Rescue of the Stargardt phenotype in *Abca4* knockout mice through inhibition of vitamin A dimerization

Short title: **Vitamin A dimerization triggers RPE lipofuscin formation**

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Supporting Information

SI Results

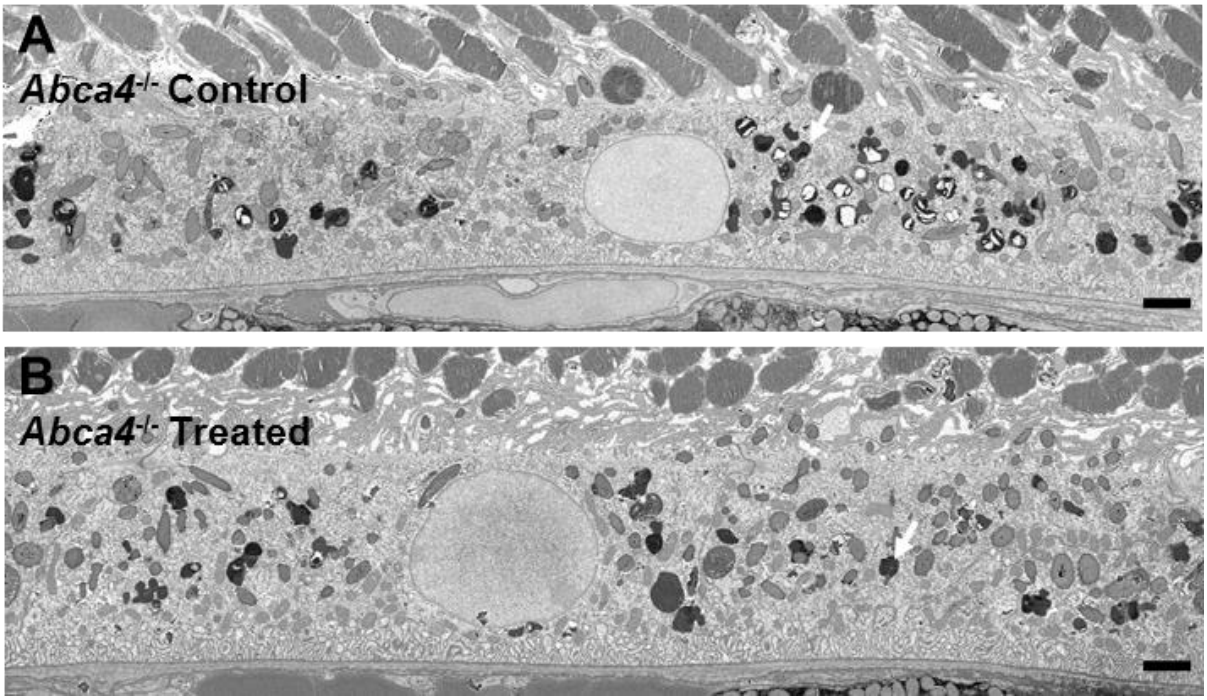

Fig. S1. Inhibiting vitamin A dimerization prevents lipofuscin granule accumulation in the RPE. Representative transmission electron micrographs of a RPE cell taken from 9-month old *Abca4*$^{-/-}$ mutant mice administered either vitamin A (control, **A**) or C20-D_3-vitamin A (treated, **B**). Lipofuscin granules were defined as particles that were electron dense, irregular shape and/or granular and limited by a single membrane. White arrows point to representative lipofuscin granule. Scale bar is approximately one micrometer.

Table S1. One-way analysis of variance results.

	WT Control	WT Treated	-/- Control	-/- Treated
Minimum	8	7	29	15
25% Percentile	8	7	29	15
Median	9	7	31	16
75% Percentile	10	7	32	16
Maximum	10	7	32	16
Mean	9	7	31	16
Std. Deviation	1	0	2	0.7
Std. Error	1	0	1	0.5
Lower 95% CI	-4	7	11	9
Upper 95% CI	22	7	50	22

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
WT Control vs WT Treated	2	2	No	ns	-4 to 8
WT Control vs -/- Control	-22	16	Yes	***	-28 to -15
WT Control vs -/- Treated	-7	5	Yes	*	-13 to -0.08
WT Treated vs -/- Control	-24	18	Yes	***	-30 to -17
WT Treated vs -/- Treated	-9	6	Yes	*	-15 to -2
-/- Control vs -/- Treated	15	11	Yes	**	9 to 21

Values represent lipofuscin volume (μM^3) per 10 μM^3 of RPE cell cytoplasm as shown in Fig. 2C.
-/-: *Abca4*$^{-/-}$ mutant mice
WT: wild type (*Abca4*$^{+/+}$) controls
Treated: C20-D_3-vitamin A diet
Control: normal rodent chow

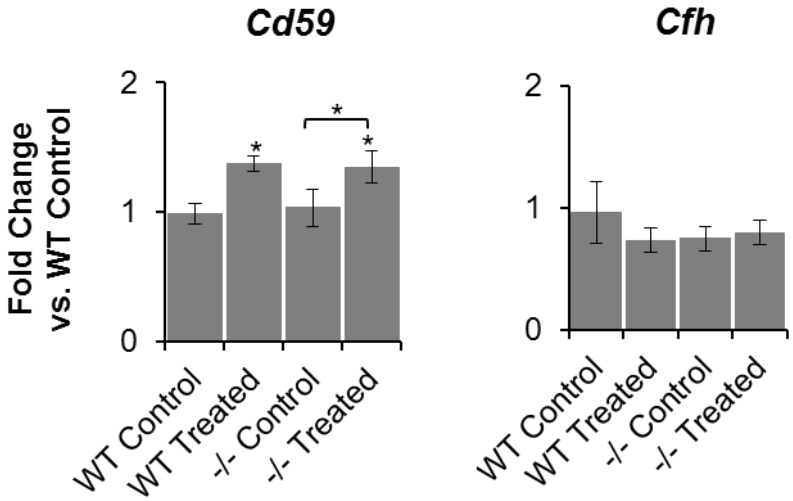

Fig. S2. C20-D$_3$-vitamin alters transcription of complement genes
Quantification of mRNA from 9-month old wild type and *Abca4*$^{-/-}$ mice administered either vitamin A (control) or C20-D$_3$-vitamin A from weaning (treated). To generate each bar, total RNA was extracted from between 5 to 10 eyes. *Cfh*: complement factor h; *Cd59*: MAC-inhibitory protein; -/-: *Abca4*$^{-/-}$ mutant mice WT: wild type controls.

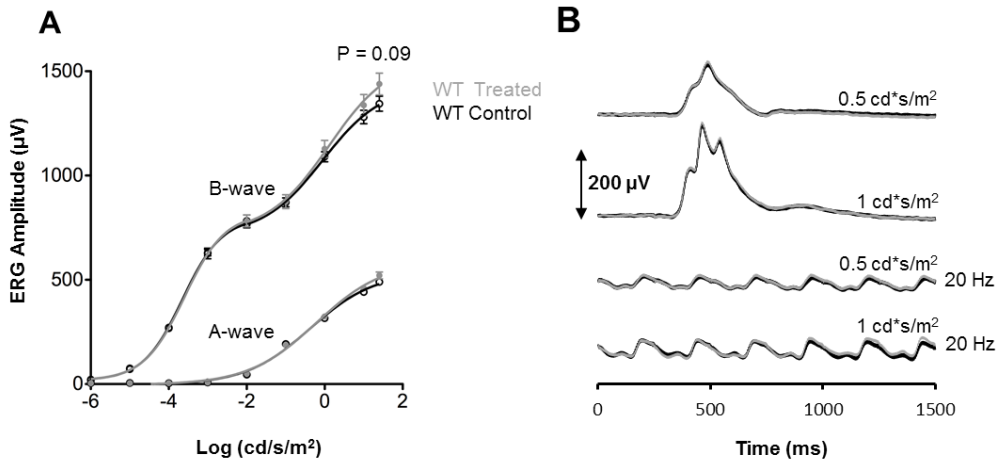

Fig. S3. Long-term inhibition of vitamin A dimerization by C20-D_3-vitamin A does not compromise retinal function in wild-type mice. (A) Average dark-adapted A- and B-wave amplitudes in response to flashes of light of increasing intensity. (**B**) Average light-adapted, B-wave amplitudes with amplitudes elicited by light flickering at 20 Hz. Mice from weaning were given a standard rodent chow (control) or one where vitamin A was replaced with C20-D_3-vitamin A (treated) to 9-month of age. All curves represent average values with standard deviations for 10 eyes (n =10). WT: wild type (*Abca4*$^{+/+}$). All ERG responses were identical between the two cohorts.

SI Methods

Table S2. Primers used for quantitative reverse transcription polymerase chain reaction (RTqPCR)

C3	Fwd	CGGCAAGTTTCTGAACACAG
	Rev	GAGTCAAAGTCTTTCAGCAGCA
CfB	Fwd	GCTCAACCAAATCAGTTATGAAGA
	Rev	CGGGTTCTATTCCAGCCTTC
Cfp	Fwd	TCTTGAGTGGCAGCTACAGG
	Rev	CAGACCAGCCACCCATCT
Cd59	Fwd	GATGTGGCTCAAGATAGTGCTG
	Rev	TTGTGCTGGGCTACCAAAT
CfH	Fwd	AAATGGGATCCTGAACCAAA
	Rev	CACTTGGGTATTTGGAATCTGC

Electroretinography. A DTL-type silver-coated nylon thread active electrode was modified to include a custom-made contact lens of clear Aclar film. This was positioned concentrically on the cornea using hypromellose eye drops (1% methylcellulose solution) for coupling. Recordings were always done from the left eye, while the right eye remained undilated and was protected by a contact lens. Platinum needles in the scruff and at the base of the tail served as reference and ground electrodes, respectively. Unfiltered signals were differentially amplified and digitized at a rate of 5 kHz using an Espion E2 system (Diagnosys LLC, Cambridge, UK). The amplitude and latency of major ERG components were measured with the Espion software (Diagnosys LLC) using automated and manual methods. Brief (4 ms) single flash stimuli were delivered in a Ganzfeld dome. Animals were placed on a heated platform, maintained at 38°C using a circulating pump-water bath. All recordings were made in a custom made light-tight Faraday cage.

For light-adapted testing, animals were pre-exposed to steady full-field white background illumination (30 cd/m2) for 10 min. Responses were then recorded to light flashes of two intensities (0.5 and 1 log cd.s/m^2) superimposed on the same background light with an ISI of 2 seconds (0.5 Hz) and 10 responses averaged per result. In addition, responses to 20 Hz flicker at the same two intensities were recorded and traces were averaged 20 times.

In single flash ERGs, the b-wave amplitude (from a-wave trough to b-wave peak) was measured for all ERGs, whereas the a-wave amplitude (from baseline to a-wave trough) was measured only when recognizable as a distinct component (stimulus intensities ≥ -1 log cd.s/m^2). Both amplitudes were measured in unfiltered recordings.

Table S3. Summary of ERG flash protocol

Flash intensity [log cd.s/m^2]	Repetitions	Inter-stimulus interval [sec]	interval after intensity step [sec]	
-6	16	5	20	Scotopic testing
-5	16	5	20	
-5	16	5	20	
-3	9	20	60	
-2	9	20	60	
-1	9	20	60	
0	4	60	120	
1	1	---	120	
1.4	1	---	120	
Pre-exposure: steady full-field white background illumination (30 cd/m2) for 10 min				
0.5	10	0.5	no fixed interval	Photopic testing
1	10	0.5	no fixed interval	
0.5	20 traces	20Hz flicker	no fixed interval	
1	20 traces	20Hz flicker	no fixed interval	