SUPPORTING INFORMATION:

MATERIALS & METHODS:

Antibodies

The following antibodies were used in this study: AKT (9272S), pAKT2 (S474) (8599S), NFκB-p65 (8242S), NFκB-p50 (3035S) and iNOS (13120S) [Cell Signaling Technologies], Actin (A2066), Lectin (L9066) and Arg-1 (ABS535) [Sigma Aldrich], Neutrophil Elastase (ab68672), LCN-2 (ab2267) and IL-10 (ab9969) [Abcam], CCL2 (sc-1785), IL-12 (sc-365389) and CD206 (sc-34577) [Santa Cruz Biotechnology], CD86 (TA326991) [Origene], GFAP (Z0334) [Daco], Iba1 (019-10741) [Wako], CRALBP (MA1-813) [Thermo Fisher] and Tmem119 (gift from Ben Barres laboratory) [19].

Complete knockout mice

βA3/A1-crystallin complete knockout (*Cryba1* KO) mice were generated by mating, over several generations, mice that had *Cryba1* deleted specifically from the RPE (*Cryba1* cKO). These conditional knockouts (*Cryba1* cKO) were produced using the *Cre*-LoxP system and the *Best1* promoter, as has been previously described [8]. When these conditional knockouts were mated together, some of the progeny underwent germline deletion of one of the floxed *Cryba1* alleles. By screening all progeny with a set of 3 PCR primers which produced distinct amplimers for the intact floxed gene and for the deleted floxed gene, it was possible to rapidly genotype each animal at the *Cryba1* locus. Animals which produced both bands were designated as 1 copy animals (i.e. they had one intact and one deleted copy of *Cryba1*). One copy animals were selected, bred together, and the progeny again screened to identify those with no intact *Cryba1* genes. Animals with no intact floxed *Cryba1* genes were

subsequently screened for the presence of *Cre*-recombinase and only those lacking the *Cre* gene were used to establish the homozygous global knockout line. This eliminated any concerns about possible deleterious effects of the *Cre*-recombinase gene itself.

LPS administration and generation of systemic inflammation in mice

The *Cryba1*$^{fl/fl}$ (floxed) and *Cryba1* KO mice (n=6; 4 months old) were injected intraperitoneally with Lipopolysaccharide (LPS) from *E. coli* (L4391-1MG) [Sigma Aldrich] at a dose of 100μg/kg body weight; twice weekly for three consecutive weeks following a previously described method [10]. This particular dose and route of administration of LPS were chosen to activate a low grade systemic inflammatory response.

Isolation, grading of human retina

The human donor eyes were obtained from the Minnesota Lions Eye Bank with the consent of the donor or donor's family for use in medical research and graded for disease severity using the Minnesota Grading System (MGS) as described [12]. The neural retina from the nasal section was processed and used for western blotting.

Immunostaining

Whole eyes from freshly dissected mice were enucleated and fixed in 2% paraformaldehyde (PFA) for 10 minutes and then the anterior parts including cornea, lens and attached iris pigmented epithelium were removed. The resulting posterior eyecups were fixed in 2% PFA for an additional hour at room temperature. Immunofluorescence was performed on frozen sections from the posterior eyecups. The sections were incubated with phosphate-buffered saline, containing 5% normal donkey or goat serum, for 30 min prior to being incubated overnight with primary antibodies

[CRALBP (1:100), GFAP (1:300) and TMEM119 (1:3), Neutrophil Elastase (1:100), LCN-2 (1:100)] at 4 °C, washed in PBS, incubated for 1 h at room temperature with secondary antibodies (1:300), and washed again with PBS. Sections were mounted using DAKO Paramount (DAKO Corporation, Carpinteria, CA). Images were acquired by a Zeiss LSM 710 confocal workstation [6].

SDS-PAGE and western blot analysis

Retinas from freshly dissected mice were sonicated in RIPA lysis buffer (Millipore, 20-188) containing 1% protease and phosphatase inhibitors (Sigma). The lysed samples were incubated on ice for 20 minutes and then they were centrifuged at 13,000g for 20 minutes. The supernatants were taken and protein concentration was evaluated by using the BCA kit (Thermo Fisher). Protein concentrations were adjusted to provide proper detection of particular proteins of interest by western blot. The samples were mixed with 4X protein sample buffer (Invitrogen) plus 5% 2-Mercaptoethanol (Sigma) and heated at 100^0C for 10 minutes. These samples were loaded onto a 4-12% Bis-Tris Nu-PAGE gel (Invitrogen) and run with MES buffer (Novex). Proteins were transferred to nitrocellulose membranes which were then blocked in 5% skim milk (Invitrogen) or 5% BSA (Sigma, for phosphorylated proteins). The membranes were incubated with appropriate primary antibody (listed above) overnight in 4^oC, followed by horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. Blots were developed by chemiluminescence (ECL) methodology (GE Life technologies). Densitometric analysis was carried out using Quantity One software (BioRad Laboratories).

RNA and Protein Arrays

The RNA array was done following the method of the Taqman Mouse Immune Panel Open Array Kit (4367786) [Thermo Fisher]. We performed a human proteome microarray 2.0 analysis as a paid service from CDI NextGen Proteomics, MD, USA.

Co-immunoprecipitation (Co-IP) and LC-MS/MS Assays

Co-IP was done using the Pierce™ Co-Immunoprecipitation Kit (26149) [Thermo Fisher]. The retinas from 3 mice from each experimental group were pooled and sonicated in IP lysis/Wash Buffer (as provided with the kit) containing 1% protease inhibitors (Sigma). The total lysates were processed according to the instructions supplied with the kit. 70μg of protein from each pooled lysate was immuno-precipitated with 10μg immobilized NFkB-p65 antibody overnight at 4°C. Rabbit IgG was used as the IgG control. Eluted samples were loaded for SDS-PAGE and western blot analysis conducted to evaluate the association of NFκB-p50. LC-MS/MS was performed as a paid service from Applied Biomics, CA, USA.

Chromatin Immuno Precipitation (ChIP) and Reverse-ChIP

ChIP was done on the LCN-2 promoter following the method described in the Chromatin Immunoprecipitation (ChIP) Assay Kit (17-295) [Millipore, Germany]. Immunoprecipitation of the DNA-protein complex was done with anti-NFκB-p65 antibody which was followed by qPCR of the DNA fragments using the following primer pairs: spanning position -266: 3'-GTGGACAGGCAGTCCAGATCTGAG-5'/ 5'-AAGATTTCTGTCCCTCTCTCCCCC-3', spanning position -619: 3'-CTGTTCCTGTAAATGGCAGTGGGG-5'/ 5'-GGGTGAGCAAGCTGAGAGTGAATG-3', spanning position -676: 3'-TAAGGACTACGTGGCACAGGAGAG-5'/5'-

GAAGTGTCCAATACCTTGAGCCCC-3', spanning position -1014: 5'-GCTTCTGCCCAAAGTAACTGGAGT-3'/ 5'-TAAGGACTGCAACCTCGGTGTCAT-3', spanning position -1822: 3'-CTGCCCTGAGTGTTGGGTTCAAAG-5'/ 5'-CTGGGGATGTAGCTCTCTGGTGTT-3', and spanning position -3171: 3'-TAGTCCTGCATTCAGTTTGCAGGC-5'/ 5'-ACCCAGGTCCAATCCACATGAAGA-3'. To determine whether pSTAT-1S727 is associated with p65, after the immuno-precipitation and ChIP assays, Reverse ChIP was performed followed by an immunoblot to evaluate the binding of pSTAT-1S727 to the p65-DNA complex [20].

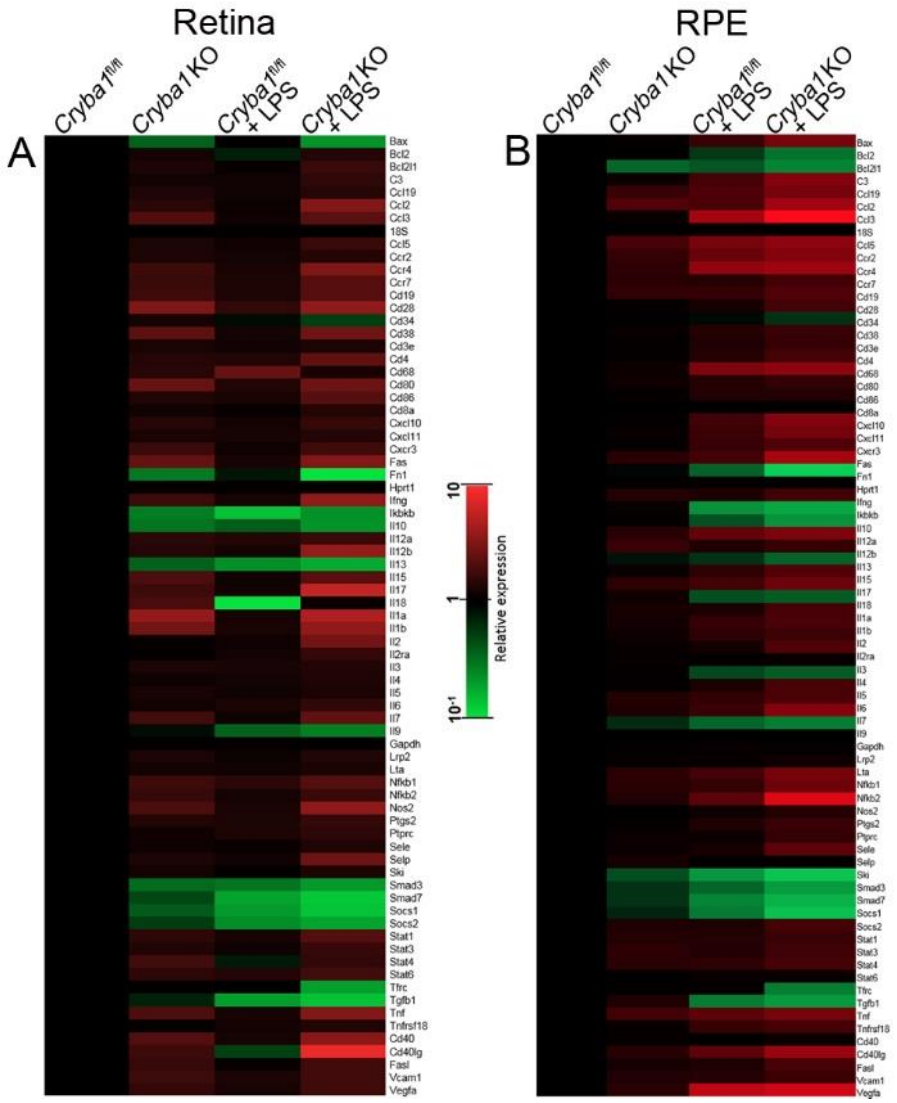
Intravitreal Injection of Triciribine:

Cryba1 $^{fl/fl}$ and *Cryba1* cKO mice were anesthetized with 0.15 ml ketamine/xylazine (ketamine 2.5 mg/ml and xylazine 0.5 mg/ml) by intraperitoneal injection. Topical anesthesia (proparacaine hydrochloride) was applied to the eye, pupils dilated with a drop of topical 2.5% phenylephrine hydrochloride ophthalmic solution, and the eye proptosed by slight depression of the lower lid with blunt curved forceps. The eye was then washed with sterile saline. For intravitreal injection of Triciribine (EMD Millipore), a 30 gauge needle was used to make a hole in the eye just posterior to the limbus and then a glass pipette on a Harvard Pico Injector (PLI-100, Harvard Apparatus, Holliston, MA) was used to inject 1 μl of a 500 μM solution in PBS containing 2.5% DMSO in PBS into the vitreous. Control animals were injected with the vehicle only (2.5% DMSO in PBS). All instruments were sterilized with a steam autoclave. Bacitracin Ophthalmic ointment was applied postoperatively. Animals were euthanized with CO_2 gas 1 hour after injection and the retinas dissected from the eyes

and homogenized. Total and phosphorylated AKT2, NFκB-p65 subunit and LCN-2 were measured in the homogenates by western blotting.

MGS1	Age	Gender	Cause of Death
1	67	M	Cancer
2	76	F	CHF
3	69	F	COPD
4	88	M	CHF
5	77	F	Anoxic Brain Injury
MGS2	**Age**	**Gender**	
1	71	M	Cancer
2	60	M	CHF
3	76	F	COPD
MGS3	**Age**	**Gender**	
1	93	F	Myocardial Infarct
2	90	M	Respiratory Failure
3	79	M	Myocardial Infarct
MGS4	**Age**	**Gender**	
1	74	F	CVA
2	84	F	Cancer
3	95	F	Cardiomyopathy

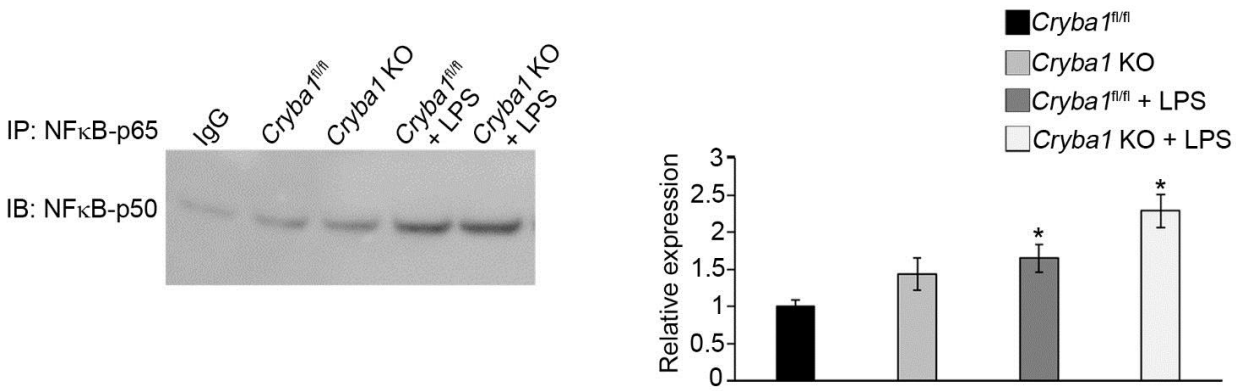
Supplementary Table 1:
Demographic information on human donors whose eyes were used in this study. Eyes were graded for AMD severity by the Minnesota Grading System (MGS).



Supplementary Figure 1:

LPS potentiates differential expression of inflammatory genes in the retina and RPE of *Cryba1* KO mice.

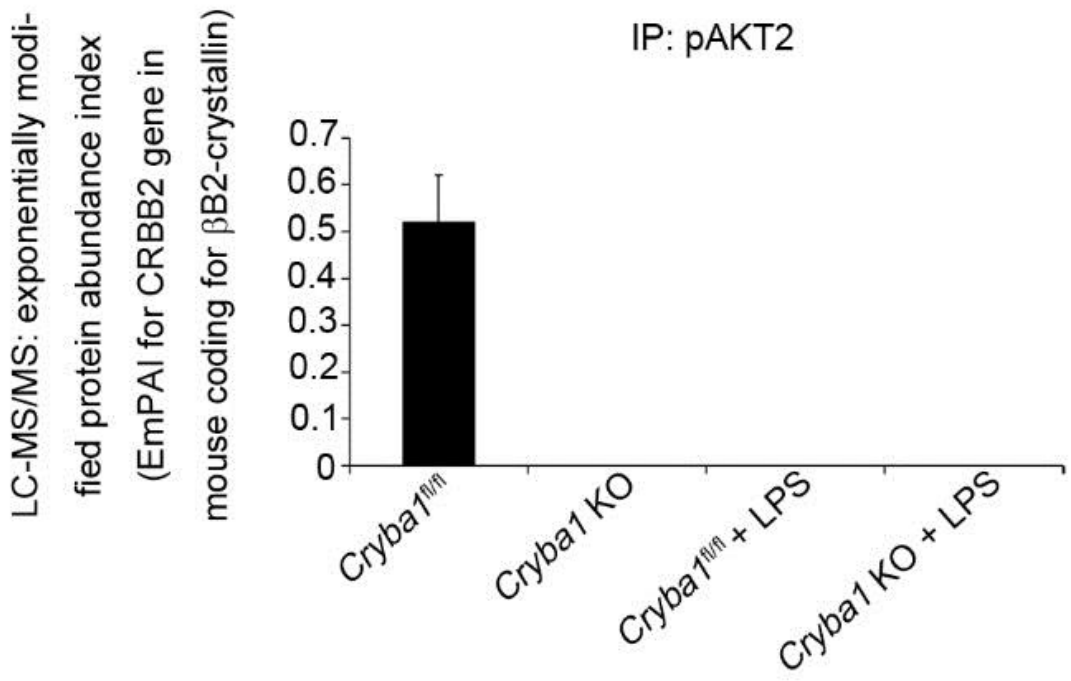
Heat maps showing mRNA profiles for different cytokines, chemokines and immune molecules from the retina (A) and RPE (B) of all the experimental mice are shown. Relative expression of 75 differentially expressed genes, including internal controls, are shown in the analysis representative for three independent experiments. Genes differentially expressed are represented as fold change with respect to *Cryba1*$^{fl/fl}$.



Supplementary Figure 2:

NFκB subunits exist as heterodimers in the nucleus of retinal cells.

Co-immunoprecipitation followed by immunoblot from nuclear lysates from the retina showed increased heterodimeric association of NFκB-p65 and p50 subunits in all LPS-treated groups. The densitometry is represented as Mean ± SD and * ($p<0.05$) depicts significant change with respect to *Cryba1*$^{fl/fl}$.



Supplementary Figure 3:

βB2-crystallin associates with AKT2 under normal conditions, but not during inflammation.

Co-immunoprecipitation followed by LC-MS/MS revealed association of βB2-crystallin with pAKT2 only in the retina of *Cryba1*$^{fl/fl}$ mice. The LC-MS/MS was done by evaluating the Exponentially Modified Protein Abundance Index (emPAI) which represents the relative quantitation of the proteins in a mixture based on protein coverage by the peptide matches in the database.