SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Flow cytometric analysis of bone marrow B cell populations

Bone marrow cells isolated from 6-8-wk-old *ship*$^{fl/fl}$*cd19*$^{cre/+}$or *ship*$^{fl/f}$ mice were stained with antibodies against different surface markers..FACS analysis was performed to identify different cell fractions: HSC (B220-, CD24+ and CD43+), Pro-B (CD25-, CD19+ and CD43+), Pre-B (CD43+, B220+ and CD24 high), immature B (B220+, IgM+ and IgD-) and recirculating B (B220+, IgM+ and IgD+). All data shown are from a representative experiment of four.

Figure S2. Flow cytometric analysis of B cell populations in spleen

Splenocytes isolated from 6-8-wk-old *ship*$^{fl/fl}$*cd19*$^{cre/+}$or *ship*$^{fl/f}$ mice were stained with antibodies against different surface markers for FACS analysis to identify different B cell populations: (A) Follicular (Fo) B cells (B220+, CD21+, CD23+) and Mz B cells (B220+, CD21+, CD23-), (B) Germinal center (GC) B cells (B220+, GL7^{high}, Fas^{high}), (C) Transitional 1 (T1) B cells (B220+, IgD^{low}, IgM^{high}), T2 (B220+, IgD^{high}, IgM^{high}) and Mature (M) B cells (B220+, IgD^{high}, IgM^{low}), (D) Transitional 3 (T3) B cells (B220+, CD93+, CD23+, IgM^{low}), (E) B1 cells (CD5int, B220int) and (F) Plasma cells (B220^{low}, IgM-, CD138+). All data shown are from a representative experiment of four.

Figure S3. NP-specific V_H gene sequence analysis

V_H gene nucleotide sequence from different B-cell clones isolated from *ship*$^{fl/fl}$*cd19*$^{cre/+}$or *ship*$^{fl/fl}$ mice 14 days after NP-CGG (alum) immunization. The sequences from different clones were aligned to the germline V_H186.2 gene sequence. A dot denoted sequence identity. Mutations that introduced an early stop codon were marked with *.

Figure S4. Sequence analysis of the CDR 3 region

The D segments in the CDR 3 regions from the V_H genes were aligned with the germline D sequences: DFL16.1, DSP2.3 and DQ52.

Figure S1

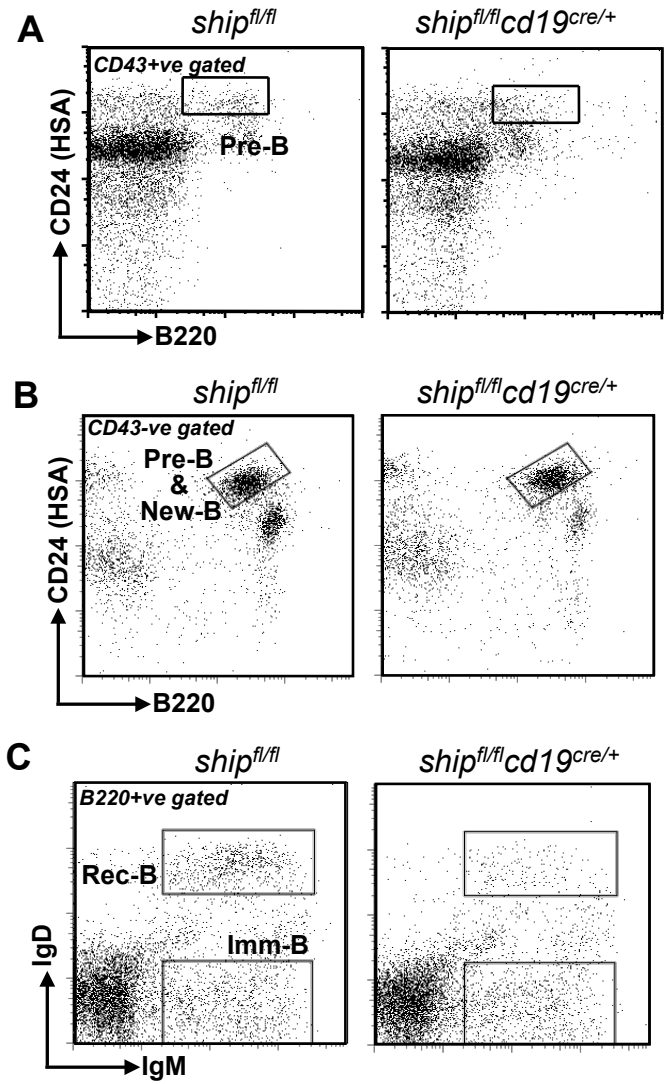

A
ship fl/fl
ship fl/fl cd19 cre/+
CD43+ve gated
Pre-B
CD24 (HSA)
B220
B
ship fl/fl
ship fl/fl cd19 cre/+
CD43-ve gated
Pre-B
&
New-B
CD24 (HSA)
B220
C
ship fl/fl
ship fl/fl cd19 cre/+
B220+ve gated
Rec-B
Imm-B
IgD
IgM

Figure S2

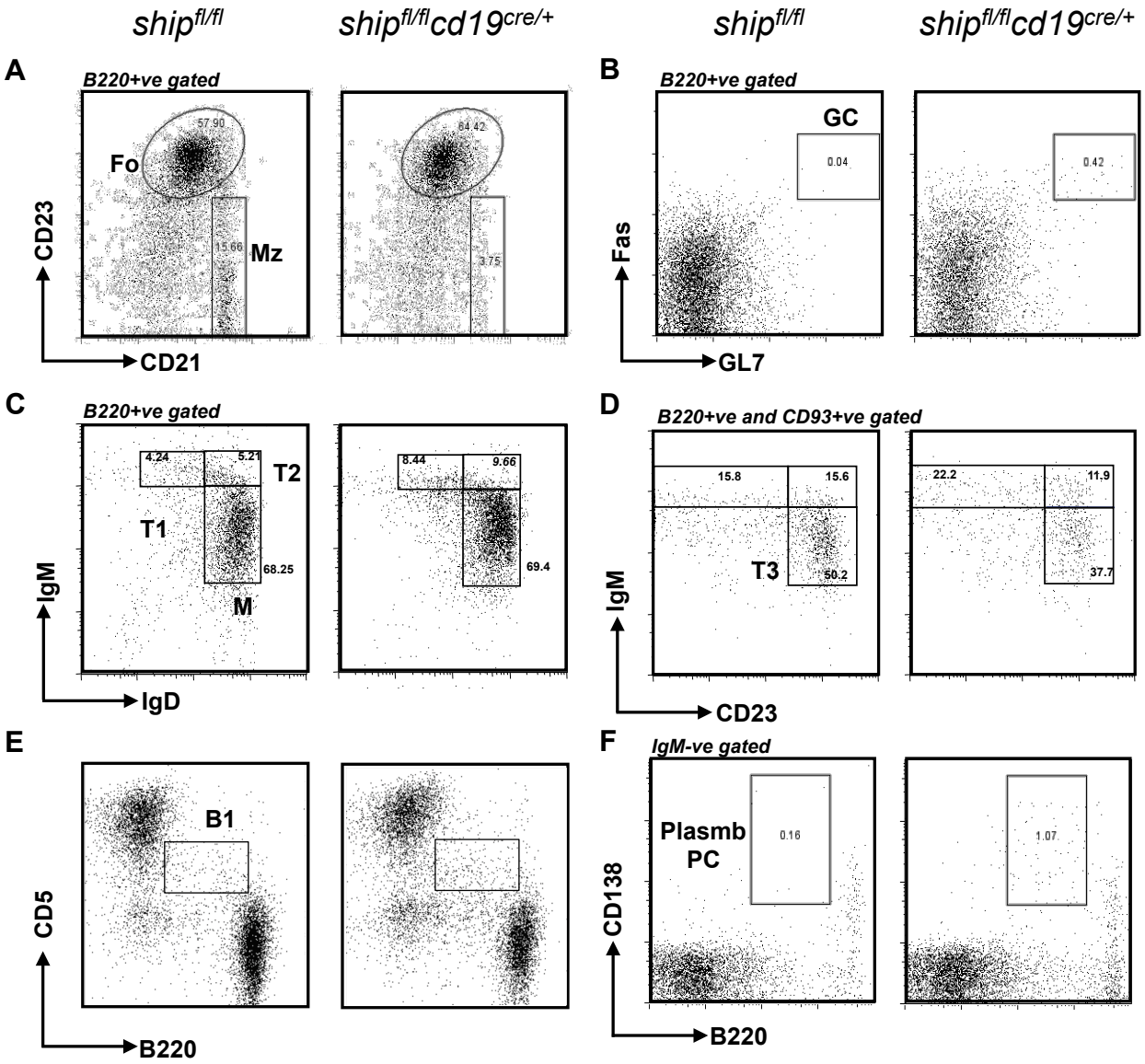

ship fl/fl
ship fl/fl cd19 cre/+
ship fl/fl
ship fl/fl cd19 cre/+
A
B220+ve gated
Fo
Mz
57.90
15.66
84.42
3.75
CD23
CD21
B
B220+ve gated
GC
0.04
0.42
Fas
GL7
C
B220+ve gated
T1
T2
M
4.24
5.21
68.25
8.44
9.66
69.4
IgM
IgD
D
B220+ve and CD93+ve gated
T3
15.8
15.6
50.2
22.2
11.9
37.7
IgM
CD23
E
B1
CD5
B220
F
IgM-ve gated
Plasmb PC
0.16
1.07
CD138
B220

Figure S3

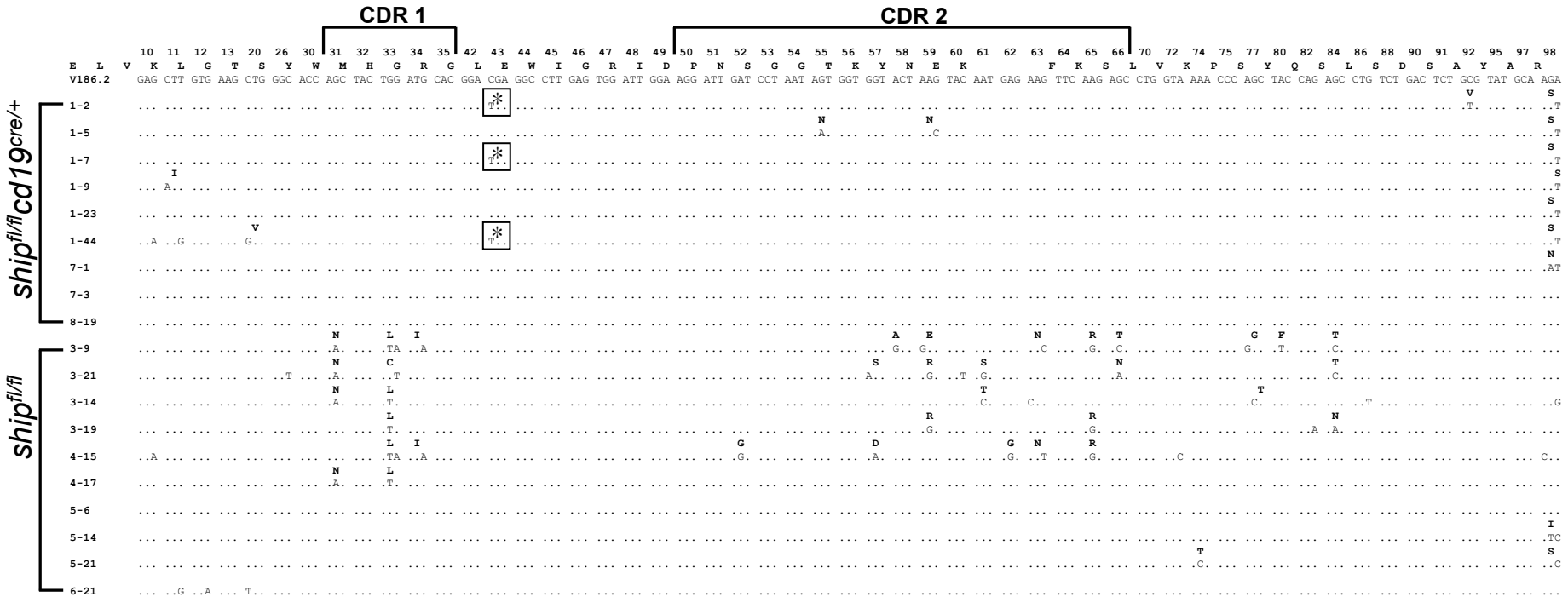
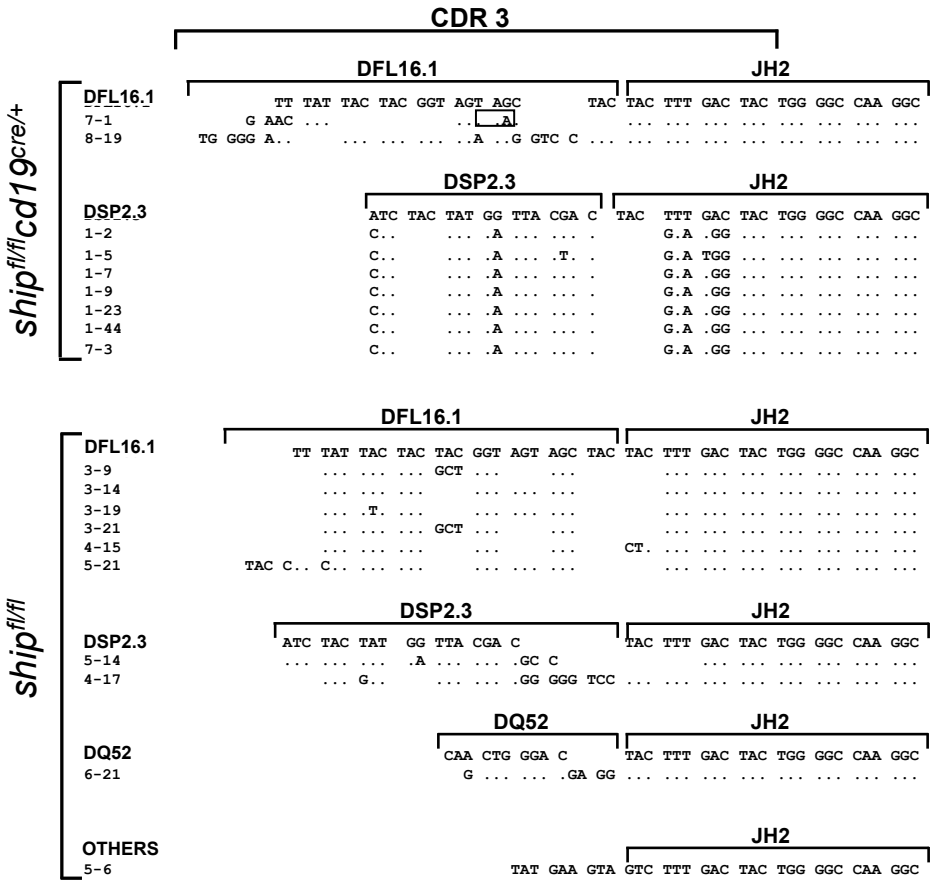

CDR 1
CDR 2
ship fl/fl cd19 cre/+
ship fl/fl
V186.2
1-2
1-5
1-7
1-9
1-23
1-44
7-1
7-3
8-19
3-9
3-21
3-14
3-19
4-15
4-17
5-6
5-14
5-21
6-21

Figure S4


CDR 3
DFL16.1
JH2
ship fl/fl cd19 cre/+
DFL16.1
7-1
8-19
TT TAT TAC TAC GGT AGT AGC TAC
G AAC ...
TG GGG A..
TAC TTT GAC TAC TGG GGC CAA GGC
DSP2.3
JH2
DSP2.3
1-2
1-5
1-7
1-9
1-23
1-44
7-3
ATC TAC TAT GG TTA CGA C
TAC TTT GAC TAC TGG GGC CAA GGC
ship fl/fl
DFL16.1
JH2
DFL16.1
3-9
3-14
3-19
3-21
4-15
5-21
TT TAT TAC TAC TAC GGT AGT AGC TAC
TAC C.. C..
TAC TTT GAC TAC TGG GGC CAA GGC
DSP2.3
JH2
DSP2.3
5-14
4-17
ATC TAC TAT GG TTA CGA C
TAC TTT GAC TAC TGG GGC CAA GGC
DQ52
JH2
DQ52
6-21
CAA CTG GGA C
TAC TTT GAC TAC TGG GGC CAA GGC
JH2
OTHERS
5-6
TAT GAA GTA GTC TTT GAC TAC TGG GGC CAA GGC