Supplementary method information

Denaturation, alkylation, reduction and tryptic digestion

Same protocol has been used before. After the proteins were extracted from tissue samples and proteins concentration was measured 0.05 M ABC, denatured with 2 % SDS and reduced with 50 mM Tris- (2-carboxyethyl) phosphine. After 60 min of incubation at + 60 °C samples were centrifuged and transferred into Pall Nanosep® 30 kDa MWCO centrifugal devices with low protein binding membrane and flushed two times with 200 µl of 8 M urea solution followed by iodoacetamide alkylation for 20 min in dark at RT. Samples were flushed three times with 8 M urea solution and 50 mM ABC before starting the enzymatic digestion with trypsin for 16 h at + 37 °C. Ratio of trypsin to protein in enzymatic digestion was 1:25. The following day samples were flushed twice with 40 µl 0.1M TEAB and once 50 µl 0.5 M NaCl to collect peptides from the filter and dried in speed vacuum. Samples were solubilized to 0.1 % TFA and desalted with C18 tips. The tips were conditioned with 50 % ACN and equilibrated with sample solvent. The clean-up was performed by aspirating and dispensing samples for 10 cycles. Tips were then rinsed with 2.5 % ACN in 0.1 % TFA. Finally, peptides were eluted with 80 % ACN in 0.1 % FA. Samples were dried in speed vacuum concentrator and stored at -20 °C until reconstituted to loading solution (5 % ACN, 0.1 % FA and HMR calibration peptides according to manufacturer guidelines) NanoRPLC-TripleTOF SWATH analysis. SWATH analysis samples were diluted to equal concentrations before injection to the instrument.

NanoRPLC-TripleTOF

Retinal proteins were analyzed by Nano-RPLC- TripleTOF instrumentation using Eksigent 425 NanoLC coupled to high speed TripleTOF™ 5600+ mass spectometer (Sciex, Concord, Canada). A microcapillary RP-LC column (cHiPLC® ChromXP C18-CL, 3 µm particle size, 120 Å, 75 µm i.d × 15 cm, Eksigent Concord, Canada) was used for LC separation of peptides. Samples were first loaded into trap column (cHiPLC® ChromXP C18-CL, 3 µm particle size, 120 Å, 75 µm i.d × 5 mm) from autosampler and flushed for 10 min at 2 µl/min (2 % ACN, 0.1 % FA). The flush system was
then switched to line with analytical column. Tear samples were analyzed with 120 min 6 step gradient using eluent A: 0.1 % FA in 1 % ACN and eluent B: 0.1 % FA in ACN (eluent B from 5 % to 7 % over 2 min, 7 % to 24 % over 55 min, 24 % to 40 % over 29 min, 40 % to 60 % over 6 min, 60 % to 90 % over 2 min and kept at 90 % for 15 min, 90 % to 5 % over 0.1 min and kept at 5 % for 13 min) at 300 nl/min.

Key parameters for TripleTOF mass spectrometer in SWATH ID library analysis were: ion spray voltage floating (ISVF) 2300 V, curtain gas (CUR) 30, interface heater temperature (IHT) +125°C, ion source gas 1 13, declustering potential (DP) 100 V. Library for SWATH analysis was created from the same samples by information dependent-acquisition (IDA) method and relative quantitation analysis was done by SWATH method. All methods were run by Analyst TF 1.5 software (Sciex, Redwood City, USA). For IDA parameters, 0.25 s MS survey scan in the mass range 350-1250 mz were followed by 60 MS/MS scans in the mass range of 100-1500 Da (total cycle time 3.302 s). Switching criteria were set to ions greater than mass to charge ratio (m/z) 350 and smaller than 1250 (m/z) with charge state 2-5 and an abundance threshold of more than 120 counts. Former target ions were excluded for 12 s. IDA rolling collision energy (CE) parameters script was used for automatically controlling CE. SWATH quantification analysis parameters were the same as for SWATH ID, with the following exceptions: cycle time 3.332 s and MS parameters set to 15 Da windows with 1 Da overlap between mass range 350-1250 Da followed by 40 MS/MS scans in the mass range of 350-1250 Da. SWATH analysis method has been published in Nättinen et al 2018. 2

Immunoblotting

17 µg of protein from retinal lysates was loaded and run into 4-12% gradient gel (NuPAGE; Invitrogen, Carlsbad, CA, USA) and transferred on polyvinylidene fluoride membranes (Amersham Biosciences, Little Chalfont, UK). Membranes were blocked with 5 % NFM/ TBST and specific proteins were detected by immunoblotting with following primary antibodies: rabbit anti-Flna
(Cat#ab76289, Abcam, Cambridge, UK), rabbit anti-Myh (Cat#11128-1-AP, Proteintech, Rosemont, IL, USA), and goat anti-GAPDH (Cat#ab9483, Abcam) followed by appropriate HRP-coupled secondary antibodies. Clarity Western ECL Substrates (Bio-Rad, Hercules, CA, USA) were used for enhanced chemiluminescence and Western blot images were captured via ImageQuant LAS 4000 software (ImageQuant; GE Healthcare, Chalfont St. Giles, UK). Proteins levels for Myh9, total Flna and Flna^{CT} were quantified by densitometry using Adobe Photoshop CS3 software, and GAPDH was used to normalize for protein loading. A calibration protein samples was included in every membrane to enable combined results from several membranes. Two sample t-test was used to analyze differences between the relative protein expression levels of control and OIR retinas.

References
