Supporting Information

Baracho et al. 10.1073/pnas.1314562111

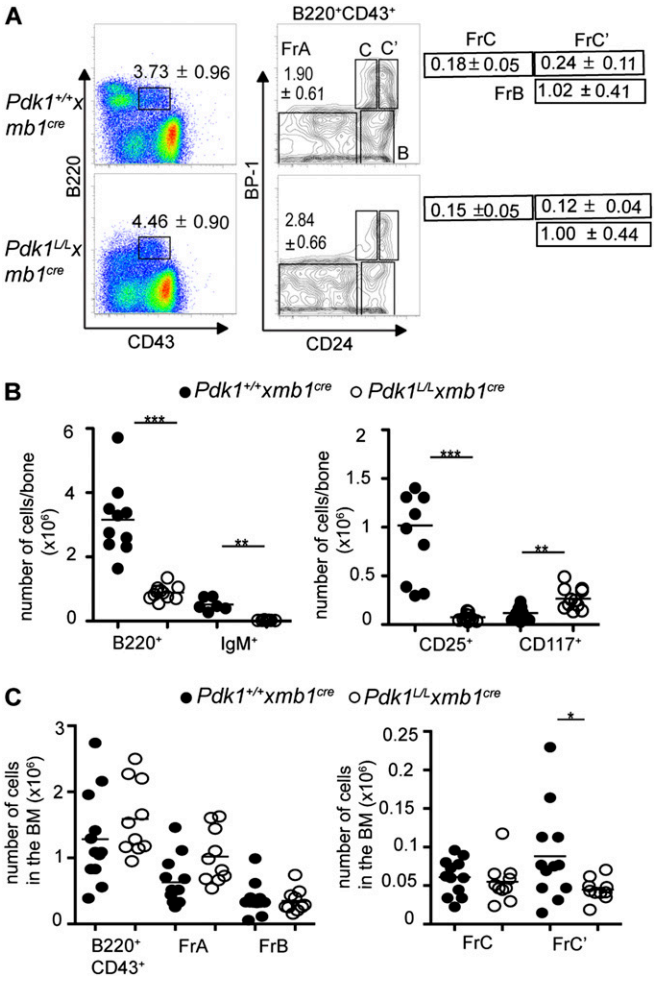

Fig. S1. Phosphoinositide-dependent protein kinase-1 (PDK1) is required for early B cell development. (*A*) Frequency of B cell progenitors based on Hardy's classification. (*B*) Absolute numbers of total B cells and of the indicated B cell subsets in the bone marrow. Data represent mean ± SD of three independent experiments. *$P < 0.05$, Mann–Whitney *U* test.

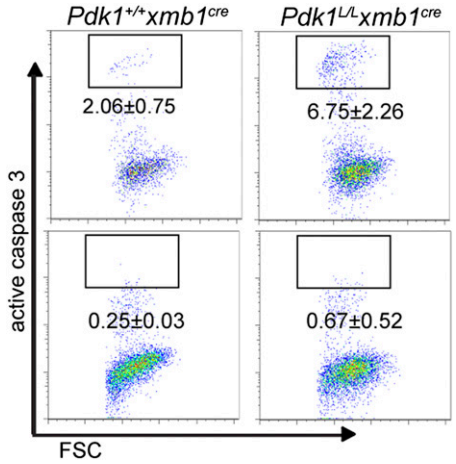

Fig. S2. PDK1 regulates pro-B cell survival. Analysis of active caspase-3 on hematopoietic stem cell-derived pro-B cells that were left untreated (*Upper*) or treated overnight with 20 mM of pan-caspase inhibitor Q-VD-OPh (R&D Systems) (*Lower*) before intracellular flow cytometric analysis.

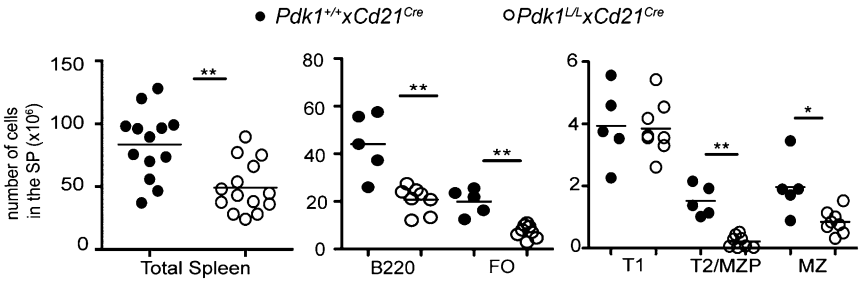

Fig. S3. PDK1 is required for peripheral B cell homeostasis. The graphs represent the absolute numbers of splenic cells, B cells, and B cell subsets in the spleens. Data represent mean ± SD of three independent experiments. *$P < 0.05$, Mann–Whitney *U* test.

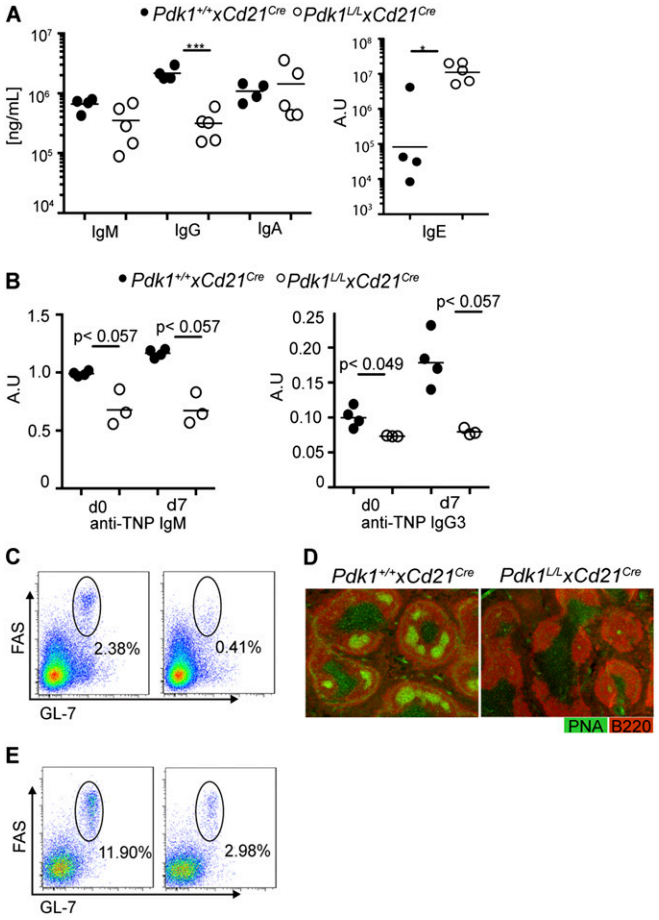

Fig. S4. PDK1 is required for antigen-driven B cell responses. (*A*) Evaluation of basal serum levels of the indicated Ig isotypes by ELISA. Data are displayed as the concentration of each Ig isotype or absorbance. (*B*) Analysis of specific anti–2,4,6-trinitrophenyl (TNP) IgM or IgG3 in the sera of unimmunized or TNP-Ficoll–immunized animals. (*C* and *D*) Analysis of splenic germinal center (GC) B cells at 7 d after immunization with sheep red blood cells (SRBCs). (*E*) Analysis of GC B cells in the Peyer's patches of unimmunized mice. Values in *C* and *E* indicate the gated GC B cell population ($GL7^+Fas^+$) as a percentage of $B220^+$ cells. The data represent one of two independent experiments.

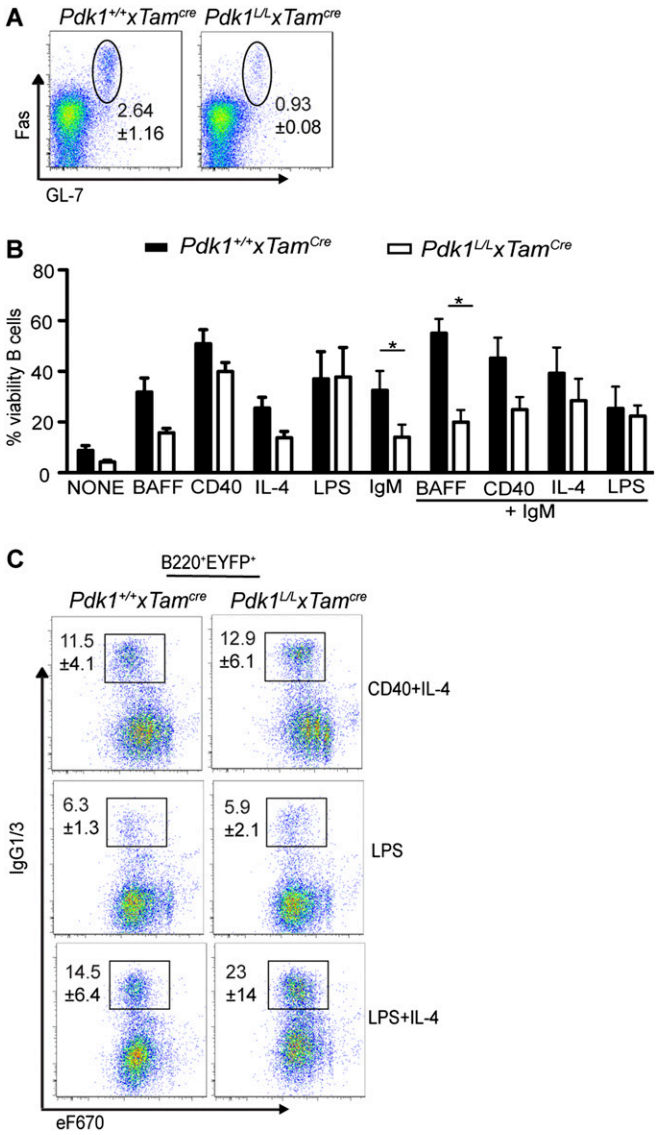

Fig. S5. Effect of acute deletion of PDK1 on peripheral B cell activation. (*A*) Analysis of splenic EYFP$^+$ GC B cells at 7 d after immunization with SRBCs. Tamoxifen was administered at days 3–5. SDs are shown ($n = 4$ per group). (*B*) Spleens were harvested at 5 d after the last injection of tamoxifen, and purified B cells were cultured for 3 d under the indicated conditions. (*C*) B cells were loaded with eF640 dye and cultured for 3 d under the indicated conditions. Cell division was assessed based on eF640 dilution by flow cytometry. The density plots show the correlated expression of IgG1 (for the cultures with IL-4) or IgG3 (for the cultures with LPS) and eF640 dilution. Data represent the mean ± SD of two or more independent experiments. *$P < 0.05$, Mann–Whitney *U* test.

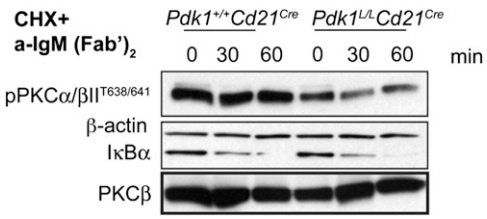

Fig. S6. PKCα/β activation in *Pdk1*$^{L/L}$ × *Cd21*^{Cre} B cells. CD43$^-$CD9$^-$ cells were stimulated with 10 μg/mL α-IgM (Fab′)$_2$ for the indicated period in the presence of cycloheximide (CHX) to block de novo protein synthesis and allow for assessment of PKC stability. Whole-cell lysates were prepared for immunoblot analysis for the indicated targets, with β-actin serving as a loading control.