

Supplementary materials & methods

Cell culture

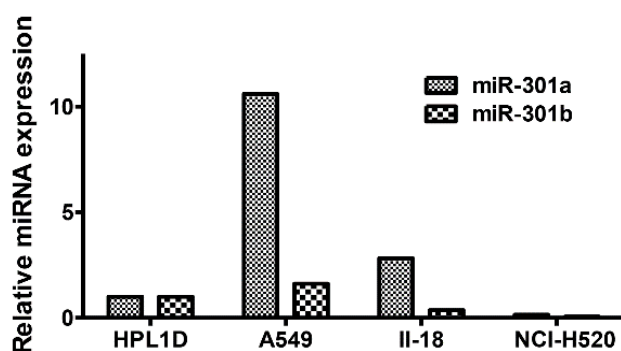
HPL1D cells were maintained in Ham's F12 medium containing 10% fetal bovine serum (FBS) and 100 mg/mL kanamycin. II-18 cells were cultured in RPMI 1640 containing 10% FBS. The cells were incubated in 37°C, 5% CO₂. HPL1D cells were kindly gifted from Department of Molecular Carcinogenesis, Nagoya University (Aichi, Japan). II-18 cells were purchased from Riken cell bank (Japan).

KEGG pathway analysis

miR-301a and miR-301b target genes obtained from miRwalk and TargetScan 7.2 database were used for pathway analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database.

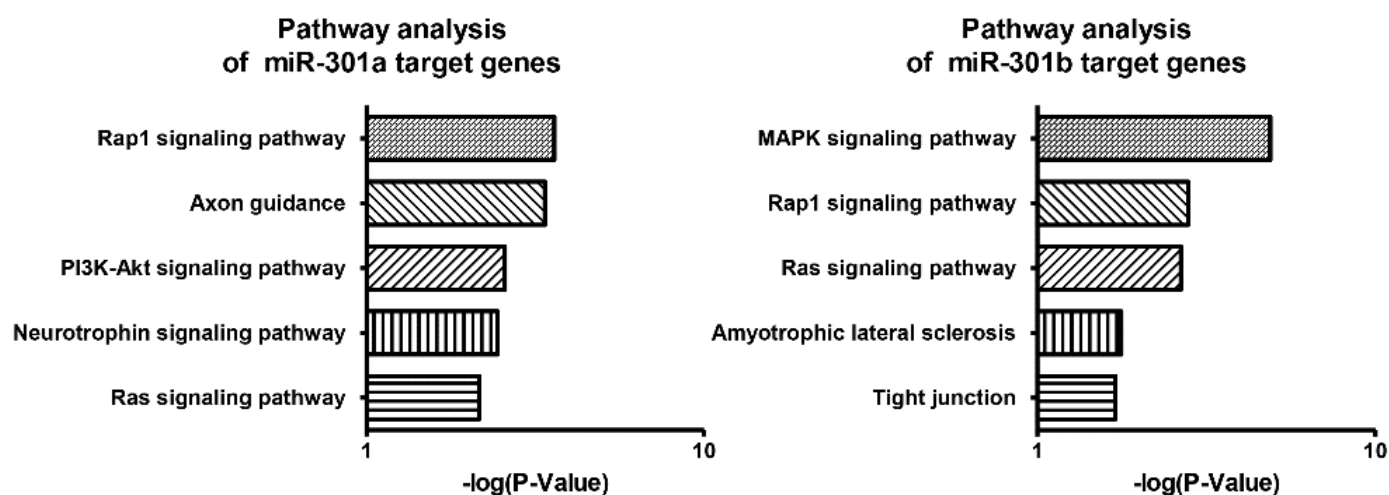
Western blot analysis

The following antibodies were used for western blot analysis in Supplementary Figure 3. anti-ERK antibody (1:5000, CST, #4695S), anti-p-ERK antibody (1:2000, CST, #4370P), and anti-β-actin polyclonal antibody (1:50000, Sigma-Aldrich, A5316). Densitometric analysis was performed using NIH ImageJ software.



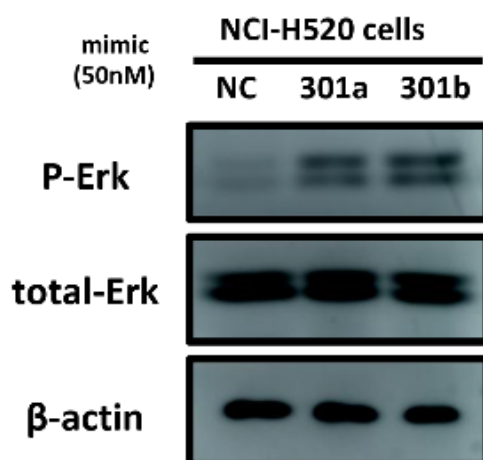
Supplementary Figure 1. miR-301a and miR-301b expressions in NSCLC cell lines and a normal lung epithelial cell line

Expressions of miR-301a and miR-301b in NSCLC cell lines (A549 cells, II-18, and NCI-H520 cells) and in a peripheral lung epithelial cell line (HPL1D) were determined by qRT-PCR. Data represents relative expression levels normalized to U6 snRNA. Data are presented mean ± S.D. of duplicate experiments



Supplementary Figure 2. Pathway analysis for predicted target genes of miR-301a and miR-301b

The miR-301a and miR-301b-predicted target genes were extracted by miRNA target prediction databases (miRwalk and TargetScan 7.2). Then, candidate target genes of miR-301a/b were subjected to functional gene enrichment analysis based on KEGG pathway database. The vertical axis provides the names of the most significantly enriched pathways ($p < 0.01$), and the horizontal axis shows the $-\log_{10}(p\text{-value})$. Values represent mean ± S.D



Supplementary Figure 3. miR-301a/b mimics enhanced phosphorylation status of ERK in NCI-H520 cells

NCI-H520 cells were transfected with miR-301a and miR-301b mimics for 48 h, and then the prepared cell lysates were subjected to western blot analysis with the indicated antibody