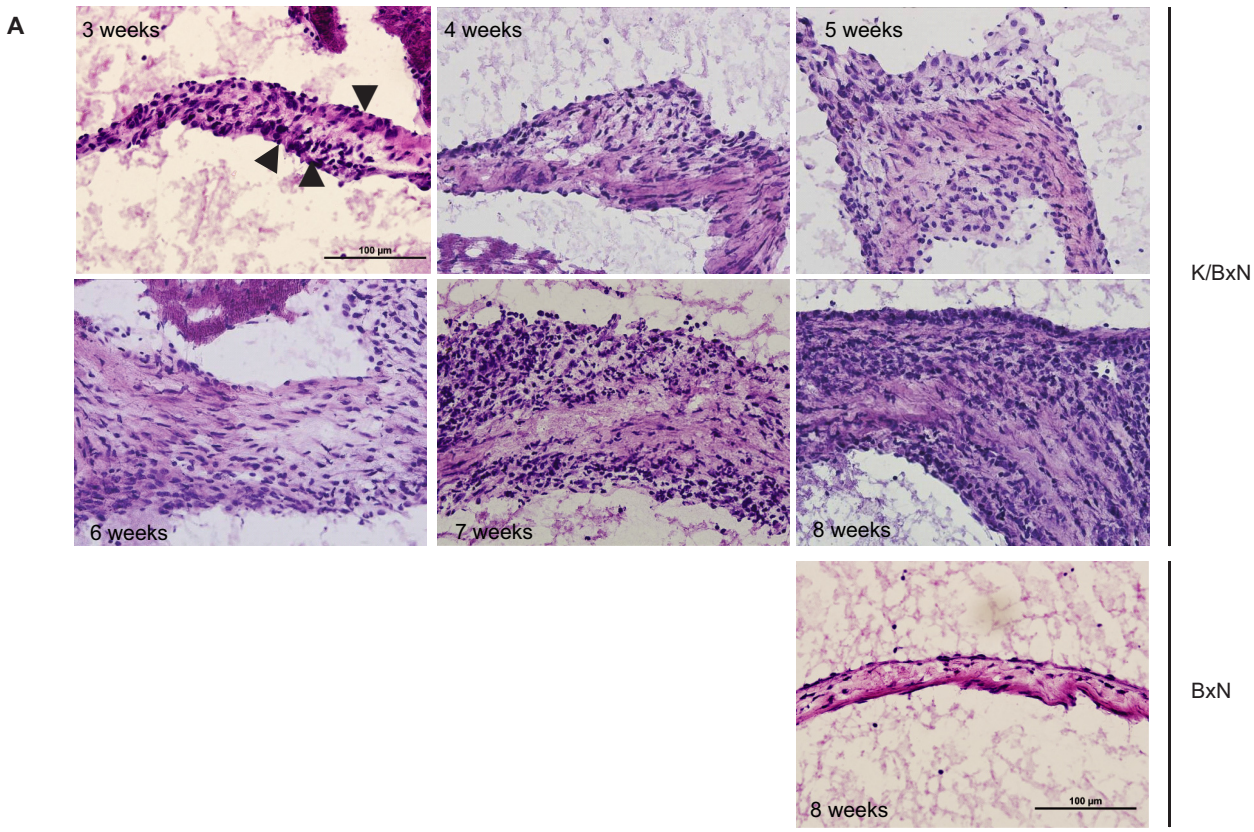
Supporting Information

Binstadt et al. 10.1073/pnas.0909132106



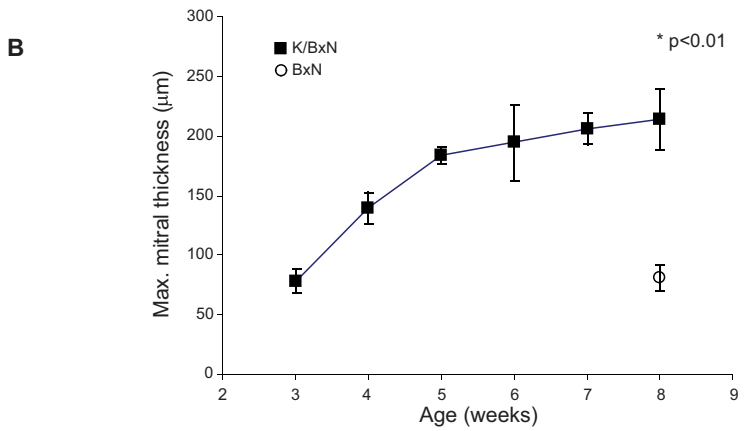


Fig. S1. Temporal development of endocarditis in K/BxN mice. (*A*) H&E staining of mitral valves from K/BxN mice at the indicated time points demonstrates progressive inflammation and thickening of the valves over time. Arrowheads (*Upper Left*) demonstrate sites of early inflammation. An example of a noninflamed mitral valve from an 8-week-old BxN mouse is shown for comparison. (Objective, 40×.) (Scale bars, *Upper Left* and *Lower Right*: 100 μm.) (*B*) The maximal thickness (mean, SEM) of the mitral valves of K/BxN mice is shown for the various time points (squares), along with the thickness of the mitral valve of 8-week-old disease-free BxN control mice (circle); $n = 3$ mice/time point. Student's t test was used to compare the data at the 8-week time point.

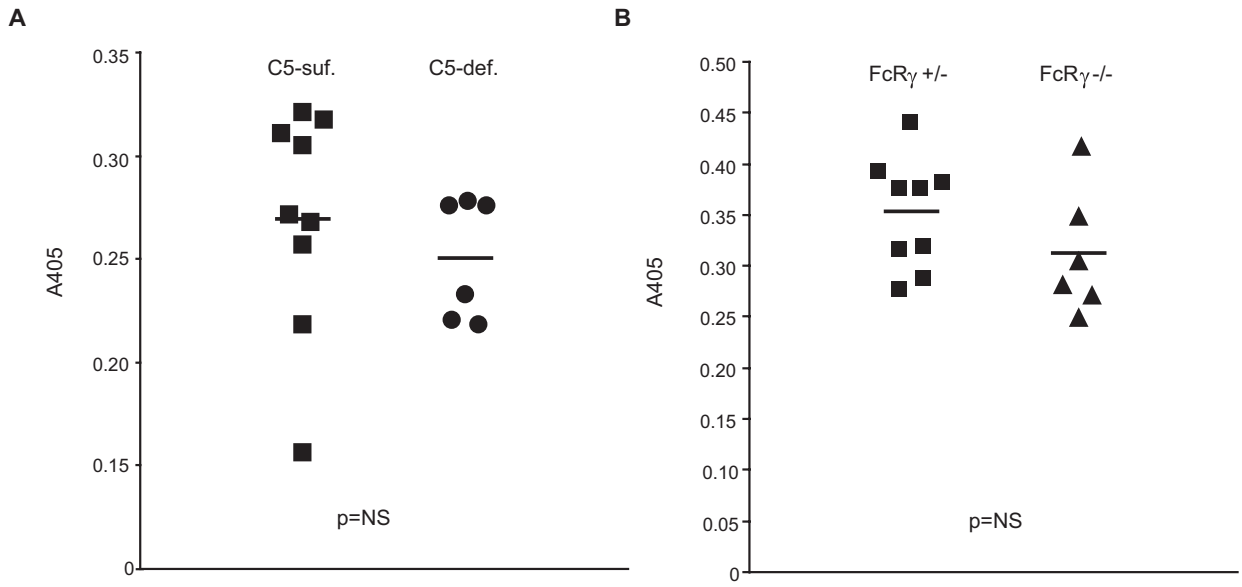


Fig. S2. Anti-GPI titers are not affected by absence of C5 or FcRγ. (*A*) The titers of anti-GPI IgG antibodies in serum (1/3,000 dilution) from C5-sufficient and C5-deficient K/BxN mice are indicated. Findings are pooled from 4 litters of mice and are representative of 4 additional litters. (*B*) The titers of anti-GPI IgG antibodies in serum (1/3,000) dilution from FcRγ+/+ and FcRγ−/− KRN+ $A^{9^{7+}}$ C57BL/6 mice are shown. Findings are pooled from 4 litters of mice. *P* values were calculated using Student's *t* test.

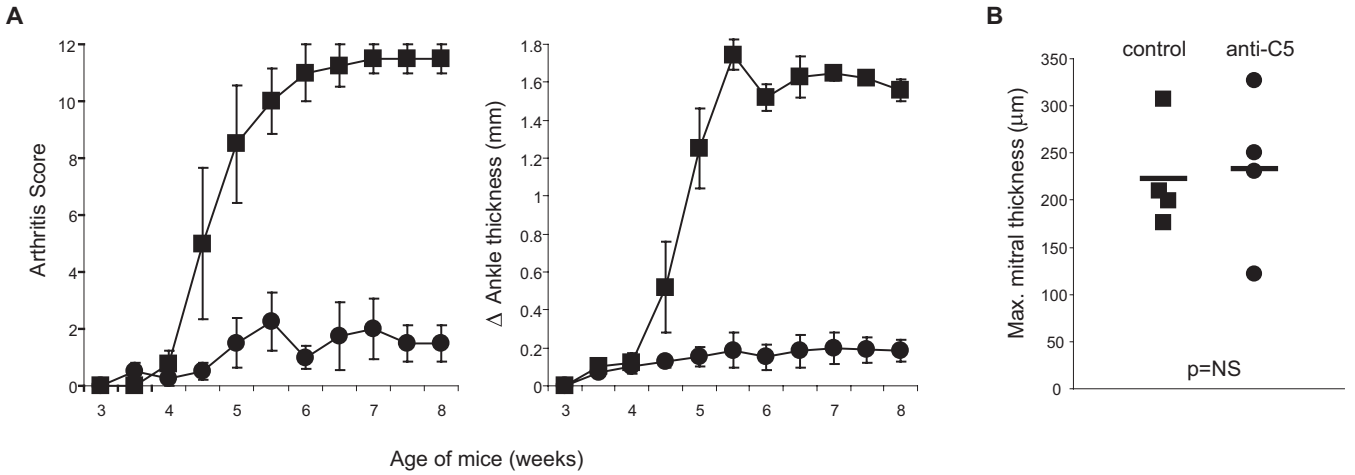


Fig. S3. Anti-C5 antibody treatment prevents arthritis without affecting cardiac valve inflammation in K/BxN mice. K/BxN mice were treated twice weekly with anti-C5 monoclonal antibody or isotype control antibody. (*A*) Arthritis scores (*Left*) and ankle thickness measurements (*Right*) show the reduction of arthritis severity in anti-C5-antibody-treated mice (circles) relative to controls (squares). (*B*) Mitral valve inflammation is not affected by anti-C5 antibody treatment (circles) relative to controls (squares); $n = 4$ mice/group; bar indicates the mean for the group.

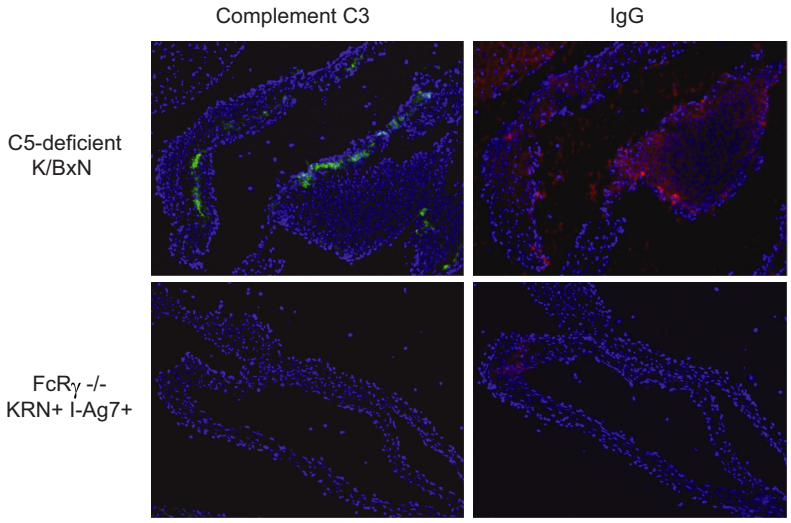


Fig. S4. Lack of IgG and C3 deposition in noninflamed mitral valves of FcRγ-deficient mice. Sections of mitral valves from C5-deficient K/BxN mice (*Top*) and FcRγ-deficient KRN+ A^{g7+} C57BL/6 mice (*Bottom*) were probed for the presence of complement (*Left*, green) and Ig (*Right*, red) as in Fig. 1. The slides are counterstained with DAPI to detect nuclei (blue). (Objective: ×10.)

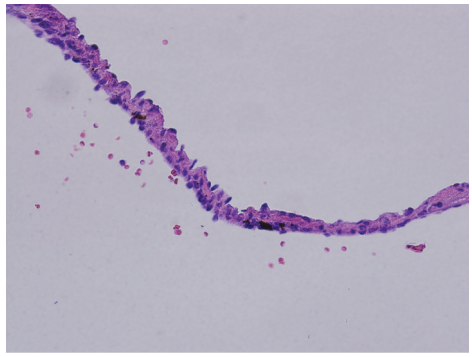


Fig. S5. Repetitive administration of arthritogenic K/BxN serum does not induce cardiac valve disease. Section of a mitral valve from a C57BL/6 mouse injected repetitively with serum pooled from K/BxN mice; 150 μL of serum was injected i.p. once weekly for 7 weeks. No inflammation is apparent. This specimen is representative of 5 similarly treated mice, none of which had cardiac valve inflammation. (Objective: $\times$40.)