

Figure S1

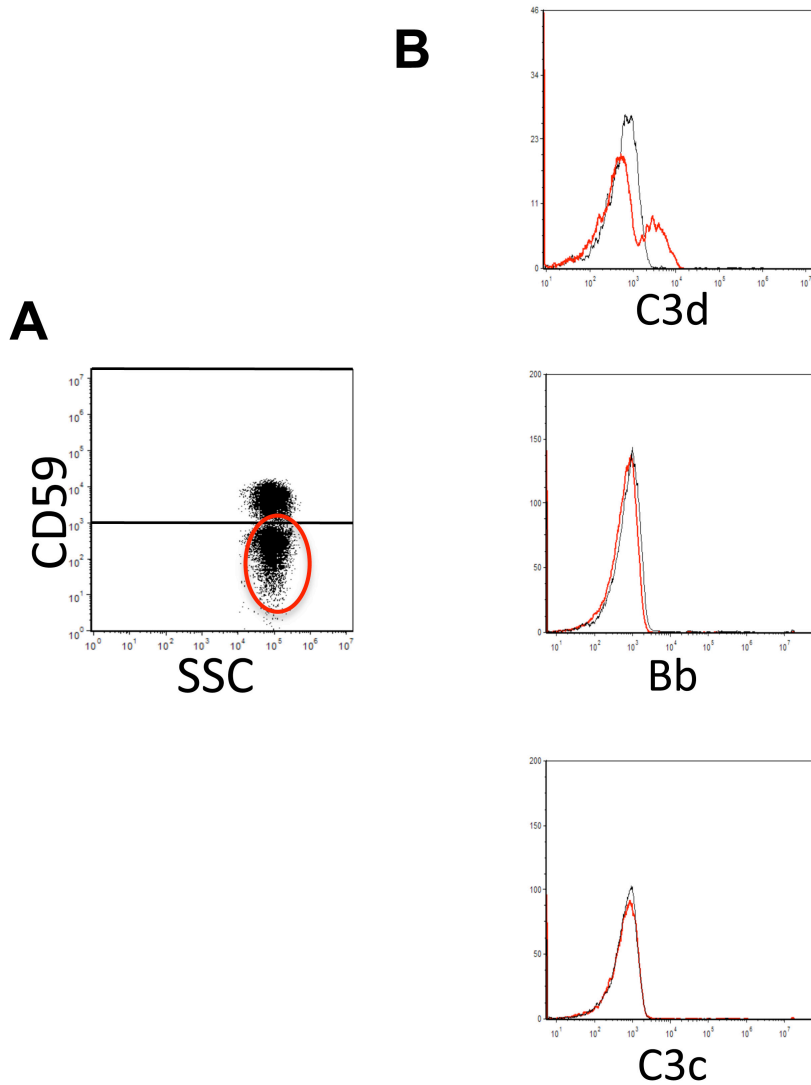


Figure S1. *In vivo* C3 binding on red cells of PNH patients on eculizumab.

(A) Normal (CD59-positive) and PNH (CD59-negative) populations (PNH RBCs are gated by the elliptic mark).

(B) Histogram plots of PNH (CD59-negative) population gated in panel A (grey line = isotypic control): Upper histogram shows anti-C3d (red line), Middle histogram shows anti-Bb (red line) and Lower histogram anti-C3c (red line).

Figure S2

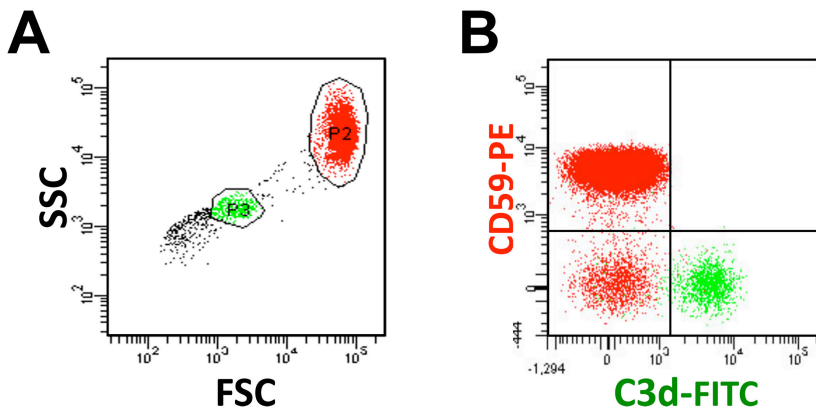


Figure S2. Effect of spontaneous complement activation on PNH red cells.

Effect of spontaneous complement activation. Red cells from PNH patients not on eculizumab are analyzed after incubation in ABO-compatible donor sera for 5 days at 37°C in sealed tube with 100% atmosphere.

(A) Ghost cells (in green) and intact red cells (in red) are identified on the basis of the physical parameters (forward –FCS- and side scatter –SSC-). [Lindorfer MA, Pawluczkwycz AW, Peek EM, Hickman K, Taylor RP, Parker CJ. Blood. 2010;115:2283-2291. DOI 10.1182/blood-2009-09-244285]

(B) Staining with anti-CD59 moAb and with anti-C3d moAb: after the spontaneous complement activation all ghost cells (in green) are PNH (CD59-negative) and almost all of them are bound with C3d fragments, whereas none of intact red cells (in red) are bound with C3d. In selected experiments the identity of intact and of ghost RBCs has been confirmed by staining with anti-Glycophorin A moAb (GA-R2, BD Becton Dickinson, NJ, USA) (data not shown).