

supplementary data

Supplementary table I: quantitative PCR on immunoprecipitated genomic DNA

Locus	Forward primer	Sequence	Reverse primer	Sequence	Annealing temp.	Enzyme ^a
<i>Chromosome 21</i>						
JB2	AL163202-315816	TCTCTCAGTCTGGTCCCAG	AL163202-316041r	TTGCAGCATTCAAAGGCCTG	52°C	<i>XbaI</i>
JB3	AL163203-176574	GTAATGGCTCAGAATCTTGCC	AL163203-176753r	GGCAGAACAAGTAGACCTTC	58°C	<i>BclI</i>
JB6	AL163203-258379	ACCAAACCCATTCCCATTAG	AL163203-258528r	GGACAACCTGACATGATGTAATAG	53.5°C	<i>AflIII</i>
JB5	AL163204-68747	TAACAGGTCAGGAGAAGTATG	AL163204-68898r	CCATCCAAGGAAGCACAG	57°C	
JB10	AL163204-93846	GTTTCCCACCAGCCAAATGAG	AL163204-94030r	GGGCAGCACGCAGATACC	59°C	<i>HphI</i>
JB4	AL163204-95600	GGTGAGACTATACTGTGGTC	AL163204-95760r	GTATTGTCTTCCATTCTCACTC	49°C	
JB8	AL163204-175374	AAACTGCTGTTATTTCAACTTCC	AL163204-175491r	ACTTCTTCTCCAAATTTTGATGTG	51°C	<i>HinfI</i>
JB9	AL163204-198686	AATGTGAAGCCCTCTTTGAGTTCC	AL163204-198891r	AGCAGGGAAATGGACAGAAATGG	59°C	<i>EcoRI</i>
JB7	AL163204-264736	CATTGGCTGTGAAGTTGAG	AL163204-264900r	GAAGGCTGAGAAGGATGAG	53.2°C	<i>HaeIII</i> ^b
STS3	AP001660-140183	ACTTATGTTATGCCCTCATTAG	AP001660-140375r	GTGTGTTTCCTTGGTGAAAC	52.5°C	
STS31	AP001660-335748	CAGGCTGTGAATATAAGTG	AP001660-335858r	AACATCAATAGCAGACAAG	53°C	
<i>Reference locus</i>						
<i>GAPDH</i> ^b	ChIPs-GAPDH.US	TAGTGTCTCTGCTGCCCCACAGTCCAG	ChIPs-GAPDH.DS	GGCGACGCAAAAGAAGATGC	68°C	

a) the PCR product from chromosome 21q is not cut with this enzyme

b) Futscher, B.W., Oshiro, M.M., Wozniak, R.J., Holtan, N., Hanigan, C.L., Duan, H., and Domann, F.E. 2002. Role for DNA methylation in the control of cell type specific maspin expression. Nat. Genet. 31: 175–179.

Supplementary table II Twenty 21q11-specific SNPs. PSV-specific detection and genotyping were combined using PSV specific primers and/or allele-specific primers and/or restriction digests.

SNP ID	NCBI(35.1) position	Genomic DNA digest	Primers	Annealing temp	PCR product digest	Fragment size (bp)
rs2261012	13528911		AGGATTTCTCCCCAGCTCT	CTGGTATTAATTTCCAAAAAAct/a	56°C	1095
JB2AluI	13575226	<i>XbaI</i>	CTCCATTACCAGGGAACATA	TACCTCAGCTTTTCTTGCTC	56°C	<i>AluI</i> 227 / 195-32 (+147-119-103-79-8)
JB11Y	13614025		GTGAATGTCTTCTGGGTGAA	TCTTCAGTTGAATCCTTAGc/t	C 60 / T 57°C	1119
JB12AflII	13688156		CAGTATGAGGACATGAAAGCA	TGCATGAGTGAATCAAGAAA	56°C	<i>AflII</i> 1090 / 650-440
JB13HinfI	13712668		TGTTATACCATGCGAGTAGTTA	TGTATGTCTTACTTCACGCTAC	56°C	<i>HinfI</i> 733 / 424-309 (+154-78-58)
rs468064	13753478		TGCCTGATTTTAAGCAAAAGA	TGGTAAAAACAAGCTTCAACG	56°C	<i>HpyCH4IV</i> 1038 / 890-148 (+20)
rs468732	13775819		GTACTAGTAAAATTATTCATCAAA	CCAATCAATGAATGACCCa/g	59°C	249
JB14M	13820507		ACTTAGAGGGCCTGGAGCA	CCTTGAAGCAACAAATTCACa/c	A 64 / C 65°C	807
JB6ApeKI	13857826	<i>AflII</i>	ACCAAACCCATTCCCATTAG	TGCTTGAGGAGCTAGCAAGA	58°C	<i>ApeKI</i> 983 / 662-321
rs2821973	13899316		ATCTGCACCACCTTTCTTCA	TCTGTTTTCAAGCCCAAGAG	62°C	<i>PvuII</i> 490 / 338-152
rs12481774	13933576		ACCTCAGAATTCATTTTCTG	CAGGTTTAACAGATGTGAa/g	A 57 / G 58°C	274
JB15Hpy4V	13958154		TGCCCCTGAGCGAAGGCA	TGGGGACTGGCAGTATCA	64°C	<i>HpyCH4V</i> 208 / 107-101 (+303)
rs8129067	13986833		TTGGGTCTCTGAGGAGGAT	TAGAGATGTTTCAAGGAGTc/c	T 63 / C 64°C	497
rs7282547	14009151		TGCCACCCATCTCAGGGTT	CTGCCTGGGGAAGAAAGg/c	64°C	223
rs2792379	14027356		CATGAAATAAGTGCATTCCTG	CTGCTTTCATCTCACTAAAc/t	T 57 / C 59°C	193
rs2792453	14034699		GTCTATGGTCTATTTAGGCAC	TCAGCAGCTTGGAGAAATc/g	C 60 / G 61°C	247
JB16AluI	14071200		CTCAAGATAAAGCTAATGAA	CCGGTTGTTGAATTTATAATTCC	54°C	<i>AluI</i> 204 / 154-50 (+94-33-12)
rs2822142	14103189		CAAAATACTGGAAGCTAAATA	TTAGCAGAAGATGTTAGATA	50°C	<i>MspI</i> 216/158-58
rs3117463	14133842	<i>EcoRI</i>	CCTTCAGCAAGCCTCAAAAC	GCACCCCAAATCAGTACCAT	56°C	<i>XhoI</i> 971 / 637-334
rs2822232	14200678		CATCAGCCTCTGCACCTTTA	CCAACCTGTGTGATAGAGGA	55°C	<i>RsaI</i> 1285/ 600-685 (+218)

Supplementary table III : Twenty five highly informative HapMap SNPs along 21q11 and their positions on the chromosome 21.

SNP ID	NCBI(35.1) position	Distance to α -sat (kb)
rs3965725	13518880	228.969
rs2261012	13528911	239.000
rs2821795	13570028	280.117
rs1148733	13599172	309.261
rs2821809	13636758	346.847
rs3843783	13666779	376.868
rs2821850	13693682	403.771
rs7409830	13703830	413.919
rs463974	13719888	429.977
rs469283	13746483	456.572
rs13047108	13801874	511.963
rs9284487	13862877	572.966
rs2821973	13899316	609.405
rs1929152	13969506	679.595
rs7282547	14009151	719.240
rs2260407	14057714	767.803
rs2822142	14103189	813.278
rs8184939	14157220	867.309
rs7276618	14197852	907.941
rs8134986	14253349	963.438
rs7278536	14297378	1007.467
rs4816917	14342603	1052.692
rs2822408	14397020	1107.109
rs2822436	14447961	1158.050
rs409070	14482031	1192.120

Supplementary table IV: Results of ChIP, positions of STS and enrichment of histone isoforms at the STS relative to *GAPDH*

STS	NCBI(35.1) position	NT_011512.10 Hs21_11669	Distance to □-sat (kb)	Average ^(a) enrichment of			
				H3K9Me3	H3K27Me	H4Ac	H3K9Ac
JB2	13575816- 13576041	315816- 316041	285.905- 286.130	1.08 ± 0.18	0.91 ± 0.32	0.41 ± 0.10	0.11 ± 0.05
JB3	13775503- 13775682	515503- 515682	485.592- 485.771	1.41 ± 0.42	1.16 ± 0.25	0.36 ± 0.06	0.34 ± 0.16
JB6	13857307- 13857456	597307- 597456	567.396- 567.545	0.55 ± 0.17	0.78 ± 0.23	0.23 ± 0.07	0.37 ± 0.04
JB5	14004389- 14004540	744389- 744540	714.478- 714.629	0.41 ± 0.22	0.68 ± 0.30	0.20 ± 0.11	0.19 ± 0.16
JB10	14029488- 14029672	769488- 769672	739.577- 739.761	5.56 ± 1.44	3.62 ± 1.49	0.84 ± 0.33	0.45 ± 0.26
JB4	14031223- 14031402	771223- 771402	741.312- 741.491	2.58 ± 0.69	2.36 ± 0.15	0.64 ± 0.06	0.20 ± 0.10
JB8	14111016- 14111133	851016- 851133	821.105- 821.222	0.22 ± 0.11	0.34 ± 0.20	0.09 ± 0.04	0.18 ± 0.09
JB9	14134328- 14134533	874328- 874533	844.417- 844.622	1.00	1.48	0.35	0.34 ± 0.28
JB7	14200378- 14200542	940378- 940542	910.467- 910.631	0.74 ± 0.38	0.57 ± 0.26	0.17 ± 0.04	0.86 ± 0.58
STS3	14414825- 14415017	1154825- 1155017	1124.914- 1125.106	0.61 ± 0.08	0.87 ± 0.22	0.34 ± 0.04	0.30 ± 0.09
STS31	14610390- 14610500	1350390- 1350500	1320.479- 1320.589	0.48 ± 0.23	0.42 ± 0.18	0.14 ± 0.05	0.09 ± 0.05

a) average of cell lines OPM-2, BT20, and BT474, and primary human fibroblasts

Supplementary table V: DNA methylation in %5mCpG

	<i>TPTE</i> <i>family</i>	<i>BAGE</i> family (a)		<i>ANKRD21</i> family		<i>RBM11</i>		<i>ABCC13</i>		<i>STCH</i>		<i>NRIP1</i>	
Tissue/cell culture	C (b)	C	S (b)	C	S <i>ANKRD21</i>	C	S	C	S	C	S	C	S
skin 1	100	42	83.7 (c)	87	87.1	24	1.3	16	25.6	0	1.1	0	0
skin 2	98	54		86	85.5	0		0		0		0	
heart 1	67	61		72		0		0		0		22	
heart 2	100	nd		68		0		0		0		0	
prostate 1	0	75		81		0		12		0		nd	
prostate 2	100	77		91		nd		0		0		0	
brain	99	52		55		0		0		0		19	
fibroblasts	99	15		64		0		24		0		0	
mean	83	54		75		3		6		0		6	
normal													
<i>std.error</i>	<i>13</i>	<i>8</i>		<i>4</i>		<i>3</i>		<i>3</i>		<i>0</i>		<i>4</i>	
LB1622-MEL	61	7	56.9	90	66.0	0	0.3	28	45.1	0		22	
SK23-MEL	0	17		97	80.4	100	74.7	98	53.6	0	0	0	0
LB23-MEL	99	11		96		100		98		0		17	
BT20	100	23		98		100		98		0		0	
BT474	0	53		93		0		78		0		nd	
CCL-155	nd	4		96		0		95		0		nd	
OPM-2	99	15		87		0		nd		0		21	
Ovarian Carc. F	100	40		92		0		0		0		0	
mean tumor	66	21		94		37		71		0		10	
<i>std.error</i>	<i>18</i>	<i>6</i>		<i>1</i>		<i>18</i>		<i>15</i>		<i>0</i>		5	
Sperm 1	0	26	31.3	0	1.0	0	0	0	0.4	0		nd	
sperm 2	0	14		0		0		0		0		0	
sperm 3	0	28		0		0		0		0		8	
sperm 4	0	19		0		0		0		0		nd	
testis 1	74	59		53		0		5		0		0	
testis 2	71	34		52		0		0		0		18	
testis 3	53	27		42		0		0		0		12	
testis 4	0	56		67		0		0		0		nd	
mean	25	44		27		0		1		0		8	
sperm/testis													
<i>std.error</i>	<i>12</i>	<i>6</i>		<i>10</i>		<i>0</i>		<i>1</i>		<i>0</i>		3	

- a) data previously published (Grunau et al. 2005) except for fibroblasts
b) C = COBRA, S = Sequencing
c) *BAGE-gf6*

Supplementary table VI: RT-PCR/restriction digest analysis of transcription

Locus	Forward primer	Sequence	Reverse primer	Sequence	Annealing temp.	Length in bp	enzyme ^a
<i>Chromosome 21</i>							
<i>TPTE</i>	TPTE EXP2	TCCAGCTACAACATCACTGGAAGT	TPTE EXP1	TGATGTATTTCGACGGTCCACCTCTG	60°C	257	<i>EaeI</i>
<i>BAGE</i>	BAGE14	GTCTCTGGTATCTCCCGCTGAG	BAGE19	TTGCTCCTGTTGAGCTGCCGTCTC	68°C	366	na
	BAGE20	CATTTTGTAAGCACTTTGGAGCCAC	BAGE21	CACTATCAGCCTCATTAGAAATCTG	60°C	488	na
	Q-BAGE-F	TTAGAGGACCAGGAGAAGGG	Q-BAGE-R	TCTGTTGCCACCACCATC	60°C	84	<i>NlaIV</i>
<i>ANKRD21</i> ^b	RT.POTE.T445	TCTCTGGCCGTCTGTCCAGATAGA	RT.POTE.T444	CACTGCCAGGAAGATGAATGTGCG	60°C	386	<i>BpuAI</i> <i>EcoRV</i>
<i>RBM11</i>	RBM1	GTGTTTGTGGGAATTTAGAGG	RBM4	CTGCACGTTAATTGGTCTTCC	60°C	216	
<i>ABCC13</i>	PRED6/6	GATTCCTTCTGCACTGCGTGTCCCATC	PRED6/8	TCATTATGAGTGGGCTAGTG	60°C	228	
<i>Reference locus</i>							
<i>GAPDH</i>	GAPDH1	TGAAGGTCGGAGTCAACGGATTTGGT	GAPDH2	CATGTGGGCCATGAGGTCCACCAC	50°C	983	
<i>ABL1</i>	Q-ABL2F	TCTTGAAGTGGGCGAATGTC	Q-ABL2R	AGGCGTGCTCTGTGAAATAC	60°C	134	

a) the transcript from chromosome 21 is not cut with this enzyme

b) (Bera et al. 2002)

Supplementary table VII: Conditions for nested PCR on bisulfite-treated DNA

Locus	Forward primer	Sequence ^a	Reverse primer	Sequence ^a	Annealing temp.	Bias ^b	COBRA enzyme
<i>TPTE</i>	BS TPTE 122f	TtgtTTTTTTaggTtgagtgTagtg	BS TPTE 937r	cactaccaAttcttActcTAcCactcc	51°C		
	BS TPTE 291f	tgtagTTaggatggtTtggaTtT	BS TPTE 735r	cctcctctAcaaAAAtAtAcctAAAc	52°C	0.017	<i>BstBI</i>
<i>BAGE</i>	BAGE.BS+32f	TttagaggaTTaggagaagggggagT	BAGE.BS+1540r	AcctAccaAttAAcattAttActAacattA	50°C		
	BAGE.BS+99f	gatggtggtggTaaTagagatggT	BAGE.BS+1371r	ccttaAAcaAtAtaAaccctAataA	50°C	0.782	<i>MboI</i>
<i>ANKRD21</i>	BS POTE 276f	tttTtTagaggaggTtgtTagTat	BS POTE 946r	aaAAAtcaatcccaAccaaaacttA	50°C		
	BS POTE 85f ^c	gTtgatggTTTtgtgtTtggg Ta	BS POTE 1002r ^c	ccaAttccaccaactaAcaAAAAacct	63°C		
	BS POTE 276f	tttTtTagaggaggTtgtTagTat	BS POTE 753r	aaattcaAAcccaAcaaaAAaatAc	60°C	0.266	<i>AccI</i>
<i>RBM11</i>	RBM11bs1	agttttTgttgtaTTaTaTtatgT	RBM11bs2	AccttAtccaAAAAatcacctAAtc	50°C		
	RBM11bs3	gtggTTTtggagTTatgTTt	RBM11bs4	caAcctctAAAAaAcccttaatc	58°C	0.086	<i>TaqI</i>
<i>ABCC13</i>	ABCC13bs1	aTtggTtaggaTtgaaggTaag	ABCC13bs2	actAaActcctctAAttcaaAttAcac	50°C		
	ABCC13bs3	gggaTtaaggtagTagTgaTtTgtT	ABCC13bs4	ccctAatctctAatctctActct	58°C	0.121	<i>RsaI</i>
<i>STCH</i>	STCHbs53	TagtgTtagTaataagaaaaatgtgTT	STCHbs1216	ccacaaaAAAAtcctAAaAAtAcac	60°C		
	STCHbs357	gaatTTtgggTTtgattaggggTa	STCHbs847	tcattctctAAccatcacAatcc	60°C	nd	<i>TaqI</i>
<i>NRIP1</i>	RIP140bs-1	gTTTTTtggTTTtagaaTtggTa	RIP140bs-2	AccctActcctttcccttAtcttAt	45°C		
	RIP140bs-3	TTtgggTagTtTTtTTtgggggAT	RIP140bs-4	aattaaccctActcctAAAtcctac	45°C	nd	<i>HpyCH4IV</i>

a) nucleotides resulting from deamination by bisulfite or mismatches to avoid CpG in upper case; chromosome 21-specific bases in bold

b) $b = (y \cdot (100 - x)) / (x \cdot (100 - y))$, with x = true methylation and y = methylation detected by COBRA

c) primers used for chromosome 21-specific PCR and cloning of PCR products