Supplementary table

Table S1. The primers of shRNA.

Gene	Primer (5'→3')
Sh-TWIST1	TCGAGGTGTCTAAATGCATTCATATTTCAAGAGAAATATGAATGCATTTAGACACTTTTTGC
Sh-SLC12A5	GATCGGCAGCACAACACTGTGCTTGTTTCAAGAGAACAAGCACAGTGTTGTGCTGCTTTTTTC
Sh-ZFHX4	CCGGCGGACAGTTCTTGCCATACTTCTCGAG-AAGTATGGCAAGAACTGTCCGTTTTT

Table S2. Sequence synthesized for the luciferase reporter assay

Purpose	Primer（5'→3'）
ZFHX4 UTR-pcDNA3.1(+)-WT	UAACAUUUUUGUUUUGCUUGCCAUGAAUUUCAACUUCCACCACCCAGUGAAUUGAUUUAUAAAUUGCUAUGCUUUGCUGUUUUUCUGUUGCUGUGGAACUUAAAGAAUGUGAAAGCUGUCAAAGGGUAUUUUACGAAUCACUUUUGUGUUUGAUAUAGUAAAACAAUGUGAUUCAUUCCAAAGUAACAGAAGGUUAUUUGUAAGAAAGUUAAAGGCUUGUGAACAAAGAAAGCUAAG
ZFHX4 UTR-psiCHECK-2-WT	UAACAUUUUUGUUUUGCUUGCCAUGAAUUUCAACUUCCACCACCCAGUGAAUUGAUUUAUAAAUUGCUAUGCUUUGCUGUUUUUCUGUUGCUGUGGAACUUAAAGAAUGUGAAAGCUGUCAAAGGGUAUUUUACGAAUCACUUUUGUGUUUGAUAUAGUAAAACAAUGUGAUUCAUUCCAAAGUAACAGAAGGUUAUUUGUAAGAAAGUUAAAGGCUUGUGAACAAAGAAAGCUAAG
TWIST1-514a-3p- psiCHECK-2-WT	UCAUGGGGAGUCUCCUUGUUCACUAGCUUGGUCUGACUAUUUGAUAUUUCAGAUUUCUUAAGGUACACCAGGAUGGAGCUGGCCAUGACUGCUGAGAAGGUAAUUGUUAUCUUAACUCUGGAUAUAAGAACUAGAUGUGUCAAAGAAAGGUAGCUCUCCAUUUGCCUUUCUUUUAUUUCUUUCUUUUUUUAAUGUGUUUUGCUUUUUCUACUUUUUUCAUAAGGGGAAACACUUUGGA
TWIST1-194-3p-psiCHECK-2-WT	UCUGCAUUCUGAUAGAAGUCUGAACAGUUGUUUGUGUUUUUUUUUUUUUUUUUUUUGACGAAGAAUGUUUUUAUUUUUAUUUUUUUUCAUGCAUGCAUUCUCAAGAGGUCGUGCCAAUCAGCCACUGAAAGGAAAGGCAUCACUAUGGACUUUCUCUAUUUUAAAAUGGUAACAAUCAGAGGAACUAUAAGAACACCUUUAGAAAUAAAAAUACUGGGAUCAAACUGGCCUGCAAAACCAU
TWIST1-UTR-pcDNA3.1(+)-WT	GUCGUGCCAAUCAGCCACUGAAAGGAAAGGCAUCACUAUGGACUUUCUCUAUUUUAAAAUGGUAACAAUCAGAGGAACUAUAAGAACACCUUUAGAAAUAAAAAUACUGGGAUCAAACUGGCCUGCAAAACCAUAGUC

	AGUUAAUUCUUUUUUUCAUCCUUCCUCUGAGGGGAAAAACAAAAAA
	AAACUUAAAAUACAAAAAACAACAUUCUAUUUAUUUAUUGAGGACC
	CAUGGUAAAAUGCAAAUAGAUCCGGUGUCUAAAUGCAUUCAUAUUU
	UUAUGAUUGUUUUGUAAAUAUCUUUGUAUAUUUUUCUGCAAUAAAU
	AAAUAUAAAAAAUUUAGAGAACCUUAGAGUUUGGUCUAUAUUUUUA
	AAACUAAAGAUUAAGUUGGUGGUAAAUACCUGCUUGUUUAAUUCUA
	GAGGCACCCAGGAGGGAGGGGGCACUAAUAUAAACAAAGCAAUGAA
	AAACUCAAAUAAAGCAGCUACUGACAGGCACAAGCAUGUUAUUUUA
	AAAGACAGCUUUAUUAUUAUUCCAGUUUGGUAUUCAGAGGGCUUAG
	UAGCAUCUCUUCAUCUCUUACUGUCUCCAAACAGCAAAAACUUAAC
	AAAUUUGCUAUUCACCUCCUUUUUAUUUUGAGGUGAUAGUUGUUAA
	AACAGUUAAAUGCAAAUAAGGAGUAUAAAACCUGCACUAUUCUCUU
	AUUAUGUACUUUUUGUCAUGCUCUUCACUCUUUGUGAAGAUUCCUA
	AAUAACAGGUUCUUUUCAGUGAAAACAUAUAUAGUGUUAUGAAAAU
	UAUCUAUACACAUAUAGAAAUGACCAGAAAUGCAUAGAAAAUAAAG
	UGUAAAUCAGCAGAAAAGAGAAAAUUCCAUUUGUAAUUUGCUUAAC
	UCUUCUCUCAUGAAUGAAUAUGACGUUUAGUGUCAUGUUUGCUAAA
	AAGAAAAGUUAAUAUAUCGCUAUAUUGACGCUAUUUGGGAAGCAUC
	AUUCUUUUUUUAUGUGUAAAGAAUAUAAGGCUGUGGAAUAAUAUAC
	AAAAAUGAAGAUCCUGAACUUUCAGUAUCCUUGGUAACUGGAUAUA
	AUCACAGUUGGAAUAAUCACAGUUUUAAAUACUACAGCUAUGCAGU
	UACUAGUCAGUUCAUUAGCGUUAUAUUUUAUCCAGAAUUACACAGA
	AUUUUUCCCUGGUGAGAUCAUACAUACACACACACACAAUCUGAAG
	UUAAGUAAAACAAUAAUGGGCAAACGGGGGUGUUUGAUUUUUUAUU
	UCAUUCUGAUUAUUUGUGGGCCAGGAACAUACUGGCUUUUAUAAUU
	AACAUUAUUAGAAAUAGUGAUUUUGUAUCAGUUCUUAGCUUUUACA
	UCCGAUUUACUGCAUCCAAGUGUAACUGGGGAAGCUUUCAAACUGG
	AAAAUCAUUUUGGUGCCUGUUUCAAACAAUGACUAAUAAGUGUAUG
	UAUCCAAACAGAUCGUUUUUGCUUAGCUCAGGUCAAAGUUAUUUUA
	UUGCCUGAAGGUUUUUGUUUUCUUCUUCACACAGCACCAUAGAAUG
	CUGACAUAAGAAUAUUAUGAAUUUAUUGUUAAUAUUUCAGACUAUA
	UUUUACAAUAAGGAGUCUGCAGCAUUGACUUGUAUUAAAAGGUUAG
	UGAAAGCUUGGAGAAGUAUUAUUAUUUUUUUAUUUUGAUAGUCCUU
	UAGCAAAUAACAGAAAGGGAAGGCGAUUAUGUGUUGGUGUAUUUGU
	UGGGGGUGUGUGUGUGUUGUGUGUAAGGCAGGUUAGAAAUGUAAGG
	AGGGUGCCCUAGGAGGAUGAGGGAAAGCAUUAGGUUGGUAAUUCUU
	UUUUGUAUGUAUCAUCCAACCACCUCCAUUUUCUAAAUUAUUCAUG
	GGGAGUCUCCUUGUUCACUAGCUUGGUCUGACUAUUUGAUAUUUCA
	GAUUUCUUAAGGUACACCAGGAUGGAGCUGGCCAUGACUGCUGAGA
	AGGUAAUUGUUAUCUUAACUCUGGAUAUAAGAACUAGAUGUGUCAA
	AGAAAGGUAGCUCUCCAUUUGCCUUUCUUUUAUUUCUUUCUUUUUU
	UAAUGUGUUUUGCUUUUUCUACUUUUUUCAUAAGGGGA
SLC12A5 UTR-pcDNA3.1(+)-	UCGCUGGGGAGCAGGCGUCUCUCCUCAGUCGGCUUGUCGCCUGCUCC CCGUAUCCCAUGGCUCCUCGCCAAAGACUGAAAUUGUGGAGCUGGA

WT	GGGCGCCCCCUCCCCGGAGUUUCCUCCCUGGGACAAGUGAGGGAGGA GGGGGCCGAUUCUGGUUUAGGGGCCGGACCCACUGAGAGGCCCCAG AGCCGCCCGUGAUGUUCCUCCCCCGUCCCCAUCUGGCAGCUCCUGUC UCGCCUGAGGGACCCAGCCGCCUUCUCCGUGCUCUGGGGCCGGGCCU CGCUGCUUAGCAGCGGCCUCUAGCUCCGUCUCCCGGGGACCUGGGCC UGAGGGAGGGCUGGAGUCGCACGCGCUUUGUCCUUAGCGCCUGUCU GCUCUCCUCUAACUAGGACCCAGGGCCUUUGGCUUCCCCAGCUCAUC CUUGGCCCUUCCGCUCCACCAGCCUGGUCUGAGGCGUGCUCUGUCCU UAGAGAAGGCGCGGUGGCCGGGUUCCCUUCCCCCUAGGGCACAUUAC UAAGGGGGUCAGGCACUGCAUGCUCGUUCCAGCACCAUCUGGGACU GGGUACAGUACCUCCAGCCCCAGGGCCCUGACCUGCGCACCUAGCUU GACAUCUCACGCACCUCCCAGAGCUGGCGCCACUGAGUAAUCCGGAC CUCACCACCUCUUUUCCUUUGAGCCCAAGGCAGAGCUGGAGCUGGCG CCACCCAGACAGCGUCAGGUGUGGCUGGGGUAGGUUUGGAGGUCUG CCAGUUACACCAAGUCCCCUCUGAGAUUCGAUCAGGGGACUGGAUA GAUUCUUUCAGGUACUCAAUCAGGAAGCU
SLC12A5 UTR-psiCHECK-2-WT	UCGCUGGGGAGCAGGCGUCUCUCCUCAGUCGGCUUGUCGCCUGCUCC CCGUAUCCCAUGGCUCCUCGCCAAAGACUGAAAUUGUGGAGCUGGA GGGCGCCCCCUCCCCGGAGUUUCCUCCCUGGGACAAGUGAGGGAGGA GGGGGCCGAUUCUGGUUUAGGGGCCGGACCCACUGAGAGGCCCCAG AGCCGCCCGUGAUGUUCCUCCCCCGUCCCCAUCUGGCAGCUCCUGUC UCGCCUGAGGGACCCAGCCGCCUUCUCCGUGCUCUGGGGCCGGGCCU CGCUGCUUAGCAGCGGCCUCUAGCUCCGUCUCCCGGGGACCUGGGCC UGAGGGAGGGCUGGAGUCGCACGCGCUUUGUCCUUAGCGCCUGUCU GCUCUCCUCUAACUAGGACCCAGGGCCUUUGGCUUCCCCAGCUCAUC CUUGGCCCUUCCGCUCCACCAGCCUGGUCUGAGGCGUGCUCUGUCCU UAGAGAAGGCGCGGUGGCCGGGUUCCCUUCCCCCUAGGGCACAUUAC UAAGGGGGUCAGGCACUGCAUGCUCGUUCCAGCACCAUCUGGGACU GGGUACAGUACCUCCAGCCCCAGGGCCCUGACCUGCGCACCUAGCUU GACAUCUCACGCACCUCCCAGAGCUGGCGCCACUGAGUAAUCCGGAC CUCACCACCUCUUUUCCUUUGAGCCCAAGGCAGAGCUGGAGCUGGCG CCACCCAGACAGCGUCAGGUGUGGCUGGGGUAGGUUUGGAGGUCUG CCAGUUACACCAAGUCCCCUCUGAGAUUCGAUCAGGGGACUGGAUA GAUUCUUUCAGGUACUCAAUCAGGAAGCU

The labeled red nucleotides were changed into its complementary nucleotides in the mutant cohort.

Table S3. The primers of RT-PCR.

Gene	Primer（5'→3'）
TWIST1-mRNA-F	GGACAAGCTGAGCAAGATTCA
TWIST1-mRNA-R	CGGAGAAGGCGTAGCTGAG
TWIST1-3'UTR specific-F	AGCCACTGAAAGGAAAGGCAT

TWIST1-3’UTR specific-R	AGCAGGTATTTACCACCAACTTA
ZFHX4-mRNA-F	GGAGAACTGTGGGCAGAGAG
ZFHX4-mRNA-R	TCTCTGCTCCTCCTGTTC
ZFHX4-3’UTR specific-F	CAACTTCCACCACCCAGTGA
ZFHX4-3’UTR specific-R	ACCTTCTGTTACTTTGGAATGAATC
SLC12A5-mRNA-F	GCAGGAGCCATGTACATCCT
SLC12A5-mRNA-R	CCATGCAGGTGAGCACACA
SLC12A5-3’UTR specific-F	CTCTCCTCAGTCGGCTTGTC
SLC12A5-3’UTR specific-F	GGCCCTGGGTCCTAGTTAGA

Table S4. Clinicopathologic parameters and expression of TWIST1/SLC12A5/ZFHX4 in 92 of LUAD patients

Variable	Cases(n)	%
Ages		
＜60	51	55.4
≥60	41	44.6
Sex		
Male	67	72.8
Female	25	
Degree of differation		
Well	25	27.2
Moderate	20	21.7
Poor	47	51.1
Tumor size		
≤3cm	34	37.0
＞3cm	58	63.0
Lymph node metastasis		
No	50	54.3
Yes	42	45.6
Pleural invasion		
No	67	72.8
Yes	25	34.7
TNM staging		
I	34	37.0
II	25	26.9
III	33	35.9
TWIST1 expression		
High	71	77.2
Low	21	22.8
SLC12A5 expression		
High	61	66.3
Low	31	33.7
ZFHX4 expression		
High	63	68.5
Low	29	31.5

Expression of TWIST/SLC12A4/ZFHX4 was normalized to paired normal tissue. High expression was defined as tumor/non-tumor>1.

Supplementary figures

Figure S1. Expression profile of TWIST1 in LUAD cancer cell lines by RT-PCR analysis

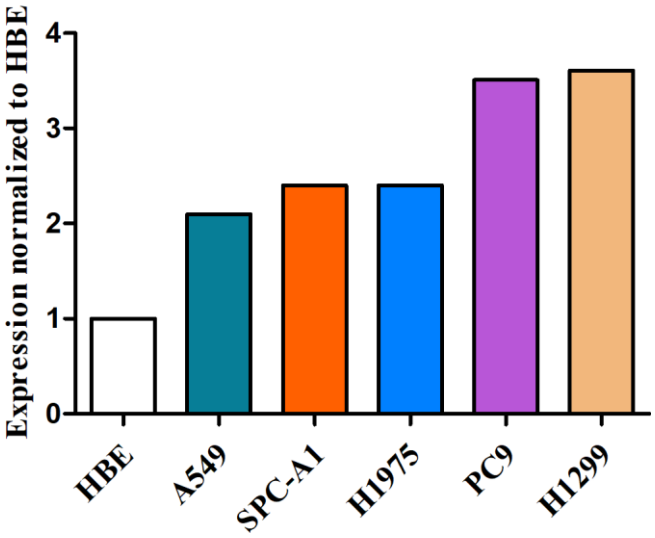

Figure S2. Overexpression of specific 3'UTR regions confirmed by q-PCR

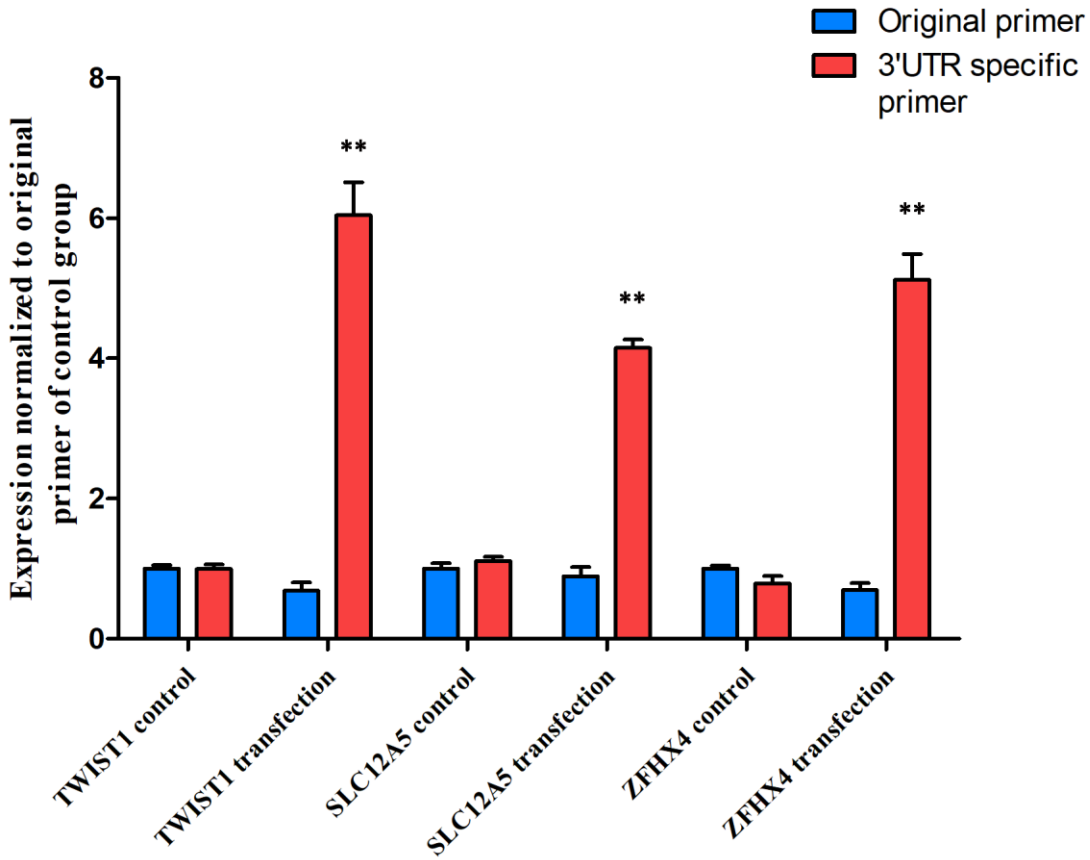

Figure S3. Biological functions of the cDNAs of TWIST1 ceRNET in A549. (A-B) Effects of TWIST1-ceRNET cDNA on proliferative capacity evaluated by 5-ethynyl-2'-deoxyuridine (EdU) proliferation assay (A) and Cell Counting Kit-8 (CCK-8) assay (B). (C) Effects of TWIST1-ceRNET cDNA on migration and invasion capacity evaluated by transwell assay and matrigel assay All data are mean ± s.d. The data statistical significances were assessed by Student's t-test compared to the NC group. *P < 0.05, **P < 0.001.

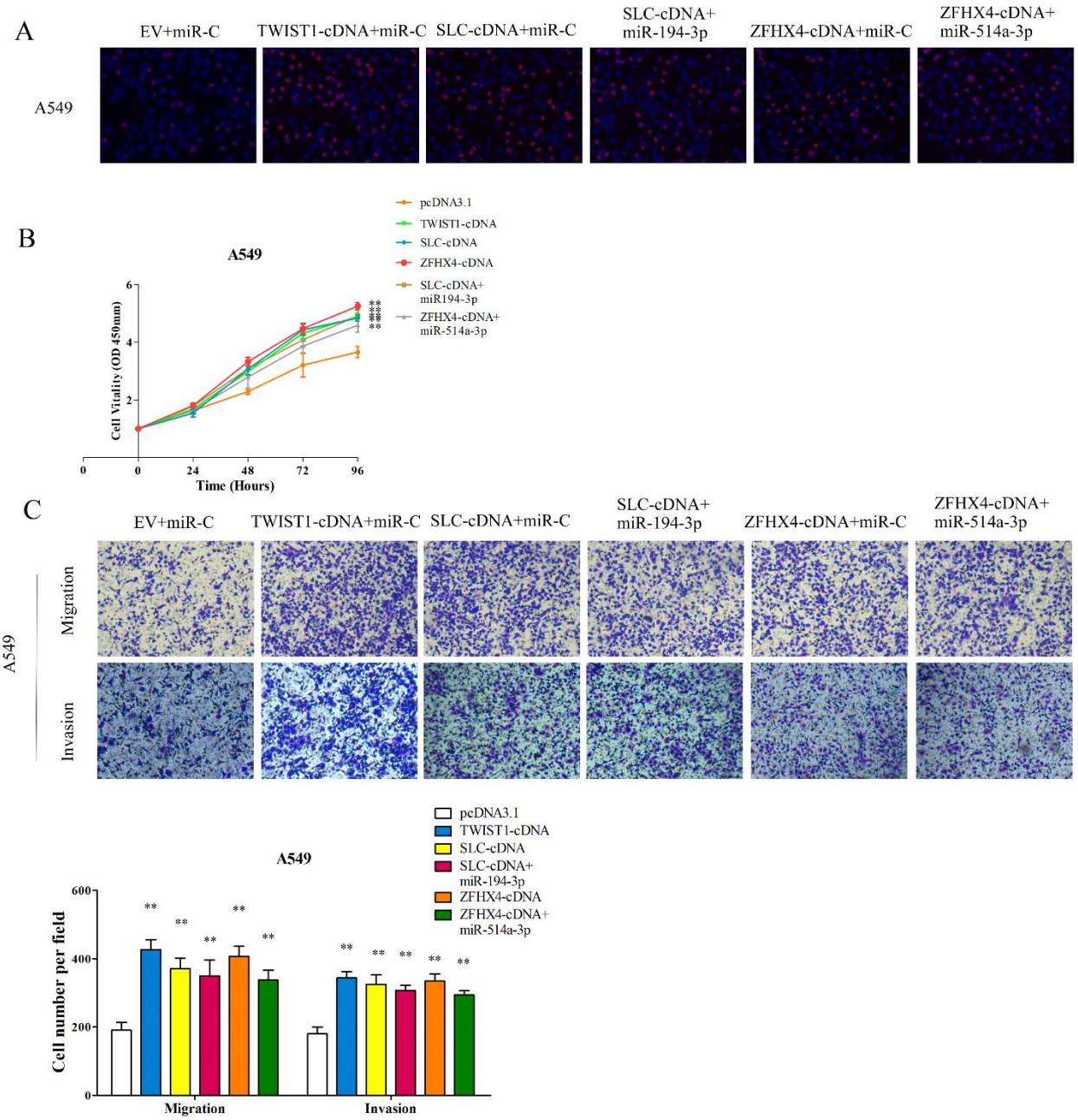

Figure S4. Q-PCR results of the RIP based on Ago2 showed that SLC12A5 and ZFHX4 can compete with the TWIST1 transcript for the binding of miRNAs in H1299 cells

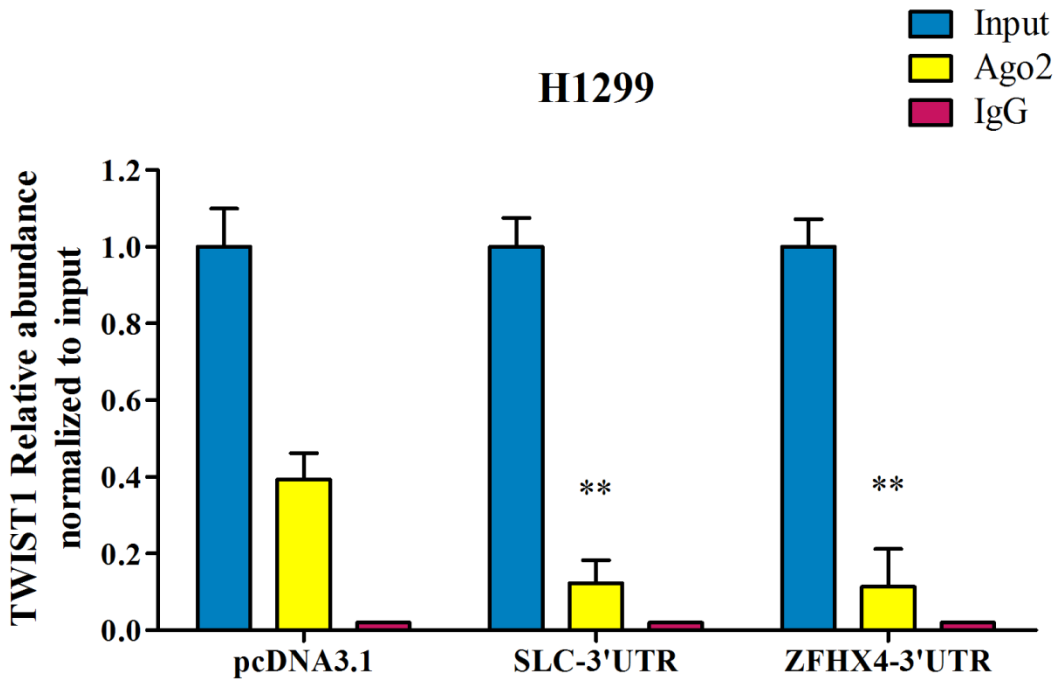

Figure S5. Q-PCR results of the RIP based on Ago2 showed that SLC12A5 and ZFHX4 can compete with the TWIST1 transcript for the binding of miRNAs in A549 cells