**Supplementary Methods**

**Immunofluorescence and confocal microscopy**

A549 cells were plated onto poly-D-lysine-coated eight well glass chamber slides (5,000 cells per well) for immunostaining. Cells were rendered quiescent by serum starvation for 36 hours, followed by treatment with 1µM nicotine for 24 hours at 37oC (experiment in Fig.1B and 2A). The cells were fixed with 10% buffered-formalin and immunofluorescence was performed as per the previously published protocols ([1](#_ENREF_1)). Primary antibodies used were Monoclonal vimentin (BD) at 1:100 dilution or monoclonal ZO1 (Abcam) at 1:100 dilution or monoclonal Ecadherin (Santa Cruz) at 1:100 dilution. Anti-mouse Alexa Fluor-488 (Molecular Probes) was used as Secondary antibody. DAPI (Vector labs) was used to stain the nuclei. Cells were visualized with a DM16000 inverted Leica TCS SP5 tandem scanning confocal microscope with a 63x/1.40NA oil immersion objective. Images were produced with three cooled photomultiplier detectors and analyzed with the LAS AF software version 1.6.0 build 1016 (Leica Microsystems, Germany). For quanification of immunofluorescence data, Definiens Developer 1.5 (Definiens AG, Munich, Germany) was used to segment nuclei based DAPI staining to enumerate number of cells.  Then the software was used to segment Alexa 488 signal to identify and quantify cellular ZO1 or ECadherin staining.  At least 200 cells within six images were analyzed for each treatment.  Data shown represents the average of these images.

**siRNA transfections and Real-time PCR**

 For siRNA transfections 50, 75 or 100 pmol of siRNAs (Santa Cruz) with Oligofectamine were added to cells. For real-time PCR, total RNA was isolated using RNeasy miniprep kit (QIAGEN) following manufacturer's protocol, followed by first-strand cDNA synthesis using iScript cDNA synthesis kit (Bio-Rad). Data was analyzed by ΔΔCT method, where gene of interested was normalized to 18s rRNA, then compared to the non-targeting siRNA control sample. Error bars represent the standard deviation of three independent experiments.

siRNAs for β-arrestin-1 was purchased from Qiagen (βarrestin siRNA#1) and Santa Cruz (βarrestin siRNA#2) and a non-targeting siRNA sequence Ambion (ControlsiRNA#1) and Santa Cruz (ControlsiRNA#2) was used as a control. siRNAs for β-arrestin-2 was purchased from Ambion, Src and α7nAChR siRNAs were obtained from Santa Cruz biotechnology. siRNAs were transfected using Oligofectamine (Invitrogen Corporation) according to manufacturer’s protocols. Eighteen hours post transfection, the cells were rendered quiescent for 36 hours using serum free medium. Cells were the treated with 1µM nicotine for 24 hours and RNA was extracted or lysates were prepared for immunoblotting.

Primer sequences used for RT-PCR are given below

βarrestin1F-AGAGTCTATGTGACGCTGACCTGC

βarrestin1R-GTTCCTGCAGCCGCGTCAG

βarrestin2F-CAAGAAGTCGAGCCCTAACTGCAAG

βarrestin2R- CGGTCCTTCAGGTAGTCAGGGTC

VimentinF-CGCCAACTACATCGACAAGGTGC

VimentinR-CTGGTCCACCTGCCGGCGCAG

FibronectinF-CCTGAGGATGGAATCCATGAGC

FibronectinR-GGCTCTCCATATCGTGCAAG

c-SrcF-TGT CCT TCA AGA AAG GCG AG

c-SrcR-ATA CCA CTC CTC AGC CTG GA

α7nAChR-F-TCG CTC CTG CAC GTG TCC CTG

α7nAChR-R-AGA CGG TGA GTG GTT GCG AGT CA

ZEB1F-AGCAGTGAAAGAGAAGGGAATGC

ZEB1R-GGTCCTCTTCAGGTGCCTCAG

ZEB2F-ATCTGCTCAGAGTCCAATGCAGCAC

ZEB2R-GAAACAGTATTGTCCACAATCTGTAG

GAPDHF- TGCACCACCACCTGCTTAGC

GAPDHR –GGCATGGACTGTGGTCATGAG

18SrRNAF 5'-CTCAACACGGGAAACCTCAC3', and

18SrRNAR 5'AAATCGCTCCACCAACTAAGAA3'.

**Constructs and transfections**

E2F1, E2F2, E2F3 constructs were obtained from Dr. W.D. Cress, Molecular Oncology, Moffitt Cancer Center. The E2F4 and E2F5 constructs have been previously described ([2](#_ENREF_2)) and were obtained from Addgene. All β-arrestin-1 constructs were a kind gift from Dr. R. J. Lefkowitz, Department of Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina. Src-CA and Src-DN constructs were purchased from Addgene ([3](#_ENREF_3)).

Fibronectin (pFN 1.2kb) and Vimentin (VimPro-1.5Kb) promoter luciferase constructs were kindly provided by Dr. Jesse Roman (Professor & Chair, Department of Medicine, University of Loiusville, Kentucky) ([4](#_ENREF_4)) and Dr. C. Gilles (Liege University, Belgium) ([5](#_ENREF_5)) respectively. A549 and H1650 cells were transfected using Fugene HD reagent (Roche) following manufacturer’s instruction. Luciferase assays were done 48 hours post transfection, using Dual Luciferase Assay system (Promega) following the manufacturer’s protocol and luciferase activity was measured with a luminometer (Turner luminometer). For each construct, relative luciferase activity was defined as the mean value of the firefly luciferase/ Renilla luciferase ratios obtained from at least three independent experiments. For promoter induction studies with either nicotine or TGF- β, cells were serum starved after transfection and subsequently stimulated with nicotine or TGF- β.

Construction of FN-promoter Mutants:-The FN promoter construct containing 1.2 kb (FN-full length promoter), FN0.5kb and FN0.2kb promoters were a kind gift from Dr. Jesse Roman, University of Louisville([4](#_ENREF_4)). The proximal E2F binding sequence in the FN promoter GAGGGGCGGGAGGGGAC (-35 to -52) was mutated to GCCTCCAGAGGATATGGAGGGGACCGTC by changing the sequence GGCG to ATAT. To incorporate this mutation, overlap extension PCR method was followed. First round of PCR was performed using FN 0.2kb promoter as template and the following primer pairs.

FN-OEF1-TCTTAACAGCTGCAAGGTCGTGG &

FN-OERM1- GACGGTCCCCTCCATATCCTCTGGAGGC and a second PCR using the following primers:-

FN-OEFM2- GCCTCCAGAGG ATAT GGAGGGGACCGTC

FN-OER3-CACCACGGTAGGCTGCGAAATGCC

The DNA fragments generated from both these PCRs are then pooled and used as template for the third PCR using the following primers

FN-OEF1-TCTTAACAGCTGCAAGGTCGTGG

FN-OER3-CACCACGGTAGGCTGCGAAATGCC

The DNA fragment obtained from this PCR was cloned into PGL3 vector. Incorporation of mutation in the resulting construct was confirmed by PCR.

Construction of Vimentin deletion mutants:- The full length vimentin construct (VimPro-1.5Kb) promoter luciferase constructs was kindly provided by Dr. C. Gilles (Liege University, Belgium) ([5](#_ENREF_5), [6](#_ENREF_6)). Deletion mutants of vimentin promoter were generated by PCR amplification using full length promoter as template and the following pairs of primers.

VimproF-280-CTGGCTCATATTATACCTTTAATGAC and

VIMproR1-AACTTCTGCAGCCTTTGGAGAGGC to generate Vimentin 269 (245+24=269) and VimChIP F386 CTGTACTGTGCATTGCTGTCC and VIMproR1-AACTTCTGCAGCCTTTGGAGAGGC for Vimentin 411(387+24=411)

Construction of ZEB1 and ZEB2 promoter constructs:-DNA was extracted from primary aortic endothelial cells using standard protocols. Primers spanning 3 Kb of the ZEB1 and ZEB2 promoter were used to PCR amplify the fragment with Hotmaster Taq (5-Prime). Primer sequences were:

ZEB1-pro-2900-GAATGATATGCTTCATGTGGAACACCG

 ZEB1-pro-R1-GTTCGCCTGCTTTCTGCGCTTACAC

 ZEB2-pro-F-3500 -GGCTTCTCAGAGAGATGCTCCTG

ZEB2 pro-R+52-Nhe2-GCTAGCATTATACCTTGAAGTCTCCGC

The fragments were then cloned into pCR2.1 using TA cloning kit (Invitrogen). The plasmids were released by restriction endonuclease digestion and ligated into pGL3-basic luciferase vector (Promega).

**Chromatin immunoprecipitation (ChIP) assays**

 Cells were treated with 1% formaldehyde for 10 minutes at room temperature for cross-linking the DNA to the proteins. The cells were scraped, washed in ice-cold PBS, and centrifuged at 1500 x g at 4°C for 5 minutes. Subsequently, the pellet was resuspended in cell lysis buffer (44 mM Tris–HCl (pH 8.1), 1% SDS, 1 mM EDTA (pH 8.0)). The cells were sonicated thrice for 15 seconds each. Subsequently, the cell lysates were centrifuged at 10,000g at 4°C for 15 minutes. An aliquot of the sheared chromatin was used as the input for the ChIP assay. The remainder of the chromatin was diluted with ChIP dilution buffer (16 mM Tris–HCl (pH 8.1), 250 mM NaCl, 0.1% SDS, 1% Triton-X-100, 1.2 mM EDTA) and rotated overnight with primary antibody. Immunoprecipitations were done using polyclonal antibodies for E2F1 and Rb (Santa Cruz), p300 (Santa Cruz), Acetylated Histone H3 (Upstate) and a Rabbit IgG (Sigma) was used as the negative control. The next day, 60 μL of 1:1 protein G-Sepharose was added to the immune complexes, and the mixture was rotated at 4°C for 2 hours. The beads were washed five times with ChIP dilution buffer and eluted with ChIP elution buffer (0.1 M sodium bicarbonate, 1% SDS, 5 mM NaCl). The cross-links were reversed by incubation at 65°C for 18 hours. DNA was isolated by ethanol precipitation. The associated proteins with the DNA were digested with 50 μg Proteinase K at 37°C for 30 minutes. DNA was purified by phenol:chloroform extraction method followed by ethanol precipitation. Purified DNA was resuspended in 30 μL water ([7](#_ENREF_7)). ChIP assays were followed by Real-time PCR to quantify recruitment of specific factors on each promoter. Data were normalized to input DNA to calculate percent DNA recovered by immunoprecipitation and expressed as percent input. Fos promoter as a negative promoter for confirming the specificity of ChIP assays.

The ChIP assays in NSCLC tissues (fresh frozen tumors and distant normal lung tissue from Moffitt Cancer Center Tissue Bank) were performed using 20mg of tissue per IP reaction. The tissue was transferred into a tube containing 10ml of PBS (containing 10µg/ml leupeptin, 10µg/ml aprotinin, 1mM PMSF) per gram of tissue. The DNA and protein were crosslinked by adding formaldehyde to a final concentration of 1% for 15 minutes ([www.genomecenter.ucdavis.edu/farnham/protocols/tissues.html](http://www.genomecenter.ucdavis.edu/farnham/protocols/tissues.html)).The crosslinking reaction was terminated by the addition of 0.125M glycine for 5 minutes. Subsequently tissue samples were disaggregated in ice-cold PBS using a Dounce homogenizer with type B pestle. The nuclei were released by addition of ice-cold nuclei lysis buffer (50mM Tris, pH 8.1, 10mM EDTA, 1% SDS, 10µg/ml leupeptin, 10µg/ml aprotinin, 1mM PMSF) for 20 minutes to the sample. 0.1g of glass beads (Sigma Chemical Company) were added to each sample and sonicated (Branson Sonifer); ChIP assays were similar to that of cultured cells as described in ([7-9](#_ENREF_7)).

ChIP sequencing:- Human NSCLC cell line A549 was maintained in Ham’s F-12K (Cellgro) supplemented with 10% FBS (Cellgro). The cells were rendered quiescent by serum starvation for 36 hours and stimulated with 1 µM nicotine for 24 hours. Cells were then fixed with 1% formaldehyde under gentle agitation for 15 minutes at room temperature. Fixation was stopped by adding 1/20th volume of 2.5M glycine and the cells were scrapped off from the plate, pelleted by centrifugation and washed with PBS three times. Finally, the pellets were snap frozen and sent to Genpathway (now Active Motif) for further analysis. ChIP lysates were prepared from quiescent as well as nicotine stimulated cells and chromatin immunoprecipitation was performed using β-arrestin-1 antibody (Novus).The detailed protocol of the assay and the data obtained are submitted to GEO (accession number GSE40689).

**Invasion Assays**

 Boyden Chamber assays were used to assess the invasive ability of cells as described previously ([10](#_ENREF_10), [11](#_ENREF_11)). The upper surface of the 6.5 mm filters (Corning) were coated with collagen (100 µg/filter) and Matrigel (BD Bioscience) (50 µg/filter). Twenty thousand cells were plated in the upper chamber with 0.1% bovine serum albumin (Sigma). Media containing 20% fetal bovine serum was placed in the lower well as chemoattractant. The cells that invaded through the filters were quantified by counting three fields under 20× objective magnifications.

**Wound healing assay or migration assay**

 Wound healing assays were performed as described earlier ([12](#_ENREF_12)). A549 cells were grown in a 6-well plate (Falcon Becton Dickinson) transfected with siRNAs. These cells were starved in serum free media for 24 hours and then washed with 1x Dulbecco’s Phosphate-buffered saline (MediaTech). The cells were scratched with a sterilized 200 μl pipette tip in three separate places in each well and medium containing 1 μM nicotine or starvation media was added to the wells. After 24 hours, the wounds were observed and images were taken in 20X magnification using Zeiss inverted phase contrast microscope.

**Statistical Analysis**

 All data have been graphically represented and statistically ana­lyzed using Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA). In all analyses, means and 95% confidence inter­vals were estimated. Statistical analysis of mRNA levels and luciferase assays were performed using Student *t* test. Values were considered significant when the *P* value was less than 0.05.

**Collagen gel culture/3-D cell culture**

Three-dimensional collagen-1 gels were prepared on ice using equal volumes of 3 mg/ml collagen solution and 2X HEPES-buffered salt solution (50.4 mM HEPES, pH 7.4, 162.6 mM NaCl, 10.6 mM KCl, 88.2 mM NaHCO3, 1.6 mM Na2HPO4 and 11 mM D(+)-glucose) yielding a concentration of 1.3 mg/ml following addition of culture medium ([13](#_ENREF_13), [14](#_ENREF_14)). The collagen gel solution (0.2 ml) was added to each well of the 96-well plate and allowed to set at 37°C for 30 min. A549 cells were collected in a single cell suspension and added on the preformed collagen gel. The plates were incubated at 37°C for 3 days, at which time complex structures had formed. The 3-D structures were visualized by phase contrast microscopy ([1](#_ENREF_1)). Matrigel assays were performed according to previously published protocols ([15](#_ENREF_15)).

**Lysate preparation, immunoprecipitation and western blotting**

Lysates from cells treated with different agents were prepared by NP-40 lysis as described earlier ([16](#_ENREF_16)). Western blots were performed using antibodies against vimentin (BD), fibronectin (Santa Cruz), E2F1 (Santa Cruz), β-arrestin-1 (BD or Epitomics), β-arrestin-2 (cell signaling) and β-Actin (Sigma). Immunoprecipitation-western blots were performed according to published protocols ([17](#_ENREF_17)). Briefly, for immunoprecipitations the cell lysates containing 250 µg of total proteins were incubated with I µg of β-arrestin-1 Ab (BD). The total reaction volume was adjusted to 100 μL with immuno­precipitation buffer (2 mM HEPES [pH 7.9], 40 mM KCl, 0.001 mM MgCl2, 25 mM EGTA, 1 mM EDTA, 1% IGEPAL-CA-630, 1 mM DTT, 5 mM PMSF, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 25 μg/mL leupeptin, 5 μg/mL pepstatin, 5 μg/mL aprotinin, 25 μg/mL trypsin–chymotrypsin inhibitor) and rotated on a nutator at 4°C for 1 hour. After 1 hour, 50 μL of 1:1 protein G-Sepharose slurry was added (GE Healthcare, Piscataway, NJ) and the mixture was rotated at 4°C for another 2 hours. The beads were washed four times in immunoprecipitation buffer. Bound proteins were eluted in sodium dodecyl sulfate (SDS) sample buffer (0.06 M Tris–HCl [pH 6.8], 10% glycerol, 2% SDS, and 100 mM DTT, 0.2% w/v bromophenol blue) and resolved on a 10% SDS–polyacrylamide gel. The proteins on the SDS–polyacrylamide gel were transferred to 0.45 μm nitrocellulose membranes (Bio-Rad Laboratories), and the interacting proteins were detected by immunoblotting. For preparing membrane fractions, subcellular fractionation kit from Thermo Scientific was used and the subcellular fractions were prepared according to the manufacturer’s protocol.

**Orthotopic Lung Tumor Model**

 For in vivo injections, shcontrol-luc cells and shβarr1-luc cells were harvested following treatment with trypsin, and washed with D-PBS solution. Single cell suspensions of 200,000 cells per 100 microliters with > 90% viability as determined by trypan blue exclusion were used for injections. Tumor cells were inoculated with 1ml syringes with 30-gauge hypodermic needles in an open technique under direct visualization into the right lung tissue of SCID mice. Specifically, SCID mice were anesthetized by gas anesthesia (3% isoflurane). Once anesthesized, mice were placed in the left lateral decubitus position with a small “bump” in the left axillary space to expose the thoracic rib cage and better define anatomical landmarks. A small posterolateral incision, approximately 5-7 mm, was performed at the lateral dorsal axillary line, ~1.5cm above the lower rib line just below the inferior border of the scapula. The 30-guage hypodermic needle was advanced between the 4th/5th intercostal spaces approximately 5-7mm into the thorax and removed after injection of cell suspension. Closure of the skin and subcutaneous tissue was performed with one staple. Mice and tumor growth/metastases with or without drug treatment were imaged weekly by In Vivo Imaging System (IVIS-200) from Xenogen Corporation as per manufacturer's protocol. The mice were injected with firefly Luciferin (150mg/kg body weight) 5-10 minutes prior to in and ex vivo tumor imaging. Mice were euthanatized when control mice become moribund. All of the mice were autopsied and tumor tissue collected with individual organs harvested for evaluation of tumor size and distant metastases as determined by luciferase expression. All animal studies were reviewed and approved by the IACUC of University of South Florida, Tampa, Florida.

**Immunohistochemistry staining**

Tumors from orthotopic lung experiment were fixed in 10% neutral-buffered formalin before processing into paraffin blocks. Paraffin sections (5µm) were rehydrated into PBS and processed using the following protocols. Tissue sections were immunostained with H&E using standard histologic techniques. Immunohistochemical staining for vimentin and fibronectin was performed using a Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ) as per manufacturer's protocol with proprietary reagents. Briefly, slides were de-paraffinized on the automated system with EZ Prep solution. Heat-induced antigen retrieval method was used in Cell Conditioning 1 for staining for vimentin. The mouse monoclonal antibody that reacts to vimentin, (#760-2917, Ventana, Tucson, AZ) was used at a predilute concentration and incubated for 12 minutes. The Ventana OmniMap anti-mouse secondary antibody was used for 16 min. Fibronectin staining utilized enzymatic retrieval method (Protease 1 for 4 minutes, Ventana). The rabbit primary antibody that reacts to fibronectin, (#ab2413, Abcam, Cambridge, MA) was used at a 1:100 concentration in Dako antibody diluent (Carpenteria, CA) and incubated for 32 minutes. The Ventana OmniMap anti-rabbit secondary antibody was used for 20 min. The detection system used was the Ventana OmniMap kit and slides were then lightly counterstained with Hematoxylin.  Slides were then dehydrated and coverslipped as per normal laboratory protocol. Stained slides were scanned on an Ariol SL-50 Automatic Scanning System, and the whole tumor sections were quantified using the Aperio™ (Vista, CA) ScanScope XT with a 200x/0.8NA objective lens at a rate of 2 minutes per slide via Basler tri-linear-array detection.

**References**

1. Dasgupta P, Rizwani W, Pillai S, Kinkade R, Kovacs M, Rastogi S, et al. Nicotine induces cell proliferation, invasion and epithelial-mesenchymal transition in a variety of human cancer cell lines. Int J Cancer. 2009;124:36-45.

2. Wang S, Nath N, Fusaro G, Chellappan S. Rb and prohibitin target distinct regions of E2F1 for repression and respond to different upstream signals. Mol Cell Biol. 1999;19:7447-60.

3. Kmiecik TE, Shalloway D. Activation and suppression of pp60c-src transforming ability by mutation of its primary sites of tyrosine phosphorylation. Cell. 1987;49:65-73.

4. Michaelson JE, Ritzenthaler JD, Roman J. Regulation of serum-induced fibronectin expression by protein kinases, cytoskeletal integrity, and CREB. Am J Physiol Lung Cell Mol Physiol. 2002;282:L291-301.

5. Gilles C, Polette M, Mestdagt M, Nawrocki-Raby B, Ruggeri P, Birembaut P, et al. Transactivation of vimentin by beta-catenin in human breast cancer cells. Cancer research. 2003;63:2658-64.

6. Zheng Y, Ritzenthaler JD, Roman J, Han S. Nicotine stimulates human lung cancer cell growth by inducing fibronectin expression. Am J Respir Cell Mol Biol. 2007;37:681-90.

7. Pillai S, Dasgupta P, Chellappan SP. Chromatin immunoprecipitation assays: analyzing transcription factor binding and histone modifications in vivo. Methods Mol Biol. 2009;523:323-39.

8. Fusaro G, Dasgupta P, Rastogi S, Joshi B, Chellappan S. Prohibitin induces the transcriptional activity of p53 and is exported from the nucleus upon apoptotic signaling. J Biol Chem. 2003;278:47853-61.

9. Wang S, Fusaro G, Padmanabhan J, Chellappan SP. Prohibitin co-localizes with Rb in the nucleus and recruits N-CoR and HDAC1 for transcriptional repression. Oncogene. 2002;21:8388-96.

10. Dasgupta P, Rastogi S, Pillai S, Ordonez-Ercan D, Morris M, Haura E, et al. Nicotine induces cell proliferation by beta-arrestin-mediated activation of Src and Rb-Raf-1 pathways. J Clin Invest. 2006;116:2208-17.

11. Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, et al. A rapid in vitro assay for quantitating the invasive potential of tumor cells. Cancer Res. 1987;47:3239-45.

12. Pillai S, Rizwani W, Li X, Rawal B, Nair S, Schell MJ, et al. ID1 facilitates the growth and metastasis of non-small cell lung cancer in response to nicotinic acetylcholine receptor and epidermal growth factor receptor signaling. Mol Cell Biol. 2011;31:3052-67.

13. Janda E, Litos G, Grunert S, Downward J, Beug H. Oncogenic Ras/Her-2 mediate hyperproliferation of polarized epithelial cells in 3D cultures and rapid tumor growth via the PI3K pathway. Oncogene. 2002;21:5148-59.

14. Janda E, Lehmann K, Killisch I, Jechlinger M, Herzig M, Downward J, et al. Ras and TGF[beta] cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. J Cell Biol. 2002;156:299-313.

15. Shin SR, Sanchez-Velar N, Sherr DH, Sonenshein GE. 7,12-dimethylbenz(a)anthracene treatment of a c-rel mouse mammary tumor cell line induces epithelial to mesenchymal transition via activation of nuclear factor-kappaB. Cancer research. 2006;66:2570-5.

16. Wang S, Ghosh RN, Chellappan SP. Raf-1 physically interacts with Rb and regulates its function: a link between mitogenic signaling and cell cycle regulation. Mol Cell Biol. 1998;18:7487-98.

17. Dasgupta P, Rizwani W, Pillai S, Davis R, Banerjee S, Hug K, et al. ARRB1-mediated regulation of E2F target genes in nicotine-induced growth of lung tumors. Journal of the National Cancer Institute. 2011;103:317-33.