Supplemental material

Dharmat et al., https://doi.org/10.1083/jcb.201712117

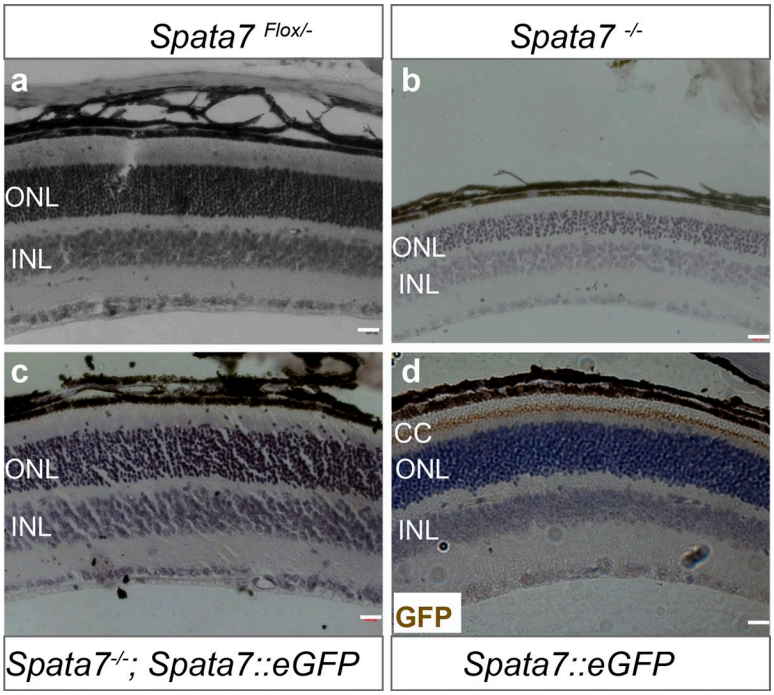


Figure S1. ***Spata7*::GFP transgenic expression can rescue the mutant phenotype of *Spata7*$^{-/-}$ mice. (a–c)** H&E staining of paraffin-embedded retinal sections of *Spata7*$^{flox/-}$ (a), *Spata7*$^{-/-}$ (b), and *Spata7*$^{-/-}$; *Spata7*::GFP (c) background were assessed at P60 for loss of photoreceptor nuclei. No loss of photoreceptors was observed in mice with transgenic GFP::*Spata7* construct. **(d)** H&E staining of *Spata7*-EGFP displaying localization at the CC in *Spata7*-BAC EGFP background. Bars, 20 µm. INL, inner nuclear layer; ONL, outer nuclear layer.

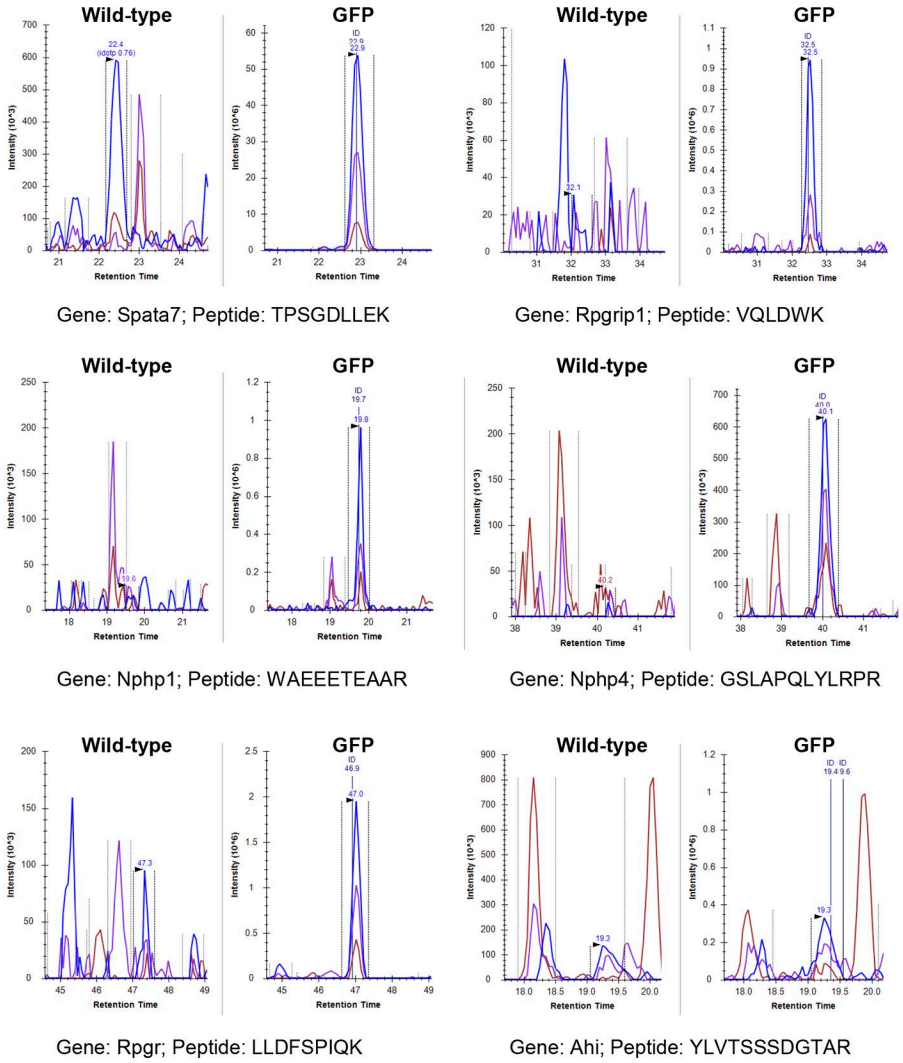


Figure S2. **MS1 peptide area using Skyline manual quantification.** Chromatograms and peak intensity traces from MS1 scan data obtained from a representative precursor ion (peptide) for each gene in the WT and GFP-tagged samples. The vertical lines and arrow mark the retention time and identification.

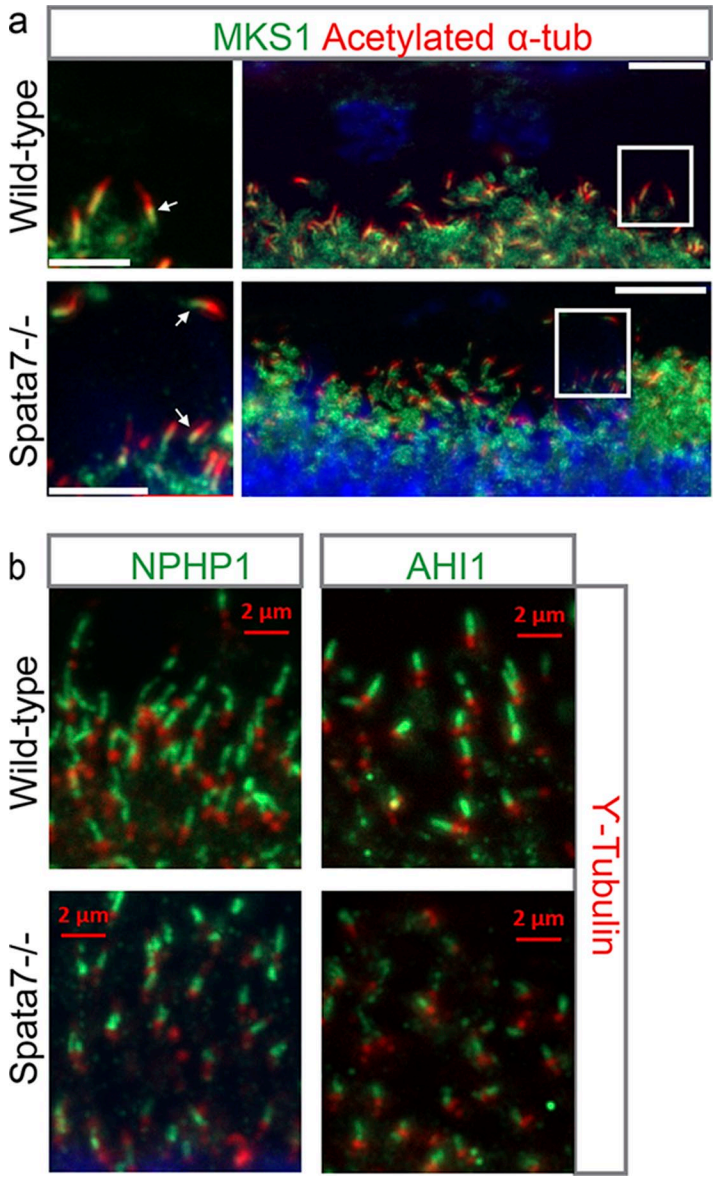


Figure S3. **Localization of MKS1, AHI1, and NPHP1 at the photoreceptor CC of WT and *Spata7* mutant photoreceptor CC. (a)** Localization of MKS1, AHI1 and NPHP1 at the photoreceptor CC. Retinal immunofluorescence displaying MKS1 (green) localized at the PCC region (indicated by arrows in insets) of the CC of both WT and *Spata7* mutant CC. Bars (main images) 10 µm; (insets) 5 µm. **(b)** NPHP1 and AHI1 (green) proteins localized in the CC and did not overlap with the basal body marked by γ-tubulin (red) in both WT and *Spata7* mutant retinae.

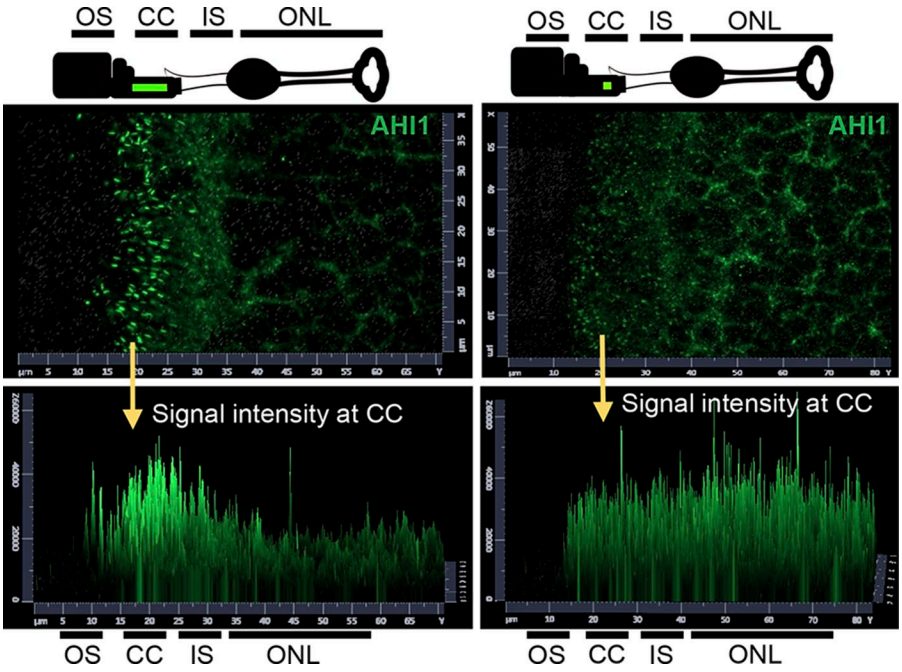


Figure S4. **AHI1 is mislocalized in *Spata7* mutant mouse photoreceptor cells.** Compared with WT (left), partial mislocalization of AHI1 (green) is detected into the outer nuclear layer (ONL), and its levels are significantly decreased in the CC in *Spata7* mutant retina immunofluorescence at P15 (right). The surface plots (bottom) quantitatively show the distribution of the fluorescent signal intensity of AHI1 in the corresponding images above. IS, inner segment; OS, outer segment.

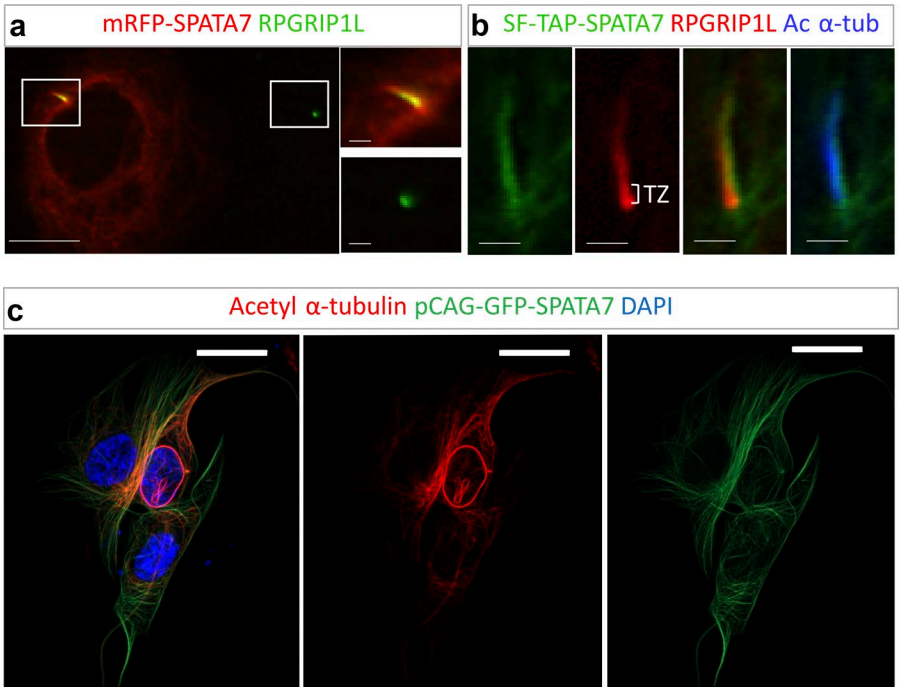
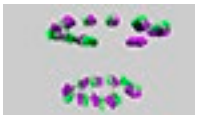


Figure S5. **Apical translocation of RPGRIP1L in the presence of mild overexpression of SPATA7. (a)** Ectopic expression of mRFP-SPATA7 in hTERT-RPE1 cells (red; left box) induced extension of the TZ marker RPGRIP1L (anti-RPGRIP1L, green; overlay is yellow) apical to the TZ, whereas RPGRIP1L (green spot; right box) remained at the TZ without expression of SPATA7. **(b)** Ectopic expression of SF-TAP-SPATA7 (anti-Flag; green) extended the location of endogenous RPGRIP1L (anti-RPGRIP1L, red; overlay is yellow) apical of the TZ along the length of the ciliary axoneme, marked by acetylated α-tubulin (blue). **(c)** High ectopic expression of SPATA7 induced cytoskeletal destabilization marked by acetylated α-tubulin, which was decorated by SPATA7-GFP. These cells cannot induce ciliogenesis and were excluded from analysis. Bars: (a, main images) 10 µm; (a, single-cilium images, and b) 2 µm; (c) 20 µm.



Video 1. **3D architecture of the DCC and PCC regions of the *Spata7* mutant photoreceptor CC.** Continuous isodense surfaces are colored as green (A-microtubule) and purple (B-microtubule). Cross-sectional segments at the DCC and PCC regions are displayed, showing the intact complex at the PCC and a progressive unraveling of the axoneme toward the DCC.

Table S1 is a separate Excel document showing Skyline, iBAQ, and specific peptide fold change for top interacting candidates (Sheet 1) as well as iBAQ and specific peptide fold change for all candidates observed across three biological repeats (Sheet 2).

Table S2 is a separate Excel document showing antibody information.