EGFR and HER2 Activate Rigidity Sensing Only on Rigid Matrices

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Supplementary Discussion

In this study, we show that EGFR and HER2 play a key role in rigidity sensing and directly affect CU formation. We find that during cell spreading, the formation of CUs is dramatically inhibited by blocking EGFR activity with the consequence that cell spread area is substantially decreased. The inhibition of SFK activity has a similar effect on CU activity and on spread area, which is consistent with earlier reports that Src kinase is responsible for non-ligand dependent activation of EGFR[1,2]. Furthermore, SFK mediated tyrosine phosphorylation can also activate overexpressed HER2 to substitute for EGFR in this pathway and enable rigidity sensing by the cell.

There is an extensive literature on EGFR because it has a major role in many cancers and growth abnormalities[3–5]. Our findings indicate that the non-ligand dependent activation of EGFR affects CU activity which we previously showed was important for rigidity sensing[6,7]. A simple hypothesis to explain the results is that EGFR is a critical part of a positive feedback system that is activated by rigid matrices. Since only a few studies of EGFR have been done on soft

surfaces[8], the lack of EGFR involvement in cell function on soft surfaces has not been well characterized. However, elements of the process can be understood.

Ligand-independent activation of EGFR is linked to Src activation of EGFR but only on rigid surfaces[9]. Our studies are consistent with SFK activation of EGFR that is dependent upon rigidity sensing and indicates that the recruitment of pEGFR to rigid adhesion sites depends upon SFK. Not only do the antibodies to the Src phosphorylation site on EGFR localize better to the adhesions than does the antibody to the autophosphorylation site, but also the inhibition of SFK has the same effect on spreading and CU activity as does the inhibition of EGFR kinase.

Our findings also highlight the transient nature of the EGFR effects. It is known that addition of EGF results in a transient (~1-3 minutes) activation of the EGFR that causes endocytosis and transport to the nucleus[10]. This addition of EGF to spread cells causes a dramatic increase in CU activity concomitant with the commonly observed rapid spreading. This effect of EGF is dependent upon myosin contractility and substrate rigidity, implying that there may be a positive feedback cycle for EGFR recruitment and activation of CU formation. The CUs are also dynamic since the contraction-relaxation cycle lasts only a minute[11]; and in stably spread cells, the level of CU activity is both very low and intermittent. However, on stiff surfaces, the CU activity causes the stabilization of adhesions and growth for many fibroblasts. If EGFR is an integral part of the system that causes CU assembly at adhesion sites, then it could also only transiently interact with the adhesions. Indeed, we find that the ratio of pEGFR to paxillin decreases upon adhesion maturation.

References

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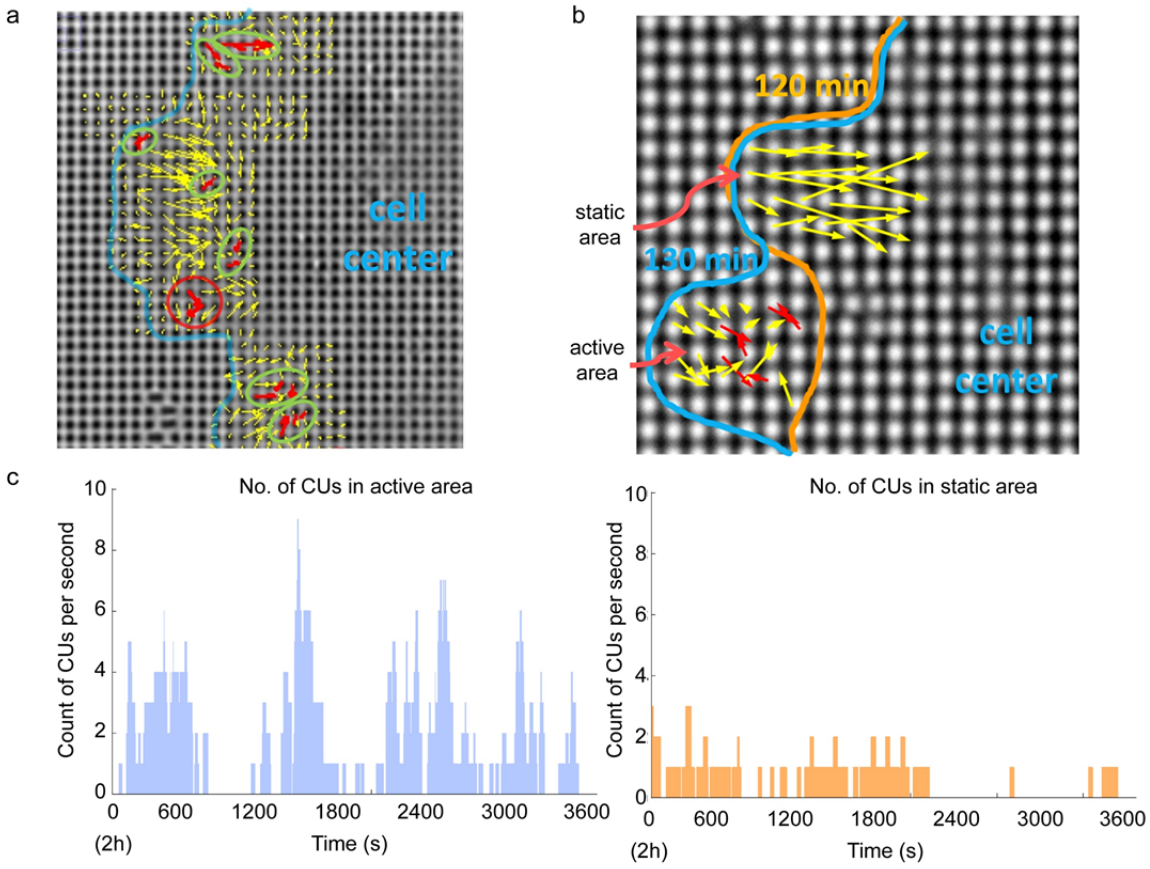
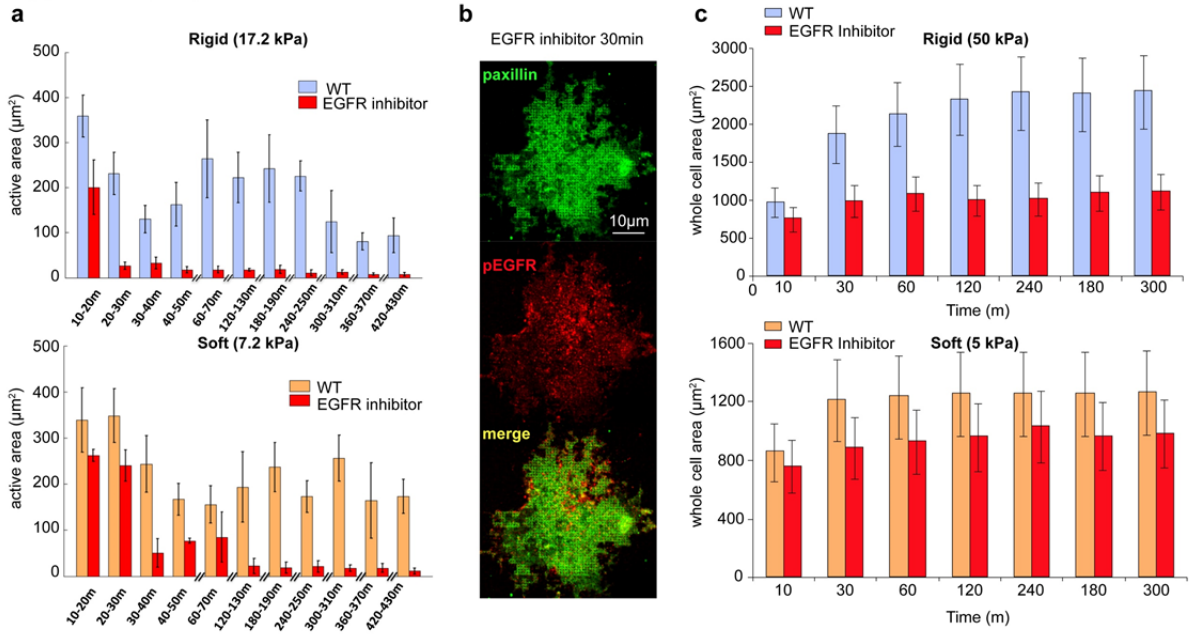
Supplementary Figure 1



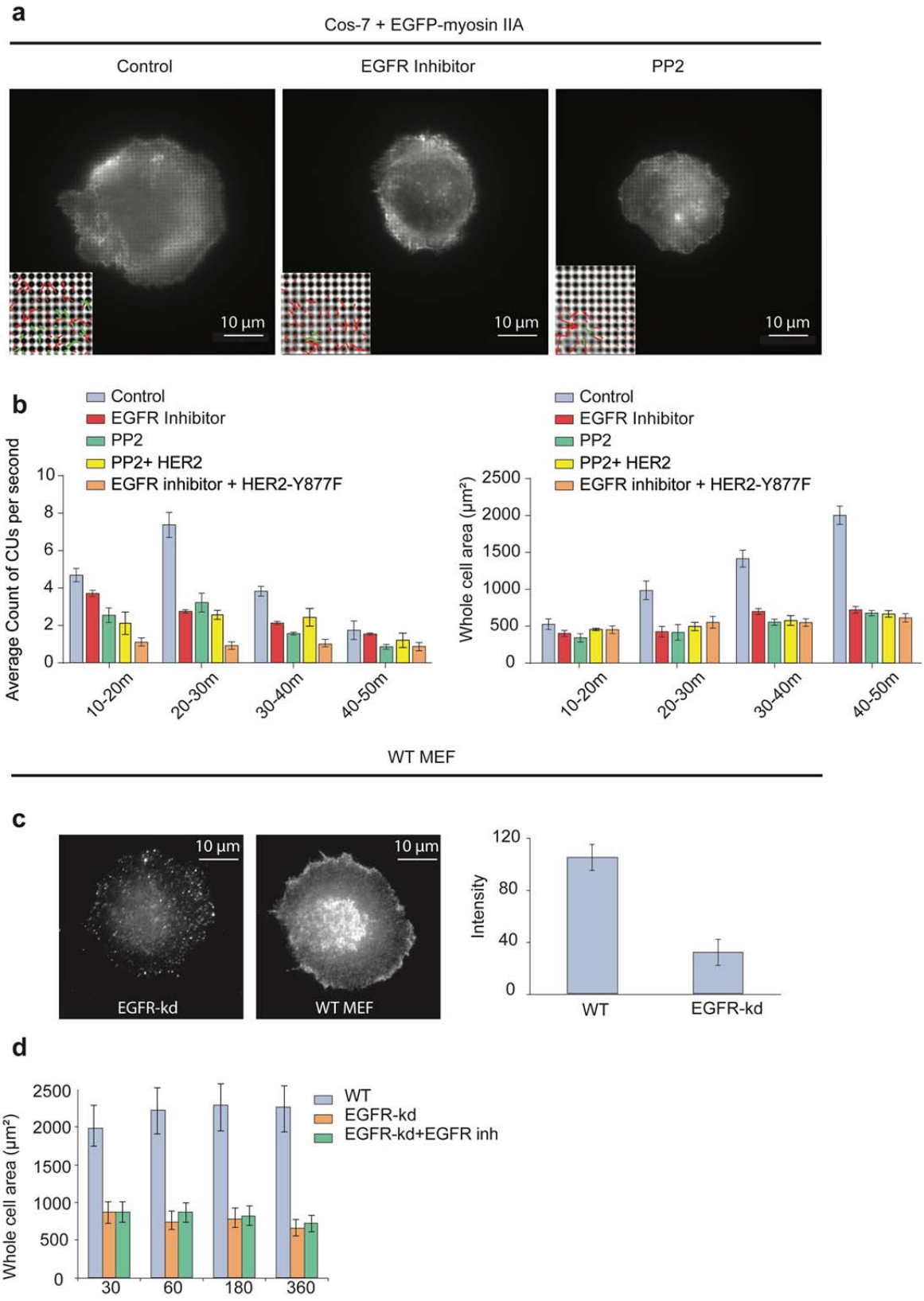
Supplementary Figure 1 CUs detection method and definition of active area. **a,** actual CUs detected in the active area by the automated program. Shown is a frame from a time-lapse movie of a cell spreading on FN-coated 17.2 kPa pillars. Arrows represent pillar movement: red, detected CUs; yellow, non-CUs. Green circles marked correctly identified CU; red circle marked incorrectly detected CU. Success rate=97.47+/-0.75%. Cell edge marked in blue. **b,** cell edge marked in orange and blue correspond to time 120 min and 130 min respectively. Active area is defined as the area where the cell edge moves outwards. **c,** number of CUs per second of the cell in **b** monitored from 2h to 3h**.** The left and the right plots show the number of CUs in the active and static area, respectively.

Supplementary Figure 2

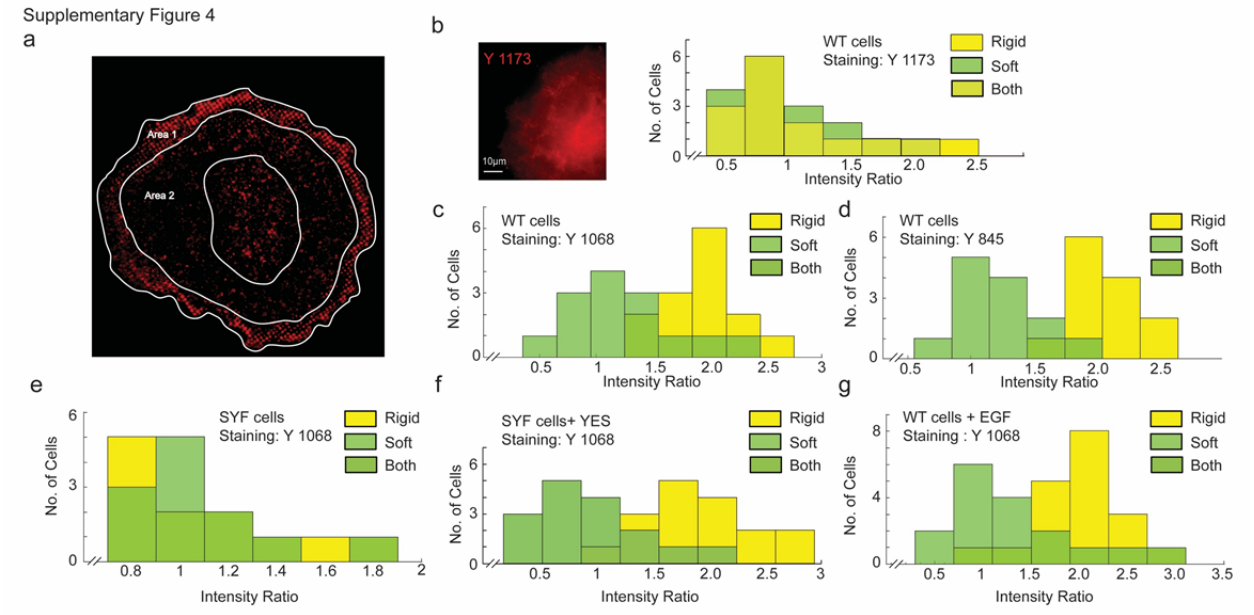


Supplementary Figure 2 Active area and pEGFR distribution disrupted by EGFR inhibitor and whole cell area on FN coated flat PDMS substrates. **a,** active area was measured in intervals of 10 minutes. Cells with and without EGFR inhibitor treatment in medium lacking serum were monitored on rigid (top panel) and soft (bottom panel) pillar substrates for over 7 hours. **b,** phosphorylated EGFR lost co-localization with paxillin with EGFR inhibitor (gefitinib, 10 nM) treatment on rigid pillar substrate. **c,** Whole cell area of WT MEFs when spread on FN coated soft and stiff flat PDMS substrate with or without EGFR inhibitor. Error bars show standard error of the mean.

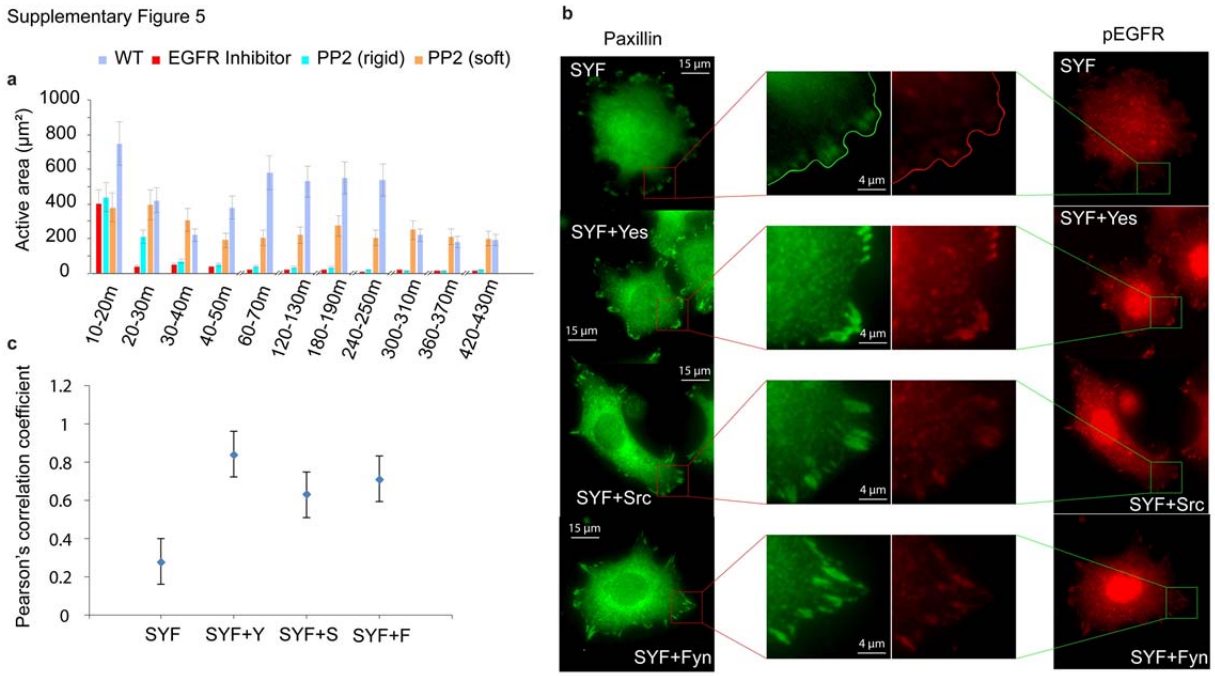
Supplementary Figure 3



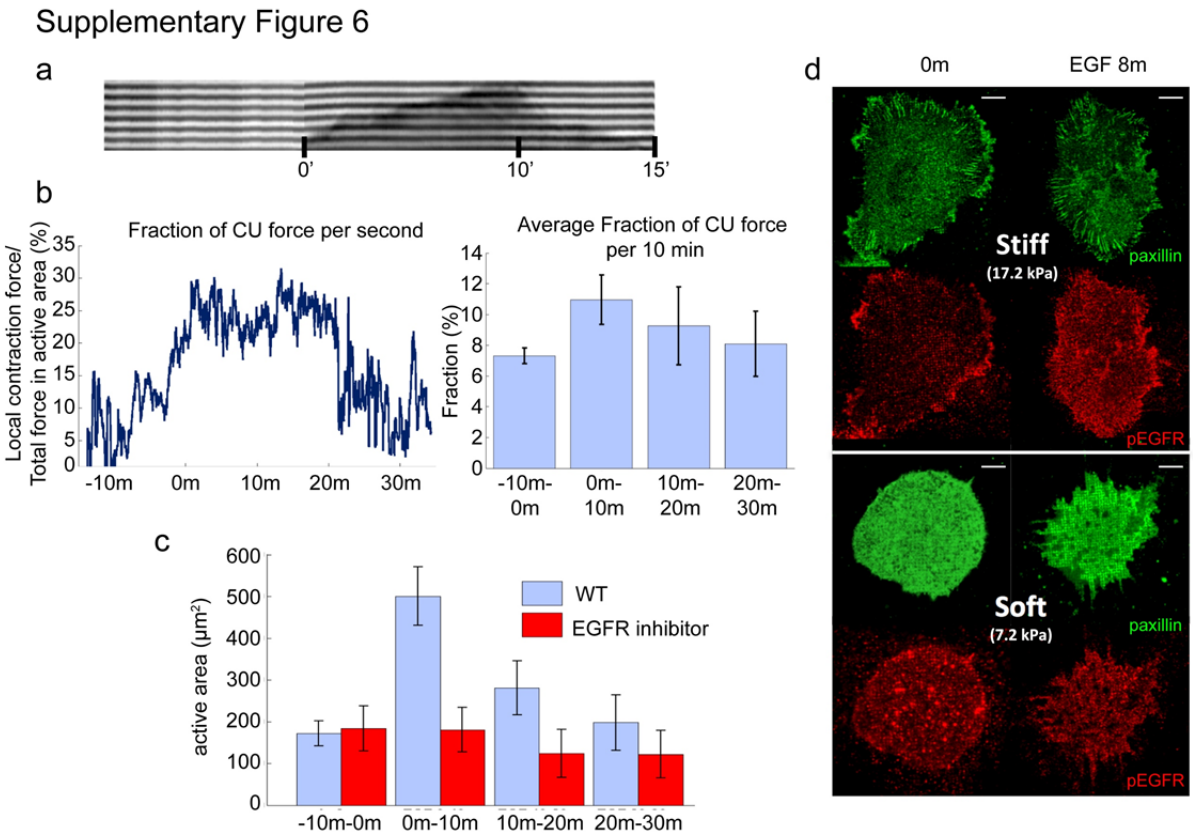
Supplementary Figure 3 Confirmation of EGFR and HER2 involvement in Cos-7 and WT MEFs using small molecule inhibitors and inactive HER2 point mutant. **a,** Cos-7 cells expressing myosin IIA were spread on rigid pillars with or without EGFR and/or HER2 inhibitor or PP2**.** Further, Cos-7 cells were transfected with HER2 mutant (Y877F) in combination with the mentioned inhibitors. The arrows in the inset show pillar displacements. **b**, Whole cell area and contractile unit count for the first hour of spreading. **c,** WT MEFs and cells with knock-down of EGFR stained for total EGFR. Average total intensity is also shown to signify the extent of knock-down. 22 cells were analyzed for each case. **d,** Whole cell area of EGFR knock-down cells as a function of time. 18 cells were analyzed from 3 independent experiments. Error bars show standard error of the mean.



Supplementary Figure 4 Quantification of pEGFR distribution at cells edges. **a,** Average Intensity of pEGFR was calculated in Area 1 and Area 2 as shown. Area 1 is defined as a band across the cell edge with a width of ~4-5 μm. Intensity ratio is calculated by dividing the mentioned average from Area 1 by that of Area 2. This calculated Intensity ratio is a measure of the peripheral localization of pEGFR. In the following plots intensity ratio is plotted for the mentioned cells on stiff and soft substrates. **b,** WT cells stained for pEGFR Y1173. **c,** WT cells stained for pEGFR Y1068. **d,** WT cells stained for pEGFR Y845. **e,** SYF cells stained for pEGFR Y1068. **f,** SYF stably expressing YES stained for pEGFR Y1068. **g,** WT cells stimulated with EGF and then stained for pEGFR Y1068.



Supplementary Figure 5 Establishing the connection between SFKs and EGFR in cell spreading and adhesions. **a,** Active area of WT MEFs on stiff and soft pillars, with or without PP2. **b,** SYF cells and respective knock ins stained for Paxillin and pEGFR after 30 min of spreading on glass. **c,** Pearson correlation coefficient between paxillin and pEGFR in peripheral zone of cells as defined by Area 1 in Supplementary Figure 4a. n>10. 3 independent experiments. Error bars represent standard error of the mean.



Supplementary Figure 6 EGF stimulation of cell edge and CU activity. **a,** Kymograph of cell edge after EGF addition at t=0 minutes. **b,** Plot of fraction of contractile unit force in total force exerted by the active area of a cell upon EGF addition. **c,** Active area of the cell with or without EGFR inhibitor when stimulated by EGF. **d,** Staining for paxillin and phosphorylated EGFR on rigid and soft substrates before (0 min) and after (8 min) addition of EGF. Scale bar is 10 μm. Error bars show standard error of the mean.