

## Article

# Supplementary material: Complement Factor H loss in RPE cells causes retinal degeneration in a human RPE-porcine retinal explant co-culture model

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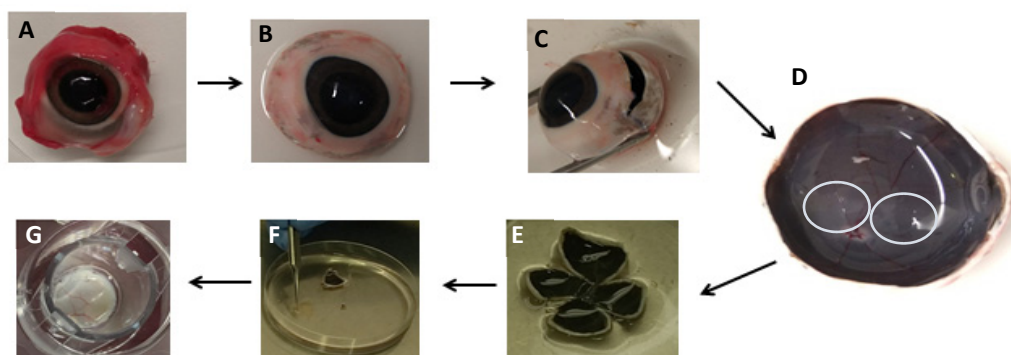
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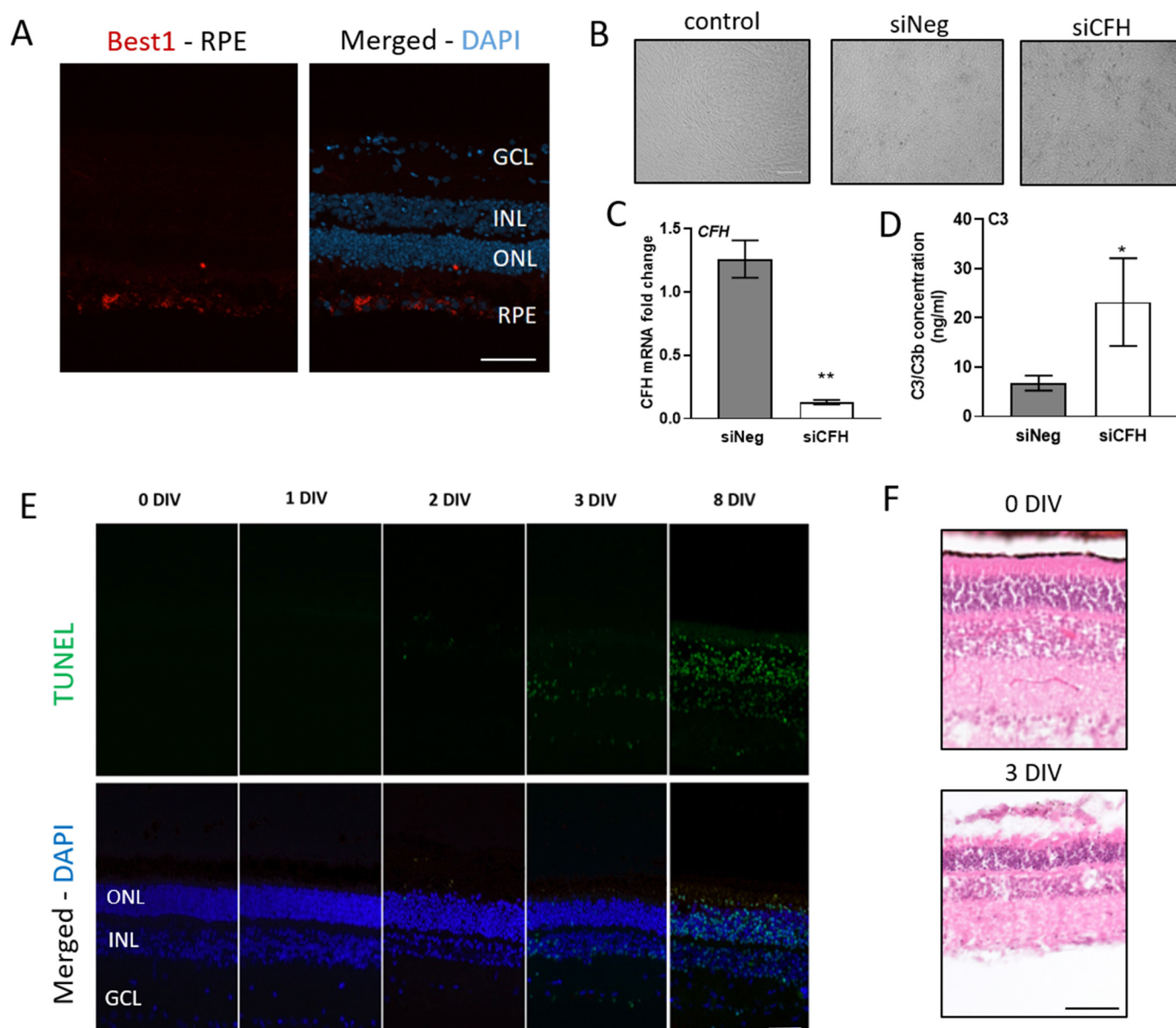
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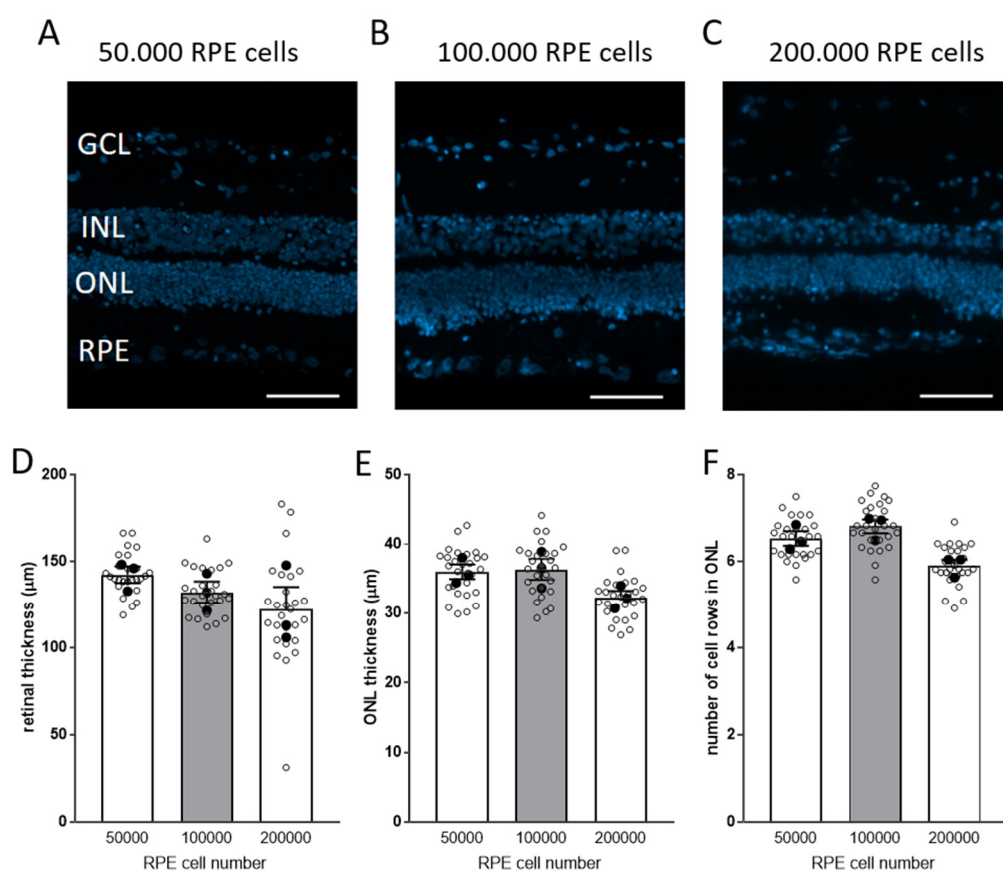
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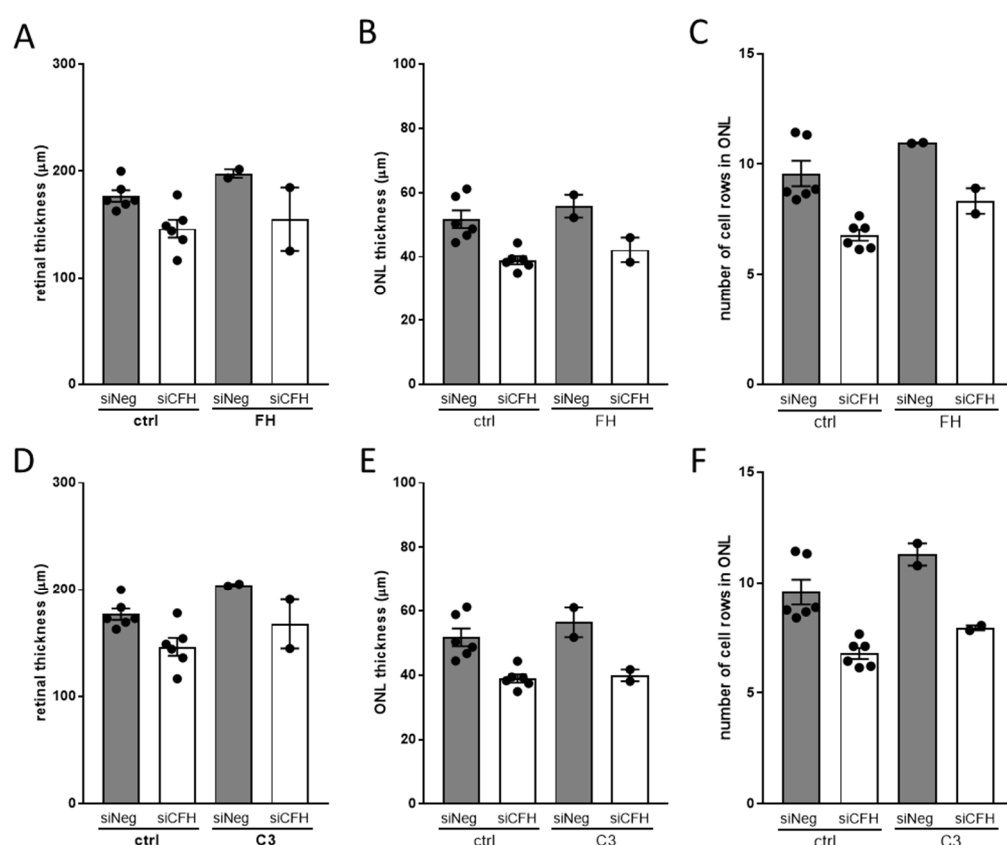
**Figure S1:** Preparation of porcine retinal explants for co-culture with RPE cells. **A.** Porcine eyecups were obtained from the slaughter house. **B.** Porcine eyecups after muscles and optic nerve removal. **C.** A small incision at about 5mm from the iris. **D.** The anterior segment of the eyecup including cornea, iris, lens, and ciliary body were removed. The avascular cone-rich region of the retina was identified as indicated by white circles. **E.** Flattened eyecup. **F.** Retinal fragments gently peeled off from the RPE-choroid-sclera layers. **G.** Retinal explant placed on the transwell containing the RPE cells for co-culture.



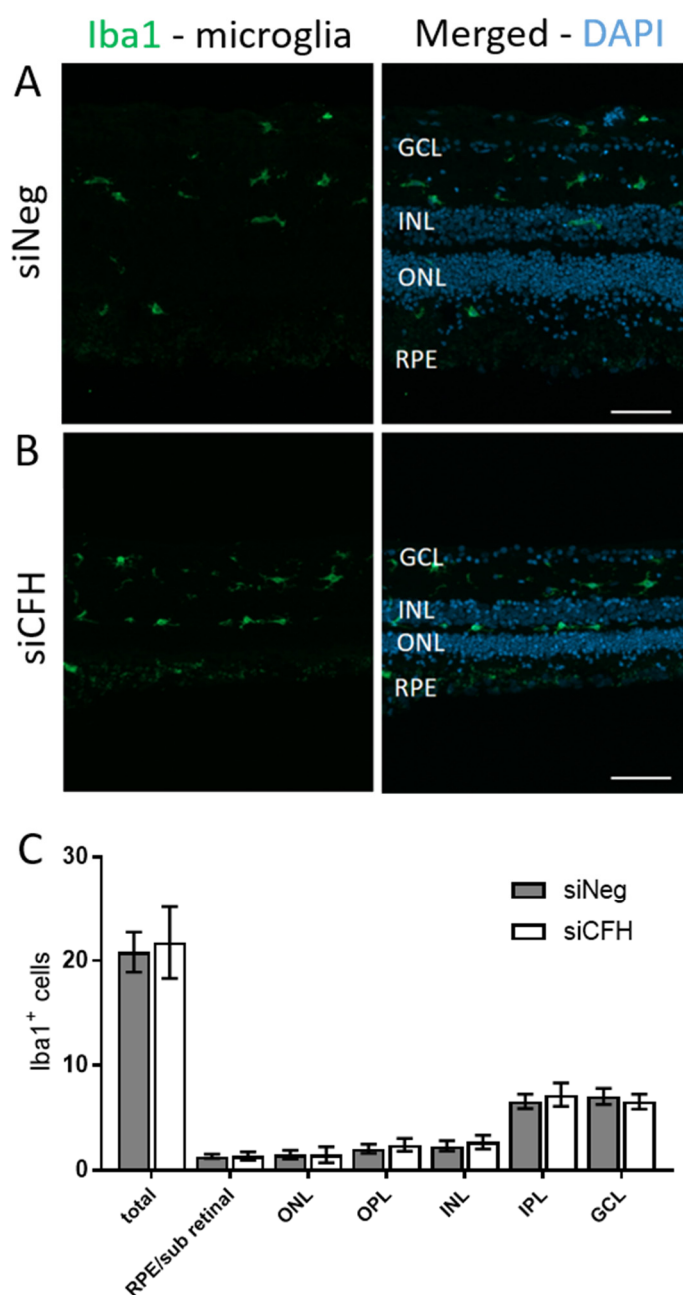
**Figure S2:** Characterization of hTERT-RPE1 cells and retinal explants used in the co-culture model. **A** porcine retinal explant in co-culture with hTERT-RPE1 cells. Cryosections were stained for RPE marker Best1 (red) and DAPI staining for nuclei. **B** Photographs of control hTERT-RPE1 cells and hTERT-RPE1 cells silenced with siNeg or siCFH siRNA. **C–D** Cell pellets and cell culture supernatants from hTERT-RPE1 cells siNeg and siCFH were collected after 72 hours. **C** Evaluation of *CFH* expression after 72 hours by qRT-PCR analyses. Data are normalized to the housekeeping genes *RPLP0* and *ACTB* using  $\Delta\Delta Ct$  methods. SEM is shown,  $n=4$ . **D** C3/C3b ELISA analyses of cell culture supernatants of hTERT-RPE1 cells. SEM is shown,  $n=4$ . **E** Tunel (green) and Dapi (blue) staining of porcine retinal explants at different time points in culture (DIV-days *in vitro*). **F** H&E staining of retinal sample 0 DIV and 3 DIV.



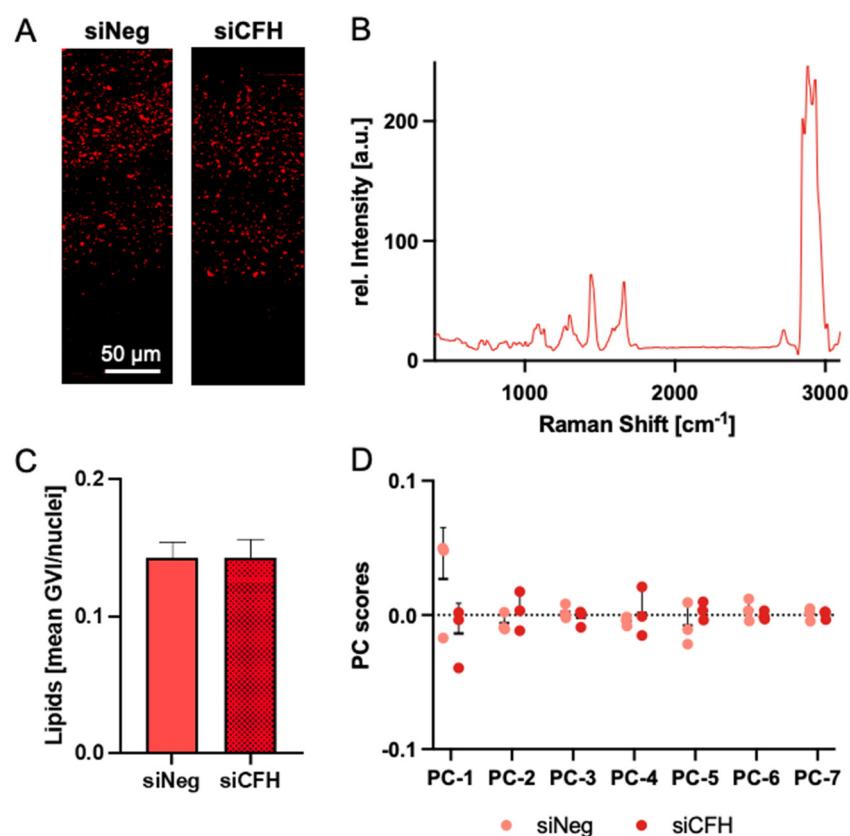
**Figure S3:** Effects of RPE cells seeding density on the co-cultured porcine retinae. Retinal explants were exposed to hTERT-RPE1 cells seeded at increasing cell density: **A** 50.000 cells, **B** 100.000 cells and **C** 200.000 cells. Retinal architecture parameters were assessed with the help of DAPI staining for nuclei. Quantification of the parameters are shown in D-F: retinal thickness (D), ONL thickness (E), number of cell rows in the ONL (F). Mean  $\pm$  SEM is shown. N=3.



**Figure S4:** Exogenous FH or C3 does not rescue or exacerbate the effects of FH loss in RPE cells on the co-cultured porcine retinae. Retinal explants from the visual streak of porcine donor eyes are exposed to either siNeg or siCFH hTERT-RPE1 cells for 72 hours and medium was supplemented with purified FH or C3. Explants cryosections were stained with DAPI for nuclei and retinal architecture parameters were quantified: **A, D** retinal thickness, **B-E** ONL thickness and **C,F** number of cells in ONL. Abbreviations: GCL – Ganglion cell layer, INL – Inner nuclear layer, ONL – Outer nuclear layer, RPE – Retinal pigmented epithelium cells, siNeg – silencing negative control, siCFH – silencing CFH. The controls were identical for experiments in A,B,C and D,E,F. Mean  $\pm$  SEM is shown. N=3 biological replicates. \* $p \leq 0.05$ ; \*\* $p \leq 0.005$ .



**Figure S5:** FH loss in RPE cells does not affect inflammatory cells on the co-cultured porcine retinae. Retinal explants from the visual streak of porcine donor eyes are exposed to either siNeg (A) or siCFH (B) hTERT-RPE1 cells for 72 hours. **A-B** Explants cryosections were stained for microglia marker Iba1 (green) and counterstaining was performed with DAPI for nuclei. **C** Quantification of Iba1 positive cells extrapolated from A-B. Abbreviations: GCL – Ganglion cell layer, INL – Inner nuclear layer, ONL – Outer nuclear layer, RPE – Retinal pigmented epithelium cells, siNeg – silencing negative control, siCFH – silencing CFH. Mean  $\pm$  SEM is shown. N= 6 biological replicates.



**Figure S6:** siCFH RPEs do not have an impact on the lipid expression and composition in the inner retina. **A–C** TCA identified a second lipid component localized in the inner retina (A) with a spectral signature assigned to phospholipids (B). Quantitative analysis by mean GVI/cell (C) did not show differences among both groups. **D** PCA of extracted lipid spectra expressed no significant difference in the lipid composition of the siNeg and siCFH group for any of the analyzed PCs. Data represent mean  $\pm$  SD,  $n=3$ , scale bar – 50  $\mu\text{m}$

Raman Shift [cm <sup>-1</sup> ]	Molecular assignment	Literature
720	C-N (lipids)	34
750	pyrrole ring breathing	53
792	DNA	56, 57
1098	Phosphate backbone (DNA)	56,57
1130	C-C stretching	53
1270	C-H deformation	34
1450	CH <sub>2</sub> scissoring / CH <sub>3</sub> bending (lipids)	34
1580	NH <sub>2</sub> (DNA bases)	55
1589	C=C, C=N	53
1655	C=O stretching (lipids)	34
2840	CH <sub>2</sub> (lipids)	34
2850	CH <sub>2</sub> (lipids)	34
2940	CH <sub>3</sub> (lipids)	34

**Table S1:** Overview of Raman peaks and their molecular assignments.