Supplementary

Table S1 Antibodies used for immunohistochemistry

Marker	Clone	Catalog number	Company	Dilution	Positive reactivity location
ASCL1	24B72D11.1	#556604	BD Biosciences, San José, CA, USA	1:50	Nucleus
NEUROD1	EPR17084	#ab205300	Abcam, Cambridge, U.K.	1:200	Nucleus
POU2F3	E5N2D	#36135	Cell Signaling Technology, Leiden, The Netherlands	1:300	Nucleus
YAP1	EP1674Y	#ab52771	Abcam, Cambridge, U.K.	1:300	Nucleus, cytoplasm
MHC I	EMR8-5	#ab70328	Abcam, Cambridge, U.K.	1:500	Cell membrane
MHC II	LGII-612.14	# 68258S	Cell Signaling Technology, Leiden, The Netherlands	1:400	Cell membrane
PD-L1	22C3	M3653	Agilent DaKo, Denmark	1:50	Partial or complete membrane
Rb	EPR17512	#ab181616	Abcam, Cambridge, U.K.	1:1000	Nucleus
CD3	LN10	ZM-0417	ZSGB-BIO	Working solution	Cytoplasm, cell membrane
CD4	UMAB64	ZM-0418	ZSGB-BIO	Working solution	Cell membrane
CD8	SP16	ZA-0508	ZSGB-BIO	Working solution	Cell membrane
CD20	L26	ZM-0039	ZSGB-BIO	Working solution	Cell membrane
CD68	KP1	ZM-0060	ZSGB-BIO	Working solution	Cytoplasm

ASCL1, achaete-scute homologue 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes-associated protein 1; MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II; PD-L1, programmed death-ligand; Rb, retinoblastoma.

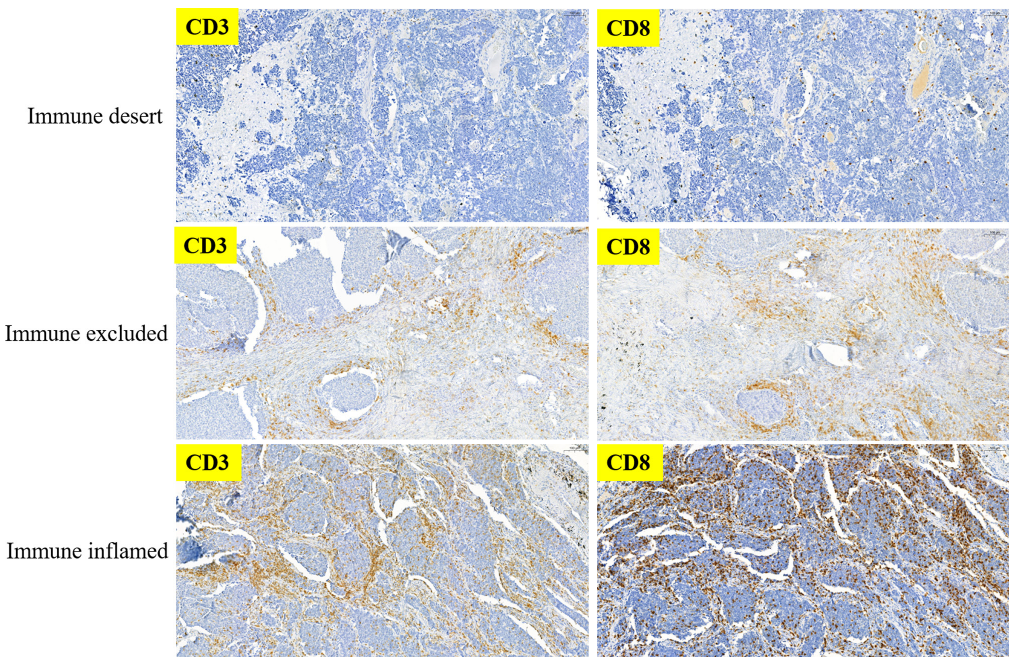

Figure S1 Representative histopathological images of tumor immune phenotypes: desert, excluded, and inflamed. "Desert" characterized by low prevalence of immune cells, "excluded" when immune cells were predominantly found in the stroma adjacent to or within the tumor, or "inflamed" when immune cells were in direct contact with tumor cells, either as spillover from stromal infiltrates into tumor cell aggregates or as diffuse infiltration of tumor cells (magnification: ×100).

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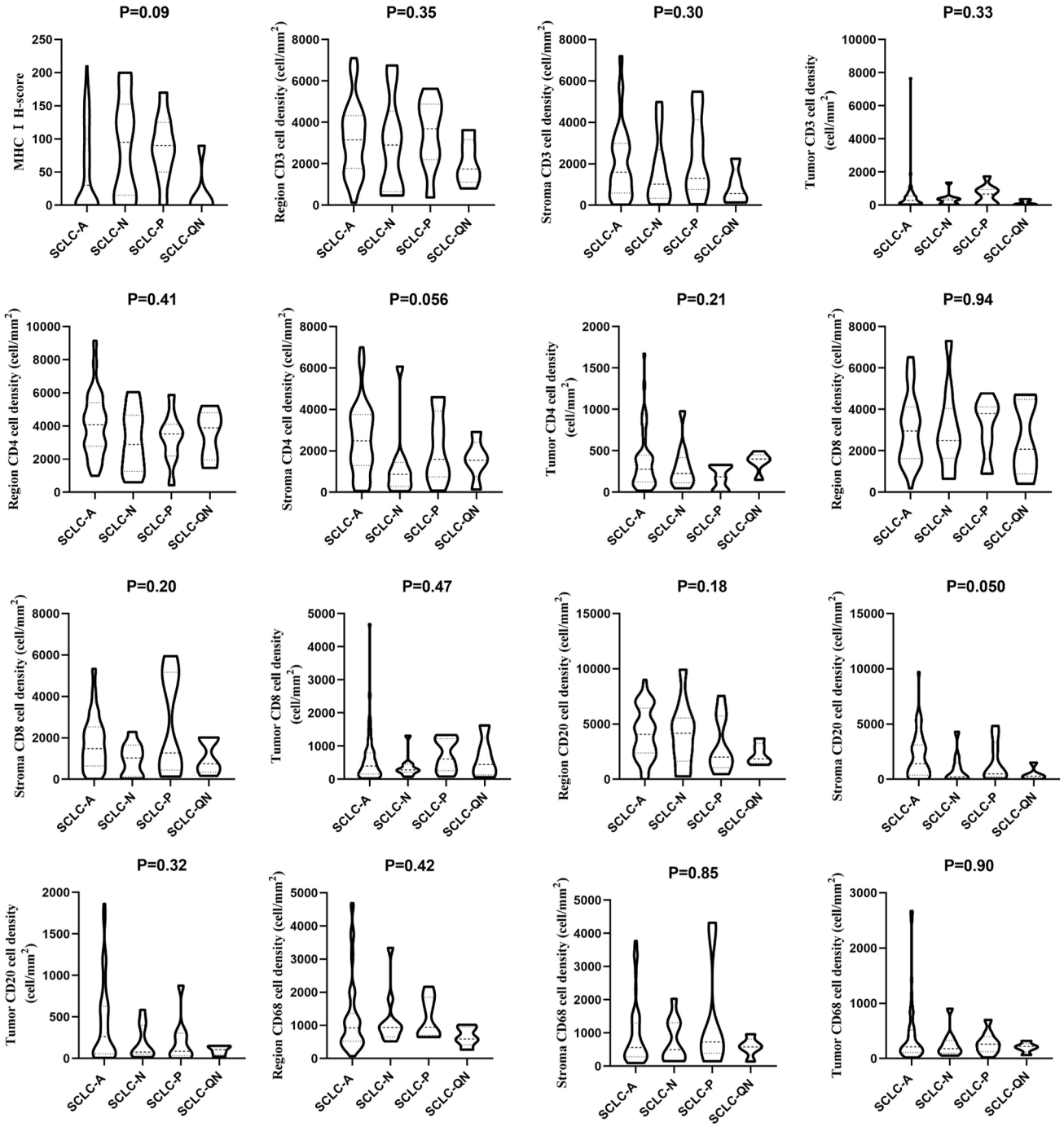

Figure S2 There were no significant differences in the expression of MHC I among the four molecular subtypes. SCLC, small cell lung carcinoma; SCLC-A, small cell lung carcinomas with ASCL1 dominant expression; SCLC-N, small cell lung carcinomas with NEUROD1 dominant expression; SCLC-P, small cell lung carcinomas with POU2F3 dominant expression; SCLC-QN, small cell lung carcinomas characterized by the low expression of all four investigated transcription factors; MHC I, major histocompatibility complex class I.

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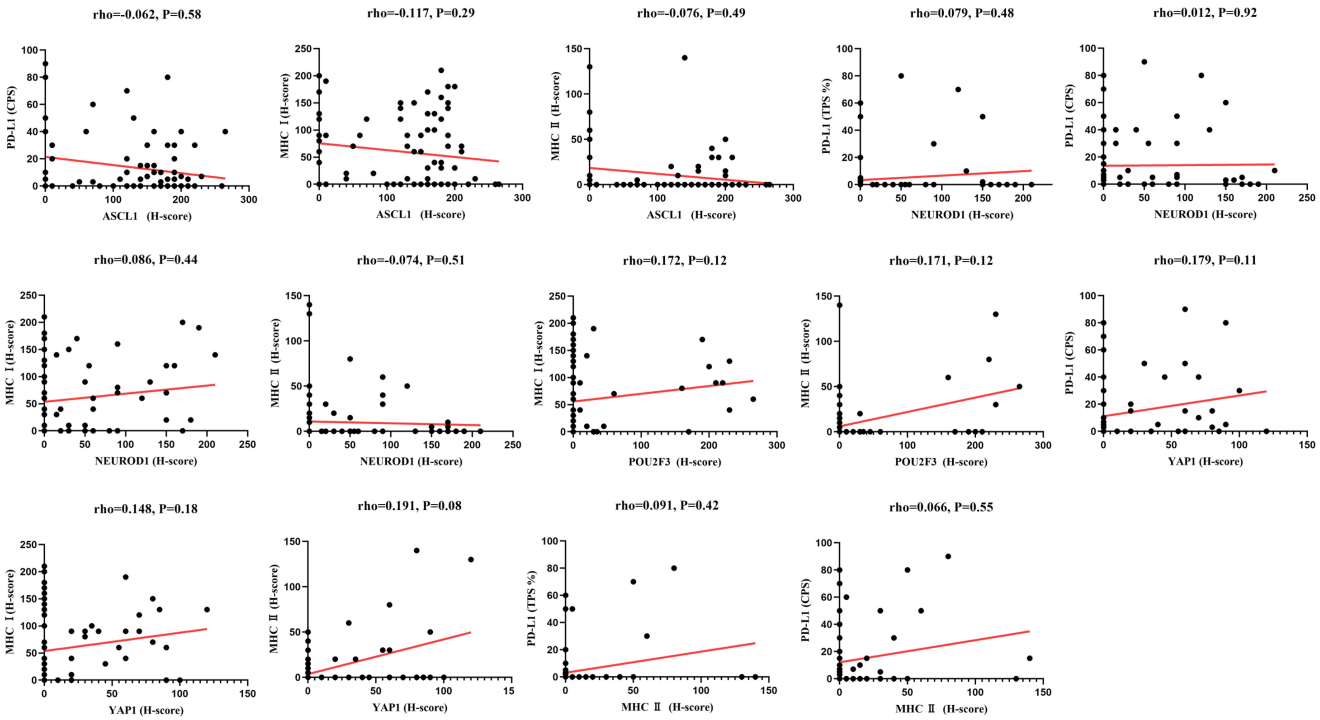

Figure S3 The relationship of expression level between subtype-specific protein and MHC I, MHC II, PD-L1 and Rb expression was analyzed by Spearman's correlation, and no significant correlations were observed between these groups. ASCL1, achaete-scute homologue 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes-associated protein 1; MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II; PD-L1, programmed death-ligand.

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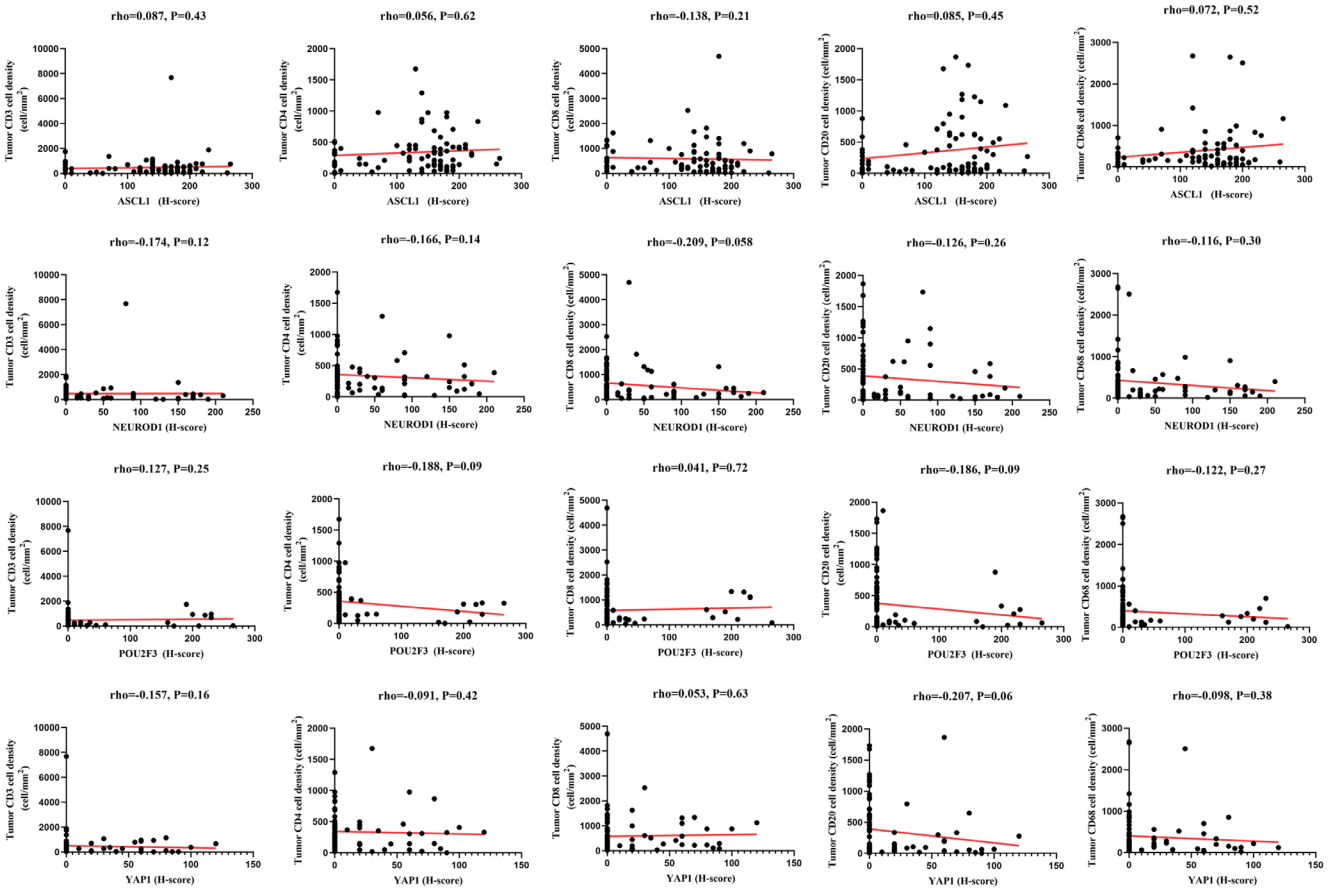

Figure S4 No significant correlations were detected between immune marker density and subtype-specific protein expression. ASCL1, achaete-scute homologue 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes-associated protein 1.

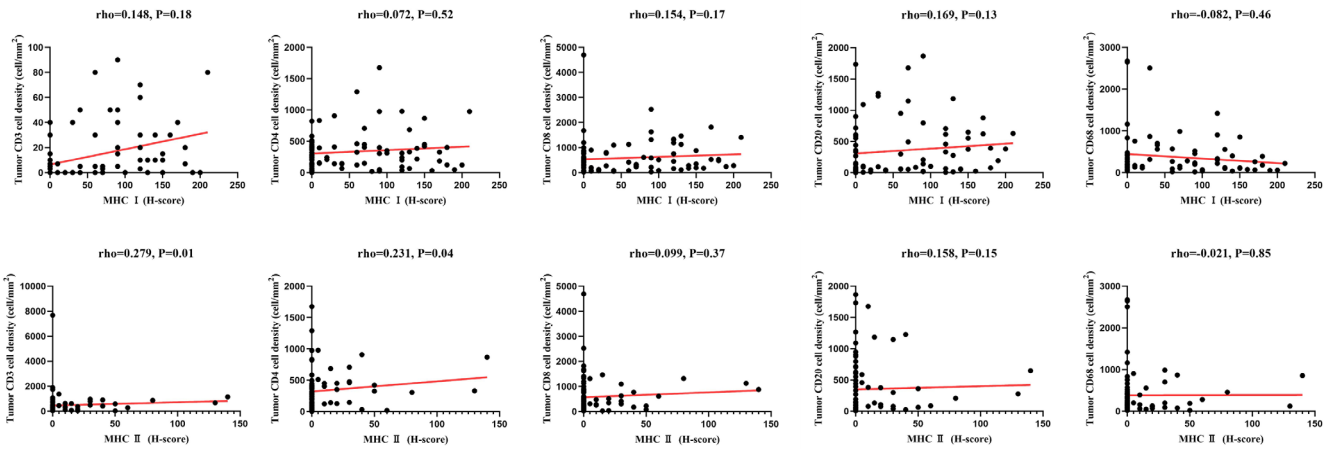

Figure S5 Significant positive correlations were presented between MHC II expression and tumor CD3 cell density, and between MHC II expression and tumor CD4 cell density, while no significant correlations were detected between other immune marker density and MHC I/II expression. MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II.

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Table S2 Immune cells infiltration density and spatial distribution in different molecular subtypes of small cell lung carcinoma

Parameter	SCLC-A	SCLC-N	SCLC-P	SCLC-QN	P value
Tumor CD3 density					0.56
Low	30 (50.8%)	4 (40.0%)	4 (44.4%)	4 (80.0%)	
High	29 (49.2%)	6 (60.0%)	5 (55.6%)	1 (20.0%)	
Stroma CD3 density					0.47
Low	27 (45.8%)	6 (60.0%)	5 (55.6%)	4 (80.0%)	
High	32 (54.2%)	4 (40.0%)	4 (44.4%)	1 (20.0%)	
Region CD3 density					0.65
Low	29 (49.2%)	5 (50.0%)	4 (44.4%)	4 (80.0%)	
High	30 (50.8%)	5 (50.0%)	5 (55.6%)	1 (20.0%)	
Tumor CD4 density					0.56
Low	30 (50.8%)	6 (60.0%)	5 (55.6%)	1 (20.0%)	
High	29 (49.2%)	4 (40.0%)	4 (44.4%)	4 (80.0%)	
Region CD4 density					0.29
Low	27 (45.8%)	6 (60.0%)	7 (77.8%)	2 (40.0%)	
High	32 (54.2%)	4 (40.0%)	2 (22.2%)	3 (60.0%)	
Tumor CD8 density					0.44
Low	30 (50.8%)	7 (70.0%)	3 (33.3%)	2 (40.0%)	
High	29 (49.2%)	3 (30.0%)	6 (66.7%)	3 (60.0%)	
Stroma CD8 density					0.52
Low	27 (45.8%)	7 (70.0%)	5 (55.6%)	3 (60.0%)	
High	32 (54.2%)	3 (30.0%)	4 (44.4%)	2 (40.0%)	
Region CD8 density					0.91
Low	29 (49.2%)	6 (60.0%)	4 (44.4%)	3 (60.0%)	
High	30 (50.8%)	4 (40.0%)	5 (55.6%)	2 (40.0%)	
Tumor CD20 density					0.47
Low	27 (45.8%)	6 (60.0%)	5 (55.6%)	4 (80.0%)	
High	32 (54.2%)	4 (40.0%)	4 (44.4%)	1 (20.0%)	
Stroma CD20 density					0.14
Low	25 (42.4%)	7 (70.0%)	6 (66.7%)	4 (80.0%)	
High	34 (57.6%)	3 (30.0%)	3 (33.3%)	1 (20.0%)	
Region CD20 density					0.39
Low	27 (45.8%)	5 (50.0%)	6 (66.7%)	4 (80.0%)	
High	32 (54.2%)	5 (50.0%)	3 (33.3%)	1 (20.0%)	

Table S2 (*continued*)

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Table S2 (*continued*)

Parameter	SCLC-A	SCLC-N	SCLC-P	SCLC-QN	P value
Tumor CD68 density					0.91
Low	30 (50.8%)	6 (60.0%)	4 (44.4%)	2 (40.0%)	
High	29 (49.2%)	4 (40.0%)	5 (55.6%)	3 (60.0%)	
Stroma CD68 density					0.78
Low	30 (50.8%)	6 (60.0%)	3 (33.3%)	3 (60.0%)	
High	29 (49.2%)	4 (40.0%)	6 (66.7%)	2 (40.0%)	
Region CD68 density					0.65
Low	29 (49.2%)	5 (50.0%)	4 (44.4%)	4 (80.0%)	
High	30 (50.8%)	5 (50.0%)	5 (55.6%)	1 (20.0%)	

SCLC, small cell lung carcinoma; SCLC-A, small cell lung carcinomas with ASCL1 dominant expression; SCLC-N, small cell lung carcinomas with NEUROD1 dominant expression; SCLC-P, small cell lung carcinomas with POU2F3 dominant expression; SCLC-QN, small cell lung carcinomas characterized by the low expression of all four investigated transcription factors. We determined the median number of immune cells density as the cut-off point for low and high expression of CD3, CD4, CD8, CD20 and CD68.

Table S3 Univariate analyses for prognostic significance of clinicopathologic parameters in surgically resected small cell lung cancer patients

Clinicopathologic parameters	OS		PFS	
	P value	HR (95% CI)	P value	HR (95% CI)
Age, ≤60 *vs.* >60 years	0.57	0.857 (0.506-1.453)	0.79	0.928 (0.535-1.609)
Sex, Male v. Female	0.003*	0.404 (0.223-0.731)	0.02*	0.499 (0.275-0.907)
Smoking story, yes v. no	0.09	0.490 (0.218-1.103)	0.61	0.799 (0.339-1.885)
TNM stage, I v. II-III	0.03*	0.556 (0.327-0.946)	0.19	0.691 (0.397-1.204)
Tumor location, central v. peripheral	0.91	0.942 (0.335-2.646)	0.90	1.068 (0.384-2.974)
Postoperative chemotherapy, yes v. no	0.12	0.644 (0.371-1.117)	0.95	0.981 (0.555-1.732)
Postoperative radiotherapy, yes v. no	0.06	0.574 (0.323-1.020)	0.62	0.863 (0.486-1.534)
prophylactic cranial irradiation, yes v. no	0.92	1.028 (0.605-1.749)	0.59	1.164 (0.666-2.036)
ASCL1 expression, Neg v. Pos	0.96	1.015 (0.541-1.903)	0.74	1.115 (0.582-2.136)
NEUROD1 expression, Neg v. Pos	0.81	1.078 (0.586-1.983)	0.26	1.450 (0.756-2.779)
POU2F3 expression, Neg v. Pos	0.94	0.969 (0.436-2.155)	0.55	1.296 (0.551-3.051)
YAP1 expression, Neg v. Pos	0.53	0.793 (0.387-1.627)	0.66	1.187 (0.557-2.528)
Tumor PD-L1 expression, Neg v. Pos	0.24	0.651 (0.317-1.339)	0.99	0.995 (0.465-2.129)
Stromal PD-L1 expression, Neg v. Pos	0.26	1.360 (0.800-2.311)	0.02*	1.909 (1.087-3.355)
MHC I expression, Neg v. Pos	0.82	1.066 (0.626-1.815)	0.95	1.019 (0.585-1.776)
MHC II expression, Neg v. Pos	0.73	0.891 (0.461-1.725)	0.28	0.692 (0.354-1.355)
NE differentiation, NE v. non-NE	0.95	0.976 (0.476-2.004)	0.97	0.986 (0.478-2.031)
Region CD3 density, low v. high	0.88	0.961 (0.567-1.627)	0.85	1.057 (0.609-1.834)

Table S3 (*continued*)

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Table S3 (*continued*)

Clinicopathologic parameters	OS		PFS	
	P value	HR (95% CI)	P value	HR (95% CI)
Stroma CD3 density, low v. high	0.82	0.940 (0.552-1.601)	0.55	0.841 (0.480-1.475)
Tumor CD3 density, low v. high	0.77	1.081 (0.637-1.835)	0.79	1.076 (0.620-1.869)
Region CD4 density, low v. high	0.34	0.771 (0.453-1.313)	0.20	0.693 (0.395-1.216)
Stroma CD4 density, low v. high	0.55	0.846 (0.493-1.453)	0.12	0.635 (0.360-1.121)
Tumor CD4 density, low v. high	0.06	0.594 (0.348-1.013)	0.03*	0.530 (0.300-0.936)
Region CD8 density, low v. high	0.85	0.950 (0.562-1.607)	0.72	1.106 (0.637-1.920)
Stroma CD8 density, low v. high	0.80	0.934 (0.552-1.582)	0.90	1.037 (0.597-1.800)
Tumor CD8 density, low v. high	0.75	1.089 (0.641-1.849)	0.64	1.143 (0.657-1.988)
Region CD20 density, low v. high	0.17	0.689 (0.405-1.174)	0.04*	0.546 (0.309-0.964)
Stroma CD20 density, low v. high	0.65	0.883 (0.517-1.509)	0.22	0.700 (0.396-1.238)
Tumor CD20 density, low v. high	0.36	0.781 (0.457-1.332)	0.04*	0.556 (0.315-0.982)
Region CD68 density, low v. high	0.97	0.989 (0.582-1.681)	0.92	1.028 (0.592-1.786)
Stroma CD68 density, low v. high	0.45	0.815 (0.482-1.379)	0.89	0.963 (0.555-1.671)
Tumor CD68 density, low v. high	0.88	0.959 (0.562-1.637)	0.55	1.185 (0.680-2.066)
CD3 immunophenotypes, desert v. inflamed	0.54	0.761 (0.317-1.825)	0.52	1.337 (0.550-3.247)
CD3 immunophenotypes, excluded v. inflamed	0.37	0.752 (0.401-1.407)	0.45	0.778 (0.404-1.500)
CD4 immunophenotypes, desert v. inflamed	0.40	0.533 (0.122-2.320)	0.39	0.521 (0.119-2.282)
CD4 immunophenotypes, excluded v. inflamed	0.48	0.811 (0.453-1.452)	0.28	0.718 (0.393-1.310)
CD8 immunophenotypes, desert v. inflamed	0.61	0.834 (0.418-1.666)	0.79	0.907 (0.447-1.842)
CD8 immunophenotypes, excluded v. inflamed	0.68	0.880 (0.477-1.622)	0.44	0.778 (0.409-1.477)
CD20 immunophenotypes, desert v. excluded	0.99	0.995 (0.584-1.696)	0.43	1.252 (0.715-2.190)
CD68 immunophenotypes, desert v. inflamed	0.35	0.639 (0.248-1.649)	0.29	0.565 (0.198-1.614)
CD68 immunophenotypes, excluded v. inflamed	0.23	0.706 (0.402-1.241)	0.40	0.776 (0.430-1.400)
TLS, absent v. present	0.20	1.661 (0.770-3.582)	0.28	1.525 (0.706-3.295)
Intra-TLS density, low v. high	0.57	1.187 (0.659-2.139)	0.48	1.247 (0.674-2.309)
Peri-TLS density, low v. high	<0.001*	0.369 (0.215-0.633)	0.048*	0.563 (0.319-0.995)

*, significant P values. OS, overall survival; PFS, progression-free survival; HR, hazard ratio; Pos, positive; Neg, negative; ASCL1, achaete-scute homologue 1; NEUROD1, neuronal differentiation 1; POU2F3, POU class 2 homeobox 3; YAP1, Yes-associated protein 1; NE, neuroendocrine; TLS, tertiary lymphoid structure; intra-TLS, intra-tumoral tertiary lymphoid structure; peri-TLS, peri-tumoral tertiary lymphoid structure.

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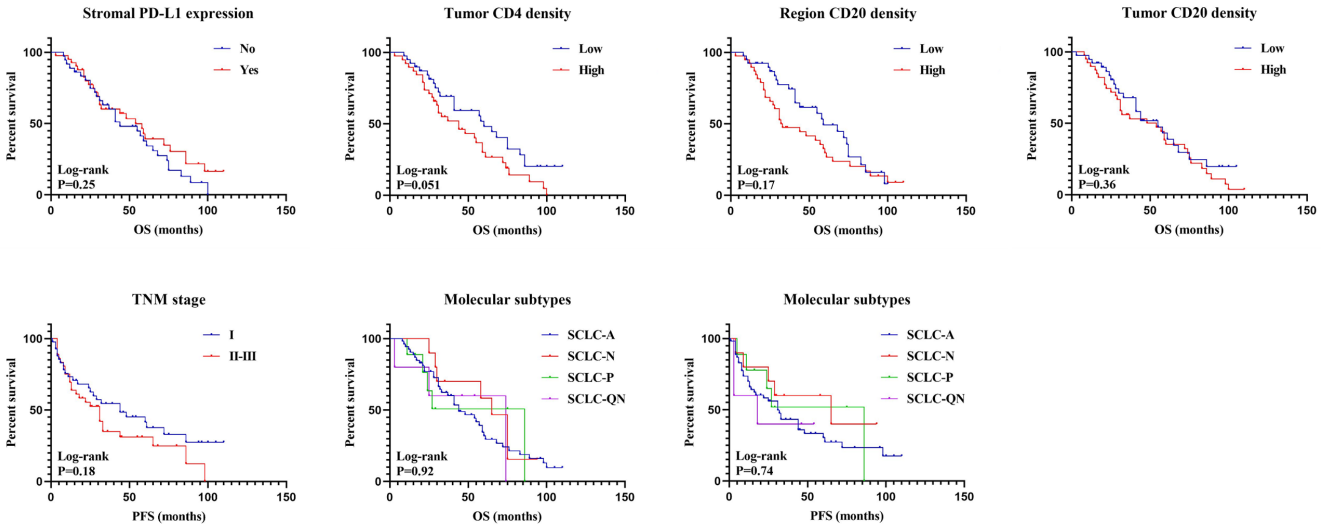

Figure S6 Kaplan-Meier analysis was performed to compare risk stratification in subgroups according to these prognostic factors, included in the multivariate analysis. We found that stromal PD-L1 expression, tumor CD4 density, region CD20 density and tumor CD20 density did not affect survival outcomes, and the difference between the PFS of patients with TNM stage I and TNM stage II-III did not reach statistical significance. In addition, Kaplan-Meier curves for OS and PFS according to molecular subtypes were examined, but no significant differences were found among molecular subtypes.

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