Supplementary Materials
Molecular Biology of the Cell
Palicharla *et al*.

Supplemental Materials

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Supplemental Information includes key resource table, 7 figures and legends.

KEY RESOURCE TABLE

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies and related		
Mouse monoclonal anti-Acetylated tubulin	Sigma	Cat# T6793 RRID: AB_477585
Rabbit polyclonal anti-ARL13B (IF)	Tamara Caspary (Emory University)	
Mouse monoclonal anti-ARL13B (IF, WB)	NeuroMab Facility	Cat# N295B/66 RRID: AB_2750771
Chicken anti-GFP (IF)	Abcam	Cat# ab13970 RRID: AB_300798
Rabbit polyclonal anti-SMO	Kathryn Anderson (MSKCC)	(Ocbina and Anderson, 2008)
Rabbit polyclonal anti-TULP3	Peter Jackson (Stanford University)	(Mukhopadhyay et al. 2010)
Rabbit polyclonal anti-GPR161	Custom-made	(Pal et al. 2016)
Mouse monoclonal anti-S tag	EMD Millipore	Cat# MAC112
Goat polyclonal anti-Myc tag	Abcam	Cat# ab9132 RRID: AB 307033
Rat monoclonal anti-HA	Roche	Cat# 11867423001 RRID: AB 390918
Goat polyclonal anti-DDDDK (Flag) tag	Abcam	Cat# ab1257 RRID: AB 299216
Mouse monoclonal anti-MBP	NEB	Cat# E8032S
Mouse monoclonal anti-GST	Sigma	Cat# G1160 RRID: AB 259845
Rabbit polyclonal anti-ADCY3	LSBio	Cat# LS-C204505
Rabbit polyclonal anti-AQP2	Sigma	Cat# A7310 RRID: AB 476762
Mouse monoclonal anti-AQP2	Santa Cruz	Cat# sc-515770 RRID: AB 2810957
Rabbit polyclonal anti-INPP5E	Proteintech	Cat# 17797-1-AP RRID: AB 2167120
Rabbit polyclonal anti-NPHP3	Proteintech	Cat# 22026-1-AP RRID: AB 2878975
Rabbit Monoclonal anti-LKB1	CST	Cat# 13031T
hFAB Rhodamine anti-Tubulin	Bio-Rad	Cat# 12004165 RRID: AB 2884950
IRDye 680 Streptavidin	LI-COR	Cat# 926-68079
Alexa Fluor 488-, 594-, 647- conjugated secondary antibodies	Life Technologies	
IRDye tagged secondary antibodies	LI-COR	
Hoechst 33342	Life Technologies	H3570
BG-Block	NEB	S9106
TMR Star	NEB	S9105
Chemicals, Media, and Kits		
Penicillin/Streptomycin	Sigma	P4333
Glutamine	Sigma	G7513

Polyfect	Qiagen	301105
PEI-Max	Polysciences	24765-2
DMEM-High Glucose	Sigma	D5796
DMEM F12	Sigma	D6421
Bovine calf serum	Sigma	12133C
Fetal bovine serum	Sigma	F0926
Cosmic serum		
Paraformaldehyde	Electron microscopy solutions	15710
Normal donkey serum	Jackson Immuno Research, West Grove, PA	Cat# 017-000-121 RRID: AB_2337258
Fluoromount-G	Southern Biotech	0100-01
Permount	ThermoFisher Scientific	SP15-100
Biotin	Sigma	Cat# B4639
GenElute mammalian total RNA purification kit	Sigma	RTN350
DNase I	Sigma	D5307
SYBR Green Quantitative RT-qPCR Kit	Sigma	QR0100
Kicqstart One-Step Probe RT-qPCR ReadyMix	Sigma	KCQS07
TNT Sp6 high-yield wheat germ protein expression system	Promega	L3261
Synthetic mounting media	Fisher Chemical	SP15
Mini-PROTEAN TGX Precast Protein Gels	Bio-Rad	
Experimental Models: Organisms		
Mouse: *Tulp3$^{f/f}$*	MRC, Harwell	(Hwang et al, 2019)
Mouse: *HoxB7-Cre*	O'Brien Kidney Research Core of UT Southwestern	
Mouse: *Ksp-Cre*	O'Brien Kidney Research Core of UT Southwestern	
Mouse: *Nestin-Cre*	JAX	Stock No. 003771
Experimental Models: Cell Lines		
IMCD3 Flp-In	Peter Jackson (Stanford University)	(Torres et al, 2009)
NIH 3T3 Flp-In	Thermo-Fisher	R76107
3T3-L1	Peter Michaely (UT Southwestern)	
TRex-293 FLP-In	Life Technologies	R78007
Phoenix A	National Gene Vector Biorepository, Indiana University	

Experimental Models: Primary cells		
Tulp3 knockout MEFs	This study	
Experimental Models: Immortalized MEFs		
Arl13b^{hnn} MEFs and wild type controls	Tamara Caspary (Emory University)	(Larkins et al, 2011)
Arl13b^{V358A} MEFs and wild type controls	Tamara Caspary (Emory University)	(Gigante et al, 2020)
Recombinant DNA		
pGLAP5	Addgene	19706
pG-LAP1	Addgene	19702
pENTR223-ARL13B	DNAsu	HsCD00511796
pENTR221-INPP5E	Life Technologies	IOH40212
NPHP3 (1-203 aa)	GeneArt	Synthesized
Gateway PLUS shuttle clone for CYS1	GeneCopoeia	GC-Y0203-CF
Gatewaytized LAP1 or LAP5 in pBABE	This study	
pQXIN-Myc-TULP3	This study	
pQXIN-HA-INPP5E	This study	
pcDNA5-BirA-FLAG N-DEST	Anne-Claude Gingras	
pcDNA5-BirA-FLAG C-DEST	Anne-Claude Gingras	
pCS2-Myc-DEST		
pLenti CRISPR Puromycin		(Shalem *et al.*, 2014)
GFP-SNAP-ARL13B in pBABE	This study	
Software and Algorithms		
ImageJ software	National Institutes of Health, Bethesda, MD	
GraphPad Prism	GraphPad, La Jolla, CA	
Bio-Rad Image lab software	Bio-Rad	
Other		
Thermo-Fisher Excelsior Automated Tissue Processor	ThermoFisher Scientific	A82300001
Paraplast Plus paraffin bath	Leica	39602004
Thermo-Shandon Histocenter 2 Embedding Workstation	ThermoFisher Scientific	6400012D
Leica stereomicroscope (M165 C) with digital camera (DFC500)	Leica	
Zeiss stereomicroscope (Discovery.V12)	Zeiss	
Zeiss LSM780 confocal microscope	Zeiss	
Nikon CSU-W1 SoRa	Nikon	
Leica DM2000 photomicroscope	Leica	
Sakura DRS-601 x-y-z robotic-stainer	Sakura-FineTek, Torrance, CA	DRS-601
CFX96 thermocycler	Bio-Rad	
Superfrost® Plus slides	Fisher Scientific	12-550-15

Figure S1 (Related to Figure 1). TULP3 determines ciliary trafficking of ARL13B.

(A) MEFs from wild type or *Tulp3* knockout (ko) mice were serum starved upon confluence for 24 h before fixation. Fixed cells were immunostained for GPR161 (green) along with acetylated tubulin (AcTUB, red) and counterstained for DNA (blue). Quantification shown in Fig. 1B.

(B-C) Wildtype and *Tulp3* ko NIH 3T3 cells were grown to confluency and starved further for 48 h to promote ciliation before fixing. The fixed cells were immunostained for Arl13b (green) along with acetylated tubulin (red) and counterstained for DNA. Total counted cells are >200 for each condition (D). Data represent mean ± SD.

(D-E) Wild type or *Tulp3* knock out 3T3-L1 cells were grown to confluency and further cultured for 72 hours to promote ciliation. The cells were treated with 500 nM Smo agonist (SAG) for 24 hours to induce Smo ciliary localization before fixing. Immunostaining was performed for Smo, Acetylated tubulin and DNA. Arrows indicate cilia positive for Smo while arrow heads indicate Smo negative cilia (E). Smo positive cilia were counted from two experiments (F), and n>100 cilia were counted for each condition. Data represent mean ± SD.

Scale, 5 μm. ****, $p<0.0001$; ns, not significant. Arrows indicate cilia positive for the indicated proteins (Gpr161 (A) or Arl13b (C) or Smo (E)), while arrow heads indicate negative cilia.

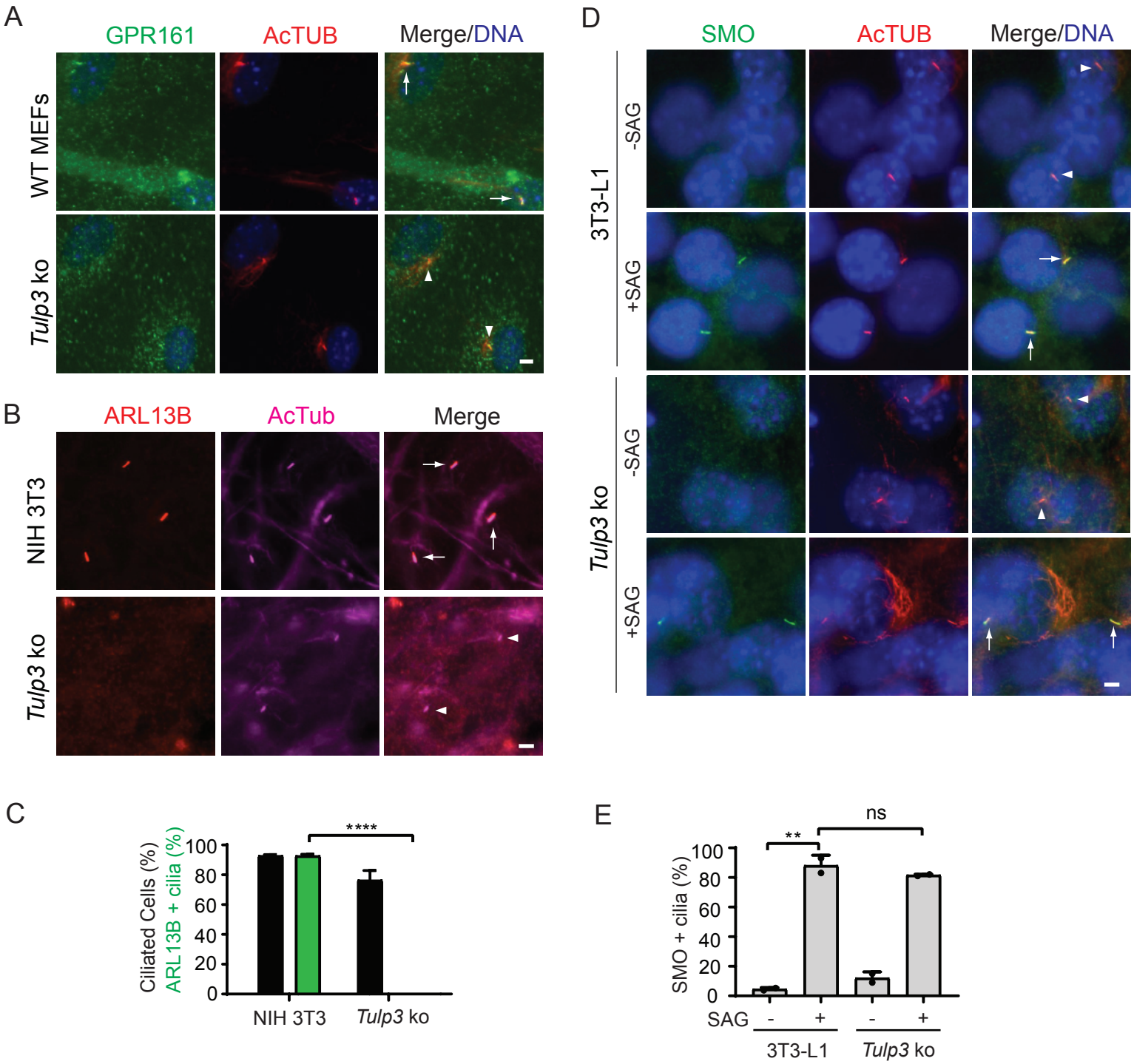

A
GPR161
AcTUB
Merge/DNA
WT MEFs
Tulp3 ko
B
ARL13B
AcTub
Merge
NIH 3T3
Tulp3 ko
D
SMO
AcTUB
Merge/DNA
3T3-L1
-SAG
+SAG
Tulp3 ko
-SAG
+SAG
C
Ciliated Cells (%)
ARL13B + cilia (%)
100
80
60
40
20
0
****
NIH 3T3
Tulp3 ko
E
SMO + cilia (%)
100
80
60
40
20
0
**
ns
SAG
-
+
-
+
3T3-L1
Tulp3 ko

Figure S2 (Related to Figure 1 and 2). Sequencing data from *Tulp3* ko cell lines.

(A) Alleles in the *Tulp3* ko CRISPR lines are shown. CRISPR/Cas9 ko lines were generated using guide RNA targeting sequences targeting exon 3 in mouse *Tulp3* using a pLenti-CRISPR Puromycin construct (Shalem *et al.*, 2014). Initial sequencing in controls and ko lines were performed on PCRs using primers flanking Exon 3 of *Tulp3*. We next performed TOPO cloning to determine the sequences of the allelic disruptions. Two IMCD3 *Tulp3* ko lines (#89 and #83) are shown. Rescue of cargo trafficking in line #89 is shown in Fig 2 and 4, and that in line #83 is shown in Figure S4D.

(B) Immunoblotting of *Tulp3* ko cell lines and MEFs show lack of TULP3 without affecting ARL13B. Immunoblotting in IMCD3 *Tulp3* ko line #89 is shown in Figure 2.

Palicharla_Figure S2

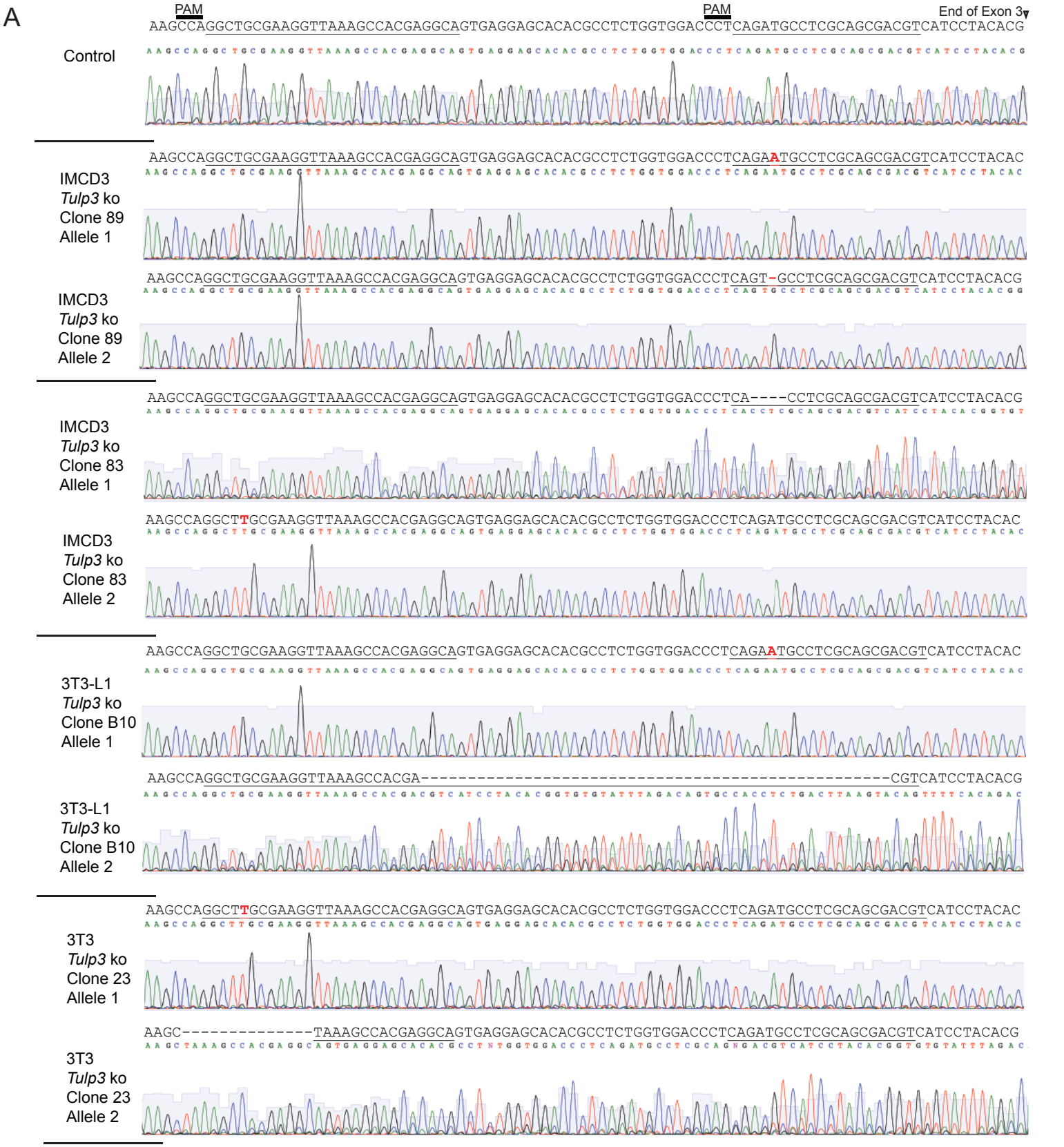


Figure S3 (Related to Figure 1). Ciliary localization of ARL13B was unaffected in *Nestin-Cre*; *Tulp3*$^{f/f}$ brain regions.

(A) Brain sections from CA1 regions in hippocampus from either control or *Nestin-Cre*; *Tulp3*$^{f/f}$ mice at P7 were immunostained for ARL13B and AC3 and counterstained for DNA. Quantification shown in **Figure 1H**.

(B) Cilia (AC3) positive cells were counted from CA1 regions in hippocampus and caudate-putamen (CPu) regions of brains from either control or *Nestin-Cre; Tulp3*$^{f/f}$ mice. Total counted cells were >200 from multiple fields from one mouse for control and two mice for *Nestin-Cre; Tulp3*$^{f/f}$. Data represent mean ± SD.

(C) Representative images for Figure 1J.

Scale, 5 μm. ns, not significant.

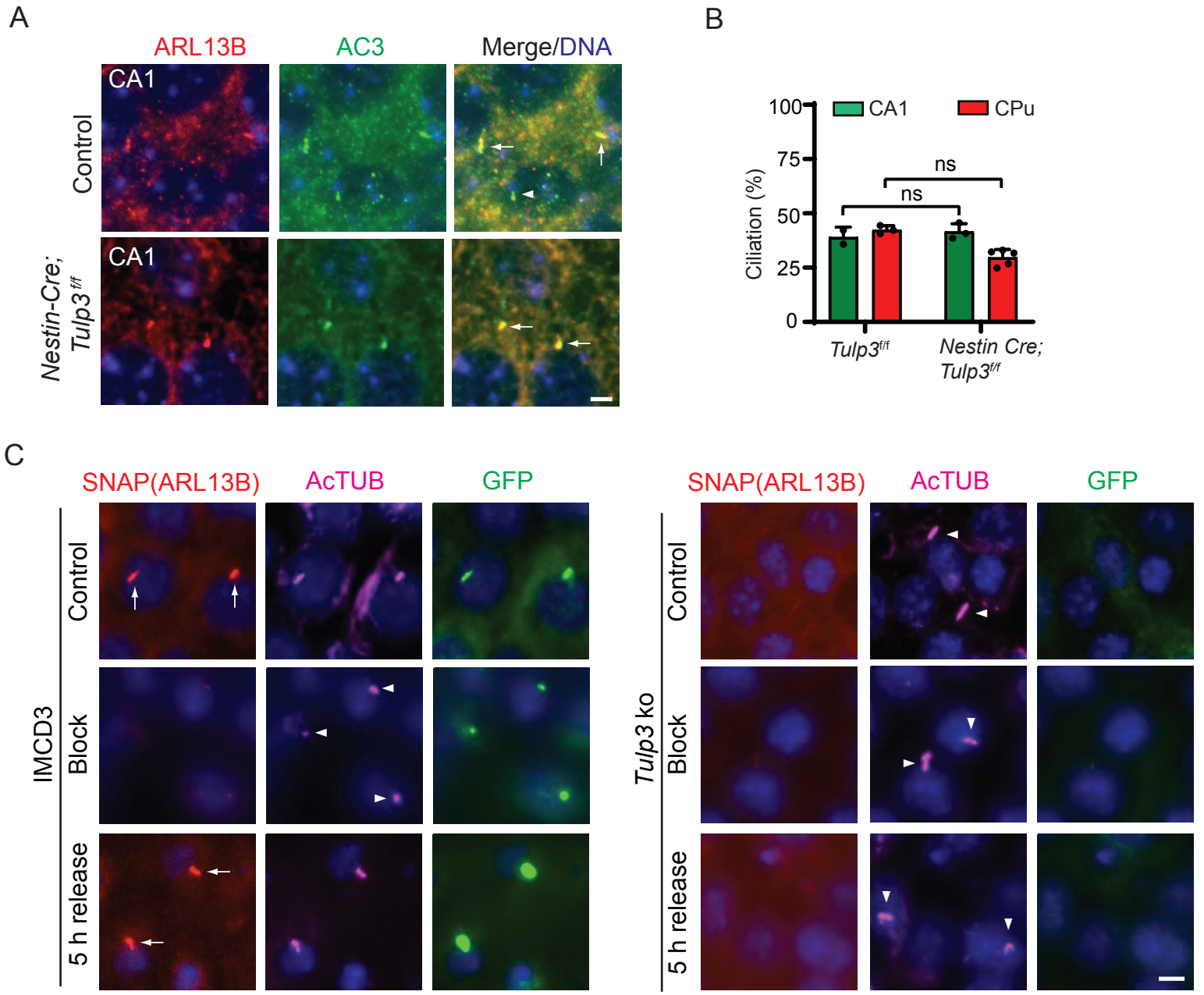

A
ARL13B
AC3
Merge/DNA
CA1
Control
CA1
Nestin-Cre; Tulp3 f/f
B
100
75
50
25
0
Ciliation (%)
CA1
CPu
ns
ns
Tulp3f/f
Nestin Cre; Tulp3f/f
C
SNAP(ARL13B)
AcTUB
GFP
IMCD3
Control
Block
5 h release
Tulp3 ko
Control
Block
5 h release

Figure S4 (Related to Figure 2, 4, and 5). Tagged ARL13B is functional.

(A) N term- or C term-GFP-Stag (LAP) tagged ARL13B stably expressed in immortalized *Arl13b*^{hnn} MEFs along with ^{HA}INPP5E were starved for 48 h and fixed. Fixed cells were immunostained for GFP (ARL13B), HA (INPP5E), acetylated tubulin (AcTUB) and counterstained for DNA. Both N- and C-tagged ARL13B fusions trafficked to cilia in *Arl13b*^{hnn} background. ^{HA}INPP5E levels in cilia were also restored by either fusion, suggesting rescue from their stable expression.

(B) Cells from (A) were counted from 3 experiments, and total counted cells are >100 for each condition.

(C) Cilia (n>30) were counted for cilia lengths. Data represent mean ± SD.

(D) Rescue of ARL13B and GPR161 trafficking to cilia in IMCD3 *Tulp3* ko line #83 upon expressing ^{LAP}TULP3 WT. Cells from were counted from 2 experiments, and total counted cells are >200 for each condition. Rescue in IMCD3 *Tulp3* ko line #89 upon expressing ^{LAP}TULP3 WT is shown in Figure 4B.

Scale, 5 μm. ****, p<0.0001; ***, p<0.001; ns, not significant.

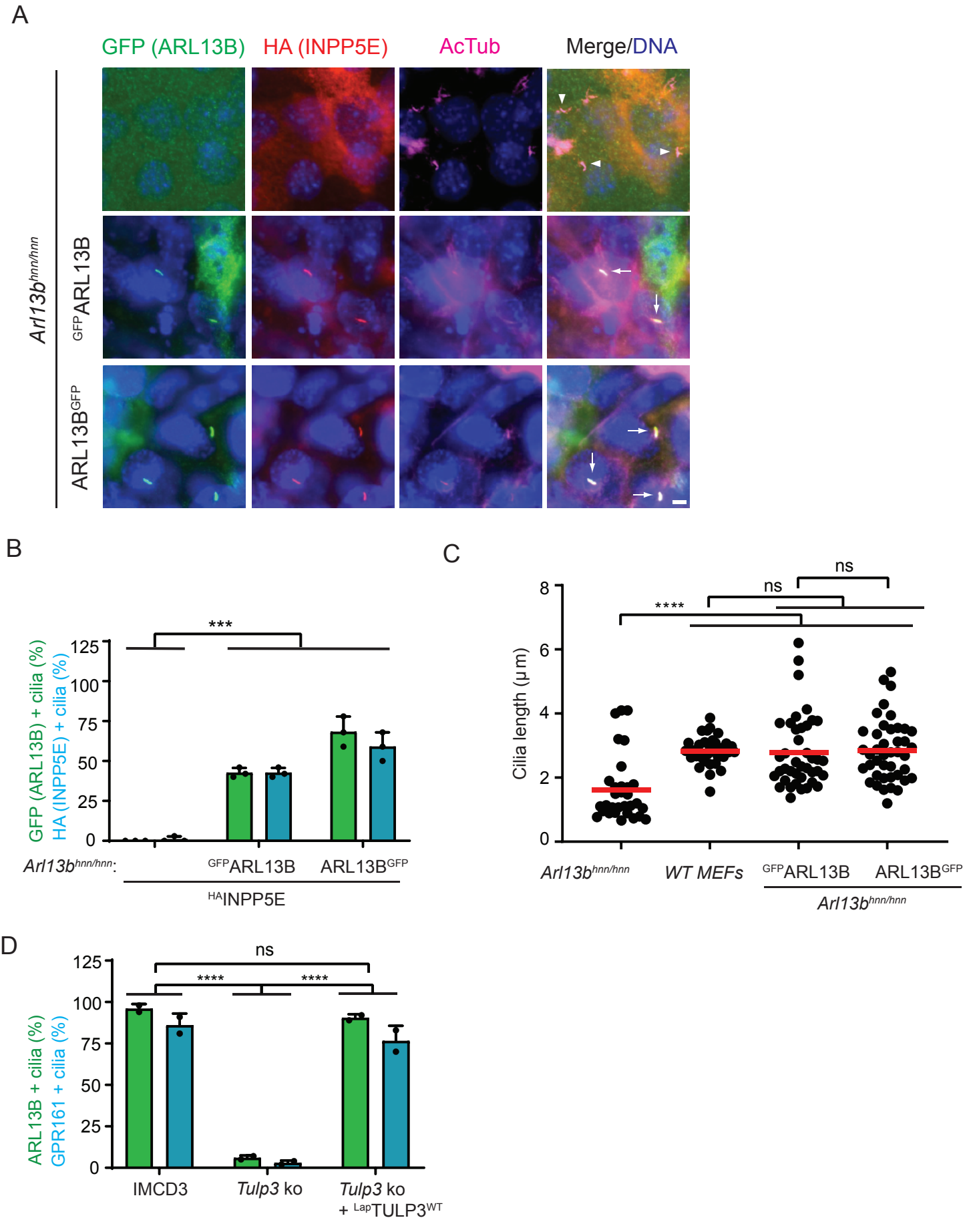

A
GFP (ARL13B)
HA (INPP5E)
AcTub
Merge/DNA
Arl13b hnn/hnn
GFP ARL13B
ARL13B GFP
B
GFP (ARL13B) + cilia (%)
HA (INPP5E) + cilia (%)
***
125
100
75
50
25
0
Arl13b hnn/hnn:
GFP ARL13B
ARL13B GFP
HA INPP5E
C
Cilia length (µm)
ns
ns
****
8
6
4
2
0
Arl13b hnn/hnn
WT MEFs
GFP ARL13B
ARL13B GFP
Arl13b hnn/hnn
D
ARL13B + cilia (%)
GPR161 + cilia (%)
ns
****
****
125
100
75
50
25
0
IMCD3
Tulp3 ko
Tulp3 ko
+ Lap TULP3 WT

Figure S5 (Related to Figure 3). TULP3 is required for trafficking of ARL13B and ARL13B-dependent lipidated cargoes to mouse kidney epithelial cilia.

(A) Kidney sagittal sections at postnatal days 0 or 24 were immunostained for ARL13B, acetylated tubulin (AcTUB), Aquaporin 2 (AQP2) and counterstained for DNA. Asterisks refer to ARL13B immunostaining in cilia of adjacent tubules lacking AQP2. Scale, 50 μm.

(B) Kidney sagittal sections at postnatal days 0 or 24 were immunostained for INPP5E, acetylated tubulin (AcTUB), and counterstained for DNA. Scale, 5 μm.

Arrows and arrowheads indicate cilia positive and negative for the indicated proteins, respectively.

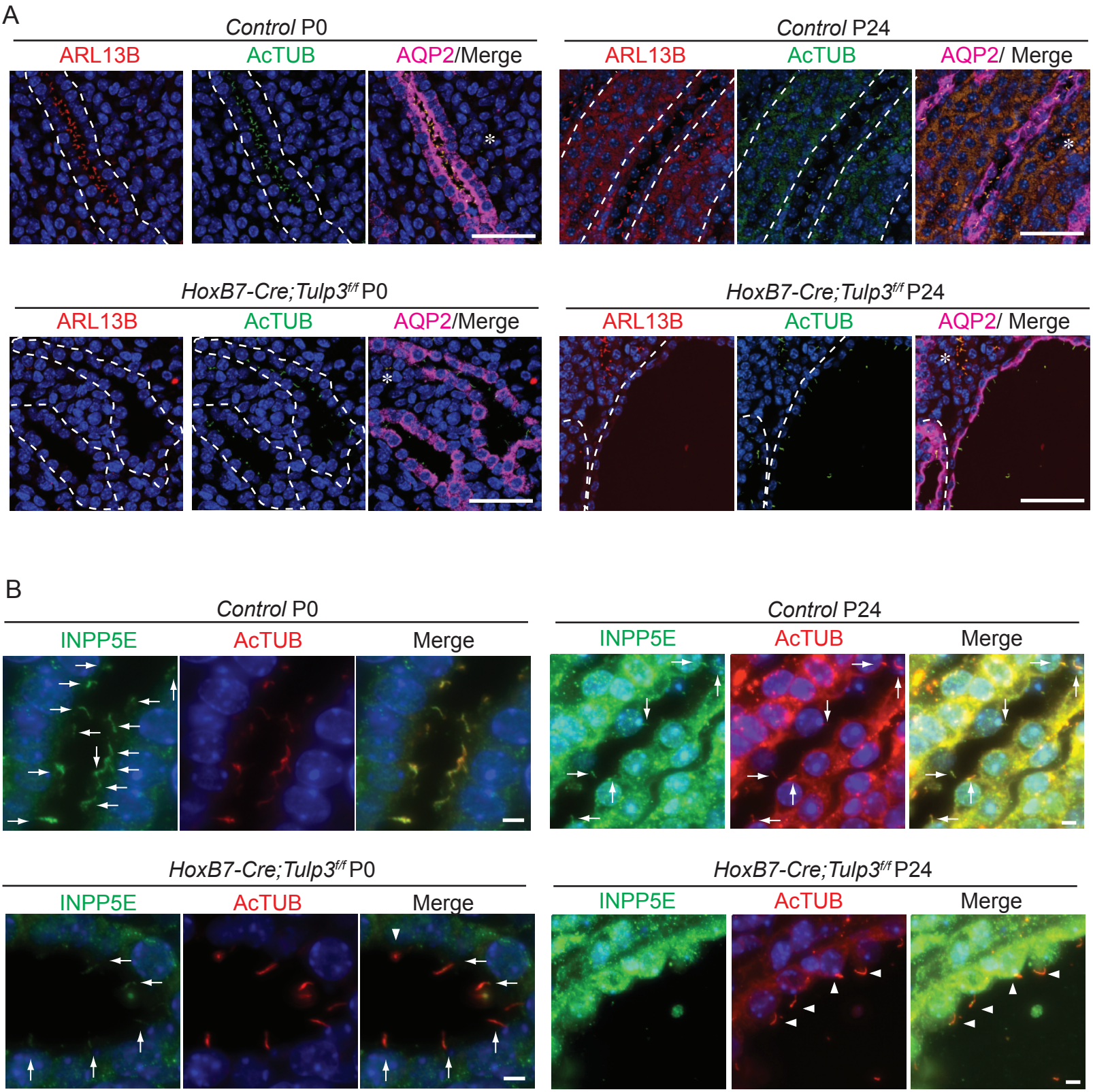

A
Control P0
ARL13B
AcTUB
AQP2/Merge
Control P24
ARL13B
AcTUB
AQP2/ Merge
HoxB7-Cre;Tulp3$^{f/f}$ P0
ARL13B
AcTUB
AQP2/Merge
HoxB7-Cre;Tulp3$^{f/f}$ P24
ARL13B
AcTUB
AQP2/ Merge
B
Control P0
INPP5E
AcTUB
Merge
Control P24
INPP5E
AcTUB
Merge
HoxB7-Cre;Tulp3$^{f/f}$ P0
INPP5E
AcTUB
Merge
HoxB7-Cre;Tulp3$^{f/f}$ P24
INPP5E
AcTUB
Merge

Figure S6 (Related to Figure 5 and 6). ARL13B domains regulating TULP3 binding.

(A) Binding between GST, ^{GST}TULP3 or ^{GST}Tubby bound to Glutathione Sepharose beads and *in vitro* translated (IVT) Myc tagged ARL13B or ARL13B^{11A} fragment as in Figure 6B.

(B) Bacterially purified GST or ^{GST}TULP3 protein bound to glutathione Sepharose beads was incubated with *in vitro* translated (IVT) Myc tagged truncations of ARL13B in Lap150N buffer at room temperature for 1 h. Beads were washed, eluted and immunoblotted with Myc antibody to show interaction. Ponceau stain shows GST or ^{GST}TULP3 on beads. Input shows flowthrough immunoblotted with Myc antibody to show the presence of ^{Myc}ARL13B in the reaction. Lack of the coiled coil in ARL13B did not prevent ^{GST}TULP3 binding.

(C) Binding between GST or ^{GST}TULP3 bound to Glutathione Sepharose beads and *in vitro* translated (IVT) Myc tagged ARL13B D2 or D2^{11A} fragment as in Figure 6B.

ns, not significant.

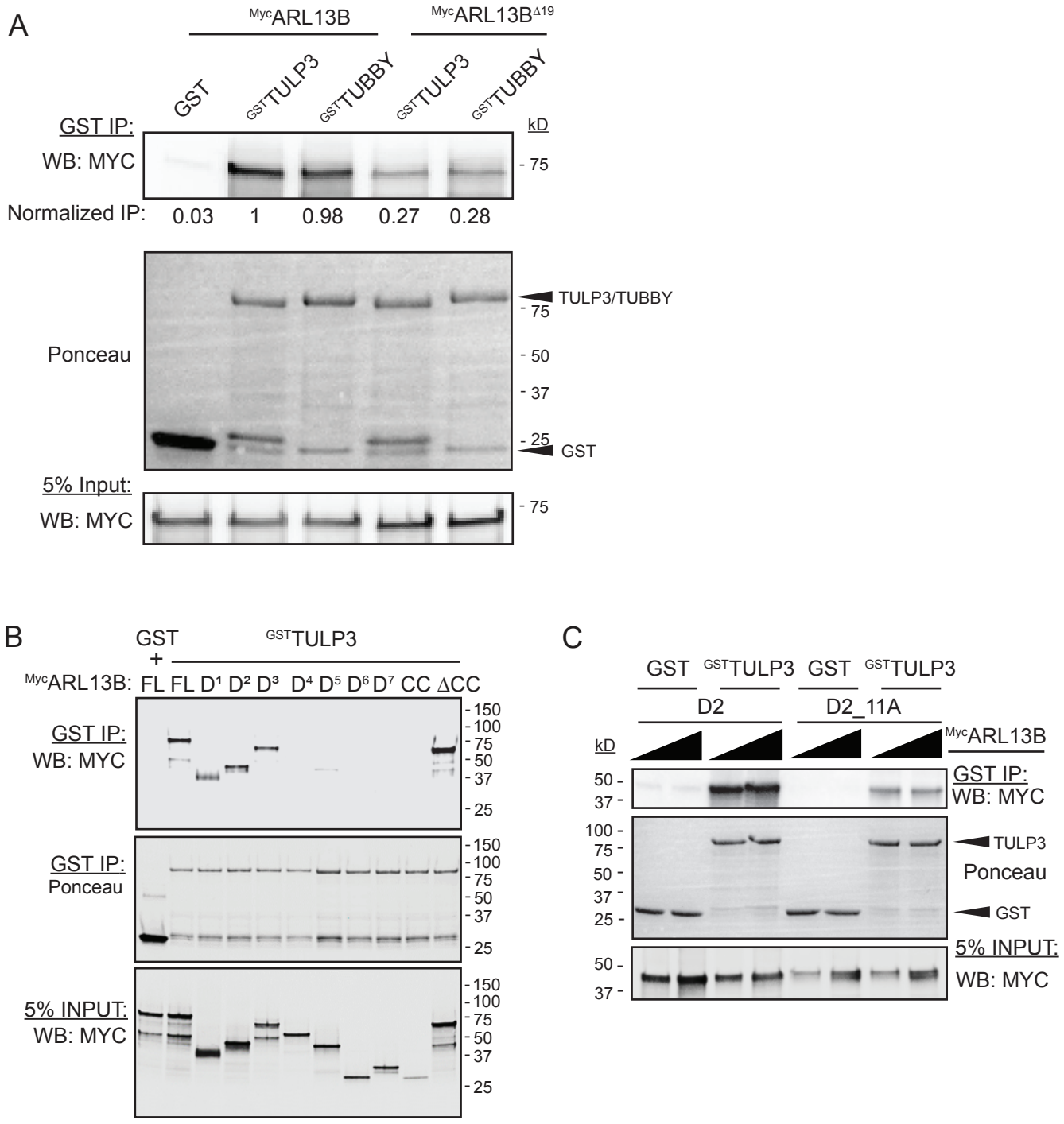

A
MycARL13B
MycARL13BΔ19
GST
GSTTULP3
GSTTUBBY
GSTTULP3
GSTTUBBY
GST IP:
WB: MYC
kD
75
Normalized IP:
0.03
1
0.98
0.27
0.28
Ponceau
TULP3/TUBBY
75
50
37
25
GST
5% Input:
WB: MYC
75
B
GST
+
GSTTULP3
MycARL13B: FL FL D1 D2 D3 D4 D5 D6 D7 CC ΔCC
GST IP:
WB: MYC
150
100
75
50
37
25
GST IP:
Ponceau
150
100
75
50
37
25
5% INPUT:
WB: MYC
150
100
75
50
37
25
C
GST
GSTTULP3
GST
GSTTULP3
D2
D2_11A
kD
MycARL13B
50
37
GST IP:
WB: MYC
100
75
50
37
25
TULP3
Ponceau
GST
5% INPUT:
50
37
WB: MYC

Figure S7 (Related to Figure 7). ARL13B domains regulating TULP3 mediated ciliary trafficking.

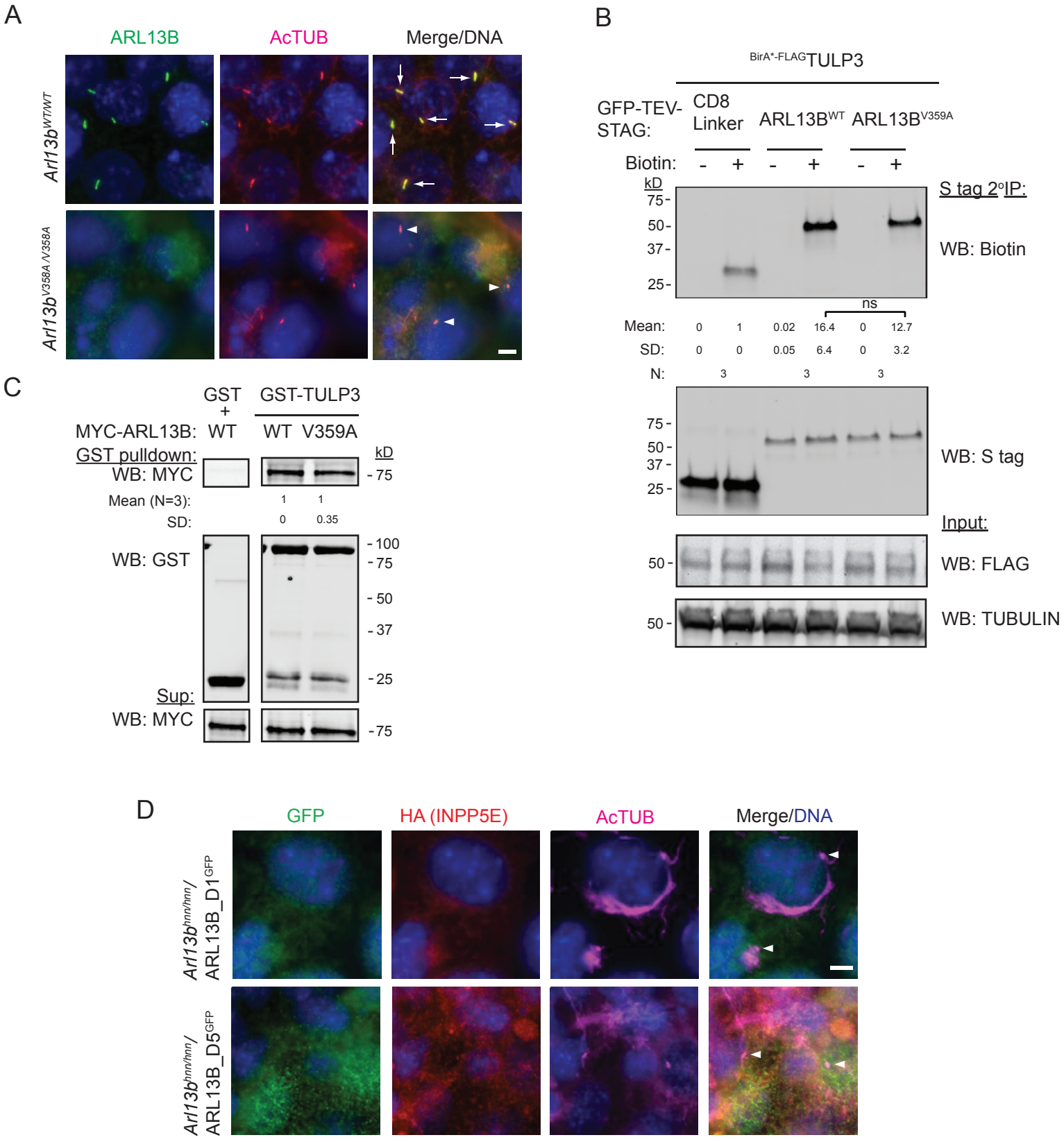
(A) Immortalized *Arl13b*$^{V358A/V35A}$ MEFs were starved for 48 h and immunostained for Arl13b, acetylated tubulin (AcTub) and counterstained for DNA.

(B) T-Rex 293 cells were co-transfected with BirA* tagged TULP3 along with LAP tagged ARL13B or V359A mutant and processed as shown in Figure 5A. Mean ± SD values indicate Biotin/S-tag ratios normalized to CD8 inker control. “n” indicates the number of experiments performed

(C) Bacterially purified GST or ^{GST}TULP3 protein bound to glutathione Sepharose beads was tested for binding with *in vitro* translated (IVT) Myc full length ARL13B and *V359A* mutant as in Figure 5D.

(D) *Arl13b*^{hnn} cells stably expressing C-LAP tagged D1 or D5 were serum starved for 48 h before fixing and immunostained for GFP, HA, acetylated tubulin (AcTUB) and counterstained for DNA.

Scale, 5 μm. Arrows indicate cilia positive for ARL13B fragments/variants (B), while arrowheads indicate cilia negative for the respective proteins.


A
ARL13B
AcTUB
Merge/DNA
Arl13b WT/WT
Arl13b V358A /V358A
B
BirA*-FLAG TULP3
GFP-TEV-STAG:
CD8 Linker
ARL13B WT
ARL13B V359A
Biotin:
kD
75
50
37
25
S tag 2°IP:
WB: Biotin
ns
Mean: 0 1 0.02 16.4 0 12.7
SD: 0 0 0.05 6.4 0 3.2
N: 3 3 3
WB: S tag
Input:
WB: FLAG
WB: TUBULIN
C
GST
+
MYC-ARL13B:
GST-TULP3
WT
WT
V359A
GST pulldown:
WB: MYC
Mean (N=3): 1 1
SD: 0 0.35
WB: GST
100
75
50
37
25
Sup:
WB: MYC
D
GFP
HA (INPP5E)
AcTUB
Merge/DNA
Arl13b hnn/hnn/ ARL13B_D1 GFP
Arl13b hnn/hnn/ ARL13B_D5 GFP