Supplemental information

CFHR5-Hybrid Proteins Anchor Properdin and Activate Complement at Self-Surfaces

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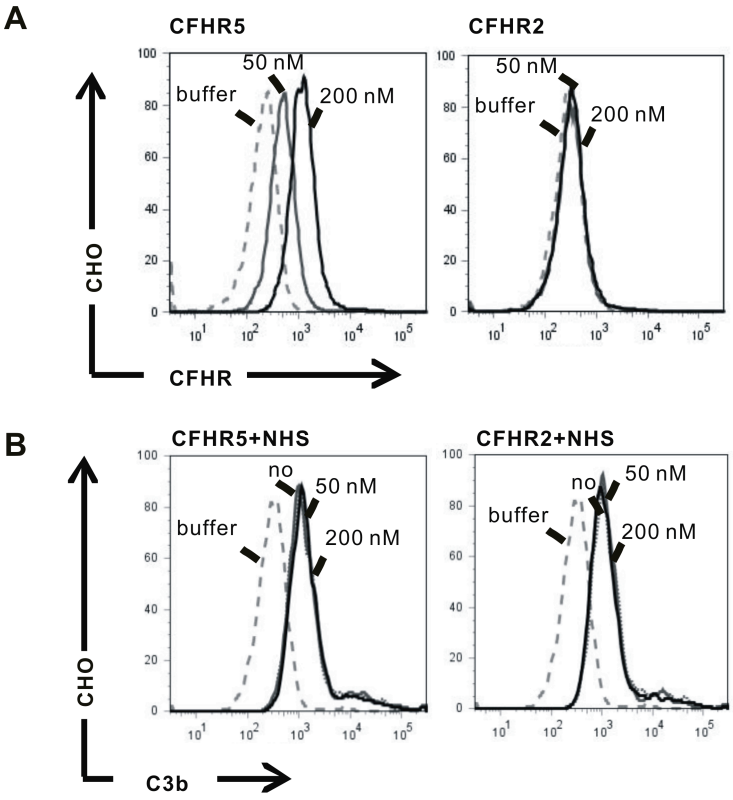
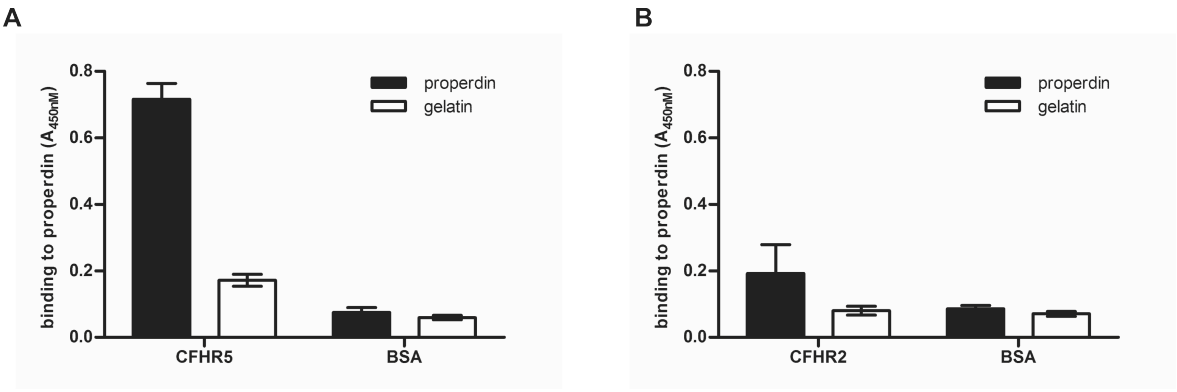
Running title: CFHR5 an anchor for properdin

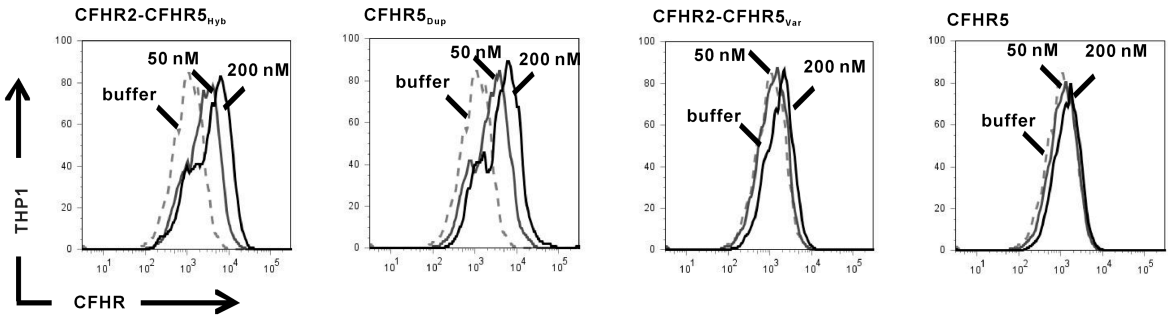
Supplemental Figures



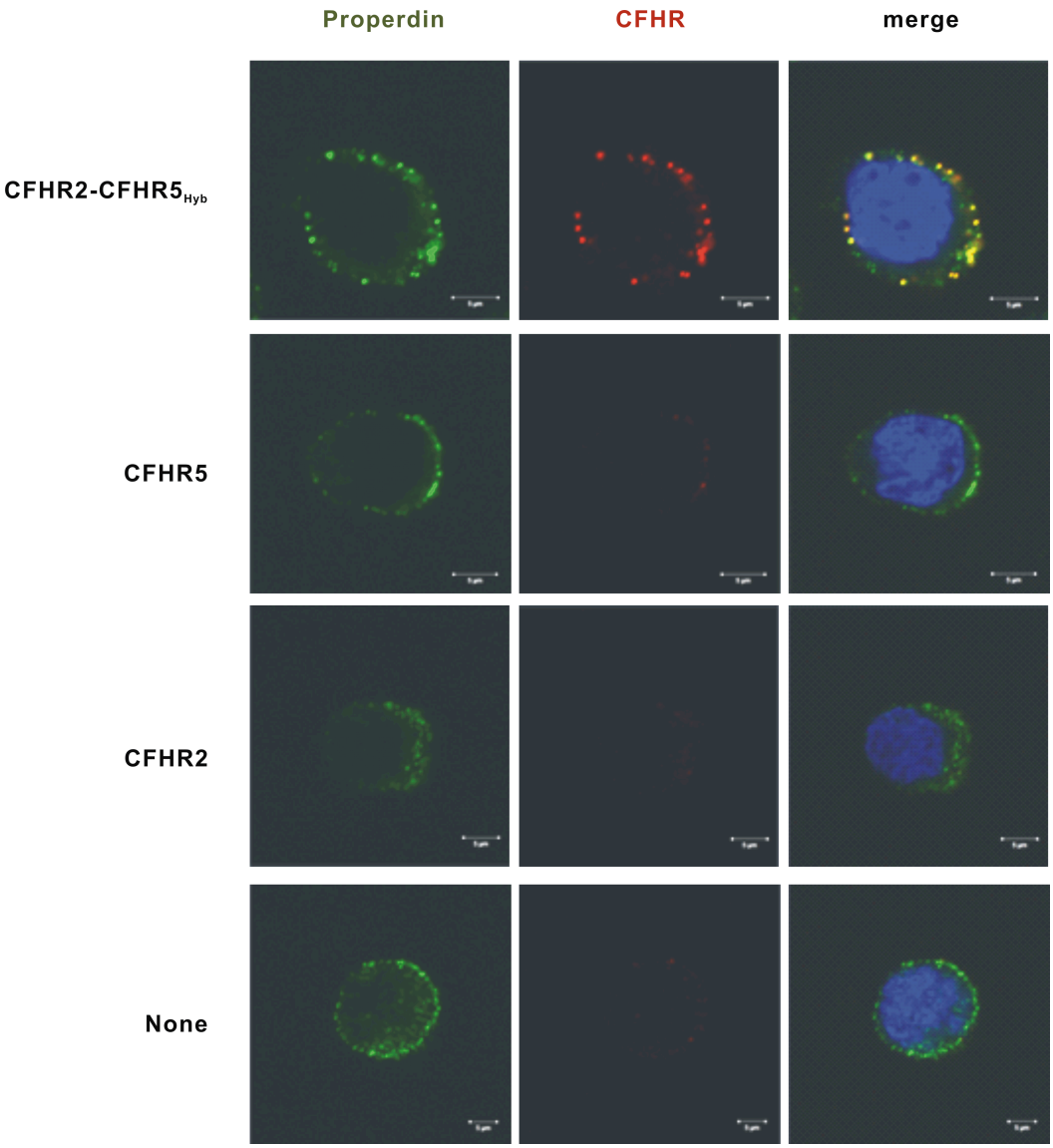
Supplemental Figure 1. Recombinant CFHR5 binds to CHO surface and enhances C3b deposition. (**A**) CHO cells were incubated with recombinant CFHR5 or CFHR2. Bound CFHR5 (left) or CFHR2 (right) was detected with either CFHR5 mAB or CFHR2 mAB by flow cytometry. Antibody binding (buffer) in absence of CFHR5 or CFHR2 was used as the negative control (dashed lines). (**B**) Intact CHO cells loaded with CFHR5 or CFHR2 were challenged with NHS and following incubation C3b deposition was detected by flow cytometry. C3b deposition of untreated CHO cells is shown in the dotted histogram. Surface-attached CFHR5 (left) and CFHR2 (right) did not affect C3b deposition on the CHO surface. Antibody binding signal on non NHS-treated CHO cells was shown with a dashed line (buffer). Data showed representative results from three independent experiments.



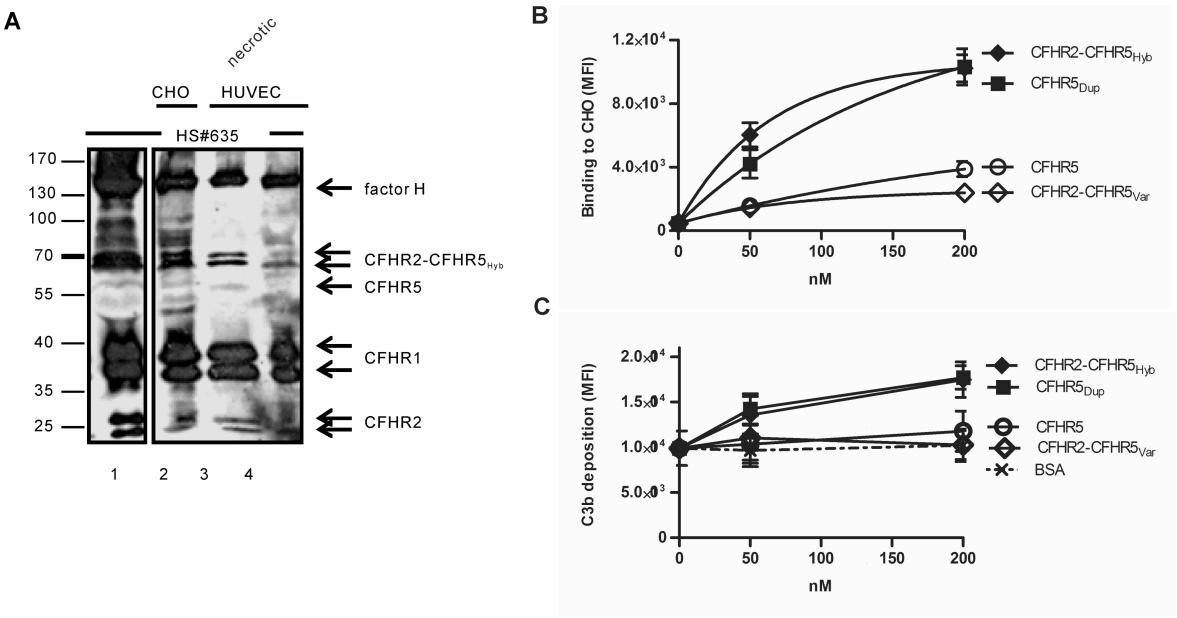
Supplemental Figure 2. CFHR5 and CFHR2 bind to immobilized properdin. Binding of recombinant CFHR5 or CFHR2 to properdin immobilized onto MaxiSorp microtiter plates was assayed by ELISA (black columns). CFHR5 (column 1, **panel A**) bound to properdin. CFHR2 binding to Properdin was of relative weak intensity (column 1, **panel B**). For BSA in both cases no binding was detectable. Binding to gelatin immobilized wells is shown as background (open columns). Results represented mean ± SEM of three independent experiments.



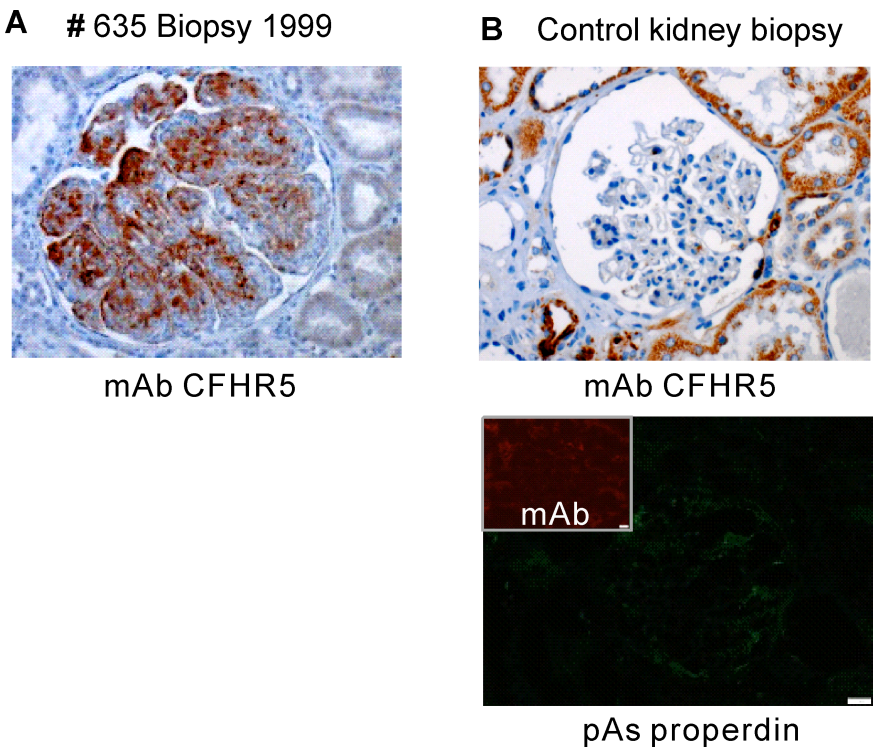
Supplemental Figure 3. Disease associated CHFR5 mutants bind to human THP1 cells. Premonocytic THP1 cells were incubated with recombinant disease proteins (CFHR2-CFHR5$_{Hyb}$ and CFHR5$_{Dup}$), CFHR2-CFHR5$_{Var}$, or CFHR5 (each at 50 and 200 nM), respectively. Bound proteins were detected with CFHR5 mAB by flow cytometry. The antibody binding in absence of proteins was used as the negative control (dashed lines, buffer). Both disease proteins bound stronger than either CFHR2-CFHR5$_{Var}$ or CFHR5. The results are representative of three independent experiments.



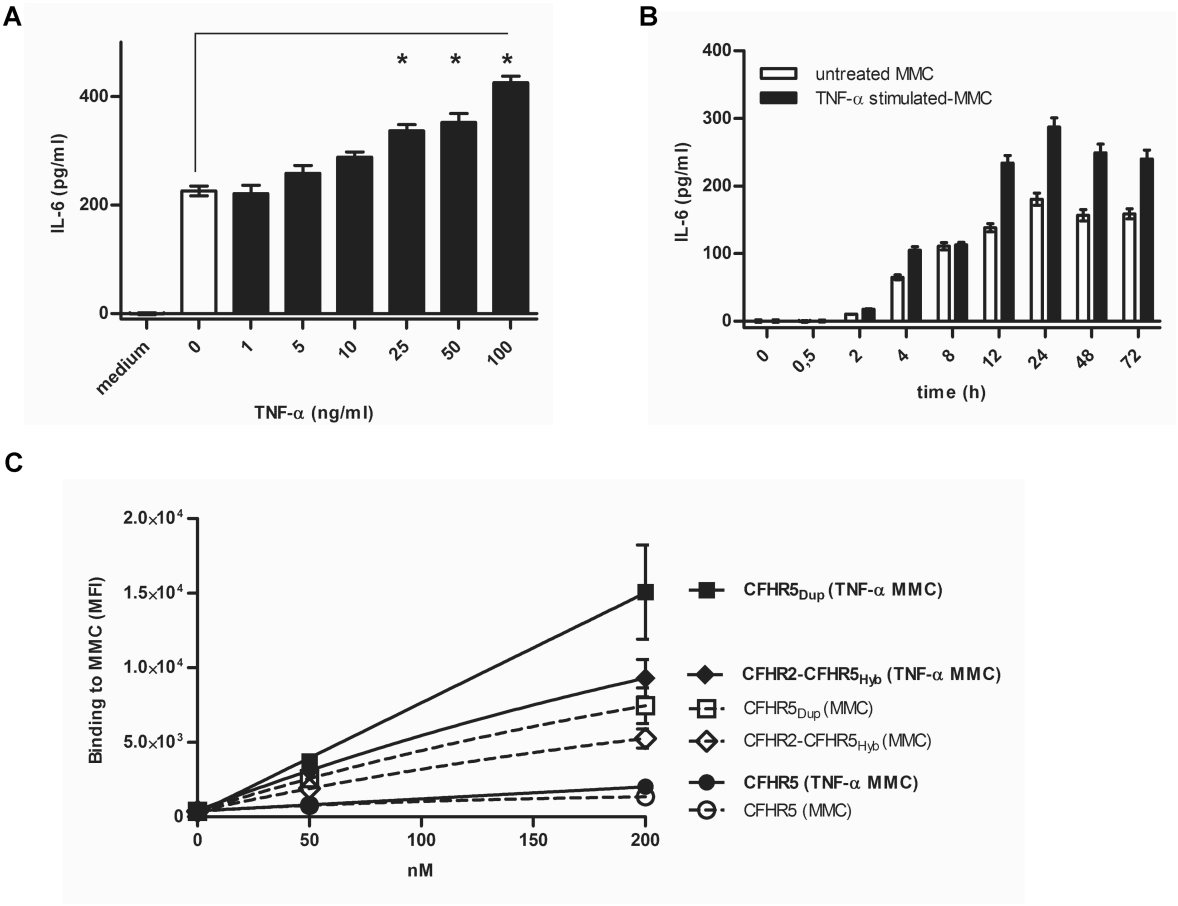
Supplemental Figure 4. CFHR2-CFHR5$_{Hyb}$ colocalizes with properdin on the THP1 surface. When analyzed by confocal microscopy, properdin (green fluorescence) was evenly distributed on the surface of human THP1 cells (left panels). Bound CFHR2-CFHR5$_{Hyb}$, CFHR5, CFHR2 (200 nM each, red fluorescence) were identified with CFHR5 mAB or CFHR2 mAB in combination with Alexa 647 mouse antiserum. CFHR2-CFHR5$_{Hyb}$ and properdin colocalize on the surface of THP1 cell (upper row, left panel). In this setting, binding from CFHR5 was hardly visualized (second row, middle panel). CFHR2 did not bind to THP1 cells (third row, middle panel). Representative results of three independent experiments are shown.



Supplemental Figure 5. Disease associated CHFR5 mutants bind to cell surfaces and augment C3b deposition. (**A**) Intact CHO cells (lane 2), necrotic (lane 3) and living HUVEC cells (lane 4) were incubated with serum from patient #635 (HS#635). Following washing the cells were lysed and the lysate was separated by SDAS-PAGE. Bound CFHR2-CFHR5$_{Hyb}$, CFHR5, CFHR2, as well as CFHR1 and factor H were visualized by Western blotting (arrows). The blot is representative of three independent experiments. (**B**) Binding of recombinant CFHR2-CFHR5$_{Hyb}$, CFHR5$_{Dup}$, CFHR2-CFHR5$_{Var}$, CFHR5 to intact CHO cells was analyzed by flow cytometry. CFHR2-CFHR5$_{Hyb}$ (diamonds) and CFHR5$_{Dup}$ (squares) bound stronger to CHO cells than CFHR2-CFHR5$_{Var}$ (open diamonds) and CFHR5 (open circles). (**C**) C3b deposition upon challenged with NHS was measured on CFHR5 mutants/CFHR5-loaded CHO surfaces. CFHR2-CFHR5$_{Hyb}$ (diamonds) and CFHR5$_{Dup}$ (squares) used at 50 and 200 nM augmented C3b deposition in a dose dependent manner. CFHR2-CFHR5$_{Var}$ (open diamonds) and CFHR5 (open circles) used at the same molar levels and also BSA (dashed line) did not influence C3b deposition on the CHO surface. The results showed median fluorescence intensity (MFI) of three independent experiments.



Supplemental Figure 6. Histology of kidney biopsies of the German C3G patient #635 and of a non-neoplastic renal parenchyma from tumour-nephrectomies. (**A**) Deposition of CFHR2-CFHR5$_{Hyb}$, and CFHR5 was detected on the basement membrane and in mesangium by immunohistochemistry (400x) with mAb-CFHR5 in kidney biopsy of patients #635 taken in 1999. (**B**) No reactivity to CFHR5 mAb was detected in kidney biopsies prepared from a non-neoplastic renal parenchyma from tumour-nephrectomies (upper panel). Similarly, no reactivity to anti-properdin antibodies in the control kidney biopsy (bottom panel).



Supplemental Figure 7. Effect of TNF-α on murine mesangial cells (MMCells) and binding of CFHR5 disease mutants and CFHR5 to the cells. (**A**) Effect of TNF-α on MMCells expression of IL-6. After incubation of MMCells with TNF-α (0-100 ng/ml) for 24 h, the culture supernatants were collected and IL-6 levels were determined by ELISA. (**B**) Effect of TNF-α on MMCells time kinetics of IL-6 expression. MMCells were incubated in the presence (filled columns) or absence (open columns) of TNF-α (25 ng/ml) for varying time periods (0.5-72 h) and the culture supernatants were collected and IL-6 levels were quantitated by ELISA. Results represented mean ± SEM of three independent experiments. (**C**) Binding of recombinant CFHR2-CFHR5$_{Hyb}$, CFHR5$_{Dup}$ and CFHR5 to both untreated and TNF-α stimulated MMCells was analyzed by flow cytometry. CFHR2-CFHR5$_{Hyb}$ (diamonds) and CFHR5$_{Dup}$ (squares) bound stronger to both cells than CFHR5 (circles). All proteins bound stronger to TNF-α stimulated MMCells (filled symbols) than to untreated cells (white open symbols). The results shown represent mean fluorescence intensity (MFI) of three independent experiments.

Supplemental Table 1. Binding of CFHR5 and CFHR2 to CHO cells

	*MFI (nM)		
	50	200	-
CFHR5	486	1190	
CFHR5-mAB ctr			201
CFHR2	305	316	
CFHR2-mAB ctr			271

*MFI: median fluorescence intensity

Supplemental Table 2. Binding of CFHR5 and CFHR2 to necrotic HUVECells

	MFI (nM)		
	50	200	-
CFHR5	2878	6218	
CFHR5-mAB ctr			387
CFHR2	970	2783	
CFHR2-mAB ctr			529

Supplemental Table 3. CFHR5 and CFHR2 influence C3b deposition on CHO cells

	MFI (nM)		
	50	200	-
CFHR5	1178	1263	
CFHR2	1112	1081	
no protein			1186
C3b-mAB ctr			296

Supplemental Table 4. CFHR5 and CFHR2 influence C3b deposition on necrotic HUVECells

	MFI (nM)		
	50	**200**	**-**
CFHR5	**1792**	**3179**	
CFHR2	**1821**	**1895**	
no protein			**1819**
C3b-mAB ctr			**335**

Supplemental Table 5. Properdin is essential for CFHR5-mediated C3b deposition on necrotic HUVECells

	MFI			
	NHS	HSΔp	HSΔp+P	-
CFHR5	3291	1747	4077	
CFHR2	2348	1375	3362	
no protein	1970	1642	3041	
C3b-mAB ctr				342

Supplemental Table 6. Binding of CFHR5 mutants and variants to THP1 cells

	MFI (nM)		
	50	200	-
CFHR2-CFHR5$_{Hyb}$	2468	4111	
CFHR5$_{Dup}$	2556	4572	
CFHR2-CFHR5$_{Var}$	1122	1424	
CFHR5	1165	1735	
CFHR5-mAB ctr			161

Supplemental Table 7. Properdin is essential for CFHR2-CFHR5$_{Hyb}$-mediated C3b deposition on necrotic HUVECells

	MFI			
	NHS	HS$_{\Delta P}$	HS$_{\Delta P}$+P	-
CFHR2-CFHR5$_{Hyb}$	2583	584	3693	
CFHR5	1322	558	2017	
no protein	1387	608	1841	
C3b-mAB ctr				261

Supplemental Table 8. Primers used in the study

primer	sequence
CFHR5$_{12}$-F	TCCAATTTGTTCCTTCACCAGAACCTTGTGTGACTTTCCA
CFHR5$_{12}$-R	TGGAACATGACACTCTCCCTTGGTGAAGGAACAAATTGGA
CFHR2-5$_{del34}$-F	CTCCCAAATGCAGGTCCACTAAGGGAGAGTGTCATGTTCC
CFHR2-5$_{del34}$-R	GGAACATGACACTCTCCCTTAGTGGACCTGCATTTGGGAG
CFHR5$_{N}$-F	GCGGTACCACCATGTGGCTCCTGGTC
CFHR5$_{N}$-R	CCTCTAGAAAGGTGAAGGAACAAATTGGAGG