

Supplementary Materials: Molecular pathogenesis of gene regulation by the *miR-150* duplex: *miR-150-3p* regulates *TNS4* in lung adenocarcinoma

Shunsuke Misono, Naohiko Seki, Keiko Mizuno, Yasutaka Yamada, Akifumi Uchida, Hiroki Sanada, Shogo Moriya, Naoko Kikkawa, Tomohiro Kumamoto, Takayuki Suetsugu and Hiromasa Inoue

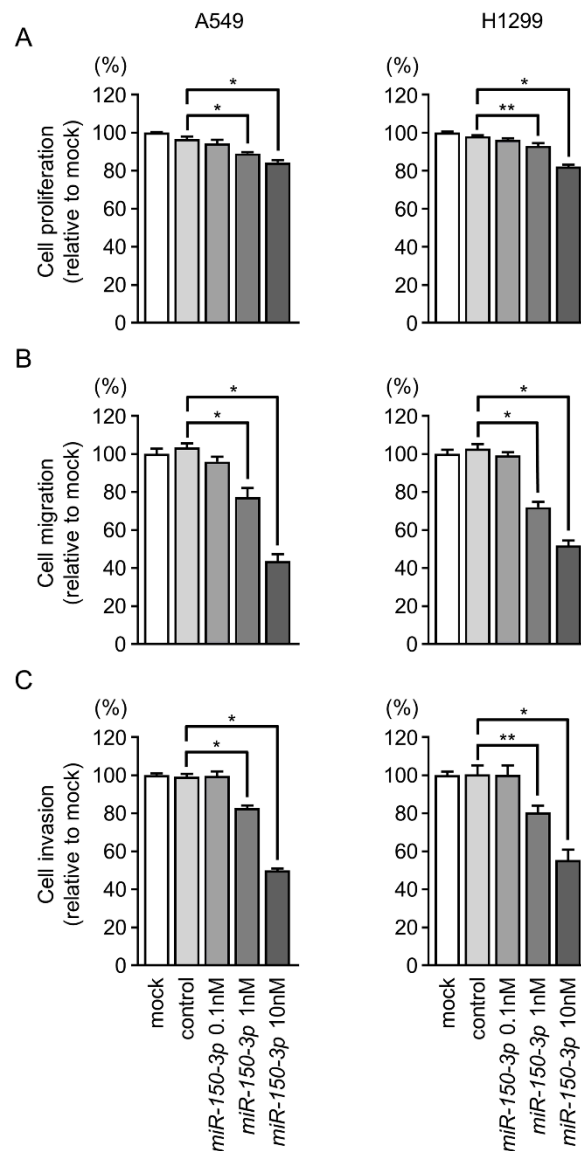


Figure S1. Functional assays of *miR-150-3p* (0.1 nM, 1 nM, 10 nM) in LUAD cells (A549 and H1299). (A-C) Cell proliferation, migration, and invasive activities were significantly blocked by ectopic expression of *miR-150-3p* with 1 nM and 10 nM concentration. * $p < 0.01$, ** $p < 0.05$.

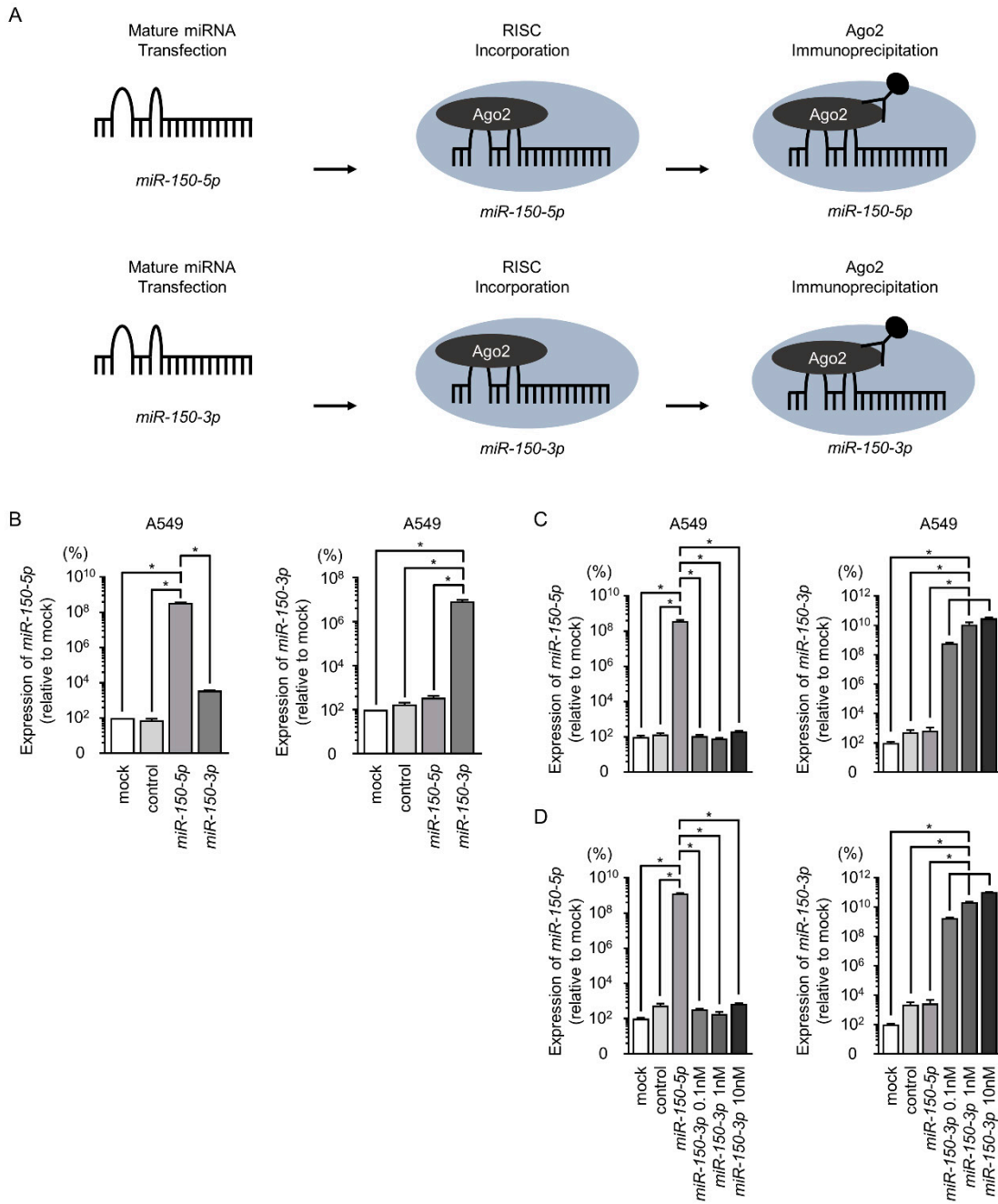


Figure S2. Both strands of *miR-150-5p* and *miR-150-3p* were incorporated into the RISC. (A) Schematic diagram for isolation of the Ago2-miRNA complex from the RISC. (B–D) Expression levels of *miR-150-5p* or *miR-150-3p* bound to Ago2 were measured by TaqMan RT-qPCR and normalized to the expression of *miR-26a* (B), *miR-16-5p* (C) and *miR-21-5p* (D), which had no effect upon *miR-150-5p* and *miR-150-3p*. * $p < 0.01$.

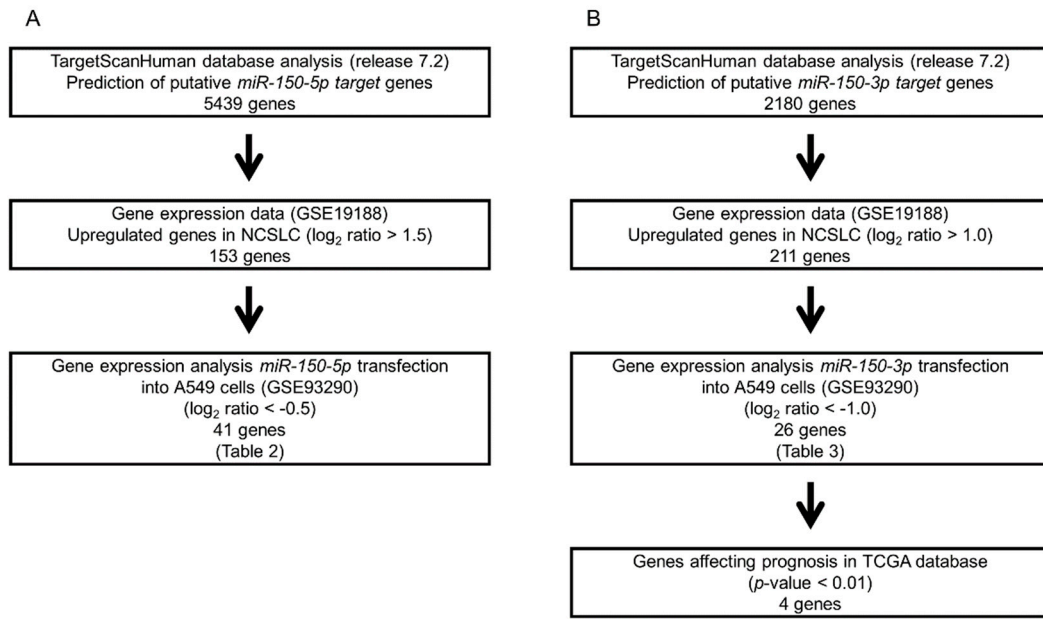


Figure S3. The strategy for identification of genes regulated by *miR-150-5p* and *miR-150-3p*. We obtained putative genes regulated by *miR-150-5p* and *miR-150-3p* using the TargetScanHuman Release 7.2 database and selected upregulated genes in non-small cell lung cancer clinical expression profiles from the GEO database (GSE19188). Then, we combined the gene expression analyses using A549 cells transfected with *miR-150-5p* or *miR-150-3p* and inspected the downregulated genes (GSE93290).

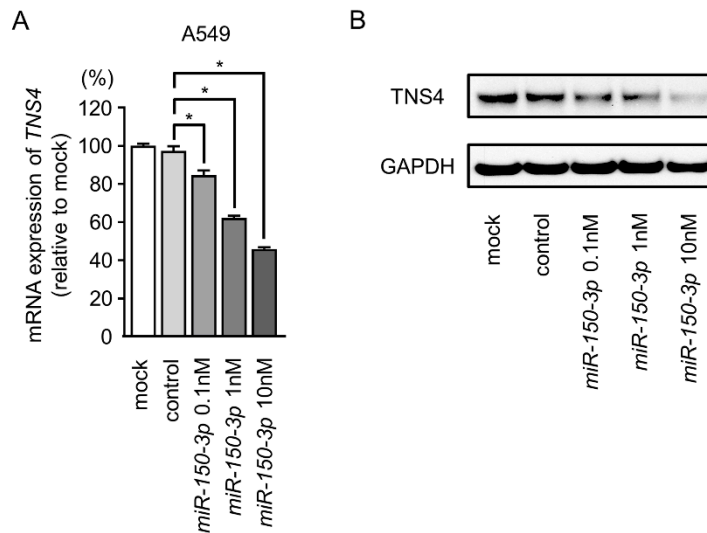


Figure S4. *TNS4* was directly suppressed at the diluted concentration of *miR-150-3p* precursor (1 nM and 0.1 nM). (A,B) *TNS4* mRNA and protein expression was reduced by *miR-150-3p* ectopic expression (48h after transfection). *GUSB* was used as an expression control. GAPDH was used as a loading control. * $p < 0.01$.

TNS4 3'UTR variant 1

GACAGAGCCTCAGGAGAACGTATGCCACCTCTTTGCGGAGTATGACATGGTCCAGCCAGC
 CTCGCAGGTCATCGGCCTGGTGACTGCTCTGCTGCAGGACGCAGAAAGGATGTAGGGGAG
 AAACCTGCCTGTGCACCTAACCAACACCTCCAGGGGCCCGCTAAGGAGCCCCCTCCACCC

CCTGAATGGGTGTGGCTTGTGGCCATATTGACAGACCAATCTATGGGACTAGGGGGATTG
 GCATCAAGTTGAAACCCCATCTCTACAAAGAATACAAAAATTAGCCGGGCAAGGTAGCGC
 ACCTGTGGTCCCAGGTA CTCTCGGGAGACTGAGGCATGAGAATCCCTTGAAACTGGGAGGCG
 GAAGTTGCAGTGAGCTGTGATCGTGCCACTGCACTCCAGCCTGGTTGACAGAGCAACATG
 CTATCTCTAAAACAAACAAACAAACAAAACTCAGGTTCCACACCCTCTAAACCCTGCC
 TCCTCTCAGGCTACAGAGACCTCTCCAGGAGGCTGAAGTGCCCTTACCCCGACCATCTGA
 CCAGCCACCGCCCCATGCCCGTGCCCCACCGAGGGCGGAGGCTGCTCACTGCTCTGTTTT
 ATCTCTGGCCTCTGATCCTGCATTCTTGTGCCAGGGCTTAGACCCAGGGCAAGGTCTTAG
 ACCCAGGGCAGGGGTAGGGTTAAAGGCTTTCAACCCAGGGGCCAGTGCCTTAATTCATGC
 AACAAATGTTTTCTGGCTGTGTGCTTTATTCATGTGAACCAGGAAAACAGAAAAATATGA
 CAGTGTTCACACAGAGGTTTATAAGTGCTATGCAGGGCTGGTGAAAGAGTAGAAACTG
 AGAAAGATAAACTTTACCCACTTGAAGGGAAGGAGGGCAATGTTACCAAGAAGGTAACAT
 TTGAGTTGGTCTTCAAGGATGAATAGGAGTTCGGCATGCAAAGAGAGTTAGAAACCAAC
 TTTTAGGAGTGGGAGGGGCTCTCATGTGCTACATACAATCTGAGGCACATTATATATGC
 CTAATCCCATTTTACAGATTAGAAAACCTGGGGCTCAGAGGGTTAACTTGCCACATTAC
 CTAAGTGTAAATGGCAGAGAAACAGGATTTCAAGTCCATGCCCATCTATTGCCCCAGCA
 TTCACAGAAAGCAGATGGAGACATTCGTGTGTGAAGCACACAGGTATGAAAAGATGTACC
 AAGTTTTGGTGTGGCTCAAGTACATGGTACCTAAGGGAGTAGGTGAGAGAGAAAGCAGAAG
 TNS4 3'UTR variant 2

GACAGAGCCTCAGGAGAACGTATGCCACCTCTTTGCGGAGTATGACATGGTCCAGCCAGC
 CTCGCAGGTCATCGGCCTGGTACTGCTCTGCTGCAGGACGCAGAAAGGATGTAGGGGAG
 AGACTGCCTGTGCACCTAACCAACACCTCCAGGGGCTCGCTAAGGAGCCCCCTCCACCC
 CCTGAATGGGTGTGGCTTGTGGCCATATTGACAGACCAATCTATGGGACTAGGGGGATTG
 GCATCAAGTTGACACCCTTGAACCTGCTATGGCCTCAGCAGTCACCATCATCCAGACCC
 CCCGGCCTCAGTTTCTCAATCATAGAAGAAGACCAATAGACAAGATCAGCTGTTCTTA
 GATGCTGGTGGGCATTTGAACATGCTCCTCCATGATTCTGAAGCATGCACACCTCTGAAG
 ACCCCTGCATGAAAATAACCTCCAAGGACCCTCTGACCCCATCGACCTGGGCCCTGCCCA
 CACAACAGTCTGAGCAAGAGACCTGCAGCCCCGTTCGTGGCAGACAGCAGGTGCCTGG
 CGGTGACCCACGGGGCTCCTGGCTTGACGCTGGTGTGTTCAAGAACTGACTACAAAACA
 GGAATGGATAGACTCTATTTCTTCCATATCTGTTCTCTGTTCCTTTTCCCACTTTCTG
 GGTGGCTTTTTGGGTCCACCCAGCCAGGATGCTGCAGGCCAAGCTGGGTGTGGTATTTAG
 GGCAGCTCAGCAGGGGGA ACTTGTCCCCATGGTCAGAGGAGACCCAGCTGTCTCTGCACCC
 CTTTGCAGATGAGTATCACCCATCTTTTCTTCCACTTGTTTTTTATTTTTATTTTTT
 TGAGACAGAGTCTCACTGTCAACCAGGCTGAACTGCAGTGGTGTGATCTAGGCTCACTGC
 AACCTCCACCTCCCAGGTA CTCTCAGGAGACTGAGGCATGAGAATCCCTTGAAACTGGGAGG
 CGGAAGTTGCAGTGAGCTGTGATCGTGCCACTGCACTCCAGCCTGGTTGACAGAGCAACA
 TGCTATCTCTAAAACAAACAAACAAACAAAACTCAGGTTCCACACCCTCTAAACCCTG
 CCTCTCTCAGGCTACAGAGACCTCTCCAGGAGGCTGAAGTGCCCTTACCCCGACCATCT
 GACCAGCCACCGCCCCATGCCCGTGCCCCACCGAGGGCGGAGGCTGCTCACTGCTCTGTT
 TTATCTCTGGCCTCTGATCCTGCATTCTTGTGCCAGGGCTTAGACCCAGGGCAAGGTCTT
 AGACCCAGGGCAGGGGTAGGGTTAAAGGCTTTCAACCCAGGGGCCAGTGCCTTAATTCAT
 GCAACAAATGTTTTCTGGCTGTGTGCTTTATTCATGTGAACCAGGAAAACAGAAAAATAT

GACAGTGTTC AACAGAGGTTTATAAGTGCTATGCAGGGCTGGTGGAAAGAGTAGAAAC
TGAGAAAGATAAACTTTACCCACTTGAAGGGAAGGAGGGCAATGTTACCAAGAAGGTAAC
ATTTGAGTTGGGTCTTCAAGGATGAATAGGAGTTCGGCATGCAAAGAGAGTTAGAAACCA
ACTTTTAGGAGTGGGGAGGGGCTCTCATGTGCTACATAACAATCTGAGGCACATTATATAT
GCCTAATCCCATTTTACAGATTAGAAAAC TGGGGCTCAGAGGGTTAACTTGCCCACATTC
ACCTAACTGTAAATGGCAGAGAAACAGGATTTCAAGTCCATGCCCATCCTATTGCCCCAG
CATTACAGAAAGCAGATGGAGACATTCGTGTGTGAAGCACACAGGTATGAAAAGATGTA
CCAAGTTTGGTGTGGCTCAAGTACATGGTACCTAAGGGAGTAGGTGAGAGAGAAAGCAGA
TNS4 3'UTR variant 3

GACAGAGCCTCAGGAGAACGTATGCCACCTCTTTGCGGAGTATGACATGGTCCAGCCAGC
CTCGCAGGTCATCGGCCTGGTACTGCTCTGCTGCAGGACGCAGAAAGGATGTAGGGGAG
AGACTGCCTGTGCACCTAACCAACACCTCCAGGGGCTCGCTAAGGAGCCCCCTCCACCC
CCTGAATGGGTGTGGCTTGTGGCCATATTGACAGACCAATCTATGGGACTAGGGGGATTG
GCATCAAGTTGACACCCTTGAACCTGCTATGGCCTTCAGCAGTCACCATCATCCAGACCC
CCCGGGCCTCAGTTTCTCAATCATAGAAGAAGACCAATAGACAAGATCAGCTGTTCTTA
GATGCTGGTGGGCATTTGAACATGCTCCTCCATGATTCTGAAGCATGCACACCTCTGAAG
ACCCCTGCATGAAAATAACCTCCAAGGACCCTCTGACCCCATCGACCTGGGGCCCTGCCCA
CACAACAGTCTGAGCAAGAGACCTGCAGCCCCTGTTTCGTGGCAGACAGCAGGTGCCTGG
CGGTGACCCACGGGGCTCCTGGCTTGACAGCTGGTGTGATGGTCAAGAAGTACTACAAAACA
GGAATGGATAGACTCTATTTCTTCCATATCTGTTCTCTGTTCTTTTCCCACTTTCTG
GGTGGCTTTTTGGGTCCACCCAGCCAGGATGCTGCAGGCCAAGCTGGGTGTGGTATTTAG
GGCAGCTCAGCAGGGGAACTTGTCCCCATGGTCAGAGGAGACCAGCTGTCTGCACCC
CCTTGCAGATGAGTATCACCCATCTTTCTTTCCACTTGTTTTTATTTTTATTTTTT
TGAGACAGAGTCTCACTGTCACCCAGGCTGAACTGCAGTGGTGTGATCTAGGCTCACTGC
AACCTCCACCTCCCAGGTTCAAGCAATTATCCTGCCTCAGGCTCCCAAGTAGCTGGGATT
ACAGGCATGTGCAACTCACCCAGCTAATTTTGTATTTTATAGTAGAAACATGGTCAAACCC
CATCTCTACAAAAAATACAAAAATTAGCCGGGCAAGGTAGCGCACCTGTGGTCCCAGGTA
CTCAGGAGACTGAGGCATGAGAATCCCTTGAAACTGGGAGGCGGAAGTTGCAGTGAGCTG
TGATCGTGCCACTGCACTCCAGCCTGGTTGACAGAGCAACATGCTATCTCTAAAACAAAC
AAACAAACAAAAACTCAGGTTCCACACCCTCTAAACCCTGCCTCCTCTCAGGCTACAGA
GACCTCTCCAGGAGGCTGAAGTGCCCTTACCCGACCATCTGACCAGCCACCGCCCCATG
CCCGTGCCCCACCGAGGGCGGAGGCTGCTCACTGCTCTGTTTTATCTCTGGCCTCTGATC
CTGCATTCTTGTGCCAGGGCTTAGACCCAGGGCAAGGTCTTAGACCCAGGGCAGGGGTAG
GGTTAAAGGCTTTCAACCCAGGGGCCAGTGCCTTAATTCATGCAACAAATGTTTTCTGGC
TGTGTGCTTTATTCATGTGAACCAGGAAAACAGAAAAATATGACAGTGTTC AACAGAG
GTTTATAAGTGCTATGCAGGGCTGGTGGAAAGAGTAGAAACTGAGAAAGATAAACTTTAC
CCACTTGAAGGGAAGGAGGGCAATGTTACCAAGAAGGTAACATTTGAGTTGGGTCTTCAA
GGATGAATAGGAGTTCGGCATGCAAAGAGAGTTAGAAACCAACTTTTAGGAGTGGGGAGG
GGCTCTCATGTGCTACATAACAATCTGAGGCACATTATATATGCCTAATCCCATTTTACAG
ATTAGAAAAC TGGGGCTCAGAGGGTTAACTTGCCCACATTCACCTAACTGTAAATGGCAG
AGAAACAGGATTTCAAGTCCATGCCCATCCTATTGCCCCAGCATTACAGAAAGCAGATG
GAGACATTCGTGTGTGAAGCACACAGGTATGAAAAGATGTACCAAGTTTGGTGTGGCTCA

AGTACATGGTACCTAAGGGAGTAGGTGAGAGAGAAAGCAGAAGAAAAAGACAGCAACAGG

Figure S5. The nucleotide sequences of 3'UTR of *TNS4* in A549 cells. Several variants of the 3'UTR of *TNS4* existed in A549 cells. The putative binding site of *miR-150-3p* was found in 3'UTR of *TNS4*, respectively (highlighted in blue). Red font was stop codon. There was no binding site of *miR-150-5p* in 3'UTR of *TNS4*.

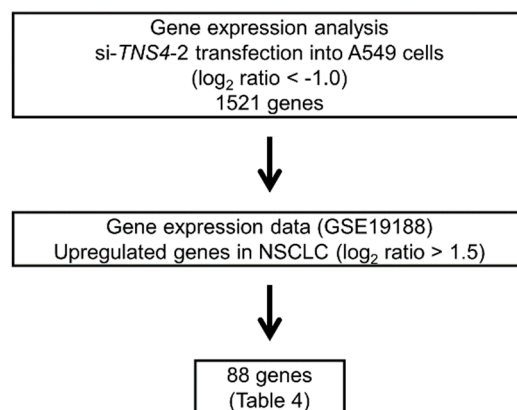


Figure S6. The strategy for identification of *TNS4*-modulated genes. We combined the gene expression analyses using A549 cells transfected with si-*TNS4* and GEO database (GSE19188). Finally, a total of 88 genes were identified as *TNS4*-modulated genes.

Table S1. Reagents used in this study.

Antibody	Dilution	Catalog Number	Company
TNS4	IHC 1:50	ab82178	Abcam, Cambridge, UK
	WB 1:500		
GAPDH	WB 1:20000	MAB374	EMD Millipore, Billerica, MA, USA
miRNA species	Concentration	Assay ID	Company
<i>miR-150-5p</i>	10 nM	PM 10070	Applied Biosystems, Foster City, CA, USA
<i>miR-150-3p</i>	10 nM	PM 12324	Applied Biosystems, Foster City, CA, USA
negative control miRNA	10 nM	AM 17111	Applied Biosystems, Foster City, CA, USA
anti-miR Negative Control #1	10 nM	AM 17010	Applied Biosystems, Foster City, CA, USA
siRNA	Concentration	Catalog number	Company
si- <i>TNS4</i>	10 nM	HSS131536	Invitrogen, Carlsbad, CA, USA
		HSS131537	
Primer and probe		Assay ID	Company
<i>miR-26a</i>		000405	Applied Biosystems, Foster City, CA, USA
<i>miR-150-5p</i>		000473	Applied Biosystems, Foster City, CA, USA
<i>miR-150-3p</i>		002637	Applied Biosystems, Foster City, CA, USA
<i>RNU48</i>		001006	Applied Biosystems, Foster City, CA, USA
<i>TNS4</i>		Hs00262662_m1	Applied Biosystems, Foster City, CA, USA
<i>GUSB</i>		Hs00939627_m1	Applied Biosystems, Foster City, CA, USA
Plasmid vector		Catalog number	Company

TNS4

RC222349

OriGene Technologies Inc., Rockville, MD, USA

Table S2. Immunohistochemical status and characteristics of the lung cancer and non-cancerous cases.

A. Immunohistochemical status and characteristics of LUAD cases							
Patient no.	Grade	T	N	M	Pathological stage	Immunohistochemical intensity	Immunohistochemical extensity
21	1	1	0	0	IA	3	3
22	1	2	1	0	IIA	3	3
23	2	1	0	0	I	3	3
24	2	2	2	0	IIIA	2	3
25	2	3	1	0	IIIA	3	3
26	2	2	0	0	IB	3	3
27	2	3	2	0	IIIA	3	3
28	2	1	0	0	IA	2	3
29	2	3	1	0	IIIA	1	3
30	-	4	0	0	IIIA	3	3
31	2	2	0	0	IIA	1	3
32	2	3	1	0	IIIA	2	3
33	2	2	1	0	IIB	1	3
34	2	3	0	0	IIB	0	0
35	2	3	1	0	IIIA	2	3
36	3	2	1	0	IIB	3	3
37	3	2	0	0	IB	1	3
38	2	2	0	0	IB	1	3
39	-	2	0	0	IB	3	3
40	2	2	1	0	IIB	1	3
B. Immunohistochemical status of non-cancerous cases							
patient no.					Immunohistochemical intensity	Immunohistochemical extensity	
87					0	0	
88					1	2	
89					1	3	
90					1	3	
91					1	2	
92					1	3	
93					1	2	
94					1	1	
95					1	3	
96					2	3	
97					1	2	

98	1	3
99	2	3
100	2	2



© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).