Supplementary Methods

Patient samples and cell lines

Consecutive patients with PTCL who were evaluated at the Mayo Clinic, Rochester, MN (between 1994 and 2009) and the University of Michigan Comprehensive Cancer Center, Ann Arbor, MI (between 1985 and 2011) were considered for study participation. Diagnostic biopsy specimens were reviewed by an expert hematopathologist (A.L.F., N.G.B., or M.S.L.) and classified according to the 2008 World Health Organization criteria. Diagnostic criteria for a defined PTCL subset (e.g. AITL, ALCL) were absent in cases classified as PTCL, NOS. These cases, while morphologically heterogenous, were generally characterized by effacement of the nodal architecture by a diffuse or paracortical expansion of medium to large T-cells exhibiting both morphologic atypia and phenotypic aberrancies. Clinical characteristics, including treatment outcomes, were available for a subset (n=66) of PTCL, NOS cases. Additional specimens for which clinical data was not uniformly available, representing CTCL and specific PTCL subsets, were utilized for gene-expression profiling (by Nanostring technology), double immunofluorescent staining, and for analysis of GATA-3 expression by immunohistochemistry, as indicated. Study approval was granted by the Institutional Review Board of each respective institution, in accordance with U.S. federal regulations and the Declaration of Helsinki. The patient-derived cutaneous and peripheral T-cell lymphoma lines utilized in this study are described in supplementary Table 1. The immunophenotype of all cell lines was verified, and *Mycoplasma* surveillance was performed to ensure that all cell lines utilized in this study were uncontaminated (data not shown). Peripheral blood mononuclear cells (PBMC) were obtained from healthy

donors and separated by density centrifugation (Ficoll-Hypaque, GE Healthcare), as previously described.[1]

Lentivirus Production and Transduction

Lentivirus packaging vectors psPAX2 (35 µg), and pC1-VSVG (35 µg) were co-transfected with 70 µg of pLKO.1-puro shRNA proviral plasmid (Sigma; supplementary Table 2) using standard PEI precipitation methods. PEI precipitation was performed by incubating the plasmids with 420 µg PEI (molecular weight 2500, Polysciences, Inc) in 10 mls Optimum (Life technologies) at room temp for 20 min, before adding to fresh 90 mls DMEM, 10% FBS media. This DNA/PEI containing media was then distributed equally to 5-T150 flasks (Falcon) containing 293T cells. Supernatants were collected and pooled after 72 hrs, filtered on a 0.45 micron HV-Durapore Stericup (Millipore), pelleted by centrifugation at 13,000 rpms on a Beckman Avanti J-E centrifuge at 4 ^{o}C for 4 hrs, and resuspended at 50X the original concentration (~5×10^{7} TU/ml) in RPMI1640 (Life technologies). The lentivirus was stored in aliquots at -80 ^{o}C. Virus transductions were performed on cell lines which were seeded on 6 well plates (~1×10^{6} cells/well) one day prior to lentiviral transduction. Cell media was changed to 1.2 ml of fresh complete media, then 0.12 ml of 50x virus and 8 µg/ml Polybrene (Sigma) were added and centrifuged at 2,500 rpm for 30 minutes at 37 ^{o}C in a Eppendorf 5810R tabletop centrifuge with a swinging bucket rotor with platform adaptor. The cells were then incubated at 37 ^{o}C, 5% CO_2 for 72 hrs prior to the start of selection with 0.5 µg/ml puromycin. After selection, the cells were expanded on a T-75 flask.

Generation of monocyte-derived macrophages and functional assays

Monocytes and T cells were positively selected from fresh PBMC using CD14 and CD3 magnetic beads, respectively (Miltenyi Biotec), as previously described.[1] Cells were cultured in RPMI 1640 supplemented with 10% FBS, 1 mM L-glutamine, and 100 µg/mL of penicillin and streptomycin at 37 ^{o}C in a humidified atmosphere with 5% CO_2. Monocytes were plated at a density of $1x10^6$/mL in flat-bottom 96 or 24-well plates in media supplemented with M-CSF (40 ng/mL) and either T-cell lymphoma conditioned media (50% v/v) or polarizing recombinant human cytokines (20 ng/mL of interferon-γ, IL-4/IL-13, or IL-10; Miltenyi Biotec). To generate T-cell lymphoma conditioned media, cell lines ($7x10^5$/well of a 6-well plate) were cultured in fresh media for 18-24 hours. Cell-free supernatants were generated by centrifugation and filtration (0.2 µm pore size) prior to use. Monocyte-derived macrophages (MDM) were utilized for immunophenotyping or functional assays after 4-5 days of culture. In some experiments, an appropriate isotype control or functional grade anti-IL-10 monoclonal antibody (1 µg/mL; clone JES3-9D7; Affymetrix eBioscience) was included. Ruxolitinib (a generous gift from M. Talpaz and N. Donato) or vehicle (DMSO) control was added to MDM cultures, where indicated. The following antibodies (and their respective isotype controls) were utilized: HLA-DR (clone L243; BioLegend), CD16a/b (clone 245536; R&D Systems), CD163 (clone GHI/61; BioLegend), pSTAT3-Y705 (4/P-STAT3; BD Biosciences). Prior to calculating the change in mean fluorescent intensity (ΔMFI) between treatment groups, the MFI observed with an appropriate isotype control was subtracted from that observed with each antigen specific antibody. For determination of macrophage-derived IL-10, adherent MDM generated (in triplicate) in 96-well plates were gently washed with fresh media. Lipopolysaccharide (100 ng/mL; Sigma-Aldrich)

was added in 200 µL volume of fresh media and cell-free supernatants were harvested after 24 hour incubation. For use as stimulators in an allogeneic mixed lymphocyte reaction, an equal number of CFSE labeled allogeneic $CD3^+$ T cells were added to washed MDM and T-cell proliferation determined by CFSE dilution, as previously described.[1]

Nanostring ncounter® Technology

The mRNA level of relevant transcripts in TCL specimens was quantified by Nanostring ncounter® Technology.[2] Total RNAs were extracted from two 20-µm sections of FFPE nodal tumors (60-90% tumor content) using RNeasy FFPE kit (Qiagen). The extracted total RNAs were measured using a Nanodrop 1000 instrument (Nanodrop) and RNA integrity was assessed using an Agilent 2100 bioanalyzer. The majority of the RNA were in 200-4000 bp, which is suitable for Nanostring ncounter® Technology. Sequence-specific probes (a 50 nt capture probe and a 50 nt reporter probe complementary to a region of 100 nt in a target gene) are constructed for genes of interest and four reference genes (supplementary Table 3). The extracted RNAs (300 ng each) were subjected to hybridization with excess amount of sets of 3'- biotinylated capture probes and 5' fluorescent barcode-tagged reporter probes at 65 ^oC for 16-20 hours. The target mRNA-probe complexes were then captured by a streptavidin coated cartridge and excess probes were washed away. The cartridge with captured mRNA-probe complexes were then imaged and counted by nCounter Digital Analyzer and analyzed by nSolver software. Negative control (no RNA) was used to correct for background. Positive RNA control and reference gene transcripts were used to correct for difference in hybridization efficiency/post hybridization processing and RNA content

using the ratio of the geometric means of individual samples to all the samples. With the normalized RNA counts of the TCL cases as input, unsupervised clustering both on the samples and the transcripts were then performed by cluster 3.0 using the centroid linkage, centered correlation metric and set on median. The gene expression heatmap with log_2 transformation were then generated by Javatree.

Quantitative RT-PCR

RNA was extracted using RNeasy Mini Kit (Qiagen) and converted to cDNA in a 20 µl reaction by reverse transcription using Quanti^{Tect} reverse transcription kit (Qiagen) in which both polyT and random primers were used. The cDNAs were then quantified by SYBR green-based qPCR on CFX96^{TM} Real-time system (BIO-RAD). Briefly,10 ng cDNA, 1x SYBR Green PCR master mix (A&B Applied Biosystems), and 0.3 µM forward/reverse primers were mixed in 20 µl volume. The cDNAs were replaced with H_2O in a no template control and with no reverse transcriptase (RT) mixture in the no RT control. Four replicates of each sample, along with “no template” and “no RT” controls were included in each experiment. PCR mixtures were loaded on a white 96-well plate (BIO-RAD) and subjected to the following thermocycle on a C1000^{TM} thermal cycler (BIO-RAD): 1 cycle of 95 ^{o}C, 10’; 45 cycles of 95 ^{o}C 15”, 60 or 55 ^{o}C 1’; melting curve generation (65-90 ^{o}C, 0.5 ^{o}C increment). The annealing/extension temperature of 55 ^{o}C was used for gene IL4 and 60 ^{o}C for the remaining transcripts. The quantification cycle (Cq) was determined by CFX Manager using default instrument setting (single threshold, Baseline substrated curve fit). Samples with Cq > 40 or a Cq difference less than 5 between a cDNA and its controls were defined as undetectable. The reference gene GAPDH was used for mRNA quantification in H9, SUDHL-1 and

SR-786 cell lines, PPIA for HH cell line and GUSB for MyLa cell lines. The reference genes were chosen based on their invariability (<15% changes between the comparison group: cell stably transfected with scrambled shRNA vs shRNA 19301) (data not shown). Relative gene expression was quantified by the $2^{-\Delta\Delta Cq}$ method. The primers used are summarized in supplementary Table 4.

Western blot

Western blot was used to determine GATA3 protein expression. Briefly, $1x10^6$ cells in log-phase growth were pelleted and suspended with 100 µl of ice-cold RIPA buffer (BBP, inc.) containing protease inhibitors (Halt TM, Thermo Scientific). Subcellular fractionation was performed using the NE-PERTM nuclear and cytoplasmic extraction kit (Thermo Scientific) according to the manufacturer's instructions with minor modifications. Protein samples were then resolved on a 10% SDS-polyacrylamide gel with Tris running buffer by electrophoresis, blotted to PVDF membrane and sequentially probed with primary antibodies against GATA3 (clone D13C9, Cell Signaling). A horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was then added, and detected through autoradiography using enhanced chemiluminescence (ECL Plus, Thermo Scientific). GAPDH (cloneD16H11, Cell Signaling) and Histone-H3 (clone 3H1, Cell Signaling) were used as protein loading controls for cytoplasmic and nuclear fractions, respectively.

Cytokine production

For cytokine determination by intracellular flow cytometry, $2x\ 10^5$ cells were fixed and permeabilized according to the manufacturer's recommendations (BD Cytofix/Cytoperm^{TM}) using a PE-conjugated IL-10 monoclonal antibody (or isotype

control; BD Biosciences). All flow cytometric analysis was performed on a Cyan 6 flow cytometer (Coulter). For IL-10 quantification in supernatants obtained from monocyte-derived macrophages, a cytometric bead array was used (BD Biosciences), according to the manufacturer's recommendations. Briefly, supernatants were mixed with capture beads, then detection reagents. After washing, samples were analyzed on a FACS Calibur using FCAP-Array software. Cytokine quantification in the cell lines indicated was performed by ELISA, according to the manufacturer's recommendations (eBioscience).

Immunohistochemistry

Paraffin tissue microarrays (TMA) were constructed from biopsy specimens and immunohistochemical staining performed, as previously described.[1,3,4] Briefly, immunohistochemical staining was performed on the DAKO Autostainer (DAKO, Carpinteria, CA) using DAKO Envision+ and diaminobenzadine (DAB) as the chromogen. Serial sections of de-paraffinized TMA sections were incubated with anti-GATA3 antibody (Santa Cruz Biotechnology, clone HG3-31 or HG3-35, 1:50) overnight at 4^{o}C, after peroxidase and protein blocking. Microwave epitope retrieval in 10 mM Tris/HCl pH9 containing 1 mM EDTA was used. Appropriate negative (no primary antibody or Karpas 299 xenografts) and positive (tonsil, H9 or MyLa xenografts, or breast adenocarcinoma) controls were stained in parallel with each TMA examined (supplementary Figure 4). In cases with insufficient tissue, whole-tissue sections were stained. The intensity of GATA-3 staining, and not the absolute number of stained tumor cells, was directly proportional to GATA-3 expression levels in T-cell lymphoma xenografts (supplementary Figure 4). Furthermore, among specimens scored as GATA-

3^+, staining was observed in the majority of tumor cells, while differences in staining intensity were noted (representative examples are shown in Figure 3). Therefore, an ROC analysis to determine the optimal cut-off for GATA-3 expression was not performed. Furthermore, the optimal cut-off for established immunohistochemical stains is highly variable. For example, the cut-offs used to define CD30 expression in PTCL range from 10-80%.[5-8] However, a 20-30% cut-off was initially entertained, as this approximates the cut-off frequently used for CTCL/PTCL-relevant markers. GATA-3 expression exceeded this cut off in the majority (85%) of cases examined. However, GATA-3 expression was observed in 10-20% of tumor cells in 5 cases. Therefore, biopsy specimens were scored by a hematopathologist (A.L.F., N.G.B., M.S.L) as GATA-3 positive if nuclear staining above background was observed in more than 10% of tumor cells. Slides were viewed with an Olympus BX51 microscope and pictures taken with an Olympus DP71 camera. Olympus BSW with DP Controller software was used for image acquisition and storage.

Double Fluorescent Staining

After deparaffinization and rehydration, slides were subjected to microwave epitope retrieval in 10 mM Tris/HCl, pH9 containing 1 mM EDTA. After rinsing several times in 10 mM Tris HCL buffer, pH 8 containing 0.154 M NaCl and 0.05% Tween-20 (TBST), non-specific binding of the antibodies was extinguished by a 30 min incubation with 'Background Sniper" (BioCare Medical, Concord, CA). Staining was performed concurrently with antibodies to pSTAT3 (Y705) (clone EPR2147Y, rabbit monoclonal antibody, 1:200, Epitomics, Burlingame, CA, AB76315,) and CD163 (mouse monoclonal antibody, clone 10D6, 1:100, Leica, Buffalo Grove IL,) at 4^oC overnight. The slides are

then washed two times with TBST for 5 minutes and incubated with a combination of goat anti mouse IgG conjugated to AF488 (Molecular probes, Carpinteria, CA, A11001, 1:200) and goat anti rabbit IgG conjugated to AF555 (Molecular probes, A31630, 1:200) for 60 minutes at room temperature in a dark humidity tray. The slides are washed with 3 changes of TBS and stained with the DNA staining dye 4',6-diaminodo-2-phenylindole (DAPI) in a non-fading mounting media (ProLong Gold, Molecular probes). The slides are allowed to dry overnight in a dark dry chamber and the edges are sealed. Immunofluorescence images were acquired using an Olympus microscope at 200x and merged to form a composite image.

Statistical Analysis

All clinical data were analyzed using JMP 8 software (SAS, Cary, NC). Progression free and overall survival were estimated using the Kaplan-Meier method and two-tailed log-rank test.[9] Progression-free survival (PFS) was calculated from the date of diagnosis to the date of disease progression, relapse, or death from any cause. Overall survival (OS) was calculated from the date of diagnosis to the date of death. Patients who did not experience any of these events were censored at the date of last follow-up. GATA-3 expression was dichotomized and the Cox proportional hazards model used to evaluate its ability to predict OS/PFS and to adjust for the Prognostic Index for PTCL, NOS (PIT).[10,11] Comparisons among groups were evaluated using a Student t-test and all p-values <0.05 were determined to be statistically significant.

References:

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Supplementary Tables

Supplementary Table 1. Cutaneous and peripheral T-cell lymphoma lines.

Cell Line	Histology	Source	Growth Medium
H9	CTCL (Sezary Syndrome)	ATCC	RPMI, 10% FBS
HH	CTCL or ATLL (HTLV-1-)	ATCC	RPMI, 15% FBS
MyLa2000	CTCL (Mycosis Fungoides)	Generous gift (R. Gniadecki)	RPMI, 10% FBS
SR-786	PTCL (ALCL, ALK+)	DSMZ	RPMI, 10% FBS
SU-DHL-1	PTCL (ALCL, ALK+)	DSMZ	RPMI, 10% FBS
Karpas 299	PTCL (ALCL, ALK+)	DSMZ	RPMI, 10% FBS

Supplementary Table 2. The sequence of shRNAs targeting human GATA3 genes and the scrambled control

shRNA	Sigma clone #	sequence	target region
scr	SHC016	CCGG*GCGCGATAGCGCTAATAATTT*CTCGAG*AAATTATTAGCGCTATCGCGC*TTTTT	none
19299	TRCN0000019299	CCGG*GCCAAGAAGTTTAAGGAATAT*CTCGAG*ATATTCCTTAAACTTCTTGGC*TTTTT	3' UTR
19301	TRCN0000019301	CCGG*CATCCAGACCAGAAACCGAAA*CTCGAGT*TTCGGTTTCTGGTCTGGATG*TTTTT	CDS
273991	TRCN0000273991	CCGG*AGCCTAAACGCGATGGATATA*CTCGAG*TATATCCATCGCGTTTAGGCT*TTTTT	3' UTR

Supplementary Table 3. Probe-sets for Nanostring nCounter® technology

Gene	Accession	Target Region	PN(CP;RP)	NSID	Comments
CCR4	NM_005508.4	35-135	302236;202236	NM_005508.4:35	
CD163	NM_004244.4	1630-1730	309101;209101	NM_004244.4:1630	
CXCR3	NM_001504.1	80-180	303037;203037	NM_001504.1:80	
GAPDH	NM_002046.3	972-1072	335205;235205	NM_002046.3:972	referece gene
GATA3_exo1a	NM_001002295_exo1a.1	56-156	393605;293605	NM_001002295_exo1a.1:56	target Exon 1a
GATA3_exo1b	NM_001002295_exo1b.1	108-208	393606;293606	NM_001002295_exo1b.1:108	target Exon 1b
GUSB	NM_000181.1	1350-1450	300976;200976	NM_000181.1:1350	referece gene
HPRT1	NM_000194.1	240-340	301669;201669	NM_000194.1:240	referece gene
IFNG	NM_000619.2	970-1070	301672;201672	NM_000619.2:970	
IL10	NM_000572.2	230-330	301231;201231	NM_000572.2:230	
IL13	NM_002188.2	516-616	315650;215650	NM_002188.2:516	
IL4	NM_000589.2	625-725	301673;201673	NM_000589.2:625	
IL5	NM_000879.2	105-205	302251;202251	NM_000879.2:105	
TBP	NM_001172085.1	587-687	326758;226758	NM_001172085.1:587	referece gene
T-bet	NM_013351.1	890-990	301952;201952	NM_013351.1:890	

PN: probe number; CP;RP: capture probe; reporter probe

Supplementary Table 4. Primers used in RT-qPCR

Gene (ref#)	primers		Amplicon		RTPrimer DB ID
	Forward (5'-3') (length, location)	Reverse (5'-3') (length, location)	length (nt)	location	
GATA-3 (NM_001002295.1)	GGGCTCTACTACAAGCTTCAC (21 nt, 1584-1604)	CTGGATGCCTTCCTTCTTCATA (22 nt, 1626-1647)	64	E4-E5	N/A
IL-4 (NM_000589.2)	GGGCTTGAATTCCTGTCCTGT (21 nt, 724-744)	TCGTCTTTAGCCTTTCCAAGAAGT (24 nt, 775-798)	75	E3-E4	N/A
IL-5 (NM_000879.2)	AGCTGCCTACGTGTATGCCA (20 nt, 83-102)	GCAGTGCCAAGGTCTCTTTCA (21nt, 133-153)	71	E1	N/A
IL-10 (NM_000572.2)	TCCCTGTGAAAACAAGAGCA (20 nt, 449-468)	CATGGCTTTGTAGATGCCTTT (21 nt, 513-533)	85	E4-E5	N/A
IL-13 (NM_002188.2)	TCTGCAATGGCAGCATGGTA (20 nt, 196-215)	GCATCCTCTGGGTCTTCTCG (20 nt, 293-312)	117	E2-E3	N/A
GAPDH (NM_001256799.1)	TCTGACTTCAACAGCGACAC (20 nt, 1048-1067)	TGTCATACCAGGAAATGAGCTT (22 nt, 1124-1145)	98	E9-E10	N/A
GUSB (NM_000181)	CTCATTTGGAATTTTGCCGATT (22 nt, 1828-1850)	CCGAGTGAAGATCCCCTTTTTA (22nt, 1887-1909)	81	E11	2929
PPIA (NM_021130)	GTCAACCCCACCGTGTTCTT (20 nt, 86-106)	CTGCTGTCTTTGGGACCTTGT (21 nt, 162-183)	97	E1-E2	2425

Note: E: exon; N/A: not available, assays developed in our laboratory not yet submitted to the database.

Supplementary Table 5. Prevalence of GATA-3 expression in TCL.

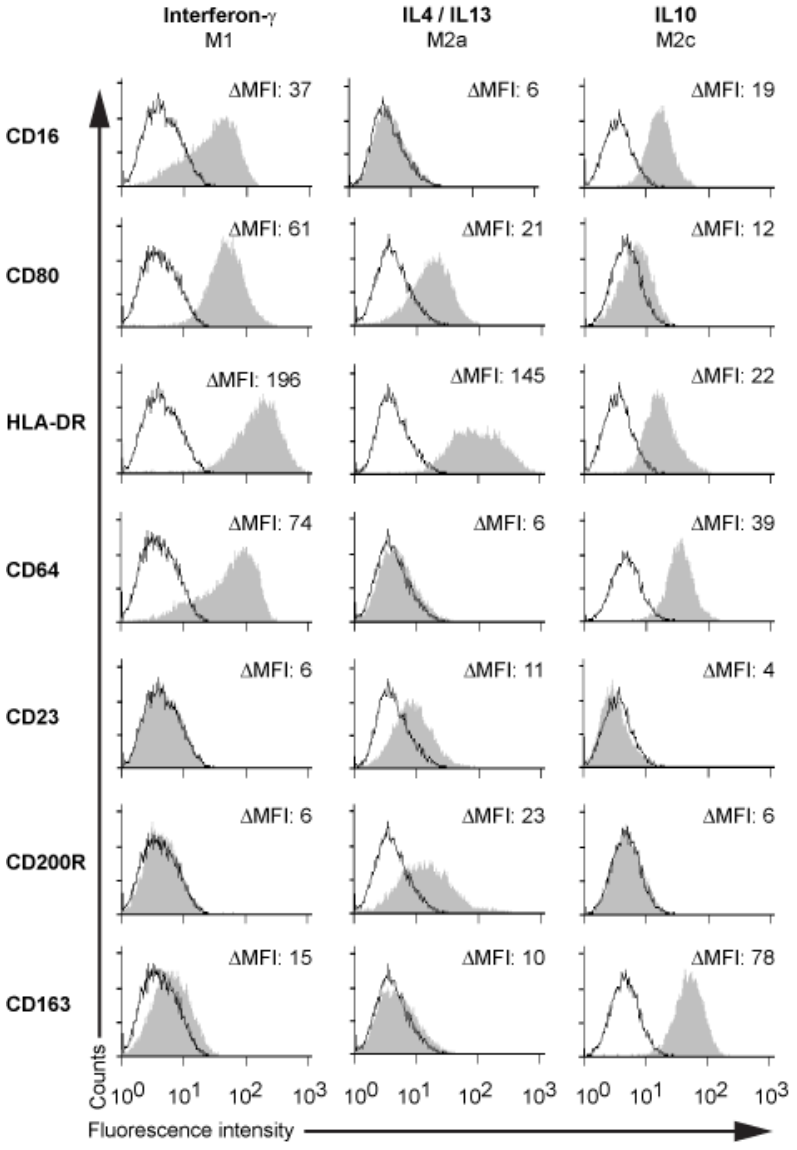
Histology	GATA-3$^+$ (%)
PTCL, NOS (n=128)	58 (45)
CTCL (n=43)	29 (67)
ALK$^-$ ALCL (n=6)	4 (67)
ALK$^+$ ALCL (n=6)	2 (33)
ALCL, ALK unknown (n=7)	1 (14)
EATL (n=6)	1 (17)
ENKTL (n=8)	0 (0)

Supplementary Table 6. Comparisons of Clinical Features by GATA-3 Expression

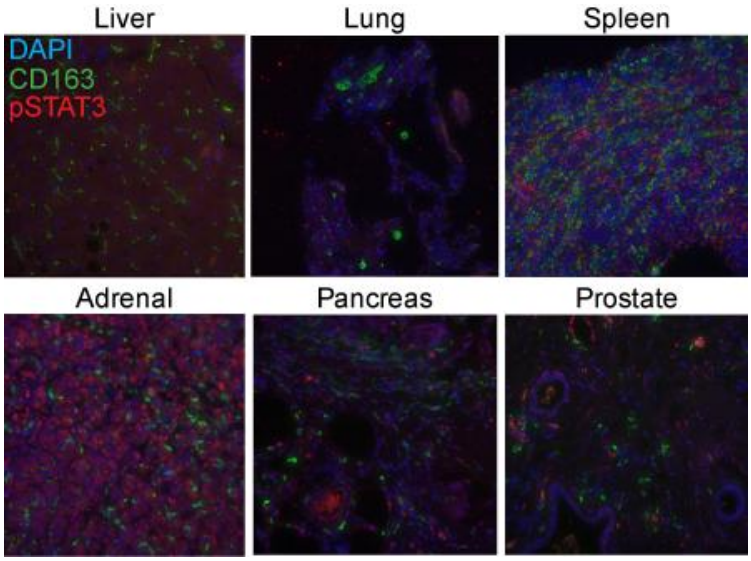
	GATA-3 Expression				
	Negative (n=32)		Positive (n=34)		
Characteristic	No.	%	No.	%	p-value
Age (years)					
Median	56		68		0.003
Range	28-85		30-90		
Stage III/IV	23	72	32	94	0.01
ECOG PS >1	13	41	15	44	0.8
LDH >normal	17	53	22	65	0.3
>1 extranodal site	9	28	16	47	0.1
Bone marrow involvement	14	44	12	35	0.5
PIT high risk (≥3 poor risk features)	12	38	16	47	0.4

Note: ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; PIT, Prognostic Index in PTCL-U. The PIT is comprised of 4 adverse prognostic factors: age >60, elevated LDH, bone marrow involvement, and stage III/IV disease.

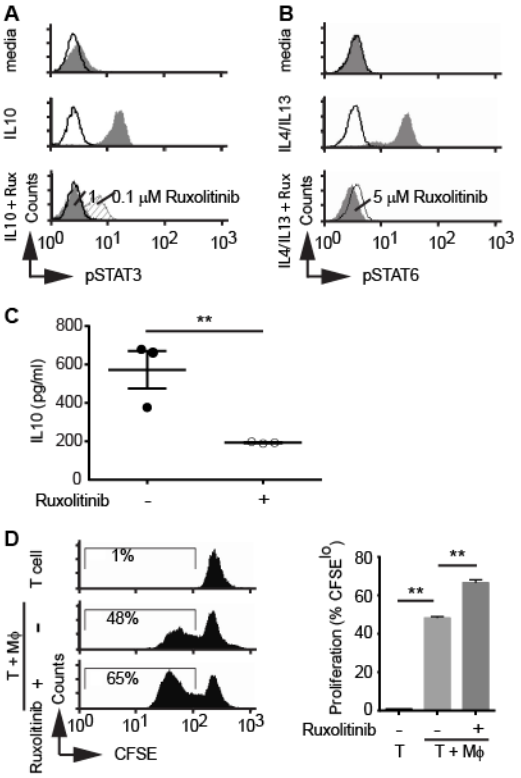
Supplementary Figures



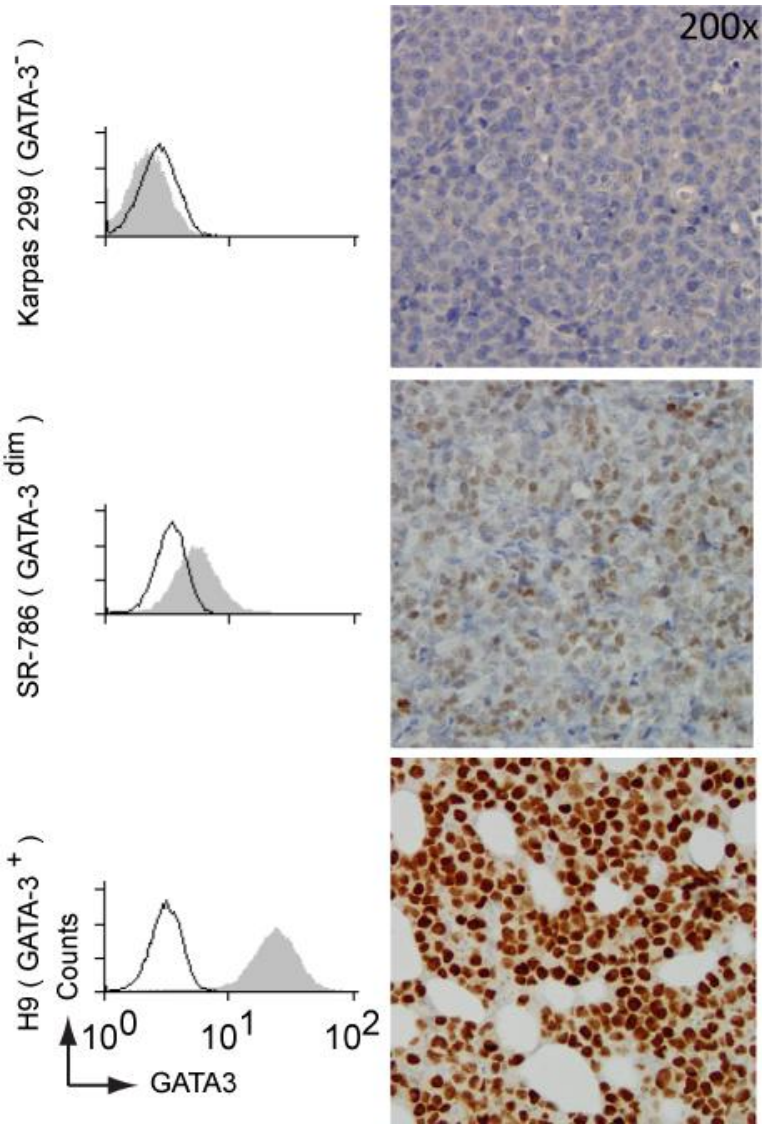
Supplementary Figure 1. Immunophenotype of IFN-γ (M1), IL-4/IL-13 (M2a), and IL-10 (M2c) polarized macrophages. Monocyte-derived macrophages were generated and polarized with the cytokines shown (20 ng/mL). Expression of the markers shown was determined by flow cytometry.



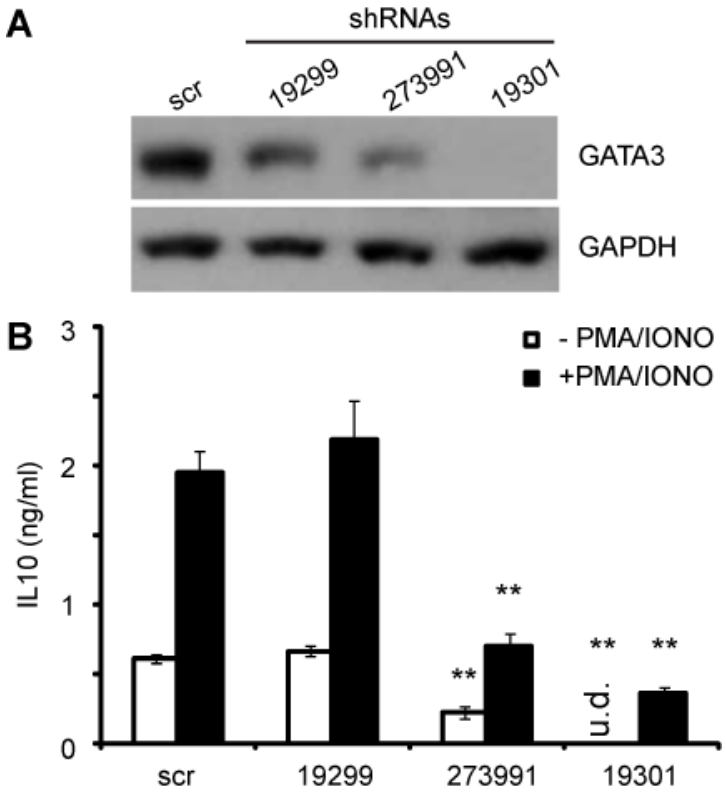
Supplementary Figure 2. The absence of pSTAT3^{+}/CD163^{+} macrophage in normal tissues. The normal tissues shown were examined for the presence of pSTAT3^{+}/CD163^{+} macrophages.



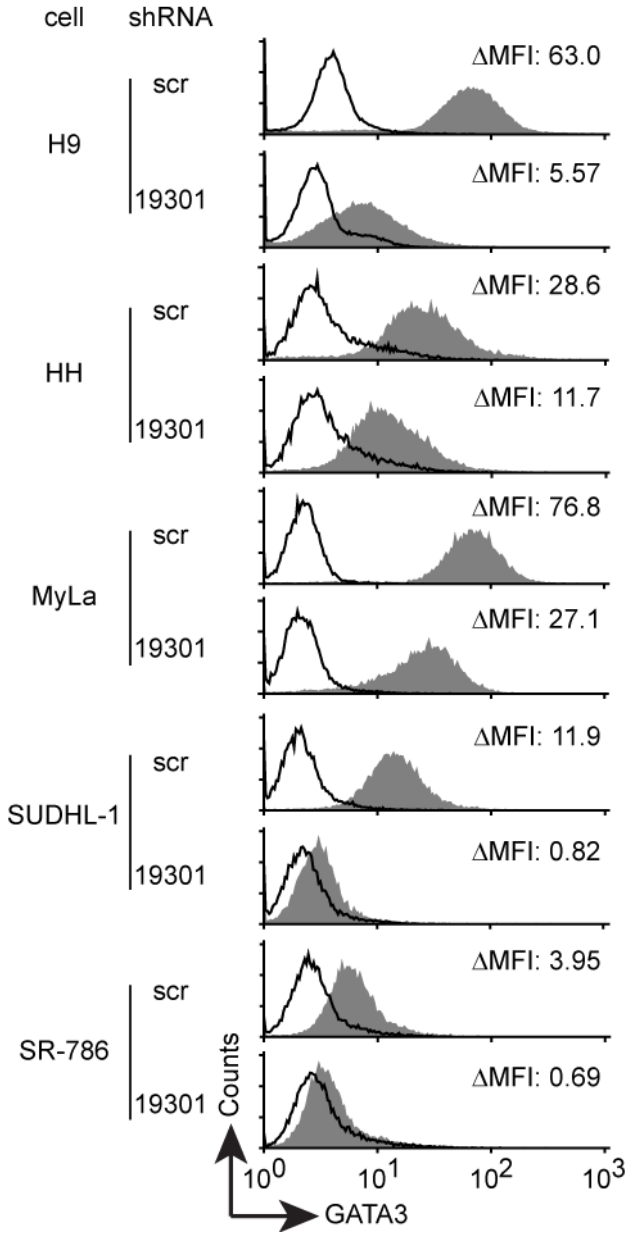
Supplementary Figure 3. Ruxolitinib inhibits STAT3 phosphorylation in IL-10 treated monocytes and alternative macrophage polarization in IL4/IL13 treated monocytes. (A) Monocytes were incubated in media alone or in media supplemented with IL-10 (20 ng/mL) (A) or IL4/IL13 (20 ng/ml) (B) in the presence of vehicle control (DMSO) or ruxolitinib (0.1, 1, or 5 μM, as indicated). STAT3 phosphorylation (Y705) (A) and STAT6 (Y641) phosphorylation (B) was determined by flow cytometry, compared with an appropriate isotype control (open histograms). Representative histograms are shown. (C, D) IL4/IL13 polarized macrophages generated in the presence or absence of ruxolitinib were washed with fresh media. Media supplemented with LPS (100 ng/mL) was added, and cell-free supernatants collected 24 hours later for determination of IL-10 production by enzyme-linked immunosorbent assay. The mean (+/- standard error) from 3 individual normal donors is shown (C). In parallel, CFSE labeled T cells were added in triplicate and T-cell proliferation determined by CFSE dilution (D). **: $p<0.01$ in unpaired two-sided t-test.



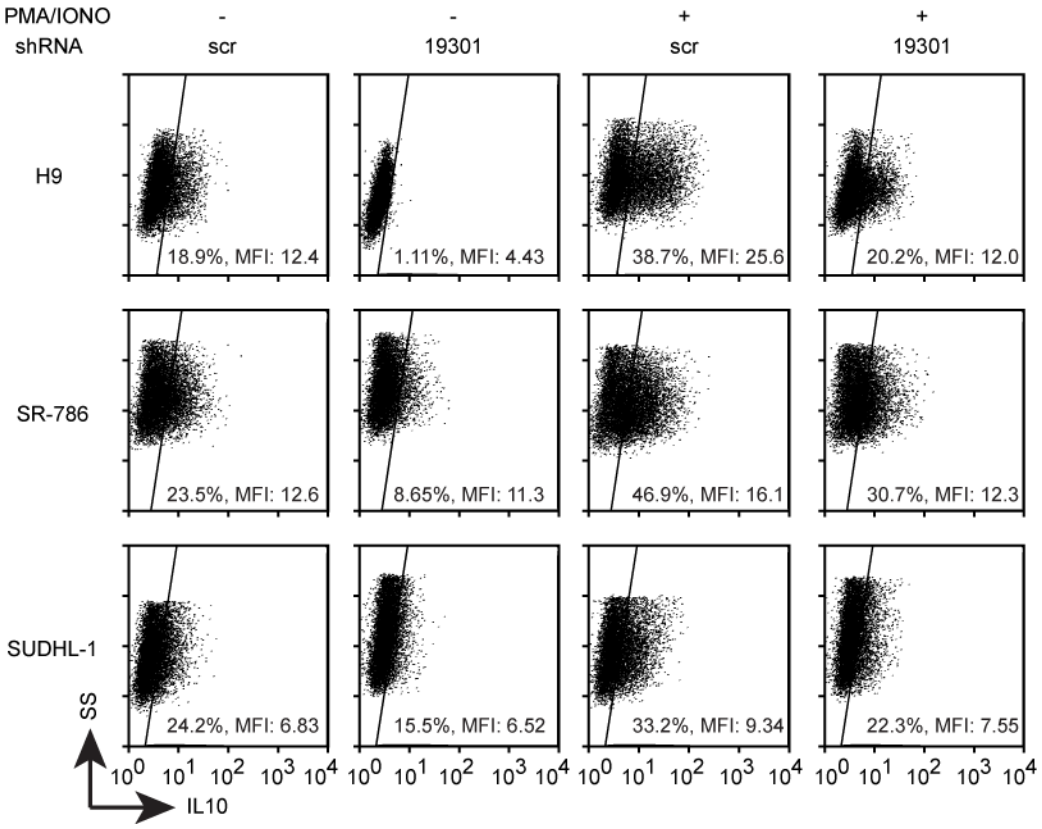
Supplementary Figure 4. GATA-3 expression in T-cell lymphoma xenografts. GATA-3 expression in the T-cell lymphoma lines shown was quantified by intracellular flow cytometry. In parallel, tumor xenografts were generated in NSG mice, and formalin-fixed, paraffin-embedded tumors generated for subsequent GATA-3 staining by immunohistochemistry.



Supplementary Figure 5. GATA-3 dependent IL-10 production in H9 cells. (A) GATA-3 expression was determined by Western blot in H9 cell lentivirally transduced with non-specific ("scramble") or with the GATA-3 targeting shRNA shown. Minimal GATA-3 knockdown was observed with 19299, whereas partial or almost complete knockdown was observed with 273991 and 19301, respectively. (B) IL-10 production was determined in unstimulated (open) or PMA/ionomycin stimulated (closed) H9 cells following transduction with the shRNA indicated. T-test was used to determine the significant difference between the groups (scr vs different shRNAs; ** $p < 0.01$).



Supplementary Figure 6. The knockdown of GATA3 expression by shRNA19301 in different T –cell lymphoma cell lines. GATA3 expression was determined by intracellular flow cytometry in T-cell lymphoma stably transfected with scrambled shRNA and GATA3-specific shRNA19301. The open histogram is isotyope control and the gray histogram is anti-GATA3 staining.



Supplementary Figure 7. IL-10 production is GATA-3 dependent. IL-10 production was determined by intracellular flow cytometry in unstimulated or PMA/ionomycin stimulated cells lentivirally transduced with the shRNA indicated.