

SUPPLEMENTARY FIGURE AND TABLE LEGENDS

Supplementary Figure 1. A comparison of promoter methylation status, as determined by bisulphite sequencing and MSP, and expression fold-increase as determined by Affymetrix U133 plus 2 array analysis in RCC-derived cell lines following global demethylation. Upper panel: genes that were frequently methylated in sporadic RCC. Lower panel: Control genes that had relatively low expression increases and no promoter methylation in cell lines.

Supplementary Figure 2. Representative traces of direct bisulphite sequencing of tumour DNA. **H:** Hemi-methylation, **M:** Complete methylation

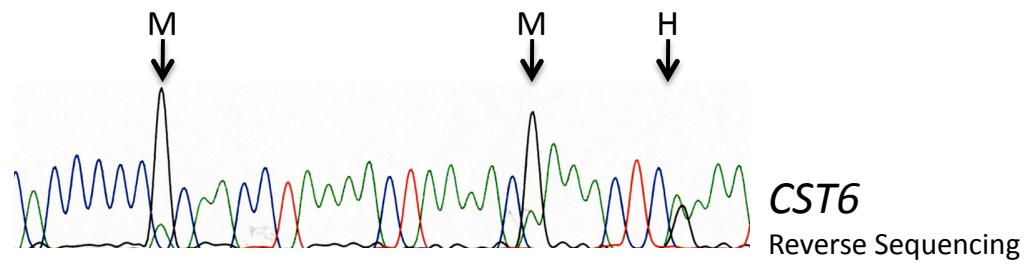
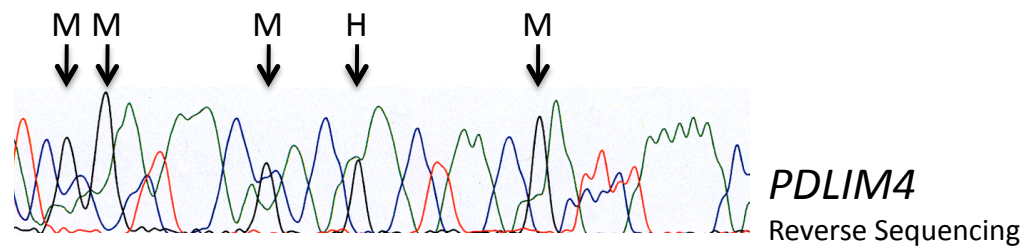
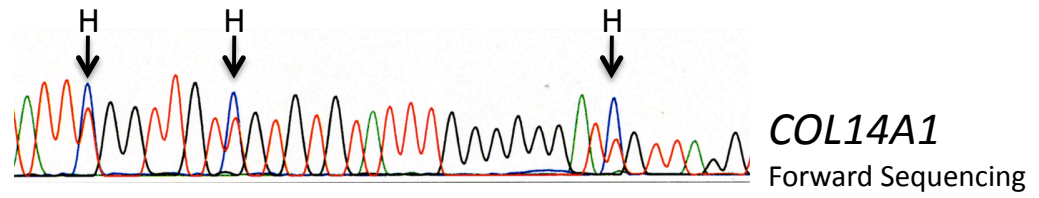
Supplementary table 1. Primers used for methylation analysis.

Supplementary table 2. Shortlist of genes (406) identified (see results for details of filtering procedure) following global demethylation of 11 RCC-derived cell lines and analysis of Affymetrix U133 plus 2 arrays. The numbers in the heat-map cells represent fold changes of expression after demethylation. The genes have been ordered into the following groups: Yellow: genes analysed in this study. Blue: genes previously known to be frequently methylated in RCC. Green: genes that we have previously analysed for promoter methylation and found low frequencies of methylation in RCC. Grey: genes with no promoter region CpG island present. Pink: genes that are not expressed in kidney tissue. Aqua: Genes not analysed thus far.

	COL14A1		BNC1		SFRP1		CST6		COL15A1		PDLIM4		GREM1		RPRM	
	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change
SKRC45	M	26.19	M	5.25	M	14.11	M	7.09	M	3.45	M	0.97	M	603.73	U	0.79
SKRC47	M	63.00	U	0.96	U	1.41	U	1.40	M	19.33	U	0.61	U	2.25	U	1.78
SKRC39	M	33.70	U	2.05	U	1.46	U	39.86	U	2.68	U	0.94	M	240.00	M	6.95
SKRC54	M	2.16	M	7.48	U	1.15	M	3.46	M	2.20	U	12.60	M	192.75	U	1.53
KTCL26	U	1.62	U	1.50	U	1.65	M	27.17	U	1.36	U	1.27	M	66.11	M	10.00
UMRC2	M	2.52	M	27.40	U	2.40	M	38.79	U	1.46	M	16.60	U	1.25	M	4.19
UMRC3	M	6.67	U	0.91	M	14.88	U	0.24	U	0.08	U	1.11	U	1.65	U	0.37
786-0	M	0.69	M	133.67	M	22.00	M	22.41	U	2.21	M	8.33	M	143.20	U	2.09
CAKI-1	U	1.67	U	4.60	U	0.28	M	35.81	U	0.06	M	21.89	M	4.41	U	0.20
KTCL140	M	16.00	M	222.00	U	0.50	U	10.50	M	20.50	U	2.80	M	395.00	U	0.39
RCC4	M	10.00	M	20.12	U	0.84	M	30.77	M	28.50	M	7.05	M	130.73	U	0.32

	LRCH1		FZD8		KLF2	
	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change	Methylation Status	Exp. Array Fold Change
SKRC45	U	0.92	U	1.11	U	3.10
SKRC47	U	2.25	U	6.82	U	2.59
SKRC39	U	4.00	U	6.70	U	3.24
SKRC54	U	0.11	U	5.27	U	0.85
KTCL26	U	0.91	U	0.22	U	1.11
UMRC2	U	0.50	U	5.06	U	0.83
UMRC3	U	0.38	U	0.08	U	0.98
786-0	U	2.89	U	0.46	U	1.97
CAKI-1	U	0.62	U	2.67	U	1.67
KTCL140	U	0.61	U	2.41	U	1.36
RCC4	U	6.58	U	2.89	U	1.30

M Methylated
U Unmethylated



Supplementary Figure 2. Representative traces of direct bisulphite sequencing of tumour DNA.
H: Hemi-methylation, **M:** Complete methylation.

Supplementary table 1

Nesteds primers for amplification and sequencing of bisulphite modified DNA
(F: Forward, R: Reverse, I: Internal)

Col14a COBRA F: 5' g tat ata gta ttt gag aat agg agg gtt yga gat 3'

Col14a COBRA IF: 5' ggg ttt ggt ttg gga gtt tgt agt agt 3'

Col14a COBRA R: 5' c taa aac tac ctc crc ctc tcc aac taa 3'

CST6 COBRA F : 5' ttt tgt gaa tyg ttt ttg tat tgg tat ttg ttg t 3'

CST6 COBRA IR: 5' ata ccr tcr aaa ccc tca aaa ccr taa ata 3'

CST6 COBRA R: 5' ata cta tta cta ccc ata tta taa cta acc acr a 3'

COL15A1 COBRA F: 5' gta ttt agg agt tta gtg tag ttt ggg at 3'

COL15A1 COBRA IF: 5' gat ttt agt ttt ttt ggg ttg tgg aga gga ttt 3'

COL15A1 COBRA R: 5' cta crc act cac rcc cra aaa ata ccr a 3'

LOXL-1 COBRA F: 5' ggg tgg tgt tga tat ttt agt tty gtt ttg ttt gt 3'

LOXL-1 COBRA IF: 5' ttg tgg ttt ggt tgg gaa ttt tag gga t 3'

LOXL-1 COBRA R: 5' cra acr aac aaa acc rcc tat aaa cct tac ata a 3'

EMX2 COBRA F: 5' g aay gta tyg att ttg gga ttt yga ggt taa gyg 3'

EMX2 COBRA IF: 5' ggt tga tgg ggt ggg agg att tyg tat 3'

EMX2 COBRA R: 5' taa atc ccr aac cta cct crc cca aac taa 3'

PDLM4 COBRA F: 5' gy ggt ttg tag att taa ggt tyg agt agg att 3'

PDLM4 COBRA IF: 5' ggt ygt tyg aat gyg ggg att ttt gag agt 3'

PDLM4 COBRA R: 5' acr aaa acc cca aaa cra aaa ccc rcr caa 3'

GREM1 COBRA F: 5' gga gyg gaa aat gtg att tgt tgt gta ttt t 3'

GREM1 COBRA IF: 5' gtt tgg tta atg gag agg ygy ggt tt 3'

GREM COBRA IR: 5' ctc acc tat caa tac rac raa tcc tcr aaa 3'

GREM COBRA R: 5' cta crt ttc cct aca aac cca aaa caa cra aa 3'

Claudin 6 COBRA F: 5' tt tgt tag tty gag gyg ggt tta gyg att 3'

Claudin 6 COBRA IF: 5' gtt gty gtt agg gat agg gtt ttt gtt 3'

Claudin 6 COBRA R: 5' acr ttt aac cct taa aaa ccc cra aaa acc 3'

FZD8 COBRA F: 5' gty gag tgg tgg tyg tta ggg ttg att 3'

FZD8 COBRA IF: 5' g tat ygy ggt ttt aga gga aat ttg ygg ygt t 3'

FZD8 COBRA R: 5' ata atc taa aat ctc cct tat act tcr ccc raa 3'

MKK6 COBRA F: 5' ggg agt ttt ttt tay gga gat ygg ggt 3'

MKK6 COBRA IF: 5' ggg ttg gag gga att ggg aag tat tgt 3'

MKK6 COBRA R: 5' ac tac rtt crc rac cca acr ttc tcc caa 3'

Noggin COB F: 5' gt tag tgg ttt tta tgg tga tgg agt 3'

Noggin COB IF: 5' att taa agg ttt gga ttt tgy gag ata gat 3'

Noggin COB R: 5' caa ctc ctc tcc cra atc tac taa 3'

ITGBL1 COBRA F: 5' g cag ygt ttg gat ttt aga gag atg tgg gtt 3'

ITGBL1 COBRA IF: 5' ggg ttg tay gtt tgt att tgg agg gga tat 3'
ITGBL1 COBRA R: 5' ccr tcr taa atc tcr cac acc cac tca taa 3'

ZFP42/REX-1 COBRA F: 5' ata gat att tat tga gyg ttt att ayg tgt t 3'
ZFP42/REX-1 COBRA IR: 5' caa taa aaa crt aaa cta ccc tac rcc 3'
ZFP42/REX-1 COBRA R: 5' cac tct act att tcc aat acc ac 3'

DMRTB1 COBRA IF: 5' gat gta gga att atg gtt ttt tgg tgt t 3'
DMRTB1 COBRA R: 5' ac crc cca aaa ccr act aaa aaa ctc 3'
DMRTB1 COBRA F: 5' t ggt tgt gtt ayg aag gtt gat ttt gtt 3'

KLF4 COBRA F: 5' g tgt ttt agt tgt tty ggg ttg tyg agg att 3'
KLF4 COBRA IR: 5' cct crt tca ata act ctt aat aac ccc aaa ac 3'
KLF4 COBRA R: 5' c ata cac aac taa acc aaa aac acr aaa act atc 3'

KLF2 COBRA F: 5' ggt ttt gta gtt ttt ttt gag gtt gga gtt 3'
KLF2 COBRA IF: 5' gg tgt ttt ttt gag agt ttt tga gag gtt 3'
KLF2 COBRA R: 5' c cra taa ata act acc cra aac cra act aa 3'
KLF2 COBRA IR: 5' c cra taa ata act acc cra aac cra act aa 3'

HERC5 COBRA F: 5' atg ttt ygt ttt tta ttg gag ttg tag gtt 3'
HERC5 COBRA R: 5' aca aca aaa tta acr crt cac ctc cac 3'
HERC5 COBRA IR: 5' ccr cra crc taa aaa aaa acc aaa act ata 3'

LRCH4 COBRA F: 5' gtg tyg gtg ttt ttt ttt ttt ggt tgg att 3'
LRCH4 COBRA IR: 5' aac cta aaa acc cra aca cra aaa tcr taa 3'
LRCH4 COBRA R: 5' cac tca cca acc taa ata ata tct aac aaa 3'

PTGIS COBRA F: 5' gta ggg ttt tta ata gag ygt tty ggt ttg att 3'
PTGIS COBRA IR: 5' ccr aaa atc tcr cct tac ccr aaa tac taa 3'
PTGIS COBRA R: 5' ct tcc cac ctt aca cct tct taa caa 3'

RANBP2L1 COBRA F: 5' ttg aat tta ttt att att att gat agt tta ggt t 3'
RANBP2L1 COBRA IF: 5' aag gtt ttt tat aga taa gat tat gtt aag ata tt 3'
RANBP2L1 COBRA R: 5' aca atc rcc act tcc aaa aaa aaa ata 3'

FBXO2 COBRA F: 5' at tyg gaa ttt agg agt ttt tty ggt agt t 3'
FBXO2 COBRA IF: 5' ta tga tty gtt ggg tyg ttt aag ttg tat 3'
FBXO2 COBRA R: 5' cta aaa aaa aat ccr att taa aac atc tcr caa 3'

TLL1 COBRA F: 5' g gtt tta gyg ttt ttt gtt gtt gta att tgt aat 3'
TLL1 COBRA IF: 5' gt agt ttt gag gat ttt agt ttt tag taa aat 3'
TLL COBRA R: 5' cat aca atc aaa atc aaa atc cra acc aca a 3'

DiCKKOPF-1 COBRA F: 5' ggg tat tta agt ttt tag agt ttt tgt tgt 3'
DiCKKOPF-1 COBRA IF: 5' gta gtt ttt att tyg aag atg agt ygg gtt 3'
DiCKKOPF-1 COBRA R: 5' caa aat aac rct cac tcc caa caa aaa ata a 3'

SYCP3 COBRA F: 5' ttg gaa ggt att agg ttt taa gtg tat 3'

SYCP3 COBRA R: 5' ta aac cct atc cta ctc aaa acc cta 3'
SYCP3 COBRA IR: 5' c aaa aac aac ctc taa cct aaa cac cta 3'

Methylation Specific primers to amplify Col15A promoter region:

Col15a MSPF: 5' gg ttg tgg aga gga ttt ttt tag cg 3'
Col15a USPF: 5' ggg ttg tgg aga gga ttt ttt tag tg 3'
Col15a MSPR: 5' tc tac tac ctc cac ccg cg 3'
Col15a USPR: 5' ctc tac tac ctc cac cca ca 3'

Probe	SK45	SK47	SK39	SK54	KT48	UM2	UM3	13860	CAK1	KT140	RCC4	Gene Symbol	Chromosomal Location	RefSeq Transcript ID
1552487_s.at	5.25	0.96	2.05	7.26	1.50	27.40	0.91	736.67	4.60	222.00	20.12	BNCL1	chr15q25.2	NM_017177
205827_at	17.30	18.00	114.67	29.29	23.44	16.74	1.11	12.91	2.55	95.50	17.54	SCYA20	chr1p36.2	NM_017185
237810_at	0.48	1.29	36.62	2.21	2.12	16.64	0.12	15.10	20.29	25.22	66.94	CLDN6	chr16p13.3	NM_012195
121865_s.at	26.19	63.00	30.70	2.16	1.62	2.52	6.67	0.69	1.67	1.09	10.00	COL14A1	chr8q23	NM_021110
203477_at	3.45	19.33	3.68	2.20	1.36	1.46	0.68	2.21	0.66	20.50	28.50	COL15A1	chr9q21-q22	NM_018555
206959_at	7.09	1.40	39.66	3.64	27.17	38.79	1.24	28.41	35.81	10.50	30.17	CSF2	chr11q13	NM_013233
204602_at	3.75	1.30	2.64	28.10	2.16	2.68	1.24	36.44	4.07	14.38	1.97	DKK1	chr10q11.2	NM_012242
240313_at	6.74	17.38	55.16	5.17	12.56	10.25	0.85	8.25	9.94	3.81	7.85	DMRTB1	chr1p32.3	NM_033067
221950_at	0.46	2.14	0.25	1.50	5.00	3.25	14.25	27.50	32.67	0.22	0.48	EMX2	chr10q26.1	NM_014098
206818_at	1.39	89.67	12.91	14.67	20.05	26.57	0.31	0.67	1.79	13.48	0.45	FOXP1	chr14q12-q13	NM_005249
181848_s.at	322.25	25.00	24.65	38.08	9.93	1.60	91.17	464.33	8.67	230.33	30.29	GREM1	chr15q13-15	NM_013372
205422_s.at	11.77	1.44	1.70	1.09	5.65	2.00	4.47	50.67	1.74	2.09	32.09	ITGBL1	chr13p33	NM_004791
202266_s.at	2.68	0.68	12.40	1.14	10.68	1.44	0.84	2.72	2.35	2.15	10.20	KLF4	chr9q31	NM_013233
203370_at	0.46	0.42	36.00	1.11	0.32	2.25	1.39	0.61	23.60	13.60	2.33	LOXL1	chr17q24	NM_005576
231798_at	2.88	1.03	5.99	26.57	1.36	1.79	1.36	3.32	1.69	12.40	11.17	NOG	chr15q21-q22	NM_005450
121564_s.at	0.97	0.61	0.94	12.60	1.27	16.68	0.11	8.33	2.89	2.80	7.52	PDLIM4	chr5q31.2	NM_003687
208131_s.at	0.36	57.33	7.37	10.66	3.27	20.38	0.74	9.11	21.00	5.50	27.52	PTGIS	chr20p13.13	NM_006961
206392_s.at	1.38	25.50	40.00	1.67	1.44	71.50	3.33	1.50	7.51	0.43	0.59	RARRRS1	chr3q25.32	NM_208663
211324_s.at	0.17	0.33	10.00	1.13	19.00	5.00	1.27	2.52	1.00	1.68	22.75	RPGD5	chr2p13	NM_005054
202037_s.at	14.11	1.41	1.46	1.15	1.65	2.40	14.88	22.00	0.28	0.50	0.84	SFRP1	chr8p12-q11	NM_003012
206415_at	3.83	18.00	7.00	0.02	0.42	0.04	13.00	0.50	1.00	10.25	1.17	TLL1	chr4q32-p33	NM_012464
204083_s.at	6.58	1.04	4.48	7.29	3.91	107.82	0.59	42.14	0.57	28.25	45.44	TPM2	chr1p13	NM_218674
202233_s.at	13.27	1.21	0.71	74.45	1.13	80.82	1.05	1.12	0.22	0.90	0.99	UCRHR	chr1p33	NM_006004
154776_at	0.45	20.00	2.63	0.56	29.33	0.52	0.78	1.59	66.47	21.40	1.10	ZFP42	chr4q35.2	NM_174900
214936_at	0.92	2.25	4.00	0.11	0.91	0.50	0.38	2.89	0.62	0.61	6.58	LRRH1	chr13q14.13	NM_015116
216587_s.at	1.11	6.82	6.70	5.27	0.22	5.06	0.98	0.46	2.67	2.41	2.89	FZD8	chr10p11.23	NM_013866
219371_s.at	3.10	2.59	3.24	0.85	1.11	0.83	0.98	1.97	1.67	1.36	1.30	KLH2	chr19p13.13	NM_016270
219370_at	0.79	1.78	6.95	15.53	10.00	1.19	0.37	2.09	2.00	0.39	0.32	REPRIMO	chr2q23.3	NM_009485
155499_s.at	9.48	2.79	10.94	20.22	18.01	41.77	1.04	19.07	8.76	69.61	32.56	COL1A1	chr17q21.33	NM_010088
205302_at	11.79	32.76	3.36	0.56	2.73	2.72	1.86	1.18	1.85	0.89	13.77	IGFBP1	chr7p12-p12	NM_000596
201650_at	33.75	8.33	216.00	0.83	174.13	99.00	1.05	1.75	1.18	317.00	0.87	KRT19	chr17q12.1	NM_002271
208352_x.at	1.00	1.62	1.18	6.17	0.72	0.28	1.09	31.50	13.13	15.45	22.38	ANKK1	chr8p11.1	NM_020481
205780_at	5.00	3.73	17.85	2.96	2.21	5.66	1.11	9.25	6.69	35.29	10.40	BIK	chr22q13.31	NM_001197
202790_at	6.06	6.00	68.45	58.67	39.63	4.07	0.53	28.08	10.33	7.86	4.02	CLDN7	chr17p13	NM_001307
206806_at	18.06	17.00	1.51	4.55	1.17	1.67	2.07	4.49	5.54	7.88	4.02	DNK1	chr7q32.3	NM_004717
204273_at	35.06	20.00	18.50	0.20	27.00	3.00	1.50	0.50	1.65	1.56	1.00	EDNBP1	chr13q22	NM_003991
201809_s.at	6.97	1.53	1.60	1.37	1.47	16.39	1.03	101.14	1.26	46.08	1.10	ENG	chr9q33-q34.1	NM_000118
215058_at	1.50	1.00	39.32	2.04	0.88	5.36	0.22	12.44	3.12	45.18	15.11	IGFBP4	chr17q12-q21.1	NM_015152
1554252_x.at	36.00	0.11	0.62	1.50	0.56	11.17	1.79	1.86	1.19	20.75	1.39	LASS3	chr15q26.3	NM_178942
202096_at	5.43	2.94	5.20	1.37	2.47	10.91	0.23	20.85	4.33	78.89	1.88	MDA2	chr21q25.3	NM_002462
229494_s.at	12.50	0.22	13.75	7.00	12.50	1.56	1.10	0.06	0.08	11.20	0.22	PHLDA2	chr11p15.5	NM_003311
206772_at	37.00	29.20	1.03	47.20	1.46	4.41	80.50	4.69	10.15	35.00	19.60	PTHFR	chr2q33	NM_005048
219395_at	1.52	2.86	1.87	12.73	9.84	3.36	0.97	2.59	1.99	32.76	1.16	FBM35B	chr2q33	NM_029399
213921_at	0.88	2.50	12.68	69.54	6.94	0.68	0.80	8.05	4.00	15.12	32.81	SST	chr9q28	NM_001408
1553599_s.at	13.04	15.31	45.56	12.48	28.89	11.04	8.07	12.54	12.93	32.38	11.88	SYCP3	chr12q	NM_153694
213969_x.at	11.75	3.01	11.34	9.90	24.39	15.26	0.94	16.90	6.42	39.88	10.32	THY1	chr11q22.31	NM_006288
230193_at	11.18	11.81	14.28	2.90	2.99	3.83	1.16	220.50	47.67	83.65	28.52	WDR66	chr12q24.33	NM_146688
1564307_s.at	0.38	8.20	0.07	1.43	7.07	4.78	1.10	32.60	1.45	12.50	12.30	AZM1L	chr12p13.31	NM_146770
207583_at	0.56	0.52	3.09	4.70	3.00	1.20	27.67	1.65	1.07	28.50	41.50	ABD2	chr17q12-q11	NM_005164
237374_at	2.00	2.00	24.50	10.10	8.22	8.25	2.09	1.73	8.17	10.63	26.00	ABHD18B	chr14q22.1	NM_181533
1568970_at	13.00	0.05	1.33	1.63	0.26	10.00	14.00	1.26	12.83	1.36	2.89	ADAM12	chr8p11.22	NM_014237
201792_at	32.08	0.36	10.73	10.33	1.49	0.79	3.82	0.52	43.14	3.32	1.91	AEBP1	chr7p13	NM_001291
210081_at	0.22	0.51	1.53	0.03	0.91	12.33	21.83	0.50	1.52	14.60	0.92	AGER	chr6p21.3	NM_172197
209173_at	12.71	3.50	39.00	2.69	5.00	1.75	0.43	44.29	0.98	2.00	0.62	AGR2	chr7p21.3	NM_006408
1560098_at	0.03	31.00	0.33	1.24	21.50	0.34	0.33	3.50	54.00	0.65	10.00	AKR1C2	chr10p15-p14	NM_001354
1562261_at	1.18	4.22	5.88	1.70	10.25	0.56	2.86	3.61	1.03	10.74	23.67	AMZ1	chr7p22.2	NM_133463
239183_at	20.50	2.43	1.18	1.94	22.00	2.75	0.60	0.60	1.06	40.00	0.66	ANGPT1	chr1q25.2	NM_004673
1564000_at	1.43	0.48	10.50	12.67	1.33	1.00	5.50	20.40	1.36	7.00	3.33	ANKRD31	chr5q13	XM_827671
203074_at	2.99	1.53	7.29	2.19	5.49	18.33	0.74	9.40	6.62	17.11	84.49	ANXA8	chr10q11.2	NM_016390
252691_at	48.00	23.67	26.33	5.00	3.00	182.00	2.83	1.96	27.00	175.00	1.00	ARL1	chr13q14.3	NM_138450
214498_at	11.27	10.13	19.57	2.38	1.43	12.09	1.50	3.53	2.66	3.63	1.00	ARL9	chr4q12	NM_206919
235503_at	72.00	3.33	19.61	1.25	20.86	5.50	2.35	26.18	14.14	5.78	8.77	ASB5	chr4q42.2	NM_080874
214498_at	11.80	8.50	3.34	3.94	1.08	11.25	0.61	2.00	27.20	11.57	0.15	ASIP	chr20q12-q12-q12	NM_016272
206783_at	0.19	0.11	12.18	4.67	0.71	9.50	0.56	2.43	4.32	20.13	12.40	B3GNT8	chr19q13.2	NM_198540
1561263_at	1.47	9.38	0.08	0.82	0.35	19.25	12.50	14.00	0.18	3.68	20.74	C10orf3	chr5p13.3	NM_181435
204865_at	1.62	1.27	69.40	2.10	1.99	11.43	0.29	2.11	3.09	28.65	10.25	CA3	chr8p13-q22	NM_005181
228083_at	4.46	4.45	34.00	32.67	0.69	3.58	1.05	11.15	1.14	11.94	0.74	CACNA2D4	chr12p13.33	NM_172364
155554_s.at	0.17	0.26	0.13	10.60	2.29	1.50	1.00	1.00	11.80	4.00	19.33	CADM2	chr3p12	NM_153184
234804_at	0.96	2.36	0.22	1.09	0.08	1.87	16.57	18.00	10.08	1.11	1.00	CCL26	chr18p12.33	NM_133459
223710_at	1.78	0.90	1.24	2.14	3.30	5.88	1.74	41.00	3.00	15.00	10.71	CCBE1	chr7q11.23	NM_006072
205114_s.at	22.14	5.65	3.78	2.26	1.99	15.33	0.58	18.42	9.00	23.46	1.07	CCL5	chr17q12-q12	NM_002985
14051_s.at	38.07	33.75	2.71											