Supplementary materials

Figure S1 TUSC7 overexpression in TNBC decreases chemoresistance of breast cancer cells

Dose-response analyses of carboplatin (A) or paclitaxel (B) effect in breast cancer cells with TUSC7 overexpression. MDA-MB-468 transfected with empty vector or TUSC7 overexpression vector were plated in 96-well plates and treated with the indicated doses of carboplatin or paclitaxel for 72 h. The viable cell number was expressed as a percentage of corresponding vehicle controls.

Figure S2 The correlation between the expression level of TUSC7 and miR-1244-3P

A. The expression level of TUSC7 was positively correlated with the expression level of miR-1244-3P ($R=0.86$, $P<0.001$).
B. Compared with the peritumor normal tissue, the expression level of miR-1244-3P was significantly lower in the tumor tissue ($P<0.001$).

Figure S3 The binding sites with miR-1224-3P in TUSC7.

Figure S4 The potentially involved signaling pathway of TUSC7

Gene expression arrays of TUSC7 shRNA downregulated MDA-MB-231 breast cancer cells and scrambled sequences control. (a) Biological pathways in TUSC7 knockdown MDA-MB-231 breast cancer cells using Pathway Studio software. (b) Genes involved in the affected pathways in TUSC7 knockdown MDA-MB-231 breast cancer cells were validated by RT-PCR.

Figure S1

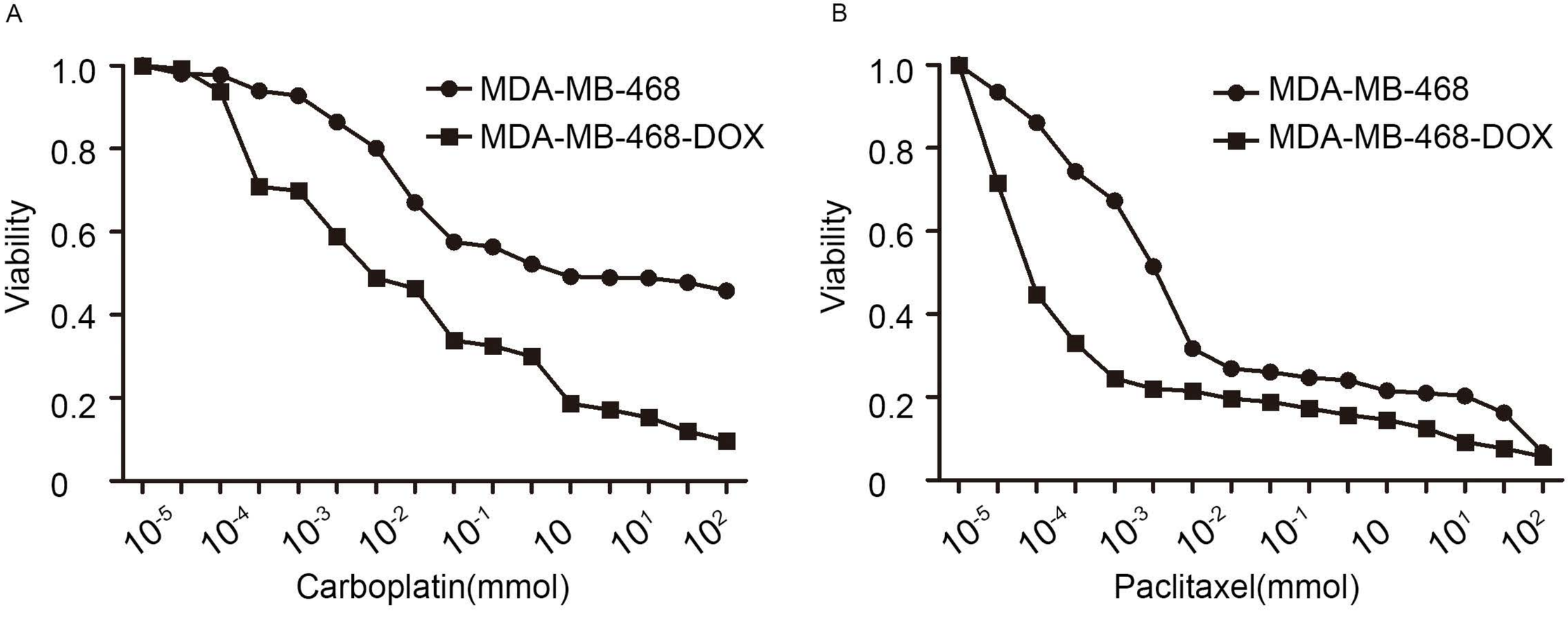

A
1.0
0.8
0.6
0.4
0.2
0
Viability
MDA-MB-468
MDA-MB-468-DOX
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10
10^{1}
10^{2}
Carboplatin(mmol)
B
1.0
0.8
0.6
0.4
0.2
0
Viability
MDA-MB-468
MDA-MB-468-DOX
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10
10^{1}
10^{2}
Paclitaxel(mmol)

Figure S2

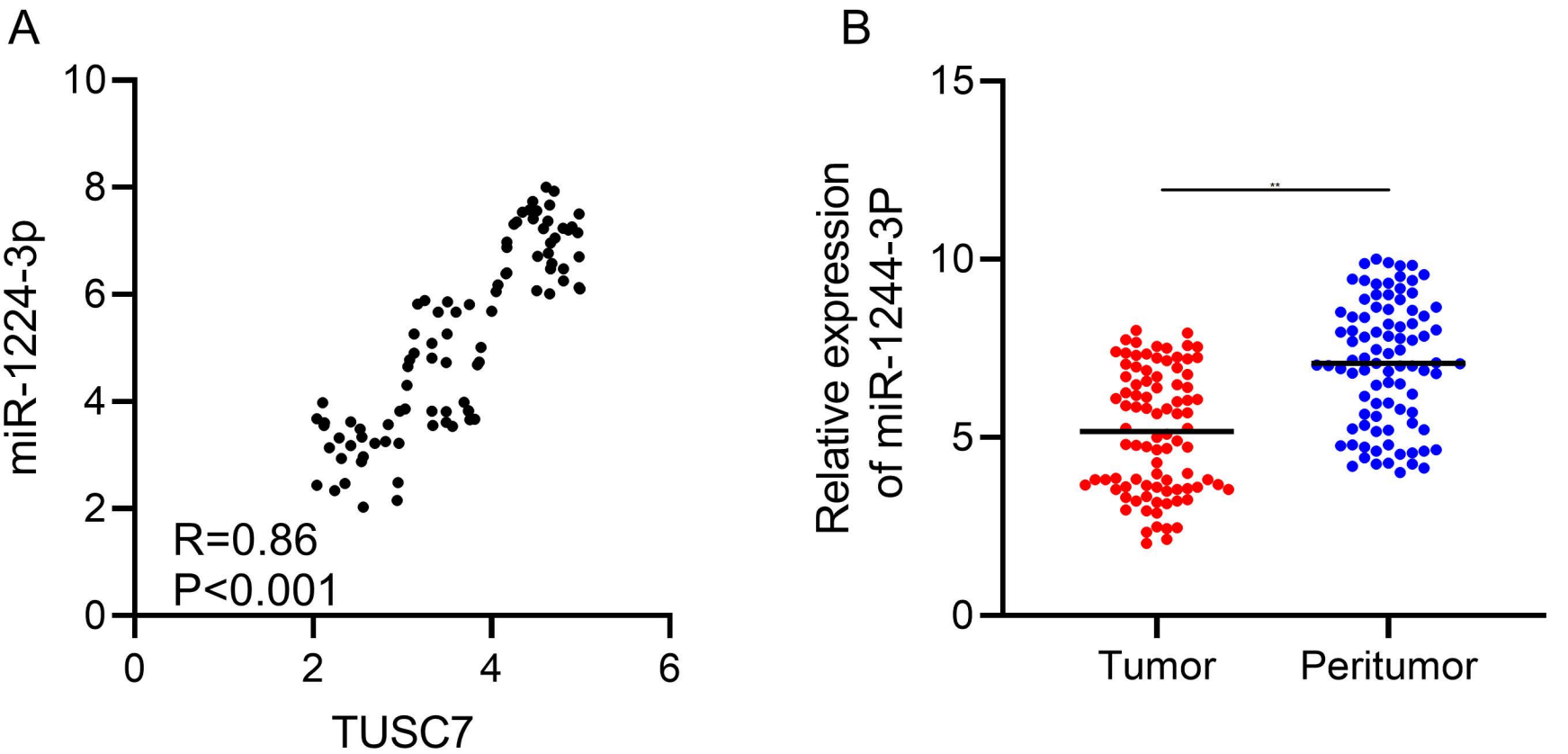

A
miR-1224-3p
TUSC7
R=0.86
P<0.001
0
2
4
6
8
10
B
Relative expression of miR-1244-3P
**
Tumor
Peritumor
0
5
10
15

Figure S3

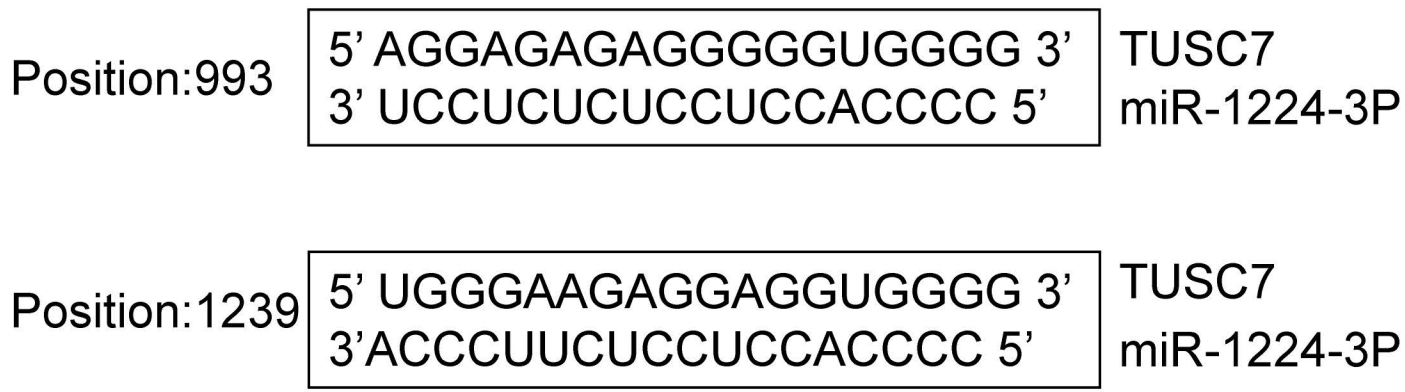
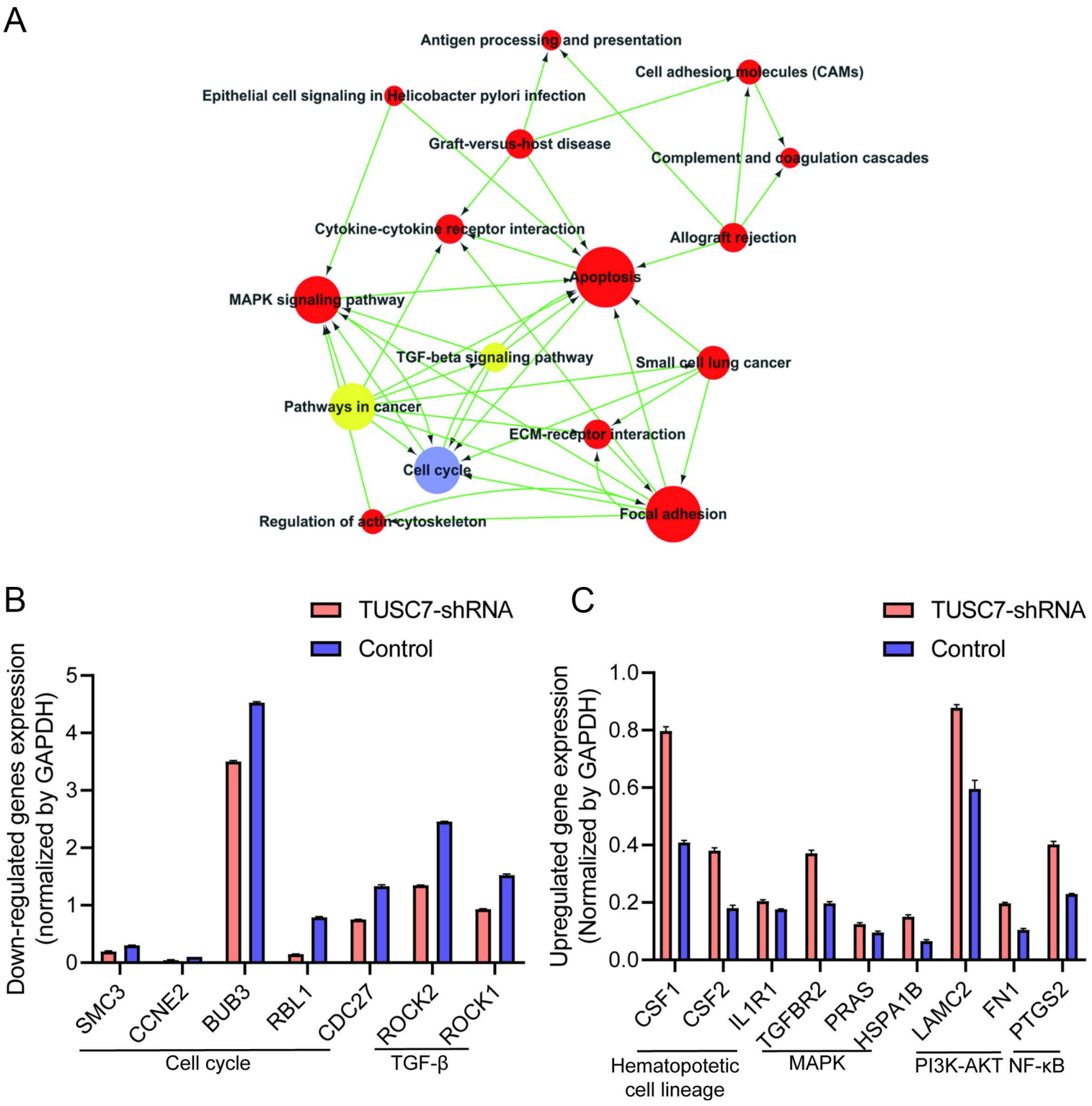

Position:993
5’ AGGAGAGAGGGGGUGGGG 3’
3’ UCCUCUCUCCUCCACCCC 5’
TUSC7
miR-1224-3P
Position:1239
5’ UGGGAAGAGGAGGUGGGG 3’
3’ACCCUUCUCCUCCACCCC 5’
TUSC7
miR-1224-3P

Figure S4


A
Antigen processing and presentation
Cell adhesion molecules (CAMs)
Epithelial cell signaling in Helicobacter pylori infection
Graft-versus-host disease
Complement and coagulation cascades
Cytokine-cytokine receptor interaction
Allograft rejection
Apoptosis
MAPK signaling pathway
TGF-beta signaling pathway
Small cell lung cancer
Pathways in cancer
ECM-receptor interaction
Cell cycle
Regulation of actin cytoskeleton
Focal adhesion
B
TUSC7-shRNA
Control
Down-regulated genes expression (normalized by GAPDH)
0
1
2
3
4
5
SMC3
CCNE2
BUB3
RBL1
CDC27
ROCK2
ROCK1
Cell cycle
TGF-β
C
TUSC7-shRNA
Control
Upregulated gene expression (Normalized by GAPDH)
0.0
0.2
0.4
0.6
0.8
1.0
CSF1
CSF2
IL1R1
TGFBR2
PRAS
HSPA1B
LAMC2
FN1
PTGS2
Hematopotetic cell lineage
MAPK
PI3K-AKT
NF-κB

Supplementary Materials

Cell culture and real-time polymerase chain reaction

Human breast cancer cell lines HCC1937 (ER-, PR-, HER2-), MDA-MB-468 (ER-, PR-, HER2-), MDA-MB-231 (ER-, PR-, HER2-), MDA-MB-453(ER-，HER2+), T47D (ER+, PR+, HER2-), SK-BR3(ER-, PR-, HER-2+), MCF7 (ER+, PR+, HER2-) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). All cells were cultured in DMEM media containing 10% FBS with 1% penicillin/streptomycin (Invitrogen, USA) in a moist 5% CO2 incubator at 37°C.

Total RNAs were extracted from tumors, their normal tissues or cells using Trizol (Invitrogen). RT (reverse transcription) reactions were performed using a PrimeScript® RT reagent kit (Takara, Dalian, China); reactions were incubated for 30 min at 37°C, 5 s at 85°C, and then maintained at 4°C. For real-time PCR, 1 μLof diluted RT products were mixed with 10 μL of 2×SYBR® Premix Ex Taq™ (Takara), 0.6-μL forward and reverse primers (10 μmol/L), and 8.4-μL nuclease-free water in a final volume of 20 μL according to the manufacturer’s instructions. The primers for TUSC7 were 5′-TGTGCCTGTTTGACCTCTGA-3′ (forward) and 5′-AGGAAGGATAAAAGACCGACCA-3′ (reverse). The primers for GAPDH were 5′-CGGAGTCAACGGATTTGGTCGTAT-3′ (forward) and 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′ (reverse). All reactions were run on the Eppendorf Mastercycler EP Gradient S (Eppendorf, Germany) using the following conditions: 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, and 60°C for 30 s. Real-time PCR was performed in triplicate, with no-template controls. Amplification of the appropriate product was confirmed by melting curve analysis following amplification. Relative expression of TUSC7 was worked out by the comparative threshold (CT) (2−ΔΔCT) method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control to normalize the data.

Vector construction and lentivirus packaging

The wild-type TUSC7 ORF (open reading frame) was isolated from human MDA-MB-231 breast cancer cell cDNA using PCR (forward primer 5′-ATCCAGCCTCTCGAGGGGGTACCAAAGTCCACTCT-3′ and reverse primer 5′-

CATGGTGGCGAATTCTTTTTCGTGAGTACACAATAGTC-3′). The fragment was cloned into the pMT38 lentiviral vector via an Xho I and EcoR I Sunbio company (Shanghai). The pMT38-TUSC7 over-expression plasmid was packaged into mature lentivirus using 293T cells; at 48 hours following the transfection, the lentivirus-containing supernatant was pulled out and went through a 0.45-mm long filter. The titer in these filters was calculated by measuring the HIV (human immunodeficiency virus) p24 gag antigen with an ELISA kit (Perkin-Elmer Life Science, Shanghai). The lentivirus was then transduced into MDA-MB-468 cells with polybrene (8 μg/ml). Controlled expression of TUSC7 was via co-expression of a tetracycline/doxycycline (DOX) responsive repressor (Tet on TUSC7-EGFP-3FLAG-Puro) from Sunbio company (Shanghai). (TUSC7-empty vector (Scramble) or TUSC7-DOX-overexpressing (TUSC Over) MDA-MB-468 cells)

ShRNAs for human TUSC7 were prepared in our laboratory and made in pLKO.1-puro vectors (Sunbio Company, Shanghai)). Three shRNA plasmids were established against diversified TUSC7 targets, encompassing a scrambled sequence, relatively negative. The three shRNA plasmids (Invitrogen, USA) were checked by sequencing. After infection with lentivirus, cells were detected for excessive expressions or decrease of the TUSC7 gene. As for this, one construct, with ≥85% knockdown efficiency, was used to further studies. A scrambled sequence was constructed as a negative control. The shRNA sequences prepared for further research include TUSC7 shRNA, 5′-CCGGGGTAGAGATAAGTCTGACATTCAAGAGATGTCAGACTTATCTCTAC CTTTTTTG-3′ and Scramble shRNA, 5’-CCGGCCTAAGGT TAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTG-3’. (TUSC7-scrambled shRNA control MDA-MB-231 cells: Scramble shRNA), TUSC7-shRNA MDA-MB-231 cells: TUSC shRNA).

The wild-type and mutant p53 vectors were obtained from Sunbio company, Shanghai. MDA-MB-468 breast cancer cells were transfected with wildtype/mutant p53 vector with various concentrations of doxorubicin (doxo), a known DNA damaging agent. Therefore, we then chose doxo at 1mg/ml for 24h to further confirm the specific effect of p53 on TUSC7 expression.

CCK8 (Cell Counting Kit-8) assays, migration, invasion, and cell cycle analysis

Cells (2×10^4) were cultivated in microtiter containers with an amount of 110 µl complete medium per well in 37°C, 5% CO_2 for 5 days. After the period of incubating cells, 10 µl of the CCK8 labeling reagent (0.5 mg/ml) (The Cell Counting Kit-8 assay; Dojindo Laboratories, Kumamoto, Japan) was added to each microtiter. Then all the cells were tested by an enzyme-labeled meter. The process of absorbing was measured at 450 nm. All experiments were performed three times in triplicate.

In vitro cell migration and invasion assays were conducted as mentioned before [18]. Images of three random fields (10×) were noticed from each membrane, and the migratory or invasive cells were counted. Triplicate assays were performed in each experimental time.

For transwell migration assays, cells were plated at 1×10^5 cells/well in the chamber on the top of a membrane (8 µm, BD Biosciences, Shanghai) with fibronectin (100 µg/ml) and 2.5% BSA (albumin from bovine serum). Cells were stored for the specified lengths of time under standard culture conditions. Tumor cells remaining on the upper side of the membrane were put away and cells that had migrated to the underside were stuck and stained in 0.1% crystal violet for half an hour, rinsed in PBS (phosphate buffer saline), and then subjected to microscopic inspection. Five fields per insert were shot. Then, the cell numbers for each field were count and the average numbers were extracted for further analysis.

The *in vitro* invasion studies were performed using a BD Bio-Coat Matrigel invasion assay system (Becton Dickinson Labware, Franklin Lakes, NJ, USA). Cells were seeded at 5×10^4 cells/well on 8-µm pore-size transwells (Corning, NY, USA). The lower chamber was filled with DMEM containing 10% FBS (fetal bovine serum). After 24 hours, the number of cells in the bottom compartment of the filter membranes was confirmed. Each experiment was performed in triplicate.

Treated cells were put in ethyl alcohol (70%) and dyed in propidium iodide (10 mg/ml, MP Biomedicals, USA), RNAse (10 kU/ml, Sigma-Aldrich, USA), and NP40 (0.01%, Sigma-Aldrich) overnight at 48°C in darkness. Then the cells were analyzed by flow cytometry (FAC Station, BD Biosciences) and FlowJo (TreeStar Inc., Ashland, OR).

MiRNA microarray and mRNA microarray

Total RNA was extracted using a mirVana TMmiRNA Isolation Kit (Ambion, Austin, TX) according to the manufacturer's instructions. The BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA) was used to check the quality of the purified RNA. The human miRNA V18.0 microarray chips (Agilent Technology, Austin, TX), containing probes for the complete Sanger miRBase 10.0, were used to screen the samples of different groups. Briefly, RNA samples were labeled and hybridized with miRNA complementary probes printed on a glass slide. After hybridizing them, the slides were scanned by an Agilent Microarray Scanner (Agilent Technologies, Santa Clara, CA) using the default settings and analyzed using Gene Spring Software 11.0 (Agilent Technologies). For mRNA microarray, HTA2.0 mRNA microarrays Chips (Affymetrix, Santa Clara, CA) were used according to the manufacturer’s instructions to identify the differences between the samples. The slides were scanned by the GeneChip Scanner 3000 and Command Console Software 3.1 (Affymetrix). The miRNA microarray and mRNA microarray were performed by the Shanghai Qiming Corporation (Shanghai, China). Gene ontology (GO) analysis and the KEGG pathway were performed for the intersected genes to identify the relevant pathway.

Luciferase reporter assays

The 3'-UTR of TUSC7 (NR__015391.1) was amplified from human genomic DNA using appropriate primers and subcloned into the region directly downstream of the Renilla gene stop codon in the psiCHECK2 vector (Promega, Madison, WI) to generate psiCHECK2-TUSC7-3'-UTR constructs. With appropriate primers, PCR amplification of the 3'UTR sequence of TUSC7 generated a series of mutant psiCHECK2-TUSC7-3'-UTR reporter vectors. miR-1224-3P、miR-7113-5p、miR-920、miR-637、miR-608、hsa-miR-6770-5p、hsa-miR-1207-5p、hsa-miR-4451 mimic duplexes were synthesized by Gene Pharma (Shanghai, China). HEK 293T cells were transfected with a mixture of reporter constructs and miR duplexes using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). After 48 hours of incubation, firefly, and Renillaluciferase activities were measured using the dual-luciferase reporter assay system (Promega, Madison, WI) from the cell lysates.

A fragment of TUSC7 3′UTR including the binding site of Mir-1224-3P.

>TUSC7 NR_015391.1

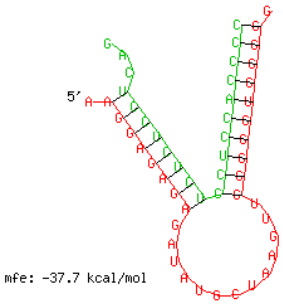
GGGGTACCAAAGTCCACTCTGAGCTTTTCCTCTGGGAACAGATCCCAGATT
ATTCCAGACTTAAGACATGGAGATATATCTTGCTAGAGGTAGGAAGATGATA
AGATCAGTTTCTATAGGTAGAGGATAATGTGTCCTAAAATGTAGGTAGAGAT
AAGTCTGACAGTGGTTTAGACAAAAAGGGCCTCTGCTAATGGTGGAGAGA
TCAAGGAAGAAAAGAGCAACGTTAAAGTGAGGCTGTACCCACAGATCATG
CTCAAGCAAATTCGAACCGTGAGCGCATTTCTCTTAAACAATGAACACTTA
GAATAAAAATTAAAATTTCCCACAAATTTCTAATTTTAAAATAGTTTCCCAA
GATTTCAGTGGTATTGTATATATGCCCATATAATTTTTTGGATTACTATTCCCA
CAAGTGTAAGATGAAAGAGGACCTGCCCTCCATTCTATCTACTCCCTCTCTG
CAAAGGCCATTTTGAAGATCTCAGTTTTACTGAAAGGGGTCTGCCACACAT
AACTTCCCTGGTGTACCAGCAACAAGTGTTCAGTGGACCATGGCAACACA
AAGAATGACGGAGGAGAGAAAATGCCATGCAGAAGTGATAGTCAAGATGA
AGATGTGGGAGAGGAGCAGCCCCCAGTATATATTCAGTGTGTCCTCTGAAG
TAACAACCATCCCATCTGGCTGAGAAGTGATGGACACGGCTCCTTAACCAC
ACTGTACCCTCTGTTCTTGGCACCTCTCTACCAGAAAGATATCAACAAATTG
GATGGAATTCAGAGAACAGTAAGAAAAATGATTAAAGAGATGGAGGCAGT
GACTTATGAGGAAAGATGAAAAGGACTAAATCTATGTAGCTTGGCAAAGCA
ACAACTGAGGTGGCGTATGAAAACTGTCTACAAGCATTTGAAGAATATAAA
CACCAAGGGAAGAGAGGGAACTTTTTAGAATGGTCTGGGGAATAAGCAGA
GTAAGAGGACAGAATTGAAGAAAGGAGAGAGATATGCTAAGTTGGGGGTG
GGGGCGTTTTCTCATCAATTGTAAATTTTGATAAATAACAAAATTAAGTCTG
GAGAGAAAGAGTTGCATCTCATGCTTTTAGGTTACAATCAGTGTTGACCTG
CACTGAGAAAATGTGACTTCCTATTATCCTTCTCAAAGTTATTGCTGCAGAG
GAAAGAAGCATACATCTTTTACCCACCAGGAAACCCCCAAAGCATCTATTA
CCATAATAGCCATGGGAAACAGAAGGCACCTCAAATAAAGGTGGGGAAAA
GAATGAAAGAAATGGCTTTGGCCTGTGCCTGTTTGACCTCTGAGAGATACT
TTTTGCAAGAAATTGTTAAGTTTTGGCCCAAAAAGTGGTCGGTCTTTTATCC
TTCCTTGTGGAGGCCAAACTGCAAACCAGGATGAAGAAACCATCAGTGGC
AGTTTGGGGAGGTGGAGGAGGGACTATCTAGACTCAGTGAATCACCAAGG
AAACATGAGGCCTATTTCTCTGTGAAGCTGAACTTCAAAAAACAATCTAAG
AGATACAAAGGGAAAAGTGTGACTGCTCTGACAGCAAAAACAAGCAAATA
TCTCCAGAGGGGATTAATCCTTCTGCTTCAAAGTAACTAAAACAAAGAAAG
CTGAGCCAGCTTCACTGGAAACAACACCTGTCCATCAGACAAGGATGAGC
TAATCAAAAGAAAACACTGCCTATGTGCACGACTCAGCAGCCTGGACAGC
TAATACCAAGGAACACGTTTTCACTATAAAGATTCAGCTTTCAAAATCCAGT
TCCCTCTTGTAGAGCCACAGGGTTAAAAGGGACCTTAGAGATCATCTACAT
ACAGGCCAAACCCTCAATGAATGCCAGTGTTCGCCTTATGTTCTTCTTGCCG
GACTCTTTTCTGGCCTCTGTTCTGTCAGGATGTTTCCTGTTTTGCAAAACAG
CTTTAATCCTTGAGTATATTTAATTGTTAGAAAACAGTTCCTCAAATTTGACC
CCAAATTTAAGTCTAATTTATGAAGAAAGTTTTGCCTTTGGAGACAGGTTTA
ATTTTACTTCCATGTGGAAAACCTTCAAATAAAATGATGACTATTGTGTACT
CACGAAAAA

Mir-1224-3P：ccccaccuccucucuccucag

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *Mir-1224-3P*
length: 21

mfe: -37.7 kcal/mol
p-value: 1.000000e+00

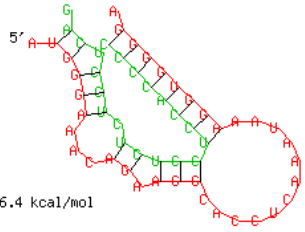
Position: 993
target 5' A GAUAUGCUAAGUU G 3'
AGGAGAGA GGGGGUGGGG
UCCUCUCU CCUCCACCCC
miRNA 3' GAC 5'



dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *Mir-1224-3P*
length: 21

mfe: -26.4 kcal/mol
p-value: 1.000000e+00

Position: 1239
target 5' A AAC A CACCUCAAAUAA A 3'
UG GGA AG AGG AGGUGGGG
AC CCU UC UCC UCCACCCC
miRNA 3' G U C 5'

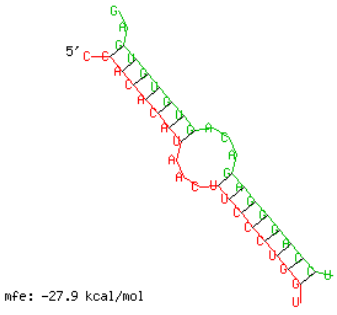


Mir-7113-5p：uccagggagacagugugugag

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *Mir-7113-5p*
length: 21

mfe: -27.9 kcal/mol
p-value: 1.000000e+00

Position: 508
target 5' C AAC U 3'
CACACAU UUCCCUGG
GUGUGUG GAGGGACC
miRNA 3' GA ACA U 5'



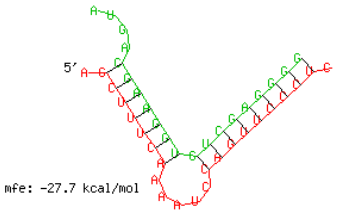
Mir-920: ggggagcuguggaagcagua

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *mir-920*
length: 20

mfe: -27.7 kcal/mol
p-value: 1.000000e+00

Position: 1768
target 5' A AAAUC C 3'
GCUUUCA CAGUUCCCU
CGAAGGU GUCGAGGGG
miRNA 3' AUGA 5'

plot as png, *jpeg* or *ps* *(in a new window)*

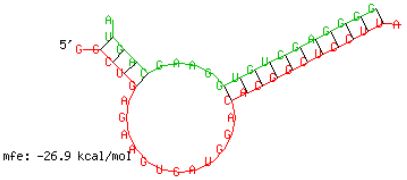


dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *mir-920*
length: 20

mfe: -26.9 kcal/mol
p-value: 1.000000e+00

Position: 684
target 5' G AGAAGUGAUGGA A 3'
GCUG CACGGCUCCUU
UGAC GUGUCGAGGGG
miRNA 3' A GAAG 5'

plot as png, *jpeg* or *ps* *(in a new window)*

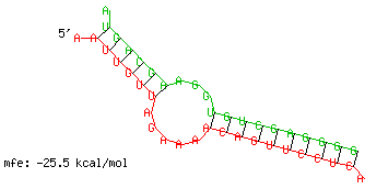


dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *mir-920*
length: 20

mfe: -25.5 kcal/mol
p-value: 1.000000e+00

Position: 1961
target 5' A AGAAA A 3'
AUUGUU ACAGUUCCUC
UGACGA UGUCGAGGGG
miRNA 3' A AGG 5'

plot as png, *jpeg* or *ps* *(in a new window)*



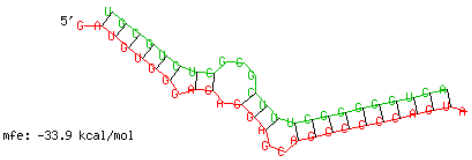
Mir-637: acuggggcuuucgggcucugcgu

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *mir-637*
length: 24

mfe: -33.9 kcal/mol
p-value: 1.000000e+00

Position: 617
target 5' G G A GC A 3'
AUGUGG AG GGA AGCCCCCAGU
UGCGUC UC CUU UCGGGGGUCA
miRNA 3' GGG 5'

plot as png, *jpeg* or *ps* *(in a new window)*

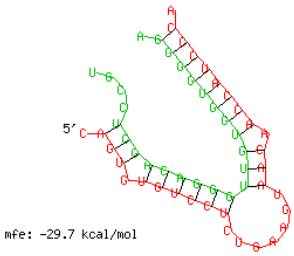


Mir-608: agggguggugguuugggacagcuccgu

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *mir-608*
length: 25

mfe: -29.7 kcal/mol
p-value: 1.000000e+00

Position: 650
target 5' C G CUGAAGU A A 3'
AGU UGUCCU AAC ACCAUCCC
UCG ACAGGG UUG UGGUGGGG
miRNA 3' UGCC A 5'



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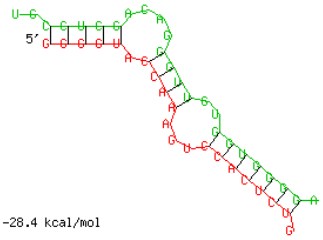
dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *mir-608*
length: 25

mfe: -28.4 kcal/mol
p-value: 1.000000e+00

Position: 1
target 5' A AGU G 3'
GGGGU CCAA CCACUCU
CCUCG GGUU GGUGGGG
miRNA 3' UG ACAG GU A 5'



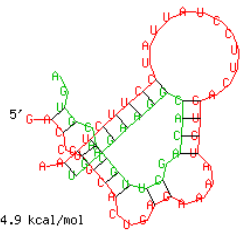
plot as png, *jpeg* or *ps* *(in a new window)*

hsa-miR-6770-5p: ugagaaggcacagcuugcacguga

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-6770-5p*
length: 24

mfe: -24.9 kcal/mol
p-value: 1.000000e+00

Position: 1119
target 5' G C CUG AAAA ACUUCCUAUUAU A 3'
AC UGCA AG UGUG CCUUCUCA
UG ACGU UC ACAC GGAAGAGU
miRNA 3' AG C G 5'

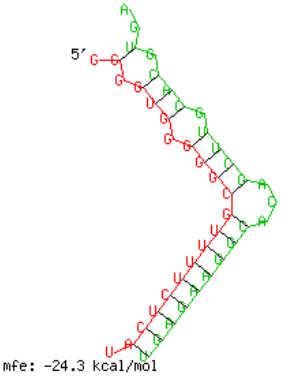


plot as png, *jpeg* or *ps* *(in a new window)*

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-6770-5p*
length: 24

mfe: -24.3 kcal/mol
p-value: 1.000000e+00

Position: 1016
target 5' G G G U 3'
G GUG GGGC GUUUUCUCA
U CAC UUCG CGGAAGAGU
miRNA 3' AG G G ACA 5'



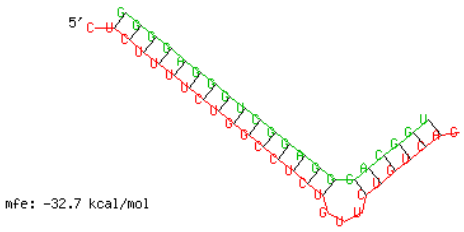
plot as png, *jpeg* or *ps* *(in a new window)*

hsa-miR-1207-5p: uggcagggaggcugggagggg

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-1207-5p*
length: 21

mfe: -32.7 kcal/mol
p-value: 1.000000e+00

Position: 1890
target 5' C GUU G 3'
UCUUUUCUGGCCUCU CUGUCA
GGGGAGGGUCGGAGG GACGGU
miRNA 3' 5'

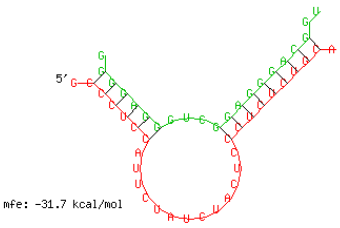


plot as png, *jpeg* or *ps* *(in a new window)*

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-1207-5p*
length: 21

mfe: -31.7 kcal/mol
p-value: 1.000000e+00

Position: 437
target 5' G AUUCUAUCUACUC A 3'
CCCUCC CCUCUCUGC
GGGAGG GGAGGGACG
miRNA 3' G GUC GU 5'

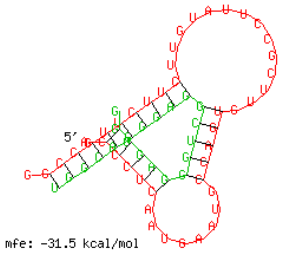


plot as png, *jpeg* or *ps* *(in a new window)*

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-1207-5p*
length: 21

mfe: -31.5 kcal/mol
p-value: 1.000000e+00

Position: 1845
target 5' A AAUGAAUG GUUCGCCUUAUGUU G 3'
CCCUC CCAGU CUUCUUGCCG
GGGAG GGUCG GAGGGACGGU
miRNA 3' G 5'



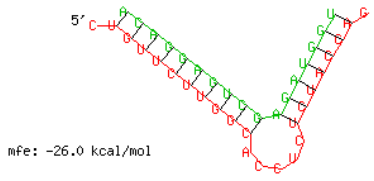
plot as png, *jpeg* or *ps* *(in a new window)*

hsa-miR-4451: ugguagagcugaggaca

dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-4451*
length: 17

mfe: -26.0 kcal/mol
p-value: 1.000000e+00

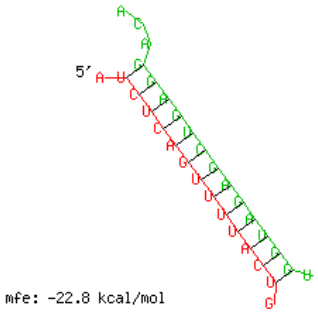
Position: 728
target 5' C ACCUC G 3'
UGUUCUUGGC UCUACCA
ACAGGAGUCG AGAUGGU
miRNA 3' 5'



dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-4451*
length: 17

mfe: -22.8 kcal/mol
p-value: 1.000000e+00

Position: 482
target 5’ A G 3’
UCUCAGUUUUACU
GGAGUCGAGAUGG
miRNA 3’ ACA U 5’



dataset: 1
Target: *TUSC7*
length: 2105
MiRNA: *hsa-miR-4451*
length: 17

mfe: -21.6 kcal/mol
p-value: 1.000000e+00

Position: 1897
target 5’ C G U G 3’
UG CCUC GUUCUGUCA
AC GGAG CGAGAUGGU
miRNA 3’ A U 5’

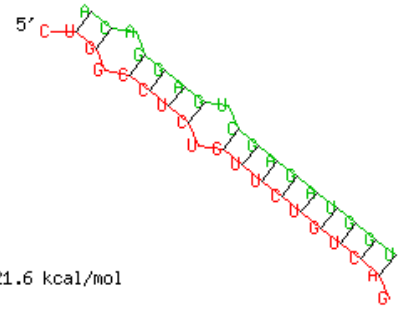


Table S1. Microarray gene expression profiling analysis identified 29 TUSC7-associated miRNAs

miRNA	Expression	miRNA	Expression
hsa-miR-30b-3p	down	hsa-miR-744-3p	up
hsa-miR-3613-5p	down	hsa-miR-6877-3p	up
hsa-miR-4701-3p	down	hsa-miR-5739	up
hsa-miR-501-3p	down	hsa-miR-7845-5p	up
hsa-miR-1303	down	hsa-miR-30e-5p	up
hsa-miR-628-3p	down	hsa-miR-3184-3p	up
hsa-miR-489-3p	down	hsa-miR-1224-5p	up
hsa-miR-891a-5p	down	hsa-let-7f-1-3p	up
hsa-miR-767-5p	down	hsa-miR-3176	up
hsa-miR-1587	down	hsa-miR-494-3p	up
hsa-miR-5585-3p	down	hsa-miR-6728-5p	up
hsa-miR-6808-5p	down	hsa-miR-5684	up
hsa-miR-660-5p	down	hsa-miR-3156-5p	up
		hsa-miR-19a-3p	up
		hsa-miR-183-3p	up
		hsa-miR-6508-5p	up

Table S2. Possible binding sites of the 3'-UTR sequence of TUSC7 and miRNAs.

miRNA	Base sequence
Mir-1224-3P	CCCCACCUCCUCUCUCCUCAG
Mir-7113-5p	UCCAGGGAGACAGUGUGUGAG
Mir-920	GGGGAGCUGUGGAAGCAGUA
Mir-637	ACUGGGGGCUUUCGGGCUCUGCGU
Mir-608	AGGGGUGGUGUUGGGACAGCUCCGAGGGGUGGU
hsa-miR-6770-5p	UGAGAAGGCACAGCUUGCACGUGA
hsa-miR-1207-5p	UGGCAGGGAGGCUGGGAGGGG
hsa-miR-4451	UGGUAGAGCUGAGGACA