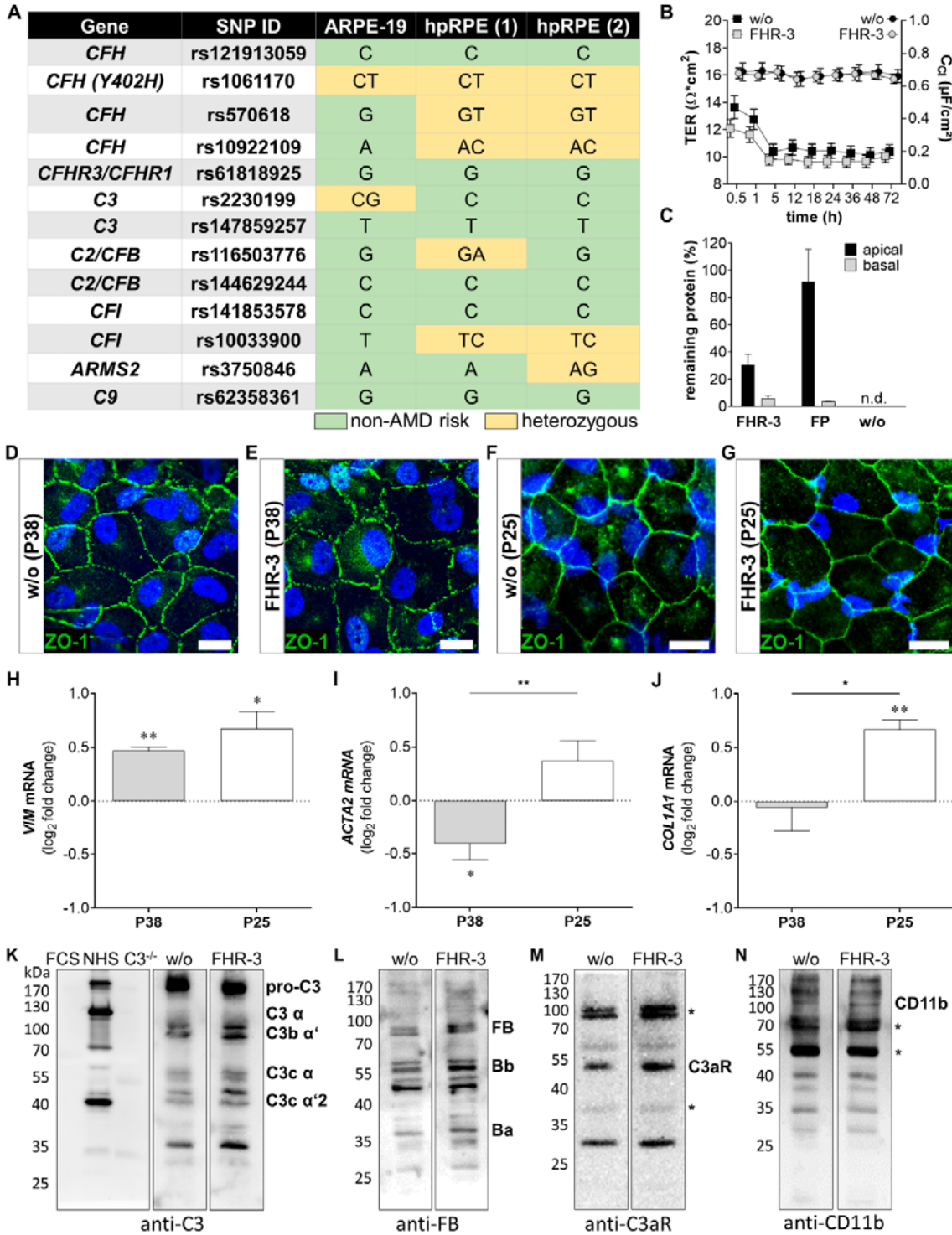
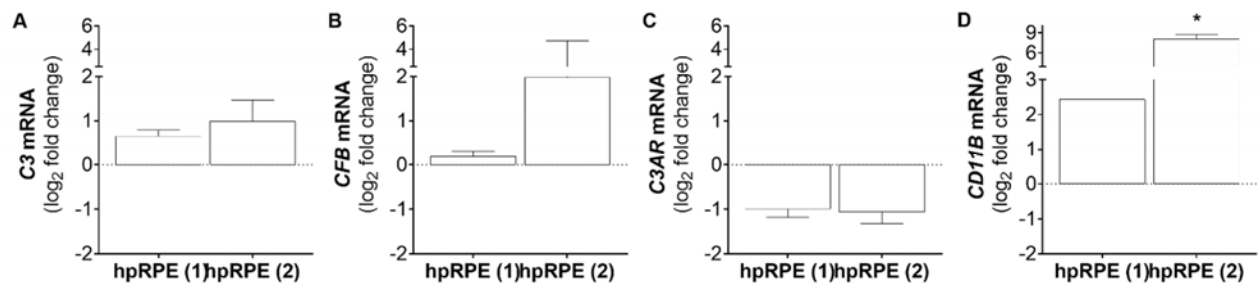


Supplementary Material



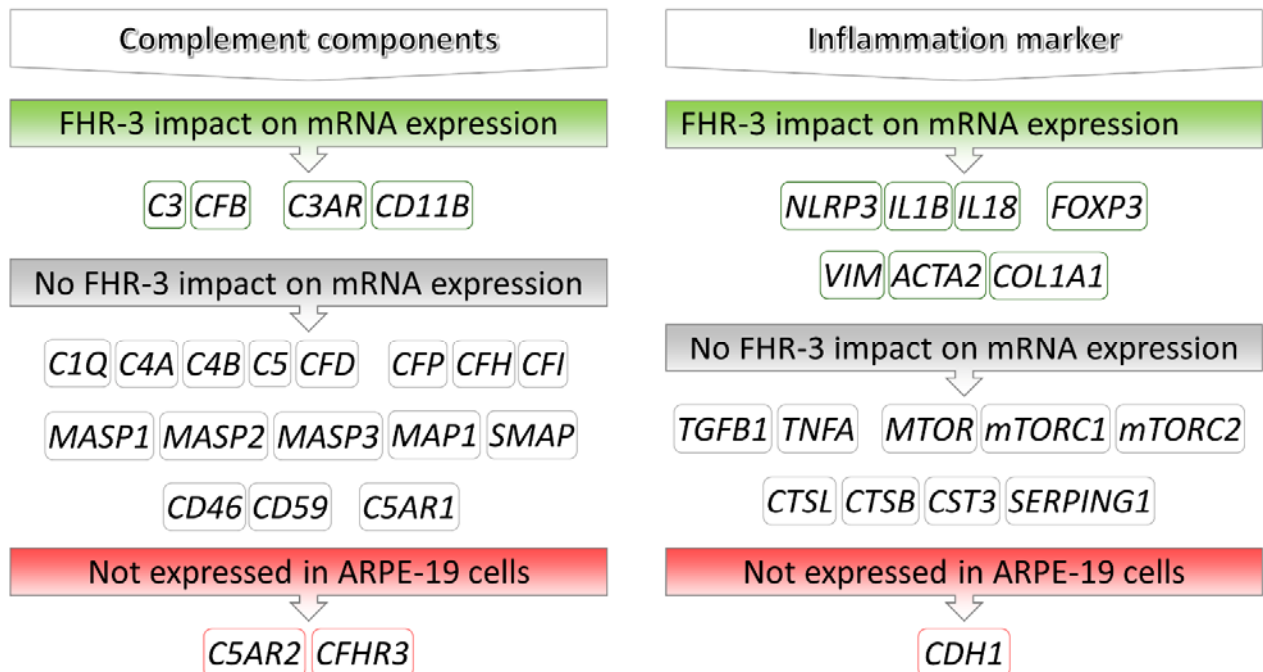
Supplementary Figure 1: Characterization of investigated ARPE-19 and hpRPE cells.

(A) Genotyping of all used cells (ARPE-19 cells, human primary RPE cells: hpRPE (1), hpRPE (2)) was performed for the most common AMD-associated complement SNPs. All typings resulted either in non- (green) or heterozygous-AMD risk SNPs (yellow). (B, square) Transepithelial resistance (TER) and (B, circle) cellular capacitance (C_{Cl}) were measured for w/o and FHR-3 treated cells for 0.5 h – 72 h. FHR-3 had no effect on (B, square) TER and (B, circle) C_{Cl} . (C) ARPE-19 cells were incubated for 24 h with either FHR-3 or Properdin (FP) as control protein. Only 30% of the incubated FHR-3 could be detected in the apical supernatant, whereas 92% of apically incubated properdin was still detectable. (D – G) Polarized ARPE-19 cells, either (D, E) high-passage (P38) or (F, G) low-passage (P25), showed an intact monolayer when tight junction protein zonula occludens-1 (ZO-1) was immunohistochemically detected (green), in both (D, F) w/o and (E, G) FHR-3 treated cells. (H – J) FHR-3 increased EMT marker mRNA expression (H) *VIM* in high-passage ARPE-19 cells (P38), and (H) *VIM*, (I) *ACTA2*, (J) *COL1A1* in low-passage ARPE-19 cells (P25) 24 h after incubation compared to w/o. (K – N) Western Blots of ARPE-19 cell lysates detected (K) pro-C3 (190 kDa), C3b (101 kDa), and C3c fragments (61 kDa, 39 kDa). No detection was observed in FCS used for cell cultivation and in C3^{-/-} Serum, (L) FB (95 kDa), and cleavage products Bb (60 kDa) and Ba (35 kDa), (M) C3aR (54 kDa) and C3aR glycosylations (stars, 40 - 100 kDa), (N) subunit CD11b (120 kDa) of complement receptor 3 (CR3) and cleavage products (stars). All proteins could be observed in w/o and FHR-3 treated cells, under serum-free conditions (exemplarily shown 5 h post-treatment). FCS fetal calf serum. NHS normal human serum. C3^{-/-} C3-depleted human serum. Scale bars 40 μ m. w/o untreated control (dotted line). Mean with standard deviation is shown. **p < 0.01, *p < 0.05. (H – J) unpaired t-test (n = 3); (C) interpolated against the respective standard curve.



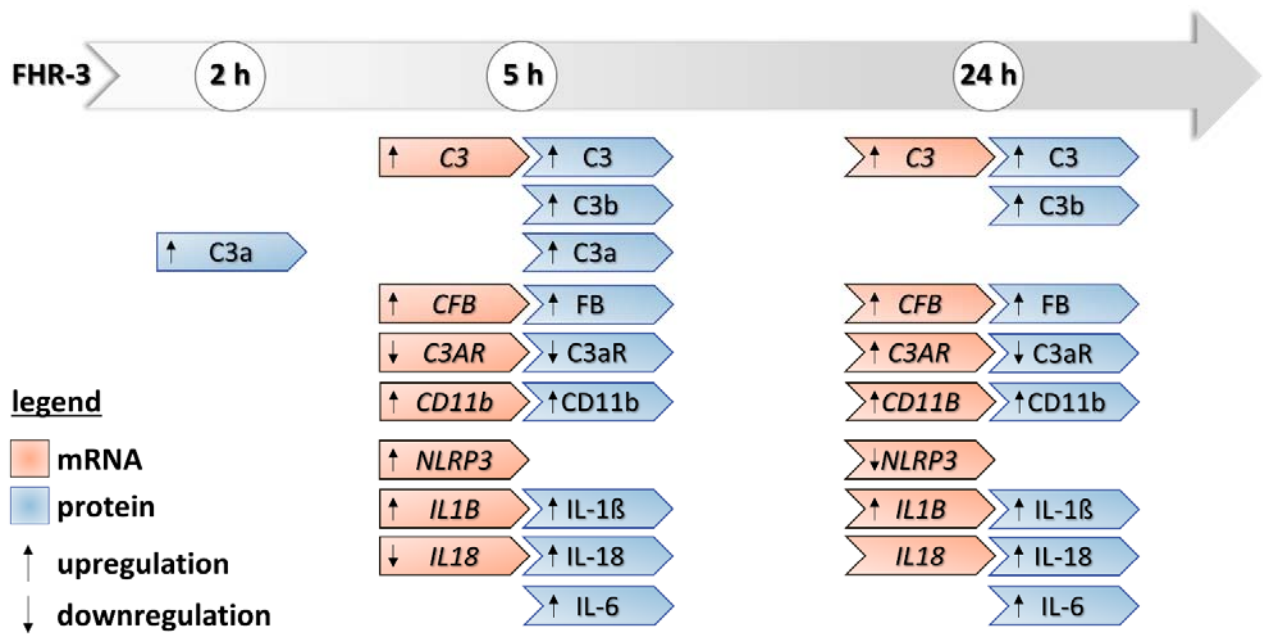
Supplementary Figure 2: FHR-3 increases mRNA expression of *C3*, *CFB*, *C3AR* and *CD11B* in hRPE cells.

(A) *C3*, (B) *CFB* and (D) *CD11B* mRNA expression was increased in two different human primary RPE cells (hpRPE (1), hpRPE (2)) after FHR-3 incubation (5 h) compared to the untreated controls (dotted line). (C) Expression of *C3AR*, in contrast, was decreased. * $p < 0.05$. Wilcoxon matched-pairs signed rank test ($n = 3$).

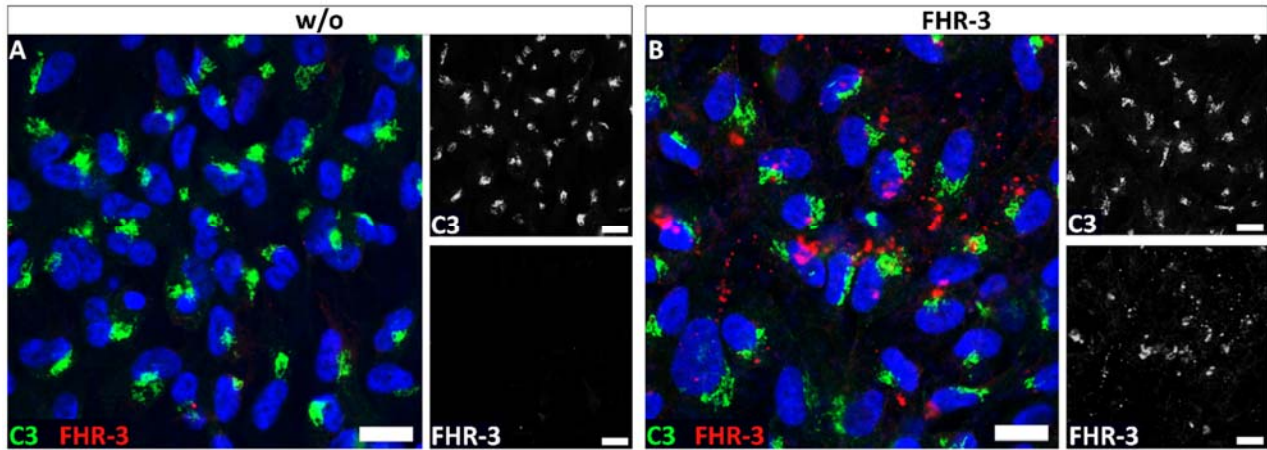


Supplementary Figure 3: Overview of investigated mRNA expression in ARPE-19 cells and impact of FHR-3 on their transcript levels.

Shown in green are all transcripts whose expression has been altered due to FHR-3 treatment. In grey are all genes whose mRNA expression has not been influenced by FHR-3 and in red are all genes that are not expressed in ARPE-19 cells at the mRNA level in this study.



Supplementary Figure 4: Timeline of FHR-3-treated ARPE-19 cells with RNA and protein regulation of complement components and inflammation markers.



Supplementary Figure 5: FHR-3 did not co-localize with C3 in ARPE-19 cells.

Intracellular C3 (green) was detected by immunofluorescence in (A) untreated and (B) FHR-3 treated (24 h) ARPE-19 cells. FHR-3 was solely detected in (B) FHR-3 treated ARPE-19 cells by using anti-FHR-3 antibody RETC-2. Co-localization of C3 and FHR-3 could not be shown in ARPE-19 cells by immunofluorescence. Scale bars 40 μm . w/o untreated control.

Supplementary Table 1: PCR and RT-qPCR primers

transcript	name or sequence	Catalogue number	Usage/Company
<i>C1QB</i>	Hs_C1QB_1_SG	QT00003493	RT-qPCR/ Qiagen
<i>C3</i>	Hs_C3_1_SG	QT00089698	
<i>C3AR</i>	Hs_C3AR1_1_SG	QT00090398	
<i>C4A</i>	Hs_C4A_1_SG	QT00237160	
<i>C5</i>	Hs_C5AR2_1_SG	QT00243971	
<i>C5AR1</i>	Hs_C5R1_1_SG	QT00997766	
<i>C5AR2</i>	Hs_C5AR2_1_SG	QT00243971	
<i>CD11B</i>	Hs_ITGAM_1_SG	QT00031500	
<i>CD46</i>	Hs_MCP_1_SG	QT00073689	
<i>CD59</i>	Hs_CD59_1_SG	QT00035952	
<i>CFB</i>	Hs_BF_1_SG	QT00012138	
<i>CFD</i>	Hs_CFD_1_SG	QT00212191	
<i>CFH</i>	Hs_CFH_1_SG	QT00001624	
<i>CFHR3</i>	Hs_CFHR3_1_SG	QT00001631	
<i>CFI</i>	Hs_CFI_1_SG	QT00213794	
<i>CFP</i>	Hs_CFP_1_SG	QT00010514	
<i>CST3</i>	Hs_CST3_1_SG	QT00046438	
<i>CTSB</i>	Hs_CTSB_1_SG	QT00088641	
<i>CTSL</i>	Hs_CTSL_1_SG	QT01664978	
<i>FOXP3</i>	Hs_FOXP3_1_SG	QT00048286	
<i>GAPDH</i>	Hs_GAPDH_1_SG	QT00079247	
<i>MBL2</i>	Hs_MBL2_1_SG	QT01004227	
<i>NLRP3</i>	Hs_NLRP3_1_SG	QT00029771	
<i>SERPING1</i>	Hs_SERPING1_1_SG	QT00053963	
<i>ACTA2</i>	fw: GCCTTGGTGTGTGACAATGG rv: AAAACAGCCCTGGGAGCAT		RT-qPCR/ in-house, Metabion
<i>CDH1</i>	fw: ACAGGCTGGCTGAAAGTGAC rv: CACGGCATGAGAATAGAGGAT		
<i>COL1A1</i>	fw: GGTGGTTTCTTGGTCGGT rv: ATGACGTGATCTGTGACGAG		
<i>IL18</i>	fw: ACTGTAGAGATAATGCACCCCG rv: AGTTACAGCCATACCTCTAGGC		
<i>IL1B</i>	fw: CTCGCCAGTGAAATGATGGCT rv: GTCGGAGATTCGTAGCTGGAT		
<i>MAP1</i>	fw: TGAAGGATGGGACGTGGAGT rv: TGCTCTGACTTGAGTTCGCT		
<i>MASP1</i>	fw: TGCAAAGGTTCCCAGAGACC rv: GCATAAGCCTTCTGGCAGGT		
<i>MASP2</i>	fw: GCGGTTTCTACTGCTCCTGCC rv: CGTGGGTATTCAGGGCTGCTGAGCTC		
<i>MASP3</i>	fw: TGCCCAAGGAGTCTGGATGA rv: CCCCCAATGATCCTCTTGACC		
<i>MTOR</i>	fw: TGCCAACTATCTTCGGAACC rv: GCTCGCTTCACCTCAAATTC		
<i>RAPTOR</i>	fw: ACTGATGGAGTCCGAAATGC		

	rv: TCATCCGATCCTTCATCCTC	
<i>RICTOR</i>	fw: GGAAGCCTGTTGATGGTGAT rv: GGCAGCCTGTTTTATGGTGT	
<i>SMAP</i>	fw: TACGTCCTGCACCGTAACAAG rv: CAGCCATCTCAGCCCAACC	
<i>TGFB1</i>	fw: CATAGCTGACTTCAAGATGTGGT rv: CCTAGTGAGACTTTGAACCGT	
<i>TNFA</i>	fw: GCTGCACTTTGGAGTGATCG rv: GGGTTTGCTACAACATGGGC	
<i>VIM</i>	fw: TGTCCAAATCGATGTGGATGTTTC rv: TTGTACCATTCTTCTGCCTCCTG	
<i>ARMS_375</i> (SNP ID: rs3750846)	fw: GCATTTCTAATATCAAATCAAACATTC AT rv: CCTGGGGCTCTGTTTGAATTG	
<i>C2_116</i> (SNP ID: rs116503776)	fw: AGCACCATCTACACTTCGCC rv: CATGGCCCTTACCTCGACAT	
<i>C2_144</i> (SNP ID: rs144629244)	fw: CAGGGGGAGATCAGAATCGTC rv: GTGGGGGCAGGGAAATTCTTT	
<i>C3_147</i> (SNP ID: rs147859257)	fw: TTGCCTCTCCTAAGCCTGTG rv: GGCTAGGGTCTCACGAGG	
<i>C3_223</i> (SNP ID: rs2230199)	fw: CTGGATGAAGAGGTACCCGC rv: CTTGGAACAGACCCCTGACA	
<i>C9_623</i> (SNP ID: rs62358361)	fw: TCTGTGACAATGGCAGACAAC rv: TTTATTCCCCACTCTCTGTTATCTT	
<i>CFH_106</i> (SNP ID: rs1061170)	fw: CCTTTGTTAGTAACTTTAGTTCGTCTT rv: GGAGTAGGAGACCAGCCATTC	PCR for genetic analysis/ in-house, Metabion
<i>CFH_109</i> (SNP ID: rs10922109)	fw: AGTCTACCTTGCTAACGGTTC rv: CCTTCTAGATTGTAGTACCTGTTGCT	
<i>CFH_121</i> (SNP ID: rs121913059)	fw: CAGATCCGTGTGTAATATCCCGA rv: TAAGAAGAGAGCCACCGGTCTCA	
<i>CFH_570</i> (SNP ID: rs570618)	fw: AGTGGTGAGGAAAAATGTAGAATGT rv: CATCTCCATCTTTCTTAAATTCTGTGA	
<i>CFH_618</i> (SNP ID: rs61818925)	fw: GTGCATTACAGGGGCATATTC rv: CCTGAAGACACAAATCTCACTGG	
<i>CFHRI</i>	fw: GGATCCATGTGGCTCCTGGTCAGTG rv: CTCGAGTCTTTTTGCACAAGTTGGATAC TC	
<i>CFI_100</i> (SNP ID: rs10033900)	fw: CCTGCAGTGTGGTCTGTGAG rv: CCAGTGCTACAAGGTGGGAA	
<i>CFI_141</i> (SNP ID: rs141853578)	fw: ATCCACTGATACAAGCGCTCA rv: TTAAAATCGTCATGATGTTCAAAGC	