

Supplementary

Table S1 Information for immunohistochemical antibodies

Antibody	Clone	Dilution	Manufacturer	Primary antibody incubation	Positive control tissue	Negative control tissue
ASCL1 (MASH1)	24B72D11.1	1:50	BD Bioscience	1 hour at room temperature	Small cell lung cancer sample	Carcinoma collection block for validation
NeuroD1	Ab60704	1:12,800	Abcam	1 hour at room temperature	Small cell lung cancer sample	Carcinoma collection block for validation
POU2F3	NBP1-83966	1:100	Novus Biologicals	1 hour at room temperature	Skin (squamous epithelium)	Normal tissue collection block for validation
YAP1	NB110-58358	1:1,600	Novus Biologicals	1 hour at room temperature	Carcinoma collection block for validation	Normal tissue collection block for validation

Table S2 Expression of transcription factors according to dominant subtype

Subtype	ASCL1-dominant	NeuroD1-dominant	POU2F3-dominant	YAP1-dominant or quadruple-negative*
No. of patients	130 (68.4)	45 (23.7)	8 (4.2)	7 (3.7)
H-score of ASCL1				
Mean ± SD	250±54	91±67	36±41	26±38
High	130 (100.0)	27 (60.0)	2 (25.0)	2 (28.6)
Low	0	12 (26.7)	3 (37.5)	1 (14.3)
Negative	0	6 (13.3)	3 (37.5)	4 (57.1)
H-score of NeuroD1				
Mean ± SD	124±68	197±76	142±67	100±70
High	105 (80.8)	42 (93.3)	7 (87.5)	4 (57.1)
Low	20 (15.4)	3 (6.7)	1 (12.5)	2 (28.6)
Negative	5 (3.8)	0	0	1 (14.3)
H-score of POU2F3				
Mean ± SD	3±14	5±18	268±60	4±7
High	1 (0.8)	1 (2.2)	8 (100.0)	0
Low	31 (23.8)	15 (33.3)	0	2 (28.6)
Negative	98 (75.4)	29 (64.4)	0	5 (71.4)
H-score of YAP1				
Mean ± SD	5±18	14±29	3±7	169±115
High	4 (3.1)	3 (6.7)	0	6 (85.7)
Low	10 (7.7)	13 (28.9)	2 (25.0)	0
Negative	116 (89.2)	29 (64.4)	6 (75.0)	1 (14.3)

The variables are presented as numbers (%) if not otherwise specified. *, only one case was quadruple-negative. SD, standard deviation.

Table S3 Treatment-related characteristics by YAP1 expression

Characteristics	YAP1-negative (n=152)	YAP1-positive (n=38)	P
Chemotherapy regimen			0.80
Etoposide/carboplatin	42 (27.6)	12 (31.6)	
Etoposide/cisplatin	100 (65.8)	23 (60.5)	
Irinotecan/cisplatin	7 (4.6)	2 (5.3)	
Etoposide/platinum/immunotherapy	3 (2.0)	1 (2.6)	
Chemotherapy cycle [§]	6 [4–6]	6 [4–6]	0.56
Relative dose intensity per cycle [†] (%)	98 [84–100]	98 [85–100]	0.98
<75	18 (11.8)	2 (5.3)	0.55
75–<85	23 (15.1)	8 (21.1)	
85–<95	24 (15.8)	7 (18.4)	
≥95	87 (57.2)	21 (55.3)	
Concurrent chemoradiotherapy			0.59
Yes	53 (34.9)	15 (39.5)	
No	99 (65.1)	23 (60.5)	
Prophylactic cranial irradiation			0.37
Yes	60 (39.5)	18 (47.4)	
No	92 (60.5)	20 (52.6)	
Early discontinuation of treatment [‡]			>0.99
Yes	32 (21.1)	8 (21.1)	
No	120 (78.9)	30 (78.9)	
Treatment-related mortality			0.60
Yes	6 (3.9)	0	
No	146 (96.1)	38 (100.0)	

The variables are presented as numbers (%) or median [IQR]. [§], data for immunotherapy were excluded; [†], relative dose intensity was calculated for etoposide and irinotecan; [‡], case of early progressive disease was excluded. IQR, interquartile range.

Table S4 Expression levels of ASCL1 and NeuroD1 among YAP1-positive patients based on treatment response

Expression level	Complete response	Non-complete response	P
ASCL1			0.97
Negative	5 (16.1)	1 (14.3)	
Low	3 (9.7)	1 (14.3)	
High	23 (74.2)	5 (71.4)	
NeuroD1			0.32
Negative	0	0	
Low	4 (12.9)	0	
High	27 (87.1)	7 (100.0)	

The variables are presented as numbers (%).

Table S5 Cox regression for overall survival

Variables	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
Age						
<70 years	Ref.			Ref.		
≥70 years	1.739	1.283–2.357	<0.001	1.675	1.223–2.294	0.001
Sex						
Male	Ref.			–	–	–
Female	0.747	0.488–1.142	0.17	–	–	–
ECOG PS						
0–1	Ref.			Ref.		
2–3	2.846	2.007–4.036	<0.001	2.509	1.741–3.616	<0.001
Stage						
Limited-stage	Ref.			Ref.		
Extensive-stage	2.446	1.752–3.415	<0.001	2.023	1.329–3.079	0.001
Metastatic lesions (presence vs. absence)						
Central nervous system	1.448	0.985–2.129	0.06	0.984	0.630–1.538	0.94
Liver	1.721	1.169–2.535	0.006	1.163	0.771–1.755	0.47
Bone	2.444	1.735–3.444	<0.001	1.565	1.075–2.278	0.01
Pleural effusion	1.648	1.178–2.305	0.004	1.004	0.680–1.482	0.98
ASCL1 expression						
Negative	Ref.			–	–	–
Positive	1.743	0.890–3.416	0.10	–	–	–
NeuroD1 expression						
Negative	Ref.			–	–	–
Positive	1.348	0.552–3.291	0.51	–	–	–
POU2F3 expression						
Negative	Ref.			–	–	–
Positive	1.052	0.763–1.452	0.75	–	–	–
YAP1 expression						
Negative	Ref.			Ref.		
Positive	0.612	0.412–0.911	0.01	0.597	0.387–0.921	0.02

ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; CI, confidence interval.

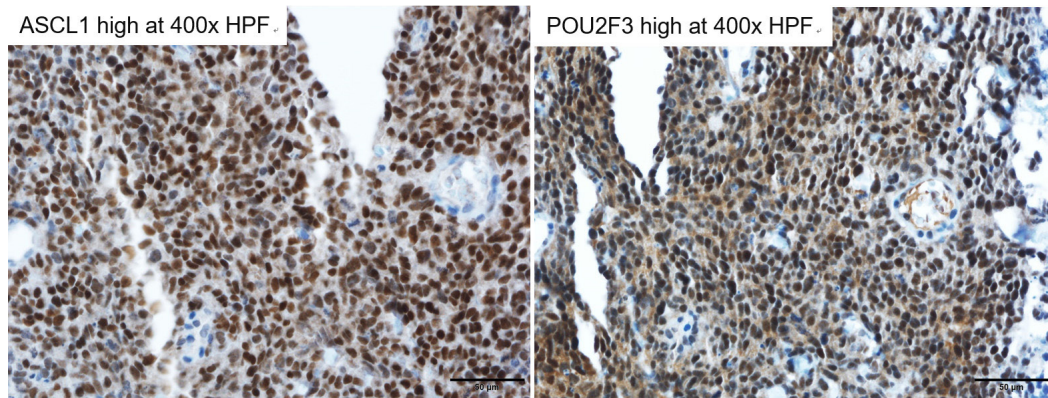


Figure S1 ASCL1-dominant small cell lung carcinoma with POU2F3-high expression. The left image shows immunohistochemical staining for ASCL1 at 400× HPE, and the right image shows immunohistochemical staining for POU2F3 at 400× HPE. HPE, high power field.

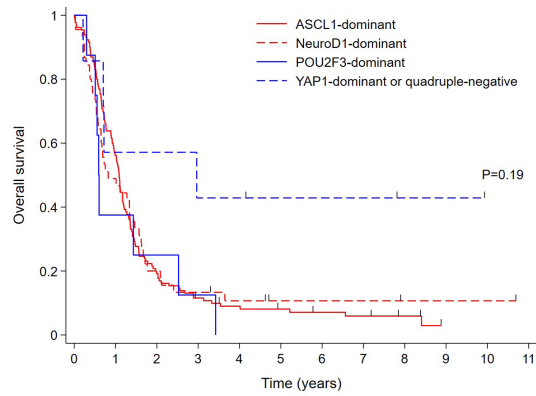
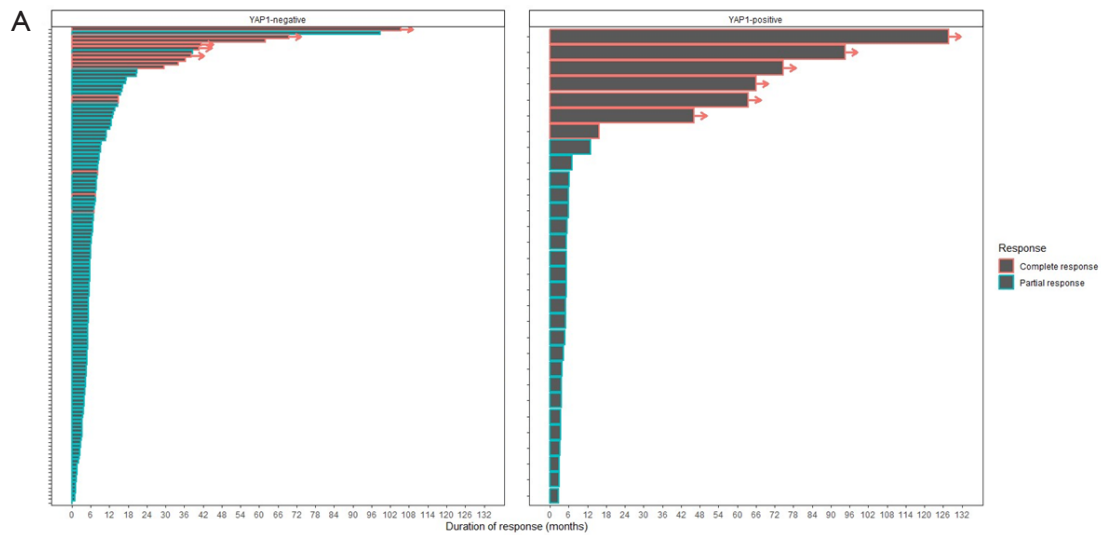
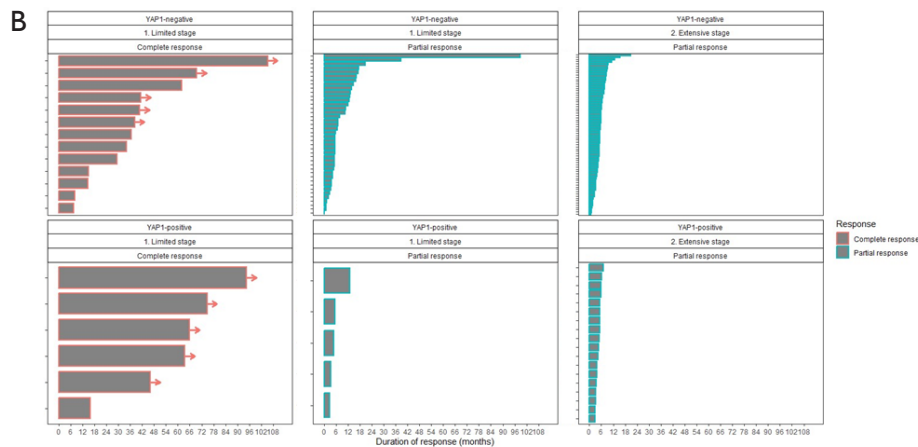


Figure S2 Overall survival by dominant subtype. Red and blue colors represent neuroendocrine and non-neuroendocrine subtypes, respectively.



Median DoR in months: YAP1-negative group, 5.8 (IQR: 4.3–9.2); YAP1-positive group, 5.2 (IQR: 3.7–12.9) (P=0.75)

Note: Arrow indicates the response maintained at the time of analysis.



Median DoR (IQR), months

	Limited-stage and complete response	Limited-stage and partial response	Extensive-stage and partial response
YAP1-negative	36.4 (15.0–41.3)	5.7 (4.3–13.2)	5.2 (3.9–7.1)
YAP1-positive	64.8 (46.1–74.8)	4.9 (3.4–5.5)	4.9 (3.5–5.2)
P	0.06	0.32	0.16

Note: Arrow indicates the response maintained at the time of analysis. Analysis for the extensive-stage and complete response subgroup was not conducted, as only one patient in each group achieved a complete response in the extensive stage.

Figure S3 DoR illustrated by swimmer plots. (A) Swimmer plots illustrate the DoR for the total population. (B) Swimmer plots illustrate the DoR for different subgroups. DoR, duration of response; IQR, interquartile range.

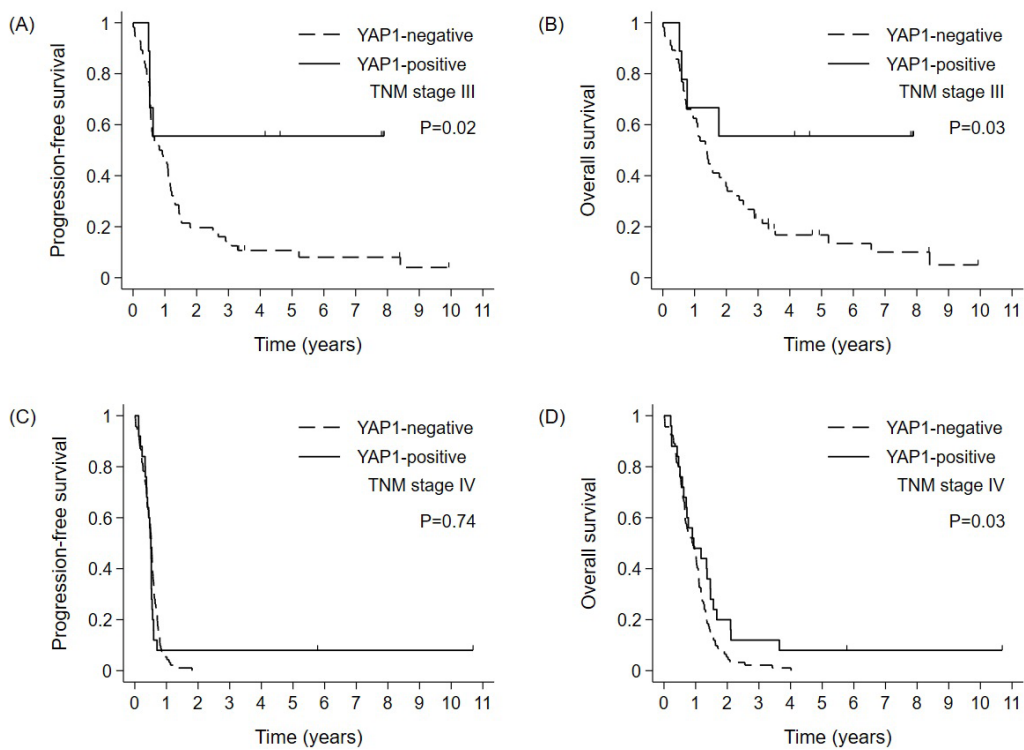


Figure S4 Survival analysis by YAP1 expression. Progression-free survival (A,C) and overall survival (B,D) in TNM stages III and IV, respectively [note: the analysis for TNM stages I–II was not performed due to the small sample size (n=8)]. TNM, tumor-node-metastasis.

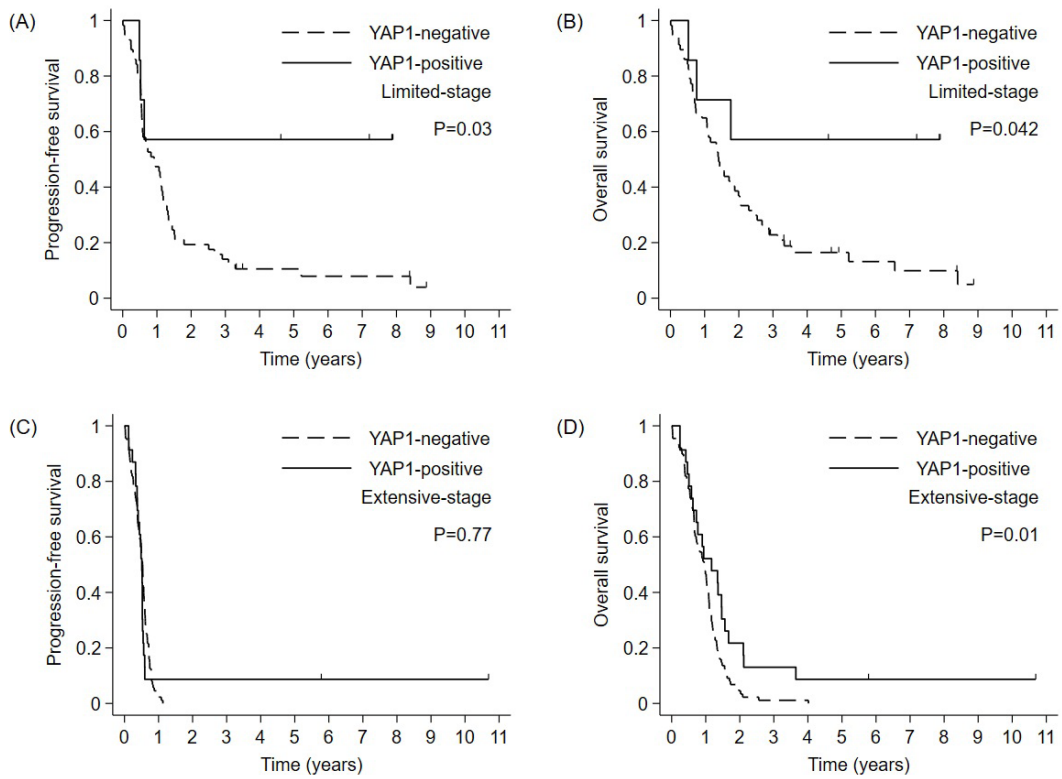


Figure S5 Survival analysis in patients with neuroendocrine subtype. Progression-free survival (A,C) and overall survival (B,D) in limited- and extensive-stage, respectively.