Figure S1
A

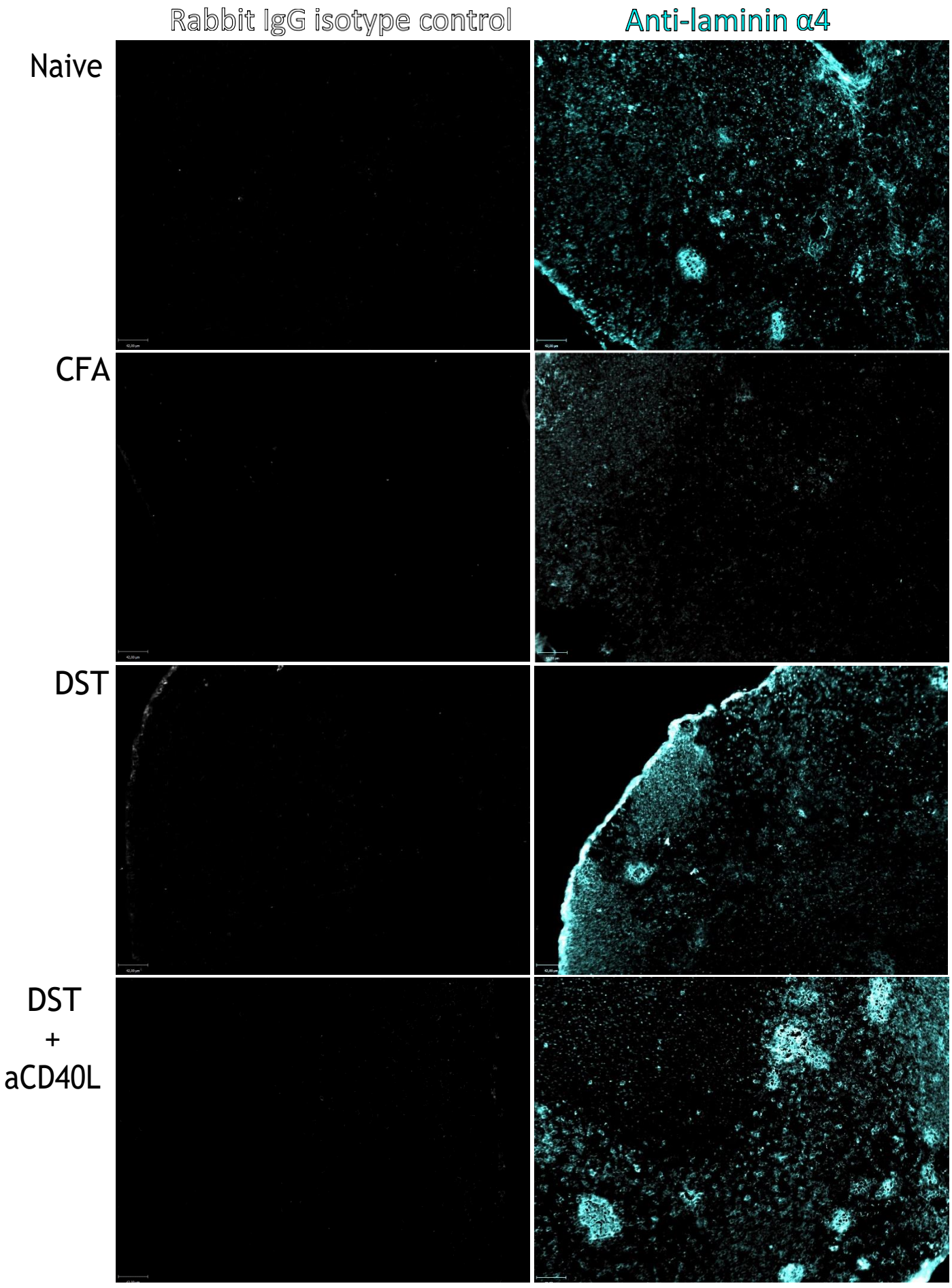

Rabbit IgG isotype control
Anti-laminin α4
Naive
CFA
DST
DST
+
aCD40L

Figure S1
B

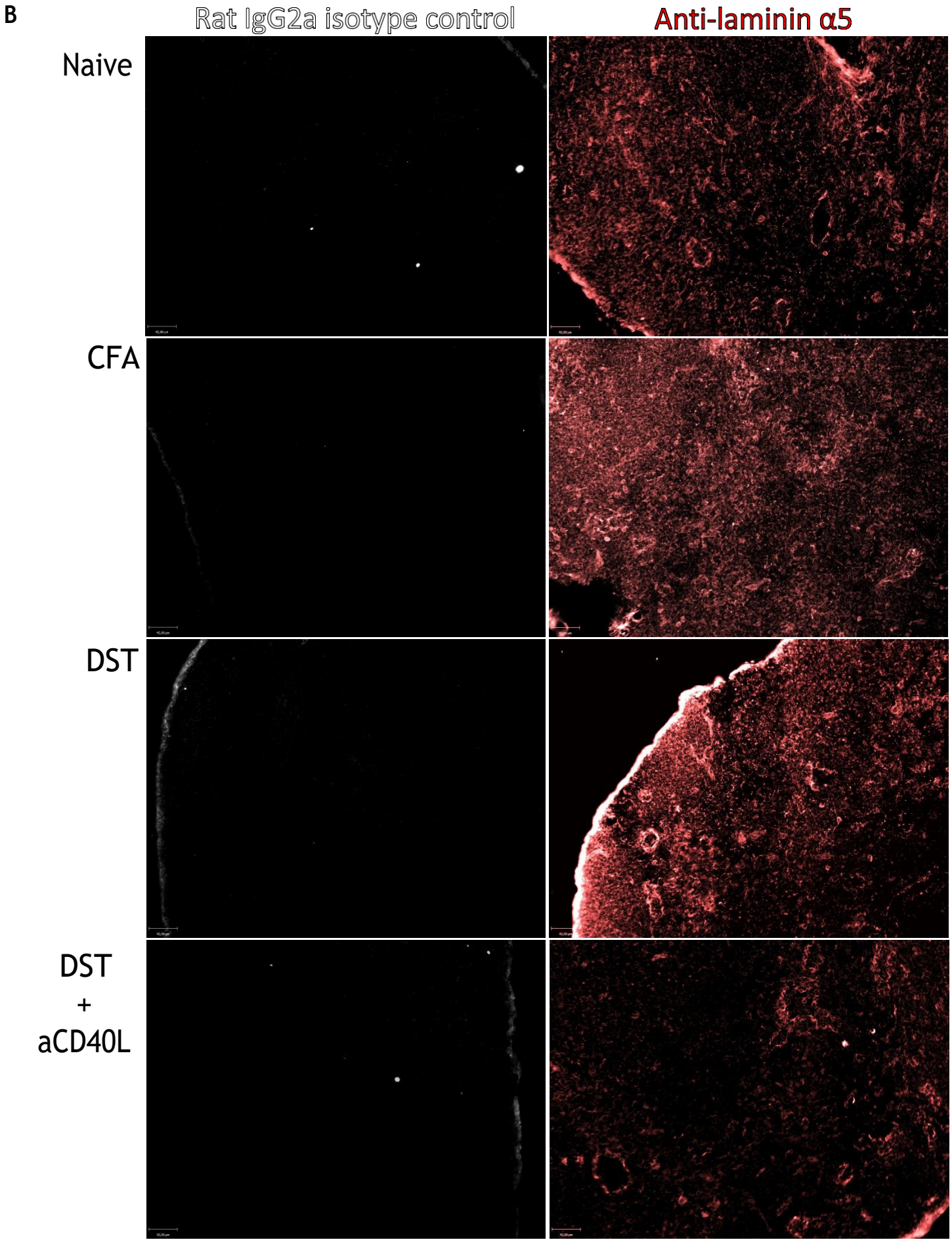
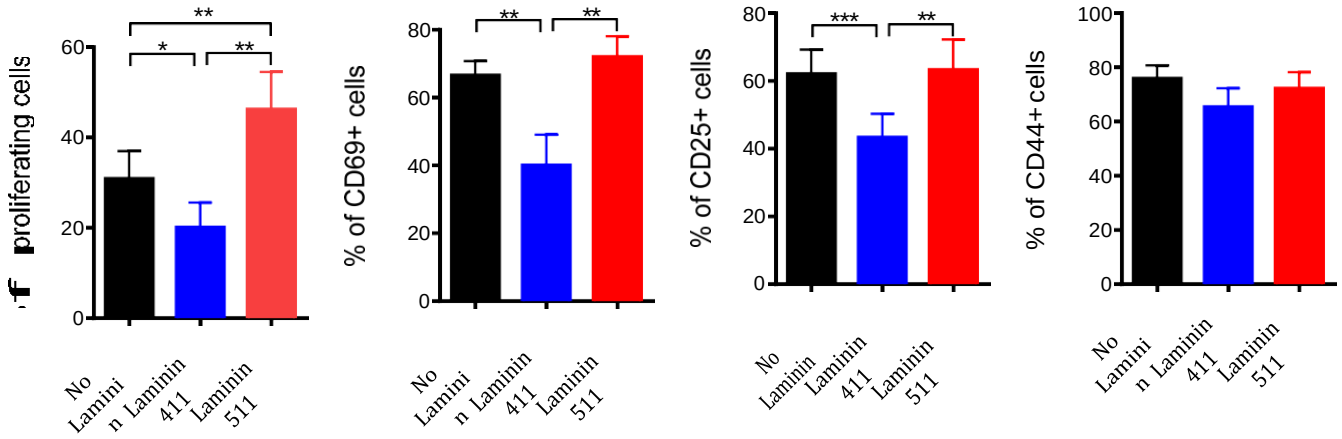
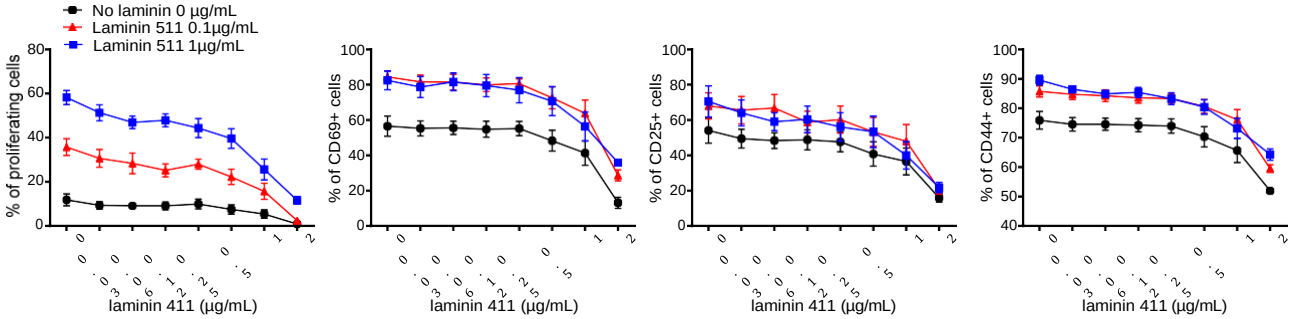

Rat IgG2a isotype control
Anti-laminin α5
Naive
CFA
DST
DST
+
aCD40L

Figure S2

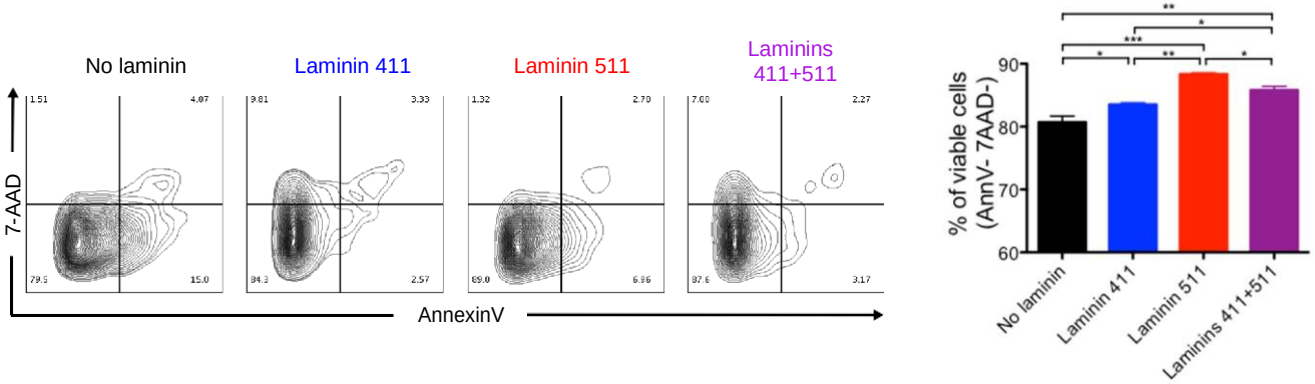
A



B



C



D

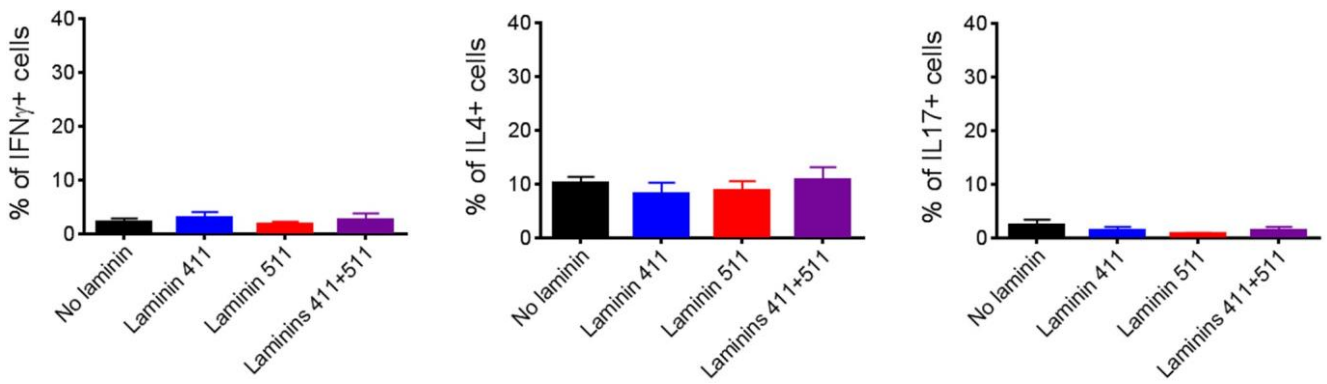
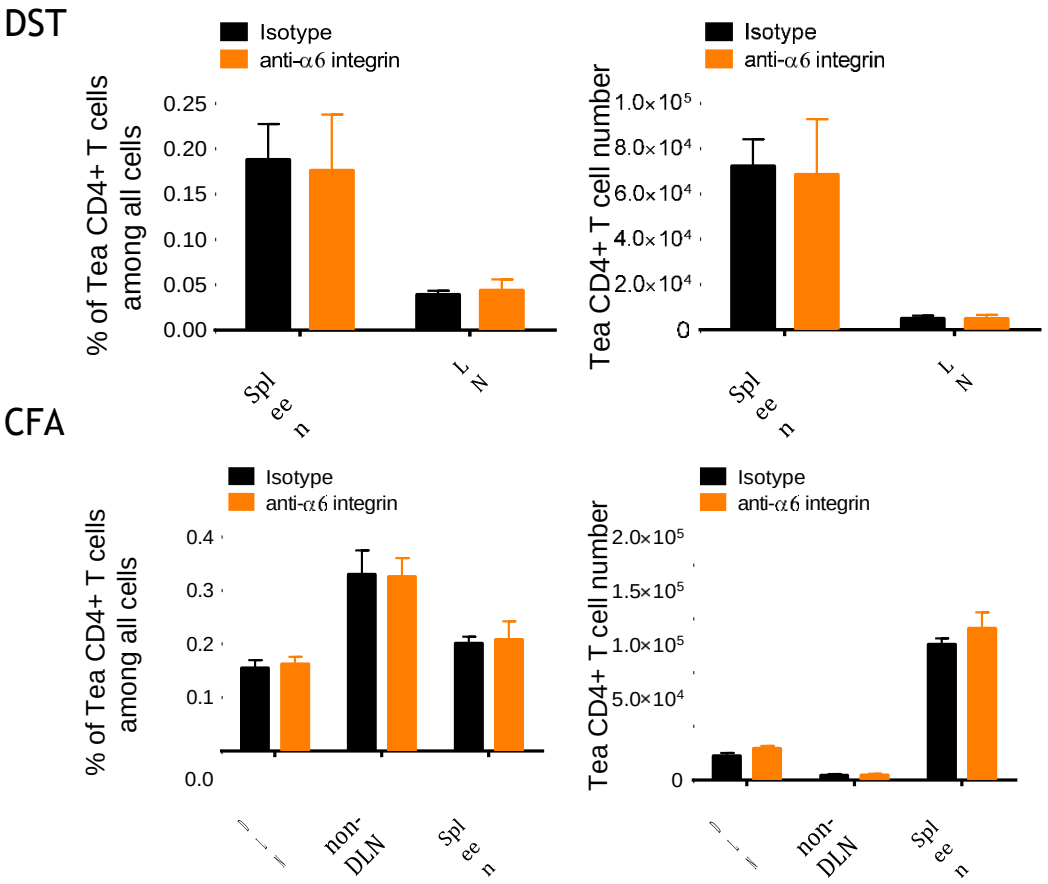


Figure S3

A


DST
Isotype
anti-α6 integrin
% of Tea CD4+ T cells among all cells
0.25
0.20
0.15
0.10
0.05
0.00
Spl ee n
L N
Isotype
anti-α6 integrin
Tea CD4+ T cell number
1.0×10⁵
8.0×10⁴
6.0×10⁴
4.0×10⁴
2.0×10⁴
0
Spl ee n
L N
CFA
Isotype
anti-α6 integrin
% of Tea CD4+ T cells among all cells
0.4
0.3
0.2
0.1
0.0
non-DLN
Spl ee n
Isotype
anti-α6 integrin
Tea CD4+ T cell number
2.0×10⁵
1.5×10⁵
1.0×10⁵
5.0×10⁴
0
non-DLN
Spl ee n

B

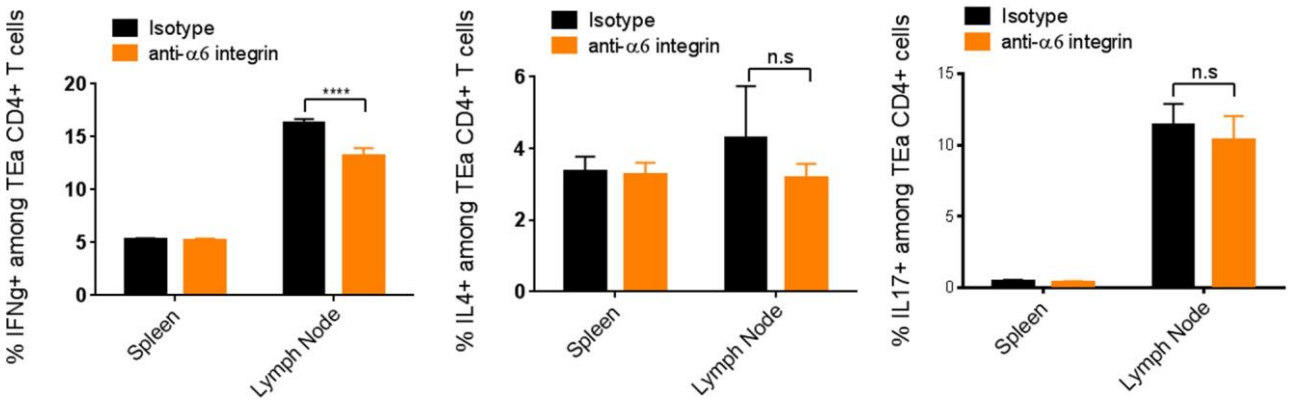
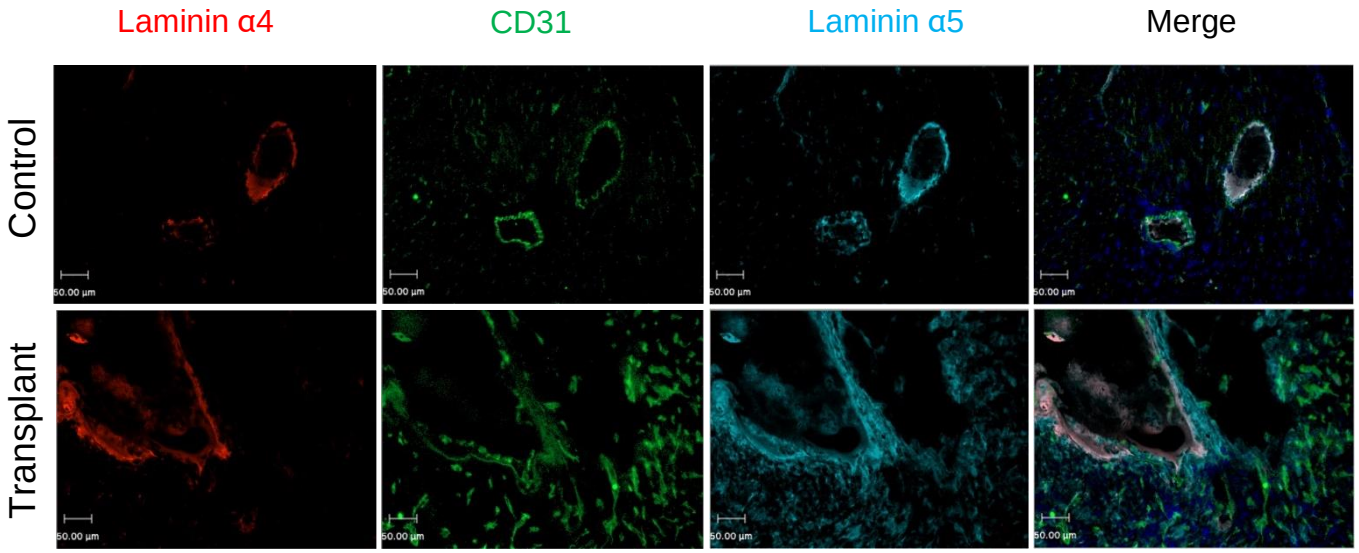

% IFNg+ among TEa CD4+ T cells
Isotype
anti-α6 integrin
****
20
15
10
5
0
Spleen
Lymph Node
% IL4+ among TEa CD4+ T cells
Isotype
anti-α6 integrin
n.s
6
4
2
0
Spleen
Lymph Node
% IL17+ among TEa CD4+ cells
Isotype
anti-α6 integrin
n.s
15
10
5
0
Spleen
Lymph Node

Figure S4

A


Laminin α4
CD31
Laminin α5
Merge
Control
Transplant

B

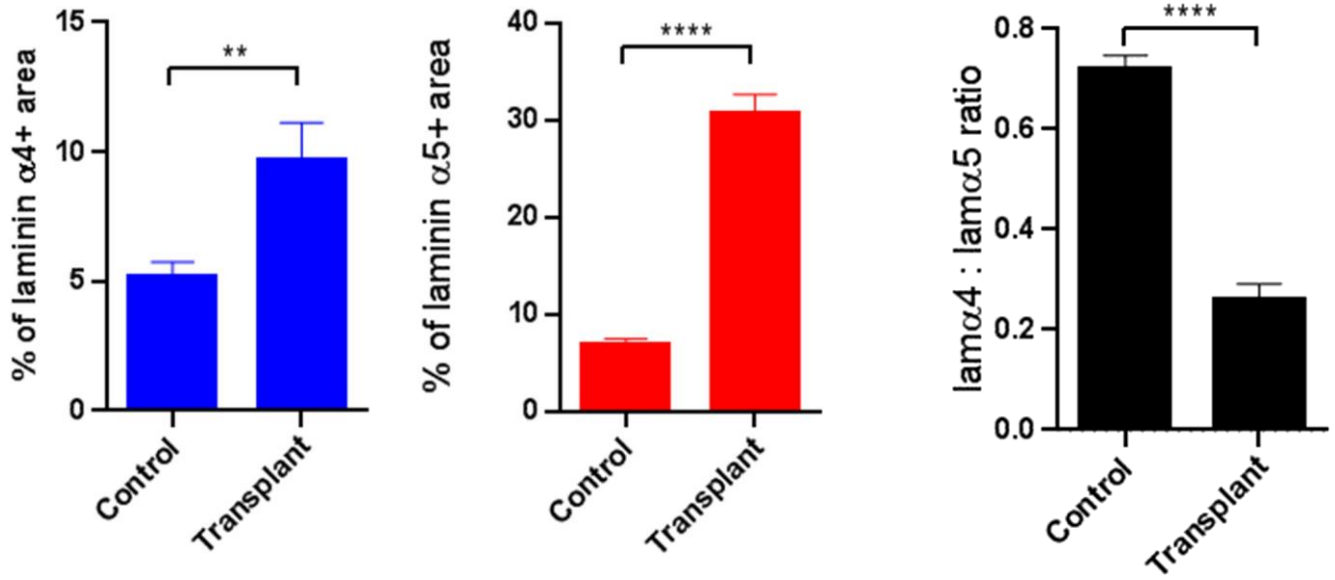

% of laminin α4+ area
15
10
5
0
**
Control
Transplant
% of laminin α5+ area
40
30
20
10
0
****
Control
Transplant
lamα4 : lamα5 ratio
0.8
0.6
0.4
0.2
0.0
****
Control
Transplant

Figure S1. Laminin α5 and laminin α4 expression differentially regulated in the lymph node following the induction of inflammation, immunity or tolerance. Skin LNs from naive, CFA, DST, DST+anti- CD40L treated C57BL/6 mice isolated 5 hours after treatment. All LNs sectioned and stained for laminin α4 or rabbit isotype control (A), laminin α5 and rat isotype control (B). Representative images: X100.

Figure S2. A, Stromal laminin 411 and 511 differentially modulate CD4+ T cell activation with anti-CD3 at 5µg/mL. CD4 T lymphocytes evaluated by flow cytometry for proliferation and CD69, CD25, CD44 expression after 3 days of culture in plates coated with anti-CD3 (5 µg/mL), with or without laminin 411 (2 µg/mL) or laminin 511 (1 µg/mL). Data are shown as mean ± SEM of 4 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001. B, The effects of laminins 411 and 511 on CD4+ T cell activation are dose dependent. CD4 T lymphocytes evaluated by flow cytometry for proliferation and CD69, CD25, CD44 expression after 3 days of culture in plates coated with anti-CD3 (1 µg/mL) and the indicated concentrations of laminin 411 or laminin 511. Data shown as mean ± SEM of 3 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001. C, Stromal laminin 411 and 511 improve CD4+ T cell viability. CD4 T lymphocytes evaluated by flow cytometry for viability after 3 days of culture in plates coated with anti-CD3 (1 µg/mL) and with laminin 411 (2 µg/mL) and/or laminin 511 (1 µg/mL). Data shown as representative profiles and mean ± SEM of 3 independent experiments with samples. *P < 0.05, **P < 0.01, ***P < 0.001. D, Laminin 411 and 511 had no impact on Th1,Th2 and Th17 polarization in a neutral environment. CD4 T lymphocytes evaluated by flow cytometry for intracellular expression of IFNγ, IL4 or IL17 after 5 days of culture with coated anti-CD3 and soluble anti-CD28, with laminin 411 (2 µg/mL) and/or laminin 511. Data are shown as mean ± SEM of at least 3 independent experiments with duplicate samples.

Figure S3. A, Targeting the laminin 511 receptors α6 integrin did not interfere with the proportion and the number of TEa CD4+ in the lymph node. DST- or CFA/Ea-treated mice received 5×10^6 CFSE-labeled CD45.1 TEa TCR transgenic CD4+ T cells. Mice were euthanized 2 days post transfer, and the proportion and the number of donor lymphocytes from spleen and skin LNs were assessed by flow cytometry. Data are shown as mean ± SEM of at least 5 mice per group from 3 independent experiments. B, Targeting the laminin 511 receptors α6 integrin reduced the induction of Th1 but not Th2 and Th17 lymphocytes. CFA/Ea-treated mice received 5×10^6 CFSE-labeled CD45.1 TEa TCR transgenic CD4+ T cells, with or without anti-α6 integrin mAb (10 µg i.v.), were euthanized three days post transfer, and donor lymphocytes from spleen and skin LNs assessed as percentage of IFNγ , IL4, or IL17+ donor cells (CD4+ CD45.1+) in different organs by flow cytometry after ex vivo activation. Data are shown as mean ± SEM of 8–10 mice per group from at least 2 independent experiments.

Figure S4. Laminin α5 and laminin α4 expression differentially regulated in the cardiac transplant following rejection. Hearts from Balb/c freshly isolated (control) or isolated 5 days after transplantation into C57BL/6 recipient (transplant). All hearts sectioned and stained for laminin α4, laminin α5, and CD31. A, Representative images, X100. B, Percentages of laminin α4 and laminin α5 positive areas, and the ratios of laminin α4: laminin α5. Data are shown as mean ± SEM. n = at least 5 mice per condition; **P < 0.01, ****P < 0.0001.

Table S1. Antibodies for immunohistochemistry

Target Molecule	Source	Clone	Company
Laminin α4	Rat	775830	RnD
Laminin α5	Rabbit	Polyclonal	Novus Biological
PNAd	Rat	MECA-79	BD Biosciences
ER-TR7 (AF488)	Rat	sc-73355	Santa Cruz
Rabbit IgG	Goat	Polyclonal	Jackson Immunoresearch
Rat IgG	Goat	Polyclonal	Jackson Immunoresearch
Rabbit IgG	Donkey	Polyclonal	Jackson Immunoresearch
Rat IgM	Goat	Polyclonal	Jackson Immunoresearch

SDC, Materials and Methods

Mice. C57BL/6 (H-2b) and BALB/c (H-2d) mice were purchased from The Jackson Laboratories (Bar Harbor, ME). T cell receptor transgenic (Tg) mice expressing the TEa TCR (recognizing I-Ae antigen in the context of I-Ab) were acquired from A.Y. Rudensky (Memorial Sloan Kettering Cancer Center, New York, NY).[1] Mice were used at 8–12 weeks of age, and all experiments were performed with age- and sex-matched mice. Animals were housed under specific-pathogen-free conditions. Animal care and experimental procedures were approved by the University

Cell preparations. LNs and spleen were collected from TEa TCR Tg or C57BL/6 mice, and tissue was passed through a 70-µm cell strainer for homogenization. CD4+ T cell isolation was completed using the EasySep Mouse CD4+ T cell Enrichment Kit according to the manufacturer's recommendations (StemCell Technologies, Vancouver, British Columbia, Canada). Purity of 95%–99% was confirmed by flow cytometry for each experiment. Cells were labeled with CFSE according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA).

Immunohistochemistry. LNs and heart were excised and immediately submerged in OCT compound (Sakura Finetek, Torrance, CA). LNs were isolated 5 hours after DST, anti-CD40L and CFA treatment and 24 hours after MC38 injection or DSS treatment. Hearts were isolated 5 days after cardiac transplant. Tissues in OCT were quickly frozen using dry ice, then kept at –80˚C for long-term storage. LN cryosections were cut in triplicate at 6 µm using a Microm HM 550 cryostat (Thermo Fisher Scientific). Sections were fixed with cold acetone/methanol (v/v: 1:1) for 5 minutes, then washed in PBS. Primary antibodies were diluted 1:100–1:200 in PBS and incubated for 1 hour in a humidified chamber (Table S1). Sections were washed twice with PBS, and secondary antibodies were applied at 1:100–1:400 dilutions for 45 minutes (Table S1). Slides were washed twice in PBS for 5 minutes, fixed with 4% paraformaldehyde, treated with 1% glycerol in PBS before coverslipping with ProLong Gold Antifade Mountant (Thermo Fisher Scientific) and imaged under a fluorescent microscope. The lymph node cortical ridge area, HEV area and laminin α4 and α5 positive area were defined as described in Ref. 2 with Volocity image analysis software (PerkinElmer, Waltham, MA). Briefly, the region of interest (ROI) corresponding to HEV is defined manually by ER-TR7 and PNAd co-staining. The ROI corresponding to the cortical ridge is defined manually by ER-TR7+ fiber density between T and/or B cell areas with exclusion of HEV area. Valuation of IHC-positively stained laminin areas in the ROIs is measured in μm^2 with Volocity Quantification module. The threshold for laminin positive area in the ROIs is defined for each LN section with laminin isotype staining on a consecutive 6 µm- thick section. The percentage of laminin area is calculated with the following formula: % laminin × area = (laminin × area × 100)/ROI area.

Human T-cell purification and culture. Human CD4+ T cells were purified from non-mobilized peripheral blood apheresis products (Memorial Blood Center, St. Paul, MN, USA) by negative selection using a kit and AutoMACS (Miltenyi Biotec, PosselD2 program). Purified CD4+ T cells were labeled with CFSE (InVitrogen), stimulated with anti-CD3/28 mAb-coated beads (Dynal), and were plated in triplicate in 96-well flat-bottom plates coated with or without laminins. Cells were cultured in X-Vivo-15 (BioWhittaker, Walkersville, MD) media supplemented with 10% human AB serum (Valley Biomedical, Winchester, VA), L-glutamine (Invitrogen), N-acetylcysteine (American Regent, Shirley, NY), and recombinant IL-2 (50 IU/ml, Novartis). Cultures were harvested on day 3 and cells were stained with a live/dead marker (Zombie-dye, BioLegend), anti-CD25 and anti-CD4 mAb (OKT4, BioLegend) and data acquired by FACScalibur or LSRII.

CD4 T cell differentiation environments. For Th1 environment, IL2 (20ng/mL, eBioscience), IL12 (20ng/mL, R & D systems, Minneapolis, MN) and anti-IL4 mAb (10 ng/mL, clone 11B11, eBioscience) were added at initiation of culture. For Th2 polarization, IL2, IL4 (0.1µg/mL, Biolegend, Dedham, MA), anti-IFNγ mAb (10ng/mL, clone XMG1.2, eBioscience) and anti-IL12 mAb (10ng/mL, clone C18.2, Biolegend) were added. For Th17 polarization, TGFβ (1 ng/mL, eBioscience) and IL6 (0.1 µg/mL, Biolegend) were added. For Treg environment, TGFβ (5ng/mL, eBioscience) and IL2 were added.

Flow cytometry analysis. Surface markers and intracellular cytokines were detected by the following anti-mouse antibodies: CD4 (clone RM4-5, eBioscience), CD69 (clone H1.2F3, eBioscience), CD25 (clone PC61.5, eBioscience), CD44 (clone IM7, eBioscience), α6 integrin (Biolegend), P-Erk1/2 (Cell Signaling Technology, Danvers, MA), P-AktT (Cell Signaling Technology), P-P38 Mapk (clone 4NIT4KK, eBioscience), P-Stat3 (clone pY705, BD Bioscience), IFNγ (clone XMG1.2, BD Bioscience), IL4 (clone 11B11, BD Bioscience), IL17 (clone eBio17B7, eBioscience), FoxP3 (clone FJK-16s, eBioscience), Rorγt (clone B2D, eBioscience), Irf4 (clone 3E4, eBioscience), Batf (clone S39-1060, BD Bioscience) and CD126 (clone M5, BD Bioscience). For intracellular cytokines, cells were fixed and permeabilized in cytofix/cytoperm buffer kit (BD Biosciences). For transcription factor staining, cells were fixed and permeabilized in Foxp3 fixation buffer kit (Invitrogen, Carlsbad, CA). Phospho-proteins were stained according to manufacturer's phosflow staining protocol (BD Biosciences). Samples were collected on a BDLSRII flow cytometry, and data analysis were generated in FlowJo (Treestar, Ashland, OR).

SDC, References

1. Grubin CE, Kovats S, deRoos P, et al. Deficient positive selection of CD4 T cells in mice displaying altered repertoires of MHC class II–bound self-peptides. *Immunity.* 1997;7(2):197-208.

2. Warren KJ, Iwami D, Harris DG, et al. Laminins affect T cell trafficking and allograft fate. *J Clin Invest.* 2014;124(5):2204-2218.