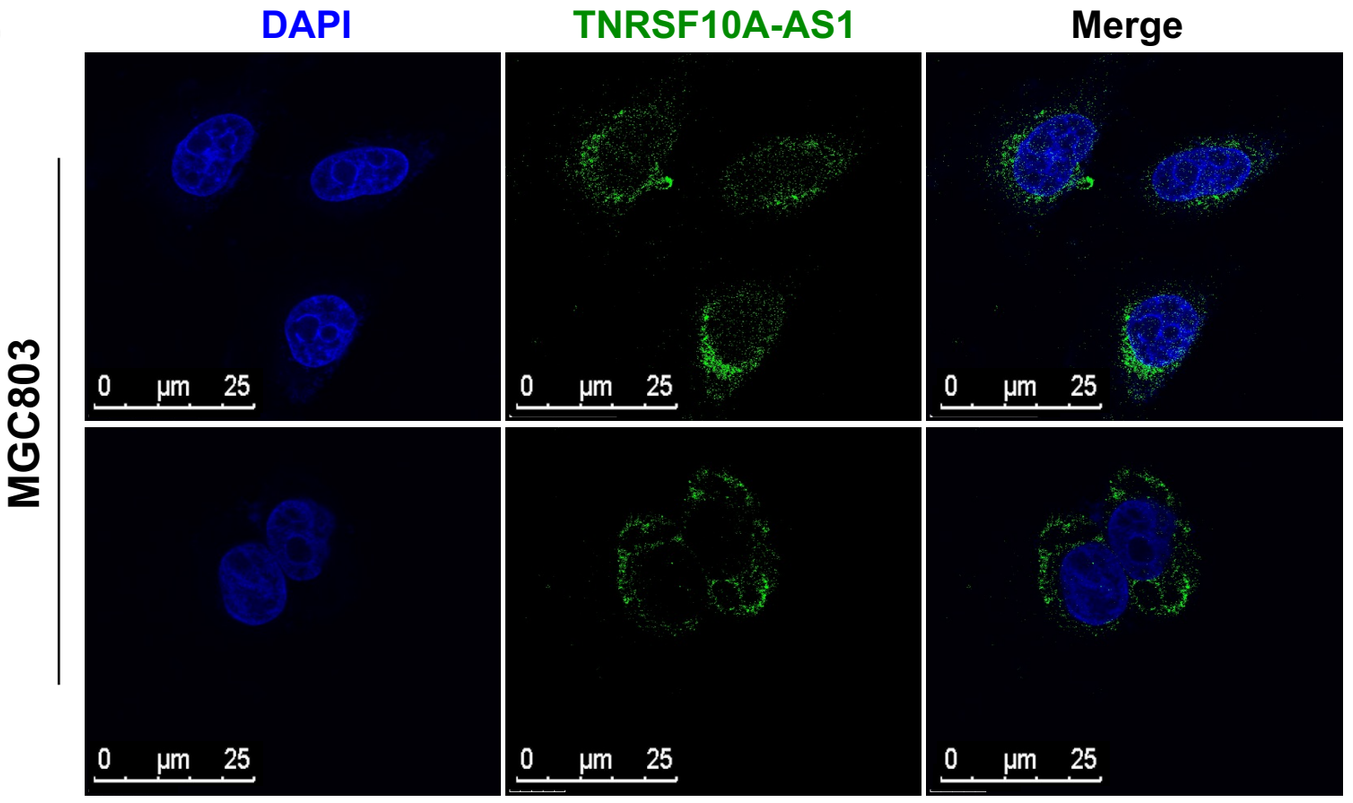
A

--------lncLocator Prediction Result --------

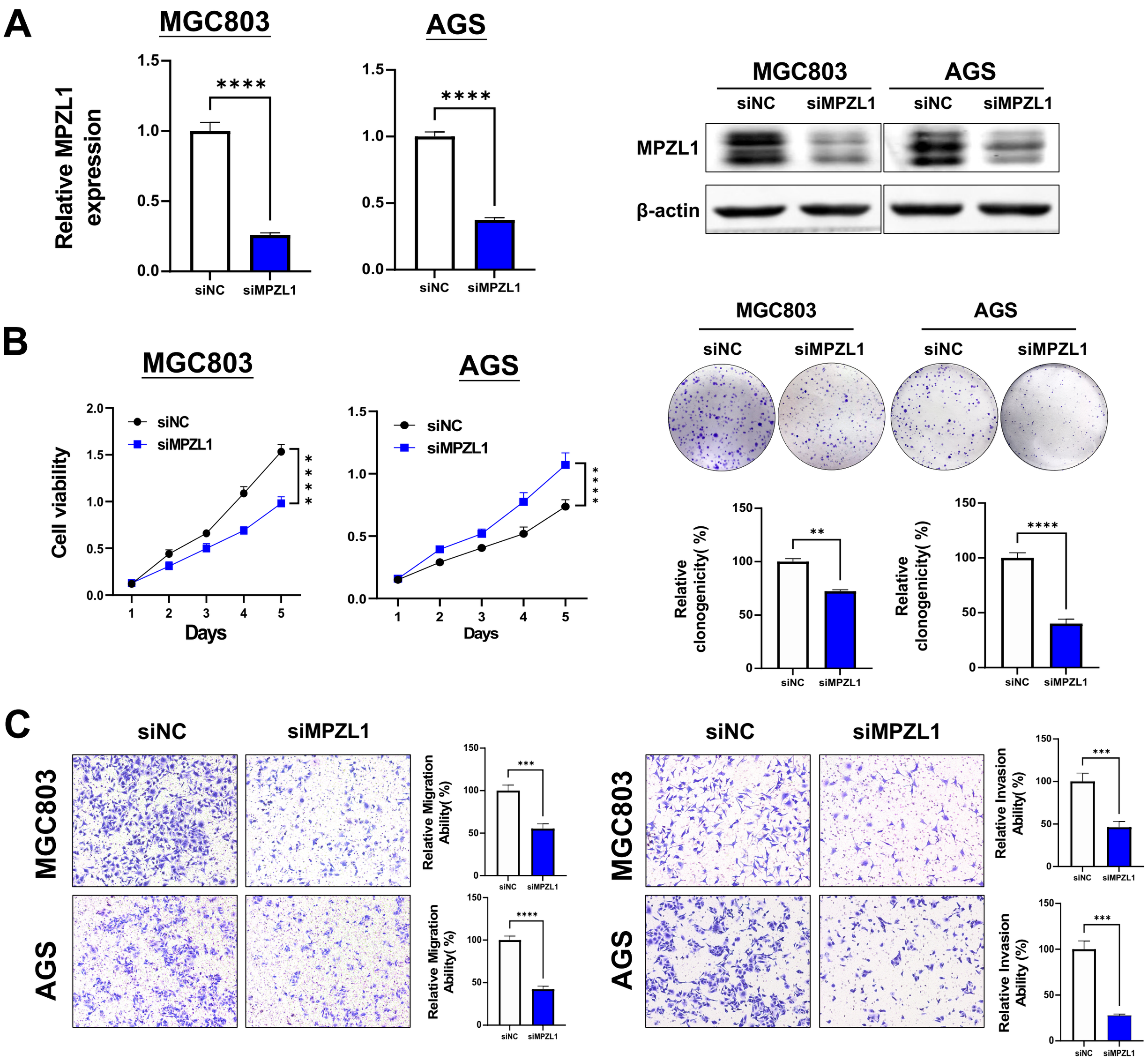
Subcellular locations	score
Cytoplasm	0.691117546327
Nucleus	0.164720143282
Ribosome	0.0237790882526
Cytosol	0.0461992240767
Exosome	0.0741839980614

Predicted location
Cytoplasm

B



Supplement Figure 1. The subcellular localization of TNFRSF10A-AS1. A, TNFRSF10A-AS1 was mainly distributed in the cytoplasm via the application site (http://www.csbio.sjtu.edu.cn/bioinf/lncLocator/index.html). **B,** RNA FISH results showed that TNFRSF10A-AS1 was distributed in both cytoplasm and nucleus, but mainly in the cytoplasm in MGC803 cell.



Supplementary Figure 2. MPZL1 showed oncogenic function in gastric cancer. **A,** Knockdown of MPZL1 in MGC803 and AGS cells was confirmed by qRT-PCR and western blot analysis. **B,** Knockdown of MPZL1 significantly inhibited cell viability (left panel) and colony formation ability (right panel) in MGC803 and AGS cells. **C,** Knockdown of MPZL1 expression inhibited cell migration (left panel) and cell invasion (right panel).

Supplementary methods

RNA interference

TNFRSF10A-AS1 siRNA (siTNFRSF10A-AS1: sense: 5'-CCAGACAGAUGGAUACCAATT-3'; antisense: 5'-UACCUAUGCAAAGAUUUGGTT-3'), MPZL1 siRNA (siMPZL1: sense: 5'-UCAAGUGGCAUAGCCAAUGTT-3'; antisense: 5'-CAUUGGCUAUGCCACUUGATT-3') and Negative Control (siNC) were ordered form Genepharma Company. 50 nmol of siMPZL1, siTNFRSF10A-AS1 or siNC were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

RNA extraction, semi-quantitative RT-PCR, and real-time PCR analyses

Total RNA was extracted from cells and tissues using TRIzol Reagent (Invitrogen). The extracted RNA was reversely transcribed into complementary DNA (cDNA) through a cDNA Reverse Transcription Kit (TransGen Biotech, Beijing). Semi-quantitative PCR was performed by AmpliTaq Gold DNA polymerase (Applied Biosystems; Thermo Fisher Scientific). Quantitative real-time PCR was performed by SYBR Green PCR Master Mix (Takara) on 7500HT Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific). Experiments were repeated twice. actin was tested for normalization. Each sample was tested in triplicate. The 2-ΔΔCt method was employed to quantify the relative gene expression levels. The sequences of primers used are listed

in Supplementary Table S4.

Protein extraction and Western blotting

Total Protein was extracted from cells using RIPA lysis buffer. Total protein extracted from tissues were using T-PER Tissue Extraction Reagent I (Applied Biosystems; Thermo Fisher Scientific). Proteins were separated on SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (GE Healthcare, Piscataway, NJ). Blots were immunostained with primary antibody and secondary antibody. Independent experiments were performed at least twice. The antibodies used are listed in Supplementary Table S5.

Colony formation and cell growth curve assays

Cells were plated in 6-well plates at 1,000 cells per well in complete DMEM. Medium was changed every 3 to 4 days. At the endpoint, cells were stained with 0.1% Crystal violet and the number of colonies consisting of >50 cells were counted. Cell growth curve was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich).

Immunocytochemistry staining

Paraffin slides from xenograft were used. Ki-67 signal was assessed. The proliferation index was determined by counting the numbers of positive staining

cells of Ki-67 as percentages of the total number of colon cells. At least 1000 cells were counted each time.

Migration and invasion assays

For the “Transwell” migration assay, 3×10^4 cells with applied genetic modification in 200 μL serum-free medium were seeded onto the upper chamber of an 8-μm Transwell filter (Corning, 3422, Shanghai, China). In the lower chamber, 600 μL complete medium containing 20% FBS was added. After incubation, cells in the lower surface were fixed by methanol, stained with 1% crystal violet, and visualized under a microscope. For the “Transwell” invasion assays, matrigel-coated chambers (Corning, 354480, Shanghai, China) were used. For all “Transwell” assays in this study, five random views were included to calculate the average number of migrated/invaded cells.

Apoptosis analyses

Cells were plated in 12-well plates and serum-starved overnight. Annexin V Apoptosis Detection Kit APC (Invitrogen, Thermo Fisher) was used to determine cell apoptosis. The experiments were conducted three times in triplicates.

Cell cycle analysis

BGC823 and GES1 cells that were stably transfected with TNFRSF10A-AS1 or

empty vector were plated in a 6-well plate, while MGC803 and AGS cells were transfected with siTNFRSF10A-AS1 or siNC. After 48h of transfection, the cells were fixed in ice-cold 70% ethanol for 24h before staining with 50μg/ml propidium iodide (BD Biosciences, Franklin Lakes, NJ). The cells were sorted by BD AccuriTM C6 (BD Biosciences), and cell cycle distributions were analyzed using the ModFitLT 5.0 software (Verity Software House, Topsham, ME). All experiments were conducted three times in triplicates.

Wound-healing assay

Confluent cultures in 6-well plates were scratched with sterile P-200 pipette tips, washed, and cultured in DMEM containing 2% FBS. Cells were photographed after 0, 24, and 48 hours, respectively. The cells migrated across the gap wound were observed and documented using an inverted microscope. Distance of the gap was quantified using Image J.

Ectopic overexpression of TNFRSF10A-AS1

Overexpression of TNFRSF10A-AS1 in BGC823 or GES1 cells were performed by lentivirus mediated transfection. GC cells were transfected with a EF-1aF/GFP&Puro-TNFRSF10A-AS1 cDNA lentiviral vector ("TNFRSF10A-AS1 vector", Genepharma Company). The EF-1aF/GFP&Puro-mock lentiviral vector ("Empty vector") were utilized as controls. Vectors were first transfected to HEK-293T cells along with the lentivirus package plasmid mix to generate lentivirus.

At 6×10^4 cells per well, GC cells were initially seeded into six-well plates in polybrene-containing complete medium. The lentivirus was added to GC cells for 48h. Afterwards, cells were cultured in 1 puromycin (3 μg/mL)-containing medium for five more passages (10-12 days). In stable cells, expression of targeted gene was assessed by qRT-PCR.

Supplementary Table 1. Clinical and pathological characteristics of patients in our cohort

TNFRSF10A-AS1 expression (2^-△ct)	Patient ID	Survival	Length	Sexy	Age	Location	TNM（7th）
0.71233216	251-9536376T	1	13	Femal	66	L	3c
0.007920765	455-9541997T	0	54	Male	57	L	2b
0.03076696	481-9542761T	1	20	Male	74	M	3b
0.002458489	467-9542415T	1	13	Femal	65	U	4
1.18609E-05	17-A25-20597T	0	60	Femal	61	M	1b
0.025441023	579-9547022T	0	47	Male	67	U	2b
0.013013894	573-9546266T	1	31	Male	62	M	3c
0.008064992	423-9541535T	1	15	Male	63	M	3c
0.001963502	187-Z11-24811T	1	22	Male	68	L	2a
5.05225E-05	167-A41-23993T	0	25	Male	66	U	2a
0.002565167	57-Z85-28072T	0	58	Male	74	L	1b
0.00585261	517-9544005T	1	4	Male	69	U	3a
0.001473082	411-9540961T	0	56	Femal	59	U	3a
0.006408494	511-9544004T	1	29	Male	59	U	3c
0.00527508	585-9547182T	1	27	Male	64	M	3a
0.004138951	525-9544427T	0	50	Femal	71	L	2b
0.01050092	211-A59-25914T	0	7	Male	55	L	3c
0.023810006	503-9532669T	1	14	Male	56	L	4
0.027349625	513-9544112T	0	51	Male	44	U	3b
0.00302101	165-Z17-24876T	0	48	Male	69	M	3b
0.05143652	151-N9-18577T	1	13	Male	56	U	3c
0.003857332	13-Z123-28886T	1	40	Femal	47	L	3c
0.016114873	389-9540514T	0	25	Male	44	L	2b
0.001024175	51-Z159-33265T	0	49	Femal	49	M	3a
0.086496998	559-9546067T	1	2	Male	71	L	4
0.02844763	171-N35-23553T	1	22	Male	57	U	3a
0.002799603	37-Z111-28681T	0	49	Male	59	L	3c

0.003689577	121-Z67-26306T	0	60	Male	68	L	3b
0.017013724	369-9539828T	1	18	Male	58	U	2a
0.100726333	19-Z153-29967T	1	33	Male	78	L	3a
0.001595925	469-9542538T	0	52	Femal	74	L	3a
0.00040513	179-Z51-26028T	1	5	Femal	65	L	4
0.026322415	535-9544115T	1	17	Male	66	L	3c
0.000473474	207-A45-24664T	0	38	Male	47	U	3c
0.108625435	509-9532869T	1	3	Femal	42	M	4
0.011326731	1-Z131-29411T	0	46	Femal	40	M	2b
0.008321937	431-9540474T	1	6	Male	63	U	3a
0.19688877	567-9546124T	1	29	Male	60	M	2a
0.00132209	169-N33-22820T	1	10	Male	60	L	2a
0.01354818	3-A79-26973T	0	36	Male	42	U	3c
0.006648973	117-Z21-24908T	0	60	Femal	60	L	3c
0.002131923	143-A35-23632T	0	49	Femal	71	M	2b
0.000470927	191-Z9-24758T	1	12	Male	60	L	3b
0.46755493	157-Z57-26261T	0	60	Femal	67	L	2b
0.022723862	501-9532666T	1	44	Femal	50	L	3c
0.004631928	43-Z129-28993T	1	16	Male	64	M	3c
0.022605443	395-9540809T	0	37	Male	60	L	3c
0.002160605	123-Z47-25943T	0	60	Femal	38	M	3a
0.004562804	435-9541634T	0	17	Male	54	U	3b
3.545386701	7-A17-19375T	0	60	Male	37	L	3a
0.01170474	447-9541880T	0	50	Male	69	L	3c
0.001784222	15-A21-19605T	0	8	Femal	79	U	3b
0.413159716	147-A51-25739T	0	22	Male	67	M	3a
0.006891285	85-A69-26043T	0	44	Male	64	U	3b
0.012235774	265-9537441T	0	60	Male	64	L	2b

0.001257128	79-Z105-28485T	0	50	Male	61	L	3c
0.014282986	415-9541385T	1	15	Male	56	U	3b
0.00724044	111-Z77-26914T	1	51	Male	75	L	2a
0.014634716	485-9542902T	0	13	Male	49	M	1b
0.0763574	105-Z25-25564T	1	9	Male	75	M	3c
0.394997114	87-N29-22640T	1	27	Femal	69	M	3a
0.01557702	119-Z71-26367T	0	60	Male	47	L	2a
0.00245678	67-Z133-29445T	1	13	Male	66	L	4
0.001274167	203-N1-15388T	1	23	Femal	63	U	3a
0.05408886	349-9539309T	0	13	Male	49	U	3a
0.064423452	27-A27-21002T	1	60	Male	69	L	2b
0.013577982	289-9537671T	1	12	Male	65	U	3c
0.006960904	255-9532010T	0	60	Male	64	U	2b
0.234007837	515-9532963T	1	38	Male	55	U	3b
0.01146916	109-Z69-26351T	1	36	Male	75	L	3b
0.014579851	453-9541959T	0	54	Male	66	L	2a
0.014382125	303-9538803T	1	5	Femal	50	L	2b
0.011912985	269-9537501T	0	9	Male	68	M	3b
0.037668656	293-9537867T	1	15	Femal	69	M	3c
0.204786773	421-9541224T	0	54	Male	51	L	2a
0.007006292	93-Z135-29520T	0	49	Male	69	U	3a
0.06237981	317-9538998T	0	60	Femal	68	U	2a
0.029996877	21-A23-19954T	0	60	Male	70	M	3c
0.046211017	249-9536435T	1	5	Femal	56	L	4
0.196504939	363-9539657T	1	9	Male	81	L	4
0.13399581	231-9534192T	1	17	Male	65	U	3c
0.138340141	25-A09-18495T	0	60	Male	60	L	2a
0.033253613	327-9539112T	0	47	Male	67	U	2a
0.048954293	47-Z95-28404T	1	36	Male	70	M	3c
0.051538287	223-N23-21184T	1	30	Femal	78	L	3b

0.045647344	9-A03-15178T	0	60	Male	68	U	2a
0.046715334	341-9539270T	0	25	Male	47	L	3c
0.089303595	353-9539589T	0	59	Femal	66	L	2a
0.358431109	425-9541358T	1	12	Male	73	U	3b
0.002774187	295-9538577T	0	60	Male	48	L	2b
0.255841015	401-9540945T	0	50	Male	69	L	3c
0.131307226	125-A39-23912T	0	8	Male	34	U	3b
0.263630223	345-9532546T	1	13	Femal	52	L	3c
0.018190799	75-Z119-28790T	0	48	Femal	54	M	3b
0.062961529	493-9540853T	1	12	Male	62	U	4
0.202941628	333-9532579T	1	29	Male	63	L	3c
0.259843623	69-N37-23830T	1	8	Male	68	U	3c
1.538732286	329-9532578T	0	39	Male	52	L	3b
0.01988476	507-9532687T	1	22	Male	74	U	3b
0.972127246	587-9547147T	0	47	Male	62	L	3b
9.7342107	589-9547138T	1	12	Femal	41	M	4
0.17330784	355-9539603T	1	30	Male	65	U	4
0.020892796	307-9538826T	0	60	Male	38	L	2b

Supplementary Table 2. The probe sequence of RNA FISH

Probe sequence name	Probe Sequence（5’-3’）
Human TNFRSF10A-AS1_1	GTTGCTATCACTGAATACCT
Human TNFRSF10A-AS1_2	GGTTATTTGTATTAGTCTGT
Human TNFRSF10A-AS1_3	AAAACACTTACGTGTGGCCG
Human TNFRSF10A-AS1_4	AAGTACTTTTTTTTGGCGGG
Human TNFRSF10A-AS1_5	ACCTTTGGAAATTTGGACTT
Human TNFRSF10A-AS1_6	CCTTGAGTTCAAGTCACAAT
Human TNFRSF10A-AS1_7	TGGCCGTCCAGTAAGCTAAG
Human TNFRSF10A-AS1_8	AGTCAAGGGAACAGCATATA

Supplementary Table 3. The TNFRSF10A-AS1 sequence and the sgRNA position of CRISPR/Cas9 knockout assay

TNFRSF10A-AS1:

AGGTATTCAGTGATAGCAACAGAAAACAGACTAATACAAATAACCATCAT
TTAACTTAGGTTAATATAAGATTTTTAAGTTACCAAAAATTAAATATAAAAA
TATTTAAAAGTAGACATCTTGGCTAGGTGCAGTGGCTCACGCCCATAATC
CCAGCACTTTGGGAGGCCGAGCCACCATGCCCGGCCACACGTAAGTGT
TTTGATTAGTAAATCCAGGTAGAAGTTGTATGTGTGAACTGTAAATAATGT
TGACAACTCTTAAGAATTGTCTGTTTTAATTAAACCAAGAAACTTAAACTA
GCTTTCTATTTACTAAAGATTATCTCAGATCACGTGACCTTGAAAAACATT
TAGATGGGCTCCAGTTTTTCTAAGAAAATGCTCCATTTATGGAAGCAATT
CTTTTCTTTCTTTTAACCAAATCTTTGCATAGGTACCAAATAACACATTTGT
TTAGGATGAGAGCTGCCCACTGCCCCCGCCAAAAAAAAGTACTTTTATAT
ACAAAAGTCCAAATTTCCAAAGGTATATGTACTTTAATTGTGACTTGAACT
CAAGGTAAATAAATTAAATAATTAATAAATTAACCTTAGCTTACTGGACGG
CCACCATCTTATATGCTGTTCCCTTGACTG

U6-sgRNA(TNFRSF10A-AS1)*4

GCTAGCGCTACCGGACTCAGGAGGGCCTATTTCCCATGATTCCTTCATA
TTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGAC
TGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATT
TCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATAT
GCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTG
GAAAGGACGAAACACCGGTAACAGTACTCAGACGTAGGTTTTAGAGCTA
GAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG
CACCGAGTCGGTGCTTTTTTACCGGTGAGGGCCTATTTCCCATGATTCCT
TCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAAT
TTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTA
ATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTAT
CATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATC
TTGTGGAAAGGACGAAACACCGATTGCAATTTGTGCGTGATAGTTTTAGA
GCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAA
GTGGCACCGAGTCGGTGCTTTTTTCTCGAGGAGGGCCTATTTCCCATGA
TTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAA
TTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAA
AGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGG
ACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTAT
ATATCTTGTGGAAAGGACGAAACACCGCTAAGAAAATGCTCCATTTAGTT
TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG
AAAAAGTGGCACCGAGTCGGTGCTTTTTTGGATCCGAGGGCCTATTTCC
CATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAT
TGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACG
TAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAA

ATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT
TTATATATCTTGTGGAAAGGACGAAACACCGCTAATCAAAACACTTACGT
GGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA
CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTCTGCAGTCGACGGTAC
CGCGG

Supplementary Table 4. DNA sequences of primers used in this study

Primer name	Sequence (5'-3')
β-actin-F	TGGACATCCGCAAAGACCTG
β-actin-R	CCGATCCACACGGAGTACTT
TNFRSF10A-AS1-F	TCTCAGATCACGTGACCTTGA
TNFRSF10A-AS1-R	GTGGGCAGCTCTCATCCTAA
MPZL1-F	GTTAAGCAGGCTCCTCGGAA
MPZL1-R	TCCGCATACACCACAGACTC
T-AS1-KO-F	CCAAGTTTGCAAGCACACGATTTC
T-AS1-KO-R	CTTCTCAACCTCCATTATCAGATGC

Supplementary Table 5. Antibodies used in this study

Antibodies	Source	Identifier
Anti-MPZL1	Cell Signaling Technology	Cat#9893
Anti-Cleaved Caspase-9	Cell Signaling Technology	Cat#9509
Anti-Cleaved Caspase-8	Cell Signaling Technology	Cat#8592
Anti-Cleaved Caspase-7	Cell Signaling Technology	Cat#8438
Anti-Caspase-9	Cell Signaling Technology	Cat#9508
Anti-Caspase-8	Cell Signaling Technology	Cat#9746
Anti-Caspase-7	Cell Signaling Technology	Cat#9492
Anti-Cyclin D1	Cell Signaling Technology	Cat#2922
Anti-CDK4	Cell Signaling Technology	Cat#12790
Anti-Snail	Cell Signaling Technology	Cat#3879
Anti-Slug	Cell Signaling Technology	Cat#9585
Anti-β-actin	Cell Signaling Technology	Cat#3700
Ki-67	Cell Signaling Technology	Cat#9449

Supplementary Table 6 KEGG pathways enriched by differentially expressed genes affected by T-AS1.

pathway_ID	pathway_des	Diff gene	UP genes	down genes	Diff gene	all_gene in pathway	rich factor	Pvalue	Qvalue
hsa04668	TNF signaling pathway	6	2	4	145	130	2.8255	0.0137	0.3564
hsa04115	p53 signaling pathway	4	1	3	145	74	3.3092	0.0170	0.3306
hsa04151	PI3K-Akt signaling pathway	12	4	8	145	375	1.9590	0.0250	0.3076
hsa04210	Apoptosis	6	2	4	145	153	2.4008	0.0310	0.3613
hsa05226	Gastric cancer	6	2	4	145	162	2.2674	0.0407	0.4126
hsa04630	JAK-STAT signaling pathway	6	1	5	145	167	2.1995	0.0469	0.4207
hsa05202	Transcriptional misregulation in cancer	8	4	4	145	195	2.5116	0.0127	0.3725