

Supplementary material

Alterations of endocannabinoid signalling and microglia reactivity in the retinas of AD-like mice precede the onset of hippocampal β -amyloid plaques

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Supplementary Table 1. Statistical analysis performed for data of Figures 2, 3, 4, 5 and 6. All data were screened for normal distribution through the Saphiro-Wilk test and data with $p > 0.05$ were considered normally distributed and were analyzed through the parametric Student's *t*-test (degrees of freedom, *t* value and *p* value are reported in the table). Data with $p < 0.05$ according to the Saphiro-Wilk test, were not considered normally distributed and were analyzed through the non-parametric Mann-Whitney test.

Figure	Statistical test	degrees of freedom	t value	p value	Saphiro-Wilk test (p value)
FIGURE 2					
2 c	Student's <i>t</i> -test	7	-4.620	0.002	0.578
2 d	Student's <i>t</i> -test	7	-0.935	0.381	0.078
2 f	Student's <i>t</i> -test	6	-2.738	0.034	0.897
FIGURE 3					
3 a (field 1)	Student's <i>t</i> -test	7	-1.104	0.306	0.666
3 a (field 2)	Student's <i>t</i> -test	7	-0.741	0.483	0.219
3 a (field 3)	Student's <i>t</i> -test	7	0.234	0.821	0.671
3 a (field 4)	Student's <i>t</i> -test	7	0.241	0.816	0.106
3 a (field 5)	Student's <i>t</i> -test	6	-0.215	0.837	0.124
3a (field 6)	Student's <i>t</i> -test	7	0.0806	0.938	0.614
3a (field 7)	Student's <i>t</i> -test	6	0.477	0.650	0.644
3a (field 8)	Student's <i>t</i> -test	7	0.398	0.702	0.185
3a (field 9)	Student's <i>t</i> -test	7	0.661	0.530	0.908
3a (field 10)	Student's <i>t</i> -test	7	0.419	0.688	0.843
3b (field 1)	Student's <i>t</i> -test	7	-0.589	0.574	0.788
3b (field 2)	Student's <i>t</i> -test	6	0.811	0.448	0.609
3b (field 3)	Student's <i>t</i> -test	7	-1.765	0.121	0.838
3b (field 4)	Student's <i>t</i> -test	7	-1.523	0.172	0.928
3b (field 5)	Student's <i>t</i> -test	7	-0.172	0.868	0.572
3b (field 6)	Student's <i>t</i> -test	7	-1.664	0.140	0.592
3b (field 7)	Student's <i>t</i> -test	7	-0.935	0.381	0.917
3b (field 8)	Student's <i>t</i> -test	7	-0.913	0.392	0.166
3b (field 9)	Student's <i>t</i> -test	6	-0.696	0.512	0.667
3b (field 10)	Student's <i>t</i> -test	7	0.580	0.580	0.315
FIGURE 4					
4 a	Student's <i>t</i> -test	11	-0.298	0.771	0.288
4 b	Student's <i>t</i> -test	11	-2.349	0.039	0.212
4 c	Student's <i>t</i> -test	11	1.424	0.182	0.261
4 d	Mann-Whitney	-	-	1	< 0.050
4 e	Student's <i>t</i> -test	11	-1.478	0.167	0.913
4 f	Mann-Whitney	-	-	0.004	< 0.050
4 g	Student's <i>t</i> -test	7	-0.593	0.572	0.498
4 h	Mann-Whitney	-	-	0.093	< 0.050
FIGURE 5					

5 b	Student's <i>t</i> -test	6	5.538	0.001	0.743
5 d	Student's <i>t</i> -test	6	-2.867	0.029	0.796
FIGURE 6					
6 a (AEA)	Student's <i>t</i> -test	5	-1.273	0.259	0.325
6 a (2-AG)	Student's <i>t</i> -test	5	7.063	0.0009	0.432
6 c (3 months)	Student's <i>t</i> -test	4	1.186	0.301	0.525
6 c (6 months)	Student's <i>t</i> -test	4	-1.519	0.203	0.824
6 c (12 months)	Student's <i>t</i> -test	5	-1.273	0.259	0.325
6 d (3 months)	Student's <i>t</i> -test	4	0.252	0.814	0.298
6 d (6 months)	Student's <i>t</i> -test	4	-0.198	0.853	0.580
6 d (12 months)	Student's <i>t</i> -test	5	7.063	0.0009	0.432

Supplementary Table 2. Linear regression analysis between CB₂ and ECS receptors/enzymes. The table reports the statistical results obtained from linear regression analysis between CB₂ and major elements of the ECS (CB₁, TRPV1, DAGL α , DAGL β , MAGL, NAPE-PLD, FAAH) for individual animals in 12-month-old WT and TG mice.

	CB ₂							
	WT				TG			
	f (x)	r	R ²	P value	f (x)	r	R ²	P value
CB₁	Y=2,226 - 1,213 x	0,459	0,211	0,436	y=0,192 + 0,539 x	0,717	0,514	0,563
TRPV1	y=1,11- 0,13 x	0,455	0,207	0,118	y=1,060 - 0,110 x	0,344	0,118	0,405
DAGLα	y=0,369 + 0,647 x	0,701	0,492	0,187	y=0,544 + 0,376 x	0,740	0,548	0,036
DAGLβ	y=-1,217 + 2,217 x	0,985	0,970	0,002	y=0,467 + 0,484 x	0,927	0,859	<0,001
MAGL	y = 0.593 + 0.414 x	0,711	0,506	0,178	y=1.730 - 0.151 x	-0,161	0.026	0.7304
NAPE-PLD	y=-0,062 + 1,102 x	0,260	0,067	0,740	y=1,209 - 0,004 x	0,003	0	0,995
FAAH	y=0,293 + 0,707 x	0,606	0,367	0,279	y=0,101 + 0,821 x	0,787	0,619	0,021

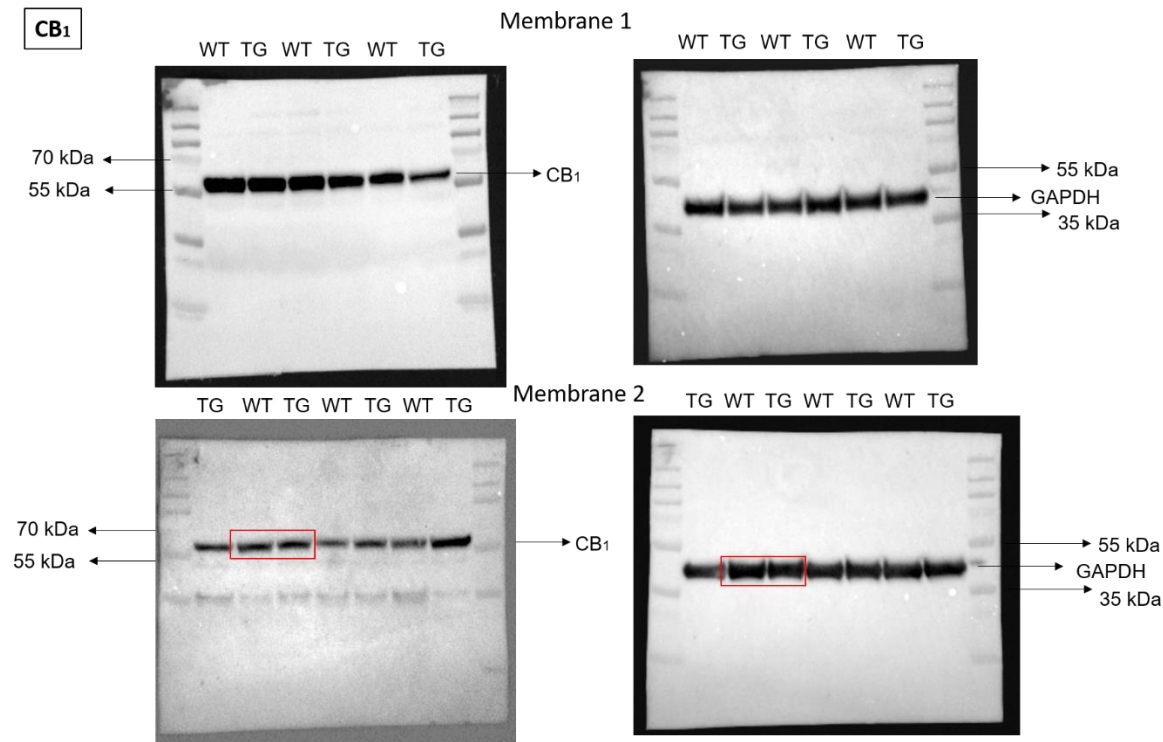
Supplementary Table 3. Linear regression analysis between MAGL and ECS receptors/enzymes. The table reports the statistical results obtained from linear regression analysis between MAGL and major elements of the ECS (CB₁, TRPV1, DAGL α , DAGL β , MAGL, NAPE-PLD, FAAH) for individual animals in 12-month-old WT and TG mice.

	MAGL							
	WT				TG			
	F(x)	r	R ²	P value	F(x)	r	R ²	P value
CB₁	y = 0.9742 + 0,026 x	0,114	0,013	0,830	y = 1,799 -0,241 x	-0,203	0,041	0,699
TRPV1	y = 1.127- 0,120 x	-0,126	0,016	0,840	y = 3,188- 1,883 x	-0,671	0,450	0,099
DAGLα	y = 0,842 + 0,151 x	0,304	0,093	0,558	y = 2.272 -0,683 x	-0,375	0,141	0,407
DAGLβ	y = 0,854 + 0,153 x	0,592	0,350	0,294	y = 1,783 -0,243 x	-0,142	0,020	0,761
NAPE-PLD	y = 0,979 + 0,023 x	0,203	0,041	0,797	y = 1,826- 0,321 x	-0,345	0,119	0,655
FAAH	y = 0,580 + 0,426 x	0,855	0,731	0,065	y = 1,669 -0,129 x	-0,148	0,022	0,752

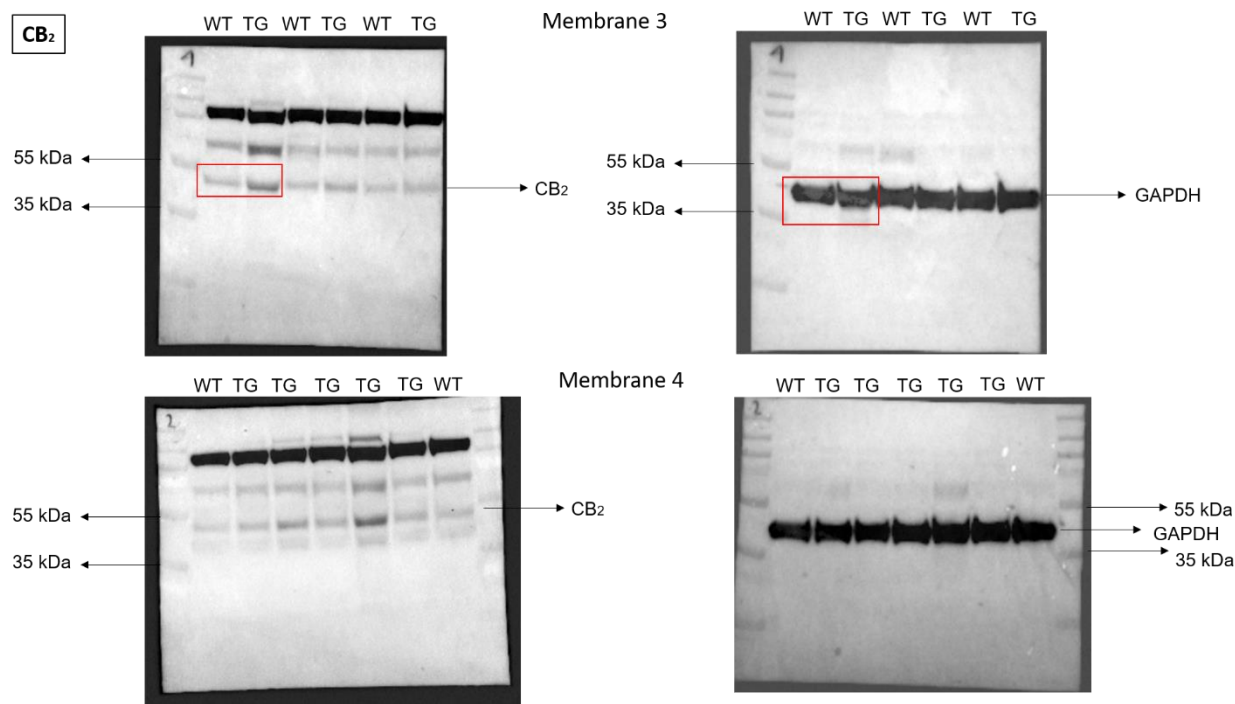
Supplementary Table 4. List of proteins and membranes used for the Western blot analysis. Proteins probed on the same membranes have similar GAPDH bands.

Membrane ID	Proteins identified by Western blot
Membrane 1	CB ₁ (Fig. 4a), NAPE-PLD (Fig. 4g)
Membrane 2	CB ₁ (Fig. 4a), NAPE-PLD (Fig. 4g)
Membrane 3	CB ₂ (Fig. 4b), FAAH (Fig. 4h), DAGL- β (Fig. 4e), TRPV1 (Fig. 4c)
Membrane 4	CB ₂ (Fig. 4b), FAAH (Fig. 4h), DAGL- β (Fig. 4e), TRPV1 (Fig. 4c)
Membrane 5	DAGL- α (Fig. 4d), MAGL (Fig. 4f), APP (Suppl. Fig. 9)
Membrane 6	DAGL- α (Fig. 4d), MAGL (Fig. 4f), APP (Suppl. Fig. 9)
Membrane 7	MAGL (Fig. 4f)

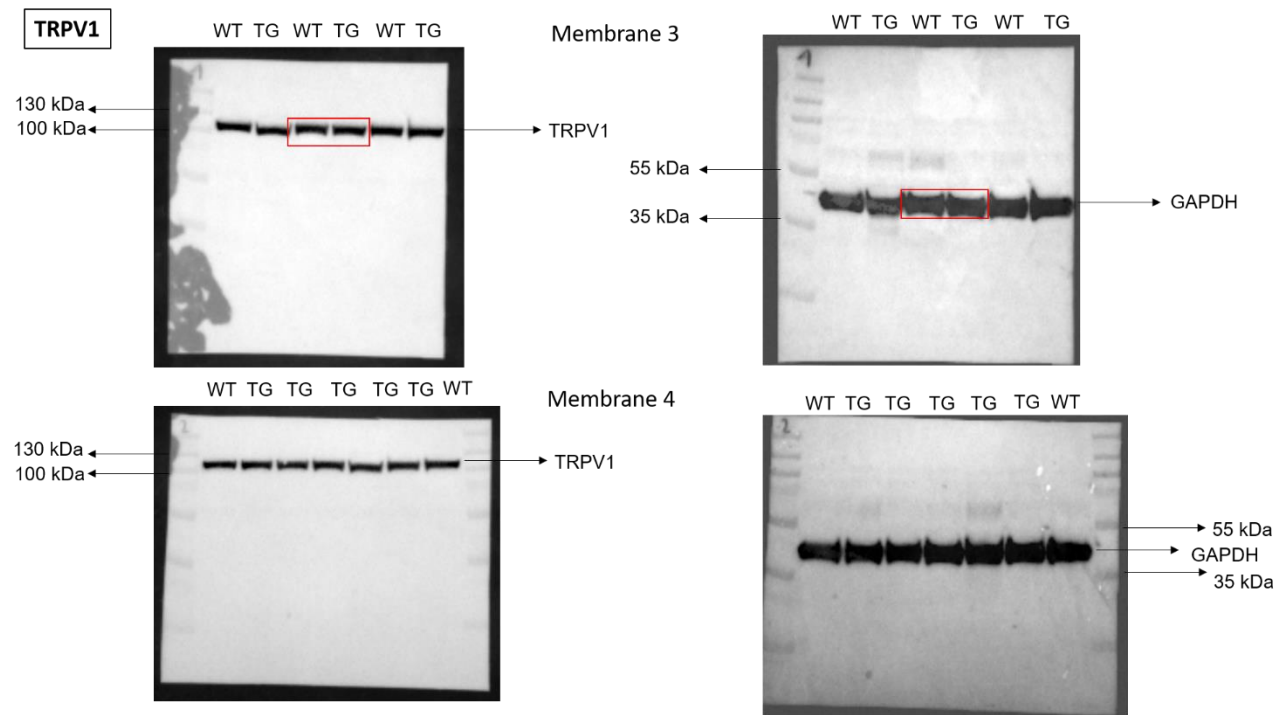
Supplementary Figure 1. Whole Western blot images of CB₁. The images show original Western blot bands of CB₁ of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 a).



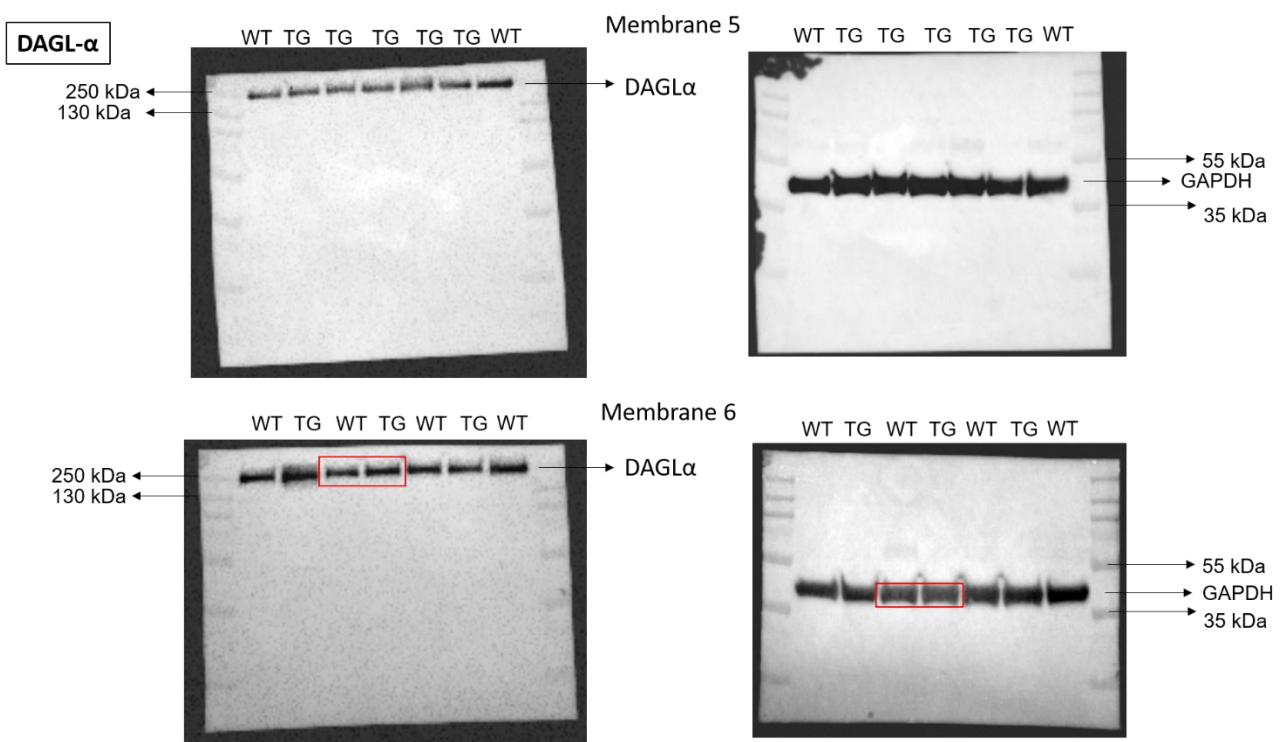
Supplementary Figure 2. Whole Western blot images of CB₂. The images show original Western blot bands of CB₂ of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 b).



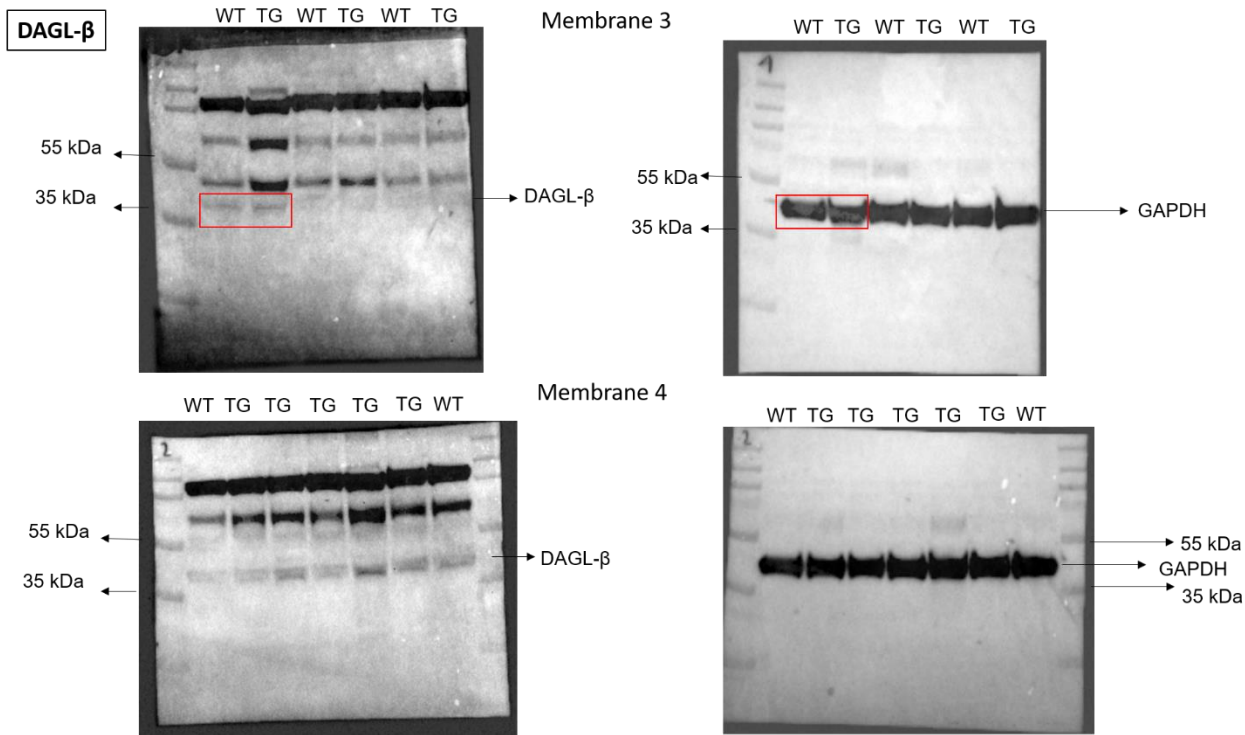
Supplementary Figure 3. Whole Western blot images of TRPV1. The images show original Western blot bands of TRPV1 of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 c).



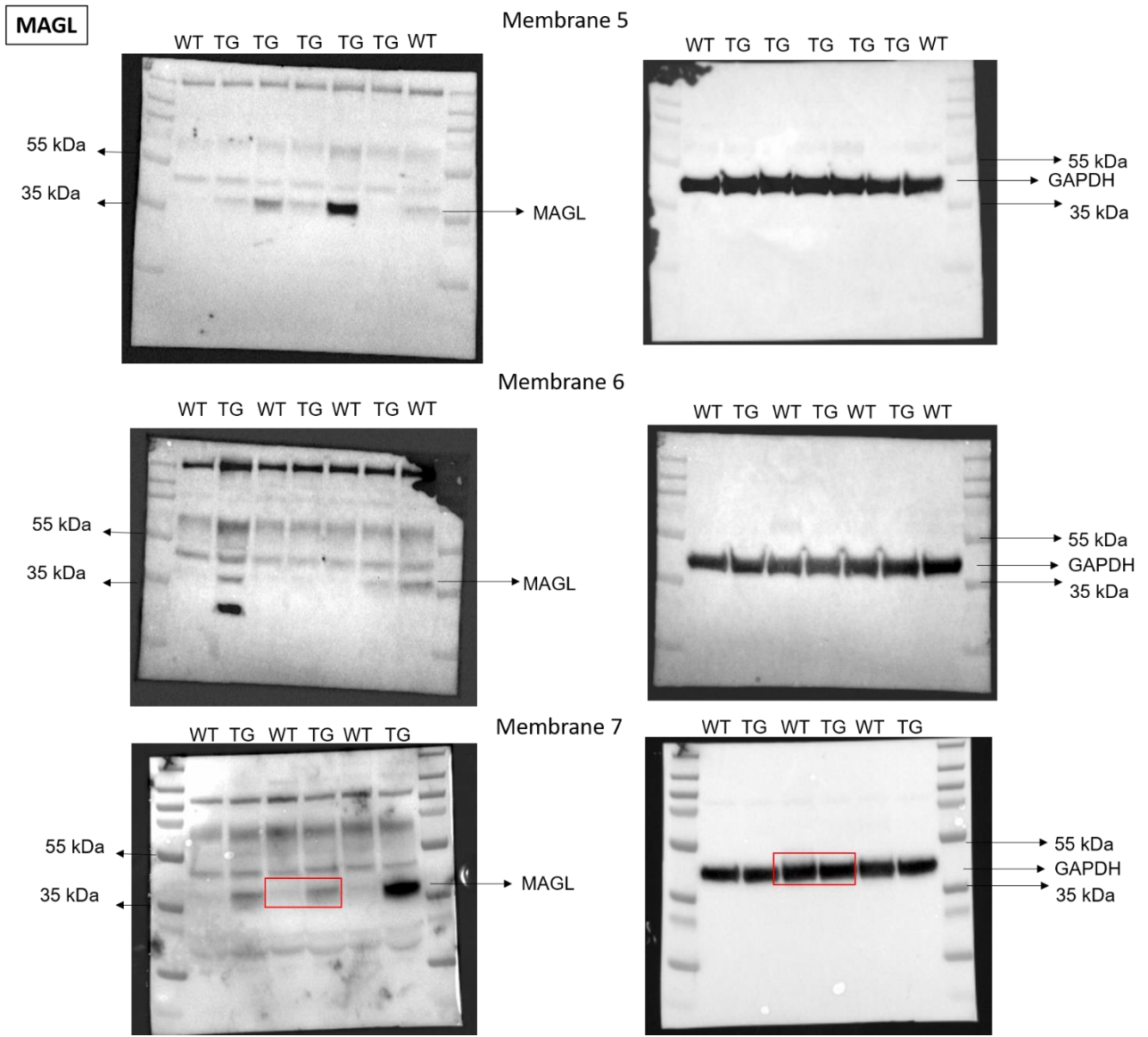
Supplementary Figure 4. Whole Western blot images of DAGL- α . The images show original Western blot bands of DAGL- α of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 d).



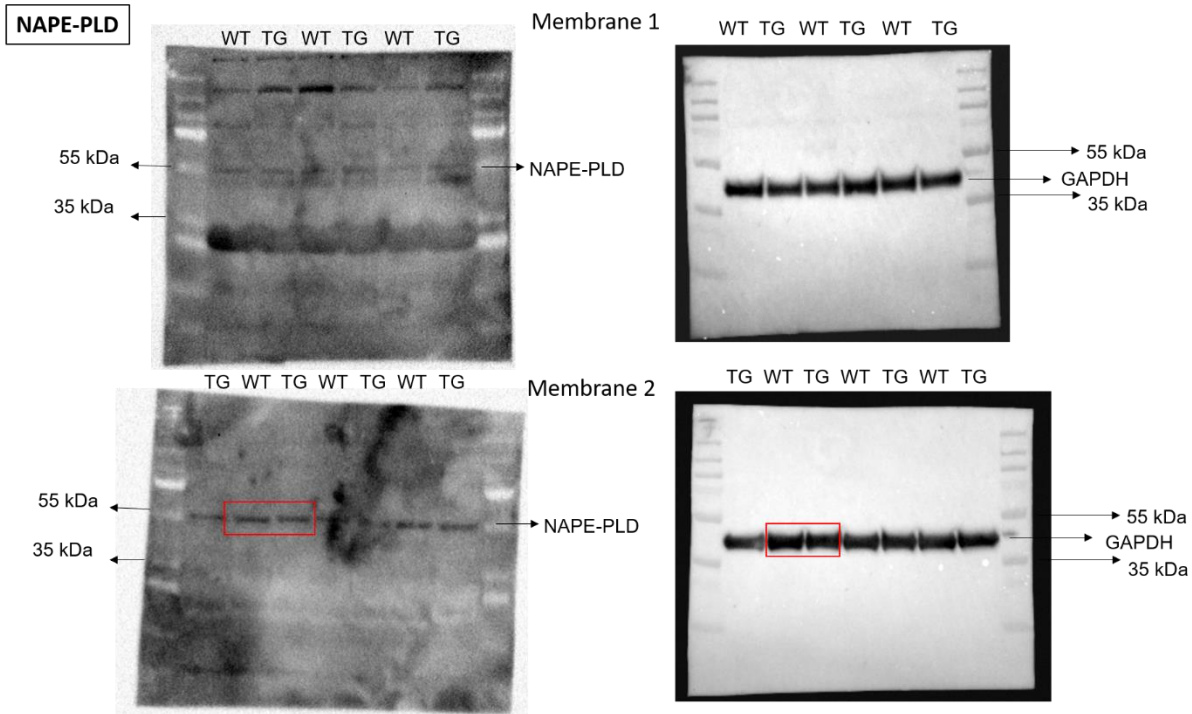
Supplementary Figure 5. Whole Western blot images of DAGL- β . The images show original Western blot bands of DAGL- β of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 e).



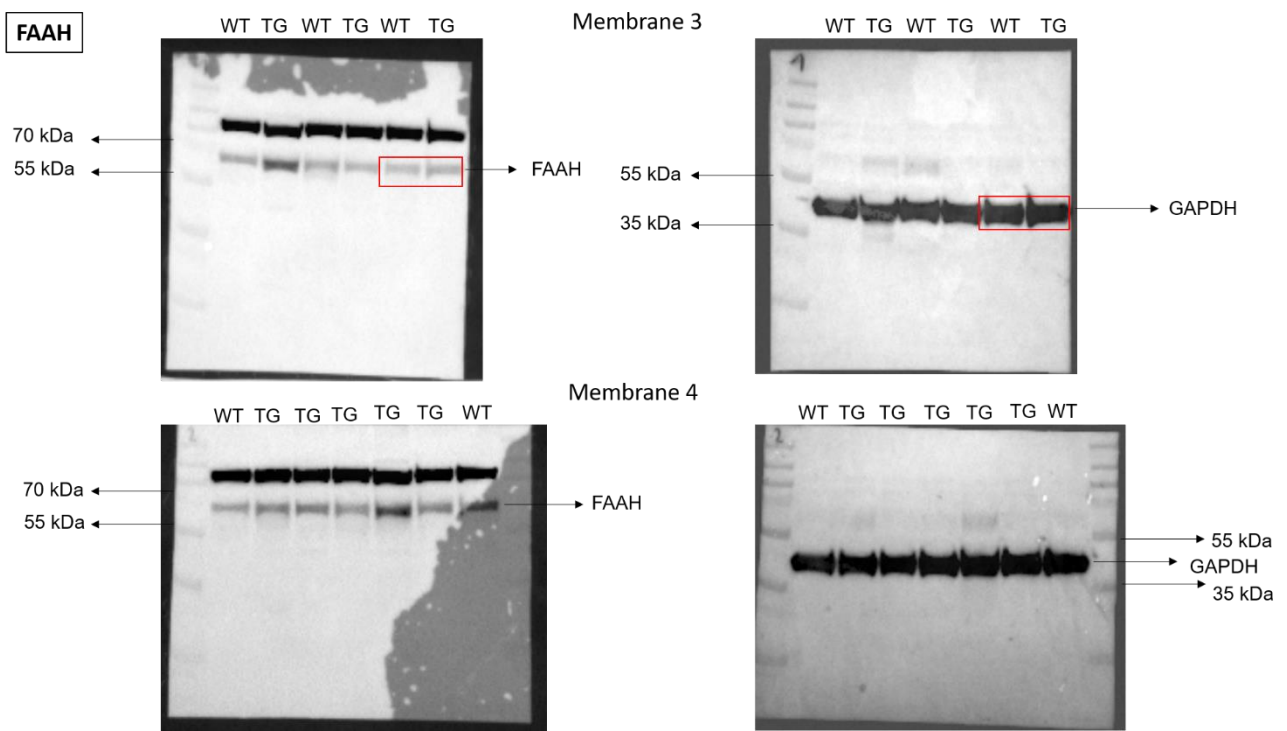
Supplementary Figure 6. Whole Western blot images of MAGL. The images show original Western blot bands of MAGL of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 f).



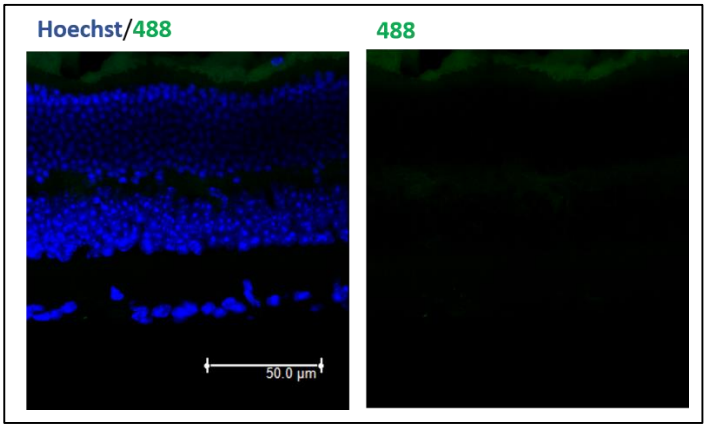
Supplementary Figure 7. Whole Western blot images of NAPE-PLD. The images show original Western blot bands of NAPE-PLD of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 g).



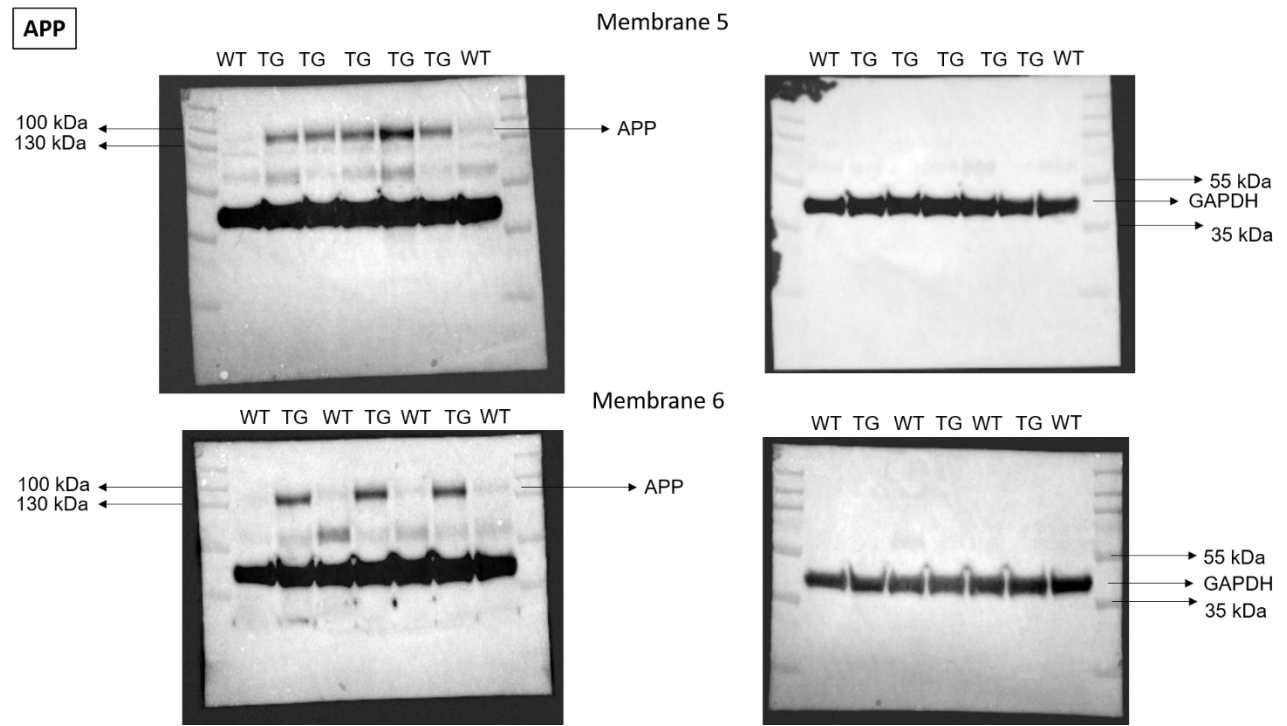
Supplementary Figure 8. Whole Western blot images of FAAH. The images show original Western blot bands of FAAH of 12-month-old WT and TG retinas with respective GAPDH housekeeping. The red boxes highlight the bands used as representative in the main text (Figure 4 h).



Supplementary Figure 9. Negative control of immunostained cryosections of Figure 5. A retinal cryosections was incubated with 488 anti-rabbit secondary antibody and counterstained with Hoechst (blue).



Supplementary Figure 10. Whole Western blot images of APP. The images show original Western blot bands of APP of 12-month-old WT and TG retinas with respective GAPDH housekeeping.



Supplementary Figure 11. Glutamate quantification in the retina of WT and TG mice.

Glutamate was quantified in the retinas of 12-month-old WT and TG mice through a bioluminescent Glutamate dehydrogenase/NADH detection system and its content was expressed as $\mu\text{mol/g}$ retina. Data are expressed as mean $\pm$ SEM. Data were normally distributed according to Saphiro-Wilk test ($p=0.880$), therefore the Student's t -test was used for statistical analysis. No statistically significant differences were found between groups ($t = 0.675$ with 6 degrees of freedom; $p = 0.525$).

