

Supplemental Figures and Legends

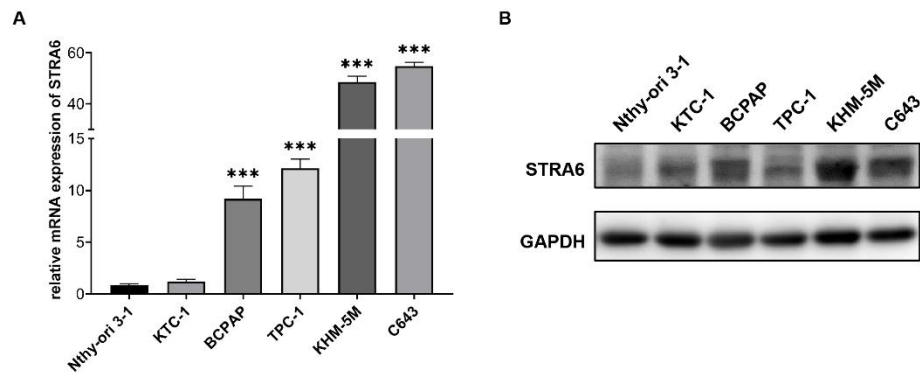


Figure S1. STRA6 is upregulated in thyroid cancer cell lines.

(A) qRT-PCR analysis of STRA6 mRNA expression in normal thyroid follicular epithelial cell line (Nthy-ori 3-1), PTC cell lines (KTC-1, BCPAP, TPC-1) and ATC cell lines (KHM-5M, C643). (B) Protein expression level of STRA6 detected by Western blot in normal thyroid follicular epithelial cell line and thyroid cancer cell lines. (***) $P < 0.001$. The data are shown as the mean \pm SD)

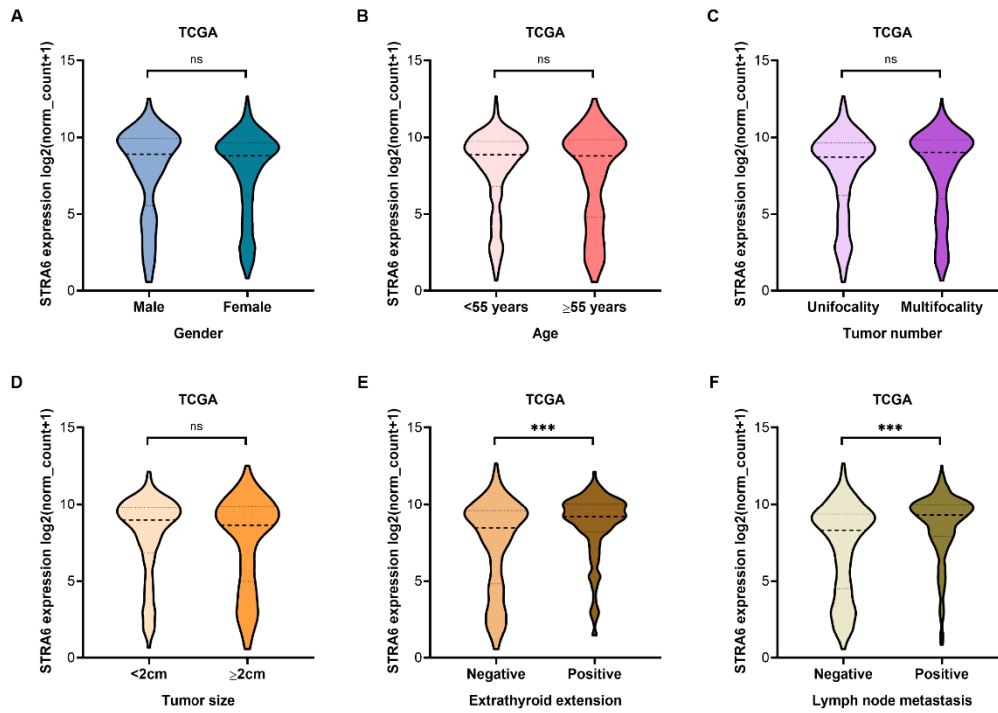


Figure S2. STRA6 is correlated with aggressive clinicopathological features in thyroid carcinoma.

(A-F) mRNA expression level of STRA6 was shown by violin plot in the TCGA cohort based on different clinicopathological features, including gender, age, tumor number, tumor size, extrathyroidal extension and lymph node metastasis. (***) $P < 0.001$; ns, not significant)

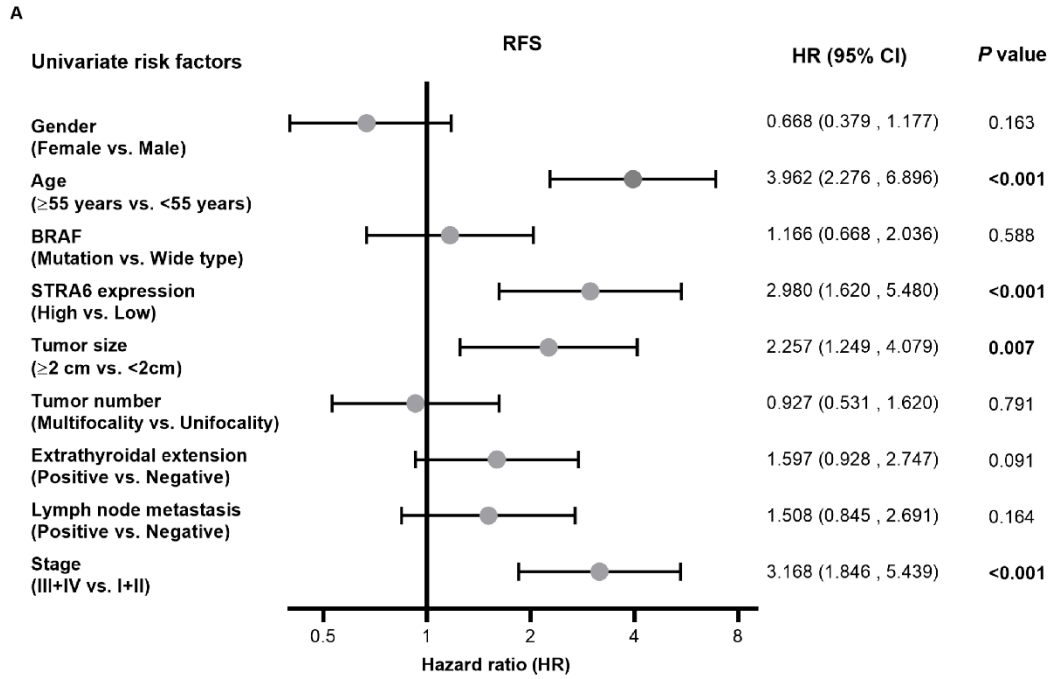


Figure S3. High STRA6 expression is an independent risk factor for RFS of PTC in TCGA cohort.

(A) Univariate Cox regression analysis for RFS of thyroid cancer patients in the TCGA cohort. All the bars corresponded to 95% confidence intervals.

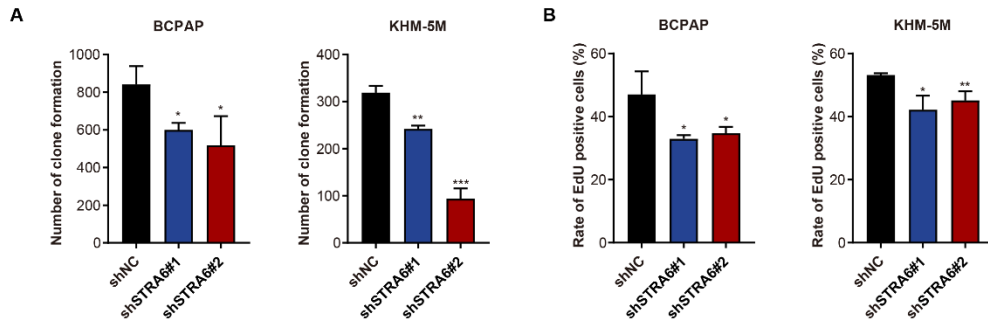


Figure S4. Knockdown of STRA6 inhibits thyroid cancer cell growth *in vitro*.

(A) The number of clone formation was counted in the control and STRA6 knockdown groups of BCPAP and KHM-

5M cells. (B) The percentage of EdU positive cells in BCPAP and KHM-5M cells transfected with indicated vectors.

(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The data are shown as the mean \pm SD)

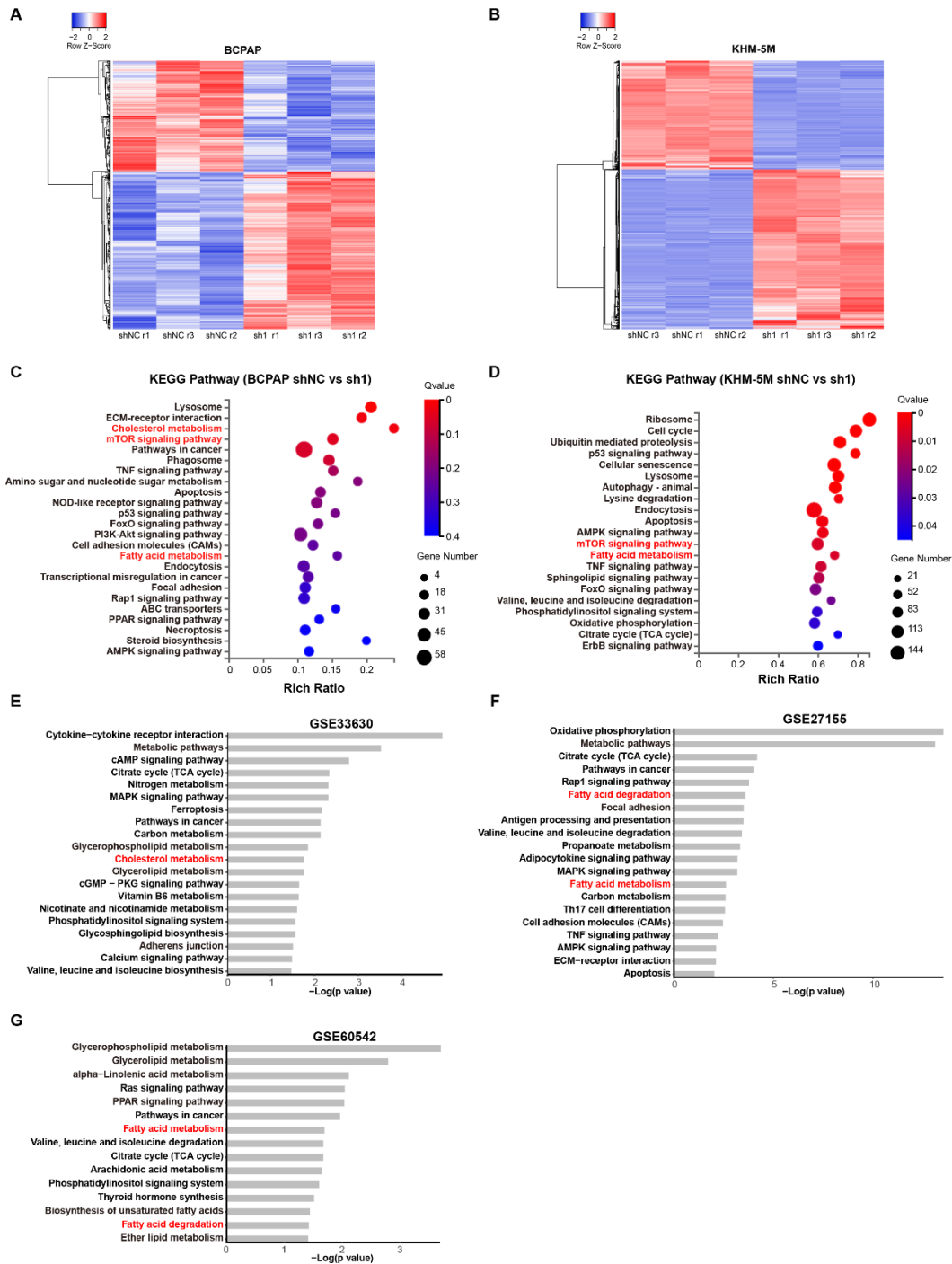


Figure S5. STRA6 regulates TC progression via the AKT/mTOR signaling pathway and lipid metabolism.

(A) The heatmap of DEGs between shNC and shSTRAR6 groups in BCPAP cells. (B) The heatmap of DEGs upon KHM-5M control and STRAR6-silenced groups. (C) The KEGG analysis based on DEGs in BCPAP cells. (D) The KEGG analysis according to DEGs in KHM-5M cells. (E-G) KEGG enrichment analysis of genes significantly correlated with STRA6 in three public datasets from GSE33630, GSE27155 and GSE60542.

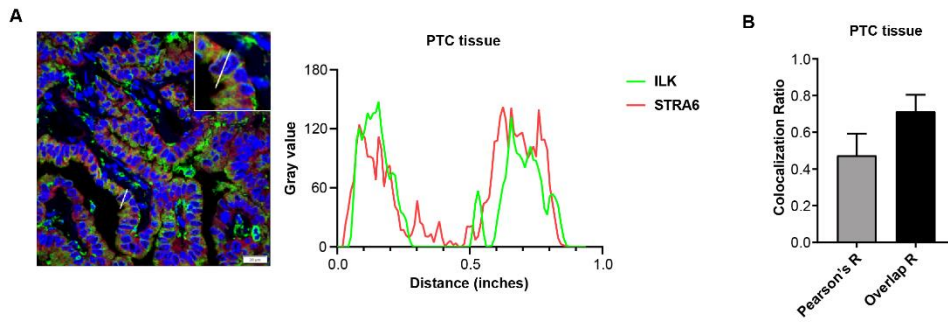


Figure S6. Colocalization of STRA6 and ILK in PTC.

(A) Plots of pixel intensity along the white line in merged images of PTC tissue. (B) The Pearson's correlation and overlap coefficient are analyzed by Colocalization Finder in Image J.

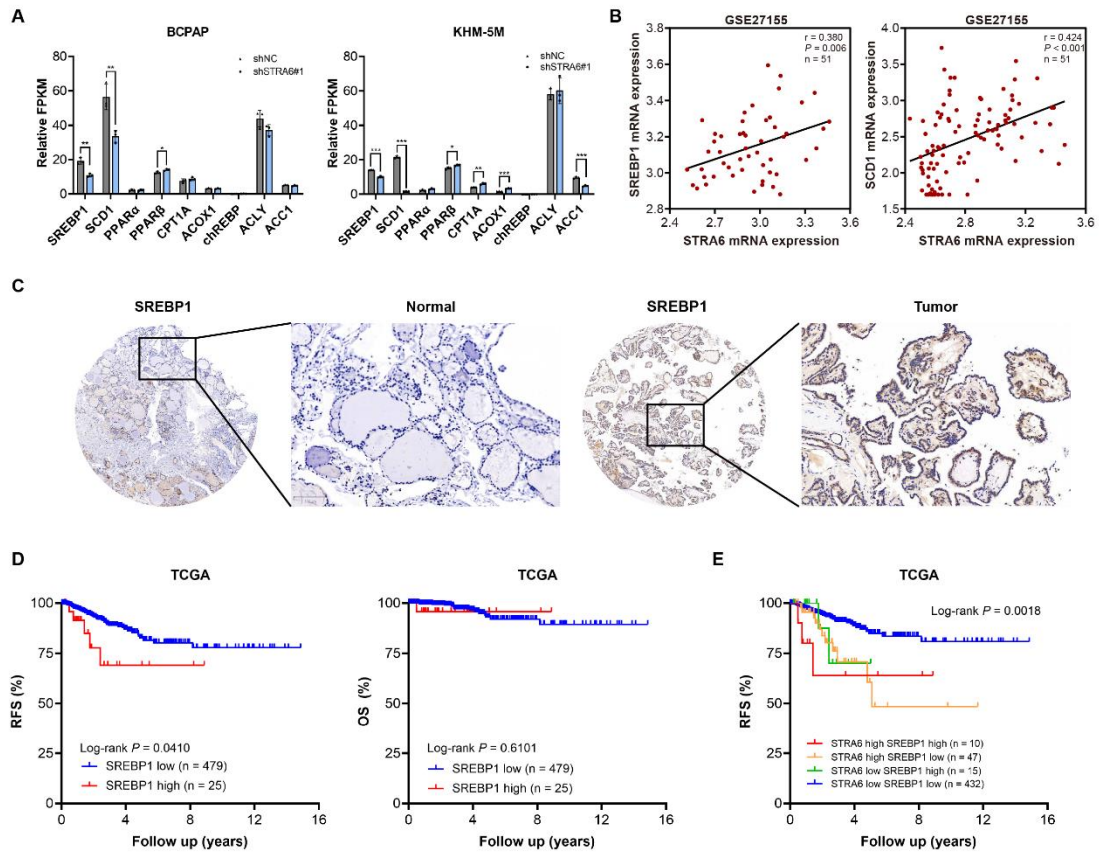


Figure S7. SREBP1 is upregulated in PTC tumor tissues and predicts poor RFS for PTC.

(A) The mRNA expression of lipid regulators from RNA-sequencing data. (B) Spearman correlation analysis of the mRNA expression level between STRA6 and SREBP1 or SCD1 in public dataset GSE27155. (C) Representative images of SREBP1 protein level in normal thyroid tissue and PTC tissue by IHC staining. (D) Kaplan–Meier survival curves of RFS P and OS according to SREBP1 mRNA expression from TCGA database. Prognostic cutoff value was identified as 12.5 by X-Tile. (E) Kaplan–Meier survival analysis for RFS combined with STRA6 expression and SREBP1 expression in the TCGA cohort. ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$. The data are shown as the mean \pm SD)

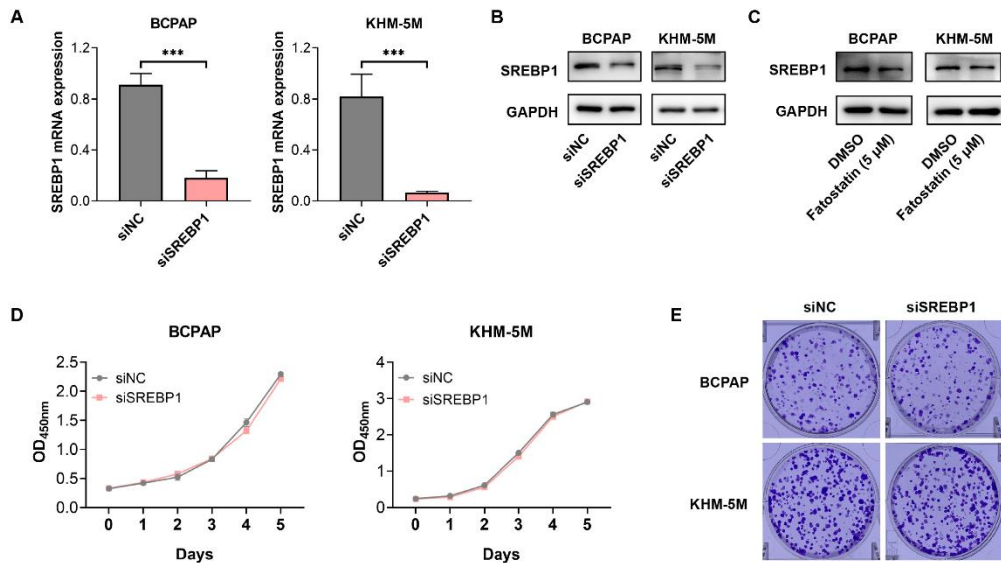


Figure S8. The effect of SREBP1 on cell proliferation in thyroid carcinoma.

(A-B) qRT-PCR and Western blot analysis of SREBP1 expression in BCPAP and KHM-5M cells with SREBP1 knockdown. (C) Western blot analysis of SREBP1 protein levels in BCPAP and KHM-5M cells with treatment of Fatostatin (5 μ M). (D) The effect of SREBP1 on cell viability was determined by CCK-8 assay in BCPAP and KHM-5M cells. (E) Colony formation assay was conducted to detect the cell proliferation in control and SREBP1-silenced TC cells. (***) $P < 0.001$. The data are shown as the mean \pm SD)

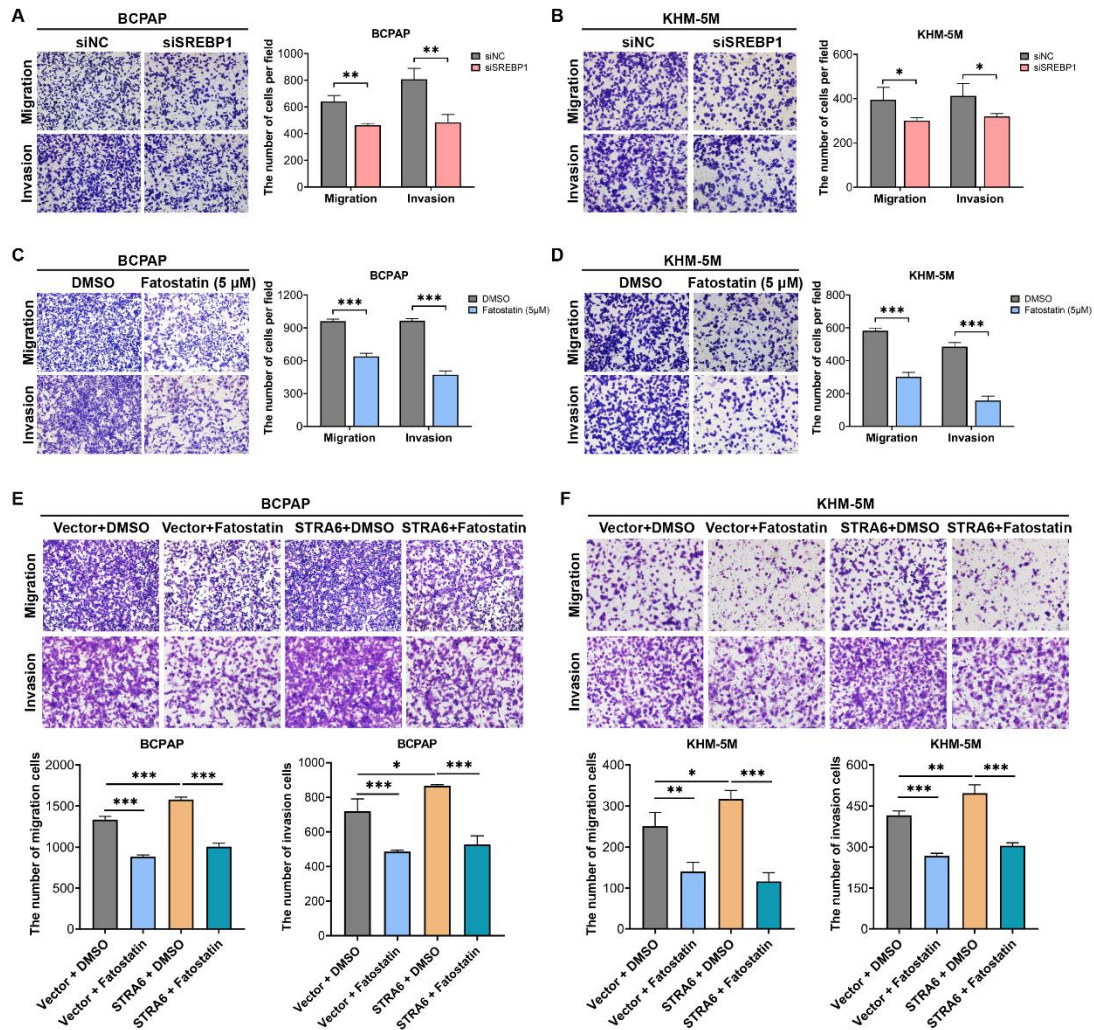


Figure S9. STRA6 promotes thyroid cancer migration and invasion via SREBP1 *in vitro*.

(A-B) Transwell migration and matrigel invasion assays were performed to compare migration and invasion capabilities of two TC cells upon silence of SREBP1. (C-D) Transwell migration and matrigel invasion assays were applied to measure their migration and invasion abilities on BCPAP and KHM-5M cells treating with 5 μM Fatostatin or DMSO. (E-F) Migration and invasion capabilities of BCPAP and KHM-5M cells with indicated treatment were estimated by Transwell assays. The migration or invasion cells in each group were counted and shown in the lower panel. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The data are shown as the mean \pm SD)

Supplemental Tables

Table S1. Primers used in qRT-PCR.

Table S2. Antibodies used in the study.

Table S3. Sequences of siRNAs and shRNAs.

Table S4. The correlation between STRA6 relative mRNA expression (PTC tissues over normal adjacent tissues) and clinicopathologic feature in 50 PTC patients.

Table S1. Primers used in qRT-PCR.

Gene	Forward Primer	Reverse Primer
STRA6	CCGGCGAGTGCTCTATG	AAGGTGGATGGCGTTGTAG
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
SREBP1c	GGAGCCATGGATTGCACATT	CAGGAAGGCTTCCAGAGAGG
SCD1	CCCCACCTACAAGGATAAGGA	CACGAGCCCATTCATAGACAT
PPAR α	ATGGTGGACACGGAAAGCC	CGATGGATTGCGAAATCTCTTGG
PPAR β	CAGGGCTGACTGCAAACGA	CTGCCACAATGTCTCGATGTC
CPT1A	TCCAGTTGGCTTATCGTGGTG	TCCAGAGTCCGATTGATTTTTGC
ACOX1	ACTCGCAGCCAGCGTTATG	AGGGTCAGCGATGCCAAAC
chREBP	GACCTCAGCTCCATGCCAG	TGTGTGATACGCCGGTTCTC
ACLY	CAGCAGGACAGCATCTTTTTC	TGGACTIONTGGGACTGAATCTTG
ACC1	AGGAAGATGGTGTCCGCTCTG	GGGAGATGTGCTGGGTCAT

Table S2. Antibodies used in the study.

Antibody	Company	Cat No.	Western blot	IHC	IP
STRA6	Novus Biologicals	H00064220- D01P	1:1000	1:200	1:50
SREBP1	Affinity Biosciences	AF6283	1:1000	1:100	
SCD1	Abcam	ab19862	1:1000		
mTOR	CST	#2983	1:1000		
p-mTOR	CST	#5536	1:1000		
AKT	CST	#4691	1:1000		
p-AKT	CST	#4060	1:2000		
p-S6K1	CST	#9234	1:1000		
S6K1	Proteintech	14485-1-AP	1:1000		
p-4E-BP1	CST	#2855	1:1000		
4E-BP1	Proteintech	60246-1-Ig	1:1000		
GAPDH	Proteintech	HRP-60004	1:10000		
p-mTOR	Proteintech	67778-1-Ig		1:1000	
Ki-67	CST	#9449		1:2000	
ILK	CST	#3856	1:1000	1:200	1:50
Anti-Flag	Sigma	F1804	1:1000		1:50
Normal Rabbit IgG	CST	#2729			1:100
Normal Mouse IgG	Millipore	12-371			1:100
Anti-mouse IgG, HRP-linked Antibody	CST	#7076	1:3000		
Anti-rabbit IgG, HRP-linked Antibody	CST	#7074	1:3000		

Table S3. Sequences of siRNAs and shRNAs.

1. Sequences of siRNAs

Gene	Sense	Antisense
siSTRA6	UCUAUUAUGCUGCCCUCUATT	UAGAGGGCAGCAUAAUAGAAC
siSREBP1	CCCACUCCAUUGAAGAUGUTT	ACAUCUCAAUGGAGUGGGTT
siNC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

2. Sequences of shRNAs

Gene	Target sequences
shSTRA6#1	gtTCTATTATGCTGCCCTCTA
shSTRA6#2	TTTCATGGTCCTCCTGAGCTC
shNC	TTCTCCGAACGTGTCACGT

Table S4. The correlation between STRA6 relative mRNA expression (PTC tissues over normal adjacent tissues) and clinicopathologic feature in 50 PTC patients.

Clinical features	n	STRA6 expression	P value
Gender			0.242
Male	17	5.13 ± 2.32	
Female	33	4.33 ± 2.21	
Age			0.650
<55years	42	4.54 ± 2.30	
≥55years	8	4.94 ± 2.11	
Multifocality			0.195
No	32	4.29 ± 2.16	
Yes	18	5.16 ± 2.38	
Tumor size			0.442
≤ 2 cm	36	4.45 ± 2.17	
> 2cm	14	5.00 ± 2.50	
Extrathyroidal extension			0.015
No	21	3.71 ± 1.93	
Yes	29	5.25 ± 2.28	
LN metastasis			0.079
No	16	3.79 ± 1.60	
Yes	34	4.99 ± 2.43	
Stage			-
I + II	50	4.60 ± 2.25	
III + IV	0	-	