Supplementary Figure Legends

Figure S1. Biochemical signature of E613R and allelic series lymphocytes

(A) Whole-cell lysates of resting thymocytes from allelic series and E613R mice were blotted with Ab to the inhibitory and activating tyrosines of Lck (Lck Y505/Src Y416). Src416 Ab binds activating tyrosines of all SFKs. The lower band represents p56 Lck; the upper band, p59 Fyn. Total Lck is detected as loading control.

(B) Whole-cell lysates of resting B cells from allelic series and E613R mice were blotted with Ab to the inhibitory and activating tyrosines of Lyn (Lyn Y507/Src Y416). Src416 Ab binds activating tyrosines of all SFKs while Lyn507 is relatively specific. Total Lyn and Erk1/2 are detected as loading controls.

Data are representative of at least three independent experiments.

Figure S2. Phenotypic and functional signature of E613R and allelic series B cells

(A) BCR-stimulated, fixed, and permeabilized lymphocytes were stained for phospho-Erk and costained for CD23 and B220 so that B cells could be identified as described in Figure 2E. Data were collected by flow cytometry. Histograms depict intracellular phospho-Erk in LN B cells from +/+, H/H, and E613R mice. Cells in bottom panel were treated identically after pre-incubation with the Mek1/2 inhibitor U0126 (10nM) for 15 minutes at 37C.

(B) Histograms representing surface expression of various markers on CD45+/+ (gray shaded histogram) and CD45-/-, H/H, or E613R follicular mature splenic B cells (CD23+AA4.1^{neg}). Data are representative of at least three independent experiments.

Figure S3. Distinct effects of Lyn deficiency and supraphysiologic CD45 expression on BCR signaling and B cell activation

(A) Histograms depict intracellular phospho-Erk in LN B cells from +/+, H/H, and Lyn-/- mice treated for 3 minutes with a range of anti-IgM doses, as described in 2D.

(B) Graph represents quantification of data in (A). Values are the mean +/- SEM of 3 biological replicates.

(C) Graph representing MFI of CD86 expression on LN B cells from CD45+/+, H/H, and Lyn-/- mice stimulated for 16 hours with varying doses of anti-IgM. Values are mean ± SEM of three biological replicates.

Figure S4. Genetic epistasis between CD45 alleles and Lyn

(A, B) Representative plots (A) or histograms (B) of splenic B cells from E613R, +/+, and H/H mice genetically sufficient or deficient for Lyn stained to identify (A) B cell developmental stages (T1 = AA4.1+CD23-; T2 = AA4.1+CD23+; FO = AA4.1-CD23+) or (B) surface expression of CD21

Figure S1.

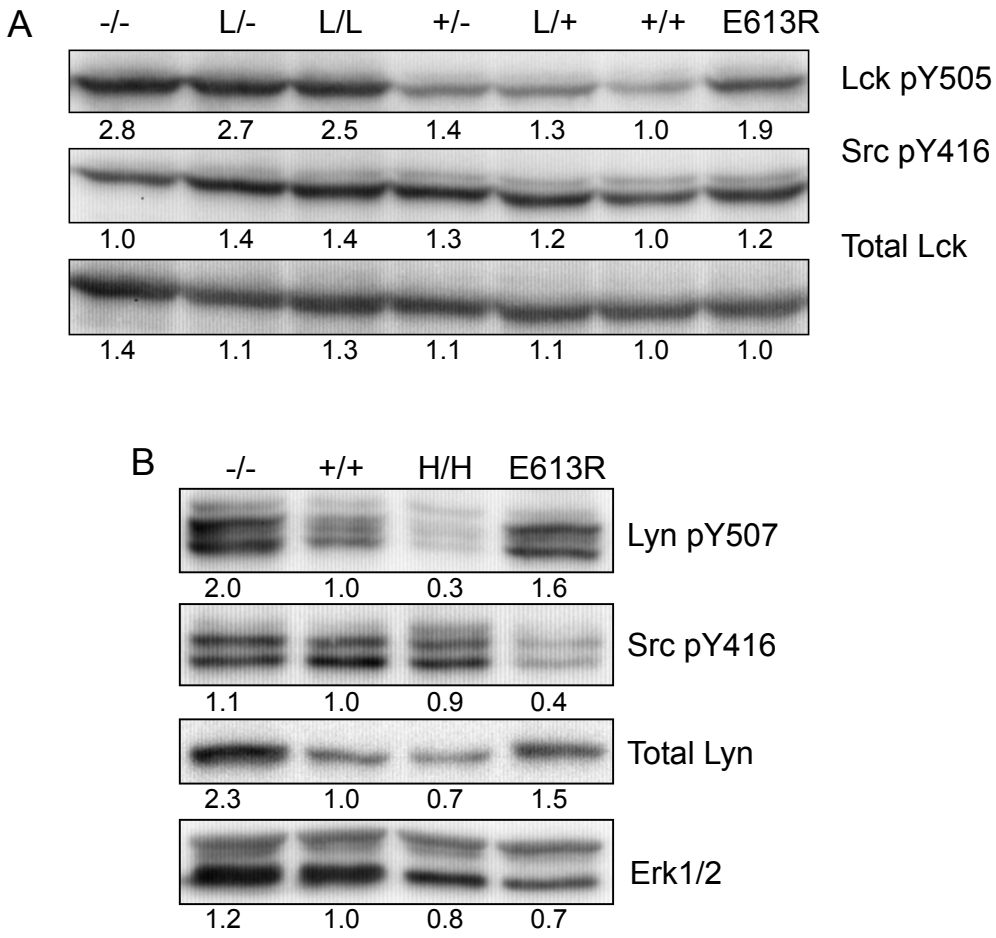
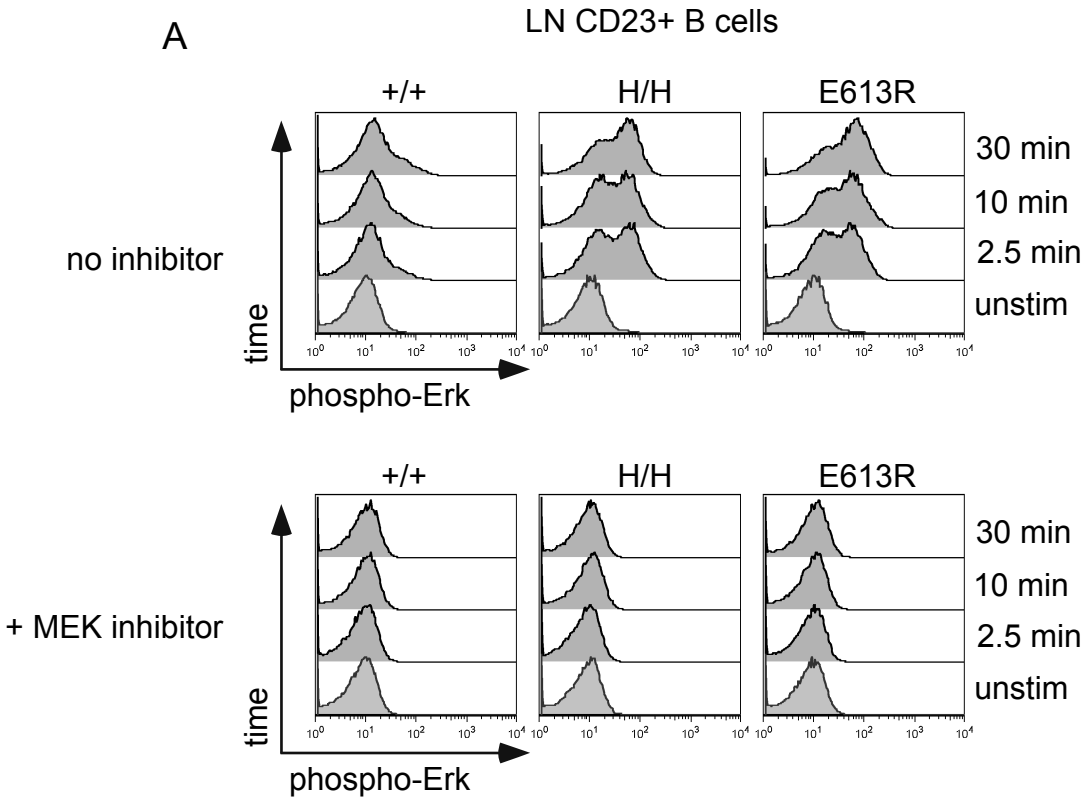

A
-/-
L/-
L/L
+/-
L/+
+/+
E613R
Lck pY505
2.8
2.7
2.5
1.4
1.3
1.0
1.9
Src pY416
1.0
1.4
1.4
1.3
1.2
1.0
1.2
Total Lck
1.4
1.1
1.3
1.1
1.1
1.0
1.0
B
-/-
+/+
H/H
E613R
Lyn pY507
2.0
1.0
0.3
1.6
Src pY416
1.1
1.0
0.9
0.4
Total Lyn
2.3
1.0
0.7
1.5
Erk1/2
1.2
1.0
0.8
0.7

Figure S2.


A
LN CD23+ B cells
+/+
H/H
E613R
30 min
10 min
2.5 min
unstim
no inhibitor
time
phospho-Erk
+/+
H/H
E613R
30 min
10 min
2.5 min
unstim
+ MEK inhibitor
time
phospho-Erk

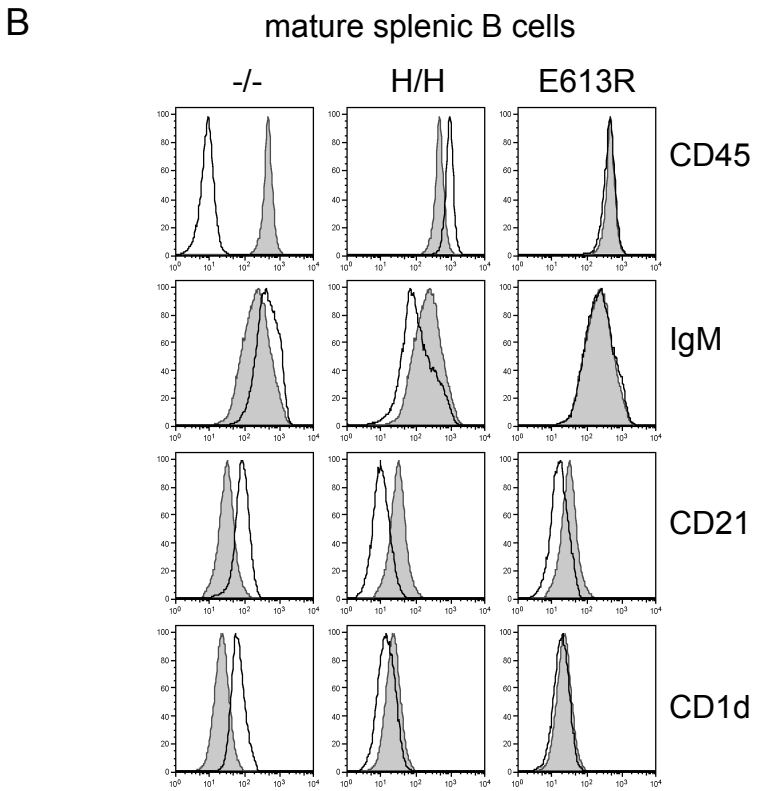

B
mature splenic B cells
-/-
H/H
E613R
CD45
IgM
CD21
CD1d

Figure S3.

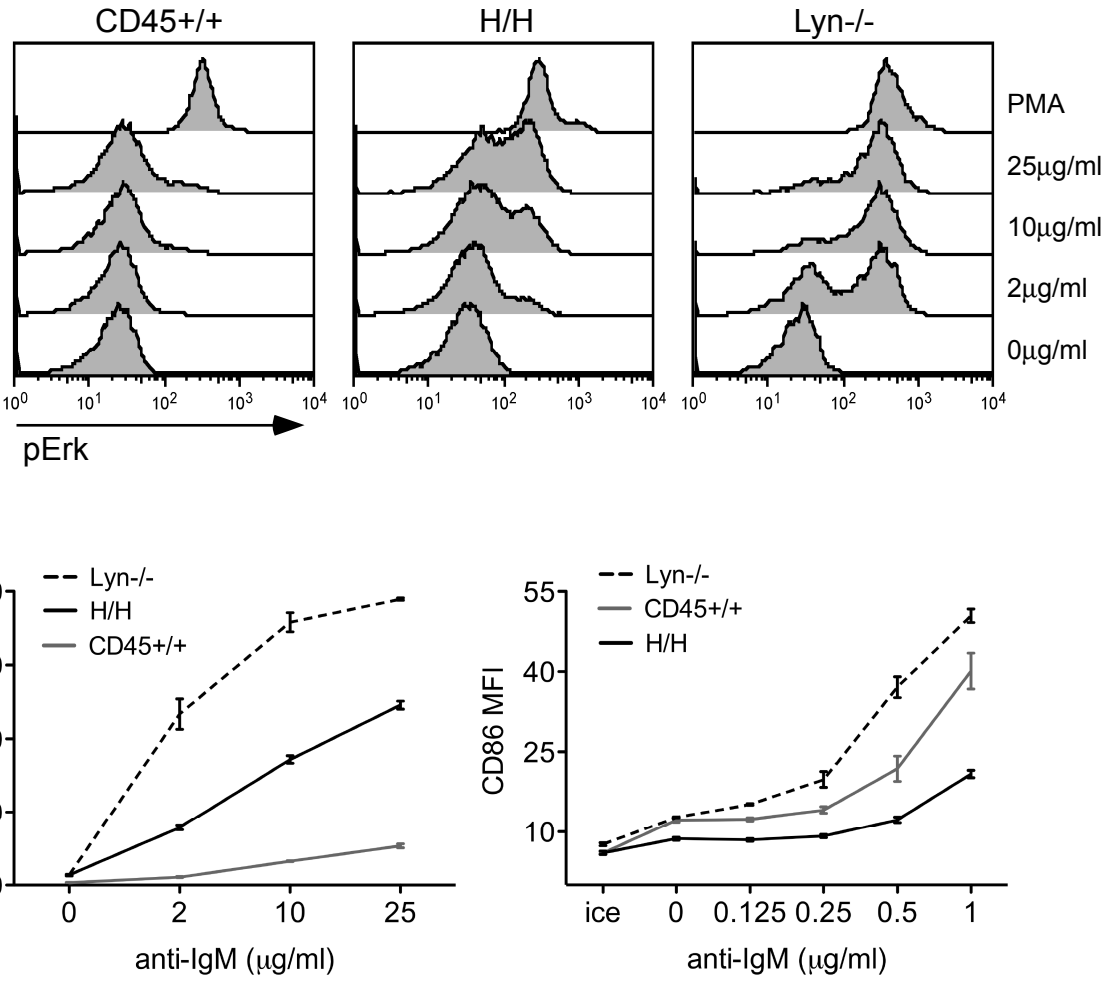
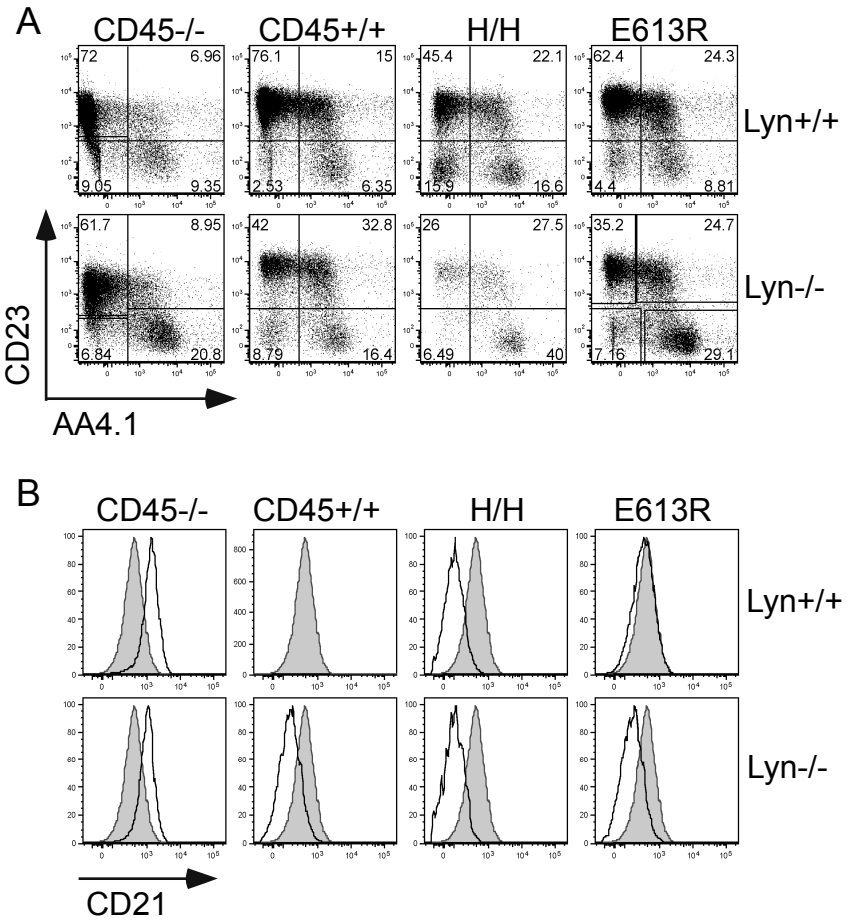

CD45+/+
H/H
Lyn-/-
PMA
25μg/ml
10μg/ml
2μg/ml
0μg/ml
pErk
Lyn-/-
H/H
CD45+/+
%pErk positive cells
anti-IgM (μg/ml)
CD86 MFI
ice
0.125
0.25
0.5

Figure S4.


A
CD45-/-
CD45+/+
H/H
E613R
Lyn+/+
Lyn-/-
CD23
AA4.1
72
6.96
19.05
9.35
76.1
15
2.53
6.35
45.4
22.1
15.9
16.6
62.4
24.3
4.4
8.81
61.7
8.95
16.84
20.8
42
32.8
8.79
16.4
26
27.5
6.49
40
35.2
24.7
7.16
29.1
B
CD45-/-
CD45+/+
H/H
E613R
Lyn+/+
Lyn-/-
CD21