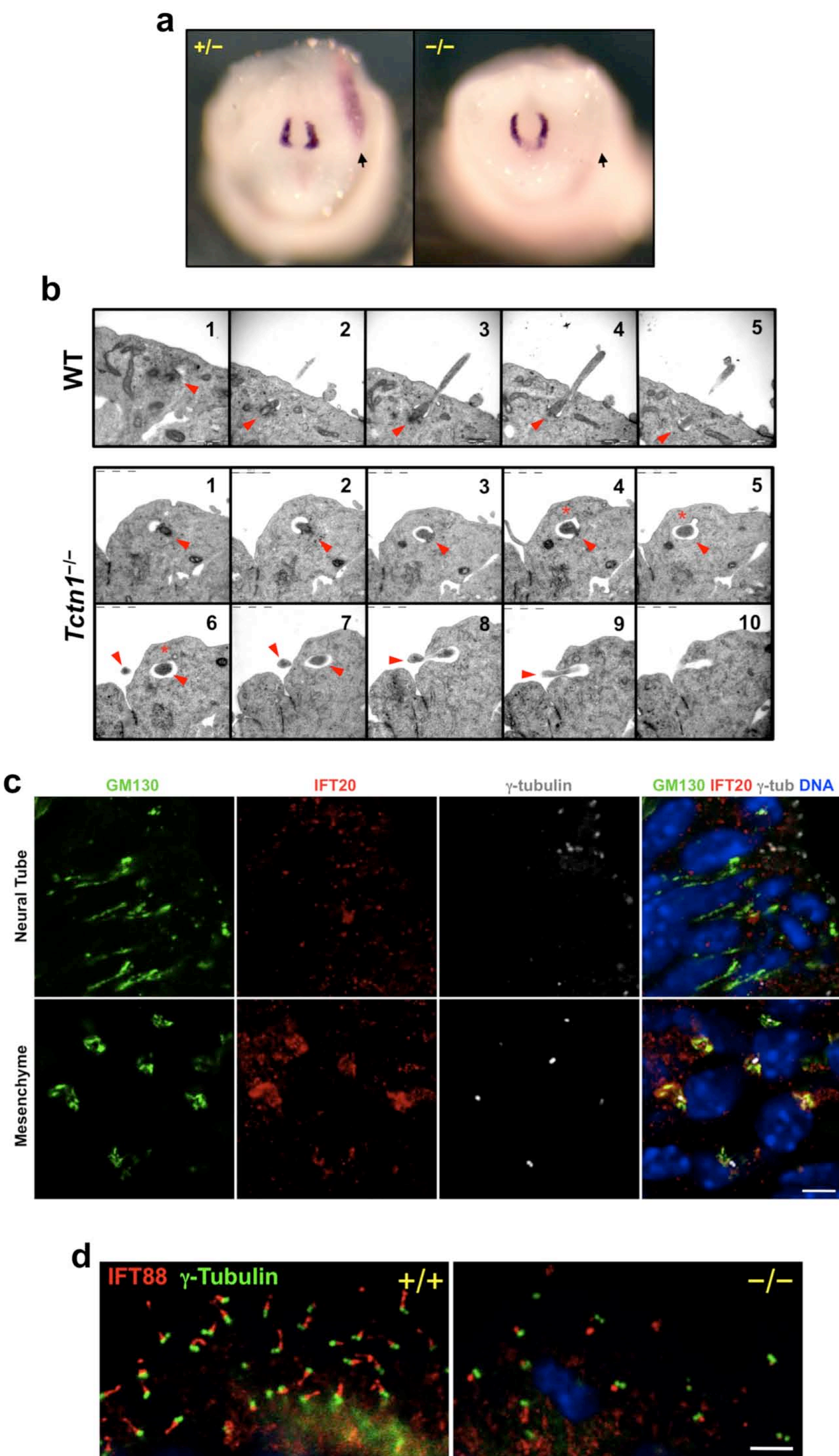


Figure S1. Phenotypes of *Tctn1*$^{-/-}$ mice (a) In situ hybridization of two-somite stage *Tctn1*$^{+/-}$ and *Tctn1*$^{-/-}$ embryos. Left-sided expression of *Nodal* in the lateral plate mesoderm is absent in *Tctn1* mutants at this stage (arrows). **(b)** TEM of serial ultrathin sections of wild type and *Tctn1*$^{-/-}$ E9.5 neural tube cilia. The mutant cilium is abnormally enlarged near the base (asterisks), and becomes thinner as it emerges from its ciliary pocket. The basal body is attached to the plasma membrane. Arrowheads indicate cilia. **(c)** Ift20 staining in an E10.5 wild type embryo shows that localization differs in neural tube and surrounding mesenchymal cells. In the latter, Ift20 colocalizes with Golgi marker GM130 around the centrosome, which is labeled by g-tubulin (bottom panels). In the neural tube, Ift20 displays a more punctate pattern, and no localization at the Golgi apparatus is observed (top panels). Similar localization is observed in *Tctn1*$^{-/-}$, *Tctn2*$^{-/-}$ and *Cc2d2a*$^{-/-}$ embryos (data not shown). Scale bar 5μm. **(d)** Ift88 localizes to the basal bodies and dysmorphic cilia of E10.5 *Tctn1*$^{-/-}$ neural tube cells. Scale bar 5μm.

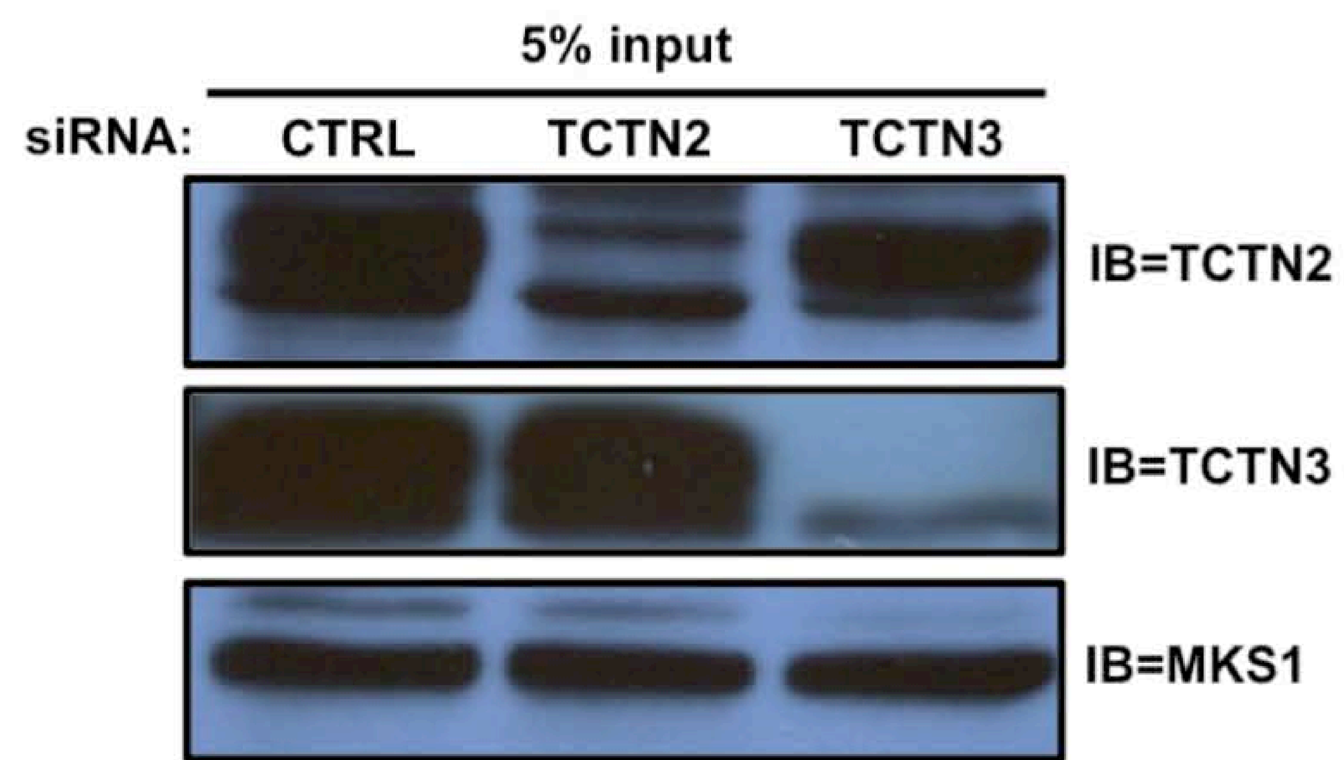
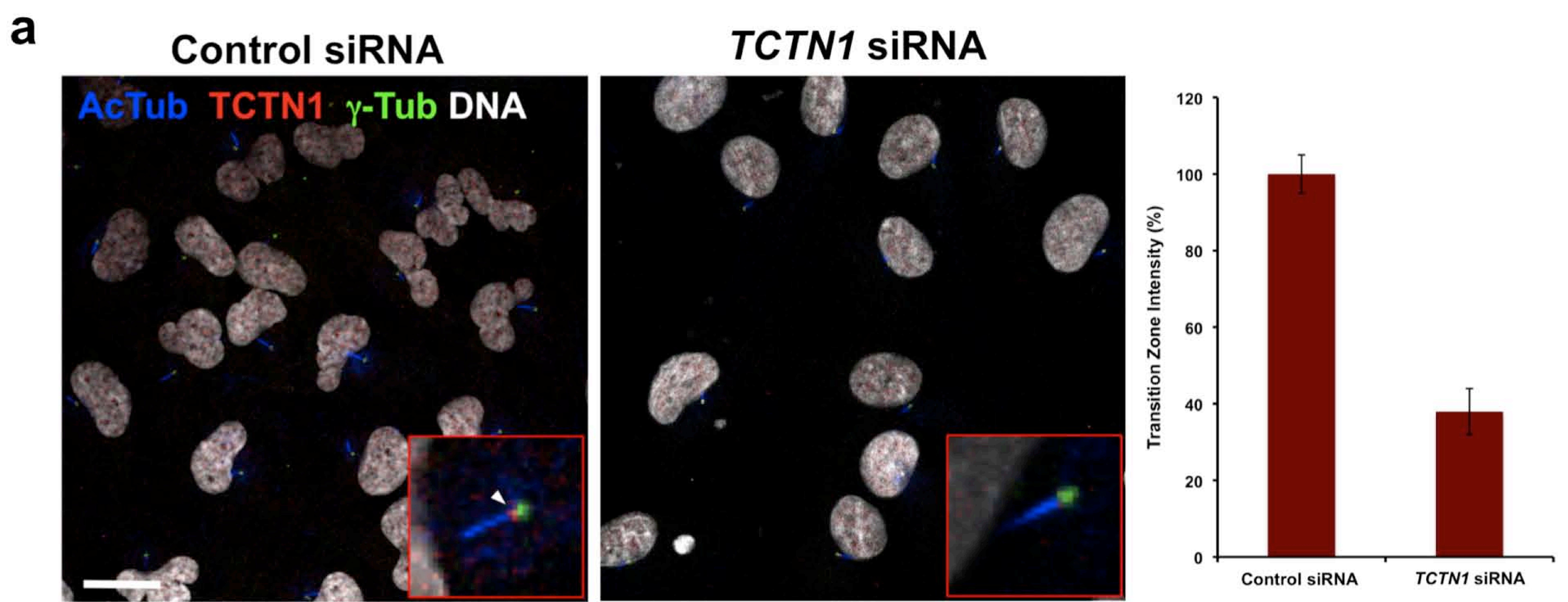
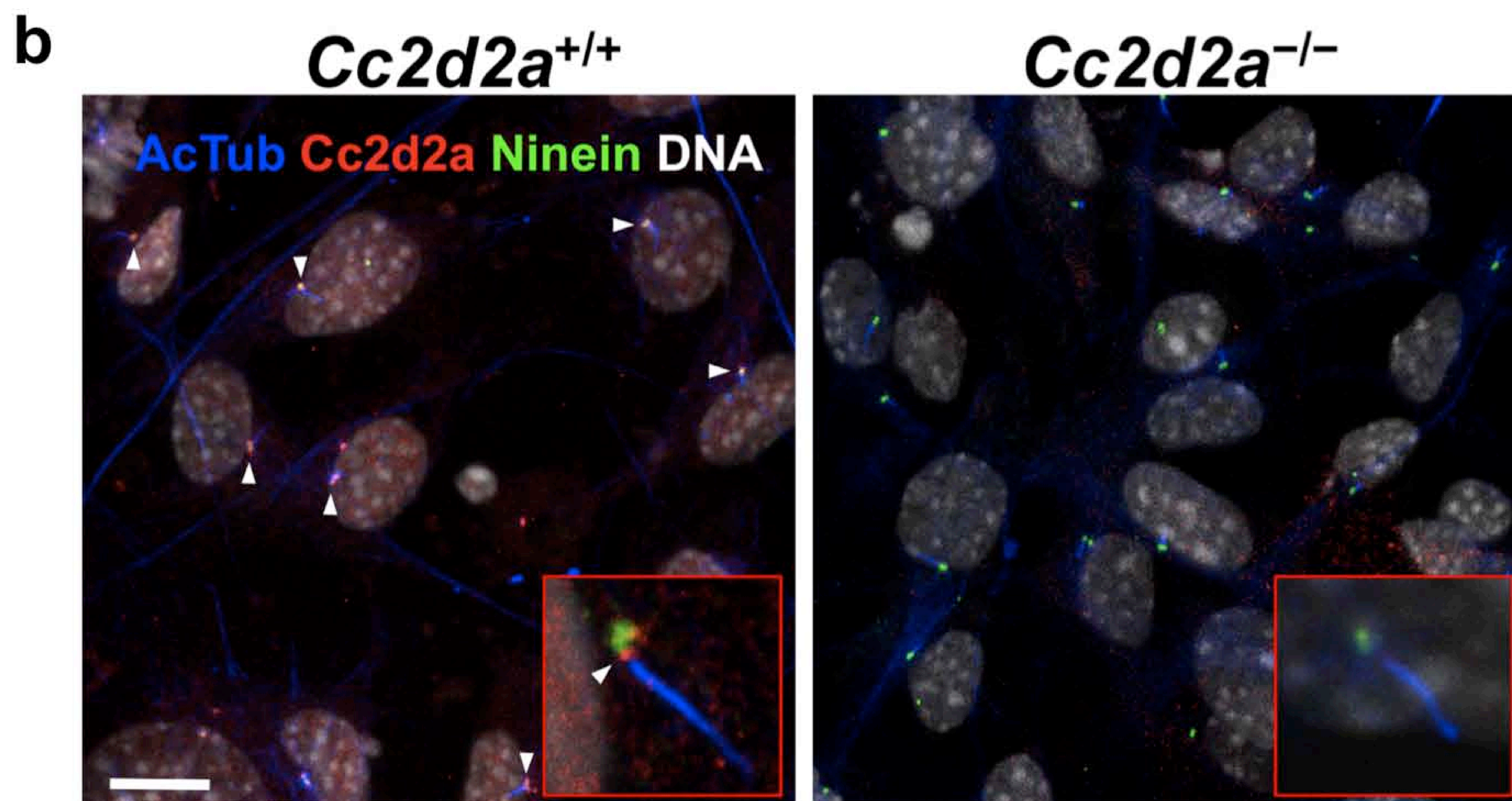


Figure S2. TCTN2 interacts with MKS1 and TCTN3 in siRNA-treated cells. (a) TCTN2 and TCTN3 are reduced by siRNA-treatment against the corresponding genes. MKS1 levels are unaffected by *TCTN2* and *TCTN3* siRNA treatments. (**b**) Endogenous MKS1, TCTN2 and TCTN3 were immunoprecipitated from lysates derived from hTERT-RPE1 cells transfected with control, *TCTN2* or *TCTN3* siRNAs, as indicated. The amount of TCTN2 in the immunoprecipitates was analyzed by immunoblot.





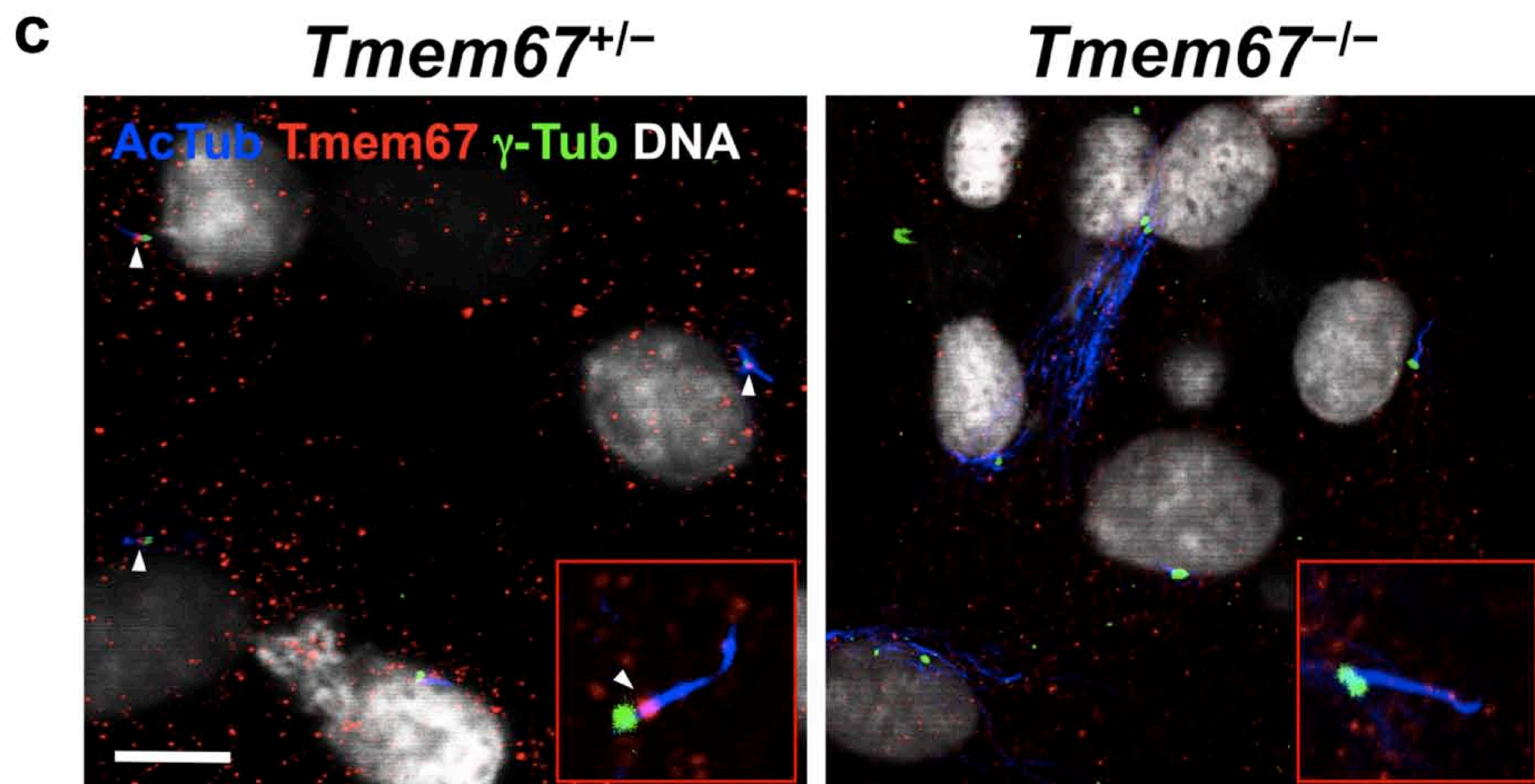


Figure S3a-c. Specificity of Tctn1, Cc2d2a and Tmem67 antibodies. (a) hTERT-RPE1 cells transfected with control or *TCTN1* siRNAs were stained as indicated. Insets show magnification of cilia demonstrating specific loss of transition zone staining in the siRNA-treated cells (arrowhead). Quantitation is shown on the right. Scale bar 10μm. **(b)** Wild type and *Cc2d2a*$^{-/-}$ MEFs were stained as indicated. Arrowheads indicate transition zone staining. Insets show magnification of cilia demonstrating specific loss of transition zone staining in the mutant cells. Scale bar 10μm. **(c)** Control and *Tmem67*$^{-/-}$ MEFs were stained as indicated. Arrowheads indicate transition zone staining. Insets show magnification of cilia demonstrating specific loss of transition zone staining in the mutant cells. Scale bar 10μm.

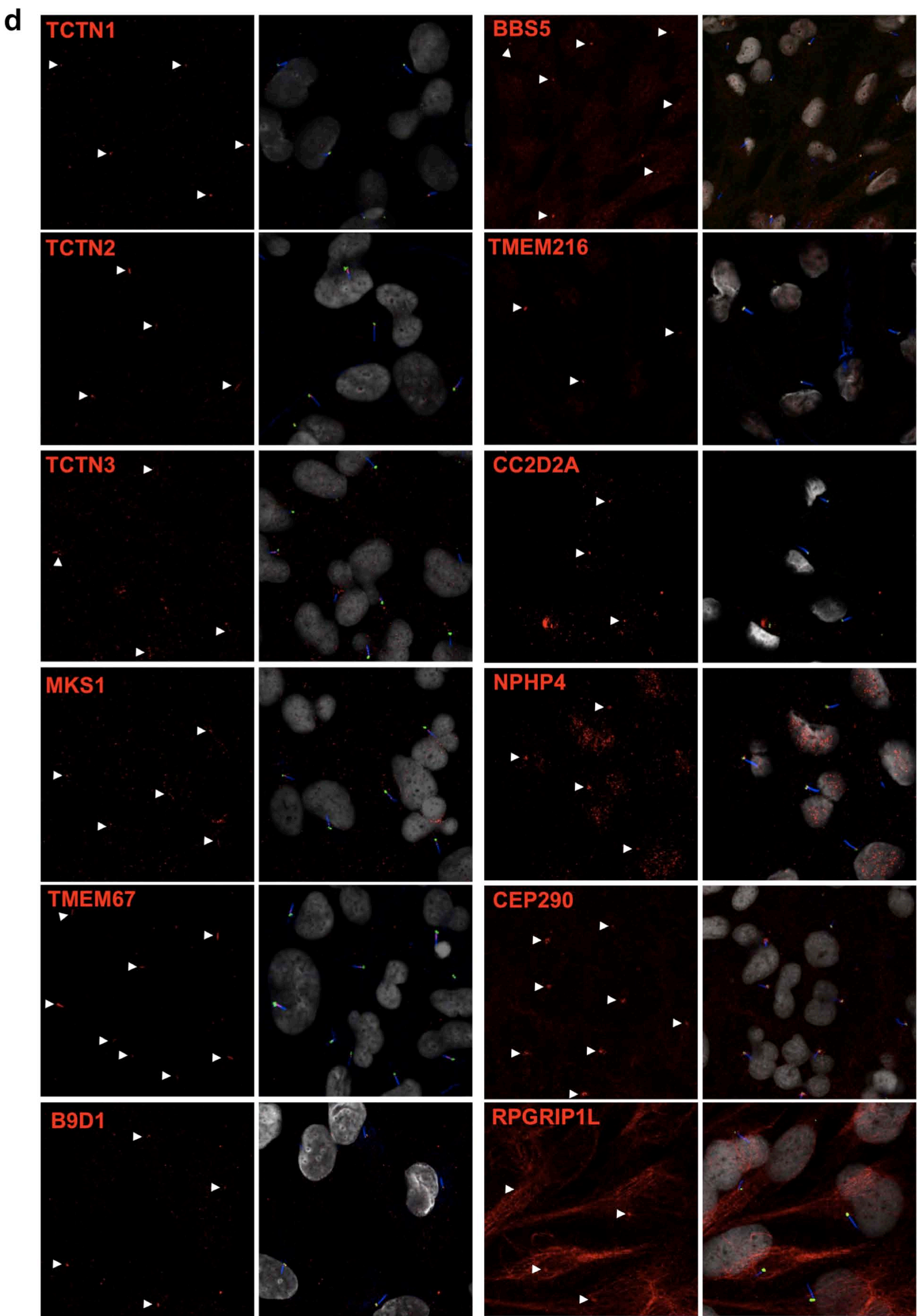


Figure S3d. Ciliary proteins in hTERT-RPE1 cells. (d) Photomicrographs of fields of hTERT-RPE1 cells corresponding to the cells depicted at higher resolution in Figure 3a. Arrowheads indicate transition zone or basal body staining.

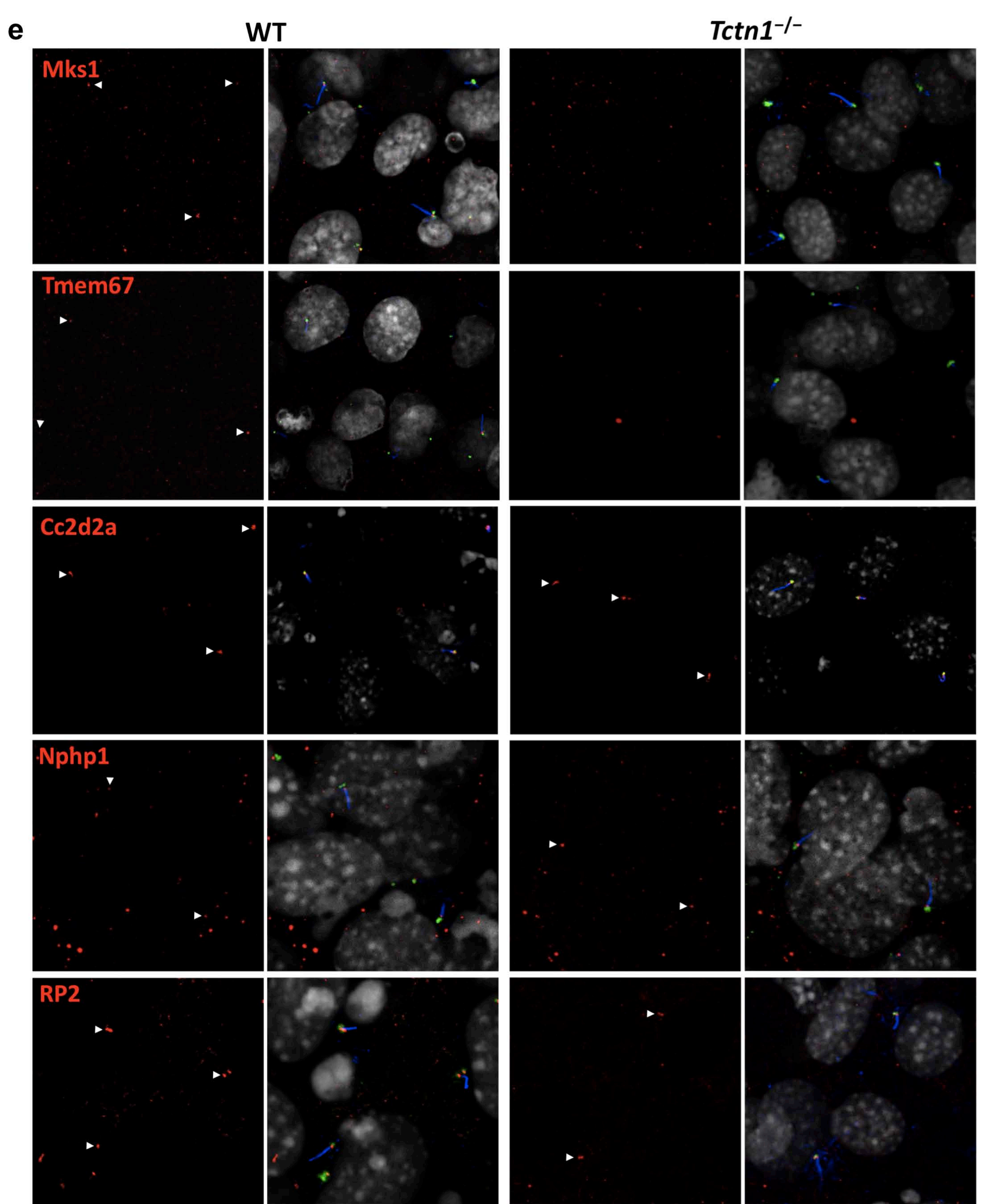


Figure S3e. Ciliary proteins in MEFs. (e) Photomicrographs of fields of MEFs corresponding to the cells depicted at higher resolution in Figure 3b. Arrowheads indicate transition zone, basal body or ciliary staining.

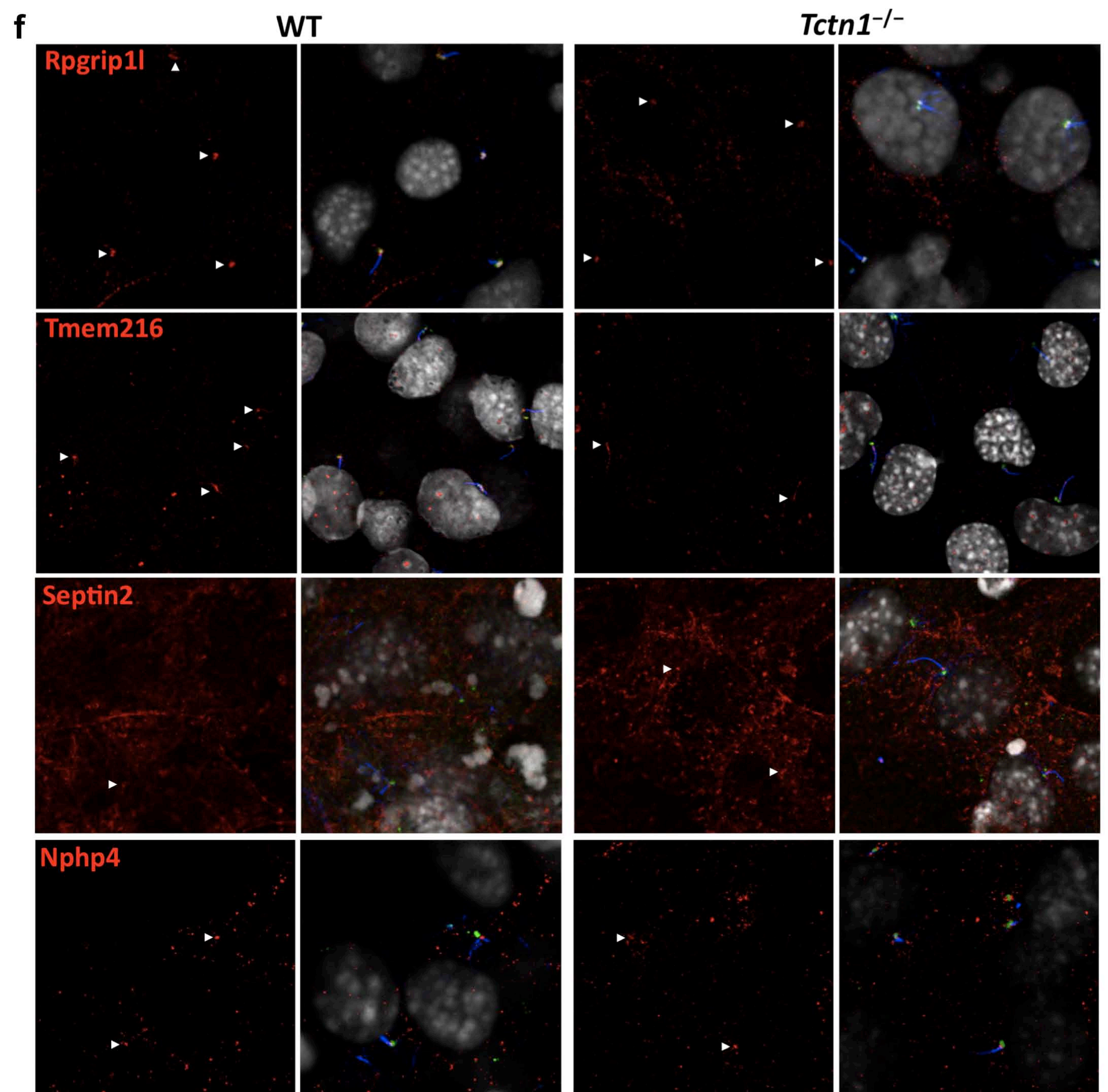


Figure S3f. Ciliary proteins in MEFs. (f) Photomicrographs of fields of MEFs corresponding to the cells depicted at higher resolution in Figure 3b. Arrowheads indicate transition zone, basal body or ciliary staining.

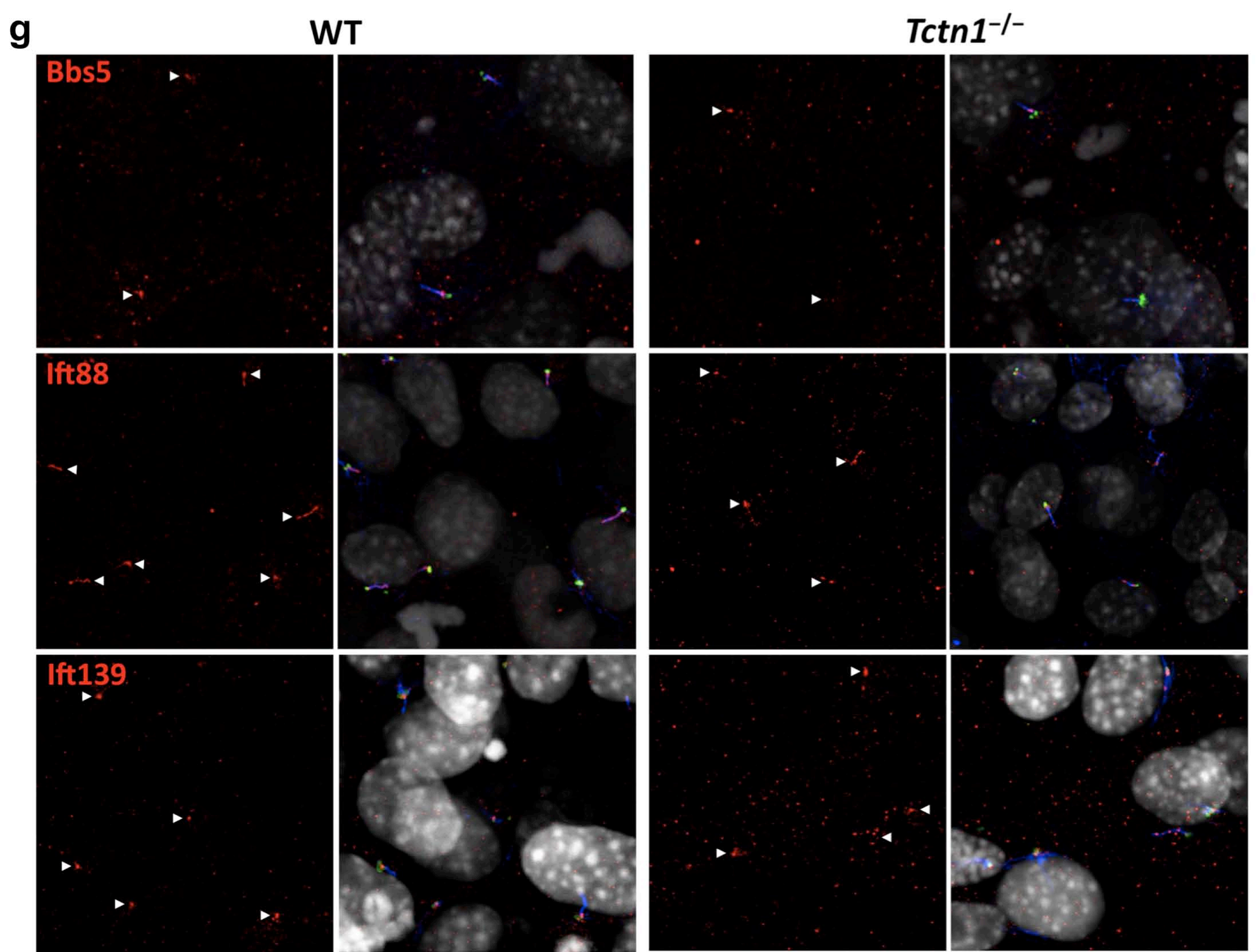
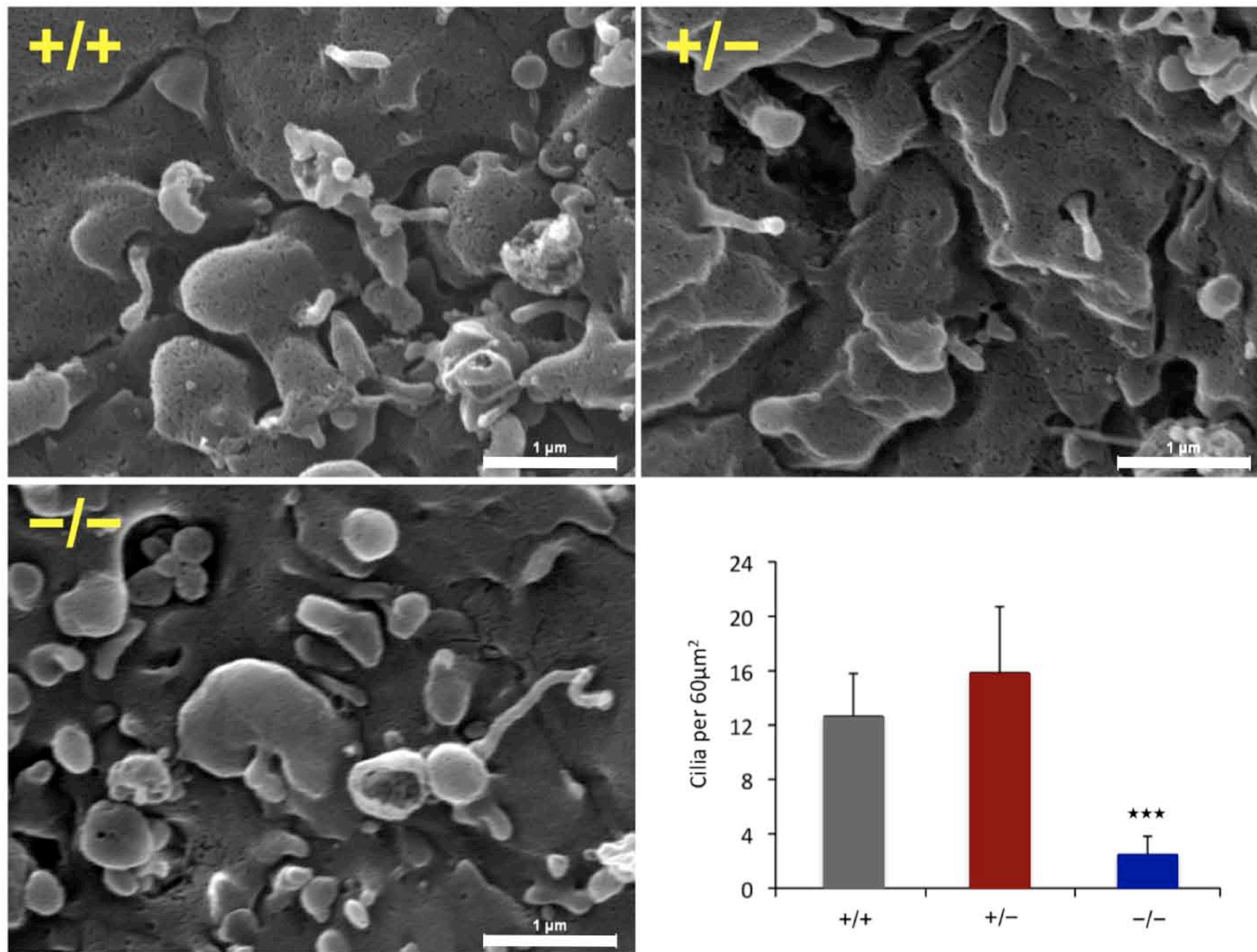


Figure S3g. Ciliary proteins in MEFs. (g) Photomicrographs of fields of MEFs corresponding to the cells depicted at higher resolution in Figure 3b. Arrowheads indicate transition zone, basal body or ciliary staining.



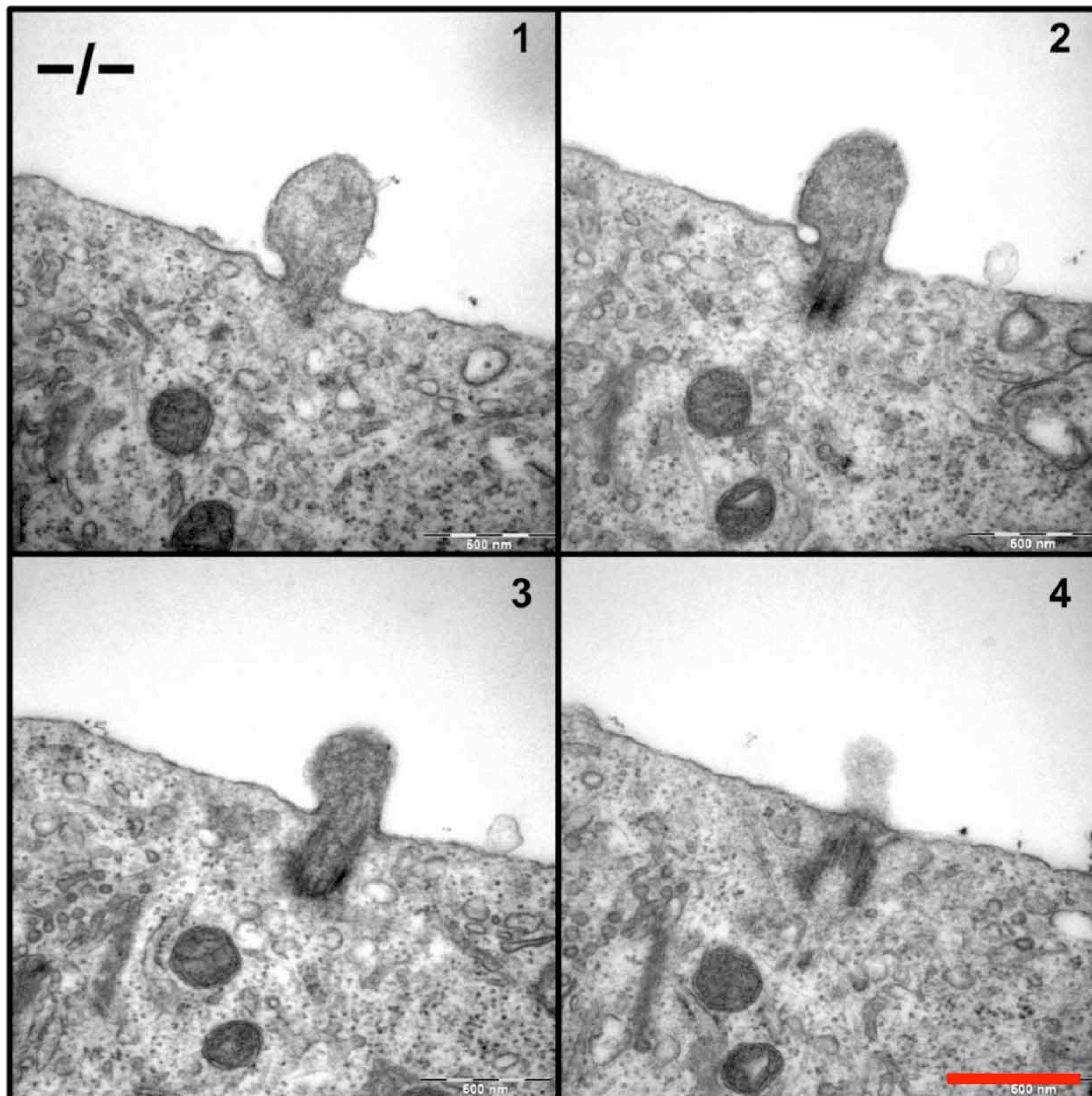


Figure S4. Cilia defects in *Tctn2*$^{-/-}$ neural tube. (a) SEM of E9.5 neural tubes shows numerous cilia in wild type and *Tctn2*$^{+/-}$ embryos, but few and dysmorphic cilia in *Tctn2*$^{-/-}$ embryos. For quantification, the number of cilia in twenty 60µm^2 areas of neural epithelium were counted per genotype (one embryo per genotype). Data are shown as mean ± standard deviation and asterisks indicate significance according to unpaired Student t-tests (*** = $p<0.001$). Scale bars 1µm. **(b)** TEM of four serial ultrathin sections of a *Tctn2*$^{-/-}$ E9.5 neural tube cilium. The basal body is attached to the membrane, and is attached to a short, bulbous dilatation lacking a discernable axoneme. Scale bar 500nm.

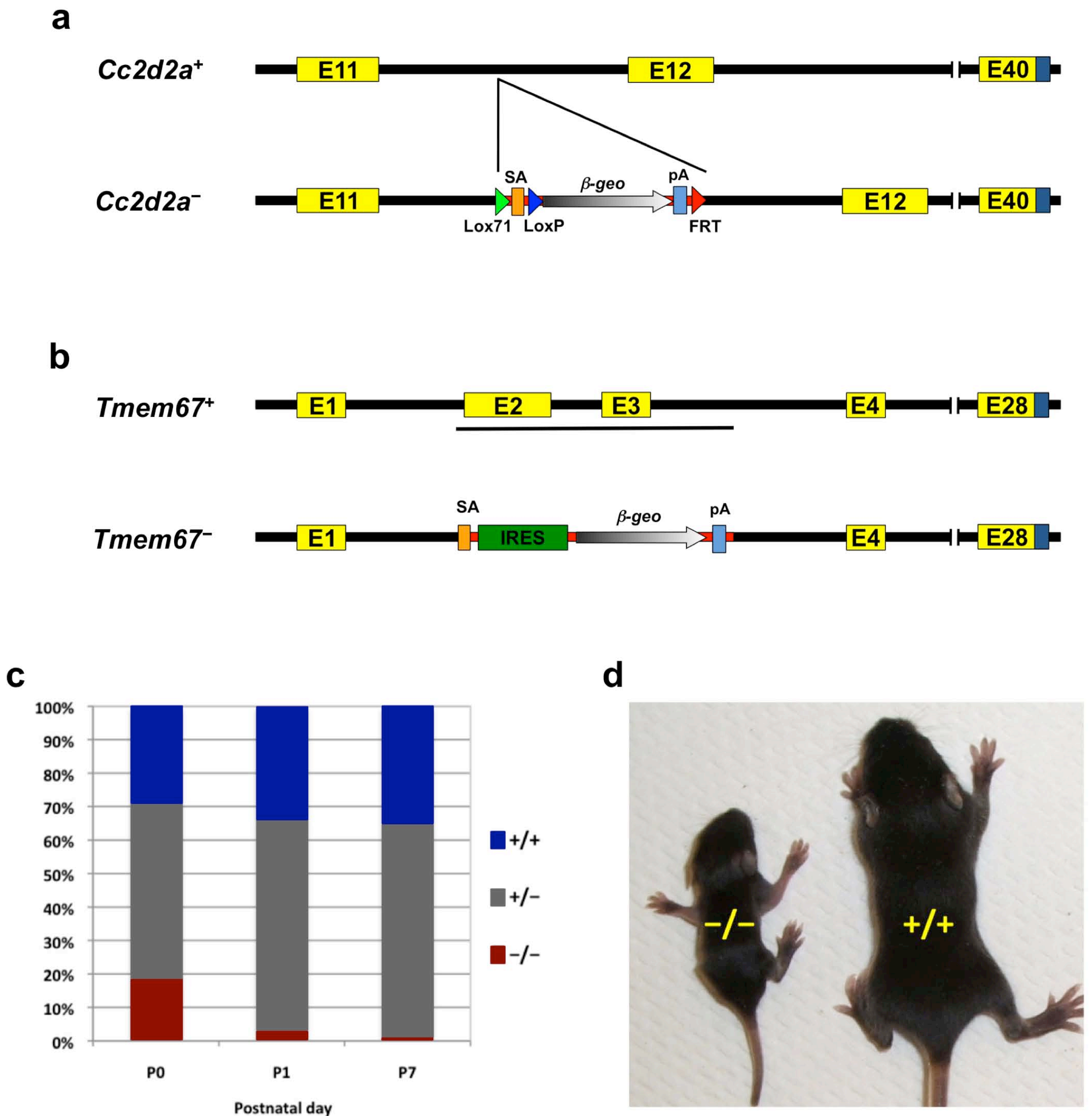


Figure S5. ***Cc2d2a* and *Tmem67* mouse mutants. (a)** Schematic of the *Cc2d2a* mutant allele used in this study, containing a gene trap insertion after exon 11, leading to a truncated gene product. **(b)** Schematic of the *Tmem67* mutant allele used in this study, which lacks exons 2 and 3. **(c)** *Tmem67*$^{-/-}$ mice die shortly after birth, as represented by the sharp decrease in the percentage of surviving *Tmem67*$^{-/-}$ pups during the first week of postnatal life. **(d)** In the rare cases in which they live beyond the first week, *Tmem67*$^{-/-}$ pups are severely growth retarded as compared to their littermates.

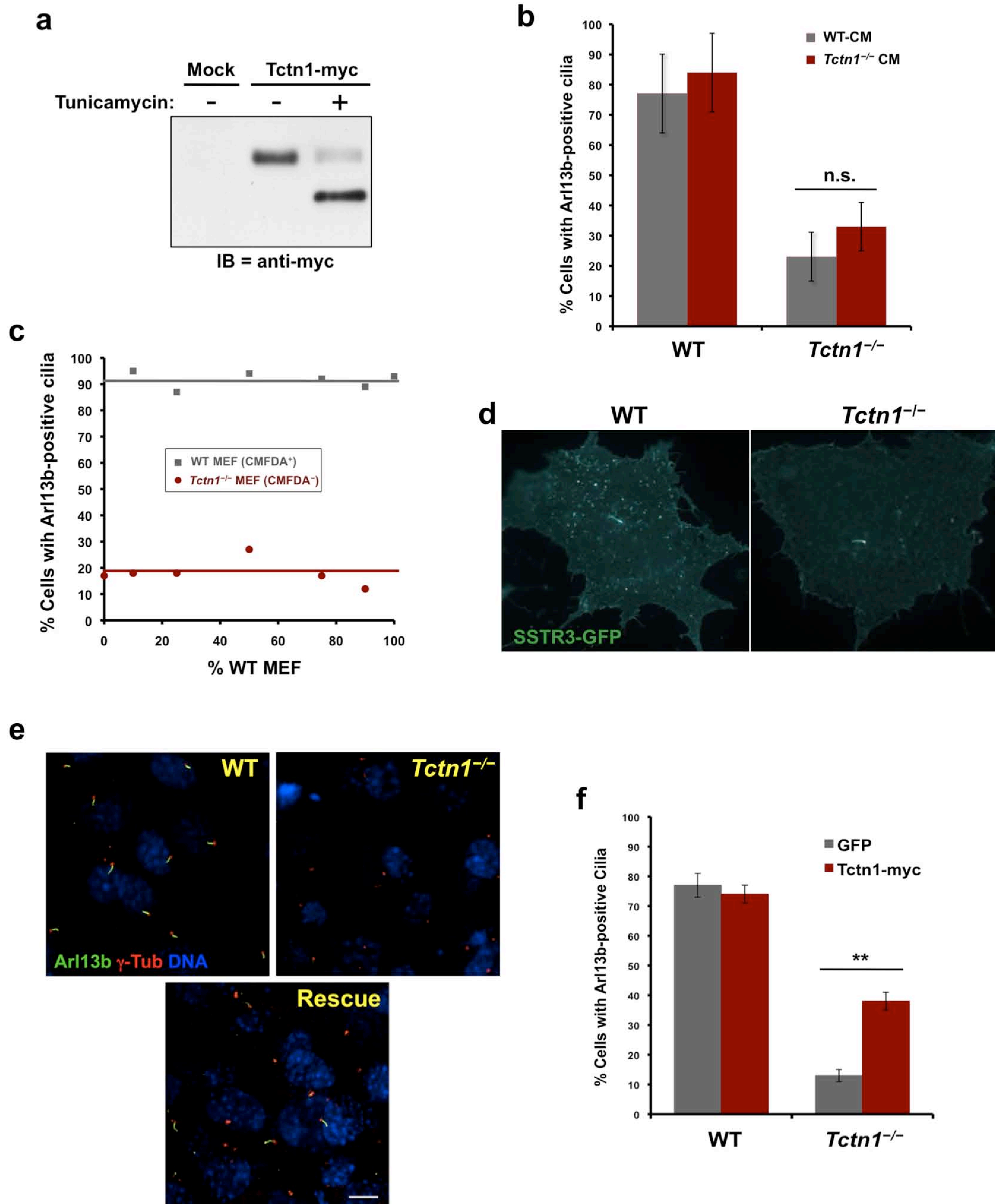


Figure S6. Tctn1 cell-autonomously regulates localization of select ciliary membrane proteins. (a) Treatment with tunicamycin, an inhibitor of N-linked glycosylation, reduces the apparent molecular weight of Tctn1-myc, indicating Tctn1 is normally N-glycosylated. **(b)** Wild type (wt) and *Tctn1*$^{-/-}$ MEFs were grown in conditioned media from both cell types. Conditioned media from wt cells did not affect the proportion of Arl13b-positive cilia possessed by *Tctn1*$^{-/-}$ MEFs. Similarly, conditioned media from *Tctn1*$^{-/-}$ MEFs did not affect the proportion of Arl13b-positive cilia possessed by wt MEFs. **(c)** Cell mixing experiments were performed by labeling wt MEFs, but not *Tctn1*$^{-/-}$ MEFs, with chloromethyl fluorescein diacetate (CMFDA), which irreversibly labels cells green. Green wt and colorless *Tctn1*$^{-/-}$ MEFs were mixed in different proportions and Arl13b-positive cilia in each cell type measured after 48h of starvation. As depicted in the graph, the proportions of Arl13b-positive cilia in both cell types are unaffected by the presence of the other cell type, further indicating that Tctn1, despite having a signal peptide, affects cilia cell-autonomously. **(d)** A plasmid encoding SSTR3-GFP was transfected into wt and *Tctn1*$^{-/-}$ MEFs. The SSTR3-GFP fusion protein is at the plasma and ciliary membrane in both cell types, as seen by GFP fluorescence. **(e)** Arl13b localization to cilia is lost in *Tctn1*$^{-/-}$ MEFs, but is restored by transfection of Tctn1-myc 24h before starving cells for 48h. Scale bar 10μm. **(f)** Quantification of Arl13b-positive cilia in wt and *Tctn1*$^{-/-}$ MEFs transfected with Tctn1-myc or GFP control. Data are mean±s.d. Asterisk indicates significance according to an unpaired Student t-test (** = p<0.01).

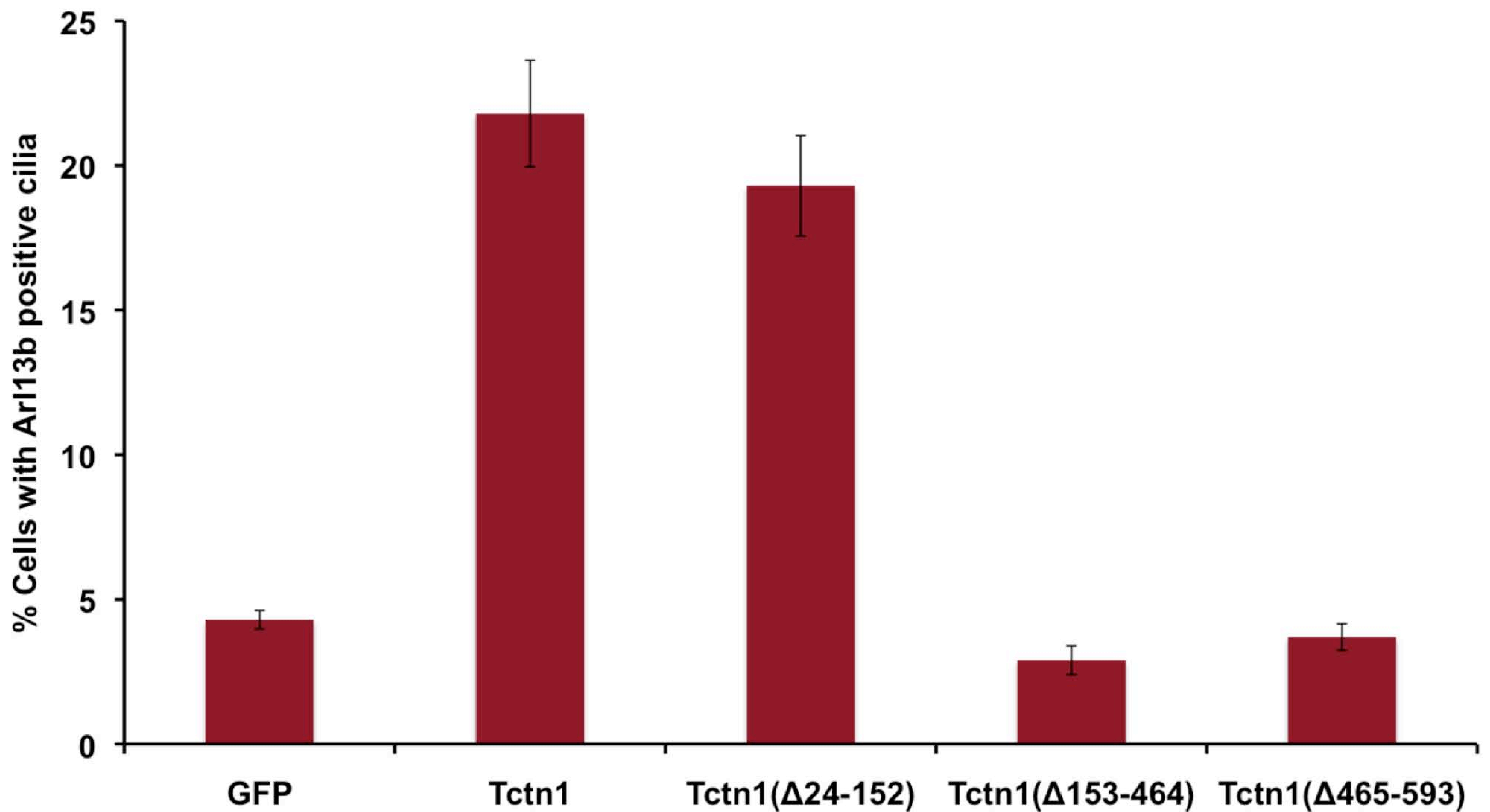


Figure S7. Structure-function analysis of Tctn1. *Tctn1*$^{-/-}$ MEFs were transfected with plasmids encoding GFP or full length or truncated versions of myc-tagged mouse Tctn1. Residues 153-464 and 465-593 of mouse Tctn1 are equally essential for the restoration of the ciliary localization of Arl13b, whereas residues 24-152 are dispensable. The IVS1-2a>g mutation in human *TCTN1* is predicted to lead to the loss of exon 2 from the mature transcript, causing a frameshift and truncation of the protein, TCTN1(D74-592)fs15X. As Tctn1(D153-464) is non-functional, we predict the IVS1-2a>g mutation to result in a loss of TCTN1 function. However, alternate *TCTN1* splicing events may produce unexpected products that retain some function. Therefore, it remains unclear whether the identified JBTS-associated mutation causes a partial or total loss of TCTN1 function.

a

	Tctn	Tmem67	Cc2d2a
Animals	1-3	1	1
Monosiga	1	1	1
Batrachochytrium	1	1	1
Tetrahymena	1	1	1
Paramecium	1	1	1
Thalassiosira	1	1	1
Naegleria	1	1	1
Trypanosoma	1	1	1
Leishmania	1	1	1
Chlamydomonas	1	1	1
Trichomonas	2	1	1
Aureococcus	1	1	1

b

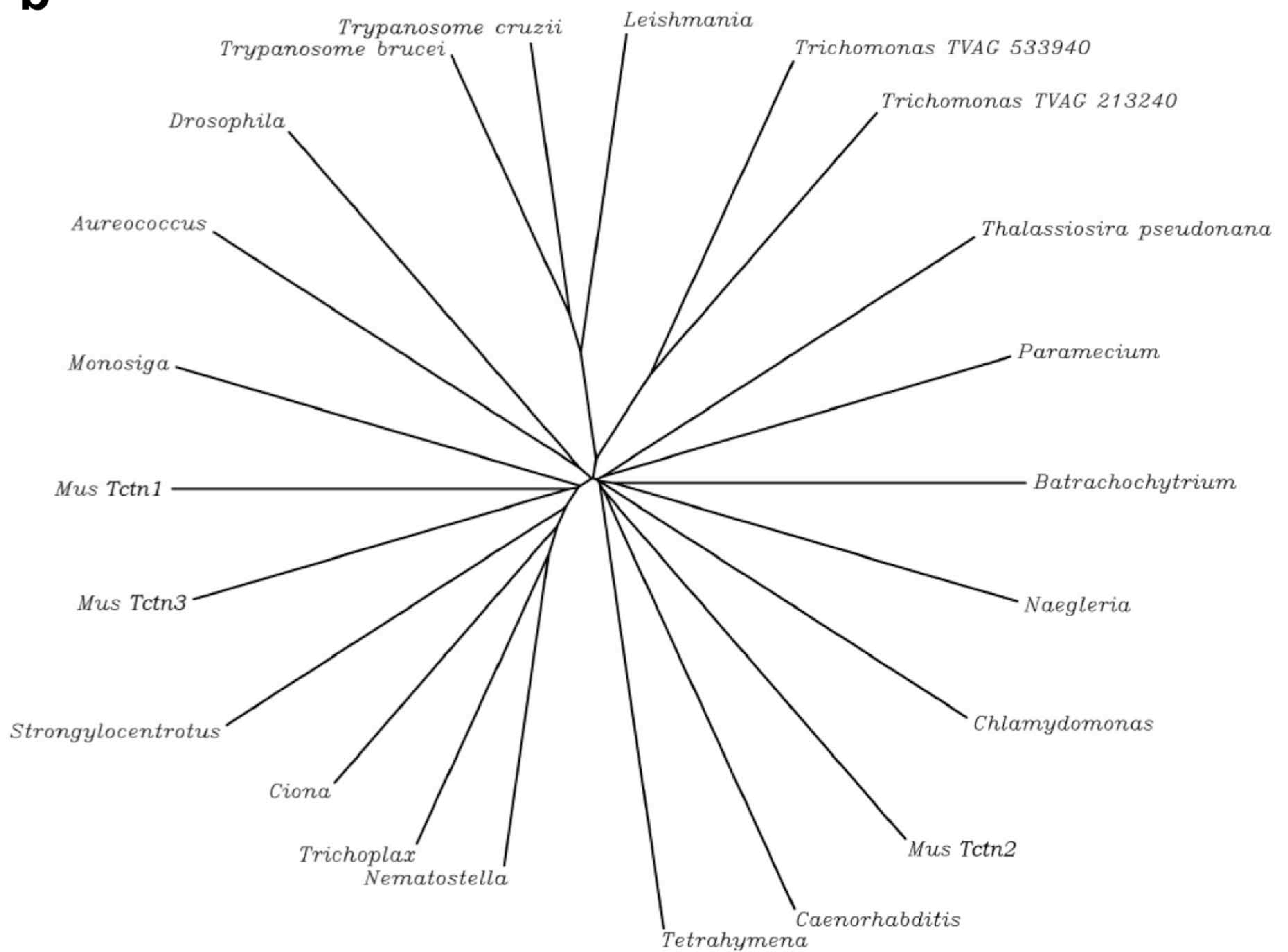


Figure S8. The Tectonic ciliopathy complex is evolutionarily conserved. (a) Table indicating number of homologs of *Tctn*, *Tmem67* and *Cc2d2a* present in the genomes of ciliated organisms including animals, *Monosiga*, *Batrachochytrium*, *Tetrahymena*, *Paramecium*, *Thalassiosira*, *Naegleria*, *Trypanosoma*, *Leishmania*, *Chlamydomonas*, *Trichomonas*, and *Aureococcus*. **(b)** Neighbor-joining analysis of the protein sequences of Tectonic homologs of the indicated ciliated organisms.

Table S1 is provided separately as an Excel (.xls) file

Table S1. Tctn1-LAP interactors. Two independent purifications of Tctn1-LAP and associated proteins were subjected to mass spectrometric analysis. The identities of 154 proteins identified specifically in the Tctn1-LAP purifications and for which at least three unique peptides were found are listed. For each of these proteins, the following features are indicated: gene symbol, description, molecular weight, species of origin, unique peptides and total spectra identified, percent sequence coverage for both Tctn1-LAP and control LAP purifications, gene ontology information (GO), ENSEMBL gene and protein IDs, number of times the protein has been identified in ciliary-related screens (references for these screens are provided as a separate sheet), number of species in which the protein has been connected to cilia function, whether the protein in question appeared as a hit on each of the screens mentioned above, and associated human diseases as listed in OMIM.