Supplementary Information

Supplementary Methods

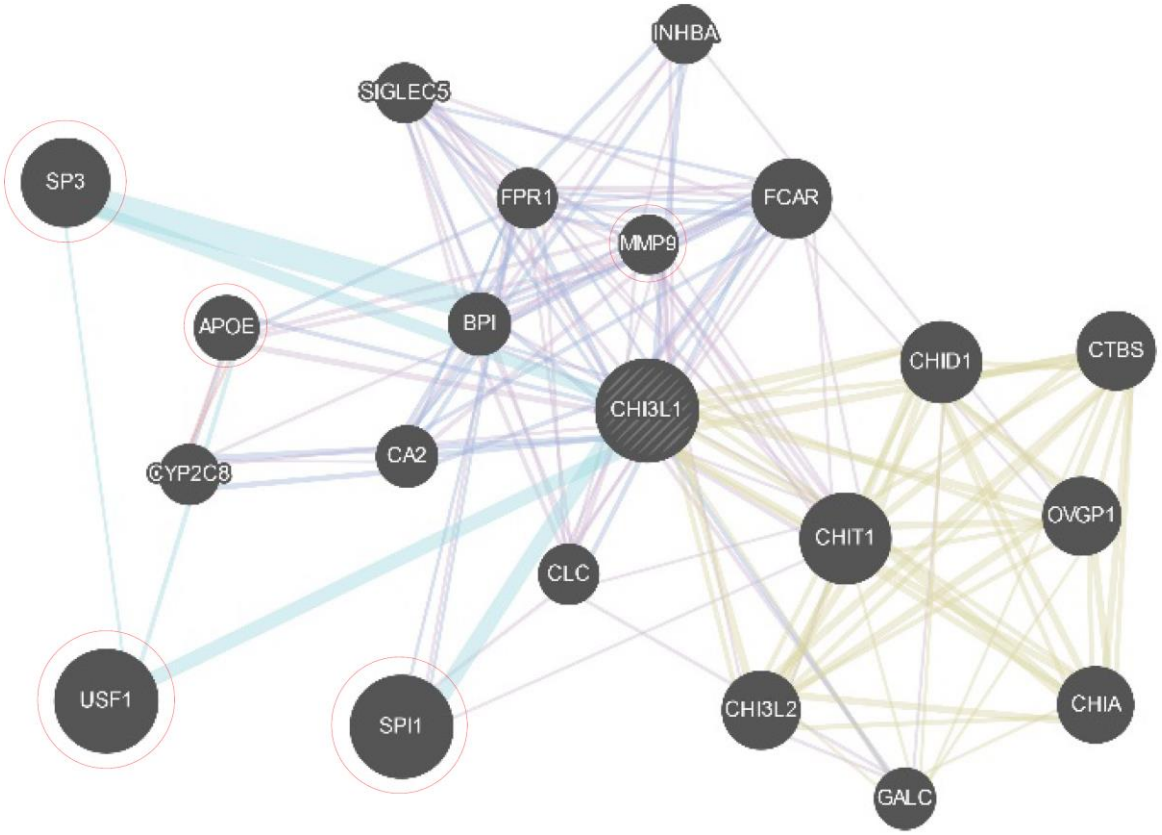
Immunohistofluorescence for relative fluorescence unit (RFU) detection

An adenoviral vector encoding mouse Chi3L1 shRNA was tagged by the red fluorescent protein gene. Lung tissues metastasized with B16F10 melanoma specimens were fixed in formalin and paraffin-embedded for examination. Sections (4 μm thick) were used for immunohistofluorescence. Paraffin-embedded sections were deparaffinized and rehydrated, washed in distilled water, mounted in Aqua-Mount with DAPI staining, and evaluated on a confocal microscope (Nanoscope Systems, Inc., Daejeon, Korea).

Wound healing migration assay

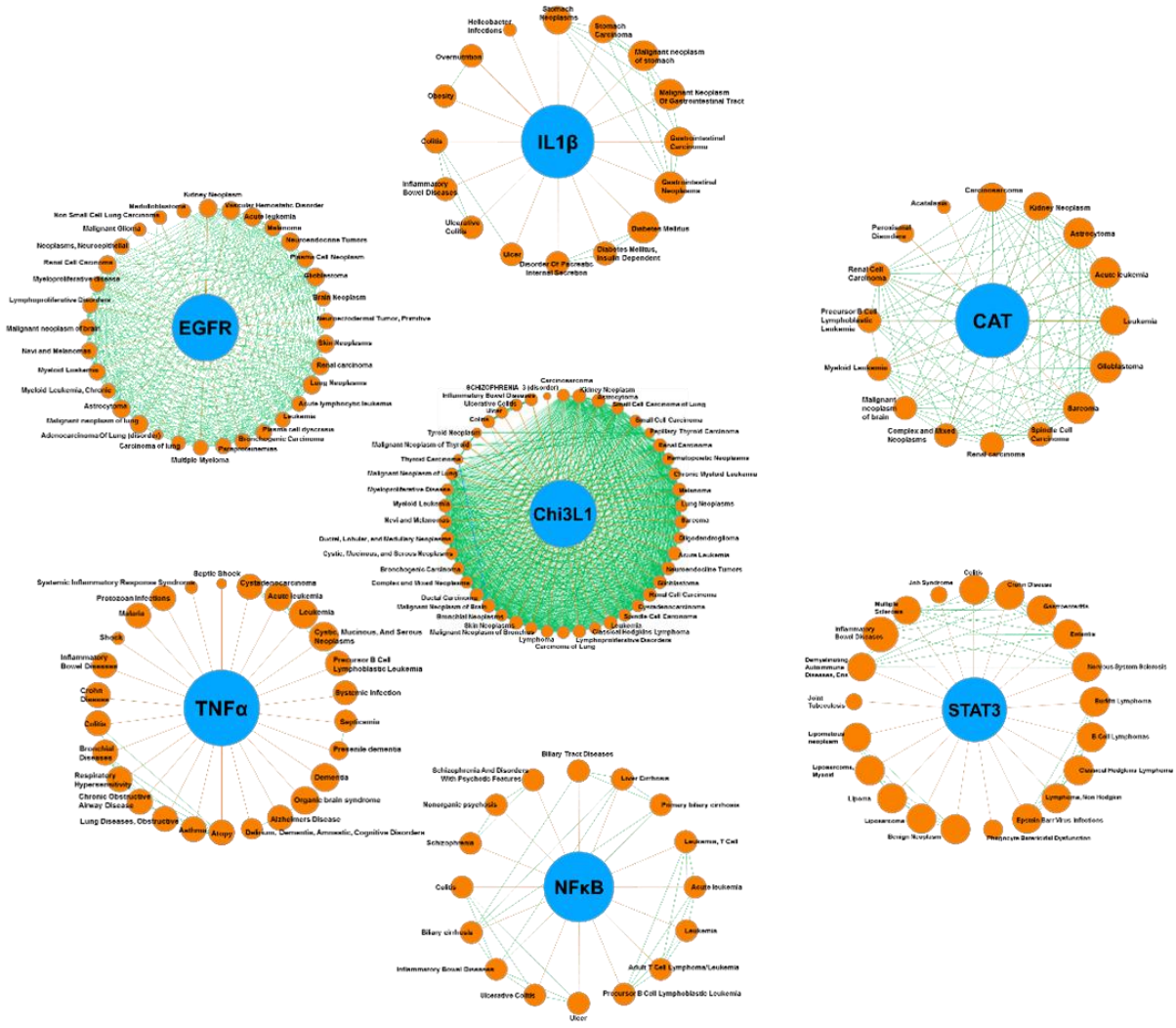
Migration of human lung cancer cells, A549 and H460, was quantified on a silicone insert (Applied BioPhysics, Inc., NY, USA). Cells were transfected with siChi3L1 or miR-125a-3p mimic before inoculation of cells (reverse transfection). Silicone inserts were discarded when the confluence of cells reached 100% and incubated at 37°C, 5% CO_2 in a humidified incubator for 12 hours. Images were captured under a light microscope (Olympus, Tokyo, Japan) at ×200 magnification and analyzed using NIH ImageJ software.

Supplementary Figures



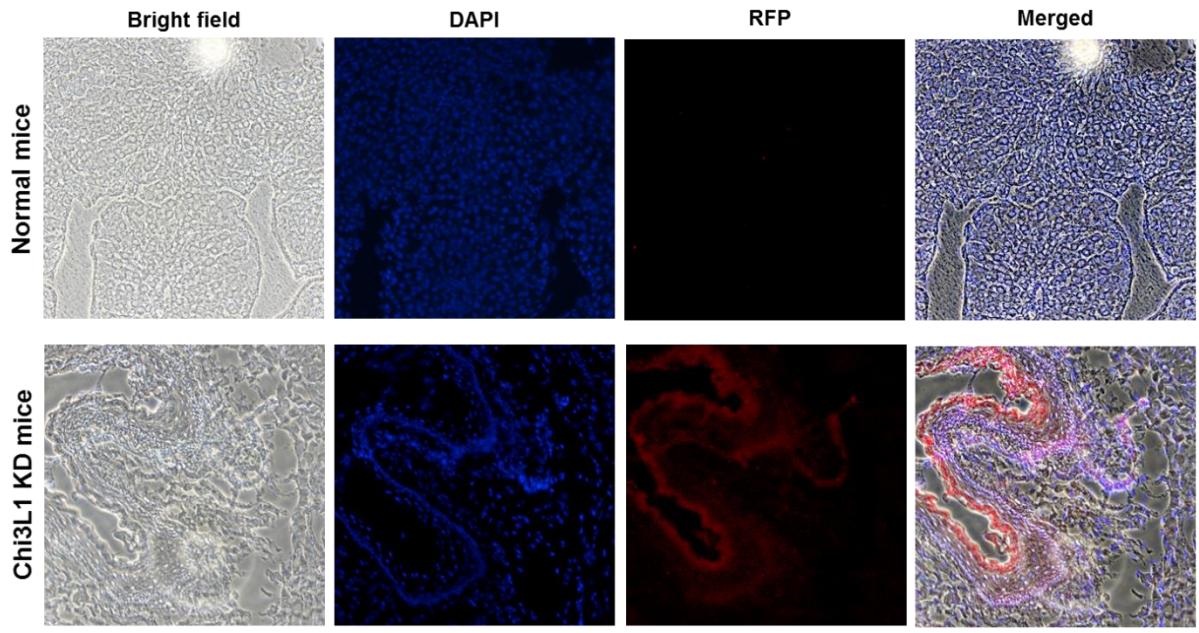
Supplementary Figure S1. Gene identifier mapping by using Biomart and Gene Expression Omnibus analysis

20 genes related to Chi3L1 based on a composite gene-gene functional interaction network. Pink lines: Physical Interactions; Purple lines: Co-expression; Blue lines: Co-localization; Cyan lines: Pathway; Yellow lines: Shared protein domains. USF1, SPI1, SP3 and APOE are related with Chi3L1 as pathway network (Cyan lines) and MMP9 is related with Chi3L1 as co-expression (Purple lines) network.



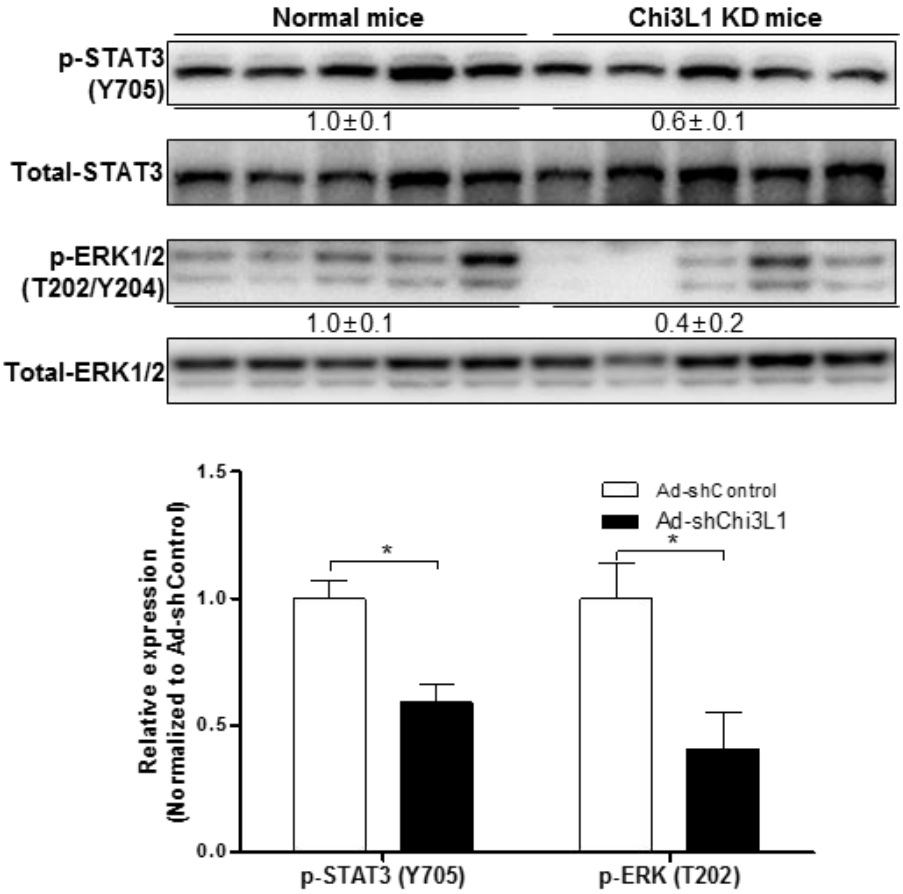
Supplementary Figure S2. Gene and disease network of representative oncogenes

Gene–disease networks were analyzed based on the GWAS/OMIM/DEG records ($p<10^{-6}$).



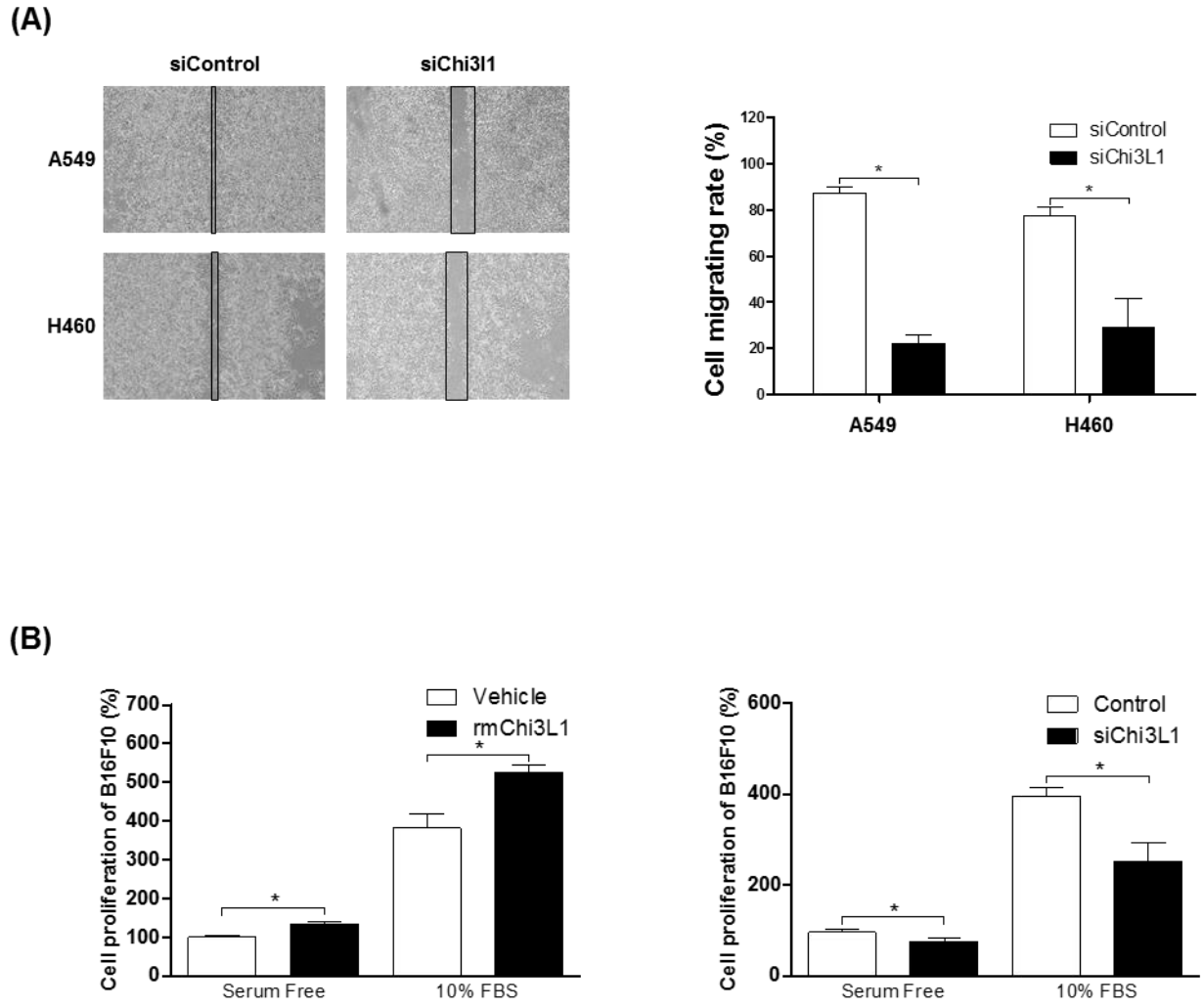
Supplementary Figure S3. Efficiency of Ad-shChi3L1 infection

Fluorescence image of Ad-shChi3L1-labeled red fluorescence units (RFU). Ad-shChi3L1-labeled red fluorescence was observed in Chi3L1 KD mice lung tissues.



Supplementary Figure S4. Effects of Chi3L1 on phospho-STAT3 and phospho-ERK in ChiL1 KD mice lung tissues

Expression of p-STAT3 and p-ERK in C57BL/6 mice lung tissues. The experiments were repeated in triplicate. Quantification of gene expression was measured and analyzed. *Significant difference from the Ad-shControl injected mice (p<0.05).

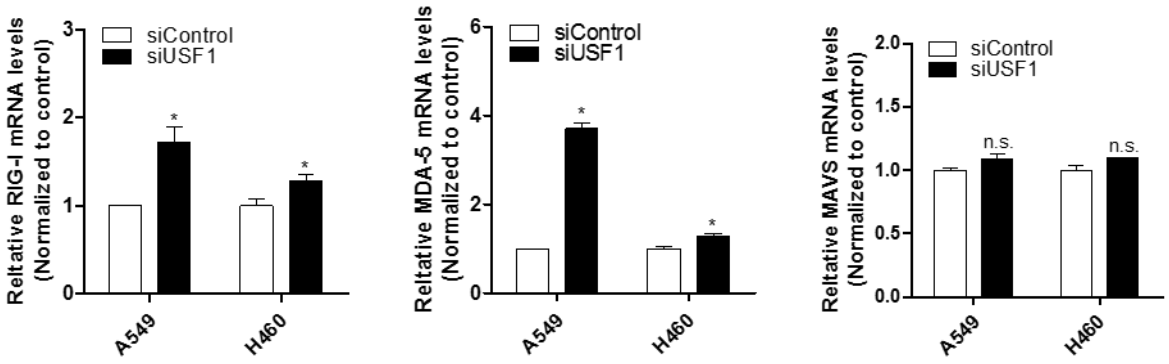


Supplementary Figure S5. Effect of Chi3L1 on migration of human lung cancer cell line

(A) Wound healing migration assay of A549 and H460 cells transfected with siChi3L1. *Significant difference from siControl-transfected cells (p<0.05).

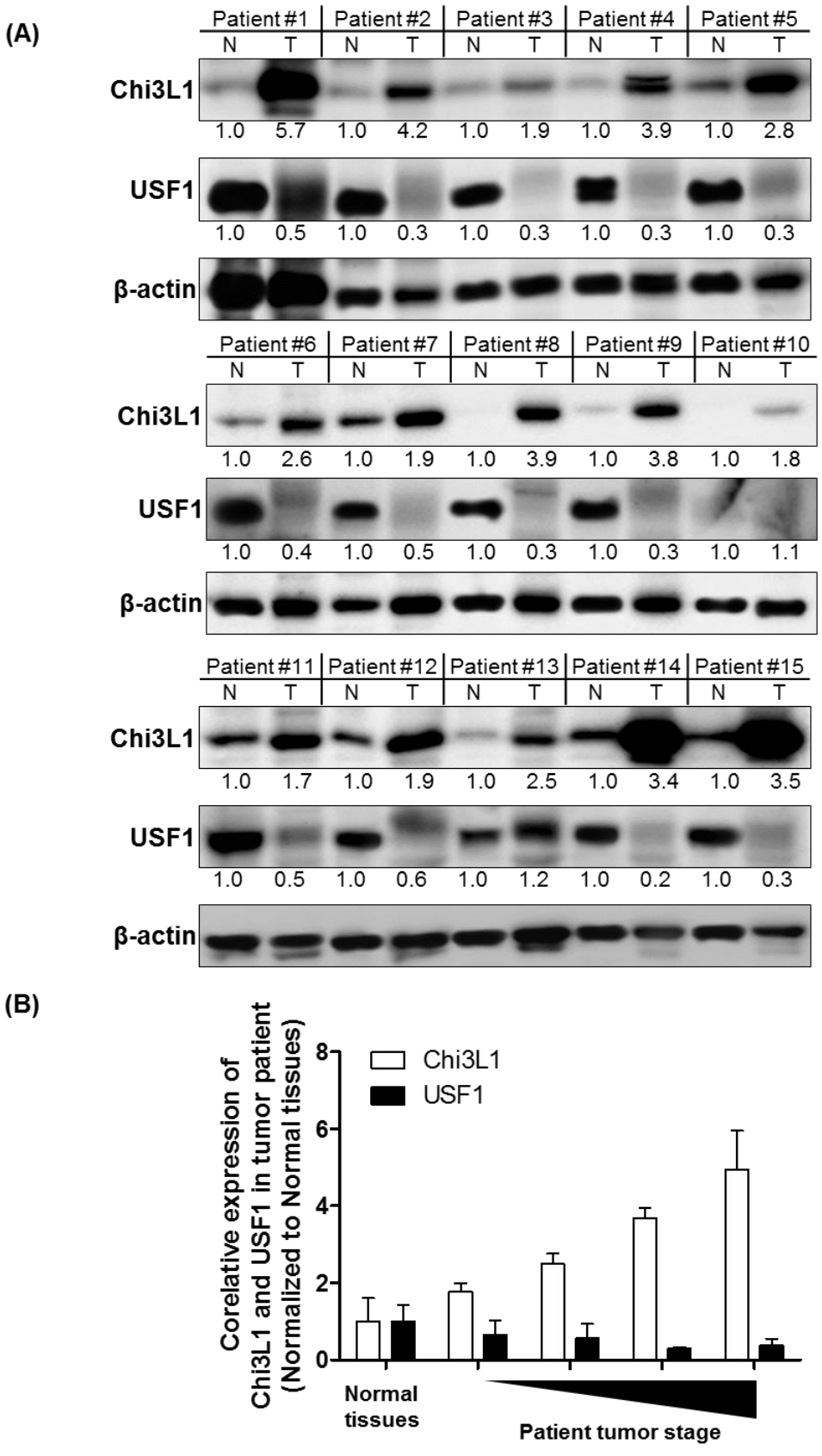
(B) Cell proliferation was measured by the MTT assay of A549 and H460 cells treated with rmChi3L1 or siChi3L1. *Significant difference from siControl-treated cells ($p < 0.05$).

All experiments were repeated in triplicate. Error bars represent SD.



Supplementary Figure S6. Effects of the RIG-like helicase activity in USF1 KD human lung cancer cells

RIG-I, MDA-5, and MAVS mRNA were measured by qRT-PCR. *Significant difference from siControl-transfected cells (p<0.05).



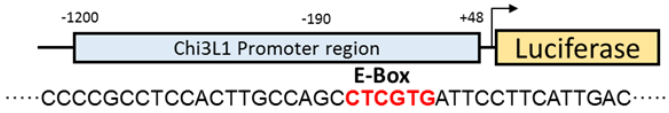
Supplementary Figure S7. Protein expression of lung tissues in lung cancer patients

(A) Expression of Chi3L1 and USF1 proteins was determined by Western blotting in the normal and tumor lung tissue of lung tumor patients (n=15).

(B) The ratio of expression levels between USF1 and Chi3L1 in 15 lung tumor patients. Patient #3, #10, #11, and #12 are stage 1. Patient #5, #6, #7, and #13 are stage 2. Patient #4, #8, #9, #14, and #15 are stage 3. Patient #1 and #2 are stage 4.

(A) Human Chi3L1 gene promoter region

AGATCTTGGA ATTCATGAAG GGAAGGCACT GGCTGAGTTT TAAAGGAAGA GAAAGGGAGA GAGGGAGAGA AGAGGGAGAG
AGCAGAGAGA CCTCACCGAG AGAGCTGCAA AACCAGCCTG GAAAAATTAG AGTATTACCT AACATTAGTG AAAAATAAAG
GTACTTTCTT GAGAAGCCCT TGGACCCATT CTGCCTCTTG GAGTTCTGAA CTTTTCACTC ACTGCCTATT AATTAATGTT
AAGCCTGCAA AGAATGGAGT TGTCCTGGAT ATTTGGCCAA AAAAAAAATG TATCCACAAA CAGGGACGTA ATCAGGCAGG
GAGCCTCGTT AAGAAGTTTT GTTCTTGTCC TAGGAGTGAT GAGAGATCAC TGAAGGATTT AGAGAGGGGC TGTATCATCA
GGCTTGGGTT CCAAAGCCTC ACTGAGAGAG TTGGGGAGCT GACTGATGTC AGATGCTCGT GCAGCCGCCC CGTAGGGCCT
GTATTTCCTC CATGGTGCCT CACTGCAGCA CCGAGCTTGC AAAAGATCCT CTCTCTTTAT GGGAATTTCA AAACAGAAGC
AAAATAGCAC CGGGGCTTAA AGCATTCTTG GGAATTTCCC TGTCTTTCCC TCTAAATAAT CAGCATGTAA ATTGCAAAAA
AAAAAAAAAA AAAAAAAAAA AAGACACGGG CCCAAAAGGG AGCGCTCAGT TTCAGGCTCT TTGCTTTCCT TCCTCCCGAG
GCTCTCTGGC CCTTACCCAG CCTGAAGACA GAAAGTGTGA GGGGGAGGGT AGGAAGGTAG GTCAAGCAGG GCAATGCTGA
GCCTGGGAAG AAAACAACAG CCTTGTTTAG GGCACTGTGG CTTACGTAAC TAAATTGTGC CCAGTTTCCA CCTGGCCAGG
GGCCTGGAGT GAATGCTGAA GATGCAAAGG TAGAGGCTGC CAGAAAAGCC AGGAAATTGC TGGCAAGAAA GGCCAGTGGT
GGGGTGCAGG AGTGGGAGGA AGGCTGGGAA ATGCGGCTGA GTCACATCTC CAGAAGCCCC CCATCATCAC CCTAGTGGCT
CTTCTGCTGG CAGGTGCCTC ATGAAGACCT GACCCAAAGT TTTCAAAACT CTGCGGTTTC TCAACCCTCC TCTGGTAATC
CATAGTACTC CCCCGCCTCC ACTTGCCAGC CTCGTGATTC CTTCATTGAC ACATAGCTCA GTTCCCATAA AAGGGCTGGT
TTGCCGCGTC GGGGAGTGGA GTGGGACAGG TATATAAAGG AAGTACAGGG CCTGGGGAAG AGGAAGCTTG GTACCGAGCT
CGGATCC



(B) Human Chi3L1 mRNA 3'UTR

CCCUCUGUUC UGCACACAGC ACGGGGGCCA AGGAUGCCCC GUCCCCCUCU GGCUCCAGCU GGCCGGGAGC CUGAUCACCU
GCCCUGCUGA GUCCCAGGCU GAGCCUCAGU CUCCCUCCCU UGGGGCCUAU GCAGAGGUCC ACAACACACA GAUUUGAGCU
CAGCCCUGGU GGGCAGAGAG GUAGGGAUGG GGCUGUGGGG AUAGUGAGGC AUCGCAAUGU AAGACUCGGG AUUAGUACAC
ACUUGUUGAU UAAUGGAAAU GUUUACAGAU CCCCAAGCCU GGCAAGGGAA UUUCUUCAAC UCCCUGCCCC CCAGCCCUCC
UUAUCAAAGG ACACCAUUUU GGCAAGCUCU AUCACCAAGG AGCCAAACAU CCUACAAGAC ACAGUGACCA UACUAAUUAU
ACCCCCUGCA AAGCCCAGCU UGAAACCUUC ACUUAGGAAC GUAAUCGUGU CCCCUAUCCU ACUUCCCCUU CCUAAUUCCA
CAGCUGCUC

Mouse Chi3L1 mRNA 3'UTR

CUCCCCCUUU CCCAUAUGGU ACCCCCACUC UCUGGCCAGG AGUUUAAUCU CUUGCAAUGU UAAGUCCCCC AACUGAGCCU
CAGUUUCUCC UUCCCUUGGC ACCUGUGUAA GGGGCCACAG CAGGCUCAGC UAUGGAGAAC AGGGAACUAG GGUAGGACGA
UGGUGGGGUU GUGAGAGUCA CAGUGUGAGC AGAUACACAA CCCUGUUAAG GAAUGCAAAU UCUCAGACUC UAACCUCCCU
UUACCCAGCC UGACCAAAGG ACACCACUUG GAUCAAGUAG GCAAAUAUCU UACAGGAUUG AGGGACCAUA CUAAUUAUAC
CCUCUGCAAA GCCCAACUUG AAUCCUUCCC UUAGGAACUU AAUCGUCCCA CUUCCCUUUC CCUAAUUCCA CAGCUGUUCA
AUAAAGCGCC AGAACCUAA

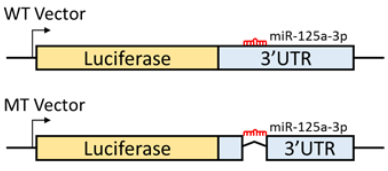
(C)

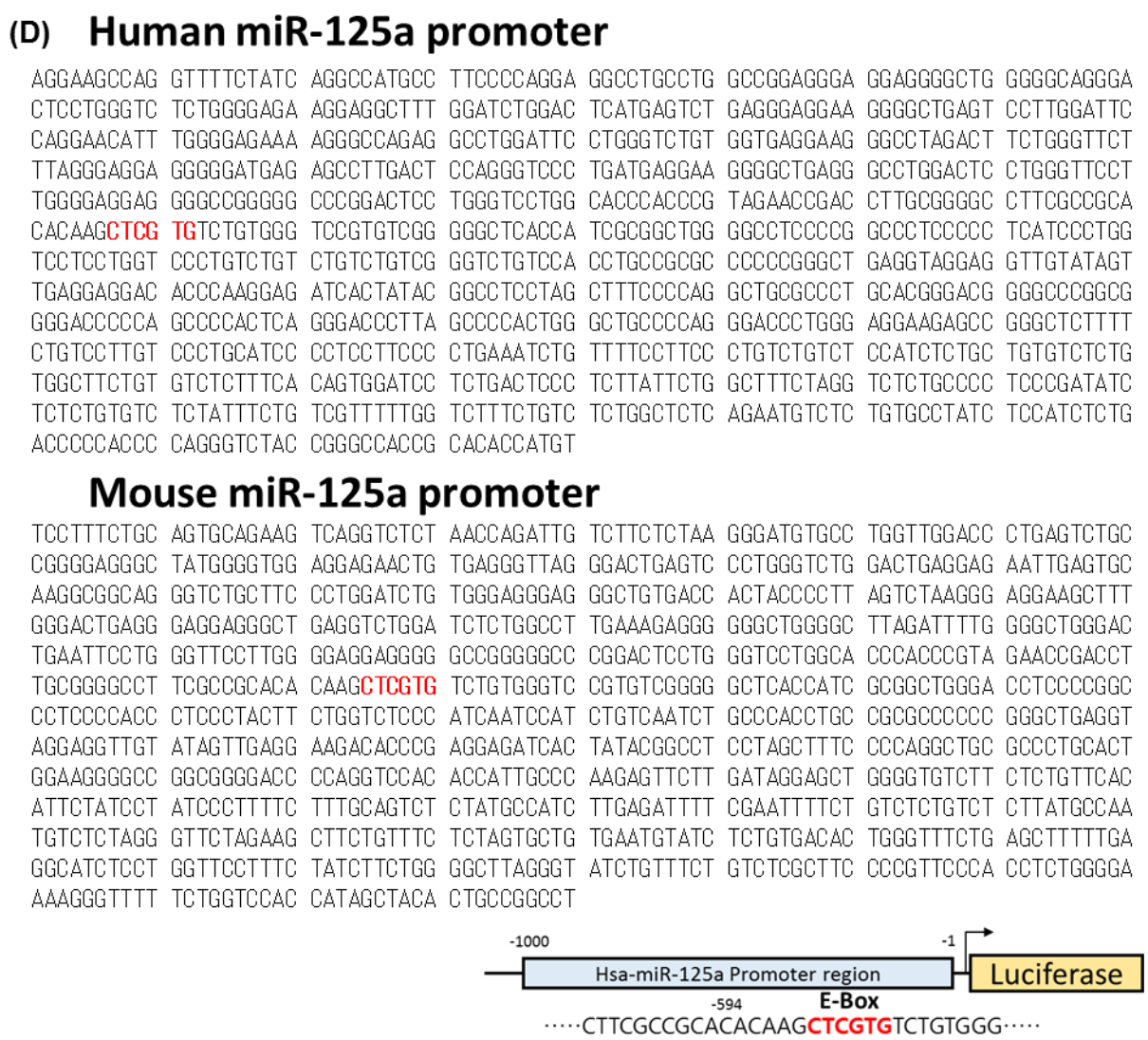
WT Vector

```
3'  ccgagGGUUCUUGG---AGUGGACa 5'  miR-125a-3p
          ||::||:||   |||||||
58:5'  gcuggCCGGGAGCCUGAUCACCUGc 3'  CHI3L1 3' UTR
```

MT Vector

```
3'  ccgagGGUUCUUGG---AGUGGACa 5'  miR-125a-3p
          ||::||:||   |||||||
58:5'  gcugg-------------------c 3'  CHI3L1 3' UTR
```





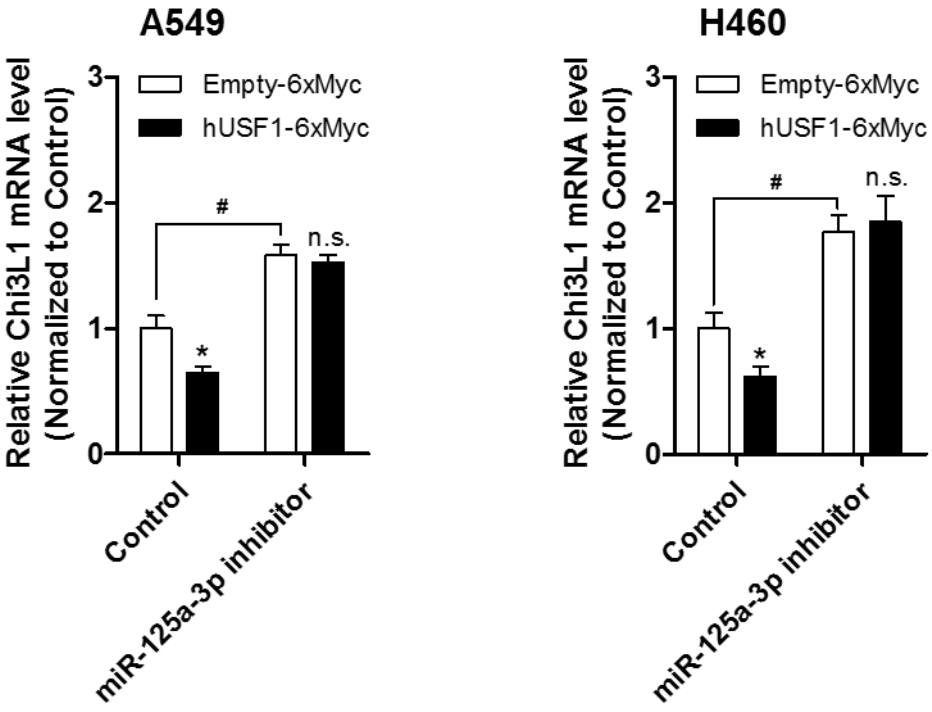
Supplementary Figure S8. Human Chi3L1 promoter region, 3'UTR of human and mouse Chi3L1 mRNA, and human miR-125a-3p promoter region mapping

(A) Chi3L1 gene promoter region. Chi3L1 promoter has pyrimidine-rich initiator elements and enhance-box (E-box) motifs. An E-box motif (CTCGTG) is located 190 bp upstream of the Chi3L1 transcription start site (bold red letters). The cloned human Chi3L1 promoter DNA fragments. This region is 1200 bases upstream of Chi3L1 transcription start site. The bHLH elements-binding site, called E-box, covers 190 base points of this region and is considered to be the USF1 binding domain.

(B) 3'UTR of matured human and mouse Chi3L1 mRNA. These gene maps show the mRNA sequences predicted to bind with miR-125a-3p (red sequences).

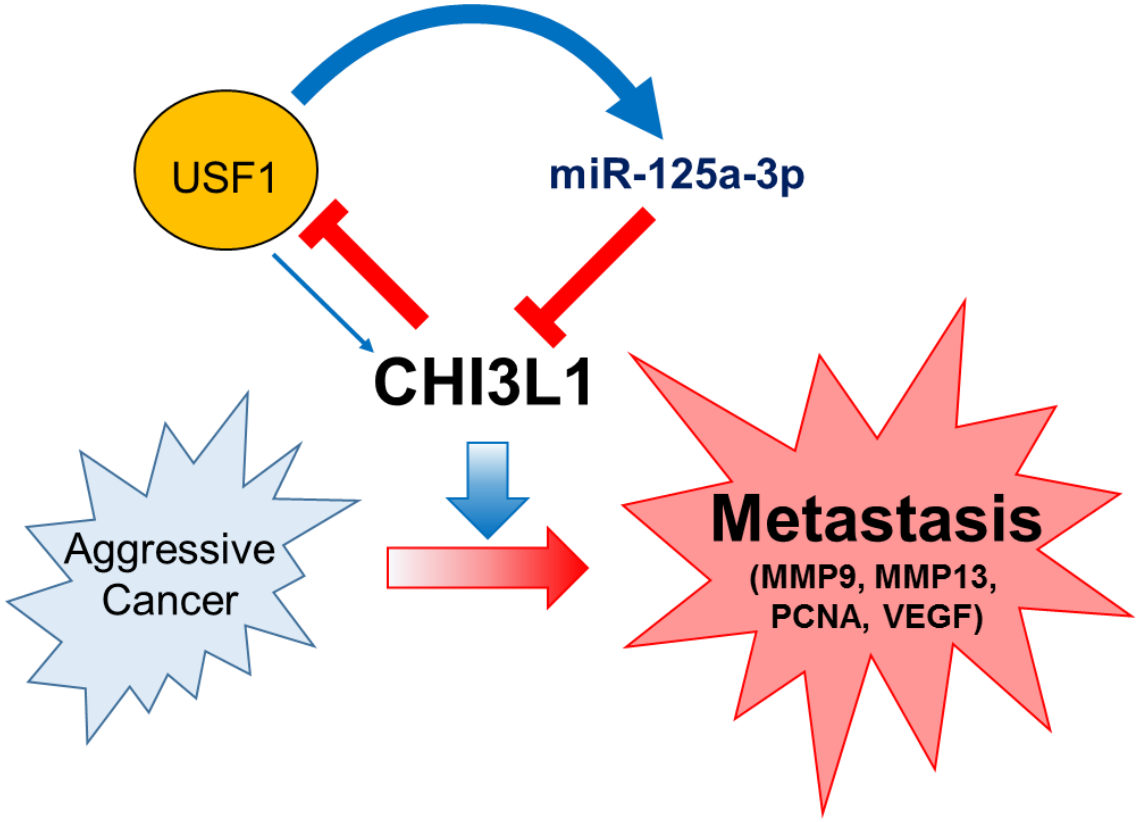
(C) Chi3L1 mRNA wild-type 3'UTR contained luciferase vector (WT Vector) and Chi3L1 mRNA miR-125a-3p binding site deleted mutant type 3'UTR contained luciferase vector (MT Vector) were subcloned and used for miR-125a-3p activity.

(D) Human and mouse *miR-125a* genes promoter (-1–1000 bp). *miR-125a* promoter has E-box motifs located at 594 bp upstream of *miR-125a* gene (bold red letters). The cloned human Chi3L1 promoter DNA fragments. This region is 1000 bases upstream of *miR-125a* gene start site. The bHLH elements-binding site, called E-box, covers 594 base points of this region and is considered to be the USF1 binding domain.



Supplementary Figure S9. Chi3L1 mRNA levels are increased and cancel the inhibition by USF1 overexpression by miR-125a-3p inhibitor

Relative expression of Chi3L1 mRNA levels in USF1-overexpressed A549 and H460 cell lines by the treatment of miR-125a-3p inhibitor. *Significant difference from empty-6×Myc vector transfected cells of control (p<0.01). $^{\#}$Significant difference between empty-6×Myc vector transfected cells of control- and empty-6×Myc vector-transfected cells of the miR-125a-3p treatment (p<0.01).



Supplementary Figure S10. Conclusions

Schematic image of the relationship among Chi3L1, USF1, miR-125a-3p, and cancer metastasis. Knock-down of Chi3L1 through siChi3L1 or miR-125a-3p significantly increases USF1 expression. Increased USF1 induces miR-125a-3p expression, but blocking USF1 expression using siUSF1 treatment inhibited miR-125a-3p. USF1 should thus strongly inhibit Chi3L1 expression through induction of miR-125a-3p. Knock-down of Chi3L1 could inhibit cancer metastasis and progression *via* miR-125a-3p-dependent increase of USF1 expression.

Supplementary Table S1. Antibody list

Resource	Source	Identifier
Anti-CHI3L1 antibody	Abcam	Cat. # ab77528
Anti-MMP9 antibody	Santa Cruz	Cat. # sc-393859
Anti-MMP13 antibody	Santa Cruz	Cat. # sc-515284
Anti-VEGF antibody	NOVUS	Cat. # NB100-664
Anti-PCNA antibody	Abcam	Cat. # ab29
Anti-USF1 antibody	Santa Cruz	Cat. # sc-390027
Anti-β-actin antibody	Santa Cruz	Cat. # sc-47778
Anti-Histone3 antibody	Cell signaling	Cat. # 4620
Anti-pSTAT3(Y705) antibody	Cell signaling	Cat. # 9131
Anti-STAT3 antibody	Cell signaling	Cat. # 9139
Anti-pERK(T202/Y204) antibody	Cell signaling	Cat. # 4376
Anti-ERK1/2 antibody	Cell signaling	Cat. # 4696

Supplementary Table S2. Primer List

Gene	Application	Primer Sequence
Chi3L1	qRT-PCR	F:GCAACACTGACTATGCTGTGG
		R:GAGTGAAGCTCCTCCCGAAG
USF1	qRT-PCR	F:CTACTGGGGAAGACCCAACC
		R:TACATCACCTGGCCCCCATTC
SPI1	qRT-PCR	F:GGAGACAGGCAGCAAGAAGA
		R:CCTTGTCCACCCACCAGATG
SP3	qRT-PCR	F:ATGACCGCTCCCGAAAAGC
		R:TCCAAGGCAGCCATTTCCTC
APOE	qRT-PCR	F:AGGAACTGAGGGCGCTGAT
		R:CGGGGTCAGTTGTTCCTCC
MMP9	qRT-PCR	F:CATTCAGGGAGACGCCCATT
		R:ACCGAGTTGGAACCACGAC
RIG-I	qRT-PCR	F:AGGAAAACTGGCCCAAAACT
		R:TTTCCCCTTTTGTCCTTGTG
MDA-5	qRT-PCR	F:GTGCATGGAGGAGGAACTGT
		R:GTTATTCTCCATGCCCCAGA
MAVS	qRT-PCR	F:GCAGCAGAAATGAGGAGACC
		R:AAAGGTGCCCTCGGACTTAT
18s	qRT-PCR	F:AGGAATTGACGGAAGGGCACCA
		R:GTGCAGCCCCGGACATCTAAG
Human Chi3L1 promoter-USF1 binding site	ChIP-qPCR	F: CCCGTAGGGCCTGTATTTCC
		R: GAAGGAATCACGAGGCTGGC

Supplementary Table S3. Oligonucleotide list

Resource	Source	Identifier
CHI3L1 (ID 1116) Trilencer-27 Human siRNA	ORIGENE	Cat. # SR300798
CHI3L1 (ID 12654) Trilencer-27 Mouse siRNA	ORIGENE	Cat. # SR412856
Control siRNA-A	Santacruz	Cat. # sc-37007
Human USF1 siRNA	Santacruz	Cat. # sc-36783
*mir*Vana™ miRNA mimic, Negative Control #1	Thermo Fisher	Cat. # 4464058
*mir*Vana® miRNA mimic hsa-miR-125a-3p	Thermo Fisher	Cat. # MC12378
*mir*Vana® miRNA mimic hsa-miR-24-3p	Thermo Fisher	Cat. # 4464066
Ambion™ In Vivo Negative Control #1 siRNA	Thermo Fisher	Cat.#: 4457287
Ambion™ In Vivo Pre-Designed siRNA against USF1	Thermo Fisher	Cat.#: 4457308

Supplementary Table S4. Material resources for experiment

Resource	Source	Identifier
Recombinant mouse Chi3L1, CF	R&D systems	Cat. # 2649-CH
Recombinant human Chi3L1, CF	R&D systems	Cat. # 2599-CH
Lipofectamine® RNAiMAX Reagent	Thermo Fisher	Cat. # 13778030
Lipofectamine™ 3000 Reagent	Thermo Fisher	Cat. # L3000001
Invivofectamine™ 3.0 Reagent	Thermo Fisher	Cat.#: IVF3005
QuantiNova™ SYBR Green PCR Kit	QIAGEN	Cat. # 208052
High-Capacity cDNA Reverse Transcription Kits	Thermo Fisher	Cat. # 4368813
miRNeasy Mini Kit	QIAGEN	Cat. # 217004
easy-BLUE™ Total RNA Extraction Kit	iNtRON	Cat. # 17061
miScript II RT Kit	QIAGEN	Cat. # 218160
miScript® SYBR® Green PCR Lit	QIAGEN	Cat. # 218073
miScript Primer Assays (has-miR-125a-3p)	QIAGEN	Cat. # MS00008554
miScript Primer Assays (mms-miR-125a-3p)	QIAGEN	Cat. # MS00011088
miScript Primer Assays (mms-miR-342-3p)	QIAGEN	Cat. # MS00002184
miScript Primer Assays (RNU6B)	QIAGEN	Cat. # MS00033740
Secrete-Pair™ Dual Luminescence Assay Kit	GeneCopoeia™	Cat. # SPDA-D010
SimpleChIP® Plus Enzymatic Chromatin IP Kit	Cell Signaling	Cat. # 9004
pO6A5 shuttle vector with U6 promoter	Sirion	Cat. # SB-P-AV-104-01
Lung tissue from human lung cancer patients and general public donors	US Biomax	Cat. # LC1503; Cat. # LC241b
B16F10	ATCC	ATCC® CRL-6475™
A549	ATCC	ATCC® CCL-185™
H460	ATCC	ATCC® HTB-177™