C3 fragment deposition was analyzed by flow cytometry LSRII (BD Biosciences) in Patient 2 (on eculizumab therapy) and Patient 6 (not on eculizumab therapy).

PNH erythrocytes were incubated at 37ºC for 1 hour with heat-inactivated eculizumab-containing serum, pH 6.4 (aEcuHS[H], Top), activated (pH 6.4) eculizumab-containing serum (aEcuHS, Middle), and activated (pH 6.4) eculizumab-containing serum plus C1INH at 6U/mL (aEcuHS + C1INH, Lower).

After incubation for 1 hour at 37ºC, C3 deposition and CD55 were assayed by staining with FITC-conjugated anti-C3/C3b/iC3b antibody (C3-FITC) and PE-conjugated anti-CD55 antibody (CD55-PE).

C3 fragment deposition on the PNH erythrocytes was negligible in aEcuHS(H) (Top). The amount of C3 fragment deposition on the erythrocytes was increased (12.0 and 16.8%) in all subjects after incubation in aEcuHS (Middle). However, co-incubation of C1INH with the acidified, eculizumab-containing serum markedly attenuated (3.7% and 4.1%) C3 fragment deposition on the CD55 deficient erythrocytes (aEcuHS + C1) (Lower).

Supplemental Figure 3S: Percent Lysis Demonstration

PNH erythrocytes were incubated with acidified normal human serum without (aNHS) and with C1INH (aNHS + C1INH); heat-inactivated normal human serum (aNHS[H]); activated (pH 6.4) eculizumab-containing serum without (aEcuHS) and with C1INH (aEcuHS + C1INH) and heat-inactivated eculizumab-containing serum (pH 6.4) (aEcuHS[H]).

After 1 hour incubation, the supernatants were collected and absorbance of hemoglobin measured at 415nm by iMark microreader (Bio-Rad).

The percentages of hemolysis were normalized based on 0% lysis (GVB, PH 6.4 only) and 100% lysis (with water).

(a). The percentage of hemolysis of patient 2 and 6 were the highest lysis in aNHS (97.6 ± 1.0 and 85.7 ± 0.8). There was no hemolysis in aNHS (H) (6.5 ± 0.1 and 6.5 ± 0.1) and in aNHS + C1INH (3.8 ± 0.4 and 1.5 ± 0.1).

(b). Hemolysis was decreased when the erythrocytes were treated with EcuHS (11.7 ± 1.2 and 7.5 ± 1.0) compared to aNHS treatment; there were baseline hemolysis in aEcuHS + C1 (14.7 ± 1.1 and 9.2 ± 0.1); and aEcuHS (H) (16.2 ± 0.9 and 5.7 ± 0.1).

Error bars are standard deviations of three separate experiments.

Supplemental Figure 4S: ***C1 Inhibition blocks APC-mediated C3 Deposition in PNH Erythrocytes***

C3 fragment deposition was analyzed by flow cytometry LSRII (BD Biosciences) in Patients 2-4 (on eculizumab therapy).

At baseline time 0, C3 fragment deposition baseline demonstrated on the PNH erythrocytes (CD55 deficient) using activated eculizumab- human serum. (aEcuHS, Top)

At time 1 hour, C3 fragment deposition was increased in all 3 subjects after incubation with 1:3 dilution of acidified (pH 6.4) eculizumab-containing human serum (aEcuHS, Middle). Then, C3 fragment deposition was decreased in all 3 subjects after co-incubation for 1 hour at 37º C with acidified (pH 6.4) eculizumab-containing human serum (1:2) and C1INH (6U/mL). (aEcuHS +C1, Lower)

At baseline (T=0) and after incubation for 1 hour at 37ºC, C3 deposition and CD55 were assayed by staining with FITC-conjugated anti-C3/C3b/iC3b antibody (C3-FITC) and PE-conjugated anti-CD55 antibody (CD55-PE).

Figure 3S

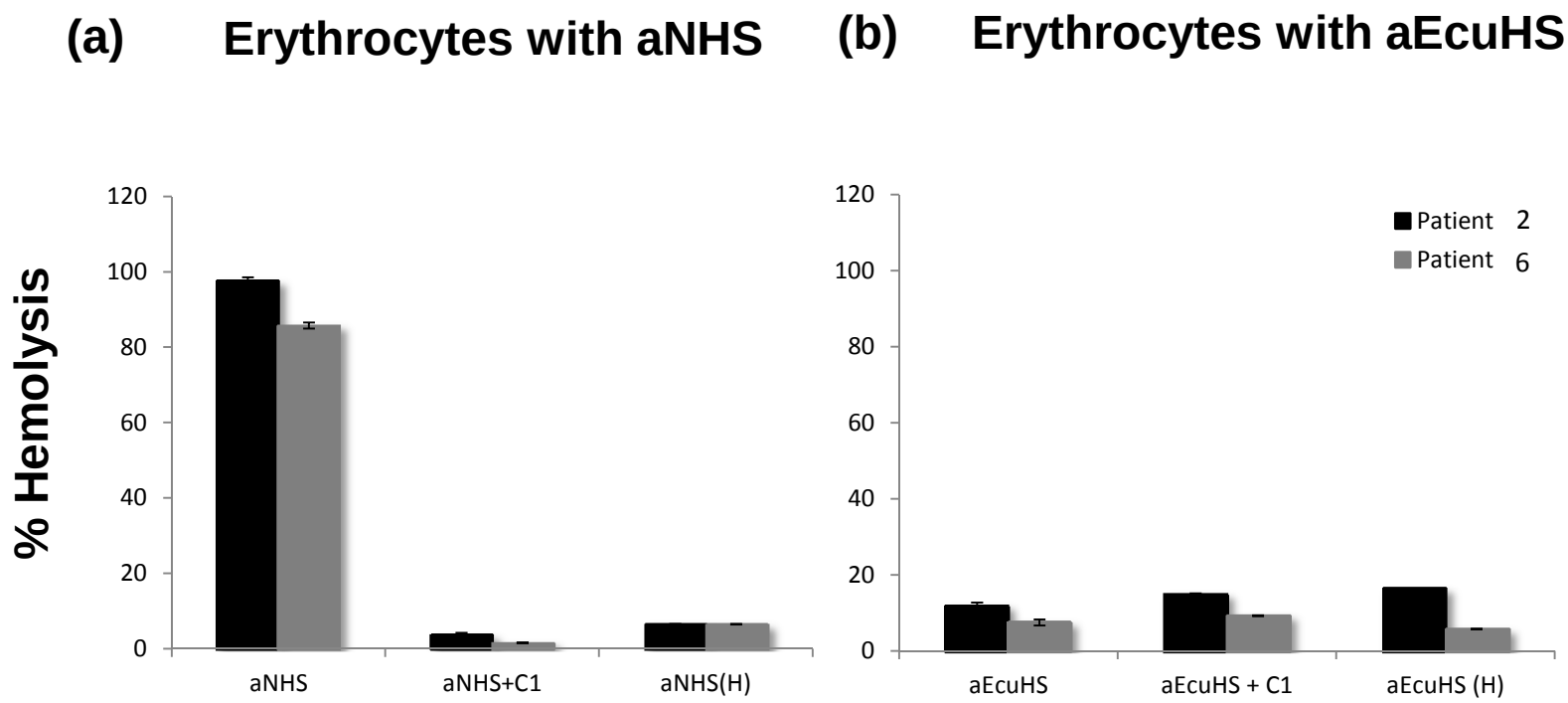
(a) Erythrocytes with aNHS
(b) Erythrocytes with aEcuHS
% Hemolysis
120
100
80
60
40
20
0
aNHS
aNHS+C1
aNHS(H)
aEcuHS
aEcuHS + C1
aEcuHS (H)
Patient 2
Patient 6

Figure 4S