Supplemental Materials
Molecular Biology of the Cell

Kast and Dominguez

Supplemental Information

IRSp53 Coordinates AMPK and 14-3-3 Signaling to Regulate Filopodia Dynamicsand Directed Cell Migration

David J. Kast[1,2] and Roberto Dominguez[1]

[1] Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[2] Current address: Department of Cell Biology and Physiology, Washington University, St. Louis, MO 63110, USA

ORCID: DK, 0000-0002-1031-1211
ORCID: RD, 0000-0003-3186-5229

Correspondence should be addressed to D.K. (kast@wustl.edu) or R.D. (droberto@pennmedicine.upenn.edu)

Supplemental Figure Legends

Figure S1. Characterization of FLS in COS-7 cells.(**A**) Western blots and quantifications of the relative abundance of IRSp53, Cdc42 and 14-3-3 in untransfected (UT) COS-7 cells and transfected (T) COS-7 cells ectopically expressing IRSp53-GFP, RFP-Cdc42(G12V) or mTagBFP2-14-3-3. Error bars are ± SD from three independent transfections. (**B**) Untransfected (top) and IRSp53-GFP transfected (bottom) COS-7 cells treated with SiR-actin (filamentous actin staining). A bar graph shows the fractions of FLS in IRSp53-GFP expressing cells that are completely filled (80-100%), partially filled (20-80%) or not filled (0-20%) with actin. (**C, D**) Quantification of the density, length and growth rate of FLS from fed or serum starved COS-7 cells in either untransfected cells treated with SiR-actin (**C**) or cells expressing IRSp53-GFP, RFP-Cdc42(G12V) and mTAGBFP2-14-3-3 **D** . The statistical significance of the measurements was determined using the Mann–Whitney rank sum test, based on the indicated number of observations (*n*) recorded from 9 or 10 cells (as indicated) and two independent transfections (n.s., not significant; *, $p < 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$).

Figure S2. Relative abundance ofEps8 and VASP in untransfected and transfected cells.Western blots and quantifications of the relative abundance ofEps8 and VASP in untransfected COS-7 cells (UT) and in transfected cells (T) ectopically expressing GFP-Eps8 orGFP-VASP. Error bars are ± SD from three independent transfections.

Figure S3. Effect of insulin on 14-3-3 binding to IRSp53. Western blots and quantifications of the relative amount of 14-3-3 that coimmunoprecipitates with IRSp53-FLAG after 2 h serum starvation,with and withoutinsulin (0.1 μM). Error bars are ± SD from three independent experiments. The statistical significance of the measurements was determined using an unpaired t-test based on three independent experiments (***, $p < 0.001$).

Figure S4. Abrogation of 14-3-3 binding to IRSp53 results in aberrant FLS formation.(**A**)Western blot and quantification of the relative abundance ofIRSp53 and mutant M234 in untransfected (UT) COS-7 cells and transfected (T) COS-7 cells ectopically expressingM234-GFP. Error bars are ± SD from three independent transfections.(**B**)M234-GFP expressing COS-7 cells treated with SiR-actin. Bar graphsshow the fractions of FLS in cells expressing IRSp53-GFP (from Figure S1B) and M234-GFP that are completely filled (80-100%), partially filled (20-80%) or not filled (0-20%) with actin.(**C**,**D**)COS-7 cellsexpressing M234-GFP (IRSp53 mutant T340A/T360A/S366A) and either RFP-Cdc42 (**C**) or mTagBFP2-14-3-3 (**D**). Scale bars throughout this figure represent 10 μm and 5 μm in whole-cell and

2

inset images, respectively.Shown on the right are the average fluorescence intensities from 10 line-scans across the cell edge (as exemplified by dashed lines in the insets). Note that FLS in these cells are extremely branched and their behavior could not be quantified.(**E**)Quantification of the density, length and growth rate of FLS from fed or serum starved COS-7 cells expressing M234-GFP, RFP-Cdc42(G12V) and mTAGBFP2-14-3-3. The statistical significance of the measurements was determined using the Mann–Whitney rank sum test, based on the indicated number of observations (*n*) reordered from 10 cellsand two independent transfections (n.s., not significant; *, p < 0.05).

**Figure S5.** Unprocessed Western blots used in the figures of this paper (as indicated).

3

# Figure S1 (Related to Figure 1)
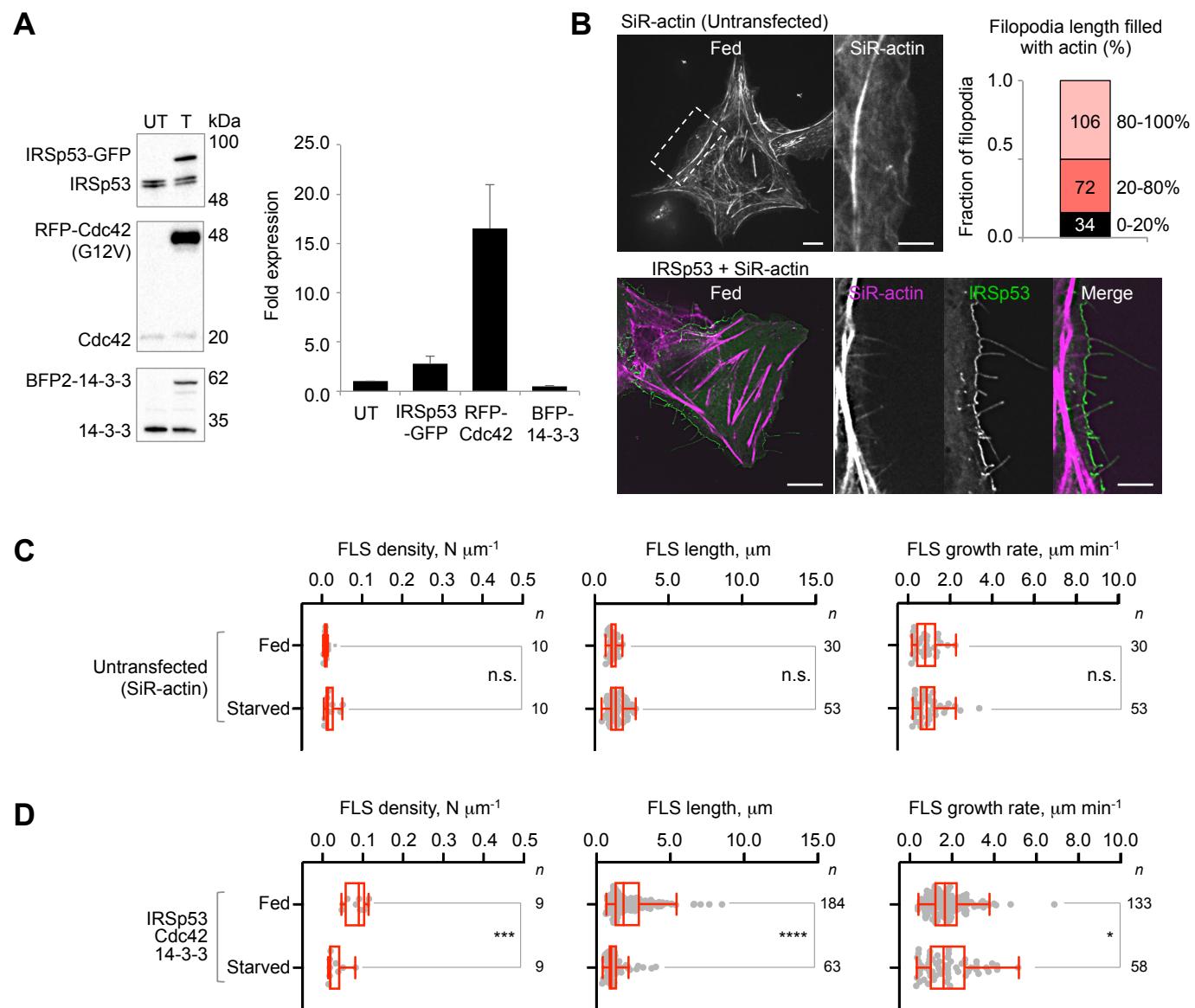
# Figure S2 (Related to Figure 2)
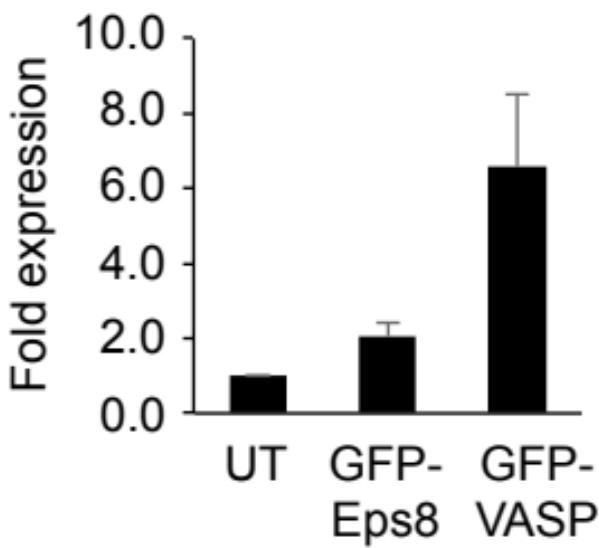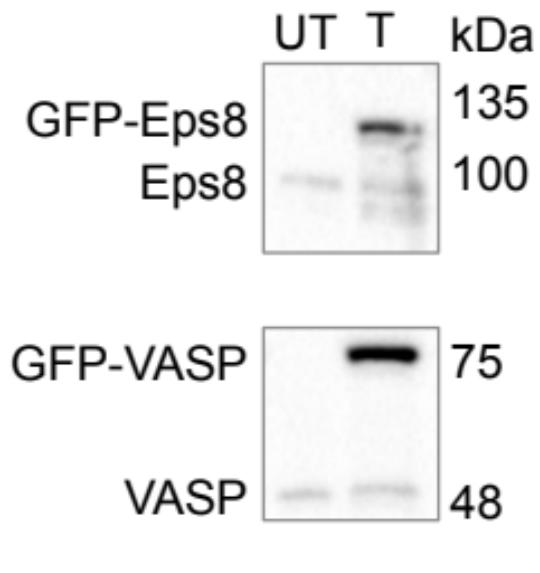
Figure S3 (Related to Figure 3)
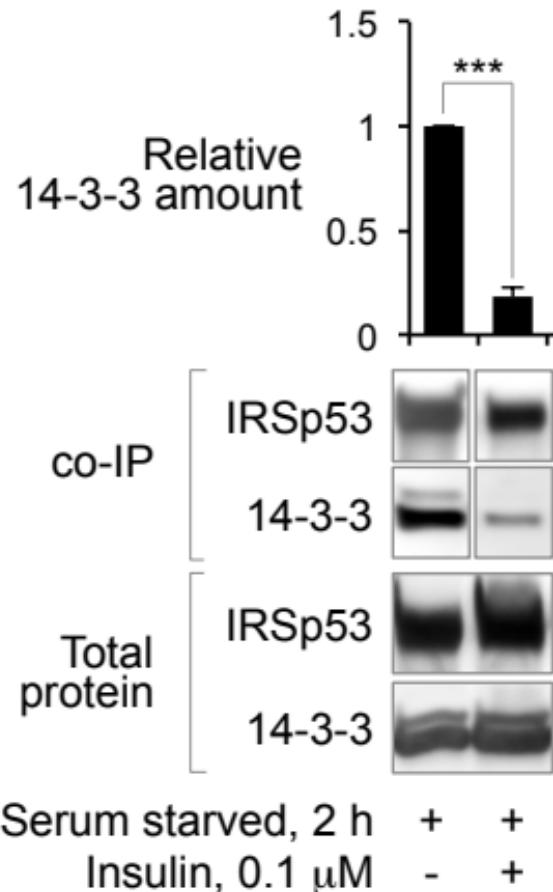
Figure S4 (Related to Figure 4)
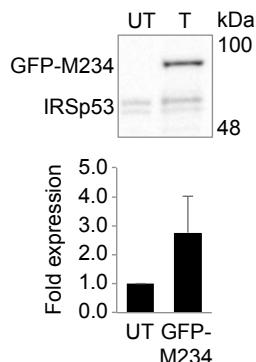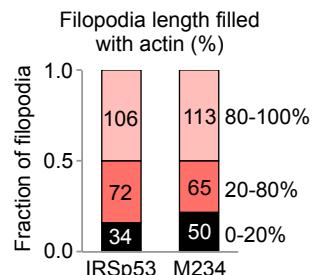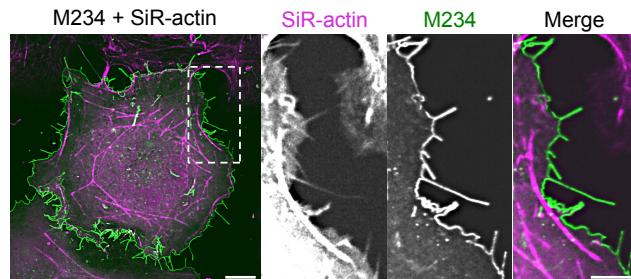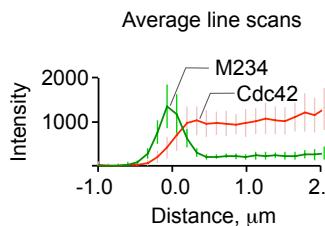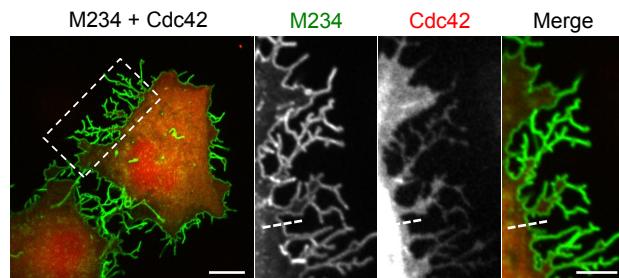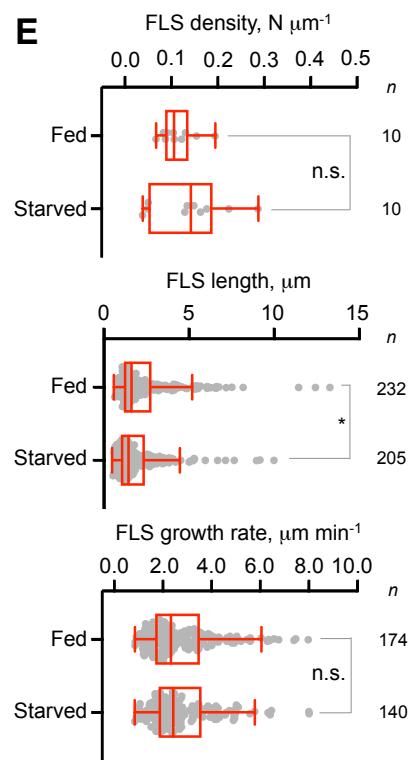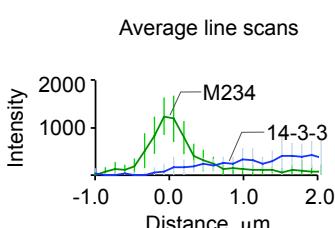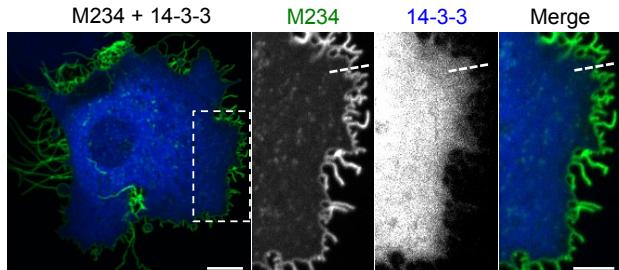
Figure S5 (Unprocessed Western Blots)

Blots used in Figure S1A (red square)
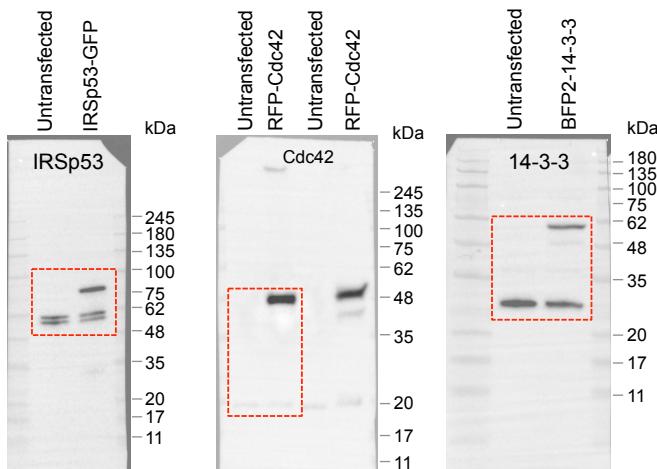
Blots used in Figure S2 (red square)
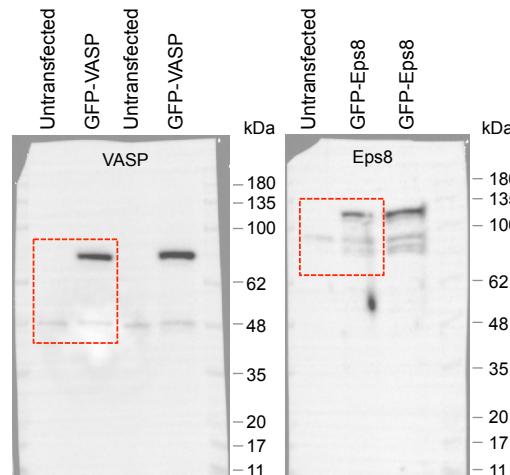
Blots used in Figure 3A (red square)
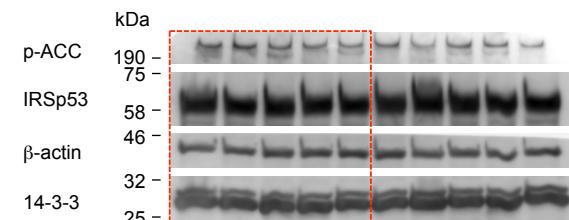
Serum starved, 2 h		+	+	+	+		+	+	+	+
Insulin, 0.1 μM						+	+	+	+	+
Dorsomorphin (μM)	0	0	1	5	10	0	0	1	5	10

Blots used in Figure 3B (red squares)
Blots used in Figure S3 (cyan squares)
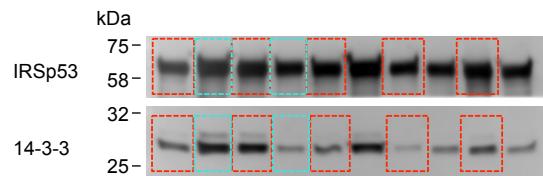
Serum starved, 2 h				+	+	+	+	+	+	+
Insulin, 0.1 μM		+		+		+		+		+
Dorsomorphin (μM)	0	0	0	0	1	1	5	5	10	10

Blots used in Figure 3E (red square)
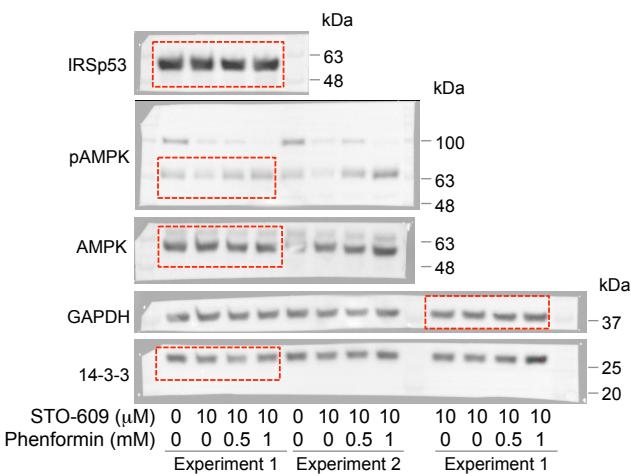
STO-609 (μM)	0	10	10	10	0	10	10	10	10	10	10	10
Phenformin (mM)	0	0	0.5	1	0	0	0.5	1	0	0	0.5	1
	Experiment 1				Experiment 2				Experiment 1			

Blots used in Figure 3F (red square)
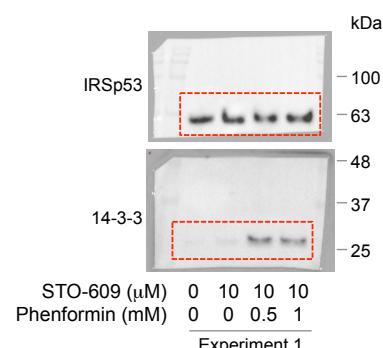
STO-609 (μM)	0	10	10	10
Phenformin (mM)	0	0	0.5	1
	Experiment 1			

Blots used in Figure S4A
(red square)
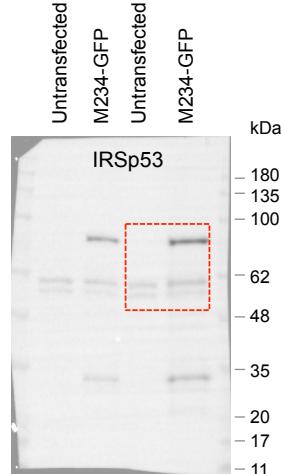
Supplemental Table S3: Oligonucleotide Sequences

Oligonucleotide	Sequence
IRSp53 constructs	
Primer: pEGFP-N1/mTAGBFP2-N1: BglII IRSp53 Forward:	GGTAGATCTATGTCTCTGTCTCGCTCA GAGGAG
Primer: pEGFP-N1/mTAGBFP2-N1: IRSp53 SalI Reverse:	GGTGTCGACCCCACTGTGGACACCAG CGTGC
Primer: pEGFP-C1: NheI-IRSp53-FLAG (ΔGFP) Forward:	GGTGCTAGCATGTCTCTGTCTCGCTC AGAGG
Primer: pEGFP-C1: IRSp53-FLAG-SalI (ΔGFP) 1 of 2 Reverse:	CGTCATCGTCCTTGTAGTCTCCGDYK DDDCACTGTGCACACCAGCGTG
Primer: pEGFP-C1: IRSp53-FLAG-SalI (ΔGFP) 2 of 2 Reverse:	ACCGTCGACCTACTTGTCGTCATCGT CCTTGTAGTCTCC
IRSp53 phospho-inhibitory mutations	
QuickChange Primer: pEGFP Site 2 Mutation (T340A) Forward:	CGACTCCTACTCCAACGCACTCCCCG TGCGCAAGAGC
QuickChange Primer: pEGFP Site 2 Mutation (T340A) Reverse:	GCTCTTGCGCACGGGGAGTGCGTTG GAGTAGGAGTCG
QuickChange Primer: pEGFP Site 3 Mutation (T360A) Forward:	GCCACCACCGAGAACAAGGCTCTGCC TCGCTCGAGC
QuickChange Primer: pEGFP Site 3 Mutation (T360A) Reverse:	GCTCGAGCGAGGCAGAGCCTTGTTCT CGGTGGTGGC
QuickChange Primer: pEGFP Site 4 Mutation (S366A) Forward:	GCCTCGCTCGAGCGCCATGGCAGCC GGCCTGG
QuickChange Primer: pEGFP Site 4 Mutation (S366A) Reverse:	CCAGGCCGGCTGCCATGGCGCTCGA GCGAGGC
Eps8 constructs	
Primer: mCherry-C1 EcoRI Eps8 Forward:	GGTGAATTCATGAATGGTCATATTTCT AATCATCCCAGTAG
Primer: mCherry-C1 Eps8 SalI Reverse:	GGTGTCGACTTAGTGACTGCTTCCTTC ATCAAAAGATT
14-3-3 constructs	
Primer: mCherry-C1/mTAGBFP2-C1: EcoRI 14-3-3 Forward:	GGTGAATTCGATGGAGAAGACTGAGC TGATCCAG
Primer: mCherry-C1/mTAGBFP2-C1 14-3-3 BamHI Reverse:	GGTGGATCCTTAGTTTTCAGCCCCTTC TGCC

Supplemental Table S4: Nucleotide Sequence of Genes(5 pages)

pEGFP-N1/mTAGBFP2-N1-IRSp53 and IRSp53-FLAG

ATGTCTCTGTCTCGCTCAGAGGAGATGCACCGGCTCACGGAAAATGTCTATAAGAC
CATCATGGAGCAGTTCAACCCTAGCCTCCGGAACTTCATCGCCATGGGGAAGAATT
ACGAGAAGGCACTGGCAGGTGTGACGTATGCAGCCAAAGGCTACTTTGACGCCCT
GGTGAAGATGGGGGAGCTGGCCAGCGAGAGCCAGGGCTCCAAAGAACTCGGAGA
CGTTCTCTTCCAGATGGCTGAAGTCCACAGGCAGATCCAGAATCAGCTGGAAGAAA
TGCTGAAGTCTTTTCACAACGAGCTGCTTACGCAGCTGGAGCAGAAGGTGGAGCT
GGACTCCAGGTATCTGAGTGCTGCGCTGAAGAAATACCAGACTGAGCAAAGGAGC
AAAGGCGACGCCCTGGACAAGTGTCAGGCTGAGCTGAAGAAGCTTCGGAAGAAGA
GCCAGGGCAGCAAGAATCCTCAGAAGTACTCGGACAAGGAGCTGCAGTACATCGA
CGCCATCAGCAACAAGCAGGGCGAGCTGGAGAATTACGTGTCCGACGGCTACAAG
ACCGCACTGACAGAGGAGCGCAGGCGCTTCTGCTTCCTGGTGGAGAAGCAGTGC
GCCGTGGCCAAGAACTCCGCGGCCTACCACTCCAAGGGCAAGGAGCTGCTGGCG
CAGAAGCTGCCGCTGTGGCAACAGGCCTGTGCCGACCCCAGCAAGATCCCGGAG
CGCGCGGTGCAGCTCATGCAGCAGGTGGCCAGCAACGGCGCCACCCTCCCCAGC
GCCCTGTCGGCCTCCAAGTCCAACCTGGTCATTTCCGACCCCATTCCGGGGGCCA
AGCCCCTGCCGGTGCCCCCCGAGCTGGCACCGTTCGTGGGGCGGATGTCTGCCC
AGGAGAGCACACCCATCATGAACGGCGTCACAGGCCCGGATGGCGAGGACTACA
GCCCGTGGGCTGACCGCAAGGCTGCCCAGCCCAAATCCCTGTCTCCTCCGCAGTC
TCAGAGCAAGCTCAGCGACTCCTACTCCAACACACTCCCCGTGCGCAAGAGCGTG
ACCCCAAAAAACAGCTATGCCACCACCGAGAACAAGACTCTGCCTCGCTCGAGCTC
CATGGCAGCCGGCCTGGAGCGCAATGGCCGTATGCGGGTGAAGGCCATCTTCTCC
CACGCTGCTGGGGACAACAGCACCCTCCTGAGCTTCAAGGAGGGTGACCTCATTA
CCCTGCTGGTGCCTGAGGCCCGCGATGGCTGGCACTACGGAGAGAGTGAGAAGA
CCAAGATGCGGGGGCTGGTTTCCCTTCTCCTACACCCGGGTCTTGGACAGCGATGG
CAGTGACAGGCTGCACATGAGCCTGCAGCAAGGGAAGAGCAGCAGCACGGGCAA
CCTCCTGGACAAGGACGACCTGGCCATCCCACCCCCCGATTACGGCGCCGCCTCC
CGGGCCTTCCCCGCCCAGACGGCCAGCGGCTTCAAGCAGAGGCCCTACAGTGTG
GCCGTGCCCGCCTTCTCCCAGGGCCTGGATGACTATGGAGCGCGGTCCATGAGCA
GTGGCAGCGGCACGCTGGTGTCCACAGTGTGA

pEGFP-N1-M234

```
ATGTCTCTGTCTCGCTCAGAGGAGATGCACCGGCTCACGGAAAATGTCTATAAGAC
CATCATGGAGCAGTTCAACCCTAGCCTCCGGAACTTCATCGCCATGGGGAAGAATT
ACGAGAAGGCACTGGCAGGTGTGACGTATGCAGCCAAAGGCTACTTTGACGCCCT
GGTGAAGATGGGGGGAGCTGGCCAGCGAGAGCCAGGGCTCCAAAGAACTCGGAGA
CGTTCTCTTCCAGATGGCTGAAGTCCACAGGCAGATCCAGAATCAGCTGGAAGAAA
TGCTGAAGTCTTTTCACAACGAGCTGCTTACGCAGCTGGAGCAGAAGGTGGAGCT
GGACTCCAGGTATCTGAGTGCTGCGCTGAAGAAATACCAGACTGAGCAAAGGAGC
AAAGGCGACGCCCTGGACAAGTGTCAGGCTGAGCTGAAGAAGCTTCGGAAGAAGA
GCCAGGGCAGCAAGAATCCTCAGAAGTACTCGGACAAGGAGCTGCAGTACATCGA
CGCCATCAGCAACAAGCAGGGCGAGCTGGAGAATTACGTGTCCGACGGCTACAAG
ACCGCACTGACAGAGGAGCGCAGGCGCTTCTGCTTCCTGGTGGAGAAGCAGTGC
GCCGTGGCCAAGAACTCCGCGGCCTACCACTCCAAGGGCAAGGAGCTGCTGGCG
CAGAAGCTGCCGCTGTGGCAACAGGCCTGTGCCGACCCCAGCAAGATCCCGGAG
CGCGCGGTGCAGCTCATGCAGCAGGTGGCCAGCAACGGCGCCACCCTCCCCAGC
GCCCTGTCGGCCTCCAAGTCCAACCTGGTCATTTCCGACCCCATTCCGGGGGCCA
AGCCCCTGCCGGTGCCCCCCGAGCTGGCACCGTTCGTGGGGCGGATGTCTGCCC
AGGAGAGCACACCCATCATGAACGGCGTCACAGGCCCGGATGGCGAGGACTACA
GCCCGTGGGCTGACCGCAAGGCTGCCCAGCCCAAATCCCTGTCTCCTCCGCAGTC
TCAGAGCAAGCTCAGCGACTCCTACTCCAACGCACTCCCCGTGCGCAAGAGCGTG
ACCCCAAAAAACAGCTATGCCACCACCGAGAACAAGGCTCTGCCTCGCTCGAGCG
CCATGGCAGCCGGCCTGGAGCGCAATGGCCGTATGCGGGTGAAGGCCATCTTCTC
CCACGCTGCTGGGGACAACAGCACCCTCCTGAGCTTCAAGGAGGGTGACCTCATT
ACCCTGCTGGTGCCTGAGGCCCGCGATGGCTGGCACTACGGAGAGAGTGAGAAG
ACCAAGATGCGGGGCTGGTTTCCCTTCTCCTACACCCGGGTCTTGGACAGCGATG
GCAGTGACAGGCTGCACATGAGCCTGCAGCAAGGGAAGAGCAGCAGCACGGGCA
ACCTCCTGGACAAGGACGACCTGGCCATCCCACCCCCCGATTACGGCGCCGCCTC
CCGGGCCTTCCCCGCCCAGACGGCCAGCGGCTTCAAGCAGAGGCCCTACAGTGT
GGCCGTGCCCGCCTTCTCCCAGGGCCTGGATGACTATGGAGCGCGGTCCATGAG
CAGTGGCAGCGGCACGCTGGTGTCCACAGTGTGA
```

mRFP-Cdc42(G12V)

```
ATGCAGACAATTAAGTGTGTTGTTGTGGGCGATGTTGCTGTTGGTAAAACATGTCTC
CTGATATCCTACACAACAAACAAATTTCCATCGGAATATGTACCGACTGTTTTTGAC
AACTATGCAGTCACAGTTATGATTGGTGGAGAACCATATACTCTTGGACTTTTTGAT
ACTGCAGGGCAAGAGGATTATGACAGATTACGACCGCTGAGTTATCCACAAACAGA
TGTATTTCTAGTCTGTTTTTCAGTGGTCTCTCCATCTTCATTTGAAAACGTGAAAGAA
AAGTGGGTGCCTGAGATAACTCACCACTGTCCAAAGACTCCTTTCTTGCTTGTTGG
GACTCAAATTGATCTCAGAGATGACCCCTCTACTATTGAGAAACTTGCCAAGAACAA
ACAGAAGCCTATCACTCCAGAGACTGCTGAAAAGCTGGCCCGTGACCTGAAGGCT
GTCAAGTATGTGGAGTGTTCTGCACTTACACAGAAAGGCCTAAAGAATGTATTTGAC
GAAGCAATATTGGCTGCCCTGGAGCCTCCAGAACCGAAGAAGAGCCGCAGGTGTG
TGCTGCTATGA
```

mCherry-C1-Eps8

```
ATGAATGGTCATATTTCTAATCATCCCAGTAGTTTTGGAATGTACCCATCTCAGATG
AATGGCTACGGATCATCACCTACCTTTTCCCAGACGGACAGAGAACATGGTTCAAA
AACAAGTGCAAAGGCCCTTTATGAACAAAGGAAGAATTATGCACGGGACAGTGTCA
GCAGTGTGTCAGATATATCTCAATACCGTGTTGAACACTTGACTACCTTTGTCCTGG
ATCGGAAAGATGCTATGATCACTGTTGATGATGGAATAAGGAAATTGAAATTGCTTG
ATGCCAAGGGCAAAGTGTGGACTCAAGATATGATTCTTCAAGTGGATGACAGAGCT
GTGAGCCTGATTGATTTAGAATCAAAGAATGAACTGGAGAATTTTCCTTTAAACACA
ATCCAGCACTGCCAAGCTGTGATGCATTCATGCAGCTATGATTCAGTTCTTGCACT
GGTGTGCAAAGAGCCAACCCAGAACAAGCCAGATCTTCATCTCTTCCAGTGTGATG
AGGTTAAGGCAAACCTAATTAGTGAAGATATTGAAAGTGCAATCAGTGACAGTAAAG
GAGGGAAACAGAAGAGGCGGCCCGACGCCCTGAGGATGATTTCCAATGCAGACCC
TAGTATACCGCCTCCACCCAGAGCTCCTGCCCCTGCGCCCCCTGGGACCGTCACC
CAGGTGGATGTTAGAAGTCGAGTGGCAGCCTGGTCTGCATGGGCAGCCGACCAAG
GGGACTTTGAGAACCAAGGCAGTATCATGAGCAGGAAGAAACACCTGAGATGAT
GGCAGCCCGCATTGACAGAGATGTGCAAATCTTAAACCACATTTTGGATGACATTG
AATTTTTTATCACAAAACTCCAAAAAGCAGCAGAAGCATTTTCTGAGCTTTCTAAAAG
GAAGAAAAACAAGAAAGGTAAAAGGAAAGGACCAGGAGAGGGTGTTTTAACGCTG
CGGGCAAAACCTCCACCTCCTGATGAATTTCTTGACTGTTTCCAAAAGTTTAAACAC
GGATTTAACCTTCTGGCCAAACTGAAGTCTCATATTCAGAATCCTAGTGCTGCAGAT
TTGGTTCACTTTTTGTTTACTCCATTAAATATGGTGGTGCAGGCAACAGGAGGTCCT
GAACTAGCCAGTTCAGTACTTAGTCCCCTATTGAATAAGGACACAATTGATTTCTTA
AATTATACTGTCAATGGTGATGAACGGCAGCTGTGGATGTCATTGGGAGGAACTTG
GATGAAAGCCAGAGCAGAGTGGCCAAAAGAACAGTTTATTCCACCATATGTTCCAC
GATTCCGCAATGGCTGGGAGCCCCCAATGCTGAACTTTATGGGAGCCACAATGGA
ACAAGATCTTTATCAACTGGCAGAATCTGTGGCAAATGTAGCAGAACATCAGCGCA
AACAGGAAATAAAAAGATTATCCACAGAGCATTCCAGTGTATCAGAGTATCATCCAG
CCGATGGCTATGCGTTCAGTAGCAACATTTACACAAGAGGATCCCACCTGGACCAA
GGGGAAGCTGCTGTTGCTTTTAAGCCAACTTCTAATCGCCATATAGATAGAAATTAT
GAACCACTCAAAACACAACCCAAGAAATATGCCAAATCCAAGTATGACTTTGTAGCA
AGGAACAACAGTGAGCTCTCGGTTCTAAAGGATGATATTTTAGAGATACTTGATGAT
CGGAAGCAATGGTGGAAAGTTCGAAATGCAAGTGGAGACTCTGGATTTGTGCCAAA
TAACATTTTGGATATTGTGAGACCTCCAGAATCTGGATTGGGGCGTGCTGATCCAC
CTTATACTCATACTATACAGAAACAAAGGATGGAGTATGGCCCAAGACCAGCTGAT
ACTCCCCCTGCTCCATCACCTCCTCCAACACCAGCTCCTGTTCCTGTTCCCCTTCC
CCCTTCCACTCCAGCACCTGTTCCTGTGTCAAAGGTCCCAGCAAATATAACACGTC
AAAACAGCAGCTCCAGTGACAGTGGTGGCAGTATCGTGCGAGACAGCCAGAGACA
CAAACAACTTCCGGTGGACCGAAGGAAATCTCAGATGGAGGAAGTGCAAGATGAA
CTCATCCACAGACTGACCATTGGTCGGAGTGCCGCTCAGAAGAAATTCCATGTGCC
ACGGCAGAACGTGCCAGTTATCAATATCACTTACGACTCCACACCAGAGGATGTGA
AGACGTGGTTACAGTCAAAGGGATTCAACCCTGTGACTGTCAATAGTCTTGGAGTA
TTAAATGGTGCACAACTTTTCTCTCTCAATAAGGATGAACTGAGGACAGTCTGCCCT
GAAGGGGCGAGAGTCTATAGCCAAATCACTGTACAAAAAGCTGCATTGGAGGATAG
CAGTGGCAGCTCCGAGTTACAAGAAATTATGAGAAGACGACAGGAAAAAATCAGTG
CTGCCGCTAGTGATTCAGGAGTGGAATCTTTTGATGAAGGAAGCAGTCACTAA
```

pEGFP-C1-VASP

ATGAGCGAGACGGTCATCTGTTCCAGCCGGGCCACTGTGATGCTTTATGATGATGG
CAACAAGCGATGGCTCCCTGCTGGCACGGGTCCCCAGGCCTTCAGCCGCGTCCA
GATCTACCACAACCCCACGGCCAATTCCTTTCGCGTCGTGGGCCGGAAGATGCAG
CCCGACCAGCAGGTGGTCATCAACTGTGCCATCGTCCGGGGTGTCAAGTATAACC
AGGCCACCCCCAACTTCCATCAGTGGCGCGACGCTCGCCAGGTCTGGGGCCTCAA
CTTCGGCAGCAAGGAGGATGCGGCCCAGTTTGCCGCCGGCATGGCCAGTGCCCT
AGAGGCGTTGGAAGGAGGTGGGCCCCCTCCACCCCCAGCACTTCCCACCTGGTC
GGTCCCGAACGGCCCCTCCCCGGAGGAGGTGGAGCAGCAGAAAAGGCAGCAGCC
CGGCCCGTCGGAGCACATAGAGCGCCGGGTCTCCAATGCAGGAGGCCCACCTGC
TCCCCCCGCTGGGGGTCCACCCCCACCACCAGGACCTCCCCCTCCTCCAGGTCCC
CCCCCACCCCCAGGTTTGCCCCCTTCGGGGGTCCCAGCTGCAGCGCACGGAGCA
GGGGGAGGACCACCCCCTGCACCCCCTCTCCCGGCAGCACAGGGCCCTGGTGGT
GGGGGAGCTGGGGCCCCAGGCCTGGCCGCAGCTATTGCTGGAGCCAAACTCAGG
AAAGTCAGCAAGCAGGAGGAGGCCTCAGGGGGGCCCACAGCCCCCAAAGCTGAG
AGTGGTCGAAGCGGAGGTGGGGGACTCATGGAAGAGATGAACGCCATGCTGGCC
CGGAGAAGGAAAGCCACGCAAGTTGGGGAGAAAACCCCCAAGGATGAATCTGCCA
ATCAGGAGGAGCCAGAGGCCAGAGTCCCGGCCCAGAGTGAATCTGTGCGGAGAC
CCTGGGAGAAGAACAGCACAACCTTGCCAAGGATGAAGTCGTCTTCTTCGGTGAC
CACTTCCGAGACCCAACCCTGCACGCCCAGCTCCAGTGATTACTCGGACCTACAG
AGGGTGAAACAGGAGCTTCTGGAAGAGGTGAAGAAGGAATTGCAGAAAGTGAAAG
AGGAAATCATTGAAGCCTTCGTCCAGGAGCTGAGGAAGCGGGGTTCTCCCTGA

mCherry-C1/mTAGBFP2-C1-14-3-3θ

ATGGAGAAGACTGAGCTGATCCAGAAGGCCAAGCTGGCCGAGCAGGCCGAGCGC
TACGACGACATGGCCACCTGCATGAAGGCAGTGACCGAGCAGGGCGCCGAGCTG
TCCAACGAGGAGCGCAACCTGCTCTCCGTGGCCTACAAGAACGTGGTCGGGGGC
CGCAGGTCCGCCTGGAGGGTCATCTCTAGCATCGAGCAGAAGACCGACACCTCCG
ACAAGAAGTTGCAGCTGATTAAGGACTATCGGGAGAAAGTGGAGTCCGAGCTGAG
ATCCATCTGCACCACGGTGCTGGAATTGTTGGATAAATATTTAATAGCCAATGCAAC
TAATCCAGAGAGTAAGGTCTTCTATCTGAAAATGAAGGGTGATTACTTCCGGTACCT
TGCTGAAGTTGCGTGTGGTGATGATCGAAAACAAACGATAGATAATTCCCAAGGAG
CTTACCAAGAGGCATTTGATATAAGCAAGAAAGAGATGCAACCCACACACCCAATC
CGCCTGGGGCTTGCTCTTAACTTTTCTGTATTTTACTATGAGATTCTTAATAACCCA
GAGCTTGCCTGCACGCTGGCTAAAACGGCTTTTGATGAGGCCATTGCTGAACTTGA
TACACTGAATGAAGACTCATACAAAGACAGCACCCTCATCATGCAGTTGCTTAGAG
ACAACCTAACACTTTGGACATCAGACAGTGCAGGAGAAGAATGTGATGCGGCAGAA
GGGGCTGAAAACTAA