

## Supplementary Information

### Supplementary Figure legends

Figure S1. LHX6 was significantly down-regulated in lung cancer cell lines. RT-qPCR and western blot analyses of LHX6 expression in lung cancer cell lines compared with a normal HBE cell line. Error bars represent the s.d. (n=3).

Figure S2. Lung adenocarcinoma patients (n=88) were divided into two groups according to the LHX6 expression: H, High expression (>median); L, Low expression ( $\leq$  median). Scale bars represent 50  $\mu$ m

Figure S3. LHX6 expression status is found to be associated with metastasis of lung adenocarcinoma patients by analyzing TCGA data-base. (A, B) Low LHX6 expression was found in lung adenocarcinoma patients with metastasis. Data retrieved from TCGA dataset. Cohort-1 lungadenocarcinoma (TCGA, provisional, n=181); Cohort-2, lung adenocarcinoma (TCGA, Nature 2014, n=167); M0, non-metastatic lung cancers; M1, metastatic lung cancers. \*\* P<0.01. ([www.cbioportal.org](http://www.cbioportal.org)).

Figure S4. LHX6 suppresses the colony formation of lung adenocarcinoma cell lines. (A) Overexpression of LHX6 reduced the colony-formation ability of LTEP-a-2 and SPC-a-1 cells. (B) Knockdown of LHX6 in LTEP-a-2-LHX6 cells recovered the colony formation ability. Error bars indicate s.d. (n=3). \* P<0.05, \*\* P<0.01.

Figure S5. A negative correlation between LHX6 and  $\beta$ -catenin was found in the TCGA (LUNG) RNAseq (IlluminaHiSeq; n=1124) and TCGA (LUAD) RNAseq (IlluminaHiSeq; n=571) data sets but not in TCGA (LUSC) RNAseq (IlluminaHiSeq; n=553) data set.

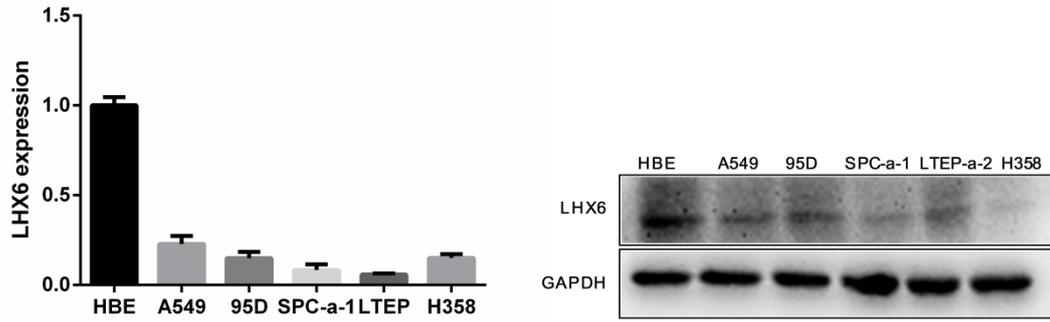
Figure S6. Re-expression of  $\beta$ -catenin reverses the inhibitory effect of LHX6. (A) The expression of LHX6 and  $\beta$ -catenin was analyzed by WB 48 h after transfection with control vector or  $\beta$ -catenin overexpression vector in LTEP-a-2-LHX6 cells. (B) MTS assays were used to examine the effect of  $\beta$ -catenin re-expression on **the relative number of viable cells based on the absorbance**. (C) Transwell assays were used to examine the effect of  $\beta$ -catenin re-expression on cell metastasis of LTEP-a-2-LHX6 cells. Error bars indicate s.d. (n=3). \*\*P<0.01.

Figure S7. Online data base ([www.jaspar.com](http://www.jaspar.com)) analysis predicts 42 LHX6 binding sites on the promoter region of  $\beta$ -catenin.

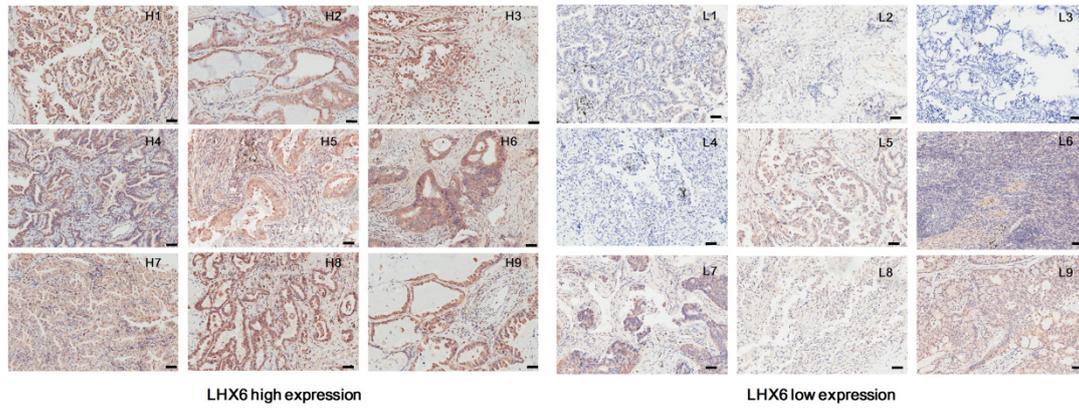
Figure S8. Public datasets were used to analyze the OS of lung cancer patients with different  $\beta$ -catenin expression status. (A) Kaplan–Meier curves for OS of patients with lung cancer (n=1145) expressing high and low expression levels of  $\beta$ -catenin. Low expression of  $\beta$ -catenin predicted longer OS of lung cancer patients compared with high  $\beta$ -catenin expression. (B) Kaplan–Meier curves for OS of patients with lung adenocarcinoma (n=673). Low expression of

$\beta$ -catenin predicted longer OS of lung cancer patients compared with high  $\beta$ -catenin expression. (C)  
Kaplan–Meier curves for OS of patients with lung squamous carcinoma: (n=524). High expression of  $\beta$ -catenin predicted longer OS of lung cancer patients compared with low  $\beta$ -catenin expression.

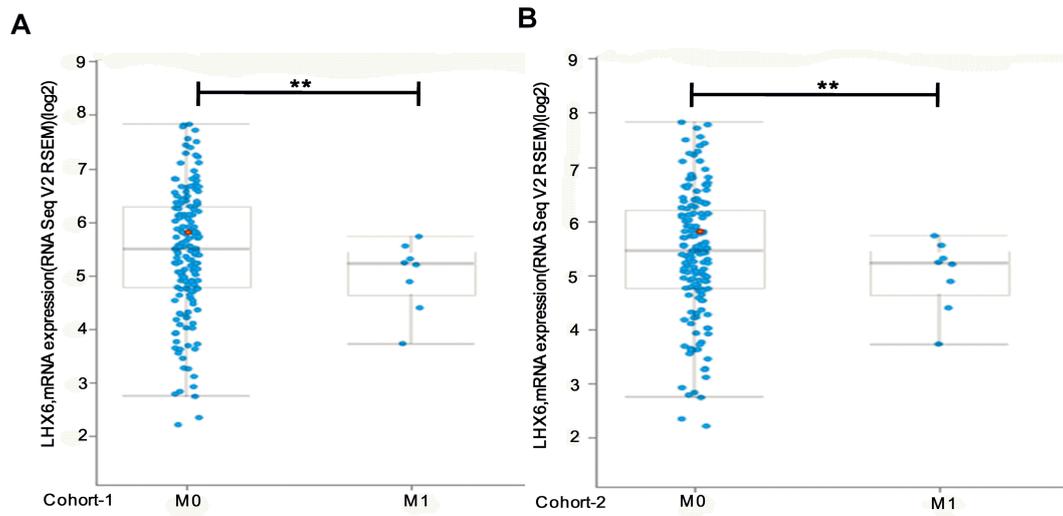
## Supplementary Figures



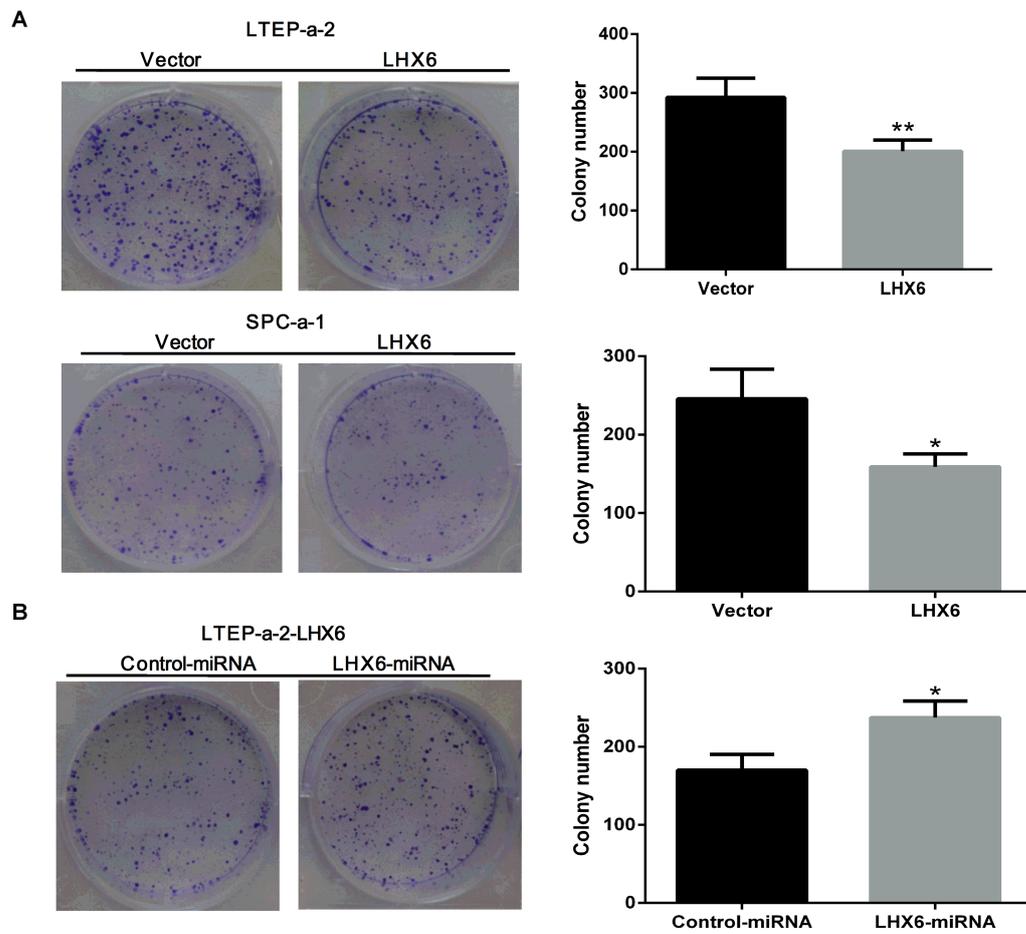
Supplementary Figure 1.



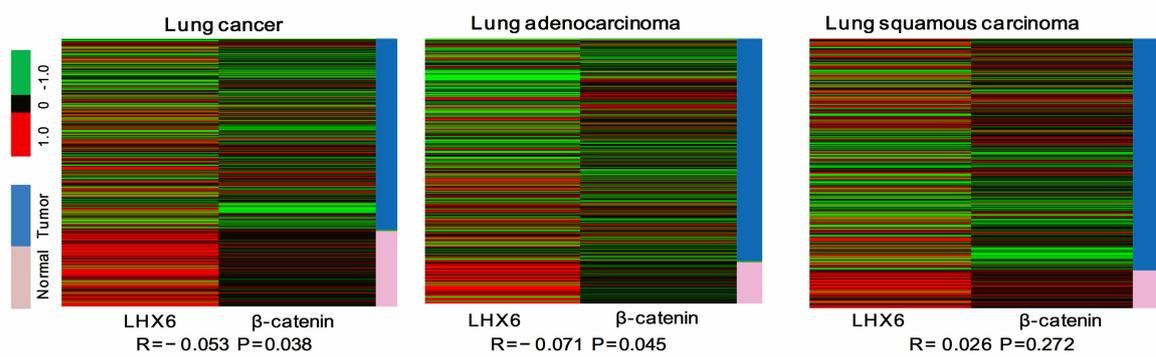
Supplementary Figure 2.



Supplementary Figure 3.

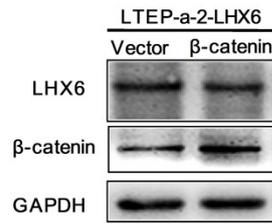


Supplementary Figure 4.

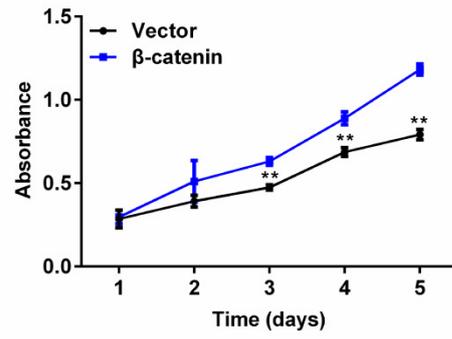


Supplementary Figure 5.

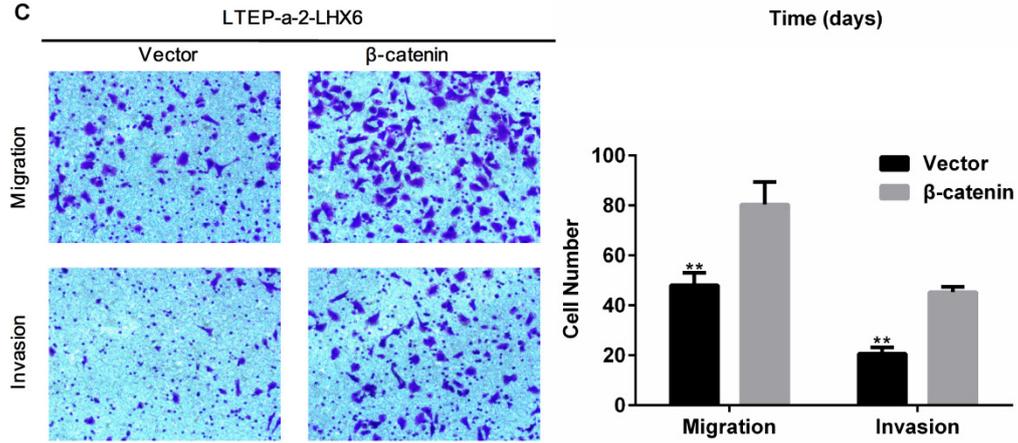
A



B



C

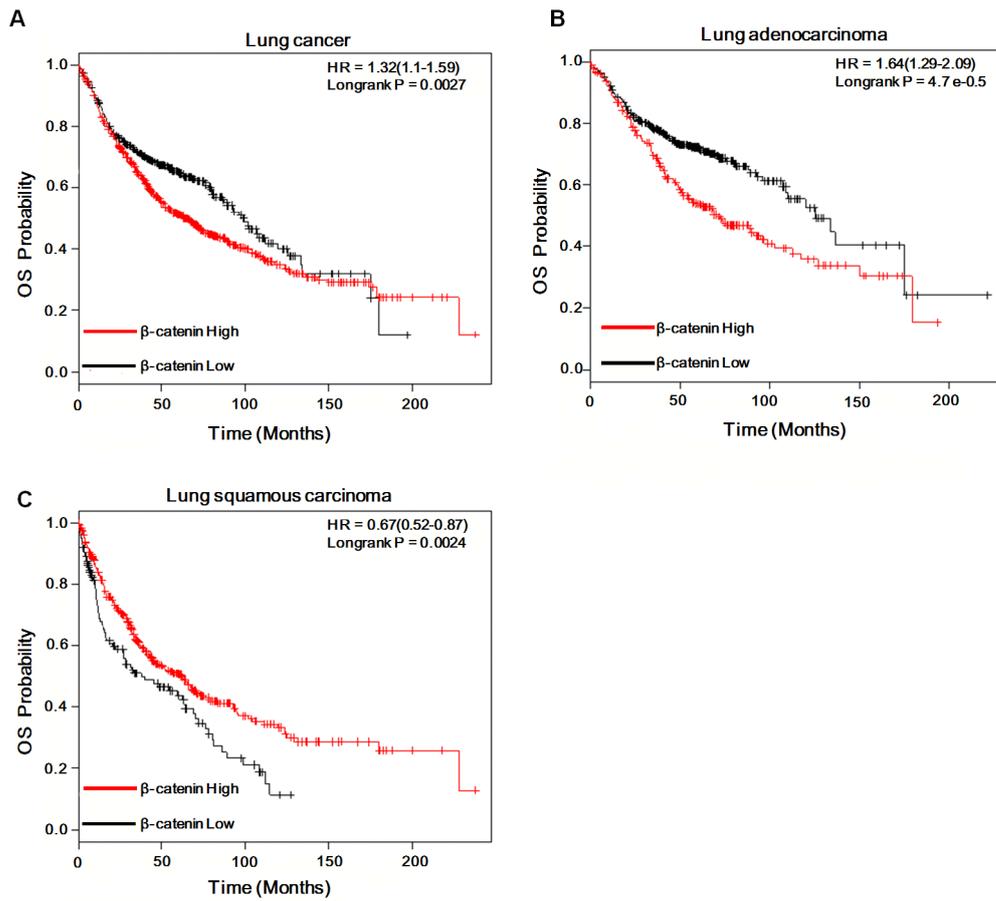


Supplementary Figure 6.

42 putative sites were predicted with these settings (80%) in sequence named *sequence*

Model ID	Model name	Score	Relative score	Start	End	Strand	predicted site sequence
MA0658.1	LHx6	5.592	0.845351123695557	102	111	-1	TTTGATTACC
MA0658.1	LHx6	7.672	0.88092080437735	102	111	1	GGTAATCAAA
MA0658.1	LHx6	4.705	0.830182706981735	290	299	-1	ATAAATTAAT
MA0658.1	LHx6	4.861	0.832850433032869	290	299	1	ATTAATTTAT
MA0658.1	LHx6	3.400	0.807866152515514	345	354	1	GATAACCAAC
MA0658.1	LHx6	4.445	0.825736496896511	395	404	-1	GTTAAATTAAT
MA0658.1	LHx6	4.838	0.832457114448407	395	404	1	ATATTTTAAC
MA0658.1	LHx6	4.203	0.821598101355648	408	417	-1	AATAAAGTAAT
MA0658.1	LHx6	3.001	0.801042930115497	555	564	1	AAAAATTAGG
MA0658.1	LHx6	3.014	0.801265240619758	577	586	-1	GTTAGTTAAA
MA0658.1	LHx6	4.829	0.832303207176226	577	586	1	TTTAACTAAC
MA0658.1	LHx6	4.887	0.833295054041392	597	606	-1	GTTGACTAGC
MA0658.1	LHx6	3.145	0.80350544647039	597	606	1	GCTAGTCAAC
MA0658.1	LHx6	3.324	0.806566491105987	608	617	-1	AAGAATTAAT
MA0658.1	LHx6	3.671	0.812500471488959	847	856	1	CTTAACCAAT
MA0658.1	LHx6	3.559	0.810585180990708	936	945	-1	ATTAACCACT
MA0658.1	LHx6	8.129	0.888735873642532	972	981	-1	ATGATTAATA
MA0658.1	LHx6	7.459	0.87727833226907	972	981	1	TTTAATCAAT
MA0658.1	LHx6	3.008	0.801162635771637	1006	1015	-1	TTTAATGTCT
MA0658.1	LHx6	5.572	0.845009107535155	1006	1015	1	GACAATTAATA
MA0658.1	LHx6	3.472	0.80909741069296	1019	1028	1	AGGAATCAGT
MA0658.1	LHx6	4.161	0.820879867418804	1148	1157	1	CAGAATTAGT
MA0658.1	LHx6	3.014	0.801265240619758	1321	1330	1	GTTATTTAAA
MA0658.1	LHx6	4.527	0.827138763154158	1449	1458	-1	TACAATTAAT
MA0658.1	LHx6	4.180	0.821204782771186	1449	1458	1	ATTAATGTATA
MA0658.1	LHx6	3.291	0.806002164441324	1640	1649	1	ATGAATTACA
MA0658.1	LHx6	3.074	0.802291289100963	1664	1673	-1	ACTAATTTTC

Supplementary Figure 7.



Supplementary Figure 8.

## Supplementary Tables

Table S1. Primers used in this study

Primer name	Primer sequence 5'-3'	Primer purpose
LHX6-qF	GCAGAACAGCTGCTACATCAAGAA	qPCR and RT-PCR
LHX6-qR	CAGTCGCTGGCGTAGATCTGTC	qPCR and RT-PCR
$\beta$ -actin-qF	CCACGAAACTACCTTCAACTCC	qPCR and RT-PCR
$\beta$ -actin-qR	GTGATCTCCTTCTGCATCCTGT	qPCR and RT-PCR
CTNNB1-F	GCGCCATTTTAAGCCTCTCG	qPCR and RT-PCR
CTNNB1-R	AAATACCCTCAGGGGAACAGG	qPCR
MMP7-qF	CATGATTGGCTTTGCGCGAG	qPCR
MMP7-qR	AGACTGCTACCATCCGTC	qPCR
MYC-qF	GCGAACACACAACGTCTTGG	qPCR
MYC-qR	TGAGCTTTTGCTCCTCTGCT	qPCR
CCND1-qF	GATGCCAACCTCCTCAACGA	qPCR
CCND1-qR	GGAAGCGGTCCAGGTAGTTC	qPCR
H- $\beta$ 2m F	GCTGTCTCCATGTTTGATGTATCTG	qPCR
H- $\beta$ 2m R	GCACGTTAACTATCTTAACAAGCTTTG	qPCR
M- $\beta$ 2m-F	AGGCTTCTCTTTTCTCCTCTGCTG	qPCR
M- $\beta$ 2m-R	TTTTCTCTCGACTTCGGTTGGATC	qPCR
CTNNB1-pF	GCTGCGATGCGGTACCGAACGAACCTTCTTTACAATACTGGCATAT	Promoter clone
CTNNB1-pR	GCGACTCGATCTCGAGACGCTGCTGCCACAGACCGAGAGGCTTAAAAT	Promoter clone
CTNNB1-pR1	GCGACTCGATCTCGAGAAATTGACGTTTGTGCGAAGCTGCAACAAGTA	Promoter clone
CTNNB1-pF1	GCTGCGATGCGGTACCGTTTTTATCCTTTAGATCTTCTAATGGTGCAGA	Promoter clone

Table S2. Correlation of LHX6 expression and clinical parameters from TMA data

Clinical Features	Total	LHX6 expression		P value
		High (n=20)	Low (n=68)	
<b>Age</b>				
≤ 60	39	8	31	0.658
>60	49	12	37	
<b>Gender</b>				
Male	47	6	41	<b>0.033</b>
Female	41	14	27	
<b>Clinical stages</b>				
I	27	9	18	0.125
II	29	6	23	
III	27	3	24	
IV	1	1	0	
NA	4			
<b>Histological grade</b>				
I	12	7	5	<b>0.006</b>
II	57	11	46	
III	19	2	17	
<b>Lymph node status</b>				
Negative	38	12	26	<b>0.039</b>
Positive	46	6	40	
NA	4			
<b>Size</b>				
≤ 3cm	34	12	22	<b>0.026</b>
>3cm	54	8	46	

\*NA: patients without relative clinical data

Table S3. Correlation of LHX6 expression and clinical stages from TCGA data base ([www.cbioportal.org](http://www.cbioportal.org)).

Clinical stages	Total	LHX6 expression		P value
		High	Low	
	n=513	n=257	n=256	<b>0.004</b>
I	170	104	66	
II	277	121	156	
III	47	24	23	
IV	19	8	11	