# Supplemental material

### Methods for video microscopy.

Human photoreceptors were examined in unstained retinal whole mounts of donor eyes obtained ≤3 hr post-mortem and prepared as described.[58](#_ENREF_58) The neurosensory retina was dissected free from the RPE, laid photoreceptors-up on a slide, cleared in 100% dimethyl sulfoxide, and mounted with 100% glycerol. Tissue was viewed with a 100X 1.4 NA oil immersion objective, Nomarski differential interference contrast optics, and analog monochrome video. Focus series through IS were obtained with a smooth manual focus, beginning near the OS, moving sclerad to the ELM, and then returning vitread to the OS. Nomarski prisms remained at the same rotation angle throughout. Video clips were selected from a 68-year-old male donor with peak cone density of 170,100 cones/mm2 and without grossly visible macular chorioretinal pathology. Analog video obtained in 1987 was converted to digital in 2008 for editing and annotation in 2011.

Litts\_Video1\_rod-free\_zone.mp4: mosaic of cone inner segments 0.149 mm nasal to the foveal center.

Litts\_Video2\_perifovea.mp4: mosaic of cone and rod inner segments, 2.5 mm temporal to the fovea on the temporal horizontal meridian.

Litts\_Video3\_foveal\_center.mp4: mosaic of cone inner segments at the foveal center

### Methods for ultrastructural morphometry of photoreceptors (Figure 3)

Fellow eyes of a 10-year-old male macaque (M. Mulatta) were obtained from the laboratory of Paul Gamlin, PhD, where the animal was euthanized by deep sodium pentobarbital anesthesia (200 mg/kg IV), followed by exsanguination by cardiac perfusion with saline followed by fixative. The eyes were preserved by immersion in 2% glutaraldehyde/1% paraformaldehyde for transmission electron microscopy (TEM). Animal work was not done for this dissertation. Previously unpublished images (Figure 4) and morphometric analysis of normal cone photoreceptors (Figure 5) are included in the introduction of this dissertation for comparison to degenerating cones discussed in this dissertation.

 Tissue blocks containing the fovea were osmicated and embedded in epoxy resin, and sectioned *en face*, and monitored by light microscopic examination of toluidine blue-stained 0.8 µm sections until IS were attained. TEM images were analyzed in 2 ISel levels, near the myoid (base), and near the OS (apex). At 6 macular locations (regions 1 to 6), 6 cones at each ISel level were analyzed. Regions 1 and 2 were rod-free (fovea) and regions 3 to 6 were rod-containing (perifovea). Measurements from 3 cones in foveal regions, and 1 cone and 3 rods in perifoveal regions, including all mitochondria and cilium (visible only near OS) were collected using ImageJ. Dimensions recorded include area, mean gray value, centroid, and fitting ellipse in the Freehand Selection mode using a digitizing tablet (Intuos 3, Wacom). For the electron density ratio, ISel cytosol was sampled adjacent to every 20th mitochondrion encountered in each cone. Only 2 cytosol samples were measured in rods due to their small cross-section.

 Data were analyzed using custom MATLAB code to generate the following parameters: cross-sectional diameter of cone ISel, rod ISel, mitochondria, and cilia; number of mitochondria in cone and rod ISel; numerical density of ISel mitochondria (number/ µm2); fraction of ISel cross-section occupied by mitochondria and cilia; ratio of electron density between the mitochondria and surrounding cytosol. To determine if mitochondrial numerical density varied across the ISel in *en face* sections, densities were calculated within 5 elliptical annular zones centered on the ISel geometric center with a major radius of 0.475 μm and a minor radius of 0.375 μm. Each cone ISel in foveal regions had ≤ 3 concentric zones. For each measure, each data point in the Figures represents an average of cones in 6 micrographs for that level. Data were similar for 2 fellow eyes sampled. Right eye data are shown in Figure 4 and 5 of the Introduction.