Immunity, Volume *54*

Supplemental information

Single-cell chromatin accessibility landscape identifies tissue repair program in human regulatory T cells

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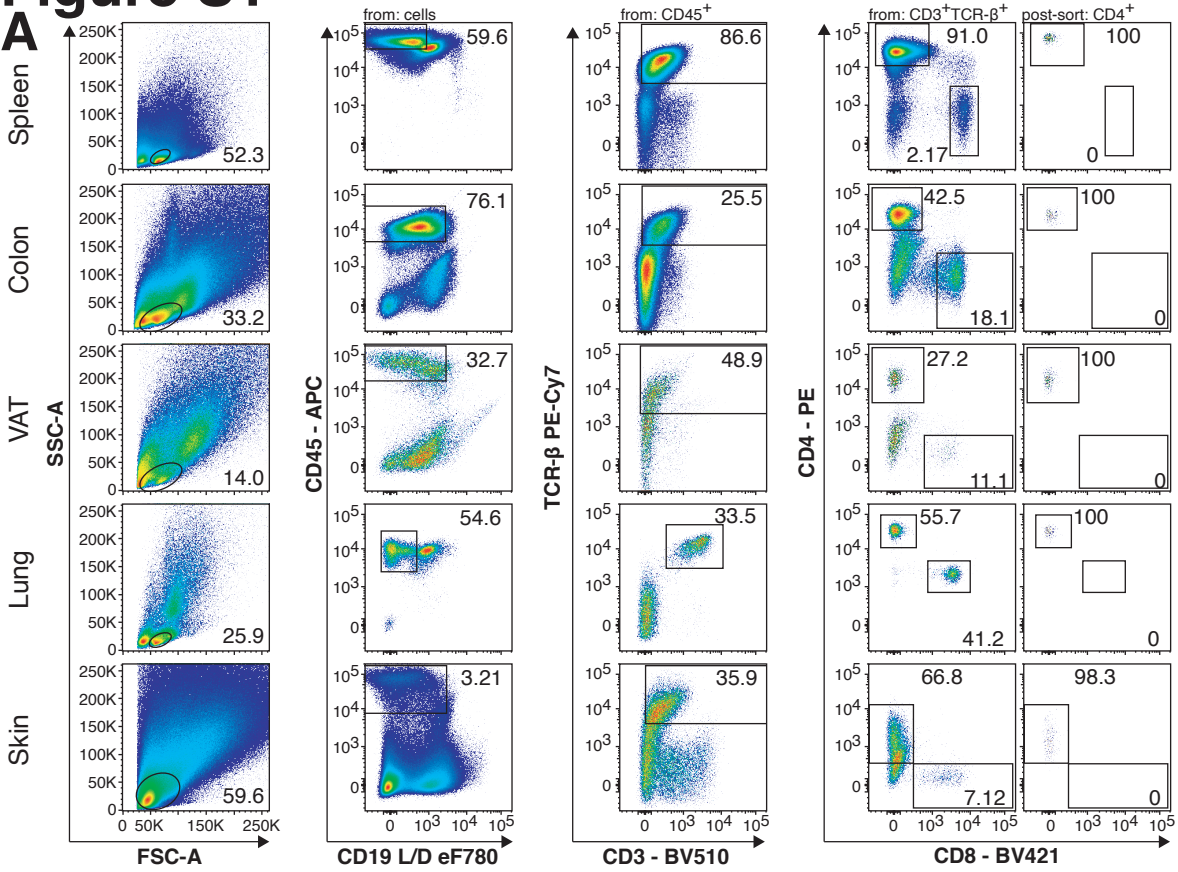
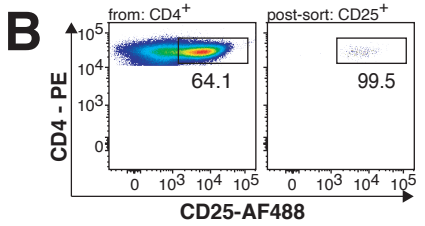
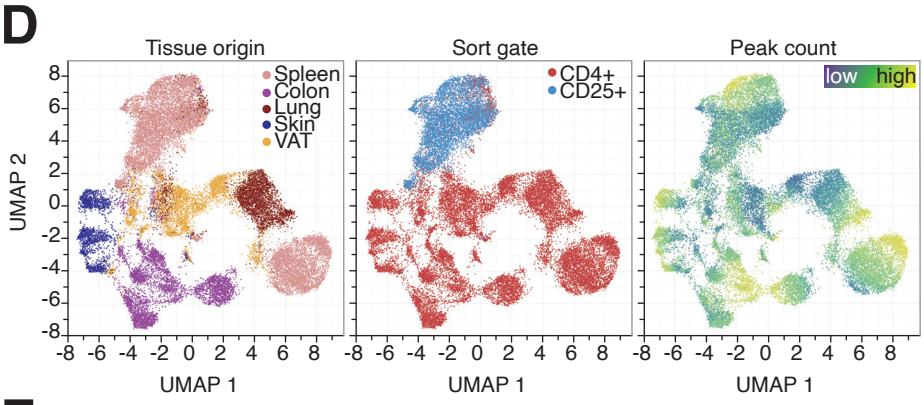
Figure S1

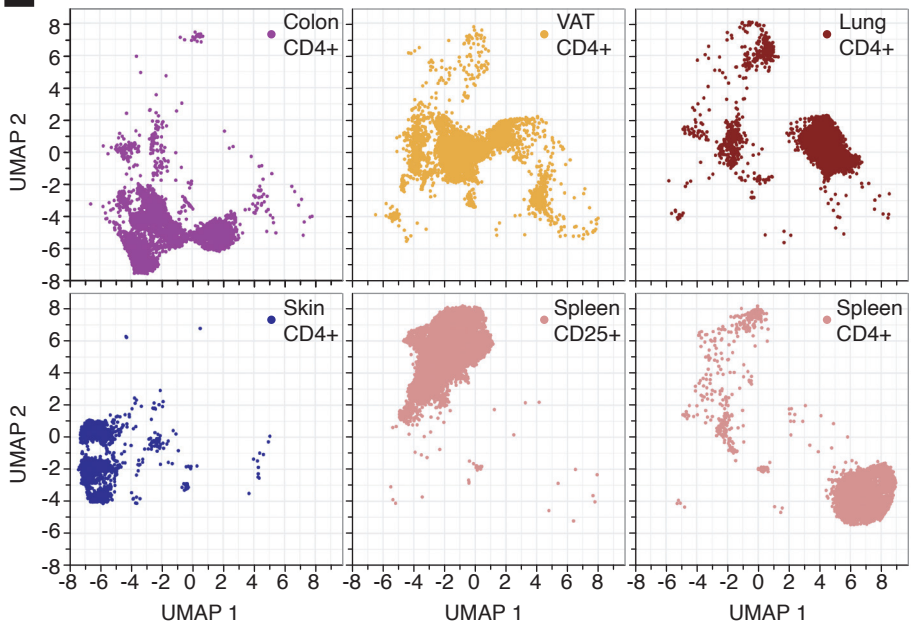




C

Tissue and cell type		Total Barcode#	Mean # of fragments
Spleen	CD4+	4,220	33,954
	CD25+	7,018	25,258
Colon	CD4+	4,976	34,215
VAT	CD4+	4,381	19,257
Skin	CD4+	2,114	28,902
Lung	CD4+	3,293	18,745





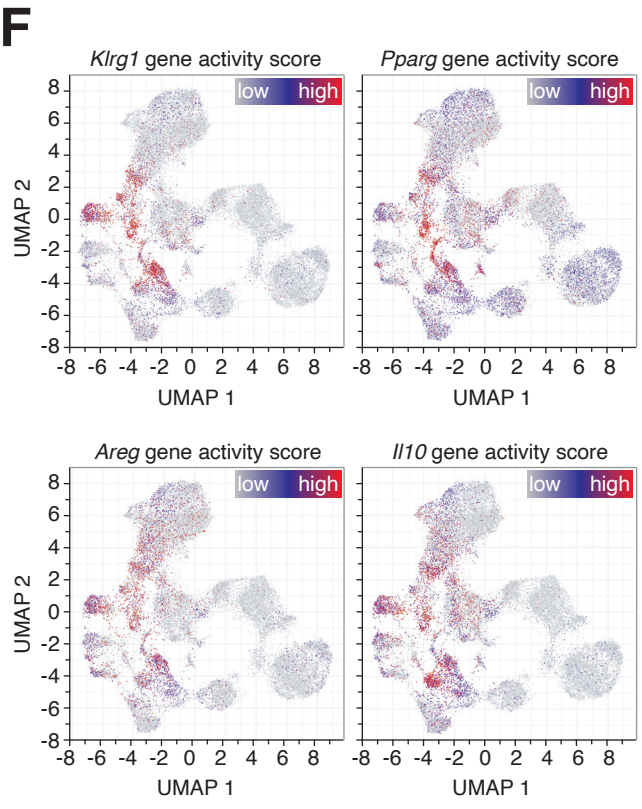


Figure S1. Single-cell ATAC landscape identifies repair signature in murine tissues and spleen. This is an extension of **Figure 1**. (**A**) Sort gates and post-sort QC for $CD4^+$ T cells from murine spleen, colon, VAT, lung and skin for scATAC-Seq. (**B**) Sort gates and post-sort QC for $CD25^+$ T cells from murine spleen for scATAC-Seq. (**C**) Total number of QC-passed and processed cells ("Barcode #") per tissue and cell type as well as mean # of fragments per cell. (**D**) UMAP of tissue T cells as in **Figure 1A** (left) with sort gate ($CD4^+$ or $CD25^+$, middle) and peak count (right). (**E**) Sample contribution to UMAP as in **Figure 1A**, with Colon $CD4^+$, VAT $CD4^+$, Lung $CD4^+$, Skin $CD4^+$, Spleen $CD4^+$ and Spleen $CD25^+$. (**F**) UMAP of scATAC-seq data with chromatin accessibility of the *Klrg1*, *Pparg*, *Areg*, and *Il10* gene loci, with blue=low and red=high accessibility.

Figure S2

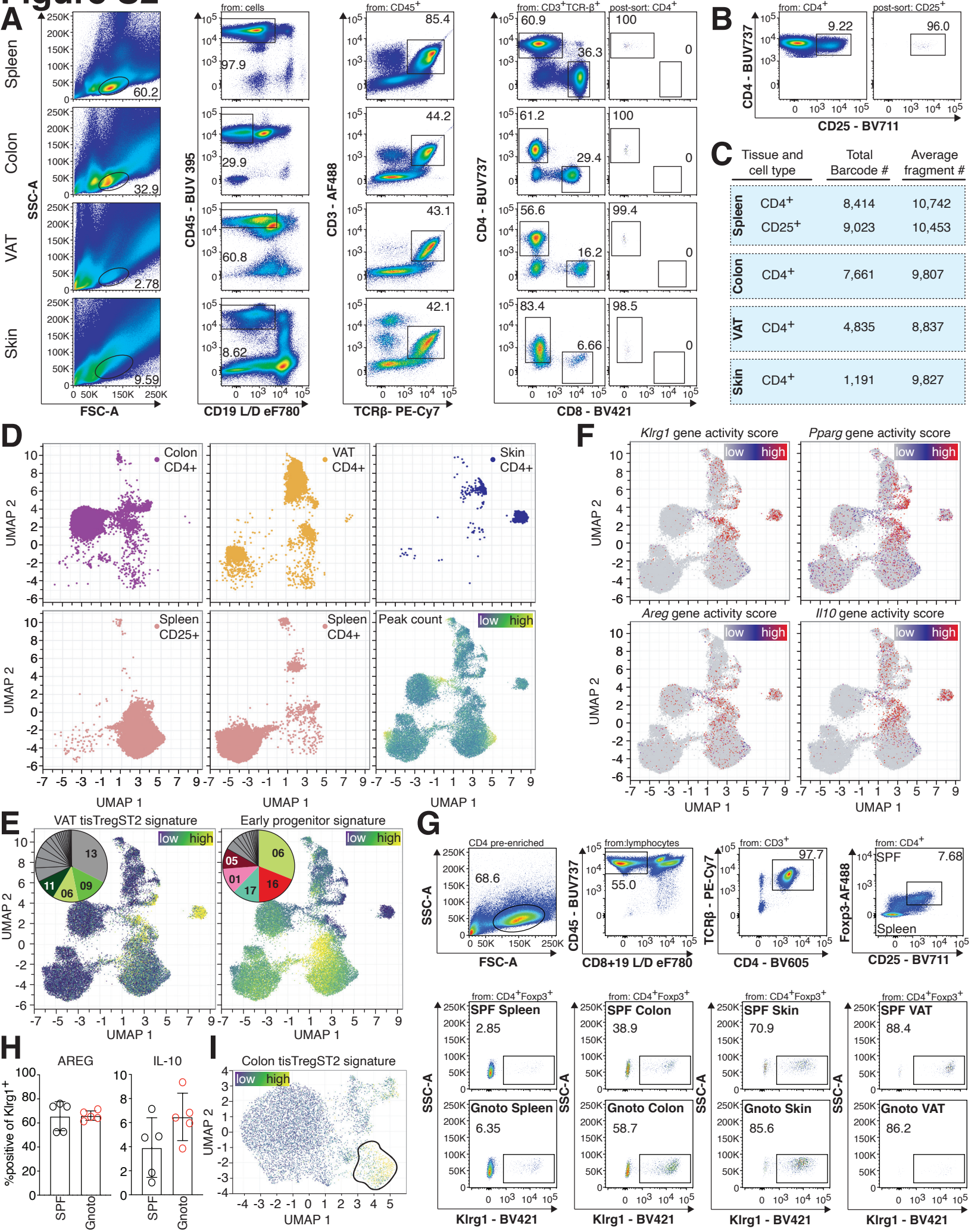

A
Spleen
Colon
VAT
Skin
SSC-A
FSC-A
CD45 - BUV 395
CD19 L/D eF780
CD3 - AF488
TCRβ- PE-Cy7
CD4 - BUV737
CD8 - BV421
from: cells
from: CD45+
from: CD3+TCR-β+
post-sort: CD4+
B
from: CD4+
post-sort: CD25+
CD4 - BUV737
CD25 - BV711
C
Tissue and cell type
Total Barcode #
Average fragment #
Spleen CD4+ 8,414 10,742
Spleen CD25+ 9,023 10,453
Colon CD4+ 7,661 9,807
VAT CD4+ 4,835 8,837
Skin CD4+ 1,191 9,827
D
Colon CD4+
VAT CD4+
Skin CD4+
Spleen CD25+
Spleen CD4+
Peak count
low high
UMAP 1
UMAP 2
F
Klrg1 gene activity score
Pparg gene activity score
Areg gene activity score
Il10 gene activity score
E
VAT tisTregST2 signature
Early progenitor signature
G
CD4 pre-enriched
from:lymphocytes
from: CD3+
from: CD4+
SPF
Spleen
CD8+19 L/D eF780
CD4 - BV605
Foxp3-AF488
CD25 - BV711
from: CD4+Foxp3+
SPF Spleen
SPF Colon
SPF Skin
SPF VAT
Gnoto Spleen
Gnoto Colon
Gnoto Skin
Gnoto VAT
Klrg1 - BV421
H
AREG
IL-10
%positive of Klrg1+
SPF
Gnoto
I
Colon tisTregST2 signature

Figure S2. Repair signature in scATAC landscape of T cells from germ-free animals. This is an extension of **Figure 2**. (**A**) Sort gates and post-sort QC for $CD4^+$ T cells from murine spleen, colon, VAT and skin for scATAC-Seq. (**B**) Sort gates and post-sort QC for $CD25^+$ T cells from murine spleen for scATAC-Seq. (**C**) Total number of QC-passed and processed cells ("Barcode #") per tissue and cell type as well as mean # of fragments per cell. (**D**) Sample contribution to UMAP as in **Figure 2A**, with Colon $CD4^+$, VAT $CD4^+$, $CD4^+$, Skin $CD4^+$, Spleen $CD4^+$, Spleen $CD25^+$ and peak count. (**E**) UMAP of scATAC-seq data where a bulk ATAC signature of VAT tisTregST2 an early tisTregST2 precursor signature were overlaid, with blue=low and yellow=high overlap. The contribution of each signature to the clusters is shown in a pie chart. (**F**) UMAP of scATAC-seq data with chromatin accessibility of the *Klrg1*, *Pparg*, *Areg*, and *Il10* gene loci, with blue=low and red=high accessibility. (**G**) Flow cytometry of VAT, skin, colon and spleen T cells from SPF vs gnotobiotic mice. Top, gating strategy. Bottom, Klrg1 expression in SPF vs gnotobiotic mice. (**H)** Intracellular expression of Areg and IL-10 in Klrg1+ Treg cells from skin of SPF vs gnotobiotic mice (n=5). (**I**) UMAP of scATAC-data with colon T where a bulk ATAC signature of colon tisTregST2 was overlaid, with blue=low and yellow=high overlap. The outlined fraction shows the localization of tisTregST2.

Figure S3

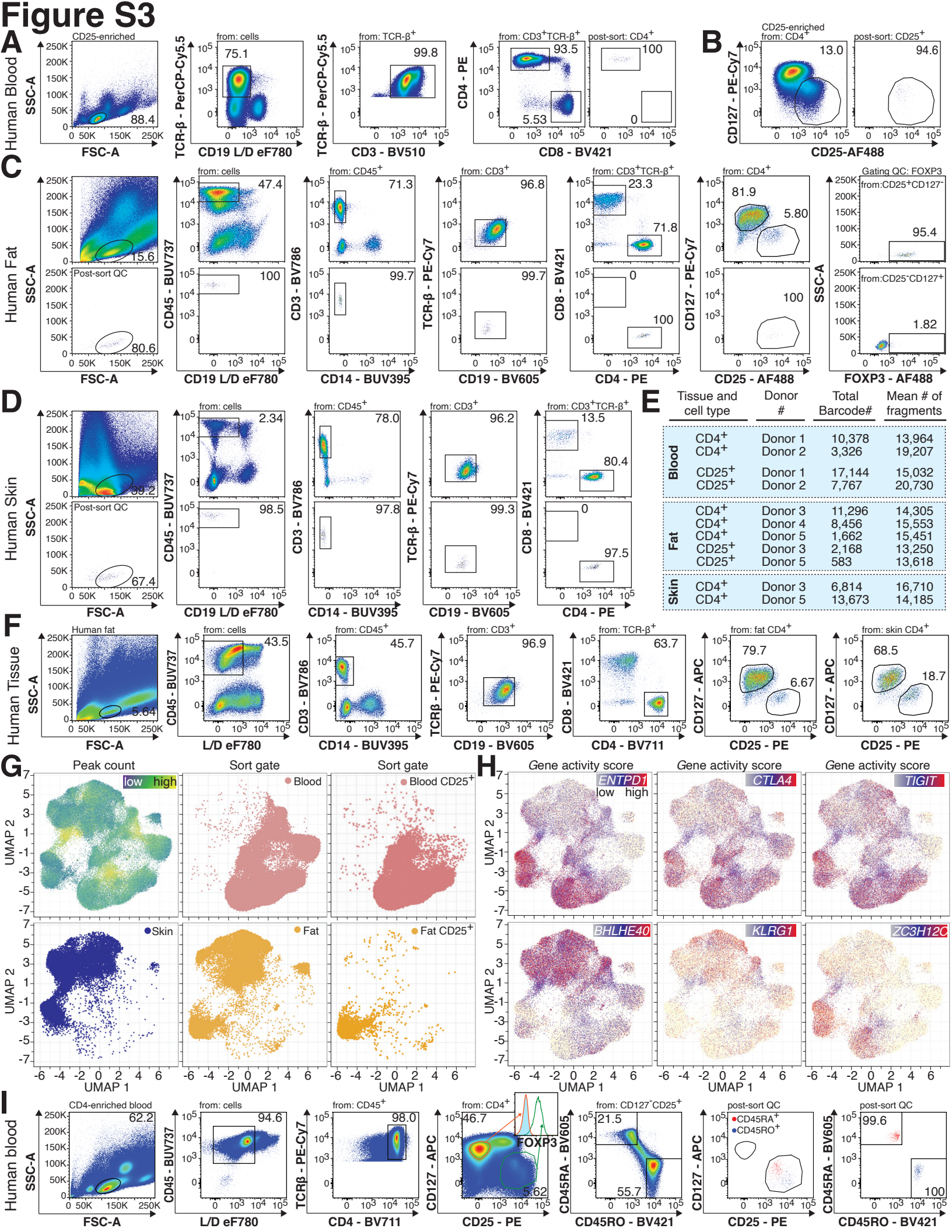

A
Human Blood
CD25-enriched
SSC-A
FSC-A
88.4
from: cells
TCR-β - PerCP-Cy5.5
CD19 L/D eF780
75.1
from: TCR-β+
TCR-β - PerCP-Cy5.5
CD3 - BV510
99.8
from: CD3+TCR-β+
CD4 - PE
CD8 - BV421
93.5
5.53
post-sort: CD4+
100
0
B
CD25-enriched
from: CD4+
CD127 - PE-Cy7
CD25-AF488
13.0
post-sort: CD25+
94.6
C
Human Fat
SSC-A
FSC-A
15.6
Post-sort QC
80.6
from: cells
CD45 - BUV737
CD19 L/D eF780
47.4
100
from: CD45+
CD3 - BV786
CD14 - BUV395
71.3
99.7
from: CD3+
TCR-β - PE-Cy7
CD19 - BV605
96.8
99.7
from: CD3+TCR-β+
CD8 - BV421
CD4 - PE
23.3
71.8
0
100
from: CD4+
CD127 - PE-Cy7
CD25 - AF488
81.9
5.80
100
Gating QC: FOXP3
from:CD25+CD127-
95.4
from:CD25-CD127+
SSC-A
1.82
FOXP3 - AF488
D
Human Skin
SSC-A
FSC-A
99.2
Post-sort QC
67.4
from: cells
CD45 - BUV737
CD19 L/D eF780
2.34
98.5
from: CD45+
CD3 - BV786
CD14 - BUV395
78.0
97.8
from: CD3+
TCR-β - PE-Cy7
CD19 - BV605
96.2
99.3
from: CD3+TCR-β+
CD8 - BV421
CD4 - PE
13.5
80.4
0
97.5
E
Tissue and cell type
Donor #
Total Barcode#
Mean # of fragments
Blood
CD4+ Donor 1 10,378 13,964
CD4+ Donor 2 3,326 19,207
CD25+ Donor 1 17,144 15,032
CD25+ Donor 2 7,767 20,730
Fat
CD4+ Donor 3 11,296 14,305
CD4+ Donor 4 8,456 15,553
CD4+ Donor 5 1,662 15,451
CD25+ Donor 3 2,168 13,250
CD25+ Donor 5 583 13,618
Skin
CD4+ Donor 3 6,814 16,710
CD4+ Donor 5 13,673 14,185
F
Human Tissue
Human fat
SSC-A
FSC-A
5.64
from: cells
CD45 - BUV737
L/D eF780
43.5
from: CD45+
CD3 - BV786
CD14 - BUV395
45.7
from: CD3+
TCRβ - PE-Cy7
CD19 - BV605
96.9
from: TCR-β+
CD8 - BV421
CD4 - BV711
63.7
from: fat CD4+
CD127 - APC
CD25 - PE
79.7
6.67
from: skin CD4+
68.5
18.7
G
Peak count
low high
Sort gate
Blood
Blood CD25+
Skin
Fat
Fat CD25+
UMAP 2
UMAP 1
H
Gene activity score
ENTPD1
low high
CTLA4
TIGIT
BHLHE40
KLRG1
ZC3H12C
I
Human blood
CD4-enriched blood
SSC-A
FSC-A
62.2
from: cells
CD45 - BUV737
L/D eF780
94.6
from: CD45+
TCRβ - PE-Cy7
CD4 - BV711
98.0
from: CD4+
CD127 - APC
CD25 - PE
46.7
FOXP3
5.62
from: CD127-CD25+
CD45RA - BV605
CD45RO - BV421
21.5
55.7
post-sort QC
CD45RA+
CD45RO+
99.6
100

Figure S3. Single-cell ATAC and gene expression landscape of human blood, fat and skin CD4$^+$ T cells. This is an extension of **Figure 3**. (**A**) Sort gates and post-sort QC for CD25-enriched T cells from human peripheral blood for scATAC-Seq. (**B**) Sort gates and post-sort QC of CD25-enriched T cells from human peripheral blood for scATAC-Seq of CD127$^-$CD25$^+$ Treg cells. (**C**) Sort gates and post-sort QC for CD4$^+$ and CD127$^-$CD25$^+$ T cells from human fat tissue, including a Foxp3 staining control to verify Treg identity. (**D**) Sort gates and post-sort QC for CD4$^+$ T cells from human skin tissue. (**E**) Total number of QC-passed and processed cells ("Barcode #") per tissue and cell type as well as mean # of fragments per cell. (**F**) Sort gates for CD127$^-$CD25$^+$ T cells from human skin and fat tissue for bulk RNA-seq. (**G**) Tissue contribution to UMAP as in **Figure 3A**, with fat, blood and skin, and peak count. (**H**) UMAP of scATAC-seq data with chromatin accessibility of the *ENTPD1*, *CTLA4*, *TIGIT*, *BHLHE40*, *KLRG1* and *ZC3H12C* gene loci. (**I**) Sort gates and post-sort QC of CD4-enriched T cells from human peripheral blood for bulk RNA-seq.

Figure S4

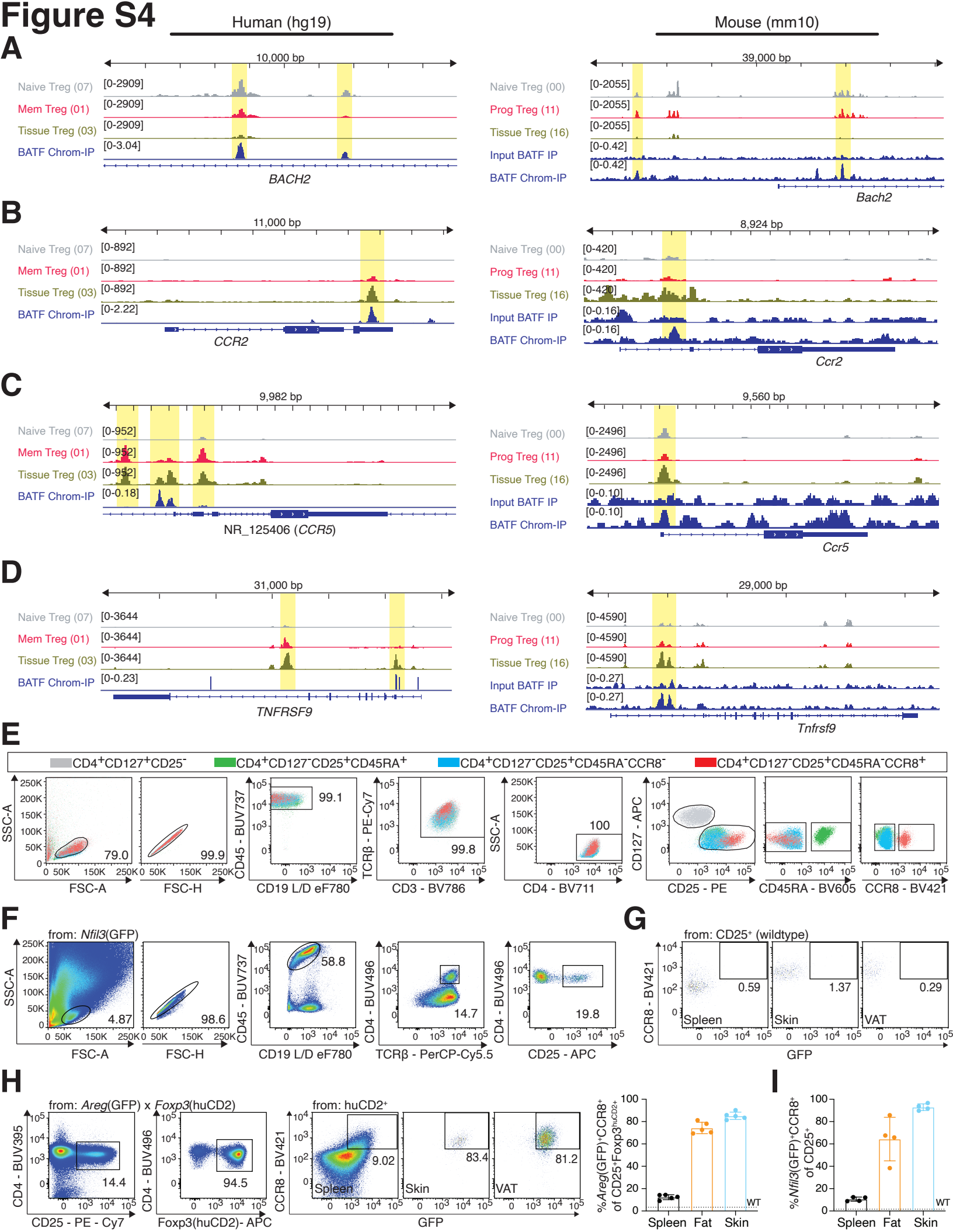

Human (hg19)
Mouse (mm10)
A
10,000 bp
Naive Treg (07)
Mem Treg (01)
Tissue Treg (03)
BATF Chrom-IP
[0-2909]
[0-3.04]
BACH2
39,000 bp
Naive Treg (00)
Prog Treg (11)
Tissue Treg (16)
Input BATF IP
BATF Chrom-IP
[0-2055]
[0-0.42]
Bach2
B
11,000 bp
[0-892]
[0-2.22]
CCR2
8,924 bp
[0-420]
[0-0.16]
Ccr2
C
9,982 bp
[0-952]
[0-0.18]
NR_125406 (CCR5)
9,560 bp
[0-2496]
[0-0.10]
Ccr5
D
31,000 bp
[0-3644]
[0-0.23]
TNFRSF9
29,000 bp
[0-4590]
[0-0.27]
Tnfrsf9
E
CD4+CD127+CD25-
CD4+CD127-CD25+CD45RA+
CD4+CD127-CD25+CD45RA-CCR8-
CD4+CD127-CD25+CD45RA-CCR8+
SSC-A
FSC-A
FSC-H
CD45 - BUV737
CD19 L/D eF780
TCRβ - PE-Cy7
CD3 - BV786
CD4 - BV711
CD127 - APC
CD25 - PE
CD45RA - BV605
CCR8 - BV421
79.0
99.9
99.1
99.8
100
F
from: Nfil3(GFP)
4.87
98.6
58.8
14.7
19.8
CD4 - BUV496
TCRβ - PerCP-Cy5.5
CD25 - APC
G
from: CD25+ (wildtype)
CCR8 - BV421
GFP
0.59
1.37
0.29
Spleen
Skin
VAT
H
from: Areg(GFP) x Foxp3(huCD2)
CD4 - BUV395
CD25 - PE - Cy7
14.4
CD4 - BUV496
Foxp3(huCD2)- APC
94.5
from: huCD2+
CCR8 - BV421
GFP
9.02
83.4
81.2
%Areg(GFP)+CCR8+ of CD25+Foxp3huCD2+
Spleen
Fat
Skin
WT
I
%Nfil3(GFP)+CCR8+ of CD25+

Figure S4. Species-conserved tissue Treg peakset identifies CCR8$^+$ Treg population in human blood. This is an extension of **Figure 4**. (**A-D**) Left, chromatin accessibility of *BACH2*, *CCR2*, *NR_125406* (*CCR5*) and *TNFRSF9* in human Treg clusters 01, 03 and 07 with BATF ChIP-Seq data (GSM803538). Right, chromatin accessibility of *Bach2*, *Ccr2*, *Ccr5* and *Tnfrsf9* in murine Treg clusters 00, 11 and 16 combined with publicly available Batf ChIP-Seq data (GSE54191). Cluster datasets group-normalized to maximum peak height. (**E**) CCR8$^+$ Treg cells (CD4$^+$CD127$^-$CD25$^+$CD45RA$^-$CCR8$^+$, red), CCR8- Treg cells (CD4$^+$CD127$^-$CD25$^+$CD45RA$^-$CCR8$^-$, blue), naive Treg cells (CD4$^+$CD127$^-$CD25$^+$CD45RA$^+$, green) and Tconv cells (CD4$^+$CD127$^+$CD25$^-$, blue) from human blood have been sorted followed by fixation, permeabilization and BATF/FOXP3 staining. Cells have been re-acquired and color code indicates cell type. (**F**) Gating strategy to identify Treg cells in *Nfil3*(GFP), *Areg*(GFP) reporter mice or wildtype animals. (**G**) CCR8 and GFP expression in wildtype animals. (**H**) Gating strategy to identify Treg cells in *Areg*(GFP) x *Foxp3*(huCD2) animals and percentage of *Areg*(GFP)$^+$CCR8$^+$ cells of Treg cells (left, n=5, 2x repeated). (**I**) Percentage of *Nfil3*(GFP)$^+$CCR8$^+$ cells of Treg cells (right, n=4, 2x repeated).

Figure S5

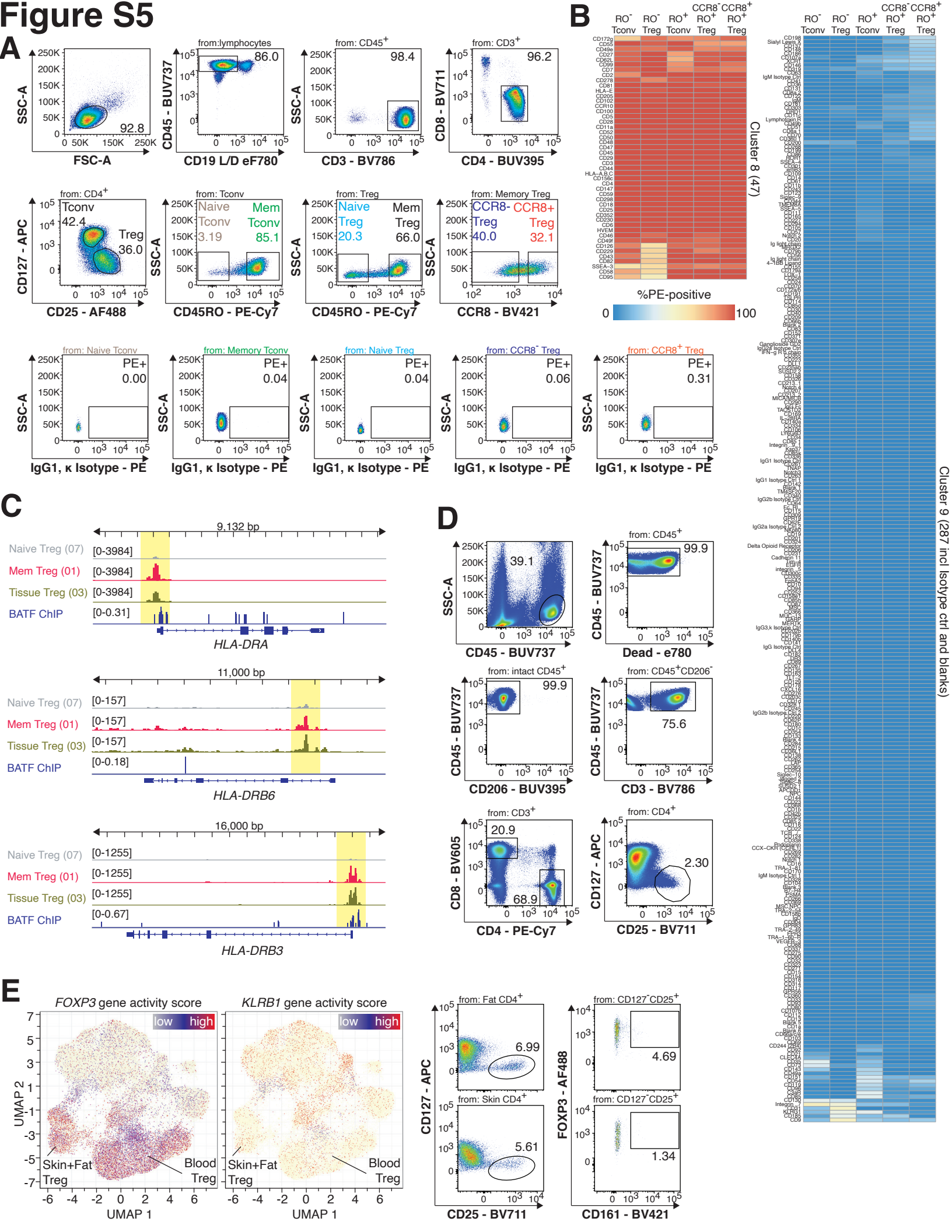
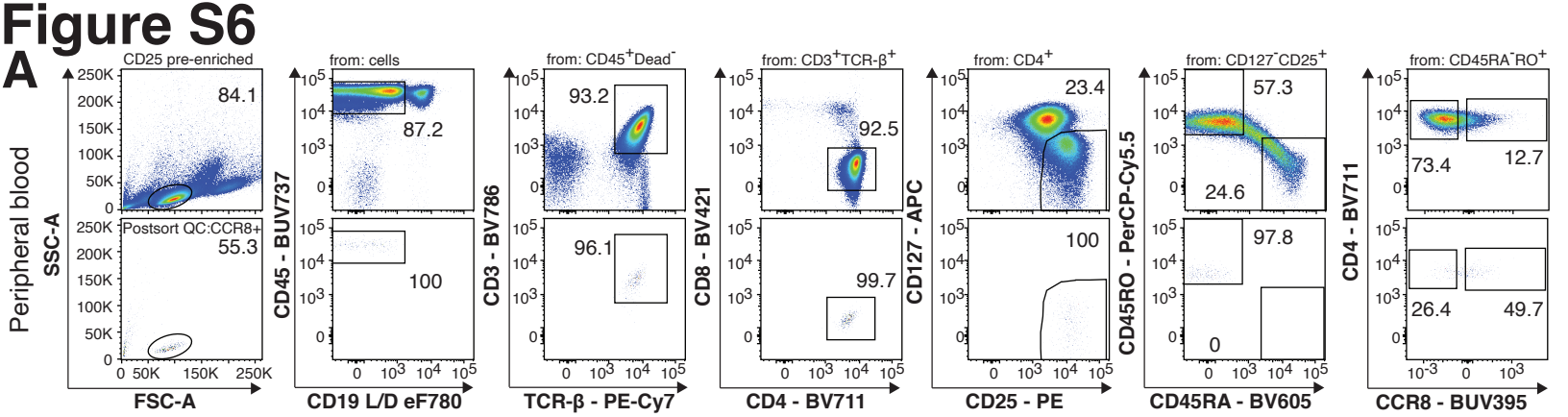
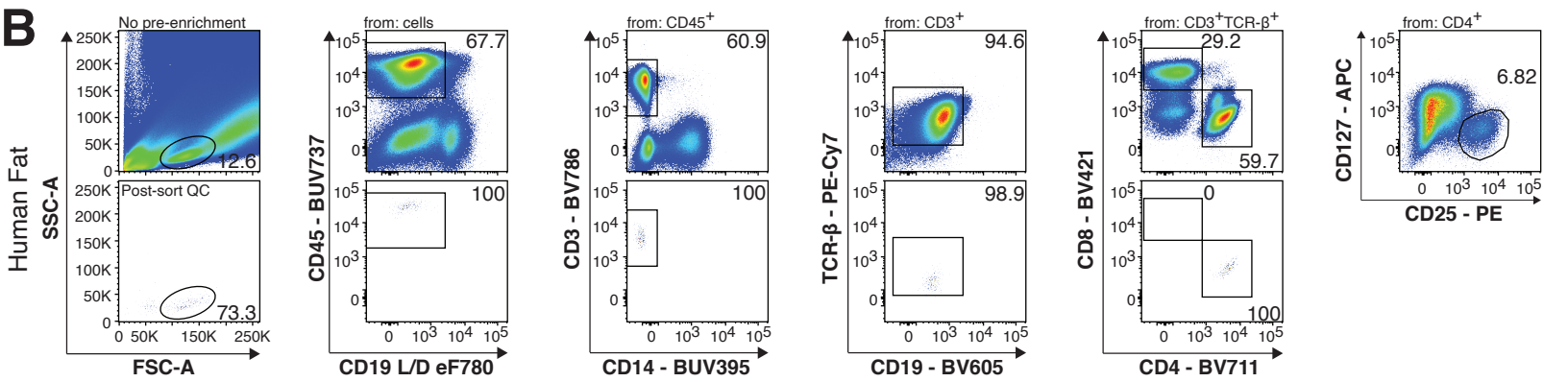
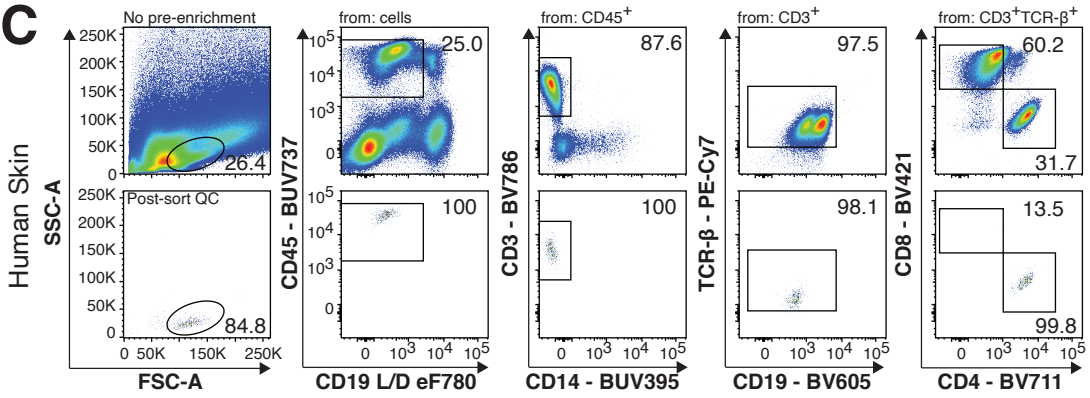

A
SSC-A
FSC-A
92.8
from:lymphocytes
86.0
CD45 - BUV737
CD19 L/D eF780
from: CD45+
98.4
SSC-A
CD3 - BV786
from: CD3+
96.2
CD8 - BV711
CD4 - BUV395
from: CD4+
Tconv
42.4
Treg
36.0
CD127 - APC
CD25 - AF488
from: Tconv
Naive Tconv
3.19
Mem Tconv
85.1
SSC-A
CD45RO - PE-Cy7
from: Treg
Naive Treg
20.3
Mem Treg
66.0
SSC-A
CD45RO - PE-Cy7
from: Memory Treg
CCR8- Treg
40.0
CCR8+ Treg
32.1
SSC-A
CCR8 - BV421
from: Naive Tconv
PE+
0.00
from: Memory Tconv
PE+
0.04
from: Naive Treg
PE+
0.04
from: CCR8- Treg
PE+
0.06
from: CCR8+ Treg
PE+
0.31
SSC-A
IgG1, κ Isotype - PE
B
RO- Tconv
RO- Treg
RO+ Tconv
CCR8-RO+ Treg
CCR8+RO+ Treg
Cluster 8 (47)
%PE-positive
0
100
Cluster 9 (287 incl Isotype ctrl and blanks)
C
9,132 bp
Naive Treg (07)
Mem Treg (01)
Tissue Treg (03)
BATF ChIP
[0-3984]
[0-3984]
[0-3984]
[0-0.31]
HLA-DRA
11,000 bp
[0-157]
[0-157]
[0-157]
[0-0.18]
HLA-DRB6
16,000 bp
[0-1255]
[0-1255]
[0-1255]
[0-0.67]
HLA-DRB3
D
SSC-A
39.1
CD45 - BUV737
from: CD45+
99.9
CD45 - BUV737
Dead - e780
from: intact CD45+
99.9
CD45 - BUV737
CD206 - BUV395
from: CD45+CD206-
75.6
CD45 - BUV737
CD3 - BV786
from: CD3+
20.9
68.9
CD8 - BV605
CD4 - PE-Cy7
from: CD4+
2.30
CD127 - APC
CD25 - BV711
E
FOXP3 gene activity score
KLRB1 gene activity score
low
high
UMAP 2
UMAP 1
Skin+Fat Treg
Blood Treg
from: Fat CD4+
6.99
from: Skin CD4+
5.61
CD127 - APC
CD25 - BV711
from: CD127-CD25+
4.69
from: CD127-CD25+
1.34
FOXP3 - AF488
CD161 - BV421

Figure S5. Surface protein, transcriptional and epigenetic analysis of human CCR8$^+$ Treg cells in blood. This is an extension of **Figure 5**. **(A)** Five populations ($CD3^+CD4^+CD127^+CD25^-CD45RO^-$ antigen-naive Tconv, $CD3^+CD4^+CD127^+CD25^-CD45RO^+$ memory Tconv, $CD3^+CD4^+CD127^-CD25^+CD45RO^-$ antigen-naive Treg, $CD3^+CD4^+CD127^-CD25^+CD45RO^+CCR8^-$ and $CD3^+CD4^+CD127^-CD25^+CD45RO^+CCR8^+$ antigen-experienced Treg were gated and the expression of PE was analyzed. Data were subjected to "pheatmap" with 9 rows, yielding 9 groups. Isotype control staining for 5 groups shown below. (**B**) Group 8 and Group 9, as in **Figure 5**, with protein labels. (**C**) Chromatin accessibility of human *HLADRA*, *HLADRB3* and *HLADRB6*. Data are derived from antigen-naive Treg cells (Cluster 07), memory Treg cells (01) and fat and skin Treg cells (03). BATF ChIP-Seq signal below (GSM803538). (**D**) Gating scheme to identify Treg cells in human fat and skin tissue for CCR8 and HLA-DR measurement. (**E**) UMAP of scATAC-seq data with chromatin accessibility of the *FOXP3* and *KLRB1* gene. Expression of CD161 on skin and fat Treg cells displayed to the right.

Figure S6

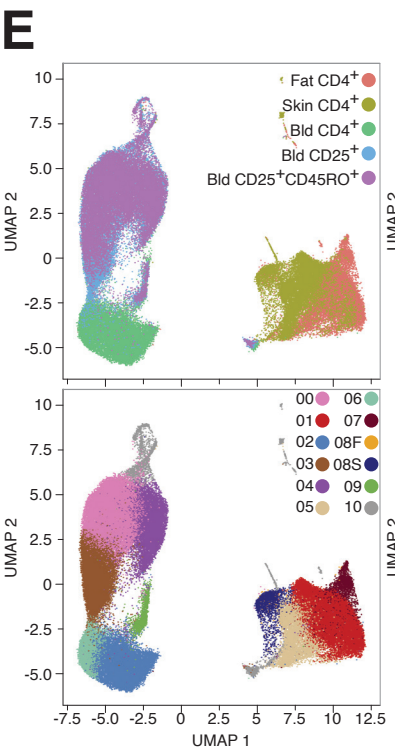


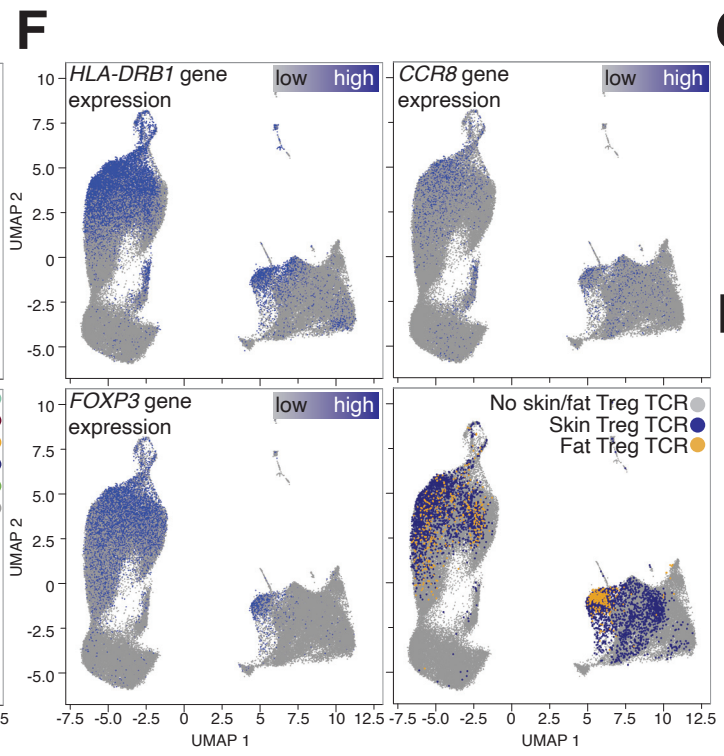




D

Tissue and cell type		Donor #	Total Barcode#	Total linked α+β TCR#
Blood	CD4+	Donor 6	14,927	10,574
	CD25+	Donor 6	20,217	14,003
	CD45RO+	Donor 6	18,967	14,367
	CCR8+	Donor 6	12,900	10,258
Blood	CD4+	Donor 7	17,769	14,765
	CD25+	Donor 7	18,066	15,013
	CD45RO+	Donor 7	20,663	15,160
Skin	CD4+	Donor 6	16,124	7,345
	CD4+	Donor 7	12,552	8,458
Fat	CD4+	Donor 6	18,570	12,345
	CD4+	Donor 7	14,007	11,890
scATAC		Donor	Barcode#	Fragment#
Blood	CCR8+	Donor 8	7,842	10,583
	CCR8	Donor 9	11,569	12,829





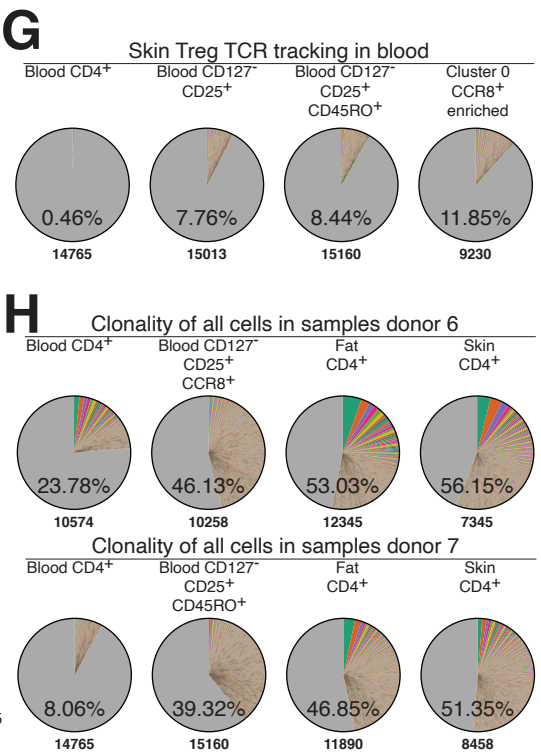


Figure S6. Single-cell RNA and TCR landscape of donor-matched human blood, fat and skin CD4$^+$ T cells. This is an extension of **Figure 6**. (**A**) Sort gates and post-sort QC for CD25 pre-enriched blood Treg (CD4$^+$CD127$^-$CD25$^+$), blood memory Treg (CD4$^+$CD127$^-$CD25$^+$CD45RA$^-$CD45RO$^+$), and blood memory CCR8 Treg (CD4$^+$CD127$^-$CD25$^+$CD45RA$^-$CD45RO$^+$CCR8$^+$) from human peripheral blood for scRNA/TCR-Seq. (**B**) Sort gates and post-sort QC for CD4$^+$ T cells from human fat tissue. (**C**) Sort gates and post-sort QC for CD4$^+$ T cells from human skin tissue. (**D**) Total number of QC-passed and processed cells ("Barcode #") per tissue and cell type (data based on CellRanger output) as well as total # of linked alpha and beta TCRs per cell (data after additional internal QC filtering steps). (**E**) UMAP of scRNA-seq data derived from FACS-sorted CD4$^+$ T cell populations of human peripheral blood, skin and fat of one individual donor (Donor 7). Top, cells color-coded based on sort strategy. Bottom, cells clustered and color-coded according to cluster. (**F**) Gene expression of *FOXP3*, *CCR8* and *HLA-DRB1* plotted on UMAP. Color code indicates expression strength. Bottom right, TCRs derived from all skin Treg cells (blue) and all fat Treg cells (yellow) were extracted and highlighted. **(G**) Tracking of skin Treg TCR clones in different sorted populations of peripheral blood of the same donor. Percentage indicates fraction of detected clones, with total number of clones shown below. (**H**) Clonality of all cells in samples donor 6 and donor 7. All TCR sequences listed in **Table S4**.

Figure S7

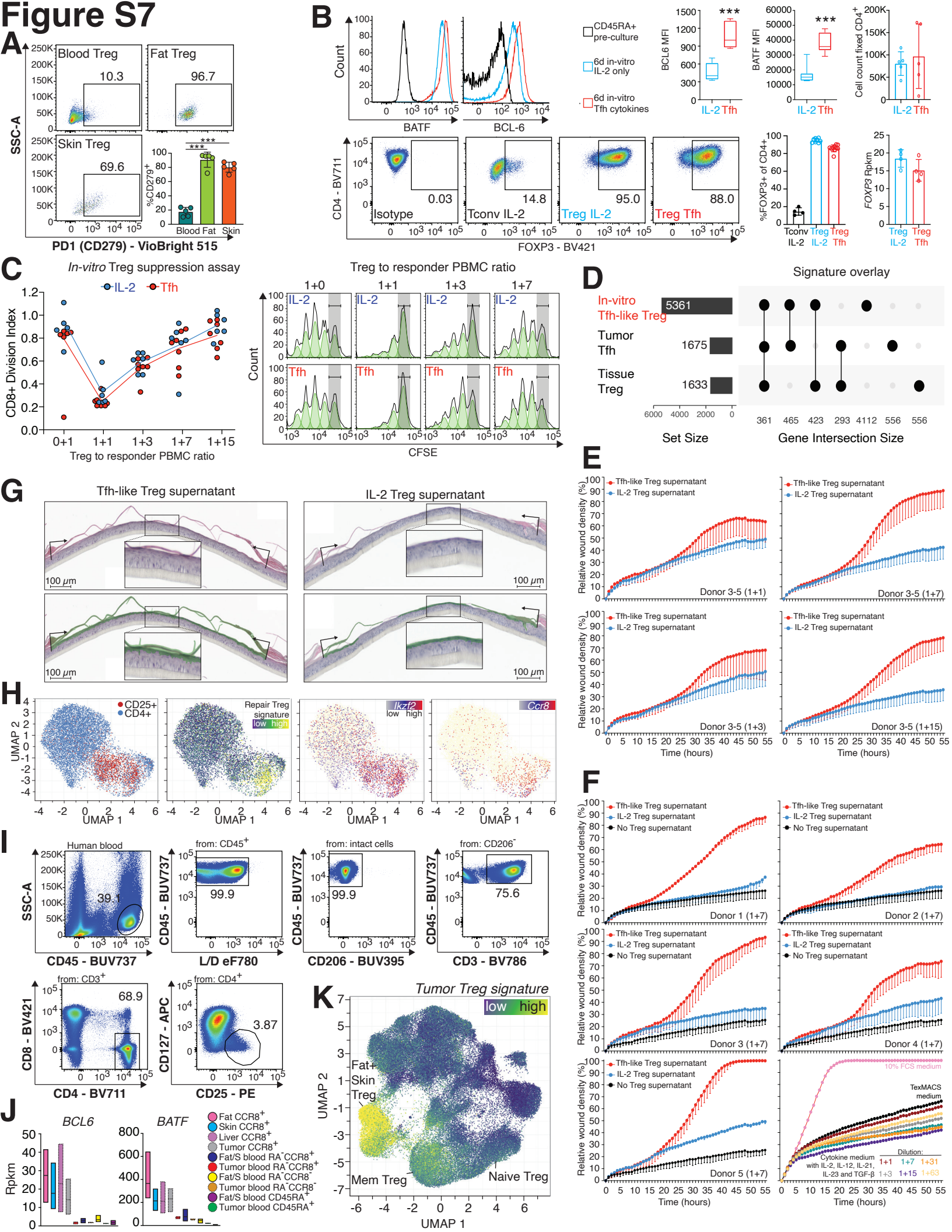

A
Blood Treg
10.3
Fat Treg
96.7
Skin Treg
69.6
SSC-A
PD1 (CD279) - VioBright 515
%CD279+
Blood Fat Skin
B
Count
BATF
BCL-6
CD45RA+ pre-culture
6d in-vitro IL-2 only
6d in-vitro Tfh cytokines
BCL6 MFI
BATF MFI
Cell count fixed CD4+
IL-2 Tfh
CD4 - BV711
FOXP3 - BV421
Isotype
0.03
Tconv IL-2
14.8
Treg IL-2
95.0
Treg Tfh
88.0
%FOXP3+ of CD4+
Tconv IL-2
Treg IL-2
Treg Tfh
FOXP3 Rpkm
C
In-vitro Treg suppression assay
IL-2
Tfh
CD8+ Division Index
Treg to responder PBMC ratio
0+1
1+1
1+3
1+7
1+15
Treg to responder PBMC ratio
1+0
1+1
1+3
1+7
Count
CFSE
D
Signature overlay
In-vitro Tfh-like Treg
5361
Tumor Tfh
1675
Tissue Treg
1633
Set Size
Gene Intersection Size
361
465
423
293
4112
556
556
E
Tfh-like Treg supernatant
IL-2 Treg supernatant
Relative wound density (%)
Donor 3-5 (1+1)
Donor 3-5 (1+7)
Donor 3-5 (1+3)
Donor 3-5 (1+15)
Time (hours)
F
No Treg supernatant
Donor 1 (1+7)
Donor 2 (1+7)
Donor 3 (1+7)
Donor 4 (1+7)
Donor 5 (1+7)
10% FCS medium
TexMACS medium
Cytokine medium with IL-2, IL-12, IL-21, IL-23 and TGF-β
Dilution:
1+1
1+7
1+31
1+3
1+15
1+63
G
Tfh-like Treg supernatant
IL-2 Treg supernatant
100 μm
H
UMAP 2
UMAP 1
CD25+
CD4+
Repair Treg signature
low high
Ikzf2
Ccr8
I
Human blood
from: CD45+
from: intact cells
from: CD206-
SSC-A
CD45 - BUV737
L/D eF780
CD206 - BUV395
CD3 - BV786
39.1
99.9
99.9
75.6
from: CD3+
from: CD4+
CD8 - BV421
CD127 - APC
CD25 - PE
68.9
3.87
J
BCL6
BATF
Rpkm
Fat CCR8+
Skin CCR8+
Liver CCR8+
Tumor CCR8+
Fat/S blood RA-CCR8+
Tumor blood RA-CCR8+
Fat/S blood RA-CCR8-
Tumor blood RA-CCR8-
Fat/S blood CD45RA+
Tumor blood CD45RA+
K
Tumor Treg signature
low high
Fat+ Skin Treg
Mem Treg
Naive Treg
UMAP 2
UMAP 1

Figure S7. Treg cells in wound healing and cancer. This is an extension of **Figure 7**. (**A**) Flow cytometry of Treg cells ($CD4^+CD25^+CD127^-$) from human blood, fat and skin tissue with CD279 (PD-1) expression (n=4-5, one-way ANOVA with Tukey post-test). (**B**) Naive Treg cells treated for six days with IL-2 or IL-2, IL-12, IL-21, IL-23 and TGF-β *in-vitro*, followed by washing, overnight re-stimulation and flow cytometry for BATF, BCL-6, FOXP3 and cell count analysis. Expression analysis to the right (n=4). (**C**) Suppression Assay for *in-vitro* generated IL-2 or Tfh-like Tregs. Left: Division index of CD8 T cells cocultured with Tfh-like (red) or IL-2 (blue) Tregs. Right: Flow Cytometry data of CFSE stained CD8 T cells cocultured with Tfh-like (red) or IL-2 (blue) Tregs, indicating division. (**D**) Peaks from tissue Treg, Tfh-like Treg and tumor Tfh signatures annotated with their closest gene. Overlaps between unique genes in these signatures counted. (**E**) Tfh-like Treg cells or IL-2 treated Treg cells of several donors stimulated overnight and supernatant collected and used in a wound healing assay with HaCaT cells (n=3, 1+3 dilution, 1+7 dilution and 1+15 dilution of supernatant). (**F**) Tfh-like Treg cells or IL-2 treated Treg in a wound healing assay with HaCaT cells (1+7 dilution, individual donors shown). Bottom right, different dilutions of cytokine medium (Tfh mix) in wound healing assay. (**G**) Histological staining of human epidermal reconstruction model treated with Tfh-like Treg supernatant or IL-2 Treg supernatant. Top, unprocessed image. Bottom, processed image with *Stratum corneum* shown in green. Box in center = magnified area of interest. (**H**) UMAP of scATAC-seq data derived from $CD4^+$ T cell populations of a murine HER2-transgenic breast carcinoma model. Far left plot indicates cell type by sort gate, left plot shows murine core tisTregST2 signature. Right; chromatin accessibility of the *Ikzf2* and *Ccr8* gene locus is shown. (**I**) Gating scheme for CCR8 vs CD45RA expression of Treg cells in skin, fat, NAT and liver tumor tissue from **Figure 7I**. (**J**)

Expression of BCL6 and BATF in all samples from **Figure 7J**. (**K**) UMAP of scATAC-seq data derived from FACS-sorted $CD4^+$ T cell populations of human peripheral blood, skin and fat of individual female donors as in **Figure 3**. A tumor Treg signature (940 peaks) was overlaid and clusters 01 (memory Treg), 03 (fat and skin tissue Treg) and 07 (naive Treg) labeled.