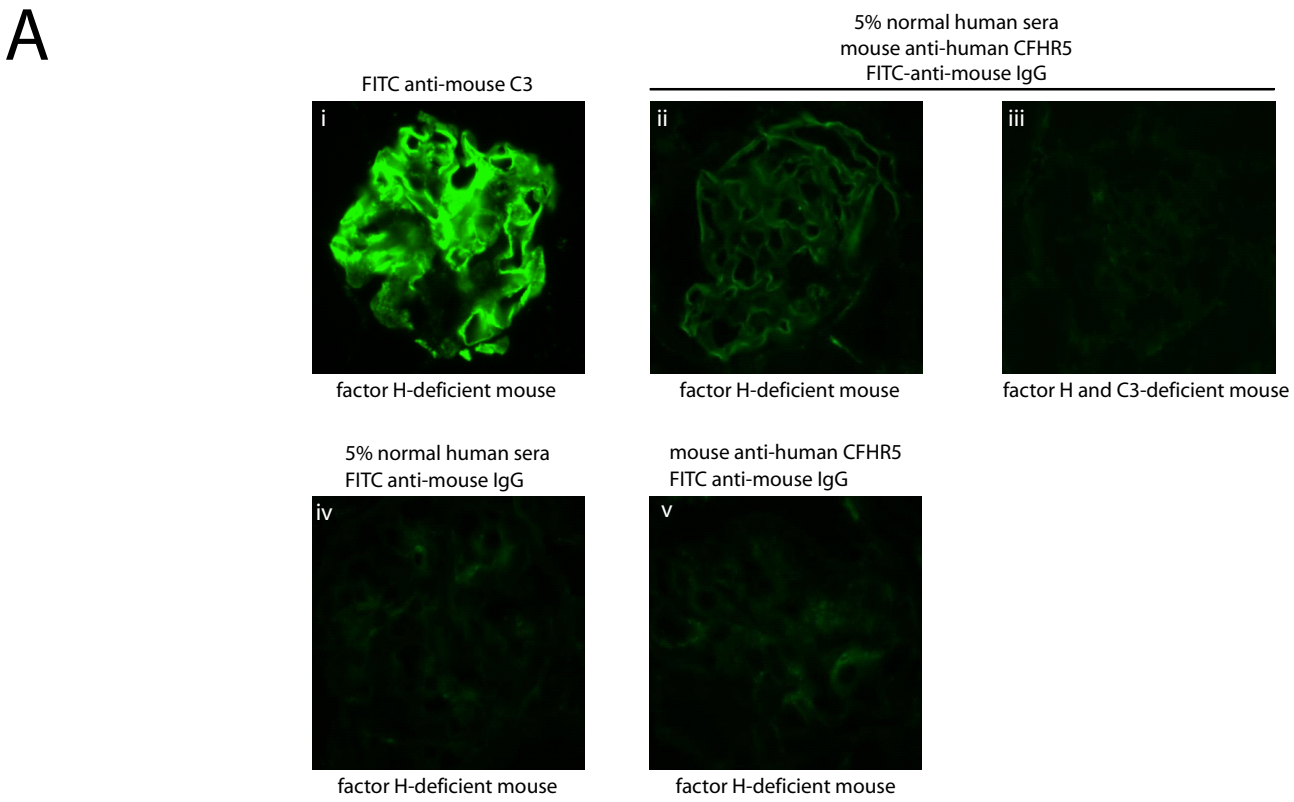
THE LANCET

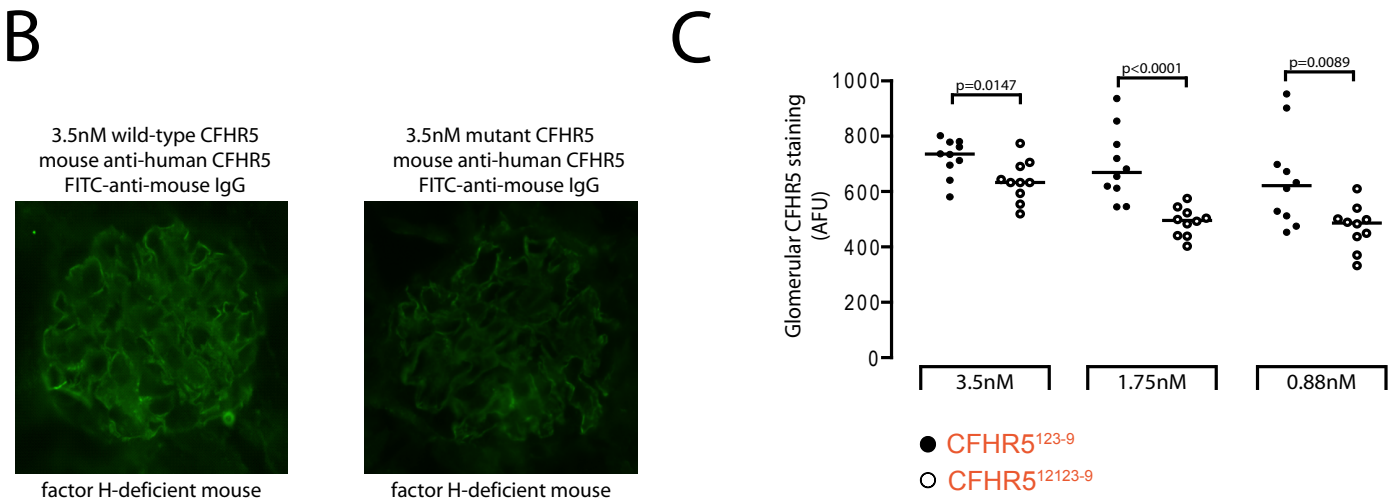
Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

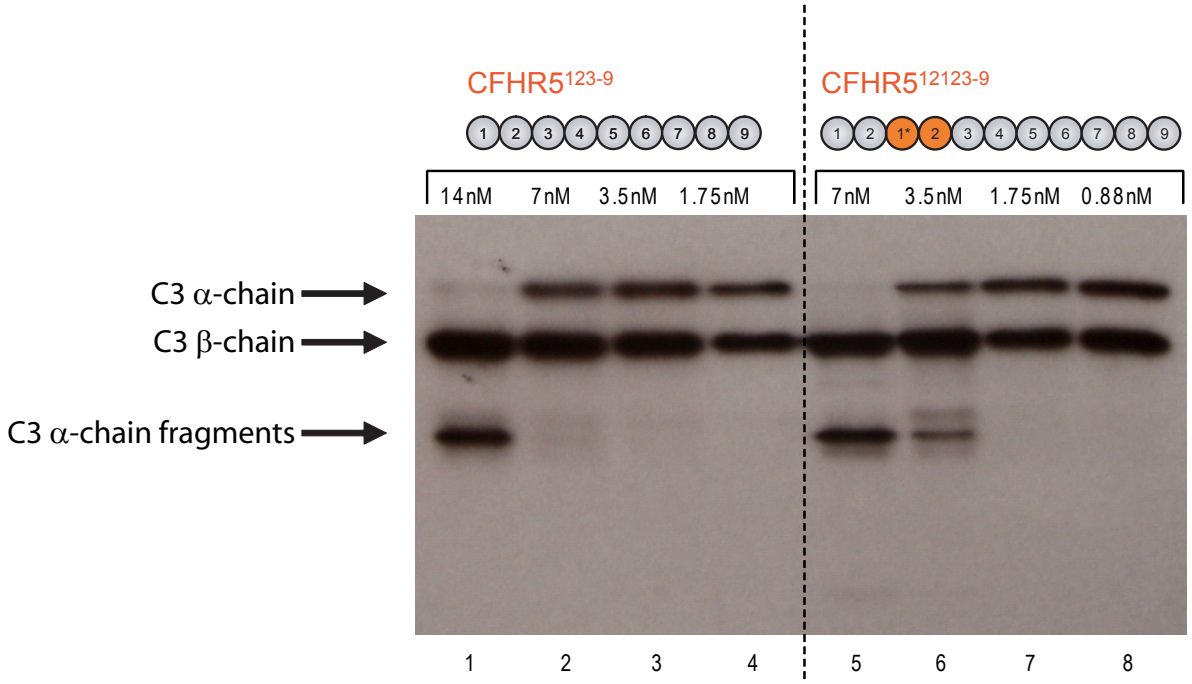
Supplement to: Gale D P*, de Jorge E G*, Cook H T, et al. Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet* 2010; published online August 26. DOI:10.1016/S0140-6736(10)60670-8.



Supplemental Figure 2. **(A). Binding of human serum CFHR5 to glomerular-bound mouse complement.** To assess the interaction of serum CFHR5 with complement in glomerular tissue we incubated unfixed frozen mouse renal sections with 5% normal human sera. The renal sections were from mice that develop spontaneous accumulation of C3 along the glomerular basement membrane as a consequence of complete genetic deficiency of complement factor H (reference 5 and image i). Sections from these animals were first incubated with 5% normal human serum, washed and then the presence of CFHR5 binding detected using a mouse anti-human monoclonal CFHR5 antibody (a gift from Dr. J. McRae, Immunology Research Centre, Melbourne, Australia) followed by application of a FITC-conjugated goat anti-mouse IgG antibody (Sigma-Aldrich, UK). CFHR5 was detected within the glomerulus in a peripheral linear staining pattern (image ii), comparable to the staining pattern seen for glomerular mouse C3 (image i). This pattern of CFHR5 reactivity was absent when the procedure was performed using renal sections from mice with combined deficiency of both factor H and C3 (image iii). Control sections included factor H-deficient sections incubated with sera followed by the FITC-conjugated goat anti-mouse IgG antibody (image iv) and sections incubated with the detection antibodies only (image v). These revealed only weak central granular glomerular staining, most likely reflecting the detection of small amounts of mesangial mouse IgG by the FITC-conjugated anti-mouse IgG antibody. These experiments demonstrated that human CFHR5 could recognise glomerular-bound mouse complement. Mouse C3 was detected with a FITC-conjugated goat anti-mouse C3 antibody (MP Biomedicals). FITC - fluorescein isothiocyanate.



Supplemental Figure 1. **(B) Binding of wild-type and mutant recombinant human CFHR5 to glomerular-bound mouse complement.** Incubation of renal sections from factor H-deficient mice with either the recombinant wild-type or mutant CFHR5 protein demonstrated that both proteins recognised glomerular-bound complement. **(C) Quantification of wild-type and mutant recombinant human CFHR5 to glomerular-bound mouse complement.** Quantification of glomerular fluorescence intensity using three different concentrations of recombinant proteins (3.5nM, 1.75nM and 0.88nM) demonstrated significantly reduced staining intensities for the mutant CFHR5 for each concentration used. This data suggests that the mutant CFHR5 protein has reduced affinity for glomerular-bound complement. Open circles denote mutant CFHR5 protein , closed circles denote wild-type CFHR5. Each point on the graph represents a single glomerular staining measurement (mean glomerular fluorescent intensity) and 10 glomeruli were quantified per section. Sections were all isolated from a single factor H-deficient mouse kidney to ensure no variation in the substrate. Images were captured using an Olympus BX40 microscope and Qimaging Retiga 200R camera (Digital Imaging Systems, UK) and quantified using Image-Pro software (MediaCybernetics, US). Original magnification x40. AFU - arbitrary fluorescent units. Mann Whitney test was used for statisitical analysis.



Supplemental Figure 2. **Factor I cofactor activity of recombinant CFHR5 proteins**. To assess the cofactor activity of the CFHR5 proteins for the factor I-mediated cleavage of C3b, serial dilutions of CFHR5 (either wild-type or mutant protein) were added to fixed amount of C3b (50ng) in the presence of factor I (10ng) in a total reaction volume of 16.5µl. After incubation overnight at 37°C the reaction was stopped by the addition of 5µl of SDS sample buffer. Samples were analysed by 10% SDS-PAGE under reducing conditions and subsequent western blotting of C3. Both wild-type and mutant CFHR5 protein displayed cofactor activity (lane 1, 5 and 6). However, the mutant CFHR5 had significantly greater activity when compared to that of the wild-type protein on an equimolar basis. Almost complete cleavage of the α-chain was seen using 7 nM concentration of mutant CFHR5 protein whilst no cleavage was seen at this concentration of wild-type protein (lane 2 and 5). Factor I and C3 were purchased from Calbiochem. C3b was generated by limited digestion with trypsin (Sigma-Aldrich).