SUPPLEMENTARY ONLINE DATA FOR

Aberrant Buildup of All-\textit{Trans}-Retinal Dimer, a Non-Pyridinium Bisretinoid Lipofuscin Fluorophore, Contributes to the Degeneration of the Retinal Pigment Epithelium

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Supplementary Figure S1. Standard calibration curves of atRAL dimer (A) and A2E (B) were constructed by plotting the peak area versus the molar quantity of each analyte detected by HPLC.

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**A**

\[ y = 0.4551x + 0.4742 \]

\[ R^2 = 0.9997 \]

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**B**

\[ y = 1.903x - 3.3819 \]

\[ R^2 = 0.9994 \]
Supplementary Figure S2. $^1$H-NMR spectrum (500 MHz, CD$_3$OD) of A2E. Inset left, the A2E structure in which the carbons are numbered.$^1$H-NMR (CD$_3$OD, 500 MHz): δ 1.05, 1.07 [each 6H, s, C5-(CH$_3$)$_2$ and C5'-(CH$_3$)$_2$], 1.53 (4H, m, C4-H$_2$ and C4'-H$_2$), 1.68 (4H, m, C3-H$_2$ and C3'-H$_2$), 1.73, 1.75 (each 3H, s, C1-CH$_3$ and C1'-CH$_3$), 2.05 (3H, s, C9-CH$_3$), 2.07 (4H, m, C2-H$_2$ and C2'-H$_2$), 2.16 (3H, s, C13-CH$_3$), 2.18 (3H, s, C9'-CH$_3$), 3.93 (2H, t, $J=5.0$ Hz, C8-H), 4.55 (2H, t, $J=4.8$ Hz, N-CH$_2$), 6.20 (1H, d, $J=16.1$ Hz, C8-H), 6.27 (1H, d, $J=12.8$ Hz, C10-H), 6.30 (1H, d, $J=16.3$ Hz, C8'-H), 6.36 (1H, d, $J=16.1$ Hz, C7-H), 6.43 (1H, d, $J=11.5$ Hz, C10'-H), 6.57 (1H, d, $J=15.9$ Hz, C7'-H), 6.63 (1H, d, $J=15.1$ Hz, C12-H), 6.70 (1H, s, C14-H), 6.78 (1H, d, $J=15.2$ Hz, C12'-H), 7.15 (1H, dd, $J=11.4$, 15.0 Hz, C11-H), 7.87 (1H, d, $J=1.5$ Hz, C16-CH), 7.94 (1H, dd, $J=1.8$, 6.7 Hz, C14'-H), 8.02 (1H, dd, $J=11.6$, 15.1 Hz, C11'-H), and 8.55 (1H, d, $J=6.8$ Hz, C15'-H).
Supplementary Figure S3. $^1$H-NMR spectrum (500 MHz, CDCl$_3$) of atRAL dimer. Inset left, the atRAL dimer structure in which the carbons are numbered. $^1$H-NMR (CDCl$_3$, 500 MHz):

$\delta$ 9.46 (1H, s, CH=O, H15'), 6.96 (1H, dd, $J$=11.5, 15.1 Hz, H11), 6.82 (1H, d, $J$=6.1 Hz, H15), 6.41 (1H, d, $J$=15.1 Hz, H12), 6.35 (1H, dd, $J$=11.1, 15.3 Hz, H11'), 6.30 (1H, d, $J$=16.1 Hz, H7), 6.19 (1H, d, $J$=12.7 Hz, H10), 6.17 (1H, d, $J$=10.8 Hz, H8), 6.16 (1H, d, $J$=11.5 Hz, H14), 6.08 (1H, d, $J$=13.7 Hz, H7'), 6.01 (1H, d, $J$=16.0 Hz, H8'), 5.99 (1H, d, $J$=10.9 Hz, H10'), 5.85 (1H, d, $J$=15.3 Hz, H12'), 2.69 (1H, d, $J$=16.9 Hz, equatorial H20), 2.42 (1H, d, $J$=16.8 Hz, axial H20), 2.01 (7H, m, H4, H19, H4'), 1.85 (3H, s, H19'), 1.73 (3H, s, H18), 1.66 (3H, s, H18'), 1.48 (3H, s, H20'), 1.45 (4H, m, H2, H2'), 1.04 (6H, s, H16, H17), 0.98 (6H, s, H16', H17).
**Supplementary Figure S4.** Reverse-phase HPLC chromatograms of synthetic atRAL dimer (A) and A2E (B). For compound elution, an Atlantis dC18 (3 µm, 4.6 mm × 150 mm) reverse-phase column was used for the stationary phase, and a gradient of acetonitrile in water with 0.1% trifluoroacetic acid was set for the mobile phase: 85–100% acetonitrile, 0.8 mL/min, 15 min; 100% acetonitrile, 0.8–1.2 mL/min, 15–20 min; 100% acetonitrile, 1.2 mL/min, 20–40 min. Detection by photodiode array was set at 430 nm. *Insets in A and B,* UV-visible absorbance spectra of atRAL dimer and A2E.
Supplementary Figure S5. The full-length blots for Fig. 4C.
Supplementary Figure S6. The full-length blots for Fig. 7B.
**Supplementary Figure S7.** The full-length blots for Figs. 8A, 8D.
Supplementary Figure S8. Cyclin B1 protein expression in the RPE of C57BL/6J, C57BL/6N and Abca4<sup>-/-</sup> Rh8<sup>-/-</sup> DKO mice. (A) Sample gel showing resolution of the Wt (220bp) genotype in C57BL/6J mice and rh8 homozygous (244bp) genotype in C57BL/6N and Abca4<sup>-/-</sup> Rh8<sup>-/-</sup> DKO mice. (B) Sequencing of Crb1 gene showed a single base deletion in both C57BL/6N and Abca4<sup>-/-</sup> Rh8<sup>-/-</sup> DKO mice at the expected position aligned with the sequence from C57BL/6J mice. (C) Expression level of Cyclin B1 in the RPE homogenates (30 µg per lane) of C57BL/6J, C57BL/6N and Abca4<sup>-/-</sup> Rh8<sup>-/-</sup> DKO mice is measured by Western blot. Relative protein band intensities were quantified by Quantity One and presented as mean ± SEM. Two mice were used for analysis of each genotype.