SUPPLEMENTAL MATERIAL

Veselits et al., https://doi.org/10.1084/jem.20161868

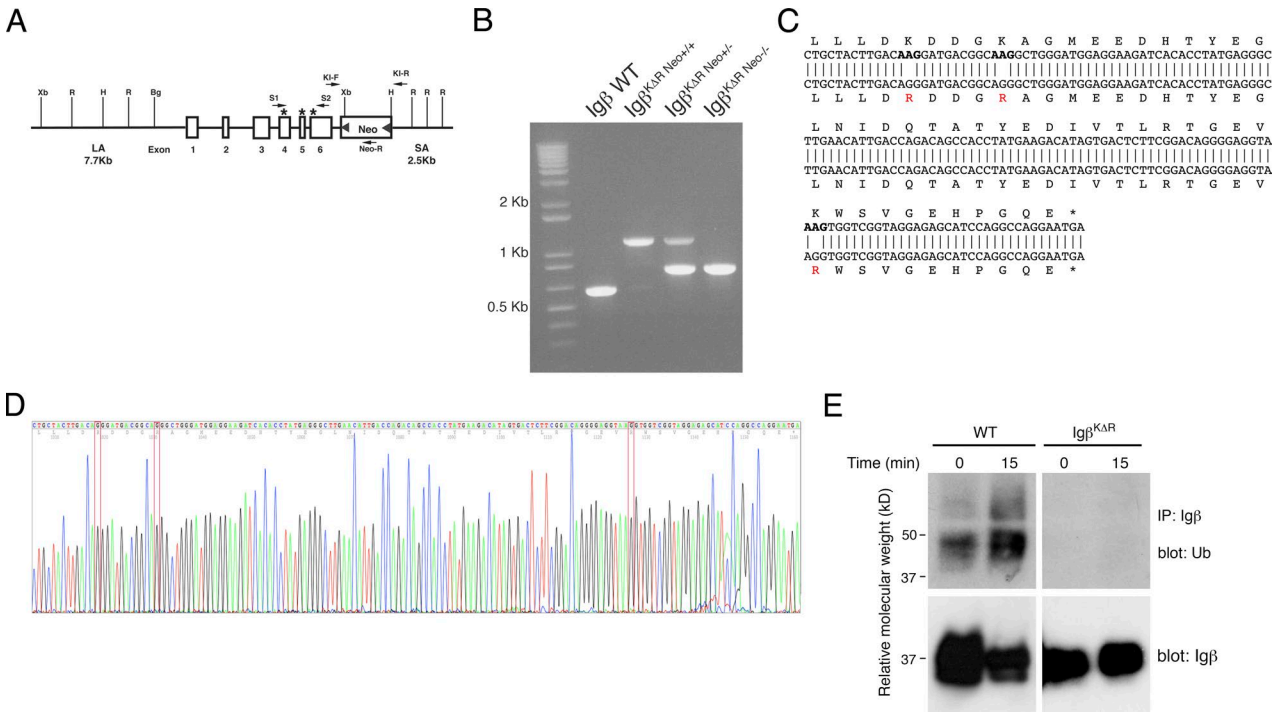

Figure S1. **Ablation of Igβ ubiquitination in vivo.** (A) Schematic representation of the targeting vector. Exons are open squares with cytoplasmic tail encoded by exons 4–6; position of targeted lysine encoding codons shown (*). Length of long arm (LA) and short arm (SA) targeting vector are provided, as are position of restriction sites for EcoRI (R), HindIII (H), BglII (Bg), and XbaI (Xb). Primers for screening (KI-F, KI-R, and Neo-R) and sequencing (S1 and S2) are shown. (B) PCR of genomic DNA from WT, *Igβ*$^{K\Delta R}$ heterozygous or homozygous knock-in (KI) mice, Neo deleted, with KI-F and KI-R primers. (C and D) Splenic B cell mRNA was PCR amplified with primers specific for Igβ (*B29*) and sequenced. Nucleotide and corresponding predicted amino acid sequences of WT (top sequence) and Igβ$^{K\Delta R}$ (bottom sequence) cytosolic tails are shown; position of lysine (K) to arginine (R) mutations shown in red (C). Electropherogram of cDNA from *Igβ*$^{K\Delta R}$ B splenocytes encoding Igβ cytoplasmic tail. At top is the nucleotide sequence. Each of the three point mutations is highlighted with a red box (D). (E) WT and *Igβ*$^{K\Delta R}$ splenic B cells (10^7 cells per sample) were stimulated with IgG- and IgM (H+L)-specific F(ab)$_2$ antibodies and then lysed. IPs with Igβ-specific antibodies were immunoblotted first with ubiquitin-specific antibodies (top) and then with Igβ-specific antibodies (bottom). Igβ$^{K\Delta R}$ lanes were exposed two times as long as WT lanes (n = 3).

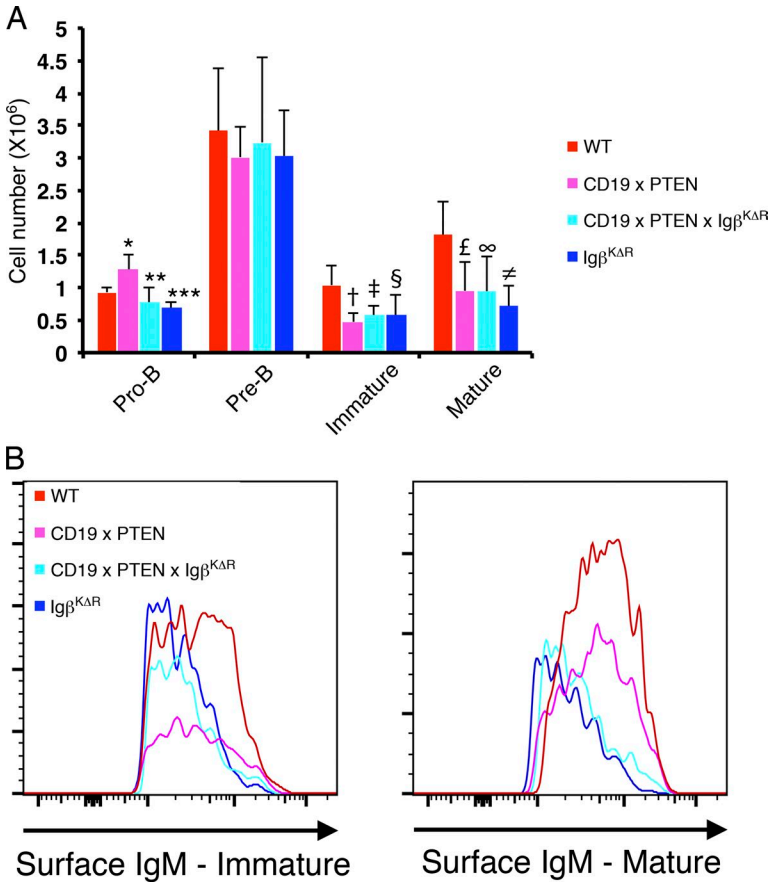

Figure S2. **Deletion of *Pten* does not restore BM B cell populations.** (A) BM cells from WT, *Pten*$^{-/-}$, *Pten*$^{-/-}$×*Igβ*$^{K\Delta R}$, and *Igβ*$^{K\Delta R}$ mice were isolated, stained with antibodies specific for B220, CD43, and IgM, and analyzed by flow cytometry. Total numbers of each population are provided for WT (red), *Pten*$^{-/-}$ (purple), *Pten*$^{-/-}$×*Igβ*$^{K\Delta R}$ (light blue), and *Igβ*$^{K\Delta R}$ (dark blue) mice; error bars indicate mean ± SD (n = 3). *, P = 0.008 versus WT; **, P = 0.0055; ***, P = 0.0005 versus *Pten*$^{-/-}$; †, P = 0.005; ‡, P = 0.019; §, P = 0.022 versus WT; £, P = 0.04; ∞, P = 0.044; ≠, P = 0.015 versus WT. (B) Mean fluorescence intensity of surface IgM on immature and mature B cells in the BM.

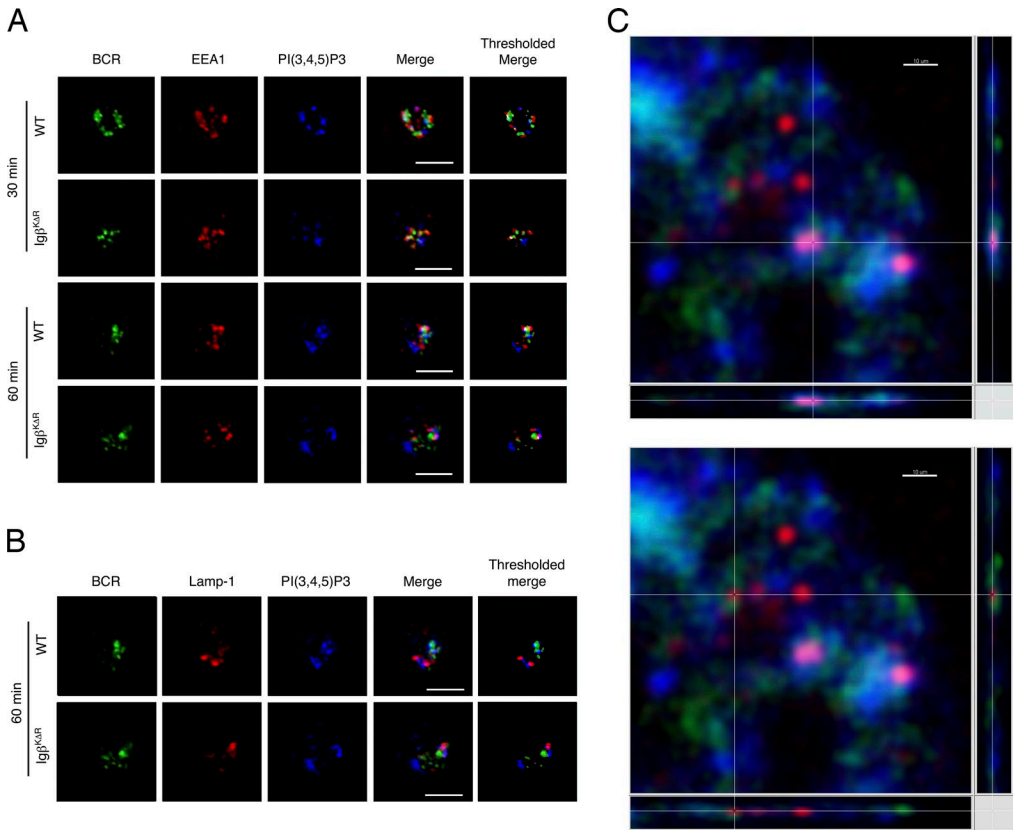

Figure S3. **Accumulation of PIP_3 on endosomes is time dependent.** (A and B) Splenocytes from WT or *Igβ*$^{K\Delta R}$ mice were stimulated with FITC-conjugated IgG- and IgM (H+L)-specific $F(ab)_2$ antibodies for the indicated times, then fixed, stained with antibodies specific for EEA1, Lamp-1, and PIP_3, and then visualized by confocal microscopy. Representative images from 30 min and 60 min of cells with EEA1 (A) and from cells at 60 min with Lamp-1 (B). The cells shown at 30 min in A are the same cells shown in Fig. 6 E visualized with Lamp-1. The cells shown in A at 60 min are the same cells in B that are visualized with Lamp-1. (C) Superresolution confocal microscopy of WT splenic B cells stimulated with Alexa Fluor 488–conjugated IgG- and IgM (H+L)-specific $F(ab)_2$ antibodies (green) for 15 min, then fixed, and stained with antibodies specific for EEA1 (red), and PIP3 (blue). Orthogonal slice of a $BCR^+EEA1^+PIP_3^+$ vesicle (top) and $BCR^-EEA1^+PIP_3^-$ vesicle (bottom). Bars, 10 µm.