Progression of Mycosis Fungoides occurs through divergence of tumor immunophenotype by differential expression of HLA-DR

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1 Supplemental Methods

LMD files were obtained from the Gallios flow-cytometer and processed in Kaluza (v1.5). Compensation was calculated from single-stained beads and was applied to all flow cytometry data. Beads were run on each day of experiments to monitor equipment performance. Manual gating was used to identify a viable CD3+ population sequentially gating on singlets, lymphocytes, PI-, CD14-, CD19-, CD56- and CD3+. Example gating can be seen in Figure S2. Further cell populations were identifed using Boolean gating as in Table S7. For each population, the geometric mean was measured for surface markers and gate percentage for transcription factors and cytokine production. Compensated fluorescence measurements were arcsinh transformed using R. Principal component analysis and heatmap, missing surface expression data (3.4%) was imputed using package 'rfImpute', and the arcsinh fold-change to peripheral blood was calculated for TIL CD4, TIL CD8, Tumor populations. Package 'RtSNE' was used for dimension reduction and visualisation of exported compensated single cell data. Cytometry data was merged with clinical data in order to analyse outcomes.

2 Supplemental Tables

Population	Boolean gating
PB CD4	vCD3+ AND CD4+ AND NOT clone AND blood
PB CD8	vCD3+ AND CD8+ AND blood
TIL CD4	vCD3+ AND CD4+ AND NOT clone AND skin
TIL CD8	vCD3+ AND CD8+ AND skin
Tumor	vCD3+ AND NOT(CD8+ CD4-) AND clone AND skin

Table S1: Boolean gating strategy for cell populations. All clonotypic samples were either CD4+ or CD4 downregulated, so the clone was not excluded for CD8 populations. Tumor could taken on an aberrant CD4 and CD8 phenotype, including downregulation of CD4 and some expression of CD8.

Fluorophore	Specificity	Clone	Source
FITC	TCR-Vβ		Beckman Coulter
PE	TCR-Vβ		Beckman Coulter
PE-dazzle594	CD14	HCD14	Biolegend
PE-dazzle594	CD19	HIB19	Biolegend
PE-dazzle594	CD56	5.1H11	Biolegend
Propridium Iodide			
PerCP-EF710	TIGIT	MBSA43	eBioscience
PE-Cy7	PD-1	EH12.2H7	Biolegend
APC	PD-L2	MIH18	Biolegend
AF700	CD4	OKT4	Biolegend
APC-Cy7	CD3	SK7	Biolegend
BV421	PD-L1	29E,2A3	Biolegend
BV510	CD8	RPA-T8	Biolegend

Table S2: Panel 1

Fluorophore	Specificity	Clone	Source
FITC	TCR-Vβ		Beckman Coulter
PE	TCR-Vβ		Beckman Coulter
PE-dazzle594	CD14	HCD14	Biolegend
PE-dazzle594	CD19	HIB19	Biolegend
PE-dazzle594	CD56	5.1H11	Biolegend
Propridium Iodide			
PerCP-Cy5.5	Galectin 9	PM1-3	Biolegend
PE-Cy7	HLA-DR	LN3	Biolegend
AF647	LAG3	11C3C65	Biolegend
AF700	CD4	OKT4	Biolegend
APC-Cy7	CD3	SK7	Biolegend
BV421	Tim3	F38-2E2	Biolegend
BV510	CD8	RPA-T8	Biolegend

Table S3: Panel 2

Fluorophore	Specificity	Clone	Source
FITC	TCR-Vβ		Beckman Coulter
PE	TCR-Vβ		Beckman Coulter
PE-dazzle594	CD14	HCD14	Biolegend
PE-dazzle594	CD19	HIB19	Biolegend
PE-dazzle594	CD56	5.1H11	Biolegend
Zombie Red	FVD		Biolegend
PerCP-Cy5.5	IL10	JES3-9D7	Biolegend
PE-Cy7	CD25	M-A251	BD Biosciences
AF647	FoxP3	259D	Biolegend
AF700	CD4	OKT4	Biolegend
APC-Cy7	CD3	SK7	Biolegend
BV421	TGF-B1	TW4-2F8	Biolegend
BV510	CD8	RPA-T8	Biolegend

Table S4: Panel 3

Fluorophore	Specificity	Clone	Source
FITC	TCR-Vβ		Beckman Coulter
PE	TCR-Vβ		Beckman Coulter
PE-dazzle594	CD14	HCD14	Biolegend
PE-dazzle594	CD19	HIB19	Biolegend
PE-dazzle594	CD56	5.1H11	Biolegend
Propridium Iodide			
PE-Cy7	MHC I	W6/32	Biolegend
APC	FasL	NOK1	BD Biosciences
AF700	CD4	OKT4	Biolegend
APC-Cy7	CD3	SK7	Biolegend
BV421	Fas	DX2	Biolegend
BV510	CD8	RPA-T8	Biolegend

Table S5: Panel 4

Fluorophore	Specificity	Clone	Source
FITC	TCR-Vβ		Beckman Coulter
PE	TCR-Vβ		Beckman Coulter
PE-dazzle594	CD14	HCD14	Biolegend
PE-dazzle594	CD19	HIB19	Biolegend
PE-dazzle594	CD56	5.1H11	Biolegend
Zombie Red	FVD		Biolegend
PerCP-Cy5.5	IFN-gamma	B27	Biolegend
PE-Cy7	IL-4	MP4-25D2	Biolegend
APC	IL-10	JES3-19F1	Biolegend
AF700	CD4	OKT4	Biolegend
APC-Cy7	CD3	SK7	Biolegend
BV421	IL-17A	BL168	Biolegend
BV510	CD8	RPA-T8	Biolegend

Table S6: Panel 5

Fluorophore	Specificity	Clone	Source
FITC	Granzyme B	QA16A02	Biolegend
PE	CD107a	H4A3	Biolegend
PE-dazzle594	CD14	HCD14	Biolegend
PE-dazzle594	CD19	HIB19	Biolegend
PE-dazzle594	CD56	5.1H11	Biolegend
Zombie Red	FVD		Biolegend
PerCP-Cy5.5	CD7	CD7-6B7	Biolegend
PE-Cy7	CD25	M-A251	BD Biosciences
APC	CD127	A019D5	Biolegend
AF700	CD4	OKT4	Biolegend
APC-Cy7	CD3	SK7	Biolegend
BV421	IL-2	MQ1-17H12	Biolegend
BV510	CD8	RPA-T8	Biolegend

Table S7: Panel 6

3 Supplemental Figures

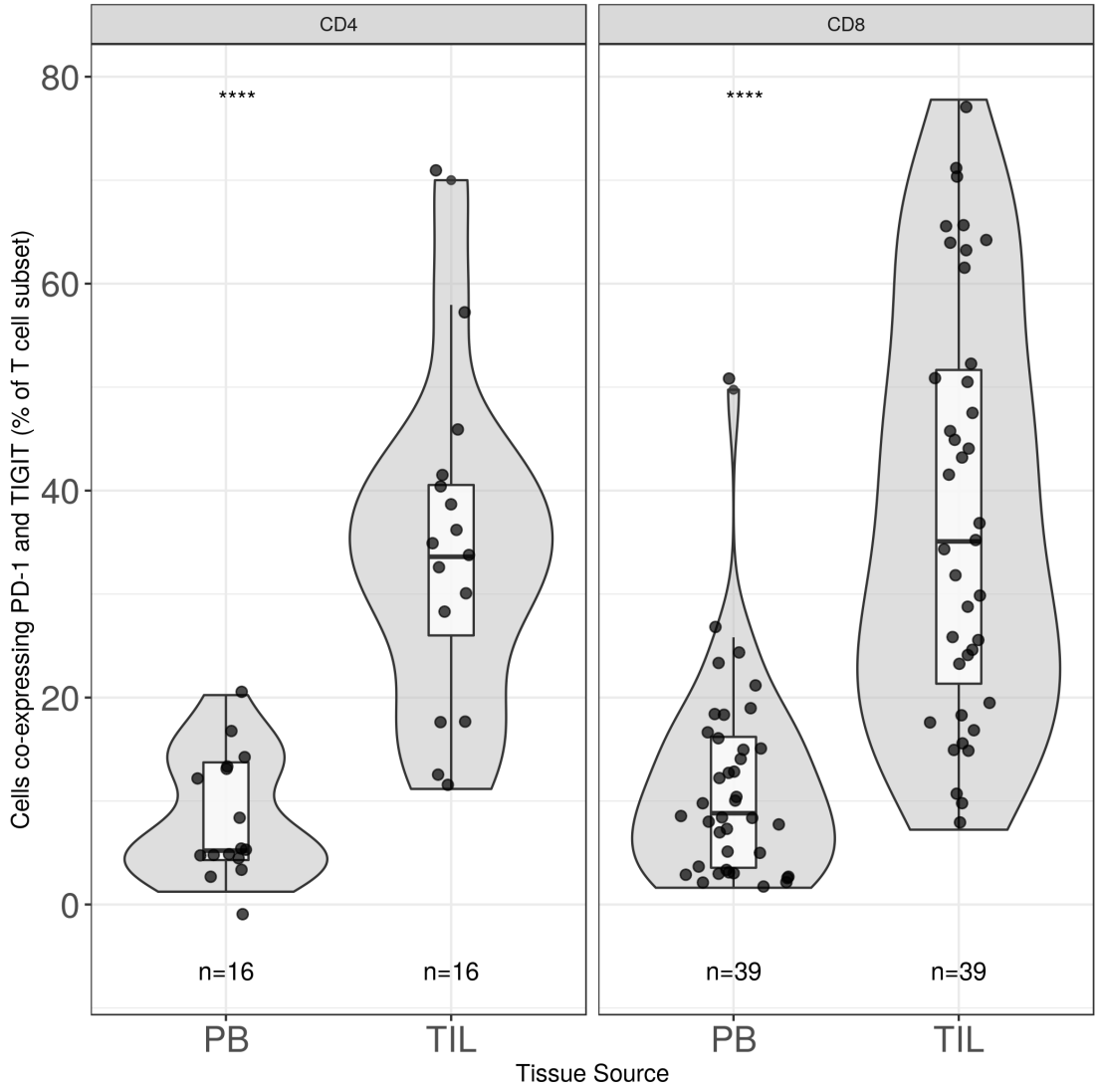

Figure S1: The pattern of of co-expression of of PD-1 and TIGIT on CD4+ and CD8+ T cells within blood and on TIL within tumor biopsies. n = 16 for CD4+ (restricted to cases with clonotypic CD4+ tumor population) and n = 39 for CD8+ analyses

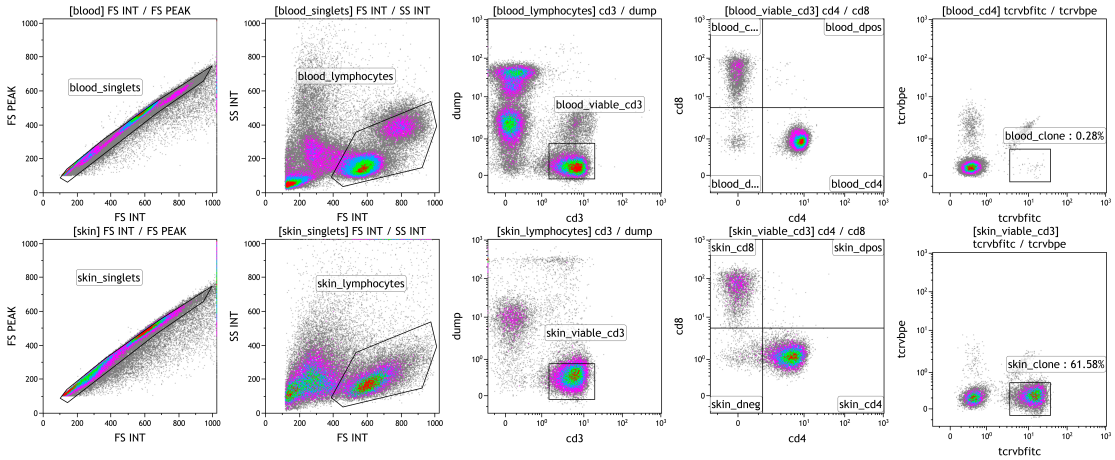

Figure S2: Example of sequential gating strategy

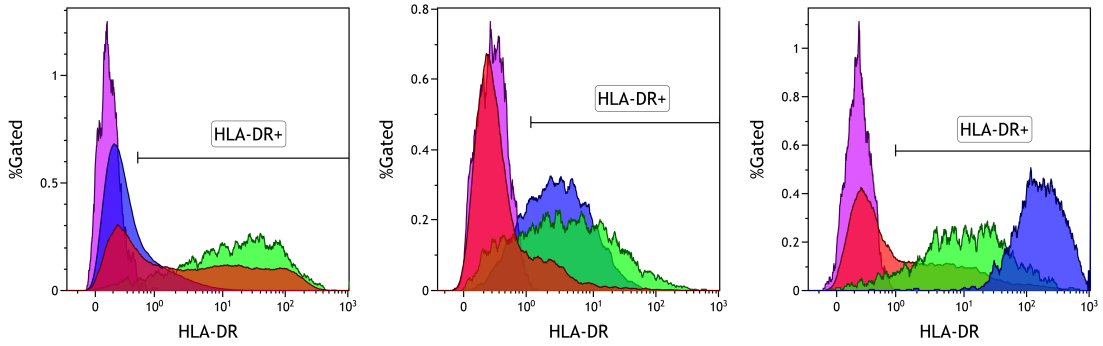

Figure S3: Example of gating from three representative samples of low, medium and high HLA-DR expression. Blue - tumor, green - CD4 TIL, red - CD4 PB, purple - unstained tissue.

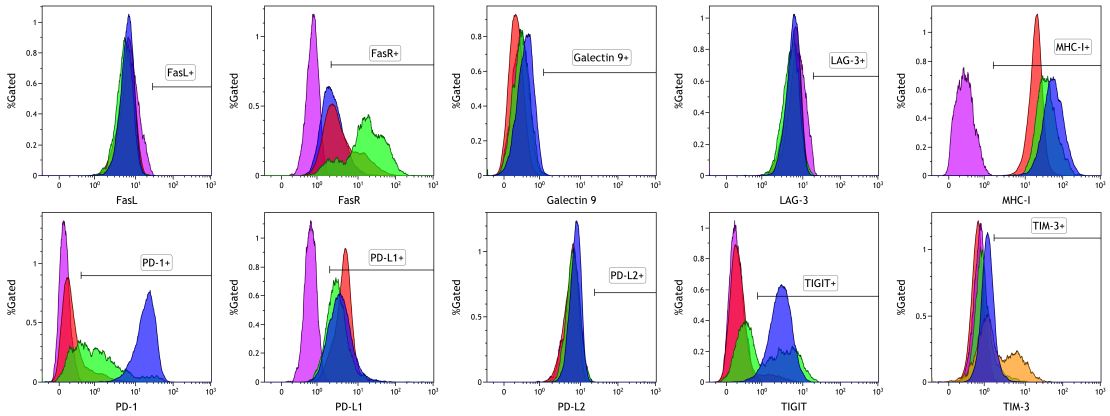

Figure S4: Example gating of other surface markers from single representative patient. Blue - tumor, green - CD4 TIL, orange - CD8 TIL, red - CD4 PB, purple - unstained tissue.

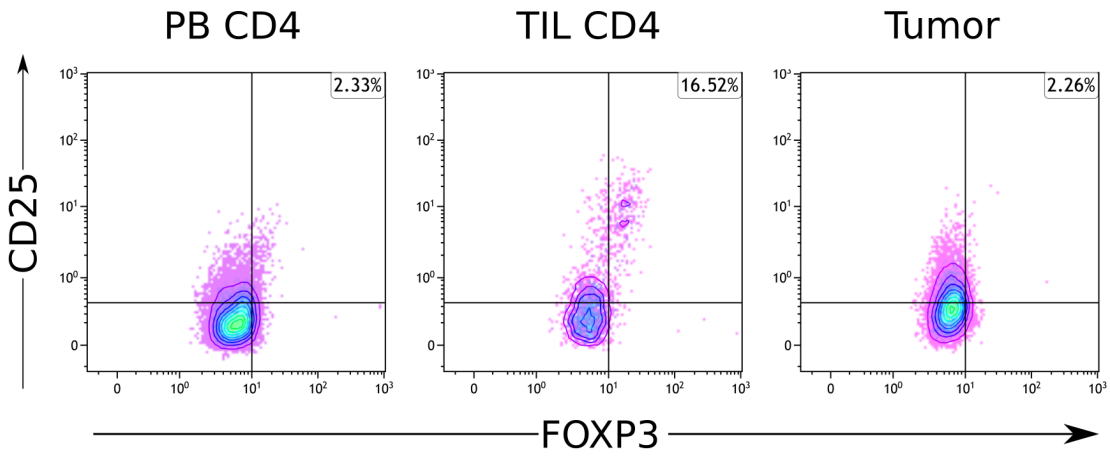

Figure S5: Example of T-regulatory cell gating for peripheral blood, TIL and Tumor

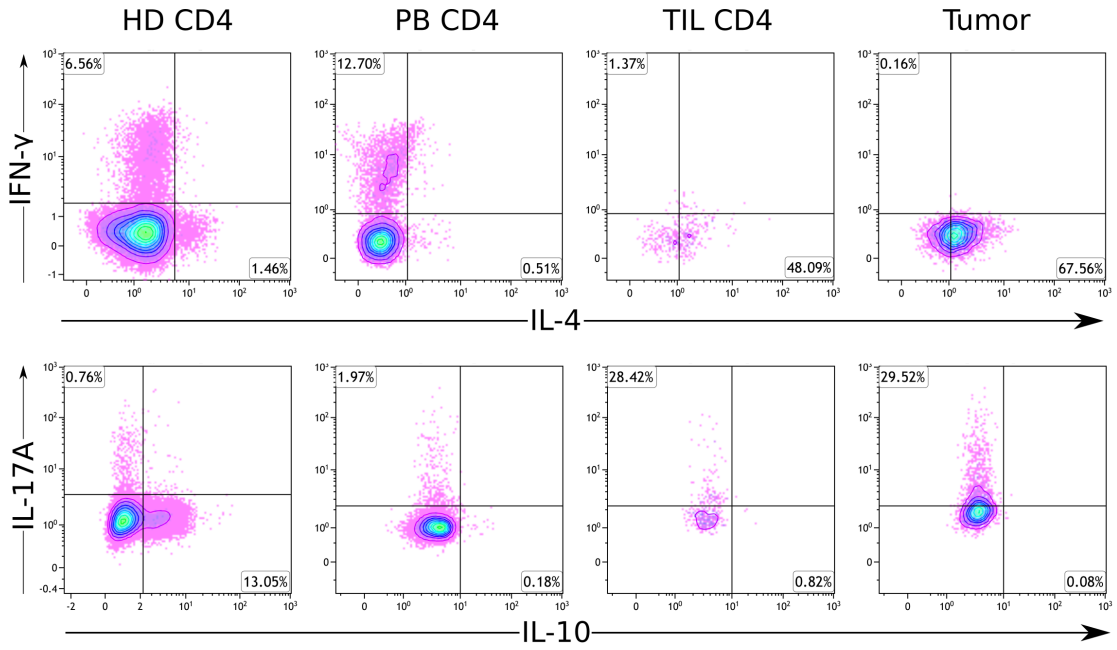

Figure S6: Example of IFN-γ, IL4, IL10 and IL17A gating in healthy donor, patient peripheral blood, TIL and Tumor populations.