

Supplementary Material

Alterations in Retinal Signaling Across Age and Sex in 3xTg Alzheimer's Disease Mice

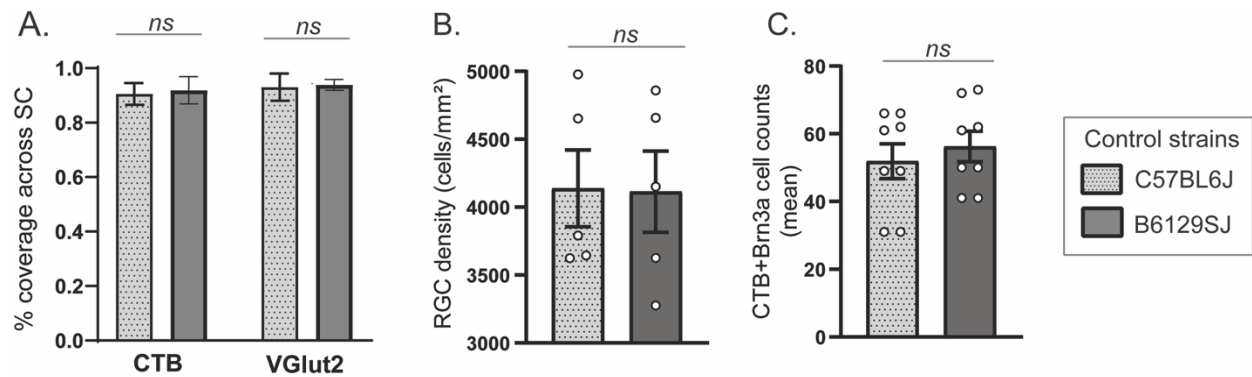
Supplementary Table 1. Product information for antibodies used for immunofluorescence studies

Antibody	Vendor/ Location	Species	Product Number	RRID Number
VGlut2	Abcam; Cambridge, UK	Mouse	Ab79157	AB_1603114
RBPMS	Millipore; Burlington, MA	Guinea Pig	ABN1376	AB_2687403
Amyloid beta	Santa Cruz; Dallas, TX	Mouse	sc-28365	AB_626669
Alexafluor-conjugated A β	Biolegend; San Diego, CA	Mouse	803013	AB_2564765
GFAP	Novus; Centennial, CO	Chicken	NBP1-05198	AB_1237006

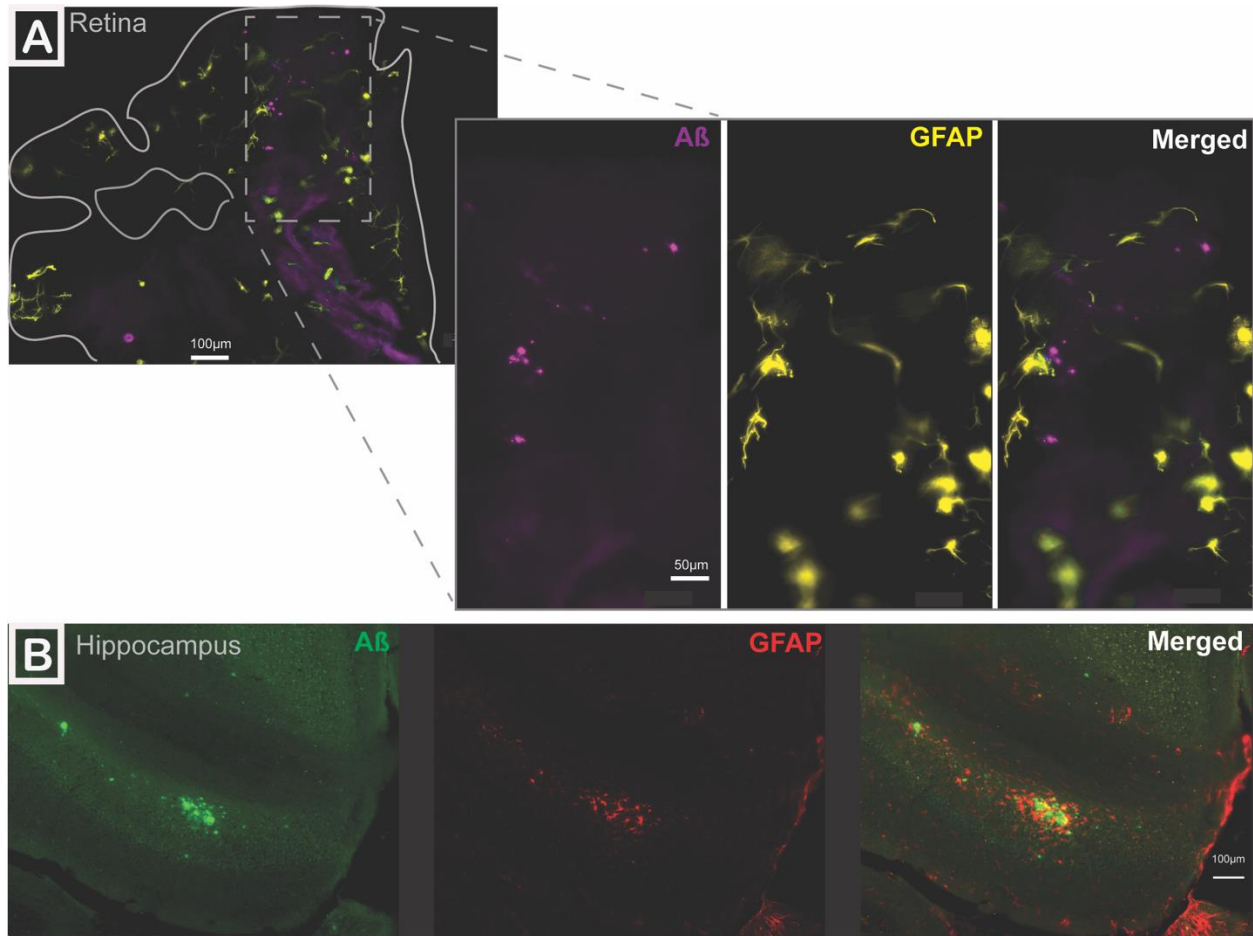
Supplementary Table 2. Statistics for factorial omnibus ANOVAs analyzing PERG amplitude data

Peak amplitude	Strain	Age	Strain x Age
Females			
N1	ns	ns	ns
P1	ns	*F _{2,78} =23.55 p<0.01	*F _{2,78} =23.55 p=0.05
N2	ns	*F _{2,77} =20.93 p<0.01	ns
Males			
N1	ns	ns	ns
P1	ns	*F _{2,81} =32.39 p<0.01	*F _{2,81} =7.54 p=0.001
N2	*F _{1,81} =19.77 p<0.01	*F _{2,81} =56.17 p<0.01	*F _{2,81} =56.17 p=0.015

Supplementary Figure 1. Data from pilot study showing that retinofugal transport and retinal ganglion cell distribution in 12-month-old C57BL/6J and B6129SJ hybrid control mice are comparable. A) Mean percent area fraction of CTB and VGlut2 coverage (measured as percent area fraction) across the superior colliculus (SC); n = 8 projections per strain; 4 mice/strain; B) Individual measurements of Brn3a+ cell density across retina (cells/mm²); C) Individual measurements of RGCs co-labeled with CTB (Brn3a+CTB). For A, error bars = s.e.m. Strains include mixed sexes. No significant differences were detected between groups on these variables.



Supplementary Figure 2. Immunohistological visualization of amyloid- β ($A\beta$) in the retina and brain of 3xTg mice. A) Micrograph of flat-mount retina that was immunohistochemically labeled with the same Alexa Fluor-conjugated $A\beta$ antibody used for in vivo retinal imaging with Micron IV ophthalmoscope. Low magnification image shows retinal borders outlined in white. Scale bar = 100 μm . Dashed lines indicate portion of retina enlarged into the three panels used to show $A\beta$, GFAP+ astrocytes, and merged label. Panels confirm presence of labeled $A\beta$ in retina surrounded by GFAP+ astrocytes. B) Conjugated $A\beta$ antibody also labels this protein in positive control tissue from 14-month-old female 3xTg hippocampus with pervasive pathology. An $A\beta$ plaque can be seen in the leftmost panel. Merged image shows GFAP+ astrocyte presence at the location of $A\beta$ plaque. The large section of non-specific signal in the low magnification image is auto-fluorescence of the retina under the channel $A\beta$ was imaged. The presence of GFAP-positive astrocytes suggests is indicative of gliosis occurring near amyloid deposits, which has also been used to corroborate the pathological nature of $A\beta$ fragments in 3xTg mouse tissue [1] as well as in other pathological AD tissue (as reviewed in Frost and Li 2017 [2]).



REFERENCES

- [1] Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ (2010) Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. *Glia* **58**, 831–838.
- [2] Frost GR, Li YM (2017) The role of astrocytes in amyloid production and Alzheimer's disease. *Open Biol* **7**, 170228.