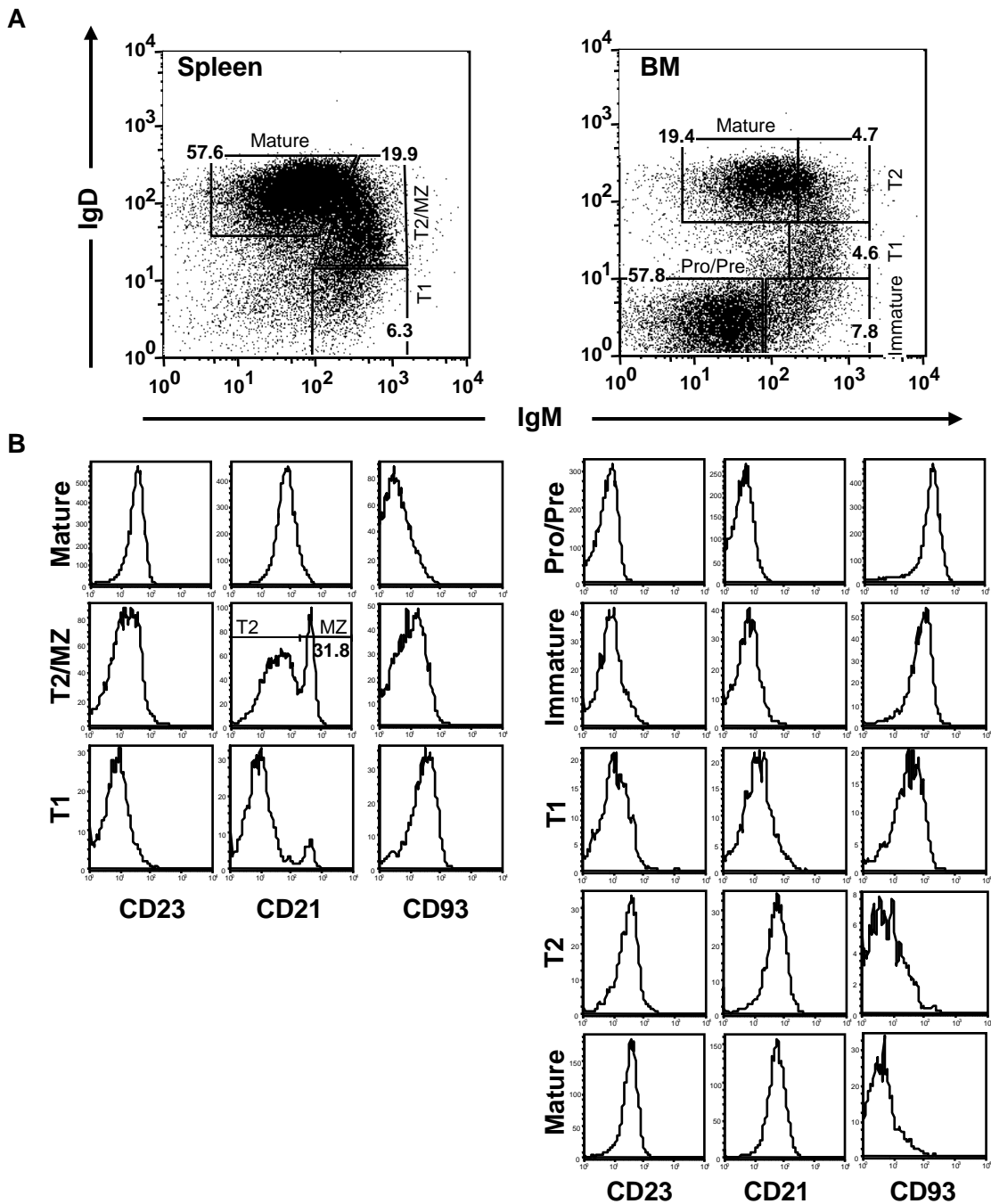


**Supplemental Figure 1. Specific labeling of B-cell lines by MPER tetramer.** 13H11 cells ( $1-1.3 \times 10^6$ ) were incubated in PBS + 3% FCS alone or buffer containing equivalent molar excess amounts of either *i*) unlabeled MPER-tetramer, *ii*) MPER peptide, *iii*) unlabeled R4A tetramer or *iv*) R4A peptide for 30 min. at  $0^\circ$  C. Unlabeled peptide and tetramer concentrations were established to represent 0.6, 1.3, 2.5 and 5.0 M excess of tetramer-associated peptide epitope. Subsequently, cells were labeled with 125 ng of APC-conjugated MPER-tetramer for 30 min. at  $0^\circ$  C. Independent aliquots of 13H11 cells were labeled with APC-conjugated Empty-tetramer as a negative control for peptide-independent binding. 13H11 cells were subsequently analyzed by flow cytometry and the fraction of tetramer binding cells (inset no.) and the M.F.I. of tetramer<sup>+</sup> cells were determined compared to unlabeled controls. Each histogram is representative of at least 3 independent measurements ( $n \geq 3$ ) compiled over 2 independent experiments. All data was acquired using a BD LSRII cytometer and histograms were created using FlowJo software.



**Supplemental Figure 2. Cell surface marker expressions on BM and splenic B cell populations.** BM and spleen cells from BL/6 mice were labeled with mAb to B220, IgM, IgD, CD23, CD21 and CD93. Flow diagrams in panel A were pre-gated on live, single B220<sup>+</sup> cells. Specific B cell compartments in the BM and/or spleen were defined as: pro/pre B cells (B220<sup>lo</sup>IgM<sup>lo</sup>IgD<sup>-</sup>CD21<sup>-</sup>CD23<sup>-</sup>CD93<sup>hi</sup>); immature B cells (B220<sup>lo</sup>IgM<sup>hi</sup>IgD<sup>-</sup>CD21<sup>-</sup>CD23<sup>-</sup>CD93<sup>hi</sup>); T1 B cells (B220<sup>lo</sup>IgM<sup>hi</sup>IgD<sup>lo</sup>CD21<sup>-</sup>CD23<sup>lo</sup>CD93<sup>hi</sup>); T2 B cells (B220<sup>hi</sup>IgM<sup>hi</sup>IgD<sup>hi</sup>CD21<sup>lo</sup>CD23<sup>hi</sup>CD93<sup>int</sup>); MZ B cells (B220<sup>hi</sup>IgM<sup>hi</sup>IgD<sup>lo</sup>CD21<sup>hi</sup>CD23<sup>lo</sup>CD93<sup>-</sup>), and mature B cells (B220<sup>hi</sup>IgM<sup>lo</sup>IgD<sup>hi</sup>CD21<sup>lo</sup>CD23<sup>hi</sup>CD93<sup>-</sup>). When B cell tetramers were used to identify Ag-specific B cells, CD93 was replaced by tetramers, and only B220, IgM, IgD, CD21, and CD23 were used to characterize B cell development. Data were acquired using a BD LSRII flow cytometer and analyzed using FlowJo software.