Methods

Patient samples

Freshly frozen and formalin-fixed, and paraffin-embedded (FFPE) lymphoma and reactive tonsillar samples were obtained from the IRB-approved lymphoma biorepository at The University of Chicago. Informed written consent was obtained from each patient prior to the banking of specimens. Each sample was reviewed by an expert hematopathologist (G.V.) to confirm the accuracy of the lymphoma diagnosis.

H&E analysis and histopathological evaluation

FFPE tissue blocks (n = 18) were cut at 4 µm thickness, mounted on slides, stained for Hematoxylin and Eosin (H&E) and scanned at 40x magnification (0.5 µm per pixel) on a Vectra Polaris scanner in *.qptiff format. The slides were then reviewed by pathologists to select the regions of interest (ROI) based on tumor content. Two tissue microarray (TMA) blocks were made. Each TMA block contained three cores (2.0 mm in diameter) each from nine samples. The TMAs were sectioned at 4 µm thickness and mounted onto 22 x 22 mm square glass coverslips (Electron Microscopy Sciences, 72204-01) precoated with 0.1% poly-L-lysine (Sigma, P8920).

CODEX® (CO-Detection by indEXing) technology (Akoya Biosciences) was used for multiplex immunofluorescence marker detections on FFPE tissue sections. This method requires antibodies to be conjugated to unique DNA barcodes and the signals are revealed by fluorophore-conjugated complementary DNA barcodes (Reporters).

Antibody conjugation

Aside from the commercially available conjugates from Akoya Biosciences, additional antibody conjugates were made in-house using barcodes and conjugation kits provided by Akoya per the manufacturer's protocol. In brief, each antibody in purified, carrier-free form was treated with the Reduction Solution (conjugation kit component) to open up the thiol groups, and then incubated with a unique DNA barcode for two hours. The successful conjugations were first confirmed via a gel run showing a shifted-up molecular weight. These conjugates were then tested at different dilutions for staining on a sample tissue expressing the target antigen. The staining was reviewed and approved by pathologists. All the antibody conjugates used in this study, including the conjugates from Akoya, are listed in **Table S1**.

Tissue staining

CODEX staining and imaging were performed per Akoya Biosciences' detailed instructions. In brief, FFPE sections of 4 µm thickness mounted on coverslips were baked at 70ºC for 60 minutes, deparaffinized in two changes of Histoclear (VWR, 97060-934), hydrated in descending series of ethanol (100% x2, 95%, 75%, 50%, 30%) and finally rinsed and incubated in distilled water. The coverslips were then submerged in a glass beaker containing antigen retrieval solution ((AR9) pH 9, Sigma) and treated in a TintoRetriever pressure cooker (BioSB) preset for 120ºC for 20 minutes. Once cooled down, the coverslips were rinsed in diH_2O, followed by rinses in Codex Hydration Buffer, and then placed in Staining Solution to incubate at room temperature for 30 minutes. The coverslips were rested with tissue side up on a home-made humidified chamber; 200 uL of antibody conjugate mix consisting of Codex Staining Solution, blocking solutions, and antibody conjugates at different dilutions (see **Table S1**) were added on the tissue section and incubated for three hours at room temperature. The tissues were completely covered

with no bubbles interfering with antibody binding. After incubation, the coverslips were washed in Staining Solution twice, post-fixed with 1.6% paraformaldehyde, cold 100% methanol, and an Akoya Final Fixative with abundant washing steps in 1x PBS in between and after each step. Coverslips were then ready for imaging or short-term storage in Storage Solution at 4ºC for up to five days.

Imaging on Codex instrument

For imaging, a 96-well reporter plate was prepared containing the corresponding reporters. Each well to be used contained three reporters that correspond to the antibodies used in each cycle, plus a Dapi nuclear stain. Two wells containing only Dapi were used as the first and last cycle for correction of background, known as blanks. The plate was sealed with foil plate seal, blocked from light, and used for imaging.

One coverslip was mounted on a perfusion stage rested on a Keyence fluorescence microscope (Osaka, Japan; model BZ-X800) equipped with a 20x/0.75 objective. The solution exchanges were performed using a microfluidics instrument (Akoya Biosciences) and controlled through a software interface. Light exposure times and the order of markers per cycle are listed in **Table S1**. After completion of imaging cycles, the raw image tiles in .tiff format were fed through the Codex image processor for stitching. The completed fluorescence images were then segmented and analyzed using a BostonGene proprietary software.

MxIF image analysis: Object-based segmentation

CNN with Mask R-CNN[39] architecture (ResNet-18 as a backbone)[40] was used to perform segmentation. Model expected 3-channel image as input: first channel corresponded to nuclei

staining (DAPI), second to dominant membrane marker (CD20) and the last one to set of additional markers stacked by maximum pixel value (CD4, CD8).

MxIF image analysis: Cell typing

Cell typing was performed based on probabilities of expression. To obtain them two CNNs for two different patterns (membrane, e.g. CD20 and nuclei, e.g. Foxp3) were trained as binary classifiers in order to predict the presence or absence of a marker expression. For preselected cell types we constructed ideal marker signatures (e.g. B-cells were defined as CD20+CD3e-CD4-CD8-PD1-TIGIT-ICOS-Foxp3-CD31-SMA-) and calculated cosine similarity between these reference vectors and vectors of CNNs outputs. The final cell type labels were then assigned as the closest match.

Significant contact interactions identified by MxIF images

Significant contact interactions between cell types of interest were determined as previously described [19]. Briefly, cell phenotypes were labeled according methods listed above. Pairwise interactions between and within cell phenotypes of interest that occurred within 5 pixels of each other constituted “cellular contact” and were calculated for each single cell with its neighbors. Pairwise interactions between and within cell phenotypes were then compared to a random distribution using permutation testing. The corresponding significance testing represents the likelihood that specific cellular interactions of interest are enriched in comparison to a randomized version of the same tissue.

Community analysis of MxIF images

Community clusters were obtained based on the graph of cells centroids generated via Delaunay triangulation algorithm. Edges with length exceeding 200 pixels were excluded. To each node we assigned corresponding cell type and median length of connected edges. Using an Adversarially Regularized Variational Graph Auto-Encoder[20] GNN (from Pytorch Geometric python package) was trained to obtain embedding vectors of cell neighborhoods in an unsupervised, generative-adversarial manner. Architecture consists of two models - variational graph autoencoder and adversarial model (3 layered perceptron), the latter model is used for regularization of the main graph autoencoder model.

Model was trained for a fixed length of 100 epochs and the model with lowest loss was selected for downstream analysis. The trained autoencoder predicted embedding vectors for all samples in the cohort and these vectors were then clustered using the K-means algorithm to obtain 15 different clusters, which represent neighborhoods of cells or communities. To describe clusters cell compositions were calculated for each community. Communities were grouped based on the dominant cell type.

Flow cytometric analysis of lymphoma specimens and ex vivo restimulation

Fresh human and A20 murine lymphoma tissue was dissociated into single cell suspensions using collagenase and mechanical digestion. Single cell isolates were then subjected to flow cytometry to assess the expression of TIGIT and PD-1 on LITs. The antibodies used for flow cytometric analyses are provided in **Table S2**. Function of $TIGIT^+$/PD-1^+ and $TIGIT^-$/PD-1^- LITs

was assessed by determining the extent of effector cytokine production following *ex vivo* restimulation with PMA/Ionomycin.

In silico data analysis of bulk tumor tissue

Correlations between *TIGIT* and *PDCD1* gene expression in DLBCL, follicular lymphoma, and cHL tumors were performed using independent large external genomic datasets containing comprehensive RNAseq or NanoString data.[21–24] Three independent DLBCL genomic datasets comprising a total of 1,200 samples were also combined to determine associations between *TIGIT* mRNA expression, biological subsets of DLBCL, and clinical outcomes following standard front-line R-CHOP therapy.[23,24] CIBERSORTx immune deconvolution was utilized to calculate inferred proportions of immune cells in the tumor environment.[25]

In vivo antibody treatment and tumor rechallenge

To test the efficacy of TIGIT blockade, balb/c mice bearing subcutaneous A20 lymphomas received intraperitoneal injections of anti-TIGIT, anti-PD-1, or combinations of these antibodies. Treatments began once tumors reached a diameter of 10 mm and were continued every 3 days for 5 doses. Isotype control (anti-rat IgG2a, clone 2A3), anti-PD-1 (BioXCell #BE0146, Rmp1-14), and anti-TIGIT (iTeos, mIgG2a, clone 31296) were each administered at a dose of 200µg. Tumor growth was monitored and compared to that in A20-bearing mice treated with isotype control antibodies. Mice that achieved complete tumor rejection following single or dual CBT were re-challenged with A20 cells to investigate immunological memory responses.

Table S1. Antibody conjugates utilized for immunofluorescence imaging

Source	Marker	Clone	Vendor	Barcode #	Fluorophore	Dilution (1:X)	Exposure time (ms)	Cycle	FluoSeq
Akoya	CD3e	EP449E	Akoya	BX045	Cy5	100	500	3	2
Akoya	CD4	EPR6855	Akoya	BX003	Cy5	200	500	1	2
BostonGene	CD8	C8/144B	BioLegend	BX029	Atto550	100	250	4	1
BostonGene	CD20	L26	ThermoFisher	BX026	Atto550	200	250	3	1
Akoya	CD21	EP3093	Akoya	BX032	Atto550	400	250	5	1
Akoya	CD31	EP3095	Akoya	BX001	Cy7	200	750	1	3
BostonGene	FOXP3	259D	BioLegend	BX035	Atto550	50	250	1	1
BostonGene	ICOS	SP98	abcam	BX024	Cy5	100	500	2	2
BostonGene	PD1	EPR4877(2)	abcam	BX014	Atto550	50	250	2	1
BostonGene	SMAa	1A4	ThermoFisher	BX004	Cy7	50	750	2	3
BostonGene	TIGIT	BLR047F	Bethyl	BX006	Cy5	100	500	4	2
BostonGene	Vimentin	O91D3	BioLegend	BX007	Cy7	400	750	3	3

Table S2. Antibodies utilized for flow cytometric analyses

		Peak Channel		Antibody	Titration	Company	Clone	Cat#
UV	BUV395	UV2	**1**	CD8	**1:100**	BD	RPA-T8	563795
	BUV661	UV11	**2**	CXCR5	**1:100**	BD	RF8B2	741559
	BUV737	UV14	**3**	CD19	**1:100**	BD	SJ25C1 (also known as SJ25-C1)	612756
Violet	BV421	V1	**4**	IFNg	**1:100**	Biolegend	4S.B3	502532
	BV711	V13	**5**	IL-2	**1:100**	Biolegend	MQ1-17H12	500346
	BV750	V14	**6**	TNFa	**1:100**	BD	MAb11	566359
	Qdot 800	V15	**7**	CD4	**1:100**	Thermo Fisher Scientific	S3.5	Q22153
Blue	FITC	B2	**8**	CD127	**1:20**	Biolegend	A019D5	351308
	Alexa Fluor 532	B3	**9**	FoxP3	**1:50**	Invitrogen	PCH101	58-4776-42
	PerCP	B8	**10**	CD3	**1:100**	Biolegend	UCHT1	300428
Yellow Green	PE	YG1	**11**	PD-1	**1:100**	Biolegend	EH12.2H7	329906
	PE-Cy7	YG9	**12**	TIGIT	**1:100**	Invitrogen	MBSA43	25-9500-42
Red	Alexa Fluor 700	R4	**13**	CD25	**1:20**	Biolegend	M-A251	356118
	Near NIR	R7	**14**	L/D	**1:1000**	Invitrogen		L34976