Supporting Information

Experimental Procedures

Bovine Retina Argonaute HITS-CLIP: Immediately after death, bovine eyes were removed and retinas were dissected out, triturated to clusters of cells and subjected to UV-induced protein-RNA crosslinking (3X 400 mJ/cm^2) with a CL1000 UV crosslinker (UVP, Upland,CA). Cells were harvested by centrifugation (1500XG, 10 min, 4 ºC). Retinal tissue from 3 animals was pooled for each library. Each pool was then used to generate libraries for both HITS-CLIP and RNAseq transcriptome analyses. Each HITS-CLIP library was derived from about 1/6 of the tissue pool. Immunoprecipitation was performed using mouse anti-Argonaute monoclonal antibody 2A8 [(1)]. Immunoprecipitation and Argonaute HITS-CLIP library preparation was performed according to Chi *et. al* [(2)]. For transcriptome libraries, bovine retina tissue was isolated within 1 h of death and placed in RNAlater stabilization solution (Qiagen, Germantown, MD). RNAseq libraries were prepared as previously described [(3)]. Both RNAseq and HITS-CLIP libraries were sequenced on an Illumina HiScan instrument using 50 bp single end sequencing (Illumina, San Diego, CA).

All sequencing reads were processed to remove adapter sequences at the 3' end. For transcriptome samples, the number of reads for each RefSeq gene in bosTau7 release was counted with the HTseq program and RPKM values were calculated by using a custom perl script. HITS-CLIP reads were then sorted according to barcodes. Only one sequence was taken from identical sequences with the same barcode and the number of such sequences was counted and recorded as part of the read name. Enrichment in HITS-CLIP samples was calculated with the MACS program by considering the corresponding transcriptome samples as controls. High confidence miRNA target sites were defined as peaks with a P-value for enrichment less than 1 X 10^{-20} present in all three biological replicates (defined as having a summit within 100 bp). Genes wherein the identified miRNA target sites were found were annotated by hand using the UCSC genome browser. Data were deposited in the GEO database (accession number: GSE59912). Gene ontology data were derived from the Ingenuity Pathway Analysis Suite (Qiagen). Transcriptome analyses for comparison of whole eye of *Opsin* knockout mice to wild type mice was performed as previously described [(3)]. These data also are deposited in the GEO database (accession number: GSE61239).

For luciferase assays, a ~1 kb fragment surrounding each identified target site was cloned into the 3'-UTR of the firefly luciferase construct in the pMirGLO vector (Promega, Madison, WI). The core mRNA sequence complementary to the seed regions of all 3 miR-183 cluster miRNAs

is ‘UGCCA’. For the mutated target site experiments, the central 3 nucleotides (GCC) of this core sequence within the identified target site were deleted using either traditional PCR mutagenesis, or inverse PCR followed by intramolecular ligation. A single site was mutated for *Map1b*, *Mbnl2*, *Slc4a7*, and *Vldlr*. Two sites were deleted for *Cds2*, *Crb1*, *Prickle2, and Tpd52.* All constructs were confirmed by Sanger sequencing. The control pMirGLO vector contained a ~1 kb fragment surrounding the identified miRNA target site from the bovine *Atp13a3* gene. This target site lacks perfect complementarity to any of the miR-183 cluster miRNAs. *Vectors* for overexpression of miR-183 cluster miRNAs were described previously [(4)]. Twenty five ng of each pMirGLO-based vector was co-transfected along with 800 ng of each miRNA overexpression plasmid or empty vector into 293T cells in 24-well plates. Forty eight hours post-transfection, renilla and firefly luciferase activity was measured with the Dual Luciferase Reporter Assay System (Promega, Madison, WI). Each day’s experiments were performed in triplicate wells. The mean (from the triplicate wells) of the ratio of firefly to renilla luciferase was calculated for each condition; then that value was divided by the corresponding value for the empty vector to generate the data presented in figure 2C. Three independent experiments (each with triplicate wells) were carried out for each condition.

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3. Mustafi, D., Kevany, B. M., Genoud, C., Okano, K., Cideciyan, A. V., Sumaroka, A., Roman, A. J., Jacobson, S. G., Engel, A., Adams, M. D., and Palczewski, K. (2011) Defective photoreceptor phagocytosis in a mouse model of enhanced S-cone syndrome causes progressive retinal degeneration, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology 25*, 3157-3176.
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