Supplemental materials and methods

Tissue specimens, cell lines and plasmid transfection. Fresh papillary thyroid carcinoma (PTC) and adjacent normal tissues were obtained from 4 patients. PTC was diagnosed by the Department of Pathology, Punan Hospital of Pudong New District (Shanghai, China). Written informed consent was provided by all patients. The present study was approved by the Institutional Ethics Committees of the Punan Hospital of Pudong New District (Shanghai, China) and followed the principles of the Declaration of Helsinki. The cell lines HeLa, TPC1, CAPAN-2, 293, HEPG2, A549, Caco-2 and MCF7 were culturedin Dulbecco's modified Eagle's medium and RT4 was cultured in McCoy's 5A medium. Cells were supplemented with 10% fetal bovine serum at 37˚C and 5% CO_2. The coding sequence of type 2 taste receptor 10 (TAS2R10) was subcloned into pcDNA3.1 to construct the TAS2R10 expression plasmid. Transfection was performed with Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientifc, Inc.) according to the manufacturer's protocol.

Reverse transcription-quantitative (RT-q)PCR. Total RNA of tissues and cell lines was extracted with TRIzol reagent (Invitrogen; Thermo Fisher Scientifc, Inc.). RT-qPCR analysis of mRNA expression was performed on a LightCycler 480 (Roche) with SYBR Green PCR master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). All reactions were run in quadruplicate. β-actin was used as the endogenous control. The $2^{-\Delta\Delta Cq}$ method was used to calculate fold changes for data analysis.

Western blot analysis. Cell lysates were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc.). Membranes were blocked in Tris-buffered saline containing 0.05% Tween-20 and 5% skimmed milk, and subsequently incubated with primary antibodies against TAS2R10 (Novus Biologicals, Ltd.) and β-actin (Santa Cruz Biotechnology, Inc.) overnight at 4˚C, followed by incubation with horseradish peroxidase-labeled secondary antibodies for 2 h. Protein expression was assessed via enhanced chemiluminescence (BOSER).

Figure S1. Expression of TAS2R10 in different cell lines detected by (A) reverse transcription-quantitative PCR and (B) western blot analysis. Lanes: 1, Hela; 2, TPC1; 3, CAPAN-2; 4, 293; 5, HEPG2; 6, RT4; 7, A549; 8, Caco-2; 9, MCF7. TAS2R10 was mainly expressed in the cell lines of Hela, TPC1 and CAPAN-2. Values are expressed as the mean ± standard deviation of four independent experiments. *P<0.05. TAS2R10, type 2 taste receptor 10.

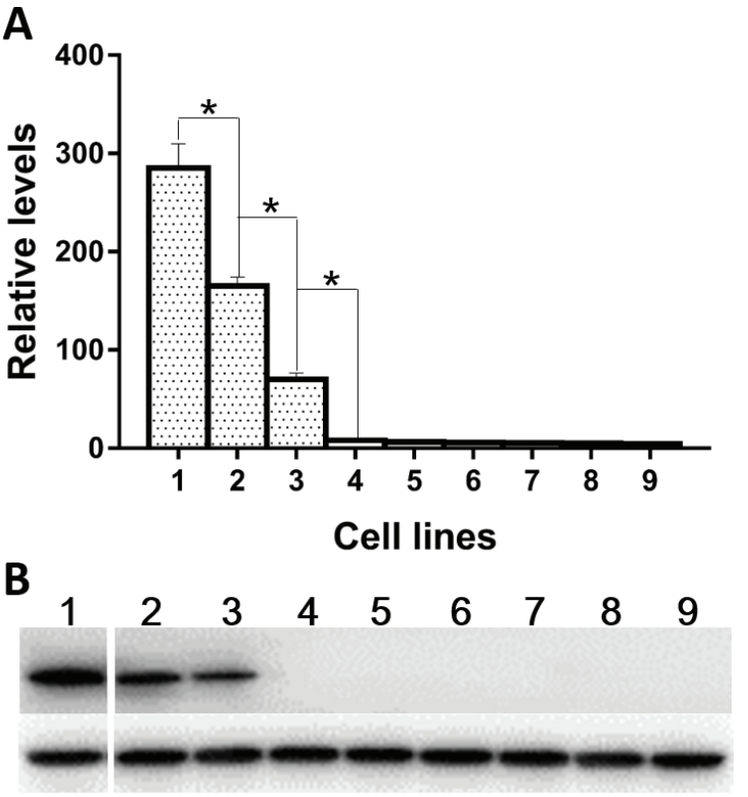

Figure S2. Expression of TAS2R10 and ANAPC5 in thyroid tissue detected by reverse transcription-quantitative PCR. (A) Analysis of 4 pairs of clinical samples of human thyroid cancer. When the expression of TAS2R10 was decreased in thyroid cancer, ANAPC5 was also decreased. (B) The thyroid cancer cell line TPC-1 was employed and overexpression experiments were performed. When TAS2R10 was overexpressed, ANAPC5 was also increased. Values are expressed as the mean ± standard deviation of four independent experiments. *P<0.05. Co, control; Ov, overexpression; No, normal; Ca, Cancer; TAS2R10, type 2 taste receptor 10; ANAPC5, anaphase promoting complex subunit 5.