

Figure S1. ERK inhibition in PtK1 cells.

(A) and (B) Representative Western blots (repeated 5 and 3 times, respectively) of ERK-mediated phosphorylation in PtK1 cells after treatment with AZD6244. (C) Average protrusion velocity and (D) persistence in cells treated with DMSO and AZD6244 for 10-20 min. The “> 75th Percentile” graphs plot the distribution of the top 25% of events in the “All” graphs. m is number of events from $n = 5$ cells for each condition tracked in 3-4 independent experiments. Error bars about the median indicate 95% confidence interval estimated from bootstrapped data. Statistical significance calculated with the permutation test. (E) Representative Western blot (repeated 2 times) of ERK-mediated phosphorylation in PtK1 cells after treatment with U0126. (F) Average protrusion velocity and (G) persistence in cells treated with DMSO and U0126 for

10-20 min. m is number of events from $n = 5$ control and $n = 4$ U0126-treated cells tracked in 2 independent experiments. For further description of the box plots see Fig. 1.

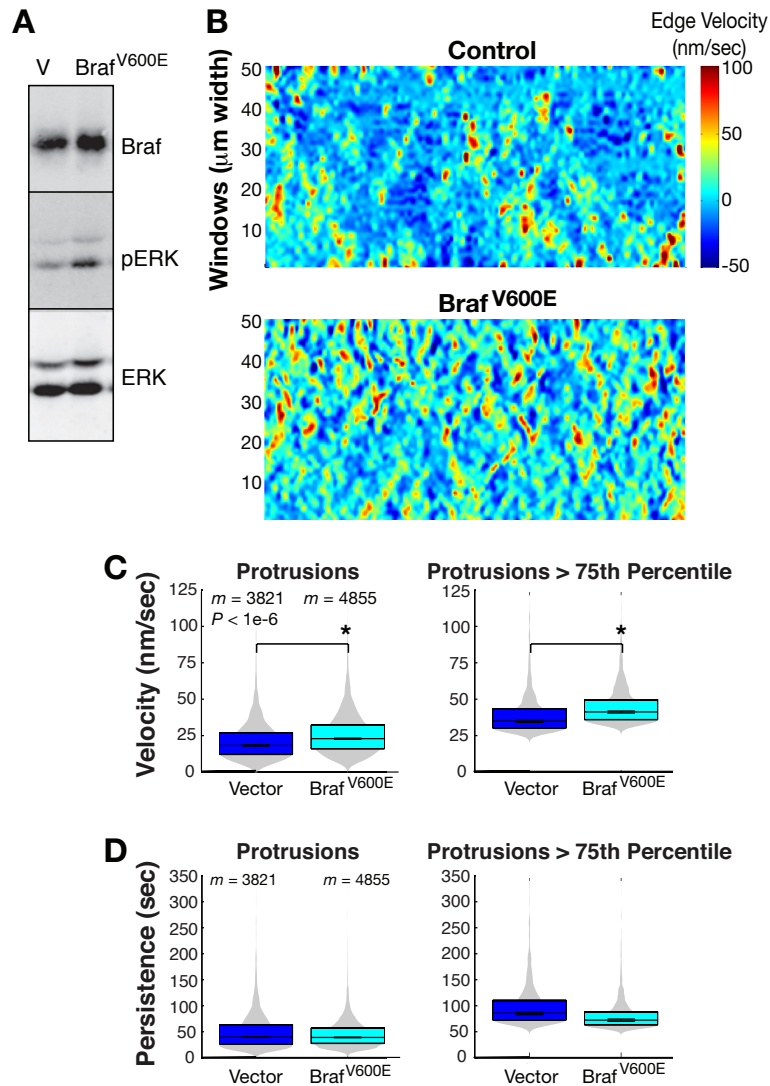


Figure S2. ERK hyperactivation induces protrusion velocity.

(A) Representative Western blot indicating Braf expression and ERK activity of PtK1 cells stably expressing empty vector (V) or Braf^{V600E} (repeated 3 times.) (B) Protrusion maps of edge dynamics in representative stable PtK1 transfectants (representative of 8 cells from 2 independent experiments). (C) Average velocity and (D) persistence time of protrusions in PtK1

cells expressing Vector or Braf^{V600E}. The “> 75th Percentile” graphs plot the distribution of the top 25% of events in the “All” graphs. *m* is number of events from *n* = 8 cells for each condition tracked in 2 independent experiments. Error bars about the median are the 95% confidence interval calculated from bootstrapped data. Statistical significance was calculated with the permutation test. For further description of the box plots see Fig. 1.

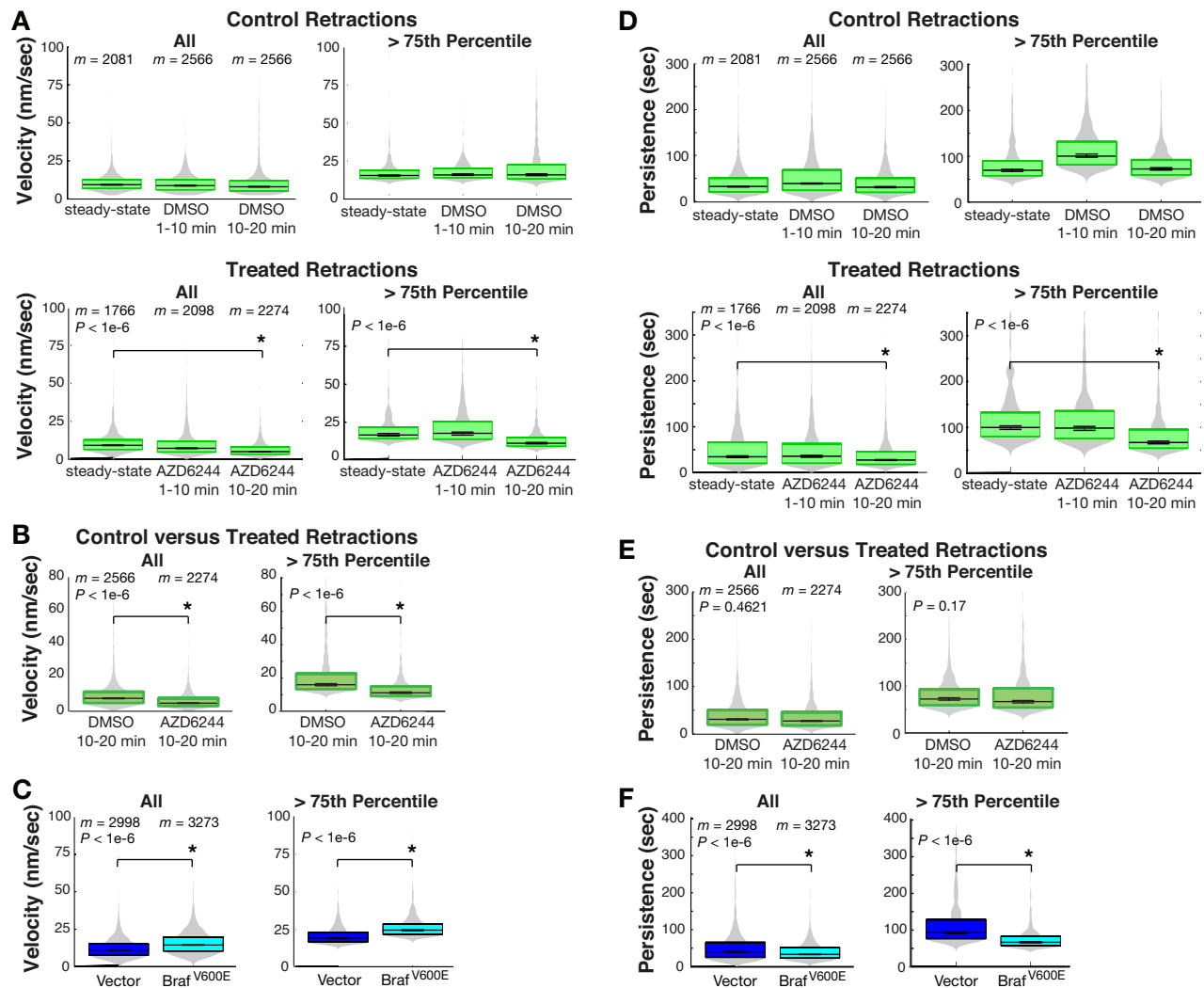


Figure S3. ERK increases retraction velocity.

(A), (B) Average retraction velocity in PtK1 cells treated with DMSO and AZD6244 and (C) expressing Vector and Braf^{V600E}. (D), (E) Average retraction persistence time in PtK1 cells treated with DMSO and AZD6244 and (F) expressing Vector and Braf^{V600E}. The “> 75th Percentile” graphs plot the distribution of the top 25% of events in the “All” graphs. m is number of events from $n = 5$ DMSO- or AZD6244-treated cells and $n = 8$ vector- or Braf^{V600E}-expressing cells tracked in 3-4 independent experiments. Error bars about the mean indicate 95% confidence interval calculated from bootstrapped data. Statistical significance was calculated with the permutation test. For further description of the box plots see Fig. 1.

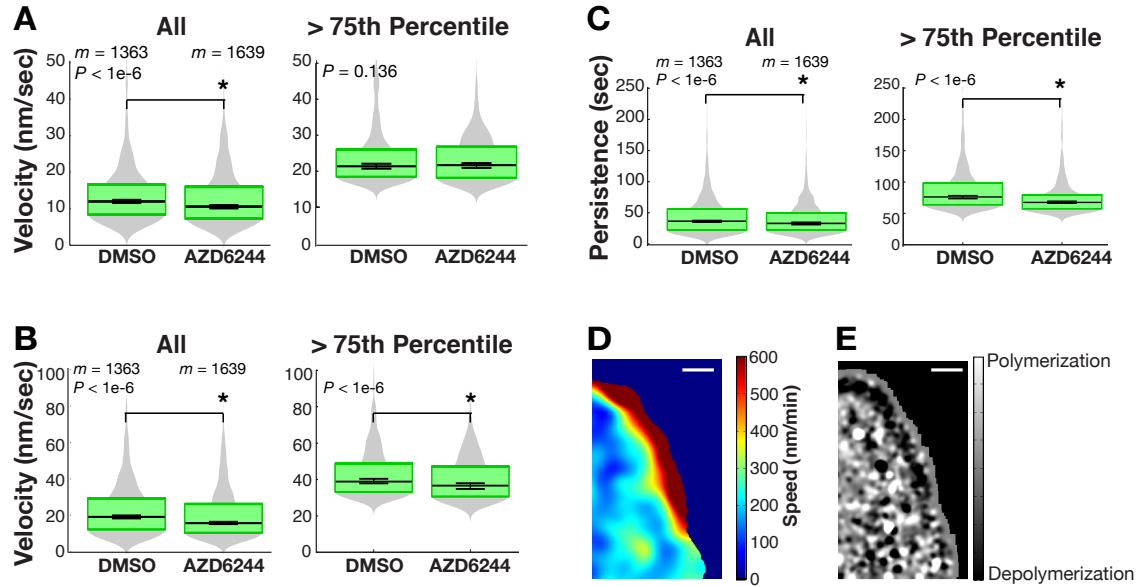


Figure S4. ERK promotes peak membrane protrusion velocity and persistence time in PtK1 cells microinjected with Alexa-568 actin monomers.

(A) Average protrusion velocity, (B) maximum protrusion velocity, and (C) persistence time in cells microinjected with Alexa-568 actin. m is number of events from $n = 7$ microinjected and DMSO-treated cells and $n = 6$ microinjected and AZD6244-treated (10-20 min) cells tracked in 4 independent experiments. The “> 75th Percentile” graphs plot the distribution of the top 25% of events in the “All” graphs. For further description of the box plots see Fig. 1. (D) Steady-state actin flow rates (temporally averaged over 1.5 min) and (E) actin polymer turnover (temporally averaged over 9 min; white indicates polymerization and black de-polymerization) for a representative cell before treatment. Images are representative of 4 DMSO-treated cells tracked in 4 independent experiments. Scale bars, 5 μ M.

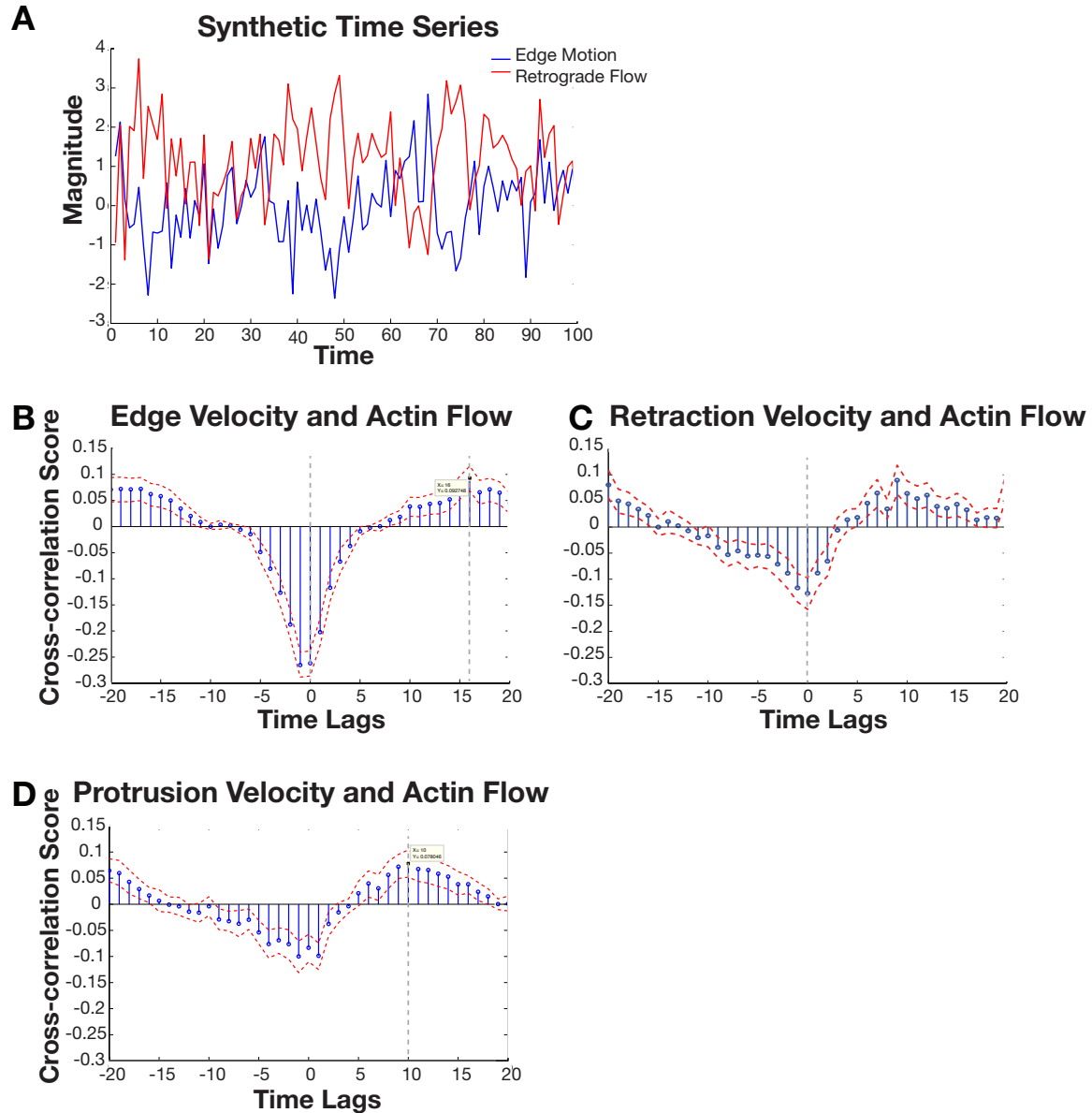


Figure S5. Simulation of cross-correlation of actin retrograde flow with cell edge velocity.

(A) Synthetic time series for edge motion and retrograde flow variables, generated using a sawtooth wave form. (B) Cross-correlation of all edge motion and actin flow and (C) edge motion specifically during retraction and (D) protrusion events show a time lag shift from 16 to 10 (arbitrary units) when the retraction component of the edge motion is extracted.

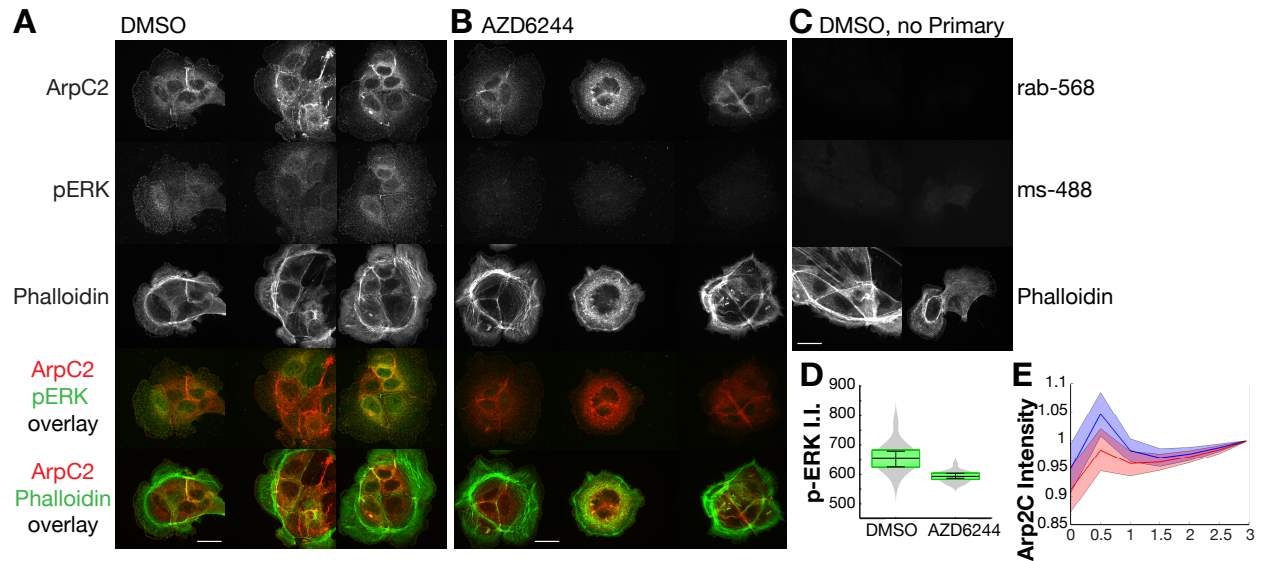


Figure S6. ERK regulates Arp2/3 localization to the cell edge.

Representative immunofluorescence images of phosphorylated ERK in steady-state PtK1 cells treated with (A) DMSO or (B) the MEK inhibitor AZD6244. (C) Staining of DMSO-treated PtK1 cells with secondary antibodies only. Scale bar, 20 μm . (D) Mean area integrated intensity of phosphorylated ERK in cells treated with DMSO ($n = 18$ cells) or MEK inhibitor ($n = 17$ cells) imaged in 3 independent experiments. (E) Mean Arp2C intensity in cells treated with DMSO (blue, $n = 18$ cells) or MEK inhibitor (red, $n = 17$ cells) imaged in 2 independent experiments. Shaded areas indicate 95% C.I.

Control cells

	Protrusion Median Velocity			Protrusion Maximum Velocity			Protrusion Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	14.8	12.9	12.4	25.2	19.5	20.0	38.2	29.9	36.2
up CI	14.5	12.5	12.0	24.0	18.7	19.5	37.4	29.2	35.2
down CI	15.3	13.2	12.8	26.0	20.4	21.0	39.1	30.6	37.3
25 percentile	9.7	8.1	7.7	14.5	11.3	12.0	24.7	19.2	22.5
75 percentile	22.2	20.0	19.3	41.2	33.4	34.9	60.0	49.0	58.6
min	0.9	1.1	1.0	1.3	1.4	1.7	2.4	4.0	2.9
max	132.1	108.2	100.0	147.1	130.1	131.4	366.9	238.2	346.5
Permutation test			*			*			equal

AZD6244-treated cells

	Protrusion Median Velocity			Protrusion Maximum Velocity			Protrusion Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	15.5	11.0	8.1	25.8	16.7	12.0	35.1	28.4	27.6
up CI	15.0	10.4	7.9	24.9	16.1	11.6	33.0	27.9	26.9
down CI	16.1	11.5	8.4	26.8	17.7	12.6	36.4	29.1	28.4
25 percentile	9.7	6.3	4.6	14.3	8.4	6.3	20.1	18.2	18.2
75 percentile	23.7	19.2	13.6	41.8	31.4	21.8	56.1	46.6	45.5
min	0.7	0.9	0.8	1.2	1.4	1.5	1.4	4.0	3.0
max	208.9	182.3	167.3	214.7	182.3	167.3	471.0	234.4	396.3
Permutation test			*			*			*

Control cells - 75th Percentile

	Protrusion Median Velocity			Protrusion Maximum Velocity			Protrusion Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	27.8	27.1	25.6	54.4	47.7	47.0	82.1	68.2	79.9
up CI	27.3	26.3	24.9	53.2	46.5	45.7	79.7	66.1	78.8
down CI	28.6	28.0	26.2	55.8	49.6	48.5	85.1	69.9	83.7
25 percentile	24.5	22.9	21.8	46.8	39.3	40.3	69.4	56.9	68.6
75 percentile	33.4	33.0	32.9	67.0	61.9	58.3	107.1	92.2	106.0
min	22.2	20.0	19.3	41.2	33.4	34.9	60.0	49.0	58.6
max	132.1	108.2	100.0	147.1	130.1	131.4	366.9	238.2	346.5
Permutation test			*			*			equal

AZD6244-treated cells-75th Percentile

	Protrusion Median Velocity			Protrusion Maximum Velocity			Protrusion Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	31.8	27.1	18.9	57.7	48.3	32.2	77.9	66.7	61.1
up CI	31.1	26.3	18.3	56.0	45.8	31.2	74.8	63.4	59.6
down CI	32.8	28.0	19.7	59.5	50.1	33.4	80.4	68.6	65.2
25 percentile	26.7	22.2	15.6	48.6	38.0	25.9	64.8	53.0	50.7
75 percentile	39.9	36.4	24.4	73.9	65.3	43.1	108.4	90.7	80.0
min	23.7	19.2	13.6	41.8	31.4	21.8	56.1	46.6	45.5
max	208.9	182.3	167.3	214.7	182.3	167.3	471.0	234.4	396.3
Permutation test			*			*			*

Control cells

	Retraction Median Velocity			Retraction Maximum Velocity			Retraction Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	9.3	8.7	7.9	13.4	13.1	11.5	32.5	38.8	30.9
up CI	9.0	8.5	7.7	13.1	12.8	11.0	30.9	37.6	29.7
down CI	9.5	8.9	8.2	13.8	13.4	11.8	33.6	39.8	32.0
25 percentile	6.9	6.0	5.1	10.0	8.9	7.9	20.5	24.1	19.5
75 percentile	12.5	12.6	11.9	18.5	19.8	17.5	50.3	68.8	50.3
min	0.9	1.0	1.0	1.6	1.4	1.8	1.8	1.6	1.6
max	92.4	92.2	81.4	92.4	110.9	81.4	280.0	330.0	247.3
Permutation test	equal			equal			equal		

AZD6244-treated cells

	Retraction Median Velocity			Retraction Maximum Velocity			Retraction Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	9.2	7.2	5.0	13.9	11.2	7.4	34.6	35.7	27.4
up CI	9.0	6.9	4.9	13.4	10.9	7.2	32.7	33.6	26.4
down CI	9.4	7.5	5.2	14.3	11.7	7.6	36.7	37.6	28.2
25 percentile	6.4	4.8	3.4	9.9	7.2	4.8	20.1	20.4	17.9
75 percentile	12.8	11.8	8.0	19.7	18.1	10.9	65.7	63.0	46.0
min	1.0	0.8	0.7	1.3	1.3	1.3	1.5	4.1	3.3
max	133.4	77.7	78.2	133.4	192.0	82.8	460.0	410.0	420.9
Permutation test	*			*			*		

Control cells - 75th Percentile

	Retraction Median Velocity			Retraction Maximum Velocity			Retraction Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	15.5	15.9	16.0	22.9	26.8	24.4	69.6	100.5	72.5
up CI	15.2	15.6	15.4	22.4	26.3	23.5	67.4	98.7	70.0
down CI	15.9	16.6	16.7	23.4	27.8	25.3	71.7	105.3	75.2
25 percentile	13.7	14.0	13.3	20.2	22.6	20.3	57.5	81.4	59.3
75 percentile	19.0	20.2	22.6	27.6	34.3	34.2	89.5	132.0	92.8
min	12.5	12.6	11.9	18.5	19.8	17.5	50.3	68.8	50.4
max	92.4	92.2	81.4	92.4	110.9	81.4	280.0	330.0	247.3
Permutation test	equal			equal			equal		

AZD6244-treated cells-75th Percentile

	Retraction Median Velocity			Retraction Maximum Velocity			Retraction Persistence Time		
	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min	steady-state	1-10 min	10-20 min
mean	16.6	17.7	11.1	26.2	27.7	15.3	99.7	98.4	67.0
up CI	15.9	16.5	10.7	25.0	26.4	14.7	95.7	92.6	64.1
down CI	17.5	18.4	11.7	27.0	29.7	15.8	102.6	102.0	69.2
25 percentile	14.2	13.8	9.2	22.0	21.6	12.6	80.1	75.7	54.4
75 percentile	21.6	25.3	15.0	32.0	44.2	20.4	132.4	136.1	95.2
min	12.8	11.8	8.0	19.7	18.1	10.9	65.7	63.0	46.0
max	133.4	77.7	78.2	133.4	192.0	82.8	460.0	410.0	420.9
Permutation test	*			*			*		

Table S1. Event velocity averages.

Mean and median protrusion/retraction rates and durations for PtK1-Lifeact cells. * *p* value between steady-state and 10-20 min time point is $< 1e-6$ according to the permutation test on the distributions of the mean velocities and persistence time.

Movie S1. Spontaneous protrusion/retraction cycles.

PtK1 cells expressing Emerald-Lifeact. 10 sec frame rate for 30 min. Cells were treated with DMSO at 10 min of imaging, when the brightness was increased.

Movie S2. MEK inhibition dampens spontaneous edge motion.

PtK1 cells expressing Emerald-Lifeact as in Supplementary Movie 1. Cells were treated with AZD6244 at 10 min of imaging, when the brightness was increased.

Movie S3. Edge motion analysis.

Protrusion and retraction vectors from edge motion calculations of cell in Supplementary Movie 1. Vector length indicates velocity intensity.

Movie S4. Analysis of spontaneous edge motion upon MEK inhibition.

Protrusion calculations for cell in Supplementary Movie 2. Vector length indicates velocity intensity; scale bar for vectors, 1 $\mu\text{m}/\text{min}$.

Movie S5. Actin speckles.

Alexa568-actin in PtK1 cells treated with DMSO for 10 min.

Movie S6. Actin speckles with MEK inhibition.

Alexa568-actin in PtK1 cells treated with AZD6244 for 10 min.

Movie S7. Flow tracking.

Interpolated vectors from qFSM flow analysis of Supplementary Movie 5. Flow vectors are temporally averaged over 9 frames (90 sec) and have correlation length of 2 microns. Vector color and length indicate flow rate; scale bar for vectors, 1 $\mu\text{m}/\text{min}$, redder color is fastest.

Movie S8. Actin flow under MEK inhibition. Interpolated flow vectors from qFSM flow analysis of Supplementary Movie 6. Flow vectors are temporally averaged over 9 frames (90 sec) and have correlation length of 2 microns. Vector color and length indicate flow rate; scale bar for vectors, 1 $\mu\text{m}/\text{min}$, redder color is fastest.