SUPPLEMENTAL MATERIAL

Rowland et al., http://www.jem.org/cgi/content/full/jem.20091673/DC1

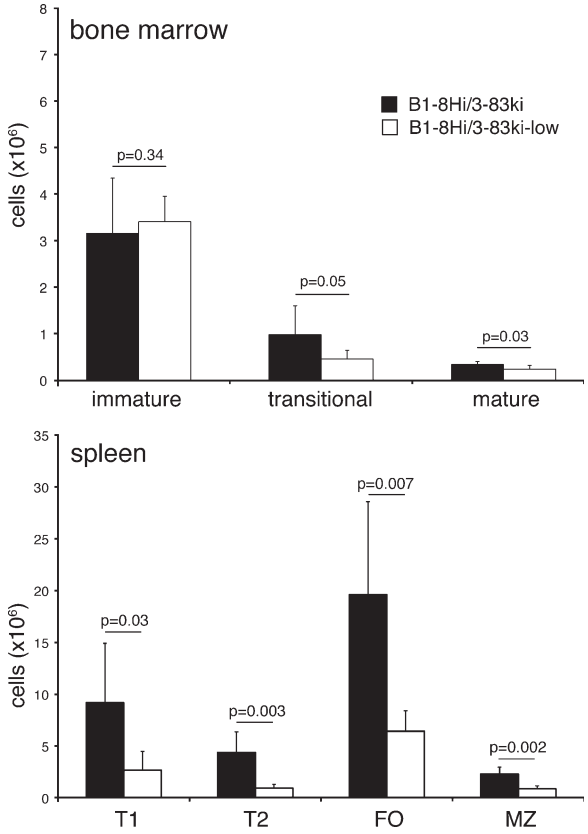

Figure S1. Generation of transitional and mature B cells is impaired in B1-8Hi/3-83ki-low mice. Bone marrow and spleen cells from B1-8Hi/3-83ki and B1-8Hi/3-83ki-low mice were analyzed by flow cytometry to determine numbers of immature ($B220^+IgM^+IgD^-$), transitional ($B220^+IgM^+IgD^{low}$), and mature ($B220^+IgD^{high}$) B cells in the bone marrow (top), and of T1 ($B220^+CD24^{high}CD23^{low}$), T2 ($B220^+CD24^{high}CD23^{high}$), follicular (FO; $B220^+CD24^{low}CD23^{high}$), and marginal zone (MZ; $B220^+CD21^{high}CD1d^{high}$) B cells in the spleen (bottom). Bar graphs represent mean cell numbers (in millions) and SDs of six mice in each group analyzed over the course of six independent experiments.

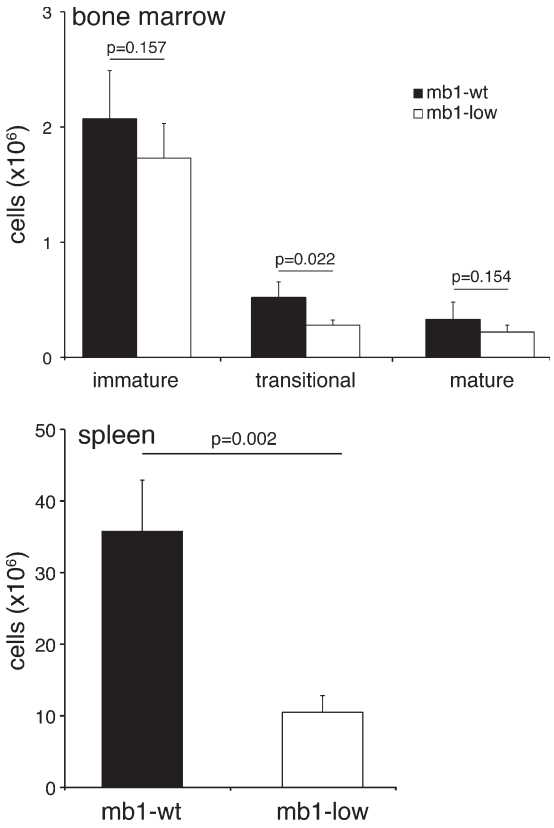

Figure S2. Generation of bone marrow transitional and spleen mature B cells is impaired in BCR-low mice carrying a normal Ig repertoire. Bone marrow and spleen cells from wild-type (mb1-wt; *Igh*$^{+/+}$*Igk*$^{+/+}$*mb-1*$^{+/+}$) and mb1-low (*Igh*$^{+/+}$*Igk*$^{+/+}$*mb-1*$^{-/mb\text{-}1\text{-}mEGFPinv}$) mice were analyzed by flow cytometry to determine numbers of immature (B220^{+}IgM^{+}IgD^{-}), transitional (B220^{+}IgM^{+}IgD^{low}), and mature (B220^{+}IgD^{high}) B cells in the bone marrow (top), and of mature follicular (B220^{+}CD21^{low}CD23^{high}) B cells in the spleen (bottom). Bar graphs represent mean cell numbers (in millions) and SDs of three mice in each group analyzed over the course of three independent experiments.

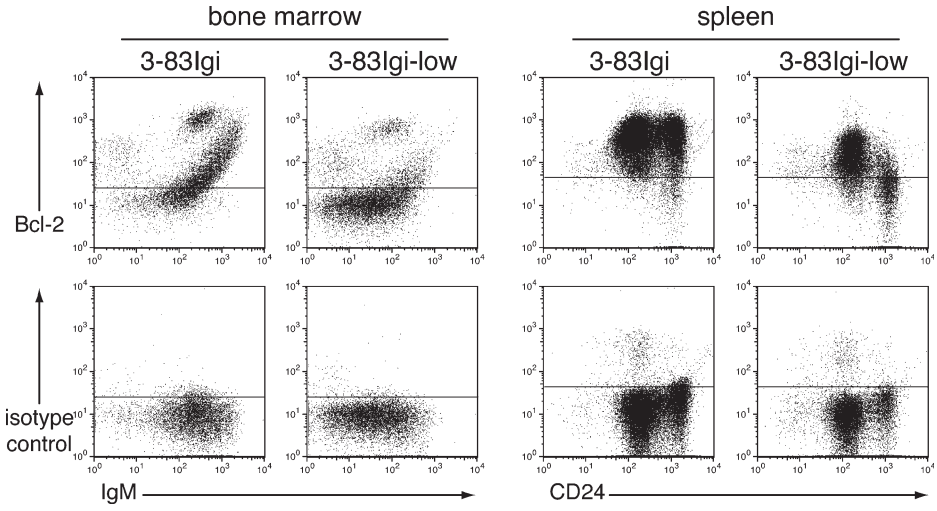

Figure S3. Representative flow cytometric analysis of Bcl-2 expression. Bone marrow and spleen cells from 3-83Igi and 3-83Igi-low mice were stained with anti–Bcl-2 (top) or isotype control (bottom) antibodies and for other indicated markers. Dot plots show B220^{+} lymphocytes. Events falling above the lines have Bcl-2–positive staining above isotype control. Data are representative of analyses of six mice from six individual experiments.

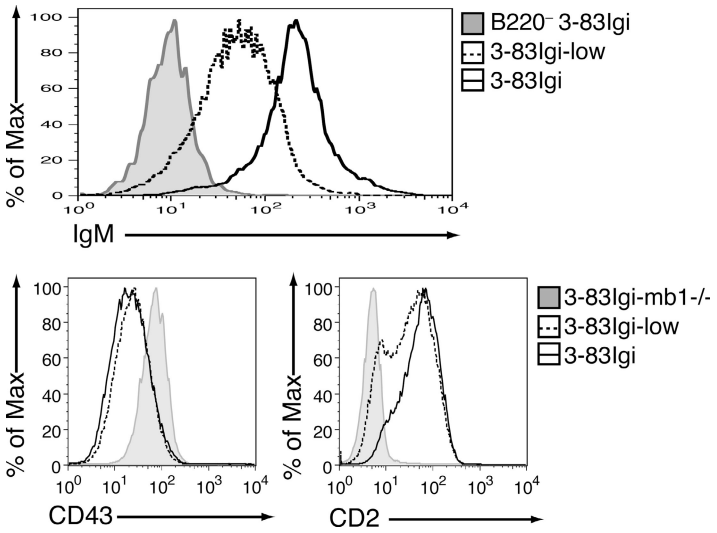

Figure S4. Characterization of B cells generated from IL-7 bone marrow cultures. Bone marrow from 3-83Igi (*Igh*$^{3\text{-}83/3\text{-}83}$*Igk*$^{3\text{-}83/3\text{-}83}$*H-2*$^{d/d}$*mb-1*$^{+/+}$), 3-83Igi-low (*Igh*$^{3\text{-}83/3\text{-}83}$*Igk*$^{3\text{-}83/3\text{-}83}$*H-2*$^{d/d}$*mb-1*$^{-/mb\text{-}1\text{-}mEGFPinv}$), and 3-83Igi-mb1$^{-/-}$ (*Igh*$^{3\text{-}83/3\text{-}83}$*Igk*$^{3\text{-}83/3\text{-}83}$*H-2*$^{d/d}$*mb-1*$^{-/-}$) mice were cultured for 4 d in the presence of IL-7 and analyzed by flow cytometry for the expression of IgM, CD43, and CD2. Gates for the analyses were drawn on live B220^{+} cells, except for those of B220^{-} cells in the shaded histogram (top). Data are representative of three independent experiments.

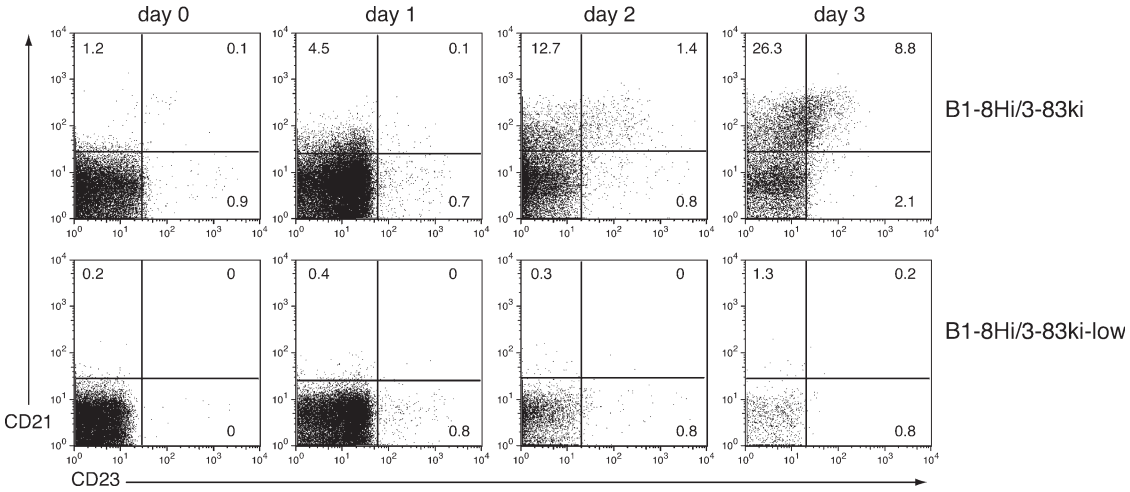

Figure S5. B1-8Hi/3-83ki-low immature B cells have impaired differentiation in vitro. Bone marrow IL-7 cultures from B1-8Hi/3-83ki and B1-8Hi/3-83ki-low mice containing >85% B220^{+} B cells were cultured in complete medium supplemented with 10 ng/ml of recombinant BAFF for up to 3 d. Before (day 0) and during each day (days 1, 2, and 3) of BAFF culture, cells were analyzed for the expression of CD21 and CD23 by flow cytometry. Dot plots show CD21 and CD23 staining of live (PI-negative) cells. Numbers represent the frequency of cells in the indicated quadrant gates. Data are representative of two individual experiments.

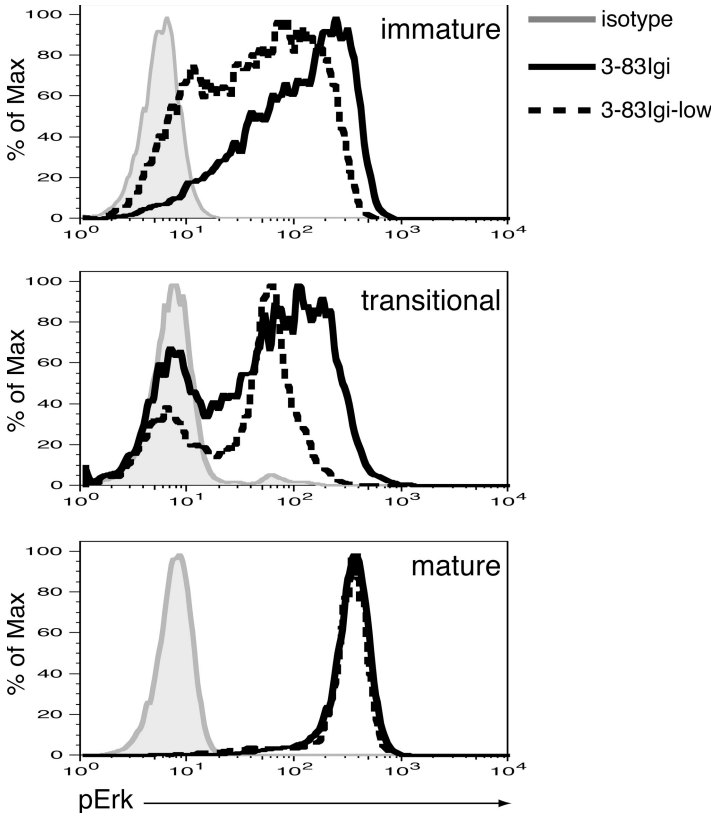

Figure S6. Representative flow cytometric analysis of pErk staining. Bone marrow (top) and spleen (middle and bottom) cells from 3-83Igi and 3-83Igi-low mice were stained with anti-pErk antibodies and for specific B cell markers. pErk staining is shown for $B220^+$ small lymphocytes in the immature (IgD^-), transitional ($CD24^{high}$), and mature ($CD24^{low}$) B cell populations. Shaded histograms are 3-83Igi B cells stained with isotype control antibodies. Data are representative of analyses from four mice in four individual experiments.

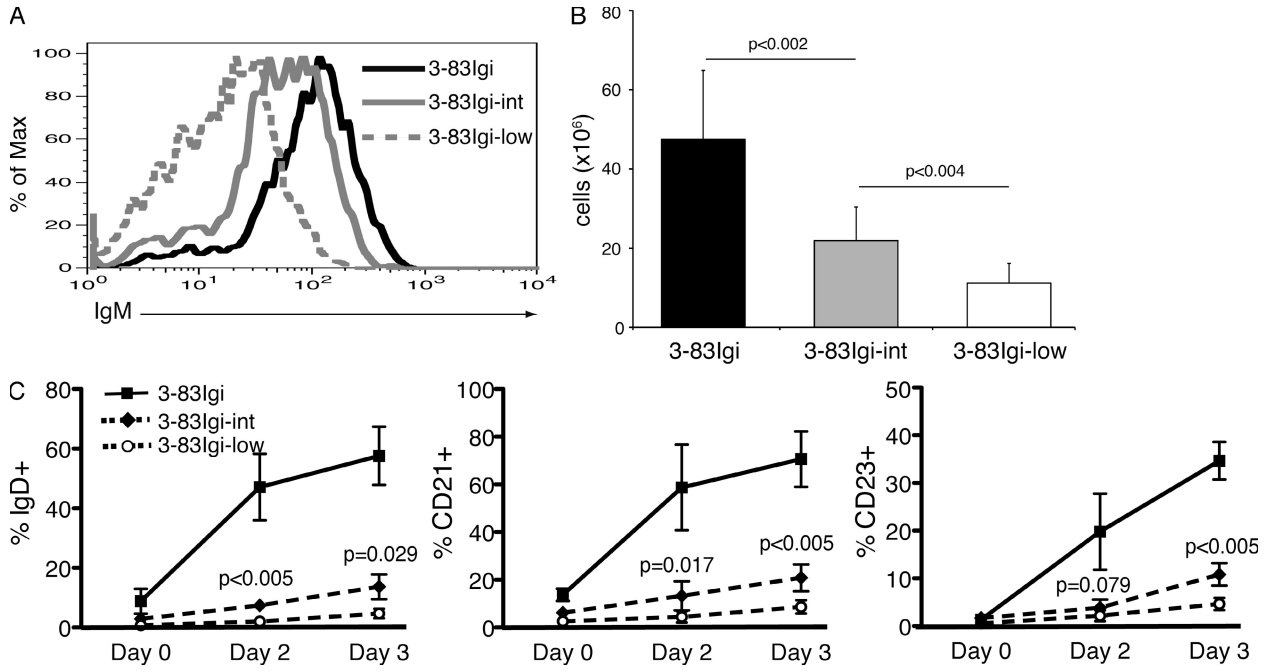

Figure S7. Generation of mature B cells is impaired in 3-83Igi-int mice. (A) Bone marrow and (B) spleen cells from 3-83Igi ($Igh^{3\text{-}83/3\text{-}83}Igk^{3\text{-}83/3\text{-}83}H\text{-}2^{d/d}mb\text{-}1^{+/+}$), 3-83Igi-int ($Igh^{3\text{-}83/3\text{-}83}Igk^{3\text{-}83/3\text{-}83}H\text{-}2^{d/d}mb\text{-}1^{mb\text{-}1\text{-}mEGFPinv/mb\text{-}1\text{-}mEGFPinv}$), and 3-83Igi-low ($Igh^{3\text{-}83/3\text{-}83}Igk^{3\text{-}83/3\text{-}83}H\text{-}2^{d/d}mb\text{-}1^{-/mb\text{-}1\text{-}mEGFPinv}$) mice were analyzed by flow cytometry to determine IgM expression on immature ($B220^+IgD^-IgM^+$) B cells (A), and numbers of mature follicular ($B220^+CD21^{low}CD23^{high}$) B cells (B). Bar graphs represent mean cell numbers (in millions) and SDs of five to seven mice in each group. (C) Representative kinetics of immature B cell differentiation in vitro. Bone marrow cells from 3-83Igi, 3-83Igi-int, and 3-8Igi-low mice were cultured in the presence of IL-7 for 4 d. After IL-7 culture (day 0), cells were recultured in the presence of BAFF, and $B220^+$ cells were analyzed by flow cytometry for expression of IgD, CD21, and CD23 at the indicated times. Data represent the mean frequencies (±SD) of live $B220^+$ B cells from four mice from four individual experiments. Statistics shown represent p-values for a Student's *t* test of 3-83Igi-int and 3-83Igi-low B cell samples.

Table S1. Absolute cell numbers in mixed bone marrow chimeras

Bone marrow mixed chimeras	Donor-derived B cells	Immature (BM)	Transitional (BM)	Transitional (SP)	Mature (SP)
3-83Igi: CB17	3-83Igi	0.78 ± 0.43	0.2 ± 0.11	2.53 ± 1.5	6.6 ± 4.48
	CB17	1.37 ± 1.03	0.34 ± 0.24	3.99 ± 2.39	12.82 ± 9.22
3-83Igi-low: CB17	3-83Igi-low	0.7 ± 0.38	0.06 ± 0.02	0.67 ± 0.35	1.01 ± 0.34
	CB17	1.24 ± 0.47	0.29 ± 0.14	5.51 ± 1.81	18.94 ± 5.81
3-83Igi-low + Bcl-2: CB17 + Bcl2 ($Thy1.1^+$)	3-83Igi-low	1.7 ± 0.38	0.14 ± 0.05	0.47 ± 0.12	0.4 ± 0.22
	CB17	0.95 ± 0.23	0.17 ± 0.02	3.68 ± 2.19	23.68 ± 8.71
3-83Igi-low + Bcl-2: CB17 + Bcl2 ($Thy1.1^-$)	3-83Igi-low	0.61 ± 0.21	0.01 ± 0.004	0.3 ± 0.06	0.05 ± 0.02
	CB17	0.44 ± 0.03	0.02 ± 0.002	1.14 ± 0.51	3.55 ± 0.43

Bone marrow chimeras were established using donor cells from the indicated mice. The following surface markers were used to discriminate B cell subsets: immature (bone marrow), IgM^+IgD^-; transitional (bone marrow), $IgM^{high}IgD^{low}$; transitional (spleen), $B220^+CD24^{high}$; and mature (spleen), $B220^+CD24^{low}$. Absolute cell numbers ($x10^6$) ± SD from at least five mice for each set of chimeras are shown. BM, bone marrow; SP, spleen.