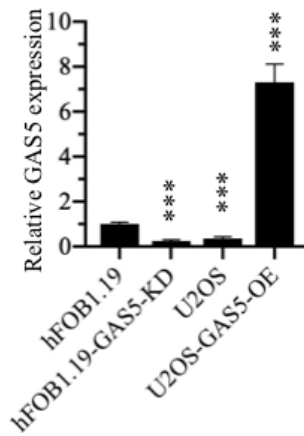


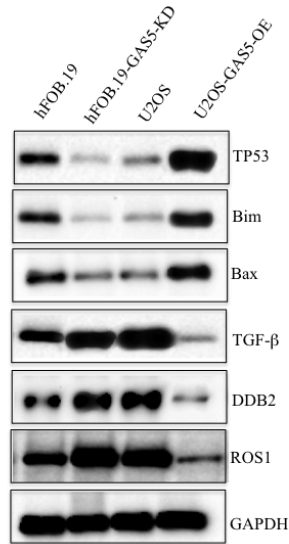
Supplementary Figure 1. GAS5 was significantly downregulated in osteosarcoma cells.

qRT-PCR analysis was performed to measure the relative GAS5 levels in osteosarcoma cell lines including U2OS, HOS, Saos2, 143B and MG63 using hFOB1.19 as a control. ** $P < 0.01$ and *** $P < 0.001$.



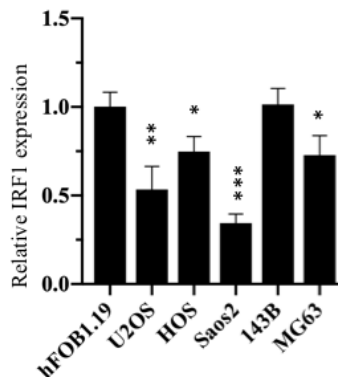
Supplementary Figure 2. The expression of GAS5 and its knockdown and overexpression cell lines.

qRT-PCR analysis was performed to measure GAS5 expression in hFOB1.19 cells expressing control-shRNA (Ctrl) and shGAS5 (GAS5-KD) and in U2OS cells expressing pCDNA3 (Ctrl) and pCDNA3-GAS5 (GAS5-OE). The expression of GAS5 in hFOB1.19-Ctrl cells was defined as 1-fold. *** $P < 0.001$.



Supplementary Figure 3. The protein levels of GAS5 targets in osteosarcoma cells.

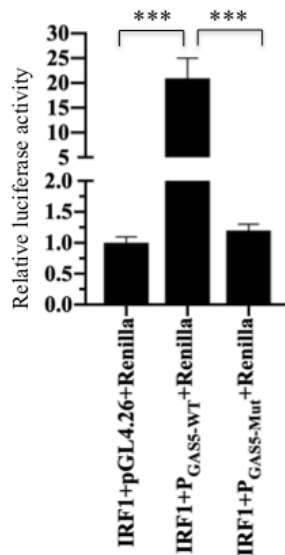
Total protein extracts from hFOB1.19 cells expressing control-shRNA (Ctrl) and shGAS5 (GAS5-KD) and U2OS cells expressing pCDNA3 (Ctrl) and pCDNA3-GAS5 (GAS5-OE) were applied for Western blot analyses to determine the protein levels of p53, Bax, Bim, TGF-β, DDB2 and ROS1. GAPDH was used as a loading control.



Supplementary Figure 4. IRF1 was significantly downregulated in osteosarcoma cells.

qRT-PCR analysis was performed to measure relative *IRF1* mRNA levels in osteosarcoma cell lines including U2OS, HOS, Saos2, 143B and MG63 using hFOB1.19 as a control. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

$P_{GAS5-WT}$...5' -CGGTTTCCCTTTGGGGT-3' -...
 $P_{GAS5-Mut}$...5' -CGGCCCAAACCCGGGGT-3' -...



Supplementary Figure 5. Overexpression of *IRF1* induced the luciferase activity of wild-type GAS5 promoter.

hFOB1.19 cells were co-transfected with the pCDNA3-IRF1-Flag, pRL-TK (Renilla luciferase) and pGL4.26- $P_{GAS5-WT}$ or pGL4.26- $P_{GAS5-Mut}$ firefly luciferase vectors. After incubation at 37 °C for 24 hr, the cells were applied to luciferase assays using a Dual Luciferase Reporter Assay System. The relative luciferase activity in cells expressing pGL4.26 (empty vector) was defined as 1-fold. $**P < 0.001$.

Supplementary Table 1. The clinicopathological characteristics of osteosarcoma patients (n=12 in each group)

Parameter	MSTS I	MSTS II	MSTS III	MSTS IV
Mean age	16.2±1.2	15.1±1.8	19.5±1.5	21.4±2.3
Gender	12M/12F	12M/12F	12M/12F	12M/12F
Average tumor diameter (cm)	2.2	4.8	6.5	7.9

F, female; M, male.

Supplementary Table 2. Primers used for qRT-PCR analysis

Gene	Forward Primers	Reverse primers
GAS5	5'-CAAGCATGCAGCTTACTG-3'	5'-GGGAGGCTGAGGATCACT-3'
IRF1	5'-ACTATCACCAGGGCTGT-3'	5'-TGACTGTGTCATATCAA-3'
TP53	5'-GGGTCTCACAGTGTGTC-3'	5'-ACCCTTCCAGCTGGACGT-3'
Bax	5'-GCTGGACATTGGACTT-3'	5'-CACAAAGATGGTCACGGT-3'
Bim	5'-TACGGCCTATTCTCAGA-3'	5'-CAGAGAGGCAATAGTAGG-3'
TGFB1	5'-GGAACACTACTGTAGT-3'	5'-GTGTTATCAGAGTCCCTG-3'
DDB2	5'-CAAGCAGAGGTGGCGAT-3'	5'-TCTCTGGAGGCAAGTCCA-3'
ROS1	5'-TGAGGGTCCTCTAGGC-3'	5'-TAGTTCAGGCCTTCAGGC-3'
CtBP1	5'-TGCAGAGACTGGTCCGG-3'	5'-GAAGGACACAGGGCAG-3'

Supplementary Table 3. Genes regulated by GAS5 in osteosarcoma cells

Gene	hFOB1.19-Ctrl	hFOB1.19-GAS5-KD	U2OS-Ctrl	U2OS-GAS5-OE
TP53	1.1	-9.4	-10.8	9.3
Bim	1.3	-8.9	-6.7	4.9
Bax	1.4	-8.8	-7.8	7.3
S100A8	1.0	-8.5	-5.7	4.8
NOL9	1.1	-7.8	-5.4	4.7
COL11A1	1.2	-6.7	-5.2	4.7
Runx1	1.4	-6.2	-5.0	4.6
TCF3	1.0	-6.0	-4.8	4.5
INSL6	1.2	-4.8	-4.8	4.5
GCNT1	1.0	-4.5	-4.7	4.4
IBD22	1.1	-4.4	-4.6	4.3
NXPH3	1.3	-4.3	-4.6	4.2
DDX52	1.4	-4.0	-4.4	4.1
NUPL2	1.5	-3.8	-4.3	4.0
KDM7A	1.1	-3.7	-4.2	3.9
ING3	1.1	-3.6	-4.0	3.7
POP5	1.2	-3.5	-3.9	3.6
PRB4	1.1	-3.3	-3.9	3.4
SARNP	1.1	-3.3	-3.6	3.2
IL6	1.3	13	15	-9.5
PHF2	1.4	4.5	5.7	-4.9
TGFB1	1.2	11.3	13.2	-8.5
CIDEB	1.0	10.5	6.5	-5.6
EGLN3	1.1	6.2	5.7	-5.5
ZNF839	1.5	5.4	5.5	-4.8
EMP2	1.1	8.9	5.4	-4.5
ROS1	1.2	5.6	5.4	-4.3
NUBP2	1.3	5.2	5.2	-4.2
RIAS1	1.2	4.9	5.2	-4.0
NFATC2	1.1	4.6	5.2	-3.8
ZBTB16	1.3	4.3	5.0	-3.8
RNF114	1.4	4.2	4.7	-3.7
BCL9L	1.5	4.1	4.5	-3.6
NOL4L	1.2	3.9	4.3	-3.4
RNF34	1.1	3.8	4.1	-3.3
ALXO5	1.1	3.8	4.1	-3.3
CDNF	1.3	3.6	3.9	-3.2

FRAT2	1.4	3.5	3.8	-3.1
TCTN3	1.2	3.4	3.7	-3.0
UTF1	1.1	3.3	3.6	-3.0
ATG4D	1.2	3.2	3.5	-2.9
IFI30	1.4	3.1	3.3	-2.7
NWD1	1.1	2.9	3.3	-2.6
PRR12	1.4	2.7	3.2	-2.6
NIPA2	1.0	2.6	3.1	-2.5
RMDN3	1.1	2.5	3.1	-2.4
SEN8	1.2	2.4	3.0	-2.4
MESP1	1.1	2.4	3.0	-2.3
NRBP1	1.3	2.3	2.9	-2.2
POLR1A	1.6	2.2	2.8	-2.2
TTC7A	1.3	2.2	2.7	-2.1

Supplementary Table 4. Proteins associated with IRF1 in osteosarcoma cells by mass spectrometry

Protein	Protein description	Molecular weight (kDa)	MASCOT scores
IRF1	Interferon Regulatory Factor 1	37	1885
VCAM1	Vascular Cell Adhesion Molecule 1	81	1754
GTF3A	General Transcription Factor IIIA	42	1667
HDAC1	Histone Deacetylase 2	55	1632
HDAC2	Histone Deacetylase 2	55	1588
GTF2F1	General Transcription Factor IIF Subunit 1	58	1521
CtBP1	C-Terminal Binding Protein 1	48	1433
GPR108	G Protein-Coupled Receptor 108	61	1398
TRIM45	Tripartite Motif Containing 45	64	1365
SIN3A	SIN3 Transcription Regulator Family Member A	145	1335
MTA1	Metastasis Associated 1	81	1227
RBBP4	RB Binding Protein 4	48	1212
RBBP7	RB Binding Protein 7	48	1186
TCF7L1	Transcription Factor 7 Like 1	63	1165
SUMO1	Small Ubiquitin-Like Modifier 1	12	1124
UBE3A	Ubiquitin Protein Ligase E3A	101	1065
MRE11A	Meiotic Recombination 11 Homolog 1	81	990
TMEM150	Transmembrane protein 150A	29	976
FBXO11	F-box protein 11	104	932
IFITM2	Interferon induced transmembrane protein 2	15	870
KDM2A	Lysine demethylase 2A	133	856
GLB1L3	Galactosidase, beta 1-like 3	75	844
CCL15	Chemokine (C-C motif) ligand 15	12	821
NPRL2	Nitrogen permease regulator 2-like protein	44	814
MITF	Microphthalmia-associated transcription factor	59	794
TDGF1	Teratocarcinoma-derived growth factor 1	21	763
TRAK1	Trafficking kinesin-binding protein 1	106	755
TFAP2A	Transcription Factor AP-2 Alpha	48	744
ERLEC1	Endoplasmic reticulum lectin 1	55	719
CTLA4	Cytotoxic T-Lymphocyte antigen 4	25	683
PLGLB2	Plasminogen-related protein B	11	665
SMN1	Survival motor neuron 1	32	636
NOS3	Nitric Oxide Synthase 3	133	625
STT3B	Catalytic subunit of the	94	583

	oligosaccharyltransferase complex		
PHF7	PHD finger protein 7	44	572
MEAF6	MYST/ESA1 associated factor 6	22	551
NBPF3	Neuroblastoma breakpoint family member 3	73	510
PSRC1	Proline/serine-rich coiled-coil protein 1	39	482
EXTL2	Exostosin like glycosyltransferase 2	37	471
GBP5	Guanylate binding protein 5	67	442
STAT1	Signal Transducer And Activator Of Transcription 1	87	405
NRBP1	Nuclear receptor-binding protein 1	60	398
WBP1	WW domain-binding protein 1	29	354
SUZ12	Suppressor of Zeste 12 protein homolog	83	336
BGLAP	Bone Gamma-Carboxyglutamate Protein	11	334
SPINK6	Serine protease inhibitor Kazal-type 6	9	327
HIPK2	Homeodomain Interacting Protein Kinase 2	131	302
SPZ1	Spermatogenic leucine zipper protein 1	49	293
RB1	Retinoblastoma 1	106	266
AFT1	Activating Transcription Factor 1	29	251
SPRYD7	SPRY domain-containing protein 7	22	245
ZEB1	Zinc Finger E-Box Binding Homeobox 1	124	233
SMAD4	SMAD Family Member 4	60	212
TM9SF2	Transmembrane 9 superfamily member 2	76	195
ZMYM2	Zinc finger MYM-type protein 2	155	167
ZNF79	Zinc finger protein 79	55	105