Figure S1: (A) Western Blot analysis showing expression levels for the ErbB family of receptors (ErbB 1-4) in Cos-7 cells with or without EGF stimulation probed using monoclonal antibodies against the ErbB family members. GAPDH staining was used as a loading control. **(B)** Flow chart illustrating the step-wise fabrication process for the TGT-based tension surface (see methods for full description). **(C)** Cell adhesion and tension are specific to the cRGDfK-integrin interaction. Representative brightfield, RICM, and tension images of Cos-7 cells plated on 56 pN or 12 pN TGT surfaces with or without cRGDfK conjugated to the TGT probe. Images were acquired 60 min after cell plating on the TGT surfaces. Cells adhere and spread and exert force on the TGT probes with cRGDfK. On surfaces without cRGDfK no cell spreading or tension was observed, but the presence of cells is verified by the brightfield image. Without cRGDfK, cells did not attach to the surfaces and the TGT probes were not opened, scale bar = 10 µm.
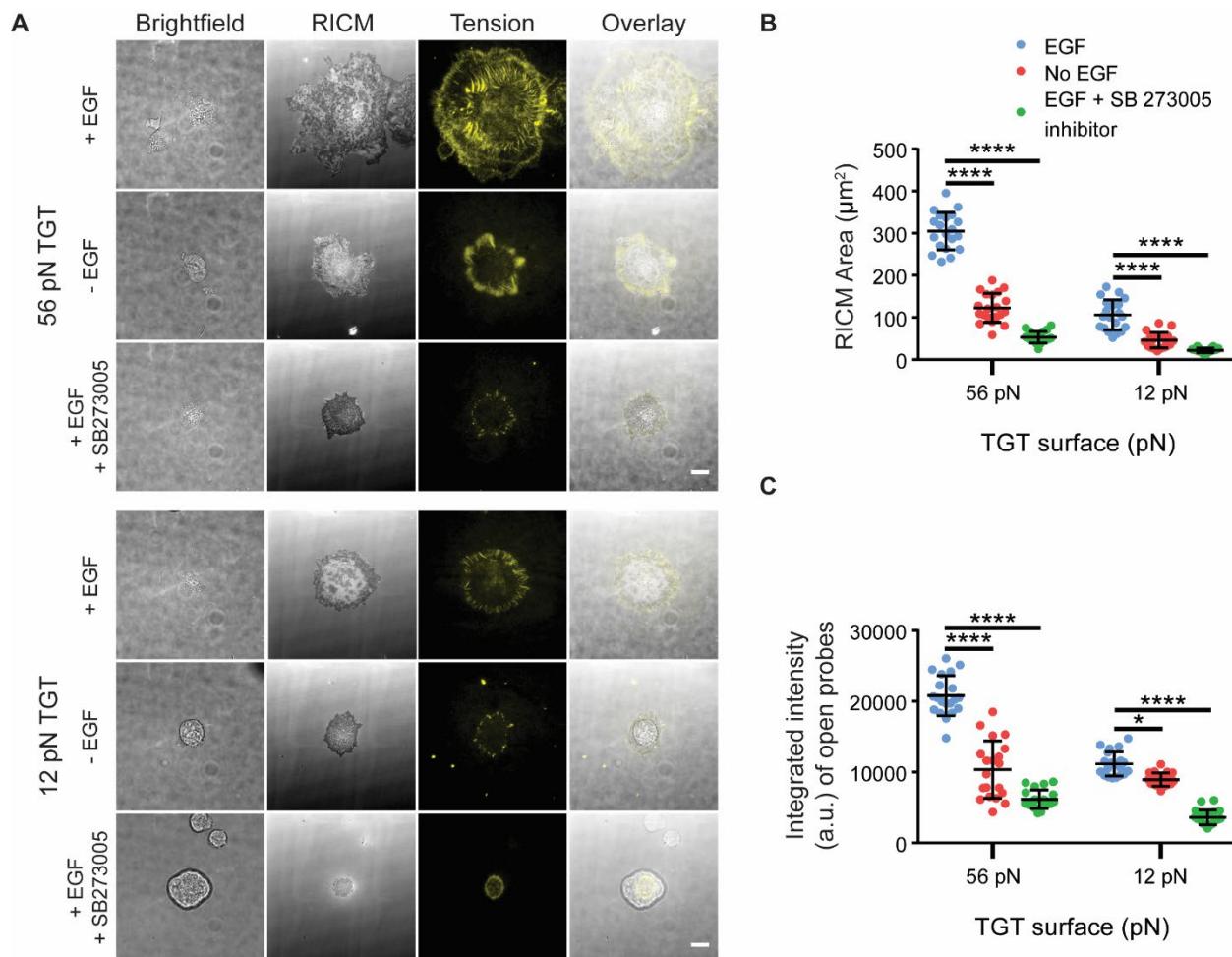
Figure S2: Cell adhesion and tension generation are specific to the $\alpha_v\beta_3/\alpha_v\beta_5$ integrin heterodimers.
(A) Cos-7 cells treated with control (DMSO – no inhibitor), or SB 273005 ($\alpha_v\beta_3/\alpha_v\beta_5$ inhibitor) in the presence or absence of EGF on both the 56 pN and 12 pN TGT surface fixed 1 hour after plating. Shown here, RICM (grayscale) and integrin tension (yellow), scale bar = 10 μm. **(B-C)** Scatter plots for the cell footprint – RICM area **(B)** and the integrated intensity of open probes **(C)** for Cos-7 cells in each treatment group with or without EGF stimulation. (bar = mean ± SD, n = 20 cells across three independent experiments; $^{ns}p > 0.05$, $^*p < 0.05$, $^{****}p < 0.0001$; one-way ANOVA).
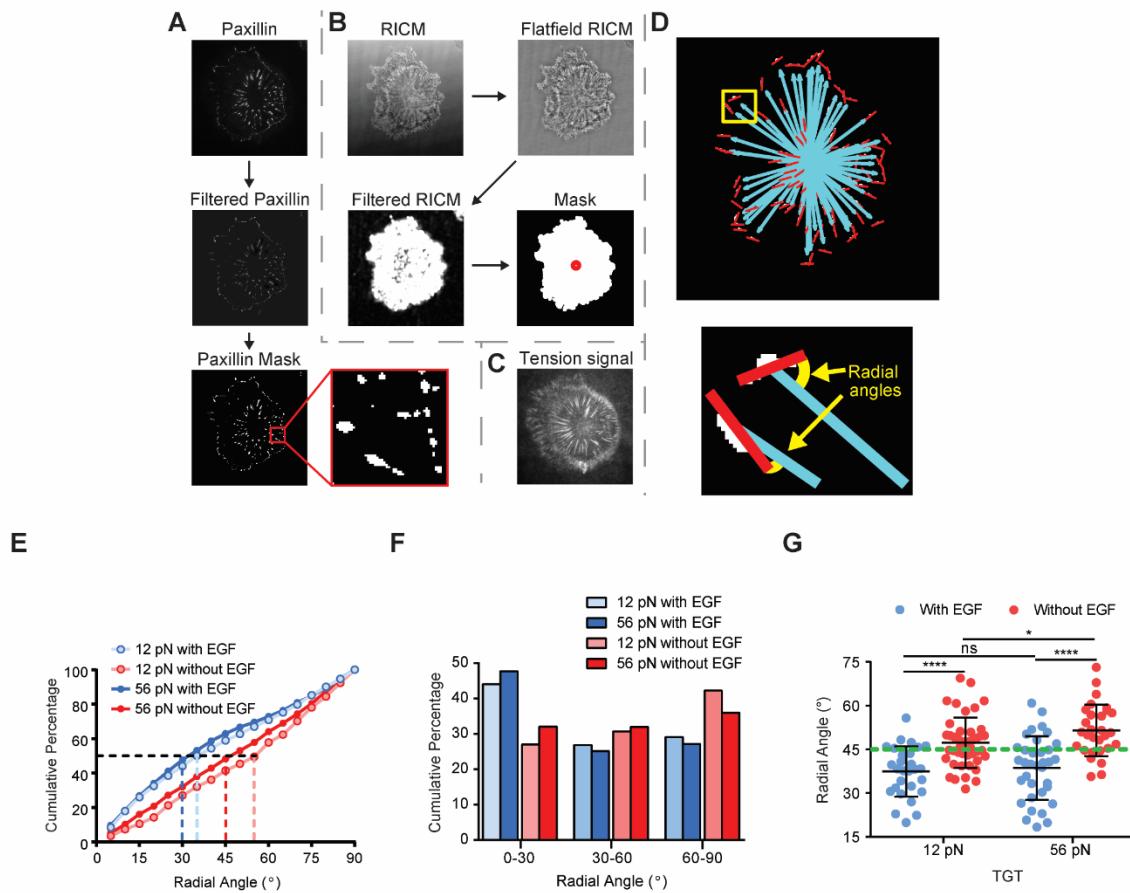
Figure S3: EGF stimulation leads to radial organization of focal adhesions. (A) First paxillin data is filtered using rolling ball background normalization (25-pixel radius), and a cutoff threshold of 2 is applied to select for bright objects within the image. **(B)** RICM image is used to create a mask to identify cell boundary. The median value of the image is subtracted and rolling ball background subtraction applied (20-pixel radius). The image is normalized to its standard deviation and subsequently squared. It is then smoothed with a rolling ball filter (5-pixel radius) and subjected to a threshold cutoff of 1 to generate a mask. All objects except for the largest are discarded, and the mask is finally subjected to a 3-pixel dilation. The centroid of the cell is calculated as the geometric centroid of the object in the mask. **(C)** The tension image acquired for this cell is shown for reference. **(D)** All objects in the paxillin mask obtained in **(A)**, not located in the cell mask in **(B)** are discarded. Small objects fewer than ten pixels are discarded. Finally, a centripetal axis (blue), which points from the cell to the object's centroid, is obtained for each object. A long axis orientation is also obtained for each object using MATLAB's region props command. The two axes are compared, and the small angle between is used to obtain the radial angle of each object. **(E)** Cumulative frequencies for the radial angles marking the FA orientation was plotted as a percentage distribution curve on both the 56 and 12 pN TGT surfaces. **(F)** Bar graph for comparative cumulative percentage distribution for radial angles binned into 30-degree bins in presence or absence of EGF on both TGT surfaces. **(G)** Scatter plots show the distribution of radial angles when grouped using each cell with at least 10 FAs as an independent sample. Error bars represent the standard deviations across the mean. Differences between the groups were assessed statistically with one-way analysis of variance - ANOVA, (n = 35 cells on 12 pN TGT with EGF, n = 29 cells on 12 pN TGT without EGF, n = 28 cells on 56 pN TGT with EGF, n = 44 cells on 56 pN TGT without EGF, $^{ns}p > 0.05$, $*p < 0.05$, $****p < 0.0001$).
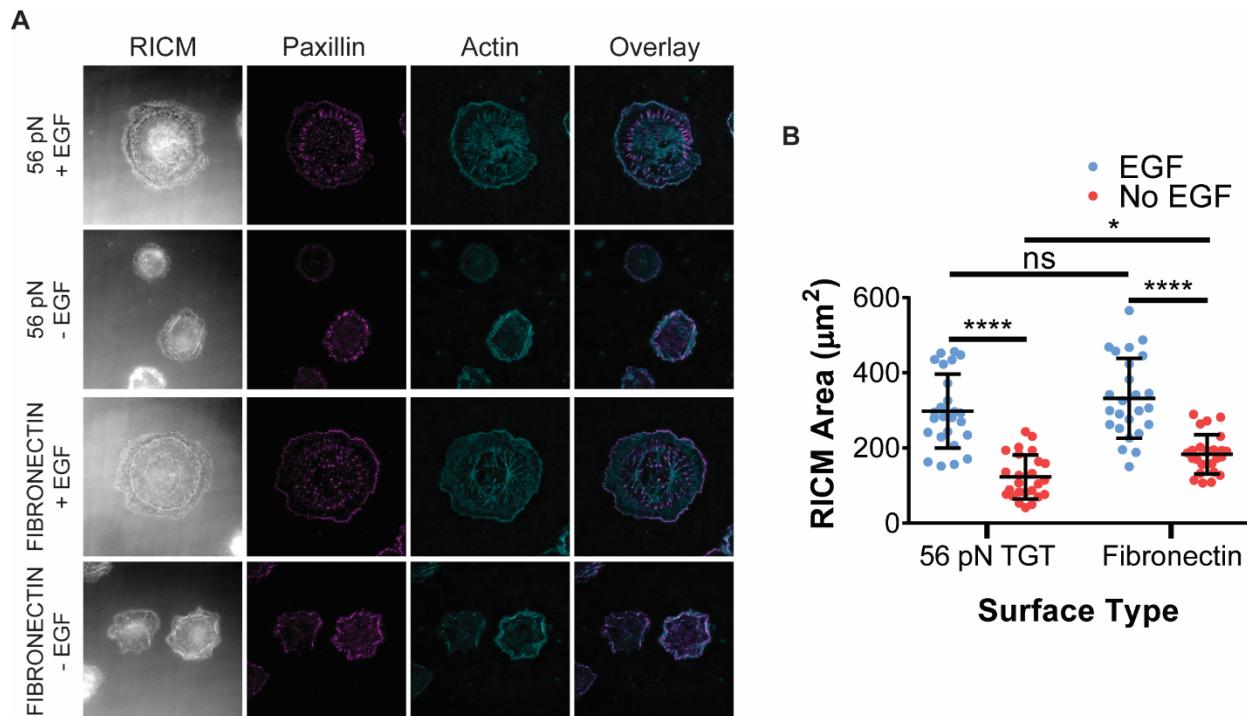
Figure S4: Complimentary analysis of Cos-7 cell spreading and FA formation on 56 pN TGT and fibronectin coated surfaces. (**A**) Representative RICM, paxillin (magenta) and actin (cyan) images for Cos-7 cells on a 56 pN TGT and fibronectin coated surfaces fixed and stained with or without EGF stimulation 1 h post plating, scale bar = 10 μm. (**B**) Scatter plots of the RICM cell spread area on both the surface**s** with or without EGF stimulation. Error bars represent the standard deviation across the means. Differences between the groups were assessed statistically with the one-way analysis of variance - ANOVA (n = 25 cells, $^{ns}p > 0.05$, $^*p < 0.05$, $^{****}p < 0.0001$).
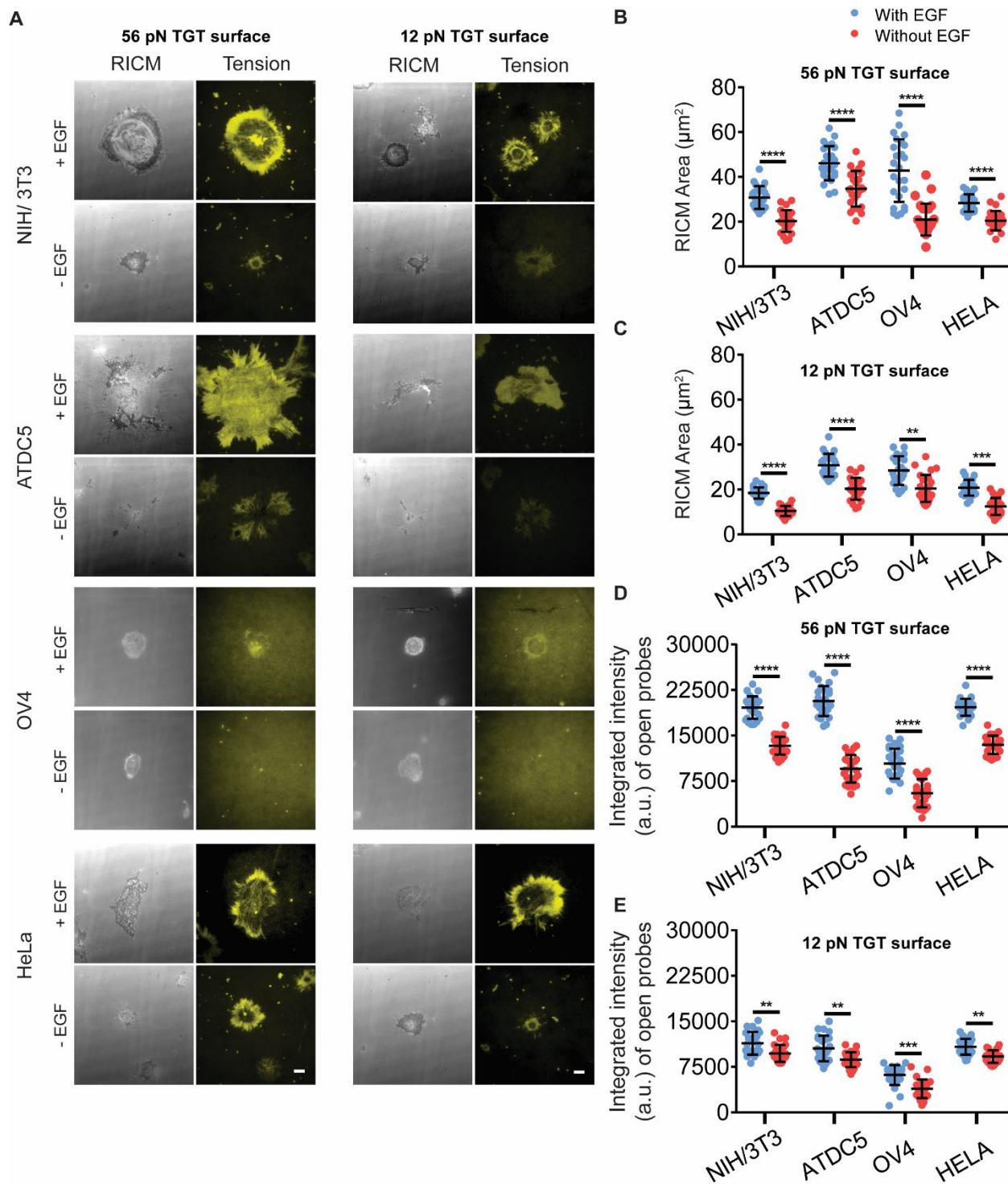
Figure S5: EGF regulates cell spreading and integrin tension across cell types. Representative RICM, and tension images for NIH/3T3, ATDC5, OV4 and HELA cells plated on 56 pN or 12 pN TGT surfaces with or without EGF stimulation. **(B-E)** Scatter plots of the RICM cell spread area (**B, C**) and integrated tension intensity for the open probes **(D, E)** for the cells above with or without EGF stimulation. Error bars represent the standard deviation across the means. Differences between the groups were assessed statistically with the one-way analysis of variance - ANOVA (n = 25 cells, $^{ns}p > 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$).
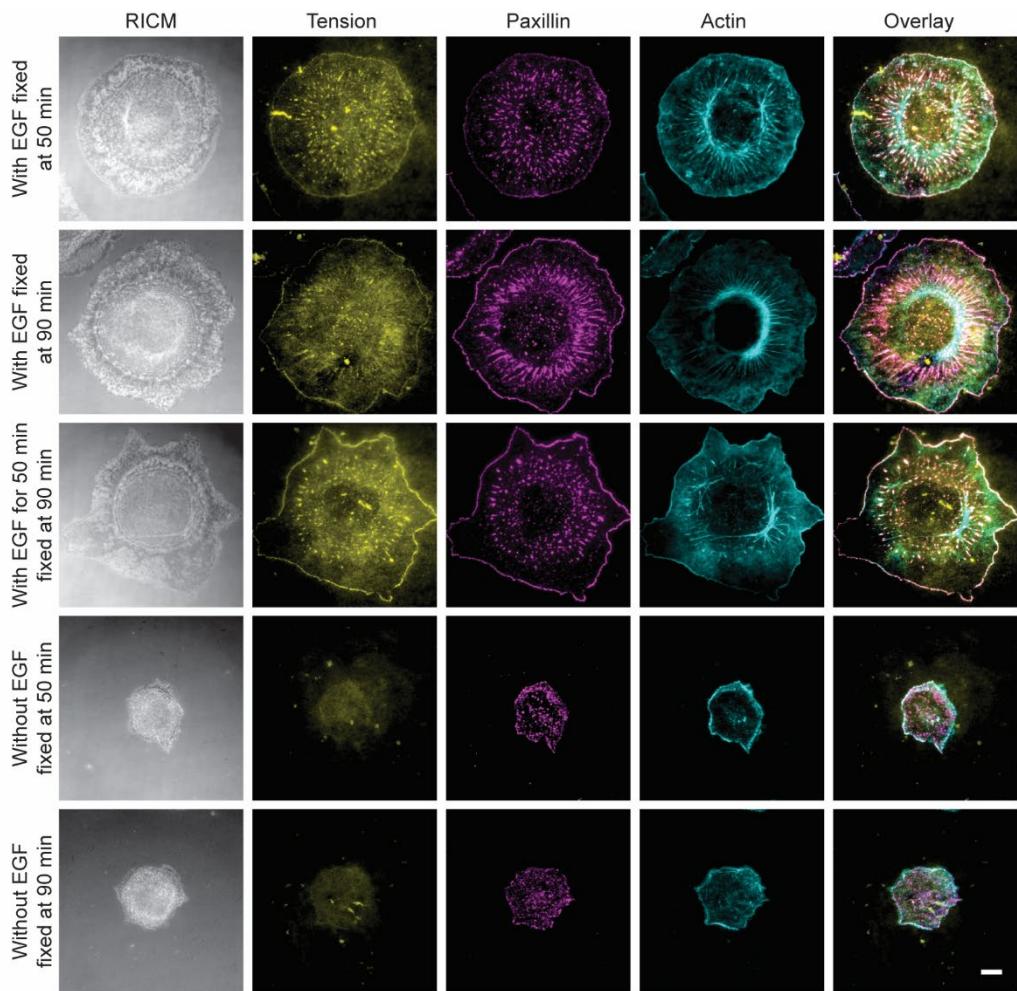
Figure S6: EGF is required for focal adhesion maturation but not maintenance. Integrin tension profiles (yellow) for Cos-7 cells on a 56 pN TGT surface fixed and stained at specific timepoints after EGF treatment (same treatment as in Figure 2 and 3). The cells were stained with paxillin antibody and phalloidin to visualize FAs (magenta) and stress fibers (cyan), scale bar = 10 μm.
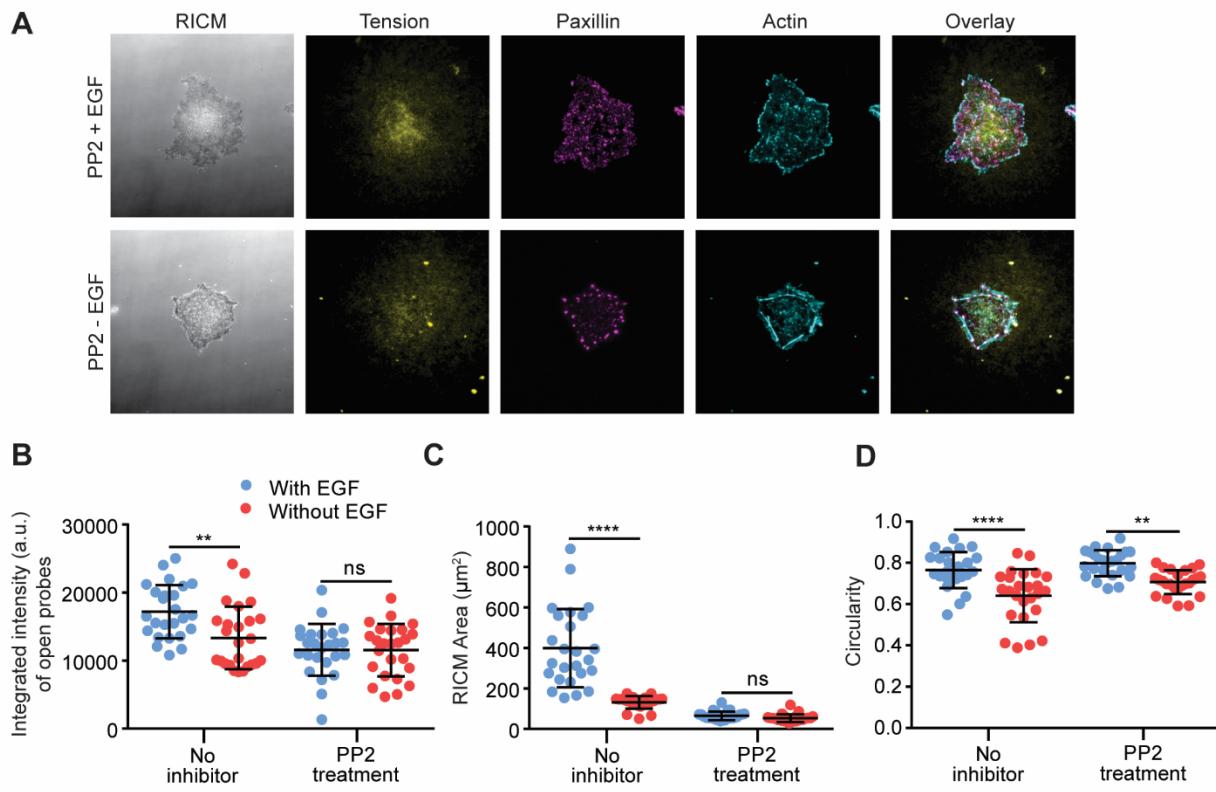
Figure S7: Src inhibitor treatment leads to decreased integrin tension and reduced cell spreading. **(A)** Integrin tension profile (yellow) for Cos-7 cells treated with PP2 (Src kinase inhibitor) in presence or absence of the EGF ligand. Cells were fixed and stained 1 h following plating on a TGT tension surface, with paxillin antibody and phalloidin to visualize focal adhesions (magenta) and stress fibers (cyan), scale bar is 10 µm. **(B-D)** Scatter plots representing the integrated tension of open probes **(B)**, RICM spread area **(C)**, and the circularity **(D)** for the control (images not shown) and inhibitor treated cells with or without ligand stimulation. **(B-D)** Error bars represent the standard deviations across the means. Differences between the groups were assessed statistically with one-way analysis of variance - ANOVA (n = 25 cells, $^{ns}p > 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$).

Supplementary Tables:

Table S1: One-way ANOVA multiple comparison table for integrated tension following rescue with WT or mutant EGFR

Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Control (EGFR siRNA) with EGF vs. Control (EGFR siRNA) without EGF	619.6	-2420 to 3659	No	ns	0.9997
Control (EGFR siRNA) with EGF vs. WT EGFR with EGF	-13696	-16735 to -10656	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. WT EGFR without EGF	-263.4	-3303 to 2776	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. Triple mutant with EGF	-8479	-11519 to -5439	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Triple mutant without EGF	-155.8	-3195 to 2884	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. Penta mutant with EGF	-1294	-4334 to 1745	No	ns	0.9379
Control (EGFR siRNA) with EGF vs. Penta mutant without EGF	290.7	-2749 to 3330	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. Src mutant with EGF	-6647	-9686 to -3607	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Src mutant without EGF	248.9	-2791 to 3289	No	ns	> 0.9999
Control (EGFR siRNA) without EGF vs. WT EGFR with EGF	-14315	-17355 to -11276	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. WT EGFR without EGF	-883.0	-3923 to 2157	No	ns	0.9954
Control (EGFR siRNA) without EGF vs. Triple mutant with EGF	-9099	-12138 to -6059	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. Triple mutant without EGF	-775.3	-3815 to 2264	No	ns	0.9983
Control (EGFR siRNA) without EGF vs. Penta mutant with EGF	-1914	-4954 to 1126	No	ns	0.5921
Control (EGFR siRNA) without EGF vs. Penta mutant without EGF	-328.9	-3369 to 2711	No	ns	> 0.9999
Control (EGFR siRNA) without EGF vs. Src mutant with EGF	-7266	-10306 to -4226	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. Src mutant without EGF	-370.7	-3410 to 2669	No	ns	> 0.9999
WT EGFR with EGF vs. WT EGFR without EGF	13432	10393 to 16472	Yes	****	< 0.0001

WT EGFR with EGF vs. Triple mutant with EGF	5217	2177 to 8256	Yes	****	< 0.0001
WT EGFR with EGF vs. Triple mutant without EGF	13540	10500 to 16580	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant with EGF	12401	9362 to 15441	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant without EGF	13986	10947 to 17026	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mutant with EGF	7049	4010 to 10089	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mutant without EGF	13945	10905 to 16984	Yes	****	< 0.0001
WT EGFR without EGF vs. Triple mutant with EGF	-8216	-11255 to -5176	Yes	****	< 0.0001
WT EGFR without EGF vs. Triple mutant without EGF	107.6	-2932 to 3147	No	ns	> 0.9999
WT EGFR without EGF vs. Penta mutant with EGF	-1031	-4071 to 2009	No	ns	0.9859
WT EGFR without EGF vs. Penta mutant without EGF	554.1	-2486 to 3594	No	ns	> 0.9999
WT EGFR without EGF vs. Src mutant with EGF	-6383	-9423 to -3344	Yes	****	< 0.0001
WT EGFR without EGF vs. Src mutant without EGF	512.3	-2527 to 3552	No	ns	> 0.9999
Triple mutant with EGF vs. Triple mutant without EGF	8323	5284 to 11363	Yes	****	< 0.0001
Triple mutant with EGF vs. Penta mutant with EGF	7185	4145 to 10224	Yes	****	< 0.0001
Triple mutant with EGF vs. Penta mutant without EGF	8770	5730 to 11809	Yes	****	< 0.0001
Triple mutant with EGF vs. Src mutant with EGF	1832	-1207 to 4872	No	ns	0.6518
Triple mutant with EGF vs. Src mutant without EGF	8728	5688 to 11767	Yes	****	< 0.0001
Triple mutant without EGF vs. Penta mutant with EGF	-1139	-4178 to 1901	No	ns	0.9723
Triple mutant without EGF vs. Penta mutant without EGF	446.4	-2593 to 3486	No	ns	> 0.9999
Triple mutant without EGF vs. Src mutant with EGF	-6491	-9530 to -3451	Yes	****	< 0.0001
Triple mutant without EGF vs. Src mutant without EGF	404.7	-2635 to 3444	No	ns	> 0.9999
Penta mutant with EGF vs. Penta mutant without EGF	1585	-1454 to 4625	No	ns	0.8136

Penta mutant with EGF vs. Src mutant with EGF	-5352	-8392 to -2312	Yes	****	< 0.0001
Penta mutant with EGF vs. Src mutant without EGF	1543	-1496 to 4583	No	ns	0.8364
Penta mutant without EGF vs. Src mutant with EGF	-6937	-9977 to -3898	Yes	****	< 0.0001
Penta mutant without EGF vs. Src mutant without EGF	-41.76	-3081 to 2998	No	ns	> 0.9999
Src mutant with EGF vs. Src mutant without EGF	6895	3856 to 9935	Yes	****	< 0.0001

Table S2: One-way ANOVA multiple comparison table for RICM Area following rescue with WT or mutant EGFR

Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Control (EGFR siRNA) with EGF vs. Control (EGFR siRNA) without EGF	4.928	-32.46 to 42.31	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. WT EGFR with EGF	-222.3	-259.7 to -184.9	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. WT EGFR without EGF	-7.594	-44.98 to 29.79	No	ns	0.9997
Control (EGFR siRNA) with EGF vs. Triple mutant with EGF	-154.3	-191.7 to -116.9	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Triple mutant without EGF	-8.794	-46.18 to 28.59	No	ns	0.9991
Control (EGFR siRNA) with EGF vs. Penta mutant with EGF	-8.335	-45.72 to 29.05	No	ns	0.9994
Control (EGFR siRNA) with EGF vs. Penta mutant without EGF	-6.794	-44.18 to 30.59	No	ns	0.9999
Control (EGFR siRNA) with EGF vs. Src mutant with EGF	-158.3	-195.7 to -120.9	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Src mutant without EGF	-7.594	-44.98 to 29.79	No	ns	0.9997
Control (EGFR siRNA) without EGF vs. WT EGFR with EGF	-227.3	-264.6 to -189.9	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. WT EGFR without EGF	-12.52	-49.91 to 24.86	No	ns	0.9871
Control (EGFR siRNA) without EGF vs. Triple mutant with EGF	-159.3	-196.6 to -121.9	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. Triple mutant without EGF	-13.72	-51.11 to 23.66	No	ns	0.9759
Control (EGFR siRNA) without EGF vs. Penta mutant with EGF	-13.26	-50.65 to 24.12	No	ns	0.9808
Control (EGFR siRNA) without EGF vs. Penta mutant without EGF	-11.72	-49.11 to 25.66	No	ns	0.9920
Control (EGFR siRNA) without EGF vs. Src mutant with EGF	-163.3	-200.6 to -125.9	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. Src mutant without EGF	-12.52	-49.91 to 24.86	No	ns	0.9871
WT EGFR with EGF vs. WT EGFR without EGF	214.7	177.4 to 252.1	Yes	****	< 0.0001
WT EGFR with EGF vs. Triple mutant with EGF	68.00	30.61 to 105.4	Yes	****	< 0.0001

WT EGFR with EGF vs. Triple mutant without EGF	213.5	176.2 to 250.9	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant with EGF	214.0	176.6 to 251.4	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant without EGF	215.5	178.2 to 252.9	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mutant with EGF	64.00	26.61 to 101.4	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mutant without EGF	214.7	177.4 to 252.1	Yes	****	< 0.0001
WT EGFR without EGF vs. Triple mutant with EGF	-146.7	-184.1 to -109.4	Yes	****	< 0.0001
WT EGFR without EGF vs. Triple mutant without EGF	-1.200	-38.59 to 36.19	No	ns	> 0.9999
WT EGFR without EGF vs. Penta mutant with EGF	-0.7407	-38.13 to 36.64	No	ns	> 0.9999
WT EGFR without EGF vs. Penta mutant without EGF	0.8000	-36.59 to 38.19	No	ns	> 0.9999
WT EGFR without EGF vs. Src mutant with EGF	-150.7	-188.1 to -113.4	Yes	****	< 0.0001
WT EGFR without EGF vs. Src mutant without EGF	0.0	-37.39 to 37.39	No	ns	> 0.9999
Triple mutant with EGF vs. Triple mutant without EGF	145.5	108.2 to 182.9	Yes	****	< 0.0001
Triple mutant with EGF vs. Penta mutant with EGF	146.0	108.6 to 183.4	Yes	****	< 0.0001
Triple mutant with EGF vs. Penta mutant without EGF	147.5	110.2 to 184.9	Yes	****	< 0.0001
Triple mutant with EGF vs. Src mutant with EGF	-4.000	-41.39 to 33.39	No	ns	> 0.9999
Triple mutant with EGF vs. Src mutant without EGF	146.7	109.4 to 184.1	Yes	****	< 0.0001
Triple mutant without EGF vs. Penta mutant with EGF	0.4593	-36.93 to 37.84	No	ns	> 0.9999
Triple mutant without EGF vs. Penta mutant without EGF	2.000	-35.39 to 39.39	No	ns	> 0.9999
Triple mutant without EGF vs. Src mutant with EGF	-149.5	-186.9 to -112.2	Yes	****	< 0.0001
Triple mutant without EGF vs. Src mutant without EGF	1.200	-36.19 to 38.59	No	ns	> 0.9999
Penta mutant with EGF vs. Penta mutant without EGF	1.541	-35.84 to 38.93	No	ns	> 0.9999
Penta mutant with EGF vs. Src mutant with EGF	-150.0	-187.4 to -112.6	Yes	****	< 0.0001

Penta mutant with EGF vs. Src mutant without EGF	0.7407	-36.64 to 38.13	No	ns	> 0.9999
Penta mutant without EGF vs. Src mutant with EGF	-151.5	-188.9 to -114.2	Yes	****	< 0.0001
Penta mutant without EGF vs. Src mutant without EGF	-0.8000	-38.19 to 36.59	No	ns	> 0.9999
Src mutant with EGF vs. Src mutant without EGF	150.7	113.4 to 188.1	Yes	****	< 0.0001

Table S3: One-way ANOVA multiple comparison table for tension occupancy per unit area following rescue with WT or mutant EGFR

Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Control (EGFR siRNA) with EGF vs. Control (EGFR siRNA) without EGF	0.5176	-6.463 to 7.498	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. WT EGFR with EGF	-27.67	-34.65 to -20.69	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. WT EGFR without EGF	-13.06	-20.04 to -6.076	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Triple mutant with EGF	-19.03	-26.01 to -12.05	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Triple mutant without EGF	-8.923	-15.90 to -1.943	Yes	**	0.0024
Control (EGFR siRNA) with EGF vs. Penta mutant with EGF	2.800	-4.180 to 9.780	No	ns	0.9570
Control (EGFR siRNA) with EGF vs. Penta mutant without EGF	4.918	-2.063 to 11.90	No	ns	0.4258
Control (EGFR siRNA) with EGF vs. Src mutant with EGF	-17.09	-24.07 to -10.11	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Src mutant without EGF	-3.627	-10.61 to 3.353	No	ns	0.8168
Control (EGFR siRNA) without EGF vs. WT EGFR with EGF	-28.19	-35.17 to -21.21	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. WT EGFR without EGF	-13.57	-20.55 to -6.594	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. Triple mutant with EGF	-19.54	-26.52 to -12.56	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. Triple mutant without EGF	-9.441	-16.42 to -2.460	Yes	***	0.0009
Control (EGFR siRNA) without EGF vs. Penta mutant with EGF	2.282	-4.698 to 9.263	No	ns	0.9891
Control (EGFR siRNA) without EGF vs. Penta mutant without EGF	4.400	-2.580 to 11.38	No	ns	0.5906
Control (EGFR siRNA) without EGF vs. Src mutant with EGF	-17.61	-24.59 to -10.63	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. Src mutant without EGF	-4.145	-11.13 to 2.835	No	ns	0.6715
WT EGFR with EGF vs. WT EGFR without EGF	14.62	7.638 to 21.60	Yes	****	< 0.0001
WT EGFR with EGF vs. Triple mutant with EGF	8.648	1.668 to 15.63	Yes	**	0.0039

WT EGFR with EGF vs. Triple mutant without EGF	18.75	11.77 to 25.73	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant with EGF	30.47	23.49 to 37.45	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant without EGF	32.59	25.61 to 39.57	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mutant with EGF	10.58	3.604 to 17.56	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mutant without EGF	24.05	17.07 to 31.03	Yes	****	< 0.0001
WT EGFR without EGF vs. Triple mutant with EGF	-5.970	-12.95 to 1.011	No	ns	0.1672
WT EGFR without EGF vs. Triple mutant without EGF	4.134	-2.847 to 11.11	No	ns	0.6750
WT EGFR without EGF vs. Penta mutant with EGF	15.86	8.876 to 22.84	Yes	****	< 0.0001
WT EGFR without EGF vs. Penta mutant without EGF	17.97	10.99 to 24.95	Yes	****	< 0.0001
WT EGFR without EGF vs. Src mutant with EGF	-4.034	-11.01 to 2.946	No	ns	0.7053
WT EGFR without EGF vs. Src mutant without EGF	9.429	2.449 to 16.41	Yes	***	0.0010
Triple mutant with EGF vs. Triple mutant without EGF	10.10	3.123 to 17.08	Yes	***	0.0003
Triple mutant with EGF vs. Penta mutant with EGF	21.83	14.85 to 28.81	Yes	****	< 0.0001
Triple mutant with EGF vs. Penta mutant without EGF	23.94	16.96 to 30.92	Yes	****	< 0.0001
Triple mutant with EGF vs. Src mutant with EGF	1.936	-5.044 to 8.916	No	ns	0.9968
Triple mutant with EGF vs. Src mutant without EGF	15.40	8.419 to 22.38	Yes	****	< 0.0001
Triple mutant without EGF vs. Penta mutant with EGF	11.72	4.743 to 18.70	Yes	****	< 0.0001
Triple mutant without EGF vs. Penta mutant without EGF	13.84	6.860 to 20.82	Yes	****	< 0.0001
Triple mutant without EGF vs. Src mutant with EGF	-8.167	-15.15 to -1.187	Yes	**	0.0087
Triple mutant without EGF vs. Src mutant without EGF	5.296	-1.685 to 12.28	No	ns	0.3169
Penta mutant with EGF vs. Penta mutant without EGF	2.118	-4.863 to 9.098	No	ns	0.9937
Penta mutant with EGF vs. Src mutant with EGF	-19.89	-26.87 to -12.91	Yes	****	< 0.0001

Penta mutant with EGF vs. Src mutant without EGF	-6.427	-13.41 to 0.5531	No	ns	0.1003
Penta mutant without EGF vs. Src mutant with EGF	-22.01	-28.99 to -15.03	Yes	****	< 0.0001
Penta mutant without EGF vs. Src mutant without EGF	-8.545	-15.53 to -1.565	Yes	**	0.0046
Src mutant with EGF vs. Src mutant without EGF	13.46	6.483 to 20.44	Yes	****	< 0.0001

Table S4: One-way ANOVA multiple comparison table for circularity following rescue with WT or mutant EGFR after EGFR siRNA silencing

Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Control (EGFR siRNA) with EGF vs. Control (EGFR siRNA) without EGF	0.05604	-0.04787 to 0.1600	No	ns	0.7817
Control (EGFR siRNA) with EGF vs. WT EGFR with EGF	-0.1055	-0.2094 to -0.001569	Yes	*	0.0434
Control (EGFR siRNA) with EGF vs. WT EGFR without EGF	0.1188	0.01493 to 0.2228	Yes	*	0.0116
Control (EGFR siRNA) with EGF vs. Triple mutant with EGF	0.008640	-0.09527 to 0.1126	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. Triple mutant without EGF	0.1251	0.02114 to 0.2290	Yes	**	0.0059
Control (EGFR siRNA) with EGF vs. Penta mutant with EGF	0.1120	0.008129 to 0.2160	Yes	*	0.0233
Control (EGFR siRNA) with EGF vs. Penta mutant without EGF	0.05604	-0.04787 to 0.1600	No	ns	0.7817
Control (EGFR siRNA) with EGF vs. Src mutant with EGF	-0.01852	-0.1224 to 0.08539	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. Src mutant without EGF	0.1068	0.002929 to 0.2108	Yes	*	0.0383
Control (EGFR siRNA) without EGF vs. WT EGFR with EGF	-0.1615	-0.2654 to -0.05761	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. WT EGFR without EGF	0.0628	-0.04111 to 0.1667	No	ns	0.6485
Control (EGFR siRNA) without EGF vs. Triple mutant with EGF	-0.0474	-0.1513 to 0.05651	No	ns	0.9074
Control (EGFR siRNA) without EGF vs. Triple mutant without EGF	0.06901	-0.03490 to 0.1729	No	ns	0.5145
Control (EGFR siRNA) without EGF vs. Penta mutant with EGF	0.0560	-0.04791 to 0.1599	No	ns	0.7825
Control (EGFR siRNA) without EGF vs. Penta mutant without EGF	0.0	-0.1039 to 0.1039	No	ns	> 0.9999
Control (EGFR siRNA) without EGF vs. Src mutant with EGF	-0.07456	-0.1785 to 0.02935	No	ns	0.3983
Control (EGFR siRNA) without EGF vs. Src mutant without EGF	0.0508	-0.05311 to 0.1547	No	ns	0.8652
WT EGFR with EGF vs. WT EGFR without EGF	0.2243	0.1204 to 0.3282	Yes	****	< 0.0001
WT EGFR with EGF vs. Triple mutant with EGF	0.1141	0.01021 to 0.2180	Yes	*	0.0189

WT EGFR with EGF vs. Triple mutant without EGF	0.2305	0.1266 to 0.3344	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant with EGF	0.2175	0.1136 to 0.3214	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mutant without EGF	0.1615	0.05761 to 0.2654	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mutant with EGF	0.08696	-0.01695 to 0.1909	No	ns	0.1909
WT EGFR with EGF vs. Src mutant without EGF	0.2123	0.1084 to 0.3162	Yes	****	< 0.0001
WT EGFR without EGF vs. Triple mutant with EGF	-0.1102	-0.2141 to -0.006289	Yes	*	0.0279
WT EGFR without EGF vs. Triple mutant without EGF	0.006212	-0.09770 to 0.1101	No	ns	> 0.9999
WT EGFR without EGF vs. Penta mutant with EGF	-0.006800	-0.1107 to 0.09711	No	ns	> 0.9999
WT EGFR without EGF vs. Penta mutant without EGF	-0.0628	-0.1667 to 0.04111	No	ns	0.6485
WT EGFR without EGF vs. Src mutant with EGF	-0.1374	-0.2413 to -0.03345	Yes	**	0.0014
WT EGFR without EGF vs. Src mutant without EGF	-0.0120	-0.1159 to 0.09191	No	ns	> 0.9999
Triple mutant with EGF vs. Triple mutant without EGF	0.1164	0.01250 to 0.2203	Yes	*	0.0150
Triple mutant with EGF vs. Penta mutant with EGF	0.1034	-0.0005105 to 0.2073	No	ns	0.0523
Triple mutant with EGF vs. Penta mutant without EGF	0.0474	-0.05651 to 0.1513	No	ns	0.9074
Triple mutant with EGF vs. Src mutant with EGF	-0.02716	-0.1311 to 0.07675	No	ns	0.9980
Triple mutant with EGF vs. Src mutant without EGF	0.0982	-0.005711 to 0.2021	No	ns	0.0817
Triple mutant without EGF vs. Penta mutant with EGF	-0.01301	-0.1169 to 0.09090	No	ns	> 0.9999
Triple mutant without EGF vs. Penta mutant without EGF	-0.06901	-0.1729 to 0.03490	No	ns	0.5145
Triple mutant without EGF vs. Src mutant with EGF	-0.1436	-0.2475 to -0.03966	Yes	***	0.0006
Triple mutant without EGF vs. Src mutant without EGF	-0.01821	-0.1221 to 0.08570	No	ns	> 0.9999
Penta mutant with EGF vs. Penta mutant without EGF	-0.0560	-0.1599 to 0.04791	No	ns	0.7825
Penta mutant with EGF vs. Src mutant with EGF	-0.1306	-0.2345 to -0.02665	Yes	**	0.0032

Penta mutant with EGF vs. Src mutant without EGF	-0.005200	-0.1091 to 0.09871	No	ns	> 0.9999
Penta mutant without EGF vs. Src mutant with EGF	-0.07456	-0.1785 to 0.02935	No	ns	0.3983
Penta mutant without EGF vs. Src mutant without EGF	0.0508	-0.05311 to 0.1547	No	ns	0.8652
Src mutant with EGF vs. Src mutant without EGF	0.1254	0.02145 to 0.2293	Yes	**	0.0057

Table S5: One-way ANOVA multiple comparison table for FA size following rescue with WT or mutant EGFR after EGFR siRNA silencing

Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Control (EGFR siRNA) with EGF vs. WT EGFR with EGF	-0.6925	-0.9564 to -0.4285	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Triple mut with EGF	-0.3323	-0.5486 to -0.1161	Yes	***	0.0006
Control (EGFR siRNA) with EGF vs. Penta mut with EGF	0.05964	-0.1217 to 0.2410	No	ns	0.9744
Control (EGFR siRNA) with EGF vs. Src mut with EGF	-0.3481	-0.5480 to -0.1483	Yes	***	0.0001
Control (EGFR siRNA) with EGF vs. Control (EGFR siRNA) without EGF	0.008188	-0.1487 to 0.1651	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. WT EGFR without EGF	0.01815	-0.1386 to 0.1749	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. Triple mut without EGF	0.02924	-0.09857 to 0.1571	No	ns	0.9980
Control (EGFR siRNA) with EGF vs. Penta mut without EGF	0.01763	-0.1241 to 0.1593	No	ns	> 0.9999
Control (EGFR siRNA) with EGF vs. Src mut without EGF	0.04307	-0.08903 to 0.1752	No	ns	0.9757
WT EGFR with EGF vs. Triple mut with EGF	0.3601	0.1107 to 0.6095	Yes	**	0.0014
WT EGFR with EGF vs. Penta mut with EGF	0.7521	0.4843 to 1.020	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mut with EGF	0.3443	0.1102 to 0.5785	Yes	**	0.0011
WT EGFR with EGF vs. Control (EGFR siRNA) without EGF	0.7006	0.5075 to 0.8938	Yes	****	< 0.0001
WT EGFR with EGF vs. WT EGFR without EGF	0.7106	0.5079 to 0.9133	Yes	****	< 0.0001
WT EGFR with EGF vs. Triple mut without EGF	0.7217	0.5034 to 0.9400	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mut without EGF	0.7101	0.4697 to 0.9505	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mut without EGF	0.7355	0.5011 to 0.9700	Yes	****	< 0.0001
Triple mut with EGF vs. Penta mut with EGF	0.3920	0.1697 to 0.6142	Yes	****	< 0.0001
Triple mut with EGF vs. Src mut with EGF	-0.01578	-0.1315 to 0.09990	No	ns	> 0.9999

Comparison	Mean diff	95% CI	Significant?	Summary	Adjusted P Value
Triple mut with EGF vs. Control (EGFR siRNA) without EGF	0.3405	0.1082 to 0.5729	Yes	**	0.0011
Triple mut with EGF vs. WT EGFR without EGF	0.3505	0.1161 to 0.5849	Yes	***	0.0009
Triple mut with EGF vs. Triple mut without EGF	0.3616	0.1283 to 0.5949	Yes	***	0.0006
Triple mut with EGF vs. Penta mut without EGF	0.3500	0.1266 to 0.5733	Yes	***	0.0005
Triple mut with EGF vs. Src mut without EGF	0.3754	0.1570 to 0.5938	Yes	***	0.0001
Penta mut with EGF vs. Src mut with EGF	-0.4078	-0.6200 to -0.1956	Yes	****	< 0.0001
Penta mut with EGF vs. Control (EGFR siRNA) without EGF	-0.05145	-0.2293 to 0.1264	No	ns	0.9890
Penta mut with EGF vs. WT EGFR without EGF	-0.04149	-0.2133 to 0.1303	No	ns	0.9970
Penta mut with EGF vs. Triple mut without EGF	-0.03040	-0.2228 to 0.1620	No	ns	0.9999
Penta mut with EGF vs. Penta mut without EGF	-0.04201	-0.2338 to 0.1498	No	ns	0.9986
Penta mut with EGF vs. Src mut without EGF	-0.01657	-0.2097 to 0.1766	No	ns	> 0.9999
Src mut with EGF vs. Control (EGFR siRNA) without EGF	0.3563	0.1562 to 0.5564	Yes	****	< 0.0001
Src mut with EGF vs. WT EGFR without EGF	0.3663	0.1694 to 0.5632	Yes	****	< 0.0001
Src mut with EGF vs. Triple mut without EGF	0.3774	0.1742 to 0.5805	Yes	****	< 0.0001
Src mut with EGF vs. Penta mut without EGF	0.3658	0.1765 to 0.5550	Yes	****	< 0.0001
Src mut with EGF vs. Src mut without EGF	0.3912	0.1924 to 0.5900	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. WT EGFR without EGF	0.009960	-0.03983 to 0.05975	No	ns	0.9993
Control (EGFR siRNA) without EGF vs. Triple mut without EGF	0.02105	-0.06755 to 0.1097	No	ns	0.9973
Control (EGFR siRNA) without EGF vs. Penta mut without EGF	0.009440	-0.1031 to 0.1220	No	ns	> 0.9999
Control (EGFR siRNA) without EGF vs. Src mut without EGF	0.03488	-0.06363 to 0.1334	No	ns	0.9595
WT EGFR without EGF vs. Triple mut without EGF	0.01109	-0.07929 to 0.1015	No	ns	> 0.9999
WT EGFR without EGF vs. Penta mut without EGF	-0.0005200	-0.1136 to 0.1125	No	ns	> 0.9999

WT EGFR without EGF vs. Src mut without EGF	0.02492	-0.08208 to 0.1319	No	ns	0.9977
Triple mut without EGF vs. Penta mut without EGF	-0.01161	-0.08728 to 0.06405	No	ns	> 0.9999
Triple mut without EGF vs. Src mut without EGF	0.01383	-0.05002 to 0.07767	No	ns	0.9987
Penta mut without EGF vs. Src mut without EGF	0.02544	-0.04335 to 0.09423	No	ns	0.9477

Table S6: One-way ANOVA multiple comparison table for FA number following rescue with WT or mutant EGFR after EGFR siRNA silencing

Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Control (EGFR siRNA) with EGF vs. WT EGFR with EGF	-24.84	-37.56 to -12.12	Yes	****	< 0.0001
Control (EGFR siRNA) with EGF vs. Triple mut with EGF	-9.440	-19.35 to 0.4682	No	ns	0.0709
Control (EGFR siRNA) with EGF vs. Penta mut with EGF	5.960	-3.804 to 15.72	No	ns	0.5309
Control (EGFR siRNA) with EGF vs. Src mut with EGF	-13.32	-25.14 to -1.500	Yes	*	0.0187
Control (EGFR siRNA) with EGF vs. Control (EGFR siRNA) without EGF	3.240	-5.191 to 11.67	No	ns	0.9348
Control (EGFR siRNA) with EGF vs. WT EGFR without EGF	4.800	-3.997 to 13.60	No	ns	0.6713
Control (EGFR siRNA) with EGF vs. Triple mut without EGF	1.640	-8.471 to 11.75	No	ns	0.9999
Control (EGFR siRNA) with EGF vs. Penta mut without EGF	5.400	-3.736 to 14.54	No	ns	0.5729
Control (EGFR siRNA) with EGF vs. Src mut without EGF	3.960	-6.231 to 14.15	No	ns	0.9306
WT EGFR with EGF vs. Triple mut with EGF	15.40	4.695 to 26.11	Yes	**	0.0014
WT EGFR with EGF vs. Penta mut with EGF	30.80	20.03 to 41.57	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mut with EGF	11.52	0.7305 to 22.31	Yes	*	0.0298
WT EGFR with EGF vs. Control (EGFR siRNA) without EGF	28.08	17.13 to 39.03	Yes	****	< 0.0001
WT EGFR with EGF vs. WT EGFR without EGF	29.64	19.57 to 39.71	Yes	****	< 0.0001
WT EGFR with EGF vs. Triple mut without EGF	26.48	16.15 to 36.81	Yes	****	< 0.0001
WT EGFR with EGF vs. Penta mut without EGF	30.24	19.13 to 41.35	Yes	****	< 0.0001
WT EGFR with EGF vs. Src mut without EGF	28.80	17.88 to 39.72	Yes	****	< 0.0001
Triple mut with EGF vs. Penta mut with EGF	15.40	5.848 to 24.95	Yes	***	0.0003
Triple mut with EGF vs. Src mut with EGF	-3.880	-12.52 to 4.756	No	ns	0.8533

Triple mut with EGF vs. Control (EGFR siRNA) without EGF	12.68	2.629 to 22.73	Yes	**	0.0062
Triple mut with EGF vs. WT EGFR without EGF	14.24	6.056 to 22.42	Yes	***	0.0001
Triple mut with EGF vs. Triple mut without EGF	11.08	1.295 to 20.87	Yes	*	0.0179
Triple mut with EGF vs. Penta mut without EGF	14.84	4.422 to 25.26	Yes	**	0.0016
Triple mut with EGF vs. Src mut without EGF	13.40	3.656 to 23.14	Yes	**	0.0024
Penta mut with EGF vs. Src mut with EGF	-19.28	-26.95 to -11.61	Yes	****	< 0.0001
Penta mut with EGF vs. Control (EGFR siRNA) without EGF	-2.720	-7.920 to 2.480	No	ns	0.7185
Penta mut with EGF vs. WT EGFR without EGF	-1.160	-6.943 to 4.623	No	ns	0.9993
Penta mut with EGF vs. Triple mut without EGF	-4.320	-11.44 to 2.799	No	ns	0.5387
Penta mut with EGF vs. Penta mut without EGF	-0.5600	-5.020 to 3.900	No	ns	> 0.9999
Penta mut with EGF vs. Src mut without EGF	-2.000	-7.033 to 3.033	No	ns	0.9215
Src mut with EGF vs. Control (EGFR siRNA) without EGF	16.56	7.384 to 25.74	Yes	****	< 0.0001
Src mut with EGF vs. WT EGFR without EGF	18.12	8.972 to 27.27	Yes	****	< 0.0001
Src mut with EGF vs. Triple mut without EGF	14.96	6.469 to 23.45	Yes	****	< 0.0001
Src mut with EGF vs. Penta mut without EGF	18.72	10.07 to 27.37	Yes	****	< 0.0001
Src mut with EGF vs. Src mut without EGF	17.28	9.252 to 25.31	Yes	****	< 0.0001
Control (EGFR siRNA) without EGF vs. WT EGFR without EGF	1.560	-3.580 to 6.700	No	ns	0.9848
Control (EGFR siRNA) without EGF vs. Triple mut without EGF	-1.600	-9.114 to 5.914	No	ns	0.9988
Control (EGFR siRNA) without EGF vs. Penta mut without EGF	2.160	-3.163 to 7.483	No	ns	0.9122
Control (EGFR siRNA) without EGF vs. Src mut without EGF	0.7200	-4.968 to 6.408	No	ns	> 0.9999
WT EGFR without EGF vs. Triple mut without EGF	-3.160	-10.86 to 4.543	No	ns	0.9070
WT EGFR without EGF vs. Penta mut without EGF	0.6000	-4.781 to 5.981	No	ns	> 0.9999

					>
WT EGFR without EGF vs. Src mut without EGF	-0.8400	-7.335 to 5.655	No	ns	0.9999
Triple mut without EGF vs. Penta mut without EGF	3.760	-2.875 to 10.39	No	ns	0.6260
Triple mut without EGF vs. Src mut without EGF	2.320	-3.189 to 7.829	No	ns	0.8934
Penta mut without EGF vs. Src mut without EGF	-1.440	-6.529 to 3.649	No	ns	0.9906

Table S7. DNA sequence for the TGT strands.

All DNA strands were custom synthesized by Integrated DNA Technologies (Coralville, IA).
Note that six T bases were added to the 56 pN TGT (bottom) sequence at the 5' end. These bases
serve as a spacer to separate the internal amine from the 5' biotin.

Name	Sequence (5' to 3')
Alkyne-21-BHQ2 (Top)	/5Hexynyl/GTG AAA TAC CGC ACA GAT GCG/3BHQ_2/
12 pN TGT (bottom)	/AmMC6/CGC ATC TGT GCG GTA TTT CAC TTT /3BioTEG/
56 pN TGT (bottom)	/5Biosg/TTT TTT/iUniAmM/CGC ATC TGT GCG GTA TTT CAC TTT

Table S8. MALDI-TOF MS analysis for the labelled oligonucleotides.

Product name	Calculated molecular weight	Found molecular weight
cRGDfK-1-BHQ2 (Top)	8157.9	8160.1
Cy3B labelled 12 pN TGT (bottom)	8592.7	8632.4
Cy3B labelled 56 pN TGT (bottom)	10272.7	10295.8

Table S9. Primer sequences for Site Directed Mutagenesis of WT EGFR EGFP.

All DNA oligonucleotides were custom synthesized by Integrated DNA Technologies
(Coralville, IA).

Name	Sequence (5' to 3')
EGFR 845	For - GCGGAAGAGAAAGAATTCCATGCAGAAGGAGGC
	Rev - GCCTCCTTCTGCATGGAATTCTTTCTCTTCCGC
EGFR 992	For - GGATGCCGACGAGTTCCTCATCCCACAGC
	Rev - GCTGTGGGATGAGGAACTCGTCGGCATCC
EGFR 1068	For - CCTCCCAGTGCCTGAATTCATAAACCAGTCCGTTC
	Rev - GAACGGACTGGTTTATGAATTCAGGCACTGGGAGG
EGFR 1086	For - TGTGCAGAATCCTGTCTTTCACAATCAGCCTCTGA
	Rev - TCAGAGGCTGATTGTGAAAGACAGGATTCTGCACA
EGFR 1148	For - CTGGACAACCCTGACTTCCAGCAGGACTTCTTT
	Rev – AAAGAAGTCCTGCTGGAAGTCAGGGTTGTCCAG
EGFR 1173	For - GCTGAAAATGCAGAATTCCTAAGGGTCGCGCC
	Rev - GGCGCGACCCTTAGGAATTCTGCATTTTCAGC