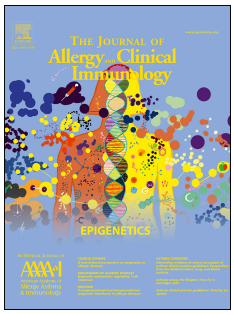
# Journal Pre-proof




Single cell transcriptomics reveal polyclonal memory T cell responses in abacavir patch test positive skin

Alec James Redwood, PhD, Francois Rwandamuriye, MS, Abha Chopra, PhD, Shay Leary, BSc, Ramesh Ram, PhD, Wyatt McDonnell, PhD, Katherine Konvinse, PhD, Katie White, MD, PhD, Rebecca Pavlos, PhD, David M. Koelle, MD, Simon Mallal, MBBS, Elizabeth Phillips, MD

PII: S0091-6749(19)31249-7

DOI: https://doi.org/10.1016/j.jaci.2019.09.013

Reference: YMAI 14191

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 9 July 2019

Revised Date: 22 September 2019

Accepted Date: 26 September 2019

Please cite this article as: Redwood AJ, Rwandamuriye F, Chopra A, Leary S, Ram R, McDonnell W, Konvinse K, White K, Pavlos R, Koelle DM, Mallal S, Phillips E, Single cell transcriptomics reveal polyclonal memory T cell responses in abacavir patch test positive skin, *Journal of Allergy and Clinical Immunology* (2019), doi: https://doi.org/10.1016/j.jaci.2019.09.013.

# Single cell transcriptomics reveal polyclonal memory T cell responses in abacavir patch test positive skin.

Alec James Redwood, PhD[a], Francois Rwandamuriye, MS[a], Abha Chopra, PhD[a], Shay Leary, BSc[a], Ramesh Ram, PhD[a], Wyatt McDonnell, PhD[b], Katherine Konvinse, PhD[c], Katie White, MD, PhD[b], Rebecca Pavlos, PhD[a], David M. Koelle, MD[d,e,f,g,h], Simon Mallal, MBBS[a,b,c] and Elizabeth Phillips, MD[a,b,c,i]

**Author affiliations**:

[a]Institute for Immunology and Infectious Diseases, Murdoch University, Murdoch, WA, Australia

[b]Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[c]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center. Nashville, Tennessee, USA

[d]Department of Medicine, University of Washington, Seattle, WA, USA

[e]Department of Global Health, University of Washington, Seattle, WA, USA

[f]Benaroya Research Institute, Seattle, WA, USA

[g]Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, USA

[h]Department of Laboratory Medicine, University of Washington, Seattle, WA, USA

[i]Department of Pharmacology, Vanderbilt University Medical Center. Nashville, Tennessee, USA

**Author academic degrees**. Alec J. Redwood **PhD**, Francois Rwandamuriye **M.S**., Abha Chopra **PhD**, Shay Leary **B.Sc.**, Ramesh Ram **PhD**, Wyatt McDonnell **PhD**, Katherine Konvinse **PhD**, Katie White **MD, PhD**, Rebecca Pavlos **PhD**, David M. Koelle **MD**, Simon Mallal **MBBS** and Elizabeth Phillips, **MD**

**Corresponding Author**:

Elizabeth J. Phillips, MD, FRCPC, FRACP, FIDSA, FAAAI

Professor of Medicine, Pharmacology, Microbiology and Immunology

1161 – 21st Avenue South

A-2200 Medical Center North

Nashville, TN 37232-2582

(615) 322-9174; (615) 343-6160

elizabeth.j.phillips@vanderbilt.edu

**Running head**: Ex vivo single cell profiling of abacavir-responsive T cells

**Conflicts of Interest:** The authors have no conflicts of interest relevant to this manuscript. However, EP receives consulting fees from Biocryst. EP and SM receive royalties from UpToDate and have equity in IIID Pty Ltd that holds a patent for HLA-B*57:01 testing for abacavir hypersensitivity. EP, SM, KCK and AC have a patent pending for HLA-A*32:01 testing for vancomycin drug reaction and eosinophilia and systemic symptoms.

**Funding Sources**:

Supported through National Institutes of Health grants P50 GM115305, K12 HL143956, R21 AI139021, P30 AI110527, F30 AI131780, and T32 GM7347, the National Health & Medical

Research Council (Australia) and Australian Centre for HIV & Hepatitis Research (ACH2).

**Capsule Summary**. Single-cell responses in HLA-B*57:01 abacavir patch test positive skin remote to the acute hypersensitivity reaction demonstrate polyclonal T-cell activation and proliferation characterized by a transcriptional and cellular response consistent with memory responses to altered peptides.

**Key words**. Abacavir, drug hypersensitivity, T cell, T-cell receptor, HLA, CD103

**To the Editor**

Abacavir is a guanosine analogue used as part of combination antiretroviral therapy for the treatment of HIV-1 infection. Abacavir hypersensitivity syndrome (AHS) was the major treatment-limiting toxicity of abacavir characterized by fever, malaise, gastrointestinal and respiratory symptoms, and a generalized rash that occurs later in 70% of cases. AHS requires carriage of HLA-B*57:01, providing a 100% negative predictive value and a 55% positive predictive value(1-3). AHS is now a preventable disease that has been addressed by routine HLA-B*57:01 screening prior to abacavir prescription. Research has shown that abacavir non-covalently binds to the floor of the peptide binding groove of HLA-B*57:01, thus generating a vastly different immunopeptidome presented to CD8+ T cells and the concurrent generation of CD8+ T cells with multiple specificities(4, 5).

AHS is a CD8+ T-cell cell dependent reaction and does not elicit an antibody response. Although the mechanism by which abacavir alters antigen presentation is understood, the nature of the specific T-cell responses is less well defined. Both $CD8^+$ and $CD4^+$ T cells are recruited to the skin in response to abacavir (6). Polyclonal HLA-B*57:01-restricted $CD8^+$ T-cell responses to abacavir within the peripheral blood are a feature of AHS(4, 7). However, it is unknown if this polyclonal response is a feature of tissue responses to abacavir, or if a restricted subset of T cells is recruited to sites of inflammation. Although it is known that HLA-B*57:01-restricted $CD8^+$ T cells produce interferon gamma (IFN$\gamma$) and tumour necrosis factor alpha (TNF$\alpha$) in response to abacavir (7), the full functionality of these cells has not been assessed. Here we combine single cell (sc) T-cell receptor (TCR) sequencing (scTCRseq) and scRNAseq to define the landscape of abacavir-specific T cells within a positive skin patch test to abacavir applied fourteen years after AHS.

A 67 year old HLA-B*57:01 positive man had a history of AHS in February 2002 which was characterized by onset of headaches, arthralgia, myalgia, fever and nausea 11 days following commencement of a fixed dose combination of abacavir, lamivudine and zidovudine, and resolution within 48 hours of discontinuation of all drugs. In March 2002, he commenced and tolerated zidovudine, lamivudine and efavirenz. In March 2003, CD4+ T cell count was 1080/$mm^3$ (40%) and viral load (VL) 80 copies/ml and abacavir patch tests to 1% and 10% abacavir in petrolatum were positive. In May 2004, he was changed to zidovudine, lamivudine, lopinavir and ritonavir due to efavirenz central nervous system side effects. In April 2005 he was changed to tenofovir, lamivudine atazanavir/ritonavir due to fat wasting. In November 2016 he was simplified to tenofovir, emtricitabine and dolutegravir. For the last 12 years he had remained stable with normal CD4+ T cell counts and undetectable viral load. In July 2017, after informed consent 1% and 10% abacavir and petrolatum control were applied to the flat surface of the back. The positive 1% and 10% patch tests and unaffected skin distant to the patch tests were biopsied 96 hours later. At the time of sampling in July 2017, he remained on tenofovir, emtricitabine and dolutegravir and CD4+ T cell count was 828/$mm^3$ (42%); viral load <40 copies/ml. T cells were isolated by collagenase digestion from all biopsies. $CD3^+$ T cells were single cell sorted and subjected to immediate *ex vivo* primer-based scTCRseq and scRNAseq. In addition, T-cell lines created from the collagenase digested tissue or isolated by explant culture, were exposed to drug to confirm specificity to abacavir.

T cells within normal skin were predominantly of the effector memory T-cell ($T_{EM}$) phenotype (CD45RO+CCR7-). In normal skin, CD4+ T cells were the major cell type expressing the skin homing marker C-C chemokine receptor 10 (CCR10) (52%), the residency marker CD103 (11%); few cells expressed CD69. In abacavir-exposed skin, 56% of CD3+ cells were CD4+ T cells. Of these, the majority (98%) were of the $T_{EM}$ phenotype; 34% expressed CCR10 and the majority were negative

for CD103 and CD69 suggesting recent recruitment to the site. CD8α was poorly expressed on CD3+ T cells, possibly as a consequence of collagenase P digestion. $CD4^+$ and $CD8^+$ T cells were both present in explant cultures from 1% abacavir-exposed skin, *i.e.* those not exposed to collagenase digestion (Sup. Fig. 1).

Direct *ex vivo* single cell sorting and sequencing was employed to map TCR usage and measure the clonality of abacavir-responsive T cells. Similar to responses that have been previously demonstrated to abacavir in the peripheral blood (Sup. Fig. 2), T-cell responses to abacavir in a positive patch test remote to the acute AHS were highly polyclonal (Fig. 1). Circos plots show variable T-cell receptor alpha variable (TRAV) and T-cell receptor beta variable (TRBV) gene expression, with associated joining (TRAJ or TABJ) chain usage for each TCR V alpha and V beta gene (Fig. 1). TRAV-26.1, -38, -12.2 and -9.2 were the most common TCR V alpha genes used by T cells in abacavir-exposed skin, with TRBV20.1 the most common V beta gene. In normal skin TRAV30 and TRBV20.1 were the most common V alpha and V beta genes, respectively.

T cells isolated from the skin of normal and abacavir-exposed skin by collagenase digestion were used to create T-cell lines using two rounds of non-specific stimulation (phytohemagglutinin P followed by anti-CD3 treatment). These lines were tested for specificity to abacavir by IFNγ enzyme-linked immunospot (ELISpot) assay. T-cell lines from 1% and 10% abacavir-exposed skin but not those from normal skin produced IFNγ when exposed to 35 uM of drug (Sup. Fig. 3A). Similar to what we had demonstrated in non-expanded T-cell populations, bulk TCR sequencing of the T-cell line from the 1% abacavir-exposed skin showed a highly diverse TCR repertoire (Sup. Fig 3B). Explant cultured T cells isolated from 1% abacavir-exposed, but not normal skin, proliferated in culture without additional exogenous stimulation. This spontaneously expanded line was also tested for specificity to abacavir. Following stimulation of the T-cell line with abacavir, CD8+ T cells

up-regulated both CD69 and CD137, indicating continued specificity for the drug in culture. This T-cell line expressed a more restricted, but still, polyclonal TCR repertoire. (Sup. Fig. 1).

Single cell transcriptional profiling was used to assess the phenotype of T cells in normal versus abacavir-exposed skin directly *ex vivo* (Fig. 2). T cells derived from skin exposed to 1% or 10% abacavir demonstrated a common transcriptional signature (Fig. 2A) and these data were therefore pooled for subsequent analysis. The top 20 differentially regulated genes in abacavir-exposed and non-exposed skin-derived T cells are shown in Figure 2B (see also Sup. Fig. 4). Differentially expressed genes fall into several categories. T cells in normal skin were marked by genes known to be enriched or suppressed in $T_{RM}$ cells and included those that either regulate T-cell migration or commitment to tissue residency such as S1PR1, SELL (L-selectin), CD44 and DUSP1 (8) . Genes significantly elevated in T cells from abacavir-exposed skin compared to abacavir-unexposed, unaffected skin included those involved in T-cell metabolism such as LAT1 (9) and those involved in T-cell activation and effector function such as superoxide dismutase 2, interferon-induced transmembrane genes 1 and 2. The latter two genes regulate immunity to RNA viruses, including HIV. A Broad Institute gene set enrichment analysis (GSEA) identified several hallmark gene sets that were differentially expressed in T cells derived from normal versus abacavir-exposed skin. The IFNγ response pathway ($p=1.06 \times 10^{-11}$) and the IFNα response pathway ($p=1.03 \times 10^{-8}$), were significantly up-regulated in T cells from abacavir-exposed skin. In contrast, in T cells derived from normal skin the TNFα signalling via NFκb pathway ($p=9.57 \times 10^{-17}$) and the UV response - down pathway ($p=2.76 \times 10^{-8}$) were significantly up-regulated.

Our findings in abacavir-exposed patch test-positive skin in a patient 14 years following acute AHS are consistent with the altered peptide model of hypersensitivity. We propose that in keeping

with this model, as applied to the skin patch test, abacavir is taken up by dendritic cells (DC) in the epidermis and presented to $T_{RM}$ cells. This interaction leads to endothelial activation and recruitment of polyclonal $T_{EM}$ to the site of abacavir exposure. It is also likely that migratory DC present the antigen to $T_{EM}$ cells in skin- draining lymph nodes and that these $T_{EM}$ lead to additional populations of polyclonal $T_{EM}$ cells that home to the skin. Collectively these $T_{EM}$ recognize the complex of HLA-B*57:01 plus abacavir complexed with self-antigens to form novel structures. Altered peptide presentation leads to T-cell activation and proliferation and the expression of a transcriptional profile consistent with memory responses to viral pathogens.

Alec J. Redwood Ph.D[a]
Francois Rwandamuriye MS[a]
Abha Chopra, PhD[a]
Shay Leary[a]
Ramesh Ram, Ph.D[a]
Wyatt McDonnell, Ph.D[b]
Katherine Konvinse, Ph.D[c]
Katie White, MD, Ph.D[b]
Rebecca Pavlos, Ph.D[a]
David M. Koelle, MD[d,e,f,g,h]
Simon Mallal, MBBS[a,b,c]
Elizabeth Phillips, MD[a,b,c,i]

[a]Institute for Immunology and Infectious Diseases,
Murdoch University, Murdoch, WA, Australia
[b]Department of Medicine, Vanderbilt University
Medical Center, Nashville, Tennessee, USA
[c]Department of Pathology, Microbiology and Immunology,
Vanderbilt University Medical Center. Nashville, Tennessee, USA
[d]Department of Medicine, University of Washington, Seattle, WA, USA
[e]Department of Global Health, University of Washington, Seattle, WA, USA

[f]Benaroya Research Institute, Seattle, WA, USA
[g]Vaccine and Infectious Diseases Division,
Fred Hutchinson Cancer Research Center, Seattle, USA
[h]Department of Laboratory Medicine, University of Washington, Seattle, WA, USA
[i]Department of Pharmacology, Vanderbilt University
Medical Center. Nashville, Tennessee, USA

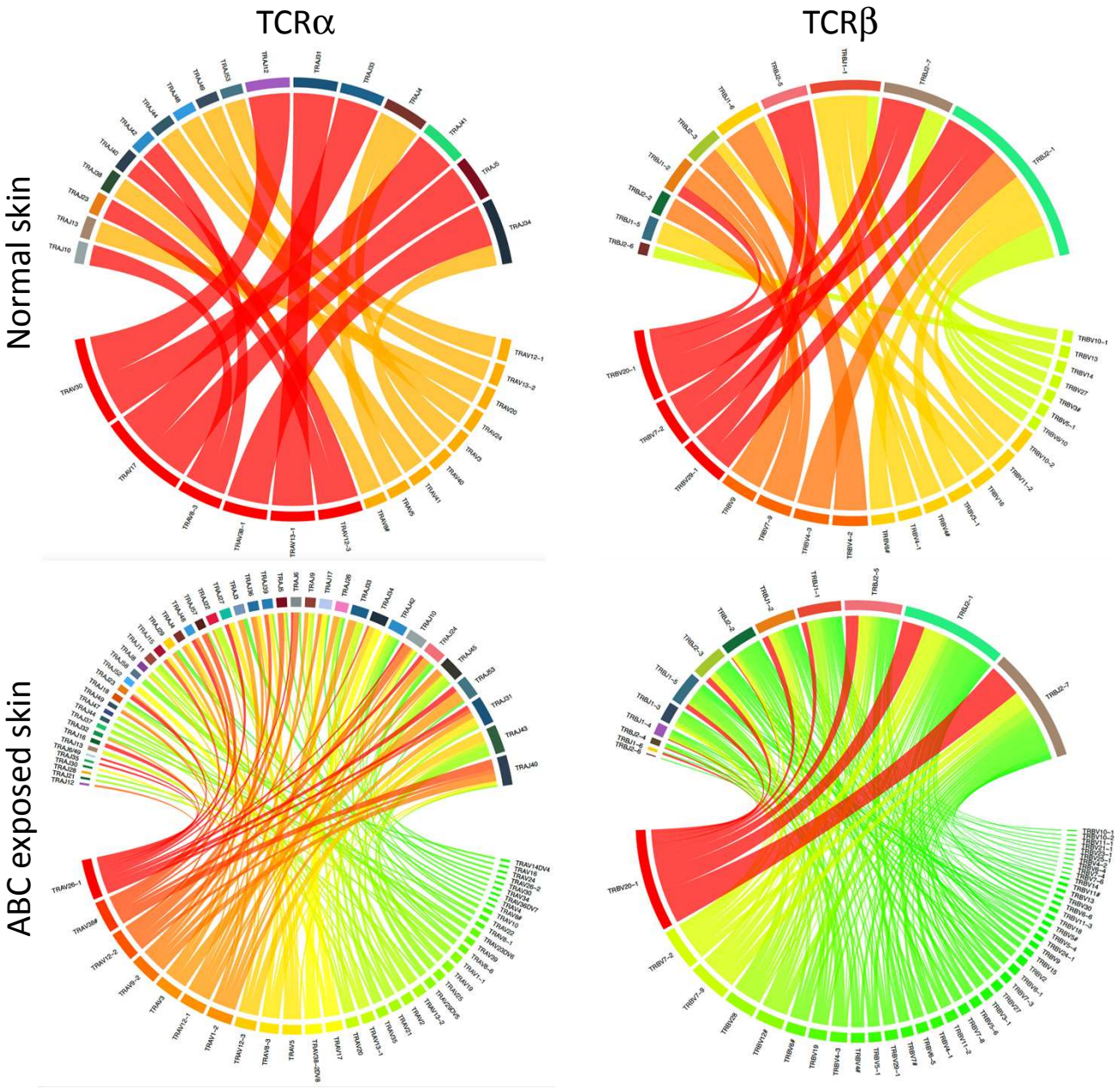
## Figure legends

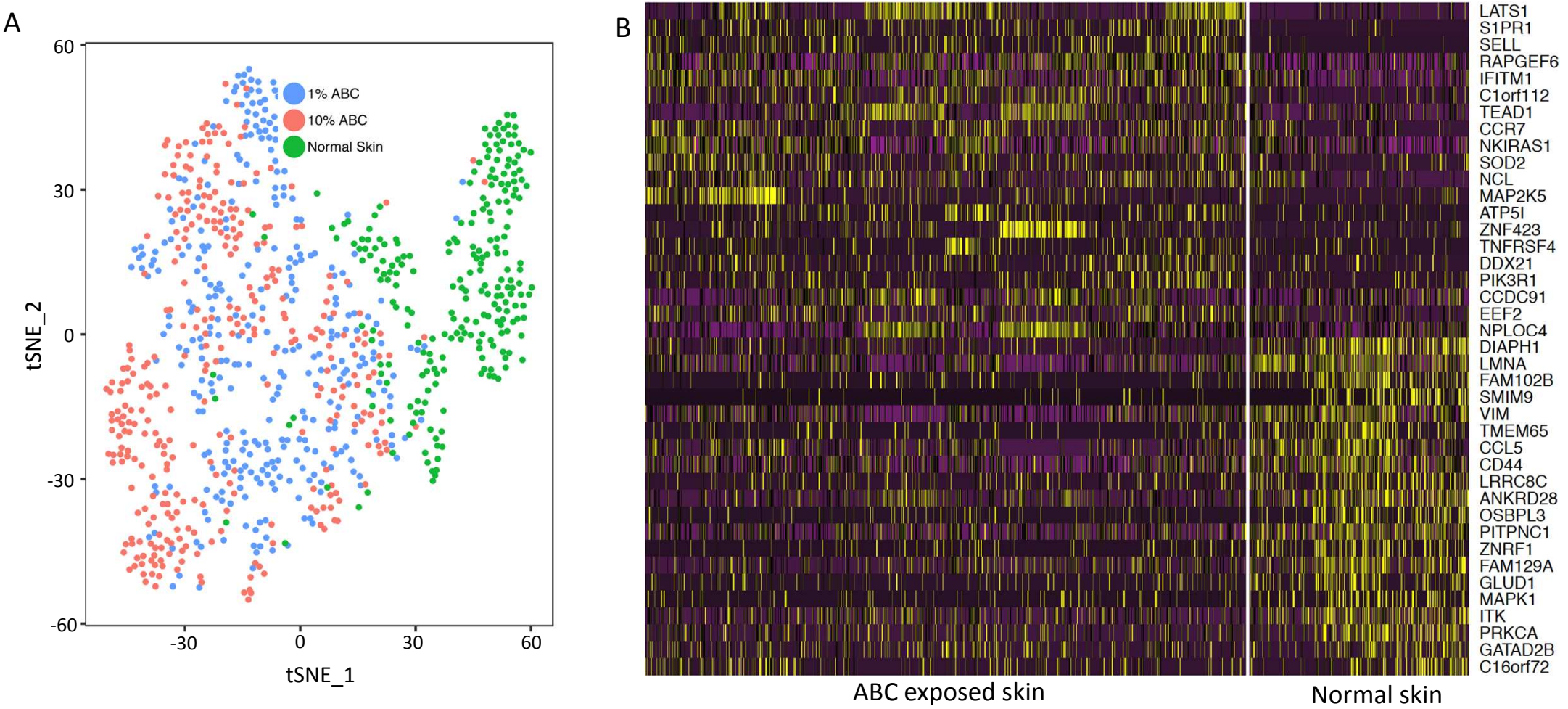
**Figure 1**. **TCR usage in normal or abacavir exposed skin**. A patient with a prior history of abacavir hypersensitivity was patch tested with 1 or 10% abacavir for 96 hours. Each 4 mm punch biopsy, 1 from normal skin, 1 from 1% abacavir exposed skin and 2 from 10% abacavir exposed skin was digested with collagenase P. $CD3^+$ cells were single cell sorted and subjected to TCR sequencing. Shown are TCR variable and joining chains making up each CDR3 region. Data are from normal skin and combined data from 1 and 10% abacavir exposed skin.

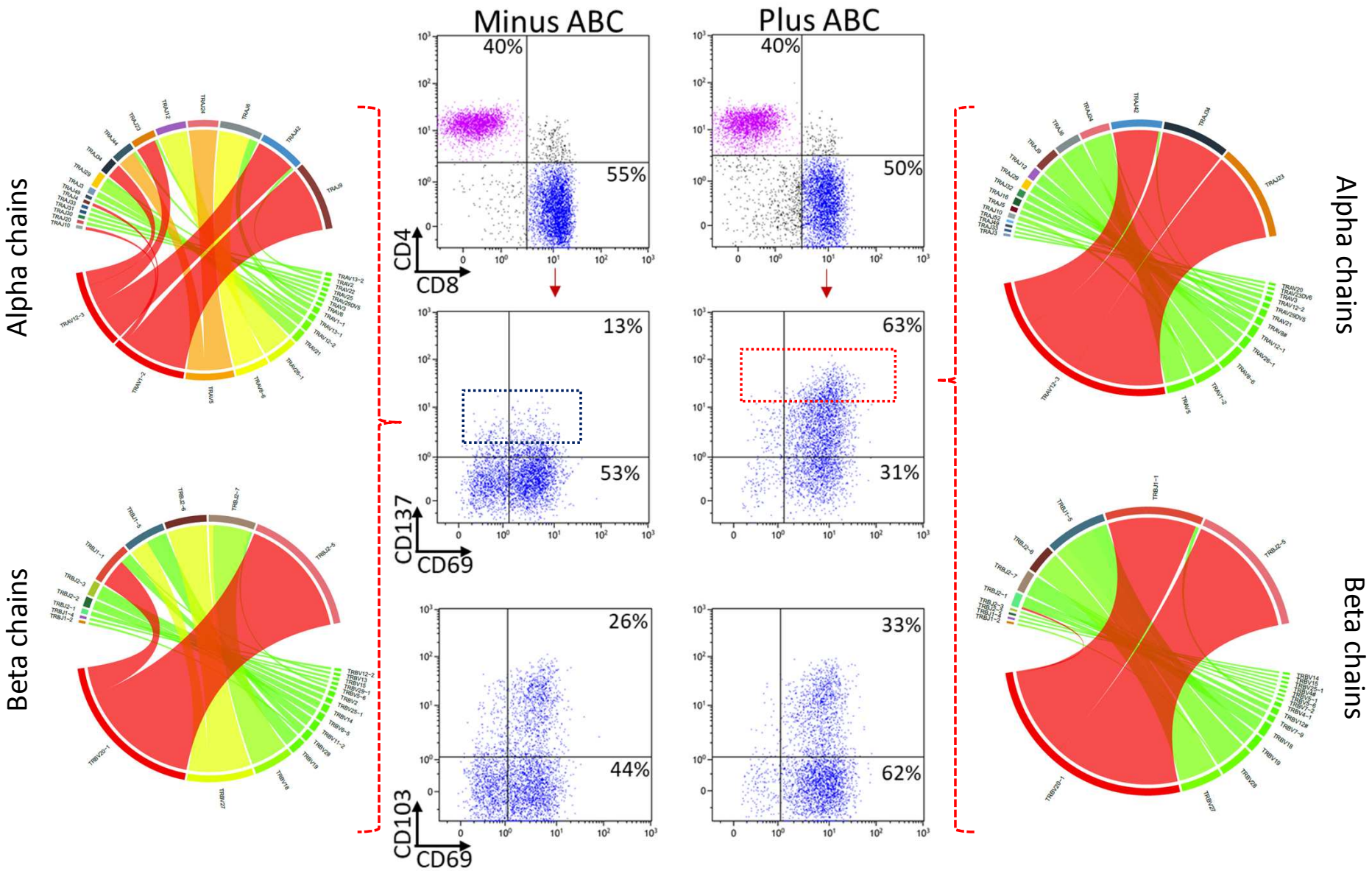
**Figure 2**. **Single cell transcriptional analysis**. **A**. tSNE plot comparing transcriptional profile of T cells isolated from normal skin and 1% and 10% abacavir exposed skin. **B**. Top 20 differentially expressed genes in abacavir exposed and normal skin. Abacavir exposed skin data are combined single cell data from 1% and 10% abacavir exposed T cells.

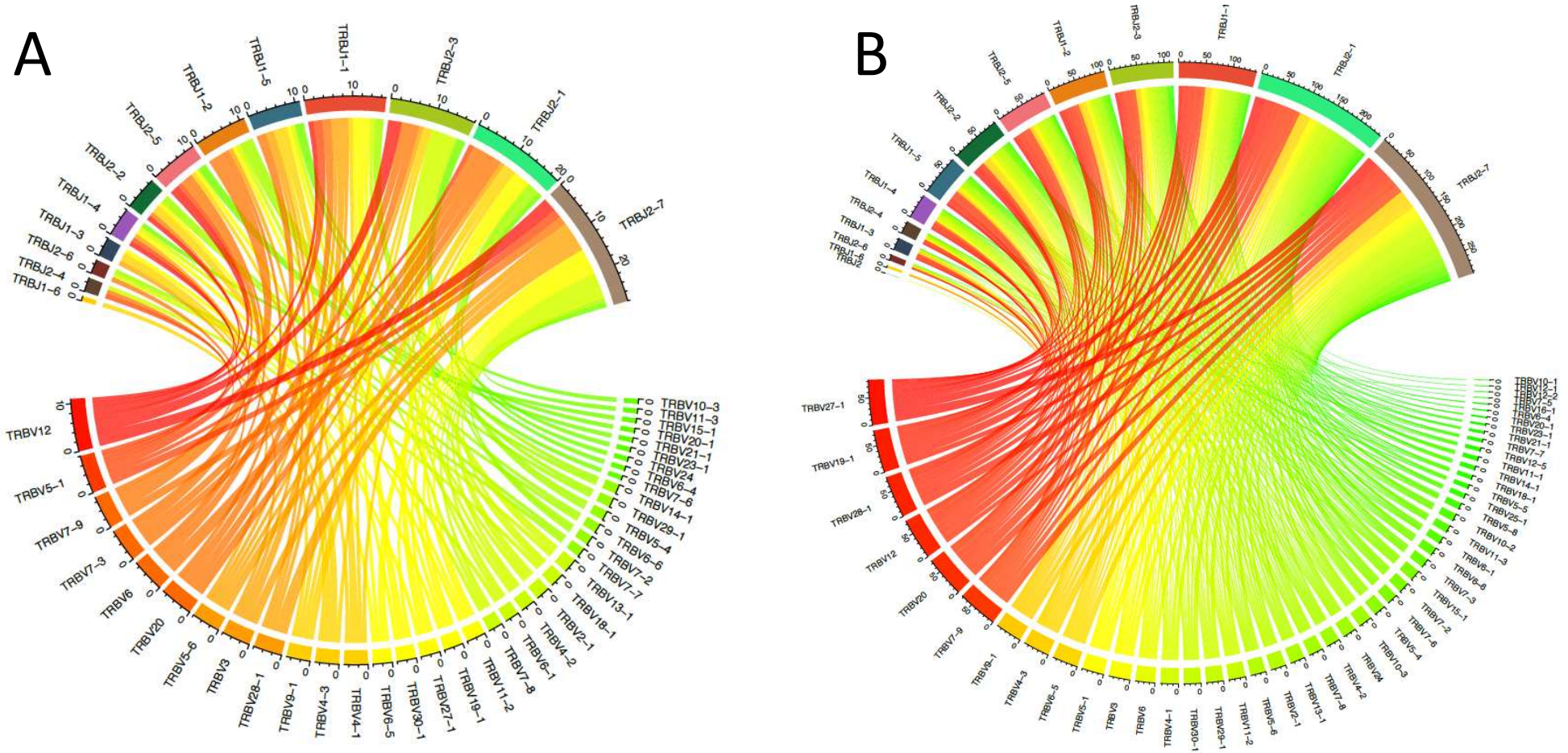
## References

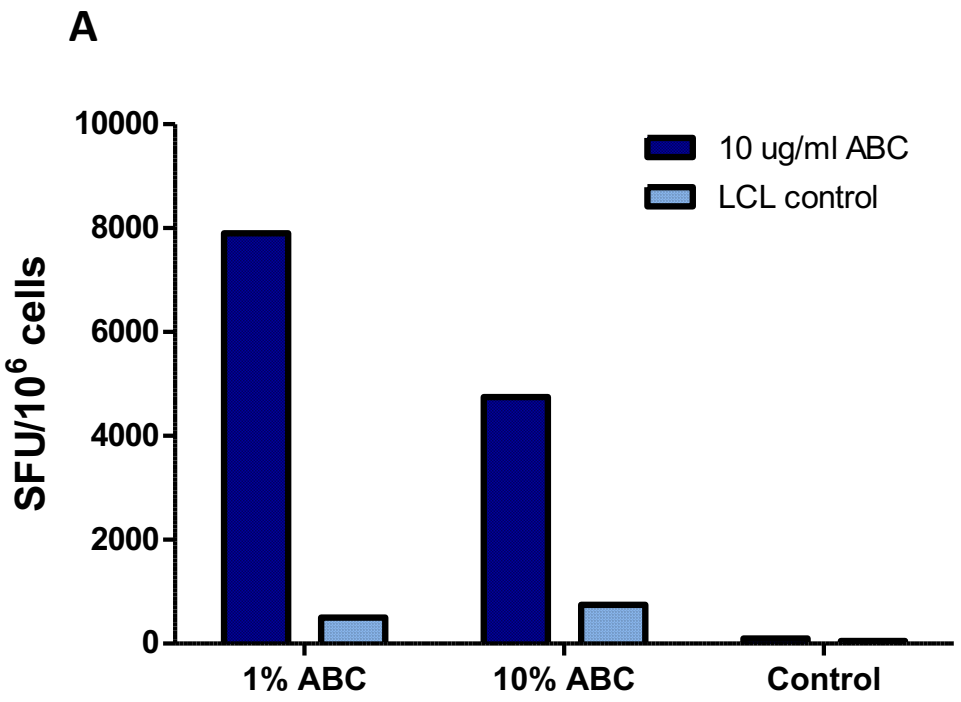

1. Saag M, Balu R, Phillips E, Brachman P, Martorell C, Burman W, et al. High sensitivity of human leukocyte antigen-b*5701 as a marker for immunologically confirmed abacavir hypersensitivity in white and black patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;46(7):1111-8.
2. Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, et al. HLA-B*5701 screening for hypersensitivity to abacavir. The New England journal of medicine. 2008;358(6):568-79.
3. Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet. 2002;359(9308):727-32.
4. Illing PT, Vivian JP, Dudek NL, Kostenko L, Chen Z, Bharadwaj M, et al. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. Nature. 2012;486(7404):554-8.
5. Ostrov DA, Grant BJ, Pompeu YA, Sidney J, Harndahl M, Southwood S, et al. Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. Proceedings of the National Academy of Sciences. 2012;109(25):9959-64.
6. Phillips EJ, Sullivan JR, Knowles SR, Shear NH. Utility of patch testing in patients with hypersensitivity syndromes associated with abacavir. AIDS. 2002;16(16):2223-5.
7. Chessman D, Kostenko L, Lethborg T, Purcell AW, Williamson NA, Chen Z, et al. Human leukocyte antigen class I-restricted activation of CD8+ T cells provides the immunogenetic basis of a systemic drug hypersensitivity.[Erratum appears in Immunity. 2008 Jul;29(1):165]. Immunity. 2008;28(6):822-32.
8. Li J, Olshansky M, Carbone FR, Ma JZ. Transcriptional Analysis of T Cells Resident in Human Skin. PloS one. 2016;11(1):e0148351-e.
9. Sinclair LV, Rolf J, Emslie E, Shi Y-B, Taylor PM, Cantrell DA. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. Nat Immunol. 2013;14:500.

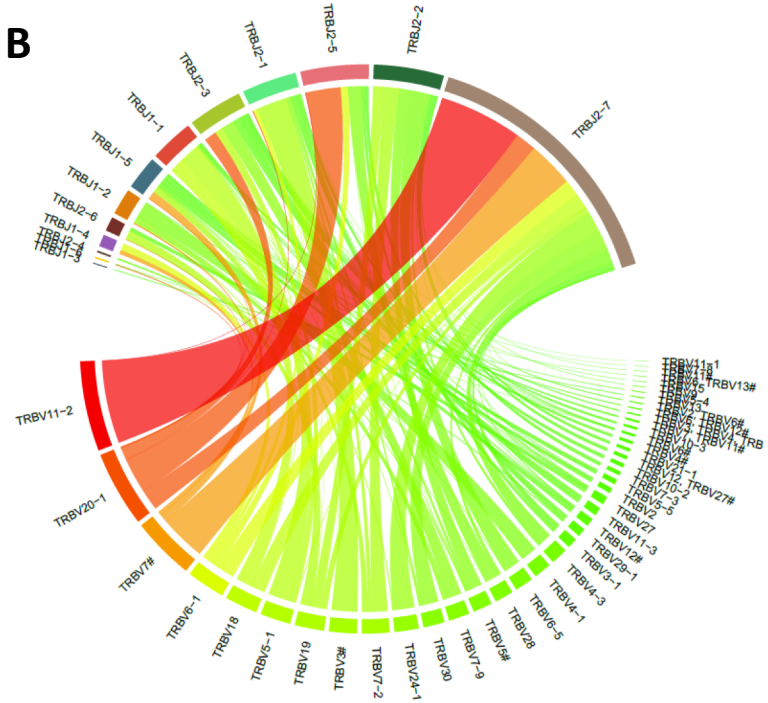

TCRα
TCRβ
Normal skin
ABC exposed skin

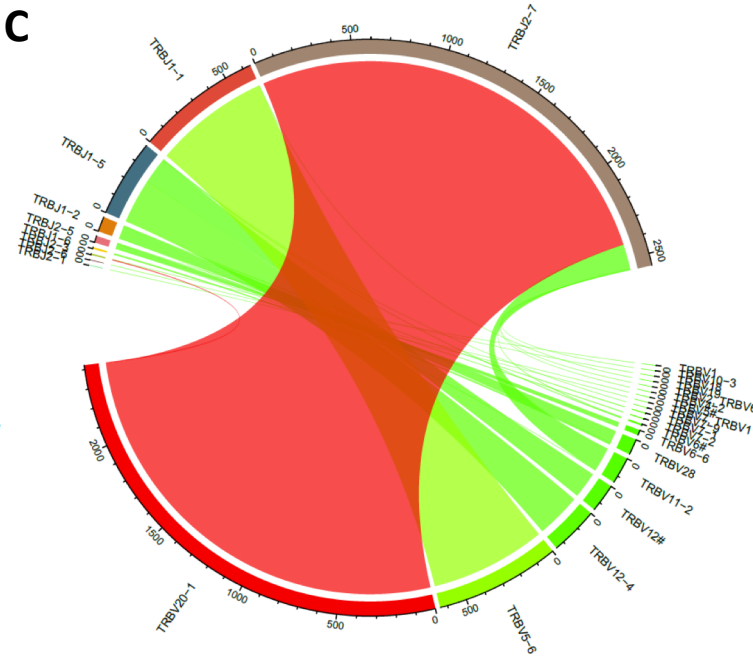

A
60
30
0
-30
-60
tSNE_2
-30
0
30
60
tSNE_1
1% ABC
10% ABC
Normal Skin
B
LATS1
S1PR1
SELL
RAPGEF6
IFITM1
C1orf112
TEAD1
CCR7
NKIRAS1
SOD2
NCL
MAP2K5
ATP5I
ZNF423
TNFRSF4
DDX21
PIK3R1
CCDC91
EEF2
NPLOC4
DIAPH1
LMNA
FAM102B
SMIM9
VIM
TMEM65
CCL5
CD44
LRRC8C
ANKRD28
OSBPL3
PITPNC1
ZNRF1
FAM129A
GLUD1
MAPK1
ITK
PRKCA
GATAD2B
C16orf72
ABC exposed skin
Normal skin

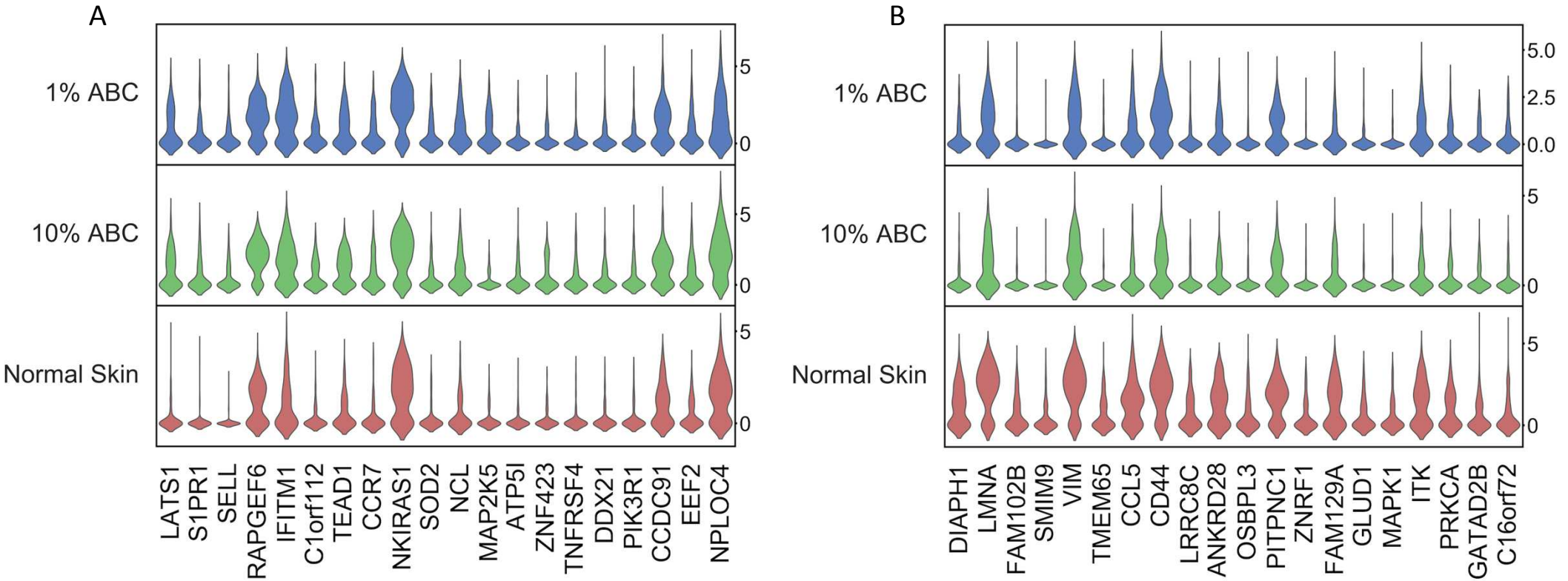

Minus ABC
Plus ABC
Alpha chains
Beta chains
Alpha chains
Beta chains
CD4
CD8
CD137
CD69
CD103
CD69
40%
55%
40%
50%
13%
53%
63%
31%
26%
44%
33%
62%

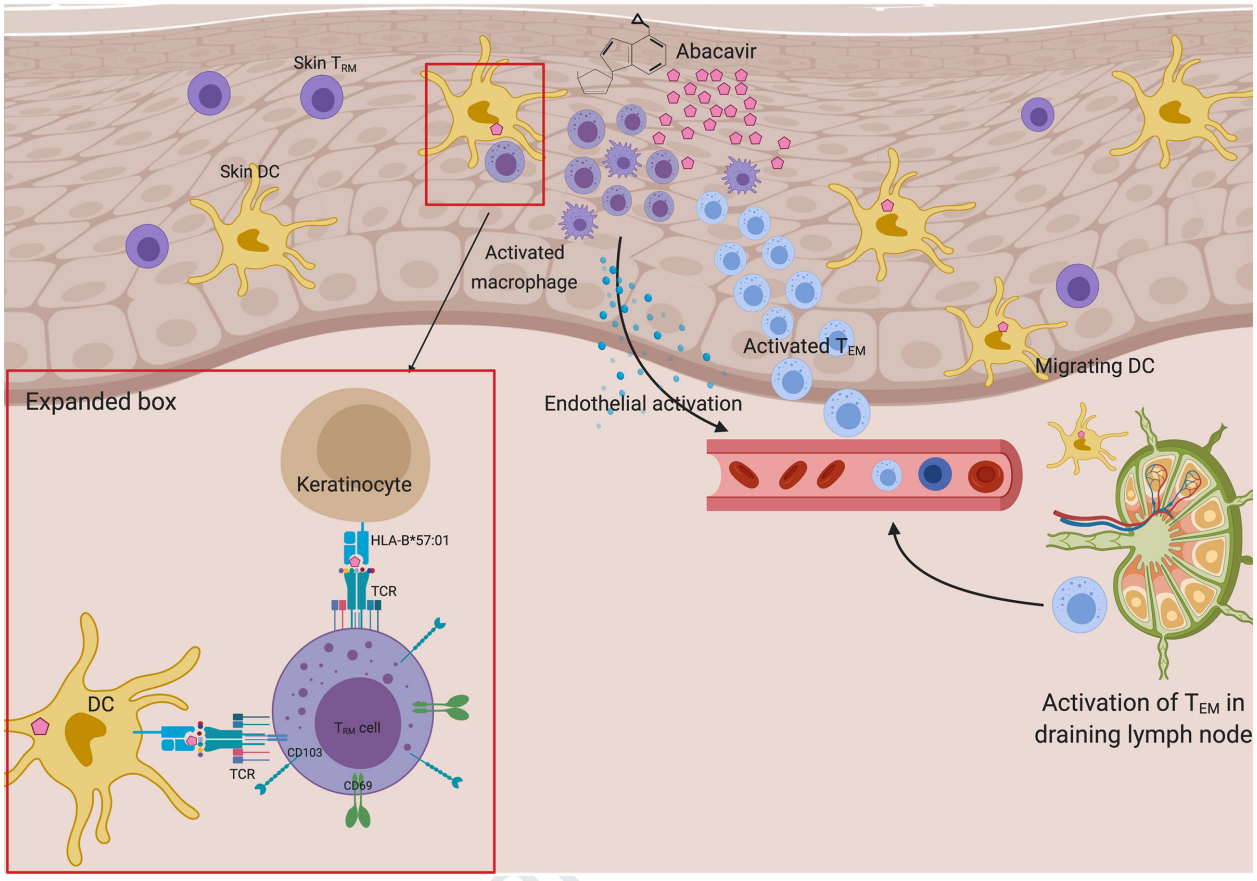

A
TRBJ1-1
TRBJ1-2
TRBJ1-3
TRBJ1-4
TRBJ1-5
TRBJ1-6
TRBJ2-1
TRBJ2-2
TRBJ2-3
TRBJ2-4
TRBJ2-5
TRBJ2-6
TRBJ2-7
TRBV10-3
TRBV11-3
TRBV15-1
TRBV20-1
TRBV21-1
TRBV23-1
TRBV24
TRBV6-4
TRBV7-6
TRBV14-1
TRBV29-1
TRBV5-4
TRBV6-6
TRBV7-2
TRBV7-7
TRBV13-1
TRBV18-1
TRBV2-1
TRBV4-2
TRBV6-1
TRBV7-8
TRBV11-2
TRBV19-1
TRBV27-1
TRBV30-1
TRBV4-1
TRBV6-5
TRBV9-1
TRBV28-1
TRBV3
TRBV5-6
TRBV20
TRBV6
TRBV7-3
TRBV7-9
TRBV5-1
TRBV12
B
TRBJ1-1
TRBJ1-2
TRBJ2-3
TRBJ2-5
TRBJ2-2
TRBJ1-5
TRBJ1-4
TRBJ2-4
TRBJ1-3
TRBJ2-6
TRBJ1-6
TRBJ2
TRBJ2-1
TRBJ2-7
TRBV10-1
TRBV13-2
TRBV7-5
TRBV16-1
TRBV6-4
TRBV20-1
TRBV29-1
TRBV21-1
TRBV7-7
TRBV12-5
TRBV11-1
TRBV14-1
TRBV18-1
TRBV5-5
TRBV25-1
TRBV5-8
TRBV10-2
TRBV11-3
TRBV6-1
TRBV6-8
TRBV7-3
TRBV15-1
TRBV7-2
TRBV7-6
TRBV6-4
TRBV10-3
TRBV24
TRBV14-2
TRBV7-8
TRBV13-1
TRBV2-1
TRBV5-6
TRBV11-2
TRBV29-1
TRBV30-1
TRBV4-1
TRBV6
TRBV3
TRBV5-1
TRBV6-5
TRBV4-3
TRBV6-1
TRBV7-9
TRBV20
TRBV12
TRBV28-1
TRBV19-1
TRBV27-1


A
10000
8000
6000
4000
2000
0
SFU/10^6 cells
10 ug/ml ABC
LCL control
1% ABC
10% ABC
Control


B
TRBJ2-7
TRBJ2-2
TRBJ2-5
TRBJ2-1
TRBJ2-3
TRBJ1-1
TRBJ1-5
TRBJ1-2
TRBJ2-6
TRBJ1-4
TRBV11-2
TRBV20-1
TRBV7#
TRBV6-1
TRBV18
TRBV5-1
TRBV19
TRBV7-2
TRBV24-1
TRBV30
TRBV7-9
TRBV5#
TRBV28
TRBV6-5
TRBV4-1
TRBV4-3
TRBV3-1
TRBV29-1
TRBV12#
TRBV11-3
TRBV27
TRBV2
TRBV5-3
TRBV10-2
TRBV27#
TRBV13#


C
TRBJ2-7
TRBJ1-1
TRBJ1-5
TRBJ1-2
TRBV20-1
TRBV5-6
TRBV12-4
TRBV12#
TRBV11-2
TRBV28
TRBV6-6


A
1% ABC
10% ABC
Normal Skin
5
0
LATS1
S1PR1
SELL
RAPGEF6
IFITM1
C1orf112
TEAD1
CCR7
NKIRAS1
SOD2
NCL
MAP2K5
ATP5I
ZNF423
TNFRSF4
DDX21
PIK3R1
CCDC91
EEF2
NPLOC4
B
1% ABC
10% ABC
Normal Skin
5.0
2.5
0.0
5
0
DIAPH1
LMNA
FAM102B
SMIM9
VIM
TMEM65
CCL5
CD44
LRRC8C
ANKRD28
OSBPL3
PITPNC1
ZNRF1
FAM129A
GLUD1
MAPK1
ITK
PRKCA
GATAD2B
C16orf72


Skin $T_{RM}$
Skin DC
Abacavir
Activated macrophage
Activated $T_{EM}$
Migrating DC
Endothelial activation
Expanded box
Keratinocyte
HLA-B*57:01
TCR
DC
$T_{RM}$ cell
CD103
CD69
TCR
Activation of $T_{EM}$ in draining lymph node

## Supplementary Materials and Methods

***Patch testing and biopsy***. Skin patch tests were prepared and applied in duplicate according to the procedure previously reported by Phillips et al. 2002 (1) and were adapted to use 1% and 10% concentrations of abacavir and excipient and petrolatum controls (Oxford compounding Pty Ltd). Test results were read and recorded at 48 h after application and then, because of the large time interval between last patch test exposure to ABC, 14 years, were re-applied for a further 48 hours. Two biopsies were taken from 1% and 10% ABC patch test skin and one biopsy was taken from control patch test, normal skin.

***T cell isolation***. Two 4 mm biopsies from each of the 1% and 10% ABC patch test skin and one biopsy from normal skin were collected directly into RPMI plus 10% fetal calf serum (FCS) and processed within 30 minutes of collection. Biopsies were finely minced in 1 ml of PBS and then added to 5 ml of 50% T-cell media (TCM, Iscove's Modified Dulbecco's Medium, 20% FCS, 2mM L-glutamine, 100 Units/ml penicillin, 100 ug/ml of streptomycin and 3.4 uM beta 2-mercaptoethanol).

For enzymatic digestion cells in T-cell media were incubated with 1 mg/ml of collagenase P and placed a rotator for 90 minutes at 37°C. DNA was digested by the addition of 200 Kuntz units/ml of DNAse I for 15 minutes at 37°C before the cells were strained through a 70 uM filter and washed with 25 ml of Hank's buffered salt solution (HBSS)/HEPES/10 mM EDTA at 4°C. Cells were centrifuged at 400 x g and washed with a further 10 ml of PBS. Skin derived T cells were then stained immediately for single cell sorting.

For the isolation of T cells via explant culture, one 1% abacavir skin biopsy was minced and placed in TCM plus 100 units/ml of rIL-2 on a cellfoam matrix grid (Nohla Therapeutics, Australia, CY-903) for 2 weeks. T cells that migrated into the media proliferated without the

addition of additional stimulation and were frozen as a T cell line. This line was used to assess specificity for abacavir. 200, 000 T cells were cultured overnight in TCM, TCM plus 20, 000 autologous LCLs or TCM plus 20, 000 autologous LCLs (lymphoblastoid cell line) and 10 ug/ml of abacavir. Cells were stained for CD3 (BD, AF700, clone UCHT1), CD4 (BD, PerCPCy5.5, clone OKT4), CD8a (Biolegend, APC Fire 750, clone RPA-T8), CD137 (PE, clone 4B4-1), CD69 (BD, APC, clone FN50), CD103 (BD, PE/Cy7 clone Ber-ACT8), CCR7 (BV421, clone 150503), CD45RO (BV510, clone UCHL1) and CCR10 (BB515, clone 1B5). Flow cytometry was employed to determine specificity by assessing up-regulation of CD137 and CD69.

***T-cell expansion***. For cloning of enzymatically extracted T cells, tissues were re-extracted as described above for an additional 90 minutes. Cells extracted from normal and abacavir exposed tissues were non-specifically expanded as described previously (2). Briefly, in a single U bottomed well of a 96 well plate, washed T cells were provided with 150, 000 irradiated allo-feeder cells (PBMCs) and cultured in T-cell cloning medium (RPMI, 5% human serum (Sigma-Aldrich), 5% FCS, 2mM L-glutamine, 100 Units/ml penicillin and 100 ug/ml of streptomycin) plus PHA to a final concentration of 1.6 ug/ml. Two days later, and then every 2-3 days, cells were supplemented with 10% natural IL-2 (ZeptoMetrix, USA). Two weeks later 50, 000 T cells were further expanded by the addition of anti-CD3 (30 ng/ml of OKT3 plus 5 x $10^6$ irradiated (8000 Rad) autologous LCLs and 50 x $10^6$ irradiated (3300 Rad) autologous PBMCs in a total volume of 25 ml. Two days later T cells were provided with 100 units/ml of rIL-2. Cells were washed on day 4 to remove anti-CD3 and expanded for an additional 10 days with the rIL-2 being replaced every 2-3 days. T cells were frozen until required.

***Single cell sorting***. Collagenase P purified T cells were stained with CD3 (BD, AF700, clone UCHT1), CD4 (BD, PerCPCy5.5, clone OKT4), CD8 (Biolegend, APC Fire 750, clone RPA-T8), CD137 (PE, clone 4B4-1), CD69 (BD, APC, clone FN50), CD103 (BD, PE/Cy7 clone Ber-ACT8), CCR7 (BV421, clone 150503), CD45RO (BV510, clone UCHL1) and CCR10 (BB515, clone 1B5). All antibodies were obtained from Becton Dickinson (BD), Australia, unless otherwise stated. Dead cells were excluded with the use of the fixable viable Stain, FVS620 (BD). Single cells were index sorted into 96 well plates on a FACSAria III (Telethon Kids Institute, Perth, Australia) directly into 3 ul of reverse transcriptase buffer. Immediately following sorting cells were frozen on dry ice and then plates were stored at -80°C until required.

***Single cell TCR sequencing***. Using our novel TCR/RNAseq platform single cells undergo oligodT-primed reverse transcription during which, individual well's (cell's) cDNA products were barcoded and generically tagged with both 3' oligo-dT and 5' biotin labelled template switching oligonucleotides (TSO). Subsequent amplification of the cDNA derived from a single cell was amplified using the generic tags. Specific transcripts were targeted with a combination of nested generic tags and suitable gene specific primers (e.g. TCR alpha or beta conserved regions). The transcriptome was analysed using a modified Nextera transposon-mediated tagging-and-fragmentation ("tagmentation") with subsequent amplification of the 5' and 3' ends of the transcripts between the introduced Nextera-tag and the generic tags from cDNA conversion. Samples were multiplexed for next generation library preparation and sequencing - single cell sequences are bioinformatically separated.

***IFNγ ELISpot***. ELISpot were performed as previously described (3). Briefly, 96 well ELISpot plates (MultiScreen-IP Filter Plate, Millipore) were coated with 100 ul/well of anti-IFNγ (1-DIK, 2µg/mL - Mabtech) overnight at 4°C. Coating antibody was removed by 6x washes in

200 ul of PBS and the wells blocked with 100 ul of RPMI plus 10% FCS (R10) for 30-180 minutes at room temperature (RT). 10, 000 to 20, 000 T cells were added to each well in a total volume of 100 ul of R10 (RPMI, 10% FCS, 2mM L-glutamine, 100 Units/ml penicillin and 100 ug/ml of streptomycin). Autologous LCLs were incubated for 24 hours with 35 uM abacavir and then washed 3 times before adding them to T cells. Cloned skin derived T cells were incubated in T cell cloning media plus 10 units/ml of rIL-2 with abacavir pulsed LCLs overnight. Controls (anti-CD3-positive control, 100 ul of R10-negative control and pulsed LCLs negative control) were treated similarly. Plates were washed as described and incubated with 100 ul/well of diluted (1/1000 in PBS/0.5% FCS) biotinylated anti-IFNγ (7-B6, Mabtech) at RT for 2 hrs. Plates were washed as described and incubated with 100 ul/well of streptavidin horseradish peroxidise (1/1000 in PBS/0.5% FCS) for one hour at RT in the dark. Plates were washed as described and developed with 100 ul/well of TMB for 12 minutes in the dark. Colour reaction was stopped by 6 washes in water and the plates air dried for 24 hrs and read on a plate reader (AID).

***Bioinformatics*.** 3’ and 5’ RNA libraries were sequenced using the 2 x 75 bp paired end Illumina NextSeq. Reads for the individual single-cells were demultiplexed using plate id (30 nt), and cell barcode (6 nt) from either Read 1 or Read 2. The other read was further demultiplexed as either 3’ or 5’ using primer sequence (30 nt) and the reminder 45 nt sequences were aligned with CLC Bio (v.2018), using the GRCh38 human reference genome (Ensembl rel. 92). Gene-specific read counts were calculated using HTSeq-count using latest Gencode annotations and the 3’ and 5’ counts were summed. Cells with less than 200 genes and more than 5% mitochondrial content were removed. Furthermore, genes with > 0 counts in fewer than three cells were also removed. Downstream analyses (normalization,

PCA, differential expression and visualization) were performed in Seurat v.2.3.4 R package and Scanpy. TSNE analysis was performed using following settings: theta=0, max_iter=10000, perplexity=100. Differential expression analysis was performed using the Wilcoxon rank-sum test. The P values were adjusted for multiple testing using the False Discovery Rate (FDR) correction. Pathway analysis was done using MSigDB.

References

1. Phillips EJ, Sullivan JR, Knowles SR, Shear NH. Utility of patch testing in patients with hypersensitivity syndromes associated with abacavir. AIDS. 2002;16(16):2223-5.
2. Jing L, Laing KJ, Dong L, Russell RM, Barlow RS, Haas JG, et al. Extensive CD4 and CD8 T Cell Cross-Reactivity between Alphaherpesviruses. The Journal of Immunology. 2016;196(5):2205.
3. Trubiano JA, Strautins K, Redwood AJ, Pavlos R, Konvinse KC, Aung AK, et al. The Combined Utility of Ex Vivo IFN-γ Release Enzyme-Linked ImmunoSpot Assay and In Vivo Skin Testing in Patients with Antibiotic-Associated Severe Cutaneous Adverse Reactions. J Allergy Clin Immunol Pract. 2018;6(4):1287-96.e1.