

***MicroRNA-130b* functions as an oncomiRNA in non-small cell lung cancer by targeting tissue inhibitor of metalloproteinase-2**

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Supplementary Material and Methods

Cell culture

HPL1D, HLC-1, and EBC-1 cells were maintained in Ham's F12 medium (Wako, Japan). PC-9, NCI-H23, NCI-H2228, NCI-H1437, II-18, NCI-H1792, RERF-LC-KJ, LC-2/ad, NCI-H1838, NCI-H226, and Sq-1 cells were cultured in RPMI 1640 medium (Wako). Calu-3 cells and RERF-LC-AI cells were maintained in Eagle's minimum essential medium (Wako) and minimum essential medium, respectively. The medium was supplemented with 10% FBS and 100 mg/mL kanamycin. The cells were incubated at 37°C in an atmosphere containing 5% CO₂. HLC-1, EBC-1, Sq-1, LC-2/ad, RERF-LC-AI, and II-18 cells were purchased from Riken Cell Bank (Japan). PC-9 cells were purchased from Cell Resource Center for Biomedical Research and Cell Bank, Tohoku University (Japan). NCI-H23, NCI-H2228, NCI-H1650, NCI-H1437, NCI-H1792, RERF-LC-KJ, Calu-3, NCI-H1838, and NCI-H1226 cells were purchased from American Type Culture Collection (USA).

Water-soluble tetrazolium salt-8 (WST-8) cell proliferation assays

Cell proliferation was examined by WST-8 assays. NCI-H1755 cells transfected with miR-130b inhibitor were reseeded in 96-well plates (3000 cells/well) for anchorage-dependent assays. A549 cells stably overexpressing *miR-130b* were seeded

in 96-well plates (500 cells/well) for anchorage-dependent assays or Nunclon Sphera 96F-well plates (1000 cells/well; Nunc) for anchorage-independent assays. Cells were then incubated for the indicated times, and WST-8 reagent (Dojindo, Japan) was then added. Cells were incubated for 2 h at 37°C in an atmosphere containing 5% CO₂, and the optical density was determined at a wavelength of 450/630 nm (measurement/reference) using an iMark microplate reader (Bio-Rad, Hercules, CA, USA).

Wound healing assay

A549 cells stably overexpressing *miR-130b* were seeded in 24-well plates (1×10^5 cells/well) and incubated for 48 h. A wound was created in the cell monolayer at ~90% confluency using a sterile 1-ml pipette tip. Images were acquired at 0 and 48 h after wound creation using an OLYMPUS IX71 fluorescence microscope (Olympus, Japan).

RNA extraction

miRNAs were purified from NSCLC cell line 24h after transfection using an miRNeasy mini kit (Qiagen). Total RNA was isolated 24h after transfection using TRIzol reagent (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions.

Cell invasion assay

NCI-H520 cells were reseeded into inserts in 96-well plates (1.25×10^4 cells/well) in

serum-free conditions 24 h after transfection. NCI-H520 cells were incubated for 24 at 37°C in an atmosphere containing 5% CO₂.

Ago2-IP and gene array analysis

Ago2-IP was performed using an IP kit (RNA-binding protein IP-assay kit for microRNA; MBL, Japan) following the manufacturer's instructions. Briefly, RNAs immunoprecipitated with anti-ago2 antibodies were eluted from beads with hemagglutinin peptide (Wako), and QIAzol reagent (Qiagen) was added to extract RNAs. Ago2-bound RNAs were cleaned using a miRNeasy column and then subjected to microarray analysis. After confirming the purity of total RNAs by Experion (Bio-Rad), cDNA was synthesized using a WT expression kit (Applied Biosystems, Foster City, CA, USA), biotinylated using a GeneChip WT terminal labeling kit (Affymetrix, USA), and then injected into a GeneChip Human 1.0 ST array (Affymetrix). After hybridization, the GeneChip was washed and stained using a GeneChip hybridization wash and stain kit (Affymetrix) with a GeneChip fluidics station (Affymetrix). The stained GeneChip was then scanned using a GeneChip scanner 3000 (Affymetrix). Partek Genomics Suite 6.6 (Molsis, Japan) was used for data analysis (GEO accession number: GSE118274).

Western blot analysis

A549, NCI-H520 and NCI-H1975 cells were transfected with the negative control mimic or *miR-130b* mimic for 48 h, and whole-cell lysates were used for western blot analysis. The following antibodies were used in this study: anti-TIMP-2 (1:1000; cat. no. SAB1400279; Sigma-Aldrich, St. Louis, MO, USA), and anti- β -actin (polyclonal; 1:50000; cat. no. A5316; Sigma-Aldrich). Densitometric analysis was performed using NIH Image J software.

Supplementary Table 1

| Sample | mock | 130b |
|--|---------------------------|---------------------------|
| Cell number (cells) | 167.5±0.5×10 ⁴ | 161.5±3.5×10 ⁴ |
| Protein amount in cultured-medium (µg) | 453.3±9.1 | 437.1±6.7 |

Supplementary Table 2**List of genes identified by Ago2-IP and gene array analysis**

| Gene symbol | Gene name |
|--------------------|---|
| ACSL4 | acyl-CoA synthetase long-chain family member 4 |
| ACVR1C | activin A receptor, type IC |
| ADAM12 | ADAM metalloproteinase domain 12 |
| ALX4 | ALX homeobox 4 |
| ANKFY1 | ankyrin repeat and FYVE domain containing 1 |
| ANKRD13C | ankyrin repeat domain 13C |
| ANKRD52 | ankyrin repeat domain 52 |
| APCDD1 | adenomatous polyposis coli down-regulated 1 |
| AQPEP | laeverin |
| ARHGAP24 | Rho GTPase activating protein 24 |
| ARID4B | AT rich interactive domain 4B (RBP1-like) |
| ARL4A | ADP-ribosylation factor-like 4A |
| ARL6IP1 | ADP-ribosylation factor-like 6 interacting protein 1 |
| ARRDC3 | arrestin domain containing 3 |
| ATP11A | ATPase, class VI, type 11A |
| ATP13A3 | ATPase type 13A3 |
| ATP2B2 | ATPase, Ca ⁺⁺ transporting, plasma membrane 2 |
| B4GALT5 | UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide5 |
| BACH2 | BTB and CNC homology 1, basic leucine zipper transcription factor 2 |
| BAG5 | BCL2-associated athanogene 5 |
| BAHD1 | bromo adjacent homology domain containing 1 |
| BARHL2 | BarH-like homeobox 2 |
| BAZ1A | bromodomain adjacent to zinc finger domain, 1A |
| BCL2L11 | BCL2-like 11 (apoptosis facilitator) |

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|---------|--|
| BHLHE41 | basic helix-loop-helix family, member e41 |
| BMPR2 | bone morphogenetic protein receptor, type II |
| BPTF | bromodomain PHD finger transcription factor |
| BRWD1 | bromodomain and WD repeat domain containing 1 |
| BZRAP1 | benzodiazapine receptor (peripheral) associated protein 1 |
| C5orf30 | chromosome 5 open reading frame 30 |
| C7orf60 | chromosome 7 open reading frame 60 |
| CAST | calpastatin |
| CBX6 | chromobox homolog 6 |
| CCDC88A | coiled-coil domain containing 88A |
| CCNA2 | cyclin A2 |
| CCND3 | cyclin D3 |
| CD69 | CD69 molecule |
| CDK19 | cyclin-dependent kinase 19 |
| CENPO | centromere protein O |
| CEP55 | centrosomal protein 55kDa |
| CFL2 | cofilin 2 |
| CHD5 | chromodomain helicase DNA binding protein 5 |
| CHRM2 | cholinergic receptor, muscarinic 2 |
| CHST1 | carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 |
| CMPK2 | cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial |
| CNOT6 | CCR4-NOT transcription complex, subunit 6 |
| CNOT6L | CCR4-NOT transcription complex, subunit 6-like |
| COL19A1 | collagen, type XIX, alpha 1 |
| COL9A3 | collagen, type IX, alpha 3 |
| COX8C | cytochrome c oxidase subunit 8C |
| CPEB2 | cytoplasmic polyadenylation element binding protein 2 |
| CREB5 | cAMP responsive element binding protein 5 |
| CSMD1 | CUB and Sushi multiple domains 1 |
| CUL3 | cullin 3 |
| CYP2U1 | cytochrome P450, family 2, subfamily U, polypeptide 1 |
| DCUN1D3 | DCN1, defective in cullin neddylation 1, domain containing 3 |
| DDX6 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 6 |
| DEPDC1 | DEP domain containing 1 |
| DGCR14 | DiGeorge syndrome critical region gene 14 |
| DICER1 | DICER1 ribonuclease III |

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|---------|---|
| DLC1 | deleted in liver cancer 1 |
| DSEL | dermatan sulfate epimerase-like |
| DYNLL2 | dynein, light chain, LC8-type 2 |
| DYRK2 | dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 |
| E2F2 | E2F transcription factor 2 |
| E2F7 | E2F transcription factor 7 |
| EFNA5 | ephrin-A5 |
| EFNB2 | ephrin-B2 |
| EGR3 | early growth response 3 |
| EIF4E3 | eukaryotic translation initiation factor 4E family member 3 |
| ENPP5 | ectonucleotide pyrophosphatase/phosphodiesterase 5 |
| ENPP6 | ectonucleotide pyrophosphatase |
| EPB41L1 | erythrocyte membrane protein band 4.1-like 1 |
| ESR1 | estrogen receptor 1 |
| ESR1 | estrogen receptor 1 |
| ETV3 | ets variant 3 |
| EXOC5 | exocyst complex component 5 |
| EXOC5 | exocyst complex component 5 |
| EXOC5 | exocyst complex component 5 |
| FAM107B | family with sequence similarity 107, member B |
| FAM13A | family with sequence similarity 13, member A |
| FAM43A | Nfamily with sequence similarity 43, member A / |
| FANCA | Fanconi anemia, complementation group A |
| FGF10 | fibroblast growth factor 10 |
| FMNL3 | formin-like 3 |
| FMR1 | fragile X mental retardation 1 |
| FOSL2 | FOS-like antigen 2 |
| FRZB | frizzled-related protein |
| FUBP1 | far upstream element (FUSE) binding protein 1 |
| FXR1 | fragile X mental retardation, autosomal homolog 1 |
| G3BP2 | GTPase activating protein (SH3 domain) binding protein 2 |
| GAN | gigaxonin |
| GATAD2B | GATA zinc finger domain containing 2B |
| GBX2 | gastrulation brain homeobox 2 |
| GGA2 | golgi-associated, gamma adaptin ear containing, ARF binding protein 2 |
| GLRA3 | glycine receptor, alpha 3 |

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| GMEB1 | glucocorticoid modulatory element binding protein 1 |
| GMFB | glia maturation factor, beta |
| GPC6 | glypican 6 |
| GPR158 | G protein-coupled receptor 158 |
| HADHA | Hydroxyacyl-Coenzyme A Dehydrogenase/3-Ketoacyl-Coenzyme A Thiolase/Enoyl-Coenzyme A Hydratase, Alpha Subunit |
| HOXA5 | homeobox A5 |
| HOXB1 | homeobox B1 |
| HOXB2 | homeobox B2 |
| HOXD1 | homeobox D1 |
| HSPA8 | heat shock 70kDa protein 8 |
| IMPDH1 | IMP (inosine 5'-monophosphate) dehydrogenase 1 |
| INSIG1 | insulin induced gene 1 |
| IRF1 | interferon regulatory factor 1 |
| ITGA11 | integrin, alpha 11 |
| ITGA4 | integrin, alpha 4 |
| ITPK1 | inositol 1,3,4-triphosphate 5/6 kinase |
| ITPKB | inositol 1,4,5-trisphosphate 3-kinase B |
| JARID2 | jumonji, AT rich interactive domain 2 |
| KALRN | kalirin, RhoGEF kinase |
| KCNA4 | potassium voltage-gated channel, shaker-related subfamily A, member 4 |
| KCNJ15 | potassium inwardly-rectifying channel, subfamily J, member 15 |
| KCNJ6 | potassium inwardly-rectifying channel, subfamily J, member 6 |
| KCNK10 | potassium channel, subfamily K, member 10 |
| KCNN3 | potassium intermediate/small conductance calcium-activated channel subfamily N member 3 |
| KDM2A | lysine (K)-specific demethylase 2A |
| KIAA1217 | KIAA1217 |
| KIF13A | kinesin family member 13A |
| KLF6 | Kruppel-like factor 6 |
| KLHDC8A | kelch domain containing 8A |
| LBR | lamin B receptor |
| LBR | lamin B receptor |
| LDLR | low density lipoprotein receptor |
| LIMD2 | LIM domain containing 2 |
| LRCH2 | leucine-rich repeats and calponin homology (CH) domain containing 2 |

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| LRP2 | low density lipoprotein receptor-related protein 2 |
| LRP4 | low density lipoprotein receptor-related protein 4 |
| LRP6 | low density lipoprotein receptor-related protein 6 |
| LRP8 | low density lipoprotein receptor-related protein 8, apolipoprotein E receptor |
| LZIC | leucine zipper and CTNNBIP1 domain containing |
| MAP4K4 | mitogen-activated protein kinase kinase kinase kinase 4 |
| MAP7 | microtubule-associated protein 7 |
| MAPK1 | mitogen-activated protein kinase 1 |
| MDGA2 | MAM domain containing glycosylphosphatidylinositol anchor 2 |
| MECP2 | methyl CpG binding protein 2 (Rett syndrome) |
| MEMO1 | mediator of cell motility 1 |
| MEMO1 | mediator of cell motility 1 |
| MEOX2 | mesenchyme homeobox 2 |
| MIER3 | mesoderm induction early response 1, family member 3 |
| MPPED2 | metallophosphoesterase domain containing 2 |
| MTMR12 | myotubularin related protein 12 |
| MYBL1 | v-myb myeloblastosis viral oncogene homolog (avian)-like 1 |
| MYO10 | myosin X |
| MYT1L | myelin transcription factor 1-like |
| NAA30 | N(alpha)-acetyltransferase 30, NatC catalytic subunit |
| NAA50 | N(alpha)-acetyltransferase 50, NatE catalytic subunit |
| NACC2 | NACC family member 2, BEN and BTB (POZ) domain containing |
| NAP1L3 | nucleosome assembly protein 1-like 3 |
| NCKAP5 | NCK-associated protein 5 |
| NCOA1 | nuclear receptor coactivator 1 |
| NDRG2 | NDRG family member 2 |
| NEUROD1 | neurogenic differentiation 1 |
| NEUROG1 | neurogenin 1 |
| NFATC2 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 |
| NFIA | nuclear factor I/A |
| NFIC | nuclear factor I/C (CCAAT-binding transcription factor) |
| NHLH2 | nescient helix loop helix 2 |
| NHSL2 | NHS-like 2 |
| NPAT | nuclear protein, ataxia-telangiectasia locus |
| NPNT | nephronectin |
| NPTX1 | neuronal pentraxin I |

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| NRARP | NOTCH-regulated ankyrin repeat protein |
| NRP1 | neuropilin 1 |
| NUP133 | nucleoporin 133kDa |
| OSTF1 | osteoclast stimulating factor 1 |
| OTUD3 | OTU domain containing 3 |
| PCYT1B | phosphate cytidylyltransferase 1, choline, beta |
| PCYT2 | phosphate cytidylyltransferase 2, ethanolamine |
| PCYT2 | phosphate cytidylyltransferase 2, ethanolamine |
| PDE4D | NM_001165899 // PDE4D // phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 dunc |
| PDE5A | phosphodiesterase 5A, cGMP-specific |
| PEX5L | peroxisomal biogenesis factor 5-like |
| PFKFB3 | 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 |
| PGM2L1 | phosphoglucomutase 2-like 1 |
| PHF14 | PHD finger protein 14 |
| PHF20 | PHD finger protein 20 |
| PITPNM2 | phosphatidylinositol transfer protein, membrane-associated 2 |
| PMEPA1 | prostate transmembrane protein, androgen induced 1 |
| POU3F2 | POU class 3 homeobox 2 |
| POU6F1 | POU class 6 homeobox 1 |
| PPP2R5E | protein phosphatase 2, regulatory subunit B', epsilon isoform |
| PRKACB | protein kinase, cAMP-dependent, catalytic, beta |
| PRKD3 | protein kinase D3 |
| PRKG1 | protein kinase, cGMP-dependent, type I |
| PRR5L | proline rich 5 like |
| PSD | pleckstrin and Sec7 domain containing |
| PTBP2 | polypyrimidine tract binding protein 2 |
| PTEN | phosphatase and tensin homolog |
| PTGES3 | prostaglandin E synthase 3 (cytosolic) |
| PTGFRN | prostaglandin F2 receptor negative regulator |
| PTP4A1 | protein tyrosine phosphatase type IVA, member 1 |
| PTPN14 | protein tyrosine phosphatase, non-receptor type 14 |
| PURG | purine-rich element binding protein G |
| PVRL3 | poliovirus receptor-related 3 |
| QSER1 | glutamine and serine rich 1 |
| RAB14 | RAB14, member RAS oncogene family |

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| RAB9B | RAB9B, member RAS oncogene family |
| RACGAP1 | Rac GTPase activating protein 1 |
| RALBP1 | ralA binding protein 1 |
| RAPGEF4 | Rap guanine nucleotide exchange factor (GEF) 4 |
| RASD1 | RAS, dexamethasone-induced 1 |
| RASSF2 | Ras association (RalGDS/AF-6) domain family member 2 |
| RBM17 | RNA binding motif protein 17 |
| RFX7 | regulatory factor X, 7 |
| RLIM | ring finger protein, LIM domain interacting |
| RND2 | Rho family GTPase 2 |
| RNF213 | ring finger protein 213 |
| RNF38 | ring finger protein 38 |
| ROCK2 | Rho-associated, coiled-coil containing protein kinase 2 |
| RPA2 | replication protein A2, 32kDa |
| RPS6KA5 | ribosomal protein S6 kinase, 90kDa, polypeptide 5 |
| RTN1 | reticulum 1 |
| RUNDC1 | RUN domain containing 1 |
| RXRA | retinoid X receptor, alpha |
| S1PR2 | sphingosine-1-phosphate receptor 2 |
| SAMD8 | sterile alpha motif domain containing 8 |
| SERBP1 | SERPINE1 mRNA binding protein 1 |
| SESTD1 | SEC14 and spectrin domains 1 |
| SGCB | sarcoglycan, beta (43kDa dystrophin-associated glycoprotein) |
| SHC3 | SHC (Src homology 2 domain containing) transforming protein 3 |
| SIK1 | salt-inducible kinase 1 |
| SIX4 | SIX homeobox 4 |
| SKP1 | S-phase kinase-associated protein 1 |
| SLC13A2 | solute carrier family 13A2 |
| SLC24A3 | solute carrier family 24 memberA3 |
| SLC25A12 | solute carrier family 25 member A12 |
| SLC25A44 | solute carrier family 25, member 44 |
| SLC39A10 | solute carrier family 39 member 10 |
| SLC44A1 | solute carrier family 44, member 1 |
| SLC6A6 | solute carrier family 6 member A6 |
| SMAD5 | SMAD family member 5 |
| SMOC2 | SPARC related modular calcium binding 2 |

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| SMURF2 | SMAD specific E3 ubiquitin protein ligase 2 |
| SNIP1 | Smad nuclear interacting protein 1 |
| SOCS6 | suppressor of cytokine signaling 6 |
| SOX21 | SRY (sex determining region Y)-box 21 |
| SOX5 | SRY (sex determining region Y)-box 5 |
| SP1 | Sp1 transcription factor |
| SPOCK1 | osteonectin, cwcv and kazal-like domains proteoglycan1 |
| ST18 | suppression of tumorigenicity 18 |
| ST8SIA5 | ST8 alpha-N-acetylneuraminide alpha-2,8-sialyltransferase 5 |
| STAT3 | signal transducer and activator of transcription 3 |
| STC1 | stanniocalcin 1 |
| STX6 | syntaxin 6 |
| SULF1 | sulfatase 1 |
| SYNCRIP | synaptotagmin binding, cytoplasmic RNA interacting protein |
| SYNM | synemin, intermediate filament protein |
| SYT10 | synaptotagmin X |
| TACC1 | transforming, acidic coiled-coil containing protein 1 |
| TAF4 | TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor |
| TBL1XR1 | transducin (beta)-like 1 X-linked receptor 1 |
| TCF4 | transcription factor 4 |
| TESK2 | testis-specific kinase 2 |
| TEX2 | testis expressed 2 |
| TEX261 | testis expressed 261 |
| TFDP2 | transcription factor Dp-2 (E2F dimerization partner 2) |
| TGFB2 | transforming growth factor, beta 2 |
| TGFBR1 | transforming growth factor, beta receptor 1 |
| TGFBR2 | transforming growth factor, beta receptor II (70/80kDa) |
| TGFBRAP1 | transforming growth factor, beta receptor associated protein 1 |
| TGOLN2 | trans-golgi network protein 2 |
| THRA | thyroid hormone receptor, alpha |
| THSD7A | thrombospondin, type I, domain containing 7A |
| TIMP2 | TIMP metalloproteinase inhibitor 2 |
| TMEM151B | transmembrane protein 151B |
| TNFRSF1B | tumor necrosis factor receptor superfamily, member 1B |
| TNFSF10 | tumor necrosis factor (ligand) superfamily, member 10 |
| TNRC6A | trinucleotide repeat containing 6A |

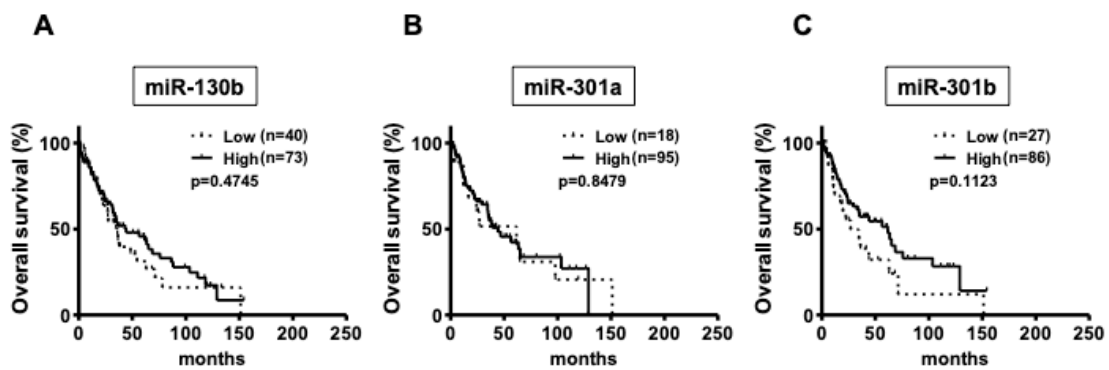
| | |
|---------|--|
| TNRC6B | trinucleotide repeat containing 6B |
| TNRC6C | trinucleotide repeat containing 6C |
| TP63 | tumor protein p63 |
| TRERF1 | transcriptional regulating factor 1 |
| TRERF1 | transcriptional regulating factor 1 |
| TRIM3 | tripartite motif-containing 3 |
| TSC22D1 | TSC22 domain family, member 1 |
| TSHZ1 | teashirt zinc finger homeobox 1 |
| UBAP2L | ubiquitin associated protein 2-like |
| UCP3 | uncoupling protein 3 (mitochondrial, proton carrier) |
| UHMK1 | U2AF homology motif (UHM) kinase 1 |
| USP28 | ubiquitin specific peptidase 28 |
| USP33 | ubiquitin specific peptidase 33 |
| USP46 | ubiquitin specific peptidase 46 |
| VAV2 | vav 2 guanine nucleotide exchange factor |
| VCPIP1 | valosin containing protein (p97)/p47 complex interacting protein |
| VSIG10 | V-set and immunoglobulin domain containing 10 |
| WDR20 | WD repeat domain 20 |
| WNK3 | WNK lysine deficient protein kinase 3 |
| WNT2B | wingless-type MMTV integration site family, member 2B |
| ZBTB20 | zinc finger and BTB domain containing 20 |
| ZBTB37 | zinc finger and BTB domain containing 37 |
| ZBTB7A | zinc finger and BTB domain containing 7A |
| ZCCHC14 | zinc finger, CCHC domain containing 14 |
| ZCCHC24 | zinc finger, CCHC domain containing 24 |
| ZFC3H1 | zinc finger, C3H1-type containing |
| ZMAT3 | zinc finger, matrin type 3 |
| ZNF275 | zinc finger protein 275 |
| ZNF3 | zinc finger protein 3 |
| ZNF652 | zinc finger protein 652 |
| ZNF704 | zinc finger protein 704 |
| ZNF784 | zinc finger protein 784 |
| ZNRF3 | zinc and ring finger 3 |

Supplementary Table 3

NSCLC clinical samples used in Supplementary Figs. 1A-C

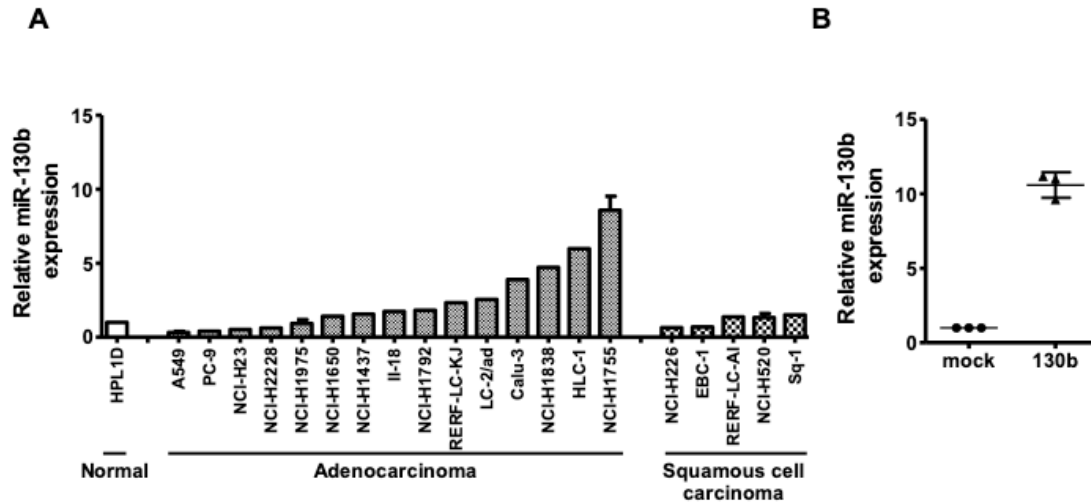
| Age (y) | | Clinical stage | |
|---------------|-------|----------------|----|
| median | 68 | I | 24 |
| range | 39-90 | II | 71 |
| Gender | | III | 10 |
| male | 84 | IV | 8 |
| female | 29 | | |

Supplementary Figures



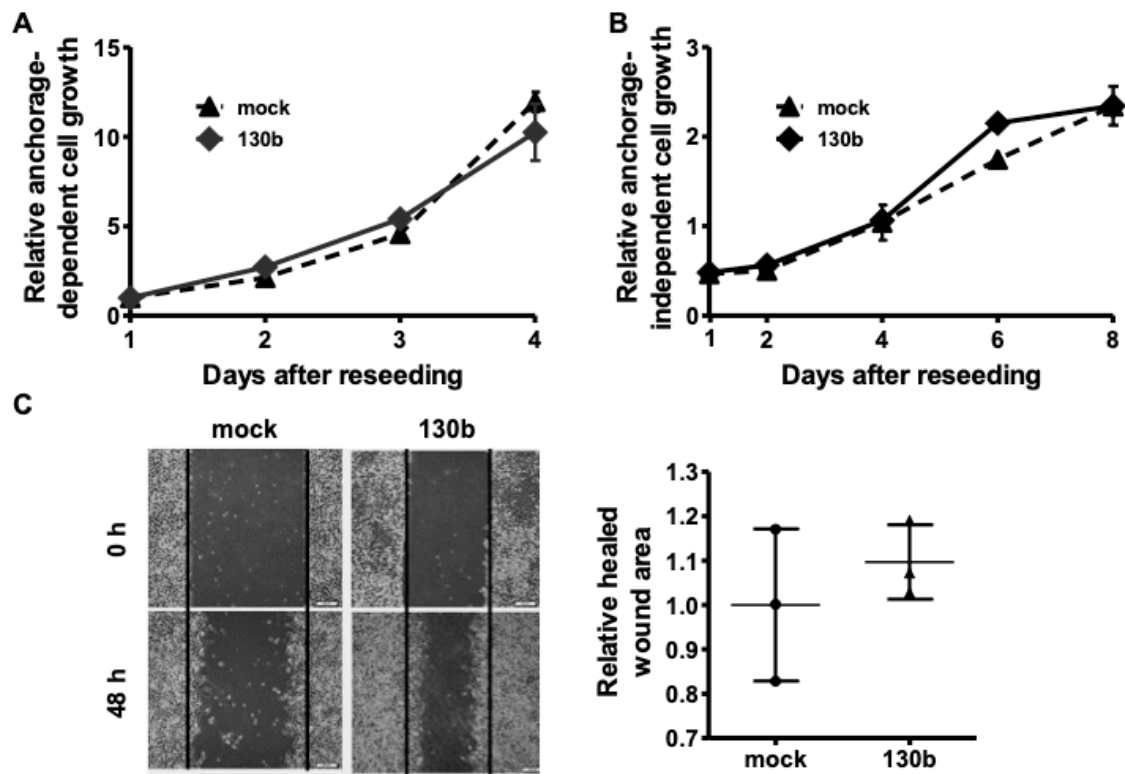
Supplementary Figure 1. Relationship between miR-130 family expression levels with overall survival of patients with squamous cell carcinoma.

(A–C) TCGA database analysis of *miR-130b*, *miR-301a*, and *miR-301b* in patients with squamous cell carcinoma. Overall survival analysis of patients with high (copy number: 1) and low (copy number: -1) *miR-130* family expression was analyzed by Kaplan-Meier analysis with log-rank tests. The number of patients analyzed is indicated in parentheses.



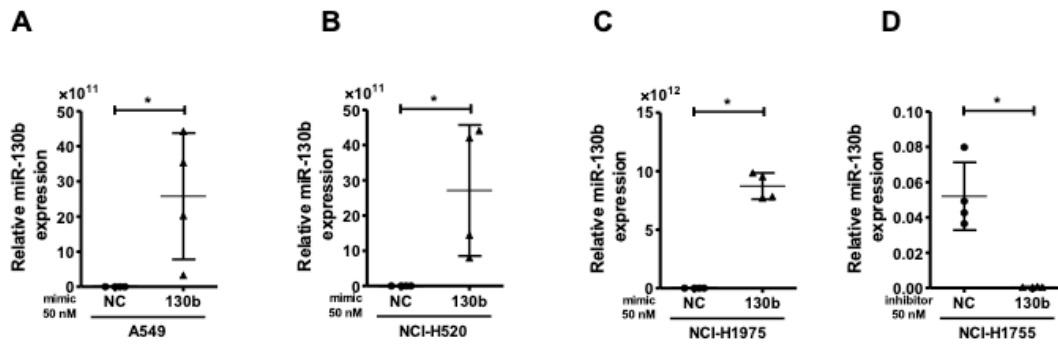
Supplementary Figure 2. *miR-130b* expression in NSCLC cell lines.

Expression of *miR-130b* in NSCLC cell lines (A) and in a clone isolated from A549 cells stably expressing *miR-130b* (B) was examined by real-time qPCR. Relative expression of *miR-130b* normalized to U6 snRNA is shown from duplicate experiments. In A549, NCI-H1975, NCI-H1755, and NCI-H520 cells, data show the means \pm standard deviations of three independent experiments.



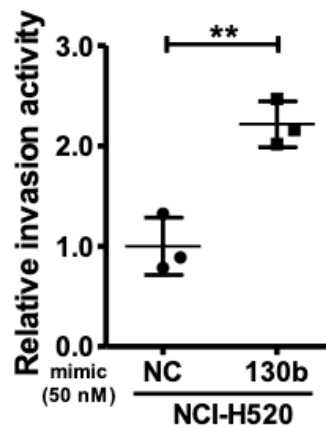
Supplementary Figure 3. Effects of *miR-130b* on the growth and migration of A549 cells.

(A, B) A549 cells stably overexpressing *miR-130b* were seeded in 96-well plates (A) or Nunclon Sphera 96F-well plates (B) and incubated for the indicated times. Relative cell proliferation was measured by WST-8 assays. Data are means \pm standard deviations of three independent experiments. (C) Relative cell motility was measured 48 h using scratch wound assays. Representative results of cell motility are shown in the left panels. Data are means \pm standard deviations of three independent experiments (right panel).



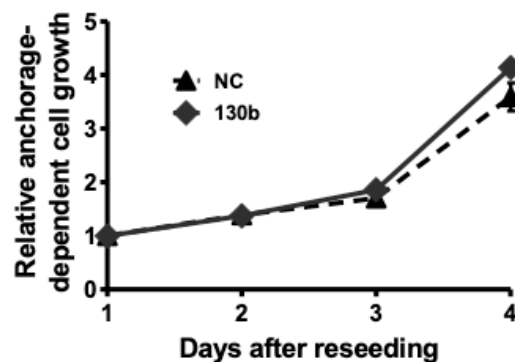
Supplementary Figure 4. *miR-130b* expression in miR-130b mimic or miR-130b inhibitor-transfected NSCLC cell lines.

miR-130b expression in miR-130b mimic or inhibitor-transfected NSCLC cell lines (A549, NCI-H520, NCI-H1975, and NCI-H1755 cells) were determined by real-time qPCR. Data are shown as the mean \pm S.D. of three independent experiments.



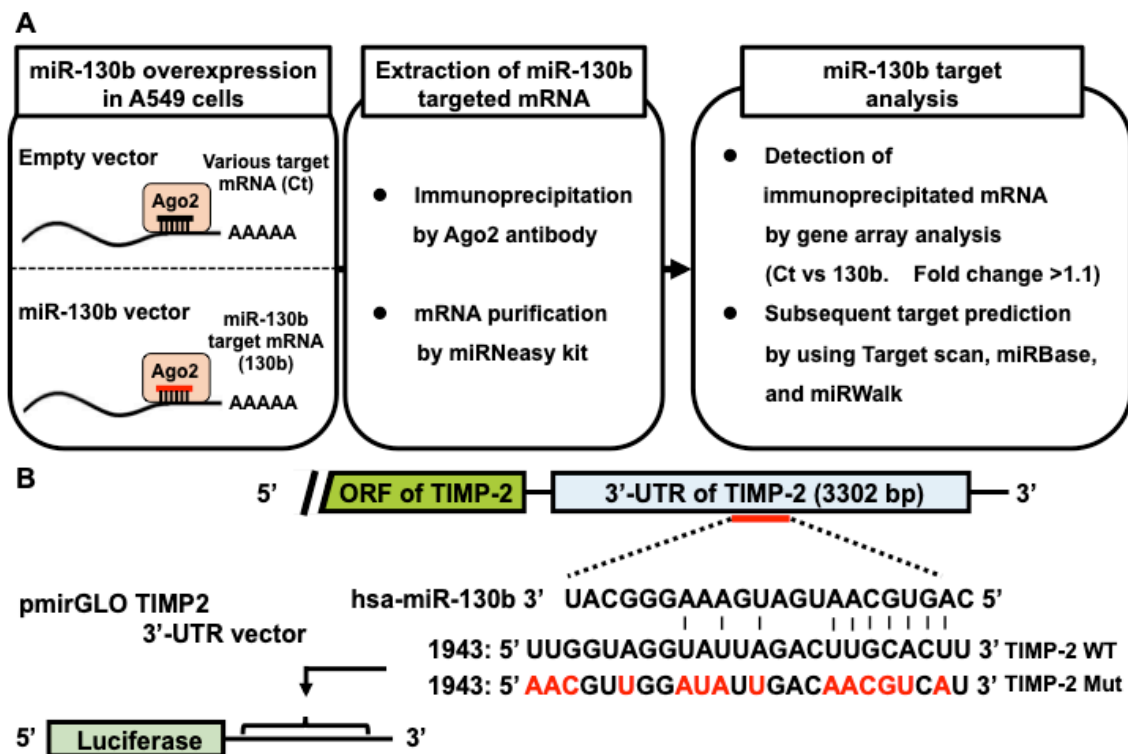
Supplementary Figure 5. Effect of *miR-130b* mimic on invasion activity in NCI-H520 cells.

NCI-H520 cells transfected with the *miR-130b* mimic (130b) or negative control miRNA mimic (NC) were subjected to invasion assays. The transfected cells were added to the upper chambers of Matrigel-coated transwell membrane inserts, and the lower chambers were filled with medium. Cells were cultured for 24 h. Fluorescence derived from invasive cells was measured. Data are the means \pm standard deviations of three independent experiments. $**p < 0.01$ for t-tests.



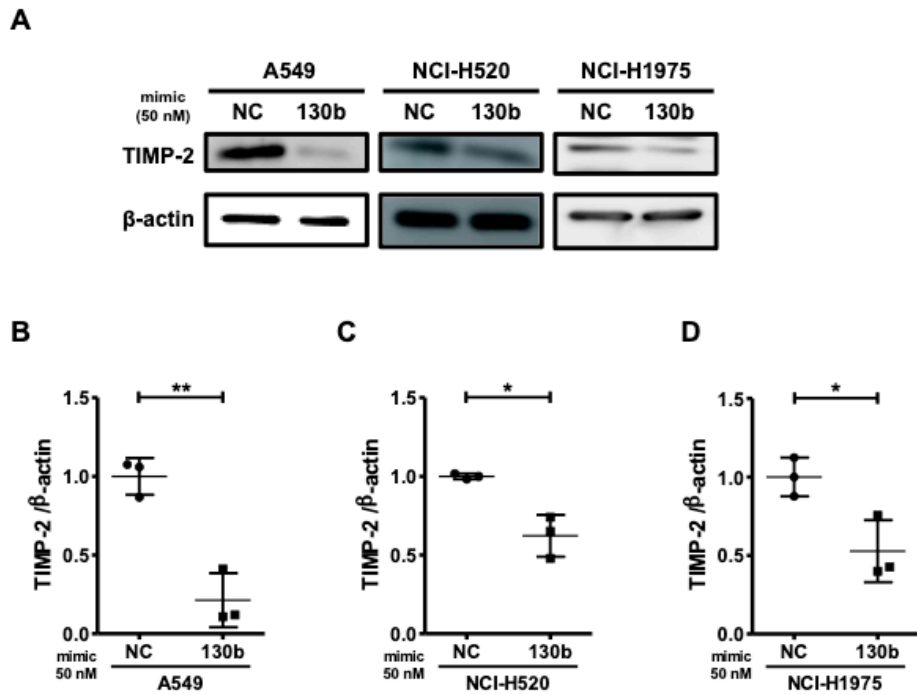
Supplementary Figure 6. Effects of *miR-130b* inhibitor on growth of NCI-H1755 cells.

NCI-H1755 cells transfected with the *miR-130b* inhibitor (130b) or negative control miRNA inhibitor (NC) were seeded into 96-well plates and incubated for the indicated times. Relative cell proliferation was measured by WST-8 assays. Data are the means \pm standard deviations of three independent experiments.



Supplementary Figure 7. Schematic illustration of the method used for identification of *miR-130b* target molecules by Ago2-IP.

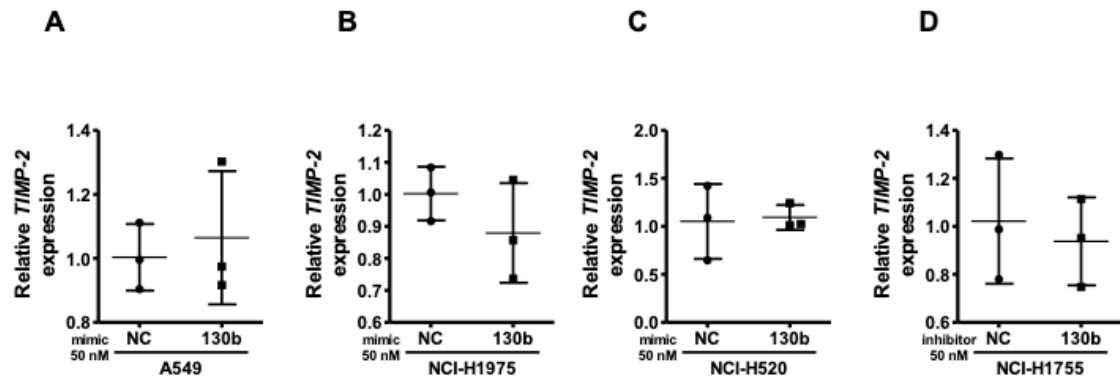
miR-130b targets were analysed using a combination of Ago2-IP, gene array analysis, and database search. (B) Sequences of predicted *miR-130b* binding sites (wild-type TIMP-2) and the mutated *miR-130b*-binding site (mutant TIMP-2) in the human TIMP-2 gene are shown. Nucleic acid bases mutated in the TIMP-2 3'-UTR are shown in red.



Supplementary Figure 8. Effect of miR-130b mimic on TIMP-2 expression in NSCLC cells.

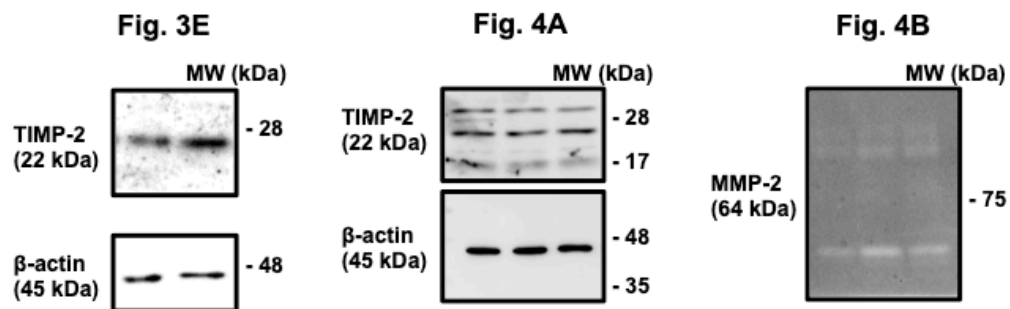
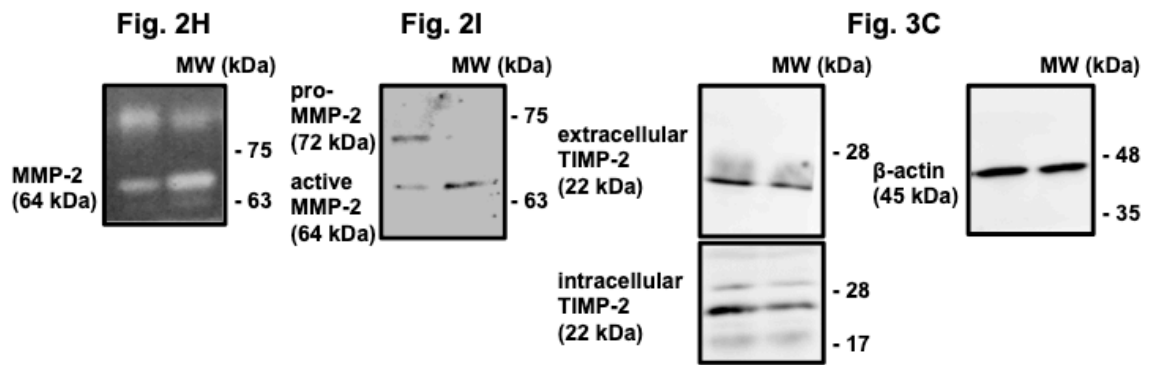
(A) A549, NCI-H520 and NCI-H1975 cells were transfected with the negative control mimic (NC) or *miR-130b* mimic (130b) for 48 h, and whole-cell lysates were subjected to western blotting analysis with anti-TIMP-2 antibodies. (B-D) Densitometric analysis of the western blot results was performed using NIH ImageJ software and the results are shown as the TIMP-2 / β -actin ratio. * $p < 0.05$, ** $p < 0.01$ for Student's t-test.

Uncropped western blot data is shown in Supplementary Fig. 10.

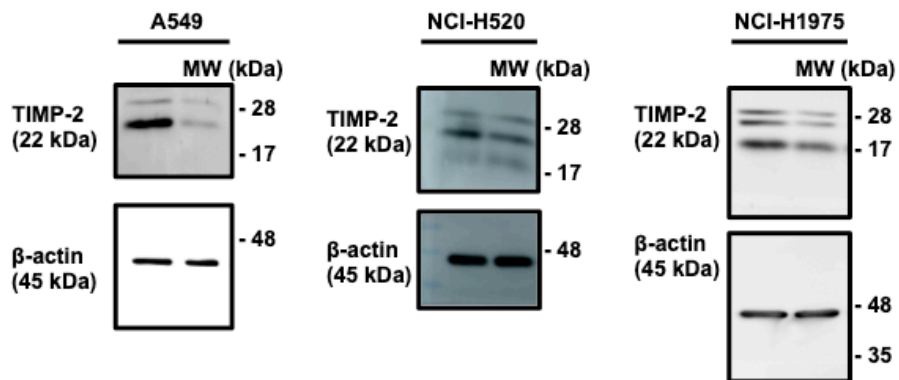


Supplementary Figure 9. Effect of miR-130b mimic or miR-130b inhibitor on *TIMP-2* mRNA expression in NSCLC cells.

(A-D) A549, NCI-H520 and NCI-H1975 cells transfected with the *miR-130b* mimic (130b) or negative control miRNA mimic (NC), or NCI-H1755 cells transfected with the *miR-130b* inhibitor (130b) or negative control miRNA inhibitor (NC) were subjected to real-time qPCR analysis of *TIMP-2*. Relative expression of *TIMP-2* normalized to *GAPDH* is shown as the means \pm standard deviations of three independent experiments.



Supplementary Fig. 8A



Supplementary Figure 10. Uncropped western blot data for Fig. 2H, 2I, 3C, 3E, 4A, 4B and Supplementary Fig. 8A.