Supplemental Materials (Figures and Legend)

Figure S1. RPE cells have epithelial cell markers. Membrane-associated ZO-1 and cytoplasmic cytokeratin-18 were detected in ARPE-19 and RPE cells from a representative donor. A similar staining pattern was observed on the RPE cells used in experiments from the other two donors used in these studies (not shown). Cells were stained on day 6 after plating (red: cytokeratin-18. green: ZO-1). Blue: DAPI (nuclei stain). Scale bar: 20 μM.
Figure S2. Anti-ARPE-19 IgG S-58 recognizes ARPE-19 cells and leads to cell-surface MAC deposition. 

A. ARPE-19 cells were probed with sheep polyclonal IgG against whole ARPE-19 cells (S-58, 1.2 mg/ml) for 30min at 37 °C and detached with TrypLE. The level of bound antibody was determined using Alexa 488 conjugated anti-sheep secondary antibody (1:1000 in 1% BSA/PBS). IgG from sheep 58 recognized ARPE-19 cells.

B. ARPE-19 cells were primed with S-58 (1.2 mg/ml) for 30 minutes at 37 °C, washed and incubated with 6% HiC1q-Dep or 6% C1q-Dep for 20 minutes at 37 °C. Cells were detached with 1x TrypLE and fixed in 0.5% PFA for 10min followed by chilling on ice for 1 minute. S-58 produced surface MAC in the presence of C1q-Dep measured as described in Methods.
Figure S3. S-58 recognizes donor RPE cells and generates cell-surface MAC. A. S-58 binds to donor RPE cells in a dose-dependent manner similar to that observed in ARPE-19 cells. (a) Total proteins (10 µg) were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with anti-sheep secondary antibody. (b) The blot (a) was stripped and re-probed with an antibody to GAPDH. B. RPE cells were primed with or without S-58 (1.2 mg/ml) and cell membrane-bound S-58 was detected by Alexa 488 donkey anti sheep secondary antibody (green). Scale bar: 20µM. Blue: DAPI (nuclei stain). C. Donor RPE cells were primed with or without S-58 (1.2 mg/ml) and then incubated with either 6% C1q-Dep, NHS or 6% HiC1q-Dep for MAC deposition using Alexa 568. Red: MAC deposited on cell surface in donor RPE cells. Scale bar: 50µM in low magnification and 10 µM in high magnification. Blue: DAPI (nuclei stain).
Figure S4. Specificity of S-58-induced cell lysis in the presence of C1q-Dep. A. and B. RPE cells were primed with either S-58 or normal sheep IgG at indicated concentrations for 30 minutes, washed once and then incubated with either 6% C1q-Dep or 6% HiC1q-Dep for evaluation of cell permeability and cell viability determined by LDH assay (A) and WST-1 assay (B), respectively, in RPE cells from a 62 year-old donor. * (P<0.05) vs medium alone.
Figure S5. FACS analysis of mCRPs expressed on ARPE-19 cell surface. ARPE-19 cells in suspension were probed with the indicated anti-mCRP primary antibody followed by detection with FITC labeled secondary antibody. Solid dark line denotes secondary antibody only while the light gray line indicates cells treated with both primary and secondary antibodies. ARPE-19 cells express CD46 (A), CD55 (B) and CD59 (C) but not CR1 (D) on their cell surface. X-axis shows the intensity of the FITC-stained cells.
Figure S6. FACS analysis of mCRP knockdown on ARPE-19 cells. ARPE-19 cells were transfected for 6 hours with either individual or combined siRNA (2 nM) against mCRP or non-targeting control siRNA followed by media replacement. Transfected cells were cultured for 72 hours, re-plated and cultured for 24 hours. Cells were then harvested and processed for FACS analysis. X-axis shows the intensity of the FITC-stained cells. The percentage of knockdown is shown on corresponding histographs and was calculated based on mean fluorescence intensity: (control siRNA value - targeted siRNA value)/control siRNA value × 100.