

Supplementary data

A novel humanized mouse model to study the function of human cutaneous memory T cells *in vivo* in human skin

Short title: *Cutaneous T cells in a skin-humanized mouse model*

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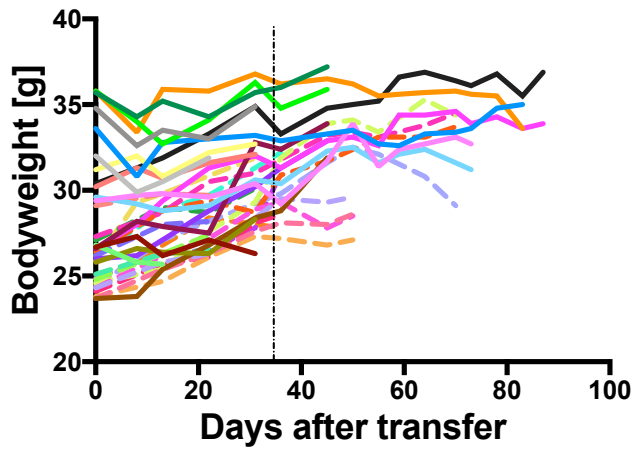
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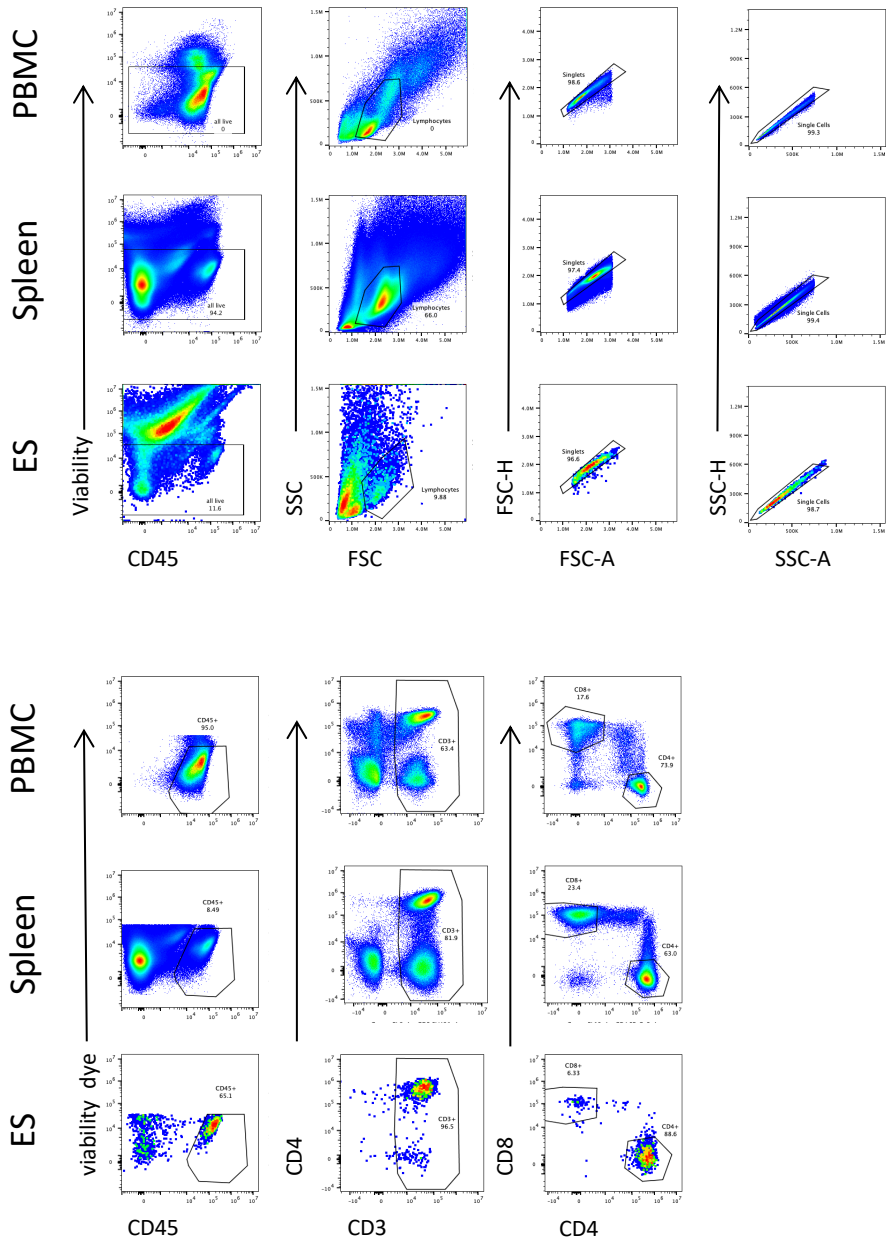
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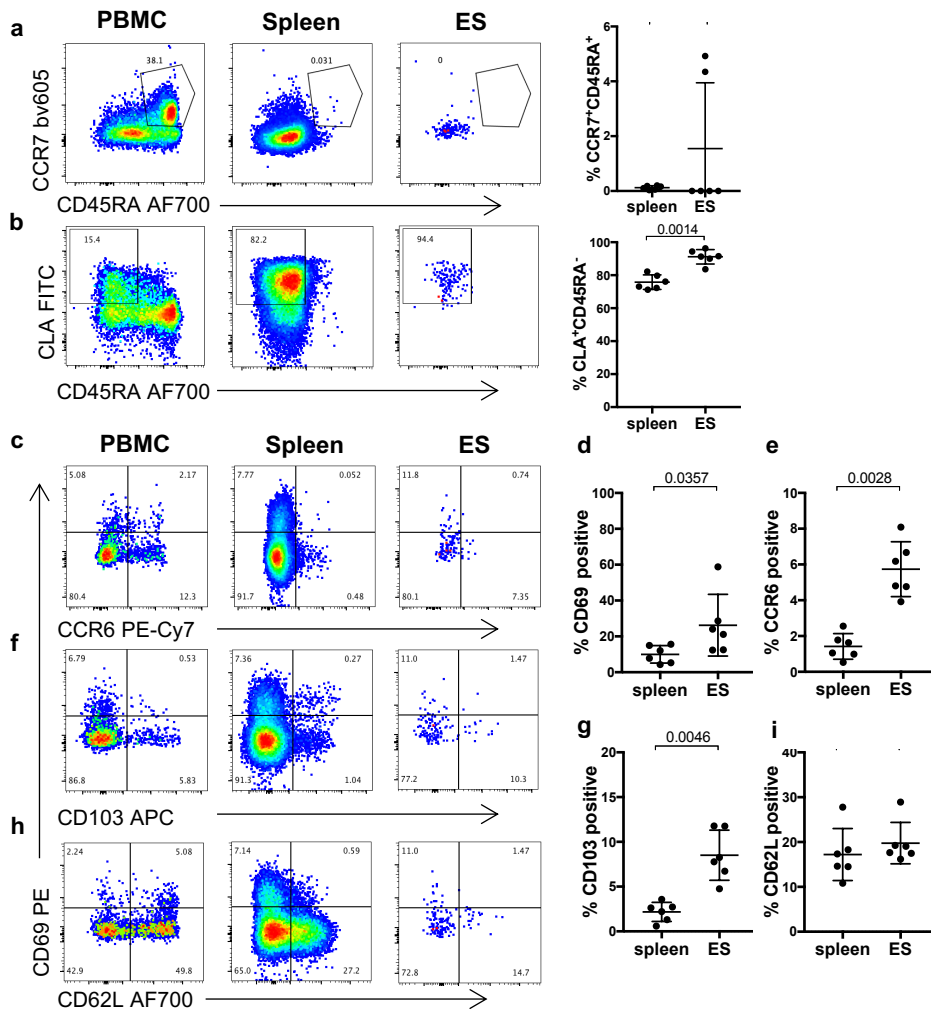
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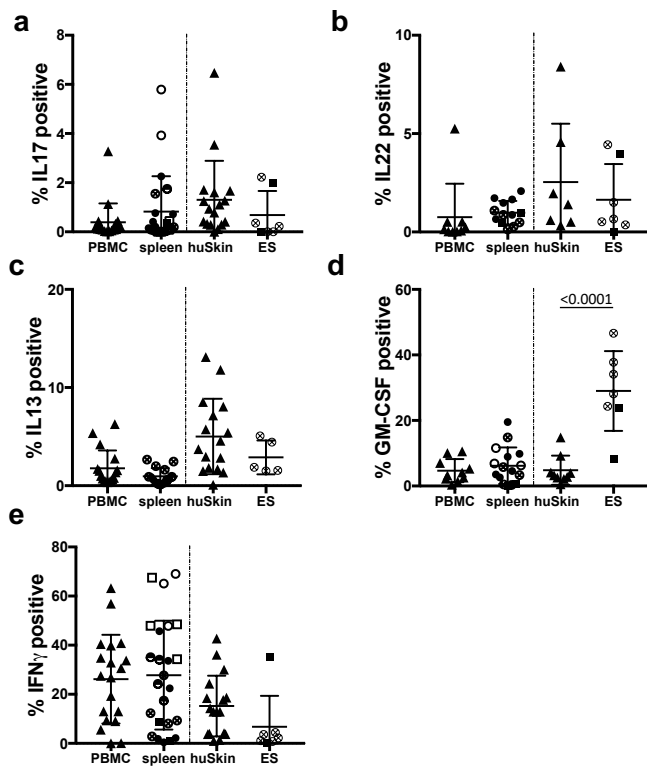
Supplementary Figure 1: Body weight curves of experimental mice after adoptive transfer human PBMC. NSG mice were adoptively transferred with human PBMC and weight was measured at indicated time points. Graph shows 2 representative experiments of 2 different PBMC donors (full lines and dotted lines) after adoptive transfer of 2.5×10^6 PBMC; $n=36$. Vertical black dotted line represents the limit of 35 days for all further experiments using huPBMC-ES-NSG mice.



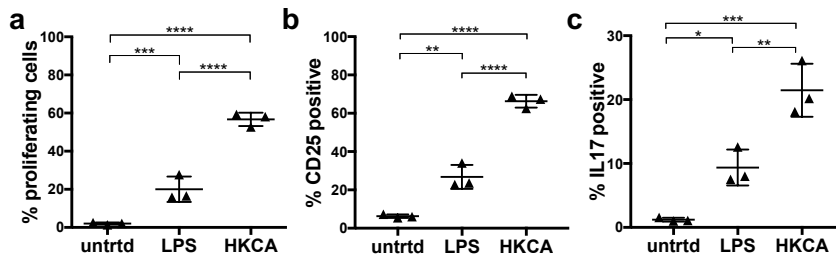
Supplementary Figure 2: Gating strategy for flow cytometric analysis of human PBMC, spleen and ES single-cell suspensions



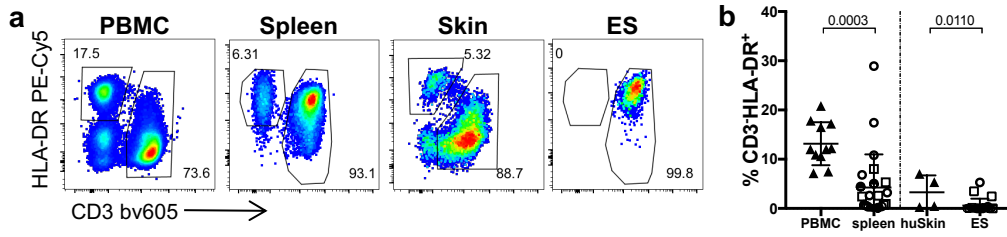
Supplementary Figure 3: Skin and spleen infiltrating CD8⁺ T cells show skin-homing memory phenotype and upregulate markers of tissue residency and skin-tropism in human ES. Representative flow cytometry analysis of (a) CCR7 and CD45RA expression, and (b) CLA and CD45RA expression by gated CD4⁺CD3⁺CD45⁺ live leukocytes from blood of healthy donors, spleen and ES of huPBMC-ES-NSG mice and graphical summary of the proportions of indicated cells by gated CD4⁺CD3⁺CD45⁺ live leukocytes. n=5-6/experiment; cumulative data of 2 independent experiments. (c, f, h) Representative flow cytometry analysis for expression of (c) CCR6 and CD69; (f) CD103 and (h) CD62L in indicated tissues by gated CLA⁺CD45RA⁺CD4⁺CD3⁺ live cells from blood of healthy donors, spleen and ES of huPBMC-ES-NSG mice. (d, e, g, i) Graphical summary of the expression of the indicated markers by CLA⁺CD45RA⁺CD4⁺CD3⁺ living cells isolated from spleen and ES. Each dot represents an individual animal; Significance determined by paired student's t test; mean +/- SD



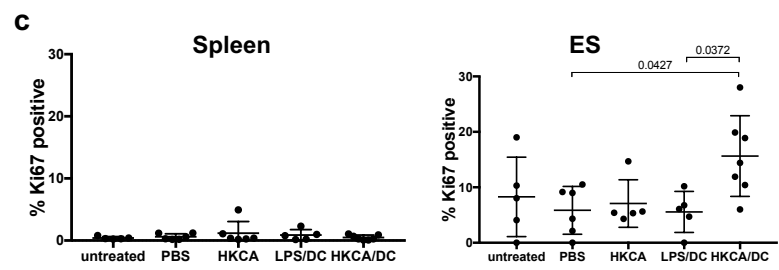
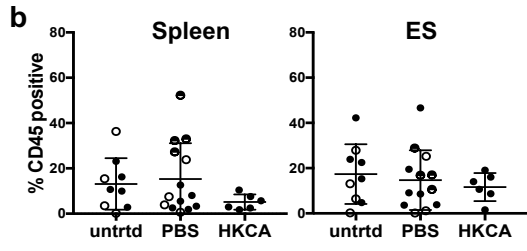
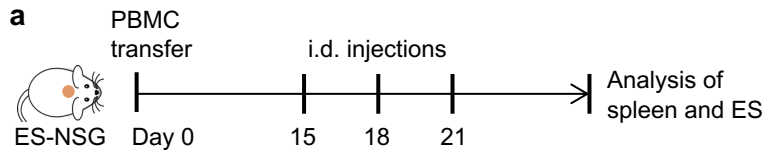
Supplementary Figure 4: Engrafted splenic and cutaneous human CD8⁺ T cells reflect diverse phenotypes of T cells in human tissues. Single cell suspensions of blood and skin of healthy donors, and spleen and ES of huPBMC-ES-NSG mice were prepared 18-35 days after PBMC transfer, stimulated *ex vivo* with PMA/ionomycin and intracellular cytokine production was analyzed by flow cytometry. (a-e) Graphical summary of flow cytometry analysis of IL17, IL22, IL13, GM-CSF and IFN γ producing CD8⁺CD3⁺CD45⁺ gated live leukocytes from blood and skin of healthy donors and spleen and ES of huPBMC-ES-NSG mice as indicated upon *ex vivo* stimulation with PMA/Ionomycin and intracellular staining. n=3-6/ experiment; combined data of 1-4 independent experiments, as indicated by the different fillings of the symbols.



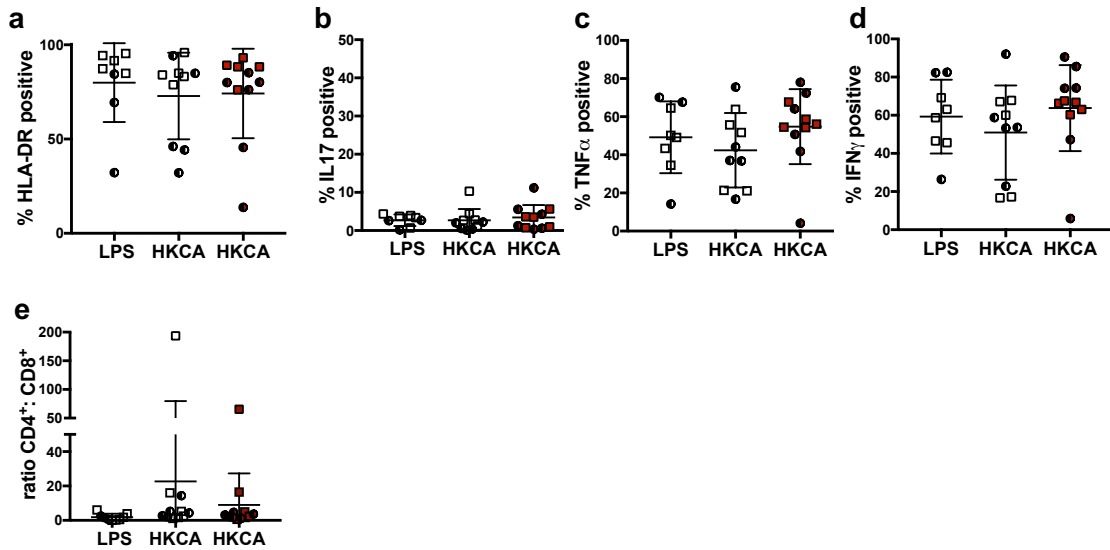
Supplementary Figure 5: Heat killed *C.alibicans* presented by moDCs leads to proliferation and activation of CD4⁺ T cells. PBMC were cultured *in vitro* in the presence of monocyte-derived DCs (moDC) for 7 days and the proliferation, CD25 expression and cytokine production of CD4⁺ T cells gated by CD3⁺CD45⁺ live leukocytes was assessed by flow cytometry. (a) Graphical summary of proliferating CD4⁺ T cells and expression of the indicated markers by of human PBMC with autologous non-activated moDC (untrtd), LPS loaded moDC (LPS) or HKCA loaded moDC (HKCA). PBMC were stained with the cell proliferation dye eFluor 450 to identify proliferating cells. n=3/group. P values indicated as asterisk: * \leq 0.05; ** \leq 0.01, *** \leq 0.001, **** \leq 0.0001



Supplementary Figure 6: CD3⁺HLA-DR⁺ antigen presenting cells do not engraft well within huPBMC-ES-NSG. a) Representative flow cytometry analysis of CD3⁺HLA-DR⁺ cells in human PBMC, skin and spleen and ES of huPBMC-ES-NSG and **(b)** graphical summary of CD3⁺HLA-DR⁺ cells gated on live CD45⁺ leukocytes. n=4-6/group, combined data of 1-5 independent experiments, as indicated by the fillings of the symbols.



Supplementary Figure 7: Free heat killed *C.alibicans* fails to elicit proliferation and activation of CD4⁺ T cells. (a) Experimental procedure of the injection of 1×10^6 /ml free HKCA cells into the ES. Single cell suspensions of indicated organs were analyzed by flow cytometry. **(b)** Graphical summary of human CD45⁺ cell proportions of live leukocytes in spleen and ES of mice that were left untreated (untrtd), injected with PBS or free HKCA. n=3-5/group, combined data of 1-3 independent experiments Statistical significance determined by ANOVA and Tuckey's test for multiple comparison; mean +/-SD. **(c)** The ES of huPBMC-ES-NSG mice were injected with PBS, free HKCA, LPS-activated moDC or HKCA-loaded moDC and harvested 7 days after the last injection similar to (a). Graphical summary of the fraction of Ki67⁺ CD4⁺ cells within the spleen and ES after i.d. injections of the ES as indicated, gated on viable CD45⁺CD3⁺ cells.



Supplementary Figure 8: Splenic T cell are not activated by an allogeneic ES or by HKCA presented in the ES.

NSG mice bearing fully healed ES of one of two different skin donors (A and B) were adoptively transferred with either skin donor-matched PBMC or skin donor-mismatched PBMC. Intradermal injections of donor A derived LPS/moDC or HKCA/moDC were performed as depicted in Fig. 6 (i.e. leukocytes were matched). Single cell suspensions of spleens were analyzed by flow cytometry after *ex vivo* stimulation with PMA/Ionomycin and intracellular staining. **(a)** Graphical summary of the expression of HLA-DR by CD4⁺ T cells of CD3⁺CD45⁺ live leukocytes isolated from the spleen of huPBMC-ES-NSG mice after intradermal injection of LPS/moDC (LPS) or HKCA/moDC (HKCA) into ES matched (black or empty symbols) or mismatched (red symbols) to the leukocytes. **(a-d)** Splenocytes were stimulated with PMA/ionomycin *ex vivo* and intracellular cytokine production of the indicated cytokines analyzed by flow cytometry. **(e)** Ratio of CD4⁺ to CD8⁺ cells in spleens of huPBMC-ES-NSG mice, gated on CD3⁺CD45⁺ live leukocytes. Each symbol represents a single animal, data compiled from 2-5 independent experiments as indicated by the fillings of the symbols.

Table S1: Detailed list of antibodies and reagents**Tissue preparation**

Reagent	Company	Catalog number
Collagenase Type 4	Worthington	LS004186
DNAse	Sigma-Aldrich	DN25
RPMI 1640	Gibco	31870074
human serum	Sigma-Aldrich	H5667/H4522
Penicillin/streptavidin	Sigma-Aldrich	P0781
L-Glutamine	Gibco	A2916801
NEAA	Gibco	11140035
Sodium-Pyruvat	Sigma-Aldrich	S8636
β-Mercaptoethanol	Gibco	31350-010
PBS	Gibco	14190169
Ficoll Paque Plus	GE-Healthcare	GE17-1440-02
Protease Inhibitor Cocktail	Sigma-Aldrich	P8340

Cellular activation

Reagent	Company	Catalog number
Brefeldin A	Sigma-Aldrich	B6542
Cytofix/Cytoperm kit	BD	RUO 554714
Foxp3 / Transcription Factor Staining Buffer Set	Invitrogen	00-5523-00
Ionomycin	Sigma-Aldrich	I06434
PMA	Sigma-Aldrich	P8139
Recombinant human IL2	Immunttools	11340023
Recombinant human IL4	Immunttools	11340047
Recombinant human GM-CSF	Immunttools	11343127
Purified NA/LE Mouse anti-human CD28 (CD28.2)	BD	555725
Functional Grade, CD3 monoclonal Antibody (OKT3)	eBioscience	16-0037-85
Cytokine/Chemokine/Growth Factor 45-Plex Human ProcartaPlex™	Invitrogen	EPX-450-12171-901

Skin cell culture and transplantation

Reagent	Company	Catalog number
Epilife	Gibco	MEPICF500
DMEM	Gibco	11960-044
MEM	Gibco	11380037
TrypLE express	Gibco	12604021
Primocin	invitrogen	ant-pm-1
X-VIVO 15	Lonza	881028

Tissue preparation from mice

Reagent	Company	Catalog number
BD Pharm Lyse	BD	555899
Collagenase from Clostridium histolyticum	Sigma-Aldrich	C9407
Hyaluronidase	Sigma-Aldrich	H3506

Histology

Reagent	Company	Catalog number
Cell Conditioning 1 (CC1)	Roche	950-124
Eosin Y aqueous solution	Sigma	HT110232
Hemalum solution acid acc. to Mayer	Carl Rorth	T865.1
Human Cytokeratin 5/6 (D5/16B4)	Roche	790-4554
ProLong™ Gold Antifade Mountant with DAPI	Invitrogen	P36931
TissueTek O.C.T. Compound	Sakura	TTEK
ultraView Universal DAB Detection kit	Roche	760-500

Antibodies

Reagent	Company	Catalog number
CLA FITC (HECA-452)	Biolegend	321306
CLA bv605 (HECA-452)	BD	563960
CD1a FITC (HI149)	BioLegend	300104
CD3 PerCP-Cy5.5 (OKT3)	eBioscience	45-0037-42
CD3 BV421 (UCHT1)	BioLegend	300434
CD3 bv605 (SK7)	BioLegend	344835
CD3 PE (HIT3a)	BD	561803
CD3 PE-Cy5.5 (UCHT1)	eBioscience	15-0038-42
CD4 PE-594 (RPA-T4)	BioLegend	300548
CD4 PE-Cy5 (RPA-T4)	BioLegend	300510
CD4 Alexa Fluor 700 (RPA-T4)	Biolegend	300526
CD4 bv500 (RPA-T4)	BD	560768
CD8 PE (OKT8)	eBioscience	12-0086-42
CD8 Pacific Orange (3B5)	eBioscience	MHCD0830
CD8 bv510 (RPA-T8)	BioLegend	301048
CD14 bv421 (M5E2)	BioLegend	301830
CD25 PE-Cy5	BioLegend	302608
CD25 PE-Cy7 (BC96)	eBioscience	302612
CD45 PerCP-Cy5.5 (HI30)	BD	564105
CD45 APC (HI30)	eBioscience	17-0459-42
CD45 BV785 (HI30)	BioLegend	304048
CD45RA PE-e610 (HI100)	eBioscience	61-0458-42
CD45RAPerCP-Cy5.5 (HI100)	BioLegend	304122
CD45RA Alexa Fluor 700 (HI100)	BioLegend	304120
CD62L AF700 (DREG-56)	Biolegend	304820
CD69 PE (FN50)	BioLegend	310906
CD69 PerCP/eFluor710 (FN50)	eBioscience	46-0699-42
CD86 PE (B7-2)	eBioscience	12-0869-41

CCR6 PE-Cy7 (G034E3)	BioLegend	353418
CCR7 bv605 (G043H7)	BioLegend	353224
GM-CSF bv421 (BVD2-21C11)	BD	562930
HLA-DR AF700 (L243)	BioLegend	307626
HLA-DR eFluor 780 (LN3)	eBioscience	47-9956-42
IFNg PE-Cy7 (B27)	BioLegend	506518
IFN-g BV605 (4S.B3)	BD	563731
IFN-g AF700 (4S.B3)	BioLegend	502520
IL-2 PE (MQ1-17H12)	eBioscience	12-7029-42
IL17A APC (BL168)	BioLegend	512334
IL-17A PerCP-Cy5.5 (eBio64DEC17)	eBioscience	45-7179-42
IL-13 PE (JES10-5A2)	BD	554571
IL-22 PE-Cy7 (22URTI)	eBioscience	25-7229-42
IL-22 PE-PE (22URTI)	eBioscience	12-7229-42
ki67 bv605 (ki67)	BioLegend	350521
ki-67 PE-Cy7 (SolA15)	eBioscience	25-5698-82
TNFa FITC (Mab11)	BioLegend	502915
TNFa PE-594 (Mab11)	BioLegend	502946
TCRgd PerCP-eFluor710 (B1.1)	eBioscience	46-9959
Fixable Viability Dye eFluor™ 780	eBioscience	65-0865-14
Fixable Viability Dye eFluor™ 520	eBioscience	65-0867-14
eBioscience™ Fixable Viability Dye eFluor™ 506	eBioscience	65-0866-14
IF primary antibody: rabbit anti-human NC-1 domain of type VII collagen (LH7.2)	kindly provided by Dr. Alexander Nyström, University of Freiburg, Germany	
IF secondary antibody: goat anti-rabbit A488	ThermoFisher	A11008
mLy6G (Gr-1) InVivoMab RB6-8C5	BioXcell	BE0075
Cell Proliferation Dye eFluor™ 450	ThermoFisher	65-0842-85