Supplementary Information:

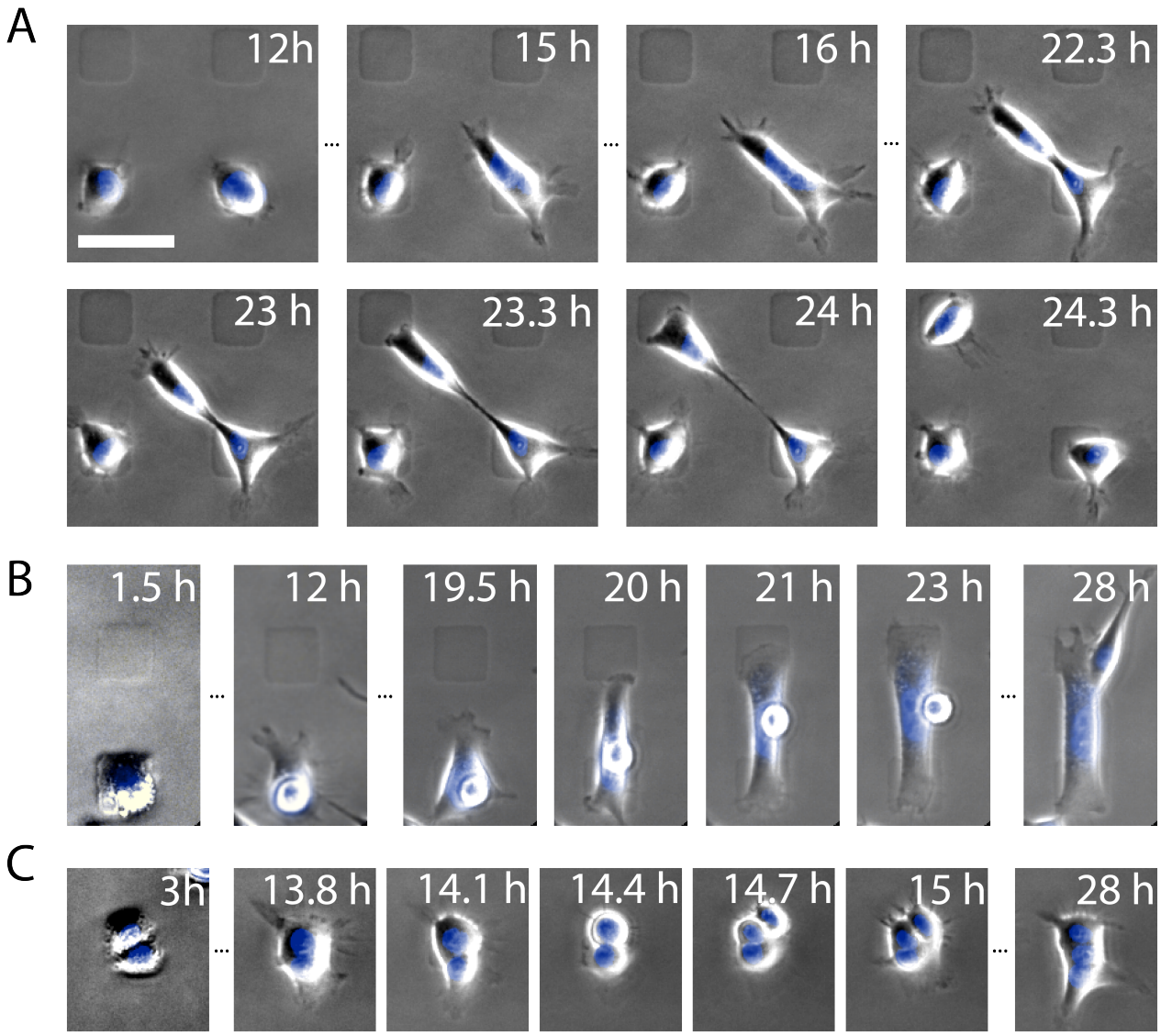

Fig. S1 Events during the self-organization process. (A) Two cells occupy the lower right adhesion site (12 h). As the adhesion site is optimized for one cell and the tension of the cell-cell junction is low in comparison to their mobility, the cells try to explore adjacent adhesion sites by stretching out their filopodia (15-23 h). After 23 h one of the cells has sensed a neighboring site. It moves away from the other cell and relocates to the upper left site. (B) A cell forms a bridge between two adhesion islands: This was observed for the smallest lattice distance d_l = 70 µm. (C) Cell division on a double occupation site (3-15 h): As there are no other sites in reaching distance, cells share the space on the same site by reducing the contact area to the surface and getting increased in height. Scale bar for all images: 100 µm.

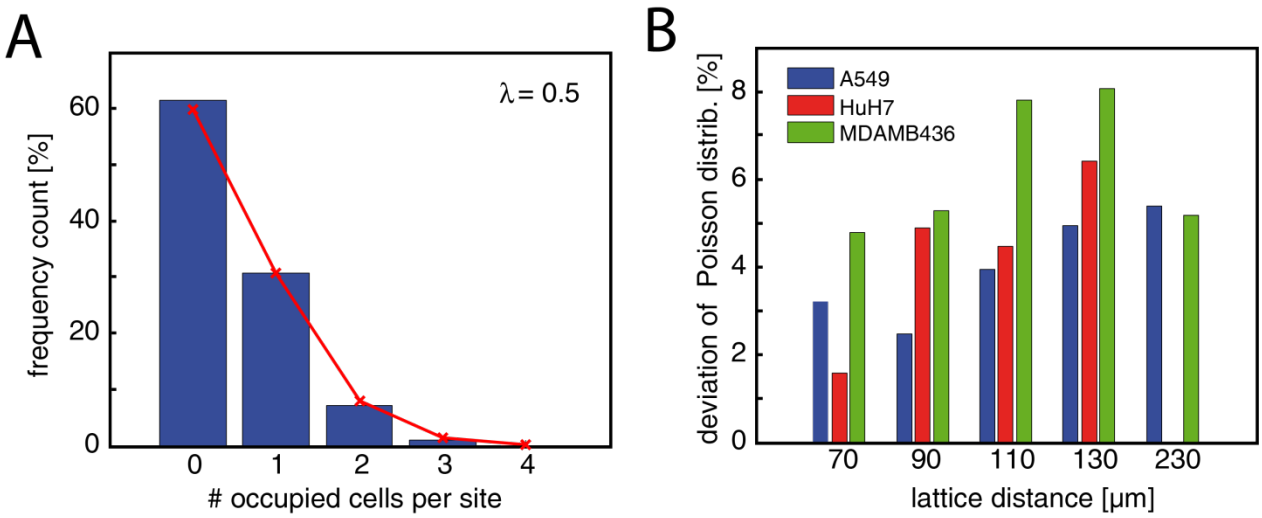

Fig. S2 Initial occupation after seeding. (A) Poisson distribution of an exemplary image position. Blue bars represent the percentage of occupied sites, the red curve displays the calculated distribution (see Eq. 3) for an expected value of $\lambda = 0.5$. The initial occupation is in good agreement with a Poisson distribution. (B) For all cell types and lattice distances, the measured occupation numbers deviates by ≤8 % from the calculated Poisson distribution.

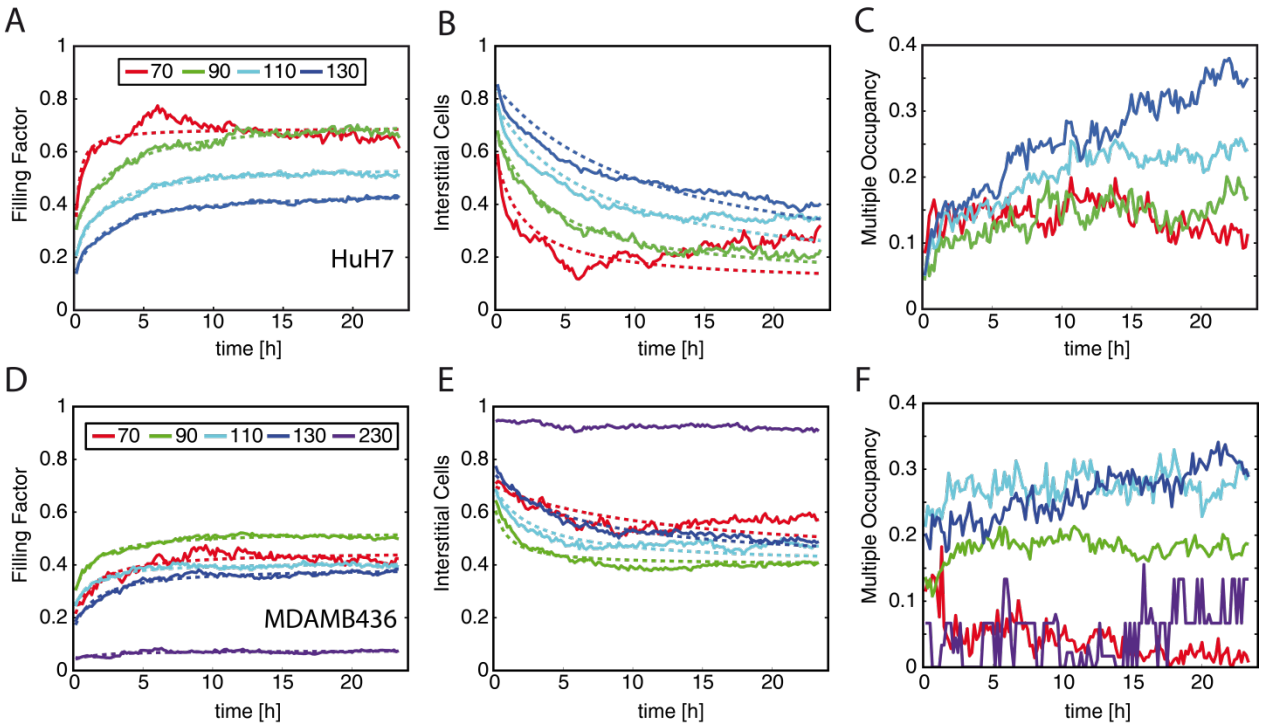

Fig. S3 Dependence of order parameters on lattice spacing. HuH7 (A-C) and MDA-MB-436 (D-F) are different in cell adhesion, motility and viability. Nevertheless the generic phenomenon of cellular self-organization was observed for all three cell lines. (A) Due to faster adhesion and spreading, HuH7 features higher initial occupation numbers than A549. (B) The time course of the "Interstitial Cells" and (C) the "Multiple Occupancy" parameters of HuH7 are similar to A549 (cf. Fig. 3). (D) The total filling of MDA-MB-436 is lower compared to A549 and (E) also the number of misplaced cells is higher since the MDA-MB-436 are less viable. Eq. 6 is fitted only to d_I = 70-130 µm (d_I = 200 µm did not converge properly; it is not included in further analysis). (F) Values of "Multiple Occupancy" are higher for MDA-MB-436 than for A549, as the cells could not be separated as efficiently before seeding.

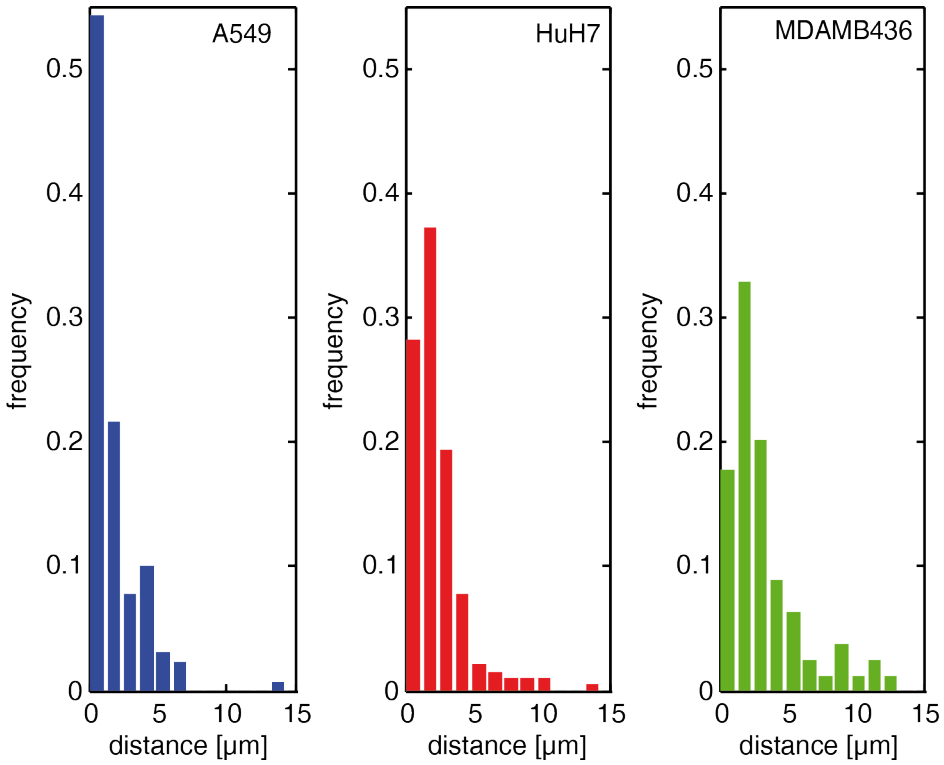

Fig. S4 Covered Distance after Adhesion. Frequency distribution of the initial covered distance represented in a bar plot for the three cell lines A549 (blue), HuH7 (red) and MDA-MB436 (green) after 50 min after seeding. The distribution for HuH7 is shifted towards longer distances in contrast to A549. As both cell lines have similar cell motilities, this shift can be explained by the faster adhesion to the substrate, followed by an earlier start of migration.

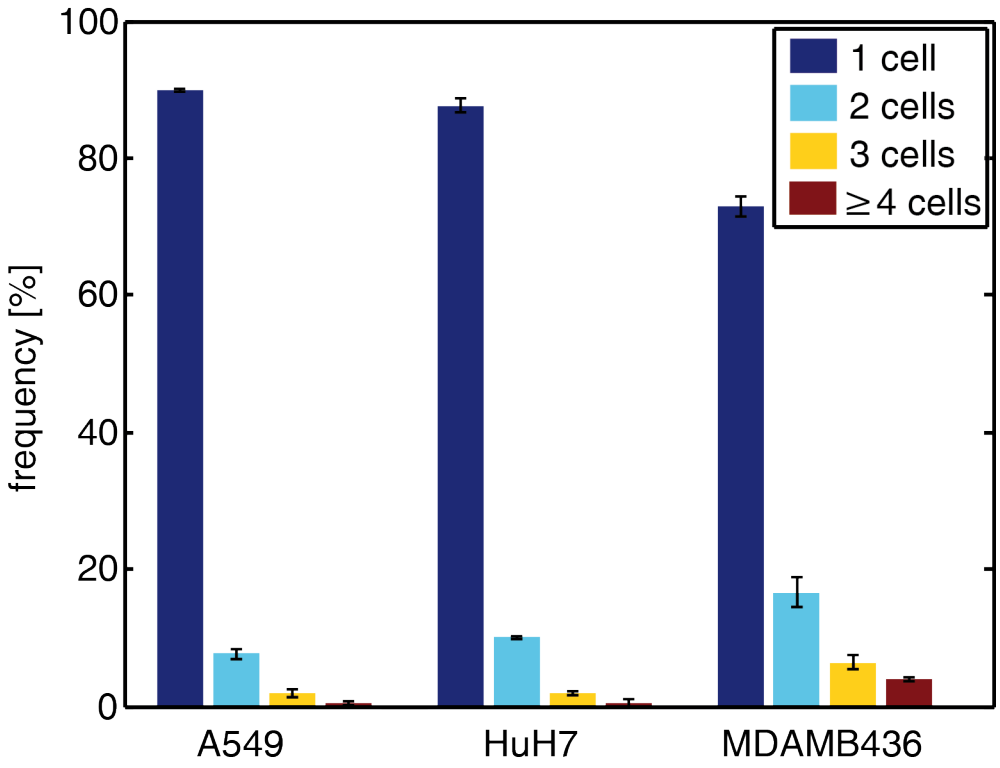

Fig. S5 Distribution of cell clusters. Frequency distribution for single cells (dark blue) and cell clusters (2 cells (light blue), 3 cells (yellow) and ≥ 4 cells (red)) is represented in a bar plot for the three cell lines A549, HuH7 and MDA-MB-436. The ratio of separated single cells for A549 and HuH7 (90 % and 88 %) is similar, whereas for the MDA-MB-436 only 73 % of the cells are separated and 27 % are clustered. As the MDA-MB-436 could not be trypsinized but only scraped prior to seeding, they were not separated as efficiently.

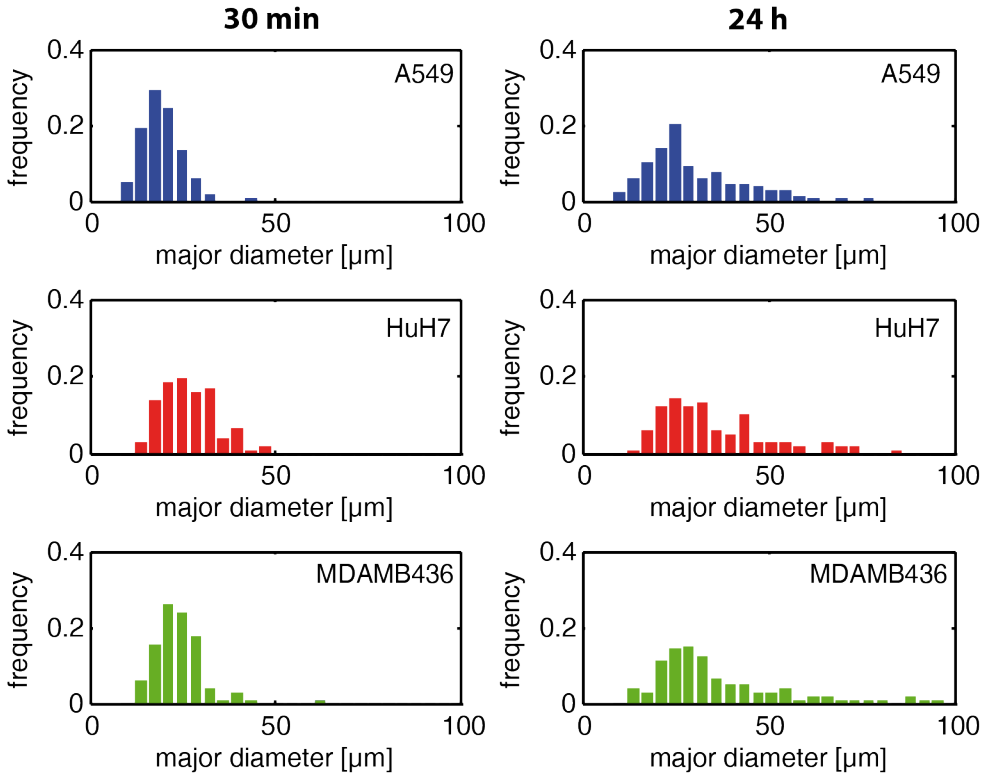

Fig. S6 Spreading area on PLL-g-PEG surface. Frequency distribution for the major diameter of elongated, spread cells on a PLL-g-PEG surface are visualized in bar plots for the three cell lines A549 (blue), HuH7 (red) and MDA-MB-436 (green) after 30 min (left) and after 24 h (right). Since A549 and MDA-MB-436 are often elongated in shape the major diameter reveals these cell types' larger reaching distances of these cells. The number of cell adhesion points is drastically decreased compared to culture treated surfaces: 24 µm (mean diameter after 24 h) vs. 55 µm (mean diameter on culture treated surface) for A549, 29 µm vs. 65 µm for HuH7 and 28 µm vs. 105 µm for MDA-MB-436. Thus, the spreading area does not play a significant role regarding the filling of the lattice. In general, cells are more spread on the PLL-g-PEG surface after 24 h than after 30 min.