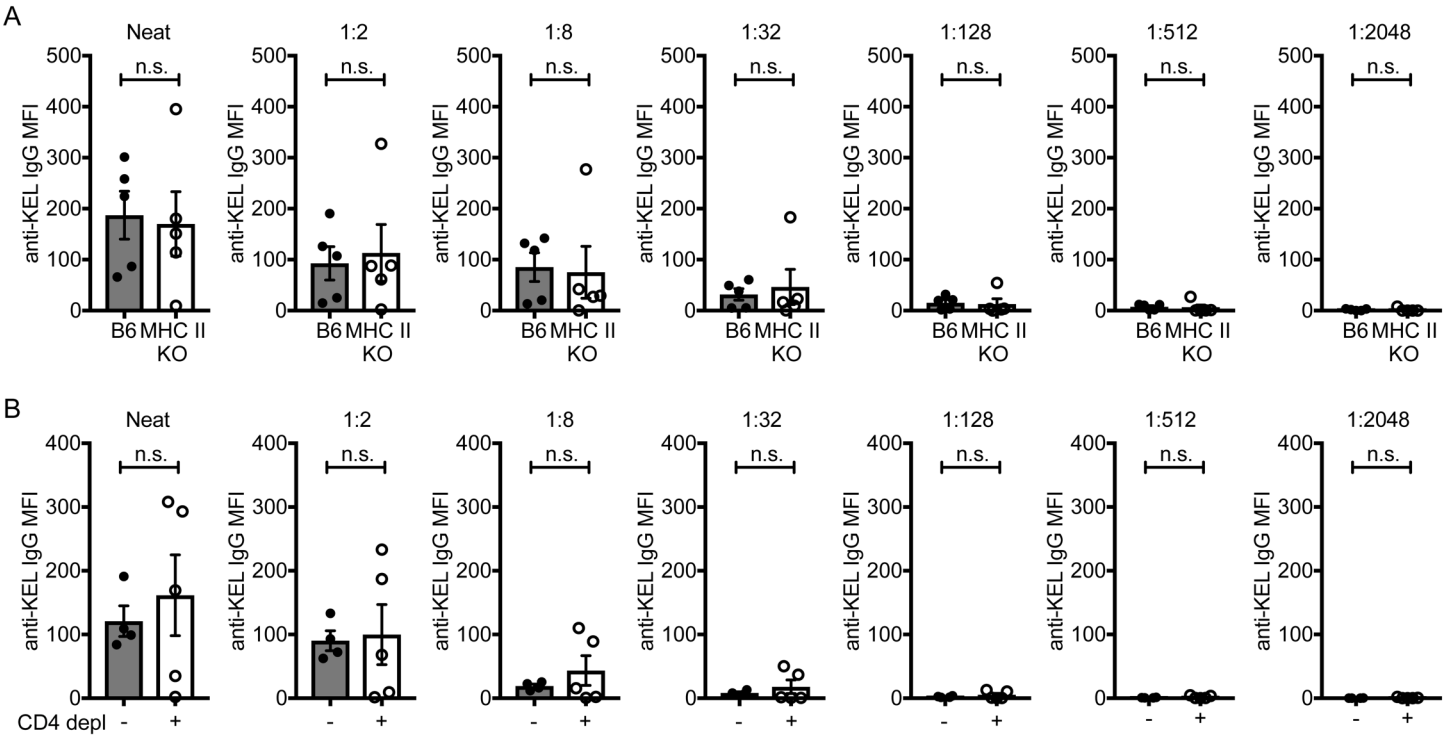
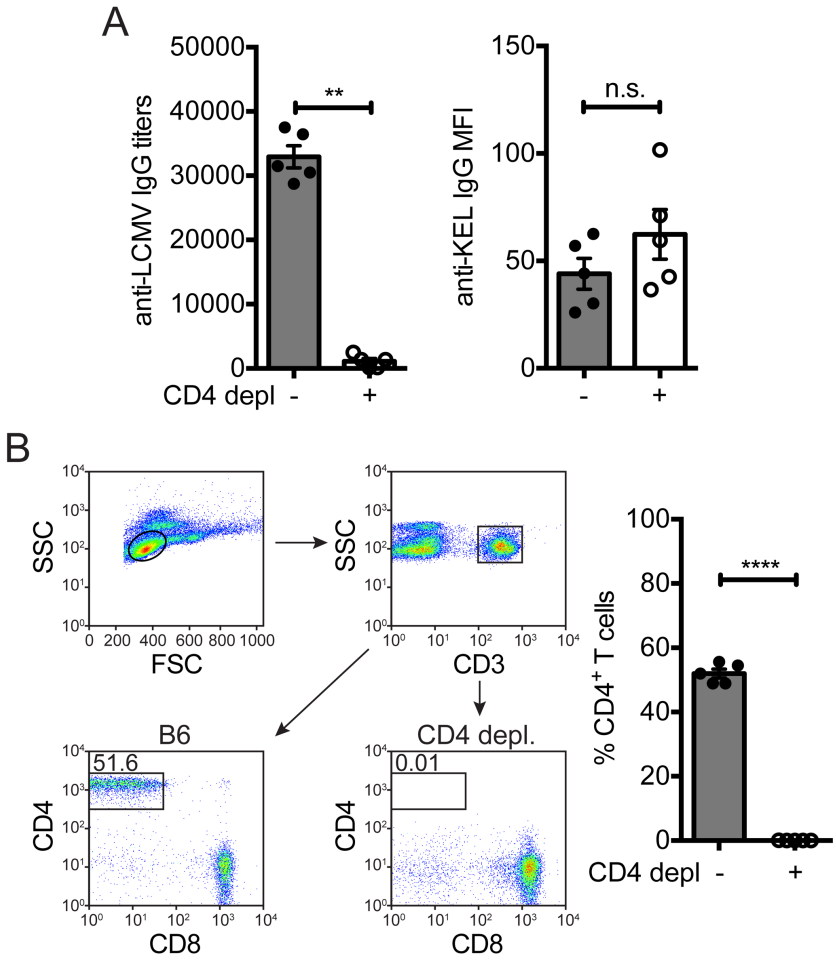
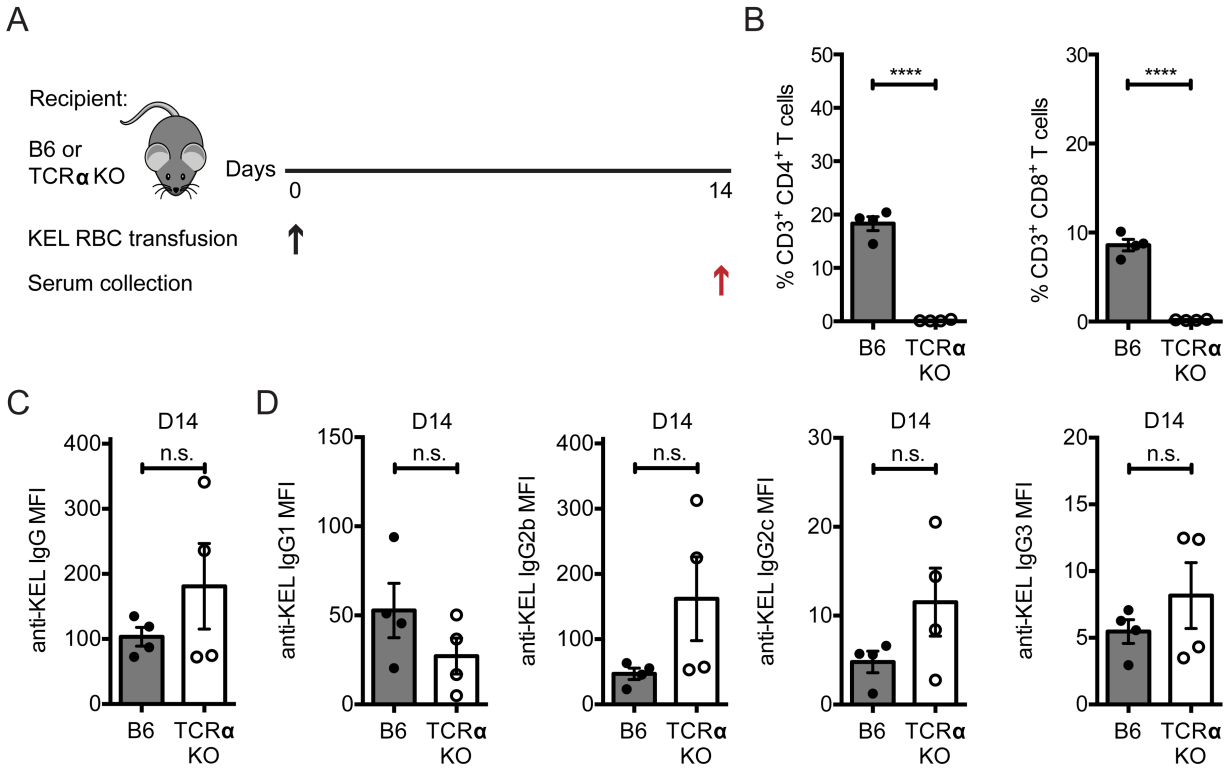


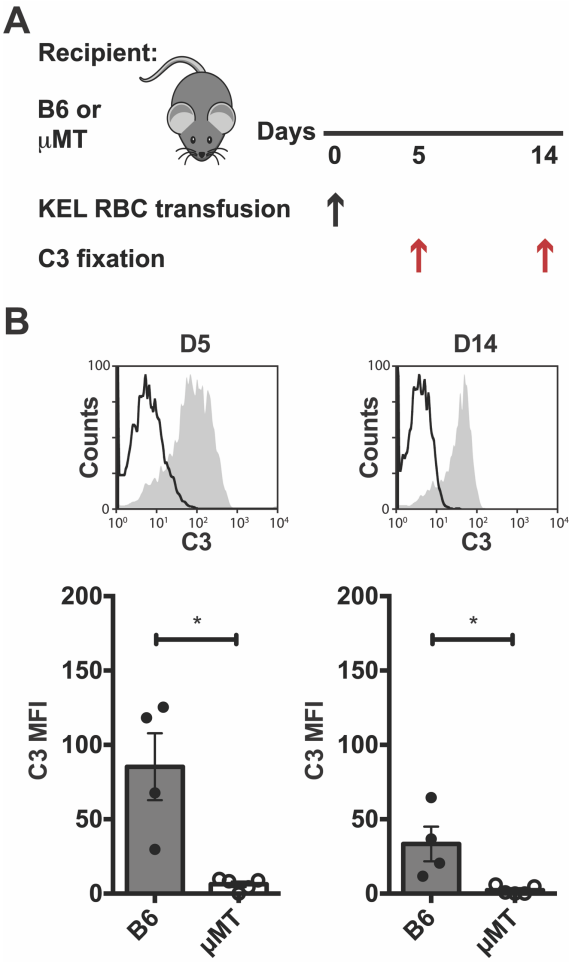
Supplementary Figure 1. Anti-KEL alloantibody class switching occurs independent of CD4$^+$ T cell help. **(A)** KEL specific IgG titration for serum isolated from B6 and MHC Class II KO (MHC II KO) recipients transfused with KEL RBCs. **(B)** Titration of anti-KEL IgG formation in PBS (-) or monoclonal anti-mouse CD4 depleting antibody (+) treated B6 recipients transfused with KEL RBCs. Serum was collected on day 14 post transfusion in panels **(A)** and **(B)**, and serological analysis for anti-KEL IgG alloantibodies in panels **(A)** and **(B)** was examined by indirect immunofluorescence staining using KEL and B6 RBCs, and neat serum. The MFI in all panels was computed by normalizing the MFI of samples incubated with KEL RBCs to background control B6 RBCs. There were 5 mice per group. All panels show representative data from experiments reproduced 3 times. Errors bars represent mean ± SEM. Statistics were generated using a Mann Whitney test in panels. n.s. indicates not statistically significant.



Supplementary Figure 2. Treatment with anti-CD4 depleting antibody prevents anti-LCMV antibody formation. **(A)** B6 recipients were treated with PBS (-) or monoclonal anti-mouse CD4 depleting antibody (+) 4 and 2 days prior to LCMV (clone: 13) infection or transfusion of KEL RBCs. Serum was collected 15 days post infection or transfusion. ELISA was used to examine sera for anti-LCMV IgG, and indirect immunofluorescence staining using neat serum as well as KEL and B6 RBCs was performed to test for anti-KEL IgG antibodies. **(B)** Representative flow plots with graphical illustration of percent peripheral blood $CD3^+$ $CD4^+$ T cells in KEL negative B6 recipients treated with PBS (-) or monoclonal anti-mouse CD4 depleting antibody (+). Error bars represent mean $\pm$ SEM. Statistics were generated using an unpaired Mann Whitney test in panel **(A)** and an unpaired parametric test in panel **(B)**. There were 5 mice per group. All panels show representative data from experiments reproduced 3 times. **, $p < 0.001$; ****, $p < 0.0001$ and n.s. indicates not statistically significant.



Supplementary Figure 3. TCRα KO mice generate an anti-KEL alloantibody response following KEL RBC transfusion. (A) Experimental schematic of anti-KEL antibody formation analysis in B6 and TCRα KO recipients transfused with KEL RBCs. **(B)** Percent $CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$ T cells in the peripheral blood of TCRα KO recipients. **(C)** Serological analysis of anti-KEL IgG (day 14 post transfusion = D14) in B6 recipients and TCRα KO recipients transfused with KEL RBCs. **(D)** Anti-KEL IgG subtype (IgG1, IgG2b, IgG2c, and IgG3) analysis in the serum of wild type B6 and $CD4^+$ T cell deficient TCRα KO recipients 14 days (D14) post transfusion of KEL RBCs. Serological analysis of anti-KEL antibody formation in panels **(C)** and **(D)** was examined by indirect immunofluorescence staining using KEL and B6 RBCs, and neat serum. The MFI in panels **(C)** and **(D)** was computed by normalizing the MFI of samples incubated with KEL RBCs to background control B6 RBCs. Error bars represent mean ± SEM. Statistics were generated using an unpaired parametric test in panel **(B)** and an unpaired Mann Whitney test in panels **(C)** and **(D)**. There were 4 mice per group. All panels show representative data from experiments reproduced 3 times. ****, $p < 0.0001$ and n.s. indicates not statistically significant.



Supplementary Figure 4. C3 does not fix on KEL RBCs transfused into recipients deficient in B cells and thereby antibodies. **(A)** B6 and μMT recipients were transfused with KEL-DiI RBCs and evaluated for C3 deposition on transfused KEL RBCs on 5 (D5) and 14 (D14) days post transfusion. **(B)** Representative and graphical illustration of C3 deposition on transfused KEL RBCs in B6 and μMT recipients 5 and 14-days following RBC transfusion. The MFI for C3 in panel **(B)** was calculated by normalizing the MFI of KEL-DiI RBCs stained for C3 to the background control KEL-DiI RBCs incubated with a streptavidin secondary only. Errors bars represent mean ± SEM. Statistics were generated using a Kruskal-Wallis with Dunn's multiple comparison test. There were 5 mice per group. All panels show representative data from experiments reproduced 3 times. *, $p < 0.05$; **, $p < 0.01$ and n.s. indicates not statistically significant.

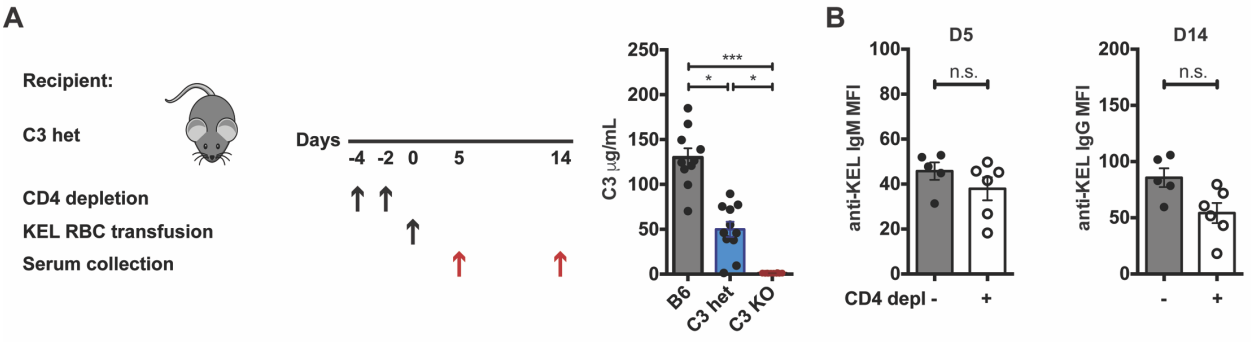
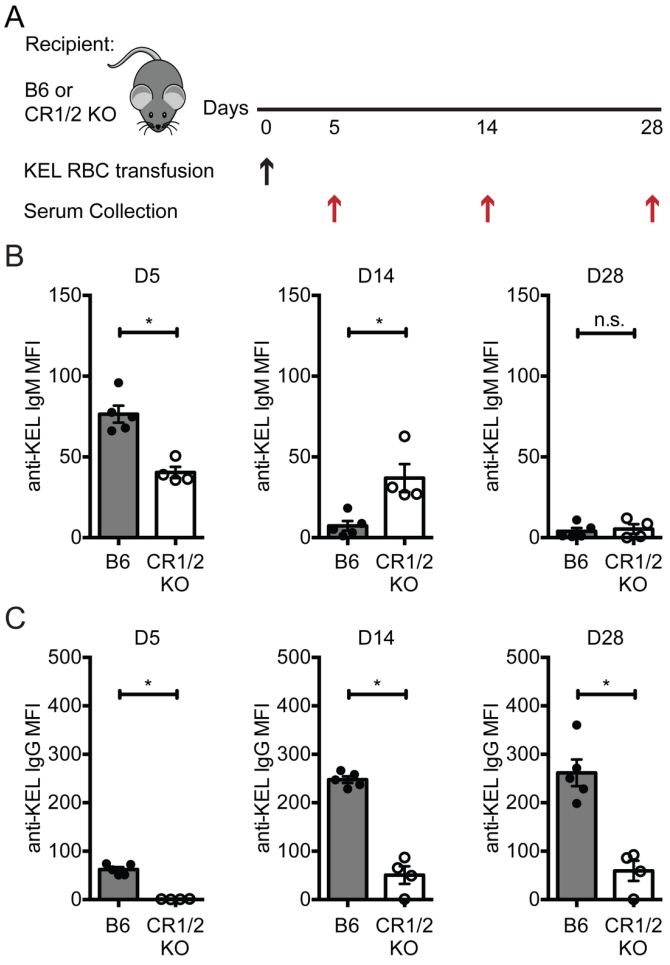


Figure 5. CD4$^+$ T cell depleted recipients that are partially deleted of C3 develop qualitatively and functionally similar anti-KEL alloantibodies as CD4$^+$ T cell depleted B6 recipients. **(A)** C3 heterozygous (C3 het) recipients were treated with PBS (-) or monoclonal anti-mouse CD4 depleting antibody (+) 4 and 2 days prior to collection of baseline serum and transfusion of KEL RBCs. ELISA was used to determine baseline C3 serum levels. **(B)** Serological analysis of anti-KEL IgM and IgG development was evaluated on days 5 (D5) and 14 (D14) post-transfusion. Anti-KEL antibody formation was examined through indirect immunofluorescence staining using KEL and B6 RBCs, and neat serum. The MFI in panel **(B)** was computed by normalizing the MFI of samples incubated with KEL RBCs to background control B6 RBCs. Error bars represent mean $\pm$ SEM. Statistics were generated using Kruskal-Wallis with Dunn's multiple comparison-test in panel **(A)** or an unpaired Mann Whitney test in panel **(B)**. There were either 10 **(A)** or 5 **(B)** mice per group. All panels show representative data from experiments reproduced 2 times. *, $p < 0.05$; **, $p < 0.01$ and n.s. indicates not statistically significant.



Supplementary Figure 6. Alloantibody response to KEL is decreased in the absence of CR1/2. (A) Experimental schematic of anti-KEL antibody formation analysis in B6 and CR1/2 KO recipients transfused with KEL RBCs. Ant-KEL IgM **(B)** and IgG **(C)** was examined on 5 (D5), 14 (D14) and 28 (D28) days post transfusion through indirect immunofluorescence staining using KEL and B6 RBCs, and neat serum. Normalizing the MFI of samples incubated with KEL RBCs to background control B6 RBCs generated the MFI in both panels. There were 5 (B6) or 4 (CR1/2 KO) mice per group. All panels show representative data from experiments reproduced 3 times. Errors bars represent mean $\pm$ SEM. Statistics were generated using unpaired Mann Whitney test. *, $p < 0.05$ and n.s. indicates not statistically significant.