Supplemental file

Stromal cell-derived DEL-1 inhibits Tfh cell activation and inflammatory arthritis

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This file includes:

Supplemental figure 8. Transplantation of DEL-1-sufficient (WT) lymph nodes to DEL-1-deficient hosts (*Del1*^{KO}) restrains Tfh and GC-B cell responses.
Supplemental figure 9. LN stromal cell-derived DEL-1 fails to inhibit Tfh and GC B cells in recipient *Lfa1*^{KO} mice.
Supplemental figure 10. (A,B) *Del1*^{KO} mice and WT littermate controls exhibit comparable numbers of Tfh cells and GC B cells in steady state. (C) Expression levels of DEL-1 and LFA-1 in the lymph nodes (LNs) in steady state and upon induction of CIA.
Supplemental figure 11. (A) *Del1*^{KO} and WT littermate control mice exhibit similar frequencies of Th1 and Th17 cells in inguinal LNs upon CIA. (B) The frequencies of PD-1^{+}CXCR5^{-} T cells are not altered by DEL1 overexpression or deletion in the CIA model.
Supplemental figure 12. DEL-1 inhibits activation of non-Tfh cells in vitro.
Supplemental figure 13. WT, *Del1*^{KO}, EC-Del1, and ColVI-Del1 mice have similar articular cartilage thickness at steady state.

Supplemental Methods

Reagents

MAbs to the following mouse molecules were purchased from Biolegend: CD45 (clone 30-F11, catalog 103110, 103106), CD19 (clone 6D5, catalog 115510), B220 (clone RA3-6B2, catalog 103204), CD3 (clone 17A2, catalog 100216), CD4 (clone GK1.5, catalog 100406, 100408, 100412, 100430 or 100410), PD-1 (clone 29F.1A12, catalog 135216 or 135218), ICOS (clone C398.4A, catalog 313506), CD11b (clone M1/70, catalog 101212), Ly6G (clone 1A8, catalog 127654), F4/80 (clone BM8, catalog 123110), ICOSL (clone HK5.3, catalog 107405), CD80 (clone 16-10A1, catalog 104706), CD86 (clone GL-1, catalog 105026), OX40L (clone RM134L, catalog 108812), IL-17A (clone TC11-18H10.1, catalog 506908), IFN-γ (clone XMG1.2, catalog 505826), Ki-67 (clone 16A8, catalog 652410 or 652406), TNF-α (clone MP6-XT22, catalog 506328), IL-2 (clone JES6-5H4, catalog 503824), CD146 (clone ME-9F1, catalog 134704, 134718), CD31 (clone 390, catalog 102410) and isotype controls isotype controls (IgG1, clone RTK2071, catalog 400406, 400416; IgG2a, clone RTK2758, catalog 400509, 400522, 400536, 400506, 400530, 400508; IgG2b, clone RTK4530, catalog 400610, 400628, 400606 or 400612). MAbs to CXCR5 (clone 2G8, catalog 551960), BCL6 (clone K112-91, catalog 563363 or 561522), FAS (clone Jo2, catalog 565130), phospho-ZAP70 (clone 17A/P-ZAP70, catalog 557881) and isotype controls (IgG1, IgG2a and IgG2; clones X40, R35-95 and Ha4/8 respectively; catalog 562438, 340761, 553928 or 565777) were obtained from BD Bioscience. Fluorescein-labeled peanut agglutinin (PNA, catalog FL-1071-5) and

Vector Blue alkaline phosphatase substrate kit were purchased from Vector Laboratories. Peroxidase-labeled goat anti-mouse IgG1(catalog 1071-05), IgG2a (catalog 1081-05) or IgG2b (catalog 1091-05) antibodies were purchased from SouthernBiotech. Alkaline phosphatase-conjugated streptavidin was obtained from Jackson ImmunoResearch. MAbs to IL-21 (clone FFA21, catalog 12-7211-82), IgG2b isotype control (clone eBMG2b, catalog 11-4732-81) and phospho-STAT3 (clone LUVNKLA, catalog 11-9033-42) were from eBioscience. Recombinant anti-NG2 antibody (clone EPR23976-145, catalog ab275024) was purchased from Abcam. PE-conjugated goat anti-human IgG-Fc (catalog 12-4998-82) was purchased from Life Technologies. Full-length human DEL-1 as a fusion protein with the human IgG1-Fc fragment (DEL-1−Fc) and a point mutant of DEL-1, in which Asp [D] was replaced by Glu [E] in the RGD motif of the second EGF repeat (DEL-1[RGE]-Fc), were constructed and purified as previously described (1). Fc protein control was purchased from R&D Systems. As human and mouse DEL-1 share 96% a.a. sequence and the two proteins have similar functions (1-4), human DEL-1 was used in mouse experimental systems in the current study, as done earlier (1,4). Recombinant human DEL-1 and recombinant mouse ICAM-1-Fc chimeric protein were obtained from R&D Systems.

Isolation of primary synovial fibroblasts from mouse joints

Synovial fibroblasts were isolated using a modification of a previously described protocol (5). Briefly, mice were euthanized and knee joints were placed in Hank's Balanced Salt Solution (HBSS) without $CaCl_2$ and $MgCl_2$, supplemented with 1% penicillin/streptomycin. Joints were then placed in Dulbecco's Modified Eagle Medium (DMEM) with 10 % fetal calf serum, 1% L-glutamine and 1 mg/ml of freshly prepared collagenase type IV, and incubated at 37ºC for 1h with shaking. After vigorous vortexing, samples were centrifuged and cells were placed in culture. Change of medium was performed every three days and cells were trypsinized upon reaching 90-100% of confluence for use in downstream analysis.

Histology

Inguinal LNs and joints were collected and fixed in 4% paraformaldehyde for 20 min or 3 days, respectively, and joints were decalcified in formic acid for 2 to 3 weeks. The tissues were

immersed stepwise in 10%, 20% and 30% sucrose in PBS. The tissues were embedded in Optimal Cutting Temperature (OCT) media and 6- to 10-μm-thick sections were prepared, which were mounted on SuperFrost Plus slides. Pathology of joints was evaluated by hematoxylin and eosin (H&E) staining and observation by bright-field microscopy. Cartilage erosion was evaluated by Safranin-O and Fast Green staining according to the manufacturer's protocol (ScienCell).

ELISA

Mouse peripheral blood was collected from the orbital venous sinus and sera were analyzed by ELISA for CII-specific total IgG according to the manufacturer's protocol (Chondrex). CII-specific IgG1, IgG2a and IgG2b antibodies were measured by ELISA as previously described (6). Briefly, the plates were coated with 1μg/ml chick CII overnight at 4°C, washed and then blocked with ELISA/ELISPOT Diluent (PBS supplemented with fetal bovine serum; eBioscience) at room temperature for 1 h. Samples were then incubated for 2 h at room temperature. The plates were washed and subsequently incubated with peroxidase-labeled goat anti-mouse IgG1 (catalog 1071-05, 1/2000 dilution, SouthernBiotech), IgG2a (catalog 1081-05, 1/2000 dilution, SouthernBiotech) or IgG2b (catalog 1091-05, 1/2000 dilution, SouthernBiotech) for 1h at room temperature. The reaction was developed by adding tetramethylbenzidine (TMB) and stopped with 2M H_2SO_4. OD values were measured at 450 nm. The concentrations of antibodies in test samples were calculated by interpolation on standard curves generated by using known concentrations of isotype-specific mouse antibodies to chick Type II Collagen (IgG1, clone 35, catalog 7048; IgG2a, clone A2-10, catalog 7050; and IgG2b, clone D1-2G, catalog 7052; all from Chondrex). The concentrations of antibodies were calculated using regression analysis (log/log plot, Microsoft Excel) followed by multiplication by the dilution factors to obtain the original sample concentrations. The concentration of DEL-1 in the culture supernatants of synovial fibroblasts, pericytes and $CD4^+$ T cells were measured using a sandwich ELISA as previously described (7,8).

Flow cytometry

Inguinal LNs were collected and single-cell suspensions were prepared by gently pressing the

tissues through a 70-μm nylon mesh (Fisherbrand) with the aid of a plastic syringe piston. For staining intracellular cytokines (IFNγ, IL-17, IL-21) in effector T cells, the cells were first stimulated with PMA (50 ng/ml; Sigma) and ionomycin (500 ng/ml; Sigma) in the presence of GolgiPlug Protein Transport Inhibitor (BD) for 5 h. No stimulation was performed for staining intracellular molecules in Tfh cells. Before staining, mononuclear cells were incubated with purified anti-mouse CD16/32 (clone 93, 1/100 dilution, catalog 101302, Biolegend) for 10 min at 4°C to block Fcγ III/II receptors. For cell surface marker staining, the cells were incubated with antibodies against various markers (CD3 (clone 17A2, 1/100 dilution, catalog 100216, Biolegend), CD4 (clone GK1.5, 1/100 dilution, catalog 100406, 100412, 100430 or 100410, Biolegend), CD19 (clone 6D5, 1/100 dilution, catalog 115510, Biolegend), CXCR5 (clone 2G8, 1/50 dilution, catalog 551960, BD Bioscience), PD-1 (lone 29F.1A12, 1/100 dilution, catalog 135216 or 135218, Biolegend), PNA (catalog FL-1071-5, Vector Laboratories) and FAS (clone Jo2, 1/100 dilution, catalog 565130, BD Bioscience)) as indicated in the figures. In case the cells were also stained intracellularly, the cells were fixed and permeabilized in Cytofix/Cytoperm (BD) followed by staining with antibodies against IL-17 (clone TC11-18H10.1, 1/100 dilution, catalog 506908, Biolegend), IFNγ (clone XMG1.2, 1/100 dilution, catalog 505826, Biolegend), BCL6 (clone K112-91, 1/20 dilution, catalog 563363 or 561522, BD Bioscience), or Ki-67 (clone 16A8, 1/100 dilution, catalog 652410 or 652406, Biolegend) for 1 h and flow cytometric analysis. For intracellular phospho-Stat3 or phospho-ZAP70 staining, the cells were fixed with IC Fixation Buffer (eBioscience) and ice-cold 100% methanol, followed by staining with anti-phospho-STAT3 (clone LUVNKLA, 1/20 dilution, catalog 11-9033-42, eBioscience) and anti-phospho-ZAP70 (clone 17A/P-ZAP70, 1/5 dilution, catalog 557881, BD Bioscience) for 1 h and flow cytometric analysis.

Synovial tissues from joints were digested with 2 mg/mL collagenase type IV (Worthington) and 0.1 mg/mL DNase I (Roche) in DMEM containing 10% fetal bovine serum and penicillin/streptomycin for 30 min at 37 °C. Cells were incubated with Live/Dead fixable dye (Invitrogen) to exclude dead cells, and then were incubated with purified anti-mouse CD16/32 (clone 93, 1/100 dilution, catalog 101302, Biolegend) for 10 min at 4°C to block Fcγ III/II receptors. Cells were then stained with antibodies against CD45 (clone 30-F11, 1/100 dilution, catalog 103110, Biolegend), CD11b (clone M1/70, 1/100 dilution, catalog 101212, Biolegend), Ly6G

(clone 1A8, 1/100 dilution, catalog 127654, Biolegend) and F4/80 (clone BM8, 1/100 dilution, catalog 123110, Biolegend), and subjected to flow cytometry. Cell acquisition was performed on a NovoCyte flow cytometer (ACEA Biosciences). Data were analyzed with NovoExpress software (ACEA Biosciences).

Cell sorting

LN stromal cells were isolated as previously described (9). Briefly, inguinal lymph nodes were collected and digested with 1 mg/ml collagenase IV and 40 µg/ml DNase I for 30 min. The non-stromal cell floating fraction was collected, and the fragments were then digested with 3.5 mg/ml collagenase IV and 40 µg/ml DNase I for 5 min; the lymph node tissue fragments were subsequently disaggregated by pipetting. The cells were stained with appropriate stromal cell markers to identify pericytes ($CD45^{-}CD31^{-}NG2^{+}CD146^{+}$). Cell sorting was performed by using BD FACS Aria II SORP. $CD4^{+}$ T cells (present in the non-stromal floating fraction) were stained with PE-CD4 (clone GK1.5, 1/100 dilution, catalog 100408, Biolegend) and $CD45^{-}$ stromal cells (derived from further digestion of LN fragments and preparation of cell supsensions) were stained with PE-CD45 (clone 30-F11, 1/100 dilution, catalog number: 103106, Biolegend) for 15 minutes and sorted into $CD4^{+}$ T cells and $CD45^{-}$ stromal cells by using EasySep™ Mouse PE positive selection kit (Stemcell, 17684).

Quantitative real-time PCR

Total RNA was extracted using the GeneJET RNA Purification Kit (Thermo-Fisher Scientific) and quantified by NanoDrop spectrometry at 260 and 280 nm. The RNA was reverse-transcribed using the High-Capacity cDNA Archive kit (Applied Biosystems) and real-time PCR with cDNA was performed using the ABI 7500 Fast System, according to the manufacturer's protocol (Applied Biosystems). Data were analyzed using the comparative (ΔΔCt) method. TaqMan probes for detection and quantification of genes investigated in this paper (mouse *Del1* (*Edil3*) Mm01291247_m1; mouse *Itgal*, Mm00801807_m1) were purchased from Thermo-Fisher Scientific.

RNA in situ hybridization

RNA in situ hybridization was performed with an RNAscope 2.0 Brown kit or RNAscope fluorenscent multiplex assay kit according to the manufacturer's protocols (Advanced Cell Diagnostics). Briefly, for RNAscope 2.0 kit, the tissues were pretreated with H_2O_2 for 10 min, boiled for 30 min in retrieval buffer, followed by 30 min of protease digestion (RNAscope Protease Plus). For RNAscope fluorenscent multiplex assay kit, the tissues were baked for 30 min at 60°C, fixed with 4% PFA for 15 min, and boiled for 5 min in retrieval buffer, followed by 30 min of protease III digestion. The tissues were then incubated with *Edil3* probe (Advanced Cell Diagnostics) for 2 h at 40°C. *DapB* (a bacterial gene coding for dihydrodipicolinate reductase) and *PPIB* (a housekeeping gene coding for peptidylprolyl isomerase B) probes (Advanced Cell Diagnostics) were used as negative and positive controls respectively. The signal was amplified with sequential hybridization of amplifiers and label probes, then detected by 3,3'-Diaminobenzidine (DAB), or fluorescent color modules. *Del1* mRNA was visualized by bright-field microscopy or confocal fluorescence microscopy.

Immunohistochemistry and immunofluorescence

Sections of LNs were blocked with 5% mouse normal serum in in Tris-buffered saline and 0.05% Tween 20 (TBST) before incubation with biotin-conjugated Ab against B220 (clone RA3-6B2, 1/50 dilution, catalog 103204, Biolegend). Alkaline phosphatase-conjugated streptavidin was used as secondary reagent and incubated for 1 h at room temperature. Color was developed by using Vector Blue alkaline phosphatase substrate kit. Images were captured using Nikon Eclipse NiE automated fluorescent microscope. Sections of inguinal LNs were blocked with 5% mouse normal serum in Tris-buffered saline and 0.05% Tween 20 (TBST) before incubation with AlexaFluor 647-conjugated anti-CD146 (clone ME-9F1, 1/50 dilution, catalog 134718, Biolegend) and anti-NG2 (clone EPR23976-145, 1/25 dilution, catalog ab275024, Abcam). AlexaFluor 488-conjugated goat anti-rabbit IgG was used as secondary antibody and incubated for 1 h at room temperature. Images were captured using a Nikon C2 & Ti2E confocal microscope.

In vitro T cell activation and differentiation

Naïve $CD4^+$ T cells were isolated from the spleen of mice using EasySep Mouse Naïve $CD4^+$ T Cell isolation kit (StemCell). Mouse BMDCs were generated by culturing bone marrow cells with 20 ng/ml IL-4 and 20 ng/ml GM-CSF (Biolegend) as previously described (10). On day 6, BMDCs were stimulated with LPS (100 ng/ml) for 6 h, followed by washing three times. The LPS-activated BMDCs were then co-cultured with naïve $CD4^+$ T cells stimulated with anti-CD3 (clone 145-2C11, 5 μg/ml, catalog 100302, Biolegend) and anti-CD28 (clone 37.51, 5 μg/ml, catalog 102102, Biolegend) at a ratio of 1:10, in the presence or not of 10 μg/ml DEL-1-Fc or DEL-1[RGE]-Fc, or equal molar concentration of Fc control for 3 days (to investigate activation markers and proliferation) or 7 days (to investigate Tfh differentiation). To determine Tfh differentiation, the cells were fixed and permeabilized in Cytofix/Cytoperm (BD) followed by staining with antibodies against CD4 (clone GK1.5, 1/100 dilution, catalog 100406, 100412, 100430 or 100410, Biolegend), CXCR5 (clone 2G8, 1/50 dilution, catalog 551960, BD Bioscience), PD-1 (clone 29F.1A12, 1/100 dilution, catalog 135216 or 135218, Biolegend), ICOS (clone C398.4A, 1/100 dilution, catalog 313506, Biolegend) and BCL6 (clone K112-91, 1/20 dilution, catalog 563363 or 561522, BD Bioscience) and flow cytometric analysis. To determine the effect of DEL-1 on T cell activation markers, the cells were fixed and permeabilized with IC Fixation Buffer (eBioscience) and ice-cold 100% methanol (11), followed by staining with antibodies to pSTAT3 (clone LUVNKLA, 1/20 dilution, catalog 11-9033-42, eBioscience) or pZAP70 (clone 17A/P-ZAP70, 1/5 dilution, catalog 557881, BD Bioscience) and flow cytometric analysis. To determine the effect of DEL-1 on T cell proliferation or activation, the cells were fixed and permeabilized in Cytofix/Cytoperm (BD) and stained with antibody to Ki67 (clone 16A8, 1/100 dilution, catalog 652406, Biolegend), TNF-α (clone MP6-XT22, 1/100 dilution, catalog 506328, Biolegend) or IL-2 (clone JES6-5H4, 1/100 dilution, catalog 503824, Biolegend).

Some experiments determined the ability of DEL-1 to influence Tfh differentiation by direct action on T cells. In this case, naive splenic $CD4^+$ cells (isolated as above) were cultured for 24h on plates coated with anti-CD3/anti-CD28 (as above) under Tfh differentiation conditions: anti-IL-4 (clone 11B11, 10 μg/ml, catalog 504121, Biolegend), anti-IFNγ (clone R4-6A2, 10 μg/ml, catalog 505708, Biolegend), anti-TGF-β (clone TW7-20B9 20 μg/ml, catalog 141302, Biolegend) and IL-6

(100 ng/ml) or under non-differentiation conditions (anti-IL-4, anti-IFNγ, and anti-TGF-β without IL-6), in the presence of 10 μg/ml DEL-1-Fc or equal molar concentration of Fc control.

Binding assay

An ICAM-1 binding assay was performed essentially as previously described (12). Briefly, inguinal LNs were harvested on day 10 from mice subjected to CIA (i.d. injection of 2 mg/ml CII emulsified with 2 mg/ml CFA into the tail of WT mice) and single-cell suspensions were prepared by gently pressing the tissues through a 70-μm nylon mesh (FisherBrand) with the aid of a plastic syringe piston. Cells were resuspended in RPMI medium containing 0.1% BSA, 1mM EGTA and 5mM $Mg2^{+}$ at a concentration of $5x10^{6}$ cells/ml and incubated for 30 mins at 37° C in the presence of 2 μg/ml ICAM-1-Fc chimeric protein (R&D Systems) and increasing concentrations of DEL-1 (R&D Systems) as a potential inhibitor. The cells were washed twice and stained with Tfh markers and PE-conjugated goat anti-human IgG-Fc followed by flow cytometry. Tfh cells were identified as $CD4^{+}CD19^{-}CXCR5^{+}PD\text{-}1^{high}$.

SRBC immunization

10% packed sheep red blood cells (MP Biomedicals) were washed and resuspended in PBS. Then 2×10^{8} SRBCs were injected i.v. into the orbital sinus of mice. On day 7, mononuclear cells from inguinal LNs were collected, and stained for markers of GC B cells (CD19, PNA, Fas) and Tfh cells (CD4, PD-1, CXCR5 and BCL6), followed by flow cytometry. On days 0, 7 and 14, mouse peripheral blood was collected from the orbital venous sinus and serum was analyzed by ELISA for anti-SRBC IgG antibodies (Abnova) as well as for the levels of total IgG1, IgG2a and IgG2b (Invitrogen), according to the protocols of the manufacturers.

References for Supplemental Methods

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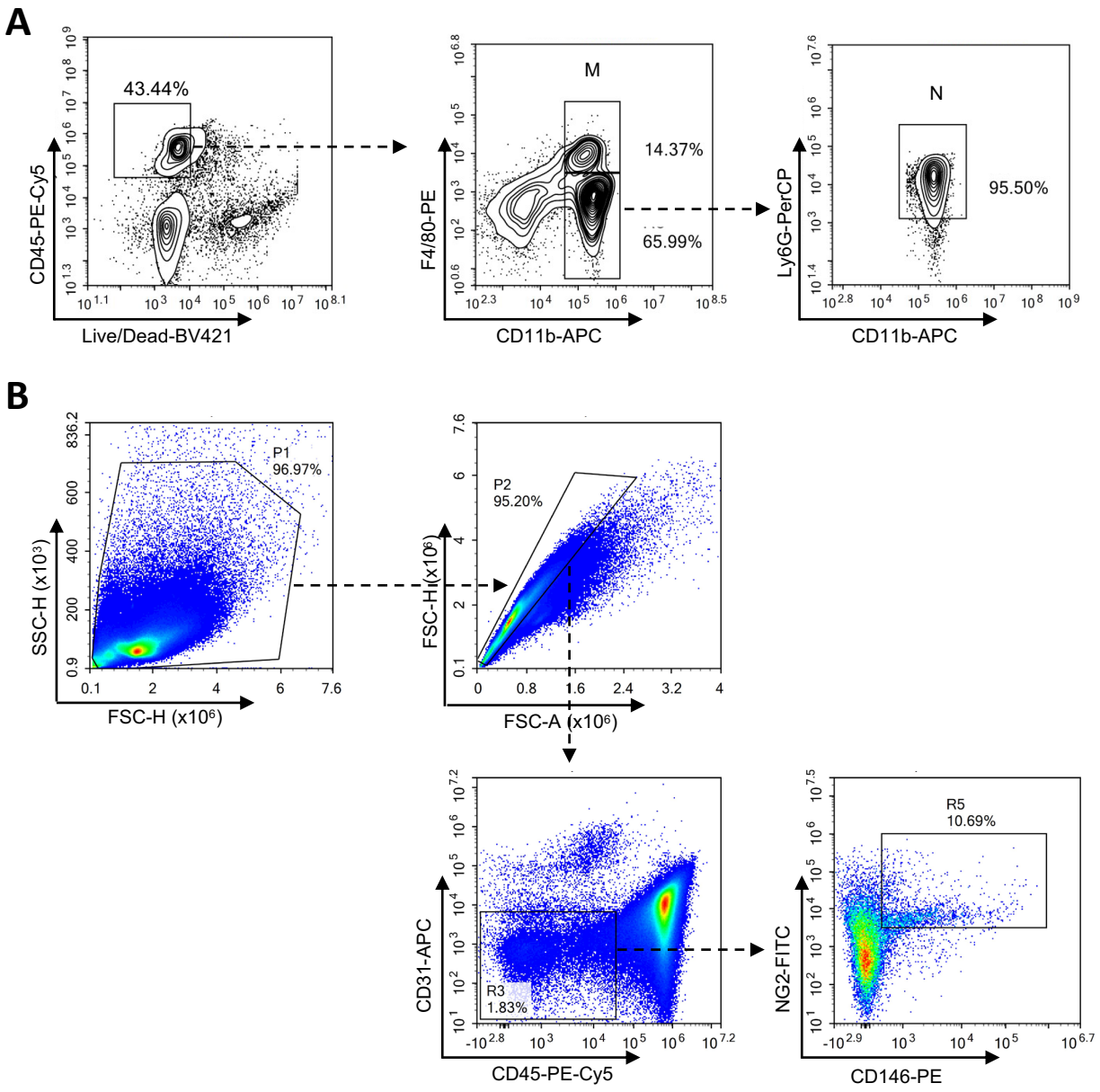
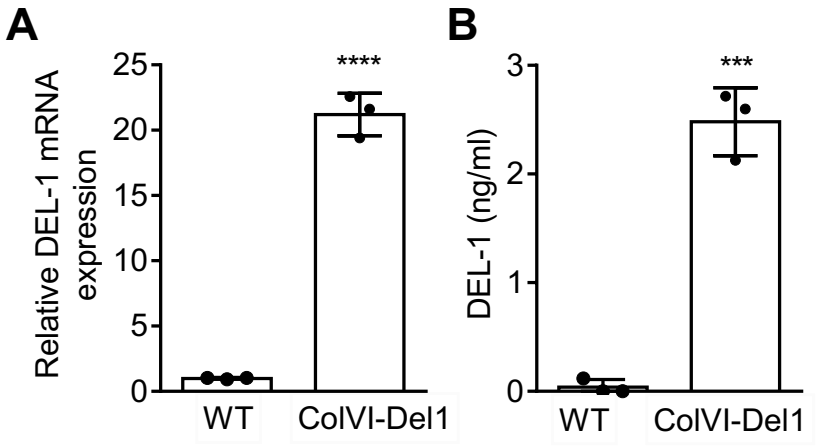
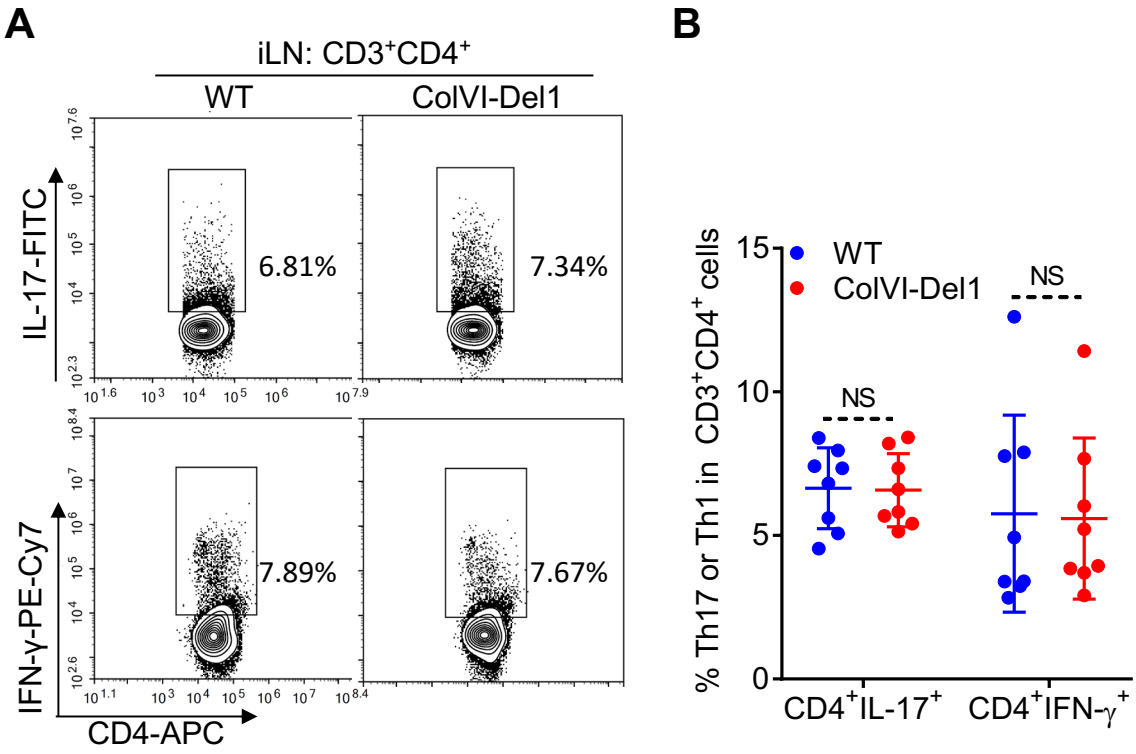


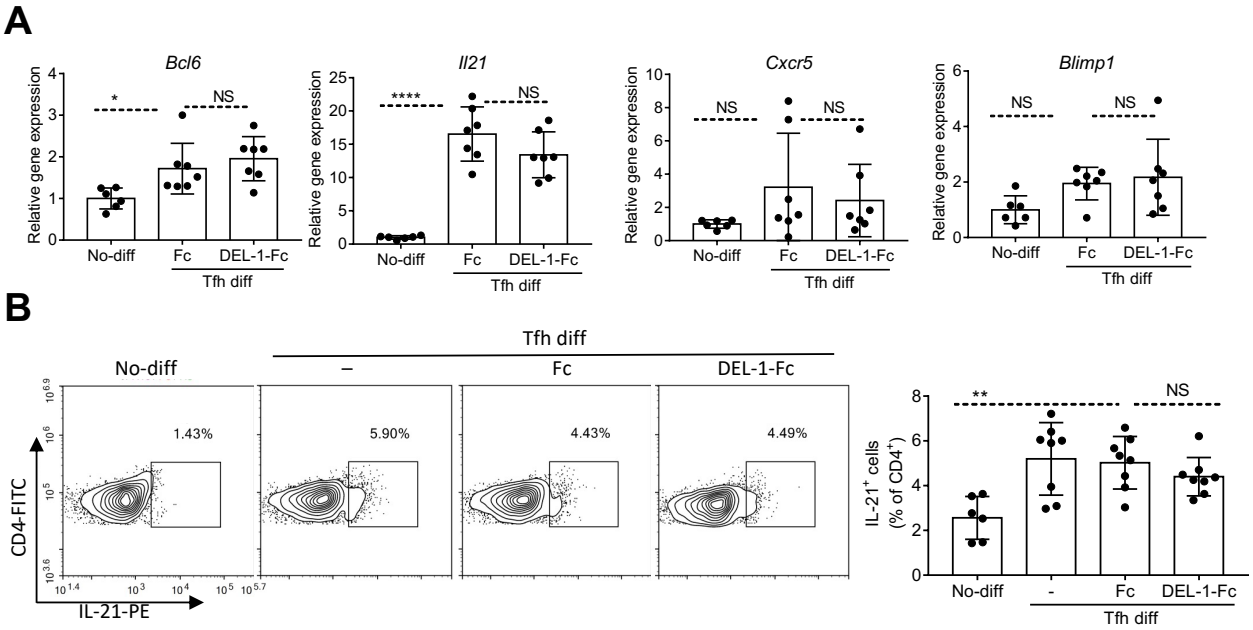
Supplemental figure 1: Flow cytometry gating strategies. (**A**) Gating strategy to identify macrophages (live $CD45^+CD11b^+F4/80^+$) and neutrophils (live $CD45^+CD11b^+F4/80^-Ly6G^+$) in the synovium of knee joints. M, macrophages; N, neutrophils. (**B**) Gating strategy of cell sorting of pericytes ($CD45^-CD31^-NG2^+CD146^+$).



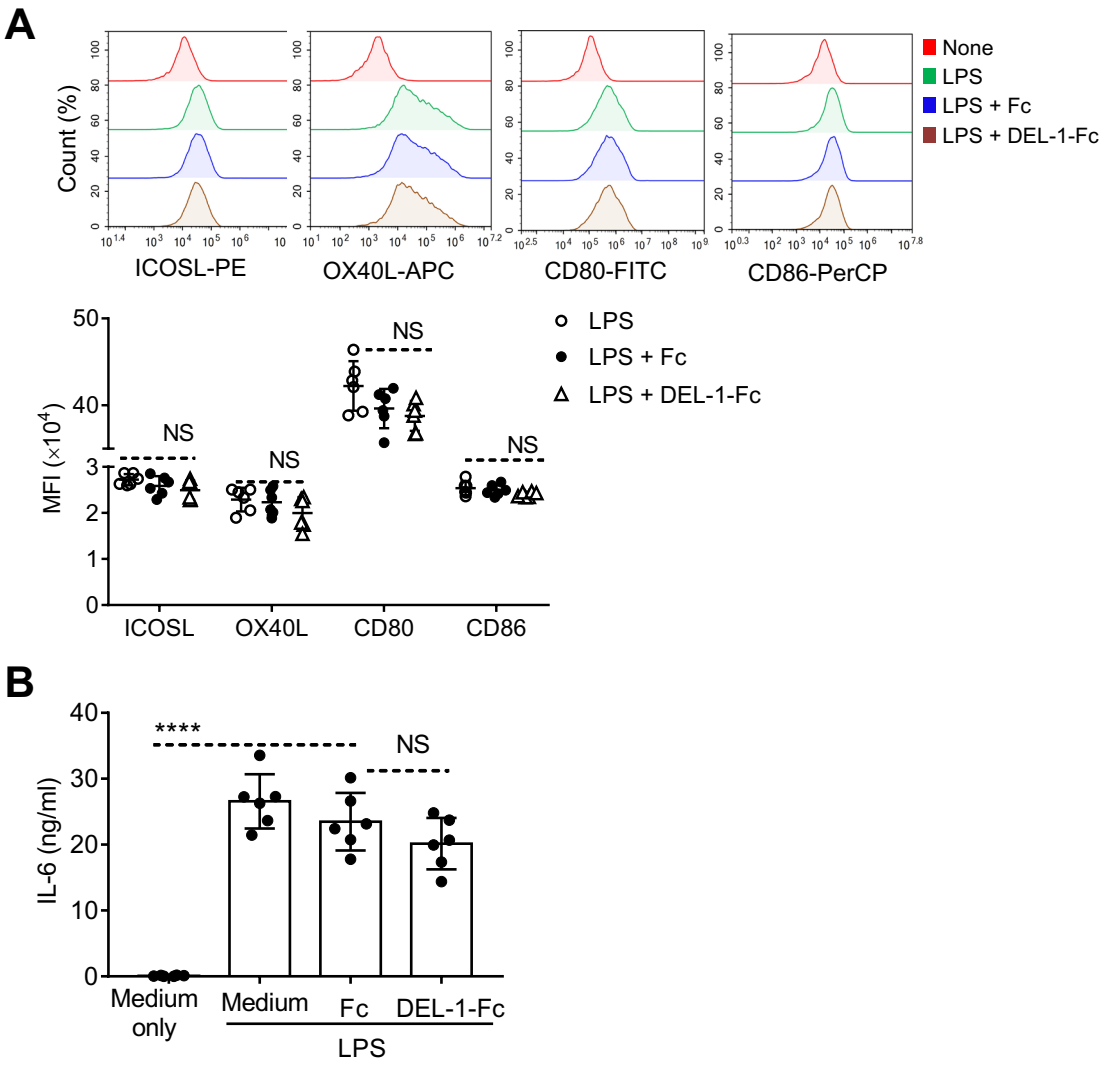
Supplemental figure 2. DEL-1 is overexpressed in synovial fibroblasts of ColVI-Del1 mice as compared to WT littermate controls. Synovial fibroblasts were isolated from the joints of WT or ColVI-Del1 mice and were cultured for 24h to determine, respectively, (**A**) *Del1* mRNA expression by qPCR or (**B**) DEL-1 protein release in culture supernatants using ELISA. Data are means ± SD (*n*= 3 cultures/group). ***$P < 0.001$; ****$P < 0.0001$. Student's unpaired *t-test*.



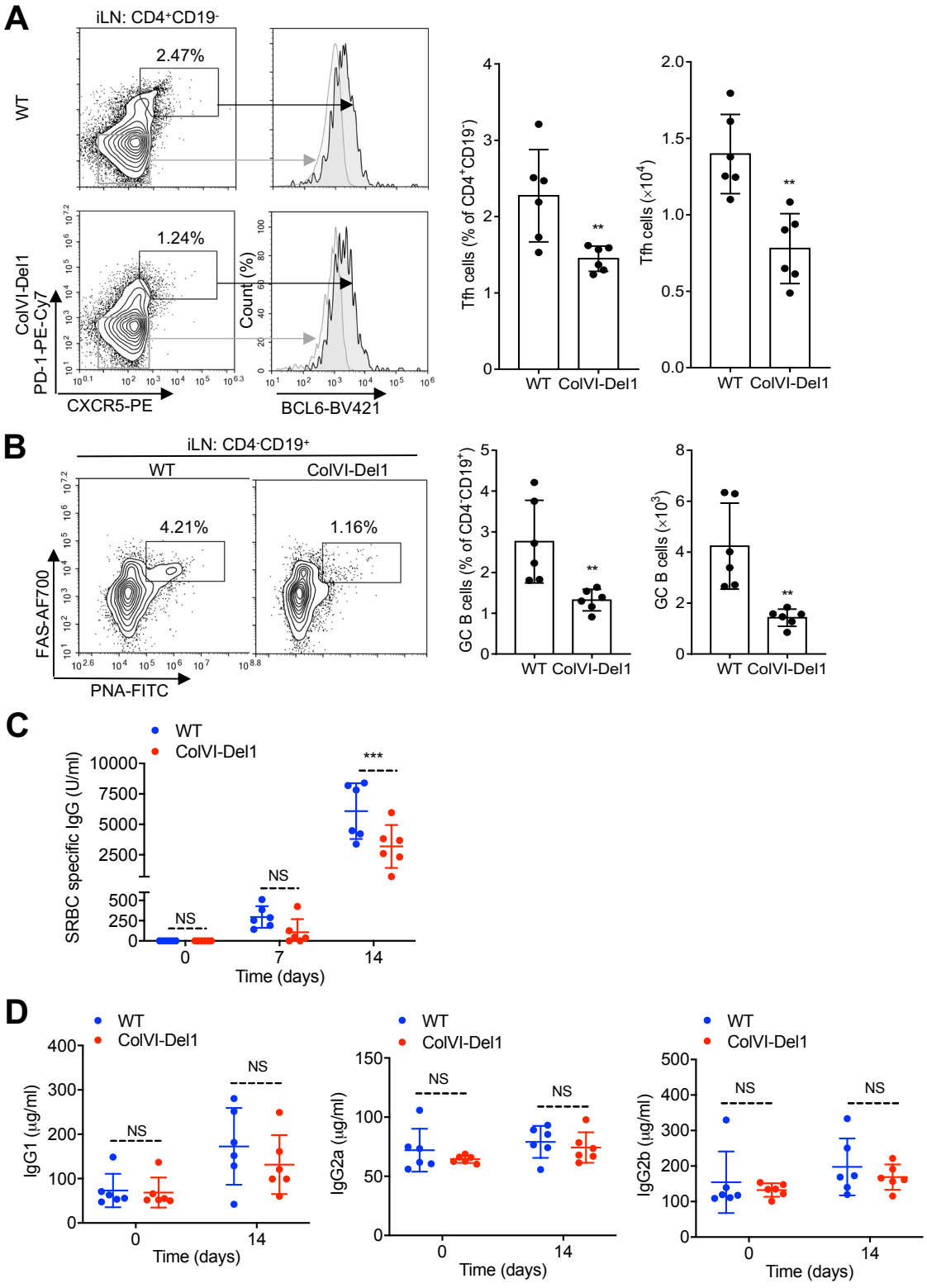
Supplemental figure 3. ColVI-Del1 mice and WT littermate display similar Th1 and Th17 cell frequencies in inguinal LNs upon CIA. CIA was induced by i.d. injection of 2 mg/ml CII emulsified with 2 mg/ml CFA into the tail at day 0 in groups of ColVI-Del1 mice and WT littermate controls. On day 10, mononuclear cells from inguinal LNs were harvested and processed for staining for CD3, CD4 as well as IFN-γ and IL-17, markers for Th1 and Th17 cells, respectively. (**A**) Representative FACS plots and (**B**) frequencies of indicated Th cell subsets. Data are means ± SD (*n* = 8 mice/group) from two independent experiments. NS, not significant. Student's unpaired *t*-test.



Supplemental figure 4. DEL-1 does not inhibit induction of Tfh differentiation markers by direct action on T cells. Naive splenic CD4$^+$ cells were cultured for 24h on plates coated with anti-CD3/anti-CD28 under Tfh differentiation conditions (anti-IL-4, anti-IFN-γ, anti-TGF-β and IL-6; 'Tfh diff') or under non-differentiation conditions (anti-IL-4, anti-IFN-γ, and anti-TGF-β without IL-6; 'No-diff'), in the presence of 10 μg/ml DEL-1-Fc or equal molar concentration of Fc control. (**A**) Cells were harvested and total RNA was extracted and processed for quantitative real-time PCR to determine expression of the indicated molecules. Data were normalized to *Gapdh* mRNA and presented as fold change in the transcript levels relative to those of controls (set as 1). (**B**) Naive splenic CD4$^+$ cells were cultured for 72h on plates coated with anti-CD3/anti-CD28 under the above-specified differentiation conditions ('No-diff' vs 'Tfh diff') and analyzed for IL-21 protein expression by flow cytometry. Shown are representative FACS plots (left) and data analysis of the percentage of IL-21$^+$ cells in CD4$^+$ T cells from the in vitro culture system. Data are means ± SD (*n*= 6-8 cultures/group, from two independent experiments). NS, not significant. One-way ANOVA and Dunnett's multiple-comparisons test except for A, *Cxcr5* panel (Kruskal-Wallis test and Dunn's multiple comparisons test).

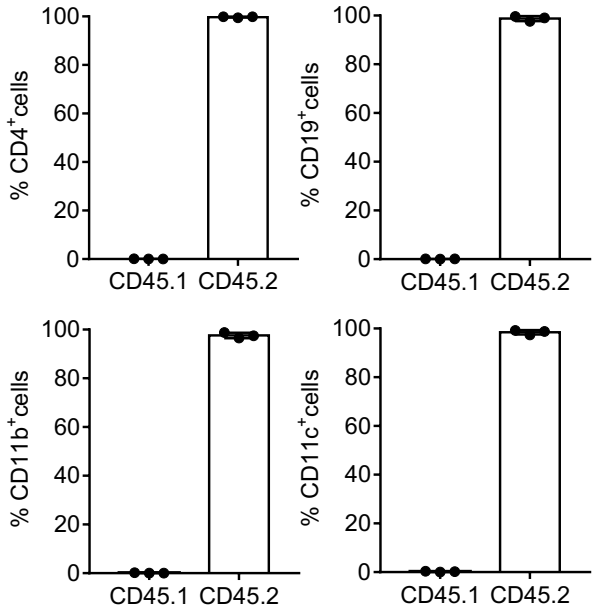


Supplemental figure 5. DEL-1 does not affect induction of costimulatory factors in DCs in vitro. BMDC were treated with LPS (100 ng/ml) in the presence or not of 10 µg/ml Del-1-Fc or equal molar concentration of Fc control for 48h. (**A**) The cells were stained for the indicated cell surface molecules and analyzed by flow cytometry. Shown are representative FACS histograms (top) and bar graphs showing individual MFI values (bottom). (**B**) The culture supernatants were collected to measure IL-6 by ELISA. Data are means ± SD (*n*= 6 cultures/group, from two independent experiments). NS, not significant. One-way ANOVA and Dunnett's multiple-comparisons test. ICOSL, ICOS ligand.

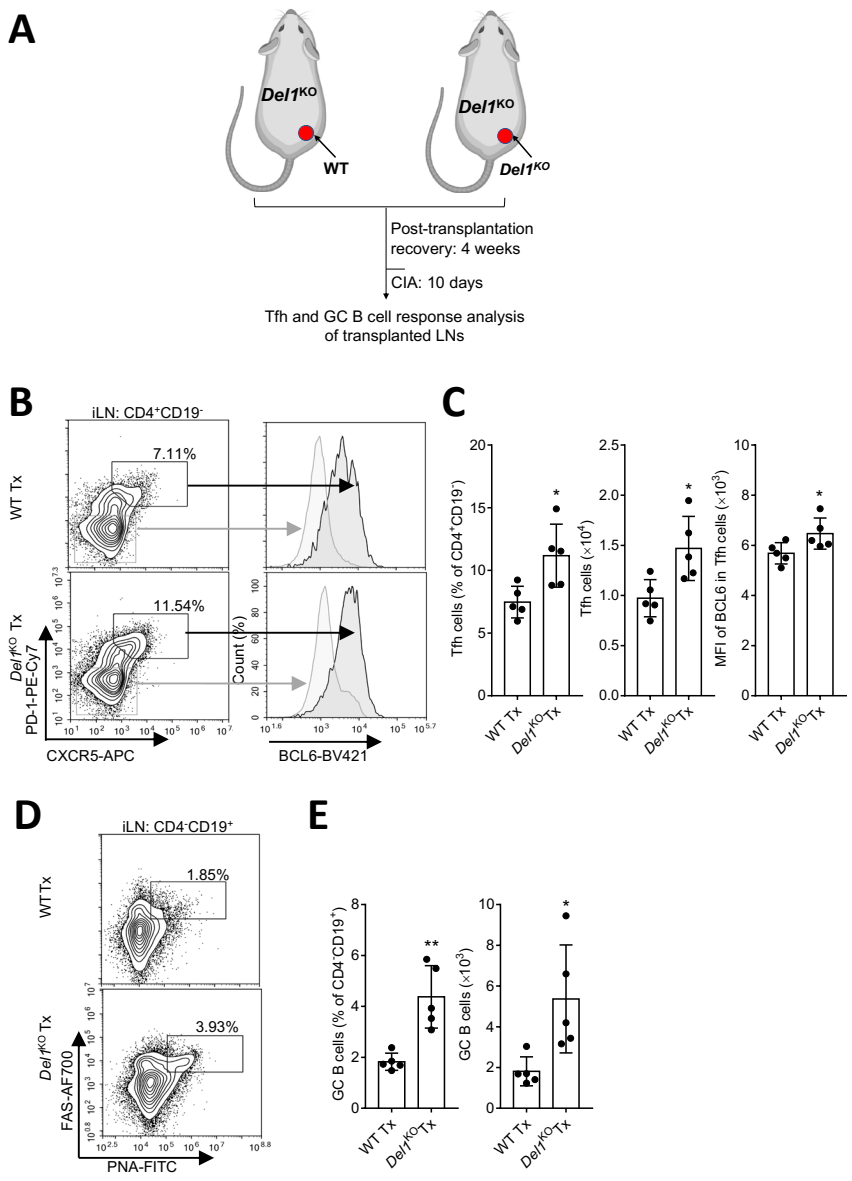


Supplemental figure 6. ColVI-Del1 mice display reduced Tfh cell and germinal center (GC) B cell responses in inguinal LNs upon sheep red blood cell (SRBC) immunization. ColVI-Del1 mice and WT littermate controls were immunized with 2 × 10^8 SRBCs i.v. On day 7, mononuclear cells from inguinal LNs were harvested and stained for the indicated Tfh and GC B cell markers followed by

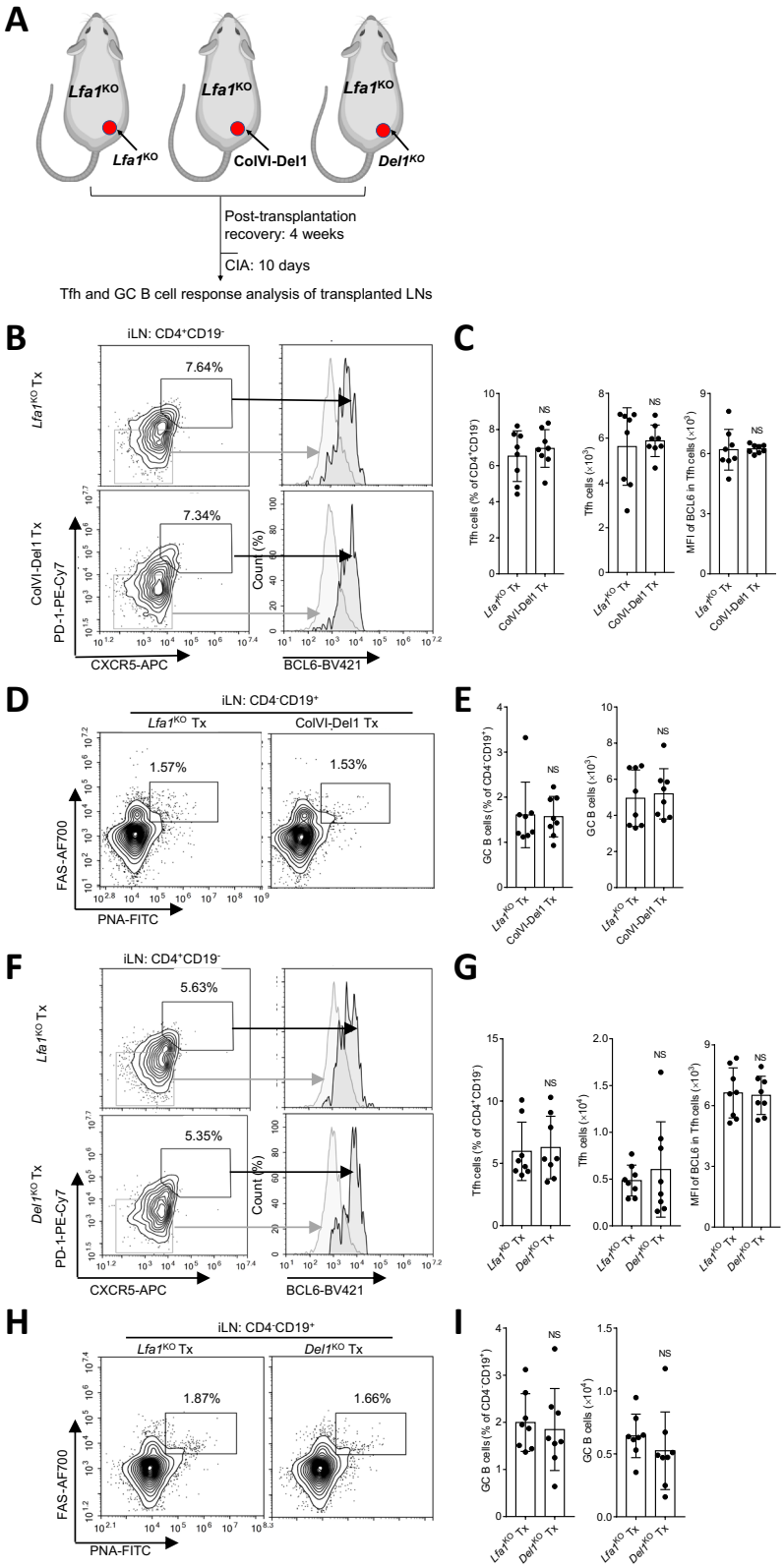
flow cytometry. (**A)** Representative FACS plots (left)and frequencies and numbers of Tfh cells defined as $CD4^{+}CD19^{-}$ $CXCR5^{+}PD\text{-}1^{high}BCL6^{high}$ cells. (**B**) Representative FACS plots (left) and frequencies and numbers of GC B cells defined as $CD4^{-}CD19^{+}PNA^{+}FAS^{+}$ cells (right). (**C**) IgG anti-SRBC Ab responses in the serum collected at the indicated timepoints. (**D**) Serum concentrations of indicated IgG isotypes in ColVI-Del1 and WT littermates sampled at day 0 (steady state) and 14 days after immunization. Data are means ± SD (*n*= 6 mice/group). $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant. Student's unpaired *t*-test (**A,B**); two-way ANOVA with repeated measures and Sidak's multiple comparisons test (**C,D**). U, units.



Supplemental figure 7. Hematopoietic cells in transplanted inguinal lymph nodes are predominantly of host (recipient) origin. Inguinal LNs from donor C57BL/6.SJL CD45.1$^+$ mice (which differ from the C57BL/6 CD45.2$^+$ strain only in expression of CD45.1 vs. CD45.2, thus allowing the distinction of donor and recipient cells) were unilaterally transplanted into CD45.2$^+$ recipient mice. After 4 weeks, LNs were collected and analyzed for CD4$^+$, CD19$^+$, CD11b$^+$ and CD11c$^+$ cells by using flow cytometry. Data are means ± SD (*n*= 3 mice/group).

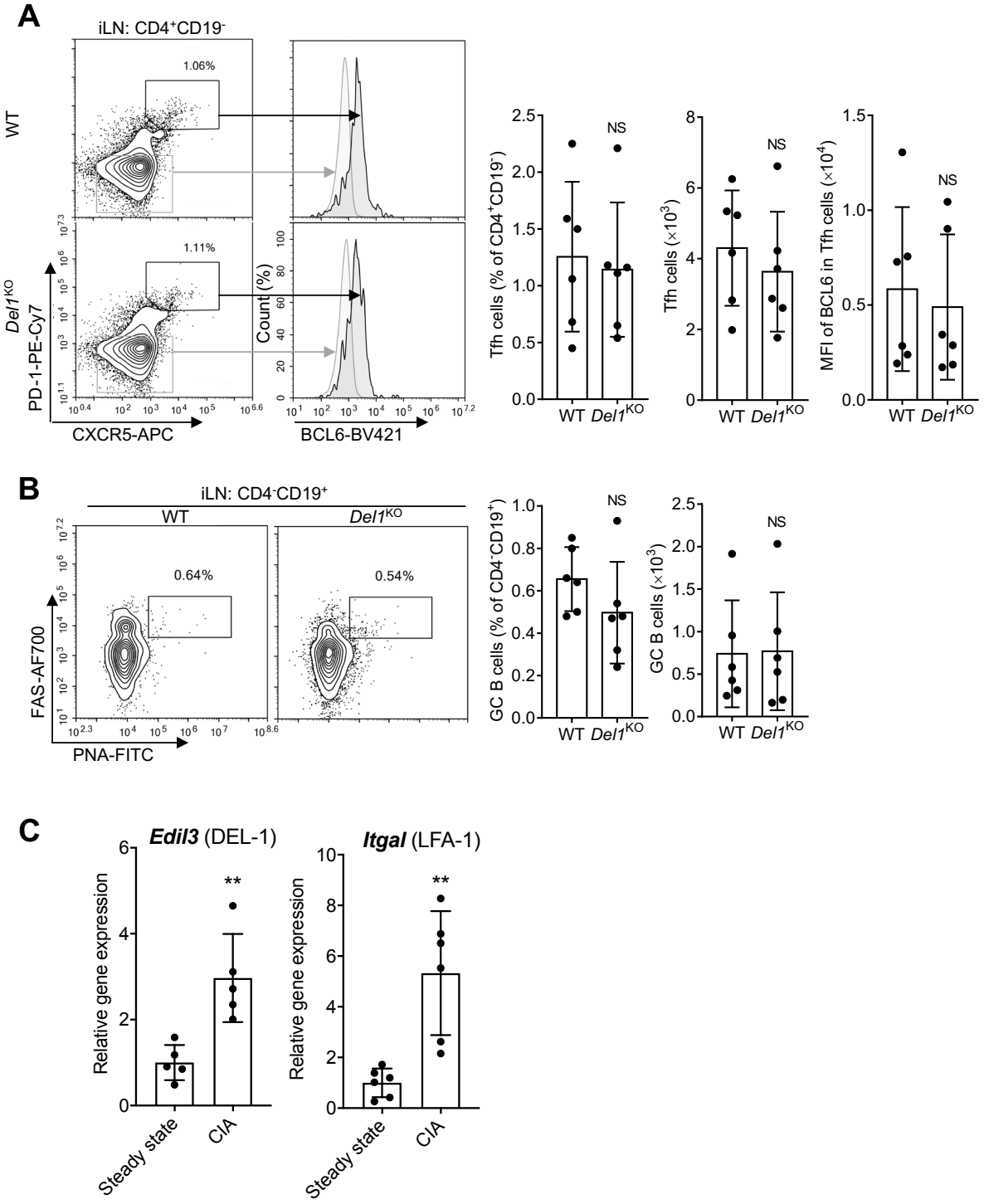


Supplemental figure 8. Transplantation of DEL-1-sufficient (WT) lymph nodes to DEL-1-deficient hosts (*Del1*^{KO}) restrains Tfh and GC-B cell responses. (**A**) LN transplantation-based experimental design to study the function of LN stromal cell-derived DEL-1 in recipient *Del1*^{KO} mice. (**B-E**) Inguinal LNs of *Del1*^{KO} recipient mice were surgically replaced unilaterally by inguinal LNs from WT or *Del1*^{KO} mice. After 4 weeks, CIA was induced in the recipient mice by i.d. injection into the tail of 2 mg/ml CII emulsified with 1 mg/ml CFA. On day 10, mononuclear cells from the transplanted inguinal LNs were harvested and analyzed for the indicated Tfh and GC B cell markers by flow cytometry. (**B**) Representative FACS plots and (**C**) frequencies and numbers of Tfh cells defined as CD4$^+$CD19$^-$ CXCR5$^+$PD-1^{hi}BCL6^{hi} cells (left and middle, respectively) and MFI of BCL6 expression (right) in CD4$^+$CD19$^-$CXCR5$^+$PD-1^{hi} cells. (**D**) Representative FACS plots and (**E**) frequencies and numbers of GC B cells defined as CD4$^-$CD19$^+$PNA$^+$FAS$^+$ cells. Data are means ± SD (n = 5 mice/group). *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Student's unpaired t-test.



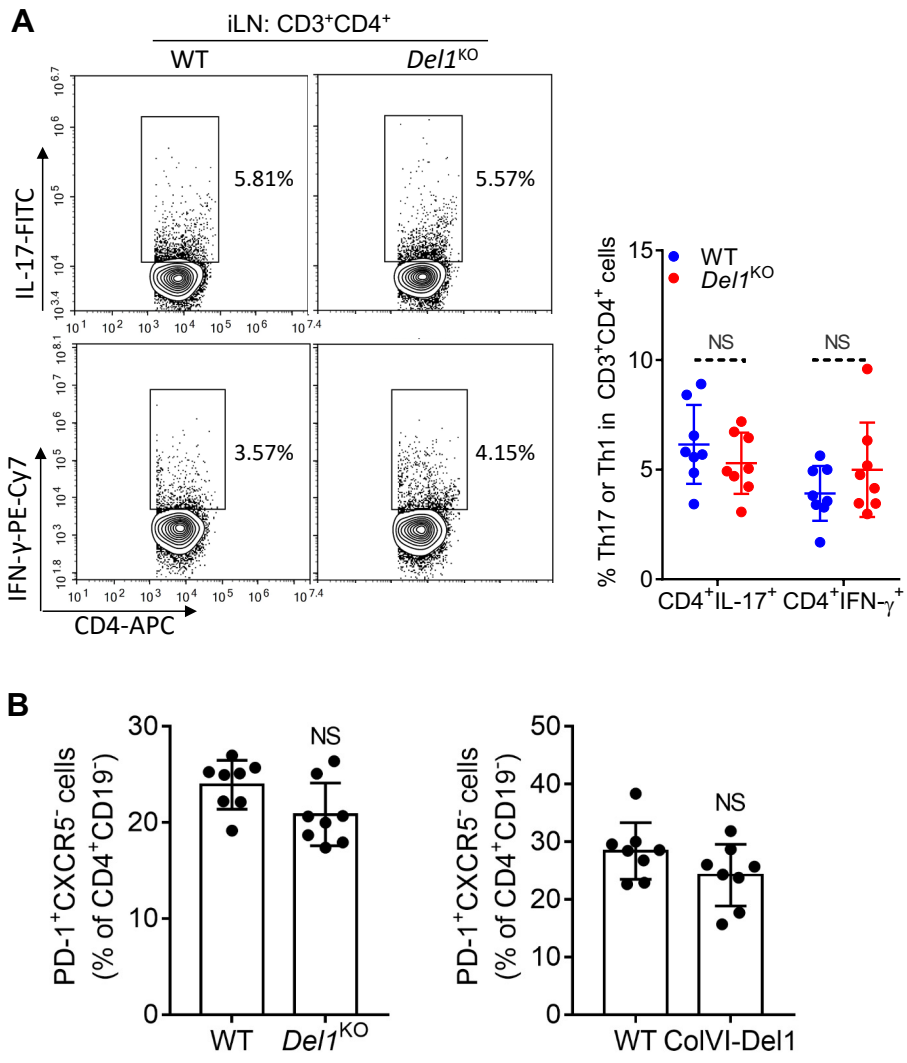
Supplemental figure 9. LN stromal cell-derived DEL-1 fails to inhibit Tfh and GC B cells in recipient *Lfa1*^{KO} mice. (A) LN transplantation-based experimental design to study the function of

LN stromal cell-derived DEL-1 in recipient *Lfa1*^{KO} mice. Inguinal LNs of *Lfa1*^{KO} recipient mice were surgically replaced unilaterally by inguinal LNs from ColVI-Del1 (**B-E**) or *Del1*^{KO} (**F-I**) mice or from *Lfa1*^{KO} donors (**B-I**), which served as control (for the surgical treatment and for expression of WT levels of DEL-1). (**B-I**) After 4 weeks, CIA was induced in the recipient mice by i.d. injection into the tail of 2 mg/ml CII emulsified with 2 mg/ml (**B-E**) or 1 mg/ml (**F-I**) CFA. On day 10, mononuclear cells from the transplanted inguinal LNs were harvested and analyzed for the indicated Tfh and GC B cell markers by flow cytometry. (**B,F**) Representative FACS plots and (**C,G**) frequencies and numbers of Tfh cells defined as $CD4^+CD19^-CXCR5^+PD\text{-}1^{hi}BCL6^{hi}$ cells (left and middle) and MFI of BCL6 expression in $CD4^+CD19^-CXCR5^+PD\text{-}1^{hi}$ cells (right). (**D,H**) Representative FACS plots and (**E,I**) frequencies and numbers of GC B cells defined as $CD4^-CD19^+PNA^+FAS^+$ cells. Data are means ± SD (*n* = 8 mice/group, from two independent experiments). NS, not significant. Student's unpaired *t*-test (**C**, **E** right, **G** middle & right, **I**); Mann-Whitney test (**E** left, **G** left).

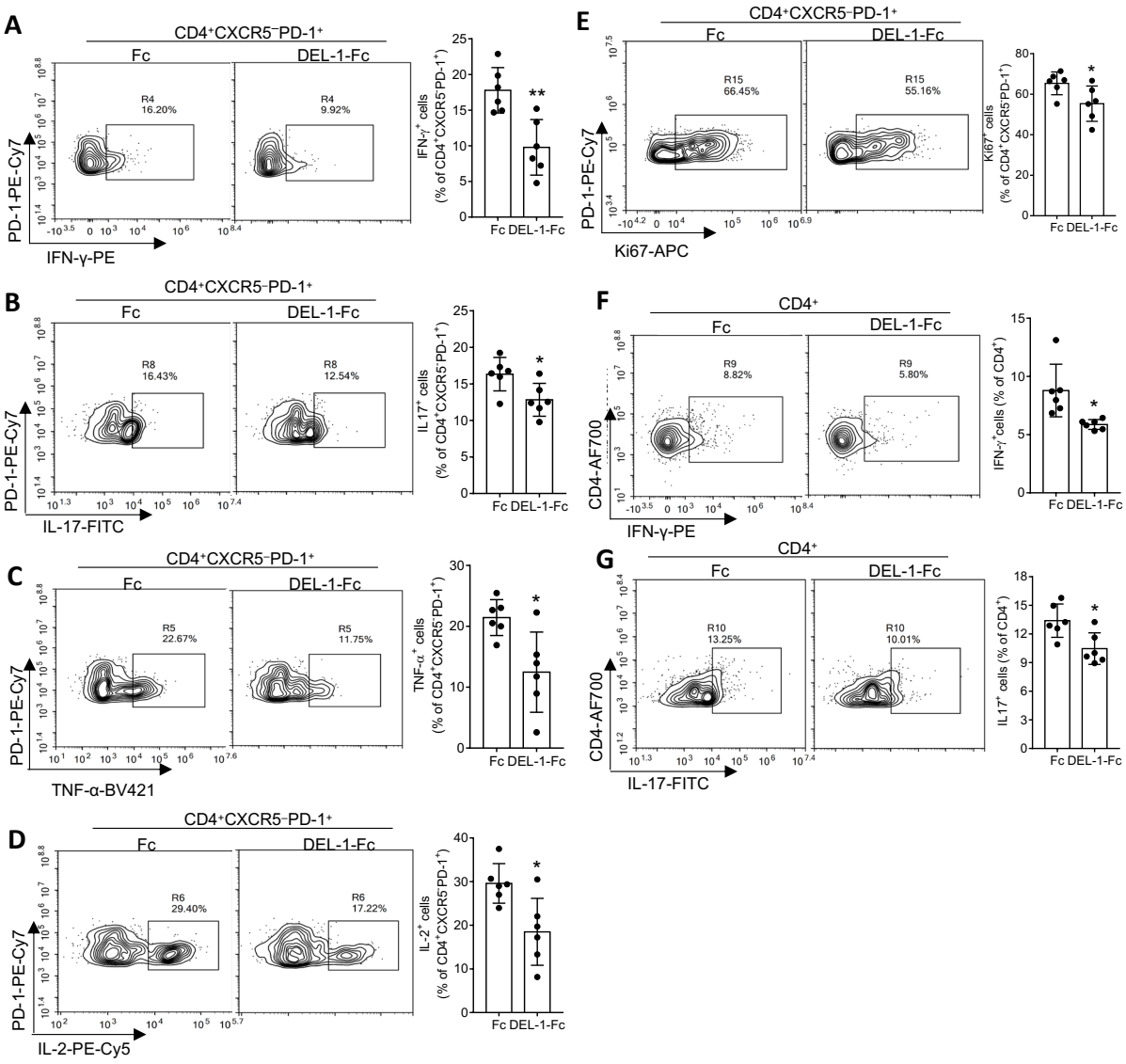


Supplemental figure 10. (A-B) *Del1*^{KO} mice and WT littermate controls exhibit comparable numbers of Tfh cells and GC B cells in steady state. Mononuclear cells of inguinal LNs from 7- to 8- week-old WT and *Del1*^{KO} mice were harvested and analyzed by flow cytometry for the indicated Tfh and GC B cell markers. (**A**) Representative FACS plots (left) and frequencies and numbers of Tfh cells defined as CD4^{+}CD19^{-}CXCR5^{+}PD-1^{high}BCL6^{high} cells (middle) and MFI of BCL6

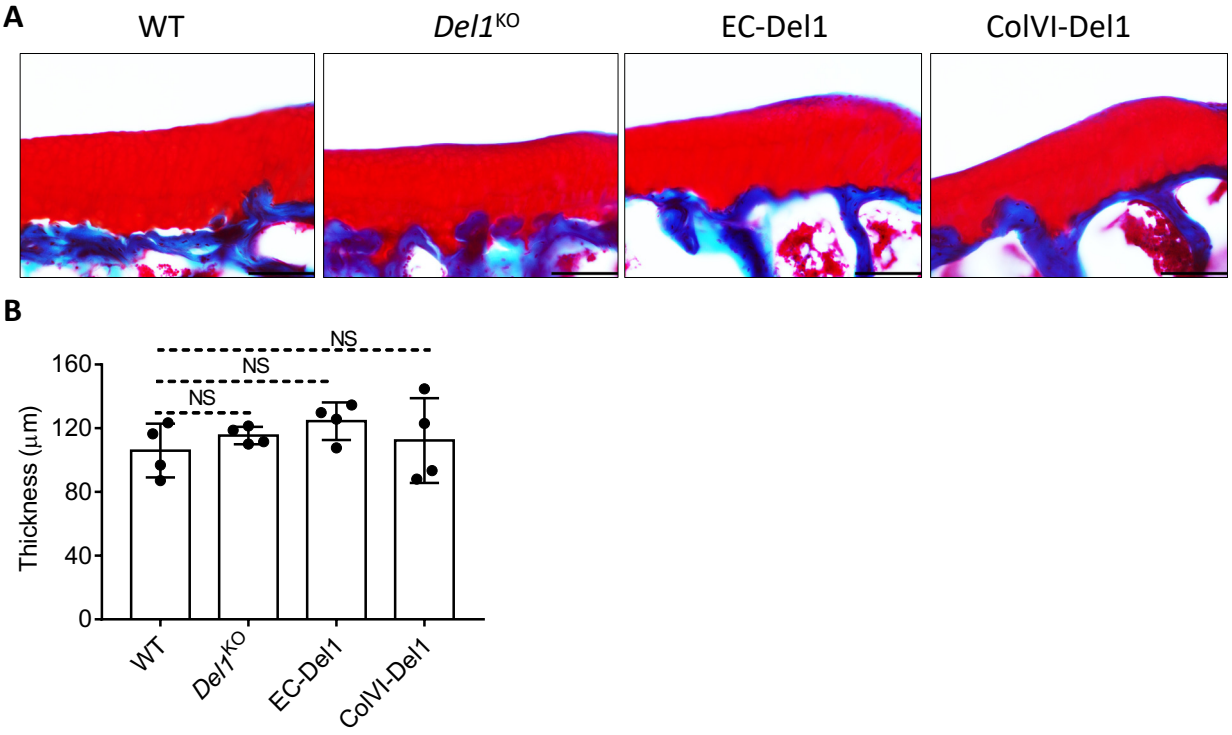
expression in $CD4^+CD19^-CXCR5^+PD\text{-}1^{high}$ cells (right). **(B)** Representative FACS plots (left) and frequencies and numbers of GC B cells (right). Data are means ± SD (*n* = 6 mice/group). NS, not significant. Student's unpaired *t*-test. **(C) Expression levels of DEL-1 and LFA-1 in the lymph nodes (LNs) in steady state and upon induction of CIA.** Inguinal LNs from 7- to 8- week-old WT mice, in steady state or upon induction of CIA for 10 days, were digested with collagenase IV and cell suspensions were prepared. Total RNA was extracted from sorted $CD45^-$ stromal cells and $CD4^+$ T cells and quantitative real-time PCR was performed to determine the expression of *Edil3* (the gene encoding DEL-1) and of *Itgal* (the gene encoding the CD11a subunit of LFA-1). Results were normalized to *Gapdh* mRNA and presented as fold change in the transcript levels relative to those at steady state (set as 1). Data means ± SD (*n* = 5-6 mice/group, from two independent experiments). $^{**}P < 0.01$. Student's unpaired *t*-test.



Supplemental figure 11. (A) *Del1*^{KO} and WT littermate control mice exhibit similar frequencies of Th1 and Th17 cells in inguinal LNs upon CIA. CIA was induced by i.d. injection of 2 mg/ml CII emulsified with 1 mg/ml CFA into the tail at day 0 in groups of *Del1*^{KO} mice and WT littermate controls. On day 10, mononuclear cells from inguinal LNs were harvested and processed for staining for CD3, CD4 as well as IFN-γ and IL-17, markers for Th1 and Th17 cells, respectively. Representative FACS plots (left) and frequencies of indicated Th cell subsets (right). **(B) The frequencies of PD-1^{+}CXCR5^{-} T cells are not altered by DEL-1 overexpression or deletion in the CIA model.** CIA was induced by i.d. injection of 2 mg/ml CII emulsified with CFA (1 mg/ml, left panel; 2 mg/ml, right panel) into the tail at day 0 in groups of *Del1*^{KO} or ColVI-Del1 mice and WT littermate controls. On day 10, mononuclear cells from inguinal LNs were harvested and analyzed by flow cytometry for the indicated cell markers. Data are means ± SD (*n* = 8 mice/group from two independent experiments). NS, not significant. Student's unpaired *t*-test.



Supplemental figure 12. DEL-1 inhibits activation of non-Tfh cells in vitro. BMDCs were treated with LPS (100 ng/ml) for 6 h, washed and then co-cultured with naïve CD4$^+$ T cells stimulated with anti-CD3 and anti-CD28, in the presence of 10 µg/ml DEL-1-Fc or equal molar concentration of Fc control for 3 days (IL-2, TNF-α, and Ki67 assays) or 7 days (all other assays). Cells were harvested and stained with the indicated markers and analyzed by flow cytometry. (**A-E**) Representative FACS histograms (left) and frequencies of IFN-γ$^+$, IL-17$^+$, TNF-α^+, IL-2$^+$ and Ki67$^+$ cells in CD4$^+$CXCR5$^-$PD-1$^+$ T cells (right). (**F,G**) Representative FACS histograms (left) and frequencies of Th1 (IFN-γ$^+$) and Th17 (IL-17$^+$) cells in CD4$^+$ T cells (right). Data are means ± SD (*n*= 6 co-cultures/group, from two independent experiments). **P* < 0.05; ***P* < 0.01 vs. Fc control. Student's unpaired *t*-test.



Supplemental figure 13. WT, *Del1*^{KO}, EC-Del1, and ColVI-Del1 mice have similar articular cartilage thickness at steady state. Knee joints from 10-week-old WT, *Del1*^{KO}, EC-Del1, and ColVI-Del1 mice were harvested, sectioned and stained with safranin-O. (**A**) Representative safranin-O-stained sections of tibial articular cartilage. Scale bars, 100 µm. (**B**) Thickness of tibial articular cartilage of the indicated genotypes, measured morphometrically (13). Data are means ± SD (*n* = 4 mice/group). NS, not significant. One-way ANOVA and Dunnett's multiple comparisons test.