

Supplemental materials and methods

CTCL cell lines

MyLa (MF-derived cell line), H9 (SS-derived cell line), HH (MF-derived cell line), Mac1 and Mac2a (CD30+ T-cell lymphoma-derived cell lines), and HuT78 (SS-derived cell line) were cultured in RPMI-1640 or Dulbecco's medium (IMDM) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. All cell lines utilized for the experiments herein were maintained at 37°C, 5% CO₂ and were tested negative for mycoplasma contamination. All media and supplements were obtained from Thermo Fisher Scientific (Irwindale, CA).

Nucleofection

The 4D-Nucleofector device (Lonza, Cologne, Germany) and the SF Cell Line 4D-Nucleofector™ X Kit L (Lonza) was used for the nucleofection of the MyLa and HuT78 cells. 4 or 6 × 10⁶ cells were mixed with the following miR inhibitors or scr: hsa-miR-155-5p miRCURY LNA miR inhibitor (Cat# YI04101510-DDA, Qiagen), hsa-miR-130b-3p miRCURY LNA miR inhibitor (Cat# YI04100279-DDA, Qiagen), hsa-miR-21-3p miRCURY LNA miR inhibitor (Cat# YI04100689-DDA, Qiagen), and miRCURY LNA miR inhibitor control (Cat# YI00199006-DDA, Qiagen) and 100 μl nucleofector solution and nucleofected according to manufacturer's instructions with the recommended programs DJ100 and DF110.

RNA extraction

Total RNA, which contains miR and mRNA, were extracted from 2-4 μ m whole skin sections of patch, plaques and tumor lesions or CTCL cell lines using a commercially available kit (RNeasy FFPE Kit, Qiagen) according to the manufacturer's protocol. RNA concentrations were measured at 260/280 nm. H&E staining with immunophenotyping was used for diagnosis and pathology staging before RNA extraction.

Single cell RNA-seq and analysis

CTCL lesional skin crawl out cells were counted and loaded onto the Chromium Controller (10x Genomics) targeting 2000-5000 cells per lane. The Chromium v2 single cell 3' RNA-seq reagent kit (10x Genomics) was used to process samples into single cell RNA-seq libraries according to the manufacturer's protocol. Libraries were sequenced with a HiSeq 2500 instrument (Illumina) with a depth of 50k-100k reads per cell. Raw sequencing data were processed using the 10x Genomics Cell Ranger pipeline (version 2.0) to generate FASTQ files and aligned to mm10 genome to gene expression count. The subsequent data analysis was performed using the Seurat package and R scripts. Normalized and scaled data were clustered using the top principal components of HVGs. The t-SNE algorithm was used to visualize the resulting clusters. Cluster-specific markers were identified to generate heatmap and feature plots in the identified cell clusters. Enrichment scores of cells in each cluster were averaged and used to generate hierarchical clustering diagram using Cluster v and Java TreeView.

***In situ* hybridization (ISH) assay**

The expression of miR-155, miR-130, and miR-21 *in situ* was determined using miRCURY LNA miRNA ISH optimization kit (Product# 90009, Exiqon). Briefly, slides were covered by

hybridization mix with 40 nM hsa-miR-155-5p, hsa-miR-130b-3p, and hsa-miR-21-3p miRCURY LNA miRNA detection probes (3' and 5' digoxigenin labeled) and scramble LNA negative control probe placed in an oven overnight at hybridization temperature. After hybridization, slides were rinsed and incubated with blocking solution for 15 min at RT. Then, slides were incubated with anti-DIG reagent for 60 min at RT. For detection, anti-digoxigenin-conjugated alkaline phosphatase (AP) Fab fragments were added and incubated for 120 min at 30°C in a humidified chamber. Slides were mounted following the stop reaction and washing. The pictures were taken by EVOS® FL Auto Imaging System (Thermo Fisher Scientific, Irwindale, CA).

Quantitative real-time reverse transcription-PCR gene expression analysis

Gene expression was tested via RT-PCR in CTCL cell lines and patient skin samples. Primer pair sequences for tested genes are miR-155 (YP00204308, Qiagen, GmbH), miR-130b (YP00204317, Qiagen, GmbH), miR-21 (YP00204230, Qiagen, GmbH), and U6 (YP00203907, Qiagen, GmbH). RT-PCR was performed utilizing the TaqMan Fast Advanced Master Mix (Cat# 4444964, Thermo Fisher Scientific, Waltham, MA) by CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) as previously described (Park et al., 2016). Target-miRs expression levels were normalized to the expression of U6 gene to obtain relative quantification of the expression. The normalized data were then transformed with \log_2 using an offset of 1. Averages of the transformed values from each sample were then used to compare the relative gene expression with the healthy control.

Cell viability

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-triazolium bromide (MTT) assay (ab211091, Abcam, Cambridge, MA). Briefly, transfected MyLa or HuT78 cells (5×10^3 cells/well) were seeded into wells of a 96-well flat-bottomed plate, and 50 μ l of MTT reagent was added. After 3 h of incubation, the medium was removed and 150 μ l MTT solvent was added. The plates were centrifuged at $1,000 \times g$, and the absorbance was quantified at 490 nm in a Cytation™ 5 Cell Imaging Multi-Mode Reader (Copyright BioTek Instruments, Inc., Highland Park, VT). Each MTT assay was performed in triplicate.

Western blot assays

Control and treated MyLa or HuT78 cell extracts were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a nitrocellulose membrane, and subjected to immunoblot with the following primary antibodies: LAG3 (Cat# 80282S; Cell Signaling), PD-L1 (Cat# 13684S; Cell Signaling), SOCS1 (Cat# 3950; Cell Signaling), SOCS2 (Cat# 2779S; Cell Signaling), SOCS3 (Cat# 52113; Cell Signaling), SOCS4 (Cat# PA5-21599; Thermo Fisher Scientific), SOCS6 (Cat# VF3004903, Invitrogen), pSTAT-3 (Cat# 9131S, Cell Signaling), STAT-3 (Cat# 4904S, Cell Signaling), PTEN (Cat# 9188S, Cell Signaling), pNF- κ B (Cat# S468, Cell Signaling), NF- κ B (Cat# L8F6, Cell Signaling), and GAPDH (Cat# 5174S, Cell Signaling). The secondary antibodies were goat anti-rabbit IgG-HRP (Cat#7074, Cell Signaling) or horse anti-mouse IgG-HRP (Cat#7076, Cell Signaling) and visualized by the enhanced chemiluminescence method (Cat#32209, Pierce ECL Western Blotting Substrate, Thermo Fisher Scientific).

Multiplex immunofluorescence analysis

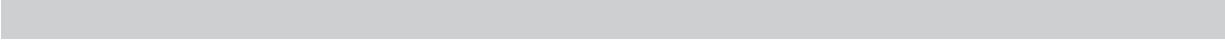
FFPE sections of MF/SS were subjected to CD3 (1:1000, Poly; Agilent), CD4 (1:300, SP35; Cell Marque), PD-1 (1:3000, UM800091 Origene) and PD-L1 for multiplex IF staining as previously described (Querfeld et al., 2018). Images were acquired with a Vectra 3 microscope and different channels were separated with Inform® Image/Cell Analysis software (ver 2.3).

⁵¹Chromium-release assay

The anti-miRs or scramble control transfected CTCL cell lines were labeled with ⁵¹Cr for one hour at 37°C. Labeled cells were washed 3 times with PBS or re-suspended in complete RPMI medium and plated in 96-well round bottom plates. Effector T cells were added in 100:1,50:1 and 25:1 effector-to-target (E: T) ratios and plates incubated at 37°C for 4 hours. Supernatants were collected after 12h, 24h and the released ⁵¹Cr was measured as counts per minute (cpm) with a MicroBeta² microplate radiometric counter (Perkin Elmer, Waltham, MA). Target cells incubated in complete media alone were subject to spontaneous release controls and target cells in complete media with 1% SDS media were subject to maximal ⁵¹Cr release controls. The cell lysis percentages were calculated using the standard formula: $100 \times (\text{cpm experimental release} - \text{cpm spontaneous release}) / (\text{cpm maximal release} - \text{cpm spontaneous release})$.

CD107a/IFN- γ analysis

Assays for analysis of cell surface-mobilized CD107a and intracellular IFN- γ were described previously (Betts et al., 2003, Rubio et al., 2003). Briefly, CD8⁺ T cells were isolated from pooled peripheral blood lymphocytes from healthy human donors and co-cultured with anti-miRs or scramble control transfected CTCL cell lines at 50:1 effector-to-target (E: T) ratio for 48h at 37°C. After co-culture CD8⁺ T cells were labeled with FITC anti-human CD107a Ab for 4h at 37°C and

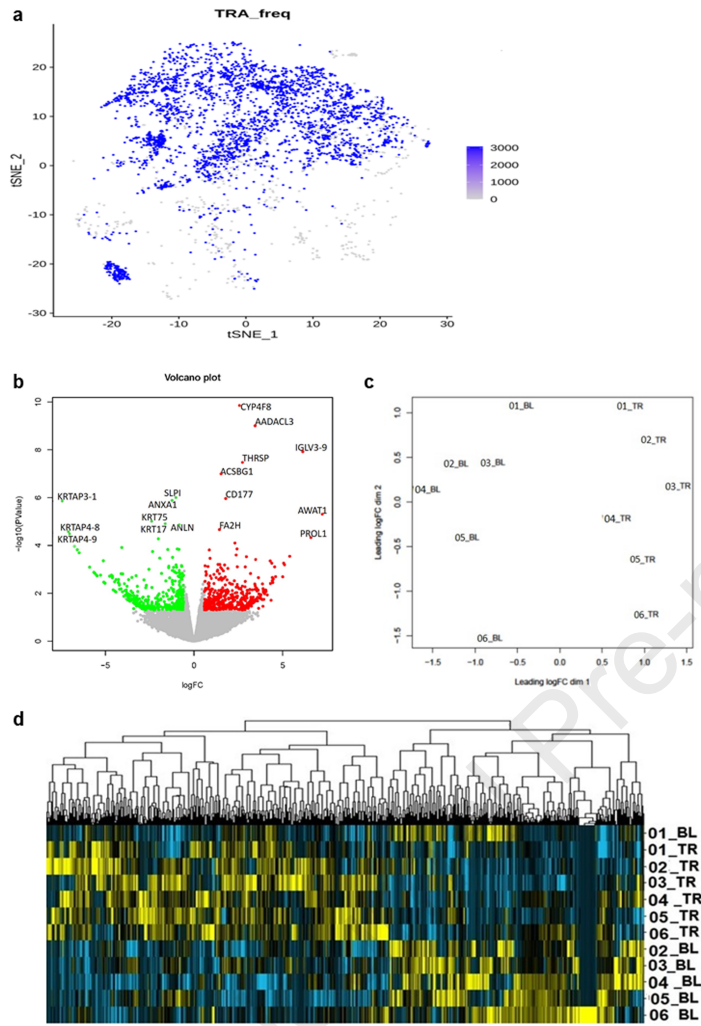


fixed and permeabilized using fixation /permeabilization kit for 20 min at room temperature.

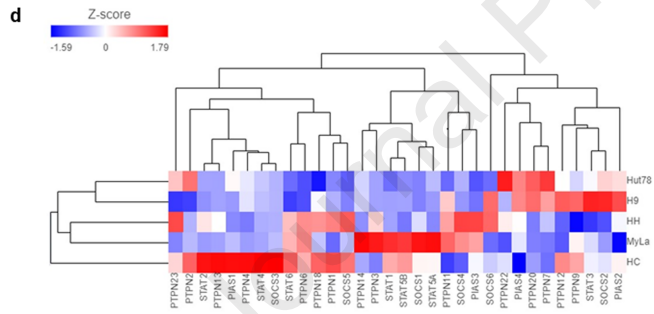
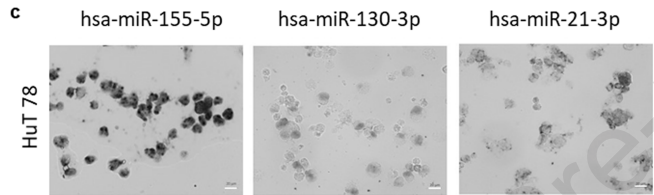
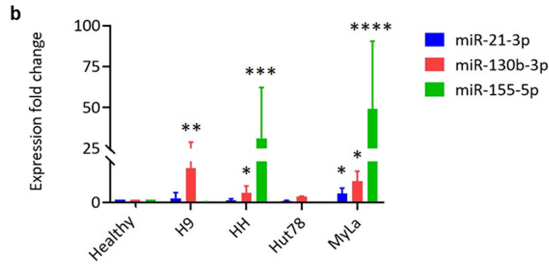
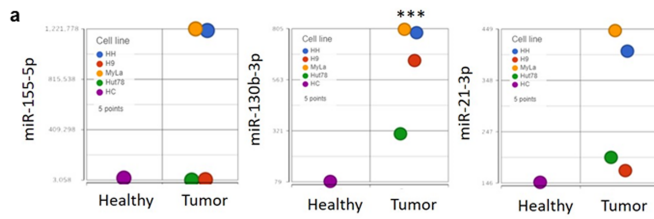
Intracellular staining for IFN- γ was performed according to the manufacturer instructions.

Journal Pre-proof

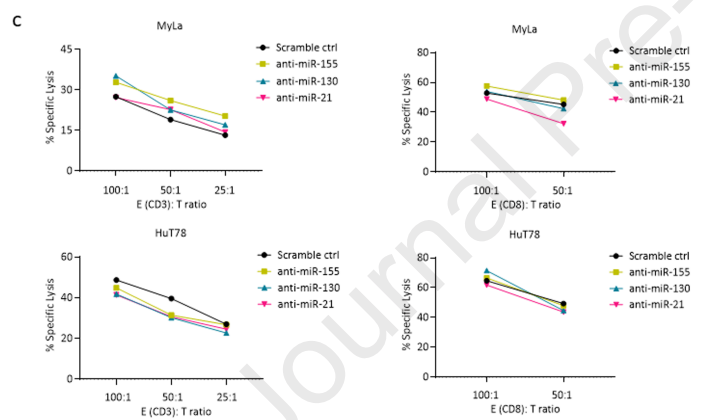
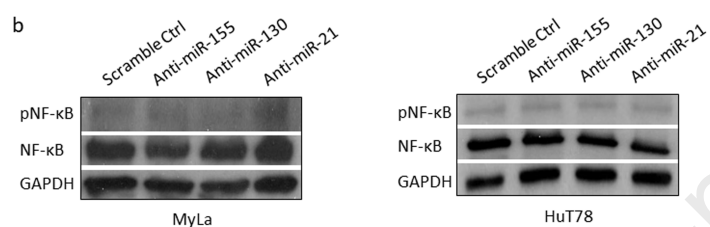
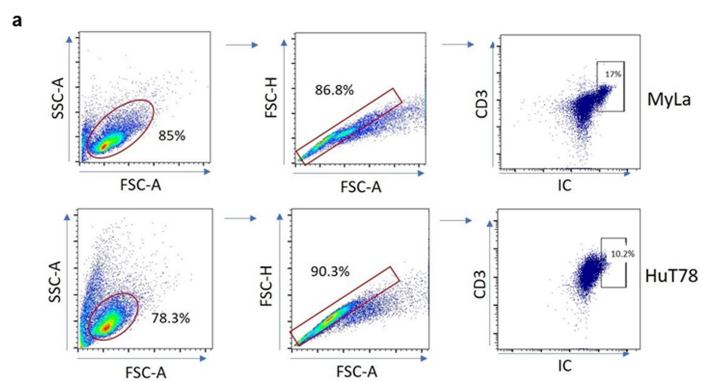
Supplemental figures



Supplemental Fig 1.



Supplemental Fig 2.



Supplemental Fig 3.