# The chromosomal instability 25 gene signature is identified in clear cell renal cell carcinoma and serves as a predictor for survival and sunitinib response

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## SUPPLEMENTARY MATERIAL

Include Table S1 – S6 and Figure S1, S2 and S2.

***Table S1-1**. Characteristic of Qilu Hospital Cohort of 10 ccRCC patients for RNA seq.*

| Qilu Hospital Cohort | ***N=10*** |
|---|---|
| Age, mean (SEM[a]) | 52.7 (10.3) |
| Sex, n (%) | |
| Female | 3 (30.0%) |
| Male | 7 (70.0%) |
| AJCC Stage[b], n (%) | |
| I | 5 (50.0%) |
| II | 1 (10.0%) |
| III | 4 (40.0%) |
| T, n (%) | |
| T1 | 5 (50.0%) |
| T2 | 1 (10.0%) |
| T3 | 4 (40.0%) |
| Lymph node metastasis, n (%) | |
| N0 | 9 (90.0%) |
| N1 | 1 (10.0%) |
| Grade, n (%) | |
| G1/G2 | 5 (50.0%) |
| G3/G4 | 5 (50.0%) |
| Tumor max diameter, n (%) | |
| <4 | 2 (20.0%) |
| 4-7 | 4 (20.0%) |
| 7-10 | 2 (40.0%) |
| >10 | 2 (20.0%) |

[a]SEM, standard error of mean.

[b]Tumor AJCC stages according to the American Joint Committee on Cancer (AJCC) 7th edition.

Table S1-2. Characteristics of 9 ccRCC patients with matched tumor and non-tumorous tissues for qPCR analysis.

| Variable | N |
|---|---|
| Age, mean (SEM[a]) | 55.2 (3.4) |
| Sex, n (%) | |
| Male | 6 (66.7) |
| Female | 3 (33.3) |
| Grade, n (%) | |
| Low | 4 (44.4) |
| High | 5 (55.6) |
| AJCC Stage[b], n (%) | |
| I | 4 (44.4) |
| II | 1 (11.1) |
| III | 4 (44.4) |
| T, n (%) | |
| < T2 | 4 (44.4) |
| ⩾ T2 | 5 (55.6) |
| Lymph node metastasis, n (%) | |
| Positive | 1 (11.1) |
| Negative | 8 (88.9) |

[a]SEM, standard error of mean.

[b]Tumor AJCC stages according to the American Joint Committee on Ca

**Table S2. qPCR primer sequences used in the present study**

| | |
|---|---|
| H-FOXM1-194F | GGCCATCCCCAACAATGCTA |
| H-FOXM1-194R | AGGTCTCCAGGGTCACTTCT |
| H-CDC2-112F | AGTCAGTCTTCAGGATGTGCT |
| H-CDC2-112R | CATGTACTGACCAGGAGGGAT |
| H-PRC1-152F | TGTGGCAGAACAATGGGAGAT |
| H-PRC1-152R | GTGTATTGGGAGCCAGTCCTC |
| H-TPX2-236F | CCAGAGAAAGCCAAGGGTAGA |
| H-TPX2-236R | TCTGTGCGGAAGTGGAAGTC |
| H-MCM2-116F | ATCTACGCCAAGGAGAGGGT |
| H-MCM2-116R | GTAATGGGGATGCTGCCTGT |
| H-MCM7-171F | GAACACAAGGATTGCCCAGC |
| H-MCM7-171R | CCAGCCCCAGACTCATCATC |
| H-KIF20A-165F | AAGGGCAGAACTGGCTCATC |
| H-KIF20A-165R | GCAAGGGCTTCAGATCAGGT |
| H-TOP2A-168F | TCAGGCGTTTGATGGATGGA |
| H-TOP2A-168R | TCCATGTTCTGACGGGAAGC |
| H-TTK-130F | TGCCCATTTGGAAGAGTCCC |
| H-TTK-130R | GCACAACCAAATCTCGGCAT |
| H-ESPL1-105F | TGATTTGGCTGACCTGACCC |
| H-ESPL1-105R | ATGTGGTCCGTCAGCTCTTG |
| H-NCAPD2-177F | GATGCGTAATGCTGTGCTGG |
| H-NCAPD2-177R | ATTCGGGTGAAGAGCTGCAA |
| H-MELK-92F | AGGTGGACCCAAAGAAACGG |
| H-MELK-92R | GCTTTGCCACTCAACAGGAT |
| H-UBE2C-134F | CCTTGAACACACATGCTGCC |
| H-UBE2C-134R | AAGACGACACAAGGACAGGC |
| H-FEN1-166F | CCAACAAGTACCCTGTGCCA |
| H-FEN1-166R | CGCTCCTCAGAGAACTGCTT |
| H-RFC4-190F | TTACGGACCACCTGGAACTG |
| H-RFC4-190R | CTTCCCATCTGAGCGACTTC |
| H-CDC45-133F | GATGTTTGAGCTGGCTTGGAT |
| H-CDC45-133R | CAGGACACCAACATCAGTCAC |
| H-RAD51AP1-92F | GATGTTGGTGGTGTTCAAGGGA |
| H-RAD51AP1-92R | CTATCACCATCACTGCCTTCCA |
| H-H2AZ1-211F | TCGAAATGGCTGGCGGTAAG |
| H-H2AZ1-211R | AGTTCAAGTACCTCTGCGGTG |
| H-PCNA-71F | GCTCCATCCTCAAGAAGGTGT |
| H-PCNA-71R | GGAGCTAATATCCCAGCAGGC |
| H-RNASEH2A-F | TCAGGCTACCCCAATGATCC |
| H-RNASEH2A-R | CCTCCTGATTCTCGGATGCT |
| H-TGIF2-206F | GATCTAGGTGAGGACGAAGGC |

| | |
|---|---|
| H-TGIF2-206R | CGGGCATTGATGAACCAGTTAC |
| H-CCT5-151F | CAAGATCAGCGATAGCGTCCT |
| H-CCT5-151R | CGGCATTCACAGCAATCTCAG |
| H-TRIP13-115F | TGGCACTGGAAAAACATCCCT |
| H-TRIP13-115R | AGAAAAGAGGCTGTGGCTGTT |
| H-MAD2L1-222F | TCTCATTCGGCATCAACAGCA |
| H-MAD2L1-222R | CCAGGACCTCACCACTTTCAA |
| H-CCNB2-172F | CGACGGTGTCCAGTGATTTG |
| H-CCNB2-172R | TTGGTGGGTTGAACTGGAACT |
| homoβ-actin-250 F | CATGTACGTTGCTATCCAGGC |
| homoβ-actin-250 R | CTCCTTAATGTCACGCACGAT |

***Table S3****. Clinic-Pathological characteristic of TCGA ccRCC Cohort*

| | **CIN-C1** ***N=350*** | **CIN-C2** ***N=180*** | **P** |
|---|---|---|---|
| Age, mean (SEM[a]) | 61.4 (12.2) | 59.3 (11.8) | 0.055 |
| Sex, n (%) | | | 0.063 |
| Male | 217 (62.0%) | 127 (70.6%) | |
| Female | 133 (38.0%) | 53 (29.4%) | |
| AJCC Stage[b], n (%) | | | <0.001 |
| I | 196 (56.0%) | 70 (39.5%) | |
| II | 43 (12.3%) | 14 (7.91%) | |
| III | 74 (21.1%) | 49 (27.7%) | |
| IV | 37 (10.6%) | 44 (24.9%) | |
| Grade, n (%) | | | <0.001 |
| G1 | 12 (3.49%) | 2 (1.12%) | |
| G2 | 167 (48.5%) | 61 (34.3%) | |
| G3 | 133 (38.7%) | 73 (41.0%) | |
| G4 | 32 (9.30%) | 42 (23.6%) | |

[a]SEM, standard error of mean.

[b]Tumor AJCC stages according to the American Joint Committee on Cancer (AJCC) 7th edition.

***Table S4****. Clinic-Pathological characteristic of E-MTAB-1980 ccRCC Cohort*

| | **CIN-C1** ***N=64*** | **CIN-C2** ***N=37*** | **P** |
|---|---|---|---|
| Age, mean (SEM[a]) | 62.3 (11.6) | 65.5 (11.1) | 0.172 |
| Sex, n (%) | | | 0.010 |
| Male | 43 (67.2%) | 34 (91.9%) | |
| Female | 21 (32.8%) | 3 (8.11%) | |
| AJCC Stage[b], n (%) | | | 0.125 |
| I | 46 (71.9%) | 20 (54.1%) | |
| II | 7 (10.9%) | 3 (8.11%) | |
| III | 5 (7.81%) | 8 (21.6%) | |
| IV | 6 (9.38%) | 6 (16.2%) | |
| Grade, n (%) | | | 0.007 |
| G1 | 10 (15.9%) | 3 (8.33%) | |
| G2 | 43 (68.3%) | 16 (44.4%) | |
| G3 | 9 (14.3%) | 13 (36.1%) | |
| G4 | 1 (1.59%) | 4 (11.1%) | |

[a]SEM, standard error of mean.

[b]Tumor AJCC stages according to the American Joint Committee on Cancer (AJCC) 7th edition.

***Table S5****. Characteristic of IMmotion151 Cohort of Sunitinib treatment*

| | **CIN-C1** ***N=273*** | **CIN-C2** ***N=143*** | **P** |
|---|---|---|---|
| Age, mean (SEM[a]) | 60.0 (10.1) | 59.3 (9.63) | 0.470 |
| Sex, n (%) | | | 0.223 |
| Female | 62 (22.7%) | 41 (28.7%) | |
| Male | 211 (77.3%) | 102 (71.3%) | |
| Histology | | | <0.001 |
| ccRCC_nonSarc | 245 (89.7%) | 101 (71.1%) | |
| ccRCC_Sarc | 26 (9.52%) | 30 (21.1%) | |
| nonccRCC_Sarc | 2 (0.73%) | 11 (7.75%) | |
| Response: | | | <0.001 |
| CR/PR | 107 (42.0%) | 32 (26.0%) | |
| PD | 38 (14.9%) | 38 (30.9%) | |
| SD | 110 (43.1%) | 53 (43.1%) | |

[a]SEM, standard error of mean.

***Table S6****. Characteristic of IMmotion151 Cohort of Sunitinib treatment*

| | **CIN-C1** ***N=58*** | **CIN-C2** ***N=27*** | **P** |
|---|---|---|---|
| Age, mean (SEM[a]) | 60.1 (9.88) | 60.9 (12.0) | 0.763 |
| Sex, n (%) | | | 1.000 |
| Female | 10 (17.2%) | 5 (18.5%) | |
| Male | 48 (82.8%) | 22 (81.5%) | |
| Tumor Mutation Burden | 26.0 (106) | 11.4 (10.8) | 0.385 |
| Response: | | | 0.001 |
| CR/PR | 24 (41.4%) | 4 (14.8%) | |
| PD | 8 (13.8%) | 13 (48.1%) | |
| SD | 26 (44.8%) | 10 (37.0%) | |

[a]SEM, standard error of mean.

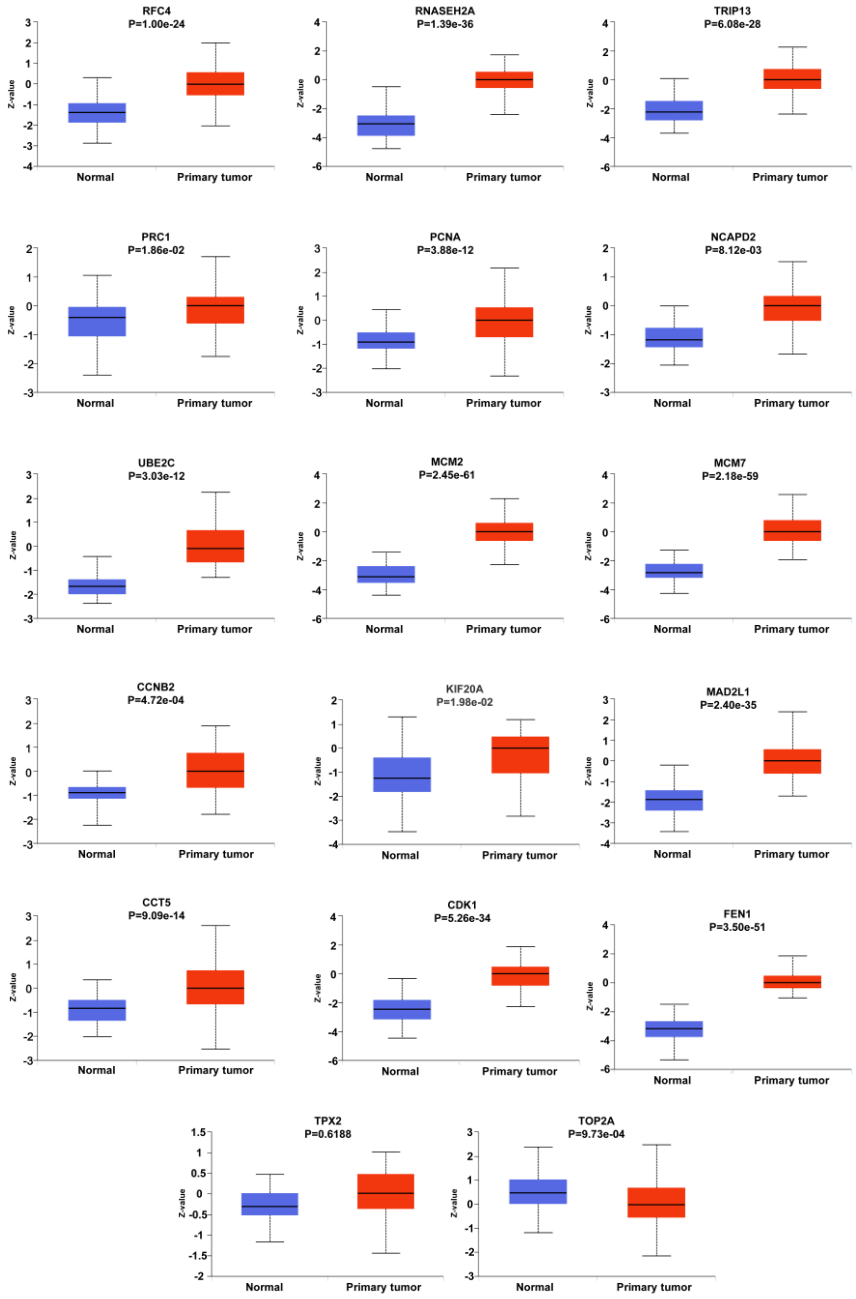


**Figure S1. Expression of CIN25 genes at protein levels in ccRCC tumors and renal nontumorous tissues in the TCGA cohort**. The data were obtained from Clinical Proteomic Tumor Analysis Consortium (http://ualcan.path.uab.edu/index.html), and 17 of 25 genes were available. Higher protein levels in tumors were observed in 16/17 genes.

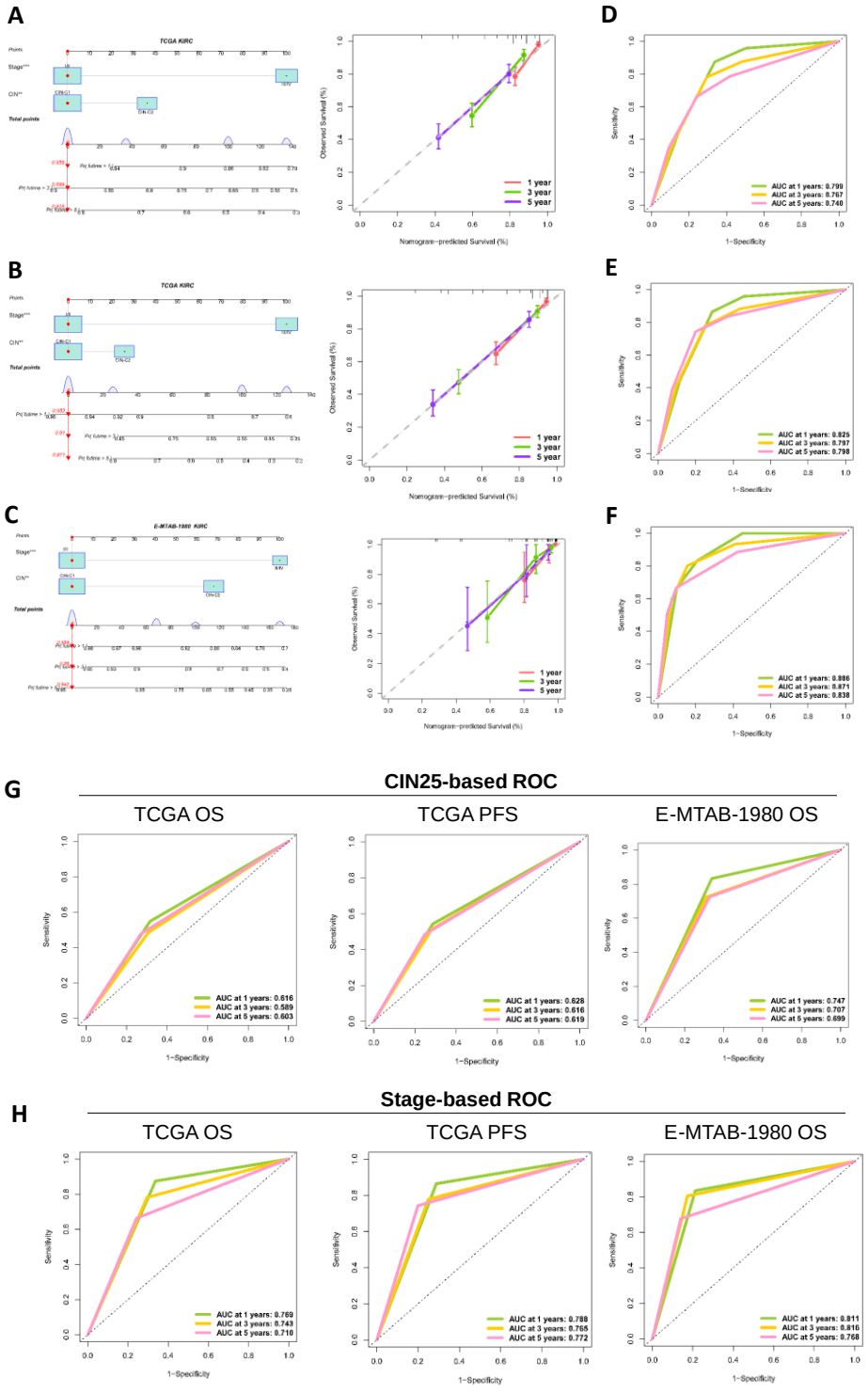


**Figure S2. CIN25 subtype- and stage-based nomograms and time-dependent Receiver Operator Characteristic (ROC) to predict overall and progression-free survival (OS and PFS) in TCGA and E-MTAB-1980 cohorts of ccRCC patients.** (A) Nomogram prediction of 1-, 3- and 5-year OS in TCGA cohort. (B) Nomogram prediction of 1-, 3- and 5-year PFS in TCGA cohort. (C) Nomogram prediction of 1-, 3- and 5-year OS in E-MTAB-1980 cohort. (D) ROC of CIN25- and stage-based 1-, 3- and 5-year OS prediction in TCGA cohort. (E) ROC of CIN25- and stage-based 1-, 3- and 5-year PFS prediction in TCGA cohort. (F) ROC of CIN25- and stage-based 1-, 3- and 5-year OS prediction in E-MTAB-1980 cohort. (G) ROC of CIN25-based 1-, 3- and 5-year survival prediction in both cohorts. (H) ROC of stage-based 1-, 3- and 5-year survival prediction in both cohorts.

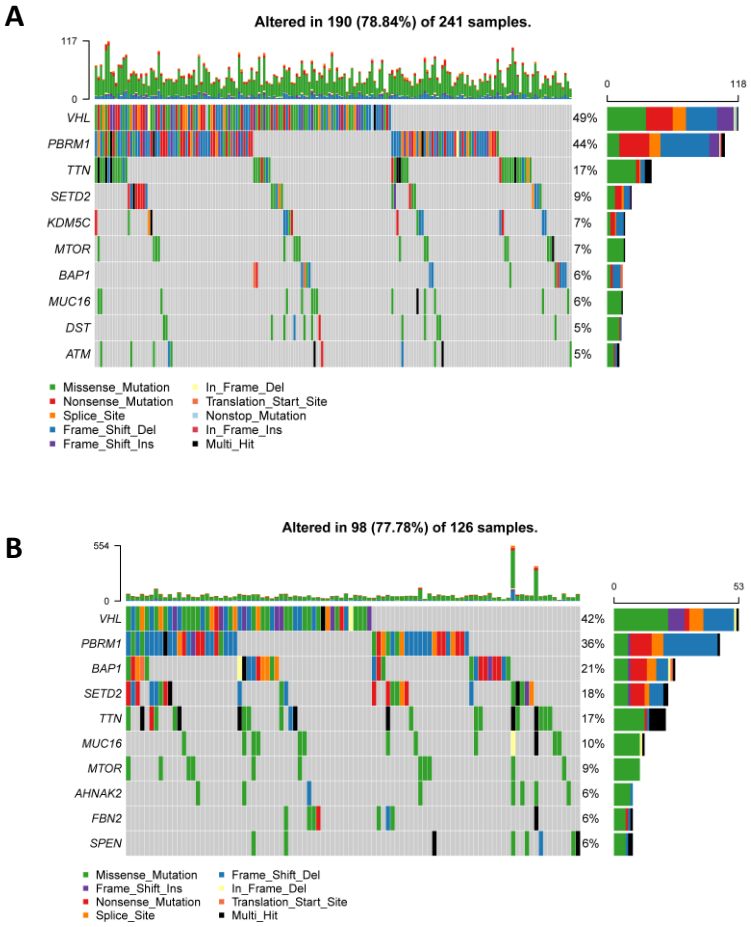


**Figure S3. The top 10 mutated genes in CIN25-C1 and C2 tumors from the TCGA ccRCC cohort. (A) CIN25-C1. (B) CIN25-C2.**