

Supplementary Materials

High-dimensional profiling of regulatory T cells in psoriasis reveals an impaired skin-trafficking property

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Supplementary Table S1. CyTOF antibody panel information.

Supplementary Table S2. Simple and multiple linear regression analyses of frequency and expression of skin-homing markers in circulating Treg in people with psoriasis compared to healthy controls.

Supplementary Figure S1. Comparison of manual gating vs. heatmap-based cell-type annotation.

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Supplementary Figure S4. Comparison of PASI score to production of IL-17A⁺ by psoriatic CD4⁺ROR γ t⁺ T cells and CCR7⁺ Tregs by FACS.

Supplementary Table S1. CyTOF antibody panel information.

Metal	Marker	Clone	Vendor	Catalog
89 Y	CD45	HI30	Fluidigm	3089003B
113 In	CD57	HNK-1	Biologend	359602
115 In	CD11c	Bu15	Biologend	337202
141 Pr	CLA	HECA-452	Biologend	321302
142 Nd	CD19	REA675	Miltenyi	130-122-301
143 Nd	CD45RA	REA562	Miltenyi	130-122-292
144 Nd	KLRG1	SA231A2	Biologend	367702
145 Nd	CD4	REA623	Miltenyi	130-122-283
146 Nd	CD8	REA734	Miltenyi	130-122-281
147 Sm	ICOS	C398.4A	Biologend	313502
148 Nd	CD16	REA423	Miltenyi	130-108-027
149 Sm	CD127	A019D5	Fluidigm	A019D5
150 Nd	CD1c	REA694	Miltenyi	130-122-298
151 Eu	CD123	REA918	Miltenyi	130-122-297
152 Sm	CD66b	REA306	Miltenyi	130-108-019
153 Eu	TIGIT	MBSA43	Fluidigm	MBSA43
154 Sm	CD86	IT2.2	Biologend	305449
155 Gd	CD27	REA499	Miltenyi	130-122-295
156 Gd	CCR5	J418F1	Biologend	359102
158 Gd	CCR6	G034E3	Biologend	353402
159 Tb	CTLA4 (CD152)	L3D10	Biologend	349902
160 Gd	CD14	REA599	Miltenyi	130-122-290
161 Dy	CD56	REA196	Miltenyi	130-108-016
162 Dy	FoxP3	PCH101	Fluidigm	3162011A
163 Dy	CXCR5	REA103	Miltenyi	130-122-325
164 Dy	CCR10	314306	R&D	MAB3478
165 Ho	CRTH2	REA598	Miltenyi	130-122-305
166 Er	CD117	104D2	Biologend	313202
167 Er	CCR7	G043H7	Fluidigm	3167009A
168 Er	CD3	REA613	Miltenyi	130-122-282
169 Tm	CD25	REA570	Miltenyi	130-122-302
170 Er	CD38	REA671	Miltenyi	130-122-288
171 Yb	CD39	A1	Biologend	328202
172 Yb	Ki67	B56	Fluidigm	3172024B
173 Yb	CXCR3	REA232	Miltenyi	130-108-022
174 Yb	HLADR	REA805	Miltenyi	130-122-299
175 Lu	PD-1	EH12.2H7	Fluidigm	329926
176 Yb	CCR4	REA279	Miltenyi	130-122-323
209 Bi	CD11b	ICRF44	Fluidigm	3209003B

Supplementary Table S2. Simple and multiple linear regression analyses of frequency and expression of skin-homing markers in circulating Treg in patients with psoriasis compared to healthy controls.

Variables	Simple Linear Regression		Multiple Linear Regression	
	β	P value	β	P value
Treg frequency				
Group	0.018	<0.001	0.019	<0.001
Age	0.000	0.325	0.000	0.269
Sex	-0.003	0.568	0.004	0.309
Treg-CCR4 expression				
Group	-5.946	<0.001	-5.755	<0.001
Age	-0.046	0.190	-0.031	0.167
Sex	2.518	0.013	0.530	0.440
Treg-CCR7 expression				
Group	4.712	<0.001	4.717	<0.001
Age	0.014	0.695	0.005	0.854
Sex	-1.448	0.153	0.047	0.957
Treg-CXCR3 expression				
Group	-1.765	0.041	-2.083	0.019
Age	-0.050	0.112	-0.057	0.067
Sex	-0.429	0.645	-1.455	0.127
CLA^{hi} Treg frequency				
Group	-0.215	<0.001	-0.208	<0.001
Age	-0.003	0.092	-0.002	0.091
Sex	0.093	0.045	0.014	0.720
CLA^{hi} Treg-CCR4 expression				
Group	-6.047	<0.001	-5.889	<0.001
Age	-0.051	0.182	-0.037	0.172
Sex	2.451	0.027	0.381	0.664
CLA^{hi} Treg-CCR7 expression				
Group	4.683	<0.001	4.530	<0.001
Age	0.014	0.725	0.001	0.975
Sex	-1.958	0.084	-0.549	0.597
CLA^{hi} Treg-CXCR3* expression				
Group	-0.215	0.039	-0.267	0.011
Age	-0.007	0.077	-0.008	0.032
Sex	-0.096	0.392	-0.231	0.041

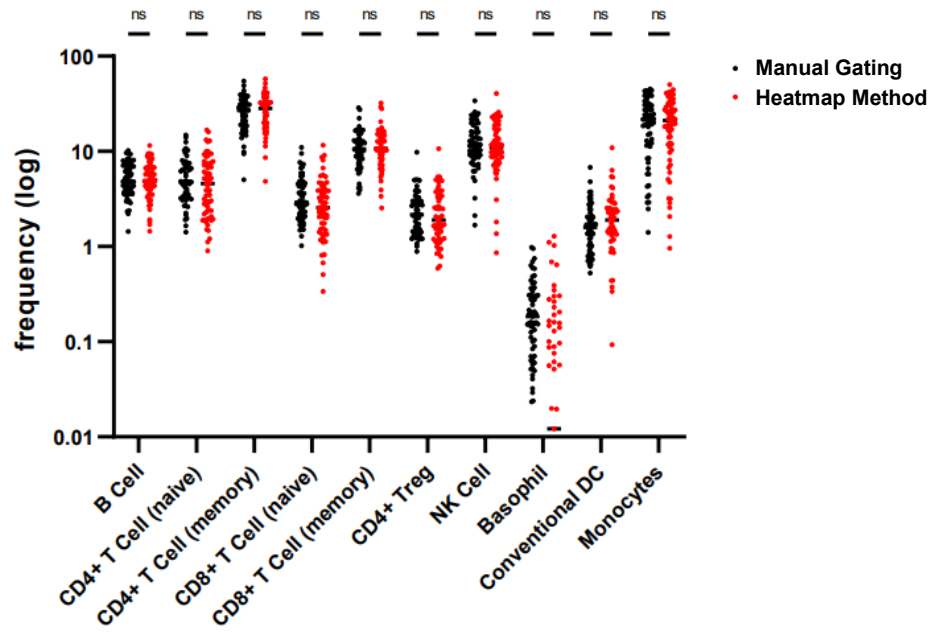
Treg, regulatory T cell; β , standardized regression coefficient; CLA, cutaneous lymphocyte antigen

Group indicates psoriasis vs healthy control cohorts

*Arcsinh (cofactor=5) transformation was performed for linear regression

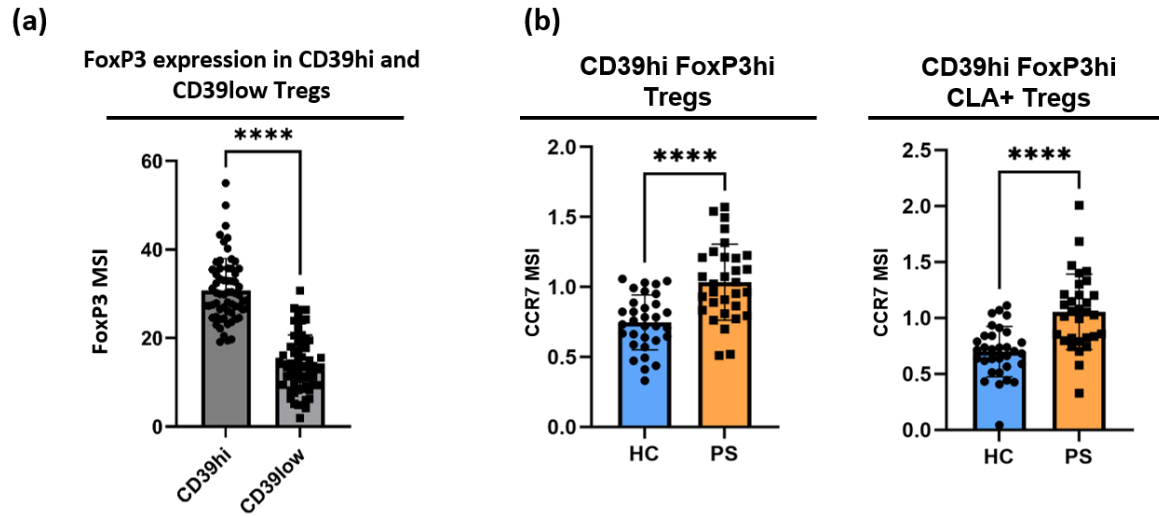
Supplementary Figure S1. Comparison of manual gating vs. heatmap-based cell-type annotation.

Frequency swarm plots of annotated immune cell types using Cytobank for manual gating compared to the described heatmap annotation-based method. Frequencies are reported as percent of total live, singlet, CD45+ immune cells.

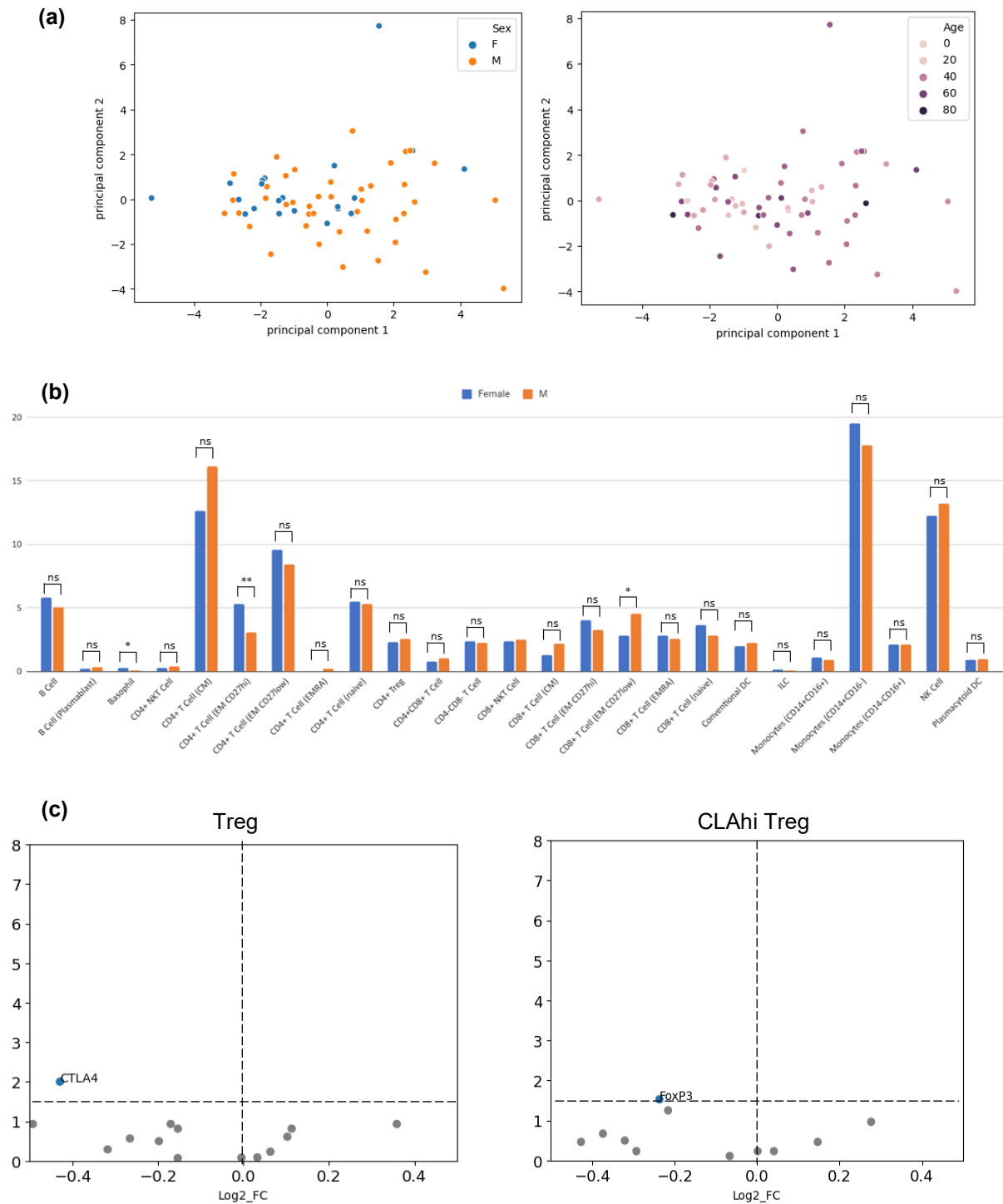


Supplementary Figure S2. Comparison of FoxP3 expression in CD39hi and CD39low Tregs. (a)

Comparison of FoxP3 MSI on CD39hi and CD39 low Tregs with each dot representing a single sample in the CyTOF PBMC dataset (both psoriasis and healthy donor cohorts). (b) Comparison of CCR7 MSI expression (arcsinh transformed) on CD39hi FoxP3hi Tregs and CD39hi FoxP3hi CLA+ Tregs between healthy controls and psoriasis samples (****student T-test p-value < 0.0001).



Supplementary Figure S3. PCA and sex-confounder analysis of CyTOF PBMC data. (a) PCA conducted on cell-type frequencies for each sample in the CyTOF PBMC dataset. Each dot represents a single sample coloured by sex or age. (b) Comparison of cell type frequencies in the CyTOF dataset by sex using the student's T-test. An asterisk indicates a significantly differing cell type frequency between sexes with a p-value between 0.01 and 0.05. (c). Volcano plots of differentially expressed Treg markers male and female samples on total Tregs and CLAhi Tregs. Blue dots represent markers with increased expression in female samples. The Y-axis denotes $-\log_{10}$ adjusted p-values and the X-axis denotes \log_2 fold change values based on arcsinh-transformed median signal intensity values (MSI).



Supplementary Figure S4. Comparison of PASI score to production of IL-17A+ by psoriatic CD4+RORgt+ T cells and CCR7+ Tregs by FACS. Psoriasis PBMCs (n=6) were activated for 4 hours with PMA+I and analysed by FACS. (a) Scatterplot of PASI score and frequency of production of IL-17A by CD4+RORgt+ Th17 cells. (b) Scatterplot of PASI score and frequency of production of IL-17A by CCR7+ Tregs.

