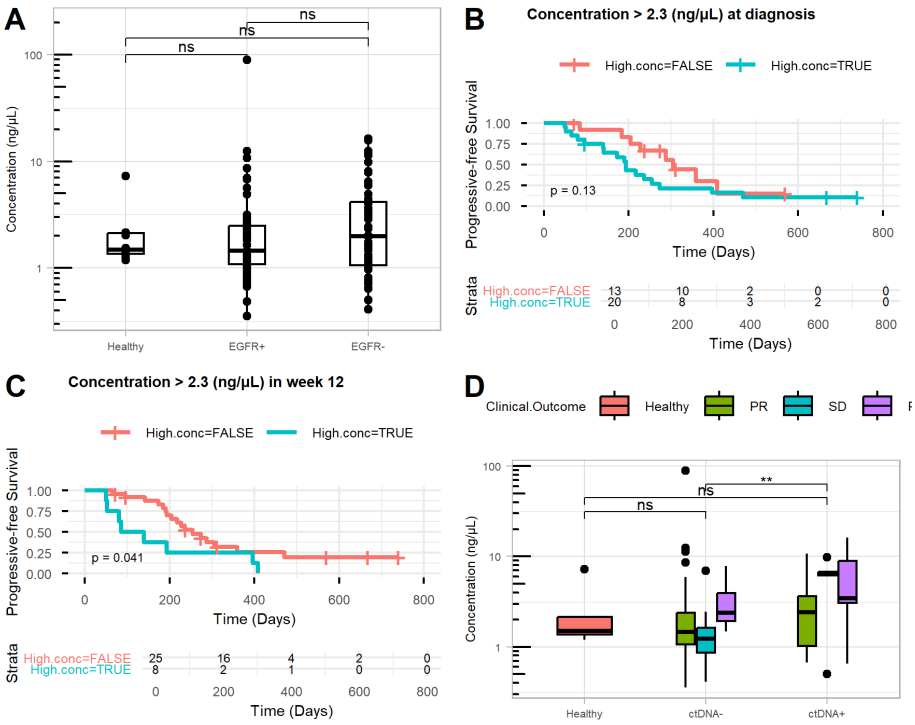
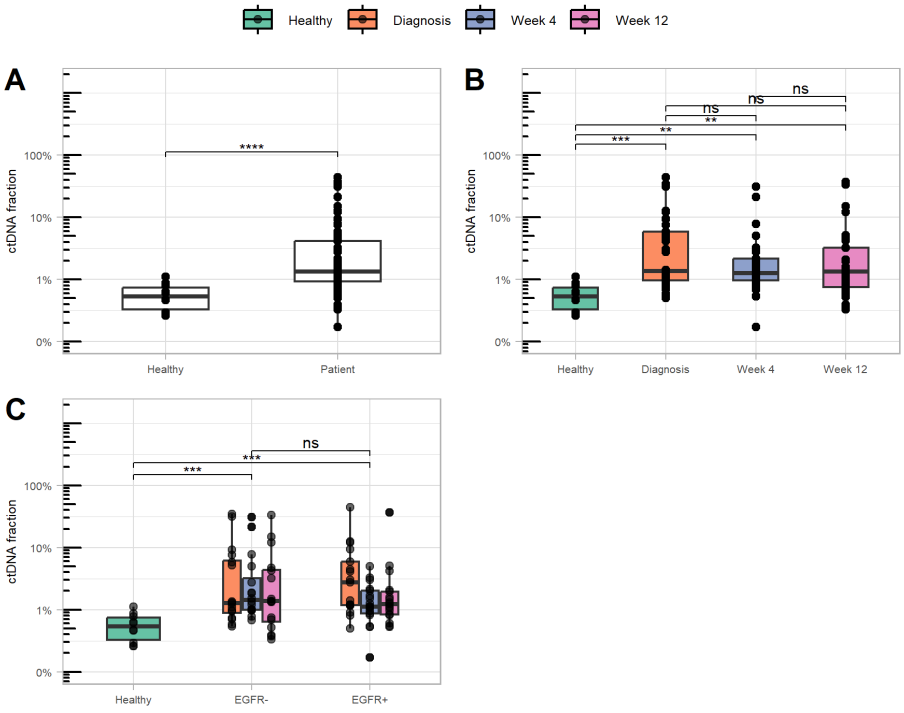
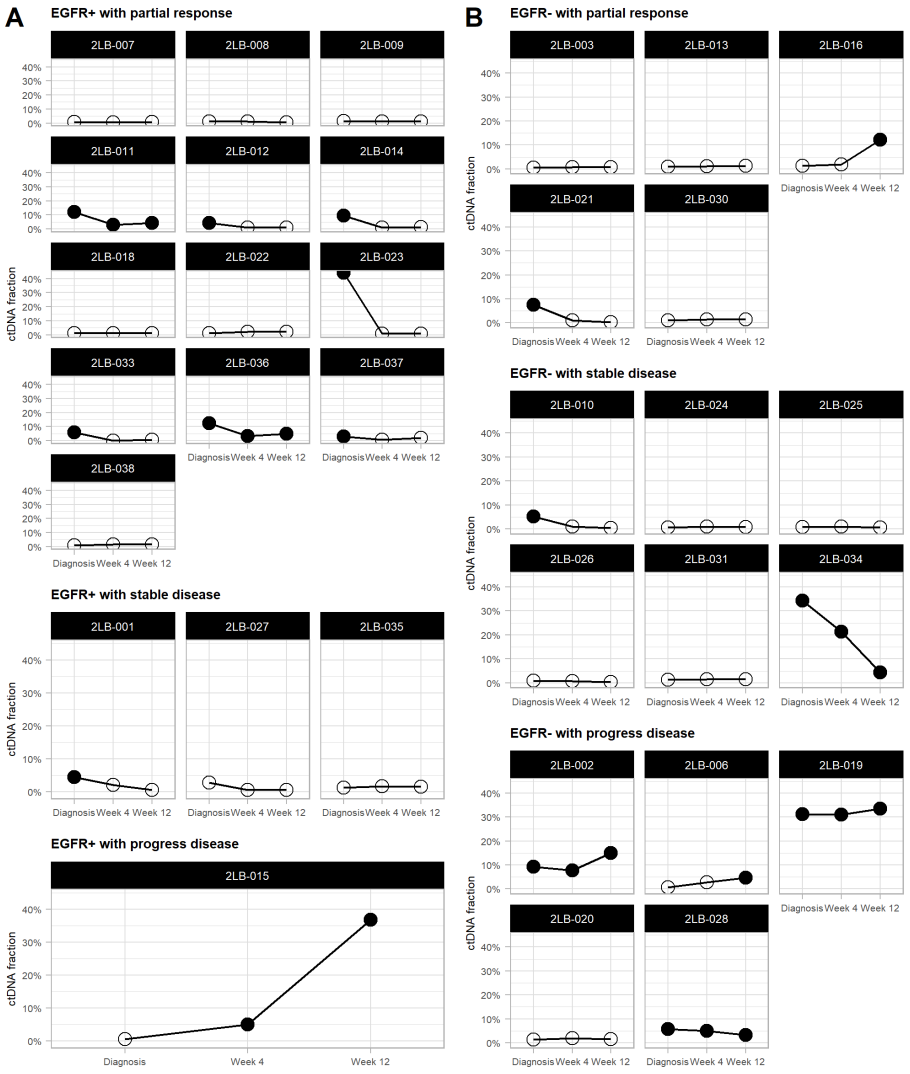
Supplementary Figures



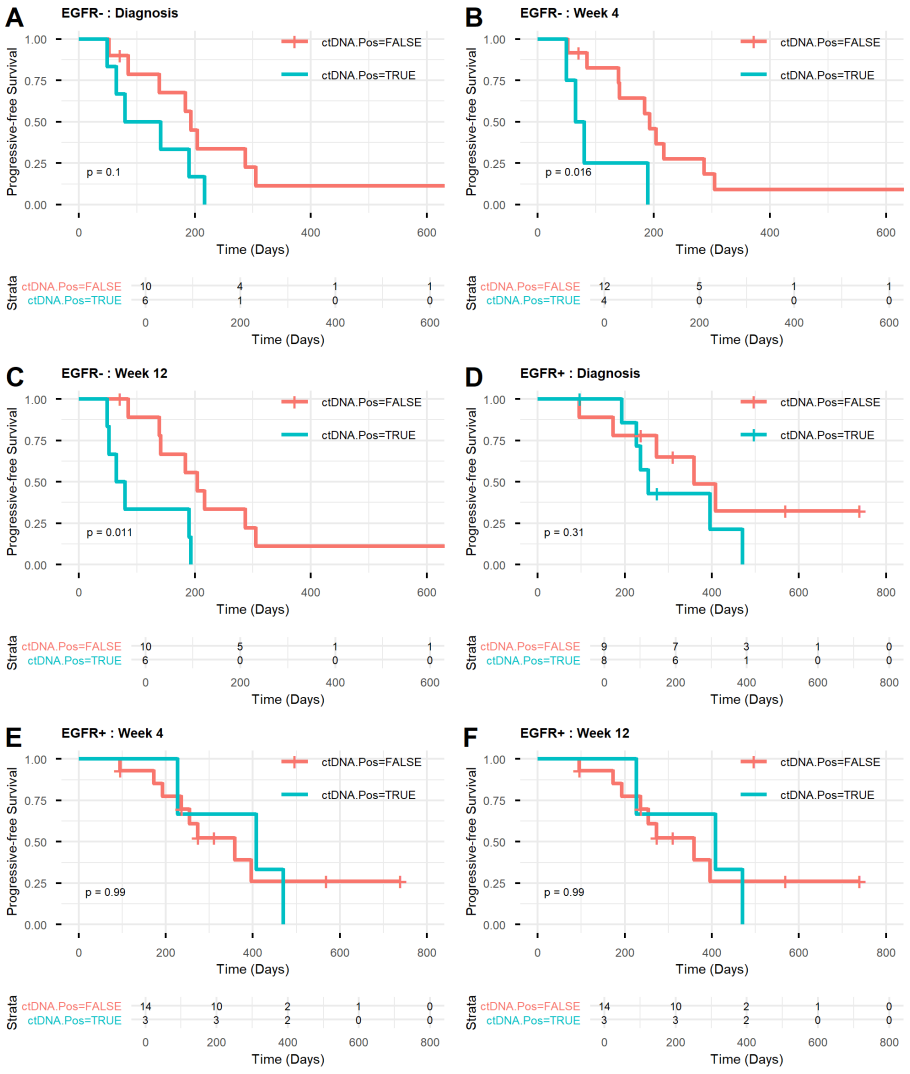
Supplementary Figure 1: The median cfDNA concentrations in patients with EGFR+ and EGFR- tumors were similar (A). The cfDNA concentration at diagnosis was not significantly associated with PFS (B). It was significantly associated with PFS at week 4 (Figure 1D) and week 12 (C). Higher cfDNA concentrations were found among ctDNA+ patients, especially those with PD (D). PR: partial response, SD: stable disease, PD: progressive disease.



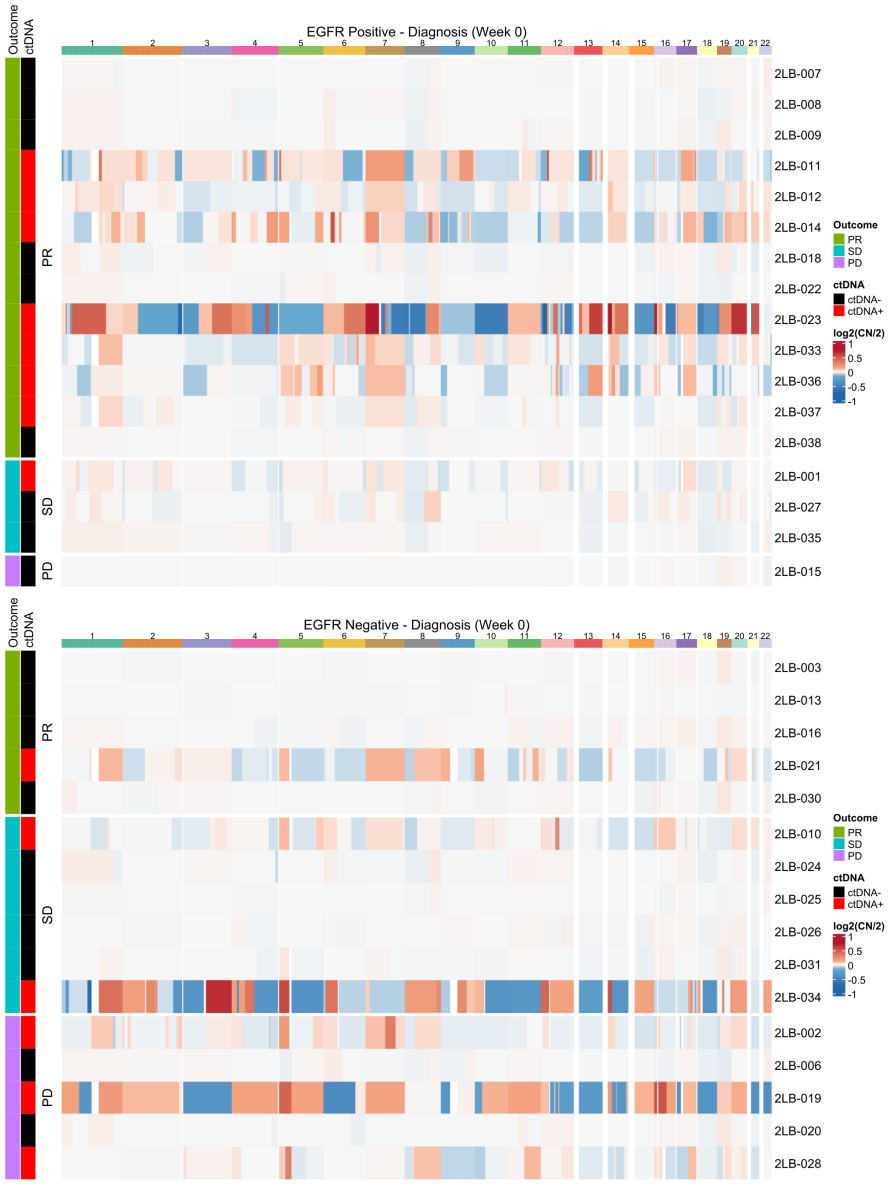
Supplementary Figure 2: Differences in the ctDNA fractions among the patients. The ctDNA fraction was significantly larger in the patients than in the controls (A). There were no significant differences in the size of the ctDNA fraction between the time points (B) and EGFR status (C). PR: Partial Response, SD: Stable Disease, PD: Progressive Disease.



Supplementary Figure 3: Longitudinal changes in the size of the ctDNA fraction in individual patients, estimated by ichorCNA. (A) Patients with EGFR+ NSCLC treated with EGFR-TKIs. (B) Patients with EGFR– NSCLC treated with chemotherapy. The estimated tumor fractions (% ctDNA) are shown as connected line plots. Patients were grouped according to the best clinical response using the Response Evaluation Criteria in Solid Tumors (version 1.1) (PR, SD, or PD). In patients with EGFR+ tumors (A), most of the patients with PR exhibited early clearance or a sustained reduction of the tumor fraction; however, one patient (2LB-015) exhibited an increasing ctDNA fraction and PD. In patients with EGFR– tumors (B), most of them with a PR or SD showed reductions in the tumor fraction; however, most of the patients with PD had detectable ctDNA levels throughout the study.

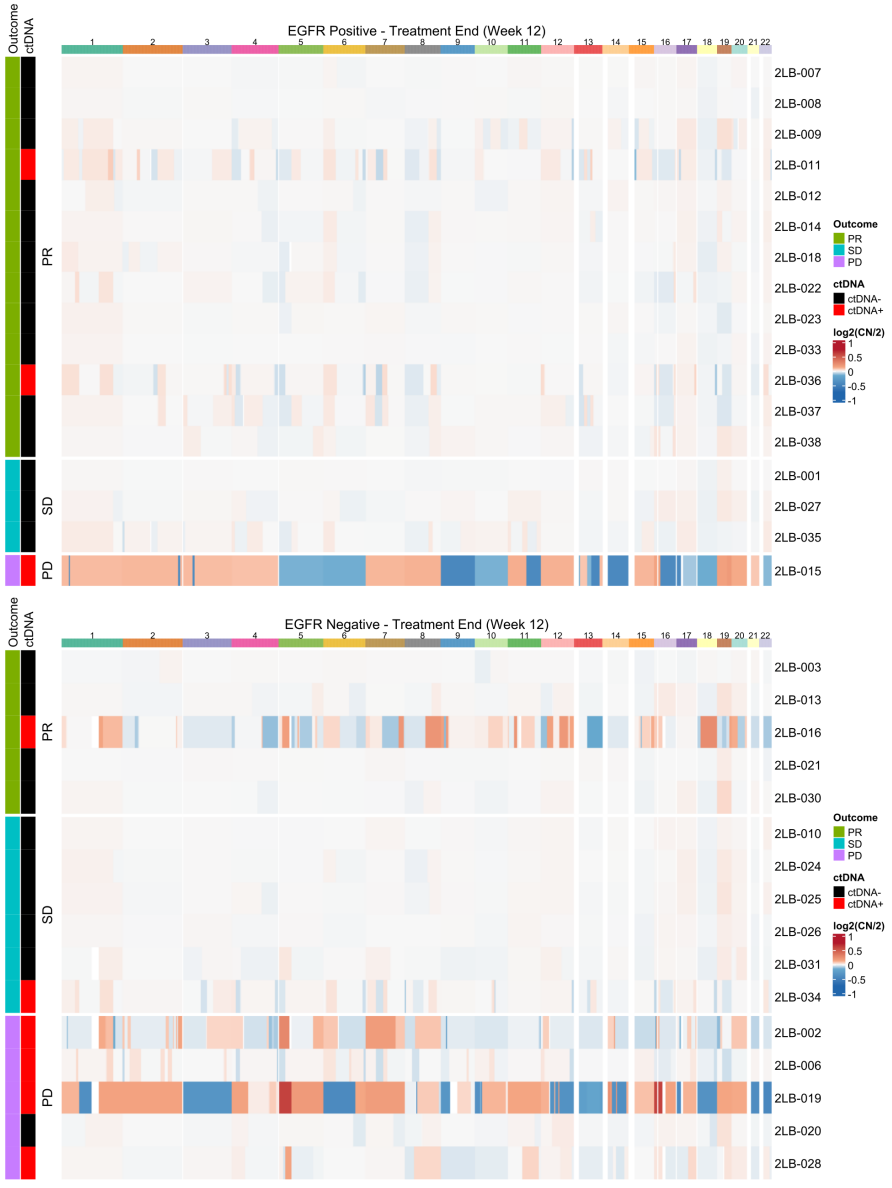


Supplementary Figure 4: Kaplan–Meier plots showing the probability of PFS of patients with EGFR+ tumors (A–C) and EGFR- tumors (D–F) at diagnosis, week 4, and week 12, based on the ctDNA fraction. Strong associations were observed in patients with EGFR- tumors at weeks 4 and 12.

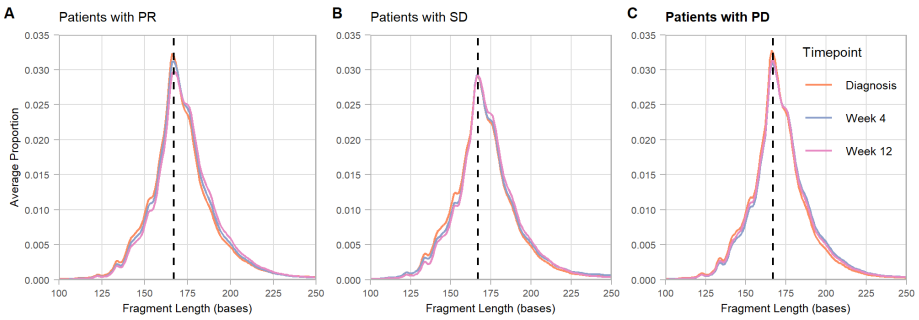


Supplementary Figure 5: The genome-wide CNAs detected in the cfDNA samples obtained at diagnosis from patients with EGFR+ (top) and EGFR- (bottom) NSCLC. The patients were grouped by clinical outcome.

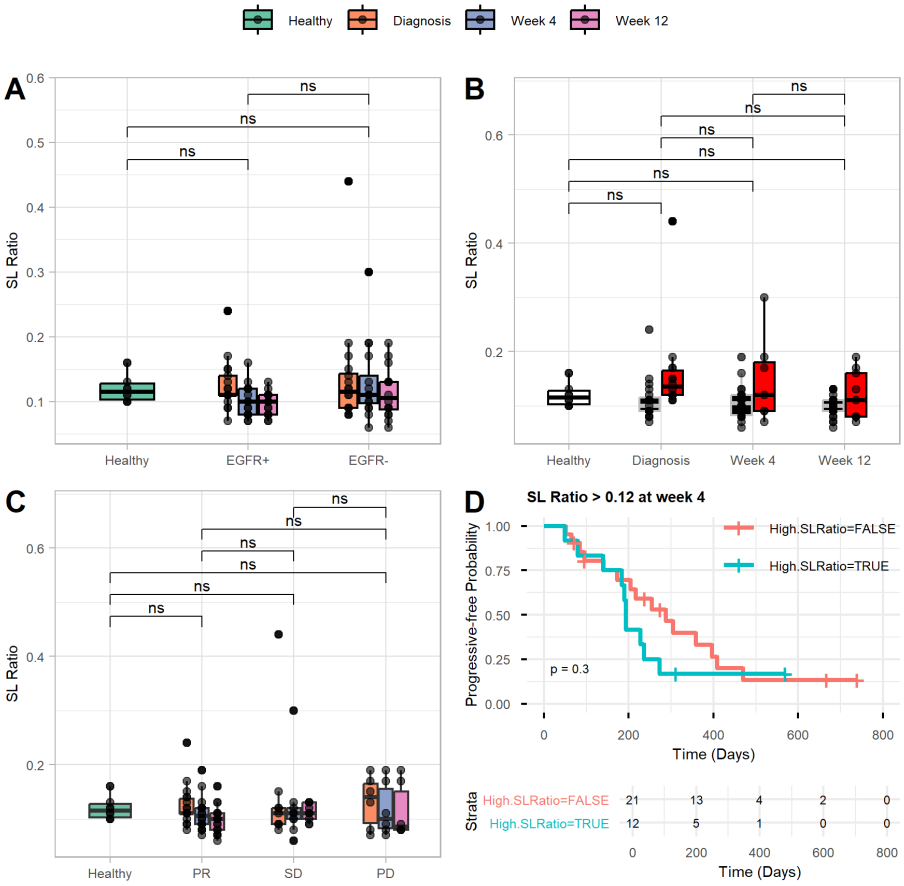
Supplementary Figure 6: The genome-wide CNAs detected in the cfDNA samples obtained at week 4 from patients with EGFR+ (top) and EGFR- (bottom) NSCLC. The patients were grouped by clinical outcome. The patients who received EGFR-TKI showed almost no signs of ctDNA. The ctDNA in patients with PD remained detectable.



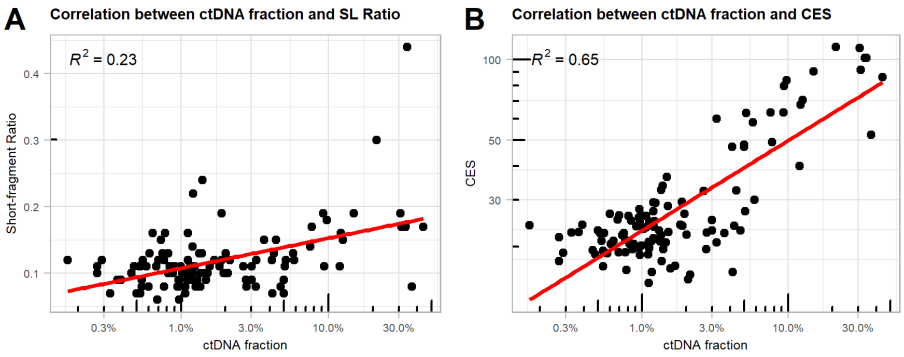
Supplementary Figure 7: The genome-wide CNAs detected in the cfDNA samples obtained at week 12 from patients with EGFR+ (top) and EGFR- (bottom) NSCLC. The patients were grouped by clinical outcome. The amount of ctDNA increased in patients with PD.



Supplementary Figure 8: Analysis of the cfDNA fragments in the samples from patients with PR (A) or SD (B) showed that the proportion of short fragments (¡ 150 bp) decreased from diagnosis (red line) to week 4 (blue line) and week 12 (pink line). In patients with PD, the proportion of short fragments peaked at diagnosis, was substantially lower at week 4, and was higher at week 12 (C).



Supplementary Figure 9: The SL ratios derived from the cfDNA samples obtained from NSCLC patients. The patient and control cfDNA samples had comparable SL ratios, regardless of the EGFR status (A), clinical time point (B), or clinical outcome (C). (D) An SL ratio ¿ 0.12 at 4 weeks did not indicate a significantly shorter PFS period.



Supplementary Figure 10: Correlations between the SL ratio and ctDNA fraction (A) and between the CES and ctDNA fraction (B). The CES was positively correlated ($R^2 = 0.65$) with the ctDNA fraction.