

Table S1. The primers and sequence of the *FABP3* amplicon

Forward primer	aggaagagagTTATAGTGATGTTGGGTTAGGTGA
Reverse primer	cagtaatacgactcactatagggagaaggctCAACCCCTCCTAAATAAACCCCT
Sequence*	CCATAGTGATGCTGGGCTAGGCTGAGAGAAGCTACAAGAGAGC AGGCGTGCAAGGGCTCCGACGGCGGCTCCCTGCCCCGGGCTGCC GCTTTAAATAGCCCT <u>CG</u> CATCACATGAGGAGACGTGGCCCCACG CCCCCGGCTCCCGAAATAGGAAGCCCCAGGCTAGGGCTCACCC AGGAGGGGCTG

* Upper case letters indicated the sequence-specific primer regions and non-specific tags were shown in lower case letters. The EpiTyper assay detected the methylation levels of 7 CpG sites and yielded 5 distinguishable mass peaks. The CpG sites that could be measured are in bold. CpG_7/cg18368411 is underlined.

Table S2. The association between *FABP3* methylation and TC in two independent validations

Variates	BTN median (IQR)	TC median (IQR)	OR (95% CI) per - 10% methylation ^s	<i>P</i> value ^s
Validation I (221 BTN subjects vs. 222 TC cases)				
CpG_1	0.67 (0.57 - 0.77)	0.62 (0.51 - 0.70)	1.26 (1.09 - 1.45)	0.002
CpG_2	0.52 (0.40 - 0.65)	0.42 (0.32 - 0.54)	1.24 (1.09 - 1.41)	0.001
CpG_7/cg18368411	0.43 (0.32 - 0.58)	0.32 (0.22 - 0.42)	1.45 (1.26 - 1.67)	2.43E-07
CpG_8	0.27 (0.20 - 0.41)	0.17 (0.12 - 0.24)	1.81 (1.50 - 2.19)	5.83E-10
CpG_9.10.11	0.22 (0.16 - 0.33)	0.15 (0.10 - 0.22)	1.67 (1.36 - 2.04)	7.06E-07
Validation II (191 BTN subjects vs. 256 TC cases)				
CpG_1	0.80 (0.70 - 0.86)	0.72 (0.59 - 0.81)	1.18 (1.04 - 1.35)	0.012
CpG_2	0.67 (0.53 - 0.76)	0.57 (0.43 - 0.70)	1.17 (1.04 - 1.32)	0.009
CpG_7/cg18368411	0.55 (0.42 - 0.69)	0.43 (0.29 - 0.55)	1.31 (1.16 - 1.47)	7.00E-06
CpG_8	0.37 (0.26 - 0.48)	0.21 (0.14 - 0.31)	1.79 (1.51 - 2.11)	6.30E-12
CpG_9.10.11	0.26 (0.19 - 0.37)	0.19 (0.13 - 0.26)	1.52 (1.29 - 1.80)	7.05E-07

^sLogistic regression adjusted for age, gender, TSH, FT3, and FT4. Significant *P* values are in bold.

Abbreviation: BTN, benign thyroid nodule; TC, thyroid cancer; IQR, interquartile range; CI, confidence interval; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free tetraiodothyronine acid.

Table S3. The association between *FABP3* methylation and TC stratified by gender combining Validation I and Validation II

Variates	BTN median (IQR)	TC median (IQR)	OR (95% CI) per - 10% methylation [§]	<i>P</i> value [§]
male	(n = 90)	(n = 120)		
CpG_1	0.72 (0.58 - 0.82)	0.68 (0.53 - 0.77)	1.11 (0.93 - 1.31)	0.260
CpG_2	0.57 (0.41 - 0.72)	0.50 (0.35 - 0.65)	1.08 (0.91 - 1.28)	0.378
CpG_7/cg18368411	0.47 (0.31 - 0.64)	0.40 (0.24 - 0.53)	1.15 (0.97 - 1.36)	0.105
CpG_8	0.29 (0.20 - 0.39)	0.20 (0.12 - 0.29)	1.56 (1.22 - 1.98)	3.36E-04
CpG_9.10.11	0.23 (0.16 - 0.34)	0.19 (0.11 - 0.25)	1.39 (1.08 - 1.78)	0.011
female	(n = 322)	(n = 358)		
CpG_1	0.72 (0.62 - 0.83)	0.67 (0.54 - 0.75)	1.21 (1.09 - 1.36)	0.001
CpG_2	0.59 (0.45 - 0.72)	0.49 (0.36 - 0.63)	1.20 (1.10 - 1.32)	1.12E-04
CpG_7/cg18368411	0.50 (0.37 - 0.63)	0.36 (0.25 - 0.50)	1.41 (1.27 - 1.56)	7.96E-11
CpG_8	0.32 (0.21 - 0.47)	0.19 (0.13 - 0.26)	1.85 (1.61 - 2.14)	2.19E-17
CpG_9.10.11	0.23 (0.18 - 0.35)	0.17 (0.12 - 0.24)	1.66 (1.43 - 1.93)	6.14E-11

[§]Logistic regression adjusted for age, TSH, FT3, and FT4. Significant *P* values are in bold.

Abbreviation: BTN, benign thyroid nodule; TC, thyroid cancer; IQR, interquartile range; CI, confidence interval; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free tetraiodothyronine acid.

Table S4. The association between *FABP3* methylation and TC stratified by age combining Validation I and Validation II

Variates	BTN median (IQR)	TC median (IQR)	OR (95% CI) per - 10% methylation [§]	<i>P</i> value [§]
< 55 years old	(n = 257)	(n = 316)		
CpG_1	0.74 (0.63 - 0.83)	0.67 (0.54 - 0.75)	1.30 (1.15 - 1.47)	3.70E-05
CpG_2	0.60 (0.46 - 0.71)	0.49 (0.37 - 0.61)	1.26 (1.13 - 1.40)	2.00E-05
CpG_7/cg18368411	0.50 (0.36 - 0.64)	0.36 (0.25 - 0.50)	1.39 (1.25 - 1.54)	1.52E-09
CpG_8	0.31 (0.20 - 0.46)	0.19 (0.13 - 0.25)	1.82 (1.57 - 2.11)	5.28E-15
CpG_9.10.11	0.22 (0.17 - 0.35)	0.17 (0.12 - 0.23)	1.63 (1.39 - 1.90)	1.28E-09
≥ 55 years old	(n = 155)	(n = 161)		
CpG_1	0.71 (0.60 - 0.82)	0.67 (0.54 - 0.77)	1.03 (0.89 - 1.20)	0.693
CpG_2	0.57 (0.43 - 0.72)	0.50 (0.34 - 0.67)	1.04 (0.92 - 1.19)	0.524
CpG_7/cg18368411	0.47 (0.35 - 0.62)	0.37 (0.25 - 0.54)	1.22 (1.05 - 1.41)	0.009
CpG_8	0.32 (0.22 - 0.45)	0.20 (0.13 - 0.30)	1.72 (1.39 - 2.12)	4.57E-07
CpG_9.10.11	0.24 (0.18 - 0.35)	0.19 (0.11 - 0.26)	1.49 (1.19 - 1.86)	4.77E-04

[§]Logistic regression adjusted for age, gender, TSH, FT3, and FT4. Significant *P* values are in bold.

Abbreviation: BTN, benign thyroid nodule; TC, thyroid cancer; IQR, interquartile range; CI, confidence interval; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free tetraiodothyronine acid.

Table S5. *FABP3* methylation in BTN and TC subtypes

Clinical characteristics	Group (n)	median of methylation levels				
		CpG_1	CpG_2	CpG_7/cg18368411	CpG_8	CpG_9.10.11
Classification of BTN	adenoma (196)	0.75 (0.65 - 0.84)	0.62 (0.47 - 0.73)	0.53 (0.38 - 0.65)	0.35 (0.22 - 0.49)	0.26 (0.18 - 0.39)
	goiter (195)	0.71 (0.61 - 0.82)	0.57 (0.44 - 0.71)	0.46 (0.35 - 0.62)	0.29 (0.21 - 0.43)	0.22 (0.17 - 0.33)
	subacute thyroiditis (3)	0.75 (0.51 - 0.98)	0.46 (0.34 - 0.70)	0.55 (0.06 - 0.63)	0.34 (0.03 - 0.47)	0.24 (0.05 - 0.31)
	lymphatic thyroiditis (18)	0.63 (0.51 - 0.73)	0.46 (0.34 - 0.50)	0.32 (0.26 - 0.43)	0.19 (0.14 - 0.28)	0.18 (0.12 - 0.19)
	<i>P</i> value ^a	0.111	0.245	0.061	0.058	0.008
	<i>P</i> value ^b	0.793	0.382	0.646	0.582	0.407
	<i>P</i> value ^c	0.002	0.001	1.41E-04	0.001	2.02E-04
Classification of TC	PTC (368)	0.68 (0.56 - 0.76)	0.51 (0.41 - 0.64)	0.38 (0.29 - 0.51)	0.19 (0.14 - 0.27)	0.18 (0.13 - 0.24)
	FTC (50)	0.72 (0.54 - 0.80)	0.57 (0.40 - 0.71)	0.50 (0.33 - 0.62)	0.32 (0.20 - 0.56)	0.25 (0.15 - 0.53)
	MTC (51)	0.54 (0.40 - 0.71)	0.28 (0.14 - 0.41)	0.14 (0.10 - 0.23)	0.09 (0.06 - 0.15)	0.08 (0.06 - 0.12)
	ATC (9)	0.30 (0.09 - 0.55)	0.22 (0.05 - 0.37)	0.10(0.06-0.25)	0.07(0.03-0.14)	0.06 (0.01 - 0.15)
	<i>P</i> value ^d	2.00E-06	4.00E-06	3.04E-13	5.51E-24	1.14E-15
	<i>P</i> value ^e	0.095	0.232	0.275	0.659	0.782
	<i>P</i> value ^f	1.63E-08	2.97E-15	2.76E-19	1.27E-18	4.56E-19
	<i>P</i> value ^g	1.40E-05	1.80E-05	3.00E-06	7.00E-06	5.90E-05

All the *P* values were calculated by the Mann-Whitney U test. Significant *P* values are in bold.

P value^a: adenoma vs. goiter; *P* value^b: adenoma vs. subacute thyroiditis; *P* value^c: adenoma vs. lymphatic thyroiditis; *P* value^d: adenoma vs. PTC; *P* value^e: adenoma vs. FTC; *P* value^f: adenoma vs. MTC; *P* value^g: adenoma vs. ATC.

Abbreviation: BTN, benign thyroid nodule; TC, thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; MTC, medullary thyroid cancer; ATC, anaplastic thyroid cancer.

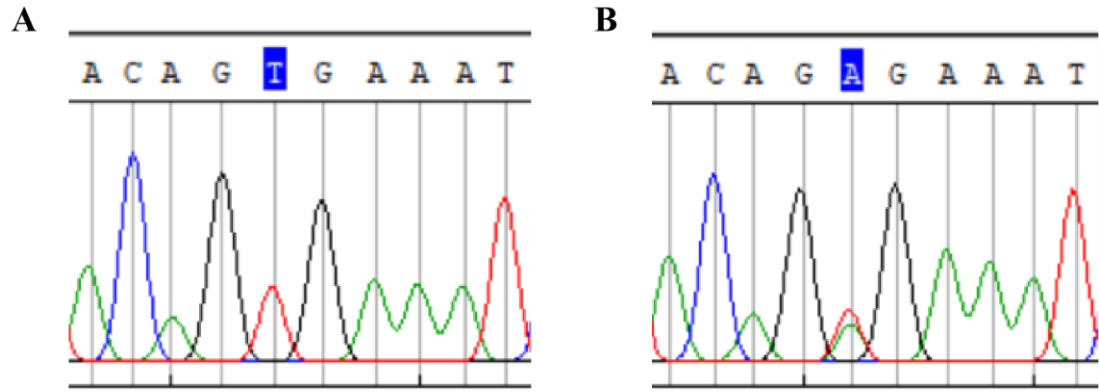


Figure S1. Representative Sanger-sequencing chromatogram. An amplicon covering the nucleotide 1799 of the BRAF gene (marked in blue, corresponding to p.V600E) was sequenced in all the 711 thyroid tumor tissues. (A) The representative sequencing chromatogram of homozygote wildtype 1799TT. (B) The representative sequencing chromatogram of heterozygote 1799TA, indicating the existence of the T>A non-synonymous mutation (V600E mutation).