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Supplemental information

Super-enhancer-driven lncRNA-DAW promotes liver cancer cell proliferation through activation of Wnt/β-catenin pathway

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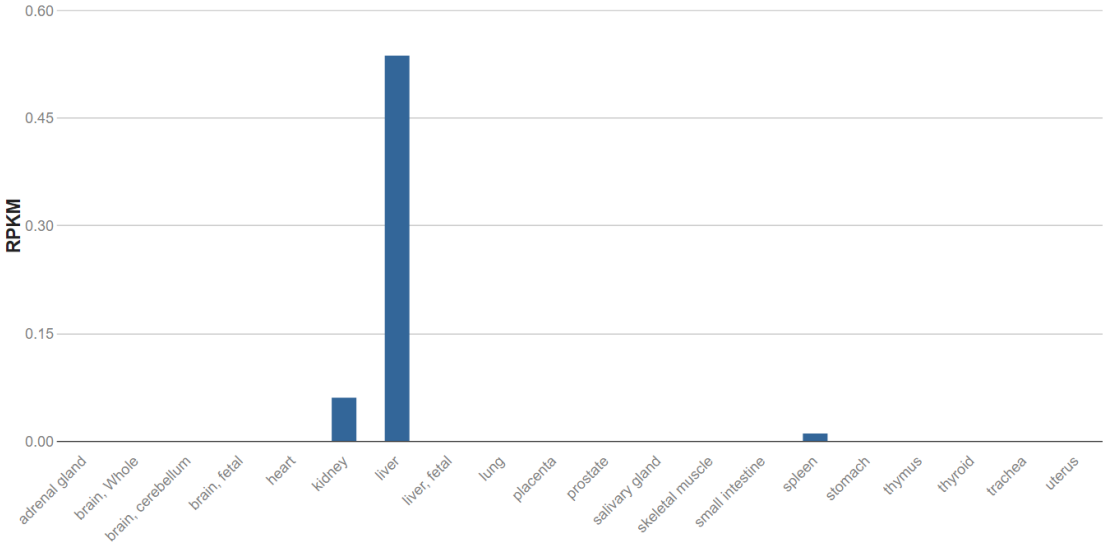
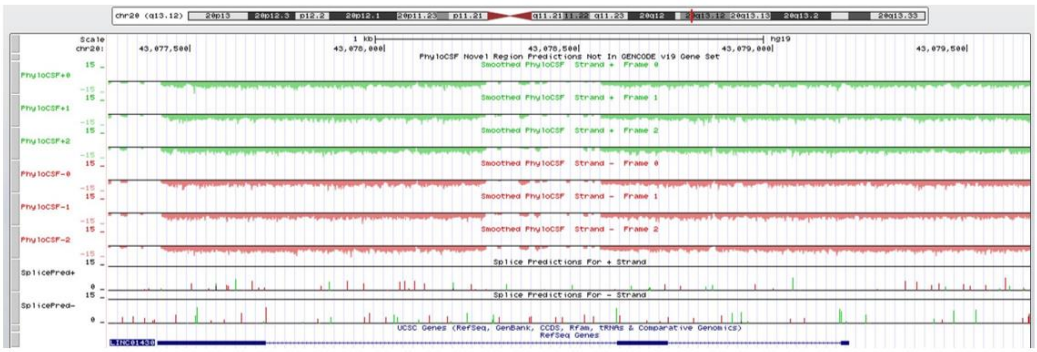
Supporting documents



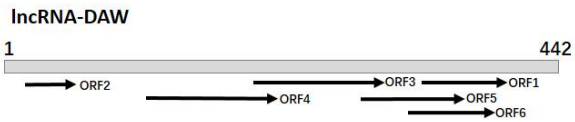
Supplementary Fig. 1. The expression profiles of lncRNA-DAW in 20 human tissues (https://www.ncbi.nlm.nih.gov/gene/?term=linc01430).

A

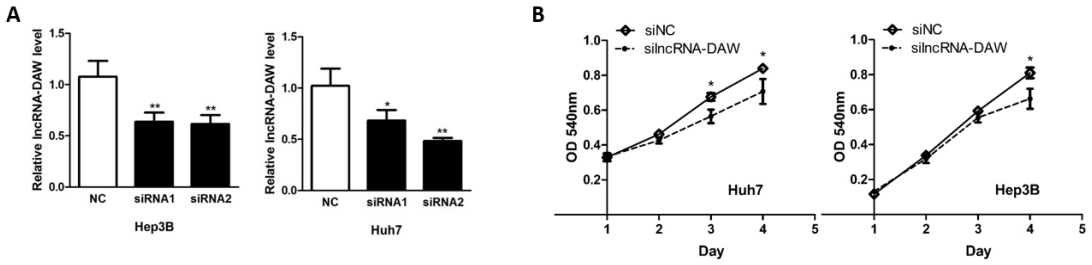


B

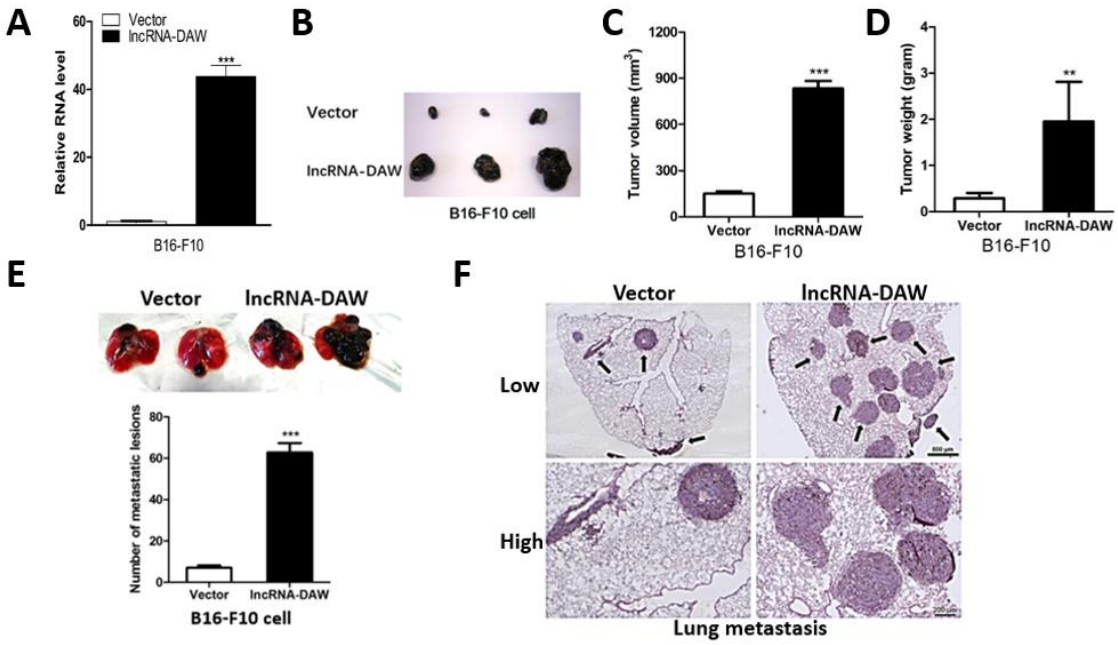
Label	Strand	Frame	Start	Stop	Length (nt/aa)
ORF3	+	3	192	302	111 | 36
ORF4	-	1	205	107	99 | 32
ORF5	-	2	351	268	84 | 27
ORF6	-	3	371	306	66 | 21
ORF1	+	1	319	381	63 | 20
ORF2	+	2	17	52	36 | 11



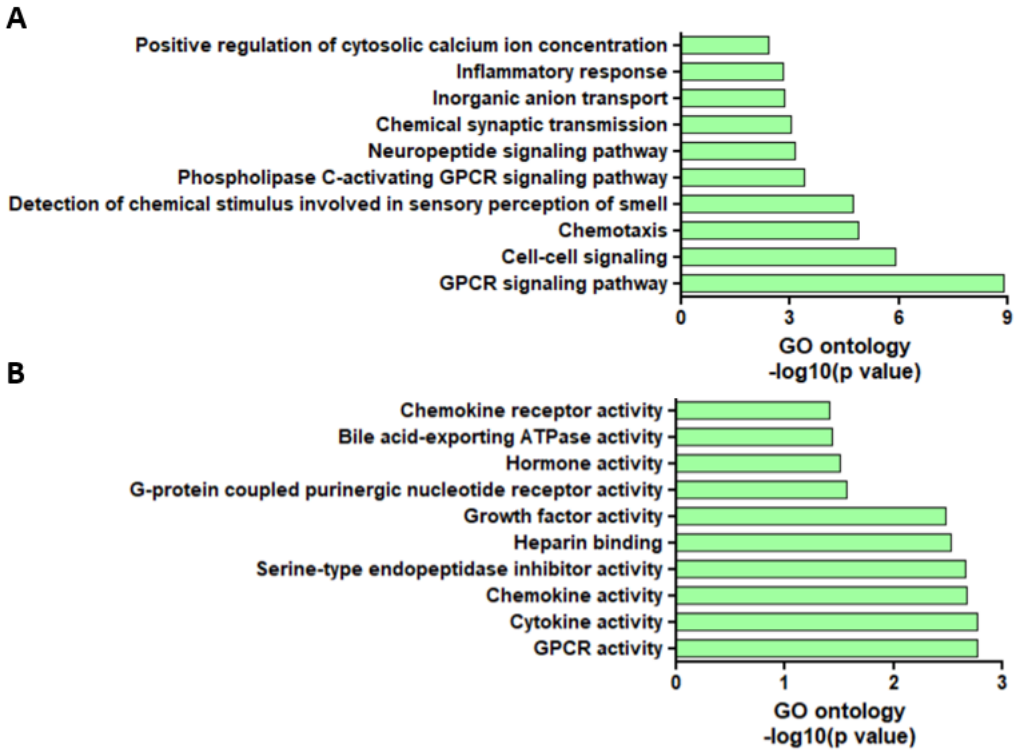
Supplementary Fig. 2. Analysis of potential ORFs expressed from lncRNA-DAW. (A)The codon substitution frequency scores (CSF) of lncRNA-DAW and negative score of CSF indicated that lncRNA-DAW did not have protein-coding potential. (B)The prediction with ORF Finder (NCBI) showed the potential peptides encoded by lncRNA-DAW (https://www.ncbi.nlm.nih.gov/orffinder).



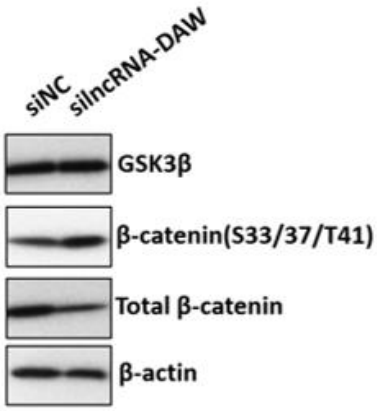
Supplementary Fig. 3. Silencing of lncRNA-DAW suppress liver cancer cell growth. (A) The RNA levels of lncRNA-DAW were evaluated after transient silencing of siRNAs targeting lncRNA-DAW. (B) MTT assays showed that knockdown of lncRNA-DAW impaired cancer cell proliferation. (*, $P < 0.05$; **, $P < 0.01$)



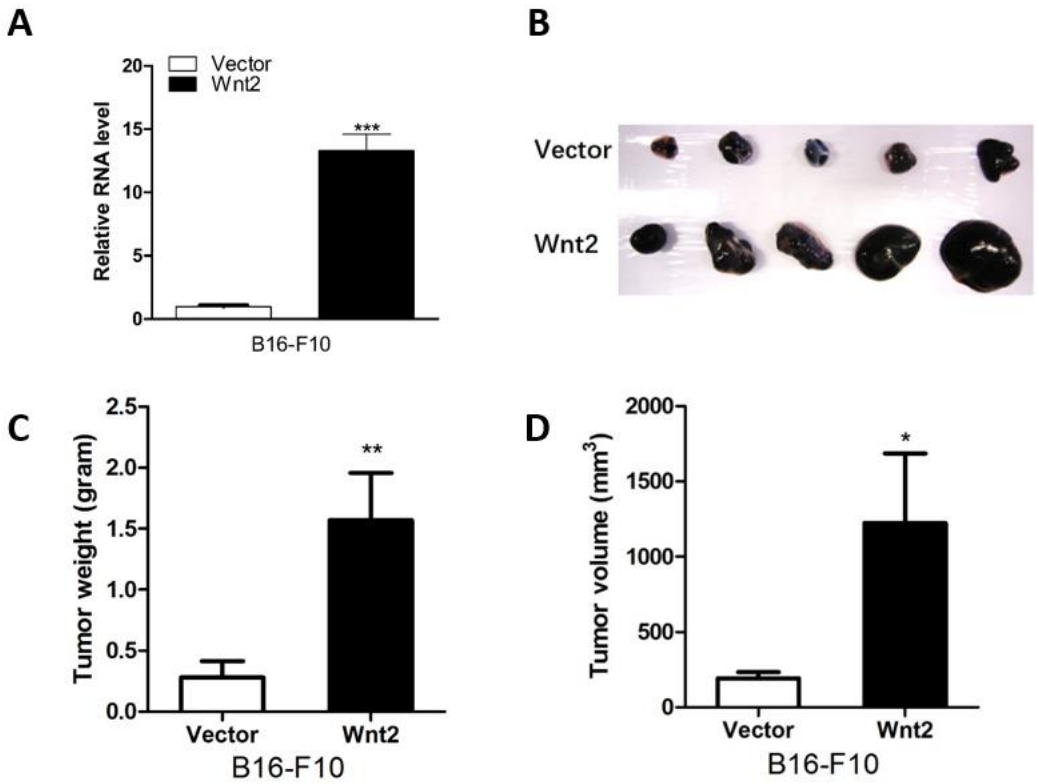
Supplementary Fig. 4. Reinforced expression of lncRNA-DAW enhanced *in vivo* tumor growth and tumor metastasis. (A) The RNA level of lncRNA-DAW was evaluated after stable expression of lncRNA-DAW. (B) The lncRNA-DAW and vector-transfected stable B16-F10 cells were subcutaneously injected into nude mice (n=5). The nude mice were sacrificed at the indicated time points and the tumor tissues were harvested. (C&D) Tumor weight and volumes were measured and calculated. (E) The lncRNA-DAW and vector-transfected stable cells were introduced into nude mice through hydrodynamic tail vein injection. The lung tissues were collected at the indicated time. And the metastatic sites under the microscope were counted and calculated. (F) The lung tissues were subjected to H&E staining. Representative pictures were captured and showed. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.)



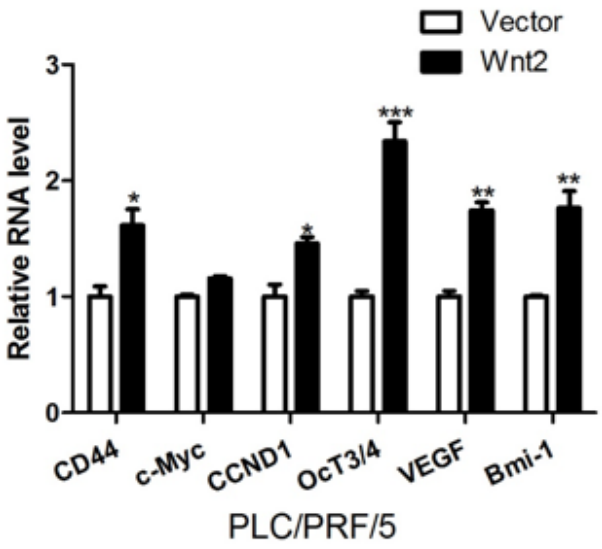
Supplementary Fig. 5. Gene ontology enrichment analysis was conducted by using DAVID website. (A) Gene ontology analysis was performed on the upregulated genes after ectopic expression of lncRNA-DAW. (B) Gene ontology analysis was performed on the downregulated genes after ectopic expression of lncRNA-DAW.



Supplementary Fig. 6. Transient silencing of lncRNA-DAW reduced protein level of β-catenin.



Supplementary Fig. 7. Stable overexpression of Wnt2 promoted *in vivo* tumor growth. (A) The RNA level of Wnt2 was evaluated after stable expression of Wnt2. (B) The Wnt2 and corresponding vector-transfected stable B16-F10 cells were subcutaneously injected into nude mice (n=5). The nude mice were sacrificed and the tumor tissues were collected. (C) Tumor weight was measured and calculated. (D) Tumor volumes were measured and calculated. (*, $P < 0.05$; **, $P < 0.01$)



Supplementary Fig. 8. Overexpression of Wnt2 in PLC/PRF/5 cells significantly activated the expression of β-catenin target genes. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.)

Supplementary Table 1 The primer sequence used in this study.

Name	**Primer sequences used for plasmid construction**
pbabe-lncRNA-DAW-F	CGCGGATCCGACCACTCGTGTGTGGATGA
pbabe-lncRNA-DAW-R	ACGCGTCGACAAAATAAAGTAAAATTCTCTGATTCTGT
pBabe-Wnt2-F	CGCGGATCCATGAACGCCCCTCTCGGT
pBabe-Wnt2-R	ACGCGTCGACTCATGTAGCGGTTGTCCAG
Name	**Primer sequences used for RT-PCR**
lncRNA-DAW-F	CTAAGCCCAACCCTGATCCA
lncRNA-DAW-R	CGTGTTTGTCTGGAAGTGCT
U1_F	TGATCACGAAGGTGGTTTTCC
U1_R	GCACATCCGGAGTGCAATG
β-actin_F	AAGATGACCCAGATCATGTTTGAG
β-actin_R	GCAGCTCGTAGCTCTTCTCCAG
RPLPO_F	CCGGATATGAGGCAGCAGTT
RPLPO_R	GAAGGCTGTGGTGCTGATGG
Bmi1_F	GTGCTTTGTGGAGGGTACTTCAT
Bmi1_R	TTGGACATCACAAATAGGACAATACTT
MYOD1-F	CGGACGTGCCTTCTGAGTC
MYOD1-R	AGCACCTGGTATATCGGGTTG
MMP1-F	AGCTAGCTCAGGATGACATTGATG
MMP1-R	GCCGATGGGCTGGACAG
WISP1-F	CCAGCCTAACTGCAAGTACAA
WISP1-R	GGCGTCGTCCTCACATACC
Wnt2-F	GATGCGTGCCATTAGCCAG
Wnt2-R	AGATTCCCGACTACTTCGGAG
Wnt9b-F	TGTGCGGTGACAACCTCAAG
Wnt9b-R	ACAGGAGCCTGATACGCCAT
DKK4-F	ACGGACTGCAATACCAGAAAG
DKK4-R	CGTTCACACAGAGTGTCCCAG
CCL8-F	TGGAGAGCTACACAAGAATCACC
CCL8-R	TGGTCCAGATGCTTCATGGAA
Name	**siRNA sequences**
silncRNA-DAW-1	GUUGAGCACUUCCAGACAATT
silncRNA-DAW-2	CAAGGACAGUGUUAAUGAUTT
Name	**CHIP primer sequence**
Wnt-2-CHIP-F	TGCTTTGGCAGATACTGCTG
Wnt-2-CHIP-R	CTGAAGCTGGGATGAAGAGC