

Supplementary Materials

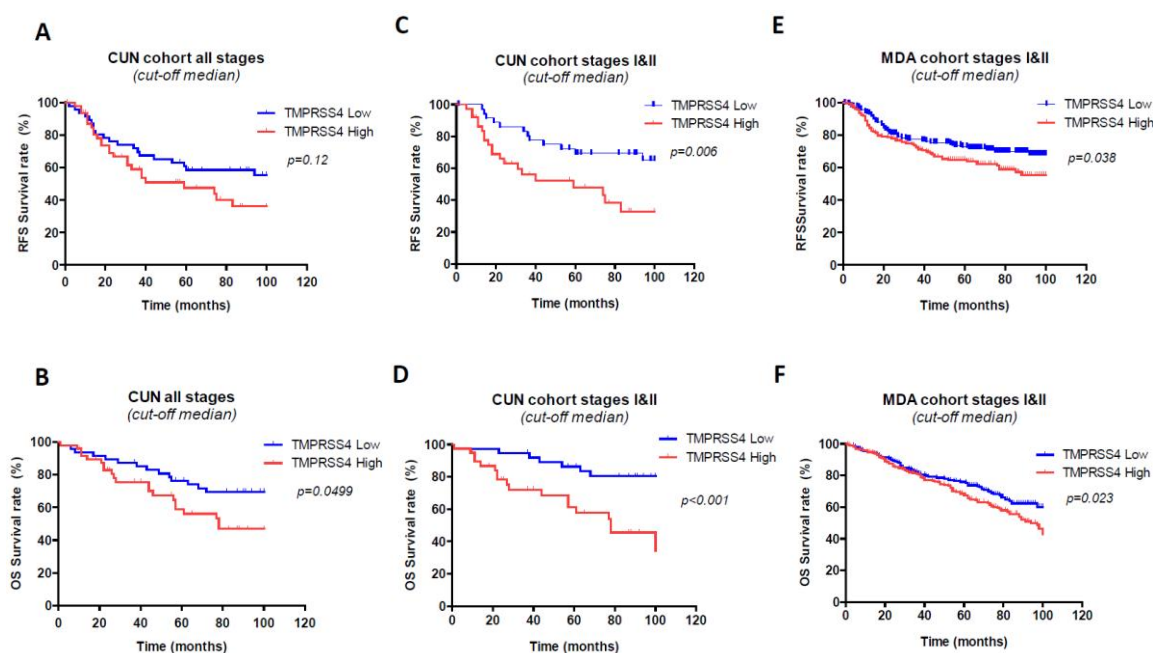
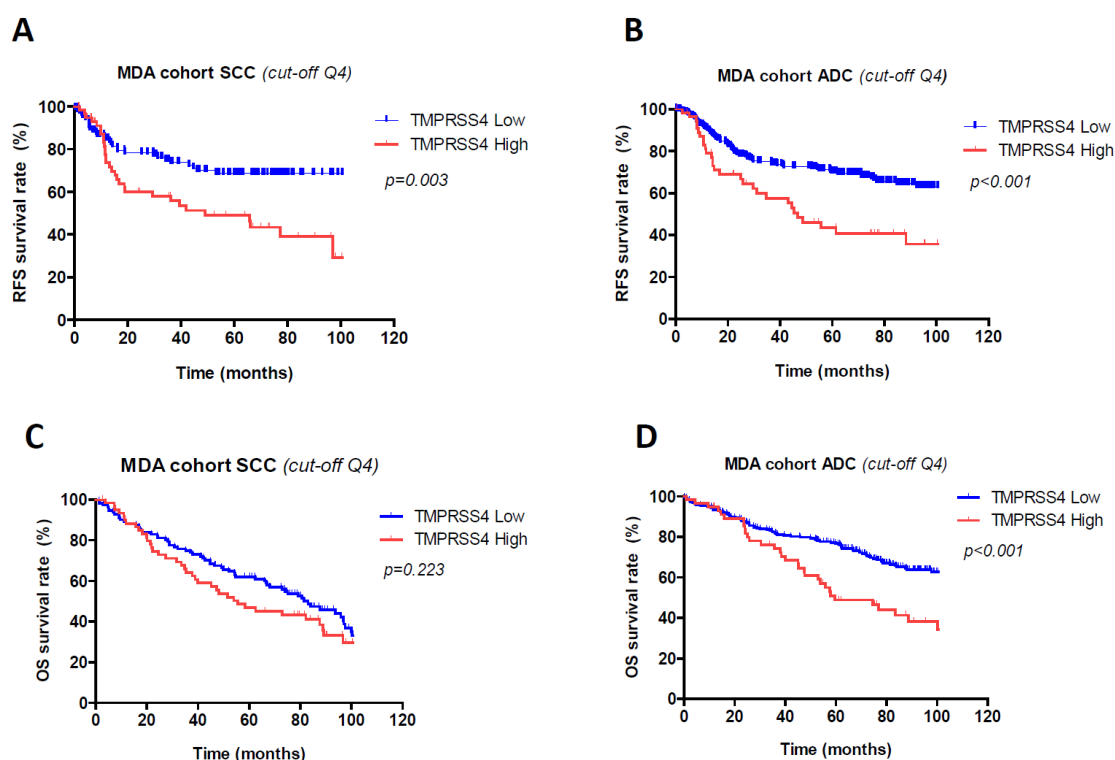


Figure S1. Prognostic value of TMPRSS4 including patients from all stages (I–IV) and early stages (I–II) in the CIMA-CUN cohort, and early stages (I–II) in the MDA cohort. RFS (A) and OS (B) in patients from CIMA-CUN stratified by the median value of TMPRSS4 protein expression. Differences between good/bad prognosis was clearly appreciated in stage I-II patients: $p=0.006$ for RFS (C) and $p<0.001$ for OS (D). RFS (E) and OS (F) for stages I-II from the MDA cohort stratified by the median.



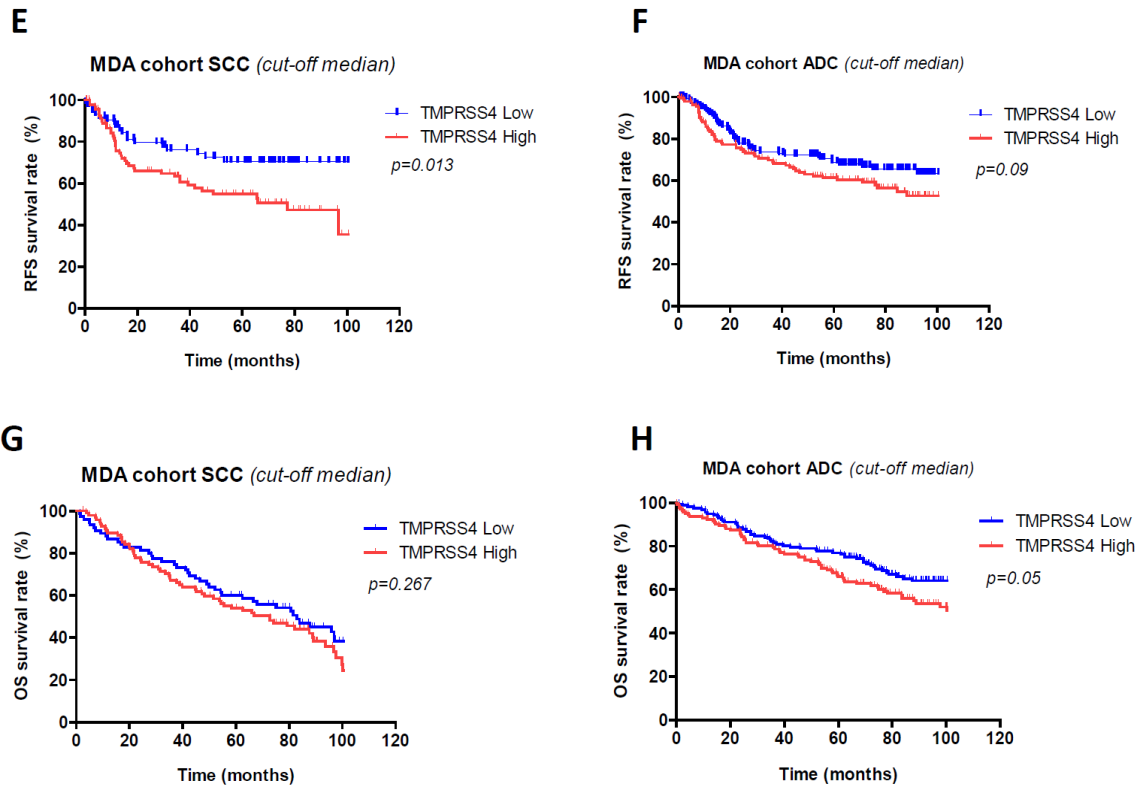


Figure S2. Prognostic value of TMPRSS4 from the MDA cohort in adenocarcinomas (ADC) or squamous cell carcinomas (SCC), considering all stages. In A-D, the top quartile (Q4) was considered as cut-off value. (A) RFS in SCC; (B) RFS in ADC; (C) OS in SCC; (D) OS in ADC. In E-H, the median was also evaluated as cut-off. (E) RFS in SCC; (F) RFS in ADC; (G) OS in SCC; (H) OS in ADC. Separation of the patients in high/low risk and p-values of the differences were improved when quartiles were considered for the stratification.

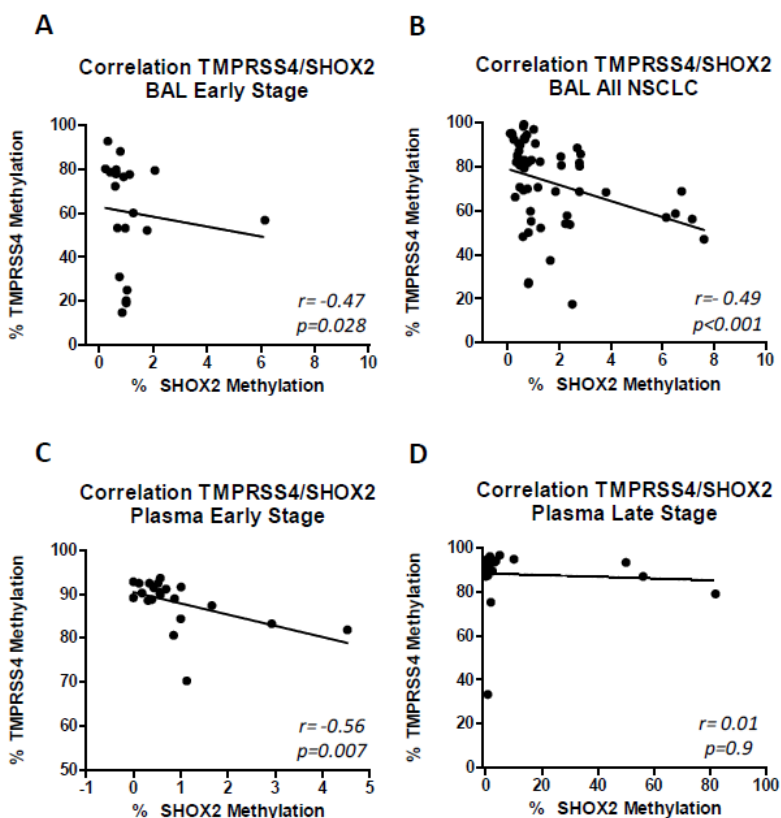


Figure S3. Inverse correlation between *TMPRSS4* methylation status and *SHOX2* methylation status in BAL (A,B) and plasma (C,D).

Table S1. Cohort of patients from Hospital General Universitario de Valencia (HGU) used to study the methylation status of *TMPRSS4* and *SHOX2* in tumors and matched non-malignant lung samples by ddPCR.

HGUV cohort (n=59)	
Age	n (%)
<65	27 (45.8)
≥65	32 (54.2)
Gender	
Female	1 (1.7)
Male	58 (98.3)
Smoking habit	
Current	30 (50.8)
Former	27 (45.8)
Never	2 (3.4)
Histology	
SCC	57 (96.6)
Others	2 (3.4)
Stage	
I	27 (45.8)
II	14 (23.7)
III	18 (30.5)
Adjuvant therapy	
No	32 (54.2)
Yes	22 (37.3)
NA *	5 (8.5)

*Not available; SCC: squamous cell carcinoma

Table S2. Cohort of patients used to quantify methylation status of the *TMPRSS4* and *SHOX2* promoters in bronchoalveolar lavages (BAL) from NSCLC patients.

BAL cohort (n=79)	
Age	n (%)
<65	51 (64.6)
≥65	28 (35.4)
Gender	
Female	14 (17.7)
Male	65 (82.3)
Smoking status	
Current	21 (26.6)
Former	43 (54.4)
Never	14 (17.7)
NA *	1 (1.3)
Histology	
ADC	22 (27.8)
SCC	38 (48.1)
Others	16 (20.3)
NA *	3 (3.8)
Stage	
I	15 (19.0)
II	8 (10.0)
III	20 (25.4)
IV	18 (22.8)
NA *	18 (22.8)

*Not available; ADC: adenocarcinoma; SCC: squamous cell carcinoma

Table S3. Cohort of patients used to quantify methylation status of the *TMPRSS4* and *SHOX2* promoters in plasmas from NSCLC patients.

Plasmas cohort (n=89)	
Age	n (%)
<65	56 (62.9)
≥65	33 (37.1)
Gender	
Female	23 (25.8)
Men	66 (74.2)
Smoking status	
Current	26 (29.3)
Former	48 (53.9)
Never	14 (15.7)
NA*	1 (1.1)
Histology	
ADC	51 (57.3)
SCC	38 (42.7)
Stage	
I	8 (9.0)
II	8 (9.0)
III	19 (21.4)
IV	52 (58.4)
NA*	2 (2.2)

*Not available; ADC: adenocarcinoma; SCC: squamous cell carcinoma

Table S4. Sequence of primers and probes for ddPCR analysis.

Gene	Sequence (5' - 3') bisulfite-treated DNA		Sequence (5' - 3') untreated DNA	
<i>TMPRSS4</i>	Fw:	GTTGGGTGGAAGTTATTTAG	Fw:	CCTGTTCTGAGGCCGAAG
	Rev:	ACTAAAACAACACCTAAAATC	Rev:	GTGTCTTGTAACCTGACACC
	Probe Met (FAM):	TGAGGT <u>CG</u> AAGGTTTTATG	Probe (FAM):	ACTTCAGGTGTTGTTCCAGCC
	Probe non-Met (HEX):	TGAGGT <u>TG</u> AAGGTTTTATG		
<i>SHOX2</i>	Fw:	AGGTTTTTTGGATAGTTAGG	Fw:	AGGCGGAAAACCAGCCTC
	Rev:	ACCCTTTAAACAACCAAC	Rev:	TGCGTTTCCACGAGGGAG
	Probe Met (FAM):	<u>ACG</u> AGTATAGG <u>CG</u> TTT <u>ACG</u> T	Probe (FAM):	CCGCTCTTTCTGTTCTCTCTTCGC
	Probe non-Met (HEX):	TAT <u>G</u> AGTATAGG <u>TG</u> TTTATGTTAT		

Table S5. Relationship between TMPRSS4 protein levels and clinicopathological characteristics of the patients from the MDA and CIMA-CUN cohorts. The median was used as cut-off value to define high vs. low expression.

MDA Cohort (n=489)				CIMA-CUN Cohort (n=95)			
	TMP Low	TMP High	<i>p</i>		TMP Low	TMP High	<i>p</i>
Age	<i>n</i> (%)	<i>n</i> (%)		Age	<i>n</i> (%)	<i>n</i> (%)	
< 65	94 (52.2)	86 (47.8)	0.356	< 65	27 (57.4)	20 (42.6)	0.124
≥65	148 (47.8)	161 (52.1)		≥65	20 (41.7)	28 (58.3)	
Gender				Gender			
Female	111 (48.5)	118 (51.5)	0.673	Female	8 (61.5)	5 (38.5)	0.349
Male	131 (50.4)	129 (49.6)		Male	39 (47.6)	43 (52.4)	
Smoking habits				Smoking habits			
Current	69 (41.3)	98 (58.7)	0.029	Current	9 (42.9)	12 (57.1)	0.787
Former	139 (54.5)	116 (45.5)		Former	34 (51.5)	32 (48.5)	
Never	34 (50.7)	33 (49.3)		Never	4 (50.0)	4 (50.0)	
Histology				Histology			
ADC	157 (52.5)	142 (47.5)	0.189	ADC	29 (64.4)	16 (35.5)	0.019
SCC	75 (43.9)	96 (56.1)		SCC	17 (37.0)	29 (63.0)	
Other	10 (52.6)	9 (47.4)		Other	1 (25.0)	3 (75.0)	
Stage (8th Edition)				Stage (8th Edition)			
I	148 (52.5)	134 (47.5)	0.245	I	23 (52.3)	21 (47.7)	0.459
II	48 (45.3)	58 (54.7)		II	14 (45.2)	17 (54.8)	
III	40 (43.5)	52 (56.5)		III	8 (44.4)	10 (55.6)	
IV	6 (66.7)	3 (33.3)		IV	2 (100.0)	0 (0.0)	
Adjuvant therapy				Adjuvant therapy			
No	157 (49.5)	160 (50.5)	0.992	No	27 (50.0)	27 (50.0)	0.906
Yes	59 (49.6)	60 (50.4)		Yes	20 (48.8)	21 (51.2)	

ADC: adenocarcinoma; SCC: squamous cell carcinoma.

Table S6. Relationship between TMPRSS4 protein levels and clinicopathological characteristics of the patients from the MDA cohort. The top quartile was used as cut-off value to define high vs. low expression.

MDA Cohort (n = 489)			
	TMP Low	TMP High	p
Age	n (%)	n (%)	
< 65	132 (73.3)	48 (26.7)	0.503
≥65	235 (76.1)	74 (23.9)	
Gender			
Female	184 (70.8)	76 (29.2)	0.02
Male	183 (79.9)	46 (20.1)	
Smoking habits			
Current	56 (83.6)	11 (16.4)	0.009
Former	119 (78.0)	56 (22.0)	
Never	44 (93.6)	3 (6.4)	
Histology			
ADC	243 (81.3)	56 (18.7)	<0.001
SCC	111 (64.9)	60 (35.1)	
Other	13 (68.4)	6 (31.6)	
Stage (8th Edition)			
I	229 (81.2)	53 (18.8)	0.001
II	72 (67.9)	34 (32.1)	
III	58 (63.0)	34 (37.0)	
IV	8 (88.9)	1 (11.1)	
Adjuvant therapy			
No	247 (77.9)	77 (22.1)	0.474
Yes	82 (68.9)	37 (31.1)	

ADC: adenocarcinoma; SCC: squamous cell carcinoma.

Table S7. Univariable Cox proportional hazards analysis of TMPRSS4 protein expression and RFS or OS in the MDA cohort. Dichotomization of TMPRSS4 expression was based on quartiles (considering the top 25%, Q4 as high). TMPRSS4 was found to be an independent prognostic factor for both RFS and OS. For the multivariable analysis, only variables whose p value was ≤ 0.1 were considered.

MDA Cohort (n=489)						
	RFS			OS		
	HR	95% CI	p	HR	95% CI	p
Age						
< 65	1			1		
≥ 65	0.88	(0.65-1.2)	0.456	1.61	(1.21-2.16)	<0.001
Gender						
Female	1			1		
Male	0.94	(0.70-1.28)	0.727	0.65	(0.50-0.86)	0.002
Smoking habits						
Never	1			1		
Former	1.48	(0.88-2.50)	0.133	1.76	(1.09-2.86)	0.021
Current	1.82	(1.06-3.12)	0.028	2	(1.21-3.31)	0.006
Histology						
ADC	1			1		
SCC	1.17	(0.85-1.62)	0.308	1.77	(1.35-2.32)	<0.001
Other	1.13	(0.5-2.59)	0.755	1.72	(0.90-3.3)	0.098
Stage (8th Edition)						
I	1			1		
II	1.99	(1.35-2.84)	<0.001	2.32	(1.69-3.17)	<0.001
III-IV	2.81	(1.96-4.02)	<0.001	2.59	(1.88-3.56)	<0.001
Adjuvant therapy						
No	1			1		
Yes	1.35	(0.96-1.90)	0.082	1.1	(0.80-1.5)	0.55
TMPRSS4						
Low	1			1		
High	2.09	(1.53-2.87)	<0.001	1.82	(1.38-2.41)	<0.001

Table S8. Clinicopathological characteristics of stage IA/IB NSCLC patients from the MDA cohort that were used to study the prognostic value of TMPRSS4.

MDA Cohort Stage I (n = 282)	
Age	n (%)
<65	101 (35.8)
≥ 65	181 (64.2)
Gender	
Female	143 (50.7)
Male	181 (49.3)
Smoking habit	
Current	91 (32.3)
Former	142 (50.4)
Never	46 (16.3)
Histology	
ADC	188 (66.7)
SCC	84 (29.8)
Other	10 (3.5)
Stage (8th Edition)	
IA1	100 (35.5)
IA2	14 (4.9)
IA3	73 (25.9)
IB	95 (33.7)
Adjuvant therapy	
No	230 (81.6)
Yes	23 (8.2)
NA *	29 (10.2)

* Not available; ADC: adenocarcinoma; SCC: squamous cell carcinoma.