SUPPLEMENTARY APPENDIX

T-cell inflamed tumor microenvironment predicts favorable prognosis in primary testicular lymphoma

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Supplementary Information

Supplementary Methods

Total RNAs were isolated with RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Life Technologies, Thermo Fisher Inc., Waltham, MA). Two or three 20 µM sections were cut from the FFPE blocks and processed according to the manufacturer's protocol. A total of 100 ng of RNA was hybridized overnight at 65°C with the Human PanCancer Immunoprofiling Panel codesets (XT-CSO-HIP1-12, NanoString Technologies, Seattle, WA). Purification and binding of the hybridized probes to the cartridge were performed on the nCounter Prep Station, followed by scanning the cartridge on the nCounter Digital Analyzer (Nanostring Technologies). The data were analyzed with nSolver 3.0 software (NanoString Technologies). The quality of the data was confirmed by using the default QC settings, and normalization done using the geNorm algorithm[1]. The data were log2 transformed for subsequent analyses. For hierarchical clustering, the data were z-score transformed, and clustering was done with the JExpress 2012 software[2] using Euclidean distance with average linkage. Pathway analysis was done with DAVID Bioinformatics Resources 6.8[3, 4] using genes from the PanCancer Immunoprofiling Panel as a background.

Immunohistochemistry (IHC)

After deparaffinization, heat-induced epitope retrieval (121°C, 3 min), and blocking of endogenous peroxidase, the TMA sections were incubated with antibodies for HLA-ABC, HLA-DR and B2M at 4°C overnight. Anti-HLA Class 1 ABC antibody (clone EMR8-5) and Anti-HLA-DR antibody (clone LN3) were from Abcam (Cambridge, UK). Beta-2-microglobulin antibody (A0072) was from Dako

(Agilent Technologies, Santa Clara, CA). Staining was completed with Vectastain ABC-HRP (Peroxidase) Kit reagents (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions, and slides were counterstained with hematoxylin.

Multiplex Immunohistochemistry (mIHC)

TMA blocks were cut in 3.5 µm sections, which were deparaffinized in xylene and rehydrated in graded ethanol series and H_2O. Heat-induced epitope retrieval (HIER) was carried out in 10 mM Tris-HCl - 1 mM EDTA buffer (pH 9) in +99°C for 20 min (PT Module, Thermo Fisher Scientific, Waltham, MA). Peroxide activity was blocked in 0.9% H_2O_2 solution for 15 min, and protein block performed with 10% normal goat serum (TBS-NGS) for 15 min.

Primary antibodies diluted in protein blocking solution and secondary anti-mouse or anti-rabbit horseradish peroxidase-conjugated (HRP) antibodies (Immunologic, Netherlands) diluted 1:1 with washing buffer were applied for 1h 45min and 45 min, respectively. Tyramide signal amplification (TSA) Alexa Fluor 488 (PerkinElmer, Waltham, MA) diluted in TBS was applied on the slides for 10 min. Primary antibodies were denaturated and enzymatic activity of secondary antibody HRP was quenched by repeating HIER. Thereafter, peroxide and protein block were repeated, followed by application of a different primary antibody, and matching HRP-conjugated secondary antibody diluted 1:3 with washing buffer and TSA Alexa Fluor 555 (PerkinElmer). Again, HIER, peroxide block and protein block were repeated. Thereafter, the slides were incubated with two additional primary antibodies immunized in different species overnight in +4°C. Next, AlexaFluor647 and AlexaFluor750 fluorochrome-conjugated secondary antibodies (Thermo Fisher Scientific) diluted in 1:150 and DAPI counterstain (Roche) diluted 1:250 in washing buffer were applied for 45 min. Last, ProLong Gold mountant (Thermo Fisher Scientific) and a coverslip were applied on the slides.

In order to minimize false positive signal from antibody cross-reactions during the mIHC procedure, we required that primary antibodies selected for mIHC must be completely denatured during the HIER step between staining rounds. Therefore, the denaturation properties of all primary antibodies were examined by performing an additional HIER step between primary and secondary antibody incubation. Antibodies not denaturing completely were detected with Cy5 and Cy7 fluorescence probes, which do not require denaturation.

Fluorescent images were acquired with the AxioImager.Z2 (Zeiss, Germany) microscope equipped with Zeiss Plan-Apochromat 20x objective (NA 0.8), CoolCube1 CCD camera (MetaSystems, Germany), PhotoFluor LM-75 (89 North) metal-halide light source and Zeiss EPLAX VP232-2 power supply. DAPI, FITC, Cy3, Cy5, and Cy7 filters with compatible LED light sources were used and exposure times for all fluorescence channels were optimized visually for fluorescence imaging.

In the image analysis, DAPI-counterstained nuclei were segmented with adaptive Otsu thresholding, clumped objects separated by intensity patterns and cells segmented with nuclei contour expansion. CellProfiler 2.1.2 was used for cell segmentation, intensity measurements (upper quartile intensity) and immune cell classification. Marker colocalization was computed with the single-cell analysis software FlowJo v10 (FlowJo LLC.,). The optimal gate coordinates were ensured by visualizing matching cells with CellProfiler. Spots with less than 5000 cells were excluded from analysis. Duplicate spots from the same patient were merged.

References

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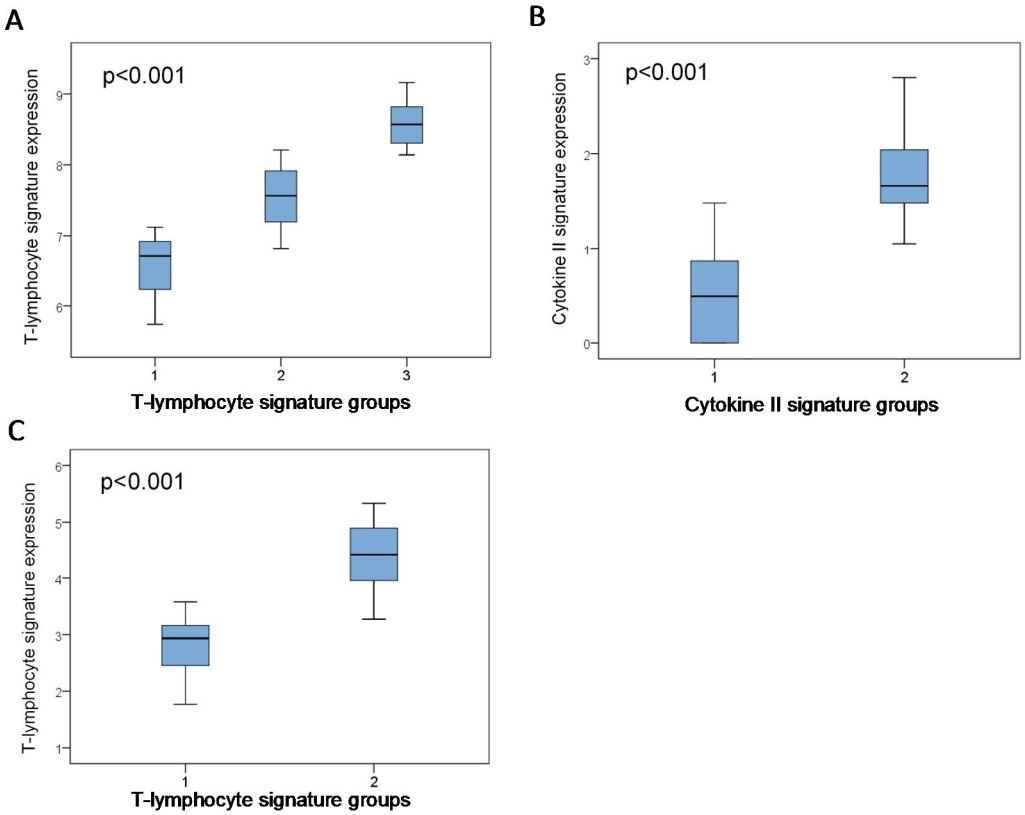
2. Dysvik B, Jonassen I. J-Express: exploring gene expression data using Java. Bioinformatics. 2001;17(4):369-370.

3. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols. 2009;4(1):44-57.

4. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic acids research. 2009;37(1):1-13.

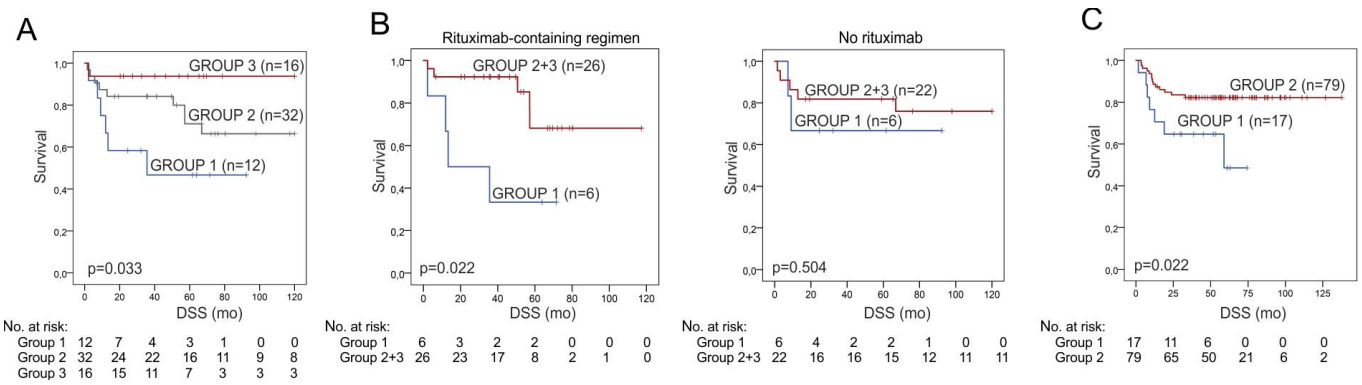
Supplementary Figures

Supplementary Figure S1



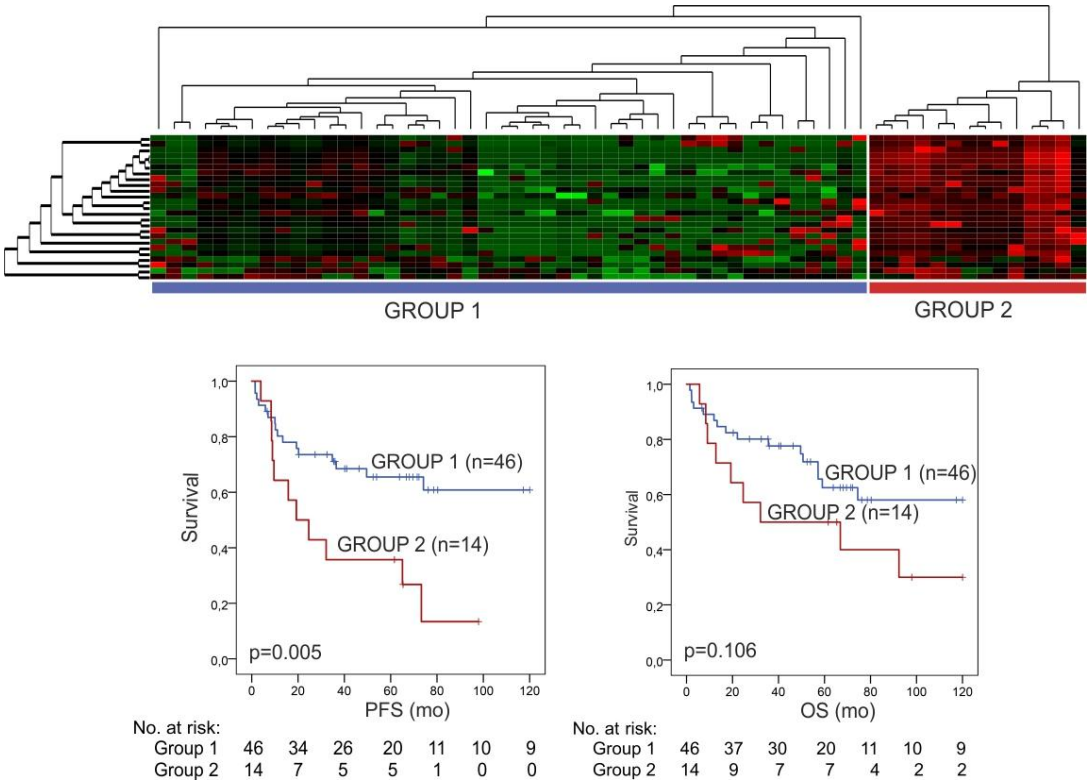
Supplementary Figure S1. Absolute expression of the T-lymphocyte and Cytokine II signatures. Median expression levels of the signature genes in the distinct patient groups were calculated and are shown as box plots. A) T-lymphocyte signature in PTL. 1=low expression, 2=intermediate expression, 3=high expression. B) Cytokine II signature in PTL. 1=low expression, 2=high expression. C) T-lymphocyte signature in DLBCL. 1=low expression, 2=high expression. The values on the y-axis are $\log_2$. Statistical significance was calculated with Kruskal-Wallis (A) and Mann-Whitney U (B-C) tests.

Supplementary Figure S2



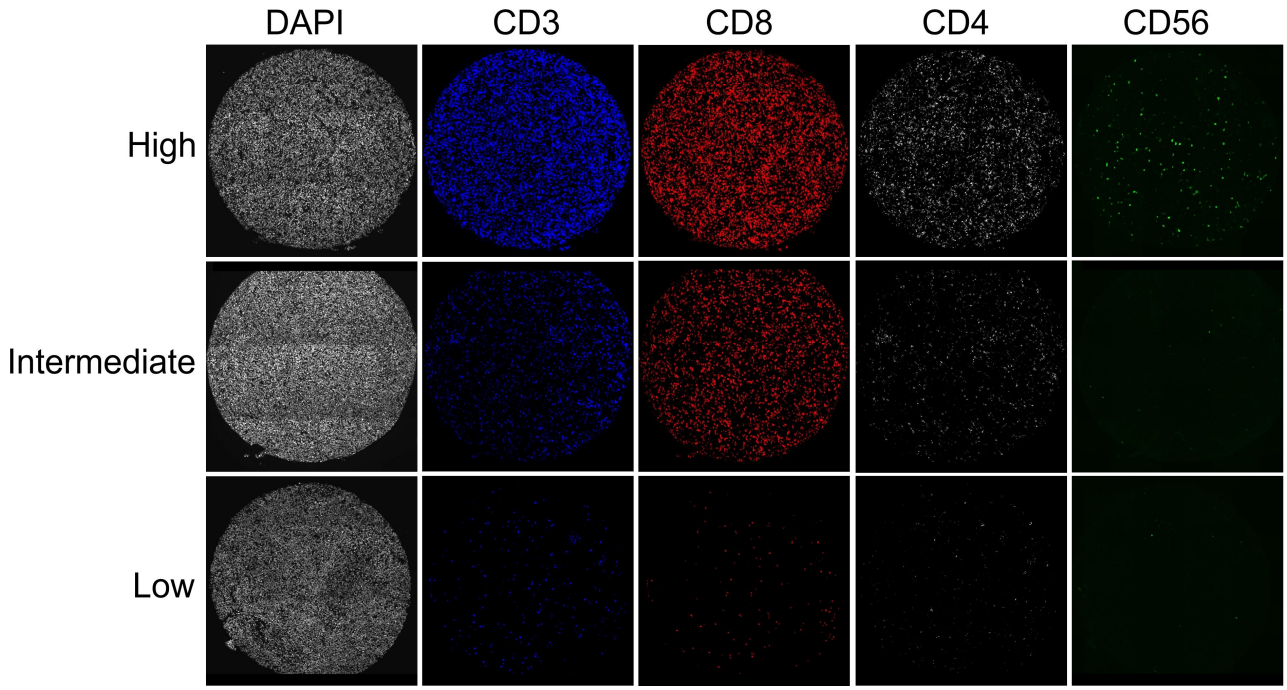
Supplementary Figure S2. T-lymphocyte signature is associated with survival in PTL. A) Kaplan-Meier survival plots (log-rank test) depicting disease-specific survival (DSS) in the three PTL patient groups based on the expression of the T-lymphocyte signature (Group 1=low expression, Group 2=intermediate expression, Group 3=high expression). B) Kaplan-Meier plots show the DSS of PTL patients stratified for the treatment (rituximab-containing regiment vs. no rituximab). Group 1=low expression, Group 2+3= intermediate+high expression. C) RNA-seq data from the CGCI DLBCL cohort was clustered based on the T-lymphocyte signature gene expression. This divided patients into two groups with higher and lower expression. Kaplan Meier-plots depict DSS in the two groups (Group 1=low expression, Group 2= high expression).

Supplementary Figure S3



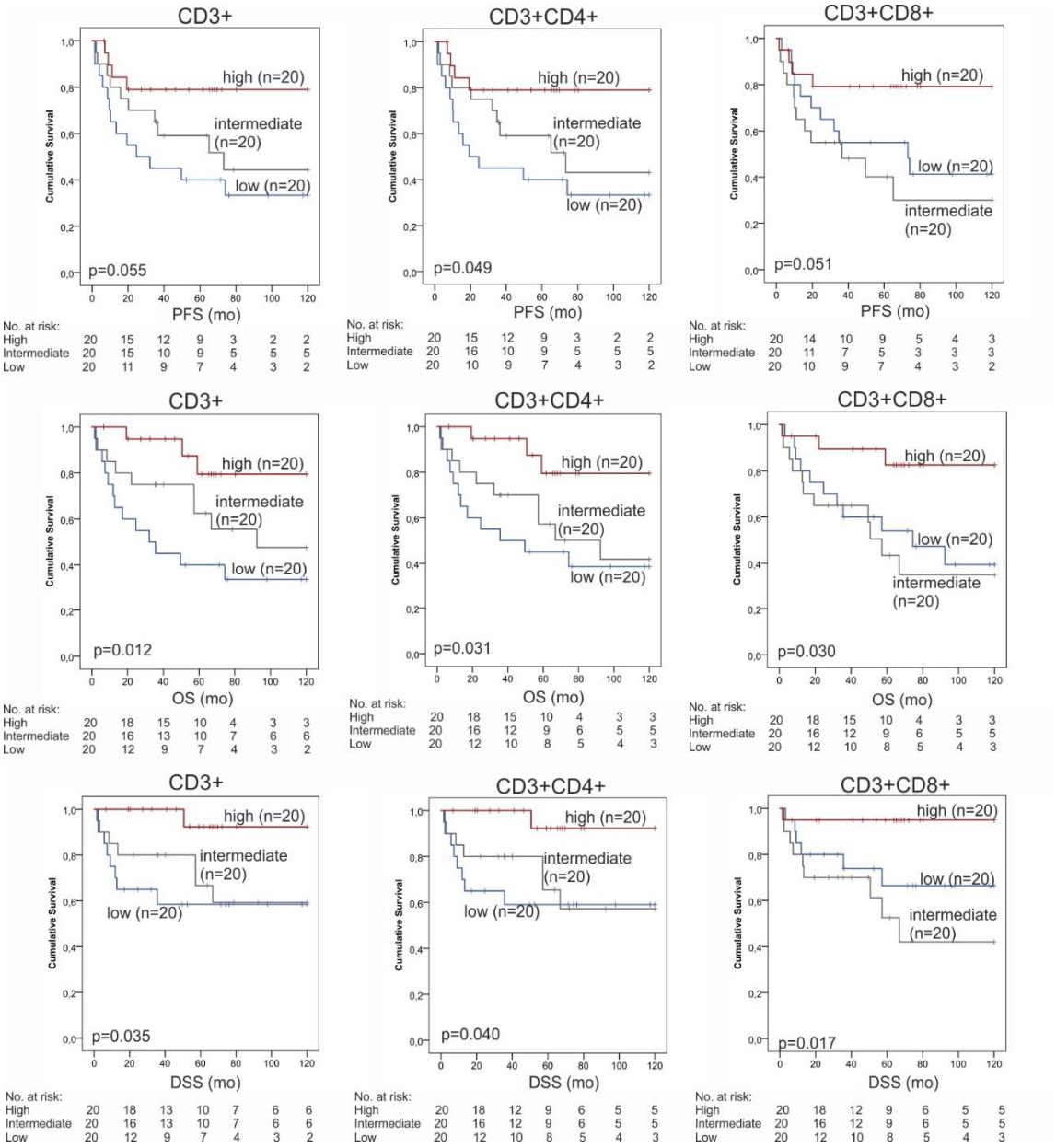
Supplementary Figure S3. Cytokine II signature is associated with survival in PTL. Re-clustering the gene expression data according to the expression of the Cytokine II signature genes divided the patients into two groups (Group 1=low expression, Group 2=high expression). Kaplan-Meier plots (log-rank test) depict PFS and OS in the two groups.

Supplementary Figure S4



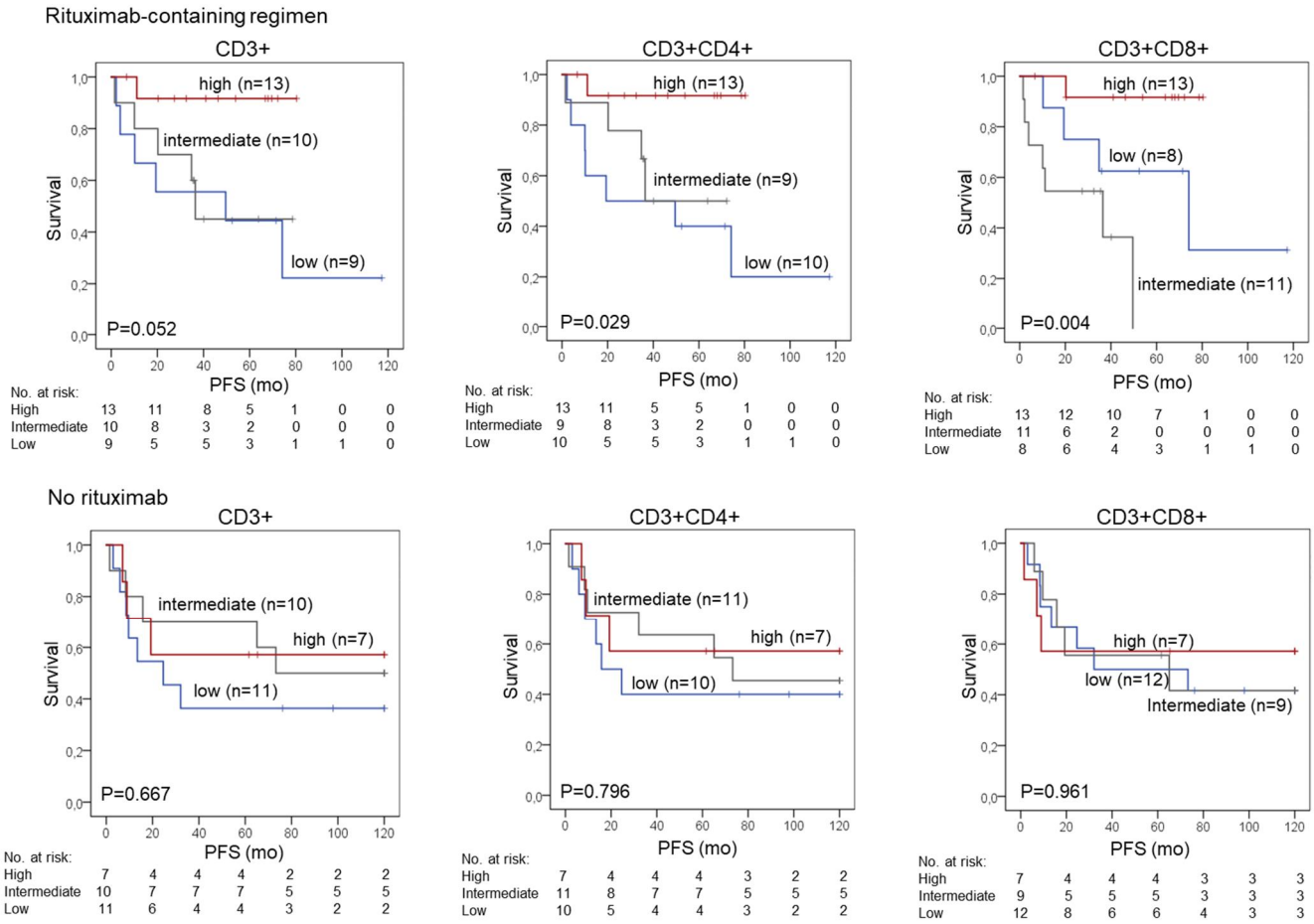
Supplementary Figure S4. Individual channels from the representative mIHC images (high, intermediate, low) of PTL TMA probed with a 4-plex panel of T-cell markers. Blue=CD3, red=CD8, white=CD4, green=CD56, grey=DAPI.

Supplementary Figure S5



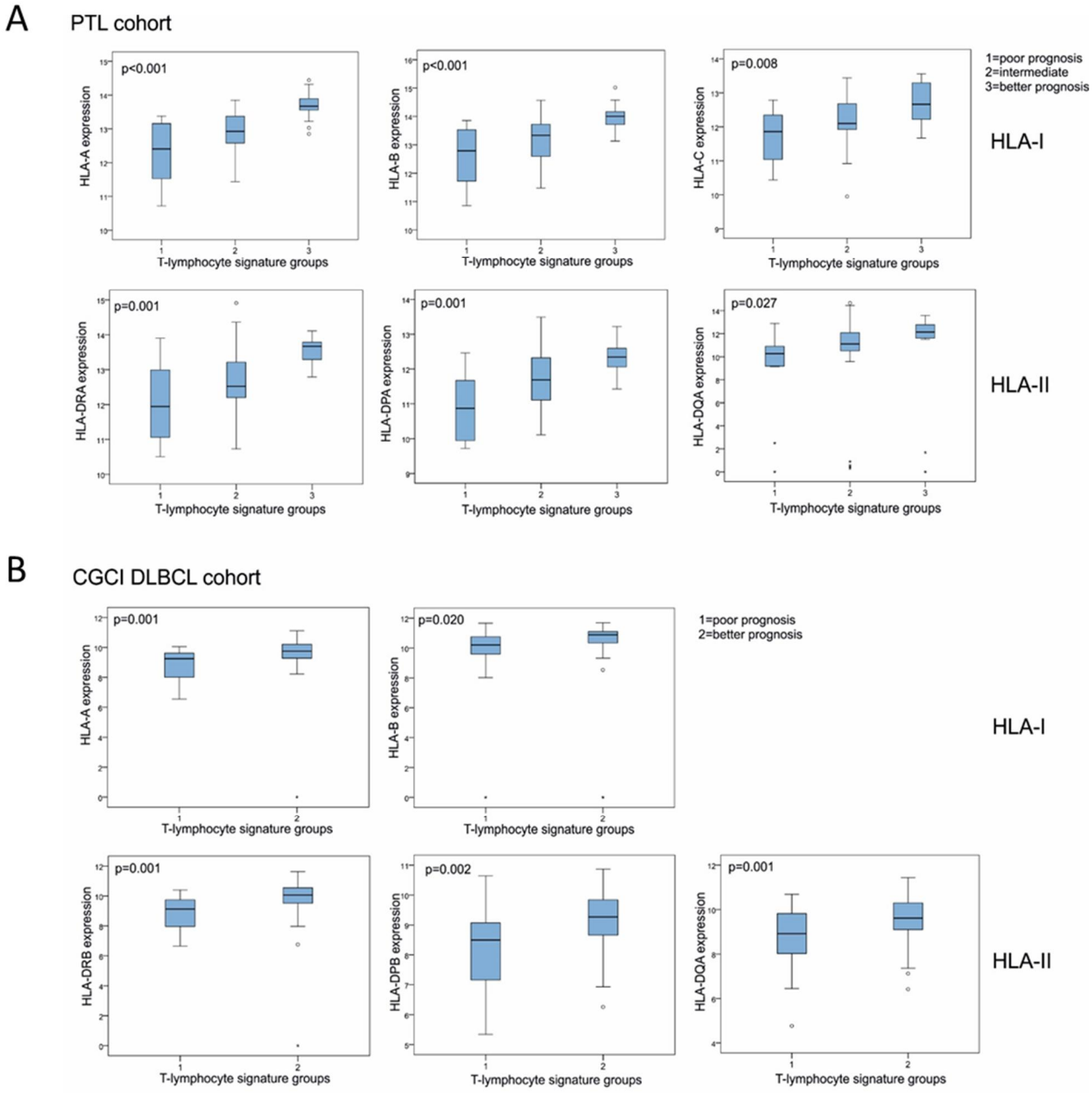
Supplementary Figure S5. Lower amount of tumor-infiltrating T-cells is associated with poor survival in PTL. Kaplan-Meier plots (log-rank test) visualizing survival associations of distinct T-cell subpopulations. The samples were divided by equally-sized tertiles of the T-cell counts.

Supplementary Figure S6



Supplementary Figure S6. High T-cell counts predict better survival in PTL patients treated with rituximab-containing regimen. Kaplan-Meier estimates (log-rank test) visualizing survival of distinct T-cell subpopulations when stratified by treatment (rituximab-containing regimen vs. no rituximab). The samples were divided by tertiles of the T-cell counts.

Supplementary Figure S7



Supplementary Figure S7. HLA I and II class genes have lower expression in PTL patients with poor prognosis. A-B) Expression of HLA I and II class genes in the PTL (A) and DLBLC (B) cohorts, as determined by the Nanostring analysis (PTL) or RNA-seq (DLBCL).

Supplementary Tables

Supplementary Table S1. T-lymphocyte signature genes.

Official Symbol	Accession	Official Full Name
ANXA1	NM_000700.1	annexin A1
BST1	NM_004334.2	bone marrow stromal cell antigen 1
BST2	NM_004335.2	bone marrow stromal cell antigen 2
C1QA	NM_015991.2	complement component 1, q subcomponent, A chain
C1QB	NM_000491.3	complement component 1, q subcomponent, B chain
C2	NM_000063.3	complement component 2
C3AR1	NM_004054.2	complement component 3a receptor 1
CARD9	NM_052813.4	caspase recruitment domain family, member 9
CASP1	NM_001223.3	caspase 1, apoptosis-related cysteine peptidase
CCL2	NM_002982.3	chemokine (C-C motif) ligand 2
CCL3	NM_002983.2	chemokine (C-C motif) ligand 3
CCL3L1	NM_021006.4	chemokine (C-C motif) ligand 3-like 1
CCL4	NM_002984.2	chemokine (C-C motif) ligand 4
CCL5	NM_002985.2	chemokine (C-C motif) ligand 5
CCL8	NM_005623.2	chemokine (C-C motif) ligand 8
CCR1	NM_001295.2	chemokine (C-C motif) receptor 1
CCR5	NM_000579.1	chemokine (C-C motif) receptor 5 (gene/pseudogene)
CD14	NM_000591.2	CD14 molecule
CD163	NM_004244.4	CD163 molecule
CD2	NM_001767.3	CD2 molecule
CD244	NM_016382.2	CD244 molecule, natural killer cell receptor 2B4
CD274	NM_014143.3	CD274 molecule
CD33	NM_001177608.1	CD33 molecule
CD3D	NM_000732.4	CD3d molecule, delta (CD3-TCR complex)
CD3E	NM_000733.2	CD3e molecule, epsilon (CD3-TCR complex)
CD3G	NM_000073.2	CD3g molecule, gamma (CD3-TCR complex)
CD4	NM_000616.4	CD4 molecule
CD6	NM_006725.3	CD6 molecule
CD68	NM_001251.2	CD68 molecule
CD7	NM_006137.6	CD7 molecule
CD84	NM_001184879.1	CD84 molecule
CD8A	NM_001768.5	CD8a molecule
CD8B	NM_004931.3	CD8b molecule
CD96	NM_005816.4	CD96 molecule
CD97	NM_078481.2	CD97 molecule
CEBPB	NM_005194.2	CCAAT/enhancer binding protein (C/EBP), beta
CFB	NM_001710.5	complement factor B
CKLF	NM_181640.2	chemokine-like factor
CMKLR1	NM_004072.1	chemokine-like receptor 1
CSF1	NM_000757.4	colony stimulating factor 1 (macrophage)
CSF1R	NM_005211.2	colony stimulating factor 1 receptor
CSF3R	NM_156038.2	colony stimulating factor 3 receptor (granulocyte)
CTSL	NM_001912.4	cathepsin L
CTSS	NM_004079.3	cathepsin S
CTSW	NM_001335.3	cathepsin W

CXCL10	NM_001565.1	chemokine (C-X-C motif) ligand 10
CXCL11	NM_005409.4	chemokine (C-X-C motif) ligand 11
CXCL16	NM_001100812.1	chemokine (C-X-C motif) ligand 16
CXCL9	NM_002416.1	chemokine (C-X-C motif) ligand 9
CXCR3	NM_001504.1	chemokine (C-X-C motif) receptor 3
CXCR6	NM_006564.1	chemokine (C-X-C motif) receptor 6
DUSP6	NM_001946.2	dual specificity phosphatase 6
EOMES	NM_005442.2	eomesodermin
FCER1G	NM_004106.1	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
FCGR1A	NM_000566.3	Fc fragment of IgG, high affinity Ia, receptor (CD64)
FCGR2A	NM_021642.3	Fc fragment of IgG, low affinity IIa, receptor (CD32)
FCGR3A	NM_000569.6	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)
FLT3LG	NM_001459.3	fms-related tyrosine kinase 3 ligand
FPR2	NM_001462.3	formyl peptide receptor 2
GZMA	NM_006144.2	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)
GZMB	NM_004131.3	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)
GZMH	NM_033423.3	granzyme H (cathepsin G-like 2, protein h-CCPX)
GZMK	NM_002104.2	granzyme K (granzyme 3; tryptase II)
GZMM	NM_005317.2	granzyme M (lymphocyte met-ase 1)
HAVCR2	NM_032782.3	hepatitis A virus cellular receptor 2
HLA-E	NM_005516.4	major histocompatibility complex, class I, E
HLA-G	NM_002127.4	major histocompatibility complex, class I, G
HSD11B1	NM_181755.1	hydroxysteroid (11-beta) dehydrogenase 1
IDO1	NM_002164.3	indoleamine 2,3-dioxygenase 1
IFIH1	NM_022168.2	interferon induced with helicase C domain 1
IFNG	NM_000619.2	interferon, gamma
IL17RA	NM_014339.6	interleukin 17 receptor A
IL1B	NM_000576.2	interleukin 1, beta
IL1RN	NM_000577.3	interleukin 1 receptor antagonist
IL32	NM_001012633.1	interleukin 32
IRAK2	NM_001570.3	interleukin-1 receptor-associated kinase 2
IRF1	NM_002198.1	interferon regulatory factor 1
ITGA1	NM_181501.1	integrin, alpha 1
ITGAL	NM_002209.2	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
ITGB2	NM_000211.2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
ITK	NM_005546.3	IL2-inducible T-cell kinase
KLRB1	NM_002258.2	killer cell lectin-like receptor subfamily B, member 1
KLRG1	NM_005810.3	killer cell lectin-like receptor subfamily G, member 1
KLRK1	NM_007360.3	killer cell lectin-like receptor subfamily K, member 1
LAG3	NM_002286.5	lymphocyte-activation gene 3
LAIR2	NM_002288.3	leukocyte-associated immunoglobulin-like receptor 2
LCK	NM_005356.2	lymphocyte-specific protein tyrosine kinase
LGALS3	NM_001177388.1	lectin, galactoside-binding, soluble, 3
LILRA5	NM_181879.2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5
LILRB1	NM_001081637.1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
LILRB2	NM_005874.1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2

LILRB3	NM_006864.2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3
LY96	NM_015364.2	lymphocyte antigen 96
NLRP3	NM_001079821.2	NLR family, pyrin domain containing 3
PDCD1LG2	NM_025239.3	programmed cell death 1 ligand 2
PECAM1	NM_000442.3	platelet/endothelial cell adhesion molecule 1
PRF1	NM_005041.3	perforin 1 (pore forming protein)
PTPRC	NM_080921.3	protein tyrosine phosphatase, receptor type, C
PYCARD	NM_013258.3	PYD and CARD domain containing
SELPLG	NM_001206609.1	selectin P ligand
SERPING1	NM_000062.2	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
SH2D1A	NM_001114937.2	SH2 domain containing 1A
SIGIRR	NM_021805.2	single immunoglobulin and toll-interleukin 1 receptor (TIR) domain
SIGLEC1	NM_023068.3	sialic acid binding Ig-like lectin 1, sialoadhesin
STAT1	NM_007315.2	signal transducer and activator of transcription 1, 91kDa
STAT4	NM_003151.2	signal transducer and activator of transcription 4
TARP	NM_001003799.1	TCR gamma alternate reading frame protein
TBX21	NM_013351.1	T-box 21
TLR1	NM_003263.3	toll-like receptor 1
TLR8	NM_016610.2	toll-like receptor 8
TNFRSF14	NM_003820.2	tumor necrosis factor receptor superfamily, member 14
TNFRSF1A	NM_001065.2	tumor necrosis factor receptor superfamily, member 1A
TNFRSF1B	NM_001066.2	tumor necrosis factor receptor superfamily, member 1B
TNFRSF9	NM_001561.4	tumor necrosis factor receptor superfamily, member 9
TNFSF10	NM_003810.2	tumor necrosis factor (ligand) superfamily, member 10
TNFSF12	NM_003809.2	tumor necrosis factor (ligand) superfamily, member 12
TNFSF13B	NM_006573.4	tumor necrosis factor (ligand) superfamily, member 13b
TNFSF14	NM_003807.3	tumor necrosis factor (ligand) superfamily, member 14
TNFSF15	NM_001204344.1	tumor necrosis factor (ligand) superfamily, member 15
UBC	NM_021009.3	ubiquitin C
VCAM1	NM_001078.3	vascular cell adhesion molecule 1

Supplementary Table S2. Cytokine I signature genes.

Official Symbol	Accession	Official Full Name
AIRE	NM_000383.2	autoimmune regulator
AMBP	NM_001633.3	alpha-1-microglobulin/bikunin precursor
ARG1	NM_000045.2	arginase, liver
C4BPA	NM_000715.3	complement component 4 binding protein, alpha
C8A	NM_000562.2	complement component 8, alpha polypeptide
C8B	NM_000066.2	complement component 8, beta polypeptide
CCL1	NM_002981.1	chemokine (C-C motif) ligand 1
CCL15	NM_032965.3	chemokine (C-C motif) ligand 15
CCL16	NM_004590.2	chemokine (C-C motif) ligand 16
CCL25	NM_005624.2	chemokine (C-C motif) ligand 25
CCL26	NM_006072.4	chemokine (C-C motif) ligand 26
CCL27	NM_006664.2	chemokine (C-C motif) ligand 27
CD1A	NM_001763.2	CD1a molecule
CD1E	NM_001042583.1	CD1e molecule
CD207	NM_015717.2	CD207 molecule, langerin
CEACAM6	NM_002483.4	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)
CLU	NM_001831.2	clusterin
CRP	NM_000567.2	C-reactive protein, pentraxin-related
CSF2	NM_000758.2	colony stimulating factor 2 (granulocyte-macrophage)
CSF3	NM_000759.3	colony stimulating factor 3 (granulocyte)
CTSG	NM_001911.2	cathepsin G
CXCL5	NM_002994.3	chemokine (C-X-C motif) ligand 5
CXCR2	NM_001557.2	chemokine (C-X-C motif) receptor 2
IFNA1	NM_024013.1	interferon, alpha 1
IFNA2	NM_000605.3	interferon, alpha 2
IFNA8	NM_002170.3	interferon, alpha 8
IFNL2	NM_172138.1	interferon lambda 2
IL11	NM_000641.2	interleukin 11
IL17F	NM_052872.3	interleukin 17F
IL1RL2	NM_003854.2	interleukin 1 receptor-like 2
IL2	NM_000586.2	interleukin 2
IL22	NM_020525.4	interleukin 22
IL23R	NM_144701.2	interleukin 23 receptor
IL25	NM_022789.2	interleukin 25
IL26	NM_018402.1	interleukin 26
IL3	NM_000588.3	interleukin 3 (colony-stimulating factor, multiple)
IL5	NM_000879.2	interleukin 5 (colony-stimulating factor, eosinophil)
ITGA2B	NM_000419.3	integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41)
LBP	NM_004139.2	lipopolysaccharide binding protein
MBL2	NM_000242.2	mannose-binding lectin (protein C) 2, soluble
PTGDR2	NM_004778.1	prostaglandin D2 receptor 2
RAG1	NM_000448.2	recombination activating gene 1
S100A12	NM_005621.1	S100 calcium binding protein A12
SSX4	NM_005636.3	synovial sarcoma, X breakpoint 4

Supplementary Table S3. Cytokine II signature genes.

Official Symbol	Accession	Official Full Name
CCL24	NM_002991.2	chemokine (C-C motif) ligand 24
CCL28	NM_148672.2	chemokine (C-C motif) ligand 28
CD1B	NM_001764.2	CD1b molecule
CLEC5A	NM_013252.2	C-type lectin domain family 5, member A
CMA1	NM_001836.2	chymase 1, mast cell
CTAG1B	NM_001327.2	cancer/testis antigen 1B
CXCR1	NM_000634.2	chemokine (C-X-C motif) receptor 1
DMBT1	NM_007329.2	deleted in malignant brain tumors 1
ELANE	NM_001972.2	elastase, neutrophil expressed
FCER1A	NM_002001.2	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide
IFNL1	NM_172140.1	interferon lambda 1
IGLL1	NM_020070.2	immunoglobulin lambda-like polypeptide 1
IL17A	NM_002190.2	interleukin 17A
IL17B	NM_014443.2	interleukin 17B
IL4	NM_000589.2	interleukin 4
IL9	NM_000590.1	interleukin 9
KIR3DS1	NM_001083539.1	killer cell immunoglobulin-like receptor, three domains, short cytoplasmic tail, 1
LCN2	NM_005564.3	lipocalin 2
MAGEA12	NM_001166386.1	melanoma antigen family A, 12
NOS2A	NM_153292.1	nitric oxide synthase 2
PPBP	NM_002704.2	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)
SERPINB2	NM_002575.1	serpin peptidase inhibitor, clade B (ovalbumin), member 2
TNFRSF11A	NM_003839.2	tumor necrosis factor receptor superfamily, member 11a, NFKB activator
TNFSF11	NM_003701.2	tumor necrosis factor (ligand) superfamily, member 11
TNFSF18	NM_005092.2	tumor necrosis factor (ligand) superfamily, member 18

Supplementary Table S4. Multivariate Cox regression analysis with the low T-lymphocyte signature and individual IPI factors.

	Progression-free survival			Overall survival			Disease-specific survival		
	HR[a]	CI	p-val	HR	CI	p-val	HR	CI	p-val
Low T-lymphocyte signature	2.570	1.069-6.181	0.035	3.281	1.336-8.060	0.010	2.404	0.716-8.077	0.156
Age	1.101	1.043-1.162	0.000	1.123	1.055-1.194	<0.001	1.100	1.027-1.178	0.007
Stage	0.810	0.256-2.563	0.720	0.891	0.345-2.298	0.811	1.526	0.458-5.085	0.491
ECOG	5.347	1.789-15.979	0.003	2.355	1.450-3.823	0.001	2.717	1.563-4.723	<0.001
LDH	3.902	1.472-10.344	0.006	2.697	1.026-7.087	0.044	7.878	1.993-32.965	0.005
No. of extranodal sites	1.408	0.401-4.941	0.593	1.303	0.959-1.722	0.091	1.407	1.016-1.949	0.040

[a]HR,hazard-ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase

Supplementary Table S5. Baseline characteristics of the T-lymphocyte signature patient groups in the PTL and DLBCL cohorts.

	PTL cohort			DLBCL cohort		
Characteristics	Signature Group 1 n (%)	Signature Group 2+3 n (%)	P-val[a]	Signature Group 1 n (%)	Signature Group 2 n (%)	P-val
Number of patients	12 (20)	48 (80)		17 (18)	79 (82)	
Age						
<60 years	4 (33)	12 (25)	0.716	9 (53)	32 (40)	0.250
≥60 years	8 (67)	36 (75)		8 (47)	47 (60)	
Gender						
Men	12 (100)	48 (100)		12 (71)	51 (65)	0.781
Women				5 (29)	28 (35)	
Molecular subgroup						
GCB	4 (33)	11 (23)	0.468	8 (47)	47 (60)	0.250
non-GCB	7 (58)	33 (69)		9 (53)	32 (40)	
Nd[b]	1 (9)	4 (8)				
Stage						
I-II[c]	7 (58)	32 (67)	0.737	2 (12)	16 (20)	0.515
III-IV	5 (42)	16 (33)		15 (88)	63 (80)	
IPI score						
0-2	7 (58)	35 (73)	0.482	4 (24)	21 (27)	1.000
3-5	5 (42)	13 (27)		13 (76)	58 (73)	
Elevated LDH	6 (50)	16 (33)	0.505	n/a	n/a	
CNS prophylaxis	5 (42)	29 (60)	0.332	n/a	n/a	
IV-prophylaxis	5 (42)	26 (54)	0.527	n/a	n/a	
IT-prophylaxis	1 (9)	7 (15)	1.000	n/a	n/a	
Treated with rituximab-containing regimen	6 (50)	26 (54)	1.000	15 (94)	76 (96)	0.528
Relapses	6 (50)	12 (25)	0.156	9 (53)	18 (23)	0.018
Deaths	9 (75)	16 (33)	0.004	7 (41)	17 (22)	0.122
Lymphoma-specific	6 (50)	10 (21)	0.025	7 (41)	14 (18)	0.050
Other	3 (25)	6 (12)		0	3 (4)	

[a]p-value determined by the Chi square test

[b]nd, not determined; LDH, lactate dehydrogenase; n/a, not assigned; CNS, central nervous system; IV, intravenous; IT, intrathecal

[c]Isolated bilateral testicular involvement was defined as stage IAE

Supplementary Table S6. Univariate analysis for T-lymphocyte signature genes with continuous variables.

	OS				PFS				DSS			
Gene symbol	HR[a]	CI(95%) Lower	Higher	p-val	HR	CI(95%) Lower	Higher	p-val	HR	CI(95%) Lower	Higher	p-val
ANXA1	0.52	0.31	0.87	0.01	0.67	0.40	1.11	0.12	0.62	0.33	1.17	0.14
BST1	0.67	0.48	0.94	0.02	0.73	0.52	1.02	0.07	0.72	0.47	1.10	0.13
CCL2	0.57	0.37	0.88	0.01	0.70	0.46	1.07	0.10	0.57	0.33	0.98	0.04
CCL3	0.71	0.47	1.07	0.10	0.83	0.55	1.23	0.35	0.60	0.36	1.00	0.05
CCL3L1	0.68	0.45	1.03	0.07	0.79	0.53	1.18	0.25	0.57	0.33	0.97	0.04
CCL4	0.57	0.39	0.82	0.00	0.64	0.45	0.92	0.01	0.51	0.32	0.81	0.00
CCL5	0.62	0.43	0.90	0.01	0.74	0.52	1.06	0.10	0.59	0.37	0.94	0.03
CCR1	0.64	0.45	0.92	0.02	0.73	0.51	1.03	0.07	0.65	0.41	1.01	0.05
CCR5	0.70	0.53	0.94	0.02	0.78	0.59	1.04	0.09	0.63	0.44	0.89	0.01
CD2	0.77	0.64	0.93	0.01	0.81	0.67	0.98	0.03	0.77	0.61	0.97	0.03
CD244	0.77	0.62	0.95	0.01	0.85	0.69	1.05	0.14	0.71	0.55	0.93	0.01
CD274	0.74	0.59	0.92	0.01	0.76	0.62	0.95	0.02	0.71	0.54	0.94	0.01
CD33	0.66	0.46	0.96	0.03	0.79	0.55	1.14	0.21	0.61	0.40	0.93	0.02
CD3D	0.66	0.52	0.84	0.00	0.72	0.56	0.92	0.01	0.64	0.50	0.83	0.00
CD3E	0.66	0.53	0.84	0.00	0.72	0.57	0.91	0.01	0.64	0.49	0.83	0.00
CD3G	0.82	0.69	0.99	0.03	0.86	0.72	1.03	0.10	0.79	0.63	0.98	0.03
CD4	0.44	0.23	0.83	0.01	0.55	0.30	1.00	0.05	0.46	0.22	0.97	0.04
CD6	0.67	0.52	0.86	0.00	0.73	0.57	0.93	0.01	0.62	0.47	0.83	0.00
CD68	0.51	0.29	0.88	0.02	0.63	0.37	1.07	0.09	0.60	0.31	1.17	0.14
CD7	0.76	0.57	1.01	0.06	0.84	0.63	1.12	0.24	0.70	0.50	0.97	0.03
CD84	0.50	0.28	0.90	0.02	0.58	0.34	1.01	0.05	0.40	0.19	0.81	0.01
CD8A	0.78	0.63	0.97	0.03	0.85	0.68	1.07	0.16	0.76	0.58	0.99	0.04
CD8B	0.83	0.71	0.98	0.03	0.88	0.74	1.03	0.12	0.84	0.68	1.03	0.09
CD96	0.71	0.58	0.87	0.00	0.76	0.62	0.95	0.01	0.68	0.53	0.86	0.00
CKLF	0.32	0.15	0.68	0.00	0.34	0.17	0.71	0.00	0.29	0.12	0.75	0.01
CSF1	0.70	0.53	0.92	0.01	0.74	0.56	0.97	0.03	0.74	0.52	1.06	0.10
CTSL	0.58	0.37	0.94	0.03	0.67	0.43	1.04	0.07	0.72	0.40	1.27	0.25
CTSW	0.72	0.54	0.96	0.03	0.78	0.58	1.04	0.09	0.61	0.43	0.86	0.00
CXCL11	0.72	0.54	0.94	0.02	0.78	0.59	1.03	0.08	0.66	0.48	0.91	0.01
CXCL16	0.66	0.47	0.93	0.02	0.72	0.51	1.02	0.06	0.78	0.49	1.25	0.30
CXCL9	0.79	0.62	1.01	0.06	0.86	0.67	1.09	0.21	0.75	0.56	1.00	0.05
CXCR3	0.66	0.51	0.86	0.00	0.73	0.57	0.95	0.02	0.64	0.47	0.88	0.01
CXCR6	0.66	0.49	0.87	0.00	0.74	0.55	0.98	0.04	0.61	0.44	0.83	0.00
EOMES	0.71	0.58	0.89	0.00	0.77	0.63	0.95	0.02	0.73	0.56	0.95	0.02
GZMA	0.65	0.50	0.84	0.00	0.72	0.55	0.94	0.01	0.62	0.45	0.84	0.00
GZMK	0.66	0.49	0.88	0.00	0.72	0.54	0.95	0.02	0.61	0.44	0.86	0.01
GZMM	0.77	0.63	0.94	0.01	0.83	0.68	1.01	0.07	0.72	0.57	0.92	0.01
HAVCR2	0.61	0.39	0.96	0.03	0.71	0.46	1.10	0.13	0.55	0.32	0.97	0.04
HLA-G	0.70	0.52	0.93	0.02	0.74	0.56	0.99	0.04	0.76	0.52	1.11	0.16
HSD11B1	0.63	0.43	0.91	0.01	0.72	0.51	1.02	0.07	0.57	0.36	0.91	0.02
IDO1	0.73	0.58	0.93	0.01	0.77	0.61	0.98	0.04	0.70	0.52	0.94	0.02
IFNG	0.63	0.42	0.94	0.02	0.77	0.53	1.12	0.17	0.49	0.29	0.83	0.01
IL32	0.44	0.28	0.70	0.00	0.53	0.34	0.82	0.00	0.45	0.26	0.79	0.01

IRAK2	0.55	0.41	0.75	0.00	0.61	0.45	0.82	0.00	0.60	0.42	0.86	0.01
IRF1	0.60	0.39	0.93	0.02	0.73	0.47	1.14	0.16	0.58	0.34	1.01	0.06
ITGAL	0.58	0.38	0.90	0.01	0.69	0.45	1.06	0.09	0.50	0.29	0.86	0.01
ITGB2	0.53	0.34	0.82	0.00	0.63	0.41	0.97	0.04	0.58	0.35	0.98	0.04
ITK	0.60	0.42	0.84	0.00	0.69	0.49	0.96	0.03	0.53	0.35	0.80	0.00
KLRB1	0.83	0.71	0.97	0.02	0.85	0.73	0.99	0.03	0.77	0.64	0.93	0.01
KLRG1	0.75	0.62	0.91	0.00	0.79	0.65	0.96	0.02	0.71	0.56	0.90	0.01
KLRK1	0.69	0.55	0.87	0.00	0.74	0.59	0.94	0.01	0.65	0.50	0.84	0.00
LAIR2	0.56	0.34	0.93	0.02	0.70	0.43	1.15	0.16	0.48	0.26	0.87	0.02
LCK	0.53	0.34	0.85	0.01	0.63	0.41	0.99	0.04	0.39	0.21	0.71	0.00
LGALS3	0.51	0.28	0.94	0.03	0.57	0.32	1.00	0.05	0.62	0.29	1.31	0.21
NLRP3	0.70	0.51	0.97	0.03	0.80	0.57	1.11	0.18	0.71	0.48	1.05	0.08
PDCD1LG2	0.69	0.51	0.94	0.02	0.74	0.55	1.01	0.06	0.74	0.51	1.07	0.11
PRF1	0.71	0.52	0.96	0.03	0.81	0.59	1.10	0.18	0.65	0.45	0.94	0.02
PTPRC	0.65	0.45	0.94	0.02	0.71	0.50	1.01	0.06	0.62	0.39	0.97	0.04
SELPLG	0.52	0.32	0.84	0.01	0.63	0.39	1.01	0.05	0.53	0.29	0.97	0.04
SH2D1A	0.79	0.66	0.94	0.01	0.82	0.68	0.99	0.04	0.78	0.62	0.97	0.03
SIGIRR	0.54	0.36	0.81	0.00	0.65	0.43	0.99	0.04	0.50	0.31	0.81	0.01
STAT1	0.61	0.40	0.92	0.02	0.68	0.46	1.03	0.07	0.56	0.34	0.92	0.02
STAT4	0.74	0.59	0.93	0.01	0.79	0.63	1.00	0.05	0.69	0.52	0.90	0.01
TARP	0.64	0.46	0.88	0.01	0.73	0.53	1.01	0.06	0.56	0.37	0.83	0.00
TLR1	0.51	0.28	0.91	0.02	0.57	0.32	1.03	0.06	0.39	0.20	0.78	0.01
TLR8	0.86	0.67	1.10	0.23	0.89	0.70	1.15	0.38	0.75	0.56	0.99	0.04
TNFRSF1A	0.49	0.25	0.95	0.04	0.67	0.35	1.30	0.24	0.64	0.28	1.46	0.29
TNFRSF9	0.57	0.39	0.83	0.00	0.64	0.44	0.92	0.02	0.54	0.35	0.82	0.00
TNFSF14	0.63	0.48	0.81	0.00	0.69	0.53	0.90	0.01	0.65	0.48	0.89	0.01
TNFSF15	0.67	0.48	0.92	0.01	0.67	0.49	0.92	0.01	0.62	0.42	0.92	0.02
UBC	0.30	0.11	0.86	0.02	0.39	0.14	1.05	0.06	0.41	0.11	1.49	0.17
VCAM1	0.49	0.31	0.78	0.00	0.58	0.37	0.90	0.02	0.50	0.29	0.88	0.02

[a]HR, hazard ratio; CI, confidence interval