Supporting Information

Browne et al. 10.1073/pnas.1007558107

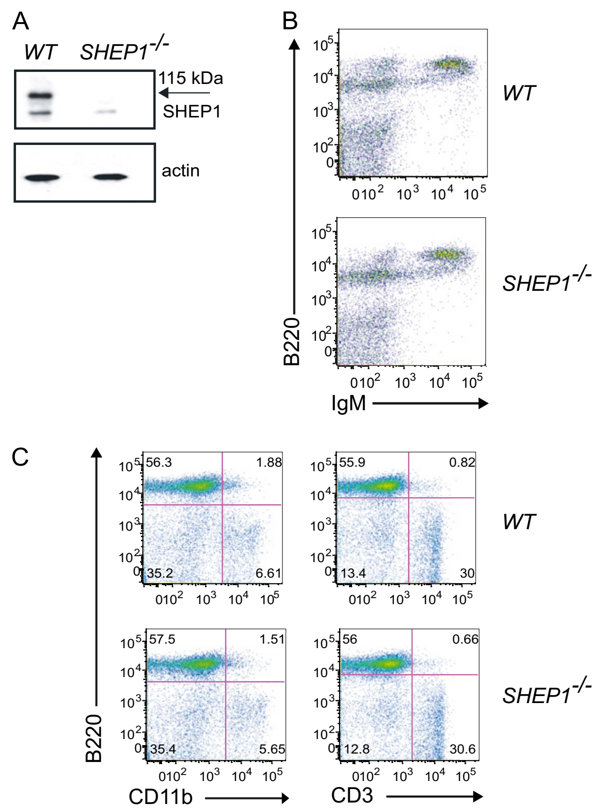

Fig. S1. B cells express SHEP1; *SHEP1*$^{-/-}$ mice have normal bone marrow B-cell compartments. (*A*) Splenic B cells from WT and *SHEP*$^{-/-}$ mice were purified, lysed, and immunoblotted with antibodies that recognize the SH2 domain of SHEP1 or actin. (*B*) Bone marrow B cells from WT and *SHEP1*$^{-/-}$ mice were isolated and stained for B220 and IgM. Mature, B220^{hi}, IgM^{+}; immature, B220^{med}, IgM^{+}; pre/pro-B, B220^{med}, IgM^{-}. (*C*) Splenic cells from WT and *SHEP1*$^{-/-}$ mice were isolated and stained for B220, CD3 and CD11b. B, T, and myeloid cells were analyzed by flow cytometry.

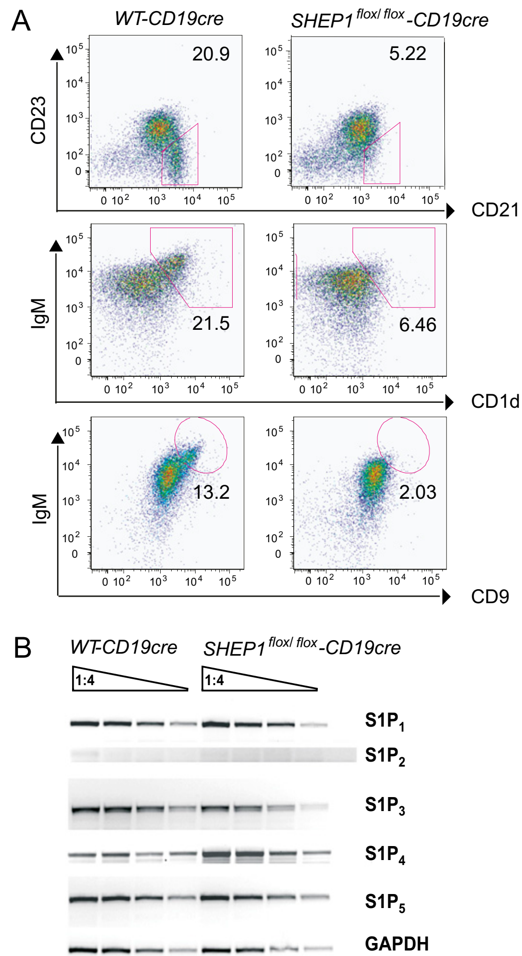

Fig. S2. Marginal zone B-cell compartment is reduced in *SHEP1*$^{flox/flox}$*-CD19cre* as analyzed by staining for other surface markers. (*A*) WT-*CD19cre* and *SHEP1*$^{flox/flox}$*-CD19cre* splenic B cells were stained for CD23, CD21, IgM, CD1d, and CD9. Frequencies of MZ B cells (IgM^{hi}, CD1d^{hi} or IgM^{hi}, CD9^{hi}) are shown. (*B*) WT-*CD19cre* and *SHEP1*$^{flox/flox}$*-CD19cre* splenic B cells were purified. The levels of S1P receptor transcripts were determined by RT-PCR on 1:4 serially diluted cDNA.

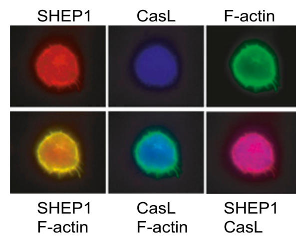

Fig. S3. Subcellular localization of SHEP1 and CasL. BAL17 cells were plated on VCAM-coated slides overnight, fixed, permeabilized, and stained with phalloidin, anti-SHEP1, and anti-CasL and analyzed by fluorescence microscopy. SHEP1 (red), F-actin (green); CasL (blue).

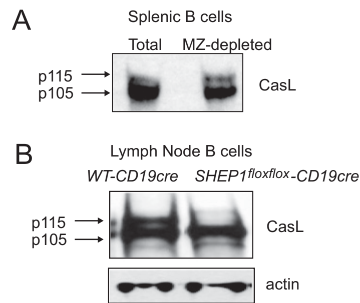

Fig. S4. CasL expression in MZB-depleted splenic B cells and in lymph node B cells. (*A*) WT-*CD19cre* splenic B cells were purified and depleted of $CD9^+$ cells. Total and CD9-depleted cells were lysed and immunoblotted with CasL antibodies. (*B*) Lymph node B cells from WT-*CD19cre* and *SHEP1*$^{flox/flox}$-*CD19cre* mice were purified, lysed, and immunoblotted with CasL and actin antibodies.

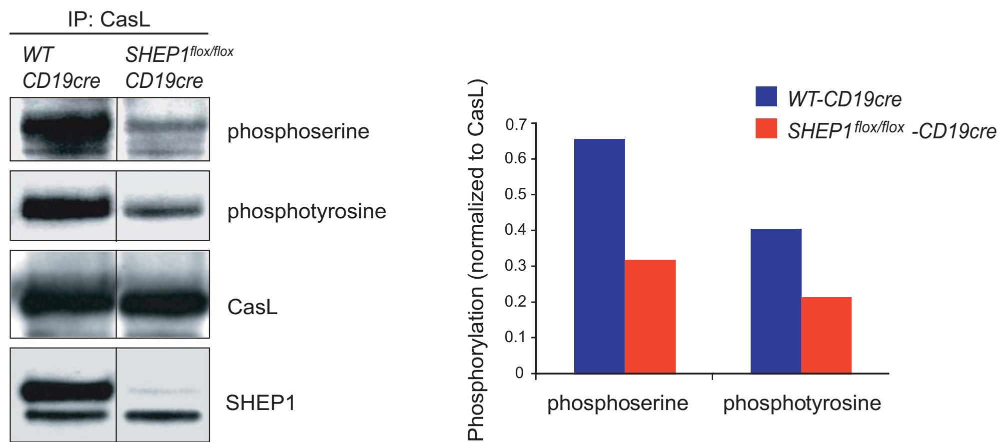

Fig. S5. Serine and tyrosine phosphorylation of CasL are reduced in SHEP1-deficient B cells. Purified splenic B cells from WT-*CD19cre* and *SHEP1*$^{flox/flox}$-*CD19cre* mice were lysed, immunoprecipitated with anti-CasL, and immunoblotted for phosphoserine, phosphotyrosine, CasL, and SHEP1 (*Left*). Phosphoserine and phosphotyrosine bands were quantified by densitometry and normalized against total CasL. Normalized CasL phosphorylation is depicted in the bar graph (*Right*).

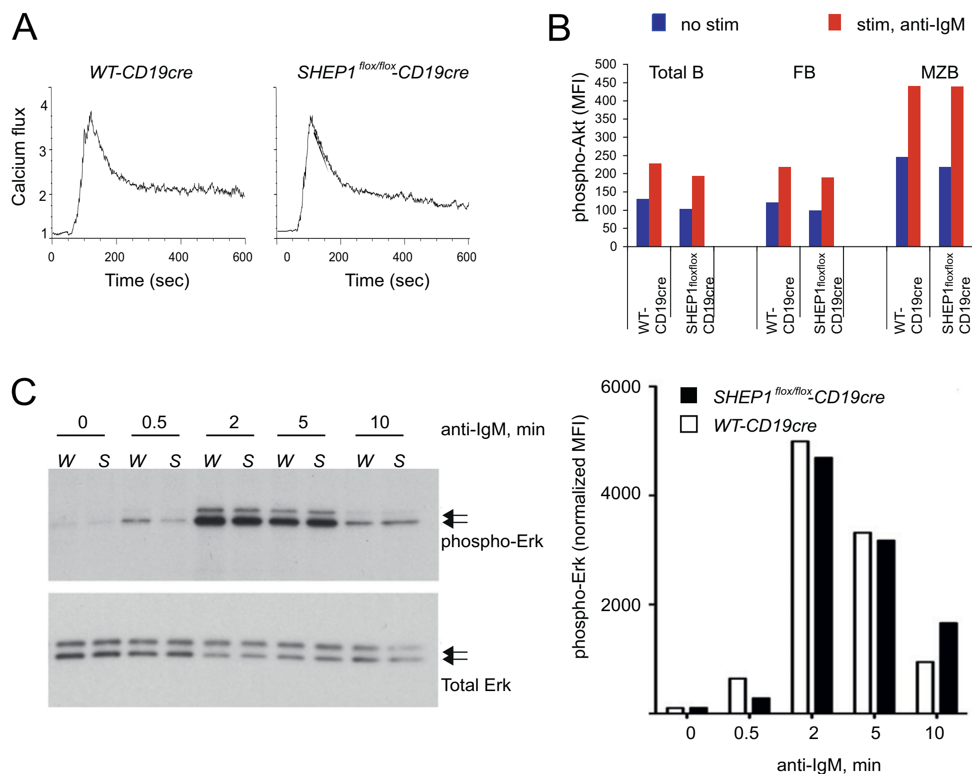

Fig. S6. SHEP1-deficient B cells can mobilize calcium and activate Akt downstream of BCR stimulation. (*A*) Purified splenic B cells were loaded with Fluo-4 and Fura Red and stimulated with 10 μg/mL anti-IgM F(ab′)$_2$. Calcium flux was measured by flow cytometry. (*B*) Purified splenic B cells were stimulated with 10 μg/mL anti-IgM F(ab′)$_2$, fixed, permeabilized, and stained for phospho-Akt, B220, CD21 and CD23. Mean fluorescence intensities are shown for total, follicular, and marginal zone B cells. (*C*) Purified splenic B cells were stimulated with 10 μg/ml anti-IgM F(ab′)$_2$. B-cell lysates were lysed and immunoblotted for phospho-Erk and total Erk (*Left*; W, WT-*CD19cre;* S, *SHEP1$^{flox/flox}$-CD19cre*). Erk phosphorylation was quantified by densitometry and depicted in the bar graph (*Right*).

Table S1. RT-PCR, genotyping, cloning, and mutagenesis primers

Cloning of SHEP1*	Forward 5′-CCA CAT GCT AGC AAA CCA GAG
	Reverse 5′-ATC TCC GGG GTC ACA GCT CGC
Mutagenesis of SHEP1†	Forward 5′-ACC ACG GGG GCC TCG AAC ACA CCA ACG CTG
	Reverse 5′-CAG CGT TGG TGT GTT CGA GGC CCC CGT GGT
Genotyping of SHEP1‡	Forward 5′-AAC AGG TCA GCC AGA TCC CCA CTT
	Reverse (wt allele) 5′-TTC TAG TCA CCT CCC TCC CTC AAT
	Reverse (floxed allele) 5′-TGC TTC CTC TTG CAA AAC CAC A
$S1P_1$§	Forward 5′-GTG TAG ACC CAG AGT CCT GCG
	Reverse 5′-AGT ACA TGG GCC GGT GGA ACT
$S1P_2$§	Forward 5′-GGC CTA GCC AGT GCT CAG C
	Reverse 5′-CCT TGG TGT AAT TGT AGT GTT CCA GA
$S1P_3$§	Forward 5′-CCG GGT GCT GAG CAA GGT GG
	Reverse 5′-CAG CCC AGG ATT GGC AGG GC
$S1P_4$§	Forward 5′-GCA AGC CCC GGG TTC CTT CC
	Reverse 5′-CCA CAG CTC CCT GCC ACA GC
$S1P_5$§	Forward 5′-GGC GCA AGG GCC TCG AAC TT
	Reverse 5′-ACG CGC AAA CCA AAG CGC AG

*SHEP1 was cloned from cDNA; accession no. AF168364.
†Y787E mutation of SHEP1 was introduced by using QuikChange Lightning Site Directed Mutagenesis Kit (Stratagene).
‡PCR primer.
§RT-PCR primer.