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. S11: Clinical impact of immune checkpoint expressing macrophages. A-B) Forest plot visualizing the impact of macrophages and CD163+ macrophages on OS (A) and PFS (B) in the entire mIHC cohort and in the T cell high and low groups, as evaluated by Cox regression univariate analyses with continuous variables. Bad cores were removed from the analyses. C) Forest plot visualizing the impact of immune checkpoint expressing macrophages on PFS in the mIHC cohort, as evaluated by Cox regression univariate analyses with continuous variables. Bad cores were removed from the analyses. D) Kaplan-Meier (log-rank test) survival plot depicts PFS in patients with high and low proportion of PD-L1+TIM3+CD163- macrophages using median cut-off in the mIHC cohort. E-G) Forest plots visualizing the impact of PD-L1+TIM3+CD163- macrophages on PFS in Cox regression multivariate analyses with IPI (E), IPI and molecular subtype (F), and IPI, molecular subtype, and double protein expression (DPE) (G) in the mIHC cohort. H-I) Forest plots visualizing the impact of immune checkpoint expressing macrophages on PFS in the T cell high (H) and low (I) groups, as evaluated by Cox regression univariate analyses with continuous variables in the mIHC cohort. Bad cores were removed from analyses. J) Forest plot visualizing the impact of the gene expression of immune checkpoint molecules in macrophages, inferred with CIBERSORTX, on OS in the T cell low group in the Reddy et al. cohort, as evaluated by Cox regression univariate analyses with continuous variables. *P≤0.05 after FDR correction.