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## Supplemental Information

# Toll-like Receptor 2 Facilitates Oxidative Damage-Induced Retinal Degeneration

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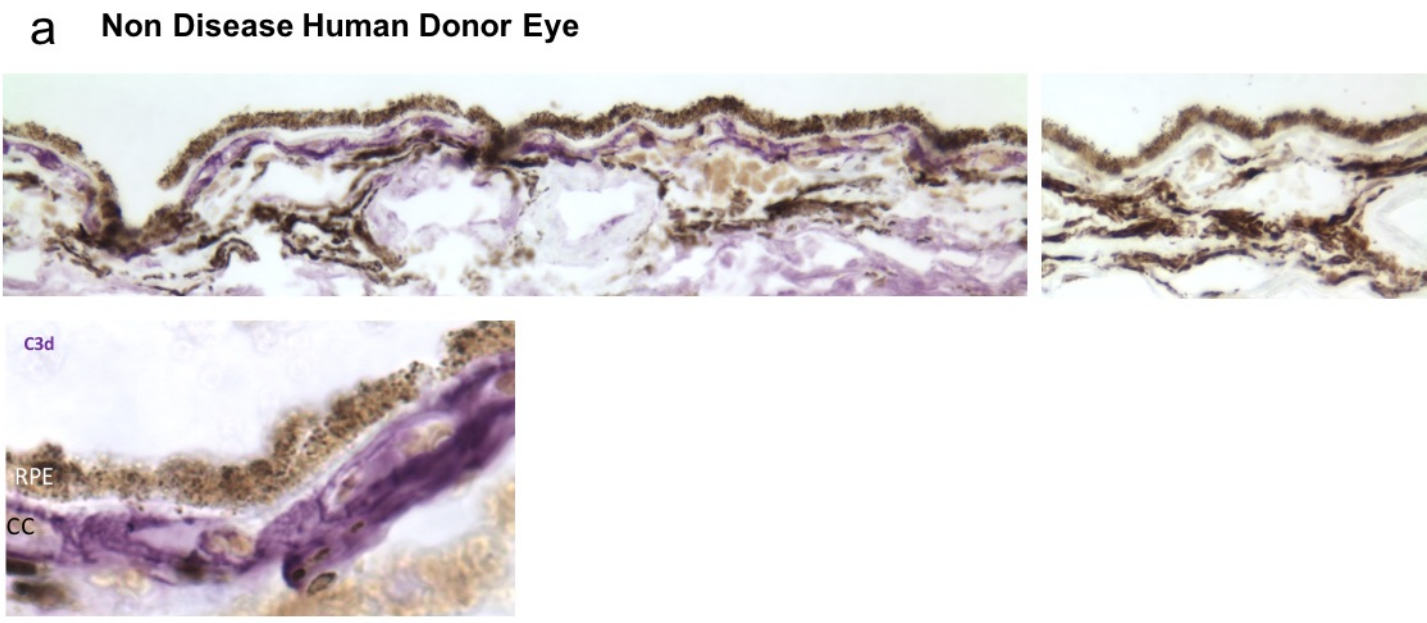
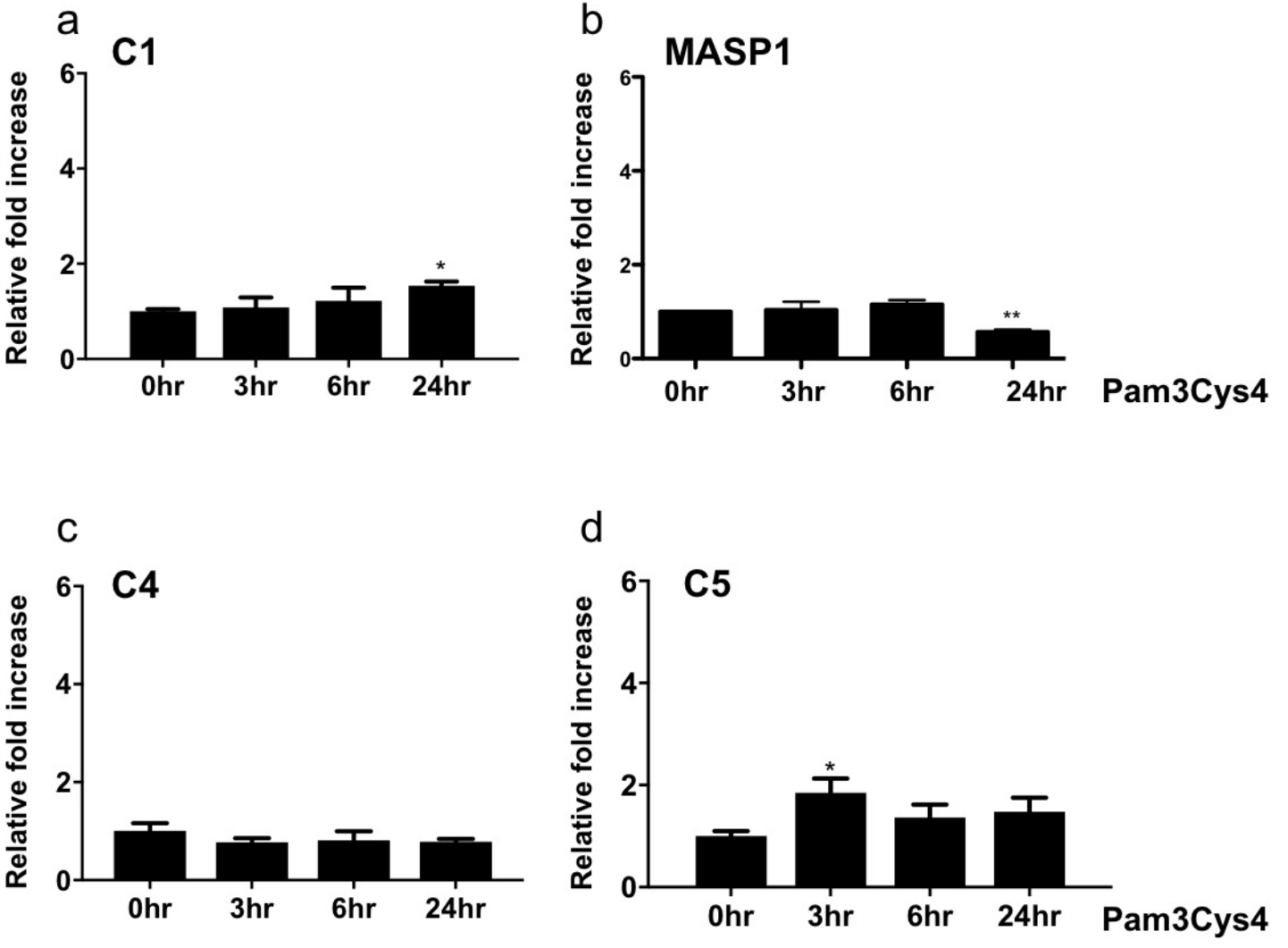
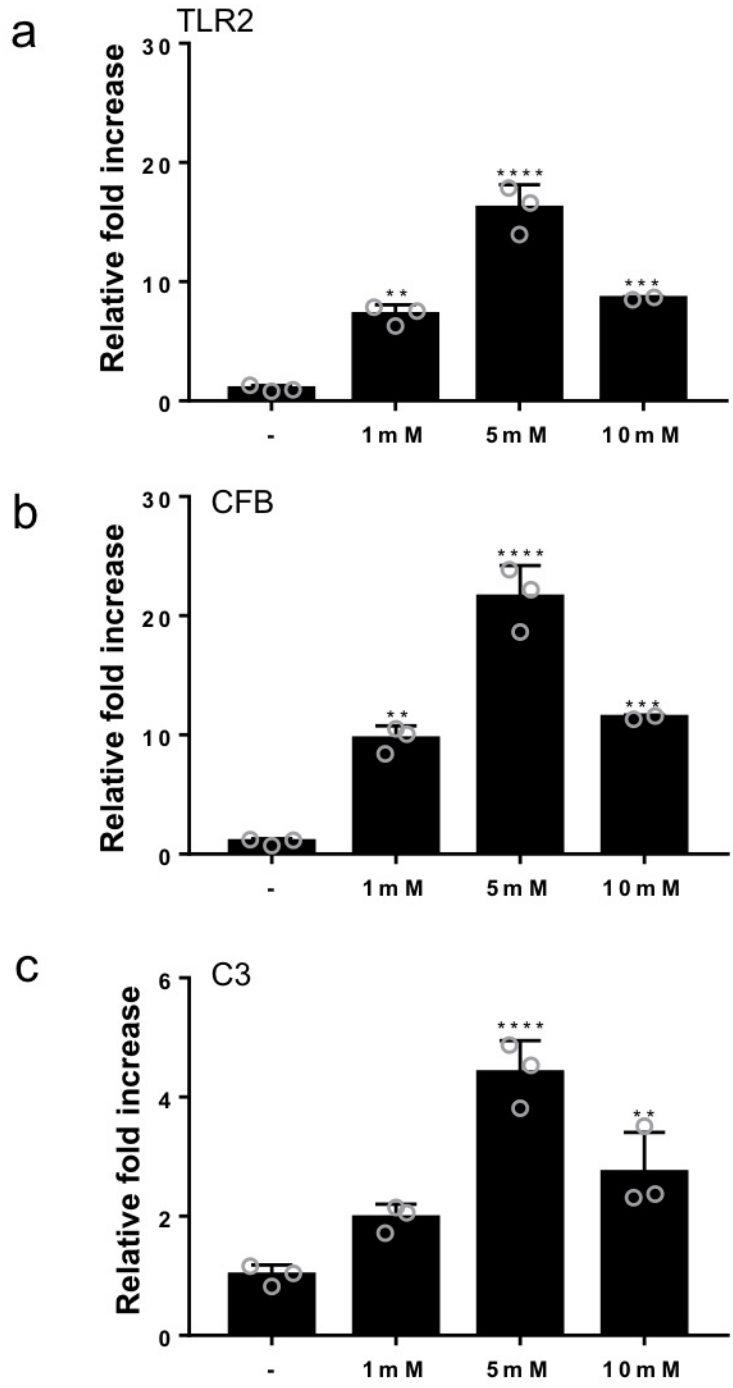


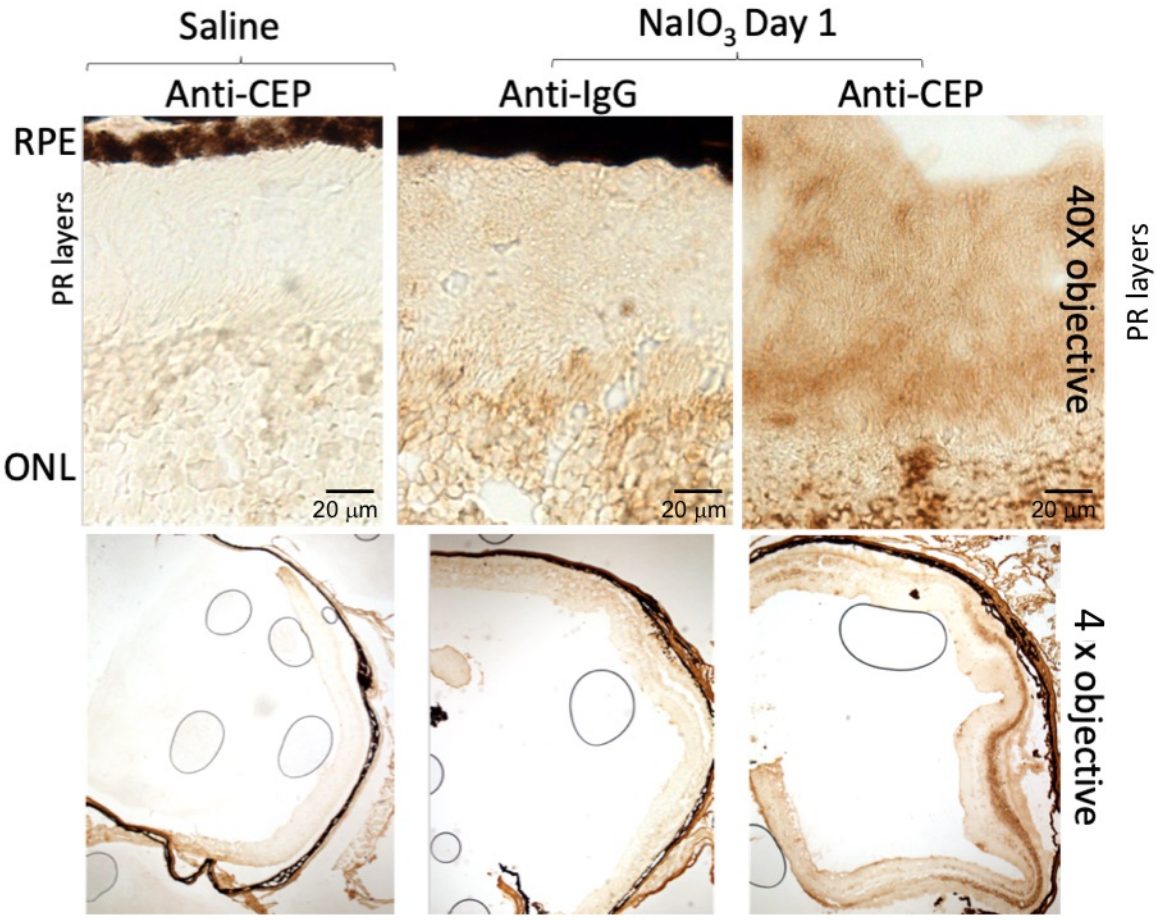

**Supplementary Figure 1. Pattern of deposition of C3d differs in non-disease and AMD donor eyes. Related to Fig. 1** Immunohistochemistry of C3d in (a) healthy non-disease donors and (b) AMD donor eyes. Immunoreactivity is visible in the choriocapillaris (CC) of both healthy and disease eyes and sub-RPE in basal laminar deposits (BLD) and drusen in the AMD donor eyes. Immunoreactivity is also visible in linear deposits (black arrow*) and in migrating cells (white arrows) CC: choriocapillaris; RPE: retinal pigment epithelium; BM: Bruch membrane.



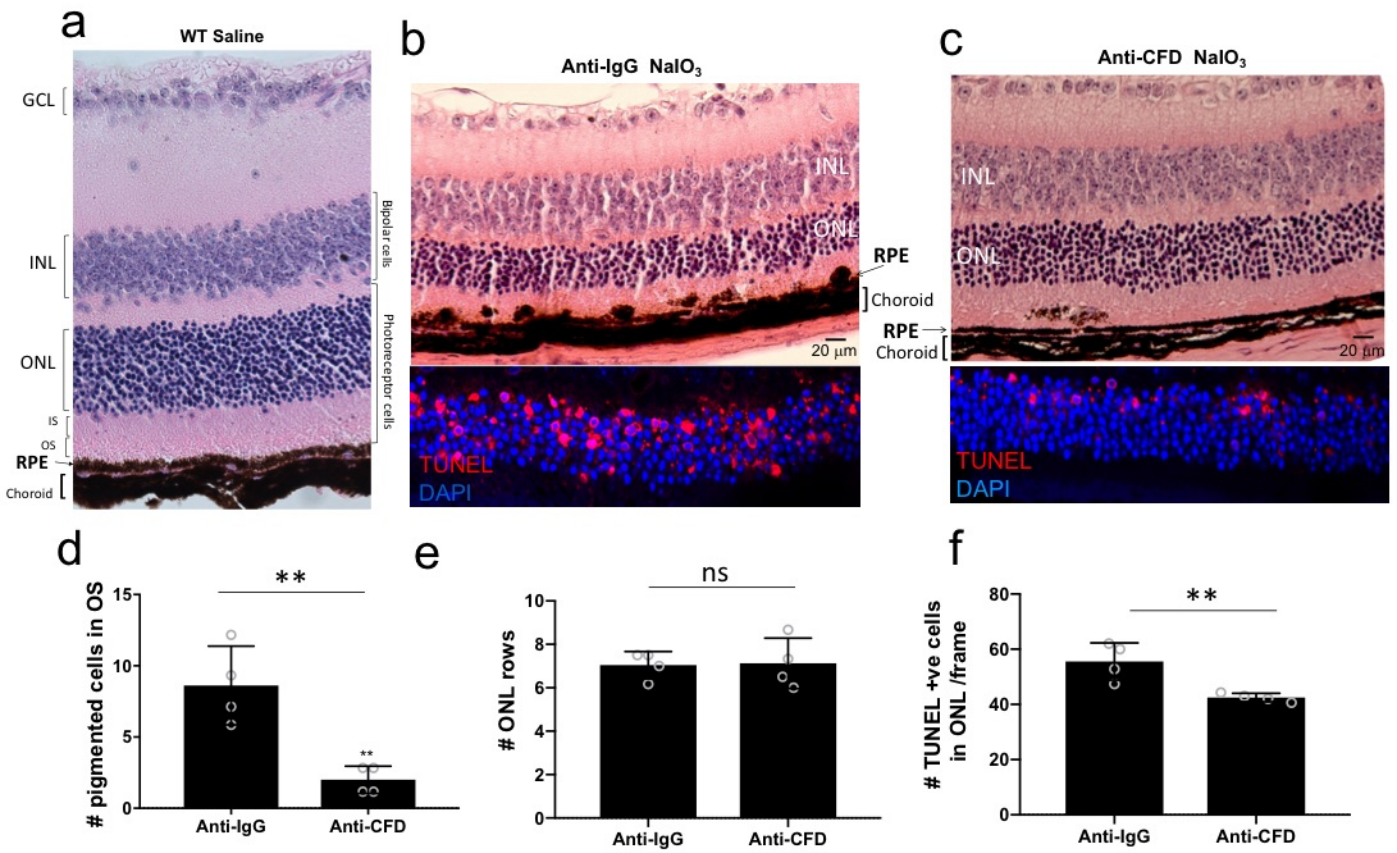

**Supplementary Figure 2. TLR2 activation of RPE weakly induces C1 and C5 but not MASP1 or C4. Related to Fig. 1** (a-d) ARPE-19 cells were treated with 20nm Pam3Cys4 over 24 h, complement factors C1, MASP1, C5 and C4 were assayed by RT-PCR. Experiments were carried out in triplicate and data are mean ± SEM for 3 separate experiments.



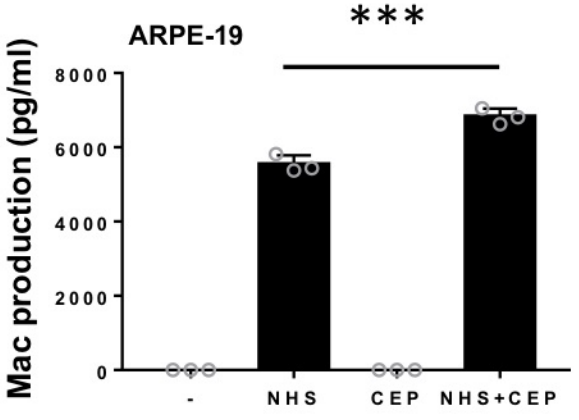

**Supplementary Figure 3. $NaIO_3$ induces TLR2, CFB and C3 expression in ARPE-19 cells. Related to Fig 3** (a-c) ARPE-19 cells were treated with 1, 5 or 10mM $NaIO_3$ for 24 h expression of (a) TLR2, (b) CFB and (c) C3 were assayed by quantitative RT-PCR.

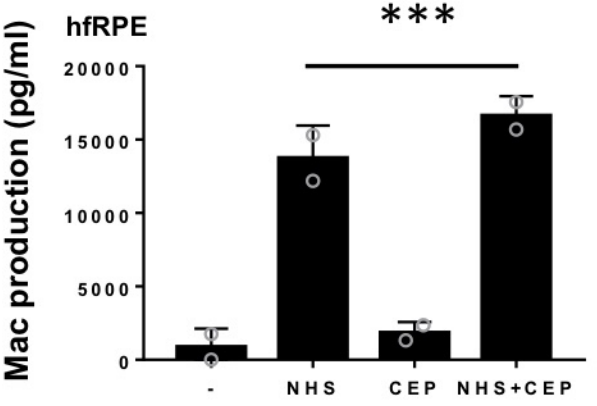



**Supplementary Figure 4. The $NaIO_3$ model of retinal degeneration generates CEP-adducts. Related to Fig 3** Wildtype C57Bl6 mice were injected intravenously (IV), via tail vein, with either saline (NaCl) or $NaIO_3$ (50mg/kg). Eyes were enucleated 24 h later and prepared for histological analysis of CEP (brown DAB stain). Immunoreactivity is visible in the ONL in mice injected with $NaIO_3$ and absent in mice injected with NaCl when compare to an IgG control.

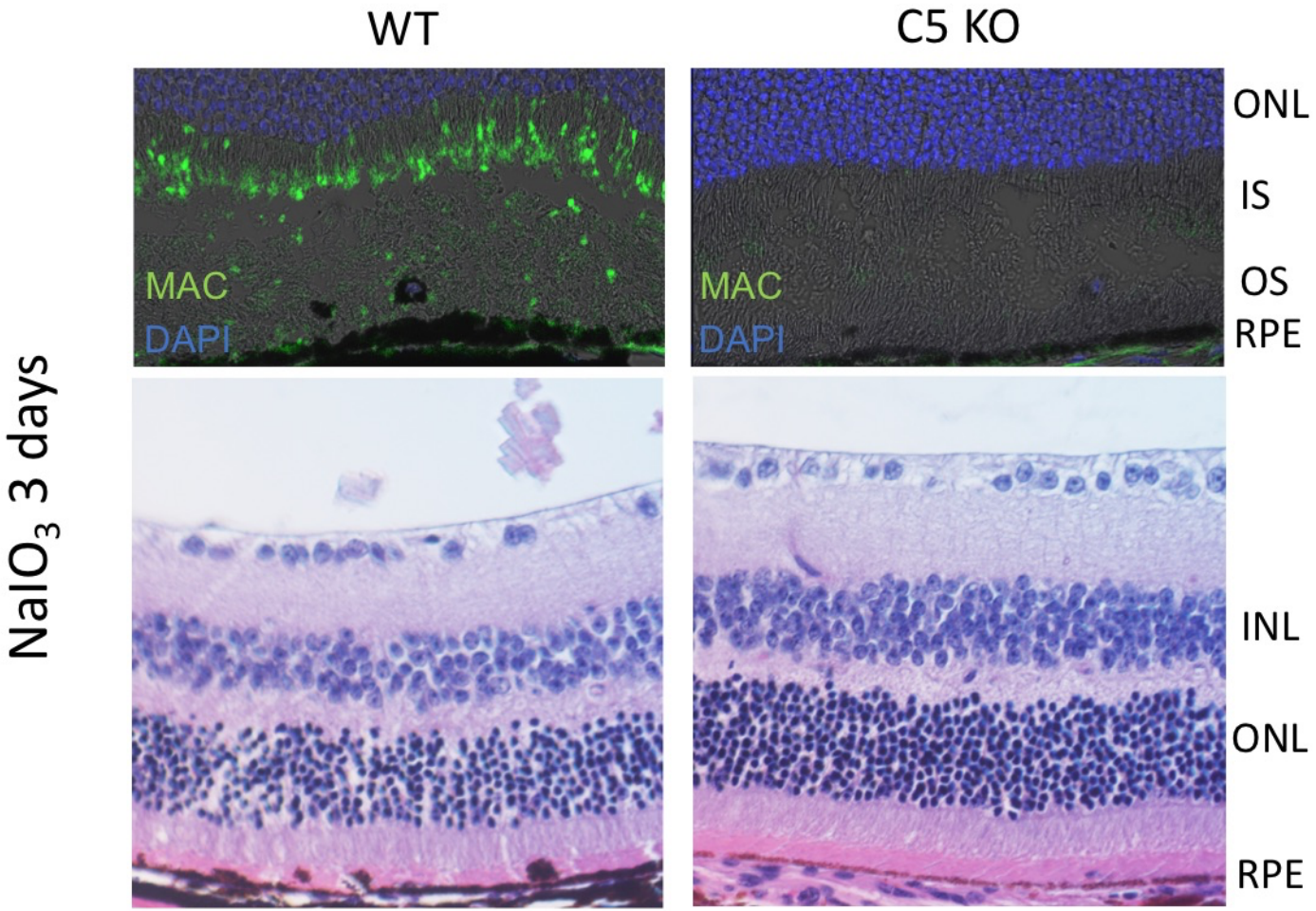



**Supplementary Figure 5. The alternative complement pathway causes RPE blebbing and photoreceptor cell death in the $NaIO_3$ model of retinal degeneration. Related to Fig 3 (a)** H&E stain of wildtype C57Bl6 mice (n=4) injected intravenously (IV) via tail vein with saline (NaCl), eyes were enucleated 72 h later and prepared for immunohistochemistry. (b-f) Wildtype C57Bl6 mice (n=4) were injected intravenously (IV) via tail vein with $NaIO_3$; at the same time, mice were injected intravitreally (IVT) with anti-CFD antibody or anti-IgG. Eyes were enucleated 72 h later and prepared for immunohistochemistry: (b,c) top panels; H&E stained, bottom panels; red = TUNEL, blue = DAPI. (d) Quantification of the number of pigmented cells in the photoreceptor outer segments. (e) Quantification of the number of photoreceptor ONL rows. (f) The number of TUNEL positive cells in the ONL per frame. Data shown are mean ± SEM p value was determined by nonparametric t test, $p < 0.01$ (**).




**Supplementary Figure 6. Related to Fig 5** (a, b) Polarised hfRPE cells or (c-e) ARPE-19 cells were treated with 10% Heat inactivated (Hi) normal human serum (NHS) or NHS in combination with human serum albumin (HSA) or CEP-HSA for 24 h Soluble MAC production was quantified in the cell culture supernatants by ELISA.




**Supplementary Figure 7. Oxidative stress induced MAC formation is blocked in C5 deficient mice. Related to Fig 5** Wildtype C57Bl6 and DBA/2J (C5 KO) mice were injected intravenously (IV), via tail vein, with $NaIO_3$ (50mg/kg), eyes were enucleated at 72 h and prepared for MAC immunofluorescence and H&E stain; (f) quantification of ONL.

| I.D. | Disease Stage | Age (years) | Gender | Cause of death | Death-Processing (time) |
|---|---|---|---|---|---|
| CTL 1 | Control | 87 | Male | Cancer | 02:57 |
| CTL 2 | Control | 91 | Female | Unavailable | 03:08 |
| CTL 3 | Control | 90 | Female | Carcinoma | 05:40 |
| CTL 4 | Control | 79 | Male | Unavailable | 05:32 |
| AMD 1 | Early AMD | 99 | Female | Acute Pancreatitis | 04:49 |
| AMD 2 | Early AMD | 89 | Female | Unavailable | 03:54 |
| AMD 3 | Early AMD | 89 | Female | Ischemic Cardiomyopathy | 06:46 |
| AMD 4 | Early AMD | 95 | Female | Respiratory Failure, COPD | 05:59 |
| AMD 5 | Early AMD | 79 | Female | COPD | 05:55 |
| AMD 6 | Early AMD | 91 | Male | Aspiration Pneumonia | 07:48 |

**Table S1. Characteristics of Donor Tissue used for Immunohistochemistry** Related to STAR Methods.