

Supplemental Files

Supplementary Table 1. The gene-specific assay details, including primers purchased, region examined for CpG sites, and PCR cycling conditions.

Gene	Primer names	CpG coverage region; Ensembl reference sequence	PCR amplicon (base-pairs)	PCR conditions
<i>KITLG</i>	ADS1344 FS1 ADS1344 FS2	-332 to -155 from ATG; ENSG00000049130	243	95°C 15 min; 45 x (95°C 30 s; 53°C 30 s; 72°C 30 s); 72°C 5 min
<i>PDE11A</i>	ADS1346 FS1 ADS1346 FS2	-743 to -618 from ATG; ENSG00000128655	176	95°C 15 min; 45 x (95°C 30 s; 53°C 30 s; 72°C 30 s); 72°C 5 min
<i>SPRY4</i>	ADS997 FS1_re ADS997 FS2	-13 to +130 from TSS; ENST00000344120	226	95°C 15 min; 45 x (95°C 30 s; 45°C 30 s; 72°C 30 s); 72°C 5 min
<i>BAK1</i>	ADS1345FS1 ADS1345FS2 ADS1345FS3	-97 to +99 from TSS; ENSG00000030110	253	95°C 15 min; 45 x (95°C 30 s; 53°C 30 s; 72°C 30 s); 72°C 5 min
<i>DND1</i>	ADS1347FS1 ADS1347FS2	-254 to -82 from ATG; ENSG00000183403	256	95°C 15 min; 45 x (95°C 30 s; 56°C 30 s; 72°C 30 s); 72°C 5 min

TSS = transcription start site

45 x = 45 cycles

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Supplementary Table 2. Mean percent methylation (standard deviation) at each CpG site in the promoter region of each gene.

CpG*	KITLG			PDE11A			SPRY4			BAK1			DND1		
	% methylation (SD)			% methylation (SD)			% methylation (SD)			% methylation (SD)			% methylation (SD)		
	Controls	TGCT	<i>P</i> _{wilcoxon}	Controls	TGCT	<i>P</i> _{wilcoxon}	Controls	TGCT	<i>P</i> _{wilcoxon}	Controls	TGCT	<i>P</i> _{wilcoxon}	Controls	TGCT	<i>P</i> _{wilcoxon}
1	3.1 (2.7)	2.7 (1.9)	0.861	17.8 (4.8)	19.0 (6.7)	0.150	0.7 (1.0)	0.9 (1.2)	0.177	1.5 (1.4)	2.0 (5.6)	0.649	69.8 (2.3)	69.5 (2.0)	0.214
2	2.4 (1.9)	2.1 (1.5)	0.129	21.9 (6.4)	24.0 (8.8)	0.026	1.2 (1.2)	1.3 (1.3)	0.575	3.3 (1.6)	3.1 (1.4)	0.932	75.6 (2.3)	75.2 (2.3)	0.584
3	1.5 (1.6)	1.6 (1.5)	0.635	15.7 (5.2)	16.8 (6.0)	0.081	1.0 (1.2)	1.0 (1.3)	0.715	2.0 (1.4)	2.0 (1.4)	0.745	72.7 (25.3)	69.4 (26.3)	0.297
4	5.5 (3.6)	5.0 (2.6)	0.276	11.5 (3.5)	12.1 (4.0)	0.328	1.8 (1.7)	1.7 (1.8)	0.743	2.0 (1.4)	2.2 (1.2)	0.252	95.7 (6.0)	96.6 (4.3)	0.653
5	2.5 (2.7)	1.8 (2.1)	0.012	9.2 (2.6)	9.8 (2.9)	0.058	4.0 (1.5)	4.0 (1.6)	0.765	2.5 (1.7)	2.6 (1.6)	0.632	62.7 (7.2)	63.4 (6.1)	0.988
6	2.3 (1.4)	2.2 (1.1)	0.590	12.7 (3.6)	13.1 (4.3)	0.420	0.9 (1.4)	1.2 (1.7)	0.302	1.4 (1.3)	1.6 (1.5)	0.090	95.2 (10)	95.6 (4.3)	0.373
7	3.1 (3.5)	2.5 (2.3)	0.510	5.9 (3.3)	6.1 (3.4)	0.458	2.0 (1.5)	2.3 (1.6)	0.027	1.3 (1.4)	1.5 (1.5)	0.350	59.9 (6.3)	60.2 (6.1)	0.798
8	2.3 (2.7)	1.5 (1.8)	0.018				3.1 (1.8)	3.1 (1.8)	0.481	2.5 (1.8)	2.7 (2.0)	0.675	93.6 (0.9)	93.5 (0.9)	0.707
9	2.6 (3.3)	2.1 (2.5)	0.447				1.0 (1.2)	1.2 (1.8)	0.733	0.4 (0.8)	0.5 (1.0)	0.148	98.9 (2.1)	98.9 (1.4)	0.936
10	3.1 (2.8)	2.8 (2.6)	0.307				2.7 (1.3)	2.7 (1.4)	0.869	0.7 (1.5)	1.0 (1.7)	0.108	87.7 (1.6)	87.8 (1.4)	0.671
11	1.0 (1.3)	0.9 (1.4)	0.241				2.7 (2.0)	2.8 (1.8)	0.642				91.5 (1.4)	91.5 (1.7)	0.714
12	0.6 (1.5)	0.4 (1.1)	0.570				0.8 (1.1)	1.1 (1.3)	0.192				79.8 (2.5)	79.8 (2.1)	0.751
13	4.6 (1.9)	4.2 (1.6)	0.106				2.3 (1.3)	2.4 (1.5)	0.769				87.1 (1.4)	86.9 (1.8)	0.077
14	5.7 (2.1)	5.3 (1.6)	0.195				2.1 (1.5)	2.3 (1.6)	0.472				91.1 (3.3)	90.7 (2.6)	0.173
15	1.9 (1.2)	1.7 (1.0)	0.214				1.3 (1.6)	1.5 (1.7)	0.216				81.9 (9.1)	81.4 (9.3)	0.732
16	2.0 (1.4)	1.9 (1.1)	0.924				3.4 (1.8)	3.5 (2.1)	0.955						
17	3.9 (2.2)	3.2 (2.0)	0.006												
18	2.7 (1.9)	2.4 (1.9)	0.354												
19	2.2 (1.3)	2.0 (1.1)	0.167												
20	2.5 (1.3)	2.2 (1.1)	0.024												
21	1.6 (1.3)	1.5 (1.2)	0.625												
22	1.9 (1.4)	1.6 (1.1)	0.125												
23	2.5 (1.3)	2.6 (1.2)	0.555												
24	2.5 (1.3)	2.4 (1.3)	0.419												

* CpGs were numbered in the order they occur in the promoter region of each gene.

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Supplementary Table 3. Relative risk of TGCT associated with promoter methylation levels across the promoter region and for select individual CpG sites by gene.

Category ^s	Controls <i>n</i> (%)	TGCT Cases <i>n</i> (%)	OR [†] (95% CI)	<i>P</i> _{trend}
<i>KITLG</i>				
Combined levels [‡]				
1st (low)	36 (32.7)	62 (41.6)	2.15 (1.12, 4.14)	0.031
2nd (middle)	37 (33.6)	60 (40.3)	2.15 (1.12, 4.11)	
3rd (high)	37 (33.6)	27 (18.1)	1.00 (ref)	
CpG #5				0.061
1st (low)	36 (33.3)	65 (44.8)	1.86 (0.98, 3.54)	
2nd (middle)	37 (34.3)	49 (33.8)	1.46 (0.76, 2.79)	
3rd (high)	35 (32.4)	31 (21.4)	1.00 (ref)	
CpG #8				0.065
1st (low)	36 (33.3)	66 (45.5)	1.78 (0.94, 3.37)	
2nd (middle)	37 (34.3)	46 (31.7)	1.18 (0.61, 2.27)	
3rd (high)	35 (32.4)	33 (22.8)	1.00 (ref)	
CpG #17				0.022
1st (low)	38 (34.6)	70 (46.9)	2.12 (1.13, 3.98)	
2nd (middle)	36 (32.7)	48 (32.2)	1.71 (0.89, 3.31)	
3rd (high)	36 (32.7)	31 (20.8)	1.00 (ref)	
CpG #20				0.041
1st (low)	37 (33.6)	66 (44.3)	1.97 (1.04, 3.73)	
2nd (middle)	37 (33.6)	53 (35.6)	1.59 (0.83, 3.04)	
3rd (high)	36 (32.7)	30 (20.1)	1.00 (ref)	
<i>PDE11A</i>				
Combined levels				
1st (low)	36 (33.0)	34 (22.5)	1.00 (ref)	0.042
2nd (middle)	38 (34.9)	54 (35.8)	1.55 (0.82, 2.93)	
3rd (high)	35 (32.1)	63 (41.7)	1.94 (1.03, 3.65)	
CpG #2				0.049
1st (low)	36 (33.0)	32 (21.2)	1.00 (ref)	
2nd (middle)	38 (34.9)	58 (38.4)	1.69 (0.90, 3.19)	
3rd (high)	35 (32.1)	61 (40.4)	1.94 (1.02, 3.67)	
CpG #5				0.010
1st (low)	36 (33.0)	34 (22.5)	1.00 (ref)	
2nd (middle)	38 (34.9)	47 (31.1)	1.43 (0.75, 2.73)	
3rd (high)	35 (32.1)	70 (46.4)	2.27 (1.21, 4.27)	
<i>SPRY4</i>				
Combined levels				
1st (low)	38 (33.3)	46 (30.9)	1.00 (ref)	0.353
2nd (middle)	39 (34.2)	44 (29.5)	0.90 (0.49, 1.67)	
3rd (high)	37 (32.5)	59 (39.6)	1.32 (0.72, 2.40)	
CpG #7				

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1st (low)	37 (33.6)	35 (23.9)	1.00 (ref)	
2nd (middle)	37 (34.6)	49 (33.6)	1.53 (0.81, 2.91)	
3rd (high)	36 (32.8)	62 (42.5)	1.99 (1.06, 3.76)	0.033
BAK1				
Combined levels				
1st (low)	38 (33.3)	38 (25.2)	1.00 (ref)	
2nd (middle)	39 (34.2)	46 (30.4)	1.24 (0.66, 2.32)	
3rd (high)	37 (32.5)	67 (44.4)	1.86 (1.01, 3.42)	0.042

n, number of subjects (percent of subjects);

† odds ratios (95% confidence intervals), adjusted for age;

§ categorized into tertiles based on the distribution in controls;

‡ for the average % methylation across the entire promoter CpG island.

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Supplemental Table 4. Relative risk of TGCT by type associated with average promoter methylation.

Tumor type	Controls <i>n</i> (%)	TGCT cases <i>n</i> (%)	OR [†] (95% CI)	<i>P</i> value
Seminoma				
<i>KITLG</i>				
Low [§]	55 (50.0)	48 (68.6)	2.19 (1.16, 4.128)	0.015
High	55 (50.0)	22 (31.4)	1.00 (ref)	
<i>PDE11A</i>				
Low	55 (50.5)	30 (41.7)	1.00 (ref)	0.25
High	54 (49.5)	42 (58.3)	1.42 (0.78, 2.59)	
<i>SPRY4</i>				
Low	57 (50.0)	34 (47.2)	1.00 (ref)	0.73
High	57 (50.0)	38 (52.8)	1.12 (0.62, 2.01)	
<i>BAK1</i>				
Low	57 (50.0)	25 (34.7)	1.00 (ref)	0.041
High	57 (50.0)	47 (65.3)	1.89 (1.03, 3.47)	
<i>DND1</i>				
Low	57 (50.0)	50 (67.6)	2.08 (1.13, 3.83)	0.019
High	57 (50.0)	24 (32.4)	1.00 (ref)	
Non-seminoma				
<i>KITLG</i>				
Low	55 (50.0)	47 (62.7)	1.37 (0.73, 2.56)	0.33
High	55 (50.0)	28 (37.3)	1.00 (ref)	
<i>PDE11A</i>				
Low	55 (50.5)	28 (37.3)	1.00 (ref)	0.09
High	54 (49.5)	47 (62.7)	1.68 (0.91, 3.12)	
<i>SPRY4</i>				
Low	57 (50.0)	32 (43.8)	1.00 (ref)	0.50
High	57 (50.0)	41 (56.2)	1.23 (0.67, 2.26)	
<i>BAK1</i>				
Low	57 (50.0)	31 (41.3)	1.00 (ref)	0.18
High	57 (50.0)	44 (58.7)	1.53 (0.83, 2.80)	
<i>DND1</i>				
Low	57 (50.0)	38 (51.4)	0.93 (0.51, 1.71)	0.81
High	57 (50.0)	36 (48.6)	1.00 (ref)	

[§] average % methylation across the entire promoter CpG island dichotomized at the median, based on the distribution in controls for each gene;

n, number of subjects (percent of subjects);

[†] odds ratios (95% confidence intervals), adjusted for age.