Supplementary Experimental Procedures

Mice

KLF2^{fl/fl}/TEa mice were bred with ERT2-Cre and ROSA26-floxed STOP-YFP (Rosa26-YFP) mice to generate KLF2^{fl/fl}/ERT2-Cre/Rosa26-YFP/TEa (KLF2^{fl/fl}) and KLF2^{+/+}/ERT2-Cre/TEa (KLF2^{+/+}) control mice. In KLF2^{fl/fl}/ERT2-Cre/Rosa26-YFP animals, administration of tamoxifen induces ERT2-Cre to translocate to the nucleus, leading to deletion of KLF2 and expression of the YFP reporter. For adoptive co-transfer studies, combinations of CD45.2^{+}, CD45.1^{+} and CD45.1^{+}CD45.2^{+} mice were used as donor and host strains to allow discrimination between each donor population and the host cells. Six to eight week old mice were used for all experiments.

Infections and MHC II-tetramer based cell enrichment

Mice were injected intravenously with 1×10^7 colony-forming units of ActA-deficient LM-2W1S bacteria or intraperitoneally (i.p.) with 2×10^5 plaque-forming units of the LCMV Armstrong strain. Tetramers composed of I-A^b and either 2W1S, LLO190-201, or LCMV glycoprotein (GP) 66-77 peptides were made as described previously(Moon et al., 2009; Tubo et al., 2013). Single cell suspensions of spleen and LN cells were stained for 1 hour at room temperature with the PE or APC-conjugated tetramers and 2 µg of CXCR5-BV421 antibody (2G8; BD bioscience). Samples were then enriched and enumerated as described previously(Moon et al., 2009; Tubo et al., 2013). For identification of surface markers, the sample was stained on ice with antibodies specific for MHCII (M5/114.15.2), F4/80 (BM8), B220 (RA3-6B2), CD11c (N418), CD8α (53-6.7), PD-1 (J43), CD4^+ (RM4-5), CD3ε (145-2C11), CD44 (IM7), CD45.1 (A20), CD45.2 (104) and CD69 (H1.2F3). Intracellular staining for T-bet (4B10, BioLegend), GATA3 (TWAJ), RORγT (Q31-378, BD bioscience), FoxP3 (FKJ-16s) and Bcl-6 (K112-91, BD bioscience) was performed as described previously(Lee et al., 2013; Tubo et al., 2013). For determining reporter signal in CD4^+ T cell lineage subpopulations, intracellular staining for anti-GFP antibody
(rabbit, Life technologies) followed by AlexaFluor488 goat anti-rabbit IgG antibody (Life technologies) was performed. All antibodies were from eBioscience (San Diego) unless otherwise noted. Cells were then analyzed on an LSR II or Fortessa (Becton Dickinson) flow cytometer. Data were analyzed with FlowJo (TreeStar).

**Adoptive transfer and Eα-SA-DEL immunization.**

For adoptive transfer experiments, 1x10⁵ TEa CD4⁺ T cells were typically co-transferred with 5x10⁴ MD4 B cells into WT B6, B6.SJL, or Tcra⁻/⁻ mice depending on the CD45 congenic marker expression of the donor cells. Naive CD4⁺ T cells from various TEa strains mice were isolated by using mouse CD4⁺ T Cell Isolation Kit (Miltenyi Biotec), and MD4 B cells were isolated by using mouse B Cell Isolation Kit (Miltenyi Biotec). For reporter studies, background reporter signal was evaluated by mixing reporter and congenic WT TEa CD4⁺ T-cells at a ratio of 1:1, and 5x10⁴ of each TEa CD4⁺ T cells were transferred into congenic recipient mice. After 12 hours, the recipients were immunized subcutaneously (7µM in 50 µl per mouse) with a conjugate antigen bearing antigens for both TEa and MD4 cells (Eα-SA-DEL) emulsified in CFA (0.5 mg/ml). On the day of harvest, TEa CD4⁺ T cells were enriched based on CD45 expression as described previously(Skon et al., 2013).

To generate the conjugated antigen (Eα-SA-DEL), biotinylated Eα peptide (Eα-Bio) and Streptavidin (SA) were purchased from New England Peptide and ProZyme respectively. Purified duck egg lysozyme (DEL) was obtained by special order from Worthington Biochemical Corporation, and biotinylated using an EZ-link Sulfo-NHS-LC Biotinylation kit (Thermo Fisher Scientific) using a 1:1 ratio of biotin to protein. After removal of free biotin by use of desalting columns (GE Healthcare), the molar amount of biotinylated DEL (DEL-Bio) was measured by Western blot, as previously described(Taylor et al., 2012). The prepared Eα-Bio and DEL-Bio were then conjugated by incubation with SA (Eα-Bio:DEL-Bio:SA= 2:2:1 ratio) at room temperature for 30 minutes.
Anti-ICOSL Treatments

Anti-ICOSL (rat IgG2a, clone HK5.3) and isotype control (rat IgG2a) mAbs were purchased from BioXcell. C57BL/6 recipients that congenic KLF2-GFP TECa and MD4 B cells were adoptively transferred were given 100 µg of anti-ICOSL or isotype control mAb via both i.p. and i.v. 7 days post Eα-SA-DEL/CFA immunization.

Inducible KLF2 deletion and B cell germinal center reaction

In vivo KLF2 deletion of KLF2 fl/fl (KLF2 fl/fl/ERT2-Cre/Rosa26-YFP/TEa) cells in WT B6.SJL or Tcra-/- recipient mice was achieved by administering tamoxifen (2 mg/mouse) in sunflower seed oil i.p. for 5 consecutive days from day 2 post-immunization. At day 7 post-immunization, the spleen and inguinal, axillary, brachial, cervical and mesenteric LNs were harvested and analyzed. KLF2 fl/fl or KLF2 +/- control cells were enriched through their congenic markers, and KLF2 KO populations were identified by YFP expression (Rosa26-YFP reporter). For in vitro KLF2 deletion, primed KLF2 fl/fl cells (cultured with recombinant IL-2 (20ng/ml), anti-CD3 (8 µg/ml per well; 145-2C11; BioXCell), and anti-CD28 (8 µg/ml per well; 37.51; BioXCell) coated plate for 24 hours) were cultured with 4-OH tamoxifen (100nM) and IL-15 (20ng/ml) for 48 hours in anti-CD3/CD28 coated wells, followed by 48 hours of additional culture in IL-15 in non-coated plates (see Supplementary Fig. 5A).

Antigen specific B cells were identified and enriched using Eα-SA-PE tetramer. Eα-SA-PE tetramer was prepared by incubation of Eα-Bio and SA-PE (4:1 ratio) at room temperature for 30 minutes. Antigen specific endogenous B cells from pooled spleen and LNs of the Tcra-/- or Tcrb-/- recipients were stained with Eα-SA-PE tetramer, and enriched by anti-PE magnetic beads pull-down(Pape et al., 2011). Sera from immunized mice were collected at day 0, 2 and 7 post immunization, and antigen-specific IgM, IgG1, IgG2a, IgG2b, IgG2c, IgG3, IgA and IgE antibodies were measured by ELISA as previously described(Pape et al., 2011). Briefly, sera were titrated into ELISA plates that had been coated with Eα-SA-DEL (20µg/ml in PBS, overnight) then blocked with
1% BSA. HRP conjugated anti-mouse-antibody isotype antibodies (Southern Biotech) were applied and detected using ABTS Peroxidase Substrate (KPL). Titers were presented as the maximum serum dilution exceeding 1.5-fold above the average background.

**Retroviral transduction approaches**

Some retrovirus constructs (Empty control, KLF2 and S1PR1) were cloned using MSCV-IRES-Thy1.1 (MiT) backbone vector as described previously (Skon et al., 2013). Cre expressing retrovirus construct (MSCV-Cre-IRES-mAmetrine) was provided from Dr. Shane Crotty. The plasmid MiT-S1PR1, MiT-KLF2, MiT (empty vector) or MSCV-Cre-IRES-mAmetrine was transfected together with the retroviral packaging vector pCL-Eco into 293T human embryonic kidney cells with Lipofectamine 2000 (Life Technology). Supernatants were collected at 48 h after transfection.

For *in vitro* over-expression of KLF2 or S1PR1, the retrovirally transduced CD4⁺ T cells were cultured with IL-15 (20ng/ml) in anti-CD3/CD28 coated plate for 2 days, and were further cultured in non-coated plate for 2 additional days (see Supplementary Fig. 5A).

Naive CD4⁺ T cells from TEa or Blimp1-YFP mice were isolated and activated by plate-bound anti-CD3 and anti-CD28 with recombinant IL-2 (20ng/ml). 24 hours after activation, cells were spin-infected by retroviruses MiT-KLF2, MiT-S1PR1 or control empty vector (MiT-Empty) as described previously (Skon et al., 2013).

Some transduced cells were transferred with MD4 B cells into congenic recipients that were then immunized with Ea-SA-DEL/CFA. In some experiment, S1PR1 function was inhibited by i.p. injection (200 µl) of FTY720 (20µg/mouse) or vehicle control (10% EtOH in PBS) at day 2, 4 and 6 post immunization.

**In vitro polarization and cytokine production assay**

Primed KLF2 inducible KO (KLF2fl/fl ERT2-Cre) or KLF2/S1PR1 retrovirus tansduced TEa cells (cultured with recombinant IL-2 (20ng/ml), anti-CD3 (8 µg/ml
per well; 145-2C11; BioXCell), and anti-CD28 (8 µg/ml per well; 37.51; BioXCell) coated plate for 24 hours) were with (for KLF2 deletion)/ without 4-OH tamoxifen (100nM) under non-polarizing (“Th0”; IL-15 (20ng/ml)) or Th1 polarizing (IL-2 (10ng/ml), IL-12 (5ng/ml), anti-IL-4 mAb (10µg/ml)) culture condition for 2 days in anti-CD3/CD28 coated wells, followed by 2 days of additional culture with the same cytokine combinations in non-coated plates.

To examine cytokine production in the KLF2 knock-out or KLF2/S1PR1 over-expressing TEa cells, cells were re-stimulated with PMA (50ng/ml, SIGMA) and ionomycin (1.5µM, SIGMA) in the presence of Monensin for 3 h and intracellular staining for IFN-γ (XMG1.2), IL-4 (11B11) and IL-17 (eBio17B7) was performed.

**Chromatin immunoprecipitation (ChIP)**

ChIP was performed as previously described (Li et al., 2013). Briefly, approximately 10^7 naïve or in vitro activated KLF2-GFP TEa cells were cross-linked with 1% formaldehyde at room temperature for 10 min and neutralized with 0.125 M glycine for 10 minutes. Chromatin was fragmented using Bioruptor®. Subsequently, the soluble chromatin was incubated with 5 µg of anti-GFP (Life technologies) or control rabbit IgG antibodies at 4°C overnight. Immunoprecipitated complexes were collected using 30ul Dynabeads G (Invitrogen) per reaction. Final ChIP DNA was extracted and purified using QIAquick spin columns (Qiagen). Precipitated DNA and input DNA were assessed by quantitative real-time PCR with SYBR Green PCR Master Mix (Applied Biosystems).

The promoter regions of each gene were determined based on histone 3 lysine 4 trimethylation (H3K4me3) and histone 3 lysine 27 acetylation (H3K27ac) pattern around transcriptional start site of the gene (see Supplementary Fig. 5B). The sequences of the primer pairs used were as follows: for the prdm1 promoter region, forward, 5’-TTTTGTTGTCCCTGCCTCTC-3’, and reverse, 5’-CCCCTTTTTTAACTGGGAAGC-3’; for the s1pr1 promoter region, forward, 5’-ACCAGCTCACTCGCAAAGTT-3’, and reverse, 5’-
GCGCTCAGAGACTTCGTCTT-3'; for the bcl6 promoter region, forward, 5'-
GCGAGCAACAGCAATAATCA-3', and reverse, 5'-
CGAGAATTGAGCTGTGTA-3'; for the tbx21 promoter region, forward, 5'-
CGTCCGAAGACCAATGAAAC-3', and reverse, 5'-
TCATAAAGCCACAGCAAGG-3'; and for the gata3 promoter region, forward,
5'- GGGTTTGGGTGCGATTTTCTC-3', and reverse, 5'-
GCGACGCAACTTAAGGAGTTCTA-3'.

Quantitative RT-PCR.

After in vitro deletion of KLF2 or retroviral overexpression of KLF2
(S1PR1), cells were sorted on a FACSAria (Becton Dickinson) on the basis of the
YFP (Rosa26-YFP Cre reporter) or Thy1.1 transduction markers respectively. In
all cases, RNA was isolated with an RNeasy microkit (Qiagen), followed by
reverse transcription (SuperScript® III Reverse Transcriptase, Life technologies).
Gene expression was assessed (in triplicate for cultured cells) with an ABI 7700
sequence-detection system, and amplification was detected with SYBR Green
PCR Master Mix (Applied Biosystems).

Cycling threshold values for the control target gene (Gapdh) were
subtracted from cycle threshold values for the gene of interest. Next, the mean
values (averaged across all repeated experiments) for gene expression of WT
(KLF2 +/+ ) or non-overexpressing control (Empty) TEa cells were used for
normalization between experiments. The sequences of the primer pairs used
were as follows: klf2, forward, 5'- ACCAATGCGGAAGACCTA-3', and reverse,
5'- CATCCTTCCAGGGCAATGA-3'; s1pr1, forward, 5'
-GTGAGACCATCTTTCTGCG-3', and reverse, 5'
AGCTTTTCTGGGTGGAGAG-3'; prdm1, forward, 5'
-GACGGGGGTACTTCTGTTCA-3', and reverse, 5'
GGCATTCTTGTGGGAACTGTGT-3'; bcl6, forward, 5'
CACACCGTCCATCATTGAA-3', and reverse, 5'
TGTCCCTACCGTGCTTTT-3'; tbx21, forward, 5'
CAACACCCCTTGGCAAGAG-3', and reverse, 5'
TCCCCCAAGCAGTTGACAGT-3'; gata3, forward, 5’-
AGAACCAGCCCTATGAA-3', and reverse, 5’- AGTTGGCAGGATGTCC-
3’; rorc, forward, 5’-GGAGGTGACCAGCTACAGA-3’, and reverse, 5’-
TGGCAAACTCCACCACACAG-3’; foxp3, forward, 5’-
GGCCCTTCTCCAGACAGA-3’, and reverse, 5’-
GCTGATCATGGCTGTTG-3’; ascl2, forward, 5’-
CGCTGCCAGACTCATGCCC-3’, and reverse, 5’-
GCTTTACGCGGTGCGCTCG-3’; CXCR5, forward, 5’-
ACTCCTTACCACAGGTACCTT-3’, and reverse, 5’-
GGAAACGGGAGGTGAACCA-3’; GAPDH, forward, 5’-
TGGCCTACATGGCCTCCA -3’, and reverse, 5’-
TCCCTAGGCCCCTCCTATT-3’;

**Immunohistochemistry**

KLF2-GFP or WT B6 mice were subcutaneously immunized with PE (15µg in CFA) at the base of the tail and were sacrificed after 14 days. Draining LNs were fixed with 4% PFA and incubated in 30% sucrose solution. Five micrometer sections were cut and stained with anti-GFP antibody (Life technology). GFP fluorescence intensities were quantified in GC (GL7 and B220 abundant) or T cell zone (CD4⁺ abundant and B220 negative) CD4⁺ T cells using ImageJ software according to histocytometric algorithms as previously described(Gerner et al., 2012).

**Statistical analysis.**

Data were analyzed with Prism software 4.0 (GraphPad). For standard data sets, an unpaired two-tailed Student’s t-test was used. For values that differed by over tenfold, the data was log10-transformed before t-test analysis. When data were normalized (by the appropriate control samples), normalization involved division of all values by the overall mean of the control values to avoid type I and II errors during calculation of significance through the t-test. Data sets (in Prism format) are available on request.
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Figure S1

KLF2^{GFP} mice

DAPI  B220  TCR\beta  PE  GL7

200\mu m
Figure S2

A

TEa TCR tg CD4 T cells
(Specific for Eα/I-A^b^)

MD4 BCR tg B cells
(Specific for HEL/DEL)

Eα Bio;DEL-Bio;SA complex
tail S.C. immunization with CFA

B

% of GC T_{FH} in total TEa

C

Number of GC T_{FH}

D

Eα-SA-DEL/CFA
s.c. immunization

Transfer
KLF2-GFP/TEa CD4
with MD4 B cells

ICOS-L
blocking Ab
i.p. and i.v.

Harvest
2ndary lymphoid
organs

E

Con Ig

ICOS-L blocking

D+9
(2 days post blocking)

D+12
(5 days post blocking)

PD-1

CXCR5
Figure S3

A. Timeline of experimental procedures:
- WT TEa or Cre-ERT2/KLF2^fl/fl /YFP(ROSA)/TEa
- Eα-DEL CFA
- Tamoxifen i.p. (2mg/mouse)
- Harvest

B. Flow cytometry plots showing YFP expression in CD4+ TEa cells:
- KLF2^fl/fl CD4+ TEa
- KLF2^+/+ CD4+ TEa

D. Flow cytometry plots showing PD-1 and CXCR5 expression:
- KLF2^+/+ CD4+ TEa
- KLF2^fl/fl CD4+ TEa (KLF2 KO)

E. Graphs showing anti-Eα-DEL IgG2b and anti-Eα-DEL IgG3 levels:
- D+2 and D+7
- ns, * indicates statistical significance

F. Flow cytometry plots showing antigen-specific and background responses:
- Eα-SA+ PE-AF647
- Intracellular (IC) Ig

G. Flow cytometry plots showing IgD and IgM levels:
- KLF2^+/+ recipient
- KLF2^fl/fl recipient

Prior gating:
- Live cells, and "dump" (CD90, F4/80, Gr-1, NK1.1) negative.
- B220 positive and CD38 high.
Figure S5

A

![Graph showing KLF2-GFP gMFI over time with pre-activation and D+1, D+3, D+5 labels.](grafico.png)

- IL2 and IL15 are indicated.
- gMFI with background subtraction.
- Retrovirus spin infection for gene over-expression or 4-OH tamoxifen treatment for KLF2 KO.

B

- H3K4me3
- H3K27ac

C

- WT (Cre-), Blimp-1 KO (Cre+)
- Thy1.1, mAmetrine (Cre RV)

D

- WT (Cre-), Blimp-1 KO (Cre+)
- KLF2, PD-1, CXCR5
Figure S6

A

| Empty   | KLF2  | S1Pr1 |
|---------|-------|-------|
| Th0     | 0.0185 | 0.0107 | 0.0213 |
| Th1     | 0.0132 | 0.106 | 0.112 |
| IL4     | 24     | 75.8  | 3.89 |
| IFN-γ   | 99.5   | 48.8  | 99.3 |

B

| WT       | KLF2 KO |
|----------|----------|
| Th0      | 0        | 0.0304 |
| Th1      | 0        | 0.0127 |
| IL4      | 22.3     | 77.7  |
| IFN-γ    | 97.9     | 0.486 |

C

Non-stim. control vs PMA/Iono

| Empty   | KLF2  |
|---------|-------|
| T-bet   | 0.59  | 1.36  |
| IFN-γ   | 3.84  | 12.5  |

D

Non-Treg (FoxP3-) & non-Th17 (RORγT-)

| Empty   | KLF2  | S1Pr1 |
|---------|-------|-------|
| GATA3   | 0.19  | 5.56  | 2.16  |
| T-bet   | 11.3  | 83    | 8.64  |
| IFN-γ   | 19.2  | 64.3  | 38.5  |

E

FoxP3- vs RORγT- vs T-Bet low

| FoxP3 | RORγT | T-Bet | GATA3 | Bcl6 |
|-------|-------|-------|-------|------|
| 0.64  | 4.09  | 12.1  | 61.1  | 17.4 |
| 3.21  | 24.9  | 61.1  | 22.5  | 40.1 |
| 20.8  | 75.3  | 15    | 11.7  | 18.6 |
| 15    | 20.8  | 24.9  | 35.6  | 33.5 |

Donor CD4 TEa cell gating
Supplementary Figure Legends

Supplementary Figure 1 (related to Figure 2). Immunohistochemistry analysis of KLF2-GFP expression in PE specific germinal centers. PE specific GCs were determined by GL7+B220+PE+TCRβ- area in dLNs (inguinal and lumbar LNs) from mice immunized subcutaneously with PE (15µg)/CFA at day 14 post immunization. See Figure 2 for more details. Gray, DAPI; Yellow, B220; Green, TCRβ; Red, PE; Blue, GL7; scale bar, 200µm. The image is representative of the staining pattern from three independent experiments.

Supplementary Figure 2 (related to Figure 3). TEa adoptive transfer system. (A) Experimental scheme. To enhance antigen-specific B cell help (required for optimal GC-Tfh cell differentiation), TEa TCR transgenic CD4+ T cells, specific for an Eα-derived peptide (amino acid residues 52–68) in the context of MHC class II molecule I-Ab, were co-transferred with BCR transgenic B cells (MD4) specific for hen/duck egg lysozyme (HEL/DEL), and the recipient mice were immunized with a conjugate antigen bearing antigens for both TEa and MD4 cells (Eα-SA-DEL) in CFA. (B, C) Quantification of GC-Tfh differentiation in donor TEa populations at various time points with/without MD4 cells. Graphs show accumulated data from 3 individual recipients at each time point, represented as mean ±s.e.m., two-tailed t-test. *, P < 0.05; and ***, P < 0.001. (D, E) ICOS-L blocking assay. (D) Experimental scheme. To block ICOS ans ICOS-L interaction, ICOS-L blocking mAbs (100µg/injection) were injected into recipients by both i.p. and i.v. at day 7 post immunization. At day 9 (2 days blocking) or day 12 (5 days blocking), KLF2-GFP TEa cells were enriched and analyzed. (E) Phenotypic analysis of KLF2-GFP TEa cells from the mice receiving ICOS-L blocking mAbs (right) or control Ig (left) at day 9 (top) or 12 (bottom) post immunization. The data are representative of three independent experiments.

Supplementary Figure 3 (related to Figure 4). Inducible deletion of KLF2 in peripheral CD4+ T cells enhances generation of GC-Tfh and the antigen
**specific germinal center response.** (A) Experimental scheme. WT KLF2 TEa cells (KLF2+/+; Cre-ERT2 / TEa) or KLF2 inducible KO TEa cells (KLF2 fl/fl; KLF2fl/fl / Cre-ERT2 / Rosa-YFP / TEa) were transferred into WT B6 (with MD4 B cells) or TCRα−/−/TCRβ−/− (without MD4 B cells) recipients and primed by subcutaneous immunization of Eα-SA-DEL/CFA. Tamoxifen was administered via intra-peritoneal injection daily from days 2 to 6 post-immunization. At day 7 after immunization, pooled spleen and LN cells from each recipient were stained with APC conjugated anti-CD45.1 (congenic marker specific for transferred TEa CD4+ T cells) antibody, and TEa CD4+ T cells enriched by anti-APC magnetic beads pull-down. (B) Representative data on the frequency of YFP induction of donor KLF2 inducible KO TEa cells from tamoxifen treated animals at day 7 post immunization. (C) Absolute numbers of donor TEa CD4+ T cells recovered from TCRα−/− recipients that received KLF2+/+ or KLF2 inducible KO TEa cells, determined 7 days after immunization and 5 days after tamoxifen treatment. (D) Representative flow cytometric analysis of TEa CD4+ T cells at day 7 post immunization in tamoxifen treatmentd TCRα−/− recipients. The top panel shows analysis of KLF2+/+ TEa cells. The lower panels are from KLF2 inducible KO TEa cells, shown as the bulk population or the populations gated for YFP+ and YFP− (the former being designated “KLF2 KO”). Note that we observe fewer TEa cells differentiating into Tfh subsets in TCRα−/− host animals without MD4 B cells. (E) Shows the anti-Eα/DEL specific titer of antibodies of the indicated isotypes from animals receiving WT KLF2 (“KLF2 +/+”) or KLF2 inducible KO (“KLF2 fl/fl”) TEa CD4+ T cells, measured at the indicated time points (see Figure 4 for more information). (F) At day 7 after immunization, pooled spleen and LN cells from each TCRα−/− recipient were stained with Eα-SA-PE tetramer, and cells enriched by anti-PE magnetic beads pull-down. Host antigen specific B cells were identified based on the following criteria: Eα-SA-PE tetramer+ PE-AF647- Thy1-Gr-1- CD11c- NK1.1- and F4/80-. Plasma blast/cells were determined as IC-Ighigh/B220low, and GC B cells were determined as C-Ighigh/B220low/GL7high. GC B cells were further validated by surface expression of IgD and IgM. After enrichment, B cells were gated as negative for non-B cell lineage markers.
(“dump”: Thy1, Gr-1, CD11c, NK1.1 and F4/80), and the antigen specific B cells were determined by PE positive gating (Eα-SA-PE tetramer bound fraction). In order to exclude B cells specific for PE (which was not a component of the immunogen) cells were also stained with PE-AF647: Cells stained with Eα-SA-PE but not PE-AF647 were designated as Eα-SA “antigen” specific. Indeed, plasma cells - B220lo, intracellular immunoglobulin (IC-Ig)hi - were found in the Eα-SA specific but not PE specific (“Background”) population, as expected. (F) Representative flow cytometric analysis of the isotype-switched endogenous memory B cell population (defined by antigen+/B220+/CD38high/IgD-/IgM-) in TCRβ-/- recipients at day 14 or 30 post immunization. Data are compiled from at least 3 separate experiments. Graphs show accumulated data from the independent experiments as mean ±s.e.m., two-tailed t-test. ns, not significant (P > 0.05); *, P < 0.05; **, P < 0.01.

Supplementary Figure 4 (related to Figure 5). Effects of KLF2 and S1PR1 overexpression and FTY720 treatment on differentiation of TEa CD4+ T cells. TEa cells were activated in vitro and then transduced with retroviruses encoding S1PR1 or KLF2, or the transduction marker (Thy-1.1) alone (“Empty”). Cells were co-transferred with MD4 B cells into mice that were then primed sc with Eα-SA-DEL in CFA. (A) shows CXCR5 and PD-1 expression on donor TEa cells at day 7 post immunization: The upper panels represent cells bearing the transduction marker (Thy-1.1) while the lower panels represent cells that did not express Thy-1.1 and so are designated non-transduced. (B) The gating strategy of CD4+ helper T cell lineages. At day 7 after subcutaneous immunization of Eα-SA-DEL/CFA, donor CD4+ TEa cells were enriched and analyzed for lineage specific transcription factor expression. First, the Treg population was identified by FoxP3 and CD25 expression. FoxP3- (non-Treg) cells were characterized for RORγT+ expression (identifying presumed Th17 cells). Next, the RORγT+ population was further sub-populated into T-bet+ and GATA3+/T-bet- populations. Finally, the Bcl6+/CD25- Tfh cells were identified within the T-betlow/GATA3+ population. (C) TEa CD4+ T cells were transduced and transferred as in (A) except in these experiments, the recipient animals were treated with FTY720 (“FTY720”:
20μg/mouse) or Vehicle alone (“Veh.”; 10% EtOH in PBS) by intra-peritoneal injection on days 2, 4 and 6 post immunization. These data are gated on Thy-1.1+ (transduced) TEa CD4+ T cells. The change in frequency of the GC-Tfh population in S1PR1 retrovirus transduced cells with or without FTY720 treatment is indicated. Note that treatment with either FTY720 or vehicle control led to low frequencies of GC-Tfh TEa T cells – this was a consistent finding, presumably related to ethanol treatment. All data are representative of three independent experiments.

Supplementary Figure 5 (related to Figure 6). Effect of KLF2 in CD4+ T cell lineage specific transcription factor expression and Tfh differentiation. (A) For in vitro culture studies, isolated KLF2-GFP CD4+ T cells were initially primed with IL-2 (20ng/ml) for 24 hours and further cultured with non-polarizing condition (IL-15 (20ng/ml)) for 48 hours in anti-CD3/CD28 coated plates. Cells were then transferred to non-coated plates and cultured to recover KLF2-GFP expression for an additional 48 hours. The timing for retroviral transduction and 4-OH tamoxifen (100nM) treatment (for KLF2fl/fl inducible knockout) is indicated. Graph shows expression of KLF2-GFP in KLF2GFP reporter TEa CD4+ T cells during in vitro culture, presented as experimental gMFI minus background gMFI of co-cultured non-transgenic TEa CD4+ T cells. (B) Histone modification pattern and the PCR amplified region of ChIP-PCR target genes. The promotor of each gene was determined based on histone3 lysine4 trimethylation (orange) and histone3 lysine27 acetylation (purple) pattern. PCR amplified region is indicated (red bar). (C, D) KLF2 overexpression in wile type (WT) or Blimp-1 KO SMARTA CD4+ T cells. For investigating the mechanism of KLF2 in regulating Tfh differentiation in vivo, prdm1fl/flBcl6+/−-SMARTA cells and LCMV were used, and Blimp-1 deletion was achieved by co-transduction of Cre expressing retrovirus (Cre RV) with KLF2 (or empty control) expressing retrovirus. (C) KLF2 overexpression was determined by Thy-1.1 expression, and Cre mediated Blimp-1 deletion was determined by mAmetrine expression. (D) Representative data of Tfh differentiation upon KLF2 overexpression in WT (Cre−; mAmetrineneg) or Blimp-1 KO (Cre++;mAmetrinepos) SMARTA cells in vivo during LCMV specific
immune respond. All data are representative of at least three independent experiments.

Supplementary Figure 6 (related to Figure 7). Analysis of Th subsets. (A, B) At day 5 of in vitro culture (described in Supplement figure 5A and experimental procedure), KLF2/S1PR1 overexpressing (A) or KLF2 knock-out (B) cells were re-stimulated with PMA and ionomycin for 3 h, and the production of IFN-γ and IL-4 were measured by flow cytometric analysis. This in vitro culture experiment was repeated three times with similar results. (C) To investigate KLF2 function in Th1 differentiation in vivo, KLF2 overexpressing (or Empty control) TEa cells were enriched using their congenic marker at day 7 post immunization and re-stimulated ex vivo with PMA and ionomycin for 3 h, and the IFN-γ and T-bet expression were measured by flow cytometric analysis. (D) Flow cytometric analysis of CD4+ helper T cell lineage defining transcription factor expression in TEa cells transduced with the indicated retroviruses assayed at day 7 post immunization in vivo. (E) At day 7 after subcutaneous immunization of Ea-SA-DEL/CFA (as in Figure 3), donor CD4+ KLF2GFP TEa cells were enriched, and the KLF2-GFP expression was compared with the level of lineage specific transcription factors, following the serial gating scheme indicated. All data are representative of at least three independent experiments.