Supplemental material

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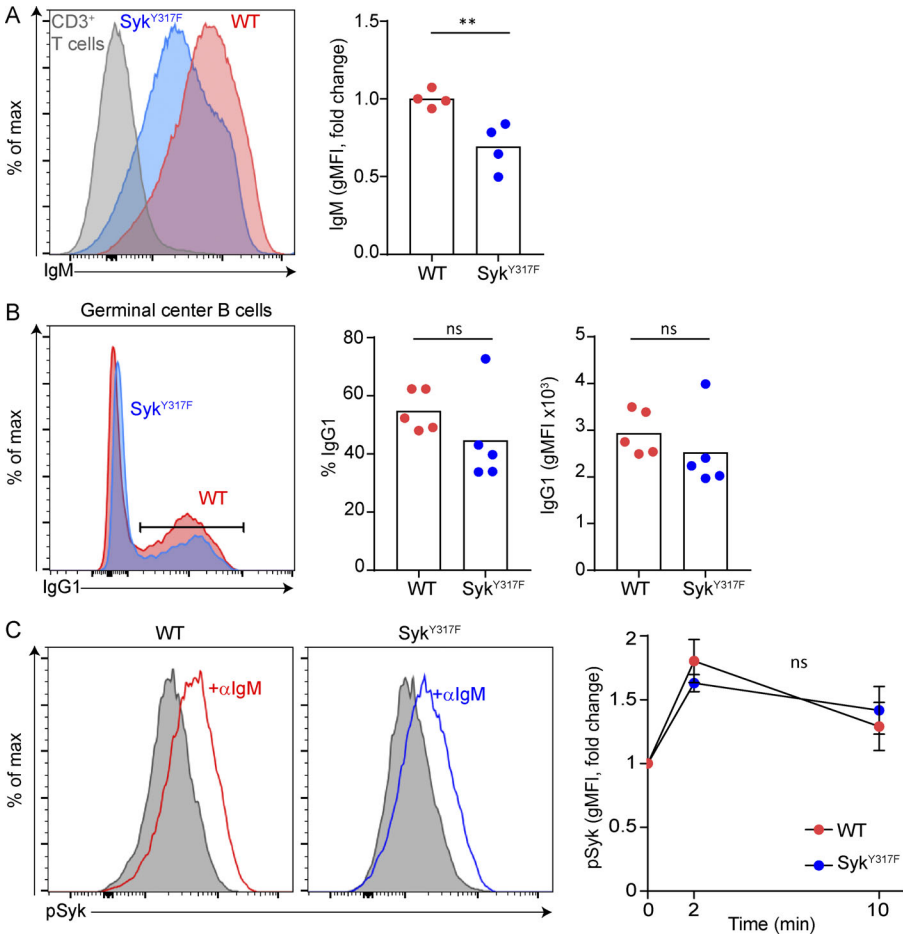

Figure S1. **IgM and IgG1 expression and Syk phosphorylation at position Y348 in WT and Syk^{Y317F} mice. (A and B)** Flow cytometry analysis and quantification of IgM expression on WT and Syk^{Y317F} follicular B cells (A) and IgG1 expression on WT and Syk^{Y317F} GC B cells (B220^{+} FAS^{+} CD38^{low}; B; *n* = 4 in A and *n* = 5 in B, two independent experiments, two-tailed Student's *t* test). Each dot in the graphs represents a single mouse; bars represent the mean. **(C)** Flow cytometry histograms and quantification of pSyk in WT and Syk^{Y317F} B cells that were left unstimulated or stimulated with anti-IgM at different time points. The gMFI of pSyk (Tyr 348) after 2 and 10 min of stimulation was normalized to the gMFI at time 0. Each graph represents the mean of ± SEM (*n* = 3, three independent experiments, two-tailed Student's *t* test). **, P ≤ 0.01; ns, not significant.

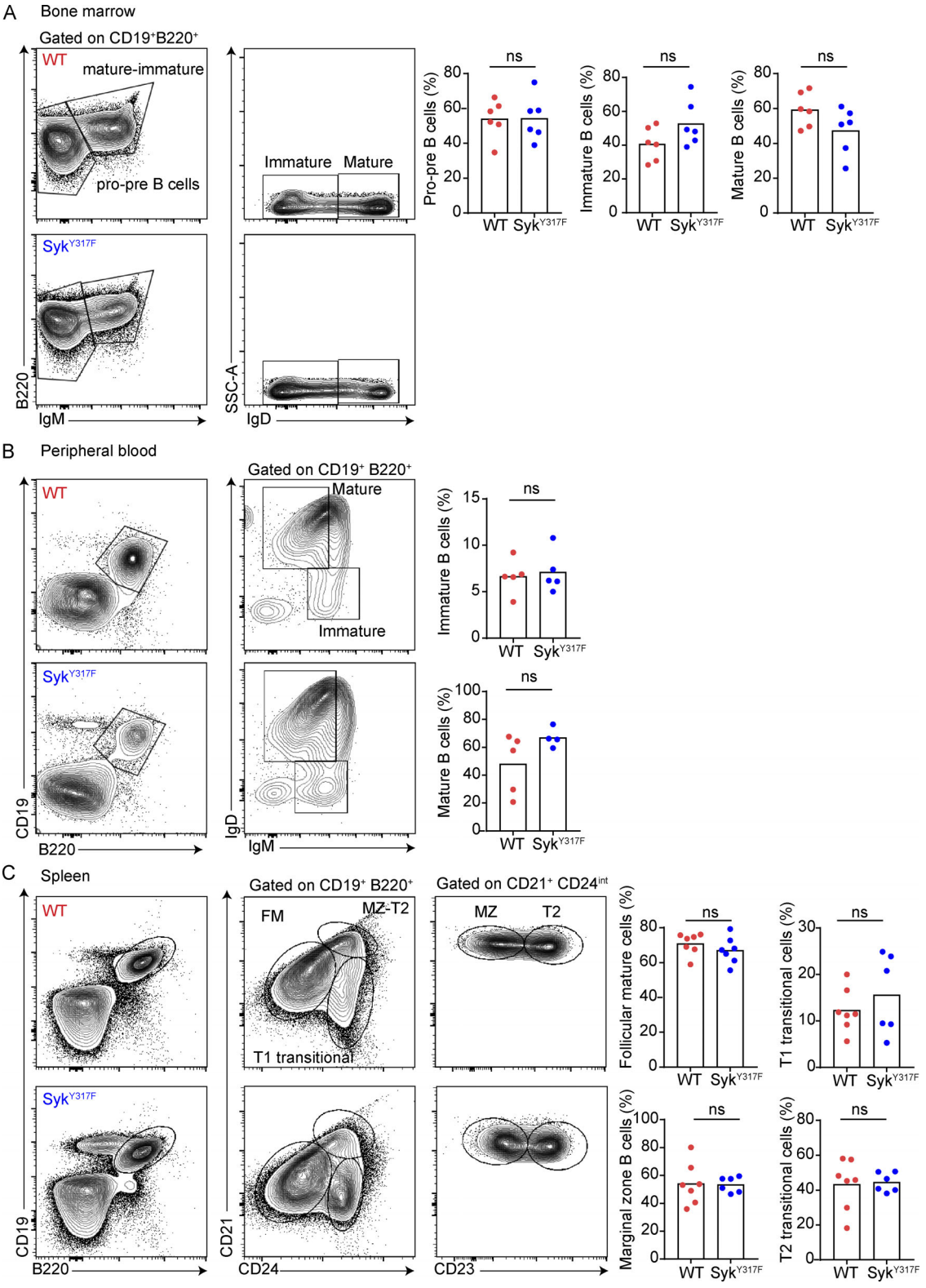

Figure S2. **Syk^{Y317F} mice exhibit normal B cell development. (A–C)** Representative flow-cytometric analysis and quantification of Syk^{Y317F} and WT B cells in different developmental stages in the BM (A), peripheral blood (B), and spleen (C). Each dot represents a single mouse; bars represent the mean (n = 4–7, two independent experiments, two-tailed Student's t test). ns, not significant.

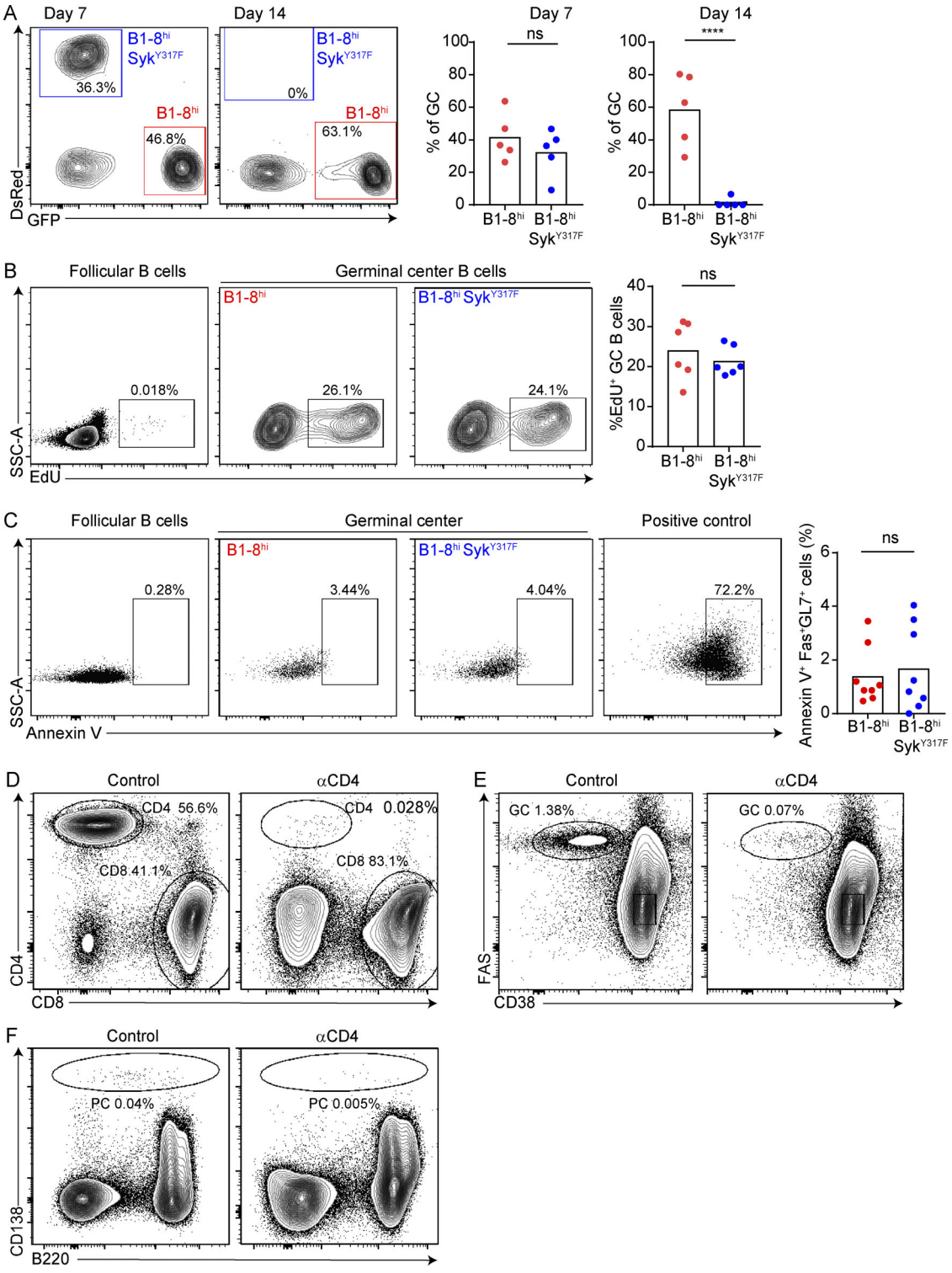

Figure S3. **Syk degradation is required for B cell persistence in GCs independently of their relative BCR affinity in a T cell–dependent manner. (A)** Analysis of transferred B1-8^{hi} and B1-8^{hi} Syk^{Y317F} B cells in GCs (B220^{+} FAS^{+}CD38^{low}) in MD4 recipient mice 7 and 14 d after immunization with NP-OVA. Each dot in the graphs represents a single mouse; bars represent the mean (n = 5, two independent experiments, two-tailed Student's t test). **(B and C)** Representative flow-cytometric analysis and quantification of EdU^{+} (B) and Annexin V^{+} (C) in B1-8^{hi} and B1-8^{hi} Syk^{Y317F} B cells that were cotransferred into WT hosts. Mice were immunized with NP-OVA, and GCs were analyzed 7 d later. Each dot in the graphs represents a single mouse; bars represent the mean (n = 6–8, two independent experiments, two-tailed Student's t test). **(D–F)** Gating strategy for CD4^{+} T cells (D), GC B cells (E), and PCs (F) following CD4-depleting antibody or PBS injection into NP-KLH–immunized chimeric mice (~50% GFP^{+}, ~50% Syk^{Y317F}). Quantification of the data are shown in Fig. 4 C. ****, P < 0.0001; ns, not significant.

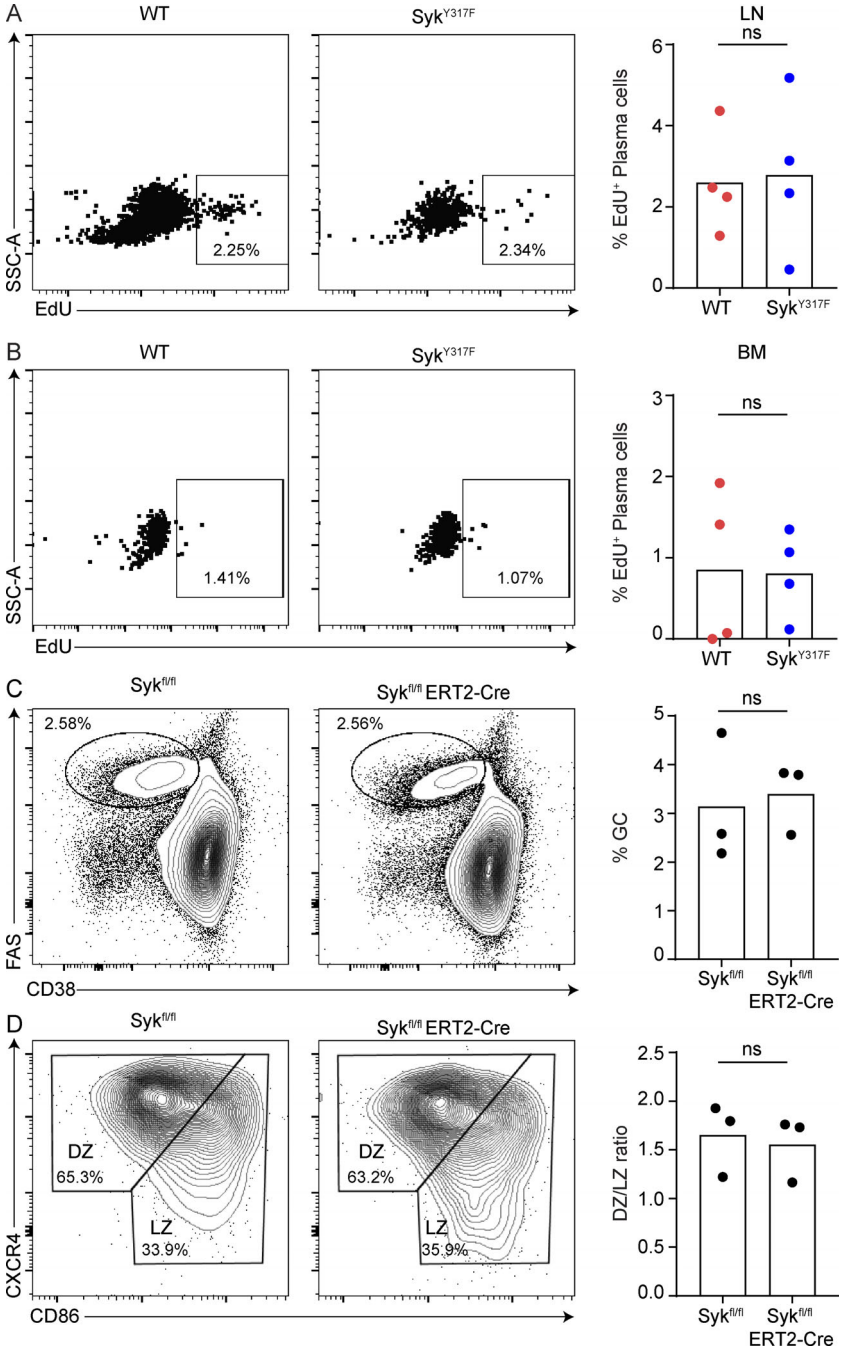

Figure S4. **Short-term EdU incorporation in PCs and ERT2-Cre expression effect on the GC reaction. (A and B)** Representative flow-cytometric plots and quantification of EdU+ PCs (CD138+) in popliteal lymph nodes (A) of NP-KLH immunized mice (day 14) and in the BM (B) 2.5 h after injection of EdU into immunized mice (n = 4, two independent experiments, two-tailed Student's t test). **(C and D)** Representative flow-cytometric plots and quantification of GC size (C), DZ, and LZ (D) GC B cell distribution in Syk$^{fl/fl}$ and Syk$^{fl/fl}$ ERT2-Cre mice 7 d after immunization without tamoxifen treatment. Each dot in the graphs represents a single mouse; bars represent the mean (n = 3, one experiment, two-tailed Student's t test). ns, not significant; SSC-A, side scatter–area.

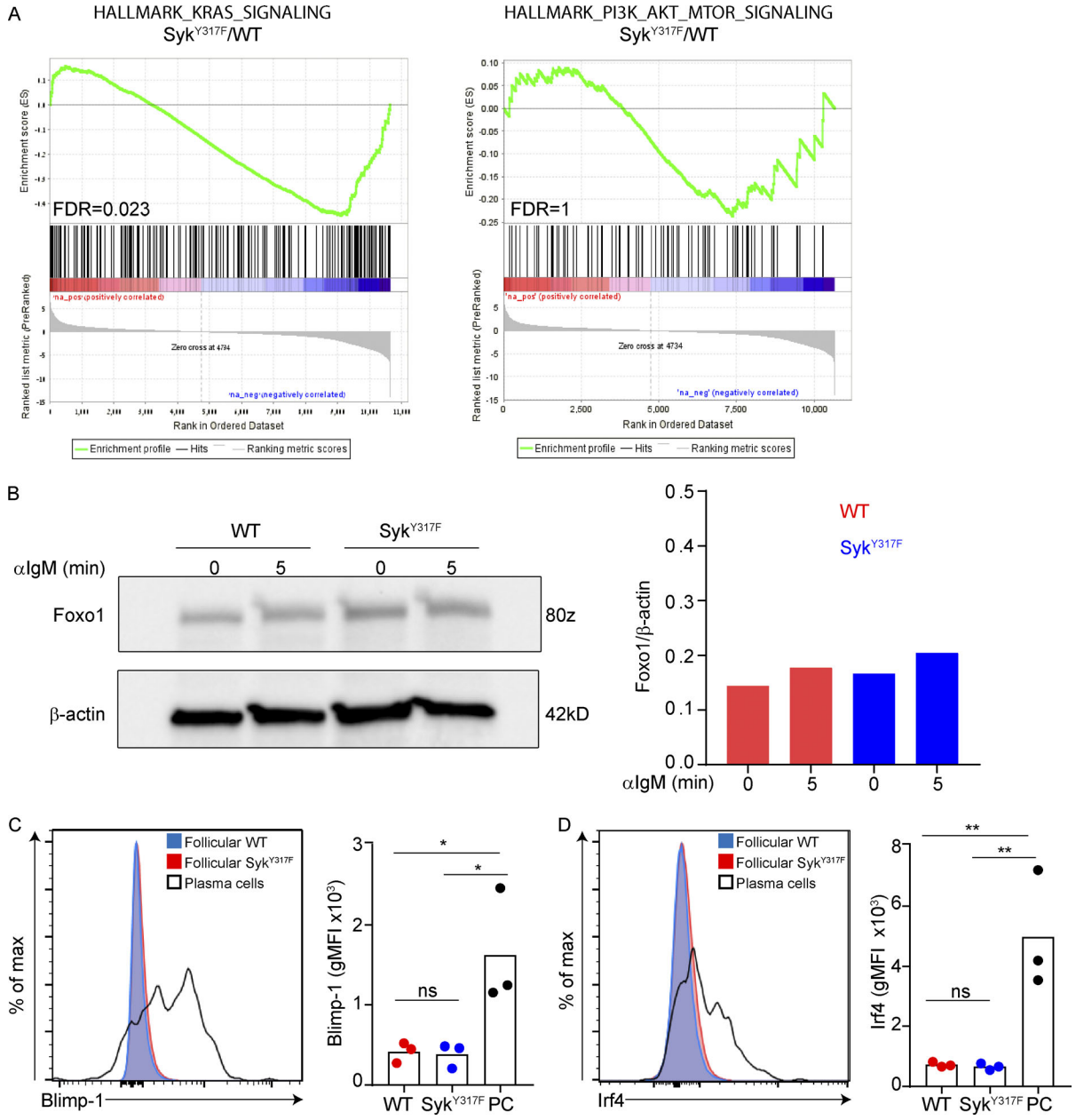

Figure S5. **Changes in gene expression that are related to the Kras and PI3K pathways in WT and Syk^{Y317F} mice. (A)** GSEA plot showing enrichment of the Kras and PI3K/Akt/MTOR signaling pathways in LZ GC B cells. False discovery rate q value = 0.023 and false discovery rate q value= 1, respectively. Analysis is based on the gene expression data shown in Fig. 6. **(B)** Foxo1 protein levels were determined by Western blot analysis of B cells derived from WT and Syk^{Y317F} mice that were stimulated with anti-IgM for the indicated time. Signals were normalized to β-actin (n = 1, one experiment). **(C and D)** Representative histograms and quantification of Blimp-1 (C) and Irf4 (D) expression in follicular B cells (B220$^+$FAS$^+$CD38^{hi}) and PCs (CD138$^+$) of WT and Syk^{Y317F} mice. Each dot in the graphs represents a single mouse; bars represent the mean (n = 3, one experiment, two-tailed Student's t test). ns, not significant; *, P = 0.05; **, P ≤ 0.01.

Table S1. **List of antibodies used for flow cytometry**

Antigen	Fluorophore	Clone	Manufacturer	Concentration, µg/ml
B220	V500	RA3-6B2	BD	1
B220	APC-Alexa Fluor 780/e450	RA3-6B2	eBioscience	1
CD138	BV605	281-2	Biolegend	0.5
CD38	FITC	90	Biolegend	1
CD38	Alexa Fluor 700	90	eBioscience	1
F4/80	APC-Alexa Fluor 780	BM8	eBioscience	1
FAS	PE-Cy7	Jo2	BD	0.33
GL-7	Alexa Fluor 647/PerCP-Cy5.5	GL7	Biolegend	2.5
Gr-1	APC-Alexa Fluor 780	RB6-8C5	eBioscience	1
Igλ	PE	RML-42	Biolegend	1
Streptavidin	Alexa Fluor 647		Biolegend	1
IgM	Biotin	RMM-1	Biolegend	1
IgM	PerCP-CeFluor710	II/41	eBioscience	1
IgG1	BV421/FITC	RMG1-1	Biolegend	1
IgD	Alexa Fluor 647	11-26c.2a	Biolegend	1
CD4	Alexa Fluor 488/Alexa Fluor 780	GK1.5	Biolegend	1
CD8	Pacific Blue	53-6.7	Biolegend	1
CD8	Alexa Fluor 780	53-6.7	eBioscience	1
CD19	Pacific Blue	6D5	Biolegend	1
CD19	FITC	MB19-1	Biolegend	1
CD3	PE	145-2C11	Biolegend	1
CD21	APC-Cy7	7E9	Biolegend	1
CD24	PeCy7	M1/69	Biolegend	1
CD23	FITC	B3B4	Biolegend	1
CXCR4	BV421	L276F12	Biolegend	1
CD86	PE/FITC	GL-1	Biolegend	0.08
pERK	Alexa Fluor 488	4B11B69	Biolegend	2.5
CD45.1	BV421	A20	Biolegend	1
CD45.1	Alexa Fluor 780	A20	eBioscience	1
CD45.2	PE	104	Biolegend	1
Blimp-1	Alexa Fluor 647	5E7	Biolegend	1
Irf4	Alexa Fluor 488/PerCP-Cy5.5	IRF4.3E4	Biolegend	1
p-S6 (Ser235/236)	Alexa Fluor 647	D57.2.2E	Cell Signaling Technology	1
pSyk ab	PE	moch1ct	eBioscience	1

Table S2. **List of primers used for Igγ1 sequencing**

Primers	**Primer sequence 5′-3′**
Variable region primers	
HP1F-mLEADER-1	GTAACACTTTTAAATGGTATCCAGTGT
HP1F-mLEADER-2	GTCCTAATTTTAAAAGGTGTCCAGTGT
HP1F-mLEADER-3	AGCAACAGCTACAGGTGTCCACTCC
HP1F-mLEADER-4	TCTTCTTCCTGTCAGTAACTRCAGG
HP1F-mLEADER-5	TTGCTATTCCTGATGGCAGCTGCCCAA
HP1F-mLEADER-6	GTGACATTCCCAAGCTGTGTCCTRTCC
HP1F-mLEADER-7	TGTACCTGTTGACAGTCGTTCCTGG
HP1F-mLEADER-8	GTTTTTTATCAAGGTGTGCATTGT
HP1F-mLEADER-9	CTATTCCTGATGGCAGCTGCCCAAAG
VH186.2 variable region primer	CTAGTAGCAACTGCAACCGGTGTACATTCTCAGGTGCAGCTGCAGGAGTC
Constant region primers	
Cγ1-outer	GGAAGGTGTGCACACCGCTGGAC
Cγ1-inner	GCTCAGGGAAATAGCCCTTGAC