

48h

oh

NC

CD24-146 CD24-1934

### Figure S2

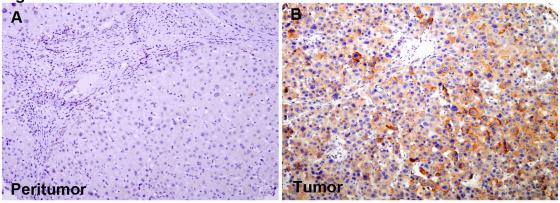
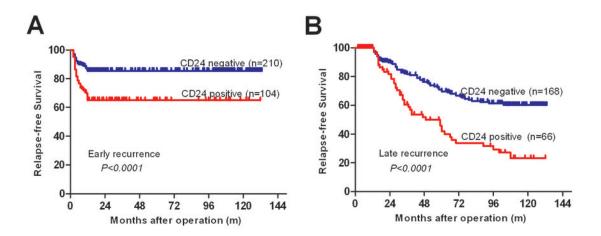
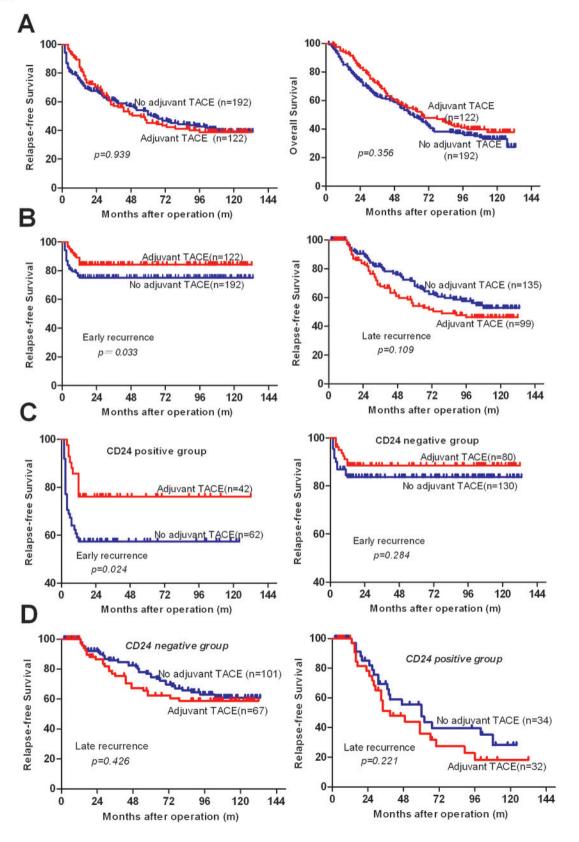


Figure S3



**Figure S4** 



#### Figure S1. Function analysis after CD24-siRNA in HCCLM3

CD24 expression in HCCLM3 was decreased significantly after treated by siRNA for 72h according to western blot analysis (**A**); Cells proliferation was detected by MTT assay (The graphs depict means ± SD of results generated from individual experiments with 3 replicate assays) (**B**); Monolayer of negative siRNA or CD24-siRNA HCCLM3 cells was abraded and then monitored at 0, 24, and 48 h for wound channel closure. The cleaned area was measured and plotted as the percentage of the original time point (0h); Data shown are mean (±SD) from three independent experiments (**C**); invasive behavior tested by trans-well Matrigel invasion assays (**D**). (Magnification×100 in **C** and ×200 in **D**)

#### Figure S2. Expression of CD24 in peritumoral and tumor tissues

The represent pictures for CD24 expression in peritumoral tissues (**A**) and tumor tissues (**B**); Most of peritumoral tissues was negative or low staining for CD24 and there were only 13 cases with positive score (13%, 13/100) in 100 case peritumoral tissues. There has 33.12% (104/314) with positive score in 314 HCC patients. There has significant difference expression of CD24 was between tumor and peritumoral tissues (p<0.0001). (Magnification, ×100)

## Figure S3. Kaplan-Meier analysis for the prognostic significance of CD24 in early recurrence and later recurrence

Kaplan-Meier analysis of RFS for CD24 expression in early recurrence groups (**A**) and later recurrence group (**B**); the prognostic significance of CD24 existed in both early recurrence and late recurrence groups (p<0.0001 for both).

# Figure S4. Kaplan-Meier analysis for adjuvant TACE in early recurrence and later recurrence stratified by CD24

Kaplan-Meier analysis of RFS and OS for adjuvant TACE in 314 cases (**A**); The effect of TACE in early recurrent and later recurrent groups (**B**); The effect of TACE in CD24-negative and CD24-positive HCC patients with early recurrence (**C**); The effect of TACE in CD24-negative and CD24-positive HCC patients with later recurrence (**D**).

**Abbreviations**: qRT-PCR, quantitative real-time polymerase chain reaction; siRNA, small interference RNA; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis; TACE, transcatheter arterial chemoembolization; RFS, Relapse-free survival; OS, overall survival.

Clinical and pathologica	al indexes	Number	Percentage (%)	
	≤52	165	52.55	
Age (y)	>52	149	47.45	
Cov	Female	48	15.29	
Sex	Male	266	84.71	
1.1. so states sta	No	26	8.28	
Liver cirrhosis	Yes	288	91.72	
	А	312	99.36	
Child-Pugh	В	2	0.64	
	Negative	55	17.52	
HBsAg	Positive	259	82.48	
	Negative	309	98.41	
HCV	Positive	5	1.59	
	≤54	152	48.41	
GGT (U/I)	>54	162	51.59	
	≤75	274	87.26	
ALT (U/I)	>75	40	12.74	
	≤20ng/ml	163	51.91	
AFP	>20ng/ml	151	48.09	
+	Complete	174	55.41	
umor encapsulation <sup>†</sup>	None	137	43.63	
	1-11	227	72.29	
Tumor differentiation	III-IV	87	27.71	
<b>_</b> .	≤5cm	171	54.46	
Tumor size	>5cm	143	45.54	
<b>-</b> .	Single	267	85.03	
Tumor number	Multiple	47	14.97	
	No	267	85.03	
Vascular invasion	Yes	47	14.97	
	NO	286	91.08	
Satellite lesion	Yes	28	8.92	
	0+1	248	78.98	
CLIP Stage	2+3+4	66	21.02	
	I	228	72.61	
TNM Stage	11-111	86	27.39	
	No	192	61.15	
Adjuvant TACE	Yes	122	38.85	
000	Negative	210	66.88	
CD24	Positive	104	33.12	
	Negative	188	59.87	
β-catenin	Positive	126	40.13	

Table S1.The clinical/pathological characteristics of 314 cases of HCC

PCNA	Negative	226	71.97	
	Positive	88	28.03	

**Abbreviations**: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; GGT, gammaglutamyl transferase; TACE, transcatheter arterial chemoembolization; TNM, tumor-node-metastasis; CLIP, Cancer of Liver Italian Program.

<sup>†</sup>3 cases without capsulation information were not calculated.

Clinicopathologic	al indexes	CD24 <sup>-</sup> (n=210)	CD24 <sup>+</sup> (n=104)	P value
Sov	Female	31	17	0.714
Sex	Male	179	87	
Age (y)	≤52	111	54	0.876
	>52	99	50	
HBsAg	Negative	39	16	0.484
	Positive	171	88	
HCV <sup>†</sup>	Negative	208	101	0.198
	Positive	2	3	
Liver cirrhosis <sup>†</sup>	No	22	4	0.051
LIVEI CITTIOSIS	Yes	188	100	
ALT (U/I)	≤75	185	89	0.529
	>75	25	15	
Child Pugh score <sup>†</sup>	А	209	103	0.611
Child-Pugh score <sup>†</sup>	В	1	1	
GGT(U/I)	≤54	100	52	0.691
661(0/1)	>54	110	52	
AFP (ng/ml)	≤20	96	67	0.002
Air (lig/ill)	>20	114	37	
Tumor encapsulation§	complete	110	64	0.159
	no	97	40	
Tumor size (cm)	≤5	120	51	0.175
	>5	90	53	
Tumor number	Single	184	83	0.068
	Multiple	26	21	
Vascular invasion	No	176	91	0.388
vascular invasion	Yes	34	13	
satellite	No	191	95	0.908
Satemic	Yes	19	9	
TNM stage	I	156	72	0.344
This stage	-	54	32	
Tumor differentiation	1-11	149	78	0.451
	III-IV	61	26	
CLIP stage	0+1	163	85	0.400
OEn Stage	2+3+4	47	19	
Adjuvant TACE	No	130	62	0.695
	Yes	80	42	
Recurrence time(m)	≤12	30	36	0.104
	>12	60	43	
Recurrence types <sup>‡</sup>	Туре І	69	49	0.039
Necurrence types	Type II and III	21	30	
β-catenin	negative	135	53	0.023

Table S2. Correlation between CD24 and clinicopathological characteristics

	positive	75	51	
PCNA	≤50%	160	66	0.018
FONA	>50%	50	38	

Abbreviations: ALT, alanine aminotransferase; AFP, alpha-fetoprotein; GGT,

gammaglutamyl transferase; TACE, transcatheter arterial chemoembolization; TNM, tumor-node-metastasis; CLIP, Cancer of Liver Italian Program.

<sup>†</sup>Fisher's exact tests; Chi-square tests for all the other analyses.

§ 3 cases without capsulation information were not calculated.

<sup>‡</sup> Type I was local recurrence in remnant liver with a single or double lesion (n = 119); Type II was multinodular ( $\geq$ 3) or diffuse pattern consisting of many nodules scattered throughout the remaining liver (n = 23); Type III was extrahepatic metastasis (n = 27).

Clinicopathological	indexes	CD24 <sup>-</sup> (n=87)	CD24 <sup>+</sup> (n=13)	P value
Sex <sup>†</sup>	Female	7	2	0.331
Sex '	Male	80	11	
Age (y)	≤52	48	6	0.543
	>52	39	7	
HBsAg <sup>†</sup>	Negative	24	3	1.000
	Positive	63	10	
HCV <sup>†</sup>	Negative	84	12	0.432
HCV	Positive	3	1	
lines simble sist	No	11	2	0.676
Liver cirrhosis <sup>†</sup>	Yes	76	11	
	≤75	77	13	0.351
ALT (U/I) <sup>†</sup>	>75	10	0	
	А	86	13	1.000
Child-Pugh score <sup>†</sup>	В	1	0	
	≤54	41	5	0.559
GGT(U/I)	>54	46	8	
	≤20	46	7	0.948
AFP (ng/ml)	>20	41	6	
<b>T</b>	complete	46	8	0.559
Tumor encapsulation	no	41	5	
<b>T</b>	≤5	36	6	0.745
Tumor size (cm)	>5	51	7	
<b>T</b>	Single	76	13	0.350
Tumor number <sup>†</sup>	Multiple	11	0	
Mana 14.14 - 414.1	No	75	11	1.000
Vascular invasion <sup>†</sup>	Yes	12	2	
, t	No	75	13	0.356
satellite <sup>†</sup>	Yes	12	0	
	I	64	11	0.508
TNM stage <sup>†</sup>	11-111	23	2	
Tumor differentiation <sup>†</sup>	1-11	66	7	0.173
	III-IV	21	6	
	0+1	65	10	1.000
CLIP stage <sup>†</sup>	2+3+4	22	3	
	≤12	23	4	1.000
Recurrence time(m) <sup>†</sup>	>12	10	11	

 Table S3. Correlation between peritumoral CD24 expression and clinicopathological characteristics in 100 patients.

Abbreviations: ALT, alanine aminotransferase; AFP, alpha-fetoprotein; GGT, gammaglutamyl transferase; TACE, transcatheter arterial chemoembolization; TNM, tumor-node-metastasis; CLIP, Cancer of Liver Italian Program.

<sup>†</sup>Fisher's exact tests; Chi-square tests for all the other analyses.