

Supplementary materials

Tissues were fixed with 10% neutral buffered formalin for 96 h at room temperature (RT). H&E staining was performed with Tissue-Tek DRS 2000 automated slide stainer (Sakura Finetek Japan Co., Ltd.). Slides were stained with hematoxylin for 3 min and with eosin for 2 min at RT. Immunohistochemistry was performed with Benchmark XT immunostainer (Roche Diagnostics). The specimens were incubated for 32 min at 37°C with the following primary antibodies (Abs): anti-CK7 Ab (790-4462; Roche Diagnostics), anti-CK20 Ab (790-4431; Roche Diagnostics),

anti-CDX2 Ab (760-4380; Roche Diagnostics) and anti-TTF-1 Ab (790-4756; Roche Diagnostics). The processes of blocking and labeled streptavidin biotin method were performed using I-View DAB universal kit (760-041; Roche Diagnostics). All the protocols including reagent dilution were performed according to manufacturer instructions. The blocking and incubation time with the biotinylated second antibody was 8 min at RT, respectively. Images of stained specimens were acquired with the BX53 microscope system (Olympus). The PCR mutation analysis experiments including BRAF V600E mutations were performed by an external laboratory (SRL, Inc.).