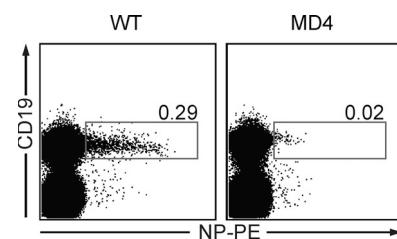
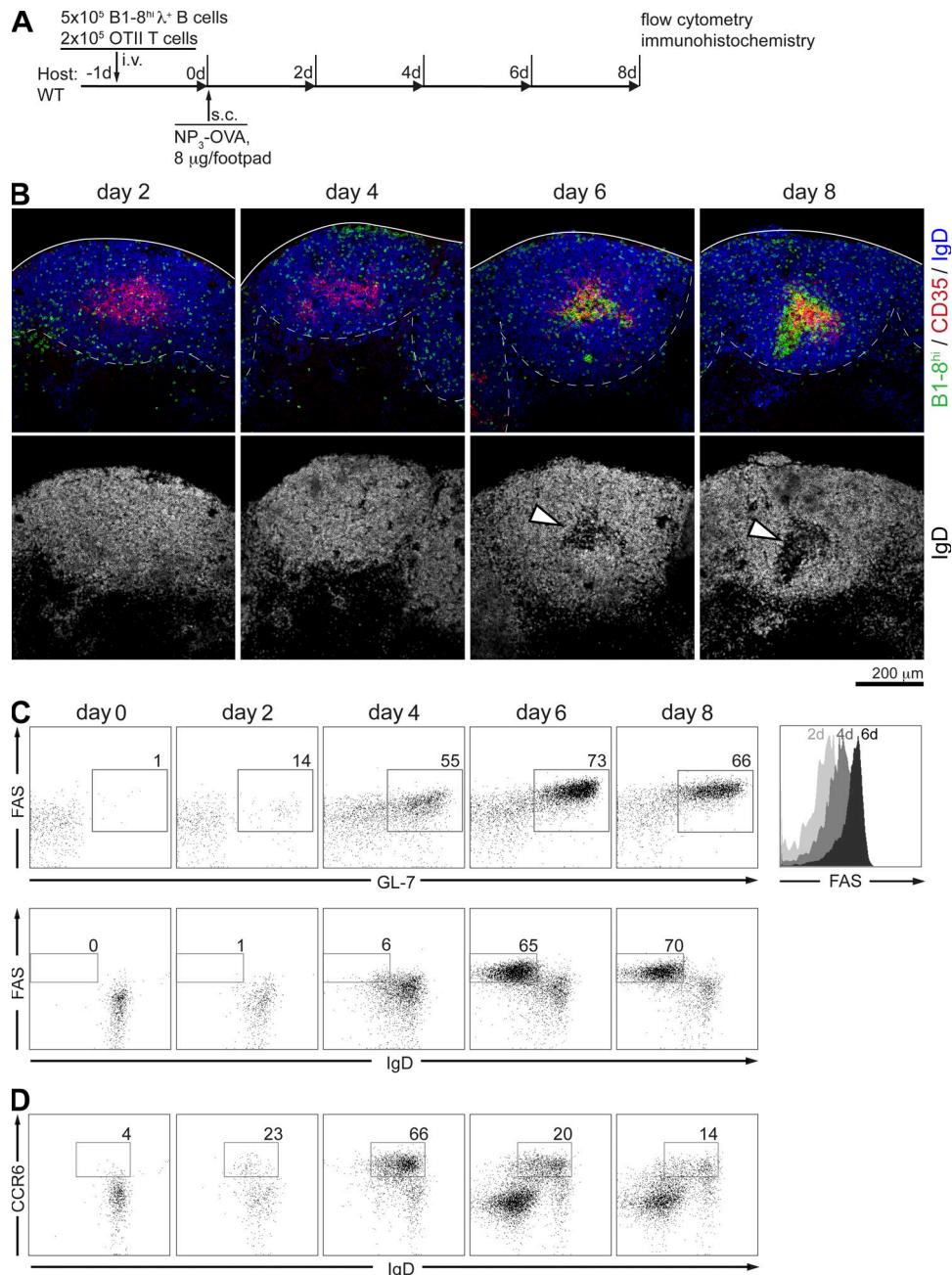


## SUPPLEMENTAL MATERIAL

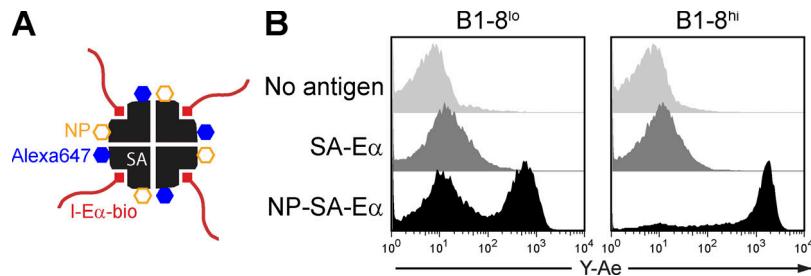
Schwickert et al., <http://www.jem.org/cgi/content/full/jem.20102477/DC1>



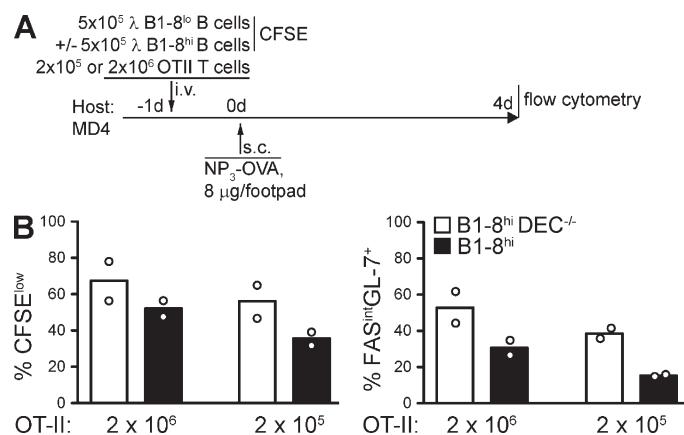
**Figure S1. Low level of NP-PE binding in MD4 mice.** Flow cytometric analysis of NP-PE binding to WT and MD4 B cells. Mice were injected into the footpads and base of tail with a total of 50 µg NP-PE, and draining lymph nodes were extracted 16 h later for flow cytometry analysis. Data are representative of two independent experiments with one to two mice per condition per experiment.



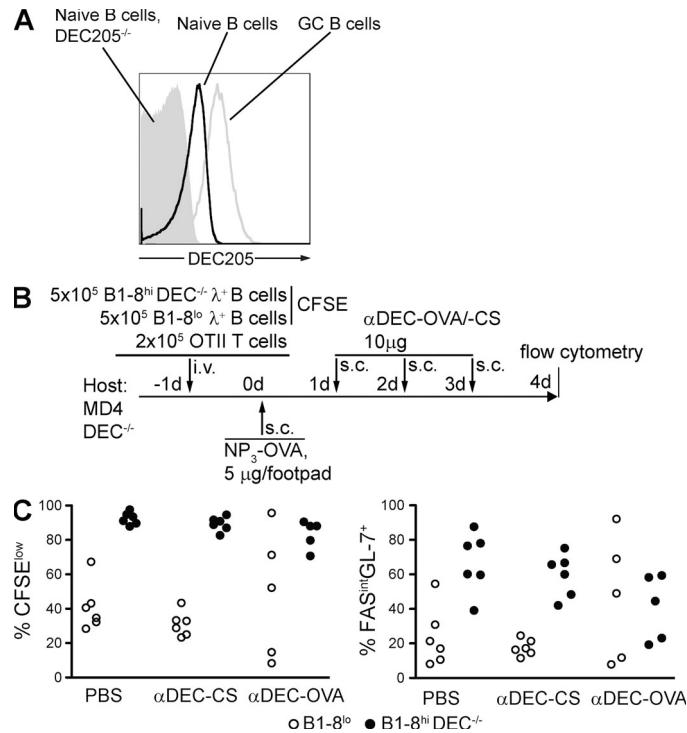
**Figure S2. Kinetics of GC formation.** (A) Diagrammatic representation of the experimental protocol. (B) Histology of B cell follicles in draining lymph nodes at day 0, 2, 4, 6, and 8 after immunization. (top) Representative images of B1-8<sup>hi</sup>CFP<sup>+</sup> B cells (green) in B cell follicles, identified by CD35 (red) and IgD (blue) staining. The dashed line indicates the border between the B cell follicle and T cell zone. (bottom) GCs can be identified by the absence of IgD<sup>+</sup> B cells, indicated by arrowheads. (C) Flow cytometry of transferred B1-8<sup>hi</sup> B cells. (top) FAS/GL-7 expression. (bottom) FAS/IgD expression. (D) Flow cytometry of transferred B1-8<sup>hi</sup> B cells; CCR6/IgD expression. All data are representative of at least two independent experiments.



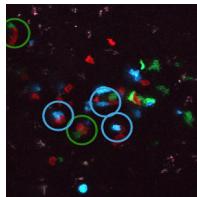
**Figure S3.** Measuring BCR-mediated antigen presentation using NP-E $\alpha$ . (A) Cartoon representing the NP-E $\alpha$  reagent. (B) Flow cytometry plot showing antigen presentation (binding of Y-Ae anti-pMHC antibody) by purified splenic B cells cultured in vitro for 16 h with 1  $\mu$ g/ml NP-SA-E $\alpha$  or control SA-E $\alpha$  reagents. Gated on live CD19 $^+$ Ig $\kappa^-$  cells. Data are representative of three experiments.



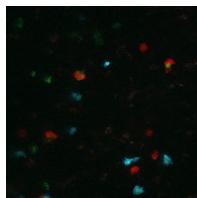
**Figure S4.** Effect of the number of T cell precursors on pre-GC competition. (A) Diagrammatic representation of the experimental layout. (B) Flow cytometric analysis of proliferation (CFSE dilution) and activation (expression of GL-7 and FAS) among transferred B1-8<sup>hi</sup>CFP<sup>+</sup> and B1-8<sup>lo</sup>CD45.1<sup>+</sup> CFSE-labeled B cells in draining lymph nodes 4 d after immunization. The number of T cells transferred is indicated below the chart. Each symbol represents one mouse. Data are from one experiment with two mice per condition.



**Figure S5. Antigen delivery to B cells via DEC205.** (A) Flow cytometric analysis of DEC205 expression on naive and GC B cells. DEC205 $^{-/-}$  B cells are shown as a negative control. Data are representative of multiple experiments. (B) Diagrammatic representation of the experimental protocol in C. (C) Flow cytometric analysis of proliferation (CFSE dilution) and activation (expression of GL-7 and FAS) among transferred B1-8<sup>lo</sup>DEC205 $^{+/+}$ CFP $^{+}$  and B1-8<sup>hi</sup>DEC205 $^{-/-}$ CD45.1 $^{+}$  CFSE-labeled B cells in draining lymph nodes 4 d after immunization. Each symbol represents one mouse. Data are from three independent experiments with one to two mice per condition per experiment.



**Video 1. T cell-B cell contacts in mice treated with anti-DEC205-CS.** Red indicates OT-II T cells, green indicates B1-8<sup>hi</sup> B cells, and cyan indicates B-18<sup>hi</sup>DEC205 $^{-/-}$  B cells. Long-lasting (>10 min) motile conjugates are marked by circles colored according to B cell genotype. This video is shown at 7 frames/s.



**Video 2. T cell-B cell contacts in mice treated with anti-DEC205-OVA.** Red indicates OT-II T cells, green indicates B1-8<sup>hi</sup> B cells, and cyan indicates B-18<sup>hi</sup>DEC205 $^{-/-}$  B cells. Long-lasting (>10 min) motile conjugates are marked by circles colored according to B cell genotype. This video is shown at 7 frames/s.

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

Surface molecule	Fluorochrome	Clone	Manufacturer	Final concentration μg/ml
220	PerCP	RA3-6B2	BD	1.0
CR6	PE	29-2L17	BioLegend	4.0
D19	APC-Cy7	1D3	BD	2.0
CD45	PE	30-F11	eBioscience	1.0
D45.1	APC	A20	BD	1.0
CD45.1	APC-eFluor780	A20	eBioscience	1.0
CD45.2	PE	104	BD	1.0
CD45.2	PerCP-Cy5.5	104	eBioscience	0.5
CD138	PE	281-2	BD	1.0
DEC205	APC	205yekta	eBioscience	0.2
FAS	PE-Cy7	Jo2	BD	0.25
GL-7	Biotin	GL-7	BD	1.0
GL-7	FITC	GL-7	BD	1.0
GL-7	Alexa Fluor 647	GL-7	eBioscience	0.5
I-A <sup>b</sup> (I-Eα <sub>52-68</sub> )	Biotin	Y-Ae	eBioscience	2.5
IgD	Alexa Fluor 647	11-26	eBioscience	2.5
IgG <sub>1</sub>	APC	X56	BD	1.0
Igκ	PE	187.1	BD	0.067
Igλ <sub>1-3</sub>	Conjugated to Alexa Fluor 700	R26-46	BD	0.625
SA	PerCP	NA	BD	0.67
SA	PE	NA	BD	0.5

NA, not applicable.