Costanza *et al*., Preclinical *in vitro* and *in vivo* evidence for targeting CD74 as an effective treatment strategy for cutaneous T-cell lymphomas

SUPPLEMENTARY MATERIALS

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Immunofluorescence stainings of skin biopsies

Immunofluorescence stainings were performed on 1 μm FFPE tissue sections. After deparaffinizing and rehydrating, MaxBlock Autofluorescence Reducing Reagent Kit (MVB-MB-M, Biozol) was used as autofluorescence bock. Heat-induced antigen retrieval was performed in 1 x HIER TRIS-EDTA buffer (pH 9) (ZUC029-500; Zytomed Systems) for 20 min at 95 °C and the sections were blocked with 5% donkey serum diluted in PBS for 30 min at room temperature. Primary antibody against CD4 (ab133616, dilution 1:500, Abcam; or M7310, 1:50, Dako) and CD74 (ab9514, 1:200; or ab108393, 1:200, both Abcam) were used, followed by incubation with fluorophore-conjugated secondary antibodies Alexa488-anti-rabbit (711-545-152) and Cy3-anti-mouse (715-165-151, both Dianova) diluted 1:400, respectively. DAPI (10184322, Invitrogen) was used in a 1:1000 dilution to stain nuclei. All antibodies were diluted in fluorescence antibody diluent (BRR901AL, Zytomed). Slides were mounted with fluorescence mounting medium (S302380-2, Agilent). Images were obtained by a Keyence BZ-X810 Fluorescence Microscope with a Keyence Plan Apochromat 20x/0.75 objective lens and the Keyence BZ-X filters DAPI, GFP and TRITC.

Primer sequences for PCR analyses

Primers used for RT-PCR analyses were *CD74* sense (s) 5′-CCAGCACCGACTTTAAGAGG-3′, *CD74* antisense (as) 5′-TACAGGAAGTAGGCGGTGGT-3′; *MIF* s 5′-CCGAGAAGTCAGGCACGTAG-3′, *MIF* as

5′-ATAGTTGATGTAGACCCTGTCCG-3′; *CD44* s 5′-AATATAACCTGCCGCTTTGC-3′, *CD44* as 5′-CAGGTCTCAAATCCGATGCT-3′; *CXCR4* s 5′-AGCATGACGGACAAGTACAGG-3′, *CXCR4* as 5′-ATACCAGGCAGGATAAGGCC-3′; *GAPDH* s 5′-ATGCTGGCGCTGAGTAC-3′, *GAPDH* as 5′-TGAGTCCTTCCACGATAC-3′. Primers used for quantitative PCR analyses were *CD74* s 5′-GCACTCCTTGGAGCAAAAGC-3′, *CD74* as 5′-GGGATGTGGCTGACCTCTTC-3′. All PCR products were verified by sequencing.

CO-Detection by indEXing (CODEX)

Human samples and CTCL tissue microarray (TMA) construction. FFPE tissue blocks were retrieved from the tissue archive at the Department of Dermatology, University Hospital Tübingen, Germany. For the CTCL TMA, 16 patient tissues were selected (three SS, five advanced stage MF, three pcALCL and five LyP tumor tissues). All patients had clinicopathologically confirmed diagnoses, as assessed by experienced clinicians and dermatopathologists.

2.5 μm H&E-stained sections of tissue blocks were digitized using a Pannoramic Midi II digital slide scanner (3DHISTECH), and dermal infiltrates were digitally annotated in the Case Viewer software (3DHISTECH). A TMA of 35 cores with 1.5 mm diameter (two cores per CTCL biopsy sample as well as two tonsil cores and one liver core as positive and negative controls for antibodies, respectively) was constructed using a TMA Grand Master automated tissue microarrayer (3DHISTECH). 4.0 μm sections of the TMA were mounted on Vectabond™-treated (Vector Labs, SP-1800) glass coverslips (22 X 22 mm, #1 1/2, Electron Microscopy Sciences, #72204-01).

CODEX experiments. Buffers and solutions used for CODEX are listed in **Table S1**. All pipetting steps were performed using filter tips.

Antibody conjugation. Maleimide-modified short DNA oligonucleotides were purchased from Biomers. Purified, carrier-free anti-human antibodies were purchased from different companies (for details, see Table S2). Conjugations were performed as previously described,[1] under a 1:2 weight/weight ratio of antibody to oligonucleotide. Purified antibodies were concentrated on 50 kDa filters (Thermo Fisher Scientific, UFC505096). Then sulfhydryl groups were activated by the mixture of 2.5 mM tris(2-carboxyethyl) phosphine (TCEP, Sigma, C4706) and 2.5 mM EDTA (pH 8.0; Invitrogen, AM9261) in 1x PBS (Thermo Fisher Scientific, 70011069) for 30 min at room temperature. Then, antibodies were washed with buffer C. Oligonucleotides were resuspended in 400 µl of buffer C and then added to the antibodies. After incubation at RT for 2 h, the conjugated antibodies were washed and spun down three times using high-salt PBS. Finally, the conjugated antibodies were recovered from the filter using antibody stabilizer solution and stored at 4 °C.

CODEX FFPE tissue staining and imaging. CODEX highly multiplexed tissue imaging was performed as previously described.[1-3] The coverslip was baked at 70 °C in an oven for 1 h and the tissue was deparaffinized three times in xylene (Thermo Fisher Scientific, X5-4) at RT for 10 min. Then, sections were rehydrated by descending concentrations of ethanol (Sigma, E7023; 100% twice, 95% twice, 80% one time, 70% one time), followed by double-distilled H_2O (ddH_2O) twice (each step for 3 min). Heat-induced epitope retrieval was performed using Dako target retrieval solution (pH 9.0; Agilent, S236784-2) for 10 min at 97 °C. After cooling to RT for 1 h, autofluorescence was quenched in bleaching solution inside an LED lamp sandwich (Aibecy A5 Ultra Bright 25’000 Lux LED Light Box-Tracing Pads; AliExpress) for 45 min twice, according to the manufacturer´s instructions (Akoya Biosciences). Then, the coverslip was washed in 1x PBS four times for 3 min, followed by 1x TBS wash buffer with Tween 20 (Cell Marque, 935B-09) for 10 min. The tissue was encircled by Bondic polyacrylamide gel (Amazon, B018BEHQU), and nonspecific binding was blocked for 1 h at RT with 100 µl blocking solution. Subsequently, the tissue was stained with 100 µl of antibody

cocktail at 4 ºC overnight in a sealed humidity chamber on a shaker. On the next day, the coverslip was washed two times in S2 for 2 min and then fixed with 1.6% paraformaldehyde (Thermo Fisher Scientific, 50-980-487) for 10 min, washed with 1x PBS for 1 min, fixed with ice-cold 100% methanol (Thermo Fisher Scientific, A412-4) for 5 min at 4 ºC, washed again, and finally fixed with BS3 fixative solution for 20 min at RT on a shaker. Finally, after washing in PBS, the coverslip was stored in S4 buffer for up to two weeks at 4 ºC. The multicycle reaction was performed by using a Keyence BZ-X810 inverted fluorescence microscope equipped with a CFI Plan Apo λ 20x/0.75 objective (Nikon) and a CODEX PhenoCycler microfluidics instrument (Akoya Biosciences). An H&E staining of the TMA was performed after the multicycle experiment.

CODEX images data processing. Raw TIFF image files were processed by CRISP-CODEX-Processor (https://github.com/will-yx/CRISP-CODEX-Processor) (https://www.biorxiv.org/content/10.1101/2022.06.10.494732v1). Each antibody staining quality was assessed visually in each TMA core. Cell segmentation was performed on the DRAQ5 nuclear stain (Thermo Fisher Scientific, 65-0880-96) using CellSeg. All the marker expression values were quantified, and single cell data were saved into FCS files. Cell gating was performed by CellEngine (https://cellengine.com), and the frequencies of cell types were verified by comparing the gating to the processed CODEX images in ImageJ/Fiji. Figures and statistics were created using Prism 9.0 software (GraphPad), and 7-color overlay images were created in ImageJ/Fiji.

scRNA- and scTCR-sequencing

Data collection (scRNA seq). For generation of single-cell RNA sequencing (scRNA-seq) and single-cell TCR sequencing (scTCR-seq), native lesional skin samples of 5 patients were immediately (i.e., within 2 hours after excision) transferred to single-cell suspension. Briefly, tissue cubes of 2 mm edge length were minced in gentleMACS (Miltenyi Biotec) using

program „h_tumor_01“, followed by program „h_tumor_02“ twice with 30 min intervals in an enzyme mix consisting of 200 µl Enzyme H, 100 µl Enzyme R and 25 µl Enzyme A (#130-095-929; Miltenyi Biotec). After three washes with PBS/0.05% bovine serum albumine (BSA), cells were passed through a 100 µm cell strainer. The number and viability of T cells in the single-cell suspension was assessed by flow cytometry using antibodies against CD3 (#1P-514-T025; EXBIO Praha) and CD45 (#1A-222-T100; EXBIO Praha) together with 7-aminoactinomycin (7AAD; #SML1633-1ML; Merck) in a CytoFLEX (Beckman Coulter). More than 80% living cells containing at least 30% cells were required for scRNA and scTCRseq using the Single Cell 5` Library & Gel Bead Kit v1.1 (#1000165; 10xGenomics) for 12,000 cells at a concentration of 600 cells/µl. After Gel Bead-In Emulsions (GEM) generation, the mRNA was reversely transcribed and cDNA amplified for library construction. The gene expression libraries were sequenced at the DKFZ Genomics and Proteomics core facility on a NovaSeq 6000, paired-end with 26 cycles of the forward and 74 cycles on the reverse read, the TCR libraries on a NextSeq 550 platform with Paired-End 150bp Mid-Output.

The data is available at the Genome-phenome Archive (EGA; https://ega-archive.org) under accession EGAS50000000226. scRNA-seq and scTCR-seq data on $CD45^+$ PBMCs of 5 patients with SS were obtained from GEO.[4]

scRNA- and scTCR-seq data analysis. The SS and MF datasets underwent separate processing using the Cell Ranger pipeline (version 6.1, 10x Genomics), employing default settings specific to gene expression and utilizing the T2T-CHM13v2.0 reference genome; TCR libraries were managed accordingly. The scRNA-seq expression matrix was read using the Seurat R package, and cells with a mitochondrial ratio exceeding 35% and a housekeeping gene ratio below 5% were excluded. For subsequent analysis, the Seurat R package (v3.2.2) was employed. Data normalization was executed using the SCTransform function, followed by dimensionality reduction through principal component analysis (PCA). The top 30 principal components were then utilized for Uniform Manifold Approximation and Projection for Dimension Reduction

(UMAP). Further unsupervised clustering was performed using the FindNeighbors and FindClusters functions. Cell clusters were annotated into known cell lineages based on the expression of typical marker genes. The scTCR-seq expression matrix was read using the scRepertoire R package, and T cells expressing the most abundant TCR clonotype were identified as the malignant clone.

DNA methylation analysis

A total of 500 ng of genomic DNA of the CTCL cell lines Se-Ax, HuT 78, My-La and HH was bisulfite-converted using the EZ DNA Methylation kit (Zymo Research, Irvine, CA, USA) according to manufacturer's instructions. DNA methylation was interrogated by the Infinium® MethylationEPIC (EPIC) BeadChips (Illumina Inc., San Diego, CA, USA) following the manufacturer's guidelines. Raw intensity files (idat) were imported into the R programming environment (v4.3.1) using the minfi package. Data were normalized to controls using the preprocess Illumina function without background correction. The methylation level at each CpG site was calculated as a beta value, which varied from 0 (no methylation) to 1 (complete methylation). Additionally, we analyzed publicly available DNA methylation array data derived from Infinium® HumanMethylation450 (450K) or MethylationEPIC (EPIC) BeadChips from a collection of benign B cells and T cells and hematopoietic cell lines (Table S3). The control cell lines included an ALK positive (Karpas-299) and negative (FE-PD) ALCL cell line as well as T-ALL (Jurkat, KE-37) cell lines. In order to cross-compare all studies, we converted samples run on EPIC arrays into virtual 450K arrays by filtering for CpGs present in both versions. The 15 CpG loci mapping to the *CD74* gene locus were extracted from the dataset and visualized as a heatmap generated using the pheatmap package or boxplots visualized with ggplot.

References

1 Black S, Phillips D, Hickey JW *et al.* CODEX multiplexed tissue imaging with DNA-conjugated antibodies. *Nat Protoc* 2021; **16**:3802-35.
2 Schurch CM, Bhate SS, Barlow GL *et al.* Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front. *Cell* 2020; **182**:1341-59 e19.
3 Phillips D, Matusiak M, Gutierrez BR *et al.* Immune cell topography predicts response to PD-1 blockade in cutaneous T cell lymphoma. *Nat Commun* 2021; **12**:6726.
4 Borcherding N, Severson KJ, Henderson N *et al.* Single-cell analysis of Sezary syndrome reveals novel markers and shifting gene profiles associated with treatment. *Blood Adv* 2023; **7**:321-35.

LEGENDS TO SUPPLEMENTARY FIGURES 1-5

Supplementary Figure 1. CD74 immunohistochemistry of various CTCL entities; CD74 flow cytometry of circulating $CD4^+$ SS cells; CD4 and CD74 double-immunofluorescence stainings of skin biopsies from SS patients. (a) Representative immunohistochemistry stainings of skin biopsies from various CTCL entities, as indicated on the top. Each triplet from top to bottom represents stainings from serial sections of the same skin biopsy of the respective patient. Top row, H&E staining; central row, CD3, CD4 or CD30 stainings, as indicated; bottom row, CD74 staining. Original magnifications: x100; bar, 50 μm. (b) CD74 flow cytometry of circulating $CD4^+$ SS cells from peripheral blood of 5 SS patients (SS_pt4; SS_pt5; SS_pt6; SS_pt7; SS_pt8). Gating strategy was as in Figure 1d. The filled and the open histogram represent SS cells stained with IgG1 isotype control (IC) and CD74 antibody, respectively. Based on the $CD3^+CD4^+CD7^-$ phenotype, tumor cell contents within the CD4- population were 46% (SS_pt4), 94% (SS_pt5), 80% (SS_pt6), 92% (SS_pt7), 90% (SS_pt8), respectively. (c) Representative CD4 and CD74 double-immunofluorescence stainings of skin biopsies from three SS patients (SS_pt1; SS_pt2; SS_pt5). Original magnification: far left, x200; center left and right, far right, x800; bar, 50 μm. White arrows indicate CD74 and CD4 double-positive cells.

Supplementary Figure 2. Tissue microarray used for CODEX multiplexed tissue imaging; seven-color overview of pcALCL and MF cases analyzed by CODEX. (a) Overview of the tissue microarray used for CODEX analysis. Left, H&E staining. Bar, 1 mm. Numbers refer to the annotation depicted on the right. Right, annotation of the skin biopsy samples on the microarray shown on the left. Note, that these patient samples are different to patient samples depicted in Figure 1d. (b, c) Seven-color overviews of a representative No.25 pcALCL (b) and No.11 MF (c) case from the multi-tumor TMA depicted in (a), imaged using a 39-marker CODEX panel. Left and center represent seven-color overviews for markers including

CD74 and CD3, CD4, CD8, CD30, CD138, Ki-67, Podoplanin, CD138 and DRAQ5 nuclear stain, with individual markers shown on the right. Original magnifications: x20; scale bar left 100 µm, center and right 50 µm.

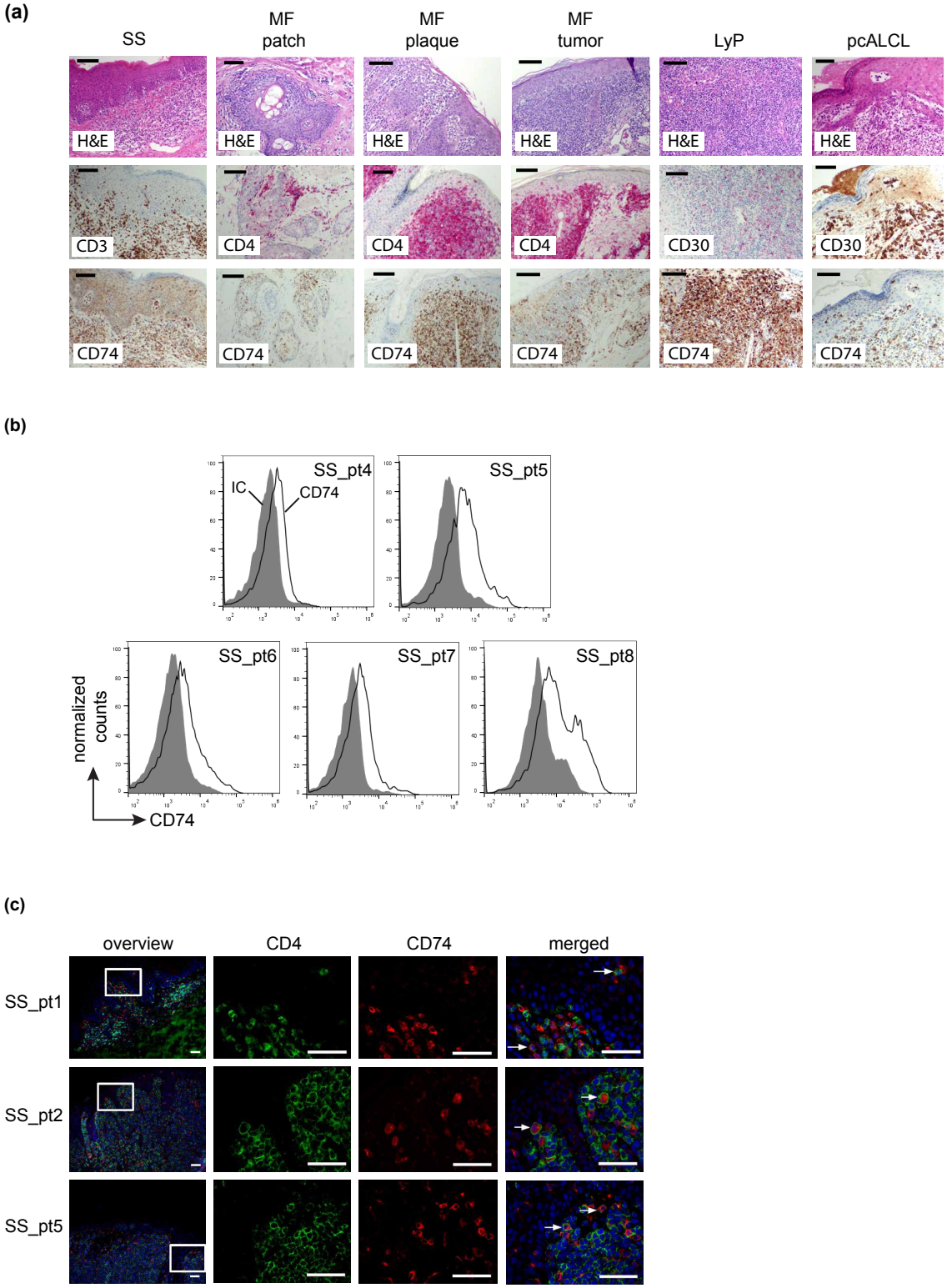
Supplementary Figure 3. CODEX multiplexed tissue imaging. (a) Images of skin biopsy sample No.10 core depicted in grayscale for each antibody. Original magnification: x20; scale bar, 200 µm. (b) Strategy for tumor cell gating following CODEX for quantification of $CD74^+$ tumor cells within representative SS, MF, LyP and pcALCL samples, corresponding to the Figures 2a-b and Supplementary Figure 2b-c.

Supplementary Figure 4. Single cell analyses of MF skin biopsies for expression of *MIF*, *CD44* and *CXCR4*. Single cell (sc) RNA and T-cell receptor sequencing of 5 MF skin biopsies. Top, UMAP of all cells within the respective skin biopsies according to the similarity of their transcriptome and annotated by the individual patient and cell type (top). Bottom panels, *MIF* (top panels), *CD44* (center) and *CXCR4* (bottom panels) mRNA expression levels in the various cell types, as indicated, in the same skin biopsies as in the top panel. MF, mycosis fungoides tumor cells; T, T cells; NK, NK cells; B, B cells; Mφ, macrophages; pDC, plasmacytoid dendritic cells; FB, fibroblasts; EN, endothelial cells; EP, epithelial cells.

Supplementary Figure 5. Single cell analyses of SS skin biopsies and analysis of CTCL cell lines for expression of *MIF*, *CD44* and *CXCR4*. (a) Single cell (sc) RNA and T-cell receptor sequencing of 5 SS skin biopsies. Top, UMAP of all cells within the respective skin biopsies according to the similarity of their transcriptome and annotated by the individual patient and cell type (top). Bottom panels, *MIF* (top panels), *CD44* (center) and *CXCR4* (bottom panels) mRNA expression levels in the various cell types, as indicated, in the same skin biopsies as in the top panel. SS, Sézary syndrome tumor cells; T, T cells; NK, NK cells; B, B cells; Mφ,

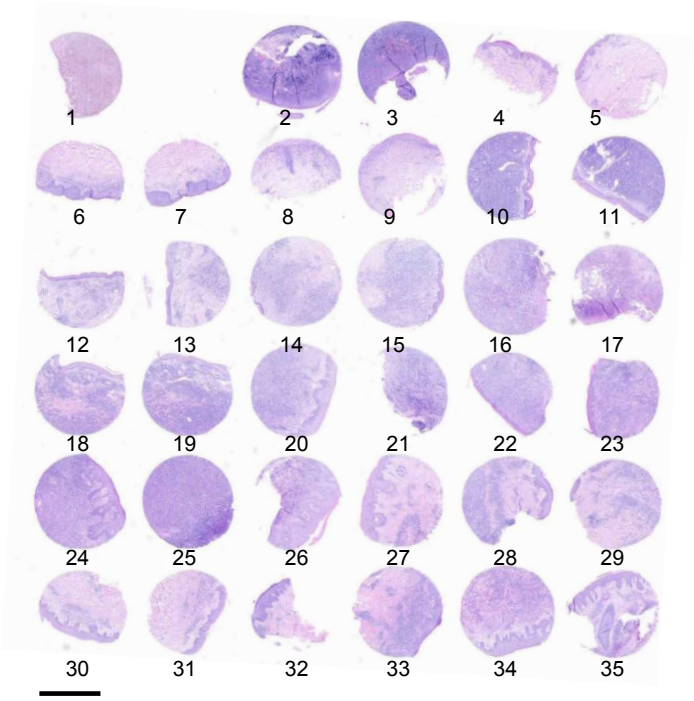
macrophages; pDC, plasmacytoid dendritic cells; FB, fibroblasts; EN, endothelial cells; EP, epithelial cells. (b) *MIF*, *CD44* and *CXCR4* expression in CTCL cell lines. Analysis of *MIF, CD44 and CXCR4* mRNA expression in various cell lines by RT-PCR. Analysis of *GAPDH* is shown as a control.

Supplementary Figure 1



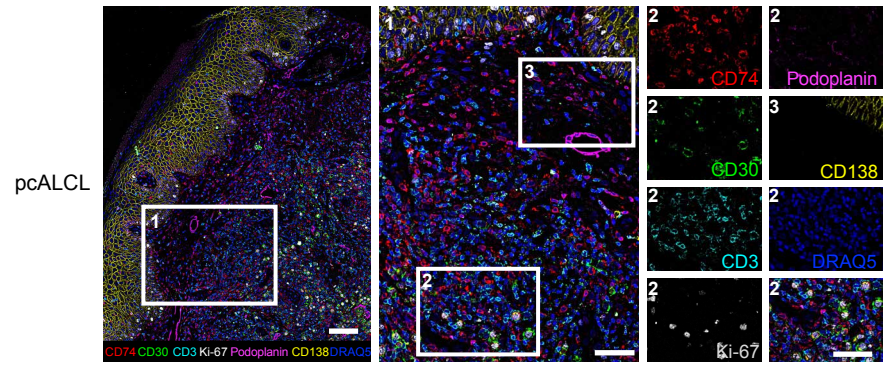
Supplementary Figure 2

(a)

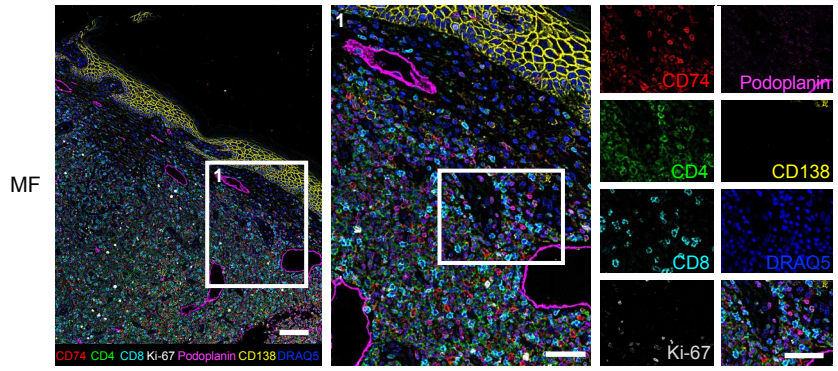


Number	Group
1	Liver
2,3	Tonsil
4,5	SS_pt1
6,7	SS_pt2
8,9	SS_pt3
10,11	MF advanced stage_pt1
12,13	MF advanced stage_pt2
14,15	MF advanced stage_pt3
16,17	MF advanced stage_pt4
18,19	MF advanced stage_pt5
20,21	pcALCL_pt1
22,23	pcALCL_pt2
24,25	pcALCL_pt3
26,27	LyP_pt1
28,29	LyP_pt2
30,31	LyP_pt3
32,33	LyP_pt4
34,35	LyP_pt5
Total: 16 patients	

(b)

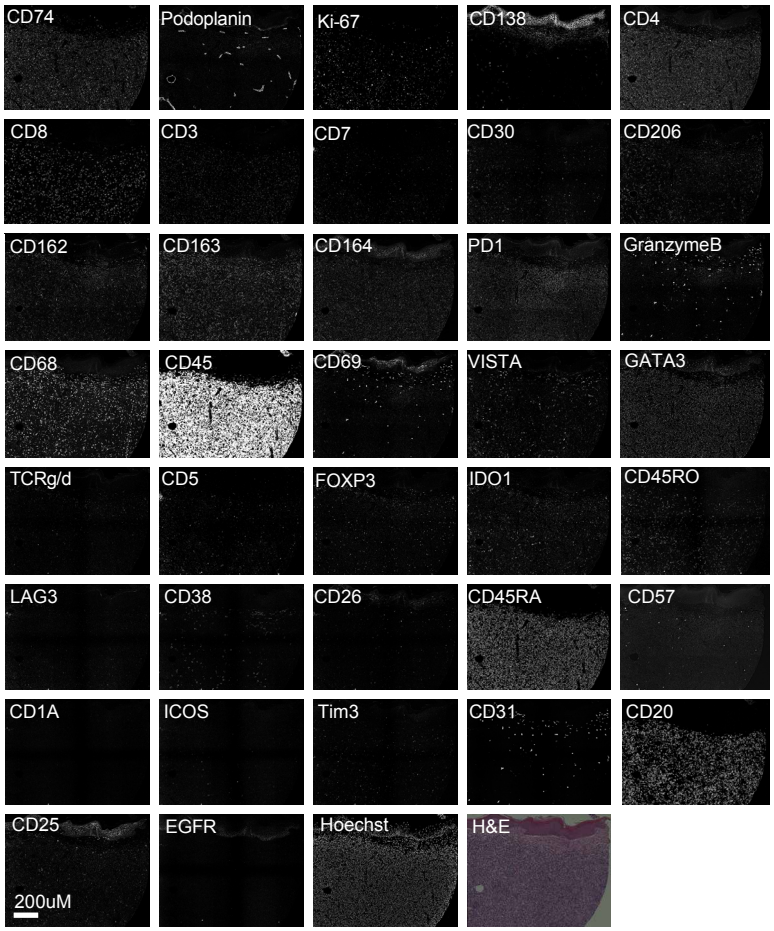


(c)

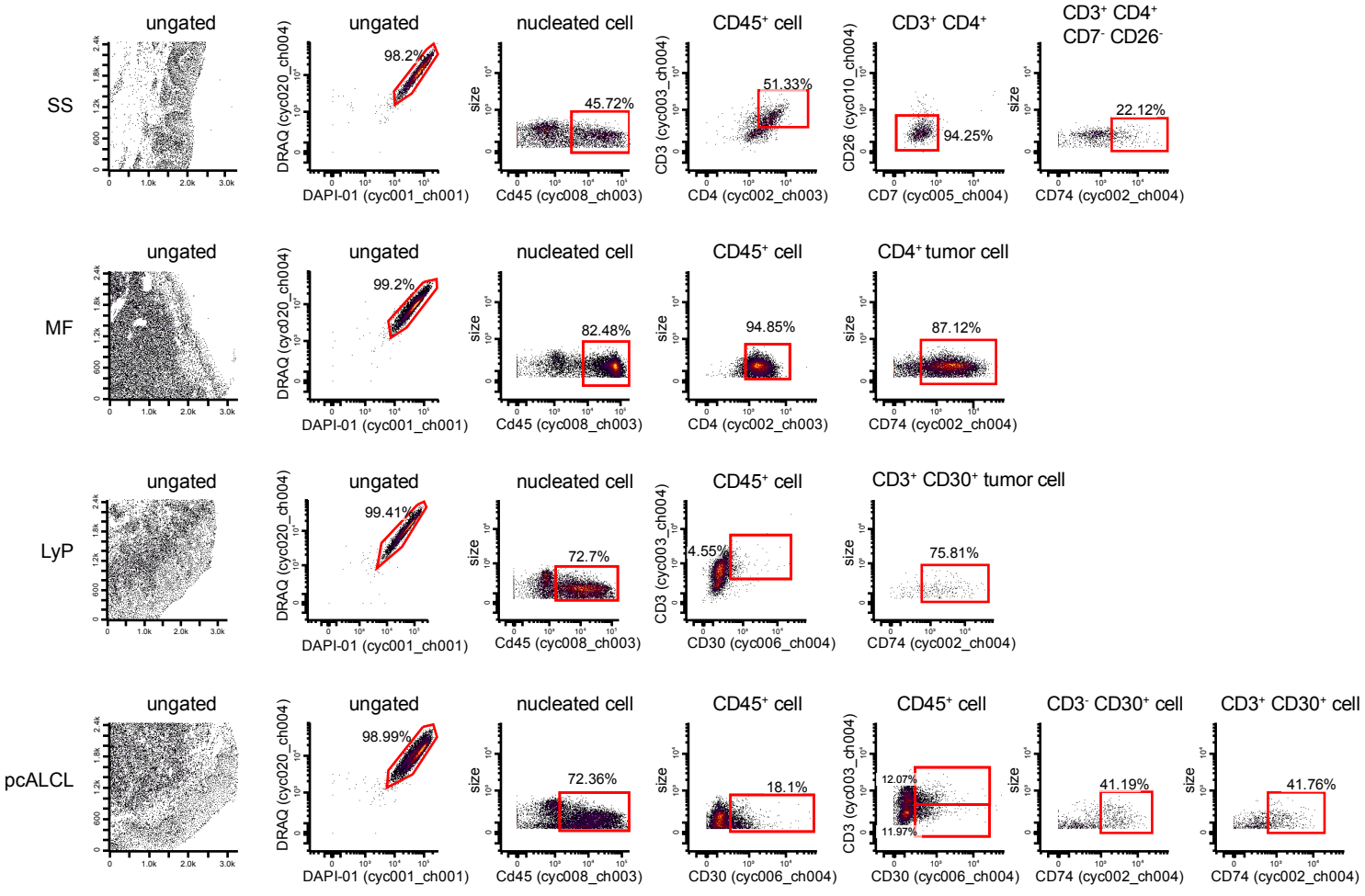


Supplementary Figure 3

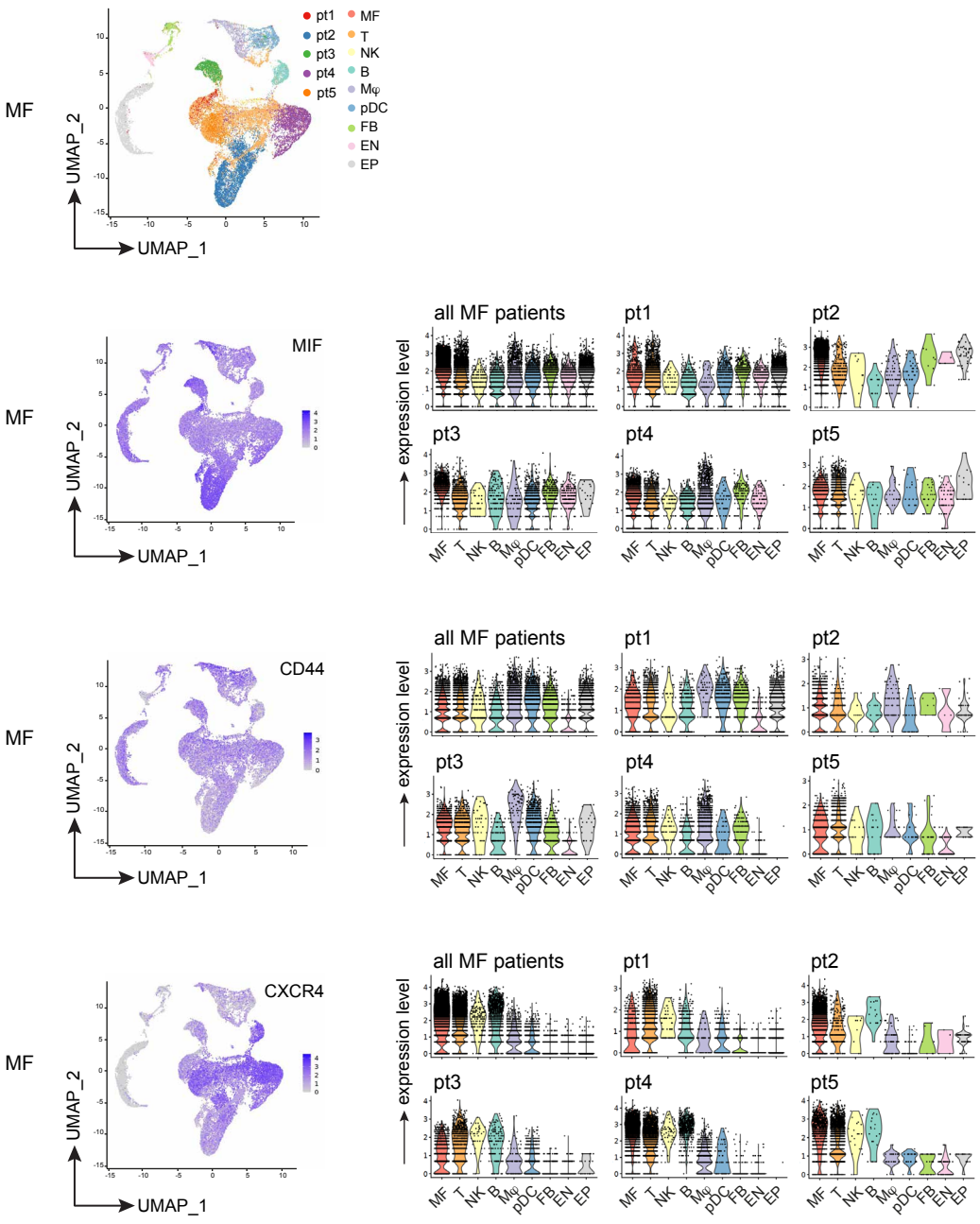
(a)



(b)

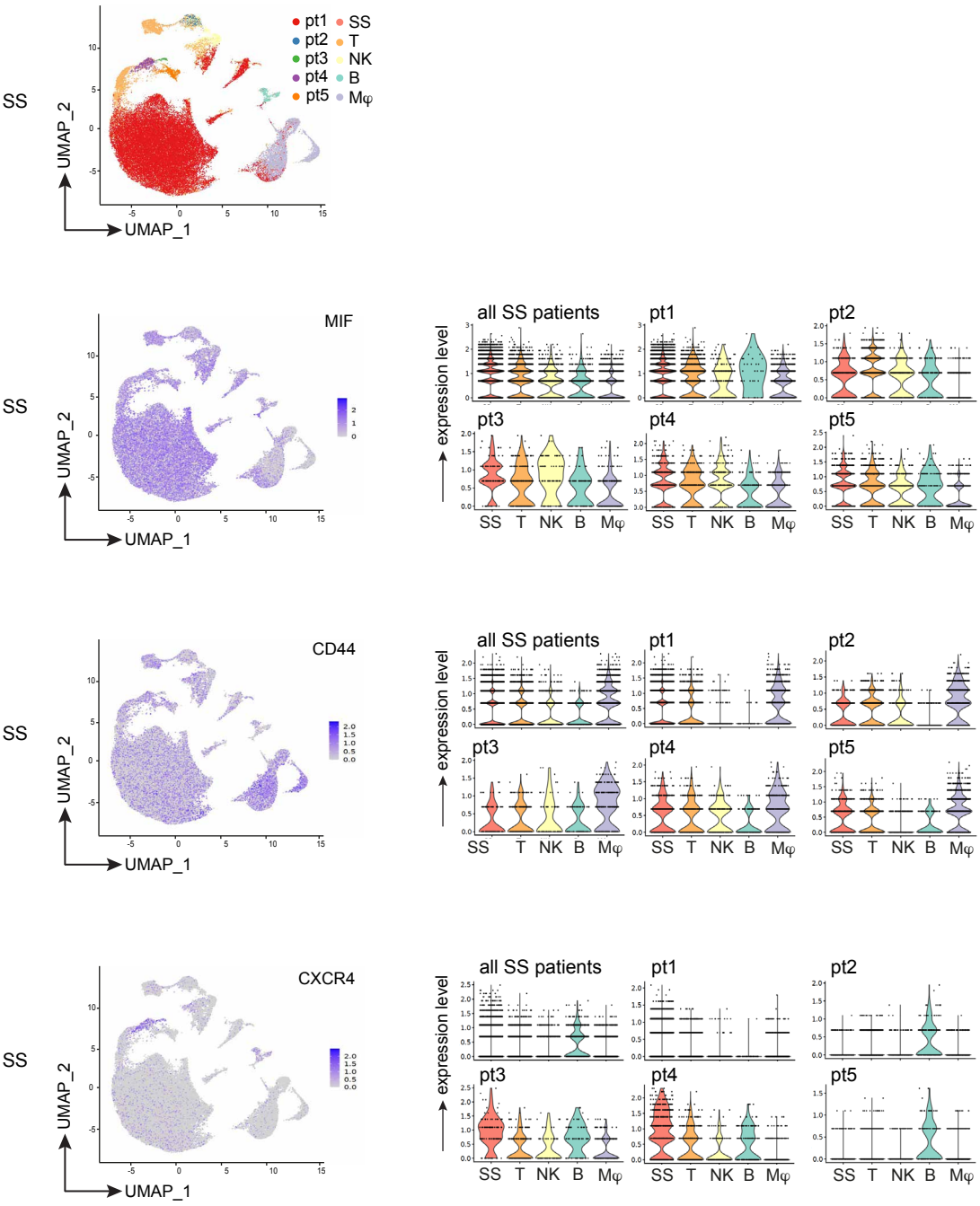


Supplementary Figure 4

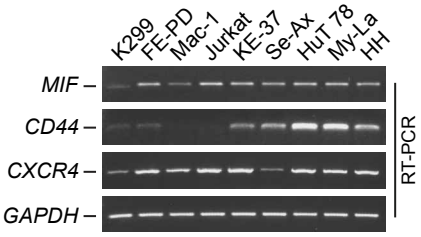


Supplementary Figure 5

(a)



(b)



Supplementary Table 1. CODEX buffers and solutions

Buffer	Ingredient
Buffer C	1 ml 1 M Tris (pH 7.0), 1 ml 1 M Tris (pH 7.5, Teknova, T1080), 30 ml 5 M NaCl (Thermo Fisher Scientific, S271-10), 2 ml 500 mM EDTA (pH 8.0), 200 mg 0.02% (wt/vol) sodium azide (NaN3, Sigma, S-8032) in 966 ml ddH_2O.
High-salt PBS	45 ml 5 M NaC1 solution, 25 ml 10x DPBS in 180 ml ddH_2O.
Tris- EDTA (TE) buffer	1 ml 1 M Tris (pH 8.0), 200 µl 500 mM EDTA (pH 8.0) and 0.02% (wt/vol) NaN3 in 98.8 ml ddH_2O.
Antibody stabilizer solution	1 ml 5 M NaCl solution, 100 µl 500 mM EDTA, and 0.02% (wt/vol) NaN3 in 9 ml Candor PBS-based antibody stabilizer (Thermo Fisher Scientific, NC0436689).
Bleaching solution	9 ml 30% hydrogen peroxide (Sigma, 107210), 1.6 ml 1 M Sodium hydroxide (Sigma, S8263) in 50 ml 1x PBS.
Staining solution 1 (S1)	50 ml 10x DPBS, 5 ml 500 mM EDTA (pH 8.0), 2.5 g bovine serum albumin (BSA, Sigma, A3059) and 100 mg 0.02% (wt/vol) NaN3 in 445 ml ddH_2O.
Staining solution 2 (S2)	250 ml S1, 19.5 ml 1M NaH_2PO_4 (Sigma, S9390), 30.5ml 1M NaH_2PO_4 (Sigma, S7907) and 25 ml 5 M NaCl with 175 ml ddH_2O.
Staining solution 4 (S4)	50 ml 5 M NaCl in 450 ml S1.
Blocking reagent 1 (B1)	10 mg mouse IgG (Sigma, I5381) in 10 ml S2.
Blocking reagent 2 (B2)	10 mg rat IgG (Sigma, I4131) in 10 ml S2.
Blocking reagent 3 (B3)	Sheared salmon sperm DNA (Thermo Fisher Scientific, AM9680), 10 mg/ml in ddH_2O.
Blocking component 4 (BC4)	Mixture of 57 non-modified CODEX oligonucleotides (0.5 mM each) in TE buffer.
Blocking solution	S2 containing B1 (1:20), B2 (1:20), B3 (1:20), BC4 (1:15).
Antibody cocktail	Blocking solution with different dilution of conjugated antibodies, final volume 100 µl.
BS3 fixative solution (BS3)	50 mg BS3 (Thermo Fisher Scientific, 21580) in 250 µl DMSO (Sigma, S8263).

Supplementary Table 2. CODEX antibodies and their usage conditions.

Antibody target	Company	Catalog #	Clone	Oligonuleotide	Flourophore	Working dilution	Exposure time (ms)
CD4	Abcam	ab181724	EPR6855	20	ATTO550	1:50	666
CD8	Cell Marque	custom	C8/144B	8	ATTO550	1:50	500
Foxp3	Invitrogen	14-4777-80	236A/E7	2	ATTO550	1:100	500
IDO1	Cell Signaling Technology	custom	D5J4E	59	ATTO550	1:50	500
GATA3	Cell Marque	custom	L50-823	60	ATTO550	1:25	500
CD25	Cell Marque	custom	4C9	24	ATTO550	1:100	500
CD45	Novus Biologicals	NBP2-34528	2B11 + PD7/26	56	ATTO550	1:400	500
CD162	Novus Biologicals	NBP2-80921	HECA-452	33	ATTO550	1:100	500
CD163	Novus Biologicals	NB110-40686	EDHu-1	45	ATTO550	1:50	500
CD164	BD Biosciences	551296	N6B6	75	ATTO550	1:400	500
PD-1	Cell Signaling Technology	custom	D4W2J	23	ATTO550	1:100	1000
CD206	Abcam	ab64693	MM0820-48L31	55	ATTO550	1:100	500
CD57	Biolegend	322325	HCD57	30	ATTO550	1:200	500
CD68	Biolegend	916104	KP1	70	ATTO550	1:100	500
CD69	Novus Biologicals	AF2359	Polyclonal	36	ATTO550	1:50	500
VISTA	Cell Signaling Technology	custom	D1L2G	79	ATTO550	1:50	500
Granzyme B	Abcam	ab219803	EPR20129-217	81	ATTO550	1:100	500
Podoplaninn	Biolegend	916606	D2-40	32	ATTO550	1:100	500
TCR-g/d	Santa Cruz Biotechnology	custom	H-41	52	ATTO550	1:100	500
CD74	Biolegend	326802	LN2	53	Alexa488	1:100	500
CD3	Cell Marque	custom	D7A6E	77	Alexa488	1:25	666
CD5	Novusbio	NBP2-34583-0.1mg	C5/473+CD5/54/F6	148	Alexa488	1:25	500
CD7	Cell Marque	custom	MRQ56	63	Alexa488	1:25	500
CD30	Cell Marque	custom	Ber-H2	57	Alexa488	1:25	500
CD20	Novus Biologicals	NBP2-54591-100ug	rIGEL/773	48	Alexa488	1:400	500
LAG3	Cell Signaling Technology	custom	D2G4O	42	Alexa488	1:50	500
CD38	Abcam	ab176886	EPR4106	55	Alexa488	1:100	500
CD26	Thermo Fisher Scientific	CF500733	OTI11D7	21	Alexa488	1:200	500
CD45RA	BD Biosciences	555486	HI100	72	Alexa488	1:100	500
CD45RO	Biolegend	304202	UCHL1	61	Alexa488	1:100	500
Ki-67	BD Biosciences	556003	B56	6	Alexa488	1:50	500
CD1a	Novus Biologicals	NBP2-34698	O10 + C1A/711	43	Alexa488	1:100	500
ICOS	Cell Signaling Technology	custom	D1K2T	74	Alexa488	1:50	500
Tim3	R&D Systems	AF2365	Polyclonal	44	Alexa488	1:25	500
CD31	Novus Biologicals	NBP2-47785-0.1mg	31.3 + C31.7 + C31.1	68	Alexa488	1:200	500
CD138	Thermo Fisher Scientific	MA1-10091	B-A38	76	Alexa488	1:50	500
EGFR	CellSignaling	26038SF	D38B1	58	Alexa488	1:50	500
DRAQ5	Thermo Fisher Scientific	65-0880-96	N/A	N/A	-	1:100	117
Hoechst 33342	Thermo Fisher Scientific	62249	N/A	N/A	-	1:2000	6.667

Supplementary Table 3. Publicly available DNA methylation array data used in this study

AUTHOR	PMID	SAMPLES	ARRAY	n
Kulis	26053498	benign_B	450K	35
Oakes	26780610	benign_B	450K	18
Lee	23074194	benign_B	450K	22
ICGC	26437030	benign_B	450K	17
Touzart	34039737	benign_T	EPIC	12
Hassler	27705804	benign_T	450K	5
Bergmann	30337361	benign_T	450K	20
Rodriguez	25539926	benign_T	450K	11
Wurster	34638496	Cell_lines	450K	3
Iorio	27397505	Cell_lines	450K	1
This study		Cell_lines	EPIC	4