

SUPPLEMENTRY MATERIAL**Supplementary Table 1. Antibodies used in the study**

	Antibody	Company¹	Catalog Number	Fluorochrome²
Surface	anti-mouse CD19	BL	115530	APC-Cy7
	anti-mouse CD19	SB	1575-17	PE-Cy7
	anti-mouse CD19	SB	1575-09L	PE
	anti-mouse IgM, F(ab') ₂	SB	1140-02	FITC
	anti-mouse IgM, F(ab') ₂	JI	115-006-020	-
Intracellular	anti-Akt (phospho T308)	CST	13842	PE
	anti-Akt (phospho S473)	CST	4075	AF647
	anti-Btk (phosphoY223)/Itk (phospho Y180)	BD	562753	PE
	anti-Btk (total)	CST	8547	-
	anti-CD79 α (phospho Y182)	CST	5173S	-
	anti-Erk 1/2 (phospho T202/Y204)	CST	9101	-
	anti-Myc (total)	CST	14426	PB
	anti-Nf κ B p65 (phospho S536)	CST	5733	PE
	anti-p38 MAPK (phospho T180/Y182)	CST	4511	-
	anti-PLC γ 2 (phospho Y759)	BD	558498	AF647
	anti-PLC γ 2 (phospho Y1217)	CST	3871	-
	anti-S6 ribosomal protein (phospho S235/236)	CST	8520	PB
	anti-Syk (phospho Y525/526)	CST	2710P	-
2°	anti-rabbit IgG (H+L), F(ab') ₂	CST	4414	AF647

¹Biologend (BL); Southern Biotech (SB); Jackson ImmunoResearch (JI); Cell Signaling Technology (CST); BD Biosciences (BD).

²allophycocyanin (APC); cyanine (CY), R-phycoerythrin (PE); fluorescein isothiocyanate (FITC); AlexaFluor 647 (AD647); Pacific Blue (PB).

Supplementary Figure Legends

Supplementary Figure S1. Myc levels are elevated in E μ -myc B cells. Intracellular flow cytometry of Myc protein levels in IgM⁺CD19⁺ splenic B cells from E μ -myc mice and wild-type littermates (n=3 of each genotype). Mean fluorescence intensity, MFI; error bars are SEM; *p=0.004.

Supplementary Figure S2. Increased phosphorylation of deep downstream signaling molecules in E μ -myc B cells. Activation of deep downstream signaling molecules were evaluated in splenic B cells from E μ -myc mice and non-transgenic littermates by intracellular phospho-flow cytometry at intervals after ligation of IgM with or without the addition of H₂O₂. Mean fluorescence intensity (MFI) for Erk1/2 pT203/Y205 (top), p38MAPK pT180/Y182 (middle), and NF- κ B p65 pS536 (bottom) are indicated; error bars are SEM; p-values compare levels of phospho-protein in E μ -myc and wild-type splenic B cells with H₂O₂ (dashed lines); *p \leq 0.01, **p \leq 0.03, and ***p \leq 0.05 (p values for other comparisons not denoted).

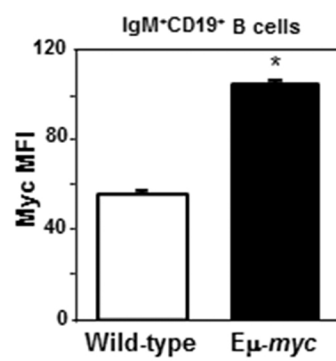
Supplementary Figure S3. Dose-dependent inhibition of Plc γ 2 and Erk1/2 phosphorylation by ibrutinib. Splenic B cells from E μ -myc mice or wild-type littermates were incubated with increasing concentrations of ibrutinib. Levels of Plc γ 2 pY759 were assessed by intracellular phospho-flow cytometry in cells at rest (A) or 10 minutes after BCR ligation (B). Basal (C) and anti-IgM stimulated (D) levels of Erk1/2 pT203/Y205 were similarly assessed, but H₂O₂ was added immediately after BCR ligation to inhibit phosphatases. Each experiment was performed at least 3 times with 2-3 mice per genotype in each experiment with mean fluorescence intensity

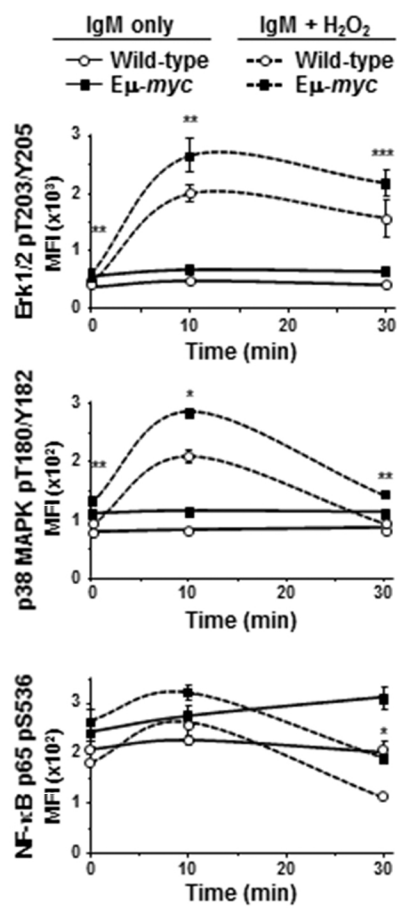
(MFI) from one representative experiment shown. Error bars represent SEM; * $p \leq 0.01$, ** $p \leq 0.03$, *** $p \leq 0.05$.

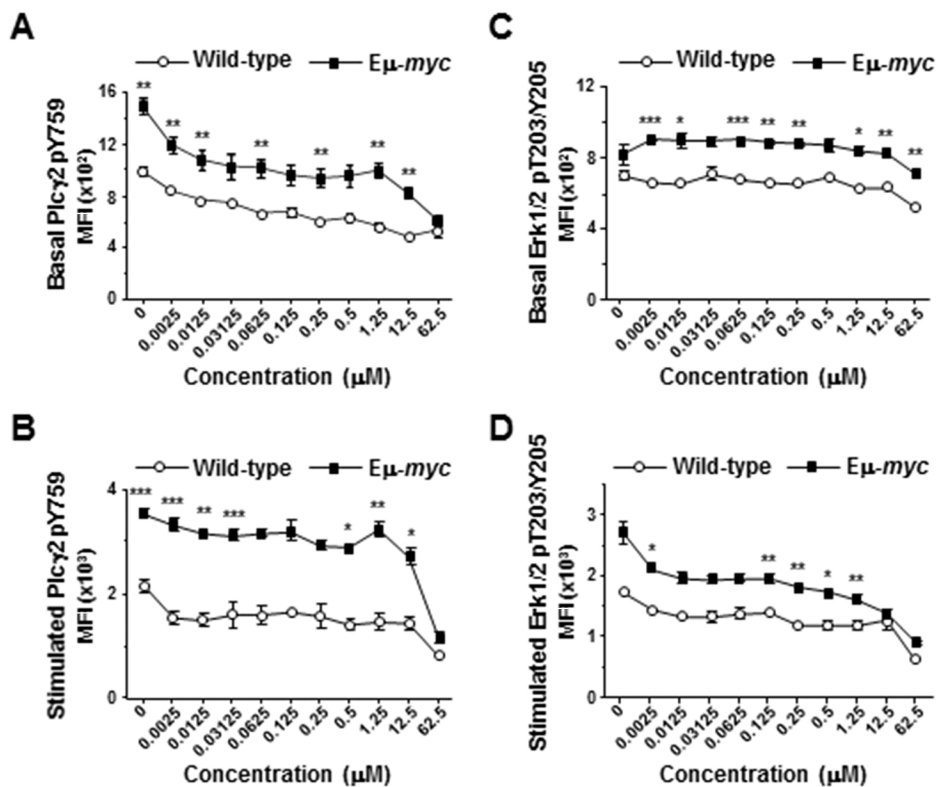
Supplementary Figure S4. Myc overexpressing non-transformed B cells have increased Plc γ 2 Y1217 phosphorylation. Plc γ 2 pY1217 was measured by intracellular phospho-flow cytometry in splenic B cells incubated without or with ibrutinib prior to IgM ligation without (A) or with (B) H₂O₂. (A) Mean fluorescence intensities (MFI) prior to and 30 min after IgM ligation from one representative experiment. (B) MFI without and intervals after BCR ligation and H₂O₂ addition (left) and histograms showing one representative mouse per genotype (right). Each protein was measured in at least three experiments with 3-4 mice of each genotype per experiment. Error bars represent SEM; A, * $p \leq 0.002$; B, * $p \leq 0.004$. For B, p-values compare levels of phospho-protein in ibrutinib-treated E μ -myc and ibrutinib-treated wild-type splenic B cells (dashed lines); p values for other comparisons not denoted.

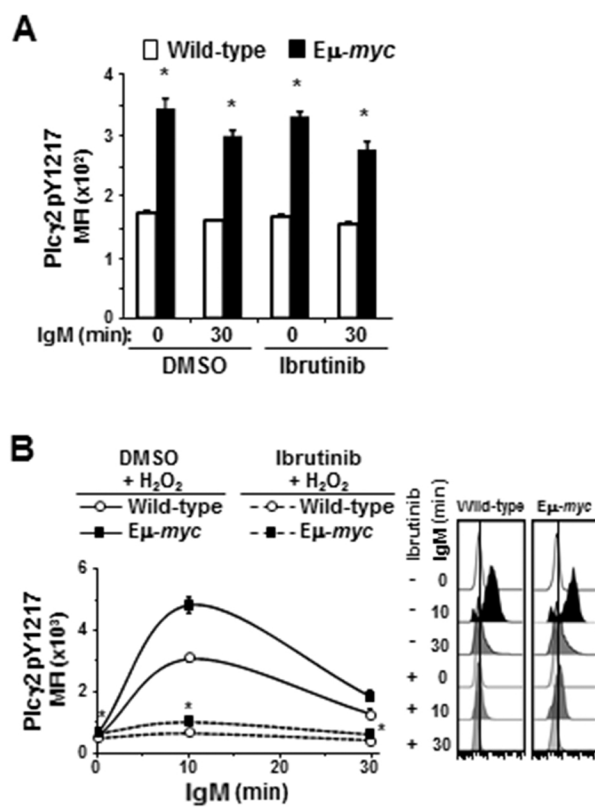
Supplementary Figure S5. Dose dependent inhibition of Syk phosphorylation by ibrutinib. Splenic B cells from E μ -myc mice or wild-type littermates were incubated with increasing concentrations of ibrutinib. Syk pY519/520 was assessed by intracellular phospho-flow cytometry either without (A) or 10 minutes after IgM ligation (B). Each experiment was performed at least 3 times with 2-3 mice per genotype in each experiment with mean fluorescence intensity (MFI) from one representative experiment shown. Error bars represent SEM; * $p \leq 0.02$, ** $p \leq 0.002$, *** $p \leq 0.0003$.

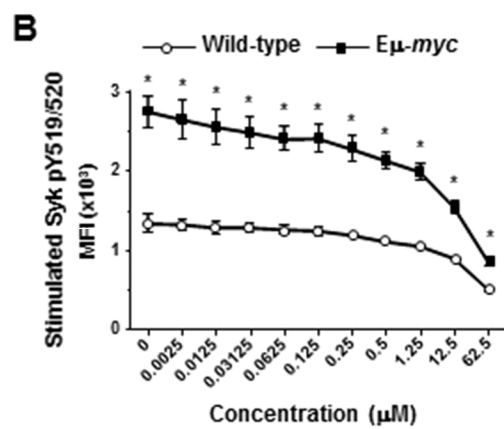
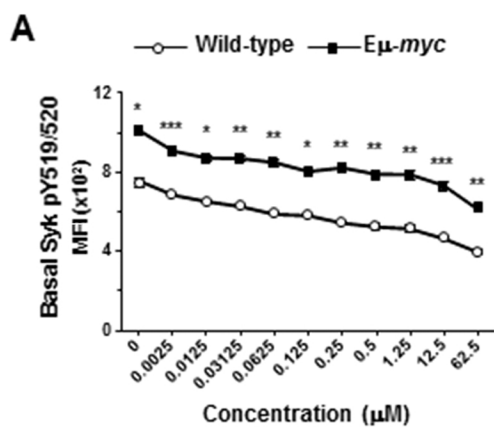
Supplementary Figure S6. Evaluation of Syk inhibition by ibrutinib. (A) Phospho-Syk levels were measured before (basal) and 10 minutes after BCR ligation (IgM-stimulated) in splenic B cells from *Btk*^{-/-} mice pre-treated with the indicated ibrutinib concentration. Error bars represent SEM; lines denote the samples being compared for p-values; *p≤0.035, ** p≤0.002. (B) Myc protein levels were assessed by intracellular flow cytometry 30 minutes after ligation of the BCR in wild-type and Eμ-*myc* B cells pre-treated with DMSO or 12.5 μM ibrutinib; error bars represent SEM. These experiments were performed at least twice with 3 mice per genotype in each experiment. A representative experiment is shown.

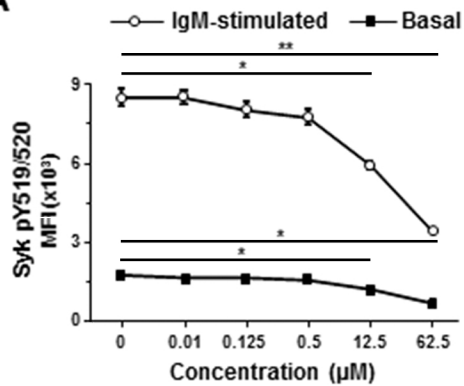










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