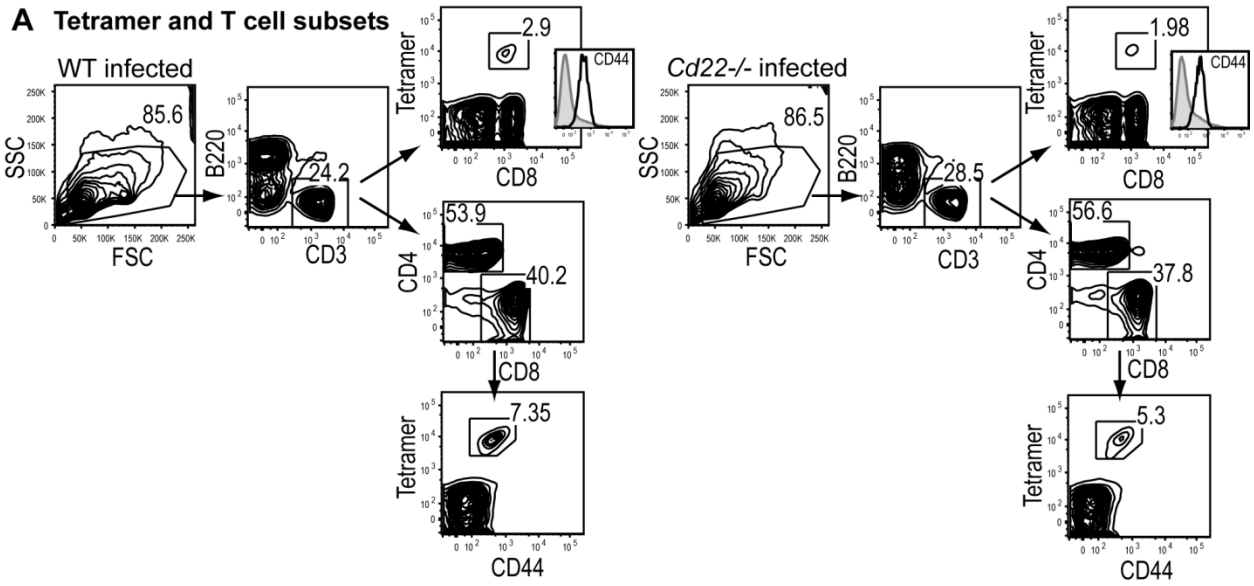
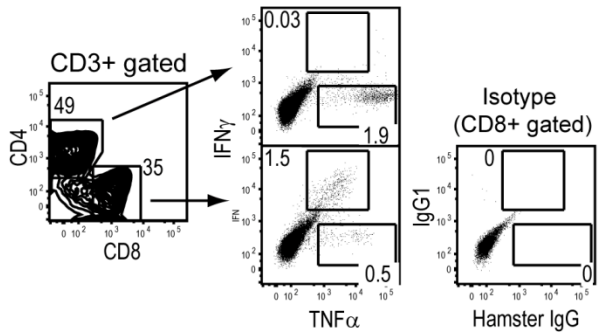
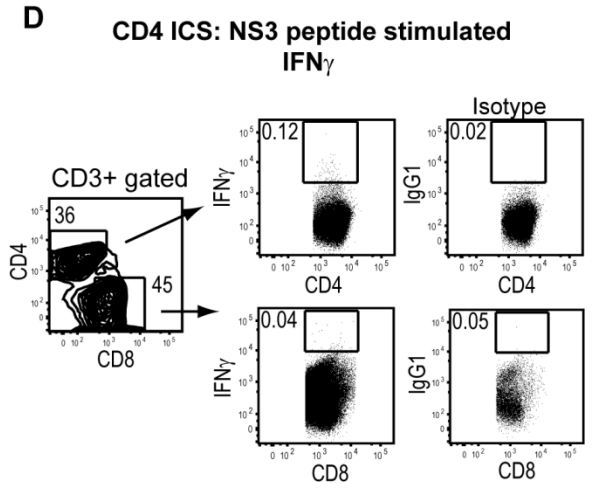

A Tetramer and T cell subsets
WT infected
SSC
FSC
85.6
B220
CD3
24.2
Tetramer
CD8
2.9
CD44
CD4
53.9
40.2
CD8
Tetramer
7.35
CD44
Cd22-/- infected
86.5
28.5
1.98
56.6
37.8
5.3


B
CD8 ICS: NS4B peptide stimulated
IFNγ, TNFα
CD3+ gated
CD4
49
35
CD8
IFNγ
0.03
1.9
1.5
0.5
TNFα
Isotype
(CD8+ gated)
IgG1
0
0
Hamster IgG


D
CD4 ICS: NS3 peptide stimulated
IFNγ
CD3+ gated
CD4
36
45
CD8
IFNγ
0.12
0.04
CD8
Isotype
IgG1
0.02
0.05
CD8

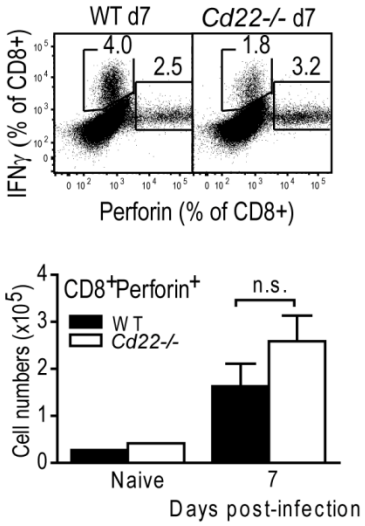

C
CD8 ICS: NS4B peptide stimulated
Perforin
WT d7
Cd22-/- d7
IFNγ (% of CD8+)
4.0
2.5
1.8
3.2
Perforin (% of CD8+)
CD8+Perforin+
n.s.
WT
Cd22-/-
Cell numbers (x10^5)
Naive
7
Days post-infection

Figure S1. Gating strategies for T cells in the spleen

Representative flow plots show gating strategies for distinguishing T cell subsets, tetramer staining, and ICS. All plots represent staining on infected mice. Numbers indicate frequency of parent populations. A) NS4B tetramer staining. Inset histograms show staining of CD44 (bold) on $CD3^+CD8^+Tetramer^+$ populations over isotype (shaded). B) ICS staining on NS4B peptide stimulated splenocytes for $IFN\gamma^+TNF\alpha^+$ and $TNF\alpha^+$ subsets of $CD8^+$ T cells with respective controls. C) ICS staining on NS4B peptide-stimulated splenocytes for perforin-producing $CD8^+$ T cells (top) and quantification of perforin-producing $CD8^+$ T cells at day 7 p.i. (bottom) D) ICS staining on NS3 peptide-stimulated splenocytes for IFNγ with respective controls in $CD4^+$ T cells.

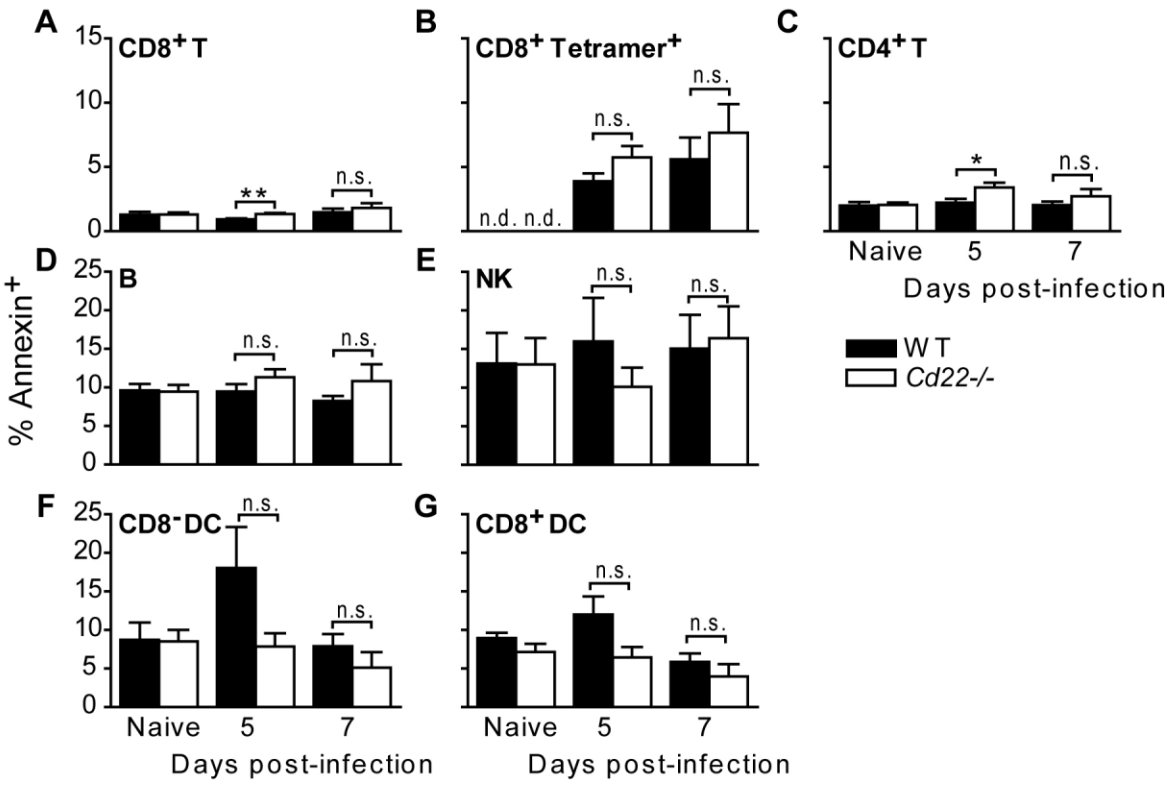


Figure S2. Annexin V staining of splenocyte populations

Splenocytes were isolated at various timepoints from WT and *Cd22*$^{-/-}$ mice, and stained with surface markers, Annexin V and Live/Dead stain as described in Materials and Methods. Cells were gated on A) total CD8^{+} T cells; B) CD8^{+}Tetramer^{+}; C) total CD4^{+} T cells; D) B cells; E) NK cells; F) total CD8^{-} DCs; G) CD8^{+} DCs. Bar graphs show the frequency of Annexin^{+}Live/Dead^{-} cells. Data are from three independent experiments with 3-4 mice per timepoint per group. Statistics were performed using a Student's t-test where *p<0.05, **p<0.01 and n.s. = not significant; n.d. = not detected.

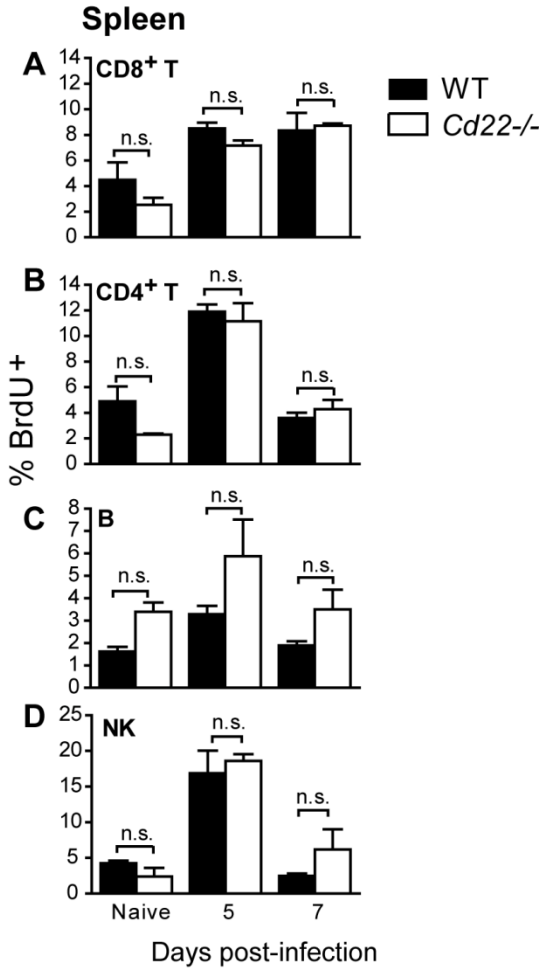


Figure S3. BrdU incorporation in splenic lymphocyte populations

WT and *Cd22*$^{-/-}$ mice were inoculated s.c. with 10^3 PFU as described. 24 hours prior to tissue harvest at indicated timepoints, mice were given one dose of 1 mg BrdU by i.p. injection. Spleens were harvested as described, and surface stained for A) $CD8^+$ T cells, B) $CD4^+$ T cells, C) B cells and D) NK cells prior to ICS to detect BrdU incorporation. Data show one representative of three independent experiments with at least three mice per group. Statistics were performed using a Student's t-test where n.s. = not significant.

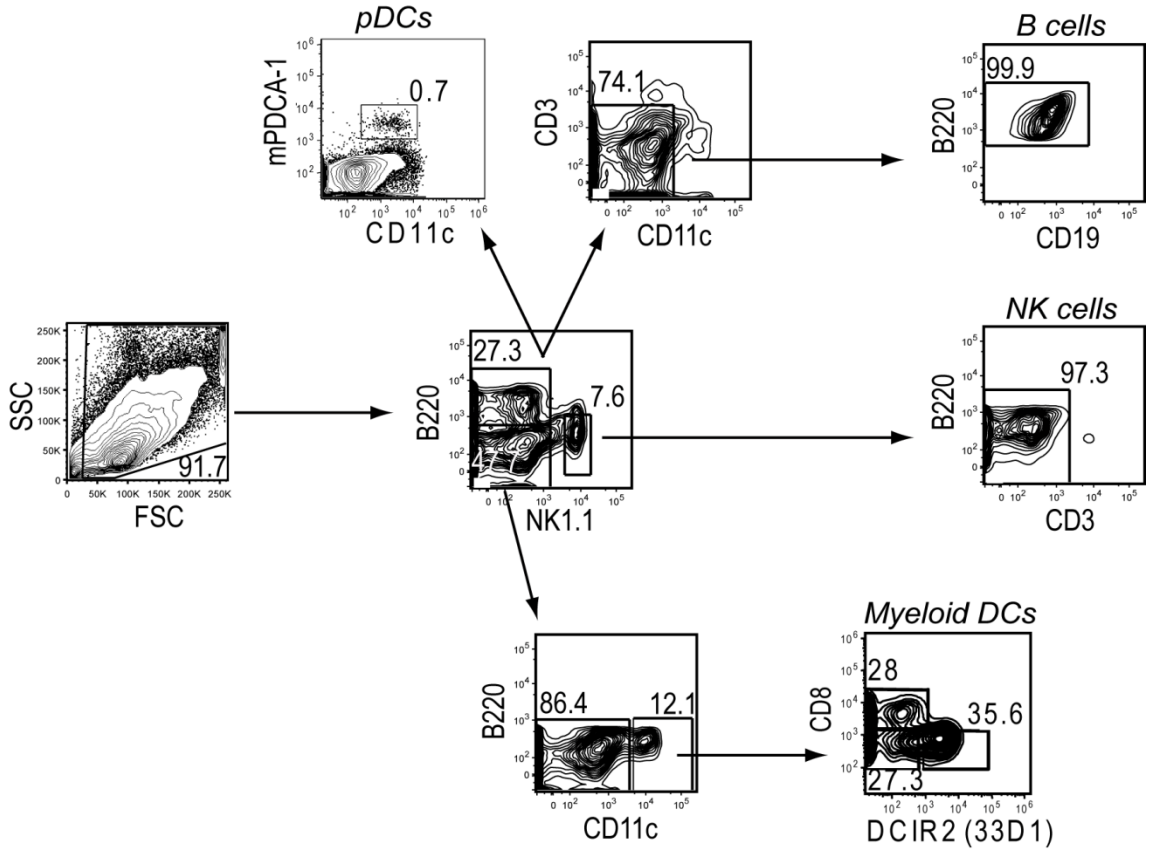


Figure S4. Gating strategy for B cells, pDCs, NK cells and myeloid DC subsets in the spleen

Representative plots showing gating strategy for various cell types in the spleen. Numbers show frequency of parent populations.