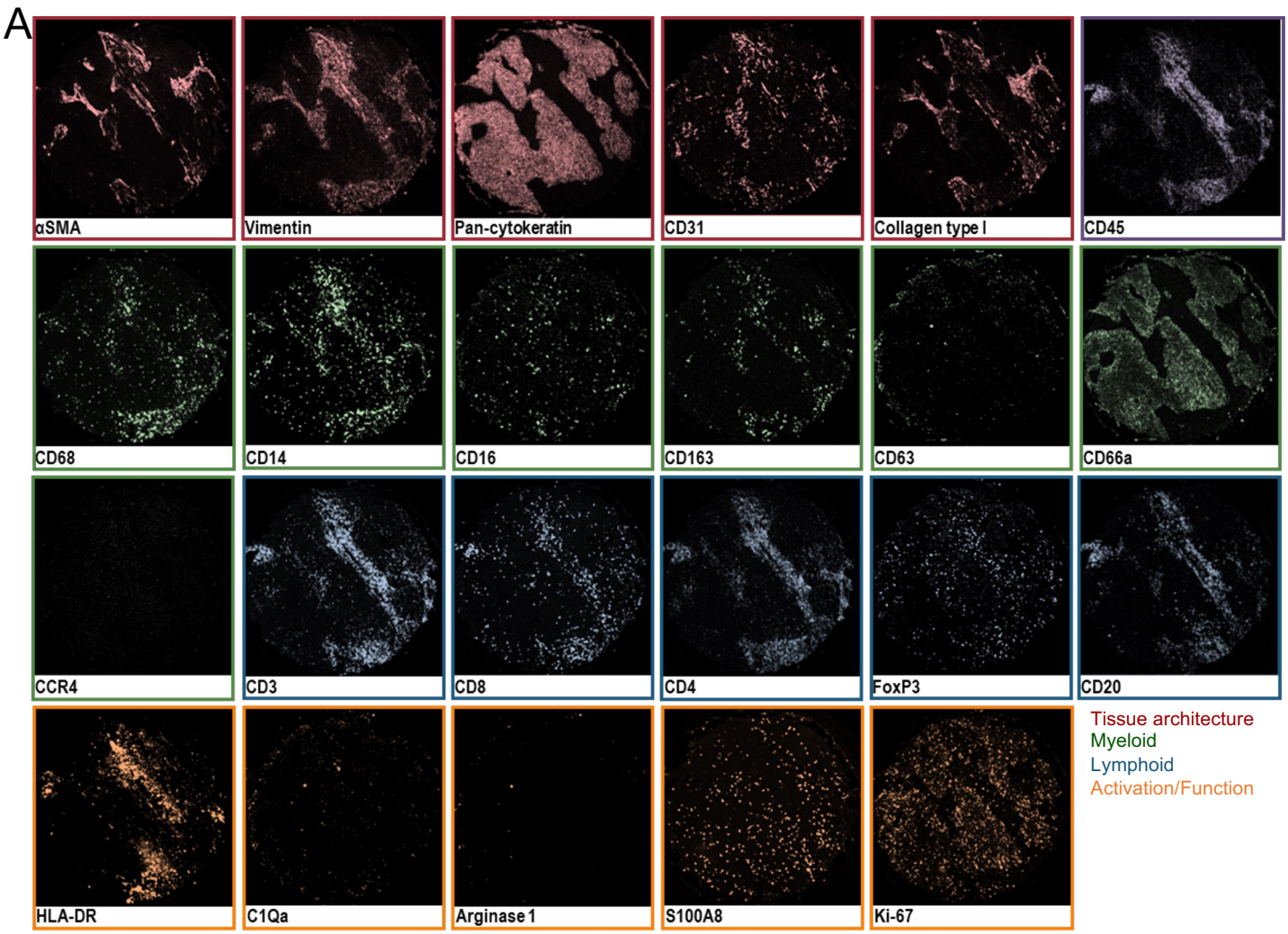
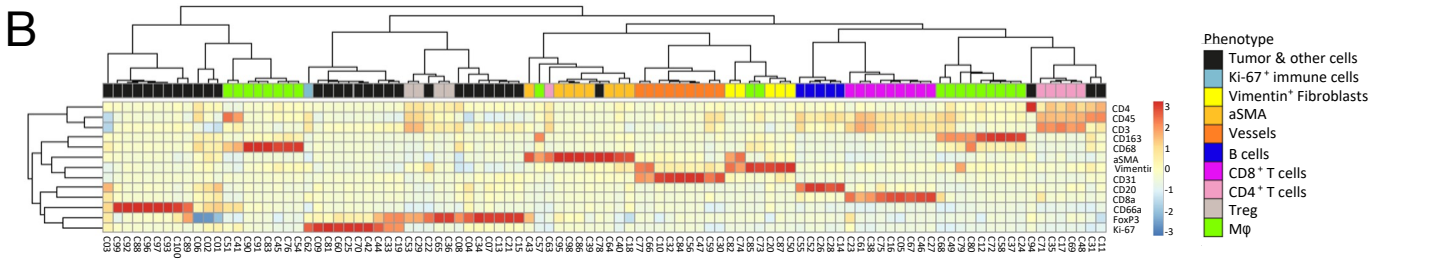
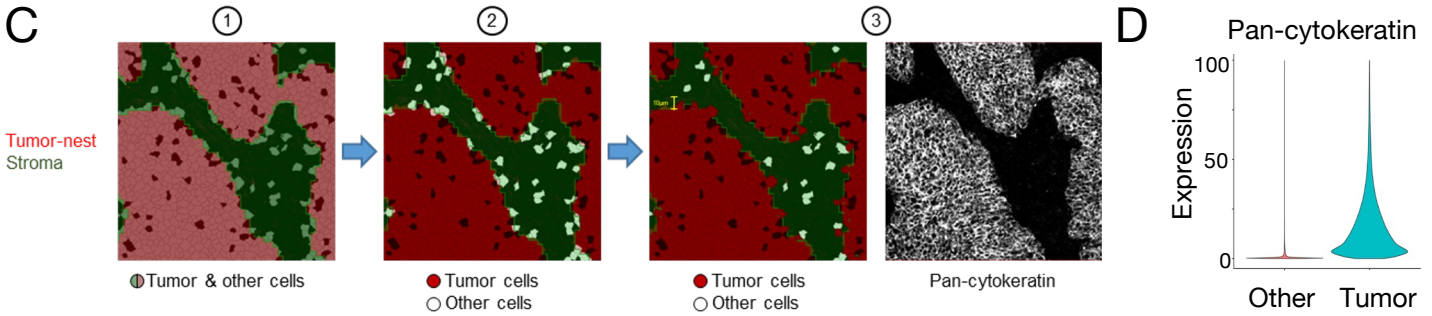
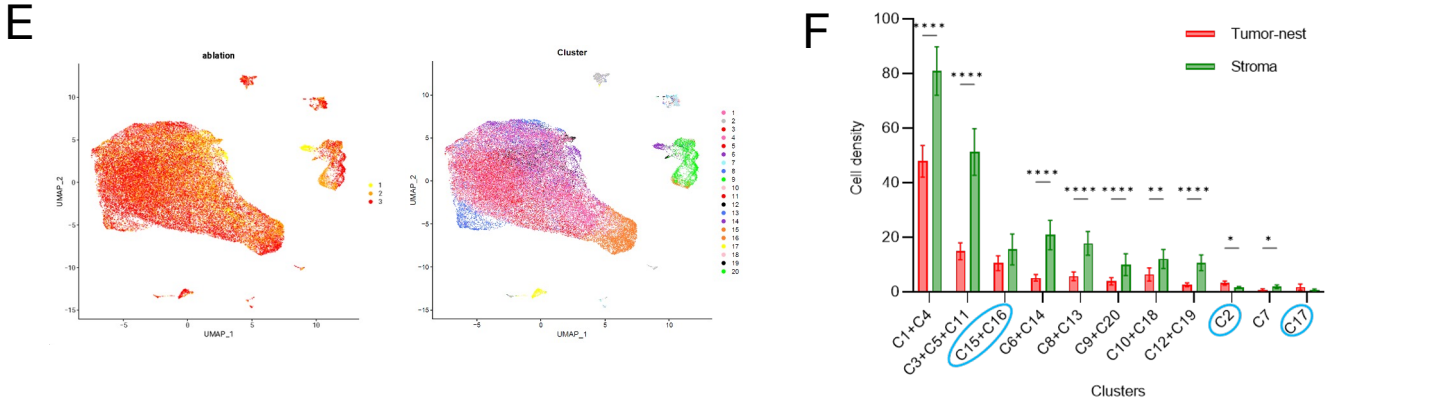

A
aSMA
Vimentin
Pan-cytokeratin
CD31
Collagen type I
CD45
CD68
CD14
CD16
CD163
CD63
CD66a
CCR4
CD3
CD8
CD4
FoxP3
CD20
HLA-DR
C1Qa
Arginase 1
S100A8
Ki-67
Tissue architecture
Myeloid
Lymphoid
Activation/Function


B
Phenotype
Tumor & other cells
Ki-67+ immune cells
Vimentin+ Fibroblasts
aSMA
Vessels
B cells
CD8+ T cells
CD4+ T cells
Treg
Mφ


C
Tumor-nest
Stroma
Tumor & other cells
Tumor cells
Other cells
Tumor cells
Other cells
Pan-cytokeratin
D
Pan-cytokeratin
Expression
100
50
0
Other
Tumor


E
ablation
Cluster
UMAP_1
UMAP_2
F
Tumor-nest
Stroma
Cell density
Clusters
C1+C4
C3+C5+C11
C15+C16
C6+C14
C8+C13
C9+C20
C10+C18
C12+C19
C2
C7
C17

Supplementary Figure 2. (**A**) IMC images of one representative TMA spot, stained with 23 markers related to tissue architecture (red), myeloid cells (green), lymphoid cells (blue) and immune activation (orange). (**B**) Heatmap showing the mean values of 13 selected lineage markers adopted for cell cluster annotation. Cells were over-clustered into 100 groups by the NN Self Organizing Map algorithm, on the basis of their marker expression. Proteins and cell clusters are ordered by hierarchical clustering with the Pearson's correlation distance. Protein expression is colored-coded from blue (lower) to red (higher) and scaled by column. Each cluster, annotated according to the phenotype, is then merged with the ones similar, resulting into 11 final cell populations. (**C**) Adjustement of the phenotyping to avoid misclassification of cells along the borders of the tumor region. After tissue segmentation (1), cells in the stromal region and located at a distance less than 10 μm from the tumor border (2-3), which had been annotated as "tumor" or "other" were re-classified as tumor cells. Pan-cytokeratin staining confirms the annotation (4). (**D**) Pan-cytokeratin expression level in cells classified as "other" and "tumor". (**E**) Batch effect analysis, to evaluate the effect of different laser ablations. Samples were classified as 1, 2 or 3, according to the level of ablation visually estimated (1, poorly; 2, average; or 3, highly ablated, respectively). UMAP plots show the cells classified according to the abalation level (left) or to cluster assignment (right). (**F**) Tissue localization of macrophage clusters (c1-c20) in the tumor or stroma regions. *$p<0.05$; ** $p<0.01$; ***$p< 0.005$; ****$p<0.001$ by Mann Whitney test.