

Figure S1. Specificity of C3(H₂O) ELISA. C3(KBr) and C3(MA) are recognized comparably in the C3(H₂O) ELISA (**A**). Increasing concentrations (1-80 ng/ml) of C3(MA) and C3(KBr) were compared in the C3(H₂O) ELISA. n=4, representative of 2 independent experiments. (**B**) Following treatment with FH or CR1 and FI, the proteins were assessed by WB under reducing conditions.

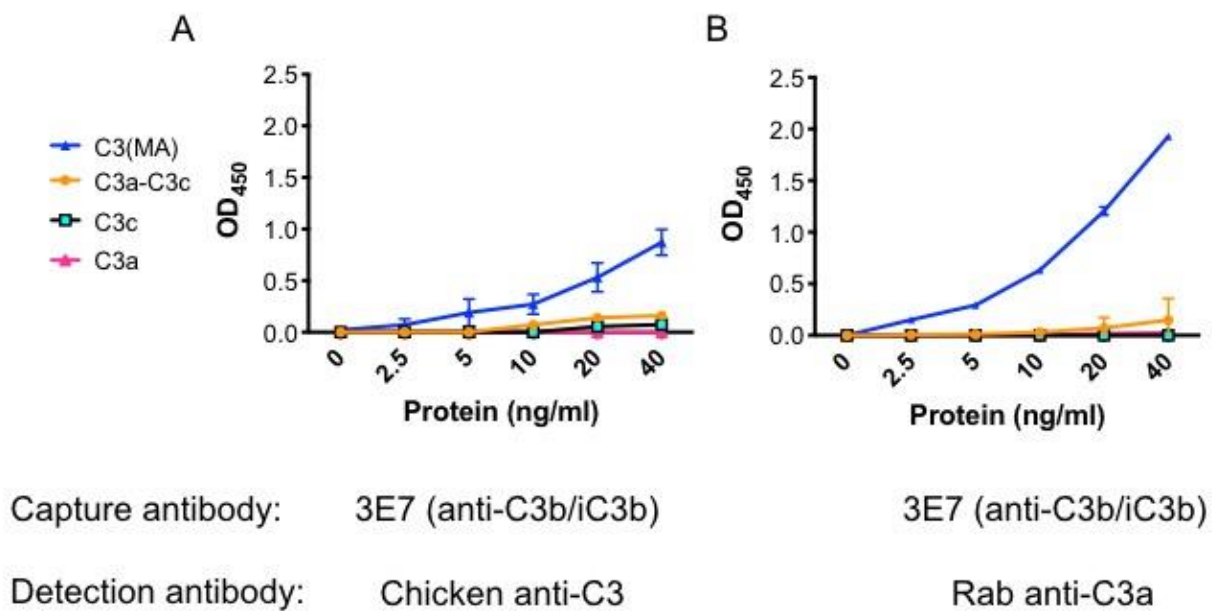


Figure S2. Specificity of the C3(H₂O) ELISA. (A) Binding comparison of proteins using anti-C3 pAb for detection. (B) Specificity of ELISA employing anti-C3a pAb. C3a-C3c, C3c and C3a are not detected.

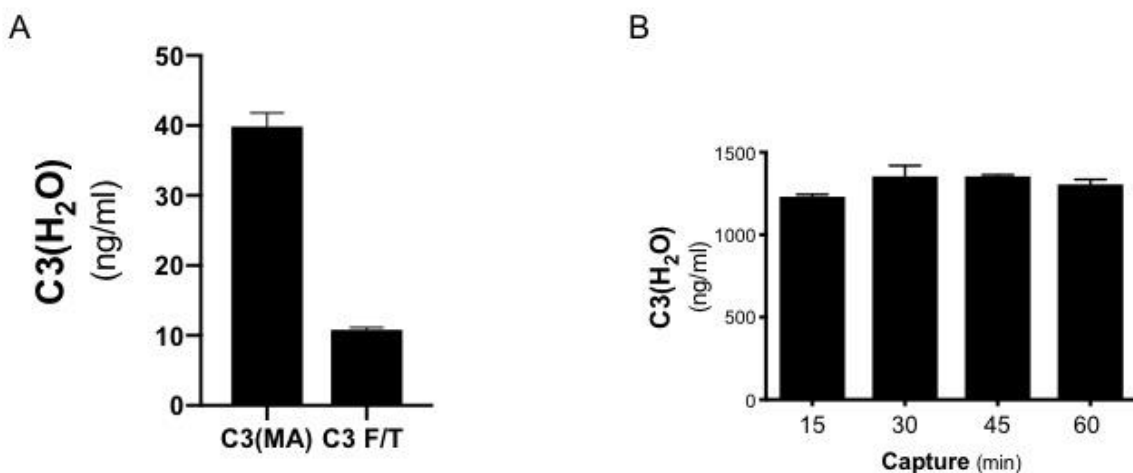


Figure S3. ELISA optimization. (A) Purified C3 (1 mg/ml) was subjected to 6 f/t cycles (C3 F/T). The C3(H₂O) content of C3 F/T was determined by loading 40 ng/ml of the protein and standard [C3(MA)] in the ELISA. Following 6 f/t cycles 25% of the purified C3 preparation has converted to C3(H₂O) [i.e. ~10 ng of the 40 ng C3 F/T loaded is C3(H₂O)], thus approximately 250 µg/ml of the undiluted 1 mg/ml C3 F/T preparation is C3(H₂O). n=2. (B) Generation of C3(H₂O) in 1:100 dilution of NHS over a 1 h capture incubation step of the ELISA was evaluated by measuring C3(H₂O) content by ELISA every 15 min during the capture step; n=2.

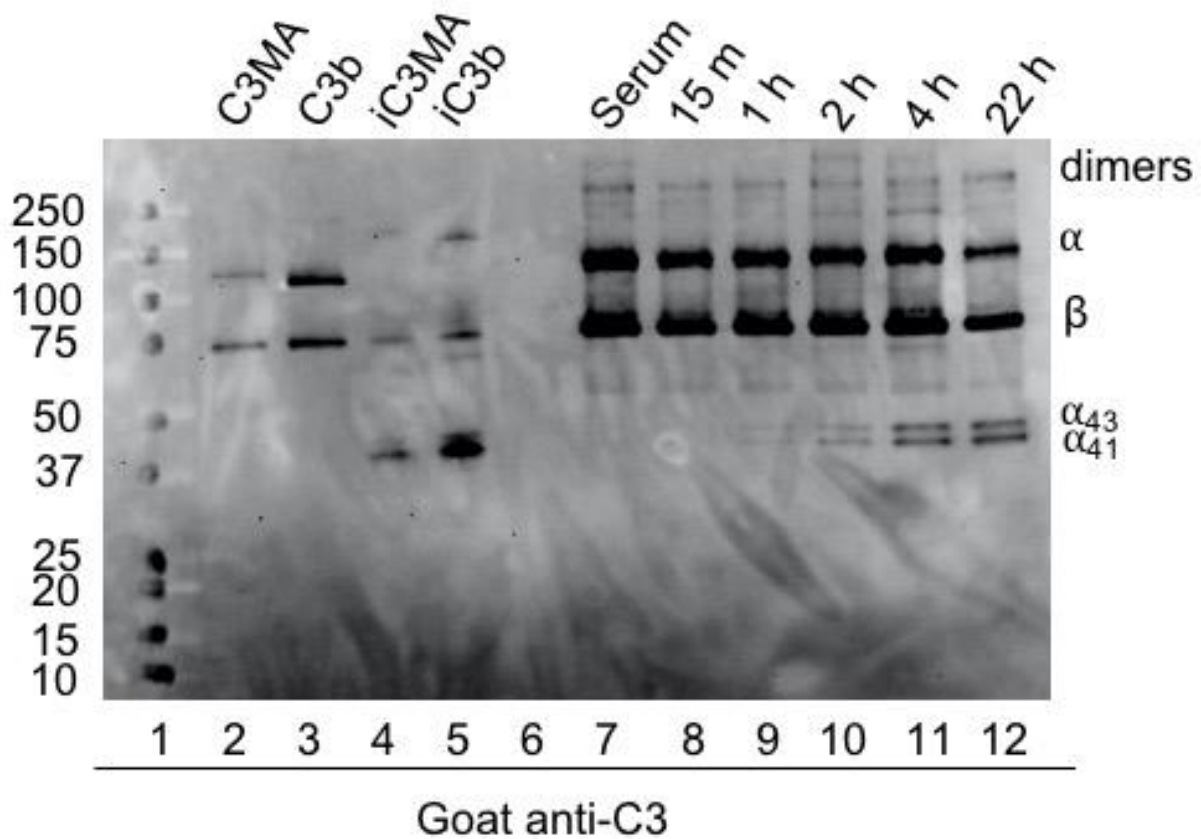


Figure S4. C3c generation is not detected after 22 h at 37°C. Uncropped and overexposed image of the WB shown in Figure 4E, demonstrating the absence of the α_{24} band that is diagnostic of C3c.