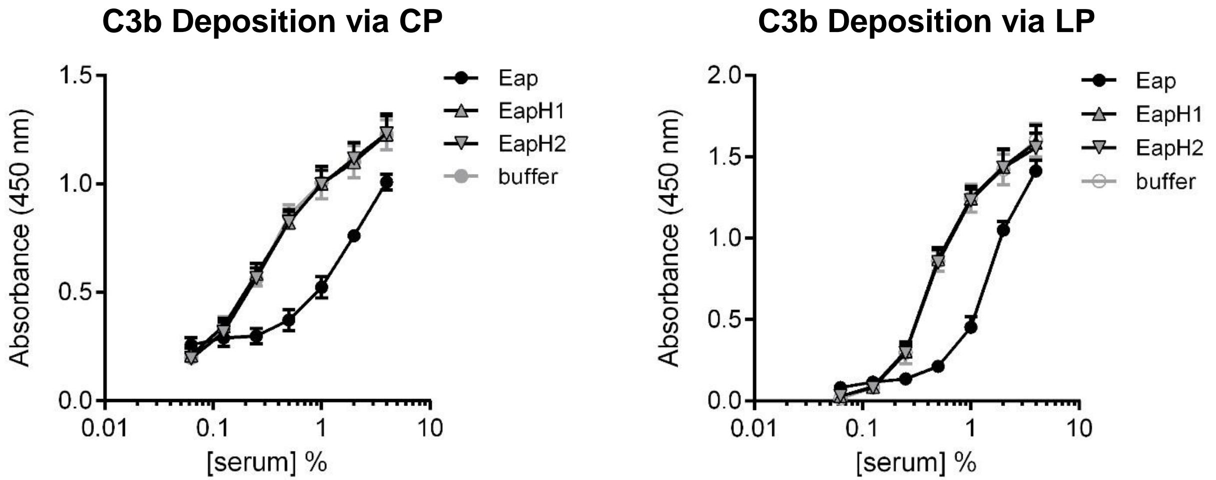
Woehl et al., Supplemental Figure 1

A



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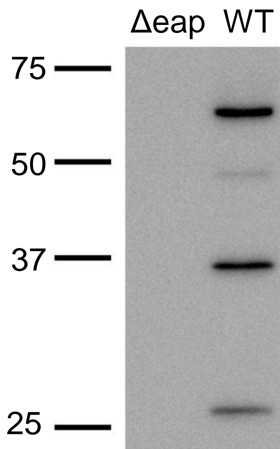
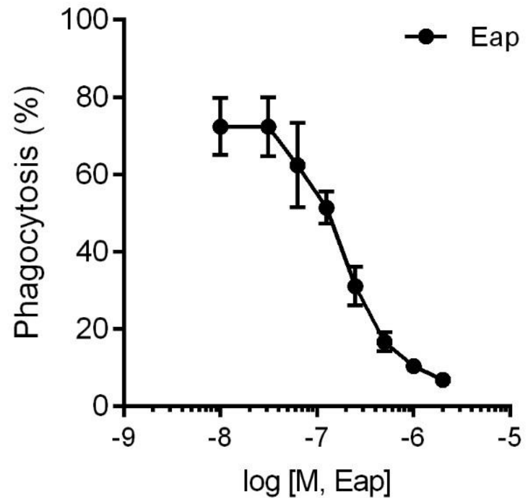
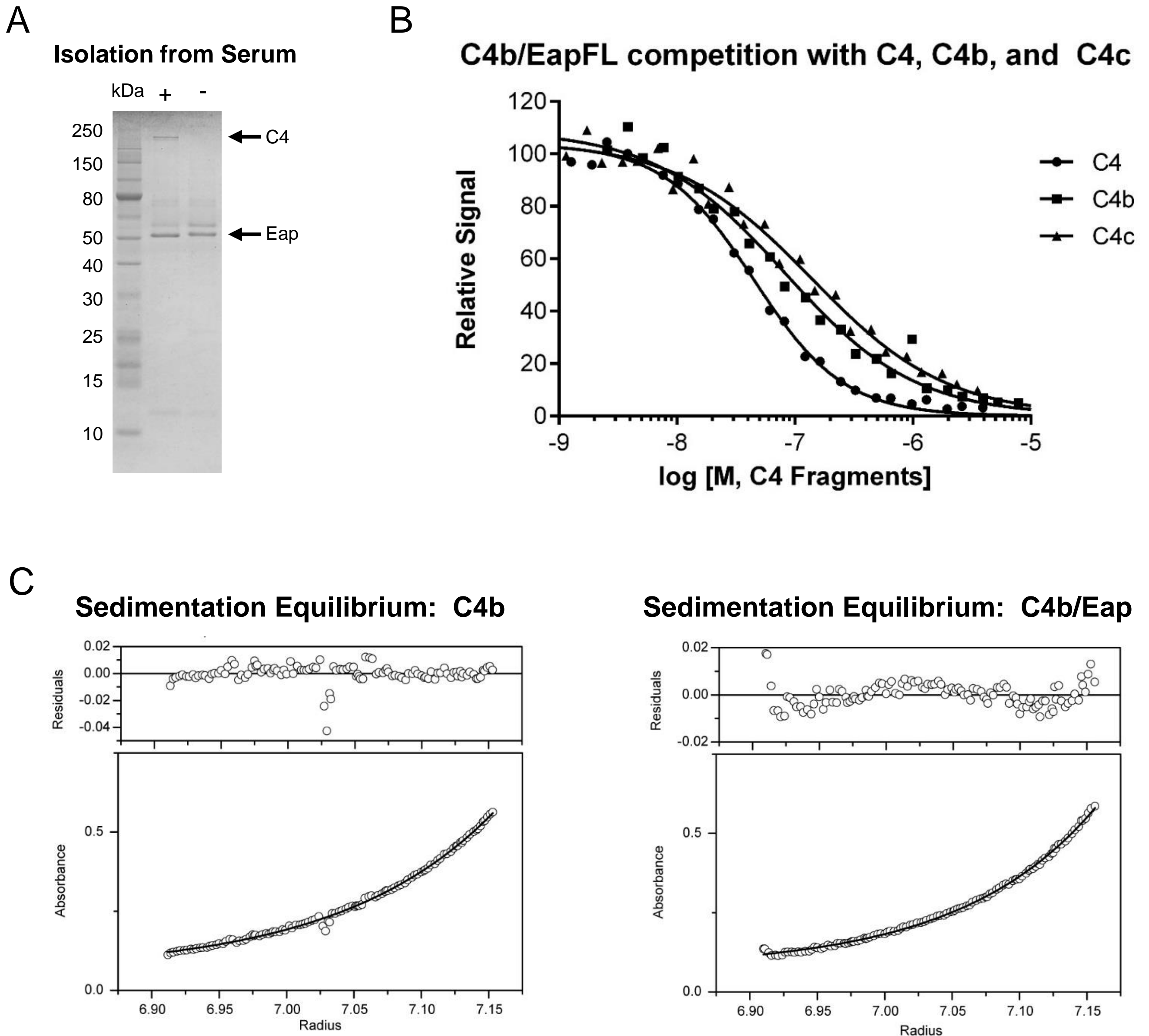


C



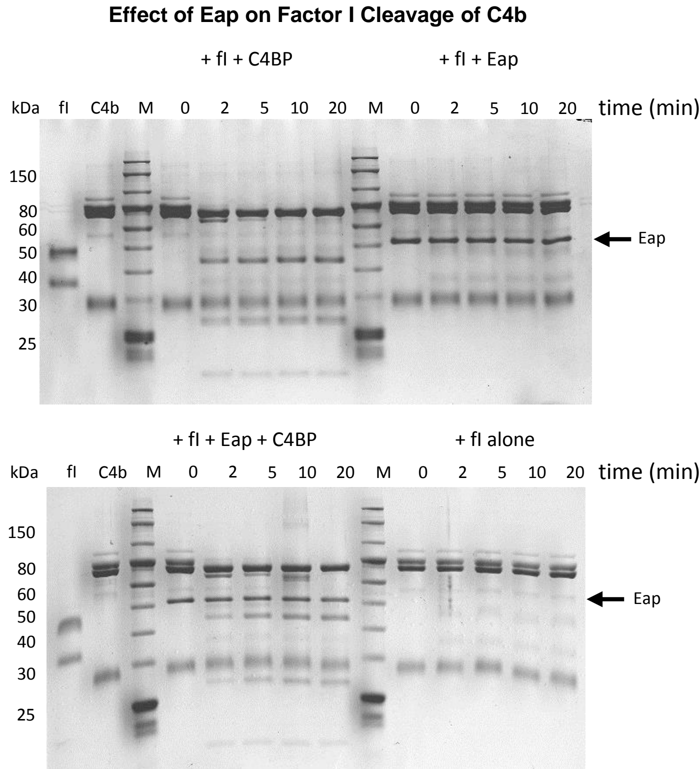
Supplemental Figure 1. Supporting Information on Eap Inhibition of CP/LP and Phagocytosis. (a) Eap inhibits the murine CP and LP. The effect of 1 μM Eap, EapH1, or EapH2 on CP (left) and LP-mediated (right) complement activation was measured across a dilution series of serum concentrations. Activation was detected as C3b deposition on an ELISA plate surface. Legends are inset. (b) Eap is present on the surface of cells of a WT but not a Δeap *S. aureus* strain. Bacteria were grown to stationary phase in liquid culture and harvested by centrifugation. After washing, Laemmli sample buffer with DTT was added to an equal volume of cell suspension and the samples were analyzed on a 12.5% (w/v) glycine polyacrylamide gel. Eap was detected by Western blot with 1:20.000 diluted rabbit-α-Eap serum and an HRP-labeled secondary antibody. A control experiment was performed to ensure that the same amount of bacteria were present in each sample by plating serial dilutions of each sample immediately prior to adding the Laemmli buffer. Note that proteolytic degradation of Eap into various combinations of adjacent subdomains has been reporter elsewhere, and is due to protease sensitivity of the interdomain linkers (27). (c) Phagocytosis of *S. aureus* Newman Δ*eap* using 1% (v/v) NHS as a source of complement components and the indicated concentrations of Eap.

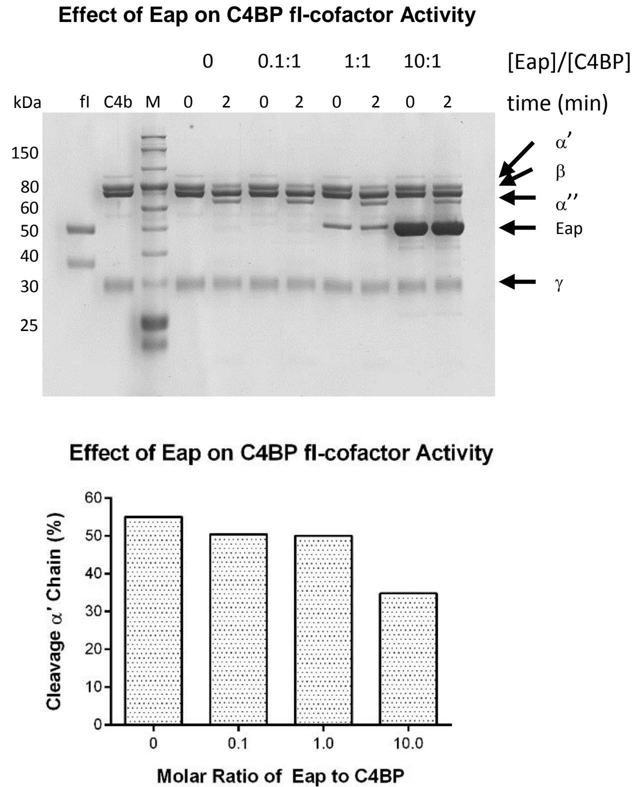
Woehl et al., Supplemental Figure 2



Supplemental Figure 2. Supporting Information on Eap Binding to C4 and its Derivatives. (a) Affinity isolation of C4 from human serum. Biotinylated Eap was used as an affinity reagent to identify potential binding partners in both normal and C4-depleted human serum. Following capture of Eap-biotin by magnetic streptavidin beads and a series of washes, the bound proteins in both samples were separated by SDS-PAGE under non-reducing conditions. A band of approximately 200 kDa was found in the lane corresponding to normal but no C4-depeleted serum, strongly suggesting that Eap binds to native C4. (b) The ability of C4, C4b, and C4c to compete the AlphaScreen signal generated by myc-Eap and C4b-biotin was assessed over a logarithmic dilution series. While three independent trials were carried out, the data presented here are from a single representative titration. The smooth line indicates the outcome of fitting all points to a dose-response curve. (c) Sedimentation equilibrium analytical ultracentrifugation analysis of C4b and C4b/Eap.Experimental data were obtained as described in *Materials & Methods* for C4b (left panel) and an equimolar mixture of C4b/Eap (right panel). Equilibrium profiles were fit to a single particle model to yield the observed molecular weight for both C4b (268 kDa) and C4b/Eap (308 kDa). The top plots in both panels show the random residuals for the respective fits.

Woehl et al., Supplemental Figure 3





Supplemental Figure 3. Eap Lacks FI-cofactor Activity and Only Weakly Impacts C4BP Cofactor Activity. (a) The effect(s) of including Eap, C4BP, both, or neither on FI-mediated proteolysis of C4b was investigated. Aliquots of each reaction series (indicated) were withdrawn over the course of 20 min and the proteins were analyzed by SDS-PAGE. (b) The effect of varying the molar ratio of Eap to C4BP on FI-mediated proteolysis of C4b was investigated. Aliquots of each reaction series (indicated) were withdrawn at 0 and 2 min, and the proteins were analyzed by SDS-PAGE. The identity of various bands, as determined by mass-spectrometry, is indicated. The ability of Eap to inhibit C4BP-dependent cleavage of C4b by FI was quantified by densitometry of the α' chain bands before and after the reaction. Addition of equimolar concentrations of both C4BP and Eap to this assay appeared to slow the rate of FI proteolysis (a, bottom panel left). This likely resulted from competition between Eap and C4BP for the same binding site on C4b, which consequently reduced the effective concentration of substrate available to FI since Eap does not have intrinsic cofactor activity (a, top panel right). To explore the functional consequences of Eap competition with C4BP in more detail, we carried out a set of studies wherein the molar ratio of Eap to C4BP was varied between 0, 0.1, 1.0, and 10 (b, top panel). Significant inhibition of FI-mediated proteolysis appeared to occur only when Eap was present at 10-fold higher concentration than C4BP (b, bottom panel). The requirement of such a high level of Eap to disrupt C4BP activity was most likely due to the weaker affinity of Eap for C4b and its lack of polyvalency, as it forms equimolar complexes with C4b. Thus, while Eap competes with C4BP for the same recognition site on C4b, it has no cofactor activity of its own, nor does competition with C4BP appear to be essential to its effects on the CP/LP of complement.