

Figure S1. Semi-quantitative digital immunohistochemical analysis. Following immunohistochemistry, digital analysis was performed averaging the percentage of positive cells compared with all cells on 8 random x40 magnification fields taken from each scanned slide. (A) Representative immunohistochemistry images and (B) representative images of digital analysis. The following formula was used: Percentage of positive cells=(number of positive cells/number of total cells) x100. (A) Scale bar, 800 μm ; magnification, x0.5; (B) scale bar, 20 μm ; magnification, x20. PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T lymphocyte-associated protein-4.

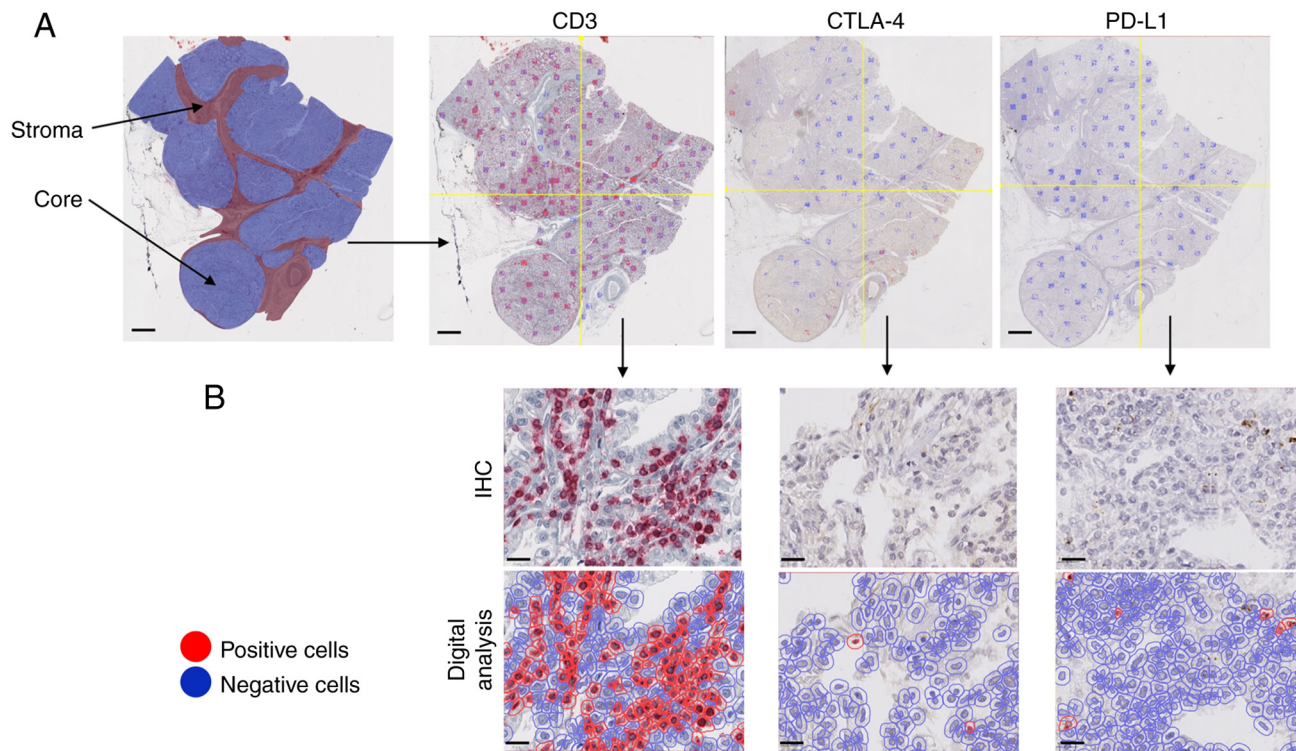


Figure S2. Percentage leukocyte analysis using flow cytometry. Percentages of (A) leukocytes and (B) lymphocytes were evaluated in the blood from healthy controls. (C) Leukocytes and (D) lymphocytes in the blood from patients with clear cell renal cell carcinoma. (E) Infiltrating leukocytes and (F) infiltrating lymphocytes from the tumor tissue. GRA, granulocytes; MO, monocytes, LY, lymphocytes.

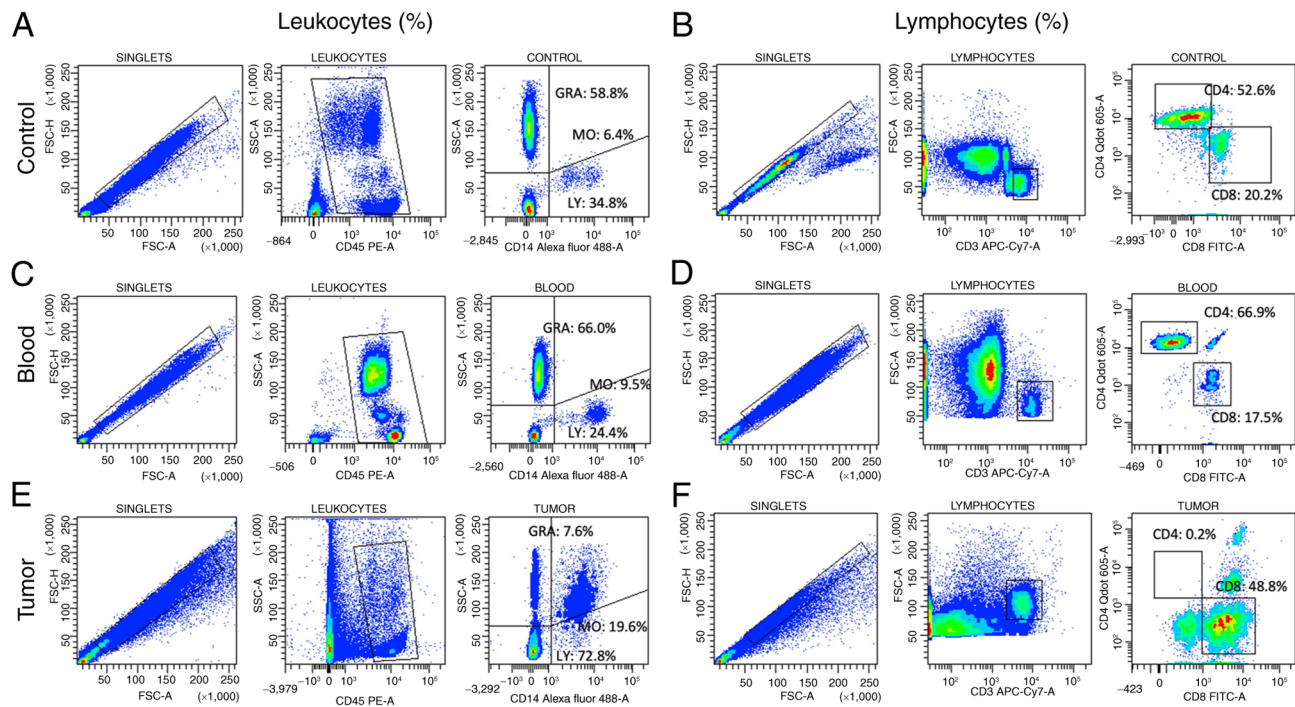


Figure S3. Expression of exhaustion-associated markers using flow cytometry. The expression of exhaustion markers was evaluated using the mean fluorescence intensity gated on lymphocytes, monocytes and granulocytes. Representative histograms are shown for lymphoid markers (A) PD-1, (B) LAG-3, (C) TIM-3 and (D) CTLA-4. (E) PD-L1 expression was evaluated in monocytes and granulocytes. PD-L1, programmed death-ligand 1; PD-1, programmed cell death protein-1; LAG-3, lymphocyte activation gene-3; TIM-3, T-cell immunoglobulin and mucin domain-containing protein-3; CTLA-4, cytotoxic T lymphocyte-associated protein-4; ccRCC, clear cell renal cell carcinoma; Gran, granulocytes; Mono, monocytes.

