Myc

Alb

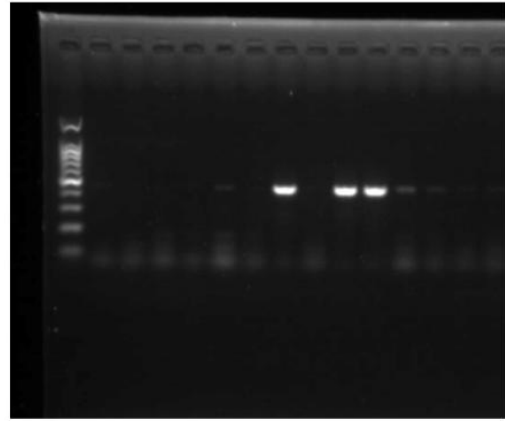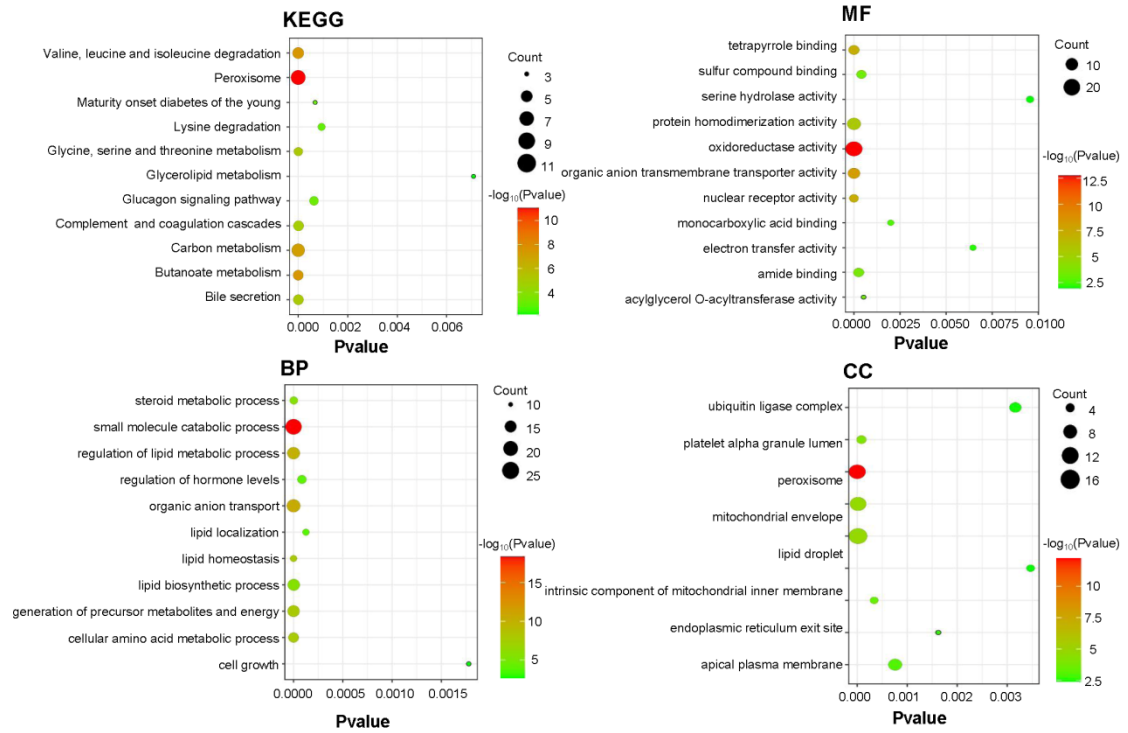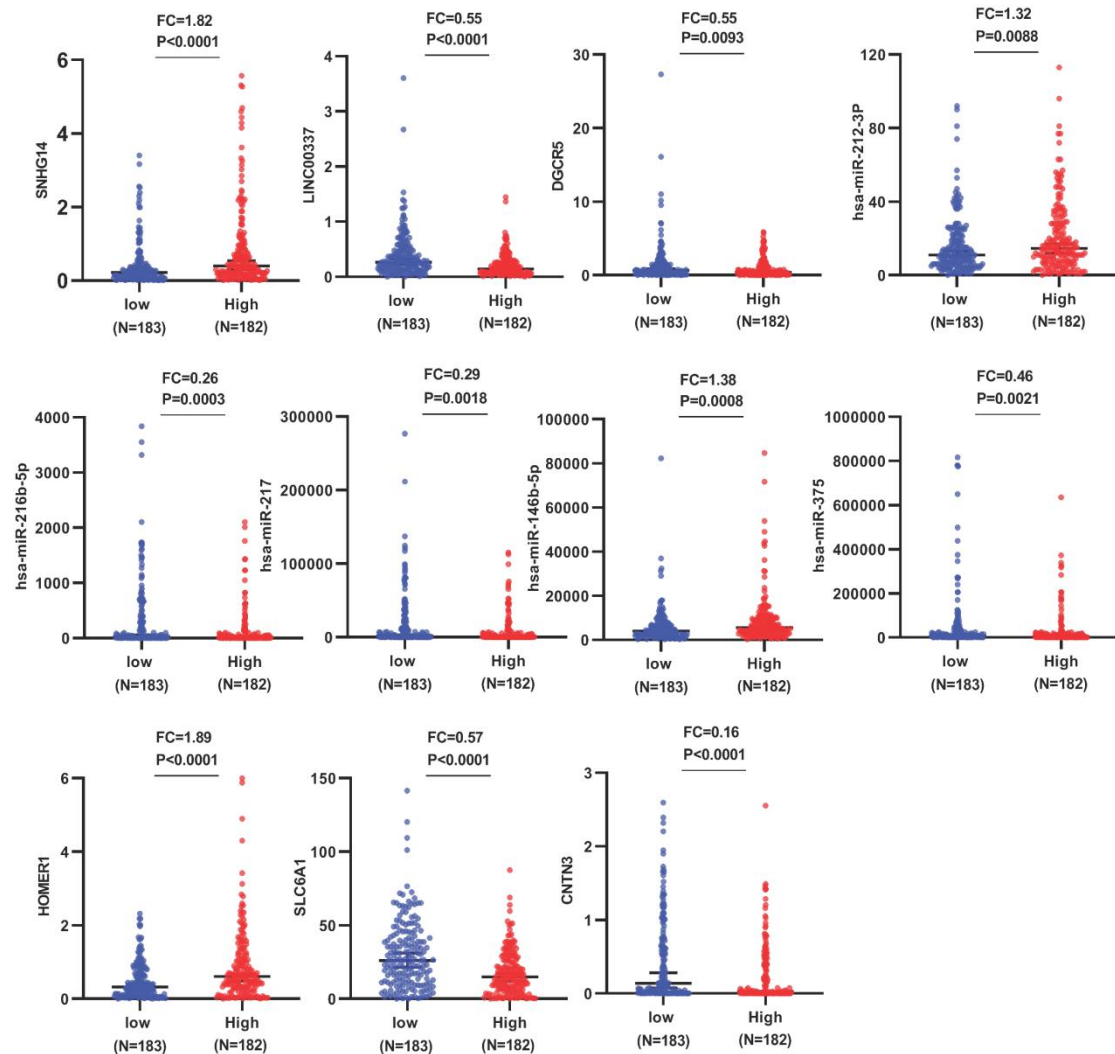**Supplemental Figure 1 The gene identification results of Alb-cre;Myc mice and wild-type (WT) mice.**

Supplemental Figure 2 Functional enrichment analysis of SLC6A1:

Functional enrichment analysis (including Gene ontology and KEGG pathway)

of SLC6A1-related 200 genes from the GEPIA database.

Supplemental Figure 3 The RNA expression analysis of a hub triple regulatory network:
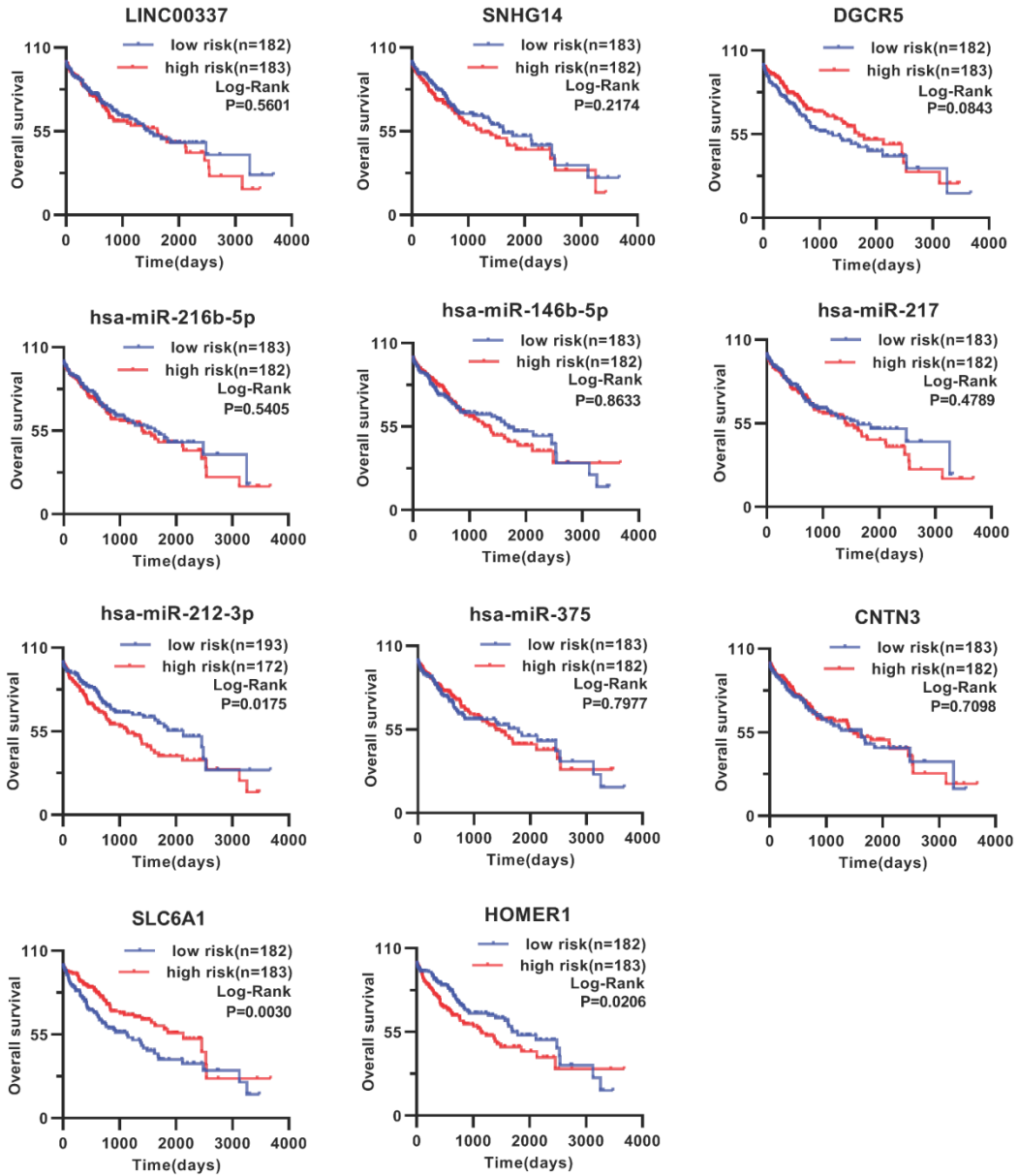
Comparison between low-Myc expression and high-Myc expression groups:

1 up-regulated (SNHG14) and 2 down-regulated (LINC00337, DGCR5)

lncRNAs, 2 up-regulated (miR-212-3p, miR-146b-5p) and 3 down-regulated

(miR-216b-5p, miR-217, miR-375) miRNAs, 1 up-regulated (HOMER1)and 2

down-regulated (SLC6A1, CNTN3) mRNAs in HCC samples.

Supplemental Figure 4 The overall survival (OS) analysis of a hub triple regulatory network:

Comparison between low-Myc expression and high-Myc expression groups: 1DEmiRNAs (miR-212-3p) and 2 DEmRNAs (SLC6A1, HOMER1) were found to be significantly associated with prognosis in HCC samples.

A RNA Expression of SLC6A1 in Cancer Cell Lines

B
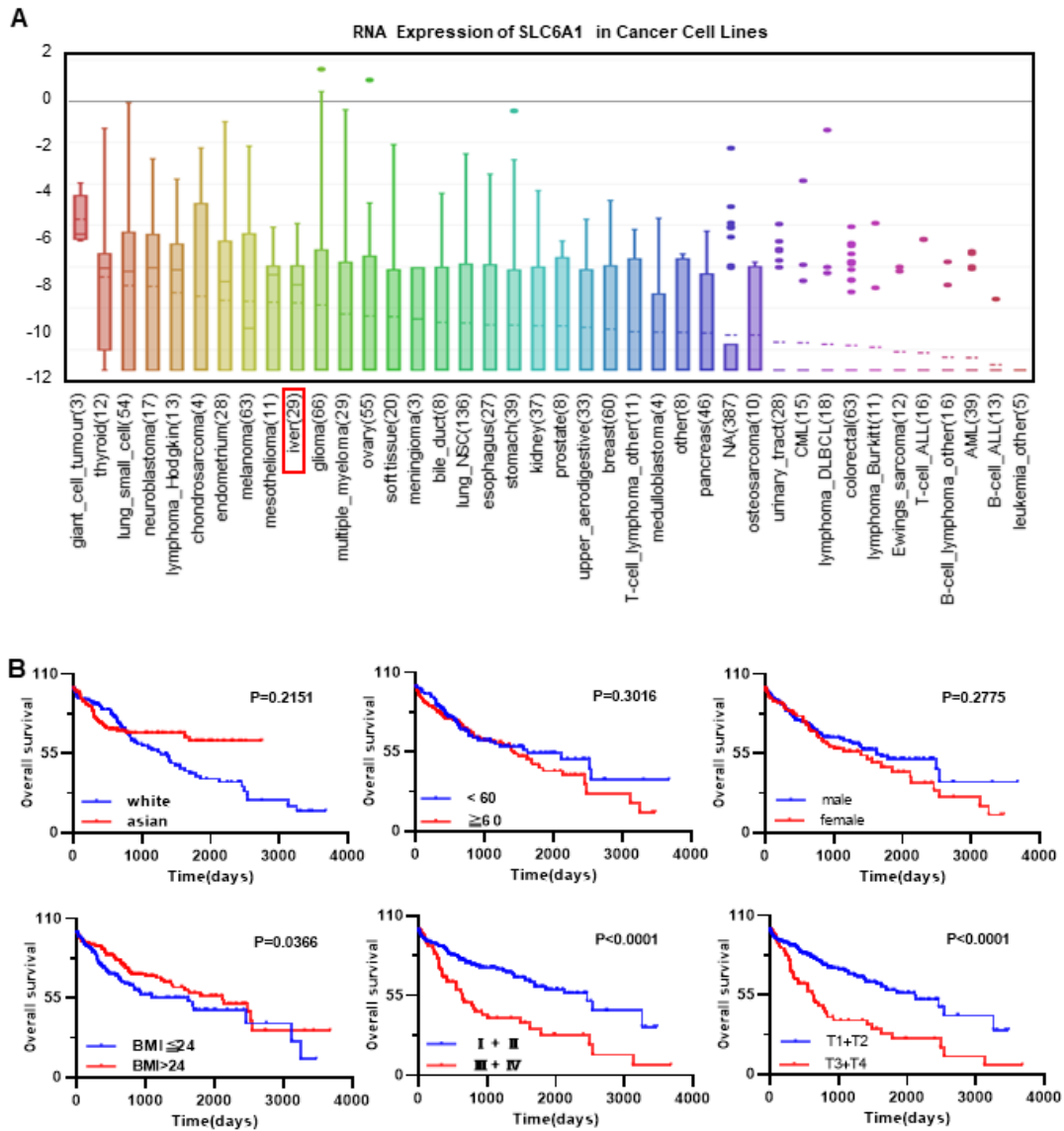
Supplemental Figure 5

(A) The RNA expression distribution of SLC6A1 in pan-cancer cell lines by CCLE database. (B)The prognostic significance of the clinicopathologic features (incluing race, age, gender, BMI, TNM stage and tumor diameter).
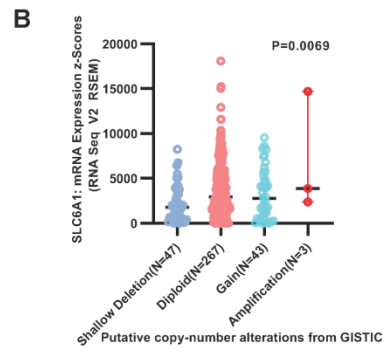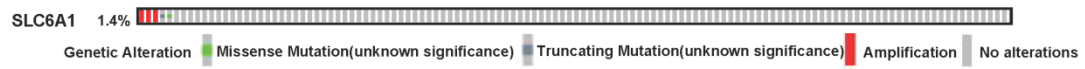
Supplemental Figure 6 The genetic alterations distribution of SLC6A1 in 360 HCC samples by cbioportal database.
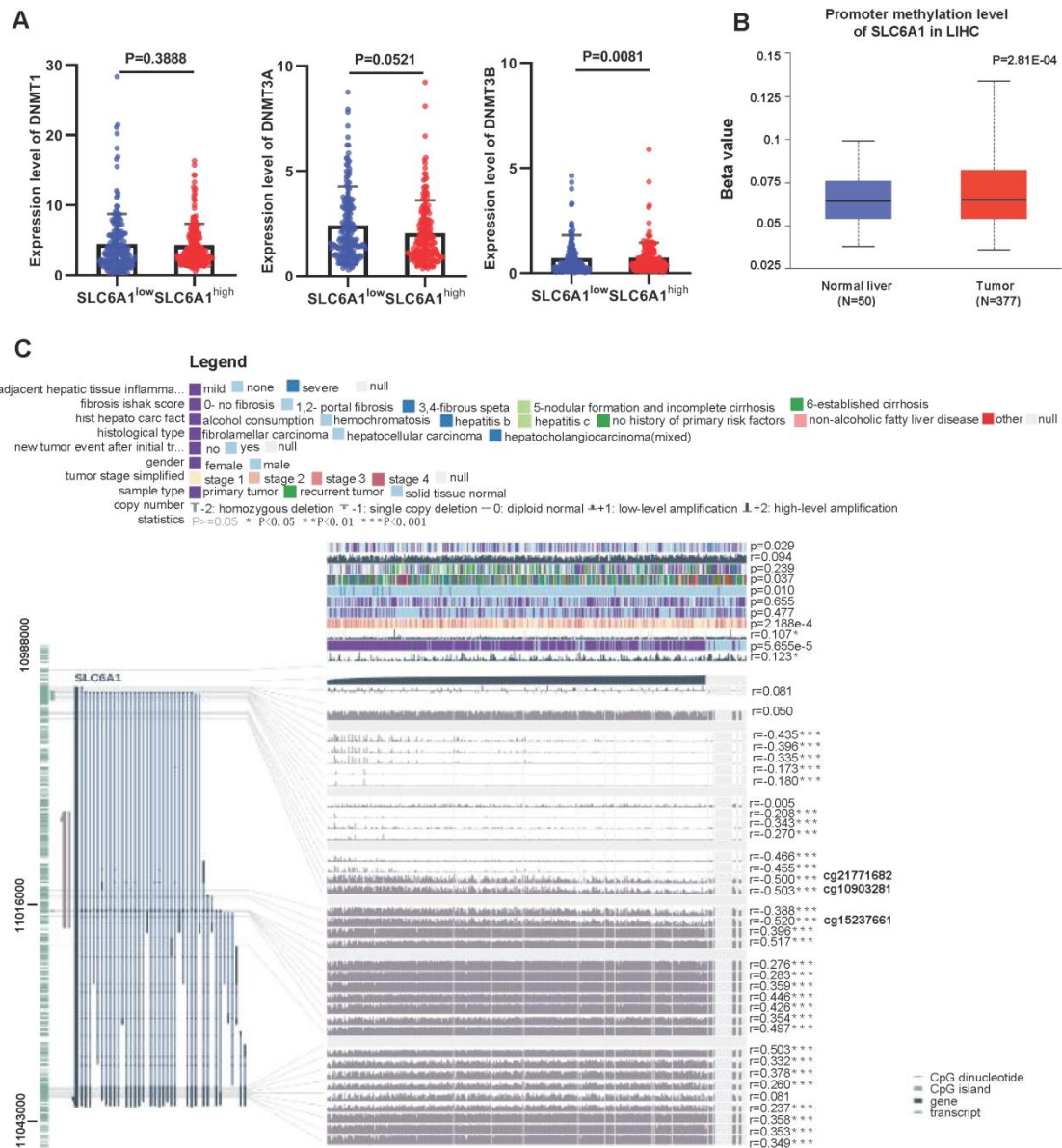
Supplemental Figure 7 Associated SLC6A1 with methylation analysis:

(A) The methylation expression of SLC6A1 in HCC tissues using the UALCAN

database (B) The SLC6A1 expression was negatively correlated with DNA

methylation, Pearson's correlation coefficients and P values for methylation

sites are shown on the right side.

Table S1 Top 10 genes by twelve ranked methods respectively in cytoHubba

mRNA	Betweenness	BottleNeck	Closeness	Clustering Coefficient	Degree	DMNC	EcCentricity	EPC	MCC	MNC	Radiality	Stress
SEC14L2	0	1	18.33333	0	1	0	0.25	7.653	1	1	2.06122	0
SLC26A7	73.29091	1	22	0	2	0	0.25	12.276	2	1	2.59184	430
TAF4B	0	1	18.33333	0	1	0	0.25	8.063	1	1	2.06122	0
SLC6A1	**55.39394**	**1**	**21.75**	**0**	**2**	**0**	**0.25**	**12.898**	**2**	**1**	**2.55102**	**376**
E2F5	0	1	18.33333	0	1	0	0.25	8.092	1	1	2.06122	0
NCKAP5	0	1	18.33333	0	1	0	0.25	8.202	1	1	2.06122	0
FAM167A	0	1	18.33333	0	1	0	0.25	8.012	1	1	2.06122	0
CNTN3	**54.23636**	**1**	**21**	**0**	**2**	**0**	**0.25**	**12.403**	**2**	**1**	**2.42857**	**326**
CNTNAP3	0	1	18.33333	0	1	0	0.25	8.101	1	1	2.06122	0
HOMER1	**55.39394**	**1**	**21.75**	**0**	**2**	**0**	**0.25**	**12.544**	**2**	**1**	**2.55102**	**376**

Table S2 The IHC data were yielded and analysed for liver cancer tissues from the Human Protein Atlas database

Group	The number of samples	Percentage (%)
SLC6A1 staining		
High	0	0
Medium	5	5 (5 of 28)
Low	3	3 (3 of 28)
Not detected	20	20 (20 of 28)
SLC6A1 intensity		
Strong	0	0
Moderate	7	7 (7 of 28)
Weak	3	3 (3 of 28)
Negative	18	18 (18 of 28)
SLC6A1 quantity		
>75%	4	4 (4 of 28)
75%-25%	2	2 (2 of 28)
<25%	4	4 (4 of 28)
None	18	18 (18 of 28)
SLC6A1 location		
Nuclear	0	0
Cytoplasmic/membranous	10	10 (10 of 28)
Cytoplasmic/membranous, nuclear	0	0
None	18	18 (18 of 28)

Table. S3 IHC of SLC6A1 in Human Protein Atlas database.

NO.	Tissue type	ID	Age	Gender	Staining
1	Normal	1720	67	Male	Medium
2	Normal	3402	54	Female	Medium
3	Tumor	2177	58	Female	Not detected
4	Tumor	3196	65	Male	Not detected

Table S4 Univariate analysis of overall survival in LIHC patients stratified based on clinical characteristics

Factor	Variable	N	SLC6A1	P value	Overall survival

		median		Months (median)	95%CI	P value (Log-rank test)
Age						
≧60	198	19.72	0.517	21.02	17.30-23.10	0.302
<60	167	18.29		18.53	15.73-20.90	
Gender						
male	247	17.96	0.186	19.13	15.93-21.20	0.278
female	118	22.87		20.98	18.50-25.67	
BMI						
<18.5	21	12.00	**0.006**	19.57	12.17-30.33	0.087
18.5-24	136	16.82		15.62	13.53-19.60	
24-28	82	23.29		21.37	18.73-25,50	
≥ 28	92	21.05		23.70	18.60-27.63	
TNM stage						
I - II	252	20.01	**0.024**	20.17	18.50-22.67	**0.000**
III-IV	89	16.27		13.60	11.63-18.60	
Diameter						
< 5cm	270	20.57	**0.010**	21.02	19.17-23.10	**0.000**
≥ 5cm	92	15.07		13.60	11.63-18.60	
Lymph-node metastasis						
Negative	249	18.29	0.227	19.37	17.07-21.83	0.115
Positive	4	15.77		21.75	0.50-33.60	
Unknown	111	21.93		20.03	14.60-23.10	
Distant metastasis						
Negative	263	17.36	0.062	19.37	16.00-21.63	**0.004**
Positive	4	22.90		13.02	0.00-31.03	
Unknown	98	22.51		20.38	16.67-23.53	

Table S5 Correlation analysis between SEC14L2/SLC6A1 and biomarkers

of immune cells in HCC

Description	Gene markers	SLC6A1	
		Cor	P
CD8+ T cell	CD8A	-0.129	1.32e-02
	CD8B	-0.239	3.19e-06
T cell (general)	CD3D	-0.3	4.3e-09
	CD3E	-0.195	1.6e-04
	CD2	-0.203	8.81e-05
B cell	CD19	-0.121	1.96e-02
	CD79A	-0.136	8.84e-03
Monocyte	CD86	-0.235	5.04e-06
	CD115 (CSF1R)	-0.253	8.76e-07
TAM	CCL2	-0.133	1.05e-02
	CD68	-0.292	1.21e-08
	IL10	-0.156	2.51e-03
M1 Macrophage	INOS (NOS2)	0.243	2.15e-06
	IRF5	0.139	7.13e-03
	COX2 (PTGS2)	-0.099	5.79e-02
M2 Macrophage	CD163	-0.061	2.38e-01
	VSIG4	-0.15	3.92e-03
	MS4A4A	-0.148	4.37e-03
Neutrophils	CD66b (CEACAM8)	-0.073	1.61e-01
	CD11b (ITGAM)	-0.14	7.09e-03
	CCR7	-0.042	4.2e-01

Natural killer cell	KIR2DL1	-0.004	9.37e-01
	KIR2DL3	0.003	9.59e-01
	KIR2DL4	-0.171	9.55e-04
	KIR3DL1	0.039	4.59e-01
	KIR3DL2	-0.05	3.33e-01
	KIR3DL3	-0.003	9.58e-01
	KIR2DS4	0.017	7.48e-01
Dendritic cell	HLA-DPB1	-0.256	6.23e-07
	HLA-DQB1	-0.23	8.06e-06
	HLA-DRA	-0.208	5.7e-05
	HLA-DPA1	-0.164	1.6e-03
	BDCA-1(CD1C)	-0.043	4.12e-01
	BDCA-4(NRP1)	-0.046	3.79e-01
	CD11c(ITGAX)	-0.156	2.6e-03
Th1	T-bet (TBX21)	-0.071	1.7e-01
	STAT4	0.006	9.05e-01
	STAT1	0.05	3.41e-01
	IFN-γ (IFNG)	-0.149	4.01e-03
	TNF-α (TNF)	-0.146	4.92e-03
Th2	GATA3	-0.145	5e-03
	STAT6	0.159	2.19e-03
	STAT5A	-0.09	8.2e-02
	IL13	0.189	2.56e-04
Tfh	BCL6	0.081	1.19e-01
	IL21	-0.026	6.24e-01
Th17	STAT3	0.075	1.5e-01
	IL17A	0.04	4.43e-01

Treg	FOXP3	0.276	6.83e-08
	CCR8	0.044	3.97e-01
	STAT5B	0.423	0e+00
	TGF β (TGFB1)	-0.243	2.34e-06
T cell exhaustion	PD-1 (PDCD1)	-0.245	1.75e-06
	CTLA4	-0.301	3.4e-09
	LAG3	-0.159	2.08e-03
	TIM-3 (HAVCR2)	-0.248	1.49e-06
	GZMB	-0.196	1.48e-04

Table S6 Correlation analysis between SLC6A1 and biomarkers of T cell (general), monocyte, TAM and T cell exhaustion in GEPIA

Description	Gene markers	LIHC(SLC6A1)			
		Tumor		Normal	
		R	P	R	P
T cell (general)	CD3D	-0.33	1.4e-10	-0.00	0.99
	CD3E	-0.18	0.0005	0.09	0.55
	CD2	-0.19	0.0003	0.11	0.44
Monocyte	CD86	-0.20	0.0002	-0.03	0.83
	CD115 (CSF1R)	-0.19	0.0002	0.11	0.43
TAM	CCL2	-0.12	0.027	-0.37	0.008
	CD68	-0.22	2.5e-05	0.015	0.92
	IL10	-0.05	0.3	0.13	0.38
Treg	STAT5B	0.46	2.2e-20	0.39	0.006
	TGF β (TGFB1)	-0.31	1e-09	-0.1	0.48
T cell exhaustion	PD-1 (PDCD1)	-0.22	1.7e-05	0.13	0.36
	CTLA4	-0.27	1.8e-07	0.03	0.82
	LAG3	-0.17	0.00095	0.09	0.55
	TIM-3 (HAVCR2)	-0.23	9.4e-06	-0.16	0.27
	GZMB	-0.23	7.4e-06	-0.015	0.92