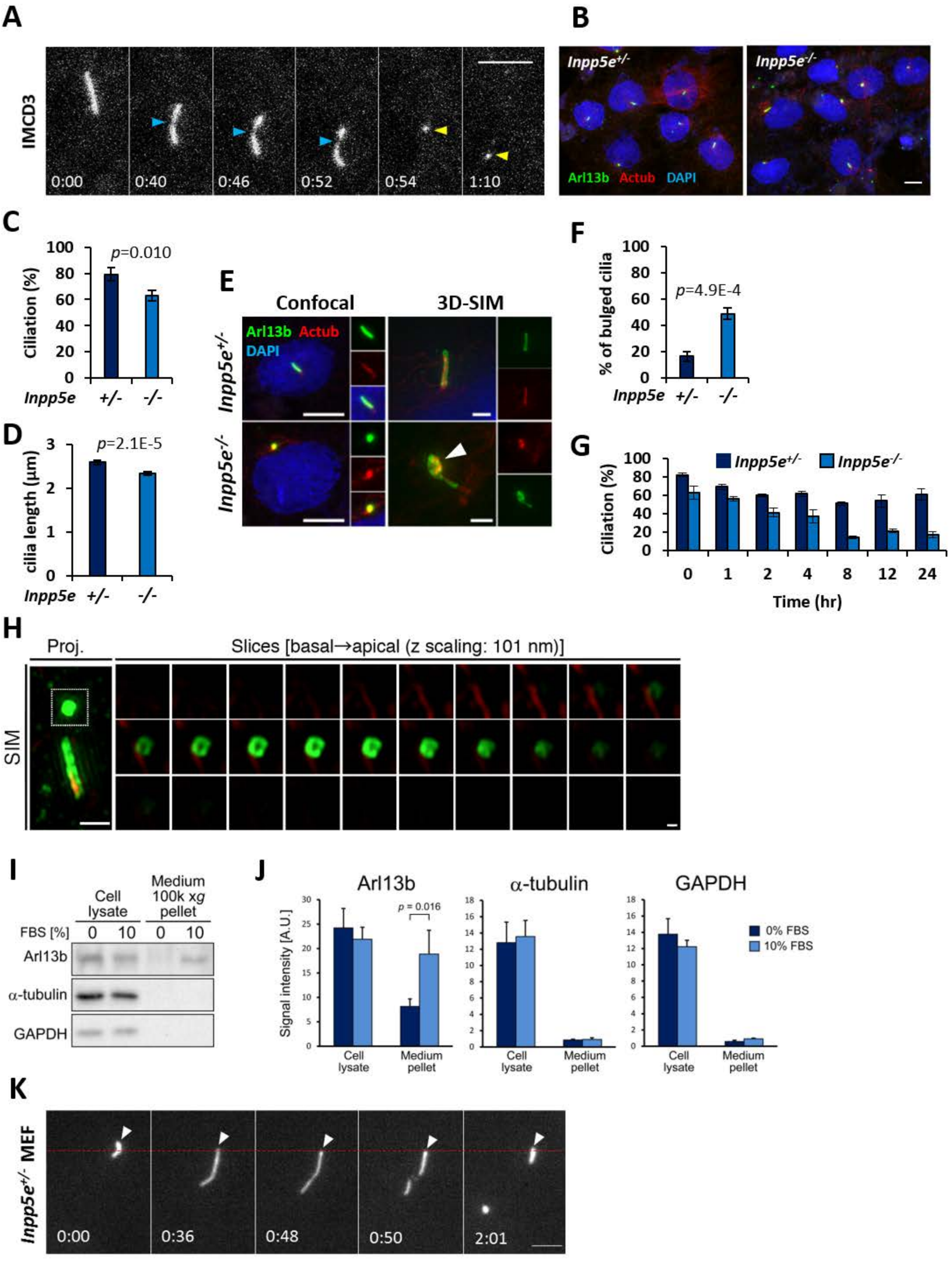
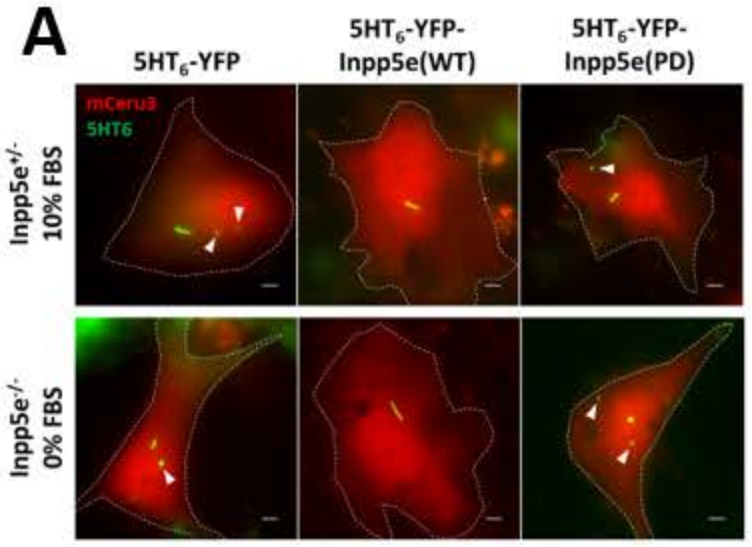
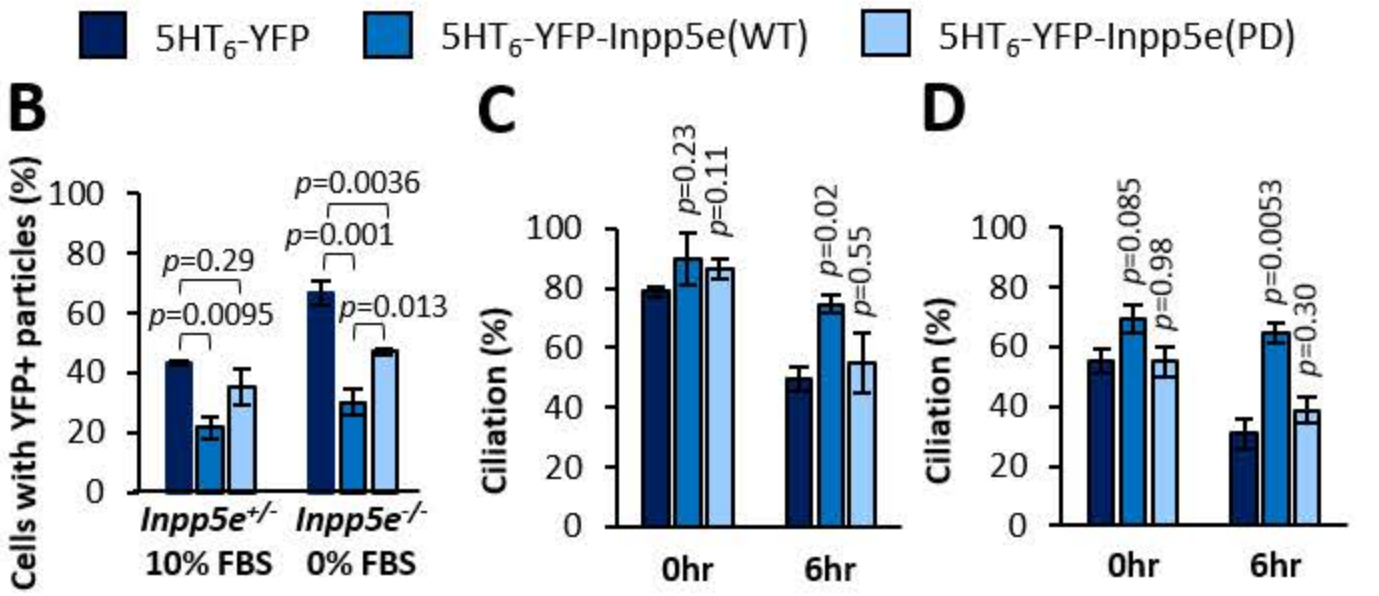
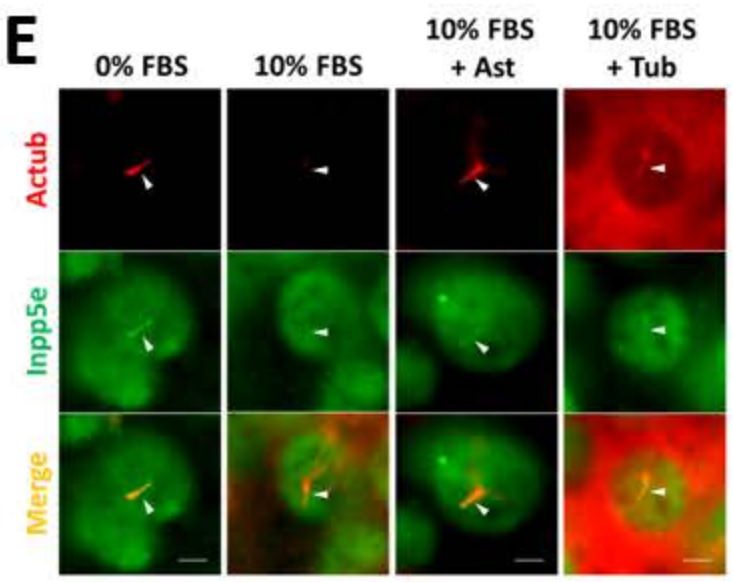
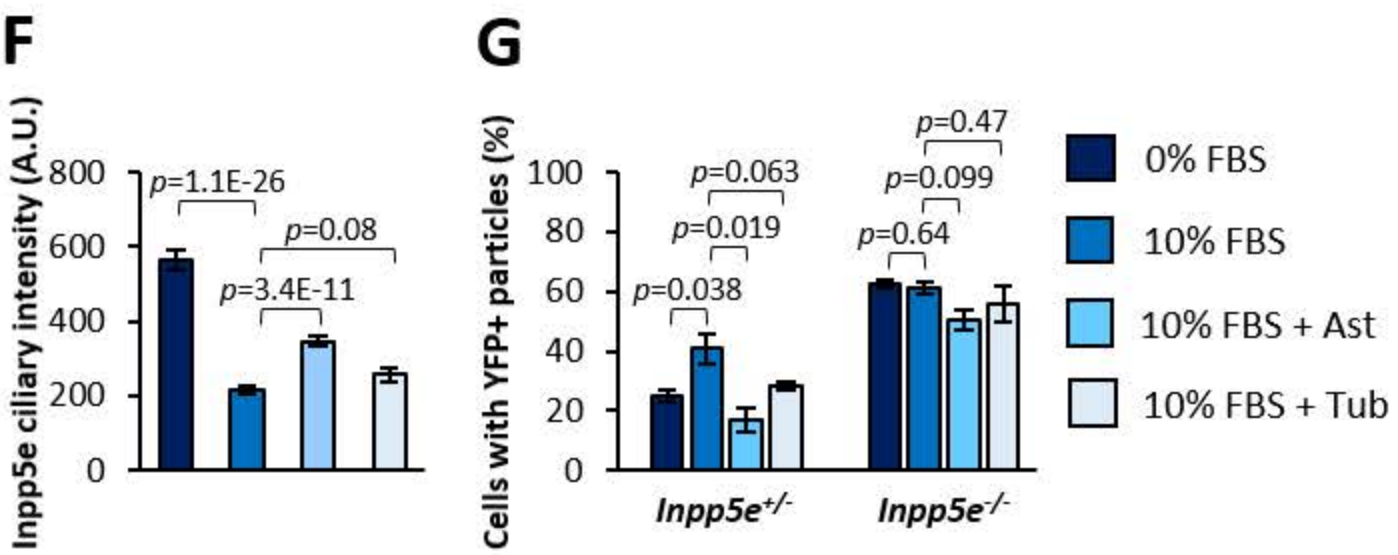

A
IMCD3
0:00
0:40
0:46
0:52
0:54
1:10
B
Inpp5e+/-
Inpp5e-/-
Arl13b Actub DAPI
C
Ciliation (%)
p=0.010
Inpp5e +/- -/-
D
cilia length (μm)
p=2.1E-5
Inpp5e +/- -/-
E
Confocal
3D-SIM
Inpp5e+/-
Inpp5e-/-
Arl13b Actub
DAPI
F
% of bulged cilia
p=4.9E-4
Inpp5e +/- -/-
G
Ciliation (%)
Inpp5e+/-
Inpp5e-/-
Time (hr)
H
Proj.
Slices [basal→apical (z scaling: 101 nm)]
SIM
I
Cell lysate
Medium 100k xg pellet
FBS [%] 0 10 0 10
Arl13b
α-tubulin
GAPDH
J
Arl13b
α-tubulin
GAPDH
Signal intensity [A.U.]
p = 0.016
Cell lysate
Medium pellet
0% FBS
10% FBS
K
Inpp5e+/- MEF
0:00
0:36
0:48
0:50
2:01

A

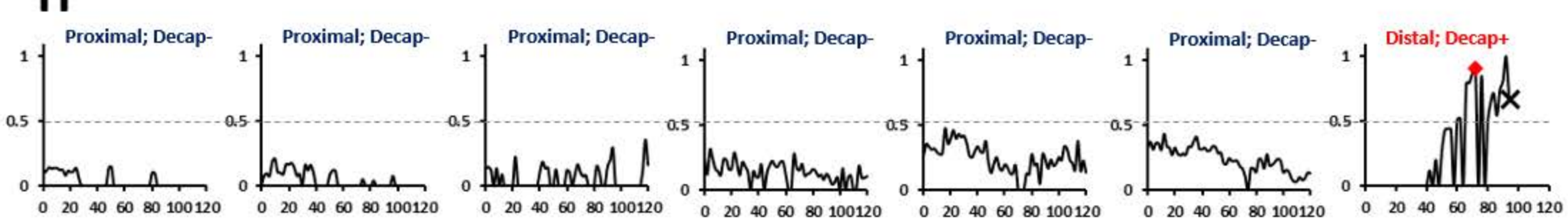

5HT6-YFP
5HT6-YFP-Inpp5e(WT)
5HT6-YFP-Inpp5e(PD)
mCerus3
5HT6
Inpp5e+/- 10% FBS
Inpp5e-/- 0% FBS


5HT6-YFP
5HT6-YFP-Inpp5e(WT)
5HT6-YFP-Inpp5e(PD)
B
Cells with YFP+ particles (%)
p=0.29
p=0.0095
p=0.0036
p=0.001
p=0.013
Inpp5e+/- 10% FBS
Inpp5e-/- 0% FBS
C
Ciliation (%)
p=0.23
p=0.11
p=0.02
p=0.55
0hr
6hr
D
Ciliation (%)
p=0.085
p=0.98
p=0.0053
p=0.30
0hr
6hr

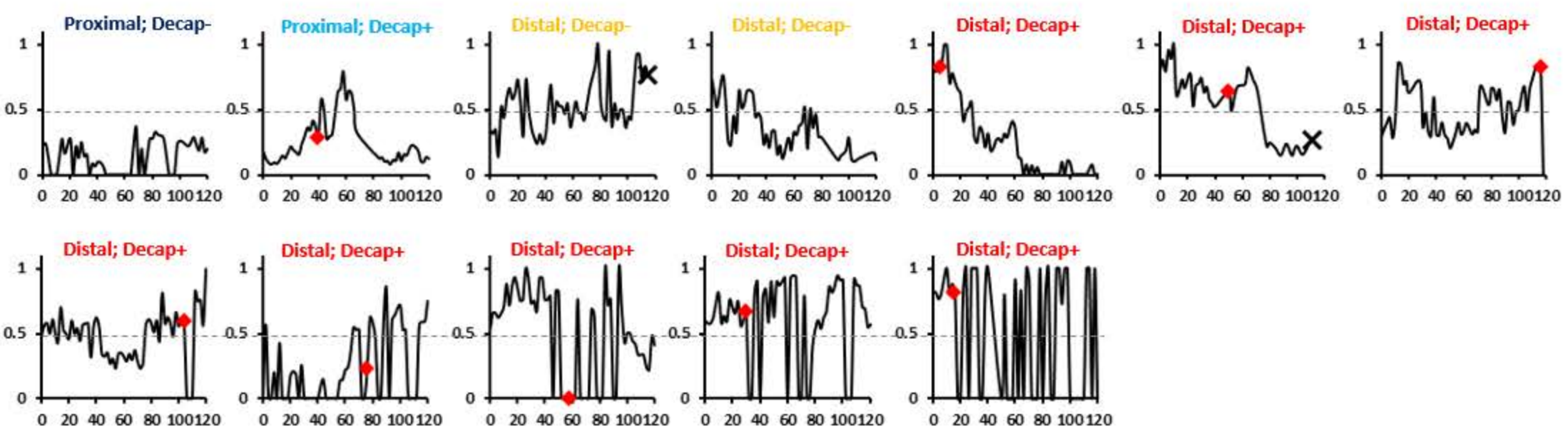

E
0% FBS
10% FBS
10% FBS + Ast
10% FBS + Tub
Actub
Inpp5e
Merge


F
Inpp5e ciliary intensity (A.U.)
p=1.1E-26
p=3.4E-11
p=0.08
G
Cells with YFP+ particles (%)
p=0.038
p=0.019
p=0.063
p=0.64
p=0.099
p=0.47
Inpp5e+/-
Inpp5e-/-
0% FBS
10% FBS
10% FBS + Ast
10% FBS + Tub

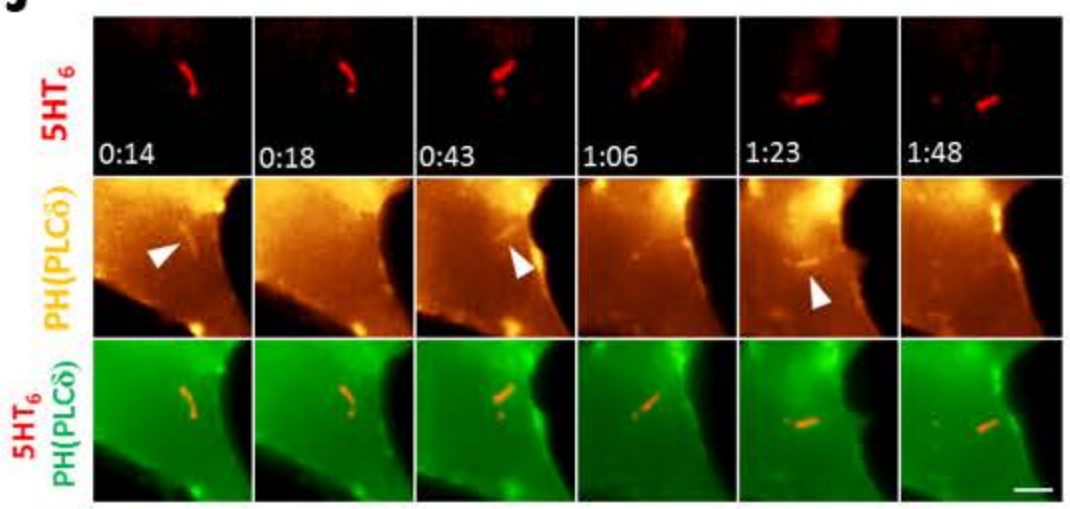
H


Proximal; Decap-
Proximal; Decap-
Proximal; Decap-
Proximal; Decap-
Proximal; Decap-
Proximal; Decap-
Distal; Decap+

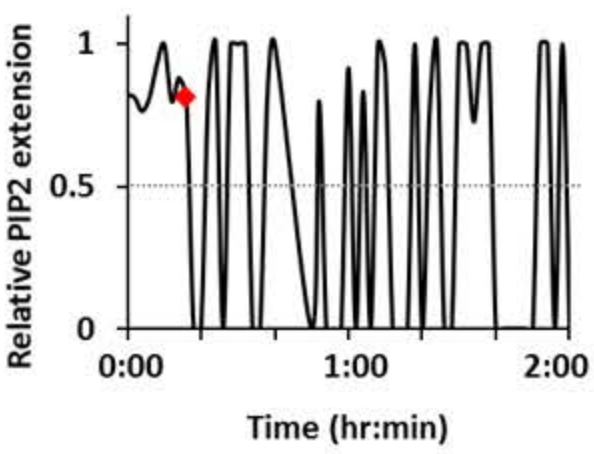
I

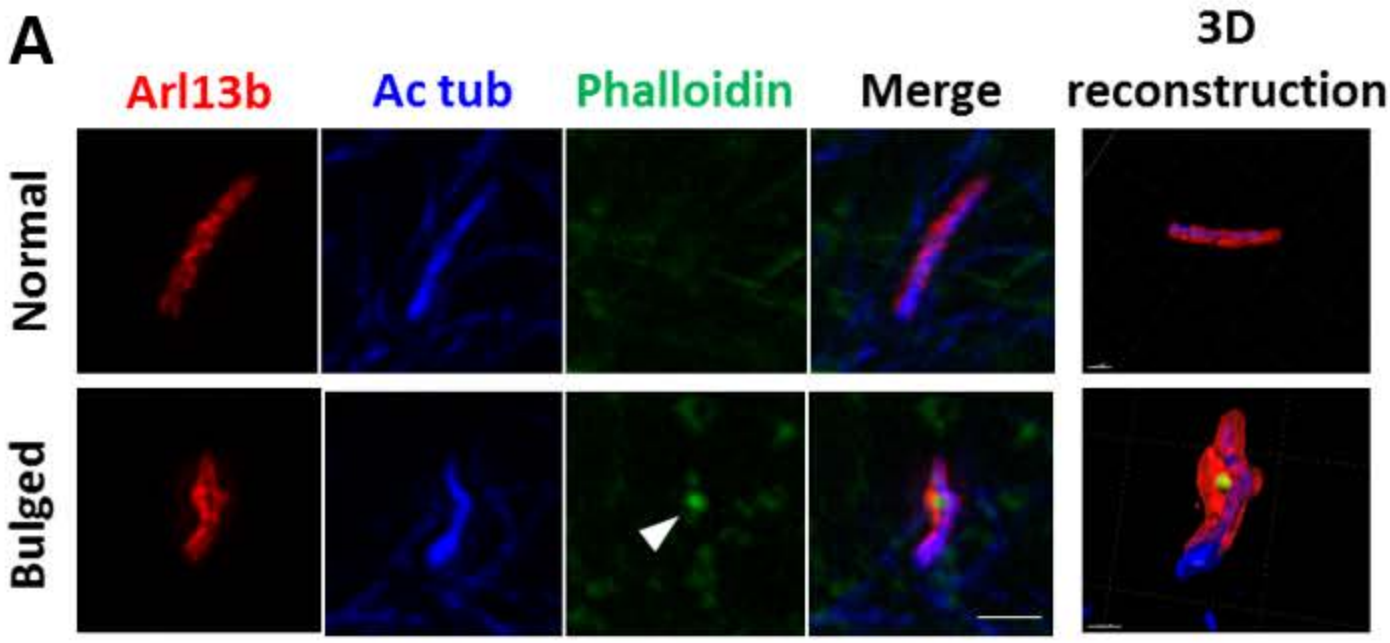

Proximal; Decap-
Proximal; Decap+
Distal; Decap-
Distal; Decap-
Distal; Decap+
Distal; Decap+
Distal; Decap+
Distal; Decap+
Distal; Decap+
Distal; Decap+
Distal; Decap+

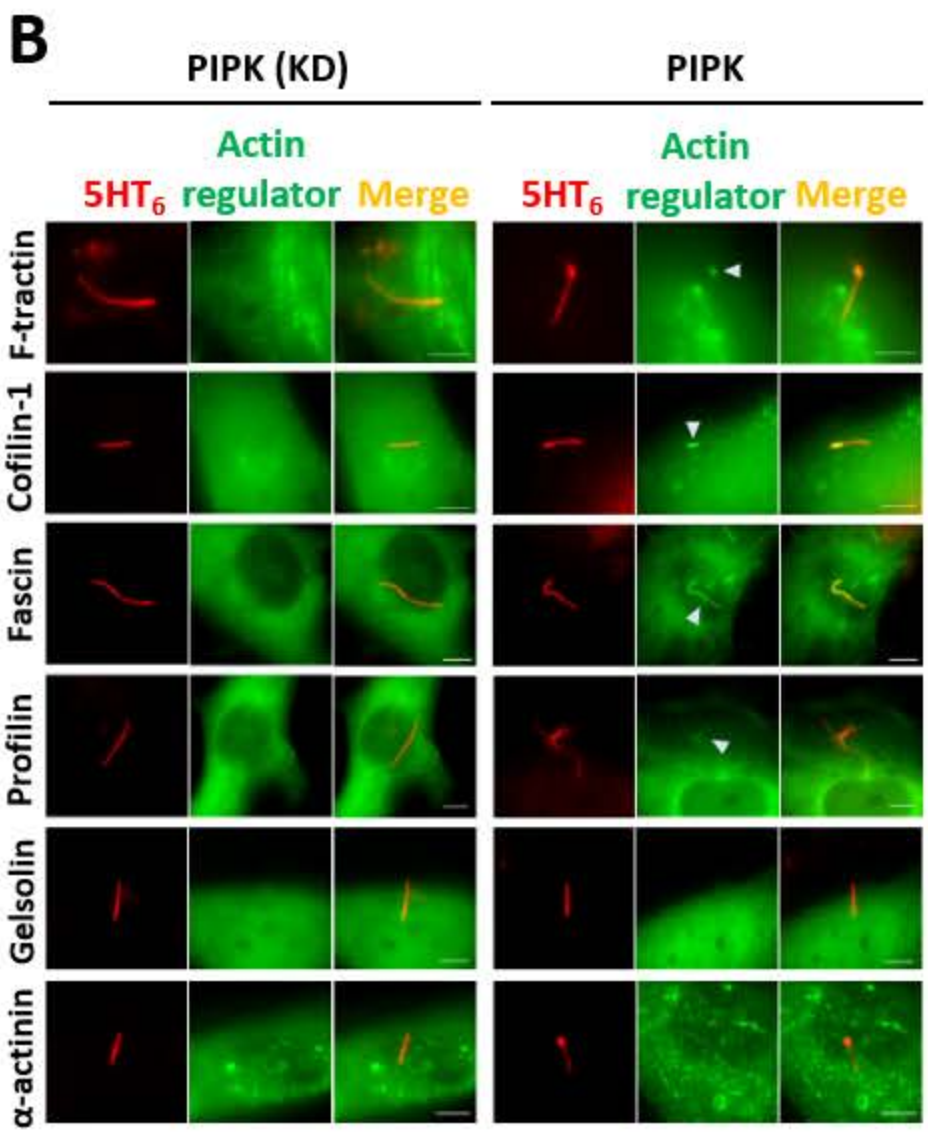
J


5HT6
0:14
0:18
0:43
1:06
1:23
1:48
PH(PLCδ)
5HT6
PH(PLCδ)

K

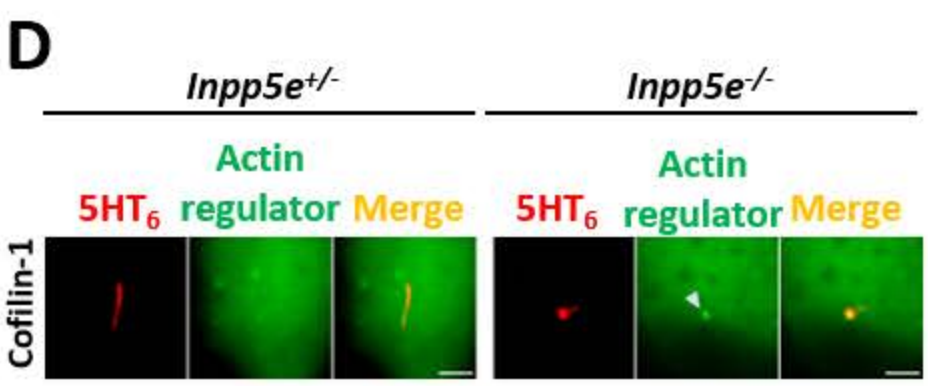

Relative PIP2 extension
Time (hr:min)
0:00
1:00
2:00





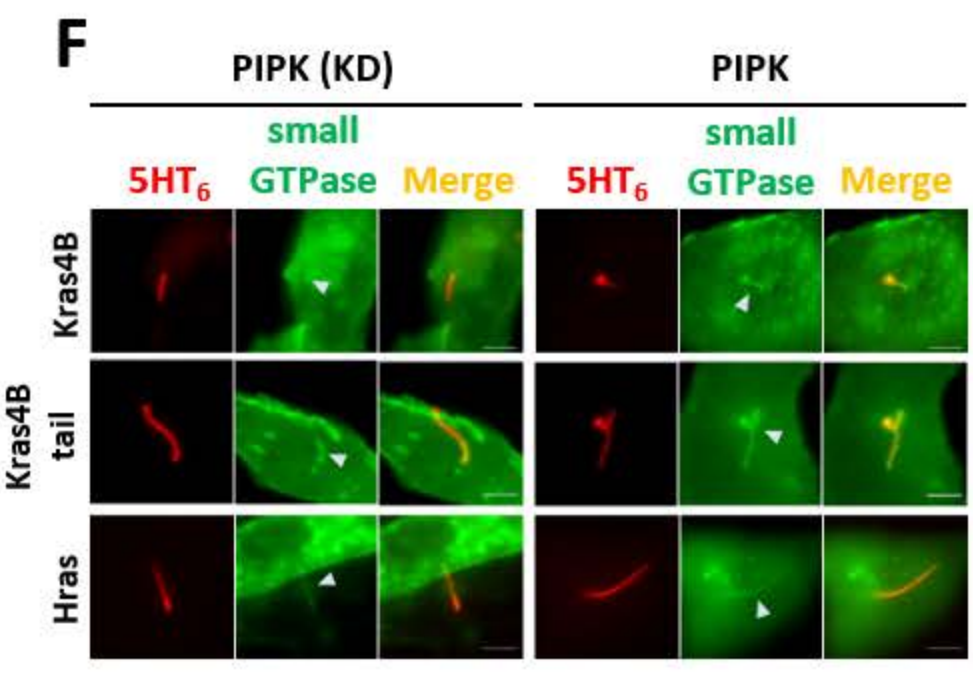
C

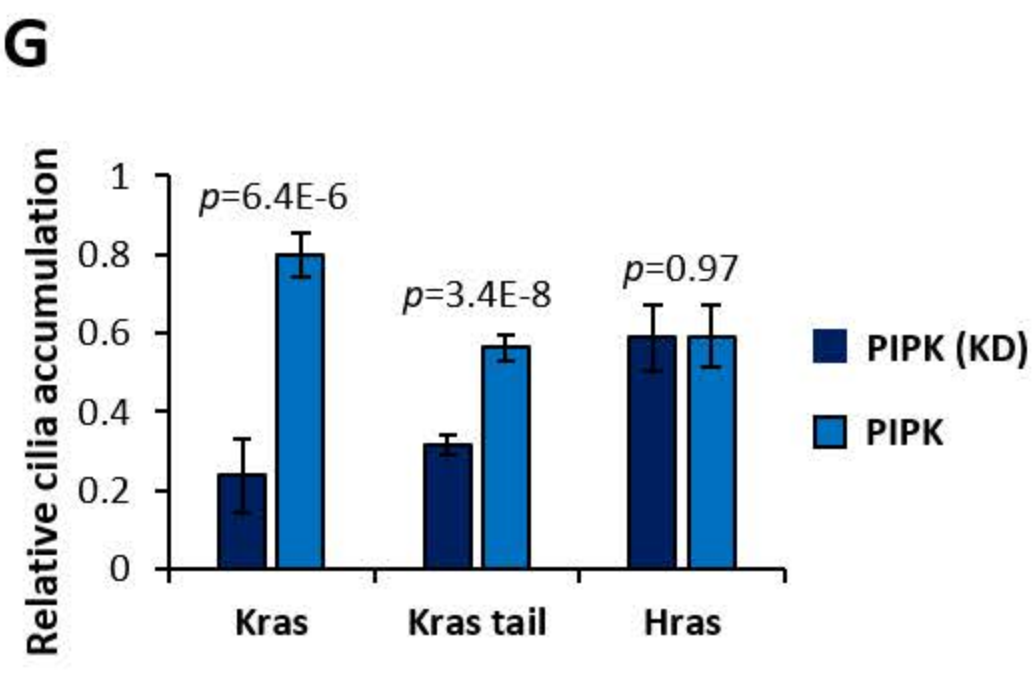
Condition	N	Probability	Relative risk	95% C.I	*p*
PIPK (KD)	38	0	15.44	0.93-257.01	0.056
PIPK	47	0.19			
PIPK (KD)	48	0	33.25	2.01-549.91	0.014
PIPK	27	0.33			
PIPK (KD)	122	0.016	17.82	4.34-73.14	0.0001
PIPK	89	0.29			
PIPK (KD)	38	0.053	2.65	0.57-12.36	0.21
PIPK	43	0.14			
PIPK (KD)	63	0.032	1.086	0.16-7.46	0.93
PIPK	58	0.034			
PIPK (KD)	13	0.077	1.00	0.10-10.038	1.00
PIPK	26	0.077			

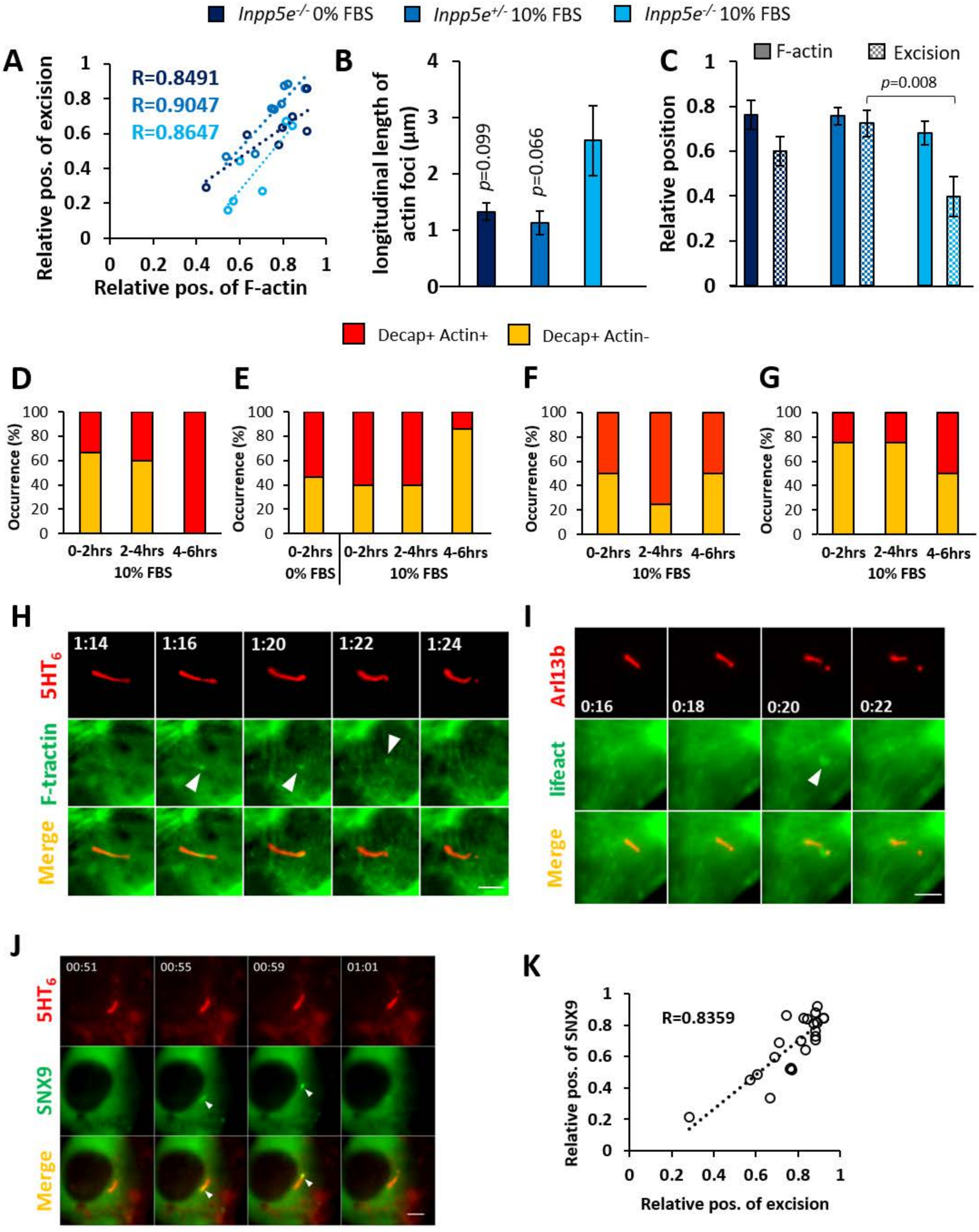


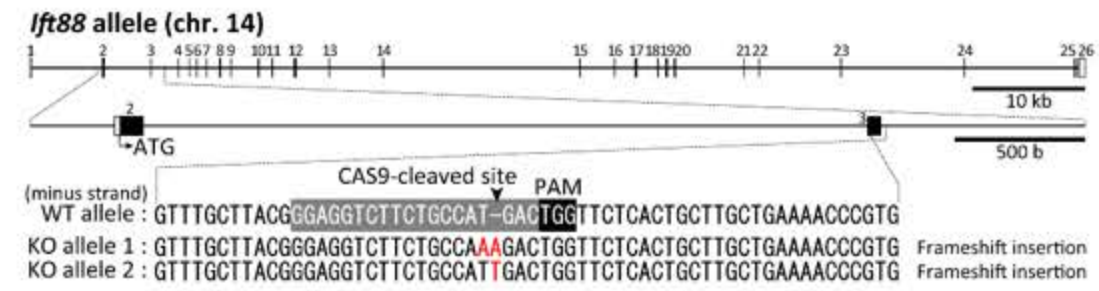
E

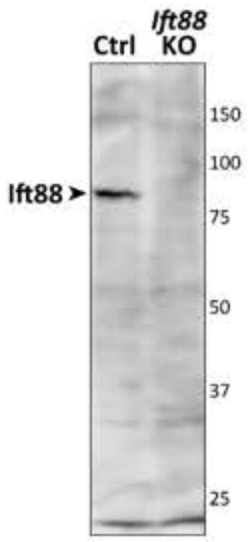
Condition	N	Probability	Relative risk	95% C.I	*p*
Inpp5e$^{+/-}$	26	0	15.30	0.93-252.72	0.057
Inpp5e$^{-/-}$	29	0.28			

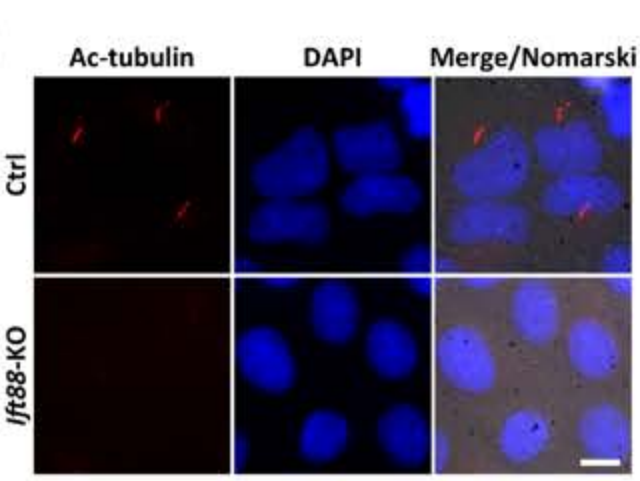


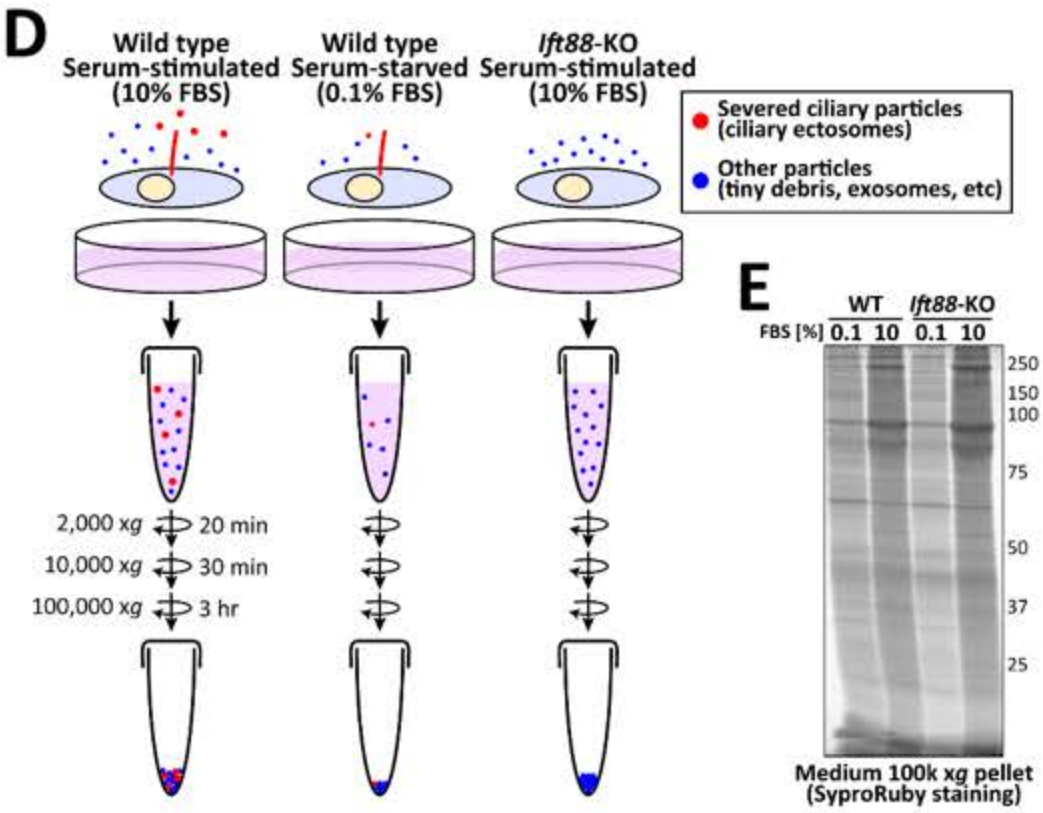



Inpp5e-/- 0% FBS
Inpp5e+/- 10% FBS
Inpp5e-/- 10% FBS
A
Relative pos. of excision
Relative pos. of F-actin
R=0.8491
R=0.9047
R=0.8647
B
longitudinal length of actin foci (μm)
p=0.099
p=0.066
C
F-actin
Excision
p=0.008
Relative position
Decap+ Actin+
Decap+ Actin-
D
E
F
G
Occurrence (%)
0-2hrs 2-4hrs 4-6hrs
10% FBS
0-2hrs
0% FBS
H
5HT6
F-tractin
Merge
1:14
1:16
1:20
1:22
1:24
I
Arl13b
lifeact
Merge
0:16
0:18
0:20
0:22
J
5HT6
SNX9
Merge
00:51
00:55
00:59
01:01
K
R=0.8359
Relative pos. of SNX9
Relative pos. of excision

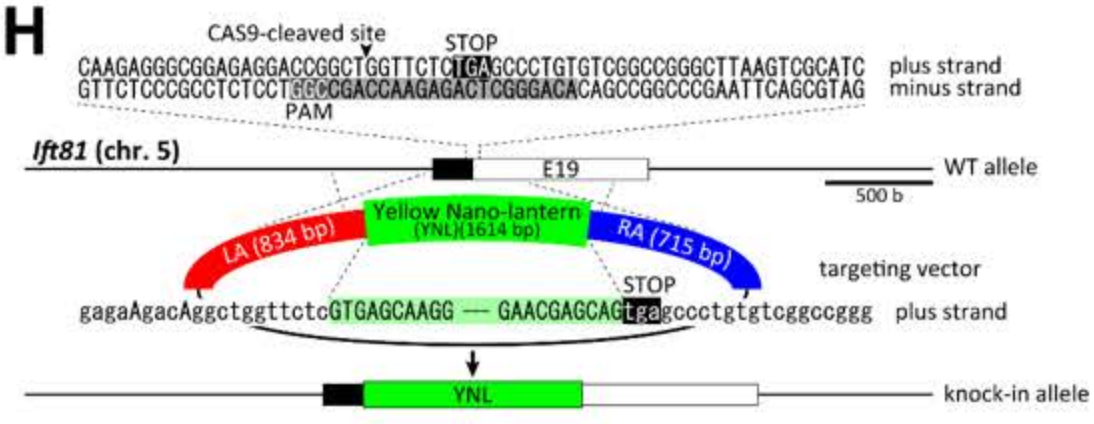

A
Ift88 allele (chr. 14)
10 kb
500 b
ATG
CAS9-cleaved site
PAM
(minus strand)
WT allele : GTTTGCTTACGGGAGGTCTTCTGCCAT-GACTGGTTCTCACTGCTTGCTGAAAACCCGTG
KO allele 1 : GTTTGCTTACGGGAGGTCTTCTGCCAAAGACTGGTTCTCACTGCTTGCTGAAAACCCGTG Frameshift insertion
KO allele 2 : GTTTGCTTACGGGAGGTCTTCTGCCATTGACTGGTTCTCACTGCTTGCTGAAAACCCGTG Frameshift insertion

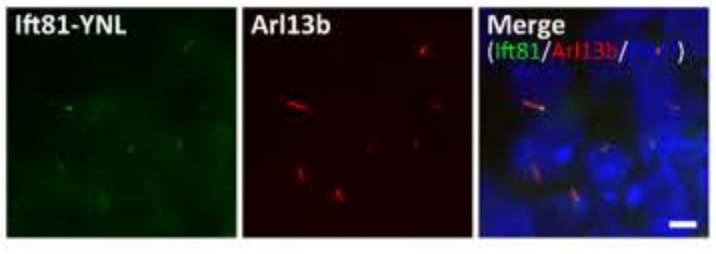

B
Ctrl
Ift88 KO
Ift88
150
100
75
50
37
25

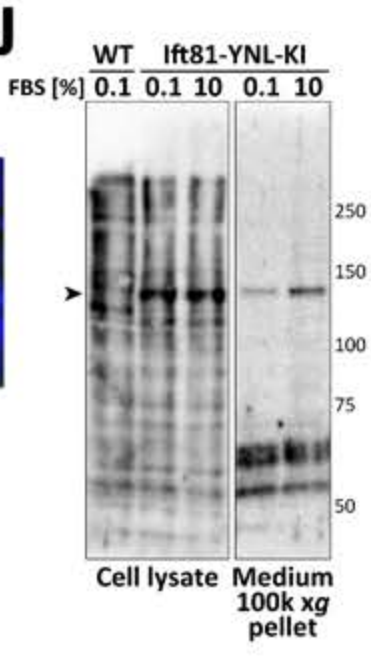

C
Ac-tubulin
DAPI
Merge/Nomarski
Ctrl
Ift88-KO

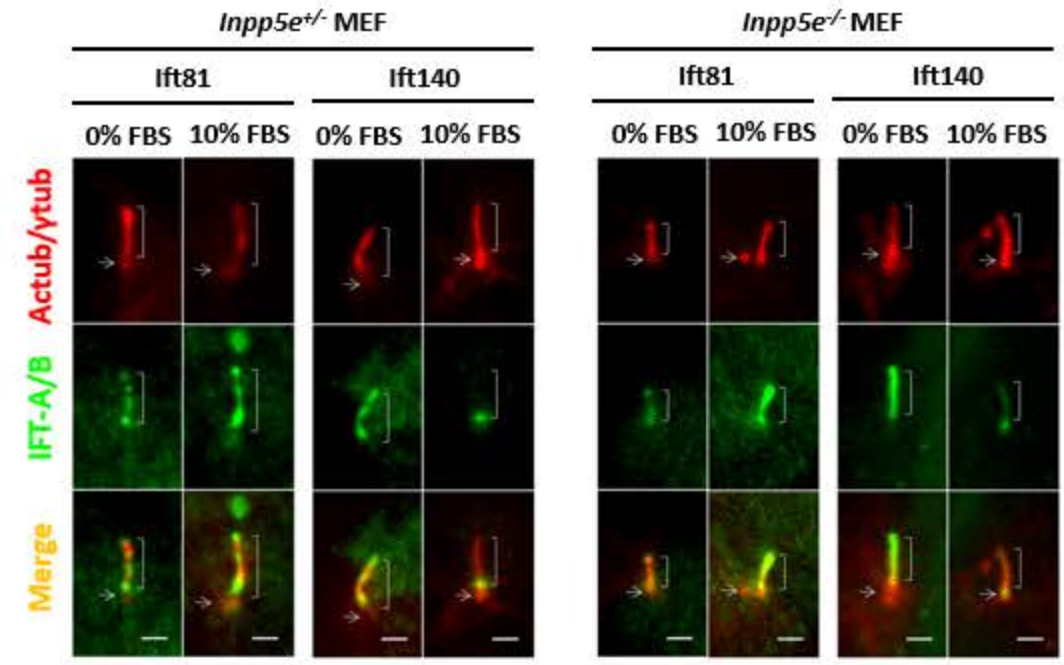

D
Wild type Serum-stimulated (10% FBS)
Wild type Serum-starved (0.1% FBS)
Ift88-KO Serum-stimulated (10% FBS)
Severed ciliary particles (ciliary ectosomes)
Other particles (tiny debris, exosomes, etc)
2,000 xg 20 min
10,000 xg 30 min
100,000 xg 3 hr

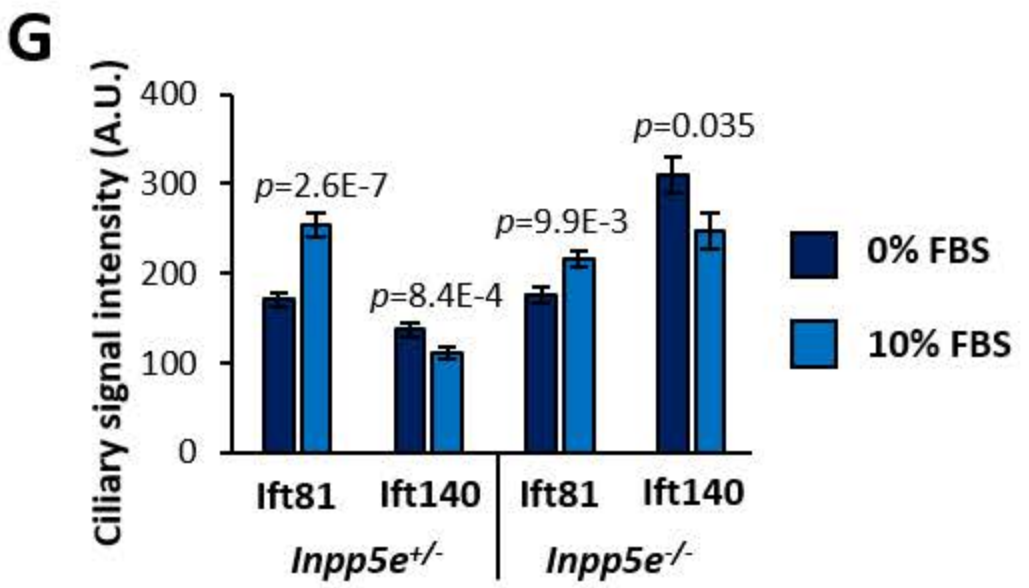
E
WT
Ift88-KO
FBS [%] 0.1 10 0.1 10
250
150
100
75
50
37
25
Medium 100k xg pellet (SyproRuby staining)

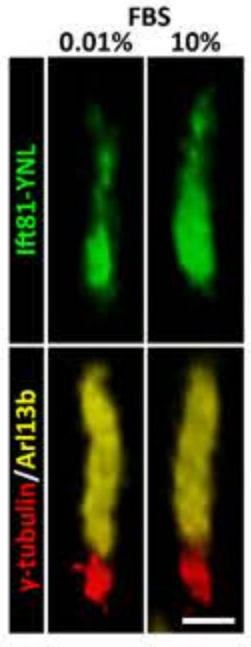

H
CAS9-cleaved site
STOP
CAAGAGGGCGGAGAGGACCGGCTGGTTCTCTGAGCCCTGTGTCGGCCGGGCTTAAGTCGCATC plus strand
GTTCTCCCGCCTCTCCTGGCCGACCAAGAGACTCGGGACACAGCCGGCCCGAATTCAGCGTAG minus strand
PAM
Ift81 (chr. 5)
E19
WT allele
500 b
Yellow Nano-lantern (YNL)(1614 bp)
LA (834 bp)
RA (715 bp)
targeting vector
STOP
gagaAgacAggctggttctcGTGAGCAAGG — GAACGAGCAGTgagccctgtgtcggccggg plus strand
YNL
knock-in allele

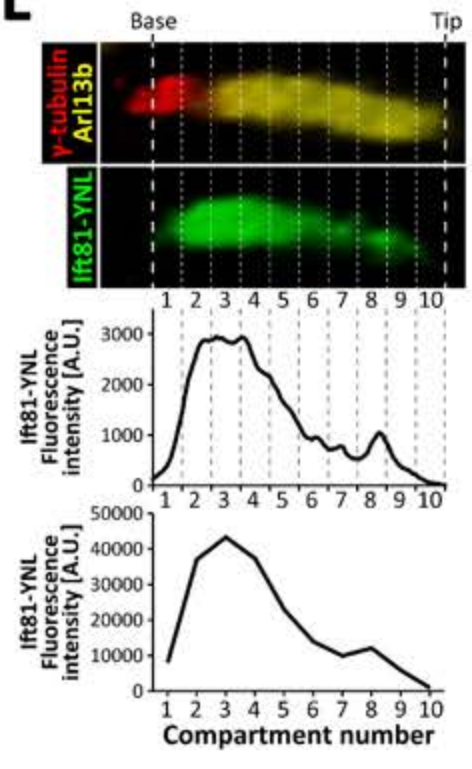

I
Ift81-YNL
Arl13b
Merge
(Ift81/Arl13b/)

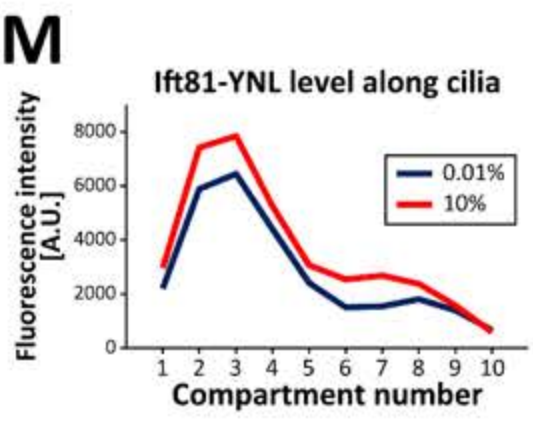

J
WT
Ift81-YNL-KI
FBS [%] 0.1 0.1 10 0.1 10
250
150
100
75
50
Cell lysate
Medium 100k xg pellet

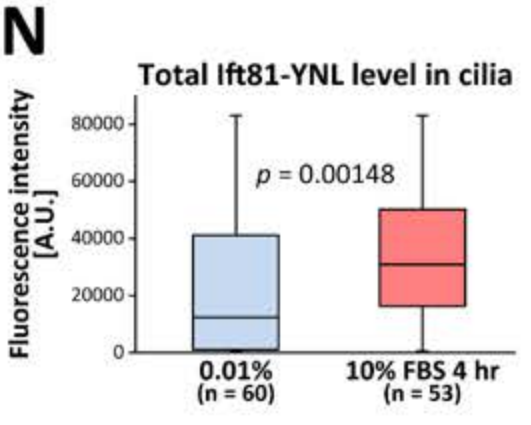

F
Inpp5e+/- MEF
Inpp5e-/- MEF
Ift81
Ift140
0% FBS
10% FBS
Actub/ytub
IFT-A/B
Merge

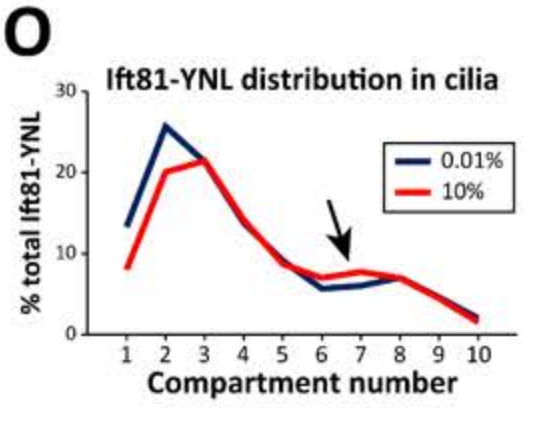

G
Ciliary signal intensity (A.U.)
400
300
200
100
0
p=2.6E-7
p=8.4E-4
p=9.9E-3
p=0.035
Ift81
Ift140
Ift81
Ift140
Inpp5e+/-
Inpp5e-/-
0% FBS
10% FBS


K
FBS
0.01%
10%
Ift81-YNL
γ-tubulin/Arl13b

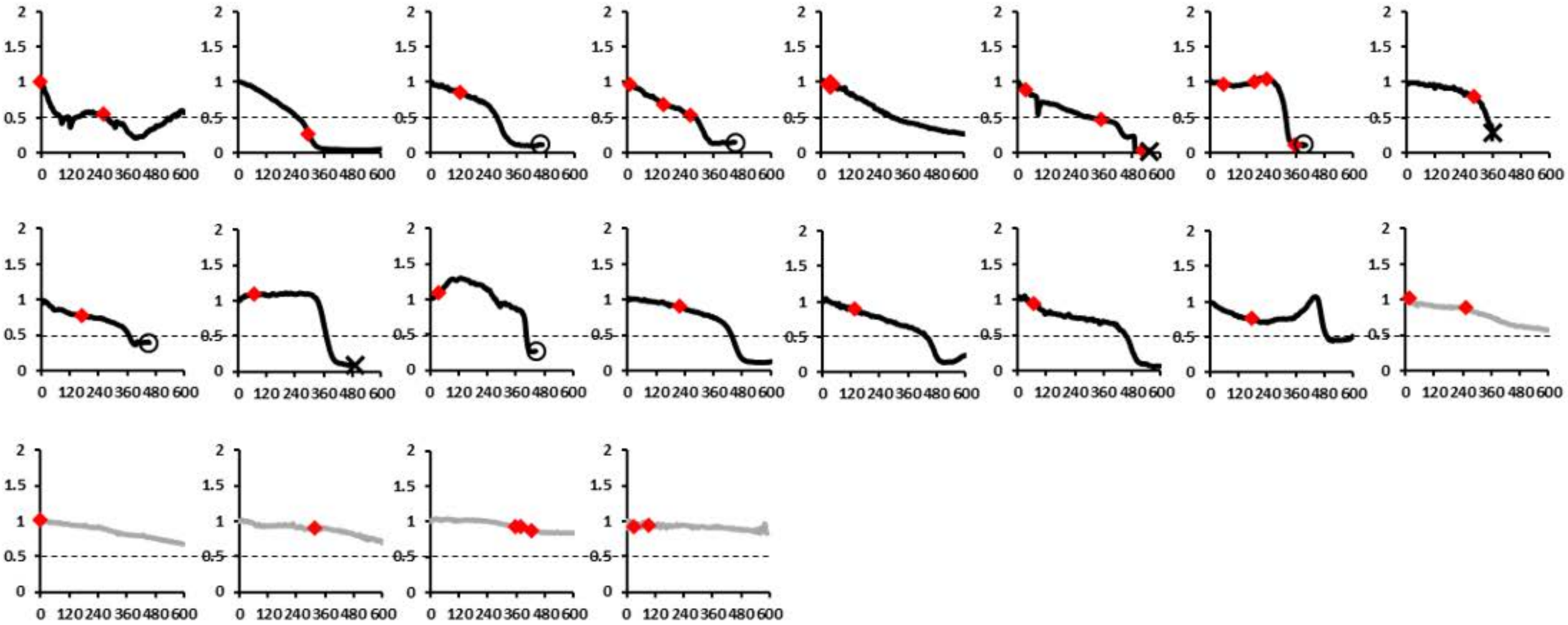

L
Base
Tip
γ-tubulin Arl13b
Ift81-YNL
Ift81-YNL Fluorescence intensity [A.U.]
Compartment number


M
Ift81-YNL level along cilia
Fluorescence intensity [A.U.]
8000
6000
4000
2000
0
0.01%
10%
1 2 3 4 5 6 7 8 9 10
Compartment number

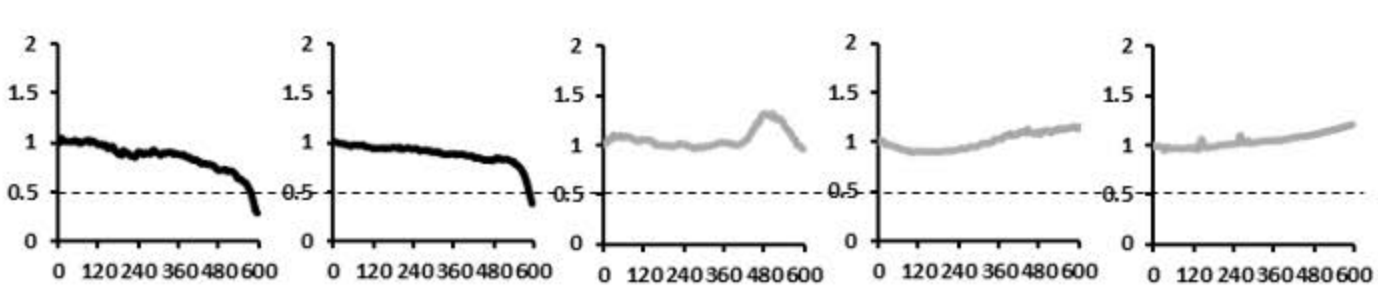

N
Total Ift81-YNL level in cilia
Fluorescence intensity [A.U.]
80000
60000
40000
20000
0
p = 0.00148
0.01% (n = 60)
10% FBS 4 hr (n = 53)


O
Ift81-YNL distribution in cilia
% total Ift81-YNL
30
20
10
0
0.01%
10%
1 2 3 4 5 6 7 8 9 10
Compartment number

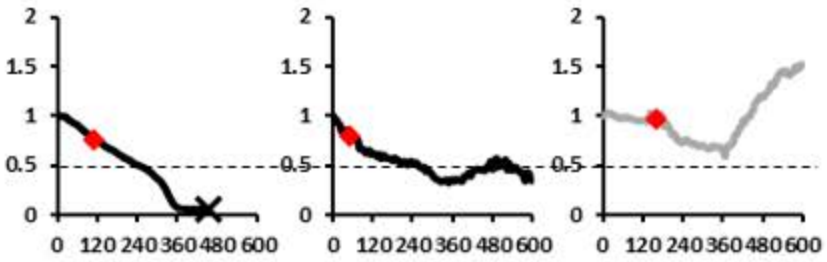
A


2
1.5
1
0.5
0
0 120 240 360 480 600

B


2
1.5
1
0.5
0
0 120 240 360 480 600

C


2
1.5
1
0.5
0
0 120 240 360 480 600

D

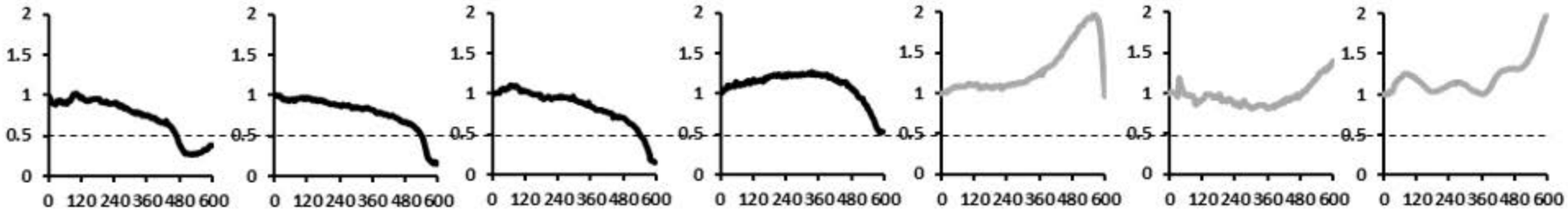

2
1.5
1
0.5
0
0 120 240 360 480 600

Figure S1. Characterization of cilia decapitation and *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEF, Related to Figure 1

(A) Time-lapse images of a mIMCD-3 primary cilium expressing Arl13b-GFP at quiescent state (0% FBS). Blue arrows indicate membrane thinning prior to excision. Yellow arrowheads indicate excised cilium tip.

(B) Representative confocal images of Arl13b and Ac-tub immunofluorescence in *Inpp5e*$^{+/-}$ MEF *and* *Inpp5e*$^{-/-}$ MEF at quiescent state. Nuclei labeled with DAPI.

(C) Quantification of % ciliation in quiescent *Inpp5e*$^{+/-}$ *and Inpp5e*$^{-/-}$ MEF. A primary cilium is defined by positive labeling with Arl13b and Ac tub immunofluorescence. Data are represented as mean ± SEM. A two-tailed Student's T-test was performed with *p* values indicated. (n=323, 374 cells; 3 experiments)

(D) Quantification of Arl13b-labeled cilia lengths in quiescent *Inpp5e*$^{+/-}$ *and Inpp5e*$^{-/-}$ MEF. Data are represented as mean ± SEM. A two-tailed Student's T-test was performed with *p* values indicated. (n=271, 353 cells; 3 experiments)

(E) Confocal and SR-SIM images of Arl13b and Ac-tub immunofluorescence in *Inpp5e*$^{+/-}$ MEF *and* *Inpp5e*$^{-/-}$ MEF. Insets are magnified images of respective cilia. White arrowhead indicates bulged ciliary tip in *Inpp5e*$^{-/-}$ MEF.

(F) Quantification of % cells displaying bulged ciliary tips as in (E). Data are represented as mean ± SEM. A two-tailed Student's T-test was performed with *p* values indicated. (n=323, 374 cells; 3 experiments as in (B))

(G) Quantification of % ciliation in *Inpp5e*$^{+/-}$ *and Inpp5e*$^{-/-}$ MEF at indicated time points post-stimulation with 10% FBS. Data are represented as mean ± SEM. (n=271, 353 cells; 3 experiments)

(H) Z-series of SR-SIM images of an Arl13b+ and Ac tub particle.

(I) Representative Western blots of Arl13b, α-tubulin and GAPDH proteins in collected cell lysates and pelleted conditioned culture media from mIMCD-3 cells treated with 0% or 10% FBS for 12 hours. Related to Figure 1H.

(J) Quantification of respective band signal intensities in (I). Data were shown as mean ± SEM. A two-tailed Student's T-test was performed with *p* values indicated. (n = 4 experiments)

(K) Representative time-lapse images of a ventral-positioned *Inpp5e*$^{+/-}$ MEF primary cilium passively breaking as a result of non-specific adhesion and immobilization to underlying substratum. Cell was expressed with 5HT$_6$-YFP. Arrowheads indicate cilia tip. Red dotted line marks position of immobilization. See also Methods under "Quantitative image analyses".

Time in min:sec in (A), hr:min in (K). Scale bars indicate 5 um in (A) and (K), 10um in (E) left panel, 1um in (E) right panel, 1μm in (H) left panel and 0.2 μm in (H) right panel.

Figure S2. AurA-dependent ciliary Inpp5e depletion re-organizes ciliary PI(4,5)P$_2$, Related to Figure 2.

(A) Live fluorescence images of *Inpp5e*$^{+/-}$ MEF or *Inpp5e*$^{-/-}$ MEF expressing 5HT$_6$-YFP, 5HT$_6$-YFP-Inpp5e(WT) or 5HT$_6$-YFP-Inpp5e(CD) with cytosolic mCeru3 respectively after 4 hours of 10% FBS stimulation or at quiescent state (0% FBS). White dashed lines delineate cells. White arrowheads indicate cell-associated YFP+ particles that were likely vesicles released from primary cilia.

(B) Quantification of % cells with associated extracellular YFP+ particles under indicated conditions, as in (A). Color coding as in panel above. Data are represented as mean ± SEM. Two-tailed Student's T-tests were performed with *p* values indicated. (n= 111, 73, 92, 94, 88, 74 cells for respective data from left to right; 4 experiments)

(C) Quantification of % ciliation in *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-YFP, 5HT$_6$-YFP-Inpp5e(WT) or 5HT$_6$-YFP-Inpp5e(CD) with cytosolic mCeru3 at 0 hour and 6 hours of 10% FBS stimulation. Color coding as in panel above. Data are represented as mean ± SEM. Two-tailed Student's T-tests were performed with respect to each 5HT$_6$-YFP condition, *p* values indicated. (n=88, 54, 49, 66, 35, 44 cells for respective data from left to right; 2 experiments)

(D) Quantification of % ciliation in *Inpp5e*$^{-/-}$ MEF expressing 5HT$_6$-YFP (control), 5HT$_6$-YFP-Inpp5e(WT) or 5HT$_6$-YFP-Inpp5e(CD) with cytosolic mCeru3 at 0 hour and 6 hours of 10% FBS stimulation. Color coding as in panel above. Data are represented as mean ± SEM. Two-tailed Student's T-tests were performed with respect to each 5HT$_6$-YFP condition, *p* values indicated. (n=94, 49, 57, 100, 55, 70 cells for respective data from left to right; 3 experiments)

(E) Ac tub and Inpp5e immunofluorescence of *Inpp5e*$^{+/-}$ MEF treated with 0% FBS, 10% FBS, 10% FBS +200nM Alisertib (Ast), or 10% FBS + 2μM Tubacin (Tub) for 4 hours. Images within Ac tub

and Inpp5e image panels are scaled to same intensity range, while Ac tub signals are adjusted in Merge to visualize axoneme. Arrowheads indicate cilia.

(F) Signal intensity measurements of Inpp5e immunofluorescence in primary cilia (cilia - vicinity background) under indicated conditions, as in (E). Color coding as in panel on the right. Data are represented as mean ± SEM. A two-tailed Student's T-test was performed with *p* values indicated. (n= 190, 169, 173, 106 cells for respective data from left to right; 3 experiments)

(G) Quantification of % cells with associated extracellular YFP+ particles in *Inpp5e*$^{+/-}$ MEF and *Inpp5e*$^{-/-}$ MEF expressing 5HT$_6$-YFP with cytosolic mCeru3 after 4 hours in 0% FBS, 10% FBS, 10% FBS + 200nM Ast, or 10% FBS + 2μM Tub. Color coding as in panel on the right. Data are represented as mean ± SEM. A two-tailed Student's T-test was performed with *p* values indicated. (n= 124, 131, 112, 121, 48, 54, 47, 37 cells for respective data from left to right; 2-3 experiments)

(H-I) Time-lapse measurements of ciliary PH(PLCδ) accumulation in *Inpp5e*$^{+/-}$ MEF at (H) quiescent state (as shown in Figure 2D) or (I) growth-stimulated state (as shown in Figure 2F). Horizontal axes indicate time(min); vertical axes indicate ciliary PH(PLCδ) accumulation expressed as a ratio of cilium length. Color coding as used in Figure 2G. Dotted line divide proximal half (0-0.5) and distal half (0.5-1.0) of cilium. "X" indicates end of imaging period. Data for (I) were between 2hrs and 4hrs, or 4hrs and 6hrs post-stimulation with 10% FBS.

(J) An example of growth-stimulated primary cilia exhibiting PI(4,5)P$_2$ oscillation post-decapitation. White arrowheads indicate ciliary PH(PLCδ).

(K) Time-lapse measurements of PH(PLCδ) accumulation in cilium in (C), expressed as relative ratio of cilium length. Red diamond marks time point of decapitation.

Time in hr:min. Scale bars indicate 5μm.

Figure S3. PI(4,5)P$_2$ regulates ciliary localization of F-actin, actin regulatory proteins and small GTPases, Related to Figure 3.

(A) SR-SIM images of Arl13b, Ac tub and phalloidin immunofluorescence in normal and bulged cilia of *Inpp5e*$^{-/-}$ MEF. Rightmost panel are IMARIS 3D reconstructions of "Merge" images. Arrowhead indicates ciliary phalloidin signals.

(B) Live fluorescence images of NIH/3T3 expressing 5HT$_6$-mCeru3-PIPK or 5HT$_6$-mCeru3-PIPK(KD) and respective YFP-tagged F-actin sensor (F-tractin) or actin regulatory proteins. White arrowheads indicate ciliary localization of respective proteins. Cofilin-1 was generally observed to accumulate at ciliary tip, while fascin was detected along ciliary length.

(C) Relative risk ratio analyses on the effect of differential ciliary PI(4,5)P$_2$ in influencing intraciliary F-actin or actin regulatory protein incidence, for corresponding horizontal image rows in (A).

(D) Live fluorescence images of *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEF expressing 5HT$_6$-mCeru3 and cofilin1-YFP. White arrowhead indicates tip accumulation of cofilin-1.

(E) Relative risk ratio analyses on the effect of differential ciliary $PI(4,5)P_2$ in influencing intraciliary cofilin-1 incidence, as in (C).

(F) Live fluorescence images of NIH/3T3 expressing $5HT_6$-mCeru3-PIPK or $5HT_6$-mCeru3-PIPK(KD) and respective YFP-tagged (top) phosphoinositide-binding Kras small GTPase, (middle) Kras tail anchor harboring phosphoinositide-binding module, or (bottom) non-phosphoinositide-binding Hras small GTPase. White arrowheads indicate ciliary localization. While Kras was restricted to the proximal cilia in $5HT_6$-FP-PIPK(KD)-expressing cells, $5HT_6$-FP-PIPK expression was sufficient to accumulate Kras into distal cilia. In contrast, Hras accumulated to distal cilia regardless of ciliary $PI(4,5)P_2$.

(G) Quantification of small GTPase or tail anchor accumulation in cilia, expressed as a relative ratio of cilium length. Data are represented as mean ± SEM. Two-tailed Student's T-tests were performed with respect to each PIPK (KD) condition, *p* values indicated. (n=12, 20, 71, 84, 25, 24 cells; 2-3 experiments)

Scale bars indicate 1μm in (A) and 5μm in (B), (D) and (G).

Figure S4. F-actin and SNX9 localize in primary cilia prior to decapitation, Related to Figure 3.

(A) Correlation plot of intraciliary F-actin position with excision position, each expressed as relative ratio of cilia length. Color coding as in panel above. Position of F-actin was measured at estimated centroid position of F-actin foci prior to excision. In each case, linear regression is drawn in dashed line, with Pearson correlation coefficient R value indicated. (n=7, 8, 6 cells from left to right; 2-3 experiments from (D) and (E))

(B) Quantification of actin foci longitudinal length just prior to excision. Color coding as in panel above. Data shown as mean ± SEM. Student's T-tests were performed with respect to *Inpp5e*$^{-/-}$ 10% FBS condition, *p* values indicated. (n= 7, 8, 6 cells from left to right; 2-3 experiments from (D) and (E))

(C) Quantification of relative position of cilia excision and F-actin. Color coding as in panel above. Data shown as mean ± SEM. Student's T-test was performed with *p* value indicated. (n= 7, 8, 6 cells from left to right; 2-3 experiments from (D) and (E))

(D) Percentage of cilia decapitation events with or without co-observed intraciliary F-actin in *Inpp5e*$^{+/-}$ MEF expressing $5HT_6$-YFP and mCeru3-Lifeact between 0-2, 2-4 and 4-6 hours of 10% FBS stimulation, related to Figure 3C. Of note, the transient nature of intraciliary F-actin could render inefficient capturing by the two-minute experimental imaging intervals, and a high F-actin marker intensity in the cell body could also limit visualization of specific signals in primary cilia; also applies to (E-G). Color coding as in panel above. (n=6 ,5, 4 cells; 2-3 experiments (D) and (E))

(E) Percentage of cilia decapitation events with or without co-observed intraciliary F-actin in *Inpp5e*$^{-/-}$ MEF $5HT_6$-YFP and mCeru3-Lifeact between 0 and 2 hours in 0% FBS, or between 0-2, 2-4 and

4-6 hours of 10% FBS stimulation, related to Figure 3D. Color coding as in panel above. (n=13, 5, 5, 7 cells; 2-3 experiments)

(F) Percentage of cilia decapitation events with or without co-observed intraciliary F-actin in *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-mCeru3 and alternative F-actin sensor, F-tractin-Citrine, at respective time periods of 10% FBS stimulation, see (H) below. Color coding as in panel above. (n=2, 4, 2 cells; 2 experiments)

(G) Percentage of cilia decapitation events with or without co-observed intraciliary F-actin in *Inpp5e*$^{+/-}$ MEF expressing Lifeact-mCeru3 and alternative ciliary membrane marker, Arl13b-YFP, at respective time periods of 10% FBS stimulation, see (I) below. Color coding as in panel above. (n=4, 4, 4 cells; 2 experiments)

(H) Time-lapse images of *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-mCeru3 and F-tractin-citrine between 0hrs and 2hrs post-stimulation with 10% FBS, as in (F). White arrowheads indicate F-actin assembly at site of cilia excision.

(I) Time-lapse images of *Inpp5e*$^{+/-}$ MEF expressing Arl13b-YFP and Lifeact-mCeru3 between 4hrs and 6hrs post-stimulation with 10% FBS, as in (G). White arrowheads indicate F-actin assembly at site of cilia excision.

(J) Time-lapse images of *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-mCeru3 and YFP-SNX9 between 4hrs and 6hrs post-stimulation with 10% FBS. Arrowheads indicate SNX9 propagation from proximal cilia to distal site of cilia excision.

(K) Correlation plot of SNX9 intraciliary position with position of excision, each expressed as relative ratio of cilia length. Position of SNX9 was measured at estimated centroid position of SNX9 foci just prior to excision. Linear regression is drawn in dashed line with Pearson correlation coefficient R value indicated. (n=23 cells; 2 experiments)

Time in hr:min. Scale bars indicate 5μm.

Figure S5. Experimental scheme for ciliary vesicle proteomic profiling and analyses of ciliary IFT distribution, Related to Figure 4.

(A) Generation of *Ift88*KO mIMCD-3 cell line using CRISPR/CAS9-based genome editing technique. The target sequence of guide-RNA (gRNA) used was placed on the minus strand of the genome corresponding to the exon 3 of *Ift88*. Generated *Ift88*KO mIMCD-3 cell line had bi-allelic frameshift insertion at the CAS9-cleaved site.

(B) Loss of Ift88 expression was verified using Western blot analysis with an anti-Ift88 antibody.

(C) Ac tub immunofluorescence revealed that *Ift88*KO mIMCD-3 cells lack primary cilia.

(D) Schematic illustrating the collection and processing of conditioned culture media under indicated conditions. Culture media were first pelleted at low centrifugal force to remove large cell debris, followed by higher centrifugal forces to concentrate smaller ciliary vesicles and exosomes.

(E) Representative SDS-PAGE of 100k xg pelleted conditioned media under conditions illustrated in (D), as visualized by SyproRuby staining.

(F) Actub/γtub and Ift81/Ift140 immunofluorescence of *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEF treated with 0% FBS or 10% FBS for 4 hours. Images within Ac tub/γtub and Ift81/Ift140 image panels in each column group are scaled to same intensity range. Brackets indicate axoneme, while arrows mark centrioles.

(G) Signal intensity measurements of Ift81 or Ift140 immunofluorescence in primary cilia (cilia - vicinity background) under indicated conditions, as in (F). Data are represented as mean ± SEM. Two-tailed Student's T-tests were performed with respect to each 0% FBS condition, *p* values indicated. (n= 114, 118, 90, 88, 14, 113, 87, 79 cells for respective data from left to right; 3 experiments)

(H) Schematic illustration of yellow Nano-lantern (YNL) knock-in into 3'-end of *Ift81* to generate Ift81:YNL knock-in mIMCD-3 cell line.

(I) Ift81-YNL co-localizes with Arl13b immunofluorescence in Ift81:YNL knock-in mIMCD-3 cell line.

(J) Western blot detection of YNL-tagged endogenous Ift81 (arrowhead) using anti-GFP polyclonal antibody in total cell lysates or particle-enriched 100k-g culture media pellets of WT or Ift81:YNL knock-in mIMCD-3 after a 24-hour stimulation with either 0.1% FBS or 10% FBS.

(K) Fluorescence images of Ift81-YNL with Arl13b and γtub immunofluorescence upon 4-hour 0.01% FBS or 10% FBS stimulation of Ift81:YNL knock-in mIMCD-3.

(L) Schematic illustration of cilia length compartmentalization used for ciliary Ift81 analyses in (M)-(O). For each primary cilium, YNL fluorescence intensities along cilia length was first obtained using a line scan spanning from base to tip of cilium (middle), and total fluorescence intensity within each compartment was derived via signal integration (bottom).

(M) Quantification of mean Ift81-YNL fluorescence intensities along cilia length of primary cilia subjected to 4-hour stimulation with 0.01% FBS or 10% FBS. (n = 60 for 0.01% FBS; n = 53 for 10% FBS)

(N) Quantification of total Ift81-YNL fluorescence signals of primary cilia subjected to 4-hour stimulation of either 0.01% FBS or 10% FBS. Total Ift81-YNL level in each cilium was calculated via integration of fluorescence signals across all compartments described in (L). Data are shown as median ± quartile (box) and 90 percentile (bars). Mann-Whitney U-test was performed with *p* values indicated. (n = 60 for 0.01% FBS; n = 53 for 10% FBS)

(O) Quantification of mean Ift81-YNL fluorescence signal distribution along cilia length, expressed as a percentage of total Ift81-YNL fluorescence signals as in (N).

Scale bars indicate 10μm in (C), 2 μm in (F), 5μm in (I) and 1 μm in (K).

Figure S6. Time-lapse measurements of nuclear Venus-p27K^{-}/mCherry-hCdt1(30/120) signal intensity ratios under growth stimulation, Related to Figure 6.

Time-lapse measurements of nuclear Venus-p27K$^-$/mCherry-hCdt1(30/120) signal intensity ratios in:

(A) Decap+ *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-mCeru3;

(B) Decap- *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-mCeru3;

(C) Decap+ *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-mCeru3-Tβ4(WT);

(A) Decap- *Inpp5e*$^{+/-}$ MEF expressing 5HT$_6$-mCeru3-Tβ4(WT).

Horizontal axes indicate time (min); vertical axes indicate basal normalized ratios of Venus-p27K$^-$/mCherry-hCdt1. Black dashed lines indicate arbitrary G_0-G_1 transit mid-point. Red diamonds mark time points of cilia decapitation events. Open circles indicate beginning of mCherry-hCdt1(30/120) signal decrease (signifying start of S-phase transit). Crosses indicate end of imaging period. Responses plotted in grey were cells which maintained ratio values >0.5 throughout the 10-hour imaging period (these cells did not demonstrate a sharp decrease in Venus-p27K$^-$ i.e. not determined for quiescence exit), and were excluded from time measurements in Figure 6E.