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Supplemental Information

Impaired Expression of Rearranged Immunoglobulin Genes and Premature p53 Activation Block B Cell Development in BMI1 Null Mice

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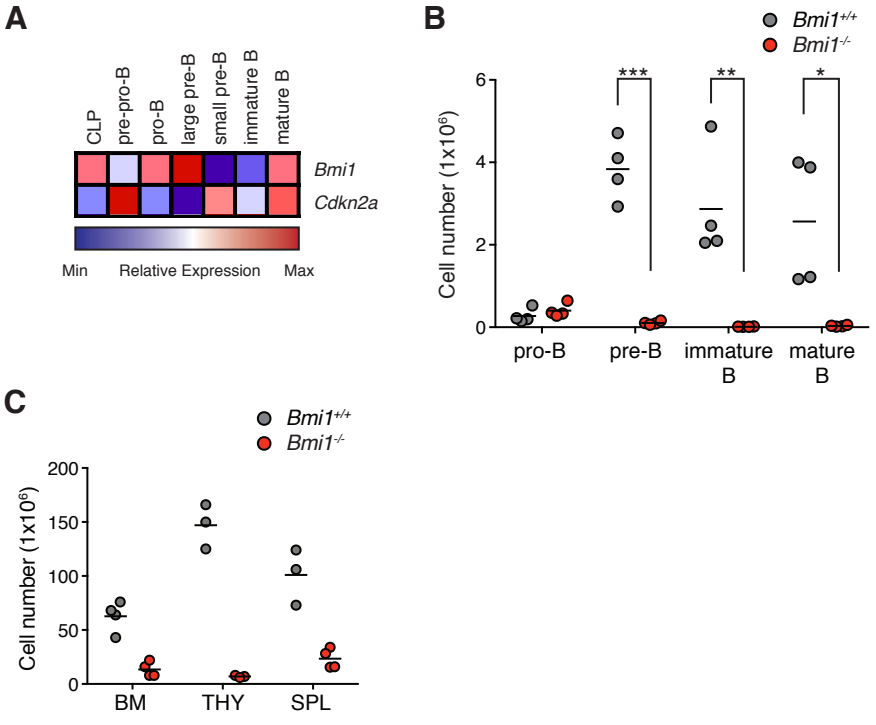

Figure S1, related to Figure 1. BMI1 is highly expressed and functions at the pro-B to pre-B cell transition.

(A) Expression pattern of *Bmi1* and *Cdkn2a* in developing B cells from the Immunological Genome Project (Painter et al., 2011). **(B)** Cell number of developing B cells in the bone marrow of indicated mice. Each point represents one animal; line represents the mean. n=4 for each genotype. **(C)** Cellularity of bone marrow, thymus, and spleen from indicated mice. Each point represents one animal; line represents the mean. $n \geq 3$ for each genotype. ***$p<0.001$, **$p<0.01$, *$p<0.05$. BM: bone marrow. THY: thymus. SPL: spleen.

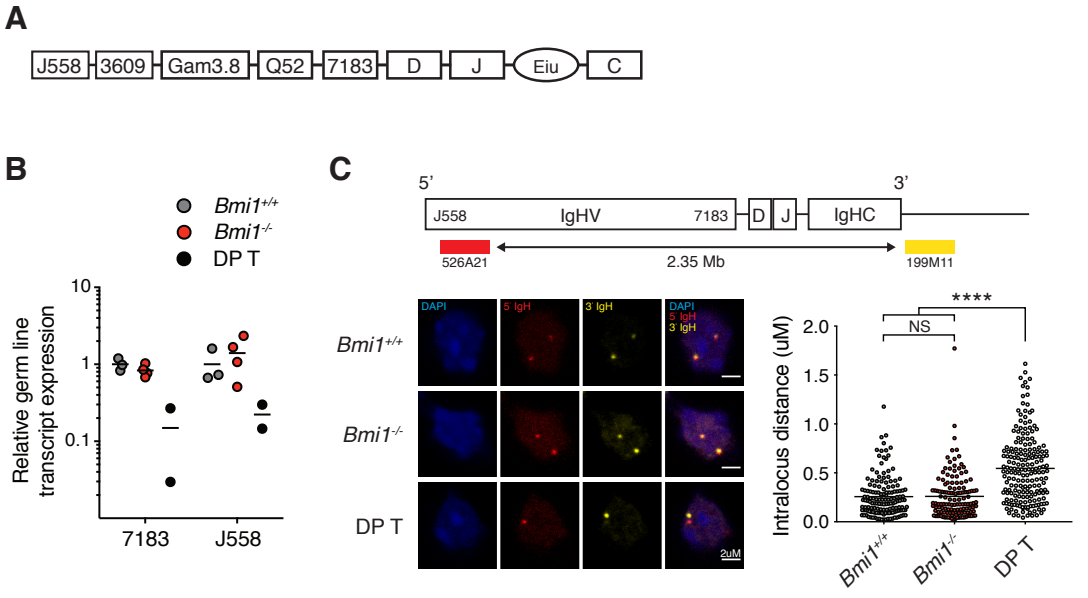

Figure S2, related to Figure 2. BMI1 is not required for *Igh* germ line transcription or *Igh* contraction. **(A)** Diagram of *Igh* locus with distal and proximal VH families indicated. **(B)** RT-qPCR expression analysis of germline transcription of both proximal (7183) and distal (J558) VH gene families from sorted pro-B cells from indicated mice. DP T were used as a negative control. Each point represents one animal; line represents the mean. n=3 for *Bmi1*$^{+/+}$ animals; n=4 for *Bmi1*$^{-/-}$ animals; n=2 for DP T. **(C)** Analysis of *Igh* contraction in pro-B cells using 3-D microscopy with proximal and distal DNA FISH probes as depicted in the diagram. 2.35 Mb separate the two DNA FISH probes. Left: representative images. Scale bars, 2 μM. Right: quantification of intralocus distance. DP T were used as a negative control. 2 independent experiments were done with >100 cells calculated for each genotype. Each point represents one cell; line represents the mean. ****p<0.0001, *p<0.05. NS: not significant. DP T: double positive thymocytes.

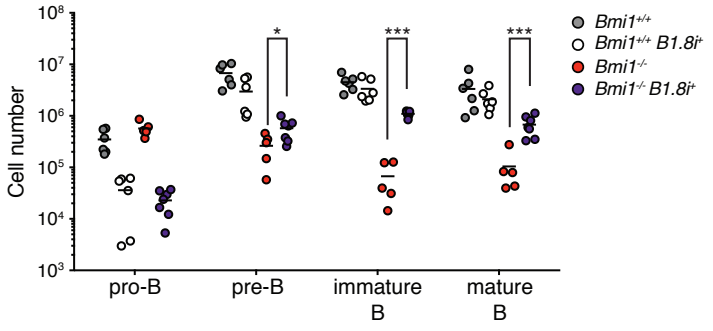

Figure S3, related to Figure 3. Bypassing VDJ_H rearrangement rescues B cell development in *Bmi1*$^{-/-}$ mice.

Cell number of indicated progenitor B cell type in the bone marrow of indicated mice. Each point represents one animal; line represents the mean. $n \geq 5$ for each genotype. ***$p<0.001$, *$p<0.05$.

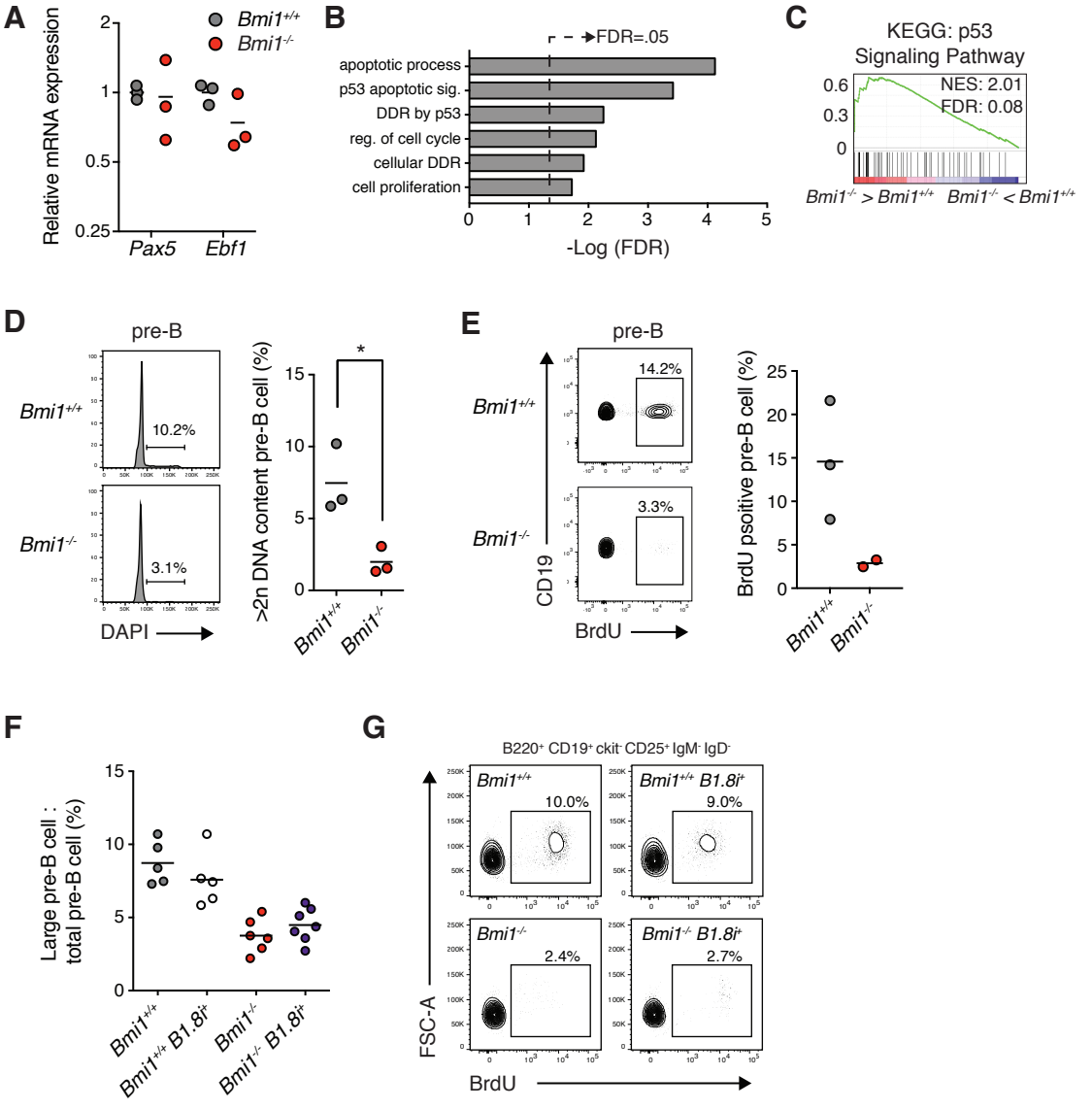

Figure S4, related to Figure 4. BMI1 prevents inappropriate p53 signaling in pro-B cells.
(A) mRNA expression levels as determined by RT-qPCR of indicated genes from pro-B cells of indicated mice. Each point represents one animal; line represents the mean. n=3 for each genotype. **(B)** Gene ontology analysis of upregulated genes in *Bmi1*$^{-/-}$ pro-B cells. **(C)** Gene set enrichment analysis identified that the p53 signaling pathway is enriched in *Bmi1*$^{-/-}$ pro-B cells. **(D)** Representative FACS plot and frequency of pre-B cells that have >2n DNA content (cells in S-G2-M phases of cell cycle) in indicated mice. Each point represents one animal; line represents the mean. n=3 for each genotype. **(E)** Representative FACS plot and frequency of BrdU^{+} pre-B cells in indicated mice. Animals received an i.p. injection of 1mg BrdU and were analyzed two hours later. Each point represents one animal; line represents the mean. n=3 for *Bmi1*$^{+/+}$ animals; n=2 for *Bmi1*$^{-/-}$ animals. **(F)** The frequency of large pre-B cells present in the pre-B cell compartment of indicated mice. Each point represents one animal; line represents the mean. n ≥ 5 for each genotype. **(G)** FACS plot of BrdU^{+} pre-B cells in indicated animals. Animals received an i.p. injection of 1mg BrdU and were analyzed two hours later. *p<0.05.

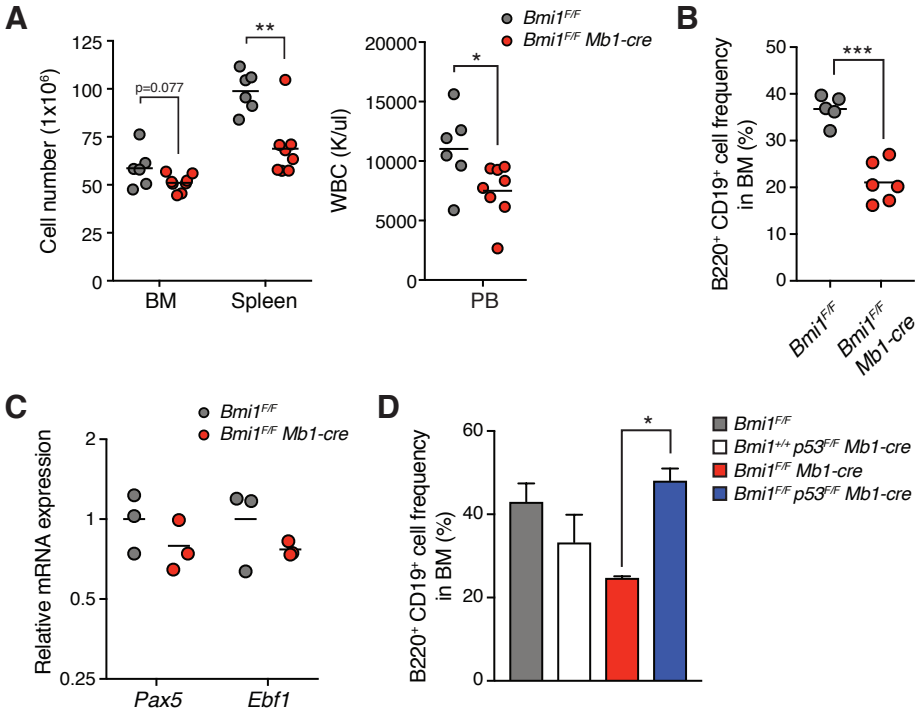

Figure S5, related to Figure 5. BMI1 functions in a cell autonomous manner to regulate B cell development. **(A)** Cell number in the spleen, bone marrow, and peripheral blood of indicated mice. Each point represents one animal; line represents the mean. n=6 for *Bmi1*$^{F/F}$ animals; n=8 for *Bmi1*$^{F/F}$ *Mb1-cre* animals. **(B)** Frequency of B220^{+} CD19^{+} cells in the bone marrow of indicated mice. Each point represents one animal; line represents the mean. n=5 for *Bmi1*$^{F/F}$ animals; n=6 for *Bmi1*$^{F/F}$ *Mb1-cre* animals. **(C)** mRNA expression levels as determined by RT-qPCR of indicated genes from pro-B cells of indicated mice. Each point represents one animal; line represents the mean. n=3 for each genotype. **(D)** Frequency of B220^{+} CD19^{+} cells in the bone marrow of indicated mice. Data represents mean ± sem. n=3 for each genotype. ***p<0.001, **p<0.01, *p<0.05. BM: bone marrow. PB: peripheral blood. WBC: white blood cell.

Cell Population	Cell-surface phenotypes
Pro-B	$B220^{+}$ $CD19^{+}$ $ckit^{+}$ $CD25^{-}$ IgM^{-} IgD^{-}
Pre-B	$B220^{+}$ $CD19^{+}$ $ckit^{-}$ $CD25^{+}$ IgM^{-} IgD^{-}
Large Pre-B	$B220^{+}$ $CD19^{+}$ $ckit^{-}$ $CD25^{+}$ IgM^{-} IgD^{-} FSC^{high}
Small Pre-B	$B220^{+}$ $CD19^{+}$ $ckit^{-}$ $CD25^{+}$ IgM^{-} IgD^{-} FSC^{low}
Immature B	$B220^{+}$ $CD19^{+}$ IgM^{+} IgD^{-}
Mature (recirculating) B	$B220^{+}$ $CD19^{+}$ IgM^{+} IgD^{+}
DP Thymocytes	Lin (B220, TER119, Gr1, CD11b)$^{-}$ $CD4^{+}$ $CD8^{+}$

Table S2, related to STAR methods. Flow cytometry cell surface phenotypes.

Gene	5’ oligo	3’ oligo
Hprt	AGG TTG CAA GCT TGC TGG T	TGA AGT ACT CAT TAT AGT CAA GGG CA
Bmi1	TGC AGA TGA GGA GAA GAG GA	TCA TTC ACC TCT TCC TTA GGC
p19^{Arf}	GCT CTG GCT TTC GTG AAC ATG	TCG AAT CTG GAC CGT AGT TGA G
Bbc3	CTG GAG GGT CAT GTA CAA TCT C	GGT GTC AGA AGG CGG AG
Pmapi1	CGG ACA TAA CTG TGG TTC TGG	ACA CTC GTC CTT CAA GTC TGC
Bax	AAA CTG GTG CTC AAG GCC C	CTT GGA TCC AGA CAA GCA GC
Phlda3	AGA CAT GTC AGC TTC TCT GTC C	CTA CTG GCT TCT GCT CTC CG
C*dkn1a*	AGA GAC AAC GGC ACA CTT TG	CAG ACA TTC AGA GC CACA GG
Trp53	TGA ACC GCC GAC CTA TCC TTA CC	CCC AGG GCA GGC ACA AAC AC
Pax5	GGA CCA TCA GGA CAG GAC AT	ACA GCA ATG GGA TAC GGA CA
Ebf1	ACA GCA ATG GGA TAC GGA CA	CGT GTG TGA GCA ATA CTC GG
D_H-Cμ	TTC AAA GCA CAA TGC CTG GCT	ATG CAG ATC TCT GTT TTT GCC TCC
V_H7183-Cμ	CGG TAC CAA GAA SAM CCT GTW CCT GCA AAT GAS C	ATG CAG ATC TCT GTT TTT GCC TCC
V_HQ52-Cμ	CGG TAC CAG ACT GAR CAT CAS CAA GGA CAA YTC	ATG CAG ATC TCT GTT TTT GCC TCC
V_HGam3.8-Cμ	CAA GGG ACG GTT TGC CTT CTC TTT GGA A	ATG CAG ATC TCT GTT TTT GCC TCC
V_H3609-Cμ	KCY YTG AAG AGC CRR CTC ACA ATC TCC	ATG CAG ATC TCT GTT TTT GCC TCC
V_HJ558-Cμ	CGA GCT CTC CAR CACA GC CTW CAT GCA RCT CAR C	ATG CAG ATC TCT GTT TTT GCC TCC
V_H7183 GLT	GCC TCT GGA TTC ACT TTC AG	CAT TGT CTC TGG AGA TGG TG
V_HJ558 GLT	CTT CTG GCT ACA CCT TCA C	CTG AGC TGC ATG TAG GCT G

K: G,T M: A,C R: A,G S: C,G W: A,T Y: C,T

Table S3, related to STAR methods. mRNA expression RT-qPCR primer pairs.

DNA product	**5' oligo**	**3' oligo**
$D_H - J_{H3}$	TTC AAA GCA CAA TGC CTG GCT	GTC TAG ATT CTC ACA AGA GTC CGA TAG ACC CTG G
$V_H7183 - J_{H3}$	CGG TAC CAA GAA SAM CCT GTW CCT GCA AAT GAS C	GTC TAG ATT CTC ACA AGA GTC CGA TAG ACC CTG G
$V_HJ558 - J_{H3}$	CGA GCT CTC CAR CACA GC CTW CAT GCA RCT CAR C	GTC TAG ATT CTC ACA AGA GTC CGA TAG ACC CTG G
Cμ	TGG CCA TGG GCT GCC TAG CCC GGG ACT T	GCC TGA CTG AGC TCA CAC AAG GAG GA
MsVHe - J_{H3}	GGG AAT TCG AGG TGC AGC TGC AGG AGT CTG G	GTC TAG ATT CTC ACA AGA GTC CGA TAG ACC CTG G

R: A,G S: C,G W: A,T

Table S4, related to STAR methods. Primer pairs for analysis of *VDJ_H* joints.