Figure S1. Larger overviews of H&E-stained slices. Each section to be stained with H&E was required to contain both clear cell renal cell carcinoma and para-cancer normal tissues. While determining TLS location heterogeneity, at least one occurrence of TLS in the H&E slices was confirmed to be TLS-positive. TLS were defined as tumor-proximal TLS when they were located within the invasive tumor margin, and TLS were defined as tumor-distal TLS when they were located in normal tissue >10 mm from the invasive margin. This identification process was performed independently by a urological surgeon and two pathologists.

H&E, hematoxylin and eosin; TLS, tertiary lymphoid structures.

Figure S2. Impact of TLS presence on the prognosis in patients with ccRCC from the FUSCC and external validation cohorts. Kaplan–Meier and log-rank tests identifying the predictive value of the overall presence of TLS in terms of the prognosis for patients with ccRCC from the FUSCC training (n=290) and external validation cohorts (n=105).

TLS, tertiary lymphoid structures; ccRCC, clear cell renal cell carcinoma; FUSCC, Fudan University Shanghai Cancer Center.

Figure S3. Heterogeneity of TLS maturation and cellular components assessed by mIHC at low magnification. Cellular composition of TLS in heterogeneous ccRCC samples was detected using mIHC in 58 ccRCC tissue samples with two sets of 7-marker multispectral fluorescent immunohistochemistry applied for further analysis at low magnification.

TLS, tertiary lymphoid structures; mIHC, multiplex immunohistochemistry; ccRCC, clear cell renal cell carcinoma.

Figure S4. Tumor-specific TLS properties in localization and maturation heterogeneity associated with differential infiltration of CD8+ T cells in tumor-proximal and tumor-distal areas. Tissue slides that were bound with primary and secondary antibodies but not fluorophores were included as negative controls to assess autofluorescence. Multiplex stained slides were scanned using a Vectra Polaris Quantitative Pathology Imaging System (Akoya Biosciences) at 20 nm wavelength intervals ranging from 440 nm to 780 nm with a fixed exposure time and an absolute magnification of 200×. All scans for each slide were then superimposed to obtain a single image. Multilayer images were imported to inForm v.2.4.8 (Akoya Biosciences) for quantitative image analysis. Tumor-proximal and tumor-distal regions were differentiated using Pan-CK staining. The quantities of various cell populations were expressed as the number of stained cells per square millimeter and as the percentage of positively stained cells in all nucleated cells. The Vectra multi-spectral imaging analysis system combined with the inForm image analysis software was used to obtain images with a high signal-to-noise ratio (excluding background spontaneous fluorescence) and conduct accurate batch quantitative analysis, provide accurate quantification of various biomarkers in each cell, and analyze the percentage of specific cell types in the number of DAPI-positive cells. Non-hierarchical clustering
of the heatmap was used to show the fraction of CD8+ T cells infiltration (proportion per hundred DAPI-positive cells) in tumor-proximal and tumor-distal areas, as well as the CPS score of PD-L1 expression and heterogeneity of TLS in localization and maturation.

TLS, tertiary lymphoid structures; CD, cluster of differentiation; CK, cytokeratin; DAPI, 4′,6-diamidino-2-phenylindole; CPS, combined positive score; PD-L1, programmed death-ligand 1.

**Figure S5. The heatmap of tumor-specific TLS associated with different TME characteristics vertically ordered by the TLS maturation group.** Non-hierarchical clustering in the heatmap reveals the fraction of lymphocytic infiltration (proportion per hundred cells) in the tumor-proximal and tumor-distal regions, as well as the heterogeneity of TLS in terms of the localization and maturation stages.

TLS, tertiary lymphoid structures; TME, tumor microenvironment

**Figure S6. The heatmap of tumor-specific TLS associated differential infiltration of CD8+ T cells vertically ordered by the TLS maturation group.**

TLS, tertiary lymphoid structures