Science
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Supplementary Materials for

Conditioning of naïve CD8$^+$ T cells for tissue-resident memory formation

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This PDF file includes:

Figs. S1 to S6

Table S2

Other Supplementary Material for this manuscript includes the following:

Table S1 (as *.txt-file)

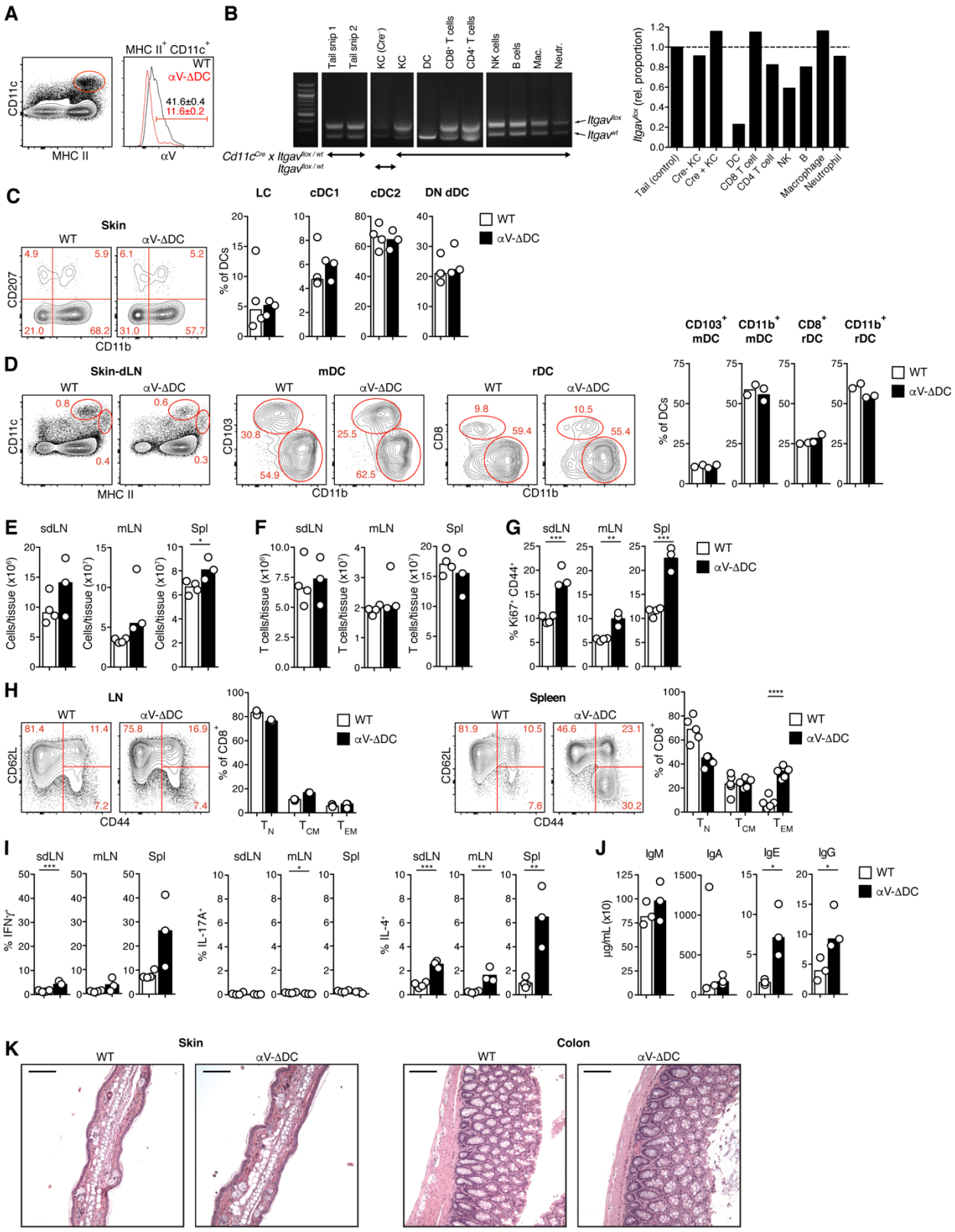

Fig. S1. **(A)** αV protein cell surface expression in splenic CD11c^{+} MHC II^{+} DC from αV-ΔDC or littermate control WT mice. Numbers indicate Means±SEM (n=3/group) (**B**) PCR analysis of

floxed (400-bp product) and WT (150-bp product) *Itgav* alleles in tail snip samples, keratinocytes (KC) and various immune cells of *Cd11c*^{Cre} × *Itgav*$^{fl/wt}$ or (Cre^{-}) *Itgav*$^{fl/wt}$ mice control. Quantification (right) of proportion of floxed alleles in *Cd11c*^{Cre} × *Itgav*$^{fl/wt}$ normalized to proportion in *Itgav*$^{fl/w}$ mice. Data are representative of three independent experiments. **(C)** Frequency of CD11c^{+} MHC II^{+}, CD11b^{+} CD207^{+} Langerhans cells, dermal CD11b^{-} CD207^{+} cDC1, CD11b^{+} CD207^{-} cDC2, and CD11b^{-} CD207^{-} DN dDC in skin of αV-ΔDC or WT mice. Data are representative of four independent experiments. **(D)** Frequency of CD11c^{hi} MHC II^{int} resident DC (rDC), CD11c^{int} MHC II^{hi} migratory DC (mDC), as well as their CD11b^{+} and CD8^{+} or CD103^{+} subsets in LNs of αV-ΔDC or WT mice. Data are means and replicates and representative of three independent experiments. (**E-G**) Total cell counts (E), T cell counts (F), and frequency of Ki67^{+} CD44^{+} CD4^{+} T cells (G) in skin-draining LNs (sdLNs), mesenteric LNs (mLNs), and spleens (Spl) of 7 week-old αV-ΔDC or WT mice. Data are means and replicates and representative of two independent experiments. **(H)** Frequencies of CD44$^{lo/int}$ naive, CD44^{hi} CD62L^{hi} central memory, and CD44^{hi} CD62L^{lo} effector/effector memory phenotype CD8^{+} T cells in spleens and LNs of 10 week-old αV-ΔDC or WT mice. Data are means and replicates and representative of six independent experiments. (**I-K**) Ex vivo-stimulated expression of indicated cytokines in CD4^{+} T cells from indicated tissues (I), serum concentrations of indicated immunoglobulins (J), and histological appearance of ear skin and colon tissue of 7 week-old αV-ΔDC or WT mice. Data are means and replicates and representative of two independent experiments. Scale bars = 100 μm. */**/***/****: $p<0.05/0.01/0.001/0.0001$ (Two-tailed unpaired Student's *t*-tests in (C-J)).

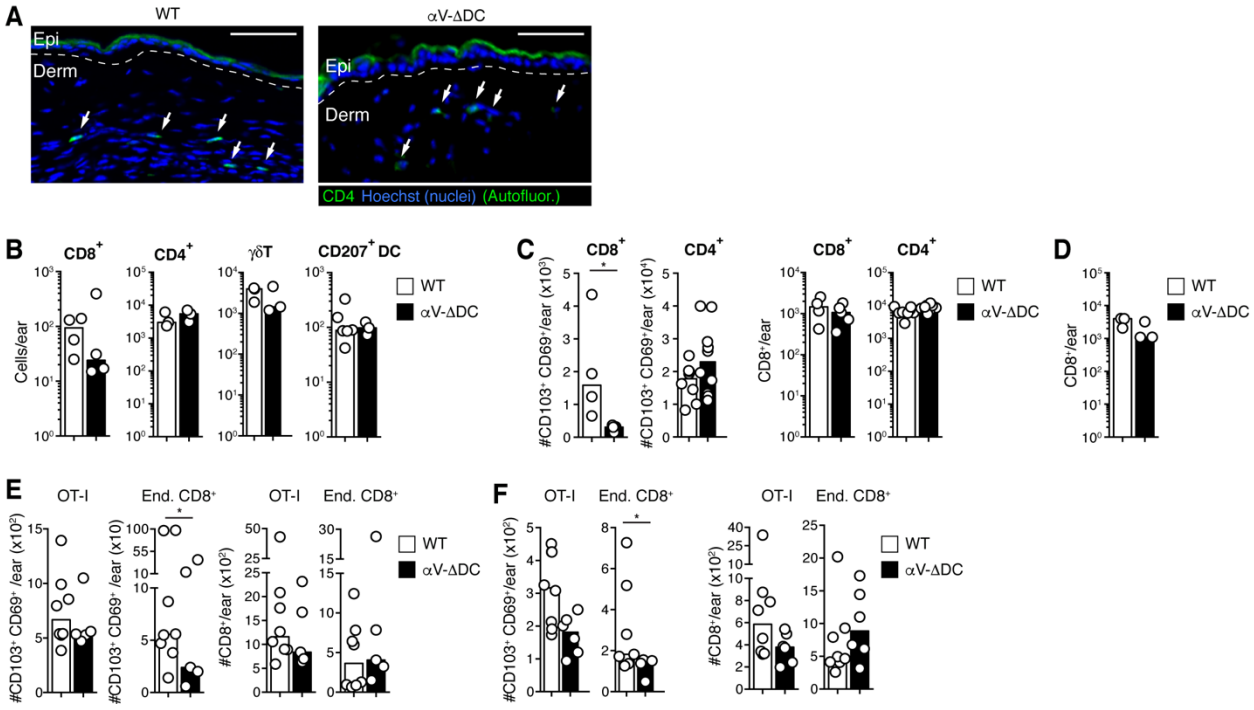

Fig. S2. **(A)** Histological cross-sections from ear skin of αV-ΔDC and WT littermate control mice. Arrows indicate $CD4^+$ T_{RM} cells in the dermis of both WT and αV-ΔDC mice. Dashed lines indicate dermal-epidermal border. Data are representative of three independent experiments. Scale bars = 50 μm. Epi: epithelium, Derm: dermis. (**B**) Absolute numbers of indicated immune cell subsets in ears of αV-ΔDC and WT mice. Data are means and replicates and representative of two independent experiments. (**C**) Absolute numbers of $CD103^+$ $CD69^+$ (left) and of total (right) $CD4^+$ and $CD8^+$ T cells in skin 4 weeks after sterile inflammation induced by mechanical irritation with a tattooing device. Data are means and replicates and representative of five independent experiments. (**D**) Absolute numbers of $CD8^+$ T cells in skin 4 weeks after topical DNFB treatment. Data are means and replicates and representative of three independent experiments. (**E-F**) Absolute numbers in skin of $CD103^+$ $CD69^+$ (left) and of total (right) $CD8^+$ T cells derived from OT-I or from polyclonal endogenous cells 4 weeks after adoptive i.v. transfer of activated OT-I cells and sterile inflammation induced by mechanical irritation with a tattooing device (E) or 4 weeks following OVA DNA vaccination after adoptive transfer of naive OT-I cells (F). Numbers above graphs indicate fold reduction in T cell numbers. Data in (E) and (F) are means and replicates and representative of six independent experiments. *: $p<0.05$ (Two-tailed unpaired Student's *t*-tests in (B-F)).

A

DARs ↑ in WT

Motif	Motif name	p-value	q-value (Benjamini)	% of target sequences with motif	% of background sequences with motif
	**Klf4(Zf)/mES-Klf4-ChIP-Seq(GSE11431)/Homer**	1.00E-21	0	31.20%	15.68%
	**KLF3(Zf)/MEF-Klf3-ChIP-Seq(GSE44748)/Homer**	1.00E-21	0	42.40%	24.77%
	**Klf9(Zf)/GBM-Klf9-ChIP-Seq(GSE62211)/Homer**	1.00E-17	0	35.52%	20.63%
	**RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer**	1.00E-16	0	36.16%	21.31%
	**RUNX2(Runt)/PCa-RUNX2-ChIP-Seq(GSE33889)/Homer**	1.00E-16	0	31.04%	17.15%
	**RUNX(Runt)/HPC7-Runx1-ChIP-Seq(GSE22178)/Homer**	1.00E-16	0	28.80%	15.57%
	**RUNX-AML(Runt)/CD4+-PolII-ChIP-Seq(Barski_et_al.)/Homer**	1.00E-14	0	27.52%	15.11%
	Sp1(Zf)/Promoter/Homer	1.00E-13	0	34.40%	21.08%
	Sp5(Zf)/mES-Sp5.Flag-ChIP-Seq(GSE72989)/Homer	1.00E-10	0	56.80%	43.39%
	**KLF10(Zf)/HEK293-KLF10.GFP-ChIP-Seq(GSE58341)/Homer**	1.00E-10	0	32.48%	21.18%
	ETV1(ETS)/GIST48-ETV1-ChIP-Seq(GSE22441)/Homer	1.00E-09	0	50.56%	38.11%
	**KLF5(Zf)/LoVo-KLF5-ChIP-Seq(GSE49402)/Homer**	1.00E-09	0	59.84%	47.33%
	GABPA(ETS)/Jurkat-GABPa-ChIP-Seq(GSE17954)/Homer	1.00E-09	0	39.04%	27.78%
	**KLF6(Zf)/PDAC-KLF6-ChIP-Seq(GSE64557)/Homer**	1.00E-08	0	53.44%	41.58%
	Etv2(ETS)/ES-ER71-ChIP-Seq(GSE59402)/Homer(0.967)	1.00E-08	0	36.16%	25.40%
	EHF(ETS)/LoVo-EHF-ChIP-Seq(GSE49402)/Homer	1.00E-08	0	43.84%	32.61%
	**EKLF(Zf)/Erythrocyte-Klf1-ChIP-Seq(GSE20478)/Homer**	1.00E-08	0	12.48%	6.15%
	ERG(ETS)/VCaP-ERG-ChIP-Seq(GSE14097)/Homer	1.00E-07	0	50.88%	40.22%
	Tcf4(HMG)/Hct116-Tcf4-ChIP-Seq(SRA012054)/Homer	1.00E-07	0	15.68%	9.08%
	ELF3(ETS)/PDAC-ELF3-ChIP-Seq(GSE64557)/Homer	1.00E-06	0	26.40%	18.02%

B

DARs ↑ in αV-ΔDC

	**IRF2(IRF)/Erythroblas-IRF2-ChIP-Seq(GSE36985)/Homer**	1.00E-33	0	16.17%	2.61%
	**ISRE(IRF)/ThioMac-LPS-Expression(GSE23622)/Homer**	1.00E-31	0	12.47%	1.50%
	**IRF1(IRF)/PBMC-IRF1-ChIP-Seq(GSE43036)/Homer**	1.00E-25	0	16.63%	3.66%
	**IRF8(IRF)/BMDM-IRF8-ChIP-Seq(GSE77884)/Homer**	1.00E-23	0	25.17%	8.71%
	**IRF3(IRF)/BMDM-Irf3-ChIP-Seq(GSE67343)/Homer**	1.00E-18	0	23.33%	9.03%
	**PU.1:IRF8(ETS:IRF)/pDC-Irf8-ChIP-Seq(GSE66899)/Homer**	1.00E-15	0	16.63%	5.53%
	**Tbet(T-box)/CD8-Tbet-ChIP-Seq(GSE33802)/Homer**	1.00E-13	0	50.81%	33.22%
	**bZIP:IRF(bZIP,IRF)/Th17-BatF-ChIP-Seq(GSE39756)/Homer**	1.00E-11	0	25.87%	13.39%
	**IRF4(IRF)/GM12878-IRF4-ChIP-Seq(GSE32465)/Homer**	1.00E-11	0	24.48%	12.36%
	ETS1(ETS)/Jurkat-ETS1-ChIP-Seq(GSE17954)/Homer	1.00E-09	0	42.03%	28.39%
	ETS:RUNX(ETS,Runt)/Jurkat-RUNX1-ChIP-Seq(GSE17954)/Homer	1.00E-08	0	7.16%	2.08%
	**Tbr1(T-box)/Cortex-Tbr1-ChIP-Seq(GSE71384)/Homer**	1.00E-08	0	53.35%	39.80%
	PRDM1(Zf)/Hela-PRDM1-ChIP-Seq(GSE31477)/Homer	1.00E-07	0	26.33%	15.97%
	Tcf4(HMG)/Hct116-Tcf4-ChIP-Seq(SRA012054)/Homer	1.00E-07	0	24.25%	14.37%
	GABPA(ETS)/Jurkat-GABPa-ChIP-Seq(GSE17954)/Homer	1.00E-07	0	35.57%	24.27%
	**Tbx5(T-box)/HL1-Tbx5.biotin-ChIP-Seq(GSE21529)/Homer**	1.00E-06	0	82.91%	72.27%
	Eomes(T-box)/H9-Eomes-ChIP-Seq(GSE26097)/Homer	1.00E-06	0	68.13%	56.24%
	Tcf3(HMG)/mES-Tcf3-ChIP-Seq(GSE11724)/Homer	1.00E-06	0	15.24%	8.06%
	ETV1(ETS)/GIST48-ETV1-ChIP-Seq(GSE22441)/Homer	1.00E-06	0	47.81%	36.39%
	RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer	1.00E-05	0	41.34%	30.45%

Fig. S3. Transcription factor motifs enriched in DARs found in naive $CD8^+$ T cells from WT mice (A) and αV-ΔDC mice (B). Motif are ranked based on statistical significance of their enrichment. KLF and Runt motifs are highlighted in bold font in (A), whereas IRF and T-box motives are highlighted in (B). Data representative of two animals/group.

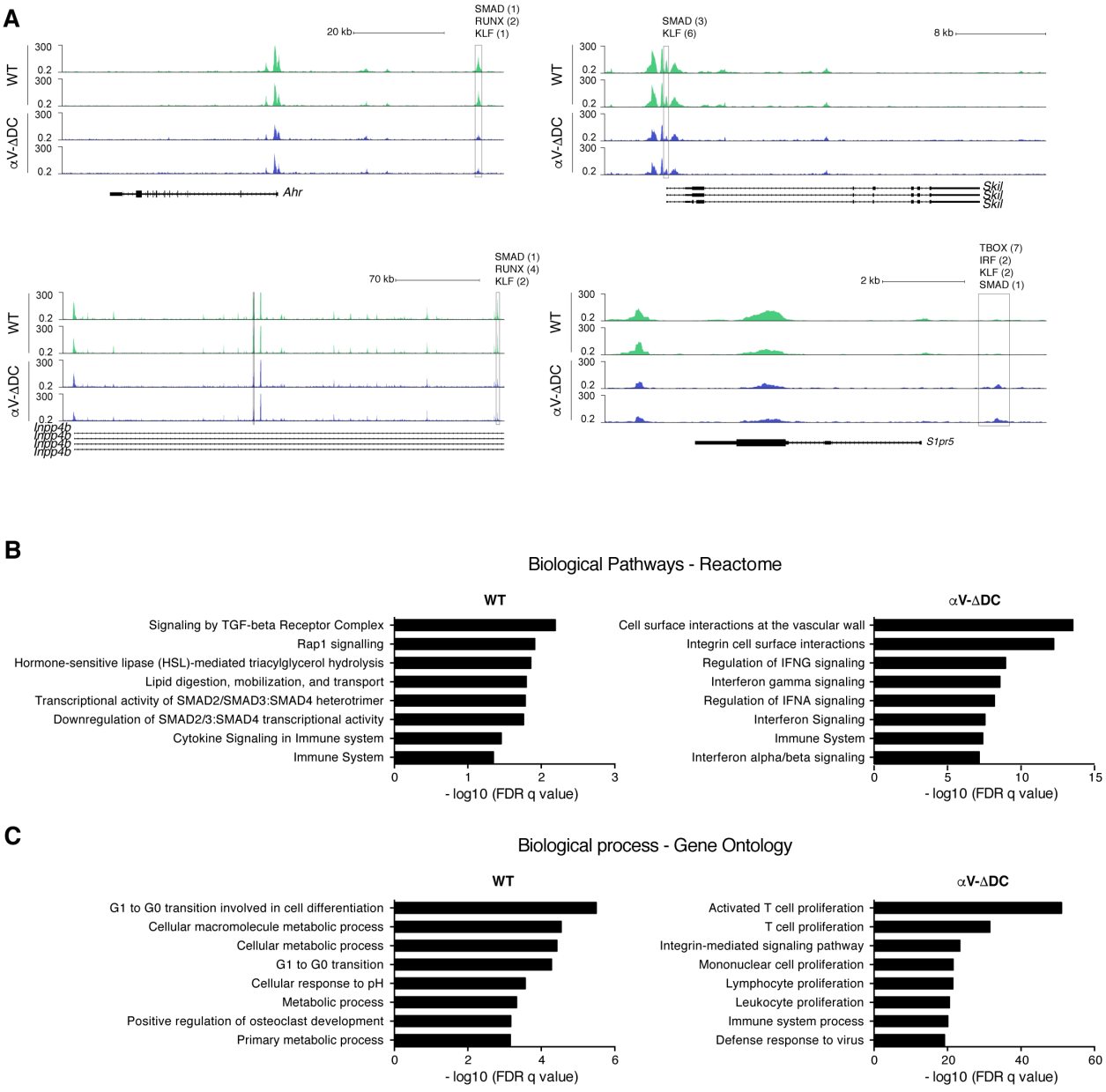

Fig. S4. **(A)** Normalized chromatin accessibility near the *Ahr* and *Skil* (top), and the *Inpp4b* and *S1pr5* loci (bottom). Rectangles mark detected DARs. Transcription factor motifs and their number of occurrences (in parentheses) in the respective DAR are indicated above. **(B, C)** GREAT analysis of DARs in cells from WT and αV-ΔDC mice. Top 8 enrichment hits each from the MSigDB Reactome database (B) and the Gene Ontology (GO) Biological Process database (C), ranked by FDR q-value and plotted as $-\log_{10}$(FDRq). Data are representative of two animals/group.

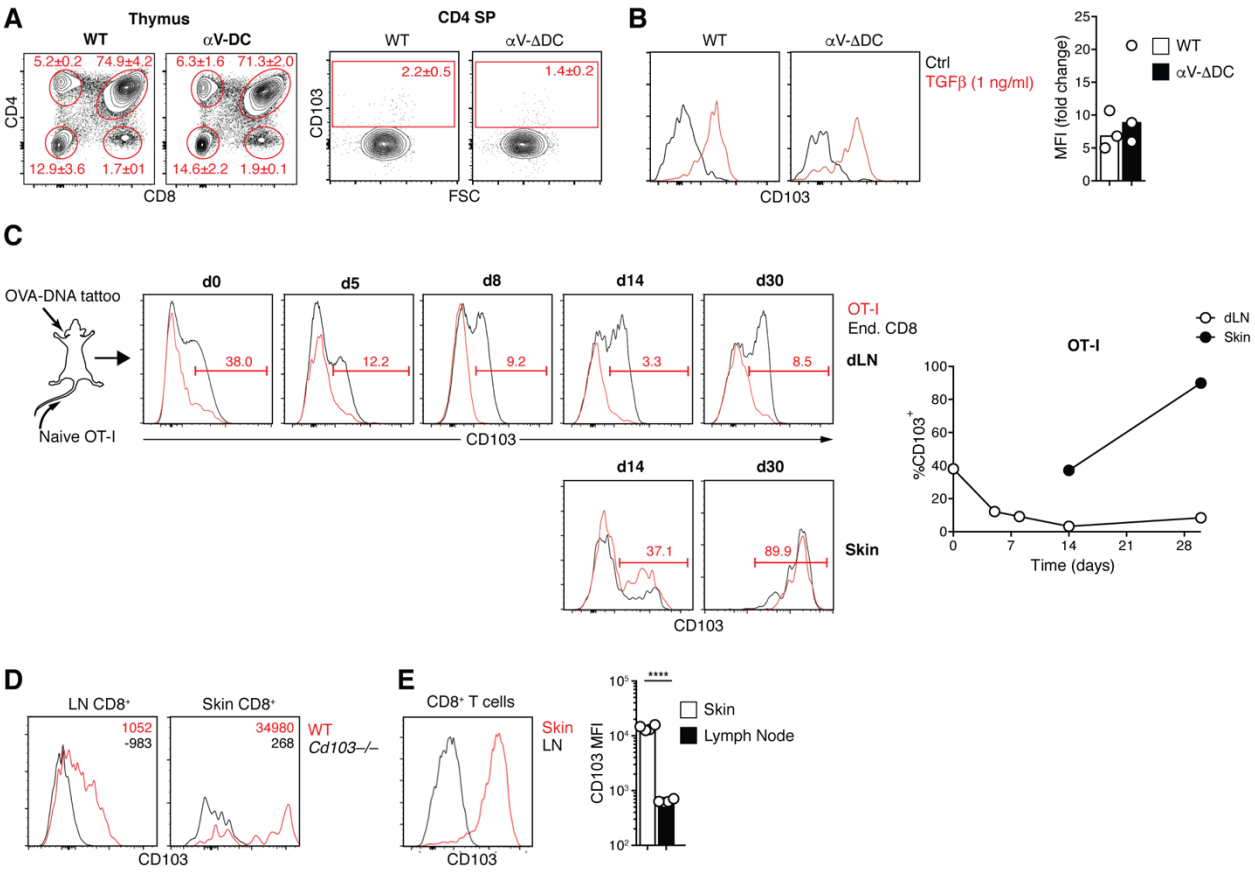

Fig. S5. **(A)** Frequencies of CD4 SP, CD8 SP, DN and DP thymocytes and CD013 expression on CD4 SP thymocytes in αV-ΔDC or littermate control WT mice. Numbers by gates indicate mean±SEM of frequencies. Data are representative of four independent experiments. **(B)** CD103 expression of naive $CD8^+$ T cells from αV-ΔDC or WT mice following 3 days of culture in 100 ng/mL of IL-15 and 5 ng/mL of IL-7 in the presence or absence of 1 ng/ml of activated TGF-β1. Graph shows fold change of MFI in response to TGF-β1. Data are means and replicates and representative of three independent experiments. **(C)** Naïve OT-I were adoptively transferred into WT mice, which were vaccinated with OVA-plasmid DNA delivered through tattoo of the ear skin. CD103 expression on OT-I cells was assayed in individual mice at indicated time-points after vaccination in the draining cervical LN (top row) and ear skin (bottom row). Endogenous $CD8^+$ T cell at each site are shown for reference. Data are representative of two independent experiments. (**D**) CD103 expression on naive and skin $CD8^+$ T cells in C57BL/6 mice, shown in direct comparison to cells from CD103-deficient mice. Data are representative of two animals/group. (**E**) CD103 expression on naive $CD8^+$ T cells in sdLNs and on total $CD8^+$ T cells in skin, 4 weeks following local DNFB challenge. Data are means and replicates and representative of six independent experiments. ****: $p<0.0001$ (Two-tailed unpaired Student's *t*-tests in (B), (E)).

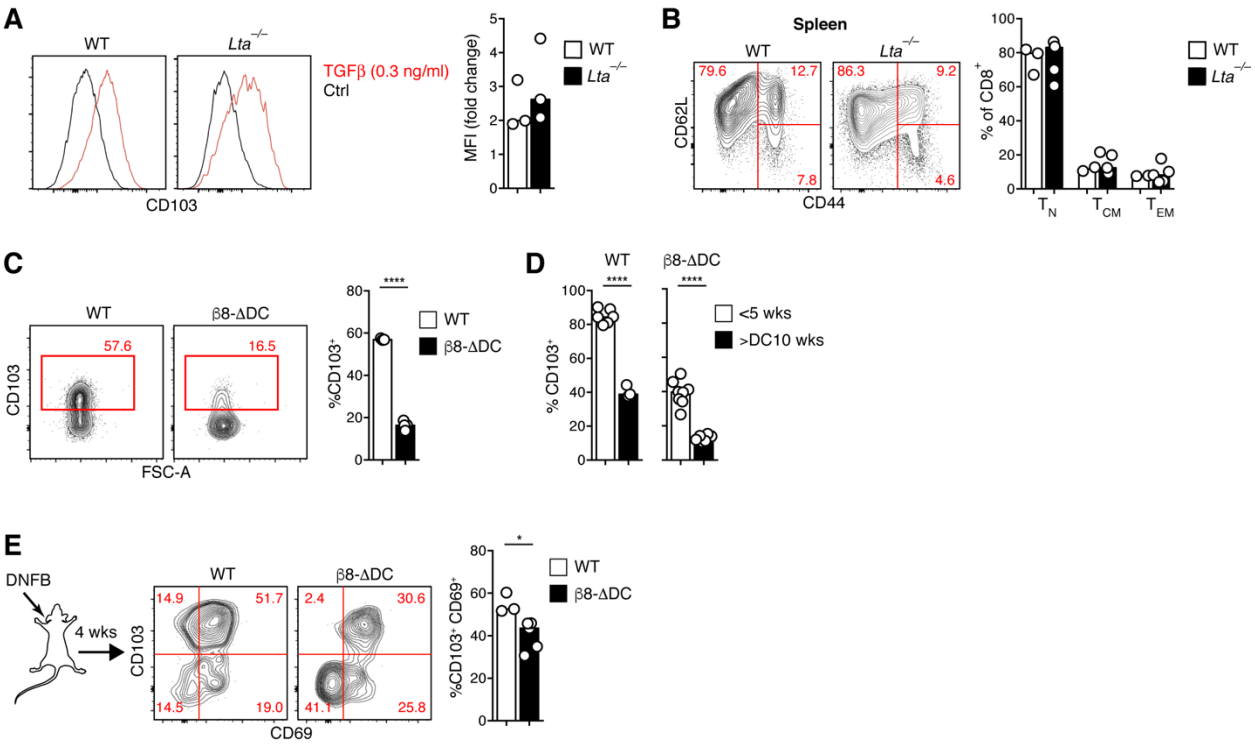

Fig. S6. **(A)** CD103 expression of naive CD8$^+$ T cells from *Lta*$^{-/-}$ or WT mice following 3 days of culture in IL-7 and IL-15 in the presence or absence of 0.3 ng/ml of activated TGF-β1. WT CD8$^+$ T cells lose CD103 expression during 3 days of culture in IL-7 in absence of TGF-β, resulting in similar baseline expression as in LTα KO cells. Graph shows fold change of MFI in response to TGF-β1. Data are medians and replicates and representative of two independent experiments. **(B)** Frequencies of CD44$^{lo/int}$ naive, CD44^{hi} CD62L^{hi} central memory, and CD44^{hi} CD62L^{lo} effector/effector memory phenotype CD8$^+$ T cells in spleens of *Lta*$^{-/-}$ or WT mice. Data are means and replicates and representative of three independent experiments. (**C-E**) Frequency of CD103$^+$ cells among naïve CD8$^+$ T cells in LNs (C), among naïve CD8$^+$ T cells in peripheral blood of very young (<5 wks) and older (>10 wks) mice (D), and of CD103$^+$ CD69$^+$ eT$_{RM}$ cells among total CD8$^+$ T cells in skin 4 week following DNFB challenge (E) in WT and β8-ΔDC mice. Data are means (C-D) or medians (E) and replicates and in each case representative of two independent experiments. */****: p<0.05/0.0001 (Mann–Whitney *U* test in (A and E) and two-tailed unpaired Student's *t*-tests in (B-D)).

Antibodies for flow cytometry

Antigen	Clone	Vendor	Formats	Conc. (μg/mL)
B220	RA3-6B2	Biolegend	FITC	1.25
CD103	2E7	Biolegend	APC, AF488, PacBlue, PerCP-Cy5.5	1
CD11b	M1/70	Biolegend	PE-Cy7, APC-Cy7	1
CD11c	HL3	BD Biosciences	PE-Cy7	1
CD19	6D5	Biolegend	BV421	1
CD207	4C7	Biolegend	APC	1
CD3	17A2	Biolegend	APC, FITC	1
CD4	RM4-5	Biolegend	PacBlue, APC-Cy7, AF700	1
CD44	IM7	Biolegend	BV605, PerCP-Cy5.5, AF700	1
CD45	30-F11	Biolegend	FITC, APC-Cy7, AF700	1.25
CD45.1	A20	Biolegend	FITC, PE-Cy7, BV605	1.25
CD45.2	104	Biolegend	FITC, PacBlue	2
CD51	RMV-7	Biolegend	PE	2.5
CD62L	MEL-14	Biolegend	AF700, APC, APC-Cy7, BV421	1
CD69	H1.2F3	Biolegend	PE-Cy7, BV421, PE	1
CD8α	5H10	Thermo Fisher	AF700, AF488, FITC	1.25
CD8β	YTS156.7.7	Biolegend	PE, AF700, FITC, PE-Cy7	1
F4/80	BM8	Biolegend	PE	1
IA/IE	M5/114.15.2	Biolegend	FITC, AF700, APC-Cy7	1
Ly6G	1A8	Biolegend	BV605, FITC	1
NK1.1	PK136	Biolegend	AF488	1.25
Thy1.1	OX-7	Biolegend	AF700, PE-Cy7, PacBlue	0.5
Thy1.2	30-H12	Biolegend	AF700, PacBlue, BV605, FITC	0.5
(Viability)	-	Biolegend	Zombie Yellow	unit "1 test"
(Viability)	-	eBioscience	eFluor506	unit "1 test"

Antibodies for immunohistochemistry

Antigen	Clone	Vendor	Formats	Conc. (μg/mL)
CD3	17A2	Biolegend	Biotin	5
CD4	RM4-5	Biolegend	AF647	2
CD8β	YTS156.7.7	Biolegend	PE	2
(Streptavidin)	-	Biolegend	AF647	2.5

Table S2: Immunoreagents for flow cytometry and immunohistochemistry. AF = Alexa Fluor, PacBlue = Pacific Blue