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Supplemental Information

Mature Retina Compensates Functionally for Partial Loss of Rod Photoreceptors

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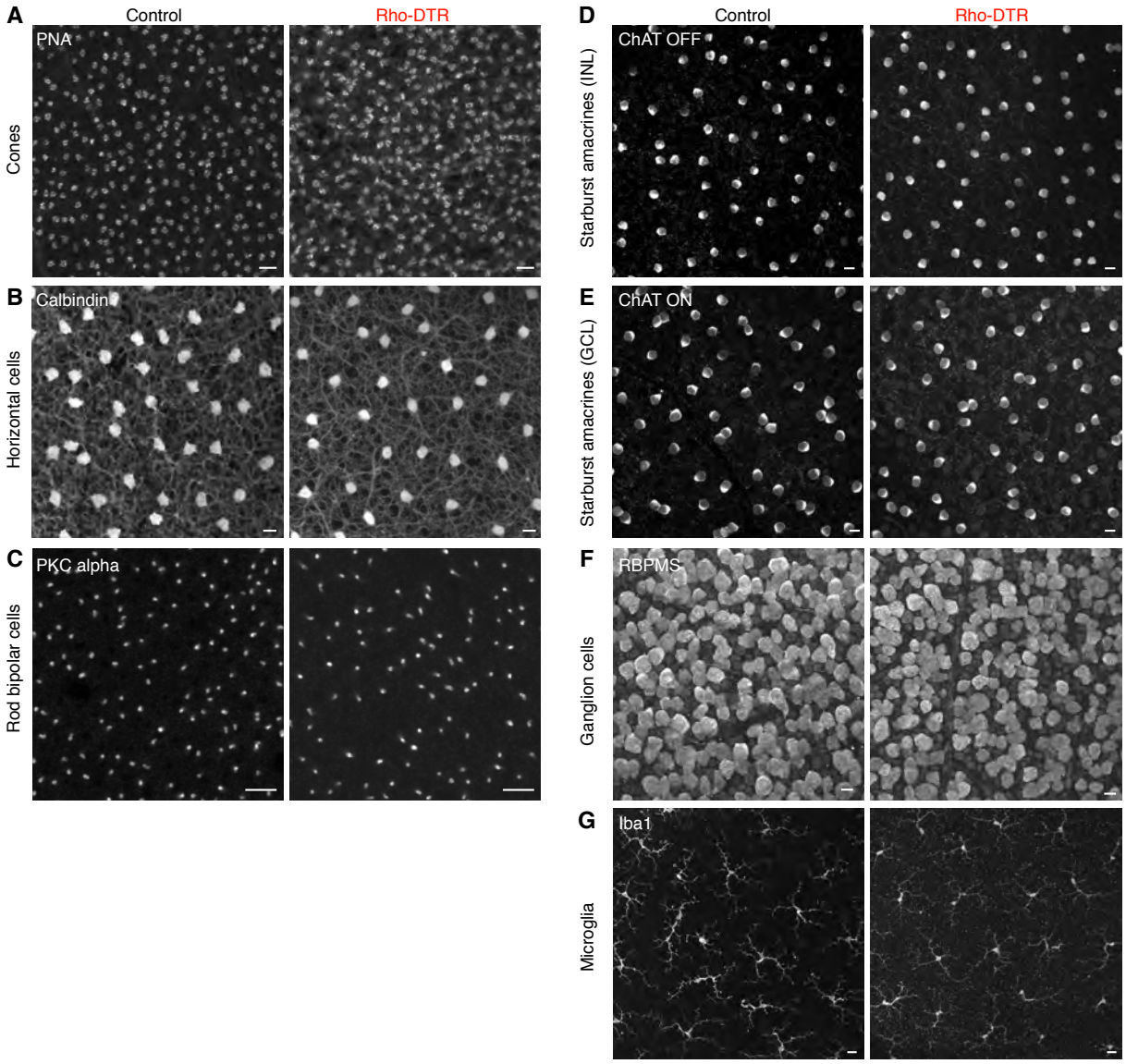

Figure S1. Diphtheria toxin system does not significantly affect other non-rod retinal cell types 1 month after rod ablation. Related to Figure 1.

Example images of cell types under control and Rho-DTR conditions. Quantification was done in flat mount retina on regions larger than shown (area of image quantified: 386 μm^2; Table S1). (A) Cone photoreceptor pedicles labeled by peanut agglutinin (PNA). (B) Horizontal cell somas labeled by calbindin. (C) Rod bipolar cell axons stalks labeled by protein kinase C (PKC) alpha. (D) Starburst amacrine cell somas in the inner nuclear layer (INL) labeled by choline acetyltransferase (ChAT) whose dendrites stratify in the OFF sublamina. (E) Starburst amacrine cell somas in the ganglion cell layer (GCL) labeled by ChAT whose dendrites stratify in the ON sublamina. (F) Ganglion cell somas labeled by RNA-binding protein with multiple splicing (RBPMS). (G) Microglia labeled by ionized calcium binding adaptor molecule (Iba1) at the level of the outer plexiform layer. Scale bars = 10 μm.

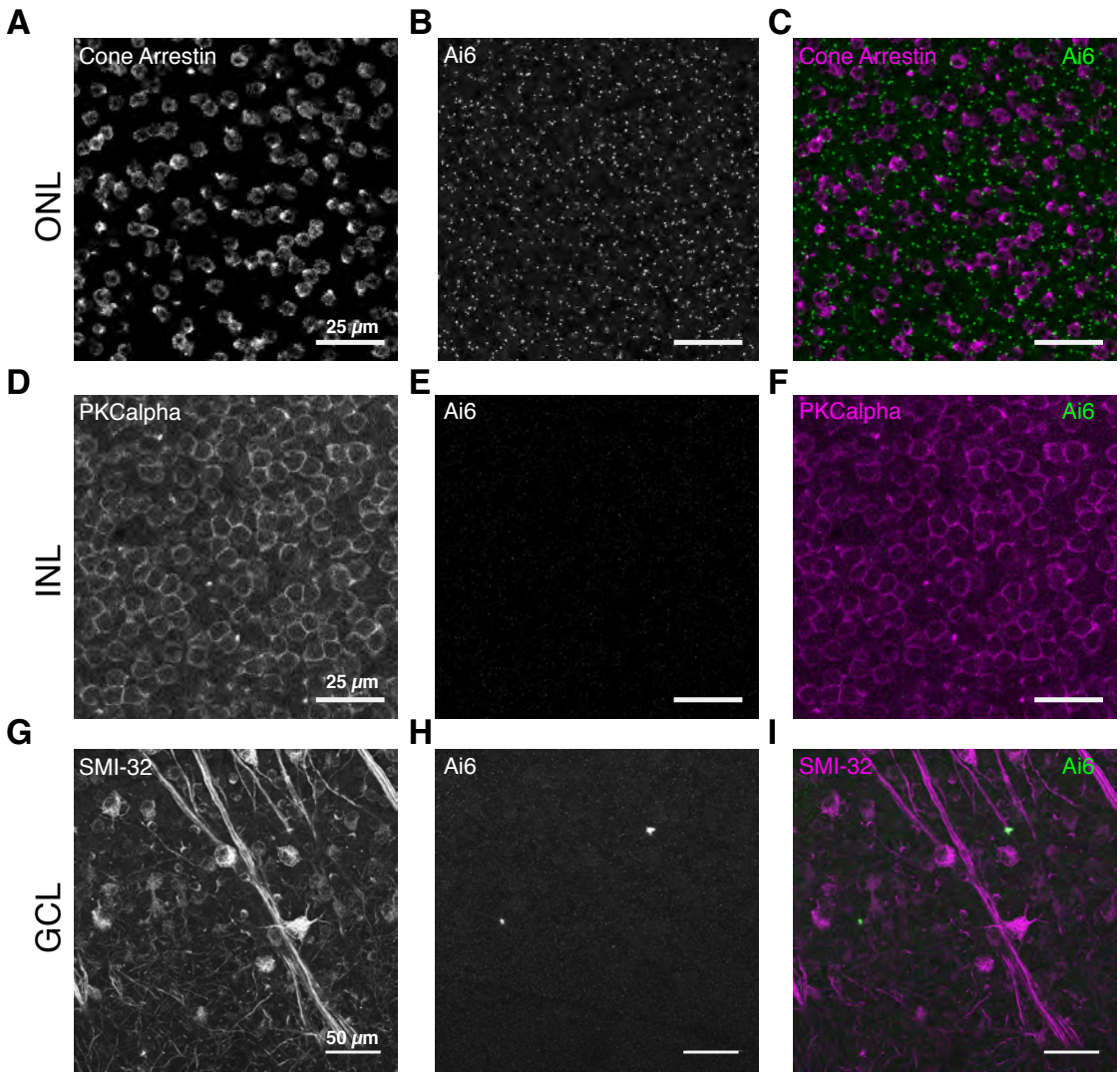

Figure S2. Diphtheria toxin system restricts diphtheria toxin receptor expression to rods. Related to Figure 1.

Confocal images of cell somas in the indicated layer from the *Rho-iCre* x *Ai6* mouse line. (A) Cone somas labeled by cone arrestin in the outer nuclear layer (ONL). (B) Rod somas that express Ai6 fluorescence. (C) Overlay of cone arrestin (magenta) and Ai6 fluorescence (green). Ai6 expression is restricted to rods and not present in cones. (D) Rod bipolar cell somas labeled by protein kinase C (PKC) alpha in the inner nuclear layer (INL). (E) Ai6 expression is absent from the inner nuclear layer. (F) Overlay of PKCalpha (magenta) and Ai6 fluorescence (green). (G) Alpha-type ganglion cells labeled by neurofilament protein SMI-32 in the ganglion cell layer (GCL). (H) Ai6 expression is absent from the ganglion cell layer. (I) Overlay of SMI-32 (magenta) and Ai6 fluorescence (green).

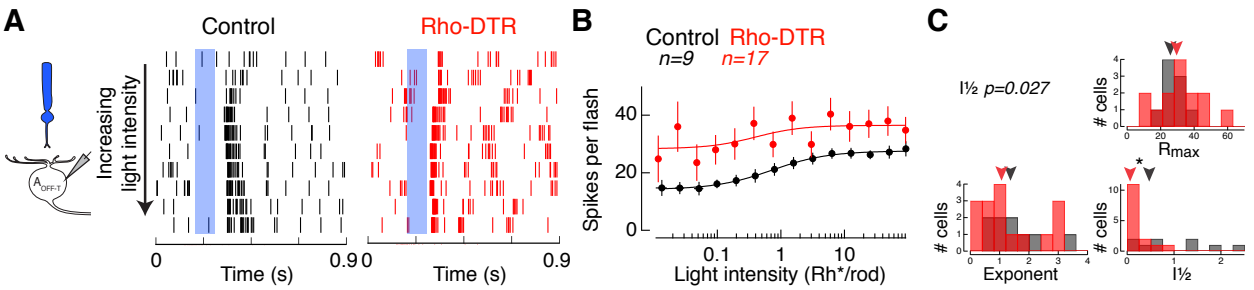

Figure S3. Rod-mediated light responses in $A_{OFF\text{-}T}$ ganglion cells have partially recovered spike output. Related to Figure 2.

(A) Spike rasters from cell-attached recordings of $A_{OFF\text{-}T}$ ganglion cells in response to a rod-preferring stimulus: 100ms flash at time 0 with the 470nm LED on a dark background (blue rectangle). Each row shows the response to a flash doubling in intensity from top to bottom in control (black) and Rho-DTR (red) conditions. (B) Average intensity-response relationship for the total number of spikes in response to each flash intensity, after the offset of the stimulus. Points are mean±SEM, *n* is number of cells. Data points fit with a Hill equation, which can be captured by a maximum response (R_{max}), intensity at half maximum response ($I_{½}$), and exponent. (C) Histogram of these three parameters of the fits for the population of $A_{OFF\text{-}T}$ ganglion cells in control and Rho-DTR conditions. Triangles above represent the median of each distribution and asterisks denote significant differences between control and Rho-DTR populations by the rank sum test. Significant p-values are reported in the upper left corner. Statistics in Table S3.

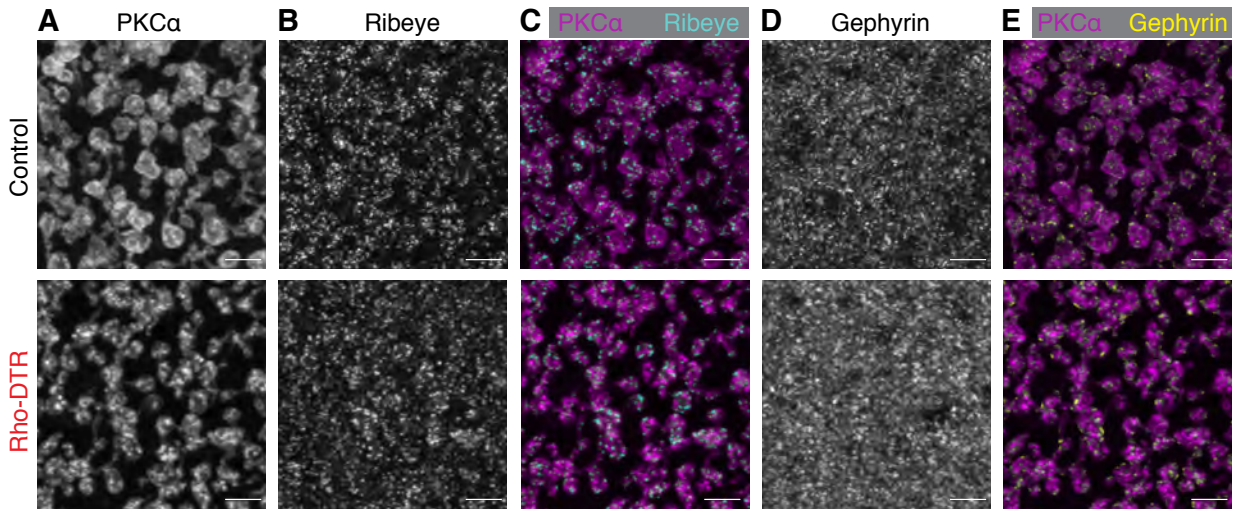

Figure S4. Partial ablation of rods has no effect on the density of excitatory output synaptic puncta and inhibitory input synaptic puncta in rod bipolar cell axons. Related to Figure 5. Confocal images of flat mount retina from control (top row) and Rho-DTR (bottom row) with (A) rod bipolar cell axons labeled by protein kinase C alpha (PKCα), (B) excitatory presynaptic release sites, i.e., ribbons, throughout the inner plexiform layer labeled by ribeye, (C) presynaptic ribbons within the rod bipolar cell axons, (D) inhibitory postsynaptic sites throughout the inner plexiform layer labeled by gephyrin, and (E) gephyrin within the rod bipolar cell axons. Amira was used to mask the volume of rod bipolar cell axons. ObjectFinder was used to quantify ribeye and gephyrin puncta within the masked rod bipolar cell axons. No significant differences were found in ribeye nor gephyrin densities between control and Rho-DTR retina: ribeye density as median±IQR for control (Rho-DTR) 0.529±0.413 puncta/μm^3 within PKC (0.492±0.190), p=0.182, rank sum test [n=10 (9) images; 4 (3) animals]; gephyrin density as median±IQR for control (Rho-DTR) 0.392±0.188 puncta/μm^3 within PKC (0.418± 0.139), p=0.860, rank sum test [n=11 (10) images; 4 (3) animals]. Scale bars = 10 μm.

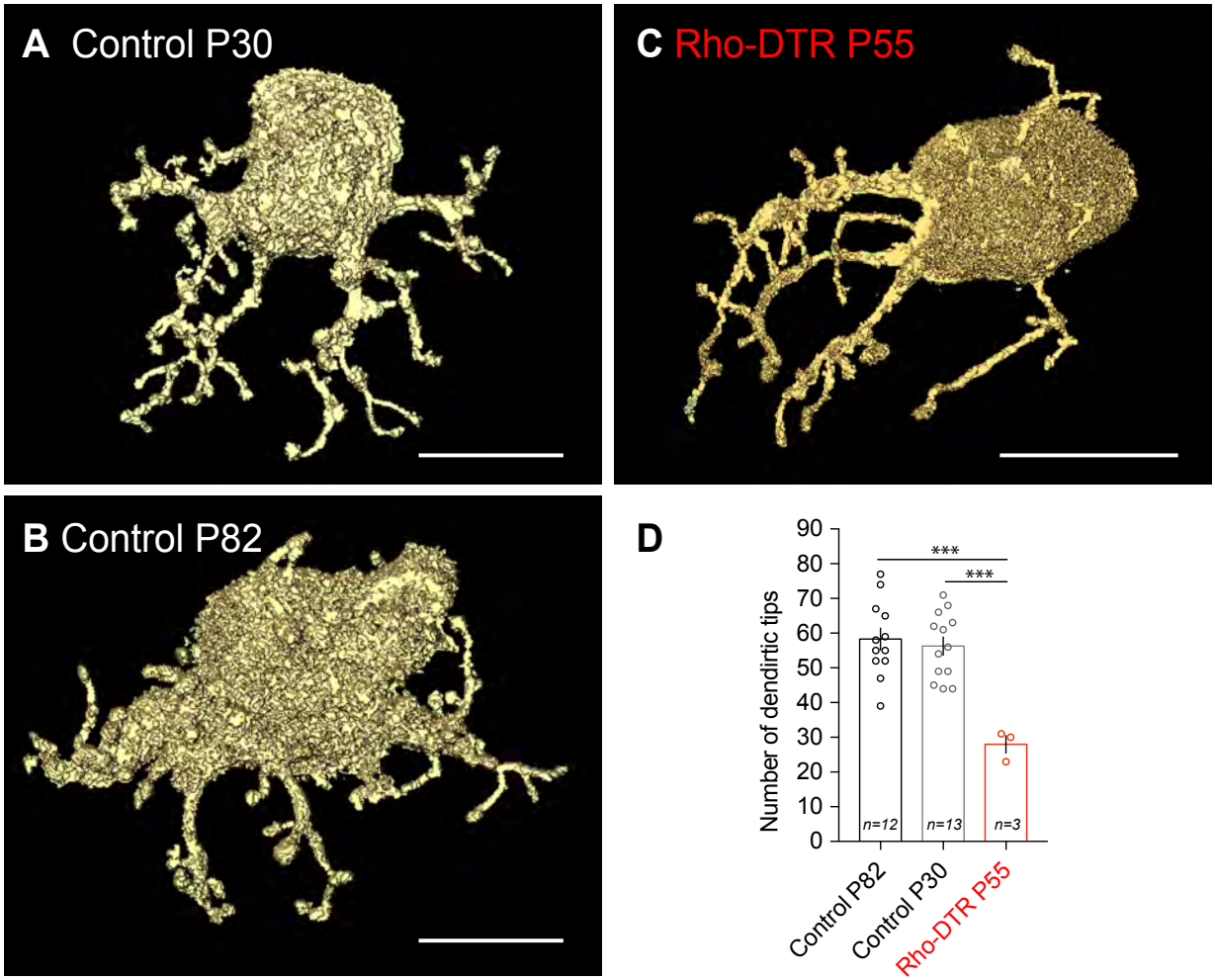

Figure S5. Partial ablation of rods reduces the number of dendritic tips in rod bipolar cells. Related to Figure 5.

En face view of rod bipolar cell somas and dendrites reconstructed from 3-dimensional binary masks. Masks were constructed using the fluorescent signal of TdTomato expression in individual rod bipolar cells in the *Grm6-TdTomato* mouse line. Each dendritic tip is a site of potential rod contact. (A) Example of a control rod bipolar cell at postnatal day (P) 30 with 56 dendritic tips. (B) Example of a control rod bipolar cell at P82 with 58 dendritic tips. (C) Example of a Rho-DTR rod bipolar cell at P55 and injected with DT at P30 with 28 dendritic tips. (D) Quantification of rod bipolar cell dendritic tips at two ages in control retina (data from Anastassov et al., 2019) and in Rho-DTR retina. The decrease in the number of dendritic tips is consistent with the reduction in the number of rods after ablation. One-way ANOVA with Dunnett's multiple comparisons correction; p=0.0001 for Control P82 vs. Rho-DTR P55, p=0.0003 for Control P30 vs. Rho-DTR P55. Scale bars = 5 μm

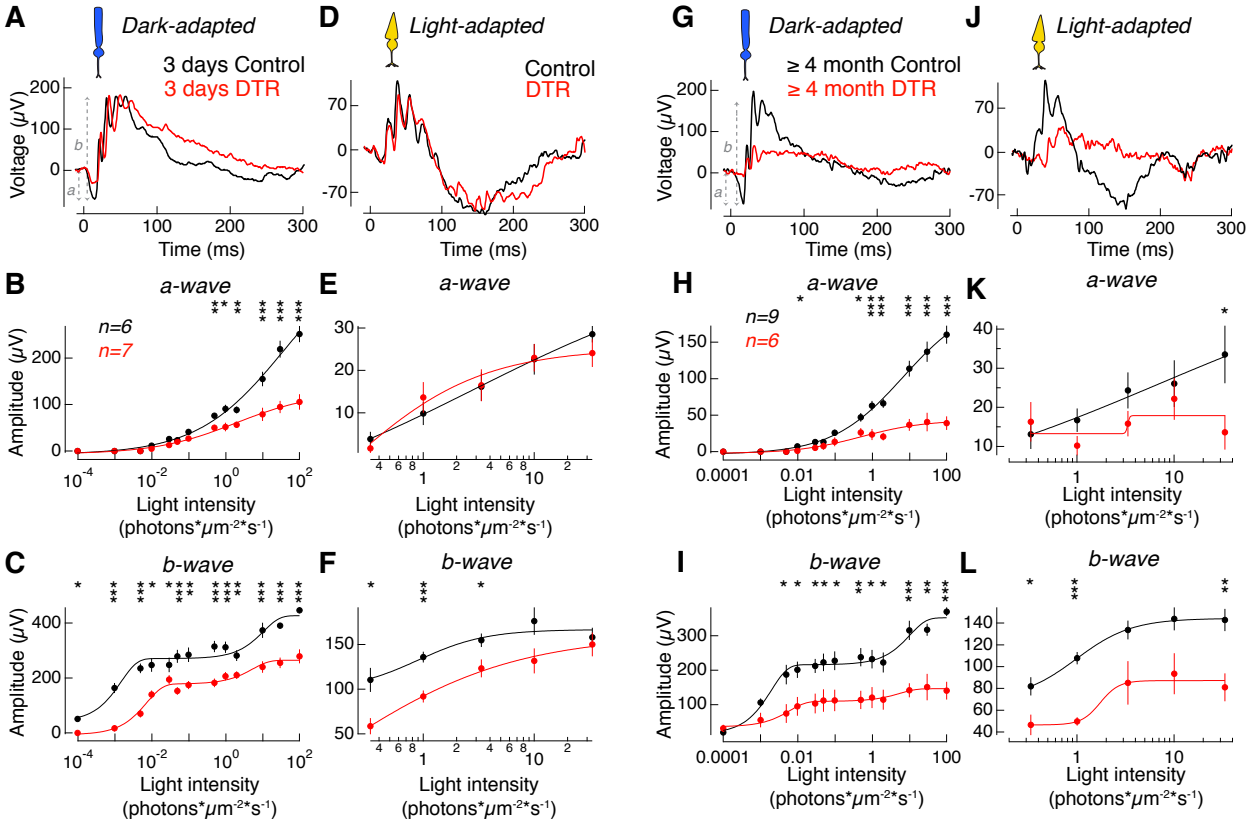

Figure S6. Compensation of the rod-mediated b-wave not yet present at 3 days and stable at ≥4 months following rod ablation. Related to Figure 4.

(A, G) Example *in vivo* electroretinogram of control (black) and Rho-DTR (red) mice injected with diphtheria toxin at P30 and P37 and recorded (A) 3 days and (G) ≥4 months after the second injection. Responses recorded in the dark-adapted, rod-mediated condition at 2.919 photons*μm^{-2}*s^{-1}. Amplitude of a-wave was measured from baseline to the trough of the first negative peak. Amplitude of b-wave was measured from the trough of the first negative peak to the second highest positive peak.

(B, H) Average amplitude of the dark-adapted a-wave, which is the rod-mediated voltage response in the waveform, as a function of light intensity at (B) 3 days and (H) ≥4 months after DT injections. Points are mean±SEM. Significant differences between response amplitudes at each light intensity are denoted by asterisks above each pair of points (t test). Light intensities [p-value for 3 days; ≥4 months]: 0.00973 photons*μm^{-2}*s^{-1} [0.096; 0.0359]; 0.4865 [0.006; 0.03055]; 0.973 [0.010; 0.0024]; 1.946 [0.009; 8.9788E-05]; 9.73 [0.004; 7.8214E-05]; 29.19 [0.0003; 0.0002]; 97.3 [9.12E-05; 2.811E-06].

(C, I) Average amplitude of the dark-adapted b-wave, which is the rod bipolar cell-mediated voltage response in the waveform, as a function of light intensity at (C) 3 days and (I) ≥4 months after DT injections. Points are mean±SEM. Light intensity [p-value for 3 days; ≥4 months]: 9.73E-05 photons*μm^{-2}*s^{-1} [0.0122; 0.437]; 9.73E-04 [0.002; 0.091]; 0.004865 [0.002; 0.0198]; 0.0097 [0.010; 0.0198]; 0.0292 [0.029; 0.0183]; 0.0487 [0.005; 0.0327]; 0.0973 [0.009; 0.0258]; 0.4865 [0.002; 0.0096]; 0.973 [0.003; 0.0275]; 1.946 [0.006; 0.0305]; 9.73 [0.003; 0.0004]; 29.19 [0.0002; 0.0086]; 97.3 [0.0003; 0.0001]. The ratio of the a- and b-waves at 9.73 photons*μm^{-2}*s^{-1} was not significantly different between control and Rho-DTR at 3 days but was at 4 months, demonstrating that the

b-wave amplitude could be predicted from simple propagation of the a-wave amplitude loss at 3 days, but not at later time points in the Rho-DTR condition. These results suggest that the b-wave has recovered amplitude only at the longer interval.
(D, J) Example *in vivo* electroretinogram of control (black) and Rho-DTR (red) mice taken in the light-adapted, cone-mediated condition at (D) 3 days and (J) ≥4 months after the second DT injection.
(E, K) Average amplitude of the light-adapted a-wave, which is the cone-mediated voltage response in the waveform, as a function of light intensity at (E) 3 days and (K) ≥4 months after DT injections. Points are mean±SEM. Light intensities [p-value for 3 days; ≥4 months]: 33.4 photons*μm^{-2}*s^{-1} [0. 269; 0.0386].
(F, L) Average amplitude of the light-adapted b-wave, which is the ON cone bipolar cell-mediated voltage response in the waveform, as a function of light intensity at (F) 3 days and (L) ≥4 months after DT injections. Points are mean±SEM. Light intensities [p-value for 3 days; ≥4 months]: 0.334 photons*μm^{-2}*s^{-1} [0.011; 0.0240]; 1.002 [0.005; 2.8803E-06]; 3.34 [0.03; 0.082]; 33.4 [0.646; 0.0054].

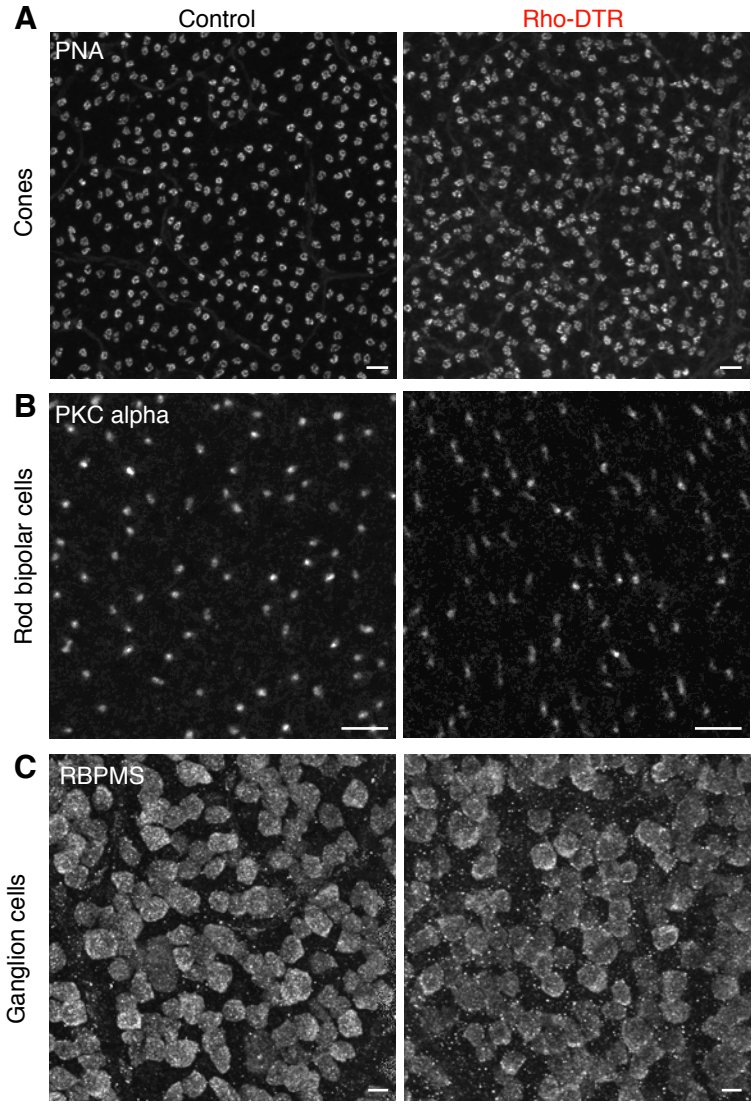

Figure S7. Effects of the diphtheria toxin system on the densities of retinal cell types ≥4 months after rod ablation. Related to Figure 1.

Example images of cell types under control and Rho-DTR conditions at intervals ≥4 months after ablation. Quantification was done in flat mount retina on regions larger than shown (area of image quantified: 386 μm^2; Table S7). (A) Cone photoreceptor pedicles labeled by peanut agglutinin (PNA). (B) Rod bipolar cell axons stalks labeled by protein kinase C (PKC) alpha. (C) Ganglion cell somas labeled by RNA-binding protein with multiple splicing (RBPMS). Scale bars = 10 μm.

Cell Type	Methods: antibody, retinal layer imaged, flat mount or section, retinal topography if specific	Control Density (median±IQR)	DTR Density (median±IQR)	n = images for control (& DTR) N = mice for control (& DTR)	Rank sum test p-value
Cone photoreceptors	PNA or Cone Arrestin, ONL , flat mount, dorsal-nasal	1.41E+04 ± 3.13E+03 cells/mm^2	1.36E+04 ± 2.83E+03 cells/mm^2	n = 17 (10) N = 9 (8)	p = 0.940
Horizontal cells	Calbindin, ONL, flat mount, dorsal-nasal	1.05E+03 ± 275.68 cells/mm^2	9.55E+02 ± 263.06 cells/mm^2	n = 15 (16) N = 6 (9)	p = 0.244
Rod bipolar cells	PKCalpha, IPL, flat mount, dorsal-nasal	1.53E+04 ± 3.51E+03 cells/mm^2	1.68E+04 ± 3.54E+03 cells/mm^2	n = 12 (15) N = 5 (6)	p = 0.251
Starburst amacrine cells (OFF)	ChAT, INL, flat mount, nasal	1.06E+03 ± 431.54 cells/mm^2	1.08E+03 ± 549.19 cells/mm^2	n = 17 (17) N = 7 (9)	p = 1
Starburst amacrine cells (ON)	ChAT, GCL, flat mount, nasal	9.86E+02 ± 431.51 cells/mm^2	9.31E+02 ± 231.61 cells/mm^2	n = 15 (16) N = 7 (9)	p = 0.782
Ganglion cells	RBPMS, GCL, flat mount, nasal	2.98E+03± 569.11 cells/mm^2	3.47E+03± 1.72E+03 cells/mm^2	n = 16 (14) N = 7 (6)	p = 0.350
Microglia	Iba1, OPL, flat mount, dorsal-nasal	116.38 ± 26.60 cells/mm^2	119.71 ± 23.28 cells/mm^2	n = 6 (11) N = 3 (3)	p = 0.508

Table S1. Effects of the diphtheria toxin system on the densities of non-rod retinal cell types 1 month after rod ablation. Related to Figure 1.

Quantification of major cell types in the retina under control and Rho-DTR conditions 1 month after DT injections. Number of cell bodies in flat mount retina was quantified as area density (area of image quantified: 386 μm^2). None of the cell types examined show a change in density. Retinal layers: OPL = outer plexiform layer, ONL = outer nuclear layer, INL = inner nuclear layer, IPL = inner plexiform layer, and GCL = ganglion cell layer.

Table S2. Fit parameters and statistics for rod-mediated responses in A_{ON-S} ganglion cells. Related to Figure 2.

		Control			DTR				
Figure number	**Figure panel**	**Measure**	**Median**	**IQR**	**Measure**	**Median**	**IQR**	**Statistical test**	**p-value**
2	C	R_{max}	32.85	14.6	R_{max}	25.49	12.2	Rank sum	0.022
		I½	5.54	8.52	I½	2.78	6.86		0.023
		Exponent	1.78	0.91	Exponent	2.44	1.56		0.083
	F	R_{max}	132.58	65.0	R_{max}	88.45	90.0		0.041
		I½	14.02	20.4	I½	11.94	46.5		0.924
		Exponent	1.63	0.97	Exponent	1.36	1.73		0.801
	I	R_{max}	124.82	316	R_{max}	99.65	419		0.706
		I½	48.00	42.9	I½	42.81	27.8		0.450
		Exponent	1.50	0.54	Exponent	1.38	0.47		1.000

Table S3. Fit parameters and statistics for rod-mediated responses in A_{OFF-T} ganglion cells in Figure S3. Related to Figure 2.

		Control			DTR				
Figure number	**Figure panel**	**Measure**	**Median**	**IQR**	**Measure**	**Median**	**IQR**	**Statistical test**	**p-value**
S3	C	R_{max}	26.337	7.14	R_{max}	32.925	20.78	Rank sum	0.1958
		I½	0.630	1.06	I½	0.154	0.195		0.0271
		Exponent	1.371	0.75	Exponent	1.058	2.068		0.6663

Table S4. Fit parameters and statistics for rod bipolar cells. Related to Figure 5.

		Control			DTR				
Figure number	**Figure panel**	**Measure**	**Median**	**IQR**	**Measure**	**Median**	**IQR**	**Statistical test**	**p-value**
5	C	R_{max}	10.31	9.41	R_{max}	10.12	21.6	Rank sum	0.955
		I½	4.28	4.42	I½	3.21	2.79		0.281
		Exponent	1.83	0.43	Exponent	1.70	0.89		1.000
	F	R_{max}	150.02	266	R_{max}	70.87	86.4		0.025
		I½	4.36	1.76	I½	4.81	2.08		0.777
		Exponent	1.65	0.60	Exponent	2.35	0.72		0.048
	I	R_{max}	56.52	16.8	R_{max}	3.48	7.34		0.001
		I½	0.93	0.63	I½	34.67	1.6e4		0.001
		Exponent	1.53	0.29	Exponent	6.41	164		0.170

Table S5. Fit parameters and statistics for cone-mediated responses in $A_{ON\text{-}S}$ ganglion cells. Related to Figure 6.

		Control			DTR				
Figure number	**Figure panel**	**Measure**	**Median**	**IQR**	**Measure**	**Median**	**IQR**	**Statistical test**	**p-value**
6	C	R_{max}	11.99	2.64	R_{max}	23.29	16.4	Rank sum	7.7e-7
		I½	251.89	232	I½	384.52	315		0.018
		Exponent	2.76	1.74	Exponent	1.76	1.39		0.005
	F	R_{max}	71.75	60.5	R_{max}	122.03	140		0.001
		I½	692.73	1.0e3	I½	1168.7	3.5e3		0.233
		Exponent	1.69	0.83	Exponent	1.23	0.95		0.013
	I	R_{max}	105.96	569	R_{max}	148.46	388		0.141
		I½	1473.5	1.5e3	I½	1543.4	485		0.940
		Exponent	1.36	0.34	Exponent	1.62	1.48		0.910

Table S6. Fit parameters and statistics for full and partial stimulation of $A_{ON\text{-}S}$ ganglion cells. Related to Figure 7.

		Full Stimulation			Half Stimulation				
Figure number	**Figure panel**	**Measure**	**Median**	**IQR**	**Measure**	**Median**	**IQR**	**Statistical test**	**p-value**
7	B	R_{max}	39.44	18.4	R_{max}	20.16	27.0	Sign rank	1.2e-4
		I½	1.24	1.38	I½	2.60	3.20		0.002
		Exponent	1.55	0.59	Exponent	1.66	0.88		0.332
	E	R_{max}	17.13	9.55	R_{max}	21.70	4.05		0.079
		I½	842.65	1414	I½	201.34	190		0.550
		Exponent	1.15	0.681	Exponent	1.53	0.47		0.502

Cell Type	Methods: antibody, retinal layer imaged, flat mount or section, retinal topography if specific	Control Density (median±IQR)	DTR Density (median±IQR)	n = images for control (& DTR) N = mice for control (& DTR)	Rank sum test p-value
Photoreceptors	H&E, ONL rows of somas per column, section	10.25 ± 0.33 cells/column	4.42 ± 0.958 cells/column	n = 22 (12) N = 11 (6)	p = 1.91E-06
Bipolar and amacrine cells	H&E, INL rows of somas per column, section	4.42 ± 0.417 cells/column	4.25 ± 0.333 cells/column	n = 22 (12) N = 11 (6)	p = 0.023
Cone photoreceptors	PNA or Cone Arrestin, ONL , flat mount, dorsal-nasal	1.36E+04 ± 1.66E+03 cells/mm^2	1.19E+04 ± 1.76E+03 cells/mm^2	n = 9 (10) N = 4 (4)	p = 0.022
Rod bipolar cells	PKCalpha, IPL, flat mount, dorsal-nasal	1.57E+04 ± 4.73E+03 cells/mm^2	1.64E+04 ± 905.7232 cells/mm^2	n = 8 (7) N = 4 (3)	p = 0.536
Ganglion cells	RBPMS, GCL, flat mount, nasal	3.07E+03 ± 299.69 cells/mm^2	3.40E+03 ± 665.97 cells/mm^2	n = 8 (6) N = 4 (3)	p = 0.029

Table S7. Effects of the diphtheria toxin system on the densities of retinal cell types ≥4 months after induced rod death. Related to Figure 1.

Quantification of major cell types in the retina under control and Rho-DTR conditions ≥4 months after DT injections. Number of cell bodies was quantified either in sections stained with Hematoxylin and Eosin (H & E) or in flat mount retina and quantified as area density (area of image quantified: 386 μm^2). The cells in the inner plexiform layer, cone photoreceptors, and ganglion cells show a significant change in density at these longer intervals that was not observed at shorter intervals (1 month). Retinal layers: OPL = outer plexiform layer, ONL = outer nuclear layer, INL = inner nuclear layer, IPL = inner plexiform layer, and GCL = ganglion cell layer.