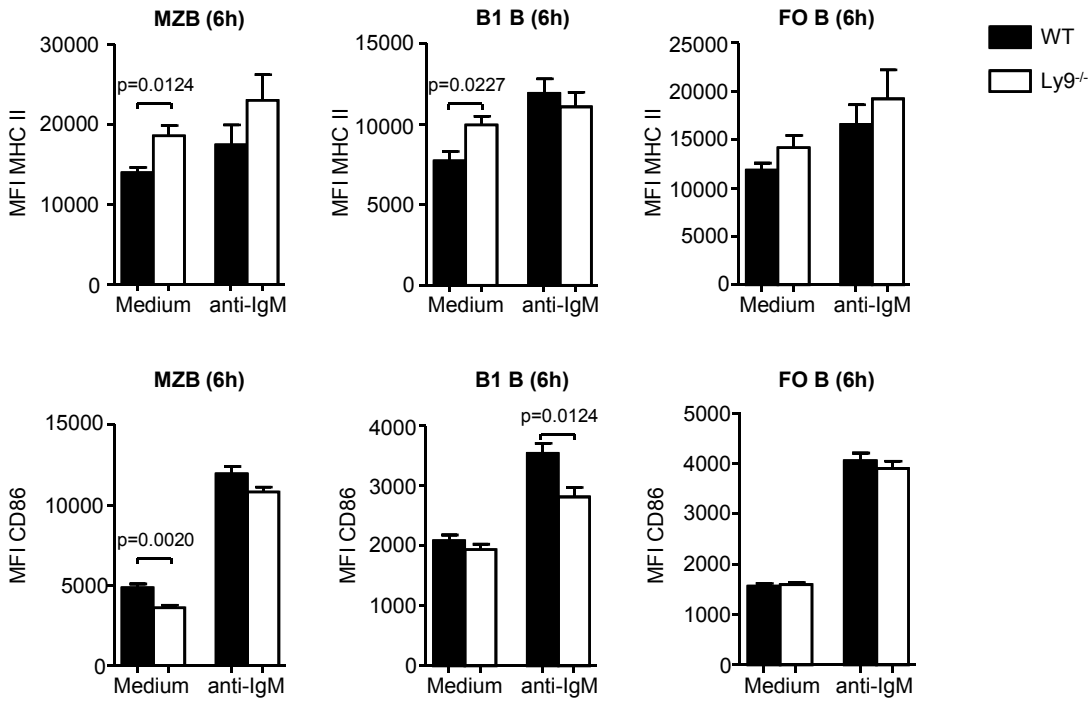
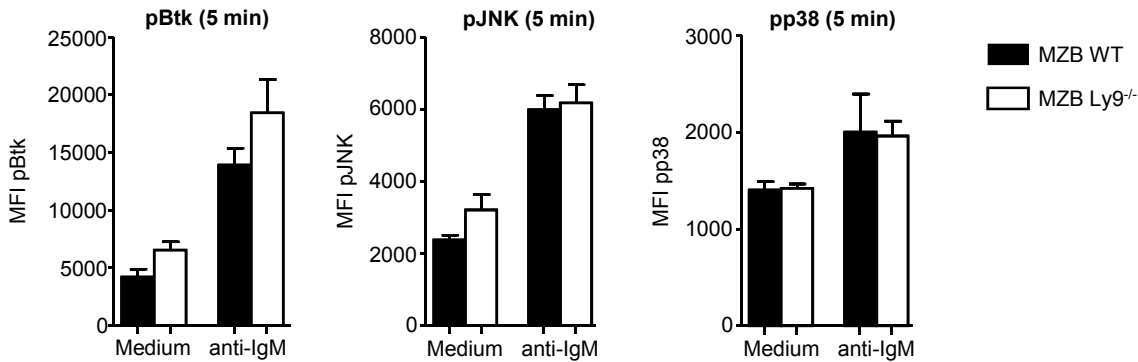


Supplemental Figure 1

A



B

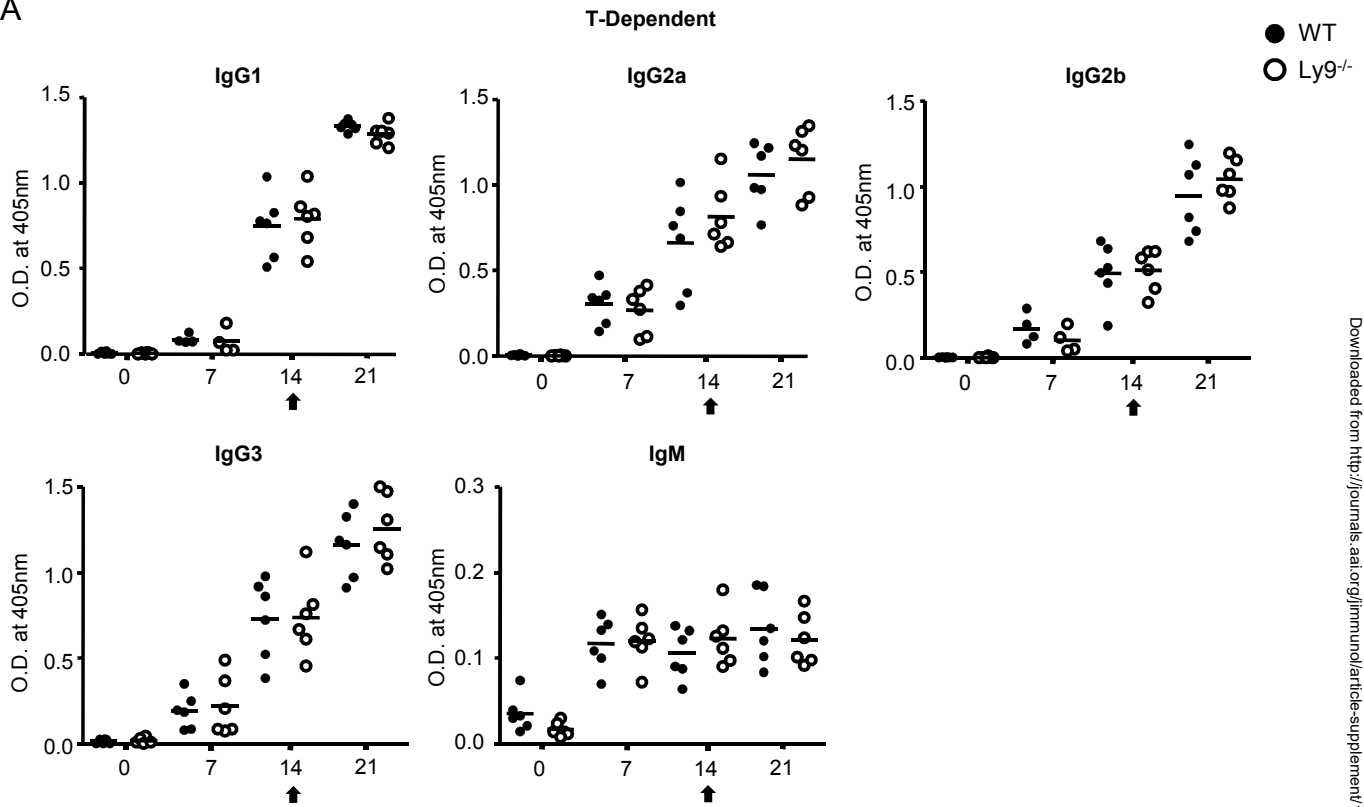


Supplemental Figure 1. MZB expansion in Ly9^{-/-} spleen is not due to an increased activation state.

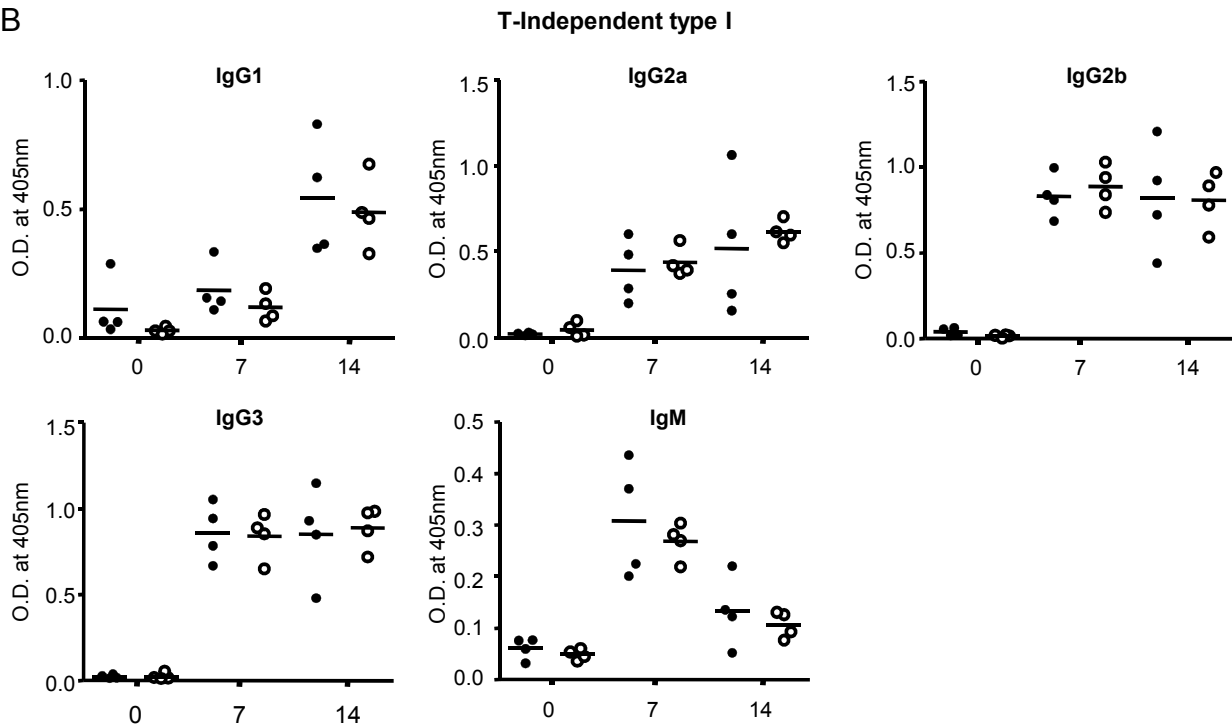
(A) Spleen cells from WT or Ly9^{-/-} mice were cultured *in vitro* in the presence or absence of 10 μ g/mL F(ab')₂ anti-mouse IgM. Mean fluorescence intensity (MFI) of MHC class II (*top panels*) or CD86 (*bottom panels*) in gated MZ (CD21^{hi} CD23^{lo}), B1 (CD19^{hi} B220⁻), or follicular (CD21^{lo} CD23^{hi}) B cells after 6h of culture. (B) Phospho flow analysis of BCR signaling events after stimulation. WT or Ly9^{-/-} MZ B cells were isolated by negative immunomagnetic separation and stimulated *in vitro* during five minutes with 10 μ g/mL F(ab')₂ anti-mouse IgM. Levels of phosphorilated Btk (pBtk) (*left*), pJNK (*middle*) and pp38 (*right*) are expressed as MFI. Data are representative of two independent experiments with four to five mice per genotype.

Supplemental Figure 2

A



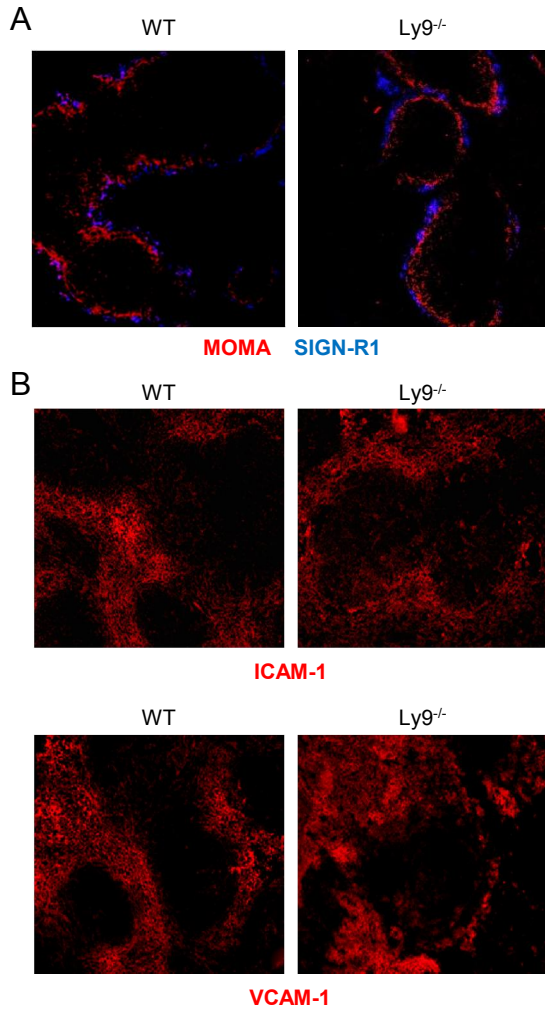
B



Supplemental figure 2. *Ly9* absence does not lead to impaired T-Dependent and T-Independent type I responses.

WT and *Ly9*^{-/-} mice were immunized i.p. with TNP₃₁-KLH (A) or TNP_{0,3}-LPS (B). Sera was collected at the indicated time points, and anti-TNP specific antibody levels were measured by ELISA. For TNP-KLH immunization, mice received a boost in day 14. Data are from two independent experiments with 2-4 mice/group.

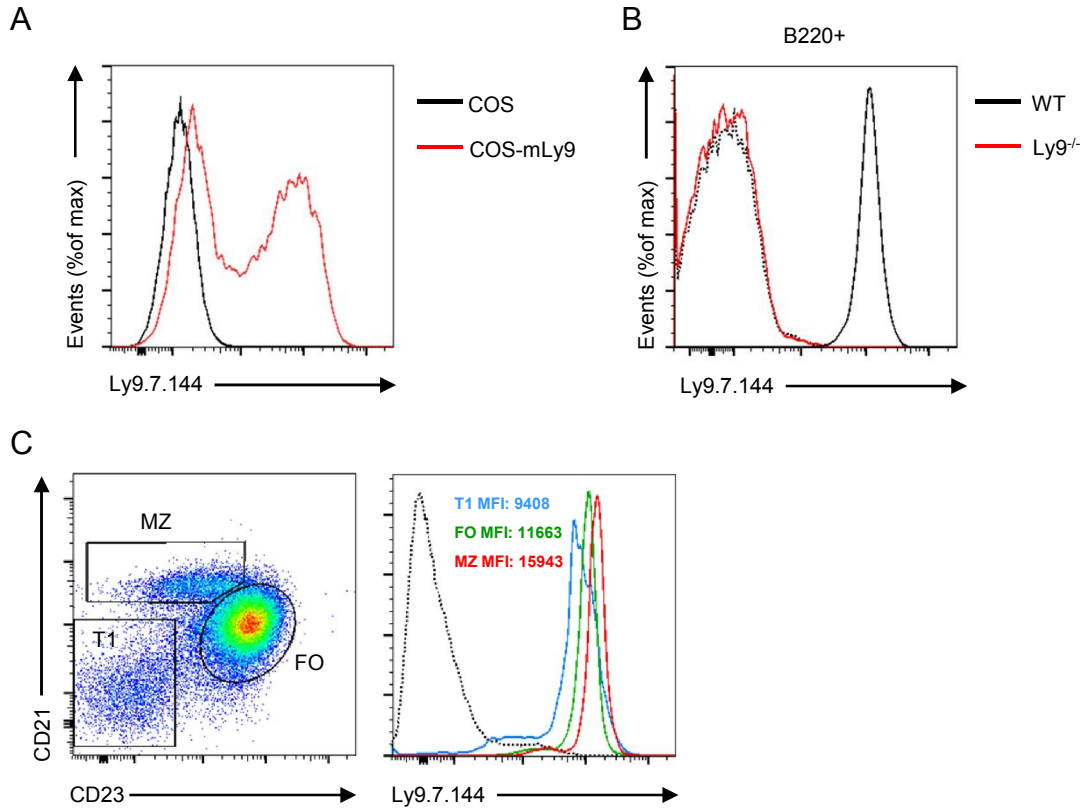
Supplemental Figure 3



Supplemental Figure 3. Ly9 deficiency does not lead to major changes in the distribution of marginal zone macrophages or in the expression of adhesion molecules in the spleen.

(A) Immunofluorescence analysis of spleen sections from WT and Ly9^{-/-} mice stained with anti-MOMA-1 (red) and anti-SIGN-R1 (blue) to detect metallophillic and marginal zone macrophages, respectively. Original magnification, 10x. (B) Analysis of ICAM-1 (top) and VCAM-1 (bottom) expression in spleens from WT or Ly9^{-/-} mice. Image magnification, 20x. Data are representative of two independent experiments with three to five mice per genotype.

Supplemental Figure 4



Supplemental Figure 4. Validation of Ly9.7.144 antibody and Ly9 expression in spleen B cell subsets.

(A) Reactivity of Ly9.7.144 antibody with mouse Ly9 (mLy9)-transfected (red) and untransfected COS cells (black). (B) Binding of Ly9.7.144 antibody to WT (black) or Ly9^{-/-} (red) spleen B220⁺ cells. (C) Ly9 expression in gated MZ (CD21^{hi} CD23^{lo}; red line), FO (CD21^{lo} CD23^{hi}; green line), or transitional 1 (CD21⁻ CD23⁻; blue line) spleen B cells. The mean fluorescence intensity (MFI) of Ly9 in each subset is indicated.