Methods

Wound-healing assay

Cells were seeded into 6-well plates for 24 hours incubation, followed by transfection. The transfected cells were incubated for another 48 hours to allow the cells reaching 90% confluence. Cell monolayer was carefully scratched using a sterile 200 μL pipette tip through the center of wells. Detached cells were removed by washing twice with 1xPBS. Cells were maintained in fresh medium for the indicated time periods. Images of the wound were recorded with a phase contrast microscope.

Transwell assay

Cell invasion was performed using modified Boyden chambers in 24-well plates with 8 μm pore inserts (BD, Biosciences) coated with 1 mg/mL Matrigel. In the upper chamber, 5×10^4cells were plated in 100 μL of starving medium after 24 hours transfection. The lower chamber contained 600 μL of complete medium. After 24h of incubation, invaded cells were fixed with 4% paraformaldehyde and were stained with 0.5% Crystal Violet.

Cell apoptosis

The apoptosis rate was evaluated using the AnnexinV-FITC/PI Apoptosis Detection kit according to theinstructions from the manufacturer. The cells wereseeded into 6-well plates. Following transfection for 24h and starvation for 24h (serum-free medium), the cells were collected,washed with PBS, and resuspended in 500μL bindingbuffer. Then, 5μL Annexin V-FITC and 5μLPI wereadded to the buffer and incubated at room temperaturefor 15 min in the dark. Cells were analyzed by flowcytometry (BD FACSCanto) within 1h.

Enzyme-Linked Immunosorbent Assay

Serum samples were obtained from 54 individuals, including 26 thyroid nodule patients, and 28 thyroid cancer patients. The levels of ANGPTL4 in serumwere determined by Human ANGPTL4 assay kit according to themanufacturer's instruction (Cat.27749, IBL International GMBH, Japan).

Figure legends

Supplemental Fig.1 Serum level of ANGPTL4 in thyroid nodule and thyroid cancer patients.

Supplemental Fig.2 The effect of ANGPTL4 on TPC-1 cell migration, invasion and apoptosis.

A. Overexpression of ANGPTL4 had no effect on thyroid cancer cell migration by the wound-healing assay. **B.** The level of ANGPTL4 had no effect on thyroid cancer cell invasion by the transwell assay. **C.** The level of ANGPTL4 had no effect on thyroid cancer cell apoptosis rate by flowcytometry.

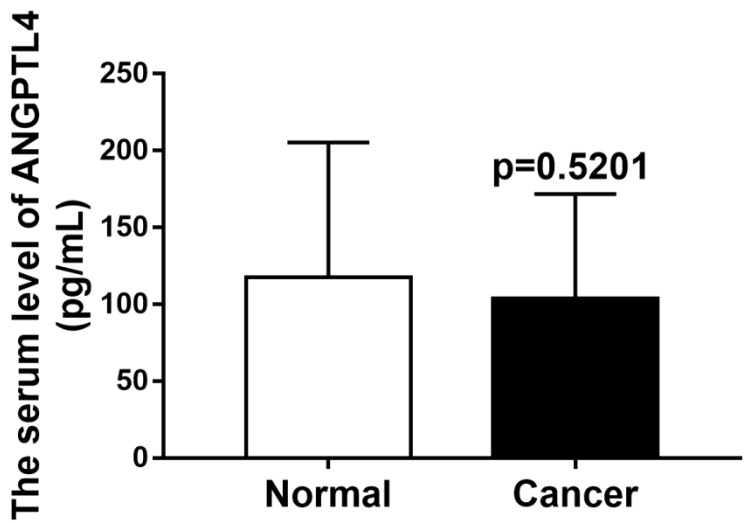
Supplemental Fig.3 The relation of ANGPTL4 protein level and pERK1/2 level in thyroid cancer.

Supplemental Fig.4 The relation of HIF1A and ANGPTL4 in thyroid cancer.

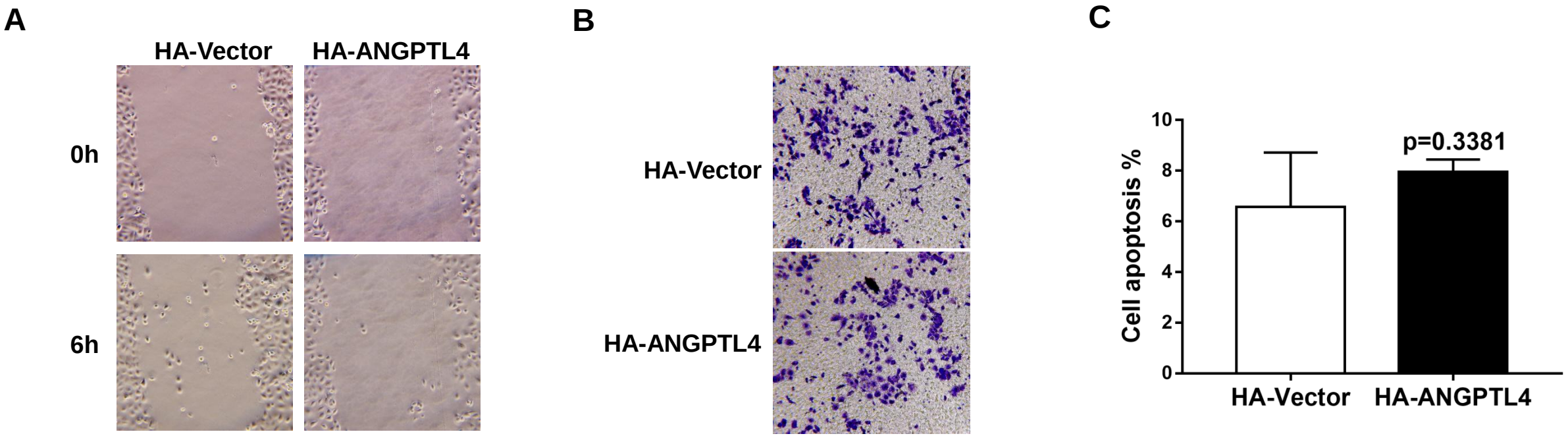
A. HIF1A mRNA level was increased in cancer tissue compared with adjacent cancer tissue in TCGA. **B.** ANGPTL4 mRNA level was positively correlated with HIF1A mRNA in thyroid cancer in TCGA.

Supplemental Fig.1

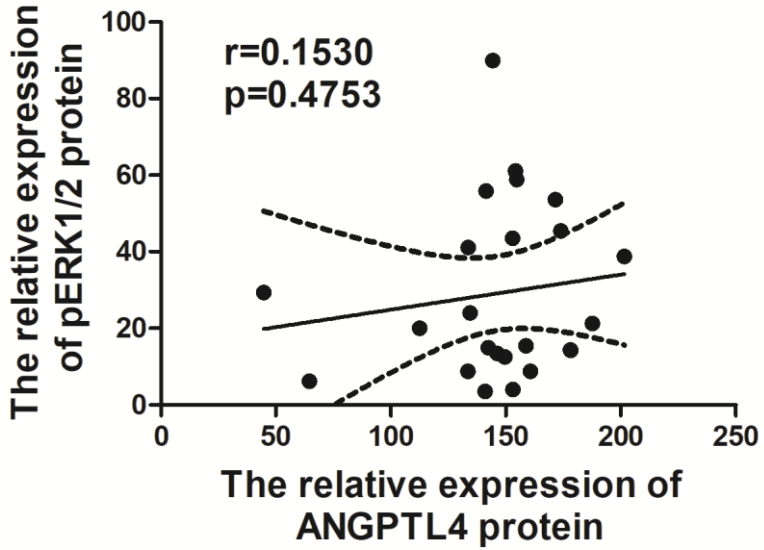
A



Supplemental Fig.2



Supplemental Fig.3



Supplemental Fig.4