Figure S1. Difference in mutation burden between LN-positive and LN-negative groups. (A) Difference in baseline mutation burden between LN-positive and LN-negative groups. One column contains a repeated value, preventing application of the Mann-Whitney test. (B) Differences in mutation burden for the initial post-radiation plasma samples of LN-positive and LN-negative groups. The P-value was determined using a Mann-Whitney test. The bars represent the median mutation burden. LN, lymph node.

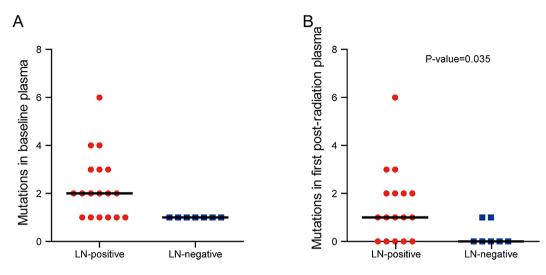


Figure S2. PFS and OS time analysis of patients stratified by baseline mutation burden. Kaplan-Meier plots of (A) PFS and (B) OS times for patients with esophageal squamous cell carcinoma treated with radiation therapy stratified by mutation burden in baseline plasma. PFS, progression free survival; OS, overall survival; HR, hazard ratio.

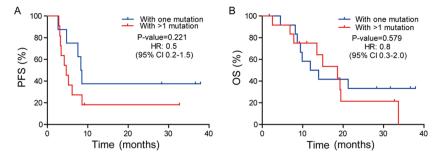


Figure S3. ctDNA tracking in plasma samples prior to and following radiation therapy. The mutation and biomarker (CEA, CA19-9, and CA72-4) tracking profiles in 6 patients (P01, P10, P13, P14, P15 and P19) are shown. For all patients, the initial time point was set at baseline and the other time points were set during follow-up visits. The solid-colored lines indicate ctDNA or biomarkers and the black dotted line indicates the threshold of the ctDNA or biomarker. The light blue block represents the radiation period. The black arrow indicates progressive disease confirmed by CT at that time point. ct, circulating tumor; VAF, variant allele fraction.

