Online Supplemental Materials

Müller glial expression of REDD1 is required for retinal neurodegeneration and visual dysfunction in diabetic mice. *Miller et al, 2022*

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Generation of Muller glia specific REDD1 knockout (mgKO) mice. Mice were rederived from cryopreserved homozygous floxed Neomycin (Neo) cassette-containing REDD1 embryos. Eventual homozygous floxed REDD1-Neo mice were crossed with a homozygous *Flp* recombinase mouse (Jackson Laboratories; # 009086), excising the Neo-cassette on the flanking FRT sites. Eventual C57BL/6J homozygous floxed/Flp REDD1 mouse is identified as REDD1$^{fl/fl}$. C57BL/6J hemizygous Pdgfra-cre mice with cre expression directed to retinal Müller glia by the *Pdgfra* (platelet derived growth factor receptor, alpha polypeptide) promoter were obtained from The Jackson Laboratory (stock #013148). Hemizygous Pdgfra-cre mice were crossed with REDD1$^{fl/fl}$ mice to achieve Müller cell specific recombination (Fig S1A). Pdgfra-cre^{+}; REDD1$^{fl/fl}$ mice are identified as REDD1 Müller glia knockout (REDD1 mgKO) mice. PCR analysis of genomic DNA was used to confirm homozygous expression of the floxed REDD1 gene and hemizygous cre in REDD1 mgKO mice (Fig. S1B). REDD1$^{fl/fl}$ mice were crossed with REDD1 mgKO to produce littermates for all experimental studies.

Figure S1

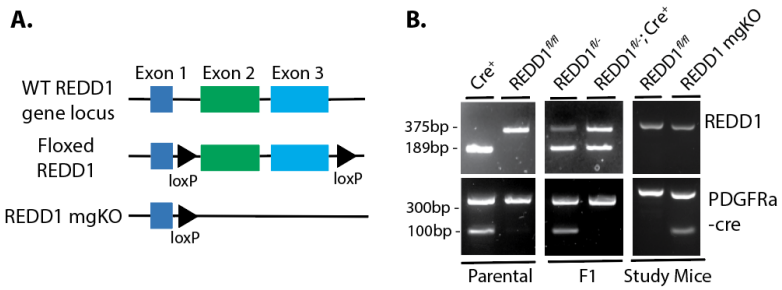

Figure S1. Müller glia specific REDD1 deletion was achieved by genomic manipulation. *A.*, Schematic of Cre-lox recombination at REDD1 gene locus to achieve conditional Müller cell specific REDD1 knockout (REDD1 mgKO). Cre-lox recombination of the floxed REDD1 gene upon Cre-recombinase expression results in deletion of exons 2 and 3. *B.*, PCR products from genotyping mouse ear punches shows the wild-type REDD1 PCR product at 189 bp and the REDD1 mutant at 375 bp. The PDGFRa-Cre PCR band is 100 bp and the reaction internal positive control is 300 bp.

Figure S2

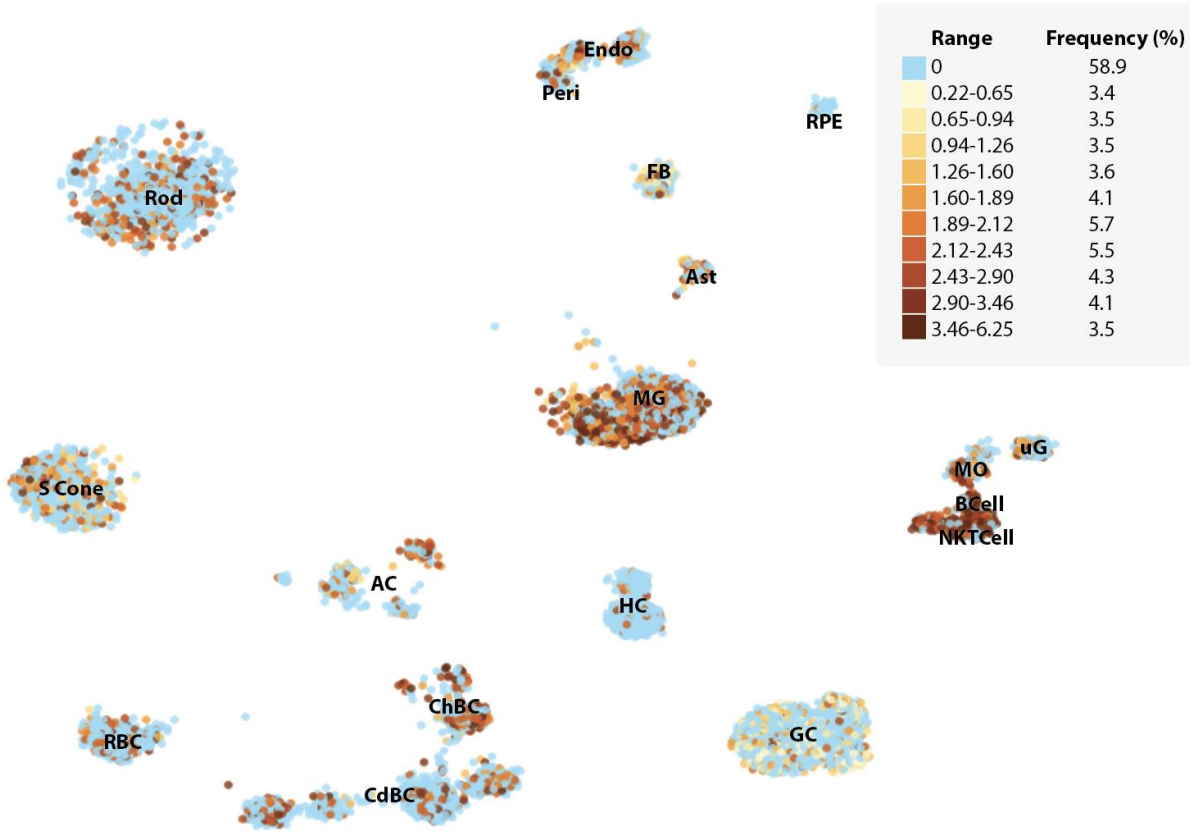

Figure S2. Transcriptomic analysis of REDD1 expression in human retinal cells. REDD1 expression was evaluated in the single cell sequencing analysis of adult retina that was performed by Cowan *et al* (28) using UCSC Cell Browser. Transcriptomic cell types are visualized by UMAP plot with each dot representing the transcriptome of a single cell. REDD1 expression for each cell is visualized by heatmap. AC, Amacrine Cell; Ast, Astrocyte; CdBC, Cone OFF bipolar cell (hyperpolarizing); ChBC, Cone ON bipolar cell (depolarizing); Endo, Endothelial cell; FB, Fibroblast; GC, Ganglion cell; HC, Horizontal cell; MG, Muller glia; MO, Monocyte; NKT Cell, Natural Killer T cell; Peri, Pericyte; RBC, Rod bipolar cell; RPE, Retinal Pigment Epithelia; uG, Microglia. Image credit: UCSC Cell Browser (https://data.iob.ch/?ds=Adult_human_foveal_retina&gene=DDIT4).

Figure S3

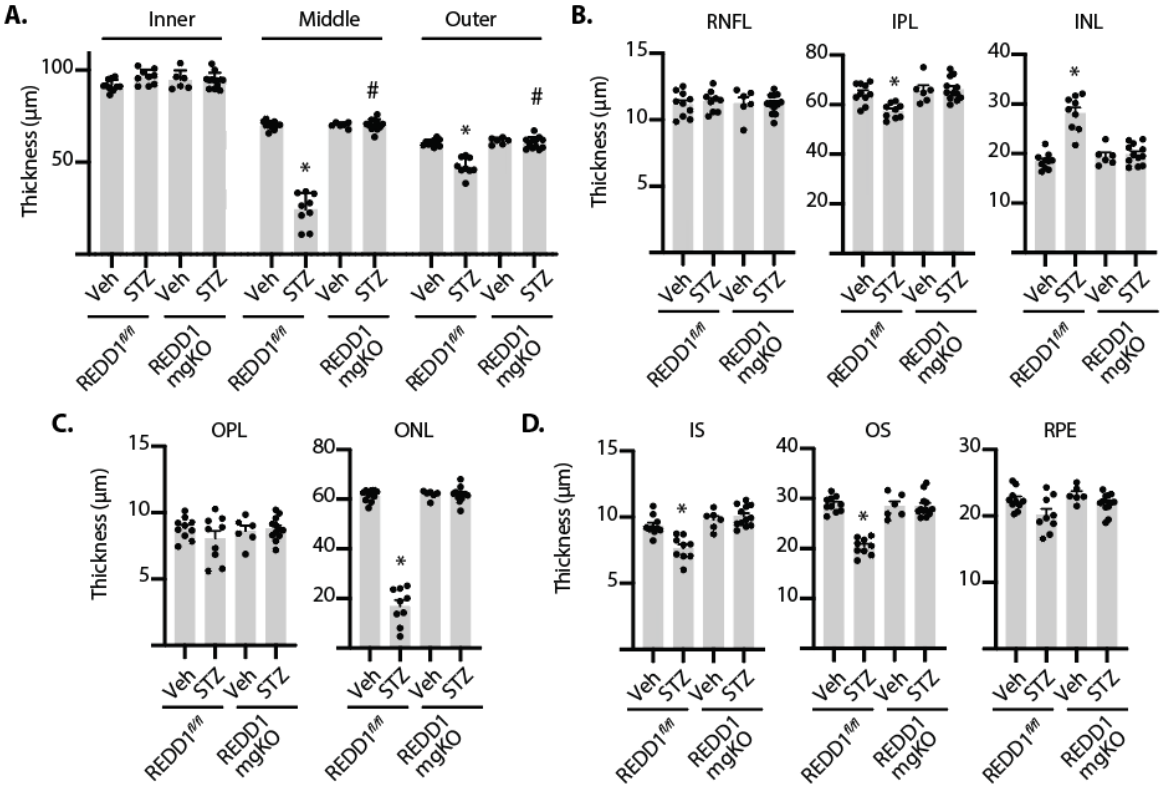

Figure S3. Diabetes-induced retinal thinning was absent in mice with Müller cell specific REDD1 deletion. Diabetes was induced in REDD1$^{fl/fl}$ and REDD1 mgKO mice by administration of streptozotocin (STZ). Control mice received a vehicle (Veh). All retinal analysis was performed after 6 weeks of diabetes. Automated retinal segmentation was computationally performed with InVivoVue software (Bioptigen). The auto segmentation feature provides an average thickness of retinal layers from 8 different measurements. Average thickness of individual layers was quantified. *A*. Thickness of inner [RNFL (retinal nerve fiber layer + ganglion cell layer), IPL (inner plexiform layer), INL (inner nuclear layer)], middle [OPL (outer plexiform layer), ONL (outer nuclear layer)], and outer [IS (inner segments), OS (outer segments), RPE (retinal pigmented epithelium) retinal layers was compared. *B-D*. Thickness of individual retinal layers within the inner (*B*.), middle (*C*.) and outer (*D*.) retina were compared. Graphs within in *B*., *C*., and *D*. share y-axis labels. Values are means ± SD. Data were analyzed overall with two-way ANOVA, and pairwise comparisons were conducted with the Dunnett's or Tukey test for multiple comparisons. *, $p \leq 0.05$ vs Veh. #, $p \leq 0.05$ vs REDD1$^{fl/fl}$.

Figure S4

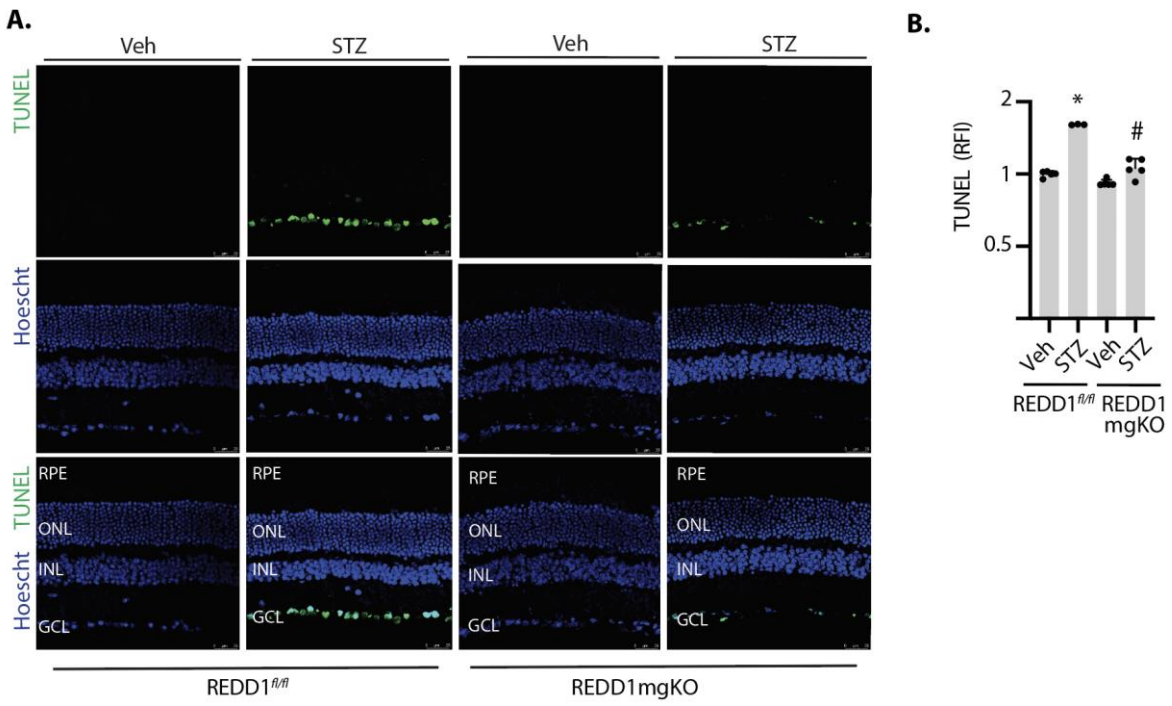

Figure S4. Apototic nuclei were reduced in the retina of diabetic REDD1 mgKO mice. Diabetes was induced in REDD1$^{fl/fl}$ and REDD1 mgKO mice by administration of streptozotocin (STZ). Control mice received a vehicle (Veh). All retinal analysis was performed after 6 weeks of diabetes. *A.*, Whole eyes were isolated, cryosectioned into sagitally oriented longitudinal cross sections, and labeled with TUNEL-Fluorescein. Hoechst was used to visualize nuclei. *B.*, Intensity of staining for TUNEL was quantified. Values are means ± SD, N = 3-5. *, $p \leq 0.05$ vs Veh. #, $p \leq 0.05$ vs REDD1$^{fl/fl}$. RPE, retinal pigment epithelium; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer; RFI, relative fluorescent intensity.

Genotyping		
Target	**Forward primer**	**Reverse primer**
Flox REDD1	5'-GCCATCAGAGCTCCTGGACT-3'	5'-GCCTTCTCACCTGGAGAGCA-3'
PDGFRa-cre	5'-GCGGTCTGGCAGTAAAAACTATC-3'	5'-GTGAAACAGCATTGCTGTCACTT-3'
qPCR		
Gene Symbol	**Forward primer**	**Reverse primer**
AQP4	5’-CTTTCTGGAAGGCAGTCTCAG-3’	5’-CCACACCGAGCAAAACAAAGAT-3’
CRALBP	5’-CAAGAGGCAGTATGTCAGAC-3’	5’-GAAGAGTTCAGGGTACTGGA-3’
DDIT4	5'-GGGATCGTTTCTCGTCCTCC-3'	5'-ATGAGGAGTCTTCCTCCGGC-3'
DDIT4L	5'-CCAGCCTCAAGGACTTCTTC-3'	5'-TCTTCAATGACTGTCGTTCC-3'
GLUL	5'-CACCCCTGGTTTGGAATGGA-3'	5'-GTAATACGGGCCTTGGGGTC-3'
GPR37	5’-GCCCTATATCGAGGTGGCTT-3’	5’-CTCCAACCCAGATAACGGCA-3’
SOX9	5’-GCCACGGAACAGACTCACAT-3’	5’CTGAGATTGCCCAGAGTGC-3’
GAPDH	5'-GTTGTCTCCTGCGACTTCA-3'	5'-TGCTGTAGCCGTATTCATTG-3'

Table S1. Mouse Oligonucleotide List. Sequences of primer used in genotyping and qPCR are listed.

Immunofluorescence				
Antibody	**Company**	**Cat #**	**Lot #**	**Dilution**
Glutamine Synthetase	Sigma	G2781	096M4864V	1 to 1000
HA-tag	Nouvs	NB600-362	A1	1 to 2000
GFAP	Agilent Dako	Z0334	20053562	1 to 1000
Cleaved Caspase 3	Cell Signalling	9661	21	1 to 1000
Western Blotting				
Antibody	**Company**	**Cat #**	**Lot #**	**Dilution**
REDD1	Protein Tech	10638-1-AP	51272	1 to 500
Nrf2	Cell Signalling	12721	8	1 to 1000
GFAP	Agilent Dako	Z0334	20053562	1 to 1000
α-tubulin	Santa Cruz	sc-32292	C0112	1 to 1000
GAPDH	Santa Cruz	sc-32233	K0315	1 to 5000

Table S2. Antibodies used for Western blotting. Primary and secondary antibodies are listed by manufacturer and catalogue number.