Supplementary Figures

		BMAL1		*CLOCK*		*CRY1*		*CRY2*	
Genes	**Types**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**
MYC	**SARC**	0.05	0.42	0.099	0.11	0.035	0.58	-0.15	1.40E-02
SOX2	**SARC**	0.19	2.20E-03	0.082	0.18	0.18	4.40E-03	0.015	0.017
CD44	**SARC**	0.064	0.30	0.096	0.12	-0.055	0.37	0.00072	0.99
CD133	**SARC**	-0.059	0.34	0.13	3.90E-02	0.13	3.10E-02	0.04	0.52
NANOG	**SARC**	0.014	0.82	-0.0099	0.87	0.097	0.12	-0.0031	0.96
POU5F1	**SARC**	0.053	0.39	0.12	0.06	0.096	0.12	0.0086	0.89
CDH2	**SARC**	0.046	0.46	0.19	2.00E-03	0.12	4.40E-02	-0.075	0.23
ZEB1	**SARC**	0.14	2.90E-02	0.44	5.20E-14	0.45	1.9E-14	0.34	2.4E-08
ZEB2	**SARC**	0.19	2.40E-03	0.22	2.50E-04	0.36	3.60E-04	0.18	2.90E-02
VIM	**SARC**	0.12	0.06	0.013	0.84	-0.0084	0.89	-0.13	3.30E-02

R: Pearson correlation coefficient

Neg. Corr. Pos. Corr.
-0.5 0 0.5

Figure S1. Differential gene correlations between core clock regulators and CSC/EMT-associated factors in human sarcoma. Table showing the Pearson's correlation coefficients (*R* values) and corresponding *p*-values to indicate correlations between the expressions of *BMAL1*, *CLOCK*, *CRY1*, and *CRY2* and genes associated with cancer stem cell (CSC) properties (*MYC*, *SOX2*, *CD44*, *CD133* [*PROM1*], *NANOG*, *POU5F1* [*OCT4*]) or epithelial-mesenchymal transition (EMT) (*CDH2*, *ZEB1*, *ZEB2*, *VIM*) in sarcoma (SARC) patient samples. Positive correlations ($R > 0$) are shown in red shades, while negative correlations ($R < 0$) are in blue shades. Significant correlations ($p < 0.05$) are indicated with red or blue *p*-values in scientific notation, while non-significant correlations are displayed with black *p*-values in general notation. The data were sourced from GEPIA (http://gepia.cancer-pku.cn/).

		BMAL1		*CLOCK*		*CRY1*		*CRY2*	
Genes	**Types**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**
DGAT1	**SARC**	0.053	0.39	-0.014	0.82	0.13	3.40E-02	0.061	0.32
DGAT2	**SARC**	-0.044	0.48	0.04	0.52	-0.0025	0.97	0.12	0.047
ACSL4	**SARC**	0.13	3.50E-02	0.18	2.70E-03	0.13	4.70E-02	-0.09	0.15
FASN	**SARC**	0.067	0.28	0.025	0.68	0.14	2.20E-02	-0.1	0.09
CHKA	**SARC**	-0.013	0.83	0.067	0.28	0.12	0.05	0.046	0.46
SREBF1	**SARC**	0.11	0.09	0.066	0.29	0.055	0.380	0.082	0.18
SLC2A1	**SARC**	0.086	0.16	0.3	9.10E-07	0.17	4.80E-03	-0.1	0.096
SLC16A1	**SARC**	0.071	0.25	0.38	1.60E-10	0.35	3.9E-09	0.11	0.081
LDHA	**SARC**	0.039	0.53	0.14	1.90E-02	0.022	0.72	-0.11	0.08
HK1	**SARC**	0.089	0.15	0.18	4.20E-03	0.16	8.10E-03	-0.017	0.78
HK2	**SARC**	0.078	0.21	0.058	0.35	0.013	0.83	-0.21	7.50E-04
PFKFB3	**SARC**	0.093	0.13	0.078	0.21	0.096	0.12	-0.091	0.14
PKM	**SARC**	0.047	0.45	0.11	0.07	0.077	0.22	-0.21	5.60E-04
HIF1A	**SARC**	0.13	4.30E-02	0.27	7.70E-06	0.18	3.10E-03	0.096	0.12
PRKAA1	**SARC**	0.18	3.80E-03	0.52	2.00E-29	0.39	6.80E-11	0.32	1.10E-07
PRKAA2	**SARC**	0.052	0.40	0.19	2.30E-03	0.17	5.10E-03	0.23	2.20E-04
PPARGC1A	**SARC**	-0.01	0.87	0.11	0.07	0.083	0.18	0.16	1.10E-02
TFAM	**SARC**	0.2	8.40E-04	0.38	1.90E-10	0.36	2.90E-09	0.13	3.80E-02
SIRT3	**SARC**	0.29	1.50E-06	0.089	0.15	0.2	1.30E-03	0.39	3.90E-11

R: Pearson correlation coefficient

Figure S2. Differential gene correlations between core clock regulators and onco-metabolic genes in human sarcoma. Table showing the Pearson's correlation coefficients (*R* values) and corresponding *p*-values to indicate correlations between the expressions of *BMAL1*, *CLOCK*, *CRY1*, and *CRY2* and the indicated genes associated with lipid metabolism (light blue), glycolysis (light orange), and mitochondrial metabolism (light green) in sarcoma patient samples. Positive correlations ($R > 0$) are shown in red shades, while negative correlations ($R < 0$) are in blue shades. Significant correlations ($p < 0.05$) are indicated with red or blue *p*-values in scientific notation, while non-significant correlations are displayed with black *p*-values in general notation. The data were sourced from GEPIA (http://gepia.cancer-pku.cn/).

		BMAL1		*CLOCK*		*CRY1*		*CRY2*	
Genes	**Types**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**
SKP2	**SARC**	0.058	0.35	0.35	5.1E-09	0.37	1.10E-09	-0.0038	0.95
KIF20A	**SARC**	0.048	0.44	0.31	2.1E-07	0.39	4.60E-11	0.043	0.48
CCNF	**SARC**	0.11	0.09	0.31	4.80E-07	0.37	6.20E-10	-0.01	0.87
TROAP	**SARC**	-0.018	0.77	0.13	3.00E-02	0.22	2.90E-04	-0.14	2.40E-02
PHB	**SARC**	0.022	0.72	0.018	0.70	0.036	0.57	-0.15	1.50E-02
CKS1B	**SARC**	0.11	0.06	0.15	1.90E-02	0.28	3.6E-06	-0.015	0.81
MCM3	**SARC**	0.11	0.07	0.38	1.2E-10	0.42	1.5E-12	0.09	0.15
CCNA2	**SARC**	0.049	0.43	0.34	1.4E-08	0.45	3.7E-14	-0.011	0.86
TRIP13	**SARC**	0.003	0.96	0.28	4.50E-06	0.26	2.70E-05	-0.071	0.25
CENPM	**SARC**	-0.033	0.60	0.11	0.07	0.16	8.10E-03	-0.2	1.20E-03
HSP90AB1	**SARC**	0.18	3.50E-03	0.13	3.90E-02	0.25	4.30E-05	0.13	3.00E-02
JUN	**SARC**	0.078	0.21	0.063	0.31	0.059	0.34	0.067	0.28
CKS2	**SARC**	-0.0049	0.94	0.17	5.90E-03	0.2	9.70E-04	-0.19	2.50E-03
TK1	**SARC**	-0.077	0.21	0.084	0.08	0.13	3.30E-02	-0.19	1.90E-03
KIF4A	**SARC**	0.15	1.80E-02	0.36	1.90E-09	0.44	4.20E-14	-0.035	0.57

R: Pearson correlation coefficient

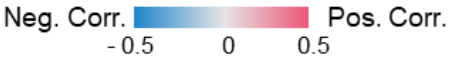
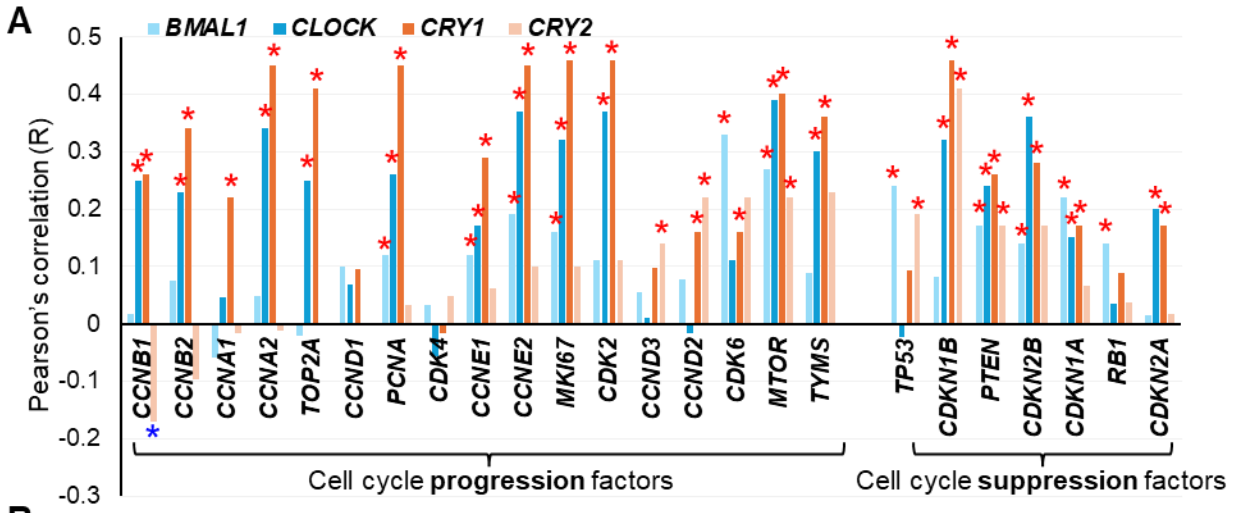


Figure S3. Differential gene correlations between core clock regulators and metastasis-associated genes in human sarcoma. Table showing the Pearson's correlation coefficients (R values) and corresponding *p*-values to indicate correlations between the expressions of *BMAL1*, *CLOCK*, *CRY1*, and *CRY2* and the indicated pro-metastatic genes in sarcoma patient samples. Positive correlations ($R > 0$) are shown in red shades, while negative correlations ($R < 0$) are in blue shades. Significant correlations ($p < 0.05$) are indicated with red or blue *p*-values in scientific notation, while non-significant correlations are displayed with black *p*-values in general notation. The data were sourced from GEPIA (http://gepia.cancer-pku.cn/).



B

			BMAL1		CLOCK		CRY1		CRY2	
	Genes	Types	R	p-value	R	p-value	R	p-value	R	p-value
Cell cycle progression factors	*CCNB1*	SARC	0.016	0.8	0.25	3.70E-05	0.26	2.20E-05	-0.17	7.30E-03
	CCNA1	SARC	-0.058	0.35	0.045	0.47	0.22	4.40E-04	-0.016	0.8
	CCNB2	SARC	0.075	0.23	0.23	1.70E-04	0.34	1.20E-08	-0.096	0.12
	CCNA2	SARC	0.049	0.43	0.34	1.40E-08	0.45	3.70E-14	-0.011	0.86
	TOP2A	SARC	-0.022	0.72	0.25	4.50E-05	0.41	2.80E-12	0.00048	0.99
	CCND1	SARC	0.1	0.098	0.068	0.27	0.096	0.12	0.0017	0.98
	PCNA	SARC	0.12	4.90E-02	0.26	2.40E-06	0.45	3.60E-14	0.032	0.60
	CDK4	SARC	0.032	0.6	-0.056	0.37	-0.016	0.8	0.049	0.43
	CCNE1	SARC	0.12	1.30E-04	0.17	4.70E-03	0.29	2.30E-06	0.061	0.33
	CCNE2	SARC	0.19	2.40E-03	0.37	8.7E-10	0.45	1.10E-14	0.099	0.11
	MKI67	SARC	0.16	1.10E-02	0.32	7.60E-08	0.46	6.00E-15	0.099	0.11
	CDK2	SARC	0.11	0.064	0.37	5.00E-10	0.46	2.70E-15	0.11	0.076
	CCND3	SARC	0.056	0.37	0.011	0.86	0.097	0.12	0.14	2.50E-02
	CCND2	SARC	0.078	0.21	-0.017	0.79	0.16	9.60E-03	0.22	3.30E-04
	CDK6	SARC	0.33	3.70E-08	0.11	0.072	0.16	9.20E-03	0.22	3.80E-04
	MTOR	SARC	0.27	7.20E-06	0.39	4.20E-11	0.4	1.00E-11	0.22	3.10E-04
	TYMS	SARC	0.089	0.15	0.3	9.30E-07	0.36	1.30E-09	0.23	0.08
Cell cycle suppression factors	*TP53*	SARC	0.24	6.20E-05	-0.024	0.7	0.092	0.14	0.19	2.60E-03
	CDKN2A	SARC	0.014	0.82	0.2	1.20E-03	0.17	7.00E-03	0.018	0.77
	RB1	SARC	0.14	2.90E-02	0.034	0.58	0.089	0.15	0.036	0.56
	CDKN1A	SARC	0.22	3.00E-04	0.15	1.90E-02	0.17	5.00E-03	0.066	0.29
	PTEN	SARC	0.17	6.20E-03	0.24	6.20E-05	0.26	1.50E-05	0.17	6.10E-03
	CDKN2B	SARC	0.14	2.80E-02	0.36	2.40E-09	0.28	5.00E-06	0.17	5.00E-03
	CDKN1B	SARC	0.082	0.19	0.32	1.70E-07	0.46	4.40E-15	0.41	5.10E-12

R: Pearson correlation coefficient

Neg. Corr. Pos. Corr.

-0.5 0 0.5

Figure S4. Differential gene correlations between core circadian regulators and cell cycle regulatory factors in human sarcoma. (**A**) Bar graph showing the correlations between the expressions of *BMAL1*, *CLOCK*, *CRY1*, and *CRY2* and the indicated genes associated with cell cycle progression or suppression in sarcoma patient samples. The Pearson's correlation

coefficient (R) was used to assess linear relationships between the gene pairs. Significant positive correlations ($R > 0$, $p < 0.05$) are marked with red asterisks, and significant negative correlations ($R < 0$, $p < 0.05$) are marked with blue asterisks. (**B**) Table showing the numerical R values and corresponding p-values for the comparisons shown in (A). Positive correlations ($R > 0$) are shown in red shades, while negative correlations ($R < 0$) are in blue shades. Significant correlations ($p < 0.05$) are indicated with red or blue p-values in scientific notation, while non-significant correlations are displayed with black p-values in general notation. The data were sourced from GEPIA (http://gepia.cancer-pku.cn/). CCNB1: cyclin B1, CCNA1: cyclin A1, CCNB2: cyclin B2, CCNA2: cyclin A2, TOP2A: topoisomerase II alpha, CCND1: cyclin D1, PCNA: proliferating cell nuclear antigen, CDK4: cyclin-dependent kinase 4, CCNE1: cyclin E1, CCNE2: cyclin E2, MKI67: marker of proliferation Ki-67, CDK2: cyclin-dependent kinase 2, CCND3: cyclin D3, CCND2: cyclin D2, CDK6: cyclin-dependent kinase 6, MTOR: mechanistic target of rapamycin kinase, TYMS: thymidylate synthetase, TP53: tumor protein p53, CDKN2A: cyclin-dependent kinase inhibitor 2A, RB1: retinoblastoma 1, CDKN1A: cyclin-dependent kinase inhibitor 1A, PTEN: phosphatase and tensin homolog, CDKN2B: cyclin-dependent kinase inhibitor 2B, CDKN1B: cyclin-dependent kinase inhibitor 1B.

		BMAL1		CLOCK		CRY1		CRY2	
Genes	Types	R	p-value	R	p-value	R	p-value	R	p-value
WISP1	SARC	0.093	0.13	0.092	0.14	0.3	1.00E-06	0.16	1.00E-02
VEGFA	SARC	0.14	2.10E-02	0.035	0.57	0.13	3.80E-02	0.011	0.86
POSTN	SARC	0.14	2.50E-02	0.023	0.72	0.15	1.40E-02	0.12	0.06
MICB	SARC	0.22	3.40E-04	0.14	2.40E-02	0.2	9.10E-04	-0.013	0.83
CD276	SARC	0.13	3.70E-02	0.065	0.29	0.13	3.70E-02	0.07	0.26
TNC	SARC	0.11	0.07	0.21	5.10E-04	0.3	8.90E-07	0.07	0.26
HAS3	SARC	0.093	0.13	0.2	1.50E-03	0.21	5.10E-04	0.082	0.19
ITGA4	SARC	0.073	0.24	0.091	0.14	0.24	7.00E-05	0.096	0.12
CD39	SARC	-0.028	0.65	0.21	7.80E-04	0.2	9.80E-04	0.21	5.00E-04
ADORA2B	SARC	0.083	0.18	0.18	3.10E-03	0.11	0.07	-0.056	0.36

R: Pearson correlation coefficient

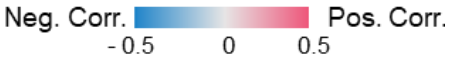


Figure S5. Differential gene correlations between core circadian regulators and pro-tumoral immunomodulatory factors in human sarcoma. Table showing the Pearson's correlation coefficients (*R* values) and corresponding *p*-values to indicate correlations between the expressions of *BMAL1*, *CLOCK*, *CRY1*, and *CRY2* and the indicated pro-tumoral immunomodulatory genes in sarcoma patient samples. Positive correlations ($R > 0$) are shown in red shades, while negative correlations ($R < 0$) are in blue shades. Significant correlations ($p < 0.05$) are indicated with red or blue *p*-values in scientific notation, while non-significant correlations are displayed with black *p*-values in general notation. The data were sourced from GEPIA (http://gepia.cancer-pku.cn/).

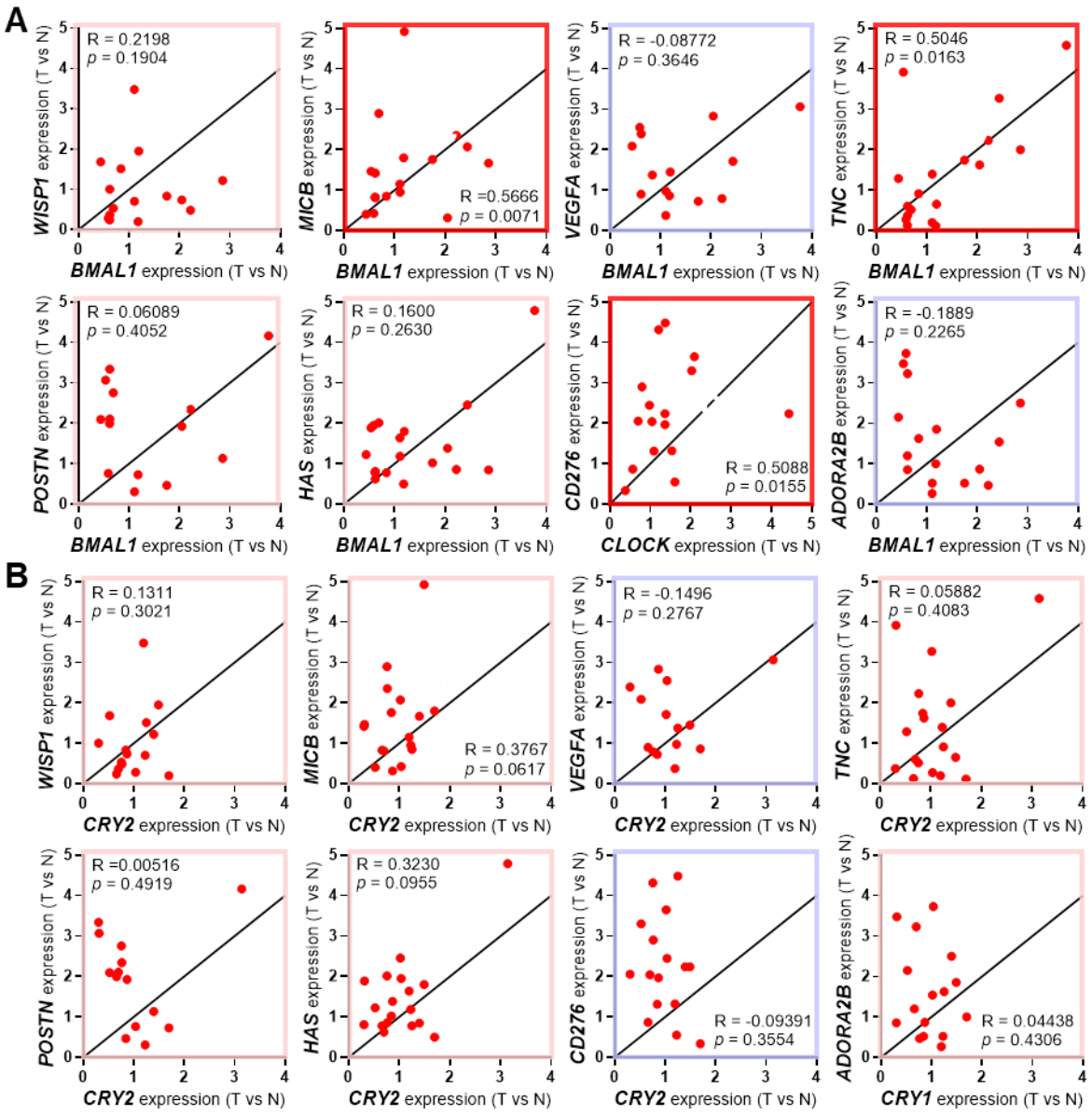


Figure S6. Differential gene correlations between *BMAL1*/*CRY2* and pro-tumoral immunomodulatory genes in human osteosarcoma. (**A, B**) Representative pairwise gene correlation plots illustrating distinct correlation patterns between *BMAL1* (A) or *CRY2* (B) and the indicated pro-tumoral immunomodulatory genes. The Spearman's correlation coefficient (*R*) was used to evaluate the linear relationships between the selected gene expression pairs in publicly available RNA sequencing data (GSE99671) by calculating the differential expression ratios of the core clock components and immunomodulatory genes in tumor (T) versus normal (N) tissues from human osteosarcoma (OS) patients. Significant positive correlations are boxed in bold red ($R > 0$, $p < 0.05$), and significant negative correlations are boxed in bold blue ($R < 0$, $p < 0.05$). WISP1: WNT1 inducible signaling pathway protein 1, MICB: MHC class I polypeptide-related chain B, POSTN: periostin, VEGFA: vascular endothelial growth factor A, TNC: tenascin

C, ITGA4: integrin subunit alpha 4, HAS3: hyaluronan synthase 3, CD276: cluster of differentiation 276 (also known as B7-H3), ADORA2B: adenosine receptor A2B.

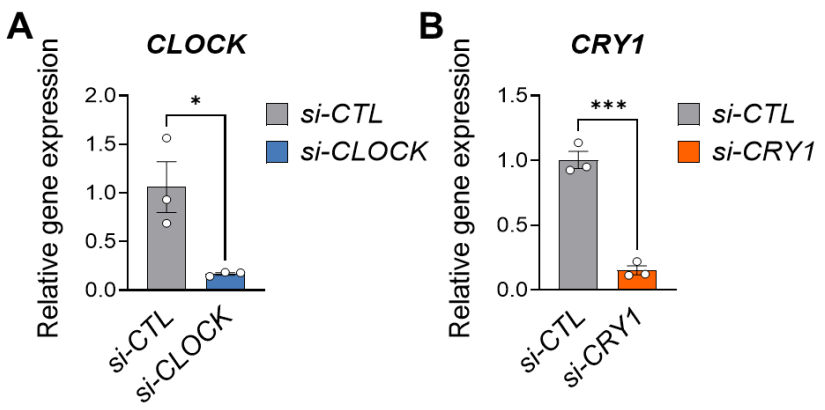


Figure S7. Knockdown efficiency of siRNAs targeting *CLOCK* and *CRY1* in 143B OS CSCs. (**A**, **B**) qPCR analysis validating the knockdown of *CLOCK* (A) and *CRY1* (B) gene expression in 143B OS CSCs 48 hours after transfection with siRNAs specifically targeting each clock gene.

A Association of immune cell infiltration with selected gene expression human SARC

Genes	CD8+ T cells	CD4+ T cells	Dendritic cells	Macrophages	Tregs	CAFs	MDSCs
SOX2	n.s.	-0.183	-0.174	-0.211	n.s.	n.s.	0.328
CD133	n.s.	-0.2	-0.352	-0.353	n.s.	n.s.	0.333
ZEB1	n.s.	-0.278	-0.237	n.s.	0.134	0.414	0.199
SLC16A1	n.s.	-0.379	-0.19	-0.216	n.s.	0.185	0.255
WISP1	0.303	-0.21	0.215	n.s.	n.s.	0.3	0.19
VEGFA	n.s.	-0.203	n.s.	0.141	n.s.	0.17	0.21
TNC	0.158	-0.271	-0.317	-0.317	n.s.	0.116	0.335

Spearman's ρ : positive correlation ($p < 0.05$, $\rho > 0$)
Spearman's ρ : negative correlation ($p < 0.05$, $\rho < 0$)
Spearman's ρ : Not significantl n.s. ($p > 0.05$)

- 0.5 0 0.5

B

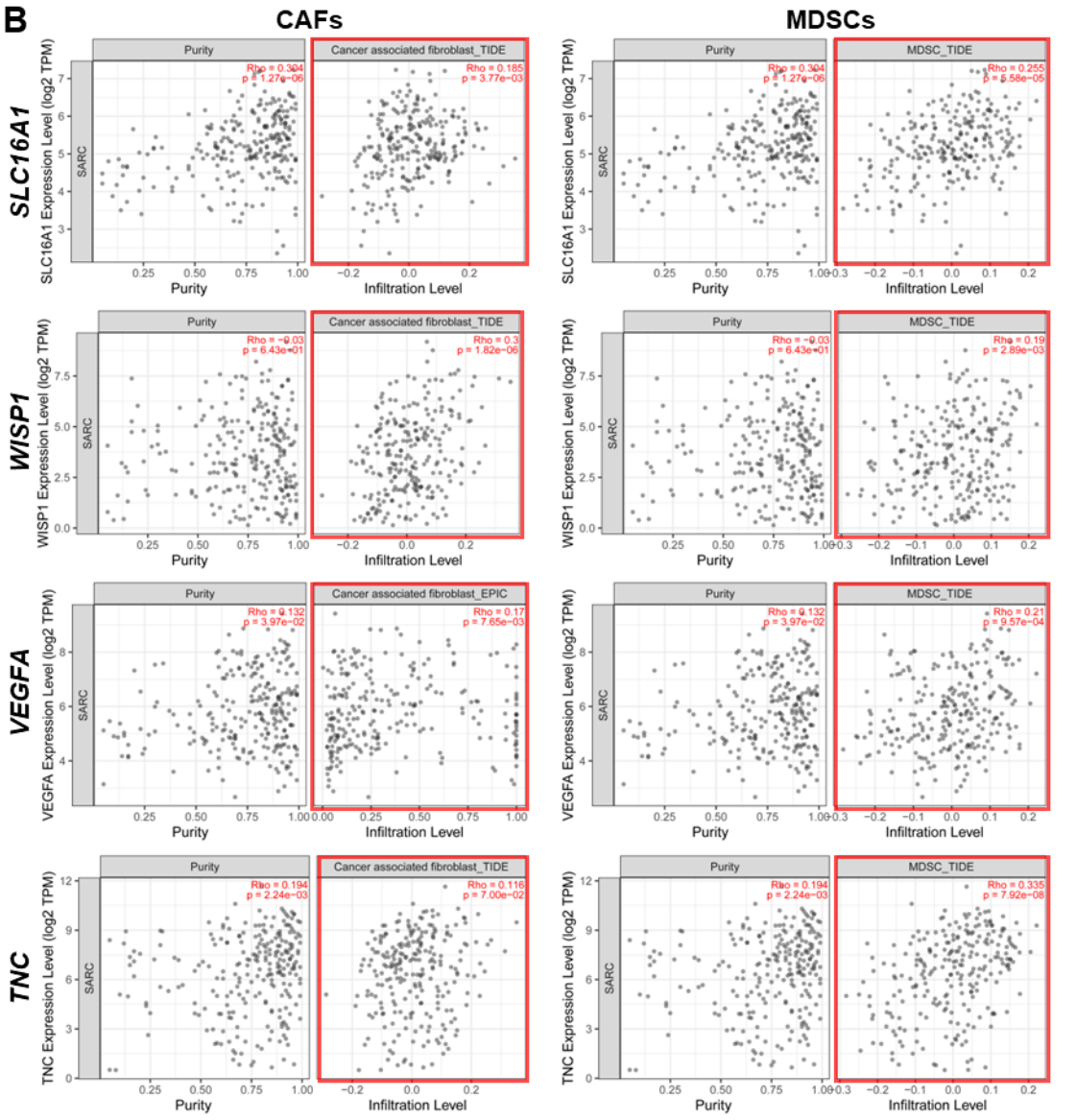


Figure S8. CSC/EMT-associated onco-metabolic and immune factors positively correlated with *CLOCK* and *CRY1* expression shape a pro-tumoral immune microenvironment in

human osteosarcoma by promoting immunosuppressive cell infiltration. (**A**) Table showing the associations between the expressions of CSC/EMT markers (*SOX2*, *CD133*, *ZEB1*) and related glycolytic (SLC16A1) and immunomodulatory factors (*WISP1*, *VEGFA*, *TNC*) that are strongly positively correlated with *CLOCK* and *CRY1* expression (Figs. 2–5) as well as the infiltration of immune cells ($CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, macrophages, Tregs, CAFs, and MDSCs) in human sarcoma (SARC). (**B**) Representative Kaplan-Meier (KM) curves illustrating significantly positive (boxed in red) correlations between the expressions of CSC/EMT-associated genes (*SLC16A1*, *WISP1*, *VEGFA*, *TNC*) and the infiltration of immunosuppressive cells (CAFs, MDSCs) in SARC. The purity-adjusted Spearman's rank correlation test was applied to determine the *p*-values and partial correlation (cor) values. Significantly positive and negative correlations were determined, based on purity-adjusted Spearman's rho values ($p < 0.05$). The data were sourced from the TIMER database (http://timer.cistrome.org/). T cell $CD8^+$: cytotoxic t lymphocytes, T cell $CD4^+$: helper T lymphocytes, Treg: regulatory T cells, CAF: cancer-associated fibroblasts, MDSC: myeloid-derived suppressor cells.

Supplementary Table. qPCR primers for onco-metabolic and immune modulatory genes.

Primers	Forward sequence	Reverse sequence
WISP1	CAAGAGGCCACGCAAGAC	ACAGGAACTGCATAGCCTAC
POSTN	AGCAGACACACCTGTTGGAA	ACGATTAAGGGAAGGTCGTTC
VEGFA	TCTTCAAGCCATCCTGTGTG	TCTGCATGGTGATGTTGGAC
SLC16A1	TTTCTTTGCGGCTTCCGTTGTTG	CCATGGGGCTGAAGGGTAAATTGA
HK1	CCATGGGGCTGAAGGGTAAATTGA	CCAGACGGTGAAGGAACTGT
TNC	GAGATTTAGCCGTGTCTGAGGTTG	GCTTCAATCTCTCCTGGATGGC
CD276	AGGGCAGCCTATGACATTCC	AGGGAGAAGGCTCCAAGACA
ITGA4	GCATACAGGTGTCCAGCAGAGA	AGCTGCTTACCACCTTGGTCCT
MYC	TGAGGAGACACCGCCCAC	CAACATCGATTTCTTCCTCATCTTC
PTN	ATGCAGGCTCAACAGTACCAGCA	ACTCCACTGCCATTCTCCAC