

Supplementary materials and methods

Cell Culture and Stable Transfection of shRNA

Cell lines A549 (RRID:CVCL_0023), H1299 (RRID:CVCL_0060), BEAS-2B (RRID:CVCL_0168), H1373 (RRID:CVCL_1465), and H1573 (RRID:CVCL_1478) were acquired from the Type Culture Collection Cell Bank at the Chinese Academy of Sciences in Shanghai, China. The cells were maintained in DMEM medium (Corning, USA) supplemented with 10% fetal bovine serum (Pan Biotechnology, Germany), grown at 37 °C in a 5% CO₂ atmosphere, and not passaged more than 25 times post-thawing. Authentication of the cells was conducted through assessment of their growth characteristics, morphologic examination, and short tandem repeat (STR) analysis, with periodic evaluations to ensure they remained Mycoplasma-free. Exponentially growing cells were utilized for the experimental procedures.

Stable knockdown of ARNTL2 was achieved in A549 and H1299 cells via transduction with pre-prepared lentiviral short hairpin RNA (shRNA) from TranSheepBio in Shanghai, China. The gene silencing efficiency was assessed through Western blot analysis. The shRNA constructs consisted of TRCN0000418980, designated sh1ARNTL2 (target sequence: 5'-GTTCAACACTTGAGATCTTTA-3'), TRCN0000021327, referred to as sh2ARNTL2 (target sequence: 5'-CCCTTACCTATCTTCTTTCAA -3'), and TRCN0000021324, labeled as sh3ARNTL2 (target sequence: 5'-GCGCGTAAACTGGACAAACTT-3'). The sequence for the scrambled control shRNA (shNC) was 5'-TTCTCCGAACGTGTCACGTAAT-3'. To introduce the recombinant lentiviral vector, an envelope plasmid (pMD2.G), and a packaging vector (psPAX2) into HEK293T cells, Lipofectamine 2000 was utilized according to the manufacturer's guidelines. Following a 48-hour transduction period, the culture supernatant was collected, filtered with a 0.45 µm filter, and subsequently employed to infect H1299 and A549 cells. Infected cells were selected using puromycin at a concentration of 9.2 µmol/L.