

Table S1. Bisulfite-PCR and sequencing primer sequences for pyrosequencing.

Gene (RefSeq accession number)	Sequence, 5'→3'	Amplicon size, bp
<i>AATF</i> (NC_000017)	FP: GGTTGTTGAATTATTTAGTAGTTTTATTGT RP: Biotin-CAAACCTCCTCCTTAAAACCTT SP: ATTTAGTAGTTTTATTGTGTAAT	217
<i>ABCC9</i> (NC_000012)	FP: GTGGGTGTTTTATTTAGAATGAGTTT RP: Biotin-ACTCTATAAACACCCCTTTATTAAC SP: AGAATGAGTTTTTTATTTT	113
<i>ADAMTS20</i> (NC_000012)	FP: AGATAGGAGGTGTGGTTGGATTA RP: Biotin-AACCACTTAACCACCCACATAAT SP: AGGAGGAGAGGGAT	196
<i>FOXC2</i> (NC_000016)	FP: GGGGTTAGTTTGTTAGAGGGAATT RP: Biotin-ATAACTCTACTATTTCCCCTTT SP: GTTGAAGTTGGTAGGT	117
<i>HELT</i> (NC_000004)	FP: GGAAATTAATAGTGTGTATGGAATGAA RP: Biotin-AACCCTTATCATCCAAATTAATAAC SP: GTTTAAAAATGGGAGTGG	181
<i>NKX2-5</i> (NC_000005)	FP: GGGTTTTTAAGTTAGGTTAATTTATAGTT RP: Biotin-CCCAACCCAAATTCTCCTTA SP: GGTAAGTGGTTAAAAGTTTAG	116
<i>OLIG3</i> (NC_000006)	FP: AGTTTGGGTGTGGGAAGGATA RP: Biotin-CCACCTCCCTAACCTAAAT SP: GTGTGGGAAGGATAGA	54
<i>ONECUT1</i> (NC_000015)	FP: Biotin-GAGTAGAGTGGTTTTGGTGTTTTG RP: CTAATTCTTACCCTCCATATCC	143

	SP: AAAAACTATCAACTAATACCAT	
<i>PCDHGA12</i> (NC_000005)	FP: AGAAAAGGTTGTTTATTATTTG RP: Biotin-TACCCAAAACCAAATTCTC SP: TGTTTATTATTTGGTTTTTA	167
<i>PRRX1</i> (NC_000001)	FP: TGGGGAGATTGATTAGTTTAAAGG RP: Biotin-AAATTTCCAACCTCAACTACCTCC SP: TTTAGTATAGTAGATGGTA	175

The primer sequences are designed to bind complementarily to the bisulfite-converted sequences of the interested regions. FP, forward primer; RP, reverse primer; SP, sequencing primer. *AATF*, apoptosis antagonizing transcription factor; *ABCC9*, ATP-binding cassette subfamily C member 9; *ADAMTS20*, ADAM metalloproteinase with thrombospondin type 1 motif 20; *FOXC2*, forkhead box C2 (mesenchyme forkhead 1); *HELT*, hey-like transcriptional repressor; *NKX2-5*, NK2 transcription factor-related locus 5 (*Drosophila*); *OLIG3*, oligodendrocyte transcription factor 3; *ONECUT1*, one cut domain family member 1; *PCDHGA12*, protocadherin γ subfamily A 12; *PRRX1*, paired-related homeobox 1.

Table SII. Primer and probe sequences for linear target enrichment-quantitative methylation-specific PCR.

Gene (RefSeq accession number)	Sequence (5'-3')	Concentration, μ M
<i>ADAMTS20</i> (NC_000012)	RP: <u>AAAGATTCGGCGACCACCGA</u> CTACCCCTTACGCCCCGCG FP: TTAGTCGGAGAGGTCGC P: FAM-GCGTCGGGTTTCGAGGAATCGCG	RP: 0.04 FP: 0.2 P: 0.2
<i>FOX2</i> (NC_000016)	RP: <u>AAAGATTCGGCGACCACCGA</u> TCGAAAACGCGCGCAATAAACGCG FP: TTAGGGTTCGGTGTAG P: HEX-TTGGGTTACGGGAAACGTTTACGTT	RP: 0.04 FP: 0.2 P: 0.2
<i>NKX2-5</i> (NC_000005)	RP: <u>AAAGATTCGGCGACCACCGA</u> CCCGCAAAACAACCCAAAAAACGCG FP: TCGAGGCGTGATTCGGC P: FAM-ACGGTTGGCGTTTAGAAGTCGGGGT	RP: 0.04 FP: 0.2 P: 0.2
<i>OLIG3</i> (NC_000006)	RP: <u>AAAGATTCGGCGACCACCGA</u> ACGTACCCCGAACTCCGAAAACCTCAACG FP: GTTCGACGTCGTACGGC P: Texas Red-GTGGATTTATTTCGCGTAGGTCGGGGT	RP: 0.04 FP: 0.2 P: 0.2
<i>PCDHGA12</i> (NC_000005)	RP: <u>AAAGATTCGGCGACCACCGA</u> CTCGCGGATACTCGAACTAAACAAACG FP: CGGTGCGTATAGGTATCGC P: FAM-CGCGTGATGGTTTTGGATGCGAACGAT	RP: 0.04 FP: 0.2 P: 0.2
<i>PRRX1</i> (NC_000001)	RP: <u>AAAGATTCGGCGACCACCGA</u> CCTAAACTAAACGCCGCACGTACTCG FP: CGGTTTATATCGAATTGGATGTC P: HEX-CGTTATTAGATTGCGTTTAGTATAGTAGATG	RP: 0.04 FP: 0.2 P: 0.2
<i>COL2A1</i> (NC_000012)	RP: CTACCCCAAAAAACCCAATCC FP: TAGGAGTATTAGTAATGTTAGGAGTA P: Cy5-AGAAGAAGGGAGGGGTGTTAGGAGAGG	RP: 0.1 FP: 0.1 P: 0.1

UTS	AAAGATTCGGCGACCACCGA	0.4
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The primer and probe sequences are designed to bind complementarily to the bisulfite-converted sequences of the target regions. Underlines indicate the UTS. RP, reverse primer; FP, forward primer; P, probe; UTS, universal tag sequence. ***ADAMTS20***, ADAM metalloproteinase with thrombospondin type 1 motif 20; ***FOXC2***, forkhead box C2 (mesenchyme forkhead 1); ***NKX2-5***, NK2 transcription factor-related locus 5 (*Drosophila*); ***OLIG3***, oligodendrocyte transcription factor 3; ***PCDHGA12***, protocadherin γ subfamily A 12; ***PRRX1***, paired-related homeobox 1; ***COL2A1***, collagen type II $\alpha 1$.

Table III. Selected methylation candidate genes by CpG methylation microarray analysis.

Gene symbol	GenBank accession no.	Description	Chromosome
<i>AATF</i> ^a	NM_012138	Apoptosis antagonizing transcription factor	17
<i>ABCC9</i> ^a	NM_020297	ATP-binding cassette subfamily C member 9	12
<i>ADAMTS20</i> ^a	NM_025003	ADAM metallopeptidase with thrombospondin type 1 motif 20	12
<i>AVPR1A</i>	NM_000706	Arginine vasopressin receptor 1A	12
<i>BARHL2</i>	NM_020063	BarH-like homeobox 2	1
<i>C20orf39</i>	NM_024893	Chromosome 20 open reading frame 39	20
<i>CBLN1</i>	NM_004352	Cerebellin 1 precursor	16
<i>CDX2</i>	NM_001265	Caudal type homeobox transcription factor 2	13
<i>DLX1</i>	NM_001038493	Distal-less homeobox 1	2
<i>DLX2</i>	NM_004405	Distal-less homeobox 2	2
<i>DLX4</i>	NM_138281	Distal-less homeobox 4	17
<i>EVX1</i>	NM_001989	Even-skipped homeobox 1	7
<i>FLJ32447</i>	NM_153038	Hypothetical protein LOC151278	2
<i>FOXC2</i> ^a	NM_005251	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)	16
<i>FOXD3</i>	NM_012183	Forkhead box D3	1

<i>FOXE1</i>	NM_004473	Forkhead box E1 (thyroid transcription factor 2)	9
<i>FOXL2</i>	NM_023067	Forkhead box L2	3
<i>GAD1</i>	NM_013445	Glutamate decarboxylase 1 (brain, 67 kDa)	2
<i>GHSR</i>	NM_198407	Growth hormone secretagogue receptor	3
<i>GRIK2</i>	NM_001166247	Glutamate receptor, ionotropic, kainate 2	6
<i>GSH2</i>	NM_133267	GS homeobox 2	4
<i>HAND1</i>	NM_004821	Heart and neural crest derivatives expressed 1	5
<i>HAND2</i>	NM_021973	Heart and neural crest derivatives expressed 2	4
<i>HELT^a</i>	NM_001029887	Hey-like transcriptional repressor	4
<i>HOXA11</i>	NR_002795	Homeobox A11	7
<i>HOXA7</i>	NM_006896	Homeobox A7	7
<i>HOXB13</i>	NM_006361	Homeobox B13	17
<i>HOXC12</i>	NR_003716	Homeobox C12	12
<i>HOXC13</i>	NM_017410	Homeobox C13	12
<i>HOXD12</i>	NM_021193	Homeobox D12	2
<i>HOXD3</i>	NM_006898	Homeobox D3	2
<i>HOXD8</i>	NM_001199747	Homeobox D8	2

<i>HOXD9</i>	NM_014213	Homeobox D9	2
<i>IPF1</i>	NM_000209	Pancreatic and duodenal homeobox 1	13
<i>IRX1</i>	NM_024337	Iroquois homeobox protein 1	5
<i>LBX1</i>	NM_006562	Ladybird homeobox 1	10
<i>LHX2</i>	NM_004789	LIM homeobox 2	9
<i>LHX8</i>	NM_001001933	LIM homeobox 8	1
<i>LMX1A</i>	NM_001174069	LIM homeobox transcription factor 1 α	1
<i>MOS</i>	NM_005372	V-mos Moloney murine sarcoma viral oncogene homolog	8
<i>MSX2</i>	NM_002449	Msh homeobox 2	5
<i>NKX2-2</i>	NM_002509	NK2 transcription factor-related, locus 2 (<i>Drosophila</i>)	20
<i>NKX2-5^a</i>	NM_001166175	NK2 transcription factor-related, locus 5 (<i>Drosophila</i>)	5
<i>NR2E1</i>	NM_003269	Nuclear receptor subfamily 2, group E, member 1	6
<i>OLIG3^a</i>	NM_175747	Oligodendrocyte transcription factor 3	6
<i>ONECUT1^a</i>	NM_004498	One cut domain, family member 1	15
<i>OTX2</i>	NM_021728	Orthodenticle homeobox 2	14
<i>PAX6</i>	NM_000280	Paired box gene 6 (aniridia, keratitis)	11
<i>PAX7</i>	NM_002584	Paired box gene 7	1

<i>PCDHGA12</i> ^a	NM_032094	Protocadherin gamma subfamily A, 12	5
<i>PRRX1</i> ^a	NM_006902	Paired-related homeobox 1	1
<i>PTGER4</i>	NM_000958	Prostaglandin E receptor 4 (subtype EP4)	5
<i>RAX</i>	NM_013435	Retina and anterior neural fold homeobox	18
<i>SALL3</i>	NM_171999	Sal-like 3 (<i>Drosophila</i>)	18
<i>SIMI</i>	NM_005068	Single-minded homolog 1 (<i>Drosophila</i>)	6
<i>SIX6</i>	NM_007374	Sine oculis homeobox homolog 6 (<i>Drosophila</i>)	14
<i>TAL1</i>	NM_003189	T cell acute lymphocytic leukemia 1	1
<i>TBX20</i>	NM_001077653	T-box 20	7
<i>TFAP2D</i>	NM_172238	Transcription factor AP-2Δ(activating enhancer binding protein 2Δ)	6
<i>TMEFF2</i>	NM_016192	Transmembrane protein with EGF-like and two follistatin-like domains 2	2
<i>TWIST1</i>	NM_000474	Twist homolog 1 (acrocephalosyndactyly 3; Saethre-Chotzen syndrome; <i>Drosophila</i>)	7
<i>VAX1</i>	NM_199131	Ventral anterior homeobox 1	10
<i>WNT2</i>	NM_003391	Wingless-type MMTV integration site family member 2	7
<i>WT1</i>	NM_001198551	Wilms tumor 1	11
<i>ZIC1</i>	NM_003412	Zic family member 1 (odd-paired homolog, <i>Drosophila</i>)	3

^aMethylation candidate genes selected for lung cancer diagnosis in this study.