SUPPLEMENTARY MATERIALS AND METHODS

Cell culture

Human LC cell lines (A549, H441, H1792, H1975, H520, H1703, and H2170) were obtained from the American Type Culture Collection (ATCC). LC cells were cultured in RPMI-1640 medium (Invitrogen), supplemented with 10% (volume/volume) heat-inactivated fetal bovine/serum (FBS; Sigma Aldrich, St. Louis, MO), 100 U/ml of penicillin G sodium and 100 μg/ml of streptomycin sulfate (Invitrogen). NHTBE cells were obtained from the Lonza Walkersville, Inc. and cultured in BEGM™ with provided supplements. The 1198 human bronchial epithelial cell line was obtained from Dr. R. Lotan (The University of Texas M. D. Anderson Cancer Center, Houston, TX) and Dr. A. Klein-Szanto (Fox Chase Cancer Center, Philadelphia, PA) and grown in Keratinocyte Serum-Free Medium (Life Technologies, Inc., Gaithersburg, MD) containing epidermal growth factor and bovine pituitary extract. Human lung fibroblasts cell lines (MRC5, BJ1, and WI38) were obtained from the ATCC and were cultured in DMEM, supplemented with 10% (volume/volume) heat-inactivated FBS, 100 U/ml of penicillin G sodium and 100 μg/ml of streptomycin sulfate. All cells have been passaged directly from original low-passage stocks and were used before passage 30. The cells were also tested within the last three months for correct morphology by microscope and tested to detect mycoplasma contamination using a MycoAlert mycoplasma detection kit (Lonza Walkersville, Inc.). All of the cells were cultured at 37°C in humidified atmosphere of 95% air and 5% CO_2.

Antibodies/Chemicals

NASTRp (N6125) and monoclonal anti-β-actin antibody (A2228) were purchased from Sigma Aldrich. Rabbit polyclonal antibody against E2F1 (NM100-92030) and mouse monoclonal E2F8 Antibody (3E9-2F5) were purchased from Novus Biologicals. Anti-E2F2 (ab-138515) and anti-E2F8 (ab-109596) were obtained from Abcam. Rabbit polyclonal antibody against UHRF1 (A301-470A) was

purchased from Bethyl Laboratories, Inc. Anti-PCNA (2586), anti-cyclin A2 (4656), anti-cyclin B1 (4138), and anti-cyclin E2 (4132) were obtained from Cell Signaling Technology. Rabbit monoclonal cyclin D1 antibody (2261-1) was purchased from Epitomics.

Knockdown of Genes

Silencer E2F siRNAs were purchased from Santa Cruz or Invitrogen; E2F1 siRNA (sc-29297, Santa Cruz Biotechnology), E2F2 siRNA (s4409, Invitrogen), E2F8 siRNA-1 (31292, Invitrogen), and E2F8 siRNA-2 (31481, Invitrogen). BLOCK-it Fluorescent Oligo (Invitrogen) was used as a control. Each siRNA was transfected using Lipofectamine RNAiMAX (Invitrogen). Sequences targeted by E2F8 shRNA or UHRF1 shRNA are listed in the Supplementary Table 4. HEK293T cells were plated in 10 cm dishes and transfected 24 h later with DNA from each lentiviral vector (Sigma Aldrich) and packaging plasmids (VSVG and dR8.91) according to Lipofectamin 2000 (Invitrogen) protocol. Medium was changed 24 h after transfection, the viral supernatant was harvested for the subsequent 48 h and filtered using 0.45 μm filters. NHTBE, human fibroblasts, and LC cell lines were infected with lentiviral supernatant with polybrene (Sigma-Aldrich, 8 μg/ml) and the cells were selected 48 h later with 1~2 μg/ml puromycin (MP Biomedicals).

Cell Proliferation

Proliferation of cells was evaluated by the cell-counting method or the MTT assay. After cells were transfected with siRNAs for 48 h, cells were harvested by trypsinization and counted using Trypan blue staining. For MTT assay, cells transfected with siRNAs for 24 h were transferred to the 96-well plates to allow growing for further 48 h. The cells were incubated with MTT (final concentration 0.5 mg/ml) for 4 h at 37°C incubator. Following MTT incubation, 150 μl of 100% DMSO was added to dissolve the crystals. Viable cells were counted by reading the absorbance at 570 nm using a microplate

reader SpectraMax (Molecular Devices).

Overexpression of gene in cells

The 1198-E2F8 and A549-E2F8 was created using PiggyBac Transposon system (Systembio Science, Inc). The gene of *E2F8* was obtained by OriGene, amplified by PCR, and sub-cloned into PB-CMV-MCS vector (PB513B-1, System Bioscience, Inc.). The construct was transfected with Super PiggyBac Transpotase expression vector (PB200PA-1, System Bioscience, Inc.) to 1198 and A549 cells using Lipofectamine 2000 (Invitrogen) and selected using puromycin (1 ~ 2 μg/ml).

Colony Formation Assay

At 48 h after transfection by the indicated siRNAs, 2 x 10^3 cells were transferred in the 6-well plates and allowed to grow for 7-14 days. Medium was removed, fixed with 10% formalin for 15 min and followed by staining with crystal violet to visualize the colonies.

Transwell Migration Assay

At 72 h after transfection by the indicated siRNAs, cells were trypsinized, and 5 x 10^4 cells were seeded on the transwell inserts with 8 μm micropore filters (Corning Costar) in 500 μl medium. Medium containing 10% FBS was added to the lower chamber as a chemoattractant. After 24 h, cells on the upper side of the filter were removed with a cotton swab after fixation and staining of the cells. Two or three random fields were imaged per transwell insert and the number of cells that had migrated to the bottom side of the membrane was counted using the particle counting module of ImageJ. Each assay was repeated in three independent experiments.

Quantitative Real-time PCR

Total RNA was purified from cells using an RNeasy Mini Kit (Qiagen). Reverse transcription of total RNA was performed using the M-MLV reverse transcriptase (Promega). Quantitative PCR was performed using *SYBR* Green PCR Core Reagents (Applied Biosystems) and iCycler thermal cycler (Bio-Rad Laboratories). Primer sequences are listed in the Supplementary Table 4 (available online).

Western Blot Analysis

Standard SDS-PAGE and western blotting procedures were used to analyze the expression of various proteins. Whole cell lysates from each of the LC cell lines tested were prepared using SDS lysis buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 0.02% bromophenol blue) containing protease inhibitors and phosphatase. All proteins were visualized using a horseradish peroxidase-conjugated secondary antibody and Amersham ECL™ Western Blotting Detection Reagents (GE Healthcare Life Sciences).

Chromatin Immunoprecipitation (ChIP)

The SimpleChIP Enzymatic kit (Cell Signaling) was used as described by the manufacturer. PCR was performed with primers specific for the indicated promoter regions and the reactions were performed in triplicate and 1% of total input sample was used as a control. Primer sequences are listed in the Supplementary Table 4 (available online).

ChIP sequencing

For ChIP-seq, two replicates and two controls (IgG and input) were used. Sequences were carried on a HiSeq 2000 generating 76 bp single-end reads. The first 2 and last 4 nucleotides were trimmed with fastx-toolkit (unpublished, http://hannonlab.cshl.edu/fastx_toolkit/index.html) to remove low quality bases. Trimmed reads were mapped to the human reference genome (hg19) using BWA-

MEM (1). Only reads with mapping quality scores equal or higher than 20 were kept. Peak and motif finding was performed using HOMER (2). For peak finding, we used transcription factor mode requiring each putative peak to have at least 2-fold normalized tags than input or IgG control samples. Putative peaks were defined with at least 10 tags. In addition, only peaks that were significant in both replicates compared with both controls were reported. Peaks were annotated to gene products using scripts in HOMER by identifying the nearest transcription start site (TSS). Motifs of length 8, 10, and 12 bp were identified for the significant peaks.

Comet assay

The alkaline comet assay was done according to the manufacturer's instruction (Cell Biolabs, Inc.). Briefly, siRNA-treated cells were pelleted and resuspended in ice-cold PBS (1 x 10^5/mL). The cells were combined with Comet agarose at 1:10 ratio (v/v) and immediately transferred onto the OxiSelect™ Comet Slide (75 µl/well) at 4ºC in the dark for 15 min. After 15 min, the slide was transferred into pre-chilled lysis buffer for 1 h at 4ºC in the dark. Next, the slide was transferred into pre-chilled alkaline solution for 30 min at 4ºC in the dark. The slide was moved to a horizontal electrophoresis chamber, filled with cold alkaline electrophoresis solution, and voltage was applied for 30 min at 1 volt/cm. Then, the slide was transferred into pre-chilled DI H_2O for 2 min, aspirated, and then repeated twice more. After the final wash, cold 70% ethanol was added on the slide for 5 min and removed. Once the agarose and slide was completely dry, diluted Vista green DNA dye (100 µl/well) was added and incubated at room temperature for 15 min, followed by microscopy using a FITC filter. DNA damage was quantified in at least 12 randomly selected comets per slide as the Tail Extent Moment variable (the percentage of DNA in the tail x the tail length) using Comet Assay IV software (http://www.perceptive.co.uk/cometassay).

TUNEL assay

TdT-mediated dUTP Nick End Labeling (TUNEL) assay was performed using ApopTag Fluorescein Direct *In Situ* Apoptosis Detection Kit (EMD Millipore). LC cells were transfected with siRNAs for 72 h, followed by fixation with 1% paraformaldehyde in PBS, pH 7.4. Cells were washed with PBS, applied in an equilibration buffer, and incubated with TdT enzyme in a humidified chamber at 37ºC for 1 h. Finally, the cells were incubated in working strength stop/wash buffer for 10 min and mounted with ProLong® Gold Antifade Reagent with DAPI (Invitrogen).

Luciferase Reporter Assay

Initially, *UHRF1*-promoter (GeneCopoeia) was sub-cloned into pGL3-Enhancer (Promega). The cells were transfected with pGL3-*UHRF1*-promoter construct and Renilla vector using Lipofectamine 2000. Luciferase activity or Renilla activity was determined by using a Dual-Glo luciferase assay kit (Promega) and followed the manufacturer's instruction.

Immunostaining

Primary antibodies against E2F8 (H00079733-M01, Novus), UHRF1 (A301-470A, Bethyl Laboratories, Inc), and PCNA (2586, Cell Signaling Technology) were used. For immunofluorescence, detection of primary antibodies was done using fluorescent conjugates of Alexa Fluor® 488 antibody (Invitrogen) along with ProLong® Gold Antifade Reagent with DAPI (Invitrogen). Before staining of fixed paraffin-embedded tissues, we followed the standard protocol including the steps of deparaffinization, antigen retrieval, and permeabilization.

Analysis by Metacore™

The set of genes affected by E2F8 siRNA in LC cell lines were uploaded into the

MetaCore+MetaDrug® version 6.16 (GeneGo, Inc.). MetaCore™ is a web-based computational platform primarily designed for the analysis of experimental data (https://portal.genego.com). A list of affected genes by E2F8 knockdown was analyzed for relative enrichment for GO processes in MetaCore™ and the results were ranked by p-value. The output p-values reflect scoring, prioritization, and statistical significance of networks according to relevance of input data.

Flow Cytometry

For cell cycle flow cytometry, the cells were fixed in 70% ethanol and stained with propidium iodine staining (BD Pharmingen) for DNA content. Apoptosis was measured using the FITC Annexin V Apoptosis Detection Kit (BD Pharmingen) following the manufacturer's instruction.

SUPPLEMENTARY TABLES

Supplementary Table 1. Genes listed in the heatmap

Symbol * (From the left)	Category
CDC23 /// KIF20A	Cell cycle DNA replication Kinetochore DNA repair (Downregulated by NASTRp)
DTL	
ASF1B	
MYBL2	
ANLN	
NCAPD2	
TK1	
UBE2T	
GAS2L3	
CCNA2	
MCM3	
MELK	
RFC3	
DLGAP5	
KIF11	
BUB1B /// PAK6	
NCAPH	
BRI3BP	
ARHGAP11A	
HJURP	
CCND3	
SKA2	
TYMS	
AURKA	
CCNB1	
MKI67	
KIF23	
PRC1	
C15orf42	
ZWINT	
CDK1	
PTTG1	
MCM2	
ATAD2	
MAD2L1	
CDK2	
TMEM14A	
CKAP2L	
MCM6	
NCAPG2	
ASPM	
CENPK	
KIAA1524	
SGOL1	

CDC45	
CENPI	
MCM5	
PLK4	
WDHD1	
DEPDC1	
FBXO5	
CDCA3	
CENPE	
SGOL2	
CSNK1G1 /// KIAA0101	
FAM64A	
MCM10	
BRIP1	
NUF2	
EXO1	
TTK	
XRCC2	
MCM4	
RRM2	
FAM83D	
UHRF1	
ESCO2	
PCNA /// PCNA-AS	
GINS1	
NEIL3	
SHCBP1	
CCPG1	**Cell death** **Cell cycle arrest** (Upregulated by NASTRp)
CTAGE5	
PABPC1L	
PTPDC1	
DNAJB9	
LPXN	
ACAD11 /// NPHP3	
C5orf41	
C6orf48	
DDIT4	
CEBPG	
WIPI1	
ASNS	
GTPBP2	
FAM129A	
LAMP3	
SESN2	
C20orf69 /// PCMTD2	
JMY	
PCK2	
NCRNA00219	
SLC1A4	
PPP1R15A	

FBXO25 /// LOC728323	
TRIB3	
ARHGEF2	
SLC6A9	
CHAC1	
DDIT3	
HIST1H2AH	**DNA repair Nucleus** (Downregulated by NASTRp)
POLQ	
FANCM	
POP1	
ERCC6L	
CNIH2	
CDC7	
CENPW	
CTSL2	
KIFC1	
NRM	
CDKN2C	**Transcription corepressor activity** (Upregulated by NASTRp)
LOC375010	
BEST1	
INHBE	
ATF3	
C6orf225 /// TUBE1 /// WISP3	
RAD54L	**DNA packaging Cell proliferation Nucleus** (Downregulated by NASTRp)
TCF19	
CDC25A	
CLSPN	
DSCC1	
DNA2	
OIP5	
HIST1H4A	
ORC1	
SPC25	
E2F8	
HELLS	
GINS4	
ZNF367	
FAM111B	
CCNE2	
SPC24	
LMNB1	
GTSE1	
DEPDC1B	
KNTC1	
C11orf82	
POLA2	
FEN1	
SLC39A10	

GMNN	
RAD51	
ATAD5	
RAD51AP1	
DUT	
ESPL1	
CCNF	
TACC3	
HIST1H3A	
PBK	
BIRC5	
KIF18B	
KIF14	
KIF20B	

Supplementary Table 2. DAVID-down/upregulated gene sets by NASTRp treatment

Downregulated Pathways

Category	Term	P-Value
GOTERM_BP_FAT	GO:0000279~M phase	5.32E-038
GOTERM_BP_FAT	GO:0007049~cell cycle	9.97E-035
SP_PIR_KEYWORDS	cell division	1.23E-033
GOTERM_BP_FAT	GO:0007067~mitosis	6.04E-032
GOTERM_BP_FAT	GO:0048285~organelle fission	3.27E-031
GOTERM_CC_FAT	GO:0044427~chromosomal part	2.50E-021
GOTERM_BP_FAT	GO:0006260~DNA replication	3.96E-019
GOTERM_BP_FAT	GO:0006259~DNA metabolic process	5.04E-017
GOTERM_CC_FAT	GO:0000775~chromosome, centromeric region	2.10E-016
SP_PIR_KEYWORDS	kinetochore	1.29E-013
GOTERM_BP_FAT	GO:0007051~spindle organization	4.80E-012
GOTERM_BP_FAT	GO:0006974~response to DNA damage stimulus	2.13E-009
GOTERM_BP_FAT	GO:0006323~DNA packaging	6.65E-009
GOTERM_CC_FAT	GO:0015630~microtubule cytoskeleton	2.29E-008
GOTERM_BP_FAT	GO:0051276~chromosome organization	2.50E-008
GOTERM_BP_FAT	GO:0065004~protein-DNA complex assembly	2.72E-008
GOTERM_BP_FAT	GO:0000075~cell cycle checkpoint	2.72E-008
GOTERM_BP_FAT	GO:0006281~DNA repair	2.08E-007
GOTERM_BP_FAT	GO:0007126~meiosis	5.56E-007
INTERPRO	IPR019821:Kinesin, motor region, conserved site	2.30E-005
GOTERM_MF_FAT	GO:0003777~microtubule motor activity	1.18E-004
GOTERM_CC_FAT	GO:0005577~fibrinogen complex	1.21E-004
GOTERM_MF_FAT	GO:0004386~helicase activity	9.45E-004

Upregulated Pathways

Category	Term	P-Value
KEGG_PATHWAY	hsa04060:Cytokine-cytokine receptor interaction	0.0004

GOTERM_BP_FAT	GO:0034976~response to endoplasmic reticulum stress	0.0019
GOTERM_BP_FAT	GO:0042981~regulation of apoptosis	0.0052
GOTERM_BP_FAT	GO:0007050~cell cycle arrest	0.0066
GOTERM_BP_FAT	GO:0034620~cellular response to unfolded protein	0.0100
GOTERM_BP_FAT	GO:0008285~negative regulation of cell proliferation	0.0158
GOTERM_BP_FAT	GO:0006836~neurotransmitter transport	0.0223
GOTERM_BP_FAT	GO:0006984~ER-nuclear signaling pathway	0.0265
GOTERM_BP_FAT	GO:0009404~toxin metabolic process	0.0357
GOTERM_MF_FAT	GO:0005125~cytokine activity	0.0362

Supplementary Table 3. GSEA-Gene sets negatively enriched by NASTRp

A. Curated gene sets

GENE SET NAME	ES	NES	NOM p-value	FDR q-value
FURUKAWA_DUSP6_TARGETS_PCI35_DN	-0.87	-2.19	0	0.007
SHEPARD_BMYB_MORPHOLINO_DN	-0.67	-2.14	0	0.014
ZHENG_GLIOBLASTOMA_PLASTICITY_UP	-0.72	-2.13	0	0.012
CHIANG_LIVER_CANCER_SUBCLASS_PROLIFERATION_UP	-0.80	-2.12	0	0.010
VECCHI_GASTRIC_CANCER_EARLY_UP	-0.72	-2.09	0	0.012
WHITFIELD_CELL_CYCLE_G2	-0.74	-2.07	0	0.011
BASAKI_YBX1_TARGETS_UP	-0.74	-2.06	0	0.010
VANTVEER_BREAST_CANCER_METASTASIS_DN	-0.74	-2.05	0	0.010
LINDGREN_BLADDER_CANCER_CLUSTER_1_DN	-0.64	-2.05	0	0.010
SARRIO_EPITHELIAL_MESENCHYMAL_TRANSITION_UP	-0.85	-2.04	0	0.010
SENGUPTA_NASOPHARYNGEAL_CARCINOMA_UP	-0.69	-2.03	0	0.010
SHEDDEN_LUNG_CANCER_POOR_SURVIVAL_A6	-0.74	-2.03	0	0.009
OLSSON_E2F3_TARGETS_DN	-0.79	-2.02	0	0.010
PID_E2F_PATHWAY	-0.74	-2.01	0	0.010
PUJANA_BREAST_CANCER_LIT_INT_NETWORK	-0.74	-2.00	0	0.010
AFFAR_YY1_TARGETS_DN	-0.67	-2.00	0	0.010
TANG_SENESCENCE_TP53_TARGETS_DN	-0.91	-1.99	0	0.010
KOBAYASHI_EGFR_SIGNALING_24HR_DN	-0.87	-1.99	0	0.010
LEE_LIVER_CANCER_SURVIVAL_DN	-0.64	-1.97	0	0.010
CHANG_CYCLING_GENES	-0.89	-1.97	0	0.010
KAUFFMANN_DNA_REPLICATION_GENES	-0.72	-1.95	0	0.011
LINDGREN_BLADDER_CANCER_CLUSTER_3_UP	-0.72	-1.95	0	0.011
BOYAULT_LIVER_CANCER_SUBCLASS_G23_UP	-0.77	-1.95	0	0.011
GEORGES_CELL_CYCLE_MIR192_TARGETS	-0.74	-1.95	0	0.011
MARKEY_RB1_ACUTE_LOF_DN	-0.80	-1.95	0	0.011
MATZUK_MEIOTIC_AND_DNA_REPAIR	-0.66	-1.95	0	0.011
MORI_MATURE_B_LYMPHOCYTE_DN	-0.70	-1.95	0	0.011
PAL_PRMT5_TARGETS_UP	-0.68	-1.95	0	0.011
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	-0.86	-1.94	0	0.011
WHITFIELD_CELL_CYCLE_S	-0.70	-1.94	0	0.011

B. Oncogenic driver signatures

GENE SET NAME	ES	NES	NOM p-value	FDR q-value
RB_P107_DN.V1_UP	-0.83	-2.07	0	0.007
VEGF_A_UP.V1_DN	-0.60	-2.01	0	0.007
E2F1_UP.V1_UP	-0.63	-1.94	0	0.007
RB_P130_DN.V1_UP	-0.53	-1.87	0	0.008
PRC2_EZH2_UP.V1_UP	-0.61	-1.85	0	0.008
RB_DN.V1_UP	-0.55	-1.77	0	0.010
SRC_UP.V1_DN	-0.51	-1.67	0	0.016
EGFR_UP.V1_DN	-0.47	-1.63	0	0.022
ERB2_UP.V1_DN	-0.42	-1.53	0	0.053
MTOR_UP.V1_UP	-0.42	-1.47	0	0.091

C. Transcription factor targets

GENE SET NAME	ES	NES	NOM p-value	FDR q-value
V$E2F_Q6_01	-0.67	-2.03	0	0.001
V$E2F_Q3	-0.68	-2.03	0	0.001
V$E2F_03	-0.67	-2.01	0	0.001
V$E2F1_Q6_01	-0.68	-2.01	0	0.001
V$E2F1_Q3	-0.69	-2.01	0	0.001
V$E2F_Q4_01	-0.66	-2.00	0	0.001
V$E2F4DP1_01	-0.71	-1.99	0	0.001
V$E2F1_Q6	-0.72	-1.99	0	0.001
SGCGSSAAA_V$E2F1DP2_01	-0.74	-1.97	0	0.001
V$E2F_02	-0.71	-1.96	0	0.001
V$E2F_Q4	-0.70	-1.96	0	0.001
V$E2F1DP1_01	-0.70	-1.96	0	0.001
V$E2F1DP2_01	-0.70	-1.96	0	0.001
V$E2F4DP2_01	-0.70	-1.96	0	0.001
V$E2F1DP1RB_01	-0.69	-1.96	0	0.001
V$E2F_Q6	-0.72	-1.95	0	0.001

Supplementary Table 4. Primer sequences for qPCR or ChIP assay and shRNA sequences

Primer Name	Sequence
E2F1_FW	CCATCAGTACCTGGCCGAGAGC
E2F1_RV	CGCTTCTGCACCTTCAGCACCT
E2F2_FW	GGCCAAGAACAACATCCAGT
E2F2_RV	TGTCCTCAGTCAGGTGCTTG
E2F3_FW	GAGCTAGGAGAAAGCGGTCA
E2F3_RV	GGAGTTTTTGGACTATCTGGAC
E2F7_FW	ACAGATGCAGAAACATCCACC
E2F7_RV	AAGAGCGAGGTCGTAAACCA
E2F8_FW	CCACCACAGCAAATATCGTG
E2F8_RV	CTTTGGCCTCAGGTAATCCA
UHRF1_FW	AGGAGCTGGATGGTGTCATT
UHRF1_RV	GCCTGCAGAGGCTGTTCTAC
POM121_FW	TGGATCGGATAGCGTCTTCT
POM121_RV	AACGGGAGGTGAATTTCCAT
NDUFB11_FW	GACAAGTCGCATGTTCCAGA
NDUFB11_RV	AGAACCCGAGGACGAAAACT
HAX1_FW	GGGTCCATAGGCCATACATC
HAX1_RV	TAGTCACCAGCCCAGGATCT
EIF4EBP1_FW	AGTTCCGACACTCCATCAGG
EIF4EBP1_FW	CGGGGACTACAGCACGAC
HIST2H2AB_FW	TGACTCTCCGTTTTCTTGGG
HIST2H2AB_RV	CAACAAGTTACTCGGGGGTG
HIST1H2BM_FW	TCCCATAGCCTTGGAAGAGA
HIST1H2BM_FW	GGCCATTAACAAGGCTCAGA
mE2F8_FW	CTGTTTGCACGAACACTTATCAG
mE2F8_RV	GTACCGCGCTAGGAATTTGTG
mUHRF1_FW	CCACACCGTGAACTCTCTGTC
mUHRF1_RV	GGCGCACATCATAATCGAAGA
mPCNA_FW	TTTGAGGCACGCCTGATCC
mPCNA_RV	GGAGACGTGAGACGAGTCCAT

UHRF1_#0_5' ChIP	CACCCTCTTTCTCGCTTCC
UHRF1_#0_3' ChIP	TGGGGATGGCGATGAAAC
UHRF1_#1_5' ChIP	AATAAGAGGCGGCTCAAGTG
UHRF1_#1_3' ChIP	AGTGCCACTGAGAGGGAAAA
Control shRNA (shctrl) (TRC ID: SHC002)	CCGGCAACAAGATGAAGAGCACCAACTC GAGTTGGTGCTCTTCATCTTGTTGTTTTT
E2F8 shRNA-1 (ID: TRCN0000017428)	GCCGCAAAGACAAGTCTTTAA
E2F8 shRNA-3 (ID: TRCN0000017430)	CGCCGAGCAGATTATGATGAT
E2F8 shRNA-5 (ID: TRCN0000017432)	CATAAGTTCTTAGCACGATAT
UHRF1 shRNA-1 (ID: TRCN0000004352)	CCGGCCGCACCAAGGAATGTACCATCTCGA GATGGTACATTCCTTGGTGCGGTTTTT
UHRF1 shRNA-2 (ID: TRCN0000004353)	CCGGGCCTTTGATTCGTTCCTTCTTCTCGAG AAGAAGGAACGAATCAAAGGCTTTTT

Supplementary Table 5. Genes from heatmap in Figure 5

	FC in H1975	FC in H441	FC in H520	FC in 3 cell lines
E2F8	-3.23	-5.66	-1.80	-3.62
UHRF1	-4.03	-2.71	-2.31	-3.01
SFXN2	-1.41	-2.79	-3.14	-2.60
HAX1	-3.84	-3.66	-1.47	-2.53
TTYH3	-3.32	-2.85	-1.84	-2.50
GMNN	-2.33	-3.58	-1.65	-2.47
EIF4EBP1	-2.99	-2.48	-2.30	-2.45
TRIM22	-2.81	-1.78	1.17	-2.43
GNPNAT1	-2.16	-2.66	-1.87	-2.34
C5orf25	-2.00	-1.16	-3.01	-2.33
STEAP4	-2.10	-2.36	1.06	-2.31
SLC38A9	-2.55	-2.64	-1.06	-2.22
SUV39H1	-3.01	-1.84	-1.79	-2.20
MX2	-2.13	-2.23	-1.62	-2.17
TMED8	-3.34	-1.87	-1.25	-2.17
FAM96A	-3.18	-2.30	-1.74	-2.15
PARP9	-1.95	-2.54	1.06	-2.11
RAB38	-1.77	-2.19	-1.41	-2.10
TRAM2	-2.68	-1.73	-1.77	-2.08
COX8A	-3.05	-1.77	-1.79	-2.08
MRPL45	-2.87	-2.07	-1.77	-2.07
SMARCA4	-2.22	-2.33	-1.67	-2.04
LOC146336	-1.88	-1.58	-3.46	-2.04
LEPROTL1	-2.36	-2.01	-1.83	-2.03
POLD2	-2.57	-1.92	-1.67	-2.01
OXA1L	-2.51	-1.71	-1.75	-2.00
HMOX1	-2.77	-2.01	-1.11	-2.00
TAB2	-2.31	-2.33	-1.44	-1.99
HCP5	-2.33	-2.17	-1.23	-1.98
PLAGL2	-1.80	-3.10	-1.47	-1.98
CXCL10	-2.39	-1.99	-1.14	-1.97
RIPK2	-1.92	-2.64	-1.58	-1.96
HIST1H2BK	-2.71	-1.88	-1.28	-1.96
SHISA5	-2.11	-2.01	-1.41	-1.95
ZNF382	-1.80	-2.64	1.09	-1.94
TMEM8A	-2.36	-1.96	-1.59	-1.94
PLAUR	-2.39	-1.79	-1.12	-1.94

C11orf24	-2.28	-1.91	-1.38	-1.92
MOBKL2A	-2.03	-1.97	-1.51	-1.92
RTCD1	-2.13	-2.43	-1.37	-1.91
COX10	-2.19	-2.10	-1.40	-1.91
SLC19A2	-2.19	-2.27	-1.39	-1.91
MMGT1	-2.01	-2.53	-1.43	-1.91
SNRPC	-2.14	-1.91	-1.65	-1.90
BMP4	-1.87	-1.59	-1.95	-1.90
ZFAND3	-2.00	-2.22	-1.48	-1.89
USP41	-2.08	-1.79	-1.24	-1.88
NDUFB11	-2.01	-1.54	-1.97	-1.86
OAS2	-1.78	-2.08	1.02	-1.86
POM121	-1.72	-2.04	-1.81	-1.84
POU2F1	-2.03	-2.35	-1.34	-1.84
MX1	-1.87	-2.17	-1.03	-1.84
POM121C	-1.72	-2.01	-1.79	-1.83
MBOAT1	-2.25	-1.92	-1.52	-1.83
TMEM180	-1.97	-2.11	-1.42	-1.83
BCL9	-2.06	-2.08	-1.25	-1.82
PIGU	-2.00	-2.01	-1.47	-1.82
CENPV	-2.03	1.38	-1.84	-1.81
HIST1H2BM	-3.53	-2.99	-1.41	-1.81
HERC6	-1.93	-1.75	1.13	-1.80
MED6	-2.00	-2.00	-1.23	-1.79
C13orf23	-2.08	-1.95	-1.56	-1.79
PLSCR1	-1.88	-1.77	-1.35	-1.77
ABR	-1.75	-1.88	-1.17	-1.76
SP110	-1.83	-1.85	-1.03	-1.76
CHAC1	-1.85	-1.48	-2.04	-1.76
CCDC21	-1.89	-2.07	-1.47	-1.75
CDC34	-2.00	-2.11	-1.47	-1.75
MOCS3	-2.03	-2.01	-1.18	-1.75
NHEJ1	-2.41	-1.84	-1.35	-1.74
PTPN9	-1.84	-2.19	-1.42	-1.71
SLC37A4	-1.83	-1.28	-1.93	-1.71
SS18	-1.79	-1.95	-1.36	-1.70
PCTP	-2.08	-1.85	-1.28	-1.69
MICB	-1.85	-1.82	-1.29	-1.68
PLDN	-1.85	-1.29	-1.79	-1.66
WBP2	-2.22	-1.83	-1.06	-1.66

MRPL36	-2.75	-1.75	-1.19	-1.65
HSPC072	-2.36	-2.66	1.72	-1.65
SMG7	-2.19	-2.22	-1.20	-1.64
VARS2	-1.28	-1.83	-1.80	-1.63
TAF9B	-2.30	-2.28	-1.14	-1.63
RTKN	-2.10	-1.82	-1.31	-1.62
NIPA2	-1.81	-1.98	-1.35	-1.62
WBP11	-1.82	-1.87	-1.09	-1.58
SNAP23	-1.77	-1.93	-1.17	-1.57
CX3CL1	-1.85	1.16	-1.77	-1.48
CCL20	-1.80	-1.84	-1.20	-1.47
C1orf83	-1.97	-1.80	1.27	-1.40
VPS52	-2.23	1.64	-2.47	-1.27
CDSN	-2.36	1.39	-1.84	-1.18
TMPRSS11E	-4.20	1.93	-1.89	-1.03
DDAH1	2.36	1.26	1.85	1.41
LRRC37A	2.24	-1.86	1.82	1.52
HIPK3	1.99	1.21	1.77	1.57
LOC100132426	1.80	1.77	1.11	1.62
LOC100133034	1.80	1.77	1.11	1.62
SORL1	2.30	-1.05	1.97	1.73
C6orf15	2.76	1.13	1.93	1.73
PIK3R3	1.88	1.30	2.03	1.82
ANGPTL4	1.83	2.58	-1.52	1.96
ENPP5	-1.02	2.23	1.95	2.11
SESN3	1.77	2.53	2.10	2.14
LOC728888	3.45	1.40	2.10	2.49

FC: Fold Change

Supplementary Table 6. Metacore-GO processes in E2F8-deficient LC cells.

Network	GO processes
DPM2 (reg), JPO1, Exportin 5, HIST1H2BN, IDI1	Ferric iron transport (26.5%; 3.001e-24) ATP hydrolysis coupled proton transport (26.5%; 4.109e-24)
UHRF1, ELF4, 4E-BP1, MASTL, CYP2R1	Regulation of transcription from RNA polymerase II promoter (68.8%; 3.22e-24) Positive regulation of gene expression (64.6%; 2.192e-23)
C2orf24, Plakophilin 3, OXA1L, UHRF1, PLAGL2	Regulation of transcription from RNA polymerase II promoter (39.1%; 2.33e-08) Positive regulation of transcription, DNA-dependent (32.6%; 2.587e-07)
E2F8, Geminin, RBM9, PTP-MEG2, BMP4	Positive regulation of tyrosine phosphorylation of Stat4 protein (14.6%; 2.4e-18) Canonical Wnt receptor signaling pathway (25.0%; 4.736e-17) Positive regulation of NK T cell activation (14.6%; 1.014e-16)
BMP4, COX VIII-2, BGAL, SLC19A2, SP1	Ionotropic glutamate receptor signaling pathway (24.5%; 1.014e-24) Cell surface receptor signaling pathway (67.3%; 3.161e-13)
ISG15, UHRF1, 4E-BP1, U1C, Ubiquitin	Chromatin assembly or disassembly (28.6%; 3.844e-18) DNA packaging (24.5%; 4.728e-14)
BRG1, CARM1, FLJ32252, MBOAT1, CCL2	Positive regulation of macromolecule biosynthetic process (83.7%; 4.007e-37) Positive regulation of nitrogen compound metabolic process (79.6%; 5.295e-33)
VDAC 1, Suv39H1, UHRF1, CDC34, Ubiquitin	Response to lipid (43.8%; 2.064e-13) Response to chemical stimulus (75.0%; 5.596e-13)
Cyclin D1, ID3, MSH2, ATF-2/c-Jun, 08p22/MSR1(CD204)	Chromatin assembly or disassembly (48.0%; 5.470e-37) Protein-DNA complex assembly (46.0%; 1.087e-35)
CARM1, E2F8, RBM9, CDCA4, E2F1	Regulation of cell cycle (38.8%; 1.238e-12)
TAB2, TTYH3, CDC34, NF-kB, Ubiquitin	Positive regulation of immune system process (81.6%; 1.067e-45) Regulation of immune system process (83.7%; 1.118e-38)
VAMP3, PTP-MEG2, hnRNP C, c-Myc, KARS	Cell-substrate adhesion (28.6%; 2.314e-17) Metanephric glomerular epithelial cell development (12.2%; 1.163e-15)
BRG1, GPT, BCL9-2, Beta-catenin, GSK3 beta	Cell surface receptor signaling pathway (82.0%; 1.825e-21) Signal transduction (86.0%; 4.023e-17)

Supplementary Table 7. List of genes in the Venn diagrams of Supplementary Figure 10

Overlapped genes in	Number	Symbol
Sup. Fig. 10B	1	ATRX
	2	PIK3R3
Overlapped genes in	**Number**	**Symbol**
Sup. Fig. 10C	1	CDCA7
	2	DCK
	3	DUSP7
	4	GMNN
	5	HIST1H2BK
	6	POLE2
	7	SPRED2
	8	SUV39H1
	9	VAMP3

Supplementary Table 8. List of 204 genes identified by E2F8 ChIP-sequencing

Entrez ID	Gene Name	Gene Description
4173	**MCM4**	minichromosome maintenance complex component 4
25896	**INTS7**	integrator complex subunit 7
84961	**FBXL20**	F-box and leucine-rich repeat protein 20
5925	**RB1**	retinoblastoma 1
6224	**RPS20**	ribosomal protein S20
7374	**UNG**	uracil-DNA glycosylase
9179	**AP4M1**	adaptor-related protein complex 4, mu 1 subunit
4172	**MCM3**	minichromosome maintenance complex component 3
7884	**SLBP**	stem-loop binding protein
9221	**NOLC1**	nucleolar and coiled-body phosphoprotein 1
4174	**MCM5**	minichromosome maintenance complex component 5
51659	**GINS2**	GINS complex subunit 2 (Psf2 homolog)
8462	**KLF11**	Kruppel-like factor 11
1869	**E2F1**	E2F transcription factor 1
10714	**POLD3**	polymerase (DNA-directed), delta 3, accessory subunit
84915	**FAM222A**	family with sequence similarity 222, member A
5902	**RANBP1**	RAN binding protein 1
5292	**PIM1**	Pim-1 proto-oncogene, serine/threonine kinase
285600	**KIAA0825**	KIAA0825
51602	**NOP58**	NOP58 ribonucleoprotein
2629	**GBA**	glucosidase, beta, acid
57592	**ZNF687**	zinc finger protein 687
116028	**RMI2**	RecQ mediated genome instability 2
7083	**TK1**	thymidine kinase 1, soluble
29893	**PSMC3IP**	PSMC3 interacting protein
2146	**EZH2**	enhancer of zeste 2 polycomb repressive complex 2 subunit
4609	**MYC**	v-myc avian myelocytomatosis viral oncogene homolog
3157	**HMGCS1**	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
1871	**E2F3**	E2F transcription factor 3
442075	**EMC3-AS1**	EMC3 antisense RNA 1
29028	**ATAD2**	ATPase family, AAA domain containing 2
2956	**MSH6**	mutS homolog 6
5933	**RBL1**	retinoblastoma-like 1
55183	**RIF1**	replication timing regulatory factor 1
83638	**C11orf68**	chromosome 11 open reading frame 68
79027	**ZNF655**	zinc finger protein 655
116225	**ZMYND19**	zinc finger, MYND-type containing 19
55298	**RNF121**	ring finger protein 121

83879	**CDCA7**	cell division cycle associated 7
1111	**CHEK1**	checkpoint kinase 1
81844	**TRIM56**	tripartite motif containing 56
7341	**SUMO1**	small ubiquitin-like modifier 1
3268	**AGFG2**	ArfGAP with FG repeats 2
5883	**RAD9A**	RAD9 homolog A (S. pombe)
11073	**TOPBP1**	topoisomerase (DNA) II binding protein 1
114781	**BTBD9**	BTB (POZ) domain containing 9
57657	**HCN3**	hyperpolarization activated cyclic nucleotide-gated potassium channel 3
1870	**E2F2**	E2F transcription factor 2
100874282	**MLIP-IT1**	MLIP intronic transcript 1 (non-protein coding)
4175	**MCM6**	minichromosome maintenance complex component 6
5984	**RFC4**	replication factor C (activator 1) 4, 37kDa
7516	**XRCC2**	X-ray repair complementing defective repair in Chinese hamster cells 2
5965	**RECQL**	RecQ helicase-like
220032	**GDPD4**	glycerophosphodiester phosphodiesterase domain containing 4
8270	**LAGE3**	L antigen family, member 3
51147	**ING4**	inhibitor of growth family, member 4
10421	**CD2BP2**	CD2 (cytoplasmic tail) binding protein 2
101060544	**HP08942**	uncharacterized LOC101060544
4176	**MCM7**	minichromosome maintenance complex component 7
3795	**KHK**	ketohexokinase (fructokinase)
7481	**WNT11**	wingless-type MMTV integration site family, member 11
79019	**CENPM**	centromere protein M
6434	**TRA2B**	transformer 2 beta homolog (Drosophila)
4171	**MCM2**	minichromosome maintenance complex component 2
51631	**LUC7L2**	LUC7-like 2 (S. cerevisiae)
990	**CDC6**	cell division cycle 6
2177	**FANCD2**	Fanconi anemia, complementation group D2
55798	**METTL2B**	methyltransferase like 2B
5558	**PRIM2**	primase, DNA, polypeptide 2 (58kDa)
10428	**CFDP1**	craniofacial development protein 1
2990	**GUSB**	glucuronidase, beta
9873	**FCHSD2**	FCH and double SH3 domains 2
7465	**WEE1**	WEE1 G2 checkpoint kinase
29128	**UHRF1**	ubiquitin-like with PHD and ring finger domains 1
23344	**ESYT1**	extended synaptotagmin-like protein 1
643836	**ZFP62**	ZFP62 zinc finger protein
64946	**CENPH**	centromere protein H

3070	**HELLS**	helicase, lymphoid-specific
6426	**SRSF1**	serine/arginine-rich splicing factor 1
2810	**SFN**	stratifin
1854	**DUT**	deoxyuridine triphosphatase
9531	**BAG3**	BCL2-associated athanogene 3
6631	**SNRPC**	small nuclear ribonucleoprotein polypeptide C
81620	**CDT1**	chromatin licensing and DNA replication factor 1
79971	**WLS**	wntless Wnt ligand secretion mediator
993	**CDC25A**	cell division cycle 25A
85236	**HIST1H2BK**	histone cluster 1, H2bk
146956	**EME1**	essential meiotic structure-specific endonuclease 1
29119	**CTNNA3**	catenin (cadherin-associated protein), alpha 3
4678	**NASP**	nuclear autoantigenic sperm protein (histone-binding)
30849	**PIK3R4**	phosphoinositide-3-kinase, regulatory subunit 4
677765	**SCARNA18**	small Cajal body-specific RNA 18
195828	**ZNF367**	zinc finger protein 367
57695	**USP37**	ubiquitin specific peptidase 37
9735	**KNTC1**	kinetochore associated 1
55120	**FANCL**	Fanconi anemia, complementation group L
4522	**MTHFD1**	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1
9156	**EXO1**	exonuclease 1
26046	**LTN1**	listerin E3 ubiquitin protein ligase 1
55159	**RFWD3**	ring finger and WD repeat domain 3
83990	**BRIP1**	BRCA1 interacting protein C-terminal helicase 1
51631	**LUC7L2**	LUC7-like 2 (S. cerevisiae)
672	**BRCA1**	breast cancer 1, early onset
8660	**IRS2**	insulin receptor substrate 2
23352	**UBR4**	ubiquitin protein ligase E3 component n-recognin 4
388182	**SPATA41**	spermatogenesis associated 41 (non-protein coding)
23677	**SH3BP4**	SH3-domain binding protein 4
23548	**TTC33**	tetratricopeptide repeat domain 33
54892	**NCAPG2**	non-SMC condensin II complex, subunit G2
4439	**MSH5**	mutS homolog 5
55924	**FAM212B**	family with sequence similarity 212, member B
8697	**CDC23**	cell division cycle 23
374393	**FAM111B**	family with sequence similarity 111, member B
4436	**MSH2**	mutS homolog 2
25923	**ATL3**	atlastin GTPase 3
4605	**MYBL2**	v-myb avian myeloblastosis viral oncogene homolog-like 2
57405	**SPC25**	SPC25, NDC80 kinetochore complex component
6921	**TCEB1**	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin

		C)
8914	**TIMELESS**	timeless circadian clock
79717	**PPCS**	phosphopantothenoylcysteine synthetase
283385	**MORN3**	MORN repeat containing 3
9675	**TTI1**	TELO2 interacting protein 1
8533	**COPS3**	COP9 signalosome subunit 3
148645	**LINC00337**	long intergenic non-protein coding RNA 337
3925	**STMN1**	stathmin 1
100529241	**HSPE1-MOB4**	HSPE1-MOB4 readthrough
9631	**NUP155**	nucleoporin 155kDa
9994	**CASP8AP2**	caspase 8 associated protein 2
84905	**ZNF341**	zinc finger protein 341
28231	**SLCO4A1**	solute carrier organic anion transporter family, member 4A1
9592	**IER2**	immediate early response 2
9782	**MATR3**	matrin 3
2630	**GBAP1**	glucosidase, beta, acid pseudogene 1
102464817	**MIR5787**	microRNA 5787
8364	**HIST1H4C**	histone cluster 1, H4c
7918	**GPANK1**	G patch domain and ankyrin repeats 1
266743	**NPAS4**	neuronal PAS domain protein 4
5982	**RFC2**	replication factor C (activator 1) 2, 40kDa
55833	**UBAP2**	ubiquitin associated protein 2
487	**ATP2A1**	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1
54962	**TIPIN**	TIMELESS interacting protein
5928	**RBBP4**	retinoblastoma binding protein 4
6015	**RING1**	ring finger protein 1
5983	**RFC3**	replication factor C (activator 1) 3, 38kDa
274	**BIN1**	bridging integrator 1
5359	**PLSCR1**	phospholipid scramblase 1
3834	**KIF25**	kinesin family member 25
54487	**DGCR8**	DGCR8 microprocessor complex subunit
150776	**LOC150776**	sphingomyelin phosphodiesterase 4
5888	**RAD51**	RAD51 recombinase
6510	**SLC1A5**	solute carrier family 1 (neutral amino acid transporter), member 5
81624	**DIAPH3**	diaphanous-related formin 3
401233	**HTATSF1P2**	HIV-1 Tat specific factor 1 pseudogene 2
5631	**PRPS1**	phosphoribosyl pyrophosphate synthetase 1
11144	**DMC1**	DNA meiotic recombinase 1
348235	**SKA2**	spindle and kinetochore associated complex subunit 2

463	**ZFHX3**	zinc finger homeobox 3
9031	**BAZ1B**	bromodomain adjacent to zinc finger domain, 1B
9902	**MRC2**	mannose receptor, C type 2
101926926	**LOC101926926**	uncharacterized LOC101926926
100151684	**RNU6ATAC**	RNA, U6atac small nuclear (U12-dependent splicing)
140766	**ADAMTS14**	ADAM metallopeptidase with thrombospondin type 1 motif, 14
81930	**KIF18A**	kinesin family member 18A
79733	**E2F8**	E2F transcription factor 8
9768	**KIAA0101**	KIAA0101
8294	**HIST1H4I**	histone cluster 1, H4i
729852	**RPA3OS**	RPA3 opposite strand
79075	**DSCC1**	DNA replication and sister chromatid cohesion 1
51278	**IER5**	immediate early response 5
407008	**MIR223**	microRNA 223
6241	**RRM2**	ribonucleotide reductase M2
23244	**PDS5A**	PDS5, regulator of cohesion maintenance, homolog A (S. cerevisiae)
10558	**SPTLC1**	serine palmitoyltransferase, long chain base subunit 1
100302739	**PCNA-AS1**	PCNA antisense RNA 1
348793	**WDR53**	WD repeat domain 53
284454	**LOC284454**	uncharacterized LOC284454
3843	**IPO5**	importin 5
5427	**POLE2**	polymerase (DNA directed), epsilon 2, accessory subunit
23082	**PPRC1**	peroxisome proliferator-activated receptor gamma, coactivator-related 1
23595	**ORC3**	origin recognition complex, subunit 3
79665	**DHX40**	DEAH (Asp-Glu-Ala-His) box polypeptide 40
89894	**TMEM116**	transmembrane protein 116
79892	**MCMBP**	minichromosome maintenance complex binding protein
11201	**POLI**	polymerase (DNA directed) iota
4521	**NUDT1**	nudix (nucleoside diphosphate linked moiety X)-type motif 1
100302213	**MIR1181**	microRNA 1181
113622	**ADPRHL1**	ADP-ribosylhydrolase like 1
4507	**MTAP**	methylthioadenosine phosphorylase
5424	**POLD1**	polymerase (DNA directed), delta 1, catalytic subunit
9134	**CCNE2**	cyclin E2
25914	**RTTN**	rotatin
9955	**HS3ST3A1**	heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1
56941	**HMCES**	5-hydroxymethylcytosine (hmC) binding, ES cell-specific

5426	**POLE**	polymerase (DNA directed), epsilon, catalytic subunit
10036	**CHAF1A**	chromatin assembly factor 1, subunit A (p150)
1039	**CDR2**	cerebellar degeneration-related protein 2, 62kDa
84939	**MUM1**	melanoma associated antigen (mutated) 1
84219	**WDR24**	WD repeat domain 24
124222	**PAQR4**	progestin and adipoQ receptor family member IV
163	**AP2B1**	adaptor-related protein complex 2, beta 1 subunit
27131	**SNX5**	sorting nexin 5
9401	**RECQL4**	RecQ protein-like 4
119587	**CPXM2**	carboxypeptidase X (M14 family), member 2
64782	**AEN**	apoptosis enhancing nuclease

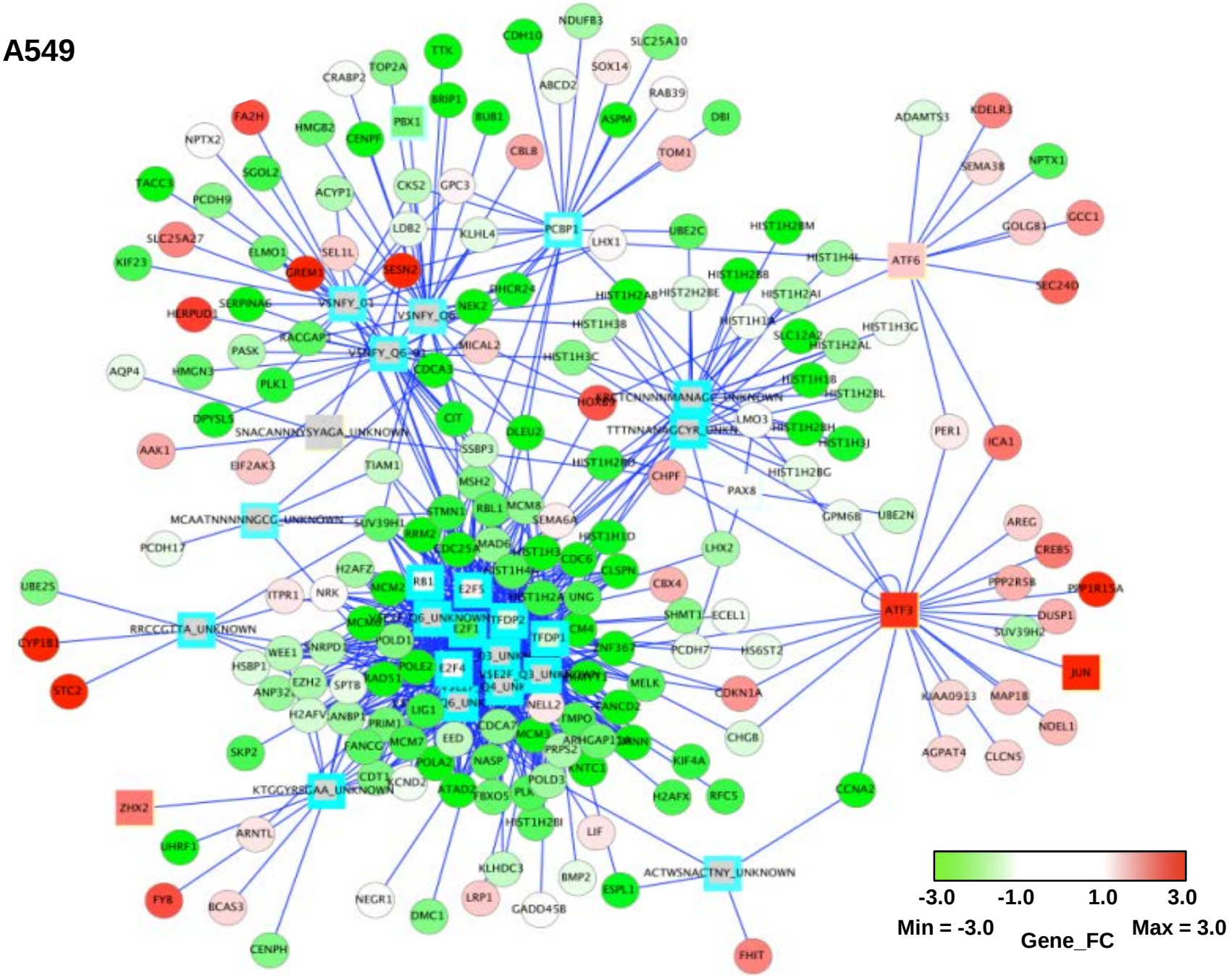
SUPPLEMENTARY FIGURES

Supplementary Figure 1, Related to Figure 1: Transcription factor networks affected by NASTRp in each different LC cell line.

(A-E) Differential expression of transcription factors and their target genes by NASTRp treatment in A549, H441, H1975, H520, and H1703 cells. Red: upregulated genes, green: downregulated genes, square: transcription factor, circle: target gene, cyan border: negative enrichment of targets, yellow: positive enrichment. Thickness of border is proportional to the magnitude of enrichment.

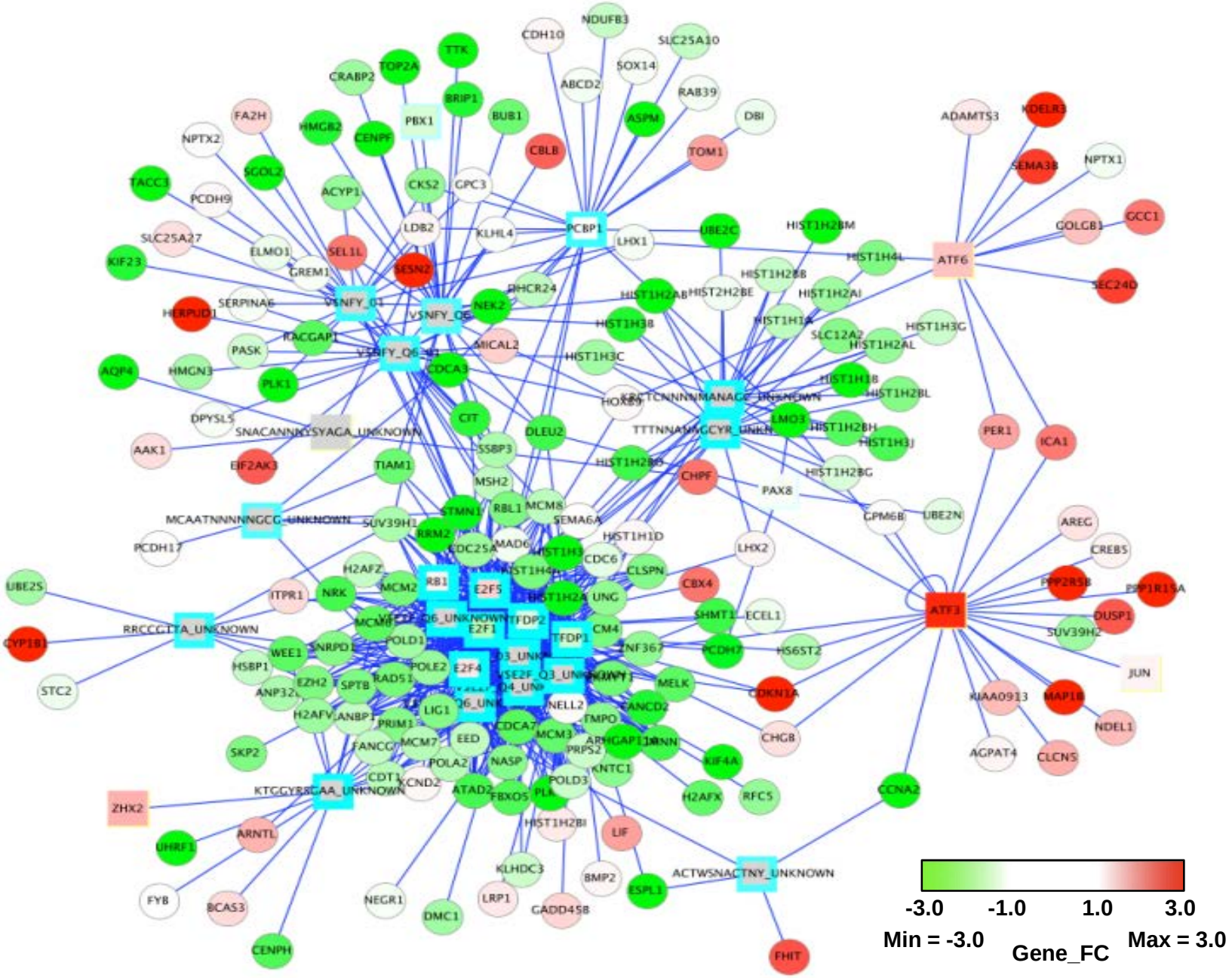
Supplementary Figure 1A

A. A549



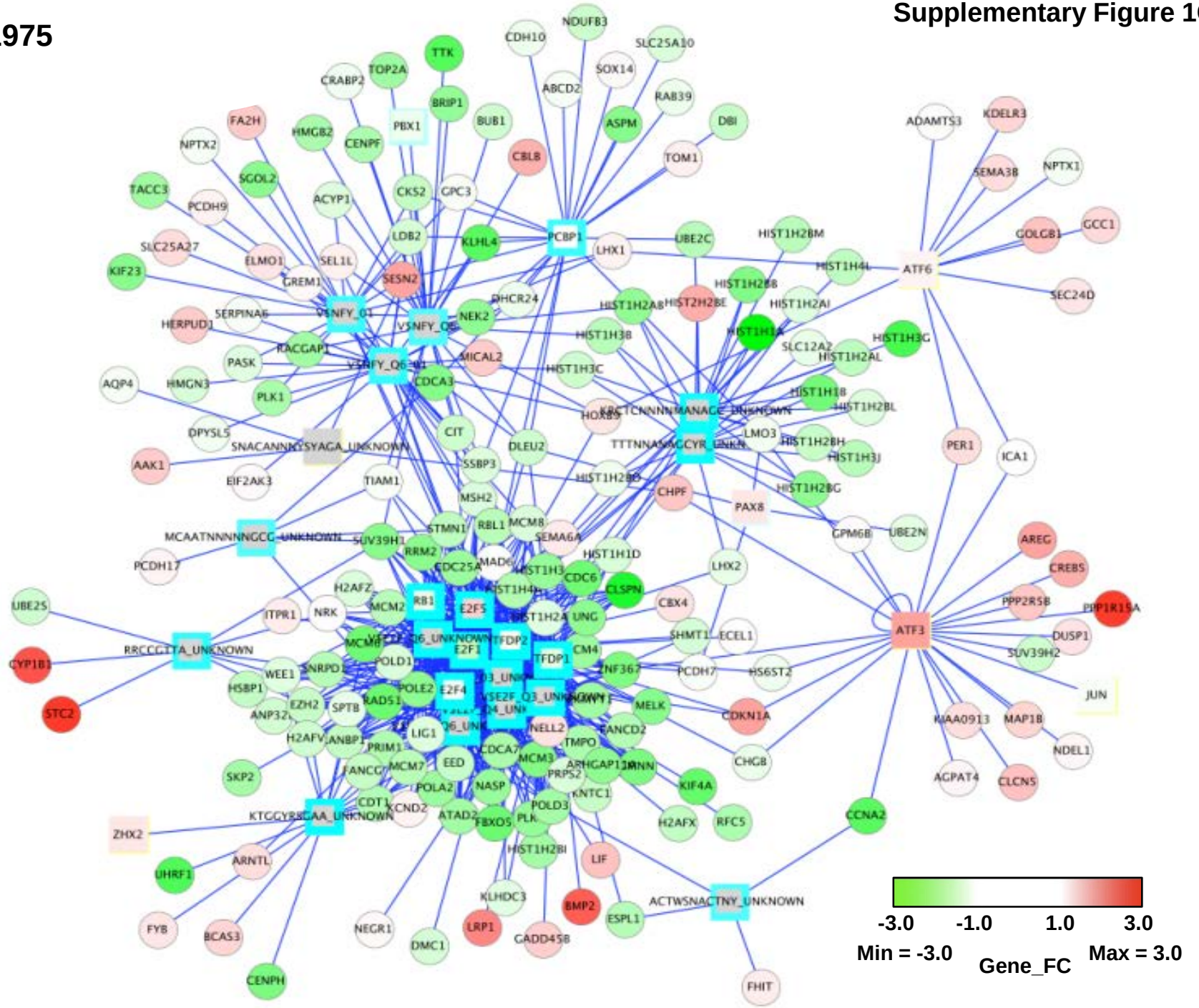
Supplementary Figure 1B

B. H441



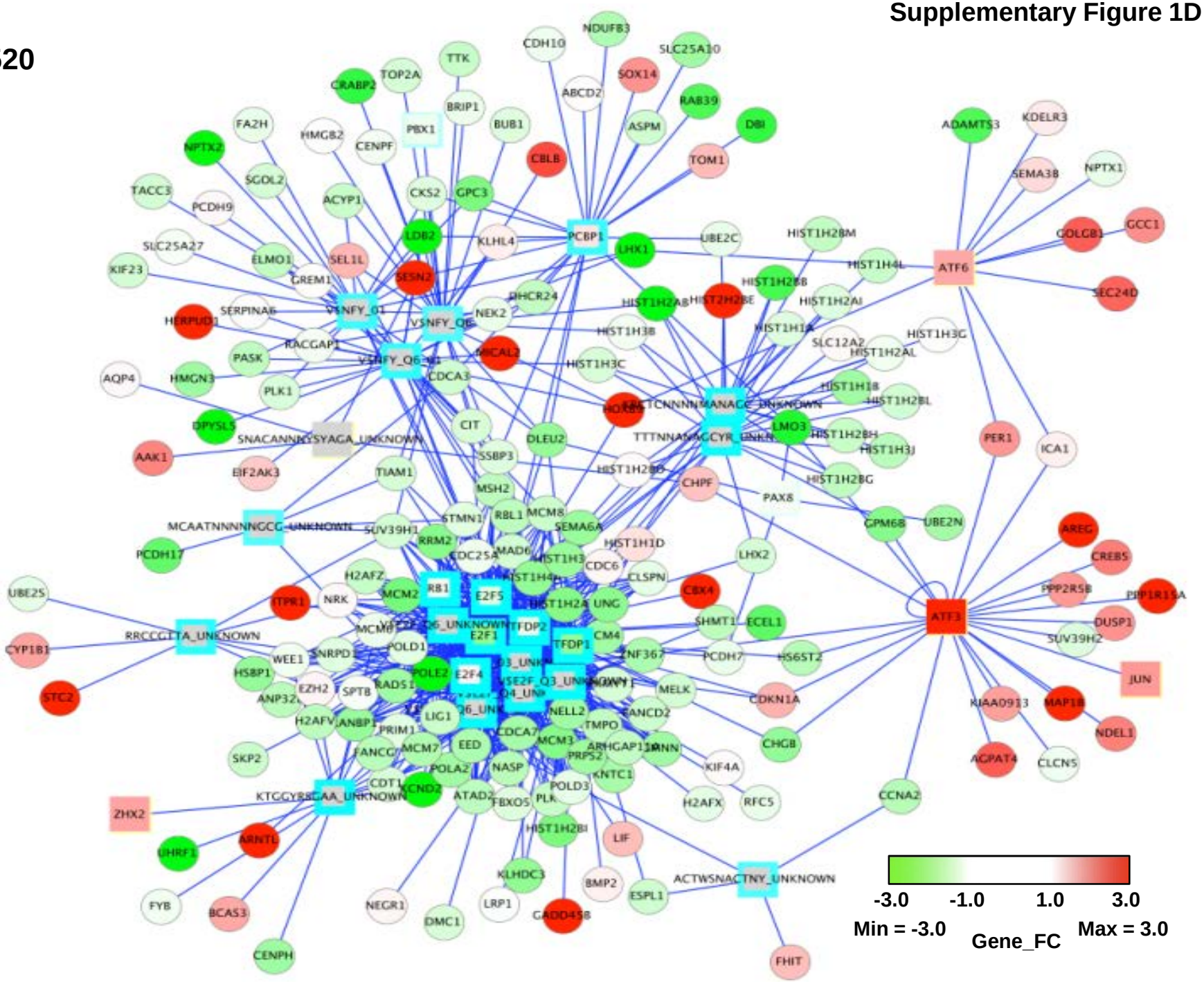
C. H1975

Supplementary Figure 1C



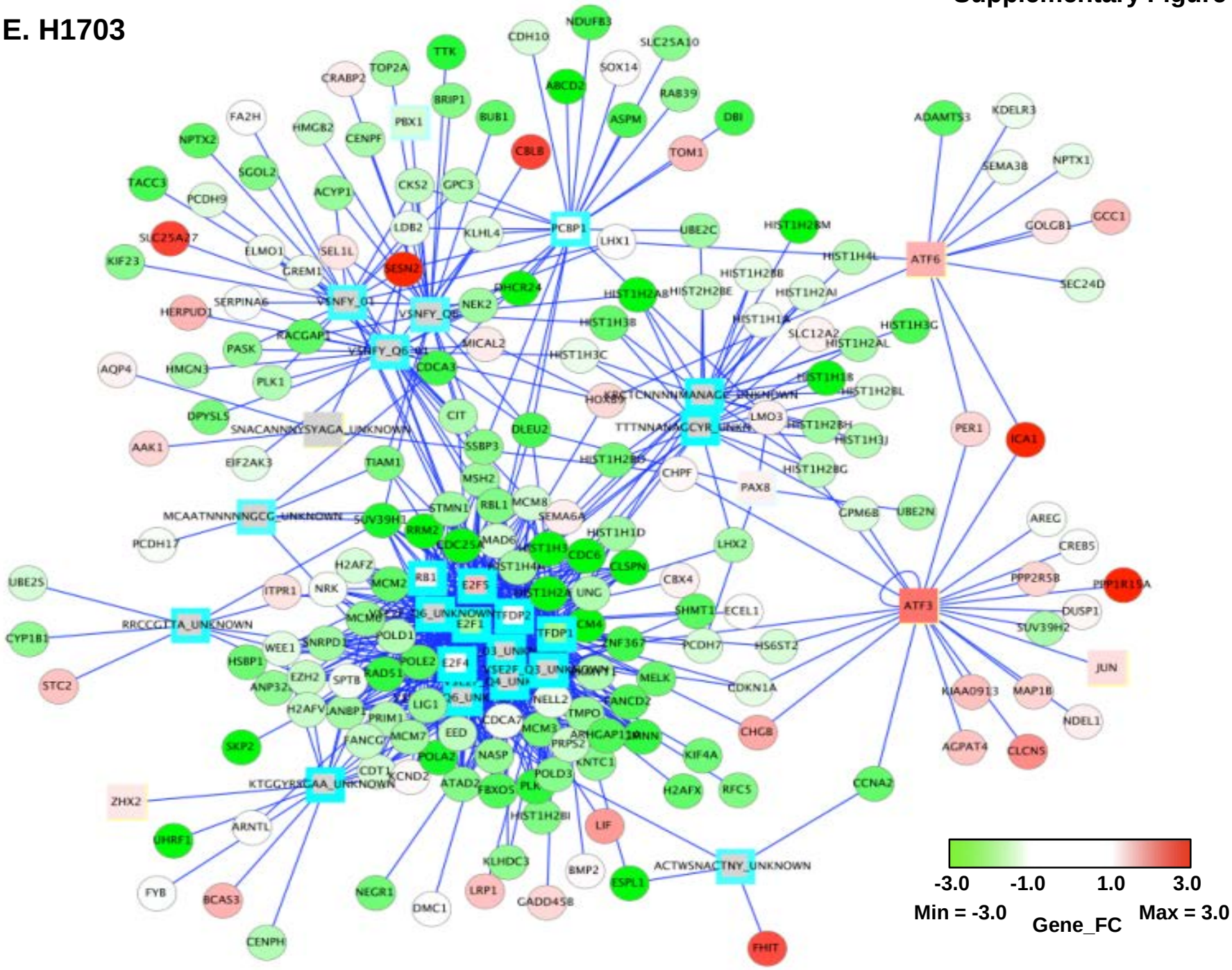
Supplementary Figure 1D

D. H520



Supplementary Figure 1E

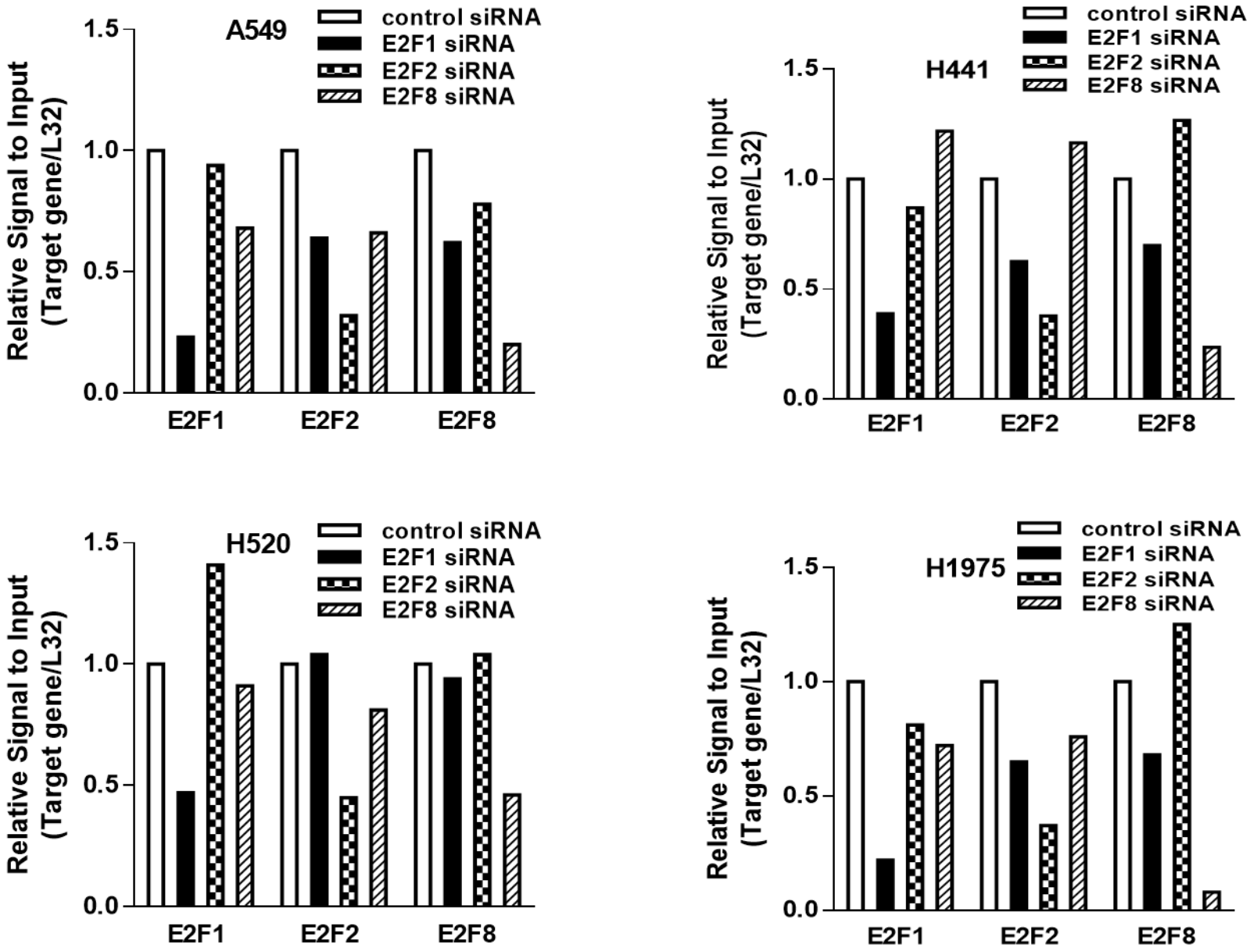
E. H1703



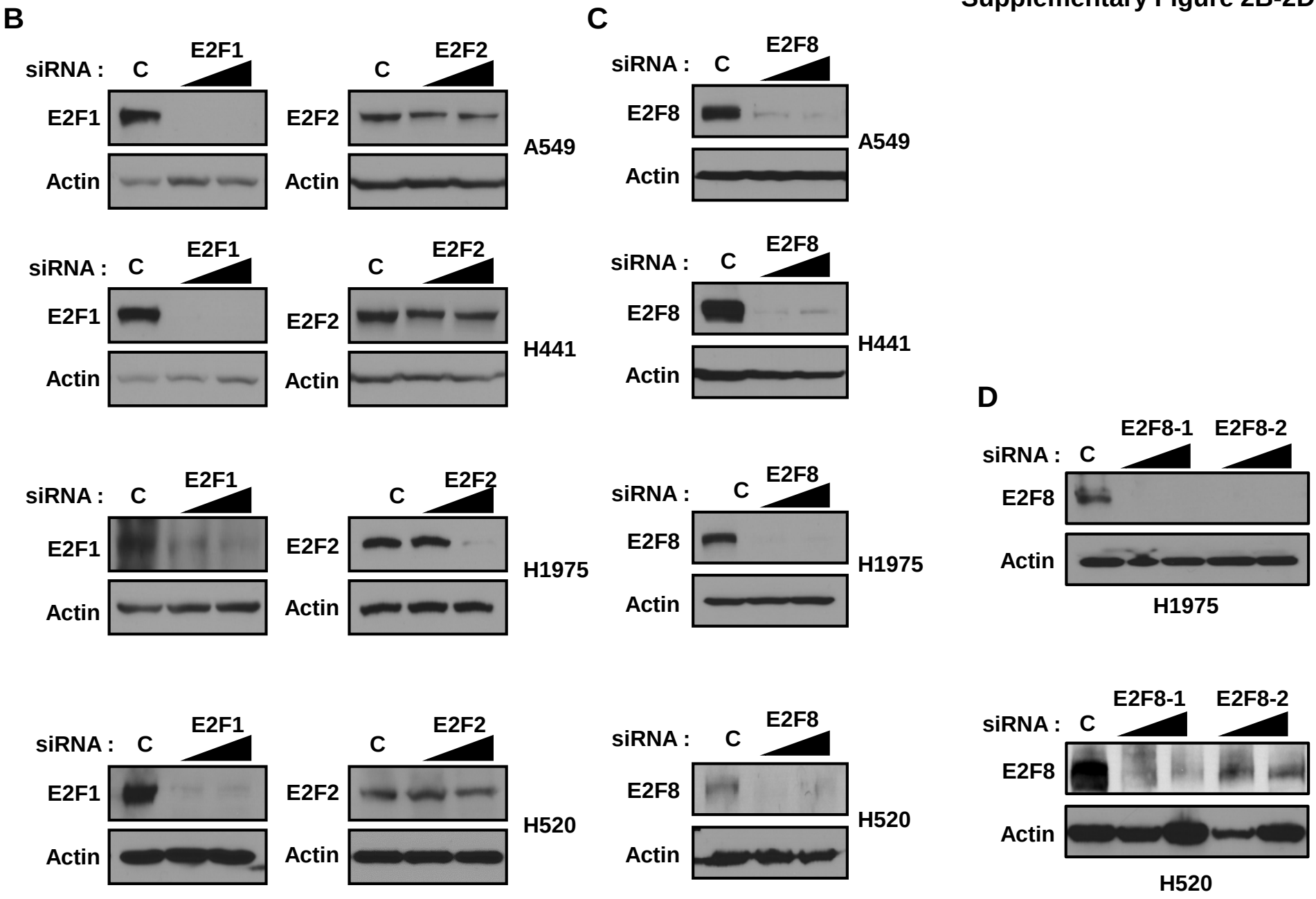
Supplementary Figure 2, Related to Figure 2 and 3: The validation of siRNAs on expression of E2Fs in LC cells.

(A) Each of the indicated cells were transiently transfected with siRNAs (40 nM, each) for 48 h and performed by qPCR analysis. L32 was used as a control. (B) Each of the indicated cells were transfected with E2F1 siRNA or E2F2 siRNA (10 and 50 nM, each) for 72 h, followed by western blot analysis. (C) Each of the indicated cells were transfected with E2F8 siRNA-1 (10 and 50 nM, each) for 72 h, followed by western blot analysis. (D) Two different E2F8 siRNAs were confirmed the effect of E2F8 knockdown in LC cells. Each of the indicated cells were transfected with control siRNA, E2F8 siRNA-1 or E2F8 siRNA-2 (10 and 50 nM, each) for 72 h, followed by western blot analysis. Further *in vitro* experiments were performed using E2F8 siRNA-1.

A

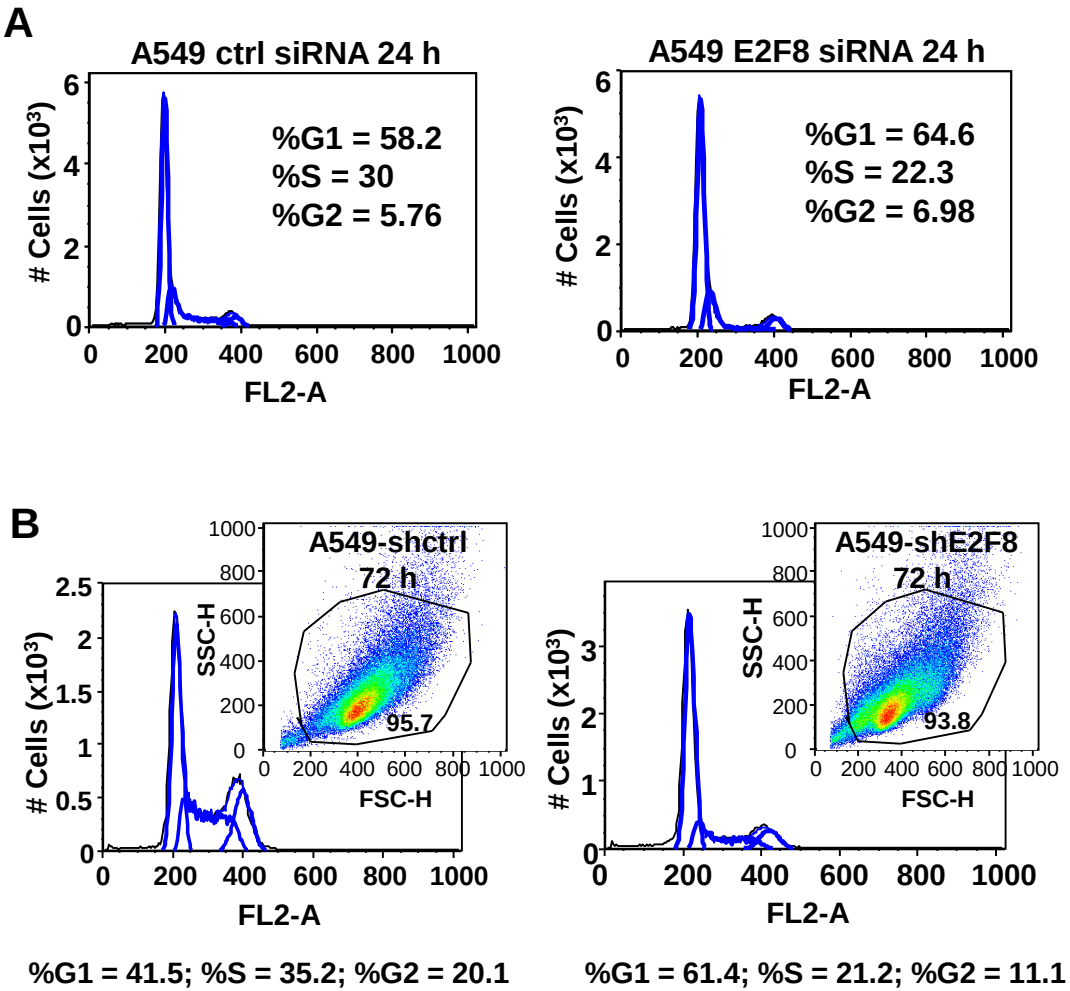

A549
H441
H520
H1975
control siRNA
E2F1 siRNA
E2F2 siRNA
E2F8 siRNA
Relative Signal to Input
(Target gene/L32)
1.5
1.0
0.5
0.0
E2F1
E2F2
E2F8

Supplementary Figure 2B-2D



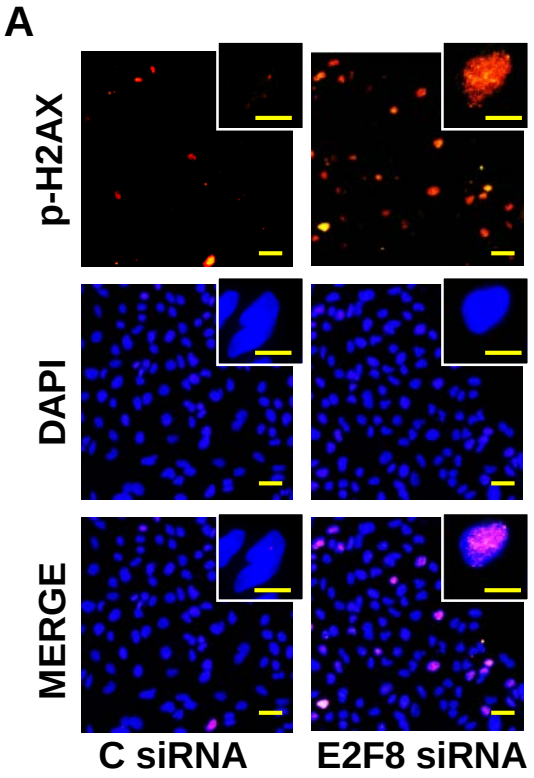
Supplementary Figure 3, Related to Figure 3: The effect of E2F8 depletion on the cell cycle progression.

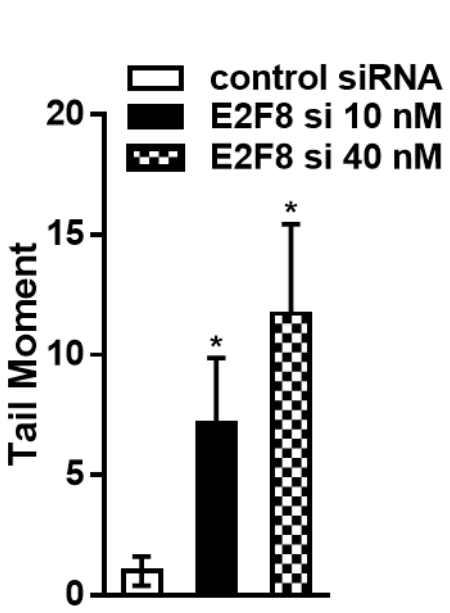
(A) A549 cells were transfected with control siRNA or E2F8 siRNA (20 nM) for 24 h. The cells were stained with propidium iodide and analyzed by flow cytometry. Percentage of cells in each phase of the cell cycle represent the corresponding histograms. (B) A549-shctrl and A549-shE2F8 cells were plated in 10 cm dishes for 3 days and stained with propidium iodide analyzed by flow cytometry. Inner panels show the increased number of contracted cells in A549-shE2F8 compared to its control cells and percentage of cells in each phase of the cell cycle represent the corresponding histograms.


A
A549 ctrl siRNA 24 h
%G1 = 58.2
%S = 30
%G2 = 5.76
Cells (x10³)
FL2-A
A549 E2F8 siRNA 24 h
%G1 = 64.6
%S = 22.3
%G2 = 6.98
Cells (x10³)
FL2-A
B
A549-shctrl
72 h
SSC-H
FSC-H
95.7
Cells (x10³)
FL2-A
%G1 = 41.5; %S = 35.2; %G2 = 20.1
A549-shE2F8
72 h
SSC-H
FSC-H
93.8
Cells (x10³)
FL2-A
%G1 = 61.4; %S = 21.2; %G2 = 11.1

Supplementary Figure 4, Related to Figure 3: The effect of E2F8 depletion on the DNA damage response in LC cells.

(A) Effect of E2F8 knockdown on the incidence of p-H2AX foci in H520 cells. Representative photographs show immunofluorescence staining of p-H2AX after 48 h control siRNA or E2F8 siRNA treatment (20 nM). Scale bar, 100 μm. **(**B) DNA damage as detected using an alkaline comet assay. H520 cells were transfected with control siRNA (40 nM) or E2F8 siRNA (10 or 40 nM) for 24 h. The panel shows representative images of cells and quantification of the tail moment of 12 randomly selected cells per slide. Two-sided *t*-test. *, $P < 0.001$.

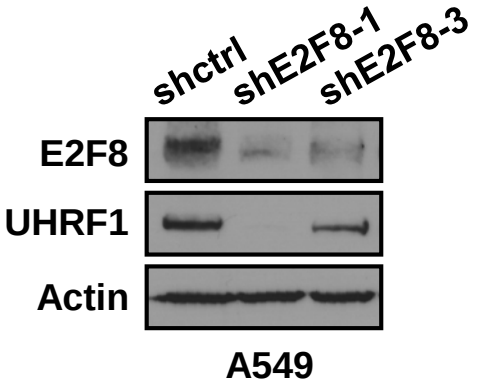

A
p-H2AX
DAPI
MERGE
C siRNA
E2F8 siRNA

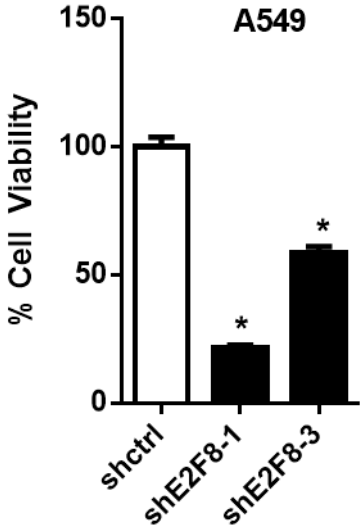

B
control siRNA
E2F8 si 10 nM
E2F8 si 40 nM
Tail Moment
20
15
10
5
0
*
*

Supplementary Figure 5, Related to Figure 3: The effect of E2F8 depletion on the cell growth in A549 cells and lung fibroblasts cell lines.

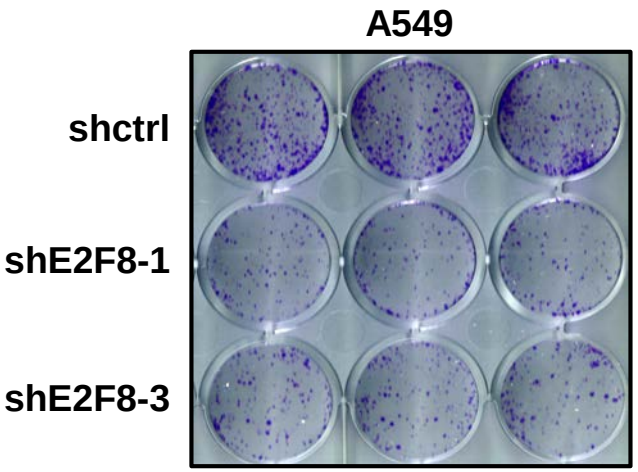
(A) Effect of E2F8 stable knockdown on LC cell proliferation. A549-shctrl, A549-shE2F8-1, and A549-shE2F8-3 cells were selected with puromycin (1.5 μg/ml) and the cells were performed by western blot analysis to confirm the expression levels of E2F8 and UHRF1. The cells were plated in 96 wells (2 x 10^3/ well) for 5 days and analyzed cell proliferation using MTT assay. (B) Again, the cells were seeded in 12 wells with low density (1 x 10^3/ well) and incubated for 7 days. The cells were fixed with 10% formalin and stained with crystal violet. (C) Effect of E2F8 stable knockdown on cell proliferation in human lung fibroblasts cell lines. MRC5-shctrl, MRC5-shE2F8-1, BJ1-shctrl, and BJ1-shE2F8-1 cells were selected with puromycin (1 μg/ml) and the cells were plated in 96 wells (2 x 10^3/ well) and analyzed cell proliferation using MTT assay at each day after cell seeding (top). The cells were performed by western blot analysis to confirm the expression level of E2F8 (bottom). Mean ± SD in three independent experiments. Two-sided *t*-test. *, $P < 0.001$.

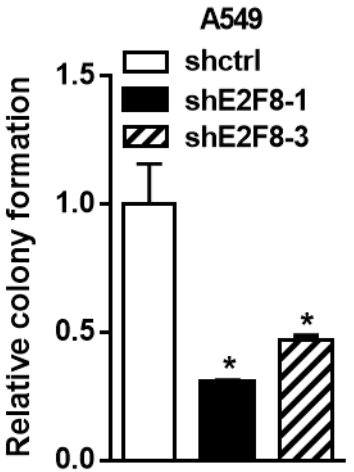
A


shctrl
shE2F8-1
shE2F8-3
E2F8
UHRF1
Actin
A549

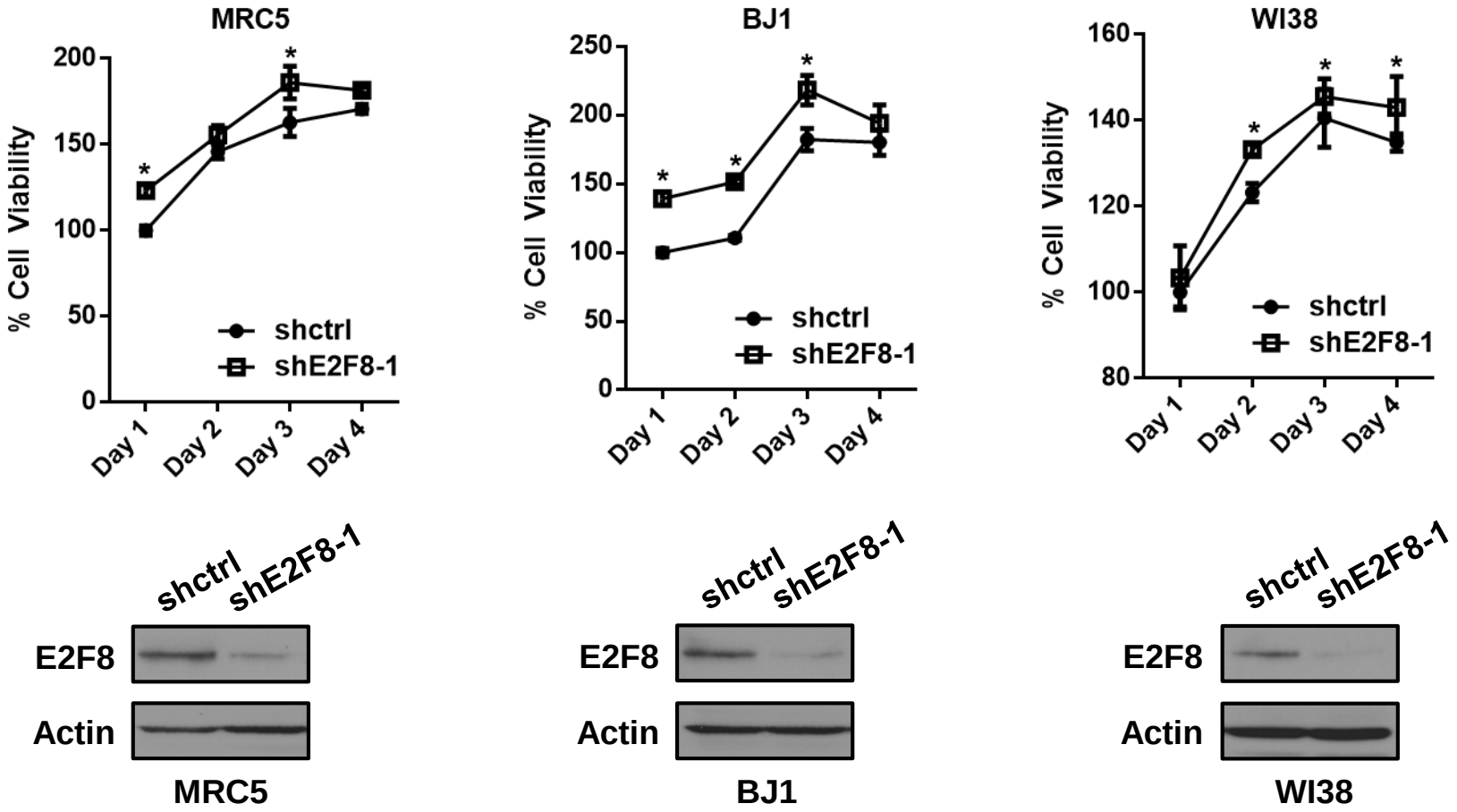

A549
% Cell Viability
150
100
50
0
*
*
shctrl
shE2F8-1
shE2F8-3

B


A549
shctrl
shE2F8-1
shE2F8-3


A549
shctrl
shE2F8-1
shE2F8-3
Relative colony formation
1.5
1.0
0.5
0.0
*
*

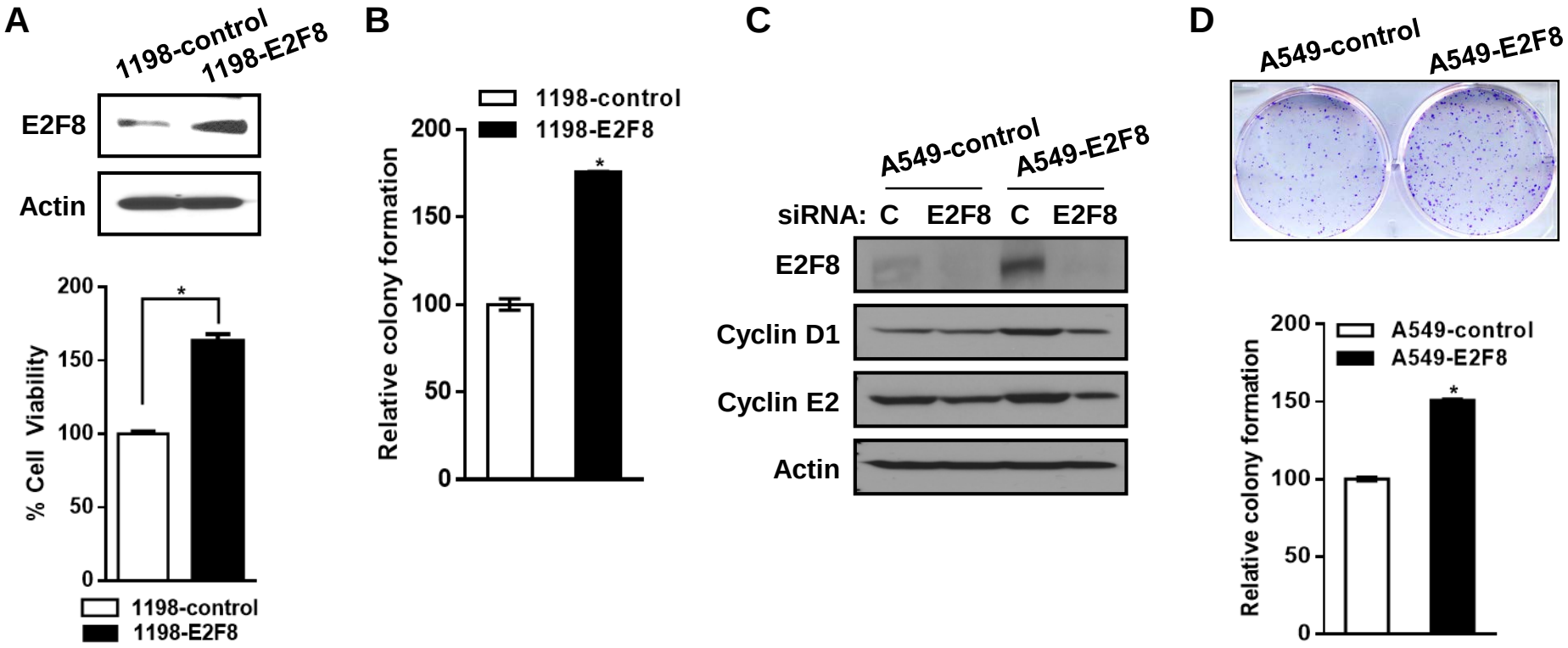
C


MRC5
% Cell Viability
200
150
100
50
0
shctrl
shE2F8-1
Day 1
Day 2
Day 3
Day 4
BJ1
250
200
150
100
50
0
WI38
160
140
120
100
80
shctrl
shE2F8-1
E2F8
Actin
MRC5
BJ1
WI38

Supplementary Figure 6, Related to Figure 3: The effect of E2F8 overexpression on cell proliferation.

(A) Effect of E2F8 overexpression on cell proliferation in 1198 cells. The stable overexpression of E2F8 was confirmed by western blot analysis. 1000 cells were seeded in the 96-well plate and cultured for 72 h, followed by MTT assay. Two-sided *t*-test. *, $P < 0.001$. (B) Effect of E2F8 overexpression on colony formation of 1198 cells. 1000 cells were seeded on the 6-well plate and cultured for 7 days. Mean ± SD in three independent experiments. Two-sided *t*-test. *, $P < 0.001$. (C-D) Effect of E2F8 overexpression in A549 cells. (C) The cells were established with stable overexpression of E2F8 and confirmed by western blot analysis. Overexpression of E2F8 was confirmed by its specific E2F8 siRNA. (D) Effect of E2F8 overexpression on colony formation of A549 cells. 1000 cells in each group were seeded on the 6-well plate and cultured for 7 days. Mean ± SD in three independent experiments. Two-sided *t*-test. *, $P < 0.001$.

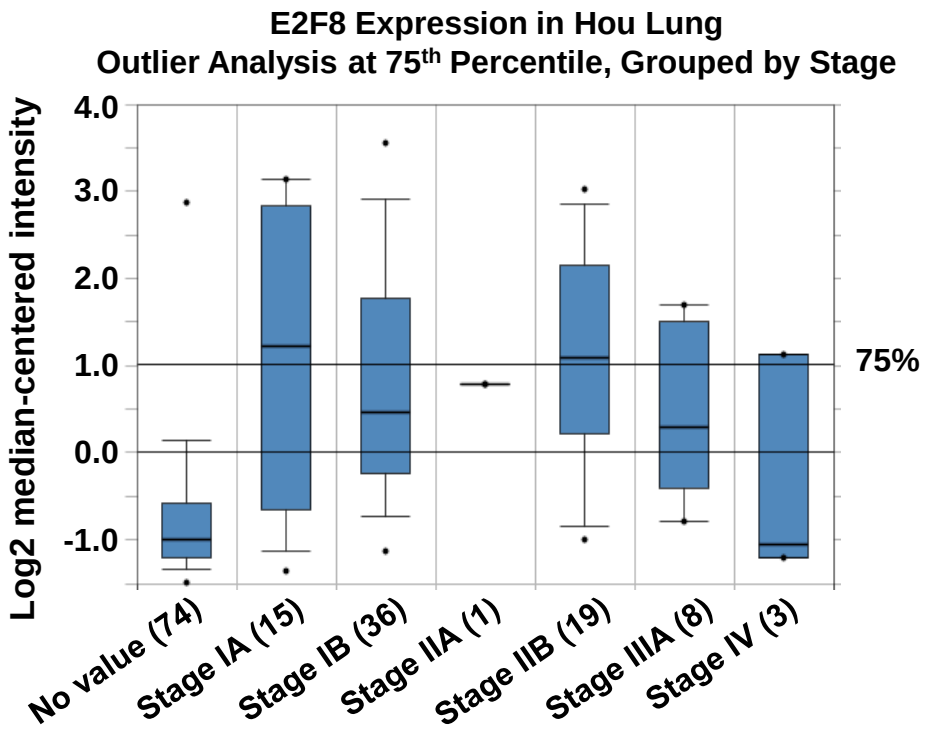
Supplementary Figure 6A-6D



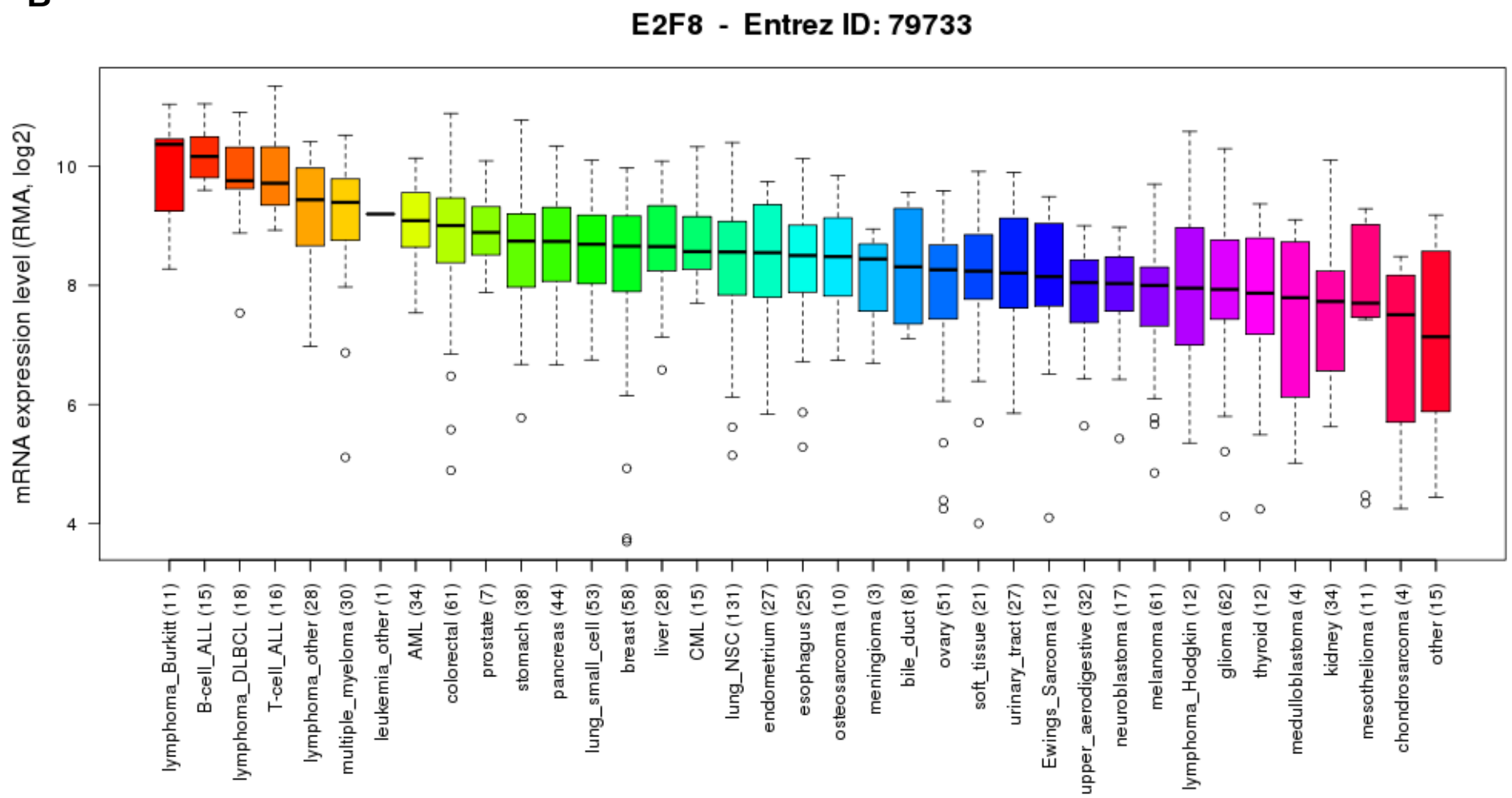
Supplementary Figure 7, Related to Figure 4: Effects of E2F8 overexpression on LC prognosis.

(A) Association of *E2F8* mRNA levels with tumor subtype grouped by stage (Hou Lung). Each data set was identified and the normalized (by ONCOMINE) data was obtained. The 25th–75th percentiles are indicated by a closed box with the median indicated by a line. (B) *E2F8* mRNA expression profile (RMA, log2) in 1,036 cancer cell lines (by CCLE; http://www.broadinstitute.org/ccle/home).

A

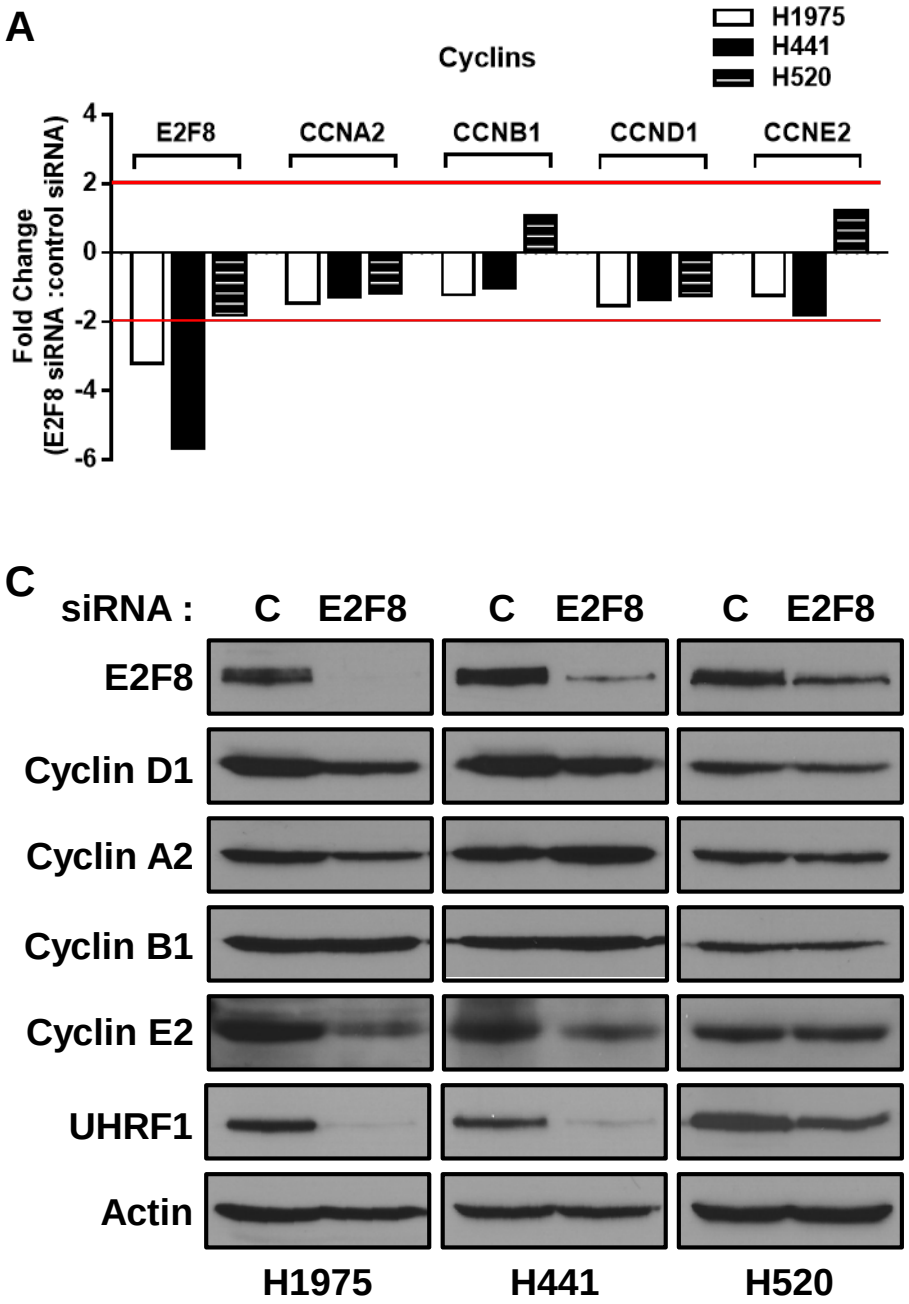

E2F8 Expression in Hou Lung
Outlier Analysis at 75th Percentile, Grouped by Stage
Log2 median-centered intensity
4.0
3.0
2.0
1.0
0.0
-1.0
75%
No value (74)
Stage IA (15)
Stage IB (36)
Stage IIA (1)
Stage IIB (19)
Stage IIIA (8)
Stage IV (3)

B


E2F8 - Entrez ID: 79733
mRNA expression level (RMA, log2)
10
8
6
4
lymphoma_Burkitt (11)
B-cell_ALL (15)
lymphoma_DLBCL (18)
T-cell_ALL (16)
lymphoma_other (28)
multiple_myeloma (30)
leukemia_other (1)
AML (34)
colorectal (61)
prostate (7)
stomach (38)
pancreas (44)
lung_small_cell (53)
breast (58)
liver (28)
CML (15)
lung_NSC (131)
endometrium (27)
esophagus (25)
osteosarcoma (10)
meningioma (3)
bile_duct (8)
ovary (51)
soft_tissue (21)
urinary_tract (27)
Ewings_Sarcoma (12)
upper_aerodigestive (32)
neuroblastoma (17)
melanoma (61)
lymphoma_Hodgkin (12)
glioma (62)
thyroid (12)
medulloblastoma (4)
kidney (34)
mesothelioma (11)
chondrosarcoma (4)
other (15)

Supplementary Figure 8, Related to Figure 5 and 6: Effects of E2F8 knockdown on the regulation of cyclins and UHRF1.

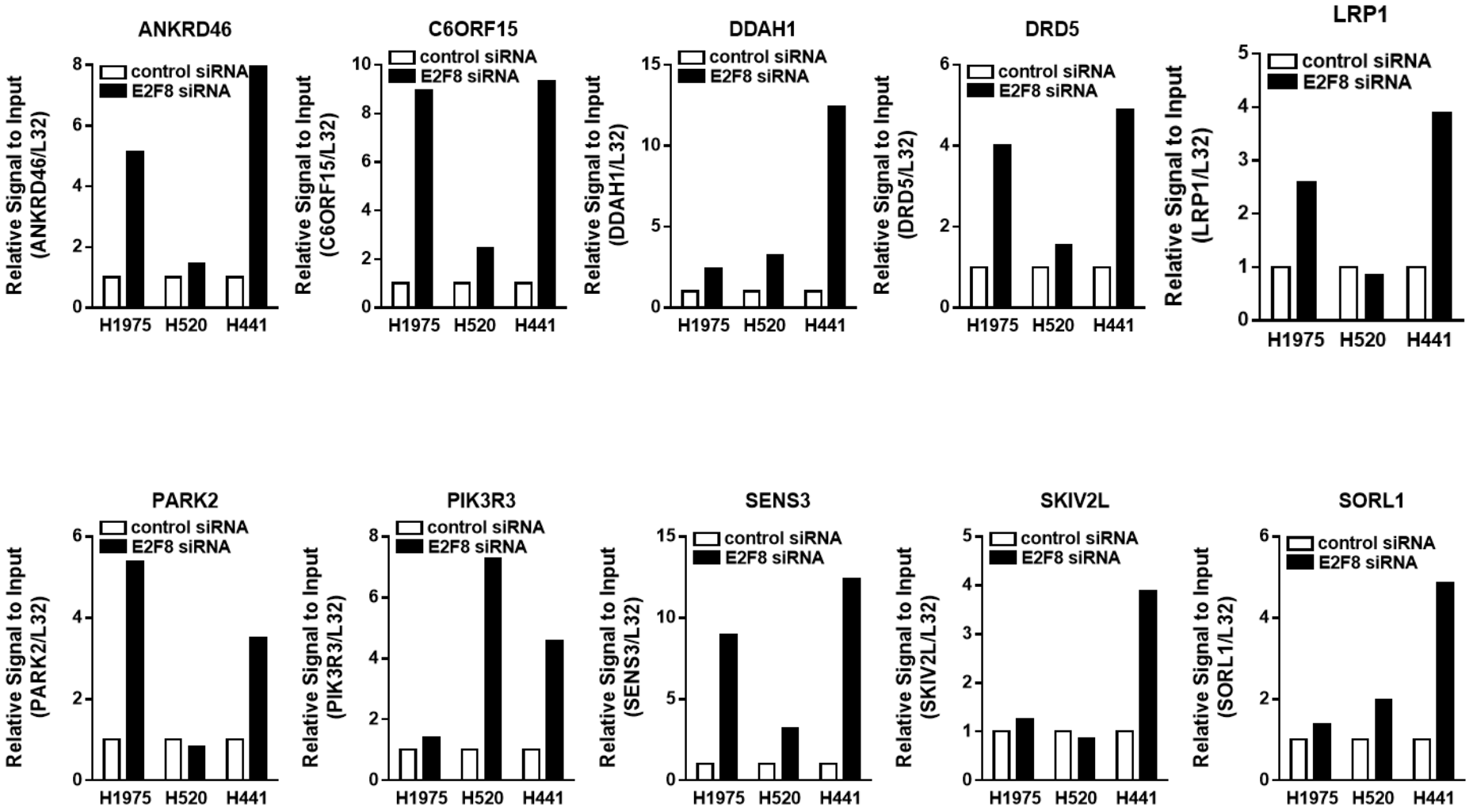
(A) Effect of E2F8 knockdown on the expression of cyclins in three LC cell lines from the microarray data. Y axis = Fold change of siE2F8-treated vs. sicontrol-treated cells; X-axis = the individual genes. (B) Indicated LC cells were transfected with control siRNA or E2F8 siRNA (40 nM, each) for 48 h and total RNAs were extracted, followed by qPCR using specific primers for each indicated gene. L32 was used as a control. Mean ± SD in three independent experiments. Two-sided *t*-test. *, $P < 0.001$. (C) Effects of E2F8 knockdown on the protein level of each cyclin and UHRF1 in LC cells. Indicated LC cells were transfected with control siRNA or E2F8 siRNA (40 nM, each) for 72 h and performed by western blot analysis.


A
H1975
H441
H520
Cyclins
E2F8
CCNA2
CCNB1
CCND1
CCNE2
Fold Change
(E2F8 siRNA :control siRNA)
4
2
0
-2
-4
-6
B
CCNA2
CCNB1
CCND1
CCNE2
UHRF1
control siRNA
E2F8 siRNA
Relative Signal to Input
(CCNA2/L32)
(CCNB1/L32)
(CCND1/L32)
(CCNE2/L32)
(UHRF1/L32)
1.5
1.0
0.5
0.0
H1975
H441
H520
*
C
siRNA :
C
E2F8
E2F8
Cyclin D1
Cyclin A2
Cyclin B1
Cyclin E2
UHRF1
Actin
H1975
H441
H520

Supplementary Figure 9, Related to Figure 5: The validation of upregulated genes by E2F8 knockdown.

Each of the indicated cells were transiently transfected with control siRNA or E2F8 siRNA (40 nM, each) for 48 h. Based on the microarray data, selected upregulated genes by E2F8 knockdown was confirmed by qPCR analysis. L32 was used as a control.

Supplementary Figure 9

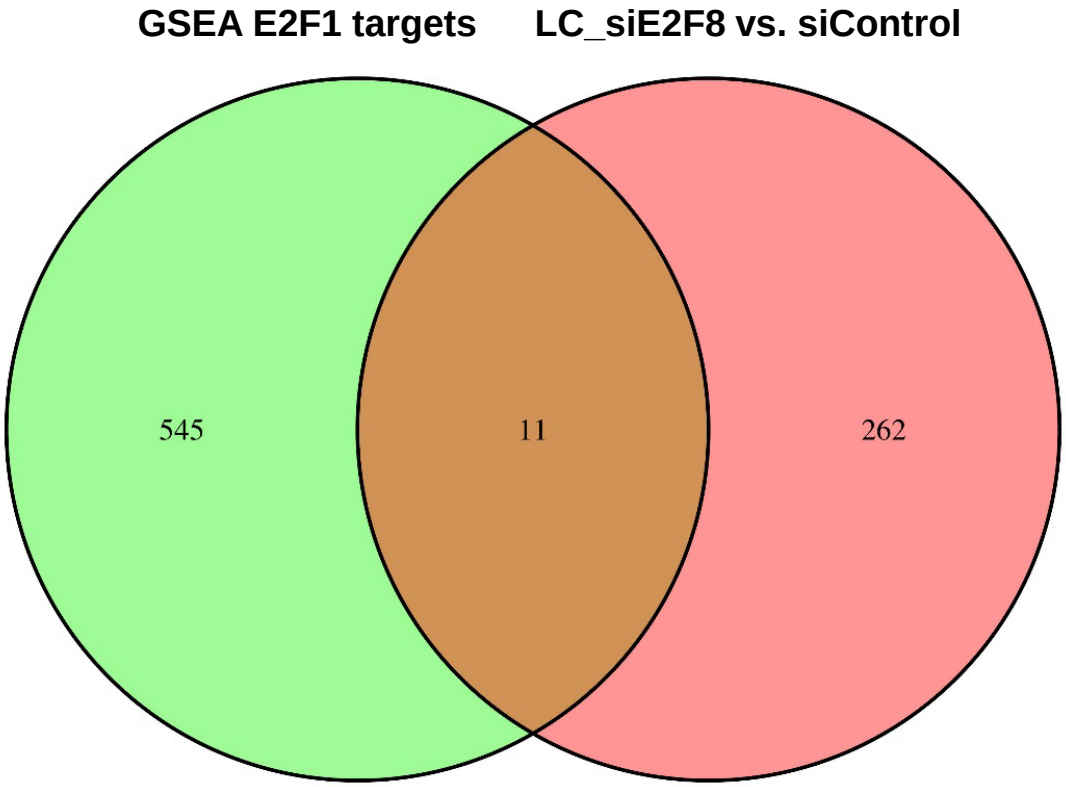


Supplementary Figure 10, Related to Figure 5: Overlap of E2F8 siRNA-affected genes with E2F1 targets.

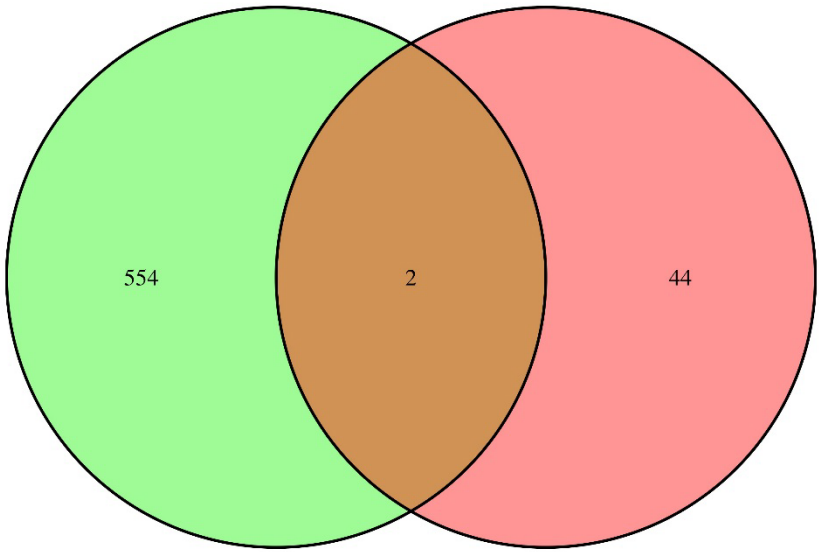
(A-C) GSEA E2F1 targets contain genes from the GSEA Molecular Signatures Database, c3.tft.v3.0.symbols matching E2F1. These are genes which have an E2F1 binding site in their proximal promoter conserved in humans, mice, rats, and dogs. (A) LC – siE2F8 vs siControl shows genes up or downregulated 1.5 fold or more between the log2 means of the three LC cell lines (H1975, H441, and H520) treated with siE2F8 versus siControl. (B and C) The same comparison is repeated for upregulated and downregulated genes respectively. Venn diagrams were created using the VennDiagram R package.

Supplementary Figure 10A

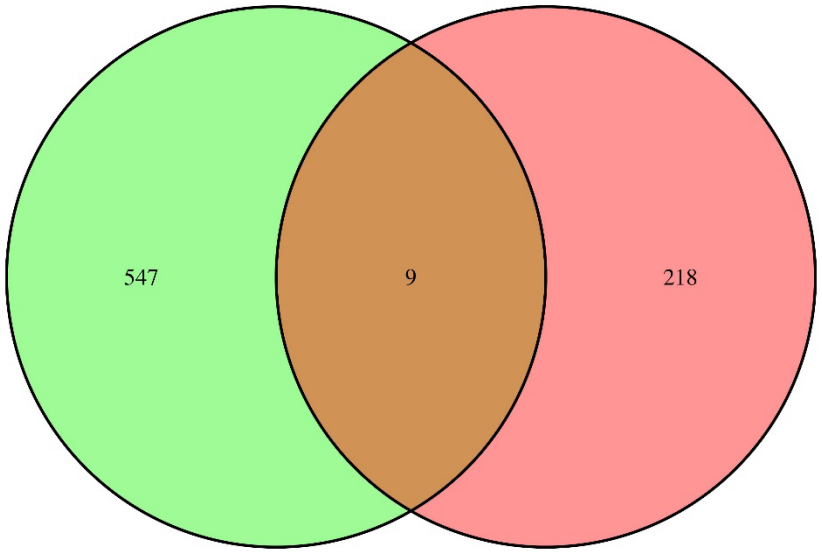
A



B


GSEA E2F1 targets
LC_siE2F8 vs. siControl
: Upregulated only
554
2
44

C


GSEA E2F1 targets
LC_siE2F8 vs. siControl
: Downregulated only
547
9
218

References

1. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 2010;26(5):589-595.
2. Heinz S, Benner C, Spann N*, et al.* Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 2010;38(4):576-589.