Supporting Information

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SI Materials and Methods

Generation of Cell Lines. Primary human lymph node fibroblasts were purchased from ScienCell (2530) and cultured in fibroblast medium (2301) according to the manufacturer's instructions. Conditioned media were generated by incubating plates of 90% confluent cells with serum-free media for 24 h. A549 and H322, H2030 and H2030-BrM3, and LKR10 and LKR13 cells were obtained from the American Type Culture Collection, Joan Massague laboratory, and Tyler Jacks laboratory, respectively (1, 2). T_{Met} (392T5, 482T1) and T_{nonMet} (368T1, 394T4, 802T4) cells were used as previously described (3). CK1750 and SC241 cells were generated by culturing tumor cells from Kras;p53 donor mice in DMEM supplemented with 10% FBS, L-glutamine, and penicillin/streptomycin. All other cell lines were cultured in RPMI-1640 media supplemented with 10% FBS, L-glutamine, and penicillin/streptomycin. Lentiviral shRNAs against tropomyosin-related kinase B (TrkB) (clones TRCN0000002243, TRCN0000002244), hypoxia-inducible factor-1 (HIF-1α) (clones TRCN0000010819, TRCN0000003808), nerve growth factor receptor (NGFR) (clones TRCN0000058154, TRCN0000058155, TRCN0000058156), or GFP (SHC005) were used to make stable knockdown cell lines according to The RNAi Consortium (TRC) instructions (Sigma). For overexpression of wild-type and kinase dead TrkB, 4 μg of pcDNA3-EmptyVector, pcDNA3-TrkB-flag, or pcDNA3-TrkB-K571M-flag constructs, gifts from the Rosalind Segal laboratory (4), were transfected into 95% confluent six-well dishes with Lipofectamine 2000 (Invitrogen). Twenty-four hours later, transfected cells were treated with 400 μg/mL G418 (Sigma) for 2 wk to select resistant colonies and make stable cell lines. For retroviral overexpression of constitutively active Akt, VSVG plasmid and pWZL-Neo-Myr-Flag-DEST or pWZL-Neo-Myr-Flag-AKT1 constructs, gifts from the Jean Zhao laboratory (5), were transfected in a 1.2:0.8 ratio into PlatE cells with FuGene (Promega). A total of 6 μg of DNA was used per 10-cm plate. The resulting virus-containing media were used to infect target cells in the presence of 8 μg/mL polybrene (Sigma) on days 2 and 3 after PlatE cell transfections. Target cells were treated with 400 μg/mL G418 (Sigma) for 2 wk to select resistant colonies.

Brain-Derived Neurotrophic Factor Immunoprecipitations and ELISAs. For the brain-derived neurotrophic factor (BDNF) immunoprecipitations, 2 μg/mL sheep IgG control (12-515, Millipore) or BDNF antibody (AB1513P, Millipore) was incubated with the conditioned media on a shaker for 4 h at 4 °C. Protein G Sepharose 4 Fast Flow Beads were suspended in PBS according to the manufacturer's instructions, and 50 μL/mL protein G slurry was added to each sample and incubated on a shaker overnight at 4 °C (17-0618-01, GE Healthcare). The samples were centrifuged and a RayBio Human BDNF ELISA kit was used to measure supernatant conditioned media BDNF concentrations (ELH-BDNF-001, RayBiotech). For the rescue experiments, 200 pg/mL human recombinant BDNF was added to the BDNF-depleted conditioned media (450-02, PrepoTech). Alternatively, conditioned media from serum-starved cell lines was run on the BDNF ELISA Kit.

FACS. Primary mouse lung or lymph node cells were isolated from 8- to 10-wk-old wild-type 129 mice or from 18-wk-old mice bearing lung tumors as previously described (6) using Mac1-APC-Cy7, pan CD45-PE, CD31-APC, and EpCAM-PE-Cy7 (BioLegend) with 4′, 6-diamidino-2-phenylindole (DAPI) (Sigma) staining to eliminate dead cells. For lymph node cell isolation, 10–11 mice were used per group. Cell sorting was performed with a BD FACS Aria and data were analyzed with FlowJo software (Tree Star, Inc.).

Quantitative RT-PCR Gene Expression Analysis. RNA was extracted using the Absolutely RNA Microprep Kit (Stratagene). cDNA was made using the SuperScript III Kit (Invitrogen) and analyzed using Taqman Assays (Applied Biosystems) for human *TRKB*/*NTRK2* (Hs00178811_m1), *TRKA*/*NTRK1* (Hs01021011_m1), *TRKC*/*NTRK3* (Hs00176797_m1), *NGFR* (Hs00609976_m1), *HIF1A* (Hs00153153_m1), *ECADHERIN* (Hs01023894_m1), *TWIST* (Hs01675818_s1), and *SNAIL* (Hs00195591_m1) and for mouse *BDNF* (Mm04230607_s1), *MAC1*/*ITGAM* (Mm00434455_m1), *CD45*/*PTPRC* (Mm01293577_m1), *PDGFRB* (Mm00440677_m1), *SMA*/*ACTA2* (Mm00725412_s1), *SPC*/*SFTPC* (Mm00488144_m1), and *TRKB*/*NTRK2* (Mm00435422_m1) with a StepOnePlus Real-Time PCR System (Applied Biosystems) and software as per the manufacturer's recommendations. Human (4326317E) or mouse (4352339E) *GAPDH* was used as an endogenous control for normalization (Applied Biosystems).

In Vitro Assays. Migration assays were performed with 24-well transwell plates according to the manufacturer's instructions (Corning). Briefly, cells were serum-starved overnight, harvested with Accutase (Millipore), and counted. The bottom chambers of transwells were filled with 600 μL serum-free, serum, or lymph node fibroblast conditioned media, and 50,000 cells per well in 100 μL were plated in serum-free media in the top chambers in triplicate. After a 24-h incubation, nonmigratory cells were removed with a cotton swab, and the migratory cells were fixed with formalin and stained with crystal violet. Five 10× images of each transwell were taken, the number of crystal violet+ cells were counted manually using ImageJ, and the average number of cells per 10× image was calculated. Alternatively, cells were stained with DAPI, 2× images of each transwell were taken with a BD Biosciences Pathway Imager, and the number of DAPI+ cells in each well was determined with an automated ImageJ macro. The average number of control (e.g., shGFP or DMSO-treated) migratory cells per 2× field was set to 1. For migration assays with ANA-12, 0.1% DMSO or 10, 30, or 50 μM ANA-12 (Maybridge, BTB06525) was incubated in both the 100-μL cell suspension in the top chambers and media in the bottom chambers of the transwell plates. For the hypoxia experiments, cells were placed in an incubator with either 21 or 1% oxygen for 16 h. For the hypoxia migration assays, the cells were suspended in 100 μL serum-free media in the top chambers, and the media in the bottom chambers of the transwell assays were treated with 0.1% DMSO or 200 nM K252A (EMD Millipore). For the proliferation assays, 50,000 cells per well were plated in triplicate in 96-well plates, and CellTiter-Glo Luminescent Cell Viability Assays were performed after 24 h (Promega). For anoikis assays, 500,000 cells per well were plated in six-well ultra-low attachment plates (VWR) in 2 mL lymph node fibroblast conditioned media. Twenty-four hours later, cells were digested into a single-cell suspension with Accutase (Sigma), and viable cell counts were determined using trypan blue staining (Sigma).

Phospho-Kinase Arrays and Immunoblots. For the phospho-kinase arrays and immunoblots, cells were serum-starved for 4–5 h; treated with 50 ng/mL BDNF (450-02, PrepoTech) for 0, 10, or 30 min; or treated with 50 ng/mL BDNF and DMSO or 200 nM K252A for 3 h. Cells were washed with PBS, harvested with cell scrapers in RIPA protein extraction buffer supplemented with

protease and phosphatase inhibitor mixture tablets (Roche), and rotated at 4 °C for 1 h and vortexed every 15 min. Samples were spun at 13,500 × *g* for 20 min at 4 °C. The protein supernatants were incubated on Proteome Profiler Human Phospho-Kinase Arrays (R&D Systems) or separated by SDS/PAGE, transferred to nitrocellulose membranes, and blotted with antibodies recognizing Akt (1:3,000 dilution, clone C67E7; Cell Signaling), pAkt (1:1,000 dilution, clone C31E5E; Cell Signaling), ERK (1:2,000- dilution, clone 3A7; Cell Signaling), pERK (1:2,000 dilution, 9101; Cell Signaling), or pan-actin (1:5,000 dilution, clone D18C11; Cell Signaling), and HRP-conjugated secondary antibodies (1:5,000 dilution, Santa Cruz). For the HIF-1α and HIF-2α immunoblots, cells were washed with PBS and lysed using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific). Nuclear proteins were separated by SDS/PAGE, transferred to PVDF membranes, and blotted with antibodies recognizing HIF-1α (1:200 dilution, clone 54, BD), HIF-2α (1:500 dilution, NB100-122, Novus Biologicals), or TATA-binding protein (1:2,000 dilution, clone 1TBP18, AbCam) and HRP-conjugated secondary antibodies (1:5,000 dilution, Santa Cruz).

Mice and Histology. All animal studies were approved by the Boston Children's Hospital Institutional Animal Care and Use Committee. *Lox-Stop-Lox-KrasG12D* (Kras), *Lox-Stop-Lox-KrasG12D;p53flox/flox* (Kras;p53), and *TrkBflox/flox* (TrkB) mice were heterozygous for Kras and homozygous for p53 and TrkB alleles. Six- to 8-wk-old Kras;p53 (129/C57) and Kras;p53;TrkB (129/C57/ICR) mice were infected with adenoviral-Cre intranasally, and mice were euthanized 17–18 wk after infections. Standard procedures were used to harvest and fix organs. Lung, liver, spleen, kidney, lymph nodes, chest wall, and brain were dissected from the animals, and tissues were fixed in 4% (vol/vol) paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) in accordance with standard procedures and analyzed for tumors by at least two investigators, including a pathologist with expertise in mouse lung cancer (R.T.B.). Briefly, grade 1 tumors were uniform with normal nuclei; grade 2 tumors were uniform but had enlarged nuclei and prominent nucleoli; grade 3 tumors had enlarged cells with pleomorphic nuclei, prominent nucleoli, and nuclear molding; and grade 4 tumors had very large pleomorphic nuclei with a high degree of nuclear atypia, giant multinucleated cells, and invasion into the blood or lymphatic vessels or through the basement membrane.

Transplantation Assays. For s.c. injections, recipient NOD scid gamma (NSG) mice were injected with 500,000 cells resuspended in 100 μL 1:1 PBS:Matrigel under the skin on their hind flank. Subcutaneous tumors were measured weekly with calipers and mice were euthanized when tumor volumes (length × width × height) reached a size of 1,000 mm^3. For i.v. injections, recipient NSG mice were injected with 500,000 cells resuspended in 200 μL PBS in the lateral tain vein. Mice were euthanized 5–6 wk after injection. Quantification of lung tumor nodules was performed by scanning H&E lung sections and using ImageJ software to calculate tumor burden (tumor volume divided by total lung volume). Quantification of the number of lung tumors was performed by a pathologist (R.T.B.) by counting the number of tumors per H&E lung section from each animal. A Cochran–Armitage test was performed with mStat for tumor grade percentage statistical analysis.

Immunohistochemistry and Tissue Microarray. For Ki67 immunohistochemistry, an antibody to Ki67 (1:20,000 dilution, NCL-Ki67p, Leica Biosystems) was used with conditions including citrate buffer antigen retrieval with a PT Module (Thermo Scientific), peroxidase block, and processing with the Vectastain Elite ABC rabbit IgG kit (Vector Laboratories) and DAB substrate kit (Vector Laboratories). Sections were blindly scored by a pathologist to determine the percentage of Ki67+ lung tumor cells (L.M.S.).

For CD31 immunohistochemistry, an antibody to CD31 (1:100 dilution, CM303, Biocare) was used with conditions including protease digestion (E2, Leica Bond Enzyme Pretreatment Kit), peroxidase and protein block (DAKO), and processing with rabbit anti-rat secondary antibody (DAKO, 1:750) and the Envision+ System-HRP (DAB) rabbit kit (K4010, DAKO). Sections were blindly scored on a 0–3 scale by a pathologist to determine the amount of CD31+ cells in each tumor (R.T.B.). Staining intensity was scored as "no expression" (absent staining = 0), "low expression" (weak staining intensity = 1), "moderate expression" (medium staining intensity = 2) or "high expression" (strong staining intensity = 3). A Cochran–Armitage test was performed with mStat for CD31 immunohistochemistry (IHC) score statistical analysis.

For TrkB immunohistochemistry, an antibody to TrkB (1:50 dilution, clone 80G2, Cell Signaling) was used with conditions including EDTA pH8 antigen retrieval (Invitrogen) with a pressure cooker, peroxidase and protein block (DAKO), and visualization with the EnVision+ System-HRP (DAB) rabbit kit (K4010, DAKO). Formalin-fixed paraffin-embedded lung adenocarcinoma specimens were obtained from the Brigham and Women's (BWH) Department of Pathology archives following approval by the BWH Institutional Review Board. Targeted genotyping of KRAS codons 12 and 13 by pyrosequencing and epidermal growth factor receptor codons 19 and 21 by sizing assays and real-time PCR, respectively, was performed as previously described in a Clinical Laboratory Improvement Amendment-certified clinical laboratory (the Center for Advanced Molecular Diagnostics at BWH) (7). These sections or a well-characterized lung adenocarcinoma tissue microarray with matched normal lung and adenocarcinoma triplicate core sections from 89 patients seen at BWH between 1997 and 1999 (8), obtained with an approved BWH Institutional Review Board protocol, were stained for TrkB. Cores were blindly scored by a pathologist (L.R.C. or L.M.S.) without access to clinical data, and staining intensity was scored as "no expression" (absent staining = 0), "low expression" (weak staining intensity = 1), or "high expression" (strong staining intensity = 2). Tissue microarray statistical analysis was performed with a 2 × 2 contingency table with a Fisher's exact test or larger contingency table with a χ^2 test, and Kaplan–Meier survival curves were generated with log-rank statistical analysis with GraphPad Stats Software. Unpaired two-tailed Student *t* tests were performed to determine significance between average values of age, smoking pack years, and tumor size.

Chromatin Immunoprecipitation. Seventy to eighty percent of confluent plates of cell lines were incubated in 1 or 20% oxygen for 8 h. The HIF-1α stabilizer deferoxamine (Sigma) was added to the media of the cells incubated in hypoxic conditions for 30 min. All media and subsequent chromatin immunoprecipitation (ChIP) solutions used on the hypoxic cells contained 1 mM deferoxamine. Cells were cross-linked with 1% formaldehyde, and the reaction stopped addition of glycine to 1 mM concentration (Sigma). After one wash with PBS, the cells were resuspended in ChIP sonication buffer (1%Triton X-100, 0.1% deoxycholate, 50 mM, Tris pH 8.1, 5 mM EDTA, 150 mM NaCl). The lysate was chilled on ice for 10 min and then sonicated for six rounds for a total of 3 min using a MISONIX 3000 sonicator. The lysate was cleared by centrifugation at 13,000 × *g* for 15 min at 4 °C. ChIPs were performed using a polyclonal rabbit antibody for HIF-1α (NB100-134) or no antibody with a mix of Protein A and Protein G beads (Amersham). For the analysis of the ChIP samples, immunoprecipitated DNAs, and 10% input DNAs were purified via a PCR purification kit (Qiagen). DNA (5 ng/μL) was analyzed by RT-PCR with SYBR Green (Applied Biosystems) to assess binding of TrkB target sequences. The data are presented as 2-ΔΔCt, normalizing for the

input and for β-actin. The sequences of primers used for qPCR are as follows: VEGF negative control region 5 kb upstream of the promoter (9)—Forward TGAGGGTTCATCAAGCTGGTGTCT and Reverse TTGGAGAGGGCAGTGCTTAACTCA; VEGF positive control promoter region (10)—Forward ACAGACGTTCCTTAGTGCTGG and Reverse AGCTGAGAACGGGAAGCTGTG; TrkB promoter region with three HREs—Forward AGACAGCCTCTACCGCGATT and Reverse ACACCCTCCAGACAAAAGCA; β-actin—Forward TCGAGCCATAAAAGGCAACT and Reverse TCTCCCTCCTCCTCTTCCTC.

Mouse Wild-Type Tissue and Human Tumor Samples. A wide variety of tissues from wild-type 129 mice or lung tumors isolated after i.v. injections were flash-frozen and pulverized on dry ice into a fine powder using a Bio-Pulverizer (Research Products International), incubated in RIPA protein extraction buffer supplemented with protease and phosphatase inhibitor mixture tablets (Roche), rotated at 4 °C for 1 h, and vortexed every 15 min. Samples were spun at 13,500 × *g* for 20 min at 4 °C, and the protein supernatants were run on BDNF ELISAs (EK000004-EK0309-1, Syd Labs). Alternatively, powdered samples were incubated in RNA lysis buffer (Stratagene) and processed for quantitative PCR (qPCR). Human lung adenocarcinoma tumor samples were obtained from surgical specimens according to a Boston Children's Hospital institutional review board-approved human subjects research protocol and pulverized. Half of each powdered sample was incubated in RNA lysis buffer (Stratagene) and processed for qPCR, and half was used to harvest protein, as described above. Protein supernatants were run on Total or Phospho-TrkB DuoSet IC ELISAs (DYC397-2, DYC688-2, R&D Systems).

Statistics. Unpaired two-tailed Student *t* tests or ANOVA were performed unless otherwise noted. Graph-Pad Prism (GraphPad Software, Inc.), Excel, and Mstat (McArdle Laboratory for Cancer Research, University of Wisconsin) were used for graphing and statistical analyses. All experiments were performed independently at least two to three times, and pooled data are represented by the mean and SEM.

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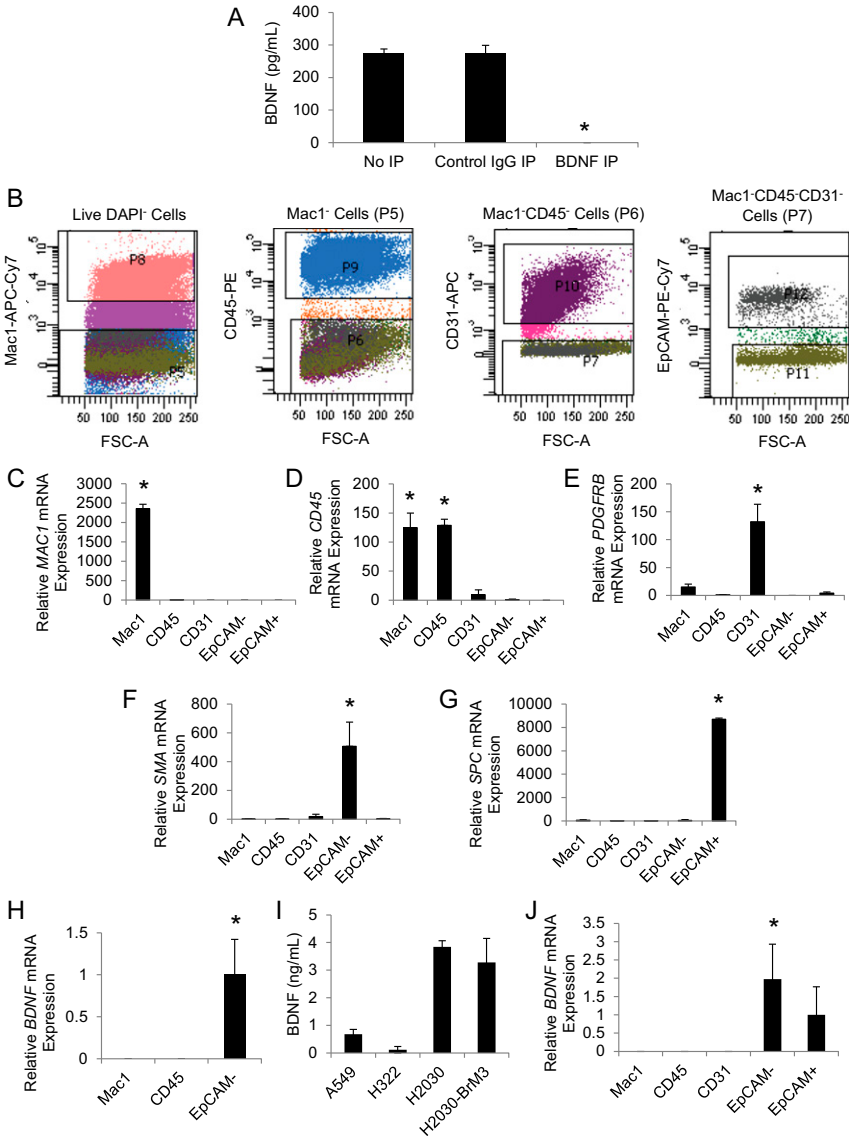


Fig. S1. Mac1^{-}CD45^{-}CD31^{-}EpCAM^{-} cells isolated from mouse lungs are fibroblasts. (*A*) BDNF ELISAs were performed on lymph node fibroblast conditioned media in which control IgG or BDNF antibodies were used for immunoprecipitations (IPs). $n = 2$. *$P < 0.01$. (*B*) Representative FACS strategy used to isolate macrophages (Mac1^{+}CD45^{+}) and hematopoietic (Mac1^{-}CD45^{+}), endothelial (Mac1^{-}CD45^{-}CD31^{+}), fibroblast (Mac1^{-}CD45^{-}CD31^{-}EpCAM^{-}) and epithelial (Mac1^{-}CD45^{-}CD31^{-}EpCAM^{+}) cells from wild-type mouse lungs. $n = 3$. (*C*) qPCR analysis showing macrophage marker *MAC1* expression of sorted mouse lung cell populations isolated in *A* relative to the CD45^{+} population, which was set to 1. $n = 3$. *$P < 0.01$. (*D*) qPCR analysis showing leukocyte marker *CD45* expression of sorted mouse lung cell populations isolated in *A* relative to the EpCAM^{-} population, which was set to 1. $n = 3$. *$P < 0.01$. (*E*) qPCR analysis showing pericyte marker *PDGFRB* expression of sorted mouse lung cell populations isolated in *A* relative to the CD45^{+} population, which was set to 1. $n = 3$. *$P < 0.01$. (*F*) qPCR analysis showing fibroblast marker *SMA* expression of sorted mouse lung cell populations isolated in *A* relative to the Mac1^{+} population, which was set to 1. $n = 3$. *$P < 0.01$. (*G*) qPCR analysis showing AT2 cell marker *SPC* expression of sorted mouse lung cell populations isolated in *A* relative to the CD45^{+} population, which was set to 1. $n = 3$. *$P < 0.01$. (*H*) qPCR analysis showing *BDNF* expression of the indicated sorted cell populations from wild-type mouse lymph nodes from 10 to 11 mice relative to the EpCAM^{-} population, which was set to 1. $n = 2$. *$P < 0.01$. (*I*) BDNF ELISAs were performed on the conditioned media of the indicated cell lines. $n = 3$. (*J*) qPCR analysis showing *BDNF* expression of the indicated sorted cell populations from the lungs of mice with tumors relative to the EpCAM^{+} population, which was set to 1. $n = 3$. *$P < 0.05$. All error bars represent mean ± SEM.

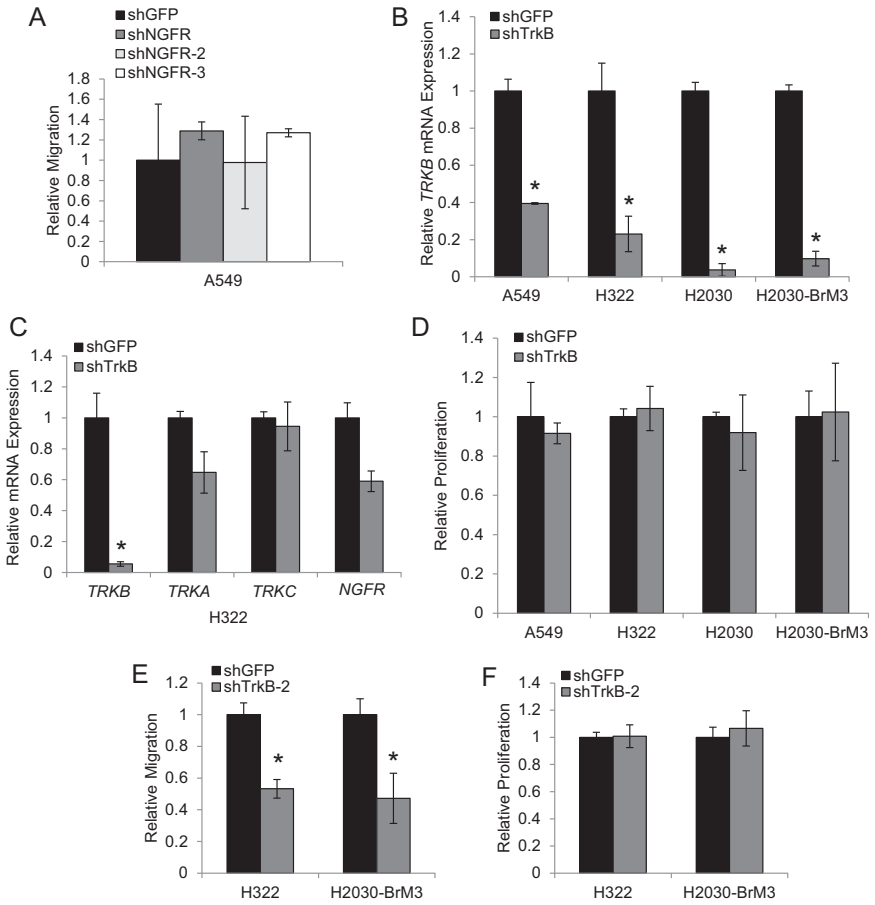


Fig. S2. Loss of TrkB decreases lung adenocarcinoma cell line migration. (*A*) Transwell assays showing migration of A549 cells expressing the indicated hairpins against GFP or NGFR toward lymph node fibroblast conditioned media. The average number of shGFP migratory cells per 2× field was set to 1. $n = 2$. (*B*) qPCR analysis showing *TRKB* expression of the indicated cells expressing shRNA targeted against TrkB (shTrkB) relative to control shRNA (shGFP), which was set to 1. $n = 3$. *$P < 0.02$. (*C*) qPCR analysis showing *TRKB*, *TRKA*, *TRKC*, and *NGFR* expression of H322 cells expressing shTrkB relative to control shGFP, which was set to 1. $n = 2$–3. *$P < 0.01$. (*D*) Relative proliferation assays comparing shGFP and shTrkB cell density using CellTiter-Glo viability assays 24 h after plating. $n = 3$. (*E*) Transwell assays showing migration of H322 and H2030-BrM3 cells expressing the indicated hairpins against GFP (shGFP) or TrkB (shTrkB-2) toward lymph node fibroblast conditioned media. $n = 2$–3. *$P < 0.02$. (*F*) Relative proliferation assays comparing H322 and H2030-BrM3 shGFP and shTrkB-2 cell density using CellTiter-Glo viability assays 24 h after plating. $n = 2$–3. All error bars represent mean ± SEM.

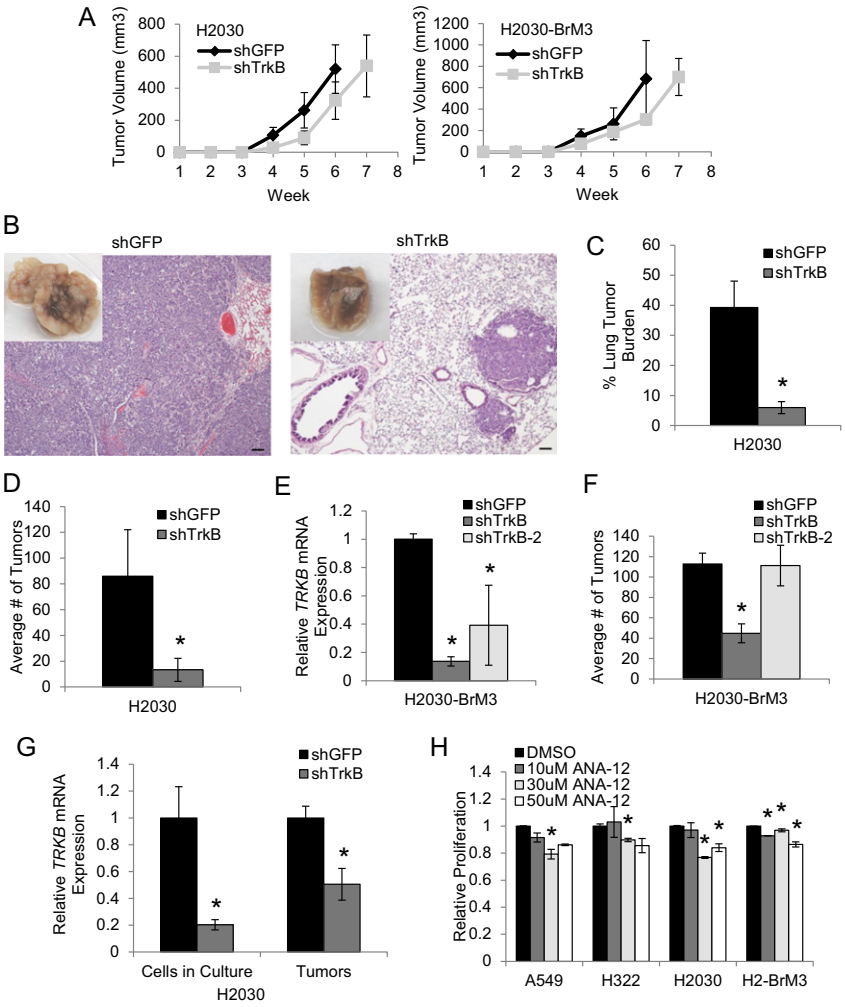


Fig. S3. Loss of TrkB decreases lung adenocarcinoma cell line metastasis. (*A*) Representative s.c. tumor growth curves of the indicated shGFP and shTrkB cell line xenografts with three mice per group. $n = 3$. (*B*) Representative lung histology from three to four mice injected i.v. with H2030-BrM3 shGFP or shTrkB cells. $n = 4$. (*C*) Quantification of lung tumor burden from three mice injected i.v. with H2030 shGFP or shTrkB cells. $n = 4$. $^*P < 0.01$. (*D*) Average number of lung tumors from the mice injected in *C*. $^*P < 0.01$. (*E*) qPCR analysis showing *TRKB* expression of i.v. injected H2030-BrM3 cells expressing shRNA targeted against TrkB (shTrkB, shTrkB-2) relative to control shRNA (shGFP), which was set to 1. $n = 2$–4. $^*P < 0.03$. (*F*) Average number of lung tumors from three to five mice per group i.v. injected with H2030-BrM3 shGFP, shTrkB, or shTrkB-2 cells ($n = 2$–4). $^*P < 0.01$. (*G*) qPCR analysis showing TrkB expression of the indicated cells expressing shRNA targeted against TrkB (shTrkB) relative to control shRNA (shGFP), which was set to 1, of H2030 cells in culture or from lung tumors propagated after injection of cells i.v. into mice. $n = 3$–4. $^*P < 0.02$. (*H*) Relative proliferation assays comparing cell density of the indicated cell lines treated with DMSO or the TrkB inhibitor ANA-12 using CellTiter-Glo viability assays 24 h after plating. $n = 2$–3. $^*P < 0.02$. All error bars represent mean ± SEM.

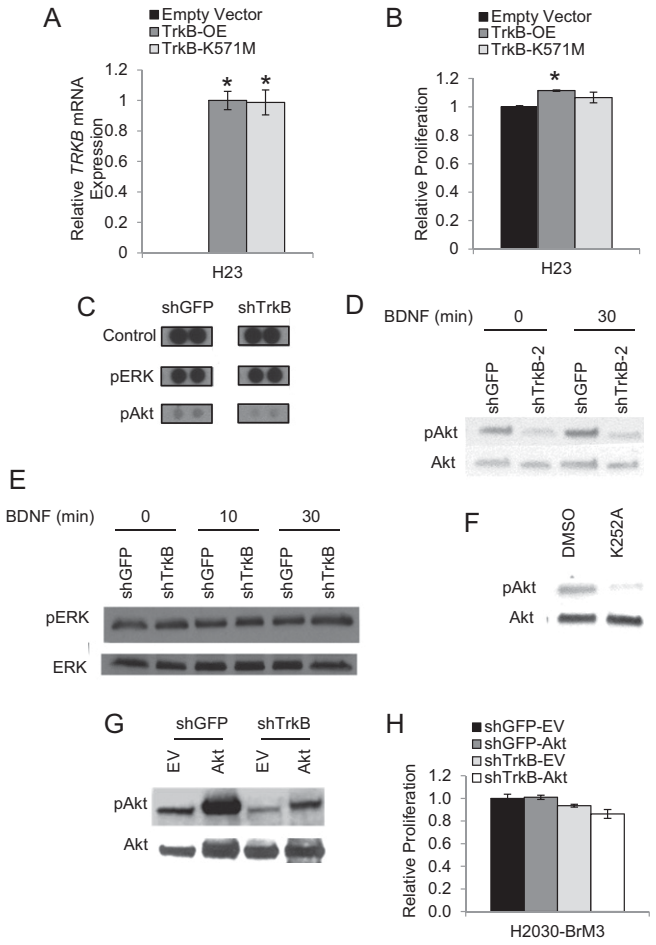


Fig. S4. Phosphorylation of Akt by TrkB drives lung cancer cell line migration. (*A*) qPCR analysis showing TrkB expression of the H23 cells overexpressing wild-type TrkB (TrkB-OE) or kinase dead TrkB (TrkB-K571M) relative to empty vector control. $n = 3$. $^*P < 0.01$. (*B*) Relative proliferation assays comparing cell density of H23 cells overexpressing wild-type TrkB (TrkB-OE) or kinase dead TrkB (TrkB-K571M) relative to Empty Vector control using CellTiter-Glo viability assays 24 h after plating. $n = 2$. $^*P < 0.02$. (*C*) Protein lysates from shGFP and shTrkB H2030-BrM3 cells stimulated with BDNF for 30 min and incubated on Human Phospho-Kinase Antibody Arrays from R&D Systems, which show the relative levels of site-specific phosphorylation of 43 kinases. $n = 1$. Control, pERK, and pAkt antibody sections of the membrane are shown. (*D*) Representative pAkt and Akt immunoblots of the protein extracts from H2030-BrM3 shGFP and shTrkB-2 cells stimulated with BDNF for 30 min. $n = 3$. (*E*) Representative pERK and ERK immunoblots of the protein extracts from H2030-BrM3 shGFP and shTrkB cells stimulated with BDNF for 10 or 30 min. $n = 3$. (*F*) Representative pAkt and Akt immunoblots of the protein extracts from H2030-BrM3 cells treated with BDNF and DMSO or the pan-Trk inhibitor K252A for 3 h. $n = 3$. (*G*) Representative pAkt and Akt immunoblots of the protein extracts from H2030-BrM3 shGFP and shTrkB cells expressing empty vector control (EV) or constitutively active Akt. $n = 3$. (*H*) Relative proliferation assays comparing cell density of shGFP and shTrkB H2030-BrM3 cells expressing empty vector (EV) control or constitutively active Akt 24 h after plating. $n = 3$. All error bars represent mean ± SEM.

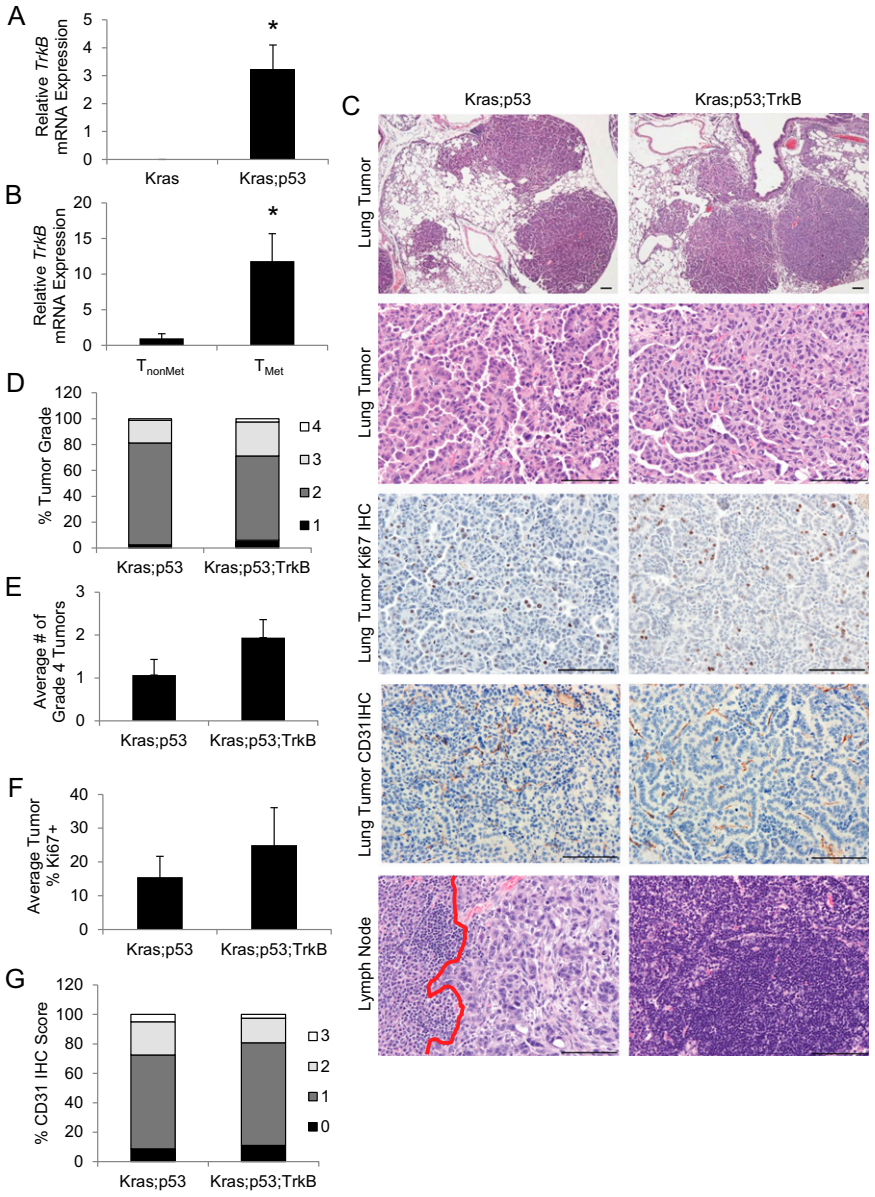


Fig. S5. Kras;p53 and Kras;p53;TrkB mice have similar lung tumors. (*A*) qPCR analysis of *TrkB* expression in Kras;p53 lung tumor cell lines (CK1750, SC241) relative to Kras lines (LKR10, LKR13). $n = 2$. *$P < 0.01$. (*B*) qPCR analysis of *TrkB* expression in Kras;p53 T_{Met} lung tumor cell lines (T_{Met}-1, T_{Met}-2) relative to T_{nonMet} lines (T_{nonMet}-1, T_{nonMet}-3). $n = 2$. *$P < 0.01$. (*C*) Representative histology of the lung tumors and lymph nodes from Kras;p53 ($n = 14$) and Kras;p53;TrkB ($n = 18$) mice. Representative Ki67 IHC of lung tumors from Kras;p53 ($n = 5$) and Kras;p53;TrkB ($n = 5$) mice. Representative CD31 IHC of lung tumors from Kras;p53 ($n = 3$) and Kras;p53;TrkB ($n = 4$) mice. Cells to the right of the red line in the Kras;p53 lymph node section are metastatic lung tumor cells. (Scale bar, 100 μm.) (*D*) Tumor-grade percentages from the mice analyzed in *C*. (*E*) Average number of invasive grade 4 lung tumors of the mice analyzed in *C*. (*F*) Quantification of the percentage of Ki67+ lung tumor cells shown in *C*. (*G*) Quantification of the CD31+ IHC score of the lung tumor cells shown in *C*. All error bars represent mean ± SEM.

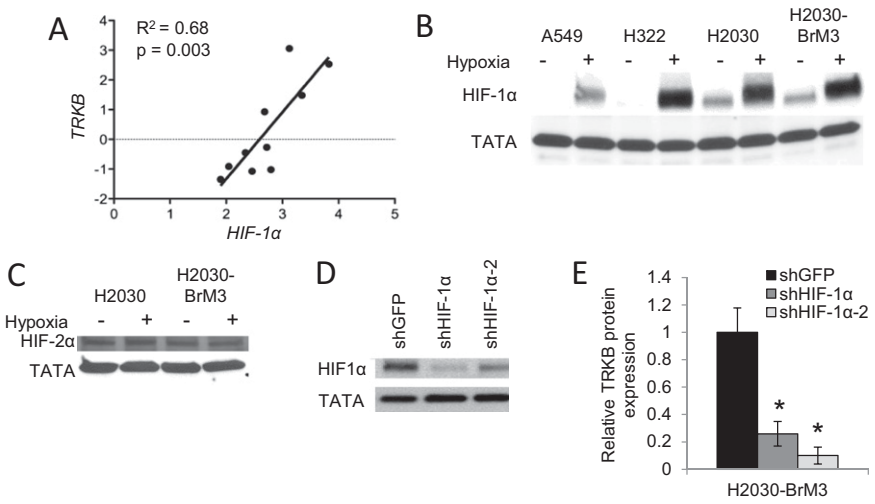
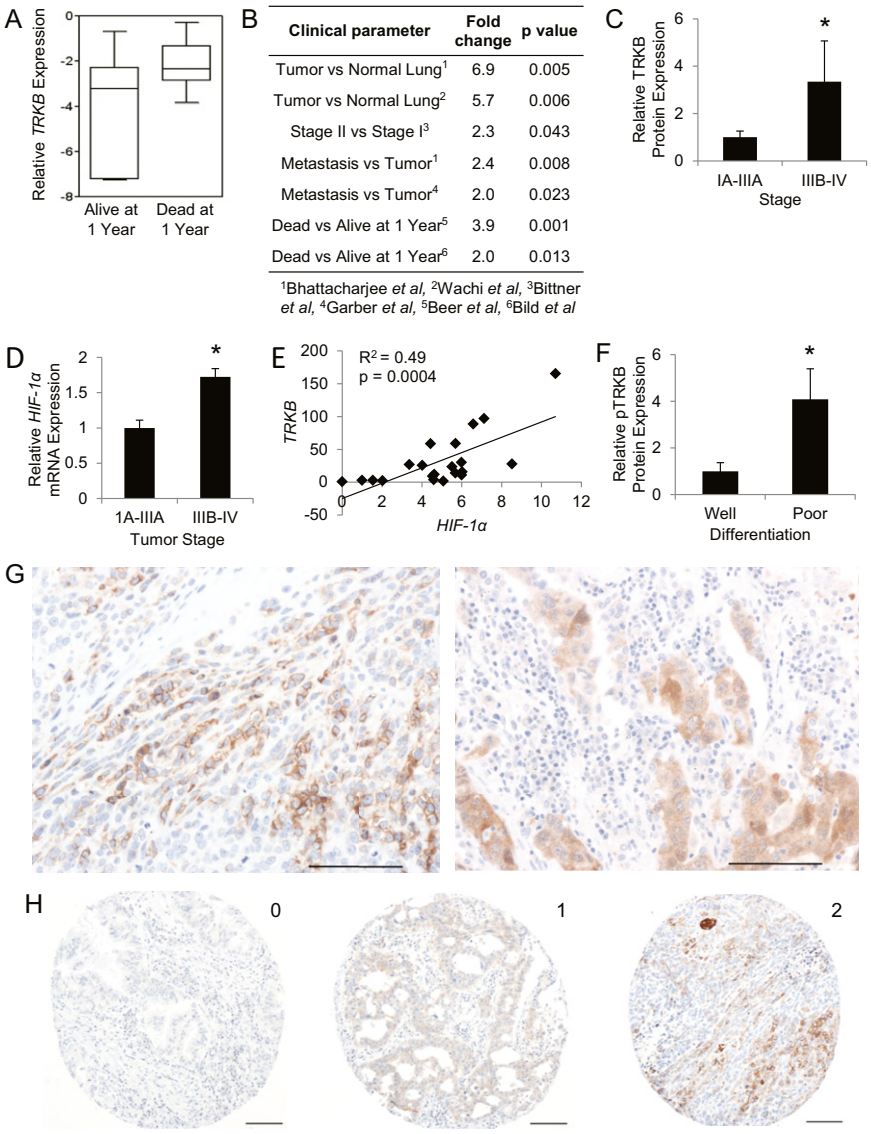


Fig. S6. HIF-1α induces TrkB expression in human lung adenocarcinoma cell lines. (*A*) Linear regression of *HIF-1α* and *TRKB* across a panel of non-small-cell lung cancer samples determined using the Oncomine cancer database (www.oncomine.org). (*B*) Representative HIF-1α immunoblot of the nuclear extracts from the indicated cell lines cultured in normoxia (21% O2) or hypoxia (1% O2). $n = 3–4$. (*C*) Representative HIF-2α immunoblot of the nuclear extracts from the indicated cell lines cultured in normoxia (21% O2) or hypoxia (1% O2). (*D*) Representative HIF-1α immunoblot of the nuclear extracts from H2030-BrM3 cells expressing control shRNA (shGFP) or shRNA targeted against HIF-1α (shHIF-1α, shHIF-1α-2). $n = 3$. (*E*) TrkB ELISA showing TRKB protein levels in H2030-BrM3 cells expressing control shRNA (shGFP) or shRNA targeted against HIF-1α (shHIF-1α, shHIF-1α-2). $n = 5$. $^*P < 0.01$. All error bars represent mean ± SEM.



Clinical parameter	Fold change	p value
Tumor vs Normal Lung[1]	6.9	0.005
Tumor vs Normal Lung[2]	5.7	0.006
Stage II vs Stage I[3]	2.3	0.043
Metastasis vs Tumor[1]	2.4	0.008
Metastasis vs Tumor[4]	2.0	0.023
Dead vs Alive at 1 Year[5]	3.9	0.001
Dead vs Alive at 1 Year[6]	2.0	0.013

[1]Bhattacharjee *et al*, [2]Wachi *et al*, [3]Bittner *et al*, [4]Garber *et al*, [5]Beer *et al*, [6]Bild *et al*

Fig. S7. Human lung tumor TrkB expression. (*A*) Log2 median-centered intensity of *TRKB* expression in the lung adenocarcinomas of patients that were alive or dead 1 y after treatment from the Beer et al. study (1) in the Oncomine cancer microarray database (http://onocomine.org). (*B*) Expression levels of *TRKB* in human non-small-cell lung cancers were determined using the Oncomine. (*C*) TrkB ELISA showing TRKB protein levels in stage IA–IIIA tumors ($n = 18$) and stage IIIB–IV primary human lung adenocarcinomas ($n = 4$). *$P < 0.01$. (*D*) qPCR analysis showing *HIF-1α* expression in stage IA–IIIA tumors ($n = 17$) relative to stage IIIB–IV human lung adenocarcinomas tumors ($n = 4$). *$P < 0.01$. (*E*) Linear regression of *HIF-1α* and *TRKB* across the lung adenocarcinoma samples described in *D*. (*F*) pTrkB ELISA showing pTRKB protein levels in well-differentiated human lung adenocarcinomas ($n = 5$) and in poorly differentiated tumors ($n = 5$). *$P < 0.05$. (*G*) Representative TrkB immunohistochemistry on human lung adenocarcinoma tissue microarray cores illustrating brown TrkB membrane (*Left*) and cytoplasmic (*Right*) staining. (Scale bar, 100 μm.) (*H*) Representative tissue cores of the lung adenocarcinoma tissue microarray illustrating brown TrkB staining. TrkB was semiquantitatively assigned to one of three categories: "no expression" (*Left*: absent staining = 0, $n = 76$), "low expression" (*Center*: weak staining = 1, $n = 10$), or "high expression" (*Right*: strong intensity = 2, $n = 3$). (Scale bar, 100 μm.) All error bars represent mean ± SEM.

1. Beer DG, et al. (2002) Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med* 8(8):816–824.

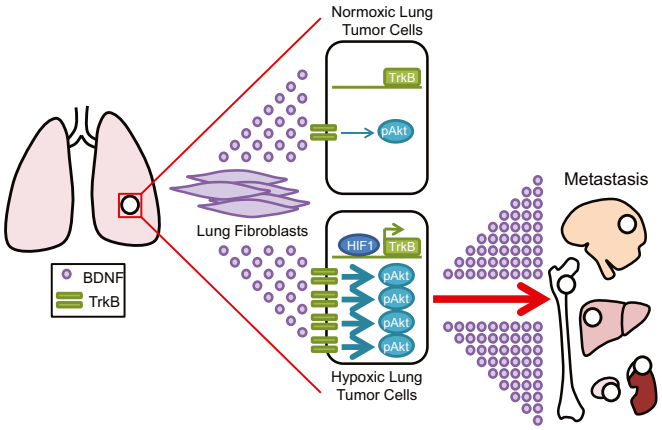


Fig. S8. Model showing how BDNF stimulates TrkB-expressing lung tumor cells to metastasize.

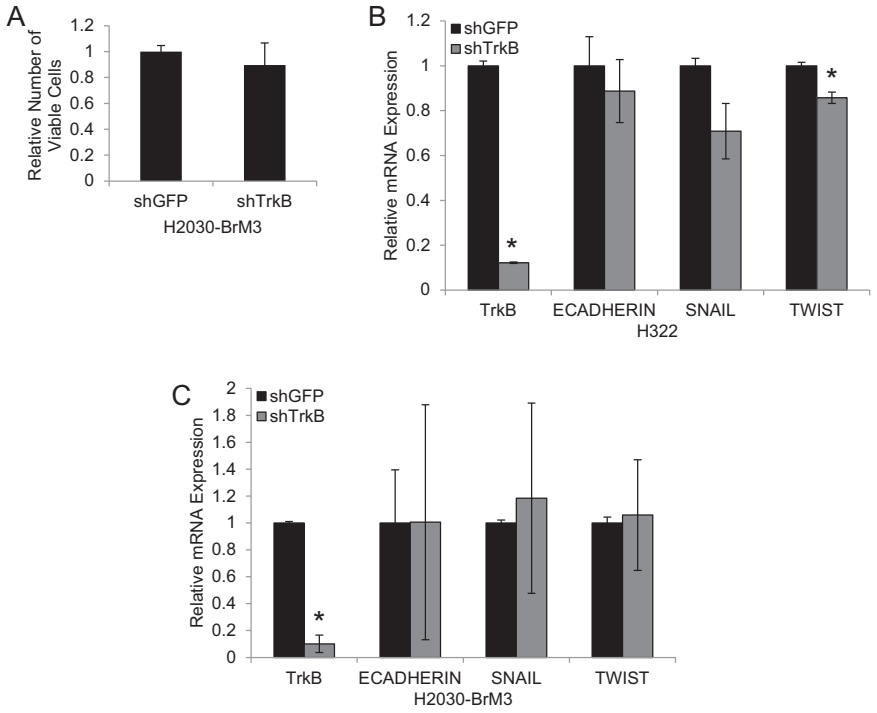


Fig. S9. Loss of TrkB does not affect anoikis or EMT of lung adenocarcinoma cells. (*A*) Anoikis assays showing the number of viable shGFP and shTrkB H2030-BrM3 cells after incubation on low-attachment plates. $n = 3$. (*B*) qPCR analysis showing *TRKB*, *ECADHERIN*, *SNAIL*, and *TWIST* expression of H322 cells expressing shTrkB relative to control shGFP, which was set to 1. $n = 2$. $^*P < 0.04$. (*C*) qPCR analysis showing *TRKB*, *ECADHERIN*, *SNAIL*, and *TWIST* expression of H2030-BrM3 cells expressing shTrkB relative to control shGFP, which was set to 1. $n = 2$–3. $^*P < 0.01$. All error bars represent mean ± SEM.

Table S1. Lung adenocarcinoma patient characteristics according to TrkB expression

Characteristic	Adenocarcinomas without TrkB expression (n = 76)	Adenocarcinomas with TrkB expression (n = 13)	P value
Sex			1.000
Male	27/76 (36%)	5/13 (38%)	
Female	49/76 (64%)	8/13 (62%)	
Age			0.480
Average	64.2 y	66.7 y	
Range	(34–84)	(52–80)	
Smoking status			0.341
Nonsmoker	11/68 (16%)	0/10 (0%)	
Smoker	57/68 (84%)	10/10 (100%)	
Pack years: average	37.4 y	59.4 y	0.037
Range	(0–150)	(15–100)	
Resection type			0.748
Wedge	23/76 (30%)	3/13 (23%)	
Lobectomy/pneuomonectomy	53/76 (70%)	10/13 (77%)	
Tumor location			0.384
Left	40/76 (53%)	5/13 (38%)	
Right	36/76 (47%)	8/13 (62%)	
Tumor size			0.671
Average	2.7 cm	2.5 cm	
Range	(0.8–6.5)	(0.6–5.5)	
Pathological stage			0.246
IA	19/76 (25%)	3/13 (23%)	
IB	30/76 (39%)	2/13 (15%)	
IIA	2/76 (3%)	1/13 (8%)	
IIB	6/76 (8%)	3/13 (23%)	
IIIA	7/76 (9%)	0/13 (0%)	
IIIB	5/76 (7%)	2/13 (15%)	
IV	7/76 (9%)	2/13 (15%)	
Tumor status			0.669
PT1	26/76 (34%)	6/13 (46%)	
PT2	39/76 (51%)	5/13 (38%)	
PT3	3/76 (4%)	0/13 (0%)	
PT4	8/76 (11%)	2/13 (15%)	
Dominant subtype			1.000
Bronchioloalveolar/acinar/papillary	55/76 (72%)	9/13 (69%)	
Solid	21/76 (28%)	4/13 (31%)	
Tumor differentiation			0.342
Well	11/76 (15%)	0/13 (0%)	
Moderate	35/76 (46%)	7/13 (54%)	
Severe	30/76 (39%)	6/13 (46%)	
Tumor heterogeneity			1.000
Pure	24/76 (32%)	4/13 (31%)	
Mixed	52/76 (68%)	9/13 (69%)	
Nodal status			0.747
Negative	47/67 (70%)	10/13 (77%)	
Positive	20/67 (30%)	3/13 (23%)	
Distant Metastasis Status			0.049
Detected	19/76 (25%)	7/13 (54%)	
Not detected	57/76 (75%)	6/13 (46%)	