

Supplemental material to:

Christopher J. Ricketts, Mark R. Morris, Dean Gentle, Michael Brown, Naomi Wake,
Emma R. Woodward, Noel Clarke, Farida Latif and Eamonn R. Maher.

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<http://www.landesbioscience.com/journals/epigenetics/article/19103>

Supplementary Material

Supplementary Figure 1: Comparison Graphs of Infinium HumanMethylation27 array total probe β -values.

A - An experimental replicate demonstrating high repeatability between HumanMethylation27 arrays with a correlation co-efficient of $R^2 = 0.9955$; B and C – Comparison of methylation scores from normal kidney samples; D, E and F – Comparison of methylation scores in paired normal and renal cancer samples demonstrating large amounts of both aberrant hypermethylation and hypomethylation in the cancer samples resulting in low levels of correlation with the normal kidney samples.

Supplementary Figure 2: Methylation levels within Normal Kidney Controls related to Age

These lists demonstrate the number of Infinium Probes/Genes either methylated in normal kidney samples aged >60 years but unmethylated in normal kidney samples aged <45 years or methylated in normal kidney samples aged <45 years but unmethylated in normal kidney samples aged >60.

Supplementary Figure 3: Schematic Diagram of the Gene Selection Process and the Selected Genes

The flow diagram demonstrates the selection criteria and the number of selected probes/genes. The X chromosome genes and imprinted genes were removed along with the probes for which one or more normal kidney samples were methylated. The functional analysis selection of the *combined analysis* list was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) software (<http://david.abcc.ncifcrf.gov/>) and the Ingenuity® Systems Pathway Analysis Software (<http://www.ingenuity.com/>).

Supplementary Figure 4: *Methylation only* Methylated Probes

This demonstrates which tumours were methylated (red dots represent methylation) for which of the 60 probes selected by the *methylation only* criteria. The distance to transcription start site was provided by Illumina annotation and does not differentiate between upstream or downstream distance. All probes were deemed to be within “true” CpG islands in accordance with an Illumina algorithm.

Supplementary Figure 5: Gene Selection Breakdown

The Venn diagrams show the number of genes selected by either criteria for the *methylation only* selection (A) and the *combined analysis* selection (B) and the number of genes that satisfied both criteria.

Supplementary Figure 6: *Methylation only* Genes with Multiple Methylated Probes

This demonstrates which tumours were methylated (red dots represent methylation) for each of the genes with multiple probes selected by the *methylation only* criteria and level of concordance between the methylation seen between two probes for the same genes CpG island. The multiple probes were mapped to the CpG islands and the first exon of the gene to demonstrate their distance apart.

Supplementary Figure 7: CoBRA Confirmation of β -values

A – Examples of CoBRA showing methylation in the assessed tumours with high β -values for *OVOL1* and *DLEC1* (U = undigested, D = digested) with no methylation in normal kidney samples.

B – *FBN2*, a recently identified gene methylated in kidney cancer and present within the high stringency list demonstrated methylation in the assessed tumours with high β -values (U = undigested, D = digested). The Infinium primer set (surrounding the Infinium probe) and the MeDIP primer set

(from the previous study) demonstrated strong, but not perfect, correlation and their positions were mapped against the CpG island. Neither primer sets showed methylation in normal kidney samples.

Supplementary Figure 8: HumanMethylation27 array β -values for genes identified by previous MeDIP study

This figure shows the HumanMethylation27 array β -values and Difference values (tumour probe β -value – average normal kidney β -value) for genes identified by a previous MeDIP study²³. Probes shaded dark grey demonstrate high levels of normal methylation and light grey demonstrate levels of methylation in the normals only slightly above accepted levels. The percentage of methylation is shown for each level of selection criteria.

Supplementary Figure 9: Multiple comparison analysis for clustered probe groups

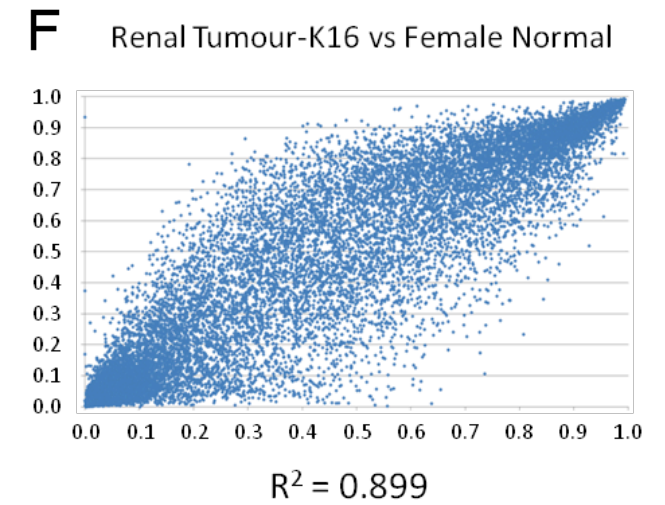
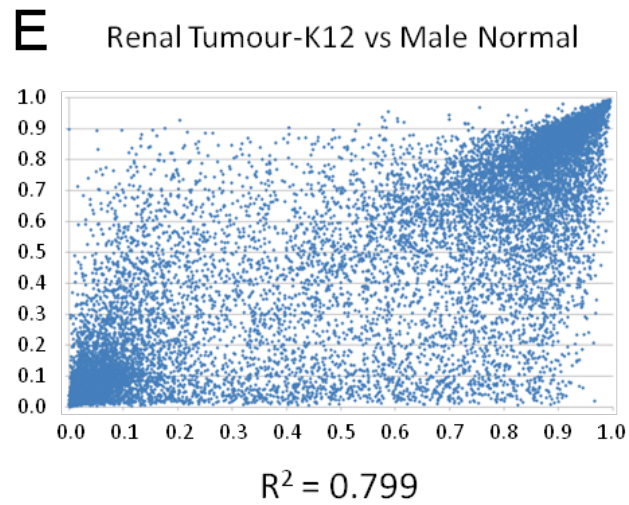
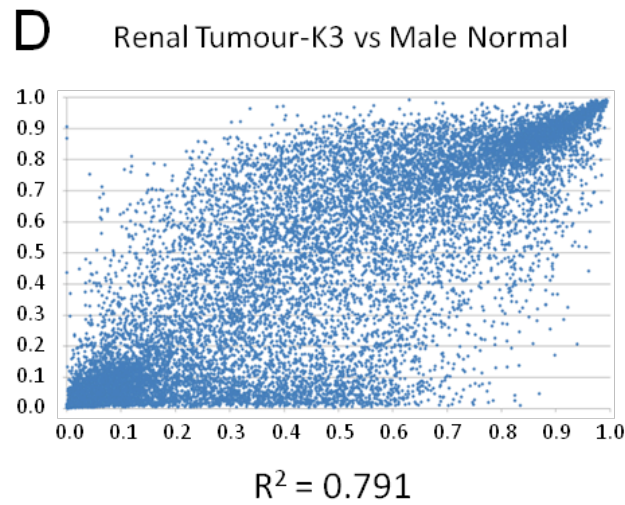
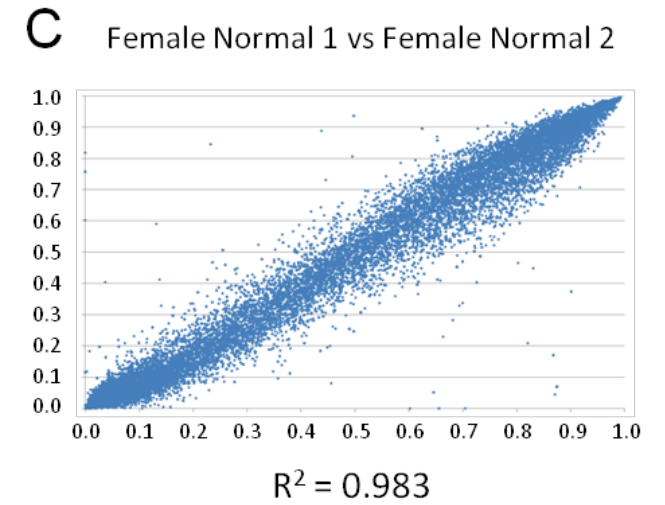
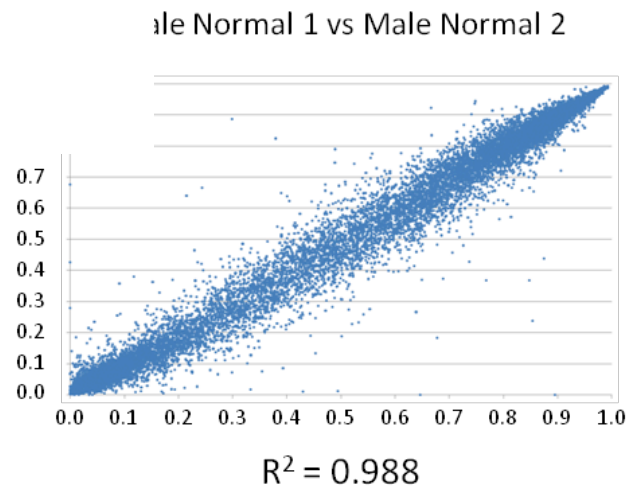
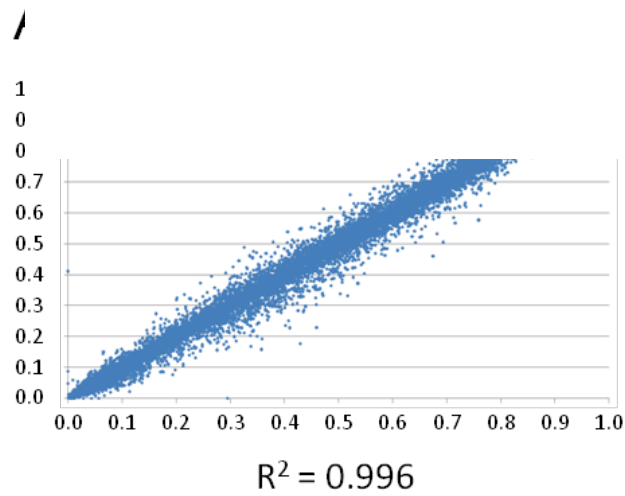
A - Multiple comparison graph of the average β -value for selected genes for clustered groups using the average *methylation only* probe β -values.

B - Student-Newman-Keuls test for all pairwise comparisons for the clustered groups using the average *methylation only* probe β -values.

Supplementary Figure 10: mRNA Analysis of Methylation of *DLEC1*, *TMPRSS2* and *BMP4*

Demonstrates the loss or depletion of each methylated genes mRNA in tumour/associated normal (T/N) mRNA pairs (numbered 1-15).

Supplementary Figure 1



Supplementary Figure 2

Infinium Probes/Genes
methylated in normal kidney
samples aged <45 years but
unmethylated in normal kidney
samples aged <60 years

Gene	Infinium Probe	Gene
	cg14982472	ABCG1
	cg18353563	ABTB1
	cg25072179	AGTRL1
	cg25375764	ALDH5A1
	cg02002676	APOBEC3B
	cg26022401	APOBEC3G
	cg19220825	AQP5
	cg09527362	C6orf150
	cg11207564	CCL3L3
	cg14754581	CCRL2
	cg23654549	CD24
	cg26239233	CITA
	cg24910675	ENG
	cg27105123	EPS8L1
	cg01600189	FUJ20444
	cg17518962	GAL3ST4
	cg03852144	GLRX
	cg23244913	HCG9
	cg16983211	HOXC5
	cg00187380	HOXC5
	cg24125648	HYPK
	cg07463059	IFI16
	cg18302652	IL8
	cg16468729	IL8
	cg07638935	KAZALD1
	cg00630164	KCNQ4
	cg10737521	KIAA0676
	cg13697387	LCE2A
	cg13079059	LENG9
	cg10261589	LOC133308
	cg24046474	LRSAM1
	cg17169998	MLC1
	cg08818984	NR3C1
	cg08750326	ODZ1
	cg07211259	PDCC1LG2
	cg27208307	PRG1
	cg05628549	PRKCDBP
	cg16245261	PRKCDBP
	cg13035743	PRRT1
	cg02397720	RAB17
	cg08822227	SH3BP2
	cg06836849	SLC17A8
	cg17339202	SYNC1
	cg20955688	TMEM71
	cg08341874	VMO1
	cg17836145	VNN2
	46 Probes	43 Genes
cg21995126	BCH2	
cg03400060	BHMT2	
cg16779976	BLNK	
cg21043558	BRAF	
cg08775230	C11orf52	
cg16512727	C12orf4	
cg20022541	C14orf152	
cg18490846	C17orf73	
cg04245402	C19orf21	
cg18919097	C3orf57	
cg01126560	C9orf142	
cg24505341	CADPS	
cg24202119	CAPSL	
cg20099806	CCDC47	
cg20537992	CDC45	
cg24765079	CDH1	
cg06268694	CELSR1	
cg17387870	CHFR	
cg07066326	CHID1	
cg27190239	CLEC2A	
cg08015496	COPA	
cg20938689	COX7C	
cg03032025	CPEB4	
cg14404298	CYP8B1	
cg24754277	DAPK1	
cg24292612	DEFB1	
cg22772878	DIRAS1	
cg10942056	DISP1	
cg09971646	DLK1	
cg21836062	DMD	
cg10674793	DVL3	
cg17703212	DYDC1	
cg16858125	ELOVL1	
cg18997129	EPHA1	
cg08575537	EPO	
cg25256723	F5	
cg21075829	FAM62C	
cg02729303	FUJ20152	
cg25370441	FUJ20184	
cg15967525	FUJ20273	
cg14851685	FUJ39501	
cg03782453	FUJ90575	
cg07147350	FMR1	
cg24058132	GALC	
cg13434842	GATA4	
cg20125091	GF11	
cg22459146	GHSR	
cg11054936	GJB2	
cg18344745	GLRB	
cg24747122	GNA13	
cg10862848	GNMT	
cg04700814	HEXIM1	
cg12866859	HEXIM1	
cg10212621	HMGCS2	
cg13176979	HMGN3	
cg18236734	HTR3E	
cg20977864	HYDIN	
cg09152089	IL22RA1	
cg00995520	KCNA3	
cg27409364	KCN C1	
cg09835085	KCNE4	
cg04439215	KCTD4	
cg10150813	KIAA0746	
cg16034652	KIAA1409	
cg02838492	KIF12	
cg18087514	KMO	
cg00616135	LACTB	
cg25947945	LAD1	
cg01919208	LAMB2	
cg13689073	LARP1	
cg16944093	LIMS2	
cg15703357	LINCR	
cg15660498	LOC148137	
cg17906786	LOC400120	
cg02838877	MBNL2	
cg02037013	MC4R	
cg06959635	MCF2	
cg02994956	NEFH	
cg12978308	NEIL1	
cg02196655	NOL10	
cg23896545	NOMO1	
cg22134325	NPAS4	
cg24194775	NPR2	
cg03958979	NR2E1	
cg15572745	NRXN3	
cg26145228	NTNG1	
cg08409225	OBFC2B	
cg24812103	PABPC1	
cg15662251	PAQR7	
cg22468055	PARP15	
cg24401441	PAX1	
cg18096388	PDCD1	
cg12435792	PDE6B	
cg10098541	PDI A5	
cg27182761	PHLDA1	
cg05947740	PHYHIP	
cg27324619	PIB5PA	
cg02105856	PIGR	
cg22968401	PIPOX	
cg04818845	PLEKHG5	
cg07059360	POLR3D	
cg17791651	POU3F1	
cg11164400	PPP1R9A	
cg01112778	PPP2R2B	
cg16196812	PRLHR	
cg12555334	PSPH	
cg16177163	PTDSS1	
cg15379633	RAB36	
cg02525756	RAB42	
cg02417264	RAMP3	
cg03996822	RASSF6	
cg11719297	RIPK4	
cg24835159	RNF43	
cg02671171	RP33AL	
cg00689340	RTKN	
cg26614073	SCAP	
cg16986846	SCGB2A1	
cg11928198	SCNM1	
cg13302823	SCRT1	
cg22752533	SLC12A5	
cg09494546	SLC16A4	
cg02641676	SLC20A2	
cg08999895	SLC22A18A5	
cg07845392	SLC25A10	
cg09548084	SLC35B3	
cg20804101	SLC6A13	
cg05467458	SLC7A9	
cg12842316	SLITRK4	
cg00002426	SLMAP	
cg24132694	SNAPC2	
cg06675478	SOX1	
cg26531804	SPINT1	
cg05517572	STAP2	
cg11822772	SUHW3	
cg23950724	SYT10	
cg12958778	TBC1D14	
cg16175725	TCF1	
cg09558850	TFPI2	
cg27090087	TINAG	
cg03109701	TMEM118	
cg00687674	TMEM84	
cg13486556	TMOD2	
cg12603560	TNKS1BP1	
cg27210390	TOM1L1	
cg23322523	TRIM55	
cg27032184	TRMU	
cg04975920	TSPAN1	
cg18735146	TTC10	
cg02905245	URB	
cg01211097	USP10	
cg22980351	WDR40B	
cg13674559	WDR44	
cg06330621	ZCCHC14	
cg25766774	ZDHHC3	
cg18793806	ZNF514	
171 Probes	168 Genes	

Supplementary Figure 3

Methylation27 BeadChip

Probes for 14,475 genes

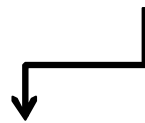
in renal cell carcinoma (RCC) and 9 normal kidney samples



Probes with 1 or more normals with β -values = 0.25 were removed

Average β -values were calculated for the normal kidney samples

Difference values for each tumour were calculated as the β -value minus the average β -value



Methylation only Selection

Selected all probes for which 7 or more RCC samples meet either of the following criteria:

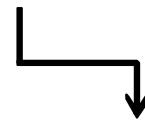
- β -values = 0.5
(43 Probes – 42 Genes)
- Difference values = 0.4
(48 Probes – 44 Genes)

Combined Selection =
60 Probes for 55 Genes



Genes selected for Analysis

- Top three methylated genes:
FOXL1 SLC34A2
TM6SF1
- Genes with 2 selected probes:
COL1A2 OVOL1
SOCS2 TNFRSF10C
ZNF154
- Genes selected by functional candidacy:
TMPRSS2
DLEC1



Combined analysis Selection

Selected all probes for which 7 or more RCC samples meet either of the following criteria:

- β -values = 0.4
(Probes 179 – Genes 166)
- Difference values = 0.3
(Probes 178 – Genes 164)

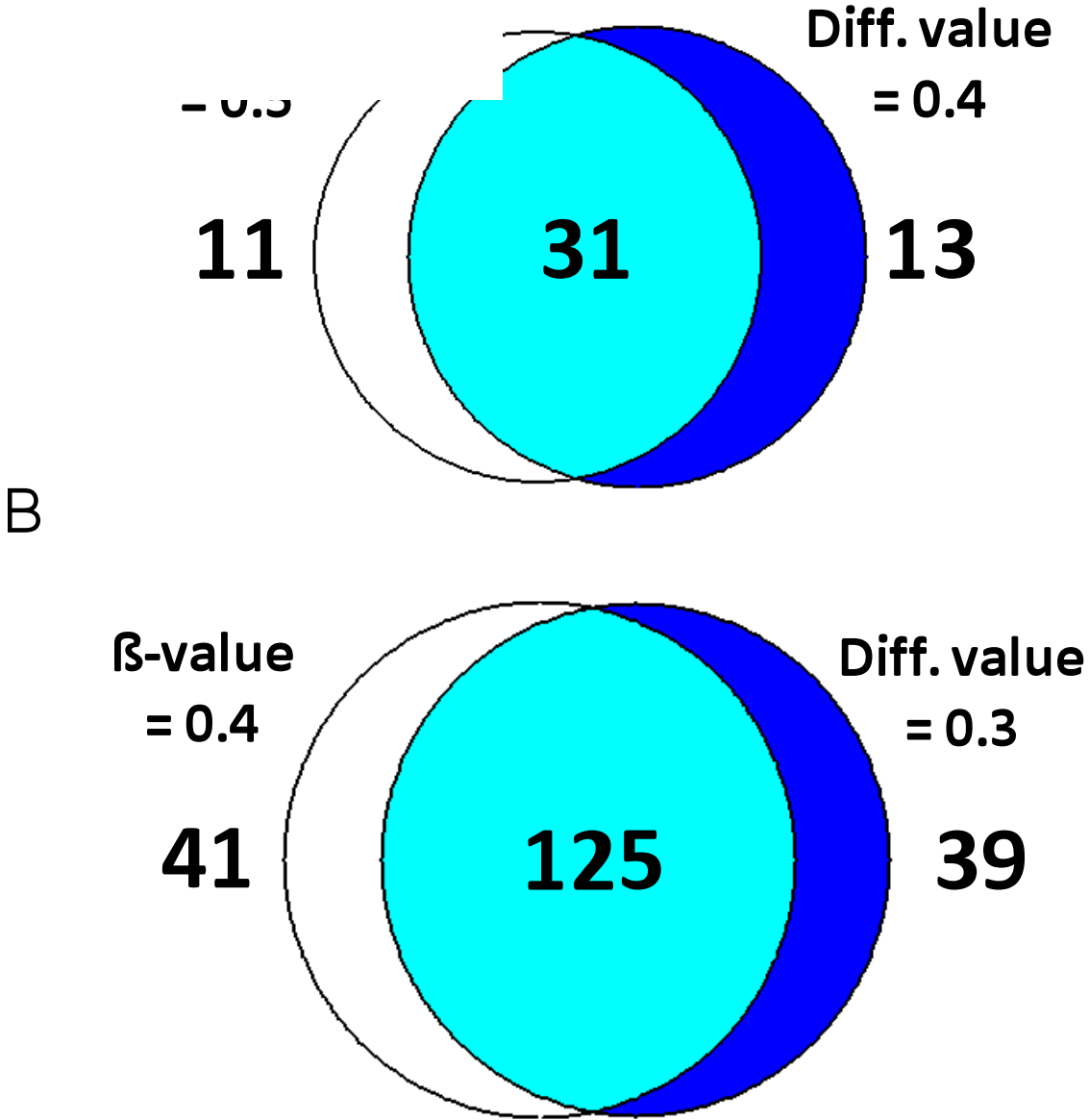
Combined Selection =
220 Probes for 205 Genes



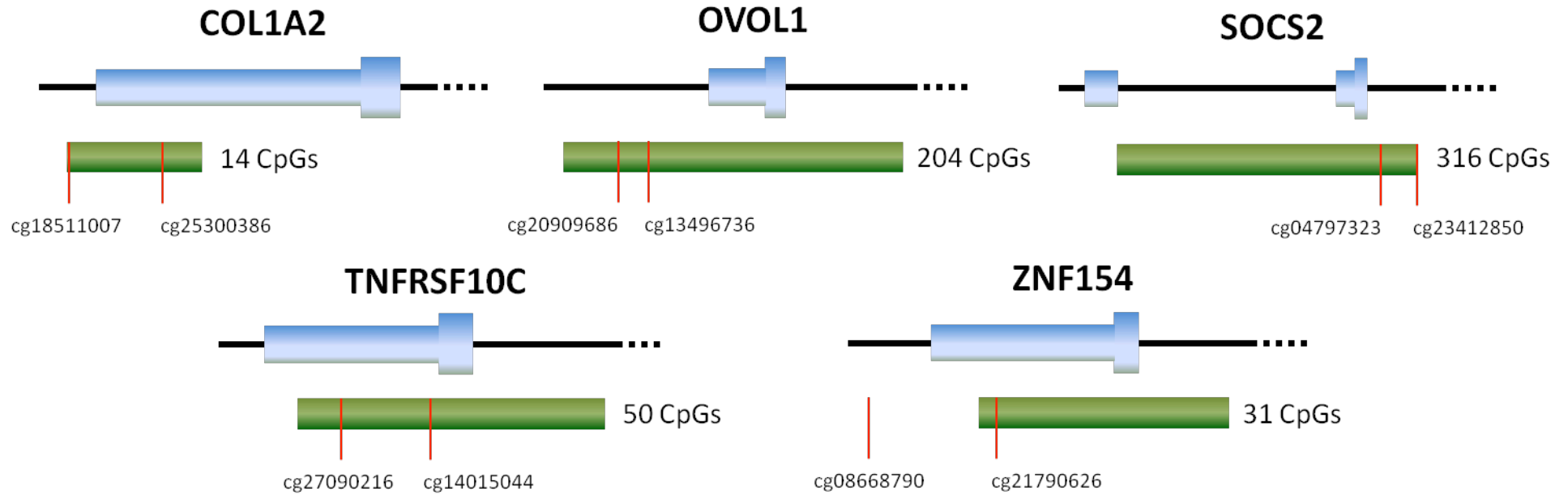
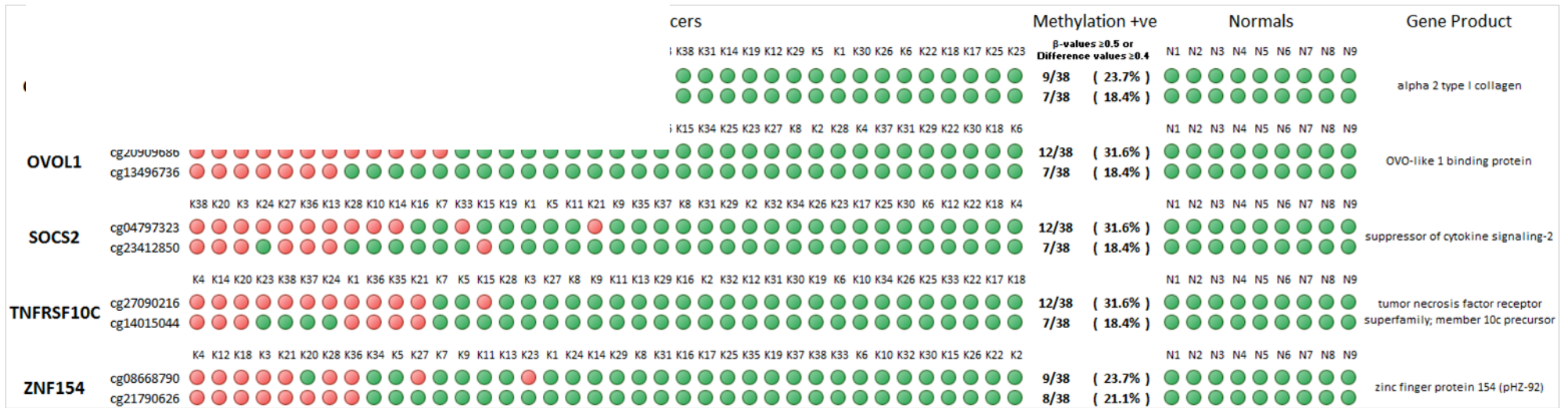
Genes selected for Analysis

- Genes selected from DAVID Functional Analysis:
BMP4
SST
CDKN2B
- Genes selected from Ingenuity[®] Systems Pathway Analysis:
BMP4
SST
CDKN2B

Supplementary Figure 5



Supplementary Figure 6



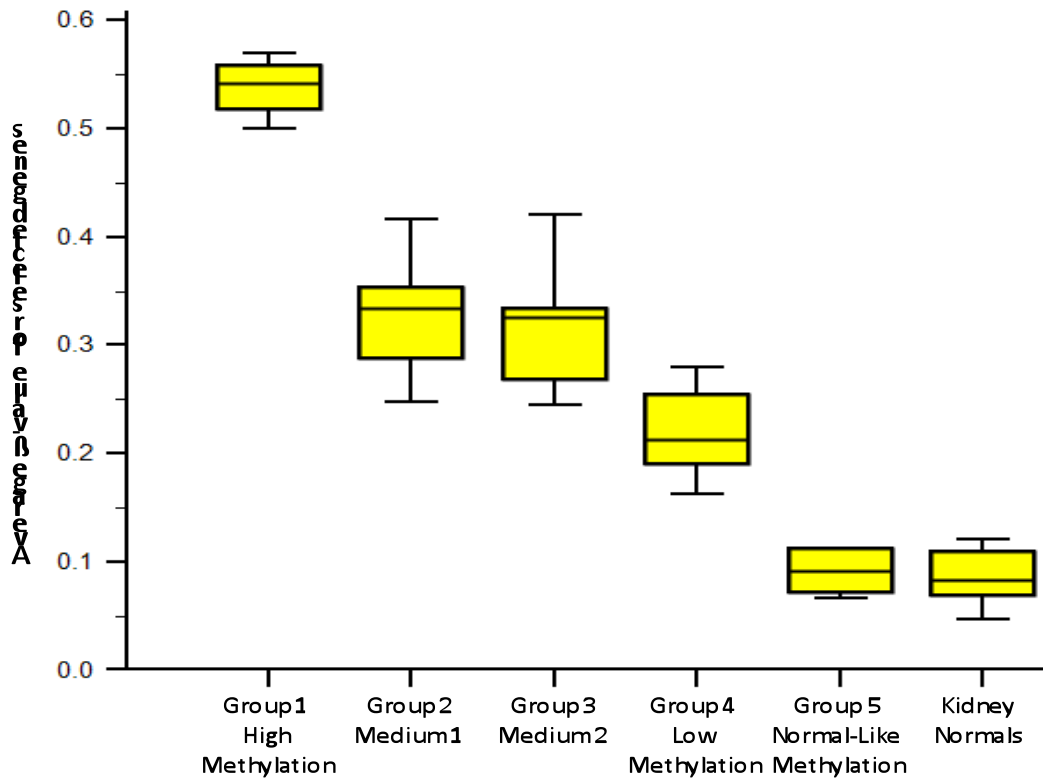
Supplementary Figure 8

SYMBOL	y Samples										PRODUCT	DISTANCE TO TSS	CPG ISLAND	
	an		Mean											
ATP5G2											0.02	ATP synthase; H+ transporting; mitochondrial F0 complex; subunit c isoform 2b precursor	462	TRUE
ATP5G2											0.05	ATP synthase; H+ transporting; mitochondrial F0 complex; subunit c isoform 2b precursor	51	TRUE
CCDC8											0.73	coiled-coil domain containing 8	414	TRUE
CCDC8	1	2.6%	6	15.8%	3	7.9%	6	15.8%	0	1	0.09	coiled-coil domain containing 8	321	TRUE
CORO6	6	15.8%	11	28.9%	1	2.6%	4	10.5%	3	9	0.24	coronin 6	98	TRUE
CORO6	1	2.6%	1	2.6%	1	2.6%	1	2.6%	0	1	0.07	coronin 6	34	TRUE
FBN2	8	21.1%	10	26.3%	5	13.2%	9	23.7%	0	9	0.17	fibrillin 2 precursor	24	TRUE
FBN2	4	10.5%	13	34.2%	3	7.9%	10	26.3%	0	6	0.13	fibrillin 2 precursor	1090	TRUE
FLJ33790	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0	0.02	hypothetical protein LOC283212	708	TRUE
FLJ33790	34	89.5%	34	89.5%	1	2.6%	5	13.2%	9	9	0.55	hypothetical protein LOC283212	426	TRUE
PCDH8	0	0.0%	0	0.0%	0	0.0%	1	2.6%	0	1	0.06	protocadherin 8 isoform 2 precursor	1353	TRUE
PCDH8	1	2.6%	7	18.4%	1	2.6%	5	13.2%	0	6	0.14	protocadherin 8 isoform 2 precursor	393	TRUE
QPCT	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0	0.06	glutaminyl-peptide cyclotransferase precursor	699	TRUE
SCUBE3	1	2.6%	3	7.9%	2	5.3%	4	10.5%	0	0	0.05	signal peptide; CUB domain; EGF-like 3	318	TRUE
SCUBE3	2	5.3%	3	7.9%	2	5.3%	4	10.5%	0	1	0.05	signal peptide; CUB domain; EGF-like 3	789	TRUE
SOX14	1	2.6%	3	7.9%	1	2.6%	2	5.3%	0	9	0.16	SRY-box 14	117	TRUE
SOX14	2	5.3%	4	10.5%	2	5.3%	5	13.2%	0	3	0.09	SRY-box 14	100	TRUE
SPINT2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	1	0.09	serine protease inhibitor; Kunitz type; 2	434	TRUE
SPINT2	0	0.0%	0	0.0%	0	0.0%	1	2.6%	0	0	0.00	serine protease inhibitor; Kunitz type; 2	85	TRUE
ZNF447	0	0.0%	0	0.0%	0	0.0%	1	2.6%	0	0	0.01	zinc finger protein 447	104	TRUE
ZNF447	0	0.0%	0	0.0%	0	0.0%	2	5.3%	0	1	0.04	zinc finger protein 447	229	TRUE

Supplementary Figure 9

A

graph of the average β -value for es for clustered groups

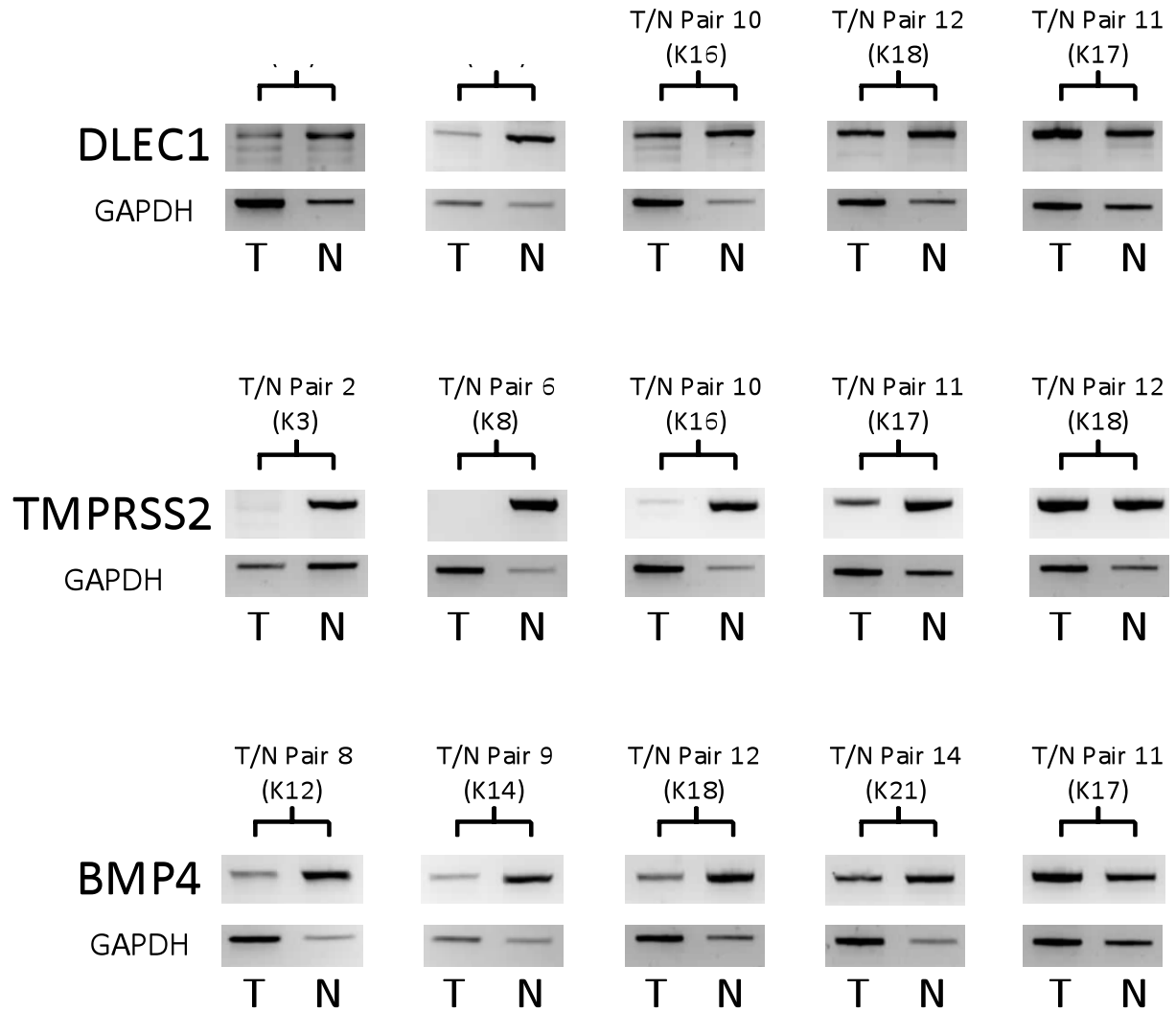


B

	Group 1 (High Methylation)	Group 2 (Medium 1)	Group 3 (Medium 2)	Group 4 (Low Methylation)	Group 5 (Normal-Like Methylation)	Normal Kidney
Normal Kidney	>0.05	>0.05	>0.05	>0.05	n.s.	
Group 5 (Normal-Like Methylation)	>0.05	>0.05	>0.05	>0.05		
Group 4 (Low Methylation)	>0.05	>0.05	>0.05			
Group 3 (Medium 2)	>0.05	n.s.				
Group 2 (Medium 1)	>0.05					
Group 1 (High Methylation)						

Student-Newman-Keuls test for all pairwise comparisons

Supplementary Figure 10



Supplementary Table 2: Combined analysis Hypermethylated Gene Probes

Selected Probes	SYMBOL	Target ID	METH+ ($\beta \geq 0.4$ or Diff ≥ 0.3)	CHR	PRODUCT	DISTANCE TO TSS (bp)	CPG ISLAND
1	FOXL1	cg06995715	21/38 (55.2%)	16	forkhead box L1	260	TRUE
2	SLC34A2	cg19616230	19/38 (50.0%)	4	solute carrier family 34 (sodium phosphate); member 2	44	TRUE
3	PENK	cg04598121	19/38 (50.0%)	8	proenkephalin	84	TRUE
4	TRIM58	cg07533148	19/38 (50.0%)	1	tripartite motif-containing 58	311	TRUE
5	MFSO7	cg24693053	17/38 (44.7%)	4	hypothetical protein LOC84179	139	TRUE
6	ZNF177	cg09643544	16/38 (42.1%)	19	zinc finger protein 177	0	TRUE
7	ZNF454	cg03355526	16/38 (42.1%)	5	zinc finger protein 454	191	TRUE
8	ICAM4	cg21494776	16/38 (42.1%)	19	intercellular adhesion molecule 4 isoform 1 precursor	100	TRUE
9	TM6SF1	cg14696396	16/38 (42.1%)	15	transmembrane 6 superfamily member 1	66	TRUE
10	SOCS2	cg04797323	15/38 (39.5%)	12	suppressor of cytokine signaling-2	311	TRUE
11	PCDHB15	cg11368643	14/38 (36.8%)	5	protocadherin beta 15 precursor	88	TRUE
12	C1orf104	cg22234962	14/38 (36.8%)	1	hypothetical protein LOC284618	38	TRUE
13	ZNF540	cg03975694	14/38 (36.8%)	19	zinc finger protein 540	164	TRUE
14	SLC15A3	cg21992250	14/38 (36.8%)	11	solute carrier family 15; member 3	548	TRUE
15	TLX3	cg25720804	14/38 (36.8%)	5	T-cell leukemia; homeobox 3	101	TRUE
16	TNFRSF10C	cg27090216	14/38 (36.8%)	8	tumor necrosis factor receptor superfamily; member 10c precursor	27	TRUE
17	GPC2	cg18691434	14/38 (36.8%)	7	glypican 2	435	TRUE
18	TNFRSF10C	cg14015044	14/38 (36.8%)	8	tumor necrosis factor receptor superfamily; member 10c precursor	185	TRUE
19	HCG9	cg04623837	13/38 (34.2%)	6	hypothetical protein LOC10255	522	TRUE
20	POU4F2	cg24199834	13/38 (34.2%)	4	POU domain; class 4; transcription factor 2	38	TRUE
21	FBN2	cg27223047	13/38 (34.2%)	5	fibillin 2 precursor	1090	TRUE
22	CCDC37	cg00891278	13/38 (34.2%)	3	hypothetical protein LOC348807	2	TRUE
23	SOX17	cg02919422	13/38 (34.2%)	8	SRV-box 17	49	TRUE
24	IRX4	cg03963198	13/38 (34.2%)	5	iroquois homeobox protein 4	9	TRUE
25	NOTCH3	cg06650786	13/38 (34.2%)	19	Notch homolog 3	337	TRUE
26	UTF1	cg09053680	13/38 (34.2%)	10	undifferentiated embryonic cell transcription factor 1	336	TRUE
27	HTR1E	cg11990309	13/38 (34.2%)	6	5-hydroxytryptamine (serotonin) receptor 1E	234	TRUE
28	PRAC	cg12374721	13/38 (34.2%)	17	small nuclear protein PRAC	242	TRUE
29	OXR1	cg17176732	13/38 (34.2%)	8	oxidation resistance 1	238	TRUE
30	FLT4	cg00489401	13/38 (34.2%)	5	fms-related tyrosine kinase 4 isoform 1	691	TRUE
31	RAMP1	cg03270167	12/38 (31.6%)	2	receptor activity-modifying protein 1 precursor	729	TRUE
32	HIST1H1A	cg10146929	12/38 (31.6%)	6	H1 histone family; member 1	77	TRUE
33	PCDHGC4	cg12145907	12/38 (31.6%)	5	protocadherin gamma subfamily C; 4 isoform 1 precursor	93	TRUE
34	CA3	cg18674980	12/38 (31.6%)	8	carbonic anhydrase III	123	TRUE
35	OVOL1	cg20909686	12/38 (31.6%)	11	OVO-like 1 binding protein	487	TRUE
36	CHODL	cg24130010	12/38 (31.6%)	21	chondrolectin precursor	26	TRUE
37	WNT2	cg01830294	12/38 (31.6%)	7	wingless-type MMTV integration site family member 2 precursor	149	TRUE
38	GCM2	cg02844545	12/38 (31.6%)	6	glial cells missing homolog 2	55	TRUE
39	PRPH	cg09595479	12/38 (31.6%)	12	peripherin	303	TRUE
40	DRD5	cg09936561	12/38 (31.6%)	4	dopamine receptor D5	20	TRUE
41	MGC39545	cg10261191	12/38 (31.6%)	11	hypothetical protein LOC403312	366	TRUE
42	TDRD5	cg12277666	12/38 (31.6%)	1	tudor domain containing 5	155	TRUE
43	IRX2	cg15433631	12/38 (31.6%)	5	iroquois homeobox protein 2	228	TRUE
44	ATP8A2	cg18236477	12/38 (31.6%)	13	ATPase; aminophospholipid transporter-like; Class I; type 8A; member 2	49	TRUE
45	COL1A2	cg18511007	12/38 (31.6%)	7	alpha 2 type I collagen	51	TRUE
46	ABCA3	cg00949442	12/38 (31.6%)	16	ATP-binding cassette; sub-family A member 3	1234	TRUE
47	COL1A2	cg25300386	12/38 (31.6%)	7	alpha 2 type I collagen	115	TRUE
48	ZNF154	cg08668790	12/38 (31.6%)	19	zinc finger protein 154 (pHZ-92)	100	TRUE
49	BNC1	cg18952647	12/38 (31.6%)	15	basonuclin 1	276	TRUE
50	ZNF154	cg21790626	12/38 (31.6%)	19	zinc finger protein 154 (pHZ-92)	68	TRUE
51	FANCE	cg03030757	11/38 (28.9%)	6	Fanconi anemia; complementation group E	616	TRUE
52	CBX4	cg04398978	11/38 (28.9%)	17	chromobox homolog 4	851	TRUE
53	SHKBP1	cg09381003	11/38 (28.9%)	19	SH3KBP1 binding protein 1	476	TRUE
54	TMPPRSS2	cg24901042	11/38 (28.9%)	21	transmembrane protease; serine 2	476	TRUE
55	PCDHGB4	cg00911351	11/38 (28.9%)	5	protocadherin gamma subfamily B; 4 isoform 1 precursor	197	TRUE
56	CDKN2B	cg10210238	11/38 (28.9%)	9	cyclin-dependent kinase inhibitor 2B isoform 2		TRUE
57	CHX10	cg11525285	11/38 (28.9%)	14	ceh-10 homeo domain containing homolog	161	TRUE
58	FLJ90650	cg15489294	11/38 (28.9%)	5	laeverin	418	TRUE
59	SPAG6	cg25802093	11/38 (28.9%)	10	sperm associated antigen 6 isoform 1	361	TRUE
60	CD38	cg26043257	11/38 (28.9%)	4	CD38 antigen	307	TRUE
61	SLC27A6	cg07103493	11/38 (28.9%)	5	solute carrier family 27 (fatty acid transporter); member 6	22	TRUE
62	MAP4K1	cg00233307	11/38 (28.9%)	19	mitogen-activated protein kinase kinase kinase kinase 1	520	TRUE
63	AEBP1	cg02126753	11/38 (28.9%)	7	adipocyte enhancer binding protein 1 precursor	38	TRUE
64	GSTP1	cg04920951	11/38 (28.9%)	11	glutathione transferase		TRUE
65	ACTL6B	cg08572611	11/38 (28.9%)	7	actin-like 6B	136	TRUE
66	UNQ9433	cg17162024	11/38 (28.9%)	8	hypothetical protein LOC389658	433	TRUE
67	FBXO39	cg20723355	11/38 (28.9%)	17	F-box protein 39	25	TRUE
68	TLX3	cg25942450	11/38 (28.9%)	5	T-cell leukemia; homeobox 3	37	TRUE
69	ZFP42	cg06274159	11/38 (28.9%)	4	zinc finger protein 42	58	TRUE
70	ZFP41	cg12680609	11/38 (28.9%)	8	zinc finger protein 41 homolog	609	TRUE
71	HLF	cg04219321	10/38 (26.3%)	17	hepatic leukemia factor	712	TRUE
72	CAB39L	cg15207619	10/38 (26.3%)	13	calcium binding protein 39-like isoform 2	144	FALSE
73	HIST1H4H	cg15599064	10/38 (26.3%)	6	H4 histone family; member H	72	TRUE
74	COL5A2	cg22774472	10/38 (26.3%)	2	alpha 2 type V collagen preproprotein	148	FALSE
75	RBP1	cg23363832	10/38 (26.3%)	3	retinol binding protein 1; cellular		TRUE

Selected Probes	SYMBOL	Target ID	METH+ ($\beta \geq 0.4$ or $\text{Diff} \geq 0.3$)	CHR	PRODUCT	DISTANCE TO TSS (bp)	CPG ISLAND
76	DLEC1	cg23881725	10/38 (26.3%)	3	deleted in lung and esophageal cancer 1 isoform DLEC1-L1	54	TRUE
77	GAS6	cg26420196	10/38 (26.3%)	13	growth arrest-specific 6	964	TRUE
78	GATA4	cg09626984	10/38 (26.3%)	8	GATA binding protein 4		TRUE
79	SLC6A3	cg13202751	10/38 (26.3%)	5	solute carrier family 6 (neurotransmitter transporter; dopamine); member 3	898	TRUE
80	PITX2	cg14056644	10/38 (26.3%)	4	paired-like homeodomain transcription factor 2 isoform b	597	TRUE
81	SCARF2	cg14785479	10/38 (26.3%)	22	scavenger receptor class F; member 2 isoform 2	389	TRUE
82	BIK	cg17179881	10/38 (26.3%)	22	BCL2-interacting killer	908	TRUE
83	HIST1H4L	cg17651821	10/38 (26.3%)	6	H4 histone family; member K	394	FALSE
84	CACNA1G	cg18454685	10/38 (26.3%)	17	voltage-dependent calcium channel alpha 1G subunit isoform 1	790	TRUE
85	BCAN	cg21475402	10/38 (26.3%)	1	brevican isoform 2	400	TRUE
86	FLJ90650	cg25044651	10/38 (26.3%)	5	laeverin	449	TRUE
87	FBN2	cg25084878	10/38 (26.3%)	5	fibrillin 2 precursor	24	TRUE
88	SALL3	cg15191648	10/38 (26.3%)	18	sal-like 3	13	TRUE
89	GRM6	cg14859460	10/38 (26.3%)	5	glutamate receptor; metabotropic 6 precursor	120	TRUE
90	SLC5A7	cg16232126	10/38 (26.3%)	2	solute carrier family 5 (choline transporter); member 7	10	TRUE
91	SOCS2	cg23412850	10/38 (26.3%)	12	suppressor of cytokine signaling-2		TRUE
92	GAS7	cg22471346	10/38 (26.3%)	17	growth arrest-specific 7 isoform a	395	TRUE
93	NEF3	cg23290344	10/38 (26.3%)	8	neurofilament 3 (150kDa medium)	159	TRUE
94	LRFN4	cg20358834	10/38 (26.3%)	11	leucine rich repeat and fibronectin type III domain containing 4	717	TRUE
95	RAB37	cg12448933	10/38 (26.3%)	17	RAB37; member RAS oncogene family isoform 1	108	TRUE
96	NEUROG1	cg14958635	10/38 (26.3%)	5	neurogenin 1		TRUE
97	INA	cg25764191	10/38 (26.3%)	10	internexin neuronal intermediate filament protein; alpha	295	TRUE
98	CPD	cg07156669	9/38 (23.7%)	17	carboxypeptidase D precursor	739	FALSE
99	ANGPT1	cg09396217	9/38 (23.7%)	8	angiopoietin 1	32	FALSE
100	ATP1A1	cg09580336	9/38 (23.7%)	1	Na+/K+ -ATPase alpha 1 subunit isoform a proprotein	714	TRUE
101	TAL1	cg19797376	9/38 (23.7%)	1	T-cell acute lymphocytic leukemia 1	32	TRUE
102	FOXJ1	cg24164563	9/38 (23.7%)	17	forkhead box J1	171	TRUE
103	SCUBE2	cg01081263	9/38 (23.7%)	11	CEGP1 protein	684	TRUE
104	TNFRSF10C	cg01407244	9/38 (23.7%)	8	tumor necrosis factor receptor superfamily; member 10c precursor		TRUE
105	UTF1	cg03755123	9/38 (23.7%)	10	undifferentiated embryonic cell transcription factor 1	270	TRUE
106	CYP26B1	cg04884908	9/38 (23.7%)	2	cytochrome P450; family 26; subfamily b; polypeptide 1	123	TRUE
107	KCNS2	cg05373457	9/38 (23.7%)	8	potassium voltage-gated channel; delayed-rectifier; subfamily S; member 2	698	TRUE
108	HTR7	cg06291867	9/38 (23.7%)	10	5-hydroxytryptamine receptor 7 isoform d	509	TRUE
109	MLLT11	cg07139440	9/38 (23.7%)	1	MLLT11 protein	1170	FALSE
110	DSCR6	cg13460409	9/38 (23.7%)	21	Down syndrome critical region protein 6	707	TRUE
111	DES	cg18182399	9/38 (23.7%)	2	desmin	76	TRUE
112	FOXE3	cg18815943	9/38 (23.7%)	1	forkhead box E3	570	TRUE
113	PPYR1	cg20491707	9/38 (23.7%)	10	pancreatic polypeptide receptor 1	251	TRUE
114	HIST1H4K	cg00634577	9/38 (23.7%)	6	H4 histone family; member D	45	TRUE
115	DGKE	cg01344452	9/38 (23.7%)	17	diacylglycerol kinase epsilon	590	TRUE
116	SST	cg02164046	9/38 (23.7%)	3	somatostatin preproprotein	53	TRUE
117	HIST1H4J	cg11037148	9/38 (23.7%)	6	H4 histone family; member E	44	TRUE
118	CNTNAP2	cg16254309	9/38 (23.7%)	7	cell recognition molecule Caspr2 precursor	323	TRUE
119	RIMS4	cg19332710	9/38 (23.7%)	20	regulating synaptic membrane exocytosis 4	47	TRUE
120	FGF4	cg19831575	9/38 (23.7%)	11	fibroblast growth factor 4 precursor	81	TRUE
121	RG57	cg21303386	9/38 (23.7%)	1	regulator of G-protein signalling 7	167	TRUE
122	ZNF454	cg23037403	9/38 (23.7%)	5	zinc finger protein 454	41	TRUE
123	BMP4	cg14310034	9/38 (23.7%)	14	bone morphogenetic protein 4 preproprotein	179	TRUE
124	OSTbeta	cg16029760	9/38 (23.7%)	15	organic solute transporter beta	411	TRUE
125	FAM3B	cg03158400	9/38 (23.7%)	21	family with sequence similarity 3; member B isoform a	225	TRUE
126	ZNF560	cg05221167	9/38 (23.7%)	19	zinc finger protein 560	118	TRUE
127	GRIK1	cg21816539	9/38 (23.7%)	21	glutamate receptor; ionotropic; kainate 1 isoform 1 precursor	46	TRUE
128	SPDY1	cg04786857	9/38 (23.7%)	2	speedy homolog 1 isoform 1	180	TRUE
129	FNDC4	cg17918501	9/38 (23.7%)	2	fibronectin type III domain containing 4	377	TRUE
130	TWIST1	cg20052718	9/38 (23.7%)	7	twist		TRUE
131	GDF7	cg05899618	8/38 (21.1%)	2	growth differentiation factor 7 preproprotein	577	TRUE
132	SCG3	cg26929536	8/38 (21.1%)	15	secretogranin III	90	FALSE
133	LOC389458	cg01870826	8/38 (21.1%)	7	hypothetical protein LOC389458	143	TRUE
134	FBXO39	cg02613386	8/38 (21.1%)	17	F-box protein 39	39	TRUE
135	NTSR1	cg03567830	8/38 (21.1%)	20	neurotensin receptor 1	318	TRUE
136	C20orf100	cg04369341	8/38 (21.1%)	20	chromosome 20 open reading frame 100	215	TRUE
137	GPR150	cg04597449	8/38 (21.1%)	5	G protein-coupled receptor 150	405	TRUE
138	DISP2	cg06595693	8/38 (21.1%)	15	dispatched B	445	TRUE
139	CTNND2	cg10331779	8/38 (21.1%)	5	catenin (cadherin-associated protein); delta 2 (neural plakophilin-related arm-repeat protein)	701	TRUE
140	CLEC11A	cg13152535	8/38 (21.1%)	19	stem cell growth factor precursor	48	TRUE
141	PLXDC1	cg14601284	8/38 (21.1%)	17	plexin domain containing 1 precursor	506	FALSE
142	CPNE7	cg16444968	8/38 (21.1%)	16	copine 7 isoform b	453	TRUE
143	ZNF83	cg17132967	8/38 (21.1%)	19	zinc finger protein 83 (HPF1)	666	TRUE
144	PRR3	cg21264055	8/38 (21.1%)	6	proline-rich protein 3	1421	TRUE
145	SLC8A2	cg22123464	8/38 (21.1%)	19	solute carrier family 8 member 2	221	FALSE
146	TCF15	cg22449114	8/38 (21.1%)	20	basic helix-loop-helix transcription factor 15	667	TRUE
147	SLC9A3	cg23061578	8/38 (21.1%)	5	solute carrier family 9 (sodium/hydrogen exchanger); isoform 3	494	TRUE
148	GBX2	cg23095584	8/38 (21.1%)	2	gastrulation brain homeo box 2	1052	TRUE
149	SLC2A14	cg05521696	8/38 (21.1%)	12	glucose transporter 14	0	TRUE
150	TBX20	cg02008154	8/38 (21.1%)	7	T-box transcription factor TBX20	295	TRUE

Selected Probes	SYMBOL	Target ID	METH+ ($\beta \geq 0.4$ or $\text{Diff} \geq 0.3$)	CHR	PRODUCT	DISTANCE TO TSS (bp)	CPG ISLAND
151	SLC6A2	cg04490714	8/38 (21.1%)	16	solute carrier family 6 member 2	8	TRUE
152	TCFL5	cg10729531	8/38 (21.1%)	20	transcription factor-like 5 protein	446	TRUE
153	GUCY2D	cg25465406	8/38 (21.1%)	17	guanylate cyclase 2D; membrane (retina-specific)	110	TRUE
154	ATP6V0C	cg05483509	8/38 (21.1%)	16	ATPase; H+ transporting; lysosomal; V0 subunit c	518	TRUE
155	GNB4	cg17483510	8/38 (21.1%)	3	guanine nucleotide-binding protein; beta-4 subunit	694	TRUE
156	ACCN4	cg19210770	8/38 (21.1%)	2	amiloride-sensitive cation channel 4 isoform 1	39	TRUE
157	KIAA1822	cg02867079	8/38 (21.1%)	14	KIAA1822 protein	211	TRUE
158	RASGRF2	cg09952204	8/38 (21.1%)	5	Ras protein-specific guanine nucleotide-releasing factor 2	106	TRUE
159	KLK10	cg11846956	8/38 (21.1%)	19	kallikrein 10 precursor		TRUE
160	NTRK3	cg14384532	8/38 (21.1%)	15	neurotrophic tyrosine kinase; receptor; type 3 isoform c precursor	963	TRUE
161	FLRT2	cg17410236	8/38 (21.1%)	14	fibronectin leucine rich transmembrane protein 2	7	TRUE
162	GATA4	cg25216696	8/38 (21.1%)	8	GATA binding protein 4		TRUE
163	OLFM2	cg00208967	7/38 (18.4%)	19	olfactomedin 2	854	TRUE
164	TUBB6	cg07307078	7/38 (18.4%)	18	tubulin; beta 6	627	TRUE
165	CHD5	cg08080029	7/38 (18.4%)	1	chromodomain helicase DNA binding protein 5	213	TRUE
166	TGIF2	cg12556134	7/38 (18.4%)	20	TGFB-induced factor 2	413	TRUE
167	PAX9	cg00509670	7/38 (18.4%)	14	paired box gene 9	592	TRUE
168	GALR1	cg00662556	7/38 (18.4%)	18	galanin receptor 1		TRUE
169	HAND2	cg01580681	7/38 (18.4%)	4	basic helix-loop-helix transcription factor HAND2	578	TRUE
170	MAFB	cg02497758	7/38 (18.4%)	20	transcription factor MAFB	682	TRUE
171	KCNC3	cg06572160	7/38 (18.4%)	19	Shaw-related voltage-gated potassium channel protein 3	733	TRUE
172	IGF2BP1	cg06638433	7/38 (18.4%)	17	insulin-like growth factor 2 mRNA binding protein 1	401	TRUE
173	CDKN2A	cg07752420	7/38 (18.4%)	9	cyclin-dependent kinase inhibitor 2A isoform 3		TRUE
174	ST3GAL4	cg08203715	7/38 (18.4%)	11	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	58	TRUE
175	DSC3	cg11832722	7/38 (18.4%)	18	desmocollin 3 isoform Dsc3a preproprotein	371	TRUE
176	KIAA0676	cg12080675	7/38 (18.4%)	5	hypothetical protein LOC23061 isoform a	365	TRUE
177	TEKT1	cg12685753	7/38 (18.4%)	17	tektin 1	479	TRUE
178	INSRR	cg12995941	7/38 (18.4%)	1	insulin receptor-related receptor precursor	711	TRUE
179	OVOL1	cg13496736	7/38 (18.4%)	11	OVO-like 1 binding protein	326	TRUE
180	PRKAR1B	cg13577076	7/38 (18.4%)	7	protein kinase; cAMP-dependent; regulatory; type I; beta	323	TRUE
181	SLC18A3	cg14008883	7/38 (18.4%)	10	solute carrier family 18 (vesicular acetylcholine); member 3	564	TRUE
182	ZFP42	cg14189571	7/38 (18.4%)	4	zinc finger protein 42	18	TRUE
183	LOC63928	cg15309006	7/38 (18.4%)	16	hepatocellular carcinoma antigen gene 520	168	TRUE
184	DLX1	cg16652259	7/38 (18.4%)	2	distal-less homeo box 1	24	TRUE
185	ITGA8	cg16902509	7/38 (18.4%)	10	integrin; alpha 8	458	TRUE
186	MYOD1	cg18555440	7/38 (18.4%)	11	myogenic differentiation 1	528	TRUE
187	GPR124	cg20011352	7/38 (18.4%)	8	G protein-coupled receptor 124	495	TRUE
188	PCDH8	cg20366906	7/38 (18.4%)	13	protocadherin 8 isoform 2 precursor	393	TRUE
189	UQCRH	cg21576698	7/38 (18.4%)	1	ubiquinol-cytochrome c reductase hinge protein	1437	TRUE
190	FGF23	cg23219570	7/38 (18.4%)	12	fibroblast growth factor 23 precursor	1	FALSE
191	STMN2	cg23326689	7/38 (18.4%)	8	superiorcervical ganglia; neural specific 10	81	TRUE
192	FLJ90166	cg23418591	7/38 (18.4%)	20	hypothetical protein LOC164284	368	TRUE
193	HSPA2	cg24642523	7/38 (18.4%)	14	heat shock 70kDa protein 2		TRUE
194	PTGDR	cg24989962	7/38 (18.4%)	14	prostaglandin D2 receptor	145	TRUE
195	C14orf39	cg27398547	7/38 (18.4%)	14	hypothetical protein LOC317761	11	TRUE
196	BSG	cg01532103	7/38 (18.4%)	19	basigin isoform 1	218	TRUE
197	HMGA1	cg03020951	7/38 (18.4%)	6	high mobility group AT-hook 1 isoform a	763	TRUE
198	GLRA1	cg14319409	7/38 (18.4%)	5	glycine receptor; alpha 1	6	TRUE
199	WBSCR17	cg01366419	7/38 (18.4%)	7	UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase-like 3	698	TRUE
200	HOXA6	cg04265576	7/38 (18.4%)	7	homeobox A6	4	TRUE
201	MDF1	cg05345286	7/38 (18.4%)	6	MyoD family inhibitor	388	TRUE
202	KCNC4	cg05675373	7/38 (18.4%)	1	Shaw-related voltage-gated potassium channel protein 4 isoform a	280	TRUE
203	SLITRK1	cg07104706	7/38 (18.4%)	13	slit and trk like 1 protein	401	TRUE
204	PAX7	cg07536847	7/38 (18.4%)	1	paired box gene 7 isoform 1	268	TRUE
205	HBQ1	cg07703401	7/38 (18.4%)	16	theta 1 globin	6	TRUE
206	POU4F1	cg08097882	7/38 (18.4%)	13	POU domain; class 4; transcription factor 1	179	TRUE
207	SLC26A5	cg09492887	7/38 (18.4%)	7	prestin isoform a	620	TRUE
208	CYYR1	cg10238818	7/38 (18.4%)	21	cysteine and tyrosine-rich 1 protein precursor	27	TRUE
209	FAM78A	cg12998491	7/38 (18.4%)	9	hypothetical protein LOC286336	625	TRUE
210	ACTA1	cg13547644	7/38 (18.4%)	1	alpha 1 actin precursor	235	TRUE
211	PITX3	cg14681055	7/38 (18.4%)	10	paired-like homeodomain transcription factor 3	30	TRUE
212	COMP	cg15784332	7/38 (18.4%)	19	cartilage oligomeric matrix protein precursor	58	TRUE
213	PHOX2A	cg18722841	7/38 (18.4%)	11	paired-like homeobox 2a	222	TRUE
214	SLC30A3	cg19461344	7/38 (18.4%)	2	solute carrier family 30 (zinc transporter); member 3	38	TRUE
215	SFRP2	cg23207990	7/38 (18.4%)	4	secreted frizzled-related protein 2 precursor		TRUE
216	EDNRB	cg23316360	7/38 (18.4%)	13	endothelin receptor type B isoform 2		TRUE
217	ZFP28	cg23850212	7/38 (18.4%)	19	zinc finger protein 28	460	TRUE
218	TWIST1	cg26312150	7/38 (18.4%)	7	twist	745	TRUE
219	GSCL	cg26599006	7/38 (18.4%)	22	gooseoid-like	425	TRUE
220	OTOP3	cg27243140	7/38 (18.4%)	17	otopetrin 3	233	TRUE

Supplementary Table 3: Pathway Analysis of the Selected Hypermethylated Genes using DAVID and Ingenuity® Systems Pathway Analysis

DAVID Functional Analysis

Cell-Cell Signalling

BMP4	DRD5	FAM3B	FGF4	GATA4	GLRA1
GRIK1	<u>GRM6</u>	HTR1E	<u>HTR7</u>	KCNC4	NTSR1
PCDH8	SLC5A7	SLC6A2	SST	WNT2	

Regulation of Cell Proliferation

ATP8A2	BMP4	BNC1	CD38	CDKN2A	<u>CDKN2B</u>
CLEC11A	<u>DLEC1</u>	EDNRB	FGF4	<u>FLT4</u>	FOXE3
FOXJ1	GATA4	SST	<u>TCFL5</u>	WNT2	

Regulation of Cell Death

BIK	BMP4	<u>CBX4</u>	CD38	CDKN2A	COMP
DLX1	EDNRB	FGF4	GCM2	<u>GSTP1</u>	PAX7
POU4F1	RASGRF2	<u>SOCS2</u>	SST		

Cell-Cell Adhesions

<u>AEBP1</u>	<u>BCAN</u>	CNTNAP2	COMP	CTNND2	DSC3
FGF4	FLRT2	<u>ICAM4</u>	ITGA8	PCDH8	PCDHB15
PCDHGB4	PCDHGC4	<u>SCARF2</u>			

Angiogenesis/Blood Vessel Development

ANGPT1	BMP4	<u>COL1A2</u>	GBX2	HAND2	
PLXDC1	SOX17	TBX20			

EGF Genes

<u>BCAN</u>	CNTNAP2	GAS6	<u>NOTCH3</u>	SCUBE2	
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Tumour Suppressor

CDKN2A	<u>CDKN2B</u>	<u>DLEC1</u>	KLK10	MAFB	
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Cadherins

PCDH8	PCDHB15	PCDHGB4	PCDHGC4		
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Ingenuity® Systems Pathway Analysis

TGF-β Signalling Pathway

BMP4	<u>CDKN2B</u>	COMP	GDF7	PITX2	
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Somatostatin Pathway

GNB4	GUCY2D	SST			
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Supplementary Table 4: Methylation Analysis Selected Genes in COSMIC (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>)

Gene	COSMIC Mutation Positive Samples		COSMIC Mutation Data		
	Kidney	Total	Nucleotide Change	Amino Acid Change	Cancer Type (frequency)
FOXL1	0/101	0/180	-	-	-
SLC34A2	1/1	2/46	c.1665 G>C c.770 G>C	p.L555L p.G257A	Kidney (1/1) Ovarian (1/1)
TM6SF1	0/0	1/45	c.1049 C>T	p.A350V	Ovarian (1/1)
COL1A2	0/0	5/470	c.2079 G>A c.2527 G>A c.2809 G>A c.2813 G>A c.688 C>T	p.R693R p.A843T p.G937S p.R938H p.P230S	Glioma (1/446) Ovarian (1/2) Ovarian (1/2) Glioma (1/446) Glioma (1/446)
SOCS2	0/101	0/180	-	-	-
OVOL1	0/0	1/44	c.402 C>T	p.N134N	Glioma (1/22)
ZNF154	0/0	0/44	-	-	-
TNFRSF10C	0/101	0/605	-	-	-
TMPRSS2	0/101	1/3700	c.1254 C>T	p.N418N	Glioma (1/23)
DLEC1	0/101	0/180	c.1052 C>G c.2532 G>A c.2595 C>T c.2614 C>T c.2651 G>A c.3296 G>A c.4035 G>A c.643 G>A	p.P351R p.S844S p.A865A p.R872C p.R884Q p.R1099H p.S1345S p.D215N	Breast (1/48) Ovarian (1/2) Glioma (1/22) Glioma (1/22) Pancreas (1/2) Pancreas (1/2) Ovarian (1/2) Malignant Melanoma (1/1)
SST	0/0	1/45	c.73 G>A	p.A25T	Pancreas (1/1)
GNB4	0/101	2/181	c.320 C>T	p.P107L	Malignant Melanoma (2/6)
GUCY2D	0/27	1/273	c.1292 G>A	p.G431D	Malignant Melanoma (1/6)

Supplementary Table 5: Mutation and Cluster Data for 38 Sporadic Renal Cell Carcinoma Patients

Group	Tumour	Sex	VHL Mutation	Methylation only Average β -value
Group 1 High Methylation CIMP	K3	M	No Mutation	0.475
	K12	M	No Mutation	0.489
	K21	F	c.349T>A, p.Trp117Arg, Exon 2	0.484
	K24	M	c.493delG, Exon 3	0.395
Group 2 Medium 1	K1	M	c.278 G>T, p.Gly93Val, Exon 1	0.299
	K5	M	c.464-1G>A, Splice error, Exon 3	0.300
	K7	M	c.233 A>G, p.Asp78Ser, Exon 1	0.354
	K9	M	No Mutation	0.325
	K11	M	c.208 G>T, p.Glu70Stop, Exon 1	0.339
	K13	F	c.464-6del474 (TTCCAGTGTATACTCTG), Exon 3	0.285
	K23	M	No Mutation	0.269
	K33	M	c.444delT, Exon 2	0.256
K34	M	c.523delT, Exon 3	0.259	
Group 3 Medium 2	K14	M	No Mutation	0.268
	K15	M	No Mutation	0.227
	K16	F	c.464-2_464-1insA, Exon 3	0.248
	K20	M	No Mutation	0.334
	K27	M	c.235del249 (CGCAGTCCGCGCGTC), Exon 1	0.291
	K35	M	c.551T>C, p.Leu184Pro, Exon 3	0.252
	K36	M	No Mutation	0.259
	K37	M	c.264G>C, Exon 1	0.220
K38	F	No Mutation	0.191	
Group 4 Low Methylation	K2	M	No Mutation	0.250
	K8	F	No Mutation	0.258
	K10	M	c.474A>T, p.Lys159Stop, Exon 3	0.161
	K19	M	No Mutation	0.170
	K28	M	No Mutation	0.201
	K29	M	c.223insA, Exon 1	0.175
	K31	F	c.406T>G, p.Phe36Val, Exon 2	0.192
K32	F	c.492insA, Exon 3	0.220	
Group 5 Normal- Like Methylation	K6	M	No Mutation	0.109
	K17	M	c.262 T>A, p.Trp88Arg, Exon 1	0.098
	K22	M	No Mutation	0.079
	K25	F	c.256 C>A, p.Pro87Thr, Exon 1	0.128
	K26	F	c.256 C>A, p.Pro86Thr, Exon 1	0.122
	K30	F	c.397delA, Exon 2	0.122
Outliers	K4	M	No Mutation	0.216
	K18	F	No Mutation	0.184