

OMTN, Volume 31

Supplemental information

Exosomal circ-PTPN22 and circ-ADAMTS6 mark

T cell exhaustion and neutrophil extracellular

traps in Asian intrahepatic cholangiocarcinoma

Xuezhu Wang, Guanqun Wang, Zilong Wu, Yucheng Dong, Yue Shi, Fan Yang, Xinyu Chen, Jun Wang, Shunda Du, Haifeng Xu, and Yongchang Zheng

Table S1 Clinical data of the Asian ICC patient cohort.

Subgroup	ID	Age	Sex	CA199	CA125	Differentiation	TNM	OP date	Lymph node	Recurrence (2021-08)	Metastases (2021-08)	Survival (2021-08)
1	2534465	72	male	13.4	NA	NA	NA	2020/9/11	none	none	none	alive
2	2537821	80	male	66.1	20.8	NA	T1N1M0	2020/9/18	metastases	none	none	alive
2	2518780	59	male	NA	NA	NA	NA	2019/12/2	none	none	none	alive
2	2287602	68	female	2907	80	G1	T4N1M1	2018/12/14	none	NA	NA	NA
2	1	66	male	1.73	6.48	G2	T1bN0M0	2020/1/11	none	recurrent (2021/4/28)	intrahepatic and lymph	alive
2	2	53	male	1592.4	8.52	G3	T2N0M0	2020/1/16	metastases	recurrent (2021/1/13)	intrahepatic and lung	alive
2	5	63	female	1988.1	1112.68	G3	T3N1M0	2020/12/17	metastases	recurrent (2021/3/5)	intrahepatic	deceased (2021/6/1)

2	7	51	male	18.15	100.81	G3	T1bN1M0	2021/1/7	metastases	none	none	alive
3	2520263	65	female	146.9	277.5	Gx	NA	2019/1/8	metastases	recurrent (2019/12/17)	intrahepatic and lymph	deceased (2019/12/26)
3	2523247	67	male	21.8	NA	G3	T3N0M0	2019/1/29	metastases	NA	NA	NA
3	2393116	60	female	119.5	NA	NA	T1N0M0	2019/3/15	none	none	none	alive
3	3	74	male	154.41	12.86	G3	T3N1M0	2020/1/18	none	NA	NA	NA
3	4	66	female	44.55	4.76	G3	T1bN0M0	2020/1/23	none	recurrent (2021/7/7)	intrahepatic	alive
3	6	75	female	510.11	157.05	G3	T1bN0M0	2020/1/29	none	recurrent (2021/3/1)	intrahepatic	deceased (2021/6/1)

Table S2 Mean size and concentration of exosomes.

	Mean size of exosomes (nm)	Concentration of exosomes (Particles/mL)
1	76.63	5.32E+8
2	80.48	5.02E+8
3	78.44	9.81E+8
4	76.97	7.22E+8
5	79.29	1.23E9

Table S3 Raw data of the size of exosomes.

These data are too big to be presented in this document.

Please find the supplemental file “TableS3.xlsx”.

Table S4 circRNAs reported by recent publications.

circRNA	Host gene	Microarray probe	ID in circBase (hg19)	ID in this study (hg38)
circ-PTPN22	PTPN22	ASCRP3004050	chr1:114377531-114397671	chr1-113834909-113855049--
circ-ADAMTS6	ADAMTS6	ASCRP3001689	chr5:64747301-64769779	chr5-65451474-65473952--
CDR1as	CDR1	ASCRP3010297	chrX:139865339-139866824	chrX-140783174-140784659-+
circ-0000284	HIPK3	ASCRP3002878	chr11:33307958-33309057	chr11-33286412-33287511-+
circ_0005230	DNM3OS	ASCRP3013585	chr1:172109619-172113577	chr1-172140479-172144437--
circRTN4IP1	RTN4IP1	ASCRP3013332	chr6:107031202-107050797	chr6-106583327-106602922--
circSETD3	SETD3	ASCRP3002403	chr14:99924615-99932150	chr14-99458278-99465813--
circ_0020256	NSMCE4A	ASCRP3009181	chr10:123718838-123721032	chr10-121959323-121961517--
circ-CCAC1	ERBB2	ASCRP3004665	chr17:37880978-37882106	chr17-39724725-39725853-+
circ-LAMP1	LAMP1	ASCRP3013337	chr13:113963957-113964177	Not captured
circ0021205	WEE1	Not captured	chr11:9597776-9611313	Not captured

Table S5 Primer sequences used for RT-qPCR validation.

	Forward	Reverse
circ-PTPN22	CCTGTATGGACACCTGAATCATT	AACACTGTCATCCTCTTGGTAAC
circ-ADAMTS6	ATTGCCACACCACGATAATGC	TCCACTGTTTAAGAGCCACTTT
GAPDH mRNA	AGGGCTGCTTTTAACTCTGGT	CCCCACTTGATTTTGGAGGGA
PTPN22 mRNA	CCTACAACCTGTGGCTGAGAA	AATCATCCTCCAGAAGTCCAG
ADAMTS6 mRNA	GCCGTGGTACTTGCCTTGATAATGA	GCTGCTGGAATACTGTTGGTGACA

Table S6 Raw data of RT-qPCR results.

circ-PTPN22

Normal without RNase R	Normal with RNase R	Subgroup2 without RNase R	Subgroup2 with RNase R	Subgroup3 without RNase R	Subgroup3 with RNase R
0.845106	1.441134	20.05075	25.43529	0.700863	1.344626
1.154449	0.29062	13.6552	25.51818	0.534849	1.033261
0.946118	0.837579	4.299362	4.770441	1.525517	0.138629
1.053882	2.719574	6.426898	7.967475	2.656079	0.202964
0.359515	2.368745	3.103428	4.48113	0.944842	1.19594
1.640485	0.415846	2.627813	4.450176	1.100489	1.691315
1.031182	0.138149	4.12041	5.94958		
0.968818	0.144016	6.202233	5.072819		
0.557926	0.419908				
1.442074	0.557926				
0.961836	0.51902				
1.03804	0.445613				
1.077575	1.246418				
0.925168	1.246418				
1.191569	1.346145				
1.000147	0.579919				

circ-ADAMTS6

Normal without RNase R	Normal with RNase R	Subgroup2 without RNase R	Subgroup2 with RNase R	Subgroup3 without RNase R	Subgroup3 with RNase R
0.943123	1.364224	8.726059	7.969826	1.182231	0.698103
1.056877	2.254259	8.801042	9.129387	1.050817	1.445443
1.027719	1.453414	6.230922	4.624975	0.077636	0.081495
0.972281	1.291856	5.462061	5.615621	0.125248	0.103871
0.651353	1.570813	2.523608	2.595477	8.485361	8.603812
1.348646	1.386562	1.817002	2.942166	8.310735	6.430701
1.058817	0.571352	1.16053	1.468948		
0.941121	0.79689	1.783589	2.470466		
0.592028	0.418237				
1.407981	0.875565				
0.982673	0.576257				
1.017327	0.832074				
1.006939	1.013943				
0.993076	0.683009				

PTPN22 mRNA

Normal without RNase R	Normal with RNase R	Subgroup2 without RNase R	Subgroup2 with RNase R	Subgroup3 without RNase R	Subgroup3 with RNase R
0.768768	0.096706	1.678219	0.340603	0.081923	0.033271
1.09308	0.003395	1.431785	0.491162	0.072313	0.029166
1.138397	0.058775	5.010523	0.704641	0.404878	0.051294
1.006928	0.341501	5.115805	0.771085	0.323635	0.044876
0.993066	0.371121	3.740912	0.506486	0.302513	0.138223
1.064369	0.926587	2.108741	0.666247	0.15659	0.174956
0.999999	0.414659	4.899345	1.720215		
1.145082	0.32949	7.074308	1.561128		
0.854918	0.295519				
0.543625	0.237907				
1.463722	0.2325				
1.226943	0.019574				
0.776505	0.019574				
0.82507	0.145853				
1.174942	0.142852				

ADAMTS6 mRNA

Normal without RNase R	Normal with RNase R	Subgroup2 without RNase R	Subgroup2 with RNase R	Subgroup3 without RNase R	Subgroup3 with RNase R
1.498709	0.632992	2.764976	0.715304	0.963277	0.072094
1.468477	0.415847	1.883026	0.700918	0.172662	0.071596
0.032463	0.00668	2.06218	1.184411	0.126477	0.050658
1.034669	0.079614	1.734079	0.97547	0.190379	0.053547
0.965381	0.072251	5.771718	4.196801	0.470508	0.083555
1.24454	0.82109	9.503187	1.548238	0.31006	0.532809
0.755557	0.380406	4.899345	1.720215		
1.243377	0.903985	7.074308	1.561128		
0.756623	1.416929				
0.82507	0.145853				
1.174942	0.142852				
0.788371	0.109344				
1.211628	0.157885				
1.139589	0.153589				
0.860392	0.167761				

Table S7 Primer sequences used PCR analysis.

	Forward	Reverse
circ-PTPN22 primer	TACCAAAAAGCAACTGCTCCA	ACAACGTACATCCCAGATGAGC
circ-ADAMTS6	ATTGCCACCACGATAATGC	TCCACTGTTTAAGAGCCACTTT
PTPN22 mRNA	CCTACAACGTGGCTGAGAA	AATCATCCTCCAGAAGTCCAG
ADAMTS6 mRNA	GCCGTGGTACTTGCCTTGATAATGA	GCTGCTGGAATACTGTTGGTGACA

Fig. S1

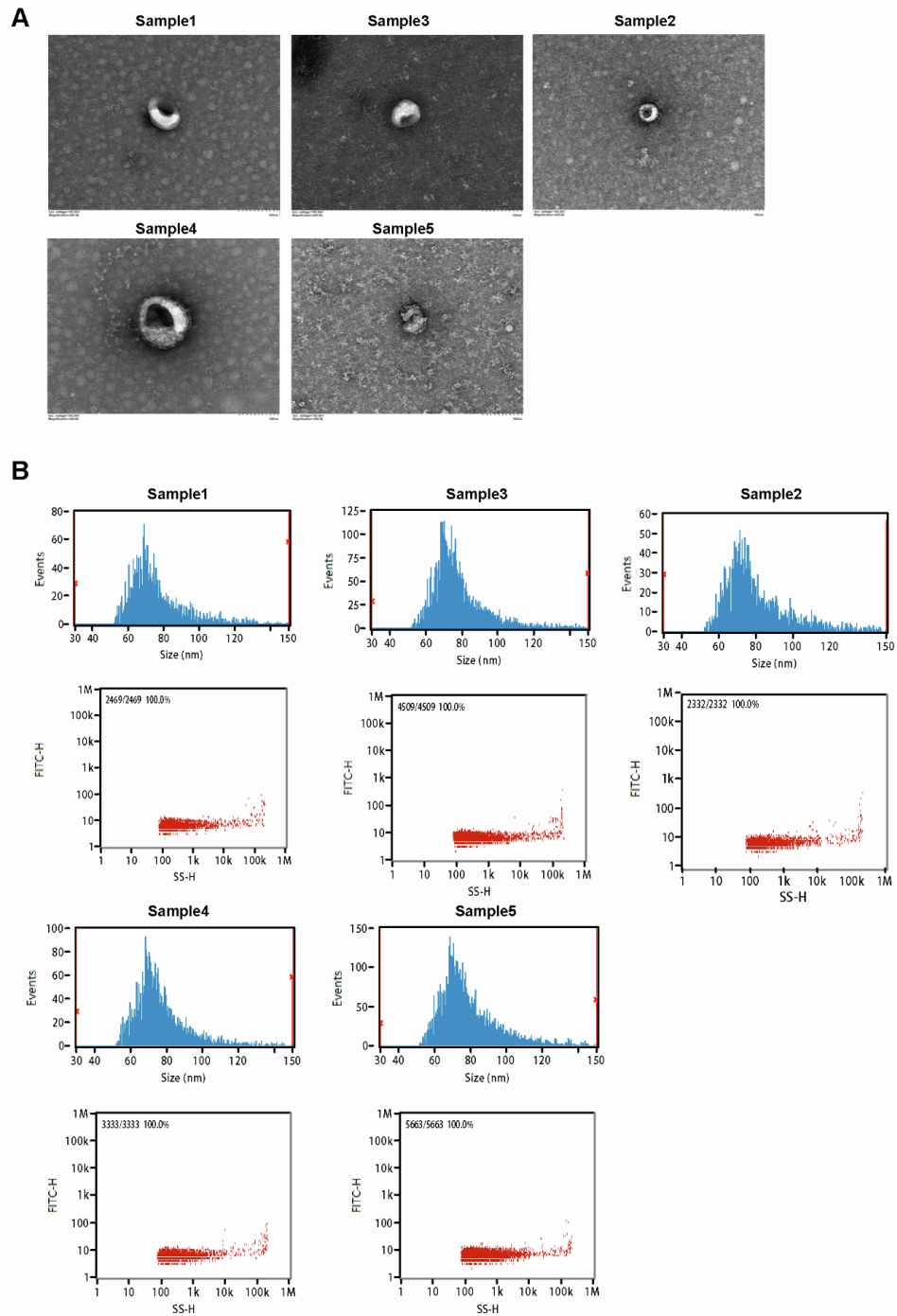


Figure S1

(A) Representative plasma exosomes detected by transmission electron microscopy. (B) Size of plasma exosomes analyzed by high sensitivity flow cytometry.

Fig. S2

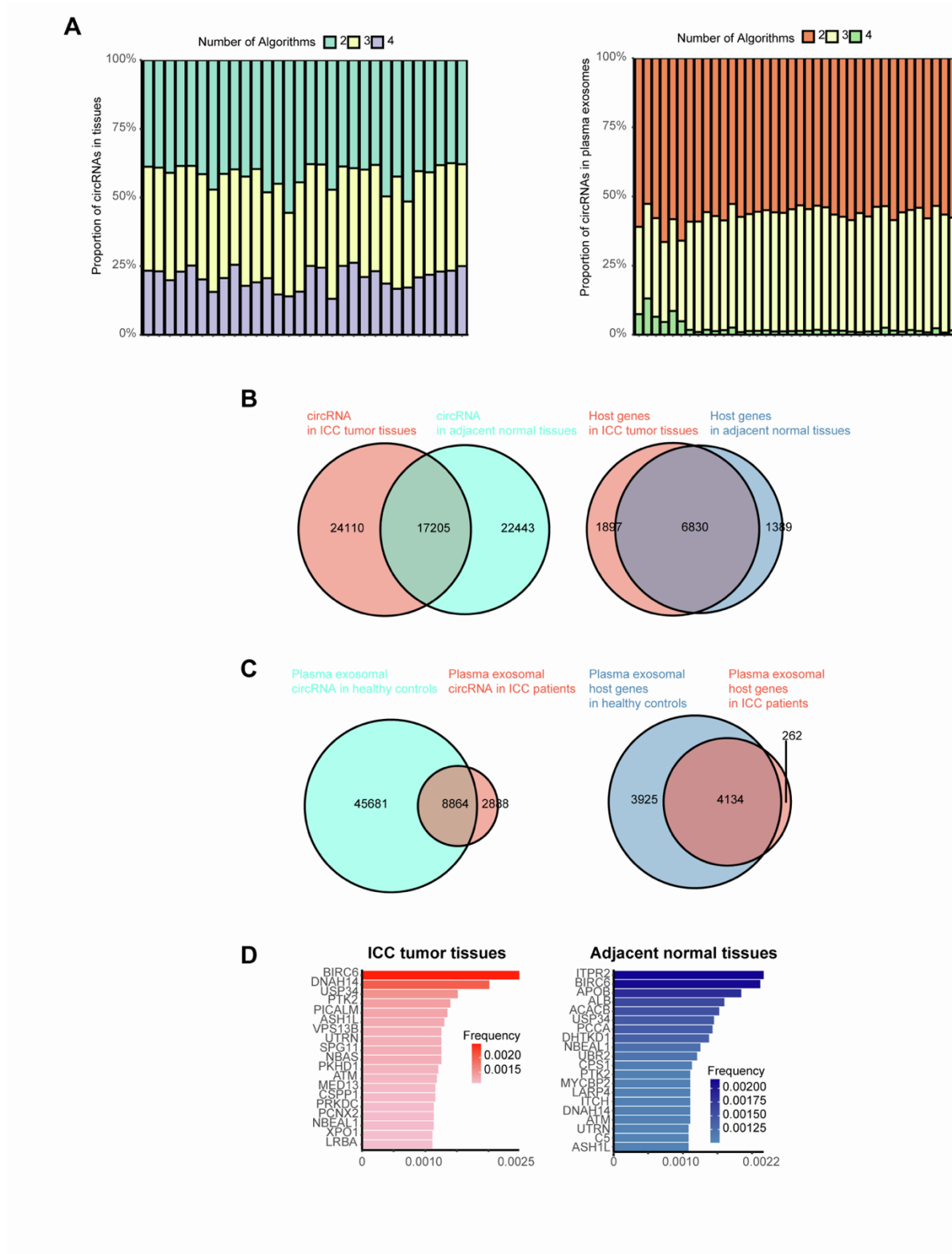


Figure S2

(A) Proportion of circRNAs predicted by circRNA_finder, find_circ, Circexplorer, and CIRI2 in ICC tumor tissues and adjacent normal tissues (left panel), and plasma exosomes of patients with ICC and healthy controls (right panel). (B) Overlapping between circRNAs (left panel) and host genes (right panel) in ICC tumor tissues and adjacent normal tissues. (C) Overlapping between circRNAs (left panel) and host genes (right panel) in plasma exosomes of patients with ICC and healthy controls. (D) Top back spliced host genes in ICC tumor tissues and adjacent normal tissues.

Fig. S3

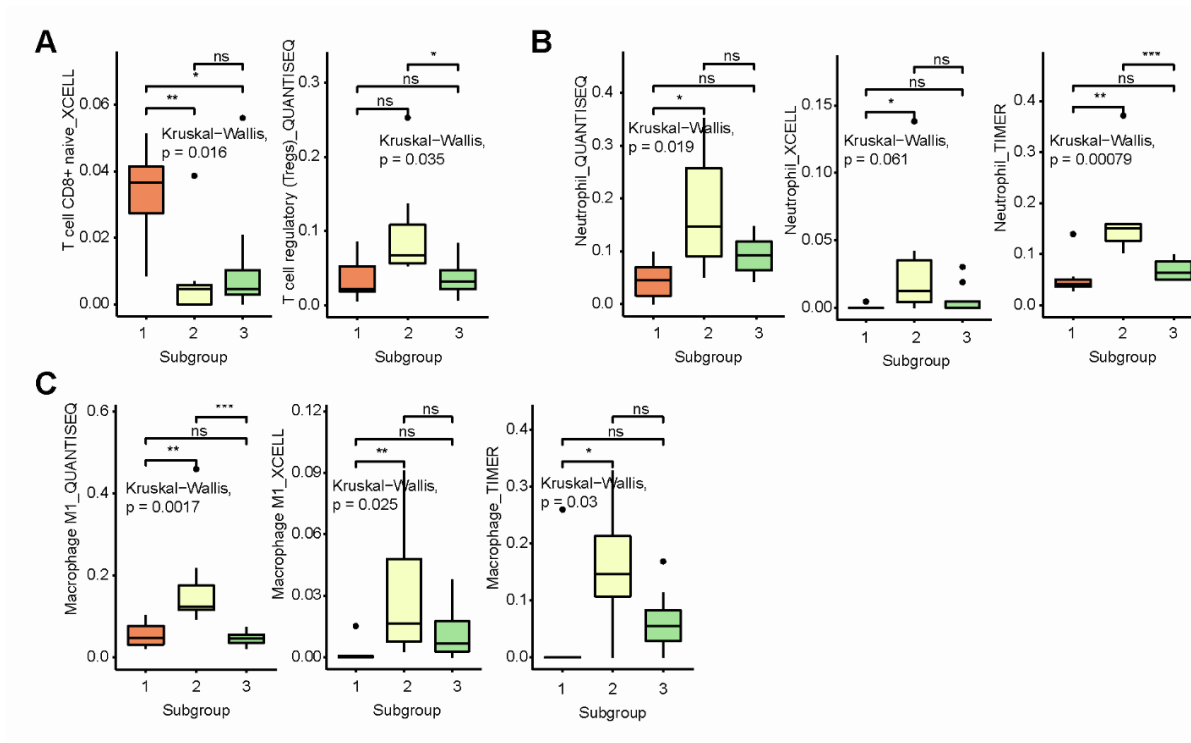


Figure S3

(A) Infiltration of CD8+ T cell and regulatory T cell in ICC subgroups. (B) Infiltration of neutrophil in ICC subgroups. (C) Infiltration of M1 macrophage in ICC subgroups.

Fig. S4

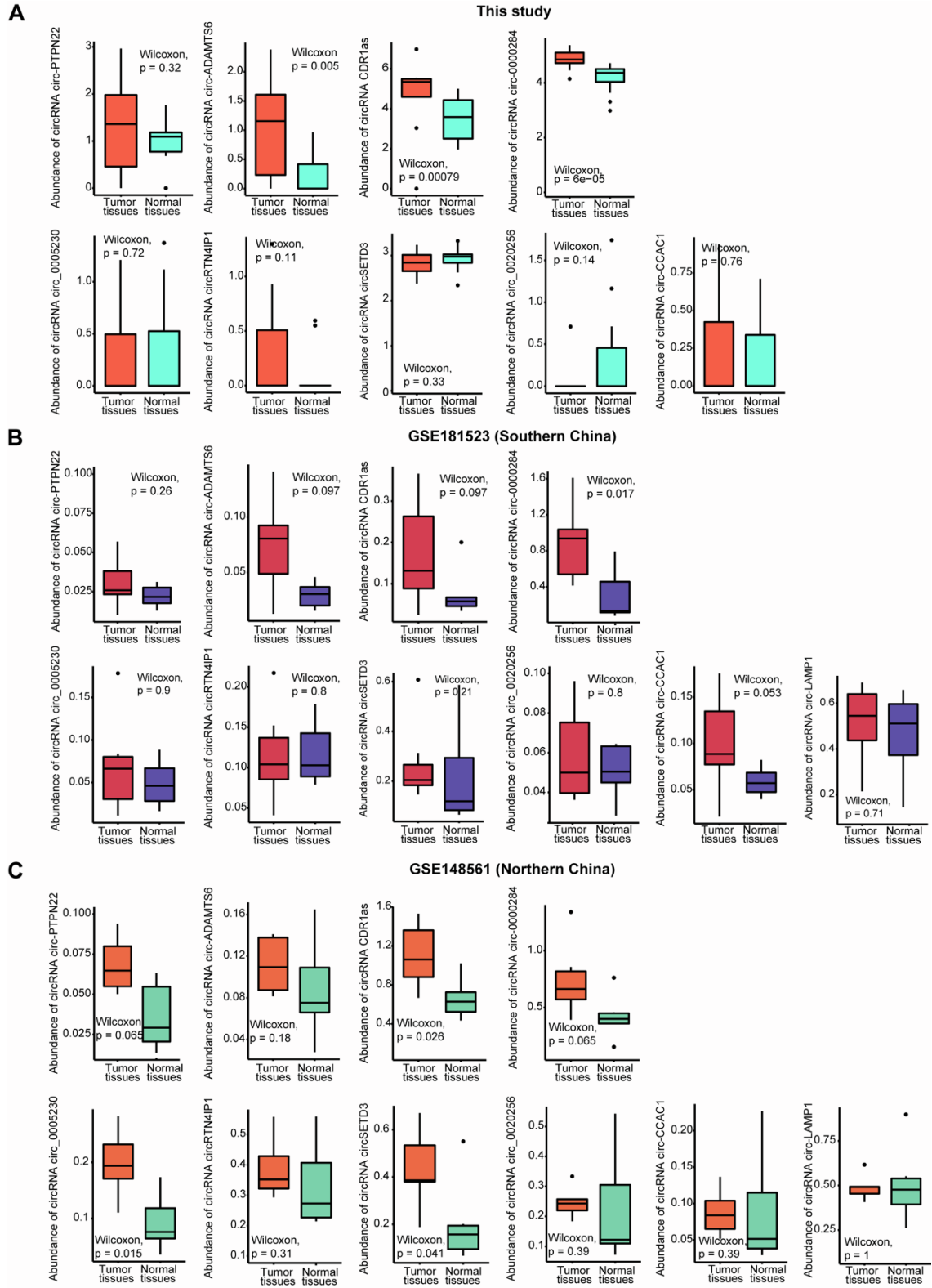


Figure S4

Differential expression of circ-PTPN22, circ-ADAMTS6, and the circRNAs reported by recent publications in (A) This study; (B) GSE181523 (Southern China) and (C) GSE148561 (Northern China).

Fig. S5

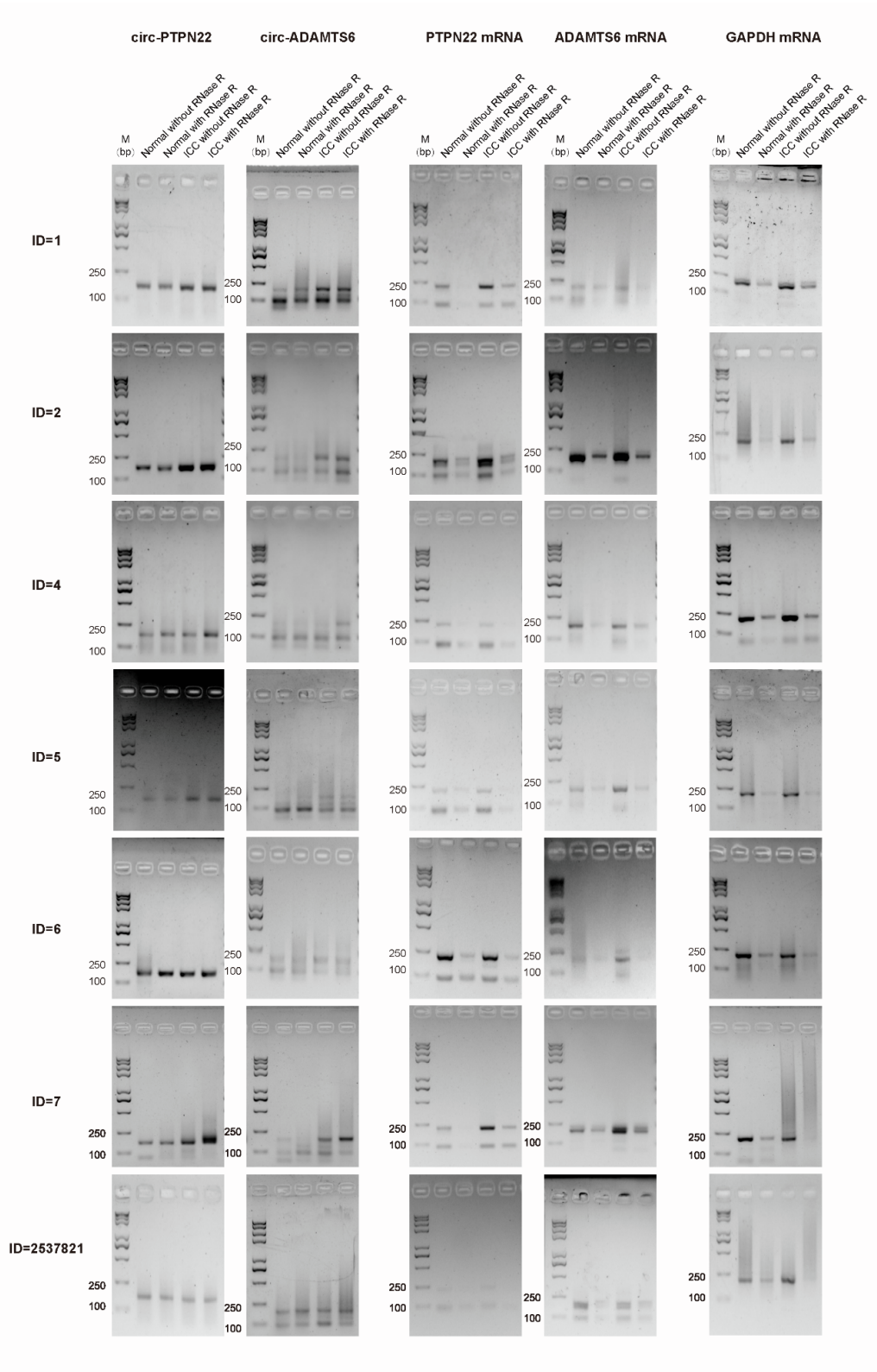


Figure S5

RT-qPCR of PTPN22 mRNA, ADAMTS6 mRNA, circ-PTPN22, and circ-ADAMTS6, analyzed by 2% agarose gel electrophoresis and stained with ExRed.

Fig. S6

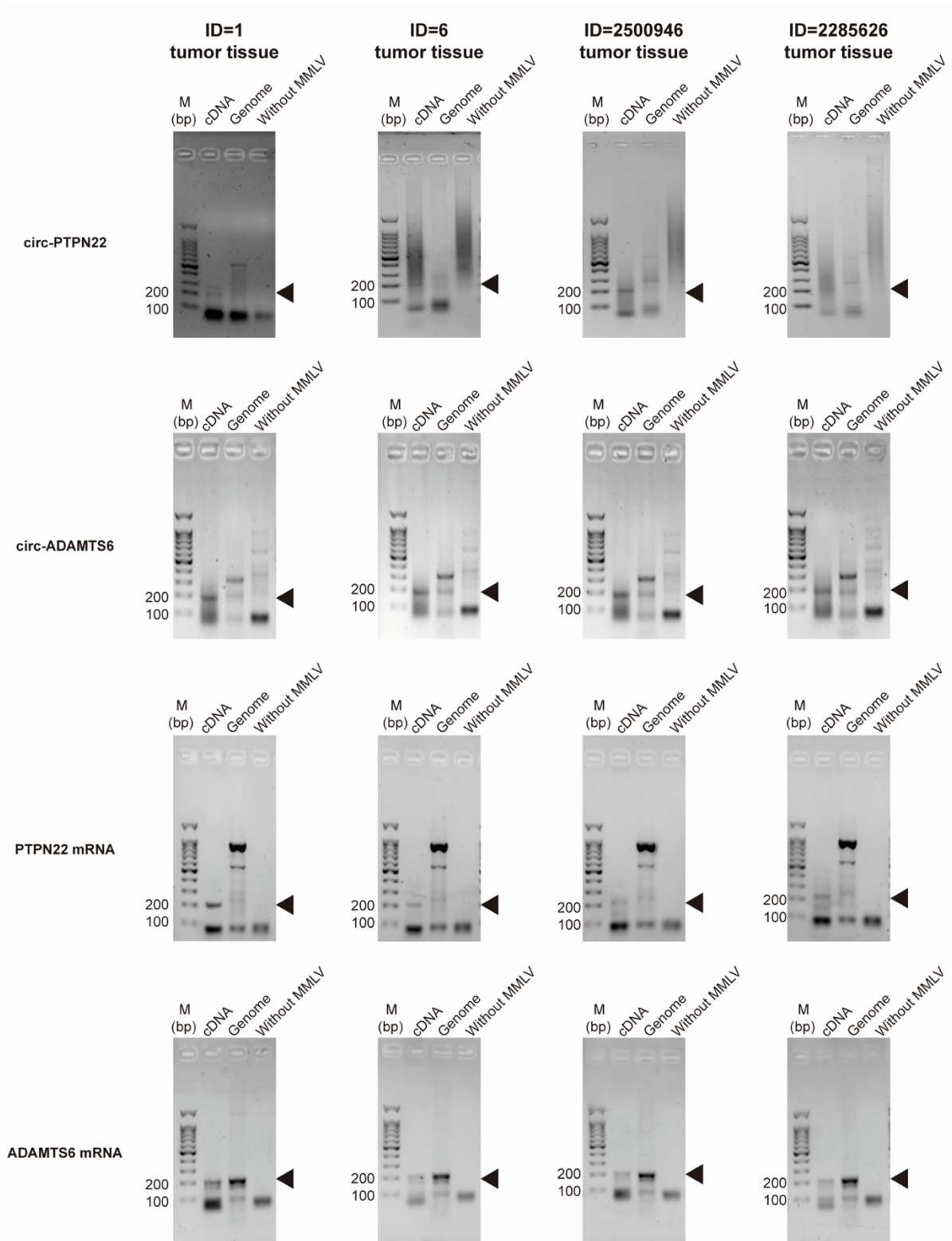


Figure S6

PCR of PTPN22 mRNA, ADAMTS6 mRNA, circ-PTPN22, and circ-ADAMTS6 from the tumor tissues, analyzed by 2% agarose gel electrophoresis and stained with ExRed.

Fig. S7

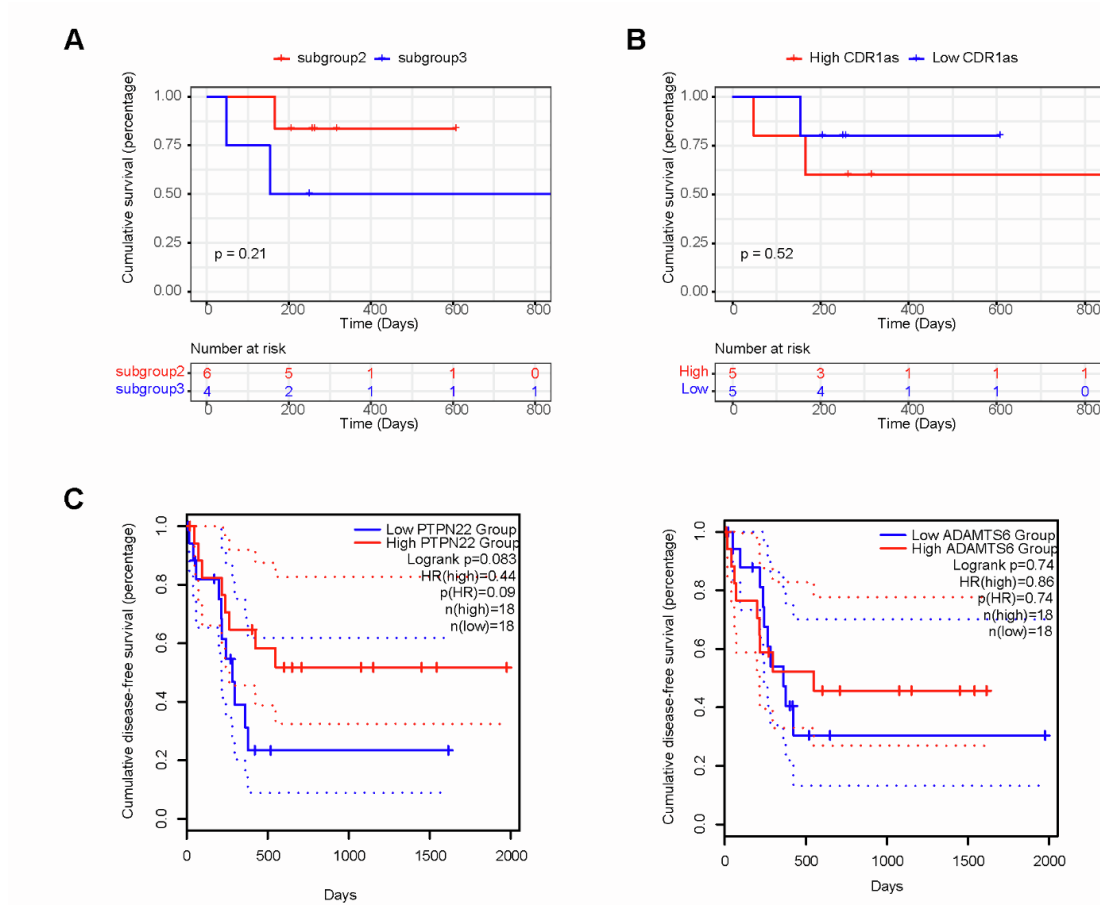


Figure S7

(A) Overall survival of patients with ICC in subgroup 2 (n=6) versus subgroup 3 (n=4). (B) Overall survival of patients whose ICC tumor tissues expressing high (n=5) versus low (n=5) levels of CDR1as. (C) Disease-free survival of TCGA-CHOL patients whose ICC tumor tissues expressing high (n=18) versus low (n=18) levels of PTPN22 and ADAMTS6, respectively.