

Supplementary Information for

Cochaperone Mzb1 is a key effector of Blimp1 in plasma cell differentiation and β 1 integrin function

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Figs. S1 to S3

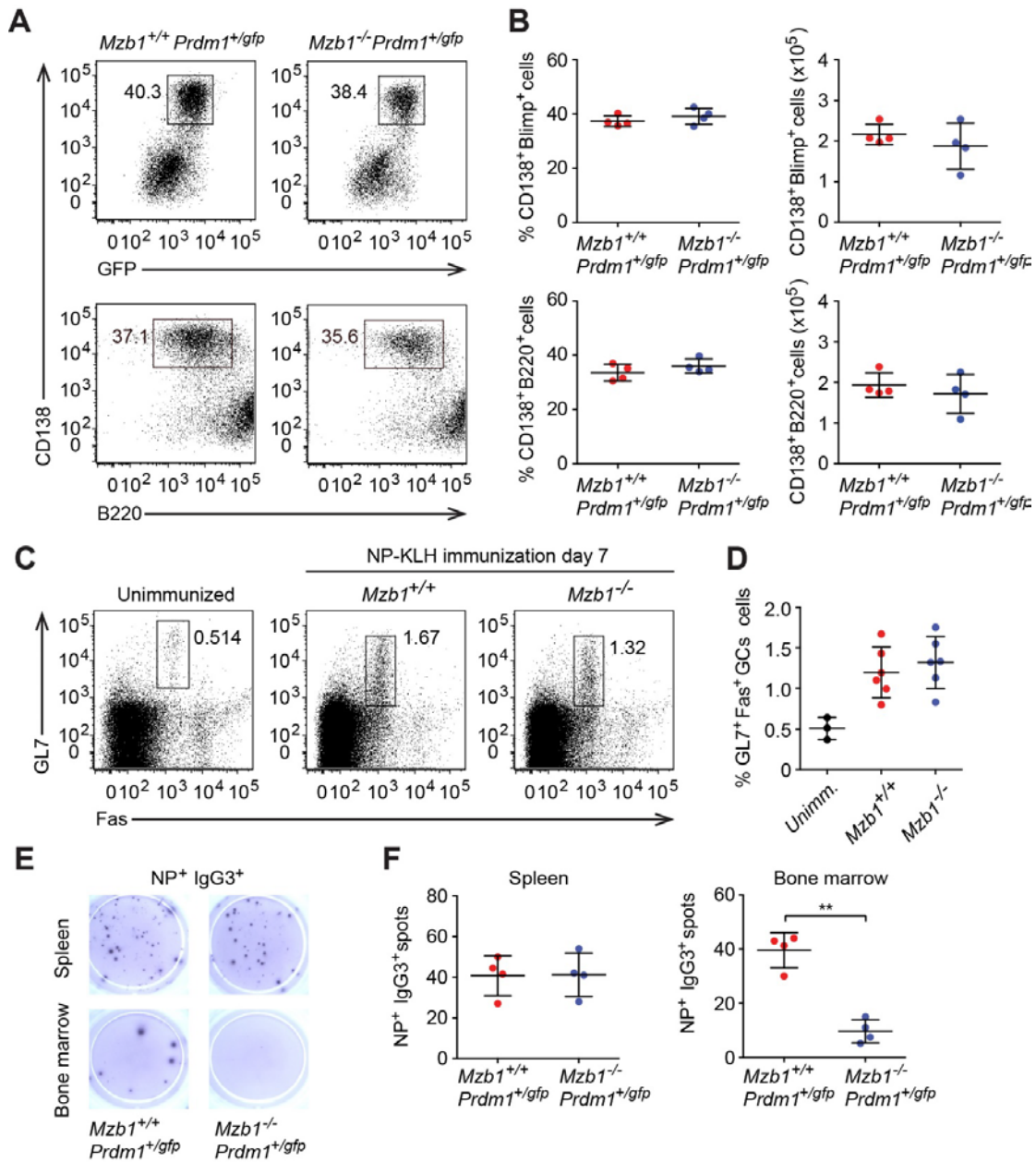


Figure S1 (related to Figure 1 and Figure 2)

(A) Flow cytometry to identify CD138⁺Blimp-GFP⁺ cells (top) and CD138⁺B220⁺ cells (bottom) among B220⁺ *Mzb1^{+/+}Prdm1^{+gfp}* and *Mzb1^{-/-}Prdm1^{+gfp}* splenocytes that were stimulated with CD40L, IL4 and IL5 in vitro for 5 d. Numbers represent cell frequencies.

(B) Mean (\pm SD) frequencies and numbers of CD138⁺Blimp-GFP⁺ cells (top) and CD138⁺B220⁺ cells (bottom), as gated in A. Data represent three independent experiments.

(C-D) Flow cytometry to determine the frequencies of GL7⁺Fas⁺ germinal center (GC) B cells in *Mzb1^{+/+}* and *Mzb1^{-/-}* mice 7 d after immunization with NP-KLH. Representative dot plots (C) and mean (\pm SD) frequencies (D) of GL7⁺Fas⁺ GC B cells in unimmunized and NP-KLH-immunized mice. Data are representative of three different experiments with 3-5 mice per group.

Representative ELISpot (E) and mean (\pm S.D.) numbers (F) of NP⁺IgG3⁺ CD138⁺Blimp-GFP⁺ cells in spleen (top) and BM (bottom) 3 d p.i. with NP-Ficoll. n = 4.

**p<0.01.

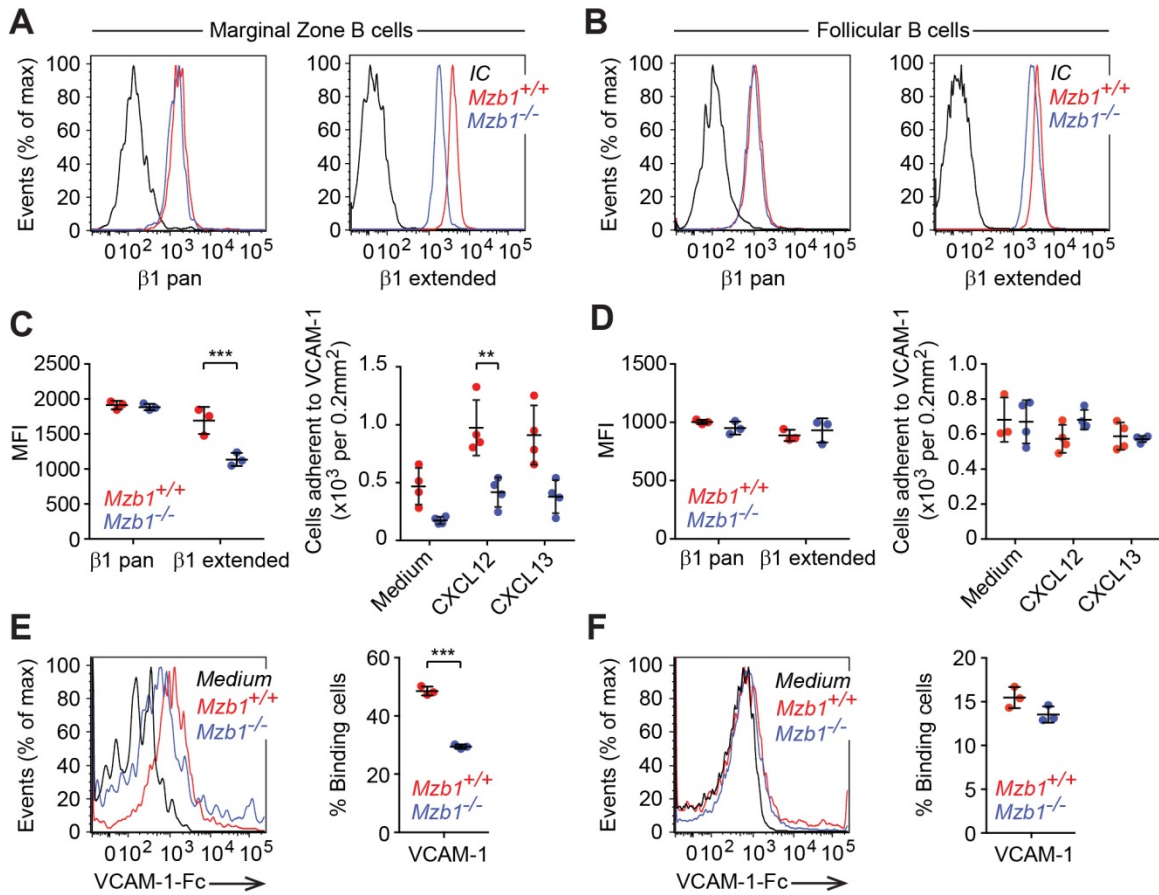


Figure S2 (related to Figure 4)

Representative histograms of total $\beta 1$ ($\beta 1$ pan) and extended $\beta 1$ integrin in $Mzb1^{+/+}$ and $Mzb1^{-/-}$ marginal zone (MZ) B cells (A) and Follicular (Fo) B cells (B). IC: Isotype Control. (C) Quantification of the data shown in A by determining the mean fluorescence intensities (MFI) \pm S.D. (left) and analysis of adhesion of $Mzb1^{+/+}$ and $Mzb1^{-/-}$ MZ B cells to slides coated with VCAM-1 after stimulation with CXCL12 and CXCL13 (right). (D) Quantification of the data shown in B (left) and adhesion of $Mzb1^{+/+}$ and $Mzb1^{-/-}$ Fo B cells to slides coated with VCAM-1 after stimulation with CXCL12 and CXCL13 (right). Representative histograms showing the ability of $Mzb1^{+/+}$ and $Mzb1^{-/-}$ MZ B cells (E) and Fo B cells (F) to bind soluble VCAM-1 (left) and quantification of the frequency of the binding cells (right). Data are representative of three different experiments. ** $p < 0.01$, *** $p < 0.001$.

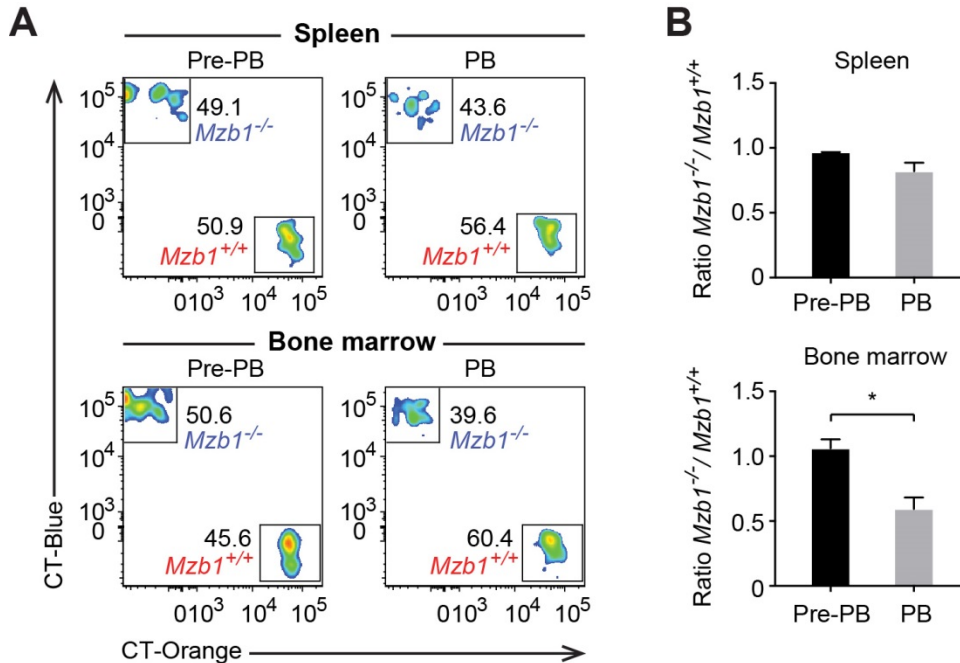


Figure S3 (related to Figure 6)

(A) Flow cytometry to assess trafficking of *in vitro* generated Pre-PB and PB after adoptive transfer into recipient mice. CD138⁻Blimp-GFP⁺ Pre-PB and CD138⁺Blimp-GFP⁺ PB were generated by LPS stimulation of B220⁺ splenic cells from *Mzb1*^{+/+}*Prdm1*^{+/gfp} and *Mzb1*^{-/-}*Prdm1*^{+/gfp} mice. After FACS sorting, cells were fluorescently labeled with cell tracker (CT) orange (*Mzb1*^{+/+}) and CT blue (*Mzb1*^{-/-}) and adoptively transferred in wild type recipients. The appearance of *Mzb1*^{+/+} and *Mzb1*^{-/-} cells in spleen (top) and BM (bottom) of the recipient mice was analyzed. The numbers in each contour plot represent cell frequencies. (B) Ratios (\pm S.D) between *Mzb1*^{-/-} and *Mzb1*^{+/+} Pre-PB and PB that migrated to the spleen (top) and BM (bottom). Data represent 2 independent experiments. n=3. *p<0.05, **p<0.01.