Supplementary Figure 1. Flow cytometry gating strategy for identifying DCs, monocytes and CD8+ T cells

Live cells or peripheral blood mononuclear cells (PBMCs) were first gated by the forward and side scatter areas, and doublets were then excluded by gating with the forward scatter area and height. Leukocytes were gated by CD45+ cells. Cells in the leukocyte gate were further gated based on lineage-HLA-DR+ cells (DCs), CD3+CD8+ T cells, and CD3-CD14+ cells (monocytes).

Supplementary Figure 2. Functional markers of CD8+ TILs after PD-1 blockade and differential gene selection of CD96+CD8+ TILs

A. The percentages of tumor apoptosis and the expression of effector cytokines, proliferation markers, and cytolytic markers on CD45+CD8+ TILs after treatment with αPD-1 or the IgG control for 24 hours (n=14).

B. Representative dot plots showing the CD96 expression on CD45+CD8+ TILs from CC specimens that were insensitive or sensitive to αPD-1. The data are shown as iR expression before αPD-1 treatment.

C. Volcano plot showing significantly differential gene expression between the single-cell CD96+CD8+ T cell cluster and the CD96-CD8+ T cell cluster.

D. Gate strategy for identification of non-immune cells, B cells, CD4+ and CD8+ T cells, and non-B/T cells.

E. The fractions of different cell populations among CD96+ or ZNF683+ cells in P39, P40, P42 IT samples.

P values were obtained by the Wilcoxon matched-pairs signed-ranks test.

Supplementary Figure 3. Schematic of the experimental strategy for Figures 2-5

A. Schematic representation of the experimental strategy for Figures 2 and 3. Fifteen IT specimens (P15-P29), 5 paired PT specimens (P17, P18, P21, P23, P26) and 5 paired blood samples (P17, P19, P21, P23, P26) were collected. The phenotypes of CD8+ T cells from blood samples, PT samples and half of the bisected IT samples were analyzed by flow cytometry.

B. Schematic of the ex vivo treatment strategy for Figure 4. Single-cell suspensions from the other half of the bisected IT specimens (P15-P29, P38-P42) were incubated ex vivo for 24 hours with αPD-1 and/or αCD96 or isotype IgG control. The treated cells were analyzed for tumor apoptosis and the CTL response (P15-P29), and for CD96 or PD-1 expression (P15-P29, P38-P42) by flow cytometry.

C. Schematic representation of the experimental strategy for Figure 5. Thirteen paired blood, IT, and PT specimens from CC patients (P30-P42) were collected. Single cells from blood specimens, PT specimens and IT specimens were analyzed by flow cytometry (P30-P42). Bulk PT (P30, P34, P35) and IT (P17, P18, P20, P21, P22, P24, P26, P27, P28, P29) tissues were analyzed by immunohistochemistry and immunofluorescence.

Wang Y, *et al. J Immunother Cancer* 2022; 10:e003667. doi: 10.1136/jitc-2021-003667

Supplementary Figure 4. The association of CD96 expression with clinical characteristics

A. Percentages of CD96+ cells in CD45+CD8+ TILs from CC patients classified by different International Federation of Gynecology and Obstetrics (FIGO) stages and histological types (n=29, P1-P29).

B. Percentages of PD-1+ and PD-1- cells in CD45+CD8+CD96+ T cells from blood (n=3, P19, P21, P26), PT (n=5, P19, P21, P26) and IT (n=15, P15-P30) samples.

C. Percentages of CD96+ cells in CD45+CD8+ T cells from αPD-1-sensitive (n=7) and αPD-1-insensitive (n=22) samples. The data are presented as CD96 expression before αPD-1 treatment.

D. Representative dot plots showing CD96 and PD-1 expression on CD45+CD8+ TILs from CC specimens that were insensitive or sensitive to αPD-1. The data are presented as iR expression before αPD-1 treatment.

P values were obtained by the Kruskal–Wallis test (A), Wilcoxon matched-pairs signed-ranks test (B), and Mann–Whitney U test (C). *P < 0.05; **P < 0.01; ***P < 0.001.

Supplementary Figure 5. Characterization of transcription and functional molecules on CD96+PD-1+CD8+ TILs in αPD-1-sensitive or αPD-1-insensitive samples

Percentages of the expression levels of the indicated markers on PD-1+CD96+CD8+ TILs from CC specimens that were insensitive (n=12) or sensitive (n=3) to αPD-1. The data are presented as marker expression before αPD-1 treatment.

P values were obtained by the Mann–Whitney U test. *P < 0.05; **P < 0.01; ***P < 0.001.

Supplementary Figure 6. Analysis of CD8+ TIL function following PD-1 and CD96 blockade treatment *ex vivo*

A. Apoptosis of tumor cells from CC specimens after treatment with αPD-1 and/or αCD96 or the IgG control for 24 hours (n=15).

B-D. Percentages of the expression levels of effector cytokines (B), proliferation markers (C), and cytolytic markers (D) on CD45+CD8+ TILs after treatment with αPD-1 and/or αCD96 or the IgG control for 24 hours (n=15).

E. Fold changes in tumor apoptosis and CTL response after treatment with αPD-1 and/or αCD96 or the IgG control for 24 hours between αPD-1-sensitive specimens (n=3) and αPD-1-insensitive specimens (n=12). Sensitivity and insensitivity were distinguished with a fold change cutoff of 1.5 in both analyses.

F. Percentages of CD96-positive cells among CD45+CD8+ TILs after treatment with αPD-1 or the IgG control for 24 hours between αPD-1-sensitive specimens (left, n=3) and αPD-1-insensitive specimens (right, n=12).
G. Percentages of PD-1-positive (left) and CD96-positive (right) cells among CD45+CD8+ TILs after treatment with αPD-1, αCD96 or the IgG control for 24 hours (n=5).
P values were obtained by the Friedman test (A, B, C, D), the Kruskal–Wallis test (E), and the Wilcoxon matched-pairs signed-ranks test (F, G). *P < 0.05; **P < 0.01; ***P < 0.001.

Supplementary Figure 7. The expression of CD111 and CD112 in CC patients
A. Quantification of the CD111 (left) and CD112 (right) positive ratios per field for PT (n=3) and IT (n=10) specimens. Three fields of each slide were randomly picked.
B. Quantification of the CD111 (left) and CD112 (right) positive ratios per field for αPD-1-sensitive IT (n=3) and αPD-1-insensitive IT (n=7) specimens. Three fields of each slide were randomly picked.
C. Representative immunohistochemistry images of CD111+ (A) and CD112+ (E) cells in PT, αPD-1-sensitive IT, and αPD-1-insensitive IT specimens. Original magnifications: X200 (upper) and X400 (bottom).
D. Representative immunofluorescence images of CD111 (D) and CD112 (H) in PT, αPD-1-sensitive IT, and αPD-1-insensitive IT specimens. Original magnifications: X200 (left) and X400 (right).
Experiments were performed independently for each specimen. P values were obtained by Mann–Whitney U test. *P < 0.05; **P < 0.01; ***P < 0.001.

Supplementary Figure 8. Flowcytometry analysis of functional markers of CD45+CD8+TILs following PD-1 and CD96 blockade treatment ex vivo
Representative histogram overlays depicting the expression of Ki67, IL-2, IFN-γ, TNF-α, CD107a, GZMB, and PRF1 in CD8+ TILs from patients who received αPD-1 and/or αCD96 or isotype IgG control treatment ex vivo.