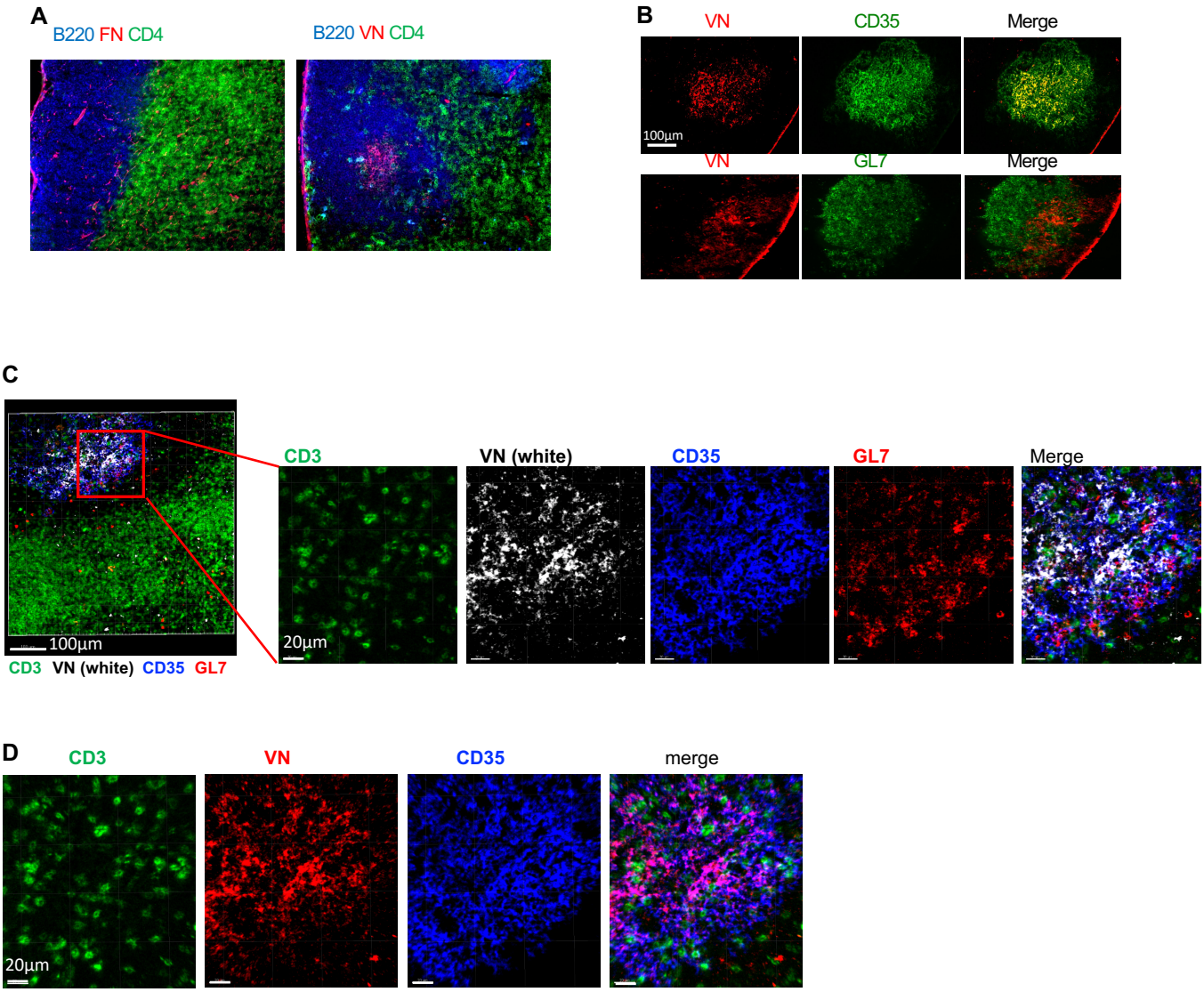

Figure S1. T cells and Vitronectin co-localization with CD35 FDC. **A, B** IHC of dLN 30 days after CFA/OVA immunization of WT C57BL/6 mice. **A** Fibronectin and vitronectin staining in relation to T (CD4) an B (B220) cell zones. **B** Vitronectin staining with $CD35^{+}$ FDC or $GL7^{+}$ GCs. **C** Confocal images of T cells in relation to FDC and VN in a $GL7^{+}$ region of the dLN, d14 post-immunization. **D** Single channel images of region highlighted in Fig 1E, d14 post-immunization (same image as **C**, pseudo-colored for better resolution).

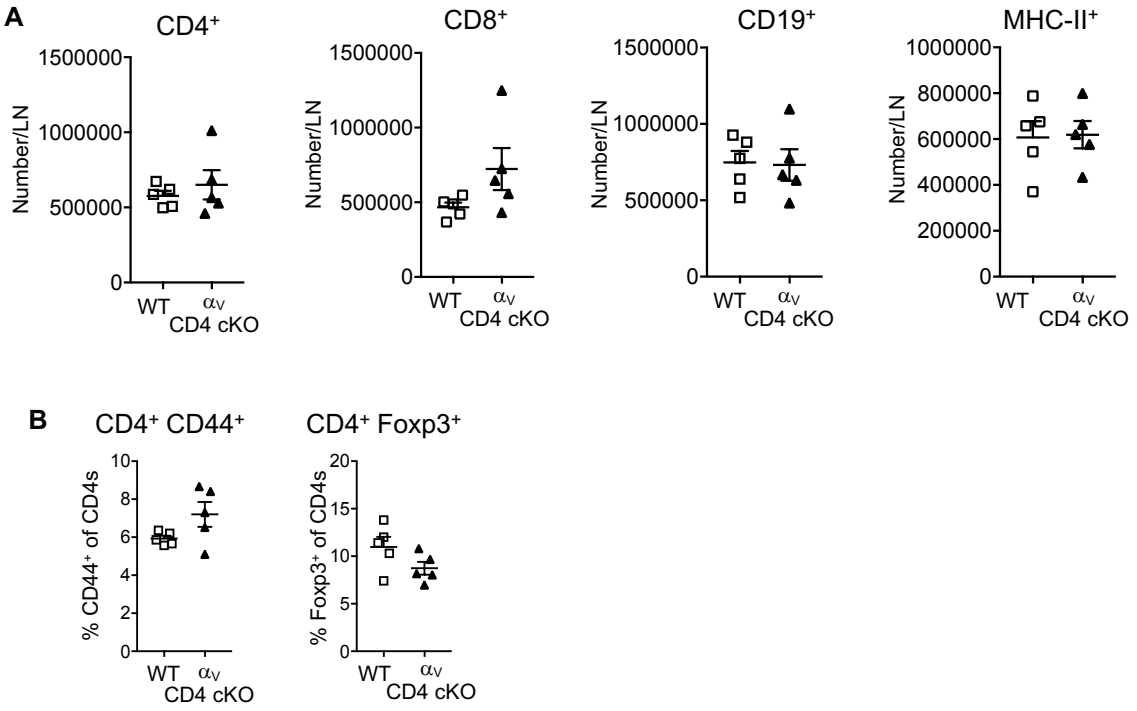

Figure S2. Steady state lymphoid populations in α_V-CD4 cKO. Flow cytometric analysis of lymphoid cell subsets in steady state (un-immunized) mice. **A** Number. **B** Frequency of CD4 subsets. No statistically significant differences between WT ($\alpha_V^{+/+}$ CD4 Cre$^+$ littermate) and α_V-CD4 cKO groups for all subsets (t test or Mann Whitney).

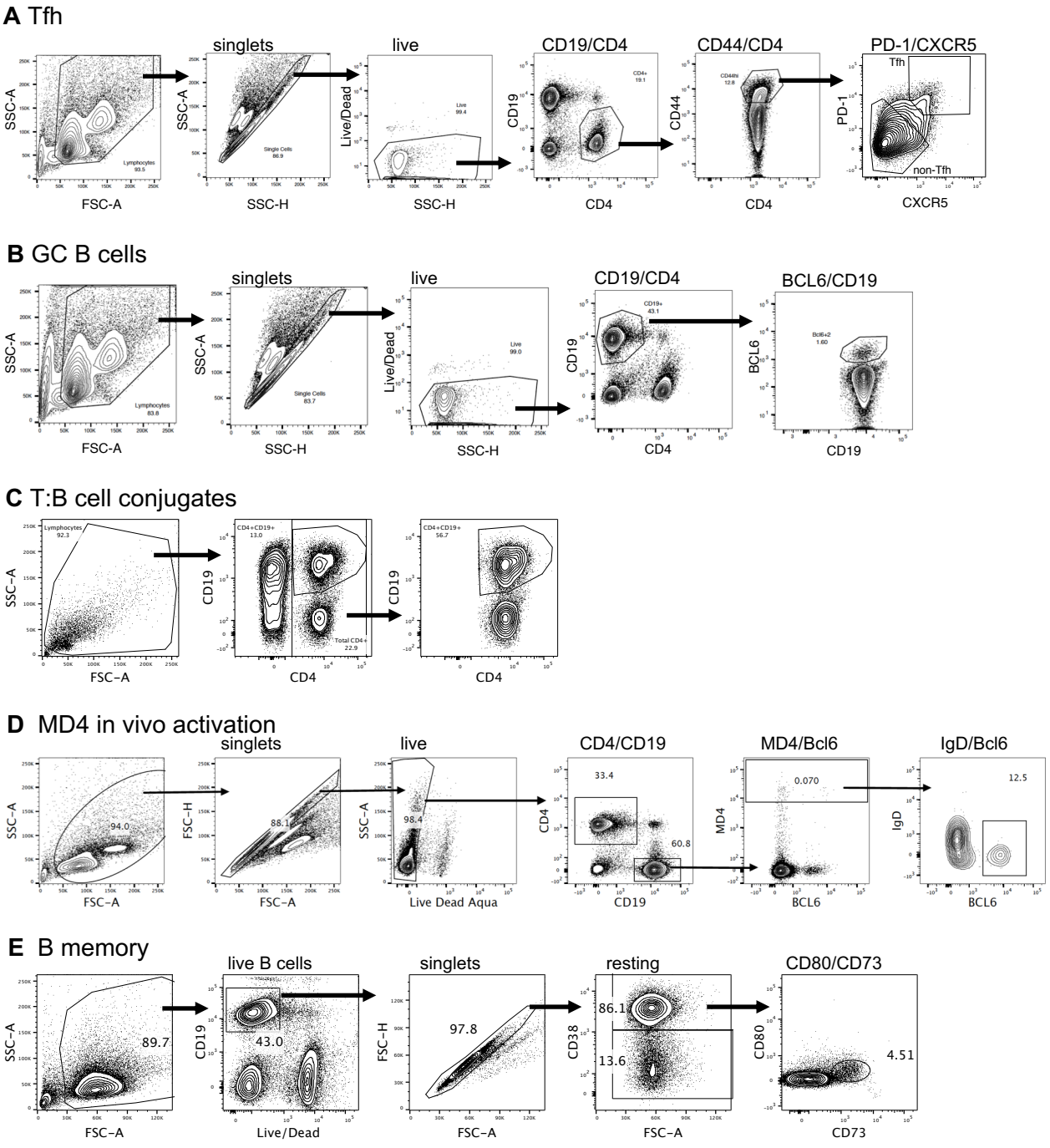

Figure S3. Gating strategies for T and B cell analysis. A Gating for CD44^{hi} Tfh and non-Tfh cells. The PD-1^{+}CXCR5^{+} population was further analyzed based on ICOS and BCL6. **B** Gating for GC B cells. The CD19+BCL6+ population was further analyzed based on Fas and GL7. **C** *In vitro* T:B cell conjugates, determining the % of CD4^{+} T cells in conjugates with CD19^{+} B cells. A-C WT, C57BL/6 or $\alpha_V^{+/+}$ CD4-Cre^{+} littermates. **D** *In vivo* T cell help based on activation of transferred MD4 Tg^{+} B cells as defined by upregulation of BCL6 expression. **E** B memory frequencies based on phenotypic analysis, resting CD38^{+} CD19^{+} cells co-expressing CD73 and CD80. WT, $\alpha_V^{+/+}$ CD4-Cre^{+} littermates.

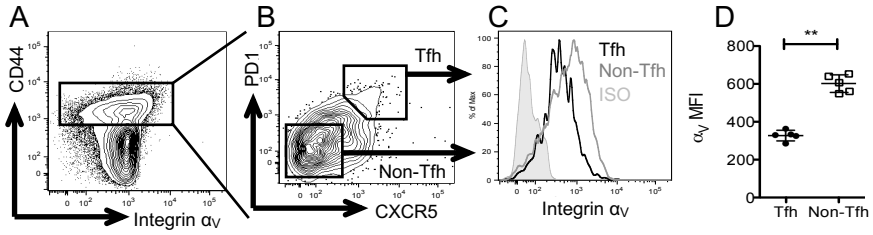

Figure S4. Integrin α_V expression by Tfh. A-D FACS analysis of α_V integrin expression by $CD4^+$ T cell populations in WT (C57BL/6) dLN 10 days post-immunization with OVA/CFA. **A** Representative FACS profile of α_V integrin and CD44 expression gated on CD4+ T cells. **B** CXCR5 and PD1 expression on $CD4^+$ $CD44^{high}$ cells from **A**, and gates used to distinguish $CXCR5^+$ $PD1^+$ Tfh and $CXCR5^-PD1^-$ non-Tfh populations. **C** Histogram showing α_V integrin levels on Tfh and non-Tfh cells from **B**. **D** α_V integrin MFI of Tfh and non-Tfh cell populations.

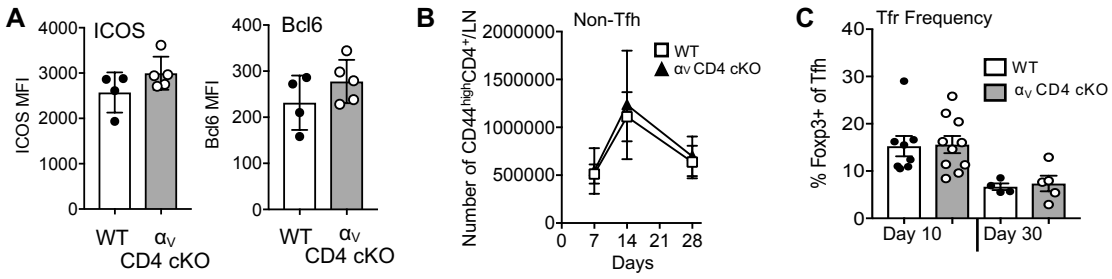

Figure S5 Tfh, non-Tfh and Tfr. A Tfh in WT ($\alpha_V^{+/+}$ CD4-Cre$^+$ littermates) and α_V-CD4 cKO dLN 10 days after OVA/CFA immunization. MFI of ICOS (left) and Bcl6 (right) expression by cells gated on CXCR5$^+$ PD1$^+$ Tfh cells. **B** Kinetics of non-Tfh CD44^{high} CD4$^+$ T cells expansion in the dLN following OVA/CFA immunization. See Figure S3 for gating strategy. WT, C57BL/6. **C** Frequency of Foxp3$^+$ Tfr cells of CXCR5$^+$ PD1$^+$ CD4$^+$ cells following CFA/OVA inflammation in dLN. Data from 3 independent experiments, 3-4 mice per group/experiment. No significant differences between WT (C57BL/6 or $\alpha_V^{+/+}$ CD4-Cre$^+$ littermates) and α_V CD4 cKO, statistics by Mann Whitney.

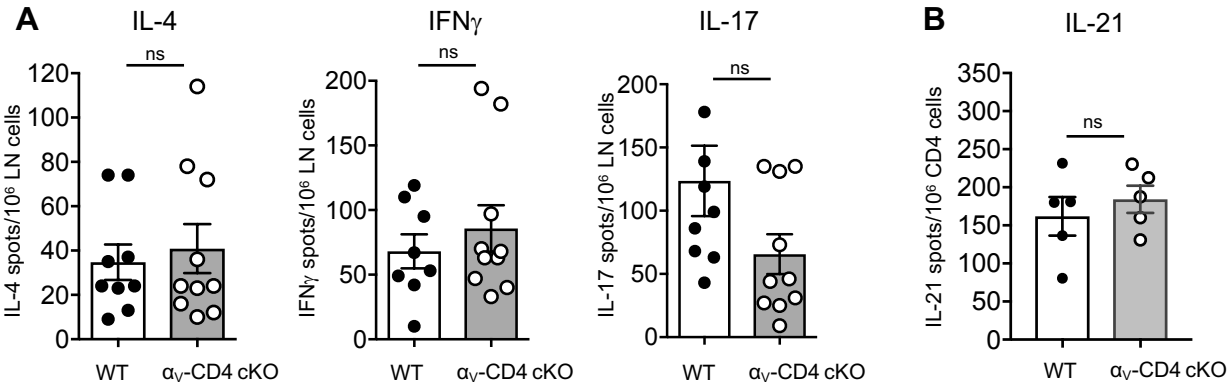

Figure S6. Antigen-specific cytokine production by WT and α_V-CD4 cKO mice. **A, B** WT ($\alpha_V^{+/+}$ CD4-Cre$^+$ littermates) and α_V-CD4 cKO mice were immunized with OVA/CFA and the dLN harvested on day 10 post-immunization. **A** OVA-specific ELISPOTS for IL-4, IFNγ and IL-17 were performed by stimulating dLN cells with/without $OVA_{323-339}$ peptide for 24h before developing and data presented as the number of cytokine secreting spots per million cells. **B** OVA-specific ELISPOTS for IL-21. Day 10 post-immunization, purified CD4 T cells from the dLN were stimulated with $OVA_{323-339}$ peptide and splenocytes as APC for 40h before developing. WT, C57BL/6. Statistics by Mann Whitney, ns P >0.05.

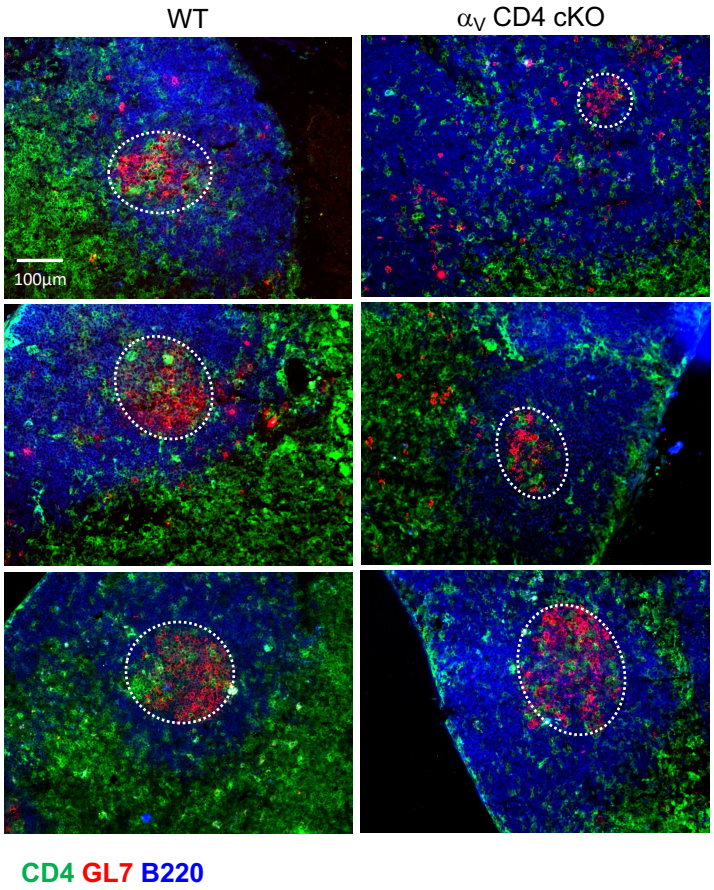

Figure S7. Mislocalization of CD4⁺ T cells to the GC in α_V-CD4 cKO mice. CD4⁺ T cell localization in the dLN of WT ($\alpha_V^{+/+}$ CD4-Cre⁺ littermates) and α_V-CD4 cKO mice d30 after CFA/OVA immunization. Representative IHC: B220⁺ (blue) B cell follicles, GL7⁺ (red) GCs, CD4⁺ (green) T cells. Dotted lines, approximate GC area defined by GL7⁺ staining.

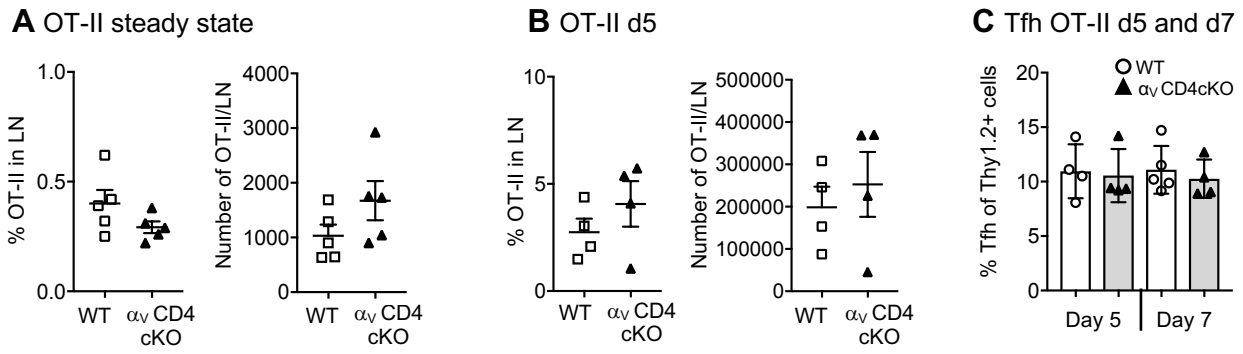

Figure S8. Tfh generation by WT and α_V-CD4 cKO OTII T cells in WT recipient mice. **A** Naïve OT-II CD62L$^+$ CD4$^+$ Thy1.2$^+$ T cells from WT ($\alpha_V^{+/+}$ CD4-Cre$^+$) and α_V-CD4 cKO mice were adoptively transferred to WT Thy1.1$^+$ congenic mice and the frequency and number of OT-II cells that homed to the lymph nodes prior to immunization as determined by flow cytometry. **B, C** Naïve OT-II transfers into congenic mice as in **A** and recipients immunized with OVA/CFA. **B** Number and frequency of Thy1.2$^+$ OT-II cells d5 post immunization. **C** Frequency of CXCR5$^+$ PD1$^+$ CD4$^+$ Tfh cells amongst the total Thy1.2$^+$ OT-II cells transferred, 5 and 7 days after immunization.

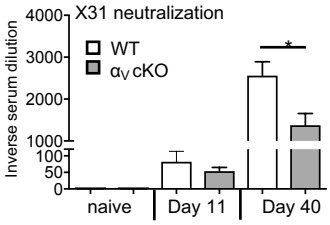

Figure S9. Viral neutralization assay. A micro-neutralization assay on sera from X31 influenza infected mice, day 11 and 40 post-infection. The inverse serum dilution represents the highest dilution of serum that was capable of protecting MDCK cell monolayers from infection. 2-3 independent experiments, 5 mice per group/experiment. WT, $\alpha_V^{+/+}$ CD4-Cre$^+$ littermates. Statistics by Mann Whitney, * P <0.05.

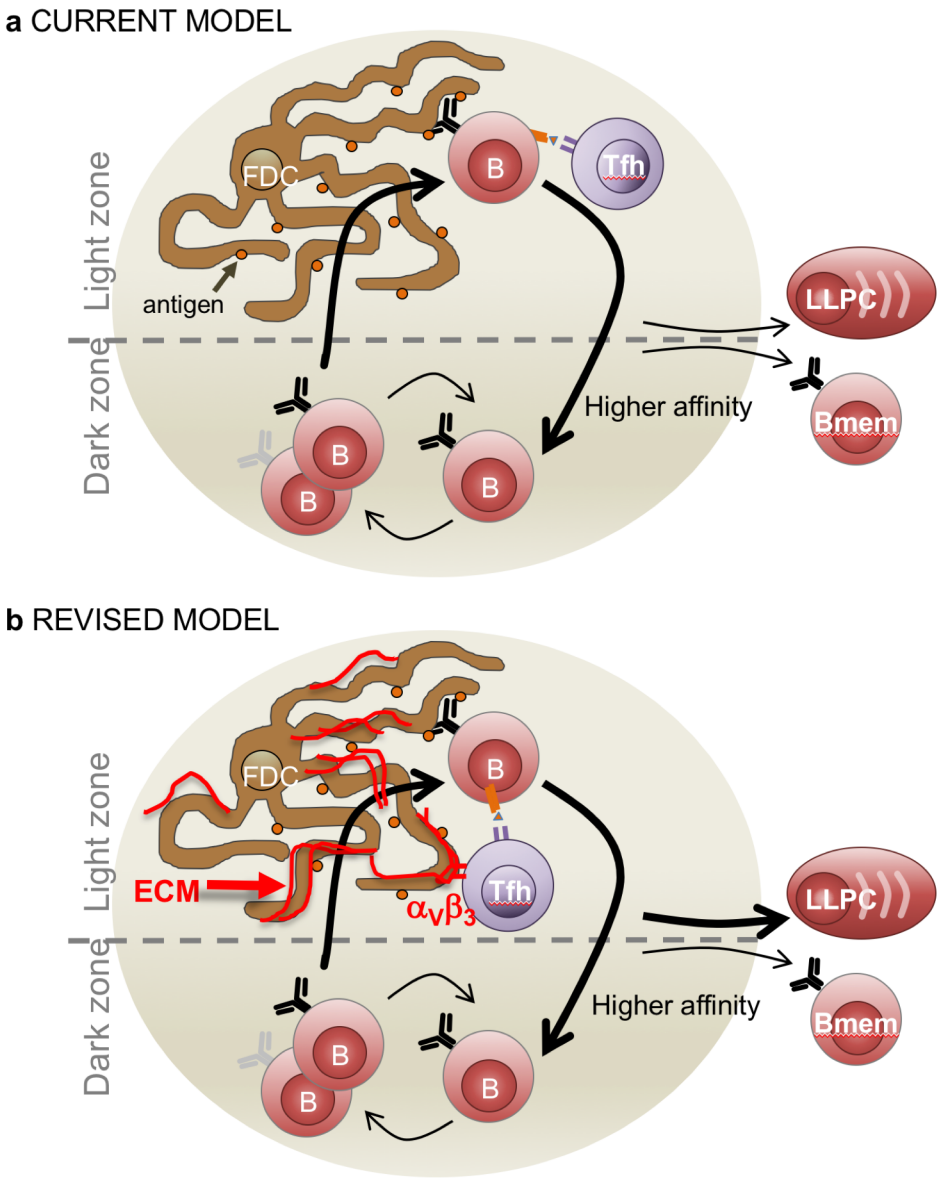

Figure S10. ECM:α_V integrin-mediated Tfh retention in the GC. **a** Current models of the GC reaction are drawn in relay: FDC interact with GC B cells for antigen exchange that is then processed and presented by GC B cells to Tfh. Most diagrams place the GC B cell between the FDC and the Tfh. In reality, the FDC form a dense network of processes that are likely to be entwined with both the GC B cells as well as the Tfh. **b** The revised model incorporates our findings on the requirement for α_V integrins for Tfh accumulation in the GC. RGD-containing ECM ligands for α_V integrin are enriched on the FDC and may provide a contact-dependent mechanism of retaining Tfh in the GC light zone. Co-localization of GC B cells and Tfh on the FDC network would facilitate T:B cell interactions for Tfh mediated selection of high-affinity GC B cells. In the absence of α_V integrins, Tfh fail to locate within the GC and their mis-localization leads to a marked defect in the generation of long-lived plasma cells (LLPC).

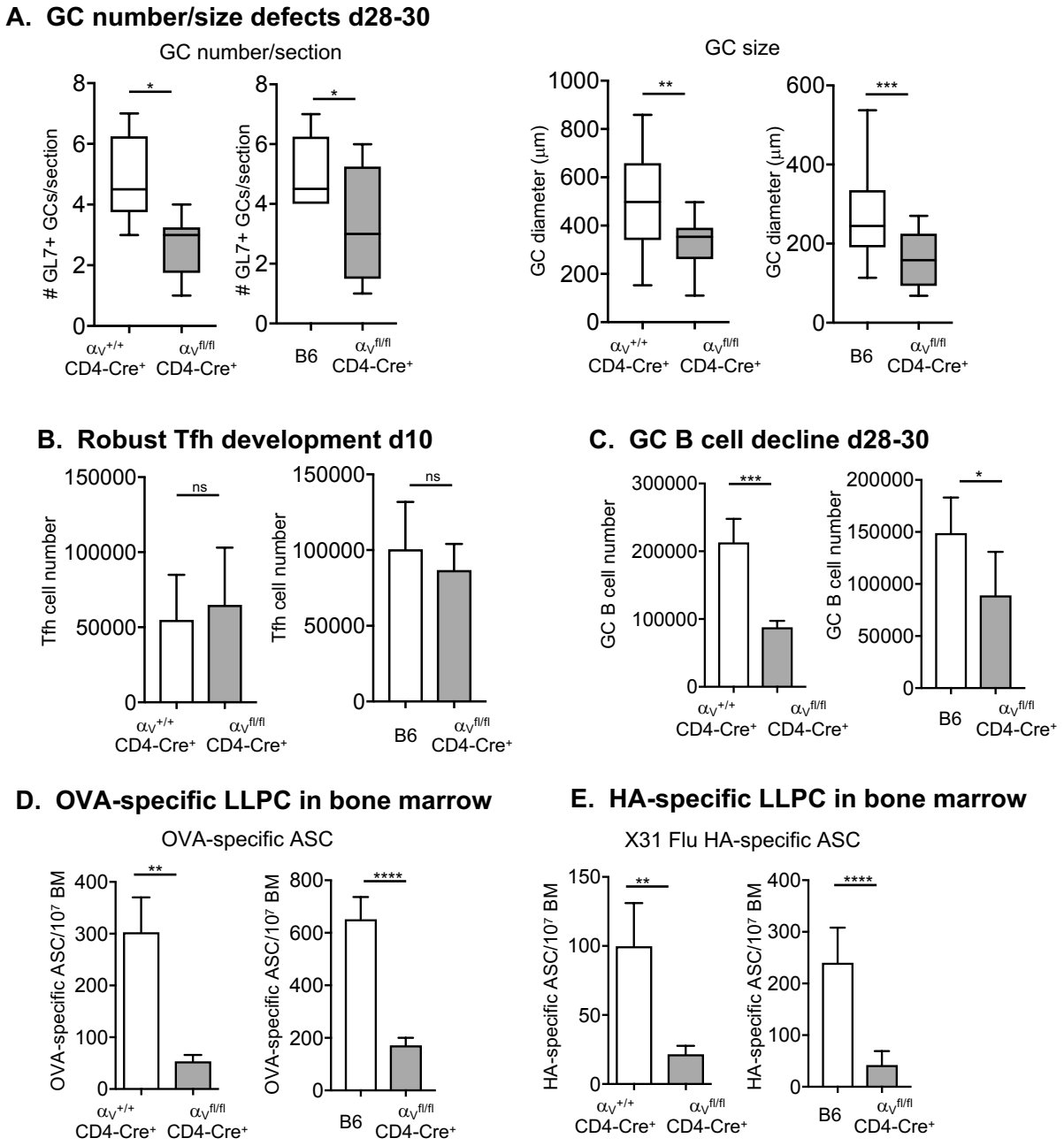

Figure S11. Comparison of control groups for major findings. For many studies of the polyclonal response to OVA/CFA in the α_V-CD4 cKO mice ($\alpha_V^{fl/fl}$ CD4-Cre$^+$) we have used either littermate controls ($\alpha_V^{+/+}$ CD4-Cre$^+$) or conventional C57BL/6 (B6) as our WT controls. To confirm that these controls can be used interchangeably, we present the data for the major findings with both control groups. Representative data from 1 of 4 independent experiments with $\alpha_V^{+/+}$ CD4-Cre$^+$ littermate controls and from 1 of 3 independent experiments with C57BL/6 (B6) controls, 4-6 mice per group per experiment. **A.** GL7$^+$ GC number and size following OVA/CFA immunization, d28-30. **B.** Tfh development following OVA/CFA immunization, d10. **C**. GC B cells following OVA/CFA immunization, d28-30. **D.** OVA-specific LLPC: d40 for $\alpha_V^{+/+}$ CD4-Cre$^+$ controls, d64 for C57BL/6 (B6) controls post-immunization, OVA/CFA. **E.** Flu HA-specific LLPC: d40 for $\alpha_V^{+/+}$ CD4-Cre$^+$ controls, d75 for C57BL/6 (B6) controls post-infection, X31 influenza. Statistics by Mann Whitney: * P <0.05. ** P <0.01, *** P <0.001, **** P <0.0001

Supplementary Information

Extended Materials and Methods

Mice and Immunizations

OT-II, Thy1.1 B6, Thy1.2 B6, MD4, and 2D2 mice purchased from Jackson Labs. *itgav* $^{fl/fl}$, α_V integrin mice were crossed to CD4-Cre B6 animals (Jackson Labs) to generate α_V-CD4 cKO mice. All mice were bred and maintained in the pathogen-free animal facility at the University of Rochester. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Rochester. Mice were immunized intradermally in both ears with a 10µl emulsion of 1µg OVA protein (Sigma, A5503) in Complete Freund's Adjuvant (Sigma, F5881) (CFA), except for experiments preformed either on naïve mice or involving MD4 transfers. Mice receiving MD4 transfers instead were immunized intradermally in both ears with a 10µl emulsion of 3.3µg OVA-HEL protein in CFA.

Immunohistochemistry

Draining cervical LNs were excised and fresh frozen in optimum cutting temperature compound (Sakura) (OCT) and stored at -80°C until sectioning. 8µm frozen sections were cut (Leica CM1950 Cryostat/Microtome) and fixed with acetone then stained for: fibronectin (AbCam ab23750), vitronectin (Santa Cruz Bio. sc-15332), MFG-E8 (LS Bio. ls-c179452), CD4 (BD RM4-5), CD3 (BD 145-2c11), B220 (BD RA3 6B2), Thy1.2 (BD 30-H12), GL7 (BD), CD35 (BD 8c12). Unconjugated antibodies were detected with donkey anti-rabbit secondary (Jackson Immuno Research) or goat anti-hamster (Biolegend), respectively. Biotinylated antibodies were detected with streptavidin (eBioscience). Imaging of stained sections was done on a Nikon Eclipse E600 (Nikon) fluorescence microscope or confocal images were acquired using a FluoView FV1000 Laser Scanning Confocal Microscope (Olympus). 15µm z-stacks with 1.64µm spacing between slices were taken with a 20x oil immersion objective lens.

Image Analysis

For analysis of ECM localization in the LN, images of reactive B cell follicles were acquired by conventional fluorescence microscopy at various time points after immunization. The B and T cell follicles were identified using B220 and CD4 staining, respectively. The integrated density of ECM staining in the B cell follicle was measured using ImageJ. For comparison, a similarly-sized region of the adjacent T cells zone was measured for each image field. To enumerate T cell infiltration of the germinal center and follicle, GL7+ regions were cropped from composite images in ImageJ. The CD3 or CD4 channel was then thresholded and binarized. Objects within the selected ROI were then counted using the ImageJ "analyze particles" function and the total number of objects per ROI area was calculated. Analysis of confocal images was performed using Imaris software (Bitplane). 3D surfaces were generated from individual channels in the Imaris "Surface" tool using a smoothing factor of 1.25 μm and a minimum cutoff of 10 voxels. Overlap was then determined by masking corresponding channels and removing non-overlapping voxels. Remaining overlapping voxels are displayed as an independent surface.

Antibodies and Flow Cytometry

All antibodies were from BD or Biolegend, unless otherwise noted. Cervical LNs were removed, mechanically separated into a single cell suspension in HBSS +2% Newborn Calf Serum (NCS) and counted. Cells were stained with Live/Dead (Invitrogen) and Fc receptors blocked with CD16/CD32 antibody (2.4G2, produced in-house). Additional markers: CD4 (RM4-5), CD44 (IM7), CXCR5 (2G8), PD1 (J43, eBioscience), ICOS (C398.4A), Bcl6 (K112-91), CD138 (281-2), CD19 (1D3), B220 (RA3 6B2), GL7 (GL7), CD95 (Jo2), Thy1.2 (30-H12), alpha V (Hma5-1, eBioscience). For memory B cells: anti-CD19, CD4, CD73 (TY/11.8), CD80 (16-

10A1) and CD38 (90/CD38). Samples were run on a BD LSR II 18 color flow cytometer (BD Biosciences) at the URMC Flow Cytometry core. Data analyzed using FlowJo (Tree Star).

CD4+ T cell transfers for T cell localization to GC

Naïve CD4+ T cells were isolated from LNs of WT or α_V-CD4 cKO OT-II Thy1.2$^+$ animals. CD4 T cells were enriched using a negative selection complement-mediated lysis cocktail containing anti-MHCII (M5/114), anti-CD24 (J11D), and anti-CD8 (3.155) and naïve CD4+ T cells isolated using CD62L-beads and a MACS magnetic column (Miltenyi Biotech). 5x10^5 purified WT or α_V-CD4 cKO CD62L$^+$ CD4$^+$ OT-II T cells were transferred i.v. to Thy1.1$^+$ C57BL/6 recipients. Recipient mice were immunized with OVA/CFA 12h hours after cell transfer. Draining LNs were collected 10 days after immunization.

CD4+ T cell transfers for T cell steady-state LN homing

1x10^6 purified WT or α_V-CD4 cKO CD62L$^+$ CD4$^+$ OT-II T cells were transferred i.v. to Thy1.1$^+$ C57BL/6 recipients. Pooled LN from recipient mice were harvested 18h hours after cell transfer (mice were not immunized).

***In vivo* B cell help**

Resting MD4 B cells were isolated from the LNs and spleen of MD4 mice by negative paramagnetic bead selection (130-090-862, Miltenyi Biotec). 100,000 MD4 cells were transfer i.v. into 2D2 hosts with or without 100,000 purified CD62L$^+$ CD4$^+$ OT-II T cells (either WT or or α_V-CD4 cKO) and immediate immunized intradermally in both ear with a 10µl emulsion of 3.3µg OVA-HEL protein in CFA. Draining LNs were harvested 5 days after immunization.

Isolation of *in vivo* generated Tfh

Cells from cervical LN of d10 CFA-OVA immunized mice were enriched for $CD4^+$ T cells via negative selection with a $CD4^+$ T cell isolation kit (130-104-454, Miltenyi Biotec). After enrichment CD4 T cells were stained with antibodies to CD4, CD19, CD44, PD-1 and CXCR5 before FACS sorting into Tfh ($CD19^-CD4^+$ $CD44^{high}$ $CXCR5^+$ $PD\text{-}1^+$) and non-Tfh populations ($CD19^-$ $CD4^+$ $CD44^{high}$ $CXCR5^-$ $PD\text{-}1^-$). At least 10 mice per experiments were pooled for Tfh isolation.

Serum antibody ELISA

Blood was collected via cardiac puncture, allowed to clot, then pelleted. Serum was extracted and stored at -80°C. ELISA plates were coated with 1 μg OVA protein per well, blocked with PBS containing 10% FCS (fetal calf serum), and diluted serum samples were incubated overnight to allow binding. Bound Ab detected with alkaline phosphatase-conjugated IgM (1020-04, Southern Biotech) or Biotin-conjugated IgG (1030-08, Southern Biotech) followed by an additional incubation with streptavidin-alkaline phosphatase (016-050-084, Jackson Immuno Research) followed by development with Phosphatase substrate (A9226, Sigma) diluted in Alkaline buffer solution (A9226, Sigma) according to manufactures instructions. Optical density measured at 450 nM with Vmax plate reader (Molecular Devices) . Dilution curves were used to generate EC50 values for each sample in MatLab. Antibody affinity was measured using a published technique (Dauner et al 2012 *Mol Cell Probes* 26:73-80): after incubation on OVA-coated plates, samples were treated with 1.5M GuHCl for 5 minutes. After GuHCl treatment, detection was performed as above and the % reduction in OD was calculated from PBS-treated controls.

T:B cell Adhesion Assay

Resting B cells were isolated from the spleens of naïve C56BL/6 mice with a B cell isolation kit (130-090-862, Miltenyi Biotec) and cultured for 40h with 1μg/mL LPS. Naïve CD4 T

cells ($CD4^+CD62L^+$) were isolated from the spleen of C56BL/6 or α_V-CD4 cKO mice as described above and cultured overnight with 10u/mL of human IL-2. LPS-stimulated B cells were loaded with OVA peptide (323-339) (0.2-20 uM) for one hour at 37˚C. 500,000 B cells and 165,000 T cells were pelleted at 1,800 RPM for 5 minutes and incubated at 37˚C for 30 minutes, and then vigorously pipetted prior to fixation (3% PFA for 20 minutes at RT). Cells were washed and Fc binding blocked with anti-CD16/32 Ab before cells were stained for CD4 and CD19. After staining cells were washed and analyzed by flow cytometry: frequency of T cells in conjugates represents the fraction of CD4+CD19+ events of the total CD4+ population.

B cell maturation assay

30,000 *in vivo* generated Tfh were FACS purified and co-cultured with 50,000 resting B cells with or without 2μg/mL anti-CD3 (2C11) and polyclonal goat anti-mouse-IgM (1020-01, Southern Biotech). On day 6 of culture, Fc binding was blocked with CD16/CD32 antibody, prior to stained cells with anti-CD4, CD19, and GL7 and analyzed by flow cytometry.

***In vitro* IgG Induction**

In vivo generated Tfh were cultured at a 1:1 ratio with purified resting B cells from the spleens of naïve C56BL/6 mice in 96-well round bottom plates (40,000-50,000 each per well). On Day 11 supernatants from co-cultures were collected and total IgG production was assayed by ELISA (see serum antibody ELISA).

Influenza A Infection

H3N2 A/Hong Kong/X31 (X31) influenza virus was grown and titered in embryonated chicken eggs. Allantoic fluid was diluted in PBS and used to infect mice intranasally (10^5 EID_{50} in 30μL). Prior to infection mice were sedated with avertin (2,2,2-tribromoethanol).

B cell ELISPOT

Plates were coated with OVA protein (100µg per well) or X-31 Hemagglutinin (100ng per well) (Sino Biological) or BSA to control for non-specific antibody capture. After coating, wells were washed with PBS and blocked with HBSS containing 10% FCS for 2 hours at room temperature. BM, spleen, or LN was lysed of red blood cells (RBC) with RBC lysis buffer and plated by limited dilution in Ag-coated plates. After incubation at 37ºC for 3-4 hours, wells were washed with PBS-Tween and anti-IgG-biotin (1030-08, Southern Biotech) added for 30 minutes. After washing, alkaline phosphatase (Jackson ImmunoResearch) was added for 30 minutes and spots detected with Vector Blue (SK-5300, Vector Laboratories). Spots were enumerated with a ImmunoSpot plate reader and software (Cellular Technology Limited, Cleveland, OH). For Bmem, spleen cells were cultured at $5x10^5$/ml and stimulated with 5µg/ml LPS (tlrl-eklps, InvivoGen) and 10U/ml rhIL-2 (Tecin, NIH) for 6 days before the OVA-specific ELISPOT.

Influenza neutralization assay

Serum samples were first Receptor Destroying Enzyme (RDE) treated per manufactures instruction (Denka Seiken) and heat inactivated at 56°C for one hour. Treated serum was diluted in serum free RPMI and incubated with 100 $TCDI^{50}$ of X-31 Influenza at 37°C for one hour. This mixture was then added to wells containing confluent MDCK monolayers for two hours at 37°C. Unbound virus was gently washed from wells and replaced with serum-free RPMI containing 0.5µg/mL TPCK trypsin. Cells were incubated 37°C for 4 days and then stained with 1% crystal violet in 10% neutral buffered formalin. Wells were scored as protective if the monolayer remained intact as revealed by crystal violet stain.