Supplementary Figures

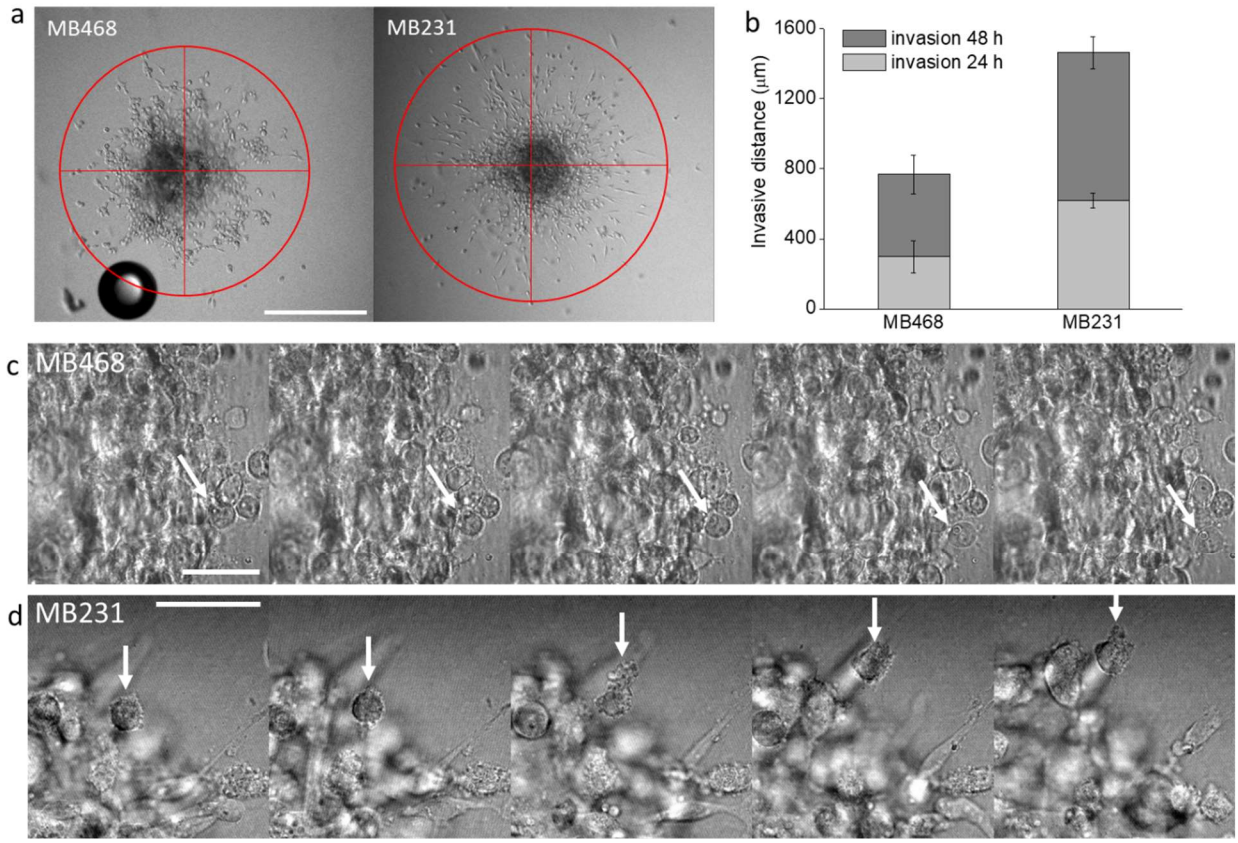


Figure S1. (a) Representative transmitted light images of (left) an MDA-MB-468 spheroid and (right) an MDA-MB-231 spheroid invading in a collagen I gel at 24 h. Red circle represents invasive distance as described in Materials and Methods. Scale bars = 500 µm. (b) MDA-MB-468 and MDA-MB-231 multicellular tumor spheroid invasion in 3D collagen I gels at t = 24 h and t = 48 h. Mean values of invasive distance ± SD are shown; n (MDA-MB-468) = 12, n (MDA-MB-231) = 9. (c, d) Transmitted light time-lapse images of spheroids invading in 1 mg/ml 3D collagen I with (c) MDA-MB-468 cells showing round cell invasion and (d) MDA-MB-231 cells showing dynamic interconversion between elongated and round cell morphology of invading cells. Images are (c) 20 min apart starting at t ≈ 7 h or (d) 30 min apart starting at t ≈ 10.5 h after collagen embedding of the spheroids. Arrows indicate emigrating bleb-bearing cells. Scale bars = 50 µm. Images in (c, d) are obtained from M1 and M2, respectively.

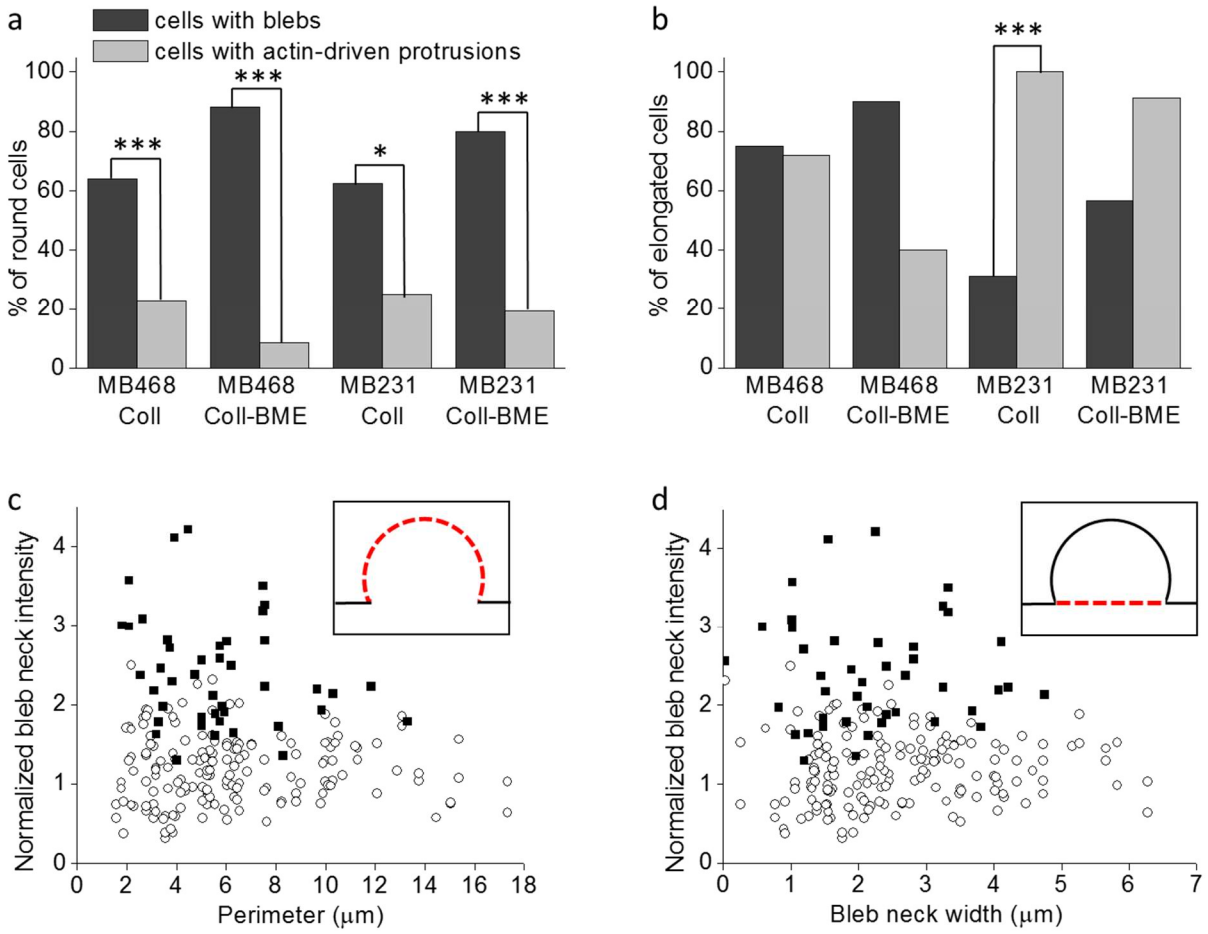


Figure S2. (a, b) Correlative analysis of the occurrence of membrane blebs and actin-driven protrusions in MDA-MB-468 and MDA-MB-231 cells of (a) round cells ($c \geq 0.75$) and (b) elongated cells ($c < 0.75$) embedded in 3D 1 mg/ml collagen I (Coll) or the composite 1 mg/ml collagen I + 3 mg/ml BME (Coll-BME) matrices. Totals may be more than 100% since some cells have both at least one bleb and at least one actin-driven protrusion. Data set is the same as that used for Fig. 1 in the main text. Data was pooled from 3-4 statistically identical biological replicates. n = 149, 103, 117, 122 for MDA-MB-468 Coll, MDA-MB-468 Coll-BME, MDA-MB-231 Coll, and MDA-MB-231 Coll-BME, respectively. Differences were assessed by t-tests between percents. In this and all subsequent figures in which statistical analysis is performed, the analyses are conducted at $\alpha = 0.05$, and significant different are marked by * (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and insignificant by †. (c, d) Mean normalized fluorescence intensity of total β1 integrin staining at individual bleb necks of the same dispersed MDA-MB-231 cells used in the analysis shown in Fig. 4 of the main text plotted against (c) perimeter of the bleb or (d) the width of the bleb neck. Blebs bearing clusters or no clusters are represented by filled and open symbols, respectively. The distributions of bleb perimeters and bleb neck widths of blebs with and without β1 clusters were compared by KS test and t-test between percents and were found to be statistically equivalent.

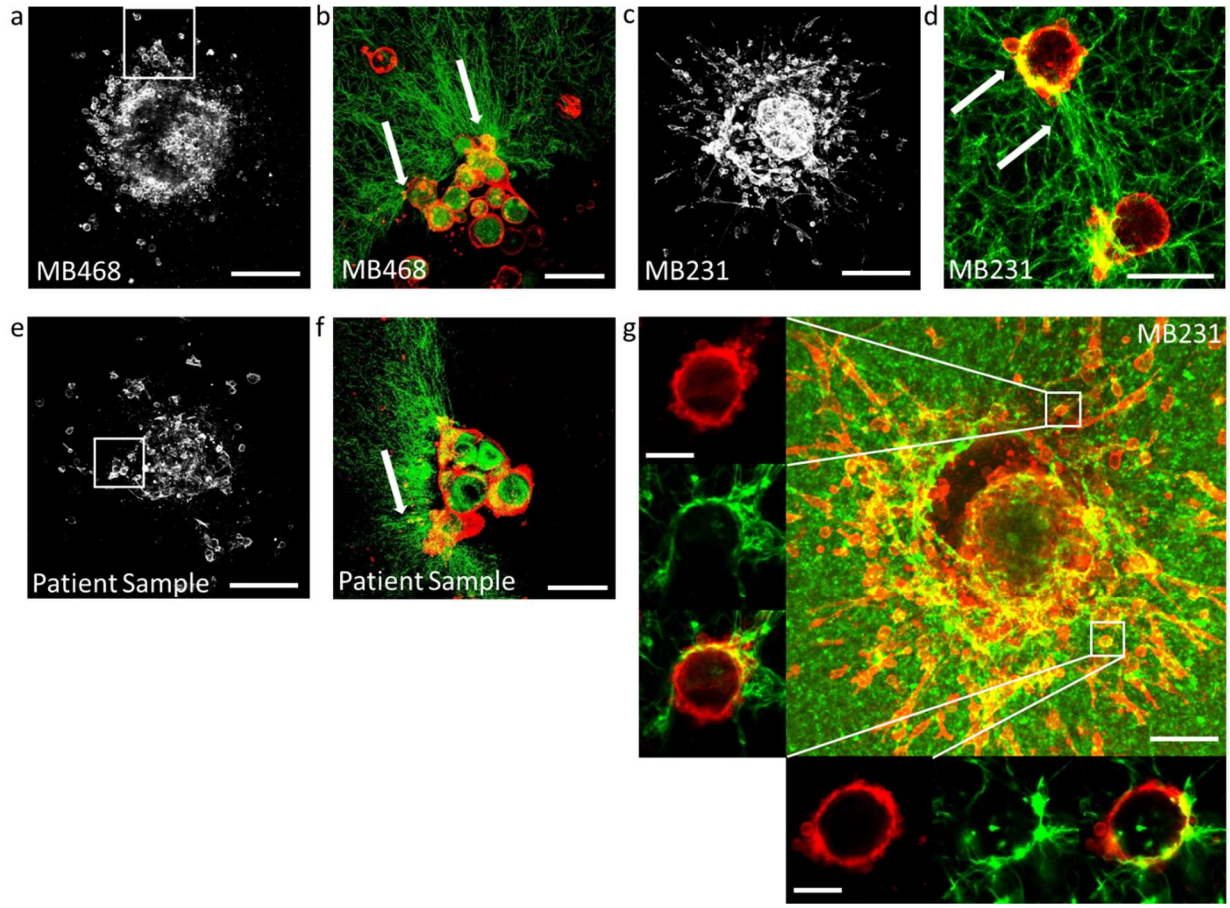


Figure S3. (a, c, e, g) Representative confocal fluorescence microscopy maximum projections over ≈ 50 µm of (a, c, e) phalloidin-stained (a) MDA-MB-468 (c) MDA-MB-231, and (e) patient-derived breast cancer organoid and (g) CellMask-stained MDA-MB-231 spheroid at t = 24 h, each in 1 mg/ml collagen I. White squares indicate regions presented in higher magnification in b, f, and g. (b, d, f) Representative images of phalloidin-stained (b) MDA-MB-468, (d) MDA-MB-231, and (f) patient-derived breast cancer cells (red) invading as clusters or individual cells and (green) their immediate collagen environment. (g) Representative images of (red) CellMask stained MDA-MB-231 cells showing polarized bleb bearing regions and (green) their immediate collagen environment. White arrows indicate sites of collagen fiber alignment adjacent to bleb-bearing regions of cells, showing efficient invasion of round bleb-bearing cells into the collagen I matrix accompanied by extensive collagen re-organization. In (b) and (f), dark regions that are apparently devoid of collagen are caused by out of plane portions of the spheroid or organoid. (a, c, e) Scale bar = 200 µm; (g) Scale bars = 100 µm on large image and 10 µm on the small images; (b, f) Scale bar = 50 µm; (d) Scale bar = 10 µm.

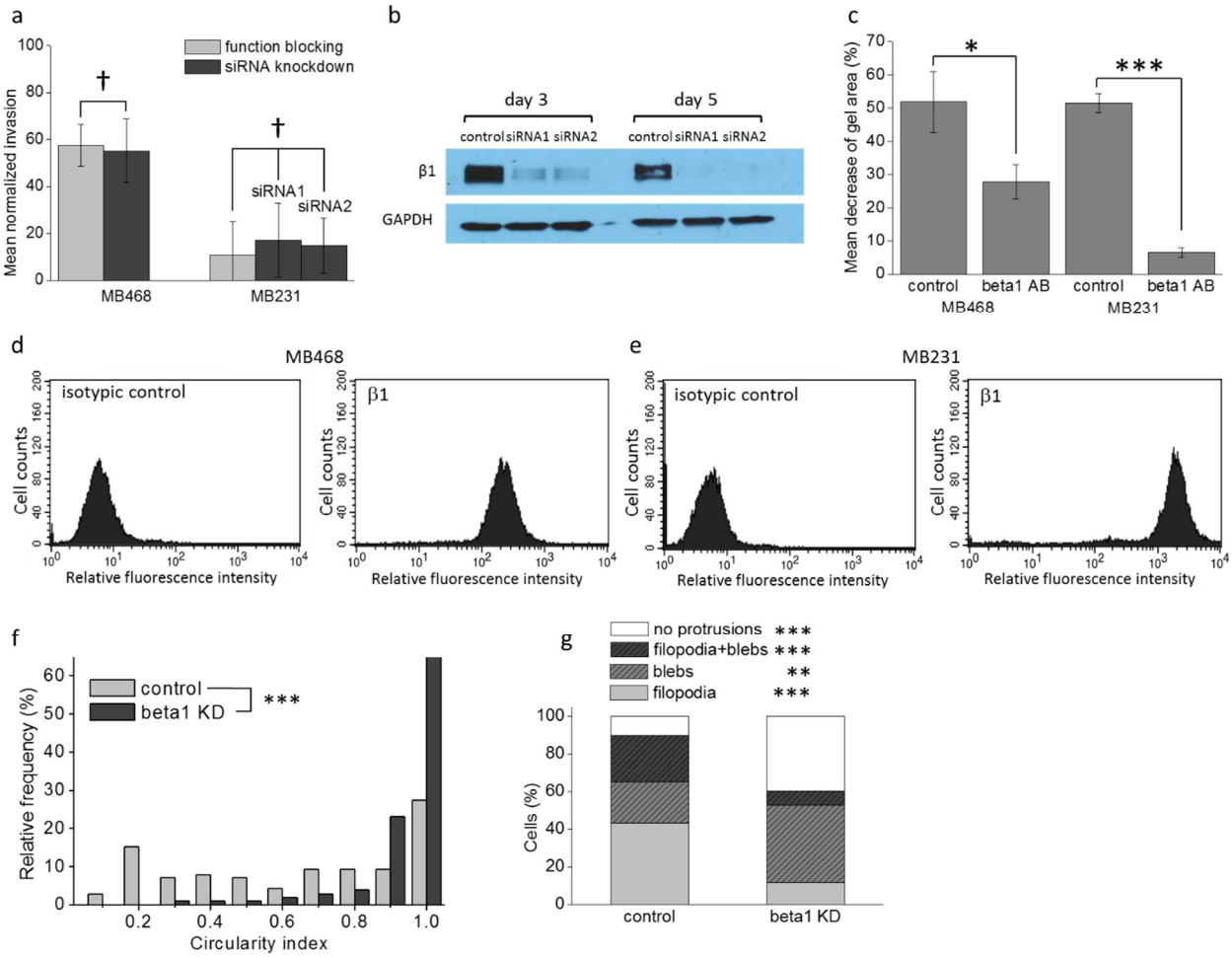


Figure S4. (a) Comparison of invasion reduction through function blocking antibody (AB) and siRNA-mediated integrin knockdown, with two siRNAs used for the MDA-MB-231 cells (siRNA1 and siRNA2). Mean invasion relative to solvent or non-targeting siRNA controls ± SD for MDA-MB-468 and MDA-MB-231 in pure collagen at t = 24 h is shown. n = 11 (MDA-MB-468 function blocking AB and control), 12 (MDA-MB-468 siRNA1 and control), 10 and 11 (MDA-MB-231 function blocking AB and control), 13 (MDA-MB-231 siRNA1 and control) and 13 and 12 (MDA-MB-231 siRNA2 and control). t-tests were used to compare normalized invasion under the function blocking antibody- and siRNA-mediated integrin inhibition (within one cell line) and the resulting inhibitory effects were found to be statistically equal. (b) SDS-PAGE and Western Blot of cell lysates from MDA-MB-231 cells transfected with either control, siRNA1, or siRNA 2 and harvested on either day 3 or day 5. Equal amounts of protein were loaded for each condition. Western Blot was probed against β1 integrin to show knockdown efficiency, and GAPDH was used as a control to show equal loading. siRNA1 is used at all other points in this paper. (c) Collagen gel contraction by MDA-MB-468 and MDA-MB-231 cells under antibody-mediated integrin inhibition compared to control shown as mean decrease of the gel area ± SD at t = 24 h. Experiment was performed in biological triplicate. (d, e) β1 integrin expression of (d) MDA-MB-468 and (e) MDA-MB-231 cells were subjected to fluorescent labeling of cell surface β1 integrin via sequential incubation with β1 integrin primary antibody and fluorescently labeled secondary antibody and fluorescence was measured by flow cytometry. Cells treated with secondary antibody only were used as a negative control (left panels). n = 10000 cells for each cell line and condition. (f, g) Analysis of changes in cellular morphology and protrusion type of MDA-MB-231 cells embedded in collagen I matrices following siRNA-mediated β1 integrin

knockdown in comparison to control group. (f) Histogram of cell circularity of β1 integrin knockdown cells to control cells. Differences in distributions were assessed by KS analysis (g) Percentage of both β1 integrin knockdown and control cells bearing at least one protrusion from each class. Actin polymerization-driven protrusions are summarized under "filopodia." Differences in occurrence of each protrusion type were assessed by t-tests. Data was pooled from 3 statistically identical biological replicates. n = 138 and 104 for the control group and the siRNA knockdown, respectively.

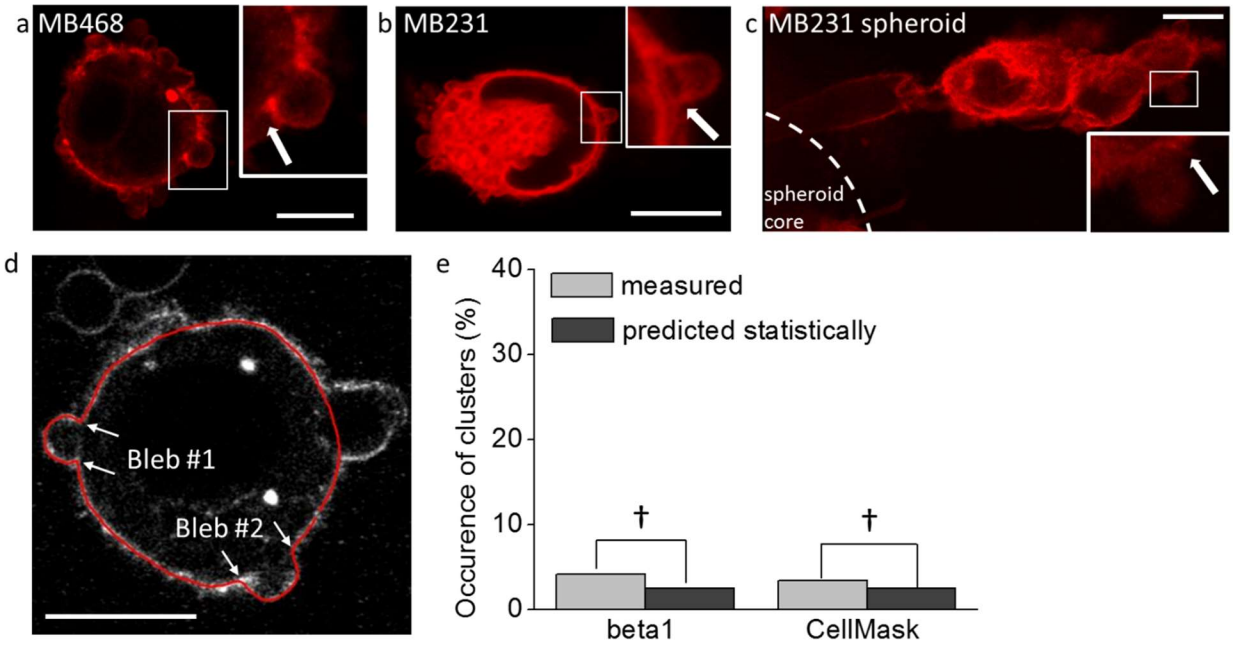


Figure S5. (a-c) CellMask images of the 1 mg/ml collagen I-embedded (a) MDA-MB-231 cell (b) MDA-MB-468 cell and (c) the edge of an MDA-MB-231 spheroid shown for each of the three images also shown in Fig. 5 in the main text. (c) White dashed lines show the edge of the spheroid. (d) Representative example of region selection for cluster analysis. Image shows a single confocal slice of a cell with the region of interest (ROI) shown in red. A more complete explanation of the protocol can be found in Materials and Methods in the main text. In (a-d), arrows indicate bleb necks. t = 6 h. Scale bar = 10 µm. (e) Analysis of integrin distribution on the cell membrane of collagen I-embedded MDA-MB-231 cells immunostained for cell surface total β1 integrins and CellMask. The measured occurrences of β1 integrin and CellMask clusters on the bulk cell membrane was compared to values predicted for normal distributions by t-test between percents and were found to be statistically equal. Data was pooled from 3 statistically identical biological replicates. n = 48 (individual, non-overlapping confocal slices were treated as independent samples).

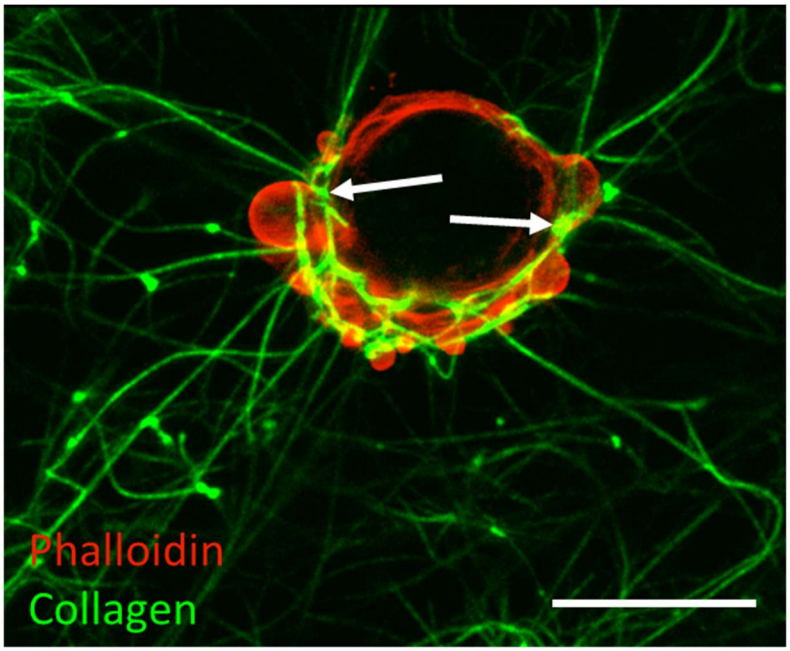


Figure S6. Representative image of an MDA-MB-231 cell stained with (red) phalloidin in a (green) 1 mg/ml collagen I matrix. Image shows polarized bleb formation as well as collagen alignment at bleb necks (white arrows). t = 6 h. Scale bar = 10 μm.

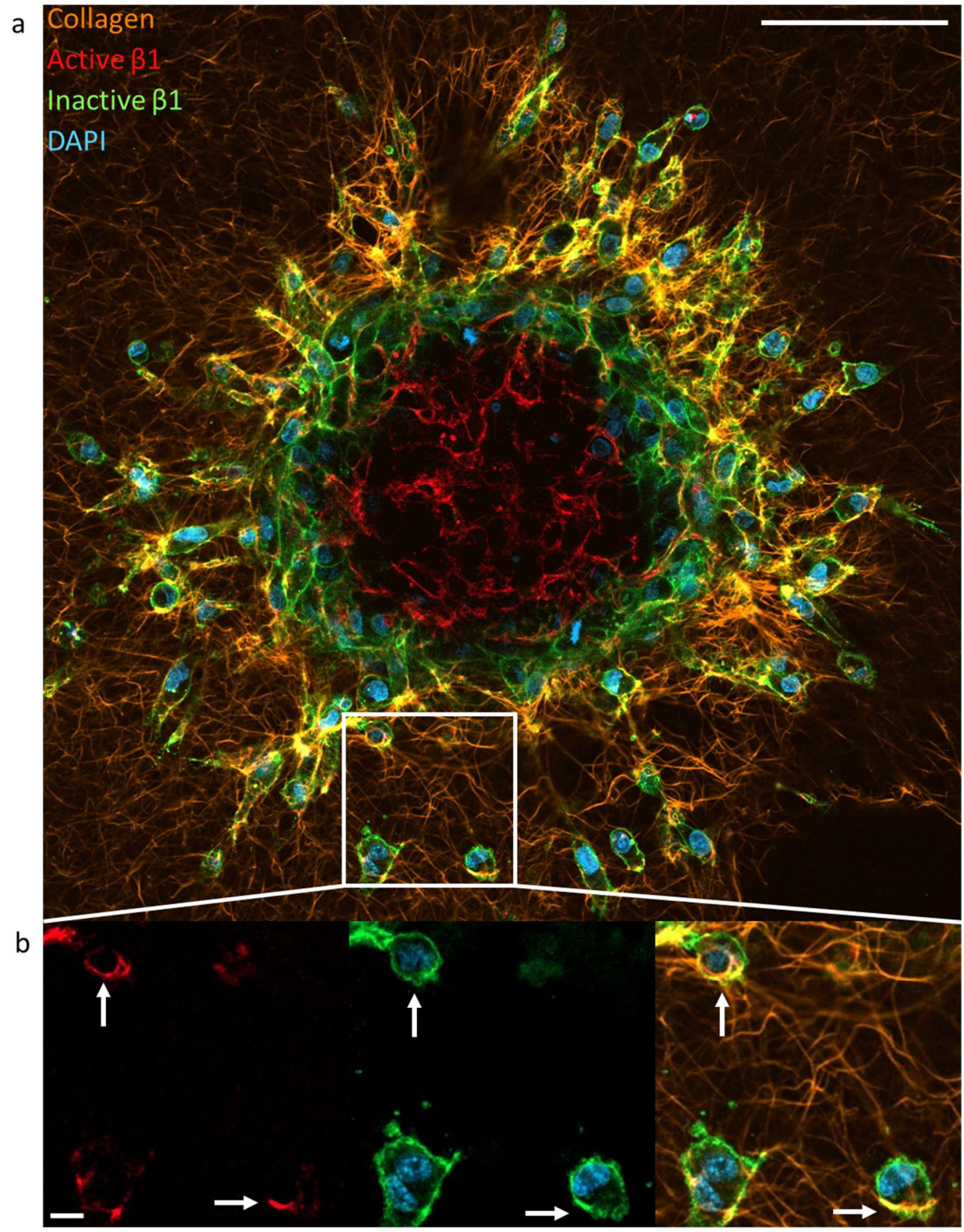


Figure S7. Representative CFM image of an MDA-MB-231 spheroid triple stained for (red) active β1 integrin, (green) inactive β1 integrin and (blue) DAPI implanted into an (orange) 1 mg/ml collagen I matrix showing round, bleb-bearing cells invading. (a) Spheroid core and invading cells. White square shows

region of interest, which is represented at higher magnification below. (b) Higher magnification images collectively show the accumulation of (left) active β1 integrin, (center) polarized, bleb-bearing cells, and (right) accumulation of collagen overlapping with both the accumulation of active β1 integrin and the polarized distribution of blebs. White arrows note round, bleb-bearing cells with active β1 integrin and collagen accumulation. t = 6 h. (a) Scale bar = 100 μm; (b) Scale bar = 10 μm.

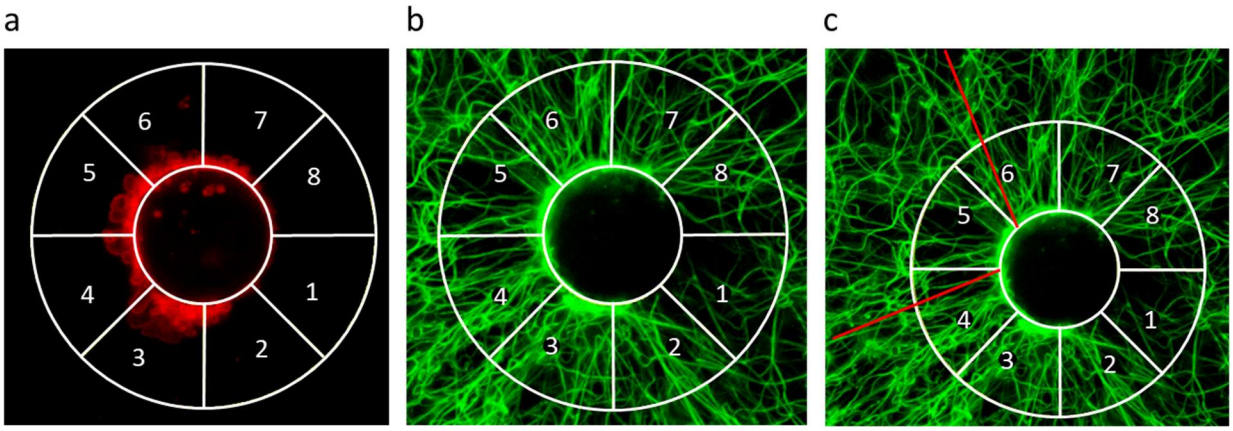


Figure S8. Representative example of segmentation for collagen orientation analysis. Measurement regions surrounding a cell for the (a) phalloidin (red) channel and (b) collagen (green) channel. Panel (a) shows the division of the cell into non-blebby regions (#1 and #8) and blebby regions (#2-7). The red lines in (c) indicate the 90° angular window corresponding to collagen alignment for region 5.

Supplementary Table

Table S1. Details of statistical analyses performed.

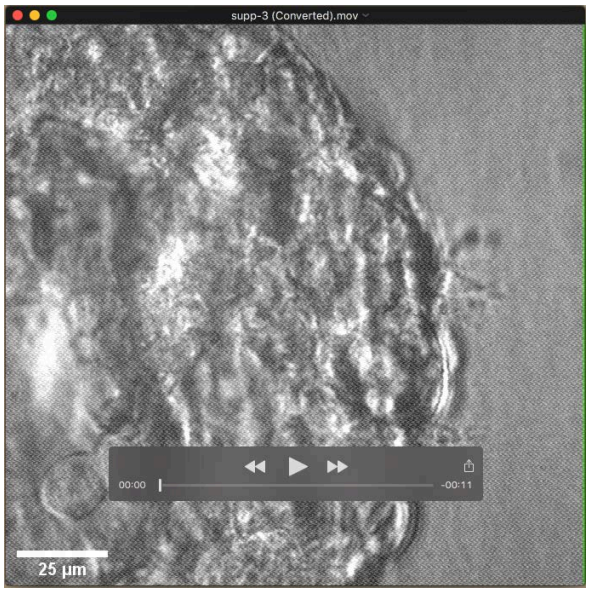
Data	Figure	Test For (Test Used)	Software Used	Test Details (α = 0.05)			Meaning of Results
Cell Morphology							
Circularity Parameter	1b	1. Compare distributions by date (KS Test)	XLSTAT	468 Coll I p > 0.05			All dates are equal
				468 Coll-BME p > 0.05 excluding one pair where p = 0.02			All dates are equal except for one date pairing
		2. Compare distributions by matrix (KS Test)	R	p > 0.05			Equal circularity
	1d	1. Compare distributions by date (KS Test)	XLSTAT	231 Coll I p > 0.05			All dates are equal
				231 Coll-BME p > 0.05 excluding two pair where p = 0.026 and p < 0.0001			All dates are equal except for one date pairing
		2. Compare distributions by matrix (KS Test)	R	p < 0.0001			231 Coll-BME is more round than 231 Coll I
	S4f	1. Compare distributions by date (KS Test)	XLSTAT	231 β1 KD p > 0.05			All dates are equal
				231 Scrambled p > 0.05			All dates are equal
		2. Compare distributions by matrix (KS Test)	R	p < 0.0001			231 β1 KD is more round than 231 Scrambled
Protrusions	1c	1. Compare Occurrence of protrusions by date (Single Factor ANOVA)	Excel	468 Coll I		p > 0.05	All dates are equal
				468 Coll-BME		p > 0.05	All dates are equal
		2. Compare occurrence of protrusions by matrix (Single Factor ANOVA)	Excel	Blebs p < 0.0001			468 Coll-BME has more
				Actin-Driven p < 0.0001			468 Coll I has more
				Both p = 0.037			468 Coll I has more
				Neither p = 0.037			468 Coll I has more
	1e	1. Compare Occurrence of protrusions by date (Single Factor ANOVA)	Excel	231 Coll I		p > 0.05	All dates are equal
				231 Coll-BME		p > 0.05	All dates are equal
		2. Compare occurrence of protrusions by matrix (Single Factor ANOVA)	Excel	Blebs p < 0.0001			231 Coll-BME has more
				Actin-Driven p < 0.0001			231 Coll I has more
				Both p > 0.05			231 Coll I has more
				Neither p = 0.001			231 Coll-BME has more
	S4g	1. Compare Occurrence of protrusions by date (Single Factor ANOVA)	Excel	231 β1 KD		p > 0.05	All dates are equal
				231 Scrambled		p > 0.05	All dates are equal
		2. Compare occurrence of protrusions by matrix (Single Factor ANOVA)	Excel	Blebs p = 0.001			β1 KD has more
				Actin-Driven p < 0.0001			Scrambled has more
				Both p = 0.0002			Scrambled has more
				Neither p < 0.0001			β1 KD has more
	6i	1. Comparison of Percents (Two Sample t-test between Percents)	Statistics Calculator	Clustered Blebs		p < 0.0001	Control has more
				Single Blebs		p < 0.0001	β1 KD has more
Circularity vs. Protrusions	S2a	1. Comparison of Percent of Round Cells Carrying Blebs vs. Actin-Driven Protrusions (One Sample t-test between percents)	Statistics Calculator	468 Coll I		p < 0.0001	Blebs are more common
				468 Coll-BME		p < 0.0001	Blebs are more common
				231 Coll I		p = 0.0386	Blebs are more common
				231 BME		p < 0.0001	Blebs are more common
	S2b	1. Comparison of Percent of Elongated Cells Carrying Blebs vs. Actin-Driven Protrusions (One Sample t-test between percents)	Statistics Calculator	468 Coll I		p > 0.05	Equal occurrence
				468 Coll-BME		p > 0.05	Equal occurrence
				231 Coll I		p < 0.0001	Actin-driven are more common
				231 BME		p > 0.05	Equal occurrence
Spheroid Invasion and Gel Contraction							
Impact of β1		1. Comparison of invasive distance under various β1 inhibition (Two-Tailed t-test)	Statistics Calculator	468		p > 0.05	Invasive distance is identical
				231		p > 0.05	Invasive distance is identical
Various Inhibitors	3a	1. Comparison of invasive distance under various treatments (Wilcoxon Rank-Sum)	R	468 Coll I	Rac	p > 0.05	Invasion is not impacted
					ROCK	p = 0.0014	Invasion is decreased
					β1 KD	p < 0.0001	Invasion is decreased
					MMPs	p = 0.0034	Invasion is decreased
	3b	1. Comparison of invasive distance under various treatments (Wilcoxon Rank-Sum)	R	231 Coll I	Rac	p = 0.0413	Invasion is decreased
					ROCK	p = 0.0095	Invasion is decreased
					β1 KD #1	p < 0.0001	Invasion is decreased
					β1 KD #2	p = 0.0002	Invasion is decreased
					MMPs	p > 0.05	Invasion is not impacted
	3c	1. Comparison of invasive distance under various treatments (Wilcoxon Rank-Sum)	R	231 Coll-BME	Rac	p < 0.0001	Invasion is decreased
					ROCK	p < 0.0001	Invasion is decreased
					β1 KD	p = 0.0002	Invasion is decreased
					MMPs	p < 0.0001	Invasion is decreased
	S4a	1. Comparison of function blocking β1 antibody to β1 siRNA knockdown (t test between percents)	Statistics Calculator	468	siRNA1	p > 0.05	Invasion is impacted equally
				231	siRNA1	p > 0.05	Invasion is impacted equally
				231	siRNA2	p > 0.05	Invasion is impacted equally
		2. Comparison of siRNA #1 to siRNA #2 (t-test between percents)	Statistics Calculator	231		p > 0.05	Invasion is impacted equally
Gel Contraction	S4c	1. Comparison of Gel Contraction under β1 antibody inhibition (Wilcoxon Rank-Sum)	R	231		p = 0.0265	KD decreases contraction
				468		p = 0.0284	KD decreases contraction

Data	Figure	Test For (Test Used)	Software Used	Test Details ($\alpha = 0.05$)			Meaning of Results
Occurrence of β1 Clusters							
Removal of Outlier	NA	1. Removal of outlying bleb based on bleb perimeter (Two Tailed Grubb's Test for Outliers)	Excel	$p = 0.0023$			Bleb is an outlier
β1 vs CellMask Clusters at the Bleb Neck - 468	4d	1. Determination of Skew and Kurtosis of distributions (Skew and Kurtosis)	Excel	β1	Skew	$\bar{s} = 1.223$	Distribution is normal
					Kurtosis	$\bar{k} = 3.690$	Distribution is normal
				CellMask	Skew	$\bar{s} = 0.764$	Distribution is normal
					Kurtosis	$\bar{k} = 2.301$	Distribution is normal
		2. Comparison of Occurrence of Clusters by Date (Two Sample t-test between percents)	Statistics Calculator	β1		$p > 0.05$	All dates are equal
				CellMask		$p > 0.05$	All dates are equal
		3. Comparison of Occurrence of β1 Clusters vs CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	$p < 0.0001$			β1 has more clusters
		4. Comparison of actual vs expected Occurrence of β1 and CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	β1		$p < 0.0001$	There are more β1 Clusters than expected
				CellMask		$p = 0.023$	There are more CellMask Clusters than expected
β1 vs CellMask Clusters at the Bleb Neck - 231	4d	1. Determination of Skew and Kurtosis of distributions (Skew and Kurtosis)	Excel	β1	Skew	$\bar{s} = 0.715$	Distribution is normal
					Kurtosis	$\bar{k} = 0.814$	Distribution is normal
				CellMask	Skew	$\bar{s} = 0.207$	Distribution is normal
					Kurtosis	$\bar{k} = -0.001$	Distribution is normal
		2. Comparison of Occurrence of Clusters by Date (Two Sample t-test between percents)	Statistics Calculator	β1		$p > 0.05$	All dates are equal
				CellMask		$p > 0.05$	All dates are equal
		3. Comparison of Distribution by Date (Single Factor ANOVA)	Excel	β1	Skew	$p > 0.05$	All dates are equal
					Kurtosis	$p > 0.05$	All dates are equal
				CellMask	Skew	$p > 0.05$	All dates are equal
					Kurtosis	$p > 0.05$	All dates are equal
		4. Comparison of Occurrence of β1 Clusters vs CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	$p = 0.0012$			β1 has more clusters
		5. Comparison of actual vs expected Occurrence of β1 and CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	β1		$p = 0.028$	There are more β1 Clusters than expected
				CellMask		$p > 0.05$	There are equal CellMask Clusters to expected
β1 vs CellMask Clusters on the Bulk Membrane - 231	S5e	1. Comparison of actual vs expected Occurrence of β1 and CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	β1		$p > 0.05$	There are equal β1 Clusters to expected
				CellMask		$p > 0.05$	There are equal CellMask Clusters to expected
Dependence of Bleb Size on β1 Cluster Occurrence - 231	S2c	1. Dependence of bleb perimeter on the Occurrence of β1 clusters (Two-Tailed t-test)	Excel	$p > 0.05$			β1 Clusters occur independently of perimeter
	S2d	1. Dependence of bleb neck width on the Occurrence of β1 clusters (Two-Tailed t-test)	Excel	$p > 0.05$			β1 Clusters occur independently of neck width
β1 vs CellMask Clusters at the Bleb Neck - 231 Spheroids	4d	1. Determination of Skew and Kurtosis of distributions (Skew and Kurtosis)	Excel	β1	Skew	$\bar{s} = 0.895$	Distribution is normal
					Kurtosis	$\bar{k} = 1.416$	Distribution is normal
				CellMask	Skew	$\bar{s} = 0.324$	Distribution is normal
					Kurtosis	$\bar{k} = 0.253$	Distribution is normal
		2. Comparison of Occurrence of Clusters by Date (Two Sample t-test between percents)	Statistics Calculator	β1		$p > 0.05$	All dates are equal
				CellMask		$p > 0.05$	All dates are equal
		3. Comparison of Occurrence of β1 Clusters vs CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	$p < 0.0001$			β1 has more clusters
		4. Comparison of actual vs expected Occurrence of β1 and CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	β1		$p < 0.0001$	There are more β1 Clusters than expected
				CellMask		$p = 0.0001$	There are more CellMask Clusters than expected

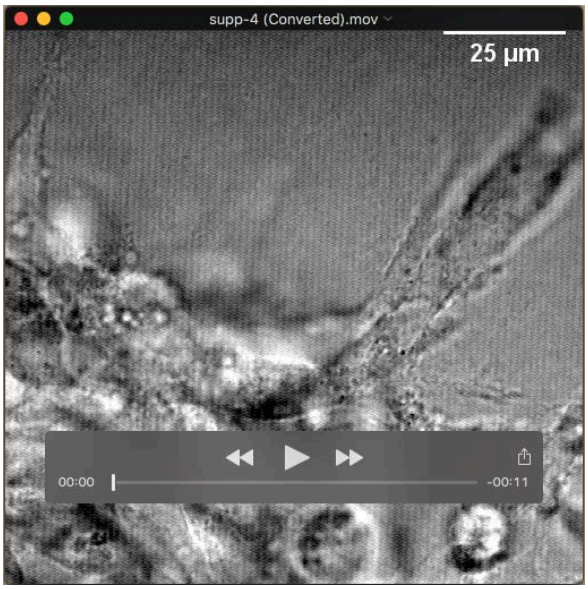
Data	Figure	Test For (Test Used)	Software Used	Test Details ($\alpha = 0.05$)			Meaning of Results
β1 vs Talin Clusters at the Bleb Neck - 231	5c	1. Determination of Skew and Kurtosis of distributions (Skew and Kurtosis)	Excel	β1	Skew	$\bar{s} = 0.682$	Distribution is normal
					Kurtosis	$\bar{k} = 1.003$	Distribution is normal
				Talin	Skew	$\bar{s} = 0.906$	Distribution is normal
					Kurtosis	$\bar{k} = 1.604$	Distribution is normal
		2. Comparison of Occurrence of Clusters by Date (Two Sample t-test between percents)	Statistics Calculator	β1		p > 0.05	All dates are equal
				Talin		p > 0.05	All dates are equal
		3. Comparison of Occurrence of β1 Clusters vs Talin Clusters (One Sample t-test between Percents)	Statistics Calculator	p > 0.05			β1 has equal clusters to Talin
		4. Comparison of actual vs expected Occurrence of β1 and Talin Clusters (One Sample t-test between Percents)	Statistics Calculator	β1		p < 0.0001	There are more β1 Clusters than expected
				Talin		p = 0.0001	There are more Talin Clusters than expected
		5. Comparison of actual vs expected Occurrence of β1 and Talin Co-clusters (One Sample t-test between Percents	Statistics Calculator	p = 0.0025			There are more β1 and Talin Co-clusters than expected
β1 vs Vinculin Clusters at the Bleb Neck - 231	5c	1. Determination of Skew and Kurtosis of distributions (Skew and Kurtosis)	Excel	β1	Skew	$\bar{s} = 1.000$	Distribution is normal
					Kurtosis	$\bar{k} = 1.614$	Distribution is normal
				Vinculin	Skew	$\bar{s} = 1.107$	Distribution is normal
					Kurtosis	$\bar{k} = 2.161$	Distribution is normal
		2. Comparison of Occurrence of Clusters by Date (Two Sample t-test between percents)	Statistics Calculator	β1		p > 0.05	All dates are equal
				Vinculin		p > 0.05	All dates are equal
		3. Comparison of Occurrence of β1 Clusters vs Vinculin Clusters (One Sample t-test between Percents)	Statistics Calculator	p < 0.0001			β1 has more clusters than Vinculin
		4. Comparison of actual vs expected Occurrence of β1 and Vinculin Clusters (One Sample t-test between Percents)	Statistics Calculator	β1		p < 0.0001	There are more β1 Clusters than expected
				Vinculin		p > 0.05	There are equal Vinculin Clusters to expected
		5. Comparison of actual vs expected Occurrence of β1 and Vinculin Co-clusters (One Sample t-test between Percents	Statistics Calculator	p < 0.0001			There are more β1 and Vinculin Co-clusters than expected
Active β1 vs Inactive β1 vs CellMask Clusters at the Bleb Neck - 231	6c	1. Determination of Skew and Kurtosis of distributions (Skew and Kurtosis)	Excel	Active β1	Skew	$\bar{s} = 0.608$	Distribution is normal
					Kurtosis	$\bar{k} = 0.533$	Distribution is normal
				Inactive β1	Skew	$\bar{s} = 2.697$	Distribution is not normal
					Kurtosis	$\bar{k} = 11.836$	Distribution is not normal
				CellMask	Skew	$\bar{s} = 0.217$	Distribution is normal
					Kurtosis	$\bar{k} = -0.096$	Distribution is normal
		2. Comparison of Occurrence of Clusters by Date (Two Sample t-test between percents)	Statistics Calculator	Active β1		p > 0.05	All dates are equal
				Inactive β1		p > 0.05	All dates are equal
				CellMask		p > 0.05	All dates are equal
		3. Comparison of Occurrence of Active β1	Statistics	p = 0.001			Active β1 has more clusters than
		4. Comparison of Occurrence of Inactive β1 Clusters vs CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	p > 0.05			Inactive β1 has equal clusters to CellMask
		5. Comparison of Occurrence of Active β1 Clusters vs Inactive β1 Clusters (One Sample t-test between Percents)	Statistics Calculator	p = 0.011			Active β1 has more clusters than Inactive β1
		6. Comparison of actual vs expected Occurrence of β1 and CellMask Clusters (One Sample t-test between Percents)	Statistics Calculator	Active β1		p < 0.0001	There are more Active β1 Clusters than expected
				Inactive β1		p = 0.005	There are more Inactive β1 Clusters than expected
				CellMask		p = 0.004	There are more CellMask Clusters than expected
Active β1 vs Inactive β1 against Collagen I Pearson's R Values	6f	1. Comparison of Pearson's R Values between Active β1 and Collagen and Inactive β1 and Collagen (Wilcoxon Rank-Sum)	R	p = 0.0016			Active β1 is more correlated with collagen accumulation than inactive β1

Data	Figure	Test For (Test Used)	Software Used	Test Details ($\alpha = 0.05$)		Meaning of Results
Collagen Pulling						
Collagen Density	8e	1. Data resampling to determine normality (Bootstrap Resampling)	R	Cell-Free Gels	Distribution is Normal	
				β1 KD	Distribution is Normal	
				Non-Blebby Region	Distribution is Normal	
				Blebby Region	Distribution is Normal	
		2. Analysis of Density of Collagen I in Bleb Bearing Regions of Cells as compared to other regions (Two Tailed t-test)	R	Blebby to Non-blebby	$p < 0.0001$	Blebby Regions have higher
				Blebby to β1 KD	$p < 0.0001$	Blebby Regions have higher
				Blebby to Cell Free	$p < 0.0001$	Blebby Regions have higher collagen density
Collagen Alignment	8f	1. Data resampling to determine normality (Bootstrap Resampling)	R	Cell-Free Gels	Distribution is Normal	
				β1 KD	Distribution is Normal	
				Non-Blebby Region	Distribution is Normal	
				Blebby Region	Distribution is Normal	
		2. Analysis of Collagen Alignment in Bleb Bearing Regions of Cells as compared to other regions (Two Tailed t-test)	R	Blebby to Non-blebby	$p = 0.0166$	Blebby Regions have more
				Blebby to β1 KD	$p = 0.0023$	Blebby Regions have more
				Blebby to Cell Free	$p < 0.0001$	Blebby Regions have more aligned collagen I
468 Refers to MDA-MB-468						
231 Refers to MDA-MB-231						

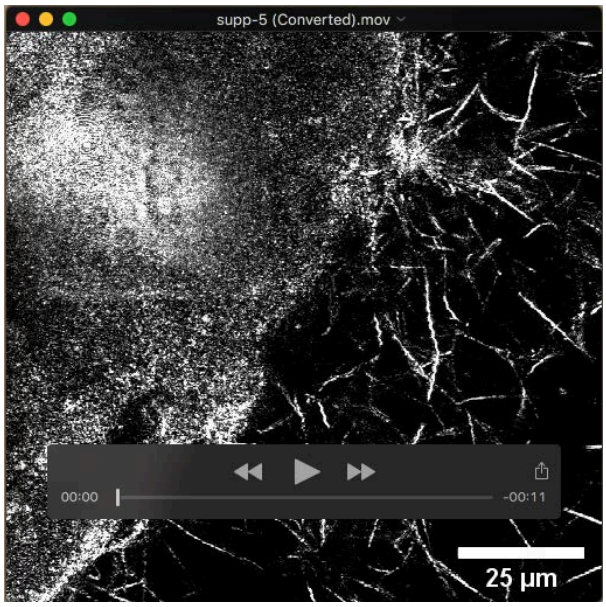
Supplementary Movies



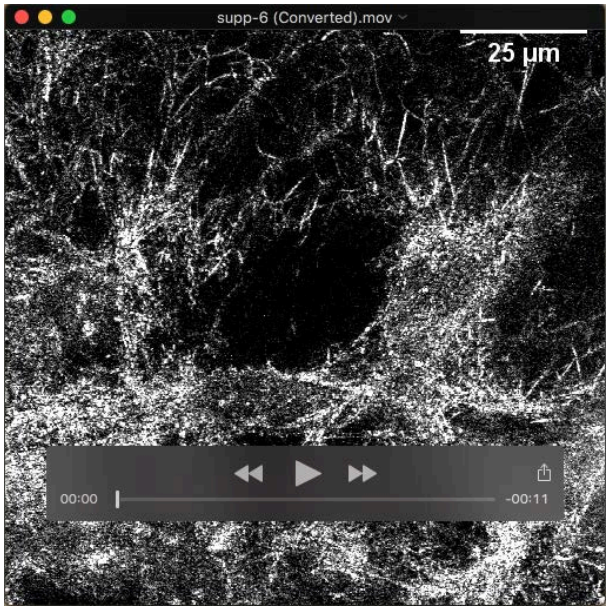
Movie 1. Transmitted light images of an MDA-MB-468 spheroid showing round cell invasion into a 1 mg/ml collagen I gel. Images are taken starting at t = 2.25 h after collagen embedding of the spheroid. Frames were taken every 5 minutes. Length of movie is 6.25 hours. Movie is played back at 7 frames/s. Frames from this movie are also shown in Figs. S1c and Fig. 2 in the main text. Scale bar = 25 µm.



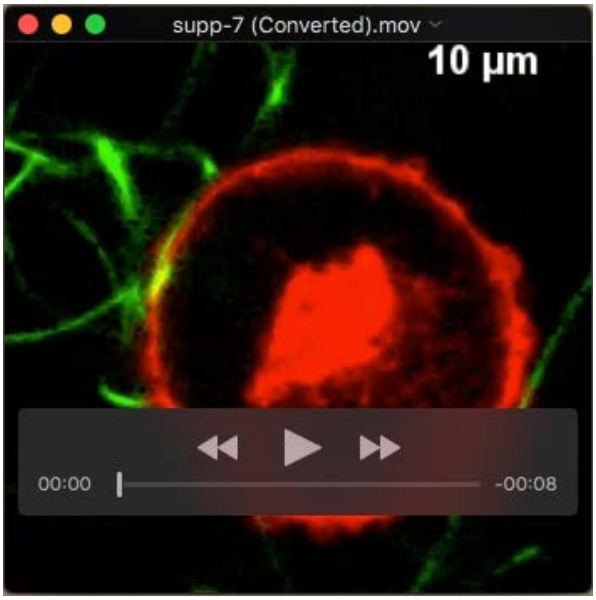
Movie 2. Transmitted light images of an MDA-MB-231 spheroid showing invasion of cells with dynamically changing morphology into a 1 mg/ml collagen I gel. Images are taken every 5 minutes starting at t = 7.75 h after collagen embedding of the spheroid. Length of movie is 6.25 hours. Movie is played back at 7 frames/s. Frames from this movie are also shown in Fig. S1d and Fig. 2 in the main text. Scale bar = 25 µm.



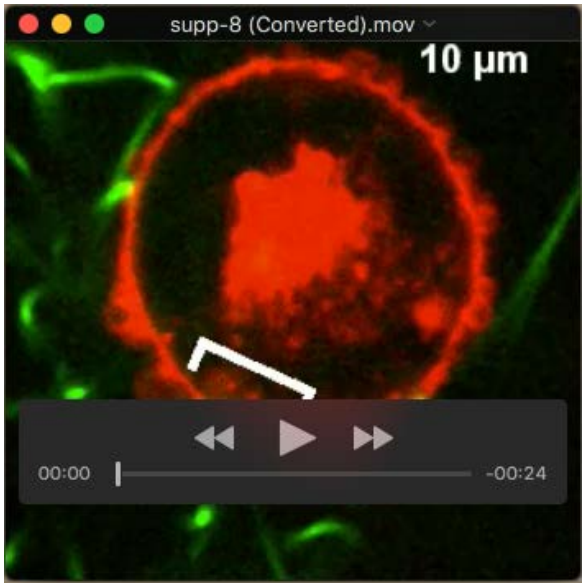
Movie 3. Confocal reflectance images of the collagen matrix surrounding the MDA-MB-468 spheroid shown in M1 (at a slightly different location on the spheroid). Locations of apparent lack of collagen I that develop over the time course of the movie are caused primarily by out of plane spheroid and invading cells limiting excitation light reaching the imaging plane. Images are taken every 5 minutes starting at t ≈ 2.25 h after collagen embedding of the spheroid. Length of movie is 6.25 hours. Movie is played back at 7 frames/s. Frames from this movie are shown in Fig. 2 in the main text. Scale bar = 25 µm.



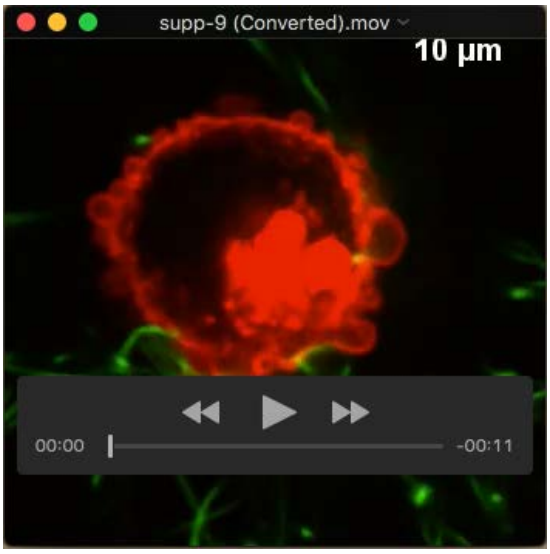
Movie 4. Confocal reflectance images of the collagen matrix surrounding the MDA-MB-231 spheroid shown in M2. Locations of apparent lack of collagen I that develop over the time course of the movie are caused primarily by out of plane spheroid and invading cells limiting excitation light reaching the imaging plane. Images are taken are taken every 5 minutes starting at t ≈ 7.75 h after collagen embedding of the spheroid. Length of movie is 6.25 hours. Movie is played back at 7 frames/s. Frames from this movie are shown in Fig. 2 in the main text. Scale bar = 25 µm.



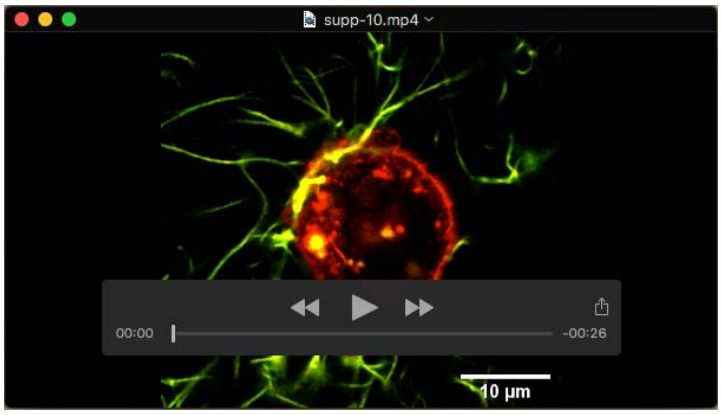
Movie 5. MDA-MB-231 cell labeled with (red) CellMask and embedded in (green) fluorescently labeled collagen I imaged shortly after completion of collagen gelation (t = 36 min after collagen embedding). The cell shows initial attachment to and pulling of a collagen fiber by a single bleb (white arrows). Images were taken every 10 seconds. Length of movie is 560 seconds. Movie is played back at 7 frames/s. Frames from this movie are also shown in Fig. 7A in the main text. Scale bar = 10 µm.



Movie 6. MDA-MB-231 cell also shown in V4 shown over a longer time period starting immediately after completion of collagen gelation (t = 81 min after collagen embedding). The cell shows persistent attachment of collagen fibers at the site of multiple recurrent blebs (bracketed region). Images were taken every 10 seconds. Length of movie is 28.5 minutes. Movie is played back at 7 frames/s. Frames from this movie are also shown in Fig. 7B in the main text. Scale bar = 10 µm.



Movie 7. MDA-MB-231 cell labeled with (red) CellMask and embedded in (green) fluorescently labeled collagen I imaged starting at t = 6 h after cells were embedded in collagen. The cell shows how bleb formation becomes increasingly confined to a particular cell region over time and how the coincident collagen reorganization is spatiotemporally coordinated with the recurrent bleb formation. Images were taken every 10 seconds. Length of movie is 12.5 minutes. Movie is played back at 7 frames/s. Frames from this movie are also shown in Fig. 8B in the main text (rotated by 180°). Scale bar = 10 µm.



Movie 8. MDA-MB-231 cell labeled with (red) CellMask and embedded in (green) fluorescently labeled collagen I imaged starting at t = 5.75 h after cells were embedded in collagen. The cell shows the strongest collagen reorganization at the site of polarized, recurrent clustered blebs. Images were taken every 9.7 seconds. Length of movie is 30 minutes. Movie is played back at 7 frames/s. Frames from this movie are also shown in Fig. 8C,D in the main text. Scale bar = 10 µm.