Supporting Information

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SI Text

A simple version of the MiDReG program is available at the following web site: http://genepyramid.stanford.edu/microarray/MiDReG/.

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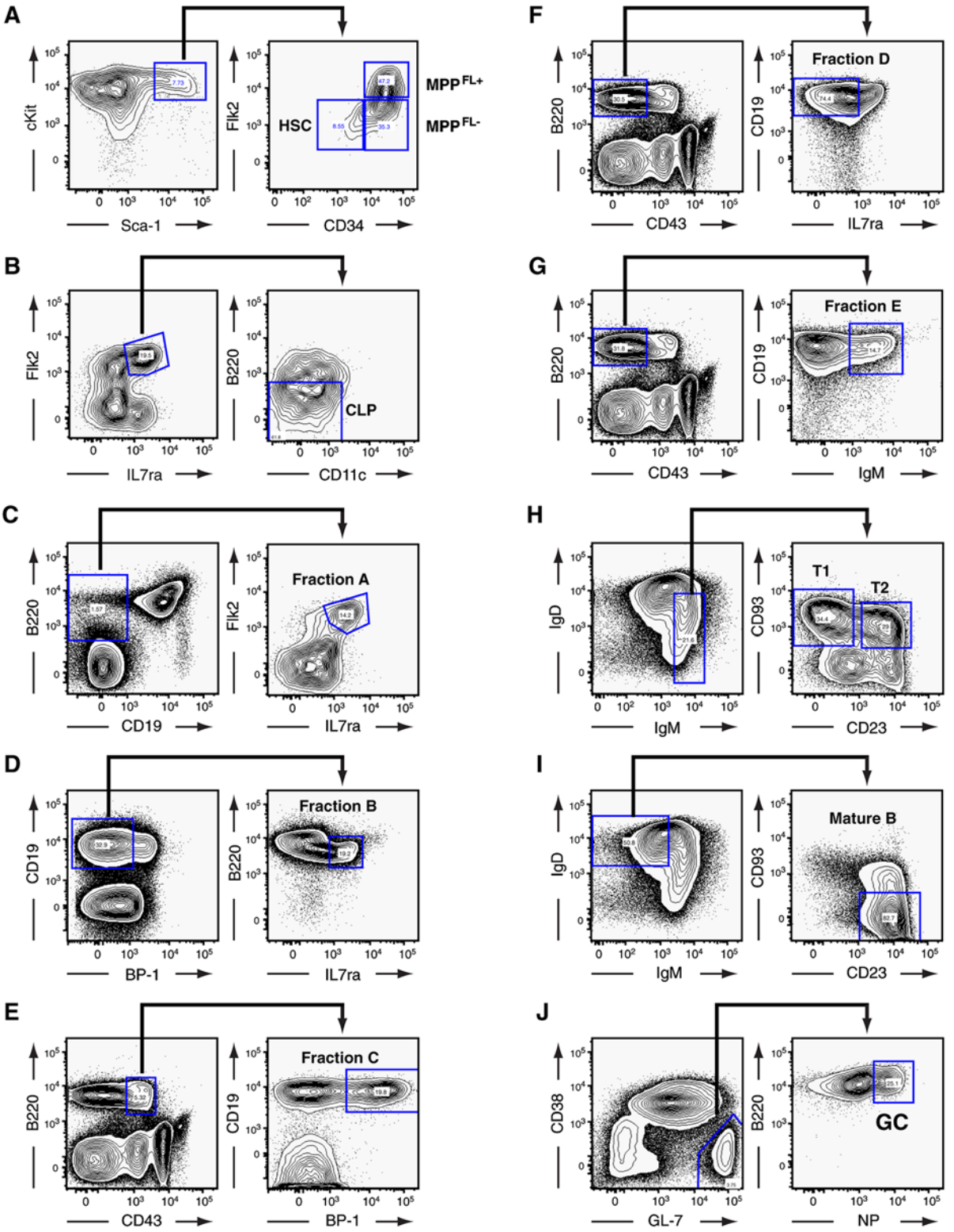

Fig. S1. Purification of B-cell precursors. Precursor populations on B-cell development pathway were purified by fluorescent-activated cell sorting from either the marrow (*A*–*G*) or the spleen (*H*–*I*) of naive mice or the spleen from immunized mice (*J*). Definition of cell surface markers for populations are as follows: (*A*) HSC, *CD34*$^-$*Flk2*$^-$*cKit*$^+$*Sca-1*$^+$*Lin*(*Mac-1*, *Gr-1*, *B220*, *CD4*, *CD8*, *IL-7Rα*, and *Ter119*)$^-$; MPP^{FL-}, *CD34*$^+$*Flk2*$^-$*cKit*$^+$*Sca-1*$^+$*Lin*$^-$; MPP^{FL+}, *CD34*$^+$*Flk2*$^+$*cKit*$^+$*Sca-1*$^+$*Lin*$^-$; (*B*) CLP, *Ly6c*$^-$*NK1.1*$^-$*CD11c*$^-$*CD27*$^+$*CD19*$^-$*B220*$^-$*Flk2*$^+$*IL-7Rα*$^+$*BP-1*$^-$; (*C*) fraction A, *Ly6c*$^-$*NK1.1*$^-$*CD11c*$^-$*CD27*$^+$*CD19*$^-$*B220*$^+$*Flk2*$^+$*IL-7Rα*$^+$*BP-1*$^-$; (*D*) fraction B, *Ly6c*$^-$*NK1.1*$^-$*CD11c*$^-$*CD19*$^+$*B220*$^+$*IL-7Rα*$^+$*BP-1*$^-$; (*E*) fraction C, *IgD*$^-$*NK1.1*$^-$*IgM*$^-$*B220*$^+$*CD43*$^+$*CD19*$^+$*IL-7Rα*$^+$*BP-1*$^+$; (*F*) fraction D, *IgD*$^-$*NK1.1*$^-$*IgM*$^-$*B220*$^+$*CD43*$^-$*CD19*$^+$*IL-7Rα*$^-$; (*G*) fraction E, *IgD*$^-$*NK1.1*$^-$*IgM*$^+$*B220*$^+$*CD43*$^-$*CD19*$^+$*IL-7Rα*$^-$; (*H*) T1, *B220*$^+$*AA4.1*$^+$*IgM*$^+$*CD23*$^-$*Mac-1*$^-$*Gr-1*$^-$*CD3*$^-$*CD4*$^-$*CD8*$^-$*Ter119*$^-$, T2, *B220*$^+$*AA4.1*$^+$*IgM*$^+$*CD23*$^+$*Mac-1*$^-$*Gr-1*$^-$*CD3*$^-$*CD4*$^-$*CD8*$^-$*Ter119*$^-$; (*I*) mature B, *B220*$^+$*AA4.1*$^-$*IgM*^{low}*CD23*$^+$*IgD*^{high}*Mac-1*$^-$*Gr-1*$^-$*CD3*$^-$*CD4*$^-$*CD8*$^-$*Ter119*$^-$; (*J*) germinal center (GC) B cells, *B220*$^+$*CD38*$^-$*GL7*$^+$*NP*$^+$*Mac-1*$^-$*Gr-1*$^-$*CD3*$^-$*CD4*$^-$*CD8*$^-$*Ter119*$^-$.

Table S1. Predicted B-cell genes

Human gene	Affymetrix ID	Mouse gene	Affymetrix ID
ARHGAP4	204425_at	*Arhgap4*	1419296_at
BACH2	227173_s_at	*Bach2*	1441657_at
BLK	206255_at	*Blk*	1422775_at
BTLA	236226_at	*Btla*	1455656_at
CCR6	206983_at	*Ccr6*	1450357_a_at
CCR7	206337_at	*Ccr7*	1423466_at
CD180	206206_at	*Cd180*	1421547_at
CD22	217422_s_at	*Cd22*	1419769_at, 1419768_at
CD53	242946_at	*Cd53*	1459744_at
CD72	215925_s_at	*Cd72*	1426112_a_at
CD79A	205049_s_at, 1555779_a_at	*Cd79a*	1418830_at
CD80	1554519_at	*Cd80*	1427717_at, 1432826_a_at
CD84	211192_s_at, 211190_x_at	*Cd84*	1446505_at
CD86	205686_s_at, 205685_at	*Cd86*	1420404_at, 1449858_at
CENTB1	205213_at	*Centb1*	1455125_at, 1434874_x_at
CLEC2D	220132_s_at, 233500_x_at, 235522_at	*Clec2d*	1419477_at
DENND1C	221080_s_at	*Dennd1c*	1431840_at
DOK3	223553_s_at, 220320_at	*Dok3*	1418096_at
FAIM3	221602_s_at	*Faim3*	1429889_at
FCER2	206759_at, 206760_s_at	*Fcer2a*	1422122_at
FCRLM1	235372_at, 235401_s_at, 235400_at	*Uqcrb*	1419908_at
FLT3LG	206980_s_at	*Flt3l*	1422115_a_at
GCET2	235310_at	*Gcet2*	1442582_at
GPR132	223887_at	*Gpr132*	1444233_at
IL12RB1	1552584_at	*Il12rb1*	1418166_at
IL21R	221658_s_at, 219971_at	*Il21r*	1450456_at
IRF4	204562_at, 216986_s_at	*Irf4*	1421173_at
ITGA4	205884_at, 205885_s_at	*Itga4*	1450155_at
ITGB7	205718_at	*Itgb7*	1418741_at
KIAA0674	76897_s_at	*C430014M02Rik*	1441486_at
KYNU	204385_at, 210663_s_at	*Kynu*	1451903_at, 1430570_at
LAT2	221581_s_at	*Lat2*	1426169_a_at
LCK	204890_s_at	*Lck*	1457917_at
LNPEP	207904_s_at	*Lnpep*	1443907_at
LY9	210370_s_at, 231124_x_at	*Ly9*	1449156_at
MAP4K2	204936_at	*Map4k2*	1434833_at
MGC10986	218600_at	*0610025L06Rik*	1460738_at
NCOA3	209062_x_at	*2010305B15Rik*	1448027_at
NUP153	1559064_at	*Nup153*	1441689_at
PAX5	221969_at	*Pax5*	1459916_at
PIK3R5	227645_at, 220566_at	*Pik3r5*	1434980_at
PTPRCAP	204960_at	*Ptprcap*	1448511_at
RAB8B	219210_s_at	*Rab8b*	1446103_at
RAC2	207419_s_at	*Rac2*	1440208_at
RHOF	1554539_a_at, 222812_s_at	*LOC330189*	1441510_at
SLAMF1	206181_at	*Slamf1*	1425571_at
SLAMF7	234306_s_at	*Slamf7*	1453472_a_at
SP100	210219_at	*Sp100*	1451821_a_at
SP110	208392_x_at	*5031415C07Rik*	1429562_at, 1429563_x_at
SPI-B	205861_at	*Spi-b*	1460407_at
SYK	209269_s_at	*Syk*	1457239_at
Sept1	227552_at	*Sept1*	1449898_at
Sept6	212415_at, 241093_at, 212413_at	*Sept6*	1445665_at
TBC1D1	1568713_a_at	*Tbc1d1*	1447016_at
TBX21	220684_at	*Tbx21*	1449361_at
TRAF1	205599_at	*Traf1*	1423602_at
TRAF3IP3	205804_s_at	*Traf3ip3*	1434573_at
TREML2	219748_at	*Treml2*	1444718_at
WASPIP	202665_s_at	*Waspip*	1446905_at
ZC3H12D	1559263_s_at	*Zc3h12d*	1458504_at
ZC3HAV1	220104_at	*Zc3hav1*	1431909_at
ZNFN1A3	221092_at	*Zfpn1a3*	1441088_at, 1453378_at, 1436649_at

This table lists all 62 predicted B-cell precursor genes using MiDReG with their Affymetrix ID in human U133 Plus 2.0 and mouse 430 2.0 platform.

Table S2. Knockout phenotypes show defects in B-cell function and differentiation

Bach2	Failed to undergo efficient class-switch recombination (1)
Ccr6	B-cell numbers are reduced in Peyer's patches from *Ccr6-/-* mice (2)
Ccr7	Impaired migration of lymphocytes (3)
Cd180	Impaired proliferative and humoral immune responses of *RP105*-deficient B cells to LPS (4)
Cd22	B cells from *Cd22*-deficient mice exhibited the cell surface phenotype and augmented intracellular calcium responses characteristic of chronically stimulated B cells, as occurs in *SHP1*-defective mice (5)
Cd72	In the bone marrow of *Cd72-/-* mice, there was a reduction in the number of mature recirculating B cells and an accumulation of pre-B cells (6)
Cd79a	In the absence of Ig-alpha, B-cell development does not progress beyond the progenitor stage (7)
Cd80	Provide costimulatory signal (8, 9)
Cd86	Mice lacking only *B7-1* or *B7-2* mounted high-titer antigen-specific IgG responses when immunized in complete Freund's adjuvant (10)
Dok3	*Dok-3* restricts the intensity of Ca2+ signaling in B cells (11)
Fcer2a	*Fcer2a*-deficient mice show exaggerated IgE responses and airway hyper-responsiveness (12)
Flt3l	Significantly reduced numbers of myeloid and B-lymphoid progenitors were noted in the bone marrow of *Flt3l-/-* mice (13)
Gpr132	Abnormal expansion of both T and B lymphocytes (14)
Il21r	Mice deficient in the receptor for *Il-21* (*Il-21r*) have normal lymphoid development; after immunization, these animals have higher production of the immunoglobulin IgE (15)
Irf4	*Irf4*-deficient B cells had impaired expression of activation-induced deaminase and lacked class-switch recombination (16, 17)
Itgb7	Lymphocyte trafficking to the gut (18, 19)
Lck	Reduced threshold for B-cell receptor (BCR) signaling in B1 cell (20, 21)
Ncoa3	*Ncoa3*-deficient mice have B-cell and T-cell hyperproliferative disorder, have hyperactivated NF-κB, and develop B-cell lymphomas (22)
Pax5	Complete block of early B-cell differentiation and altered patterning of the posterior midbrain in mice lacking *Pax5*/*BSAP* (23)
Ptprcap	T and B lymphocytes showed reduced proliferation in response to antigen receptor stimulation (24)
Rac2	*Rac2* knockout mice show decreased B-cell lymphopoiesis, reduced BCR signaling, and reduced chemotaxis (25, 26)
Spi-B	*Spi-B-/-* mice exhibit severe abnormalities in B-cell function. *Spi-B* is essential for antigen-dependent expansion of B cells (27)
Syk	*Syk*-deficient fetal liver showed a block in B-cell development at the pro-B to pre-B-cell transition (28–31)
Tbx21	*Tbx21* knockout mice show abnormal IgG2a class switching and IFN-gamma production (32, 33)
Waspip	Both *Wip*-deficient T and B cells show a profound defect in their subcortical actin filament networks. *Wip* is important for immunologic synapse formation and T-cell activation (34)
Znfn1a3	Aiolos-null mice have hyperactivated B cells (35)

41 genes were knocked out in mice out of the total 62 predicted B-cell precursor genes. Comprehensive review of the literatures identified 26 genes whose knockout phenotype show defects in B-cell function and differentiation. A short summary of this phenotype is listed here for each of the 26 genes.

Table S3. Detailed classification of predicted B-cell genes

Gene	Mouse knockout	B-cell phenotype	Category	Pubmed	Comment
ARHGAP4			Signal transducer	12181357,12115726, 18489790	*ARHGAP4* can inhibit *MEKK1*, which can activate NF-κB–mediated cell survival (36–38)
BACH2	Yes	Yes	Transcription factor	15152264	Failed to undergo efficient class-switch recombination (1)
BLK	Yes		Signal transducer	14662906, 10648608	Involved in signaling downstream of the pre-BCR (39, 40)
BTLA	Yes		Cell surface receptor	19155498, 12796776, 19342624, 15128774	*BTLA* regulates B-cell receptor signaling by targeting *Syk* and *BLN*K (41–44)
CCR6	Yes	Yes	Cell surface receptor	10843382	B-cell numbers are reduced in Peyer's patches from *CCR6-/-* mice (2)
CCR7	Yes	Yes	Cell surface receptor	10520991	Impaired migration of lymphocytes (3)
CD180 (*RP105*)	Yes	Yes	Cell surface receptor	10880523	Impaired proliferative and humoral immune responses of *RP105*-deficient B cells to LPS (4)
CD22	Yes	Yes	Cell surface receptor	8986715	B cells from *CD22*-deficient mice exhibited the cell surface phenotype and augmented intracellular calcium responses characteristic of chronically stimulated B cells, as occurs in *SHP1*-defective mice (5)
CD53			Cell surface receptor	17429843	*CD53* is a genetic target for early B-cell factor (45)
CD72	Yes	Yes	Cell surface receptor	10549631	In the bone marrow of *CD72-/-* mice, there was a reduction in the number of mature recirculating B cells and an accumulation of pre-B cells (6)
CD79A	Yes	Yes	Cell surface receptor	12097390	In the absence of Ig-alpha, B-cell development does not progress beyond the progenitor stage (7)
CD80	Yes	Yes	Cell surface receptor	11169445, 7694362	Provide costimulatory signal (8, 9)
CD84			Cell surface receptor	12115647	*CD84* is expressed in B cells. Member of the *CD2* subset of the Ig superfamily (46)
CD86	Yes	Yes	Cell surface receptor	9075931	Mice lacking only *B7-1* or *B7-2* mounted high-titer antigen-specific IgG responses when immunized in complete Freund's adjuvant (10)
CENTB1			Metabolic process	16256741, 17005562	Inhibits NF-κB and activates *Itgb1* recycling to control cell migration (47, 48)
CLEC2D	Yes		Cell surface receptor	18453569, 18782774	Expressed in B cells and can be stimulated through *TLR9*, surface Ig, or *CD40*. Possible role in B cells (49, 50)
DENND1C			Unknown		No known function for this gene
DOK3	Yes	Yes	Signal transducer	17290227, 17363732	*Dok-3* restricts the intensity of Ca2+ signaling in B cells (11)
FAIM3			Signal transducer	19703010	Inhibits Fas-mediated apoptosis (51). GC B cells are sensitive to Fas-mediated cell death. Indirectly connected to B cells
FCER2	Yes	Yes	Cell surface receptor	8041705, 8176203	*FCER2A*-deficient mice show exaggerated IgE responses and airway hyper-responsiveness (12)
FCRLM1			Cell surface receptor	12202404	Whereas no function is known for this, it appears to be only expressed in B cells. Previously predicted to have a B-cell function (52)
FLT3LG	Yes	Yes	Cytokine activity	10828034	Significantly reduced numbers of myeloid and B-lymphoid progenitors were noted in the bone marrow of *flt3L-/-* mice (13)
GCET2	Yes		Metabolic process	17489982, 16493007	Expressed in GC B cells and a very useful marker of B-cell lymphoma (53, 54)
GPR132	Yes	Yes	Cell surface receptor	11371358	Abnormal expansion of both T and B lymphocytes (14)
IL12RB1	Yes		Cell surface receptor	8977288, 9257825	Previously predicted to have a functional role in B cells. May have a functional role in IL12 signaling during the B-cell response (55, 56)
IL21R	Yes	Yes	Cell surface receptor	12446913	Mice deficient in the receptor for *IL-21* (*IL-21R*) have normal lymphoid development; after immunization, these animals have higher production of the immunoglobulin IgE (15)
IRF4	Yes	Yes	Transcription factor	16767092, 8999800	*IRF4*-deficient B cells had impaired expression of activation-induced deaminase and lacked class-switch recombination (16, 17)

Gene	Mouse knockout	B-cell phenotype	Category	Pubmed	Comment
ITGA4	Yes		Cell surface receptor	12130787, 14645544	Role in marginal zone B-cell localization (57, 58)
ITGB7	Yes	Yes	Cell surface receptor	17786243, 8684468	Lymphocyte trafficking to the gut (18, 19)
KIAA0674			Metabolic process	16756961	Involved in neuronal development. No connection to B cells (59)
KYNU			Metabolic process	14592712	Involved in tryptophan degradation. No connection to B cells (60)
LAT2	Yes		Signal transducer	15477348, 15477350, 12486104, 12514734	*LAT2* interacts with *Grb2* to enhance BCR signaling (61–64)
LCK	Yes	Yes	Signal transducer	15972627, 15357954	Reduced threshold for BCR signaling in B1 cells (20, 21)
LNPEP	Yes		Metabolic process	11884418	GLUT4 glucose uptake pathway. No obvious connection to B cells (65)
LY9	Yes		Cell surface receptor	16365421	Expression on B cells, T cells, macrophage, DCs, granulocytes. B-cell development, and activation appear normal. This gene may be redundant with other *SLAM* family members (66)
MAP4K2	Yes		Signal transducer	7477268, 7515885, 19246396	*GCK* is important for JNK/p38-mediated inflammation in macrophages. This gene is expressed in GC B cells (67–69)
MGC10986			Signal transducer	17699795	This gene has unknown function according to literature (70)
NCOA3	Yes	Yes	Signal transducer	16675958	*NCOA3*-deficient mice have B-cell and T-cell hyperproliferative disorder and hyperactivated NF-κb and develop B-cell lymphomas (22)
NUP153			Metabolic process		
PAX5	Yes	Yes	Transcription factor	8001127	Complete block of early B-cell differentiation and altered patterning of the posterior midbrain in mice lacking *Pax5/BSAP* (23)
PIK3R5	Yes		Signal transducer	17041586	*PI3K* signaling defects in neutrophils. B cells never examined before. Possible B-cell function (71)
PTPRCAP	Yes	Yes	Cell surface receptor	9607926	T and B lymphocytes showed reduced proliferation in response to antigen receptor stimulation (24)
RAB8B			Signal transducer	18701652	GLUT4 glucose uptake pathway. No obvious connection to B cells (72)
RAC2	Yes	Yes	Signal transducer	11907095, 18579797, 18579797, 18191593	*RAC2* knockout mice show decreased B-cell lymphopoiesis, reduced BCR signaling, and reduced chemotaxis (25, 26)
RHOF			Signal transducer	15877735	Expressed in B cells and lymphomas (73)
SLAMF1	Yes		Cell surface receptor	18055867	It is expressed on activated B cells. It is also expressed in LT-HSC (74). No identified B-cell function
SLAMF7	Yes		Cell surface receptor	17878365	*SLAMF7* is up-regulated on activated B cells. Also, it induces proliferation and autocrine cytokine expression in B cells (75)
SP100			Transcription factor	16177824, 10921892	Associated with Bright, a B-cell-specific protein known to up-regulate BCR transcription in activated cells (76, 77)
SP110			Transcription factor	15308735, 16648851, 15815631	Mutations in human *SP110* cause severe T- and B-cell immunodeficiency (78–80)
SPI-B	Yes	Yes	Transcription factor	9384589	*Spi-B-/-* mice exhibit severe abnormalities in B-cell function. *Spi-B* is essential for antigen-dependent expansion of B cells (27)
SYK	Yes	Yes	Signal transducer	7477353, 12563261, 7477352, 18356083	*Syk*-deficient fetal liver showed a block in B-cell development at the pro-B to pre-B-cell transition (28–31)
Sept1			Transcription factor	19043408	Known role in T-cell trafficking, but unknown function in B cells. Expressed in B cells (81)
Sept6	Yes		Transcription factor	16314519	Common translocation partner to *MLL* in acute myeloid leukemia. No obvious role in B-cell function (82)
TBC1D1			Signal transducer	19740738	Involved in GLUT4 glucose uptake pathway. No obvious connection to B cells (83)

Gene	Mouse knockout	B-cell phenotype	Category	Pubmed	Comment
TBX21	Yes	Yes	Transcription factor	12882831, 15905519, 12766768, 18339657	*TBX21* knockout mice show abnormal IgG2a class switching and IFN-gamma production (32, 33)
TRAF1	Yes		Cell surface receptor	17405906, 19234489, 16622006, 11672546	*TRAF1* knockout has T-cell hyperactivation (84). *TRAF1* may be redundant for B-cell activation with other *TRAF*s (85, 86). It enhances *CD40* signaling in combination with *TRAF2* (87)
TRAF3IP3			Cell surface receptor	17868742, 18313334	Interacts with *Traf3*, a nuclear factor known to suppress B-cell survival by inhibiting NF-κB2 activation (88). Also associated with cell proliferation (89)
TREML2			Cell surface receptor	16670310	Expressed on all B-cell subsets, particularly high in B1 cells. Also expressed in neutrophils, peritoneal macrophages (90)
WASPIP	Yes	Yes	Metabolic process	11869681	Both *WIP*-deficient T and B cells show a profound defect in their subcortical actin filament networks. *WIP* is important for immunologic synapse formation and T-cell activation (34)
ZC3H12D			Metabolic process	17854321, 18178554	Some tumor suppressor activity, and associated with B-cell lymphomas (91, 92)
ZC3HAV1			Metabolic process		No known function
ZNFN1A3	Yes	Yes	Transcription factor	9806640	Aiolos-null mice have hyperactivated B cells (35)

This table describes the detailed association of predicted B-cell genes to B-cell function and differentiation. Out of 62 predicted genes, 41 mouse knockouts were published in the literature. Out of 41 knockouts, 26 genes have a B-cell phenotype. Out of 62 genes, 9 other genes have B-cell functions according to other experiments, and 5 genes have indirect connections to B-cell functions.

Table S4. Predicted role of GLUT4 pathway in B cell

Gene	Mouse knockout	B-cell phenotype	Category	Pubmed	Comment
TBC1D1			Signal transducer	19740738	Involved in GLUT4 glucose uptake pathway. No obvious connection to B cells (83)
RAB8B			Signal transducer	18701652	GLUT4 glucose uptake pathway. No obvious connection to B cells (72)
LNPEP	Yes		Metabolic process	11884418	GLUT4 glucose uptake pathway. No obvious connection to B cells (65)

The most interesting genes identified by MiDReG were those with unexpected connections to B-cell development and function. For example, three genes (*TBC1D1*, *RAB8B*, and *LNPEP*) identified by MiDReG to be up-regulated in B-cell development are known components of a pathway seemingly unrelated to B-cell function, the GLUT4 glucose uptake pathway. GLUT4 is a glucose transporter normally sequestered in vesicles in muscle and adipose tissue. Upon insulin signaling, these vesicles are quickly fused to the plasma membrane leading to GLUT4 surface expression and rapid glucose uptake (93). GLUT4 surface levels are regulated by endocytic recycling (94). This endocytic recycling pathway bears some similarity to internalization of the BCR complex upon antigen encounter. When the BCR of a naïve B cell first encounters antigen, it is internalized through receptor-mediated endocytosis, and the antigen is processed and presented by MHC class II molecules on the cell surface for activation by Th2 helper T cells. Both BCR and GLUT4 endocytosis have clathrin-mediated pathways. In addition, both insulin and BCR signaling involve tyrosine kinases, PI-3 kinase, Protein Kinase C, Akt, Rho, and Rab GTPases, and Ca^{2+} flux (93, 95). The prediction of genes specific to the GLUT4 exocytic pathway in B cells leads to the surprising but testable hypothesis that genetic components important for GLUT4 endocytosis are also functionally important for BCR endocytosis. The identification of these genes and the prediction of similarities between GLUT4 and BCR endocytosis would not have been possible without MiDReG.

Table S5. Antibodies used

Antigen	Clone	Conjugate	Source	Population
Ly6c	AL-21	FITC	BD Pharmingen	CLP, Fr.A, B
Flk2	A2F10	PE	eBioscience	CLP, Fr.A, B
CD19	1D3	PECy5.5	eBioscience	CLP, Fr.A, B
B220	6B2	PECy7	eBioscience	CLP, Fr.A, B
CD27	LG.7F9	APC	eBioscience	CLP, Fr.A, B
CD11c	N418	Alexa750-APC	eBioscience	CLP, Fr.A, B
BP-1 (Ly51)	6C3	Biotin	BD Pharmingen	CLP, Fr.A, B
IL7Ra	A7R34	Biotin	House	CLP, Fr.A, B
NK1.1	PK136	PECy5	eBioscience	CLP, Fr.A, B
	PK136	PECy7	eBioscience	CLP, Fr.A, B
IgD	11-26c	FITC	eBioscience	Fr.C, D, E
CD43	1B11	PE	BD Pharmingen	Fr.C, D, E
IgM		APC	eBioscience	Fr.C, D, E
CD23	B3B4	PE	eBioscience	T1, T2, mature B
IgM	II/41	PECy5	eBioscience	T1, T2, mature B
CD93 (AA4.1)	AA4.1	APC	eBioscience	T1, T2, mature B
CD3	2C11	PECy5	eBioscience	T1, T2, mature B
CD4	GK1.5	Pacific Blue	House	T1, T2, mature B
CD8	53.6.7	Pacific Blue	House	T1, T2, mature B
Ter119	TER119	Pacific Blue	House	T1, T2, mature B
CD11b		Pacific Blue	House	T1, T2, mature B
Gr1		Pacific Blue	House	T1, T2, mature B
Mac1	M1/70	PECy5	eBioscience	T1, T2, mature B
GL7	GL-7	biotin	eBioscience	GC
CD38	90	APC	eBioscience	GC
NP	NP	PE	Biosearch	GC
ckit	2B8	PECy7	eBioscience	HSC, MPP
	2B8	APCCy7	eBioscience	HSC, MPP
Sca1	E13-161-7	Alexa680	House	HSC, MPP
CD34	RAM34	Biotin	House	HSC, MPP
	RAM34	FITC	House	HSC, MPP
Streptavidin		Qdot605	Invitrogen	
Goat anti-rat		Alexa488	Invitrogen	

House: conjugates made within the Weissman lab.

Table S6. Primers used for real-time PCR

Gene name	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
Beta-actin	GTCTGAGGCCTCCCTTTTT	GGGAGACCAAAGCCTTCATA
Aicda	GGGAAAGTGGCATTCACCTA	GAACCCAATTCTGGCTGTGT
Arhgap4	CCAGTCTGAGGCAGCTTACC	GCTGAGGTGAAGCAATAGGC
Bach2	CAGCCAAACTGTTCCAGTGA	GGTGATTATAAGCGCCTGGA
Cd19	TAGGTTCACAGGTCCCAAGG	ATCACCCCTTGCCTAAGACC
Cd53	CTGACTTGCTTTAAGGAAAACTGG	TTCCCTATAGAGCACCACGAA
Cd72	CTACGGCCTGCCAATTTTTA	AGAGTGAGGGAGTGCTGGAG
cKit	CATCCATCCAGCACAATCAG	TGACAAAGTCGGGATCAATG
Fcrlm1	GAAGCTGCATCTGGTGATGA	CACAGAAGCTCTCGGTCCTC
Il21r	ATGTGTCCATGTCCAGACCA	ATTTGCAAAGGAAGGGGACT
Itga4	CAGAGCCACACCCAAAAGTT	GGGCCACGTTCTCATCTTTA
Itgb7	GTACAGGCGCTTTGAGAAGG	TGTTGTCCTGCTTCCAGTTG
Ly9	AAAAAGACGGCACAAACACC	GGTAGGGGCTTTCAGGAGAC
Nup153	ATACTGGGGAGTTGGTGCTG	GGGCTTCTTCAACATGAGAAA
Pax5	TTGGAGGATCCAAACCAAAG	CTGATCTCCCAGGCAAACAT
Ptprcap	CCCATGCATGTTATGGTGAA	TGATGTAAGGGTGGGGTGAC
Sept6	TGCAGTCACAGAAGGGTTGA	AGGCCAACCATGAAAATGAT
Spi-B	CATGGGGTTGCAAAGAGTTT	GCAGAATCAGAACCCAGAGC
Syk	CCTCTCTGCACCCCAGACTA	ATCTGCAGTGGGTCAGTGTG
Tbc1d1	TGGGGAGTAATGAGGAAACG	CCCCCTTAAGTCCCAAATTC
Traf3ip3	TCTGAAGGAGGACTGGAGGA	GCTCTCCAGCTCTCTGTGCT
Waspip	CTCCAGCAGGGAGAAGATGA	CATTGCACATTTGGATCGTC
Zc3h12d	AGGCACCAAGAATGGATCAG	CAAGGCCGGCAAGTTTACTA
Ebf1	CCCTCCAACTGCAGTAGCTC	TCTGACAACTGGTGCGAAAG
Pax5	TTGGAGGATCCAAACCAAAG	CTGATCTCCCAGGCAAACAT
VpreB	CATGCTGCTGGCCTATCTCA	TACAGGAGAGGCGGATGGTG
Rag1	GAGGTTCCGCTACGACTCTG	TCTTGGGATCTCATGCCTTC
Lambda5	TGCTGCTGTTGGGTCTAGTG	CAGCTCTGCTCCTTTCTGCT
E2A	ACGGTTCTGGATGAGACAGG	AAGCAGGGAGCTGTTCAGTG

Cells were sorted into TRIzol (Invitrogen Life Technologies), and RNA was isolated according to manufacturer's instruction. cDNA was synthesized by using the Superscript III kit (Invitrogen Life Technologies) using random hexamers. Amplifications were performed by using SYBR Green PCR core reagents (Applied Biosystems), and transcript levels were quantified by using an ABI 7900 Sequence Detection Systems (Applied Biosystems). The mean ct value of triplicate reactions was normalized against the mean ct value of β-actin. Primers were used at 400 nM.