Biophysical Journal, Volume *122*

Supplemental information

Fidgetin-like 2 depletion enhances cell migration by regulating GEF-H1, RhoA, and FAK

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Supplementary Information for:

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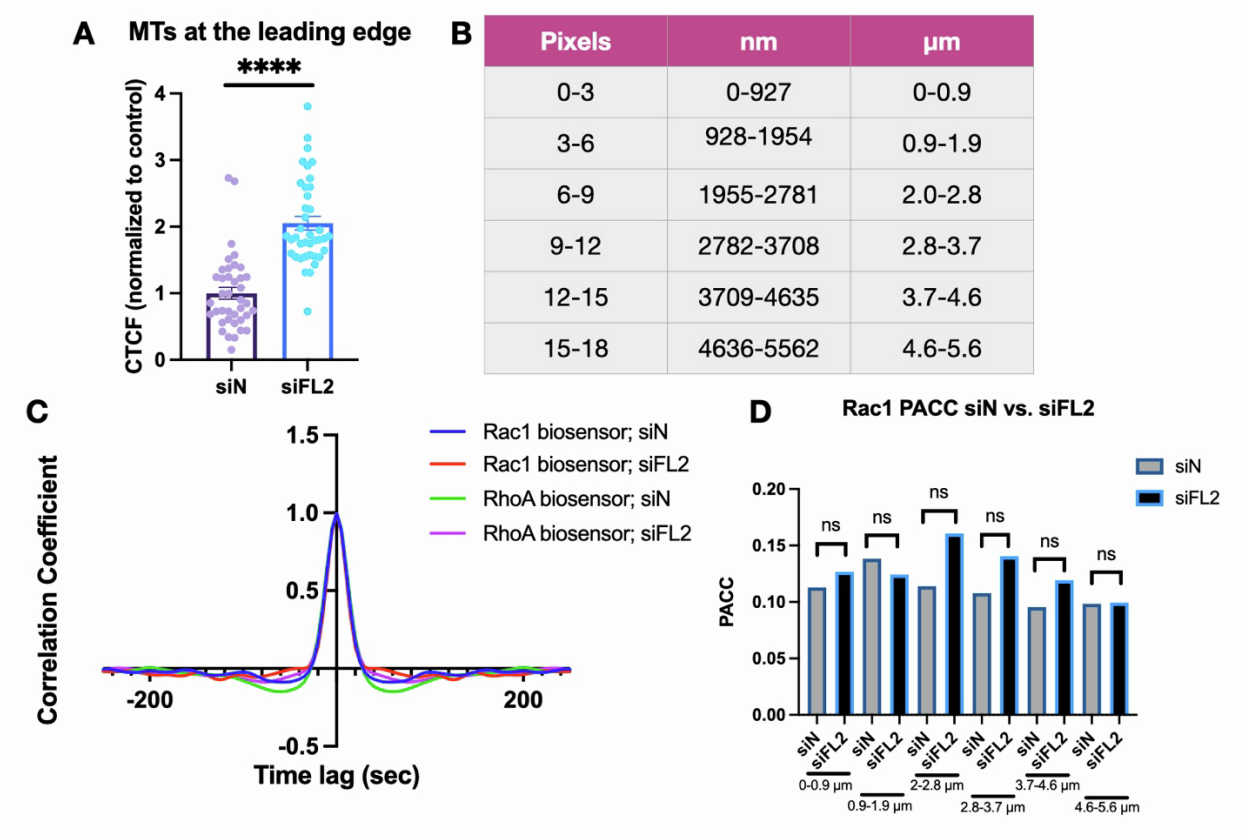
Supplementary Figures



Pixels	nm	µm
0-3	0-927	0-0.9
3-6	928-1954	0.9-1.9
6-9	1955-2781	2.0-2.8
9-12	2782-3708	2.8-3.7
12-15	3709-4635	3.7-4.6
15-18	4636-5562	4.6-5.6

Figure S1. Protrusion velocity autocorrelation. (A) Scatterplot of MT fluorescence (indicating FL2 KD) in U2OS cells along a scratch. Datapoints represent individual cells. All analysis performed within 5.6 µm of cortex. (n=39,39, mean+/-SEM; Welch's t-test, ****p<0.0001). **(B)** Conversion from distance in pixels (used in FRET analysis) to nm and µm. The 18-pixel-wide region from the leading edge analyzed in the FRET assays defines the 5.6 µm-wide region analyzed in all subsequent studies. **(C)** Temporal autocorrelation of the leading edge protrusion velocity from experiments where RhoA and Rac1 fluctuations were measured at the leading edge. **(D)** Bar graph showing PACC of Rac1 FRET biosensor activation irrespective of time to

edge protrusion at various distances (up to 5.6 µm) away from the cortex after control or FL2 siRNA treatment. (n=401 windows/17 cells (siN), 334 windows/17 cells (siFL2); Student's t-test.)

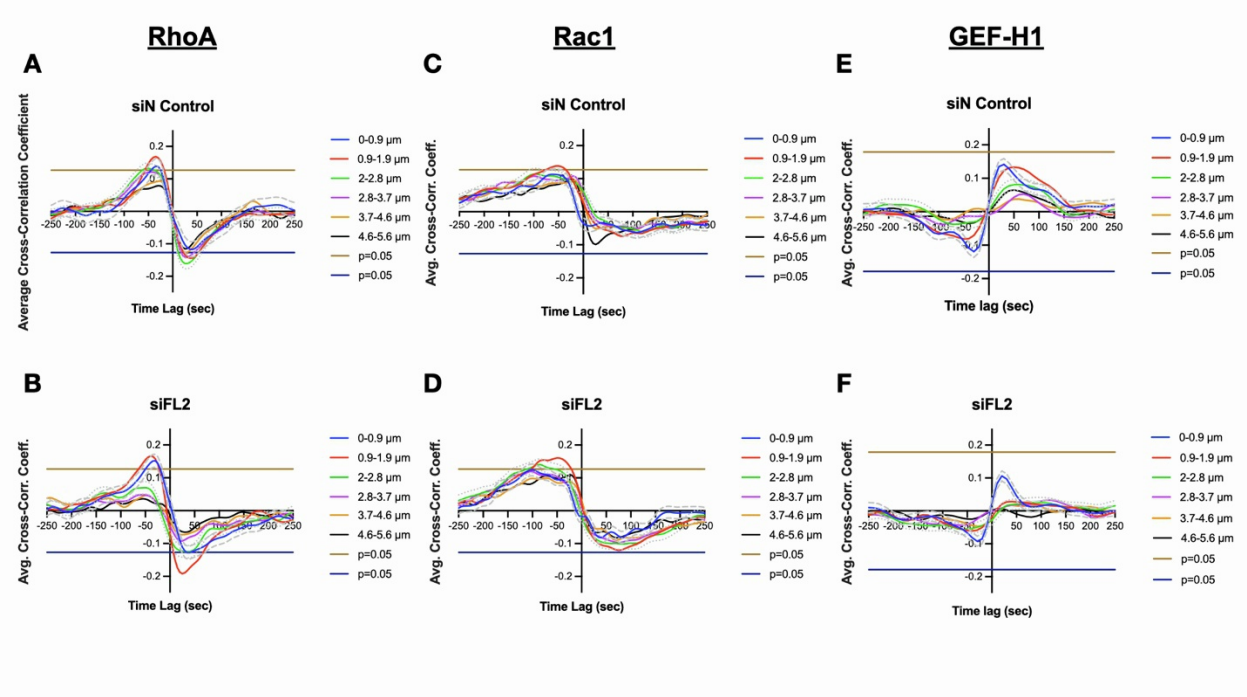


Figure S2. RhoA, Rac1, and GEF-H1 involvement in edge protrusion shift to differing degrees after FL2 knockdown. (A- F) Curves showing correlation between FRET biosensor readouts (RhoA (A,B); Rac1 (C,D); GEF-H1 (E,F)) and edge velocity relative to edge protrusion initiation (x=0) after either control siRNA (A,C,E) or FL2 siRNA (B,D,F) treatment. Curves plotted are spline fits from pooled correlation coefficients of individual cells. Individual curves represent various distances (up to 5.6 µm) away from the cell edge. Dashed and dotted lines show 95% confidence intervals, estimated by non-parametric bootstrapping. Horizontal lines indicate significance of Pearson's correlation coefficient at $p<0.05$. (A: n=440 windows, 21 cells; B: 462 windows, 21 cells; C: n=401 windows, 17 cells; D: 334 windows, 17 cells; E: 341 windows, 22 cells; F: 457 windows, 25 cells).

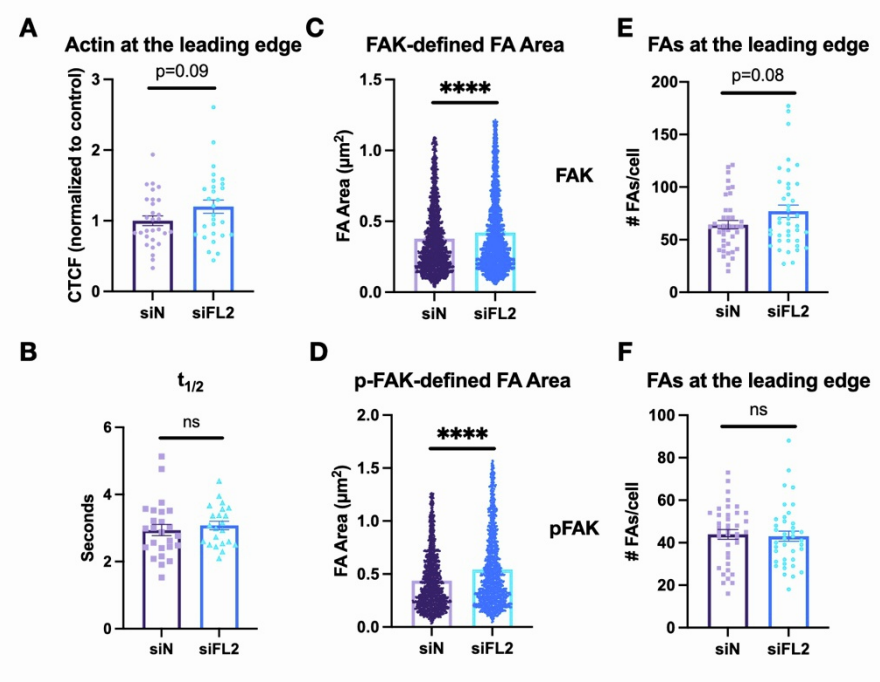


Figure S3. FL2 knockdown increases FA area but not the number of FAs or actin polymerization at the leading edge. (A) Scatterplot of phalloidin fluorescence intensity at the leading edge. Datapoints represent individual cells. (n=29,28 cells, mean+/-SEM; Welch's t-test). **(B)** Scatterplot of half-time to fluorescence recovery ($t_{1/2}$) of F-tractin-eGFP at the leading edge using FRAP. Datapoints represent individual cells. (n=25,22 cells, mean+/-SEM; Welch's t-test.) All experiments performed in triplicate. **(C-D)** Scatterplots of FA area as defined by (C) FAK or (D) p-FAK fluorescence. Datapoints represent individual FAs. (C: n=2298,2733; D: n=1508,1481, mean+/-SEM; Welch's t-test, *p<0.05, ****p<0.0001). **(E-F)** Scatterplots of number of FAs at the leading edge in U2OS cells along a scratch. FAs defined by either (E) FAK or (F) p-FAK immunofluorescence. Datapoints represent individual cells. (E: n=40,40; F: n=38,38, mean+/-SEM; Welch's t-test, ****p<0.0001). All analyses performed within 5.6 µm of cortex.

Supplemental Video Captions

Video S1. Representative RhoA activity in a siN control-treated U2OS cell stably expressing the RhoA FRET biosensor. Linear pseudocolor map indicates RhoA activity. Images taken in 5-sec intervals for 20-minute time-lapse on a confocal microscope using a 40x oil objective. Scale bar indicates 10 µm. Video plays at 20 frames per sec (fps).

Video S2. Representative RhoA activity in a siFL2-treated U2OS cell stably expressing the RhoA FRET biosensor. Linear pseudocolor map indicates RhoA activity. Images taken in 5-sec intervals for 20-minute time-lapse on a confocal microscope using a 40x oil objective. Scale bar indicates 10 µm. Video plays at 20 frames per sec (fps).