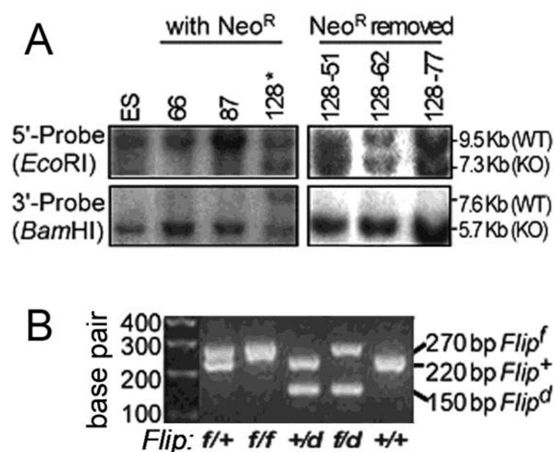


Figure S1. Generation of *Flip* conditional Knock out mouse. (A). Southern blot analysis of *Flip* targeted mouse ES cells. Genomic DNA of neomycin resistant ES clones was digested by EcoRI and hybridized with ^{32}P-labeled 5'- probe (top panel), or digested by *Bam*HI and hybridized with 3'-probe (lower panel). Clone #128 displays the 7.3-kb targeted allele identified by the 5'-probe and the 7.6-kb targeted allele identified by the 3'-probe. The presence of the wild-type (WT) allele (9.5Kb by 5'-probe and 5.7 Kb by 3'-probe) indicates these cells are heterozygous for the targeted *Flip* allele. In clones #66 and #87 the targeting construct was randomly inserted. The *neo*^{R} cassette was removed from 3 clones derived from clone 128 (clones 51, 62, 77), indicated by the 3'-probe that hybridized with a 5.7 Kb fragment, which were employed to generate chimeric mice by blastocyst injection and embryo re-implantation in C57BL/C mice. (B) PCR genotyping of *Flip* by genomic DNA extracted from tail biopsies. Three *Flip* alleles were identified: 270bp for floxed (*Flip*^{f}), 220bp for WT (*Flip*$^+$) and 150bp for deleted (*Flip*^{d}).

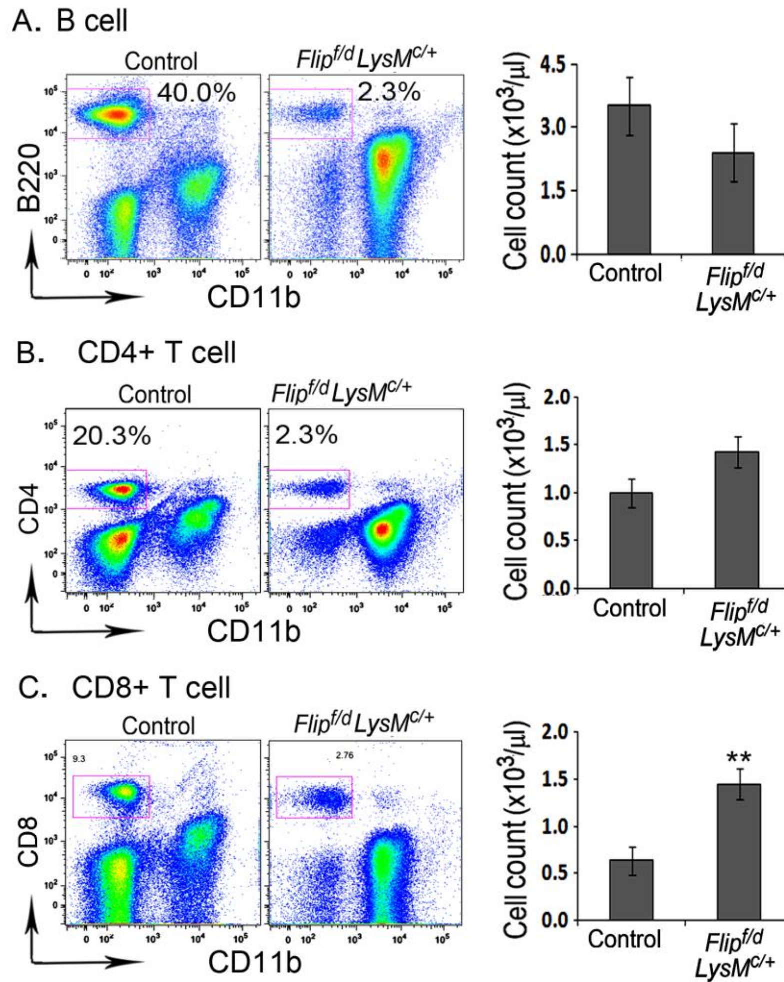


Figure S2. Analysis of lymphocytes in peripheral blood. The B cells (A) and CD4+ (B) or CD8+ (C) T cells in the peripheral blood of control or *Flip*$^{f/d}$*, LysM* $^{c/+}$ mice were assessed by flow cytometry. Lymphocytes were identified as CD45+, CD11b-, NK1.1- and CD11c-, plus B220+, CD4+, CD8+ for B cells and CD4+ or CD8+ T cells, respectively (n = 8 for each group). A representative example of the distribution of B cells and CD4+ or CD8+ T cells in total CD45+ population is presented on the right. The absolute counts for each cell type is presented based on the total white blood cell counts which were 7.9 ± 1.3 (± 1 SE) for the controls and 49.1 ± 7.3 (x10^4/µl blood) for the *Flip*$^{f/d}$*, LysM* $^{c/+}$ mice (p=0.002). ** represents $p < 0.01$ compared with controls.

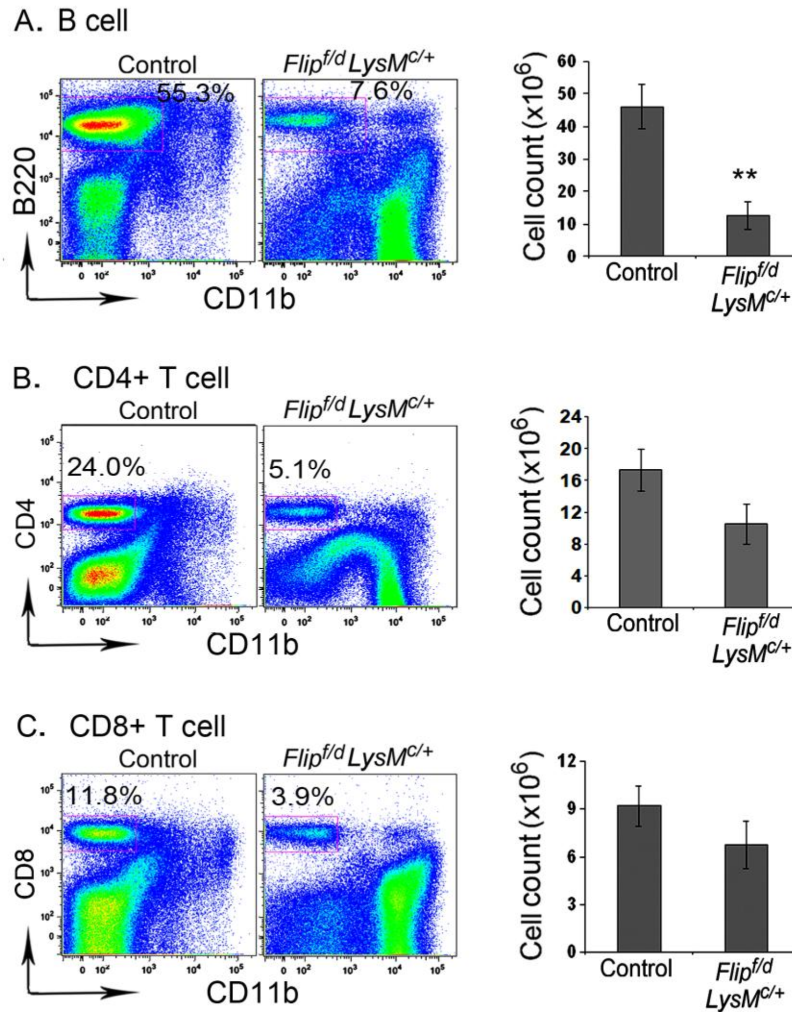


Figure S3. Analysis of lymphocytes in spleen. The presence of B cells (A) and CD4+ (B) or CD8+ (C) T cells in the spleens of control and *Flip*$^{f/d}$, *LysM* $^{c/+}$ mice was determined by flow cytometry (n = 4 for each represented group). Lymphocytes were identified as indicated in Supplemental Figure 2. Representative examples of the distributions of B cells and CD4+ and CD8+ T cells are presented in the panels on the left. The total numbers of each cell type were determined based on the number of live splenocytes in each group (87.5 ± 11.6 and 147.5 ± 25.9 (x10^6) for control and *Flip*$^{f/d}$, *LysM* $^{c/+}$ mice). ** represents $p < 0.01$ compared with controls.

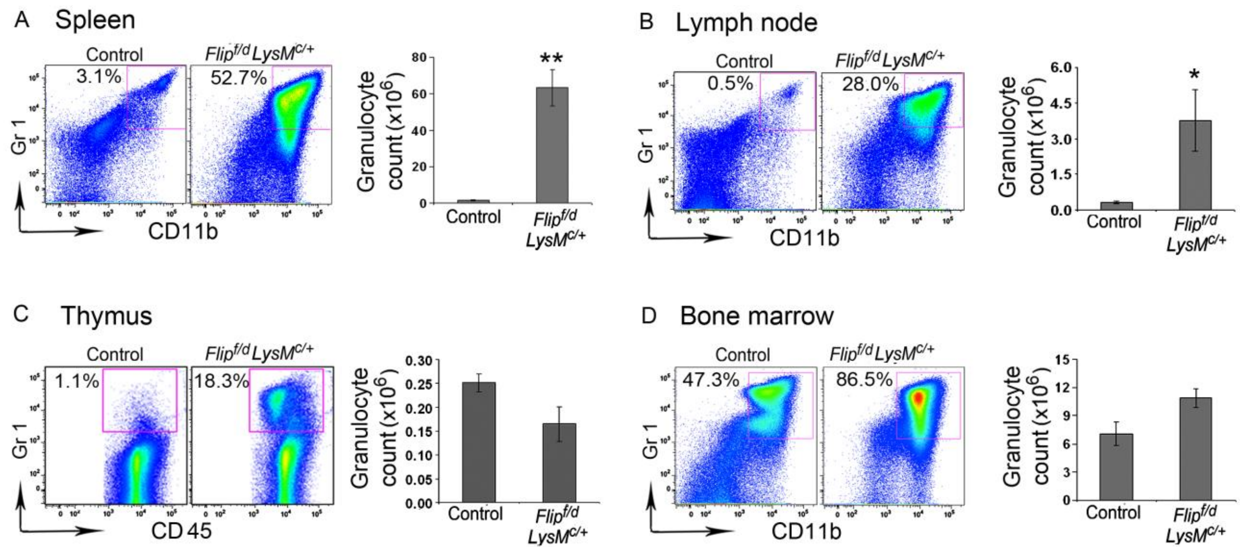


Figure S4. Analysis of granulocytes in the lymphoid organs. Granulocyte infiltration in spleen(A), lymph node (B), thymus(C) and bone marrow (D) of control and *Flip$^{f/d}$*, *LysM* $^{c/+}$ mice were assessed by flow cytometry. Granulocytes were identified as CD45+, CD11b+, NK1.1-, CD11c-, B220-, CD4-, CD8- and Gr1+ (n = 4 for each represented group). A representative example of the distribution of Gr1+ and 11b+ granulocytes in total CD45+ population is presented on the left of each panel. The total numbers (x10^6) of live cells in the littermate controls and *Flip$^{f/d}$*, *LysM* $^{c/+}$ mice were: 87.5 ± 11.6 and 147.5 ± 25.9 (splenocytes); 141.3 ± 12.8 and 25.4 ± 10.1 (cells from lymph nodes, $p < 0.01$); 222.5 ± 31.7 and 5.1 ± 2.4 (thymocytes, $p < 0.001$) and 16.0 ± 2.3 and 12.8 ± 1.2 (cells from tibias). * represents $p < 0.05$ and ** $p < 0.01$ compared with control groups.

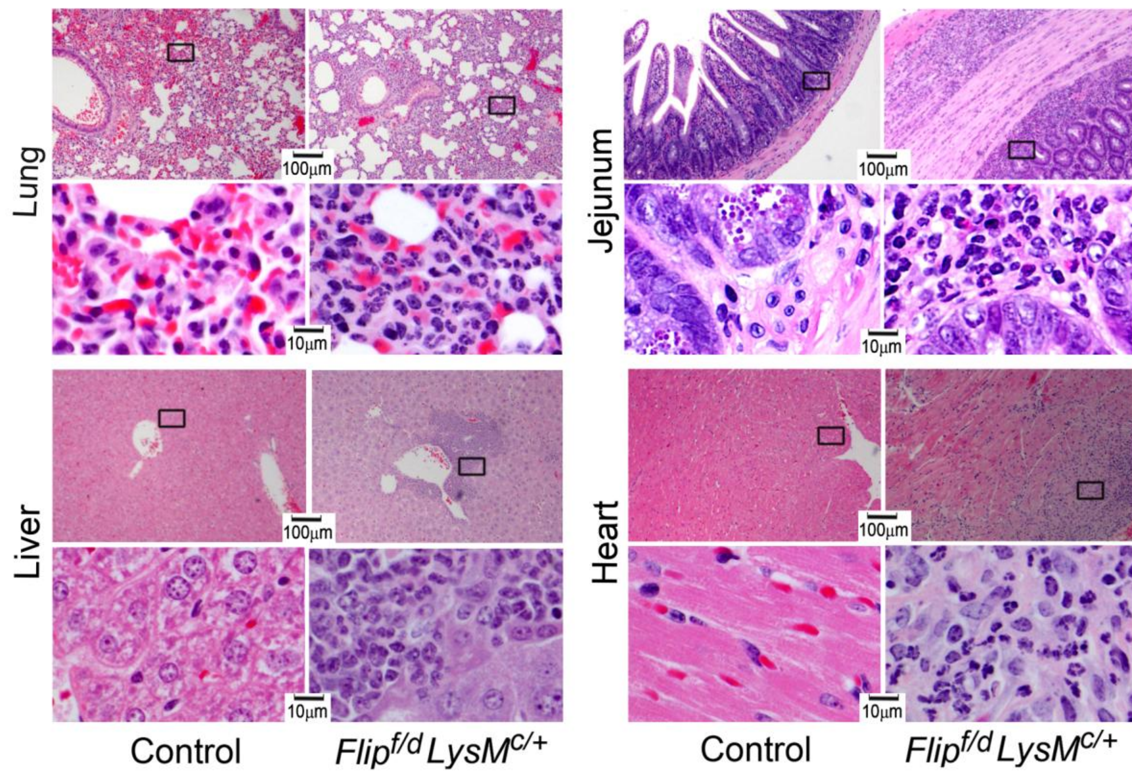


Figure S5. Increased leukocyte infiltration in non-lymphoid organs.

Representative tissue sections from the indicated organs from *Flip*$^{f/d}$, *LysM* $^{c/+}$ and littermate control mice were stained with hematoxylin and eosin. The area identified by the box is enlarged in the panel below.

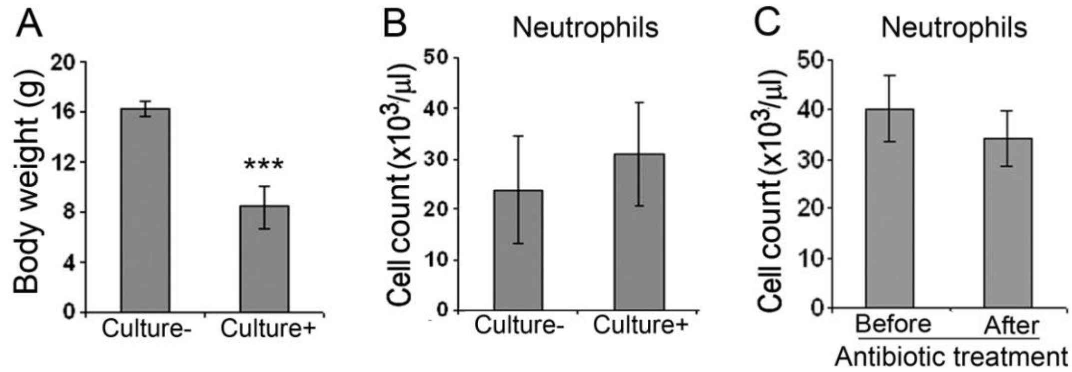
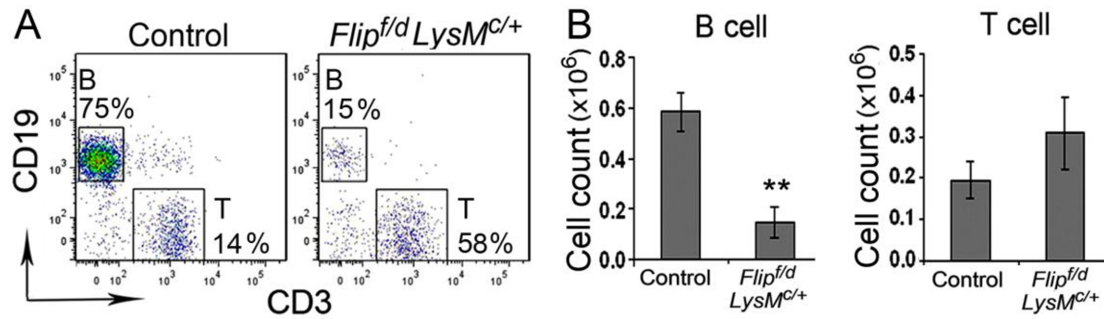


Figure S6. Analysis of infection in *Flip$^{f/d}$, LysM$^{c/+}$* and control mice. The peritoneal cavity, thymus, spleen, liver, and lymph nodes of *Flip$^{f/d}$, LysM$^{c/+}$* (n = 13) and control mice were cultured. Body weight (A) and neutrophil counts (B) were determined in the culture positive (n=4) and negative mice (n = 9). *Flip$^{f/d}$, LysM$^{c/+}$* mice (n = 20) were treated with ampicillin +/- gentimycin, and circulating neutrophil counts were performed before and after treatment (C). *** represents $p < 0.001$.



FigureS7. **Analysis of lymphocytes in the peritoneum.** (A) A representative example of lymphocytes ($CD11b^-$) stained with anti-CD19 and -CD3 employing control and *Flip*$^{f/d}$, *LysM* $^{c/+}$ mice. (B) The number of B and T lymphocytes present in the peritoneal cavity was determined following flow cytometry (n = 8 and 13 for control and KO, respectively). ** represents $p < 0.01$.

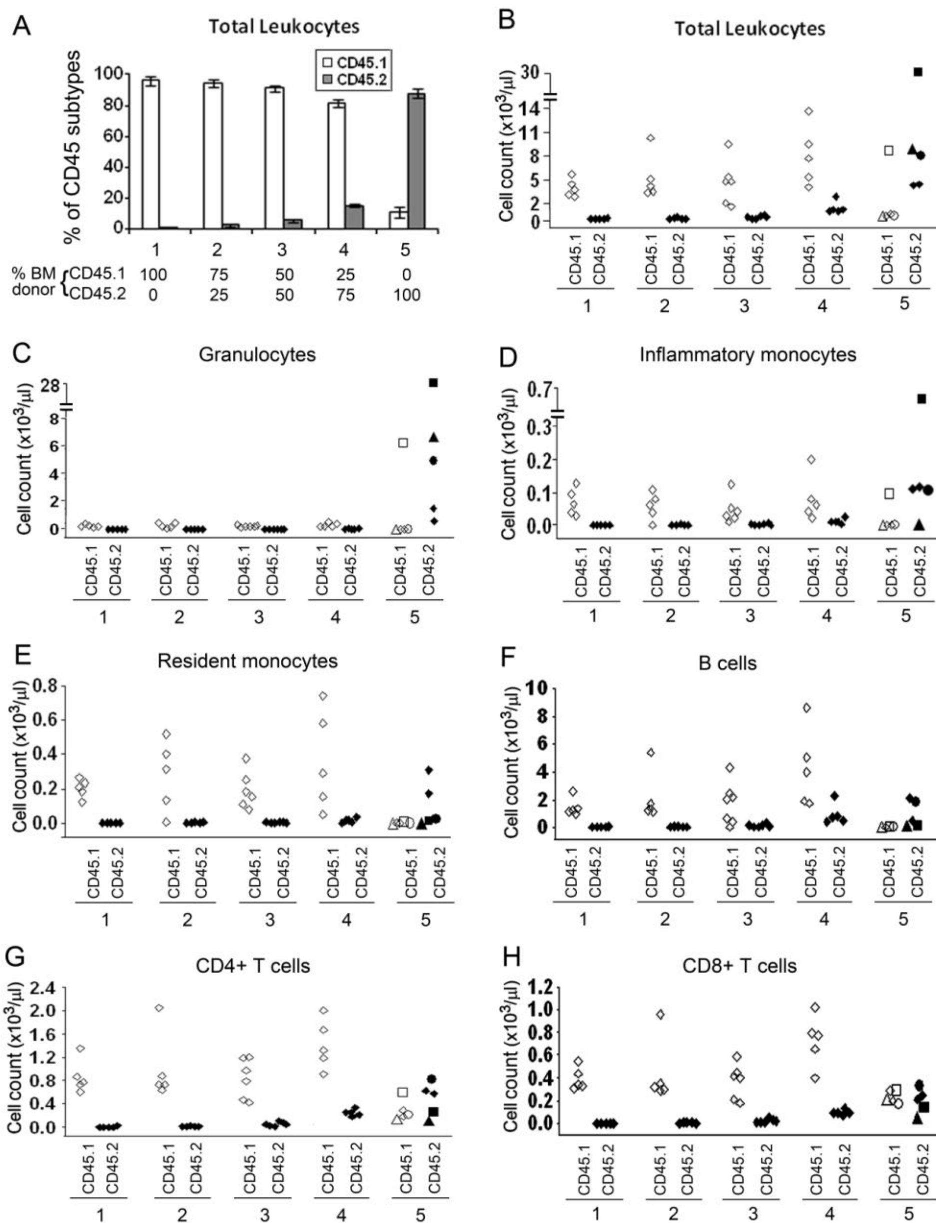


Figure S8. ***Flip***$^{f/d}$***, LysM*** $^{c/+}$ **mice demonstrate a cell intrinsic defect in bone marrow transplants.** (A) *Flip*$^{f/d}$, *LysM* $^{c/+}$ (CD45.2^{+}) bone marrow cells failed to compete with wild bone marrow cells (CD45.1^{+}), indicated by the % of CD45 subtypes in peripheral blood 8 weeks post transplantation. (B-H) The cell numbers were calculated for the indicated cell types from peripheral blood (10^3/μl) by total white blood cell count and flow cytometry in mice from groups 1-

5, which received *Flip*$^{f/d}$, *LysM* $^{c/+}$ donor BM cells at 0, 25, 50, 75 and 100%, respectively. Cells from CD45.1 and CD45.2 subtype were indicated as open and solid symbols. Three individual mice in group 5 were specified by matched symbols.