Supplemental material

Supplementary table S1. Overview of the three retrospective collections of NSCLCs used in this study

Cohort	Total number of tumors	Number of quantified tumors	Clinical annotation	Immunotherapy treated	Tumor genotype available
Cohort 1 (YTMA250)	288	258	Yes	No	No
Cohort 2 (YTMA310)	138	111	No	Unknown	Yes
Cohort 3 (YTMA404)	81	69	Yes	Yes	Yes
Total	507	438			

Supplementary table S2. Panel for multiplexed CMTM6 and PD-L1 immunofluorescence staining

	Cytokeratin	CD68	CMTM6	PDL1
Primary antibodies	Rabbit polyclonal (Agilent) 1/100, 1 hour incubation	Clone PG-M1 (mouse IgG3, Abcam), 1 ug/ml, 4ºC overnight incubation	Clone RCT6 (mouse IgG1, Absea), 0.4 ug/ml, 4ºC overnight incubation	Clone E1L3N (rabbit IgG, Cell Signaling), 1 ug/ml, 4º overnight incubation
Secondary antibodies	Anti-rabbit Alexa488 (Invitrogen), 1/100, 1 hour incubation, RT	Anti-mouse IgG3 (ab97260, Abcam), 1/1000, 1 hour incubation, RT	Anti-mouse IgG1 (M1-14D12, eBioscience), 1/100, 1 hour incubation, RT	Anti-rabbit EnVision (K4009, Agilent), 1 hour incubation, RT
Fluorescent reagents		Biotynilated tyramid/Streptavidine-Alexa750 conjugate (Perkin-Elmer), 1 hour incubation, RT	Cy3+-tyramide (Perkin-Elmer), 10 min incubation, RT	Cy5-tyramide (Perkin-Elmer), 10 min incubation, RT
Counterstain	DAPI, 1/1000, 5 min incubation, RT			

Supplementary table S3. Panel for multiplexed TILs immunofluorescence staining

	Cytokeratin	**CD8**	**CD4**	**CD20**
Primary antibodies	Clone Z0622 (rabbit IgG, Agilent) 1/100, 1 hour incubation	Clone C8/144B (mouse IgG1, Agilent), 1 ug/ml, 4ºC overnight incubation	Clone SP35 (rabbit IgG, Spring Bioscience), 1 ug/ml, 4ºC overnight incubation	Clone L26 (Mouse IgG2a, Agilent), 1 ug/ml, 4º overnight incubation
Secondary antibodies	Anti-rabbit Alexa456 (Molecular Probes), 1/100, 1 hour incubation, RT	Anti-mouse IgG1 (M1-14D12, eBioscience), 1/100, 1 hour incubation, RT	Anti-rabbit envision (K4009, Agilent), 1 hour incubation, RT	Anti-mouse IgG2 (, Abcam), 1/200, 1 hour incubation, RT
Fluorescent reagents		Fluorescein-tyramide (Perkin-Elmer), 10 min incubation, RT	Biotynilated tyramid/Streptavidine-Alexa750 conjugate (Perkin-Elmer), 1 hour incubation, RT	Cy5-tyramide (Perkin-Elmer), 10 min incubation, RT
Counterstain	DAPI, 1/1000, 5 min incubation, RT			

Supplementary table S4. CMTM6 expression in the tumor compartment and clinicopathological characteristics in two independent NSCLC cohorts

	YTMA404 cohort			YTMA250 cohort		
Characteristic	**CMTM6 low**	**CMTM6 high**	**p value**	**CMTM6 low**	**CMTM6 high**	**p value**
Gender						
Male	16	22	0.18	50	656	0.62
Female	18	13		66	65	
Age						
< 70 yo	15	20	0.27	65	67	0.97
>= 70 yo	19	15		51	53	
ECOG						
0	2	4				
1	26	28	0.29			
2	6	2				
3	0	1				
Smoking history						
Never smoker	6	7		23	15	
Current smoker	8	8	0.78	27	35	0.24
Former smoker	19	20		58	63	
Histology						
Adenocarcinoma	27	23		77	58	
Squamous-cell carcinoma	6	9	0.52	24	39	0.02
Large-cell carcinoma	1	2		3	9	
Others	0	1		11	13	
Stage						
I				75	72	
II				22	23	
III	2	0	0.52	11	19	0.46
IV (M1a)	8	10		6	4	
IV (M1b)	5	5				
IV (M1c)	19	20				
EGFR mutation status						
Wild type	22	22	0.36			
Mutant	6	3				
KRAS mutation status						
Wild type	15	17	0.08			
Mutant	13	5				
CNS metastasis						
No	26	24	0.58			
Yes	8	10				
Liver metastasis						
No	28	28				
Yes	6	6	1.00			
LIPI score						
Good	16	12				
Intermediate	12	14	0.72			
Poor	2	2				

Supplementary table S5. CMTM6 expression in the stromal compartment and clinical-pathological characteristics in two independent NSCLC cohorts

	YTMA404 cohort			YTMA250 cohort		
Characteristic	**CMTM6 low**	**CMTM6 high**	**p value**	**CMTM6 low**	**CMTM6 high**	**p value**
Gender						
Male	19	19	0.89	53	53	0.77
Female	15	16		63	68	
Age						
< 70 yo	16	19	0.54	60	72	0.15
>= 70 yo	18	16		57	47	
ECOG						
0	3	3				
1	27	27	0.80			
2	4	4				
3	0	1				
Smoking history						
Never smoker	5	8	0.63	21	17	0.52
Current smoker	8	8		27	35	
Former smoker	20	19		59	62	
Histology						
Adenocarcinoma	25	25		71	64	
Squamous-cell carcinoma	8	7	0.70	28	35	0.45
Large-cell carcinoma	1	2		4	8	
Others	0	1		13	11	
Stage						
I				77	70	
II				19	26	
III	2	0	0.38	13	17	0.50
IV (M1a)	9	9		6	4	
IV (M1b)	6	4				
IV (M1c)	17	22				
EGFR mutation status						
Wild type	22	22	0.76			
Mutant	4	5				
KRAS mutation status						
Wild type	14	18	0.42			
Mutant	10	8				
CNS metastasis						
No	28	22	0.04			
Yes	5	13				
Liver metastasis						
No	26	30	0.45			
Yes	7	5				
LIPI score						
Good	15	13				
Intermediate	10	16	0.53			
Poor	2	2				

Supplementary table S6. Objective response rates according to CMTM6 expression

		CMTM6 in the tumor compartment			**CMTM6 in the stromal compartment**			**CMTM6 in the CD68 compartment**		
	N	**Low (%)**	**High (%)**	p value	**Low (%)**	**High (%)**	p value	**Low (%)**	**High (%)**	p value
PR	8 (14.8)	3 (10.7)	5 (19.2)	0.37	2 (6.9)	6 (24)	0.078	2 (7.7)	6 (21.4)	0.15
SD+PD	46 (85.2)	25 (89.3)	21 (80.8)		27 (93.1)	19 (76)		24 (92.3)	22 (78.6)	

PR: partial response; SD: stable disease; PD: progressive disease

Supplementary table S7. Objective response rates according to PD-L1 expression

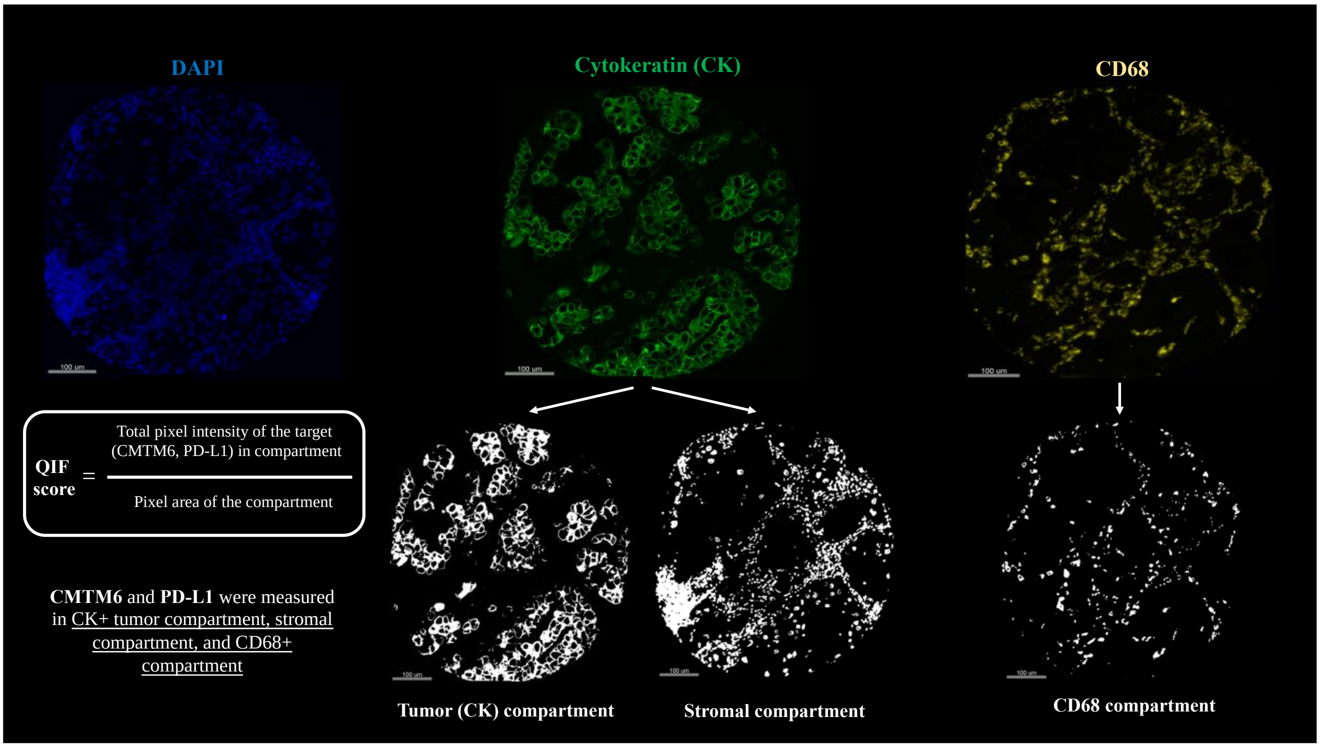
		PDL1 in the tumor compartment			**PDL1 in the stromal compartment**			**PDL1 in the CD68 compartment**		
	N (%)	**Low (%)**	**High (%)**	p value	**Low (%)**	**High (%)**	p value	**Low (%)**	**High (%)**	p value
PR	8 (14.8)	3 (11.1)	5 (18.5)	0.44	1 (3.6)	7 (26.9)	0.016	2 (7.4)	6 (22.2)	0.12
SD+PD	46 (85.2)	24 (88.9)	22 (81.5)		27 (96.4)	19 (73.1)		25 (92.6)	21 (77.8)	

PR: partial response; SD: stable disease; PD: progressive disease

Supplementary table S8. Objective response rates according to CMTM6/PD-L1 co-expression subgroups

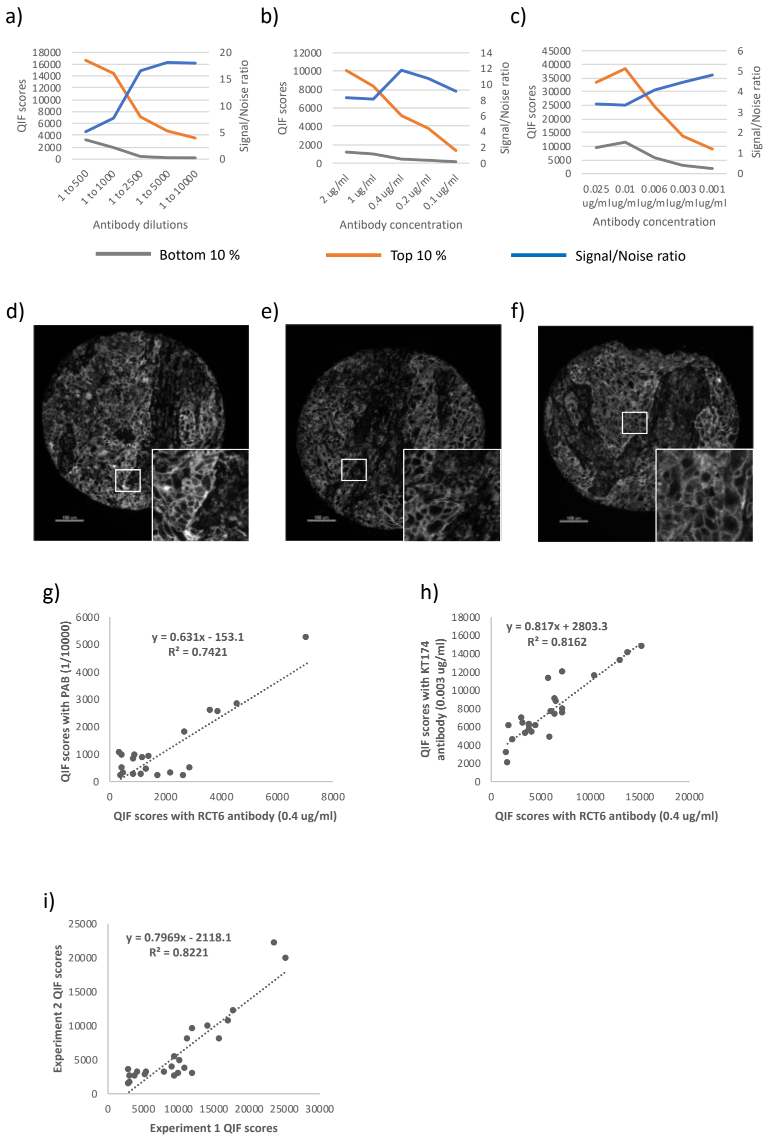
		CMTM6 AND PDL1 in the tumor compartment			**CMTM6 AND PDL1 in the stromal compartment**			**CMTM6 AND PDL1 in the CD68 compartment**		
	N	**Rest (%)**	**Double High (%)**	p value	**Rest (%)**	**Double High (%)**	p value	**Rest (%)**	**Double High (%)**	p value
PR	8 (14.8)	4 (11.1)	4 (22.2)	0.27	2 (5.6)	6 (33.3)	0.007	3 (8.1)	5 (29.4)	0.041
SD+PD	46 (85.2)	32 (88.9)	14 (77.8)		34 (94.4)	12 (66.7)		34 (91.9)	12 (70.6)	

PR: partial response; SD: stable disease; PD: progressive disease

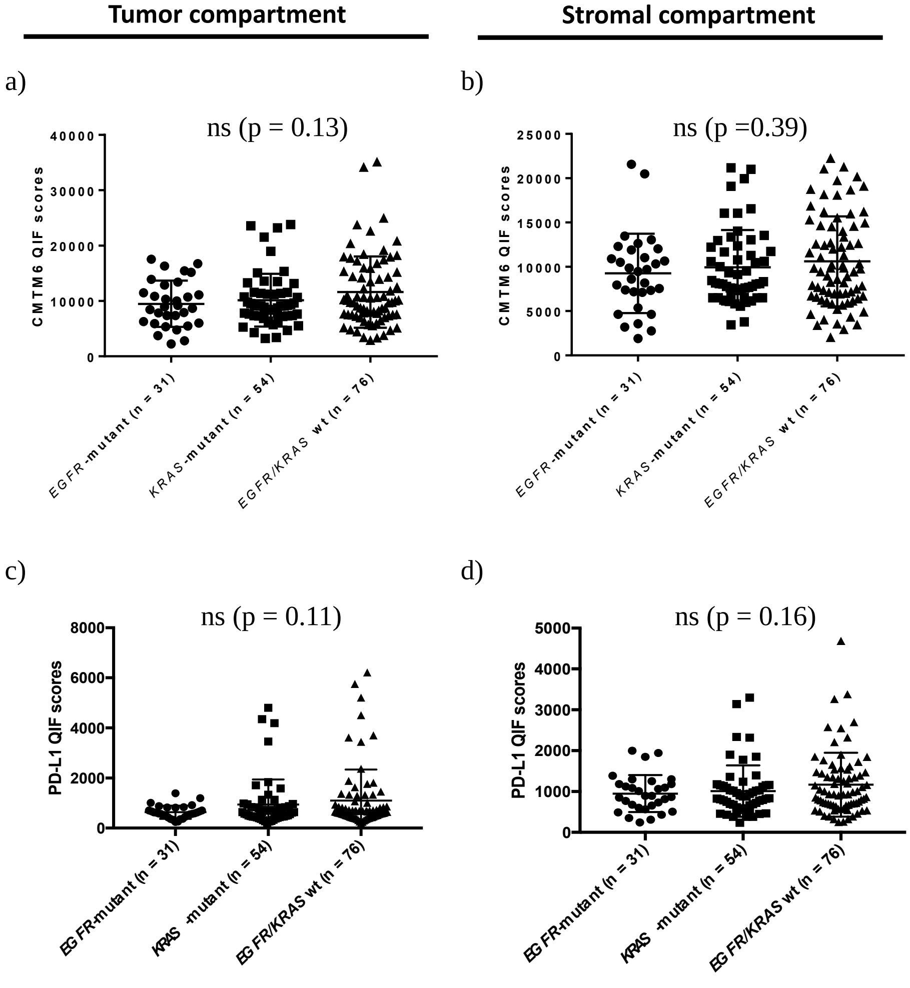


QIF: quantitative immunofluorescence

Supplementary figure S1. Representative image showing tissue compartmentalization and target quantification using the AQUA software

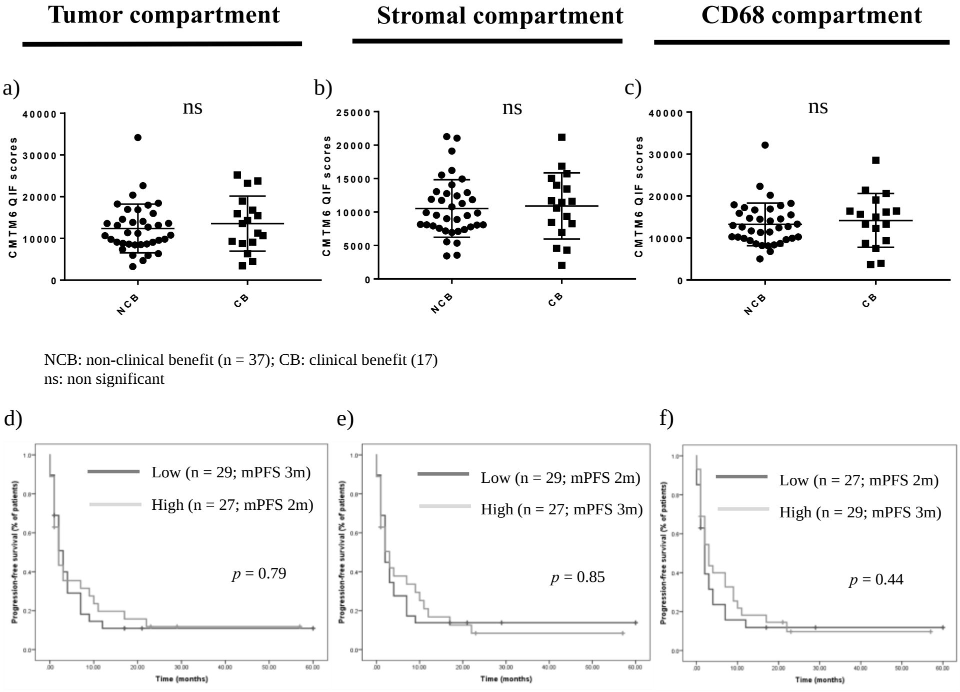


Supplementary figure S2. Validation of anti-CMTM6 antibody clone RCT6. (a)-(c) Titration curves of three different anti-CMTM6 antibodies used for assay validation plotted at five different concentrations: polyclonal antibody (ab198284) (a), monoclonal antibody clone RCT6 (b), and monoclonal antibody clone KT174 (c); (d)-(f) Representative membranous CMTM6 staining pattern with anti-CMTM6 polyclonal antibody (ab198284) (d), anti-CMTM6 monoclonal antibody clone RTC6 (e) and anti-CMTM6 monoclonal antibody clone KT174 (f). (g)-(h) Cross validation of anti-CMTM6 antibody clone RCT6 to the anti-CMTM6 polyclonal antibody (ab198284) (g) and to a second anti-CMTM6 monoclonal antibody (clone KT174) targeting non-overlapping epitopes (h). (i) Reproducibility of CMTM6 measurement with clone RCT6 in two independent multiplexed experiment



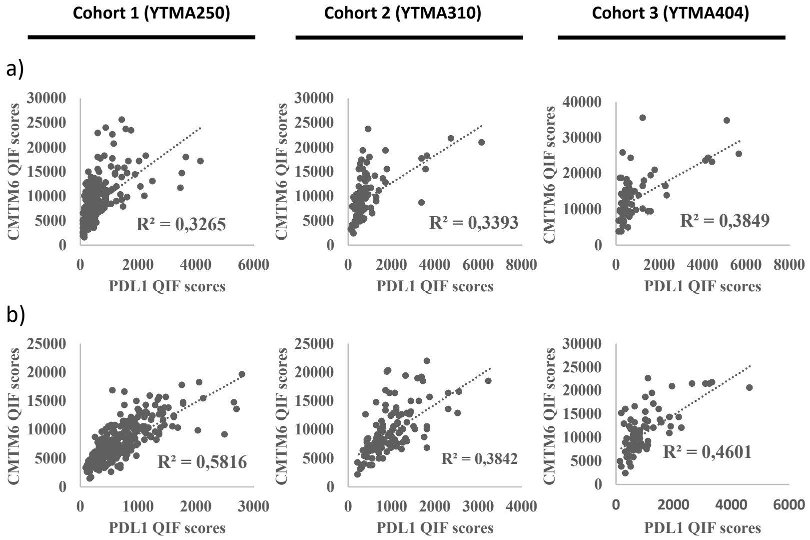
ns: non significant

Supplementary figure S3. CMTM6 and PD-L1 expression levels in the tumor compartment (a and c, respectively) and the stromal compartment (b and d, respectively) in *EGFR*- and *KRAS*-mutant NSCLCs (YTMA310 and YTMA404 cohorts combined)

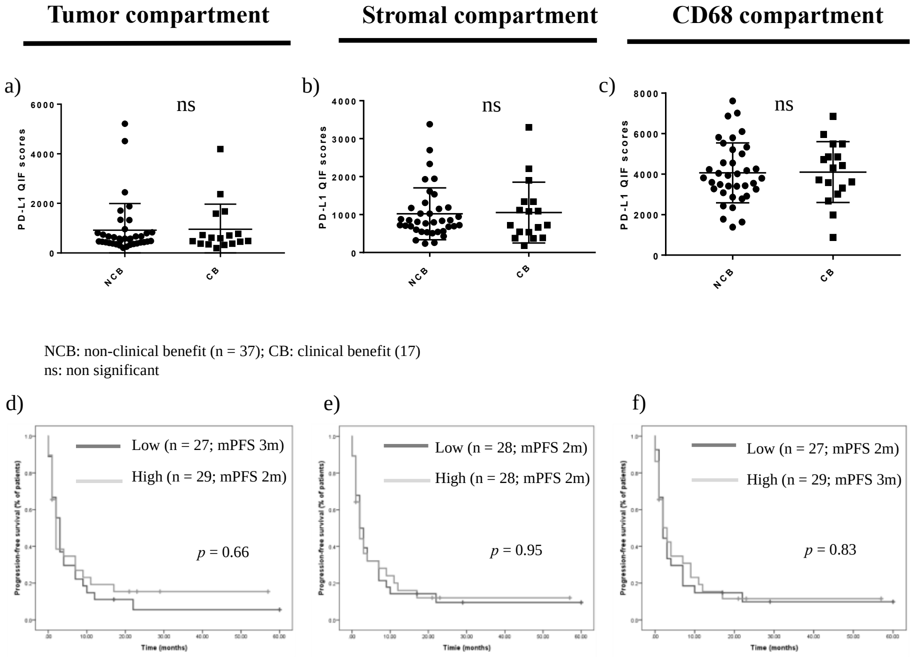


NCB: non-clinical benefit (n = 37); CB: clinical benefit (17)
ns: non significant

Supplementary figure S4. CMTM6 expression levels according to clinical benefit subgroups in the tumor compartment (a), the stromal compartment (b), and the CD68 compartment (c). PFS under PD-1 axis blockade according to CMTM6 expression in the tumor compartment (d), the stromal compartment (e), and the CD68 compartment (f)

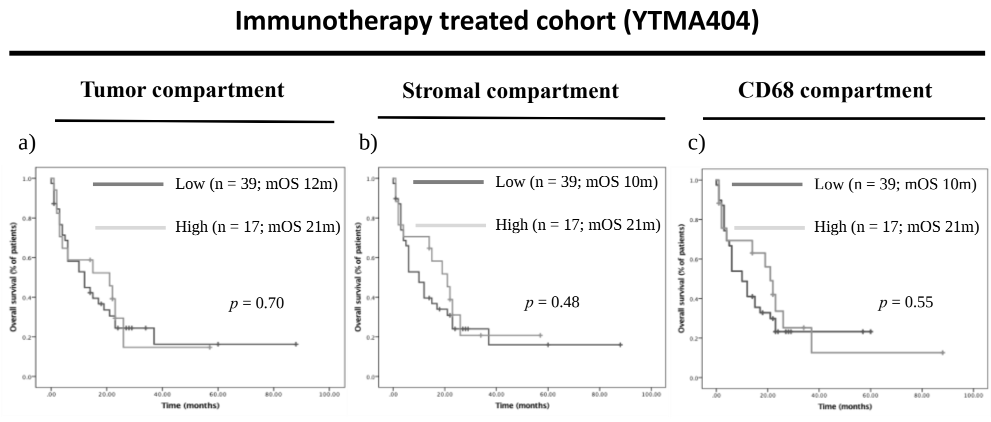


Supplementary figure S5. Correlation between CMTM6 and PDL1 expression levels in the tumor compartment (a) and the stromal compartment (b) in the three tested cohorts separately

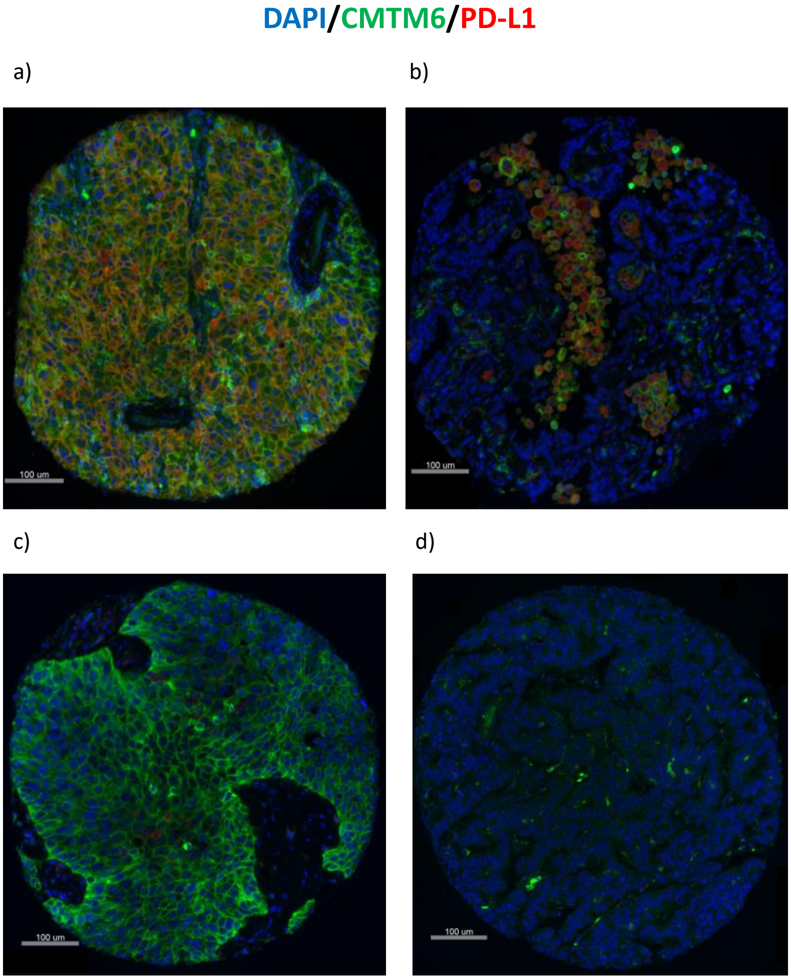


NCB: non-clinical benefit (n = 37); CB: clinical benefit (17)
ns: non significant

Supplementary figure S6. PD-L1 expression levels according to clinical benefit subgroups in the tumor compartment (a), the stromal compartment (b), and the CD68 compartment (c). PFS under PD-1 axis blockade according to PD-L1 expression in the tumor compartment (d), the stromal compartment (e), and the CD68 compartment (f)



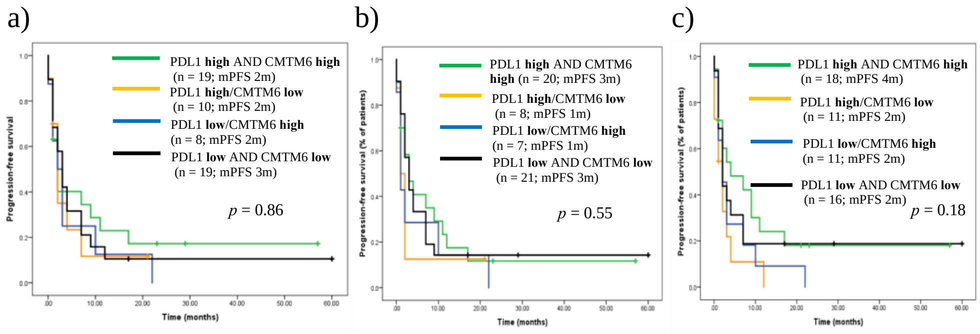
Supplementary figure S7. OS under PD-1 axis blockade in patients in the top 30th percentile of PD-L1 expression in the tumor compartment (a), the stromal compartment (b), and the CD68 compartment (c)



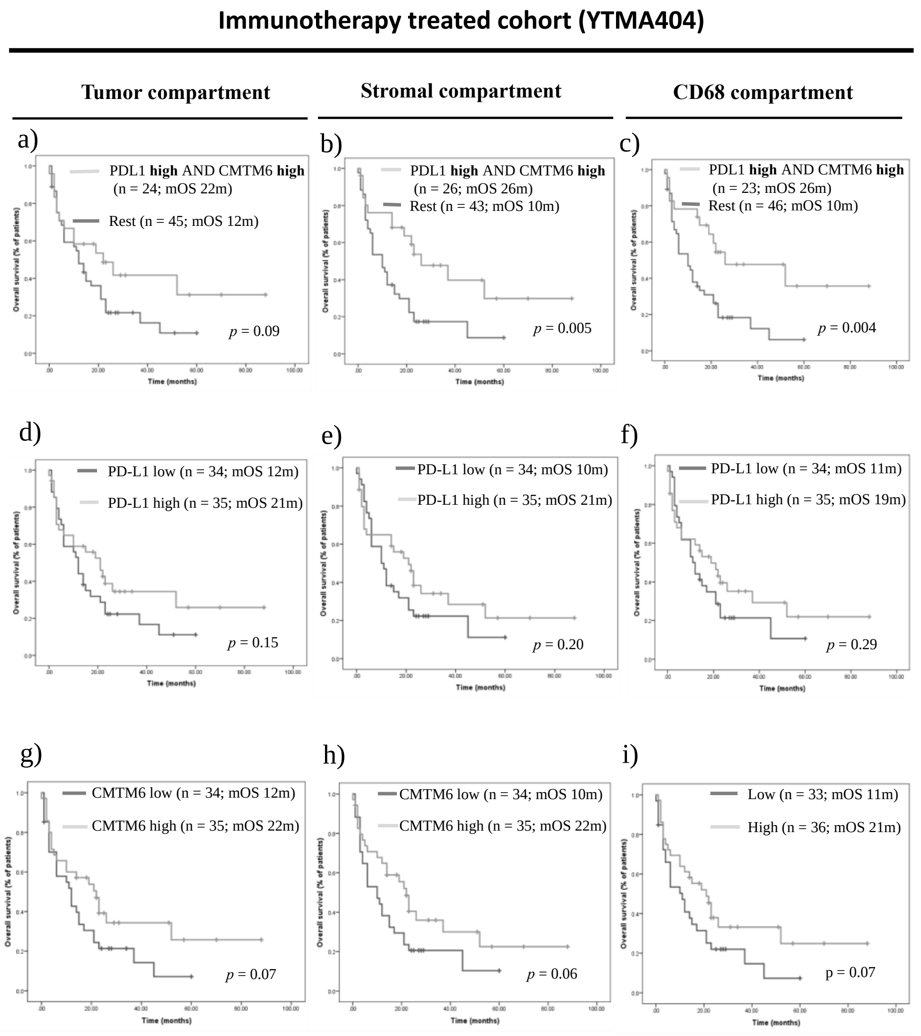
Supplementary figure S8. Representative images showing CMTM6 and PD-L1 co-expression phenotypes in NSCLC: (a) CMTM6 high / PD-L1 high; (b) CMTM6 low / PD-L1 high; (c) CMTM6 high / PD-L1 low; (d) CMTM6 low / PD-L1 low

Immunotherapy treated cohort (YTMA404)

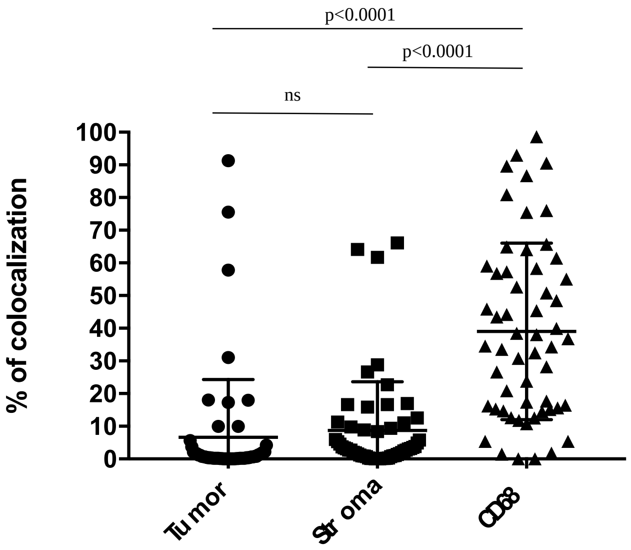
Tumor compartment **Stromal compartment** **CD68 compartment**



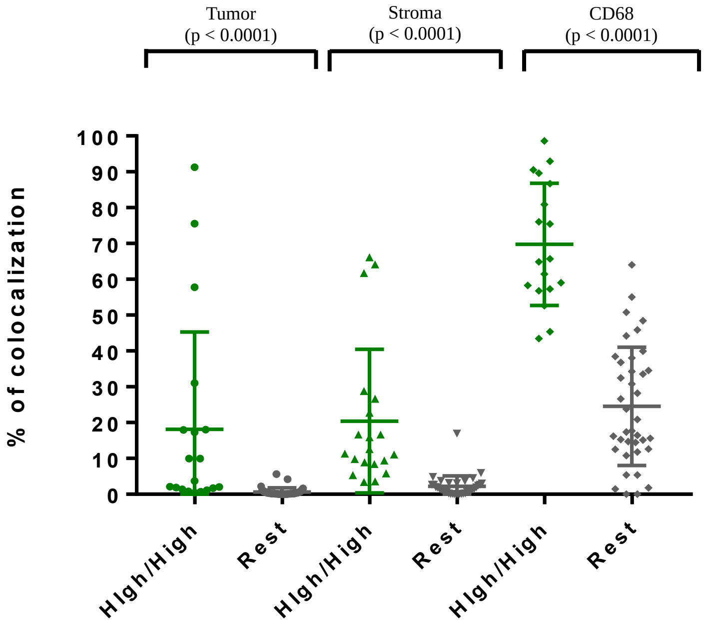
Supplementary figure S9. PFS under PD-1 axis blockade in the four CMTM6/PD-L1 co-expression phenotypes



Supplementary figure S10. Indicative performance of high CMTM6 and PD-L1 in the full immunotherapy-treated patient cohort (n = 69). (a)-(c) OS in patients with high CMTM6 and PD-L1 co-expression in the tumor compartment (a), the stromal compartment (b), and the CD68 comparment (c); (d)-(f) OS according to PD-L1 expression in the tumor compartment (d), the stromal compartment (e), and the CD68 compartment (f); (g)-(i) OS according to CMTM6 expression in the tumor compartment (g), the stromal compartment (h), and the CD68 compartment



Supplementary figure S11. Percentage of CMTM6 and PD-L1 pixel co-localization in the tumor, stroma, and CD68 compartments



Supplementary figure S12. Percentage of CMTM6 and PD-L1 colocalization in the tumor, stroma and CD68 compartments in tumors with high expression of CMTM6 and PD-L1 compared to the rest of the three tumor phenotypes combined