

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Andreani, V;Ramamoorthy, S;Pandey, A;Lupar, E;Nutt, SL;Lämmermann, T;Grosschedl, R

Title:

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

Date:

2018-10-09

Citation:

Andreani, V., Ramamoorthy, S., Pandey, A., Lupar, E., Nutt, S. L., Lämmermann, T. & Grosschedl, R. (2018). Cochaperone Mzb1 is a key effector of Blimp1 in plasma cell differentiation and β 1-integrin function. *Proceedings of the National Academy of Sciences of the United States of America*, 115 (41), pp.E9630-E9639. <https://doi.org/10.1073/pnas.1809739115>.

Persistent Link:

<https://hdl.handle.net/11343/253226>

License:

CC BY-NC-ND

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

Virginia Andreani^a, Senthilkumar Ramamoorthy^a, Abhinav Pandey^a, Ekaterina Lupar^a, Stephen L. Nutt^{b,c}, Tim Lämmermann^a, and Rudolf Grosschedl^{a,1}

^aDepartment of Cellular and Molecular Immunology, Max Planck Institute of Immunobiology and Epigenetics, 79108 Freiburg, Germany; ^bThe Molecular Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia; and ^cDepartment of Medical Biology, University of Melbourne, Parkville, VIC 3010, Australia

Edited by Klaus Rajewsky, Max Delbrück Center for Molecular Medicine, Berlin, Germany, and approved September 4, 2018 (received for review June 6, 2018)

Plasma cell differentiation involves coordinated changes in gene expression and functional properties of B cells. Here, we study the role of Mzb1, a Grp94 cochaperone that is expressed in marginal zone (MZ) B cells and during the terminal differentiation of B cells to antibody-secreting cells. By analyzing *Mzb1*^{-/-}*Prdm1*^{+gfp} mice, we find that Mzb1 is specifically required for the differentiation and function of antibody-secreting cells in a T cell-independent immune response. We find that Mzb1-deficiency mimics, in part, the phenotype of Blimp1 deficiency, including the impaired secretion of IgM and the deregulation of Blimp1 target genes. In addition, we find that *Mzb1*^{-/-} plasmablasts show a reduced activation of β 1-integrin, which contributes to the impaired plasmablast differentiation and migration of antibody-secreting cells to the bone marrow. Thus, Mzb1 function is required for multiple aspects of plasma cell differentiation.

Mzb1 | Grp94 | Blimp1 | integrin | plasma cell

The terminal differentiation of B cells into antibody-secreting cells (ASCs) is an essential process in the humoral immune response. After an encounter with antigen, B cells proliferate and differentiate into short-lived, cycling plasmablasts (PBs) that secrete antibody and reside in extrafollicular foci of secondary lymphoid organs (1). PBs can further differentiate into quiescent long-lived plasma cells (PCs) after migration to the bone marrow (BM), which provides niches that enable PC longevity (2). However, the majority of PCs are derived from activated B cells that enter the B cell follicles of secondary lymphoid organs and form germinal centers (GC) under the influence of follicular T helper cells. After extensive proliferation and affinity maturation of the B cell receptor, GC B cells differentiate into long-lived PCs or memory B cells (2).

Mature B cells include the innate-like marginal zone (MZ) B cells, B1 cells, and the dominant follicular B (Fo B) cell subset (3). MZ B and B1 cells respond rapidly to T cell-independent (TI) antigens, such as bacterial lipopolysaccharides (LPS), but they can also engage in a slower T cell-dependent (TD) immune response that is mediated primarily by Fo B cells. The generation of ASCs in a TD response involves an initial extrafollicular response step that produces PB and a subsequent GC response step that produces PC and memory B cells (4). ASCs expand their endoplasmic reticulum (ER) as a consequence of the unfolded protein response (UPR) that is induced by protein overloading and results in the activation of the transcription factor XBP-1, which regulates the UPR and secretion of immunoglobulins (Ig). The UPR can consequently regulate the folding, processing, and export of the new synthesized proteins (5, 6). Before the activation of the UPR and XBP-1, the transcription factor IRF4 initiates PB differentiation by the activation of the *Prdm1* gene, encoding the transcription factor Blimp1 (7). Blimp1 silences the expression program of B cells and contributes to the activation of genes involved in the regulation of the UPR and the migratory and sessile properties of PBs and PCs (8, 9).

The *Mzb1* (*pErp1*) gene, which encodes a B cell-specific and ER-localized protein, is abundantly expressed in MZ B cells and B1 cells, and its expression increases to even higher levels during differentiation of activated B cells to ASCs (10–14). Mzb1 functions as a cochaperone of the substrate-specific chaperone Grp94/gp96 (Hsp90b1) under ER stress conditions and enables efficient antibody secretion in vitro and in immunized mice (12, 13). Mzb1 and Hsp90b1 are also among the few genes that are bound and activated by Blimp1 during the B cell to preplasmablast (pre-PB) transition (8). Mzb1 has also been implicated in the activation of integrin β 1, which forms a heterodimer with the α 4-integrin to bind vascular cell adhesion molecule (VCAM)-1 and to mediate lymphocyte adhesion and migration (12, 15, 16). VCAM-1 is abundantly expressed in the red pulp of the spleen and facilitates integrin-mediated B cell localization in the splenic MZ and peripheral lymphoid tissue compartmentalization (17, 18). In addition, the expression of VCAM-1 in BM stromal cells is important for the maturation and retention of PCs in the BM (19–23).

Previously, the role of Mzb1 has been studied primarily in cell cultures and has focused on the secretion of antibodies. Therefore, questions arise as to whether and how the highly abundant expression of *Mzb1* in ASCs regulates the terminal differentiation of

Significance

Antibody-secreting plasma cells are effectors of the humoral immune response. Transcription factor Blimp1 (*Prdm1*) is essential for the generation and function of plasma cells, and it regulates many genes, including *Mzb1* (*pErp1*). Mzb1 protein is localized in the endoplasmic reticulum and acts as a cochaperone for the substrate-specific chaperone Grp94 (gp96). By the analysis of *Mzb1*^{-/-}*Prdm1*^{+gfp} mice, we find that Mzb1 is required for T cell-independent immune responses and differentiation of plasma cells. In *Mzb1*^{-/-}*Prdm1*^{+gfp} mice, we also observe impaired β 1-integrin activation and trafficking of plasma cells to the bone marrow. Notably, we show that Mzb1 accounts for many of the Blimp1-associated downstream functions, suggesting that Mzb1 is a key effector of the Blimp1 regulatory network in plasma cells.

Author contributions: V.A., T.L., and R.G. designed research; V.A., A.P., and E.L. performed research; V.A., S.R., A.P., S.L.N., T.L., and R.G. analyzed data; and V.A. and R.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, <https://www.ncbi.nlm.nih.gov/geo> (accession no. GSE118124).

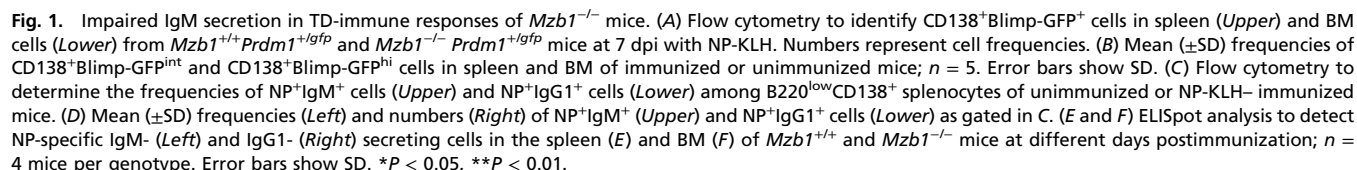
¹To whom correspondence should be addressed. Email: grosschedl@ie-freiburg.mpg.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1809739115/-DCSupplemental.

Published online September 26, 2018.

lived, cycling Blimp1^{int} PBs and long-lived, quiescent Blimp1^{hi} PCs (24). To assess the role of Mzb1 in the TD PC generation, we immunized *Mzb1*^{-/-}*Prdm1*^{+/*gfp*} and *Mzb1*^{+/+}*Prdm1*^{+/*gfp*} littermates with (4-hydroxy-3-nitrophenyl)acetyl-keyhole limpet hemocyanin (NP-KLH) and analyzed the frequencies of ASCs in spleen and BM by flow cytometry at 7 d postimmunization (dpi). Similar frequencies of Blimp1-GFP^{int} PBs and Blimp1-GFP^{hi} PCs were detected in the spleen and BM of *Mzb1*^{-/-}*Prdm1*^{+/*gfp*} mice relative to *Mzb1*^{+/+}*Prdm1*^{+/*gfp*} mice (Fig. 1 *A* and *B*). Moreover, we found that the Mzb1 deficiency had no significant effect on PC differentiation in vitro, driven by CD40L, IL-4, and IL-5 (*SI Appendix, Fig. S1 A and B*). We also did not detect any significant difference in the generation of GC B cells in *Mzb1*^{-/-} and *Mzb1*^{+/+} mice after immunization with NP-KLH

Impaired TI PC Differentiation in *Mzb1*^{-/-} *Prdm1*^{+/*gfp*} Mice. With the aim of gaining insight into the role of Mzb1 in PC differentiation and function, we crossed *Mzb1*^{-/-} mice with *Prdm1*^{+/*gfp*} reporter mice that allow for the identification and separation of short-



(SI Appendix, Fig. S1 C and D). However, the immunization of *Mzb1*^{-/-} with NP-KLH revealed a significant decrease in the frequency of NP-specific IgM⁺ ASCs relative to *Mzb1*^{+/+} mice (Fig. 1 C and D). This difference was not observed for the more abundant NP-specific IgG1⁺ ASCs (Fig. 1 C and D). ELISpot analysis showed that the secretion of NP-specific IgM antibodies in splenic and BM-derived ASCs of *Mzb1*^{-/-} mice was reduced compared with *Mzb1*^{+/+} mice (Fig. 1 E and F). Notably, no defect in the secretion of NP-specific IgG1 antibodies was observed in *Mzb1*^{-/-} mice. Thus, *Mzb1* is specifically required for the generation of IgM⁺ ASCs and proper secretion of IgM after TD immunization, but is dispensable for the generation of follicular PBs and PCs.

To assess a potential role of *Mzb1* in the differentiation and function of extrafollicular PBs, we immunized *Mzb1*^{-/-}*Prdm1*^{+/-gfp} and *Mzb1*^{+/+}*Prdm1*^{+/-gfp} mice with the TI antigen trinitrophenylated derivatives of LPS (TNP-LPS) and examined the frequencies of CD138⁺Blimp-GFP^{int} PBs and CD138⁺Blimp1-GFP^{hi} PCs in the spleen and BM by flow cytometry at 3 dpi (Fig. 2A). Similar frequencies of CD138⁺Blimp1-GFP^{int} PBs were detected in *Mzb1*^{-/-}*Prdm1*^{+/-gfp} and *Mzb1*^{+/+}*Prdm1*^{+/-gfp} mice but the frequency of CD138⁺Blimp1-GFP^{hi} PCs was reduced in

both the spleen and BM of *Mzb1*^{-/-}*Prdm1*^{+/-gfp} mice (Fig. 2A and B). ELISpot analysis detected similar numbers of antigen-specific ASCs in the spleen of *Mzb1*^{-/-}*Prdm1*^{+/-gfp} and *Mzb1*^{+/+}*Prdm1*^{+/-gfp} mice (Fig. 2C and D, Top). However, the numbers of TNP-specific IgM⁺ ASCs were markedly reduced in the BM of *Mzb1*^{-/-}*Prdm1*^{+/-gfp} relative to those in *Mzb1*^{+/+}*Prdm1*^{+/-gfp} mice (Fig. 2C and D, Lower). In comparison with *Mzb1*^{+/+}*Prdm1*^{+/-gfp} mice, immunizations of *Mzb1*^{-/-}*Prdm1*^{+/-gfp} mice with NP-Ficoll also generated fewer BM ASCs that secrete NP-specific IgG3 as a typical TI isotype (SI Appendix, Fig. S1 E and F). The equivalent numbers of TNP-specific ASCs in the spleen of *Mzb1*^{+/+} and *Mzb1*^{-/-} could be accounted for by the similar high abundance of Blimp1-GFP^{int} PBs, whereas the ASCs population in BM is dominated by the Blimp1-GFP^{hi} PCs that are diminished in *Mzb1*^{-/-} mice. It is known that PCs in the BM secrete more antibodies per cell and per minute than PBs in the spleen (25). Interestingly, we also observed a decreased frequency of CD138⁺Blimp1-GFP⁺ cells in the peripheral blood of *Mzb1*^{-/-}*Prdm1*^{+/-gfp} 3 dpi (Fig. 2E). This decreased frequency of cells was not due to diminished cell survival because we did not detect any differences in the apoptosis of *Mzb1*^{-/-} and *Mzb1*^{+/+} ASCs (Fig. 2F).

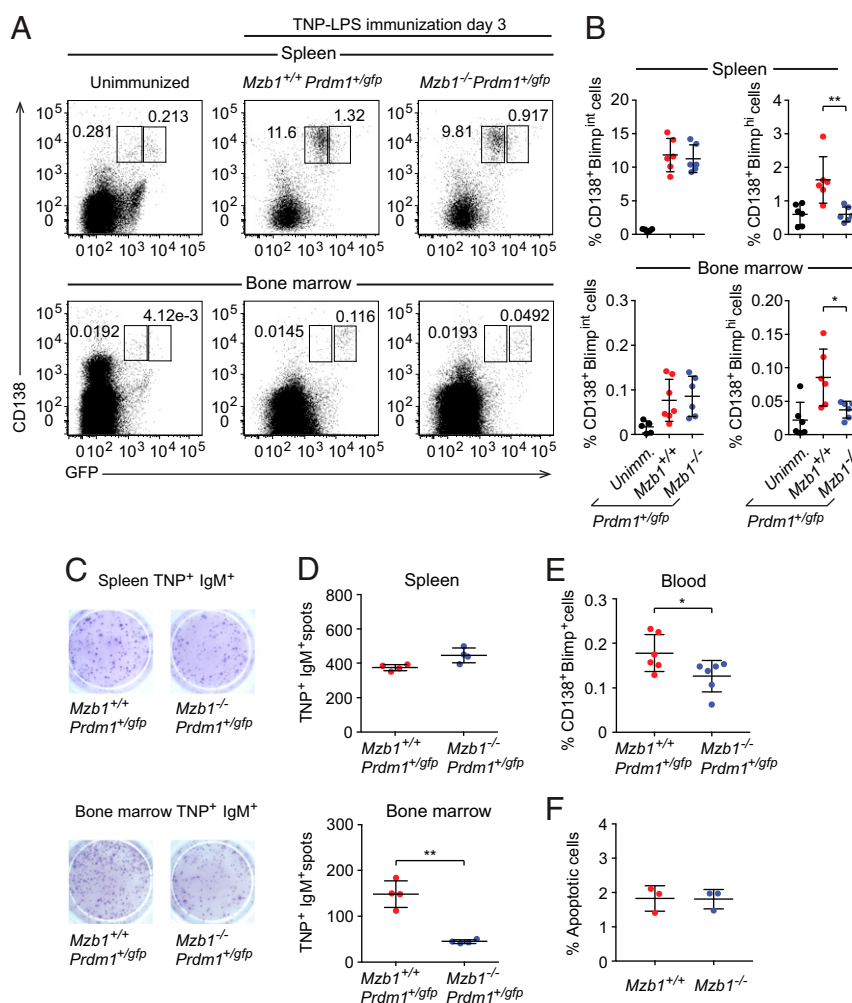


Fig. 2. Defect of PC differentiation in TI-immune responses of *Mzb1*^{-/-} mice. (A) Flow cytometry to detect CD138⁺Blimp-GFP⁺ cells in the spleen (Upper) and BM (Lower) of *Mzb1*^{+/+}*Prdm1*^{+/-gfp} and *Mzb1*^{-/-}*Prdm1*^{+/-gfp} mice 3 dpi with TNP-LPS. Numbers represent cell frequencies. (B) Mean (±SD) frequencies of *Mzb1*^{+/+} and *Mzb1*^{-/-} CD138⁺ Blimp-GFP^{int} and CD138⁺ Blimp-GFP^{hi} cells in the spleen and BM, as gated in A. Representative ELISpot (C) and mean (±SD) numbers (D) of TNP⁺IgM⁺ CD138⁺Blimp-GFP⁺ cells in the spleen (Upper) and BM (Lower) 3 dpi, *n* = 4. (E) Mean (±SD) frequencies of CD138⁺Blimp-GFP⁺ cells in blood of TNP-LPS-immunized mice 3 dpi. (F) Analysis of the frequencies (±SD) of apoptotic B220^{low} CD138⁺ cells in *Mzb1*^{+/+} and *Mzb1*^{-/-} mice 3 dpi with TNP-LPS. Data are representative of three experiments with three to six mice per group and experiment. **P* < 0.05, ***P* < 0.01.

Taken together, these data suggest that *Mzb1* is required for the generation and function of PCs after TI immunization.

Transcriptome of *Mzb1*^{-/-} PBs Resembles That of *Prdm1*^{-/-} PBs. To gain insight into the molecular basis of the deficiency in the generation of extrafollicular PBs, we conducted an RNA-seq analysis on B220⁺ cells from the spleen of *Mzb1*^{-/-}*Prdm1*^{+/-gfp} and *Mzb1*^{+/-}*Prdm1*^{+/-gfp} mice that have been stimulated with LPS in vitro for 4 d. Similar frequencies of pre-PBs (CD138⁻ Blimp1-GFP⁺) were detected in both *Mzb1* WT and mutant cell cultures (Fig. 3A). In cultures from *Mzb1*-deficient mice, however, we observed a reduced frequency of PBs (CD138⁺ Blimp1-GFP⁺) and increased frequency of activated B cells (Act B) (CD138⁻ Blimp1-GFP⁻) relative to cultures from *Mzb1*^{+/-} mice. The absolute numbers of *Mzb1*^{-/-} pre-PBs and PBs were also reduced

relative to *Mzb1*^{+/-} cells (Fig. 3B). The block of PB differentiation correlates with the robust up-regulation of *Mzb1* RNA in *Mzb1*^{+/-} pre-PBs (Fig. 3C).

RNA-seq analysis of these cell populations identified 63 up-regulated and 20 down-regulated genes in *Mzb1*-deficient pre-PBs relative to *Mzb1*^{+/-} pre-PBs (Fig. 3D). In *Mzb1*^{-/-} PBs, 77 genes were up-regulated whereby 29 genes were shared between pre-PBs and PBs (Fig. 3E). Analysis of publicly available ChIP-seq and ATAC-seq datasets for histone modifications, transcription factor occupancy, and chromatin accessibility at the *Mzb1* gene indicated that both Blimp1 and IRF4 bind the promoter region of the *Mzb1* gene (Fig. 3F) (8). Moreover, the *Mzb1* gene shows accessibility already in activated B cells and enhanced histone H3 K9 acetylation in PBs (Fig. 3F). Notably, 18 of 77 genes that were up-regulated in *Mzb1*^{-/-} PBs have been

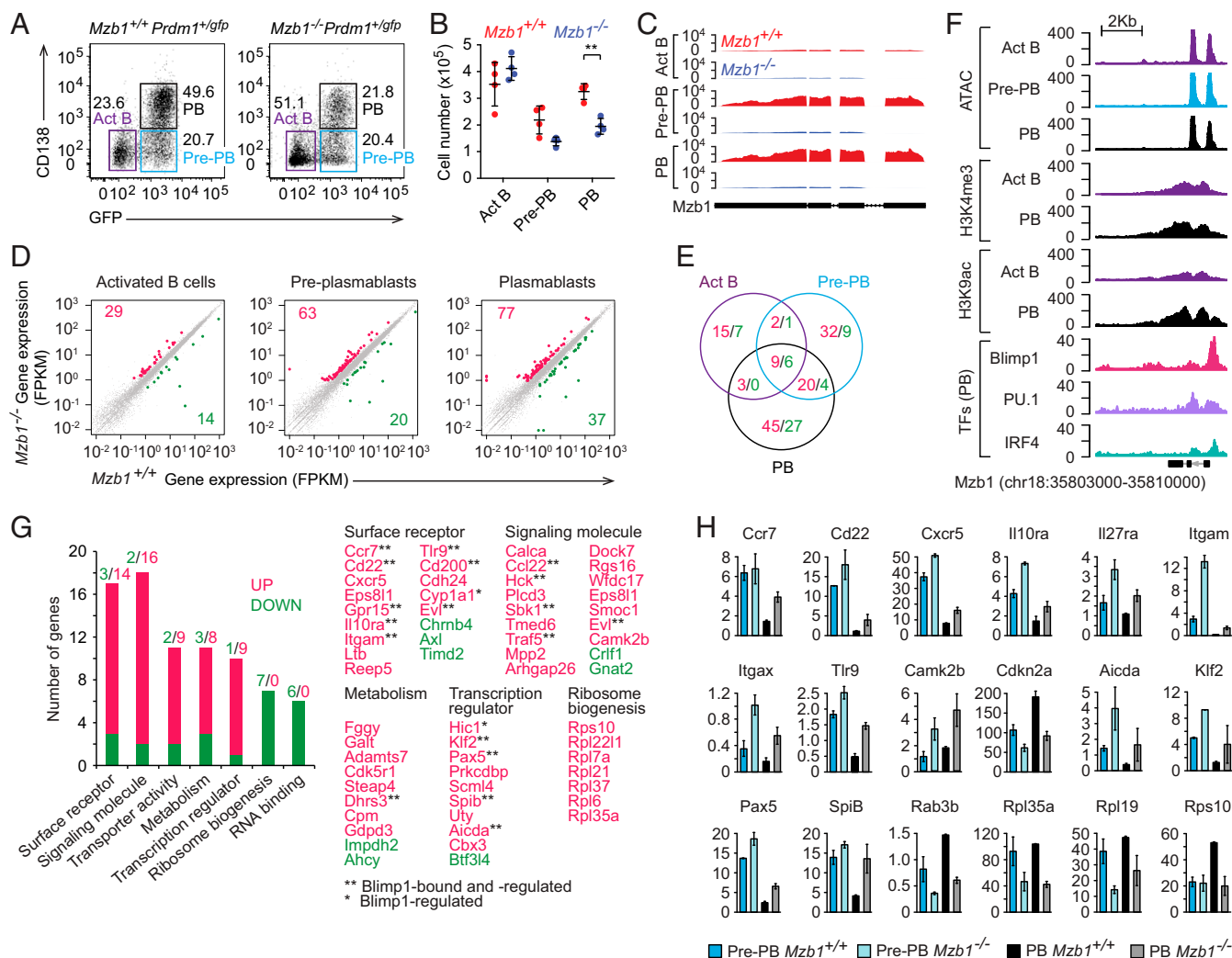


Fig. 3. Changes in the transcriptome of *Mzb1*^{-/-} PBs. Flow cytometry to detect frequencies (A) and mean (±SD) numbers (B) of CD138⁻Blimp1-GFP⁺ activated B cells (Act B), CD138⁻Blimp1-GFP⁺ Pre-PB, and CD138⁺Blimp1-GFP⁺ PB at 4 d after LPS stimulation of *Mzb1*^{+/-}*Prdm1*^{+/-gfp} and *Mzb1*^{-/-}*Prdm1*^{+/-gfp} B220⁺ splenocytes. Data are representative of three experiments; n = 4. **P < 0.01. (C) RNA-Seq reads of *Mzb1* transcripts from *Mzb1*^{+/-} (red) and *Mzb1*^{-/-} (blue) Act B, Pre-PB, and PB. (D) Scatter plot of gene-expression levels in *Mzb1*^{+/-} (x axis) and *Mzb1*^{-/-} (y axis) Act B cells (Left), Pre-PB (Center), PB (Right). The unaltered (gray), up- (red), and down- (green) regulated genes are highlighted. (E) Overlap of differentially expressed genes in Act B cells, Pre-PB, and PB. Numbers of up- (red) and down- (green) regulated genes in *Mzb1*^{-/-} relative to *Mzb1*^{+/-} are shown. (F) Tn5 transposase accessible (ATAC-seq) regions, H3K4me3, and H3K9ac marks, on the *Mzb1* locus in Act B cells (purple), Pre-PB (blue), and PB (black). The occupancy of IRF4, PU.1, and Blimp1 on the *Mzb1* locus in PB cells, is shown (Bottom). Data source, NCBI GEO accession no. GSE71698. (G) Functional classification of up- (red) and down- (green) regulated genes in *Mzb1*^{-/-} PB. Numbers above the bars indicate number of genes associated with each functional class. Selected genes from functional classes are indicated (Right). (H) Levels (fragments per kilobase of transcript per million mapped reads) of differentially expressed key genes in *Mzb1*^{+/-} and *Mzb1*^{-/-} Pre-PB and PB. Error bars show SD; n = 2.

previously identified as genes that are repressed by Blimp1 (8). These include genes encoding the surface receptors CCR7, CD22, IL-10R α , Toll-like receptor 9 (TLR-9), the signaling molecules CCL2, Traf5, and the transcription factors Pax-5, Klf-2, Spi-B, and AID (Fig. 3 *G* and *H*). The majority of these and other genes were also deregulated in pre-PBs but not in Act B cells, suggesting that the Mzb1-deficiency mimics, in part, the changes in gene expression associated with Blimp1-regulated PC differentiation.

Impaired Integrin Activation and Adhesion of Mzb1^{-/-} PBs and MZ B Cells. Recent studies established that Prdm1-deficiency leads to impaired migration and substrate adhesion of PB (8). Given the striking overlap in gene-expression changes between Mzb1- and Prdm1-deficiency, we next examined whether Mzb1-deficient PBs show similar deficits in their adhesive properties. Indeed, Mzb1-deficient pre-PBs and PBs showed impaired adhesion to intercellular adhesion molecule-1 (ICAM-1) and VCAM-1, which are ligands for α L β 2- and α 4 β 1-integrins, respectively (Fig. 4*A*). However, no significant changes in the adhesion of Mzb1-deficient Act B cells were observed. Previously, we have shown that Mzb1 knockdown in the mature B cell line K46 leads to decreased chemokine-induced adhesion in vitro (12). In these cells, Mzb1 regulates chemokine-induced integrin activation by promoting the transition of the low-affinity bent conformation to the high-affinity extended conformation of integrins. To examine whether or not Mzb1 deficiency also causes altered integrin activation in PBs, we used pan- β 1 antibodies to measure all forms of cell surface β 1-integrin or a conformation-specific anti- β 1 antibody that specifically interacts with the extended conformation of the β 1-subunit (26, 27). Our flow cytometric analysis of Act B cells, pre-PBs, and PBs generated from WT and Mzb1-deficient mice showed that Mzb1-deficient pre-PBs and PBs show reduced levels of the extended form of β 1 on the surface (Fig. 4*B*). In contrast, the levels of total β 1-integrin remained unchanged (Fig. 4*C*). In agreement with this finding, Mzb1-deficient pre-PBs and PBs showed an impaired binding of soluble VCAM-1 (Fig. 4*D*). To examine the effects of Mzb1 deficiency on cell migration, we performed a migration assay with VCAM-1-coated transwells. This assay indicated that Mzb1^{-/-} pre-PBs and PBs migrate less efficiently than their WT counterparts toward the chemokine CXCL12 (Fig. 4*E*). This impaired migration of Mzb1-deficient pre-PBs and PBs was not due to an altered chemokine sensing, as evidenced by the normal expression of CXCR4, the receptor for CXCL12 (Fig. 4*F*). Thus, Mzb1 is required for the activation and function of β 1-integrin in pre-PBs and PBs.

Given that MZ B cells present the predominant cell type for the generation of short-lived PBs and PCs during TI-dependent immune responses (4, 28), we asked whether the genetic depletion of Mzb1 already interferes with integrin functionality in MZ B cells. Similar to the observations in pre-PBs and PBs, Mzb1-deficient MZ B cells show a reduced surface expression of the extended form of β 1-integrin, relative to Mzb1-expressing MZ B cells (*SI Appendix*, Fig. S2*A*). Mzb1 low-expressing Fo B cells only show low levels of activated β 1-integrin on their surface, without obvious differences between Mzb1^{+/+} and Mzb1^{-/-} Fo B cells (*SI Appendix*, Fig. S2*B*). Moreover, Mzb1^{-/-} MZ B cells, but not Mzb1^{-/-} Fo B cells, show an impaired VCAM-1-Fc binding and adhesion to VCAM-1-coated plates, in the absence or presence of CXCL12 and CXCL13 chemokines (compare *SI Appendix*, Fig. S2*C* and *E* with *SI Appendix*, Fig. S2*D* and *F*).

To examine whether or not the impaired expression of the extended form of β 1-integrin reflects a defect in Mzb1-dependent protein folding, we performed limited trypsin digestion on Mzb1^{+/+} and Mzb1^{-/-} MZ B and Fo B cells. Gel electrophoretic separation of the partially digested proteins indicated that β 1-integrin is degraded in Mzb1-deficient MZ B

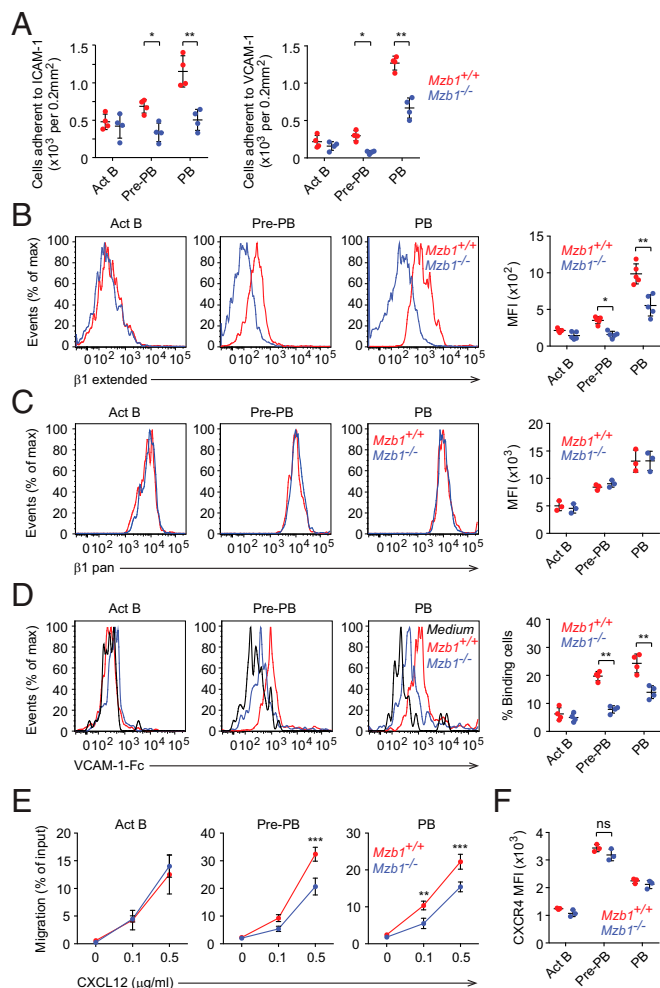


Fig. 4. Mzb1^{-/-} ASCs show impaired β 1 integrin activation and migration. (A) Adhesion of Act B, Pre-PB, and PB cells to slides coated with ICAM-1 (Left) or VCAM-1 (Right). (B and C) Representative histograms and quantification (mean fluorescent intensity, MFI) of extended β 1-integrin (B) and total β 1-integrin (β 1 pan) in Act B, Pre-PB, and PB cells. Data are representative of three experiments; $n = 3$. (D) Representative histograms showing the adhesion of Act B, pre-PB and PB cells to soluble VCAM-1, and quantification of the frequency of the binding cells. (E) Transwell assay to assess the migration of Act B, Pre-PB, and PB cells toward CXCL12 (0, 0.1 and 0.5 μ g/ml) on VCAM-1-coated plates. (F) Quantification (MFI) of CXCR4 cell surface expression in the corresponding cells. ns, nonsignificant. Data are representative of three independent experiments. $n = 3$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

cells at lower concentrations of trypsin than in Mzb1^{+/+} MZ B cells (Fig. 5*A*). In contrast, similar patterns of β 1 degradation were observed in Mzb1^{-/-} and Mzb1^{+/+} Fo B cells (Fig. 5*B*). Notably, the degradation patterns of α 4-integrin and CD19 were similar in both MZ B and Fo B cells from Mzb1^{-/-} and Mzb1^{+/+} mice. To gain some insight into the mechanism by which Mzb1 affects the conformation of β 1-integrin, we examined whether Mzb1 affects the interaction between Grp94 and β 1-integrin. To this end, we performed coimmunoprecipitation of lysates from WT and Mzb1^{-/-} B1-8 B cells with anti-Grp94 antibody, followed by immunoblot analysis to detect Grp94, β 1-integrin, and α 4-integrin. In Mzb1^{+/+} cells, we detected an association of Grp94 with the ~100-kD precursor form of β 1, termed β 1', which is localized in the ER and is not yet associated with the α 4-subunit (29) (Fig. 5*C*). In contrast, no association of the ~125-kDa mature (Golgi) form of β 1 was detected in lysates from Mzb1^{-/-} cells. Taken together, these

BD. Anti-CD29 (9EG7) in combination with secondary anti-rat PE antibody (both BD) was used to detect the active conformation state of $\beta 1$ -integrin. Anti-CD29 (Hmb1-1) antibody was used to detect the total (pan) $\beta 1$ -integrin (eBioscience). IgG1 biotinylated antibody (Southern Biotech) was conjugated with SA-BV421 (Biolegend). NP-PE antibody was from Biosearch Tech. Anti-CD16/32 (93) (BD) was used to block nonspecific binding.

Immunizations, ELISpot, and Antibody Treatments. Mice were injected intraperitoneally with 50 μ g TNP-LPS, 50 μ g NP-Ficol, or 150 μ g adsorbed NP-KLH (Biosearch Technology) 1:1 ratio onto Alu-Gel-S (Serva). Spleens, BM, and blood were taken after the indicated time points postimmunization. ASCs were analyzed by ELISpot as previously described (13). In some experiments, mice received 100 μ g of anti-VCAM-1 (429) or rat IgG2a isotype control antibodies (both BD), intravenously, 24 h postinfection. Spleens and BM were analyzed 48 h after antibody treatment.

Apoptosis Assay. ASCs (B220^{low}CD138⁺) from *Mzb1*^{+/+} and *Mzb1*^{-/-} mice were stained for Annexin V-FITC and 7AAD, according to the protocol's instructions (BD Bioscience) 3 dpi with TNP-LPS. Cells were acquired in LSR Fortessa flow cytometer and analyzed with FlowJo software.

BM Chimeras. For 50:50 BM chimeras, lethally irradiated *Rag2*^{-/-} mice (2 $\times$ 6 Gy) were reconstituted in an equal ratio with CD45.1 C57BL/6J and either CD45.2 *Mzb1*^{+/+} BM or CD45.2 *Mzb1*^{-/-} BM. Mice were rested for 10 wk before TNP-LPS immunization.

Adoptive Transfer. *Mzb1*^{+/+} and *Mzb1*^{-/-} ASCs (B220^{low}CD138⁺) or control B220⁺CD138⁻ were sorted 3 dpi with TNP-LPS, and were incubated with 0.5 μ M Cell Tracker (CT) Orange or 1 μ M CT Blue (Molecular Probes), respectively, at 37 °C for 30 min. Cells were washed and mixed in a 1:1 ratio for intravenous injection in C57BL/6 recipient mice. A total of 1 $\times$ 10⁵ cells/100 μ L were injected per tail vein. Labeled cells from spleens and BM of recipient mice were analyzed by flow cytometer 20 h after adoptive transfer. The same experiment was performed with CD138⁻ Blimp⁺ (Pre-PB) and CD138⁺ Blimp⁺ (PB) differentiated from splenic B220⁺ cells of *Mzb1*^{+/+}*Prdm1*^{+/-}*Igfp* and *Mzb1*^{-/-}*Prdm1*^{+/-}*Igfp* mice.

In Vitro Differentiation of PBs. To mimic TI immunization in vitro, splenic B cells were purified from *Mzb1*^{+/+} and *Mzb1*^{-/-}*Prdm1*^{+/-}*Igfp* mice using anti-B220 magnetic beads (Miltenyi Biotec) and cultured with 25 μ g/mL LPS (L5668; Sigma-Aldrich). After 4 d, three populations were isolated: CD138⁻ Blimp⁻ activated B (Act B) cells, CD138⁻ Blimp⁺ (Pre-PB), and CD138⁺ Blimp⁺ (PB). In some experiments cells were differentiated in 96-U-bottom-well plates, coated overnight at 4 °C with VCAM-1 (2.5, 5 and 10 μ g/mL; R&D Systems). After coating, plates were washed and blocked with Iscove's modified Dulbecco's medium (IMDM)-BSA 1% for 1 h at 37 °C. B220⁺ cells (1 $\times$ 10⁵) were added to the wells and cultured with LPS, in the absence or presence of recombinant CXCL12 (0.5 μ g/mL; R&D Systems). To differentiate CD138⁺ Blimp⁺ cells under TD conditions, B220⁺ cells were cultured for 5 d in the presence of CD40L (5 ng/mL), IL-4, and IL-5 (10 ng/mL; Peprotech).

mRNA Preparation and RNA-Seq Analysis. In vitro differentiated Act B, Pre-PB, and PB cells were sorted by flow cytometry (FACSaria; Becton Dickinson) and total RNA was isolated with an RNeasy Mini Kit (Qiagen) and treated with DNase I, according to the manufacturer's instructions. The total mRNA was enriched by Oligo-dT magnetic beads. The libraries were prepared by using a TruSeq Stranded mRNA library preparation kit. The samples were sequenced using Illumina HiSeq. 3000. The base calling was performed by using BCL2Fastq pipeline (v0.3.1) and bcl2fastq (v2.17.1.14). The paired-end RNA-Seq datasets were mapped to the mouse reference genome (mm9) using Tophat (v2.0.14) and Bowtie (v2.2.6.0) (45). The mapped reads were further

assembled by using Cufflinks (v2.2.1). The expression level of the annotated genes (University of California, Santa Cruz, mm9) was calculated by Cuffquant (46). The two biological replicates of each condition were normalized and the differential gene expression between the conditions was calculated by using Cuffdiff. The gene sets were further filtered for more than twofold up- or down-regulation. The RNA-Seq profile of the *Mzb1* locus was visualized by using MISO (47). The differentially expressed genes were curated using gene ontology, panther functional classifications, and the published literature (8, 9).

Transwell Migration Assay. Act B, Pre-PB, and PB were sorted by flow cytometry (as described above). Next, 1 $\times$ 10⁵ cells (100 μ L) were placed in the upper compartment, and IMDM medium (200 μ L) containing recombinant CXCL12 (0, 0.1 or 0.5 μ g/mL) (SDF-1 α ; R&D Systems) was placed in the lower compartment of VCAM-1 (5 μ g/mL in PBS; R&D Systems) -coated transwell chamber (5- μ m pore size; Corning). After incubation at 37 °C for 4 h, cells migrating into the lower chamber were counted by collecting events for a fixed time (60 s) on a LSR Fortessa flow cytometer, and their percentage relative to the total cells was calculated.

Static Adhesion Assay and VLA-4 Affinity Assay. Adhesion assays and a VLA-4 affinity assay was performed, as previously described (12).

Limited Proteolysis. Limited proteolysis was carried out as previously described (48). MZ and Fo B cells were lysed in 1% Triton X-100, 10 mM Hepes pH 7.4, and 150 mM NaCl. Following nuclei pelleting, protein amount was quantified and supernatant containing equal protein amounts was divided into aliquots, and trypsin was added in increasing concentrations (0, 25, 50, and 100 μ g/mL) for 15 min. Proteolysis was stopped using sample buffer. DTT was added to the samples to achieve a final concentration of 25 mM. Digested samples were heated for 5 min at 95 °C, resolved by SDS/PAGE and immunoblotted with $\beta 1$ -integrin (183666; Abcam), $\alpha 4$ -integrin (8440; Cell Signaling), and CD19 (3574; Cell Signaling) antibodies.

Coimmunoprecipitation. B1-8 B cells were lysed in 2% CHAPS (C3023; Sigma), 10 mM Hepes pH 7.4, and 150 mM NaCl, 1 mM PMSF, and protease inhibitor mixture (P8340; Sigma). Precleared lysates were transferred to Sepharose beads (GE Healthcare) preincubated for 2 h at 4 °C with rabbit polyclonal Grp94 (ab13509; Abcam) or IgG control (2729; Cell Signaling) antibodies. After 4 h, immunoprecipitates were washed and resuspended in sample buffer with DTT (25 mM). Samples were heated for 5 min at 95 °C and resolved by SDS/PAGE. After electrophoresis, samples were transferred onto a nitrocellulose membrane, and immunoblotted with $\beta 1$ -integrin (183666; Abcam) and $\alpha 4$ -integrin (8440; Cell Signaling) antibodies.

Statistics. Data are expressed as mean $\pm$ SD. Data were analyzed by two-tailed Student's *t* test or one-way ANOVA as appropriate, using the GraphPad Prism program (v7). *P* values of less than 0.05, 0.01, and 0.001 were considered significant.

Data Deposition. The RNA-seq data reported in this paper have been deposited to the National Center for Biotechnology Gene Expression Omnibus (GEO) database (accession no. GSE118124).

ACKNOWLEDGMENTS. We thank Eirini Trompouki for critical reading the manuscript; Marika Rott for the assistance with the manuscript preparation; Ingrid Falk and Franziska Ludin for technical assistance; members of R.G.'s department for the discussions; and the Deep Sequencing, Flow Cytometry Facility, and Mouse facilities of the Max Planck Institute. This work was supported by funds from the Max Planck Society and German Research Foundation.

- Shapiro-Shelef M, Calame K (2005) Regulation of plasma-cell development. *Nat Rev Immunol* 5:230–242.
- Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM (2015) The generation of antibody-secreting plasma cells. *Nat Rev Immunol* 15:160–171.
- Allman D, Pillai S (2008) Peripheral B cell subsets. *Curr Opin Immunol* 20:149–157.
- Cerutti A, Cols M, Puga I (2013) Marginal zone B cells: Virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol* 13:118–132.
- Todd DJ, Lee AH, Glimcher LH (2008) The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* 8:663–674.
- Walter P, Ron D (2011) The unfolded protein response: From stress pathway to homeostatic regulation. *Science* 334:1081–1086.
- Kallies A, et al. (2007) Initiation of plasma-cell differentiation is independent of the transcription factor Blimp-1. *Immunity* 26:555–566.

- Minnich M, et al. (2016) Multifunctional role of the transcription factor Blimp-1 in coordinating plasma cell differentiation. *Nat Immunol* 17:331–343.
- Tellier J, et al. (2016) Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response. *Nat Immunol* 17:323–330.
- Shimizu Y, Meunier L, Hendershot LM (2009) pERp1 is significantly up-regulated during plasma cell differentiation and contributes to the oxidative folding of immunoglobulin. *Proc Natl Acad Sci USA* 106:17013–17018.
- van Anken E, et al. (2009) Efficient IgM assembly and secretion require the plasma cell induced endoplasmic reticulum protein pERp1. *Proc Natl Acad Sci USA* 106:17019–17024.
- Flach H, et al. (2010) *Mzb1* protein regulates calcium homeostasis, antibody secretion, and integrin activation in innate-like B cells. *Immunity* 33:723–735.

13. Rosenbaum M, et al. (2014) MZB1 is a GRP94 cochaperone that enables proper immunoglobulin heavy chain biosynthesis upon ER stress. *Genes Dev* 28:1165–1178.
14. Shi W, et al. (2015) Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. *Nat Immunol* 16:663–673.
15. Kinashi T (2005) Intracellular signalling controlling integrin activation in lymphocytes. *Nat Rev Immunol* 5:546–559.
16. Luo BH, Carman CV, Springer TA (2007) Structural basis of integrin regulation and signaling. *Annu Rev Immunol* 25:619–647.
17. Lu TT, Cyster JG (2002) Integrin-mediated long-term B cell retention in the splenic marginal zone. *Science* 297:409–412.
18. Ulyanova T, et al. (2005) VCAM-1 expression in adult hematopoietic and non-hematopoietic cells is controlled by tissue-inductive signals and reflects their developmental origin. *Blood* 106:86–94.
19. Jacobson K, Kravitz J, Kincade PW, Osmond DG (1996) Adhesion receptors on bone marrow stromal cells: In vivo expression of vascular cell adhesion molecule-1 by reticular cells and sinusoidal endothelium in normal and gamma-irradiated mice. *Blood* 87:73–82.
20. Koni PA, et al. (2001) Conditional vascular cell adhesion molecule 1 deletion in mice: Impaired lymphocyte migration to bone marrow. *J Exp Med* 193:741–754.
21. Leuker CE, Labow M, Müller W, Wagner N (2001) Neonatally induced inactivation of the vascular cell adhesion molecule 1 gene impairs B cell localization and T cell-dependent humoral immune response. *J Exp Med* 193:755–768.
22. Minges Wols HA, Underhill GH, Kansas GS, Witte PL (2002) The role of bone marrow-derived stromal cells in the maintenance of plasma cell longevity. *J Immunol* 169: 4213–4221.
23. Tokoyoda K, Egawa T, Sugiyama T, Choi BI, Nagasawa T (2004) Cellular niches controlling B lymphocyte behavior within bone marrow during development. *Immunity* 20:707–718.
24. Kallies A, et al. (2004) Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. *J Exp Med* 200:967–977.
25. Taubenheim N, et al. (2012) High rate of antibody secretion is not integral to plasma cell differentiation as revealed by XBP-1 deficiency. *J Immunol* 189:3328–3338.
26. Lenter M, et al. (1993) A monoclonal antibody against an activation epitope on mouse integrin chain beta 1 blocks adhesion of lymphocytes to the endothelial integrin alpha 6 beta 1. *Proc Natl Acad Sci USA* 90:9051–9055.
27. Bazzoni G, Shih DT, Buck CA, Hemler ME (1995) Monoclonal antibody 9EG7 defines a novel beta 1 integrin epitope induced by soluble ligand and manganese, but inhibited by calcium. *J Biol Chem* 270:25570–25577.
28. Fagarasan S, Honjo T (2000) T-independent immune response: New aspects of B cell biology. *Science* 290:89–92.
29. Tiwari S, Askari JA, Humphries MJ, Bulleid NJ (2011) Divalent cations regulate the folding and activation status of integrins during their intracellular trafficking. *J Cell Sci* 124:1672–1680.
30. Staron M, et al. (2010) gp96, an endoplasmic reticulum master chaperone for integrins and Toll-like receptors, selectively regulates early T and B lymphopoiesis. *Blood* 115:2380–2390.
31. Cyster JG (2003) Homing of antibody secreting cells. *Immunol Rev* 194:48–60.
32. Li YF, Xu S, Ou X, Lam KP (2014) Shp1 signalling is required to establish the long-lived bone marrow plasma cell pool. *Nat Commun* 5:4273.
33. van Spriel AB, et al. (2012) The tetraspanin CD37 orchestrates the $\alpha 4 \beta 1$ integrin-Akt signaling axis and supports long-lived plasma cell survival. *Sci Signal* 5:ra82.
34. Ansa-Addo EA, et al. (2016) Clients and oncogenic roles of molecular chaperone gp96/grp94. *Curr Top Med Chem* 16:2765–2778.
35. Liu B, et al. (2010) Folding of Toll-like receptors by the HSP90 paralogue gp96 requires a substrate-specific cochaperone. *Nat Commun* 1:79.
36. Takahashi K, et al. (2007) A protein associated with Toll-like receptor (TLR) 4 (PRAT4A) is required for TLR-dependent immune responses. *J Exp Med* 204:2963–2976.
37. Wakabayashi Y, et al. (2006) A protein associated with toll-like receptor 4 (PRAT4A) regulates cell surface expression of TLR4. *J Immunol* 177:1772–1779.
38. Braakman I, Bulleid NJ (2011) Protein folding and modification in the mammalian endoplasmic reticulum. *Annu Rev Biochem* 80:71–99.
39. Pena F, Jansens A, van Zadelhoff G, Braakman I (2010) Calcium as a crucial cofactor for low density lipoprotein receptor folding in the endoplasmic reticulum. *J Biol Chem* 285:8656–8664.
40. Wehrli N, et al. (2001) Changing responsiveness to chemokines allows medullary plasmablasts to leave lymph nodes. *Eur J Immunol* 31:609–616.
41. Hauser AE, et al. (2002) Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response. *J Immunol* 169:1277–1282.
42. Kabashima K, et al. (2006) Plasma cell S1P1 expression determines secondary lymphoid organ retention versus bone marrow tropism. *J Exp Med* 203:2683–2690.
43. Good-Jacobson KL, O'Donnell K, Belz GT, Nutt SL, Tarlinton DM (2015) c-Myb is required for plasma cell migration to bone marrow after immunization or infection. *J Exp Med* 212:1001–1009.
44. Hargreaves DC, et al. (2001) A coordinated change in chemokine responsiveness guides plasma cell movements. *J Exp Med* 194:45–56.
45. Kim D, et al. (2013) TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 14:R36.
46. Trapnell C, et al. (2013) Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nat Biotechnol* 31:46–53.
47. Katz Y, Wang ET, Airolidi EM, Burge CB (2010) Analysis and design of RNA sequencing experiments for identifying isoform regulation. *Nat Methods* 7:1009–1015.
48. Thibodeau PH, et al. (2010) The cystic fibrosis-causing mutation deltaF508 affects multiple steps in cystic fibrosis transmembrane conductance regulator biogenesis. *J Biol Chem* 285:35825–35835.