

Figure S1. Spatial distribution of agonist-induced changes in contractility

(a) A heat map of strain energies on individual posts for a representative cell. **(b)** The posts in (a) are separated into 4 subsets based on quartiles of the magnitudes of strain energies on the posts. Red regions denote posts in Subset 4 that experience strain energies above the 75th percentile of strain energies on all the attached posts. Similarly, the other colors denote posts in lower ranges. **(c)** Proportion of total strain energy within a cell that is contributed by the posts in the top quartile (Subset 4) of strain energies. Shown are both pre-stimulation ($t = 0$ min) or post-stimulation ($t = 3$ min for histamine, LPA, S1P and thrombin; $t = 6$ min for BSA and VEGF) conditions. The proportions are averages of $n = 21$ (BSA; $10 \mu\text{g/mL}$), $n = 12$ (histamine; $3 \mu\text{M}$), $n = 13$ (LPA; $10 \mu\text{g/mL}$), $n = 16$ (S1P; $0.5 \mu\text{M}$), $n = 10$ (thrombin; 1 U/mL), and $n = 27$ (VEGF; 50 ng/mL) cells. **(d)** The proportion of posts that start in a particular subset pre-stimulation and finish in another particular subset post-stimulation. For example, $4' \rightarrow 4$ denotes the proportion of posts that remain in Subset 4 before and after stimulation. $4' \rightarrow 3$ denotes the proportion of posts that are in Subset 4 before stimulation and switch to Subset 3 after stimulation. The proportions are averages of the same cells in (c), and indicate that posts experiencing the highest strain energies pre-stimulation (Subset 4') often experience the highest strain energies after stimulation (Subset 4). **(e)** An image of the cell in (a) showing three subcellular zones: the Periphery which is the outermost perimeter of attached posts, the Sub-periphery which is the perimeter after the periphery is eroded, and the Interior, which contains all the posts after the Sub-periphery is eroded. **(f)** The proportion of posts in Subset 4 that are found in the three subcellular zones described in (e). Proportions are averages of $n = 21$ (BSA), $n = 5$ (histamine), $n = 8$ (LPA), $n = 9$ (S1P), $n = 4$ (thrombin), and $n = 27$ (VEGF) cells. Error bars indicate SEM.

Figure S2. Dynamic effects of vasoactive agonists on cell area

(a-d) Time-series plots of the average spread area of cells stimulated with **(a)** LPA ($10 \mu\text{g/mL}$), **(b)** S1P ($0.5 \mu\text{M}$), **(c)** histamine ($3 \mu\text{M}$) or **(d)** thrombin (10 nM) immediately after $t = 0$ min. Data for each

treatment condition is the average of $n = 17$ (LPA), $n = 16$ (S1P), $n = 16$ (histamine), and $n = 11$ (thrombin) cells from one experiment each. Error bars are \pm SEM.

Table S3. Sources of variation in single-cell measurements

Two-way ANOVA analysis testing the factors of independent experiments and time-lapse imaging on the measurement of average strain energy in serum-starved cells. Contribution of differences in experimental conditions (e.g. time of day of experiment, culture media temperature/pH) to total variability is calculated by dividing the sum-of-squares (SS) fraction of “Experimental” by the Total SS. Unexplained variability is calculated by dividing the Error SS by the Total SS. Measurements of basal contractility at different time points within the same experiment contributed negligible variability.

Figure S4. Cluster analysis of BSA-induced contractility dynamics

(a-b) Time-series plot of the two largest clusters parsed from a data set of 49 cells stimulated with BSA. The (1,11) window and a minimum Pearson correlation coefficient of 0.8 were used to cluster strain energy profiles. The clusters are qualitatively similar but have not merged at the coefficient cutoff value. The bold black line is the average while gray lines represent individual cells. The remaining 18 cells were classified into much smaller clusters (not shown).

Figure S1

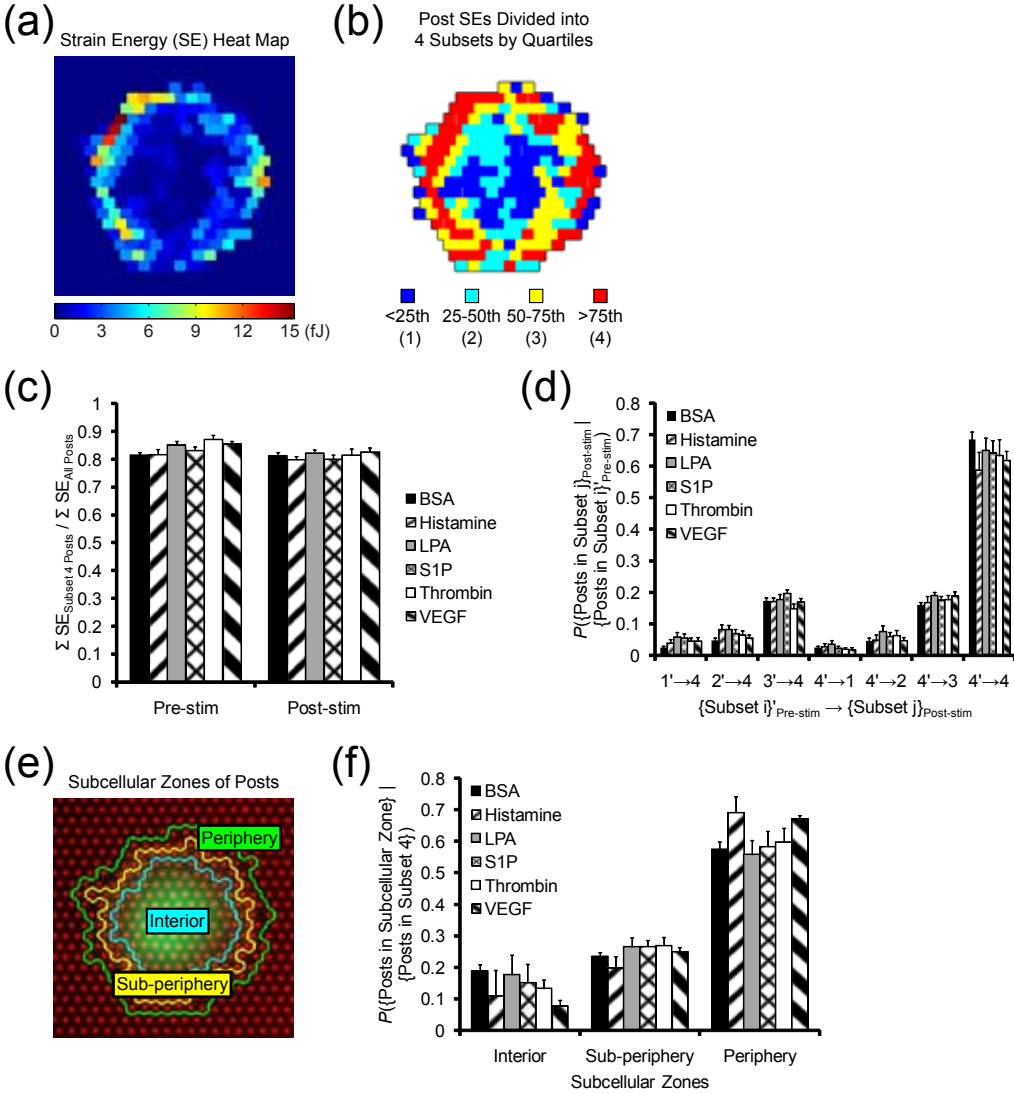


Figure S2

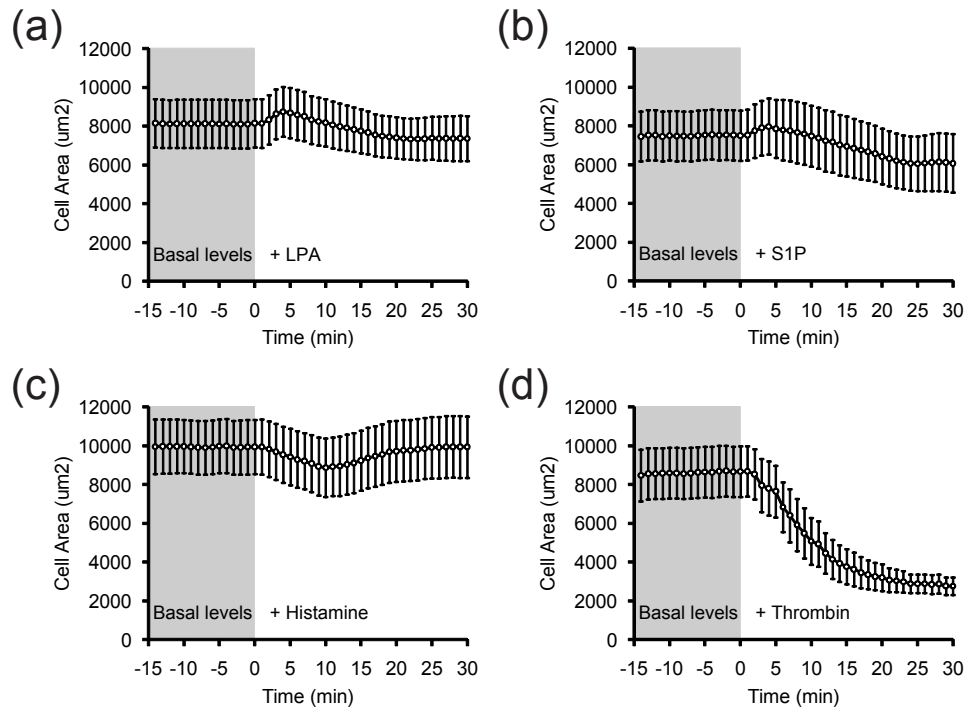


Table S3

Two-way ANOVA					
Source of Variability	SS	df	MS	F	p-value
Experimental	237813.2	5	47562.6	16.64	0
Time	267.9	5	53.6	0.02	0.9999
Interaction	5769.1	25	230.8	0.08	1
Error	1954937.1	684	2858.1		
Total	2198787.3	719			

% variability due to differences between experiments: 11%
 % variability not explained by any systematic source: 89%

Figure S4

