SUPPLEMENTARY INFORMATION

Figure S1. PIPKIγ is required for migration and lamellipodium formation

(A) MDA-MB-231 breast cancer cells were infected with lentivirus expressing short hairpin (sh) RNAs against either human PIPKIγ or scrambled control. Infected cells were selected by a cell sorter (viral vector contains GFP coding sequence). Either parental or virus infected cells maintained in normal culture conditions were photographed under an inverted microscope at 200X magnification. White arrows indicate ruffle-like structures.

(B) Either parental or virus infected cells were placed in the upper chamber of a Transwell and cells were allowed to migrate for 12-16 h towards 10% serum as a chemoattractant in the lower chamber. Cells were fixed and stained with a 0.5% crystal violet (CV) solution. CV-positive cells that had migrated across 3.0 μm pores were counted from photographs taken from at least five random fields (bottom). Expression levels of the endogenous proteins were analyzed by immunoblotting of cell lysates with isoform specific PIPKIγ antibodies (top).

(C) ShRAN-resistant DsRed-tagged PIPKIγ isoforms were stably expressed in shPIPKIγ#2 cells. Cells expressing a similar amount of PIPKIγ compared to the control cells were isolated using a cell sorter. With these reconstituted cells, serum-induced chemotaxis was measured with a Transwell as described above (top). Protein expression was confirmed by immunoblotting against the indicated molecules (bottom). WT, wild type. KD, kinase dead. Data are shown as mean ± SD of four independent experiments.

(D) The reconstituted cells allowed to migrate into a scratch wound were fixed after 3 hours and immunostained with the Arp2/3 complex component ARPC2. Images were taken at 400X magnification and the representative images are shown.

(E) Either control or shPIPKIγ#2 cells were grown to confluence. Lawn of cells was scratched and boundaries between cells and cell-free space were photographed at 5, 30,

60, 120, 180 and 240 min after scratching. At least 200 cells were counted for disk-like protrusions. Data are shown as mean ± SD of four experiments.

The experiments described above were performed independently at least four times.

Figure S2. PIPKIγ interacts with IQGAP1 regardless of kinase activity

(A) Identification of the PIPKIγ binding site on IQGAP1. Equal amounts of [^{35}S]methionine-labeled IQGAP1-N, IQGAP1-(2-764), IQGAP1-(763-864) or IQGAP1-C were incubated with 4 μg GST-PIPKIγi1 or GST alone on glutathione beads. Complexes were washed, resolved by SDS-PAGE and processed by autoradiography. An aliquot of [^{35}S]methionine-labeled TNT product that was not subjected to chromatography was processed in parallel (Input).

(B) Control vector or HA-tagged PIPKIγi1 wild type (WT) or kinase dead (KD) mutant was expressed with in MDA-MB-231 cells and endogenous IQGAP1 proteins were immunoprecipitated with an anti-IQGAP1 antibody. Immunoprecipitates were resolved by SDS-PAGE and the associated PIPKIγi1 was analyzed by immunoblotting with an anti-HA antibody.

(C) HA-PIPKIγi1 was co-transfected with Myc-IQGAP1 WT or S1441S/S1443A mutant in MDA-MB-231 cells for 36 h. Then, cells were serum starved for 12 h before treating with 10% FBS for 30 m. Myc-IQGAP1 WT or mutant was immunoprecipitated with an anti-Myc antibody and the associated PIPKIγi1 was analyzed by immunoblotting with an anti-HA antibody (top). Data are shown as mean ± SD of three independent experiments (bottom).

(D) The reconstituted MEFs were used for 5 μM lysophosphatidic acid induced cell migration using a Transwell. Data are shown as mean ± SD of three independent experiments.

(E) Myc-IQGAP1 was co-transfected with HA-PIPKIγi1 WT or E111L mutant in MDA-MB-

231 cells for 48 h. Cells were harvested and exogenous IQGAP1 was immunoprecipitated with an anti-Myc antibody and the associated PIPKIγi1 was analyzed by immunoblotting with an anti-HA antibody.

(F) MDA-MB-231 cells were transfected with the indicated IQGAP1 proteins or mock control for 48 h. Cells were harvested with a hypotonic buffer and the membrane fraction was separated from the cytosolic fraction by centrifugation. 10 μg of each protein was resolved by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies (top). The percentage of protein bound in the pellet relative to total (S+P) was calculated by quantifying the immunoblots (bottom).

The experiments described above were performed independently at least four times.

Figure S3. IQGAP1 interacts with PIP_2 and sequestration of PIP_2 blocks IQGAP1 targeting to membrane

(A) 0.01 μM of GST-tagged IQGAP1 full length (FL), N- or C-terminal half was incubated with Pip Strips (Eschelon Bioscience) for 1 h at room temperature and bound proteins were detected by immunoblotting with an anti-GST antibody. LPA, lysophosphatidic acid. LPC, lysophosphocholine. PE, phosphatidylethanolamine. PC, phosphatidylcholine. S1P, sphingosine 1-phosphate. PA, phosphatidic acid. PS, phosphatidylserine.

(B) Myc-tagged IQGAP1 wild type or mutants was co-expressed with HA-tagged PIPKIγi1 in HEK293 cells and exogenous IQGAP1 proteins were immunoprecipitated with an anti-Myc antibody. Immunoprecipitates were resolved by SDS-PAGE and the associated PIPKIγi1 was analyzed by immunoblotting with an anti-HA antibody.

(C) IQGAP1 proteins from the reconstituted MEFs were immunoprecipitated with an anti-GFP antibody. Immunoprecipitates were resolved by SDS-PAGE and the associated PIPKIγ was analyzed by immunoblotting with an anti-PIPKIγ antibody.

(D) Before plating, MDA-MB-231 cells were treated with either vehicle or 1 mM neomycin

(Calbiochem) for 10 min, or transfected with either vector control or GFP-PLCδ1-PH for 24 h. Cells were plated on 10 ng/ml collagen I-coated culture dish for 1 h and similar fractionation assay was performed as Fig. 3 A. Equal amount of proteins (10 μg each) were resolved by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies.

(E) MDA-MB-231 cells were transfected with Myc-IQ domain alone or mock control for 48 h. Cells were harvested with a hypotonic buffer and the membrane fraction was separated from the cytosolic fraction by centrifugation. 10 μg of each protein was resolved by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies (top). The percentage of protein bound in the pellet relative to total (S+P) was calculated by quantifying the immunoblots (bottom). Data are shown as mean ± SD of three independent experiments.

(F) Cell lysates from reconstituted MEFs were used for immunoprecipitating endogenous N-WASP. Immunoprecipitates were resolved by SDS-PAGE and the associated IQGAP1 was analyzed by immunoblotting with an anti-IQGAP1 antibody.

All the experiments described above were performed independently at least three times.

Figure S4. PIPKIγ- and PIP_2-binding of IQGAP1 are required for directionally persistent migration

(A) The reconstituted MEFs were plated on gelatin gel for 3 h before recording using time-lapse microscopy at 400X. To locate cells expressing GFP-positive IQGAP1 proteins, cells were first photographed under a fluorescent channel. Immediately after, cells were imaged every 5 min for 3 h to generate the movies shown in videos 1-3.

(B) MDA-MB-231 cells were transfected with mock control or increasing amount of GFP-PLCδ1-PH DNA for 4h. Cells plated on collagen I for 1h were fixed and immunostained with endogenous IQGAP1. Cells were photographed at 400X magnification.

All the experiments described above were performed independently at least three times.

Figure S5. Enhancement of actin polymerization is specific to $PI4,5P_2$

(A and B) Actin polymerization (1.5 μM of pyrene-conjugated G-actin, 12.5 nM of Arp2/3 comple and 40 nM of N-WASP-ΔB) in the presence of the indicated combinations of GST-IQGAP1-C (50 nM) or 5% phosphoinositide-liposomes (2 μM).

(C) 0.1 μM of His-C2 WT or AA3 mutant were incubated with 1 μM of GST-C1 immobilized on glutathione beads in the absence or presence of the indicated phosphoinositide-liposomes for 10 m. Liposome-bound proteins were detected by immunoblotting with an anti-His antibody.

(D) Actin polymerization was performed in the presence of 50 nM GST-IQGAP1-C with the indicated liposomes.

All the experiments described above were performed independently at least three times.

Supplementary Movie 1. Migration of wild type IQGAP1-reconstituted *Iqgap1*$^{-/-}$ MEFs

Iqgap1 KO MEFs were reconstituted with WT IQGAP1. Then, reconstituted MEFs were plated on gelatin gel for 3 h before recording using time-lapse microscopy. Images were collected every 5 min for 3 h at 400X magnification and combined into a time-lapse movie.

Supplementary Movie 2. Migration of ΔIQ-reconstituted *Iqgap1*$^{-/-}$ MEFs

Iqgap1 KO MEFs were reconstituted with the ΔIQ mutant IQGAP1. Then, reconstituted MEFs were plated on gelatin gel for 3 h before recording using time-lapse microscopy. Images were collected every 5 min for 3 h at 400X magnification and combined into a time-lapse movie.

Supplementary Movie 3. Migration of AA3-reconstituted *Iqgap1*$^{-/-}$ MEFs

Iqgap1 KO MEFs were reconstituted with the AA3 mutant IQGAP1. Then, reconstituted MEFs were plated on gelatin gel for 3 h before recording using time-lapse microscopy. Images were collected every 5 min for 3 h at 400X magnification and combined into a time-lapse movie.

Figure S1

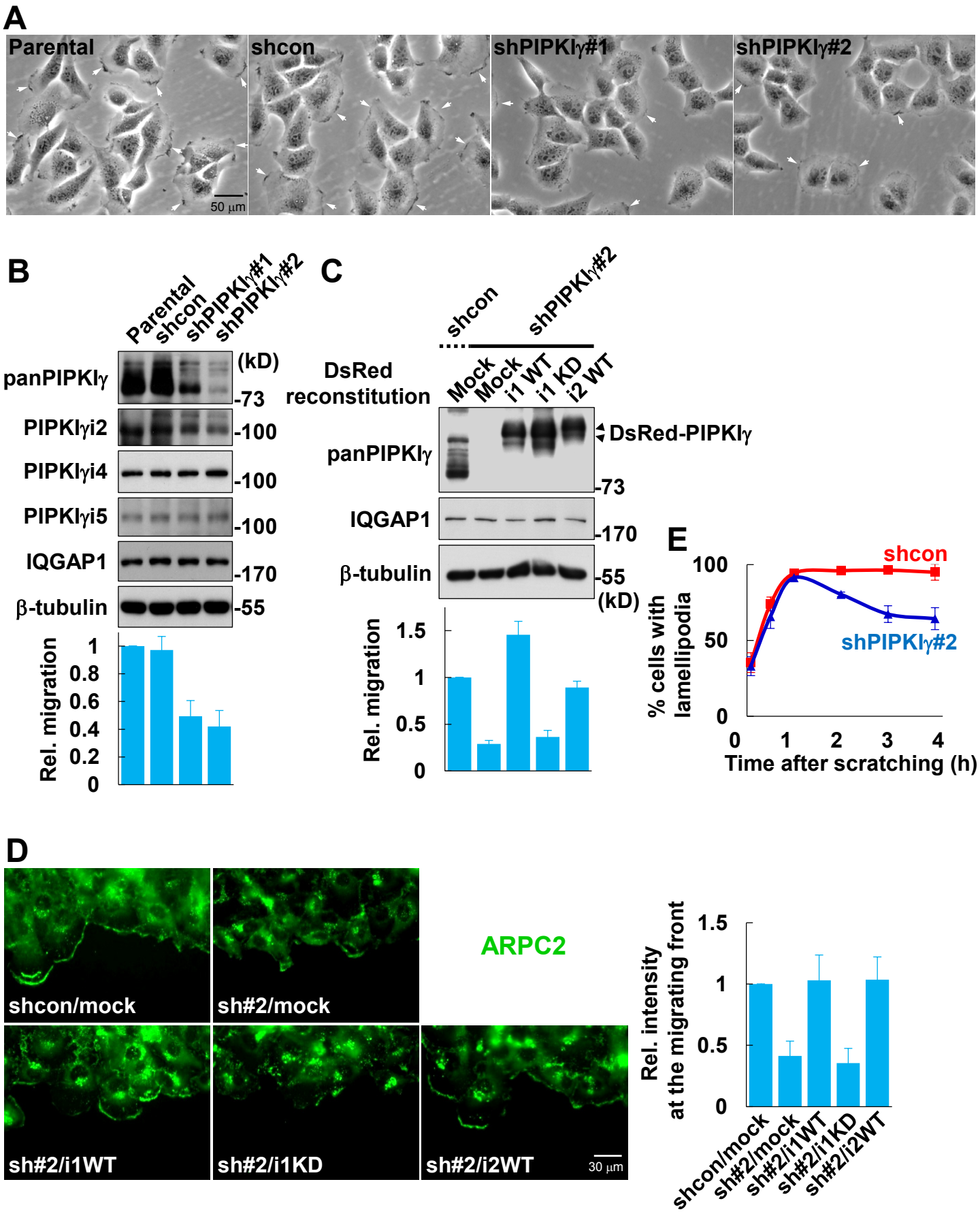
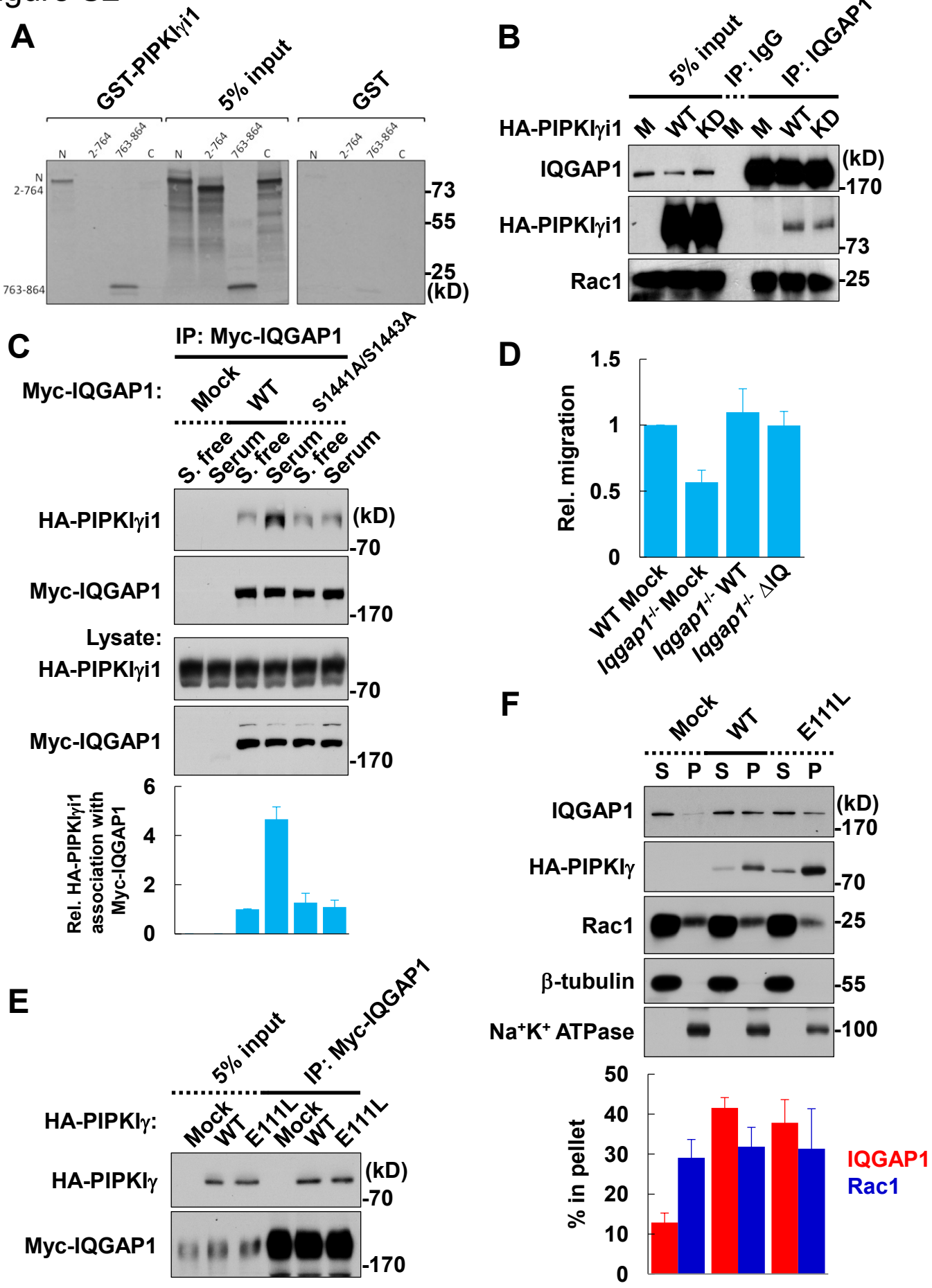

A
Parental
shcon
shPIPKIγ#1
shPIPKIγ#2
50 μm
B
Parental
shcon
shPIPKIγ#1
shPIPKIγ#2
panPIPKIγ
(kD)
-73
PIPKIγi2
-100
PIPKIγi4
-100
PIPKIγi5
-100
IQGAP1
-170
β-tubulin
-55
Rel. migration
1
0.8
0.6
0.4
0.2
0
C
shcon
shPIPKIγ#2
DsRed reconstitution
Mock
Mock
i1 WT
i1 KD
i2 WT
panPIPKIγ
DsRed-PIPKIγ
-73
IQGAP1
-170
β-tubulin
-55
(kD)
Rel. migration
1.5
1
0.5
0
E
shcon
shPIPKIγ#2
% cells with lamellipodia
100
50
0
0
1
2
3
4
Time after scratching (h)
D
shcon/mock
sh#2/mock
sh#2/i1WT
sh#2/i1KD
sh#2/i2WT
ARPC2
30 μm
Rel. intensity at the migrating front
1.5
1
0.5
0
shcon/mock
sh#2/mock
sh#2/i1WT
sh#2/i1KD
sh#2/i2WT

Figure S2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N
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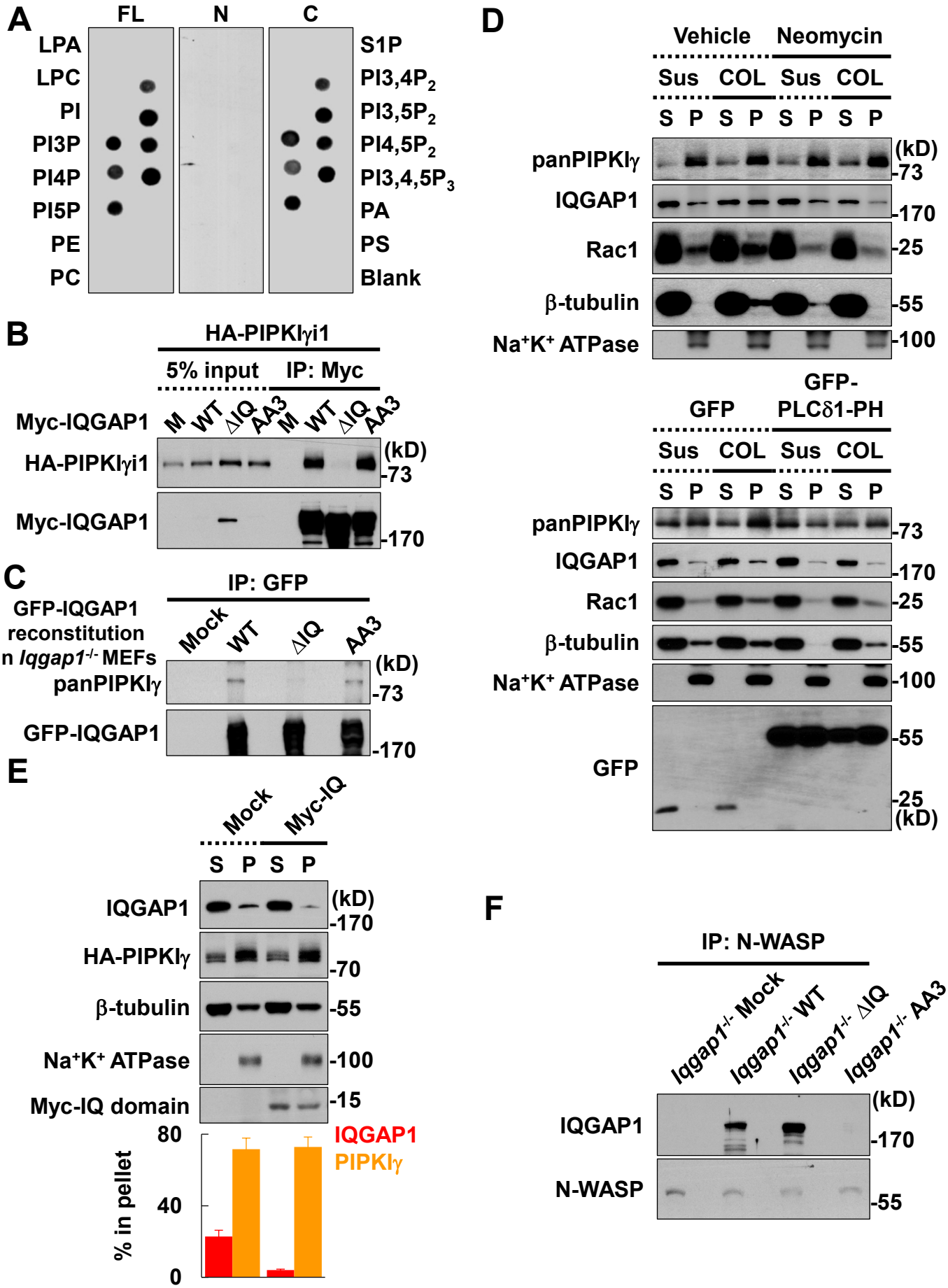
763-864
C
73
55
25
(kD)
B
5% input
IP: IgG
IP: IQGAP1
HA-PIPKIγi1
M
WT
KD
IQGAP1
Rac1
(kD)
170
73
25
C
IP: Myc-IQGAP1
Myc-IQGAP1:
Mock
WT
S1441A/S1443A
S. free
Serum
HA-PIPKIγi1
Myc-IQGAP1
Lysate:
70
170
Rel. HA-PIPKIγi1 association with Myc-IQGAP1
D
Rel. migration
WT Mock
Iqgap1-/- Mock
Iqgap1-/- WT
Iqgap1-/- ΔIQ
E
5% input
IP: Myc-IQGAP1
HA-PIPKIγ:
F
S
P
IQGAP1
HA-PIPKIγ
Rac1
β-tubulin
Na+K+ ATPase
% in pellet
IQGAP1
Rac1

Figure S3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FL
N
C
LPA
LPC
PI
PI3P
PI4P
PI5P
PE
PC
S1P
PI3,4P2
PI3,5P2
PI4,5P2
PI3,4,5P3
PA
PS
Blank
B
HA-PIPKIγi1
5% input
IP: Myc
Myc-IQGAP1
M
WT
ΔIQ
AA3
HA-PIPKIγi1
(kD)
-73
Myc-IQGAP1
-170
C
IP: GFP
GFP-IQGAP1 reconstitution in Iqgap1-/- MEFs
Mock
WT
ΔIQ
AA3
panPIPKIγ
(kD)
-73
GFP-IQGAP1
-170
D
Vehicle
Neomycin
Sus
COL
S
P
panPIPKIγ
IQGAP1
Rac1
β-tubulin
Na+K+ ATPase
(kD)
-73
-170
-25
-55
-100
GFP
GFP-PLCδ1-PH
GFP
E
Mock
Myc-IQ
IQGAP1
HA-PIPKIγ
β-tubulin
Na+K+ ATPase
Myc-IQ domain
(kD)
-170
-70
-55
-100
-15
% in pellet
80
40
0
IQGAP1
PIPKIγ
F
IP: N-WASP
Iqgap1-/- Mock
Iqgap1-/- WT
Iqgap1-/- ΔIQ
Iqgap1-/- AA3
IQGAP1
N-WASP
(kD)
-170
-55

Figure S4

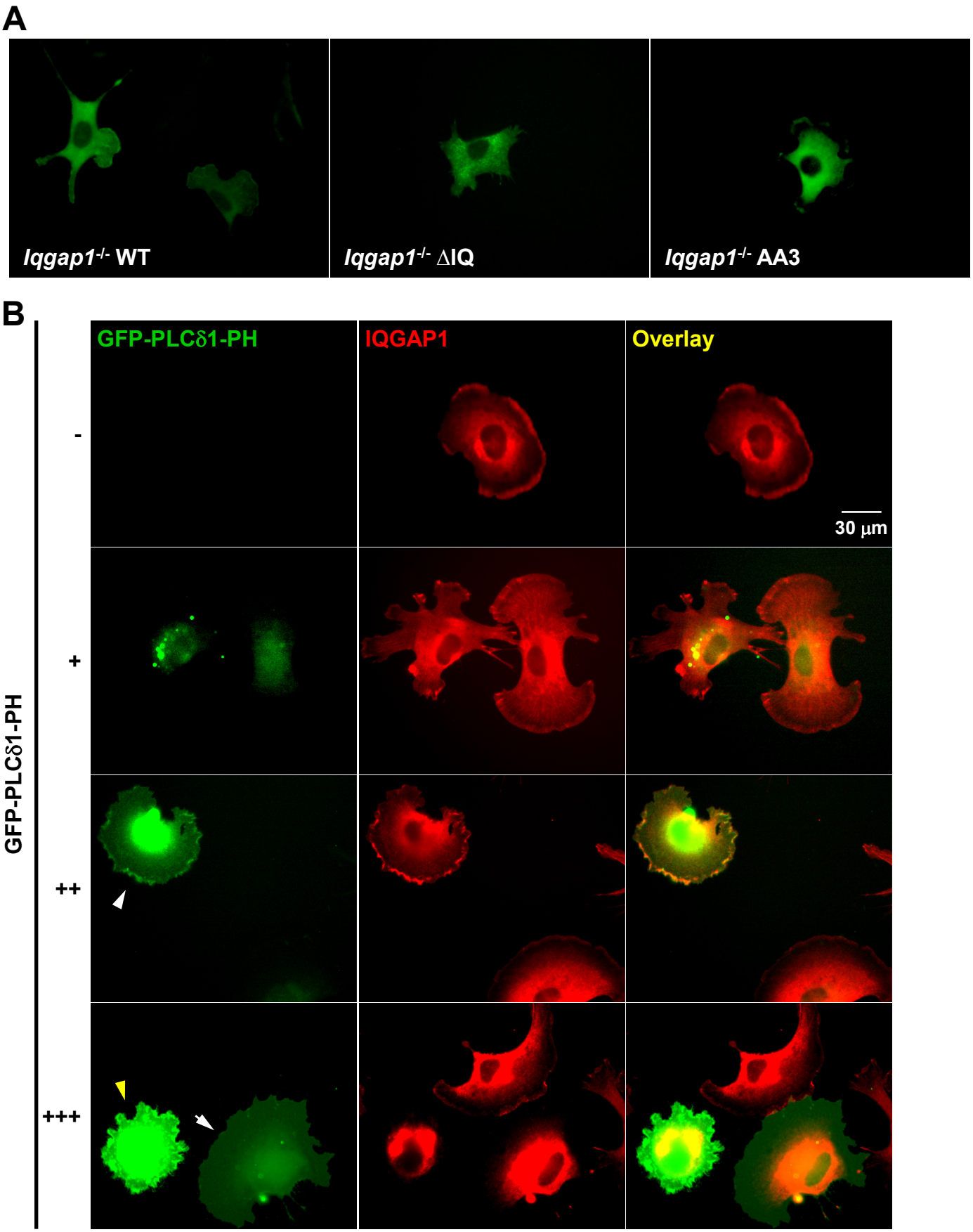
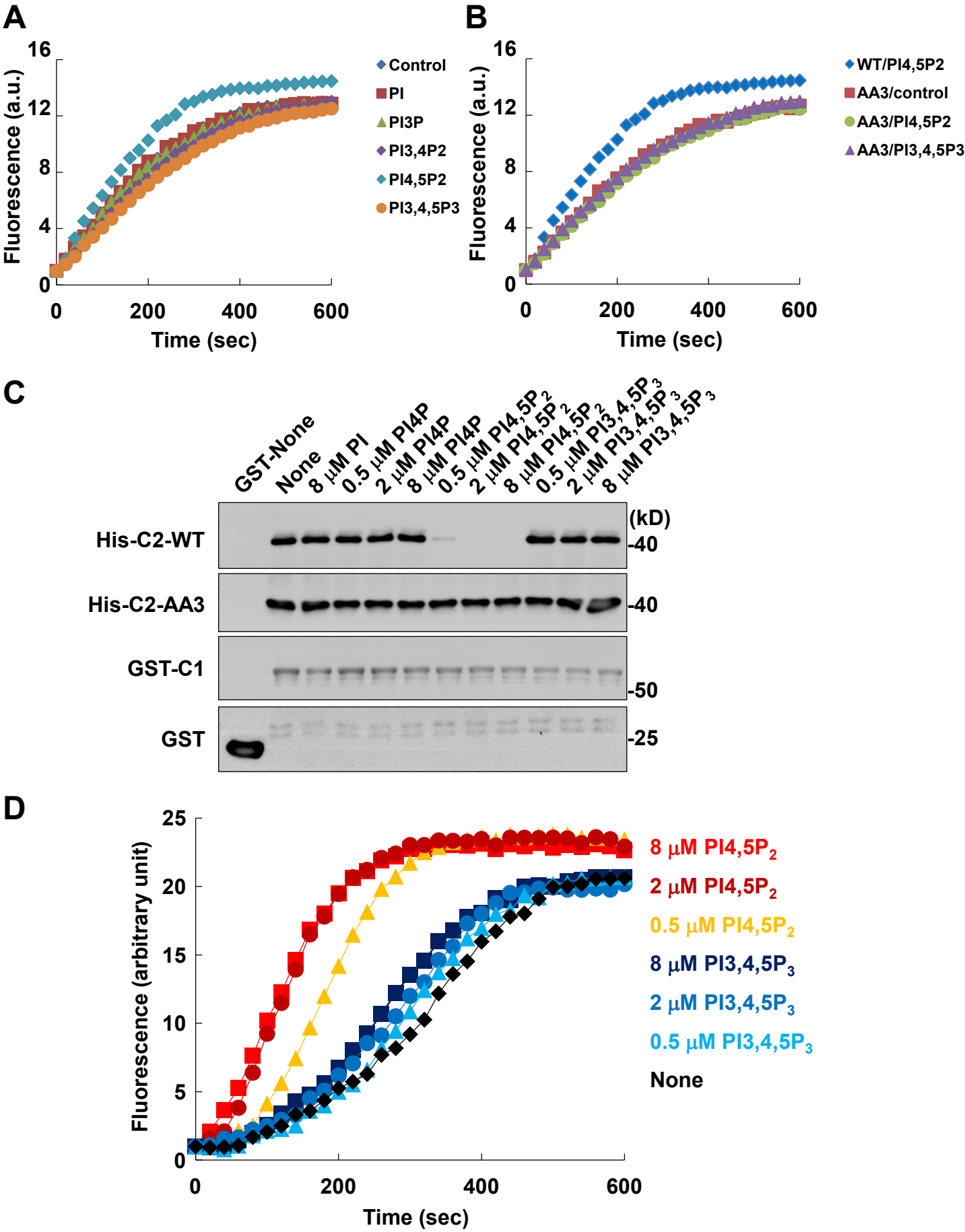

A
Iqgap1-/- WT
Iqgap1-/- ΔIQ
Iqgap1-/- AA3
B
GFP-PLCδ1-PH
GFP-PLCδ1-PH
IQGAP1
Overlay
-
+
++
+++
30 μm

Figure S5


A
Fluorescence (a.u.)
Time (sec)
Control
PI
PI3P
PI3,4P2
PI4,5P2
PI3,4,5P3
B
Fluorescence (a.u.)
Time (sec)
WT/PI4,5P2
AA3/control
AA3/PI4,5P2
AA3/PI3,4,5P3
C
GST-None
None
8 μM PI
0.5 μM PI4P
2 μM PI4P
8 μM PI4P
0.5 μM PI4,5P2
2 μM PI4,5P2
8 μM PI4,5P2
0.5 μM PI3,4,5P3
2 μM PI3,4,5P3
8 μM PI3,4,5P3
(kD)
His-C2-WT
His-C2-AA3
GST-C1
GST
-40
-40
-50
-25
D
Fluorescence (arbitrary unit)
Time (sec)
8 μM PI4,5P2
2 μM PI4,5P2
0.5 μM PI4,5P2
8 μM PI3,4,5P3
2 μM PI3,4,5P3
0.5 μM PI3,4,5P3
None