

Figure S1. Representative pathology of TETs. The tumor and thymic tissues were fixed in 10% formalin and embedded in paraffin. Histological sections (5- μ m-thick) were prepared and stained with hematoxylin for 5 min and eosin for 3 min at room temperature and examined under a microscope (Olympus BX51FL, Olympus Corp.). (A) Type A thymoma, exhibiting a spindle-shaped cell pattern and a paucity of interspersed lymphocytes. (B) Type B1 thymoma; tumor cells are surrounded by abundant lymphocytes. (C) Type B2 thymoma; tumor cells are admixed with equal amounts of lymphocytes. (D) Type B3 thymoma; the tumor comprises solid sheets and a paucity of interspersed lymphocytes. (E) Thymic carcinoma; the tumor has infiltrative sheets of polygonal cells.

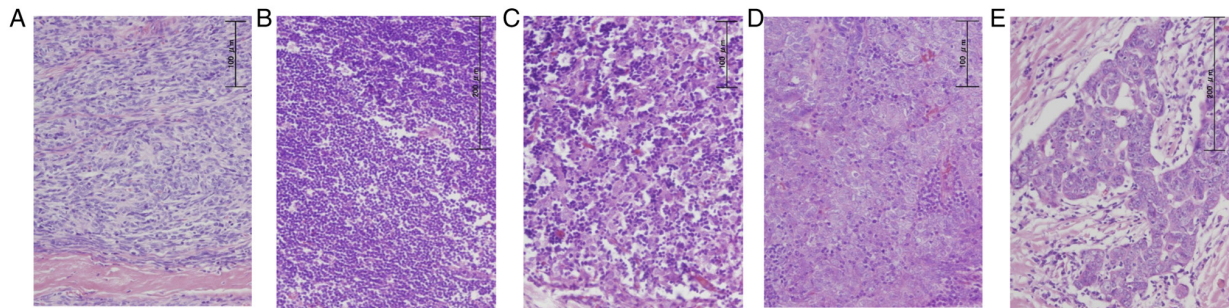


Figure S2. Volcano plot of differential CGI methylation profiles of 8 B3 thymoma and 7 thymic carcinoma samples. The x-axis indicates the average β -value difference (methylation level). The y-axis indicates the $-\log_{10}$ value of the adjusted Welch's test P-value for each CpG island (CGI). Black points are significant methylated CpG sites by Bonferroni's test. The arrow shows the plots which show more methylated CGI in thymic carcinoma than in B3 thymoma.

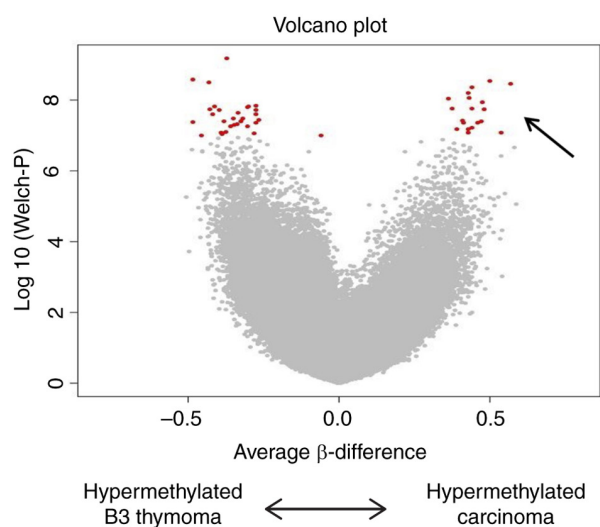


Figure S3. DNA methylation rate of 4 genes in TETs according to the Masaoka-Koga stage. (A) DNA methylation rate of the *GHSR* gene in TETs according to the Masaoka-Koga stage. The upper and lower ends of the whiskers, the upper and lower edges of the boxes, the horizontal lines across each box, 'x' marks and the circles outside the boxes represent the upper and lower extremes, the upper (75th) and lower (25th) quartiles, medians, means and data outliers, respectively. The median DNA methylation rates in Stages I, II, III, IVA and IVB were 32.4 (range, 6.6-60.4; IQR, 22.0-45.3), 37.8 (range, 22.4-69.4; IQR, 36.4-53.4), 39.8 (range, 21.4-78.0; IQR, 24.7-50.7), 40.8 (range, 34.0-61.6; IQR 38.0-45.2) and 73.0 (range, 27.4-79.4; IQR 52.2-76.2), respectively. (B) DNA methylation rate of the *GNG4* gene in TETs according to the Masaoka-Koga stage. The median DNA methylation rates in stages I, II, III, IVA and IVB were 7.6 (range, 3.2-15.2; IQR, 6.9-10.0), 11.8 (range, 4.8-55.8; IQR, 7.2-22.0), 10.2 (range, 7.2-23.8; IQR, 8.8-17.0), 10.2 (range, 6.2-42.8; IQR, 7.2-18.0) and 27.8 (range, 5.4-69.6; IQR, 8.8-50.4), respectively. There was a significant difference in DNA methylation between stage I and IVB (Tukey-Kramer test, *** $P < 0.05$ as indicated). (C) DNA methylation rate of the *HOXD9* gene in TETs according to the Masaoka-Koga stage. Median DNA methylation rates in stages I, II, III, IVA and IVB were 10.2 (range, 4.7-32.2; IQR, 7.2-20.6), 13.5 (range, 5.5-53.0; IQR, 9.8-23.7), 14.8 (range, 5.8-49.8; IQR, 8.1-24.5), 18.8 (range, 8.8-45.8; IQR, 13.8-29.0) and 8.2 (range, 4.7-63.7; IQR, 7.7-53.8) and respectively. (D) DNA methylation rate of the *SALL3* gene in TETs according to the Masaoka-Koga stage. The median DNA methylation rates in stages I, II, III, IVA and IVB were 5.0 (range, 2.1-35.3; IQR, 4.2-17.4), 6.4 (range, 1.1-47.5; IQR, 3.9-21.6), 9.3 (range, 3.9-47.0; IQR, 7.5-26.4), 32.4 (range, 5.5-56.9; IQR, 7.1-34.3) and 5.3 (range, 2.3-75.3; IQR, 3.3-57.5), respectively.

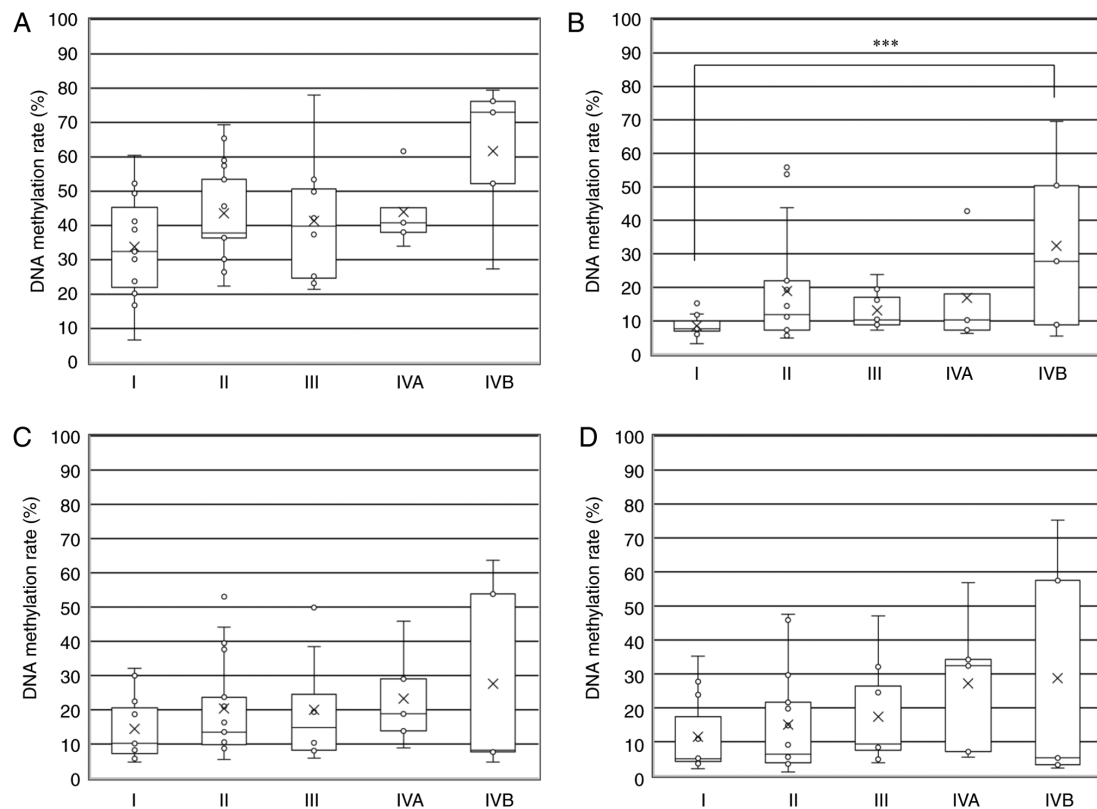


Table SI. List of patients.

Sample no.	HumanMethylation450 K BeadChip analysis	Bisulphite pyrosequencing		Age	Sex	MG	Masaoka-Koga clinical staging	Histology	WHO histological classification
		TETs	Thymus						
4		○		50	F	-	1	Thymoma	A
19		○		62	M	-	1	Thymoma	A
24		○		80	F	-	1	Thymoma	A
/0037		○	○	84	M	-	4b	Thymoma	A
/0031		○	○	57	F	+	1	Thymoma	A
/0036		○		65	F	-	1	Thymoma	AB
47		○		65	F	-	2	Thymoma	AB
36		○	○	65	F	-	1	Thymoma	B1
44		○		72	F	-	1	Thymoma	B1
42		○		51	F	+	2	Thymoma	B1
/0038		○	○	65	M	-	3	Thymoma	B1
40		○		60	F	-	1	Thymoma	B2
27		○	○	74	F	-	2	Thymoma	B2
28		○	○	65	F	-	2	Thymoma	B2
30		○	○	40	M	+	2	Thymoma	B2
31		○	○	75	F	-	2	Thymoma	B2
38		○	○	40	F	-	2	Thymoma	B2
39		○	○	52	F	+	2	Thymoma	B2
/0033		○		38	F	-	3	Thymoma	B2
/0030		○		65	M	+	4a	Thymoma	B2
29		○	○	43	M	-	4b	Thymoma	B2
9	○	○		66	M	-	1	Thymoma	B3
11	○	○		75	F	-	1	Thymoma	B3
12	○	○		64	M	-	2	Thymoma	B3
34		○		68	F	+	2	Thymoma	B3
18	○	○		47	M	-	3	Thymoma	B3
20	○	○	○	75	M	-	3	Thymoma	B3
10	○	○		28	F	+	4a	Thymoma	B3
14	○	○		36	M	+	4a	Thymoma	B3
23	○	○		72	M	-	4a	Thymoma	B3
2	○	○	○	55	M	-	2	Carcinoma	Carcinoma
3	○			51	F	-	2	Carcinoma	Carcinoma
5	○	○	○	60	M	-	2	Carcinoma	Carcinoma
25		○	○	61	F	-	2	Carcinoma	Carcinoma
33		○		68	F	-	2	Carcinoma	Carcinoma
35		○	○	69	M	-	2	Carcinoma	Carcinoma
/0039		○	○	55	F	-	2	Carcinoma	Carcinoma
1	○	○		51	F	-	3	Carcinoma	Carcinoma
16	○	○		61	M	-	3	Carcinoma	Carcinoma
37		○	○	48	F	-	3	Carcinoma	Carcinoma
6	○	○	○	58	F	-	4a	Carcinoma	Carcinoma
7	○	○		69	F	-	4b	Carcinoma	Carcinoma
15		○		50	F	+	3	Thymoma + carcinoma	Carcinoma + B2
22		○		67	M	-	4b	NECTT	Small cell carcinoma
13		○		61	M	-	1	NECTT	Typical carcinoid
/0034		○	○	68	M	-	2	NECTT	Typical carcinoid
17		○		64	M	-	4b	NECTT	Atypical carcinoid

TET, thymic epithelial tumor; MG, myasthenia gravis; M, male; F, female; NECTT, neuroendocrine tumor of the thymus. Circles indicate that samples were used in the HumanMethylation450 K BeadChip (Illumina) analysis and/or Bisulfite pyrosequencing.

Table SII. List of pyrosequencing primers.

Gene/primer name	Sequence
Pyrosequencing for <i>GNG4</i>	
Forward	5'-TGTTGAGTGAAGGGGATTAGGG-3'
Reverse	5'-CCTTTTCTACAAATCTTACCAACACTAC-3'
Sequence	5'-GGAGGAGGGGGTGTT-3'
Pyrosequencing for <i>HOX9</i>	
Forward	5'-GGGATAGAGGGTTGTAAGAAGAAG-3'
Reverse	5'-AAAACCCCCAAACCCAAATCCATATAC-3'
Sequence	5'-GAAGAAGAGAATAAATAGTTTTTTAG-3'
Pyrosequencing for <i>GHSR</i>	
Forward	5'-GAAGGTTATGTTGGATAGGTAGAG-3'
Reverse	5'-AAACATCCCTAACAACCTACTCACCATAC-3'
Sequence	5'-AGAGGTTGGTGGTGG-3'
Pyrosequencing for <i>SALL3</i>	
Forward	5'- TGGTGAAGGG GGATTAGG-3'
Reverse	5'-CTCCTCTCCCTCCCCTAC-3'
Sequence	5'-GTTTTGA GGTTTTTTTTT TTTTTTTG-3'

Table SIII. Hypermethylated genes in TC in relation to B3 thymomas.

Island	P-value	Adjusted P-value	β -difference	Gene name
chr1:112058184-112058590	2.94E-07	0.000755	0.314774	<i>ADORA3</i>
chr5:134363092-134365146	9.86E-07	0.001139	0.415723	<i>PITX1</i>
chr19:52995940-52996595	2.88E-06	0.001804	0.306899	<i>ZNF578</i>
chr2:171676552-171676980	3.89E-06	0.001845	0.350758	<i>GAD1</i>
chr2:45169505-45171884	5.91E-06	0.002194	0.302859	<i>SIX3</i>
chr19:33716312-33716751	6.80E-06	0.002241	0.346254	<i>SLC7A10</i>
chr1:235814280-235814488	8.32E-06	0.002337	0.376351	<i>GNG4</i>
chr3:27765196-27765675	8.60E-06	0.002337	0.301578	<i>EOMES</i>
chr1:224805408-224805853	1.03E-05	0.002583	0.318205	<i>CNIH3</i>
chr1:119526782-119527192	1.06E-05	0.002587	0.318005	<i>TBX15</i>
chr1:119531991-119532196	1.18E-05	0.002713	0.391201	<i>TBX15</i>
chr14:38060841-38062119	1.39E-05	0.002889	0.347624	<i>FOXA1</i>
chr2:63282514-63283122	1.52E-05	0.002889	0.386739	<i>OTX1</i>
chr7:150655108-150655643	1.41E-05	0.002889	0.35119	<i>KCNH2</i>
chr5:178003623-178004247	1.96E-05	0.003334	0.334628	<i>COL23A1</i>
chr3:172167526-172167866	2.46E-05	0.003756	0.315345	<i>GHSR</i>
chr1:38200919-38201200	2.83E-05	0.003935	0.304146	<i>EPHA10</i>
chr6:108495654-108495986	2.98E-05	0.004005	0.33114	<i>NR2E1</i>
chr20:17206528-17206952	3.04E-05	0.004035	0.360027	<i>PCSK2</i>
chr6:101846766-101847135	3.35E-05	0.004259	0.328455	<i>GRIK2</i>
chr14:57278709-57279116	3.50E-05	0.004374	0.383513	<i>OTX2OS1</i>
chr1:34642382-34643024	3.77E-05	0.004525	0.314545	<i>C1orf94</i>
chr2:176986424-176988291	4.79E-05	0.005011	0.329175	<i>HOXD9</i>
chr7:8481974-8482762	4.71E-05	0.005011	0.30506	<i>NXP1</i>
chr5:178421225-178422337	4.94E-05	0.005102	0.38488	<i>GRM6</i>
chr18:76737005-76741244	5.15E-05	0.005183	0.305268	<i>SALL3</i>
chr7:19184221-19184686	5.29E-05	0.005249	0.38878	<i>FERD3L</i>
chr19:36334994-36335321	5.76E-05	0.005526	0.317908	<i>NPHS1</i>
chr3:122296612-122296828	7.11E-05	0.006123	0.377236	<i>PARP15</i>

The 4 genes selected in the present study are presented in bold font.