Electronic Supplementary Material (ESI) for Integrative Biology.

A microfluidic dual gradient generator for conducting cell-based drug combination assays

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SUPPLEMENTARY INFORMATION

Table S1. Statistical comparison of column averages between adjacent columns in the tracer concentration dataset. P-values resulting from t-test are given for select time points.

Time (h)	B vs. C	C vs. D	D vs. E	E vs. F
0.5	0.051	0.004	2.1E-04	0.037
1	0.063	0.010	2.2E-04	0.002
2	0.024	0.007	7.4E-05	1.6E-04
4	0.011	0.002	1.6E-06	0.001
6	0.011	0.002	1.0E-05	0.009
8	0.012	0.001	3.5E-07	0.010

Table S2. Analysis of variance within individual columns in the tracer concentration dataset. P-values resulting from one-way analysis of variance are given for select time points.

Time (h)	B	C	D	E	F
0.5	0.844	0.861	0.952	0.931	0.987
1	0.846	0.610	0.865	0.973	1.000
2	0.952	0.400	0.744	0.967	0.997
4	0.849	0.429	0.912	0.992	0.576
6	0.857	0.674	0.998	0.938	0.674
8	0.871	0.710	0.997	0.980	0.690

Table S3. Results of the one-way analysis of variance in the A431 cell caspase activation control dataset (low EGF, no drugs). Variance among all 25 regions, within columns, within rows, and among column and row averages of percentage of caspase positive cells for indicated time points.

***p*-value**		**2 h**	**4 h**	**6 h**
All regions		0.827	0.662	0.534
Within columns	2	0.581	0.563	0.436
	3	0.072	0.819	0.682
	4	0.846	0.390	0.572
	5	0.890	0.922	0.787
	6	0.025	0.519	0.552
Within rows	B	0.578	0.650	0.800
	C	0.776	0.565	0.665
	D	0.612	0.797	0.725
	E	0.502	0.510	0.642
	F	0.261	0.229	0.286
Between column averages		0.908	0.787	0.847
Between row averages		0.632	0.682	0.576

Table S4. Results of the one-way analysis of variance in the MDA-MB-231 cell motility control dataset. Variance among all 25 regions, within columns, within rows, and among column and row averages of the cell speed (S) and the magnitude of the velocity vector (|V|) were analysed.

***p*-value**		**S**	**|V|**
All regions		0.163	0.900
Within columns	2	0.692	0.798
	3	0.231	0.784
	4	0.465	0.398
	5	0.115	0.366
	6	0.718	0.453
Within rows	B	0.397	0.842
	C	0.539	0.637
	D	0.937	0.918
	E	0.388	0.300
	F	0.166	0.872
Between column averages		0.155	0.719
Between row averages		0.888	0.256

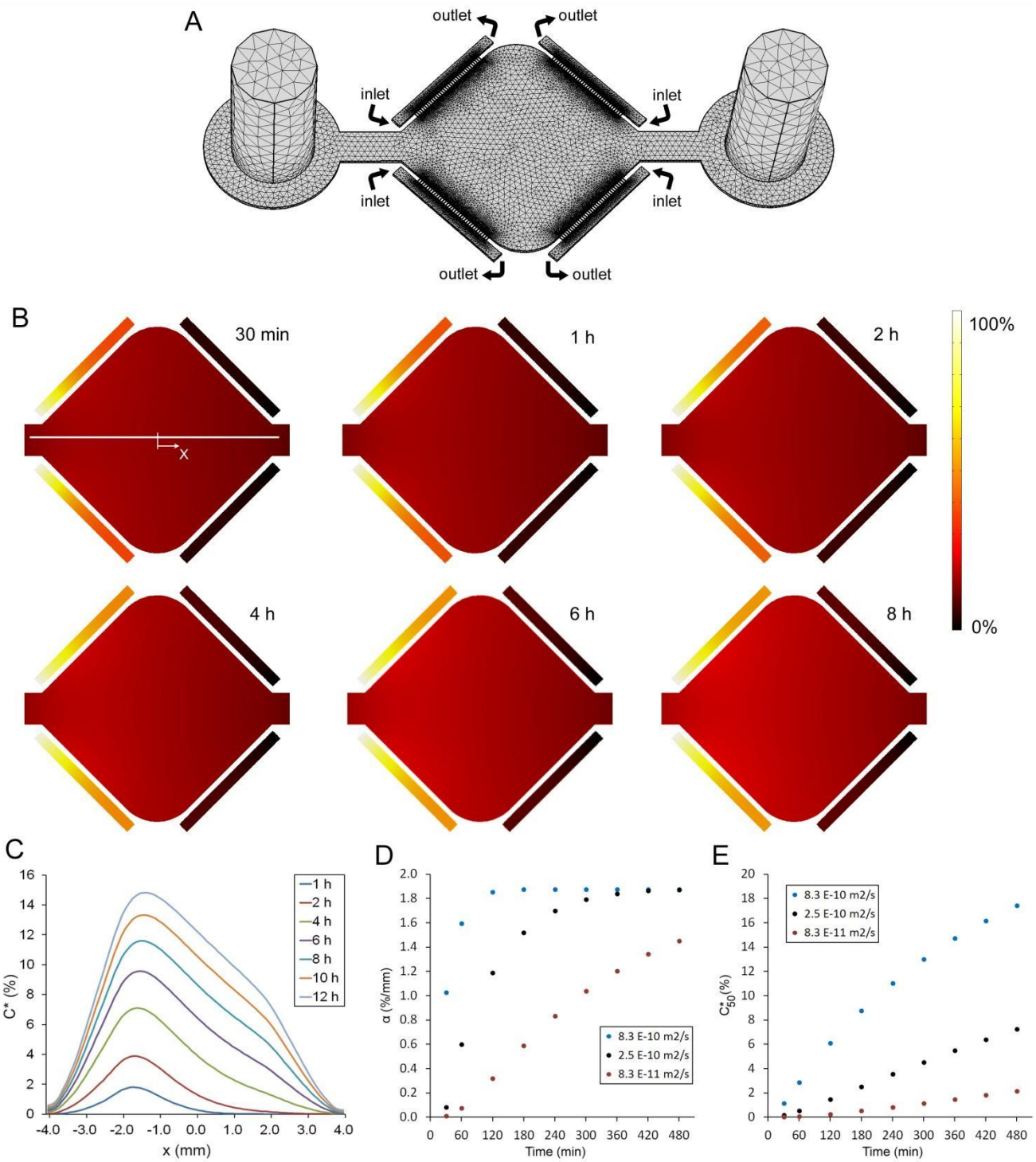


Figure S1. Results of diffusion simulations. ***A.*** Finite-elements mesh on the three-dimensional model. ***B.*** Concentration distribution of a molecule with diffusion coefficient 8.3×10^{-10} m^2/s at indicated time points after the induction of side flows. ***C.*** The concentration profile of the same molecule along the centreline of the cell chamber (white line in ***B***). ***D-E.*** Time profiles of the gradient value (α) and the mid-point concentration (C_{50}*), for varying diffusion coefficients used in the simulations.

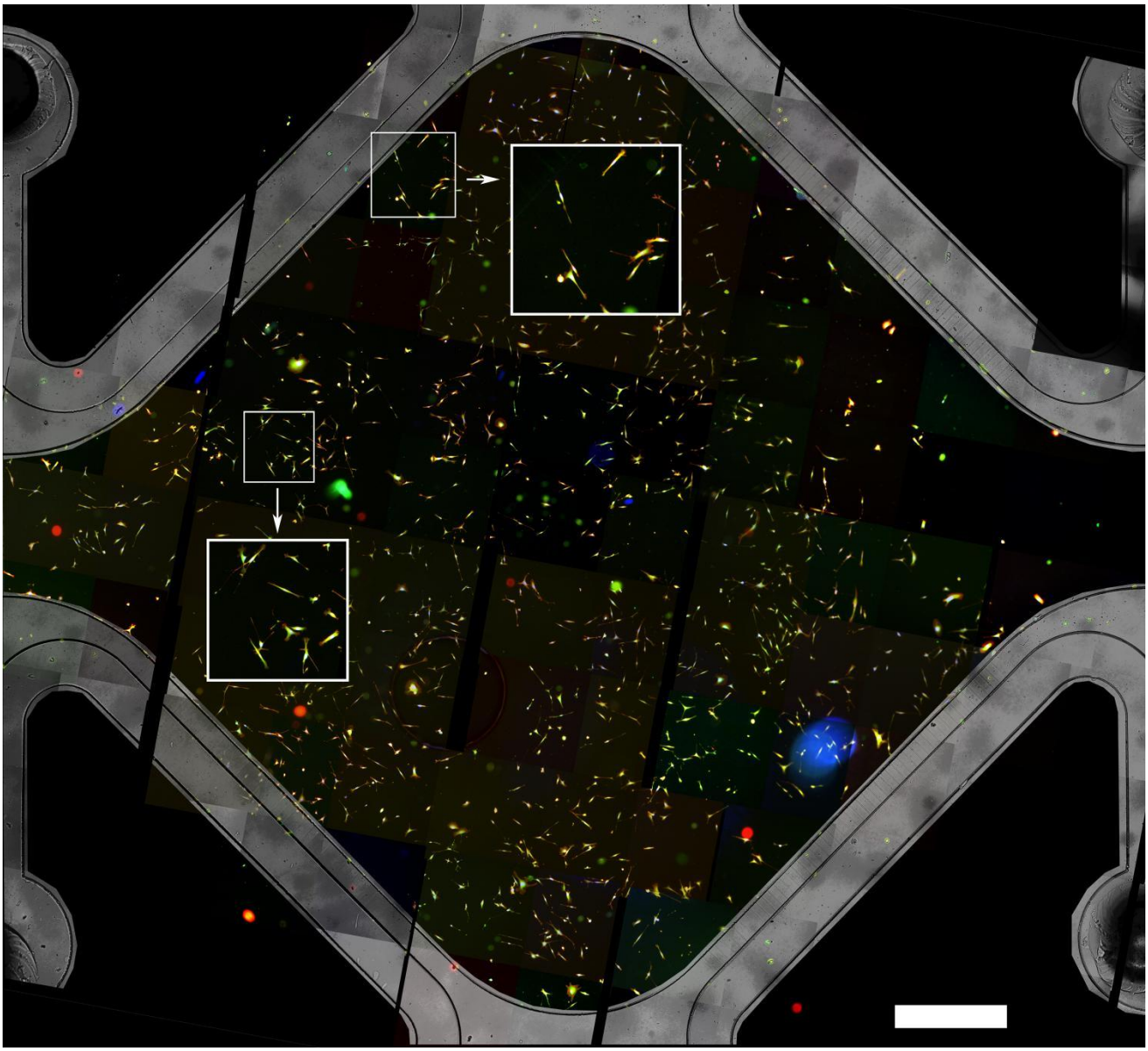


Figure S2. MDA-MB-231 cell culture in the microfluidic device. Cells were immunostained to reveal their actin filaments (red), microtubules (green), and nuclei (blue). The edges of the cell chamber and the flow channel geometry are shown as a brightfield overlay. Marked areas are 2× magnified. Scale bar = 500 µm.

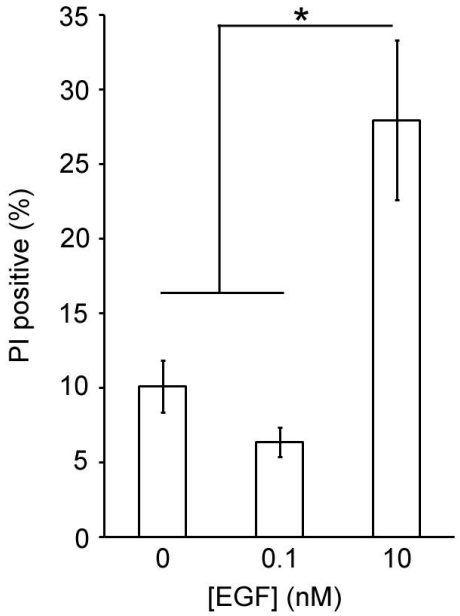


Figure S3. A431 cells were treated with the indicated concentrations of EGF for 16 h and cell death was measured by propidium iodide (PI) staining. N = 3. Error bars show standard deviation. One-way analysis of variance, followed by t-test. * $p < 0.001$.

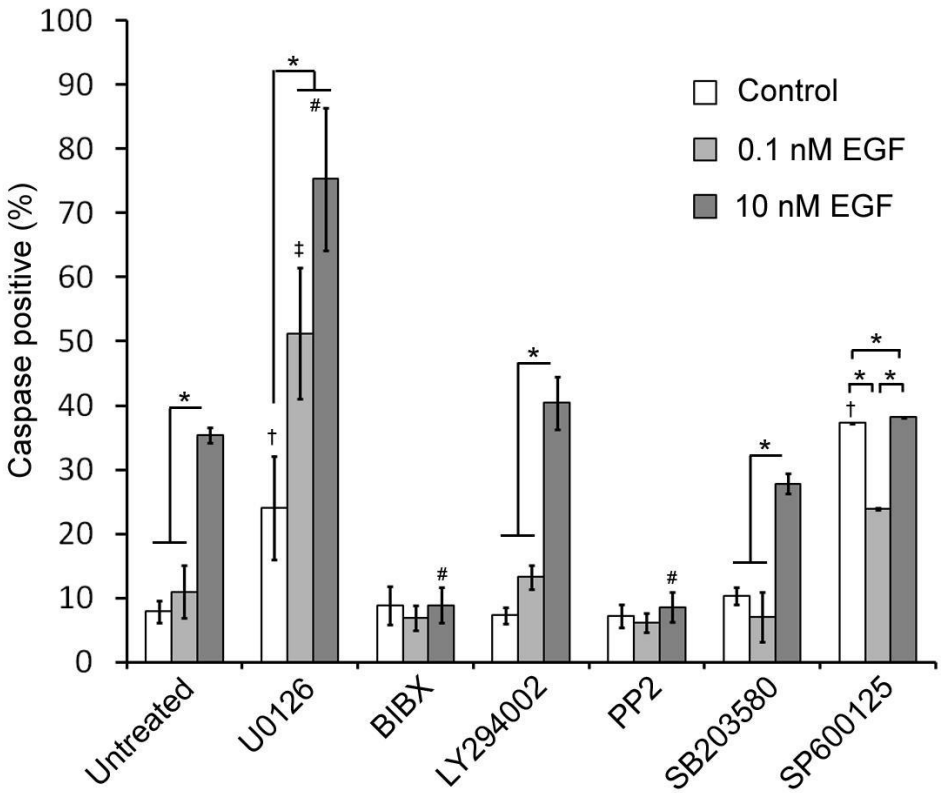


Figure S4. Off-chip experiments for exploring drug combinations that promote apoptotic induction. A431 cells were pre-incubated for 1 h where indicated with 10 µM of MEK inhibitor U0126, 5 µM EGFR inhibitor BIBX, 5 µM PI3K inhibitor LY294002, 5 µM Src inhibitor PP2, 10 µM p38 inhibitor SB203580, or 50 µM JNK inhibitor SP600125. The cells were then grown in full medium without extra EGF or were treated with low (0.1 nM) or high (10 nM) EGF concentrations for 16 h, as indicated. Caspase 3/7 activation was determined using zVAD-fmk-FITC. N = 3. Error bars show standard deviation. One-way analysis of variance, followed by t-test. * $p < 0.01$, † significantly different from (untreated) control; ‡ significantly different from (untreated) low EGF; # significantly different from (untreated)

high EGF.

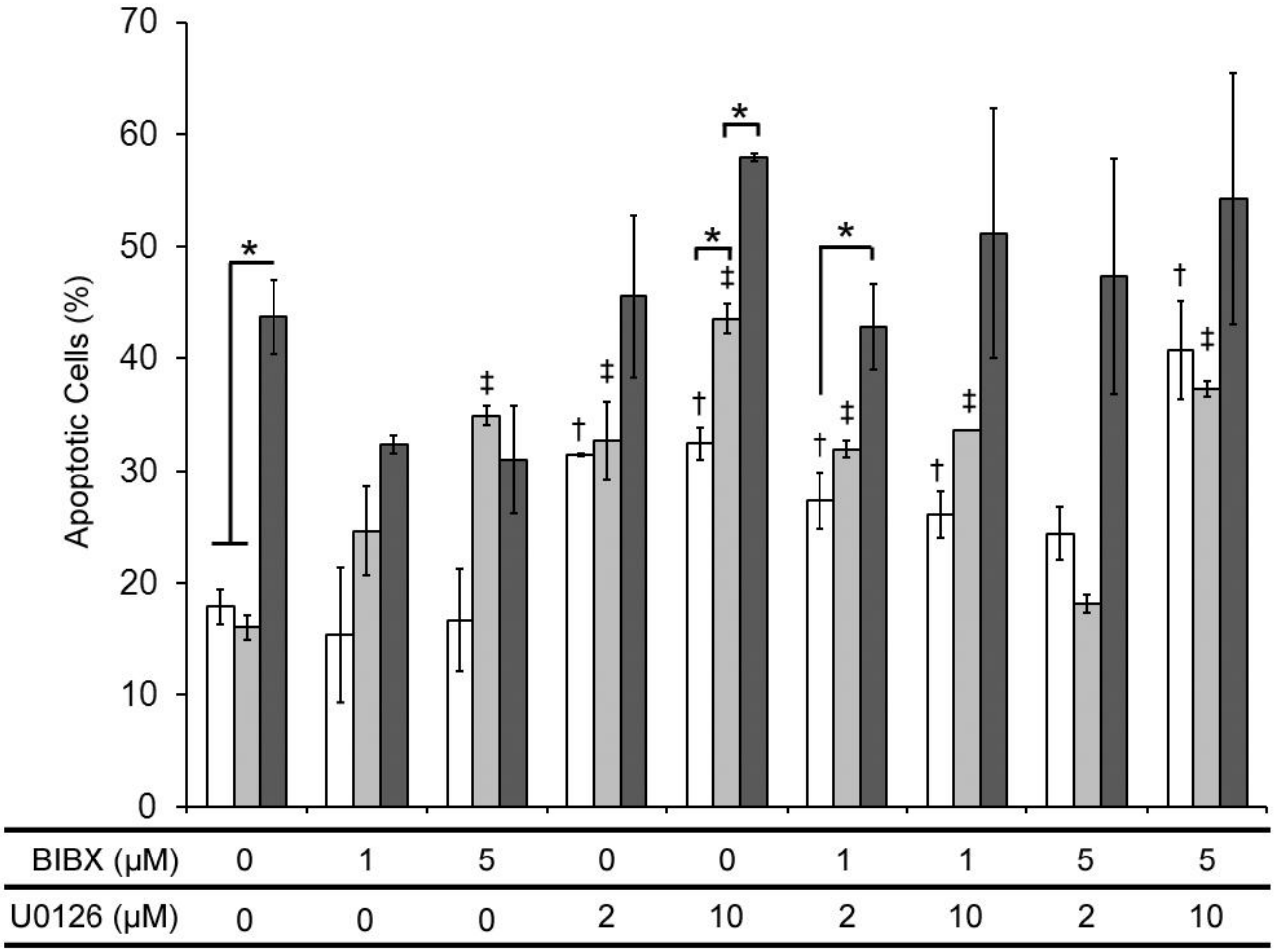


Figure S5. Off-chip titration experiments. A431 cells were incubated with the fluorogenic caspase probe for 30 min and exposed to EGF at low (0.1 nM, light gray) or high (10 nM, dark gray) concentrations and simultaneously to MEK inhibitor U0126 and EGFR inhibitor BIBX at indicated concentrations. Data represents percentage of caspase-positive cells at 3 h time point. N = 3 experiments. Error bars indicate SEM. One-way analysis of variance, followed by t-test. * $p < 0.05$; † significantly different from untreated control; ‡ significantly different from low EGF.

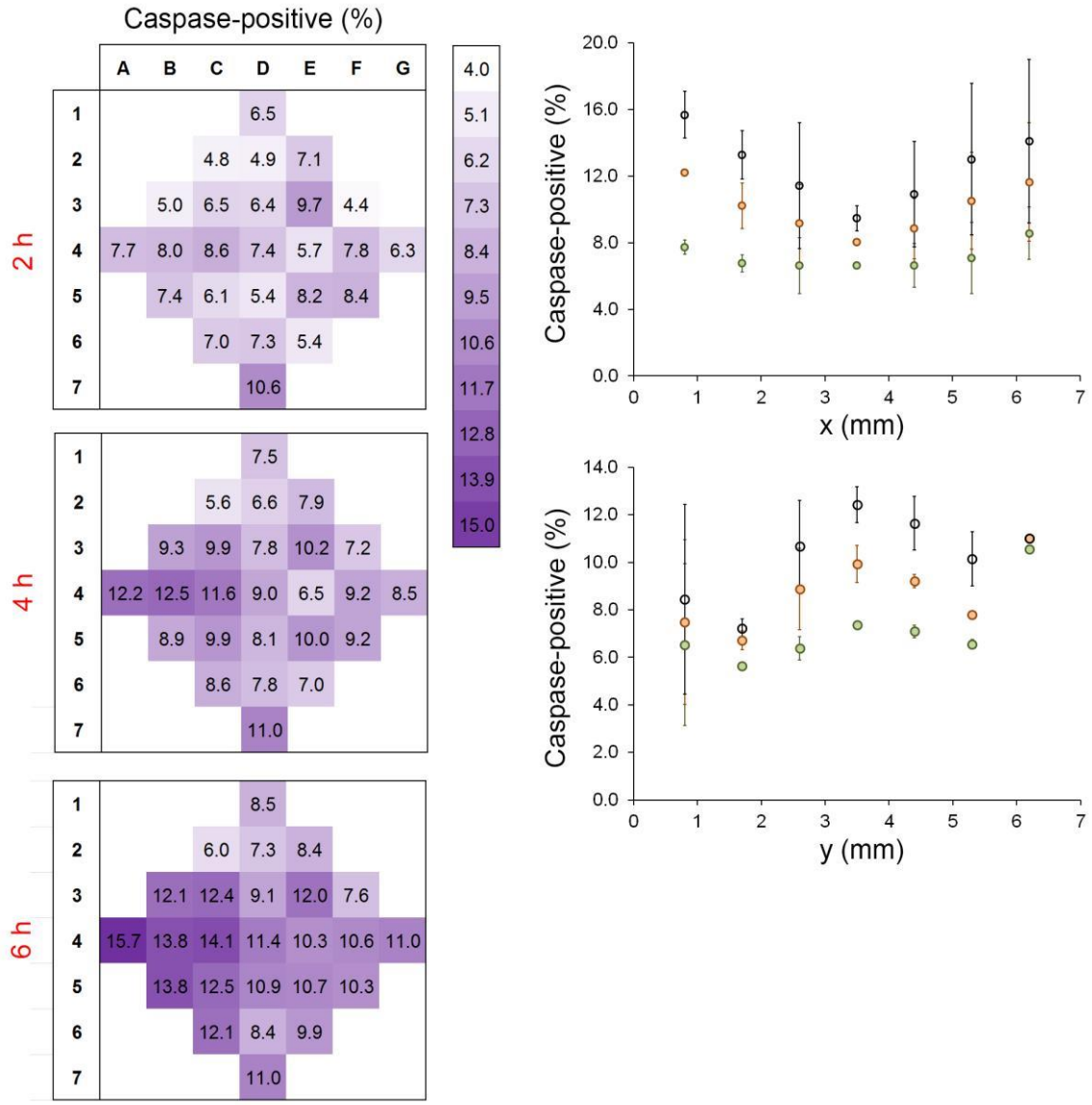


Figure S6. Caspase activation in A431 cells treated with 0.1 nM EGF in the gradient device in the absence of U0126 and BIBX. Average percentage of caspase positive cells in 25 regions of the cell chamber are mapped out. In addition, column and row averages are provided for 2 h (green), 4 h (brown), and 6 h (black) time points. The differences between regions and between column and row averages are not statistically significant (see Table S3). Error bars indicate SEM.

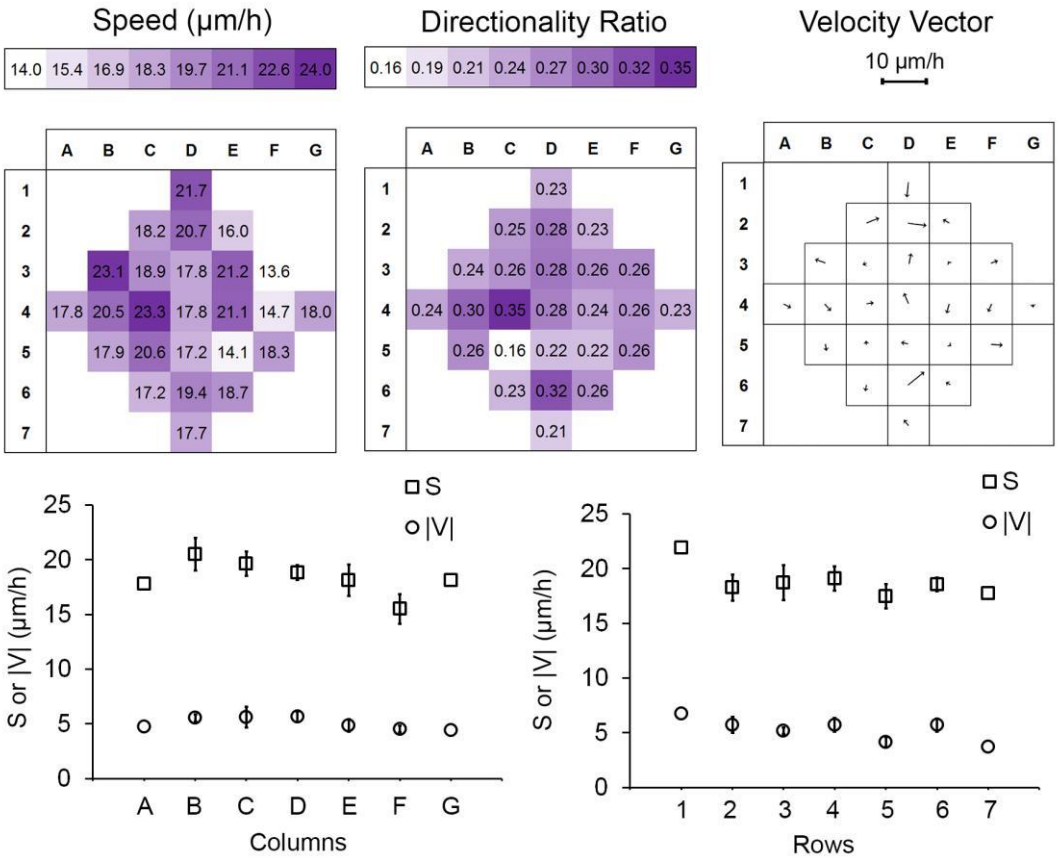


Figure S7. MDA-MB-231 cell motility in the gradient device in the absence of EGF and BIBX. Cell motility data is expressed in terms of speed, directionality ratio, and velocity vector. The mean values in 25 regions of the cell chamber are mapped out. In addition, column and row averages of the speed (S) and of the magnitude of the velocity vector (|V|) are provided. The differences between regions and between column and row averages are not statistically significant (see Table S4).

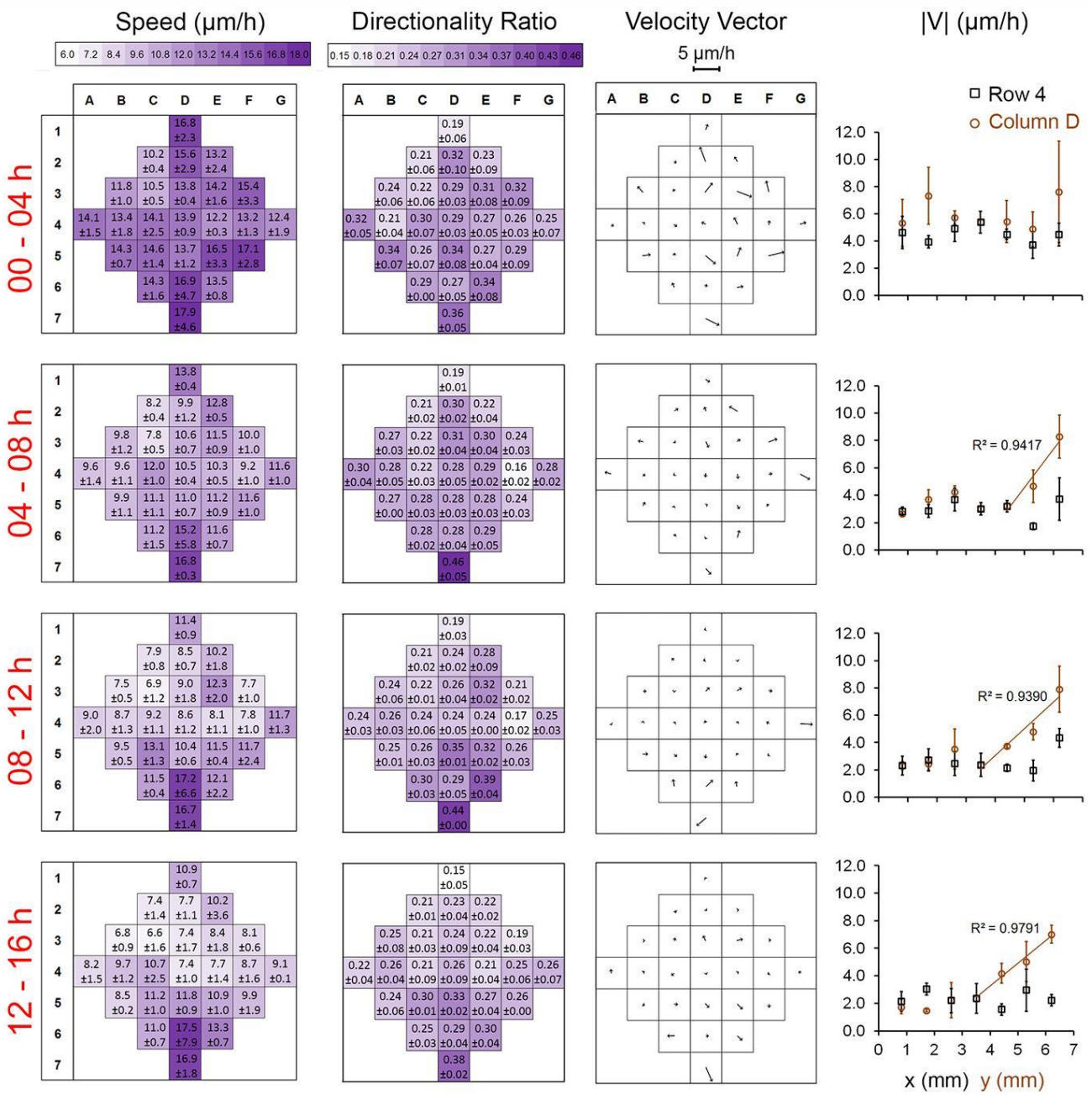


Figure S8. Heat maps of cell speed and directionality ratio, and arrows indicating the mean velocity vector are given at indicated time periods during simultaneous EGF (left-to-right, decreasing) and BIBX (top-to-bottom, decreasing) treatment. Mean velocity magnitude ($|V|$) in the central row and central column are expressed in plots in the last column; the fit line indicates the evolution of the differential motility in the *y*-direction.

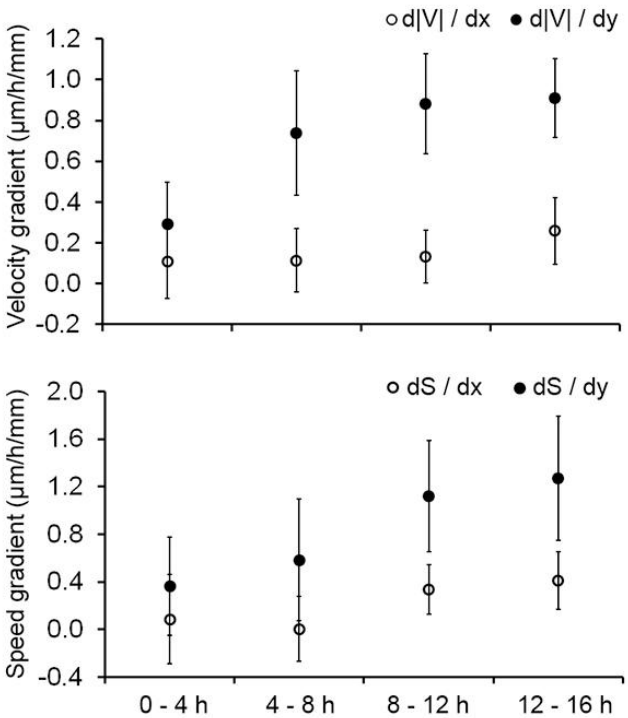
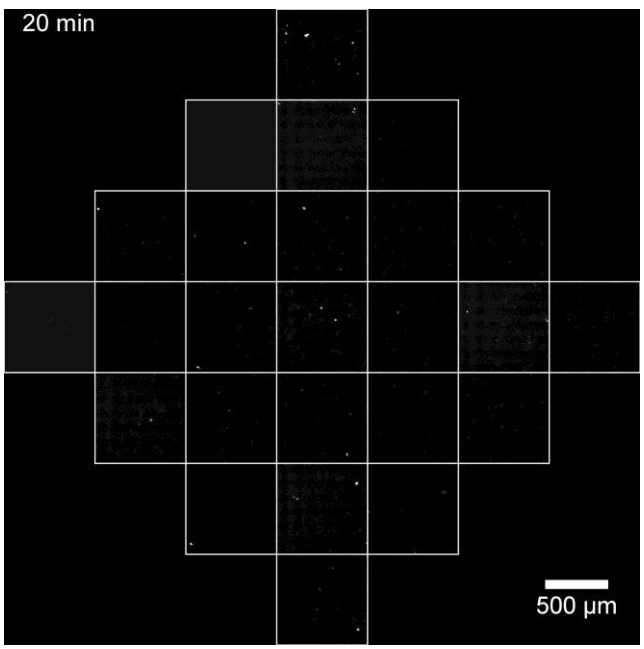
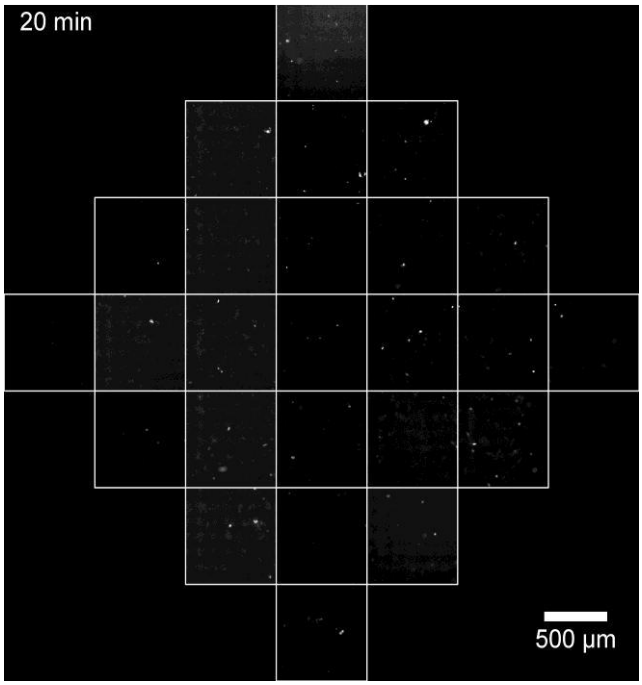


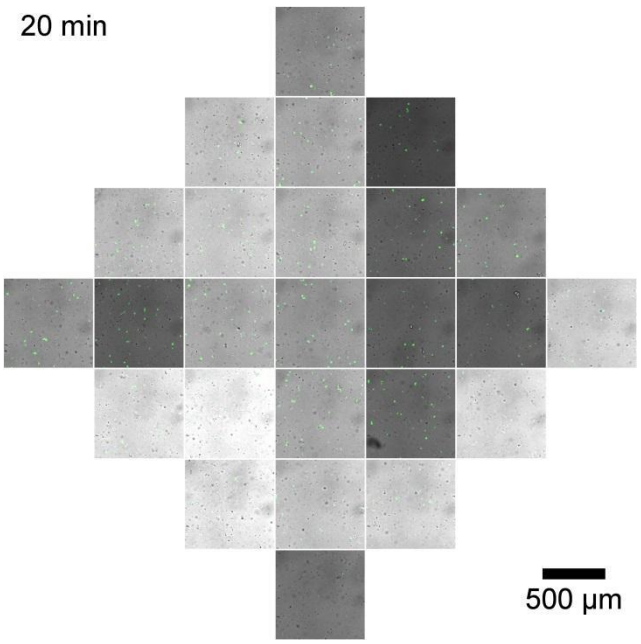
Figure S9. Column and row averages were fit with straight lines to calculate the *x*- and *y*-gradients, respectively, of velocity magnitude and cell speed.



Movie S1. Caspase activation in A431 cells treated with 0.1 nM EGF and exposed to orthogonally aligned gradients of MEK inhibitor (U0126; left-to-right, decreasing) and EGF receptor inhibitors (BIBX; top-to-bottom, decreasing).



Movie S2. Caspase activation in A431 cells treated with 10 nM EGF and exposed to orthogonally aligned gradients of MEK inhibitor (U0126; left-to-right, decreasing) and EGF receptor inhibitors (BIBX; top-to-bottom, decreasing).



Movie S3. MDA-MB-231 cell motility in response to exposure to orthogonally-aligned concentrations of EGF (left-to-right, decreasing) and EGF receptor inhibitor (BIBX; top-to-bottom, decreasing).