Predicting phenotype to mechanotype relationships in cells based on intra-cellular signaling network

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SUPPLEMENTARY INFORMATION

Supplementary Figures

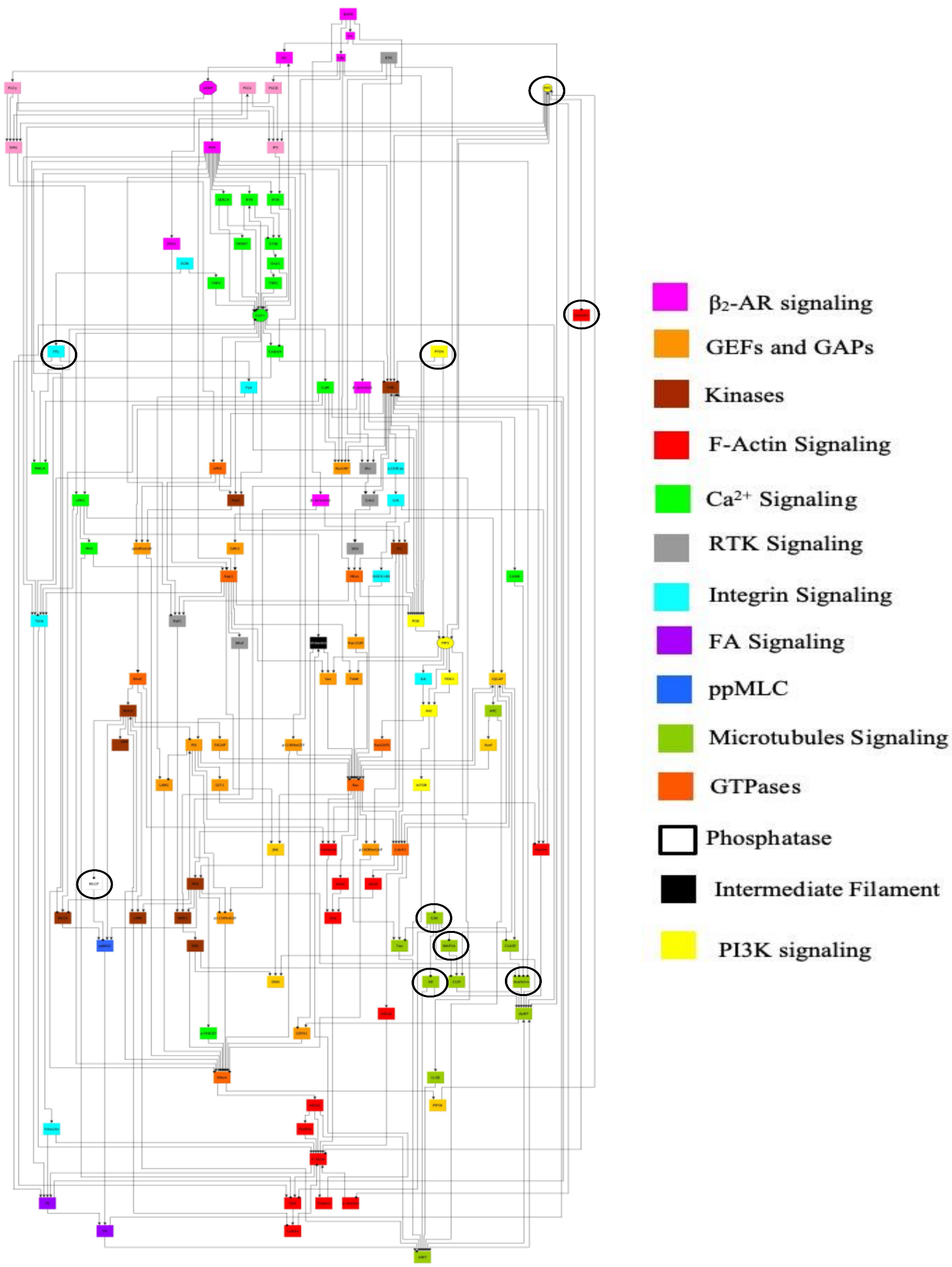
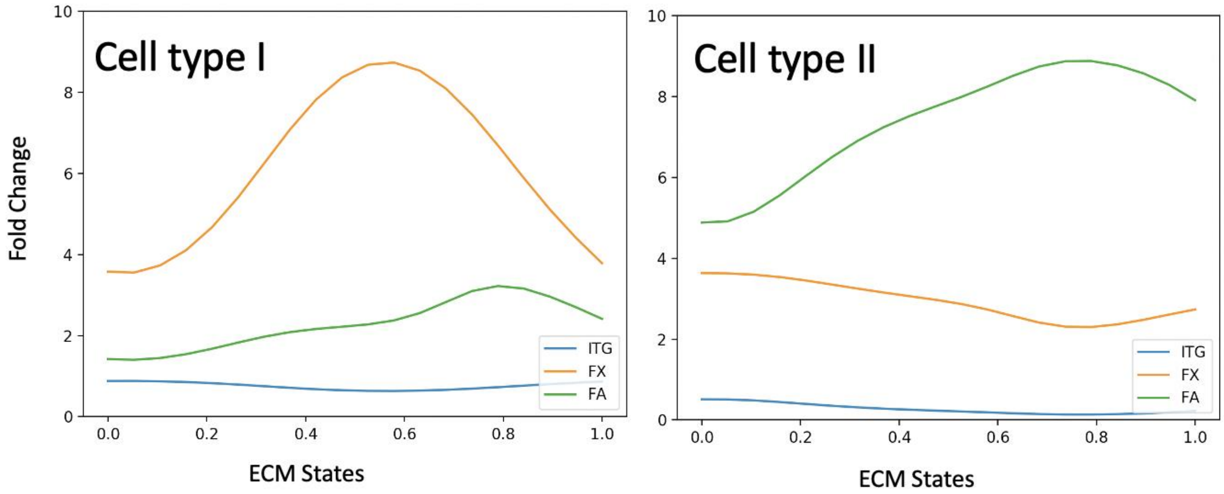


Figure S1. All cytoskeletons network.

Colors represent different signaling modules. The shapes other than rectangular shows the non-protein components such as Ca^{2+} and cAMP. Black circled nodes show the components initiated at 0.1 as mentioned methods. yEd-graph editor is used to render the interactive network.



<u>Figure S2. Focal adhesion components have biphasic response regarding ECM states.</u>

Depending on the ECM states integrins, focal adhesion complexes (FX) and focal adhesions (FA) show biphasic dynamics. Blue Line: Integrin, Orange Line: Focal adhesion complexes Green Line: Focal Adhesion

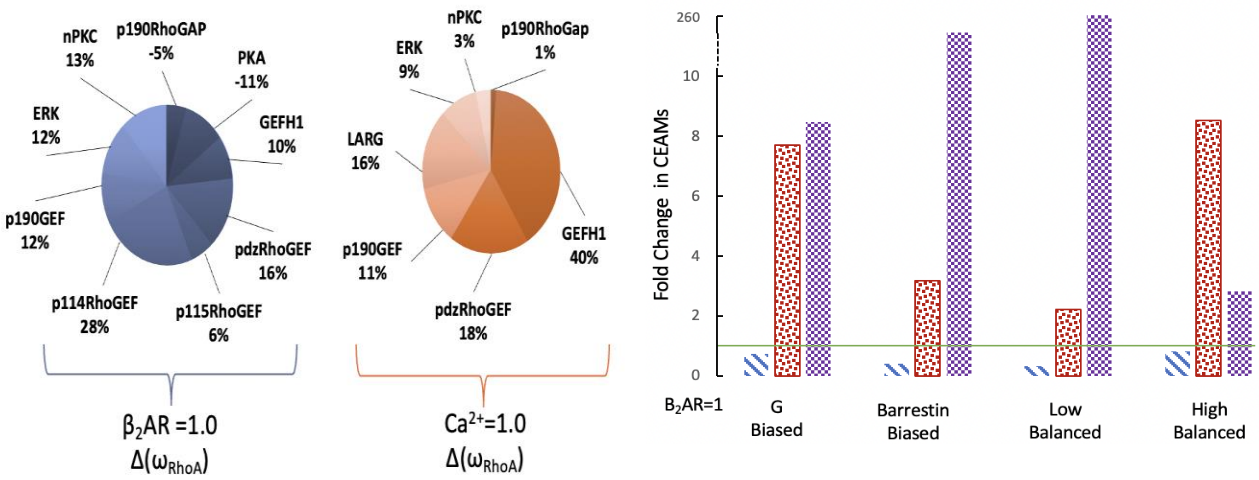


Figure S3. Cell Type I Signaling distribution profile based in situ and ligand biased activation.

(a) It shows how β_2AR and Ca^{2+} contribute to the activation of RhoA at steady states in Cell Type I. The values show what percentage of the weight factor driving RhoA signaling is being contributed by the specific preceding nodes indicated in the pie chart. (b) In β_2AR signaling response, the signaling response can be biased by varying affinities in four unique ways - increased G protein affinity, increased β-arrestin affinity, low affinity to both G protein and β-arrestin, and high affinity to both. These differences in ligand affinity can lead to diverse mechanotype profiles as dictated by CEAMs.

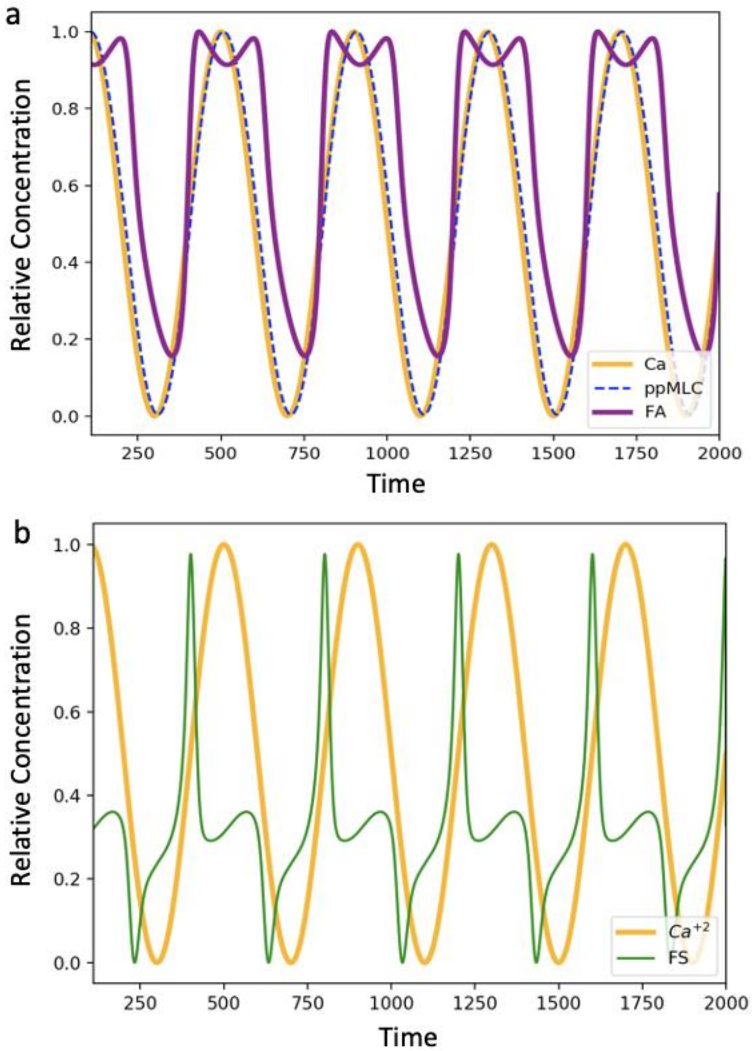


Figure S4. Cell Type II oscillatory mechanical responses based on transient signals.

Orange line: Ca^{2+}, Green line: Front speed. Migrating cell's front speed is calculated as a function of the focal adhesion turnover rate (dFA/dt) and its relationship to Ca^{2+} cycles is shown in Cell Type II

Supplementary Tables

Table S1. Cytoskeleton Directed Network

Calcium Signaling Network

Signaling Pathway	References
PIP2 → IP3	[1]
PLCg → IP3	Calcium Signaling Pathway [2]
PLCe → IP3	Calcium Signaling Pathway [2]
PLCb → IP3	Calcium Signaling Pathway[2]
PTEN --| PIP3	Calcium Signaling Pathway[2]
PIP2 → PIP3	Calcium Signaling Pathway[2]
PI3K → PIP3	Calcium Signaling Pathway[2]
DAG → nPKC	Calcium Signaling Pathway[2]
Ca2+ → cPKC	Calcium Signaling Pathway[2]
PLCg → DAG	Calcium Signaling Pathway[2]
PLCb → DAG	Calcium Signaling Pathway[2]
PLCe → DAG	Calcium Signaling Pathway[2]
PIP2 → DAG	[1]
IP3R → Ca^{2+}	Calcium Signaling Pathway [2]
Ca^{2+} → Calpain	[3]
PKA --| Calpain	[4]
PKA → TRPM7	[5]
PKA → IP3R	[6]
IP3 → IP3R	Calcium Signaling Pathway [2]
STIM → Orai1	Calcium Signaling Pathway [2]
Orai1 → Ca^{2+}	Calcium Signaling Pathway [2]
RyR → STIM	Calcium Signaling Pathway [2]
IP3R → STIM	Calcium Signaling Pathway [2]
Ca^{2+} → RyR	Calcium Signaling Pathway [2]
Ca^{2+} → CaM	Calcium Signaling Pathway [2]
Ca^{2+} → Pyk2	Calcium Signaling Pathway [2]
CaM → CAMK	Calcium Signaling Pathway [2]
SERCA --| Ca^{2+}	Calcium Signaling Pathway [2]
RyR → Ca^{2+}	Calcium Signaling Pathway [2]
PMCA --| Ca^{2+}	Calcium Signaling Pathway [2]
SERCA --|STIM	Calcium Signaling Pathway [2]
CaM → RasGRF	RasGRF Signaling Pathway[7]

ECM → TRPV	[8]
Orai1 → TRPC	[9]
CaM → PI3K	[10]
TRPV → Ca^{2+}	[8]
TRPC → Ca^{2+}	[9]
TRPM7 → Ca^{2+}	[5]
Barrestin1 --| RasGRF	[11]
FAK → RasGRF	Focal Adhesion Signaling[2]
CaM →PMCA	[12]
PKA → SERCA	Calcium Signaling Pathway [2]
Rap1 → PLCe	cAMP Signaling Pathway[2]

Microtubule Network

Stathmin → dyMT	[13]
CLIP → dyMT	[14]
CLASP → dyMT	[14]
EB →dyMT	[14]
Gs → dyMT	[15]
Ca^{2+} → dyMT	[16]
Paxilin → dyMT	[17]
PKA --| Stathmin	[18]
PAK --| Stathmin	[19]
ERK --| Stathmin	[20]
CAMK --| Stathmin	[21]
nPKC → IQGAP	[22]
Barrestin1 → IQGAP	[22]
IQGAP → CLIP	[23]
Map1b → CLIP	[24]
IQGAP → CLASP	[25]
Vimentin --| GEFH1	[26]
dyMT → GEFH1	[26]
GSK --| Tau	Alzheimer Disease Signaling Pathway[2]
Rac → Tau	[27]
Cdc42 → Tau	[28]
LL5β → stMT	[29]
CLASP → stMT	[29]
EB → stMT	[30]
mDia1 → stMT	[31]
APC → stMT	[31]
Tau → stMT	[32]
FA → stMT	[33]
LIMK --| stMT	[34]
CLIP → stMT	[35]
PIP3 → LL5β	[36]
GSK --| EB	[37]
GSK --| MAP1B	[38]

Focal Adhesion Network

ECM → ITG	Focal Adhesion Pathway[2]
ITG → Src	Focal Adhesion Pathway[2]
ITG →Fyn	Focal Adhesion Pathway[2]
Src → FAK	Focal Adhesion Pathway[2]
FAK → Src	Focal Adhesion Pathway[2]
cPKC → FAK2/Pyk2	Focal Adhesion Pathway[2]
Src → p190RhoGAP	Focal Adhesion Pathway[2]
FAK → p190GEF	[39]
PIP5K → PIP2	Focal Adhesion Pathway[2]
ROCK → ppMLC	Focal Adhesion Pathway[2]
ROCK --| MLCP	Focal Adhesion Pathway[2]
MLCP --|ppMLC	Focal Adhesion Pathway[2]
RhoA → mDia1	Focal Adhesion Pathway[2]
FAK → Paxilin	Focal Adhesion Pathway[2]
FAK →PI3K	Focal Adhesion Pathway[2]
FAK → p130Cas	Focal Adhesion Pathway[2]
PTEN --| FAK	Focal Adhesion Pathway[2]
PIP2 → Vinculin	Focal Adhesion Pathway[2]
Calpain --| Talin	Focal Adhesion Pathway[2]
Crk → Paxilin	Focal Adhesion Pathway[2]
p130Cas →Crk	Focal Adhesion Pathway[2]
PIP3 →ILK	Focal Adhesion Pathway[2]
ILK → Akt	Focal Adhesion Pathway[2]
PIP3 → PDK1	Focal Adhesion Pathway[2]
PDK1 →Akt	Focal Adhesion Pathway[2]
PIP3 → Vav	Focal Adhesion Pathway[2]
Vav → Rac	Focal Adhesion Pathway[2]
Rac → PAK	Focal Adhesion Pathway[2]
Crk → GRF2	Focal Adhesion Pathway[2]
Crk → DOCK180	Focal Adhesion Pathway[2]
DOCK180 → Rac	Focal Adhesion Pathway[2]
GRF2 → Rap1	Focal Adhesion Pathway[2]
Rap1 → B-Raf	Focal Adhesion Pathway[2]
B-Raf → MEK1	Focal Adhesion Pathway[2]
PAK → MEK1	Focal Adhesion Pathway[2]
MEK1 → ERK	Focal Adhesion Pathway[2]
Rap1 → JNK	Focal Adhesion Pathway[2]
PAK --| MLCK	Focal Adhesion Pathway[2]

RhoA → ROCK	Focal Adhesion Pathway[2]
RhoA → PIP5K	Focal Adhesion Pathway[2]
Cdc42 → PAK	Focal Adhesion Pathway[2]
Rac → JNK	Focal Adhesion Pathway[2]
PIP3 → Akt	Focal Adhesion Pathway[2]
Pyk2 → Src	Focal Adhesion Pathway[2]
Cdc42 → mDia2	[40]
Src → PI3K	[41]
PKA → Talin	[42]
cPKC → Talin	[42]
PIP2 → Talin	[42]
Rap1 → Talin	[42]
Talin → Vinculin	Focal Adhesion Pathway[2]
Paxilin → Vinculin	Focal Adhesion Pathway[2]
Vimentin --| GEFH1	[26]
Fyn → LARG	[43]
PAK → p115RhoGEF	[44]
PKA --| RhoA	cAMP Signaling Pathway[2]
GEFH1 → RhoA	[45]
pdzRhoGEF → RhoA	[46]
p115RhoGEF → RhoA	[47]
p114RhoGEF → RhoA	[48]
p190RhoGAP → RhoA	Focal Adhesion Pathway[2]
p190GEF → RhoA	[49]
LARG → RhoA	[43]
pdzRhoGEF → RhoC	[50]
F-Actin → FX	[51]
Vinculin →FX	[51]
Talin → FX	[51]
RhoC → ROCK	[52]
PKA → FAK	[53]
PIP2 → FAK	[54]
FX/FA → FAK	[54]

RTK Signaling

RTK → Shc	Focal Adhesion Pathway[2]
Shc → Grb2	Focal Adhesion Pathway[2]
FAK → Grb2	Focal Adhesion Pathway[2]
FAK → Shc	Focal Adhesion Pathway[2]
RTK → FAK	Focal Adhesion Pathway[2]
Grb2 → SOS	Focal Adhesion Pathway[2]
SOS → HRas	Focal Adhesion Pathway[2]
HRas → Raf1	Focal Adhesion Pathway[2]
Raf1 → MEK1	Focal Adhesion Pathway[2]
RTK → PI3K	Focal Adhesion Pathway[2]
H-Ras → PI3K	Focal Adhesion Pathway[2]
Fyn → Shc	Focal Adhesion Pathway[2]
ERK → DNA	Focal Adhesion Pathway[2]
mTOR → DNA	Focal Adhesion Pathway[2]
JNK → DNA	Focal Adhesion Pathway[2]
Akt → mTOR	Focal Adhesion Pathway[2]
RTK → PLCγ	Calcium Signaling Pathway[2]
Src → HRas	[55]
Src → PI3K	[56]
cPKC → Raf1	[57]
nPKC → Vimentin	[58]
Filamin → Vimentin	[59]
nPKC → PKD	Rap Signaling Pathway[2]
ERK → RhoA	[60]
nPKC → RhoA	[61]
p114RhoGEF → Rac	[48]
PAK → ppMLC	[62]

Actin Cytoskeleton Signaling

LARG → RhoA	Regulation of Actin Cytoskeleton[2]
HRas → Rac1GEF	Regulation of Actin Cytoskeleton[2]
Rac1GEF → Rac	Regulation of Actin Cytoskeleton[2]
Vav → Rac	Regulation of Actin Cytoskeleton[2]
Tiam1 → Rac	Regulation of Actin Cytoskeleton[2]
Cdc42 → Wasp	Regulation of Actin Cytoskeleton[2]
Cdc42 → IQGAP	Regulation of Actin Cytoskeleton[2]
Rac → IQGAP	Regulation of Actin Cytoskeleton[2]
PIX --| LARG	Regulation of Actin Cytoskeleton[2]
PAK → PIX	Regulation of Actin Cytoskeleton[2]
PIX → GIT1	Regulation of Actin Cytoskeleton[2]
PIX → Cdc42	Regulation of Actin Cytoskeleton[2]
PIX → Rac	Regulation of Actin Cytoskeleton[2]
mDia1 → Profilin	Regulation of Actin Cytoskeleton[2]
PIP2 → Vinculin	Regulation of Actin Cytoskeleton[2]
PIP2 --| Actinin	Regulation of Actin Cytoskeleton[2]
GIT1 --| Paxilin	Regulation of Actin Cytoskeleton[2]
ROCK → ERM	Regulation of Actin Cytoskeleton[2]
ROCK → LIMK	Regulation of Actin Cytoskeleton[2]
LIMK --| Cofilin	Regulation of Actin Cytoskeleton[2]
SSH → Cofilin	Regulation of Actin Cytoskeleton[2]
PIP2 --| Gelsolin	Regulation of Actin Cytoskeleton[2]
Rac → Wave	Regulation of Actin Cytoskeleton[2]
Asef → Rac	Regulation of Actin Cytoskeleton[2]
APC → Asef	Regulation of Actin Cytoskeleton[2]
Wave → Arp	Regulation of Actin Cytoskeleton[2]
Wasp → Arp	Regulation of Actin Cytoskeleton[2]
PIP3 → TIAM	Regulation of Actin Cytoskeleton[2]
Src → Cortactin	[63]
F-Actin → Actinin	[63]
Cortactin → Arp	[63]
Vimentin → Vav	[64]
PAK → LIMK	Regulation of Actin Cytoskeleton[2]
Rac → Cortactin	[63]
Actin → Filamin	[65]
PKD --| SSH	[66]
ROCK --| PIX	[67]

<table>
<tr><td>PKA → PIX</td><td>[68]</td></tr>
<tr><td>ROCK → FilGAP</td><td>[69]</td></tr>
<tr><td>Profilin → F-Actin</td><td>Regulation of Actin Cytoskeleton[2]</td></tr>
<tr><td>Arp → F-Actin</td><td>Regulation of Actin Cytoskeleton[2]</td></tr>
<tr><td>Rac1 -->
p190RhoGAP</td><td>[70]</td></tr>
<tr><td>Cofilin --| F-Actin</td><td>Regulation of Actin Cytoskeleton[2]</td></tr>
<tr><td>Gelsolin --| F-Actin</td><td>Regulation of Actin Cytoskeleton[2]</td></tr>
<tr><td>IQGAP → F-Actin</td><td>[23]</td></tr>
<tr><td>mDia1 → F-Actin</td><td>Regulation of Actin Cytoskeleton[2]</td></tr>
<tr><td>mDia2 → F-Actin</td><td>Regulation of Actin Cytoskeleton[2]</td></tr>
<tr><td>Rac → Cdc42</td><td>Regulation of Actin Cytoskeleton[2]</td></tr>
<tr><td>Src →Cdc42</td><td>[71]</td></tr>
<tr><td>FilGAP --| Rac</td><td>[69]</td></tr>
<tr><td>PKA → PIX</td><td>[68]</td></tr>
<tr><td>GSK --| SSH</td><td>[72]</td></tr>
<tr><td>Ca^{2+} → SSH</td><td>[73]</td></tr>
<tr><td>F-Actin → SSH</td><td>[74]</td></tr>
<tr><td>cPKC → Cdc42</td><td>[75]</td></tr>
<tr><td>RasGRF →HRas</td><td>Ras Signaling Pathway</td></tr>
</table>

β_2AR Signaling

Ca → AC	cAMP Signaling Pathway[2]
PKA → PMCA	cAMP Signaling Pathway[2]
PMCA --| Ca2+	cAMP Signaling Pathway[2]
PKA → RyR	cAMP Signaling Pathway[2]
PKA --| RhoA	cAMP Signaling Pathway[2]
PKA --| Raf1	cAMP Signaling Pathway[2]
BAR2 → Gs	cAMP Signaling Pathway[2]
Gs → AC	cAMP Signaling Pathway[2]
AC → cAMP	cAMP Signaling Pathway[2]
cAMP → PKA	cAMP Signaling Pathway[2]
cAMP → EPAC	cAMP Signaling Pathway[2]
EPAC → Rap1	cAMP Signaling Pathway[2]
Rap1 → PI3K	cAMP Signaling Pathway[2]
Rap1 → TIAM	cAMP Signaling Pathway[2]
Rap1 → Vav	cAMP Signaling Pathway[2]
Barrestin2 → Src	[76]
BAR2 → Barrestin1/2	[77]
BAR2 → Gbg	[78]
Barrestin2 → p115RhoGEF	[79]
p114RhoGEF → Rac	[48]
Gbg → p114RhoGEF	[48]
PAK → ppMLC	[80]
Barrestin1 → pdzRhoGEF	[81]
Pyk2 → p190GEF	[39]
FAK → p190RhoGEF	[39]
pyk2 → pdzRhoGEF	[46]
FAK → pdzRhoGEF	[82]
Gbg → PI3K	Pathways in Cancer[2]
Barrestin1 → PI3K	[83]
Rap1 → Raf1	Rap1 Signaling Pathway[2]
PKD → Rap1	Rap1 Signaling Pathway[2]
Gbg → PLCe	[84]
PKA → RasGRF	Ras Signaling Pathway[2]
Gbg → RasGRF	Ras Signaling Pathway[2]

Table S2. Components Names and Symbols

Symbols	Name	Also known as
RacGAP1	Rac GTPase activating protein 1	
Barrestin1	Beta Arrestin 1	
Barrestin2	Beta Arrestin 2	
RhoC	Ras Homolog Family Member C	
nPKC	Novel Protein Kinase C (δ,ε,η,θ)	
cPKC	Conventional Protein Kinase C(α ,βI,βII γ)	
Cortactin	Cortactin	
Vinculin	Vinculin	
RasGRF	Ras protein specific guanine nucleotide releasing factor 1	
TRPM7	Transient Receptor Potential Cation Channel Subfamily M Member 7	
PMCA	Plasma Membrane Ca2+ ATPase	
Filamin	Filamin	
Vimentin	Vimentin	
PKD	polycystin-1	
FS	Front Speed	
Pyk2	Protein Tyrosine Kinase 2	PTK2
Calpain	Ca2+-activated neutral cysteine proteases	
p115RhoGEF	Rho Guanine Nucleotide Exchange Factor 1	ARHGEF1
p114RhoGEF	Rho Guanine Nucleotide Exchange Factor 18	ARHGEF18
pdzRhoGEF	PDZ Rho Guanine Nucleotide Exchange Factor	
Gbg	G beta-gamma complex	
EPAC	Rap Guanine Nucleotide Exchange Factor 3	RAPGEF2
STIM	Stromal Interaction Molecule	
Orai1	Calcium Release-activated calcium channel protein 1	
RyR	Ryanodine Receptors	
SERCA	Sarco-/Endoplasmic Reticulum Ca2+ ATPase	
Fyn	Photo-oncogene tyrosine-protein kinase Fyn	
ITG	Integrins	
LARG	Leukemia-associated Rho Guanine Nucleotide Exchange Factor	ARHGEF12
Asef	Rho Guanine Nucleotide Exchange Factor 4	ARHGEF4
ERM	Ezrin, Radixin, Moesin	ARHGEF28
p190GEF	p190 Rho Guanine Nucleotide Exchange Factor	RGNEF (mouse)
FilGAP	Filamin A Binding RhoGTPase Activating Protein	

SSH	Protein Phosphatase Slingshot	
IQGAP	IQ motif-containing GTPase-activating Protein	
p190RhoGap	p190 Rho Family GTPase-activating Protein	GRLF1
GIT1	ARF GTPase-activating Protein	ARFGAP1
mDia2	Mouse Diaphanous 2	
PLCe	Phospholipase C-epsilon	
PLCg	Phospholipase C-gamma	
FA	Focal Adhesion	
FX	Focal Adhesion Complex	
APC	Adenomatous Polyposis Coli	
GEFH1	Rho Guanine Nucleotide Exchange Factor 2	ARHGEF28
ECM	Extracellular Matrix	
CLASP	CLIP-associating proteins	
dyMT	Dynamic Microtubules	
Tau	microtubule stabilizer protein	
stMT	Stable Microtubules	
LL5B	Phosphatidylinositol (3,4,5) Trisphosphate Sensor proteins	
EB	End-binding Protein	
MAP1b	Microtubule Associated Protein 1B	
CLIP	Class II-associated invariant chain peptide	
PTEN	Phosphatase and Tensin Homolog	
Rac1GEF	Rac1 GTPase Guanine Nucleotide Exchange Factor	
Gelsolin	Gelsolin	
Profilin	Profilin	
PIX	p21-actciated Protein Kinase Exchange Factor	ARHGEF7
Stathmin	Stathmin	
PDK1	Pyruvate Dehydrogenase Kinase1	
Paxilin	Paxilin	
Talin	Talin	
Actinin	Alpha-actinin-1	
ILK	Integrin-linked protein kinase	
ppMLC	di-phosphorylated non-muscle myosin light chain	
Src	proto-oncogene c-Src	
MLCK	Myosin Light Chain Kinase	
cAMP	cyclic adenosine monophosphate	
p130Cas	p130 CRK-associated Substrate	
GRF2	General Regulatory Factor 2	
IP3	Inositol Triphosphate	

MLCP	Myosin Light Chain Phosphatase	
TIAM	T-cell lymphoma invasion and metastasis-inducing protein 1	
F-Actin	Actin Filaments	
bRaf	b-RAF proto-oncogene	
Cofilin	Cofilin	
RhoA	Ras Homolog Family Member A	
PAK	p21 activated kinases	
Vav	Rho Guanine Nucleotide Exchange Factor	
GSK	Glycogen synthase kinase 3	
ERK	Extracellular signal regulated kinase	MAPK
DAG	Diglyceride	
PIP5K	Phosphatidylinositol 4-phosphate 5-kinase	
Rap1	Saccharomyces cerevisiae repressor-activator protein 1	
PIP2	Phosphatidylinositol 4.5-bisphosphate	
MEK1	Mitogen -activated protein kinase 1	MAP2K
RTK	Receptor Tyrosine Kinase	
Wave	WASP-family verprolin-homologous protein	
FAK	focal adhesion kinase	
SOS	Son of Sevenless	
Grb2	Growth Factor Receptor Bound Protein 2	
mTOR	Mammalian Target of Rapamycin	
ROCK	Rho-associated protein kinase	
AC	adenylate cyclase	
DNA	Signals change cell proliferation or cell cycle	
PIP3	Phosphatidylinositol 3,4.5-triphosphate	
PI3K	Phosphoinositide 3-kinases	
HRas	RasGTPase	
mDia1	Mouse Diaphanous 2, Formin	
Akt	Protein Kinase B	
DOCK180	Dedicator of cytokinesis	DOCK1
Shc	Shc-transforming protein 1	
PKA	Protein Kinase A	
Crk	Adapter Molecule Crk	
Raf1	Raf-1 photo-oncogene, serine/threonine kinase	
β2AR	β2- Adrenergic Receptor	
Gs	Gs alpha subunit	
Rac	Rho Family GTPases	
LIMK	LIM Domain Kinase 1	
Ca	Calcium	

Wasp	Wiskott-Aldrich Syndrome Protein	
JNK	c-Jun N-terminal Kinases	
Arp	Actin Related Protein 2/3	ARP2/3
Cdc42	Cell Division Cycle 42	
CaM	Calmodulin	
CaMK	Calmodulin Dependent Protein Kinase	
TRPV	Transient Receptor Potential Cation Channel V	
TRPC	Transient Receptor Potential Cation Channel C	
PLCb	Phospholipase C-beta	
IP3R	Inositol Trisphosphate Receptor	

REFERENCES

1. Czech, M. P. PIP2 and PIP3. *Cell* **100**, 603–606 (2000).
2. Kanehisa, M. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
3. Khorchid, A. & Ikura, M. How calpain is activated by calcium. *Nat. Struct. Biol.* **9**, 239–241 (2002).
4. Shiraha, H., Glading, A., Chou, J., Jia, Z. & Wells, A. Activation of m-Calpain (Calpain II) by Epidermal Growth Factor Is Limited by Protein Kinase A Phosphorylation of m-Calpain. *Mol. Cell. Biol.* **22**, 2716–2727 (2002).
5. Howe, A. K. Cross-talk between calcium and protein kinase A in the regulation of cell migration. *Curr. Opin. Cell Biol.* **23**, 554–561 (2011).
6. Taylor, C. W. Regulation of IP3 receptors by cyclic AMP. *Cell Calcium* **63**, 48–52 (2017).
7. Martemyanov, K. A. *et al.* RasGrf (RAS Protein-Specific Guanine Nucleotide-Releasing Factor). in *Encyclopedia of Signaling Molecules* (ed. Choi, S.) 1605–1612 (Springer New York, 2012). doi:10.1007/978-1-4419-0461-4_138.
8. Ji, C. & McCulloch, C. A. TRPV4 integrates matrix mechanosensing with Ca^{2+} signaling to regulate extracellular matrix remodeling. *FEBS J.* **288**, 5867–5887 (2021).

9. Liao, Y. *et al.* Functional interactions among Orai1, TRPCs, and STIM1 suggest a STIM-regulated heteromeric Orai/TRPC model for SOCE/Icrac channels. *Proc. Natl. Acad. Sci.* **105**, 2895–2900 (2008).

10. Nussinov, R. *et al.* Calmodulin and PI3K Signaling in KRAS Cancers. *Trends Cancer* **3**, 214–224 (2017).

11. Ma, X. *et al.* βArrestin1 Regulates the Guanine Nucleotide Exchange Factor RasGRF2 Expression and the Small GTPase Rac-mediated Formation of Membrane Protrusion and Cell Motility. *J. Biol. Chem.* **289**, 13638–13650 (2014).

12. Mohamed, T. M. A. *et al.* Measurement of Plasma Membrane Calcium–Calmodulin-Dependent ATPase (PMCA) Activity. in *Membrane Transporters in Drug Discovery and Development* (ed. Yan, Q.) vol. 637 333–342 (Humana Press, 2010).

13. Rubin, C. I. & Atweh, G. F. The role of stathmin in the regulation of the cell cycle. *J. Cell. Biochem.* **93**, 242–250 (2004).

14. de Forges, H., Bouissou, A. & Perez, F. Interplay between microtubule dynamics and intracellular organization. *Int. J. Biochem. Cell Biol.* **44**, 266–274 (2012).

15. Yu, J.-Z., Dave, R. H., Allen, J. A., Sarma, T. & Rasenick, M. M. Cytosolic Gαs Acts as an Intracellular Messenger to Increase Microtubule Dynamics and Promote Neurite Outgrowth. *J. Biol. Chem.* **284**, 10462–10472 (2009).

16. O’Brien, E. T., Salmon, E. D. & Erickson, H. P. How calcium causes microtubule depolymerization. *Cell Motil.* **36**, 125–135 (1997).

17. Efimov, A. *et al.* Paxillin-dependent stimulation of microtubule catastrophes at focal adhesion sites. *J. Cell Sci.* **121**, 196–204 (2008).

18. Tian, X., Tian, Y., Sarich, N., Wu, T. & Birukova, A. A. Novel role of stathmin in microtubule-dependent control of endothelial permeability. *FASEB J.* **26**, 3862–3874 (2012).

19. Daub, H., Gevaert, K., Vandekerckhove, J., Sobel, A. & Hall, A. Rac/Cdc42 and p65PAK Regulate the Microtubule-destabilizing Protein Stathmin through Phosphorylation at Serine 16. *J. Biol. Chem.* **276**, 1677–1680 (2001).

20. Cassimeris, L. The oncoprotein 18/stathmin family of microtubule destabilizers. *Curr. Opin. Cell Biol.* **14**, 18–24 (2002).

21. le Gouvello, S., Manceau, V. & Sobel, A. Serine 16 of stathmin as a cytosolic target for Ca2+/calmodulin-dependent kinase II after CD2 triggering of human T lymphocytes. *J. Immunol. Baltim. Md 1950* **161**, 1113–1122 (1998).

22. Noordstra, I. & Akhmanova, A. Linking cortical microtubule attachment and exocytosis. *F1000Research* **6**, 469 (2017).

23. Watanabe, T. *et al.* Interaction with IQGAP1 Links APC to Rac1, Cdc42, and Actin Filaments during Cell Polarization and Migration. *Dev. Cell* **7**, 871–883 (2004).

24. Tortosa, E., Galjart, N., Avila, J. & Sayas, C. L. MAP1B regulates microtubule dynamics by sequestering EB1/3 in the cytosol of developing neuronal cells. *EMBO J.* **32**, 1293–1306 (2013).

25. Tymanskyj, S. R., Scales, T. M. E. & Gordon-Weeks, P. R. MAP1B enhances microtubule assembly rates and axon extension rates in developing neurons. *Mol. Cell. Neurosci.* **49**, 110–119 (2012).

26. Jiu, Y. *et al.* Vimentin intermediate filaments control actin stress fiber assembly through GEF-H1 and RhoA. *J. Cell Sci.* jcs.196881 (2017) doi:10.1242/jcs.196881.

27. Nishimura, Y., Applegate, K., Davidson, M. W., Danuser, G. & Waterman, C. M. Automated Screening of Microtubule Growth Dynamics Identifies MARK2 as a Regulator of Leading Edge Microtubules Downstream of Rac1 in Migrating Cells. *PLoS ONE* **7**, e41413 (2012).

28. Borin, M. *et al.* Rac1 activation links tau hyperphosphorylation and Aβ dysmetabolism in Alzheimer's disease. *Acta Neuropathol. Commun.* **6**, 61 (2018).

29. Lansbergen, G. *et al.* CLASPs Attach Microtubule Plus Ends to the Cell Cortex through a Complex with LL5β. *Dev. Cell* **11**, 21–32 (2006).

30. Mimori-Kiyosue, Y. *et al.* CLASP1 and CLASP2 bind to EB1 and regulate microtubule plus-end dynamics at the cell cortex. *J. Cell Biol.* **168**, 141–153 (2005).

31. Wen, Y. *et al.* EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. *Nat. Cell Biol.* **6**, 820–830 (2004).

32. Kadavath, H. *et al.* Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. *Proc. Natl. Acad. Sci.* **112**, 7501–7506 (2015).

33. Garcin, C. & Straube, A. Microtubules in cell migration. *Essays Biochem.* **63**, 509–520 (2019).

34. Gorovoy, M. *et al.* LIM Kinase 1 Coordinates Microtubule Stability and Actin Polymerization in Human Endothelial Cells. *J. Biol. Chem.* **280**, 26533–26542 (2005).

35. Akhmanova, A. *et al.* CLASPs Are CLIP-115 and -170 Associating Proteins Involved in the Regional Regulation of Microtubule Dynamics in Motile Fibroblasts. *Cell* **104**, 923–935 (2001).

36. Hiepen, C. *et al.* BMP2-induced chemotaxis requires PI3K p55γ/p110α-dependent phosphatidylinositol (3,4,5)-triphosphate production and LL5β recruitment at the cytocortex. *BMC Biol.* **12**, 43 (2014).

37. Kumar, P. *et al.* GSK3β phosphorylation modulates CLASP–microtubule association and lamella microtubule attachment. *J. Cell Biol.* **184**, 895–908 (2009).

38. Trivedi, N., Marsh, P., Goold, R. G., Wood-Kaczmar, A. & Gordon-Weeks, P. R. Glycogen synthase kinase-3β phosphorylation of MAP1B at Ser1260 and Thr1265 is spatially restricted to growing axons. *J. Cell Sci.* **118**, 993–1005 (2005).

39. Lim, Y. *et al.* PyK2 and FAK connections to p190Rho guanine nucleotide exchange factor regulate RhoA activity, focal adhesion formation, and cell motility. *J. Cell Biol.* **180**, 187–203 (2008).

40. Ubukawa, K. *et al.* Cdc42 regulates cell polarization and contractile actomyosin rings during terminal differentiation of human erythroblasts. *Sci. Rep.* **10**, 11806 (2020).

41. Rexer, B. *et al.* Intracellular Src Family Kinases Mediate PI3K-Akt Pathway Activation and Resistance to Lapatinib in HER2-Overexpressing Human Breast Cancer Cells. *Cancer Res.* **69**, 707–707 (2009).

42. Das, M., Subbayya Ithychanda, S., Qin, J. & Plow, E. F. Mechanisms of talin-dependent integrin signaling and crosstalk. *Biochim. Biophys. Acta BBA - Biomembr.* **1838**, 579–588 (2014).

43. Guilluy, C. *et al.* The Rho GEFs LARG and GEF-H1 regulate the mechanical response to force on integrins. *Nat. Cell Biol.* **13**, 722–727 (2011).

44. Rosenfeldt, H., Castellone, M. D., Randazzo, P. A. & Gutkind, J. S. Rac inhibits thrombin-induced Rho activation: evidence of a Pak-dependent GTPase crosstalk. *J. Mol. Signal.* **1**, 8 (2006).

45. Birkenfeld, J. *et al.* GEF-H1 Modulates Localized RhoA Activation during Cytokinesis under the Control of Mitotic Kinases. *Dev. Cell* **12**, 699–712 (2007).

46. Ying, Z., Giachini, F. R. C., Tostes, R. C. & Webb, R. C. PYK2/PDZ-RhoGEF Links Ca $^{2+}$ Signaling to RhoA. *Arterioscler. Thromb. Vasc. Biol.* **29**, 1657–1663 (2009).

47. Chumki, S. A., van den Goor, L. M., Hall, B. N. & Miller, A. L. p115RhoGEF activates RhoA to support tight junction maintenance and remodeling. *Mol. Biol. Cell* **33**, ar136 (2022).

48. Niu, J., Profirovic, J., Pan, H., Vaiskunaite, R. & Voyno-Yasenetskaya, T. G Protein βγ Subunits Stimulate p114RhoGEF, a Guanine Nucleotide Exchange Factor for RhoA and Rac1: Regulation of Cell Shape and Reactive Oxygen Species Production. *Circ. Res.* **93**, 848–856 (2003).

49. Miller, N. L. G., Lawson, C., Chen, X. L., Lim, S.-T. & Schlaepfer, D. D. Rgnef (p190RhoGEF) knockout inhibits RhoA activity, focal adhesion establishment, and cell motility downstream of integrins. *PloS One* **7**, e37830 (2012).

50. Bagnato, A. & Rosanò, L. Endothelin-1 receptor drives invadopodia: Exploiting how β-arrestin-1 guides the way. *Small GTPases* **9**, 394–398 (2018).

51. Humphries, J. D. *et al.* Vinculin controls focal adhesion formation by direct interactions with talin and actin. *J. Cell Biol.* **179**, 1043–1057 (2007).

52. Matsuoka, T. Rho/ROCK signaling in motility and metastasis of gastric cancer. *World J. Gastroenterol.* **20**, 13756 (2014).

53. Cheng, Y. *et al.* Depression promotes prostate cancer invasion and metastasis via a sympathetic-cAMP-FAK signaling pathway. *Oncogene* **37**, 2953–2966 (2018).

54. Tapial Martínez, P., López Navajas, P. & Lietha, D. FAK Structure and Regulation by Membrane Interactions and Force in Focal Adhesions. *Biomolecules* **10**, 179 (2020).

55. Bunda, S. *et al.* Src promotes GTPase activity of Ras via tyrosine 32 phosphorylation. *Proc. Natl. Acad. Sci.* **111**, (2014).

56. Campbell, P. S. *et al.* AhR ligand aminoflavone suppresses α6-integrin–Src–Akt signaling to attenuate tamoxifen resistance in breast cancer cells. *J. Cell. Physiol.* **234**, 108–121 (2019).

57. Kolch, W. *et al.* Protein kinase Cα activates RAF-1 by direct phosphorylation. *Nature* **364**, 249–252 (1993).

58. Ivaska, J. *et al.* PKCε-mediated phosphorylation of vimentin controls integrin recycling and motility. *EMBO J.* **24**, 3834–3845 (2005).

59. Kim, H. *et al.* Filamin A is required for vimentin-mediated cell adhesion and spreading. *Am. J. Physiol.-Cell Physiol.* **298**, C221–C236 (2010).

60. Tong, J., Li, L., Ballermann, B. & Wang, Z. Phosphorylation and Activation of RhoA by ERK in Response to Epidermal Growth Factor Stimulation. *PLOS ONE* **11**, e0147103 (2016).

61. Barandier, C., Ming, X.-F., Rusconi, S. & Yang, Z. PKC is required for activation of ROCK by RhoA in human endothelial cells. *Biochem. Biophys. Res. Commun.* **304**, 714–719 (2003).

62. Brzeska, H., Szczepanowska, J., Matsumura, F. & Korn, E. D. Rac-induced increase of phosphorylation of myosin regulatory light chain in HeLa cells. *Cell Motil. Cytoskeleton* **58**, 186–199 (2004).

63. Yin, M., Ma, W. & An, L. Cortactin in cancer cell migration and invasion. *Oncotarget* **8**, 88232–88243 (2017).

64. Havel, L. S., Kline, E. R., Salgueiro, A. M. & Marcus, A. I. Vimentin regulates lung cancer cell adhesion through a VAV2–Rac1 pathway to control focal adhesion kinase activity. *Oncogene* **34**, 1979–1990 (2015).

65. Iwamoto, D. V. *et al.* Structural basis of the filamin A actin-binding domain interaction with F-actin. *Nat. Struct. Mol. Biol.* **25**, 918–927 (2018).

66. Spratley, S. J., Bastea, L. I., Döppler, H., Mizuno, K. & Storz, P. Protein Kinase D Regulates Cofilin Activity through p21-activated Kinase 4. *J. Biol. Chem.* **286**, 34254–34261 (2011).

67. Kuo, J.-C., Han, X., Hsiao, C.-T., Yates, J. R. & Waterman, C. M. Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for β-Pix in negative regulation of focal adhesion maturation. *Nat. Cell Biol.* **13**, 383–393 (2011).

68. Chahdi, A. & Sorokin, A. Protein Kinase A-Dependent Phosphorylation Modulates β_1 Pix Guanine Nucleotide Exchange Factor Activity through 14-3-3β Binding. *Mol. Cell. Biol.* **28**, 1679–1687 (2008).

69. Ohta, Y., Hartwig, J. H. & Stossel, T. P. FilGAP, a Rho- and ROCK-regulated GAP for Rac binds filamin A to control actin remodelling. *Nat. Cell Biol.* **8**, 803–814 (2006).

70. Bustos, R. I., Forget, M.-A., Settleman, J. E. & Hansen, S. H. Coordination of Rho and Rac GTPase function via p190B RhoGAP. *Curr. Biol. CB* **18**, 1606–1611 (2008).

71. Hu, J. *et al.* Cdc42-interacting protein 4 is a Src substrate that regulates invadopodia and invasiveness of breast tumors by promoting MT1-MMP endocytosis. *J. Cell Sci.* **124**, 1739–1751 (2011).

72. Tang, W. *et al.* A PLCβ/PI3Kγ-GSK3 Signaling Pathway Regulates Cofilin Phosphatase Slingshot2 and Neutrophil Polarization and Chemotaxis. *Dev. Cell* **21**, 1038–1050 (2011).
73. Wang, Y., Shibasaki, F. & Mizuno, K. Calcium Signal-induced Cofilin Dephosphorylation Is Mediated by Slingshot via Calcineurin. *J. Biol. Chem.* **280**, 12683–12689 (2005).
74. Bamburg, J., Minamide, L., Wiggan, O., Tahtamouni, L. & Kuhn, T. Cofilin and Actin Dynamics: Multiple Modes of Regulation and Their Impacts in Neuronal Development and Degeneration. *Cells* **10**, 2726 (2021).
75. Tatin, F., Varon, C., Génot, E. & Moreau, V. A signalling cascade involving PKC, Src and Cdc42 regulates podosome assembly in cultured endothelial cells in response to phorbol ester. *J. Cell Sci.* **119**, 769–781 (2006).
76. Luttrell, L. M. *et al.* β-Arrestin-Dependent Formation of β_2 Adrenergic Receptor-Src Protein Kinase Complexes. *Science* **283**, 655–661 (1999).
77. Ferguson, S. S. *et al.* Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. *Science* **271**, 363–366 (1996).
78. Vasudevan, N. T., Mohan, M. L., Goswami, S. K. & Prasad, S. V. N. Regulation of β-adrenergic receptor function: An emphasis on receptor resensitization. *Cell Cycle* **10**, 3684–3691 (2011).
79. Ma, X., Zhao, Y., Daaka, Y. & Nie, Z. Acute Activation of β2-Adrenergic Receptor Regulates Focal Adhesions through βArrestin2- and p115RhoGEF Protein-mediated Activation of RhoA. *J. Biol. Chem.* **287**, 18925–18936 (2012).
80. Chew, T.-L., Masaracchia, R. A., Goeckeler, Z. M. & Wysolmerski, R. B. [No title found]. *J. Muscle Res. Cell Motil.* **19**, 839–854 (1998).

81. Di Modugno, F. *et al.* hMENA is a key regulator in endothelin-1/β-arrestin1–induced invadopodial function and metastatic process. *Proc. Natl. Acad. Sci.* **115**, 3132–3137 (2018).

82. Iwanicki, M. P. *et al.* FAK, PDZ-RhoGEF and ROCKII cooperate to regulate adhesion movement and trailing-edge retraction in fibroblasts. *J. Cell Sci.* **121**, 895–905 (2008).

83. Jean-Charles, P.-Y., Kaur, S. & Shenoy, S. K. G Protein–Coupled Receptor Signaling Through β-Arrestin–Dependent Mechanisms: *J. Cardiovasc. Pharmacol.* **70**, 142–158 (2017).

84. Bonacci, T. M., Ghosh, M., Malik, S. & Smrcka, A. V. Regulatory Interactions between the Amino Terminus of G-protein βγ Subunits and the Catalytic Domain of Phospholipase Cβ2. *J. Biol. Chem.* **280**, 10174–10181 (2005).