

Supplementary methods

Hematoxylin and eosin staining. Tumor specimens were fixed with 4% paraformaldehyde at room temperature for 12 h. The formalin-fixed tissues were then embedded in paraffin and cut into sections of 4 μ m in thickness. The formalin-fixed and paraffin-embedded (FFPE) sections were stained with hematoxylin and eosin for 5 min at room temperature and then observed under a light microscope (Motic Corporation).

Immunohistochemical staining. Tissue specimens were fixed with 4% paraformaldehyde at room temperature for 12 h. The formalin-fixed tissues were then embedded in paraffin, and cut into sections of 4 μ m in thickness. The FFPE sections were deparaffinized, rehydrated and antigen retrieval was performed in Tris-EDTA buffer. Thereafter, the endogenous peroxidases were inactivated using 3% H₂O₂ at room temperature for 10 min. The membranes were then blocked with 5% BSA for 1 h at room temperature. Afterward, those sections were incubated with anti-CK7 (1:100; cat. no. 55658; Cell Signaling Technology, Inc.), anti-TP53 (1:200; cat. no. 2527; Cell Signaling Technology, Inc.), anti-MLH1 (1:100; cat. no. ab223844; Abcam), anti-MSH2 (1:200; cat. no. 2017; Cell Signaling Technology, Inc.), anti-MSH6 (1:500; cat. no. 12988; Cell Signaling Technology, Inc.) or anti-PMS2 (1:500; cat. no. ab110638; Abcam) antibodies at 4°C overnight. Next,

they were incubated with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit IgG; 1:1,000; cat. no. ab6721; or goat anti-mouse IgG; 1:2,000; cat. no. ab205719; both Abcam) at room temperature for 1 h, and then subjected to the DAB substrate kit (cat. no. 8059; Cell Signaling Technology, Inc.). Finally, the sections were counterstained with hematoxylin and observed under a light microscope (Motic Corporation).

Next-generation sequencing. The panel used in the present study covered 520 cancer-related genes, including nearly all targets of current cancer-targeted therapies (OncoScreen Plus[®]; Burning Rock Biotech, Ltd.). Genomic DNA was extracted from FFPE samples using the QIAamp DNA FFPE tissue kit (Qiagen, Inc.) according to the manufacturer's instructions. DNA concentrations were measured through the Qubit dsDNA assay (Life Technologies; Thermo Fisher Scientific, Inc.). The quality of the fragments was assessed with a high sensitivity DNA kit using Bioanalyzer 2100 (Agilent Technologies, Inc.). A minimum of 50 ng DNA was required for NGS library construction. The 520 genes were captured and sequenced using a TG NextSeq[™] 500/550 High Output Kit v2.5 (300 cycles; cat. no. 20024913; Illumina, Inc.) with pair-end reads. The sequence data was analyzed using proprietary computational algorithms optimized for accurately identifying somatic and germline mutations.