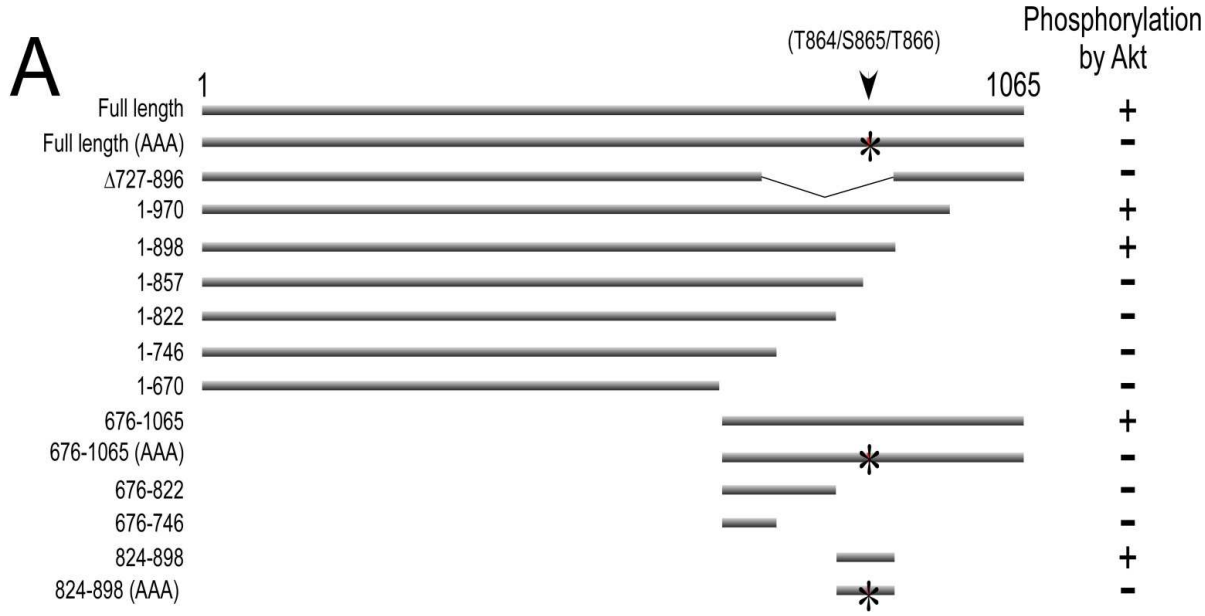
Table of Contents

Appendix figure S1



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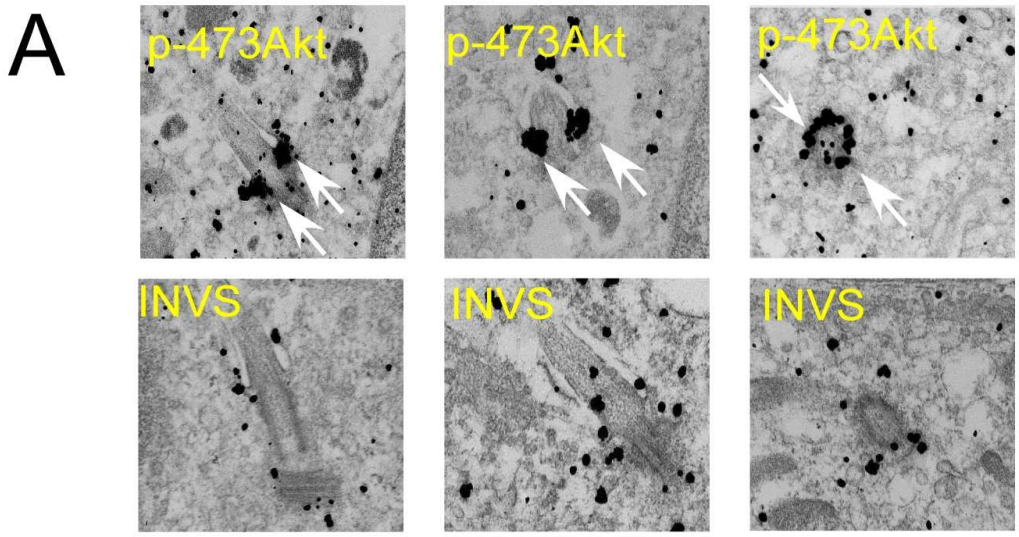
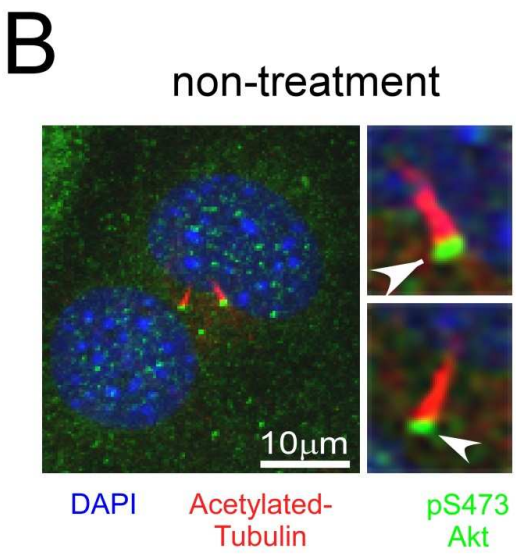
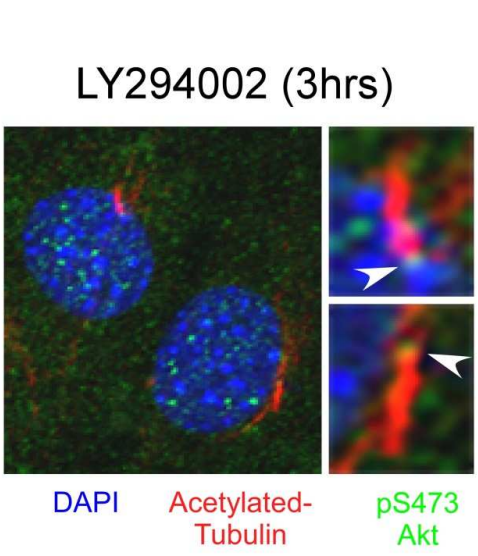
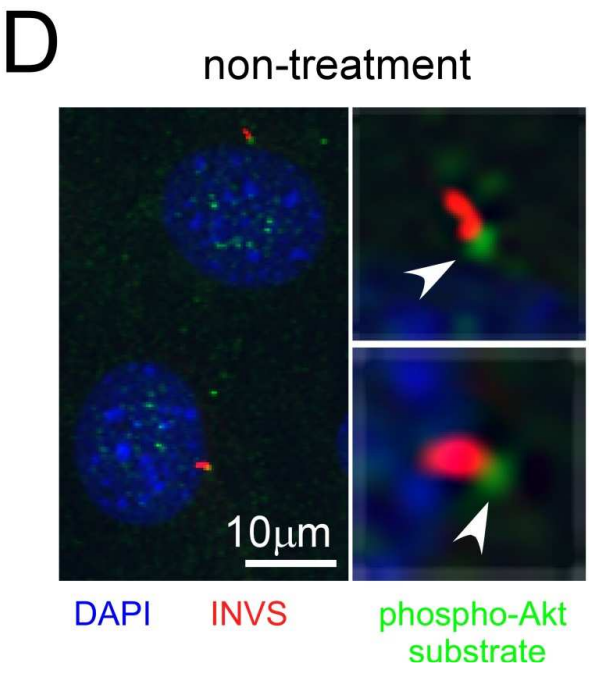
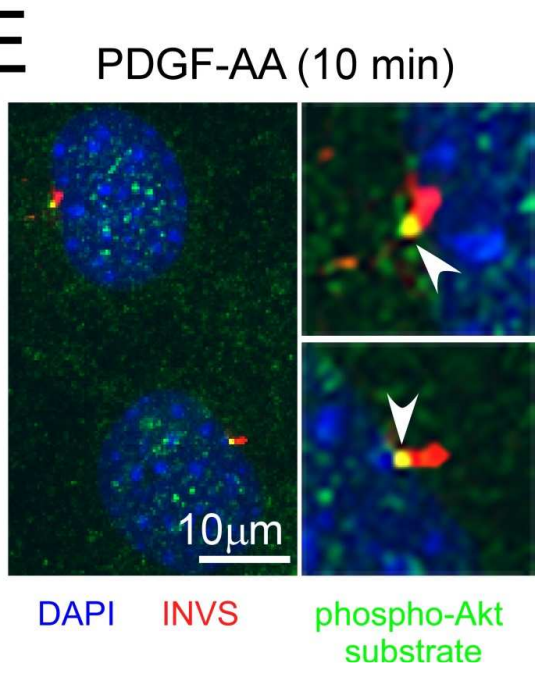
Appendix figure S2



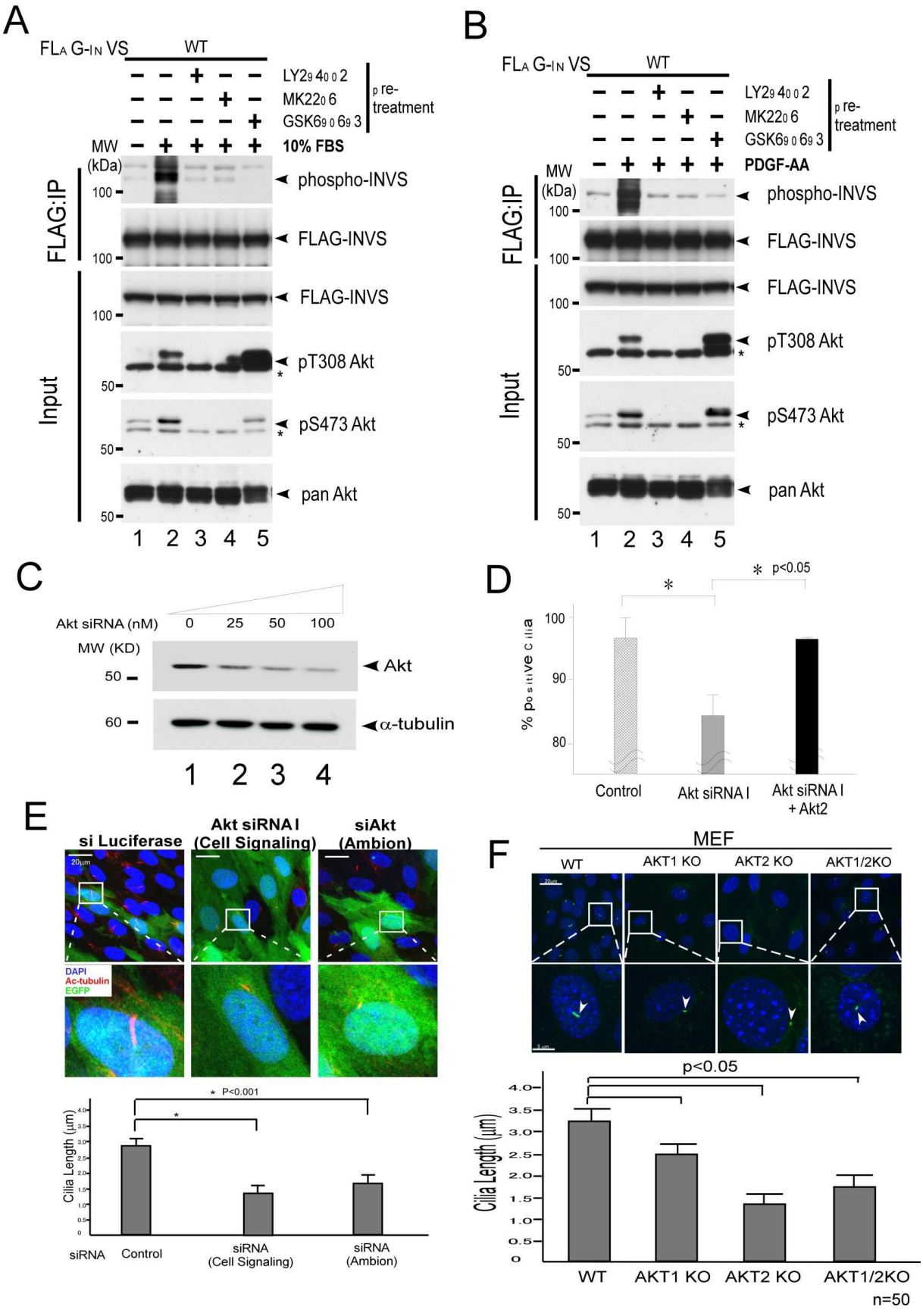




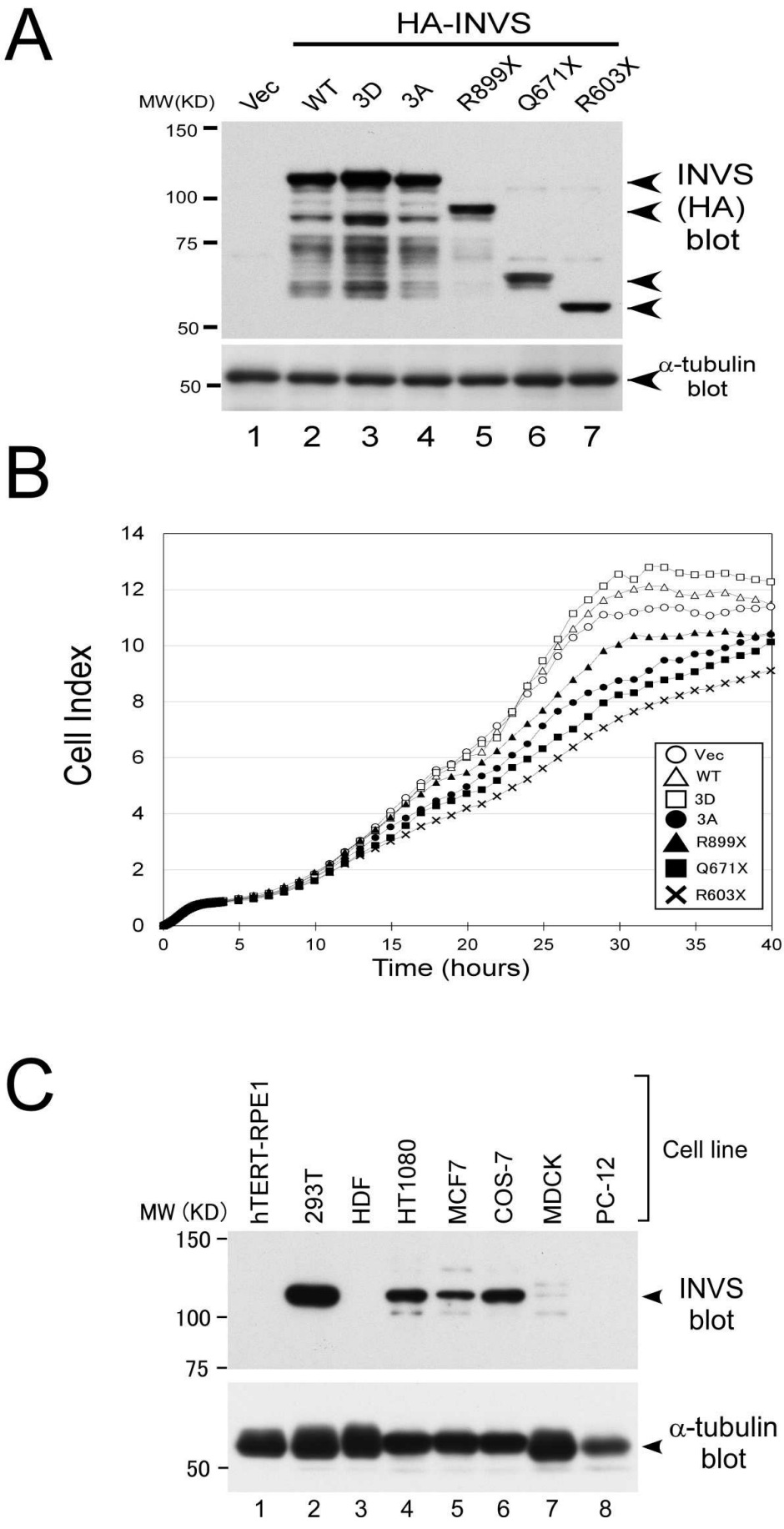




Appendix figure S3



Appendix figure S4



Appendix figure legend and method

Appendix figure S1A: Diagram of INVS constructs

Diagram showing the structure of Flag-tagged wild-type (WT), INVS subfragments, point mutant in mammalian expression vectors (N-Term, Int-, and C-Term INVS, INVS) used in the current study. Plasmid vector containing a partial cDNA fragment of human INVS was purchased from RPZ. The full-length human INVS was subcloned into the pFlagCMV2 Vectors (Sigma) or pME-18S. Other forms of INVS including subfragments of INVS were generated by restriction digest or PCR amplifications and subcloned. T864A/S865A/T866A (Serine residues are substituted into Alanine) was generated by QuikChange (Agilent technologies). The nucleotide sequences were confirmed in the final constructs used in the studies. The results of the summary of phosphorylation of INVS were shown on the right side.

Appendix figure S1B. Amino acid alignment of the putative Akt phosphorylation site among other species of mammalian species.

Using "***Scan Site***" (Obenauer, Cantley et al., 2003), three consecutive amino acids (T864, S865, and T866) were identified as putative phosphorylation targets by Akt. These consecutive serine threonine residues are conserved in other mammalian species including Pan troglodytes, Canis lupus familiaris, Bos Taurus, or Oryctolagus cuniculus.

Appendix figure S2A. Immunoelectron microscopy (IEM) of p-Akt and INVS (INVS) under unstimulated condition.

Silver-intensified immunogold method for electron microscopy showed that phospho-Akt was localized at basal body close to the ciliary pocket, however, INVS is not accumulated at ciliary pocket prior the PDGF-AA stimulation.

Method

For electron microscopic observation, NIH3T3 cells were cultured in DMEM with 10% FCS at subconfluent condition on chamber glass slide (Lab-Tek), serum-starved for 48 hr.in the absence of PDGF-AA, and fixed for 2 hr. by addition of 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). After pretreatment with goat normal serum, glass slides were incubated with mouse anti-Ser473 Akt (587F11, 4051, Cell Signaling Technology) or rabbit anti-INVS antibody (10585-1-AP, Proteintech) (1 μg/ml) overnight, and subsequently reacted with goat anti-rat IgG or anti-rabbit IgG covalently linked with 1-nm gold particles (1: 200 in dilution; Nanoprobes). Following silver enhancement using a kit (HQ silver; Nanoprobes), the cells were osmificated, dehydrated, and directly embedded in Epon (Nisshin EM). Ultrathin sections were prepared and stained with both uranyl acetate and lead citrate for observation under an electron microscope (H-7100; Hitachi). The specificity of the immunoreactions was confirmed by the disappearance of the immunolabeling when the antibody was pre-incubated with the antigen.

Appendix figure S2B and C. PI3K inhibitor LY294002 inhibited phospho-S473 Akt localization at ciliary pocket.

Treatment of the cells with PI3K inhibitor LY294002 inhibited the phosphorylation

of Akt at the ciliary pocket supporting the specificity of the phospho-Akt immunostaining.

Method:

hTERT-RPE1 cells (ATCC) were cultured confluent condition, serum starved for 48 hrs., treated with 30mM LY294002 for 3hrs., fixed with 3.7% formaldehyde, stained with anti-acetylated-tubulin (Sigma), phospho-S473 Akt (CST#4051) and DAPI (4',6-diamidino-2-phenylindole, blue, Sigma), and examined using a confocal microscopy (FLUOVIEW FV-1000, Olympus).

Appendix figure S2D and S2E. Upon PDGF-AA stimulation, INVs appeared to be the same position with phospho-Akt substrate at ciliary pocket.

Upon PDFG treatment, endogenous INVS and phosphorylated Akt substrate appeared to be the same position.

Method:

hTERT-RPE1 cells (ATCC) were cultured confluent condition, serum starved for 48 hrs., treated with 50nM PDGF-AA for 10 min., fixed with 3.7% formaldehyde, stained with anti-INVS (SantaCruz#sc-8719), anti-phospho-Akt substrate (CST#9611) and DAPI (4',6-diamidino-2-phenylindole, blue, Sigma), and examined using a confocal microscopy (FLUOVIEW FV-1000, Olympus).

Appendix figure S3A and S3B The observations INVS can be phosphorylated by Akt dependent manner are consistent if we treat the cells with serum (**Appendix figure S3A**) or using PDGF-AA stimulation (**Appendix figure S3B**).

Method: in vivo phosphorylation of INVS:

293T cells were transfected with FLAG-tagged wild type INVS (WT, INVS) or phosphorylation defective mutant INVS [T864A/S865A/T866A, (3A)]and serum starved for 4 hrs. The cells were subsequently treated with 30μM LY294002 (Sigma), 2μM MK 2206 (#11593, Cayman Chemical), or 10μM GSK690693 (#A11030-10, AdoQ BioScience) for 1hr., which were followed by treatment with either 50 ng/ml PDGF-AA (Pepro Tech) or 10% FBS for 10 min. After the treatment cells were harvested, lysed with modified RIPA buffer and FLAG-tagged INVS proteins were immunoprecipitated with FLAG(M2) agarose beads. Proteins of immunoprecipitates or of crude cell extracts were resolved onto SDS-PAGE and immunoblotted by FLAG (M2)-HRP (Sigma), phospho-Akt substrate antibody (9614, Cell Signaling Technology), anti-phospho-S473 Akt antibody (9271, Cell Signaling Technology), anti-phospho-T308 Akt antibody (9275, Cell Signaling Technology), or anti-Akt antibody (9272, Cell Signaling Technology) and detected by using ECL.

Appendix figure S3C. The expression of Akt was suppressed dose dependent manner.

Method:

hTERT-RPE cells (ATCC) were transfected with 100nM Akt siRNA I, (#6211,Cell Signaling Technology) by CUY21 Pro-vitro (NEPAGENE Co.Ltd). Seventy-two hours later, the cells were lysed with Brij cell lysis buffer and resolved onto SDS-PAGE and immunoblotted by anti-Akt antibody (#9272, Cell Signaling Technology) and detected by using ECL.

Appendix figure S3D. % presence of primary cilia (%positive) were reduced by Akt

siRNA treatment to knock down Akt1/2 expression, which can be rescued by the re-introduction of the siRNA resistant Akt.

Method:

hTERT-RPE cells were transfected with 100μM siRNA specific for Akt (#6211, siRNA I, Cell Signaling Technology) or firefly luciferase (control, Wako Nippon GENE) as a control. Akt-siRNA resistant human Akt2 was generated by QuikChange (Agilent technologies) using the following pairs of primers (HD 581: 5'-GATGTGCGG CCGCCTACCGTTT TACAACCAGGACCACG-3' and HD582: 5'-CGTGGTCCTGG TTGTAAAACG GTAGGCGGCCGCACATC-5', mutated nucleotides were underlined) (Katome, Obata et al., 2003). Seventy-two hours later, the cells were lysed with Brij cell lysis buffer and resolved onto SDS-PAGE and immunoblotted by anti-Akt antibody (#9272, Cell Signaling Technology) and detected by using ECL.

Appendix figure S3E. Ciliary length was reduced by two different siRNA for Akt.

Method.

hTERT-RPE1 cells were transfected with 100nM Akt siRNA I (#6211, Cell Signaling Technology) or Akt1 (siRNA s659, Ambion) plus Akt2 siRNA (s1217, Ambion) along with 10μg pCMV-EGFP. The cells were cultured confluent condition and serum starved for 48 hrs, fixed with 3.7% formaldehyde, stained with anti-acetylated-tubulin (Sigma) and DAPI (4',6-diamidino-2-phenylindole, blue, Sigma), and examined using a confocal microscopy (FLUOVIEW FV-1000, Olympus). The ciliary length was measured by NIH ImageJ.

Appendix figure S3F. Akt knockout MEF cells (Akt1, Akt2, and Akt1/2) showed shorter length of primary cilia.

Method:

Akt knock out MEF cells (Akt1, Akt2, and Akt1/2) were generous gift from Dr.Morris Birnbaum.The MEF cells were cultured confluent condition and serum starved for 48hrs., fixed with 3.7% formaldehyde, stained with anti-acetylated-tubulin (Sigma) and DAPI (4',6-diamidino-2-phenylindole, blue, Sigma), and examined using a confocal microscopy (FLUOVIEW FV-1000, Olympus) to measure the ciliary by NIH ImageJ.

Appendix figure S4A. Stably transfected MDCK cells used in Fig 6 and 7 [wild type (WT-INVS), triple alanine mutant (3A), phospho-mimic triple asparadic acid mutant (3D), R899X, Q691X, or R603X mutant INVS (Oud, van Bon et al., 2014, Tory, Rousset-Rouviere et al., 2009)] express similar levels of each construct by immunoblotting.

Method

MDCK cells were transduced with Lenti-virus (generous gift from Dr. Miyoshi) encoding HA-tagged WT or indicated forms of INVS (Oud et al., 2014, Tory et al., 2009) and maintained in the presence of 10μg/ml blasticidin-contained DMEM culture media with 10%FBS as described in Experimental protocol and the levels of expression of INVS were verified by HA-immunoblotting.

Appendix figure S4B.

WT and phospho-mimic INVS at S/T864-866 (3D) transduced MDCK cells proliferate

more efficiently compared to 3A or truncation mutants observed in NPHP2, which lacks the phosphorylation site at 864-866 of serine/threonine residues.

Method

MDCK cells were transduced by Lenti virus and maintained in DMEM with 10% FBS containing 10μg/ml Blasticidin as described. Proliferation assays were performed essentially used xCELLigence (Real-Time Cell Electric Sensing, ACEA, Biosciences)(Suizu, Hiramuki et al., 2009). The values shown were natural proliferative activities compared to the control out of 3 replicates.

Appendix figure S4C.

MDCK cells expresses minimal levels of INVS detected by anti-INVS western blot.

Method:

Indicated Cell lines (ATCC) were maintained in DMEM with 10 % FBS (or 10 % Horse Serum and 5 % FBS for PC-12 cells) and lysed with ice-cold Brij97 cell lysis buffer (see below). Twenty μg of proteins was resolved onto SDS-PAGE and immunoblotted by anti-INVS antibody (10585-1-AP, Proteintech) or anti-α-tubulin antibody (DM1A, #9026, Sigma) and detected by ECL.

Brij97 cell lysis buffer:

0.875% Brij97 (Sigma), 0.125% NP40, 150mM NaCl, 10mM Tris HCl pH 7.5, and 2.5 mM EDTA containing proteinase inhibitor mix (Leupeptin and AEBSF), phosphatase inhibitors (1 mM Na3VO4 and 10 mM NaF).

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Appendix Supplementary Methods

Yeast Two-Hybrid screening

Yeast two hybrid assays were essentially performed described previously(Laine, Kunstle et al., 2000, Suizu, Hiramuki et al., 2009). Y190 cells (Clontech) were transformed by the lithium acetate method with the "bait plasmid" (human Akt2/PAS2-1or human Akt3/PAS2-1) according to the manufacturer's protocol. β-gal positive clones were subsequently mated with Y187 yeast (MATα gal4 gal80 his3 trp1–901 ade2–101 ura3–52 leu2–3,-112 met2URA3::GAL–.lacZ) carrying pAS1-CYH2 without an insert or with SNF-1 or p53 to determine the specificity for the interaction. Two independent clones were identified that encode partial cDNA of human INVS which interact with either Akt2 or Akt3.

Construction of INVS expression vectors

Plasmid vector containing a partial cDNA fragment of human INVS was purchased from RPZ. The full length human INVS was subcloned into the pFLAG-CMV2 vector (Sigma), pME18s-KOZAK-HA, pEGFP-C2 (Invitrogen), or pCSII-EF1α-MCS-IRES2-Blasticidin lenti virus vector [a generous gift from Dr. H. Miyoshi (Tahara-Hanaoka, Sudo et al., 2002)]. Other forms of INVS including subfragment of INVS construct were generated by the restriction digest or PCR amplifications and subcloned into indicated plasmid. T864A/S865A/T866A (Serine or Threonine residues are substituted into Alanine) was generated by QuikChange (Agilent technologies). The nucleotide sequences were confirmed in the final constructs used in the studies.

Co-immunoprecipitation experiments

Co-immunoprecipitation experiments were essentially performed as described previously (Laine et al., 2000, Suizu et al., 2009). In brief, 293T cells (ATCC) were co-transfected with a total of 9μg of indicated plasmids per 10cm dish. 72 hours after transfection, cells were washed twice with ice-cold PBS and lysed with ice-cold Brij97 lysis buffer or modified RIPA buffer (see below) with proteinase inhibitors (Leupeptin, and AEBSF). The cells were Lysates were precleaned with protein G/protein A mixture (50% v/v, GE healthcare) for 1 hr., immunoprecipitated with anti-HA (12CA5) monoclonal antibody, anti-FLAG-M2 monoclonal antibody, or mouse IgG as a control for 3 hrs or overnight, run on SDS-PAGE 10% Tris glycine gel, and immunoblotted with indicated antibodies and detected using ECL. For endogenous Akt-INVS interaction, HEK 293 cells were harvested without transfection, harvested and lysed in the same buffer as described above and immunoprecipitated with anti-Akt and immunoblotted with anti- INVS antibody (10585-1-AP, Proteintech) and detected by ECL.

Modified RIPA buffer :

150 mM NaCl, 10 mM Tris-Hcl, 0.1% SDS, 1% TritonX-100, 1% Sodium deoxycholate, 5 mM EDTA; pH 7.5

Co-immunoprecipitation assays of endogenous interaction in 293T cells

293T cells (ATCC) were cultured in the presence of 10% FBS. The cells were treated with 100ng/ml of nocodazole for overnight. The cells were washed twice with ice-cold PBS and lysed with ice-cold Brij 97 lysis buffer (Suizu et al., 2009) in which Tris-HCl

was replaced with phosphate buffered saline (PBS pH7.4) and proteinase inhibitors (leupeptin and AEBSF), 10μM MG132, 5mM iodoacetamide, and phosphatase inhibitors (1mM Na_3VO_4 and 10mM NaF), and treated with EGS (5mM, Ethylene glycobis sulfosuccinimidylsuccinate, Pierce) for 30min. Cell lysates were stopped chemical cross linker by 1M Tris-HCl (pH7.5) for 15min at room temperature, and precleaned with protein G/protein A mixture (50% v/v, GE healthcare) for 1 hr, immunoprecipitated with anti-Akt antibody (#2966, Cell Signaling Technology) or control antibody (Mouse IgG, Dako cytomations), resolved onto SDS-PAGE (8% Tris glycine gels), and immunoblotted with anti-Akt rabbit antibody (#9272, Cell Signaling Technology) or anti-INVS antibody (10585-1-AP, Proteintech). Expression of Akt in whole cell lysate from 293T cells was shown underneath by immunoblotting using indicated antibodies.

Immunoelectron microscopy (IEM)

For silver-intensified immunogold method for electron microscopy, NIH3T3 cells were cultured in DMEM with 10% FCS at subconfluent condition (in the presence or absence of PDGF-AA (50ng/ml) for 10min. as indicated) on chamber glass slide (Lab-Tek), serum-starved for 48 hr., and fixed for 2 hr. by addition of 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). After pretreatment with goat normal serum, glass slides were incubated with mouse anti-Ser473Akt (587F11, 4051, Cell Signaling Technology) or rabbit anti-INVS antibody (10585-1-AP, Proteintech) (1μg/mL) overnight, and subsequently reacted with goat anti-rat IgG or anti-rabbit IgG covalently linked with 1-nm gold particles (1: 200 in dilution; Nanoprobes). Following silver enhancement using a kit (HQ silver; Nanoprobes), the cells were osmificated,

dehydrated, and directly embedded in Epon (Nisshin EM). Ultrathin sections were prepared and stained with both uranyl acetate and lead citrate for observation under an electron microscope (H-7100; Hitachi). The specificity of the immunoreactions was confirmed by the disappearance of the immunolabeling when the antibody was pre-incubated with the antigen.

Luciferase Reporter Assays

Luciferase reporter assays were essentially performed using Dual Luciferase kit (Promega) using 293T cells (ATCC) transfected with indicated INVS constructs, mouse Dvl1-HA [a generous gift from Dr. Patricia Salinas, University College London(Ahmad-Annuar, Ciani et al., 2006)], and Luciferase reporter vectors [M50 Super 8x TOPFlash and M51 Super 8x FOPFlash (TOPFlash mutant) were gifts from Dr. Randall Moon (Addgene plasmid #12456)] by PEI (Veeman, Slusarski et al., 2003). The levels of expression of the indicated plasmids (each form of INVS and Dvl) in the transfected cells were verified by immunoblotting.

in vitro Akt kinase assays of INVS

Indicated Flag-tagged INVS constructs were transfected into 293T cells and purified with Flag agarose beads (Sigma). In vitro kinase assays (IVK) were performed essentially using the Akt kinase assay kit (Cell Signaling) with increasing amount of wild type INVS protein. For the generation of recombinant INVS, INVS subfragments were generated by PCR amplification and subcloned into pGEX6P-3 vector, and recombinant proteins were generated according to the manufacture's instruction(GE

Health care). IVK reaction was performed by incubating in the presence or absence of recombinant active Akt (UBI) for 20min. at 30^{o}C with gentle agitation. The reactions were terminated by adding SDS sample buffer and autoradiogramed for radioisotope rabelled experiment. Otherwise, the samples were separated on SDS-PAGE and immunoblotted with anti-Akt, or Flag, or phospho-Akt substrate antibodies (#9614, Cell Signaling Technology) and detected by ECL.

Phosphorylation of INVS in cellular experiments:

HEK293T cells or COS7 cells were transfected with FLAG-tagged wild type INVS (WT) or phosphorylation defective mutant INVS (T864A/S865A/T866A, 3A) and serum starved for 4 hrs. The cells were subsequently treated with 30μM LY294002 (Sigma), 2μM MK 2206 (#11593, Cayman Chemical), or 10μM GSK690693 (#A11030-10, AdoQ BioScience) for 1hr., which were followed by treatment with either 50ng/ml PDGF-AA (Pepro Tech) or 10% FBS for 10 min. After the treatment cells were harvested, lysed with modified RIPA buffer and FLAG-tagged INVS proteins were immunoprecipitated with FLAG agarose beads (M2, Sigma). Proteins of immunoprecipitates or of crude cell extracts were resolved onto SDS-PAGE and immunoblotted by FLAG -HRP (M2,Sigma), phospho-Akt substrate antibody (#9614, Cell Signaling Technology), anti-phospho-S473 Akt antibody (#9271, Cell Signaling Technology), anti-phospho-T308 Akt antibody (#9275, Cell Signaling Technology), or anti-Akt antibody (#9272, Cell Signaling Technology) and detected by using ECL.

Phosphorylation of INVS by MyrAkt:

HEK 293T cells were transfected with FLAG-tagged wild type INVS or

phosphorylation defective mutant INVS (T864A/S865A/T866A) with HA–tagged Myristoylated-Akt (Kohn, Summers et al., 1996, Suizu et al., 2009). After serum starvation for 4 hrs. cells were lysed with modified RIPA buffer and FLAG-tagged INVS proteins were immunoprecipitated with FLAG (M2) agarose beads (Sigma). Proteins of immunoprecipitates or of crude cell extracts (for Input) were resolved onto SDS-PAGE and immunoblotted by FLAG (M2)-HRP (Sigma), phospho-Akt substrate antibody (#9614, Cell Signaling Technology), anti-phospho-S473 Akt antibody (#9271, Cell Signaling Technology), or HA-HRP (3F10, Roche) and detected by using ECL.

Measurement of primary cilia using siRNA (Fig. 5E)

hTERT-RPE1 cells were transfected with Akt siRNA I (100nM, #6211,Cell Signaling Technology) plus pCMV-EGFP (10μg), and cultured in DMEM with 10%FBS. Akt-siRNA resistant human Akt2 was generated by QuikChange (Agilent technologies) using the following pairs of primers (HD 581: 5'-GATGTGCGGCCGCCTACCGTTT TACAACCAGGACCACG-3' and HD582: 5'-CGTGGTCCTGGTTGTAAAACG GTAGGCGGCCGCACATC-5', mutated nucleotides were underlined) (Katome, Obata et al., 2003) (Matsuda-Lennikov, Suizu et al., 2014). Five microgram of siRNA resistant Akt was simultaneously transfected into the cells. The cells were cultured confluent condition and subsequently serum starved for 48 hrs, fixed with 3.7% formaldehyde, stained with anti-acetylated-tubulin (Sigma), DAPI (4',6-diamidino-2-phenylindole, Sigma), and examined using confocal microscopy (FLUOVIEW FV-1000, Olympus) to measure the ciliary length by NIH ImageJ..

Localization of INVS and Akt using confocal microscopy (Fig. 3A-D, Fig 4A)

hTERT-RPE1 cells were cultured in DMEM with 10% FBS at confluent condition and serum starved for 48 hrs. For the PDGF stimulation, cells were treated with PDGF-AA (50ng/ml) for 10min. or indicated time period. The cell were fixed with 3.7% formaldehyde, stained with anti-acetylated-tubulin and DAPI (4',6-diamidino-2-phenylindole, blue, Sigma), anti-γ-tubulin (Sigma), anti-acetylated tubulin (Sigma), p-Ser473Akt (#4051, Cell Signaling technology), ARL13B (#17711-1-AP, Proteintech), or p-Akt substrate (#9611, Cell Signaling Technology) and examined using confocal microscopy (FLUOVIEW FV-1000, Olympus).

Localization of WT and 3A INVS after PDGF stimulation (Fig4. E)

hTERT-RPE1 cells were transduced with lenti virus encoding EGFP-tagged wild type (WT) INVS or phosphorylation defective mutant INVS (3A; T864A/S865A/T866A), and maintained in DMEM with 10% FBS containing 20μg/mL Blasticidin. After serum starvation for 48 hrs, cells were stimulated 50ng/ml PDGF-AA for 10min. (or unstimulated), fixed with 3.7% formaldehyde, permeabilized with 1% NP-40, and blocked with 3% BSA in PBS. The cells were stained with anti-γ-tubulin (Sigma) and anti-acetylated tubulin (Sigma) antibodies, and the line profile of fluorescent intensity was measured by Image-Pro Plus (Media Cybernetics).

Measurement of ciliary length in hTERT-RPE1 cells (Fig5.F and **G):**

hTERT-RPE1 cells were transduced with lenti virus (WT, 3A or EGFP) as described in (Fig. 4E). After serum starvation for 48 hrs. cells were fixed with 3.7% formaldehyde, permeabilized with 1% NP-40, and blocked with 3% BSA in PBS . Fixed cells were

stained with anti-γ-tubulin (Sigma) and anti-acetylated tubulin (Sigma) antibodies, and examined (FLUOVIEW FV-1000, Olympus). The ciliary length was measured by Image-Pro Plus (Media Cybernetics, PA, USA) (n>50).

Generation of MDCK cells expressing WT, 3A, and truncated forms of INVS (Fig. 6A and B, Fig. 7A-D,)

MDCK cells were transduced with lenti-virus encoding HA-tagged WT or indicated forms of INVS (Tory, Rousset-Rouviere et al., 2009) (Oud, van Bon et al., 2014) and maintained in the presence of 10μg/ml Blasticidin-contained DMEM culture media with 10%FBS.

The levels of expression of INVS were verified by HA-immunoblotting (Appendix figure S4).

Measurement of spindle axis (Fig. 7A and B)

Spindle angle between the axis of a metaphase spindle was calculated essentially described previously (Kikuchi, Niikura et al., 2010). MDCK cells expressing various forms of INVS were grown of glass cover slips at subconfluent condition. For staining of γ-tubulin (centrosome structure), cells were washed twice with PBS and fixed with 3.7% (v/v) formaldehyde for 15 min. at room temperature. Cells were permeabilized in 1% NP-40 for 15 min., washed three times with PBS and blocked with 3% BSA in PBS for 10 min. Cells were stained with mouse anti γ-tubulin antibody (Sigma) for overnight at 4°C. After the primary antibody reaction, the cells were counterstained with Alexa Fluor488-conjugated goat anti-mouse antibody (Invitrogen) and DAPI (4',6-diamidino-2-phenylindole, blue, Sigma). On the basis of γ-tubulin intensity, the

linear distance and the vertical distance between the two poles of the metaphase spindles were measured by taking Z stack images from 0.45μm thick sections of a metaphase cell. Acquisition of Z stack image was carried out using a confocal microscopy (FLUOVIEW FV-1000, Olympus) and reconstruction of Z stack image was done by Imaris (Bitplane AG, Zurich). The linear distance and the vertical distance between the two poles of the metaphase spindles of MDCK cells were measured by taking Z-stack images and the spindle angle between the axis of a metaphase spindle was calculated by Canvas X software (ACD systems).

Three-dimensional culture of MDCK cells (Fig. 7A-D)

Three-dimensional (3D) culture of MDCK cells expressing various forms of INVS were conducted essentially described in (Yamamoto, Awada et al., 2013). 50μl of Matrigel (Corning Cat#356230) was mounted on a 15mm round glass coverslips in 24 well plate. After incubation at 37°C for 30 min. to solidify the gel, harvested MDCK cells ($5x10^4$) were resuspended in 1ml of 2% Matrigel in DMEM with 10% FBS containing 10μg/ml Blasticidin and mounted on solidified gel, and further incubation was performed for 7 or 14 days. To visualize primary cilia and cortical actin mesh in 3D Matrigel, the cells were fixed for 30 min. in PBS containing 3.7% (v/v) formaldehyde and then permeabilized with PBS containing 0.5% (v/v) Triton X-100 and 3% (w/v) BSA for 30 minutes. The cells were stained with anti-acetylated tubulin antibody (Sigma) over night at 4°C. Cells were washed with 3% BSA in PBS three times and incubated for 2 hrs. with goat Alexa-Fluor-488-conjugated anti-mouse, TRITC-phalloidin (Sigma) to visualize actin or primary cilia (mouse anti-acetylated tubulin, Sigma), and examined using a confocal microscopy (FLUOVIEW FV-1000,

Olympus) or differential interference contrast (DIC) microscope.

Statistical analysis

Statistical analysis was verified by student's *t* test or Mann-Whitney's test and $p<0.05$ considered as statistically significant.

Antibodies used in this study.

Phospho-Akt (Ser473) antibody (9271, Cell Signaling Technology), Phospho-Akt (Thr308) antibody (9275, Cell Signaling Technology), Phospho-Akt (Ser473) (587F11) XP mouse mAb (4051, Cell Signaling Technology), anti-HA (12CA5, Roche), anti-Flag M2 antibody (F3165, Sigma), anti-Mouse IgG (X0931, Dako), anti-HA-HRP High Affinity (3F10, 2013819, Roche), anti-Flag M2-HRP conjugate (A8529, Sigma), Akt antibody (9272,Cell Signaling Technology), anti-acetylated tubulin (T7451, Sigma), anti-γ-tubulin (T5326, Sigma), anti-INVS antibody (10585-1-AP, proteintech), ARL13B (17711-1-AP, Proteintech).

Other reagents used in this study:

LY294002 (L9908, Sigma), MK2206 (11593, Cayman Chemical). GSK690693 (A11030-10, AdoQ BioScience), PDGF-AA (100-13A, PeproTech), FLAG (M2) agarose beads (A2220, Sigma), Matrigel (356230, Corning), TRITC-Phalloidin (P1951, Sigma), DAPI (4',6-Diamidino-2-phenylindole) (D9542, Sigma), Polyethylenimine [(PEI), Linear (MW 25,000) (Polysciences, Inc.)], Active Akt (14-276, UBI)

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