6/8/10µm)	0

This table gives the p-values of the Kruskal-wallis statistical test used to compare the data. In the first part, the data for different sizes of patterns were compared, for a given gap width. p-values higher than 0.01 indicated populations were not significantly different and could be pooled. In the second part, data on patterns of same gap width and different areas were pooled together. We show that there is significant difference ($p<0.01$) between "below LTM" group and "above LTM". "Below LTM" group is defined as 2 and 4 µm gaps for 3T3 and 2 µm gaps for SCF. "Above LTM" group is defined as 6, 8 and 10 µm gaps for 3T3 and SCF.

Table S3

Summary of experiments parameters of presented results

	Pattern	**8 branches - small**				
Cell type		**2 µm**	**4 µm**	**6 µm**	**8 µm**	**10 µm**
		1000 µm²				
3T3	n° of cells	3	11	5	0	0
	n° of gaps	21	83	39		
	n° of bridges	6	30	34		
	mean bridging ratio	0.29	0.4	0.88		
	mean area (µm²)	711 +/- 190	737 +/- 78	749 +/- 314		

		3000 µm²				
SCF	n° of cells	21	20	29	22	16
	n° of gaps	137	147	199	171	96
	n° of bridges	36	85	177	137	82
	mean bridging ratio	0.28	0.61	0.92	0.83	0.90
	mean area (µm²)	1989 +/- 353	2293 +/- 410	2228 +/- 566	2461 +/- 427	1776 +/- 456

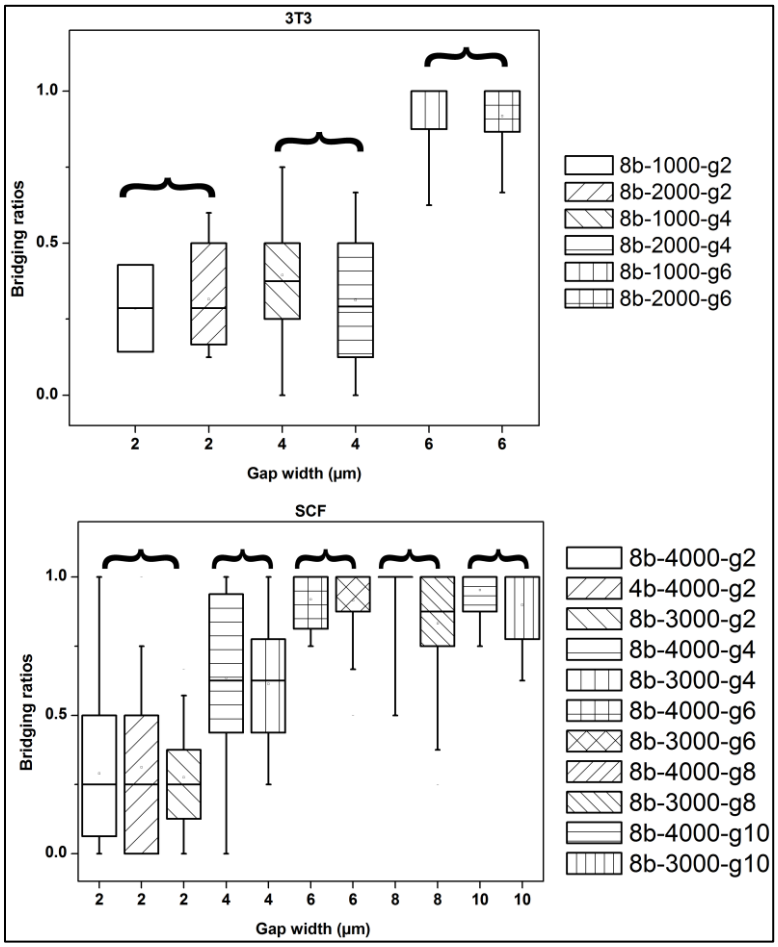
Cell type	Pattern	8 branches - large				
		2 µm	4 µm	6 µm	8 µm	10 µm
		2000 µm²				
3T3	n° of cells	11	8	12	4	4
	n° of gaps	68	34	64	32	32
	n° of bridges	21	8	57	32	32
	mean bridging ratio	0.32	0.31	0.92	1.00	1.00
	mean area (µm²)	1138 +/- 576	1021 +/- 492	1136 +/- 601	1369 +/- 269	818 +/- 264
		4000 µm²				
SCF	n° of cells	16	12	20	17	11
	n° of gaps	120	91	141	119	72
	n° of bridges	31	53	132	105	68
	mean bridging ratio	0.29	0.64	0.92	0.94	0.95
	mean area (µm²)	2725 +/- 574	2872 +/- 685	2866 +/- 673	2547 +/- 586	2804 +/- 789

Cell type	Pattern	4 branches – 4000 µm²
		2 µm
SCF	n° of cells	32
	n° of gaps	124
	n° of bridges	37
	mean bridging ratio	0.31
	mean area (µm²)	3164 +/- 437

This table gives a summary of the data treated and presented in this paper. For each type of pattern (a given size and a given gap width) and each cell type, the total numbers of cells, of covered gaps and of measured bridges are indicated. The mean bridging ratio is the average over all cells of a group of their bridging ratios. We also indicate for informational purpose, the mean spread areas of the cell with the standard deviations. This serves as a confirmation that the non-adhesive gap in our patterns does not hinder global cell spreading, since the distribution of spread areas are similar for a given pattern size.

Figure S1

Dependency of bridging ratios on the adhesive pattern



We verified that the area of the adhesive pattern did not change the distributions of bridging ratios by doing a kruskal-wallis test, with a significance value $p<0.01$. For each gap width, we checked that the data from smaller patterns (1000 µm² for 3T3 and 3000 µm² for SCF) could be pooled with data on the larger patterns (2000 µm² for 3T3 and 4000 µm² for SCF). The results showed no significant difference. We had the same approach to compare SCF on patterns with 8 non-adhesive branches of 2 µm wide and only 4 non-adhesive branches of same width to see if the adhesive area density could change our results. As discussed in the main text, for our geometry of patterns, adhesive density had no significant impact. Figure S1 shows separately the bridging ratios of cells for all patterns considered in this study, curly braces indicate those that were pooled for the analysis. Box, box whiskers, and dashes correspond respectively to the 25^{th}-75^{th} percentiles, the 5^{th} and 95^{th} percentiles and the extrema values. The median is indicated by a line, the mean by a square. The legend details the pattern category: number of non-adhesive branches, total pattern area, gap width. For example "8b-1000-g2" is a 1000 µm² pattern with 8 non-adhesive gaps of 2 µm wide.

Figure S2

Distribution of actin bridges' lengths

Histogram distribution of the lengths of actin bridges on 2 µm (A), 4 µm (B) and 6 µm(C) gaps. In each plot the bridges' lengths for each cell type are compared. Bin width is 1 µm. Whereas there is clearly a difference on the smaller gaps between 3T3 and SCF, since the latter extend bridges over distances as small as 3 µm, the distributions for 4 µm and 6 µm gaps are similar, the gaps being at least as large as the LTM both for 3T3 and SCF.