

Figure S1 Representative IHC images. PD-L1 expression was tested by anti-human PD-L1 (Dako 22C3). The cutoff value was <1% for PD-L1 positivity or negativity. The cutoff value was $\geq 50\%$ for high expression level. IHC, immunohistochemical; PD-L1, programmed cell death ligand-1.

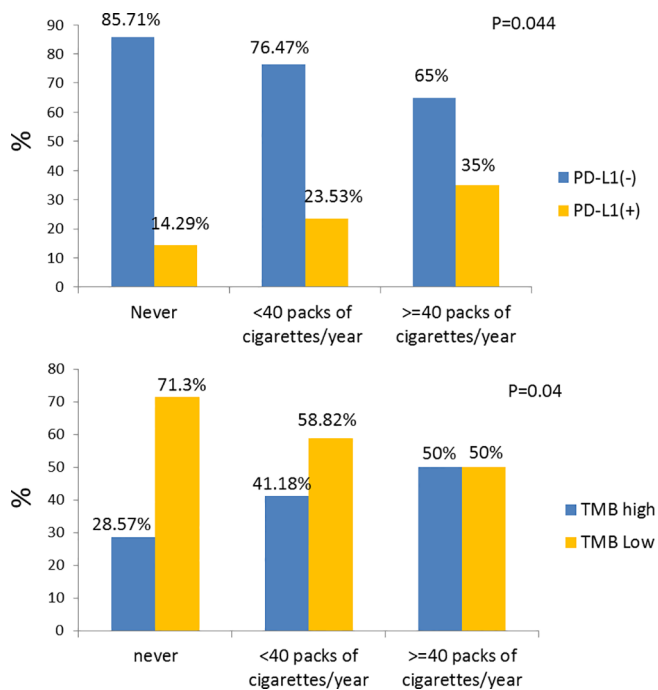


Figure S2 The relationship between smoking status, PD-L1 positivity, and TMB. (A) Patients with a history of heavy smoking (≥ 40 pack-years) were more likely to have a higher PD-L1 positive rate ($P=0.044$). The proportion of LUSC patients with positive PD-L1 increases with the addition of smoking pack-years. Positive PD-L1 expression was identified in 14.29% of never smokers, 23.53% of <40 pack-year smokers, and 35% of ≥ 40 pack-year smokers. (B) Patients with a history of heavy smoking (≥ 40 pack-years) were more likely to have a higher TMB ($P=0.04$). The proportion of LUSC patients with high TMB increases with the addition of smoking pack-years. High TMB were identified in 28.57% of never smokers, 41.18% of <40 pack-year smokers, and 50% of ≥ 40 pack-year smokers. PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden; LUSC, lung squamous cell carcinoma.

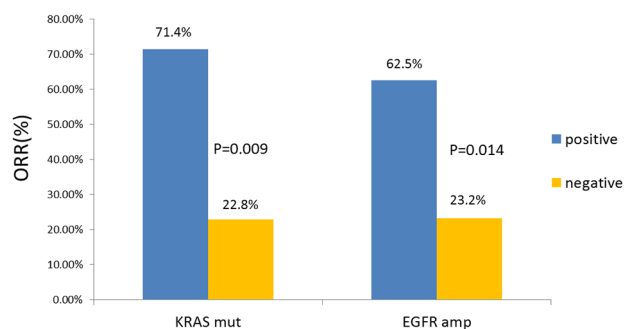


Figure S3 Correlation of gene mutation and amplification with immunotherapy response. Compared with wild-type, patients with KRAS mutation had higher ORR (71.4% vs. 22.8%, $P=0.009$). Compared with wild-type, patients with EGFR amplification had higher ORR (62.5% vs. 23.2%, $P=0.014$). ORR, objective response rates.

Table S1 Gene variations of included patients and their correlations with TMB and PD-L1 expression (N=64).

Gene	Total, N (%)	PD-L1 (-)		PD-L1 (+)		P value	TMB low*		TMB low**		P value
		n	%	n	%		n	%	N%		
TP53 mutation											
Yes	44 (68.8%)	30	68.18	14	31.82	0.5800	22	50.00	22	50.00	0.2638
No	20 (31.2%)	15	75.00	5	25.00		13	65.00	7	35.00	
CDKN2A mutation											
Yes	9 (14.1%)	6	66.67	3	33.33	0.2866	3	33.33	6	66.67	0.1129
No	55 (85.9%)	39	70.91	16	29.09		32	58.18	23	41.82	
KEAP1 mutation											
Yes	8 (12.5%)	4	50.00	4	50.00	0.1788	2	25.00	6	75.00	0.0639
No	56 (87.5%)	41	73.21	15	26.79		33	58.93	23	41.07	
CREBBP mutation											
Yes	7 (10.9%)	3	42.86	4	57.14	0.0885	3	42.86	4	57.14	0.2502
No	57 (89.1%)	42	73.68	15	26.32		32	56.14	25	43.86	
KRAS mutation											
Yes	7 (10.9%)	4	57.14	3	42.86	0.2324	5	71.43	2	28.57	0.2122
No	57 (89.1%)	41	71.93	16	28.07		30	52.63	27	47.37	
BIM mutation											
Yes	5 (7.8%)	4	80.00	1	20.00	0.3713	3	60.00	2	40.00	0.3485
No	59 (92.2%)	41	69.49	18	30.51		32	54.24	27	45.76	
AMER1 mutation											
Yes	4 (6.2%)	3	75.00	1	25.00	0.4243	1	25.00	3	75.00	0.2013
No	60 (93.8%)	42	70.00	18	30.00		34	56.67	26	43.33	
APC mutation											
Yes	4 (6.2%)	3	75.00	1	25.00	0.4243	2	50.00	2	50.00	0.3802
No	60 (93.8%)	42	70.00	18	30.00		33	55.00	27	45.00	
AR amplification											
Yes	17 (26.6%)	13	76.47	4	23.53	0.5167	9	52.94	8	47.06	0.8660
No	47 (73.4%)	32	68.09	15	31.91		26	55.32	21	44.68	
SOX2 amplification											
Yes	14 (21.9%)	9	64.29	5	35.71	0.5766	6	42.86	8	57.14	0.3144
No	50 (78.1%)	36	72.00	14	28.00		29	58.00	21	42.00	
PIK3CA amplification											
Yes	11 (17.2%)	6	54.55	5	45.45	0.2085	8	72.73	3	27.27	0.1157
No	53 (82.8%)	39	73.58	14	26.42		27	50.94	26	49.06	
EGFR amplification											
Yes	8 (12.5%)	5	62.50	3	37.50	0.2675	2	25.00	6	75.00	0.0639
No	56 (87.5%)	40	71.43	16	28.57		33	58.93	23	41.07	
RICTOR amplification											
Yes	7 (10.9%)	5	71.43	2	28.57	0.3363	5	71.43	2	28.57	0.2122
No	57 (89.1%)	40	70.18	17	29.82		30	52.63	27	47.37	
FGFR1 amplification											
Yes	6 (9.4%)	4	66.67	2	33.33	0.3398	3	50.00	3	50.00	0.3190
No	58 (90.6%)	41	70.69	17	29.31		32	55.17	26	44.83	
ZNF703 amplification											
Yes	5 (7.8%)	4	80.00	1	20.00	0.3713	3	60.00	2	40.00	0.3485
No	59 (92.2%)	41	69.49	18	30.51		32	54.24	27	45.76	
PIK3CA mutation or amplification											
Yes	23 (35.9%)	15	65.22	8	34.78	0.5040	14	60.87	9	39.13	0.4568
No	41 (64.1%)	30	73.17	11	26.83		21	51.22	20	48.78	

*TMB low, TMB \leq 10.62 (Mean TMB for all 64 subjects is 10.62); **TMB high, TMB >10.62. TMB, tumor mutational burden; PD-L1, programmed death ligand 1.

Table S2 Univariate and multivariate analyses of clinical parameters on overall response and disease control (Logistic regression) (N=64)

Factor	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
Overall response rate						
Sex (male/female)	0.607	0.093–3.962	0.6020			
Age (≥ 65 / <65)	0.760	0.243–2.376	0.6373			
Smoker (<40 pack-years/no)	0.156	0.012–2.108	0.0603			
Smoker (≥ 40 pack-years/no)	1.667	0.287–9.664	0.0512			
BMI (18.5–24/ <18.5)	0.722	0.140–3.731	0.5085			
BMI (≥ 24 / <18.5)	0.235	0.034–1.631	0.0883			
ECOG (1–2/0)	0.009	0.001–0.057	0.0000	0.027	0.005–0.142	<0.0001
Dyslipidemia (yes/no)	0.889	0.303–2.606	0.8300			
Brain metastases (yes/no)	0.765	0.140–4.180	0.7569			
Liver metastases (yes/no)	0.933	0.252–3.452	0.9176			
Radiotherapy (yes/no)	0.120	0.034–0.429	0.0011	0.241	0.045–1.280	0.0949
Lines of therapy (yes/no)	<0.001	<0.001 – >999.999	0.9402			
PD-L1 (positive/negative)	0.880	0.275–2.815	0.8297			
TMB (>10.62 / ≤ 10.62)	1.520	0.518–4.466	0.4459			
Disease control rate						
Sex (male/female)	<0.001	<0.001 – >999.999	0.9661			
Age (≥ 65 / <65)	1.152	0.306–4.338	0.8349			
Smoker (<40 pack-years/no)	0.117	0.011–1.195	0.0005	0.093	0.005–1.632	0.0036
Smoker (≥ 40 pack-years/no)	6.500	0.357–118.4	0.0121	6.327	0.298–134.5	0.0134
BMI (18.5–24/ <18.5)	0.604	0.063–5.773	0.5214			
BMI (≥ 24 / <18.5)	0.945	0.082–10.91	0.8188			
ECOG (1–2/0)	<0.001	<0.001 – >999.999	0.9565			
Dyslipidemia (yes/no)	1.261	0.359–4.432	0.7178			
Brain metastases (yes/no)	0.652	0.114–3.715	0.6299			
Liver metastases (yes/no)	0.293	0.076–1.135	0.0756			
Radiotherapy (yes/no)	1.261	0.359–4.432	0.7178			
Lines of therapy (yes/no)	0.184	0.048–0.709	0.0139	0.425	0.050–3.640	0.4345
PD-L1 (positive/negative)	0.412	0.081–2.091	0.2845			
TMB (>10.62 / ≤ 10.62)	5.400	1.076–27.09	0.0404	13.109	1.429–120.2	0.0229

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden.

Table S3 Univariate and multivariate analyses of immunotherapy-only patients on Progression-Free Survival and Overall Survival (Cox regression) (N=53)

Factor	Univariate analysis			Multivariate analysis		
	HR (log rank)	95% CI	P value	HR (log rank)	95% CI	P value
Progression-free survival						
Sex (male/female)	0.953	0.530–1.713	0.8713			
Age (≥ 65 / <65)	1.943	0.589–6.410	0.2753			
Smoker (<40 pack-years/no)	0.676	0.257–1.783	0.4292			
Smoker (≥ 40 pack-years/no)	0.708	0.256–1.959	0.5056			
BMI (18.5–24/ <18.5)	4.638	1.750–12.29	0.0020	0.288	0.098–0.845	0.0234
BMI (≥ 24 / <18.5)	2.395	0.856–6.705	0.0963	0.414	0.143–1.199	0.1041
ECOG (1–2/0)	1.099	0.420–2.881	0.8471			
Dyslipidemia (yes/no)	1.036	0.582–1.843	0.9052			
Brain metastases (yes/no)	1.273	0.564–2.873	0.5606			
Liver metastases (yes/no)	1.263	0.639–2.496	0.5012			
Radiotherapy (yes/no)	1.764	0.952–3.266	0.0711			
Lines of therapy (3–4/1–2)	2.044	1.115–3.746	0.0207	2.527	0.129–0.512	0.0045
PD-L1 (positive/negative)	0.898	0.486–1.661	0.7318			
TMB (>9.76 / ≤ 9.76)	0.339	0.183–0.629	0.0006	0.256	0.129–0.512	0.0001
Overall survival						
Sex (male/female)	3850984	0.000–.	0.9893			
Age (≥ 65 / <65)	0.859	0.385–1.917	0.7104			
Smoker (<40 pack-years/no)	5.348	0.672–42.58	0.1132			
Smoker (≥ 40 pack-years/no)	2.963	0.394–22.31	0.2916			
BMI (18.5–24/ <18.5)	1.573	0.363–6.809	0.5447			
BMI (≥ 24 / <18.5)	1.030	0.212–4.993	0.9711			
ECOG (1 – 2/0)	7.068	1.545–32.34	0.0117	3.117	0.598–16.25	0.1772
Dyslipidemia (yes/no)	0.595	0.276–1.286	0.1869			
Brain metastases (yes/no)	0.774	0.231–2.593	0.6775			
Liver metastases (yes/no)	1.328	0.534–3.303	0.5412			
Radiotherapy (yes/no)	2.712	1.140–6.455	0.0241	2.459	0.838–7.213	0.1013
Lines of therapy (yes/no)	2.210	0.995–4.905	0.0513	1.196	0.482–2.964	0.6995
PD-L1 (positive/negative)	1.074	0.470–2.457	0.8654			
TMB (>10.62 / ≤ 10.62)	0.245	0.100–0.597	0.0020	0.210	0.080–0.551	0.0015

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden.

Table S4 Univariate analyses of TMB and PD-L1 on overall survival and progression-free survival (Cox regression)

Factor	HR (log rank)	95% CI	P value
Total population (n=64)			
Overall survival			
TMB \leq 10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	0.897	0.343–2.342	0.8240
TMB >10.62 and PD-L1 (-) vs. TMB \leq 10.62 and PD-L1 (-)	0.528	0.226–1.232	0.1394
TMB >10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	0.240	0.054–1.062	0.0600
Progression-free survival			
TMB \leq 10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	0.783	0.357–1.718	0.5424
TMB >10.62 and PD-L1 (-) vs. TMB \leq 10.62 and PD-L1 (-)	0.494	0.262–0.928	0.0284
TMB >10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	0.531	0.235–1.200	0.1282
Mono-immunotherapy group (n=53)			
Overall survival			
TMB \leq 10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	1.309	0.486–3.525	0.5946
TMB >10.62 and PD-L1 (-) vs. TMB \leq 10.62 and PD-L1 (-)	0.307	0.112–0.839	0.0213
TMB >10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	0.175	0.037–0.832	0.0284
Progression-free survival			
TMB \leq 10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	1.210	0.520–2.817	0.6587
TMB >10.62 and PD-L1 (-) vs. TMB \leq 10.62 and PD-L1 (-)	0.345	0.168–0.710	0.0038
TMB >10.62 and PD-L1 (+) vs. TMB \leq 10.62 and PD-L1 (-)	0.384	0.155–0.952	0.0388

TMB, tumor mutational burden; PD-L1, programmed cell death ligand-1;

Table S5 Univariate and multivariate analyses of gene variations on progression-free survival and overall survival (Cox regression) (N=64)

Factor	Univariate analysis			Multivariate analysis		
	HR (log rank)	95% CI	P value	HR (log rank)	95% CI	P value
Progression-free survival						
TP53 mutation (yes/no)	1.061	0.599–1.877	0.8394			
CDKN2A mutation (yes/no)	0.658	0.308–1.405	0.2791			
KEAP1 mutation (yes/no)	1.848	0.857–3.986	0.1175			
CREBBP mutation (yes/no)	1.433	0.642–3.200	0.3797			
KRAS mutation (yes/no)	0.615	0.255–1.483	0.2787			
BIM mutation (yes/no)	0.644	0.233–1.785	0.3980			
AMER1 mutation (yes/no)	0.782	0.243–2.514	0.6795			
APC mutation (yes/no)	1.798	0.639–5.059	0.2663			
AR amplification (yes/no)	1.478	0.830–2.631	0.1846			
SOX2 amplification (yes/no)	1.209	0.660–2.213	0.5392			
PIK3CA amplification (yes/no)	1.233	0.638–2.384	0.5339			
EGFR amplification (yes/no)	0.671	0.314–1.432	0.3024			
RICTOR amplification (yes/no)	1.154	0.517–2.578	0.7263			
FGFR1 amplification (yes/no)	1.110	0.474–2.602	0.8102			
ZNF703 amplification (yes/no)	1.417	0.560–3.585	0.4615			
PIK3CA mutation or amplification (yes/no)	1.153	0.670–1.985	0.6073			
Overall survival						
TP53 mutation (yes/no)	1.551	0.667–3.605	0.3081			
CDKN2A mutation (yes/no)	0.631	0.219–1.818	0.3934			
KEAP1 mutation (yes/no)	2.094	0.842–5.209	0.1120			
CREBBP mutation (yes/no)	1.307	0.455–3.752	0.6187			
KRAS mutation (yes/no)	0.678	0.200–2.300	0.5334			
BIM mutation (yes/no)	0.330	0.045–2.432	0.2769			
AMER1 mutation (yes/no)	1.080	0.255–4.564	0.9170			
APC mutation (yes/no)	1.252	0.293–5.343	0.7618			
AR amplification (yes/no)	1.253	0.575–2.732	0.5702			
SOX2 amplification (yes/no)	0.939	0.404–2.182	0.8840			
PIK3CA amplification (yes/no)	0.985	0.378–2.567	0.9746			
EGFR amplification (yes/no)	0.698	0.240–2.031	0.5098			
RICTOR amplification (yes/no)	1.194	0.415–3.435	0.7416			
FGFR1 amplification (yes/no)	0.559	0.133–2.361	0.4291			
ZNF703 amplification (yes/no)	0.422	0.057–3.108	0.3968			
PIK3CA mutation or amplification (yes/no)	0.805	0.368–1.762	0.5880			

Table S6 Univariate and multivariate analyses of gene variations on overall response and disease control (Logistic regression) (N=64)

Factor	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
Overall response rate						
TP53 mutation (yes/no)	0.696	0.224–2.163	0.5315			
CDKN2A mutation (yes/no)	3.661	0.860–15.58	0.0790			
KEAP1 mutation (yes/no)	0.765	0.140–4.180	0.7569			
CREBBP mutation (yes/no)	0.361	0.040–3.225	0.3619			
KRAS mutation (yes/no)	7.678	1.338–44.06	0.0222	11.111	1.851–66.691	0.0085
BIM mutation (yes/no)	4.031	0.616–26.39	0.1459			
AMER1 mutation (yes/no)	<0.001	<0.001–>999	0.9756			
APC mutation (yes/no)	<0.001	<0.001–>999	0.9756			
AR amplification (yes/no)	0.415	0.104–1.659	0.2136			
SOX2 amplification (yes/no)	0.933	0.252–3.452	0.9176			
PIK3CA amplification (yes/no)	0.471	0.092–2.419	0.3668			
EGFR amplification (yes/no)	5.000	1.057–23.65	0.0424	7.407	1.490–36.822	0.0144
RICTOR amplification (yes/no)	0.361	0.040–3.225	0.3619			
FGFR1 amplification (yes/no)	<0.001	<0.001–>999	0.9756			
ZNF703 Amplification (yes/no)	<0.001	<0.001–>999	0.9756			
PIK3CA mutation or amplification (yes/no)	0.365	0.104–1.276	0.1144			
Disease control rate						
TP53 mutation (yes/no)	1.762	0.483–6.433	0.3913			
CDKN2A mutation (yes/no)	0.778	0.140–4.319	0.7738			
KEAP1 mutation (yes/no)	>999	<0.001–>999	0.9569			
CREBBP mutation (yes/no)	1.434	0.156–13.16	0.7498			
KRAS mutation (yes/no)	0.532	0.090–3.142	0.4861			
BIM mutation (yes/no)	>999	<0.001–>999	0.9661			
AMER1 mutation (yes/no)	>999	<0.001–>999	0.9698			
APC mutation (yes/no)	0.673	0.064–7.098	0.7419			
AR amplification (yes/no)	1.105	0.261–4.680	0.8919			
SOX2 amplification (yes/no)	1.499	0.288–7.803	0.6302			
PIK3CA amplification (yes/no)	1.047	0.195–5.613	0.9577			
EGFR amplification (yes/no)	1.711	0.190–15.39	0.6317			
RICTOR amplification (yes/no)	0.250	0.048–1.312	0.1012			
FGFR1 amplification (yes/no)	1.170	0.124–11.05	0.8909			
ZNF703 amplification (yes/no)	0.917	0.093–9.026	0.9406			
PIK3CA mutation or amplification (yes/no)	1.152	0.306–4.338	0.8349			