Supplementary Data

Genome-Wide Screen of Promoter Methylation Identifies Novel Markers in Melanoma

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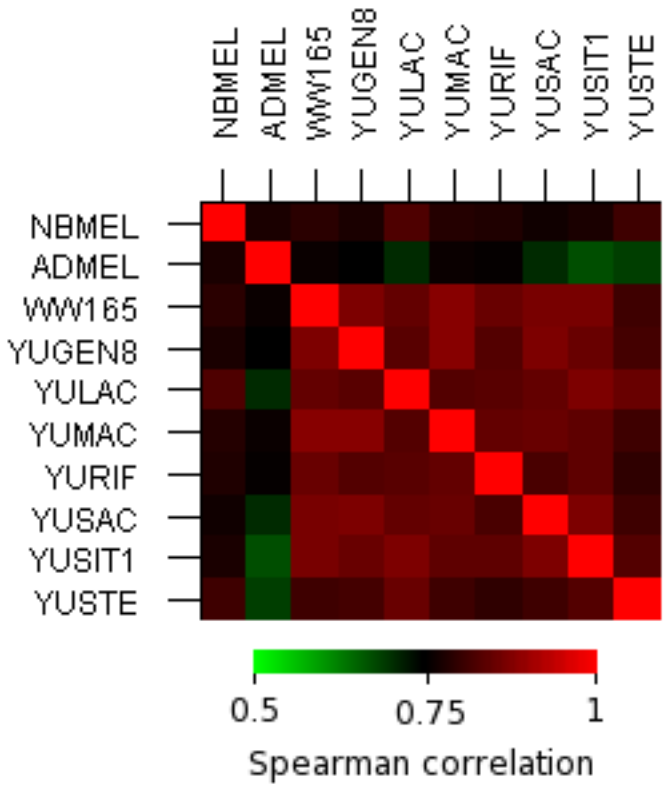
Supplementary Figure S1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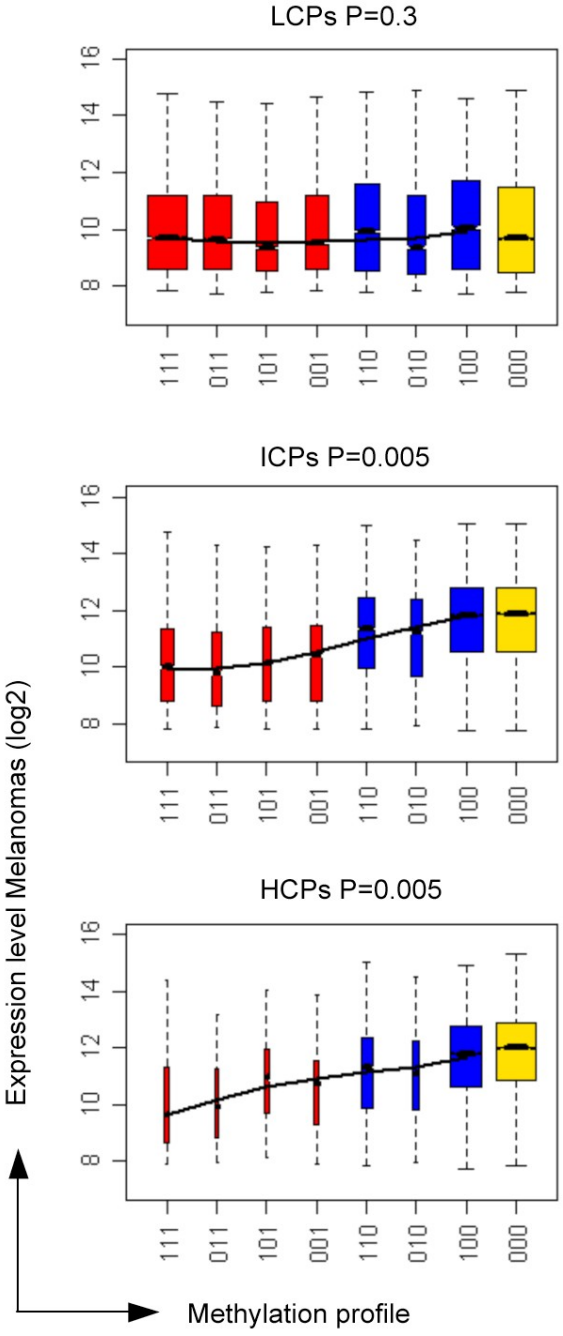
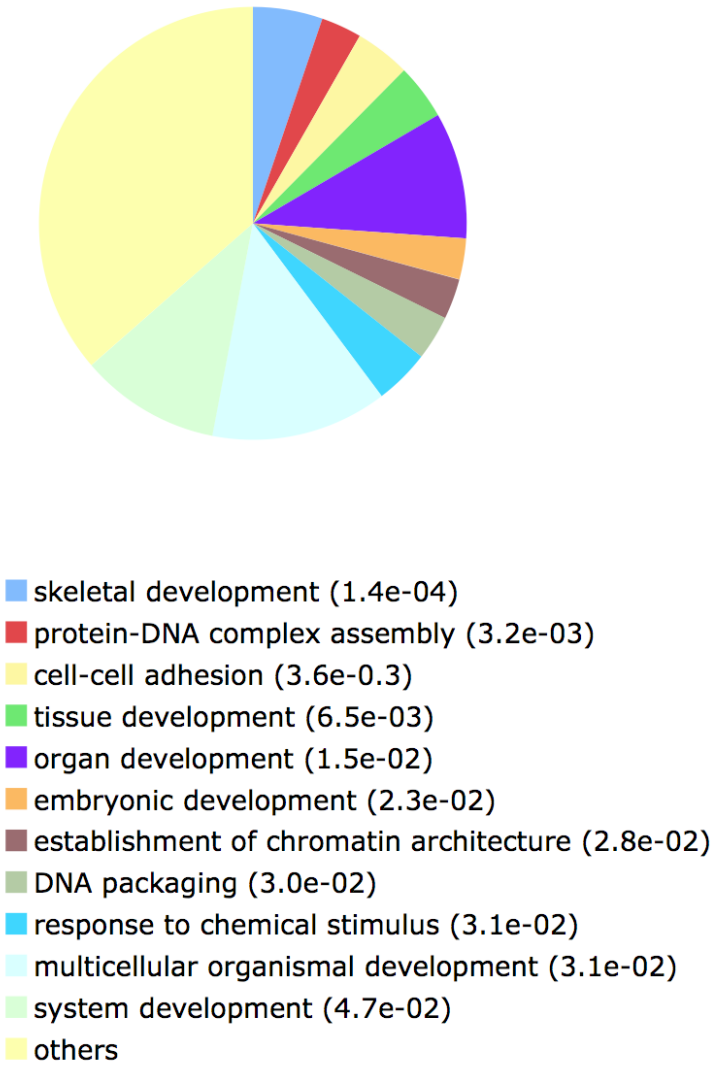
ADMEL
WW165
YUGEN8
YULAC
YUMAC
YURIF
YUSAC
YUSIT1
YUSTE
0.5
0.75
1
Spearman correlation

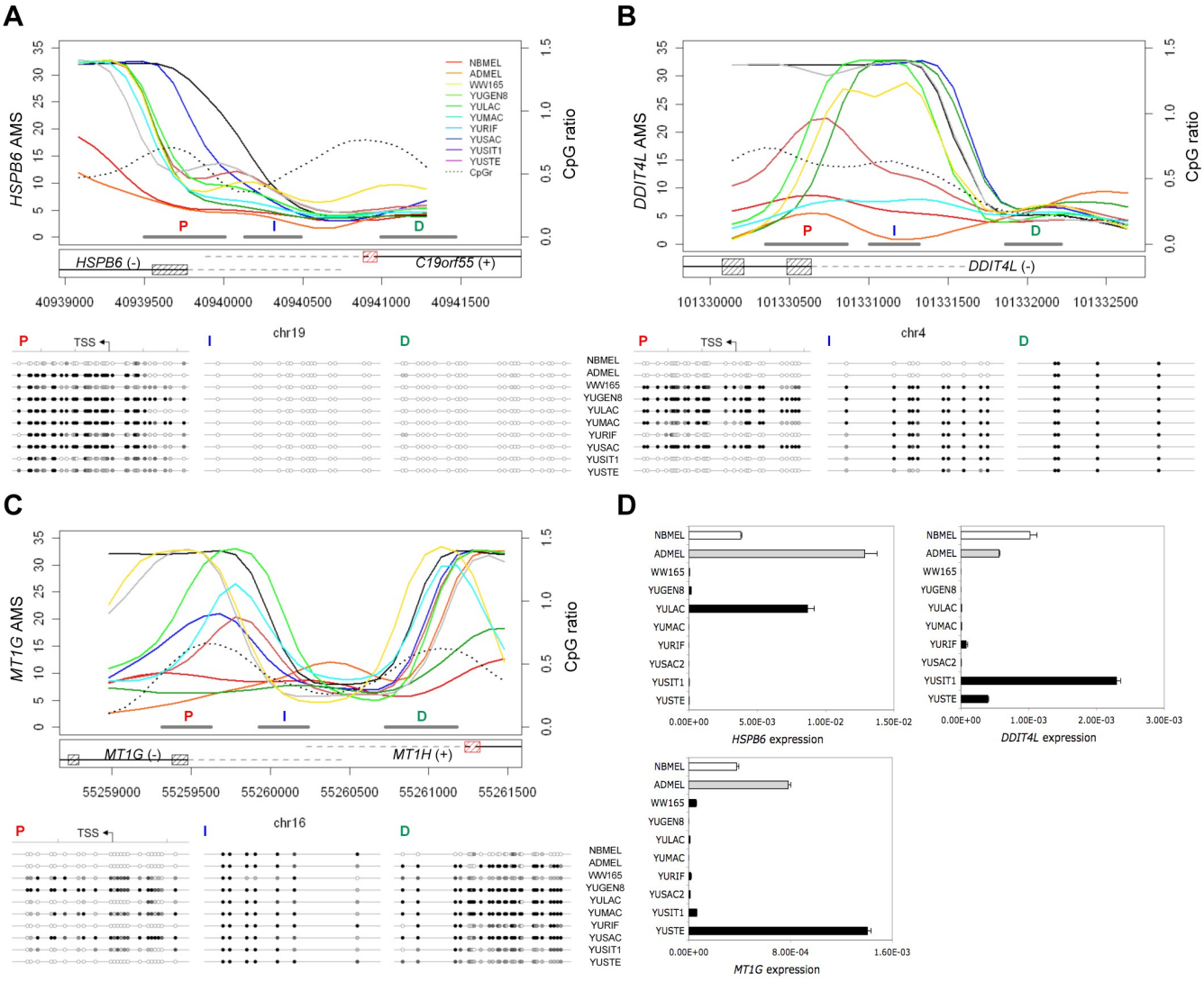
Supplementary Figure S2



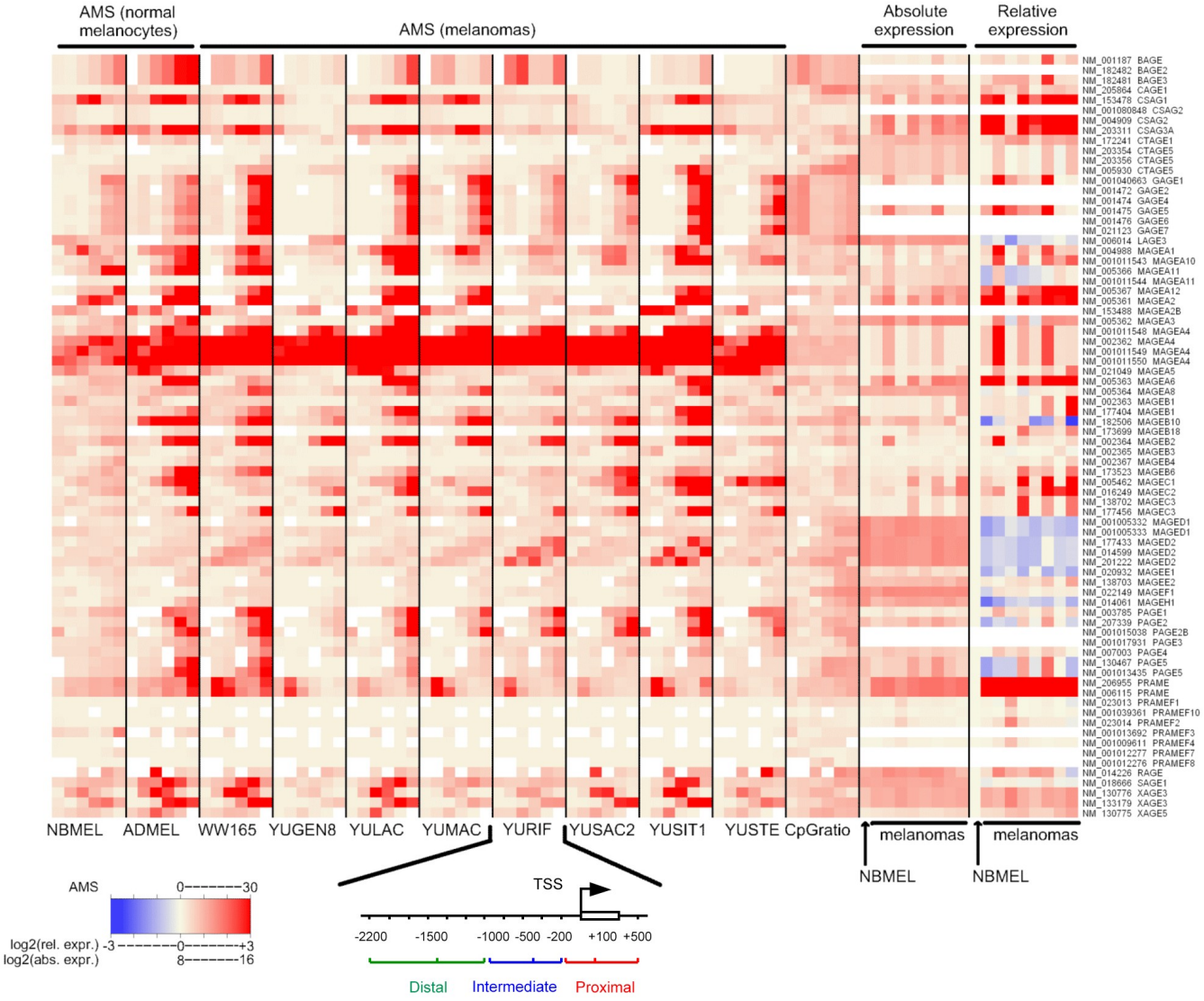
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Legends for Supplementary Figures

Figure S1 Pair-wise Similarity of Promoter Methylation

Spearman correlation coefficient of genome-wide promoter AMS is displayed according to the color scale.

Figure S2. Promoter Methylation and Transcriptional Repression in Melanoma Cells

Expression of genes under the control of promoters with methylation profile in each promoter category. Red and blue groups indicate promoters with and without proximal methylation (XX1 and XX0 profiles, respectively). Yellow indicate unmethylated promoters. From left to right are groups ranging from fully methylated to unmethylated, and the order is based on the progressive lack of methylation from distal to proximal regions. Smoothing over the median for each group is shown.

Figure S3. Over-represented GeneOntology functional families

GeneOntology terms enriched in the list of hyper-methylated markers; enrichment *p*-value is determined based on the hyper-geometric distribution and terms with $P<0.05$ are represented.

Figure S4. Promoter Methylation and Gene Expression for *HSPB6*, *DDIT4L* and *MT1G*

(A) Probe-level AMS, CpG ratio and genomic sequences of bisulfite modified DNA for the proximal (P), intermediate (I), and distal (D) promoter regions of *HSPB6* in newborn (NBMEL) and adult (ADMEL) melanocytes and eight melanoma cell strains; gray bars indicate the amplicons of BS sequencing; D, distal, I, intermediate, and P, proximal for each promoter region; 1 Kb upstream region (dashed line), exon (dashed box) and CDS (solid black line) for each RefSeq in the locus are displayed; CpGs are represented as circles and white, grey and black shades refer to the average of mCpG/CpG (0 to 1) for each

sample.

The methylation obtained by BS sequencing of HSPB6 proximal promoter in ADMEL is not concordant with the AMS profile. For this reason we repeated the same analysis in other three ADMEL cell strains validating the un-methylated status predicted by ADMEL AMS. (B) and (C) are the same as (A) for *DDIT4L* and *MT1G*, respectively. (D) *HSPB6, DDIT4L* and *MT1G* transcript levels measured by real-time RT-PCR; expression levels were normalized to that of *ACTB*.

Figure S5. Heat-map of Methylation and Expression Profiles for Cancer/Testis Antigens

Promoter AMS for newborn (NBMEL) and adult (ADMEL) melanocytes and eight melanoma cell strains as well as the CpG ratio in each of 6 regions is shown; absolute gene expression is displayed as well the expression relative to normal melanocytes (NBMEL); Each color represents the average probe-level AMS (0 to 30) in the promoter regions as well as log2 absolute (8 to16) and relative (-3 to +3) expression level, as indicated in the lower left-hand side of the figure. RefSeq IDs and gene symbols are represented on the right-hand side.

Supplementary Table S1. Selected hyper-methylated markers

RefSeq	Gene Symbol	Chr	Differential methylation[a]	LME[b] *p*-value	Ref. Methylation[c]	Ref. Melanoma[d]
NM_207446	*FAM174B*	chr15	11.7	0.000367	-	-
NM_000089	*COL1A2*	chr7	15	0.000441	[1-3]	[4]
NM_002119	*HLA-DOA*	chr6	12.1	0.000585	-	-
NM_002570	*PCSK6*	chr15	7.5	0.000599	[5]	-
NM_207306	*KIAA0495*	chr1	12.9	0.00153	-	-
NM_177476	*LYNX1*	chr8	14.4	0.00211	-	-
NM_182795	*NPM2*	chr8	12.5	0.00213	[6]	-
NM_032391	*PRAC*	chr17	13.3	0.00243	-	-
NM_145685	*BRF1*	chr14	8.45	0.00246	-	-
NM_144617	*HSPB6*	chr19	11.1	0.00332	-	-
NM_001001567	*PDE9A*	chr21	10.4	0.0035	-	-
NM_003498	*SNN*	chr16	3.02	0.00373	-	-
NM_003985	*TNK1*	chr17	12	0.00555	-	-
NM_015264	*C22orf9*	chr22	6.85	0.00579	-	-
NM_139247	*ADCY4*	chr14	8.44	0.0058	-	-
NM_024832	*RIN3*	chr14	8.65	0.00663	-	-
NM_153757	*NAP1L5*	chr4	8.94	0.00687	[7]	-
NM_015537	*NELF*	chr9	4.1	0.00832	-	-
NM_001854	*COL11A1*	chr1	14.1	0.00853	[8]	-
NM_003433	*ZNF132*	chr19	17.5	0.00889	-	-
NM_199334	*THRA*	chr17	2.79	0.00984	-	-
NM_032089	*PCDHGA9*	chr5	7.15	0.0106	-	-
NM_021828	*HPSE2*	chr10	14	0.0129	-	-
NM_177477	*LYNX1*	chr8	11.9	0.0134	-	-
NM_020896	*OSBPL5*	chr11	2.97	0.0134	-	-
NM_020682	*AS3MT*	chr10	16.2	0.0144	[9][e]	-
NM_144673	*CMTM2*	chr16	7.45	0.0145	-	-
NM_016941	*DLL3*	chr19	9.78	0.0153	-	-
NM_004273	*CHST3*	chr10	5.04	0.0155	-	-
NM_012216	*MID2*	chrX	8.18	0.0155	-	-
NM_004107	*FCGRT*	chr19	13.4	0.0156	-	-
NM_004794	*RAB33A*	chrX	12.6	0.0156	-	[10]
NM_021805	*SIGIRR*	chr11	4.39	0.0163	-	-
NM_001039999	*FAM83G*	chr17	6.95	0.0163	-	-
NM_015696	*GPX7*	chr1	9.68	0.0164	[11]	-

NM_004881	*TP53I3*	chr2	15.1	0.0179	-	-
NM_001085384	*ZNF154*	chr19	15.4	0.0187	-	-
NM_145166	*ZBTB47*	chr3	11.2	0.0195	-	-
NM_015197	*PACS2*	chr14	6	0.0196	-	-
NM_017787	*C10orf26*	chr10	1.76	0.0205	-	-
NM_006896	*HOXA7*	chr7	7.4	0.0222	[12]	
NM_020895	*GRAMD1A*	chr19	2.98	0.0239	-	-
NM_020857	*VPS18*	chr15	4.13	0.0252	-	-
NM_201252	*AKR7L*	chr1	14.6	0.0253	-	-
NM_207644	*C22orf36*	chr22	7.86	0.0253	-	-
NM_030971	*SFXN3*	chr10	2.2	0.0254	-	-
NM_007085	*FSTL1*	chr3	7.26	0.0269	-	-
NM_006868	*RAB31*	chr18	6.05	0.0271	-	-
NM_145244	*DDIT4L*	chr4	13.3	0.029	[13]	[14]
NM_174934	*SCN4B*	chr11	8.89	0.0291	-	-
NM_005101	*ISG15*	chr1	7.82	0.0295	[15]	-
NM_138383	*MTSS1L*	chr16	2.88	0.0295	-	-
NM_152456	*IL34*	chr16	7.72	0.0299	-	-
NM_005950	*MT1G*	chr16	10.2	0.033	[16,17]	-
NM_020972	*ZFYVE28*	chr4	6.63	0.0346	-	-
NM_176875	*CCKBR*	chr11	14.6	0.0349	-	-
NM_016282	*AK3*	chr9	4.69	0.0358	-	-
NM_001004434	*SLC30A2*	chr1	11.9	0.037	-	-
NM_001552	*IGFBP4*	chr17	5.27	0.0389	[18]	-
NM_017805	*RASIP1*	chr19	2.18	0.0397	-	-
NM_004823	*KCNK6*	chr19	10.2	0.0399	-	-
NM_023942	*LRRC61*	chr7	9.22	0.0402	-	-
NM_032406	*PCDHGC4*	chr5	8.36	0.0405	[13]	-
NM_001268	*RCBTB2*	chr13	1.71	0.0412	-	-
NM_002615	*SERPINF1*	chr17	10.1	0.0424	-	-
NM_021076	*NEFH*	chr22	7.37	0.0448	-	-
NM_006361	*HOXB13*	chr17	5.73	0.0475	[19]	[4]
NM_005634	*SOX3*	chrX	13.5	0.0485	[20][e]	-

[a] Average melanoma AMS – average normal melanocytes AMS
[b] Linear Mixed Effect models
[c] Supplemental references for genes already reported to be silenced by promoter methylation in human cancer studies
[d] Supplemental references for genes already reported to be silenced by promoter methylation in melanoma
[e] Gene reported to be silenced by promoter methylation in normal human cells, but not associated with cancer yet.

Supplementary Table S2. Selected hypo-methylated markers

RefSeq	Gene Symbol	Chr	Differential methylation[a]	LME[b] *p*-value	Ref. Methylation[c]	Ref. Melanoma[d]
NM_016334	*GPR89A*	chr1	-0.785	0.0412	-	-
NM_015454	*LARP7*	chr4	-0.0742	0.0303	-	-
NM_015384	*NIPBL*	chr5	-2.59	0.000416	-	-
NM_001858	*COL19A1*	chr6	-2.26	4.92E-05	-	-
NM_182562	*FAM169B*	chr15	-2.37	0.0325	-	-
NM_016937	*POLA1*	chrX	-1.83	0.00475	-	-
NM_004606	*TAF1*	chrX	-0.972	0.035	-	-
NM_182540	*DDX26B*	chrX	-1.42	0.0357	-	-

[a] Average melanoma AMS – average normal melanocytes AMS
[b] Linear Mixed Effect models
[c] Supplemental references for genes already reported to be silenced by promoter methylation in human cancer studies
[d] Supplemental references for genes already reported to be silenced by promoter methylation in melanoma

Supplementary Table S3. Primer oligos used for bisulfite DNA sequencing (P, I, D for proximal, intermediate and distal, respectively)

Gene Symbol		Position	Primer Sequences 5'-3'	Annealing Tm (° C)
COL1A2	P	chr7:93,861,707-93,862,034	AGTGGTTTATAGGGTATAGGTGAGG	55
			AACAACCACAACTAAAAAAAACCTAC	
	I	chr7:93,861,106-93,861,547	GGAGGGTTGAAGGGTTTAGGTTTAG	58
			CCCACTATTTCAACAATAATACACTCTCTT	
	D	chr7:93,860,454-93,860,793	AGAAGTTGTTGATGGAGTTAATTTTTGTAG	58
			AAAATAACATTCCAAACCCTAAATTTAAAA	
NPM2	P	chr8:21,938,061-21,938,368	GTTTTTTAATAGTTTTTTAGGTGATTTTGT	55
			AAACCTCTATACACCTCCACCAAAC	
	I	chr8:21,937,431-21,937,823	AATGAGTTTTTGTTTTAGGGGTGTG	56
			CAATTAAAAAACCCTCCTTCTTATTC	
	D	chr8:21,936,811-21,937,200	TTTTATTTAGTAGATGAGGAAATTGAGGTA	56
			AACAAACACATACATACCATACACAAAC	
HSPB6	P	chr19:40,939,499-40,940,011	TTAGATTTTTGGTAATGGAAGTGGT	58
			AAAACTCCCCCTCCTCAACCTTAAT	
	I	chr19:40,940,135-40,940,488	TTTTTAGGAGTAGATTTTTGGAGAATG	58
			CTCCATCAATTAAACCAATCAATCC	
	D	chr19:40,940,992-40,941,465	ATTATAGGAGGTTGGGGAGGATTAA	58
			ACACTCCAATCTAAACCAACAAAAC	
DDIT4L	P	chr4:101,330,348-101,330,861	ATTTAATTTGATAAAGTTTTGGGAG	60
			TCTACCCTAAAAACAACAAATATCC	
	I	chr4:101,331,006-101,331,316	TTTTTGTTTTGGGGTTTTGTTGTGT	60
			AAAATACCAAACTCACCCTTCCTAC	
	D	chr4:101,331,865-101,332,215	TGAAAGGTAGTTTTAGTTGTTTTGGTATAA	60
			TCCAACTCCTAACCATAAACCCATC	
MT1G	P	chr16:55,259,317-55,259,626	GATATTTTTTATGGTGTTTGGGAATTTG	60
			ACCTTACACTTAACCCATCTCCTAC	
	I	chr16:55,259,928-55,260,239	GTGTGTAAAGGGTAGGTTAAGGGTG	60
			TACAAAATCTTCATATCACCTTCAATAATC	
	D	chr16:55,260,725-55,261,177	TAGGTAATGTTAGAGATTTGAAGGT	60

TACAAAAACCCTACCCCACCCTTAC

Supplementary Table S4. Primer oligos used for real-time PCR

Gene Symbol	Position	Primer Sequences 5'-3'	Annealing Tm (° C)
COL1A2	chr7:93,877,511-93,877,746	TCCAAAGGAGAGAGCGGTAA	60
		CAGATCCAGCTTCCCCATTA	
NPM2	chr8:21,938,958-21,939,189	TGCAGGCTGTTGCTTCATAC	60
		GGCTGCATCTTCTTGTCCTC	
HSPB6	chr19:40,937,470-40,937,618	GGACACCAGGCCAACTAGAA	60
		GACTTGGGGGTCAAGACAAA	
DDIT4L	chr4:101,328,307-101,330,111	TGCTGGACTGTGGCTATCAC	60
		GAGGTTGGGTTCAGGAACAA	
MT1G	chr16:55,258,741-55,259,445	CCTTCTCGCTTGGGAACTCT	60
		GCATTTGCACTCTTTGCACT	

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