Supporting Information

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SI Methods

Genetic Screening of Mice. DNA was extracted from the tails of 10- to 21-day-old litters of gene-targeted and transgenic mice using the Puregene DNA Purification Kit (Flowgen Bioscience Limited) according to the manufacturer's instructions. Genetic status of the Mlh1 alleles was detected by the presence of 450-bp and/or 350-bp PCR products for the targeted allele and WT allele, respectively, at an annealing temperature of 58 °C. The Eμ-v-Abl transgene was detected via the presence of a 381-bp PCR product using an annealing temperature of 58 °C. The presence of the vavP-bcl2 transgene was confirmed by detection of a 200-bp PCR product at an annealing temperature of 58 °C. Presence of the Eμ-myc transgene was confirmed by the presence of an 830-bp PCR product using an annealing temperature of 55 °C. The sequences of the primers used for Mlh knockout allele detection were (5′→3′): TGTCAATAGGCTGCCCTAGG and (5′→3′): TGGAAGGATTGGAGCTACGG. The sequences of the primers used for Mlh WT allele detection were (5′→3′): TTTTCAGTGCAGCCTATGCTC and (5′→3′): TGGAAGGATTGGAGCTACGG. Synthetic oligonucleotide sequences for detection of the v-Abl transgene were (5′→3′): GGAACTGATGAATGGGAGCAGTGG and (5′→3′): GCAGACACTCTATGCCTGTGTGG. The primer sequences to detect the vavP-bcl2 transgene were (5′→3′): GCCGCAGACATGATAAGATACATTGATG and (5′→3′): AAAACCTCCCACACCTCCCCCTGAA. The Eμ-myc transgene was detected using the following primers (5′→3′): CAGCTGGCGTAATAGCGAAGAG and (5′→3′): CTGTGACTGGTGAGTACTCAACC. Primer sequences to ensure integrity of these 3 PCR reactions were (5′→3′): GATGTGCTCCAGGCTAAAGTT and (5′→3′): AGAAACGGAATGTTGTGGAGT, which generated a PCR product of 500 bp.

Flow Cytometry. To determine the cell surface phenotype of tumors, tissue (either splenocytes or ascites cells) were harvested after passage through C57BL/6 RAG-1$^{-/-}$ mice to obtain normal lymphocyte-free tumor populations. Single-cell suspensions were made and erythrocytes were removed using ACK lysis buffer (155 mM ammonium chloride, 10 mM potassium hydrogen carbonate, 0.1 mM EDTA, pH 7.4). One million to 2 million cells were stained for 20 min on ice in FACS buffer (0.5% wt/vol BSA, 0.04% vol/vol sodium azide in PBS) with the following reagents: anti-CD8-FITC (53–6.7), anti-CD19-FITC (1D3), anti-CD86-FITC (GL1), anti–T-cell receptor (TCR) $\gamma\delta$-FITC (GL3), anti-CD22.2-FITC (Cy34.1), anti-CD4-PE (GK1.5), anti-CD40-PE (3/23), anti-B220-PE (RA3–6B2), anti-NK1.1-PE (PK136), anti-CD138-PE (281–2), anti-IgD-PE (11–26), goat F(ab)$_2$ anti-mouse IgG-PE (catalog no. M35004–1; Caltag Laboratories), sheep polyclonal anti-mouse Ig-PE (catalog no. AQ326H; Chemicon Australia), anti-TCRβ-allophycocyanin (APC) (H57–597), anti-B220-APC (RA3–6B2), anti-CD93-APC (AA4.1), IgM-biotin (R6–60.2), anti-H2-K^b-biotin (AF6–88.5), anti-H2-D^b-biotin (28–14-8), anti-I-A^b-biotin (KH74), anti-CD80-biotin (16–10A1), or anti-CD70-biotin (FR70). Nonspecific binding was blocked by the inclusion of anti-Fc receptor (2.4G2) mAb in each mixture. Cells were then washed twice in FACS buffer and stained with 0.2 μg/mL streptavidin-APC (catalog no. 17–4314-82; eBioscience) on ice for 20 min when appropriate. Following a final wash in FACS buffer, the cells were resuspended in 300 μL of FACS buffer with 0.67 μM hydroxystilbamidine methanesulfonate (Fluoro-gold; Invitrogen) viability dye and analyzed on a BD LSRII (BD Biosciences). Antibody isotypes produced by v-Abl$^+$ plasmacytomas were determined by cytometric bead array (Mouse Ig Isotyping Kit, catalog no. 550026; BD Biosciences) as described in the manufacturer's instructions. Briefly, each plasmacytoma line was passaged through a C57BL/6 RAG-1$^{-/-}$ mouse. Ascites fluid was harvested from tumor-bearing mice and diluted 1:1,000. Samples and appropriate controls and standards were incubated with mouse Ig capture beads for 15 min at room temperature. Anti-mouse kappa light chain-PE and anti-mouse lambda light chain-FITC detector antibodies were then added to each sample for 15 min in the dark. All samples were then washed, resuspended in buffer, and analyzed on a BD LSRII.

Statistical Analysis. Kaplan-Meier survival curves were generated using MedCalc version 8.1 software. A log-rank test was used to compare survival curves and to determine P values. Disease incidence was compared among each strain using a Fisher's exact test. A P value of less than 0.05 was considered statistically significant. Average lifespans of animals that succumbed to disease are presented in the text as mean ± SEM.

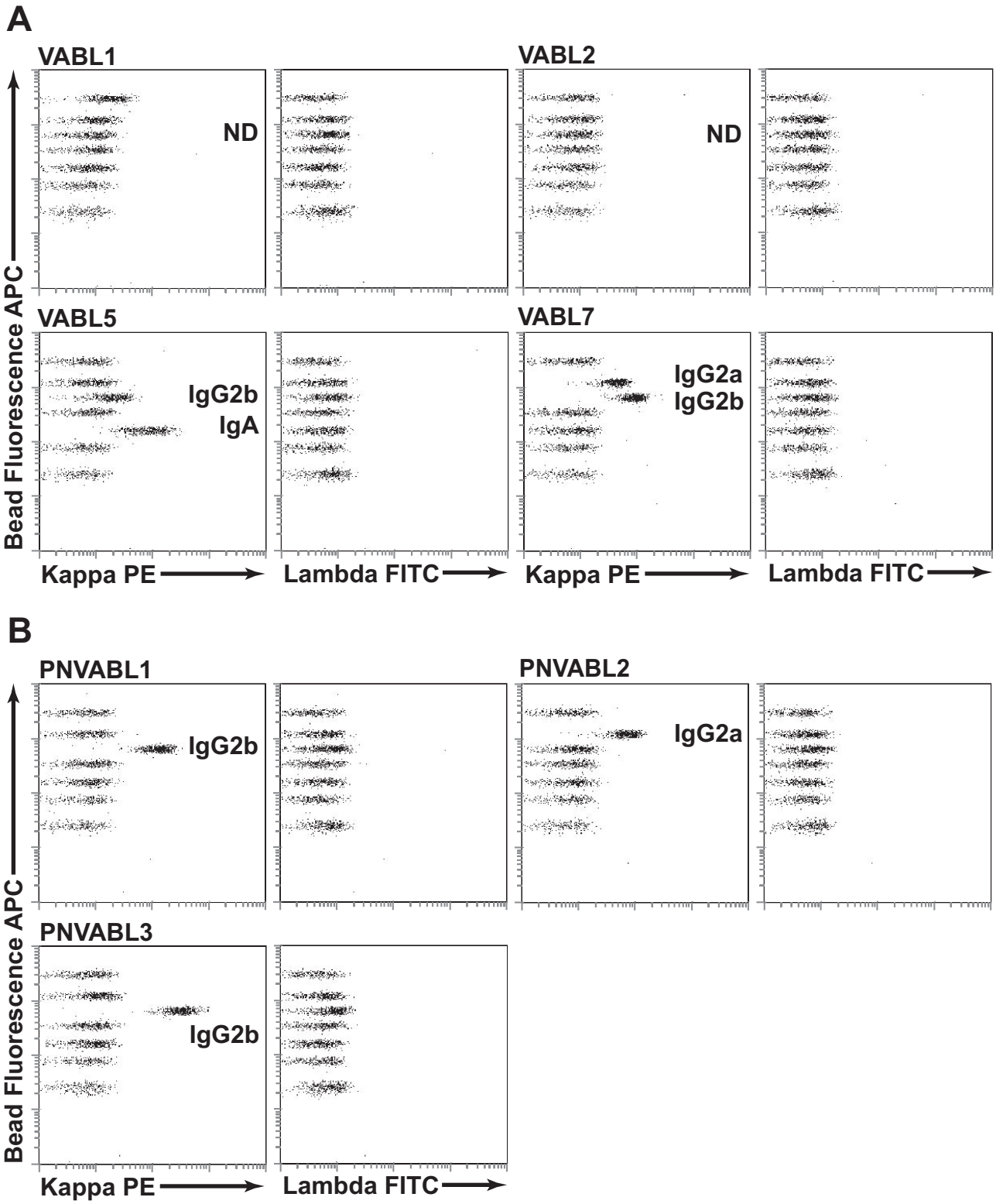

Fig. S1. v-Abl^{+} pfp$^{+/+}$ and v-Abl^{+} pfp$^{-/-}$ plasmacytomas produce IgG and/or IgA antibodies. Ig isotypes were determined by cytometric bead array (CBA) analysis of ascites from RAG1$^{-/-}$ mice after transplantation with various v-Abl^{+} pfp$^{+/+}$ (*A*) and v-Abl^{+} pfp$^{-/-}$ (*B*) plasmacytomas. Heavy chain isotypes are represented by bead clusters with decreasing APC fluorescence intensity in the following order: IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, and IgE. Light chain (kappa or lambda) type was determined by additional PE or FITC fluorescence. Text represents the isotype(s) secreted by each plasmacytoma. ND, not determined.

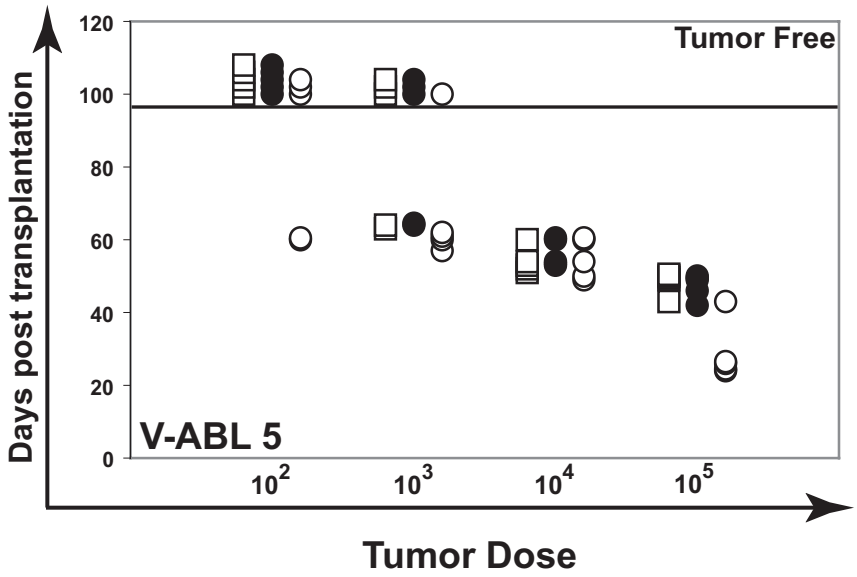

Fig. S2. Transplantation analysis of VABL5. Tumor cells were derived from ascites after in vivo passage through C57BL/6 RAG-1$^{-/-}$ mice and were then transferred by i.p. injection into WT mice (open squares), pfp$^{-/-}$ mice (shaded circles), and RAG-1$^{-/-}$ mice (open circles) at the indicated doses. Mice were monitored for plasmacytoma development (abdominal distension) for 100 days, after which they were declared tumor-free (above solid bar).

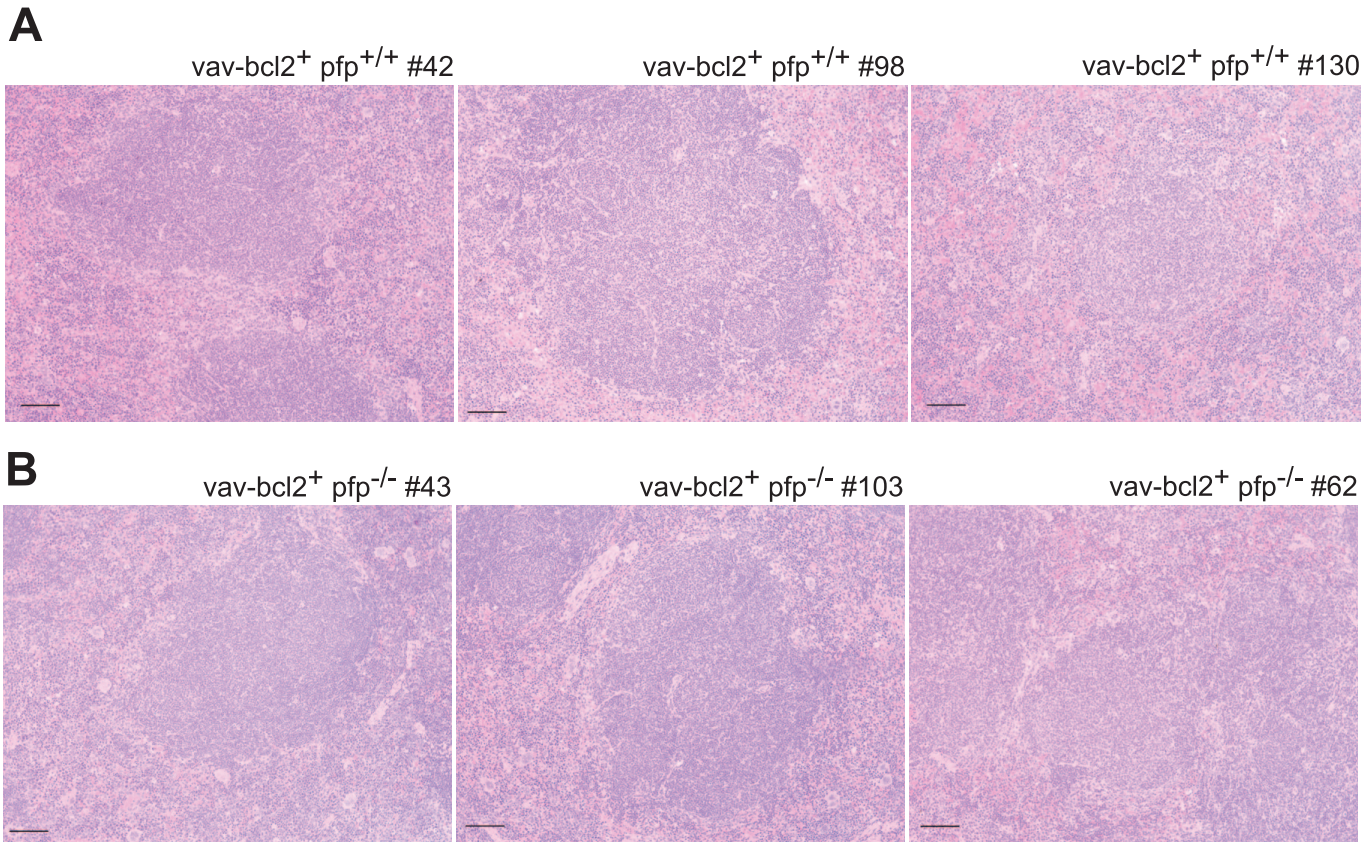

Fig. S3. Vav-bcl2^{+} pfp$^{+/+}$ and vav-bcl2^{+} pfp$^{-/-}$ mice develop follicular lymphoma. H&E-stained sections of formalin-fixed spleens from moribund vav-bcl2^{+} pfp$^{+/+}$ (*A*) and vav-bcl2^{+} pfp$^{-/-}$ (*B*) mice showing follicular lymphoma in each instance. Three representative spleens from both groups are shown. (Scale bar: 100 μm.)

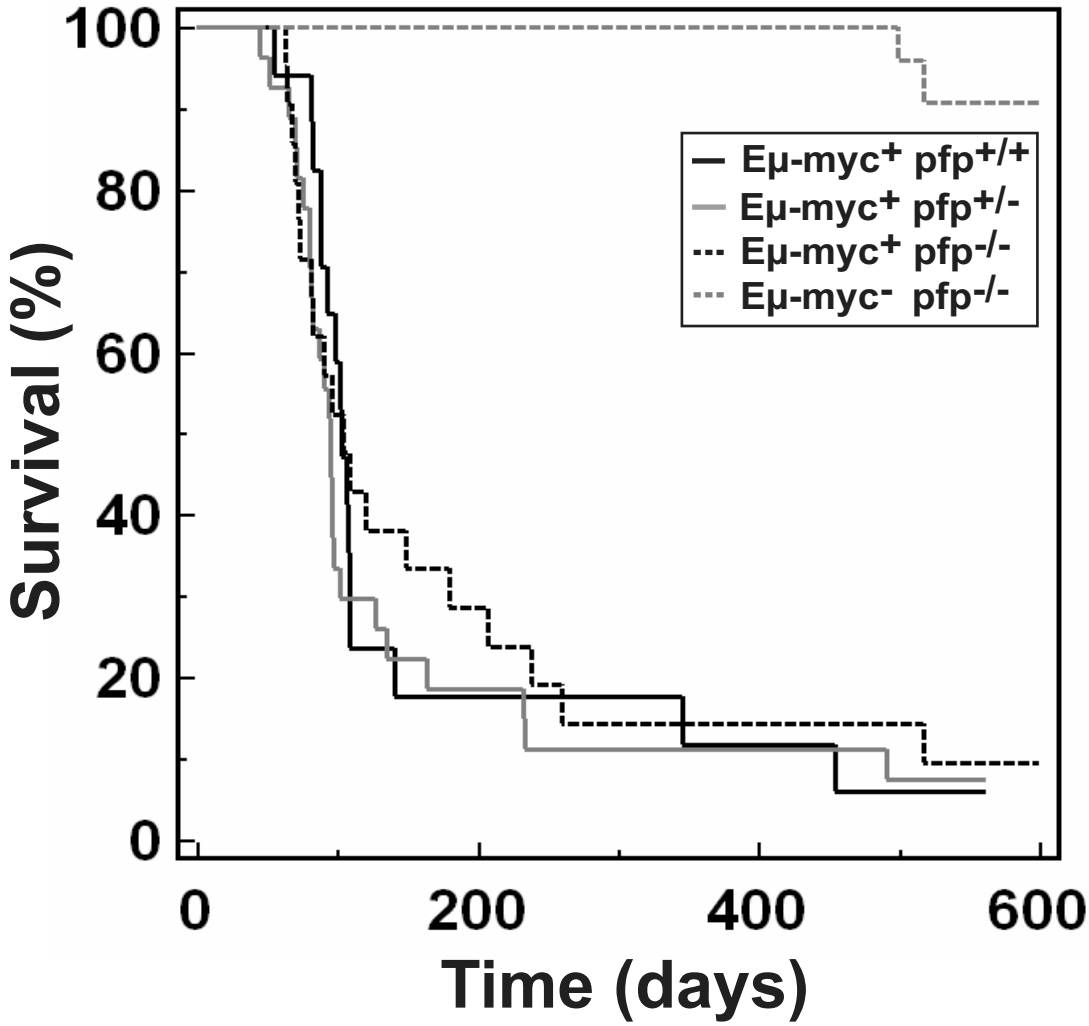

Fig. S4. Loss of pfp has no influence on Eμ-myc^{+} lymphoma development. Kaplan-Meier curve showing percentage survival of Eμ-myc^{+} pfp$^{+/+}$ ($n = 17$, black solid line), Eμ-myc^{+} pfp$^{+/-}$ ($n = 27$, gray solid line), Eμ-myc^{+} pfp$^{-/-}$ ($n = 21$, black dashed line), and Eμ-myc^{-} pfp$^{-/-}$ ($n = 24$, gray dashed line) nontransgenic littermates. Mice were monitored for at least 550 days. There are no statistically significant differences between any of the pfp genotypes ($P = 0.705$, log-rank test).

Other Supporting Information Files

Table S1
Table S2
Table S3
Table S4