SUPPLEMENTAL MATERIALS.

1. Supplemental Figures and Tables.

Figure S1. A. Genes assessed for differential expression in 344SQ versus 393P cell lines; graphs show quantification of qRT-CPR data. **B.** Western blot of MSI2 protein expression in 7 non-invasive versus 7 metastatic murine cell lines. **C.** Quantification of MSI1 mRNA and protein data in in 7 non-invasive versus 7 metastatic murine cell lines. **D.** Western blot of Msi1 protein expression in 7 non-invasive versus 7 metastatic murine cell lines. **E**. Representative AQUA images of MSI2 expression from analysis of TMAs containing 22 normal and 123 NSCLC specimens. Blue - DAPI; Green -cytokeratin; Red - MSI2. Scale bars: 100 μm **F.** Quantification of MSI1 expression in TMA of 19 normal and 120 NSCLC specimens was not significant, as determined by Mann-Whitney test. **G**. KM Plots (http://kmplot.com/) analysis of overall survival in non-small cell lung cancer expressing high versus low MSI2. **H.** Western confirmation of higher expression of MSI2 human NSCLC A549 and H358 cell lines versus H226 and H322. **I**. qRT-PCR indicates effective stable knockdown of MSI2 in 344SQ, 531LN2 murine and MSI2 in A549, H358 human cell lines by independent shRNA depleting MSI2 (- m1, -m2, -h1, -h2) or a control shRNA (SCR). **J.** Representative images of crystal violet-stained cells from Matrigel invasion assay for the 531LN2, A549, and H358 cell lines. **K.** Western blot of MSI2 expression following transfection of siRNAs targeting Msi2 in 344SQ murine and A549 and H358 human NSCLC cell lines, using 2 independent siRNAs. **L.** Quantification of Matrigel invasion assay for 344SQ and A549 cells, transfected by GL2 control or Msi2/MSI2 –targeting SiRNAs, based on three independent assays. **M., N.** Quantification of viability assay calculated by CellTiterBlue (CTB) (**M**) or direct count of DAPI-stained nuclei (**N**). P values reflect data on final day of experiment. All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001 relative to controls.

Figure S2. MSI2 depletion decreases response of murine 344SQ and human H358 cells to TGF-beta stimulation of ECM matrix sphere formation. Images (**A**) and quantitation **(B)** of control (SCR) and MSI2-depleted (-m1, -m2) 344SQ cells grown on ECM Matrix for 5 days in the presence of 0, 10, or 100 ng/ml TGFβ. All cells were imaged by confocal microscopy. Scale bar, 50 mm. All graphs: *, p≤0.05; **, p≤0.01; ***, p≤0.001 relative to indicated controls.

Figure S3. Primary and metastatic 344SQ Tumor Growth in Subcutaneous Xenografts. A. Gross tumor volume measurement of subcutaneous (S.C.) tumor growth of control (SCR) and MSI2-depleted (-m1, -m2) 344SQ xenografts. **B., C.** IHC assessment for (**B**) Ki-67 proliferation marker and (**C**) cleaved caspase apoptotic marker in 344SQ primary subcutaneous xenograft tumors using H-score, quantified by Vectra. **D.** Left, graph represents metastatic burden in lung of the mice with 344SQ subcutaneous xenografts analyzed in (**A**), expressed as ratio of metastatic area / total lung, quantified from three section levels per lung. Right, representative images of metastatic burden of SCID mice with 344SQ control (SCR) and MSI2 depleted (-m1/-m2) subcutaneous xenografts. Arrows indicate metastatic nodules in the lungs. **E, F**. Analysis of the same metastases shown in (D) for expression of Ki-67 (E) and cleaved caspase (F); H-score quantitation performed as in (**B, C**) Scale bars: shown on images respectively. All graphs: values shown are not significant between comparison groups, except ****, p≤0.0001 for **D**.

Figure S4. MSI2 overexpression, and MSI2 phenotype in migration control. A. Quantification of CellTiterBlue (CTB) proliferation assays of 393p/M2a and 393p/M2b MSI2-overexpressing clones versus 393p/cDNA control cell lines. **B**., **C**. Quantification (**B**) and representative image (**C**) of Matrigel invasion analysis of 393p/cDNA control and 393p/M2a and 393p/M2b MSI2 overexpressing cell lines. **D.** Gross pathological assessment of lung metastases of 393p xenografts (393p/cDNA, 393p/M2a and 393p/M2b). **E.** Quantification of cell migration for indicated cell lines with control (SCR) or MSI2-targeting shRNAs, based on a wound healing assay. **F.** Quantified migration for the SCR control and MSI2-depleted cells 531LN2 cell line in a transwell invasion assay. Statistical differences were calculated for each time point using Excel software, t-test. All graphs: *, p≤0.05; **, p≤0.01; ***, p≤0.001 relative to controls.

Figure S5. Reverse phase protein array (RPPA) analysis heatmap representing relative expression of 171 proteins or phosphoproteins. Comparison groups are two independent 344SQ derivatives with MSI2 knockdown (-m1 and –m2) and negative control. Three biological repeats were used for each cell line. 344SQ-SCR, 344SQ-m1 344SQ-m2 murine cells were lysed and prepared according to MD Anderson Core Facility instructions as previously described, and RPPA performed at the facility (48-50). Data were analyzed to establish significant differences in expression using the MultiExperiment Viewer (MeV, v4.9) program (www.tm4.org/mev/) (51). Scale bar represents ln values from fold change. Note, some hits scoring as positive in this assay were not validated in subsequent probe of MSI2 depletion in the 4 independent cell line models used in this study, including notably NOTCH-1.

Figure S6. Stable and transient depletion or overexpression of MSI2. A. qRT-PCR comparing expression of mRNA for FN1 and CLDN7 in 4 model NSCLC cell lines (MSI2-depleted -m1/-h1, -m2/-h2) and SCR, control scrambled shRNA. Graphs represent data from four independent runs. **B.** Western blot analysis quantification of CLDN7 protein expression in 4 model NSCLC cell lines (-m1,-m2,-h1,-h2 MSI2-depleted) and SCR, control scrambled shRNA. Graphs represent data from four independent runs. **C.** Western blot analysis of indicated proteins in 344SQ, A549 and H358 cell lines, transfected by GL2 control or MSI2-depleting (-m1, -m2,-h1, -h2) siRNAs. **D**. Western blot analysis of NUMB expression in murine and human cell lines. **E.**, **F**. qRT-PCR (**E**) and Western (**F**) quantification of expression of mRNA for CLDN5 and CLDN3 in 4 NSCLC cell lines expressing control shRNA (SCR) or MSI2-depleting shRNA (-m1,-m2,-h1,-h2). Graphs represent data from four independent experiments. **G.** qRT-PCR comparing expression of TGFβRI and SMAD3 in 4 model NSCLC cell lines expressing control shRNA (SCR) or MSI2-depleting shRNA (-m1,-m2,-h1,-h2). Graphs represent data from four independent experiments. **H.** Western blot of indicated proteins in 3 model cell lines, transfected by GL2 control or two independent MSI2-depleting siRNAs. **I.,** J. Western blot analysis of indicated proteins in the 393P cell line, overexpressing MSI2 (393p/M2a and 393p/M2b versus the 393p/cDNA control cell line). All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001 relative to controls.

Figure S7. MSI2 controls expression of CLDN7 at cell-cell junctions. A., **B**. Immuofluorescence analysis of CLDN7 co-stained with the tight junction marker ZO-1 in murine 344SQ (**A**) and 531LN2 (**B**) NSCLC cell lines, with or without MSI2 stable knockdowns. Green, CLDN7; Red, ZO-1; Blue, DAPI. Scale bars are 30μm.

Figure S8. Western blot analysis of EMT markers in MSI2 overexpressing or depleted cells. A., B. Western blot analysis (**A**) and quantification (**B**) of MSI2, ZEB-1, ZEB-2, FOXC2, SNAIL, SLUG, VMN (vimentin) versus β-actin loading control in 344SQ, A549 and H358 cell lines expressing MSI2 (SCR) or depleted of MSI2 (-m1, -m2, -h1, -h2). **C., D.** Western blot analysis (C) and quantification (D) of MSI2, E- Cadherin, vimentin, SLUG and SNAIL protein expression 393p/cDNA control and 393p/M2a and 393p/M2b MSI2 overexpressing cell lines. All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001 relative to controls.

Figure S9. Invasion by TGFβR1/SMAD3-overexpressing NSCLC 3NSCLC cell lines. A., B. Representative Western blot (**A**) and quantitation of TGFβR1 (**B**) in 344SQ (left) or A549 (right) cells expressing control (SCR) or MSI2-depleting shRNA (m1, h1), after introduction of TGFβR1 (TβR1). **C., D.** Quantified (**C**) and representative (**D**) data for invasion through Matrigel for the cell models shown in **A, B**. **D.** All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001.

Figure S10. Quantification of Western blots of control and MSI2-depleted mouse NSCLC cells, transiently depleted of TGFβRI or SMAD3. A., B. Quantified data for Western blots shown in Figure 3A, B. for 344SQ (**A**) and A549 (**B**) cells with (-m1 and -h1) or without (SCR) shRNA depletion of MSI2, and with depletion of TGFβR1 (-tβ1, -tβ2, -Tβ1 and -Tβ2), SMAD3 (-sm1, -sm2, -Sm1 and -Sm2) or neither (GL2) by siRNA depletion of TGFβR1. All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001 relative to controls based on t-test.

Figure S11. CLDN7 regulation of invasion. A., B. Representative Western blot analysis of indicated proteins (A) and quantification of CLDN7 expression (**B**) in pCMV6-control/SCR and pCLDN7/SCR stably transfected

A549 cells. **C**., **D**. Quantification (**C**) and representative images (**D**) of Matrigel invasion for pCMV-control/SCR and pCLDN7/SCR stably transfected A549 cells. **E., F.** Western blot analysis (**E**) and quantification of CLDN7 expression (**F**) in 344SQ cells expressing MSI2 (SCR) or stably MSI2-depleted (-m1) transiently transfected with GL2 control or CLDN7 siRNAs. **G.** Quantification of Matrigel invasion assay for cells in **E, F**., based on three experiments. All graphs: *, p≤0.05; *, p≤0.01; ***, p≤0.001 relative to controls.

Table S1. Primers used in RT-QPCR analysis. Expression of genes noted in left column were analyzed by Taqman or SYBR Green assays, using primers indicated in columns 2-4 as noted.

Table S2. Patient characteristics for the primary NSCLC tumors arrayed in tissue microarray (TMA).

Table S3. Patient characteristics for the analyzed grouped NSCLC samples (normal, primary tumor and lymph node metastasis).

Table S4. Sequences of siRNA mixtures and shRNA used in gene knockdowns. Data represent siRNA pools (two independent siRNAs/pool) used for depletion of the genes indicated, for function testing experiments.

Table S5. Antibodies used for RPPA analysis. A list of antibodies used with corresponding catalog numbers, validation status (2013), provided by MD Anderson Cancer Center RPPA Facility.

2. Supplemental Methods.

Quantitative RT–PCR analysis. Total RNA was isolated using a Qiagen AllPrep DNA/RNA Mini Kit (#80204) and tested for quality on a Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA concentrations were determined with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Ambion-Thermo Fisher Scientific, Waltham, MA) and a mixture of anchored oligo-dT and random decamers (Integrated DNA Technologies, Coralville, IA). Two reverse-transcription reactions were performed for each sample using either 100 or 25 ng of input RNA. Aliquots of the cDNA were used to measure the expression levels of the genes using the primers listed in Supplmentary Table S1. The assays were from Applied Biosystems (Thermo Fisher Scientific Waltham, MA) or designed with Primer Express as indicated in Table S1. They were used in combination with Taqman Universal Master mix or Power SYBR Green master mix (Applied Biosystems, Thermo Fisher Scientific Waltham, MA) and run on a 7900 HT sequence detection system (Applied Biosystems, Thermo Fisher Scientific Waltham, MA). Cycling conditions were 95°C, 15 min, followed by 40 (two-step) cycles (95°C, 15 s; 60°C, 60 s). Ct (cycle threshold) values were converted to quantities (in arbitrary units) using a standard curve (four points, four fold dilutions) established with a calibrator sample. Ppib and POLR2F were used as normalizers. For each sample, the values were averaged and standard deviation of data derived from two independent PCR experiments.

siRNA Targeting Sequences: Small interfering RNAs (siRNAs) were obtained from GE Dharmacon (Lafayette, CO). See Supplementary Table S4 for sequences used.

shRNA Targeting Sequences and lentivirus production: Short hairpin RNAs (shRNAs) were obtained from SIGMA-ALDRICH (St Louis, MO). See Supplementary Table S4 for sequences used. To prepare lentivirus for introduction of shRNAs into NSCLC cells, HEK-293T cells were transfected with shRNA lentivirus prepared in the pLKO.1 system (Addgene, Cambridge, MA), with the psPAX2 and pMD2.G packaging plasmids. Media containing lentiviral particles was collected on day 4. Subsequently, lung cancer cells were infected with lentivirus and selected by growth in RPMI 1640 with 10% FBS and puromycin, using standard methods.

***cDNA ORF Sequences*.** cDNA ORF inserts were obtained from OriGene (Rockville, MD). See Supplementary Table S4 for sequences used.

***TCGA Analysis*.** The Cancer Genome Atlas (TCGA) results shown in this study are based upon provisional data generated by the TCGA Research Network (http://cancergenome.nih.gov/). Expression data for the 59 tumor-normal paired samples for the lung adenocarcinoma study were downloaded from https://tcga-data.nci.nih.gov/. Z-score corresponds to the mRNA expression of the tumor sample minus the mean expression in the reference sample divided by the standard deviation of expression in the reference sample.

Kaplan-Meier analysis for overall survival (OS). Was performed using http://kmplot.com online software with JetSet option for the optimal MSI2 probe, auto select for the best cutoff and censore at threshold settings applied (5).

***Mouse models for tumor growth*.** All experiments involving mice were performed according to protocols approved by Institutional Animal Care and Use Committees (IACUCs) at M.D. Anderson Cancer Center or the Fox Chase Cancer Center. Orthotopic xenograft experiments were performed in syngeneic wild type 129Sv mice of at least 6 weeks of age. Intrathoracic injections of 10^6 cells into the left lung in single-cell suspension were placed in a volume of 100 µl of complete media as previously described (1). Animals were monitored regularly and euthanized on day 28. Necropsies were performed to quantify the number of metastases to mediastinal lymph nodes, chest wall and distant extrathoracic sites.

For sub-cutaneous xenografts, immunocompromised eight week-old C.B17 SCID mice were inoculated

subcutaneously with shRNA-transfected derivatives of 344SQ syngeneic lung cancer cells into the left and right flank subcutaneously using a 27G needle, 100 ul volume. Mice were palpated twice a week after tumor cells implantation to assess tumor onset. Tumor volume was determined by external caliper twice a week (body weight also was monitored twice weekly), the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined. Tumor volumes based on caliper measurements were calculated by the modified ellipsoidal formula: *Tumor volume = 1/2(length × width^2).* After three weeks mice were euthanized and tumors and lungs were collected.

***Tissue preparation, Histology, Quantitative Analysis*.** Lungs and tumors were collected. Tissues were collected and fixed in 10% phosphate-buffered formaldehyde (formalin) 24-48 hrs, dehydrated and embedded in paraffin. Tissues were processed by dehydration in a series through ethanol followed by xylene (70% ethanol, 3 hr; 95% ethanol, 2 hr; 100% ethanol, 2 hr; ethanol-xylene, 1hr; xylene, 3hr) then immersed in paraffin. Paraffin blocks were cut into 5 µm sections, mounted on microscope slides, and stored at room temperature until used. Prepared specimens were analyzed by hematoxylin and eosin (H&E) staining (Sigma-Aldrich, St. Louis, MO). Tumor sections were immunostained with antibodies to Ki-67 (DAKO, Carpinteria, CA) to allow quantitation of proliferation, and with antibodies to cleaved caspase (Cell Signaling, #9661) to allow quantitation of apoptosis. Immunohistochemistry and H&E were performed by standard protocols. Immunostained slides were scanned using an Aperio ScanScope CS scanner (Aperio, Vista, CA) and Vectra Automated Quantitative Pathology Imaging System (Perkin Elmer, Waltham, MA). Scanned images then were viewed with Aperio's image viewer software (ImageScope). Selected regions of interest were outlined manually by a pathologist (KQ Cai). Expression levels of the proliferative index marker Ki-67 or the cleaved caspase indicator of apoptosis were quantified using Vectra Automated Quantitative Pathology Imaging System specific protocols and algorithms. H-score was calculated as follows: the percentage of cells at each staining intensity level was calculated, and an H-score was assigned and calculated for each slide using the following formula: [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)](2, 3). H-scores were subsequently used for results analysis. The area of lung metastases was assessed using the Vectra automated multispectral slide analysis system, using specific protocols and algorithms designed for the identification of tumor tissue.

Tissue Microarrays (TMAs) Non-small cell lung cancer surgical specimens resected from 1997 to 2012 from the Fox Chase Cancer Center (FCCC) Biosample Repository Facility were used to construct tissue microarrays (TMA). Tissue from each tumor was placed in two unique spots on each TMA. All samples were obtained from primary tumors and/or nodal metastases at the time of initial resection. Clinical information (Supplementary Table S3) was available from the repository database and abstracted from clinical databases in an anonymized fashion. At the time of tissue acquisition, patients provided Institutional Review Board (IRB)–approved informed consent for storing tissue and reviewing de-identified clinical data. For TMAs, automated image capture was performed by the HistoRx PM-2000 (HistoRx) (New Haven, CT), using the AQUAsition software. High-resolution monochromatic digital images of the cytokeratin staining visualized with AF555, DAPI, and target staining with Cy5 were captured and saved for each tumor histospot. Tumor mask was created from the cytokeratin image of each histospot, representing areas of the epithelial tumor. Histospots were excluded if the tumor mask represented less than 5% of the total histospot area. DAPI immunoreactivity defined the nuclear compartment. Images were visually inspected and cropped for unfavorable factors such as “out of focus,” debris, or damaged specimen before automatic analysis. An AQUA score was generated by dividing the sum of target signals within the tumor mask. AQUA scores were normalized to the exposure times and bit depth at which the images were captured, allowing scores collected at different exposure times to be compared directly. The nuclear scores from two non-overlapping images were averaged for each case.

***Paired Analysis of NSCLC specimens*.** Non-small cell lung cancer surgical specimens from the University of New Mexico Human Tissue Repository Facility (HTR) were used to conduct IHC. All samples were obtained from normal lung, primary tumors and/or nodal or distant metastasis at the time of initial resection. Clinical information (Supplementary Table S3) was available from the repository database and

abstracted from clinical databases in an anonymized fashion. At the time of tissue acquisition, patients provided Institutional Review Board (IRB)–approved informed consent for storing tissue and reviewing de-identified clinical data. IHC slides stained for MSI2 were analyzed using APERIO Spectrum scanner (Leica Biosystems, Buffalo Grove, USA).

***SDS-PAGE and Western Blots*.** For Western blotting, cells were disrupted in CelLytic M lysis buffer (Sigma-Aldrich, St. Louis, MO) supplemented with protease and phosphatase inhibitor cocktails (Roche, Basel, Switzerland). Whole cell lysates were used directly for SDS–PAGE and Western blotting, using standard procedures. Primary antibodies included rabbit anti-MSI2 (Abcam #ab76148), anti-MSI1 (Abcam #ab52865), anti-NUMB (Abcam #ab14140), anti–FN1 (Abcam #ab6328), anti-TGFβR1 (Cell Signaling #3712), anti-SMAD3 (Cell Signaling #9523) and anti-SMAD3p (Cell Signaling #9520), Claudin-7 (Novus #67525), Claudin-3 (Abcam #ab15102), Claudin-5 (Abcam, #ab15106), FN1 (Abcam #ab6328), anti-E-Cadherin (Cell Signaling #3195), anti-β-Actin (Abcam #ab49900), anti-ZEB1(Cell Signaling #3396), anti-ZEB2 (Santa Cruz #271984), anti-FOXC2 (Abcam #65141), anti-SNAIL(Abcam #180714), anti-SLUG(Abcam #51772), anti-VMN (Cell Signaling #5741)*.* Secondary anti-mouse and anti-rabbit horseradish peroxidase–conjugated antibodies (GE Healthcare, Little Chalfont, UK) were used at a dilution of 1:10,000 for visualization of Western blots and blots developed by chemiluminescence using the West-Pico system (Pierce, Waltham, MA). Image analysis was done using ImageJ (National Institutes of Health, Bethesda, MD), with signal intensity normalized to β-actin or total level of detected proteins. Data was analyzed in Excel by paired t-test to determine statistical significance.

***Immunofluorescence*.** Cells were fixed with 4% paraformaldehyde (10 min) and then cold methanol (5 min), permeabilized with 1% Triton X-100 in PBS and blocked with 3–5% BSA in PBS. Samples were then incubated with the primary antibodies overnight at 4°C. Primary antibodies were: anti-Claudin-7 (Novus #67525), anti-Claudin-3 (Abcam #ab15102) and anti E-Cadherin (BD Transductions 610182). Following rinse in PBS + 0.01% Tween, samples were incubated for 1 hour with an Alexafluor 488, 568 or 647 tagged donkey anti-rabbit, mouse, or goat secondary antibody (Life Technologies, Eugene, OR), and counterstained with a 2 μmol/L 4′, 6-diamidino-2-phenylindole (DAPI) (Life Technologies, #1652731, Eugene, OR) solution. Samples were imaged using a Nikon C1 Spectral confocal microscope (Nikon, Melville, NY) equipped with a numerical aperture (NA) 1.40, oil immersion, 63x Plan Apo objective (Nikon). Images were acquired at room temperature using EZ-C1 3.8 (Nikon) software and analyzed with MetaMorph (Molecular Devices, Union City, CA).

***Analysis of cell migration*.** Time-lapse multifield experiments were performed in phase contrast on an automated inverted Nikon Eclipse TE300 microscope equipped with thermal and CO_2 regulation (Nikon, Melville, NY). 2 x10^6 cells of each clone were plated on a 6 well plate a day prior to imaging. The next morning cells were "scratched" with a p200 pipet tip. Immediately after, the cells were imaged at 15 minute intervals with a 100× objective and a CCD camera (EZ CoolSnap, Roper) and then "stacked" into movies using Metamorph (Universal Imaging) software. The videos were analyzed using the ImageJ public domain software (http://imagej.nih.gov/ij/docs/guide/146.html). Visual fields were divided into different coordinates and the distance between the invasion fronts of the cells was measured at each of the assigned longitudes at 0, 1, 3, 6, 9 and 12 hour time points. The distance was then converted from pixels into millimeters using a premeasured scale bar (1mm= 406,001 pixels) and normalized to 0 hour being 1 for each video. Statistical differences were calculated for each time point using Excel software, t-test.

As a second approach, the upper chambers of migration chambers (3465-024-K Trevigen, Gaithersburg, MD) were seeded with serum-starved cells (1 × 10^5 cells per well) in triplicate wells. Medium (RPMI 1640/DMEM), with 10% FBS, was placed in the lower and upper chambers, respectively. Mitomycin C at a concentration of 10 μg/ml was added to the upper chamber to inhibit cell proliferation. Cells were incubated at 37°C for 24 hours. After incubation, the cells remaining on the upper surface of the membrane were removed with cotton swabs. The cells on the lower surface of the membrane were fixed and stained with crystal violet and visualized under a Nikon Eclipse TE 2000-U microscope at 20X magnification and CRI Nuance Multispectral Imaging System. Four visual fields were photographed and counted per chamber, and

the results were analyzed with Image J and Excel expressed as the mean relative cell count number per visual field ± SEM with two-tailed t-test showing statistical significance.

Cell proliferation assays. Cells (1-2 × 10^3 cells/well) were plated in quadruplicate in RPMI1640 media with 10% FBS in 96-well cell culture plates for 5 days. On days 1-5, *CellTiter-Blue*® (Promega, Fitchburg, WI) or WST-1 Sigma-Aldrich (St. Louis, MO) reagent (for the 393P cell line) were added to each well; after 2 hours incubation at 37°C, optical density readings were made in the 570 – 600 and 420-480 nm wave-length range, responsively, using *Perkin*-Elmer ProXpress Visible-UV-fluorescence 16 bit scanner (Perkin-Elmer, Waltham, MA). As a second approach to measuring proliferation 1-2 x 10^3 cells for each model of interest were plated in 96-well plates, and wells fixed and stained with DAPI at 24 hour intervals from days 1-5 after plating, then scored by automated microscope (ImageXpress Micro, Downingtown, PA).

Cell invasion assay. The upper chambers of growth factor–reduced Matrigel invasion chambers (354483; BD Biosciences, Franklin Lakes, NJ or 3483-024-01, Trevigen, Gaithersburg, MD) were seeded with cells (1 × 10^5 cells per well) in triplicate wells. Medium (RPMI 1640), with or without 10% FBS, was placed in the lower and upper chambers, respectively. Mitomycin C at a concentration of 10 µg/ml was added to the upper chamber to inhibit cell proliferation. Cells were incubated at 37°C for 24 hours. Cells that had invaded through Matrigel were visualized with crystal violet. Three microscopic fields (original magnification, 10X) were photographed and counted per chamber, and the results were expressed as the mean ± SEM of invaded cells from replicate wells and multiple independent experiments. Data was analyzed in Excel by paired t-test to determine statistical significance.

Spheroidal growth assay: A 3D culture sphere assay was run using the Cultrex 3D Spheroid BME Cell Invasion Assay kit (Trevigen, catalog # 3500-096-K) by the standart protocol. 344SQ and A549 were plated 1000 per well in the Spheroid Formation Extracellular Matrix with or without TGF-β at 10 or 100 ng/ml concentration and grown for 3 days. Then the invasion matrix was added on top. On day 5 pictures were taken with the NIKON Eclipse TE 2000U microscope and CRI Nuance Multispectral Imaging System FX and then analyzed with Image J to measure the spheres' size.

Overexpression studies. For overexpression of MSI2, a full-length cDNA encoding MSI2 (for sequence information, please refer to NM_138962.2) or empty vector was obtained from the human ORFeome collection and transferred to the following viral vectors via Gateway recombination and virus production following manufacturer's recommendations: pLenti63/V5 DEST (Thermo Fisher Scientific, Waltham, MA)(4). All overexpression studies were performed using newly transduced stable cell lines generated by single clone selection. In brief, MSI2 cDNA cloned into pLenti63/V5 DEST plasmid or was empty pLenti63/V5 DEST plasmid were transfected into 393p cells. Subsequently, lung cancer cells were selected by growth in RPMI 1640 with 10% FBS and selected in blasticidin. Individual clones were picked and validated for MSI2 overexpression by Western blot. For overexpression of claudin 7 (CLDN7), a lentiviral plasmid containing a full length ORF insert encoding human CLDN7, transcript variant 1(RC200530L1) was acquired from OriGene (Rockville, MD) and expressed in A549 NCLC cells using pLKO.1 system from Addgene (Cambridge, MA). Subsequently, lung cancer cells were selected by growth in RPMI 1640 with 10% FBS with puromycin and A'lenti CLDN7 cell line was validated for CLDN7 overexpression by Western blot. For overexpression of TGFβR1, the previously generated MSI2 shRNA depleted human and mouse NSCLC A559 and 344SQ cells were transfected with lentivirally expressed ORF TGFβR1, transcript variant 1 (CW301688, a modification of RC219514L1) from Origene, using the Addgene pLKO.1 system and selected in DMEM and RPMI 1640 with 10% FBS and blasticidin. Effectiveness of targeted gene expression in 3'-m1/TβR1 and A-h1/TβR1 cell lines was confirmed by Western blot analysis.

3. Supplementary References.

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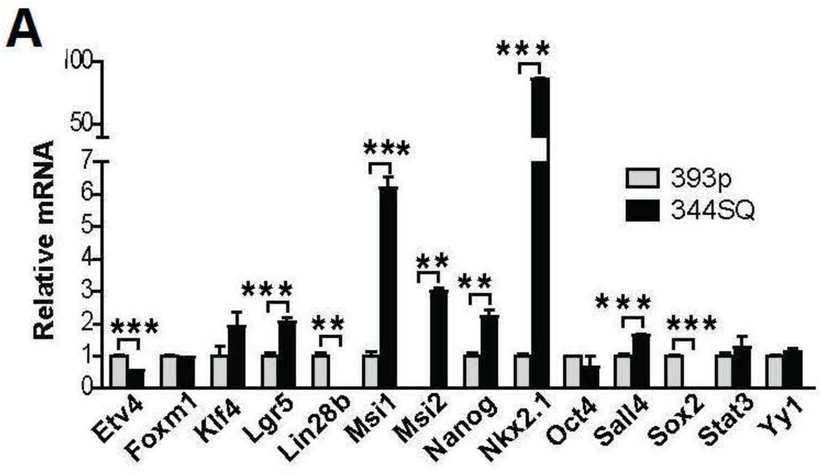
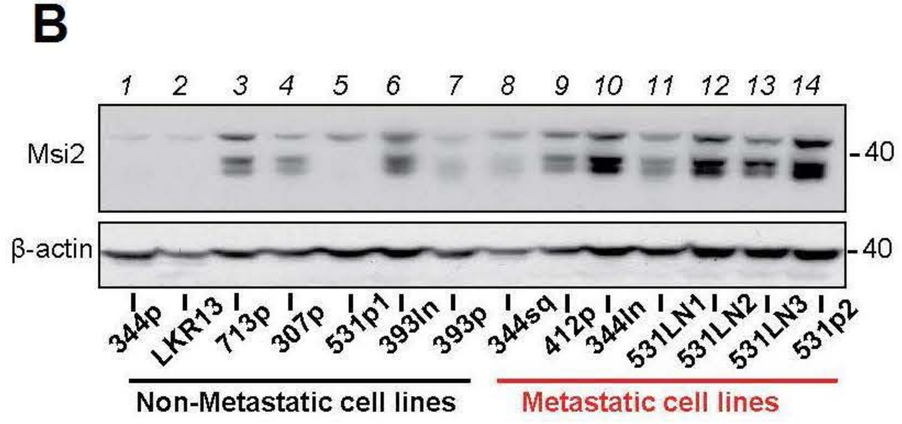
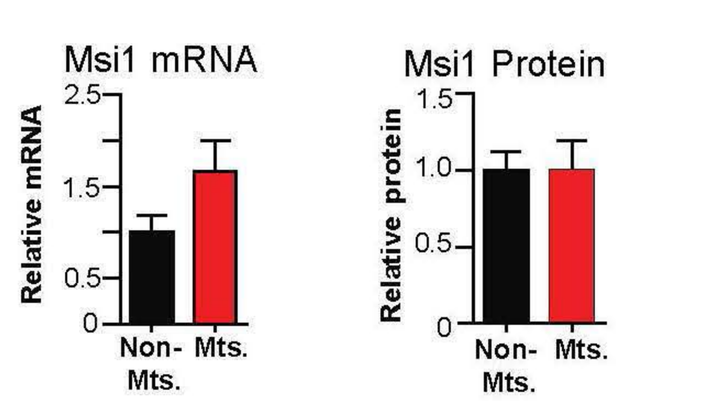
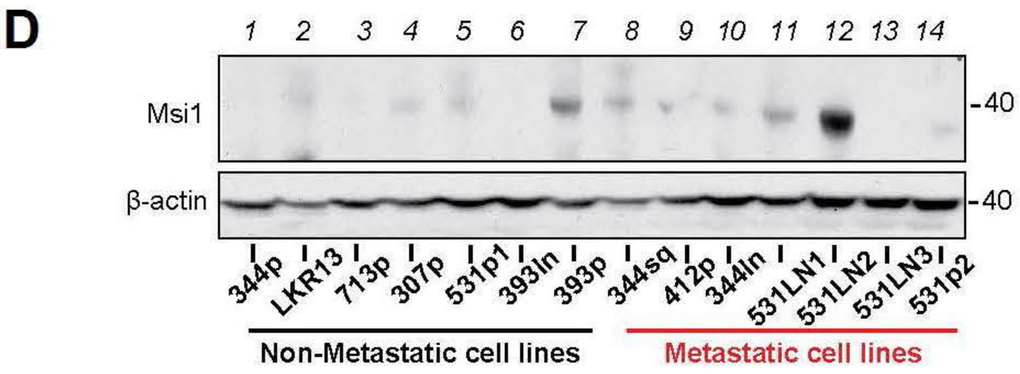
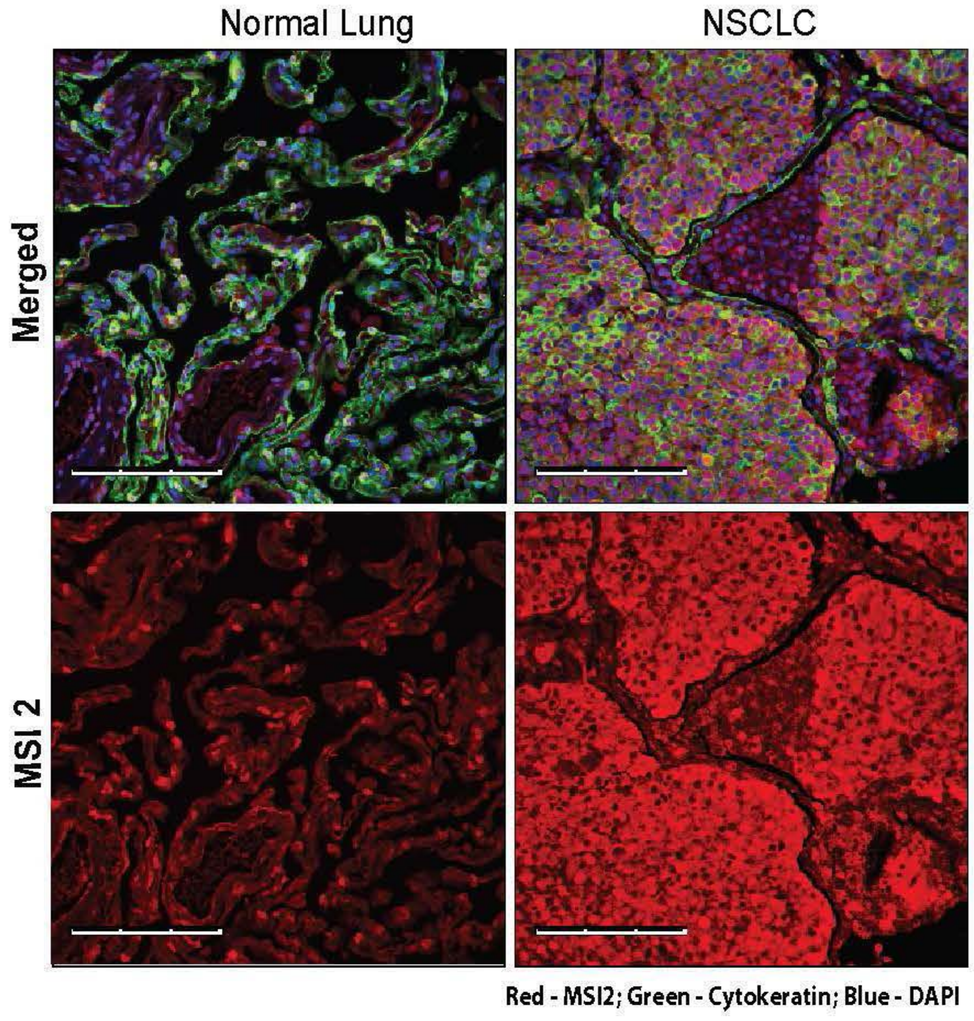
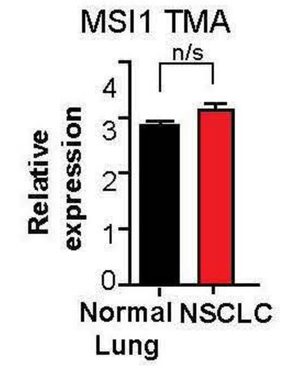


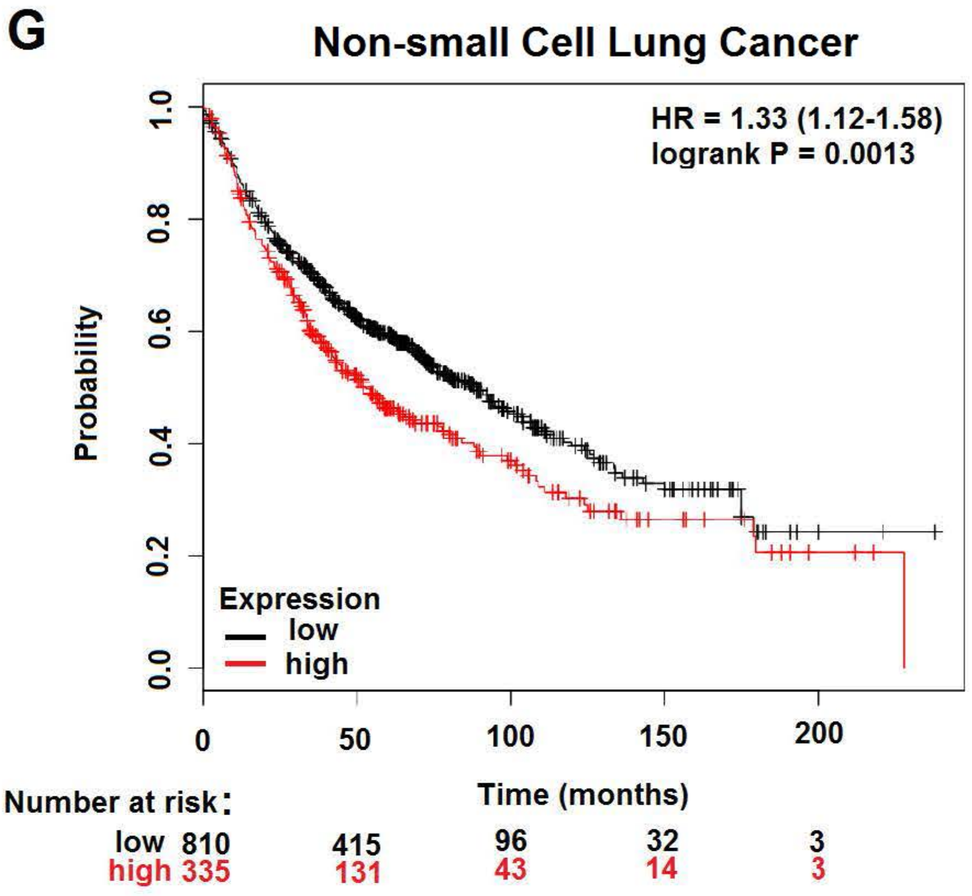


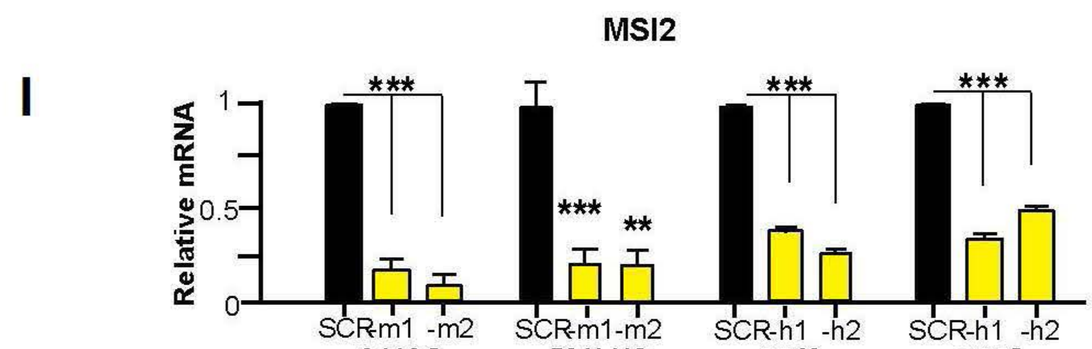


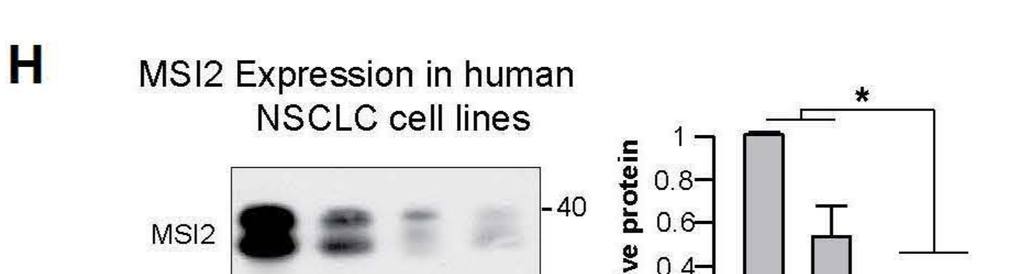


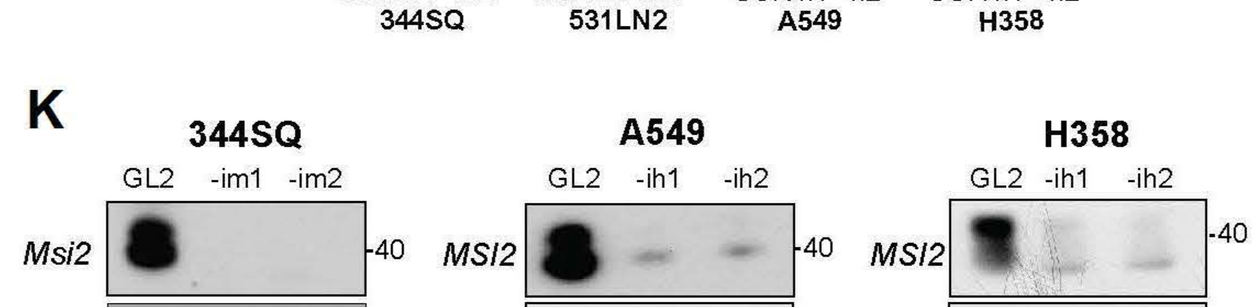


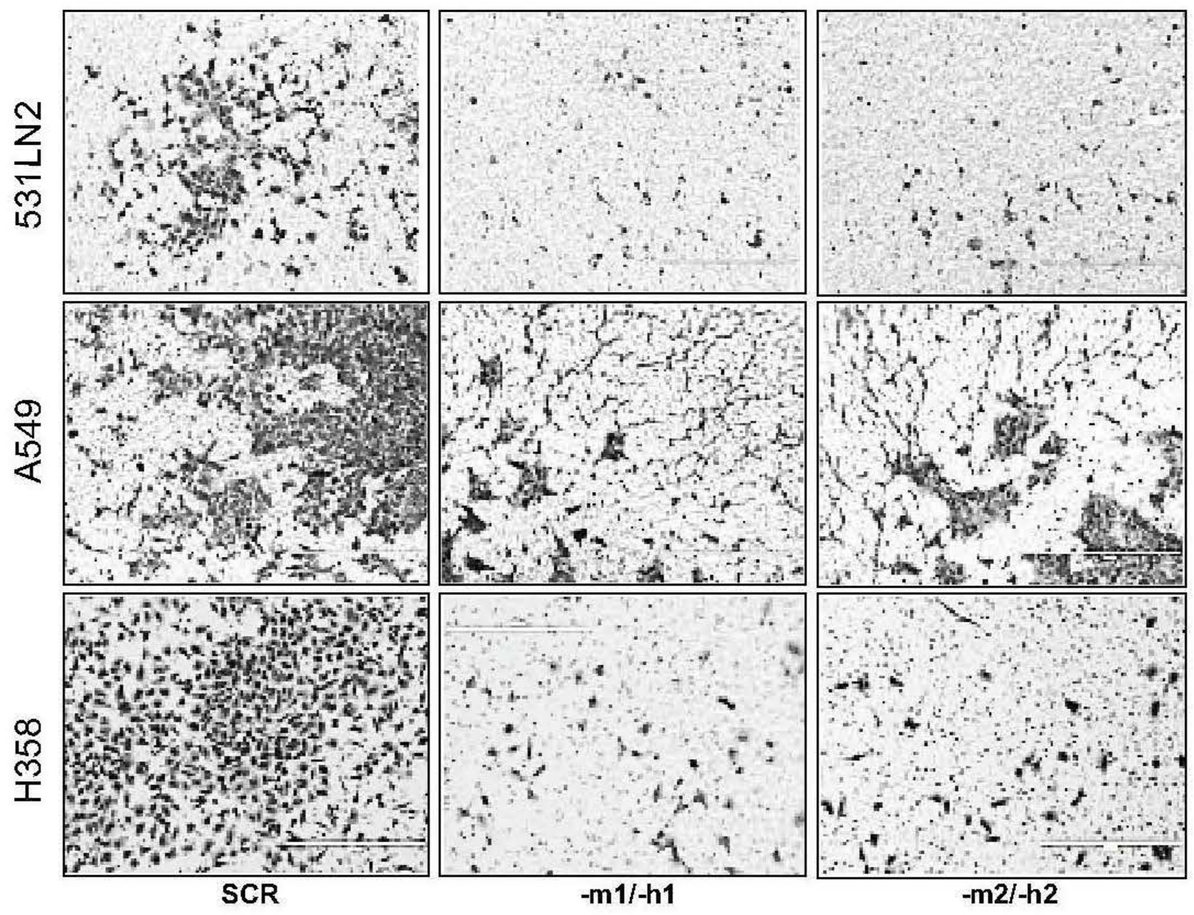


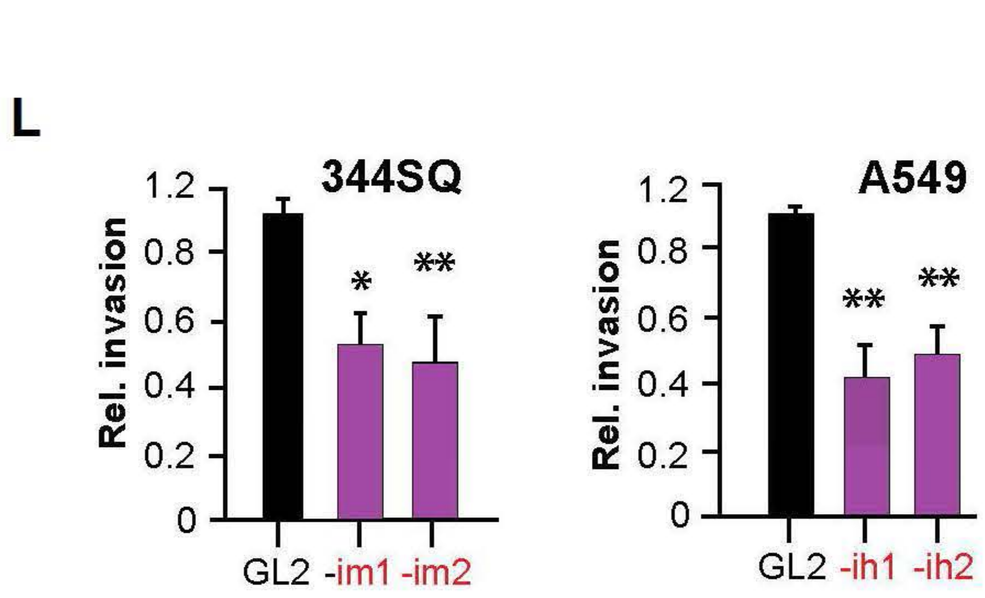


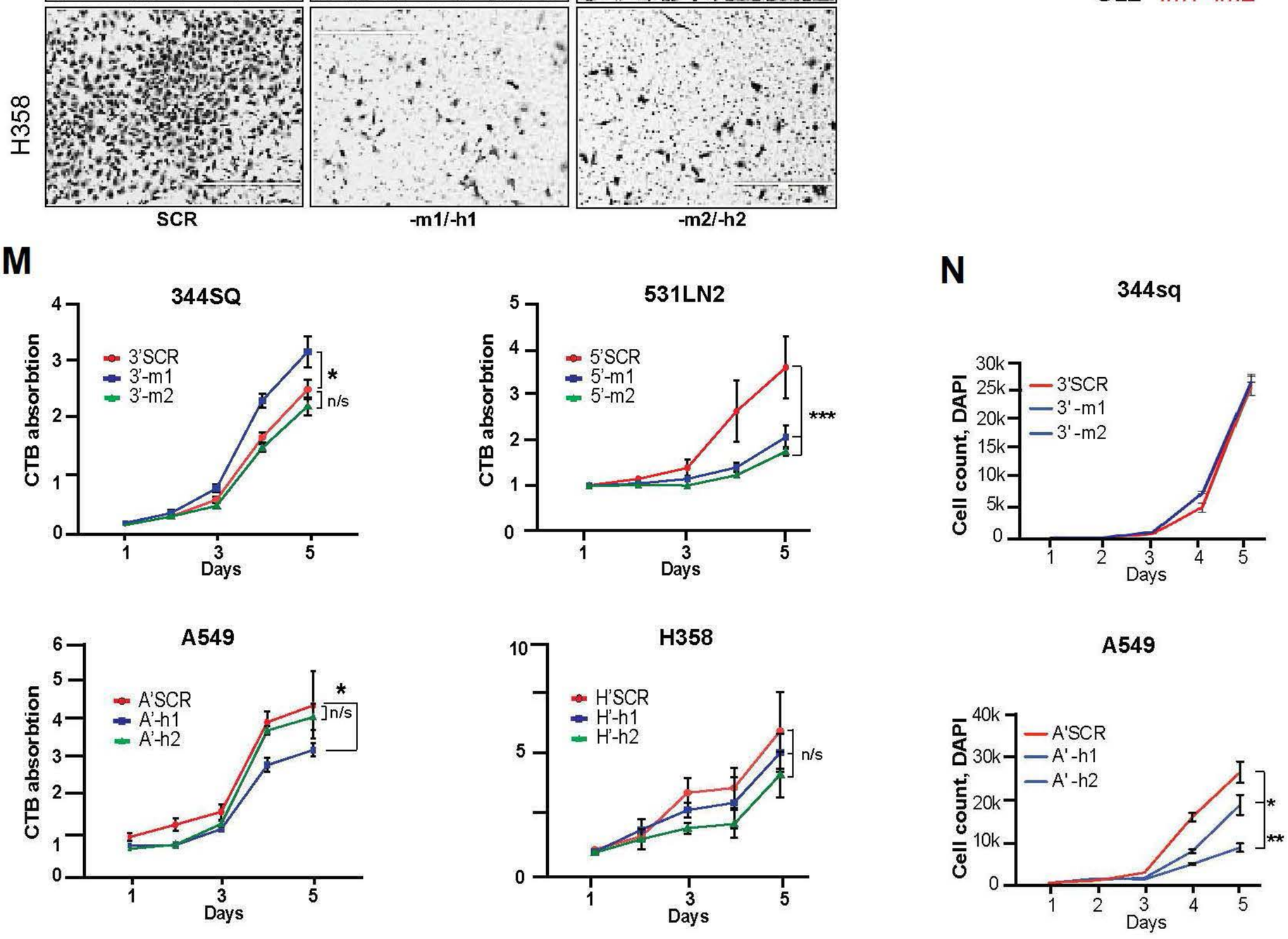


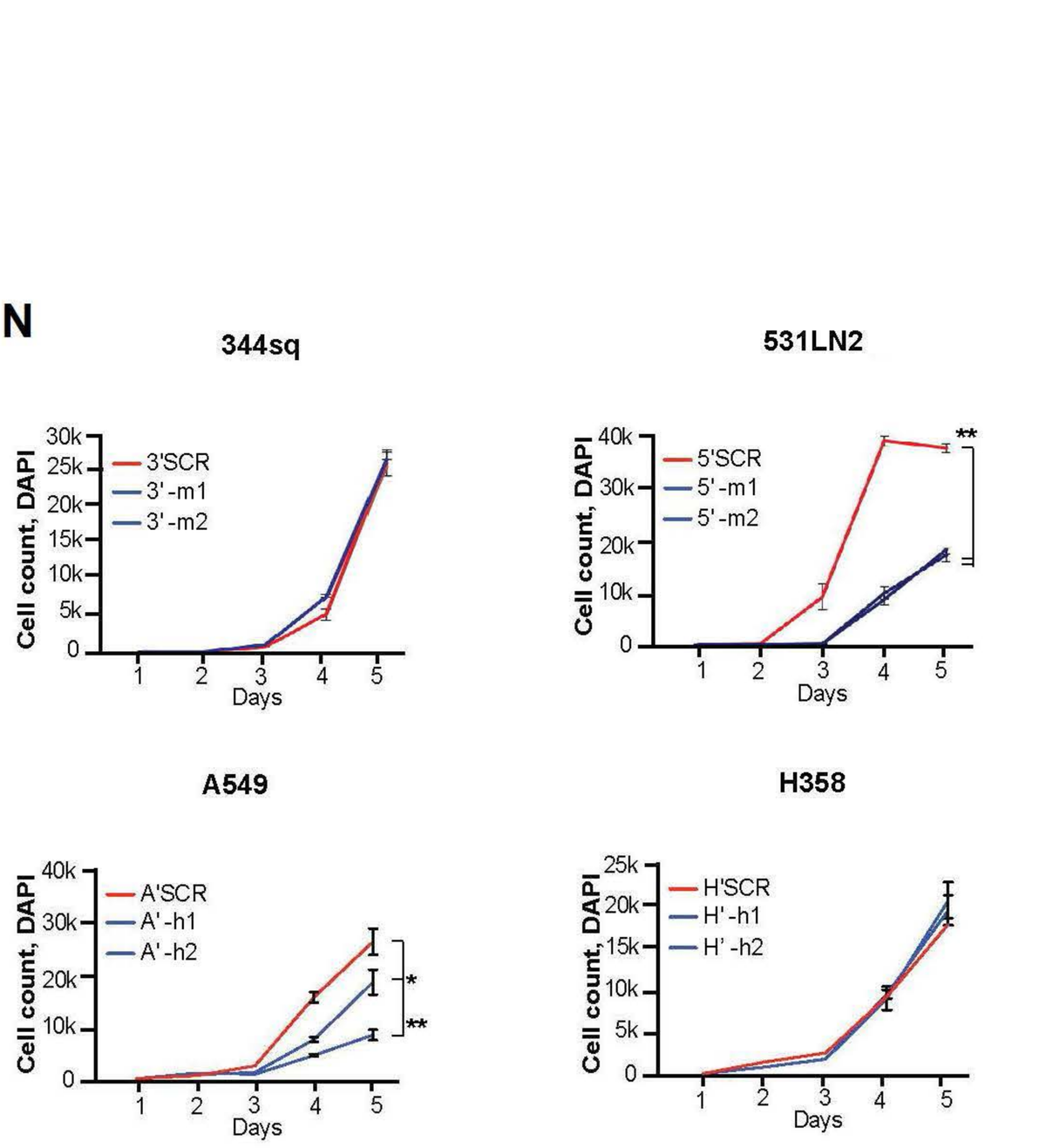












Kudinov et al, Figure S1.

A

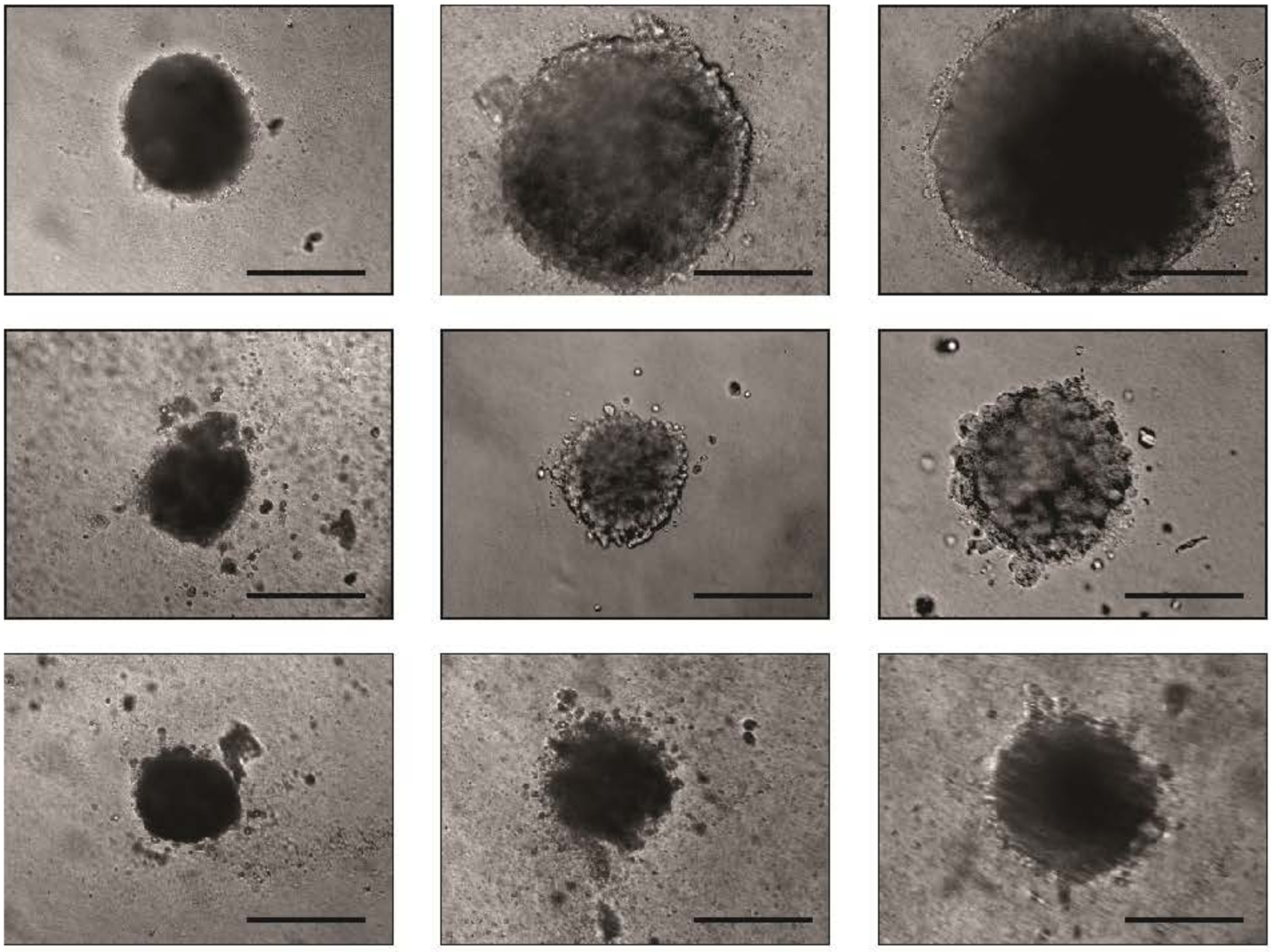
344SQ Cell Line

TGFβ 0 10 ng/mL 100 ng/mL

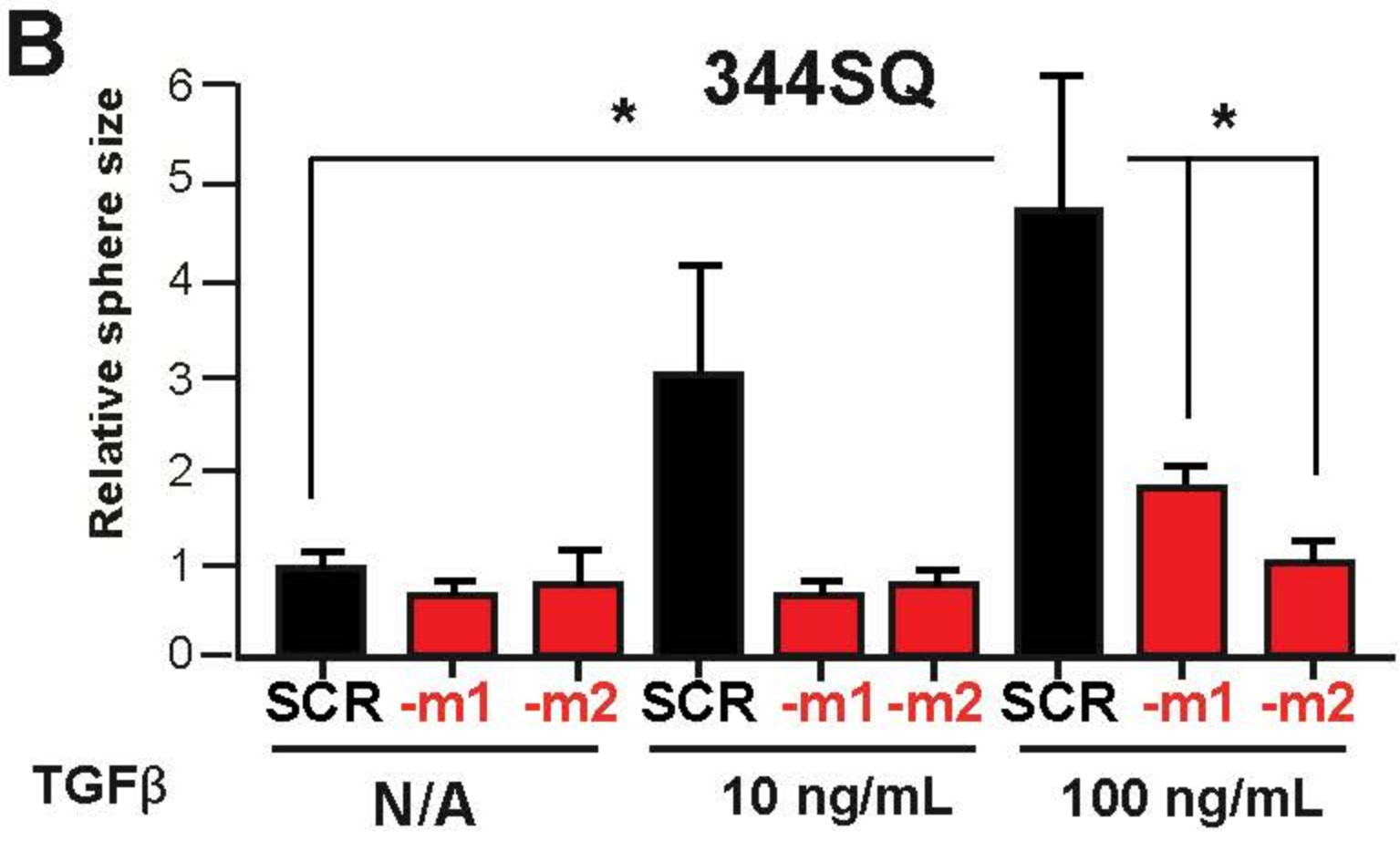
344SQ'SCR

344SQ'-m1

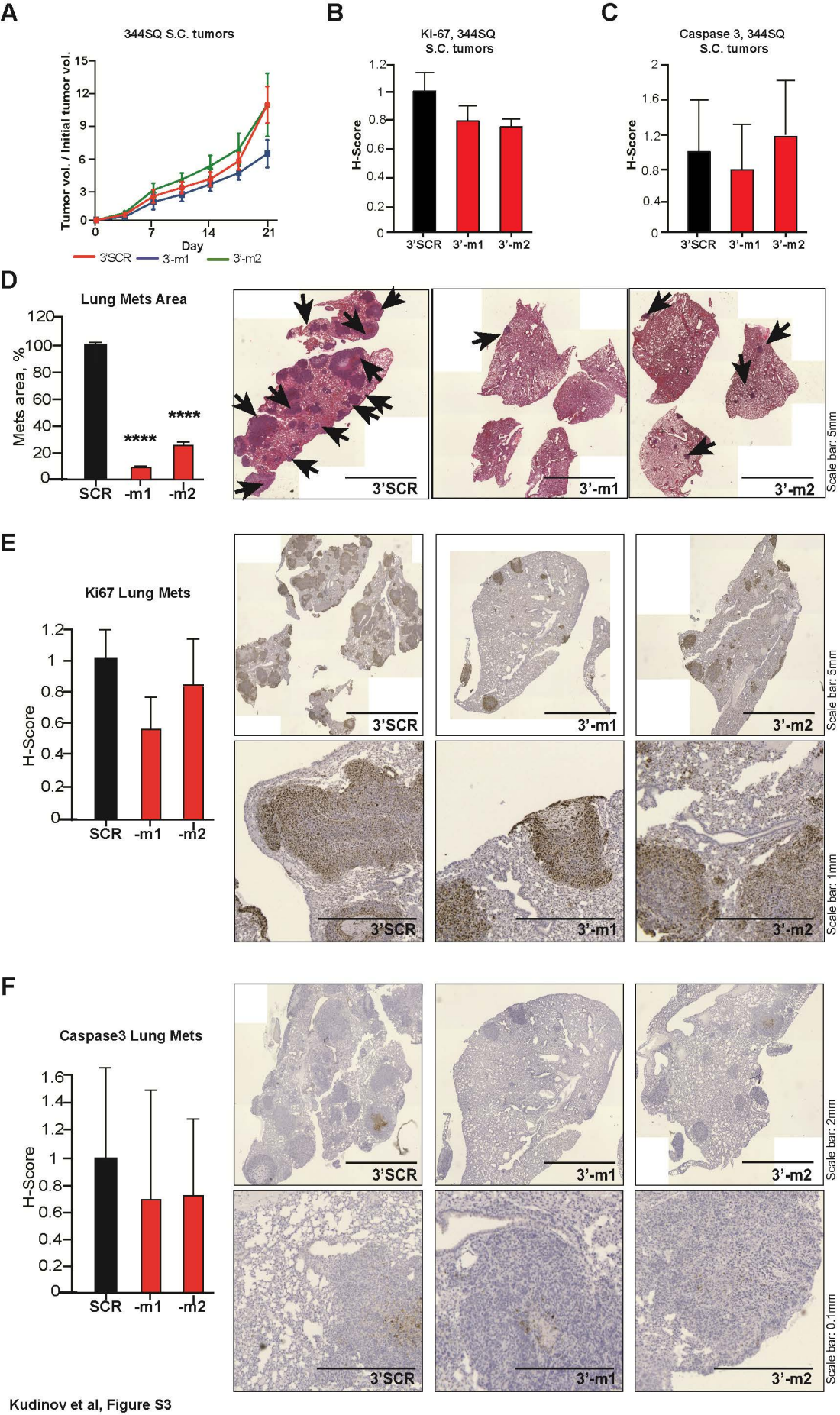
344Q'-m2



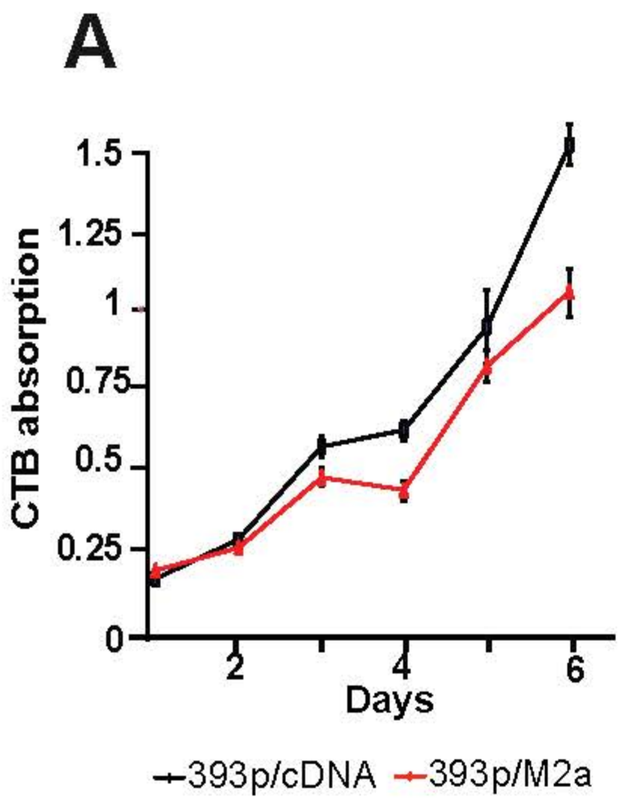
B

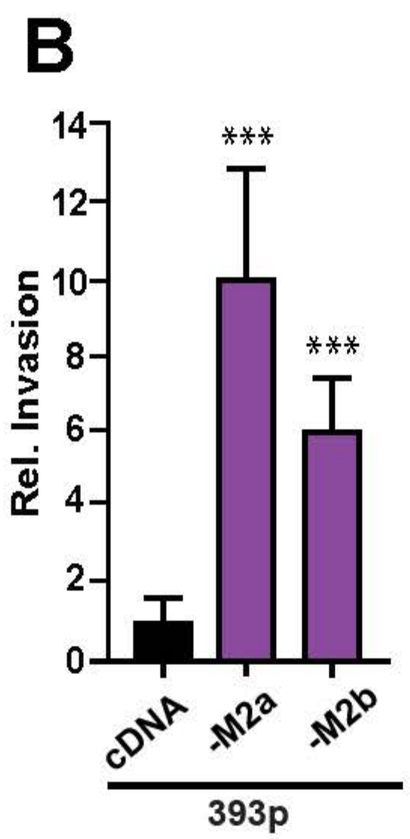


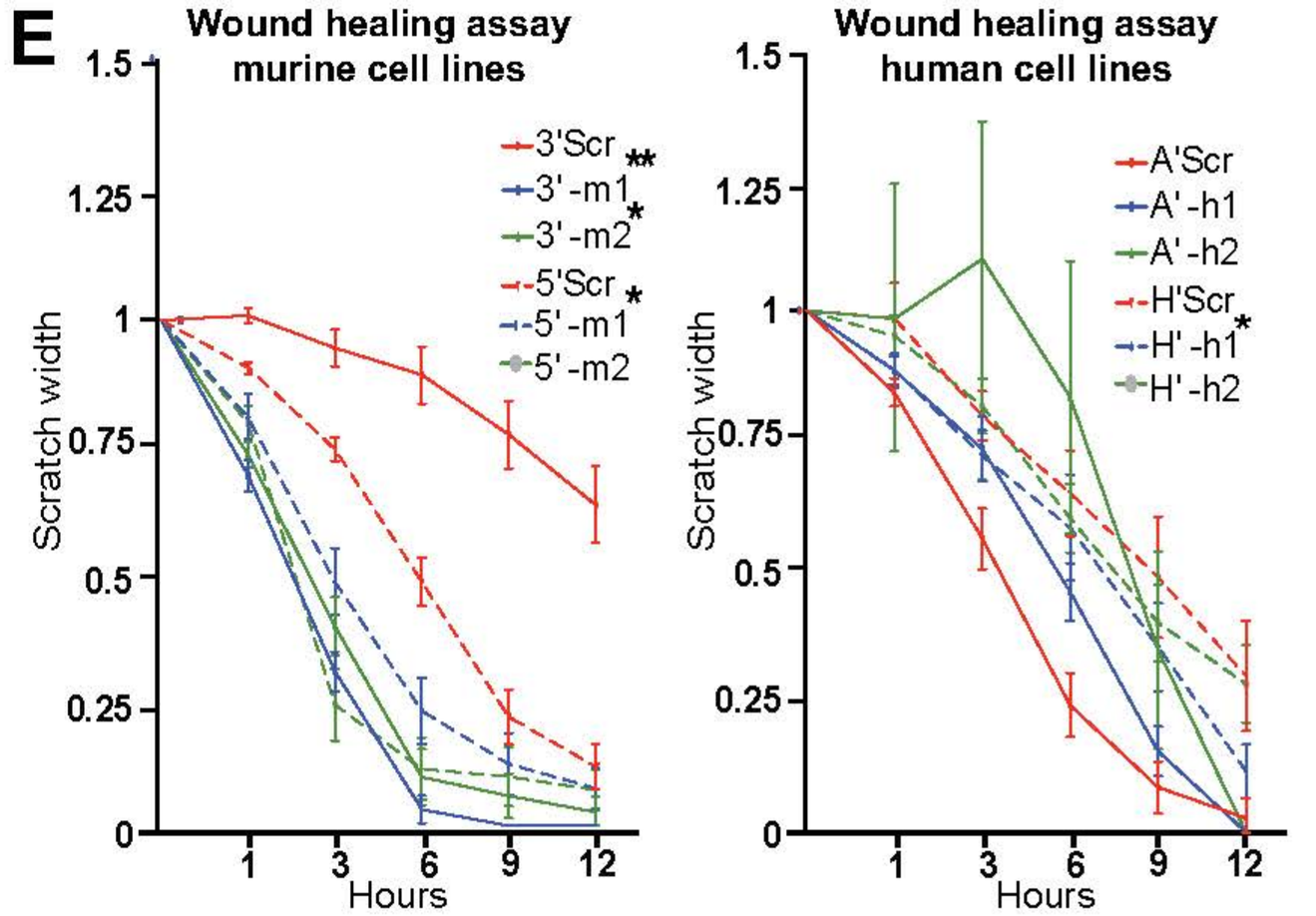
Kudinov et al, Figure S2

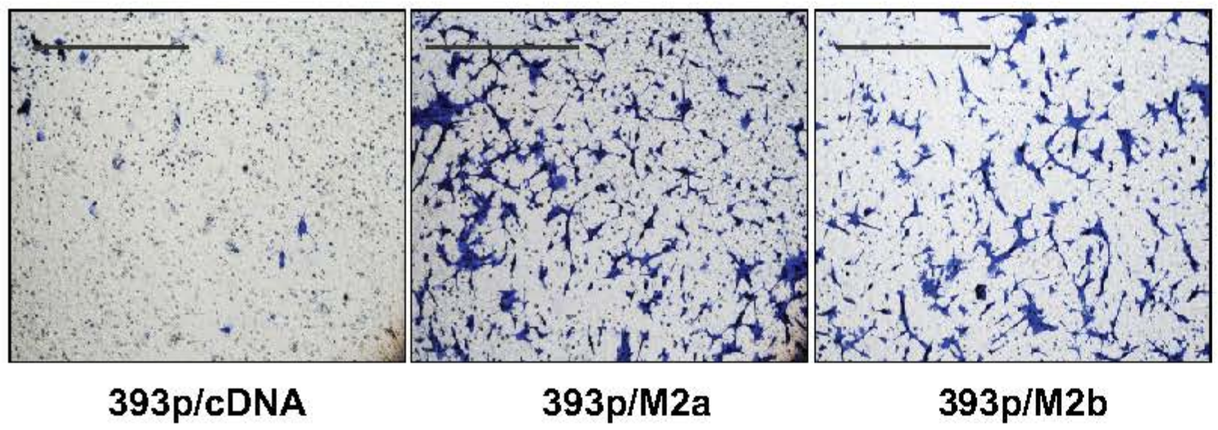


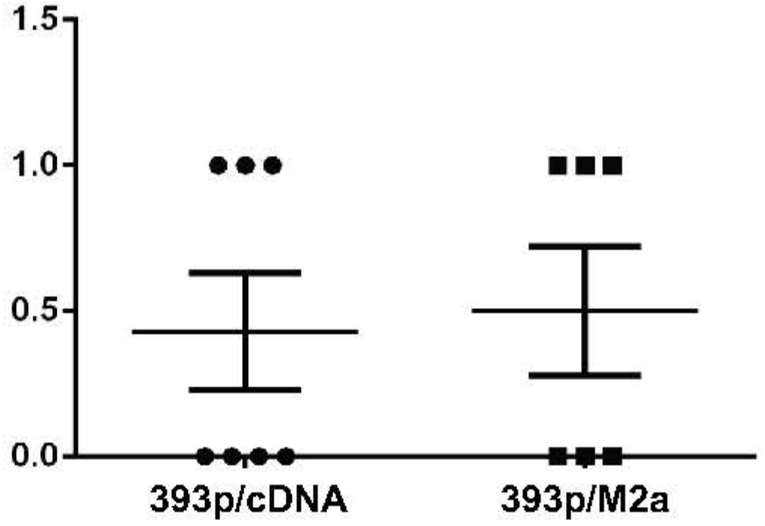
Kudinov et al, Figure S3

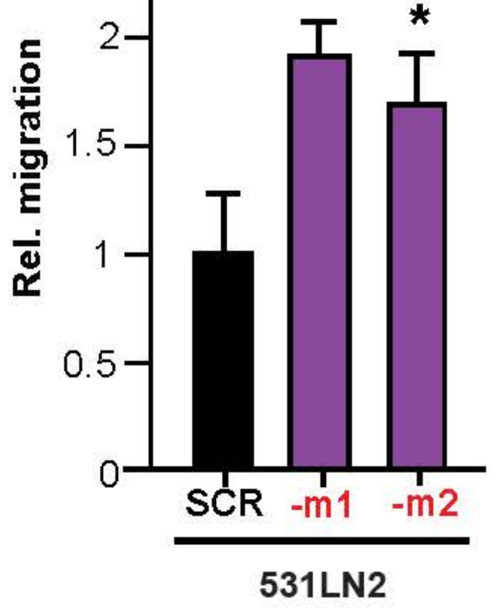




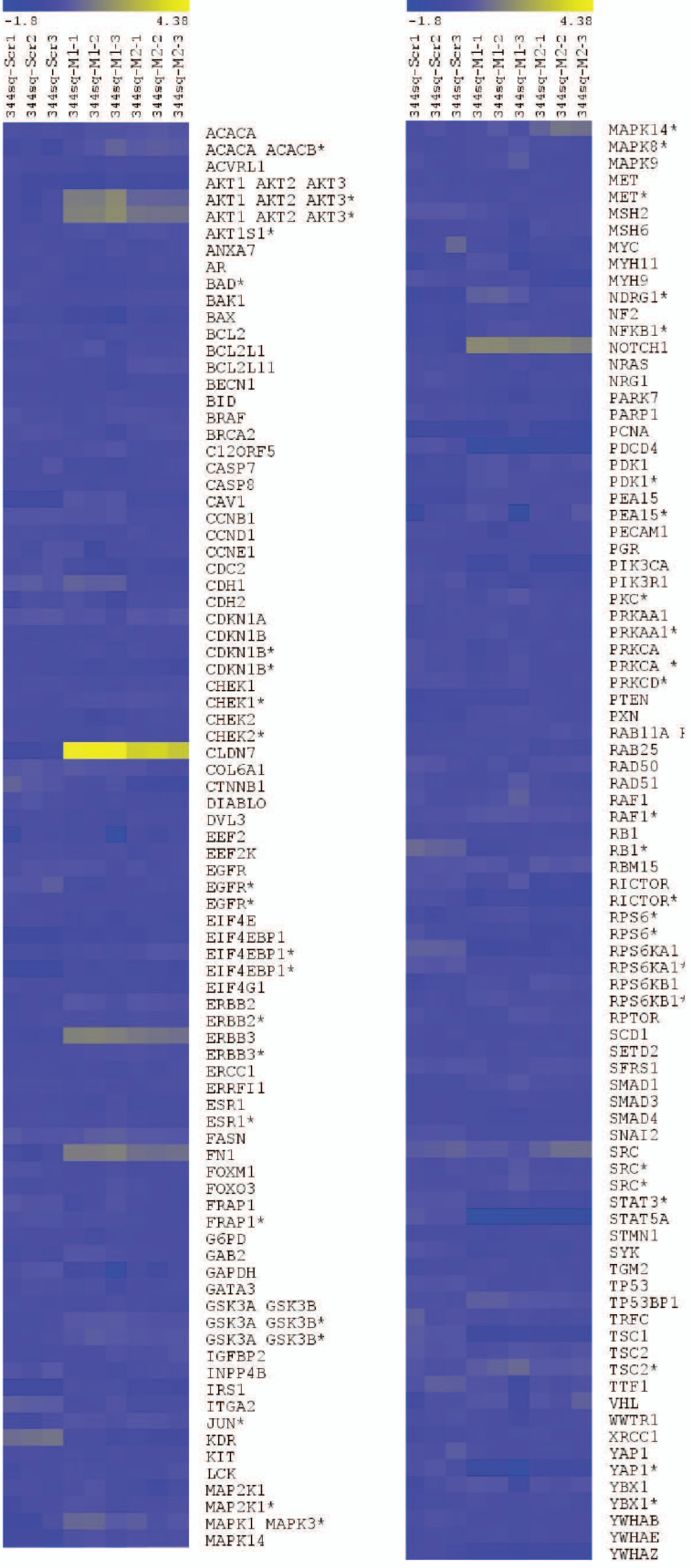




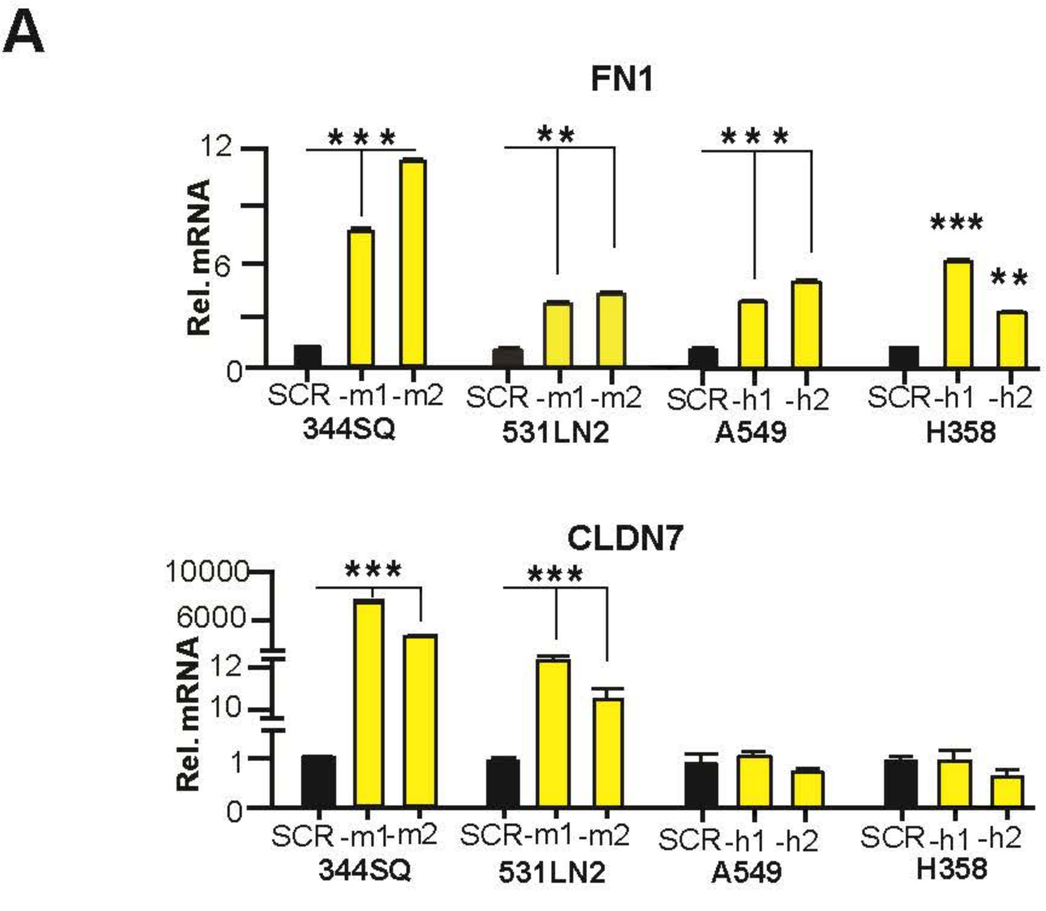


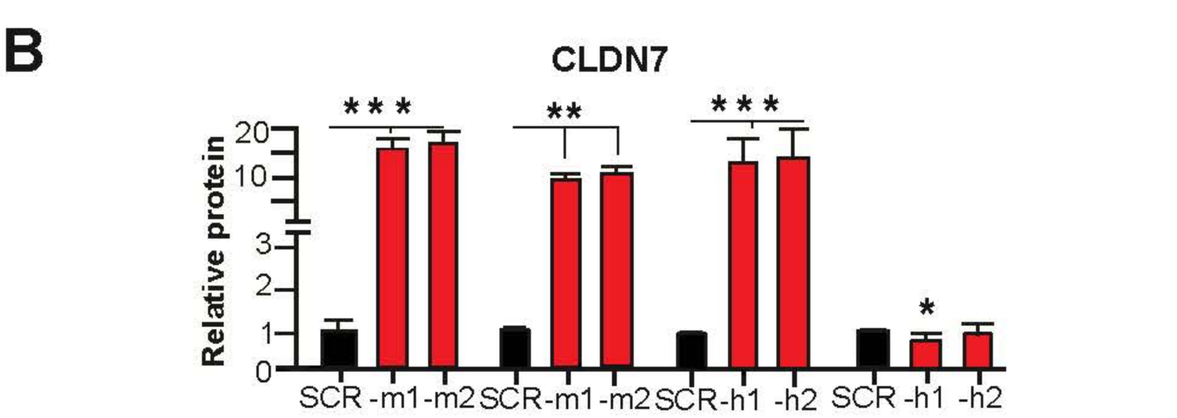


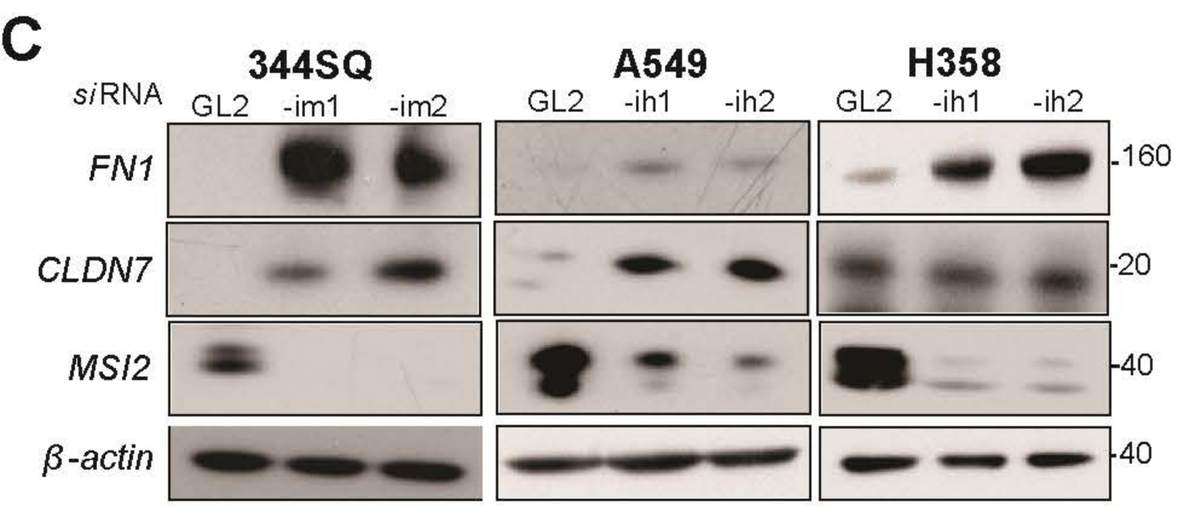
Kudinov et al, Figure S4.

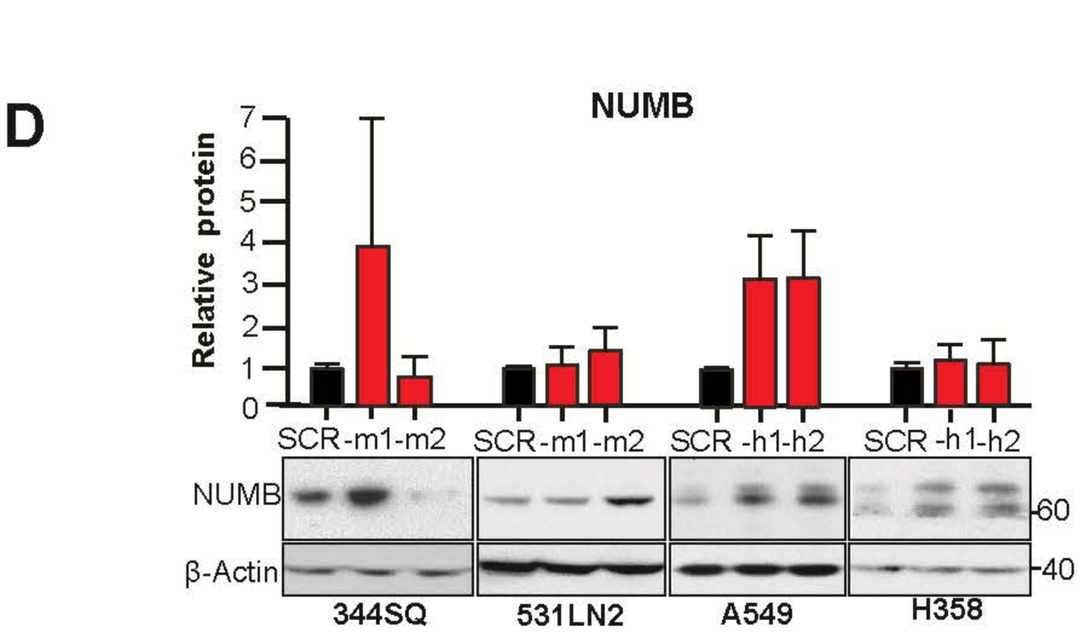


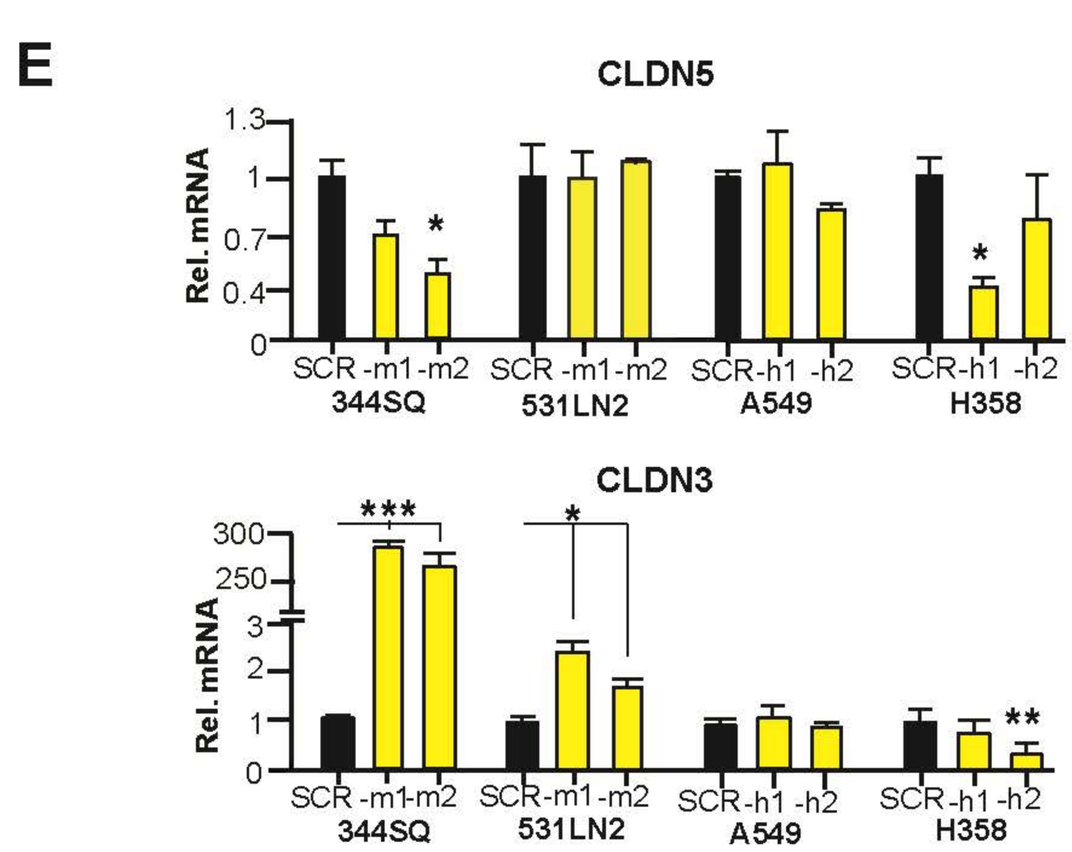
Kudinov et al, Figure S5.

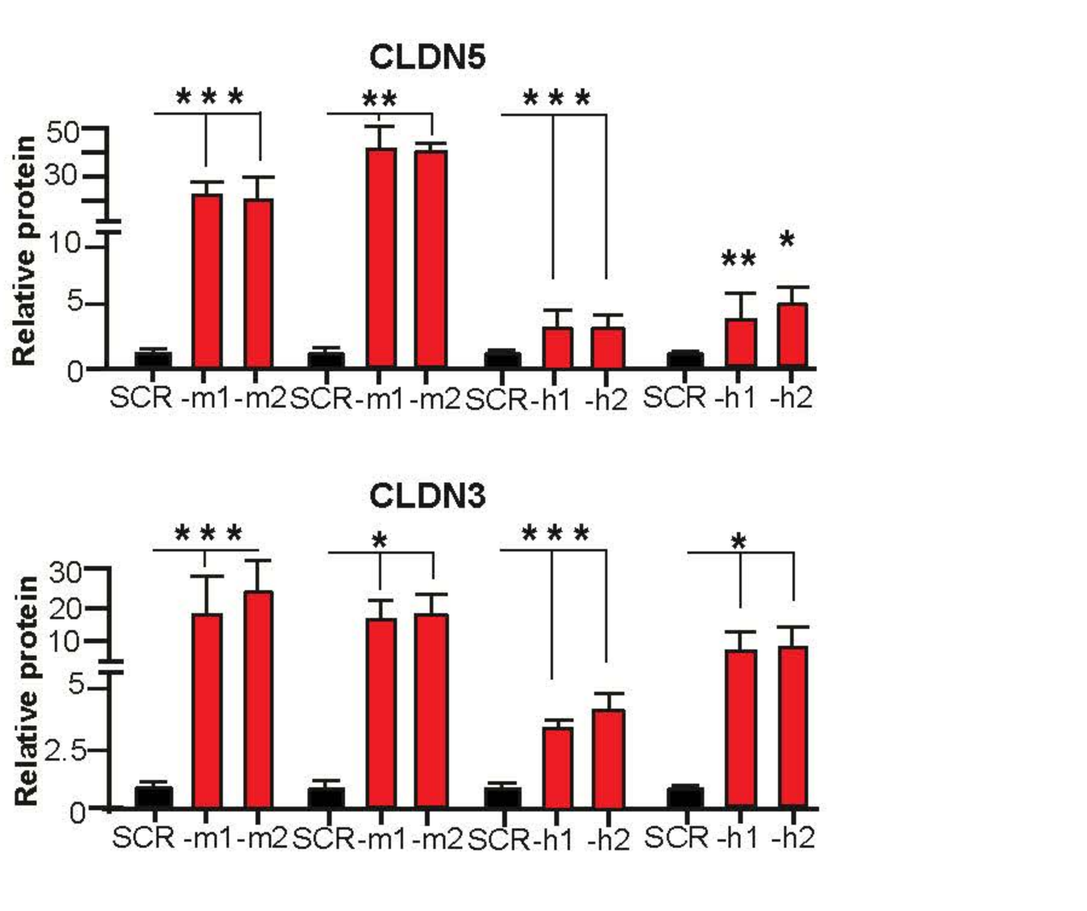


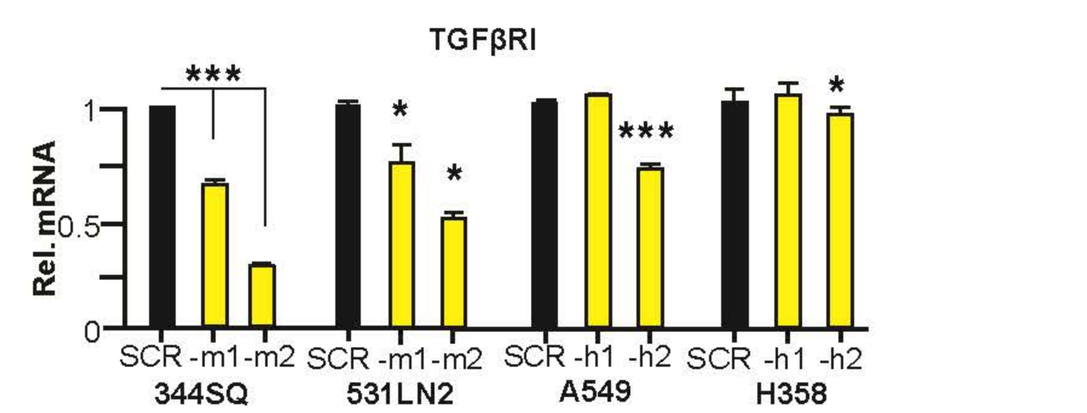


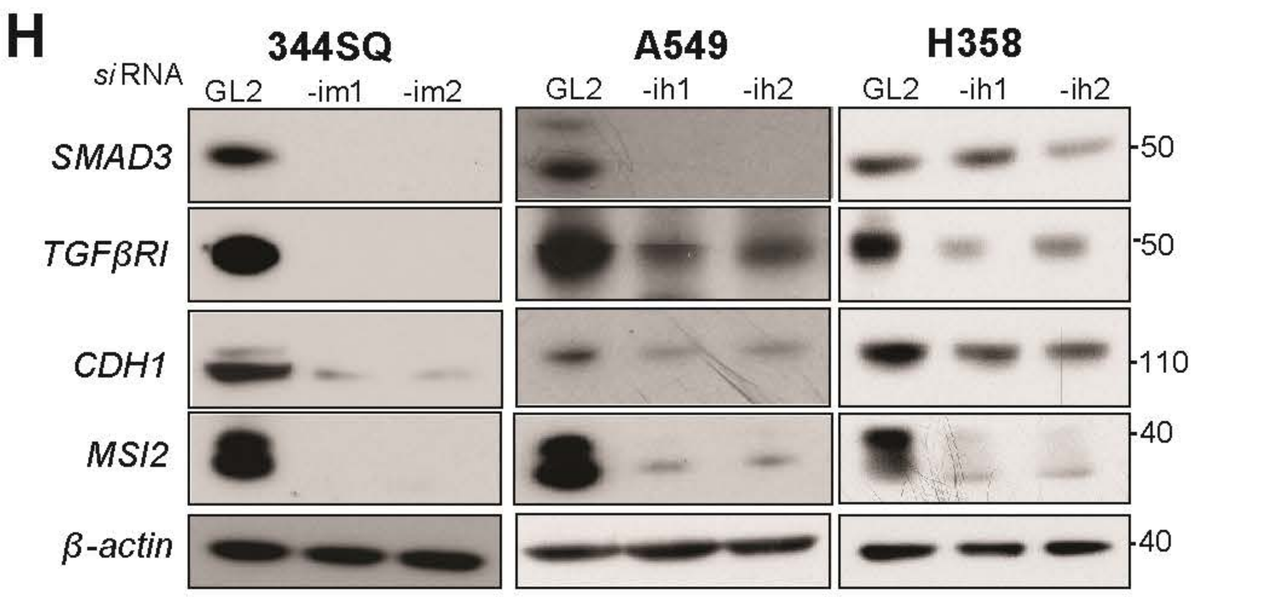


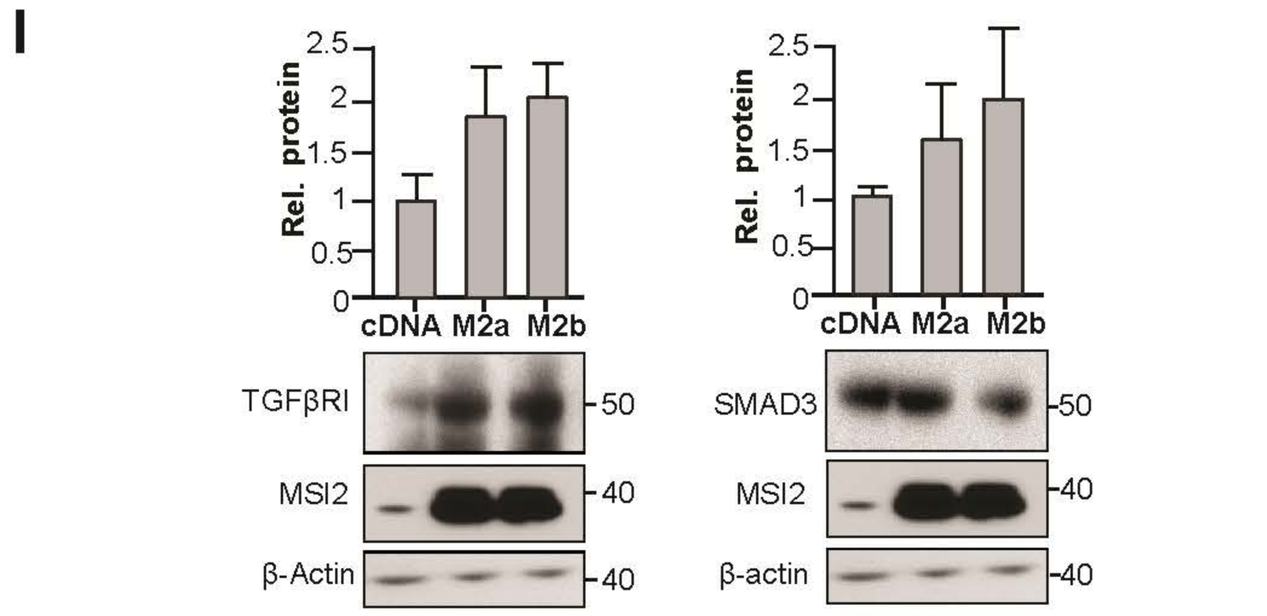


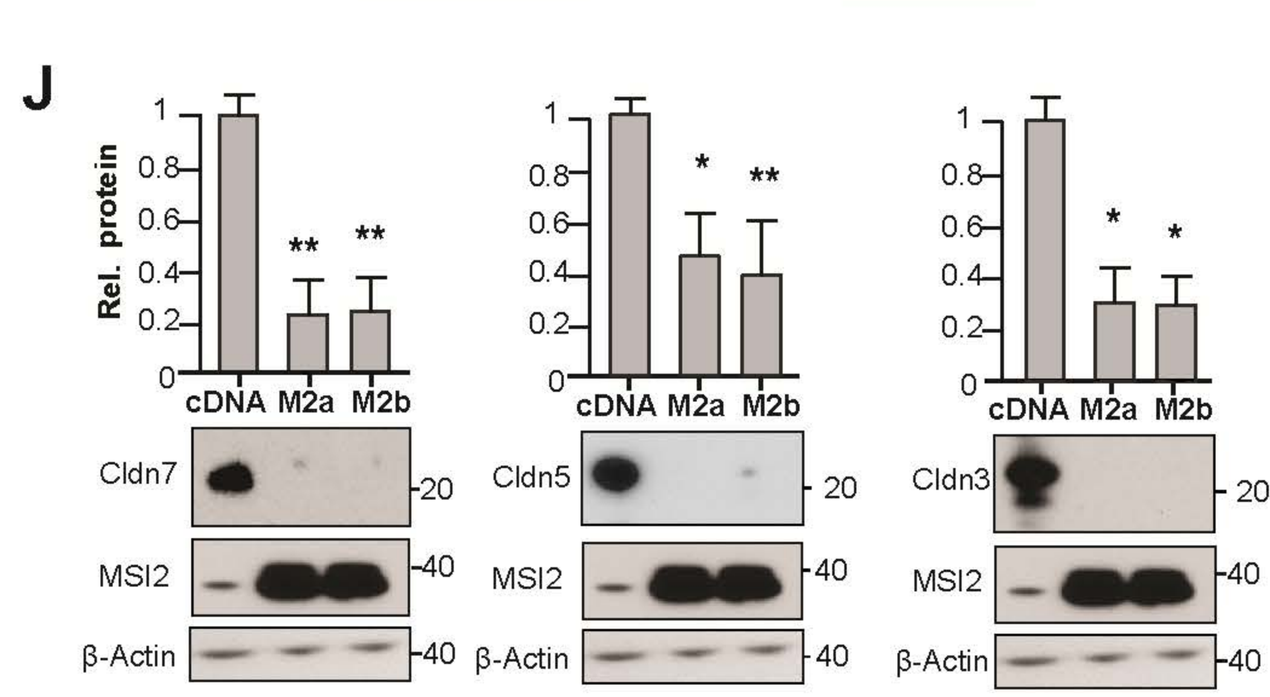




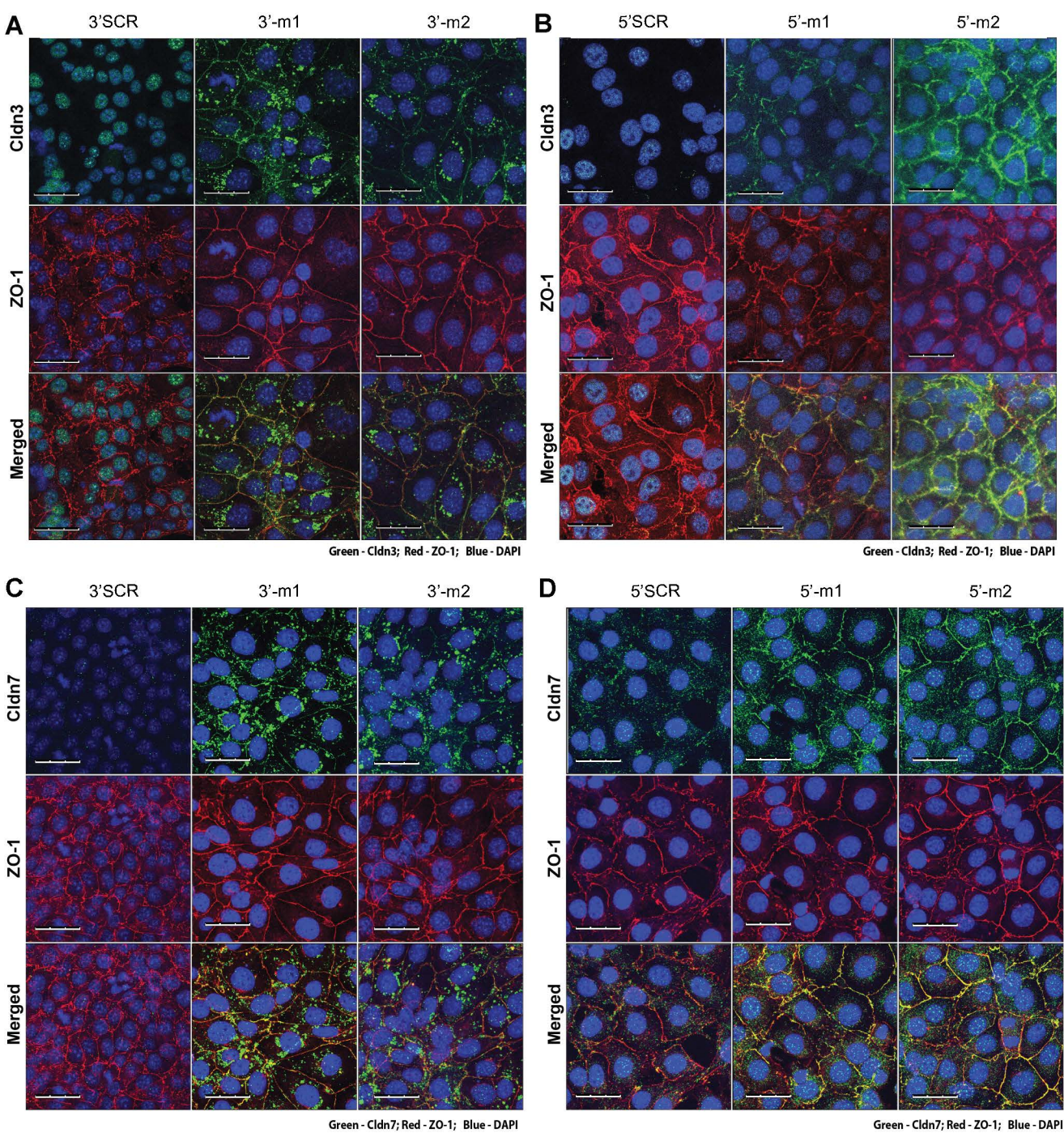




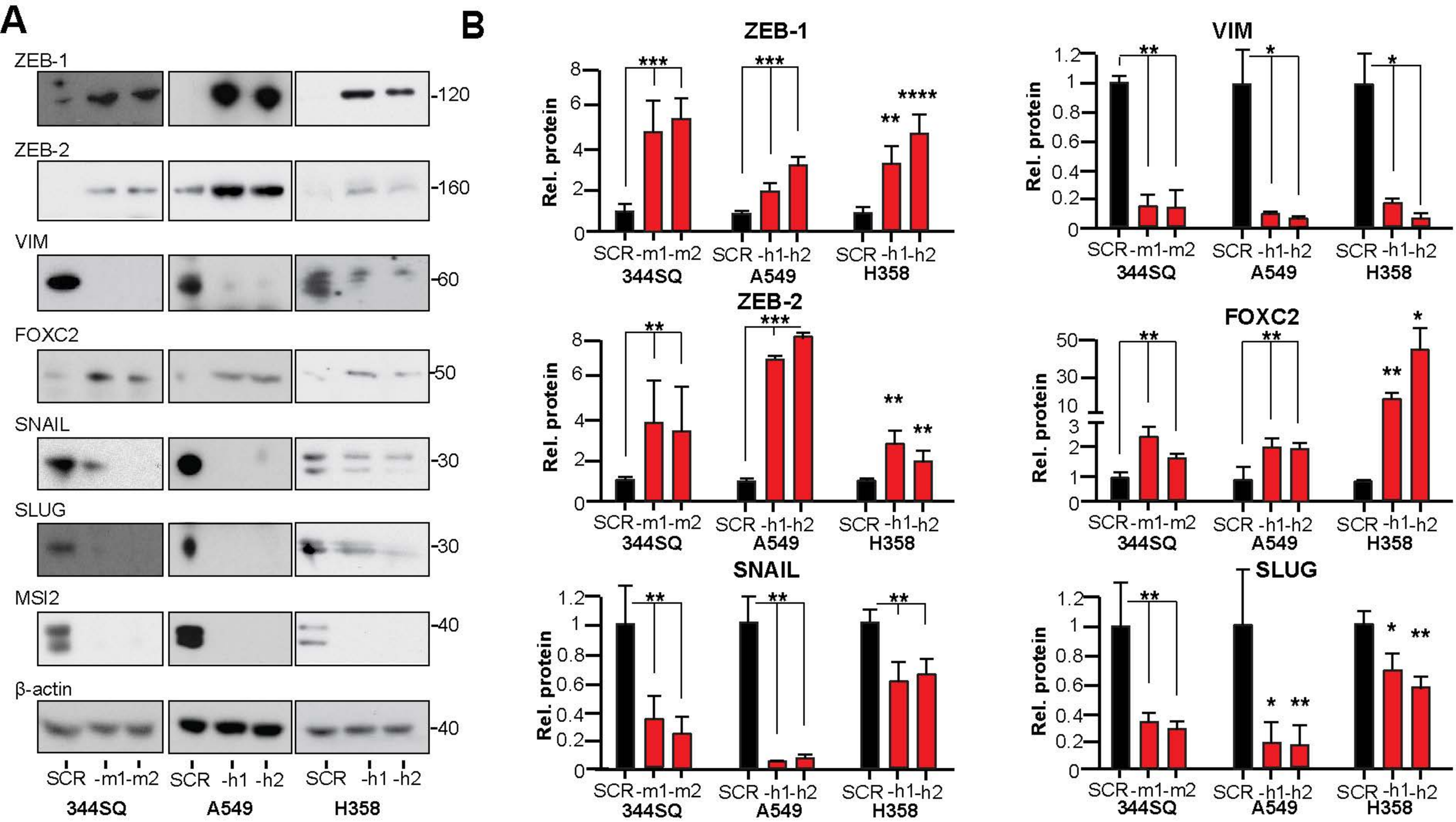




Kudinov et al, Figure S6.



Kudinov et al, Figure S7.



Kudinov et al, Figure S8

A

344SQ Cell Line

A549 Cell Line

3'SCR 3'-m1 3'-m1/TβR1

A'SCR A'-h1 A'-h1/TβR1

TGFβR1

MSI2

β-actin

50

40

40

50

40

40

B

Rel. protein TGFβRI

1.6

1.4

1.2

1

0.8

0.6

0.4

0.2

0

**

3'SCR 3'-m1 3'-m1/TβR1

Rel. protein TGFβRI

1.4

1.2

1

0.8

0.6

0.4

0.2

0

*

A'SCR A'-h1 A'-h1/TβR1

C

Rel. Invasion

1.2

1

0.8

0.6

0.4

0.2

0

***

3'SCR 3'-m1 3'-m1/TβR1

Rel. Invasion

5

4

3

2

1

0

*

A'SCR A'-h1 A'-h1/TβR1

D

3'SCR

3'-m1

3'-m1/TβR1

A'SCR

A'-h1

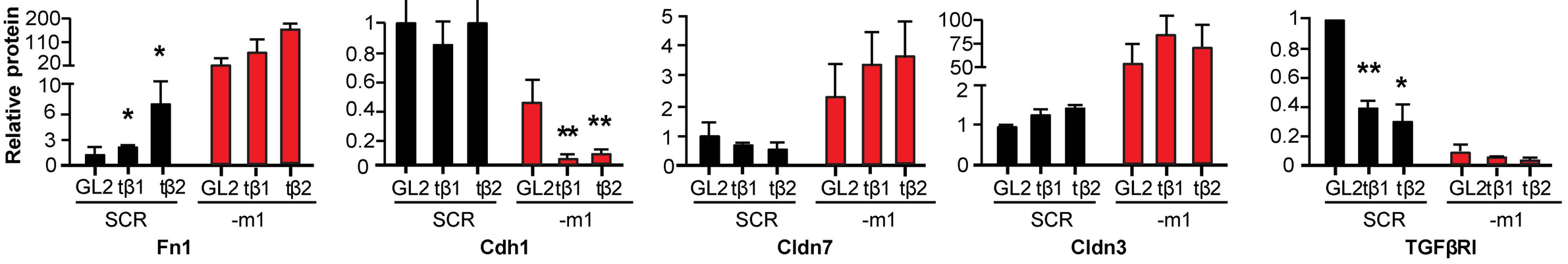
A'-h1/TβR1

Kudinov et al, Figure S9

A

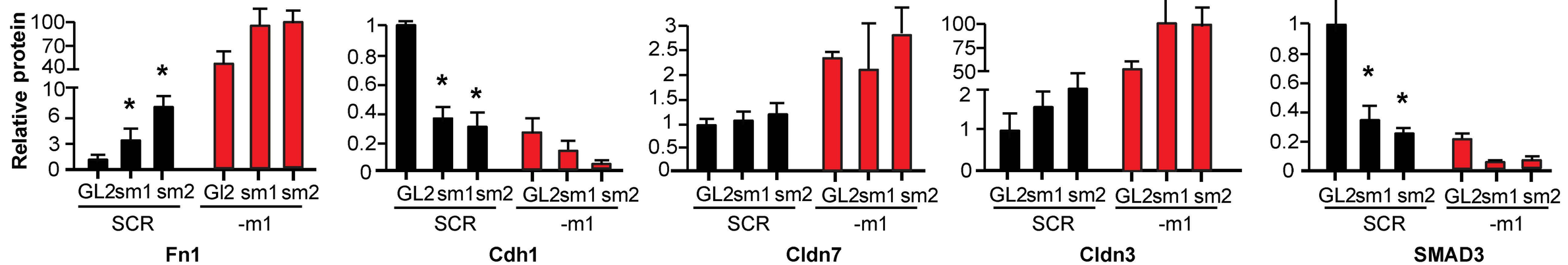
344SQ

TGFβR1 (T-) siRNA



344SQ

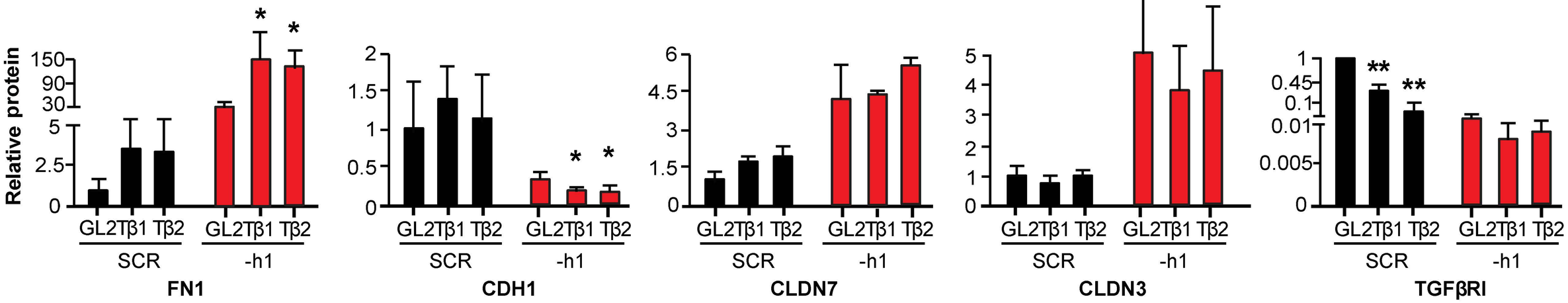
SMAD3 (S-) siRNA



B

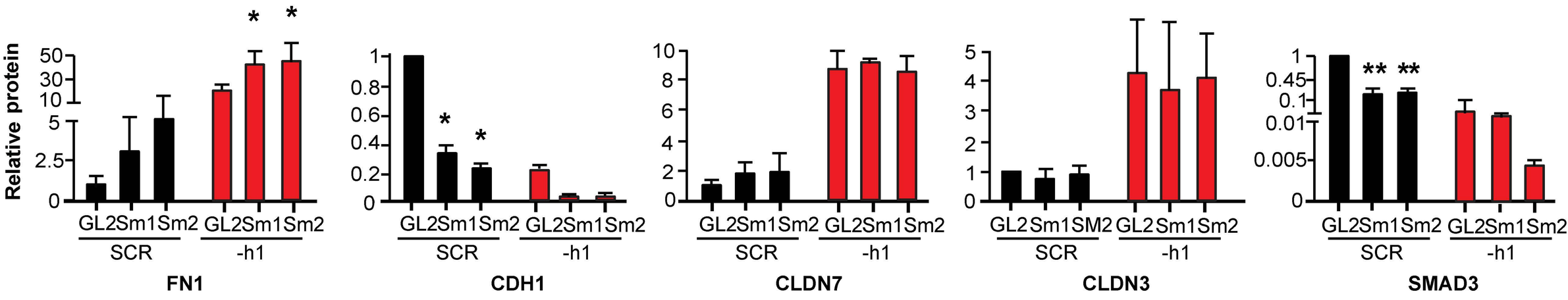
A549

TGFβR1 (T-) siRNA



A549

SMAD3 (S-) siRNA



Kudinov et al, Figure S10.

Table S1. Primers used in RT-QPCR analysis.

	Taqman assays from Life Technologies	Taqman assays designed with Primer Express	SYBR Green assays designed with Primer Express
Mouse			
Msi2			F:AGCAGTATTTCGAGCAGTTTGGCA R:TGTCGAACATCAGCATCGCATCC
Fn1	Mm01256744_m1		
Cldn7			F: AGAGCACCGGCATGATGAG R:GGCGACAAACATGGCTAAGAA
Tgfbr1	Mm00436964_m1		
Smad3	Mm01170760_m1		
Cldn3	Mm00515499_s1		
Cldn5	Mm00727012_s1		
Cdh1 (E-Cad)	Mm00486918_m1		
Ppib	Mm00478295_m1		
HUMAN			
MSI2			F:GGTCATGAGAGATCCCACTACG R:TCTACACTTGCTGGGTCTGC
FN1		F:ATGCCGACCAGAAGTTTGG R:AATGCGGTACATGACCCCTT P:6fam-CCCCATGGCTGCCCACGAG-bhq1	
CLDN7	Hs00600772_m1		
TGFBR1	Hs00610320_m1		
SMAD3	Hs00232222_m1		
CLDN3	Hs00265816_s1		
CLDN5			
CDH1	Hs00170423_m1		
POLR2F		F:TGCCATGAAGGAACTCAAGG R:TCATAGCTCCCATCTGGCAG P:6fam-CCCCATCATCATTCGCCGTTACC-bhq1	

F, forward; R, reverse; P, probe.

Table S2. Patient characteristics for the primary NSCLC tumors arrayed in tissue microarray (TMA).

Gender	
Male	48%
Female	52%
Age at diagnosis	Years
Mean	66.0
Min	41.0
Max	83.0
SD	9.1
Histology	%
Acinar cell carcinoma	12.0
Squamous cell carcinoma, NOS	23.0
Adenocarcinoma, NOS	44.0
Non-small cell carcinoma	4.0
Bronchiolo-alveolar adenocarcinoma, NOS	7.0
Papillary adenocarcinoma, NOS	4.0
Adenosquamous carcinoma	6.0
Overall stage	%
1A	7
1B	12
2B	2
3A	55
3B	13
4	12
T stage	%
1	19
2	53
3	13
4	15
Lymph nodes	%
0	22
X	78
M stage	%
0	88
X	12
Grade	%
Well differentiated	2.3
Moderately differentiated	46.6
Poor differentiated	48.8
Undifferentiated	2.4

TMAs contained specimens from 123 patients with characteristics noted in the table and for 22 normal lungs

Table S3. Patient characteristics for the analyzed grouped NSCLC samples (normal, primary tumor and lymph node metastasis).

Normal-Lung Cancer-Metastatic Matched Cases

Patient	Age at DOS	M/F	Diagnosis	Grade	Stage (pathologic)	Type of Metastasis
001	46	M	adenocarcinoma (mucinous features)	G2	T1 N1 MX (IIA)	Lymph node
002	64	F	adenocarcinoma	G2	T2 N1 MX (IIB)	Lymph node
003	68	F	adenocarcinoma	G3	T0 N2 MX (IIIA)	Lymph node
004	63	M	adenocarcinoma (conventional)	G2	T1a N1 MX (IIA)	Lymph node
005	64	M	adenocarcinoma (mixed: mucinous 80%, fetal 10%, acinar 5%, solid 5%)	G2	T2a N2 MX (IIIA)	Lymph node
006	73	M	adenocarcinoma	G2	T2a N1 MX (IIB)	Lymph node
007	70	F	invasive squamous cell with focal adenocarcinoma (nodal mets appear squamous in differentiation)	G2	T4 N2 MX (IIIB)	Lymph node
008	78	F	squamous cell carcinoma	G2	T3 N1 MX (IIIA)	Lymph node
009	54	M	squamous cell (keratinizing) carcinoma	G2	T2a N1 MX (IIB)	Lymph node
010	73	F	adenocarcinoma	G2-3 (Moderately to poorly)	T1a N2 MX (IIIA)	Lymph node
011	61	F	squamous cell carcinoma (minor component of glandular differentiation <5%)	G2	T1b N1 MX (IIA)	Lymph node
012	52	F	adenocarcinoma (predominantly solid pattern)	G3	T2a N1 MX (IIB)	Lymph node
013	65	M	adenocarcinoma	G3	T2N2MX	Lymph node
014	55	F	squamous cell carcinoma	G3	T1N2MX (IIIA)	Lymph node

Table S4. Sequences of siRNA mixtures and shRNA used in gene knockdowns.

LP_4873								
G-CUSTOM-117757								
Supplier	**Well**	**Pool Catalog Number**	**Duplex Catalog Number**	**Gene Symbol**	**GENE ID**	**Gene Accession**	**GI Number**	**Sequence**
Dharmacon	B02	J-003929-09	J-003929-09	Human TGFBR1 "Tβ1" (Mixture 1)	7046	NM_004612	66346739	GAGAAGAACGUUCGUGGUU and UGCGAGAACUAUUGUGUUA
Dharmacon	B04	J-003929-11	J-003929-11	Human TGFBR1 "Tβ2" (Mixture 2)	7046	NM_004612	66346739	GACCACAGACAAAGUUAUA and CGAGAUAGGCCGUUUGUAU
Dharmacon	B07	J-040617-05	J-040617-05	Mouse Tgfbr1 "tβ1" (Mixture 1)	21812	NM_009370	40254607	GGGCAGUUACUACAACAUA and CUAGAUCGCCCUUUCAUUU
Dharmacon	B09	J-040617-07	J-040617-07	Mouse Tgfbr1 "tβ2" (Mixture 2)	21812	NM_009370	40254607	GCGAAGGCAUUACAGUGUU and UGACAGCUUUGCGAAUUAA
Dharmacon	C02	J-020067-05	J-020067-05	Human SMAD3 "Sm1" (Mixture 1)	4088	NM_005902	52352808	CAACAGGAAUGCAGCAGUG and GAGUUCGCCUUCAAUAUGA
Dharmacon	C04	J-020067-07	J-020067-07	Human SMAD3 "Sm2" (Mixture 2)	4088	NM_005902	52352808	GGACGCAGGUUCUCCAAAC and UUAGAGACAUCAAGUAUGG
Dharmacon	C07	J-040706-05	J-040706-05	Mouse Smad3 "sm1" (Mixture 1)	17127	NM_016769	31543221	GAACUUACAAGGCGACACA and GGACGCAGGUUCUCCAAAC
Dharmacon	C09	J-040706-07	J-040706-07	Mouse Smad3 "sm2" (Mixture 2)	17127	NM_016769	31543221	CCAUGGAGCUCUGUGAGUU and GGAUUGAGCUACACCUGAA
Dhamrmacon	=D07	J-060672-09	J-060672-09	Mouse Cldn7 "Cl7-1" (Mixture 1)	53624	NM_016887	31560439	CCAUGAACGUUAAGUACGA and GGGAGAUGACAAAGCGAAG
Dhamrmacon	D09	J-060672-11	J-060672-11	Mouse Cldn7 "Cl7-2" (Mixture 2)	53624	NM_016887	31560439	CCGAAUAGCUAUGACUGGA and CUGGAUUGGUCAUCAGAUU
Qiagen		SI04236652 and SI04285834		Human MSI2 "-ih1" (Mixture 2)	124540		31560439	ATGAGAGATCCCACTACGAAA and CUGGAUUGGUCAUCAGAUU
Qiagen		SI04312665 and SI04375847		Human MSI2 "-h2" (Mixture 1)	124540		31560439	TCCCAACTTCGTGGCGACCTA and CCAGATAGCCTTAGAGACTAT
Qiagen		SI04465426 and SI04958079		Mouse Msi2 "-m1" (Mixture 2)	76626		31560439	TTCCAAGACGATTGACCCAAA and GCAAGTGTAGATAAAGTATTA
Qiagen		SI04958086 and SI04958093		Mouse Msi2 "-m2" (Mixture 1)	76626		31560439	ATGAGAGATCCCACAACGAAA and CCAGATAGCCTTAGAGACTAT

shRNA Targeting Sequences

Mouse Msi2-m1: CCGGCCCAACTTTGTGGCAACCTATCTCGAGATAGGTTGCCACAAAGTTGGGTTTTTG

Mouse Msi2-m2: CCGGCGTAGGAGGATTGTCTGCAAACTCGAGTTTGCAGACAATCCTCCTACGTTTTTG

Human MSI2-h1: CCGGGTGGAAGATGTAAAGCAATATCTCGAGATATTGCTTTACATCTTCCACTTTTTG

Human MSI2-h2: CCGGCCCAACTTCGTGGCGACCTATCTCGAGATAGGTCGCCACGAAGTTGGGTTTTTG

cDNA ORF Sequences used for rescue experiments

Human CLDN7:
ATGGCCAATTCGGGCCTGCAGTTGCTGGGCTTCTCCATGGCCCTGCTGGGCTGGGTGGGTCTGGTGGCCTGCACCGCCATCCCGCAGTGGCAGATGAGCTCCTATGCGGGT
GACAACATCATCACGGCCCAGGCCATGTACAAGGGGCTGTGGATGGACTGCGTCACGCAGAGCACGGGGATGATGAGCTGCAAAATGTACGACTCGGTGCTCGCCCTGTCCG
CGGCCTTGCAGGCCACTCGAGCCCTAATGGTGGTCTCCCTGGTGCTGGGCTTCCTGGCCATGTTTGTGGCCACGATGGGCATGAAGTGCACGCGCTGTGGGGGAGACGACA
AAGTGAAGAAGGCCCGTATAGCCATGGGTGGAGGCATAATTTTCATCGTGGCAGGTCTTGCCACCTTGGTAGCTTGCTCCTGGTATGGCCATCAGATTGTCACAGACTTTTATA
ACCCTTTGATCCCTACCAACATTAAGTATGAGTTTGGCCCTGCCATCTTTATTGGCTGGGCAGGGTCTGCCCTAGTCATCCTGGGAGGTGCACTGCTCTCCTGTTCCTGTCCTG
GGAATGAGAGCAAGGCTGGGTACCGTGCACCCCGCTCTTACCCTAAGTCCAACTCTTCCAAGGAGTATGTG

Human TGFβR1:
ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGCGGCGGCGGCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCA
CCTCTGTACAAAAGACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCG
AGATAGGCCGTTTGTATGTGCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAATAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGC
CTTGGTCCTGTGGAACTGGCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACTCATGTTGATGGTCTATATCTGCCACAACCGCACTGTCATTCACCATCGAGTGC
CAAATGAAGAGGACCCTTCATTAGATCGCCCTTTTATTTCAGAGGGTACTACGTTGAAAGACTTAATTTATGATATGACAACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAA
CAATTGCGAGAACTATTGTGTTACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGAGAAGAAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACG
TTCGTGGTTCCGTGAGGCAGAGATTTATCAAACTGTAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGACAATGGTACTTGGACTCAGCTCTGGTTGGTGTCAG
ATTATCATGAGCATGGATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAAACTTGCTCTGTCCACGGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTT
GGTACCCAAGGAAAGCCAGCCATTGCTCATAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACTTGCTGTATTGCAGACTTAGGACTGGCAGTAAGACATGATTCAGCCA
CAGATACCATTGATATTGCTCCAAACCACAGAGTGGGAACAAAAAGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAATATGAAACATTTTGAATCCTTCAAACGTGCTGACATCTAT
GCAATGGGCTTAGTATTCTGGGAAATTGCTCGACGATGTTCCATTGGTGGAATTCATGAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCTGACCCATCAGTTGAAGAAATGAG
AAAAGTTGTTTGTGAACAGAAGTTAAGGCCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGAGAGAATGTTGGTATGCCAATGGAGCAGC
TAGGCTTACAGCATTGCGGATTAAGAAAACATTATCGCAACTCAGTCAACAGGAAGGCATCAAAATG

Table S5. Antibodies used for RPPA analysis.

Ab Name	Gene Name	Company	Catalog #	Ab ID	Species	Validation Status*
14-3-3_beta	YWHAB	Santa Cruz	sc-628	882.	Rabbit	Validated
14-3-3_epsilon	YWHAE	Santa Cruz	sc-23957	913.1	Mouse	Use with Caution
14-3-3_zeta	YWHAZ	Santa Cruz	sc-1019	883.	Rabbit	Validated
4E-BP1	EIF4EBP1	CST	9452	2.8	Rabbit	Validated
4E-BP1_pS65	EIF4EBP1	CST	9456	3.1	Rabbit	Validated
4E-BP1_pT37_T46	EIF4EBP1	CST	9459	6.4	Rabbit	Validated
53BP1	TP53BP1	CST	4937	985.1	Rabbit	Validated
ACC_pS79	ACACA ACACB	CST	3661	13.4	Rabbit	Validated
ACC1	ACACA	Epitomics	1768-1	14.1	Rabbit	Under evaluation
ACVRL1	ACVRL1	Epitomics	2940-1	1086.10	Rabbit	Use with Caution
Akt	AKT1 AKT2 AKT3	CST	4691	1084.11	Rabbit	Validated
Akt_pS473	AKT1 AKT2 AKT3	CST	9271	23.10	Rabbit	Validated
Akt_pT308	AKT1 AKT2 AKT3	CST	2965	1154	Rabbit	Validated
AMPK_alpha	PRKAA1	CST	2532	39.4	Rabbit	Use with Caution
AMPK_pT172	PRKAA1	CST	2535	40.6	Rabbit	Validated
Annexin_VII	ANXA7	BD Biosciences	610668	1142.1	Mouse	Validated
AR	AR	Epitomics	1852-1	756.1	Rabbit	Validated
Bad_pS112	BAD	CST	9291	63.7	Rabbit	Validated
Bak	BAK1	Epitomics	1542-1	71.2	Rabbit	Use with Caution
Bax	BAX	CST	2772	73.5	Rabbit	Validated
Bcl-2	BCL2	Dako	Dako M0887	80.1	Mouse	Validated
Bcl-xL	BCL2L1	CST	2762	85.5	Rabbit	Validated
Beclin	BECN1	Santa Cruz	sc-10086	87.1	Goat	Use with Caution
beta-Catenin	CTNNB1	CST	9562	75.3	Rabbit	Validated
Bid	BID	Epitomics	1008-1	88.1	Rabbit	Use with Caution
Bim	BCL2L11	Epitomics	1036-1	90.1	Rabbit	Validated
B-Raf	BRAF	Santa Cruz	sc-5284	96.2	Mouse	Use with Caution
BRCA2	BRCA2	CST	9012	761.1	Rabbit	Use with Caution
Caspase-7_cleavedD198	CASP7	CST	9491	109.6	Rabbit	Use with Caution
Caveolin-1	CAV1	CST	3238	114.1	Rabbit	Validated
CD31	PECAM1	Dako	M0823	127.1	Mouse	Validated
CD49b	ITGA2	BD Biosciences	611016	937.1	Mouse	Validated
CDK1	CDC2	CST	9112	1007.5	Rabbit	Validated
Chk1	CHEK1	CST	2360	1203.3	Mouse	Use with caution
Chk1_pS345	CHEK1	CST	2348	903.7	Rabbit	Use with caution
Chk2	CHEK2	CST	3440	146.1	Mouse	Validated
Chk2_pT68	CHEK2	CST	2197	147.2	Rabbit	Use with Caution
cIAP	BIRC2	Millipore	07-759	930.1	Rabbit	Caution
c-Jun_pS73	JUN	CST	9164	155.5	Rabbit	Validated
c-Kit	KIT	Epitomics	1522	157	Rabbit	Validated
Claudin-7	CLDN7	Novus	NB100-91714	852.1	Rabbit	Validated
c-Met_pY1235	MET	CST	3129	727.5	Rabbit	Validated

c-Myc	MYC	Santa Cruz	sc-764	1143.1	Rabbit	Use with Caution
Collagen_VI	COL6A1	Santa Cruz	SC-20649	171.1	Rabbit	Validated
C-Raf	RAF1	Millipore	05-739	803	Rabbit	Validated
C-Raf_pS338	RAF1	CST	9427	179.4	Rabbit	Validated
Cyclin_B1	CCNB1	Epitomics	1495-1	192.1	Rabbit	Validated
Cyclin_D1	CCND1	Santa Cruz	SC-718	194.1	Rabbit	Validated
Cyclin_E1	CCNE1	Santa Cruz	SC-247	201.1	Mouse	Validated
DJ-1	PARK7	Abcam	ab76008	891.1	Rabbit	Validated
Dvl3	DVL3	CST	3218	940.1	Rabbit	Validated
E-Cadherin	CDH1	CST	3195	1099.10	Rabbit	Validated
eEF2	EEF2	CST	2332	1060.3	Rabbit	Use with Caution
eEF2K	EEF2K	CST	3692	1061.2	Rabbit	Validated
EGFR	EGFR	CST	2232	1120.15	Rabbit	Validated
EGFR_pY1068	EGFR	CST	2234	217.13	Rabbit	Use with caution; also sees pHer2
EGFR_pY1173	EGFR	Epitomics	1124	221.3	Rabbit	Validated
eIF4E	EIF4E	CST	9742	722.3	Rabbit	Validated
eIF4G	EIF4G1	CST	2498	1124.3	Rabbit	Use with Caution
ER-alpha	ESR1	Lab Vision	RM-9101-S	238.6	Rabbit	Validated
ER-alpha_pS118	ESR1	Epitomics	1091-1	241.1	Rabbit	Validated
FASN	FASN	Cell Signaling	3180	1156.00	Rabbit	Validated
Fibronectin	FN1	Epitomics	1574-1	262.1	Rabbit	Validated
FOX03a	FOXO3	CST	2497	1122.6	Rabbit	Use with Caution
FOXO3a_pS318_S321	FOXO3	CST	9465	270.1	Rabbit	Use with Caution
FoxM1	FOXM1	CST	5436	1123.1	Rabbit	Validated
G6PD	G6PD	Santa Cruz	sc-373887	1155	Mouse	Validated
Gab2	GAB2	CST	3239	943.1	Rabbit	Validated
GAPDH	GAPDH	Ambion	AM4300	274.11	Mouse	Caution
GATA3	GATA3	BD Biosciences	558686	764.1	Mouse	Validated
GSK3_pS9	GSK3A GSK3B	CST	9336	1082.12	Rabbit	Validated
GSK3-alpha-beta	GSK3A GSK3B	Santa Cruz	SC-7291	284.2	Mouse	Validated
GSK3-alpha-beta_pS21_S9	GSK3A GSK3B	CST	9331	285.12	Rabbit	Validated
HER2	ERBB2	Lab Vision	MS-325-P1	1038.2	Mouse	Validated
HER2_pY1248	ERBB2	R&D Systems	AF1768	1075.1	Rabbit	Use with caution; likely sees pEGFR
HER3	ERBB3	Santa Cruz	sc-285	911.1	Rabbit	Validated
HER3_pY1289	ERBB3	CST	4791	728.12	Rabbit	Use with Caution
Heregulin	NRG1	CST	2573	890.1	Rabbit	Validated
IGFBP2	IGFBP2	CST	3922	335.1	Rabbit	Validated
INPP4B	INPP4B	CST	4039	1065.1	Rabbit	Validated
IRS1	IRS1	Upstate (Millipore)	06-248	802.1	Rabbit	Validated
JNK_pT183_pY185	MAPK8	CST	4668	888.5	Rabbit	Validated
JNK2	MAPK9	CST	4672	380.1	Rabbit	Use with Caution
Lck	LCK	CST	2752	397.2	Rabbit	Validated
MAPK_pT202_Y204	MAPK1 MAPK3	CST	4377	405.3	Rabbit	Validated
MEK1	MAP2K1	Epitomics	1235-1	417.1	Rabbit	Validated
MEK1_pS217_S221	MAP2K1	CST	9154	1076.3	Rabbit	Validated
MIG-6	ERRFI1	Sigma	WH0054206M1	1062.1	Mouse	Validated
mTOR	FRAP1	CST	2983	444.3	Rabbit	Validated
mTOR_pS2448	FRAP1	CST	2971	446.14	Rabbit	Use with caution
MYH11	MYH11	SDI / Novus	21370002	1139.1	Rabbit	Validated
Myosin IIa pS1943	MYH9	CST	5026	1160	Rabbit	Validated

N-Cadherin	CDH2	CST	4061	452.1	Rabbit	Validated
NDRG1_pT346	NDRG1	CST	3217	1126	Rabbit	Validated
NF2	NF2	SDI	2271.00.02	1046.1	Rabbit	Use with Caution
NF-kB-p65_pS536	NFKB1	CST	3033	457.4	Rabbit	Use with Caution
Notch1	NOTCH1	CST	3268	1064.1	Rabbit	Validated
N-Ras	NRAS	Santa Cruz	sc-31	1136.1	Mouse	Validated
p21	CDKN1A	Santa Cruz	SC-397	470.1	Rabbit	Validated
p27	CDKN1B	Epitomics	1591-1	897.1	Rabbit	Validated
p27_pT157	CDKN1B	R&D	AF1555	842.1	Rabbit	Use with Caution
p27_pT198	CDKN1B	Abcam	ab64949	878.1	Rabbit	Validated
p38_MAPK	MAPK14	CST	9212	478.10	Rabbit	Validated
p38_pT180_Y182	MAPK14	CST	9211	479.15	Rabbit	Validated
p53	TP53	CST	9282	481.3	Rabbit	Under evaluation
p70S6K	RPS6KB1	Epitomics	1494-1	493.1	Rabbit	Validated
p70S6K_pT389	RPS6KB1	CST	9205	494.7	Rabbit	Validated
p90RSK	RPS6KA1	CST	9347	759.5	Rabbit	Caution
p90RSK_pT359_S363	RPS6KA1	CST	9344	770.2	Rabbit	Use with Caution
Paxillin	PXN	Epitomics	1500-1	505.1	Rabbit	Caution
PCNA	PCNA	Abcam	ab29	511.1	Mouse	Caution
PDCD4	PDCD4	Rockland	600-401-965	816.1	Rabbit	Use with Caution
PDK1	PDK1	CST	3062	515.5	Rabbit	Validated
PDK1_pS241	PDK1	CST	3061	516.7	Rabbit	Validated
PEA15	PEA15	CST	2780	1017.2	Rabbit	Validated
PEA15_pS116	PEA15	Invitrogen	44-836G	1018.1	Rabbit	Validated
PI3K-p110-alpha	PIK3CA	CST	4255	808.1	Rabbit	Use with Caution
PI3K-p85	PIK3R1	Upstate (Millipore)	06-195	523.3 or 523.4	Rabbit	Validated
PKC-alpha	PRKCA	Upstate (Millipore)	05-154	529.1	Mouse	Validated
PKC-alpha_pS657	PRKCA	Upstate (Millipore)	06-822	530.2	Rabbit	Use with caution
PKC-delta_pS664	PRKCD	Upstate (Millipore)	07-875	932.1	Rabbit	Validated
PKC-pan_BetaII_pS660	PKC	CST	9371	1137.	Rabbit	Validated
PR	PGR	Epitomics	1483-1	549.1	Rabbit	Validated
PRAS40_pT246	AKT1S1	Biosource	441100G	739.1	Rabbit	Validated
PTEN	PTEN	CST	9552	566.3	Rabbit	Validated
Rab11	RAB11A RAB11B	CST	3539	1083.3	Rabbit	Under evaluation
Rab25	RAB25	CST	4314	1150.1	Rabbit	Validated
Rad50	RAD50	Millipore	05-525	987.1	mouse	Validated
Rad51	RAD51	Chem Biotech	na 71	579.3	Mouse	Under evaluation
Raptor	RPTOR	CST	2280	1128.8	Rabbit	Validated
Rb_pS807_S811	RB1	CST	9308	557.9	Rabbit	Validated
RBM15	RBM15	SDI / Novus	21390002	1138.1	Rabbit	Validated
Rictor	RICTOR	CST	2114	1129.4	Rabbit	Use with Caution
Rictor_pT1135	RICTOR	CST	3806	1130.4	Rabbit	Validated
S6_pS235_S236	RPS6	CST	2211	600.8	Rabbit	Validated
S6_pS240_S244	RPS6	CST	2215	601.4	Rabbit	Validated
SCD1	SCD1	Santa Cruz	sc-58420	1127.1	Mouse	Validated
SF2	SFRS1	Invitrogen	32-4500	1131.1	Mouse	Validated
Smad1	SMAD1	Epitomics	1649-1	922.2	Rabbit	Validated
Smad3	SMAD3	Epitomics	1735-1	796.1	Rabbit	Validated
Smad4	SMAD4	Santa Cruz	sc-7966	920.1	Mouse	Validated
Src	SRC	Upstate (Millipore)	05-184	621.2	Mouse	Validated

Src_pY416	SRC	CST	2101	623.18	Rabbit	Use with caution
Src_pY527	SRC	CST	2105	626.5	Rabbit	Validated
STAT3_pY705	STAT3	CST	9131	637.6	Rabbit	Validated
STAT5-alpha	STAT5A	Epitomics	1289-1	638.1	Rabbit	Validated
Stathmin	STMN1	Epitomics	1972-1	718.1	Rabbit	Validated
Syk	SYK	Santa Cruz	sc-1240	1033.1	Mouse	Validated
TAZ	WWTR1	CST	2149	777.1	Rabbit	Validated
TIGAR	C12ORF5	Epitomics	S1711	1107.1	Rabbit	Validated
Transglutaminase	TGM2	Lab Vision	MS-224-P1	908.2	Mouse	Validated
TRFC	TRFC	SDI / Novus	22500002	1140.1	Rabbit	Validated
TSC1	TSC1	CST	4906	1125.1	Rabbit	Use with Caution
TTF1	TTF1	Epitomics	2044-1	1081.1	Rabbit	Validated
Tuberin	TSC2	Epitomics	1613-1	670.30	Rabbit	Validated
Tuberin_pT1462	TSC2	CST	3617	671.2	Rabbit	Validated
VEGFR2	KDR	CST	2479	688.4	Rabbit	Validated
VHL	VHL	BD Biosciences	556347	693.1	Mouse	Use with Caution
XRCC1	XRCC1	CST	2735	906.1	Rabbit	Under evaluation
YAP	YAP1	Santa Cruz	sc-15407	780.3	Rabbit	Under evaluation
YAP_pS127	YAP1	CST	4911	782.1	Rabbit	Under evaluation
YB-1	YBX1	SDI	1725.00.02	700.1	Rabbit	Validated
YB-1_pS102	YBX1	CST	2900	835.1	Rabbit	Validated

Validation Status*

VALID=RPPA and WB correlation > 0.7

Use with Caution=RPPA and WB correlation < 0.7

Under Evaluation=Antibody has given mixed results and / or evaluated by another lab; we are in the process of (re)validating.