

⁵⁰PFS (mo)

Supplementary Fig. S4: The clinical impact of macrophages, NK cells and T cells in the **DLBCL LME analyzed by mIHC**

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Supplementary Fig. S4: The clinical impact of macrophages, NK cells and T cells in the DLBCL LME analyzed by mIHC. A) Unsupervised hierarchical clustering of samples based on the proportion of macrophages. B) Unsupervised hierarchical clustering of samples based on the proportion of NK cells. C-E) Boxplots depicting the proportions of macrophages (C), NK cells (D), and regulatory T cells (E), in the groups with high, intermediate, and low proportions of T cells. F-O) Boxplots depicting the proportions of T cells (F), CD4+ T cells (G), CD8+ T cells (H), CD163+ macrophages (I), CD163- macrophages (J), B cells (K), PD-1+ cells (L), TIM3+ cells (M), PD-L1+ cells (N), and LAG3+ cells (O) in the groups with high and low proportion of T cells. P-Q) Kaplan-Meier (log-rank test) survival plot depicts PFS in the T cell high/macrophage low (T cell high/M low), T cell high/macrophage high (T cell high/M high), and the T cell low groups (Q).

Supplementary Fig. S6: The clinical impact of macrophages in the T cell high and low groups in non-double hit DLBCL patients in the mIHC cohort



Supplementary Fig. S7: The clinical impact of the macrophage/T cell proportion in the mIHC cohort



Supplementary Fig. S8: CIBERSORT deconvolution analysis in the Reddy et al. validation cohort



Supplementary Fig. S9: The clinical impact of macrophages, NK cells and T cells in the LME analyzed by CIBERSORT in the Reddy et al. validation cohort



Supplementary Fig. S10: Differential expression of genes and pathways between distinct LME phenotypes







Supplementary Fig. S11: Clinical impact of immune checkpoint expressing macrophages

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All patients	OS HR (95% CI)				Ρ
CD68+ (n=176)	1.08 (1.01-1.16)		⊢- ● -I		0.031
CD163+ (n=175)	1.01 (0.99-1.04)		•		0.343
T cell high group					
CD68+ (n=102)	1.11 (1.01-1.23)		⊢ • +		0.032
CD163+ (n=101)	1.02 (0.99-1.05)		1 0 1		0.248
T cell low group					
CD68+ (n=70)	1.13 (0.99-1.29)		F		0.061
CD163+ (n=70)	1.05 (0.97-1.13)		H		0.205
		0.80	1.0	1.5	

C All patients

Macrophage subtype	PFS HR (95% CI)	P
PD-L1+CD68+ (n=175)	1.12 (0.91-1.36)	F I	0.280
PD-L1+CD163+CD68+ (n=174)	0.99 (0.73-1.33)	F	0.936
PD-L1+CD163-CD68+ (n=175)	1.78 (1.18-2.68)	+ ● I	0.006
TIM3+CD68+ (n=165)	1.09 (0.98-1.21)	•	0.128
TIM3+CD163+CD68+ (n=164)	1.05 (0.89-1.25)	⊢ ● I	0.562
TIM3+CD163-CD68+ (n=165)	1.20 (1.01-1.44)	H O H	0.043
PD-L1+TIM3+CD68+ (n=165)	1.18 (0.88-1.58)	⊬-●	0.278
PD-L1+TIM3+CD163+CD68+ (n=164)	0.88 (0.51-1.53)	F4	0.662
PD-L1+TIM3+CD163-CD68+ (n=165)	2.36 (1.37-4.06)	F4	0.002
PD-1+CD68+ (n=171)	1.20 (0.98-1.46)	⊢ ●-1	0.073
PD-1+CD163+CD68+ (n=168)	1.25 (0.92-1.72)	⊢- ● I	0.155
PD-1+CD163-CD68+ (n=171)	1.36 (0.92-2.01)	F	0.121
		0.50 1.0 2.0 5.0	

E Model 1

Variable	PFS HR (95% CI)		P
PD-L1+TIM3+CD163-CD68+	1.88 (1.05-3.37)	F	0.035
IPI 3-5 (n=77/162)	2.88 (1.44-5.74)	⊢ ● I	0.003
-	0	.50 1.0 2.0 10.0	
Model 2			
Variable	PFS HR (95% CI)		P
PD-L1+TIM3+CD163-CD68+	2.22 (1.05-4.68)	F	0.036
IPI 3-5 (n=77/162)	1.62 (0.71-3.68)	H	0.249
ABC (n=53/128)	2.79 (1.21-6.46)	F	0.016
<u> </u>	0	.50 1.0 2.0 10.0	
Model 3			
Variable	PFS HR (95% CI)		P
PD-L1+TIM3+CD163-CD68+	2.02 (0.95-4.29)	+ - 4	0.068
IPI 3-5 (n=77/162)	1.75 (0.77-4.01)	+•	0.183
ABC (n=53/128)	2.22 (0.94-5.26)	F	0.070
DPE (n=48/137)	1.63 (0.74-3.57)	+	0.221
	0	50 1 0 2 0 10 0	

J T cell low group

Macrophages/Monocytes	OS HR (95% CI)		P
HAVCR2 (TIM3)	2.66 (0.65-11.0)	F4	0.176
CD274 (PD-L1)	0.80 (0.56-1.15)	H O H	0.231
PDCD1LG2 (PD-L2)	0.63 (0.24-1.62)	⊢ ● 1	0.335
		0.20 1.0.20 10.0	

В			
All patients	PFS HR (95% CI)		P
CD68+ (n=176)	1.07 (1.01-1.14)	F	0.034
CD163+ (n=175)	1.01 (0.98-1.03)	•	0.549
T cell high group			
CD68+ (n=102)	1.09 (1.00-1.19)	► ● I	0.052
CD163+ (n=101)	1.01 (0.98-1.04)		0.681
T cell low group			
CD68+ (n=70)	1.11 (0.98-1.26)	F	0.098
CD163+ (n=70)	1.06 (0.99-1.14)	H	0.103



H T cell high group

Macrophage subtype	PFS HR (95% CI)	P
PD-L1+CD68+ (n=102)	1.16 (0.92-1.47)	⊢●⊣	0.206
PD-L1+CD163+CD68+ (n=101)	1.00 (0.72-1.39)	H	1.000
PD-L1+CD163-CD68+ (n=102)	2.13 (1.31-3.47)	H	0.002*
TIM3+CD68+ (n=96)	1.12 (0.98-1.27)	•	0.100
TIM3+CD163+CD68+ (n=95)	1.05 (0.86-1.28)	H O H	0.632
TIM3+CD163-CD68+ (n=96)	1.28 (1.04-1.59)	H O H	0.021
PD-L1+TIM3+CD68+ (n=96)	1.22 (0.87-1.70)	H	0.248
PD-L1+TIM3+CD163+CD68+ (n=95) 0.91 (0.51-1.65)	+ 6 1	0.766
PD-L1+TIM3+CD163-CD68+ (n=96) 2.76 (1.44-5.28)	H	0.002*
PD-1+CD68+ (n=102)	1.20 (0.95-1.52)	⊢●⊣	0.132
PD-1+CD163+CD68+ (n=100)	1.29 (0.91-1.83)	F	0.159
PD-1+CD163-CD68+ (n=102)	1.28 (0.79-2.07)	H	0.323
		0.50 1.0 2.0	10.0

L T cell low group PFS HR (95% CI) Р Macrophage subtype PD-L1+CD68+ (n=70) 1.15 (0.70-1.91) 0.581 F-PD-L1+CD163+CD68+ (n=70) 1.09 (0.44-2.73) 0.851 1.66 (0.58-4.78) PD-L1+CD163-CD68+ (n=70) 0.345 . TIM3+CD68+ (n=68) 1.20 (0.90-1.59) 0.210 TIM3+CD163+CD68+ (n=68) 1.37 (0.84-2.24) 0.205 . TIM3+CD163-CD68+ (n=68) 1.21 (0.79-1.85) 0.373 PD-L1+TIM3+CD68+ (n=68) 2.23 (0.80-6.23) 0.126 PD-L1+TIM3+CD163+CD68+ (n=68) 1.49 (0.15-14.5) 0.730 PD-L1+TIM3+CD163-CD68+ (n=68) 6.04 (1.28-28.6) 0 024 PD-1+CD68+ (n=67) 2.13 (1.18-3.86) 0.012 PD-1+CD163+CD68+ (n=66) 3.16 (0.95-10.5) 0.061 PD-1+CD163-CD68+ (n=67) 2.78 (1.20-6.43) 0.017 . 20.0

Supplementary Fig. S12: Clinical impact of immune checkpoint expressing macrophages in non-double hit DLBCL patients

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All patients	OS HR (95% CI)				P
CD68+ (n=168)	1.09 (1.02-1.17)		F		0.018
CD163+ (n=167)	1.01 (0.99-1.04)		•		0.269
T cell high group					
CD68+ (n=97)	1.12 (1.01-1.32)		F		0.028
CD163+ (n=96)	1.02 (0.99-1.05)		1 11		0.246
T cell low group					
CD68+ (n=67)	1.14 (1.00-1.30)		F		0.050
CD163+ (n=67)	1.06 (0.98-1.14)		F		0.157
		0.80	1.0	1.5	

C All patients

Macrophage subtype	OS HR (95% CI)	Р
PD-L1+CD68+ (n=167)	1.16 (0.94-1.43)	⊨ ⊕ -+	0.155
PD-L1+CD163+CD68+ (n=166)	1.06 (0.78-1.43)	H	0.728
PD-L1+CD163-CD68+ (n=167)	1.84 (1.19-2.86)	+ - ++	0.006
TIM3+CD68+ (n=157)	1.13 (1.02-1.26)	•	0.026
TIM3+CD163+CD68+ (n=156)	1.11 (0.94–1.31)	E 🔴 H	0.221
TIM3+CD163-CD68+ (n=157)	1.25 (1.05-1.50)	H 🔴 H	0.014
PD-L1+TIM3+CD68+ (n=157)	1.28 (0.95-1.72)	⊢- ● 1	0.107
PD-L1+TIM3+CD163+CD68+ (n=156)	0.97 (0.56-1.69)	FB	0.923
PD-L1+TIM3+CD163-CD68+ (n=157)	2.85 (1.62-5.00)	F	<0.001
PD-1+CD68+ (n=163)	1.26 (1.03-1.55)	F 🔴 - 1	0.028
PD-1+CD163+CD68+ (n=160)	1.40 (1.02-1.92)	⊨ - +	0.037
PD-1+CD163-CD68+ (n=163)	1.38 (0.91–2.10)	F4	0.128
		0.50 1.0 2.0 5.0	

E Model 1

Variable	OS HR (95% CI)		P
PD-L1+TIM3+CD163-CD68+	2.18 (1.18-4.02)	●	0.013
IPI 3-5 (n=71/154)	3.54 (1.95-10.6)	+	<0.001
F	0.	50 1.0 2.0 10.0	
F Model 2			
Variable	OS HR (95% CI)		P
PD-L1+TIM3+CD163-CD68+	2.81 (1.27-6.26)	F	0.011
IPI 3-5 (n=71/154)	1.96 (0.76-5.05)	++	0.162
ABC (n=52/120)	4.42 (1.54-12.6)	F	0.007
0	0.	50 1.0 2.0 10.0	
G Model 3			
Variable	OS HR (95% CI)		P
PD-L1+TIM3+CD163-CD68+	2.56 (1.11-5.92)	F	0.028
IPI 3-5 (n=71/154)	2.08 (0.80-5.46)	+ - +	0.135
ABC (n=52/120)	3.68 (1.21-11.2)	+I	0.022
DPE (n=43/130)	1.40 (0.57-3.44)	F==	0.462
	0.	50 1.0 2.0 10.0	

3				
All patients	PFS HR (95% CI)			Р
CD68+ (n=168)	1.08 (1.01-1.16)		⊢-●	0.018
CD163+ (n=167)	1.01 (0.98-1.04)		•	0.490
T cell high group				
CD68+ (n=97)	1.10 (1.01-1.21)		H	0.032
CD163+ (n=96)	1.01 (0.98-1.04)			0.682
T cell low group				
CD68+ (n=67)	1.12 (0.98-1.27)		F	0.089
CD163+ (n=67)	1.07 (0.99-1.15)		⊢- ● I	0.078
		0.80	1.0	1.5



H T cell high group

Macrophage subtype	OS HR (95% CI)	P
PD-L1+CD68+ (n=97)	1.22 (0.95-1.57)	F 🔴-1	0.116
PD-L1+CD163+CD68+ (n=96)	1.08 (0.77-1.51)	H	0.665
PD-L1+CD163-CD68+ (n=97)	2.14 (1.28-3.60)	H4	0.004*
TIM3+CD68+ (n=91)	1.17 (1.02-1.33)	•	0.023
TIM3+CD163+CD68+ (n=90)	1.11 (0.91-1.35)	H O H	0.287
TIM3+CD163-CD68+ (n=91)	1.33 (1.07-1.64)	H O H	0.009*
PD-L1+TIM3+CD68+ (n=91)	1.33 (0.95-1.88)	H	0.100
PD-L1+TIM3+CD163+CD68+ (n=90)	1.00 (0.55-1.82)	F	0.994
PD-L1+TIM3+CD163-CD68+ (n=91)	3.40 (1.73-6.69)	+I	<0.001
PD-1+CD68+ (n=97)	1.29 (1.01-1.64)	H 🔴 - I	0.039
PD-1+CD163+CD68+ (n=95)	1.50 (1.06-2.12)	⊢-●-⊣	0.024
PD-1+CD163-CD68+ (n=97)	1.30 (0.77-2.20)	F4	0.331
		0.50 1.0 2.0	10.0

I T cell low group

Macrophage subtype	OS HR (95% CI)	Ρ
PD-L1+CD68+ (n=67)	1.23 (0.75-2.01)	H	0.422
PD-L1+CD163+CD68+ (n=67)	1.20 (0.48-2.96)	+ 0 4	0.696
PD-L1+CD163-CD68+ (n=67)	1.93 (0.67-5.55)	FI	0.224
TIM3+CD68+ (n=65)	1.29 (0.97-1.72)	H O I	0.083
TIM3+CD163+CD68+ (n=65)	1.61 (0.97-2.66)	F	0.065
TIM3+CD163-CD68+ (n=65)	1.28 (0.84-1.94)	H	0.246
PD-L1+TIM3+CD68+ (n=65)	2.55 (0.90-7.27)	►	0.079
PD-L1+TIM3+CD163+CD68+ (n=65)	2.14 (0.23-20.2)		0.507
PD-L1+TIM3+CD163-CD68+ (n=65)	6.52 (1.31-32.6)	>	0.022
PD-1+CD68+ (n=64)	2.33 (1.23-4.43)	H	0.010
PD-1+CD163+CD68+ (n=63)	3.43 (0.91-13.0)	F	0.069
PD-1+CD163-CD68+ (n=64)	3.01 (1.27-7.16)	+•	0.013
		0 20 0 50 10 20 200	1