**Supplementary Methods**

**Whole-exome sequencing**

Whole-exome sequencing reads, which were masked nucleotides with a quality value less than 20, were independently aligned to a human reference genome (hg38) using BWA (<http://bio-bwa.sourceforge.net/>) and Bowtie 2 (<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>). Both somatic synonymous and nonsynonymous mutations were called using our in-house caller and two publicly available mutation callers: Genome Analysis Toolkit (<https://gatk.broadinstitute.org/hc/en-us>) MuTect2 and VarScan 2 (<http://varscan.sourceforge.net/>). Mutations were discarded if any of the following criteria were met: total read number < 20, variant allele frequency in the tumor samples < 0.05, mutant read number in the germline control samples > 2, mutation occurring in only one strand of the genome, or the variant present in normal human genomes in either the 1000 Genomes Project dataset (<https://www.internationalgenome.org/>) or our in-house database. Gene mutations were annotated by SnpEff (<https://pcingola.github.io/SnpEff/>).

The copy number status was analyzed using our in-house pipeline, which determines the logR ratio (LRR) as follows: (1) we selected SNP positions in the 1000 Genomes Project database that were in a homozygous state (variant allele frequency (VAF) 0.05 or 0.95) or a heterozygous state (VAF 0.4–0.6) in the genomes of respective normal samples, (2) normal and tumor read depths at the selected position were adjusted based on the G+C percentage of a 100 bp window flanking the position, (3) we calculated the LRR = , where and are normal and tumor adjusted depths at position , and (4) each representative LRR was determined by the median of a moving window (1 Mb) centered at position . Based on the calculated LRR values, allele-specific copy numbers were determined with FACETS (Shen and Seshan 2016).

**Multiplex fluorescence IHC**

Tissue sections (4-μm thick) were obtained from archived FFPE tissue specimens. The levels of CD3, CD4, CD8, CD204, cytokeratin, and PTEN protein expression in FFPE samples were assessed using IHC with anti-CD3 (SP7; Abcam), anti-CD4 (4B12; Novocastra), anti-CD8 (4B11; Novocastra), anti-CD204 (SRA-E5; Abcam), cytokeratin (AE1+AE3; Dako), and anti-PTEN (6H2.1; Cascade Bioscience) antibodies. Antibodies were labeled with a horseradish peroxidase (HRP)-conjugated secondary antibody (Envision plus, DAKO), and then, fluorophore-conjugated tyramide (Opal IHC kit, PerkinElmer) was fixed on the FFPE sections by the HRP enzymatic reaction. Images were captured using an automated multisector imaging system (Vectra version 3.0; PerkinElmer). An image analysis program (Inform; PerkinElmer) was used to segment tumor tissues into carcinoma and stromal areas and to detect immune cells with specific phenotypes. Before performing the final evaluation, training sessions for tissue segmentation and phenotype recognition were carried out repeatedly until the algorithm reached the level of confidence recommended by the program supplier (at least 90% accuracy). After phenotyping typical CD4+ and CD8+ cells using Inform software, gated CD3+ populations by the mean fluorescence intensity of CD3, CD3+CD4+, and CD3+CD8+ cells were determined as CD4+ T cells and CD8+ T cells, respectively. A similar gating strategy was used for the analysis of the CD204+ population. The area of each carcinoma was evaluated to assess the density of lymphocytes, represented by (number of lymphocytes)/(mm2) in each tumor. Precise calculation methods for the density of lymphocytes was mentioned in a previous report(1). In addition, the PTEN-positive and -negative carcinoma areas in sections were segmented by Inform software, and the percentage of the PTEN-positive area in the carcinoma was assessed, calculated as the ratio of the PTEN-positive area (mm2) per total carcinoma area (PTEN-positive area plus -negative carcinoma area, mm2).

Reference

1. Imaizumi K, Suzuki T, Kojima M, Shimomura M, Sakuyama N, Tsukada Y, et al. Ki67 expression and localization of T cells after neoadjuvant therapies as reliable predictive markers in rectal cancer. Cancer Sci. 2020;111:23–35.