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 |
|-----|---------|------|-------|------|---------|--------|-------|------|
|     | 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 |
| SKRC45 | M | 26.19 | U | 5.25 | M | 14.11 | M | 7.09 | M | 3.45 | M | 0.97 | M | 289.73 | U | 0.79 |
| SKRC47 | M | 63.00 | U | 0.96 | U | 2.50 | 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 | 39.96 | U | 2.68 | U | 1.46 | U | 2.25 | U | 1.78 |
| SKRC54 | M | 2.16 | M | 7.48 | M | 1.15 | M | 3.46 | M | 2.00 | M | 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 | 56.11 | U | 10.09 |
| UMRC2 | M | 2.52 | M | 27.49 | U | 2.40 | U | 1.46 | M | 16.60 | U | 1.25 | M | 4.19 |
| UMRC3 | M | 6.67 | M | 133.67 | U | 22.00 | M | 22.11 | M | 8.33 | M | 143.20 | U | 2.09 |
| 786.0 | M | 0.69 | M | 133.67 | U | 0.91 | M | 0.24 | M | 0.08 | U | 1.11 | U | 1.65 | M | 0.37 |
| CAKI-1 | U | 1.67 | U | 4.60 | U | 0.28 | U | 35.81 | U | 0.06 | U | 21.85 | 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 | 395.09 | U | 0.39 |
| RCC4 | M | 10.00 | M | 20.12 | U | 0.34 | U | 30.77 | M | 28.50 | U | 7.05 | M | 130.73 | U | 0.32 |

|     | LRCH1 | FZD8 | KLF2 |
|-----|-------|------|------|
|     | Exp. Array | Fold Change | Methylation Status | Exp. Array | Fold Change | Methylation Status | Exp. Array | Fold Change | Methylation Status |
| 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 | 0.98 | 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**: Methylation
**U**: Unmethylated
Supplementary Figure 2. Representative traces of direct bisulphite sequencing of tumour DNA. 
H: Hemi-methylation, M: Complete methylation.
Supplementary table 1
Nestesd 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 ttg ggt ttg gga gtt tgt agt 3’
Col14a COBRA R: 5’ c taa aac tac ctc ctc ctc aac taa 3’

CST6 COBRA F: 5’ ttt tgt gaa tyg ttt ttg tat tgt tgt 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 cta acc acc r 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 tgt tgt tgt tgt gaa gaa gaa gaa gaa tgg tgt gtt tgt gtt tgg tgt gtt ggg tgt ggt ggg 3’
LOXL-1 COBRA IR: 5’ ctc acc tat caa tac rac raa tcc tcr aaa 3’
LOXL-1 COBRA R: 5’ cra acr aac aac aaa accord rcc tat aat aat cct tac ata a 3’

EMX2 COBRA F: 5’ g aay gta tyg att tgt gga ttt yga ggt taa gyy 3’
EMX2 COBRA IF: 5’ ggt tga tgt ggg gtt ggg aga att tyg tay 3’
EMX2 COBRA R: 5’ taa atc ccr aac cta cct tcc cca aac aac taa 3’

PDLM4 COBRA F: 5’ gty ggt tgt tag att tta ggt tgt ggt agt 3’
PDLM4 COBRA IF: 5’ ggt gyt tyg aat ggg ggg att tgt gag agt 3’
PDLM4 COBRA R: 5’ acr aaa acc cca aaaa cca aaa ccc rcr cca a 3’

GREM1 COBRA F: 5’ gga gyg gaa aat gtt tgt tgt gta ttt t 3’
GREM1 COBRA IR: 5’ gtt tgg tta atg gag agg ygy ggt tt ggg 3’
GREM1 COBRA R: 5’ cta ctn tct cct cta ctc ccc cca cca aac cca a 3’

Claudin 6 COBRA F: 5’ ttt tgt tag tta tag ggg tgt taa gyy ggt ggt ttt gga ttt gaa gaa ytg ttt ttt ttt ggg 3’
Claudin 6 COBRA IF: 5’ gtt gty gtt agg ggt ggt gtt ggg 3’
Claudin 6 COBRA R: 5’ acr att ttt aac cct taa aaaaa cca cca cca aac cca a 3’

FZD8 COBRA F: 5’ gty gtt gtt ttt gag ggt ttt ggg 3’
FZD8 COBRA IF: 5’ g tat ygy gtt ttt aga gga aat tgt ygg ygt gtt ggg 3’
FZD8 COBRA R: 5’ atc aac cct taa atc cct ctc tta gag act ccr cca tta gat ggt 3’

MKK6 COBRA F: 5’ ggg agt ttt tgt gaa gat ggg ggt ggg 3’
MKK6 COBRA IR: 5’ gtt ggg tgt ggg gtt gaa aag ttt tgt gtt ggg 3’
MKK6 COBRA R: 5’ cta ccc atc ctc cct tcc tcc cca aac ccr cca cca a 3’

Noggin COB F: 5’ gtt tag ggg gga gaa gaa ggg gtt ggg ttt gga 3’
Noggin COB IR: 5’ att ttt gga ttt tgg gga gtt ttt gtg ggg 3’
Noggin COB R: 5’ cta ccc ctc cct ctc cca cca aac ccr cca a 3’

ITGBL1 COBRA F: 5’ g cag ygt tgt gtt ttt aga gag atg tgt ggt 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 crr 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 aag aag tgt gtt gtt ttt 3’

KLF4 COBRA F: 5’ gtt ttt tgt tty ggg tgt tyg agg att 3’
KLF4 COBRA IR: 5’ cct cct tca ata act ctt aat aac ccc aaa ac 3’
KLF4 COBRA R: 5’ c ata cac aac taa acc aaa aac acc rra act atc 3’

KLF2 COBRA F: 5’ gtt 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 ra taa ata act acc ra aac ra act aa 3’
KLF2 COBRA IR: 5’ c ra taa ata act acc ra aac ra act aa 3’

HERC5 COBRA F: 5’ atg ttt ygt ttt tta tgt gag tag tgt 3’
HERC5 COBRA R: 5’ cca cca aab taa aac rta cca cct ccc 3’
HERC5 COBRA IR: 5’ ccr cca rra taa aab aac acc act ata 3’

LRCH4 COBRA F: 5’ gtg tyg gtt ttt ttt ttt gtt tag gtt 3’
LRCH4 COBRA IR: 5’ ccr cca cca cca aac cca ccc aca 3’
LRCH4 COBRA R: 5’ ccr cca cca cca aac cca ccc aca 3’

PTGIS COBRA F: 5’ gta ggg ttt tta ata gag gtt ttt gtt 3’
PTGIS COBRA IR: 5’ ccr aab aac tcr ctc ccc aca cca 3’
PTGIS COBRA R: 5’ crt ctc cac ctt cca ctc tca caa 3’

RANBP2L1 COBRA F: 5’ ttt aat tta att att att gat gat ttt att 3’
RANBP2L1 COBRA IF: 5’ aag gtt ttt tat aag taa gta tat gtt aag ata 3’
RANBP2L1 COBRA R: 5’ cca aac rta act cca cca aab aab ata 3’

FBXO2 COBRA F: 5’ at tyg gaa ttt agg aag gtt ttt gtt 3’
FBXO2 COBRA IF: 5’ ta tca tta gtt ggg tyg ttt aag tgg tat 3’
FBXO2 COBRA R: 5’ ata aaa aat aac ccr att taa aac atc tca ctc 3’

TLL1 COBRA F: 5’ g gtt tta gty ell ttt gtt gta att tgg aat 3’
TLL1 COBRA IF: 5’ gtt att tta ggttat gtt att tgg gtt 3’
TLL1 COBRA R: 5’ cat aca atc aac atc aac atc cca aca a 3’

DiCKKOPF-1 COBRA F: 5’ ggg tat tta agt ttt tag ttt tgt tgt 3’
DiCKKOPF-1 COBRA IF: 5’ gta gtt ttt att tyg aag atg aag gtt gtt 3’
DiCKKOPF-1 COBRA R: 5’ caa aat aac rct ctc cca cca aab ata a 3’

SYCP3 COBRA F: 5’ ttt gaa ggt att agg tta gtt tag 3’
SYCP3 COBRA R: 5' ta aac cct atc 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'
