Supplementary Materials

Immunomodulation of UVB-induced regulatory T cells prevents the establishment of squamous cell carcinoma

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This file includes:

Figures S1-S5

Table S1

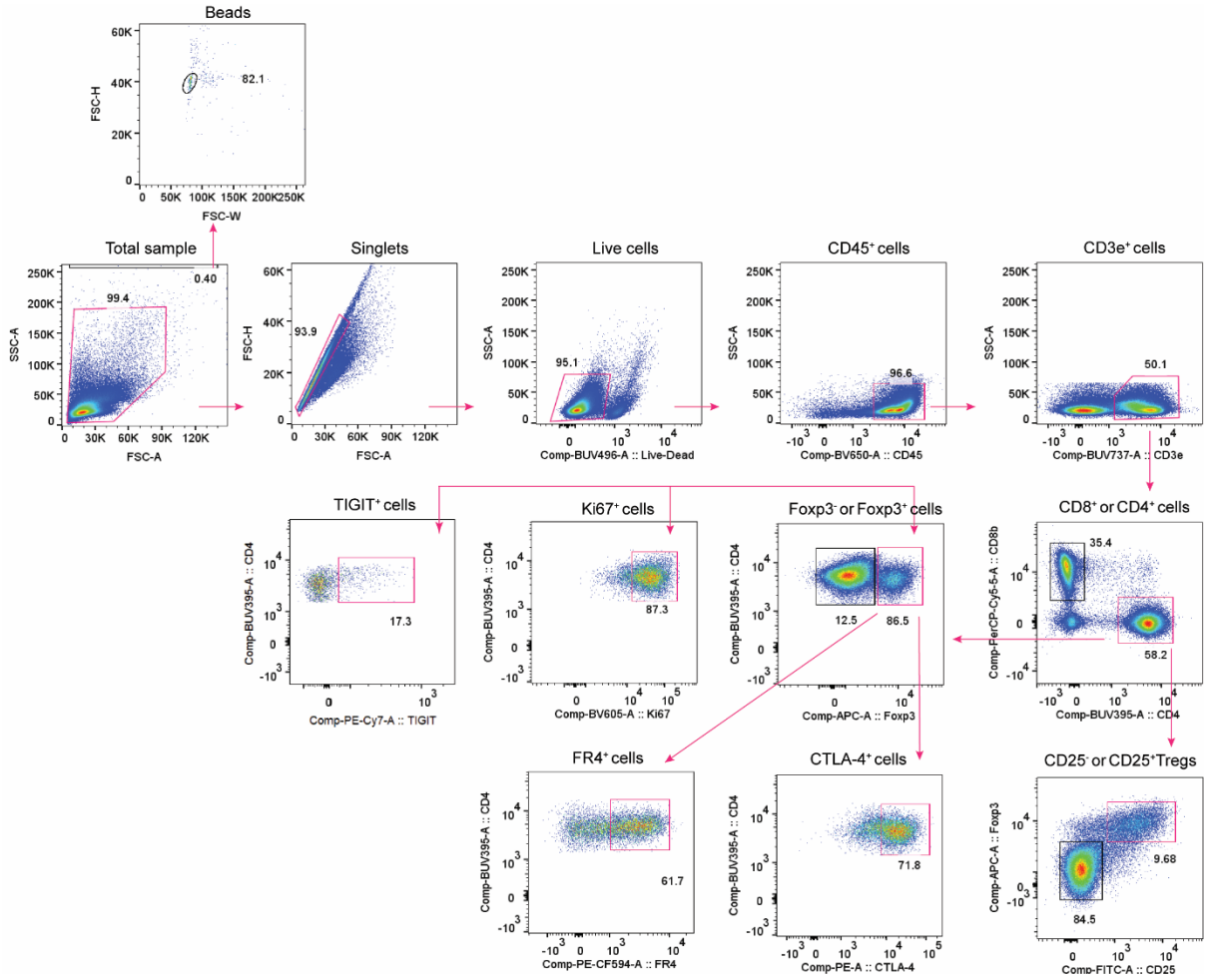

Figure S1. Gating strategy for UVB-induced Treg phenotyping. Representative flow plots illustrate the gating methodology used to identify and analyze Tregs in tissues post UVB exposure.

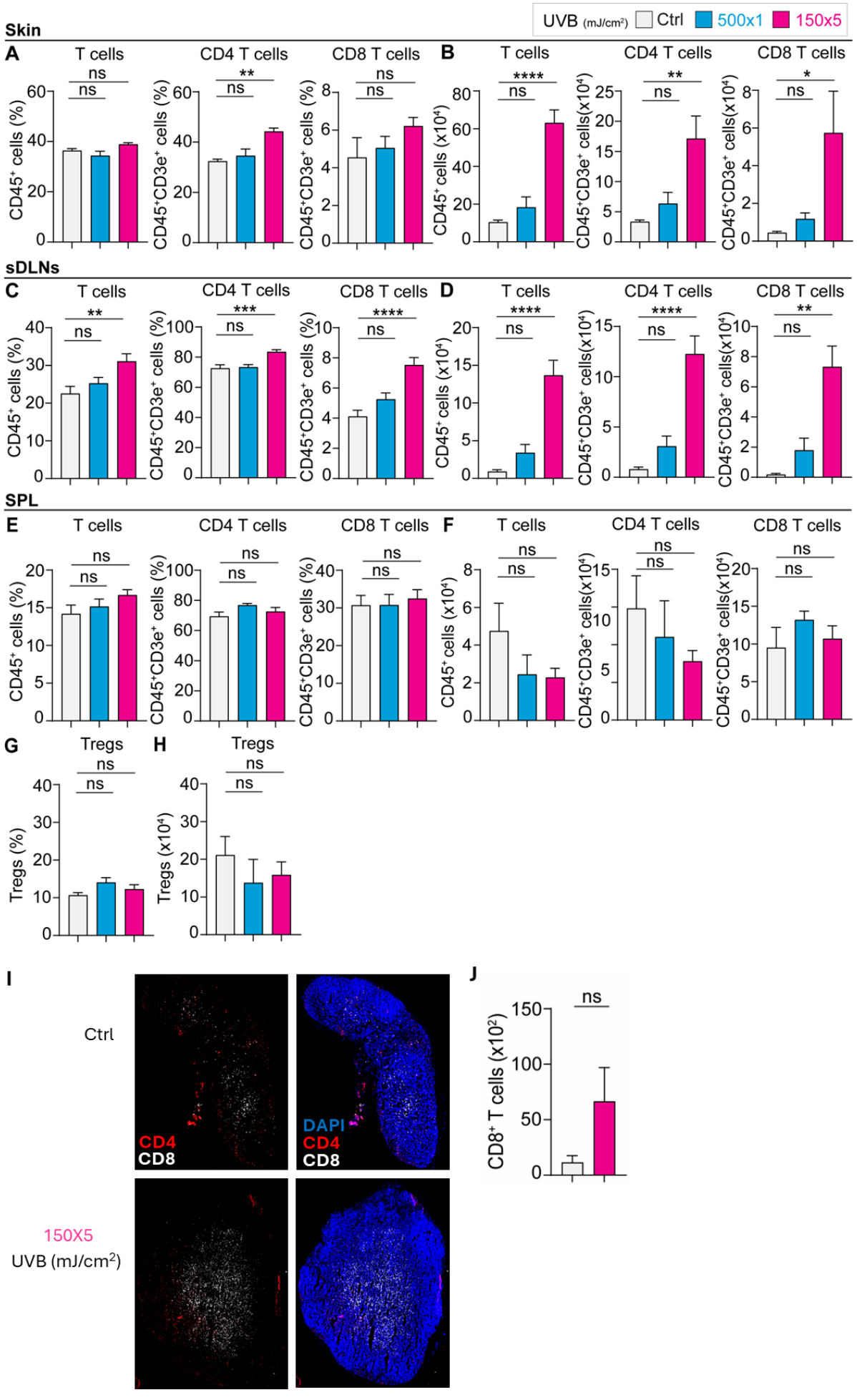
UVB (mJ/cm²) Ctrl 500x1 150x5
Skin
A
T cells
CD4 T cells
CD8 T cells
B
T cells
CD4 T cells
CD8 T cells
sDLNs
C
T cells
CD4 T cells
CD8 T cells
D
T cells
CD4 T cells
CD8 T cells
SPL
E
T cells
CD4 T cells
CD8 T cells
F
T cells
CD4 T cells
CD8 T cells
G
Tregs
H
Tregs
I
Ctrl
150X5
UVB (mJ/cm²)
CD4
CD8
DAPI
CD4
CD8
J
CD8⁺ T cells (x10²)
ns

Figure S2. Analysis of T cells and their subsets following UVB radiation. Tissue samples including skin, sDLN, and spleen from Foxp3-IRES GFP mice were assessed using flow cytometry after different UVB exposure regimens. Live $CD45^+$ cells were gated to determine the frequency and total cell number of T cells, $CD4^+$ T cells, and $CD8^+$ T cells in Skin (A; frequency, B; absolute cell count), sDLN (C; frequency, D; absolute cell count), and Spleen (E; frequency, F; absolute cell count). (G-H) Frequency and absolute cell count of Tregs in Spleen. (I-J) Representative immunofluorescence staining of sDLNs showing $CD4^+$ and $CD8^+$ T cell distribution and $CD8^+$ T cell quantification. Data are pooled from three independent experiments and represented as mean ±SEM (n=10-15). (A-H) Statistical analysis was performed using One-way ANOVA with Dunnett's multiple comparisons test. (J) Statistical significance was assessed using student's t-test. *$*p < 0.05$, $**p < 0.01$, $***$, $P< 0.001$, $****p < 0.0001$*, n.s.=not significant. UVB: Ultraviolet B radiation, sDLNs: skin-draining lymph nodes, SPL: spleen.

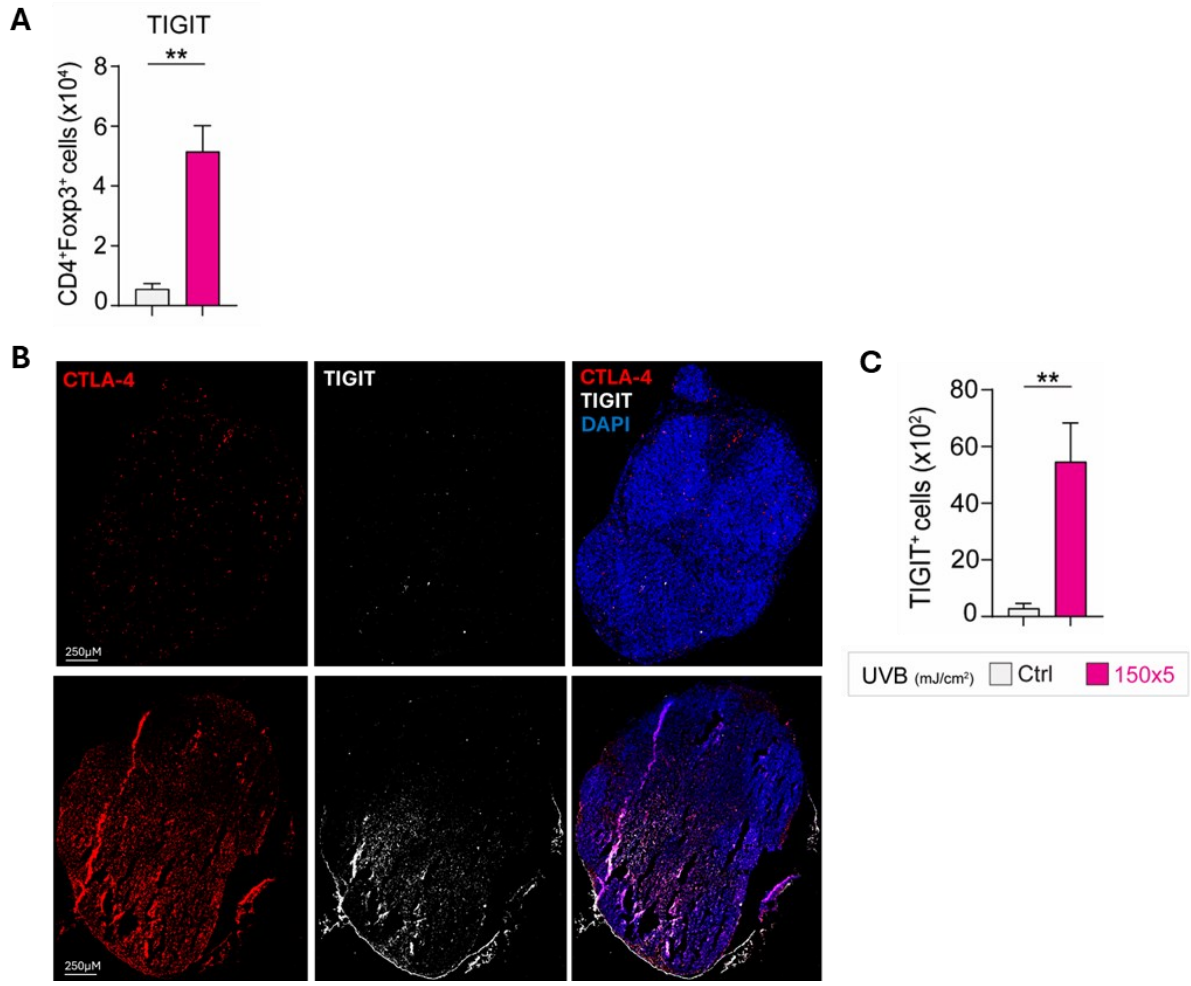

Figure S3. TIGIT expression in Tregs following UVB exposure. (A) Abundance of TIGIT$^+$ Tregs in sDLNs. (B-C) Representative immunofluorescence staining of TIGIT expression in Tregs within sDLNs, along with its quantification. Data pooled from three independent experiments (mean ±SEM). Statistical significance was assessed using student's t-test (n=3-5). **, *P< 0.01*. UVB: Ultraviolet B radiation, sDLNs: skin-draining lymph nodes.

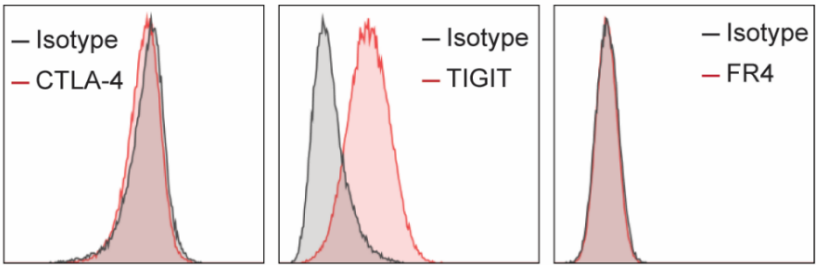

Figure S4. Intrinsic expression analysis of CTLA-4, TIGIT, and FR4 in SCC cells. Representative histograms show the expression levels of CTLA-4, TIGIT, and FR4 on SCC cells. Dark grey lines indicate isotype controls, while red lines represent staining with CTLA-4, TIGIT, and FR4 antibodies respectively.

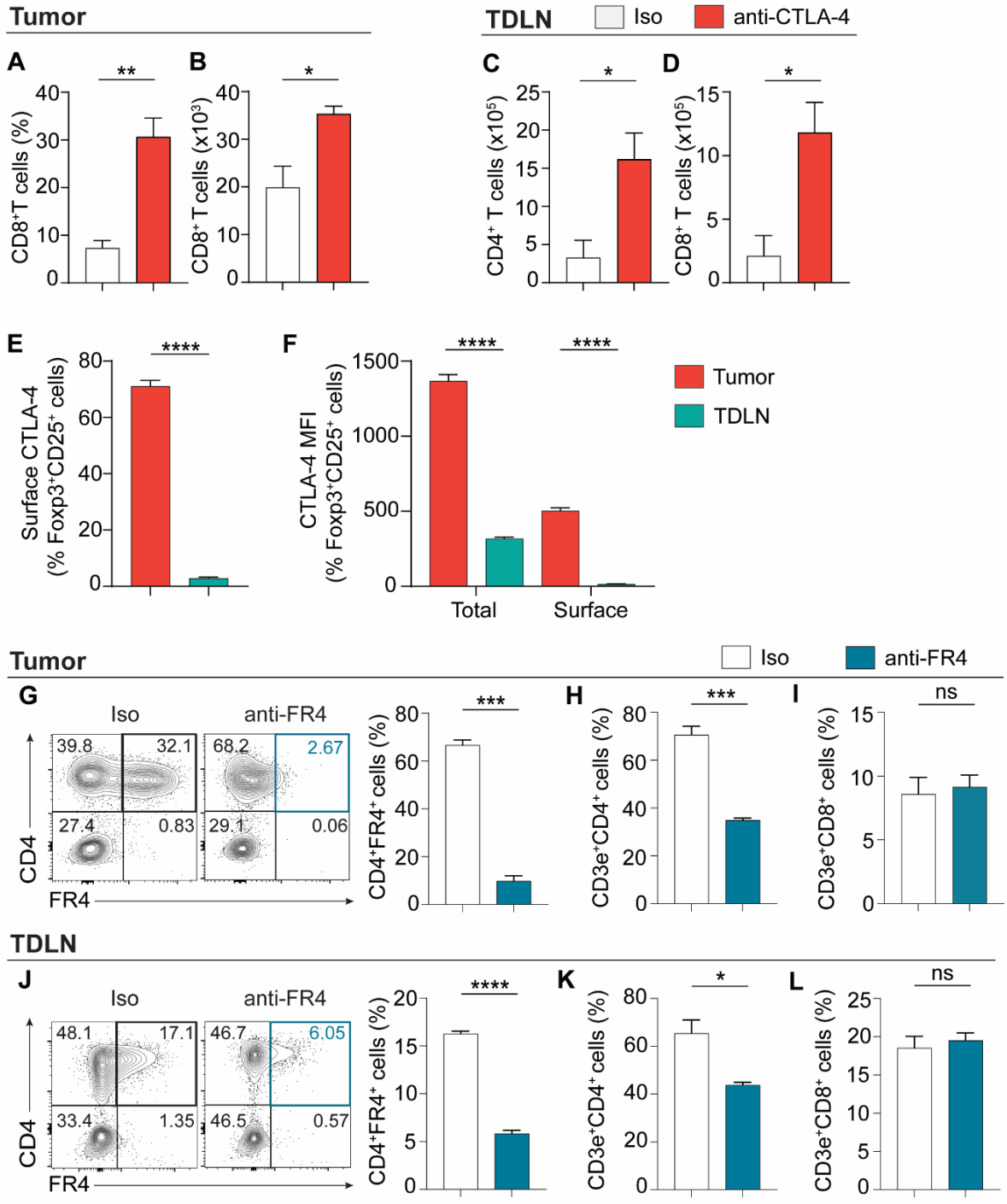

Figure S5. Immune cell profiling in the tacrolimus-induced SCC model. (A-B) Anti-CTLA-4: Frequency and total numbers of $CD8^+$ T cells in tumors; (E-F) $CD4^+$ and $CD8^+$ T cell counts in TDLN. (E-F) Surface expression and MFI of CTLA-4 in tumor and TDLN. (G, J) Anti-FR4: Flow plots of $CD4^+FR4^+$ T cells and frequencies in tumors and TDLN; (H-I, K-L) $CD4^+$ and $CD8^+$ T cell counts in tumors and TDLN. Data are derived from two independent

experiments (n=6-8). Unpaired student's t test was used to determine statistical significance. *$*p < 0.05$, **$p < 0.01$, ***, $P< 0.001$, ****$p < 0.0001$*, n.s.=not significant. TDLN: Tumor-draining lymph node, MFI: Mean fluorescent intensity.

Table S1. List of key resources used in this study.

REAGENT/RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CD45 – BV650	BD Biosciences	Cat# 563410
CD3e – BUV737	BD Biosciences	Cat# 612771
CD4 – BUV395	BD Biosciences	Cat# 563790
CD8β – APC-Cy7	BioLegend	Cat# 126619
CD8β – AF647	BioLegend	Cat# 126612
CD25 – FITC	BioLegend	Cat# 101908
CD25- APC	BioLegend	Cat# 102012
Foxp3 –AF647	BioLegend	Cat# 126408
CD152 – PE	BioLegend	Cat# 106305
TIGIT – PE-Cy7	BioLegend	Cat# 142107
FR4 – PE-CF594	BioLegend	Cat# 125016
Ki67 – BV605	BioLegend	Cat# 652413
LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit	Thermo Fisher	Cat# L34957
CD16/CD32	BD Biosciences	Cat# 553142
Anti-mCTLA4-mIgG2a InvivoFit™	InvivoGen	Cat# mctla4-mab10-1
Anti-mTIGIT-mIgG2a InvivoFit™	InvivoGen	Cat# mtigit-mab10-1
FR4 Monoclonal Antibody	Thermo Fisher	Cat# 16-5446-85
InVivoMAb anti-mouse CD8β	BioXCell	Cat# BE0223
InVivoPlus anti-mouse CD4	BioXCell	Cat# BP0003-1
Anti-β-Gal-mIgG2a InvivoFit™	InvivoGen	Cat# bgal-mab10-1
InVivoPlus rat IgG2b isotype control	BioXCell	Cat# BE0090
InVivoMAb rat IgG1 isotype control	BioXCell	Cat# BE0088
Rat IgG2b kappa isotype control	Thermo Fisher	Cat# 16-4031-81
Chemicals, peptides, and recombinant proteins		
DMEM, high glucose	Gibco	Cat# 11965092
Ham's F-10 Nutrient Mix	Gibco	Cat# 11550043
Phosphate Buffer Saline	Gibco	Cat# 10010023
Fetal Bovine Serum (FBS)	Gibco	Cat# 10099141
Trypsin-EDTA (0.05%), phenol red	Gibco	Cat# 25300062
Penicillin/Streptomycin/glutamate	Gibco	Cat# 10378016
Insulin	Sigma-Aldrich	Cat# I2643
Hydrocortisone	Sigma-Aldrich	Cat# H0888
Human EGF recombinant protein	Gibco	Cat# PHG0311
Adenine	Sigma-Aldrich	Cat# A2786
Cholera toxin from *Vibrio cholera*	Sigma-Aldrich	Cat# C8052
Collagenase D from *Clostridium histolyticum*	Roche	Cat# 11088866001
DNase I from bovine pancreas	Roche	Cat# 11284932001
Ovalbumin	Sigma-Aldrich	Cat# A5503
Saponin from quillaja bark	Sigma-Aldrich	Cat# S7900
DAPI	Thermo Fisher	Cat# 62248
Triton X-100	Sigma-Aldrich	Cat# T8787
Flow-Count Fluorospheres	Beckman Coulter	Cat# 7547053

Foxp3 /Transcription Factor Staining Buffer Set	eBioscience	Cat# 00552300
Software and Algorithms		
FlowJo software	FlowJo, LLC	www.flowjo.com.
GraphPad Prism (version 9.1)	GraphPad Software, Inc.	www.graphpad.com.
ImageJ	NIH	Version 1.50i
QuPath v.0.1.3	Queen's University, Belfast, Northern Ireland	www.qupath.github.io.