

Supplementary Table 1. Summary of promoter methylation frequency in surrounding non-neoplastic liver tissues (ST) and HCC

Gene	Description	Methylation frequency (%)		Methylation frequency (%)		P	P
		ST	HCC	A	B	(<i>t</i> test, ST vs HCC)	(<i>t</i> test, A vs B)
Inhibitors of the Jak/Stat pathway							
ARHI	A Ras Homologue Member I	0	68	93	43	1.78E-20	9.52E-06
CIS	Cytokine-inducible SH2-containing protein	25	85	87	80	3.14E-13	0.465
GRIM-19	Gene associated with retinoid-interferon mortality	0	23	40	8	4.50E-05	0.00474
SOCS1	Suppressors of cytokine signaling 1	37	83	90	77	3.82E-08	0.0816
SOCS2	Suppressors of cytokine signaling 2	90	85	83	87	0.411	0.723
SOCS3	Suppressors of cytokine signaling 3	0	25	0	50	2.08E-05	1.37E-06
SHP-1	Src homology 2 domain	93	87	90	83	0.266	0.456
PIAS-1	Protein inhibitor of activated STAT-1	95	95	93	97	1	0.561
PIAS-γ	Protein inhibitor of activated STAT gamma	77	83	87	80	0.365	0.496
SYK	Non-receptor tyrosine kinase	17	22	17	27	0.490	0.355
Inhibitors of the EGFR/FGFR/Ras pathway							
NORE 1A	Novel Ras effector 1A	0	22	43	0	9.56E-05	1.59E-05
RASSF1A	Ras-effector homologue 1A	25	77	83	70	1.52E-09	0.229
RASSF2	Ras-effector homologue 2	10	35	17	53	0.00089	0.0024
RASSF3	Ras-effector homologue 3	0	5	10	7	0.313	0.647
RASSF4	Ras-effector homologue 4	0	5	10	7	0.313	0.647
LOX	Lysyl oxidase	17	62	47	77	1.17E-07	0.0164
HRASLS	H-Ras-like suppressor	97	98	100	97	0.562	0.321
DAB2	Disabled-2	33	65	100	33	0.000425	2.7E-10
hDAB2IP	DAB2 interactive protein	42	73	100	47	0.000362	3.41E-07
GATA-4	GATA factor 4	7	58	77	40	6.63E-11	0.00343
GATA-5	GATA factor 5	3	25	40	10	0.000553	0.0067
GATA-6	GATA factor 6	8	23	40	7	0.0243	0.00183
SPRY1	Sprouty 1	0	0	0	0	0	0
SPRY2	Sprouty 2	0	40	73	0	6.09E-09	1.73E-12
SPRY4	Sprouty 4	0	30	53	10	6.03E-05	0.00011
CNK1	Connector enhancer of KSR 1	90	92	93	90	0.978	0.647
RIG	Ras-related inhibitor of cell growth	100	100	100	100	1	1
RRP22	Ras-related on chromosome 22	0	60	87	33	5.07E-16	6.97E-03
CLU	Clusterin	0	67	93	40	1.78E-19	2.47E-06
HIN-1	Secretoglobulin family 3A member 1	0	25	37	13	2.08E-05	0.0373
SOCS-4	Suppressors of cytokine signaling 4	0	10	20	0	0.0117	0.00925
SOCS-5	Suppressors of cytokine signaling 5	0	33	63	3	3.04E-07	4.63E-08
RASSF1C	Ras-effector homologue 1C	0	0	0	0	0	0
NORE 1B	Novel Ras effector 1B	0	0	0	0	0	0
TSC1	Tuberous sclerosis 1	0	0	0	0	0	0
TSC2	Tuberous sclerosis 2	0	0	0	0	0	0
Inhibitors of the Wnt/β-catenin signaling							
APC	Adenomatous polyposis coli	98	95	97	93	0.313	0.561
CDH1	E-cadherin	25	40	47	33	0.080	0.299
SFRP1	Secreted Frizzled-related Protein 1	0	20	33	7	0.0001	0.092
SFRP2	Secreted Frizzled-related Protein 2	3	27	43	10	0.00027	0.0029
SFRP4	Secreted Frizzled-related Protein 4	17	22	7	33	0.490	0.0092
SFRP5	Secreted Frizzled-related Protein 5	80	83	87	80	0.640	0.496
DKK-1	Dickkopf-1	85	87	87	87	0.795	1
DKK-3	Dickkopf-3	17	62	47	77	1.17E-07	0.0164
WIF-1	Wnt inhibitory factor-1	0	25	43	7	2.08E-05	0.00075
HDPR1	Dapper 1	10	35	53	17	0.00089	0.0024
Inhibitors of the RELN signaling							
DAB1	Disabled-1	0	35	63	7	1.20E-07	5.63E-07
RELN	Reelin	0	30	50	10	1.78E-06	0.00049

Supplementary Table 1. (continuation from page 1)

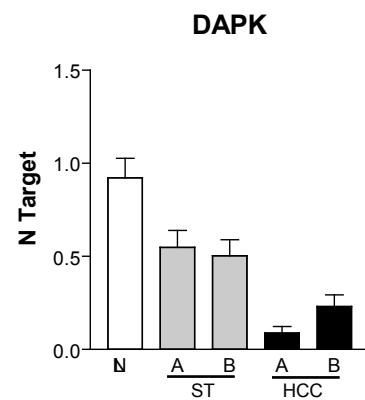
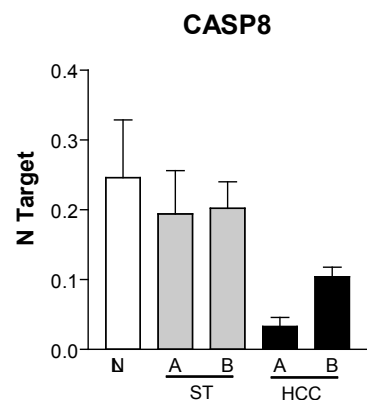
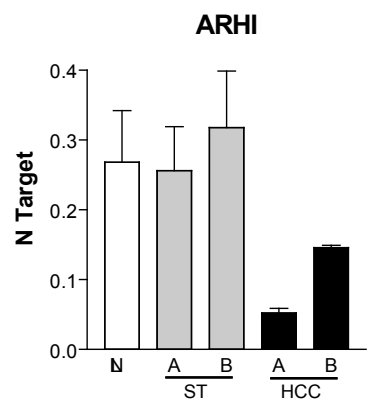
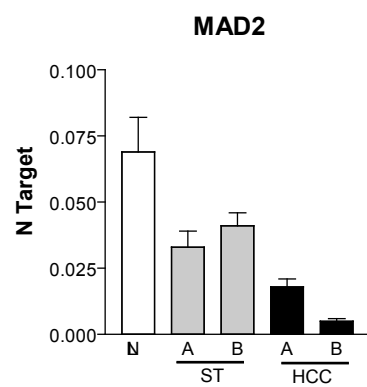
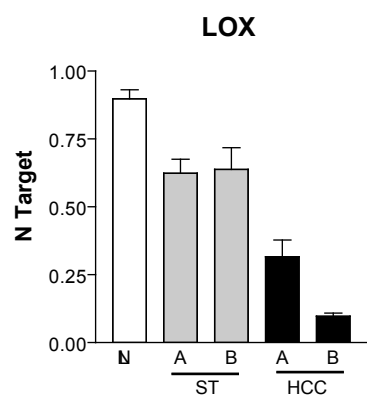
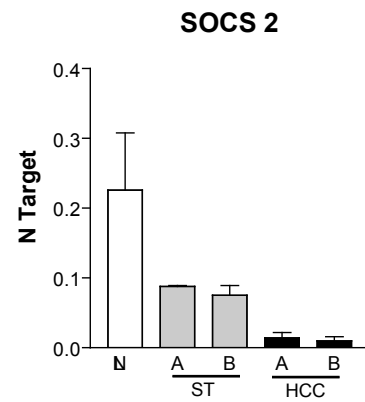
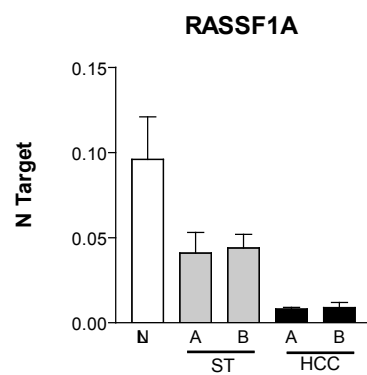
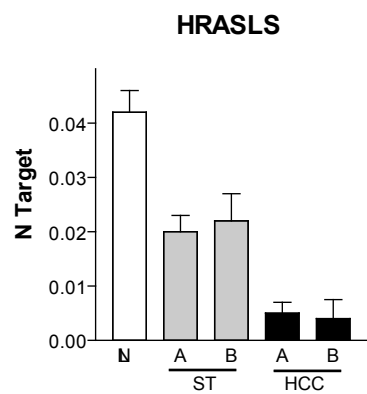
Gene	Description	Methylation frequency (%)		Methylation frequency (%)		P	P
		ST	HCC	A	B	(t test, ST vs HCC)	(t test, A vs B)
Cell cycle regulators and mitotic checkpoints							
p14	p14 ARF	0	8	7	10	0.0222	0.779
p15	p15 INK4B	10	40	60	20	0.00010	0.0012
p16	p16 INK4A	38	68	90	47	0.00084	0.00031
p57	p57 Kip2	0	63	83	43	1.19E-17	0.00097
14-3-3-σ	14-3-3 protein sigma	100	95	93	97	0.0806	0.561
MAD2	Mitotic arrest deficient 2	0	33	10	53	3.04E-07	0.00017
CHFR	Checkpoint forkhead RING finger domains protein	0	0	0	0	0	0
p18	p18 INK4C	0	0	0	0	0	0
p21	p21WAF1/cip1	0	0	0	0	0	0
p27	p27 Kip1	0	0	0	0	0	0
p73	Tumor protein p73	0	0	0	0	0	0
Rb	Retinoblastoma	0	0	0	0	0	0
Histone methyltransferases							
PRDM2	PR domain containing 2 with ZNF domain	17	72	87	57	5.36E-11	0.00935
PRDM5	PR domain containing 5	7	40	60	27	8.47E-06	0.0086
Retinoid receptor and transport							
RAR-β	Retinoic acid receptor-beta2	90	90	97	83	1	0.0879
CRBP1	Cellular retinoic acid binding protein 1	0	0	0	0	0	0
Apoptosis inducers							
DAPK	Death-associated protein kinase	45	75	87	63	0.000671	0.0373
CASP3	Caspase-3	0	5	7	3	0.0806	0.561
CASP8	Caspase-8	0	18	37	0	0.00212	0.000131
DCR1	Decoy receptor 1	0	27	33	20	0.0181	0.250
ASC	Apoptotic speck protein	0	40	57	23	6.09E-09	0.00782
ASCL	Apoptotic speck protein-like	0	25	40	10	2.08E-05	0.00670
PAR4	Prostate apoptosis response 4	0	38	63	13	1.71E-08	2.64E-05
BNIP3	BCL2/adenovirus E1B 19kDa interacting protein	0	30	53	3	1.78E-06	4.23E-06
BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like	0	63	100	27	1.19E-17	1.73E-12
IGFBP3	Insulin-like growth factor binding protein 3	7	57	73	40	2.47E-10	0.008602
ASK1	Apoptosis signal-regulating kinase 1	0	0	0	0	0	0
DCR2	Decoy receptor 2	0	0	0	0	0	0
DR4	Death receptor 4	0	0	0	0	0	0
DR5	Death receptor 5	0	0	0	0	0	0
APAF1	Apoptosis proteases-activating factor-1	0	0	0	0	0	0
NOXA	Phorbol-12-myristate-13-acetate-induced protein 1	0	0	0	0	0	0
Immune response/ interferon signaling pathway							
IRF-1	IFN regulatory factor-1	88	90	93	87	0.771	0.382
IRF-7	IFN regulatory factor-7	38	80	85	77	1.40E-06	0.464
CIITA	Class II transactivator	17	53	73	33	1.46E-05	0.00227
DNA repair							
GADD45β	Growth arrest and DNA damage 45 beta	83	90	87	93	0.270	0.398
GADD45γ	Growth arrest and DNA damage 45 gamma	47	62	67	57	0.0979	0.434
RAD23	UV excision repair protein RAD23	0	57	73	40	1.48E-14	0.00860
MGMT	Methylguanine DNA methyltransferase	0	0	0	0	0	0
hMLH1	Homo sapiens mutL homolog 1	0	0	0	0	0	0
BRCA1	Breast cancer 1 early onset	0	0	0	0	0	0
Angiogenesis inhibitors							
EGLN2	Prolyl-hydroxylase-1	0	57	93	20	1.48E-14	1.43E-11
TSP1	Thrombospondin1	17	45	57	33	0.000655	0.0713

Supplementary Table 1. (continuation from page 2)

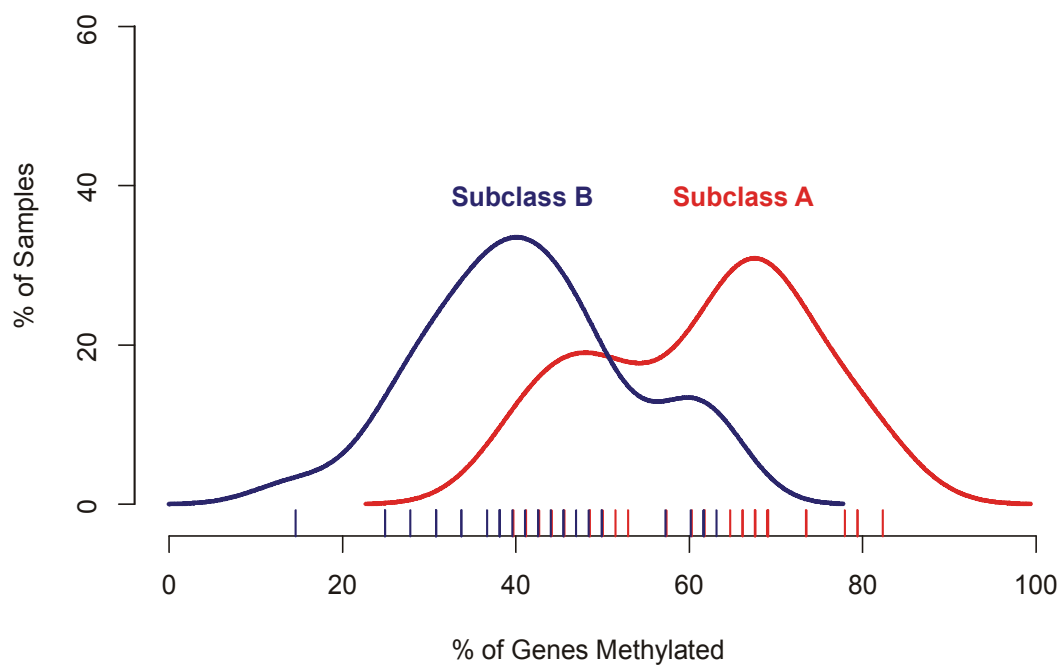
Gene	Description	Methylation frequency (%)		Methylation frequency (%)		P	P
		ST	HCC	A	B	(<i>t</i> test, ST vs HCC)	(<i>t</i> test, A vs B)
Angiogenesis inhibitors							
<i>TIMP-3</i>	Tissue inhibitor of metalloproteinase 1	7	23	27	20	0.0103	0.549
<i>TM</i>	Thrombomodulin	0	47	67	27	6.56E-11	0.00150
<i>TFPI2</i>	Tissue factor pathway inhibitor 2	0	22	37	7	9.56E-05	0.00423
<i>EDNRB</i>	Endothelin receptor type B	0	35	50	17	1.2E-07	0.00558
<i>VHL</i>	Von Hippel-Lindau protein	0	0	0	0	0	0
Phase II xenobiotic-metabolizing enzymes							
<i>NQO1</i>	NAD(P)H quinine oxidoreductase 1	0	35	20	50	1.2E-07	0.0143
<i>GSTP1</i>	Glutathione S-transferase P1	3	43	23	63	4.94E-08	0.00138
Other genes							
<i>CAV1</i>	Caveolin 1	0	67	93	40	1.78E-19	2.48E-06
<i>Bikunin</i>	Bikunin	78	90	90	90	0.0812	1
<i>CSK1</i>	Cyclin suppressing kinase	0	23	37	10	4.51E-05	0.0141
<i>PTPRO</i>	Protein tyrosine phosphatase receptor type O	92	82	83	77	0.108	0.526
<i>WT1</i>	Wilms tumor 1	0	0	0	0	0	0
<i>LKB1</i>	Serine/ threonine kinase 11	0	0	0	0	0	0
<i>PTEN</i>	Phosphatase and tensin-like chromosome 10	0	0	0	0	0	0

Supplementary Table 2. Summary of mean methylation density of 30 gene promoters determined by COBRA in surrounding non-neoplastic liver tissues (ST) and HCC

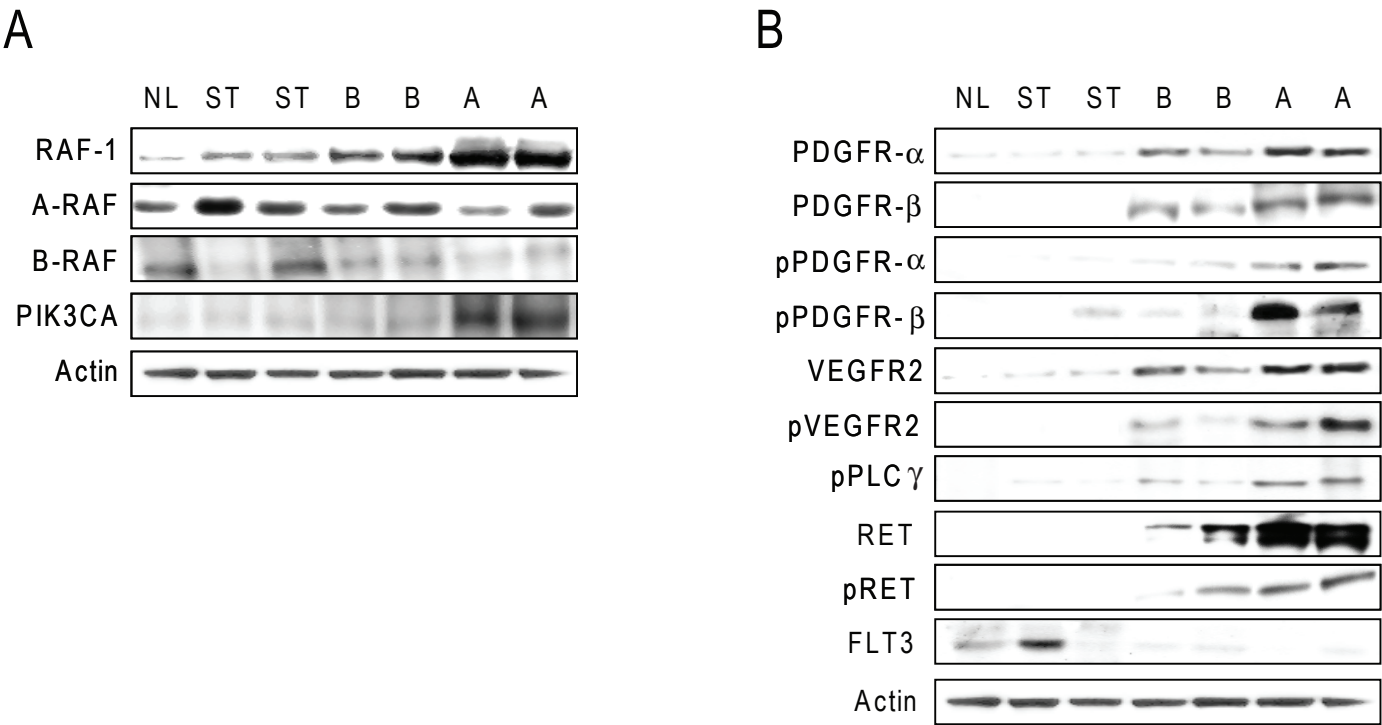
Gene	Description	Mean methylation density (%)		Mean methylation density (%)		P	P
		ST	HCC	A	B	(<i>t</i> test, ST vs HCC)	(<i>t</i> test, A vs B)
Inhibitors of the Jak/Stat pathway							
ARHI	A Ras Homologue Member 1	2.97	30.03	49.23	10.83	1.19E-14	2.33E-05
SOCS1	Suppressors of cytokine signaling 1	9.20	43.75	42.33	45.17	6.96E-16	0.933
SOCS2	Suppressors of cytokine signaling 2	25.92	55.10	51.00	59.20	2.97E-14	0.470
SYK	Non-receptor tyrosine kinase	2.63	13.50	12.83	14.17	0.004015	0.987
Inhibitors of the EGFR/FGFR/Ras pathway							
RASSF1A	Ras-effector homologue 1A	13.83	63.67	59.89	67.50	7.17E-30	0.530
RASSF2	Ras-effector homologue 2	3.33	26	10.17	40.00	1.25E-06	0.015
RASSF3	Ras-effector homologue 3	0	6.50	5.00	7.63	0.022475	0.2630
RASSF4	Ras-effector homologue 4	0	7.80	6.00	9.60	0.022515	0.243
LOX	Lysyl oxidase	6.92	44.75	14.00	75.50	4.42E-10	8.63E-05
HRASLS	H-Ras-like suppressor	21.98	73.52	73.37	73.67	1.78E-31	0.689
hDAB2IP	DAB2 interactive protein	9.45	61.05	93.60	28.50	4.20E-19	3.07E-05
CNK1	Connector enhancer of KSR 1	14.83	61.93	84.53	38.33	2.40E-26	4.97E-05
RRP22	Ras-related on chromosome 22	0	44.25	77.00	10.00	8.68E-17	1.33E-05
CLU	Clusterin	0	39.58	72.50	6.67	5.40E-14	1.38E-05
Cell cycle regulators and mitotic checkpoints							
p15	p15 INK4B	2.75	39.13	69.87	8.40	2.59E-11	2.13-E16
p16	p16 INK4A	6.42	59.42	86.00	32.83	2.27E-19	5.81E-12
MAD2	Mitotic arrest deficient 2	4.58	51.55	20.00	83.77	3.96E-16	2.59E-06
Histone methyltransferases							
PRDM2	PR domain containing 2 with ZNF domain	4.50	50.23	75.97	24.50	2.15E-15	1.25E-09
PRDM5	PR domain containing 5	1.25	28.00	41.33	14.67	6.59E-06	1.76E-13
Retinoid receptor							
RAR-β	Retinoic acid receptor-beta2	31.33	61.38	76.37	46.40	4.87E-20	2.97E-10
Apoptosis inducers							
DAPK	Death-associated protein kinase	12.62	53.07	69.30	36.83	1.50E-19	5.84E-08
CASP8	Caspase-8	0	23.38	40.10	6.67	2.15E-08	3.43E-06
ASC	Apoptotic speck protein	0	49.48	68.17	30.80	1.22E-22	4.15E-07
ASCL	Apoptotic speck protein-like	0	22.65	36.63	10.00	4.63E-09	8.38E-05
BNIP3	BCL2/adenovirus E1B 19kDa interacting protein	0	25.85	44.70	7.00	1.68E-09	1.16E-07
BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like	0	55.33	79.83	30.83	4.42E-28	6.45E-17
IGFBP3	Insulin-like growth factor binding protein 3	1.75	46.20	60.57	26.67	3.13E-16	2.62E-05
DNA repair							
GADD45β	Growth arrest and DNA damage 45 beta	23.53	60.62	75.90	45.33	2.48E-17	4.59E-07
GADD45γ	Growth arrest and DNA damage 45 gamma	10.58	51.80	43.33	60.27	7.47E-19	0.01695
Other genes							
CAV1	Caveolin 1	0	50.50	27.33	73.67	4.19E-26	1.76E-15



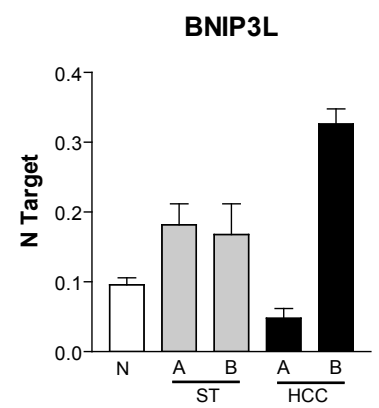
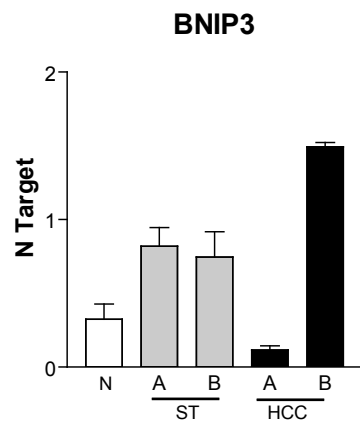
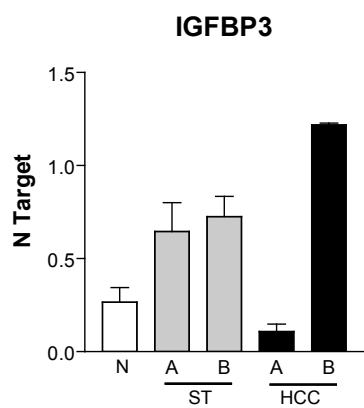
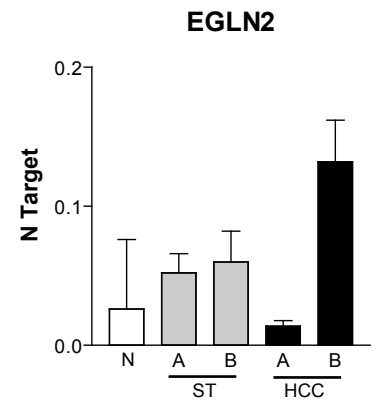
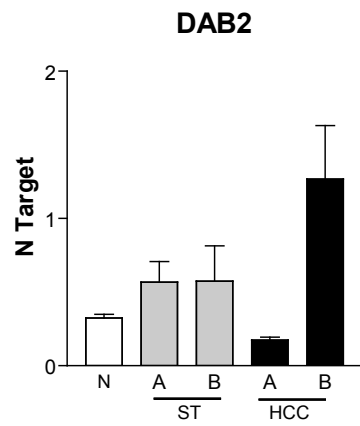
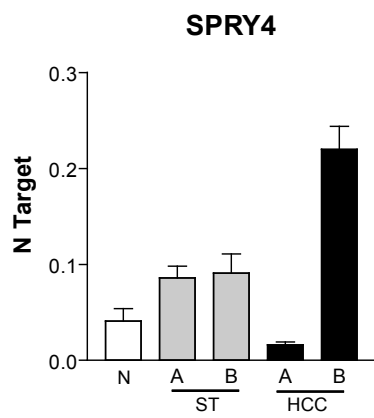
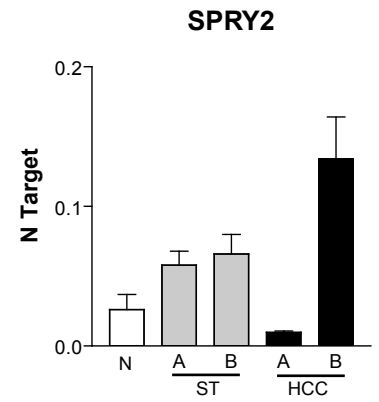
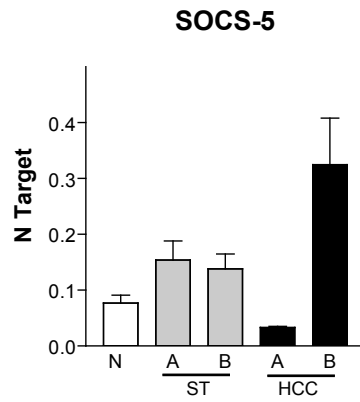
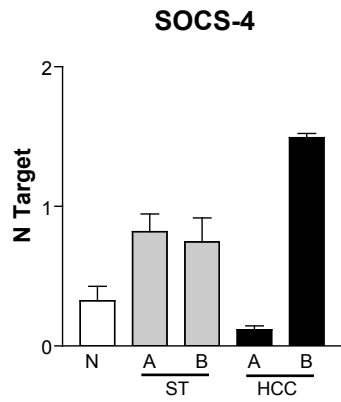
Suppl Figure 1



Suppl. Figure 2



Suppl. Figure 3



Suppl Figure 4

Supplementary Figure 1. Real-time RT-PCR analysis of genes with a similar degree of methylation in the two classes of HCC (*HRASLS*, *RASSF1A*, *SOCS2*), highest degree of promoter methylation in subclass B HCC (*LOX*, *MAD2*), and highest degree of promoter methylation in subclass A HCC (*ARHI*, *CASP8*, *DAPK*) based on COBRA. Note that mRNA levels are inversely correlated with degree of promoter methylation. Data are expressed as the mean $\pm$ SD of N target (NT). $NT = 2^{-\Delta Ct}$, where ΔCt value of the sample was calculated by subtracting the average Ct value of the target gene from the average Ct value of the *18S* gene.

Supplementary Figure 2. Distribution of genes methylated per case (expressed as a percentage) in subclass A (red line) and subclass B (blue line) HCC.

Supplementary Figure 3. Representative western blot analysis of RAF kinases, PIK3CA (A), and upstream inducers of Ras (B) in normal livers (NL), non-neoplastic surrounding tissues (ST) and HCC. (A) Highest levels of RAF-1 and PIK3CA were detected in subclass A HCC, with no differences being detected for A-RAF and B-RAF levels in the collection of samples examined. (B) Progressive rise in total and phosphorylated (activated) levels of PDGFR- α , PDGFR- β , VEGFR2, and RET from surrounding liver tissues to subclass A HCC. Activated phospholipase C (PLC) γ , a downstream effector of VEGFR2, was also highest in tumors with poor prognosis. In contrast, FLT3 expression was heterogeneous and did not correlate to tumor development or prognosis.

Supplementary Figure 4. Expression levels of *Ras* inhibitors (*SOCS-4*, *SOCS-5*, *SPRY2*, *SPRY4*, *DAB2*, *EGLN2*, *IGFBP3*, *BNIP3* and *BNIP3L*) in normal livers (NL), non-neoplastic surrounding tissues (ST), and HCC detected by real-time RT-PCR analysis. Levels of mRNA for each gene directly correlate with protein levels and inversely with promoter methylation data. Data are expressed as the mean $\pm$ SD of N target (NT). $NT = 2^{-\Delta Ct}$, where ΔCt value of the sample was calculated by subtracting the average Ct value of the target gene from the average Ct value of the *18S* gene.