Online Supplementary Material

Materials and methods

Immuno electron microscopy

Pre-embedding

Animals were anesthetized with an intraperitoneal injection of xylazine (5 mg/kg) and ketamine (70 mg/kg) before intracardiac perfusion with cold PBS, followed by perfusion with a mix of paraformaldehyde 4% and glutaraldehyde 0.5% in cold PBS. Eyes were then removed and the cornea and lens dissected out. After embedding in 4% w/v agarose in PBS, eyecups were sectioned with a VT1000S vibratome (Leica Microsystems GmbH, Wetzlar, Germany) to obtain 40 µm thick slices. After a 1 hour blocking in PBS containing 0.1% saponin, 5% NGS and 5% BSA, tissues were incubated with the primary antibody in PBS + 0.1% saponin for 48 hours at 4°C. Secondary biotinylated goat anti-rabbit antibodies (Vector Laboratories, Burlingame, CA, USA) were applied for 2 to 4 hours at room temperature. The avidin-biotin-peroxidase ABC kit (Vector Laboratories, Burlingame, CA, USA) was used at a 1/500 dilution, for 45 minutes at room temperature, and revelation was performed in DAB 0.05%, H₂O₂ 0.03% in a 0.1 M Tris buffer pH 7.8 for 6 to 8 minutes under visual control. Tissues were postfixed in glutaraldehyde 2.5% for at least one hour at room temperature, rinsed with PBS and incubated in 1% osmium tetroxide. They were then dehydrated in graded ethanol solutions and, after propylene oxide treatment, embedded in an araldite-epon mixture. Ultra-thin sections (80–100 nm) were placed on grids for direct observation on a CM12 transmission electron microscope (Philips Electron Optics, Eindhoven, The Netherlands).

Tokuyasu post-embedding
Post-embedding immunogold labeling was performed as previously described. Retinas were dissected from mouse perfused with a mixture of 4% formaldehyde and 0.3% glutaraldehyde in PB. After several rinses in buffer, the samples were cryoprotected in 2.3M sucrose overnight and frozen in liquid nitrogen. Ultrathin cryosections (60-90nm) were performed at -110°C with a Leica ultracut S ultramicrotome equipped with a FCS cryochamber (Leica Microsystems GmbH, Wetzlar, Germany). For immunogold labeling, thawed sections were first rinsed in 150 mM glycine and blocked in 1% BSA. The primary antibody (H4) was used at a 1/500 dilution and incubated for 1 hour. Colloidal gold (10 nm) conjugated protein A (University of Utrecht, Netherlands) was used for primary antibody visualization. After postfixation in 1% glutaraldehyde, sections were contrasted with 0.3% uranyl acetate in 2% methyl cellulose and observed with a Morgagni transmission electron microscope (FEI, Netherlands).