Supporting Information

Wu et al. 10.1073/pnas.1006608107

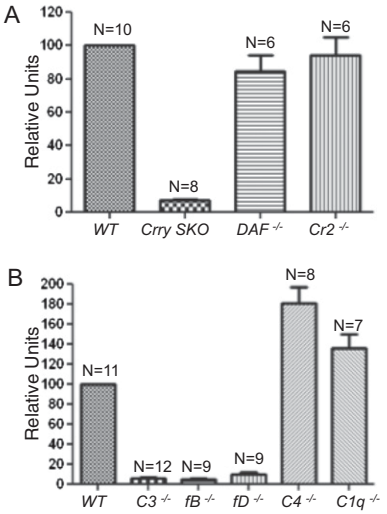

Fig. S1. Properdin concentration in WT and complement-deficient mice. After immunoprecipitation following by Western blotting, gels were scanned by densitometry. Properdin was normalized to WT controls. Western blots of mouse Ig heavy chain were used as a loading control for the C3, fB, and C4 blots. Number of mice in each group analyzed is noted. Mean ± SEM. *A* shows complement regulator/receptor deficient mice; *B* shows complement component deficient mice.

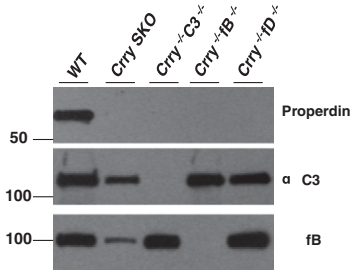

Fig. S2. Properdin concentration in $Crry^{-/-}C3^{-/-}$, $Crry^{-/-}fB^{-/-}$, and $Crry^{-/-}fD^{-/-}$ mice. Representative of four $Crry^{-/-}C3^{-/-}$, four $Crry^{-/-}fB^{-/-}$, and two $Crry^{-/-}fD^{-/-}$ mice.

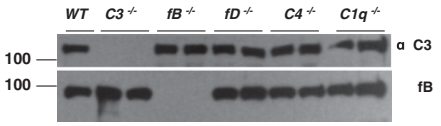

Fig. S3. C3 and fB are absent in their respective knockout mice by Western blot analysis. Representative of >10 $C3^{-/-}$ and 10 $fB^{-/-}$ mice.

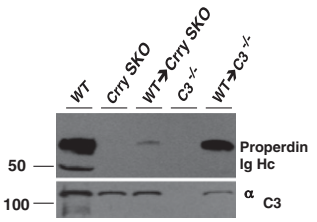

Fig. S4. Properdin reconstitution following serum infusion. WT serum (250 μL) was transferred to *Crry SKO* or $C3^{-/-}$ mice. After 2 h, samples were harvested from the recipient mice. Representative of three experiments. Densitometric scanning of the results at multiple time points from a similar experiment to that shown here are presented in Fig. 4*B*. The IgHc represents variable elution of the primary Ab used to pull down properdin (also Fig. S9).

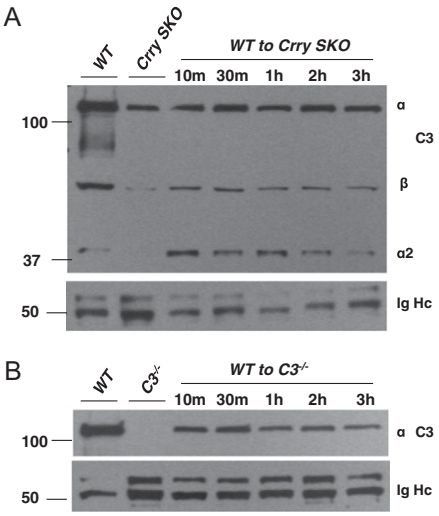

Fig. S5. Western blot analysis of C3 in *Crry SKO* (*A*) and *C3*$^{-/-}$ (*B*) mice following WT serum transfer. WT serum (200 μL) was infused i.v. into *Crry SKO* and *C3*$^{-/-}$ mice. Sera were then collected at the time points indicated. Each time-point represents a different mouse. Representative of two experiments.

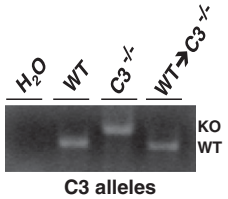

Fig. S6. Genotype of bone marrow chimeric mice. C3 alleles were examined using DNA derived from peripheral blood leukocytes of *WT*, *C3*$^{-/-}$, and *C3*$^{-/-}$ mice reconstituted with WT bone marrow. Representative of two experiments.

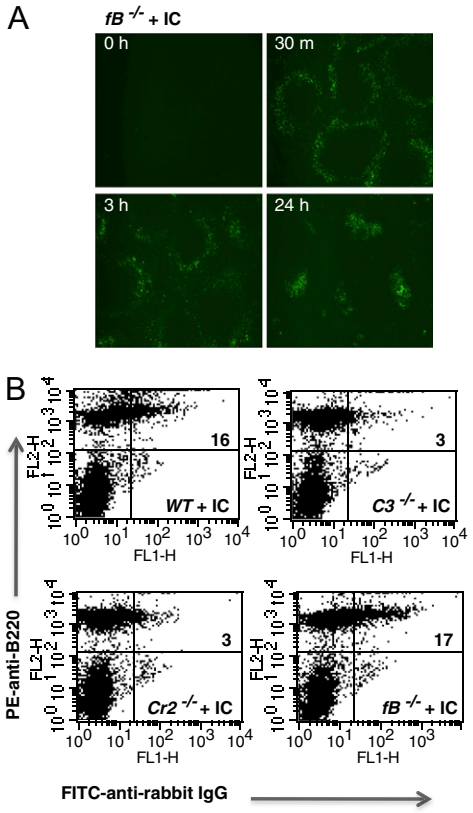

Fig. S7. Processing by the spleen of immune complexes (IC) that activate complement. (*A*) A kinetic analysis of IC deposition in the spleen of $fB^{-/-}$ mice, which is dependent upon classical pathway activation. ICs are FITC labeled. Representative of three experiments. (*B*) IC deposition on splenic marginal zone B cells of *WT*, $C3^{-/-}$, $Cr2^{-/-}$, and $fB^{-/-}$ mice. Splenocytes were isolated 30 min post-IC injection. FACS was used to analyze trapping of IC by marginal zone B cells. PE-B220 and FITC–anti-rabbit IgG antibodies were used to identify B lymphocytes and ICs, respectively. An IC trapping defect in B cells was observed in $C3^{-/-}$ and $Cr2^{-/-}$ but not in $fB^{-/-}$ or *WT* mice. Representative of two experiments.

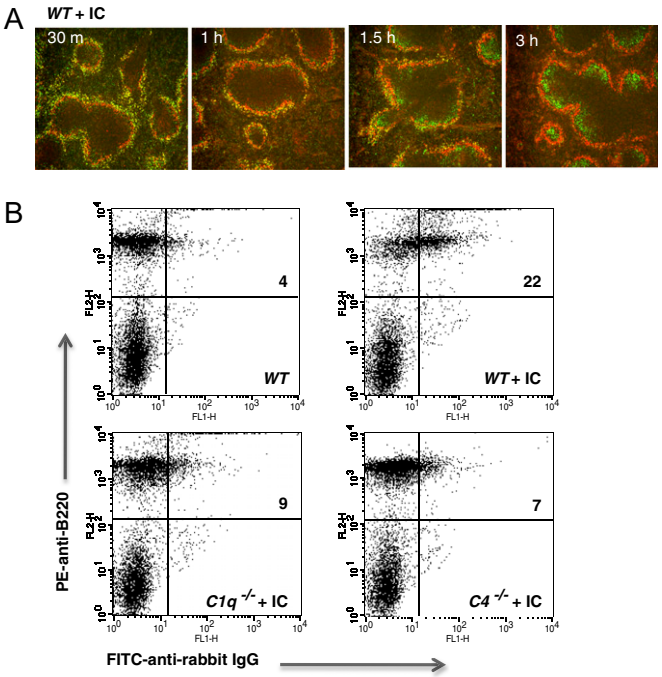

Fig. S8. Dynamics of IC deposition in the spleen. (*A*) IC deposition in *WT* mice. Note the migration of the IC (green) from marginal zone to the follicular areas, a complement-dependent process. The Moma-1 positive macrophages are labeled with a PE-conjugated antibody (red). (*B*) IC deposition on splenic marginal zone B cells of *WT*, $C1q^{-/-}$, and $C4^{-/-}$ mice. Splenocytes were isolated 30 min post-IC injection. FACS was used to analyze trapping of IC by marginal zone B cells. PE-B220 and FITC–anti-rabbit IgG antibodies were used to identify B lymphocytes and ICs, respectively. A partial IC trapping defect in B cells was observed in $C1q^{-/-}$ and $C4^{-/-}$ mice. Representative of three separate experiments.

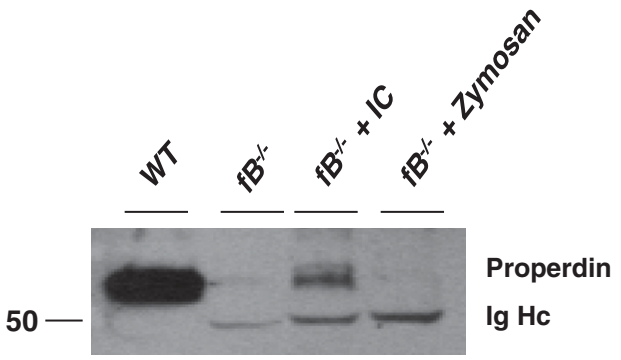

Fig. S9. Effect of immune complexes and zymosan on properdin release in $fB^{-/-}$ mice. Samples to measure properdin were obtained at 3 h.

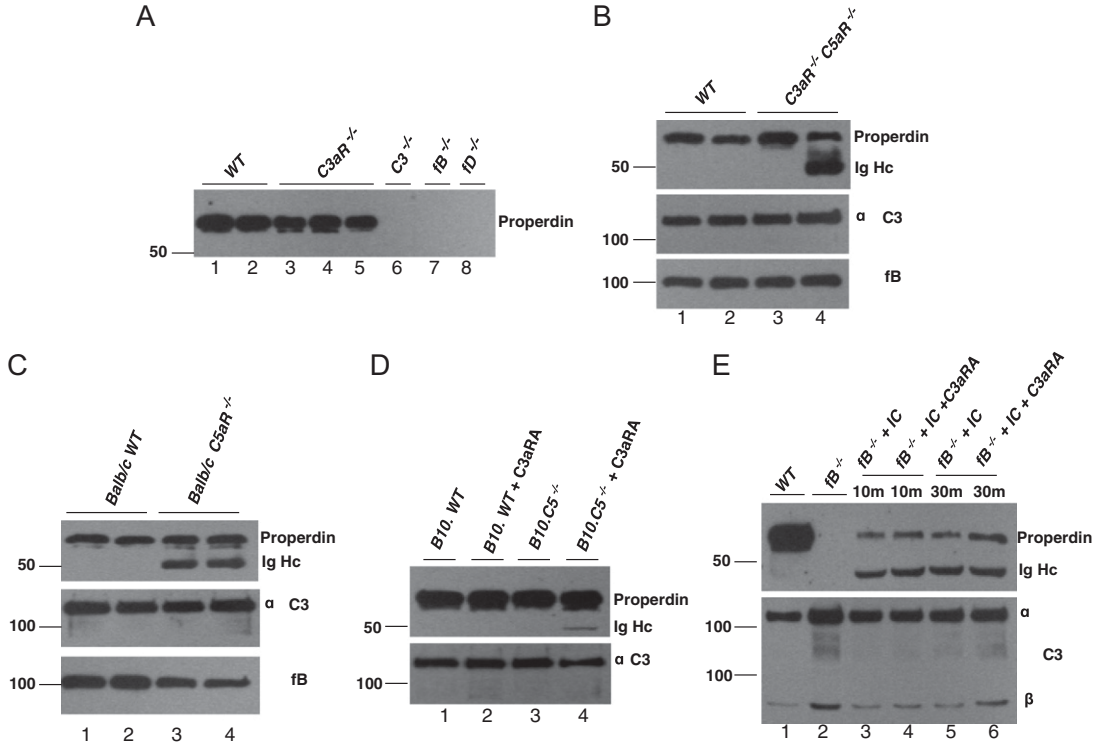

Fig. S10. Lack of a role for C3aR and/or C5aR in properdin release. (*A*) Properdin concentration in $C3aR^{-/-}$ mice. (*B*) Properdin concentration in $C3aR^{-/-}C5aR^{-/-}$ mice. (*C*) Properdin concentration in $C5aR^{-/-}$ mice. (*D*) Properdin concentration in *B10.WT* control and $C5^{-/-}$ mice treated daily for 3 d with a C3aR antagonist (500 μg/mouse, SB 290157; Calbiochem). (*E*) Effect of C3aR antagonist on immune complex-induced properdin release in $fB^{-/-}$ mice. $fB^{-/-}$ mice were treated with C3aR antagonist at a dose of 500 μg/mouse. After 30 min, ICs were infused intravenously. Serum samples were then collected at 10 or 30 min. Each lane represents a different mouse.