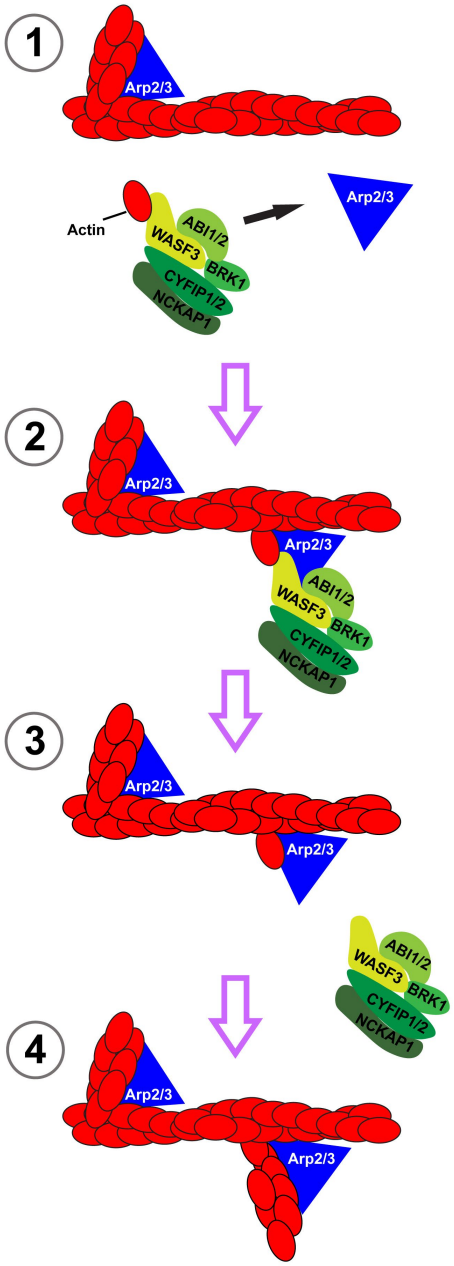
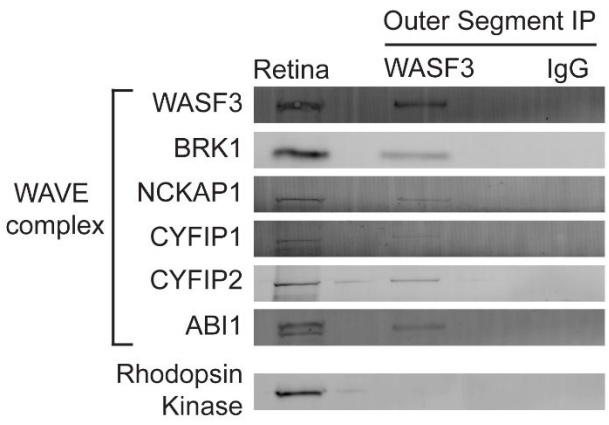
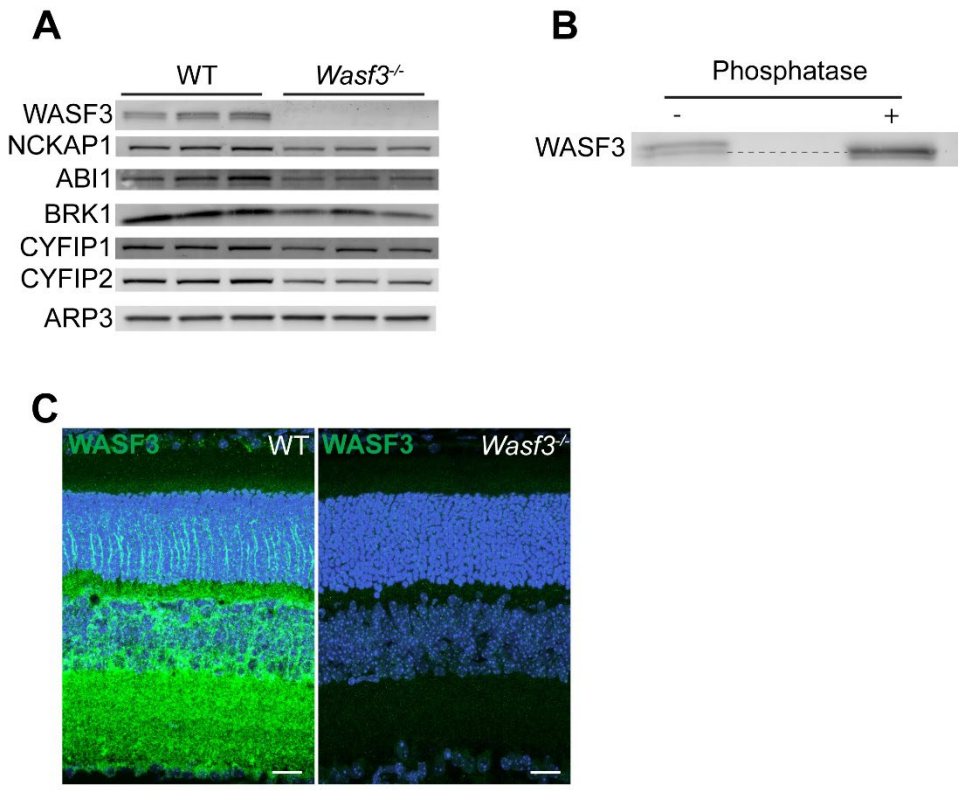
Supplementary Information



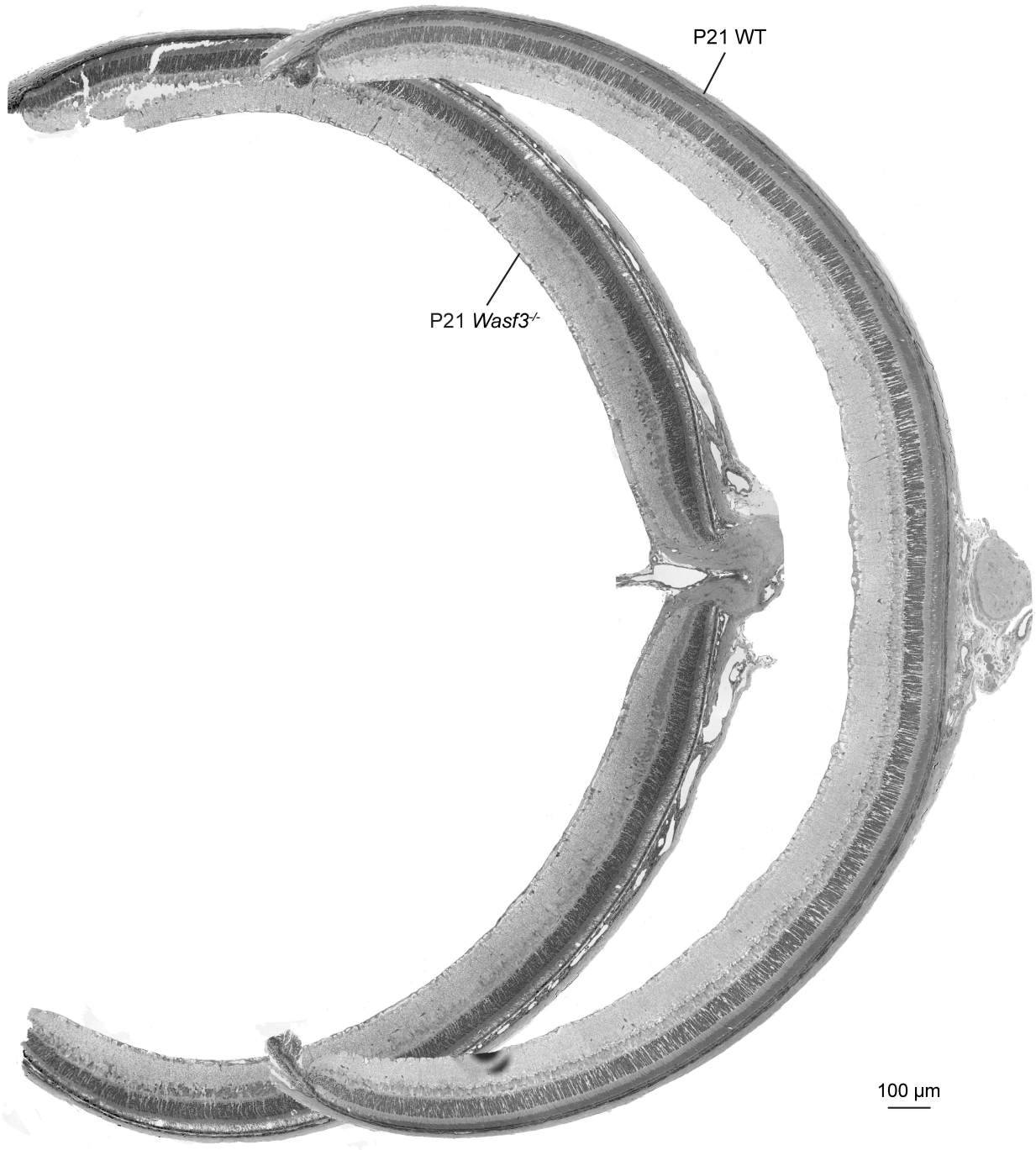
Supplementary Figure 1. The roles of the Arp2/3 and WAVE complexes in building branched actin networks. The cartoon illustrates the generally accepted mechanism by which the Arp2/3 and WAVE complexes function in building branched actin networks. See (1) for a review. Arp2/3 is labeled in blue, the WAVE complex in green and actin in red. The indicated WAVE complex subunits represent specific isoforms identified in this study. *In step 1*, the WAVE complex (whose WASF subunit is bound to an actin monomer) interacts with Arp2/3. *In step 2*, Arp2/3 associated with both monomeric actin and the WAVE complex binds to the side of a preexisting actin filament. *In step 3*, the WAVE complex disassociates from the filament leaving Arp2/3 behind. *In step 4*, the newly seeded actin branch elongates.



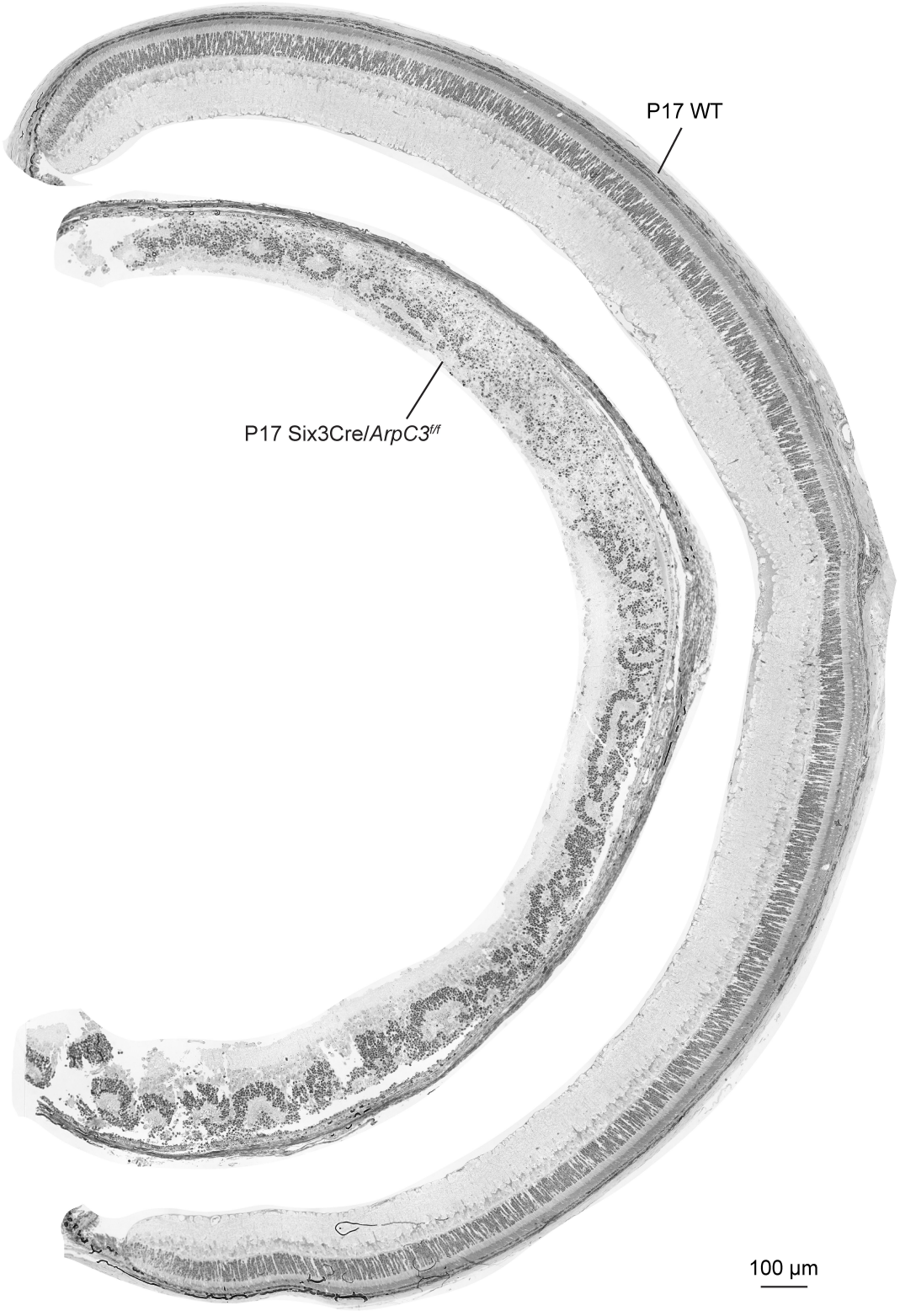
Supplementary Figure 2. Immunoprecipitation of the WAVE complex from purified mouse rod outer segments. Western blot showing co-precipitation of WASF3 with WAVE complex members BRK1, NCKAP1, CYFIP1/2, and ABI1 from lysed rod outer segments using the anti-WASF3 antibody. Immunoblotting for rhodopsin kinase, an outer segment protein not associated with the WAVE complex, was used as a negative control. Whole retinal lysate was used as a reference.



Supplementary Figure 3. Confirmation of the loss of WASF3 in the retina of *Wasf3*$^{-/-}$ mice. (A) Western blots of WAVE complex subunits from retinal lysates of WT and *Wasf3*$^{-/-}$ mice at P21. Three mice of each genotype were analyzed. Each lane was loaded with 20 µg total protein. (B) Retinal cross sections of WT and *Wasf3*$^{-/-}$ mice at P21 immunostained with anti-WASF3 antibody (green). Nuclei are stained with Hoechst (blue). The scale bars are 20 µm. (C) Western blot of WASF3 from WT retinal lysate, which was either treated or not treated with calf intestinal phosphatase. The horizontal dashed line highlights the location of the lower band.

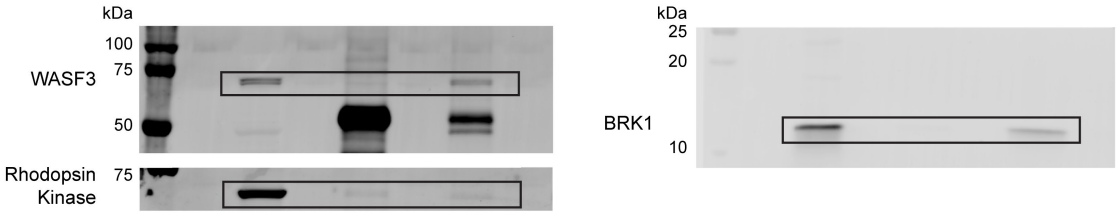


Supplementary Figure 4. Representative tile scanned images of the entire retina from P21 WT and *Wasf3*$^{-/-}$ mice.

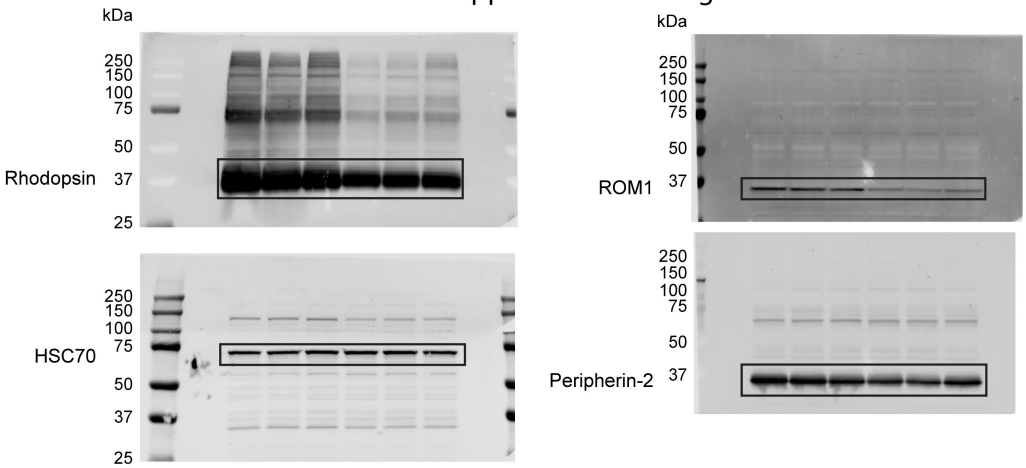


Supplementary Figure 5. Representative tile scanned images of the entire retina from P17 WT and *Six3Cre/ArpC3*$^{f/f}$ mice.

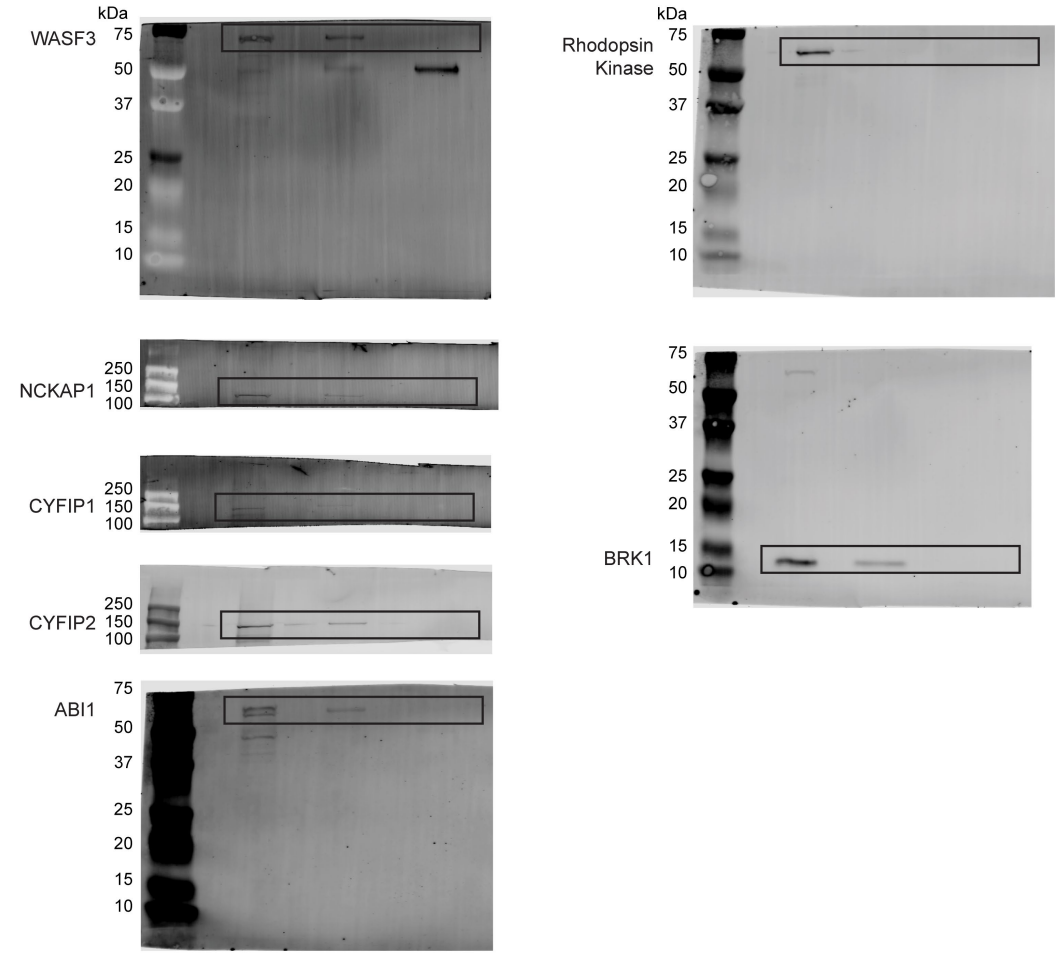
Uncropped blots from Fig. 2

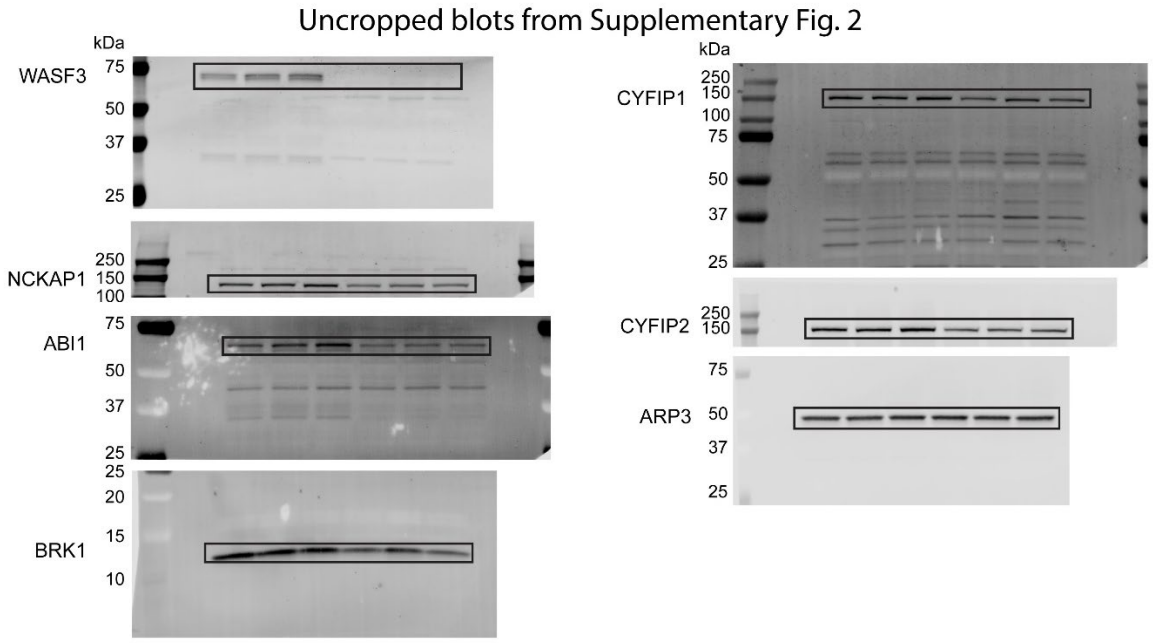


Uncropped blots from Fig. 5



Uncropped blots from Supplementary Fig. 1





Supplementary Figure 6. The uncropped images of western blots from Figs. 2, 5 and Supplementary Figs. 1,2. The black boxes indicate the cropped region shown in the main figures.

Dataset S1. Proteins confidently identified by mass spectrometry in a preparation of isolated bovine rod outer segments. Shown are proteins represented by two or more identified peptides with protein confidence value <0.05. The data are sorted by the number of spectral counts, except for the four WAVE complex subunits shown at the top and highlighted in yellow.

Dataset S2. Proteins confidently identified by mass spectrometry in immunoprecipitates from lysates of purified mouse rod outer segments obtained with anti-WASF3 or control IgG antibodies. For each protein, the total number of unique peptides, the confidence score associated with the protein's identification, and the total ion intensity of all identified peptides are shown. Protein enrichment was calculated by dividing the total ion intensity of the protein's identified peptides in the WASF3 immunoprecipitate by the protein's total ion intensity in the control immunoprecipitate. Both average and median enrichment values across four independently conducted experiments are listed. Proteins in the table are sorted from highest median enrichment to lowest. The WAVE complex subunits are highlighted in yellow. Proteins included in the table are: 1) identified in at least three experiments; 2) have at least 2 identified peptides in any given experiment; 3) enriched, on average, by at least 2-fold across all experiments.

Dataset S3. Proteins confidently identified by mass spectrometry in immunoprecipitates from lysates of purified mouse rod outer segments obtained with anti-ABI1 or control IgG antibodies. For each protein, the total number of unique peptides, the confidence score associated with the protein's identification, and the total ion intensity of all identified peptides are shown. Protein enrichment was calculated by dividing the total ion intensity of the protein's identified peptides in the WASF3 immunoprecipitate by the protein's total ion intensity in the control immunoprecipitate. Average enrichment values between two independently conducted experiments are listed. Proteins in the table are sorted from highest to lowest enrichment. The WAVE complex subunits are highlighted in yellow. Proteins included in the table are: 1) identified in both experiments; 2) have at least 2 identified peptides in at least one experiment; 3) enriched by at least 2-fold after averaging the values from both experiments.

Supplementary Movie 1. A video showing a 3D volume representation of a Z-stack confocal image of a P21 WT retinal cross section stained with phalloidin to label F-actin. The video zooms in on the inner-outer segment juncture.

Supplementary Movie 2. A video showing a 3D volume representation of a Z-stack confocal image of a P21 *Wasf3*$^{-/-}$ retinal cross section stained with phalloidin to label F-actin. The video zooms in on the inner-outer segment juncture.

References

1. T. Takenawa, S. Suetsugu, The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* **8**, 37-48 (2007).