



(51) International Patent Classification:

C12Q 1/68 (2006.01) *G01N 33/15* (2006.01)
C12N 15/113 (2010.01) *A61K 39/395* (2006.01)
G01N 33/574 (2006.01)

(21) International Application Number:

PCT/US2016/029526

(22) International Filing Date:

27 April 2016 (27.04.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/153,253 27 April 2015 (27.04.2015) US

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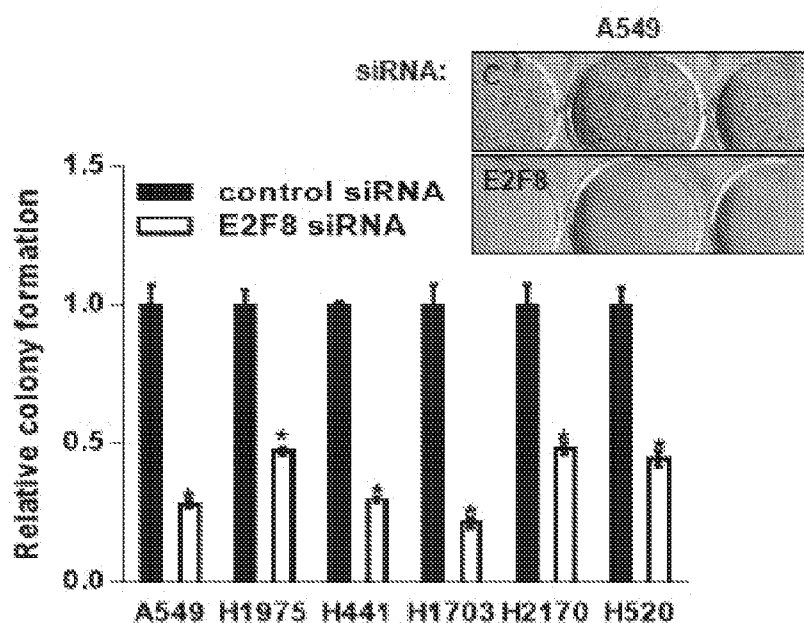
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: NOVEL COMPOSITIONS USEFUL FOR TREATING CANCER, AND METHODS USING SAME

FIG. 3B



(57) Abstract: The present invention includes a method of treating a cancer in a subject in need thereof, wherein the method comprises administering to the subject a therapeutically effective amount of composition that inhibits E2F8 expression in the subject. In certain embodiments, the subject is further administered at least one additional anti-cancer agent. The invention also includes a method of predicting the effectiveness of an anti-cancer agent in treating a subject suffering from cancer, and a method of providing the prognosis of a cancer treatment for a subject having a cancer.



Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

TITLE OF THE INVENTION

Novel Compositions Useful for Treating Cancer, and Methods Using Same

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CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/153,253, filed April 27, 2015, which application is incorporated herein by reference in its entirety.

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STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT

This invention was made with government support under grant numbers R01-CA126801 and R01-CA155196 awarded by National Cancer Institute. The government has certain rights in the invention.

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BACKGROUND OF THE INVENTION

There were about 160,000 cancer death in the USA in 2013. Lung cancer (LC) is the most frequent cause of cancer deaths worldwide with limited treatments for patients. Targeted inhibitors against receptor tyrosine kinases (RTKs) or epidermal growth factor receptor (EGFR) have shown some efficacy, but a majority of patients develop therapeutic resistance. Even though LC development is largely associated with mutations in oncogenic Kras or in the tumor suppressor p53, there are no clinically effective drugs for these patients.

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The E2F family members are divided into transcription activators (E2F1-E2F3) and repressors (E2F4-E2F8). Ectopic expression of E2F8 causes downregulation of E2F-target genes and cell-cycle arrest in fibroblasts. The synergistic function of E2F8 with E2F7 is essential for embryonic development, embryonic placental development, and embryonic angiogenesis in mice.

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Current therapies for cancer including surgery, chemotherapy, radiotherapy, and immunotherapy are effective only a certain amount of time, and eventually resistance to the treatment develops. Therefore, there is a need in the art to develop novel methods for treating a cancer. The present invention fulfills these needs.

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BRIEF SUMMARY OF THE INVENTION

The invention provides a method of treating or preventing a cancer in a subject in need thereof. The invention further provides a method of providing the prognosis of a cancer treatment for a subject having a cancer. The invention further provides a method of predicting the effectiveness of an anti-cancer agent in treating a subject suffering from cancer. The invention further provides a kit.

In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of a composition that inhibits expression of the *E2F8* gene in the subject, whereby the cancer is treated or prevented in the subject.

In certain embodiments, the method comprises detecting the level of expression of the *E2F8* gene in a biological sample from the subject. In other embodiments, the method comprises comparing the level of expression of the *E2F8* gene in the subject's biological sample to the level of expression of the *E2F8* gene in a control sample. In yet other embodiments, overexpression of *E2F8* gene in the subject's biological sample as compared to the control sample indicates an adverse outcome for the subject's cancer treatment.

In certain embodiments, the method comprises administering the anti-cancer agent to the subject. In other embodiments, the method comprises determining the expression level of the *E2F8* gene in a biological sample from the subject. In yet other embodiments, overexpression of the *E2F8* gene in the subject's biological sample as compared to a control sample indicates that the anti-cancer agent is not effective in treating the subject's cancer.

In certain embodiments, the composition comprises at least one agent selected from the group consisting of an antibody, siRNA, ribozyme, antisense RNA, modified antisense RNA, small molecule, and any combinations thereof. In other embodiments, the modified antisense RNA comprises morpholino-*E2F8* or a pharmaceutically acceptable salt thereof. In yet other embodiments, the antibody comprises an antibody selected from the group consisting of a polyclonal antibody, monoclonal antibody, humanized antibody, synthetic antibody, heavy chain antibody, human antibody, biologically active fragment of an antibody, and any combinations thereof. In yet other embodiments, the small molecule comprises naphthol AS-TR-Phosphate (NASTRp) or a pharmaceutically acceptable salt thereof.

In certain embodiments, the subject is a mammal. In other embodiments, the mammal is human.

In certain embodiments, the composition is administered to the subject by an inhalational, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, intracranial, or intravenous route of administration. In other embodiments, the composition is co-administered with at least one additional anti-cancer agent. In yet other embodiments, the at least one additional anti-cancer agent is selected from the group consisting of a nitrosourea, cyclophosphamide, adriamycin, 5-fluorouracil, paclitaxel and its derivatives, cisplatin, methotrexate, thiotepa, mitoxantrone, vincristine, vinblastine, etoposide, ifosfamide, bleomycin, procarbazine, chlorambucil, fludarabine, mitomycin C, vinorelbine, gemcitabine, and any combinations thereof. In yet other
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embodiments, the at least one additional anti-cancer agent is selected from the group consisting of a drug approved for non-small cell lung cancer, a drug combination to treat non-small cell lung cancer, and a drug approved for small cell lung cancer.

In certain embodiments, the cancer is a solid tumor. In other embodiments, the solid tumor comprises lung cancer. In yet other embodiments, the lung cancer comprises at least one selected from the group consisting of small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma. In yet other embodiments, the solid tumor comprises melanoma. In yet other embodiments, the solid tumor comprises pancreatic cancer. In yet other embodiments, the solid tumor is selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma,
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angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary
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carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma,
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neuroblastoma, and retinoblastoma.

In certain embodiments, the biological sample comprises at least one selected from the group consisting of urine, blood, and tissue. In other embodiments, the tissue comprises at least one selected from the group consisting of lung tissue, skin tissue and pancreas tissue.

In certain embodiments, the subject is counseled to receive a therapeutically effective amount of a composition that inhibits expression of the *E2F8* gene in the subject.

In certain embodiments, the subject is administered another anti-cancer agent, if the anti-cancer agent is not effective in treating the subject's cancer.

5 In certain embodiments, the subject is counseled to receive a therapeutically effective amount of a composition that inhibits expression of the *E2F8* gene in the subject.

In certain embodiments, the kit comprises a pharmaceutical composition that inhibits E2F8 expression in a subject. In other embodiments, the kit comprises an instruction manual reciting a method of treating a cancer in a subject using the pharmaceutical
10 composition.

BRIEF DESCRIPTION OF THE DRAWINGS

For the purpose of illustrating the invention, there are depicted in the drawings certain embodiments of the invention. However, the invention is not limited to the precise
15 arrangements and instrumentalities of the embodiments depicted in the drawings.

FIG. 1A illustrates the chemical structure of naphthol AS-TR-Phosphate (NASTRp) and the chemical structure of naphthol AS-E phosphate (NASEp).

FIG. 1B is a heat map of the genes regulated by NASTRp treatment at 10 μ M for 24 h in each indicated (non-small-cell lung carcinoma) NSCLC cell line. Red color (right side of Color Key) shows the upregulated genes; green color (left side of Color Key) shows the downregulated genes. Fold change ≥ 2 , $P < 0.05$. x-axis legend: (1) cell cycle, DNA replication, kinetochore, DNA repair; (2) cell death, cell cycle arrest; (3) DNA repair, nucleus; (4) transcription co-repressor activity; (5) DNA packaging, cell proliferation, nucleus. y-axis legend: a, H441-NSTR (10 μ M); b, H1975-NSTR (10 μ M); c, H1703-NSTR (10 μ M); d, A549-NSTR (10 μ M); e, H520-NSTR (10 μ M); f, H520-control; g, H441-control; h, H1975-control; i, H1703-control; j, A549-control. x-axis coordinates (from A to B): CDC23 III KIF20A, 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,
25 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 III KIAA0101, FAM64A, MCM10, BRIP1, NUF2, EXO1, TTK, XRCC2, MCM4, RRM2, FAM83D, UHRF1, ESC02, PCNA III PCNA-AS, GINS1, NEIL3, SHCBP1, CCPG1,
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CTAGE5, PABPC1L, PTPDC1, DNAJB9, LPXN, ACAD11 III NPHP3, C5orf41, C6orf48, DDIT4, CEBPG, WIP1, ASNS, GTPBP2, FAM129A, LAMP3, SESN2, C20orf69 III LOC100134822 III LOC728323 III PCMTD2, JMY, PCK2, NCRNA00219, SLC1A4, PPP1R15A, FBX025 III LOC728323, TRIB3, ARHGEF2, SLC6A9, CHAC1, DDIT3, 5 HIST1H2AH, POLQ, FANCM, POP1, ERCC6L, CNIH2, CDC7, CENPW, CTSL2, KIFC1, NRM, CDKN2C, LOC375010, BEST1, INHBE ATF3, C6orf225 III TUBE1 III WISP3, RAD54L, 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, 10 DUT, ESPL1, CCNF, TACC3, HIST1H3A, PBK, BIRC5, KIF18B, KIF14, KIF20B. In a non-limiting aspect, H441 is a human lung adenocarcinoma epithelial cancer cell line; H1975 is a human lung (non-small cell) adenocarcinoma cell line; H1703 is a human lung (non-small cell) squamous cancer cell line; A549 is a human lung epithelial cancer cell line; H520 is a human lung squamous carcinoma cell line.

15 FIGs. 1C-1D comprise a schematic network map illustrating transcription factors and their targets affected by NASTRp (FIG. 1C represents the left part of the map, and FIG. 1D represents the right part of the map). The center of the node for transcription factors (squares) and the whole node for target genes (circles) reflect the fold change in expression from the microarray analysis. Red color (right side of Gene_FC scale) shows the upregulated genes; green color (left side of Gene_FC scale) shows the downregulated genes. 20 Non-limiting illustrative up-regulated genes: CYP1B1, STC2, SESN2, HOXB9, ATF3, PPP1R15A.

FIG. 1E is a bar graph that illustrates the effects of NASTRp-mediated expression changes in each E2F family member. Y axis indicates ratio of expression values (Log 2) of NASTRp-treated vs. vehicle-treated cells; X-axis indicates the individual genes. 25

FIG. 1F is an image that illustrates the effects of NASTRp on the expression of E2F1, E2F2, and E2F8 in a dose-dependent manner (0, 5, 10, or 20 μ M; 48 h) in H441 and H520 cells.

FIG. 1G is an image that illustrates the effects of NASTRp on the expression of E2F1, E2F2, and E2F8 in a time-dependent manner (0, 4, 8, 24 h; 20 μ M) in H441 and H520 cells. 30

FIG. 2A is a set of bar graphs that illustrates protein levels of *E2F1*, *E2F2*, and *E2F8* in NSCLC cell lines compared with normal human lung tracheobronchial epithelial (NHTBE) cells. The expressions were quantitated by qPCR. All values in the graphs

represent mean $\pm$ SD in three independent experiments.

FIG. 2B is an image that illustrates protein levels of E2F1, E2F2, and E2F8 in LC cell lines compared with NHTBE cells.

FIG. 2C is a set of graphs that illustrates the effects of E2F1 or E2F2 knockdown on the LC cell growth. The cells transiently transfected with indicated siRNA (40 nM, each) were stained with trypan blue and the number of viable cells was counted at the indicated days. All values in the graphs represent mean $\pm$ SD of three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 2D is a bar graph that illustrates the effects of E2F1 or E2F2 knockdown on colony formation. Two days after knockdown of each gene (40 nM, each), the cells were seeded again in 6 wells with low density (2×10^3 /well) and incubated for 7-14 days. All values in the graphs represent mean $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 2E is a bar graph that illustrates the effects of E2F1 or E2F2 knockdown on cell invasion. Three days after knockdown of each gene (40 nM, each), the cells were transferred into transwell inserts containing matrigel-coated membranes and assessed 24 h after incubation. All values in the graphs represent mean $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 3A is a set of graphs that illustrates the effects of E2F8 knockdown on cell growth. LC cell lines (A549, H441, H1975, H2170, H1703, and H520) transiently transfected with control siRNA, or E2F8 siRNA (40 nM, each) were stained with trypan blue and the number of viable cells was counted at the indicated days. All values in the graphs represent mean $\pm$ SD of three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 3B illustrates the effects of E2F8 knockdown on colony formation. Two days after knockdown of E2F8, the cells were seeded again in 6-wells with low density (2×10^3 /well) and incubated for 7-14 days. All values in the graphs represent mean $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$. Inset shows the representative images of colonogenic assay are from the A549 cells transfected with control siRNA or E2F8 siRNA (40 nM, each).

FIG. 3C illustrates the effects of E2F8 knockdown on cell invasion. Three days after knockdown of E2F8, the cells were transferred into transwell inserts containing matrigel-coated membranes and assessed 24 h after incubation. All values in the graphs represent mean $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$. Inset shows the representative images of transwell invasion assay are from the A549 cells

transfected with control siRNA or E2F8 siRNA (40 nM, each).

FIG. 3D is a set of bar graphs that illustrates the effects of E2F8 knockdown on the cell growth in NHTBE cells. Relative mRNA level of E2F8 after NHTBE cells were transfected with control siRNA or E2F8 siRNA (50 nM, each) (left) and its effects on cell viability after 72 h incubation (right). All values in the graphs represent mean $\pm$ SD of three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 3E illustrates the level of protein expression of E2F8 after NHTBE cells were transfected with lentiviral shRNAs (left; short exposure/long exposure) and its effect on cell proliferation at each day after 24 h seeding (right). All values in the graphs represent mean $\pm$ SD of three independent experiments. Two-sided t-test. NS: non-significance.

FIG. 3F is a set of graphs that illustrates the effects of E2F8 knockdown on the cell cycle progression. A549 cells were transfected with control siRNA or E2F8 siRNA (20 nM) for 48 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.

FIG. 3G is an image that illustrates the effects of E2F8 knockdown on the phosphorylation of H2AX (Ser139) in NHTBE and LC cells. The cells were transfected with control siRNA or E2F8 siRNA (10 or 40 nM) for 48 h and performed by western blot analysis.

FIG. 3H is a set of images that illustrates effect of E2F8 depletion on the cell death as assessed by flow cytometry. Representative dot plot of Annexin V (x-axis) versus propidium iodide (y-axis) analyses of A549-shctrl (top) and A549-shE2F8 (bottom) cells.

FIG. 3I is a set of images illustrating the effects of E2F8 knockdown on the cell death in terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Indicated cells were transfected with control siRNA or E2F8 siRNA (40 nM) for 72 h and then the assay was performed. Scale bar = 100 μ m.

FIG. 3J is a bar graph that illustrates the effects of E2F8 knockdown on caspase 3/7 activity.

FIG. 3K is a set of images that illustrates expression of cleaved PARP or cleaved caspase-3. Indicated cells were transfected with control siRNA or E2F8 siRNA (40 nM, each) for 48 h and then performed each assay. Two-sided t-test. *, $P < 0.001$.

FIG. 4A is a bar graph that illustrates association of E2F8 mRNA levels with lung tumor subtype. The identified and normalized (by ONCOMINE; www.dot.oncomine.org) data was used for the analysis of the E2F8 expression in Hou Lung. 1: adjacent normal lung (n = 65), 2: large cell lung carcinoma (n = 19), 3: lung adenocarcinoma (n = 45),

4: squamous cell lung carcinoma (n = 27). The 25th-75th percentiles are indicated by a closed box, with the median indicated by a line; different degrees of outliers are indicated by the whiskers and the points, as defined for standard boxplots.

FIG. 4B is a set of images illustrating representative immunostainings for E2F8 expression in normal and LC tissues. Samples from human LC tissue microarray containing normal, adenocarcinoma, and squamous cell carcinoma tissues were examined by immunofluorescence staining with an anti-E2F8 antibody. Each H&E image was provided by US Biomax Inc. Scale bar = 200 μ m.

FIG. 4C is a graph that illustrates Kaplan-Meier analysis (kmplot dot com/analysis) of overall survival by low or high *E2F8* (E2F8 probe set 219990_s_at) expression in 1197 LC patients with adjuvant treatment. Overall survival analysis of the patients was performed by using Cox proportional hazard models and follow-up data for 7 years after surgery.

FIG. 4D is a graph that illustrates patients (n= 171) without adjuvant treatment were separated by high versus low E2F8 expression and analyzed for overall survival.

FIG. 4E is a graph that illustrates overall survival curves for the lung adenocarcinoma (LUAD) patients (n= 414).

FIG. 4F is a graph that illustrates the overall survival curves for lung squamous cell carcinoma (LUSC) patients (n= 109, GSE4573) with tumors expressing the high and low E2F8 level. All plots were analyzed in combined 10 data sets (CARRAY: n=462, GSE14814: n=90, GSE19188: n=156, GSE29013: n=55, GSE31210: n=246, GSE3141: n=110, GSE37745: n=196, GSE4573: n=130, GSE8894: n=138, and TCGA: n=133). HR = hazard ratio.

FIG. 5A is an image that illustrates a heatmap of genes deregulated in E2F8 depleted-LC cells. Microarray analysis was performed on three LC cell lines (H1975, H441, and H520) after the cells were transfected with control siRNA or E2F8 siRNA (40 nM, each) for 24 h. Genes up/downregulated more than 1.75 fold in 2 of 3 cell lines are shown. Red: right side of Raw Z-score; Green: left side of the Raw Z-score. x-axis coordinates (from A to B: SS18, SNAP23, MED6, C11orf24, SMARCA4, NDUFB11, POLD2, NIPA2, MRPL45, SHISA5, MOBKL2A, HIST1H2BM, OXA1L, HIST1H2BK, COX8A, USP41, PLSCR1, PARP9, MX1, OAS2, MX2, HERC6, PLAUR, ABR, SP110, CX3CL1, SLC37A4, CCL20, CHAC1, RTKN, CCDC21, PTPN9, PLAGL2, PLDN, CDC34, NHEJ1, RTCD1, COX10, TMED8, HCP5, POM121C, POM121, TMEM8A, GMNN, WBP2, BCL9, ZFAND3, FIGU, SNRPC, RIPK2, POU2F1, MGMT1, WBP11, TAB2, MICB, GNPAT1, PIK3R3, HIPK3,

TRAM2, LEPROTL1, TTYH3, HAX1, UHRF1, SMG7, MRPL36, EIF4EBP1, TAF9B, C13orf23, MBOAT1, DDAH1, SORL1, BMP4, SESN3, C6orf15, MOCS3, LOC146336, CXCL10, HSPC072, TRIM22, LOC100133034, LOC100132426, TMPRSS11E, STEAP4, RAB38, ENPP5, FAM96A, C5orf25, CENPV, LRRC37A, LOC728888, VARS2, C1orf83, SFXN2, VPS52, CDSN, ANGPTL4, TMEM180, PCTP, SLC19A2, SUV39H1, HMOX1, ZNF382, SLC38A9, E2F8.

FIG. 5B is a set of graphs that illustrates the four overlapping Gene set enrichment analysis (GSEA) profiles (from top 20). GSEA plots of each gene set are downregulated in the siE2F8 group compared with sicontrol group. FDR = false discovery rate. NES = normalized enrichment score.

FIG. 5C is a table that illustrates enriched gene ontology (GO) pathways generated by DAVID overrepresentation analysis in E2F8 knockdown group. Representative pathways were selected from the top 30. *P* values are shown only for the top GO pathways.

FIG. 5D is a set of bar graphs that illustrates the expression of representative genes of LC cells after treated with control siRNA or E2F8 siRNA (40 nM, each) for 48 h. qPCR analysis was performed to measure the mRNA level of the representative genes (*E2F8*, *UHRF1*, *POM121*, *NDUFB11*, *HAX1*, *EIF4EBP1*, *HIST2H2AB*, and *HIST1H2BM*). All values in the graphs represent mean $\pm$ SD of three independent experiments. Two-sided *t*-test. *, *P* < 0.001.

FIG. 5E is a graph that illustrates the distribution of the 204 ChIP-seq peaks revealed by E2F8 and categorized based on their location as promoter, CpG-island, intron, LTR (long terminal repeat), intergenic, 5UTR (5' untranslated region), TTS (transcription terminal site), exon, SINE (short interspersed elements), and LINE (long interspersed elements).

FIG. 5F is a table that illustrates most highly represented motif found by HOMER® software with its de Novo algorithm (*) and the top 10 results of HOMER® search through a library of known motifs. The identified motifs are sorted according to their *p*-values. Letter size indicates frequency.

FIG. 6A is an image that illustrates the level of UHRF1 in LC cells compared with NHTBE cells. The protein expression of UHRF1 was performed by western blot analysis.

FIG. 6B is a graph that illustrates the correlation of mRNA levels between *E2F8* and *UHRF1* in each individual LC cell line. *R* = correlation coefficient.

FIG. 6C is a set of images that illustrates the effects of E2F8 knockdown on

the expression of UHRF1. Each of the indicated cells were transfected with control siRNA or E2F8 siRNA (40 nM, each) for 72 h, followed by western blot analysis.

FIG. 6D is a set of images illustrating immunofluorescence staining of UHRF1 in A549 cells after 72 h transfection with control siRNA or E2F8 siRNA (40 nM, each).

5 Scale bar = 100 μ m. Inset bar = 20 μ m.

FIG. 6E is a schematic diagram illustrating the positions of putative E2F binding elements located in the gene promoter of human *UHRF1* ([www dot genomatrix dot de](http://www.dot.genomatix.de)) and the specific element corresponds to the region identified by ChIP sequencing. #0: the region for primers which cover E2F8 binding element, #1: the regions for primers which
10 cover putative E2F binding elements.

FIG. 6F is an image that illustrates direct binding of E2F8 on the UHRF1 promoter. ChIP assay was done with chromatin prepared from H441 cells. The binding of E2F8 to the *UHRF1* promoter was detected by visualization of the polymerase chain reaction (PCR) product. The single bands detected in input samples indicate the specificity of the
15 PCR primers.

FIG. 6G is a bar graph that illustrates relative *UHRF1* promoter activity with different UHRF1-luciferase constructs. 293T cells were transfected with pGL3-UHRF1-luc(-1088) which includes all elements in the FIG. 6E or pGL3-UHRF1-luc (-491) which lacks the region identified by ChIP sequencing, and cultured for 2 days. Luciferase activity
20 was measured and normalized by Renilla activity. pGL3-luciferase construct was used as a negative control. Two-sided t-test. *, $P < 0.001$.

FIG. 6H is a bar graph that illustrates the effects of each indicated siRNA on *UHRF1* promoter activity and expression in LC cells. The cells were transfected with indicated siRNAs (40 nM, each) for 48 h, followed by luciferase reporter assay. All values in
25 the graphs represent mean $\pm$ SD of three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 6I is a bar graph that illustrates the effects of each indicated siRNA on *UHRF1* promoter activity and expression in LC cells. The cells were transfected with indicated siRNAs (40 nM, each) for 48 h, followed qPCR analysis. All values in the graphs
30 represent mean $\pm$ SD of three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 6J is a bar graph that illustrates the effects of UHRF1 knockdown on colony formation. Each of the cells were seeded in 12 wells with low density (1×10^3 /well) and incubated for 7 days. The cells were fixed with 10% formalin and stained with crystal violet (left) and extracted those colonies with 10% acetic acid and quantitated (right).

FIG. 6K illustrates western blot analysis performed to confirm the expression levels of UHRF1, p-H2AX, and cleaved caspase-3.

FIG. 7A is a set of images of xenografts derived from control siRNA, E2F8 siRNA-1, or E2F8 siRNA-2-treated H520 cells illustrating the effects of E2F8-deficient cells on the capability of tumor growth *in vivo*. After 24 h siRNA transfection (40 nM, each), the cells were inoculated subcutaneously into the right and left side dorsal flanks of female nude mice (xenograft n = 7/group).

FIG. 7B is a graph that illustrates the tumor volume changes of xenografts derived from control siRNA, E2F8 siRNA-1, or E2F8 siRNA-2-treated H520 cells after inoculation. Tumor volume was measured with digital calipers and calculated by the formula $0.52 \times \text{length} \times \text{width}^2$.

FIG. 7C is a bar graph that illustrates the mass of xenografts derived from control or E2F8-knockdown H520 cells. Tumor mass was evaluated at 4 weeks after injection. Two-sided t-test. *, $P < 0.001$.

FIG. 8A illustrates the oligo sequence of mo-E2F8 as a translation-blocker (E2f8, SEQ ID NO:1; mo-E2f8, SEQ ID NO:2).

FIG. 8B is a set of images that illustrates the validation of mo-E2F8 on the expression of E2F8 and UHRF1 in A549 and A549-luc cells. The cells were treated with mo-control (10 μM) or mo-E2F8 (1, 5, or 10 μM) for 72 h and performed by western blot analysis.

FIG. 8C is a set of images illustrating the effects of mo-E2F8 on the cancer cell growth. A549-luc cells were treated with mo-control (10 μM) or mo-E2F8 (5 or 10 μM) for 72 h. Scale bar = 100 μm .

FIG. 8D is a bar graph that illustrates the cell viability after A549-luc cells are treated with mo-control or mo-E2F8 for 72 h. All values in the graphs represent mean $\pm$ SD of three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 8E is a set of representative images illustrating the effects of mo-E2F8 on the NHTBE cell growth. NHTBE cells were treated with mo-control (5 or 10 μM) or mo-E2F8 (5 or 10 μM) for 72 h. Scale bar = 100 μm .

FIG. 8F is a bar graph that illustrates cell viability of NHTBE cells after treated with mo-control or mo-E2F8 for 72 h. All values in the graphs represent mean $\pm$ SD of three independent experiments.

FIG. 8G is a set of images illustrating the effects of mo-E2F8 on tumor growth *in vivo*. A549-luc cells were inoculated subcutaneously into the right and left side dorsal

flanks of female nude mice (xenograft n = 8/group). Once the size of the xenograft reached about 5 x 5 mm (length x width), mice were intraperitoneally injected with mo-control or mo-E2F8. Each morpholino was treated at day 7, 10, 12, 14, and day 17 after cell inoculation. Bioluminescence images of A549-luc xenografts were captured at day 7, 14, 21, and day 28 after cell inoculation.

FIG. 8H is a graph illustrating bioluminescent intensity of tumors.

FIG. 8I is a graph illustrating volume of xenografts derived from mo-control or mo-E2F8-treated mice. Two-sided t-test. *, $P < 0.001$.

FIG. 8J is a graph illustrating the effects of mo-E2F8 on the mRNA levels of *E2F8*, *UHRF1*, and *PCNA* in vivo analyzed by qPCR analysis. Two-sided t-test. *, $P < 0.001$.

FIG. 8K is a set of immunofluorescence images of E2F8, UHRF1, and PCNA in tumors derived from mo-control or mo-E2F8-treated mice. Scale bar = 100 μ m. Inset bar = 20 μ m.

FIGs. 9A-9B are a set of images illustrating differential expression of transcription factors and their target genes in A549 cells after treated by NASTRp (FIG. 9A represents the left part of the full image, and FIG. 9B represents the right part of the full image). Red color indicates upregulated genes. Green color indicates downregulated genes. Square indicates transcription factor. Circle indicates target gene. Cyan border indicates negative enrichment of targets. Yellow color indicates positive enrichment. Thickness of border is proportional to the magnitude of enrichment. Non-limiting illustrative up-regulated genes: CYP1B1, STC2, HERPUD1, GREM1, SESN2, HOXB9, ATF3, JUN, PPP1R15A.

FIGs. 9C-9D are a set of images illustrating differential expression of transcription factors and their target genes in H441 cells after treated by NASTRp (FIG. 9C represents the left part of the full image, and FIG. 9D represents the right part of the full image). Red color indicates upregulated genes. Green color indicates downregulated genes. Square indicates transcription factor. Circle indicates target gene. Cyan border indicates negative enrichment of targets. Yellow color indicates positive enrichment. Thickness of border is proportional to the magnitude of enrichment. Non-limiting illustrative up-regulated genes: CYP1B1, HERPUD1, SESN2, KDELR3, CDKN1A, ATF3, PPP2R5B, PP1R15A, MAP18.

FIGs. 9E-9F are a set of images illustrating differential expression of transcription factors and their target genes in H1975 cells after treated by NASTRp (FIG. 9E represents the left part of the full image, and FIG. 9F represents the right part of the full

image). Red color indicates upregulated genes. Green color indicates downregulated genes. Square indicates transcription factor. Circle indicates target gene. Cyan border indicates negative enrichment of targets. Yellow color indicates positive enrichment. Thickness of border is proportional to the magnitude of enrichment. Non-limiting illustrative up-regulated genes: CYP1B1, STC2, PPP1R15A.

FIGs. 9G-9H are a set of images illustrating differential expression of transcription factors and their target genes in H520 cells after treated by NASTRp (FIG. 9G represents the left part of the full image, and FIG. 9H represents the right part of the full image). Red color indicates upregulated genes. Green color indicates downregulated genes. Square indicates transcription factor. Circle indicates target gene. Cyan border indicates negative enrichment of targets. Yellow color indicates positive enrichment. Thickness of border is proportional to the magnitude of enrichment. Non-limiting illustrative up-regulated genes: STC2, ARNTL, ITPR1, GADD45B, HERPUD1, SESN2, CBLB, MICAL2, HOXB9, HIST2H2BE, CBX4, ATF3, AREG, PPP1R15A, MAP1B.

FIGs. 9I-9J are a set of images illustrating differential expression of transcription factors and their target genes in H1703 cells after treated by NASTRp (FIG. 9I represents the left part of the full image, and FIG. 9J represents the right part of the full image). Red color indicates upregulated genes. Green color indicates downregulated genes. Square indicates transcription factor. Circle indicates target gene. Cyan border indicates negative enrichment of targets. Yellow color indicates positive enrichment. Thickness of border is proportional to the magnitude of enrichment. Non-limiting illustrative up-regulated genes: SLC25A27, SESN2, CBLB, ICA1, PPP1R15A.

FIG. 10A is a set of graphs illustrating validation of siRNAs on expression of E2Fs in LC cells. 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.

FIG. 10B is a set of images illustrating western blot analysis of each of the indicated cells transfected with E2F1 siRNA or E2F2 siRNA (10 and 50 nM, each) for 72 h.

FIG. 10C is a set of images illustrating western blot analysis of each of the indicated cells transfected with E2F8 siRNA-1 (10 and 50 nM, each) for 72 h.

FIG. 10D is a set of images illustrating western blot analysis of 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.

FIG. 11A, comprising two graphs, illustrates the effects of E2F8 depletion on the cell cycle progression. 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.

FIG. 11B, comprising two graphs, illustrates the effects of E2F8 depletion on the cell cycle progression. 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.

FIG. 12A is a set of images illustrating the effects 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 μ m.

FIG. 12B is a graph illustrating the effects of E2F8 knockdown on the DNA damage. 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. *, $P < 0.001$.

FIG. 13A, comprising an image of western blot analysis and a graph, illustrates the effects of E2F8 depletion on the cell growth in A549 cells. A549-shctrl, A549-shE2F8-1, and A549-shE2F8-3 cells were selected with puromycin (1.5 μ g/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 (2×10^3 /well) for 5 days and analyzed cell proliferation using MTT assay.

FIG. 13B, comprising an image of stained cells and a graph, illustrates the effects of E2F8 depletion on the cell growth in A549 cells. The A549 cells were seeded in 12 wells with low density (1×10^3 /well) and incubated for 7 days. The cells were fixed with 10% formalin and stained with crystal violet.

FIG. 13C, comprising three graphs and three images of western blot analysis, illustrates the effects 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 μ g/ml) and the cells were plated in 96 wells (2×10^3 /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 $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 14A, comprising an image of western blot analysis and a graph,

illustrates the effects 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. *, $P < 0.001$.

5 FIG. 14B is a graph illustrating the effects of E2F8 overexpression on colony formation of 1198 cells. 1,000 cells were seeded on the 6-well plate and cultured for 7 days. Mean $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 14C is an image of western blot analysis illustrating the effects of E2F8 overexpression in A549 cells.

10 FIG. 14D, comprising an image of stained cells and a graph, illustrates the effects of E2F8 overexpression on colony formation of A549 cells. 1,000 cells in each group were seeded on the 6-well plate and cultured for 7 days. Mean $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 15A is a graph illustrating the association of E2F8 mRNA levels with
15 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. E2F8 – Entrez ID: 79733

FIG. 15B is a graph illustrating E2F8 mRNA expression profile (RMA, log2) in 1,036 cancer cell lines (by CCLE; www.dot.broadinstitute.org/ccle/home).

20 FIG. 16A is a graph illustrating the effects 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.

FIG. 16B is a set of graphs illustrating the effects of E2F8 knockdown on the expression of cyclins of indicated LC cells. Each cell was transfected with control siRNA or
25 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 $\pm$ SD in three independent experiments. Two-sided t-test. *, $P < 0.001$.

FIG. 16C illustrates the effects of E2F8 knockdown on the protein level of each cyclin and UHRF1 in LC cells. Indicated LC cells were transfected with control siRNA
30 or E2F8 siRNA (40 nM, each) for 72 h and performed by western blot analysis.

FIG. 17, comprising a set of graphs, illustrates the validation of genes upregulated by E2F8 knockdown. Each of the indicated cells were transiently transfected with control siRNA or E2F8 siRNA (40 nM, each) for 48 h. L32 was used as a control.

FIG. 18A is a Venn diagram illustrating the overlap of genes of LC – siE2F8

vs siControl. 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 are shown.

FIG. 18B is a Venn diagram illustrating the overlap of genes of LC – siE2F8 vs siControl. Genes upregulated 1.5 fold or more between the log2 means of the three LC
5 cell lines (H1975, H441, and H520) treated with siE2F8 versus siControl are shown.

FIG. 18C is a Venn diagram illustrating the overlap of genes of LC – siE2F8 vs siControl. Genes 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 are shown.

FIG. 19 is an image and a graph illustrating the finding that miR-142-5p
10 downregulates E2F8 expression in NCI-H441 lung cancer cells and suppresses growth of the cells. Top: Expression of E2F8. Bottom: relative cell proliferation normalized to control at D3, as a function of days after transfection.

FIG. 20 is a set of immunofluorescence staining images illustrating the finding of E2f8 overexpression in lung tumors derived from *Kras*^{LSL-G12D/+}; *Trp53*^{Flox/Flox} (KP) lung
15 cancer mouse model.

FIGs. 21A-21D illustrate inhibitory effects of γ PNA targeting E2f8 on colony formation and viability of A549 cells. A549 lung cancer cells were transfected with γ PNA-E2f8 at 0, 5 and 10 μ M concentration by nucleofection using Nucleofector II and kit T reagent (Lonza, NJ). FIG. 21A: Transfected cells were subject to Western blotting with anti-E2F8 mouse monoclonal antibody (Abnova, CO) to validate the suppressive effect of γ PNA-E2f8 on E2F8 expression. FIG. 21B: Transfected A549 cells were cultured in 6 cell plates for colony formation assay. 10 days after incubation, cells were stained with 0.1% crystal violet and the number of colonies was quantitated (FIG. 21C). FIG. 21D: Transfected cells were seeded in 96 well plates and cell viability was measured by Celltiter Glo (Promega,
25 USA) in a time-course manner. ***P*<0.01.

FIGs. 22A-22B are a set of graphs illustrating the finding that E2F8 is required for the growth and survival of pancreatic cancer and melanoma cell lines. The MiaPaCa2 pancreatic cancer cell line (FIG. 22A) and 501MEL melanoma cell line (FIG. 22B) were infected with lentiviral media possessing shscrambled, shE2F8 #1 or #3. Infected cells were
30 selected with 1 μ g/ml puromycin for 4 days. 3,000 MiaPaCa2 and 2,000 501MEL cells were seeded into 96 well plates with complete media. Cell proliferation was measured by Celltiter glo in a time-course manner for 96 hrs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates, in one aspect, to the unexpected discovery that E2F8 transcription factor is overexpressed in cancer cells, and depletion of E2F8 inhibits cancer cell proliferation and tumor growth. The present disclosure supports E2F8 transcription factor as a novel therapeutic target for cancer, and also as a prognostic and predictive biomarker for cancer therapy.

In one aspect, the invention includes a method of treating a cancer in a subject in need thereof, wherein the method comprises administering to the subject a therapeutically effective amount of a composition that inhibits E2F8 expression and activity in the subject, whereby the cancer is treated in the subject. In certain embodiments, the subject is administered at least one additional anti-cancer agent. In certain embodiments, the cancer comprises lung cancer, melanoma, and/or pancreatic cancer. In other embodiments, the cancer comprises lung cancer. In yet other embodiments, the cancer comprises melanoma. In yet other embodiments, the cancer comprises pancreatic cancer. In yet other embodiments, the cancer comprises non-small-cell lung carcinoma. In yet other embodiments, the cancer comprises small-cell lung carcinoma. In yet other embodiments, the cancer comprises squamous cell lung carcinoma. In yet other embodiments, the cancer comprises lung adenocarcinoma.

In another aspect, the invention includes a method of providing prognosis of a cancer therapy for a subject.

In another aspect, the invention includes a method of predicting the effectiveness of an anti-cancer agent. In certain embodiments, the method comprises comparing the expression level of E2F8 in the subject after the treatment with the anti-cancer agent with the expression level of E2F8 in a control.

In yet another aspect, the invention includes a kit. The kit comprises a composition that inhibits E2F8 expression in a subject and an instruction manual teaching how to use the composition.

Definitions

As used herein, each of the following terms have the meaning associated with it in this section.

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics and chemistry are those well-known and

commonly employed in the art.

As used herein, the articles “a” and “an” refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

5 As used herein, the term “about” will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. As used herein when referring to a measurable value such as an amount, a temporal duration, and the like, the term “about” is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

10 As used herein, the term “adverse outcome” relating to a therapeutic treatment refers to a treatment that does not significantly (or at all) benefit the patient who is receiving and/or will receive the treatment. In certain embodiments, the patient suffers from cancer, and an adverse outcome for a treatment is characterized by therapeutically ineffective, insignificant, minimal or non-existent tumor size decrease and/or tumor growth rate decrease.
15 In other embodiments, a patient is likely to present an adverse outcome when there is overexpression of the *E2F8* gene in a patient’s tumor(s) as compared to a control sample.

The term “antibody,” as used herein, refers to an immunoglobulin molecule that specifically binds with an antigen. Antibodies may be intact immunoglobulins derived from natural sources or from recombinant sources and may be immunoreactive portions of
20 intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and F(ab)₂, as well as single chain antibodies and humanized antibodies (Harlow *et al.*, 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow *et al.*, 1989, In:
25 Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston *et al.*, 1988, Proc Natl Acad Sci USA 85:5879-5883; Bird *et al.*, 1988, Science 242:423-426).

The term “antigen” or “Ag” as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The
30 skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an “antigen” as that term is used herein. Furthermore,

one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired
5 immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

“Antisense” refers particularly to the nucleic acid sequence of the non-coding
10 strand of a double stranded DNA molecule encoding a polypeptide, or to a sequence which is substantially homologous to the non-coding strand. As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a polypeptide. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be
15 complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a polypeptide, which regulatory sequences control expression of the coding sequences.

As used herein, the term “beneficial outcome” relating to a treatment refers to a treatment that significantly benefits the patient who is receiving and/or will receive the
20 treatment. In certain embodiments, the patient suffers from cancer, and a beneficial outcome for a treatment is characterized by therapeutically effective, significant or measurable tumor size decrease and/or tumor growth rate decrease. In other embodiments, a patient is likely to present a beneficial outcome when there is normal expression or underexpression of the *E2F8* gene in a patient’s tumor(s) as compared to a control sample.

As used herein, “differentially expressed” or “differential expression” refers to any statistically significant difference ($p < 0.05$) in the level of expression of a gene between
25 two samples (e.g., two biological samples), or between a sample and a reference standard. Whether a difference in expression between two samples is statistically significant can be determined using an appropriate t-test (e.g., one- sample t-test, two-sample t-test, Welch's t-
30 test) or other statistical test known to those of skill in the art.

As used herein, the term “down-regulation” or “downregulation” as relating to a protein, ligand and/or receptor refers to any process or mechanism that reduces, or slows or prevents the increase of, the concentration, expression level and/or activity of the protein, ligand and/or receptor. The term “down-regulation” or “downregulation” includes any

process or mechanism described herein or known to those skilled in the art, including *in vivo* or *ex vivo* methods.

The terms “dsRNAi”, “RNAi”, “iRNA”, and “siRNA” are used interchangeably herein unless otherwise noted.

5 As used herein, the terms “effective amount” or “therapeutically effective amount” or “pharmaceutically effective amount” of a compound are used interchangeably to refer to the amount of the compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered. The term to “treat,” as used herein, means
10 reducing the frequency with which symptoms are experienced by a patient or subject or administering an agent or compound to reduce the severity with which symptoms are experienced. An appropriate therapeutic amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

 As used herein, “encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve
15 as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the
20 nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, may be referred to as encoding the protein or other product of that gene or cDNA.

 As used herein, the terms “gene expression” and “expression of the gene” are interchangeable. Both refer to the translation of information encoded in a gene into a gene
25 product (*e.g.*, RNA, protein). Expressed genes include genes that are transcribed into RNA (*e.g.*, mRNA) that is subsequently translated into protein, as well as genes that are transcribed into non-coding functional RNA molecules that are not translated into protein (*e.g.*, transfer RNA (tRNA), ribosomal RNA (rRNA), microRNA, ribozymes). “Level of expression,” “expression level” or “expression intensity” refers to the level (*e.g.*, amount) of one or more
30 products (*e.g.*, mRNA, protein) encoded by a given gene in a sample or reference standard.

 As used herein, the term “immunoglobulin” or “Ig” is defined as a class of proteins that function as antibodies. The five members included in this class of proteins are IgA, IgG, IgM, IgD, and IgE. IgA is the primary antibody that is present in body secretions, such as saliva, tears, breast milk, gastrointestinal secretions and mucus secretions of the

respiratory and genitor-urinary tracts. IgG is the most common circulating antibody. IgM is the main immunoglobulin produced in the primary immune response in most mammals. It is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody responses, and is important in defense against bacteria and viruses. IgD is the
5 immunoglobulin that has no known antibody function, but may serve as an antigen receptor. IgE is the immunoglobulin that mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to allergen.

The terms “inhibit” and “antagonize”, as used herein, mean to reduce a molecule, a reaction, an interaction, a gene, an mRNA, and/or a protein’s expression,
10 stability, function or activity by a measurable amount or to prevent entirely. Inhibitors are compounds that, *e.g.*, bind to, partially or totally block stimulation, decrease, prevent, delay activation, inactivate, desensitize, or down regulate a protein, a gene, and an mRNA stability, expression, function and activity, *e.g.*, antagonists.

“Instructional material,” as that term is used herein, includes a publication, a
15 recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the composition and/or compound of the invention in a kit. The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition of the invention or be shipped together with a container which contains the compound and/or composition. Alternatively, the instructional material may be
20 shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively. Delivery of the instructional material may be, for example, by physical delivery of the publication or other medium of expression communicating the usefulness of the kit, or may alternatively be achieved by electronic transmission, for example by means of a computer, such as by electronic mail, or download
25 from a website.

The term “overexpression” refers to a gene producing more gene products (*e.g.* mRNA, protein) in relative to a control.

“Parenteral” administration of a composition includes, *e.g.*, subcutaneous (*s.c.*), intravenous (*i.v.*), intramuscular (*i.m.*), or intrasternal injection, or infusion techniques.

30 As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the composition, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term “pharmaceutical composition” refers to a mixture of at least one compound useful within the invention with other chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to: intravenous, oral, aerosol, parenteral, ophthalmic, pulmonary, intracranial and topical administration.

“Primer” refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, *i.e.*, in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers may be labeled with, *e.g.*, chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

“Probe” refers to a polynucleotide that is capable of specifically hybridizing to a designated sequence of another polynucleotide. A probe specifically hybridizes to a target complementary polynucleotide, but need not reflect the exact complementary sequence of the template. In such a case, specific hybridization of the probe to the target depends on the stringency of the hybridization conditions. Probes may be labeled with, *e.g.*, chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

The term “promoter” as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

As used herein, the term “promoter/regulatory sequence” means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements that are required for expression of the gene product. The promoter/

regulatory sequence may for example be one that expresses the gene product in a tissue specific manner.

The term “recombinant DNA” as used herein is defined as DNA produced by joining pieces of DNA from different sources.

5 The term “recombinant polypeptide” as used herein is defined as a polypeptide produced by using recombinant DNA methods.

The term “RNA” as used herein is defined as ribonucleic acid.

By the term “specifically bind” or “specifically binds,” as used herein, is meant that a first molecule (*e.g.*, an antibody) preferentially binds to a second molecule (*e.g.*,
10 a particular antigenic epitope), but does not necessarily bind only to that second molecule.

As used herein, a “subject” refers to a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. In certain embodiments, the subject is human.

By the term “synthetic antibody” as used herein, is meant an antibody which is
15 generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using
20 synthetic DNA or amino acid sequence technology which is available and well known in the art.

The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one that has been transfected,
25 transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

As used herein, the term “treatment” or “treating” is defined as the application or administration of a therapeutic agent, *i.e.*, a composition useful within the invention (alone or in combination with another pharmaceutical agent), to a subject, or application or
30 administration of a therapeutic agent to an isolated tissue or cell line from a subject (*e.g.*, for diagnosis or *ex vivo* applications), who has a disease or disorder, a symptom of a disease or disorder or the potential to develop a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of the disease or disorder or the potential to develop the disease or disorder. Such

treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

The term “underexpression” refers to a gene that does not produce or produce fewer gene products (*e.g.* mRNA, protein) in relative to a control.

5 Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within
10 that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

15 **Disclosure**

The present disclosure provides the unexpected discovery that E2F8 is overexpressed in lung cancer cells and tumors from lung cancer patients. As demonstrated herein, depletion of E2F8 inhibits lung cancer cells proliferation and tumor growth, but has little effect on the viability of normal lung epithelial cells. As such, E2F8 is a novel
20 therapeutic target for cancer treatment. In certain embodiments, a composition that inhibits expression of the *E2F8* gene is administered along with at least one additional anti-cancer agent, whereby the composition increases the therapeutic efficacy of the at least one additional anti-cancer agent.

The present invention also provides the unexpected discovery that E2F8
25 expression plays a causal role in survival of lung cell patients. As demonstrated herein, E2F8 expression is high at early stage in patients. This discovery warrants that E2F8 expression can be used as a prognostic and predictive biomarker for cancer in these patients.

As demonstrated herein, E2F8 transcription factor was overexpressed in lung cancer cells compared with normal human lung tracheobronchial epithelial (NHTBE) cells
30 (FIG. 2A and 2B). Depletion of E2F8 has little effect on the normal lung epithelial cells, but significantly decreases the viability of lung cancer cells (FIG. 3A). The viability decrease is attributable to DNA damage induced by the depletion of E2F8. Therefore, inhibition of E2F8 gene expression has therapeutic effects.

In one aspect, the invention includes a method of treating a cancer in a subject

in need thereof. The method comprises administering to the subject a therapeutically effective amount of a composition that inhibits E2F8 expression in the subject. In certain embodiments, the subject is further administered at least one additional anti-cancer agent,

While the description of the invention is exemplified through lung cancer, a skilled artisan would recognize that the present invention can be used in treating various types of cancers, in addition to lung cancer. In certain embodiments, the methods of the invention can be used to treat a cancer selected from the group consisting of lung cancer, breast cancer, colon cancer, endometrial cancer, renal cell carcinoma, liver cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, skin cancer, stomach cancer, and thyroid cancer. Various cancer subtypes can also be treated using the methods of the invention. For example, subtypes of lung cancer, such as non-small cell lung cancer; small cell lung cancer; and lung carcinoid tumor, can be treated using the compositions of the invention.

The methods described herein can be used to treat many different types of solid tumor. In a particular embodiment, the methods of the invention can be used to treat a solid tumor selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Compositions

The inhibition of a targeted gene expression (*e.g.*, *E2F8*) is well known in the art. It can be achieved using, for example, DNA-binding agents, small molecules, siRNAs, antibodies, antisense RNAs, modified antisense RNAs (such as morpholino oligomers, such as *E2F8*), synthetic oligonucleotides or any combinations thereof. It may be also achieved

post-transcriptionally through RNA interference. Alternatively, the inhibition of *E2F8* gene expression can be achieved by inhibiting the protein encoded by the *E2F8* gene.

In certain embodiments, the composition comprises an agent selected from the group consisting of an antibody, siRNA, antisense RNA, modified antisense RNA, a small molecule inhibitor and any combinations thereof. In other embodiments, the antibody comprises an antibody selected from a polyclonal antibody, monoclonal antibody, humanized antibody, synthetic antibody, heavy chain antibody, human antibody, biologically active fragment of an antibody, and combinations thereof.

In certain embodiments, the inhibitor comprises an isolated nucleic acid. In other embodiments, the inhibitor is an siRNA or antisense molecule, which inhibits *E2F8* expression. In yet other embodiments, the nucleic acid comprises a promoter/regulatory sequence such that the nucleic acid is preferably capable of directing expression of the nucleic acid. Thus, the invention provides expression vectors and methods for the introduction of exogenous DNA into cells with concomitant expression of the exogenous DNA in the cells such as those described, for example, in Sambrook *et al.* (2012, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel *et al.* (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York) and as described elsewhere herein.

In certain embodiments, the subject is a mammal. In other embodiments, the mammal is human. In yet other embodiments, the composition is administered by an inhalational, oral, rectal, vaginal, parenteral, intracranial, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, or intravenous route of administration. In yet other embodiments, the agent is naphthol AS-TR-Phosphate (NASTRp), or a therapeutically effective salt or solvate thereof.

Synthetic nucleic acids are one of the ways to inhibit a targeted gene expression (*e.g.* *E2F8*). Non-limiting representatives of these synthetic nucleic acids (oligonucleotides) are antisense oligonucleotides, ribozymes, DNA enzymes and external guide sequences (EGS).

Antisense molecules and their use for inhibiting gene expression are well known in the art (*see, e.g.*, Cohen, 1989, In: Oligodeoxyribonucleotides, Antisense Inhibitors of Gene Expression, CRC Press). "Antisense oligonucleotides" are short single-stranded nucleic acid derivatives that bind to a complementary messenger ribonucleic acid (mRNA) whose translation into the corresponding protein is to be inhibited. In the cell, antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule

thereby inhibiting the translation of genes. The use of antisense methods to inhibit the translation of genes is known in the art, and is described, for example, in Marcus-Sakura, 1988, *Anal. Biochem.* 172:289. Such antisense molecules may be provided to the cell via genetic expression using DNA encoding the antisense molecule as taught by U.S. Patent No. 5,190,931. Alternatively, antisense molecules of the invention may be made synthetically and then provided to the cell. Antisense oligomers of between about 10 to about 30, and more preferably about 15 nucleotides, are preferred, since they are easily synthesized and introduced into a target cell. Synthetic antisense molecules contemplated by the invention include oligonucleotide derivatives known in the art which have improved biological activity compared to unmodified oligonucleotides (U.S. Patent No. 5,023,243).

In certain embodiments, an antisense nucleic acid sequence expressed by a plasmid vector is used to inhibit E2F8 protein expression. The antisense expressing vector is used to transfect a mammalian cell or the mammal itself, thereby causing reduced endogenous expression of E2F8.

The ability to specifically inhibit gene function in a variety of organisms utilizing antisense RNA or dsRNA-mediated interference (RNAi or dsRNA) is well known in the fields of molecular biology.

RNA interference (RNAi) is a phenomenon in which the introduction of double-stranded RNA (dsRNA) into a diverse range of organisms and cell types causes degradation of the complementary mRNA. In the cell, long dsRNAs are cleaved into short 21-25 nucleotide small interfering RNAs, or siRNAs, by a ribonuclease known as Dicer. The siRNAs subsequently assemble with protein components into an RNA-induced silencing complex (RISC), unwinding in the process. Activated RISC then binds to complementary transcript by base pairing interactions between the siRNA antisense strand and the mRNA. The bound mRNA is cleaved and sequence specific degradation of mRNA results in gene silencing. See, for example, U.S. Patent No. 6,506,559; Fire *et al.*, 1998, *Nature* 391(19):306-311; Timmons *et al.*, 1998, *Nature* 395:854; Montgomery *et al.*, 1998, *TIG* 14(7):255-258; Engelke, Ed., *RNA Interference (RNAi) Nuts & Bolts of RNAi Technology*, DNA Press, Eagleville, PA (2003); and Hannon, Ed., *RNAi A Guide to Gene Silencing*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (2003). Soutschek *et al.* (2004, *Nature* 432:173-178) describes a chemical modification to siRNAs that aids in intravenous systemic delivery. Optimizing siRNAs involves consideration of overall G/C content, C/T content at the termini, T_m and the nucleotide content of the 3' overhang. See, for instance, Schwartz *et al.*, 2003, *Cell*, 115:199-208 and Khvorova *et al.*, 2003, *Cell* 115:209-216.

Therefore, the present invention also includes methods of decreasing levels of E2F8 using RNAi technology.

dsRNA (RNAi) typically comprises a polynucleotide sequence identical or homologous to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules and should not hybridize with sequences within the hybridizing portions of the dsRNA molecule. The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. Accordingly, one method for treating retroviral infection according to the invention comprises the use of materials and methods utilizing double-stranded interfering RNA (dsRNAi), or RNA-mediated interference (RNAi) comprising polynucleotide sequences identical or homologous to a desired component of TGF- β signaling pathway. A simple injection of dsRNA, whose sense-strand sequence is identical to the target mRNA to be inhibited, can specifically inhibit expression of a target gene having the corresponding DNA sequence. This does not impair the expression of nonhomologous genes and the base sequence of the target gene is not altered.

RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence may also be used for inhibition. Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (Gribskov & Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (*e.g.*, University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase may be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an

expression construct, a regulatory region (*e.g.*, promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands); the promoters may be known inducible promoters such as baculovirus. Inhibition may be targeted by specific transcription in an organ, tissue, or cell type. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or bacteriophage RNA polymerase (*e.g.*, T3, T7, SP6). The use and production of an expression construct are known in the art (for example, WO 97/32016; U.S. Patent Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA may be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or combinations thereof. Alternatively, the RNA may be used with no, or a minimum of, purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing and/or stabilization of duplex strands.

Fragments of genes may also be utilized for targeted suppression of gene expression. These fragments are typically in the approximate size range of about 20 consecutive nucleotides of a target sequence. Thus, targeted fragments are preferably at least about 15 consecutive nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 consecutive nucleotides in length. In a more preferred embodiment, the gene fragments are at least about 25 consecutive nucleotides in length. In an even more preferred embodiment, the gene fragments are at least 50 consecutive nucleotides in length. Various embodiments also allow for the joining of one or more gene fragments of at least about 15 nucleotides via linkers. Thus, RNAi molecules useful in the practice of the instant invention may contain any number of gene fragments joined by linker sequences.

In certain embodiments, the invention provides a vector comprising an siRNA or antisense polynucleotide. In other embodiments, the siRNA or antisense polynucleotide inhibits the expression of E2F8. The incorporation of a desired polynucleotide into a vector and the choice of vectors is well-known in the art.

In certain embodiments, the expression vectors described herein encode a short hairpin RNA (shRNA) inhibitor. shRNA inhibitors are well known in the art and are

directed against the mRNA of a target, thereby decreasing the expression of the target. In certain embodiments, the encoded shRNA is expressed by a cell, and is then processed into siRNA. For example, in certain instances, the cell possesses native enzymes (*e.g.*, dicer) that cleaves the shRNA to form siRNA.

5 The siRNA, shRNA, or antisense polynucleotide can be cloned into a number of types of vectors as described elsewhere herein. For expression of the siRNA or antisense polynucleotide, at least one module in each promoter functions to position the start site for RNA synthesis.

 In order to assess the expression of the siRNA, shRNA, or antisense
10 polynucleotide, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected using a viral vector. In certain embodiments, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes
15 may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers are known in the art and include, for example, antibiotic-resistance genes, such as neomycin resistance and the like.

 Therefore, in another aspect, the invention relates to a vector, comprising the nucleotide sequence of the invention or the construct of the invention. The choice of the
20 vector will depend on the host cell in which it is to be subsequently introduced. In certain embodiments, the vector of the invention is an expression vector. Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. In certain embodiments, the expression vector is selected from the group consisting of a viral vector, a bacterial vector and a mammalian cell vector. Prokaryote- and/or eukaryote-vector based systems can be
25 employed for use with the present invention to produce polynucleotides, or their cognate polypeptides. Many such systems are commercially and widely available.

 Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in virology and molecular biology manuals. Viruses, which are useful as vectors include, but
30 are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers. (See, *e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193.

By way of illustration, the vector in which the nucleic acid sequence is introduced can be a plasmid that is or is not integrated in the genome of a host cell when it is introduced in the cell. Illustrative, non-limiting examples of vectors in which the nucleotide sequence of the invention or the gene construct of the invention can be inserted include a tet-on inducible vector for expression in eukaryote cells.

The vector may be obtained by conventional methods known by persons skilled in the art (Sambrook *et al.*, 2012). In certain embodiments, the vector is a vector useful for transforming animal cells.

In certain embodiments, the recombinant expression vectors may also contain nucleic acid molecules which encode a peptide or peptidomimetic inhibitor of invention, described elsewhere herein.

A promoter may be one naturally associated with a gene or polynucleotide sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous."

Similarly, an enhancer may be one naturally associated with a polynucleotide sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding polynucleotide segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a polynucleotide sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a polynucleotide sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," *i.e.*, containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCRTM, in connection with the compositions disclosed herein (U.S. Patent 4,683,202, U.S. Patent 5,928,906). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

It will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type, organelle, and organism chosen

for expression. Those of skill in the art of molecular biology generally know how to use promoters, enhancers, and cell type combinations for protein expression. The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is
5 advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or endogenous.

The recombinant expression vectors may also contain a selectable marker gene which facilitates the selection of transformed or transfected host cells. Suitable selectable marker genes are genes encoding proteins such as G418 and hygromycin which confer
10 resistance to certain drugs, β -galactosidase, chloramphenicol acetyltransferase, firefly luciferase, or an immunoglobulin or portion thereof such as the Fc portion of an immunoglobulin preferably IgG. The selectable markers may be introduced on a separate vector from the nucleic acid of interest.

Following the generation of the siRNA polynucleotide, a skilled artisan will
15 understand that the siRNA polynucleotide has certain characteristics that can be modified to improve the siRNA as a therapeutic compound. Therefore, the siRNA polynucleotide may be further designed to resist degradation by modifying it to include phosphorothioate, or other linkages, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and the like (see, *e.g.*, Agrwal *et al.*, 1987, Tetrahedron Lett. 28:3539-3542; Stec *et al.*, 1985 Tetrahedron Lett. 26:2191-2194; Moody *et al.*, 1989 Nucleic Acids Res. 12:4769-4782; Eckstein, 1989 Trends Biol. Sci. 14:97-100; Stein, In: Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression, Cohen, ed., Macmillan Press, London, pp. 97-117 (1989)).
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Any polynucleotide may be further modified to increase its stability *in vivo*.
25 Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiester linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine, and wybutosine and the like, as well as acetyl-, methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine, and uridine.

30 In certain embodiments, inhibiting the activity of E2F8 can be accomplished by using a transdominant negative mutant.

Ribozymes and their use for inhibiting gene expression are also well known in the art (see, *e.g.*, Cech *et al.*, 1992, J Biol Chem 267:17479-17482; Hampel *et al.*, 1989, Biochemistry 28:4929-4933; Eckstein *et al.*, WO 92/07065; U.S. Patent No. 5,168,053).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences encoding these RNAs, molecules may be engineered to recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988, J. Amer. Med. Assn. 260:3030). A major advantage of this approach is the fact that ribozymes are sequence-specific.

There are two basic types of ribozymes, namely, tetrahymena-type (Hasselhoff, 1988, Nature 334:585) and hammerhead-type. Tetrahymena-type ribozymes recognize sequences that are four bases in length, while hammerhead-type ribozymes recognize base sequences 11-18 bases in length. The longer the sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating specific mRNA species, and 18-base recognition sequences are preferable to shorter recognition sequences which may occur randomly within various unrelated mRNA molecules.

Ribozymes useful for inhibiting the expression of E2F8 may be designed by incorporating target sequences into the basic ribozyme structure that are complementary to the mRNA sequence of E2F8. Ribozymes targeting E2F8 may be synthesized using commercially available reagents (Applied Biosystems, Inc., Foster City, CA) or they may be genetically expressed from DNA encoding them.

EGS are synthetic RNA analogs which activate the cellular RNase P and bind via appropriate flanking sequences to the target mRNA and induce a specific mRNA degradation.

It is also possible to inhibit E2F8 gene expression by interaction with particular proteins with the aid of "decoy" oligomers, which mimic the binding regions for transcription factors. Treatment with decoy agents makes it possible to intercept particular proteins, in particular transcription factors, in a sequence-specific manner and thereby prevent a transcription activation.

Inhibition of the protein encoded by E2F8 can be achieved by agents such as small molecules, antibodies, peptides and enzymes. As will be understood by one skilled in the art, any antibody that may recognize and specifically bind to E2F8 is useful in the present invention. The invention should not be construed to be limited to any one type of antibody, either known or heretofore unknown, provided that the antibody may specifically bind to E2F8. Methods of making and using such antibodies are well known in the art. For example,

the generation of polyclonal antibodies may be accomplished by inoculating the desired animal with the antigen and isolating antibodies which specifically bind the antigen therefrom. Monoclonal antibodies directed against full length or peptide fragments of a protein or peptide may be prepared using any well known monoclonal antibody preparation procedures, such as those described, for example, in Harlow *et al.* (1989, Antibodies, A Laboratory Manual, Cold Spring Harbor, New York) and in Tuszynski *et al.* (1988, Blood 72:109-115). Quantities of the desired peptide may also be synthesized using chemical synthesis technology. Alternatively, DNA encoding the desired peptide may be cloned and expressed from an appropriate promoter sequence in cells suitable for the generation of large quantities of peptide. Monoclonal antibodies directed against the peptide are generated from mice immunized with the peptide using standard procedures as referenced herein. However, the invention should not be construed as being limited solely to methods and compositions including these antibodies, but should be construed to include other antibodies, as that term is defined elsewhere herein.

In certain embodiment, the composition further comprises at least one anti-cancer agent. The anti-cancer agent can be selected from the group consisting of a nitrosourea, cyclophosphamide, adriamycin, 5-fluorouracil, paclitaxel and its derivatives, cisplatin, methotrexate, thiotepa, mitoxantrone, vincristine, vinblastine, etoposide, ifosfamide, bleomycin, procarbazine, chlorambucil, fludarabine, mitomycin C, vinorelbine, gemcitabine, and the combinations thereof. In other embodiments, the anti-cancer agent is selected from the group consisting of:

Drugs Approved for Non-Small Cell Lung Cancer: Abitrexate (Methotrexate); Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation); Afatinib Dimaleate; Alimta (Pemetrexed Disodium); Avastin (Bevacizumab); Bevacizumab; Carboplatin; Ceritinib; Cisplatin; Crizotinib; Cyramza (Ramucirumab); Docetaxel; Erlotinib Hydrochloride; Folex (Methotrexate); Folex PFS (Methotrexate); Gefitinib; Gilotrif (Afatinib Dimaleate); Gemcitabine Hydrochloride; Gemzar (Gemcitabine Hydrochloride); Iressa (Gefitinib); Mechlorethamine Hydrochloride; Methotrexate; Methotrexate LPF (Methotrexate); Mexate (Methotrexate); Mexate-AQ (Methotrexate); Mustargen (Mechlorethamine Hydrochloride); Navelbine (Vinorelbine Tartrate); Nivolumab; Opdivo (Nivolumab); Paclitaxel; Paclitaxel Albumin-stabilized Nanoparticle Formulation; Paraplat (Carboplatin); Paraplatin (Carboplatin); Pemetrexed Disodium; Platinol (Cisplatin); Platinol-AQ (Cisplatin); Ramucirumab; Tarceva (Erlotinib Hydrochloride); Taxol (Paclitaxel); Taxotere (Docetaxel); Vinorelbine Tartrate; Xalkori (Crizotinib); Zykadia (Ceritinib).

Drug Combinations Used to Treat Non-Small Cell Lung Cancer: Carboplatin-Taxol; Gemcitabine-Cisplatin.

Drugs Approved for Small Cell Lung Cancer: Abitrexate (Methotrexate); Doxorubicin Hydrochloride; Etopophos (Etoposide Phosphate); Etoposide; Etoposide Phosphate; Folex (Methotrexate); Folex PFS (Methotrexate); Hycamfin (Topotecan Hydrochloride); Mechlorethamine Hydrochloride; Methotrexate; Methotrexate LPF (Methotrexate); Mexate (Methotrexate); Mexate-AQ (Methotrexate); Mustargen (Mechlorethamine Hydrochloride); Toposar (Etoposide); Topotecan Hydrochloride; VePesid (Etoposide).

Methods of Prediction and Prognosis

In one aspect, the present invention includes a method for predicting the effectiveness of an anti-cancer agent for a subject. The method comprises administering the anti-cancer agent; and determining the *E2F8* gene expression level in a biological sample from the subject. In certain embodiments, the determination the *E2F8* gene expression level is conducted after at least one treatment course of the anti-cancer agent. The term "treatment course" refers to the duration of administration of the anti-cancer agent as required for possible treatment outcomes, depending on the characteristics of the anti-cancer agent. The treatment course can be, for example, about 1, 2, 3, or 4 weeks. Alternatively, the treatment course can be, for example, about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months.

When the *E2F8* gene is overexpressed in the biological sample relative to a control, the anti-cancer agent is determined to be not effective. When the *E2F8* gene expression level is lower than that in a control (underexpression), the anti-cancer agent is determined to be effective.

In another aspect, the present invention includes a method of providing prognosis of a subject having a cancer, comprising detecting the level of expression of *E2F8* gene in a biological sample from the subject, wherein, if the *E2F8* gene is overexpressed in the biological sample as relative to a control, an adverse outcome for the subject is determined, and wherein, if the *E2F8* gene expression is underexpressed or about equally expressed in the biological sample as relative to a control, a beneficial outcome for the subject is determined.

For the prognostic and predictive methods of the invention, gene expression can be evaluated in a biological sample from a subject. A biological sample can be a tissue sample, a biological fluid sample, a cell (e.g., a tumor cell) sample, and the like. Any means

of sampling from a subject, for example, tissue biopsy, by blood draw, spinal tap, tissue smear or scrape can be used to obtain a biological sample. Thus, the biological sample can be a biopsy specimen (*e.g.*, tumor, polyp, mass (solid, cell)), aspirate, smear or blood sample. The biological sample can be a tissue from an organ that has a tumor (*e.g.*, cancerous growth) and/or tumor cells, or is suspected of having a tumor and/or tumor cells. A tumor sample can also be obtained by *in vitro* harvest of cultured human cells derived from an individual's tissue. Tumor samples can, if desired, be stored before analysis by suitable storage means that preserve a sample's protein and/or nucleic acid in an analyzable condition, such as quick freezing, or a controlled freezing regime.

The presence or absence of gene expression can be ascertained by the methods described herein or other suitable assays known to those of skill in the art.

A difference (*e.g.*, an increase, a decrease) in *E2F8* gene expression can be determined by comparison of the level of expression of the gene in a biological sample from a subject to that of a suitable control. Suitable controls include, for instance, a nonneoplastic tissue sample (*e.g.*, a non-neoplastic tissue sample from the same subject from which the cancer sample has been obtained), a sample of non-cancerous cells, non-metastatic cancer cells, non-malignant (benign) cells or the like, or a suitable known or determined reference standard. The reference standard can be a typical, normal or normalized range of levels, or a particular level, of expression of a protein or RNA (*e.g.*, an expression standard). The standards can comprise, for example, a zero gene expression level, the gene expression level in a standard cell line, or the average level of gene expression previously obtained for a population of normal human controls. Thus, the method does not necessarily require that expression of the gene be assessed in, or compared to, a control sample. A determination of difference (*e.g.*, an increase, a decrease) in the level of expression of a gene between two samples, or between a sample and a reference standard, can be accomplished using an appropriate algorithm or statistical analysis, several of which are known to those of skill in the art.

Suitable assays that can be used to assess the level of expression of a gene in a biological sample from a subject are known to those of skill in the art. For example, the level of an RNA (*e.g.*, mRNA) gene product in a sample can be determined by any technique that is suitable for detecting RNA expression levels in a biological sample. Several suitable techniques for determining RNA expression levels in cells from a biological sample (*e.g.*, Northern blot analysis, RT-PCR, *in situ* hybridization) are well known to those of skill in the art. Detection and quantification of specific RNA is accomplished using appropriately

labeled DNA or RNA probes complementary to the RNA in question. See, for example, Molecular Cloning: A Laboratory Manual, J. Sambrook *et al.*, eds., 2nd edition, Cold Spring Harbor Laboratory Press, 1989, Chapter 7, the entire disclosure of which is incorporated by reference. Other techniques for measuring gene expression in a sample are also within the skill in the art, and include various techniques for measuring rates of RNA transcription and degradation.

Gene expression on an array or gene chip can be assessed using an appropriate algorithm (*e.g.*, statistical algorithm). Suitable software applications for assessing gene expression levels using a microarray or gene chip are known in the art.

The level of expression of E2F8 can also be determined by assessing the level of a protein(s) encoded by the gene in a biological sample from a subject. Methods for detecting a protein encoded by E2F8 include, for example, immunological and immunochemical methods, such as flow cytometry (*e.g.*, FACS analysis), enzyme-linked immunosorbent assays (ELISA), chemiluminescence assays, radioimmunoassay, immunoblot (*e.g.*, Western blot), immunohistochemistry (IHC), and mass spectrometry. For instance, antibodies to a protein product of *E2F8* gene can be used to determine the presence and/or expression level of the protein in a sample either directly or indirectly *e.g.*, using immunohistochemistry (IHC).

Kits

In one aspect, the invention includes a kit. The kit comprises a composition comprising an agent that specifically binds to the protein encoded by *E2F8*, and an instruction manual. In certain embodiments, the agent is a monoclonal antibody specifically binding to the protein encoded by *E2F8*.

The kit can be used in connection with the methods described herein to make prediction on the effectiveness of an anti-cancer agent or provide the prognosis for a subject having a cancer.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-

recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

Materials and Methods

Unless otherwise noted, all cell lines, starting materials and resins were obtained from commercial suppliers and used without purification.

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 (LonzaWalkersville, Inc.). All of the cells were cultured at 37°C in humidified atmosphere of 95% air and 5% CO₂.

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.

Microarray Analysis

RNA was isolated using RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Gene expression analysis was performed on Affymetrix Human Gene 1.0 ST Genome arrays at the Yale University Keck Biotechnology Resource Laboratory. Expression values were normalized using GenePattern (www.broadinstitute.org/cancer/software/genepattern). Gene set enrichment analysis (GSEA; www.broadinstitute.org/mit-edu/gsea) was used to identify gene clusters. DAVID (david.abcc.ncifcrf.gov) functional annotation tool was used to identify gene ontology (GO) terms.

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 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 μ m filters. NHTBE, human fibroblasts, and LC cell lines were infected with lentiviral supernatant with polybrene (Sigma-Aldrich, 8 μ g/ml) and the cells were selected 48 h later with 1~2 μ g/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 μ l 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 Transposase expression vector (PB200PA-1, System Bioscience, Inc.) to 1198 and A549 cells using Lipofectamine 2000 (Invitrogen) and selected using puromycin (1 ~ 2 μ g/ml).

Colony Formation Assay

At 48 h after transfection by the indicated siRNAs, 2×10^3 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 5×10^4 cells were seeded on the transwell inserts with 8 μ m micropore filters (Corning Costar) in 500 μ l 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 Table 4.

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 ECLTM 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 Table 4 .

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 (hannonlab *dot* cshl *dot* edu/fastx_toolkit/index *dot* html) to remove low quality bases. Trimmed reads were mapped to the human reference genome (hg19) using BWA-MEM. Only reads with mapping quality scores equal or higher than 20 were kept. Peak and motif finding was performed using

HOMER®. For peak finding, transcription factor mode requiring each putative peak to have at least 2-fold normalized tags than input or IgG control samples were used. 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 (1×10^5 /mL). The cells were combined with Comet agarose at 1:10 ratio (v/v) and immediately transferred onto the OxiSelect™ Comet Slide (75 μ l/well) at 4°C in the dark for 15 min. After 15 min, the slide was transferred into pre-chilled lysis buffer for 1 h at 4°C in the dark. Next, the slide was transferred into pre-chilled alkaline solution for 30 min at 4°C 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 H₂O 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 μ l/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 ([www dot perceptiv dot co dot uk/cometassay](http://www.dot-perceptive-dot-co-dot-uk/cometassay)).

25 *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 37°C 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 (portal *dot* genego *dot* 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.

In Vivo Studies

Female J:NU nude mice were obtained from Jackson Laboratory and used when 6-7 weeks old. H520 cells were pretreated with 40 nM of control siRNA, E2F8 siRNA-1, or E2F8 siRNA-2 for 24 h, followed by transplantation (2×10^6 cells/flank,

xenograft $n = 7/\text{group}$) into the flank of mice. For A549-luc xenografts, A549 cells were transfected with pGL4.51 luciferase plasmid (Promega, E132A) using Lipofectamine 2000 and selected by culturing in the presence of 600 $\mu\text{g}/\text{ml}$ Geneticin (Invitrogen, #10131-035). Then, the mice received subcutaneous injections of cells (2×10^6 cells/flank) and were
 5 injected intraperitoneally with mo-control or mo-E2F8 (10 mg/kg) (vivo morpholino, Gene Tools, LLC) every other day, 5 times, once the size of the xenograft ($n = 8/\text{group}$) reached approximately 5×5 mm (length $\times$ width). All xenografts were transplanted in both the right and left dorsal flanks of mice. Tumor growth was monitored via bioluminescence imaging (IVIS spectrum, XENOGEN).

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Statistical Methods

Statistical analyses of microarray data were computed with R ([www dot R-project dot org](http://www.dot R-project dot org)) in combination with PostgreSQL database (www dot postgresql dot org) for data storage and retrieval.

15

NASTRp-derived top differentially expressed genes were selected with a fold change of at least 2 and a p-value of 0.05 or better as calculated with a Mann-Whitney/Wilcoxon test. E2F8 siRNA-derived top differentially expressed genes were selected with a fold change of at least 1.75. Survival was analyzed using the Kaplan–Meier method and compared by the log-rank test. The Cox proportional hazards assumption was
 20 examined by including time dependent covariables in the model (kmplot dot com/analysis). All statistical tests were two-sided and analyzed by Student's t-test.

Example 1: E2F family members are overexpressed in LC cells

Naphthol AS-TR phosphate (NASTRp) [3-((4-chloro-2-methylphenyl) carbamoyl)naphthalen-2-yl phosphoric acid or a salt thereof; FIG. 1A], an analog of naphthol
 25 AS-E phosphate [3-((4-chloro-phenyl) carbamoyl)naphthalen-2-yl phosphoric acid or a salt thereof], is an inhibitor of cAMP response element-binding protein (CREB) transcriptional activity (Best *et al.*, Proc Natl Acad Sci U S A 2004, 101:17622-7). NASEp inhibits IL-1 β -induced CXC chemokine gene expression and angiogenic activity in LC cells (Sun *et al.*,
 30 Cancer Prev Res (Phila) 2008, 1:316-28).

E2F pathway is downregulated by NASTRp. To evaluate the global effects of NASTRp, a microarray analysis was performed on five LC cell lines including three lung adenocarcinoma (LUAD; A549, H1975, and H441) and two squamous cell carcinoma (LUSC; H1703 and H520). The overall pattern of genes affected by NASTRp is shown in a

heat map (FIG. 1B) and the list of genes (*) is in Table 1. From DAVID analysis, cell cycle and cell proliferation-related genes were downregulated while cell death and cell cycle arrest-related genes were upregulated (Table 2).

GSEA was used to identify E2F transcription factor-gene sets mainly affected by NASTRp (Table 3). E2F transcription factors and their pathways were highly enriched in the overall transcription factor network perturbed by NASTRp (FIGs. 1C-1D and FIGs. 9A-9B). From the microarray data, mRNA levels of E2F1, E2F2 and E2F8 were markedly downregulated by NASTRp in all cell lines tested (FIG. 1E). Protein levels of E2F1, E2F2, and E2F8 were downregulated by NASTRp in a dose-dependent (FIG. 1F) or time-dependent (FIG. 1G) manner.

Activator E2Fs, E2F1 and E2F2 are overexpressed in LC (Eymin *et al.*, *Oncogene* 2001, 20(14):1678-1687; Coe *et al.*, *Br J Cancer* 2006, 94(12):1927-1935; Huang *et al.*, *Clin Cancer Res* 2007, 13(23):6938-6946). The present study indicated that E2F family members E2F1, E2F2, and E2F8 were overexpressed in LC cells compared with normal human lung tracheobronchial epithelial (NHTBE) cells (FIGs. 2A-2B).

To determine the relative importance of E2F family members in LC cell survival, the effects of E2F1 or E2F2 depletion on the cell growth of LC cells were examined. The knockdown of E2F1 led to decreased cell numbers with time (FIG. 2C), lower colony formation (FIG. 2D), and invasive activity of the cells (FIG. 2E). Although the effect of E2F2 knockdown on cell proliferation looked rather weak compared to the effect of E2F1 knockdown, it led to reduced cell proliferation in most cell lines.

Example 2: E2F8 is essential for LC cell survival

This study has also demonstrated that the expression of E2F8 is elevated in LC cells. Surprisingly, knockdown of E2F8 resulted in a significant decrease in the viability of all LC cell lines tested (FIG. 3A). Moreover, siE2F8 led to reduced colony formation (FIG. 3B, FIGs. 13A and 13B) and invasion abilities (FIG. 3C) of the cells. To confirm whether E2F8 affects cell proliferation, E2F8 was overexpressed in transformed 1198 cells, one of the *in vitro* lung carcinogenesis model (IVLCM) cell lines (Klien-Szanto *et al.*, *Proc Natl Acad Sci U S A* 1992, 89(15):6693-6697; Chun *et al.*, *J Natl Cancer Inst* 2003, 95(4):291-302), and A549 cells. The cells stably expressing E2F8 proliferated more rapidly than its control cells (FIGs. 14A-14D). Importantly, E2F8 knockdown had little effect on the viability of normal lung epithelial cells (FIGs. 3D-3E). Also, E2F8 depletion caused increased cell growth in normal lung fibroblasts (FIG. 13C). The validation of siRNAs was performed by qPCR and

western blot analysis (FIG. 10).

To test whether E2F8 plays a role in cell cycle progression in LC cells, flow cytometry using siRNA or lentiviral shRNA was used. There was a significant cell cycle arrest at the G1/S phase by 48 h after siE2F8 treatment (FIG. 3F) more than by 24 h treatment (FIG. 11A). It was also confirmed using cells stably transduced with E2F8 shRNA (FIG. 11B). The data has demonstrated that E2F8 depletion induced DNA damage as indicated by increased expression of p-H2AX (Ser139), a marker for double-strand breaks, in LC cells but not in normal NHTBE cells (FIG. 3G and FIG. 12A). Furthermore, DNA breaks in response to siE2F8 was confirmed by an alkaline comet assay (FIG. 12B). Increased cell death occurred in A549 cells treated with shE2F8 relative to its control cells as assessed by flow cytometry with Annexin V staining (FIG. 3H). After siE2F8 transfection, there was a significant increase in the level of TUNEL® staining (FIG. 3I) and caspase-3/7 activity in LC cells (FIG. 3J). An increase in cleaved PARP and cleaved caspase-3 expression in E2F8-depleted cells (FIG. 3K) was found. Taken together, the data clearly indicate that E2F8, while not necessary for normal cells, is essential for the growth of LC cells.

Example 3: E2F8 is overexpressed in LC and the overexpression is a worse prognosis factor

To determine the clinical relevance of E2F8 expression in human LC, the Oncomine database was used. Compared with normal tissues, the analysis revealed an elevated E2F8 expression in large cell lung carcinoma, LUAD, and LUSC (FIG. 4A). E2F8 expression was also elevated in the early stages of LC (FIG. 15A). Using cancer cell line encyclopedia (CCLE) database (Barretina *et al.*, Nature 2012;483(7391):603-607), E2F8 is found to be expressed across a wide spectrum of cancer cells (FIG. 15B).

Immunofluorescence staining in human LC tissue microarray revealed that E2F8 is highly overexpressed in LUAD and LUSC tissues compared with normal lung tissues (FIG. 4B).

Overexpression of E2F8 mRNA levels were associated with worse overall survival of LC patients (FIG. 4C). E2F8 mRNA levels were more strongly associated with worse overall survival of LC patients who did not receive any adjuvant therapy (FIG. 4D). Positive E2F8 expression was significantly correlated with shorter survival time of patients with LUAD (FIG. 4E) and LUSC (FIG. 4F) histology types. Results on clinical tumor samples have demonstrated that aberrant activation of E2F8 is associated with mortality of LC patients.

Example 4: Gene expression analysis following E2F8 knockdown reveals specific gene signatures in LC cells

To gain insight into the role of E2F8 in LC cell growth, genes perturbed by E2F8 knockdown were examined. Down/upregulated genes greater than 1.75 fold change in at least two of three cell lines are shown in a heatmap (FIG. 5A) and a list of genes (*) is in Table 5. GSEA was used to examine gene sets altered by siE2F8 and the representative gene sets were showed in FIG. 5B. DAVID (FIG. 5C) and MetaCore analysis (Table 6) revealed that knockdown of E2F8 deregulated gene sets involved in regulation of transcription, cancer progression, chromatin organization, regulation of immune system, glutamate receptor signaling, or cell surface receptor signaling. Among the top-down/upregulated genes by siE2F8, qPCR was used to confirm the expression level of selected genes (FIG. 5D and FIG. 17). Overlaps between E2F1 targets and genes deregulated by E2F8 knockdown were examined using version 3.0 of the GSEA TFT targets. As shown in FIG. 18 and Table 7, the Venn diagrams indicate that only a few genes overlap between E2F1 targets and E2F8-associated genes, suggesting that E2F8 regulates its target genes, E2F1 independently, at least in LC cells.

In order to characterize genome-wide E2F8 binding sites (BS) in the resting status, ChIP-sequencing was conducted using H441 cells, which contain high levels of endogenous E2F8. Two independent ChIP experiments were performed and statistical significant binding regions, which had at least 2-fold normalized tags than in input or IgG control were identified (E2F8-1 vs IgG; n=2328, E2F8-2 vs IgG; n=1414, E2F8-1 vs input; n=2120, and E2F8-2 vs input; n=1285). A set of 204 binding regions overlapped among all four comparisons (Table 8). FIG. 5E shows the genome-wide distribution of 204 BS in relation to the transcriptional start site, indicating that E2F8 mostly localizes to the promoter of the genes. Also, FIG. 5F shows DNA recognition sequences and statistical significance for the most highly represented E2F8-binding-motif (*) and top hit transcription factors. Taken together, these data suggest that E2F8 binds to the promoter and regulates gene transcription acting as a novel transcription activator in LC.

Example 5: UHRF1 is a potential target gene of E2F8

From the microarray data, UHRF1 was one of the top-downregulated genes by siE2F8. Compared to other cyclins, the level of UHRF1 was significantly decreased by E2F8 (FIG. 16). UHRF1 expression was increased in LC cells (FIG. 6A), resulting in a significant correlation between mRNA levels of E2F8 and UHRF1 (FIG. 6B). Suppression of UHRF1

expression by siE2F8 was confirmed using immunoblotting and immunofluorescence staining (FIG. 6C and 6D).

The UHRF1 promoter contained several putative E2F BS, including ChIP sequencing-based E2F8 BS (FIG. 6E). ChIP-PCR results involved primer set (#0), which covers nucleotides from -1072 to -1060 corresponding to the region identified by ChIP sequencing, and primer set (#1), which covers putative E2F BS, showed a strong binding of E2F8 on the UHRF1 promoter (FIG. 6F). However, deletion of the ChIP sequencing region (#0) shows that there is basically no effect on the UHRF1 promoter activity and this data suggests that the specific E2F8 BS (#0) plays a critical role in the regulation of UHRF1 expression (FIG. 6G). Surprisingly, promoter activity (FIG. 6H) and the expression of UHRF1 (FIG. 6I) were both dominantly downregulated by siE2F8. Moreover, there was no additive effect on the knockdown of UHRF1 expression in the cells transfected with combined siRNAs of E2F1 and E2F8, suggesting E2F8 directly regulates UHRF1 independent to E2F1.

Data have demonstrated that UHRF1 deletion caused suppressed colony formation (FIG. 6J) and increased expressions of p-H2AX and cleaved caspase-3 (FIG. 6K) and these results support the fact that UHRF1 contributes to the apoptotic effect of E2F8 knockdown.

Example 6: E2F8 is critical for tumor growth

As shown in FIG. 7, there was a significant suppression in tumor growth (FIGs. 7A and 7B) and mass (FIG. 7C) of the xenografts derived from E2F8-depleted cells compared to control cells. To further determine the role of E2F8 in tumor growth, a unique morpholino E2F8 (mo-E2F8) was developed to block expression of E2F8 *in vivo* (FIG. 8A) (Summerton *et al.*, Curr Top Med Chem 2007, 7(7):651-660). The treatment suppressed the expression of E2F8 and UHRF1 (FIG. 8B) and induced cell death (FIGs. 8C and 8D). However, the same amount of mo-E2F8 had no effect on the survival and growth of normal NHTBE cells (FIGs. 8E and 8F).

Mice treated with mo-E2F8 showed significantly suppressed/delayed tumor growth compared to control-treated mice (FIGs. 8G and 8H). Tumor volume further supported the suppressed tumor burden due to E2F8 knockdown (FIG. 8I). Also, there was a decrease in the mRNA levels (FIG. 8J) and expressions (FIG. 8K) of E2F8, UHRF1, and PCNA in tumors derived from mo-E2F8-treated mice. These data clearly demonstrate that E2F8 is critical for the progression of tumors *in vivo*.

Example 7: miR-142-5p downregulates E2F8 expression in NCI-H441 lung cancer cells and suppresses growth of the cells

NCI-H441 lung cancer cells were transfected with the indicated doses of
 5 mir142-5p mimic using lipofectamine RNAi max. Two days after the transfection (D2),
 10,000 cells were split into 12 well plates for cell proliferation. From D3 to D7, cells were
 fixed and followed by staining with 0.05% crystal violet. Stained crystal violet in cells were
 extracted with 10% acetic acid and measured by OD₅₄₀ absorbance to determine cell
 proliferation. Three days after the transfection, cells were lysed and cell lysates were
 10 subjected to SDS-PAGE and immunoblotting using an anti-E2F8 rabbit polyclonal antibody
 (Abcam, CA) to examine the status of E2F8 levels affected by the miR142-5p mimic (FIG.
 19).

**Example 8: E2f8 overexpression in lung tumors derived from
 15 *Kras*^{LSL-G12D/+}; *Trp53*^{Flox/Flox} (KP) lung cancer mouse model**

E2f8 expression is substantially elevated in tumor areas compared to that in
 non-tumor lung. Lungs were collected from KP mice 10 weeks after the Ad-Cre infection.
 Non-tumor lungs were collected from wild-type littermates infected with the same Ad-Cre
 expressing virus. Formalin-fixed paraffin embedded (FFPE) lung tumor tissues were subject
 20 to immunofluorescence staining with an anti-E2F8 rabbit polyclonal antibody (Abcam, CA)
 (FIG. 20).

**Example 9: Inhibitory effects of γ PNA targeting E2f8 on colony formation and viability
 of A549 cells**

A549 lung cancer cells were transfected with γ PNA-E2f8 at 0, 5 and 10 μ M
 concentration by nucleofection using Nucleofector II and kit T reagent (Lonza, NJ). FIG.
 21A: Transfected cells were subject to Western blotting with anti-E2F8 mouse monoclonal
 antibody (Abnova, CO) to validate the suppressive effect of γ PNA-E2f8 on E2F8 expression.
 FIG. 21B: Transfected A549 cells were cultured in 6 cell plates for colony formation assay.
 30 10 days after incubation, cells were stained with 0.1% crystal violet and the number of
 colonies was quantitated (FIG. 21C). FIG. 21D: Transfected cells were seeded in 96 well
 plates and cell viability was measured by Celltiter Glo (Promega, USA) in a time-course
 manner. ** $P < 0.01$.

Example 10: E2F8 is required for the growth and survival of pancreatic cancer and melanoma cell lines

The MiaPaCa2 pancreatic cancer and 501MEL melanoma cell lines were infected with lentiviral media possessing shscrambled, shE2F8 #1 or #3. Infected cells were selected with 1 µg/ml puromycin for 4 days. 3,000 MiaPaCa2 and 2,000 501MEL cells were seeded into 96 well plates with complete media. Cell proliferation was measured by Celltiter glo in a time-course manner for 96 hrs. Knockdown of E2F8 blocks cell growth and proliferation of these cancer cell lines (FIGs. 22A-22B).

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed:

1. A method of treating or preventing a cancer in a subject in need thereof, wherein the method comprises administering to the subject a therapeutically effective amount of a composition that inhibits expression of the *E2F8* gene in the subject, whereby the cancer is treated or prevented in the subject.
2. The method of claim 1, wherein the composition comprises at least one agent selected from the group consisting of an antibody, siRNA, ribozyme, antisense RNA, modified antisense RNA, small molecule, and any combinations thereof.
3. The method of claim 2, wherein the modified antisense RNA comprises morpholino-E2F8 or a pharmaceutically acceptable salt thereof.
4. The method of claim 2, wherein the antibody comprises an antibody selected from the group consisting of a polyclonal antibody, monoclonal antibody, humanized antibody, synthetic antibody, heavy chain antibody, human antibody, biologically active fragment of an antibody, and any combinations thereof.
5. The method of claim 2, wherein the small molecule comprises naphthol AS-TR-Phosphate (NASTRp) or a pharmaceutically acceptable salt thereof.
6. The method of claim 1, wherein the subject is a mammal.
7. The method of claim 6, wherein the mammal is human.
8. The method of claim 1, wherein the composition is administered to the subject by an inhalational, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, intracranial, or intravenous route of administration.
9. The method of claim 1, wherein the composition is co-administered with at least one additional anti-cancer agent.

10. The method of claim 9, wherein the at least one additional anti-cancer agent is selected from the group consisting of a nitrosourea, cyclophosphamide, adriamycin, 5-fluorouracil, paclitaxel and its derivatives, cisplatin, methotrexate, thiotepa, mitoxantrone, vincristine, vinblastine, etoposide, ifosfamide, bleomycin, procarbazine, chlorambucil, fludarabine, mitomycin C, vinorelbine, gemcitabine, and any combinations thereof.

11. The method of claim 9, wherein the at least one additional anti-cancer agent is selected from the group consisting of a drug approved for non-small cell lung cancer, a drug combination to treat non-small cell lung cancer, and a drug approved for small cell lung cancer.

12. The method of claim 1, where the cancer is a solid tumor.

13. The method of claim 12, wherein the solid tumor comprises lung cancer.

14. The method of claim 13, wherein the lung cancer comprises at least one selected from the group consisting of small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma.

15. The method of claim 12, wherein the solid tumor comprises melanoma.

16. The method of claim 12, wherein the solid tumor comprises pancreatic cancer.

17. The method of claim 12, wherein the solid tumor is selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder

carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

18. A method of providing the prognosis of a cancer treatment for a subject having a cancer, the method comprising the steps of:

detecting the level of expression of the *E2F8* gene in a biological sample from the subject; and;

comparing the level of expression of the *E2F8* gene in the subject's biological sample to the level of expression of the *E2F8* gene in a control sample, wherein overexpression of *E2F8* gene in the subject's biological sample as compared to the control sample indicates an adverse outcome for the subject's cancer treatment.

19. The method of claim 18, where the cancer comprises lung cancer.

20. The method of claim 19, wherein the lung cancer comprises at least one selected from the group consisting of small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma.

21. The method of claim 18, wherein the cancer comprises melanoma.

22. The method of claim 18, wherein the cancer comprises pancreatic cancer.

23. The method of claim 18, wherein the cancer is a solid tumor selected from a group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma,

ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

24. The method of claim 18, wherein the subject is a mammal.

25. The method of claim 18, wherein the biological sample comprises at least one selected from the group consisting of urine, blood, and tissue.

26. The method of claim 25, wherein the tissue comprises at least one selected from the group consisting of lung tissue, skin tissue and pancreas tissue.

27. The method of claim 18, wherein the subject is counseled to receive a therapeutically effective amount of a composition that inhibits expression of the *E2F8* gene in the subject.

28. A method of predicting the effectiveness of an anti-cancer agent in treating a subject suffering from cancer, the method comprising administering the anti-cancer agent to the subject; and determining the expression level of the *E2F8* gene in a biological sample from the subject, wherein overexpression of the *E2F8* gene in the subject's biological sample as compared to a control sample indicates that the anti-cancer agent is not effective in treating the subject's cancer.

29. The method of claim 28, wherein the subject is a mammal.

30. The method of claim 28, wherein the cancer comprises lung cancer.

31. The method of claim 30, wherein the lung cancer comprises at least one selected from the group consisting of small cell lung carcinoma, squamous cell lung carcinoma and lung adenocarcinoma.

32. The method of claim 28, wherein the cancer comprises melanoma.

33. The method of claim 28, wherein the cancer comprises pancreatic cancer.

34. The method of claim 28, wherein the biological sample is at least one selected from the group consisting of urine, blood, and tissue.

35. The method of claim 28, further wherein the subject is administered another anti-cancer agent, if the anti-cancer agent is not effective in treating the subject's cancer.

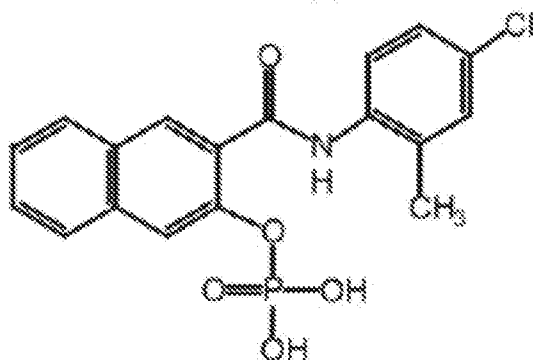
36. The method of claim 28, further wherein the subject is counseled to receive a therapeutically effective amount of a composition that inhibits expression of the *E2F8* gene in the subject.

37. A kit comprising (i) a pharmaceutical composition that inhibits E2F8 expression in a subject and (ii) an instruction manual reciting a method of treating a cancer in a subject using the pharmaceutical composition.

38. The kit of claim 31, wherein the cancer is at least one selected from the group consisting of lung cancer, pancreatic cancer and melanoma.

FIG. 1A

**Naphthol AS-TR-Phosphate
(NASTRp)**



**Naphthol AS-E-Phosphate
(NASEp)**

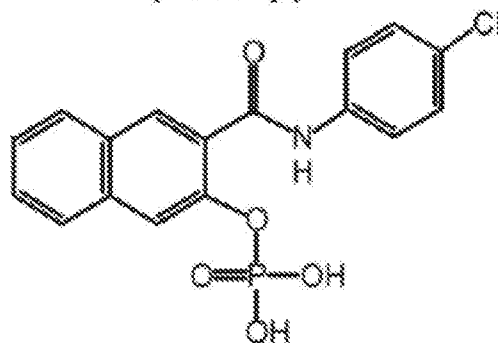


FIG. 1B

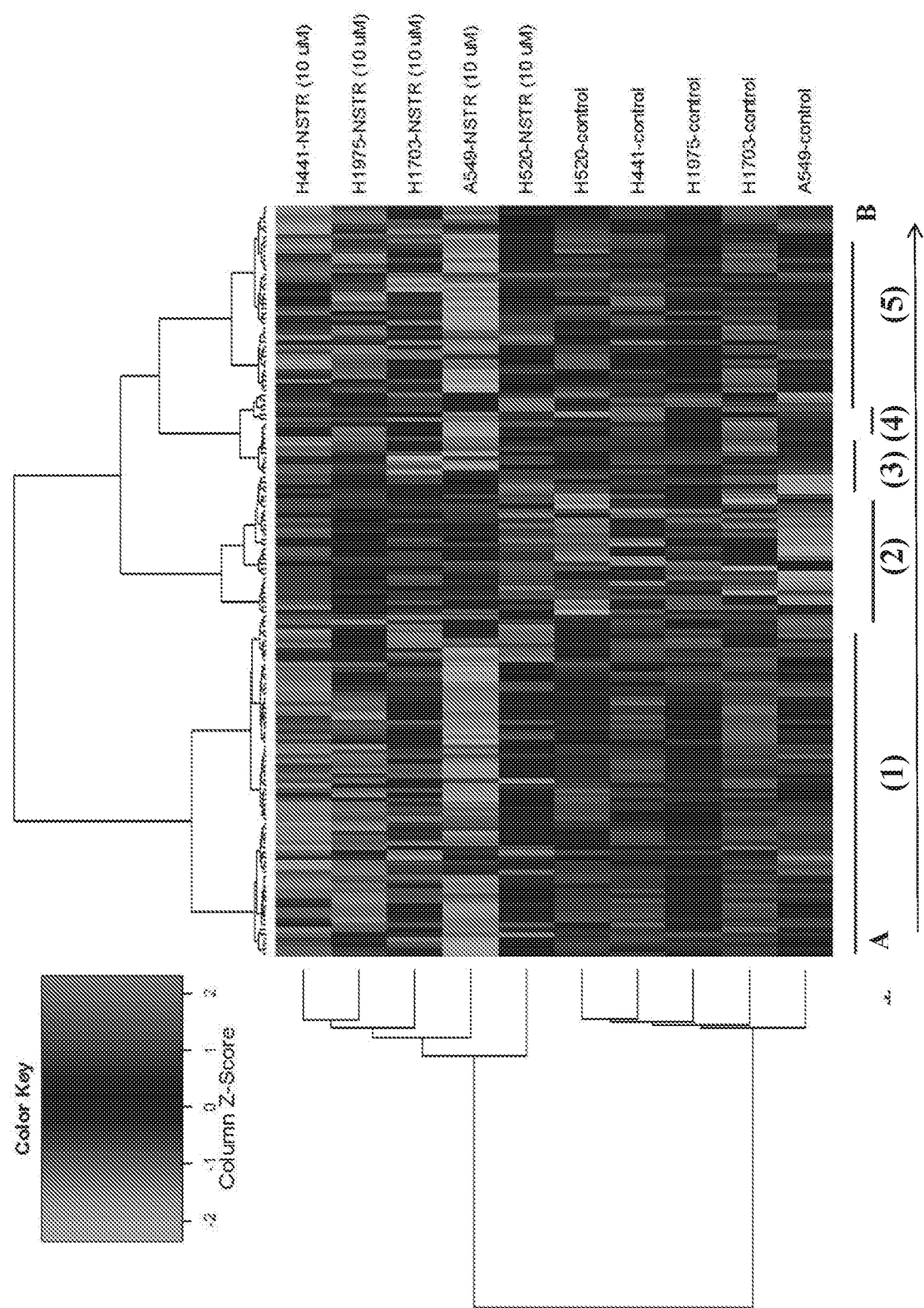


FIG. 1C

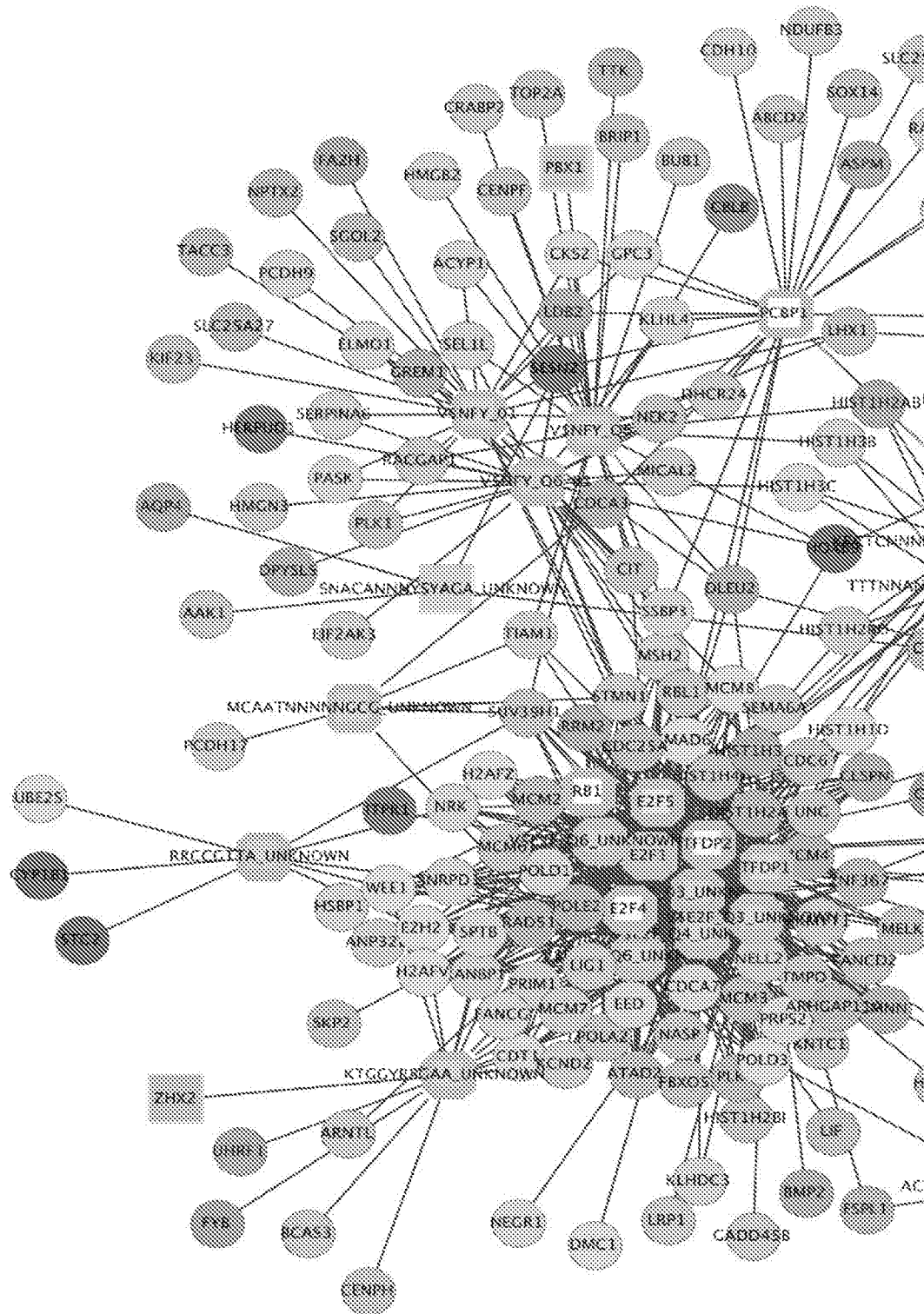


FIG. 1D

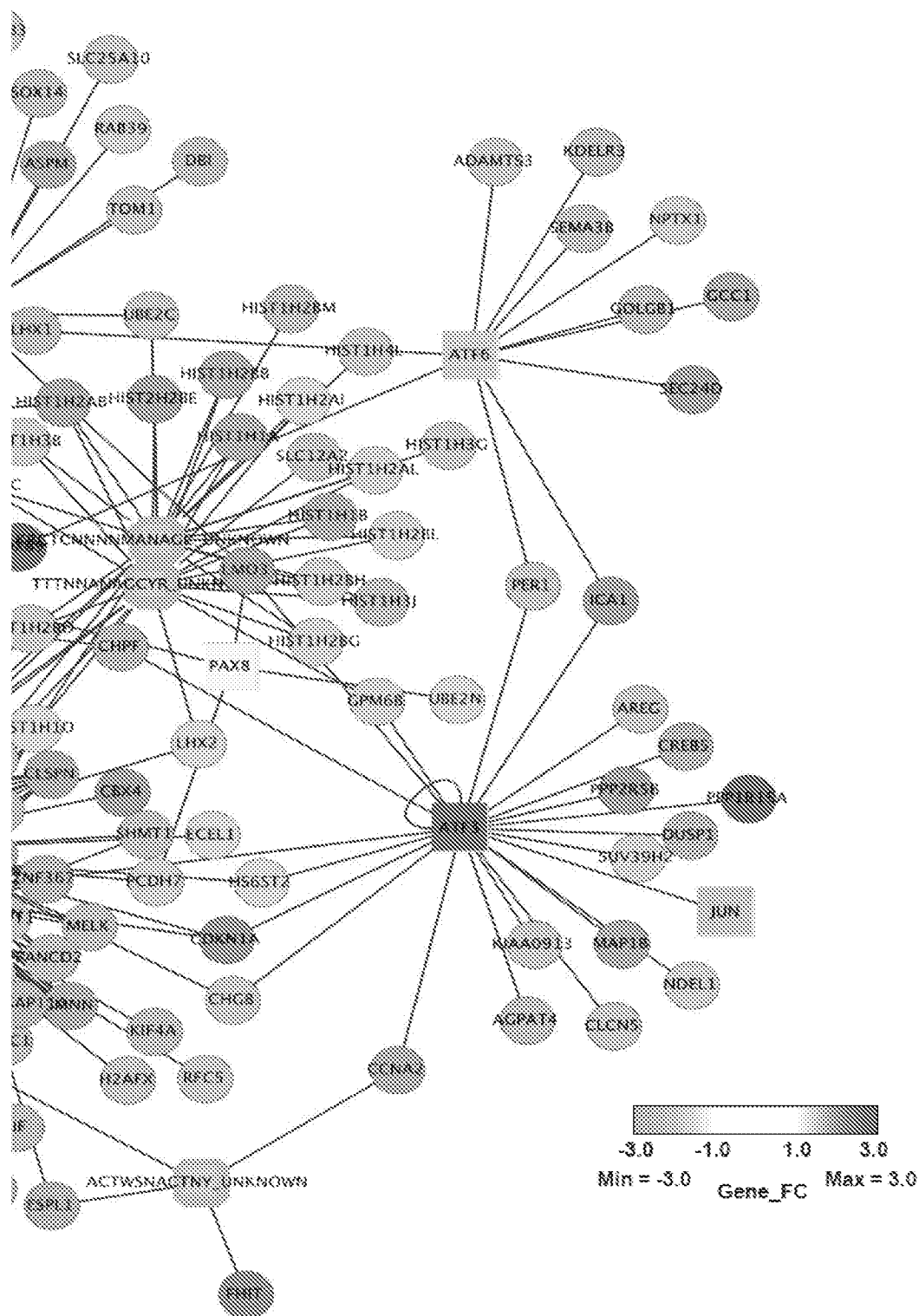


FIG. 1E

E2F family

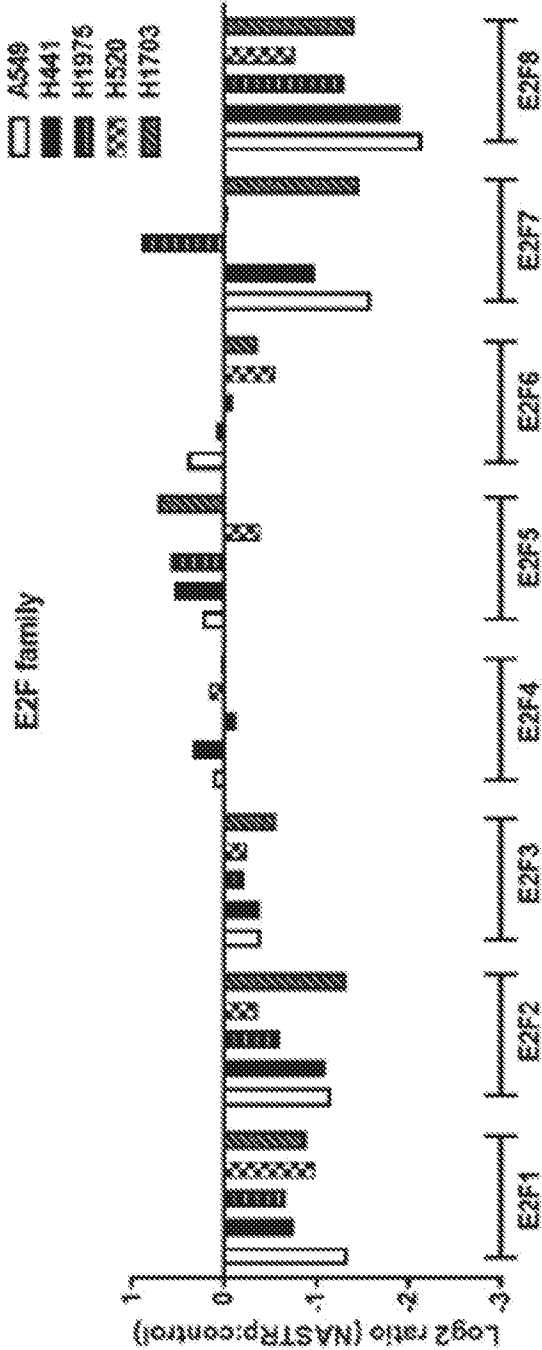


FIG. 1F

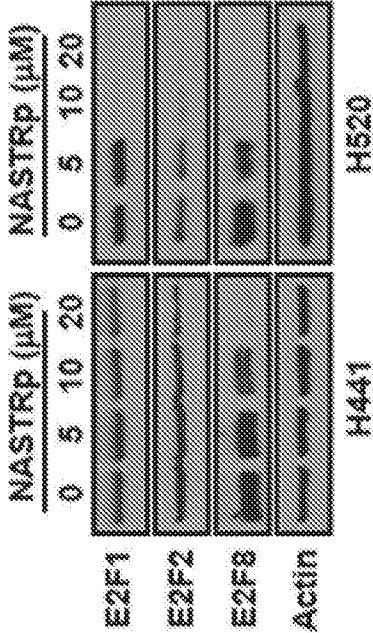


FIG. 1G

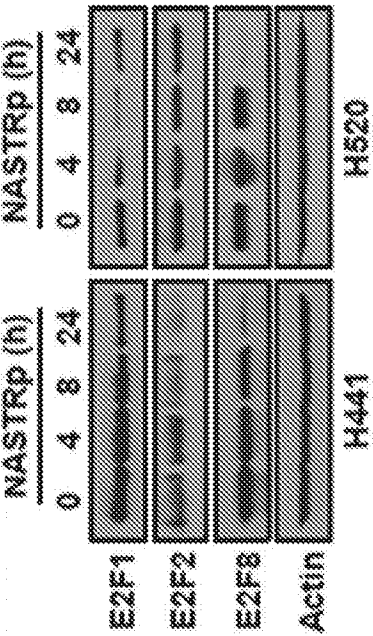


FIG. 2A

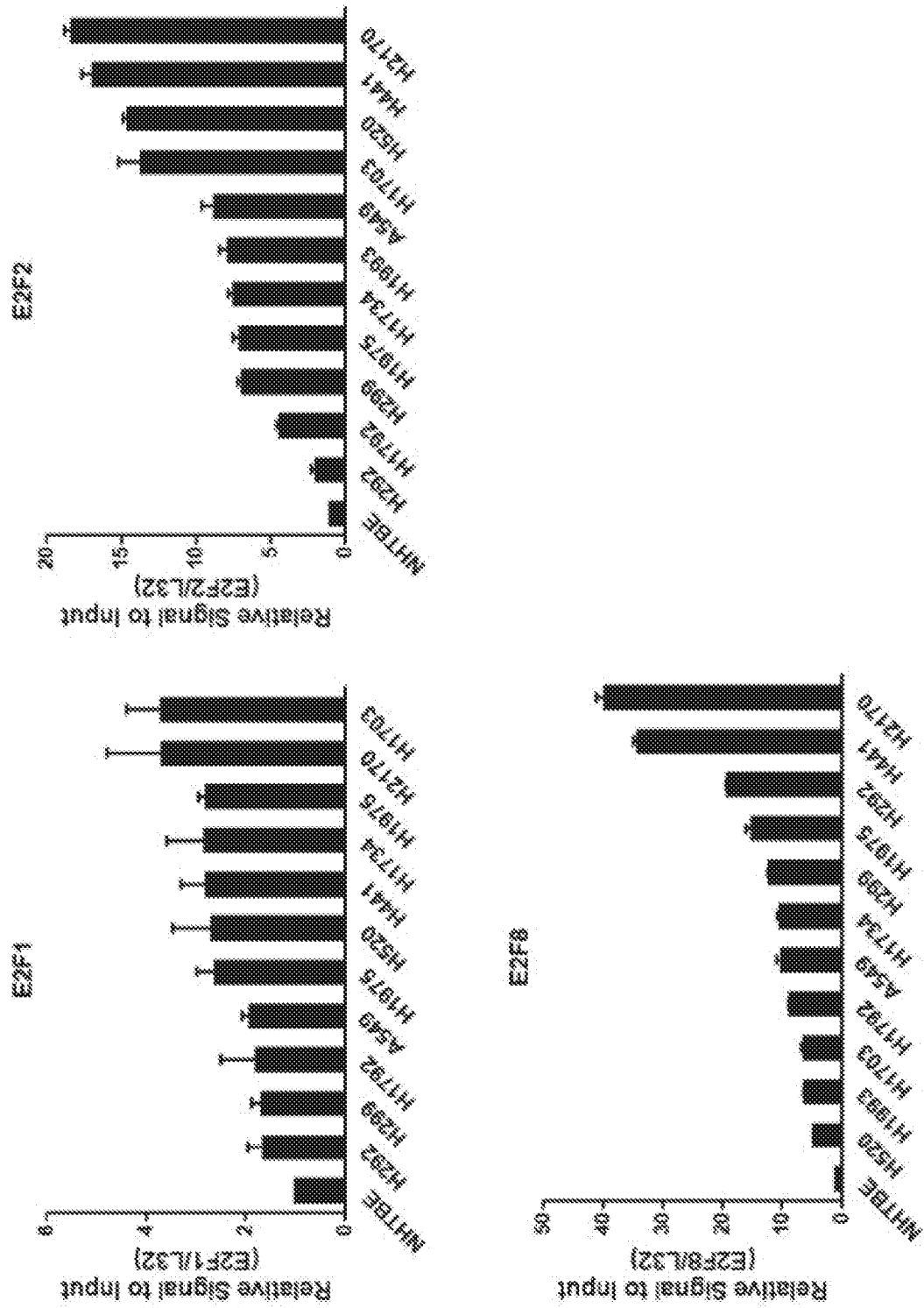


FIG. 2B

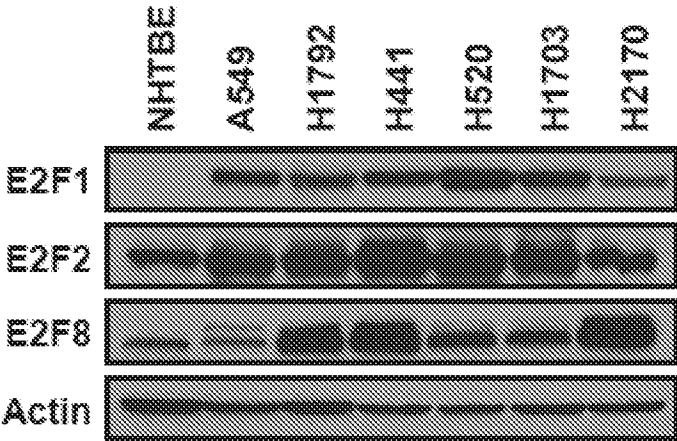


FIG. 2C

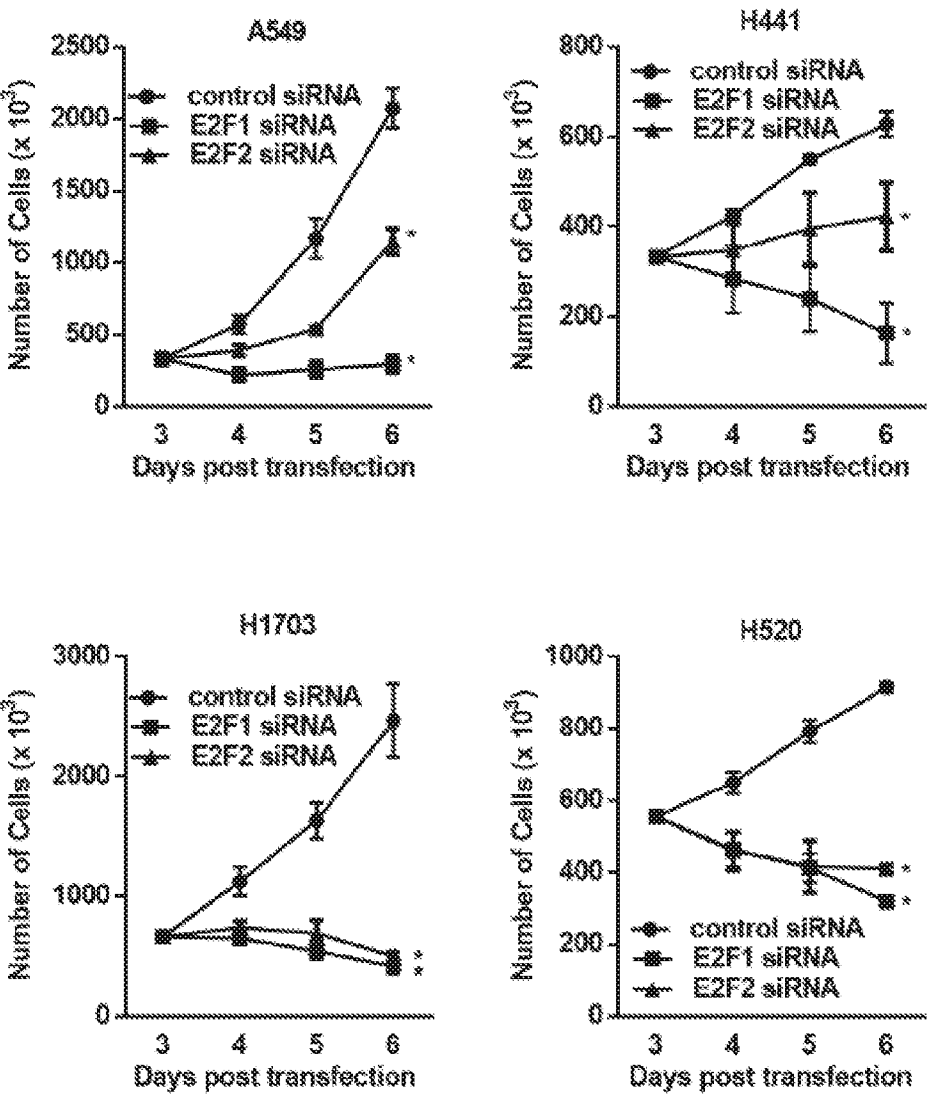


FIG. 2D

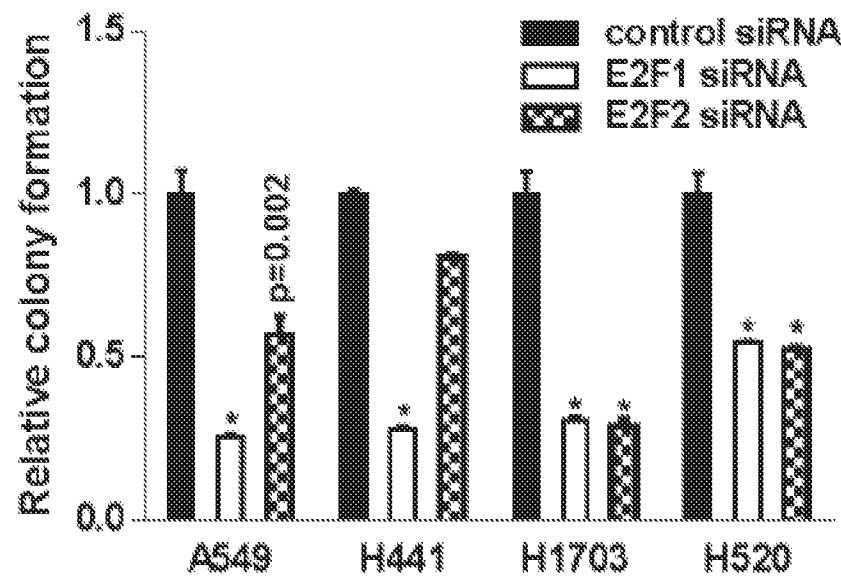


FIG. 2E

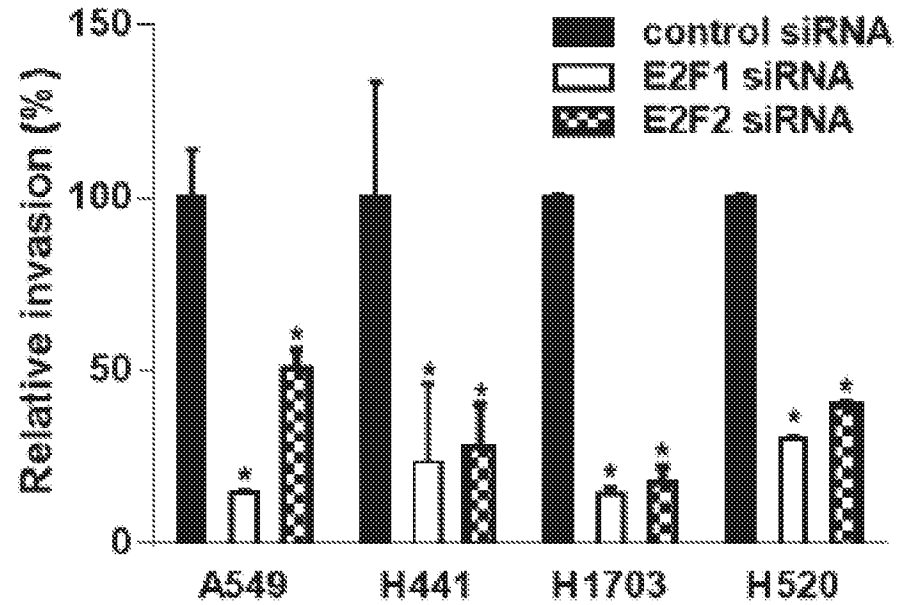


FIG. 3A

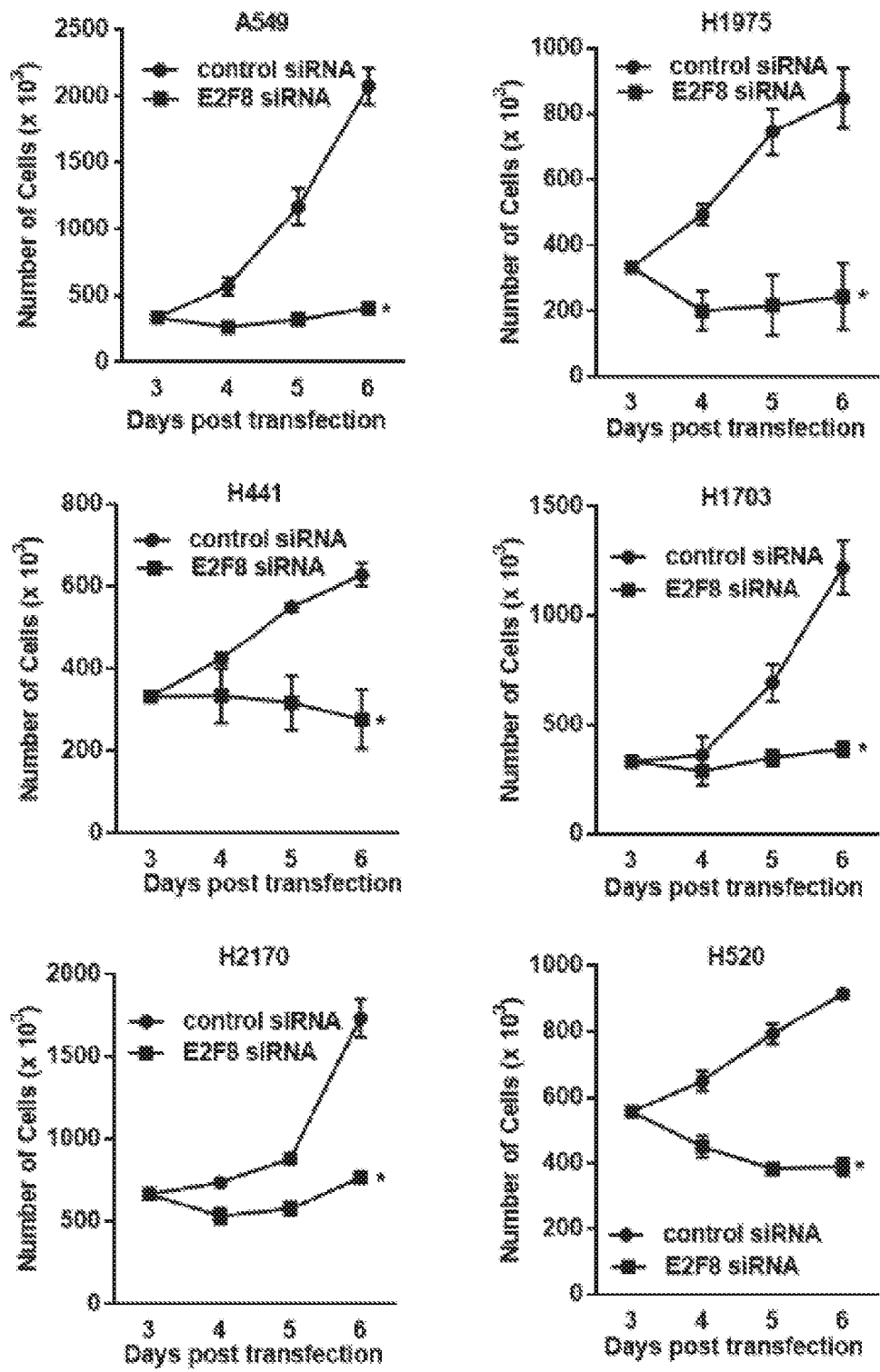


FIG. 3B

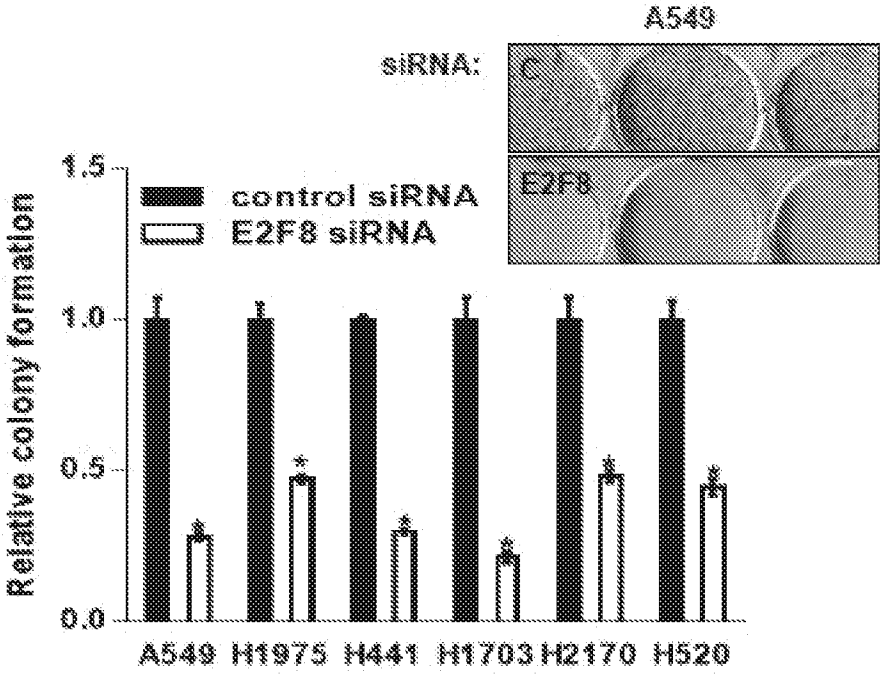


FIG. 3C

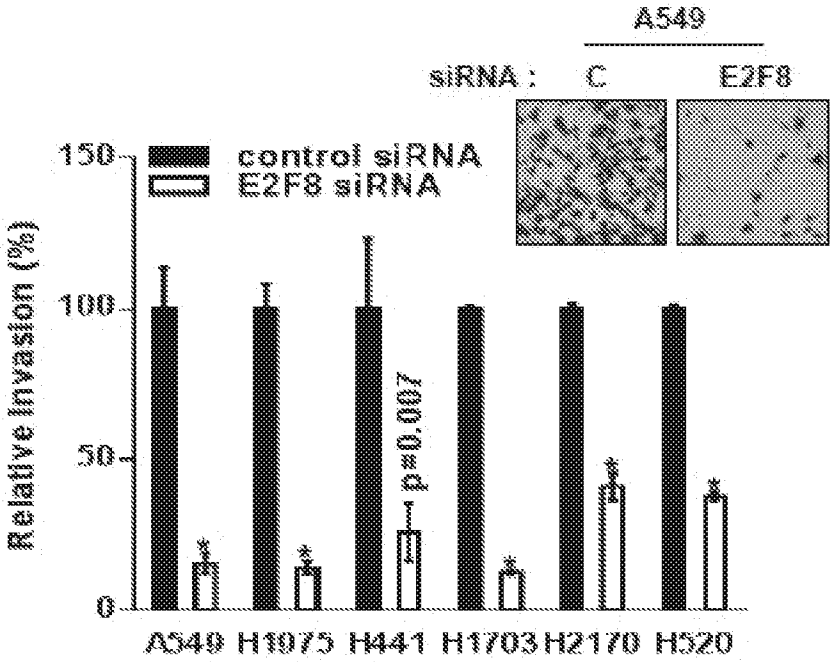


FIG. 3D

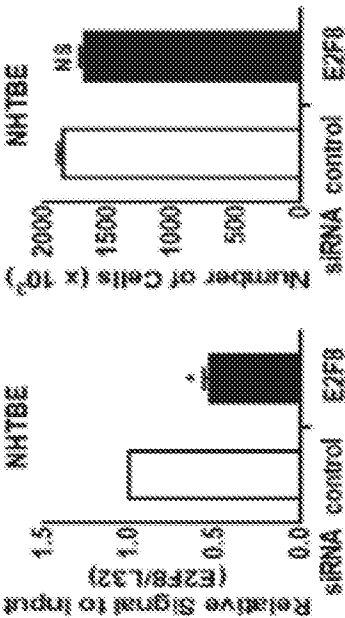


FIG. 3E

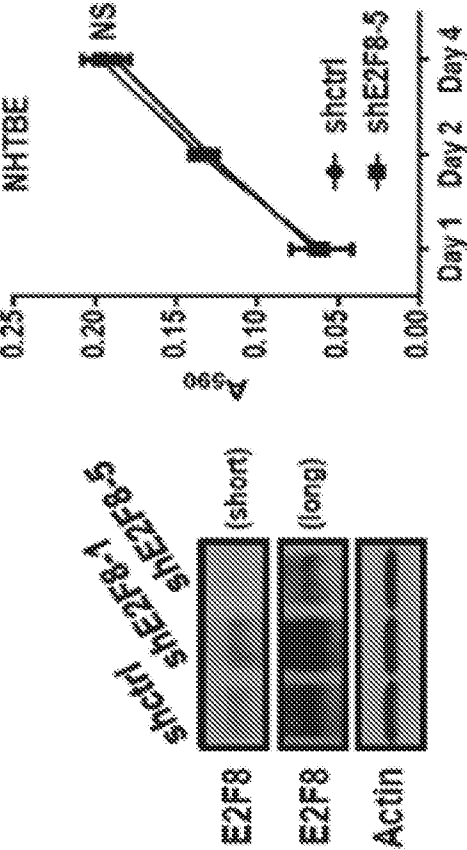


FIG. 3F

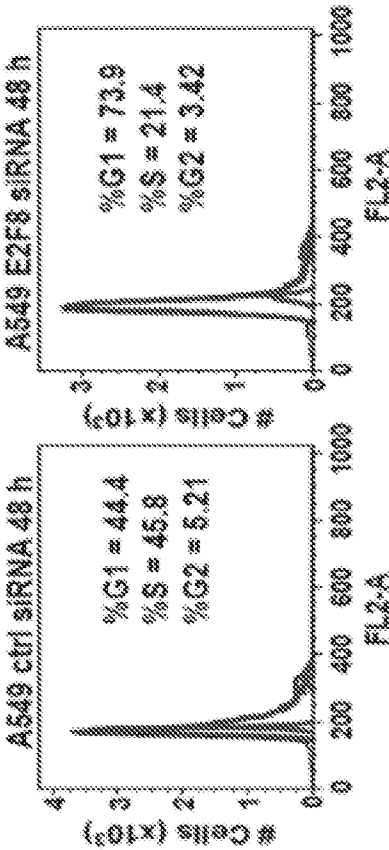


FIG. 3G

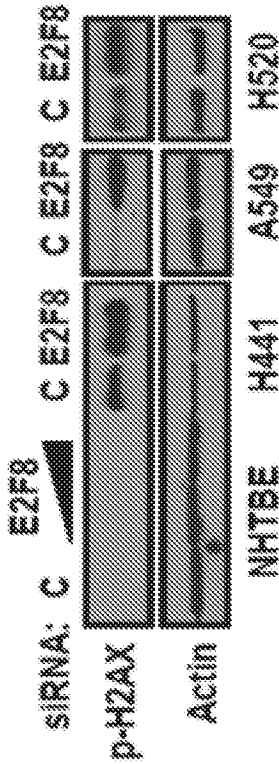


FIG. 3H

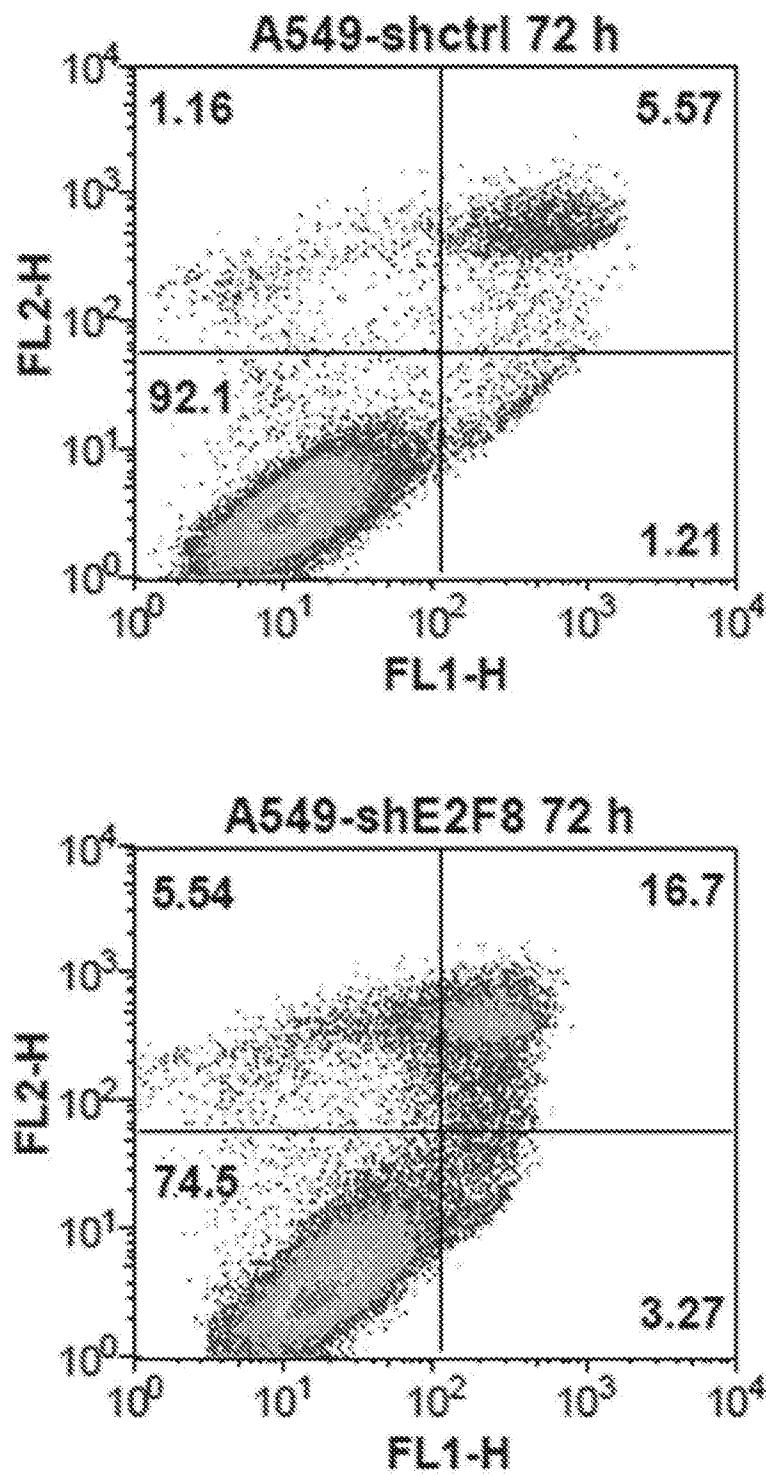


FIG. 3I

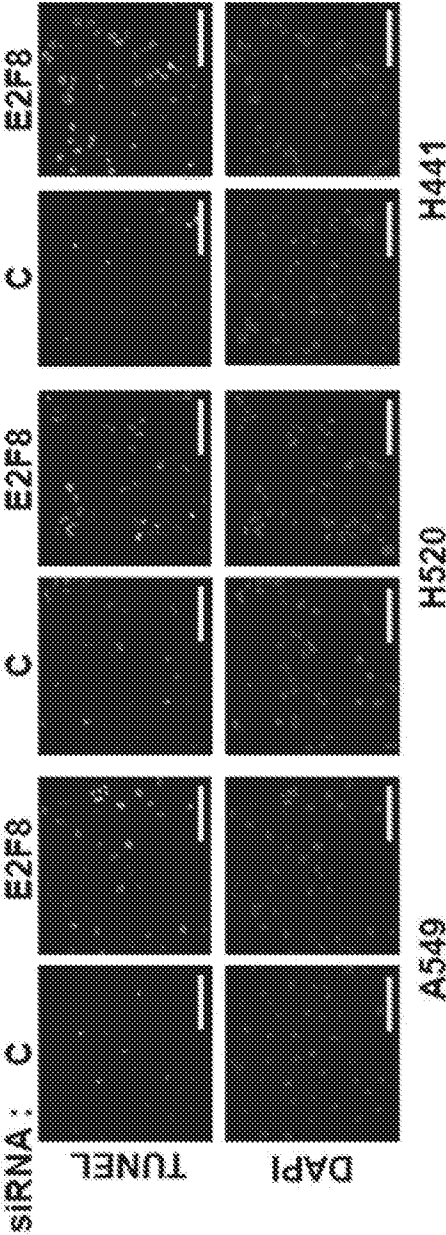


FIG. 3K

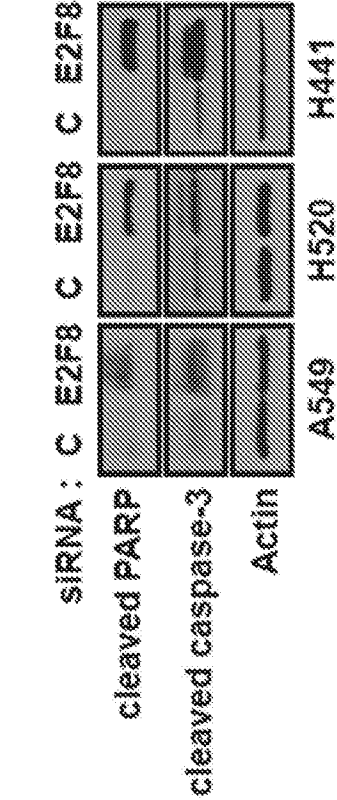


FIG. 3J

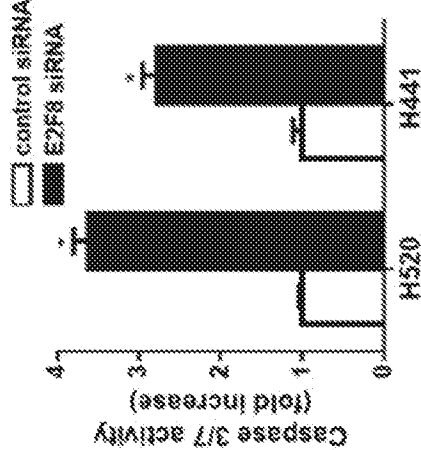


FIG. 4B

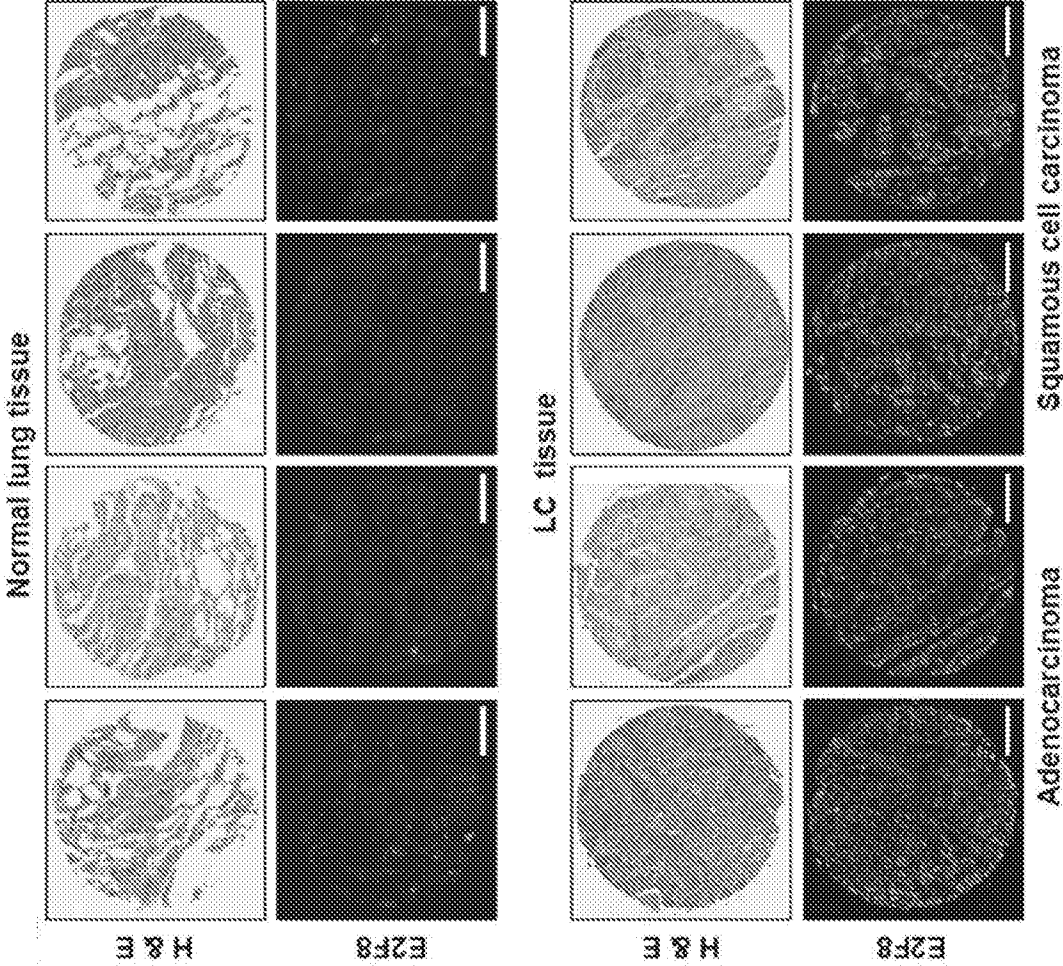


FIG. 4A

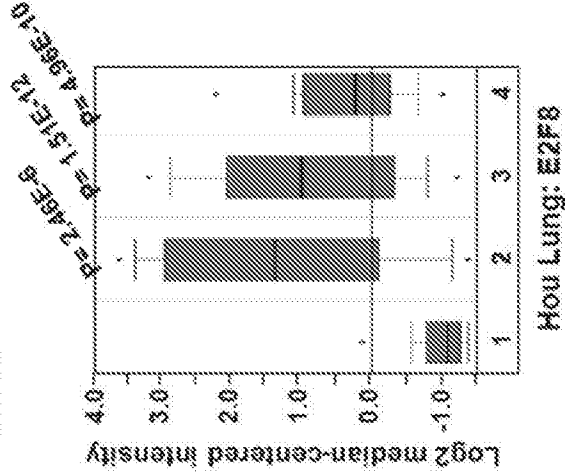


FIG. 4C

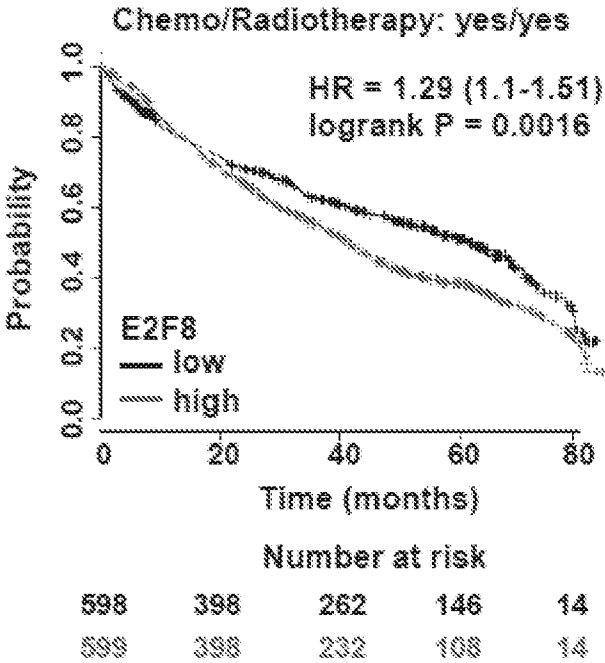


FIG. 4D

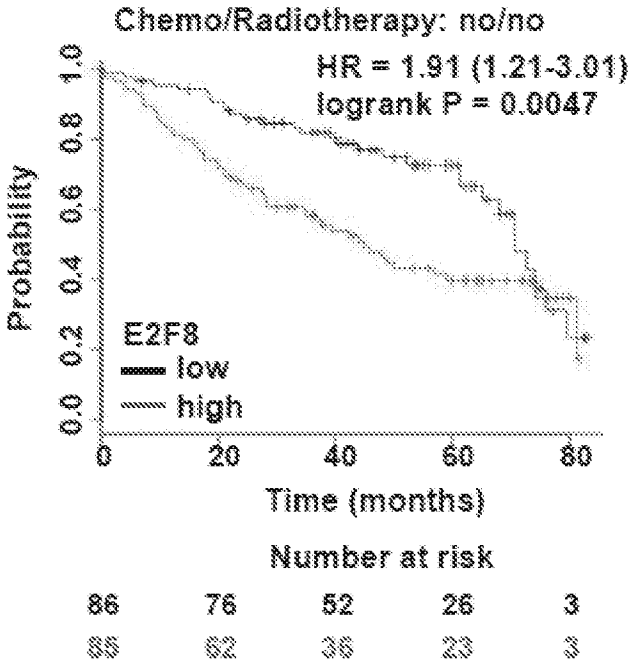


FIG. 4E

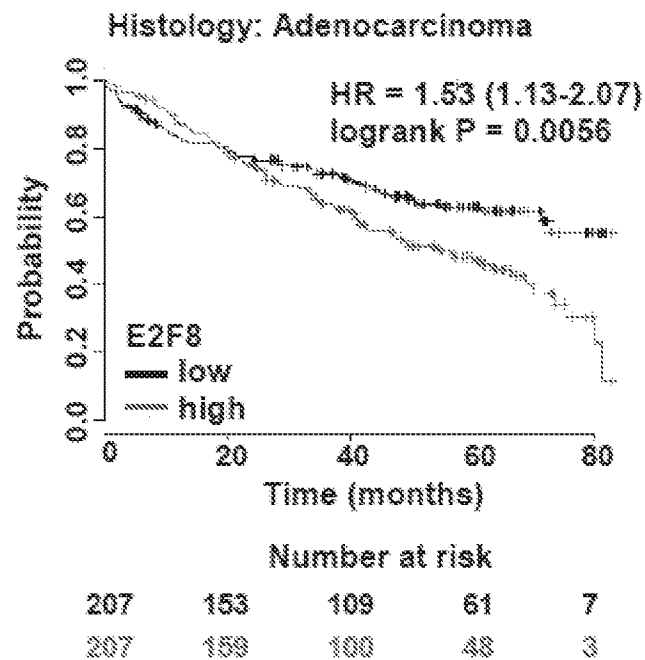


FIG. 4F

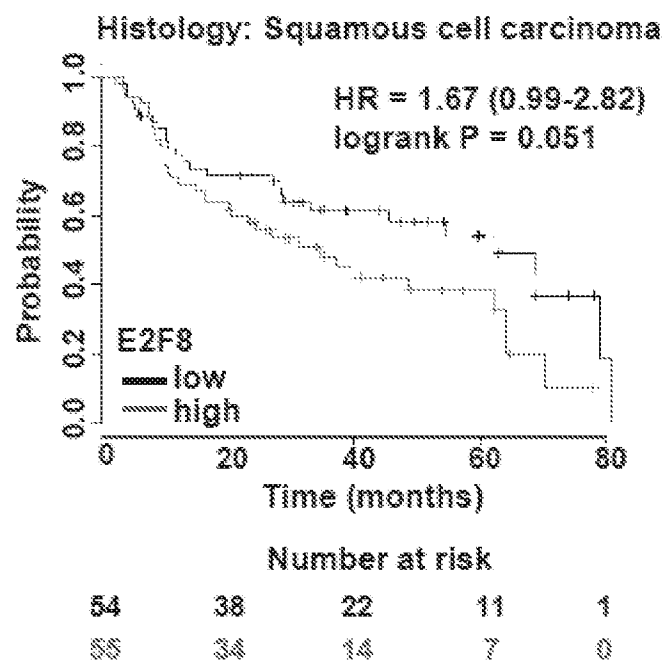


FIG. 5A

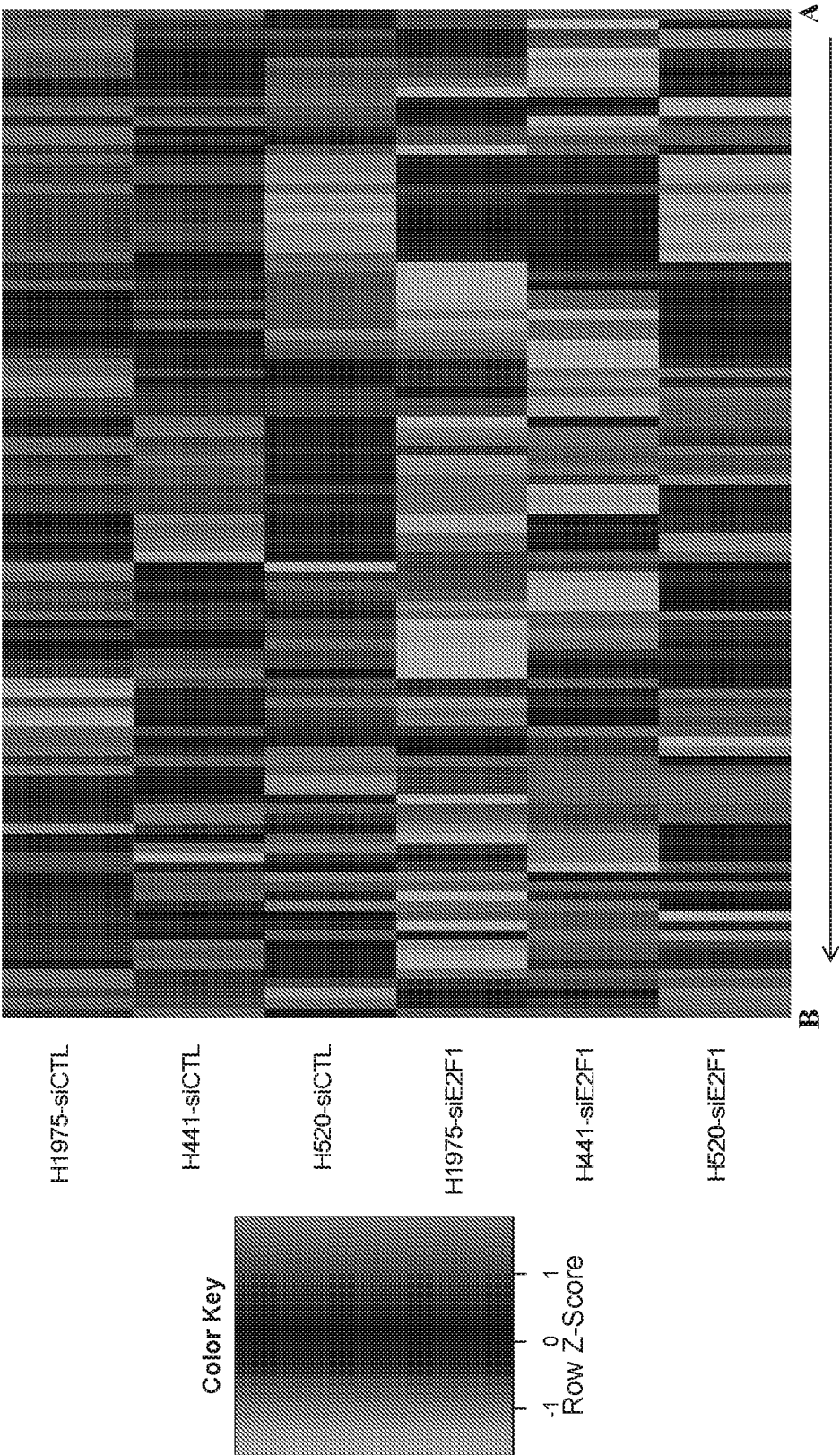


FIG. 5B

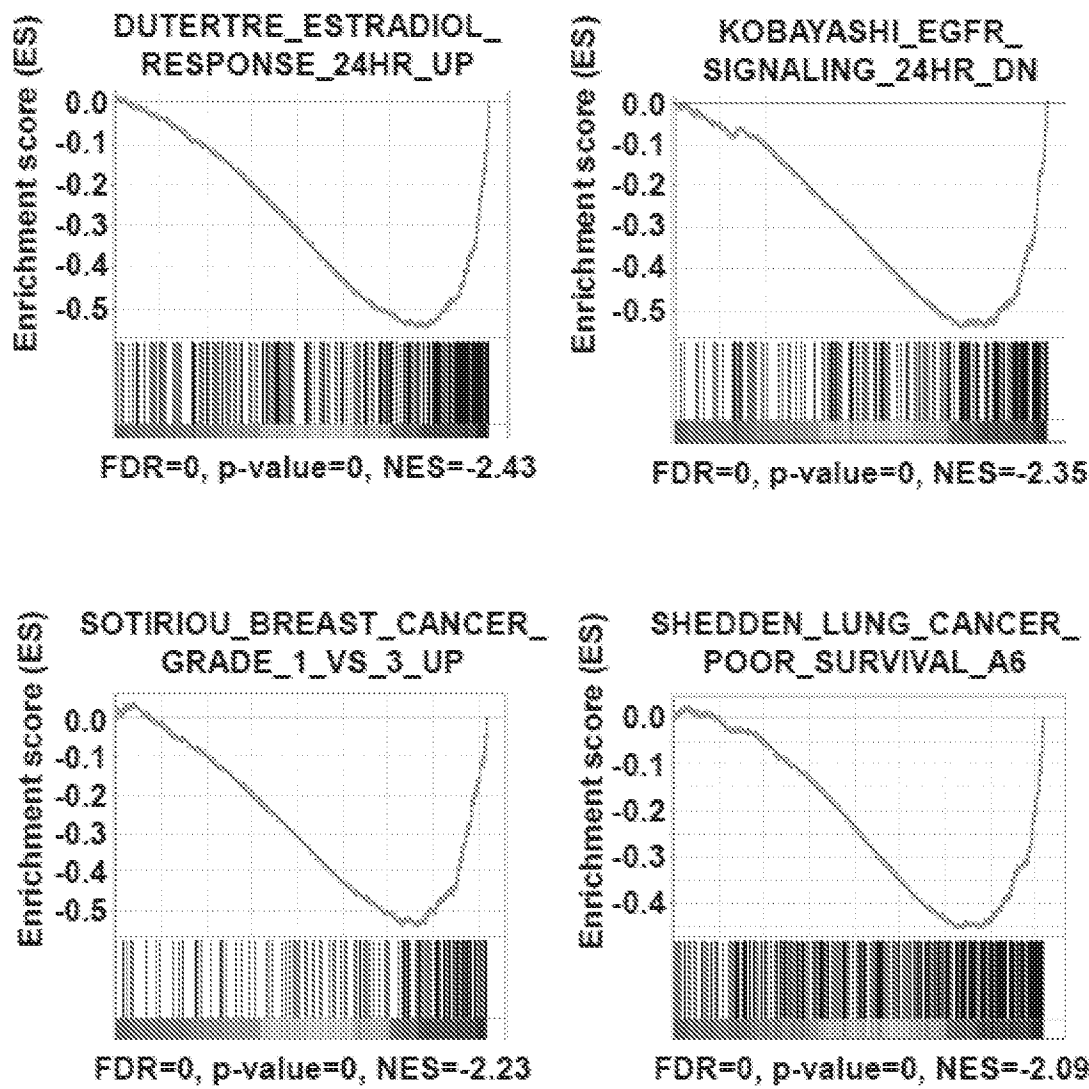


FIG. 5C

siE2F8 Gene Signature

Gene Category	p value	Selected genes
GO:0035325--chromatin organization	3.5E-04	HIST2H2AB, HIST1H2BN, HIST1H2BM, HIST1H2BH, SUV39H1, CARM1, SMARCA4
GO:0031090--organelle membrane	2.8E-03	NDUFB11, OXA1L, HAX1, COX10, SLC37A4, COX8A, DPAGT1, SHISA5, MRPL45
GO:0005783--endoplasmic reticulum	7.0E-03	WAGT1, SLC37A4, PIGL, DPAGT1, TRAM2, POM121, OPM3, RAB38, POM121C, POPDC1
SP, PIR KEYWORDS: Acetylation	1.8E-02	UHRF1, TUFM, XPO5, SYNGR2, EIF4EBP1, SCL9L, RRM10, PTPN8, GNN, ACLY, POLD2
GO:003253--cell proliferation	4.2E-02	UHRF1, E2F8, TSPT2, BMP4, ELF4, RPK2

FIG. 5D

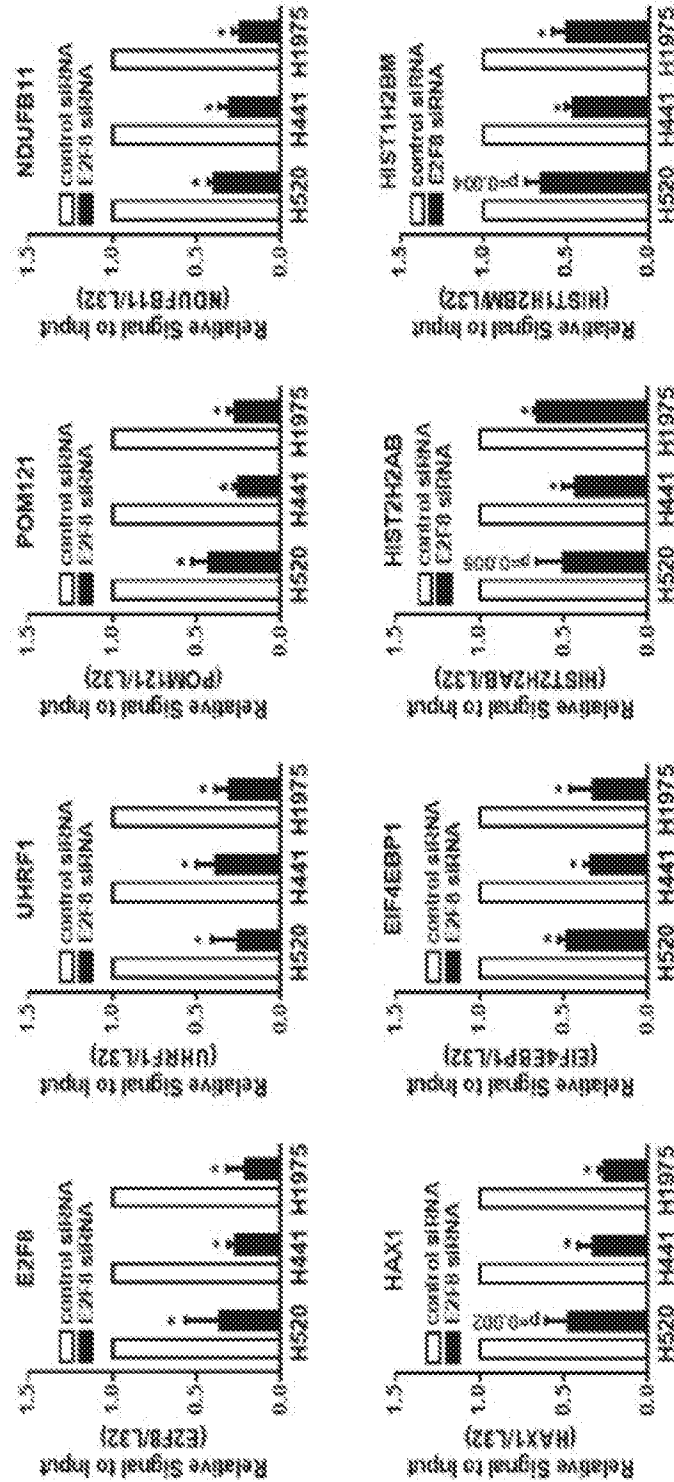


FIG. 5E

Distribution of binding regions relative to TSS

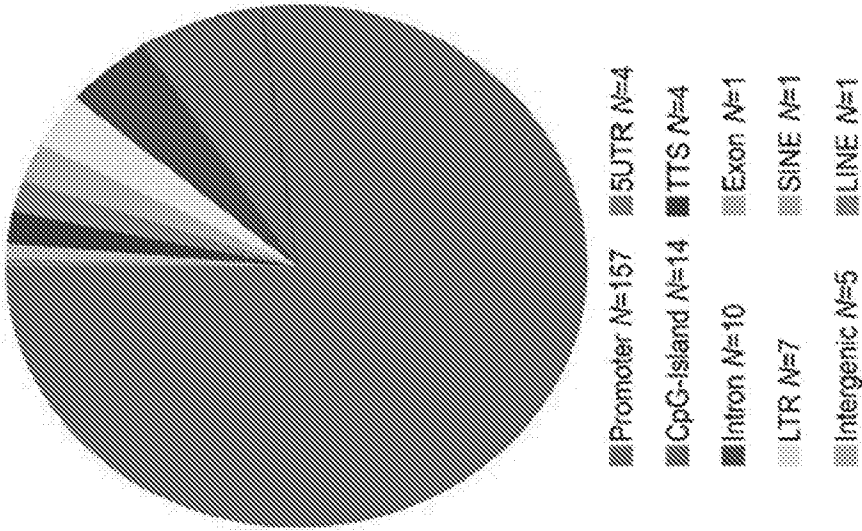


FIG. 5F

Motif	Name	P-value	% of target sequences with motif
	E2F8*	10 ⁻¹⁰⁰	75.98%
	E2F7	10 ⁻¹⁰⁰	73.04%
	E2F	10 ⁻¹⁰⁰	51.47%
	E2F1	10 ⁻¹⁰⁰	80.88%
	E2F4	10 ⁻¹⁰⁰	92.16%
	E2F6	10 ⁻¹⁰⁰	83.82%
	NFY (CCAAT)	10 ⁻²⁰	38.73%
	Elk1 (ETS)	10 ⁻¹⁰	39.22%
	Elk4 (ETS)	10 ⁻¹⁰	37.75%
	Sp1 (ZF)	10 ⁻¹⁰	39.71%
	Flt1(ETS)	10 ⁻¹⁰	42.18%

FIG. 6A

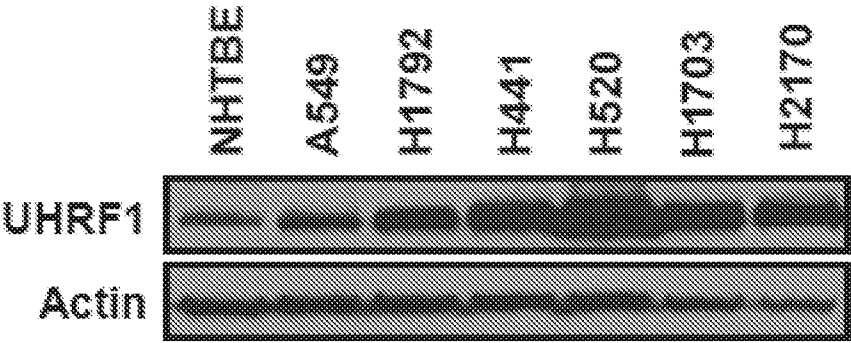


FIG. 6B

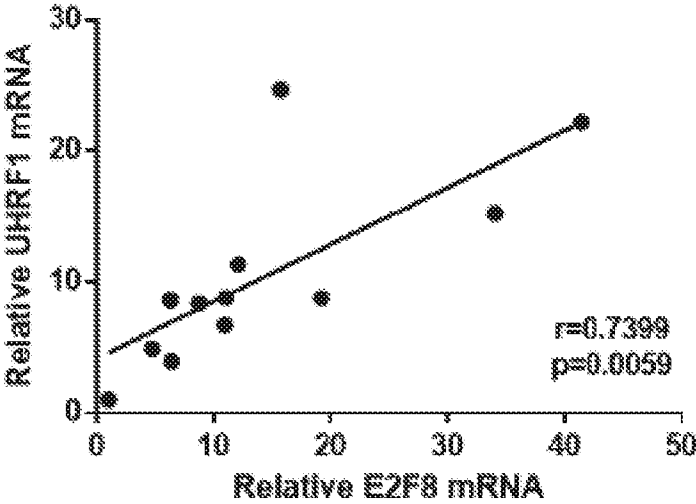


FIG. 6C

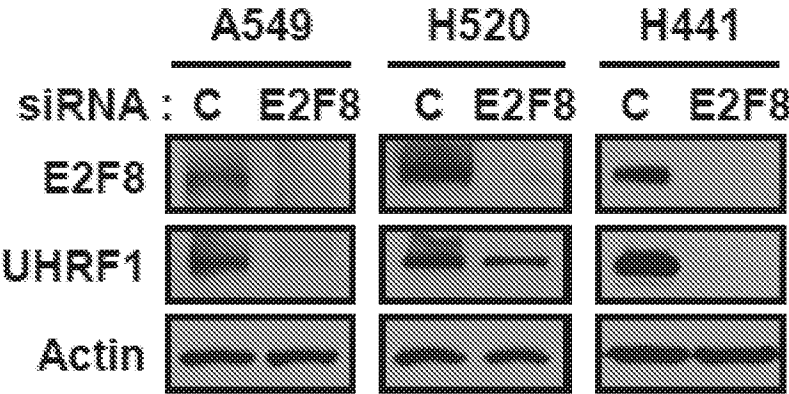


FIG. 6D

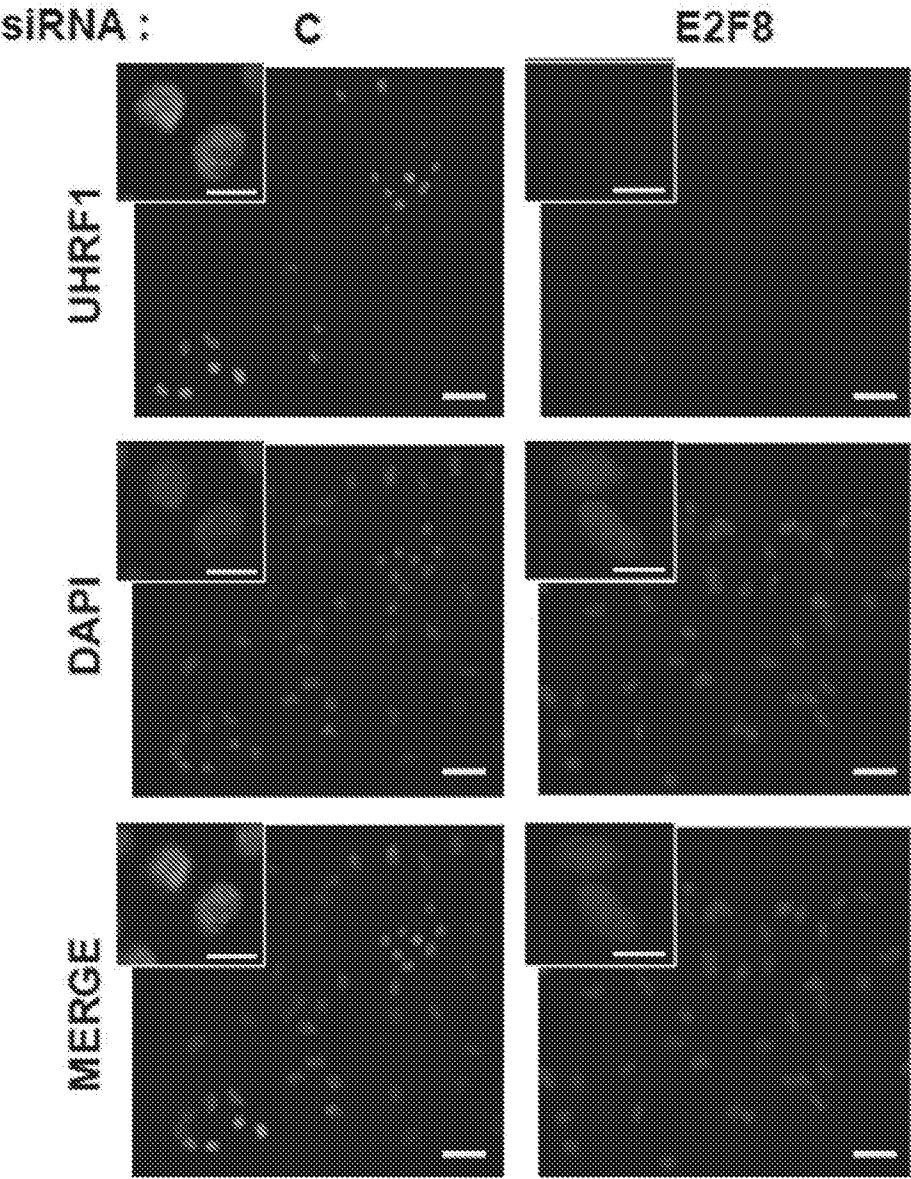


FIG. 6E

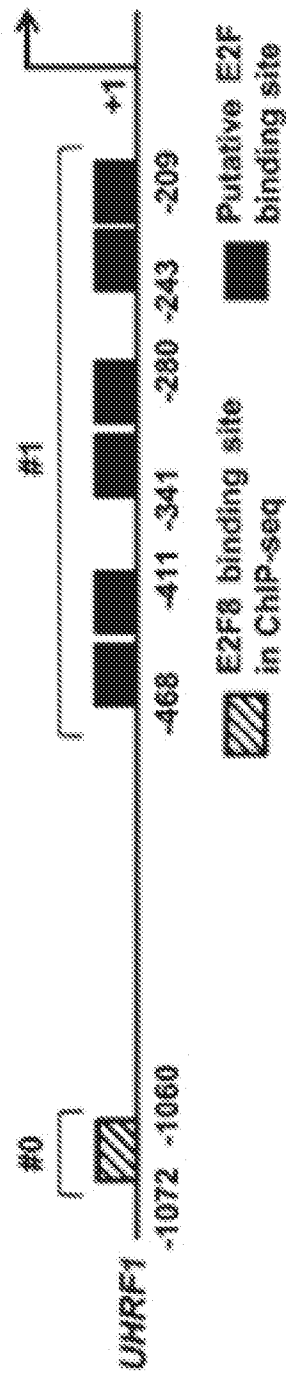


FIG. 6F

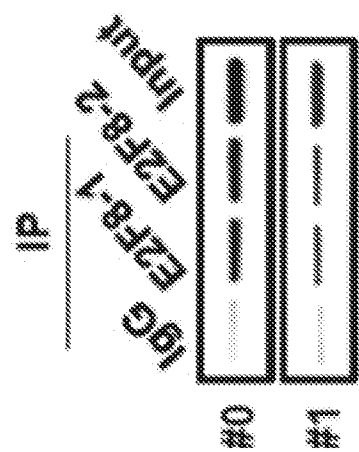


FIG. 6G

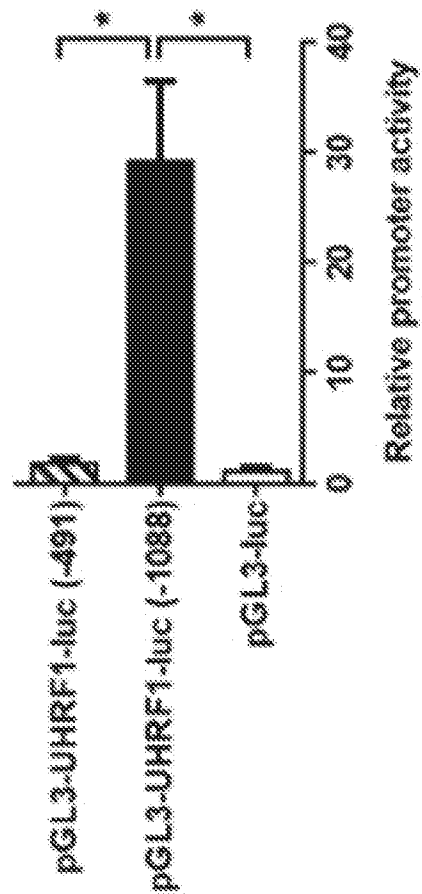


FIG. 6H

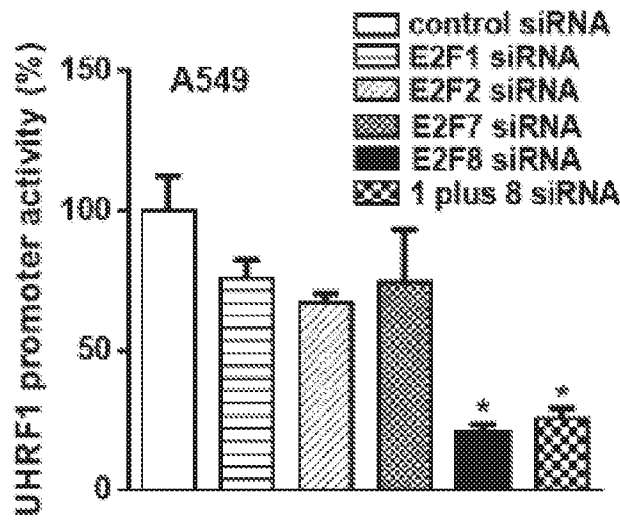


FIG. 6I

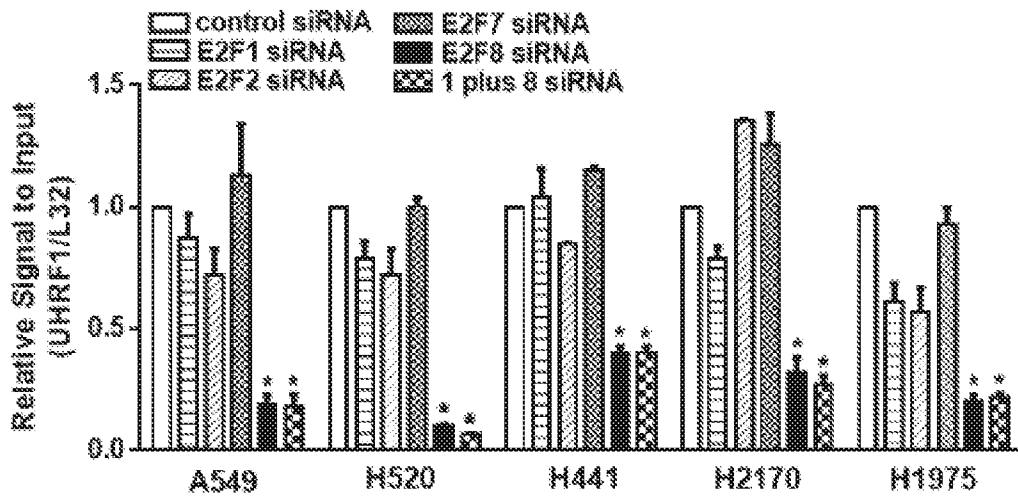


FIG. 6J

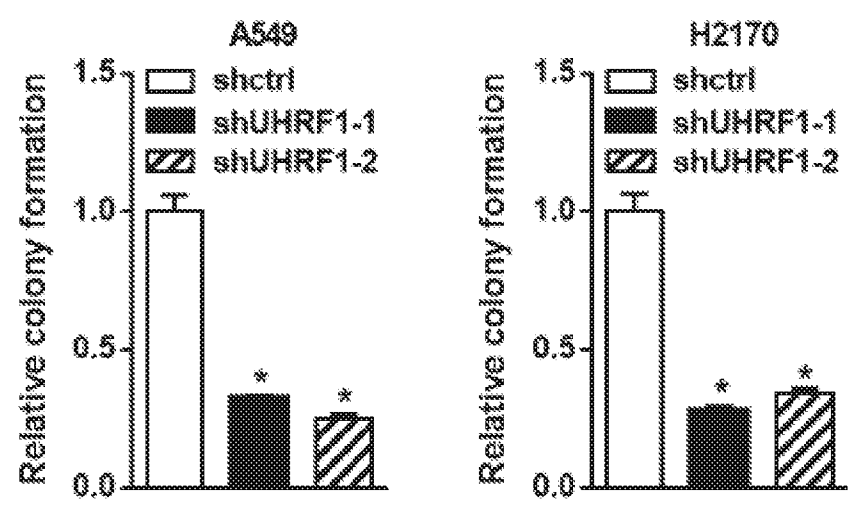


FIG. 6K

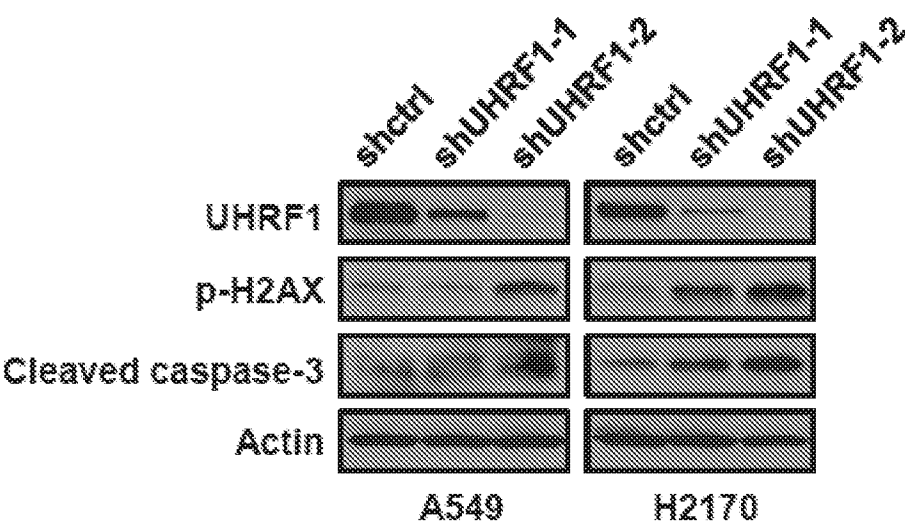


FIG. 7A
siRNA

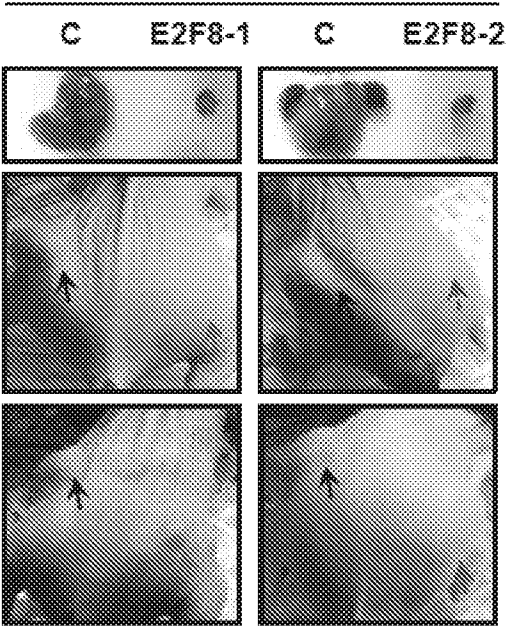


FIG. 7B

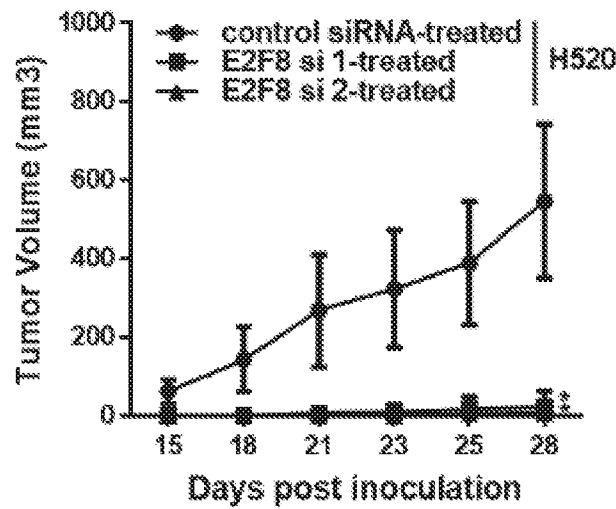


Fig. 7C

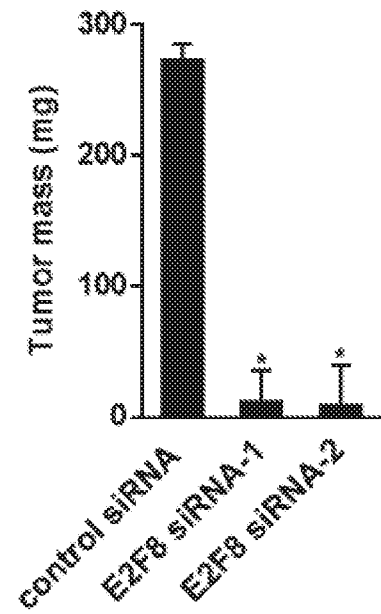


FIG. 8A

E2F8 5'...TGATGAGGAATTGACAAAATGGAGAACCAAAAGGAAACCTCT...3'
|||||
mo-E2F8 3' TACCTCTTGGTTTCCCTTTGGAGA 5'

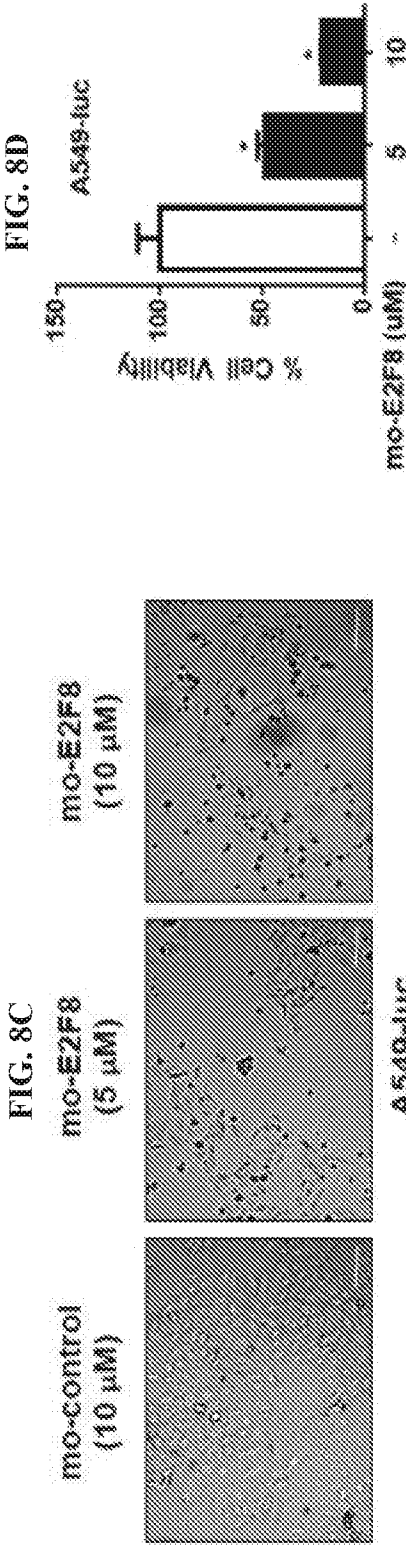
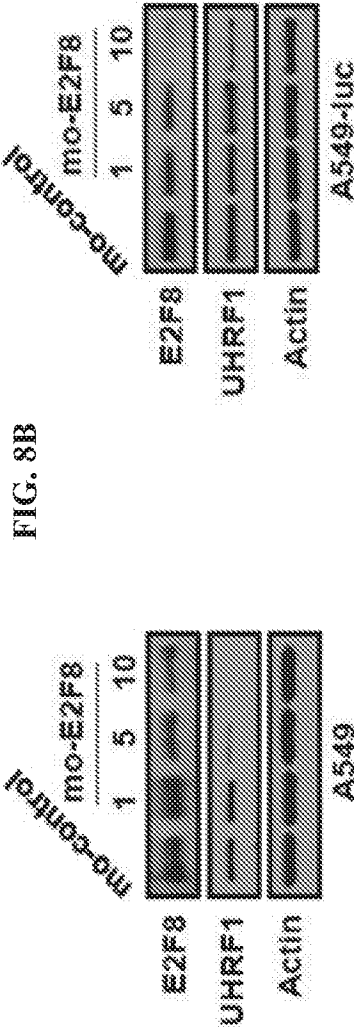


FIG. 8E

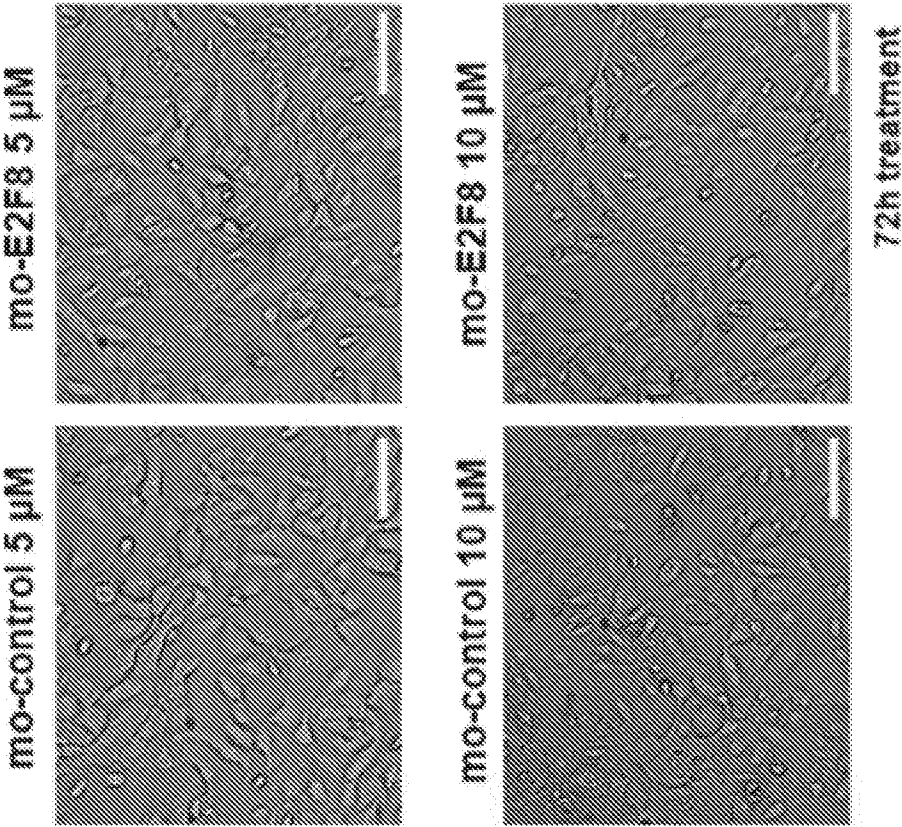


FIG. 8F

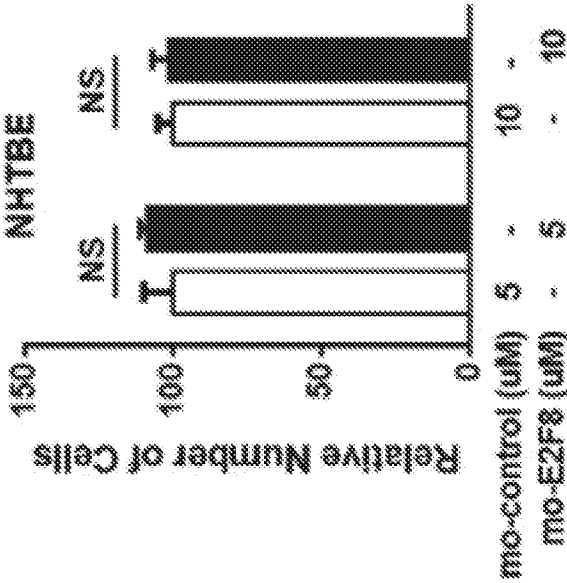


FIG. 8G

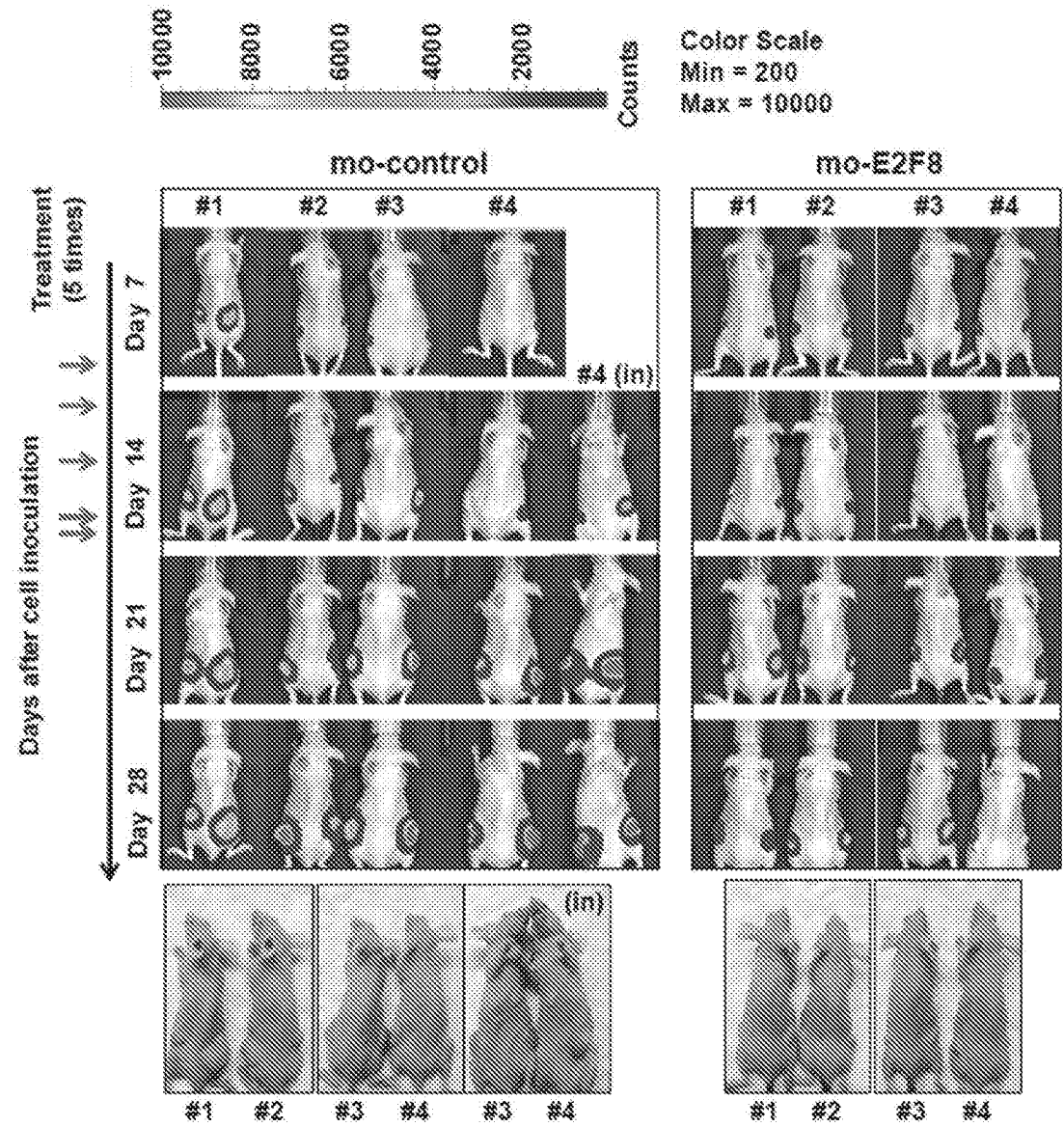


FIG. 8H

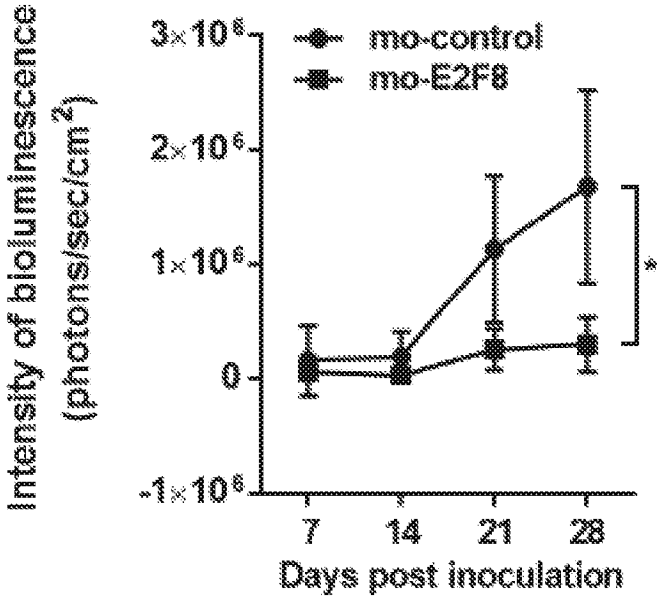
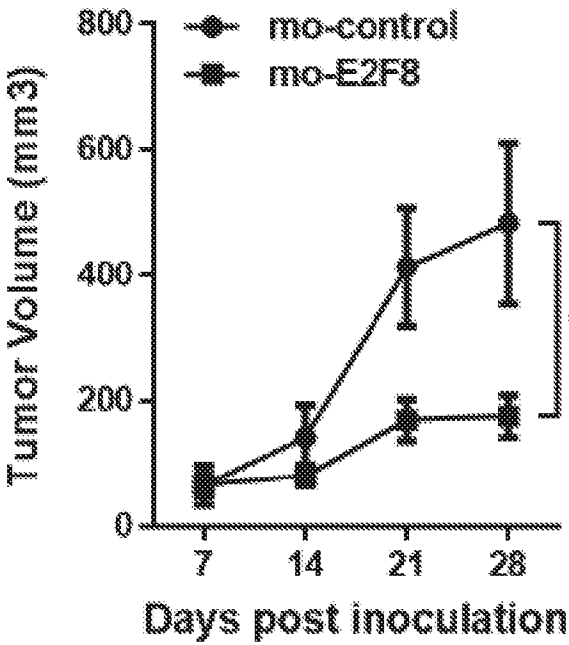


FIG. 8I



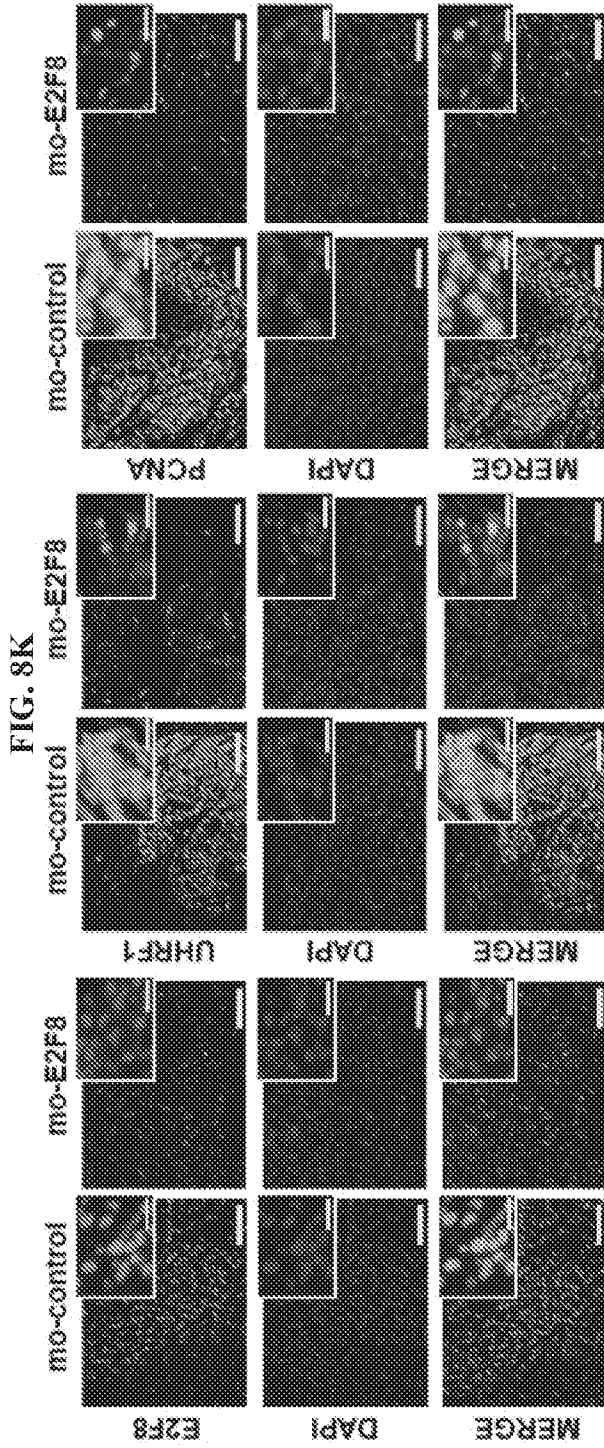
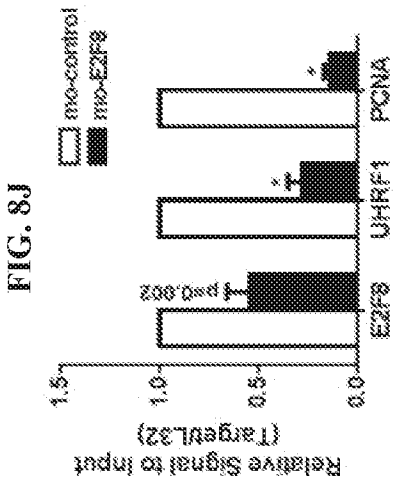


FIG. 9A

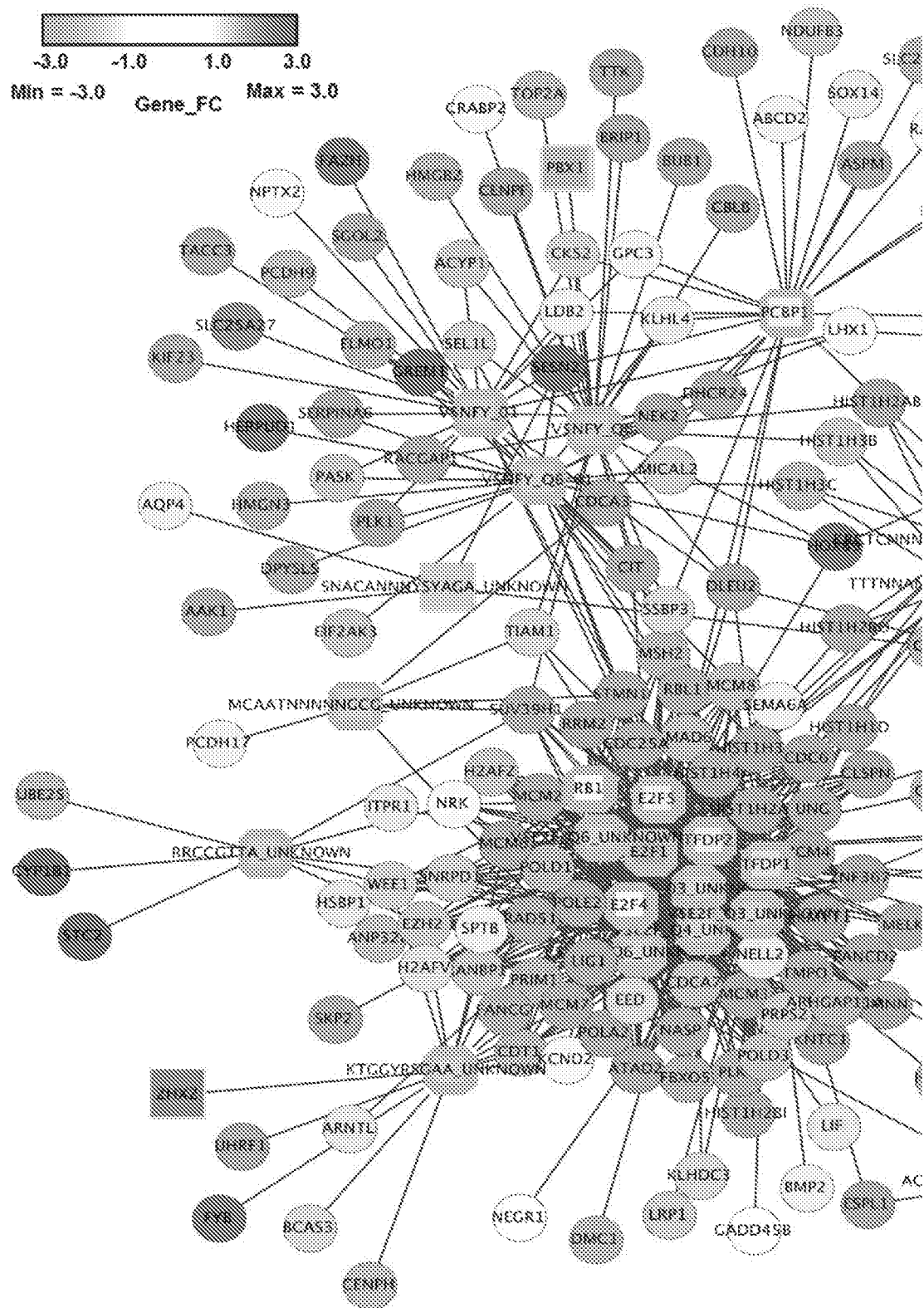


FIG. 9B

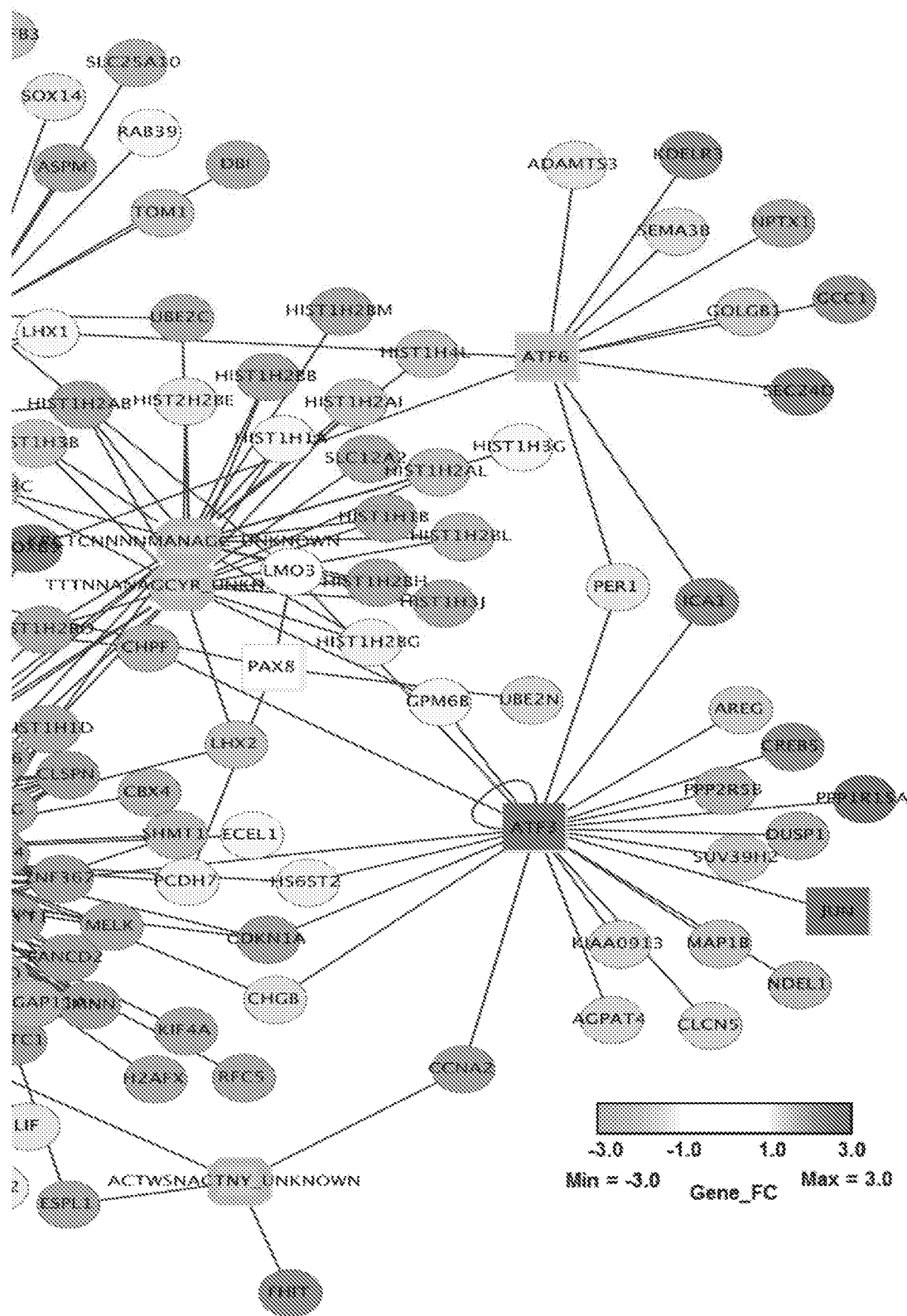


FIG. 9C

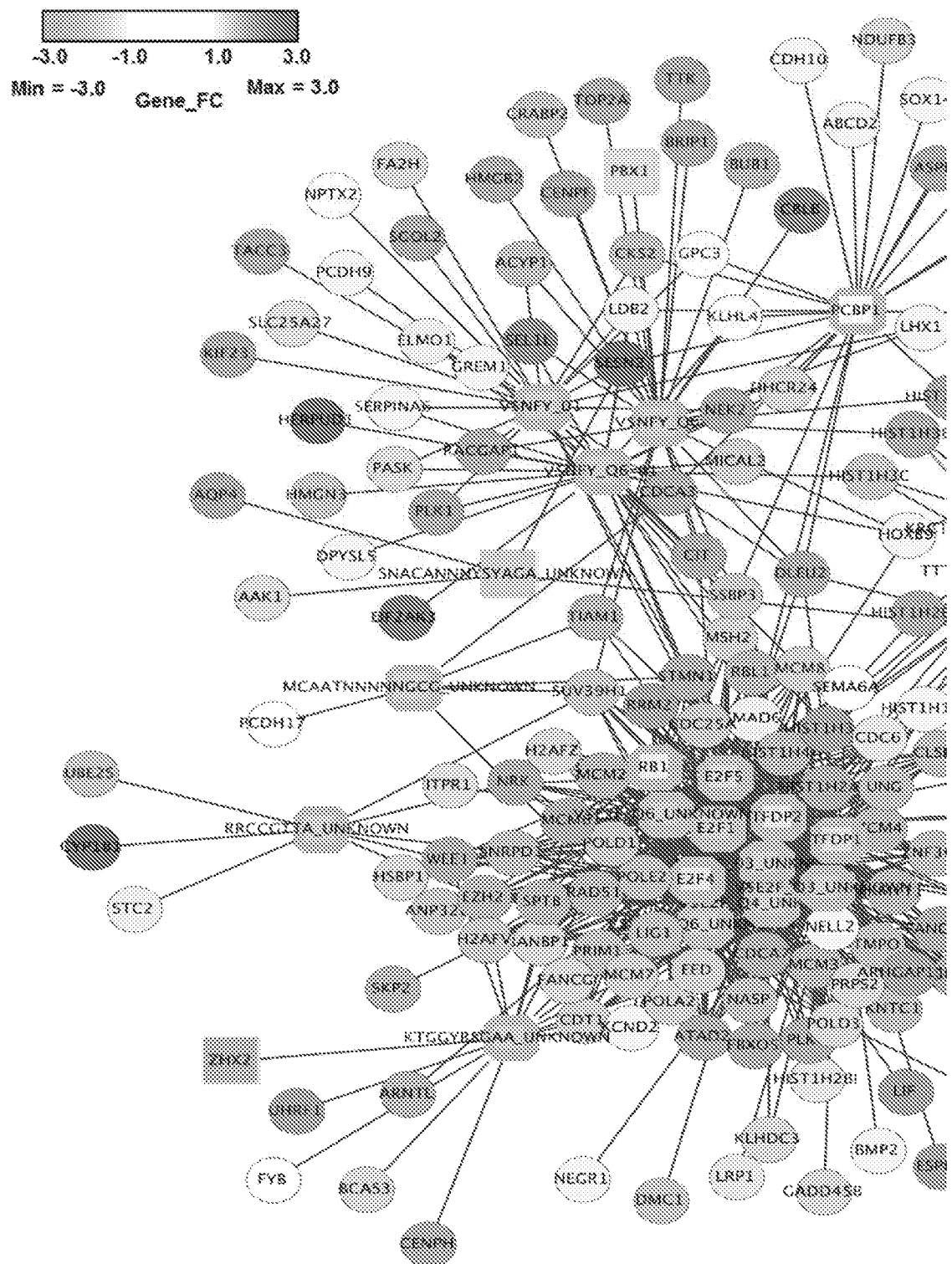


FIG. 9D

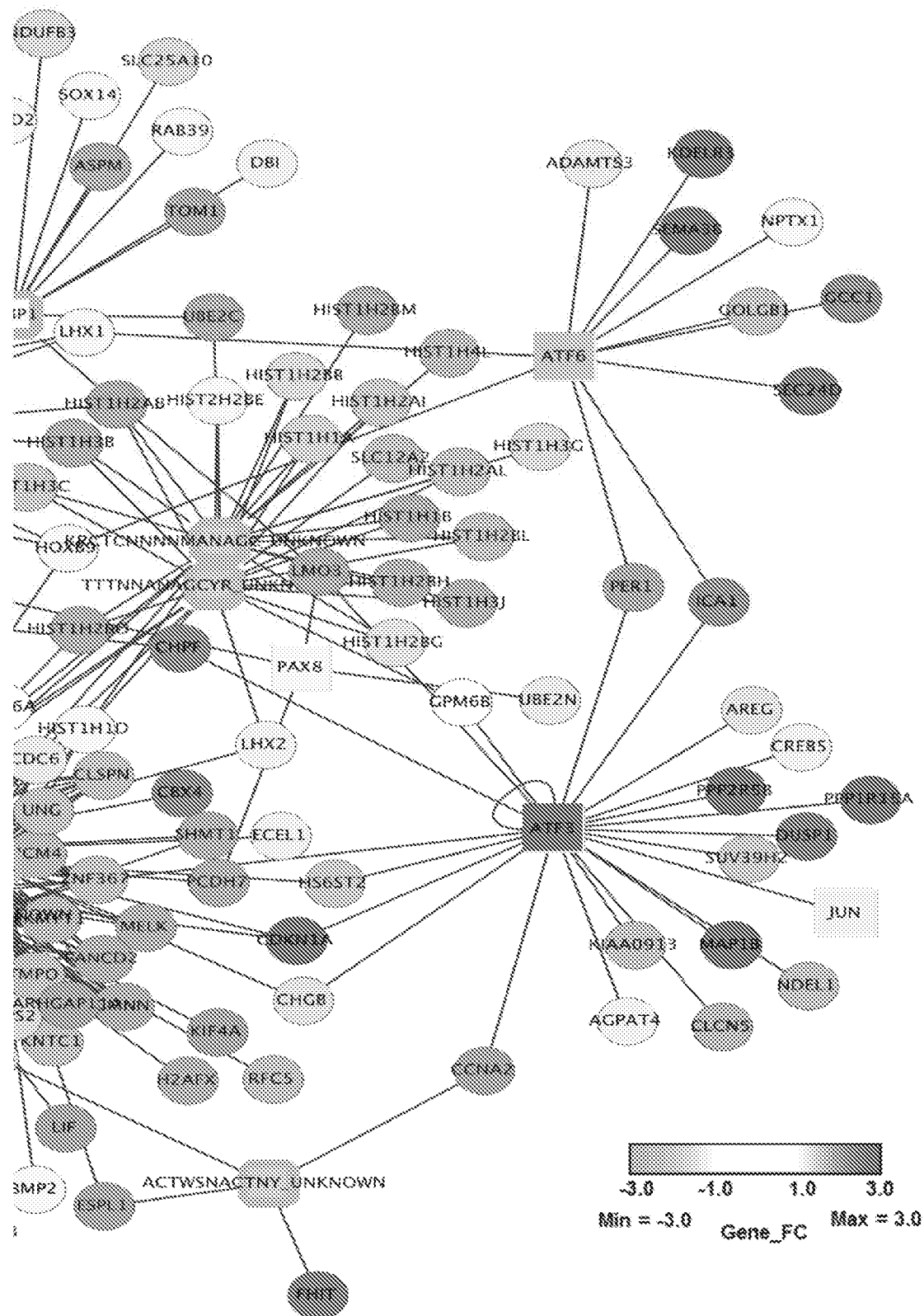


FIG. 9E

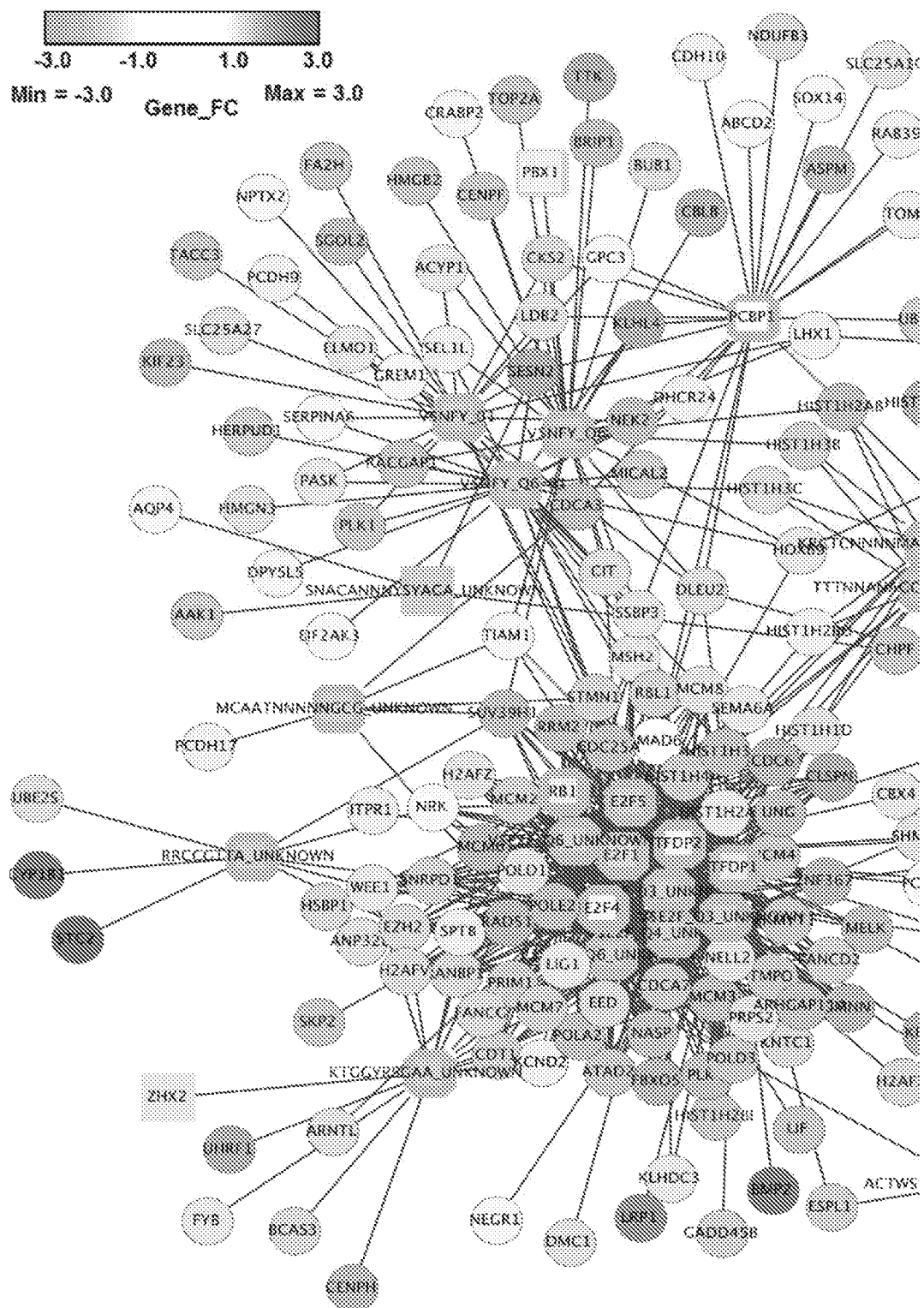


FIG. 9F

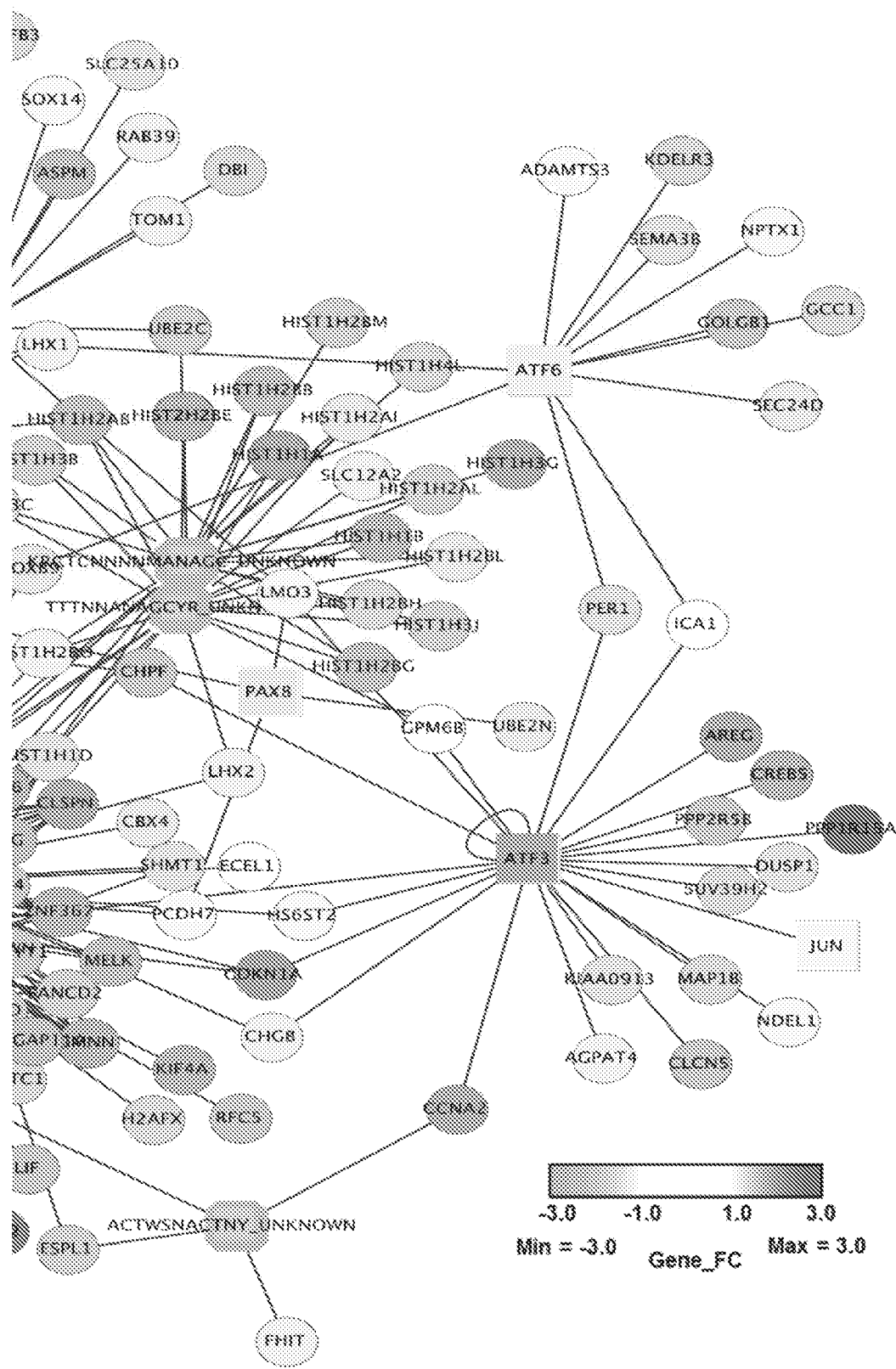


FIG. 9G

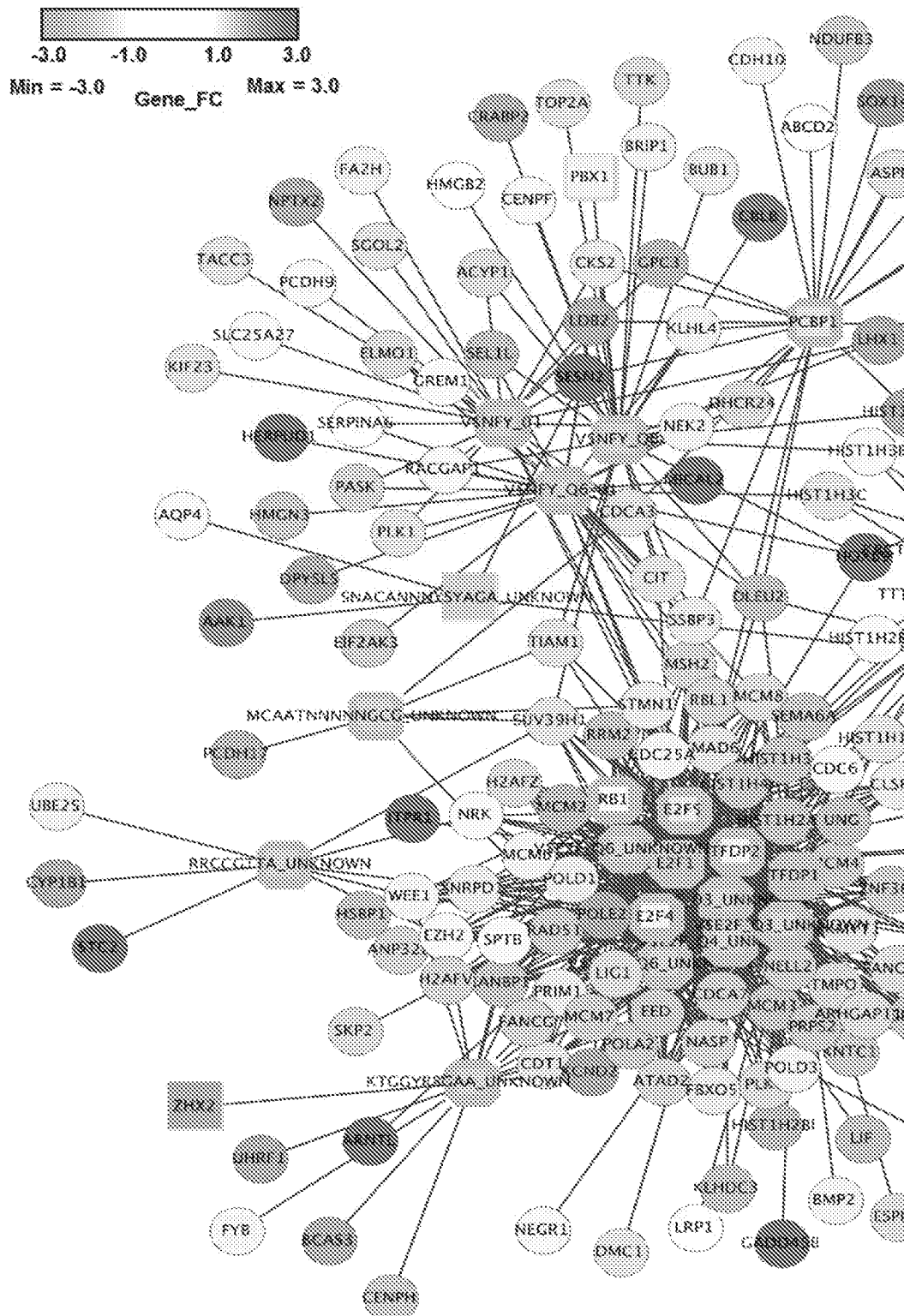


FIG. 9H

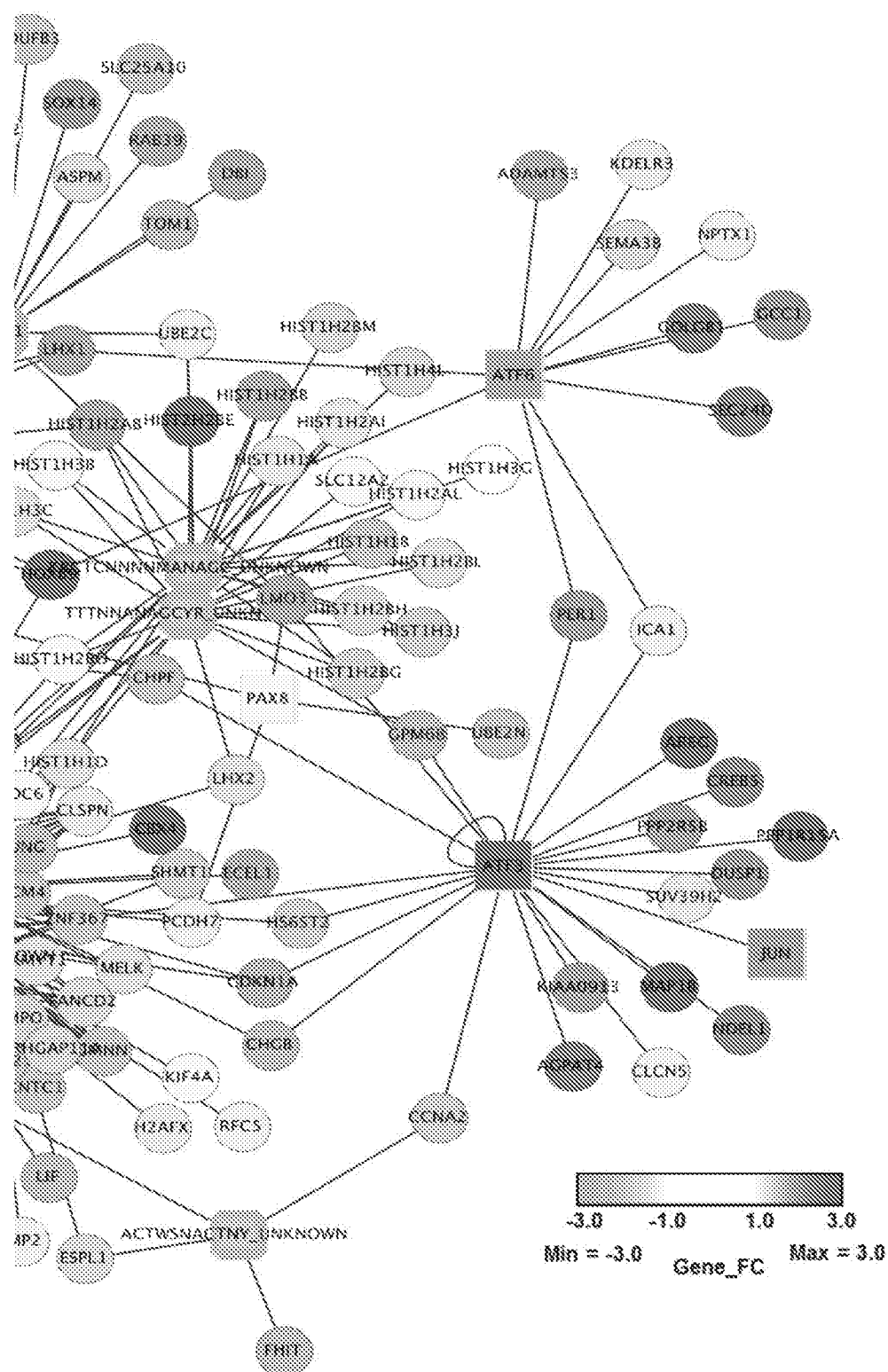


FIG. 9I

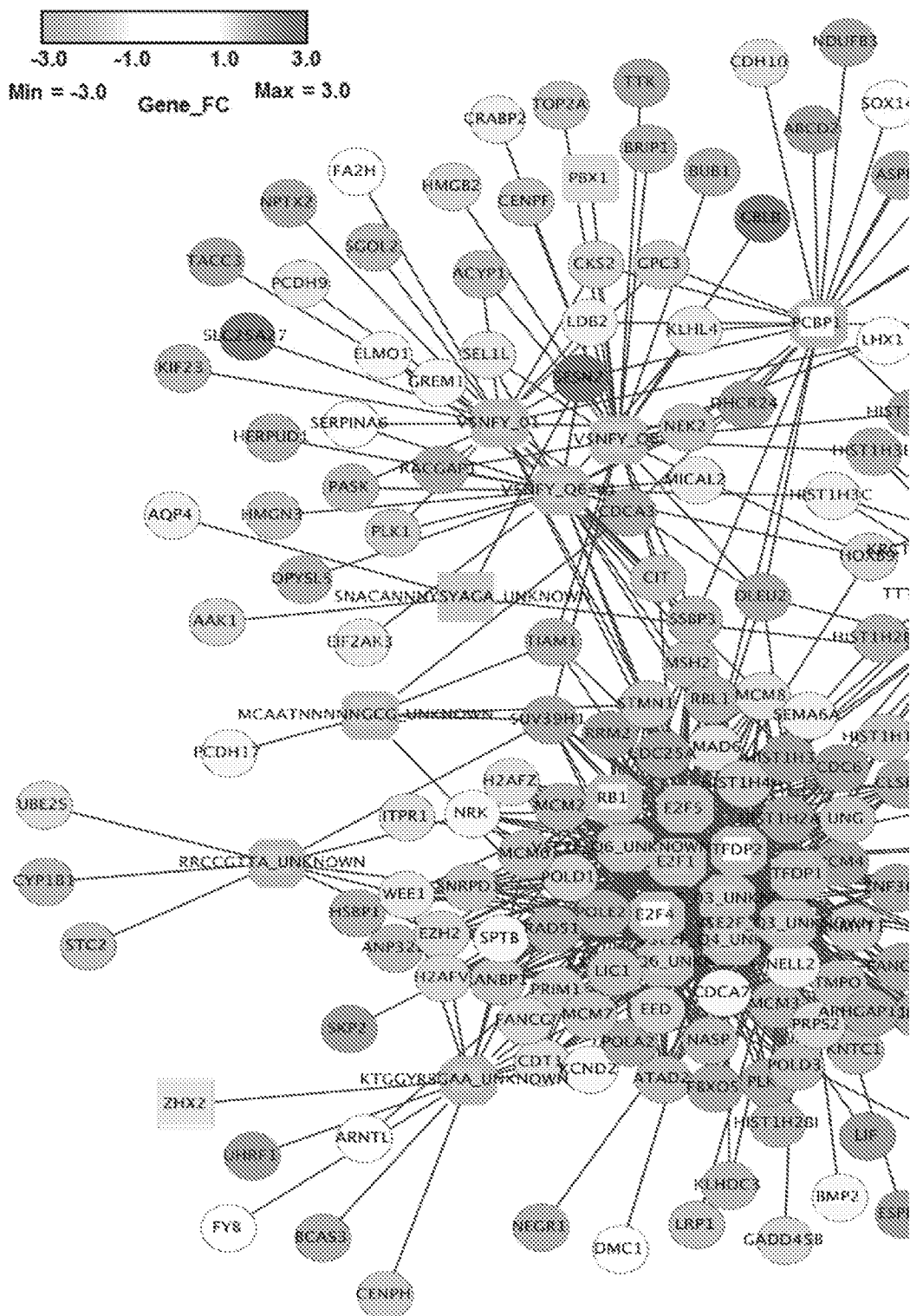


FIG. 9J

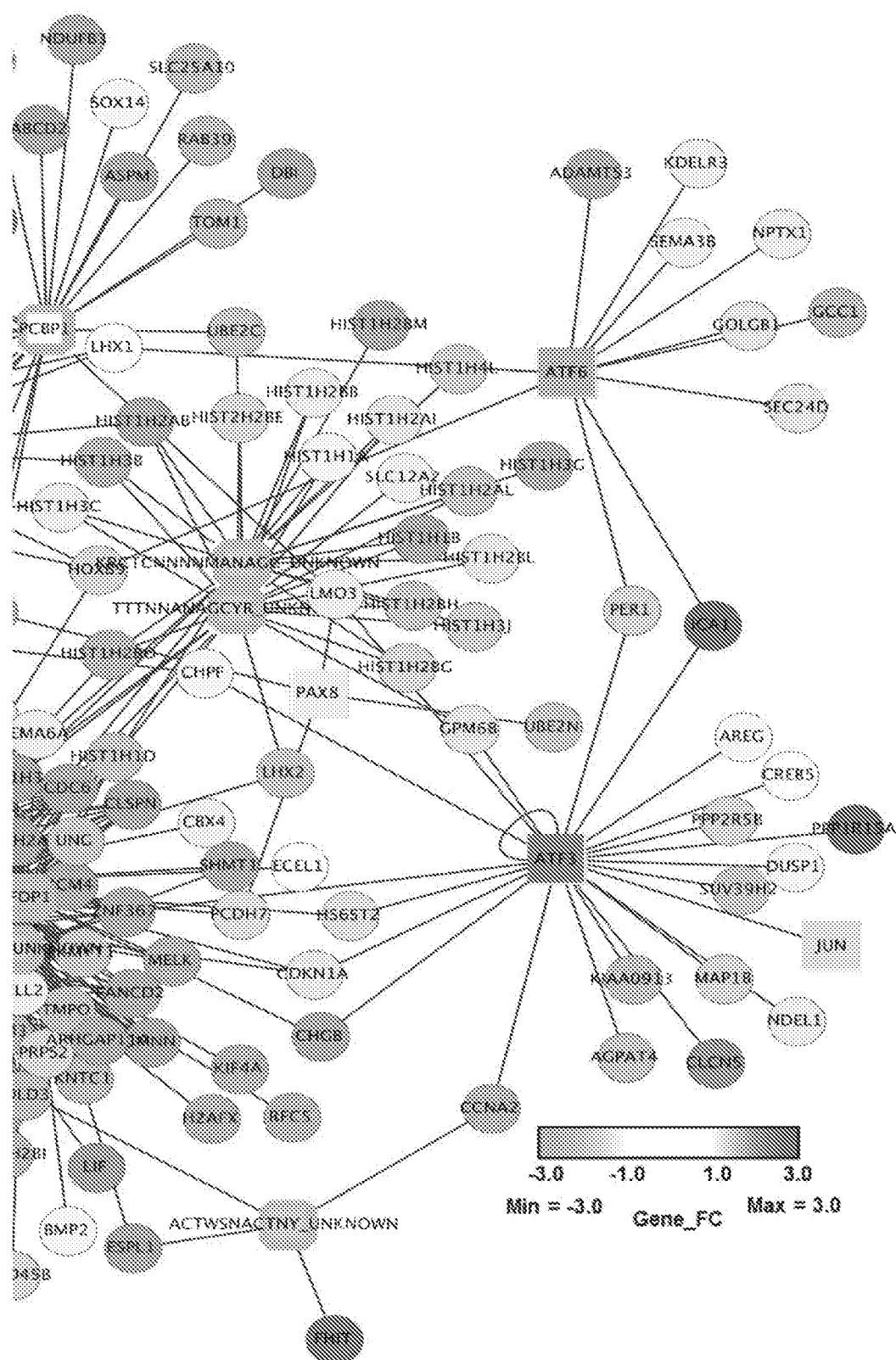
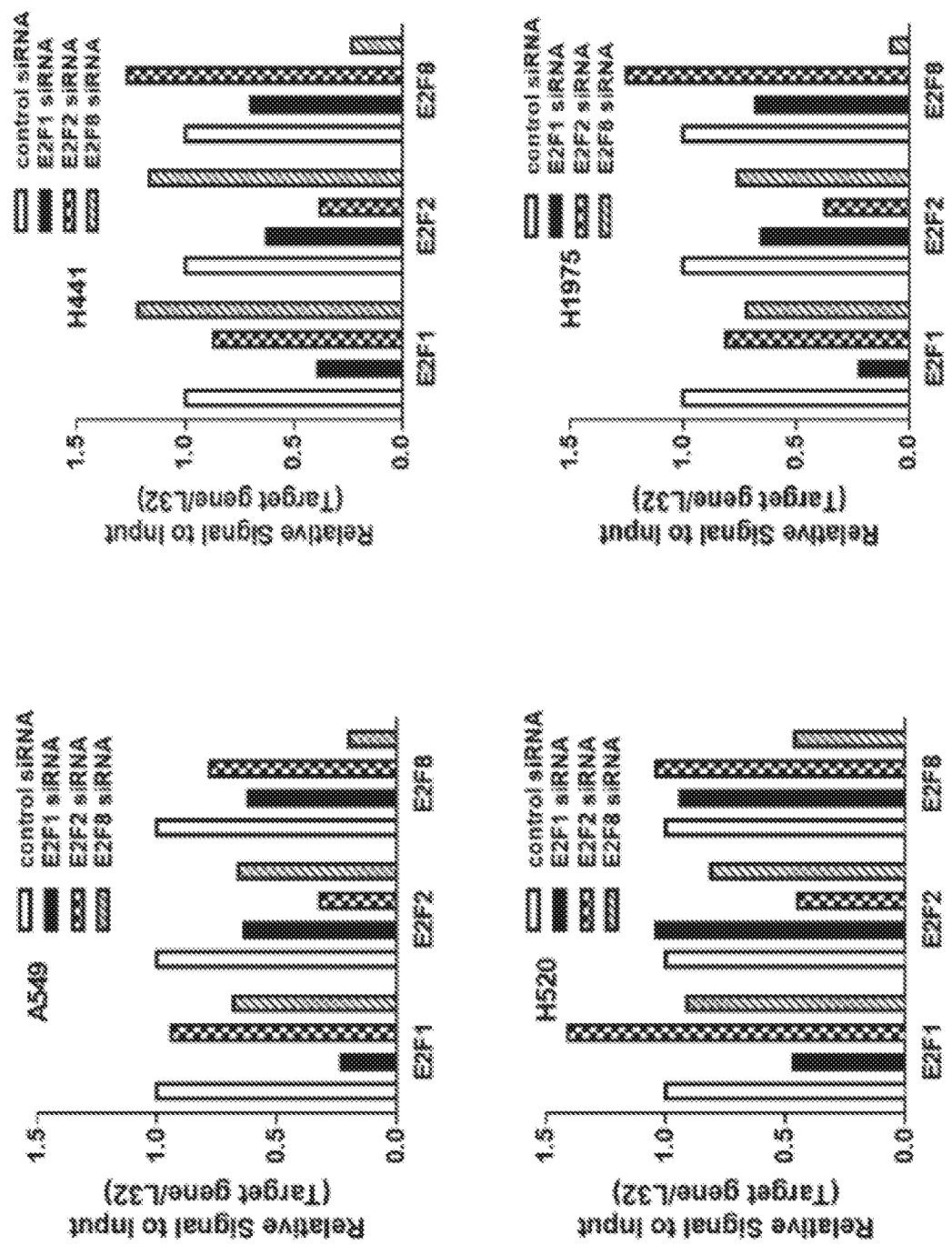


FIG. 10A



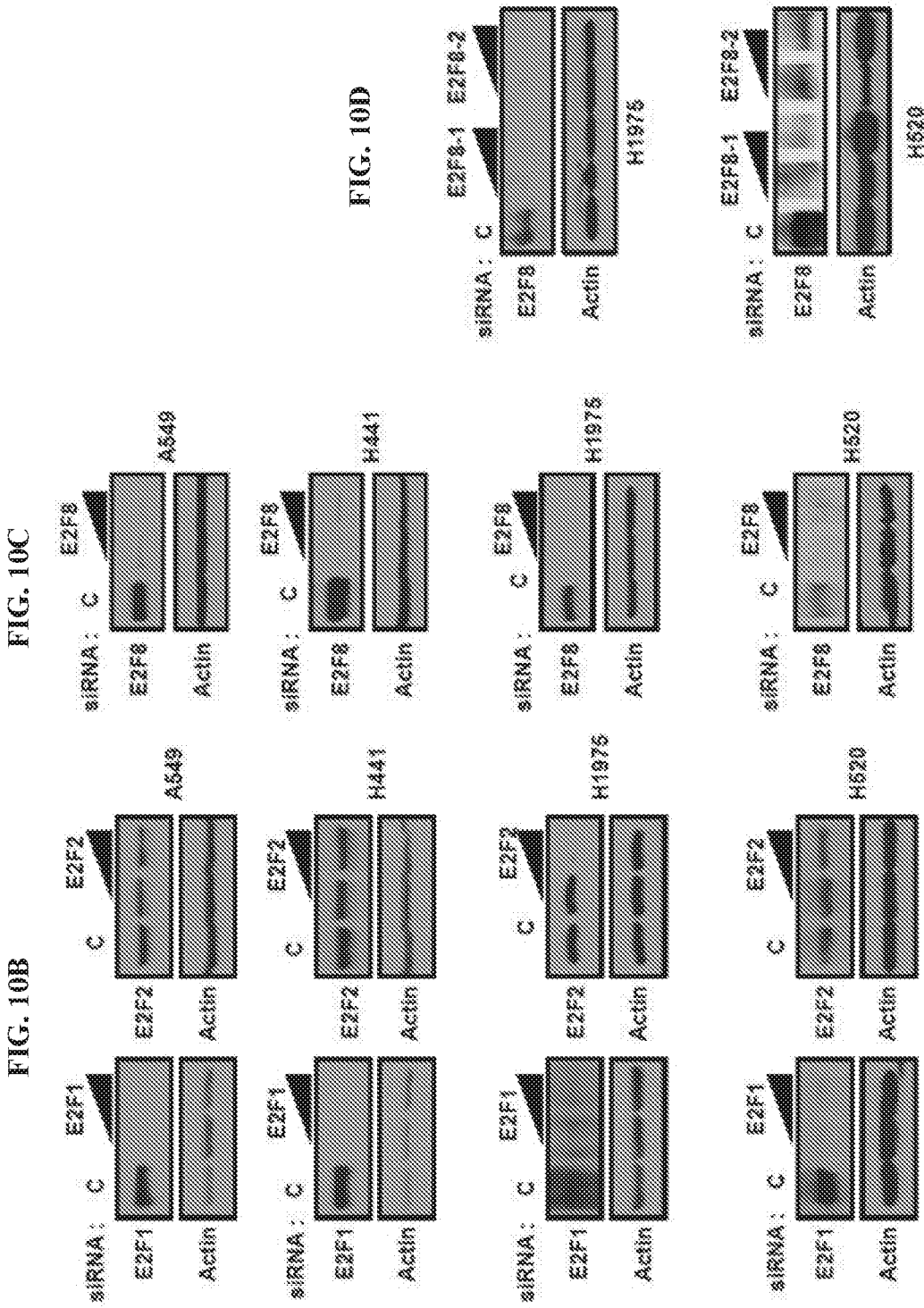


FIG. 11A

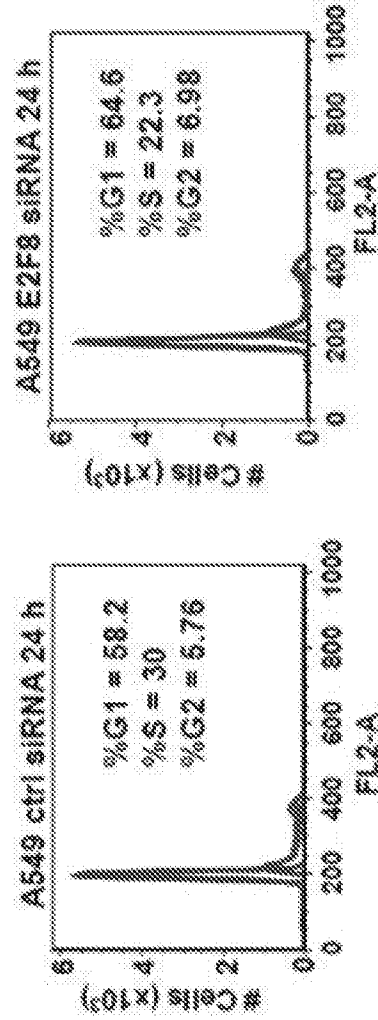


FIG. 11B

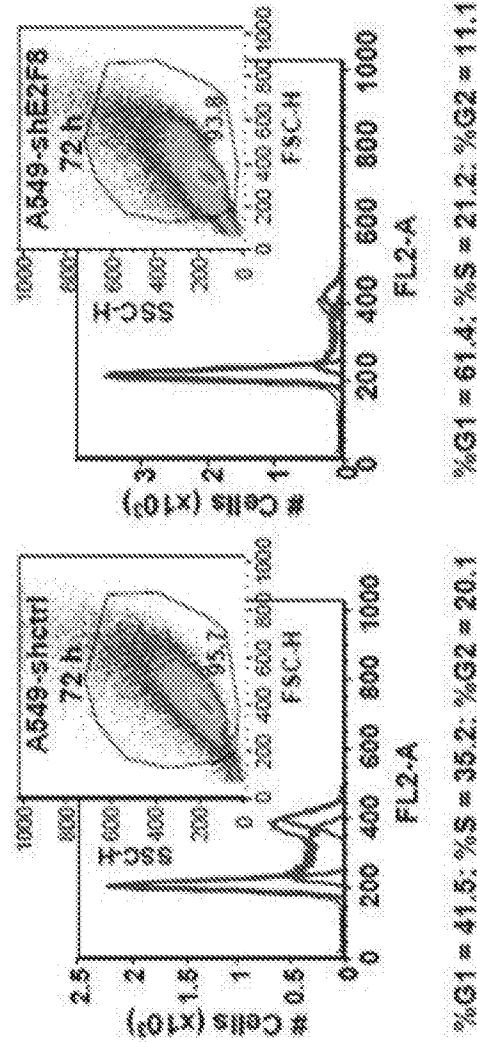


FIG. 12A

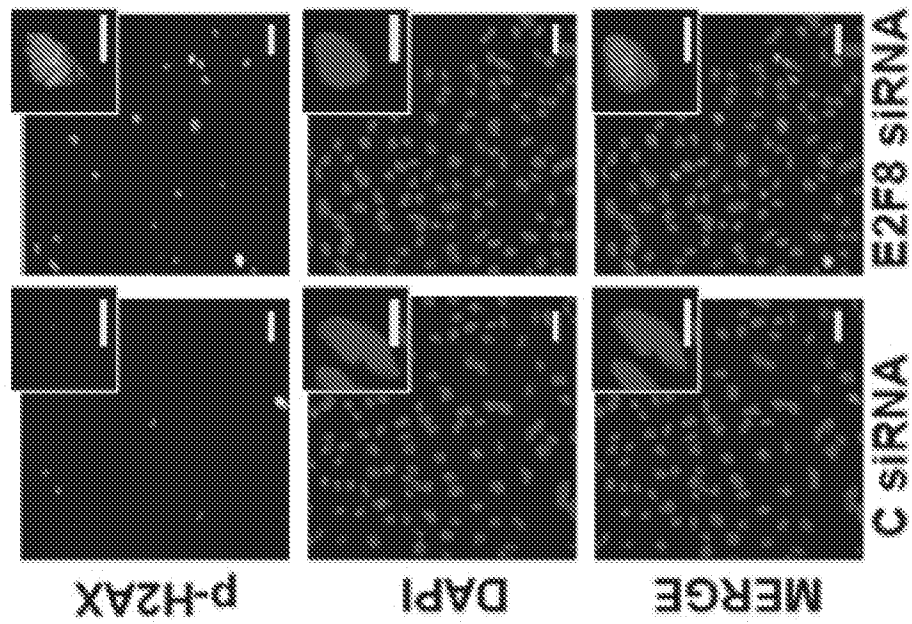


FIG. 12B

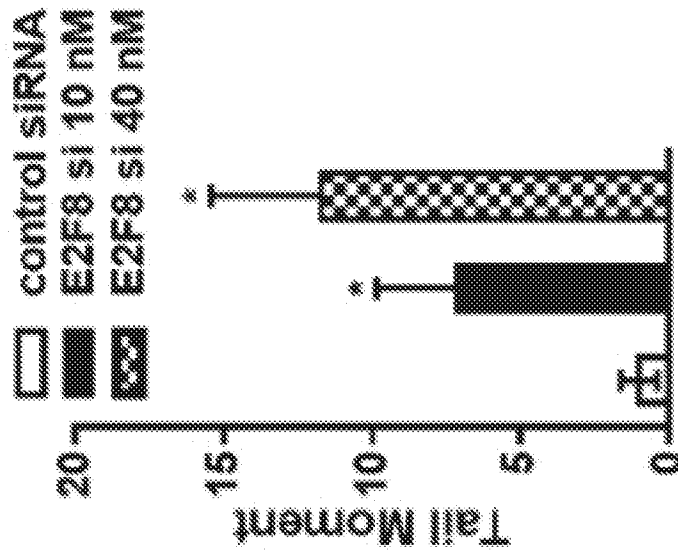


FIG. 13A

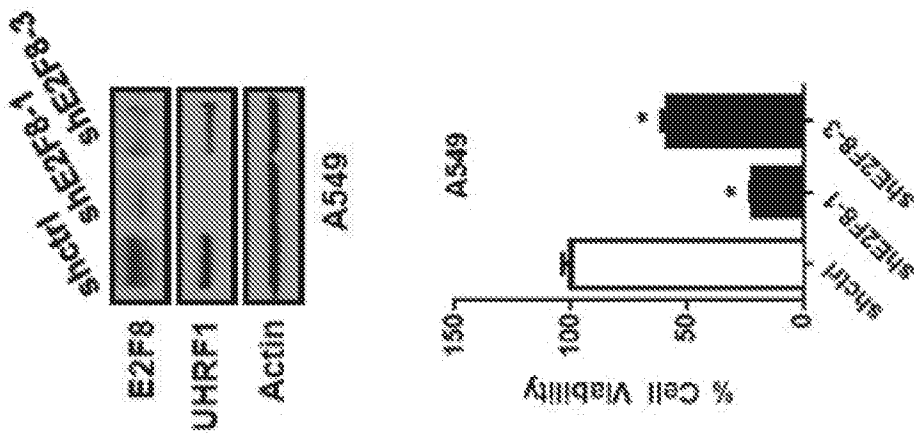


FIG. 13B

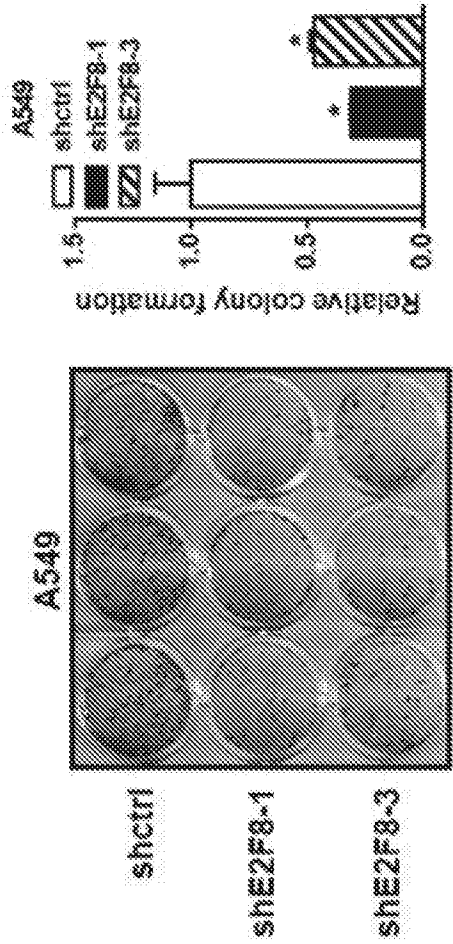
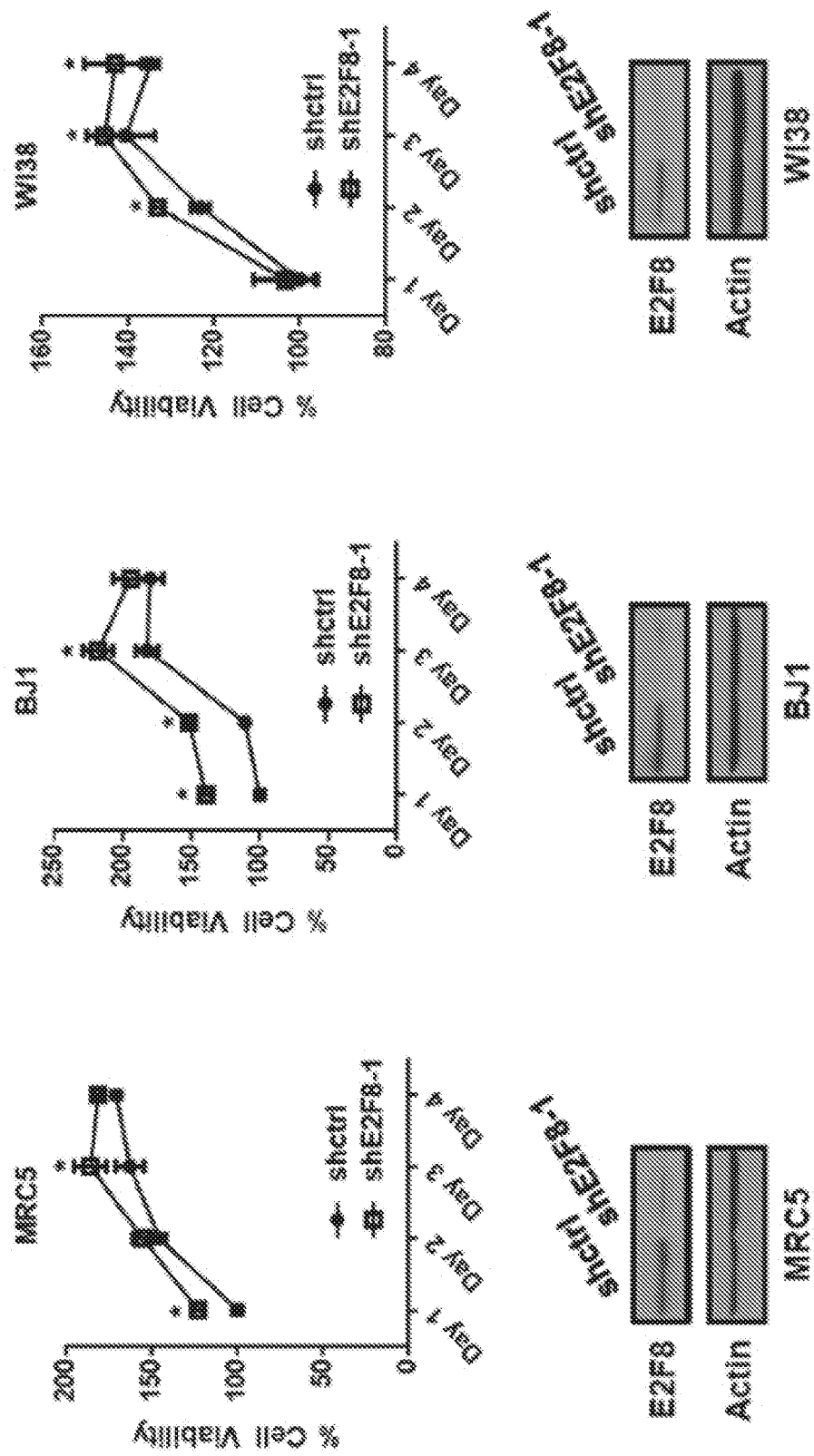


FIG. 13C



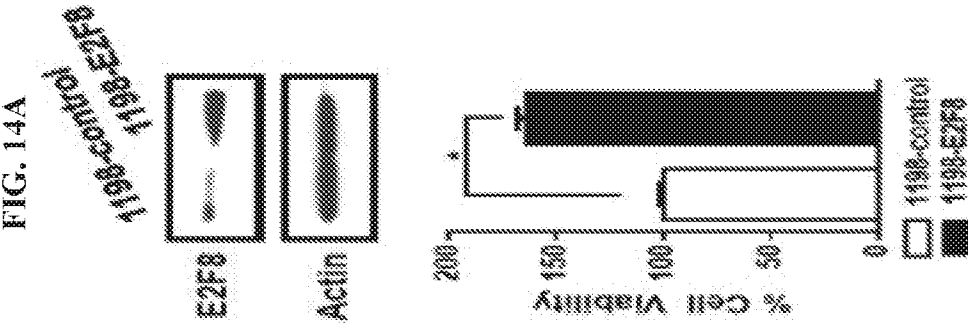
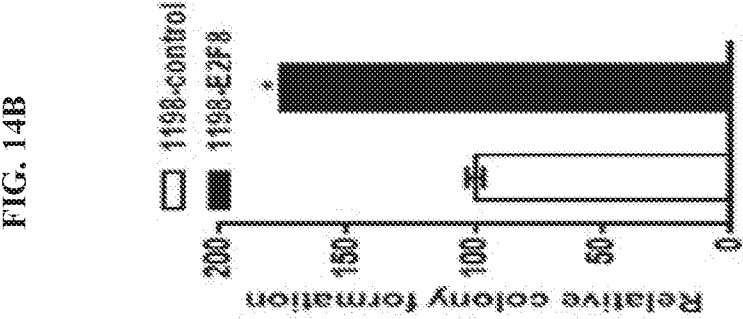
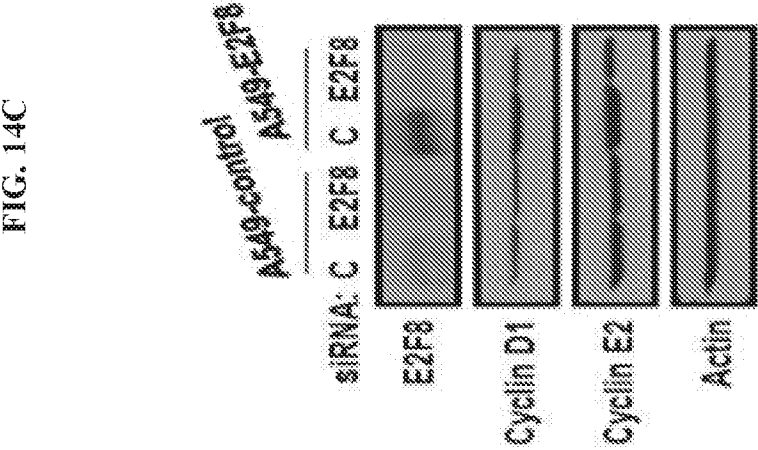
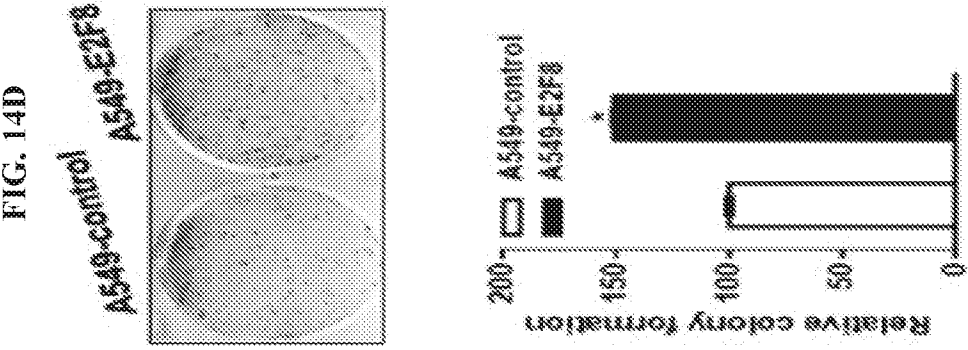


FIG. 15A

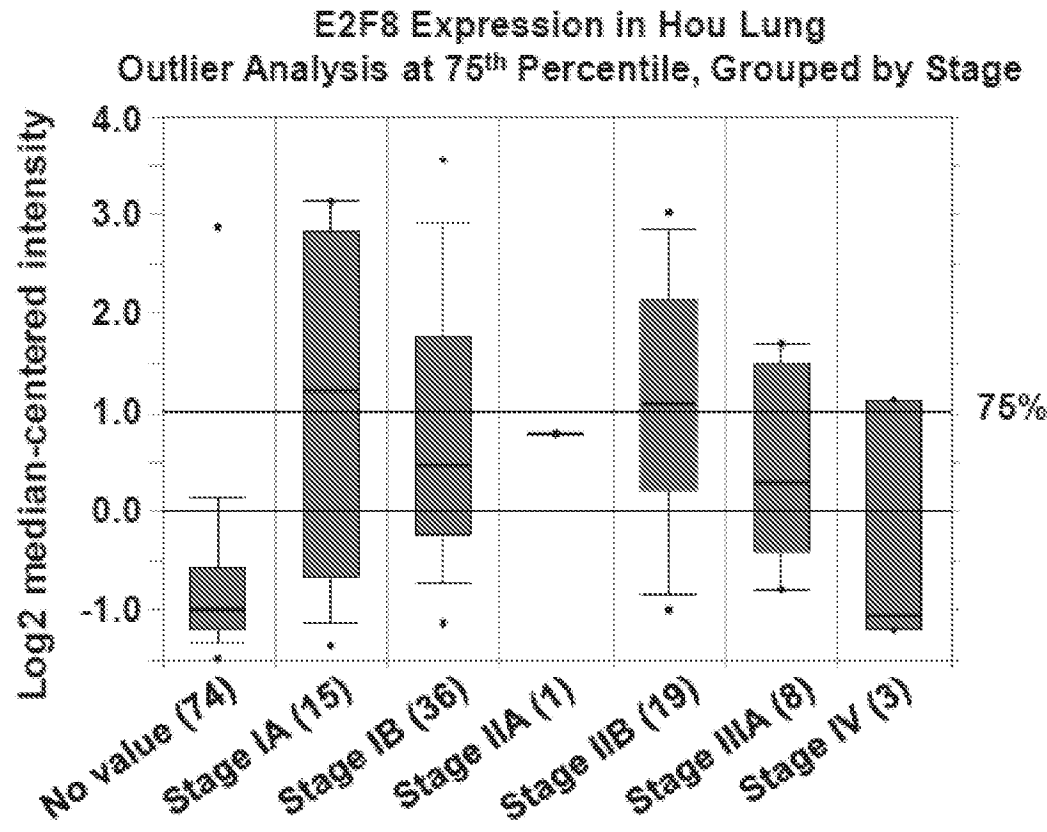


FIG. 15B

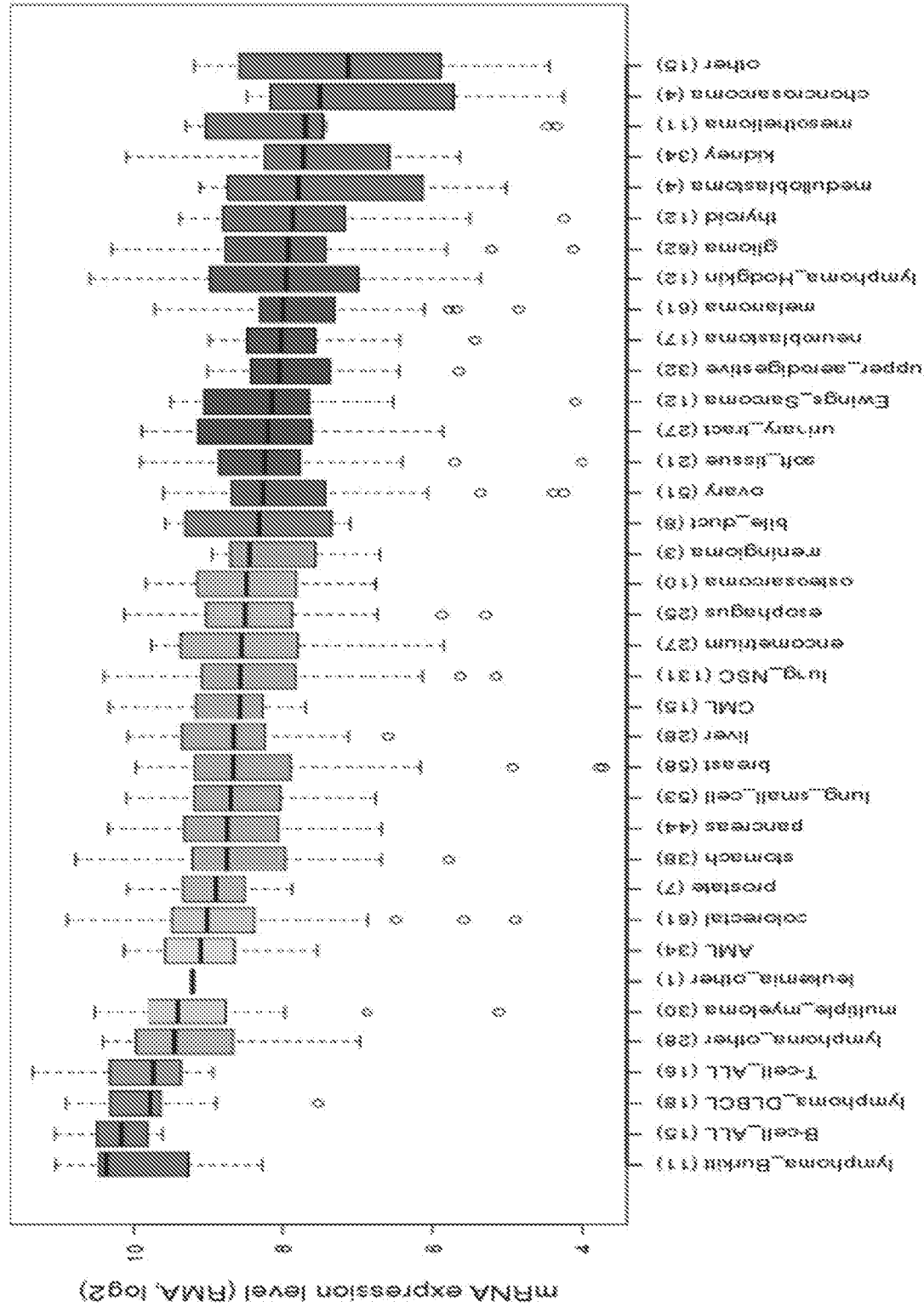


FIG. 16A

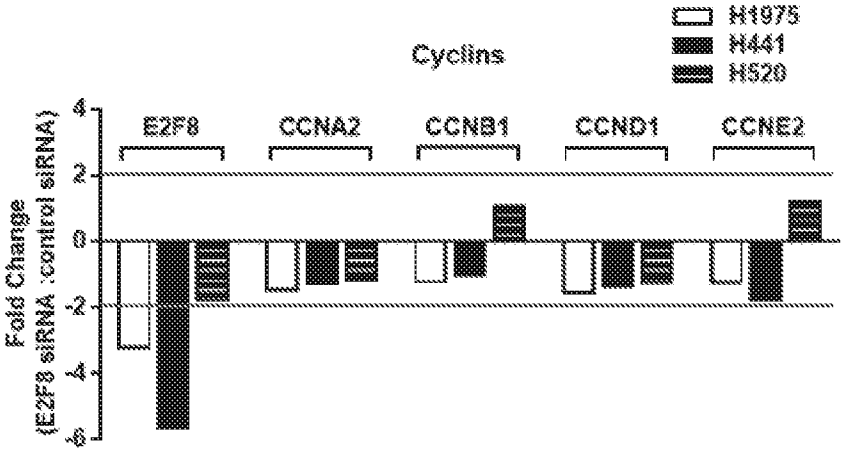


FIG. 16B

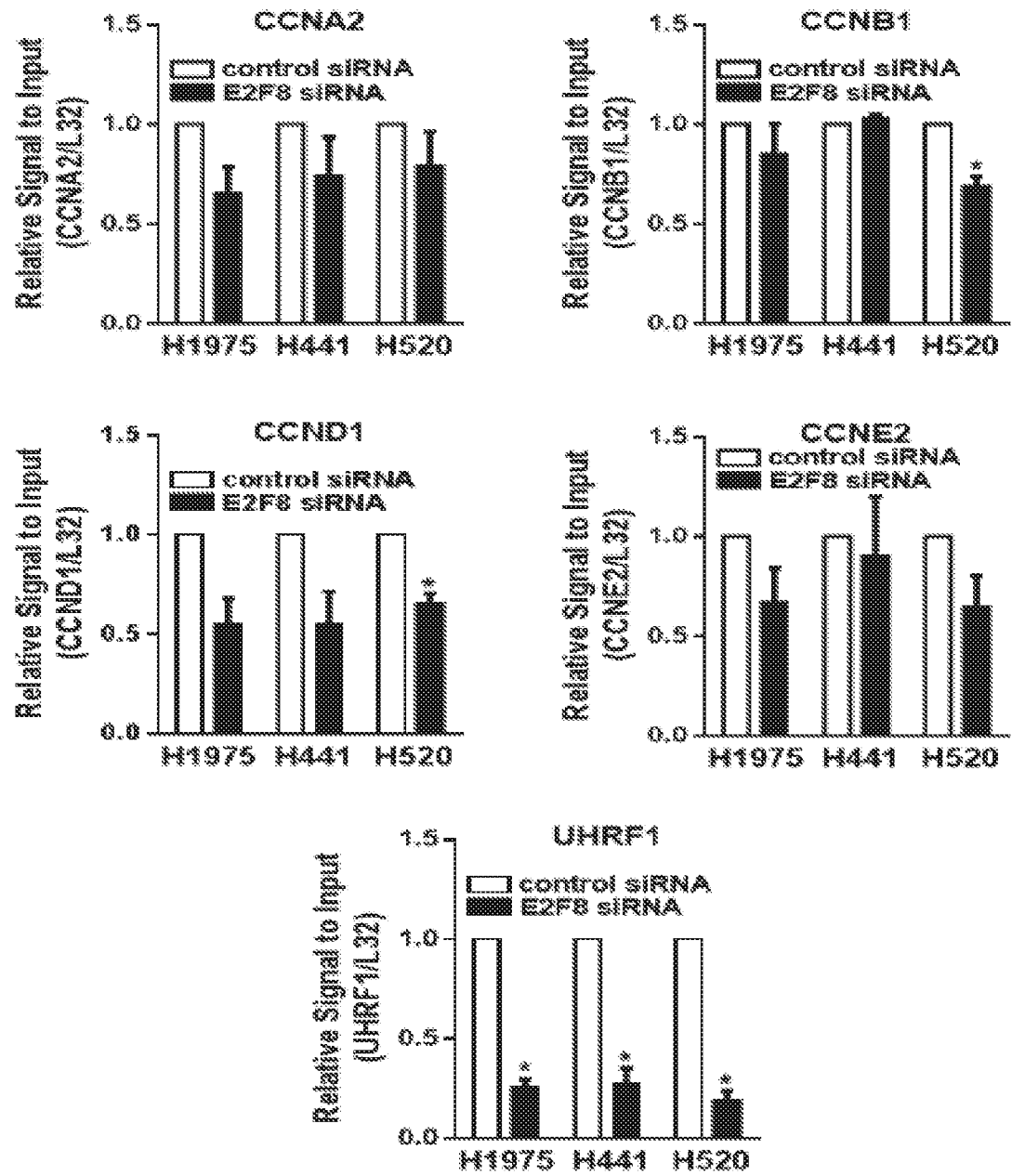


FIG. 16C

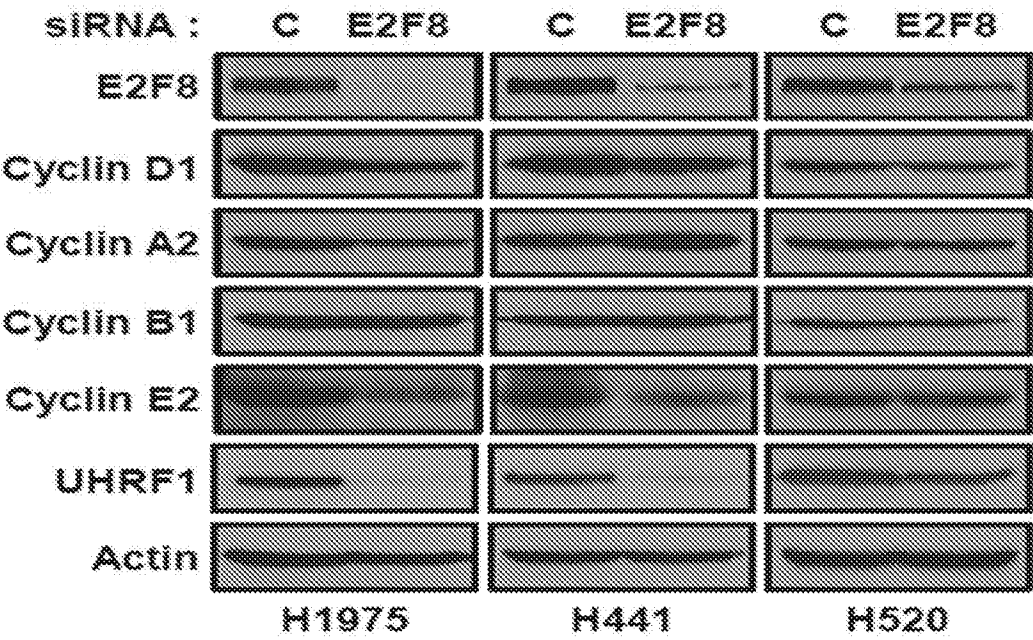


FIG. 17

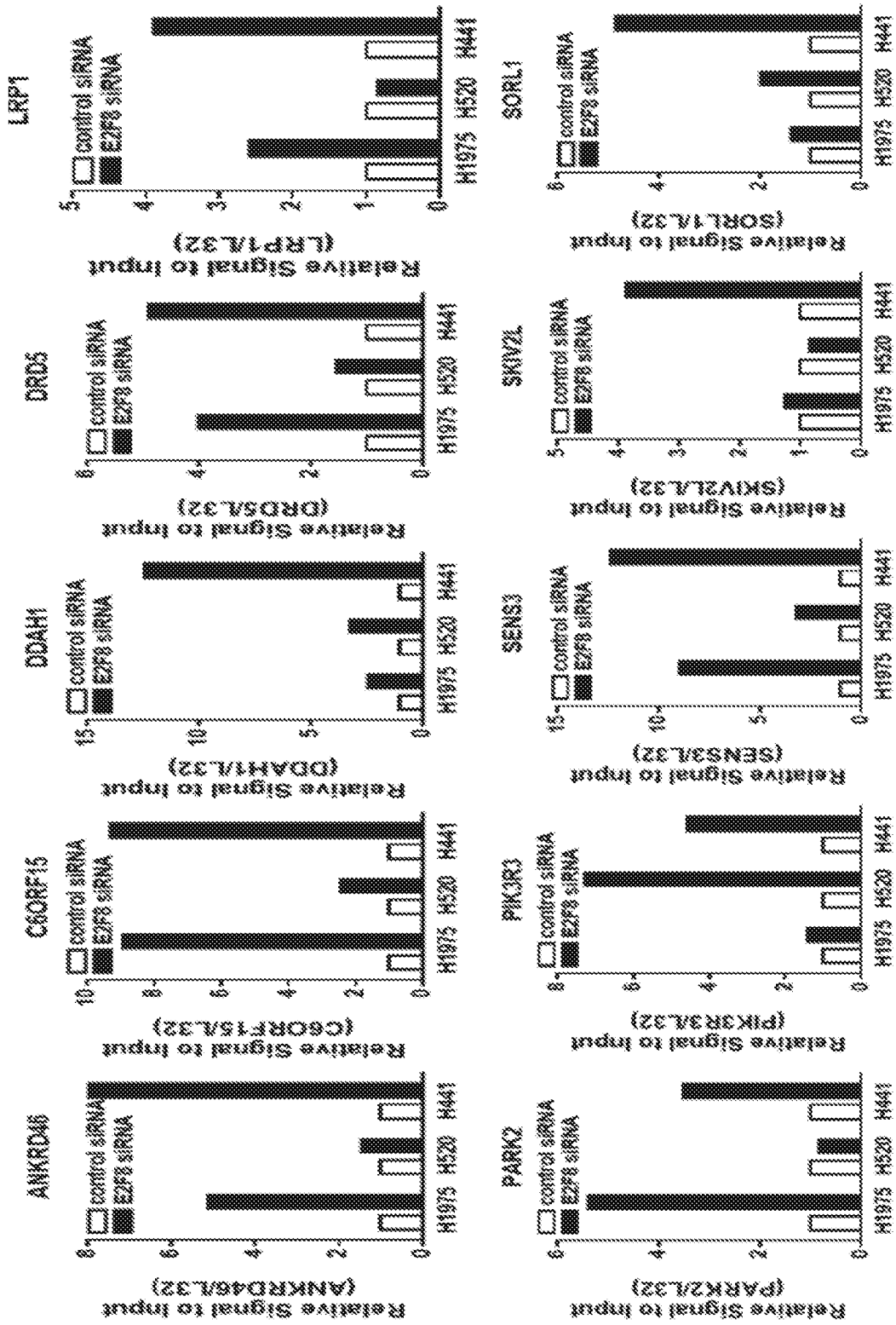


FIG. 18A

GSEA E2F1 targets LC_siE2F8 vs. siControl

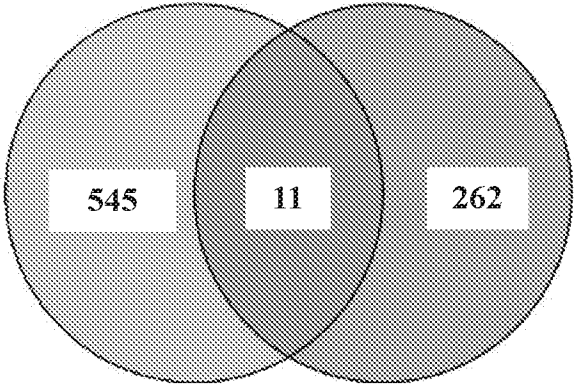


FIG. 18B

GSEA E2F1 targets LC_siE2F8 vs. siControl:
Upregulated only

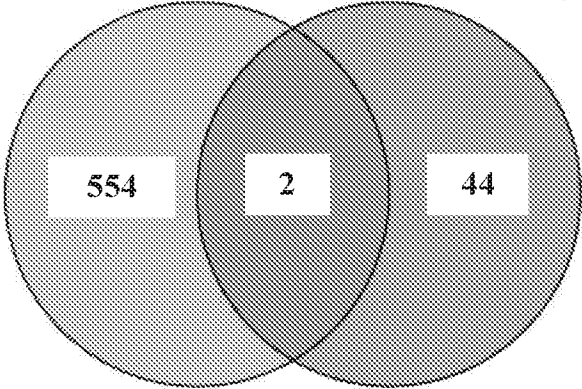


FIG. 18C

GSEA E2F1 targets LC_siE2F8 vs. siControl:
Downregulated only

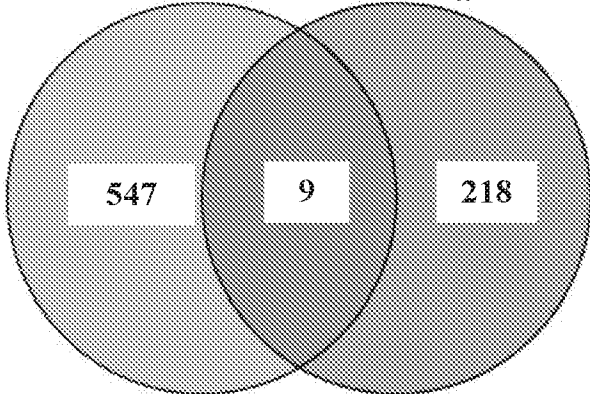


FIG. 19

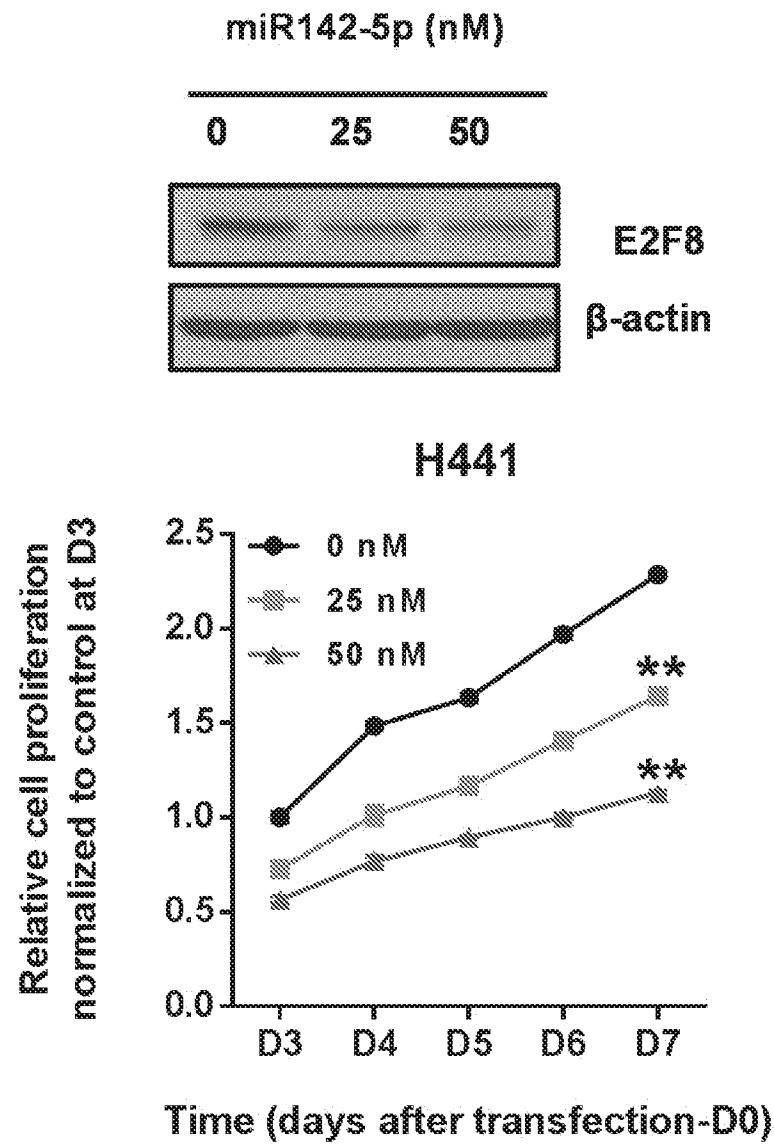


FIG. 20

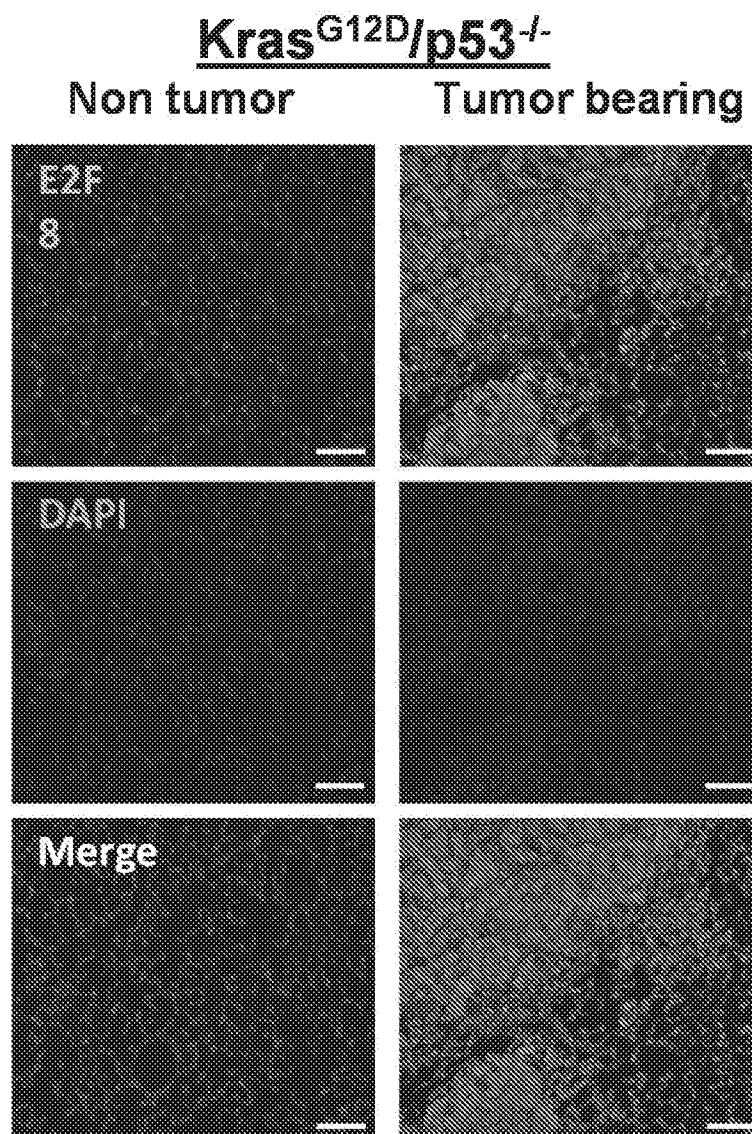


FIG. 21A

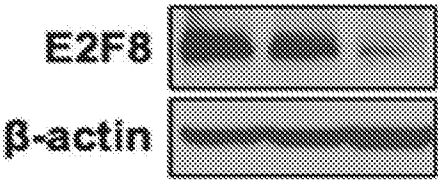


FIG. 21B

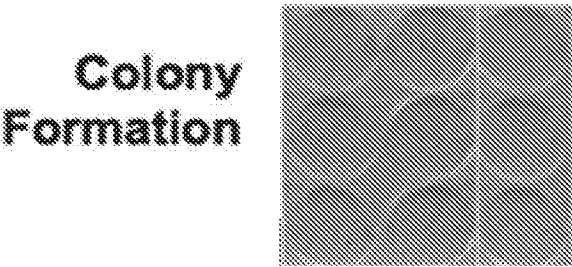


FIG. 21C

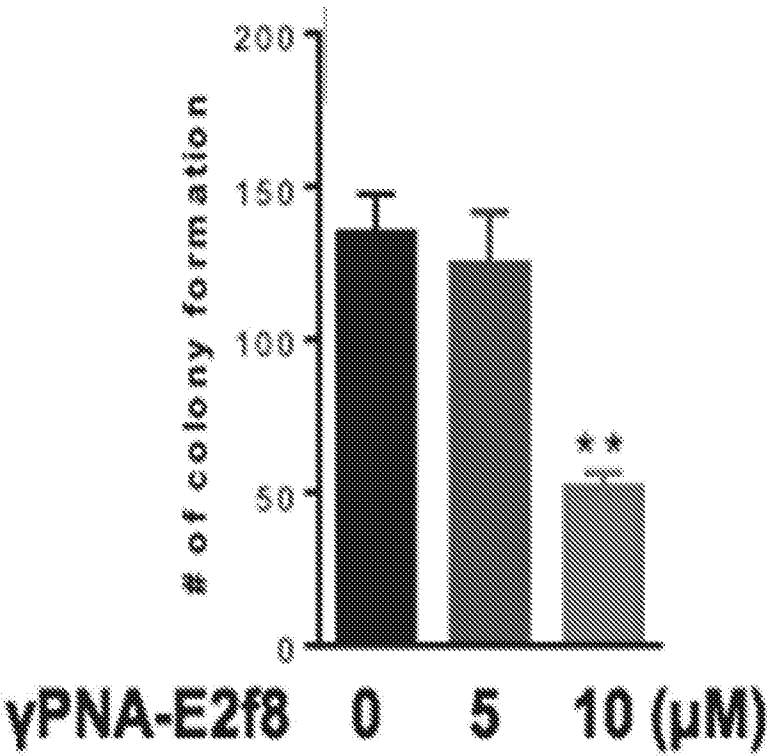


FIG. 21D

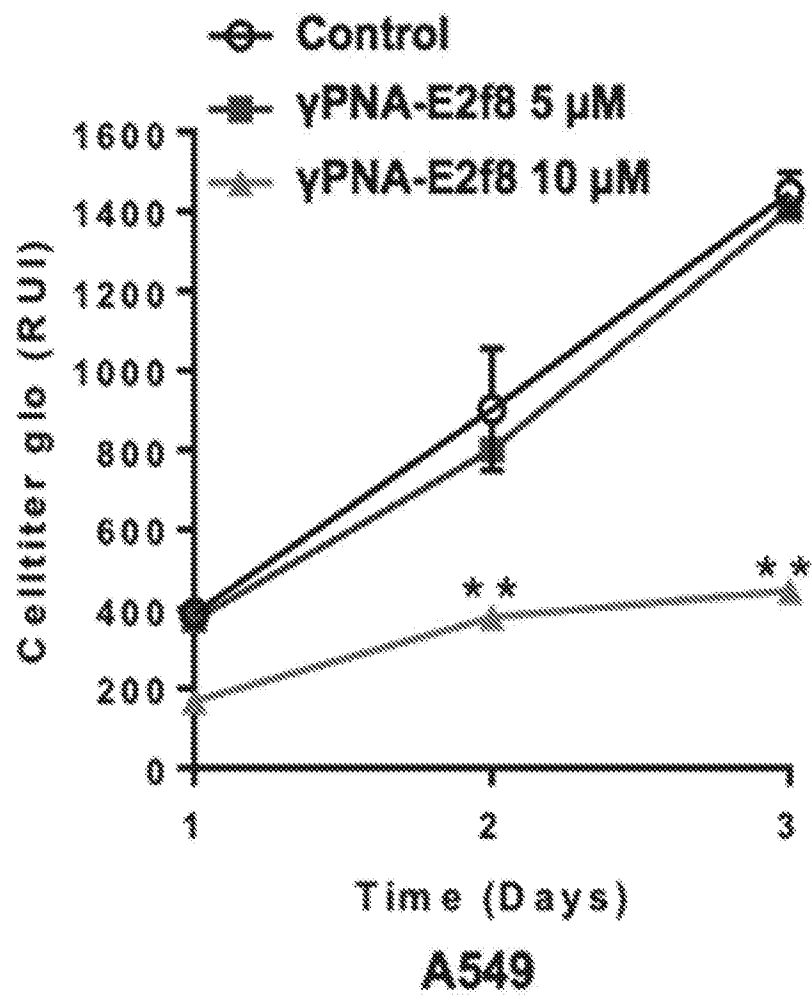


FIG. 22A

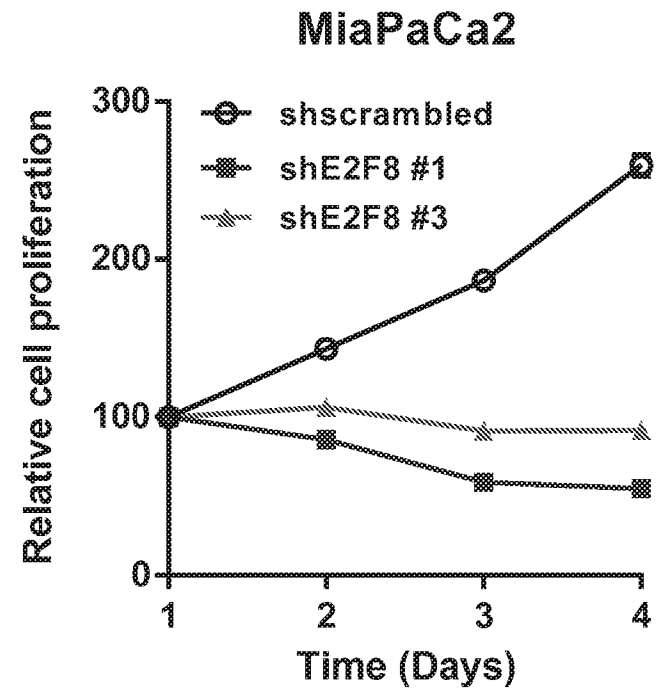
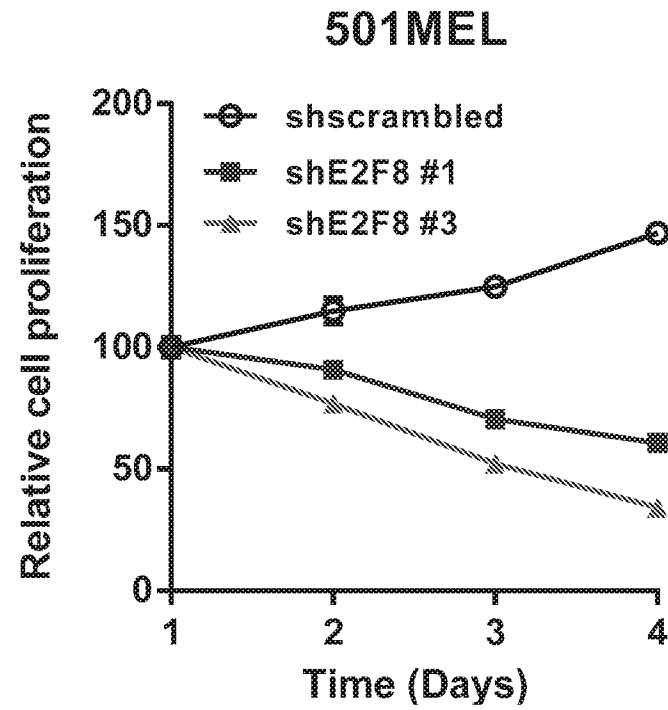


FIG. 22B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/29526

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68; C12N 15/113; G01N 33/574, 33/15; A61K 39/395 (2016.01)

CPC - C12Q 1/6886; C12N 15/1135, 15/1138; G01N 33/57423; A61K 39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12Q1/68; C12N15/113, 15/12, 5/0783; G01N33/574, 33/15, 33/53; A61K39/395, 45/06; A61P35/00; G06F19/18, 19/20

CPC: C12Q1/6883, 1/6886; C12N15/1135, 15/1136, 15/1138; G01N33/57423, 33/57438, 33/15, 33/5743; A61K39/395; C07K16/2818

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; pubmed; EBSCO

Keywords: E2F8, inhibit*, gene, cancer, tumor, lung, pancrea*, melanoma, inactivat*, repress*, express*, subject, administer, siRNA, anti-sense, morpholino

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEIJTS, BG et al. Atypical E2fs Control Lymphangiogenesis through Transcriptional Regulation of Ccbe1 and Flt4. PLoS One. 12 September 2013; Vol. 8, No. 9; pages 1-10; abstract; page 1, second column, paragraph 1; page 2, second column, paragraphs 2-3; page 7, first column, paragraph 2; page 7, second column, paragraph 2; page 8, first column, paragraph 3; page 8, second column, paragraph 1; page 9, first column, paragraph 2; figures 1, 4. DOI: 10.1371/journal.pone.0073693	1-4, 9, 12 ----- 5-8, 10-11, 13-17, 37-38
X	DENG, Q et al. E2F8 Contributes to Human Hepatocellular Carcinoma via Regulating Cell Proliferation. Tumor and Stem Cell Biology. 15 January 2010; Vol. 70, No. 2; pages 782-791; page 782, second column, paragraph 3; page 784, first column, paragraph 2. DOI: 10.1158/0008-5472.CAN-09-3082	18, 23-25, 28-29, 34 ----- 19-22, 26-27, 30-33, 35-36
Y	LEE, JW et al. A Novel Small-Molecule Inhibitor Targeting CREB-CBP Complex Possesses Anti-Cancer Effects along with Cell Cycle Regulation, Autophagy Suppression and Endoplasmic Reticulum Stress. PLoS One. 21 April 2015; Vol. 10, No. 4; pages 1-16; abstract; page 4/16, paragraph 2; DOI: 10.1371/journal.pone.0122628. eCollection 2015.	5
Y	WILKERSON, MD et al. Lung Squamous Cell Carcinoma mRNA Expression Subtypes Are Reproducible, Clinically Important, and Correspond to Normal Cell Types. Clinical Cancer Research. 19 July 2010; Vol. 16, No. 19; pages 4864-4875; page 4865, first column, paragraph 3; page 4869, first column, paragraph 1; DOI: 10.1158/1078-0432.CCR-10-0199.	6-7, 13-14, 19-20, 26, 30-31
Y	BOADU, RJ. RNA Interference and Nonviral Targeted Gene Therapy of Experimental Brain Cancer. NeuroRx. January 2005; Vol. 2, No. 1; pages 139-150; page 140, second column, paragraph 2	8



Further documents are listed in the continuation of Box C.



See patent family annex.

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later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

07 July 2016 (07.07.2016)

Date of mailing of the international search report

11 AUG 2016

Name and mailing address of the ISA/

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Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/29526

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0142784 A1 (RAYCHAUDHARI, P et al.) 06 June 2013; abstract; paragraphs [0012], [0041], [0052]	10-11, 27, 36
Y	EMMETT, MS et al. Angiogenesis and Melanoma - from Basic Science to Clinical Trials. American Journal of Cancer Research. 08 August 2011; Vol. 1, No. 7; pages 852-868; page 853, first column, paragraph 3	15, 21, 32
Y	GOONESEKERE, NCW et al. A Meta Analysis of Pancreatic Microarray Datasets Yields New Targets as Cancer Genes and Biomarkers. PLoS One. 16 April 2014; Vol. 9, No. 4; pages 1-13; page 6, second column, paragraph 1; DOI: 10.1371/journal.pone.0093046	16-17, 22, 33
Y	US 2002/0115057 A1 (YOUNG, P) 22 August 2002; abstract; paragraphs [0005], [0013], [0022], [0121]	35
Y	US 2014/0120116 A1 (THE CHINFSE UNIVERSITY OF HONG KONG) 01 May 2014; paragraph [0083]	37-38