Supplementary Material and Methods

1 Immunofluorescence staining

Tissue samples obtained prior to any treatment at initial endoscopy or surgery were immediately frozen and stored at –80°C. Frozen specimens were sectioned at 4-6 µm with a cryostat, placed on slides, air dried and fixed for 10 minutes with 100% acetone.

Before incubation with primary antibodies, the slides were treated with avidin/biotin blocker (Vector Laboratories, Burlingame USA) and Fc receptor blocked by human serum (5%).

The antibodies used for the various immunofluorescence stainings are described in Supplementary Table S2.

The various antibodies were diluted in PBS. Isotype-matched antibodies were used as negative controls. In each case, we checked that the secondary antibodies did not cross-react with the isotype or species of the other primary antibody immunoglobulin in the double immunofluorescence technique.

Fluorescent images of mounted sections were analyzed with an epifluorescence microscope DMR (Leica Microsystems, Wetzlar, Germany).

Semiquantitative assessment of CD4, CD8 and PD-L1 staining was scored as 0 (less than 10% positive cells), + (10-20% positive cells), ++ (20-50% positive cells), +++ (more than 50% positive cells) based on reference staining slides using a high magnification (x400).

For double immunofluorescence staining, a quantitative measurement of PD-1^{+}CD4^{+}, PD-1^{+}CD8^{+} and Foxp3^{+}CD4^{+} tumor-infiltrating cells was performed in at least four random high power fields (x400). For quantitative variable, the median was used as a cut-off.Two authors (CB and ET), blinded for clinical data, independently scored the slides.

2 Flow cytometry analysis

In Mice

To detect anti-$E7_{49–57}/D^b$-specific $CD8^+$ tumor-infiltrating T cells, tumors were dissociated, washed twice in PBS and incubated with the Fc receptor block CD16/CD32 (eBioscience). They were then stained with the PE labeled $E7_{49–57}/D^b$ tetramer, according to the manufacturer's recommendations (Beckman Coulter Immunomics. Marseilles. France). Briefly, cells were incubated at 4°C with the PE-labeled tetramer for 35 minutes. After incubation and washes, labeled anti-CD8 mAbs (eBioscience. Paris. France) were used to phenotype the tetramer-positive $CD8^+$ T cells. Irrelevant tetramers recognizing a LCMV derived peptide in the context of D^b molecules were used in each experiment. Naive non immunized mice were also included as controls for these experiments.

For the analysis of PD-1 expression, tetramer positive $CD8^+$T cells were co-stained with anti mouse PD-1 FITC mAb (eBioscience. Paris, France) and Live/Dead (Invitrogen. Cergy Pontoise, France) to exclude dead cells. PE labeled PD-L1 mAb (ebiosciences) were used to analyze PD-L1 expression. Isotype controls were included in each experiment.

Analyses were performed in a BD Biosciences LSR II or BD Biosciences FACSCalibur with FlowJo (Tree Star) or Cell Quest software (BD Biosciences).

In humans

After dissociation of biopsies by DNAse I (30 IU/mL, Roche) and Collagenase D (1mg/mL, Roche) for 60 min, cells were stained with a fixable viability Dye eFluor780 (eBioscience). Live T cells were then stained with CD45 PE-Cy7 (Beckman Coulter), CD3 Alexa Fluor700 (Becton Dickinson), CD4 Pacific Blue (Beckman Coulter), CD8 FITC (Becton Dickinson), PD-1 APC (Becton Dickinson), HLA-DR PE (Becton Dickinson) and CD38 PE (Becton Dickinson). Isotype control antibodies were included in each experiment.