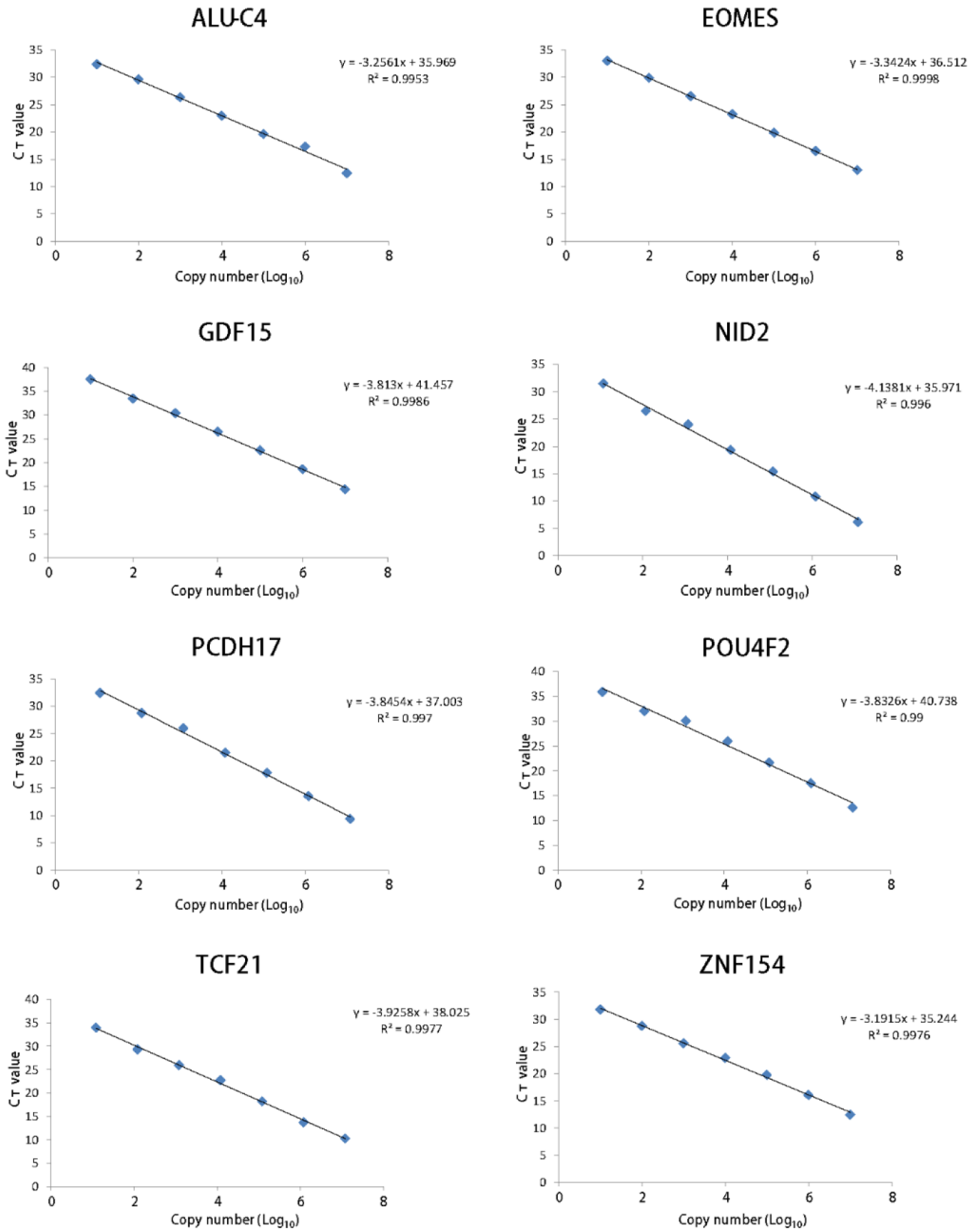
An epigenetic biomarker combination of PCDH17 and POU4F2 detects bladder cancer accurately by methylation analyses of urine sediment DNA in Han Chinese

Supplementary Materials



Supplementary Figure S1: Standard curves of the candidate genes.

Supplementary Table S1: Clinical information of the training set

Case ID	Gender	Age	Grade	TNM	Primary/ recurrent
UCC01	male	64	high	T4N1M0	primary
UCC02	male	73	high	T2bN0M0	primary
UCC03	female	63	high	TaN0M0	primary
UCC04	female	40	high	T2bN0M0	primary
UCC05	male	40	low	T1N0M0	primary
UCC06	female	46	high	TaN0M0	primary
UCC07	female	62	low	T1N0M0	recurrent
UCC08	female	33	high	T1N0M0	primary
UCC09	female	72	high	T3bN0M0	primary
UCC10	male	73	high	TaN0M0	primary
UCC11	male	70	high	T1N0M0	primary
UCC12	male	61	high	T2N0M0	primary
UCC13	male	75	high	T2bN0M0	primary
UCC14	male	50	low	T1N0M0	recurrent
UCC15	female	73	high	T2bN0M0	primary
UCC16	male	50	low	T2aN0M0	primary
UCC17	male	56	high	T1N0M0	recurrent
UCC18	male	43	low	TaN0M0	primary
UCC19	male	59	high	T3aN0M0	primary
UCC20	female	55	low	TaN0M0	recurrent
UCC21	male	58	high	T2aN0M0	primary
UCC22	male	35	low	T1N0M0	recurrent
UCC23	male	66	high	T1N0M0	recurrent
UCC24	male	59	high	T1N0M0	primary
UCC25	male	74	high	TaN0M0	primary
UCC26	male	49	high	T2aN0M0	recurrent
UCC27	female	41	high	T3bN3M1	primary
UCC28	male	68	high	TaN0M0	recurrent
UCC29	male	74	high	T2aN0M0	primary
UCC30	male	61	high	T1N0M0	primary
UCC31	male	74	high	T4aN2M0	primary
UCC32	male	54	high	T1N0M0	primary
UCC33	male	71	low	TaN0M0	primary
UCC34	male	70	high	T1N0M0	recurrent
UCC35	male	68	high	T2N0M0	recurrent
UCC36	male	87	high	T1N0M0	primary
UCC37	male	55	high	T1N0M0	primary

UCC38	male	66	high	T1N0M0	primary
UCC39	male	48	low	T1N0M0	primary
UCC40	male	54	low	TaN0M0	primary
UCC41	female	75	high	T1N0M0	recurrent
UCC42	male	79	low	TaN0M0	primary
UCC43	male	67	high	T2N0M0	primary
UCC44	female	80	low	TaN0M0	primary
UCC45	male	74	high	T2bN0M0	primary
UCC46	male	45	high	T3N0M0	primary
UCC47	female	41	low	T1N0M0	primary
UCC48	male	61	high	T1N0M0	primary
UCC49	male	74	high	T4aN2M0	primary
UCC50	male	49	low	TaN0M0	primary
UCC51	male	60	high	T2bN0M0	primary
UCC52	male	66	high	T3aN1M0	recurrent
UCC53	female	36	high	T3aN0M0	primary
UCC54	female	63	low	TaN0M0	primary
UCC55	female	72	low	T1N0M0	primary
UCC56	male	83	low	T1N0M0	primary
UCC57	male	61	high	T2bN0M0	primary
UCC58	male	35	high	T1N0M0	recurrent
HV01	female	25	NA	NA	NA
HV02	female	27	NA	NA	NA
HV03	male	27	NA	NA	NA
HV04	male	28	NA	NA	NA
HV05	female	28	NA	NA	NA
HV06	male	28	NA	NA	NA
HV07	male	27	NA	NA	NA
HV08	female	29	NA	NA	NA
HV09	female	29	NA	NA	NA
HV10	male	26	NA	NA	NA
HV11	female	30	NA	NA	NA
HV12	male	26	NA	NA	NA
HV13	male	28	NA	NA	NA
HV14	male	28	NA	NA	NA
HV15	male	28	NA	NA	NA
HV15	male	28	NA	NA	NA
HV16	male	31	NA	NA	NA
HV17	male	28	NA	NA	NA

HV18	male	26	NA	NA	NA
HV19	male	30	NA	NA	NA
HV20	male	28	NA	NA	NA
HV21	male	28	NA	NA	NA
HV22	male	29	NA	NA	NA
HV23	male	26	NA	NA	NA
HV24	male	28	NA	NA	NA
HV26	male	26	NA	NA	NA
HV27	male	30	NA	NA	NA
HV28	male	27	NA	NA	NA
HV29	male	30	NA	NA	NA
HV30	male	30	NA	NA	NA
IUC01	female	41	NA	NA	NA
IUC02	female	61	NA	NA	NA
IUC03	male	55	NA	NA	NA
IUC04	male	46	NA	NA	NA
IUC05	female	24	NA	NA	NA
IUC06	male	46	NA	NA	NA
IUC07	female	30	NA	NA	NA
IUC08	female	85	NA	NA	NA
IUC09	male	30	NA	NA	NA
IUC10	female	60	NA	NA	NA
IUC11	female	25	NA	NA	NA
IUC12	female	34	NA	NA	NA
IUC13	female	53	NA	NA	NA
IUC14	female	24	NA	NA	NA
IUC15	female	40	NA	NA	NA
IUC16	male	51	NA	NA	NA
IUC17	male	85	NA	NA	NA
IUC18	female	78	NA	NA	NA
IUC19	male	83	NA	NA	NA
IUC20	female	44	NA	NA	NA
KC01	female	79	NA	NA	NA
KC02	male	43	NA	NA	NA
KC03	male	56	NA	NA	NA
KC04	male	38	NA	NA	NA
KC05	female	71	NA	NA	NA
KC06	male	62	NA	NA	NA
KC07	female	38	NA	NA	NA

KC08	female	78	NA	NA	NA
KC09	male	82	NA	NA	NA
KC10	male	31	NA	NA	NA
KC11	male	62	NA	NA	NA
KC12	female	48	NA	NA	NA
KC13	female	59	NA	NA	NA
KC14	female	28	NA	NA	NA
KC15	female	57	NA	NA	NA
KC16	female	38	NA	NA	NA
KC17	male	62	NA	NA	NA
KC18	female	66	NA	NA	NA
KC19	female	50	NA	NA	NA
KC20	male	22	NA	NA	NA
PC01	male	72	NA	NA	NA
PC02	male	83	NA	NA	NA
PC03	male	72	NA	NA	NA
PC04	male	75	NA	NA	NA
PC05	male	68	NA	NA	NA
PC06	male	73	NA	NA	NA
PC07	male	70	NA	NA	NA
PC08	male	70	NA	NA	NA
PC09	male	68	NA	NA	NA
PC10	male	66	NA	NA	NA
PC11	male	73	NA	NA	NA
PC12	male	72	NA	NA	NA
PC13	male	74	NA	NA	NA
PC14	male	68	NA	NA	NA
PC15	male	70	NA	NA	NA
PC16	male	78	NA	NA	NA
PC17	male	67	NA	NA	NA
PC18	male	70	NA	NA	NA
PC19	male	65	NA	NA	NA
PC20	male	70	NA	NA	NA

UCC: Urothelial cell carcinoma; HV: Healthy volunteers; IUC: Infected urinary calculi; KC: Kidney cancer; PC: Prostate cancer.

Supplementary Table S2: Clinical information of the validation set

Case ID	gender	age	grade	TNM	primary/ recurrent
UCC01	male	43	high	T2bN0M0	primary
UCC02	male	75	high	T2bN0M0	primary
UCC03	female	56	high	T2aN0M0	recurrent
UCC04	male	43	low	TaN0M0	primary
UCC05	female	65	high	T2N0M0	recurrent
UCC06	male	62	high	TaN0M0	primary
UCC07	male	56	low	TaN0M0	primary
UCC08	male	70	high	T1N0M0	primary
UCC09	male	64	high	T1N0M0	recurrent
UCC10	male	49	high	T2aN0M0	recurrent
UCC11	male	72	high	T3bN2M0	primary
UCC12	male	57	high	T3aN0M0	primary
UCC13	male	51	high	T3aN0M0	primary
UCC14	male	57	high	T1N0M0	primary
UCC15	male	59	high	T1N0M0	primary
UCC16	female	81	high	T1N0M0	primary
UCC17	male	50	high	T2N0M0	recurrent
UCC18	male	59	high	T3N0M0	primary
UCC19	male	80	high	T1N0M0	primary
UCC20	male	55	high	T3bN0M0	primary
UCC21	female	55	low	T1N0M0	primary
UCC22	male	61	high	T2bN0M0	primary
UCC23	male	73	high	T1N0M0	primary
UCC24	male	57	low	TaN0M0	recurrent
UCC25	male	39	low	T2aN0M0	primary
UCC26	male	67	high	T1N0M0	primary
UCC27	female	62	low	TaN0M0	recurrent
UCC28	female	65	low	TaN0M0	primary
UCC29	male	70	high	T1N0M0	primary
UCC30	male	58	low	T1N0M0	primary
UCC31	male	80	high	T1N0M0	primary
UCC32	male	70	high	T1N0M0	primary
UCC33	male	65	high	T1N0M0	primary
UCC34	male	69	high	TaN0M0	primary
UCC35	female	53	low	T1N0M0	primary
UCC36	female	63	high	TaN0M0	primary
UCC37	male	36	high	T2aN0M0	primary
UCC38	male	71	high	TaN0M0	primary

UCC39	male	59	low	T2aN0M0	primary
UCC40	male	44	low	TaN0M0	recurrent
UCC41	male	70	high	T1N0M0	primary
UCC42	female	55	high	T4aN2M0	primary
UCC43	male	47	low	TaN0M0	primary
UCC44	male	59	high	T1N0M0	recurrent
UCC45	male	56	low	T1N0M0	primary
UCC46	male	58	high	T2N0M0	recurrent
UCC47	male	68	high	T4aN2M0	primary
UCC48	male	63	high	T1N0M0	primary
UCC49	male	79	high	T2bN0M0	primary
UCC50	female	63	low	TaN0M0	primary
UCC51	male	58	high	T1N0M0	recurrent
UCC52	male	84	high	T1N0M0	recurrent
UCC53	male	60	high	T3aN1M0	recurrent
UCC54	male	62	high	T3aN1M0	recurrent
UCC55	male	63	high	T3aN1M0	recurrent
UCC56	female	63	high	T2bN0M0	primary
UCC57	female	65	low	T1N0M0	primary
UCC58	male	63	high	T1N0M0	primary
UCC59	male	53	low	TaN0M0	primary
UCC60	male	60	high	T1N0M0	primary
UCC61	male	74	high	T1N0M0	primary
UCC62	male	67	high	TaN0M0	primary
UCC63	female	55	high	T1N0M0	recurrent
UCC64	male	59	high	T4N1M0	primary
UCC65	male	72	high	T2N0M0	primary
UCC66	male	58	low	TaN0M0	primary
UCC67	male	65	low	TaN0M0	primary
UCC68	male	70	low	TaN0M0	primary
UCC69	male	57	high	T1N0M0	primary
UCC70	male	64	high	T2bN0M0	primary
UCC71	female	52	high	T4aN2M0	primary
UCC72	male	61	high	T1N0M0	primary
HV01	female	42	NA	NA	NA
HV02	male	31	NA	NA	NA
HV03	male	37	NA	NA	NA
HV04	female	35	NA	NA	NA
HV05	male	34	NA	NA	NA

HV06	male	30	NA	NA	NA
HV07	male	45	NA	NA	NA
HV08	male	28	NA	NA	NA
HV09	male	29	NA	NA	NA
HV10	male	32	NA	NA	NA
HV11	male	43	NA	NA	NA
HV12	female	30	NA	NA	NA
HV13	female	30	NA	NA	NA
HV14	male	33	NA	NA	NA
HV15	male	39	NA	NA	NA
HV16	female	29	NA	NA	NA
HV17	male	44	NA	NA	NA
HV18	female	28	NA	NA	NA
HV19	female	29	NA	NA	NA
HV20	male	29	NA	NA	NA
HV21	male	36	NA	NA	NA
HV22	male	32	NA	NA	NA
HV23	male	35	NA	NA	NA
IUC01	female	41	NA	NA	NA
IUC02	male	44	NA	NA	NA
IUC03	female	59	NA	NA	NA
IUC04	female	27	NA	NA	NA
IUC05	female	43	NA	NA	NA
IUC06	female	38	NA	NA	NA
IUC07	male	38	NA	NA	NA
IUC08	male	56	NA	NA	NA
IUC09	male	58	NA	NA	NA
IUC10	female	39	NA	NA	NA
IUC11	female	60	NA	NA	NA
IUC12	male	51	NA	NA	NA
IUC13	male	26	NA	NA	NA
IUC14	female	32	NA	NA	NA
IUC15	male	41	NA	NA	NA
IUC16	male	56	NA	NA	NA
IUC17	male	36	NA	NA	NA
IUC18	female	60	NA	NA	NA
IUC19	female	65	NA	NA	NA
IUC20	male	31	NA	NA	NA
IUC21	female	33	NA	NA	NA

KC01	male	58	NA	NA	NA
KC02	male	66	NA	NA	NA
KC03	male	54	NA	NA	NA
KC04	male	64	NA	NA	NA
KC05	male	59	NA	NA	NA
KC06	female	67	NA	NA	NA
KC07	female	57	NA	NA	NA
KC08	female	60	NA	NA	NA
KC09	male	52	NA	NA	NA
KC10	male	44	NA	NA	NA
KC11	female	54	NA	NA	NA
KC12	female	62	NA	NA	NA
KC13	male	71	NA	NA	NA
KC14	female	60	NA	NA	NA
KC15	male	66	NA	NA	NA
KC16	female	78	NA	NA	NA
KC17	male	61	NA	NA	NA
KC18	male	52	NA	NA	NA
KC19	male	68	NA	NA	NA
KC20	female	66	NA	NA	NA
KC21	female	67	NA	NA	NA
KC22	male	41	NA	NA	NA
KC23	female	63	NA	NA	NA
KC24	male	65	NA	NA	NA
KC25	male	65	NA	NA	NA
KC26	male	64	NA	NA	NA
PC01	male	70	NA	NA	NA
PC02	male	84	NA	NA	NA
PC03	male	71	NA	NA	NA
PC04	male	70	NA	NA	NA
PC05	male	75	NA	NA	NA
PC06	male	81	NA	NA	NA
PC07	male	81	NA	NA	NA
PC08	male	83	NA	NA	NA
PC09	male	82	NA	NA	NA
PC10	male	82	NA	NA	NA
PC11	male	83	NA	NA	NA
PC12	male	81	NA	NA	NA
PC13	male	69	NA	NA	NA

PC14	male	75	NA	NA	NA
PC15	male	80	NA	NA	NA
PC16	male	75	NA	NA	NA
PC17	male	66	NA	NA	NA
PC18	male	84	NA	NA	NA
PC19	male	68	NA	NA	NA
PC20	male	72	NA	NA	NA
PC21	male	77	NA	NA	NA
PC22	male	69	NA	NA	NA

UCC: Urothelial cell carcinoma; HV: Healthy volunteers; IUC: Infected urinary calculi; KC: Kidney cancer; PC: Prostate cancer.

Supplementary Table S3: List of the primers of methylation biomarkers

Gene	Primers	Product size (bp)
ACTB-1 (Reference gene)	F:TAGGGAGTATATAGGTTGGGGAAGTT R:AACACACAATAACAAACACAAATTCAC	103
ACTB-2 (Reference gene)	F:TGGTGATGGAGGAGGTTTAGTAAGT R:AACCAATAAAACCTACTCCTCCCTTAA	133
ACTB-3 (Reference gene)	F:TGGTGATGGAGGAGGTTTAGTAGGT R:ACCCAATAAAACCTACTCCTCCCTTAA	133
ALU-C4 (Reference gene)	F:GGTTAGGTATAGTGGTTTATATTTGTAATTTTAGTA R:ATTAACTAAACTAATCTTAAACTCCTAACCTCA	89
EOMES	F:GGTTGGGGAAGTAGAGTTTCGAT R:ATAAACAATTACAAACGCCGCCA	80
GDF15	F:TCGGCGGTTATTTGTATTTGC R:CGTCGAAAACAACCGAAACA	101
HOXA9	F:GTGGTTATTATCGTGTTTAGCGT R:CCGATACCACCAAATTATTACATA	110
MYO3A	F:TCGGCGGGAGGATTTGAT R:CCCGCGAACCGAAATAAAA	93
NID2	F:GCGGTTTTTAAGGAGTTTTATTTTC R:CTACGAAATTCCCTTTACGCT	99
PCDH17–1	F:ATAGGTTTGGGATGTA R:GAAAAACAACTAACACTTA	329
PCDH17–2	F:CGGCGGAGGGCGTAGTA R:GTCCACGTCCAACAAATACGATAC	73
POU4F2	F:GTTGTGCGAAGTTGAGTTTATTC R:CCGTTCAAACTAACAACAAAAACGA	142
TCF21–1	F:AGGATTTTTAAGAGGTGG R:AAACCTTACTCAACACTC	267
TCF21–2	F:GTTAAGAGGAGGAAGGCGTTTATT R:CCTTACTCAACACTCGCATACGA	124
TMEFF2	F:GTTCGGGGTTACGCGC R:TTCGCCTCACTCTCCGCT	83

TWIST1	F:GTTAGGGTTCGGGGGCGTTGTT R:CCGTCGCCTTCCTCCGACGAA	92
VIM	F:TTCGGGAGTTAGTTCGCGTT R:ACCGCCGAACATCCTACGA	103
ZNF154	F:TTTATCGGATTAGAGATAGTAGAGCGT R:TAACGTAAATCCCCCAAAACGACG	152

F: forward primer; R: reverse primer.