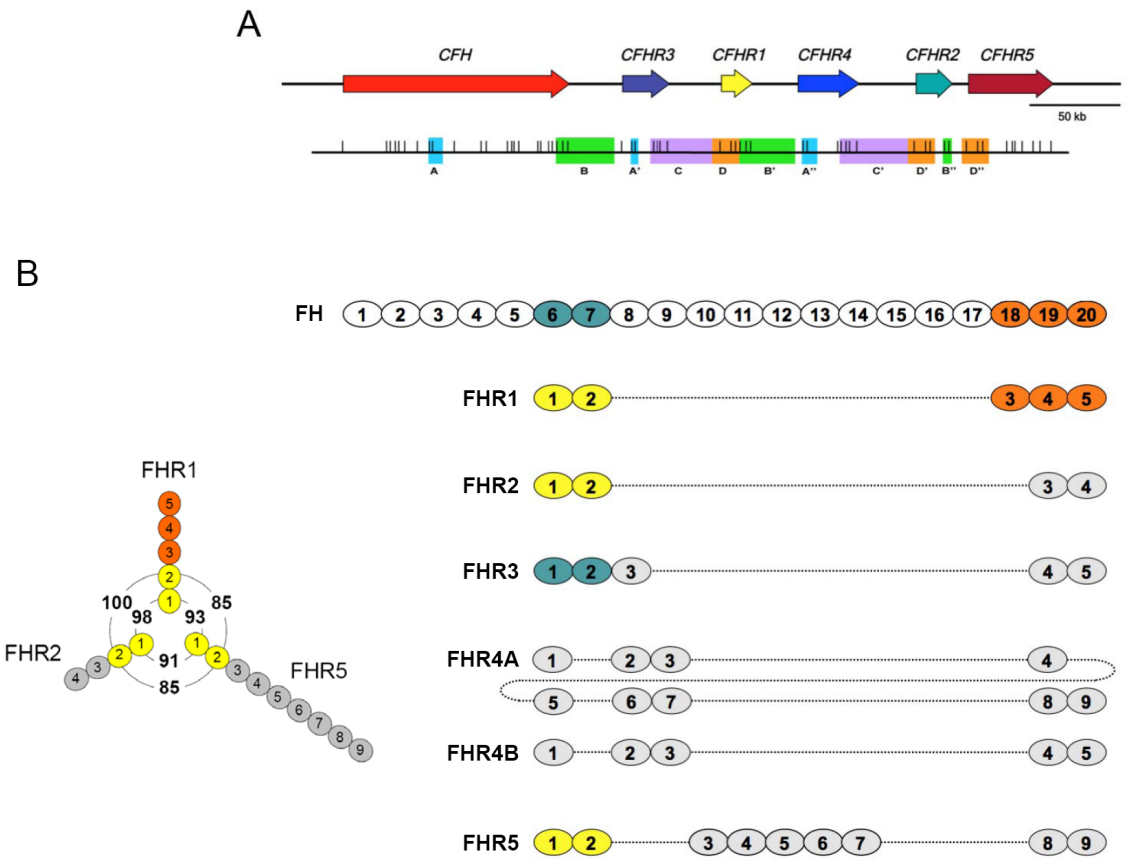
SUPPLEMENTARY FIGURES

Supplementary Figure 1

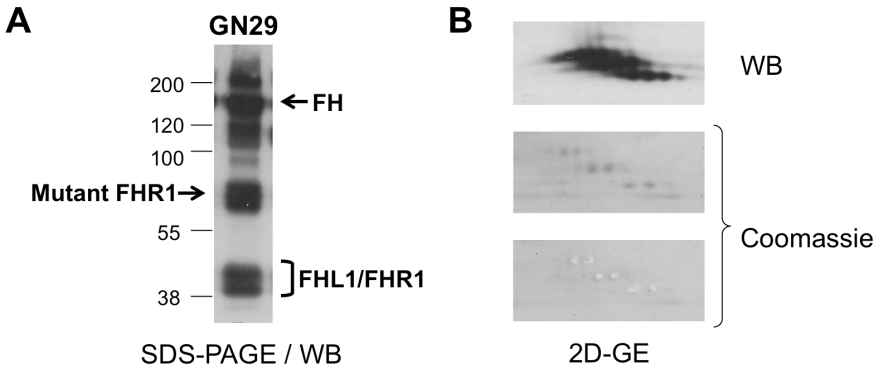


The *CFH-CFHRs* locus.

A) Genomic organization of the *CFH* and *CFHR1-5* genes. Arrows represent the genes with their names. The coloured boxes underneath indicate the sequence repeats. The vertical lines indicate the position of the exons of the *CFH* and *CFHRs* genes.

B) Structural organization of the FH and FHR proteins. Short consensus repeats (SCRs) are represented by ovals and are numbered from the N-terminal end. Homologous SCRs are aligned. Colours illustrate SCRs presenting almost complete identity of amino acid sequences. Percentages of amino acid similarities for SCRs 1 and 2 between FHR1, FHR2 and FHR5 are provided.

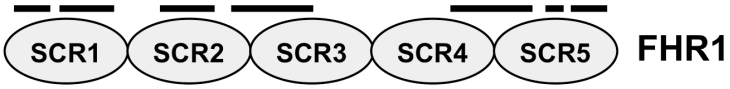
Supplementary Figure 2



C

sp|Q03591| FHR1_HUMAN

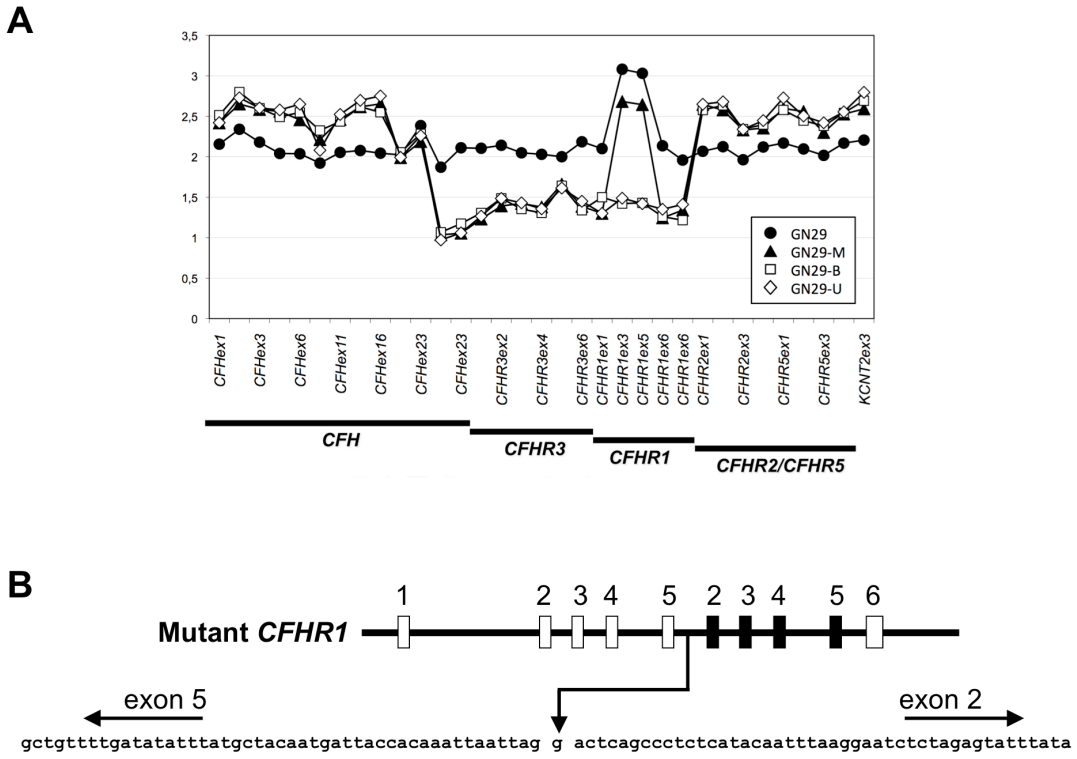
MWLLVSVILISRISSVGGEATFCDFPK**INHGILYDEEK**YKPFSQVPTGEVFYYSCEYNFVSPSKSFWTR**ITCT EEGWSPTPK**CLRLCFFPFVENGHSESSGQTHLEGDTVQIICNTGYR**LQNNENNISCVER**GWSTPPKCR**ST DTSCVNPPTVQNAHILSRQMSKYPSGERVRYECR**SPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPP PPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEGNKRITCR**NGQWSEPPKCLHPCVISREIMENYNIALR**WT AKQKLYLR**TGESAEFVCKR**GYRLSSRSHTLR**TTCWDGKLEYPTCAKR**



Proteomic analysis of the FHR anomalous band identified in the proband and his mother.

Anomalous FHR bands were partially purified by heparin chromatography, and their presence confirmed by SDS-PAGE followed by western blot (A), and further characterized by 2D gel electrophoresis . Three bands could be observed, each composed of several isoforms differing in isoelectric point (B). Several spots were picked up from the Coomassie-stained gel for Mass Spectrometric analysis (Proteomic Unit, Parque Cientifico de Madrid, Universidad Complutense de Madrid). The spots were trypsin digested, and the peptides generated were identified by MALDI-TOF (Matrix-Assisted Laser Desorption-Ionization Time-Of-Flight) spectrometry in a 4700 Proteomics Analyzer (PerSeptives Biosystems, Framingham, MA, USA). Peptides in the spots matched to “sp | FHR1_HUMAN” with a score of 100 (significant when greater than 56) Matched peptides are depicted in bold and underlined. Position of the matched peptides in FHR1 is also indicated (C).

Supplementary Figure 3

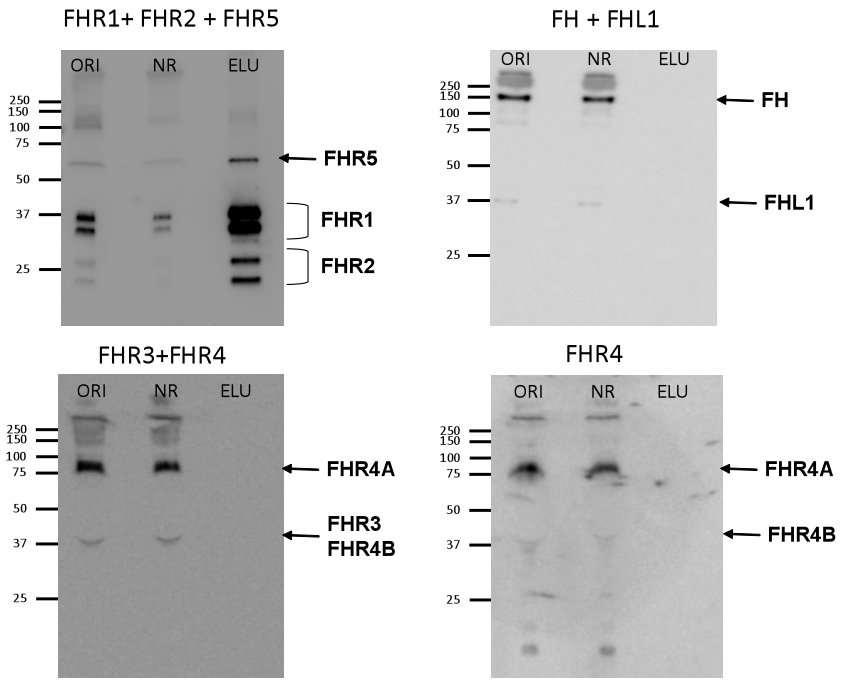


MLPA assay and PCR identification of the breakage point joining exons 5 and 2.

A) Diagram representing the results of the MLPA analysis in the four members of pedigree GN29. Notice that GN29-M, GN29-B and GN29-U carry one copy of the $\Delta_{CFHR3\text{-}CFHR1}$ allele. GN29 and GN29-M clearly show a gain of genomic material at the *CFHR1* locus.

B) Long PCR analyses allowed the identification of the breaking point within the *CFHR1* gene. The position is indicated and the flanking nucleotide sequences depicted underneath. The genomic rearrangement that generated the *CFHR1* internal duplication introduced an additional guanine that is not present in the native *CFHR1* genomic sequence.

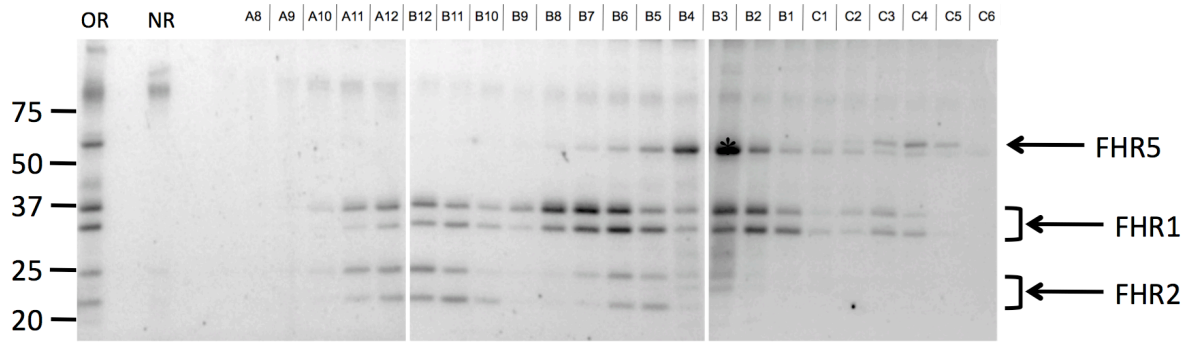
Supplementary Figure 4



FHR1, FHR2 and FHR5 do not interact with FH, FHR3 and FHR4A/B

A sample of EDTA-plasma from a *CFHR1*A/B* heterozygote was passed through the MBC125 affinity column and the presence of FH and FHR1-5 proteins analyzed by western blot using a panel of specific antibodies. FH, FHL1, FHR3 and FHR4A /FHR4B proteins are not retained by the MBC125 column, indicating that they do not interact with FHR1, FHR2 and FHR5. Antibodies used in the western blot were, MBC125 (anti FHR1, FHR2 and FHR5), OX24 (anti FH and FHL1), in house rabbit polyclonal antibody to FHR3 and FHR4 (from P. Zipfel) and in house rabbit antibody to FHR4 (from M. Jozsy).

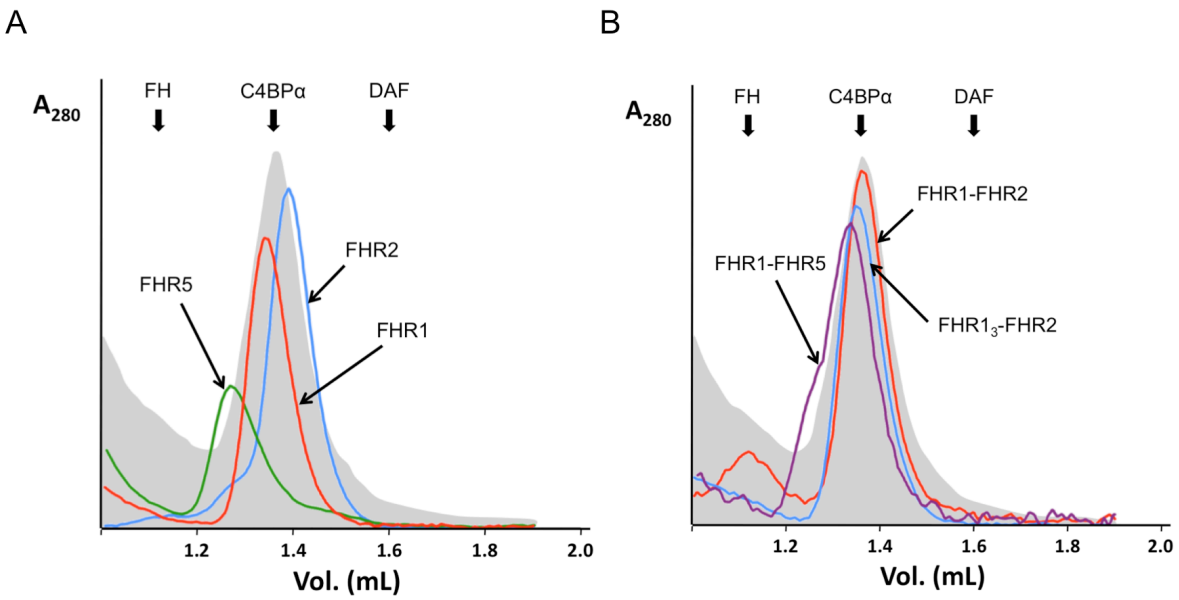
Supplementary Figure 5



Oriole-stained gel of the MBC125 affinity purified FHR1, FHR2 and FHR5 proteins eluted from the heparin columns.

Column fractions are indicated on the top. Positions of the FHR protein bands are shown on the right. Briefly, heparin fractions were separated in a 12 % SDS-Page gel and detected by fluorescence after being stained for 1h with Oriole solution (BioRad), following the manufacturer's instructions. Notice the presence of a single major contaminant protein indicated by an asterisk. This contaminant is not recognized by the MBC125 antibody by western blot. OR, original sample. NR, non retained proteins.

Supplementary Figure 6



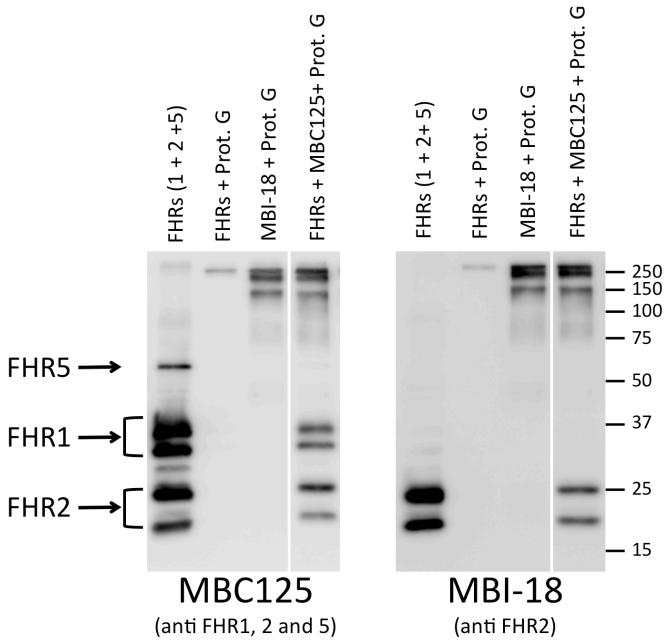
Gel filtration experiments of affinity purified FHR1, FHR2 and FHR5 proteins.

Gel Filtration of purified FHRs was carried out in Superdex 200 PC3.2/30 columns (GE Healthcare). Purified FH (20 SCRs), C4BPαΔCT(8 SCRs) and sDAF (4SCRs), all of them elongated proteins similar to FHRs, were used as molecular weight markers. Their elution volumes indicated with an arrow.

A) Elution profiles of purified FHR1 (red), FHR2 (blue) and FHR5 (green) are superimposed on the elution profile of a sample obtained from a MBC125 affinity column that contained all three FHRs shown as a grey area.

B) Elution profiles of purified FHR1-FHR2 (red), FHR1$_3$-FHR2 (blue) and FHR1-FHR5 (purple) are superposed with the profile of a MBC125 affinity column as in A.

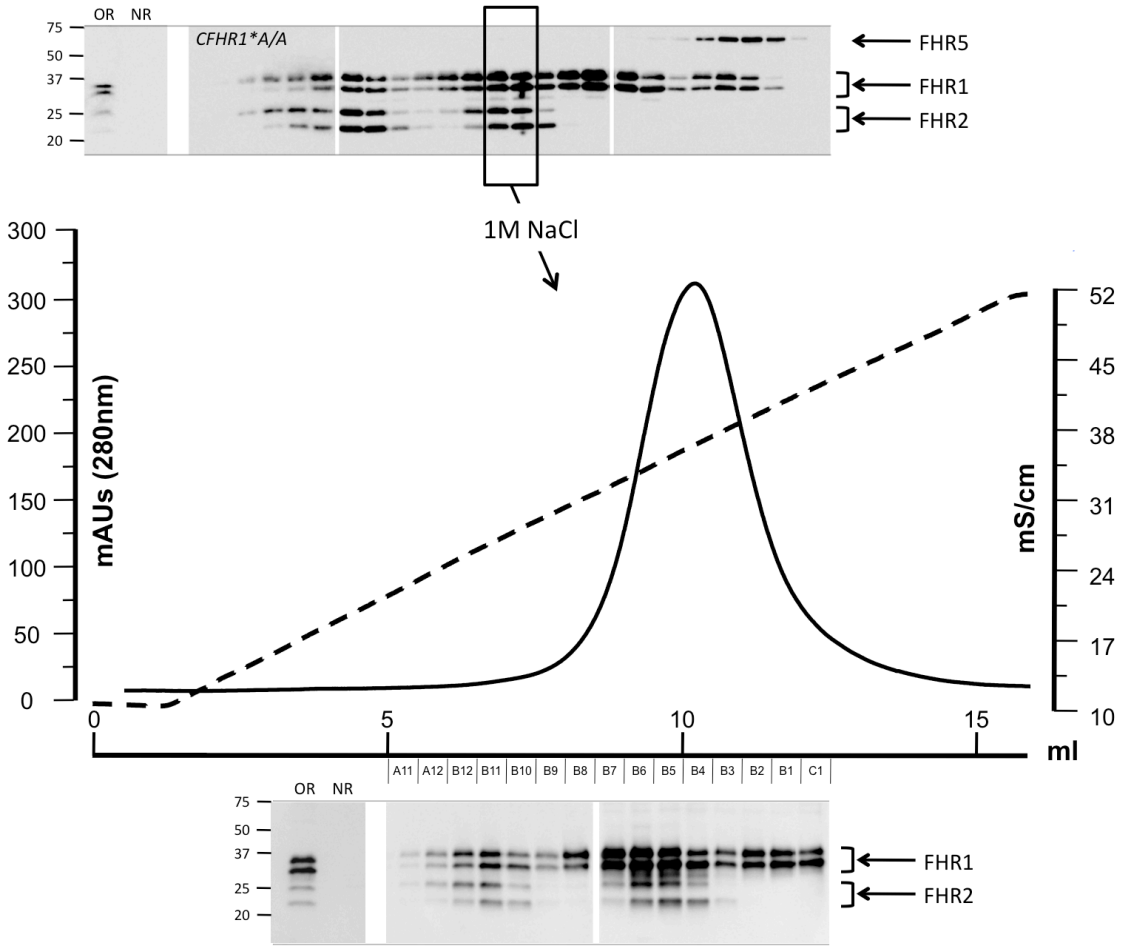
Supplementary Figure 7



Immunoprecipitation experiment illustrating the presence of FHR1-FHR2 hetero-oligomers

FHRs affinity purified with the MBC125 mAb column and dialyzed against PBS were immunoprecipitated with MBI-18, a mAb specific for FHR2 and the immunoprecipitate analyzed in western blot with both MBI-18 and MBC125. Data demonstrate that MBI-18 binds a FHR complex that contains both FHR2 and FHR1.

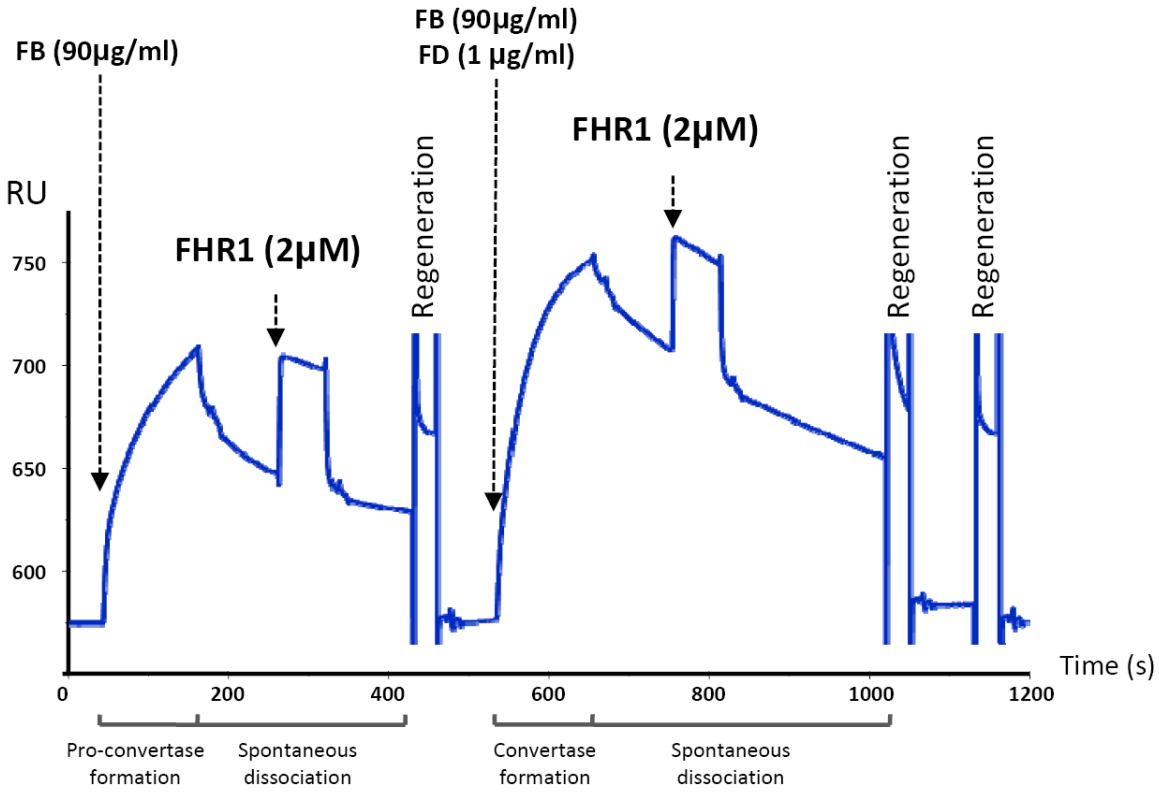
Supplementary Figure 8



Denaturation/renaturation experiments with FHR proteins included in the elution peak 2 ($FHR1_3$-FHR2).

Two fractions from the elution profile of the heparin column of a FH-depleted normal EDTA-plasma corresponding to the peak 2 ($FHR1_3$-FHR2) were pooled, incubated in 1 M NaCl, dialyzed against PBS and loaded again into a heparin column (GE Healthcare). Retained proteins were fractionated in a 50-350 NaCl gradient and analyzed by western blot using MBC125.

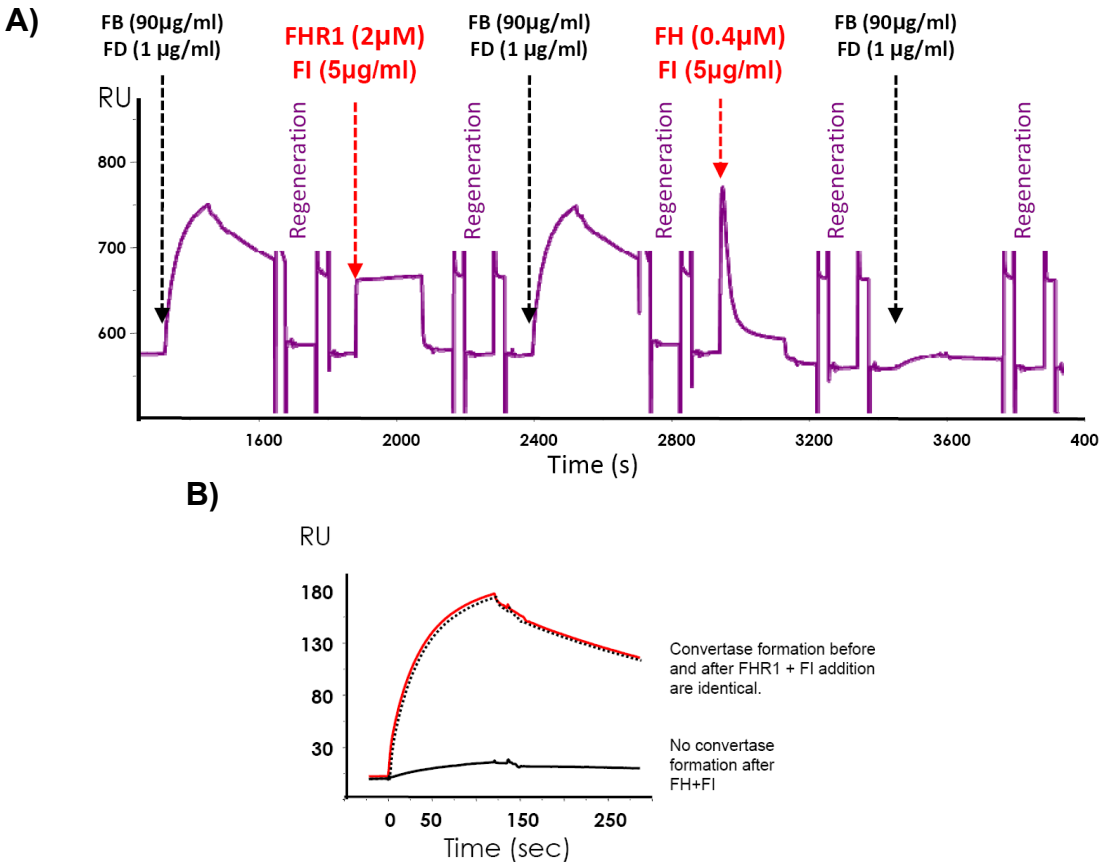
Supplementary Figure 9



FHR1 oligomers show no decay accelerating activity for the AP proconvertase or AP convertase.

C3b was deposited on the chip surfaces using thioester coupling. AP proconvertase was formed by flowing 90μg/ml of FB and then it was allowed to decay spontaneously. Addition of excess FHR1 oligomers (2μM) shows no acceleration of decay. Chip was regenerated and AP convertase formed by flowing 90μg/ml of FB and 1μg/ml of FD. Addition of excess FHR1 oligomers (2μM) shows no acceleration of the spontaneous decay of the AP convertase. Conclusion: FHR1 oligomers do not accelerate the decay of the C3bB (AP proconvertase) or C3bBb (AP convertase) complexes.

Supplementary Figure 10

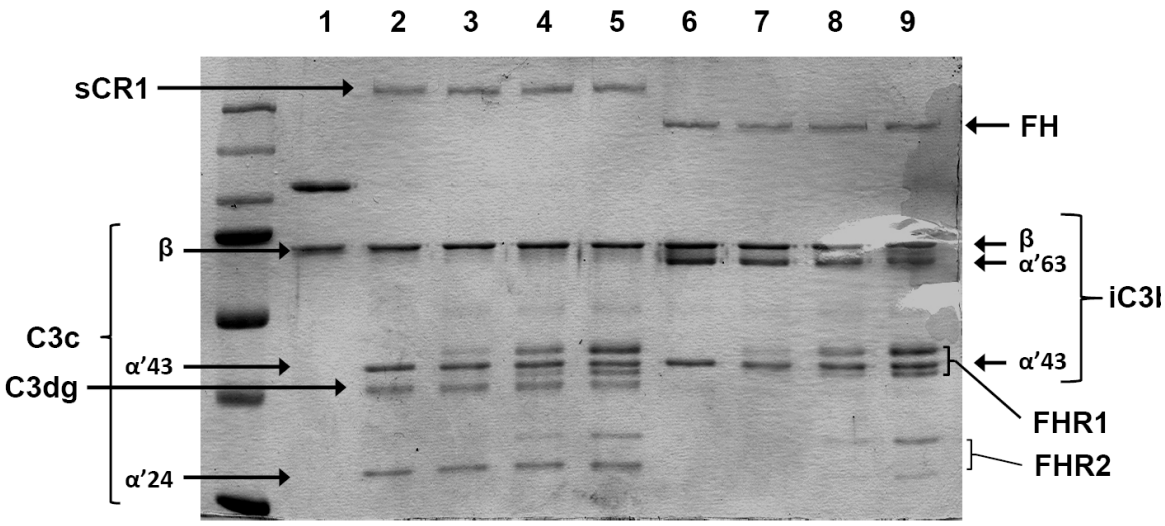


FHR1 oligomers show no cofactor activity for the FI-mediated C3b inactivation.

A) C3b was deposited on the chip surfaces using thioester coupling. AP convertase was formed by flowing 90µg/ml of FB and 1µg/ml of FD to set basal level of convertase formation. Surface was then regenerated and incubated with FHR1 oligomers (2µM) and FI (5µg/ml). After regeneration AP, flowing 90µg/ml of FB and 1µg/ml of FD resulted in identical formation of AP convertase. Surface was regenerated again and incubated with FH (0.4µM) and FI (5µg/ml). After a new regeneration cycle, 90µg/ml of FB and 1µg/ml of FD were flowed again, but this time a minimal amount of AP convertase was formed, indicating that most C3b has been converted into iC3b.

B) Comparison of AP convertase formation before and after treatment with FHR1 and FH. Conclusion: FHR1 oligomers show no capacity to act as cofactor for the FI-mediated cleavage of surface bound C3b.

Supplementary Figure 11



1- C3b + FI
2- C3b+sCR1+FI
3-, 4-, 5- C3b+sCR1+FI+FHRs (100, 200 or 400 ng)
6- C3b+FH+FI
7-, 8-, 9- C3b+FH+FI+FHRs (100, 200 or 400ng)

FHR1 does not have cofactor activity for the cleavage of C3b or iC3b.

C3b (1μg) was incubated with FI (25ng) and CR1 (300ng) or FH (200ng) in the absence or presence of increasing amounts of FHR1, 2 oligomers (100, 200 and 400ng) for 30min at 37ºC. Cleavage of C3b into iC3b or C3c + C3dg was analyzed by SDS-PAGE. FHR1 oligomers show no capacity to act as a cofactor for the FI-mediated cleavage of C3b or iC3b. They also have no effect on the cofactor activity of FH or CR1.