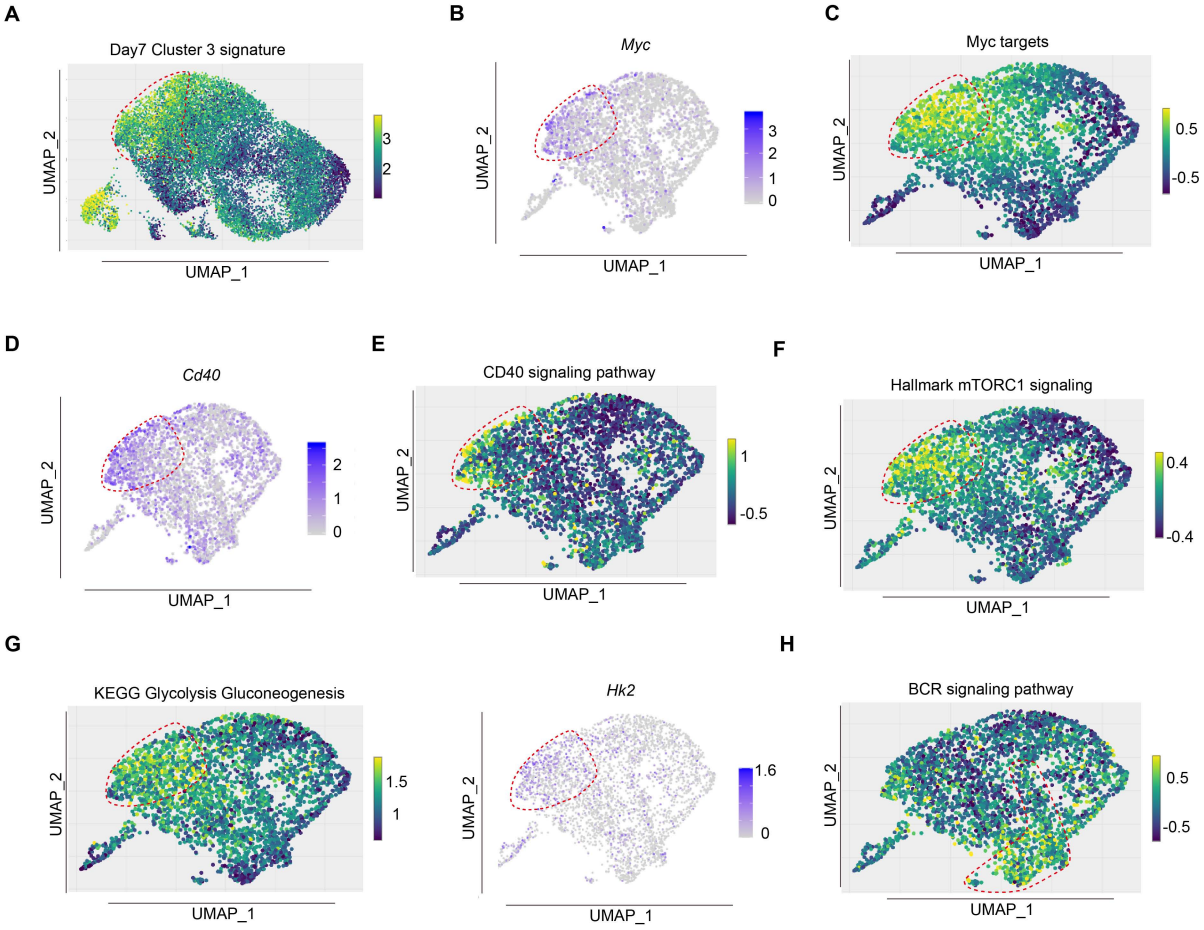
A
Day7 Cluster 3 signature
UMAP_2
UMAP_1
3
2
B
Myc
UMAP_2
UMAP_1
3
2
1
0
C
Myc targets
UMAP_2
UMAP_1
0.5
-0.5
D
Cd40
UMAP_2
UMAP_1
2
1
0
E
CD40 signaling pathway
UMAP_2
UMAP_1
1
-0.5
F
Hallmark mTORC1 signaling
UMAP_2
UMAP_1
0.4
-0.4
G
KEGG Glycolysis Gluconeogenesis
UMAP_2
UMAP_1
1.5
1
H
Hk2
UMAP_2
UMAP_1
1.6
0
BCR signaling pathway
UMAP_2
UMAP_1
0.5
-0.5

Figure S1, Related to Figure 1. CD23^{+} LZ cells showed enrichment for a BCR signaling signature.

(A) The visualize signature score of the top 50 marker genes of day7 cluster 3 (CD23^{+}) GC B cells in day 14 GC B cell dataset, calculated by VISION. Color scaled with signature score. Dashed line showed cluster 1 in day 14 dataset.

(B, C) UMAP plots of the expression of *Myc* (B) and visualize signature scores of MYC targets (C) in day 7 GC B cell dataset. Color scaled for each gene with log normalized expression level or signature score. Dashed line gated region represents cluster 2.

(D, E) UMAP plots of the expression of *Cd40* (D) and visualize signature scores of CD40 signaling (E) in day 7 GC B cell dataset. Color scaled for each gene with log normalized expression level or signature score. Dashed line gated region represents cluster 2.

(F) Visualize signature scores of hallmark mTORC1 signaling calculated by VISION in day 7 GC B cell dataset. Color scaled with signature score. Dashed line gated region represents cluster 2.

(G) Visualize signature scores of KEGG Glycolysis Gluconeogenesis pathway in day 7 GC B cell dataset (left), UMAP plots of the expression of *Hk2* (right). Color scaled for each gene with log normalized expression level or signature score. Dashed line gated region represents cluster 2.

(H) Visualize signature scores of hallmark BCR signaling pathway calculated by VISION in day 7 GC B cell dataset. Color scaled with signature score. Dashed line gated region represents cluster 3.

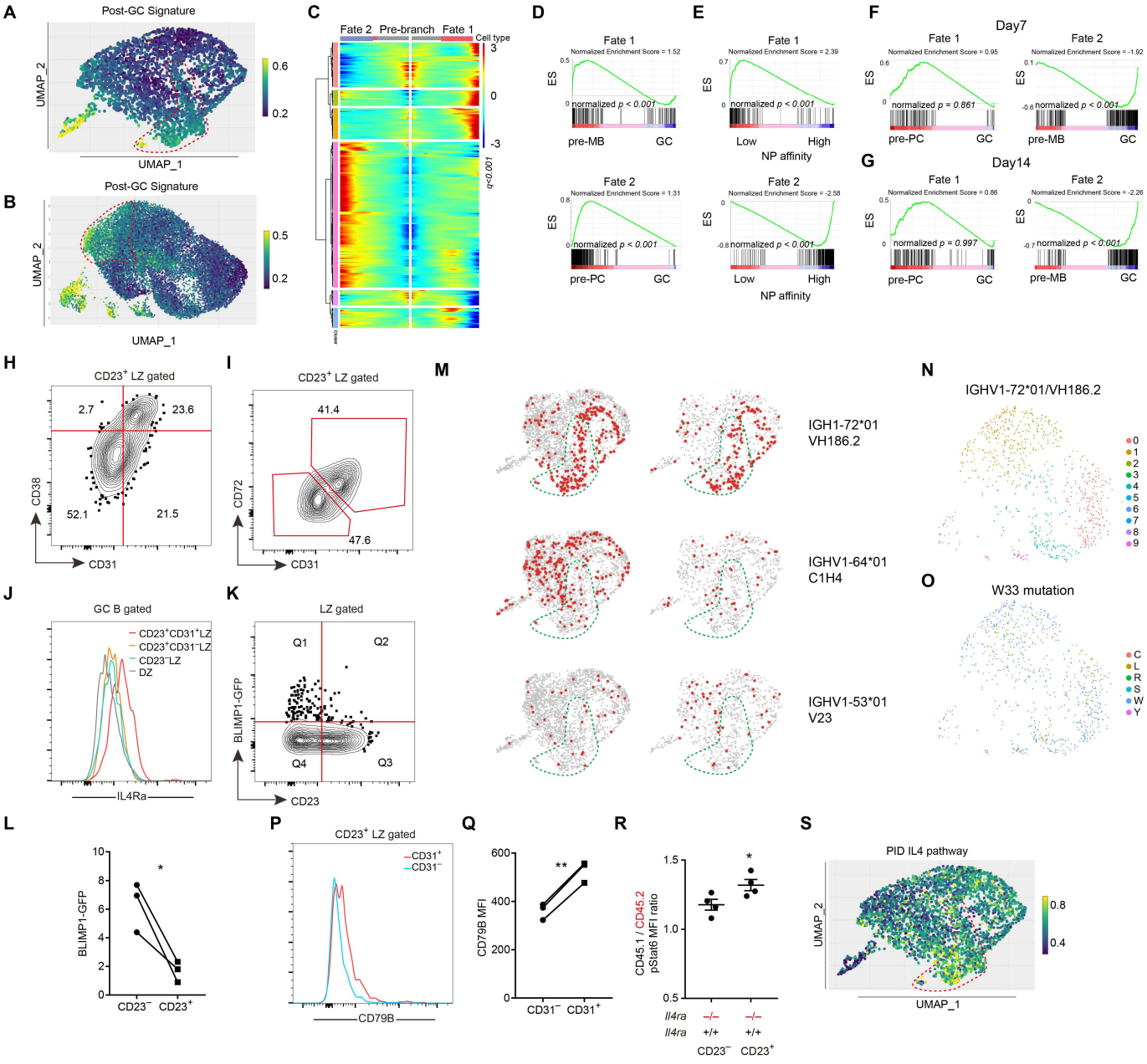
A
Post-GC Signature
UMAP_2
UMAP_1
0.6
0.2
B
Post-GC Signature
UMAP_2
UMAP_1
0.5
0.2
C
Fate 2
Pre-branch
Fate 1
Cell type
3
0
-3
q<0.001
D
Fate 1
Normalized Enrichment Score = 1.52
normalized p < 0.001
pre-MB
GC
Fate 2
Normalized Enrichment Score = 1.31
normalized p < 0.001
pre-PC
GC
E
Fate 1
Normalized Enrichment Score = 2.39
normalized p < 0.001
Low
High
NP affinity
Fate 2
Normalized Enrichment Score = -2.58
normalized p < 0.001
Low
High
NP affinity
F
Day7
Fate 1
Normalized Enrichment Score = 0.95
normalized p = 0.861
pre-PC
GC
Fate 2
Normalized Enrichment Score = -1.92
normalized p < 0.001
pre-MB
GC
G
Day14
Fate 1
Normalized Enrichment Score = 0.86
normalized p = 0.997
pre-PC
GC
Fate 2
Normalized Enrichment Score = -2.26
normalized p < 0.001
pre-MB
GC
H
CD23+ LZ gated
2.7
23.6
52.1
21.5
CD38
CD31
I
CD23+ LZ gated
41.4
47.6
CD72
CD31
M
IGH1-72*01
VH186.2
IGHV1-64*01
C1H4
IGHV1-53*01
V23
N
IGHV1-72*01/VH186.2
0
1
2
3
4
5
6
7
8
9
O
W33 mutation
C
L
R
S
W
Y
J
GC B gated
CD23+CD31+LZ
CD23+CD31−LZ
CD23−LZ
DZ
IL4Ra
K
LZ gated
Q1
Q2
Q4
Q3
BLIMP1-GFP
CD23
L
BLIMP1-GFP
CD23−
CD23+
P
CD23+ LZ gated
CD31+
CD31−
CD79B
Q
CD79B MFI
CD31−
CD31+
R
CD45.1 / CD45.2
pStat6 MFI ratio
Il4ra −/−
Il4ra +/+
Il4ra −/−
Il4ra +/+
CD23−
CD23+
S
PID IL4 pathway
UMAP_2
UMAP_1
0.8
0.4

Figure S2, Related to Figure 2 and 3. Properties of CD23^{+} LZ B cells

(A, B) Visualize signature scores of post-GC (Efnb1^{+} S1pr^{low}) B cell in day 7 GC dataset (A) and day 14 GC dataset (B).

(C) Heatmap of differentially expressed genes between cell fate 1 and cell fate 2 within cluster 2 of day 14 LZ GC B cell dataset by pseudo-time analysis (all genes were listed in Table S4).

(D) GSEA analysis of differentially expressed genes from the two cell fates compared to published pre-memory B cell and pre-plasma cell RNA sequencing datasets (Ise et al., 2018; Wang et al., 2017). Enrichment profiles for cell fate 1 compared with pre-memory (upper) and cell fate 2 compared with pre-plasma cell (lower). ES presents enrichment score.

(E) GSEA analysis of differentially expressed genes from different cell fates compared to the published RNA sequencing datasets of different NP affinity GC B cells (Shinnakasu et al., 2016). Enrichment profiles for cell fate 1 compared with low-affinity (upper) and cell fate 2 compared with high-affinity (lower). ES presents enrichment score.

(F, G) GSEA analysis of differentially expressed genes from the two cell fates in day 7 (F) and day14 (G) datasets compared to published pre-plasma B cell and pre-memory cell RNA sequencing datasets (Ise et al., 2018; Wang et al., 2017). Enrichment profiles for cell fate 1 compared with pre-plasma (left) and cell fate 2 compared with pre-memory B cell (right). ES presents enrichment score.

(H, I) Representative FACS profile displaying gates between CD31 and CD38 (H), or CD31 and CD72 (I) in CD23^{+} LZ GC B cells. Data represent three independent experiments, with at least three mice per experiment.

(J) Representative histograms of IL4Ra in DZ (B220^{+} CD95^{hi} GL7^{hi} CXCR4^{hi} CD86^{lo}), CD23^{-} LZ (B220^{+} CD95^{hi} GL7^{hi} CXCR4^{lo} CD86^{hi}), CD31^{-}CD23^{+} LZ and CD31^{+}CD23^{+} LZ

GC B cells 12-14 days after immunization. Data represent three independent experiments, with at least three mice per experiment.

(K, L) *Blimp1*$^{Gfp/+}$ reporter mice were immunized with NP-haptenated antigen. (K) Representative FACS profile displaying gates between CD23 and BLIMP1 in LZ GC B cells. (L) Percentages of BLIMP1^{+} cells in CD23^{-} and CD23^{+} LZ GC B cells on day 13 after immunization. Each symbol represents one mouse, and each line represents the same mouse. * P<0.05.

(M) The usage of different NP specific heavy chain gene in each cluster of two day 7 GC B samples.

(N) The usage of IGHV1-72*01 in each cluster in day 14 dataset.

(O) Distribution of IGHV1-72*01 W33L mutation in each cluster in day 14 dataset.

(P, Q) Representative histograms (P) and MFI (Q) of CD79b in CD31^{+} or CD31^{-} CD23^{+}LZ GC B cells. Each symbol represents one mouse, and each line represents the same mouse. One of two experiments with similar result is shown. ** P<0.01.

(R) Geometric mean fluorescence intensity (MFI) of pStat6 in CD23$^{–}$ or CD23^{+} *Il4ra*$^{+/+}$ GC B cells relative to the MFI of *Il4ra*$^{-/-}$ cells of the same type in CD45.1 *Il4ra*$^{+/+}$ / CD45.2 *Il4ra*$^{-/-}$ mixed BM chimeras. * P<0.05.

(S) Visualize signature score of PID IL4 pathway calculated by VISION in day 7 GC B cell dataset. Color scaled with signature score.

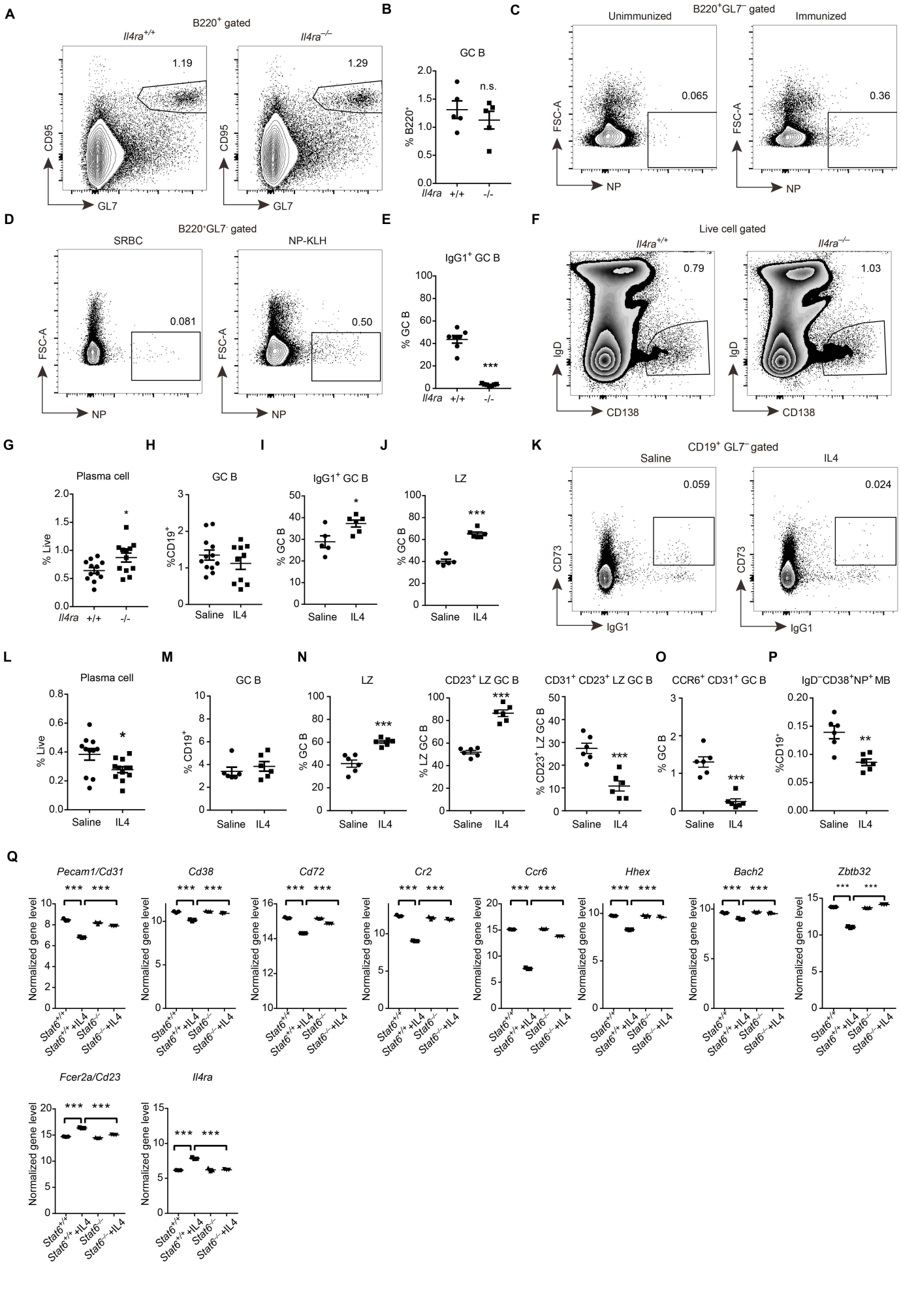
A
B220+ gated
Il4ra+/+
Il4ra−/−
1.19
1.29
CD95
GL7
B
GC B
% B220+
n.s.
Il4ra +/+ -/-
C
B220+GL7− gated
Unimmunized
Immunized
0.065
0.36
FSC-A
NP
D
B220+GL7- gated
SRBC
NP-KLH
0.081
0.50
E
IgG1+ GC B
% GC B
***
F
Live cell gated
0.79
1.03
IgD
CD138
G
Plasma cell
% Live
H
GC B
%CD19+
Saline IL4
I
IgG1+ GC B
J
LZ
K
CD19+ GL7− gated
Saline
IL4
0.059
0.024
CD73
IgG1
L
M
N
CD23+ LZ GC B
% LZ GC B
O
CD31+ CD23+ LZ GC B
% CD23+ LZ GC B
P
CCR6+ CD31+ GC B
IgD−CD38+NP+ MB
Q
Pecam1/Cd31
Cd38
Cd72
Cr2
Ccr6
Hhex
Bach2
Zbtb32
Fcer2a/Cd23
Il4ra
Normalized gene level
Stat6+/+
Stat6+/+ +IL4
Stat6−/−
Stat6−/− +IL4

Figure S3, Related to Figure 4. Influence of IL4RA signaling on GC outputs

(A, B) Representative FACS profiles (A) and frequencies mean ± SEM) (B) of $CD95^{hi}$ $GL7^{hi}$ GC B cells in $B220^{+}$ B cells 13 days after immunization in mice with indicated genotypes. Each symbol represents one mouse. One of two experiments with similar results is shown. n.s., not significant.

(C, D) Representative FACS profiles of NP^{+} memory B cells in $B220^{+}GL7^{-}$ B cells in unimmunized (C) or NP-haptenated antigen immunized (C, D) or SRBC immunized mice (D).

(E) Frequency (mean ± SEM) of $IgG1^{+}$ cells in GC B cells 13 days after immunization in mice with indicated genotypes.

(F, G) Representative FACS profiles (F) and frequencies (mean ± SEM) (G) of IgD^{low} $CD138^{hi}$ plasma cells in splenocytes 13 days after immunization in mice with indicated genotypes. Each symbol represents one mouse. Data are pooled from three independent experiments. *P<0.05.

(H-L) Immunized mice received saline or IL4-αIL4 treatment. (H-J) Frequencies (mean ±SEM) of $CD95^{hi}$ $GL7^{hi}$ GC B cells in $CD19^{+}$ B cells (H), $IgG1^{+}$ cells in GC B cells (I), and LZ cells in GC B cells (J). Each symbol represents one mouse. One of three experiments with similar results is shown. (K) Representative FACS profiles of $IgG1^{+}$ $CD73^{+}$ in $CD19^{+}GL7^{-}$ B cells in mice with indicated treatment. (L) Frequencies (mean ± SEM) of IgD^{low} $CD138^{hi}$ plasma cells in splenocytes in mice with indicated treatment. Each symbol represents one mouse. (I, J) One of three experiments with similar results is shown. (H, L) Data are pooled from three independent experiments. * P<0.05, *** P<0.001.

(M-P) NP-KLH immunized *Aicda*$^{+/+}$ and *Aicda*$^{-/-}$ BM chimeric mice received saline or IL4-αIL4 treatment. (M) Frequencies (mean ±SEM) of $CD95^{hi}$ $GL7^{hi}$ GC B cells in $CD19^{+}$

B cells. (N-P) Frequencies (mean ±SEM) of LZ cells in GC B cells (N left), $CD23^+$ cells in LZ GC B cells (N middle), $CD31^+$ cells in $CD23^+$ LZ GC B cells (N right), $CD31^+CCR6^+$ cells in GC B cells (O), and $IgD^-CD38^+NP^+$ memory B cells in $CD19^+$ B cells (P). Each symbol represents one mouse. Data are pooled from two independent experiments. ** $P<0.01$, *** $P<0.001$.

(Q) Normalized expression of different gene in *Stat6*$^{+/+}$ and *Stat6*$^{-/-}$ primary B cell after IL4 stimulation. Data are from (Mokada-Gopal et al., 2017). *** $P<0.001$ by one-way ANOVA with Sidak multiple comparisons test.

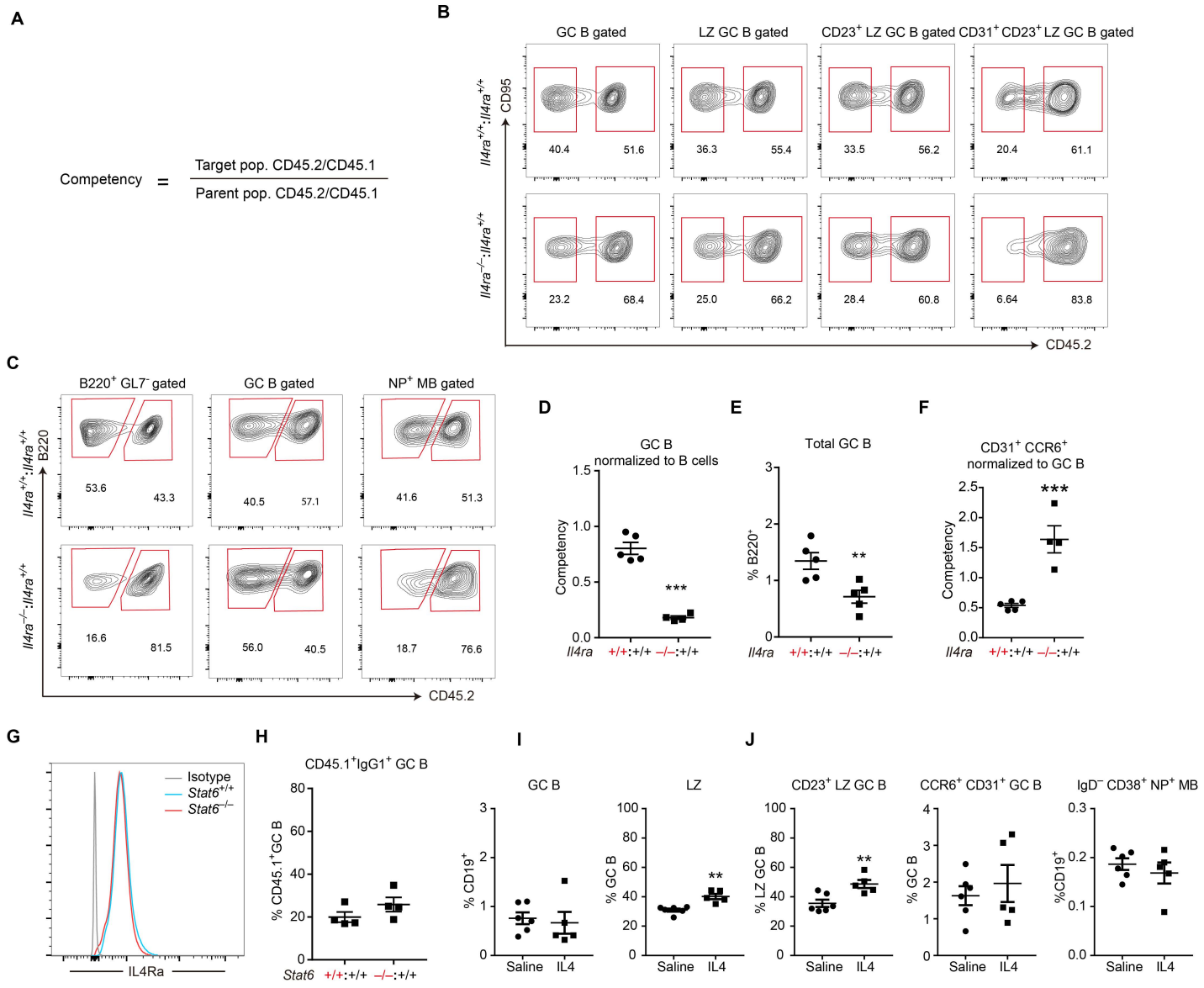
A
Competency = Target pop. CD45.2/CD45.1 / Parent pop. CD45.2/CD45.1
B
GC B gated
LZ GC B gated
CD23+ LZ GC B gated
CD31+ CD23+ LZ GC B gated
Il4ra+/+:Il4ra+/+
Il4ra−/−:Il4ra+/+
CD95
CD45.2
40.4
51.6
36.3
55.4
33.5
56.2
20.4
61.1
23.2
68.4
25.0
66.2
28.4
60.8
6.64
83.8
C
B220+ GL7- gated
GC B gated
NP+ MB gated
B220
53.6
43.3
40.5
57.1
41.6
51.3
16.6
81.5
56.0
40.5
18.7
76.6
D
GC B normalized to B cells
Competency
Il4ra +/+:+/+ −/−:+/+
E
Total GC B
% B220+
F
CD31+ CCR6+ normalized to GC B
Competency
G
Isotype
Stat6+/+
Stat6−/−
IL4Ra
H
CD45.1+IgG1+ GC B
% CD45.1+GC B
Stat6 +/+:+/+ −/−:+/+
I
GC B
LZ
% CD19+
% GC B
Saline IL4
J
CD23+ LZ GC B
CCR6+ CD31+ GC B
IgD− CD38+ NP+ MB
% LZ GC B
% GC B
%CD19+
Saline IL4

Figure S4, Related to Figure 5. IL4RA and STAT6 are intrinsically required for restraining pre-memory B cell development

(A-C) Equation for calculating competitive competencies (A) and representative FACS profiles show gating strategies (B, C) for analysis of the indicated types of mixed BM chimeras.

(D, F) Competency values (mean ± SEM) of GC B cell (D) and $CD31^+$ $CCR6^+$ (F) GC B cell compartment.

(E) Frequencies (mean ± SEM) of total GC B cells.

(G) Representative histograms of IL4RA in $B220^+$ FO B cells in *Stat6*$^{+/+}$ and *Stat6*$^{-/-}$ mice.

(H) Frequencies (mean ± SEM) of $CD45.1^+IgG1^+$ cell in $CD45.1^+$ GC B cells.

(I, J) Immunized *Stat6*$^{-/-}$ mice received IL4-αIL4 complex or saline intravenously once per day on day 11, 12, 13 post immunization and were analyzed on day 14. (I) Frequencies (mean ± SEM) of GC B cells in $CD19^+$ B cells (I left) and LZ cells in GC B cells (I right). (J) Frequencies (mean ± SEM) of $CD23^+$ cells in LZ GC B cells (J left), $CD31^+CCR6^+$ cells in GC B cells (J middle), and IgD^- $CD38^+NP^+$ memory B cells in $CD19^+$ B cells (J left). Each symbol represents one mouse. One of two experiments with similar result is shown. ** P<0.01.

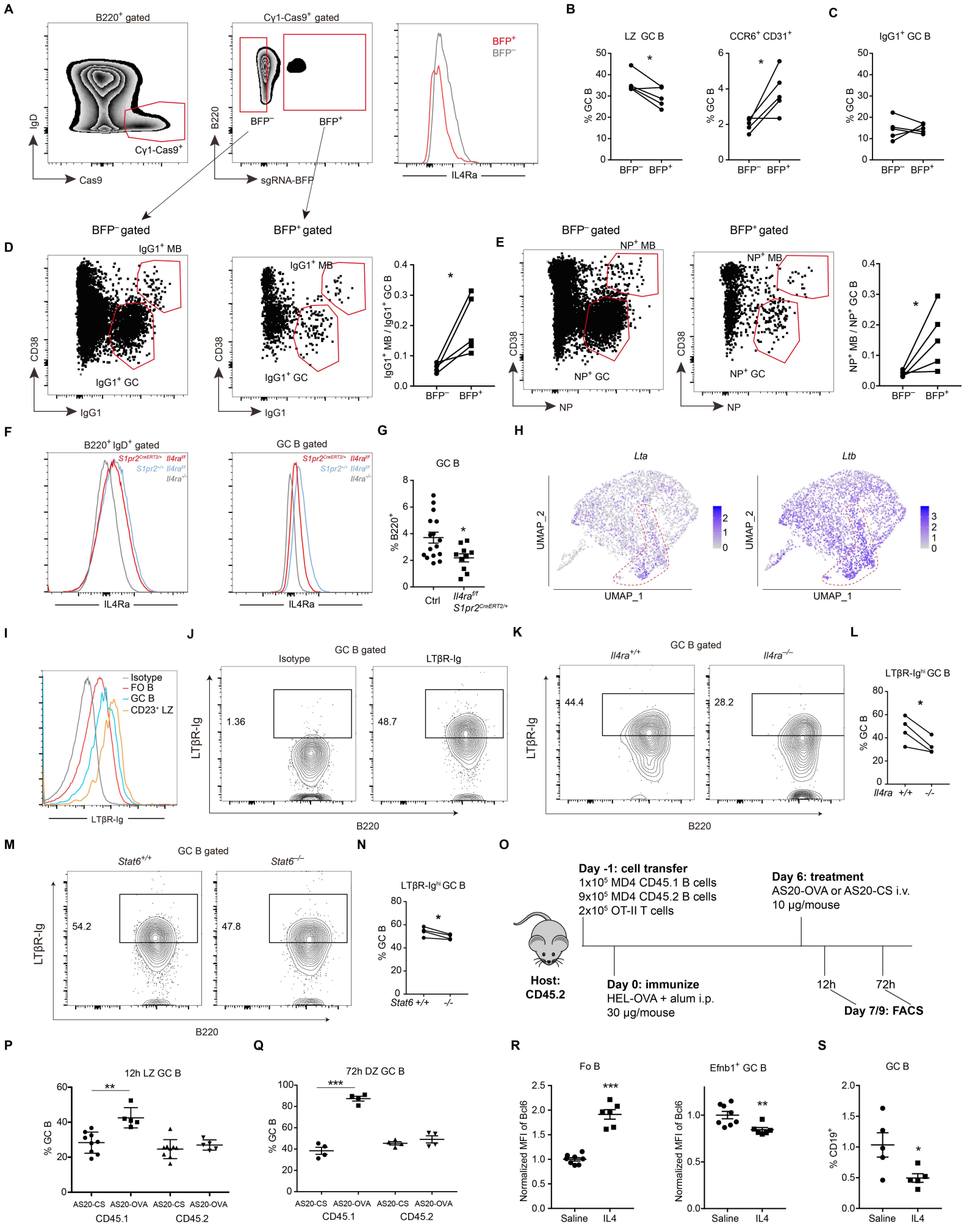
A
B220+ gated
IgD
Cγ1-Cas9+
Cas9
Cγ1-Cas9+ gated
B220
BFP−
BFP+
sgRNA-BFP
BFP+
BFP−
IL4Ra
B
LZ GC B
% GC B
BFP− BFP+
CCR6+ CD31+
C
IgG1+ GC B
BFP− gated
BFP+ gated
D
IgG1+ MB
CD38
IgG1+ GC
IgG1
IgG1+ MB / IgG1+ GC B
E
NP+ MB
NP+ GC
NP
NP+ MB / NP+ GC B
F
B220+ IgD+ gated
S1pr2CreERT2/+ Il4raf/f
S1pr2+/+ Il4raf/f
Il4ra−/−
GC B gated
IL4Ra
G
GC B
% B220+
Ctrl
Il4raf/f S1pr2CreERT2/+
H
Lta
Ltb
UMAP_2
UMAP_1
I
Isotype
FO B
GC B
CD23+ LZ
LTβR-Ig
J
GC B gated
Isotype
LTβR-Ig
1.36
48.7
B220
K
Il4ra+/+
Il4ra−/−
44.4
28.2
L
LTβR-Ighi GC B
Il4ra +/+ -/-
M
Stat6+/+
Stat6−/−
54.2
47.8
N
LTβR-Ighi GC B
Stat6 +/+ -/-
O
Day -1: cell transfer
1x10^5 MD4 CD45.1 B cells
9x10^5 MD4 CD45.2 B cells
2x10^5 OT-II T cells
Day 6: treatment
AS20-OVA or AS20-CS i.v.
10 µg/mouse
Host:
CD45.2
Day 0: immunize
HEL-OVA + alum i.p.
30 µg/mouse
12h
72h
Day 7/9: FACS
P
12h LZ GC B
AS20-CS AS20-OVA AS20-CS AS20-OVA
CD45.1
CD45.2
Q
72h DZ GC B
R
Fo B
Normalized MFI of Bcl6
Saline IL4
Efnb1+ GC B
S
GC B
% CD19+

Figure S5. Intrinsic IL4Ra requirement in GC B cells, LTα1β2 expression by CD23^{+} LZ cells, and antigen-loading of GC B cells using AS20-OVA.

(A-E) Conditional knock out *Il4ra* by Cas9-mediated gene targeting. Cas9-GFP expression is driven by *Cγ1*$^{Cre/+}$. IL4Ra-sgRNA positive cells were marked with BFP. Immunized mice were analyzed on day 14. (A, D, E) Gating strategy and representative FACS profiles of B220^{+} IgD^{-}Cas9-GFP^{+} sgRNA-BFP^{+} or $^{-}$ cells further gated on CD38^{+} IgG1^{+} memory B cell and CD38^{-} IgG1^{+} GC B cells. Representative histograms of IL4RA in B220^{+} IgD^{-} Cas9-GFP^{+} sgRNA-BFP^{+} or $^{-}$ cells (A right). Ratio of indicated memory B cell and GC B cell gates shown on right in D and E. (B, C) Frequencies amongst GC B cells (mean ± SEM) of BFP^{-} and BFP^{+} total LZ cells (B left), CD31^{+}CCR6^{+} LZ cells (B right), and IgG1^{+} GC B cells (C) gated as in A. Each symbol represents one mouse. One of two experiments with similar result is shown. *P<0.05 by paired student's t-test.

(F, G) Control and *S1pr2*$^{CreERT2/+}$ *Il4ra*$^{f/f}$ mice were immunized, treated with tamoxifen and analyzed on day 13. Representative histograms of IL4RA in B220^{+} IgD^{+} follicular B cell and B220^{+} IgD^{-}CD38^{-} GL7^{+} GC B cells (F). Frequencies (mean ± SEM) of B220^{+} IgD^{-} CD38^{-} GL7^{+} GC B cells in B220^{+} B cells (G). Each symbol represents one mouse. Data are pooled from three independent experiments. * P<0.05.

(H) UMAP plots of *Lta* and *Ltb* expression in day 7 GC B cell dataset. Color scaled for each gene with log normalized expression level.

(I) Representative histograms of LTα1β2 expression in the B220^{+} FO B cells, the CD95^{hi} GL7^{hi} GC B cells, CD23^{+} CXCR4^{lo} CD86^{hi} LZ GC B cells from B6 mice, determined by staining with LTbR-Ig. Data represent two independent experiments, with at least three mice per experiment.

(J) Representative FACS profiles of LTα1β2^{+} detected using LTβR-Ig or isotype control in CD95^{hi} GL7^{hi} GC B cells. Data represent one independent experiment with at least three mice per experiment.

(K, L) Mixed 50:50 BM chimeras were made with CD45.1 wild-type (*Il4ra*$^{+/+}$) and CD45.2 *Il4ra*$^{-/-}$ BM cells. Representative FACS profiles (K) and frequencies of LTα1β2^{+} cells in CD95^{hi} GL7^{hi} GC B cells (L) with indicated genotypes in chimeras. Each symbol represents one mouse, and each line represents the same mouse. * P<0.05.

(M, N) Mixed 50:50 BM chimeras were made with CD45.1 wild-type (*Stat6*$^{+/+}$) and CD45.2 *Stat6*$^{-/-}$ BM cells. Representative FACS profiles (M) and frequencies of LTα1β2^{+} cells in CD95^{hi} GL7^{hi} GC B cells (N) with indicated genotypes in chimeras. Each symbol represents one mouse, and each line represents the same mouse. * P<0.05.

(O-Q) AS20-OVA stimulation scheme diagram (O). Frequencies (mean ± SEM) of LZ B cells in GC 12h post indicated treatment (P) or DZ B cells in GC 72h post treatment (Q). Each symbol represents one mouse.

(R) Immunized mice received IL4-αIL4 complex or saline intravenously once per day on day 11, 12, 13 post immunization and were analyzed on day 14. Normalized geometric mean fluorescence intensity (MFI) of Bcl6 in CD19^{+}IgD^{+} follicular B cells (R left) and CD19^{+}IgD^{-}Fas^{hig}GL7^{hig}Efnb1^{+} mature GC B cells. ***P<0.01, ***P<0.001.*

(S) Immunized mice received IL4-αIL4 complex or saline intravenously once per day on day 10, 11, 12, 13 post immunization and were analyzed on day 14. Frequencies (mean ±SEM) of CD95^{hi} GL7^{hi} GC B cells in CD19^{+} B cells.

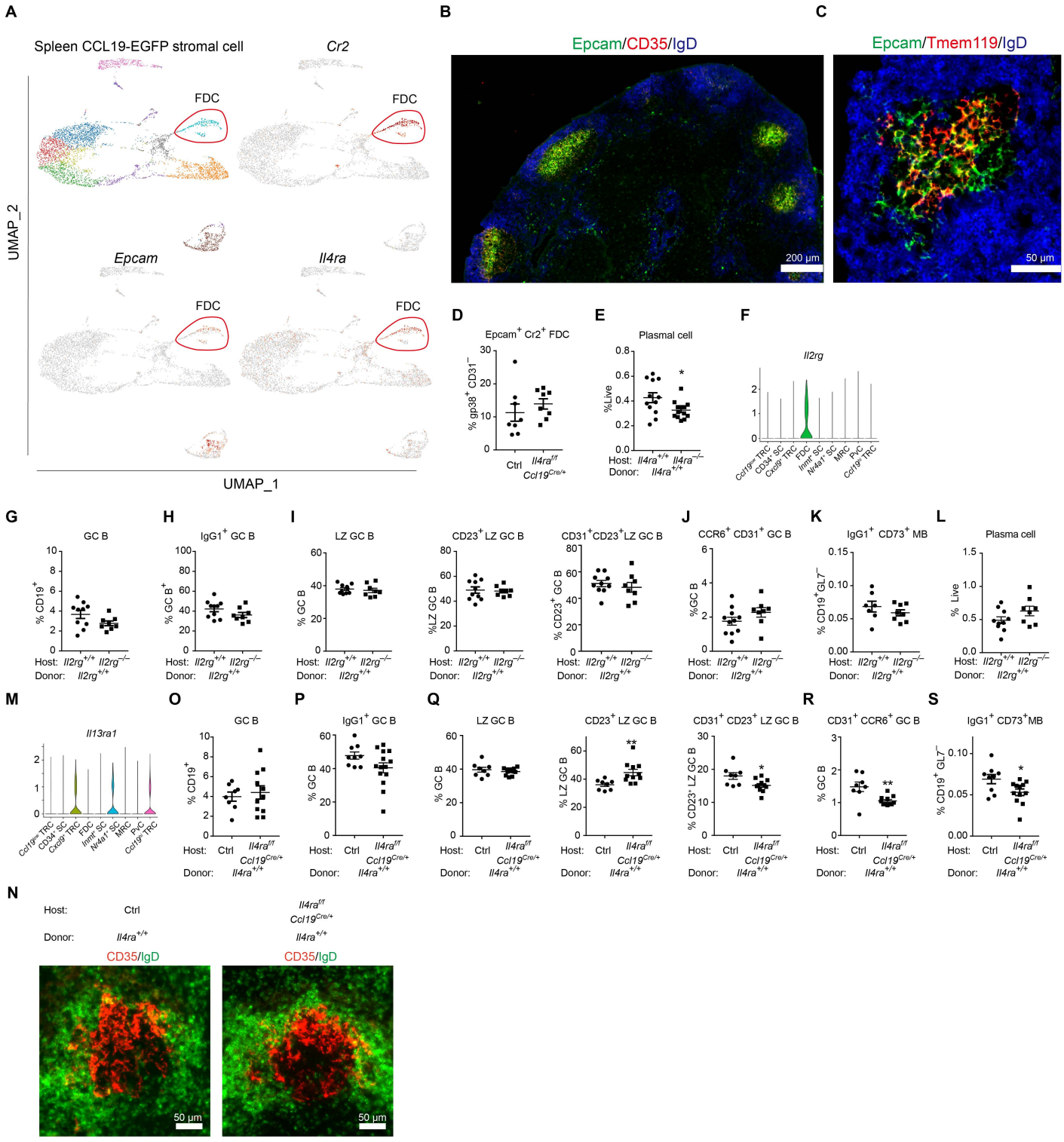
A
Spleen CCL19-EGFP stromal cell
Cr2
Epcam
Il4ra
FDC
UMAP_2
UMAP_1
B
Epcam/CD35/IgD
200 μm
C
Epcam/Tmem119/IgD
50 μm
D
Epcam+ Cr2+ FDC
% gp38+ CD31−
Ctrl
Il4ra f/f Ccl19Cre/+
E
Plasmal cell
%Live
Host: Il4ra+/+ Il4ra−/−
Donor: Il4ra+/+
F
Il2rg
Ccl19low TRC
CD34+ SC
Cxcl9+ TRC
FDC
Inmt+ SC
Nr4a1+ SC
MRC
PvC
Ccl19+ TRC
G
GC B
% CD19+
Host: Il2rg+/+ Il2rg−/−
Donor: Il2rg+/+
H
IgG1+ GC B
% GC B+
I
LZ GC B
CD23+ LZ GC B
CD31+CD23+LZ GC B
J
CCR6+ CD31+ GC B
%GC B
K
IgG1+ CD73+ MB
% CD19+GL7−
L
Plasma cell
% Live
M
Il13ra1
O
GC B
% CD19+
Host: Ctrl Il4ra f/f Ccl19Cre/+
Donor: Il4ra+/+
P
IgG1+ GC B
% GC B
Q
LZ GC B
CD23+ LZ GC B
% LZ GC B
R
CD31+ CD23+ LZ GC B
% CD23+ LZ GC B
CD31+ CCR6+ GC B
S
IgG1+ CD73+MB
% CD19+ GL7−
N
Host: Ctrl Il4ra f/f Ccl19Cre/+
Donor: Il4ra+/+
CD35/IgD
50 μm

Figure S6, Related to Figure 6. IL4RA and EPCAM are expressed by the FDCs in spleen and LNs.

(A) Expression of *Cr2*, *Il4ra*, and *Epcam* projected on UMAP plots. Data are from the published dataset (Cheng et al., 2019).

(B) Representative FDC image in mesenteric LNs co-stained with EPCAM, CD35 and IgD. Sections are representative of multiple LNs from 3 mice. Scale bar, 200 μm.

(C) Representative FDC image in mesenteric LNs co-stained with EPCAM, Tmem119 and IgD. Sections are representative of multiple LNs from 3 mice. Scale bar, 50 μm.

(D) Frequencies (mean ± SEM) of $CR2^+$ $Epcam^+$ FDC in $CD45^-$ $CD31^-$ $gp38^+$ stromal cell 13 days after immunization in mice with indicated genotypes. Each symbol represents one mouse. Data are pooled from two independent experiments.

(E) Frequencies (mean ± SEM) of IgD^{low} $CD138^{hi}$ plasma cell in splenocytes 13 days after immunization in reversed chimeras. Each symbol represents one mouse. Data are pooled from three independent experiments. * P<0.05.

(F) Violin plots of expression of *Il2rg* in different stromal cell subsets.

(G-L) Reverse chimaeras were made by reconstituting irradiated $Il2rg^{+/+}$ and $Il2rg^{-/-}$ mice with wild-type ($Il2rg^{+/+}$) BM. Frequencies (mean ± SEM) of $CD95^{hi}$ $GL7^{hi}$ GC B cells in $CD19^+$ B cells (G), $IgG1^+$ cells in GC B cells (H), LZ cells in GC B cells (I left), $CD23^+$ cells in LZ GC B cells (I middle), $CD31^+$ cells in $CD23^+$ LZ GC B cells (I right) and $CCR6^+$ $CD31^+$ cells (J) in GC B cells 13 days after immunization. (K) Frequencies (mean ± SEM) of $IgG1^+$ $CD73^+$ memory cells in $CD19^+$ $GL7^-$ non-GC B cells. (L) Frequencies (mean ± SEM) of IgD^{low} $CD138^{hi}$ plasma cell in splenocytes. Each symbol represents one mouse. Data are pooled from two independent experiments.

(M) Violin plots of expression of *Il13ra1* in different stromal cell subsets.

(N-S) Reverse chimaeras were made by reconstituting irradiated *Il4ra*$^{+/+}$ and *Ccl19*$^{Cre/+}$ *Il4ra*$^{f/f}$ mice with wild-type (*Il4ra*$^{+/+}$) BM. Representative FDC images in the spleen co-stained with CD35 and IgD. Sections are representative of multiple spleens from 3 mice. Scale bar, 50 μm (N). Frequencies (mean ± SEM) of CD95^{hi} GL7^{hi} GC B cells in CD19^{+} B cells (O), IgG1^{+} cells in the GC B cells (P), CXCR4^{lo} CD86^{hi} LZ cells in GC B cells (Q left), CD23^{+} cells in LZ GC B cells (Q middle), CD31^{+} cells in CD23^{+} LZ GC B cells (Q right), and CD31^{+}CCR6^{+} cells in the GC B cells (R), and CD73^{+} IgG1^{+} memory B cell in CD19^{+}GL7^{-} B cells (S) 13 days after NP-KLH immunization. Each symbol represents one mouse. Data are pooled from three independent experiments. ** P<0.01, * P<0.05.

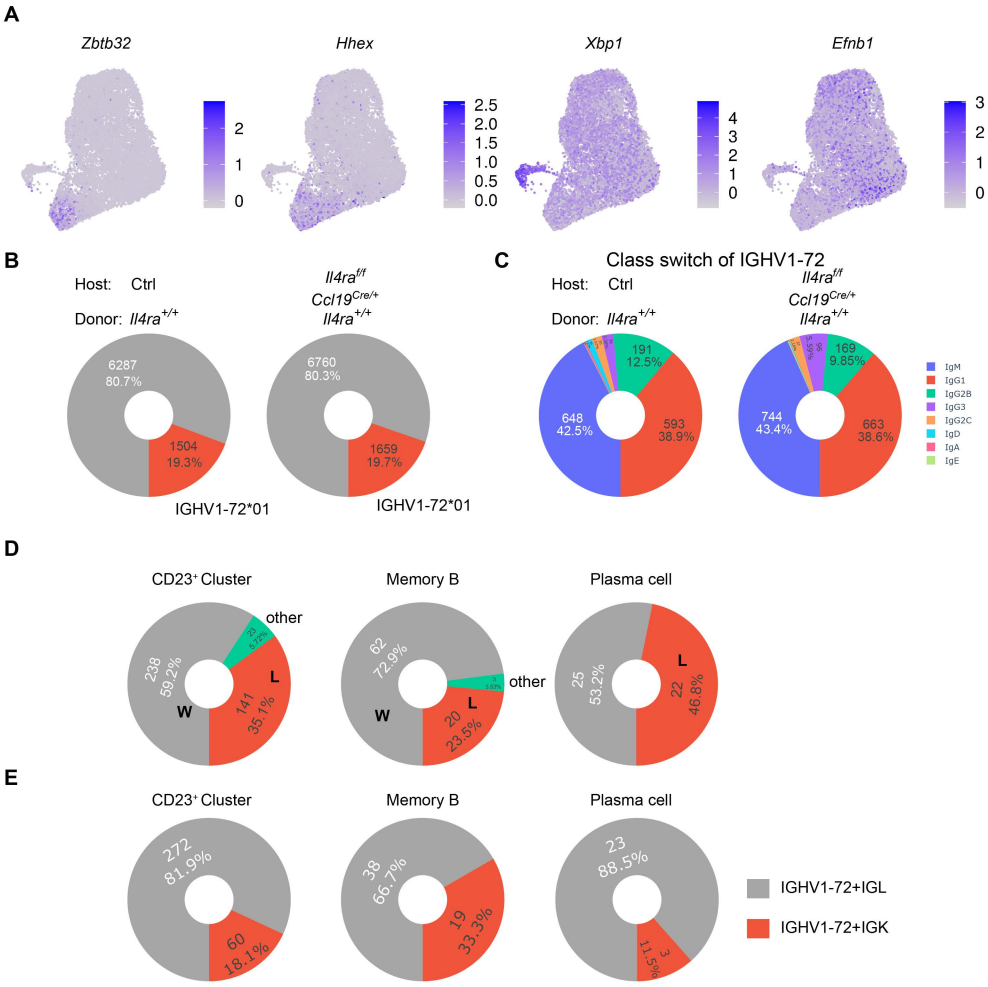
A
Zbtb32
2
1
0
Hhex
2.5
2.0
1.5
1.0
0.5
0.0
Xbp1
4
3
2
1
0
Efnb1
3
2
1
0
B
Host: Ctrl
Donor: Il4ra+/+
6287
80.7%
1504
19.3%
IGHV1-72*01
Il4raf/f
Ccl19Cre/+
Il4ra+/+
6760
80.3%
1659
19.7%
IGHV1-72*01
C
Class switch of IGHV1-72
Host: Ctrl
Donor: Il4ra+/+
191
12.5%
648
42.5%
593
38.9%
Il4raf/f
Ccl19Cre/+
Il4ra+/+
169
9.85%
744
43.4%
663
38.6%
IgM
IgG1
IgG2B
IgG3
IgG2C
IgD
IgA
IgE
D
CD23+ Cluster
other
238
59.2%
W
141
35.1%
L
Memory B
62
72.9%
W
other
20
23.5%
L
Plasma cell
25
53.2%
22
46.8%
L
E
CD23+ Cluster
272
81.9%
60
18.1%
Memory B
38
66.7%
19
33.3%
Plasma cell
23
88.5%
3
11.5%
IGHV1-72+IGL
IGHV1-72+IGK

Figure S7, Related to Figure 7. scVDJ analysis of stroma cell IL4Ra-deficient mice.

(A-E) Reverse chimaeras were made by reconstituting irradiate *Il4ra*$^{+/+}$ and *Ccl19*$^{Cre/+}$ *Il4ra*$^{f/f}$ mice with wild-type (*Il4ra*$^{+/+}$) BM. Single cell RNA sequencing and single cell V(D)J sequencing was performed on B220^{+} CD95^{hi} GL7^{hi} IgD^{-} cells sorted 14 post-immunization. Contaminating naïve B cells and non-B cells were filtered during the data analysis.

(A) UMAP plots of the expression of memory B cell markers (*Zbtb32* and *Hhex*), plasma cell marker (*Xbp1*), and GC B cell marker (*Efnb1*). Color scaled for each gene with log normalized expression level.

(B) Pie chart shows the usage of IGHV1-72*01 in different conditions.

(C) Pie chart shows the immunoglobulin isotypes of IGHV1-72*01 in different conditions.

(D) Pie chart shows the W33L mutation in different cell types. Control and stomal IL4Ra-deficient samples were pooled together.

(E) Pie chart shows the IGHV1-72*01 coupled light chain frequency in different cell types. Control and stomal IL4Ra-deficient samples were pooled together.