SUPPLEMENTARY MATERIAL

A p97/valosin-containing protein inhibitor drug CB-5083 has a potent but reversible off-target effect on phosphodiesterase-6

Henri Leinonen*, Cheng Cheng, Marja Pitkänen, Christopher L. Sander, Jianye Zhang, Sama Saeid, Teemu Turunen, Alyaa Shmara, Lan Weiss, Lac Ta, Timothy Ton, Ari Koskelainen, Jesse D. Vargas, Virginia Kimonis*, Krzysztof Palczewski*.

H.L., C.L.S., J.Z., K.P.: Gavin Herbert Eye Institute, Dept. of Ophthalmology, UC Irvine, Irvine, CA, USA.

K.P.: Dept. of Physiology & Biophysics, UC Irvine, Irvine, CA, USA.

K.P.: Dept. of Chemistry, UC Irvine, Irvine, CA, USA.

C.C., A.S., L.W., L.T., T.T., V.K.: Div. of Genetics and Genomic Medicine, Dept. of Pediatrics, UC Irvine, Irvine, CA, USA.

M.P., S.S., T.T., A.K.: Dept. of Neuroscience and Biomedical Engineering, Aalto University, Espoo, Finland.

J.D.V.: Cleave Therapeutics, Inc., San Francisco, CA, USA. (formerly Cleave Biosciences Inc.)

Running title: Off-target deactivation of PDE6 by VCP inhibitor CB-5083

*Correspondence:

Krzysztof Palczewski Ph.D., Gavin Herbert Eye Institute, Department of Ophthalmology, Gillespie Neuroscience Research Facility, room 2216, 837 Health Sciences Rd, Irvine, CA, 92617. Email: kpalczew@uci.edu, phone: (949)824-6527

Henri Leinonen, Ph.D., Gavin Herbert Eye Institute, Department of Ophthalmology, Gillespie Neuroscience Research Facility, room 2216, 837 Health Sciences Rd, Irvine, CA, 92617. Email: hleinone@uci.edu, phone: (216)502-5428.

Virginia Kimonis, MD. MRCP, Professor, Dept. of Pediatrics, Division of Genetics and Genomic Medicine, Univ. of California-Irvine Med. Center, Hewitt Hall, Rm 2038, 843 Health Sciences Rd., Irvine CA 92697 Email: vkimonis@uci.edu phone: (949)824-0571

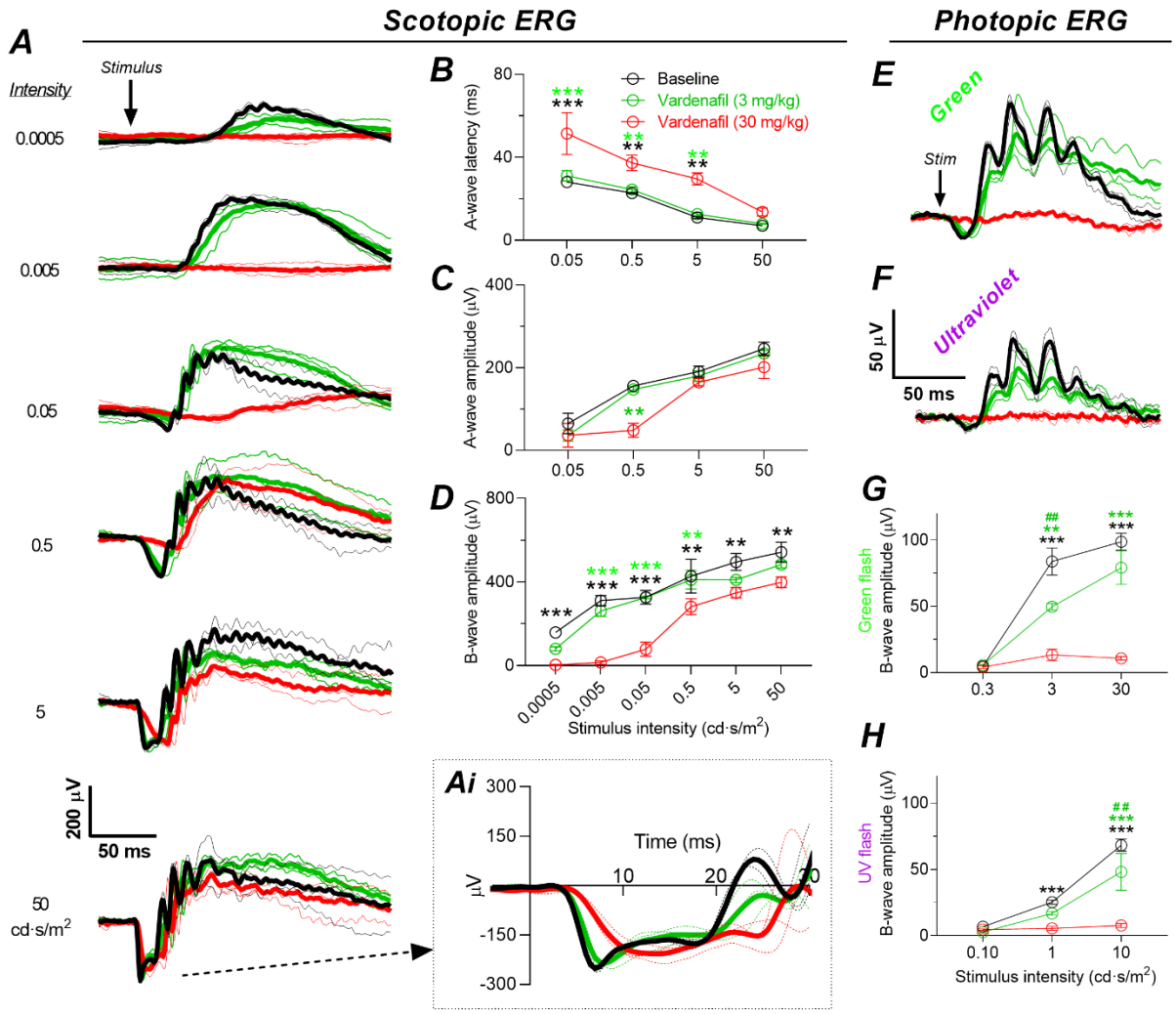

Figure S1. Single systemic administration of vardenafil strongly suppresses retinal function in mice. ERGs were first recorded in non-treated C57BL/6J mice (baseline, n=3, black), and then repeated in a separate session 45 min after intraperitoneal vardenafil. This two-step sequence was conducted for each dose of vardenafil (3 mg/kg and 30 mg/kg, green and red, respectively). The non-treatment and treatment sessions were separated by 1 week. (A) Rod-dominant scotopic ERG waveforms. Thin traces are individual mouse responses and thick lines are group averages. Inset Ai shows a magnified image of the A-wave leading edge at the highest stimulus strength. (B) A-wave latencies. (C) A-wave amplitudes. (D) B-wave amplitudes. (E-F) Photopic ERG waveforms in response to green (30 cd·s/m^2) and UV (10 cd·s/m^2) flashes. (G-H) Photopic ERG amplitudes. Statistical analysis: Repeated measures ANOVA followed by Bonferroni's post hoc tests. *P<0.05, **$^{/\#\#}P$<0.01, ***P<0.001. Asterisks mark significant difference between baseline and 30 mg/kg vardenafil (black), or 3 mg/kg vardenafil and 30 mg/kg vardenafil (green). Pound signs mark difference between baseline and 3 mg/kg vardenafil. Data are presented as mean ± SD.

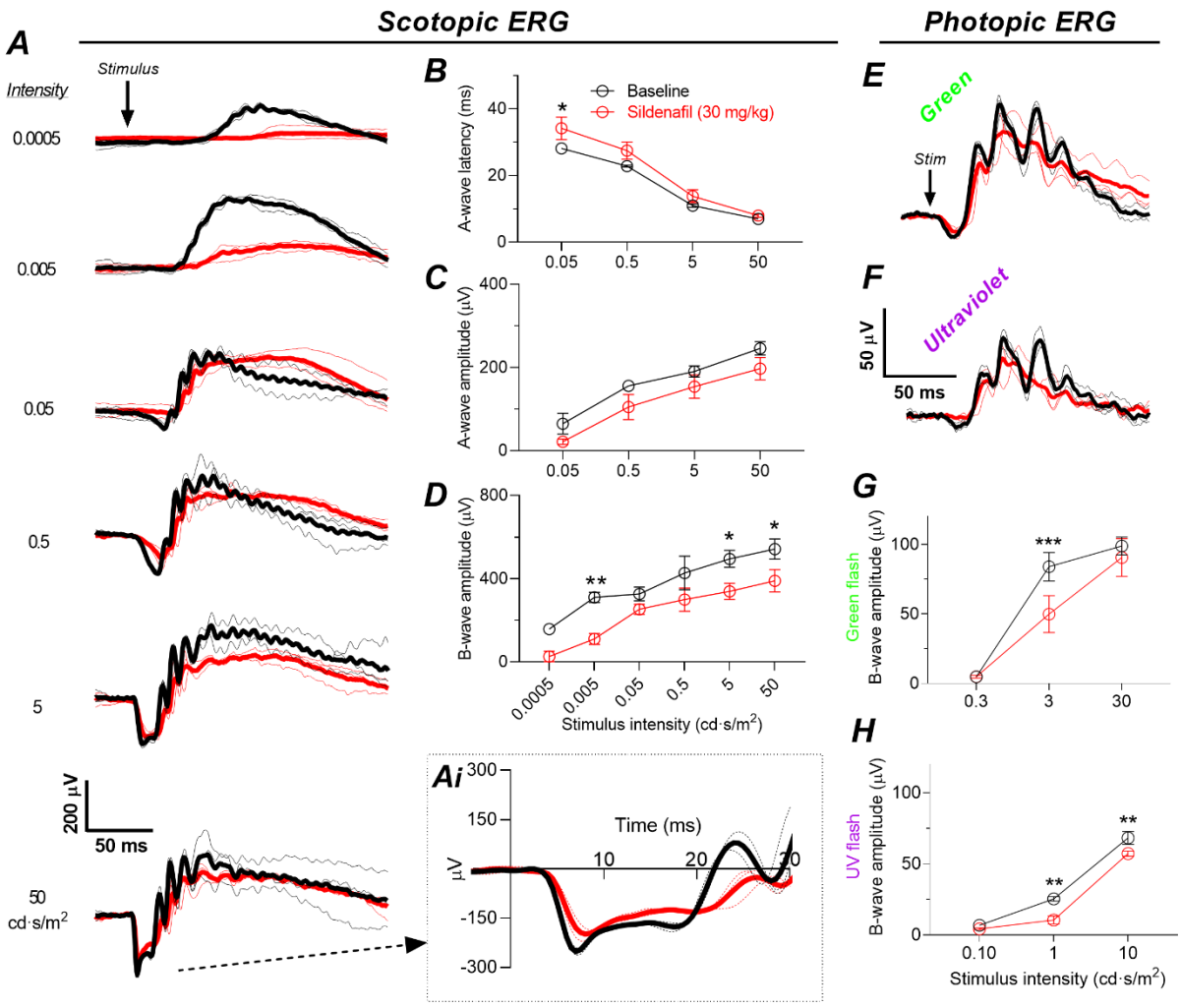

Figure S2. Single administration of sildenafil moderately suppresses retinal function in mice. ERGs were first recorded in non-treated C57BL/6J mice (baseline, n=3, black), and then repeated in a separate session 45 min after intraperitoneal sildenafil 30 mg/kg (red). The non-treatment and treatment sessions were separated by 1 week. (A) Rod-dominant scotopic ERG waveforms. Thin traces are individual mouse responses and thick lines are group averages. Inset Ai shows a magnified image of the A-wave leading edge at the highest stimulus strength. (B) A-wave latencies. (C) A-wave amplitudes. RM ANOVA between-subjects main effect: $P<0.01$. (D) B-wave amplitudes. (E-F) Photopic ERG waveforms in response to green (30 cd·s/m^2) and UV (10 cd·s/m^2) flashes. (G-H) Photopic ERG amplitudes. Statistical analysis: Repeated measures ANOVA followed by Bonferroni's post hoc tests. *$P<0.05$, **$P<0.01$. Data are presented as mean ± SD.

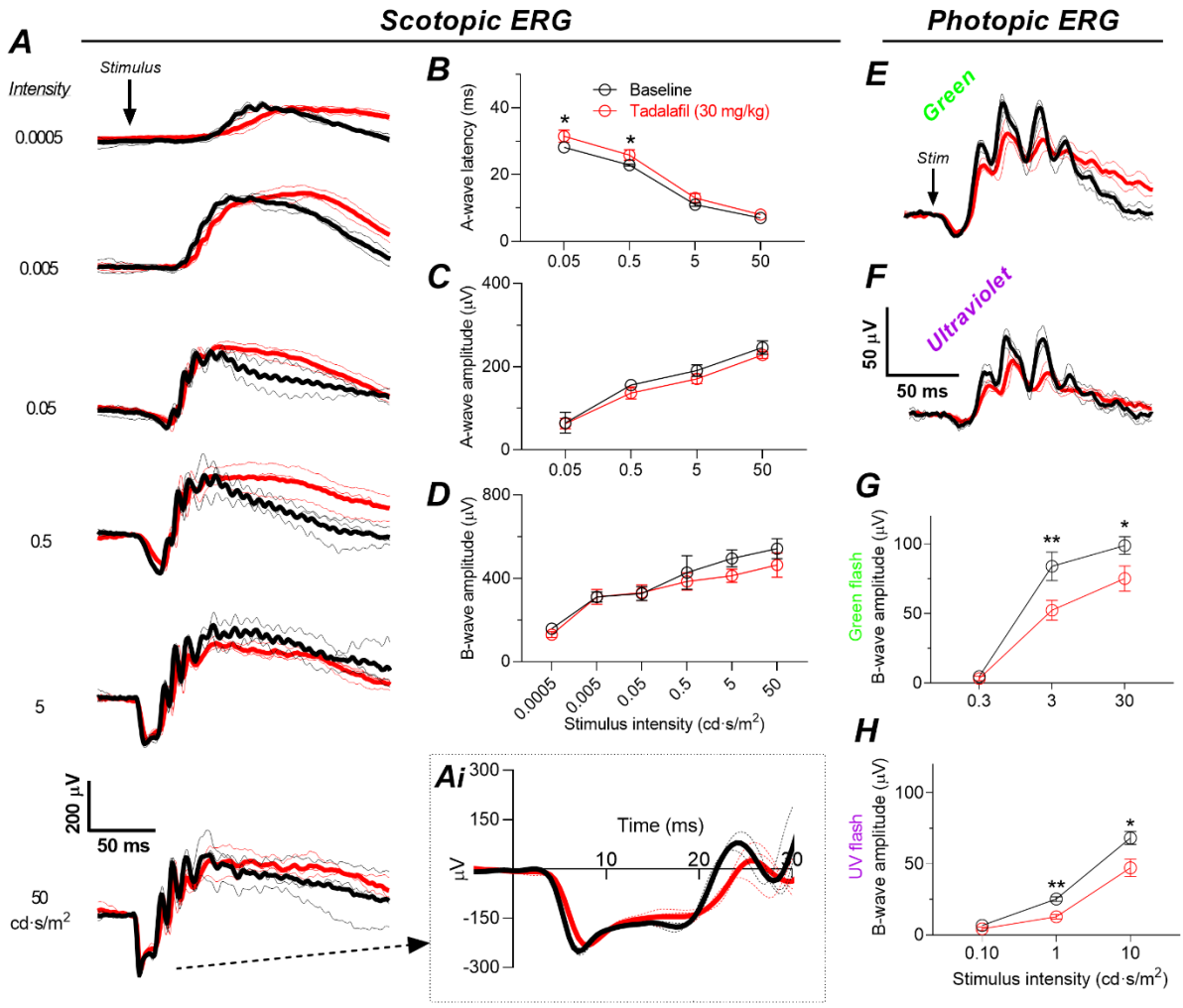

Figure S3. Single administration of tadalafil shows a mild effect on rod-dominant ERG and an intermediate effect on cone-mediated ERG. ERGs were first recorded in non-treated C57BL/6J mice (baseline, n=3, black), and then repeated in a separate session 45 min after intraperitoneal tadalafil 30 mg/kg (red). The non-treatment and treatment sessions were separated by 1 week. (A) Rod-dominant scotopic ERG waveforms. Thin traces are individual mouse responses and thick lines are group averages. Inset Ai shows a magnified image of the A-wave leading edge at the highest stimulus strength. (B) A-wave latencies. (C) A-wave amplitudes. RM ANOVA between-subjects main effect: $P<0.05$. (D) B-wave amplitudes. RM ANOVA between-subjects main effect: $P<0.01$ (E-F) Photopic ERG waveforms in response to green (30 cd·s/m^2) and UV (10 cd·s/m^2) flashes. (G-H) Photopic ERG amplitudes. Statistical analysis: Repeated measures ANOVA followed by Bonferroni's post hoc tests. *$P<0.05$, **$P<0.01$. Data are presented as mean ± SD.

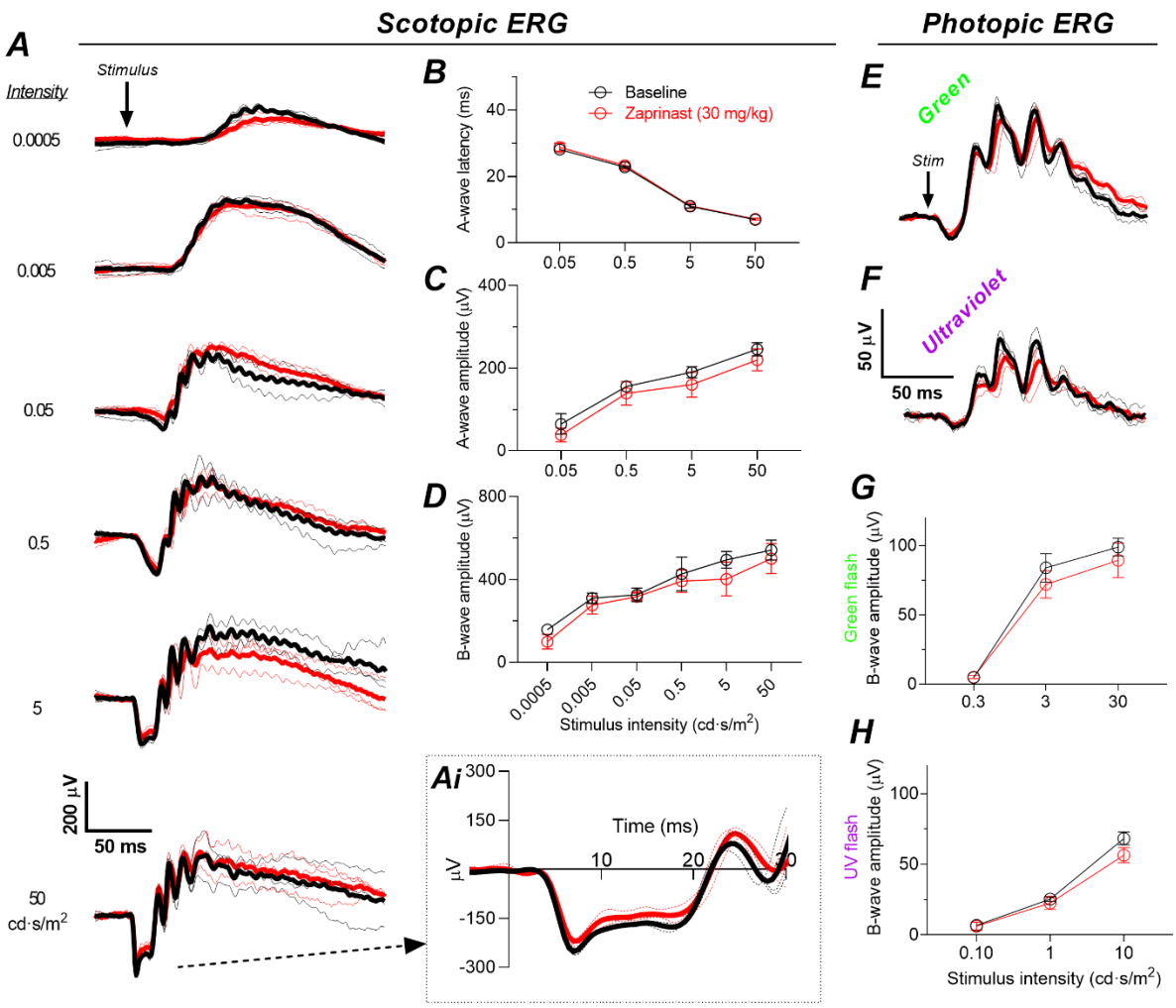

Figure S4. Single administration of a PDE6-selective inhibitor Zaprinast has a negligible effect on retinal function in mice. ERGs were first recorded in non-treated C57BL/6J mice (baseline, n=3, black), and then repeated in a separate session 45 min after intraperitoneal zaprinast 30 mg/kg (red). The non-treatment and treatment sessions were separated by 1 week. (A) Rod-dominant scotopic ERG waveforms. Thin traces are individual mouse responses and thick lines are group averages. Inset Ai shows a magnified image of the A-wave leading edge at the highest stimulus strength. (B) A-wave latencies. (C) A-wave amplitudes. (D) B-wave amplitudes. (E-F) Photopic ERG waveforms in response to green (30 cd·s/m^2) and UV (10 cd·s/m^2) flashes. (G-H) Photopic ERG amplitudes. Statistical analysis: Repeated measures ANOVA. No significant differences between groups were found although RM ANOVA between-subject effect trended towards significance in A- and B-wave amplitudes (P=0.07 and P=0.05, respectively). Data are presented as mean ± SD.

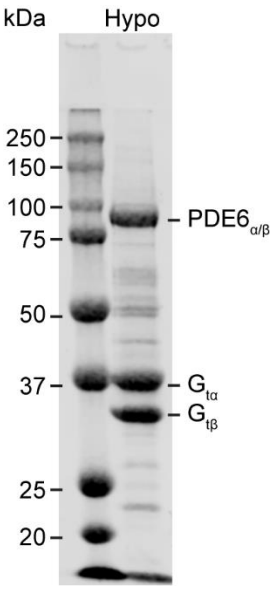

Figure S5. Coomassie staining of the hypotonic bovine ROS fraction used in the PDE6-activity assay. PDE6 is roughly one third of the protein in the hypotonic wash fraction of freshly prepared ROS, the other two thirds being the alpha and beta subunits of transducin ($G_{t\alpha}$ and $G_{t\beta}$, respectively).

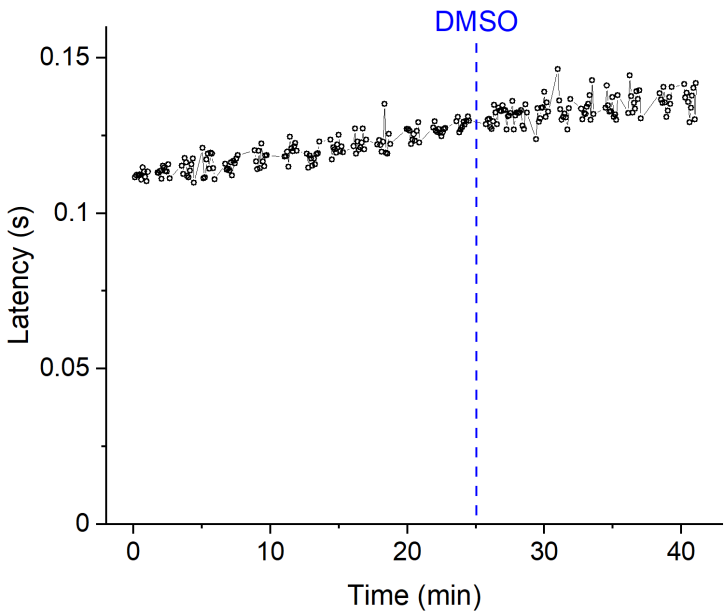

Figure S6. DMSO at a concentration of 0.00026 % in *ex vivo* ERG perfusion solution does not affect the ERG signal. The latency of the response to a dim flash stimulus in a photoreceptoral *ex vivo* ERG was followed through a ~40 min experiment. DMSO (0.00026 v/v %) was added to the perfusion solution at the 25 min time point with no discernible effect on response kinetics.

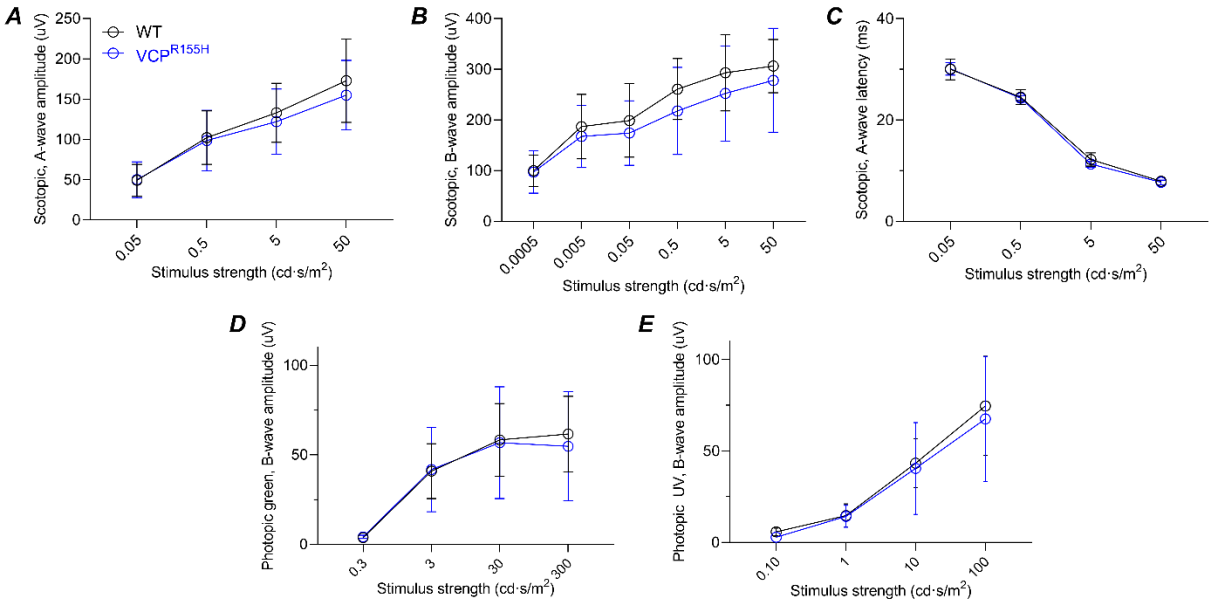

Figure S7. Retinal function is not significantly altered in aged heterozygous *VCP^{R155H}* knock-in mice. ERG recording was performed in 24-month-old VCP mice (n=5) and 19-month-old C57BL/6J wild-type mice (n=7). (A). Scotopic A-wave amplitude. (B) Scotopic B-wave amplitude. (C) Scotopic A-wave latency. (D) Photopic B-wave amplitude for green stimuli. (E) Photopic B-wave amplitude for UV stimuli. Statistical analysis: Two-way ANOVA. No significant differences between groups were found. Data are presented as mean ± SD.