Jiang et al., http://www.jcb.org/cgi/content/full/jcb.201410112/DC1

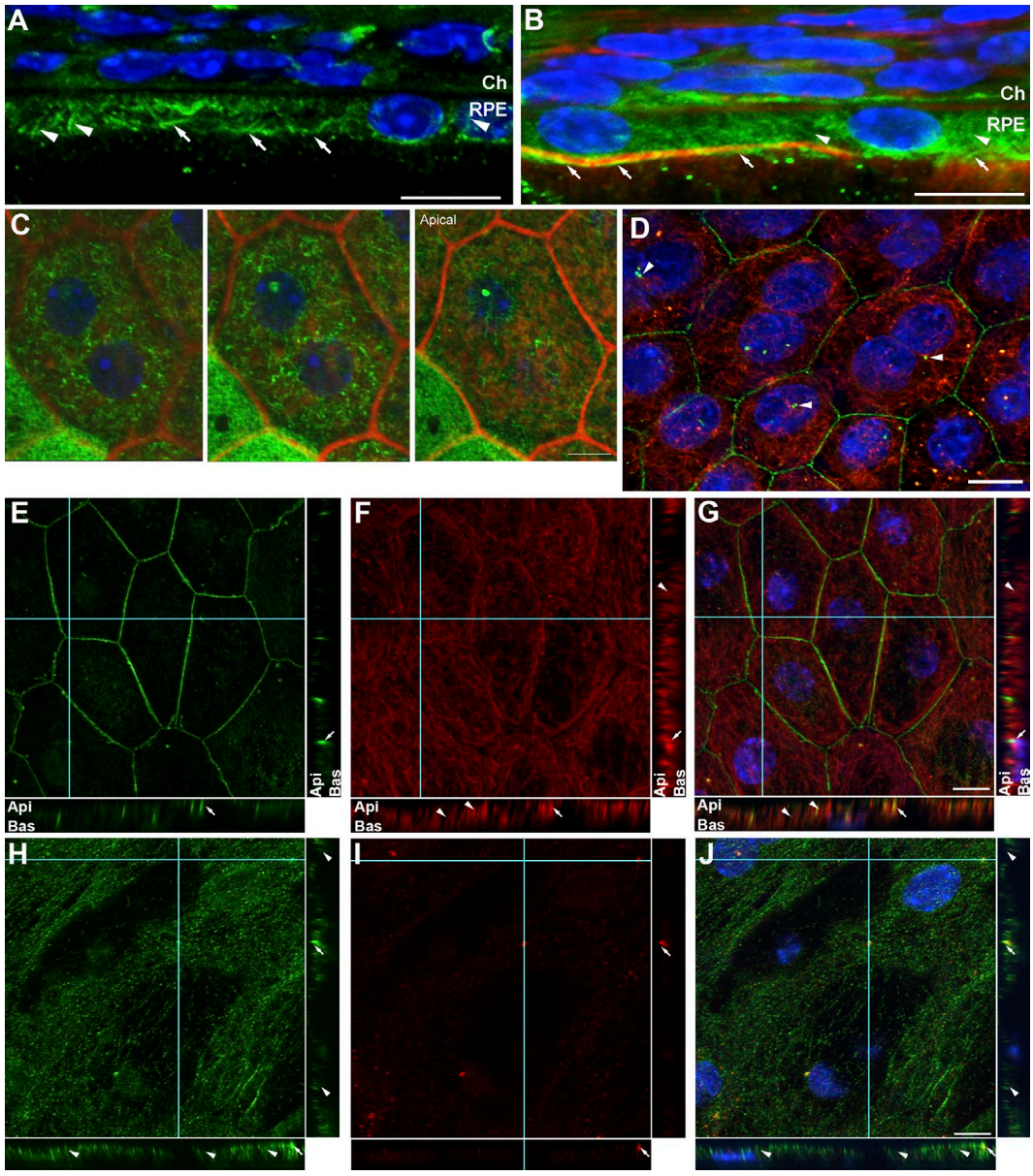


Figure S1. **Microtubule organization in the RPE in vivo and in vitro.** (A and B) Confocal images of apical-basal sections of the RPE from albino mice, showing immunolabeling of α-tubulin (green). In B, phalloidin-TRITC labeling of F-actin (red) is also shown. Horizontal (arrows) and apical-basal (arrowheads) microtubules are evident. Most of the horizontal microtubules are in the apical part of the cell body, in the same plane as the circumferential actin filaments. (C) Images from a whole mount of the retina from an albino mouse, showing three different z-planes of a single cell, all near the boundary between the apical domain and the cell body. α-Tubulin immunolabeling is green, and phalloidin labeling of F-actin is red. The bright green dot in the right panel, which shows the most apical of the three z-planes and includes part of the apical domain of the RPE (evident by the presence of F-actin throughout the plane), represents microtubules in a cilium, which projected into more apical z-planes (not depicted). (D) Image of a flat mount of RPE cells from an albino mouse, immunolabeled against PARD3 (green) and α-tubulin (red). Arrowheads indicate centrosomes from which microtubules radiate. (E–J) Superimposed stacks of confocal images from confluent cultures of primary mouse RPE cells, cultured on Transwell filters. Images were taken near the margin of the confluent sheet of cells. (E–G) Immunolabeling with antibodies against PARD3 (E), which labels tight junctions and centrosomes, and α-tubulin (F). G shows the image with merged channels. (H–J) Immunolabeling with antibodies against β-tubulin (H) and centrin (I). J shows the image with merged channels. On the right and at the bottom of each panel are orthogonal projections along the apical-basal axis indicated in cyan; the apical (Api) and basal (Bas) sides are indicated. Note the apical localization of centrosomes (arrows) and the presence of apical-basal microtubules (arrowheads). Microtubules that are oriented on the horizontal plane are also abundant (F and H). Bars, 10 µm.

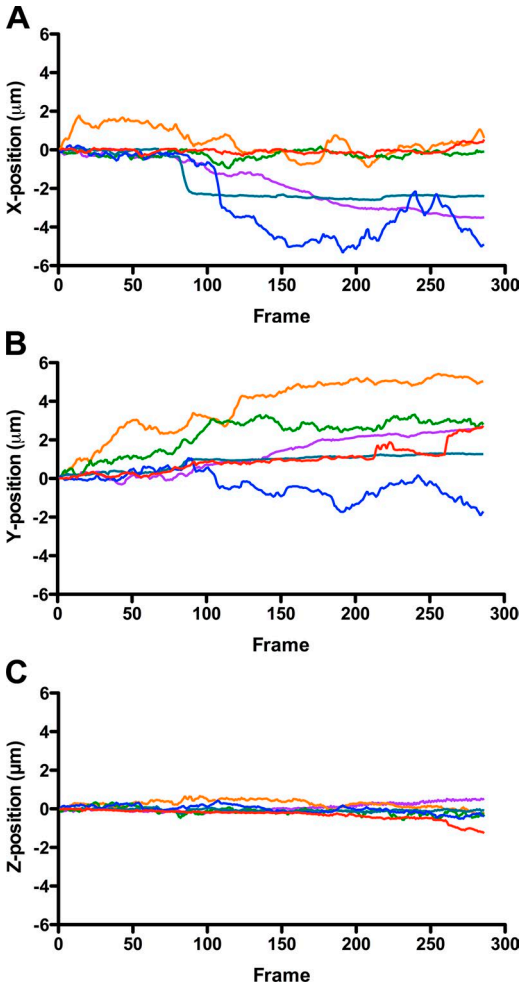


Figure S2. **3D tracking of phagosomes in polarized RPE cells in culture.** Tracks of POS phagosomes, 20 min after feeding primary RPE cells isolated from WT or *Klc1*$^{-/-}$ mice with mouse POSs. A stack of images, each containing seven different z-axis planes, was obtained every 0.6 s for 3 min, using a spinning disk confocal microscope system (UltraVIEW ERS). Displacement in the x axis (A), y axis (B), or z axis (C) is shown separately. Volocity software was used to perform the 3D analysis and determine the tracks. The tracks shown are representative of those obtained from two separate experiments, where $n \geq 18$ tracks from five or more cells for each filter.

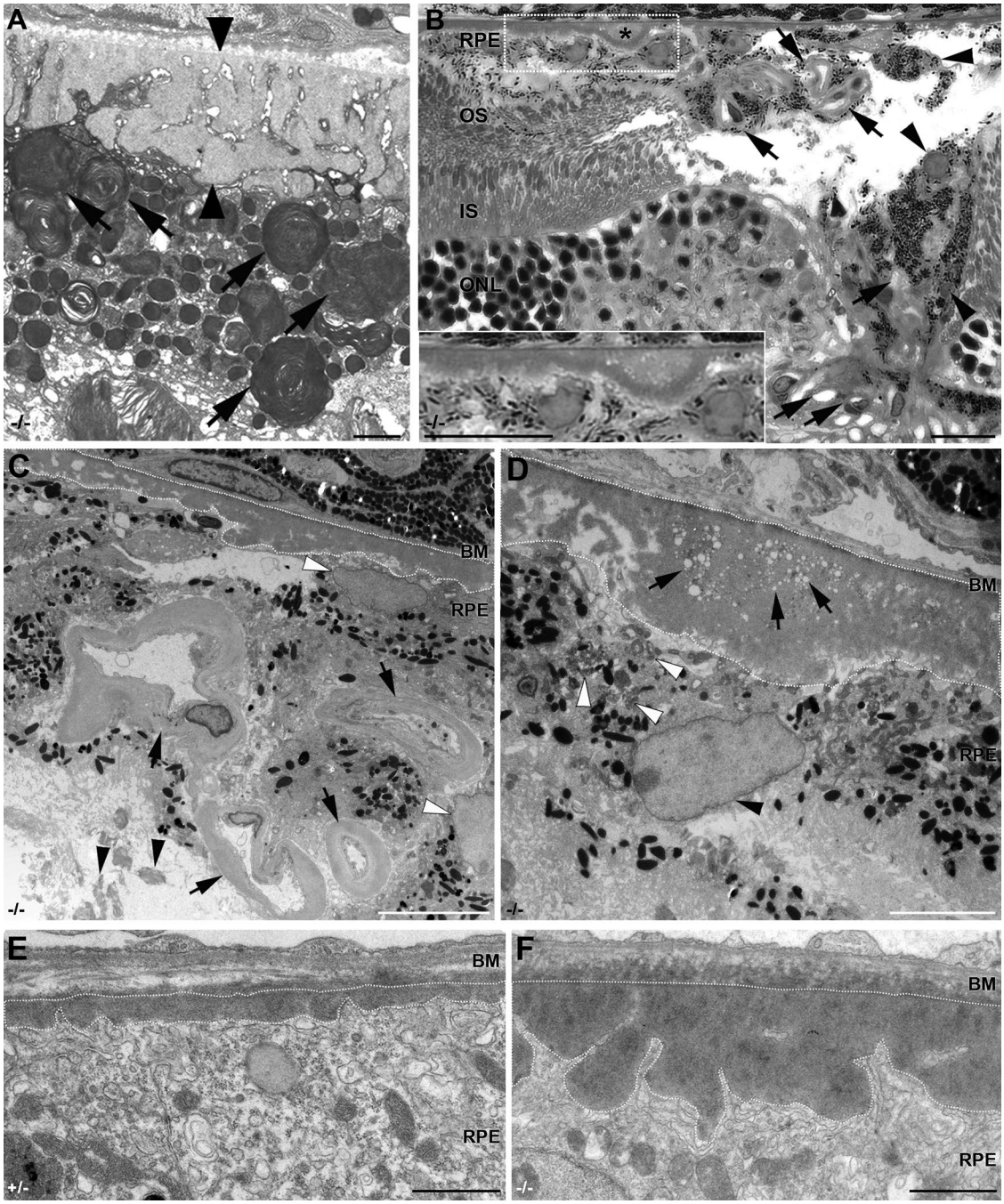
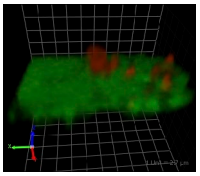
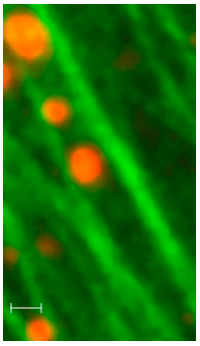


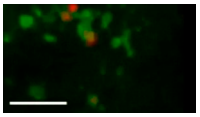
Figure S3. **Pathology in retinas of 19–22-mo-old *Klc1*$^{-/-}$ mice.** (A) EM of accumulated undigested phagosomes (arrows) in a later-stage impaired RPE cell. The enlarged Bruch's membrane is evident between the arrowheads. (B) Semithin section of a 22-mo-old *Klc1*$^{-/-}$ mouse exhibiting several characteristics of AMD. The asterisk indicates a dome-shaped deposit similar to a druse. Arrows indicate new blood vessels invading the retinal space, disrupting RPE cells (arrowheads) as well as the photoreceptor cell layer. The inset in the bottom-left of the panel corresponds to the magnified area outlined by the white dotted rectangle; it shows sub-RPE deposits, including the druse-like deposit. (C) EM of a 22-mo-old *Klc1*$^{-/-}$ mouse showing neovascularization. New blood vessels are indicated by arrows, and RPE cells (white arrowheads) are perturbed. Black arrowhead indicates disorganized disk membranes from degenerating photoreceptors. The white dotted line outlines sub-RPE deposits. (D) EM of a druse-like sub-RPE deposit from a 22-mo-old *Klc1*$^{-/-}$ mouse. The deposit appears to contain membranous debris (arrows). The black arrowhead indicates an RPE cell, adjacent to this deposit, loaded with undigested material (white arrowheads). The white dotted line outlines the area of the druse-like sub-RPE deposit plus adjacent basal laminar deposits. (E and F) EMs of 19-mo-old control (*Klc1*$^{+/-}$) (E) and *Klc1*$^{-/-}$ (F) mice, determined by PCR to lack the *Crb1*$^{rd8/rd8}$ mutation. The difference in the thickness of the sub-RPE deposits, between mutant and control, is comparable to that found with other mice used in the present report (Fig. 7). White dotted lines outline the sub-RPE deposits. BM, Bruch's membrane; RPE, retinal pigment epithelium; OS, outer segments; IS, inner segments; ONL, outer nuclear layer. Bars: (A) 1 µm; (B) 20 µm; (C and D) 5 µm; (E and F) 1 µm.



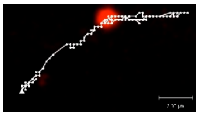
Video 1. **Ingestion of POSs by a cultured primary mouse RPE.** The cell was transfected with pEGFP and incubated with Texas red-X–labeled mouse POSs. It was analyzed by time-lapse microscopy, using a spinning disk confocal microscope system (UltraVIEW ERS). A stack of images was obtained of 102 different z-axis planes, 0.2 μm apart, every 62 s. Volocity software was used to reconstruct the 3D images. The movie comprises 3D images from seven z-stacks, and its playback rate is one 3D image per second (i.e., 62 times real speed). Images of individual frames of this video are shown in Fig. 1 A.



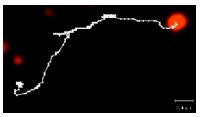
Video 2. **Movement of POS phagosomes along microtubules in an RPE cell.** Cultured primary mouse RPE cells were transfected to express GFP–α-tubulin and incubated with mouse POSs (red) labeled with Texas red-X succinimidyl esters. The cells were analyzed by time-lapse microscopy, using a spinning disk confocal microscope system (UltraVIEW ERS). Single z-plane images were collected for each color sequentially, with one dual-colored frame obtained every second for 2 min. The playback rate of the movie is 10 frames per second (i.e., 10 times real speed). Bar, 1 μm. Images of individual frames of this video are shown in Fig. 1 B.



Video 3. **Association of KLC1 with motile POS phagosome in an RPE cell.** RPE cells were transfected to express KLC1A-YFP (green) and incubated with bovine POSs (red) labeled with Texas red-X succinimidyl esters. The cells were analyzed by time-lapse microscopy, using a spinning disk confocal microscope system (UltraVIEW ERS). Single z-plane images were collected for each color sequentially, with one dual-colored frame obtained every second. The playback rate of the movie is five frames per second (i.e., five times real speed). The movie shows two different segments of the same region. A POS phagosome remains associated with KLC1-YFP as it moves back and forth, near the center of the frame. Because each frame contains red and green channel images that were acquired sequentially, the red and green fluorescence appear less colocalized when the phagosome moves at a faster speed. KLC1-YFP also labels motile endosome-like organelles. Bar, 2 μm. Images of individual frames of the first segment of this video are shown in Fig. 3 E.



Video 4. **POS phagosome tracking in an RPE cell.** Cultured primary mouse RPE cells were incubated with Texas red-X–labeled mouse POSs. The cells were analyzed by time-lapse microscopy, using a spinning disk confocal microscope system (UltraVIEW ERS). Single z-plane images were collected at 2.9 frames per second for 3 min. The playback rate of the movie is 15 frames per second (i.e., five times real speed). The trajectory was obtained by Volocity software and is shown as a white line, with the location of the phagosome at each frame indicated by a dot on the line (some dots are superimposed on each other). Arrowhead indicates the location at the end of the 3-min track. Bar, 2 μm. Image of an individual frame is shown in Fig. 4 C.



Video 5. **POS phagosome tracking in an RPE cell.** Cultured primary mouse RPE cells were incubated with Texas red-X–labeled mouse POSs. The cells were analyzed by time-lapse microscopy, using a spinning disk confocal microscope system (UltraVIEW ERS). Single z-plane images were collected at 2.9 frames per second for 3 min. The playback rate of the movie is 15 frames per second (i.e., five times real speed). The trajectory was obtained by Volocity software and is shown as a white line, with the location of the phagosome at each frame indicated by a dot on the line (some dots are superimposed on each other). Arrowhead indicates the location at the end of the 3-min track. Bar, 2 μm.