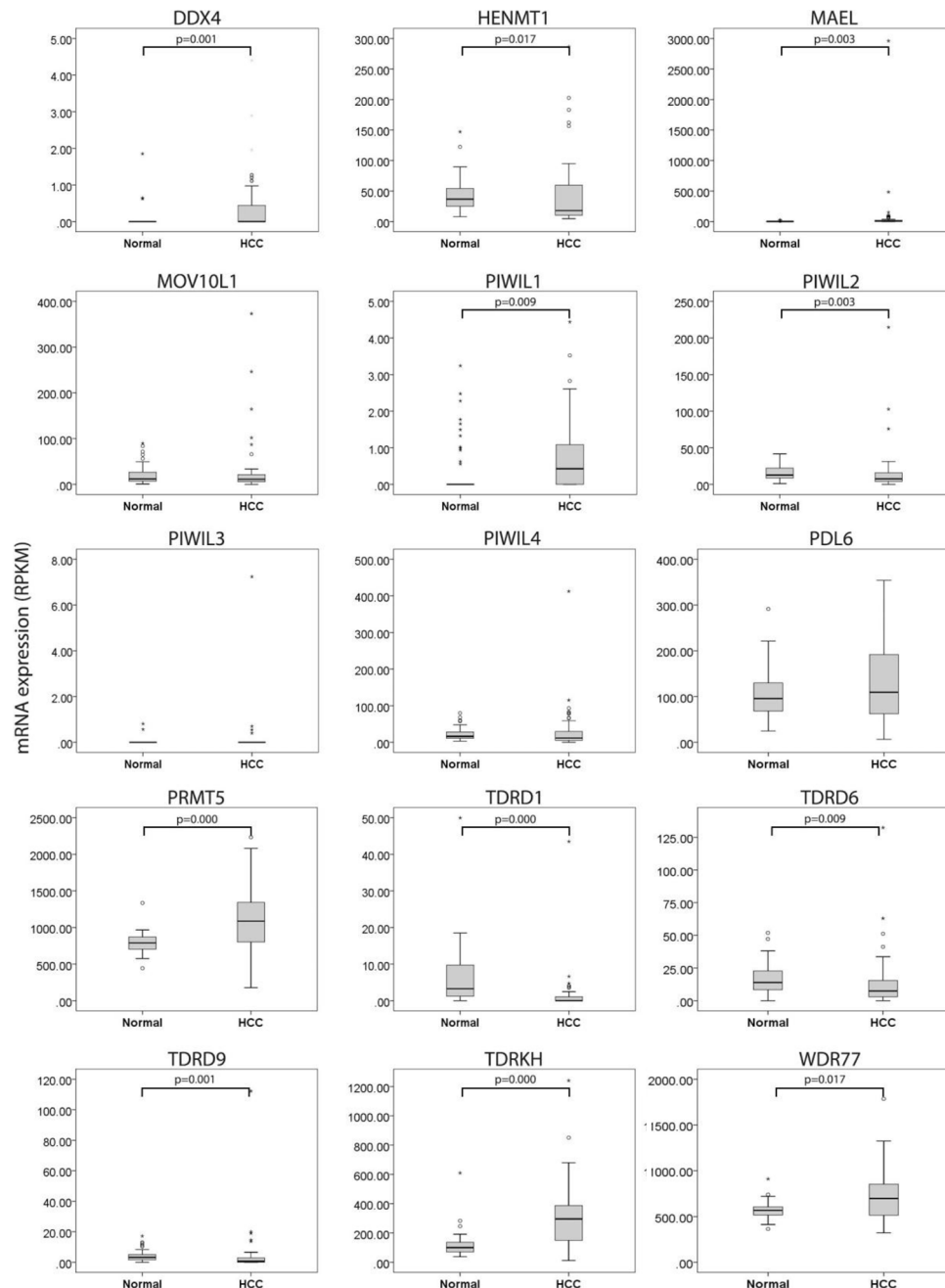
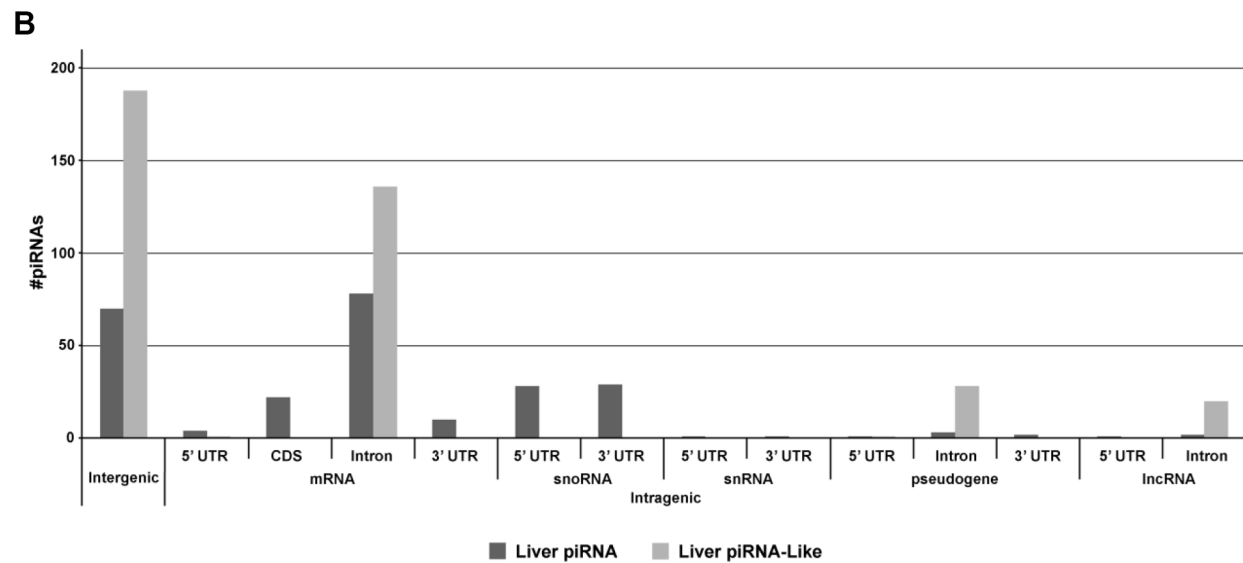
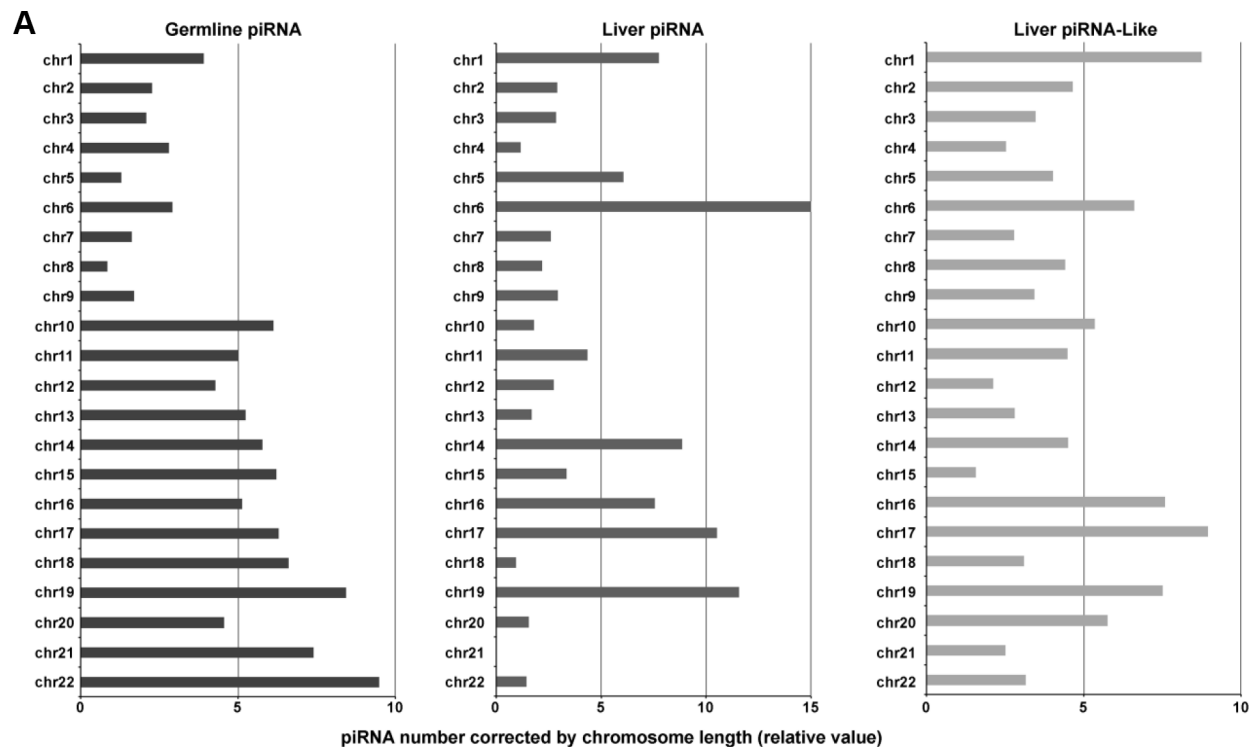


Specific patterns of PIWI-interacting small noncoding RNA expression in dysplastic liver nodules and hepatocellular carcinoma

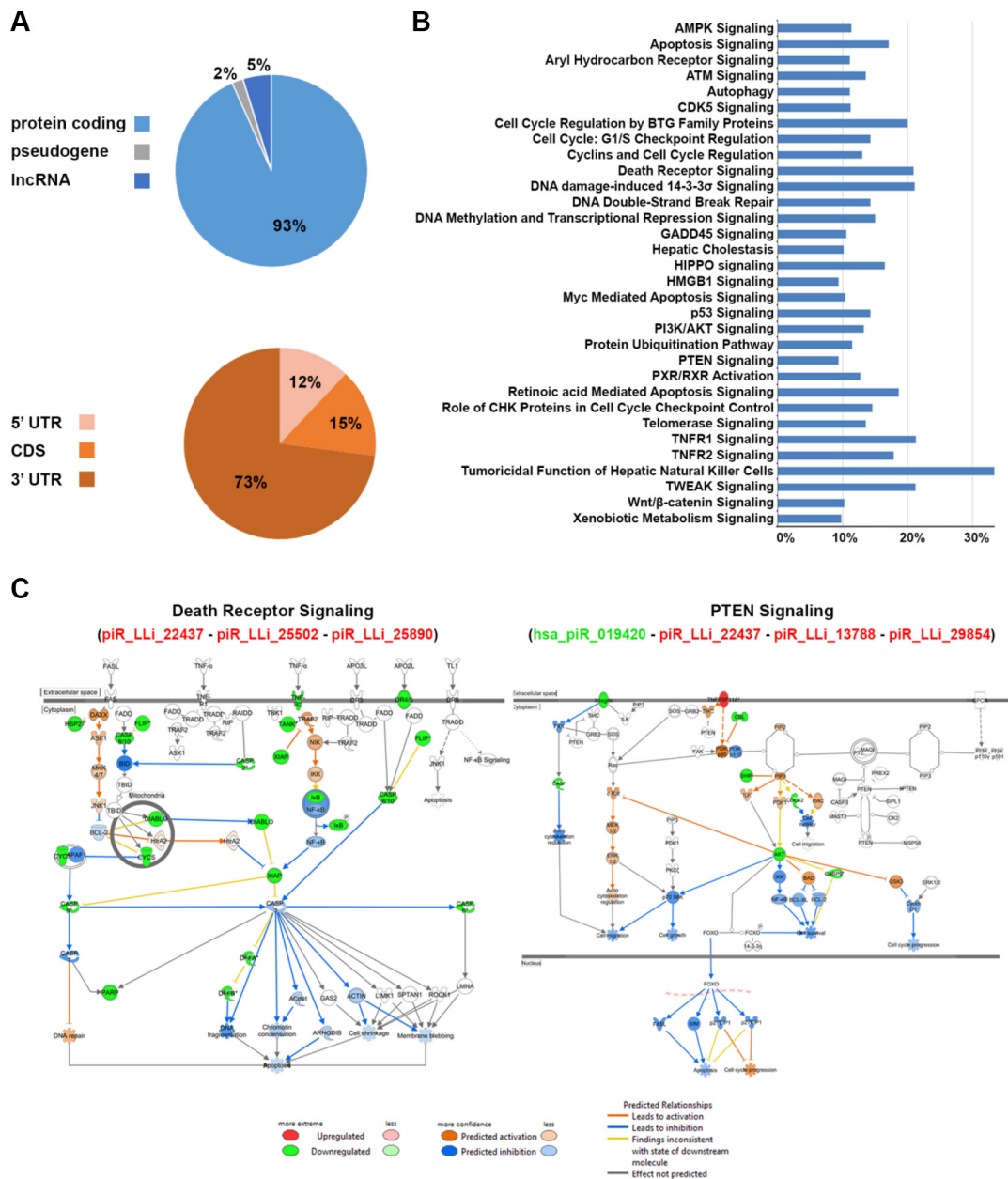
Supplementary Materials



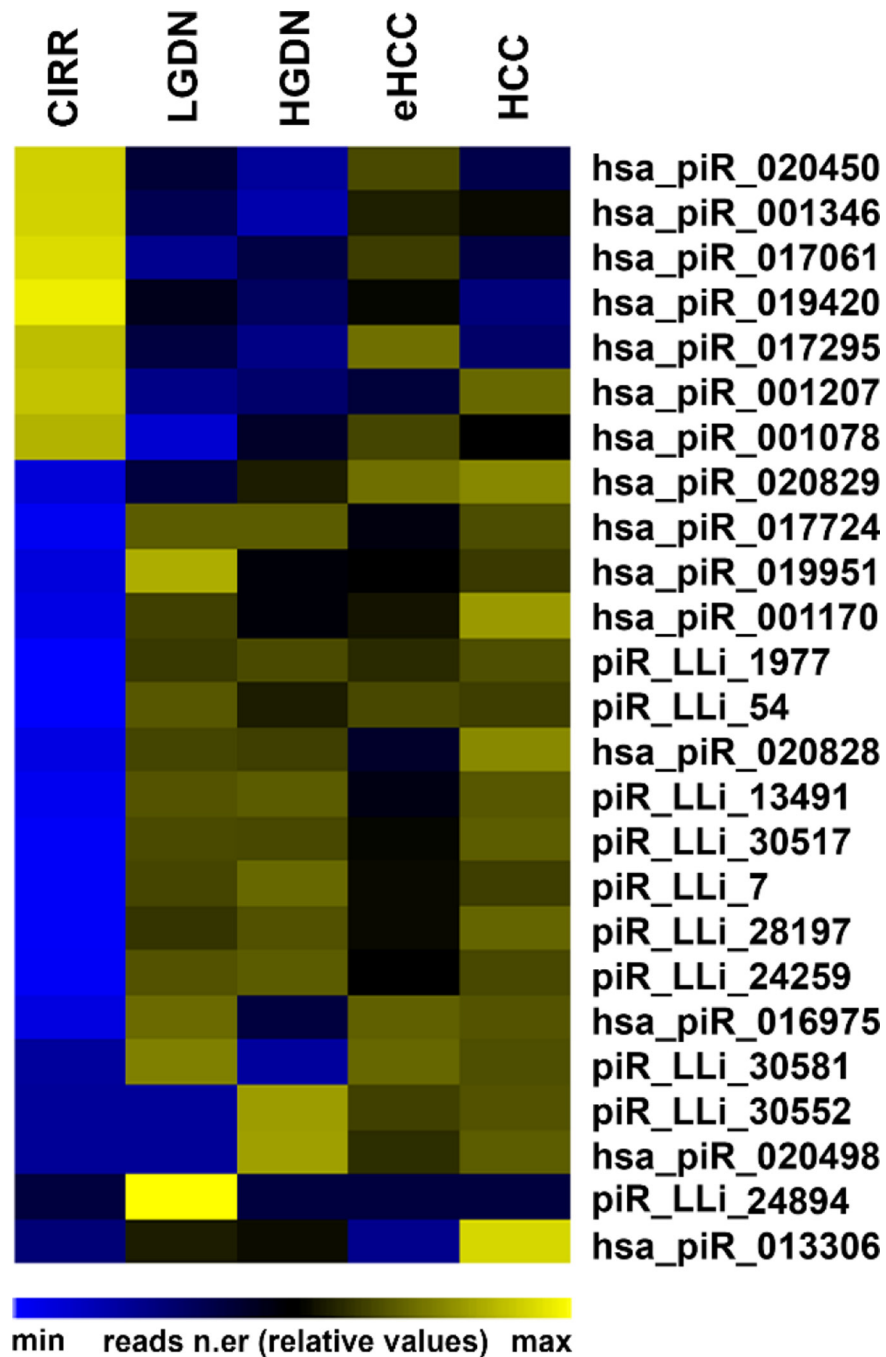
Supplementary Figure S1: Presence of the piRNA–PIWI pathway in human liver. Expression of transcripts encoding 12 known components of the piRNA biogenesis pathway in 50 matched non cancerous liver and HCC (data downloaded from TCGA, <http://cancergenome.nih.gov/>). Mann–Whitney *U* test was used in statistical comparisons and reported only when significant.



Supplementary Figure S2: Chromosomal and genomic distribution of piRNAs and piRNAs-like. (A) Bar plots of germline piRNA (piRNABank), liver piRNAs and liver piRNAs-like loci distribution between chromosomes. (B) Bar plot showing genomic annotation of expressed piRNAs and piRNA-likes, considering only piRNAs originating from the transcribed strand of genomic loci.



Supplementary Figure S3: piRNA-targeted functional pathways. (A) Pie charts representing the piRNA binding sites distribution in different transcripts type and transcripts regions. (B) The diagram shows the pathways significantly affected in HCC by the deregulated piRNAs identified here. Horizontal bars indicate the percentage of piRNA-targeted genes within each pathway. (C) IPA Molecule Activity Predictor analysis performed on death receptor (A) and PTEN signaling. The piRNAs regulating the pathways are reported, in each case, above the diagrams in red (up-regulated) or green (down-regulated), according to their behavior in HCCs. The expression of putative piRNA target-mRNAs have been considered only when their expression inversely correlated to that of the targeting piRNAs. The relationship between nodes (edges between pathway components) and the node themselves (pathway components) are color-coded based on the predicted relationships.



Supplementary Figure S4: piRNAs expression profile during hepatocarcinogenesis. Heatmap of piRNAs and piRNA-like expression profile in cirrhotic tissue, dysplastic nodules (LGDN and HGDN), eHCC and progressed HCC. Expression intensities are displayed from blue (low expression) to yellow (high expression).

Supplementary Table S1: Clinical and pathological features of the series analyzed

	Gender/ age	Aetiology	Type and number of hepatocellular nodules CN:LGDN:HGDN: eHCC:pHCC	HCC features	Surrounding liver	DFS	OS
Patient 1	M, 77	HCV	1:0:0:0:2	pT2, G2, V0, R0	cirrhosis	68	72 (<i>ad</i>)
Patient 2	F, 75	HCV	1:0:0:0:1	pT2, G2, V1, R0	cirrhosis	16	38 (<i>dod</i>)
Patient 3	M, 67	NASH	1:1:0:0:1	pT1, G1, V0, R0	cirrhosis	7	72 (<i>dod</i>)
Patient 4	F, 65	HCV	1:0:0:1:1	pT2, G2, V0, R1	severe hepatitis	34	96 (<i>ad</i>)
Patient 5	M, 47	HCV	1:1:0:1:1	pT2, G4, V0, R0	cirrhosis	48	48 (<i>and</i>)
Patient 6	M, 65	HBV	1:0:0:0:3	pT2, G2, V1, R1	cirrhosis	24	68 (<i>ad</i>)
Patient 7	M, 69	<i>uk</i>	1:0:0:1:1	pT3a, G2, V1, R0	cirrhosis	7	14 (<i>ad</i>)
Patient 8	M, 61	Alcohol	1:1:0:0:1	pT2, G1, V1, R1	cirrhosis	60	60 (<i>and</i>)
Patient 9	M, 64	Alcohol	1:0:1:0:1	pT2, G2, V1, R0	severe hepatitis	5	20 (<i>ad</i>)
Patient 10	M, 66	HCV	1:1:1:1:1	pT2, G3, V1, R0	cirrhosis	12	40 (<i>ad</i>)
Patient 11	F, 57	HCV	1:0:0:1:2	pT2, G2, V0, R0	cirrhosis	24	24 (<i>and</i>)
Patient 12	M, 60	HCV	1:3:1:0:2	pT2, G3, V0, R0	cirrhosis	84	84 (<i>and</i>)
Patient 13	M, 74	Alcohol	1:0:1:0:1	pT1, G1, V0, R0	cirrhosis	7	32 (<i>ad</i>)
Patient 14	F, 70	HCV	1:0:0:0:1	pT1, G1, V0, R1	cirrhosis	28	43 (<i>ad</i>)
Patient 15	M, 59	HBV	1:0:1:0:2	pT2, G2, V0, R0	mild hepatitis	6	6 (<i>ad</i>)
Patient 16	F, 74	<i>uk</i>	1:1:0:1:1	pT2, G1, V0, R1	severe hepatitis	<i>uk</i>	<i>uk</i>
Patient 17	M, 70	<i>uk</i>	1:1:1:0:1	pT1, G2, V0, R0	severe hepatitis	<i>uk</i>	<i>uk</i>

M: male; F: female; *ad*: alive with disease; *and*: alive without disease; *dod*: died of disease; *uk*: unknown.

Supplementary Table S2: Sequencing statistics and reads distribution

Sample	Sequencing reads	Adapter trimmed	Length and quality filtered	rRNA reads filter out	reads in analysis	mapped reads	miRNA reads	piRNA reads	tRNA reads	Rfam annotation reads	RefGenes annotation reads	Unassigned reads
CIRR_1	39,886,295	39,225,918	2,985,609	135,903	2,849,706	1,548,304	9,300	3,885	10,468	14,881	71,196	1,374,192
CIRR_2	51,489,975	50,828,200	7,464,337	3,990,547	3,473,790	2,426,400	791,165	103,962	131,203	118,421	767,014	474,322
CIRR_3	40,945,296	40,518,388	5,098,872	2,635,893	2,462,979	1,587,651	151,250	58,317	224,023	37,950	239,133	817,488
CIRR_4	40,000,084	38,882,530	4,084,493	2,151,974	1,932,519	1,141,551	177,915	50,820	169,140	55,841	211,311	432,325
CIRR_6	40,381,965	39,835,305	4,042,964	718,180	3,324,784	1,359,469	227,150	73,015	198,498	37,223	234,925	505,600
CIRR_7	34,870,179	34,252,889	10,333,629	3,968,752	6,364,877	4,355,480	1,895,221	128,848	447,510	127,327	693,909	960,965
CIRR_9	46,877,444	45,393,999	17,787,722	8,031,220	9,756,502	7,236,109	1,645,205	521,037	2,780,952	226,012	553,131	1,373,622
CIRR_11	18,768,870	18,625,875	1,570,572	1,323,383	247,189	121,170	16,194	5,507	11,894	3,444	32,014	49,147
CIRR_12	23,341,154	23,021,632	6,984,836	1,977,439	5,007,397	3,791,881	2,041,890	126,754	159,071	99,826	655,952	628,648
CIRR_13	35,756,347	35,209,513	13,312,189	4,972,095	8,340,094	6,231,543	4,015,782	152,989	422,026	126,145	579,384	819,491
CIRR_14	35,090,155	34,731,462	1,698,620	604,940	1,093,680	724,259	172,671	23,195	42,680	37,108	216,255	218,109
CIRR_15	28,795,563	28,455,139	11,336,225	1,632,476	9,703,749	8,084,226	5,722,948	411,891	618,618	130,744	397,735	677,386
CIRR_16	27,561,943	27,140,271	7,133,108	2,809,612	4,323,496	3,111,859	1,449,725	141,869	387,411	84,319	400,786	596,066
CIRR_17	27,777,226	27,407,597	8,781,463	3,436,690	5,344,773	3,555,663	1,484,299	98,382	893,586	69,071	308,650	652,793
HCC_1	37,508,700	37,071,642	4,791,383	2,165,994	2,625,389	1,671,093	290,445	52,824	310,561	48,153	152,132	727,830
HCC_1b	41,182,406	40,764,018	3,829,002	1,628,965	2,200,037	1,402,791	213,439	52,540	135,190	53,692	330,316	586,035
HCC_2	62,049,238	57,483,853	31,947,720	19,173,870	12,773,850	9,174,050	3,810,048	281,956	1,233,720	273,674	1,223,173	2,196,675
HCC_3	37,411,107	36,040,876	25,088,561	7,899,833	17,188,728	13,214,184	1,605,946	611,649	9,320,261	203,208	497,596	872,043
HCC_4	35,800,306	33,264,032	16,649,882	11,667,526	4,982,356	3,342,270	1,346,632	129,124	358,815	119,140	569,375	754,616
HCC_5	27,923,090	27,442,913	12,377,478	4,946,783	7,430,695	5,486,468	1,139,813	609,825	2,221,161	249,302	546,374	675,345
HCC_6	39,893,650	39,240,026	22,927,797	9,427,029	13,500,768	10,352,633	7,027,488	328,292	807,492	191,920	685,457	1,160,575
HCC_6b	41,600,752	38,263,542	22,461,249	12,249,960	10,211,289	7,158,257	3,889,616	247,372	701,810	166,265	764,229	1,269,057
HCC_7	36,806,364	35,164,151	15,880,386	6,537,212	9,343,174	6,960,685	3,197,051	181,627	1,018,411	153,867	574,144	1,726,659
HCC_8	31,162,799	30,117,190	15,096,303	8,188,194	6,908,109	4,551,260	2,581,396	159,097	338,858	100,791	394,978	888,424
HCC_9	41,948,446	41,378,741	11,872,833	4,570,161	7,302,672	5,186,427	1,283,320	408,912	1,954,216	191,487	343,825	922,378
HCC_10	34,195,204	33,596,761	11,180,810	5,035,308	6,145,502	4,532,665	3,044,405	157,170	220,546	92,330	326,360	630,499
HCC_11	23,512,360	23,216,346	11,649,127	4,133,579	7,515,548	5,249,527	1,590,020	309,444	880,076	203,856	694,686	1,485,000
HCC_11b	30,229,052	29,664,930	17,152,692	7,014,189	10,138,503	7,869,158	4,261,155	239,803	725,587	224,131	721,905	1,591,576
HCC_12	26,023,524	25,553,155	12,562,895	4,422,712	8,140,183	5,901,802	744,926	639,929	3,334,135	358,117	337,610	455,880
HCC_12b	30,664,580	30,019,781	15,570,970	5,418,943	10,152,027	7,491,423	1,057,392	820,608	4,100,452	360,185	444,009	675,238
HCC_13	34,189,349	32,698,076	23,348,482	3,851,814	19,496,668	15,380,555	3,625,228	572,002	9,354,847	124,849	720,756	891,802
HCC_14	51,175,779	50,424,281	22,156,136	7,576,892	14,579,244	11,177,124	3,826,041	481,468	4,623,063	235,730	941,759	984,915
HCC_15	30,227,699	29,694,810	18,313,170	1,426,157	16,887,013	13,355,602	3,673,484	415,710	5,390,310	137,609	484,294	3,117,887
HCC_15b	30,375,300	29,911,820	18,565,205	861,261	17,703,944	14,325,368	3,535,989	373,143	7,852,606	103,463	321,983	2,055,014

Small RNA sequencing statistics and distribution of reads mapped in different RNA annotation.

Supplementary Table S3A: piRNA expression in cirrhosis and HCC tissues. See Supplementary_Table_S3A

Supplementary Table S3B: Annotation of liver piRNA. See Supplementary_Table_S3B

Supplementary Table S4: piRNAs differential expressed between cirrhosis and HCC tissues. See Supplementary_Table_S4

Supplementary Table S5: piRNAs differentially expressed in different cancer tissues

piRNA ID	Breast cancer [1-S]	Endometrial cancer [2-S]	Gastric cancer [3-S]	Gastric cancer [4-S]	Myeloma [5-S]	Renal carcinoma [6-S]	Multiple cancer types [7-S]
has_piR_000823			X		X		
has_piR_001042				X			X
has_piR_001152		X		X			X
has_piR_001170		X				X	
has_piR_001205				X			X
has_piR_003728							X
has_piR_007336				X			X
has_piR_009051							X
has_piR_010894				X		X	
has_piR_011901							X
has_piR_012681							X
has_piR_013306				X			X
has_piR_016664							X
has_piR_016975							X
has_piR_017033							X
has_piR_017061				X			X
has_piR_017791				X			X
has_piR_011901				X			
has_piR_012681				X			
has_piR_014620				X			
has_piR_018165							X
has_piR_019102				X			X
has_piR_019168	X	X					
has_piR_019368							X
has_piR_019420	X			X			X
has_piR_020009		X				X	X
has_piR_020305							X
has_piR_020499	X						
has_piR_020450				X			
has_piR_020657				X			X
has_piR_020814				X			X
has_piR_020829	X						
has_piR_023057				X			X

X: piRNA differentially expressed in cancer tissues.

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Supplementary Table S6A: piRNA-like expression in cirrhosis and HCC tissues. See Supplementary_Table_S6A

Supplementary Table S6B: Annotation of liver piRNA-Like. See Supplementary_Table_S6B

Supplementary Table S7: piRNA-like differential expression analysis between cirrhosis and HCC tissues. See Supplementary_Table_S7

Supplementary Table S8: Top canonical pathways predicted to be target of piRNAs deregulated in HCC. See Supplementary_Table_S8