Supplementary

Table S1 Sequences of qRT-PCR primers

Gene	Forward (5'→3')	Reverse (5'→3')
BARX2	GGGCTGTTCCGTCTCTGAC	CCTTCCCTGCGGGCATATC
ACTIN	GTCATTCCAAATATGAGATGCGT	GCATTACATAATTTACACGAAAGCA
FOXA1	GCAATACTCGCCTTACGGCT	TACACACCTTGGTAGTACGCC
HK2	GAGCCACCACTCACCCTACT	CCAGGCATTCGGCAATGTG
GPI	TATTGTGTTCACCAAGCTCACACC	TATTGTGTTCACCAAGCTCACACC
PFKL	GGAGAAGCTGCGCGAGGTTTAC	ATTGTGCCAGCATCTTCAGCATGAG
GAPDH	ATTGTGCCAGCATCTTCAGCATGAG	CAAAGGTGGAGGAGTGGGTGTCGC
ALDOA	AGGCCATGCTTGCACTCAGAAGT	AGGGCCCAGGGCTTCAGCAGG
ENO1	GACTTGGCTGGCAACTCTG	GGTCATCGGGAGACTTGAA
PKM2	GCCCGTGAGGCAGAGGCTGC	TGGTGAGGACGATTATGGCCC
LDHA	ATGGCAACTCTAAAGGATCA	GCAACTTGCAGTTCGGGC
GLUT1	GGCCAAGAGTGTGCTAAAGAA	ACAGCGTTGATGCCAGACAG
GLUT4	TGGGCGGCATGATTTCCTC	GCCAGGACATTGTTGACCAG
PGK1	ATGTCGCTTTCTAACAAGCTGA	GCGGAGGTTCTCCAGCA
PGAM1	GGAAACGTGTACTGATTGCAGCCC	TTCCATGGCTTTGCGCACCGTCT

qRT-PCR, quantitative real-time polymerase chain reaction.

 Table S2 Sequences of siRNAs

Table B2 Bequences of si	
Gene	Target sequence $(5' \rightarrow 3')$
BARX2(si1)	GAAGCAGAAGTATTTGTCA
BARX2(si2)	GGAACCGAAAGCACGTGAT
FOXA1(si1)	CACTGCAATACTCGCCTTA
FOXA1(si2)	TCCCGGTCAGCAACATGAA

Table S3	PCR	primers
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Region (gene)	Location	Forward (5'→3')	Reverse (5'→3')
site1(HK2)	–1693bp, –1704bp	GACAAGGGCAGGGAAGAGAAGA	TGCACGTCCTCAACCCTCCT
site2(HK2)	–1388bp, –1399bp	TTGCTGAGAGGCTAGATGGATTA	TGCCATGTTCTACTGAGGAGGA
site3(HK2)	–806bp, –817bp	TCGTGCGTGTCTTCCACTCTG	ACTTTCTGGGACCTGCCTTAGA
site1(BARX2)	–1620bp, –1631bp	AGCCGCCTCTGCATGGAGAA	GCATTAGACAAGGCCCGCAAC
site2(BARX2)	–515bp, –529bp	CTCTGACCCTTTCCAACGAGGA	GGCGACAAGAGCGAGATTCTGA
site3(BARX2)	–1490bp, –1501bp	AATGCTAGGCTGTGACTTGGC	AGGCTGGTCTTGAACTCCTGAC

PCR, polymerase chain reaction.



Figure S1 Overexpression of *BARX2* promoted proliferation and progression of A549 cells. (A,B). Overexpression of *BARX2* in A549 cells was validated by (A) qRT-PCR and (B) Western blotting. (C) CCK8 assays revealed that overexpression of *BARX2* promoted proliferation of A549 cells. (D,E) Transwell and Matrigel assays indicated that overexpression of *BARX2* promoted migration and invasion of A549 cells. After migration/invasion, the remaining cells on the top filter were washed off, and the filters were fixed in 4% methanol for 30 minutes. Filters were washed again in PBS before staining in 0.1% crystal violet for 30 min and then counted under the microscope. **P<0.01; ***P<0.001. OE, overexpression; OD, optical density.

Table S4 BARX2 LUAD GSEA Reactome

Description	Set	Enrichment score	NES	P value	Rank
Sulfur amino acid metabolism	10	-0.464	-1.802	0.020	2332
Diseases associated with glycosaminoglycan metabolism	30	0.355	1.641	0.030	3402
Heparan sulfate/heparin (HS-GAG) metabolism	34	0.335	1.597	0.041	2546
Selenoamino acid metabolism	65	-0.201	-2.129	0.045	6299
Glycosaminoglycan metabolism	77	0.261	1.509	0.046	2546

LUAD, lung adenocarcinoma; GSEA, Gene Set Enrichment Analysis; NES, normalized enrichment score; HS, heparan sulfate; GAG, glycosaminoglycans.



Figure S2 Overexpression of *BARX2* promoted the Warburg effect in A549 cells. (A) ECAR were measured by Seahorse XF in A549 cells after overexpression expression of *BARX2*. (B-D) Detection of (B) glucose consumption, (C) pyruvate production, and (D) lactate production in A549 cells after overexpression expression of *BARX2*. **P<0.01. OE, overexpression; ECAR, extracellular acidification rate; OM, oligomycin; 2-DG, 2-deoxy-glucose.

A Matrix ID ↓↑ Name ↓		J↑ Scor	Score ↓↑ Relative score ↓			Sequence ID			$\downarrow \uparrow$	Start	End ↓↑	Strand 1	
(M	MA1471.1 BARX2		10.75	59	0.924323176424		NC_000002.12:74832126-74834125			125	603	614	•
(A1471.1	BARX2	9.30	0685 0.90149531146		NC_000002	.12:7483	2126-74834	125	602	613	+	
P	redicted s	equence	e TATAATAA	тттт									
MA1471.1 BARX2 6.9732		321	0.8648116954	NC_000002.12:74832126-74834125				26	37	37 -			
• M	A1471.1	BARX2	6.92	127	0.863995157411		NC_000002.12:74832126-74834125			125	297	308	+
P	redicted s	equence	e AAAAGCAG	GTTTG									
• M	A1471.1	BARX2	6.14	421	21 0.851780172912		NC_000002	2126-74834	587	598	+		
(A1471.1	BARX2	6.04	621	0.8502397580	31	NC_000002	2126-74834	591	602			
(A1471.1	BARX2	5.970	022	0.849045222163		NC_000002.12:74832126-74834125				566	577	+
(A1471.1	BARX2	5.88	926	26 0.847772506725		NC_000002.12:74832126-74834125			125	606	617	-
(A1471.1	BARX2	5.850	091	0.847169632049		NC_000002.12:74832126-74834125			125	283	294	-
(A1471.1	BARX2	5.83	968	0.846993184581		NC_000002.12:74832126-74834125			532	543	+	
A0148.1	FOXA	1	11.153	0.90	9933400065	BAR	(2-	385	395			TGTTTT	CTTTC
D	1 Nam	e↓î	Score 1	scor	re ↓₹	ID	ţĭ	Start	End ↓↑	Str	and ↓⊺	sequen	ce
					pro		noter						
IA0148.2	FOXA	1	11.1126	0.90	9824858351	BAR) pron	(2- noter	385	395			TGTTTTCTTTC	
1A0148.3	FOXA	1	7.32982	0.87	3154356737	BARX2- promoter		1472	1486	-		ACATTGTTTATAAAA	
1A0148.2	FOXA	1	7.85392	0.85	3925583843	BARX2- promoter		515	525	+		TATTTTCTCAC	
1A0148.1	FOXA	1	7.89045	0.85	3268840335	BAR) pron	(2- noter	515	525	+		TATTTTCTCAC	
IA0148.4	FOXA	1	9.0415	0.85	152950241651 B		(2- noter	387	398	+		AAGAAAACAATT	
1A0148.4	FOXA	1	9.00937	0.85	52324437944 BARX		(2- noter	1474	1485	+		TTATAAACAATG	
IA0148.2	FOXA	1	7.7373	0.85	51925027617 BAR) pron		(2- noter	805	815	+		ТАТТТТСТСТА	
IA0148.1	FOXA	1	7.79244	0.85	1566601006	BAR	(2- noter	805	815	+		TATTTTCTCTA	

Figure S3 (A) Putative *BARX2* binding sites identified in the HK2 promoter with a calculated score of 10.759-5.83968 using Jaspar Database. (B) Putative FOXA1 binding sites identified in the *BARX2* promoter with a calculated score of 11.153-7.32982 using Jaspar Database.