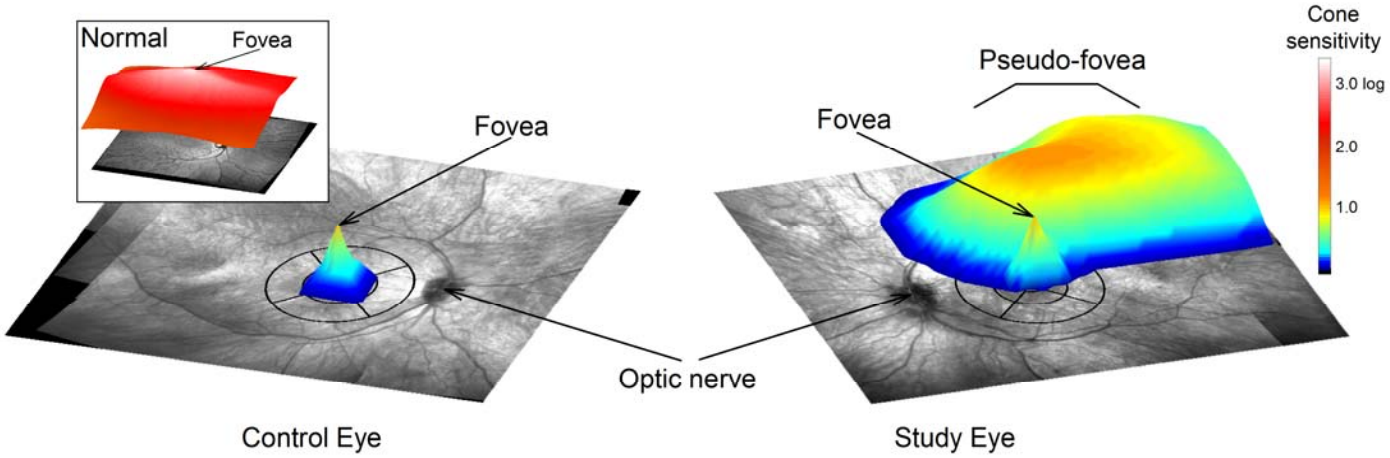
Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Cideciyan AV, Hauswirth WW, Aleman TS, et al. Vision 1 year after gene therapy for Leber's congenital amaurosis. N Engl J Med 2009;361:725-7.

Supplementary Figure



Legend to Supplementary Figure. Spatial distribution of cone vision displayed in three dimensional representation across control and study eyes of P2. 'Pseudo-fovea' region of fixation corresponded to the area of treatment and to the extrafoveal region of cone function gained shortly after treatment. It is important to note that cone function in the 'pseudo-fovea' has higher sensitivity than the cone function in the untreated fovea. Inset left, cone vision in normal eyes. Although all three patients in our trial demonstrated retinal regions with significant improvements in visual sensitivity after treatment[a,b,c] the slow emergence of an additional perceptual gain and the shift in fixation was observed only in P2. The lack of a shift in fixation in the other two patients is explained as follows: P1 has no demonstrable extrafoveal cone function;[a,b,c] P3 has treatment-related increase in cone function but his cone sensitivity in the treated region was lower than in the fovea centralis.[a,b,c]

Legend to Supplementary Videos 1-8. Video representation of fixation data of P2 at 1 month (Videos 1-4) and 12 months (Videos 5-8) after treatment. Each video shows fixation epochs of 10 second duration to one of four target luminances from brighter (2.7 log) to dimmer (1.8 log). The location of the continuously illuminated deep red target (685 nm peak; 80 nm FWHM) is represented by the small circle. The target subtended 1 degree in the visual field. Normal perceptual threshold was near 0.0 log (arbitrary) units. Video imaging of the retina was performed monocularly with near-infrared light (MP1, Nidek Technologies America, Inc, Greensboro, NC). Translation of each video frame with respect to a reference image was recorded at 25 Hz. Video representations of the fixation data were generated by applying the horizontal and vertical offset values to an NIR image of the fundus; each translated image then contributed a single frame of the video which was played back at the same 25 Hz rate as the original recording. Isosensitivity contours defining the foveal and supero-temporal regions of vision derived from microperimetric examinations are shown for reference (white lines). At the 1 month visit, P2 perceives the three highest luminance targets and fixates using the foveal region (Videos 1-3). The lowest luminance target is not perceived and P2 attempts to point straight ahead with a resulting fixation overlapping with the fovea as well as a larger extrafoveal region (Video 4). At the 12 month visit, P2 perceives all four targets including the dimmest target that was not perceived at all previous visits (Videos 5-8). For the highest luminance target, fixation is at the fovea (Video 5). At incrementally lower target luminances, fixation dwells increasingly longer periods in the supero-temporal retina that was treated (Videos 6-8).

Supplementary References.

a. Hauswirth WW, Aleman TS, Kaushal S, et al. Treatment of Leber congenital amaurosis due to *RPE65* mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase 1 trial. Hum Gene Ther 2008;19:979-90.

b. Cideciyan AV, Aleman TS, Boye SL, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. Proc Natl Acad Sci USA 2008;105:15112-17.

c. Cideciyan AV, Hauswirth W, Aleman TS, et al. Human RPE65 gene therapy for Leber congenital amaurosis: persistence of early visual improvements and safety at one year. Hum Gene Ther 2009 Jul 7. [Epub ahead of print]