

Figure S1. DNA methylation analysis of the FOXF1 gene upstream regulatory region. Pyrosequencing analysis indicates that the DNA methylation levels of FOXF1 were decreased in A549/DDP cells compared with A549, H1299 and 16HBE cells at 12 CpG sites. FOXF1, forkhead box F1.

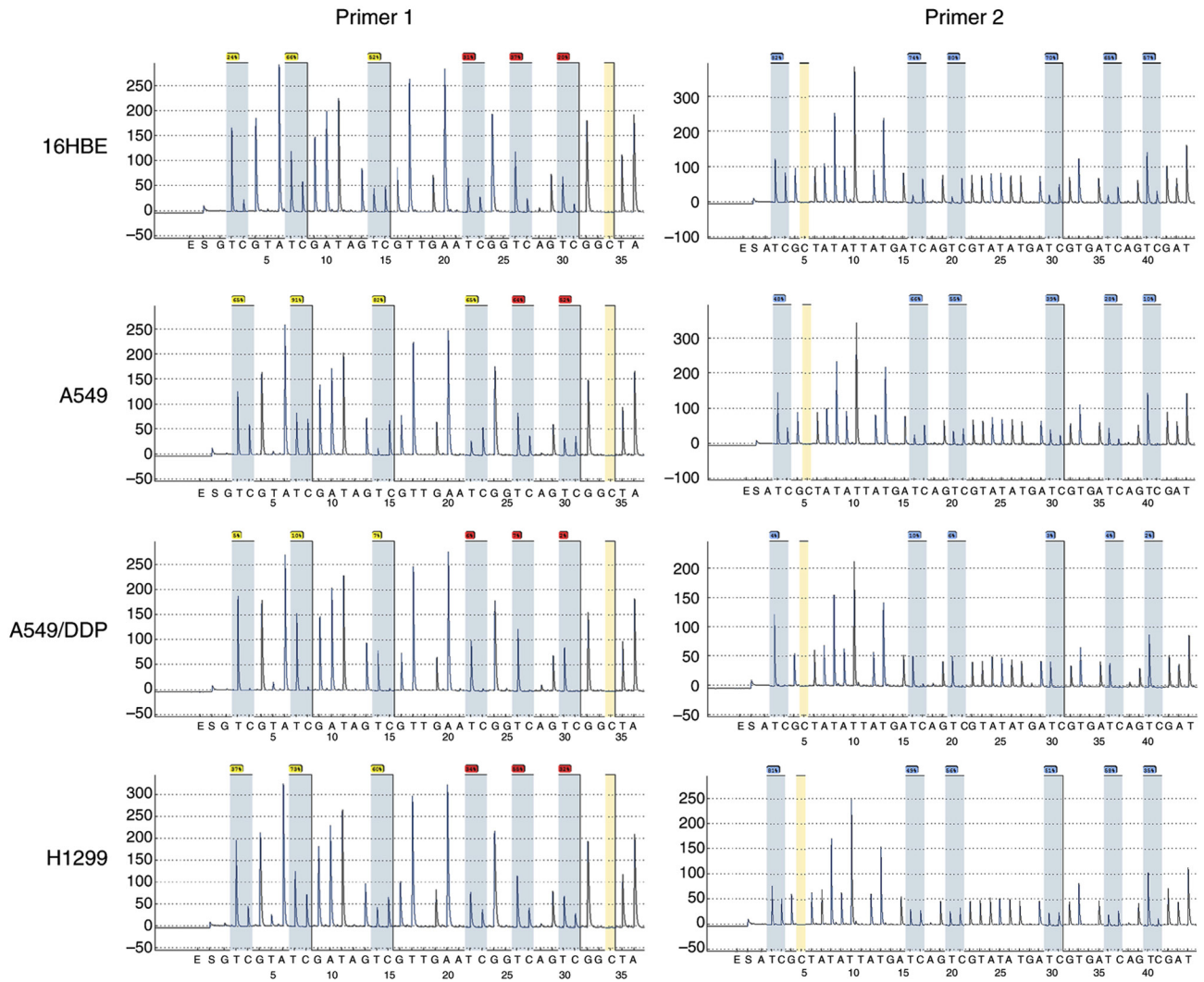


Figure S2. FOXF1 regulates cisplatin resistance by inhibiting cell apoptosis. Overexpression of FOXF1 decreased cisplatin-induced cell apoptosis in 16HBE cells (DDP treatment). The late apoptotic cells were shown in the bar graph. (n=3); *P<0.05 (FOXF1 plasmids compared to control plasmids). FOXF1, forkhead box F1.

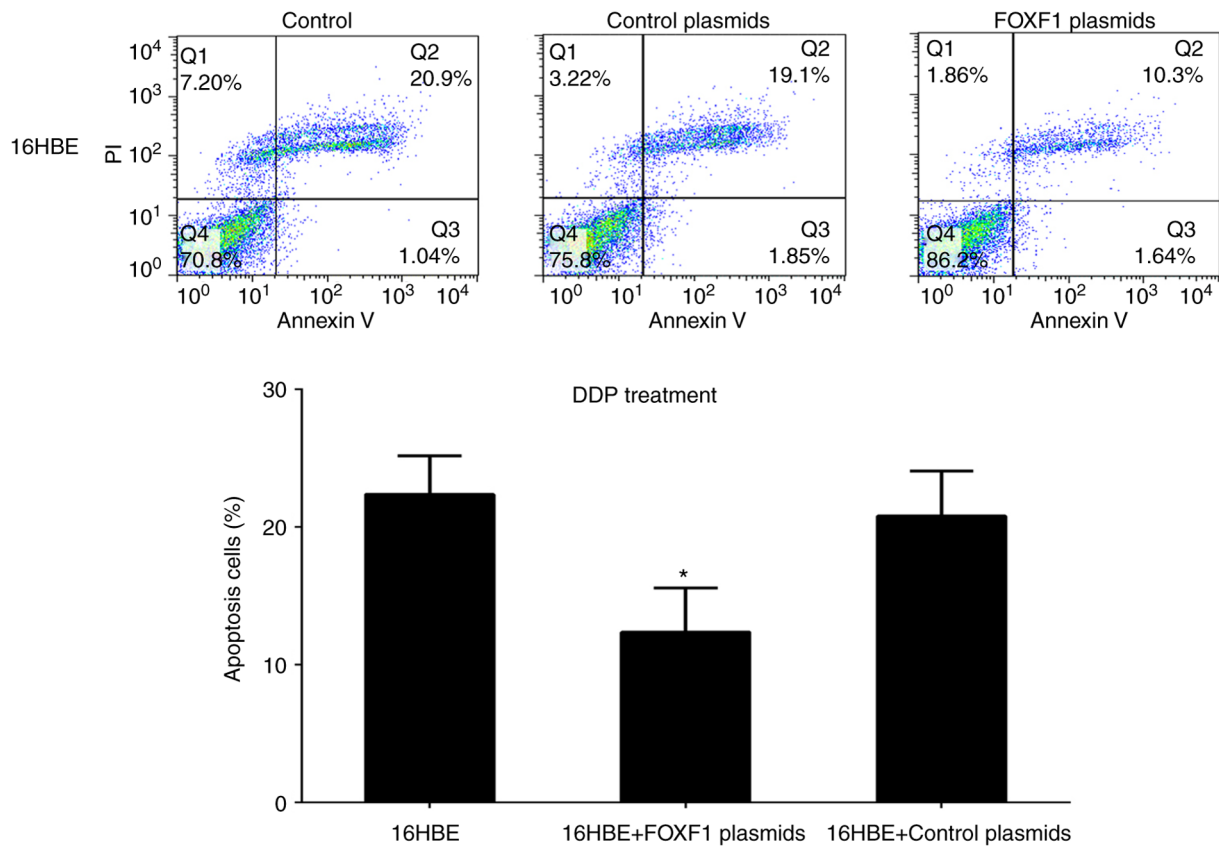


Figure S3. FOXF1 promotes the expressions of stem cell markers. Immunofluorescence detection of ALDH1 and OCT4 in 16HBE cells after transfection of FOXF1 plasmids. (n=3); *P<0.05 (FOXF1 plasmids compared to control plasmids). FOXF1, forkhead box F1.

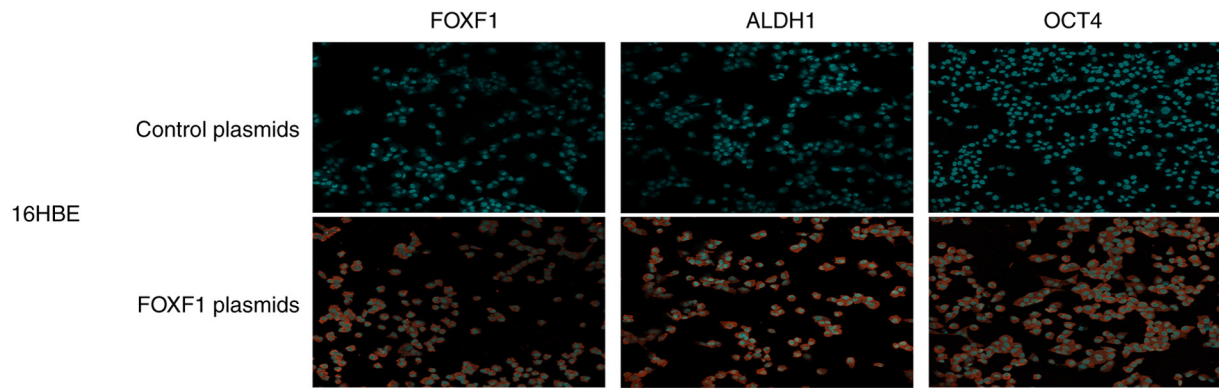


Table SI. Overall patient characteristics.

Clinicopathological factors	No. of patients
Total (n)	70
Sex, n (%)	
Male	53 (75.7%)
Female	17 (24.3%)
Age, years (range)	
Mean	57.2 (26-77)
Histological type, n (%)	
Adenocarcinoma	39 (55.7%)
Squamous carcinoma	31 (44.3%)
TNM clinical stage, n (%)	
III	43 (61.4%)
IV	27 (38.6%)

Table SII. Sequences of RT-PCR primers and pyrosequencing primers.

Primer	Sequence
FOXF1 F	5'-TACATCGCGCTCATCGTCAT-3'
FOXF1 R	5'-GTTTCATCATGCTGTACATGGGC-3'
ALDH1 F	5'-CAAGATCCAGGGCCGTACAA-3'
ALDH1 R	5'-CAGTGCAGGCCCTATCTTCC-3'
OCT4 F	5'-GCTGGATGTCAGGGCTCTTT-3'
OCT4 R	5'-AACCACACTCGGACCACATC-3'
GAPDH F	5'-TGACTTCAACAGCGACACCCA-3'
GAPDH R	5'-CACCTGTTGCTGTAGCCAAA-3'
Position 1 forward	5'-GTAAATTTTTTGTAGGGTTTTTGTATTT-3'
Position 1 reverse	5'-ACCCCCAACACACACTAA-3'
Position 1 sequencing	5'-TGTTAGGGTTTTTGTATTTT-3'
Position 2 forward	5'-GGAAGTTTAAGGTTTATAGGAGTTT-3'
Position 2 reverse	5'-CTACCAAACCTTTACCATCTC-3'
Position 2 sequencing	5'-AGGTTTATAGGAGTTTAAGT-3'

Table SIII. Different methylation of FOXF1 between A549/DDP cells and A549 cells.

Gene region	Average of β -values		Fold change (A549/DDP/A549)	P-value
	A549/DDP	A549		
TSS1500 region 1	0.137	0.502	0.273	0.001
TSS1500 region 2	0.094	0.087	1.073	0.381
1stExon	0.608	0.942	0.646	0.019
Gene body	0.774	0.929	0.833	0.086
3'UTR	0.667	0.906	0.736	0.025

Values in bold font indicate statistically significant differences ($P < 0.05$).

Table SIV. Pyrosequencing analysis of the methylation status of FOXF1 in normal lung cells (16HBE cells) and NSCLC cells (A549, A549/DDP and H1299 cells).

	Cells	Methylation percentages of position (%)											
		1	2	3	4	5	6	7	8	9	10	11	12
1	16HBE	24	66	52	31	37	20	82	74	80	70	65	57
2	A549	65	91	82	65	64	52	48	66	55	39	28	10
3	A549/DDP	5	10	7	6	7	2	4	10	6	3	4	2
4	H1299	37	73	60	34	55	32	81	49	56	51	58	35