Biophysical Journal, Volume *121*

Supplemental information

IQGAP1 scaffolding links phosphoinositide kinases to cytoskeletal reorganization

V. Siddartha Yerramilli, Alonzo H. Ross, Suzanne Scarlata, and Arne Gericke

SUPPLEMENTARY INFORMATION

Figure S1: Changes in lifetime do not occur for eGFP and mCherry lacking functional domains: A) A false color image of a representative cell (left) shows untagged eGFP expressed in HeLa cells. A phasor plot (middle) shows that eGFP has a single fluorescent lifetime indicated by a homogenous population on the phasor arc. The pixels included in the green circle of the phasor plot (lifetime center= 2.56 ns) are false colored green and overlaid on a grayscale image of the cell (right), demonstrating a uniform lifetime throughout the cell. B) Average eGFP lifetime in HeLa cells transfected with untagged eGFP, eGFP-IQGAP1, eGFP-IQGAP1 with untagged mCherry, eGFP-PIPKIγ, or eGFP-PIPKIγ with untagged mCherry. n≥5 measured in at least two independent experiments, no significant differences between with and without untagged mCherry groups. Error bars denote standard deviation. *Scale bar= 10μm*

Figure S2: IQGAP1 strongly associates with PIPKIγ: A) Distribution of each individual pixel lifetime shifts due to FRET between eGFP-IQGAP1 and dsRed-PIPKIγ (Medians 2.35 and 2.15). This histogram analysis reaches the same conclusion as the phasor plot in Fig. 1. B) Coexpression of dsRed-PIPKIγ with eGFP-IQGAP1 significantly decreases eGFP lifetime. Treatment with EGF (100 ng/ml) or with LY294002 (1 μM) does not significantly affect this interaction. C) eGFP-IQGAP1 shows evidence of FRET when co-expressed with dsRed-PIPKIγ in NIH3T3 and HepG2A cell lines, in addition to HeLa results in decreased eGFP IQGAP1 lifetimes. n≥5 measured in at least two independent experiments, * = p ≤0.05, **=p<0.01, ***=p<-0.001, ****=p<0.0001. Error bars denote standard deviation.

Figure S3: IQGAP1 associates with PI(4,5)P2 and PI(3,4,5)P3 but not PI(4P):: A) Western blot showing the knockdown of IQGAP1 in response to IQGAP1 siRNA mCherry-PH-PLCδ1 (PI(4,5)P2 sensor),. B) dsRed-PIPKIγ demonstrates FRET when co expressed with eGFP-IQGAP1 demonstrates FRET when co-expressed with mCherry-PH-PLCδ1 (PI(4,5)P2 sensor), mCherry-PH-Akt1 and mCherry-PH-Akt2 (PI(3,4,5)P3 sensor) but not mCherry-PH-SidM (PI(4)P sensor) in HeLa cells. Only the IQGAP1-PH-Akt1 and IQGAP1-PH-Akt2 interactions are altered by EGF. eGFP lifetimes of all PH-PLCδ1 and PH-Akt1 significantly different from eGFP-IQGAP1 the except IQGAP+PH PLCδ1+EGF. eGFP lifetimes in cells expressing both eGFP-IQGAP1 and mCherry PH Akt2 are significantly different from cells that express eGFP-IQGAP1 only upon EGF stimulation. n ≥5 measured in at least two independent experiments* = p ≤0.05, **=p<0.01, ***=p<-0.001, ****=p<0.0001.Error bars denote standard deviation.

Figure S4: IQGAP1 interacts with phosphoinostide kinases and mediates their association: A) A representative image showing colocalization between eGFP-IQGAP1, dsRed-PIPKIγ and LysoTracker that highlights late endosomes and lysosomes in HeLa cells. B) A representative image showing colocalization between eGFP-IQGAP1 and anti-PI3K p110α antibody tagged with Alexa 647 secondary antibody). *Scale bar= 10μm.*

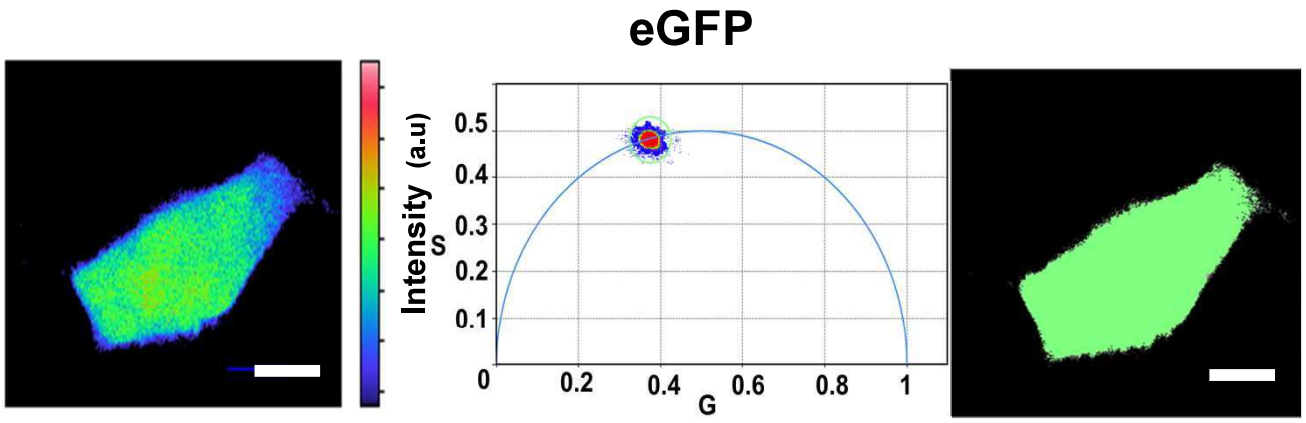
Figure S5: IQGAP1 and PI3K bind to focal adhesion and cytoskeletal proteins: A) A graph of several focal adhesion proteins that were co-immunoprecipitated with anti-IQGAP1 and anti-PI3K p110a antibodies that denotes their percent coverage which is calculated by dividing the

number of amino acids in all found peptides by the total number of amino acids in the entire protein sequence. The proteins that are known to bind to $PI(4,5)P_2$ are highlighted. B) A graph of several cytoskeletal proteins that were co-immunoprecipitated with anti-IQGAP1 and anti-PI3K p110a antibodies that denotes their percent coverage which is calculated by dividing the number of amino acids in all found peptides by the total number of amino acids in the entire protein sequence. The proteins that are known to bind to $PI(4,5)P_2$ are highlighted.

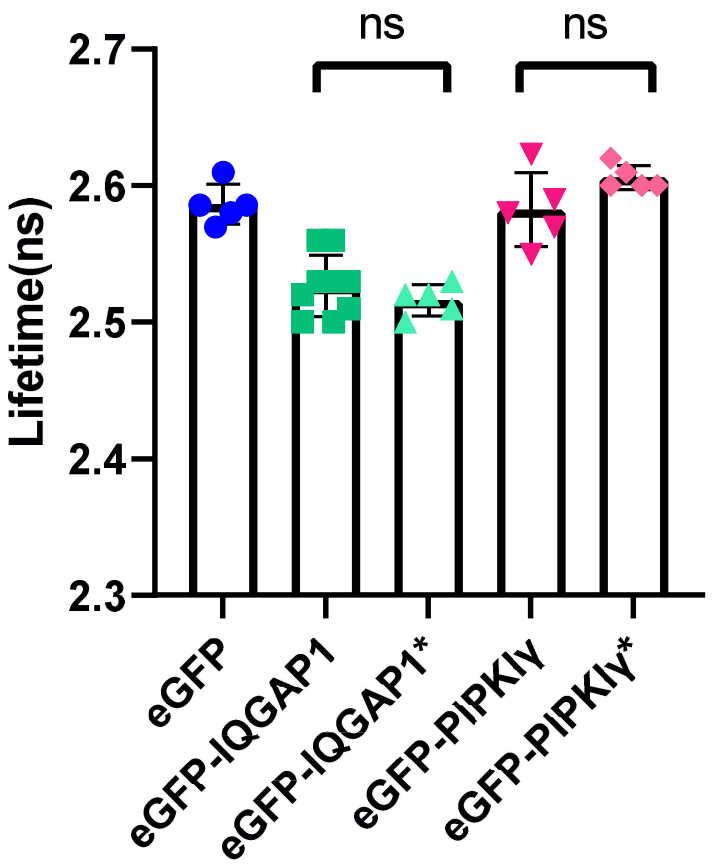
Figure S6: IQGAP1 mediates actin clustering: A) N&B results are plotted using a Brightness vs Intensity plot, where individual pixels of the images of HeLa cells expressing mCherry-β-actin are highlighted either in a green box (B<1.5) or a magenta box (B>1.5) (left panels). The distribution of these highlighted pixels can be seen overlaid (right panels) on a representative unstimulated cell. B) A Brightness vs Intensity plot, where individual pixels of the images of HeLa cells expressing mCherry-β-actin are highlighted either in a green box (B<1.5) or a magenta box (B>1.5) (left panels). The distribution of these highlighted pixels can be seen overlaid (right panels) on a representative cell that is stimulated by EGF (100 ng/ml) for 1 hour. C) A statistically larger number of pixels with higher B values (magenta pixels, B>1.5) that represent oligomeric species or clusters of mCherry-β-actin are seen after the cells are stimulated with EGF). A similar but muted response is seen when IQGAP1 expression is downregulated using siRNA but this increase is statistically lower than the amount of actin clusters in stimulated control cells. n≥4 measured in at least two independent experiments. * = p ≤0.05, **=p<0.01, ***=p<-0.001, ****=p<0.0001. Error bars denote standard deviation. *Scale bar= 10μm*

Figure S1

A

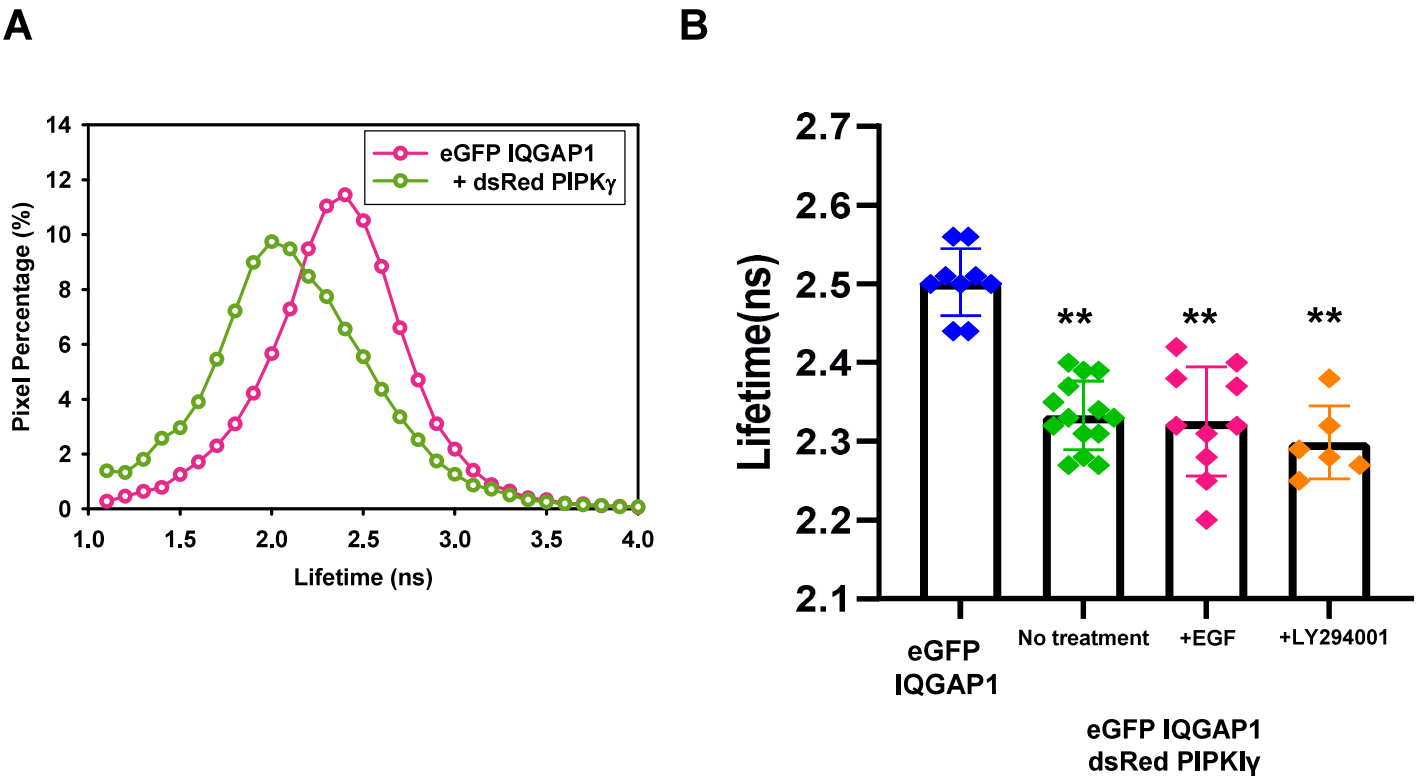


B



* = EGF stimulation

Figure S2


A
B
eGFP IQGAP1
+ dsRed PIPKγ
Pixel Percentage (%)
Lifetime (ns)
Lifetime(ns)
**
**
**
eGFP IQGAP1
No treatment
+EGF
+LY294001
eGFP IQGAP1
dsRed PIPKIγ

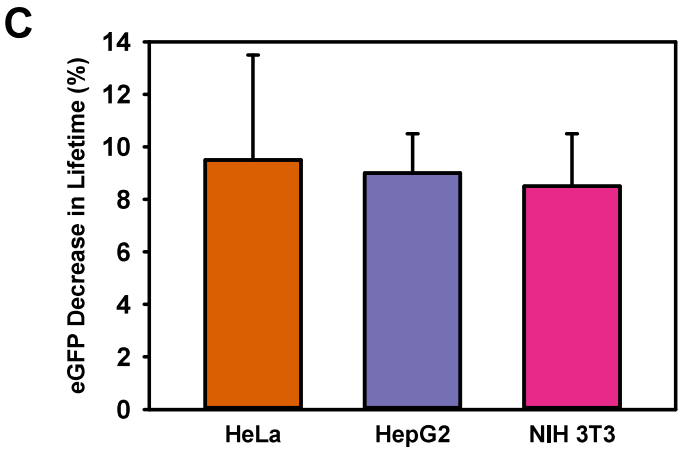

C
eGFP Decrease in Lifetime (%)
HeLa
HepG2
NIH 3T3

Figure S3

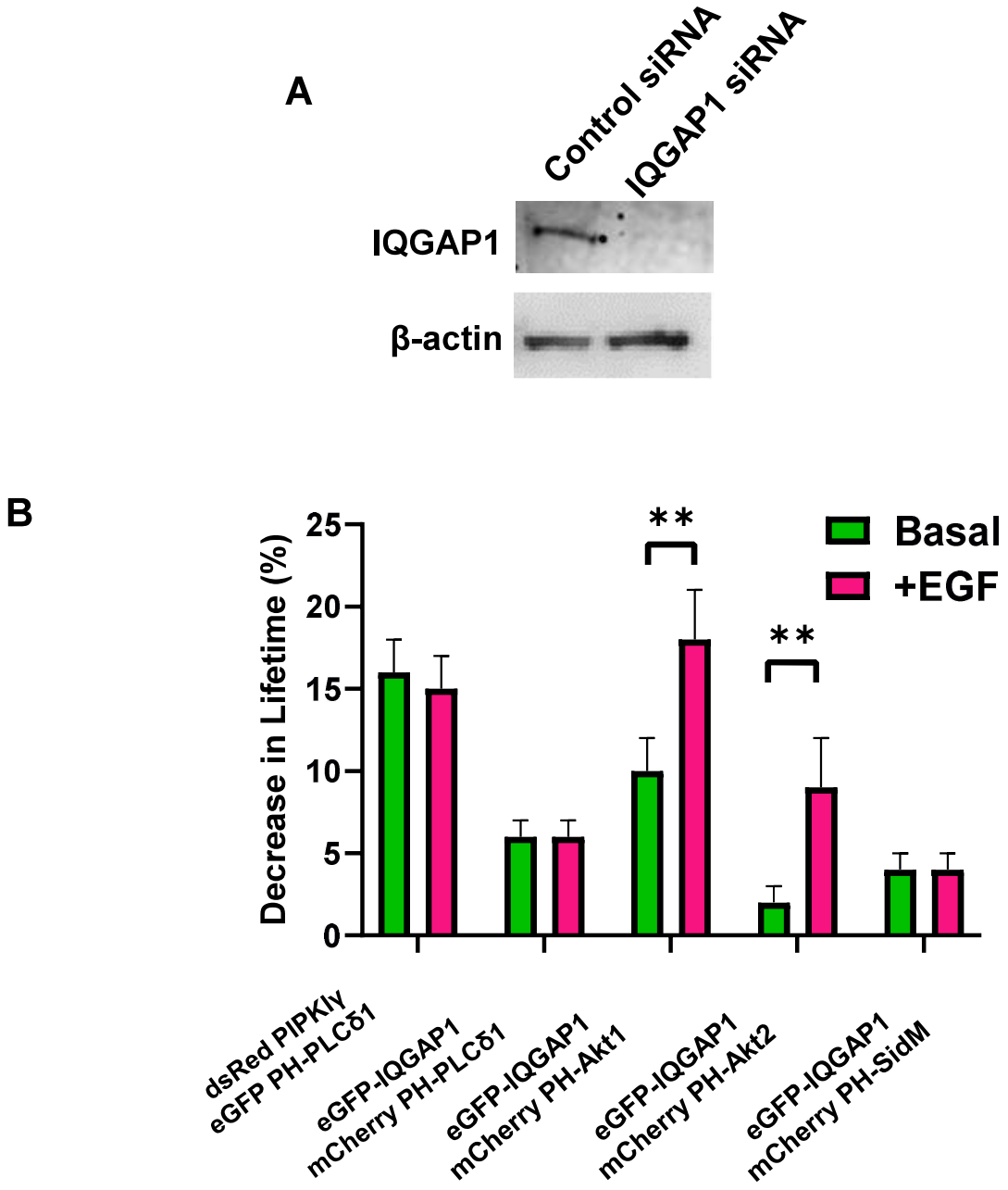

A
Control siRNA
IQGAP1 siRNA
IQGAP1
β-actin
B
Decrease in Lifetime (%)
25
20
15
10
5
0
**
**
Basal
+EGF
dsRed PIPKIγ
eGFP PH-PLCδ1
eGFP-IQGAP1
mCherry PH-PLCδ1
eGFP-IQGAP1
mCherry PH-Akt1
eGFP-IQGAP1
mCherry PH-Akt2
eGFP-IQGAP1
mCherry PH-SidM

Figure S4

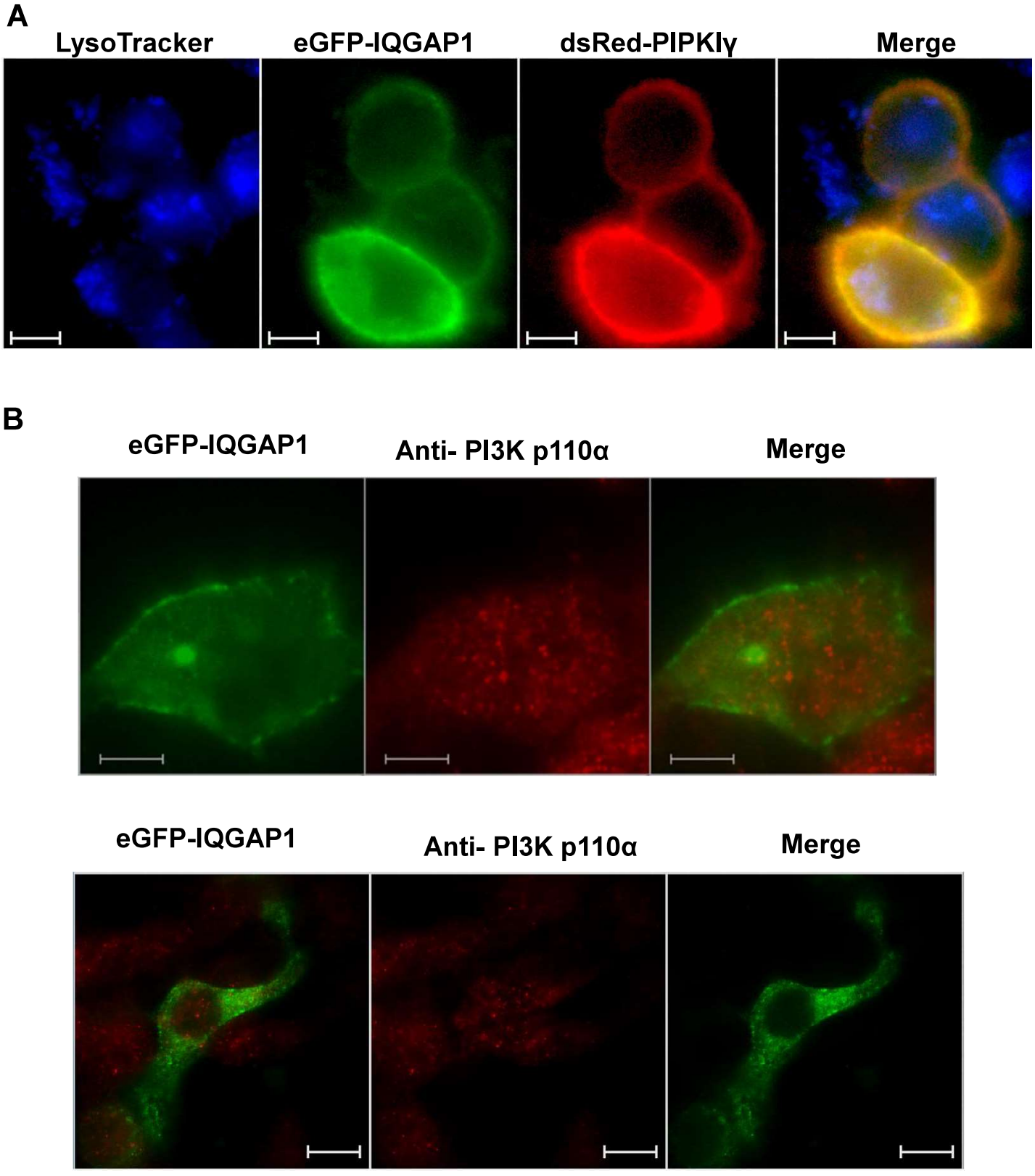

A
LysoTracker
eGFP-IQGAP1
dsRed-PIPKIγ
Merge
B
eGFP-IQGAP1
Anti- PI3K p110α
Merge
eGFP-IQGAP1
Anti- PI3K p110α
Merge

Figure S5

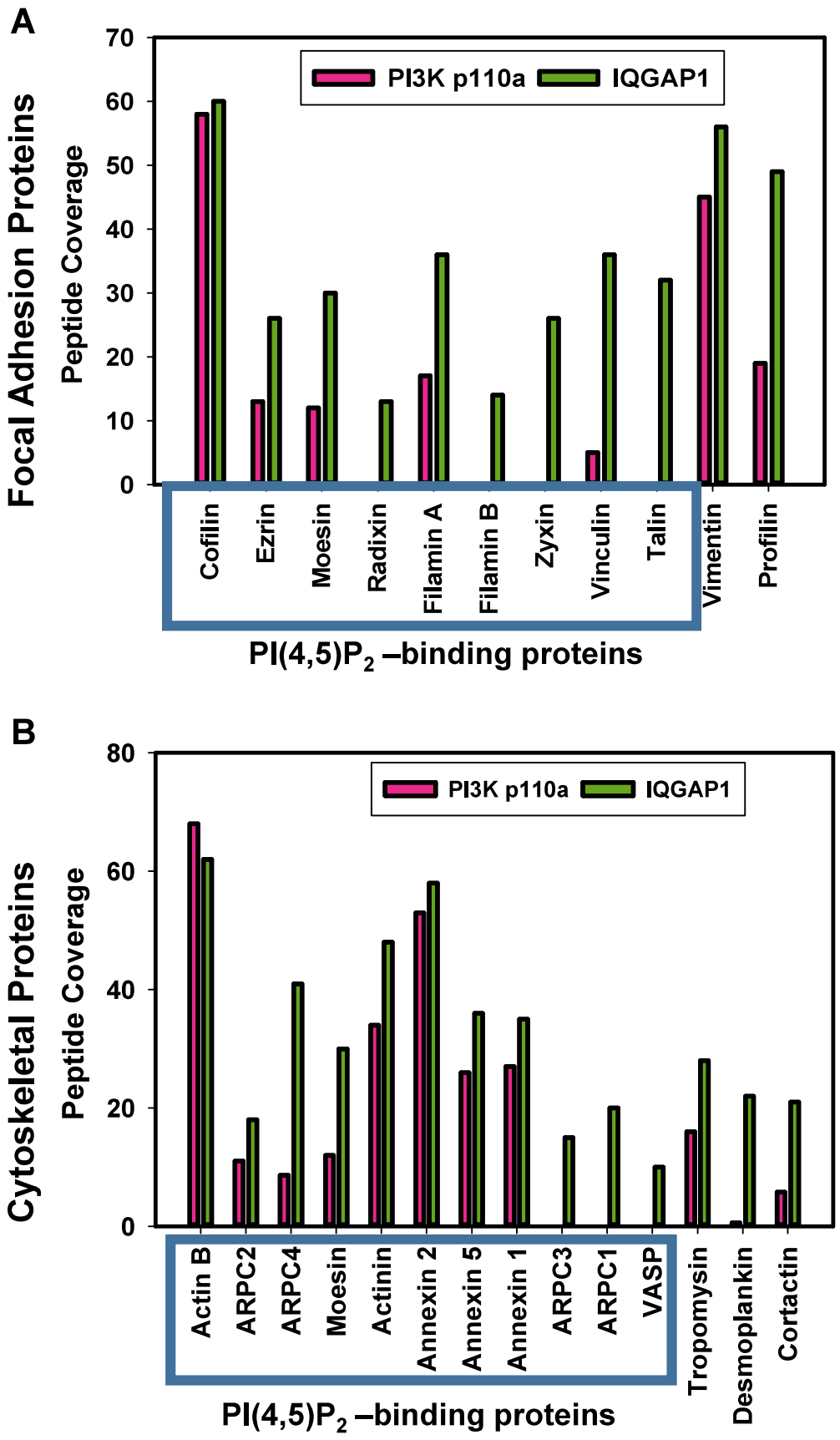
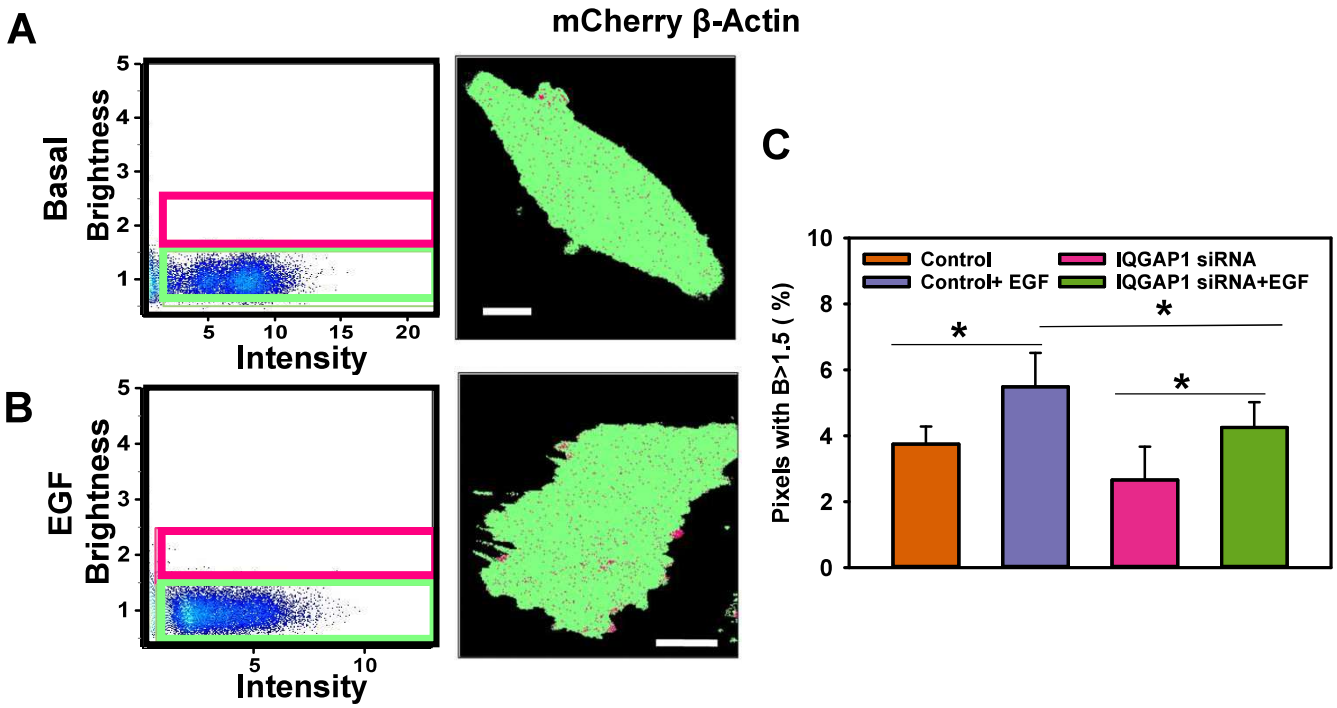

A
Focal Adhesion Proteins
Peptide Coverage
70
60
50
40
30
20
10
0
PI3K p110a
IQGAP1
Cofilin
Ezrin
Moesin
Radixin
Filamin A
Filamin B
Zyxin
Vinculin
Talin
Vimentin
Profilin
PI(4,5)P2 –binding proteins
B
Cytoskeletal Proteins
Peptide Coverage
80
60
40
20
0
PI3K p110a
IQGAP1
Actin B
ARPC2
ARPC4
Moesin
Actinin
Annexin 2
Annexin 5
Annexin 1
ARPC3
ARPC1
VASP
Tropomysin
Desmoplankin
Cortactin
PI(4,5)P2 –binding proteins

Figure S6


A
mCherry β-Actin
Basal Brightness
Intensity
B
EGF Brightness
Intensity
C
Pixels with B>1.5 (%)
Control
Control+ EGF
IQGAP1 siRNA
IQGAP1 siRNA+EGF
*
*
*