**Supplemental Materials**

**Supplemental Tables**

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| **Supplemental Table S1. Lower limits of quantification (LLOQ) of analyte (data from ThermoFisher†).** | |
| **Analyte** | **LLOQ\*** |
| BTLA | 144.65 |
| CD27 | 5.76 |
| CD28 | 42.02 |
| CD80 | 43.58 |
| CD137/4-1BB | 14.21 |
| CD152/CTLA4 | 9.52 |
| GITR | 27.2 |
| HVEM | 18.55 |
| IDO | 4.37 |
| LAG-3 | 11.21 |
| PD-1 | 7.13 |
| PD-L1 | 3.64 |
| PD-L2 | 48.71 |
| TIM-3 | 63.18 |
| \* in pg/mL;  † ProcartaPlex Human Immuno-Oncology Checkpoint Panel, <https://www.thermofisher.com/order/catalog/product/EPX14A-15803-901> | |

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| **Supplement table S2. Real-time PCR probes for T cell immune checkpoint gene expression used in this study** | | | |
| **Gene name** | **Official full name** | **Probe number** | **Catalog number** |
| *BTLA* | B and T lymphocyte associated (BTLA) | Hs00699198\_m1 | 4331182 |
| *CD27* | CD27 molecule (CD27) | Hs00609654\_g1 | 4351372 |
| *CD28* | CD28 molecule (CD28) | Hs00155512\_m1 | 4331182 |
| *CD3E* | CD3e molecule(CD3E) | Hs01062241\_m1 | 4331182 |
| *CD274* | CD274 molecule (CD274) | Hs00204257\_m1 | 4331182 |
| *CTLA4* | cytotoxic T-lymphocyte associated protein 4(CTLA4) | Hs00175480\_m1 | 4331182 |
| *HAVCR2* | hepatitis A virus cellular receptor 2(HAVCR2) | Hs00958618\_m1 | 4331182 |
| *IDO1* | Indoleamine 2,3-dioxygenase 1 (IDO1) | Hs00699198\_m1 | 4331182 |
| *LAG3* | lymphocyte activating 3(LAG3) | Hs00958444\_g1 | 4351372 |
| *PDCD1* | programmed cell death 1(PDCD1) | Hs01550088\_m1 | 4331182 |
| *PDCD1LG2* | Programmed cell death 1 ligand 2 (PDCD1LG2) | Hs00228839\_m1 | 4331182 |
| *TNFRSF14* | TNF receptor superfamily member 14 (TNFRSF14) | Hs00998605\_g1 | 4331182 |
| *TNFRSF9* | TNF receptor superfamily member 9 (TNFRSF9) | Hs00155512\_m1 | 4331182 |
| *GAPDH* | glyceraldehyde-3-phosphate dehydrogenase(GAPDH) | Hs01548420\_m1 | 4331182 |

\*All probes were purchased from Applied Biosystems, Waltham, MA

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| **Supplemental Table S3. Covariates of unconditional logistic regression model, multivariate Cox proportional hazard model for recurrence and survival** | | | | | | | | | | | | |
| Covariates | | Model for  tumor stage Coef.(95%CI)\* | | *P* value | | Model for recurrence  Coef.(95%CI)\*\* | | *P* value | | Model for  survival Coef.(95%CI)\*\*\* | | *P* value | |
| Sex | -0.04(-0.72-0.65) | | 0.92 | | 0.41 (-0.11-0.94) | | 0.12 | | -1.22 (-2.40--0.04) | | 0.04 | |
| Age | 0.01 (-0.02-0.05) | | 0.41 | | -0.03 (-0.05-0.002) | | 0.07 | | 0.05 (0.001-0.09) | | 0.04 | |
| Smoking status |  | |  | |  | |  | |  | |  | |
| Former | -0.12 (-0.75-0.51) | | 0.71 | | -0.06 (-0.59-0.47) | | 0.82 | | -1.05 (-1.98--0.12) | | 0.03 | |
| Current | -0.08 (-1.25-1.08) | | 0.89 | | 0.73 (-0.04-1.50) | | 0.06 | | 0.53 (-0.57-1.63) | | 0.35 | |
| BMI | -0.01 (-0.06-0.04) | | 0.62 | | -0.03 (-0.07-0.007) | | 0.11 | | -0.07 (-0.14--0.004) | | 0.04 | |
| Hypertension | -0.10 (-0.83-0.63) | | 0.79 | | 0.03 (-0.52-0.59) | | 0.91 | | 0.06 (-0.94-1.06） | | 0.91 | |
| Diabetes | 0.17 (-0.63-0.96) | | 0.68 | | -0.37 (-0.94-0.20) | | 0.2 | | -1.08 (-1.97--0.19) | | 0.02 | |
| Histology | NA | | NA | | 0.39 (-0.27-1.04) | | 0.25 | | 0.49 (-0.61-1.60) | | 0.38 | |
| Stage | NA | | NA | | 1.98 (1.42-2.54) | | 0 | | 0.92 (0.15-1.70) | | 0.02 | |
| Tumor grade† | NA | | NA | | 0.49 (0.30-0.67) | | 0 | | 0.27 (-0.03-0.56) | | 0.08 | |
| Treatment‡ | NA | | NA | | -0.04 (-0.71-0.64) | | 0.92 | | -0.14 (-1.20-0.92) | | 0.79 | |
| \*: covariates of unconditional logistic regression model for tumor stage;  \*\*: covariates of multivariate Cox proportional hazard model for recurrence;  \*\*\*covariates of multivariate Cox proportional hazard model for survival.  † Tumor grade based on Fuhrman criteria. ‡indicate adjuvant chemotherapy | | | | | | | | | | | | |

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| **Supplemental Table S4. Circulating levels of soluble immune checkpoint proteins in ccRCC patients** | | | | | | | | | |
|  | **Early Stagec** | | |  | **Late Stagec** | |  |  |  |
| **Marker** | **No** |  | **Median (IQR) pg/ml** |  | **No** | **Median (IQR) pg/ml** |  | ***P* value** | **cutoffb** |
| sBTLA | 76 |  | 144.65 (144.65-852.02) |  | 82 | 144.65 (144.65-469.94) |  | 0.48 | 2269 |
| sCD27 | 87 |  | 350.64 (192.04-753.37) |  | 90 | 307.57 (179.93-595.62) |  | 0.47 | 1029 |
| sCD28 | 75 |  | 42.02 (42.02-149.34) |  | 76 | 42.02 (42.02-138.74) |  | 0.86 | 42.68 |
| sCD80a | 83 |  | 43.58 (43.58-43.58) |  | 86 | 43.58 (43.58-43.58) |  | 0.54 | 43.58 |
| sCD137a | 77 |  | 14.21 (14.21-14.21) |  | 82 | 14.21 (14.21-14.21) |  | 0.96 | 110.2 |
| sCTLA4 | 76 |  | 24.78 (9.52-61.01) |  | 80 | 33.14 (9.52-66.64) |  | 0.32 | 33.14 |
| sGITRa | 79 |  | 27.20 (27.20-27.20) |  | 85 | 27.20 (27.20-27.20) |  | 0.71 | 27.2 |
| sHVEMa | 85 |  | 18.55 (18.55-18.56) |  | 85 | 18.55 (18.55-18.56) |  | 0.45 | 18.55 |
| sIDO | 76 |  | 4.37 (4.37-18.33) |  | 75 | 4.37 (4.37-12.96) |  | 0.73 | 4.37 |
| sLAG3 | 86 |  | 139.98 (81.58-216.34) |  | 90 | 160.55 (103.70-206.76) |  | 0.18 | 258 |
| sPD1a | 88 |  | 7.13 (7.13- 7.13) |  | 91 | 7.13 (7.13- 7.13) |  | - | - |
| sPDL1 | 73 |  | 7.26 (3.64-21.67) |  | 75 | 7.85 (3.64-19.72) |  | 0.94 | 13.37 |
| sPDL2 | 85 |  | 1381.38 (48.71-5328.93) |  | 87 | 1348.71(48.71-6132.34) |  | 0.92 | 3032 |
| sTIM3 | 87 |  | 3628.83 (2713.02-5176.08) |  | 90 | 4226.43 (2962.89-5696.84) |  | 0.16 | 5908 |
| Abbreviations: ccRCC, clear cell renal cell carcinoma; IQR, interquartile range.  a CD137, HVEM, GITR, CD80 and PD1 level demonstrated few variations, thus these markers were not included in the subsequent analysis.  b Levels of all soluble biomarkers and immune checkpoint genes were dichotomized into low- and high-level groups using a logistic regression spline model *(12)*.  c Early stage indicates stage I&II disease, Late stage indicate stage III disease, the staging criteria according to NCCN guideline 2019 v.2.0. | | | | | | | | |  |

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| **Supplemental Table S5. Association with sTIM3 with death risk of ccRCC patients stratified by clinical stage** | | | | | |
| **Protein name** | **Early stage (I & II)** | |  | **Late stage (III)** | |
| **High *vs* low**\* | **Adjusted HR (95%CI)**† | ***P* value** |  | **Adjusted HR (95%CI)**† | ***P* value** |
| sTIM3 | 1 (reference) |  |  | 1 (reference) |  |
|  | **36.14 (3.733-350)** | **1.95E-03#** |  | 1.62 (0.60-4.41) | 0.34 |
| Abbreviations: ccRCC, clear cell renal cell carcinoma; HR, hazard ratio; CI confidence interval.  Significant values in bold font.  \* High- and low-level groups dichotomized by the logistic regression spline model (12).  † Adjusted by age, gender, smoking, BMI, diabetes, hypertension, histology, grade, and treatment.  #Significant after Bonferroni adjustment for multiple testing. | | | | | |

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| **Supplemental Table S6. Risk scores of soluble immune checkpoint biomarkers for ccRCC survival** | | | | | | |
| **Risk score \*** | **Dead** | **Alive** | **HR (95% CI)**† | ***P* value** | **MST (month)** | ***P* log-rank** |
| Low | 11 (9.02) | 111 (90.98) | 1 (reference) |  | - |  |
| Medium | 8 (30.77) | 18 (69.23) | 3.29 (1.14-9.52) | 0.028 | - |  |
| High | 14 (41.18) | 20 (61.11) | 12.88 (3.62-45.78) | 7.88E-05 | 22.4 | 5.14E-11 |
| Abbreviations: ccRCC, clear cell renal cell carcinoma; HR, hazard ratio; CI, confidence interval; MST, median survival time.  \* Risk groups were divided into tertiles based on levels of sBTLA and sTIM3.  † Adjusted by age, gender, smoking, BMI, diabetes, hypertension, histology, grade, stage and treatment.  (-) MST greater than follow-up time. | | | | | | |

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| **Supplemental Table S7: The correlation between circulating checkpoint level and T cell functions** | | | | | | | | | | | | |
|  |  | ***CD8A*** | |  | **CYT score 1†** | |  | **CYT score 2†** | |  | ***IFNG*** | |
| **Markers** | ***N*** | **rho** | ***P*** |  | **rho** | ***P*** |  | **rho** | ***P*** |  | **rho** | ***P*** |
| sTIM3 | 46 | -0.17 | 0.26 |  | -0.14 | 0.35 |  | 0.00 | 0.99 |  | -0.10 | 0.50 |
| **sCD28** | **38** | -0.25 | 0.14 |  | **-0.33** | **0.05\*** |  | **-0.33** | **0.05\*** |  | -0.07 | 0.67 |
| sCD137 | 40 | 0.16 | 0.33 |  | 0.04 | 0.80 |  | 0.04 | 0.80 |  | -0.03 | 0.83 |
| sCD27 | 45 | -0.06 | 0.72 |  | 0.01 | 0.96 |  | 0.17 | 0.27 |  | 0.15 | 0.32 |
| sCTLA4 | 39 | -0.05 | 0.75 |  | -0.08 | 0.65 |  | -0.02 | 0.89 |  | 0.28 | 0.08 |
| sHVEM | 43 | -0.06 | 0.71 |  | -0.10 | 0.52 |  | -0.10 | 0.51 |  | -0.12 | 0.44 |
| sIDO | 35 | -0.06 | 0.73 |  | -0.05 | 0.79 |  | -0.11 | 0.52 |  | 0.13 | 0.46 |
| **sLAG3** | **46** | **-0.35** | **0.02** |  | **-0.31** | **0.04** |  | -0.25 | 0.09 |  | -0.21 | 0.17 |
| sBTLA | 39 | -0.05 | 0.76 |  | -0.09 | 0.59 |  | 0.07 | 0.68 |  | -0.05 | 0.75 |
| sPD1 | 46 | 0.08 | 0.61 |  | 0.00 | 0.97 |  | -0.08 | 0.59 |  | -0.15 | 0.33 |
| sPDL1 | 36 | 0.25 | 0.15 |  | 0.29 | 0.09 |  | 0.45 | 0.01 |  | **0.51** | **1.31E-03** |
| sPDL2 | 43 | 0.05 | 0.76 |  | 0.11 | 0.49 |  | 0.18 | 0.24 |  | 0.19 | 0.22 |

\* Before rounding, *P*=0.0464 and *P*=0.0459, respectively.

1 Cytolytic score based on *GZMA* and *PRF1* tissue expression.

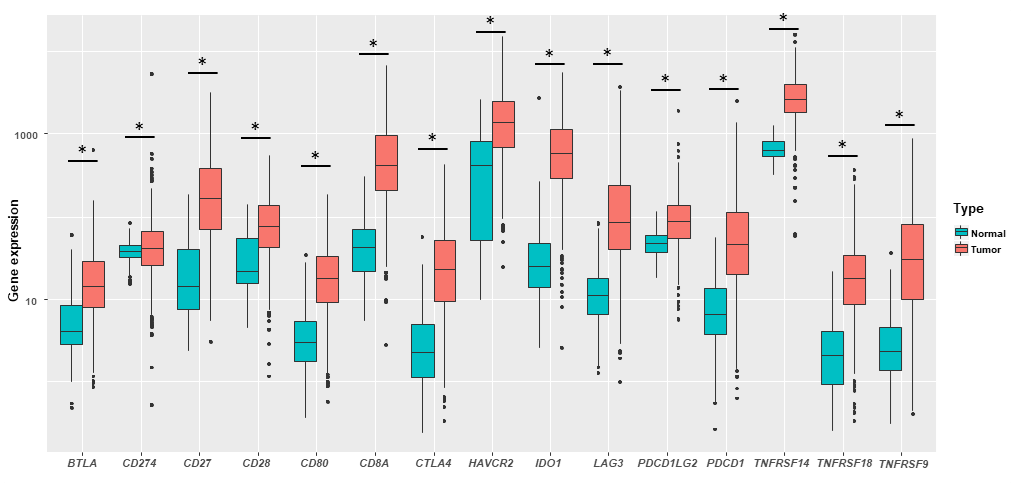
2 Cytolytic score based on *GZMB* and *PRF1* tissue expression.

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| **Supplemental Table S8. Tumor expression of immune checkpoint genes and association with ccRCC overall survival in the MDACC and TCGA cohorts** | | | | | | | | | | | | |
|  |  | **MDACC cohort (*n*=47)** | | | | |  | **TCGA cohort (*n*=382)#** | | | | |
| **Gene** | **Expression** | **Dead** | **Alive** | **HR (95%CI)\*** | ***P* value** | ***P* log-rank** |  | **Dead** | **Alive** | **HR (95%CI) †** | ***P* value** | ***P* log-rank** |
| *BTLA* | low | 7 (20.59) | 27 (79.41) | 1 (reference) |  |  |  | 49(25.65) | 142(74.35) | 1(reference) |  |  |
|  | high | 3 (23.08) | 10 (76.92) | 1.41 (0.27-7.46) | 0.69 | 0.25 |  | 43(22.51) | 148(77.49) | 0.73 (0.47-1.14) | 0.17 | 0.35 |
| *CD27* | low | 6(25.00) | 18(75.00) | 1 (reference) |  |  |  | 39(20.42) | 152(79.58) | 1(reference) |  |  |
|  | high | 4(17.39) | 19(82.61) | 0.27 (0.03-2.76) | 0.27 | 0.93 |  | 53(27.75) | 138(72.25) | 1.00(0.65-1.56) | 0.97 | 0.21 |
| *CD28* | low | 6(20.69) | 23(79.31) | 1 (reference) |  |  |  | 41(21.47) | 150(78.53) | 1(reference) |  |  |
|  | high | 4(22.22) | 14(77.78) | 1.08 (0.22-5.16) | 0.93 | 0.55 |  | 51(26.70) | 140(73.30) | 1.11 (0.72-1.71) | 0.63 | 0.47 |
| *CD274* | low | 7 (24.14) | 22 (75.86) | 1 (reference) |  |  |  | 59(30.89) | 132(69.11) | 1(reference) |  |  |
|  | high | 3 (16.67) | 15 (83.33) | 0.77 (0.13-4.59) | 0.78 | 0.48 |  | 33(17.28) | 158(82.72) | 0.51(0.33-0.80) | **3.23E-03** | **6.70E-04** |
| *CTLA4* | low | 7(21.88) | 25(78.13) | 1 (reference) |  |  |  | 39(20.42) | 152(79.58) | 1(reference) |  |  |
|  | high | 3(20.00) | 12(80.00) | 0.80(0.14-4.58) | 0.80 | 0.66 |  | 53(27.75) | 138(72.25) | 1.22 (0.78-1.89) | 0.38 | **0.05** |
| *HAVCR2* | low | 4(15.38) | 22(84.62) | 1 (reference) |  |  |  | 52(27.23) | 139(72.77) | 1(reference) |  |  |
|  | high | 6(28.57) | 15(71.43) | 5.79 (1.00-33.7) | **0.05** | 0.32 |  | 40(20.94) | 151(79.06) | 0.59(0.39-0.91) | **0.02** | 0.08 |
| *IDO1* | low | 5 (20.00) | 20 (80.00) | 1 (reference) |  |  |  | 41(21.47) | 150(78.53) | 1(reference) |  |  |
|  | high | 5 (22.73) | 17 (77.27) | 1.10 (0.20-6.14) | 0.91 | 0.54 |  | 51(26.70) | 140(73.30) | 0.94 (0.62-1.44) | 0.79 | 0.45 |
| *LAG3* | low | 6 (22.22) | 21 (77.78) | 1 (reference) |  |  |  | 35(18.32) | 156(81.68) | 1(reference) |  |  |
|  | high | 4 (20.00) | 16 (80.00) | 1.40 (0.20-10.0) | 0.74 | 0.70 |  | 57(29.84) | 134(70.16) | 1.14 (0.73-1.78) | 0.56 | 0.03 |
| *PDCD1* | low | 7 (21.21) | 26 (78.79) | 1 (reference) |  |  |  | 41(21.47) | 150(78.53) | 1(reference) |  |  |
|  | high | 3 (21.43) | 11 (78.57) | 0.98 (0.18-5.17) | 0.98 | 0.68 |  | 51(26.70) | 140(73.30) | 0.97 (0.62-1.51) | 0.89 | 0.28 |
| *PDCD1LG2* | low | 3 (10.34) | 26 (89.66) | 1 (reference) |  |  |  | 51(26.70) | 140(73.30) | 1(reference) |  |  |
|  | high | 7 (38.89) | 11 (61.11) | 8.62 (1.47-50.7) | **0.02** | **0.01** |  | 41(21.47) | 150(78.53) | 0.69 (0.44-1.06) | 0.09 | 0.17 |
| *TNFRSF14* | low | 6(20.69) | 23(79.31) | 1(reference) |  |  |  | 47(24.61) | 144(75.39) | 1(reference) |  |  |
|  | high | 4(22.22) | 14(77.78) | 3.85 (0.37-40.1) | 0.26 | 0.65 |  | 45(23.56) | 146(76.44) | 0.92 (0.60-1.42) | 0.72 | 0.79 |
| *TNFRSF9* | low | 5(21.74) | 18(78.26) | 1(reference) |  |  |  | 46(24.08) | 145(75.92) | 1(reference) |  |  |
|  | high | 5(20.83) | 19(79.17) | 0.39 (0.05-2.85) | 0.35 | 0.53 |  | 46(24.08) | 145(75.92) | 0.73 (0.47-1.16) | 0.14 | 0.95 |
| \* adjusted by age, gender, smoking, BMI, diabetes, hypertension, histology, grade and stage.  † adjusted by age, sex, grade, stage.  # Number of patients analyzed is less than total due to missing clinical outcome information. | | | | | | | | | | | | |

**Supplemental Figures**



**Supplemental Figure S1.** Schematic design of the study



**Supplemental Figure S2**. Tumor and normal tissue expression of immune checkpoint genes in ccRCC patients derived from TCGA database. Maroon boxplot indicates tumor tissues (*N*=533), and green boxplot indicates normal tissues (*N*=72). Wilcoxon rank sum test was used in the comparison analysis. \*indicates *P*<0.05.