

**Supplementary Information: Mapping rare, deleterious mutations in Factor H:
Association with early onset, drusen burden, and lower antigenic levels in familial AMD**

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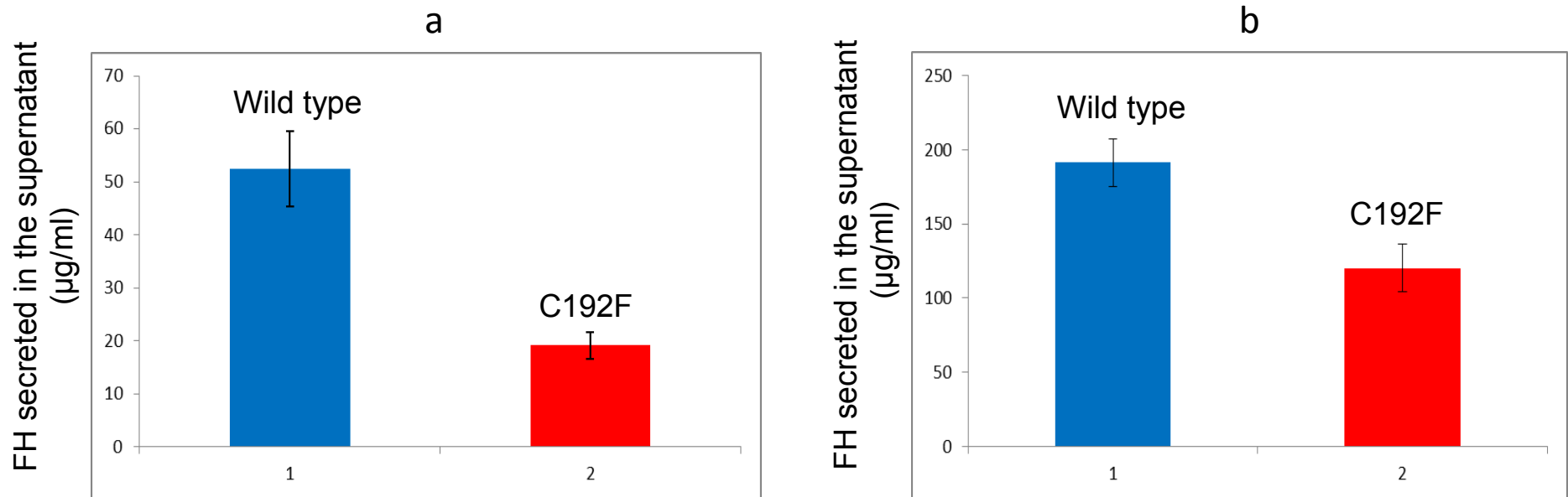
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Supplementary Table S1: Factor H levels

ID	AMD Disease Status	Genotype	Factor H Levels (µg/ml)
Pedigree A (CFH C192F)			
III:2	Affected	G/T	198 ^a
IV:1	Affected	G/T	265
IV:2	Affected	G/T	294
IV:3	Unaffected	G/G	365
IV:4	Affected	G/T	252
IV:5	Affected	G/T	186 ^a
IV:6	Affected	G/T	207 ^a
IV:7	Unaffected	G/G	312
Pedigree B (CFH IVS6+1G>A)			
II:1	Affected	G/A	147 ^a
II:2	Affected	G/A	191 ^a
Pedigree C (CFH R175P)			
II:1	Affected	G/C	205 ^a
II:2	Affected	G/C	193 ^a
Pedigree D (CFH R127H)			
II:1	Affected	G/G	541
II:2	Affected	G/A	172 ^a
III:1	Affected	G/A	271
III:2	Unaffected	G/G	493

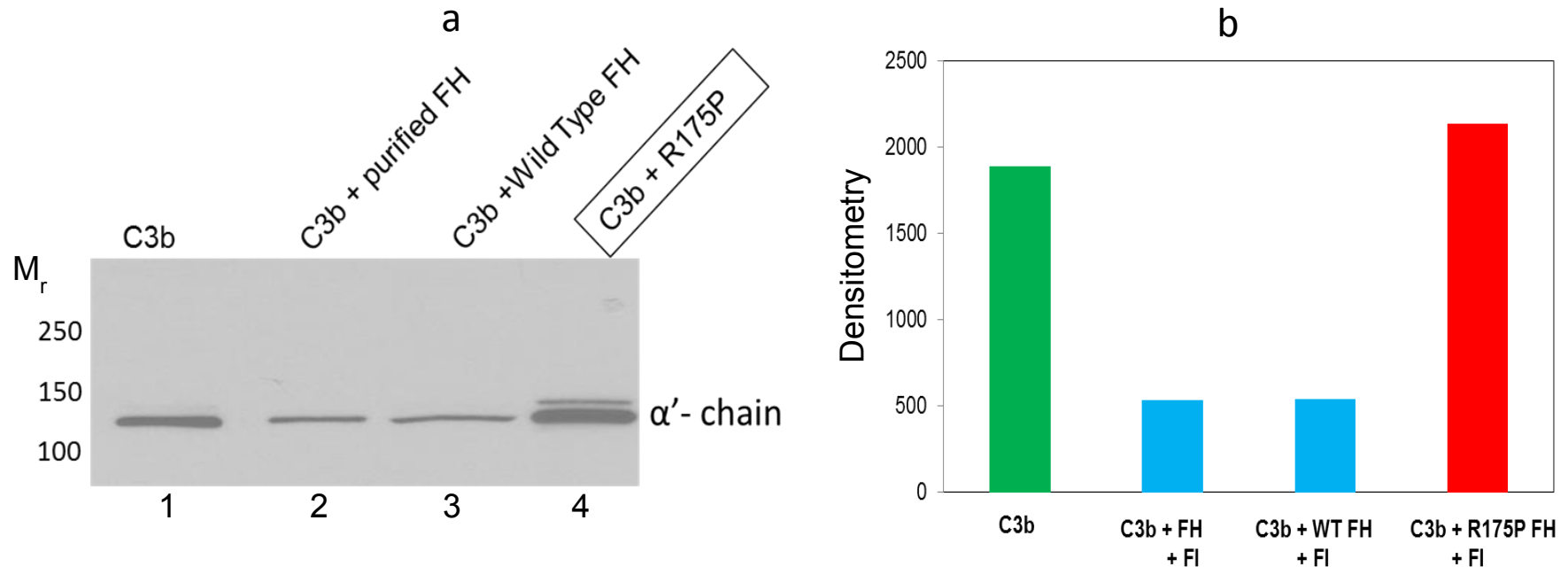
^aBelow range of serum Factor H levels measured in controls; minimum level measured in controls = 212 µg/ml. AMD: Age-related macular degeneration.



Supplementary Figure S1: Quantification of Factor H secreted into the supernatant

The wild type and Factor H (FH) variant (C192F) were recombinantly expressed in 293T cells employing two separate transfections. The quantity of wild type and variant protein was determined by ELISA.

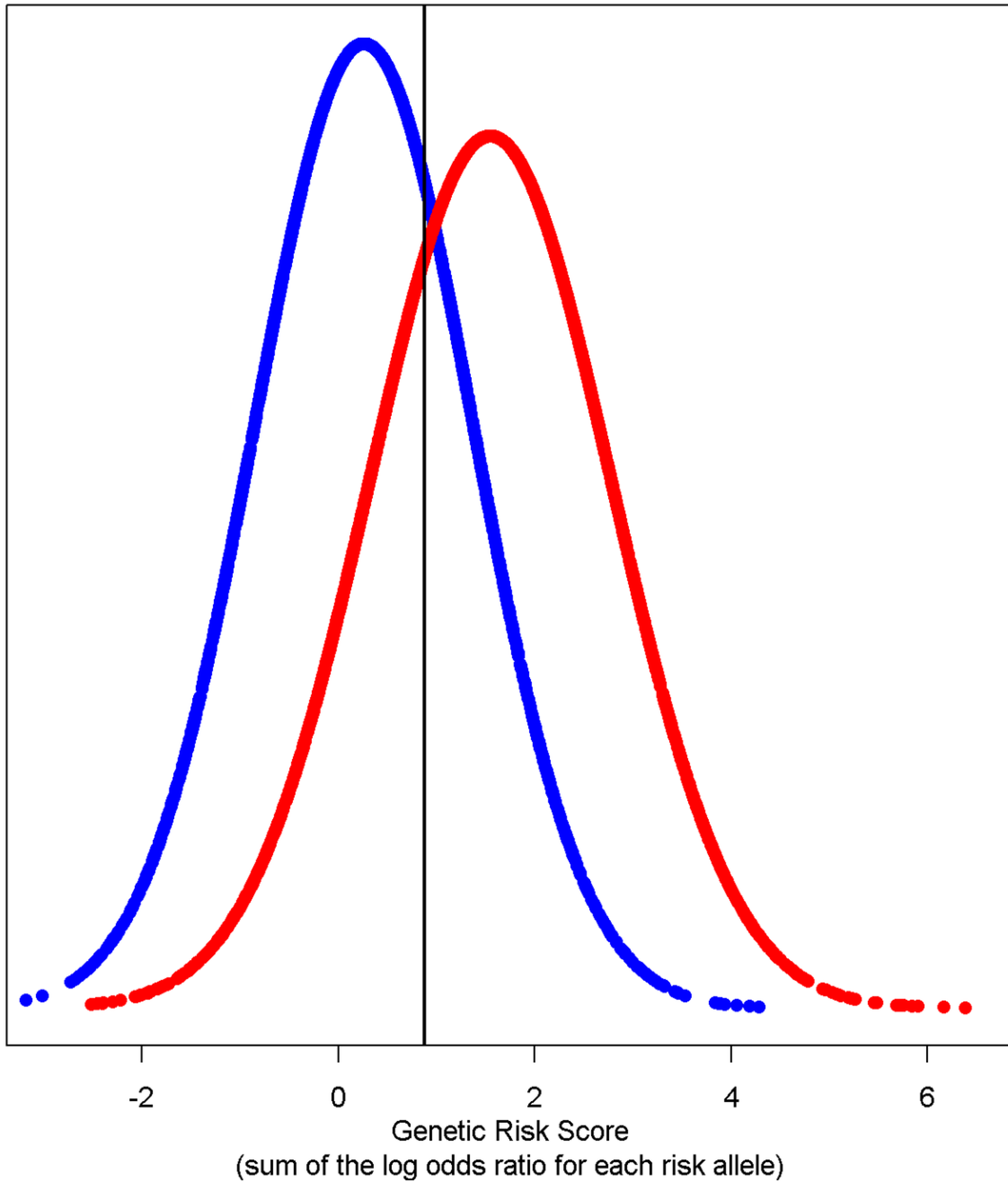
- (a) In the first transfection as shown, the mean concentration (shown as mean \pm standard deviation) of wild type FH secreted into the supernatant was 52 $\mu\text{g/ml}$ (\pm 7.1) and the mean concentration of FH C192F variant secreted was 19 $\mu\text{g/ml}$ (\pm 2.6); i.e. the FH variant was secreted at 36% compared to wild type.
- (b) In the second transfection as shown, the mean concentration of wild type FH secreted was 198 $\mu\text{g/ml}$ (\pm 29.8) and the mean concentration of FH C192F variant secreted was 114 $\mu\text{g/ml}$ (\pm 15.9); i.e. the FH variant was secreted at 57% compared to wild type.



Supplementary Figure S2: Functional evaluation of a Factor H mutation: Cofactor activity

C3b is resistant to inactivation by Factor H (FH) variant R175P. We incubated the FH proteins (wild type or R175P variant) at physiological salt concentration with C3b and Factor I (FI) at 37°C for 30 min. The reaction was stopped by addition of 3x reducing buffer. After electrophoresis and transfer to a nitrocellulose membrane, Western blots were developed using goat anti-human C3 polyclonal antibody (a). Bands were quantified using densitometry (b). There was minimal, if any, detectable cleavage of C3b by FH R175P while the two positive controls (lanes 2 and 3) cleaved ~70% of the α' -chain of C3b.

Lane 1, C3b alone (10 ng, negative control); lane 2, C3b (10 ng) with purified FH (200 ng) and FI (20 ng); lane 3, C3b (10 ng) incubated with wild type FH (200 ng, recombinantly produced in our lab) and FI (20 ng); lane 4, C3b (10 ng) incubated with R175P FH variant (200 ng, recombinantly produced in our lab) and FI (20 ng). Purified C3b, FH, and FI obtained from Complement Technologies, Inc. M_r : relative electrophoretic mobility.



Supplementary Figure S3: Distribution of genetic risk scores

● = controls (Clinical Age-Related Maculopathy Staging [CARMS]²³ grade 1); ● = AMD cases (CARMS grades 3B, 4, and 5); vertical black line indicates maximized sum of sensitivity and specificity threshold = 0.874.