Supplemental Information

Retinoic acid is the trigger for neural hyperactivity in retinal degeneration and blocking its receptor unmasks light responses and augments vision

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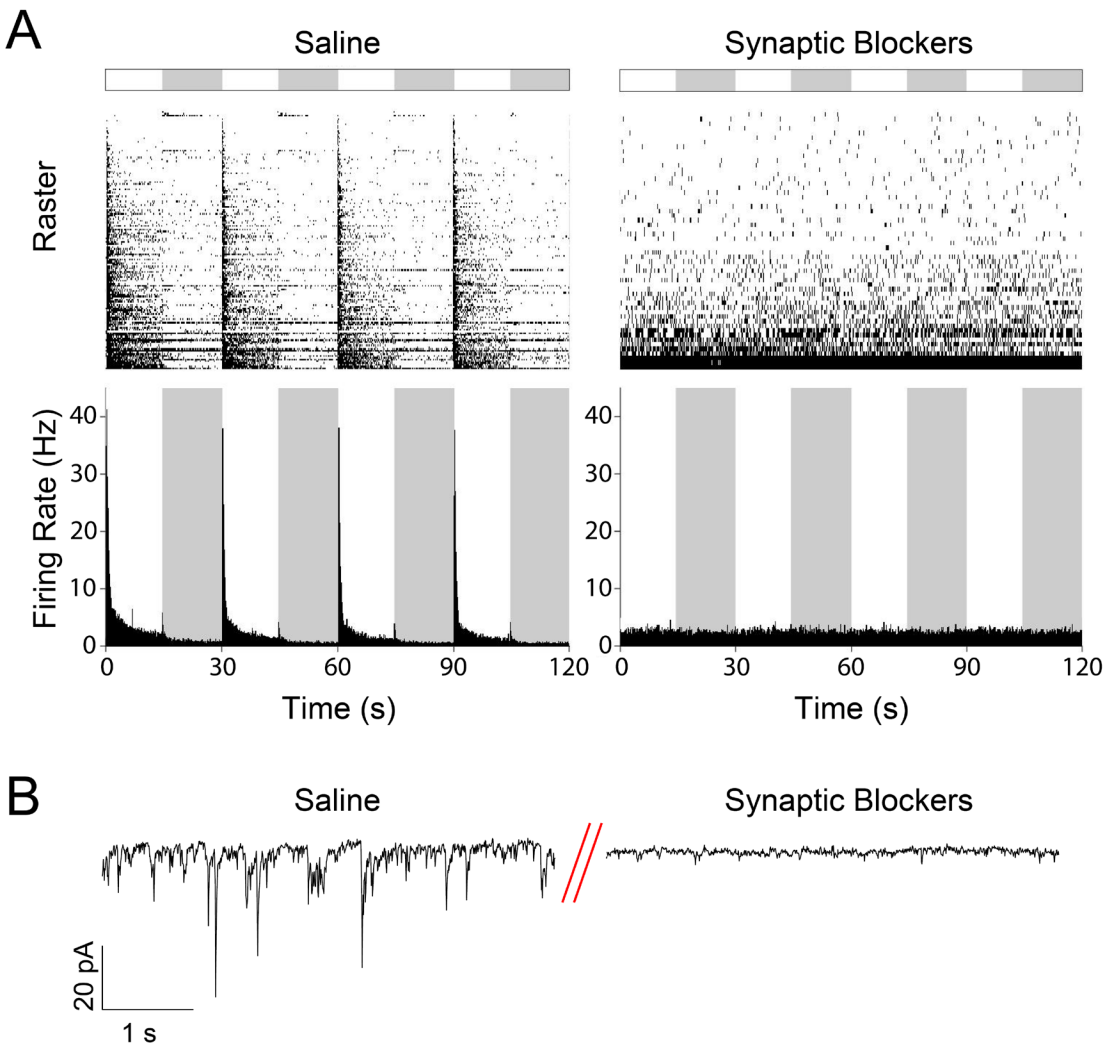
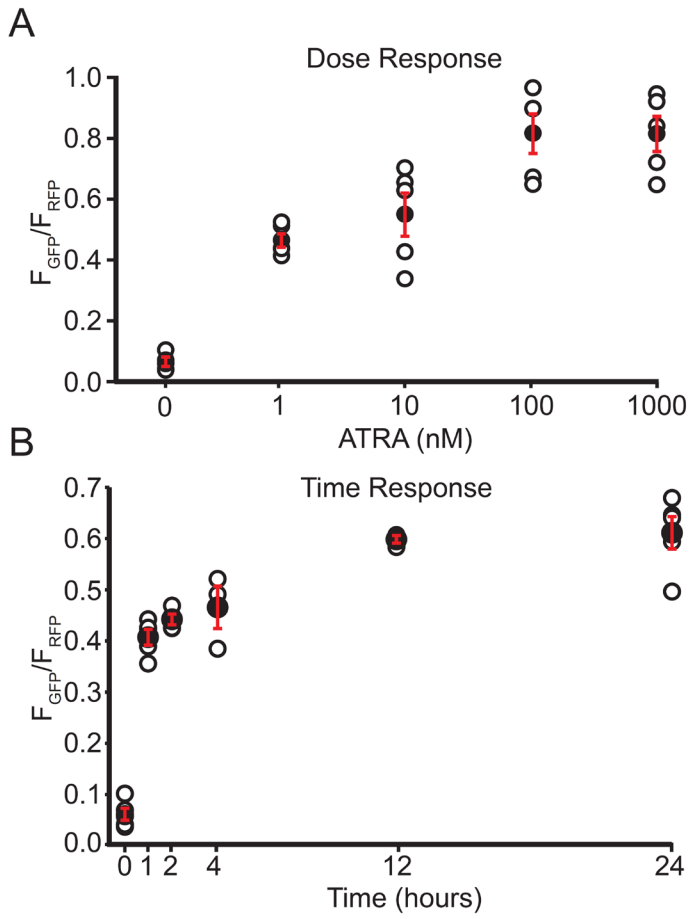
Figure S1



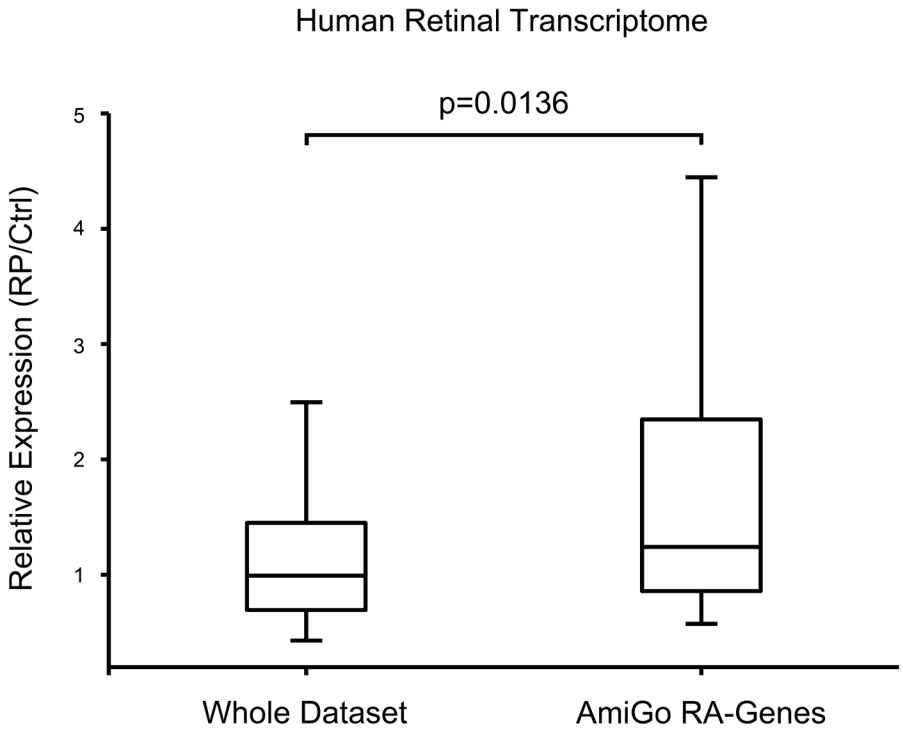
Supplemental Figure 1. Neurotransmitter receptor antagonist cocktail blocks chemical synaptic responses in RGCs (related to Fig. 1)**.**
(A) MEA recording of light responses in WT retina in saline before (left panel) or after (right panel) perfusion of a mixture of synaptic blockers, including: (in μM) 10 AP4, 40 DNQX, 30 AP5, 10 SR-95531, 50 TPMPA, 10 strychnine, and 50 tubocurarine. Light responses disappeared in 9 out of 9 recordings.
(B) Patch-clamp recordings of spontaneous excitatory postsynaptic currents in a *rd1*-RGC neuron, voltage clamped to -60 mV (normal saline, left panel). After perfusion of saline including the synaptic blocker mixture (right panel), excitatory post-synaptic currents disappeared in 23 out of 23 cells recorded.

Figure S2



Supplemental Figure 2. Dose- and time-dependence of RAR-induced gene expression in HEK-293 cells (related to Fig. 2B)**.**
(A) Dose-dependent gene expression induced by ATRA, determined by the GFP/RFP ratio. HEK-293 cells were transfected by Lipofectamin 2000 with the RAR reporter construct. Cells were treated with various doses of ATRA for 48 hrs and then fixed with paraformaldehyde. Images were separately analyzed for RFP and GFP fluorescence levels, and then the ratio of fluorescence was calculated. For all doses of ATRA, liarozole (100 μM) was added to prevent ATRA degradation. Individual data points and mean ± SEM values are shown.
(B) Ratiometric analysis of time-dependent gene expression induced by ATRA. Transfected cells were treated with 1,000 nM ATRA + 100 μM Liarozole, and fixed after 1, 2, 4, 12 or 24 hrs. Images were analyzed for RFP and GFP fluorescence levels. Individual data points and mean ± SEM values are shown.
(A,B) 3-5 separate experiments were conducted in duplicate wells of HEK cells grown in serum-free media. For full dataset, see **Table S1**.

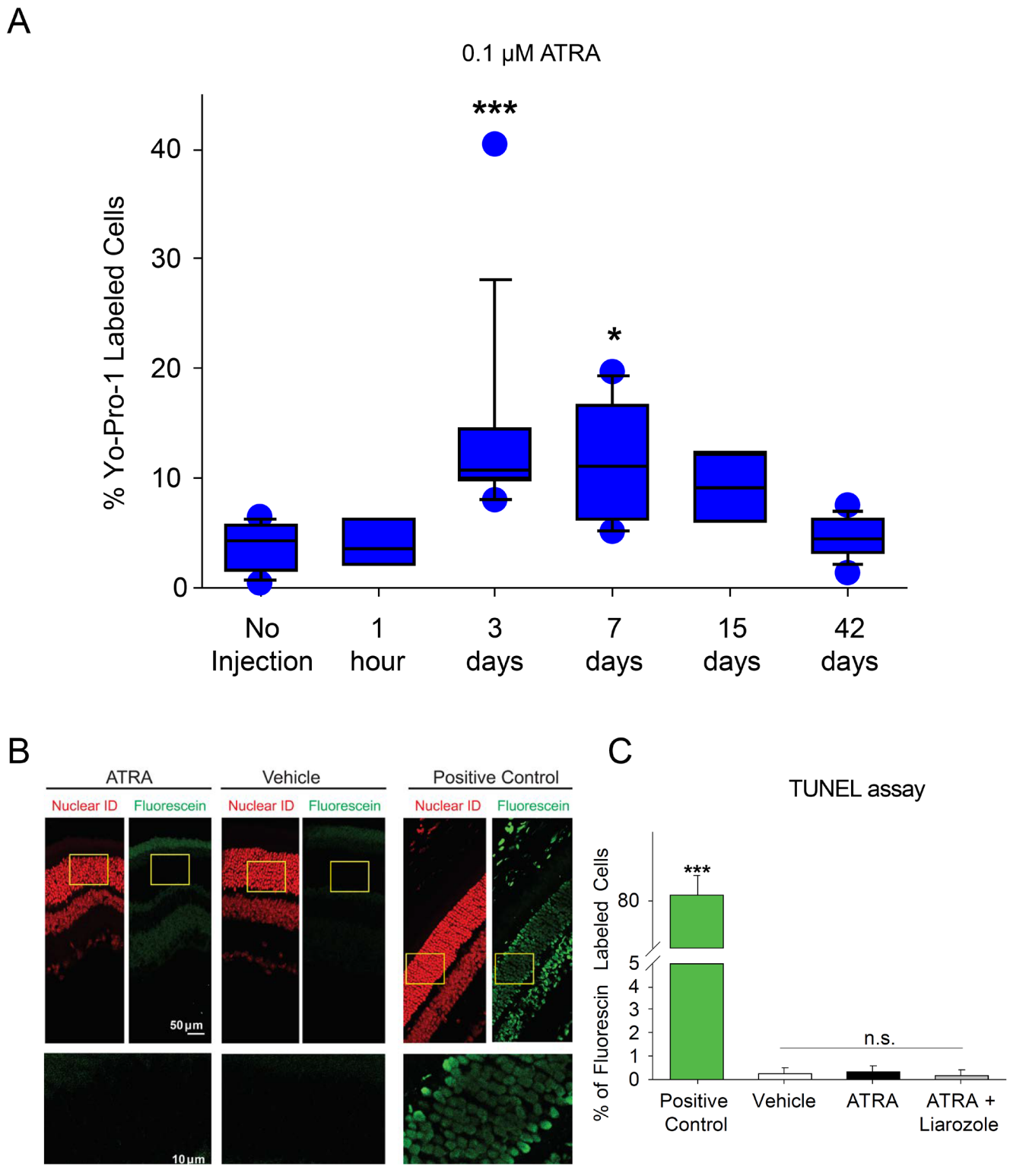
Figure S3



Supplemental Figure 3. Analysis of human transcriptome data for RA-responsive genes (related to Fig. 2C)**.**

Gene expression in a retinal sample from an RP patient compared to a sample from a control donor. Primary data were from Mullins et al., 2012[40]. Boxplots for RP/Ctrl are shown for the whole dataset (left) vs. from the subset consisting of 120 AmiGO-validated RA-responsive genes (right). RP/control for RA-responsive gene transcripts = 3.03 ± 0.71; RP/control for entire population of transcripts = 1.44 ± 0.014; p = 0.0136, Mann-Whitney Rank Sum Test.

Figure S4


A
0.1 µM ATRA
***
*
% Yo-Pro-1 Labeled Cells
0
10
20
30
40
No Injection
1 hour
3 days
7 days
15 days
42 days
B
ATRA
Vehicle
Positive Control
Nuclear ID
Fluorescein
50 µm
10 µm
C
TUNEL assay
% of Fluorescin Labeled Cells
***
n.s.
0
1
2
3
4
5
80
Positive Control
Vehicle
ATRA
ATRA + Liarozole

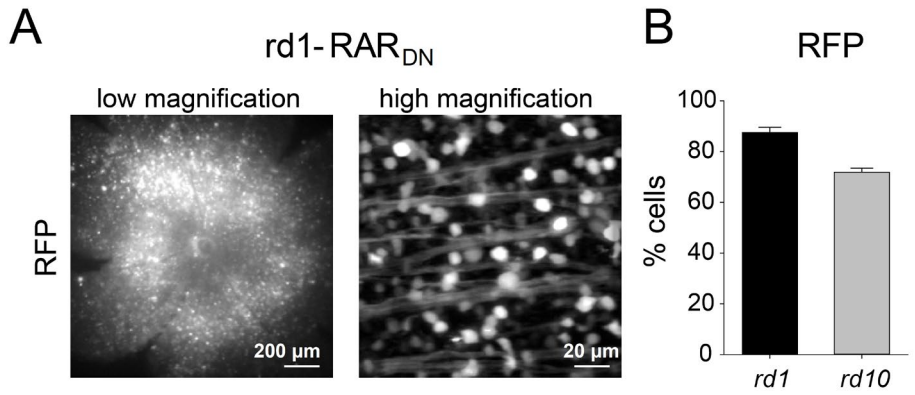
Supplemental Figure 4. Intravitreal injection of ATRA is reversible and non-toxic (related to Figures 3 and 4).

(A) Quantification of the fraction of WT RGCs (blue box plots), labeled with Yo-Pro-1 following intravitreal injection of 0.1 μM ATRA. Labeling was evaluated in WT retinas, 1 hr or 3, 7, 15, or 42 days after injection. Cell nuclei were counterstained with Nuclear I.D. All data are represented as mean ± SEM. *p<0.05, ***p<0.001, t-test and Mann-Whitney Test. For full dataset, see **Table S1**.

(B) TUNEL staining of WT retinas (green fluorescein labeling). Boxed area is over the photoreceptor nuclear layer. Positive control was obtained by treating fixed retinal sections with DNAse to induce DNA breakdown. Nuclear I.D. was used for counterstaining (red labeling). 0.1 μM ATRA was injected intravitreously, alone or with 100 μM Liarozole (final concentrations after 1:5 dilution in the retina). Vehicle was PBS with 0.1% DMSO.

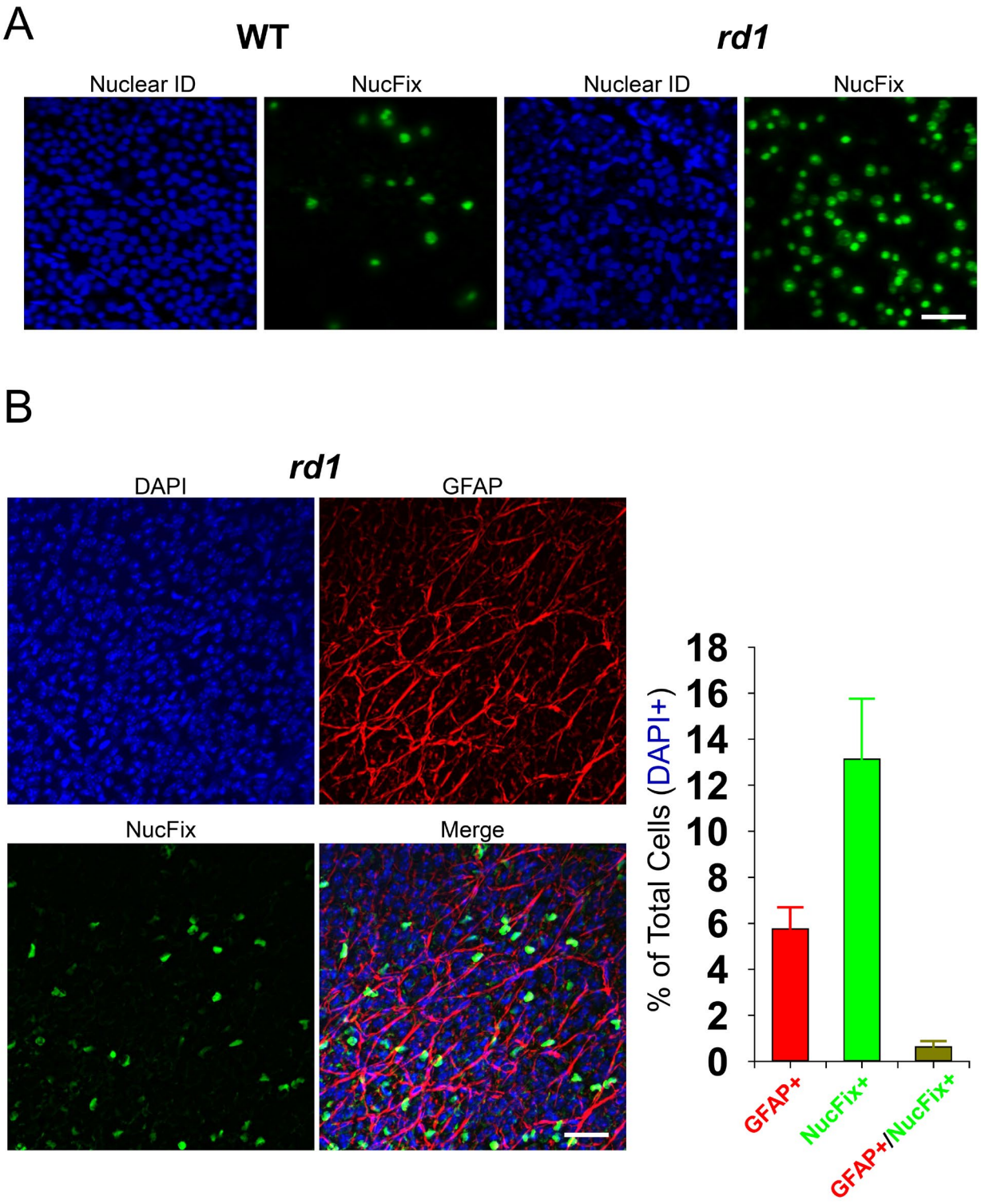
(C) Quantification of (B). Eyes were collected and stained 5-6 days post-injection. Injections were performed in 2 mice. For each mouse, one eye was injected and analyzed for each condition, and a total of 6 different fixed retinal sections were stained and analyzed for each treatment. % of fluorescin-labeled cells is shown for positive control (green), vehicle (white), ATRA (black) and ATRA + Liarozole. Values are mean ± SEM, *** $p < 0.001$, n.s. – non-significant, Kruskal-Wallis. For full dataset, see **Table S1**.

Figure S5



Supplemental Figure 5. Retinal expression of RAR$_{DN}$ (related to Fig. 3, 5 and 7)**.**
(A) Confocal fluorescent image of flat-mount live retina in a P90 *rd1* mouse injected with intravitreally with an AAV2 serotype of the RAR$_{DN}$ virus (pAAV-hSyn1-RAR$_{DN}$-RFP-WPRE) at P30. Imaging was carried out in living retinal flat-mount ganglion cell layer (GCL) at 4X (low magnification, left), and at 40X (high magnification, right), under constant perfusion of oxygenated saline. Images were taken in 20-30 µm-thick 3D Z-stacks through the GCL.
(B) Quantification of the fraction of RFP-positive cells in the GCL of *rd1* mice intravitreally injected with AAV2-RAR$_{DN}$ (as in A, related to Figures 3 and 5), and in P40-50 *rd10* mice injected in the tail vein with an AAV9-RARDN at P2-3 (related to Figure 7). Each retinal piece was imaged in 3-5 different fields. Total number of cells per field of view was established using Nuclear I.D. in naïve retinas. All data are represented as mean ± SEM. For full dataset, see **Table S1**.
(A-B) Similar results were obtained for RAR$_{DP}$ virus (pAAV-hSyn1-VP16-RAR-RFP-WPRE), data not shown (related to Figure 3).

Figure S6


A
WT
rd1
Nuclear ID
NucFix
Nuclear ID
NucFix
B
rd1
DAPI
GFAP
NucFix
Merge
% of Total Cells (DAPI+)
18
16
14
12
10
8
6
4
2
0
GFAP+
NucFix+
GFAP+/NucFix+

Supplemental Figure 6. RAR does not induce hyperpermeability in retina astrocytes (related to Figure 3).

(A) Labeling ganglion cell layer (GCL) cells in WT and *rd1* retinas with NucFix (green), a fixable analog of Yo-Pro-1. Note that a higher proportion of cells were labeled in *rd1* than in WT; hence NucFix labeling is degeneration-dependent, like labeling by Yo-Pro-1 and other dyes. Cells were co-stained with Nuclear ID (blue) to reveal all cells. Dye loading was carried out in living tissue under constant perfusion with oxygenated saline. Images were taken at 40X-maginification, in 20-30 µm-thick 3D Z-stacks through the GCL. Scale bar is 50 µm.
(B) Left: Immunostaining of fixed *rd1* retinal samples with an antibody against glia fibrillary astrocyte protein (GFAP, red) in NucFix pre-loaded cells (green). DAPI was used to reveal all nuclei. Right: Quantification of the % of cells positively stained for GFAP (red), NucFix (green) and both (yellow). The total number of cells for each field was quantified as the number of DAPI-positive nuclei (including pericytes and other non-neuronal cell types). Data were pulled from 2 mice, 4 retinal samples and 4 different imaged fields. All data are represented as mean ± SEM.

Supplemental Table Title and Legend

Supplemental Table 1. Full datasets for all experiments (related to Figures 1-7). Table S1 includes all datasets for the experiments presented in this study. Data are organized by figure. For each figure, a separate sheet displays the full dataset, including sample size, statistical test, and p values. (Online Excel File)