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Supplemental information

Night vision restored in days

after decades of congenital blindness

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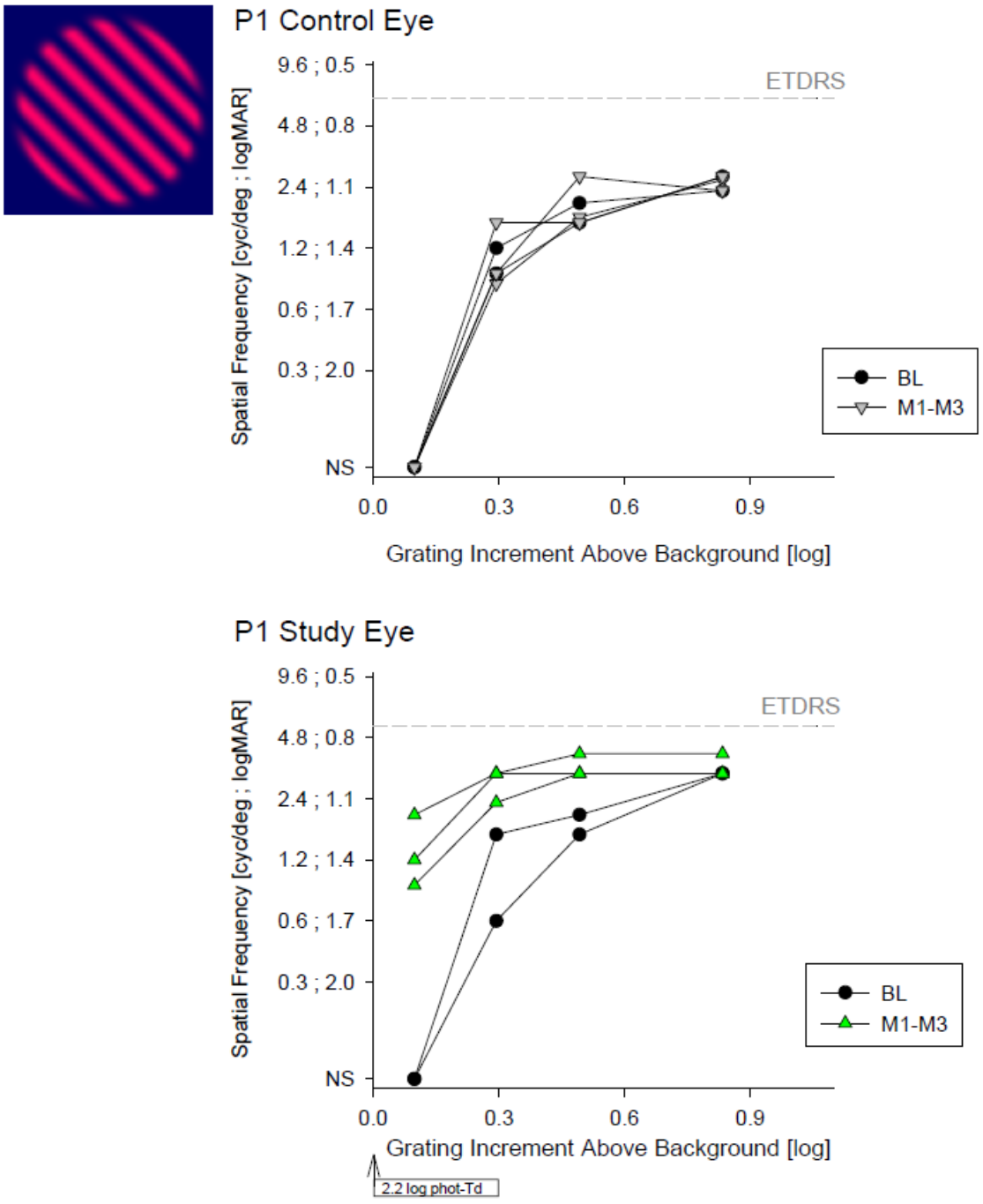
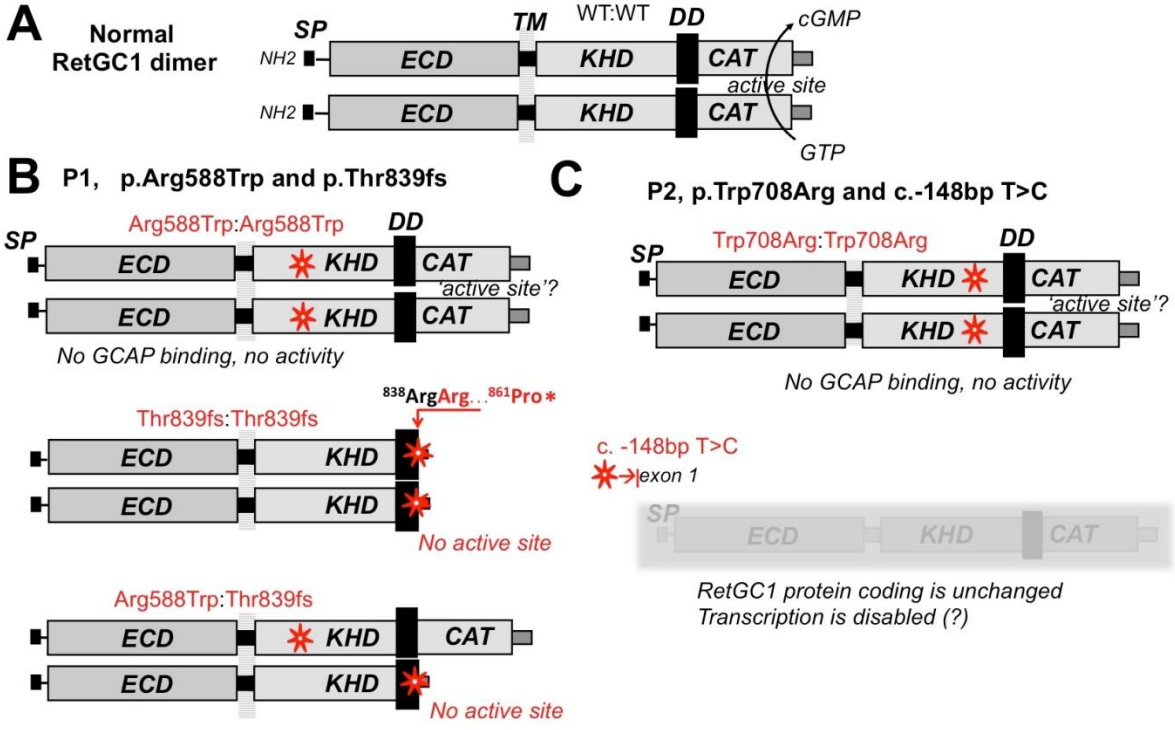
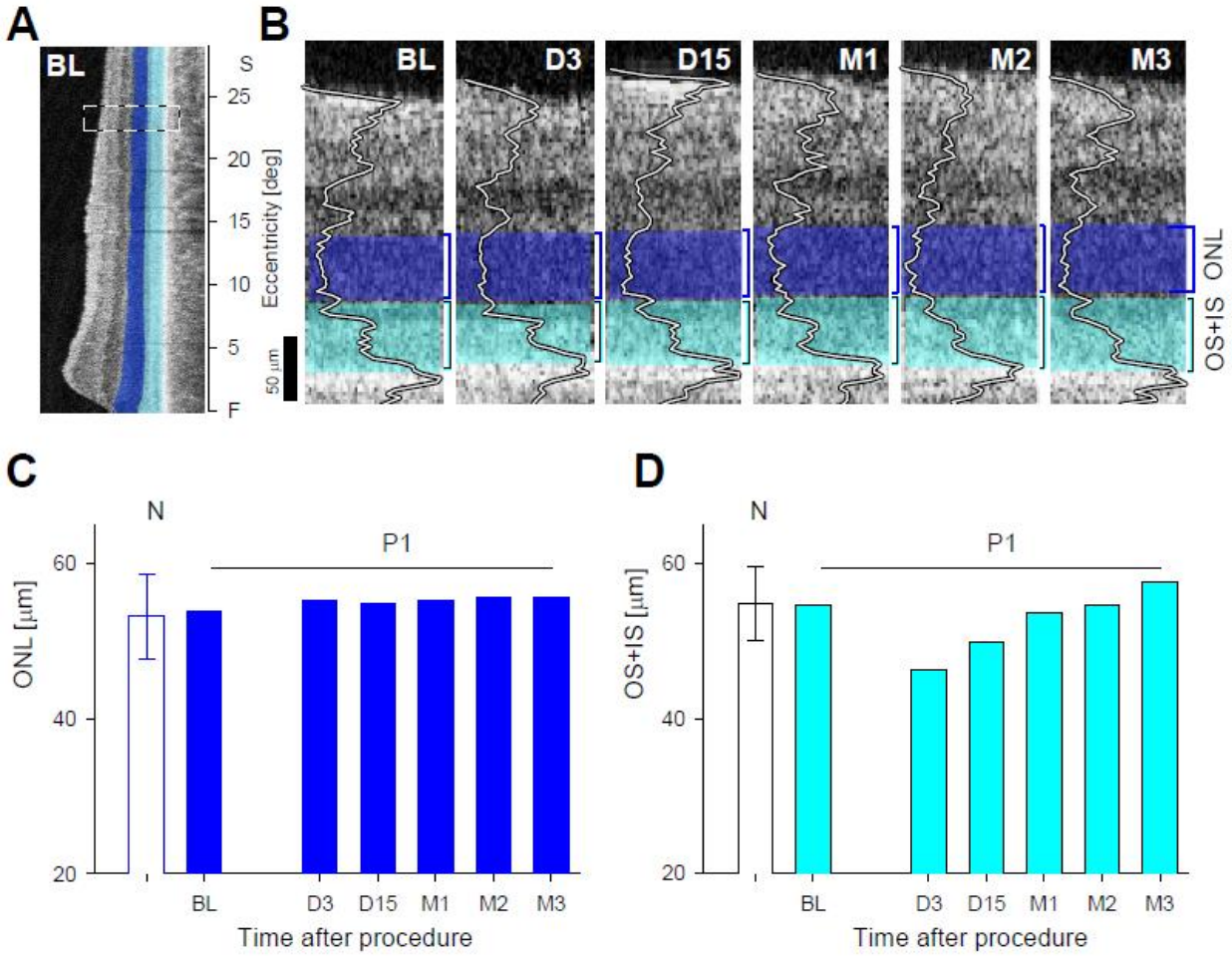


Supplemental Figure S1: Chromatic Grating Acuity in P1 at Baseline and Post-operative visits, Related to 'Changes to Visual Acuity' Results Section and STAR Methods, 'Spatial Vision.' Plotted are thresholds of spatial frequencies seen in each eye, at each visit, for each condition. BL, two baseline visits; M1-M3, months 1 through 3 post-operatively; NS, not seen. Note that the range of spatial frequencies available were 0.3 to 8 cyc/deg. Inset, schematic appearance of the red-on-blue gratings. ETDRS, standard acuities at baseline in each eye for reference.



Supplemental Figure S2: Changes of RetGC1 in P1 and P2. Related to 'Changes in RetGC1 Biochemical Properties Caused by the *GUCY2D*-LCA Mutations in P1 and P2' Results Section and STAR Methods, 'Guanylyl cyclase expression and assays.' (A) The schematics of the RetGC1 architecture. RetGC1 coded by a human *GUCY2D* gene (as well as its ortholog in rodents, GC-E, coded by *Gucy2e* gene (Yang et al., 1999) is a homodimer of a single-transmembrane polypeptide whose primary structure includes the removable N-terminal signal peptide (SP), the extracellular domain (ECD), the transmembrane region (TM), and the cytosolic segment, consisted of the kinase homology domain (KHD) and the catalytic domain (CAT) connected to each other via the dimerization domain (DD). KHD and DD domains are essential for binding GCAP (Peshenko et al., 2015a; Peshenko et al., 2015b). Dimerization of the two CAT domains is strictly required to produce the active site, as a single subunit is incapable of binding Mg^{2+}GTP as a substrate (Liu et al., 1997). (B) In P1, the p.Arg588Trp mutant CAT1 domain is preserved, yet the direct biochemical analysis shows that it fails to bind GCAP and produce active homodimer (see Figure 5); the product of the second LCA allele, Thr839fs, is a truncated polypeptide, a priori incapable of forming the active site, neither on its own, nor by dimerizing with the product of the other allele. (C) In P2, the products of the both alleles would potentially be able to form the active site. However, Trp708Trp RetGC1 fails to bind GCAP and remains inactive (see Figure 5). The c. -148T>C mutation in the promoter region does not alter the normal protein coding sequence of *GUCY2D*, but likely disables mRNA transcription from this LCA allele and thus prevents the synthesis of RetGC1 in photoreceptors from this allele.



Supplemental Figure S3: Retinal Structure at Site of Peak Sensitivity During the First Three Months after Treatment in P1, Related to Figure 3 and Discussion. (A) OCT scan along the vertical meridian starting at the fovea of P1 at baseline (BL). ONL layer is highlighted in blue and photoreceptor segments (OS+IS) lamina in cyan. White dashed lines outline the location of post-treatment peak rod sensitivity. (B) Magnifications of the retinal region of peak rod sensitivity at different times after treatment with overlaid longitudinal reflectivity profiles (D3, D15, days 3 and 15; M1, M2, M3, months 1 2, and 3). Blue and cyan brackets represent average ONL thickness and OS+IS length, respectively. (C) Measurements of ONL at location outlined in A and B. (D) Measurements of photoreceptor segment length at this location. Single values represent average of 5 measurements in the interval 22-24º at increments of 0.5º. White bar (marked N) is average normal value at the same location (N=13, error bar is the standard deviation).

Table S1. Genotype and age of *GUCY2D*-LCA patients, related to 'Experimental model and subject details' in STAR Methods

Patient ID	Age at treatment (years)/ Sex	Allele 1	Allele 2
P1	19/Male	c.1762C>T, p.Arg588Trp[a,b]	c.2516del, p.Thr839fs[a,b]
P2	32/Female	c.2122T>C, p.Trp708Arg[a]	c.-148T>C[a]

a. Originally reported in Stone, 2007; previously referred to in Stone, 2007, as -146T>C
b. Previously reported by our group in Jacobson et al., 2017

Table S2. Earliest timepoint at which rod-mediated blue FST increases were detectable in our Phase 1 *RPE65*-LCA Clinical Trial, related to Discussion section and Figure 1.

Patient ID[a]	Timepoint[b] (days)
P2	9
P5	30
P7	30
P9	8
P10	30
P11	5
P12	6
P13	5
P14	10
P15	10

a. Patient IDs match Jacobson et al., 2012. P1, P3, P4, P6 and P8 not listed did not demonstrate >0.9 l.u. rod-mediated improvement by Day 30.

b. Days post-treatment

Table S3. Magnitude of changes from baseline (CFB) compared across independent measures, related to Results section, Figures 1, 2 & 4, and Supplemental Figure S1.

	Control Eyes						Study Eyes					
	BL		M3		CFB[a]		BL		M3		CFB[a]	
Measure	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Light Detection Threshold												
DA FST threshold[b] (blue), log_{10}	-1.24	-2.38	-1.37	-2.67	-0.13	-0.29	-1.10	-2.37	-5.63	-5.47	**-4.53**	**-3.10**
DA FST threshold[b] (red), log_{10}	-1.24	0.00	-1.07	-0.60	+0.17	-0.60	-1.15	+0.54	-3.33	-3.00	**-2.18**	**-3.54**
DA Functional vision threshold, log_{10}	5.53	6.62[c]	5.79	6.01	0.29	-0.61	5.59	6.62[c]	1.18	0.22	**-4.41**	**-6.40**
DA chromatic perimetry[b,d] (blue), log_{10}	-0.40	0.00	-0.20	0.00	+0.20	0.00	-0.20	0.00	-5.30	-5.30	**-5.10**	**-5.30**
Mesopic microperimetry[b,d], log_{10}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-2.00	-1.60	**-2.00**	**-1.60**
DA TPLR threshold, log_{10}	-0.09	0.07	-0.53	-0.54	-0.44	-0.61	0.35	-0.05	-3.72	-2.29	**-4.07**	**-2.24**
Spatial Vision / Visual Acuity												
BCVA, logMAR	0.66	1.34	0.68	1.30	0.02	-0.04	0.72	1.34	0.66	1.26	-0.06	-0.08
CGA, logMAR[e]	>2.00	>2.00	>2.00	>2.00	0.00	0.00	>2.00	>2.00	1.20	>2.00	**-0.80**	0.00
LLVA (2ND), logMAR	>1.70	>1.70	1.54	>1.70	0.16	0.00	>1.70	>1.70	0.80	1.34	**-0.90**	**-0.36**

Abbreviations: BCVA, best-corrected visual acuity; BL, pre-treatment baseline; CFB, change from baseline; CGA, chromatic grating acuity; DA, dark-adapted; FST, full-field stimulus test; LLVA, low-luminance visual acuity; M3, month 3 after treatment; TPLR, transient pupillary light reflex

a. Negative CFB values represent improvement. Specific test-retest variabilities previously reported suggest changes larger than 1.00 log unit for light detection thresholds and changes larger than 0.30 log unit for spatial vision measures are thought to be statistically significant and clinically meaningful. Bolded CFB values represent substantial changes (Roman et al., 2007; Cideciyan et al., 2008; Cideciyan et al., 2012; Roman et al., 2013; Cideciyan et al., 2016; Wood et al., 2021; Roman et al., 2022a; Roman et al., 2022b).
b. Threshold equivalents of sensitivity values.
c. Imputed, see Results section.
d. Locations chosen 20° superior retina for P1 and 8° superior retina for P2, see Figures 2 and 3.
e. At 0.1 log photopic red increment above blue background