

Exploration of a Novel Circadian miRNA Pair Signature for Predicting Prognosis of Lung Adenocarcinoma

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Supplementary materials

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Figure S1. Analysis of immune cells between the high- and low-risk group.

Figure S2. Gene Set Enrichment Analysis (GSEA) between the high- and low-risk group.

Table S1. The forward primer sequences of 11 cmiRNA in the prognostic model used in quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

miRNA	forward primer sequences
hsa-miR-584-5p	AAGTTGCATTATGGTTTGCCTGG
hsa-miR-539-3p	CCTGCTGGATCATACAAGGACAA
hsa-miR-382-5p	AACGGCGAAGTTGTTCGTGG
hsa-miR-376a-3p	GCGCATCATAGAGGAAAATCCACGT
hsa-miR-335-5p	ATGCGCTCAAGAGCAATAACGA
hsa-miR-31-5p	AACACGCAGGCAAGATGCTG
hsa-miR-215-5p	GCGGCCTGATGACCTATGAATT
hsa-miR-153-3p	AGCTGGACTTGCATAGTCACAAA
hsa-miR-138-5p	AATTGCTCAGCTGGTGTTGTGA
hsa-miR-136-5p	AAGCGCCTACTCCATTTGTTTTG
hsa-miR-133b	ACCGTCTTTGGTCCCCTTCAA

Table S4. cmiRNA pairs associated with prognosis obtained by Univariate COX regression analysis ($p < 0.01$).

id	HR	HR.95L	HR.95H	<i>p</i> value
hsa-miR-1301-3p hsa-miR-31-5p	0.413999396	0.259152073	0.66137036	0.000224451
hsa-miR-590-5p hsa-miR-31-5p	0.403672516	0.252108247	0.646355296	0.000158758
hsa-miR-628-5p hsa-miR-31-5p	0.401853234	0.250326331	0.645102019	0.000159942
hsa-miR-503-5p hsa-miR-31-5p	0.498869725	0.317389351	0.784118946	0.002578261
hsa-miR-139-5p hsa-miR-584-5p	0.455970827	0.288842241	0.719802597	0.000747842
hsa-miR-539-3p hsa-miR-376a-3p	2.047587084	1.299731036	3.225754215	0.00199842
hsa-miR-153-3p hsa-miR-133b	2.044139653	1.222537035	3.417898028	0.006409482
hsa-miR-584-5p hsa-miR-335-5p	2.269786156	1.248127997	4.127725047	0.00722347
hsa-miR-409-5p hsa-miR-31-5p	0.510762588	0.326515364	0.798977476	0.00324996
hsa-miR-409-3p hsa-miR-31-5p	0.435636034	0.277673898	0.683459107	0.000298863
hsa-miR-187-3p hsa-miR-31-5p	0.423664851	0.266291945	0.674041815	0.000289022
hsa-miR-187-3p hsa-miR-215-5p	0.51737803	0.331035031	0.808615405	0.003823657
hsa-miR-508-3p hsa-miR-31-5p	0.481034758	0.308091077	0.751058553	0.001285116
hsa-miR-432-5p hsa-miR-31-5p	0.523238595	0.333715601	0.820395051	0.00476216
hsa-miR-136-5p hsa-miR-31-5p	0.425624582	0.266537943	0.679664152	0.000347522
hsa-miR-136-5p hsa-miR-382-5p	0.467632002	0.294520979	0.742492743	0.001272174
hsa-miR-138-5p hsa-miR-31-5p	0.339818315	0.210702981	0.548053409	9.60E-06
hsa-miR-196b-5p hsa-miR-151a-5p	1.853672806	1.162394144	2.956056592	0.009543416
hsa-miR-33a-5p hsa-miR-31-5p	0.415053516	0.252430082	0.682444104	0.000528495
hsa-miR-335-5p hsa-miR-31-5p	0.345515885	0.218742488	0.545761493	5.21E-06
hsa-miR-335-5p hsa-miR-215-5p	0.472672695	0.284548304	0.785172407	0.003803736
hsa-miR-758-3p hsa-miR-31-5p	0.463314163	0.29628723	0.724499714	0.000744079
hsa-miR-31-5p hsa-miR-93-3p	2.714016346	1.704354779	4.321802491	2.60E-05
hsa-miR-31-5p hsa-miR-382-5p	2.405022745	1.528351537	3.784557587	0.000148371
hsa-miR-31-5p hsa-miR-326	1.995031156	1.251141308	3.181214852	0.003718432
hsa-miR-31-5p hsa-miR-133a-3p	2.086912141	1.336857297	3.257791461	0.001205333

The cmiRAN pairs finally included in the model are marked in red.

Table S5. Corresponding miRNAs in the prognostic model in TCGA-LUAD and GEO cohort.

TCGA-LUAD cohort	GSE63805 cohort
hsa-miR-539-3p	hsa-miR-539
hsa-miR-153-3p	hsa-miR-153
hsa-miR-584-5p	hsa-miR-584
hsa-miR-136-5p	hsa-miR-136
hsa-miR-138-5p	hsa-miR-138
hsa-miR-335-5p	hsa-miR-335
hsa-miR-376a-3p	hsa-miR-376a
hsa-miR-133b	hsa-miR-133b
hsa-miR-382-5p	hsa-miR-382
hsa-miR-31-5p	hsa-miR-31
hsa-miR-215-5p	hsa-miR-215

Table S6. Expression levels of miRNA of the model and its targeted genes in tumor tissue (compared with normal tissues) in TCGA-LUAD cohort.

ID	LogFC	<i>p</i> value	ID	LogFC	<i>p</i> value
hsa-miR-539-3p	1.44	4.52E-04	NINJ1	0.26	2.99E-03
hsa-miR-153-3p	1.30	4.38E-06	CCDC85C	-0.11	5.66E-04
hsa-miR-584-5p	-1.11	2.87E-09	KDSR	-0.36	3.00E-15
hsa-miR-136-5p	2.32	1.73E-16	DDIT4	1.23	7.51E-09
hsa-miR-138-5p	-1.12	6.44E-07	OTUD4	-0.13	7.15E-04
hsa-miR-335-5p	-1.04	3.13E-13	RPL22	0.17	1.07E-02
hsa-miR-376a-3p	1.61	7.85E-05	INHBB	1.21	2.40E-04
hsa-miR-133b	-2.39	5.36E-31	QKI	-1.17	3.03E-30
hsa-miR-382-5p	1.32	1.34E-07	ZNF704	-0.65	8.09E-13
hsa-miR-31-5p	4.91	5.15E-18	GAB1	-1.16	3.80E-27
hsa-miR-215-5p	3.06	2.23E-09	ZBTB34	-0.12	4.76E-05
LHFPL6	-1.55	7.42E-31	RHOBTB1	-0.18	8.06E-06
TAGLN2	0.34	5.10E-04	FAM53B	-0.61	2.08E-14
UBA2	0.56	1.02E-09	LATS2	-1.05	8.34E-26
DUSP1	-1.46	8.44E-22	VEZT	0.55	1.33E-13
KIF3C	1.23	5.56E-08	BACH2	-0.52	1.81E-07
YES1	0.27	5.71E-02	SEPHS1	0.32	1.48E-06
RFFL	0.57	3.07E-11	FBXO28	0.63	5.24E-18
ELAVL1	0.36	1.21E-17	KDM4C	-0.03	2.67E-03
SHISA5	0.17	5.33E-03	BRWD1	0.51	4.47E-03
RABGAP1L	-0.25	1.36E-06	CRISPLD2	-0.49	1.18E-02
DOCK5	0.57	5.10E-02	XPR1	2.05	2.00E-30
RMND5A	-0.08	1.64E-03	PLAG1	0.38	7.79E-01
RARA	-0.48	3.73E-11	TOP1	0.50	7.97E-12
KLF11	-0.57	4.81E-15	ATP1B1	1.40	8.89E-16
ROCK2	-0.04	2.13E-02	NR1D2	-0.11	1.15E-01
EID1	-0.54	7.35E-19	CD200	-0.44	9.98E-05
GNG2	-0.71	5.05E-14	PPIL1	1.17	7.67E-26
SEH1L	0.52	2.38E-11	SH3PXD2A	0.47	7.15E-06
NSFL1C	0.29	2.53E-06			

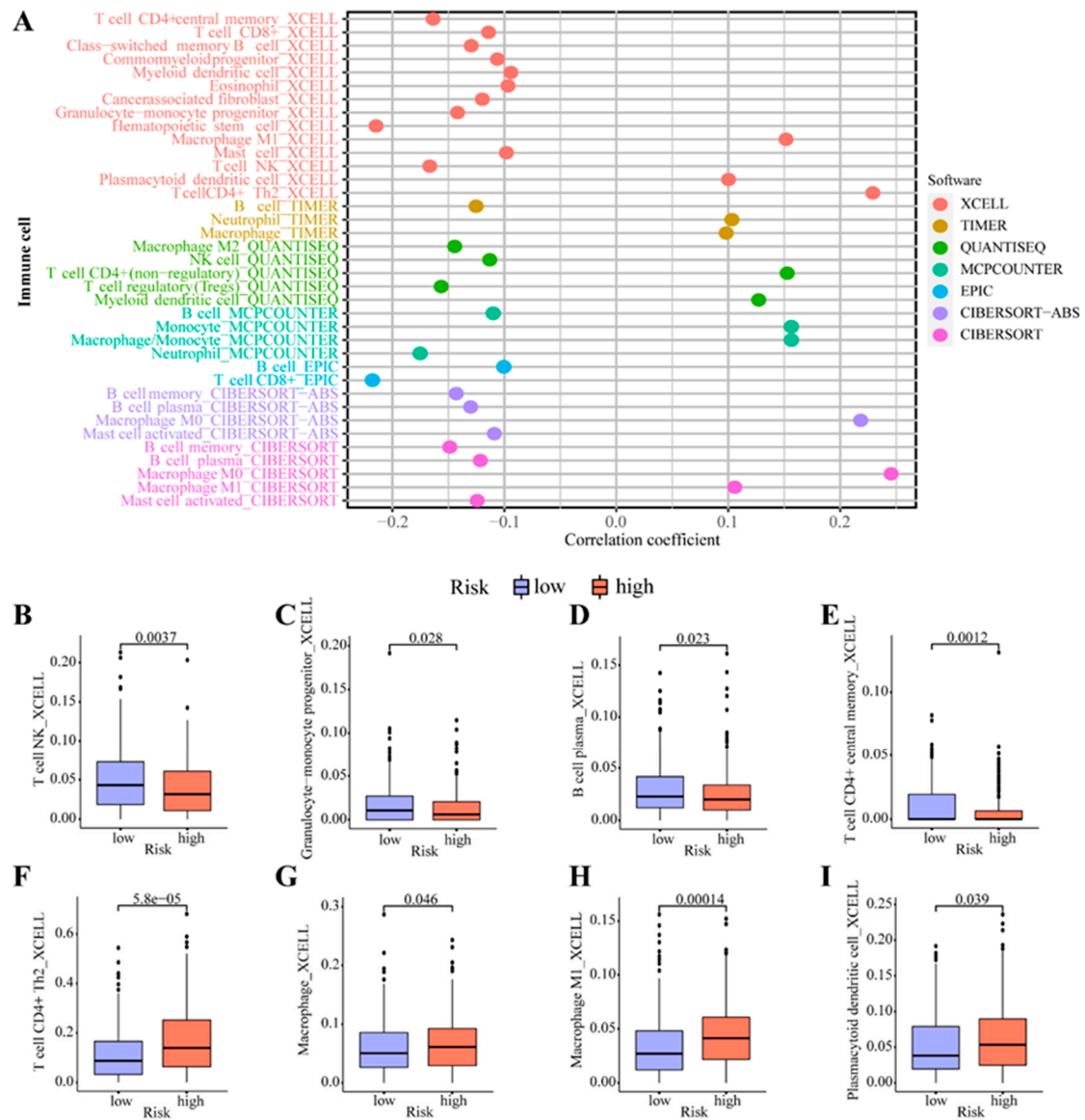


Figure S1. Analysis of immune cells between the high- and low-risk group

(A) Overview of significant correlation between risk score and immune cells by Spearman correlation analysis ($p < 0.05$). (B-I) Comparison of level of representative immune cells between the high- and low-risk group.

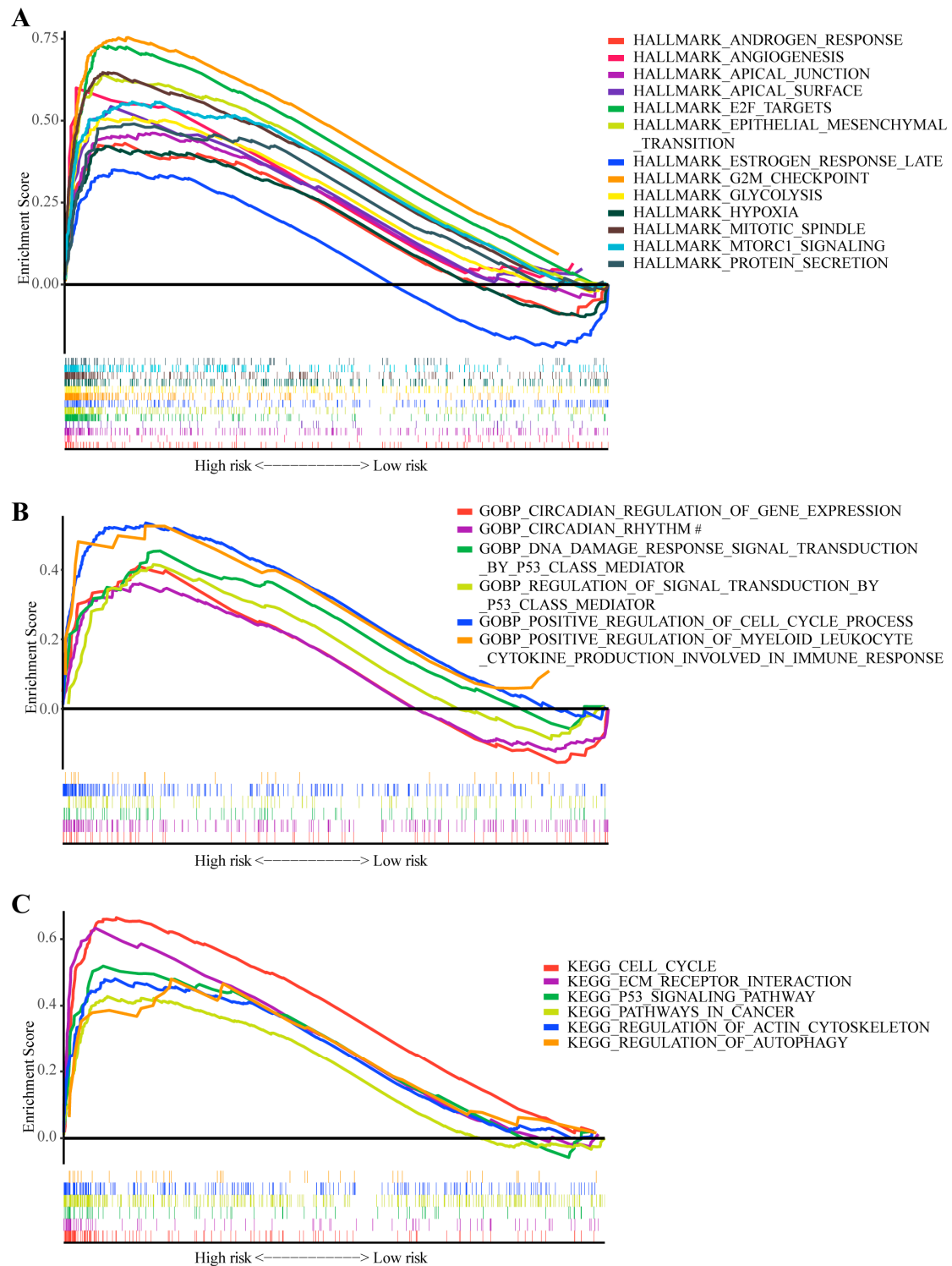


Figure S2. Gene Set Enrichment Analysis (GSEA) between the high- and low-risk group

GSEA plots depict the significant enrichment ($FDR < 0.05$) of **(A)** classical cancer hallmark gene sets and **(B, C)** other typical gene sets of tumor-related biological processes between the high- and low-risk group. (# $FDR = 0.051$).