Content of Supplemental Information

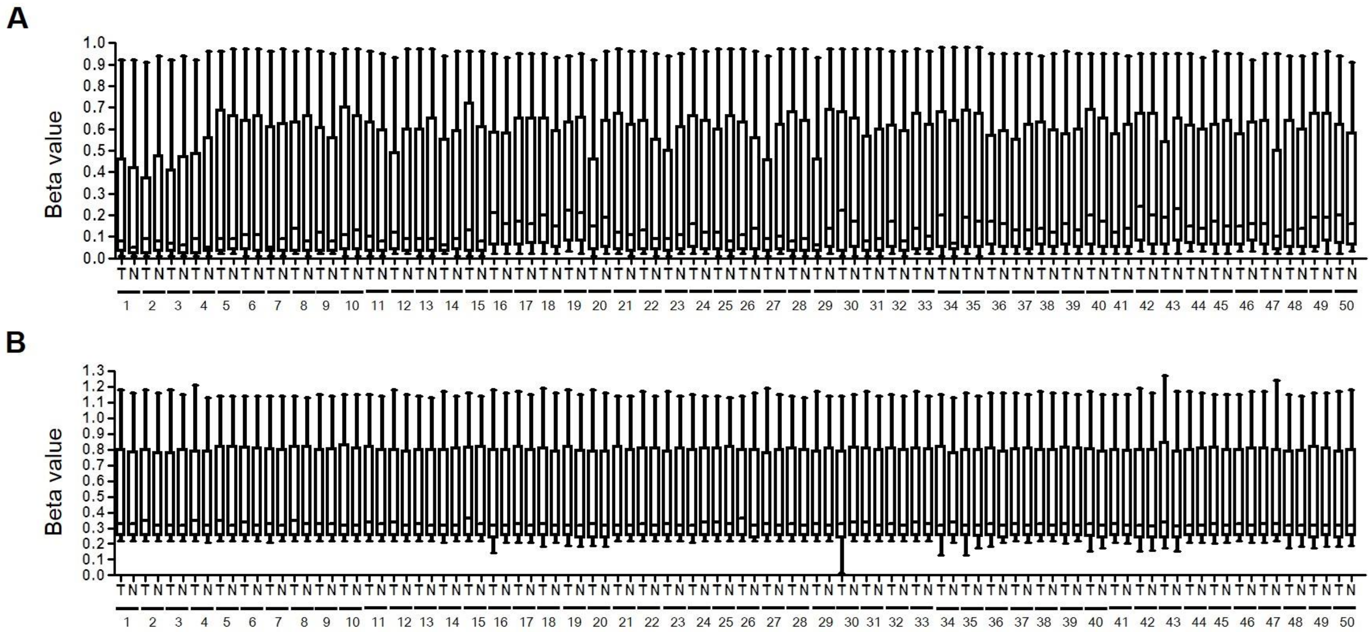

Figure S1. Distribution of beta values of DNA methylation array of the screening cohort (50 patients).

A) Un-normalized beta values. B) Normalized beta values using LOESS method. T: tumor tissue; N: normal tissue.

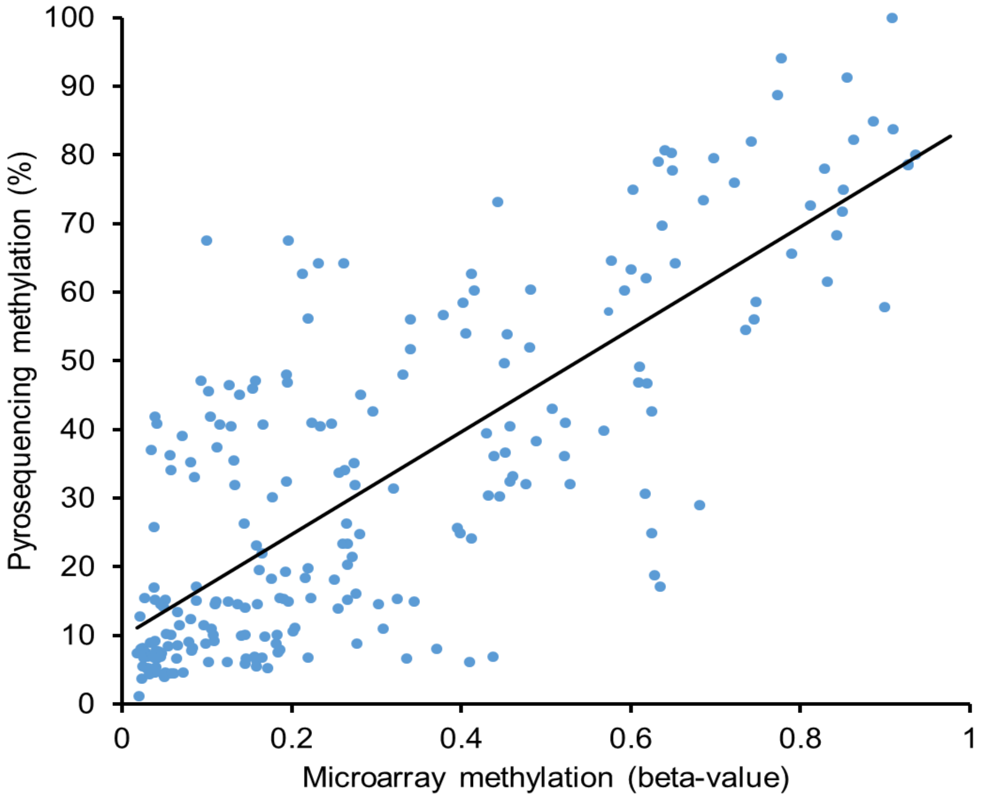

Figure S2. Pyrosequencing validation of methylated CpG sites of probes identified by array analyses. Scatterplot analysis showed a high concordance in methylation level between Illumina genome-wide methylation assay (X-axis: Beta value) and pyrosequencing validation (Y-axis). The data was collected on 20 CpG sites in 30 patients and was fitted by a linear regression line with a Pearson Correlation Coefficient 0.78.

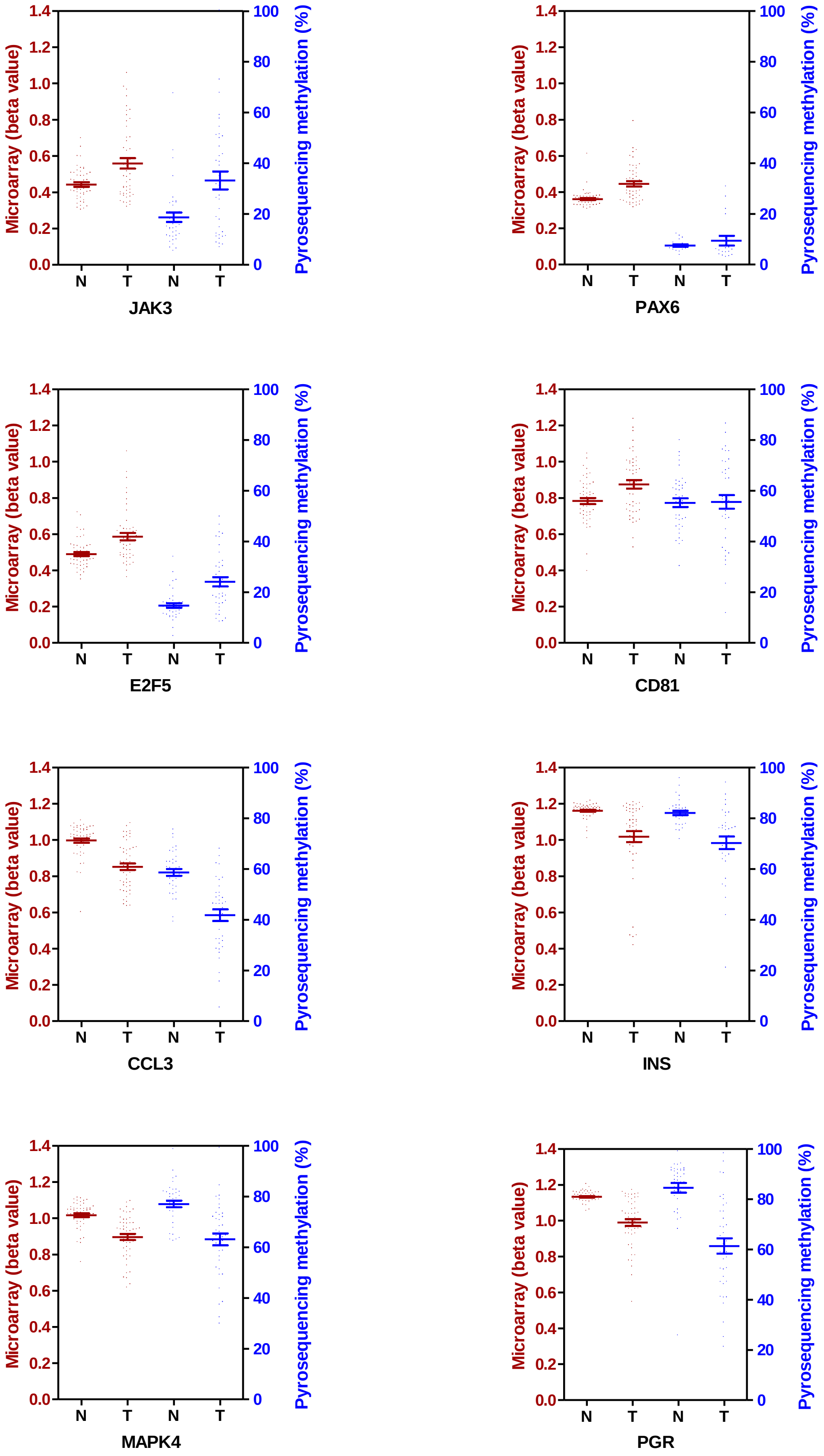

Figure S3. Distribution of beta and pyrosequencing values of 8 validated In-CpG sites in two independent cohorts.

The red color and blue color represent the array-derived beta values in screening cohort and the pyrosequencing methylation percentage in validation cohort respectively. N: normal tissue; T: tumor tissue. Error bars indicate standard error of the means.

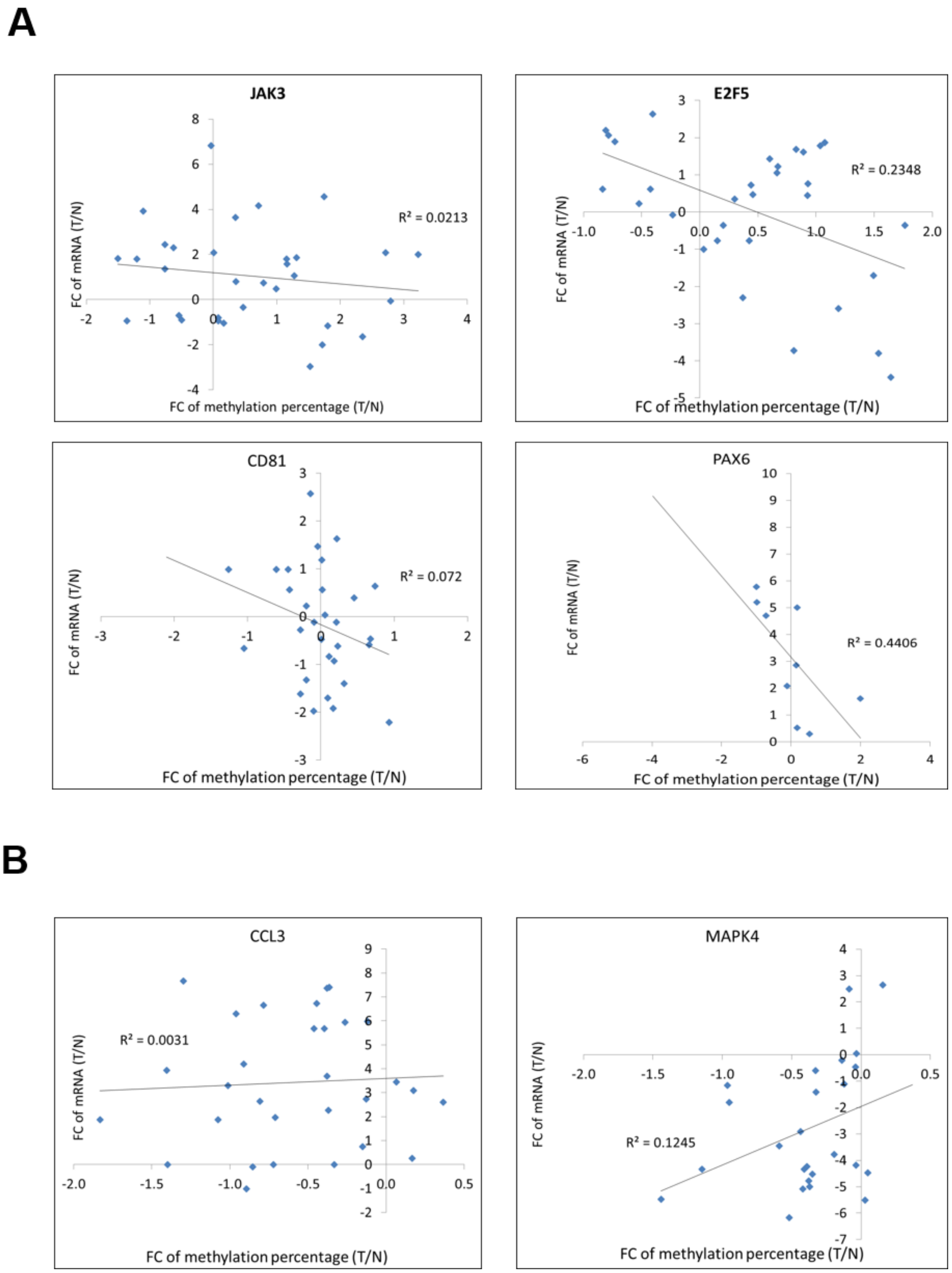

Figure S4. Correlation between pyrosequencing validation values and mRNA expressions in validation cohort of ESCC patients.

A) Increased methylation CpG sites of genes revealed from $\mathrm{GRN}_{\mathrm{escc}}$. B) Decreased methylation CpG sites of genes revealed from $\mathrm{GRN}_{\mathrm{escc}}$. FC: fold change; T/N: tumor/normal.

Table S1. Clinicopathological characteristics of the patients included in this study.

		DNA methylation microarray analysis (N = 50)		Pyrosequencing validation (N = 50)	
		N	%	N	%
Age	<60	31	62	32	64
	>60	19	38	18	36
Sex	Male	47	94	49	98
	Female	3	6	1	2
UICC tumor stage	I	4	8	3	6
	II	15	30	13	26
	III	20	40	21	42
	IV	11	22	13	26
T status	T1	5	10	4	8
	T2	6	12	2	4
	T3	33	66	37	74
	T4	6	12	7	14
N status	N0	19	38	22	44
	N1	31	62	28	56
M status	M0	38	76	37	74
	M1	12	24	13	26
Recurrence	Yes	28	85	17	85
	No	5	15	3	15

N: number of patients; T status: tumor size; N status: lymph node metastasis; M status: distant organ metastasis.

Table S2. List of 186 genes with expression changes are related to ESCC metastasis and progression (curated from the literature).

#	Gene symbol	Gene ID	Expression change associated with progression	Reference
1	ACTN1	87	Up	(Michaylira, et al., 2010)
2	ALCAM	214	Up	(Verma, et al., 2005)
3	AMIGO2	347902	Up	(Michaylira, et al., 2010)
4	ARHGDIG	398	Up	(Li, et al., 2006)
5	ATR	545	Down	(Li, et al., 2006)
6	AURKA	6790	Up	(Michaylira, et al., 2010) (Shi, et al., 2010) (Wang, et al., 2009)
7	BGN	633	Up	(Wong, et al., 2009)
8	BID	637	Up	(Michaylira, et al., 2010)
9	BIRC5	332	Up	(Michaylira, et al., 2010)
10	BMP5	653	Down	(Li, et al., 2006)
11	BNIP3	664	Up	(Li, et al., 2006)
12	CARD10	29775	Up	(Michaylira, et al., 2010)
13	CASP3	836	Down	(Li, et al., 2006)
14	CAV1	857	Up	(Kato, et al., 2002)
15	CCNB1	891	Up	(Song, et al., 2008)
16	CCND1	595	Up	(Li, et al., 2006) (Nakajima, et al., 2002)
17	CCND2	894	Down	(Li, et al., 2006)
18	CCR7	1236	Up	(Ding, et al., 2003) (Ishida, et al., 2009)
19	CCT5	22948	Up	(Uchikado, et al., 2006)
20	CD44	960	Up	(Wong, et al., 2009)
21	CD58	965	Up	(Li, et al., 2006)
22	CD82	3732	Down	(Li, et al., 2006) (Miyazaki, et al., 2000) (Uchida, et al., 1999)
23	CD86	942	Up	(Li, et al., 2006)
24	CD9	3732	Down	(Uchida, et al., 1999)
25	CDC151	977	Up	(Suzuki, et al., 2011)
26	CDC25B	994	Down	(Li, et al., 2006)
27	CDH1	999	Down	(Li, et al., 2006) (Kaihara, et al., 2001) (Chen, et al., 2011) (Zhang, et al., 2012) (Lioni, et al., 2007) (Matsushima, et al., 2011)
28	CDH2	1000	Up	(Li, et al., 2010) (Li, et al., 2009) (Li, et al., 2009)
29	CDK5	1020	Down	(Li, et al., 2006)
30	CKS2	1164	Up	(Michaylira, et al., 2010) (Uchikado, et al., 2006)
31	CLDN5	7122	Up	(Chiba, et al., 2010)
32	CLDN7	1366	Down	(Usami, et al., 2006) (Lioni, et al., 2007)
33	COL12A1	1303	Up	(Michaylira, et al., 2010)

34	COL16A1	1307	Up	(Michaylira, et al., 2010)
35	COL1A1	1277	Up	(Wong, et al., 2009)
36	COL1A2	1278	Up	(Wong, et al., 2009)
37	COL27A1	85301	Up	(Michaylira, et al., 2010)
38	COL4A2	1284	Up	(Michaylira, et al., 2010)
39	COL5A1	1289	Up	(Michaylira, et al., 2010)
40	COL5A3	50509	Up	(Michaylira, et al., 2010)
41	COL8A1	1295	Up	(Michaylira, et al., 2010)
42	CSTA	1475	Down	(Li, et al., 2005)
43	CTGF	1490	Down	(Li, et al., 2006)
44	CTNNA1	1495	Down	(Li, et al., 2006)
45	CTNNB1	1499	Up	(Hou, et al., 2011)
46	CTSB	1508	Up	(Jiao, et al., 2007)
47	CTTN	2017	Up	(Luo, et al., 2006) (Luo and Wang, 2007)
48	CYR61	3491	Up	(Xie, et al., 2011)
49	DACT1	51339	Up	(Hou, et al., 2011)
50	DCBLD1	285761	Up	(Michaylira, et al., 2010)
51	DCBLD2	131566	Up	(Michaylira, et al., 2010)
52	DEC1	50514	Down	(Wong, et al., 2011)
53	DFNA5	1687	Up	(Michaylira, et al., 2010)
54	DRD2	1813	Up	(Li, et al., 2006)
55	DSP	1832	Down	(Li, et al., 2006)
56	EDN1	1906	Up	(Jiao, et al., 2007)
57	EGFR	1956	Up	(Li, et al., 2006)
58	ENG	2022	Down	(Wong, et al., 2008)
59	EP300	2033	Down	(Zhang, et al., 2007)
60	EPHA2	1969	Up	(Li, et al., 2006)
61	EPHB6	2051	Down	(Li, et al., 2006)
62	ETS1	2113	Up	(Mukherjee, et al., 2003)
63	FERMT2	10979	Up	(Michaylira, et al., 2010)
64	FEZ1	9638	Up	(Michaylira, et al., 2010)
65	FHL1	2273	Up	(Michaylira, et al., 2010)
66	FHOD3	80206	Up	(Michaylira, et al., 2010)
67	FLNA	2316	Up	(Michaylira, et al., 2010)
68	FLRT2	23768	Up	(Michaylira, et al., 2010)
69	FN1	2335	Up	(Michaylira, et al., 2010) (Wong, et al., 2009)
70	FXYD5	53827	Up	(Michaylira, et al., 2010)
71	GJB2	2706	Down	(Uchikado, et al., 2006)
72	GLI1	2735	Up	(Mori, et al., 2006)

73	HGF	3082	Up	(Grugan, et al., 2010)
74	HIF1A	3091	Up	(Kurokawa, et al., 2003)
75	HLA-DRA	3122	Up	(Sadanaga, et al., 1994)
76	HLA-DRB1	3123	Up	(Sadanaga, et al., 1994)
77	HLA-DRB3	3125	Up	(Sadanaga, et al., 1994)
78	HLA-DRB4	3126	Up	(Sadanaga, et al., 1994)
79	HLA-DRB5	3127	Up	(Sadanaga, et al., 1994)
80	HNT	50863	Up	(Michaylira, et al., 2010)
81	HSPA4	3308	Down	(Li, et al., 2006)
82	HTRA1	5654	Up	(Michaylira, et al., 2010)
83	ID1	3397	Up	(Yuen, et al., 2007)
84	ID2	3398	Down	(Yuen, et al., 2007)
85	IGF1R	3480	Up	(Kong, et al., 2012)
86	IGFBP3	3486	Up	(Li, et al., 2006)
87	IGFBP6	3489	Down	(Li, et al., 2006)
88	IGFBP7	3490	Up	(Michaylira, et al., 2010)
89	IL8	3576	Up	(Ren, et al., 2005)
90	INHBA	3624	Up	(Michaylira, et al., 2010)
91	ITGA3	3675	Up	(Michaylira, et al., 2010)
92	ITGA5	3678	Up	(Michaylira, et al., 2010)
93	ITGAM	3684	Up	(Li, et al., 2006)
94	ITGB5	3693	Up	(Li, et al., 2006)
95	IVL	3713	Down	(Oda and Ono, 2004)
96	JUN	3725	Up	(Li, et al., 2006)
97	KISS1	3814	Down	(Li, et al., 2009)
98	LAMB1	3912	Up	(Michaylira, et al., 2010)
99	LAMB3	3914	Up	(Michaylira, et al., 2010)
100	LAMC2	3918	Up	(Michaylira, et al., 2010) (Shen, et al., 2007) (Yamamoto, et al., 2001)
101	LGALS3	3958	Down	(Shibata, et al., 2005)
102	LOX	4015	Up	(Sakai, et al., 2009)
103	LOXL2	4017	Up	(Michaylira, et al., 2010)
104	LPXN	9404	Up	(Michaylira, et al., 2010)
105	LTBP2	4053	Up	(Michaylira, et al., 2010)
106	LYPD3	27076	Up	(Hansen, et al., 2008)
107	MAP1B	4131	Up	(Michaylira, et al., 2010)
108	MAPK14	1432	Down	(Li, et al., 2006)
109	MAPK4	5596	Down	(Li, et al., 2006)
110	MAPK7	5598	Down	(Li, et al., 2006)

111	MCAM	4162	Up	(Michaylira, et al., 2010)
112	MDM2	4193	Down	(Li, et al., 2006)
113	MIF	4282	Up	(Ren, et al., 2005)
114	MKI67	4288	Up	(Nakajima, et al., 2002) (Kawamura, et al., 1996)
115	MMP1	4312	Up	(Shen, et al., 2007) (Chen, et al., 2010)
116	MMP13	4322	Up	(Li, et al., 2006) (Ye, et al., 2011)
117	MMP2	4313	Up	(Li, et al., 2006) (Zhang, et al., 2012)
118	MMP9	4318	Up	(Wong, et al., 2009)
119	MRC2	9902	Up	(Michaylira, et al., 2010)
120	MTA1	9112	Up	(Qian, et al., 2005)
121	MTSS1	9788	Up	(Xie, et al., 2011)
122	MUC1	4582	Up	(Ye, et al., 2011)
123	NDUFB9	4715	Up	(Uchikado, et al., 2006)
124	NEFL	4747	Up	(Michaylira, et al., 2010)
125	NME1	4830	Down	(Tomita, et al., 2001)
126	PALM2-AKAP2	445815	Up	(Michaylira, et al., 2010)
127	PCDH7	5099	Up	(Michaylira, et al., 2010)
128	PDCD10	11235	Down	(Li, et al., 2006)
129	PDGFRA	5156	Up	(Michaylira, et al., 2010)
130	PDPN	10630	Up	(Michaylira, et al., 2010) (Chuang, et al., 2009)
131	PIK3CA	5290	Up	(Akagi, et al., 2009)
132	PLEK2	26499	Up	(Michaylira, et al., 2010)
133	PLOD1	5351	Up	(Michaylira, et al., 2010)
134	POR	5447	Up	(Li, et al., 2006)
135	POSTN	10631	Up	(Michaylira, et al., 2010)
136	PPM1D	8493	Down	(Li, et al., 2006)
137	PPP1R1B	84152	Down	(Li, et al., 2006)
138	PRKCI	5584	Up	(Liu, et al., 2011) (Yang, et al., 2008)
139	PTHLH	5744	Up	(Michaylira, et al., 2010)
140	PTK2	5747	Up	(Miyazaki, et al., 2003)
141	PTP4A3	11156	Up	(Ooki, et al., 2010)
142	PTPN2	5771	Down	(Li, et al., 2006)
143	PTPRF	5792	Down	(Li, et al., 2006)
144	PTTG1	9232	Up	(Ito, et al., 2008) (Yan, et al., 2009)
145	PXN	5829	Up	(Wong, et al., 2009)
146	RAC3	5881	Up	(Li, et al., 2006)
147	RXRA	6256	Down	(Li, et al., 2006)
148	S100A4	6275	Up	(Zhang, et al., 2012) (Zhang, et al., 2010)
149	SDK2	54549	Up	(Michaylira, et al., 2010)

150	SEMA4D	10507	Up	(Li, et al., 2006)
151	SERPINB3	6317	Up	(Nagatani, et al., 2003)
152	SGCB	6443	Up	(Michaylira, et al., 2010)
153	SKP2	6502	Down	(Liu, et al., 2011) (Wang, et al., 2009)
154	SLIT2	9353	Down	(Kim, et al., 2008)
155	SNAI2	6591	Up	(Michaylira, et al., 2010) (Tang, et al., 2011)
156	SNCA	6622	Up	(Michaylira, et al., 2010)
157	SPARC	6678	Up	(Michaylira, et al., 2010) (Wong, et al., 2009)
158	SPHK1	8877	Up	(Michaylira, et al., 2010)
159	SPON2	10417	Up	(Michaylira, et al., 2010)
160	SPP1	6696	Up	(Uchikado, et al., 2006)
161	SRC	6714	Up	(Chen, et al., 2010)
162	SRPX	8406	Up	(Michaylira, et al., 2010)
163	STAT5B	6777	Down	(Li, et al., 2006)
164	STC2	8614	Up	(Kita, et al., 2011)
165	STMN1	3925	Up	(Uchikado, et al., 2006)
166	STOML2	30968	Up	(Cao, et al., 2010)
167	SYNPO	11346	Up	(Michaylira, et al., 2010)
168	TGFB1	7040	Up	(Wong, et al., 2009)
169	TGFB1I1	7041	Up	(Michaylira, et al., 2010)
170	TGM2	7052	Up	(Michaylira, et al., 2010)
171	THBD	7056	Down	(Matsushita, et al., 1998) (Tezuka, et al., 1995)
172	THBS1	7057	Up	(Michaylira, et al., 2010) (Zhou, et al., 2009)
173	THY1	7070	Up	(Michaylira, et al., 2010)
174	TIMP2	7077	Down	(Chen, et al., 2010)
175	TNC	3371	Up	(Michaylira, et al., 2010)
176	TNFRSF12A	51330	Up	(Michaylira, et al., 2010)
177	TNFRSF6B	8771	Up	(Xiong, et al., 2011)
178	TNS1	7145	Up	(Michaylira, et al., 2010)
179	TP53	7157	Up	(Nakajima, et al., 2002) (Han, et al., 2007)
180	TWIST1	7291	Up	(Yuen, et al., 2007) (Xie, et al., 2009)
181	TWIST2	117581	Up	(Michaylira, et al., 2010)
182	VEGFA	7422	Up	(Mukherjee, et al., 2003) (Ren, et al., 2005)
183	VEGFC	7424	Up	(Li, et al., 2006) (Han, et al., 2007) (Krzystek-Korpacka, et al., 2007)
184	VIM	7431	Up	(Chen, et al., 2010) (Jin, et al., 2010)
185	VWF	7450	Up	(Wong, et al., 2009)
186	ZEB2	9839	Up	(Matsushima, et al., 2011)

#: serial number; Up: expressed genes in ESCC progression; Down: repressed genes in ESCC progression.

Table S3. List of primers for pyrosequencing validation genes used in this study.

Gene symbol	Primer type	Sequence (from 5'-end to 3'-end)	Product size (bp)
ADCYAP1	Forward	TGGTTTTTGAGGTTTTTGTTTAGATAT	315
	Biotinylated reverse	GGGACACCGCTGATCGTTTACCCTACCACTTCTTACTTACTATAAT	
	Sequencing	GTTTTTAGGGTGGTGATTTTAG	
KCNK4	Forward	AATGGGTTTGGGAGATGTTAGATTAG	139
	Biotinylated reverse	GGGACACCGCTGATCGTTTAAAAAAAATTACAACAAAAACCAACCTTCTA	
	Sequencing	TAGAGTGGTGTTTGTT	
SLC5A8	Forward	GGGGATATTTGAGTAGATGAGAATTG	153
	Biotinylated reverse	GGGACACCGCTGATCGTTTAACCCTTTATCCCACATTCC	
	Sequencing	GGTAGGTTTTGTTTAAGTG	
CD81	Forward	TTTTAAGGGGGTGGTAGTAG	154
	Biotinylated reverse	GGGACACCGCTGATCGTTTACCAAAATACTCACCTCCTTT	
	Sequencing	GGGGTGGTAGTAGGG	
CFTR	Biotin-Forward	GGGACACCGCTGATCGTTTAGGGTGAGTAGTGGGTGGAGAAA	111
	Reverse	ACCCCTTCCTTTTACTCTTT	
	Sequencing	CCTAATCCCACCCCAAATTT	
E2F5	Biotin-Forward	GGGACACCGCTGATCGTTTAAGGGAGGAAGAAAAAAAGGAGATA	226
	Reverse	ACTCTACACTTAATCCAAATCAT	
	Sequencing	AACAATCAACCAACTTC	
HS3ST2	Forward	GTGGTGGGGGTGTTTAGT	107
	Biotinylated reverse	GGGACACCGCTGATCGTTTACCTCCCCATACAAACCACT	
	Sequencing	AAGTTGGTTTGGGGT	
JAK3	Forward	TTAGGAAATTAAGGGGTTTATATAGGAAGG	210
	Biotinylated reverse	GGGACACCGCTGATCGTTTAACTAACCTTTACCCTAACAACAC	
	Sequencing	GGTTTTTGTTAGTTAGGTAAGA	
PENK	Forward	GGGGTAGAGTAATAGTAGTTAAGTG	184
	Biotinylated reverse	GGGACACCGCTGATCGTTTAACCCCAAAAAAAAACATTCCT	
	Sequencing	TGAGAGGAGAGAGGGA	
SEZ6L	Forward	AGGTTGGGTAGGTAATGGA	188
	Biotinylated reverse	GGGACACCGCTGATCGTTTAACAAAATTTAACCCCAATTCCCCTTAC	
	Sequencing	GTTAGGTAGAGTTGTTGG	
ZIM2	Forward	TGAGGTTGTTGATTGGTTAGTATAG	148
	Biotinylated reverse	GGGACACCGCTGATCGTTTACACCAACCCAAAATAAACATCTC	
	Sequencing	GAGAAGTTTTGATAAGG	
PI3	Forward	GTTATTGAGTTGAGGTGGAGTT	174
	Biotinylated reverse	GGGACACCGCTGATCGTTTAAATAACCAAAAACTTCCACAATC	
	Sequencing	ATTAAGTTTGAAATTGAGGG	
PGR	Forward	GGTAAAGAGAATTTTGGGAAGTAGG	124

	Biotinylated reverse	GGGACACCGCTGATCGTTTAATACTTCTATACCCACACTTACTC	
	Sequencing	GGAAGTAGGTATAGAATGTTTA	
INS	Biotinylated forward	GGGACACCGCTGATCGTTTATTTTTAGTTTAGGATGGTTTAAGATGT	182
	Reverse	CCCAAATCATACCCTCCTTCT	
	Sequencing	ACCTTAACCCATCCAT	
SFTPA1	Forward	GGAGGGGTAGTGAGAGAAT	179
	Biotinylated reverse	GGGACACCGCTGATCGTTTACACTCACTAACTCACACCATCTATC	
	Sequencing	TATTTTGAGGGGGGT	
CCL3	Forward	ATGGTTAGAGAGTGGTGATATT	174
	Biotinylated reverse	GGGACACCGCTGATCGTTTACAATCCTTTCTTAACTCTACTAACAC	
	Sequencing	GTAGGTGAAGGAATGTGG	
CSF3R	Forward	AGGAAGGTTGTTAGGGTTTTATTAGT	240
	Biotinylated reverse	GGGACACCGCTGATCGTTTATACTTAACCTCCTTAATCTCTCTTCT	
	Sequencing	TTGTTAGAGGTTGAGTTAT	
TRPM5	Forward	AGGGAGTTGAGGGGTTAGTG	109
	Biotinylated reverse	GGGACACCGCTGATCGTTTACTCAACCCTCATAAAACTCATATCT	
	Sequencing	AGAGGGGTTGAGATG	
MAPK4	Forward	AGAAGGGTTTAGGAAATATAGGAAT	141
	Biotinylated reverse	GGGACACCGCTGATCGTTTAAAATCACTACCACCAACTCTCT	
	Sequencing	AGGAAATATAGGAATTAGTAG	
PAX6	Forward	TGTATTAGGAGGGGAGAGGGAGTATTTA	206
	Biotinylated reverse	GGGACACCGCTGATCGTTTATATCATCATCCTCCAACAAAACACT	
	Sequencing	GGAGAGGGAGTATTTAAT	

Table S4. List of primers used in RT-PCR

Gene symbol	Primer type	Sequence or TaqMan® Assay ID	Product size (bp)
CD81	Forward	GCCAAGGATGTGAAGCAGTT	208
	Reverse	CCTCCTTGAAGAGGTTGCTG	
E2F5	Forward	CTGGAGGTACCCATTCCAGA	168
	Reverse	GGAAGGCTGTGTGAGGTCAT	
JAK3	Forward	AGCCGCCTCCTTCTCTATTC	212
	Reverse	TACCAGAAAATGGGGCTCTG	
CCL3	TaqMan® probes	Hs00234142_m1	53
INS	TaqMan® probes	Hs02741908_m1	139
MAPK4	TaqMan® probes	Hs00177074_m1	72
PAX6	TaqMan® probes	Hs00240871_m1	76
PGR	TaqMan® probes	Hs01556702_m1	77

Table S5. List of 44 top-ranked probes containing DNA methylated CpG sites.

Rank	Probe ID	Gene ID	Gene symbol	GRN$_{escc}$	GRN$_{g\text{-}escc}$	TSS	Gene description	Methylation level (Tumor/Normal)	GOs
1	5725	9353	SLIT2[†]	Yes	No	+111	Slit homolog 2	I	GO:0045595~regulation of cell differentiation GO:0001525~angiogenesis GO:0016477~cell migration GO:0006935~chemotaxis
2	5615	3718	JAK3*	Yes	No	+64	Janus kinase 3	I	GO:0007166~cell surface receptor linked signal transduction
3	3926	6581	SLC22A3	No	Yes	+122	Solute carrier family 22 (extraneuronal monoamine transporter), member 3	D	
4	6139	1029	CDKN2A	Yes	No	Null	Cyclin-dependent kinase inhibitor 2A	I	GO:0045595~regulation of cell differentiation GO:0001558~regulation of cell growth GO:0042981~regulation of apoptosis
5	3913	6435	SFTPA1*	No	No	+340	Surfactant, pulmonary-associated protein A1	D	
6	2192	2273	FHL1[†]	Yes	No	-768	Four and a half LIM domains 1	I	
7	4002	2242	FES	Yes	No	-223	V-FES feline sarcoma viral/V-FPS fujinami avian sarcoma viral oncogene homolog	I	GO:0008283~cell proliferation
8	2003	160728	SLC5A8*	No	No	-38	Solute carrier family 5 (iodide transporter), member 8	I	
9	1072	3202	HOXA5	Yes	No	-1,324	Homeobox A5	I	
10	3752	5179	PENK*	No	Yes	+26	Proenkephalin	I	
11	1661	5080	PAX6*	Yes	No	-1,121	Paired box gene 6 isoform b	I	GO:0045595~regulation of cell differentiation GO:0016477~cell migration
12	2136	8577	TMEFF1	No	Yes	-626	Transmembrane protein with EGF-like and two follistatin-like domains 1	I	
13	1685	5266	PI3*	No	Yes	-1,394	Elafin preproprotein	D	
14	4232	3791	KDR	Yes	No	-445	Kinase insert domain receptor (a type III receptor tyrosine kinase)	I	GO:0001525~angiogenesis GO:0016477~cell migration
15	2811	5241	PGR*	Yes	No	-456	Progesterone receptor	D	
16	5968	7070	THY1[†]	Yes	No	-20	Thy-1 cell surface antigen	I	GO:0045595~regulation of cell differentiation GO:0001525~angiogenesis GO:0007155~cell adhesion
17	2868	6348	CCL3*	Yes	No	+53	Chemokine (C-C motif) ligand 3	D	GO:0006935~chemotaxis
18	2721	3569	IL6	Yes	No	-611	Interleukin 6 (interferon, beta 2)	D	GO:0045595~regulation of cell differentiation GO:0016477~cell migration GO:0006935~chemotaxis GO:0042981~regulation of apoptosis
19	1203	3630	INS*	Yes	No	-804	Proinsulin precursor	D	GO:0045595~regulation of cell differentiation GO:0001558~regulation of cell growth GO:0042981~regulation of apoptosis
20	247	1080	CFTR*	Yes	No	-115	Cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	I	
21	1087	9956	HS3ST2*	No	No	-546	Heparan sulfate D-glucosaminyl 3-O-sulfotransferase 2	I	
22	3534	50801	KCNK4*	No	No	+3	Potassium channel, subfamily K,	I	

							member 4 isoform 1		
23	5893	3651	IPF1	Yes	No	-750	Insulin promoter factor 1, homeodomain transcription factor	I	
24	3981	8794	TNFRSF10C	No	Yes	+109	Tumor necrosis factor receptor superfamily, member 10c precursor	I	
25	631	3205	HOXA9	No	Yes	+252	Homeobox protein A9 isoform b	I	
26	6131	23671	TMEFF2	No	Yes	+442	Transmembrane protein with EGF-like and two follistatin-like domains 2	I	
27	5078	4811	NID1	Yes	No	-714	Nidogen (enactin)	D	GO:0007155~cell adhesion
28	5943	64321	SOX17	No	Yes	-303	SRY-box 17	I	GO:0001525~angiogenesis
29	2153	29850	TRPM5*	No	No	-721	Transient receptor potential cation channel, subfamily M, member 5	D	
30	1088	9956	HS3ST2	No	Yes	-171	Heparan sulfate D-glucosaminyl 3-O-sulfotransferase 2	I	
31	5660	5596	MAPK4*†	Yes	No	273	Mitogen-activated protein kinase 4	D	
32	758	2070	EYA4	No	Yes	-508	Eyes absent 4 isoform a	I	
33	5602	1875	E2F5*	Yes	No	-516	E2F transcription factor 5	I	
34	1790	63968	PWCR1	No	No	-811	Prader-Willi syndrome chromosome region 1	D	
35	5345	7077	TIMP2†	Yes	No	-267	Similar to Metalloproteinase inhibitor 2 precursor (TIMP-2) (Tissue inhibitor of metalloproteinases-2) (CSC-21K)	I	GO:0045595~regulation of cell differentiation
36	950	283120	H19	No	No	-1,411	H19, imprinted maternally expressed transcript (non-protein coding)	D	
37	220	975	CD81*	Yes	No	-272	CD81 antigen	I	GO:0008283~cell proliferation GO:0044409~entry into host GO:0030260~entry into host cell
38	3463	1441	CSF3R*	Yes	No	-8	Colony stimulating factor 3 receptor isoform d precursor	D	GO:0007155~cell adhesion
39	1262	50801	KCNK4*	No	No	-171	Potassium channel, subfamily K, member 4 isoform 1	I	
40	1931	23544	SEZ6L*	No	No	-299	Seizure related 6 homolog (mouse)-like precursor	I	
41	961	338433	HBII-52	No	No	-563	Small nucleolar RNA, C/D box 115-1	D	
42	4118	3569	IL6	Yes	No	+168	Interleukin 6 (interferon, beta 2)	D	GO:0045595~regulation of cell differentiation GO:0016477~cell migration GO:0006935~chemotaxis GO:0042981~regulation of apoptosis
43	2198	23619	ZIM2*	No	No	-22	Zinc finger, imprinted 2	I	
44	41	116	ADCYAP1*	No	Yes	-455	Adenylate cyclase activating polypeptide precursor	I	

Rank: ranked genes in ascending order based on logrank-test-derived p-values; TSS: methylated CpG sites relative to transcription start site; *: available for primer design for pyrosequencing validation; †: overlapped with literature-curated significantly expressed genes associated with ESCC progression; GRN_{escc}: ESCC progression-associated gene regulatory network; $\text{GRN}_{\text{g-escc}}$: non-ESCC progression-associated gene regulatory network; I: methylation level increased in tumor; D: methylation level decreased in tumor; Yes: in network; No: not in network; GOs: cancer progression-related Gene Ontology terms.

Table S6. Univariate and multivariate analysis of 20 validated CpG sites with clinico-pathological prognostic factors in the validation cohort.

Univariate		
	HR (95% CI)	*P*-value
Gene methylation (increased vs decreased methylation)		
In-CpGs		
JAK3	2.61 (0.93-7.30)	0.068
PAX6	4.33 (0.93-20.11)	0.062
CFTR	0.48 (0.16-1.46)	0.198
E2F5	3.08 (1.10-8.63)	0.032*
CD81	2.95 (1.17-7.41)	0.021*
CCL3	8.46 (1.12-63.74)	0.038*
CSF3R	24.53 (0.03-23196.12)	0.360
INS	0.32 (0.103-0.101)	0.052
MAPK4	0.06 (0.01-0.67)	0.022*
PGR	3.70 (1.30-10.46)	0.014*
Out-CpGs		
SLC5A8	3.16 (0.60-16.67)	0.176
PENK	0.48 (0.18-1.28)	0.143
HS3ST2	0.71 (0.26-1.97)	0.509
KCNK4	28.15 (0.06-13818.94)	0.291
SEZ6L	1.69 (0.68-4.15)	0.257
ZIM2	0.04 (0.00-74.11)	0.409
ADCYAP1	1.42 (0.66-3.08)	0.373
PI3	2.06 (0.59-7.11)	0.255
SFTPA1	21.34 (0.00-1031990.42)	0.578
TRPM5	1.81 (0.68-4.83)	0.239
TNM stage (III/IV vs I/II)	0.97 (0.35-2.74)	0.957
Local lymph node metastasis (yes vs no)	0.53 (0.21-1.35)	0.181
Distant metastasis (yes vs no)	2.81 (1.08-7.34)	0.035*
Age (>=55 vs <55)	0.40 (0.14-1.14)	0.086
Drinking status (yes vs no)	1.13 (0.26-4.90)	0.876
Multivariate^c		
JAK3	2.70 (0.96-7.59)	0.060
PAX6	3.90 (0.81-18.78)	0.089
E2F5	3.18 (1.13-8.92)	0.028*
CD81	3.52 (1.35-9.18)	0.010*
CCL3	7.67 (1.01-58.12)	0.049*
INS	0.24 (0.07-0.78)	0.018*
MAPK4	0.11 (0.01-1.29)	0.079
PGR	5.26 (1.73-16.04)	0.004*

HR: hazard ratio; CI: confidence interval; c: multivariate analysis in each gene was adjusted with distant metastasis; *: p-value < 0.05.

Table S7. Array-based examination of methylated In-CpG and Out-CpG sites.

CpGs	Distance from TSS	Gene	Fold change (p-value[1])	Association with survival p-value[2]	Survival correlation direction[3]
In-CpG	+64	JAK3	1.2 (<0.0004**)	<0.0001***	−
In-CpG	-1,121	PAX6	1.2 (<0.0001***)	0.017*	−
In-CpG	-115	CFTR	1.1 (0.047*)	0.0002**	−
In-CpG	-516	E2F5	1.1 (<0.0001***)	<0.0001***	−
In-CpG	-272	CD81	1.1 (0.001*)	<0.0001***	−
In-CpG	+53	CCL3	-1.1 (<0.0001***)	0.017*	+
In-CpG	-8	CSF3R	-1.2 (<0.0001***)	0.002*	+
In-CpG	-804	INS	-1.1 (<0.0001***)	0.023*	−
In-CpG	+273	MAPK4	-1.1 (<0.0001***)	0.047*	−
In-CpG	-456	PGR	-1.1 (<0.0001***)	0.011*	+
Out-CpG	-38	SLC5A8	1.1 (0.006*)	0.0005**	−
Out-CpG	+26	PENK	1.5 (<0.0001***)	0.004*	+
Out-CpG	-546	HS3ST2	1.1 (<0.0003**)	0.130	−
Out-CpG	+3	KCNK4	1.2 (<0.0001***)	0.038*	−
Out-CpG	-299	SEZ6L	1.1 (0.0009**)	0.113	−
Out-CpG	-22	ZIM2	1.1 (<0.0001***)	0.075	−
Out-CpG	-455	ADCYAP1	1.3 (<0.0001***)	0.0002**	−
Out-CpG	-1,394	PI3	-1.2 (<0.0001***)	0.004*	+
Out-CpG	+340	SFTPA1	-1.1 (<0.0001***)	0.003*	−
Out-CpG	-721	TRPM5	-1.1 (0.0003**)	0.075	−

TSS: transcription start site; Fold change: beta values between matched ESCC and normal adjacent tissue; 1: p-value of t-test; 2: p-value of log-rank test; 3: the direction of correlation was considered as "+" (respectively "–") when the methylation increase in tumor led to a good (respectively poor) survival rate; NA: not applicable; *: p-value < 0.05; **: p-value < 0.001; ***: p-value < 0.0001.

Method S1. Detailed procedures to calculate the 6 correlation metrics.

The support *Supp* is a widely-used measure in *association rule* data mining (Liu, et al., 2011). In this study, for each probe, a *Supp* of sample set *X* is defined as the proportion of total clinical samples in the data set which contains *X*. The exact definition is given by the following equation.

$$Supp(X)=\frac{C_X}{\#\,of\ \ samples},X\subset\{hypo,hyper,N_0,N_1\}$$

Where C_X represents the occurrence times of *X* for a given probe.

$$PhiCoefficient(hypo,N_0)=\frac{Supp(hypo,N_0)\text{ - }Supp(hypo)Supp(N_0)}{\sqrt{Supp(hypo)Supp(N_0)(1\text{ - }Supp(hypo))(1\text{ - }Supp(N_0))}}$$

The domain of *PhiCoefficient* (Cramer, 1946) is given by [-1, 1], and a value of 0 implies that there is no correlation.

$$OddsRatio(hypo,N_0,hyper,N_1)=\frac{\dfrac{Supp(hypo,N_0)}{Supp(hyper,N_0)}}{\dfrac{Supp(hypo,N_1)}{Supp(hyper,N_1)}}=\frac{Supp(hypo,N_0)Supp(hyper,N_1)}{Supp(hyper,N_0)Supp(hypo,N_1)}$$

The domain of *OddsRatio* (Edwards, 1963) is given by $[0,\infty]$, and a value of 1 implies that there is no correlation.

$$PiatetskyShapiroMeasure(hypo,N_0)=Supp(hypo,N_0)\text{ - }Supp(hypo)Supp(N_0)$$

The domain of *PiatetskyShapiroMeasure* (Piatetsky-Shapiro, 1991) is given by [-0.25, 0.25], and a value of 0 implies that there is no correlation.

$$LiftMeasure(hypo,N_0)=\frac{Supp(hypo,N_0)}{Supp(hypo)Supp(N_0)}$$

The domain of *LiftMeasure* (Tufféry, 2011) is given by $[0,\infty]$, and a value of 1 implies that there is no correlation.

$$AddedValue(hypo,N_0)=\frac{Supp(hypo,N_0)}{Supp(hypo)}\text{ - }Supp(N_0)$$

The domain of *AddedValue* (Sahar and Mansour, 1999) is given by [-0.5, 1], and a value of 0 implies that there is no correlation.

$$\mathrm{KlosgenMeasure(hypo{,}N_0)}=\sqrt{Supp(hypo,\mathrm{N}_0)}\times AddedValue(hypo,\mathrm{N}_0)$$

The domain of *KlosgenMeasure* (Klösgen, 1992) is given by $[\,(2/\sqrt{3}-1)^{1/2}(2-\sqrt{3}-1/\sqrt{3}),2/3\sqrt{3}\,]$, and a value of 0 implies that there is no correlation.

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