## SI Materials and Methods

### Cell isolation and sorting.

To isolate DCs spleens digested with Collagenase B as described in SI Materials and Methods. To isolate B and T cells, spleens and Peyer's Patches were mashed through a 12-well tissue disaggregator and strained through a 70-μm filter. Red blood cells were lysed with ACK lysis buffer. To sort splenic DCs, single cell splenocyte suspensions were positively selected for CD11c expressing cells with CD11c microbeads (Miltenyi). DC2s were sorted as $B220^{-}$ $CD11c^{+}$ $MHC\text{-}II^{+}$ $CD172a^{+}$ and segregated based on ESAM expression.

### Immunizations.

Mice were immunized intraperitoneally with $2\times10^{9}$ SRBCs (Colorado Serum Company) and GC reactions were analyzed 8 days after treatment. Anti-SRBC serum titers were measured by ELISA 17 days after immunization as described in SI Materials and Methods. For blockade of Tim-3; mice were treated three times with 250μg of anti-Tim-3 or anti-trinitrophenol (isotype) i.p. every 72 hours beginning a day before SRBC immunization. Mice were immunized intravenously by tail vein injection with $2\times10^{8}$ heat-inactivated Listeria monocytogenes expressing OVA prepared as previously described (79) and analyzed for splenic GC reactions eight days later. For CD25 induction, mice were immunized with SRBCs as described above and analyzed 24 hours after treatment. For microscopy, spleens were harvested 8 days after immunization and processed as described in SI Materials and Methods.

### Soluble antigen presentation assay.

To obtain OT-II cells, spleen single cell suspensions were passed through a 70-μm filter and red blood cells were lysed with ACK lysis buffer. $B220^{+}$ cells were depleted with anti-B220 microbeads (Miltenyi). OT-II cells were sorted as $B220^{-}CD11c^{-}CD45.1^{+}TCR\text{-}V\alpha2^{+}CD4^{+}CD8\alpha^{-}$. Sorted OT-II cells were labeled with CFSE and plated at a density of $1.25\times10^{5}$cells/mL in complete media with $1.25\times10^{5}$ sorted DCs/mL and the indicated concentrations of soluble OVA (Worthington). Cells were cultured at 37ºC for 72 hours and analyzed by flow cytometry. OT-II

proliferation was determined as the percent of $CD45.1^{+}CD4^{+}$TCR-$V\alpha2^{+}CD44^{+}$ cells which had undergone at least one CFSE dilution.

**Microarray gene expression analysis.**

Total RNA was extracted from purified splenic DC2s 24 hours after mice were immunized with SRBCs using the RNAqueous-Micro Kit (Ambion). RNA was amplified using the Ovation Pico WTA Sytem (NuGEN) and hybridized to GeneChip Mouse Gene 1.0 ST microarrays (Affymetrix). Data was processed using robust multiarray average summarization and quartile normalization using ArrayStar software version 14 (DNASTAR). Microarrays from untreated mice were done in biological duplicates and from SRBC treated mice in biological triplicates in two independent experiments.

**Antibodies.**

The following antibodies were purchased from Becton Dickinson: CD8α V450 (53-6.7); B220 FITC, BV510 (RA3-6B2); CD19 APC-Cy7 (1D3); CD95 PE (Jo2); Zbtb46 PE (U4-1374). From eBioscience: CD4 APC (GK1.5); CD44 APC (IM7); CD24 PE-Cy7 (M1/69); CD172a APC, PerCP-eFluor710 (P84); IgD PE (11-26); Tim-3 PE (RMT3-23); F4/80 APC-ef780 (BM8), B220 Pe-Cy7 (RA3-6B2). From Tonbo Biosciences: CD4 PerCP-Cy5.5 (GK1.5); CD45.1 PE-Cy7 (A20); CD11c APC-Cy7 (N418); MHC-II I/A-I/E v450 (M5/114.15.2 From BioLegend: CD8α BV510 (53-6.7); CD25 AF488 (PC61); B220 Biotin (RA3-6B2); CD62L AF488 (ME44); TCR-β PE APC (H57-597); TCR Vα2 PerCP-Cy5.5 (B20.1); CD279 Pe-Cy7 (29F.1A12); CXCR5 BV421 (438D7); GL7 AF488 (GL7); Ly-6G Biotin (1A8); ESAM PE (1G8); MojoSort Streptavidin Nanobeads. From Life Technologies: TCR Vα2 PE (B20.1). From Invitrogen: IgD ef450 (11-26c). From Bio-X-Cell Tim-3 (RMT3-23) and anti-trinitrophenol (2A3). Carboxyfluorescein succinimidyl ester (CFSE) was purchased from Sigma.

**Splenic DC isolation.**

Spleens were minced and digested for 60 minutes in complete media (Iscove's modified Dulbecco's medium $^{+}$ 10% FCS, 1% Penn/Strep, 1% L-glutamine, 1% Non-essential amino acids, 1% Sodium Pyruvate, 0.1% Beta-mercaptoethanol) with 250 µg/mL Collagenase B (Roche) and 30U/mL DNase I (Sigma-Aldrich). Red blood cells were lysed with ammonium chloride-potassium bicarbonate (ACK) lysis buffer. Single cell suspensions were passed through a 70 µm-strainer. All DC flow cytometry analysis was pre-gated on singlets, live and B220– events.

**Anti-SRBC IgG1 ELISA**

To measure serum anti-SRBC IgG1 titers mice were immunized with SRBC i.p. as described above. Blood (100-200uL) was collected from the superficial temporal vein by submandibular puncture using a 5mM lancet the day of immunization and on day 17 after treatment. To isolate serum, blood was incubated for 2 hours at 25ºC followed by overnight incubation at 4ºC. Samples were then centrifuged at 2400g for 5 minutes at 25ºC and supernatants were transferred to new 1.5mL microcentrifuge tubes. Serum was stored at –80ºC until the day of analysis. Immunolon 2-HB flat bottom 96-well microtiter plates were coated overnight with 25µg/mL of SRBC cell lysates prepared as previously described(76) or BSA as control in PBS. Wells were then washed with 0.1% Tween 20 (Sigma) in PBS and blocked for 1 hour at 37ºC with 20mg/mL BSA and 0.05% Tween in PBS. After discarding blocking solution, serum samples were added at a 1/100 dilution using mouse SRBC IgG Standard (Abnova) was used as a positive control and incubated for 1hr at 25ºC. Wells were then washed 5 times and incubated with 1µg/mL of biotinylated anti- mouse IgG1 (A85-1, BD) for 1hr at 25ºC. Subsequently, wells were washed 5 times and incubated for 1 hr at 25ºC with 1µg/mL peroxidase conjugated Streptavidin in blocking solution. Wells were then washed and assay was developed with 100uL of 3,3',5,5'-tetramethylbenzidine (TMB, Dako) quenched with 2N sulfuric acid (Sigma). Peroxidase activity was measured at 450nm using a Multiskan Ascent spectrophotometer (Titertek).

**Microscopy.**

Spleens were flash frozen in Optimal Cutting Temperature (O.C.T.) compound (Sakura) with 2-methylbutane (Fischer) cooled in liquid nitrogen. Blocks were stored at –80ºC overnight and allowed to come to –20ºC for 30 minutes prior to sectioning. Eight µm sections were fixed for 5 minutes in excess 2% methanol-free paraformaldehyde (Electron Microscopy Science) and washed in excess PBS for 10 minutes. Sections were fixed with CAS-Block (Invitrogen) for 10 minutes at room temperature, and stained for the indicate markers in CAS-Block plus 0.2% Triton-X (Sigma Aldrich) overnight at 4ºC in a humidity chamber. Samples were subsequently washed with PBS three times for 5 minutes and mounted with ProLong Gold Antifade reagent containing DAPI (4,6-diamidino-2-phenylindole; Invitrogen). Four-color epifluorescence microscopy was visualized with a Zeiss AxioCam MRn microscope equipped with an AX10 camera. Monochrome images were with AxioVision software with a 10x objective at room temperature and then exported to ImageJ for color balancing and overlayering.

**SI Figure Legends**

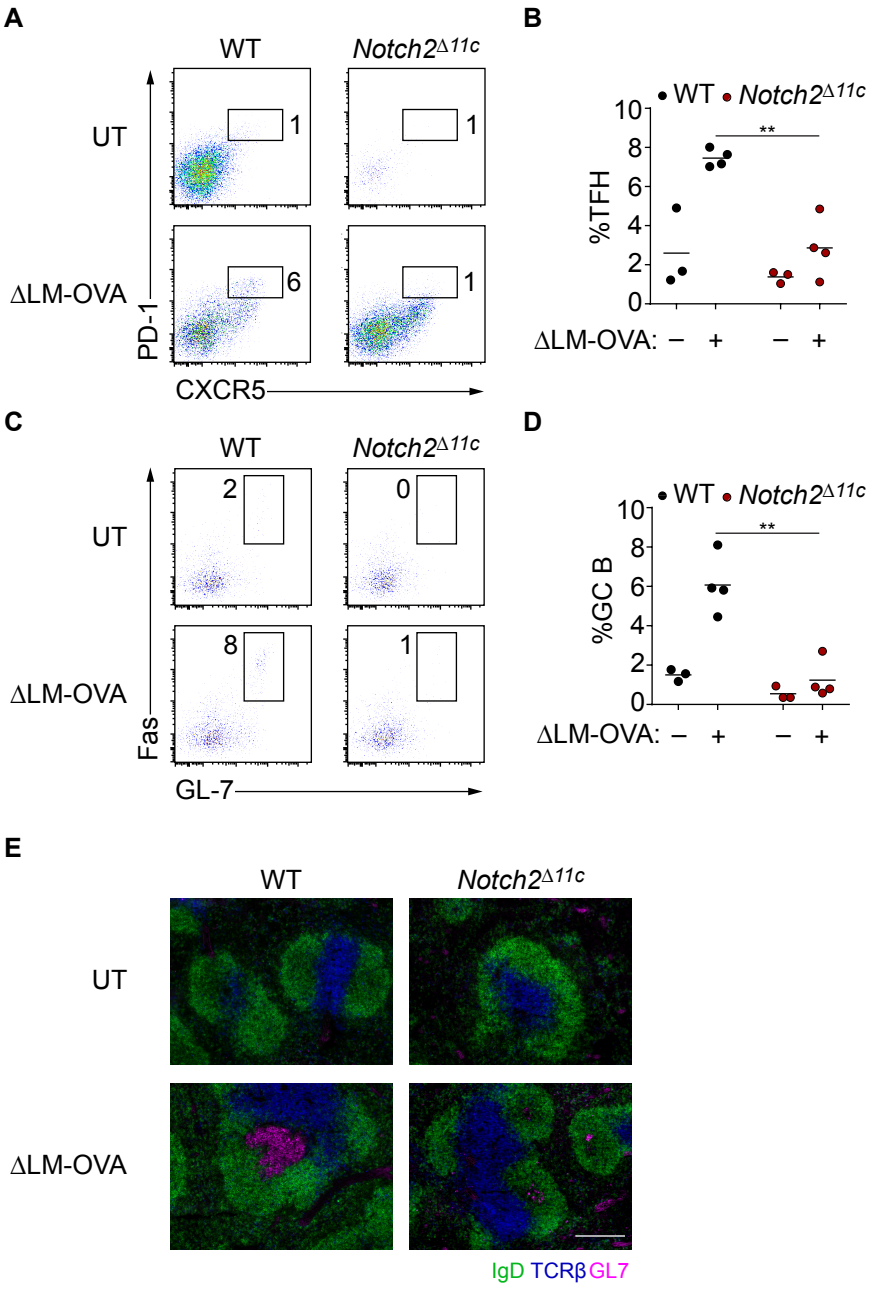
**Figure S1. Notch2 deletion in DCs impairs germinal center formation after immunization with heat killed LM-OVA.** WT and CD11c-Cre$^{+}$ *Notch2$^{f/f}$* (*Notch2Δ11c*) mice were immunized with heat inactivated L. monocytogenes expressing OVA (ΔLM-OVA) and analyzed for GC reactions 8 days after challenge. (A) Representative flow cytometry analysis of CD4 T cells (B220$^{-}$TCR-β$^{+}$CD4$^{+}$CD8$^{-}$CD44$^{+}$) from spleens of the indicated mice 8 days after immunization with ΔLM-OVA. Shown are untreated mice as controls. (B) Quantification of $T_{FH}$ percentages in the indicated mice treated as in A. (C) Representative flow cytometry analysis of splenic B cells (B220$^{+}$CD19$^{+}$IgD$^{-}$) from mice treated as in A. Shown are untreated mice as controls. (D) Quantification of GC B cells in the indicated mice treated as in A. Each dot represents a biological replicate from two independent experiments. (E) Microscopy of spleens from the

indicated genotypes from mice taken 8 days after immunization with ΔLM-OVA. Shown are untreated mice as controls.

**Figure S2. TFH and GC B cells form in Peyer's Patches from *Notch2$^{\Delta 11c}$* mice.** (A) Representative flow cytometry analysis of TFH cells in Peyer's Patches from wild-type (WT) and *Itgax*-Cre$^{+}$ *Notch2$^{f/f}$* (*Notch2$^{\Delta 11c}$*) mice. Cells are pre-gated as (B220$^{-}$, TCR-β$^{+}$, CD4$^{+}$, CD8$^{-}$, CD44$^{+}$, CD62L$^{-}$). (B) Quantification of TFH cells in Peyer's Patches from WT and *Notch2$^{\Delta 11c}$* mice. (C) Flow cytometry analysis of GC B cells in Peyer's Patches from WT and *Notch2$^{\Delta 11c}$* mice pre-gated as B220$^{+}$ IgD$^{lo}$. (D) Quantification of Peyer's Patches GC B cells from the indicated genotypes. Each dot represents a biological replicate.

**Figure S3. Notch2 signaling is dispensable for DC2 lineage commitment.** (A) Flow cytometry analysis of splenic DCs for expression of Zbtb46. DC1: CD24$^{+}$ CD172a-; DC2: CD24-CD172a$^{+}$. Data is representative of three biological replicates from two independent experiments (B) Representative single color histograms for intracellular Zbtb46 expression in DC1 (top) or DC2 (bottom) in the indicated mice. *Zbtb46$^{gfp/gfp}$* mice, which lack Zbtb46 protein expression, are shown as negative staining controls.

**Figure S4. Antibody mediated blockade of Tim-3 *in vivo.*** (A) Flow cytometry analysis of Tim-3 staining of DC2s from mice treated with anti-Tim-3 blocking antibody or isotype control 8 days after immunization with SRBC. Shown are unimmunized mice as controls. Numbers indicate the mean fluorescence intensity (geom. Mean). (B) Quantification of the MFI of Tim-3 staining on DC2s treated as in A. Each dot represents a biological replicate from two independent experiments.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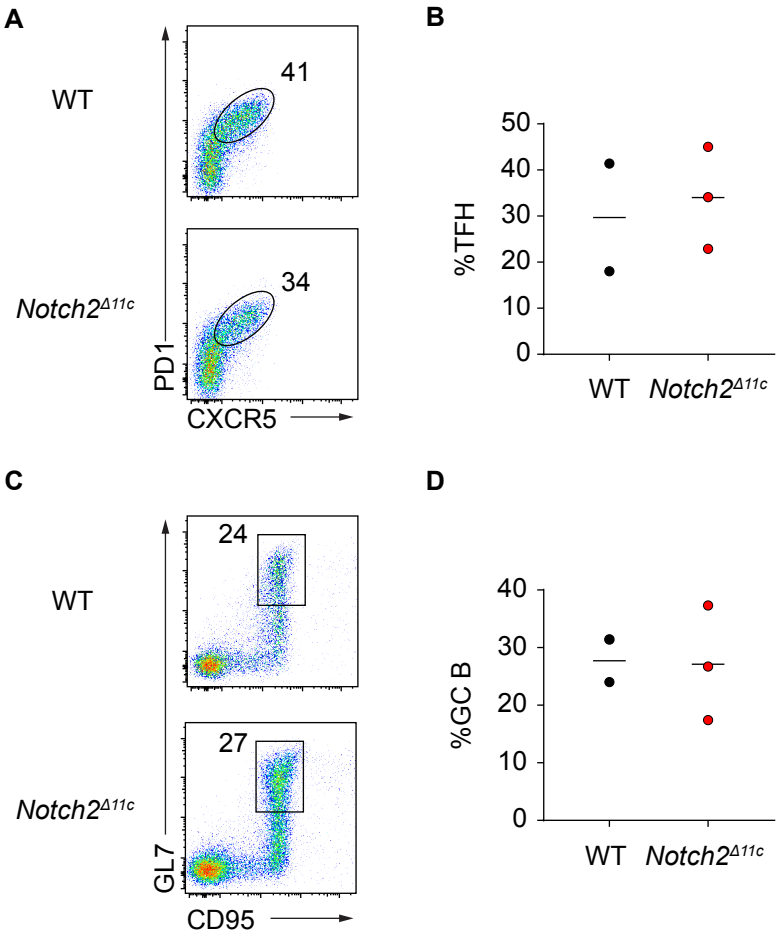
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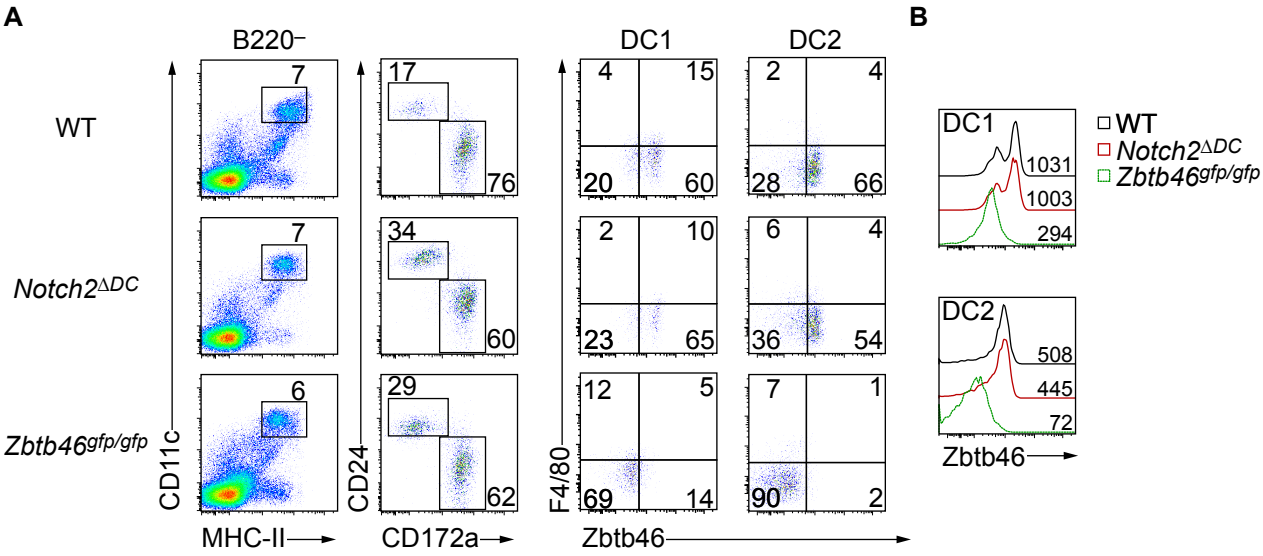
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DC2
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90
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CD11c
MHC-II
CD24
CD172a
F4/80
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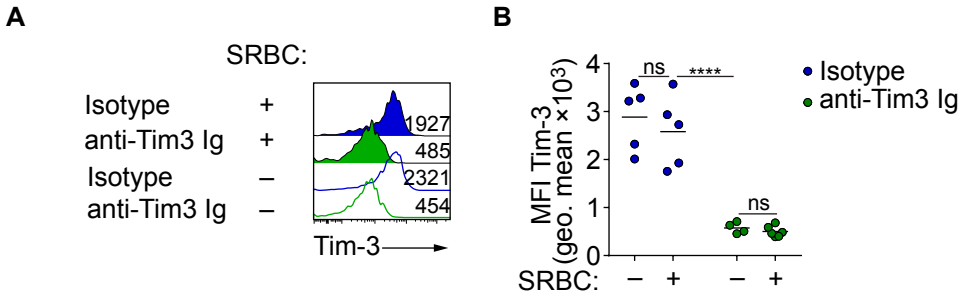
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