SUPPLEMENTAL MATERIAL

Zaretsky et al., https://doi.org/10.1084/jem.20171129

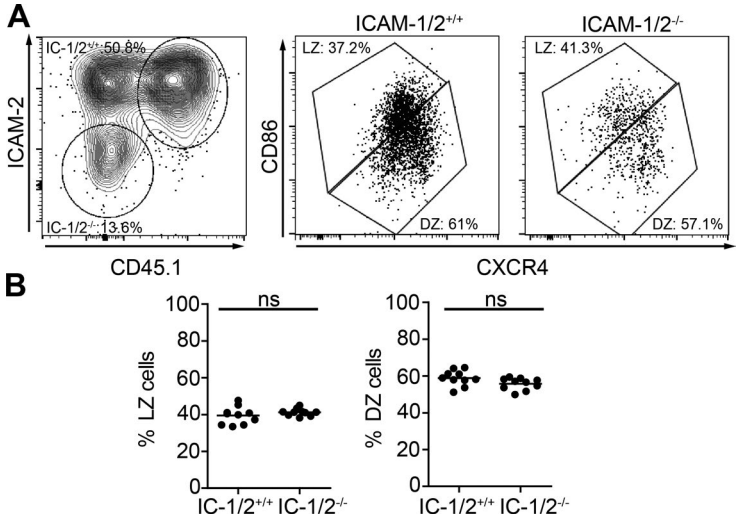


Figure S1. **B cell ICAMs are not required for normal distribution of antigen-specific B cells between the GC light and dark zone.** (A) Representative flow cytometric plots depicting gating strategy for light zone (LZ) and dark zone (DZ) B cells among adoptively transferred CD45.1$^+$ IC-1/2$^{+/+}$ and IC-1/2$^{-/-}$ B1-8^{hi} B cells in host mice, 7 d after immunization with NP-OVA. (B) Quantification of the light and dark zone distribution for each cell type is shown in the graph. Data represent two independent experiments with nine mice total for each condition. ns, not significant.

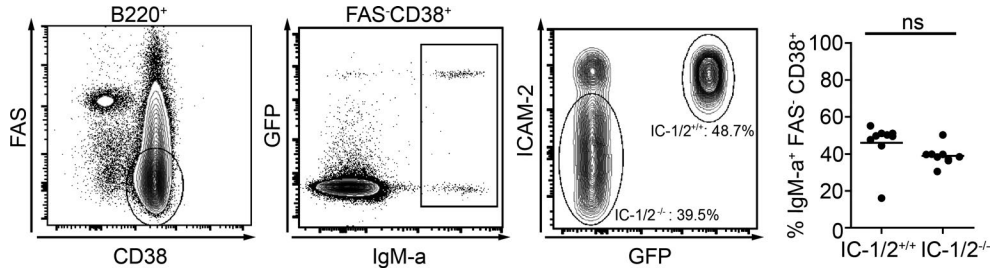


Figure S2. **B cell ICAMs are not required for homing of follicular B cells to lymph nodes.** Representative flow cytometric plots depicting gating strategy for analysis of adoptively transferred follicular IC-1/2$^{+/+}$ and IC-1/2$^{-/-}$ B1-8^{hi} IgM-a$^+$ B cells. Transferred IgM-a$^+$ B1-8^{hi} B cells that do not express activation markers (FAS$^-$ CD38$^+$) represent the non-NP-specific (mostly Igκ^+) B cells that did not participate in the immune response and competition (endogenous in WT hosts B cells express IgM-b). These cells represent the initial ratio among the transferred cells before immunization and GC formation. To clearly detect each cell population type, we transferred into WT mice GFP$^+$ ICAM-1/2$^{+/+}$ B1-8^{hi} and ICAM-1/2$^{-/-}$ B1-8^{hi} B cells before immunizing the mice with NP-OVA. Naive follicular B cells were gated as non-GC cells (FAS$^-$ CD38$^+$ IgM-a$^+$, comprising more than 90% Igκ^+ B cells). ICAM-1/2$^{+/+}$ B cells were detected by GFP expression, and ICAM-1/2$^{-/-}$ B cells were detected as transferred follicular B cells that lack GFP expression. The graph shows quantification of adoptively transferred non-GC B cells. Each dot in the graphs represents a single mouse; lines represent the mean. Graphs are representative of three independent experiments with eight mice total for each condition or time point. ns, not significant.

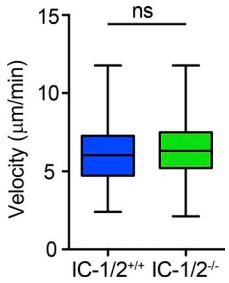
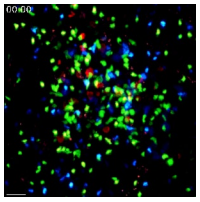
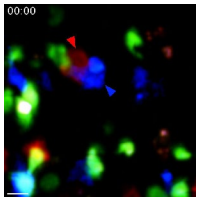


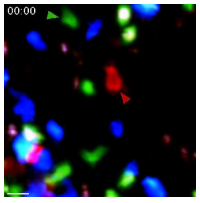
Figure S3. **B cell ICAMs are not required for B cell motility.** Automated analysis of the velocity of IC-1/2$^{+/+}$ CFP^{+} B1-8^{hi} B cells and IC-1/2$^{-/-}$ CFSE-labeled B1-8^{hi} within popliteal LNs 2 d after immunization with NP-OVA. Each bar in the graph represents the mean velocity of ∼100 B cells; lines represent the SD. Data represent two to three independent experiments with a total of three mice. ns, not significant.



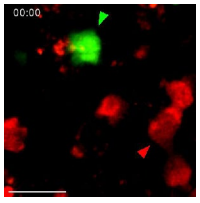
Video 1. **Congregation of Tfh cells and B cells at the T–B border in response to immunization.** Dynamics of DsRed^{+} OT-II T cells (red), CFP^{+} ICAM-1/2$^{+/+}$ B1-8^{hi} B cells (blue), and ICAM-1/2$^{-/-}$ CFSE-labeled B1-8^{hi} B cells (green) at the border between the B and T zones of a popliteal lymph node, 2 d after immunization with NP-OVA. Bar, 20 µm.



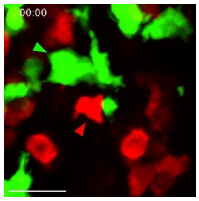
Video 2. **Tfh cell interaction with ICAM-1/2$^{+/+}$ B cell.** The video shows a magnification of a small area in Video 1. Note that Tfh cell (red arrowhead) interacts with the trailing edge of the ICAM-1/2$^{+/+}$ B1-8^{hi} B cell (blue arrowhead) for the entire length of the video. Bar, 5 µm.



Video 3. **Tfh cell interaction with ICAM-1/2$^{-/-}$ B cell.** The video shows a magnification of a small area in Video 1. Note that Tfh cell (red arrowhead) interacts with the trailing edge of the ICAM-1/2$^{-/-}$ B1-8^{hi} B cell (green arrowhead) for few minutes followed by separation of the cells. Bar, 5 µm.



Video 4. **Tfh cell interaction with ICAM-1/2$^{-/-}$ B cells under noncompetitive conditions.** Dynamics of DsRed^{+} OT-II T cell (red), and ICAM-1/2$^{-/-}$ GFP^{+} B1-8^{hi} B cell (green) at the border between the B and T zones of a popliteal lymph node, 2 d after immunization with NP-OVA. Red and green arrowheads indicate a T cell and B cell, respectively. Images were recorded ∼5 h after injection of PBS to hind footpad. Bar, 20 µm.



Video 5. **Tfh cell interaction with ICAM-1/2$^{-/-}$ B cells presenting high levels of pMHCII.** Dynamics of DsRed^{+} OT-II T cell (red), and ICAM-1/2$^{-/-}$ GFP^{+} B1-8^{hi} B cell (green) as in Video 4 ∼5 h after injection of anti–DEC205-OVA to hind foot pad. Red and green arrowheads indicate a T cell and B cell, respectively. Bar, 20 µm.

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

Antigen	Fluorophore	Clone	Manufacturer	Concentration
				µg/ml
B220	BV605	RA3-6B2	BioLegend	0.5
B220	APC–Alexa Fluor 780	RA3-6B2	eBioscience	1
B220	V500	RA3-6B2	BD	1
B220	e450	RA3-6B2	eBioscience	1
CD138	BV421	281-2	BioLegend	1
CD138	BV605	281-2	BioLegend	0.5
CD38	Alexa Fluor 700	90	eBioscience	1
CD4	APC–Alexa Fluor 780	GK1.5	eBioscience	1
CD44	V500	IM7	BD	1
CD45.1	APC–Alexa Fluor 780	A20	eBioscience	0.5
CD45.1	BV421	A20	BioLegend	1
CD45.2	Pacific Blue	A20	BioLegend	2.5
CD62L	Alexa Fluor 700	MEL-14	BioLegend	2.5
CD8	APC–Alexa Fluor 780	53-6.7	eBioscience	1
CD86	PE or APC	GL-1	BioLegend	0.08
CXCR4	BV421	L276F12	BioLegend	1
CXCR5	BV605	L138D7	BioLegend	2.5
F4/80	APC–Alexa Fluor 780	BM8	eBioscience	1
FAS	PE-Cy7	Jo2	BD	0.33
FAS	FITC	Jo2	BD	0.83
GL-7	Alexa Fluor 647	GL7	BioLegend	2.5
GL-7	FITC	FR70	BioLegend	2.5
GL-7	PerCP-Cy5.5	GL7	BioLegend	1
Gr-1	APC–Alexa Fluor 780	RB6-8C5	eBioscience	1
ICAM-1	Alexa Fluor 488	YN1/1.7.4	BioLegend	2.5
ICAM-1	Pacific Blue	YN1/1.7.4	BioLegend	2.5
ICAM-1	PE	YN1/1.7.4	BioLegend	2.5
ICAM-2	Alexa Fluor 647	3C4	BioLegend	2.5
ICAM-2	Alexa Fluor 488	3C4	BioLegend	2.5
IgD	FITC	11-26c (11-26)	eBioscience	1
IgM	eFluor 710	II/41	eBioscience	1
IgM-a	PE	MA-69	BioLegend	2.5
Igλ	PE	RML-42	BioLegend	1
Igλ	APC	RML-42	BioLegend	1