SUPPLEMENTAL MATERIAL

Identification of early disease biomarkers in a mouse model of *IMPG2*-associated retinal degeneration

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SUPPLEMENTAL FIGURES

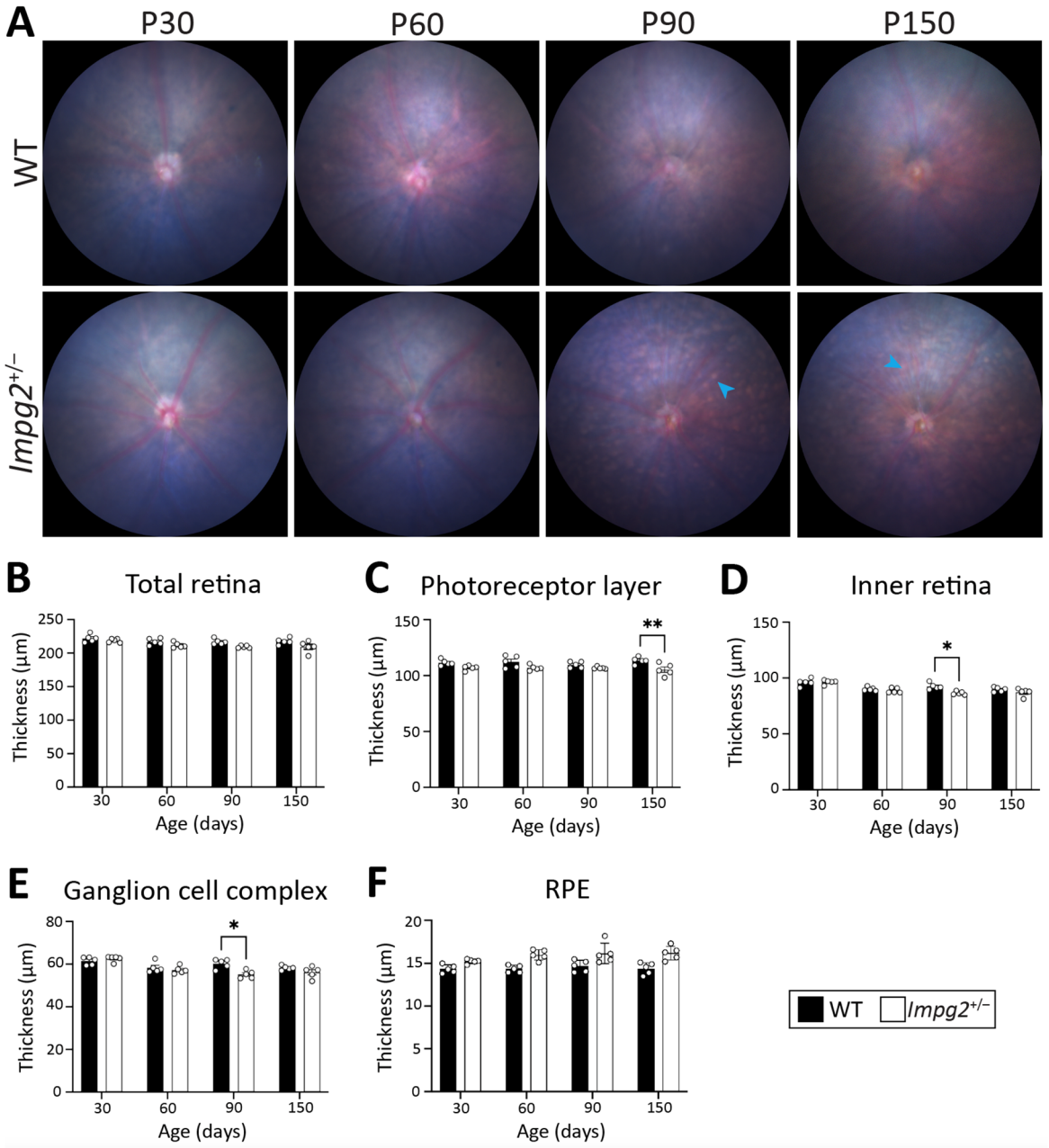

Figure S1: Fundus imaging and retinal thickness measurements reveal mild structural alterations in *Impg2*$^{+/-}$ mice.
(A) Representative WT and *Impg2*$^{+/-}$ fundus images taken at P30, P60, P90, and P150. Small, yellowish puncta (blue arrowheads) are observed in *Impg2*$^{+/-}$ mice at ages P90 and P150. WT retinas are unremarkable. **(B)** Total retina, **(C)** photoreceptor layer, **(D)** inner retina, **(E)** ganglion cell complex, and **(F)** retinal pigment epithelium (RPE) thickness measurements collected at each timepoint. Each data point represents one mouse, and data are expressed as mean ± SEM. Statistical significance, determined using a two-way ANOVA with Šidák correction for multiple comparisons, is defined as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Sample sizes (Age: WT, *Impg2*$^{+/-}$) – P30: 5, 5; P60: 5, 5; P90: 5, 5; P150: 5, 5.

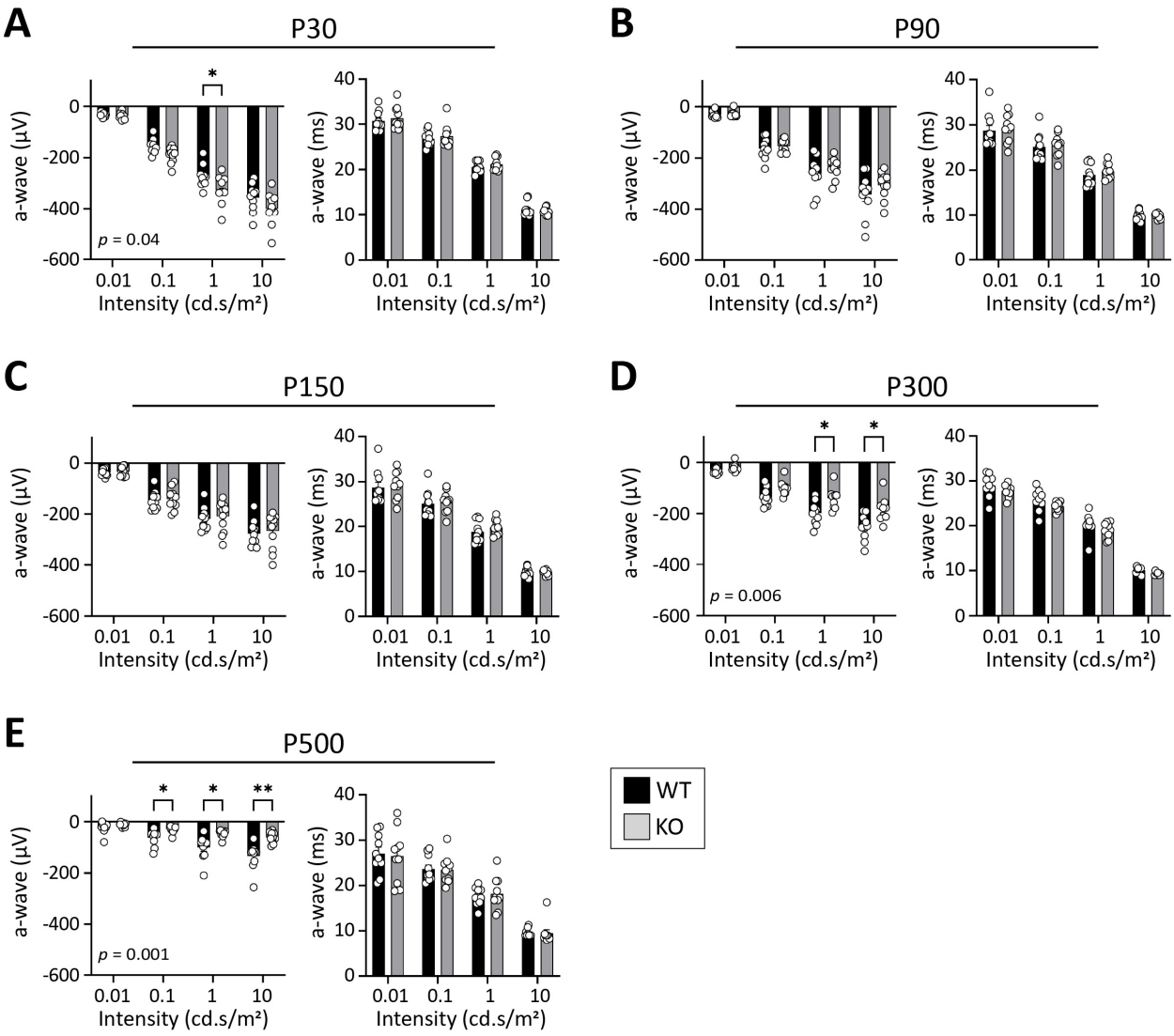

Figure S2: ERG a-wave amplitude and implicit time.
Measurement of the a-wave amplitude and implicit time for ERGs generated in response to a broadband light stimulus at 0.01 cd.s/m^2, 0.1 cd.s/m^2, 1 cd.s/m^2, and 10 cd.s/m^2 under dark adaptation in WT (black) and KO (gray) mice at ages **(A)** P30, **(B)** P90, **(C)** P150, **(D)** P300, and **(E)** P500. For each panel, a-wave amplitudes are shown on the left and implicit times are shown on the right. In comparison to WT mice, a-wave amplitude gradually declines in KO mice, and implicit time is unaffected. Each data point represents one mouse, and data are expressed as mean ± SEM. Statistical significance, determined using a two-way ANOVA with Šidák correction for multiple comparisons, is defined as *$p < 0.05$, **$p < 0.01$. Sample sizes (Age: WT, KO) – P30: 10, 10; P90: 10, 10; P150: 11, 10; P300: 10, 10; P500: 10, 10.

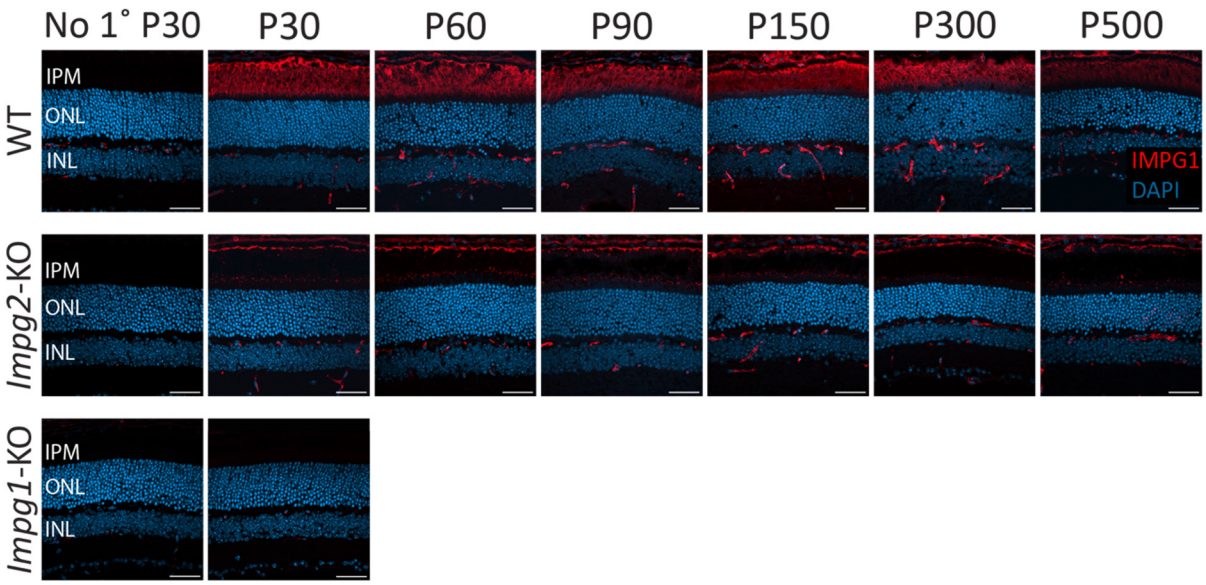

Figure S3: IMPG1 mislocalization in *Impg2*-KO mice (uncropped images).
Uncropped versions of images included in Figure 4A and representative staining performed in a P30 *Impg1* knockout (*Impg1*-KO) retina are shown**.** Confocal micrographs display non-specific IMPG1 signal in the inner retina, as well as the same IPM distribution described in the main text for *Impg2* knockout (*Impg2*-KO) and WT tissue. IMPG1 signal in the IPM is absent in *Impg1*-KO tissue. IPM, interphotoreceptor matrix; ONL, outer nuclear layer; INL, inner nuclear layer; no 1° P30, negative control in which no primary antibody was applied to P30 tissue. Scale bar, 40 µr 3

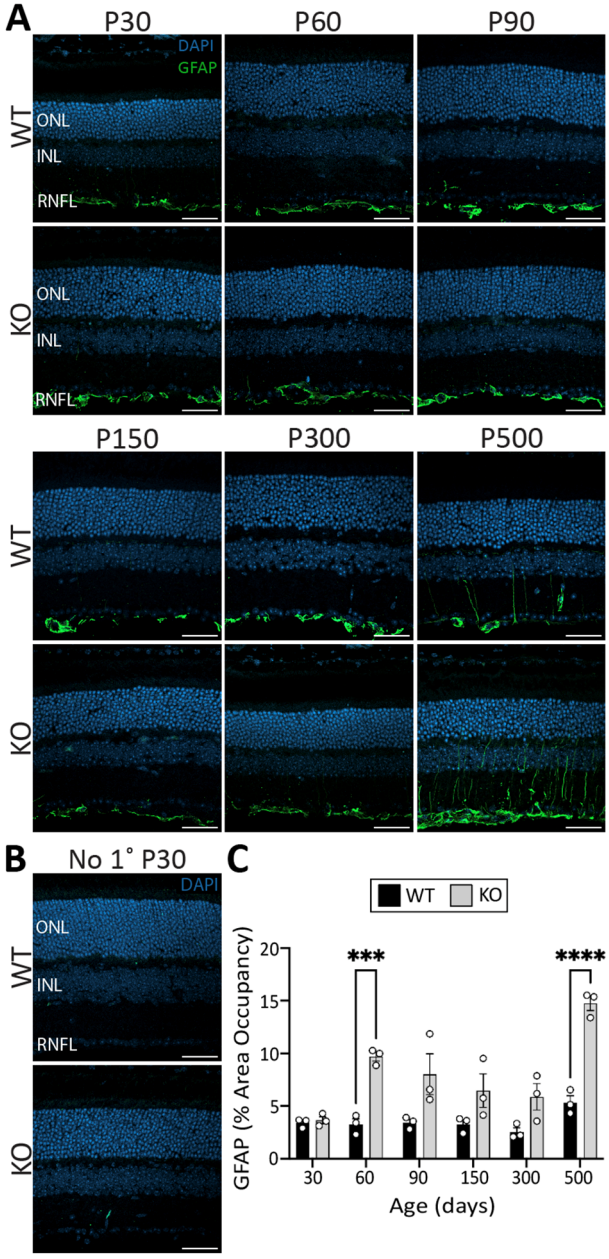

Figure S4: Reactive gliosis is modest in a central retinal region of *Impg2*-KO mice. **(A)** Representative time course of GFAP (green) expression in WT and KO retinal cross-sections collected near the optic nerve at P30, P60, P90, P150, P300, and P500 via confocal microscopy. ONL, outer nuclear layer; INL, inner nuclear layer; RNFL, retinal nerve fiber layer. Scale bar, 40 μr 3**(B)** Representative images of P30 WT and KO retinal tissue sections incubated without primary antibody, confirming the specificity of the GFAP antibody. **(C)** GFAP percent occupancy was calculated as the area of GFAP-positive retina spanning from the inner limiting membrane to the external limiting membrane in a 300 μm-wide annotation area. Except for P30, gliosis in KO tissue (gray) was slightly increased compared with WT tissue (black). Each data point represents one mouse, n = 3/age/genotype , and data are expressed as mean ± SEM. Statistical significance, determined using a two-way ANOVA with Šidák correction for multiple comparisons, is defined as ***$p < 0.001$, ****$p < 0.0001$.

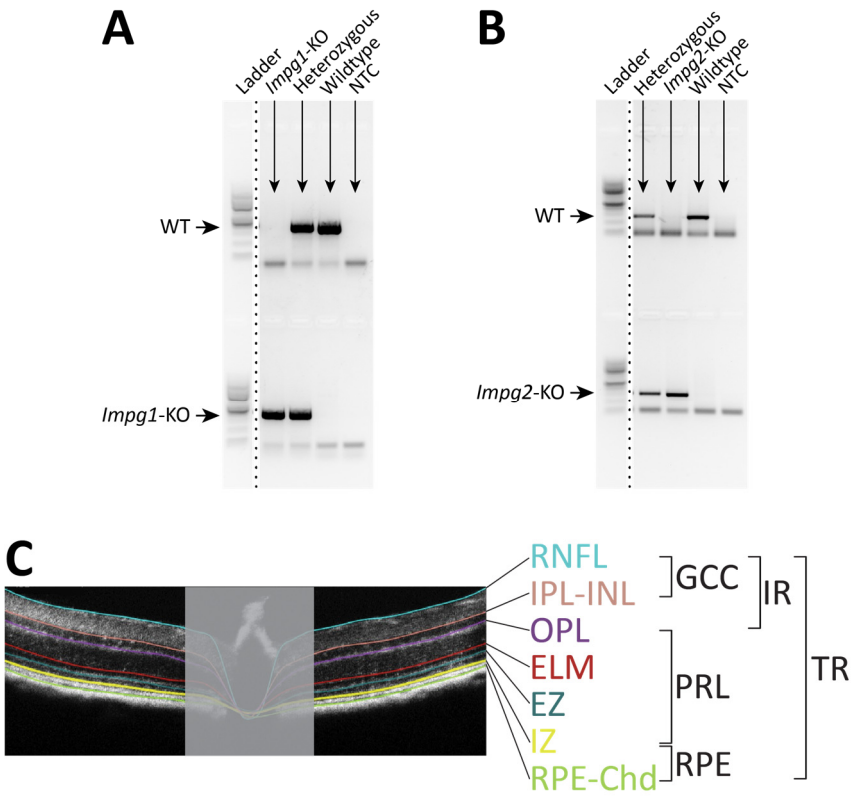

Figure S5: PCR genotyping assays for *Impg1*-KO mice and *Impg2*-KO mice and method for quantifying retinal layer thickness.
Genomic DNA samples were collected from WT, heterozygous, *Impg1* knockout (*Impg1*-KO), and *Impg2* knockout (*Impg2*-KO) mice and subjected to PCR. The amplified DNA products were visualized following electrophoresis in 1.5% agarose gels. Each gel includes a lane for the molecular weight ladder and for the no-template control (NTC) sample in which the PCR reaction contained no DNA. **(A)** *Impg1*-KO genotyping. Top gel: One PCR reaction used a primer pair that amplifies a 495 bp region within the WT allele and does not amplify the KO allele. Bottom gel: A second PCR reaction used a primer pair that amplifies a 478 bp region within the KO allele and does not amplify the WT allele. WT DNA samples produce only the band from the WT reaction, heterozygous DNA samples produce a band from both reactions, and *Impg1*-KO DNA samples produce only the band from the KO reaction. **(B)** *Impg2*-KO genotyping. Top gel: One PCR reaction used a primer pair that amplifies a 256 bp region within the WT allele and does not amplify the KO allele. Bottom gel: A second PCR reaction used a primer pair that amplifies a 250 bp region within the KO allele and does not amplify the WT allele. WT DNA samples produce only the band from the WT reaction, heterozygous DNA samples produce a band from both reactions, and *Impg2*-KO DNA samples produce only the band from the KO reaction. **(C)** Segmentation of retinal OCT images and quantification of retinal layers. All OCT images used for retinal layer measurements were acquired in the nasotemporal plane at the level of the optic nerve head. Segmentation lines correspond to the retinal nerve fiber layer (RNFL, cyan), the boundary between the inner plexiform layer and inner nuclear layer (IPL-INL, taupe), the outer plexiform layer (OPL, purple), the external limiting membrane (ELM, red), ellipsoid zone (EZ, teal), the interdigitation zone (IZ, yellow), and the junction of the retinal pigment epithelium and choroid (RPE-Chd, green). Thicknesses of the total retina (TR), photoreceptor layer (PRL), inner retina (IR), ganglion cell complex (GCC), and retinal pigment epithelium (RPE) were quantified. The final value for each outcome is the average of all measurements calculated across the retina, excluding those acquired at and immediately surrounding the optic nerve head (shaded gray region).

SUPPLEMENTAL TABLES

Table S1. Subretinal lesion area measurements from fundus images

Age	WT (μm^2) Mean ± SEM	*Impg2*-KO (μm^2) Mean ± SEM
P30	24 ± 4	1,922 ± 1,900
P60	31 ± 9	**123,122**** ± 26,968
P90	23 ± 3	**92,096*** ± 28,245
P150	25 ± 4	**57,058*** ± 15,166

Significance is defined as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Table S2. Retinal thickness measurements acquired from OCT images

Age	Total Retinal Thickness (μm) Mean ± SEM		Photoreceptor Layer Thickness (μm) Mean ± SEM		Inner Retinal Thickness (μm) Mean ± SEM		Ganglion Cell Complex Thickness (μm) Mean ± SEM	
	WT	***Impg2*-KO**	**WT**	***Impg2*-KO**	**WT**	***Impg2*-KO**	**WT**	***Impg2*-KO**
P30	212.1 ± 1.2	205.0 ± 1.2	99.3 ± 1.4	97.2 ± 1.2	93.2 ± 1.0	89.9 ± 0.8	60.0 ± 0.8	58.2 ± 0.9
P60	210.1 ± 1.5	209.1 ± 2.9	99.7 ± 1.0	100.4 ± 1.4	91.0 ± 0.9	89.2 ± 1.5	60.3 ± 0.9	57.8 ± 1.2
P90	210.7 ± 1.0	**198.8****** ± 1.4	98.0 ± 0.9	93.8 ± 0.8	89.7 ± 1.2	**84.4*** ± 1.2	57.2 ± 0.8	55.0 ± 0.8
P150	212.1 ± 1.4	**196.4****** ± 1.6	101.3 ± 1.5	**92.6***** ± 1.4	90.6 ± 0.9	**83.0****** ± 0.9	57.1 ± 0.6	54.9 ± 1.0
P300	212.7 ± 3.3	**190.7****** ± 2.1	105.7 ± 2.2	**88.2****** ± 1.6	89.3 ± 1.6	**82.1****** ± 1.1	59.5 ± 1.2	**53.3****** ± 1.0
P500	210.5 ± 1.6	**170.4****** ± 3.1	103.6 ± 1.2	**72.8****** ± 2.0	89.1 ± 0.8	**79.6****** ± 1.1	58.5 ± 1.0	**55.2*** ± 0.8

Significance is defined as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Table S3. ERG measurements

		Dark-adapted										Light-adapted			
		0.001 cd.s/m²		0.01 cd.s/m²		0.1 cd.s/m²		1 cd.s/m²		10 cd.s/m²		3 cd.s/m²		10 cd.s/m²	
		WT	KO	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO
b-wave (µV)	P30	215.6 ± 15.5	238.6 ± 22.4	396.5 ± 24.0	414.8 ± 33.2	505.3 ± 26.5	506.3 ± 43.5	614.8 ± 26.7	647.4 ± 49.9	645.0 ± 33.1	652.5 ± 48.4	73.5 ± 4.9	64.7 ± 5.1	145.1 ± 10.4	129.9 ± 10.8
	P90	245.4 ± 19.5	190.7 ± 19.1	376.4 ± 33.5	301.3 ± 30.0	447.9 ± 40.7	371.3 ± 33.4	526.5 ± 50.4	458.9 ± 41.3	568.3 ± 53.9	495.8 ± 43.3	93.3 ± 9.8	59.9 ± 5.9	183.1 ± 17.3	**142.0*** ± 12.7
	P150	187.2 ± 17.1	178.5 ± 19.0	309.0 ± 22.3	252.2 ± 22.4	377.4 ± 25.6	310.4 ± 26.8	424.1 ± 28.6	380.2 ± 32.7	445.6 ± 25.3	403.9 ± 34.6	71.1 ± 2.6	62.6 ± 8.4	142.7 ± 6.2	130.4 ± 15.8
	P300	198.0 ± 17.0	129.9 ± 18.2	258.4 ± 22.7	200.0 ± 21.8	331.7 ± 22.2	247.4 ± 23.2	375.1 ± 28.7	290.5 ± 27.4	387.7 ± 28.0	304.4 ± 28.7	59.0 ± 6.8	38.1 ± 3.7	115.5 ± 12.4	**80.2**** ± 7.3
	P500	88.9 ± 19.5	33.5 ± 8.5	125.4 ± 23.8	52.0 ± 11.4	156.7 ± 25.9	**67.8*** ± 13.3	185.3 ± 27.3	**86.1*** ± 16.6	212.5 ± 27.6	**100.5*** ± 15.1	28.8 ± 3.5	14.4 ± 2.0	59.4 ± 8.6	**25.4**** ± 3.6
a-wave (µV)	P30	n/a	n/a	-32.7 ± 2.2	-36.7 ± 3.9	-157.1 ± 9.0	-191.2 ± 9.9	-273.2 ± 13.8	**-327.9*** ± 17.8	-357.7 ± 17.7	-400.6 ± 20.5	n/a	n/a	n/a	n/a
	P90	n/a	n/a	-31.5 ± 3.9	-29.3 ± 3.8	-163.3 ± 13.1	-153.2 ± 7.9	-262.2 ± 21.6	-240.4 ± 13.1	-343.0 ± 27.3	-307.5 ± 17.4	n/a	n/a	n/a	n/a
	P150	n/a	n/a	-36.3 ± 4.1	-34.1 ± 6.0	-146.1 ± 10.7	-141.2 ± 13.6	-220.0 ± 13.4	-204.4 ± 19.2	-278.9 ± 15.1	-260.2 ± 22.2	n/a	n/a	n/a	n/a
	P300	n/a	n/a	-29.7 ± 3.9	-18.8 ± 4.8	-136.9 ± 11.1	-98.8 ± 8.7	-199.0 ± 14.2	**-140.1*** ± 12.5	-252.0 ± 16.1	**-182.0*** ± 14.9	n/a	n/a	n/a	n/a
	P500	n/a	n/a	-22.4 ± 7.1	-10.6 ± 2.4	-62.9 ± 9.6	**-29.7*** ± 4.3	-101.3 ± 14.9	**-46.6*** ± 4.5	-135.0 ± 15.9	**-61.7**** ± 6.6	n/a	n/a	n/a	n/a

Values represent the mean ± SEM. Significance is defined as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. *Impg2* knockout, KO.