Supplementary Materials

Immunofluorescence

Retinal cryosections and whole mounts were prepared as previously described (1). Briefly, sections were incubated in 5% donkey serum containing 0.3% Triton X-100 for 1 h at room temperature. The whole mounts were incubated with antibody Brn3 (sc-390780, 1:200, Santa Cruz, Dallas, TX), whereas cryosections were incubated with antibodies RLBP1 (1:200, Abcam, Cambridge, MA, ab15051) and glial fibrillary acidic protein (GFAP) (#3670, 1:200, Cell Signaling Technology, Beverly, MA) overnight at 4°C. Then, the sections were incubated with Alexa Fluor 594-conjugated donkey anti-mouse antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hour at room temperature. The nuclei in the cryosections were stained with DAPI for 5 minutes.

Western blot

Retina were lysed using a mammalian cell lysis kit (Sigma-Aldrich, St. Louis, MO) and protein concentrations were assessed using a bicinchoninic acid kit (Sigma-Aldrich) according to the kits' instructions. The samples' lysates were equally loaded and run on 10% gel for SDS-PAGE, transferred onto polyvinylidene difluoride membranes, and blocked with 5% BSA. The membranes were incubated with primary antibodies against cleaved caspase-3, β-actin (# 9664, #3700, 1:1000, Cell Signaling Technology), and cellular retinaldehyde-binding protein (CRALBP; ab243664, 1:1000, Abcam, Cambridge, MA) overnight at 4°C, followed by washing and incubation with

horseradish peroxidase-conjugated secondary anti-rabbit or anti-mouse antibodies (1:10,000, Cell Signaling Technology). After washing, the bands were exposed using the ECL Western Blotting Substrate (Millipore, Billerica, MA).

Enzyme-linked immunosorbent assay (ELISA)

Retinal tissues were lysed, and protein concentrations were assessed as western blot. Based on the manufacturer's instructions, the expression level of interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) were determined using ELISA kits (R&D Systems, Minneapolis, MN).

Electroretinogram (ERG)

After dark adaptation overnight, the mice were anesthetized with their pupils dilated and corneas anesthetized. The ground electrodes, reference electrodes, and corneal contact electrodes were then placed on the mice. The Phoenix Ganzfeld System (Phoenix Research Labs, Pleasanton, CA) was used to record the scotopic a/b-wave at a stimulus intensity of 1.0–4.0 $\log^{10}$ (cd·sec/m^2).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays

The In Situ Cell Death Detection Kit (Roche, Indianapolis, IN) was used on mouse retinal sections according to the manufacturer's instructions. Nuclei were stained with DAPI.

Trypsin digest for retinal vascular architecture

Globes were fixed in 4% formalin. The retinas were detached and digested at 37°C in 3% trypsin/0.2 M Tris-HCl (pH 7.45) for 30–60 min. Then, specimens were flat-mounted, stained with the Periodic Acid Schiff (PAS) Stain Kit (Abcam), and imaged by light microscopy (Olympus, Center Valley, PA). Acellular capillaries were counted in three high-powered fields per retinal quadrant.

Vascular Permeability

Evans blue (45 mg/kg body weight) was intravenously injected in the animals and circulated for 2 h. Animals were anesthetized through intracardial perfusion. Then, the retinas were dissociated, dried for 2 h, and weighed. The Evans blue dye was extracted by incubating with formamide for 18 h at 70°C. The absorbances of the extracts were measured with spectrophotometry at 620 and 740 nm (background). Vascular permeability was calculated in μg of Evans blue per milligrams of total protein content.

Supplementary Table 1. Characteristics of db/m and db/db mice in fasting body weights and blood glucose.

Characteristic	db/m	db/db	*P*-value
Fasting body weights			
2 months	18.1±1.2	37.1±4.2	<0.01
3 months	22.1±1.1	40.6±3.0	<0.01
4 months	24.5±0.7	45.1±2.3	<0.001
5 months	24.1±2.1	49.6±1.1	<0.001
6 months	22.6±2.6	51.2±1.3	<0.001
7 months	24.2±0.8	55.9±1.4	<0.001
8 months	22.7±1.7	57.6±2.5	<0.001
Fasting blood glucose			
2 months	99.6±21.4	322.2±35.4	<0.01
3 months	97.2±8.80	387.1±66.5	<0.01
4 months	93.0±12.8	451.6±58.2	0.001
5 months	96.6±11.0	488.1±29.1	<0.001
6 months	99.6±3.7	522.6±24.5	<0.001
7 months	96.0±9.8	557.4±22.8	<0.001
8 months	93.6±13.1	561.6±19.8	<0.001

The results are presented as mean±SD (standard deviation).

Supplementary Table 2. Characteristics of db/m mice treated with PBS and db/db mice treated with PBS, ShH10Y.RLBP1, and ShH10Y.GFP in fasting body weights and blood glucose.

Characteristic	db/m	db/db		
	PBS	PBS	ShH10Y.RLBP1	ShH10Y.GFP
Fasting body weights				
2 months	18.0±1.3	36.1±3.8*	38.7±2.5*	39.1±3.7*
3 months	20.2±1.8	41.2±4.0*	38.7±4.0*	39.7±3.7*
4 months	21.6±1.8	44.0±2.1*	44.4±2.5*	45.3±3.1*
5 months	22.2±1.9	53.3±3.0*	50.4±2.9*	50.6±1.8*
6 months	22.5±2.1	55.9±2.0*	51.8±2.5*	52.1±2.3*
7 months	22.6±2.4	57.5±4.2*	54.2±2.6*	54.2±2.6*
8 months	23.1±2.0	61.3±3.5*	61.8±2.9*	59.7±3.4*
Fasting blood glucose				
2 months	82.6±7.3	361.3±51.1*	331.4±49.3*	341.3±46.5*
3 months	88.6±6.7	419.9±34.5*	421.6±34.0*	423.9±37.8*
4 months	90.4±7.9	474.1±39.1*	481.1±27.6*	455.9±36.1*
5 months	92.9±11.3	497.0±18.6*	531.5±18.3*	525.2±29.6*
6 months	89.5±9.0	540.9±25.9*	550.3±15.1*	512.8±16.8*
7 months	91.8±10.5	547.7±22.3*	553.0±28.4*	576.7±22.2*
8 months	95.0±10.2	562.3±16.1*	560.7±27.5*	555.5±18.9*

The results are presented as mean±SD (standard deviation).

*$P < 0.001$ compared with db/m mice.

Supplementary Figure legend

Supplementary Figure 1. Glucose tolerance test of db/m and db/db mice during experiment I. Blood glucose concentrations during GTT in db/m and db/db mice at 2 months (a) and 8 months (b) and their areas under the curves (AUCs; $mg \cdot min^{-1} \cdot dL^{-1}$) were compared (c). Statistical analyses were performed on the measured AUC values. Mean ± SD were used for the AUC, 10-90 percentile, ***$P < 0.001$.

Supplementary Figure 2. scRNA library statistics and quality control features a–c: Comparison of quality control features of the six samples indicates that although sequencing depth varied across samples/libraries, the number of genes and transcripts detected per cell were similar across samples. a: Violin plot of unique molecular identifiers (UMIs) per cell. b: Violin plot of the number of unique genes detected per single cell in each sample. c: Violin plot of the number of unique transcripts detected per single cell in each sample. d: Single cells are colored by sample origin (3 db/m and 3 db/db mice) on the t-SNE plot. None of the clusters were driven exclusively by a sample, as single cells from all samples are present in each cluster.

Supplementary Figure 3. Determination of retina cell clusters a: t-SNE plots showing expression of known cell markers. b: Violin plot visualization of well-established marker genes used to identify clusters. Cells were derived from the retinas of 3 db/m and 3 db/db mice.

Supplementary Figure 4. Gene of DR (left panel) and PDR (right panel) related GWAS for different cell types. Cell types are shown in rows, columns are each sample, and the color is the average expression for all genes associated with each sample, for each cell type.

Supplementary Figure 5. Identification of genes associated with DR a–c: Heatmap (up panel) and Volcano plot (down panel) of dysregulated genes in the retinal ganglion cells (a), cones (b), microglia (c) vascular endothelial cells (d), pericytes (e), and horizontal cells (f) of db/db mice. Data of top 50 dysregulated genes (25 upregulated and 25 downregulated) were z-normalized for heatmap visualization. Each column represents an individual sample from the db/m or db/db groups. Volcano plot of dysregulated genes at $P<0.05$ and a fold change >1.2 or < -1.2. The top 50 DEGs are labeled.

Supplementary Figure 6. KEGG pathway enrichment analysis of DEGs. Bubble plot of the top 30 enriched KEGG pathway terms of different cell types. The colors of the nodes are illustrated the P-value. The sizes of the nodes are illustrated the gene counts.

Reference

1. Molday LL, Cheng CL, Molday RS: Cell-Specific Markers for the Identification of Retinal Cells and Subcellular Organelles by Immunofluorescence Microscopy.