

## Supplementary Tables

**Supplementary Table S1: Multiplex immunohistochemistry (mIHC) panels used to characterize different immunophenotypes of cells in the lymphoma microenvironment**

	Panel 1	Panel 2	Panel 3
1st round			
TSA-488	M-anti-CD68 (Abcam; ab955, clone KP1) 1:200	M-anti-PD-1 (LSBio; B12784, clone 3C6) 1:200	M-anti-CD3 (Abcam; ab17143, clone F7.2.38) 1:100; 1,5h RT
TSA-555	R-anti-PD-L1 (CST <sup>1</sup> ; 13684) 1:200	R-anti-PD-L1 (CST; 13684) 1:200	R-anti-CD56 (Cell Marque; 156R-95) 1:1000
Alexa-647	R-anti-Tim-3 (CST; 45208, clone D5D5R) 1:100	R-anti-CD4 (Abcam; ab133616, clone EPR6855) 1:50	R-anti-Tim-3 (CST; 45208, clone D5D5R) 1:100
Alexa-750	M-anti-CD20 (Thermo; MS-340-s, clone L26) 1:50	M-anti-CD68 (Abcam; ab955, clone KP1) 1:50	M-anti-CD45 (Dako; M0701, clone 2B11 + PD7/26) 1:50
Bleach boil			
2nd round			
Alexa 647		M-anti-CD3 (Abcam; ab17143, clone F7.2.38) 1:50	
Alexa-750	R-anti-CD163 (Abcam; ab188571, clone EPR14643) 1:200	R-anti-CD163 (Abcam; ab188571, clone EPR14643) 1:200	R-anti-Granzyme B (Abcam; ab4059) 1:100

<sup>1</sup>CST, Cell Signaling Technologies

**Supplementary Table S2: Univariate analysis of established risk factors in the mIHC cohort**

Variable	OS HR (95% CI)	OS <i>P</i>	PFS HR (95% CI)	PFS <i>P</i>
IPI <sup>a</sup> (3-5 vs. 0-2)	4.32 (2.06-9.09)	<0.001	3.12 (1.67-5.83)	<0.001
Molecular subtype (ABC vs. GCB) <sup>b</sup>	4.90 (1.98-12.09)	<0.001	3.22 (1.49-6.98)	0.003
MYC and BCL2 double protein expression	2.49 (1.28-4.84)	0.007	2.28 (1.22-4.29)	0.010

<sup>a</sup>IPI, international prognostic index

<sup>b</sup>ABC, activated B-cell like; GCB, germinal center B-cell like

**Supplementary Table S3: Amounts of different immune cells and their phenotypes analyzed by mIHC**

	Immunophenotype	Mean	Median	Min	Max
T cells	CD3+	16.8397	15.121	0.4	61.45
	CD4+CD3+	12.8438	10.0544	0.3	49.16
	CD4-CD3+	7.3612	6.4367	0.18	22.85
Macrophages	CD68+	10.2969	9.8532	0.58	23.75
	CD163+	10.2712	6.4109	0.04	54.93
	CD163+CD68+	4.0739	2.7442	0.02	16.21
	CD163-CD68+	6.2587	5.7123	0.38	16.7
	CD4+CD68+	6.7644	6.1136	0.49	21.13
NK cells	CD56+CD3-	0.0315	0.0072	0	0.97
	CD56+CD3-CD45+	0.0155	0.0037	0	0.26
B cells	CD20+	56.4643	60.3967	0.08	92.16
Unspecified immune cells	CD45+	44.6614	45.1574	8.54	62.53
	CD3-CD45+	36.264	36.5055	1.36	59.18
	CD4+	26.5679	25.3837	6.11	58.49
	CD3-CD4+	13.7241	12.8978	2.14	38.01
	CD56+	0.0552	0.0098	0	3.21
Cytotoxic cells	GrB+	0.9858	0.212	0	32.85
	GrB+CD45+	0.4182	0.1089	0	8.02
	GrB+CD3+	0.369	0.056	0	24.86
	GrB+CD56+CD3-	0.0011	0	0	0.11
	GrB+CD56+CD3-CD45+	0.0003	0	0	0.01
	GrB+CD3-	0.6144	0.1474	0	8
Immune checkpoint molecules	PD-L1+	2.9993	1.325	0.01	22.69
	TIM3+	9.1892	5.5684	0.16	46.8
	PD-1+	8.2321	6.3024	0.47	33.78
	PD-L1+TIM3+	1.1368	0.3799	0	12.6
	PD-L1+PD-1+	1.2925	0.6881	0	8.49
Immune checkpoint expressing T cells	PD-1+CD3+	5.148	3.9601	0.1	29.81
	TIM3+CD3+	3.3353	1.8406	0.02	22.59
	PD-L1+CD3+	1.3273	0.5554	0	11.05
	PD-L1+PD-1+CD3+	0.786	0.3494	0	5.46
	PD-1+CD4+CD3+	3.7949	2.4235	0.07	25.84
	PD-L1+CD4+CD3+	1.0719	0.476	0	9.55
	PD-L1+PD-1+CD4+CD3+	0.6634	0.2942	0	5.11
	PD-1+CD4-CD3+	1.3531	0.9965	0.02	7.73
	PD-L1+CD4-CD3+	0.2554	0.0844	0	3.95
	PD-L1+PD-1+CD4-CD3+	0.1226	0.0627	0.0003	1.0317
Immune checkpoint expressing macrophages	TIM3+CD68+	2.2954	1.536	0.01	14.63
	PD-L1+CD68+	1.0684	0.5386	0	6.8
	PD-1+CD68+	1.0459	0.7047	0.02	7.2
	PD-L1+TIM3+CD68+	0.5807	0.2541	0	5.35
	PD-L1+PD-1+CD68+	0.304	0.1474	0	3.02
	TIM3+CD163+	2.7969	0.7445	0	24.74
	PD-L1+CD163+	1.1669	0.2674	0	13.61
	PD-1+CD163+	1.2176	0.4807	0	11.35
	PD-L1+TIM3+CD163+	0.5568	0.0975	0	9.3
	PD-L1+PD-1+CD163+	0.4065	0.0859	0	4.11
	TIM3+CD163+CD68+	1.1399	0.4775	0	10.44
	PD-L1+CD163+CD68+	0.5983	0.1922	0	5.77
	PD-1+CD163+CD68+	0.4751	0.1925	0	4.9
	PD-L1+TIM3+CD163+CD68+	0.3122	0.0752	0	4.49
	PD-L1+PD-1+CD163+CD68+	0.1753	0.0457	0	2.64
	TIM3+CD163-CD68+	1.1614	0.6767	0.01	8.24
	PD-L1+CD163-CD68+	0.4742	0.2972	0	3.18
	PD-1+CD163-CD68+	0.5771	0.4006	0.01	3.79

	PD-L1+TIM3+CD163-CD68+	0.2703	0.1357	0	2.13
	PD-L1+PD-1+CD163-CD68+	0.1316	0.0807	0	0.91
	PD-L1+CD4+CD68+	0.7404	0.3338	0	6.31
	PD-1+CD4+CD68+	0.8859	0.606	0.02	6.61
	PD-L1+PD-1+CD4+CD68+	0.2743	0.1329	0	2.79
Immune checkpoint expressing NK cells	TIM3+CD56+CD3-	0.0064	0.0012	0	0.08
	TIM3+CD56+CD3-CD45+	0.0044	0.0007	0	0.08
Immune checkpoint expressing B cells	TIM3+CD20+	4.8583	2.4151	0	34.7
	PD-L1+CD20+	1.4918	0.6564	0	20.75
	PD-L1+TIM3+CD20+	0.6303	0.2138	0	11.47
Unspecified immune checkpoint expressing cells	TIM3+CD45+	6.226	3.4665	0.02	36.24
	TIM3+CD3-	6.2015	3.2318	0.01	41.05
	TIM3+CD68-	6.4251	3.0131	0.03	35.21
	TIM3+CD20-	3.8481	0.8507	0.02	40.54
	TIM3+GrB+	0.3354	0.0556	0	4.91
	PD-L1+CD3-	1.848	0.7587	0.01	18.87
	PD-L1+CD3-CD4+	0.9362	0.371	0	9.85
	PD-L1+CD68-	1.9138	0.7528	0.01	21.64
	PD-L1+CD20-	1.2581	0.4007	0.01	16.51
	PD-1+CD3-	3.0841	1.8092	0.17	23.5
	PD-1+CD3-CD4+	1.0302	0.7565	0.04	4.47
	PD-1+CD68-	7.1719	5.565	0.42	28.48
	PD-L1+PD-1+CD3-	0.5065	0.2879	0	5.89
	PD-L1+PD-1+CD3-CD4+	0.2553	0.1242	0	3.32
	PD-L1+PD-1+CD68-	0.9917	0.5837	0	5.96
Proportion T cells expressing immune checkpoint molecules	PD-1+CD3+/CD3+	25.7243	24.8914	1.51	62.82
	TIM3+CD3+/CD3+	26.3649	20.9115	0.21	97.22
	PD-L1+CD3+/CD3+	6.3508	4.222	0.08	37.33
	PD-L1+PD-1+CD3+/CD3+	3.8775	2.6883	0.02	32.07
	PD-1+CD4+CD3+/CD4+CD3+	29.0796	27.4973	1.23	61.47
	PD-L1+CD4+CD3+/CD4+CD3+	8.6076	5.3314	0.09	54.79
	PD-L1+PD-1+CD4+CD3+/CD4+CD3+	5.3417	3.4567	0.03	47.61
	PD-1+CD4-CD3+/CD4-CD3+	19.1875	16.7218	0.84	64.19
	PD-L1+CD4-CD3+/CD4-CD3+	3.1507	1.4152	0.02	25.22
	PD-L1+PD-1+CD4-CD3+/CD4-CD3+	1.6647	0.9469	0.02	12.56
Proportion of macrophages expressing immune checkpoint molecules	PD-L1+CD68+/CD68+	9.1753	5.9764	0.09	42.8
	TIM3+CD68+/CD68+	19.5377	14.584	0.09	67.96
	PD-1+CD68+/CD68+	9.271	7.5281	0.35	41.93
	PD-L1+TIM3+CD68+/CD68+	4.7154	2.43	0	27.33
	PD-L1+PD-1+CD68+/CD68+	2.7695	1.7685	0.01	19.45
	PD-L1+CD163+CD68+/CD163+CD68+	11.5686	7.6547	0	52.01
	TIM3+CD163+CD68+/CD163+CD68+	25.1285	21.0324	0	77.81
	PD-1+CD163+CD68+/CD163+CD68+	9.5549	8.1337	0	46.42
	PD-L1+TIM3+CD163+CD68+/CD163+CD68+	5.873	3.1107	0	33.56
	PD-L1+PD-1+CD163+CD68+/CD163+CD68+	3.3834	2.2892	0	25.05
	PD-L1+CD163-CD68+/CD163-CD68+	7.3976	5.2716	0.03	37.88
	TIM3+CD163-CD68+/CD163-CD68+	16.3864	11.7623	0.1	59.83
	PD-1+CD163-CD68+/CD163-CD68+	9.2283	7.4636	0.6	37.6
	PD-L1+TIM3+CD163-CD68+/CD163-CD68+	3.8634	2.0822	0	22.86
	PD-L1+PD-1+CD163-CD68+/CD163-CD68+	2.2864	1.5441	0	12.22
Proportion of NK cells expressing immune checkpoint molecules	TIM3+CD56+CD3-/CD56+CD3-	27.8687	20	0	100
	TIM3+CD56+CD3-CD45+/CD56+CD3-CD45+	29.3928	20.4133	0	100
Proportion of B cells expressing immune checkpoint molecules	PD-L1+CD20+/CD20+	3.1077	1.1314	0	37.47
	TIM3+CD20+/CD20+	9.5093	5.4205	0.02	52.19
	PD-L1+TIM3+CD20+/CD20+	1.3046	0.4396	0	21.44

**Supplementary Table S4: Patient demographics for the groups with a lymphocyte high/macrophage low and a lymphocyte high/macrophage high or a lymphocyte low LME in the mIHC and Reddy et al. cohorts**

Characteristics	mIHC cohort		<i>P</i>	Reddy et al. cohort		<i>P</i>
	T cell high / Macrophage low n (%)	Other n (%)		T cell high / Macrophage low n (%)	Other n (%)	
No of patients	28 (100)	143 (100)		185 (100)	311 (100)	
Age			0.409			0.055
<60	16 (57)	67 (47)		86 (49)	119 (40)	
≥60	12 (43)	76 (53)		89 (51)	179 (60)	
nd				10	13	
Gender			1.000			0.710
Male	16 (57)	83 (58)		103 (56)	167 (54)	
Female	12 (43)	60 (42)		82 (44)	144 (46)	
Molecular subtype			0.023			0.781
GCB <sup>a</sup>	14 (70)	47 (41)		84 (45)	133 (43)	
ABC	3 (15)	51 (44)		72 (39)	131 (42)	
Unclassified	3 (15)	17 (15)		29 (16)	47 (15)	
nd	8	28				
WHO PS			0.251			0.177
0-1	23 (82)	98 (70)		135 (79)	214 (74)	
≥2	5 (18)	42 (58)		35 (21)	77 (26)	
nd		1		15	20	
Stage			0.037			0.005
I-II	18 (64)	59 (42)		81 (45)	98 (32)	
III-IV	10 (36)	83 (58)		99 (55)	208 (68)	
nd		1		5	5	
IPI			<0.001			<0.001
0-2	23 (82)	66 (47)		99 (69)	113 (46)	
3-5	5 (18)	74 (53)		45 (31)	131 (54)	
nd		3		41	67	
Elevated LDH			0.017			0.001
Yes	12 (43)	95 (68)		95 (57)	111 (41)	
No	16 (57)	45 (32)		72 (43)	167 (59)	
nd		3		18	37	
EN			0.348			0.348
0-1	23 (82)	96 (72)		132 (77)	209 (72)	
≥2	5 (18)	38 (28)		40 (23)	83 (28)	
nd		9		13	19	
B-symptoms			0.137			
Yes	7 (25)	57 (41)				
No	21 (75)	81 (59)				
nd		5				
Double expressor			0.233			
Yes	5 (22)	43 (36)				
No	18 (78)	77 (64)				
nd	5	23				
Double hit			1.000			
Yes	1 (4)	7 (6)				
No	23 (96)	112 (94)				
nd	4	24				
Treatment			0.044			
R-CHOP	23 (82)	91 (64)				
R-CHOEP	4 (14)	50 (35)				

R-CHOP like <sup>b</sup>	1 (4)	2 (1)		
5-year PFS	92.6 %	69.8 %		
5-year OS	92.3 %	74.4 %	69.7 %	58.3 %

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<sup>a</sup>GCB, germinal center B-cell like; ABC, activated B-cell like; nd, not determined; IPI, international prognostic index; LDH, lactate dehydrogenase; EN, extranodal site; R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine, prednisone; R-CHOEP, R-CHOP+etoposide; <sup>b</sup>R-CHOP like, one R-CHOP/R-CHEP, one R-CHOP/R-CNOP, one R-CHOP/R-mini-CHOP

**Supplementary Table S5: Proportions of immune cell subtypes inferred by CIBERSORT in the Reddy et al. cohort**

Cell subtype	Mean (%)	Median (%)	Min (%)	Max (%)
T cells	23.22	21.60	4.96	65.06
CD8 T cells	6.90	5.75	0.00	36.51
CD4 T cells	15.80	14.31	2.84	50.26
Naive CD4 T cells	0.61	0.00	0.00	13.87
Resting memory CD4 T cells	5.87	4.52	0.00	34.79
Activated memory CD4 T cells	2.97	1.88	0.00	18.74
Follicular helper T cells	5.28	4.47	0.00	27.27
Regulatory T cells	1.07	0.00	0.00	11.28
Gamma delta T cells	0.53	0.00	0.00	10.26
Macrophages	18.49	17.15	3.08	49.41
M0 macrophages	6.85	5.95	0.00	26.07
M1 macrophages	4.39	3.76	0.00	19.10
M2 macrophages	7.25	6.51	0.00	29.90
Monocytes	0.56	0.00	0.00	10.72
NK cells	3.24	2.78	0.00	13.66
Resting NK cells	1.57	0.34	0.00	12.84
Activated NK cells	1.67	1.03	0.00	13.66
Naive B cells	11.01	7.83	0.00	65.95
Memory B cells	31.13	29.39	0.00	73.01
Plasma cells	2.64	0.30	0.00	29.37
Dendritic cells	1.08	0.25	0.00	10.37
Resting dendritic cells	0.37	0.00	0.00	8.47
Activated dendritic cells	0.71	0.00	0.00	10.37
Mast cells	7.62	7.39	0.00	25.07
Resting mast cells	7.22	6.99	0.00	25.07
Activated mast cells	0.40	0.00	0.00	15.02
Eosinophils	0.63	0.00	0.00	8.63
Neutrophils	0.37	0.00	0.00	7.41

**Supplementary Table S6: Differential pathways enriched in the T cell high group in the mIHC group.** Please see separate Excel file.

**Supplementary Table S7: Differential pathways enriched in the T cell high group in the Reddy et al. cohort.** Please see separate Excel file.

**Supplementary Table S8: Differential pathways enriched in the T cell high/macrophage low group in the Reddy et al. cohort.** Please see separate Excel file.

**Supplementary Table S9: Differential pathways enriched in the T cell high/Macrophage low group compared to T cell high/Macrophage high group in the Reddy et al. cohort.** Please see separate Excel file.

**Supplementary Table S10: Differently expressed genes in the T cell high/macrophage low group in the Reddy et al. cohort.** Please see separate Excel file.



**Supplementary Table S11: Patient demographics for the Chapuy et al. cohort**

Characteristics	Chapuy et al. cohort n (%)
No of patients	137 (100)
Age	
median (range)	67 (26-93)
<60	44 (34)
≥60	86 (66)
nd	7
Gender	
Male	76 (55)
Female	61 (45)
Molecular subtype	
GCB <sup>a</sup>	
ABC	
Unclassified	
nd	137
WHO PS	
0-1	87 (81)
≥2	21 (19)
nd	29
Stage	
I-II	39 (36)
III-IV	69 (64)
nd	29
IPI	
0-2	60 (58)
3-5	44 (42)
nd	33
Elevated LDH	
Yes	52 (50)
No	52 (50)
nd	33
EN	
0-1	80 (74)
≥2	28 (26)
nd	29
Treatment	
R-CHOP like	101 (78)
other	29 (22)
nd	7
5-year PFS	64.3 %
5-year OS	70.6 %

<sup>a</sup>GCB, germinal center B-cell like; ABC, activated B-cell like; nd, not determined; IPI, international prognostic index; LDH, lactate dehydrogenase; EN, extranodal site; R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine, prednisone