Supporting Information

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SI Materials and Methods

Primer Sequences for Genotyping Mice. After the mutations in *Nfkbid*, *Zeb1*, and *Ruvbl2* were identified, *bumble*, *cellophane*, and *Worker* mice were genotyped by sequencing PCR products amplified from genomic DNA using the following sets of primers: *bumble* PCR, forward: 5′TCATTTGGCACACATGAGGTCCC3′ and reverse: 5′GCTCAGCAGGTCTTCCACAATCAG3′; *bumble* sequencing, forward: 5′AGGACTCTCTGGATACCCG3′ and reverse: 5′AGCGGCGTCTGTAAGTCAC3′; *cellophane* PCR, forward: 5′TCAGGTGGAGGGCTTCACATCTAAC3′ and reverse: 5′GCTCTGTCAGCATAGACACCAAGG3′; *cellophane* sequencing, forward: 5′GTGTAGATCCCAGGGATTCAACTC3′ and reverse: 5′CACCAAGGCATTAAAGGCG3′; *Worker* PCR, forward: 5′CGGACACATGGTTCCAATGTTCCTC3′ and reverse: 5′TGTGACACGAAGCCTGTTTCCC3′; *Worker* sequencing, forward: 5′CCAATGTTCCTCAAAGTTCCAG3′ and reverse: 5′ATTAGTGATTGATGGCCCAGAG3′.

Antibody Clones for Flow Cytometry. CD16/CD32 (2.4G2), CD4 (RM4-5), CD5 (53-7.3), CD8 (53-6.7), CD19 (1D3), CD21/35 (7G6), CD23 (B3B4), CD38 (90), CD43 (S7), CD69 (H1.2F3), B220 (RA3-6B2), BP1 (BP-1), TCRβ (H57-597), CD24 (30-F1), CD44 (1M7), IgM (11/41), NK1.1 (PK136), and IgD (11-26).

Primer Sequences for RT-PCR. cDNA was amplified with the following primer sets: 5′CTCCATCTGTGAATGAGGCAGAGC3′ (forward) and 5′AGATCCACTTGAATGCCGGACTTAAAC3′ (reverse) for *bumble* and 5′TGGGCCATAGAGATGACTGGA TGAC3′ (forward) and 5′CTGCCTGCGATGGCTGTGAATG3′ (reverse) for *Worker*. Purified PCR products were sequenced using the following primer sets: 5′TTCCAAGAGACTGTGGATTCC3′ (forward) and 5′GAGTCCATAGGTGGCAGC3′ (reverse) for *bumble* and 5′TGACCGGGAGCCTAGAG3′ (forward) and 5′CATGGCAATGGCTGTCTTC3′ (reverse) for *Worker*.

Primer Sequences for Quantitative PCR. Levels of *Nfkbid* and *Zeb*1 transcripts were analyzed by real-time PCR using the following sets of primers: 5′TGATTTCTACCCTCCGTCAGACCCAG3′ (forward) and 5′TGACAGGGAAGGCTCAGGATACAG3′ (reverse) for *Nfkbid* and 5′CCTTTGCATACAGAACCCAGCTT GAAC3′ (forward) and 5′GCACTTGAACTTGCGGTTTCCC3′ (reverse) for *Zeb1*.

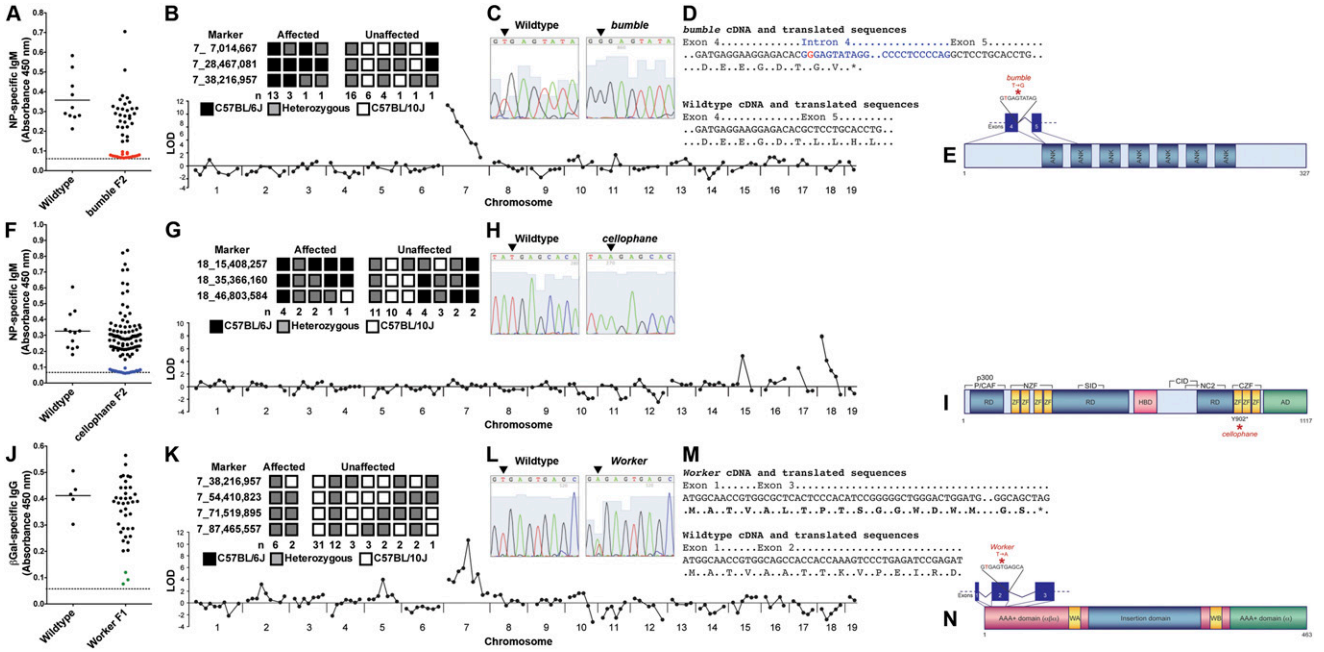

Fig. S1. Identification of the *bumble*, *cellophane*, and *Worker* mutations by bulk segregation analysis and whole-genome sequencing. (*A*) Serum 4-hydroxy-3-nitrophenylacetyl (NP)-specific IgM responses measured by ELISA in WT and *bumble* F2 mice immunized 5 d before with NP-Ficoll. (*B*) Chromosomal mapping of the *bumble* mutation by bulk segregation analysis. LOD, logarithm of odds score. (*Inset*) Results of genotyping individual mice for the C57BL/6J and C57BL/10J alleles of the markers in the critical region on chromosome 7. *n*, number of F2 mice with the indicated genotype. (*C*) DNA sequence chromatograms of the mutated nucleotide in *Nfkbid*. (*D*) *Nfkbid* cDNA and translated sequences for WT and *bumble*. Intronic sequence and the mutated nucleotide in *bumble* are highlighted in blue and red, respectively. (*E*) Schematic of the *Nfkbid* gene with the *bumble* mutation indicated in red. (*F*) Serum NP-specific IgM responses measured by ELISA in WT and *cellophane* F2 mice immunized 5 d before with NP-Ficoll. (*G*) Chromosomal mapping of the *cellophane* mutation by bulk segregation analysis. (*Inset*) Results of genotyping individual mice for the C57BL/6J and C57BL/10J alleles of the markers in the critical region on chromosome 18. (*H*) DNA sequence chromatograms of the mutated nucleotide in *Zeb1*. (*I*) Schematic of the *Zeb1* gene with the *cellophane* mutation indicated with an asterisk. (*J*) Serum βGal-specific IgG responses measured by ELISA in WT and *Worker* F1 mice immunized 14 d before with recombinant Semliki Forest virus (rSFV)–β-gal (βGal). (*K*) Chromosomal mapping of the *Worker* mutation by bulk segregation analysis. (*Inset*) Results of genotyping individual mice for the C57BL/6J and C57BL/10J alleles of the markers in the critical region on chromosome 7. (*L*) DNA sequence chromatograms of the mutated nucleotide in *Ruvbl2*. (*M*) *Ruvbl2* cDNA and translated sequences for WT and *Worker*. Note that thymic cDNA from *Worker* mice contained a mixture of correctly and aberrantly spliced cDNA. For clarity, only the aberrant exon 1:exon 3 sequence is shown. (*N*) Schematic of the *Ruvbl2* gene with the *Worker* mutation indicated in red. In *A*, *F*, and *J*, each point represents data from one mouse, and the bar indicates the mean of all values. Background (indicated by the dashed line) was determined by incubating pooled WT sera on uncoated ELISA wells.

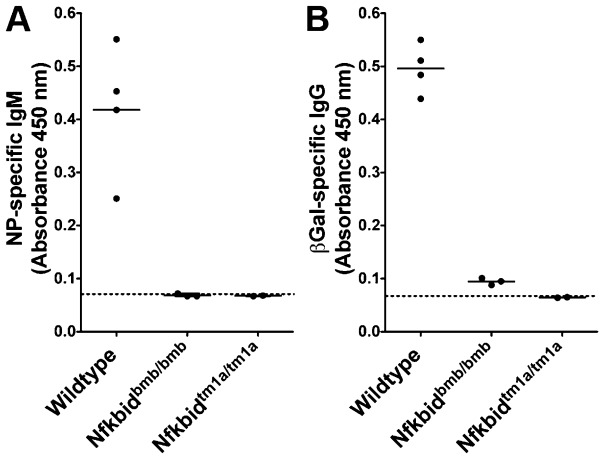

Fig. S2. Mice with a targeted mutation in *Nfkbid* phenocopy *bumble* mice. Serum NP-specific IgM (*A*) and βGal-specific IgG (*B*) responses measured by ELISA in WT mice, *bumble* homozygotes, and mice with a targeted mutation in *Nfkbid* immunized 5 or 14 d before with NP-Ficoll and rSFV-βGal, respectively. Each point represents data from one mouse, and the bar indicates the mean of all values. Background (indicated by the dashed line) was determined by incubating pooled WT sera on uncoated wells.

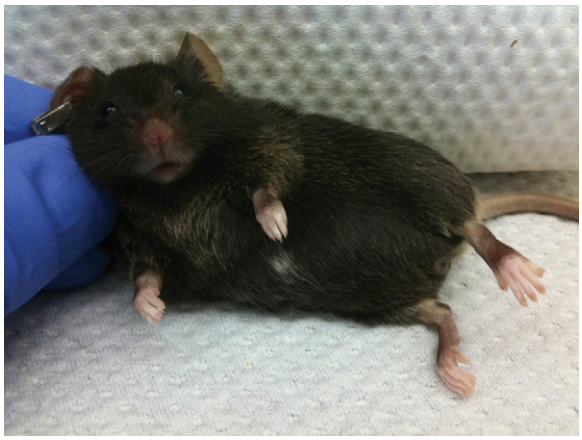

Fig. S3. Visible phenotypes in *Worker* mice. White belly spot and white paws in a *Worker* male.

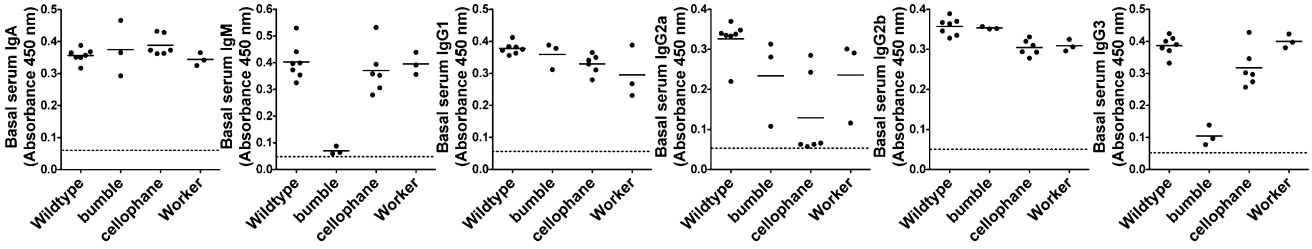

Fig. S4. Basal serum levels of IgM and IgG3 are reduced in *bumble* mice. Levels of immunoglobulins of the indicated isotypes in sera from naïve WT and mutant mice. Each point represents data from one mouse, and the bar indicates the mean of all values. Background (indicated by the dashed line) was determined by incubating a positive control sample on uncoated ELISA wells.

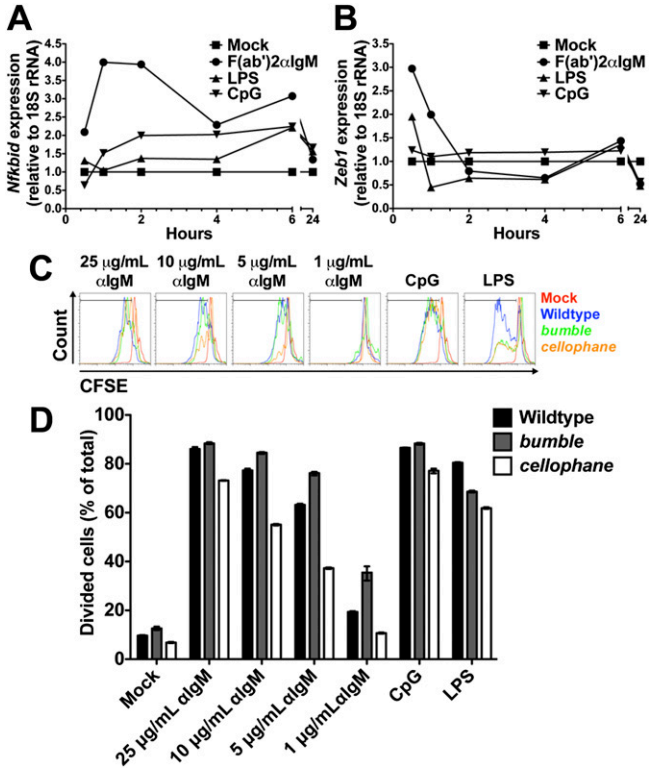

Fig. S5. *Zeb1*, but not *Nfkbid*, is required for B-cell proliferation after B-cell receptor cross-linking. Induction of (*A*) *Nfkbid* and (*B*) *Zeb1* measured by real-time PCR in WT splenic B cells stimulated for up to 24 h with the indicated stimuli. Each point represents the mean for triplicate samples. (*C*) Histograms showing carboxyfluorescein succinimidyl ester dilution peaks from mock-treated WT B cells and WT or mutant B cells stimulated with the indicated concentrations of $F(ab')_2\alpha$IgM, CpG, or LPS for 72 h. Results are representative of triplicate cultures. (*D*) Percentage of B cells that divided at least one time based on carboxyfluorescein succinimidyl ester dilution. Bars (± range) indicate the mean of triplicate cultures.

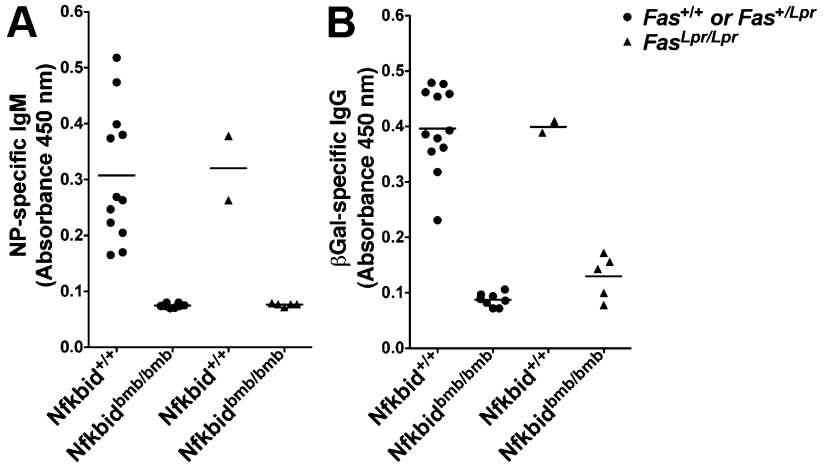

Fig. S6. Fas deficiency fails to rescue humoral defects in *bumble* mice. Serum NP-specific IgM (*A*) and βGal-specific IgG (*B*) responses measured by ELISA in *Nfkbid*$^{+/+}$ or *Nfkbid*$^{bmb/bmb}$ littermates with at least one WT *Fas* allele (circles) or homozygous for the *Lpr* mutation in *Fas* (triangles) immunized 5 or 14 d before with NP-Ficoll and rSFV-βGal, respectively. Each point represents data from one mouse, and the bar indicates the mean of all values.

Table S1. Summary of phenotypes and causative mutations

Strain	Peripheral blood lymphocyte phenotype*	IgM†	IgG‡	Inheritance	Gene§	Base change	Type of mutation¶
emptyhive	B-cell deficiency	+	+	X-linked recessive	*Atp11c*	C→T	Nonsense
spelling	B-cell deficiency	+	+	X-linked recessive	*Atp11c*	T→A	Missense
killer	B-cell deficiency	+	+	X-linked recessive	Unknown	Unknown	Unknown
ray	B-cell deficiency	+	+	Unknown	Unknown	Unknown	Unknown
Untied	None	X	+	Dominant	*Prkcb*	T→C	Missense
Bartle	None	X	+	Dominant	Unknown	Unknown	Unknown
may	Partial block in B-cell maturation	X	+	Recessive	Unknown	Unknown	Unknown
well	Partial block in B-cell maturation	X	+	Recessive	*Cd22*	C→T	Nonsense
queen	Partial block in B-cell maturation	X	+	Recessive	*Plcg2*	T→A	Missense
sothe	B cells arrested at T2	X	+	Recessive	*Pik3ap1*	A→T	Nonsense
busy	B cells arrested at T2	X	+	Recessive	*Blnk*	T→A	Nonsense
apple	B-cell deficiency	X	+	Unknown	Unknown	Unknown	Unknown
walla	None	+	X	X-linked recessive	*Cd40lg*	T→C	Missense
Worker	T-cell deficiency and belly spot	+	X	Dominant	*Ruvbl2*	T→A	Splice donor site
frizz	T-cell deficiency and low B220	+	X	Recessive	*Dock2*	T→A	Splice acceptor site
frazz	T-cell deficiency and low B220	+	X	Recessive	*Dock2*	T→A	Splice donor site
dew	T-cell deficiency	+	X	Recessive	Unknown	Unknown	Unknown
wanna	Severe T-cell deficiency	+	X	Recessive	*Zap70*	A→G	Missense
honey	None	X	X	Recessive	*Irf4*	A→T	Missense
bumble	High IgM	X	X	Recessive	*Nfkbid*	T→G	Splice donor site
cellophane	Slight block in B-cell maturation	X	X	Recessive	*Zeb1*	T→A	Nonsense
lucky	B cells arrested at T1	nd	X	Recessive	*Map3k14*	C→T	Nonsense
stinger	Slight B-cell deficiency	X	X	Recessive	Unknown	Unknown	Unknown
clover	Severe B-cell deficiency	X	X	Recessive	*Prkdc*	T→C	Splice donor site
crab	Severe B-cell deficiency	X	X	Recessive	*Cd79a*	T→A	Missense
huckle	T- and B-cell deficiency	X	X	Recessive	*Rag1*	T→A	Nonsense

*Peripheral blood lymphocyte phenotype: summary of the mutant's PBL phenotype based on flow cytometric analysis of blood cells using antibodies against CD4, CD8, NK1.1, and CD44 or CD23, IgD, B220, and IgM.

†IgM: serum titers of NP-specific IgM 5 d after i.p. immunization with NP-Ficoll were in normal range (+) or ranged from undetectable to suboptimal compared with WT mice (X).

‡IgG: serum titers of βGal-specific IgG 14 d after i.p. immunization with rSFV-bGal were in normal range (+) or ranged from undetectable to suboptimal compared with WT mice (X).

§Gene: mutations were identified by candidate gene sequencing or through a combination of bulk segregation analysis and whole-genome sequencing. Because of their lack of mature sperm, *ray* mice were unable to breed; this strain was lost, and the mutation cannot be identified.

¶Mutation: for details on the location and nature of each of the mutations listed here, refer to http://mutagenetix.utsouthwestern.edu.