

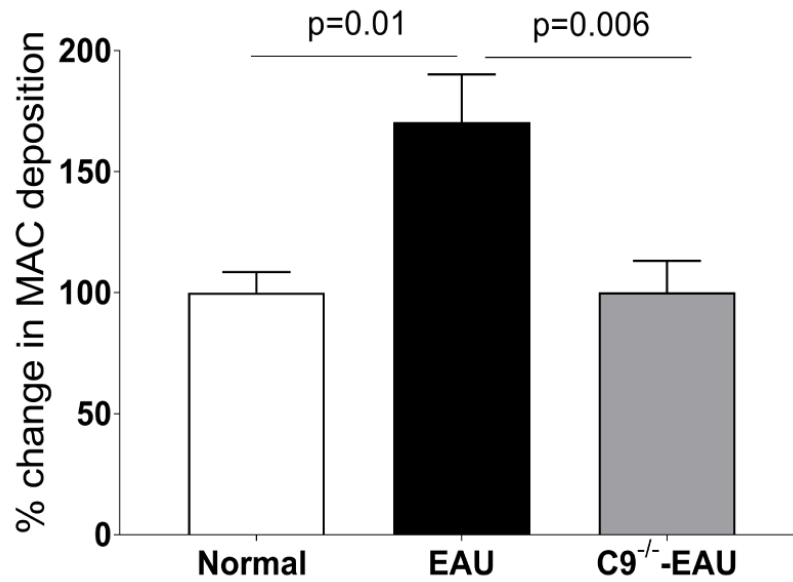
YMTHE, Volume 26

## **Supplemental Information**

### **Complement-Mediated Activation of the NLRP3 Inflammasome and Its Inhibition by AAV-Mediated Delivery of CD59 in a Model of Uveitis**

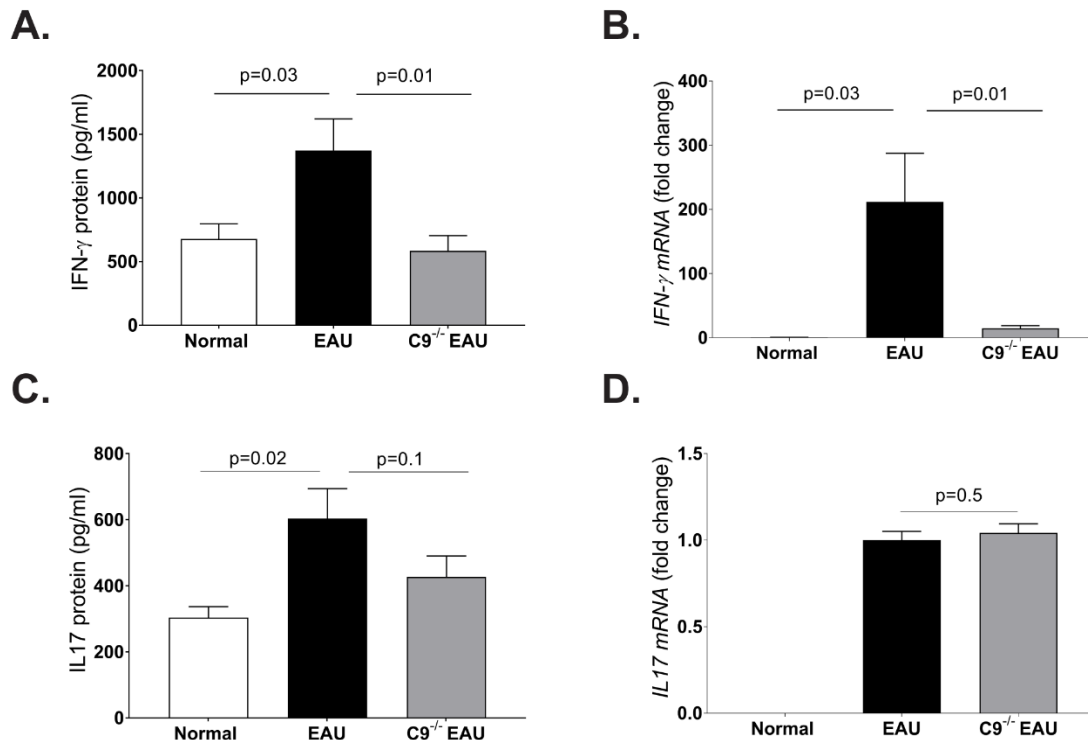
**Binil Kumar, Siobhan M. Cashman, and Rajendra Kumar-Singh**

## Supplemental Figure 1



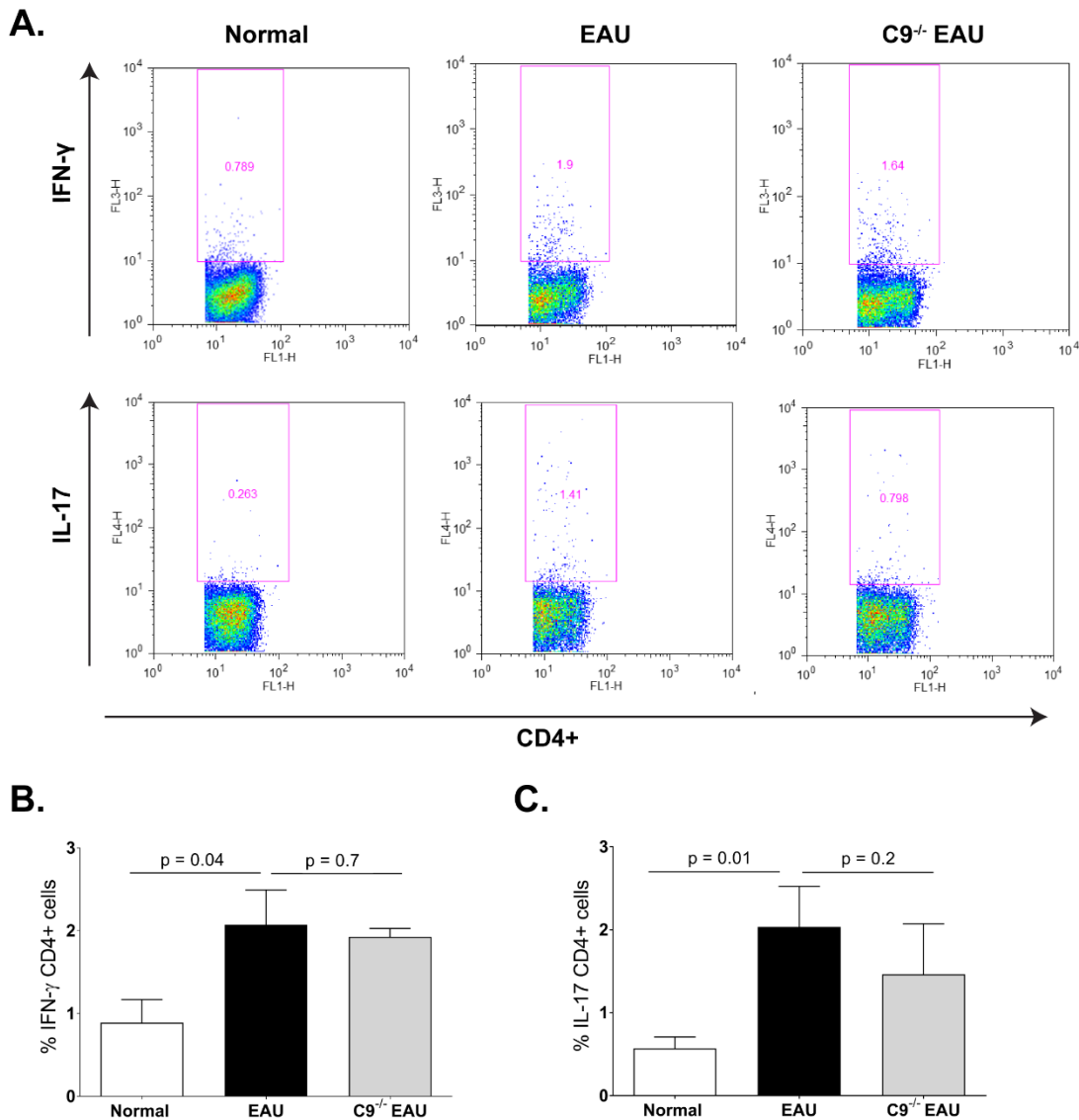
**Figure S1. Elevation of MAC in EAU retinas.** Quantification of MAC fluorescence intensity in the retina exhibits a 70% increase in MAC formation in EAU retinas relative to normal retinas with no significant elevation in MAC in C9<sup>-/-</sup> EAU retina. Values are represented as mean ± SEM.

## Supplemental Figure 2



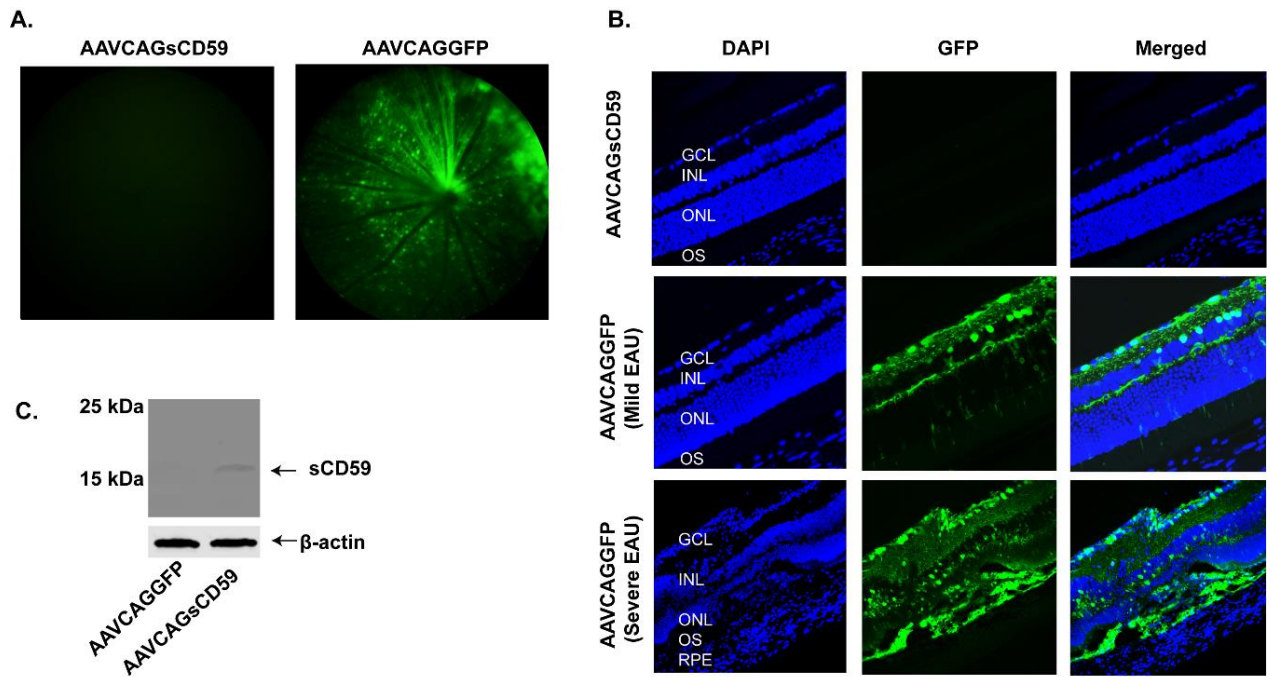
**Figure S2. Effects of MAC on the differentiation of Th1 and Th17 cells in EAU retinas.** Freshly dissected retinas were quantified for mRNA and protein levels of IL-17 and IFN- $\gamma$  using ELISA and RT-PCR in normal, EAU and C9<sup>-/-</sup> EAU retinas. (A-B). The increase in IFN- $\gamma$  protein and mRNA levels was 102% and 200 fold higher respectively in EAU retinas relative to normal control retinas. However, In C9<sup>-/-</sup> EAU retinas, IFN- $\gamma$  protein decreased by 14% and mRNA levels increased by 14 fold compared to normal control retinas. (C-D). The IL-17 protein levels were 99% higher in EAU retinas relative to normal control retinas. However, IL-17 protein levels were only 44% greater in C9<sup>-/-</sup> EAU retinas relative to normal control retinas. The IL-17 mRNA levels in normal control retinas remained below the detection limit. Each experiment was repeated two to three times. Values are represented as mean $\pm$ SEM.

## Supplemental Figure 3



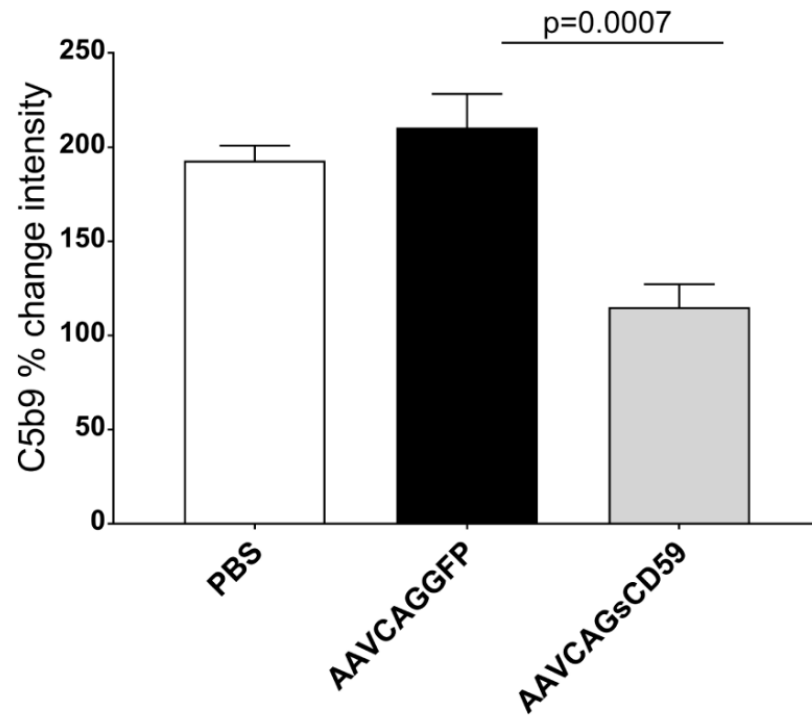
**Figure S3. Th1 and Th17 cells in draining lymph node (DLN) in EAU mice.** DLN cells were collected from EAU and C9<sup>-/-</sup> EAU mice after 24 days. (A) Cells from the DLN were stained for IL-17 and IFN- $\gamma$ , and representative scatter plots gated on CD4<sup>+</sup>. (B) We found a significant increase in IL-17 and IFN- $\gamma$  positive CD4<sup>+</sup> cells from draining lymph nodes in EAU mice compared to normal control mice. However, the percentage of IL-17 and IFN- $\gamma$  positive CD4<sup>+</sup> cells from draining lymph nodes in C9<sup>-/-</sup> EAU mice relative to EAU mice remained insignificant. Each experiment was repeated three times. Values are represented as mean $\pm$ SEM.

## Supplemental Figure 4



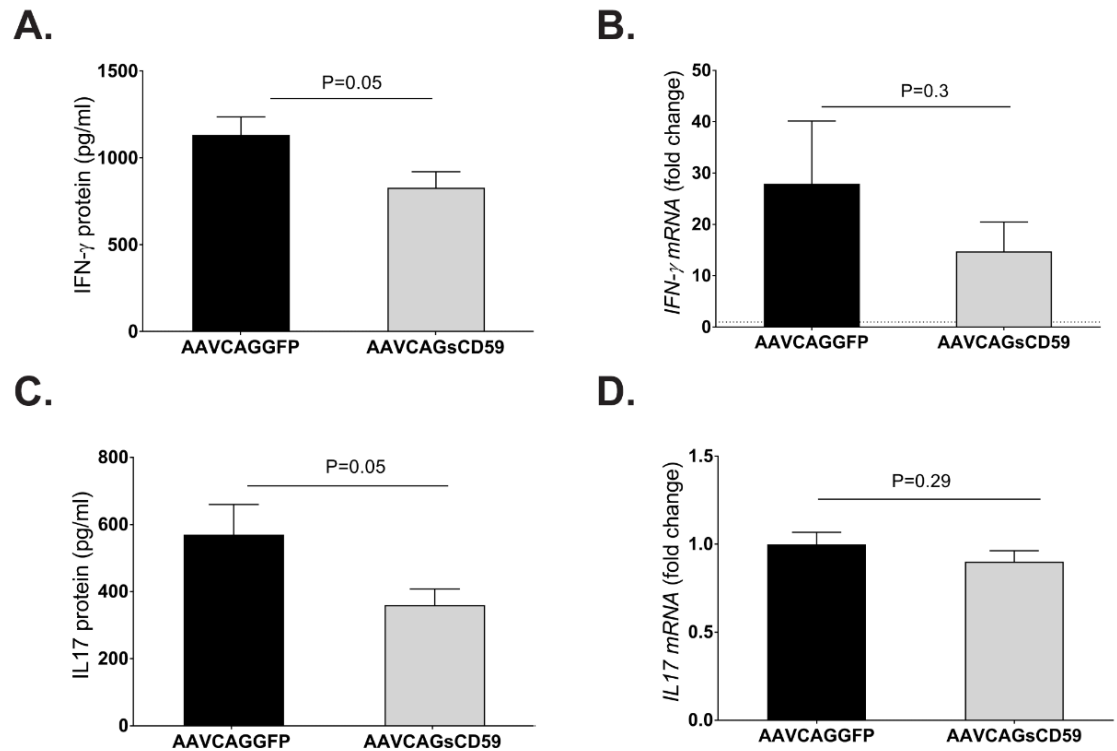
**Figure S4. AAV mediated expression of sCD59 and GFP in mouse retina.** Six-week-old C57Bl/6J mice were injected with  $3.5 \times 10^9$  genome copies/ $\mu\text{l}$  of AAVCAGsCD59 or AAVCAGGFP ( $1 \mu\text{l}$ ) and one week later challenged with EAU and maintained for 24 days. **(A)**, Fluorescence fundus imaging exhibiting expression of GFP in mouse retina. **(B)**, Retinal cryostat images from the AAVCAGGFP injected group exhibiting robust expression of GFP in the ganglion cell layer, inner plexiform layer and inner nuclear layer in retina from mild EAU mice. However, retina from severe EAU mice exhibited GFP expression also in the photoreceptors and retinal pigment epithelium. **(C)**, Western blot analyses showing expression of sCD59 from mouse retina after a single intravitreal injection of AAVCAGsCD59. GCL, Ganglion cell layer; INL, Inner nuclear layer; ONL, Outer nuclear layer; OS, Outer segments; RPE, Retinal pigment epithelium.

## Supplemental Figure 5



**Figure S5. Formation of MAC is attenuated by a single intravitreal injection of AAVCAGsCD59 in EAU retinas.** Quantification of MAC fluorescence intensity in the AAVCAGsCD59 injected retina indicating a 45% reduction in formation of MAC relative to AAVCAGGFP injected retinas. Values are represented as mean  $\pm$  SEM.

## Supplemental Figure 6



**Figure S6. Soluble CD59 inhibits differentiation of Th1 and Th17 cells in EAU retinas.** Freshly dissected retinas were quantified for IL-17 and IFN- $\gamma$  protein and mRNA using ELISA and RT-PCR respectively in AAVCAGsCD59 and AAVCAGGFP EAU retinas. (A-B). In AAVCAGsCD59 EAU retinas IFN- $\gamma$  protein and mRNA levels decreased by 25% and 47% respectively relative to AAVCAGGFP EAU retinas. (C-D). The IL-17 protein and mRNA levels in AAVCAGsCD59 EAU retinas decreased by 35% and 10% relative to AAVCAGGFP EAU retinas. However, differences in mRNA expression of IL-17 and IFN- $\gamma$  were not deemed statistically significant. Each experiment was repeated two to three times. Values are represented as mean  $\pm$  SEM.