

**Tumour grade significantly correlates with total dysfunction of tumour  
tissue-infiltrating lymphocytes in renal cell carcinoma**

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## **Supplementary Figure legends**

### **Supplementary Figure 1.** Gating and data acquisition of flow cytometry of 3 samples

analysed in RNA sequencing. Expression of CD103 were evaluated within each fraction of CD8<sup>+</sup> T cells.

### **Supplementary Figure 2.** Rarefaction analysis of T-cell receptor (TCR) repertoire

samples from 4 subsets of Fr. I, Fr. II, Fr. III, and Fr. IV of CD8<sup>+</sup> T cells of 3 patients

(Pt1–Pt3) (Left: TCRA, Right: TCRB). Solid and dashed lines mark interpolated and

extrapolated regions of rarefaction curves, respectively, and points mark exact sample

size and diversity. Shaded areas mark 95% confidence intervals **(a)**. Comparison of

TCRA and TCRB repertoire diversities using normalized Chao1 estimate **(b)**. Clonal

space homeostasis of TCRA and TCRB within each fraction of 3 patients **(c)**. Venn

diagrams representing TCRA (upper) and TCRB (lower) repertoire clones among 4

subsets for each patient **(d)**. Statistical analysis for multiple comparisons was performed

by Kruskal-Wallis analysis. The central tendency of the box plot indicates the median of

each group, and the upper and lower ranges of the box plot show the 25<sup>th</sup> and 75<sup>th</sup>

percentiles of each data set, respectively.

**Supplementary Figure 3.** Rarefaction analysis of T-cell receptor (TCR) repertoire samples from 4 subsets of Fr. I, Fr. II, Fr. III, and Fr. V of CD4<sup>+</sup> T cells of 3 patients (Pt1–Pt3) (Left: TCRA, Right: TCRB). Solid and dashed lines mark interpolated and extrapolated regions of rarefaction curves, respectively, and points mark exact sample size and diversity. Shaded areas mark 95% confidence intervals (**a**). Comparison of TCRA and TCRB repertoire diversities using normalized Chao1 estimate (**b**). Clonal space homeostasis of TCRA and TCRB within each fraction of 3 patients (**c**). Venn diagrams representing TCRA (upper) and TCRB (lower) repertoire clones among 4 subsets for each patient (**d**). Statistical analysis for multiple comparisons was performed by Kruskal-Wallis analysis. The central tendency of the box plot indicates the median of each group, and the upper and lower ranges of the box plot show the 25<sup>th</sup> and 75<sup>th</sup> percentiles of each data set, respectively.

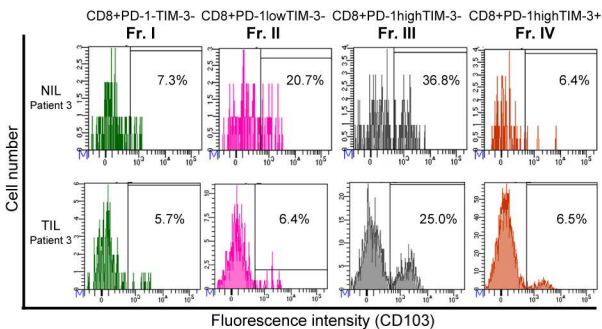
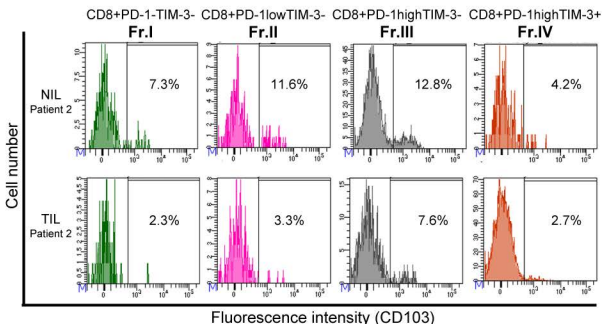
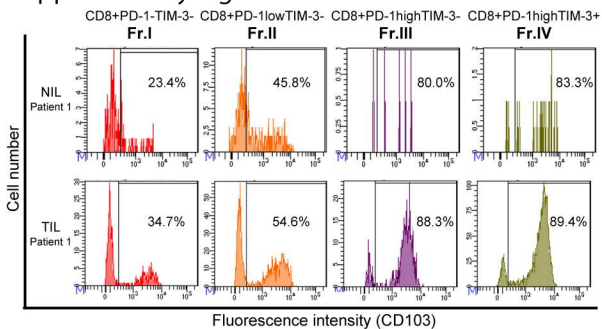
**Supplementary Figure 4.** Comparison of the multiple cytokine productivity (IFN $\gamma$ , TNF $\alpha$ , and IL-2) of CD4 TILs from 12 patients with both high CD4 Fr. V and CD8 Fr. IV stratified by tumour grade within Fr. II, Fr. III, and Fr. V. Comparison of two sample types was performed by Mann-Whitney U test. The central tendency of the box plot indicates the median of each group, and the upper and lower ranges of the box plot

show the 25<sup>th</sup> and 75<sup>th</sup> percentiles of each data set, respectively. \* $P < 0.05$ .

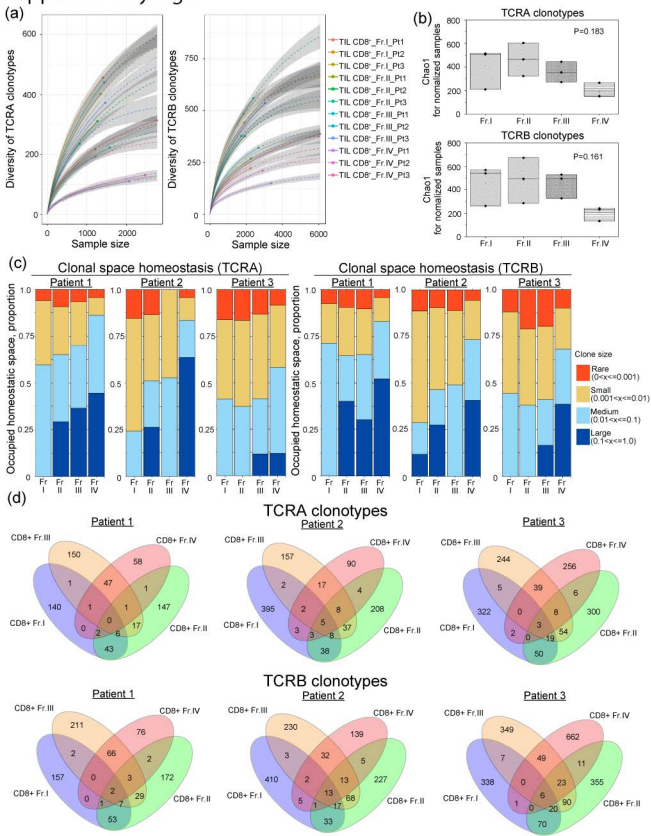
**Supplementary Figure 5. (a)** Table indicating the expression of CD8 Fr. IV and CD4 Fr. V T cells and clinical and pathological items within 10 patients treated with nivolumab monotherapy among 97 patients analysed **(b)**Probability estimates of cancer-specific survival time of the above 10 patients and stratification by expression of dysfunctional populations (upper left), combination of tumour grade and expression of dysfunctional populations (upper right), and tumour grade (lower). Statistical analysis was performed by log-rank test.

**Supplementary Figure 6.** Flowchart illustrating patient cohorts that were evaluated for this analysis.

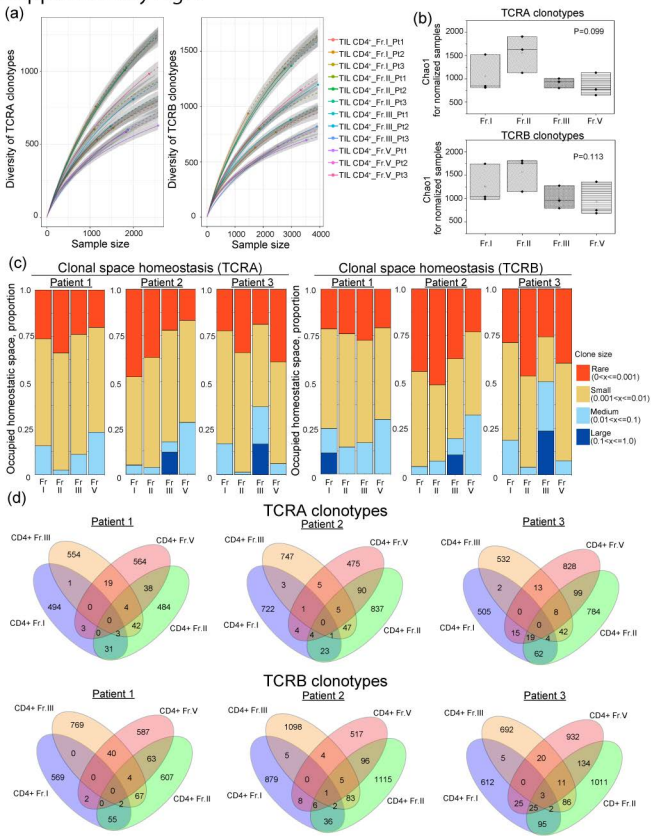
# Supplementary Fig. 1



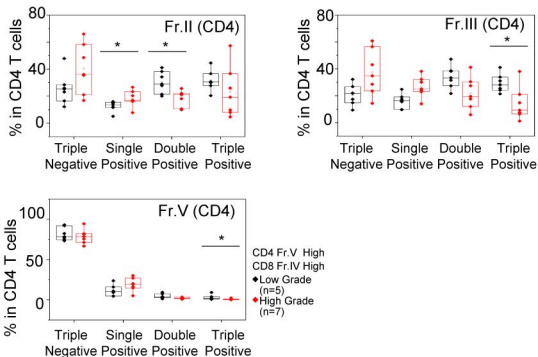
Supplementary Fig. 2



# Supplementary Fig. 3



# Supplementary Fig. 4

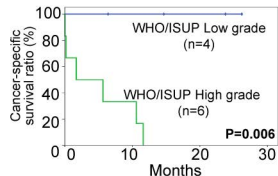
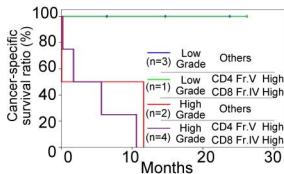
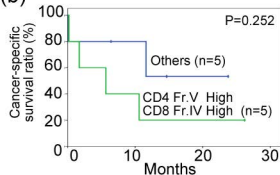


# Supplementary Fig. 5

(a)

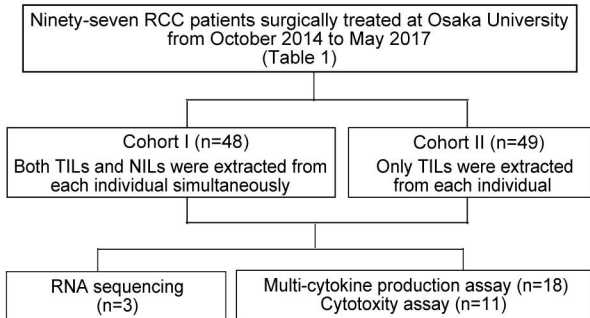
| Sample ID | Age | Gender | WHO/ISUP grade | WHO/ISUP grade | CD8 Fr. IV /CD8 (%) | CD8 Fr.IV | CD4 Fr.V /CD4 (%) | CD4 Fr.V | Coagulative Necrosis |
|-----------|-----|--------|----------------|----------------|---------------------|-----------|-------------------|----------|----------------------|
| URO42     | 63  | Male   | 3              | High           | 3.99                | Low       | 8.99              | High     | Presence             |
| URO115    | 75  | Male   | 2              | Low            | 3.50                | Low       | 8.30              | High     | Presence             |
| URO195    | 60  | Male   | 4              | High           | 68.27               | High      | 10.54             | High     | Presence             |
| URO3      | 80  | Male   | 2              | Low            | 16.88               | Low       | 2.13              | Low      | Presence             |
| URO223    | 65  | Male   | 4              | High           | 65.0                | High      | 18.0              | High     | Absence              |
| URO86     | 67  | Female | 4              | High           | 50.73               | High      | 4.66              | High     | Absence              |
| URO174    | 69  | Female | 2              | Low            | 24.89               | High      | 11.87             | High     | Absence              |
| URO181    | 67  | Female | 3              | High           | 60.01               | High      | 9.15              | High     | Presence             |
| URO204    | 66  | Male   | 3              | High           | 2.52                | Low       | 3.76              | High     | Presence             |
| URO76     | 72  | Female | 2              | Low            | 5.33                | Low       | 0.12              | Low      | Presence             |

(b)

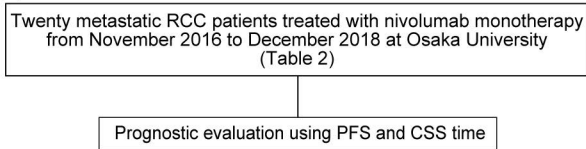


# Supplementary Fig. 6

(a)



(b)



| Supplementary Table 1. Clinical characteristics of the patients ( <i>n</i> = 97) |                      |
|----------------------------------------------------------------------------------|----------------------|
| Age (median) (years)                                                             | 27–82 (66)           |
| Gender                                                                           |                      |
| Male                                                                             | 70                   |
| Female                                                                           | 27                   |
| Histological type                                                                |                      |
| Clear cell                                                                       | 86                   |
| Clear cell with sarcomatoid change                                               | 1                    |
| Sarcomatoid                                                                      | 1                    |
| Papillary                                                                        | 4                    |
| Chromophobe                                                                      | 2                    |
| Mucinous tubular and spindle                                                     | 1                    |
| Unclassified                                                                     | 2                    |
| pT stage                                                                         |                      |
| 1a/1b                                                                            | 43 / 15              |
| 2                                                                                | 6                    |
| 3a/3b/3c                                                                         | 20 / 10 / 3          |
| WHO/ISUP grade                                                                   |                      |
| 1 / 2 / 3 / 4                                                                    | 5 / 63 / 20 / 9      |
| Coagulative necrosis                                                             |                      |
| Absence                                                                          | 72                   |
| Presence                                                                         | 25                   |
| INF                                                                              |                      |
| A                                                                                | 75                   |
| B                                                                                | 22                   |
| pN stage                                                                         |                      |
| 0                                                                                | 92                   |
| I                                                                                | 5                    |
| M stage                                                                          |                      |
| 0                                                                                | 86                   |
| I                                                                                | 11                   |
| Lymphovascular invasion                                                          |                      |
| Yes                                                                              | 31                   |
| No                                                                               | 66                   |
| NLR (mean)                                                                       | 0.82 – 9.68 (2.71)   |
| PLR (mean)                                                                       | 0.26 – 4.65 (1.05)   |
| CRP (median) (mg/dl)                                                             | 0.0 – 18.34 (0.09)   |
| Follow-up time (median) (months)                                                 | 0.49 – 44.19 (23.93) |

Abbreviations: INF = infiltration; NLR = neutrophil to lymphocyte ratio; PLR = platelet to lymphocyte ratio; CRP = C-reactive protein.

| Supplementary Table 2. Clinical characteristics of the 20 patients treated by Nivolumab |                     |
|-----------------------------------------------------------------------------------------|---------------------|
| Age (median) (years)                                                                    | 45 – 81(66)         |
| Gender                                                                                  |                     |
| Male                                                                                    | 15                  |
| Female                                                                                  | 5                   |
| Histological type                                                                       |                     |
| Clear cell                                                                              | 17                  |
| Papillary                                                                               | 1                   |
| Unclassified                                                                            | 2                   |
| WHO/ISUP grade                                                                          |                     |
| 1                                                                                       | 1                   |
| 2                                                                                       | 8                   |
| 3                                                                                       | 6                   |
| 4                                                                                       | 5                   |
| Coagulative necrosis                                                                    |                     |
| Absence                                                                                 | 10                  |
| Presence                                                                                | 10                  |
| MSKCC risk classification                                                               |                     |
| Favourable                                                                              | 2                   |
| Intermediate                                                                            | 11                  |
| Poor                                                                                    | 7                   |
| Number of prior therapies                                                               |                     |
| 1                                                                                       | 7                   |
| 2                                                                                       | 7                   |
| 3                                                                                       | 3                   |
| 4                                                                                       | 0                   |
| 5                                                                                       | 1                   |
| 6                                                                                       | 1                   |
| 7                                                                                       | 1                   |
| NLR (median)                                                                            | 1.44 – 6.90 (3.47)  |
| PLR (median)                                                                            | 0.15 – 3.76 (1.17)  |
| CRP (median) (mg/dl)                                                                    | 0.04 – 17.12 (1.11) |
| Follow-up time (median) (months)                                                        | 0.03 – 30.87 (10.6) |

Abbreviations: MSKCC = Memorial Sloan Kettering Cancer Center; NLR = neutrophil to lymphocyte ratio; PLR = platelet to lymphocyte ratio; CRP = C-reactive protein.