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Supplementary Material: CFH loss in human RPE cells leads to inflammation and complement system dysregulation *via* the NF-κB pathway

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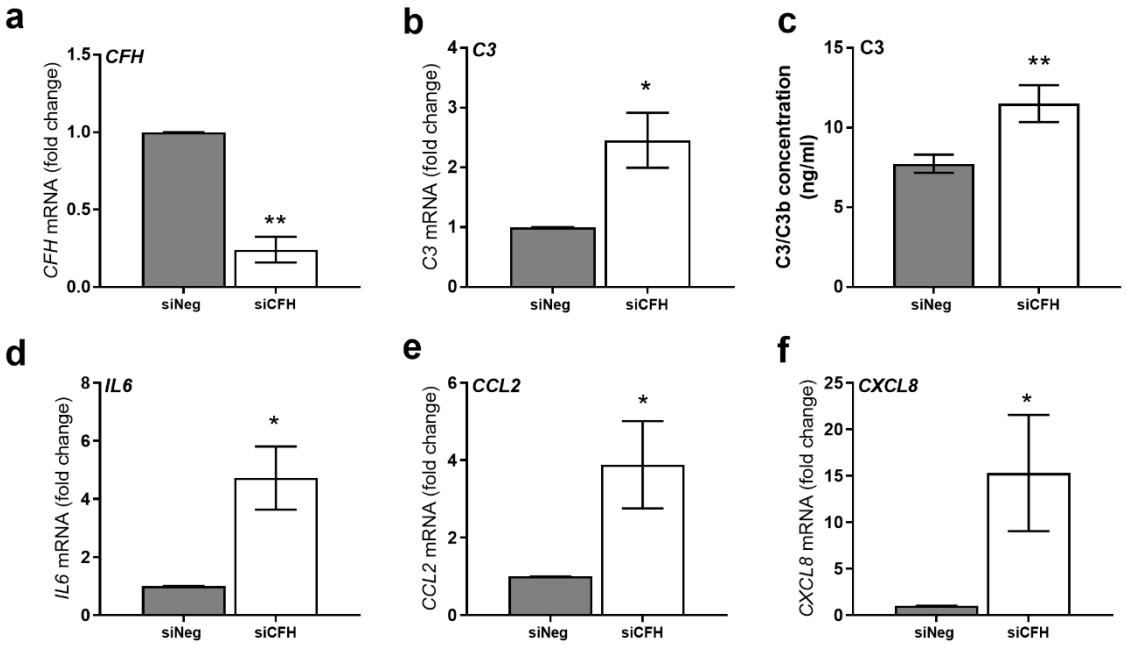

Figure S1. FH reduction leads to increased expression levels of C3 and inflammatory cytokines. a Evaluation of *CFH* expression by qRT-PCR analyses. **b** Monitoring of complement component 3 (*C3*) gene expression by qRT-PCR analyses in ARPE19 cells. **c** C3/C3b ELISA analyses of cell culture supernatants of ARPE19 cells. SEM is shown, n=4. **d-f** monitoring of gene expression of **d**

interleukin-6 (*IL6*), **e** C-C Motif Chemokine Ligand 2 (*CCL2*) and **f** interleukin-8 (*CXCL8*). qRT-PCR data are normalized to house-keeping gene *PRPL0* using Δ ΔCt method. SEM is shown, n=4. Significance was assessed by Student's t-test. *p<0.05, **p<0.01.

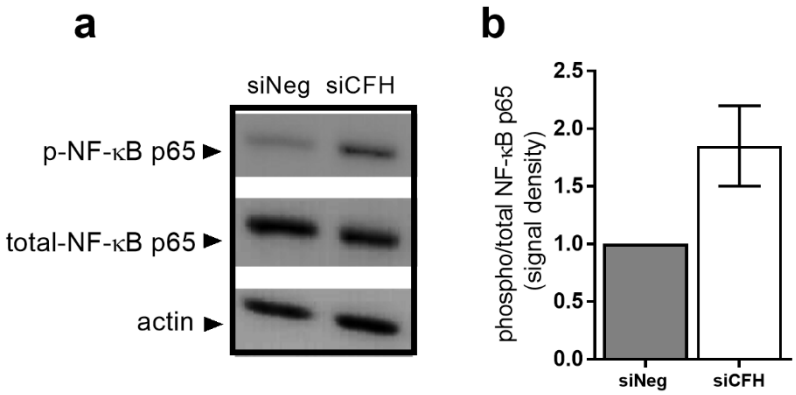

Figure S2. RPE cells deprived of FH show NF-κB activation. **a** Western blot analyses of phosphorylated and total levels of NF-κB p65 subunit in cell lysates of ARPE19 cells. Total actin was used as loading control**. b** Quantification of signal density of at least 3 independent experiments as reported in **a.** Bars indicate the signal density ratio between levels of phosphorylated and total NF-κB p65 subunit. Western Blot images were cropped, and full-length blots are provided in Supplementary Material.

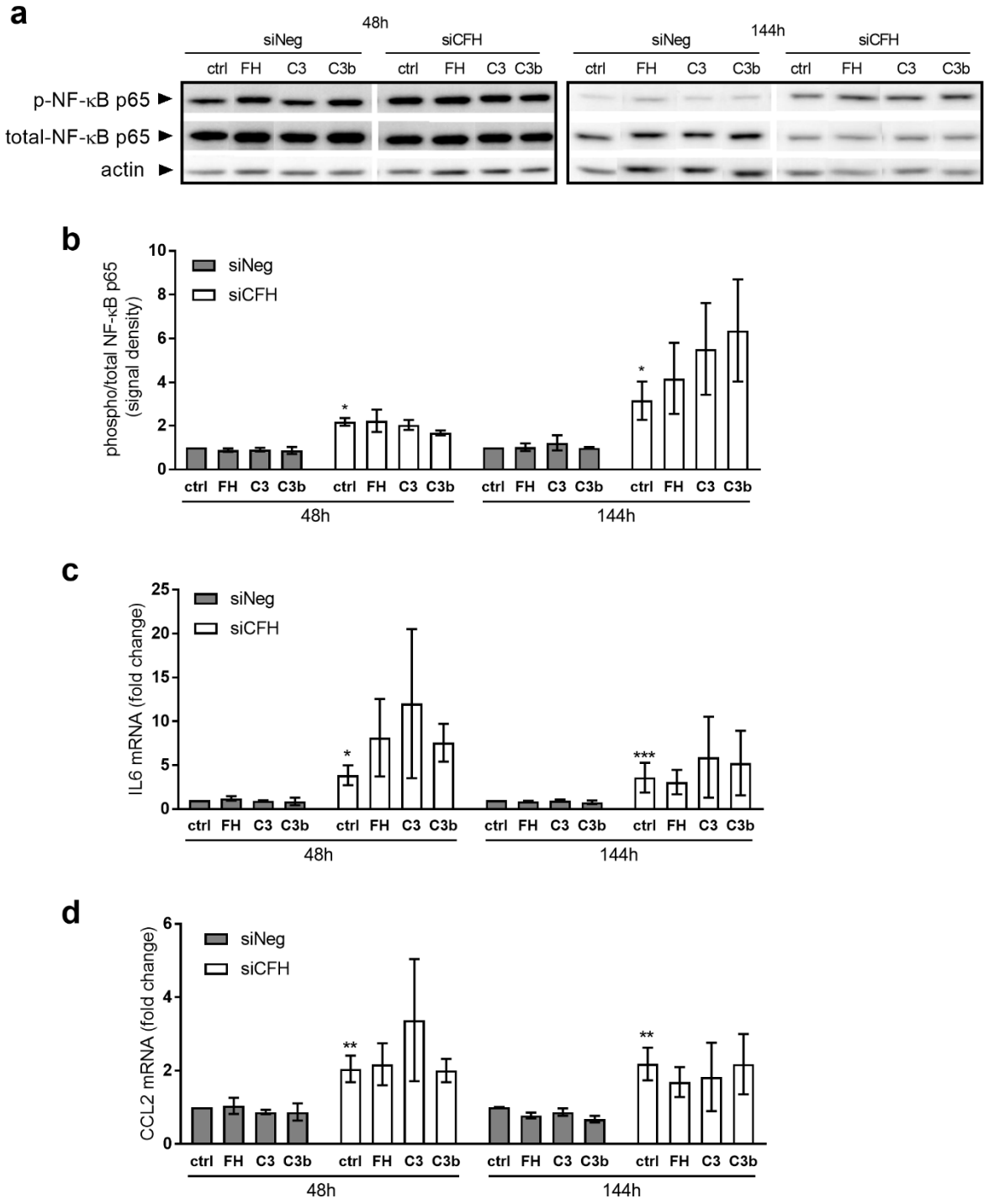

Figure S3. Impact of exogenous FH, C3 and C3b in hTERT-RPE1 cells. a Western blot analyses of phosphorylated and total levels of NF-κB p65 subunit in in cell lysates of hTERT-RPE1. Total actin was used as loading control **b** Quantification of signal density of at least 4 independent experiments in the conditions reported in a. Bars indicate the signal density ratio between phosphorylated and total levels of NF-κB p65 subunit. **c-d** gene expression analyses of hTERT-RPE1 cells in the conditions reported in a: *IL6* in panel c, *CCL2* in panel d. SEM is shown, n=4-8. Western Blot images were cropped, and full-length blots are provided in Supplementary Material. Significance was assessed by Student's t-test. *p<0.05, **p<0.01, *** p<0.001.

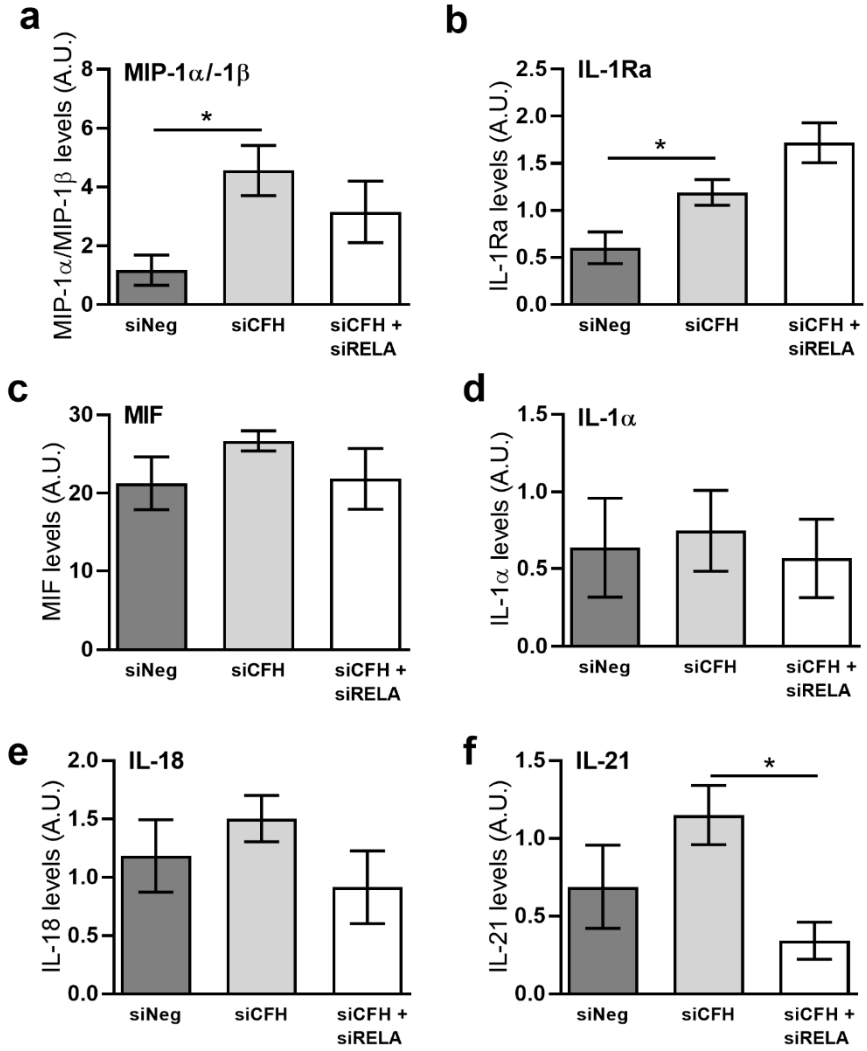

Figure S4. Cytokines profile in hTERT-RPE1 cells.. **a-f** Quantification of signal density in the conditions reported in Fig. 3a: **a** C-C motif chemokine ligand 3/4, MIP-1α/-1β; **b** interleukin 1 receptor antagonist, IL-1Ra. **c** macrophage migration inhibitory factor, MIF; **d** interleukin-1 alpha, IL-1a; **e** interleukin-18, IL-18; **f** interleukin-21, IL-21. SEM is shown. n=3. Significance was assessed by Student's t-test. *$p<0.05$.

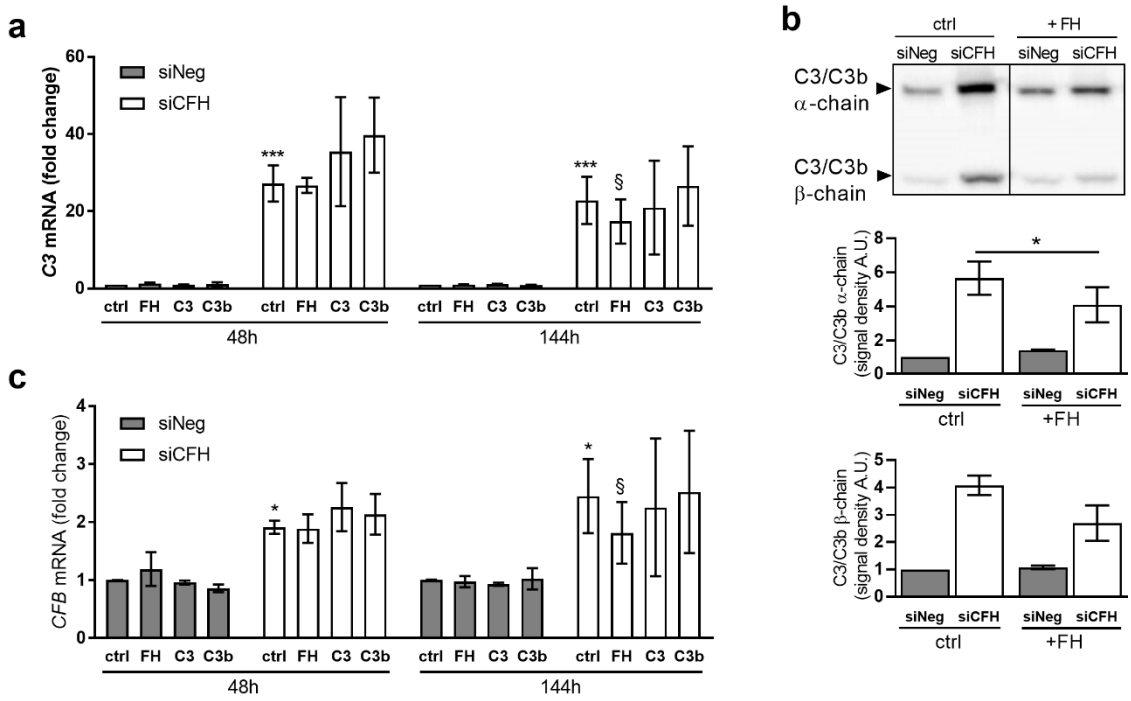

Figure S5. Impact of exogenous FH, C3 and C3b in hTERT-RPE1 cells. a *C3* gene expression analyses of hTERT-RPE1 cells **b** Western blot analyses and quantification of phosphorylated and total levels of NF-κB p65 subunit in in cell lysates of hTERT-RPE1. Total actin was used as loading control. Western Blot images were cropped, and full-length blots are provided in Supplementary Material **d**. **c** *CFB* gene expression analyses of hTERT-RPE1 cells.

SEM is shown, n=4-8. Significance was assessed by Student's t-test. Comparison between siNeg and siCFH: *p<0.05, **p<0.01, *** p<0.001. Comparison between siCFH ctrl and siCFH FH: § p <0.05.