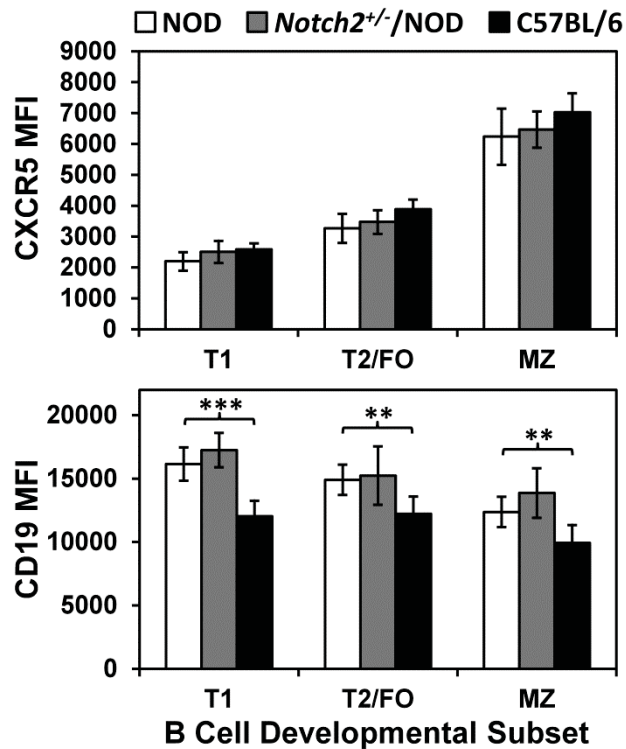
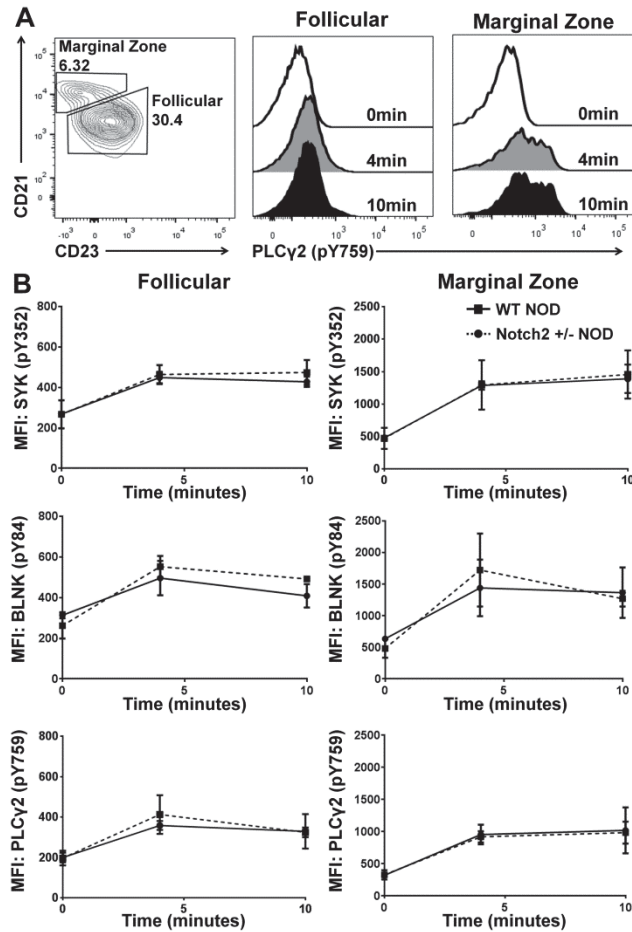


Supplemental Figure 1.



**Supplemental Figure 1: *Notch2* haploinsufficiency in NOD mice does not affect surface expression of B cell homing receptor CXCR5, or B cell signaling protein CD19.** Splenocytes were harvested from n = 6 NOD (white), n = 5 *Notch2*<sup>+/-</sup>/NOD (grey), or n = 6 B6 (black) mice. CD19<sup>+</sup> IgM<sup>+</sup> live lymphocytes were further gated as T1 (IgM<sup>high</sup> CD21<sup>low</sup> CD23<sup>low</sup>), T2/FO (CD21<sup>low</sup> CD23<sup>high</sup>), or MZ subsets (IgM<sup>high</sup> CD21<sup>high</sup> CD23<sup>low</sup>), and flow cytometry was used to assess CXCR5 and CD19 expression within each subset. Male and female mice were 14-16 weeks of age. Comparisons between groups were not significant by two-tailed t test unless specified, \*\* p < 0.01, \*\*\* p < 0.001.

Supplemental Figure 2.



**Supplemental Figure 2: *Notch2* haploinsufficiency in NOD mice does not alter proximal signaling in response to BCR-stimulation.** Splenocytes were harvested from WT NOD or *Notch2*<sup>+/-</sup>/NOD mice and rested for 30-45 minutes, then stimulated for 0, 4, or 10 minutes with 10 $\mu$ g/mL anti-IgM. Reaction was stopped with 1.6% paraformaldehyde and cells were stained for extracellular markers, then permeabilized with methanol. Cells were stained for phospho-Syk, phospho-BLNK, or phospho-PLC $\gamma$ 2. A) Gated on B220<sup>+</sup> lymphocytes, and then gated by CD21 and CD23 for marginal zone and follicular populations. N=3 mice per group, no significant differences were found between *Notch2* haploinsufficient and wild-type NOD.

Supplemental Table I. Complete comparative significance (p values) for T cell numbers in *Notch2* haploinsufficiency, and for data shown in Figure 2.

<b>T cell numbers in <i>Notch2</i><sup>+/-</sup>/NOD versus WT NOD mice</b>							
<b>Average # T Cells x10<sup>6</sup></b>							
	<b>WT NOD</b>	<b><i>Notch2</i><sup>+/-</sup>/NOD</b>		<b>P-Value</b>			
<b>CD4+</b>	23.2 ± 5.3	20.2 ± 1.8		0.2677			
<b>CD8+</b>	9.4 ± 1.9	8.99 ± 0.78		0.6790			
<b>Comparative significance (p values) for Figures 2C, percent of MZ B cells.</b>							
	<i>Notch2</i> <sup>+/-</sup> 125TgNOD	NOD	<i>Notch2</i> <sup>+/-</sup> NOD	125Tg/B6	<i>Notch2</i> <sup>+/-</sup> 125Tg/B6	B6	<i>Notch2</i> <sup>+/-</sup> B6
<b>125Tg/NOD</b>	0.006	0.02	< 0.001	0.03	< 0.001	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> <b>125Tg/NOD</b>	-	0.92	< 0.001	0.20	< 0.001	< 0.001	< 0.001
<b>NOD</b>		-	< 0.001	0.27	< 0.001	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> <b>NOD</b>			-	< 0.001	0.85	0.046	< 0.001
<b>125Tg/B6</b>				-	< 0.001	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> <b>125Tg/B6</b>					-	0.22	0.001
<b>B6</b>						-	< 0.001
<b>Comparative significance (p values) for Figure 2D, number of MZ B cells.</b>							
	<i>Notch2</i> <sup>+/-</sup> 125TgNOD	NOD	<i>Notch2</i> <sup>+/-</sup> NOD	125Tg/B6	<i>Notch2</i> <sup>+/-</sup> 125Tg/B6	B6	<i>Notch2</i> <sup>+/-</sup> B6
<b>125Tg/NOD</b>	0.013	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> <b>125Tg/NOD</b>	-	0.41	< 0.001	0.02	< 0.001	< 0.001	< 0.001
<b>NOD</b>		-	< 0.001	0.046	< 0.001	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> <b>NOD</b>			-	0.08	< 0.001	0.095	< 0.001
<b>125Tg/B6</b>				-	< 0.001	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> <b>125Tg/B6</b>					-	< 0.001	0.10
<b>B6</b>						-	< 0.001

Supplemental Table II. Comparative significance (p values) for Figures 3 through 5.

Comparative significance (p values) for Figure 3, number of MZ B cells.			
	<i>Notch2</i> <sup>+/-</sup> NOD	<i>Btk</i> -deficient NOD	<i>Btk</i> -deficient <i>Notch2</i> <sup>+/-</sup> NOD
NOD	< 0.001	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> NOD	-	0.30	< 0.001
<i>Btk</i> -deficient NOD		-	0.003

Comparative significance (p values) for Figure 3, total number of B cells.			
	<i>Notch2</i> <sup>+/-</sup> NOD	<i>Btk</i> -deficient NOD	<i>Btk</i> -deficient <i>Notch2</i> <sup>+/-</sup> NOD
NOD	0.75	< 0.001	< 0.001
<i>Notch2</i> <sup>+/-</sup> NOD	-	< 0.001	< 0.001
<i>Btk</i> -deficient NOD		-	0.16

Means and Standard Deviation for Figure 4C  
qRT-PCR for *Notch2*

	<i>Notch 2</i>							
	Wild-type B6		<i>Btk</i> <sup>null</sup> B6		Wild-type NOD		<i>Btk</i> <sup>null</sup> NOD	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MZ	2.54	2.69	1.64	1.80	19.3	8.21	13.6	7.93
T2	1.80	1.27	2.21	1.67	10.0	5.32	4.06	3.64
pMZ	0.325	0.316	0.251	0.432	19.6	6.84	5.17	5.55

Comparative Significance (multiplicity-adjusted p values) for Figure 4C  
qRT-PCR for *Notch2*

<u>Marginal Zone</u>	<i>Btk</i> <sup>null</sup> B6	NOD	<i>Btk</i> <sup>null</sup> NOD
B6	0.9923	< 0.0001	0.0167
<i>Btk</i> <sup>null</sup> B6	-	< 0.0001	0.0087
NOD		-	0.3617
<u>Transitional 2</u>	<i>Btk</i> <sup>null</sup> B6	NOD	<i>Btk</i> <sup>null</sup> NOD
B6	0.9992	0.0711	0.9154
<i>Btk</i> <sup>null</sup> B6	-	0.0932	0.9511
NOD		-	0.3354
<u>Pre-Marginal Zone</u>	<i>Btk</i> <sup>null</sup> B6	NOD	<i>Btk</i> <sup>null</sup> NOD
B6	>0.9999	<0.0001	0.5140
<i>Btk</i> <sup>null</sup> B6	-	<0.0001	0.5011
NOD		-	0.0012

**Means and Standard Deviation for Figure 4D  
qRT-PCR for *Hes5***

	<i>Hes5</i>							
	Wild-type B6		<i>Btk<sup>null</sup></i> B6		Wild-type NOD		<i>Btk<sup>null</sup></i> NOD	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MZ	1.37	0.922	2.14	2.46	5.69	1.45	1.91	0.554
T2	2.19	0.907	2.36	0.936	3.90	1.75	2.66	0.064
pMZ	4.19	2.44	4.85	3.20	5.56	1.97	2.54	0.832

**Comparative Significance (multiplicity adjusted p values) for Figure 4D  
qRT-PCR for *Hes5***

<u>Marginal Zone</u>	<i>Btk<sup>null</sup></i> B6	NOD	<i>Btk<sup>null</sup></i> NOD
B6	0.9266	0.0081	0.9774
<i>Btk<sup>null</sup></i> B6	-	0.372	0.9983
NOD		-	0.0413
<u>Transitional 2</u>	<i>Btk<sup>null</sup></i> B6	NOD	<i>Btk<sup>null</sup></i> NOD
B6	0.9991	0.5293	0.9853
<i>Btk<sup>null</sup></i> B6	-	0.6139	0.9961
NOD		-	0.7963
<u>Pre-Marginal Zone</u>	<i>Btk<sup>null</sup></i> B6	NOD	<i>Btk<sup>null</sup></i> NOD
B6	0.9531	0.6976	0.6192
<i>Btk<sup>null</sup></i> B6	-	0.9407	0.3384
NOD		-	0.1364

**Comparative significance (p values) for Figure 5B, number of VH125Tg insulin-binding B cells.**

	MZ Low Auto	FO/T2 High Auto	FO/T2 Low Auto
MZ High Auto	0.0017	0.30	0.11
MZ Low Auto	-	< 0.001	< 0.001
FO/T2 High Auto		-	0.59

**Comparative significance (p values) for Figure 5C, number of *Btk<sup>null</sup>*/VH125Tg insulin-binding B cells.**

	MZ Low Auto	FO/T2 High Auto	FO/T2 Low Auto
MZ High Auto	0.03	< 0.001	< 0.001
MZ Low Auto	-	0.001	0.04
FO/T2 High Auto		-	0.14