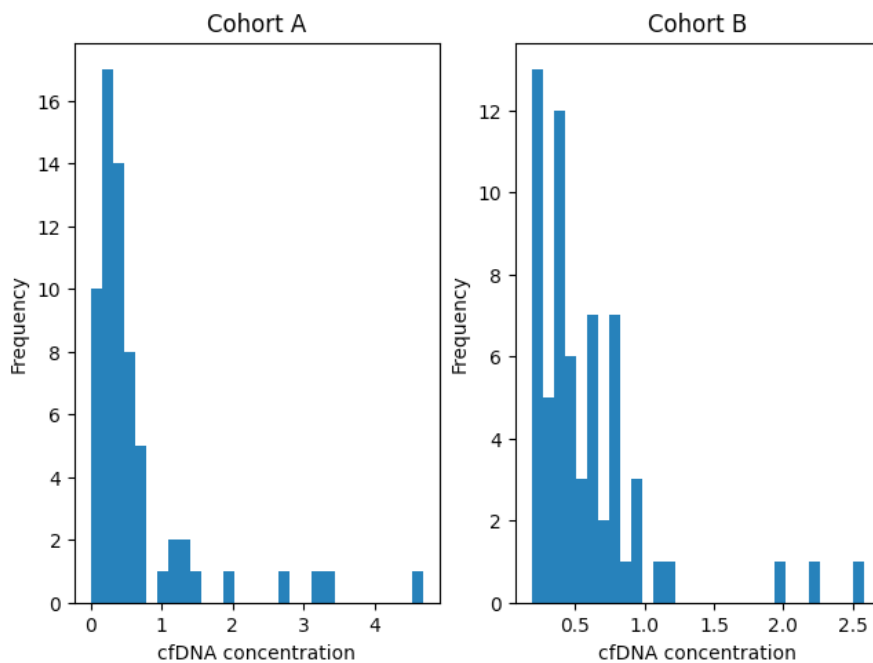


**Table S1** List of 171 genes covered by Axen Cancer Panel 2

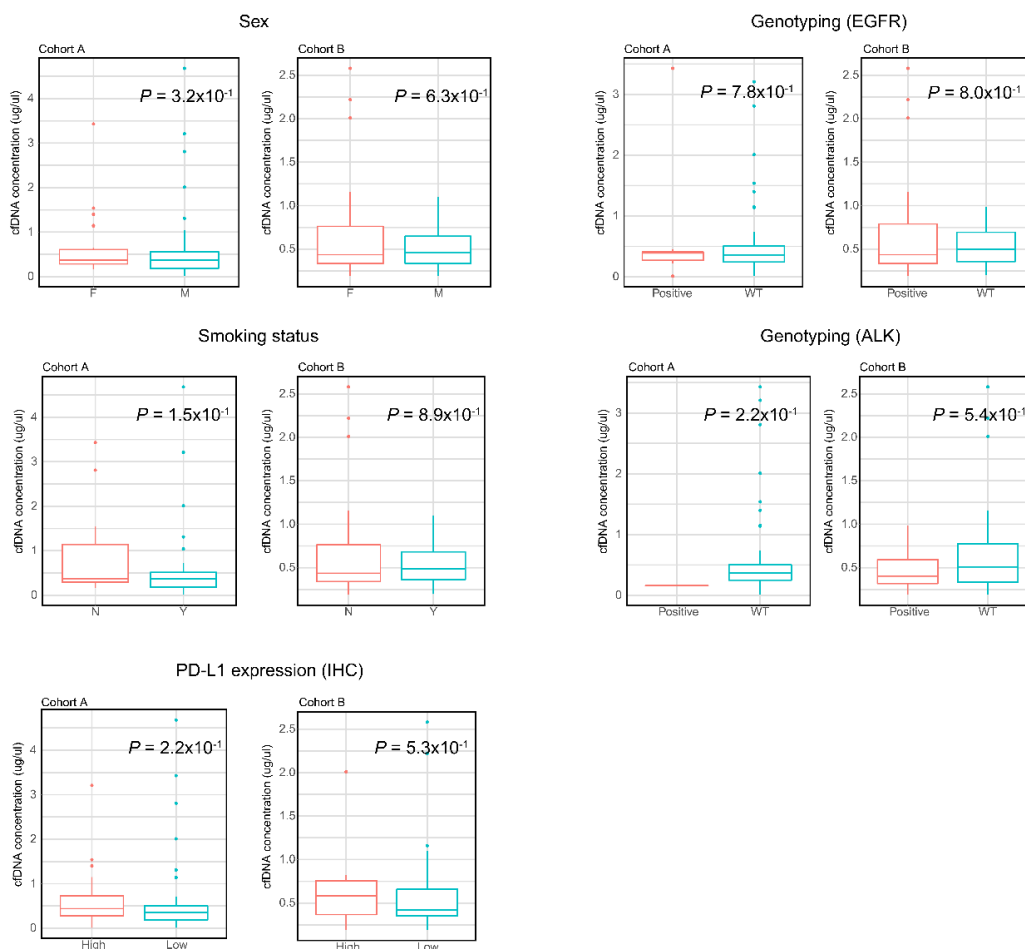
|               |               |                |                |               |               |               |               |
|---------------|---------------|----------------|----------------|---------------|---------------|---------------|---------------|
| <i>ABL1</i>   | <i>ABL2</i>   | <i>AKT1</i>    | <i>AKT2</i>    | <i>AKT3</i>   | <i>ALK</i>    | <i>APC</i>    | <i>AR</i>     |
| <i>ARAF</i>   | <i>ASXL1</i>  | <i>ATM</i>     | <i>ATR</i>     | <i>AURKA</i>  | <i>AURKB</i>  | <i>AURKC</i>  | <i>AXL</i>    |
| <i>BAP1</i>   | <i>BCL2</i>   | <i>BRAF</i>    | <i>BRCA1</i>   | <i>BRCA2</i>  | <i>BRD2</i>   | <i>BRD3</i>   | <i>BRD4</i>   |
| <i>CBFB</i>   | <i>CCND1</i>  | <i>CCND2</i>   | <i>CCND3</i>   | <i>CCNE1</i>  | <i>CDH1</i>   | <i>CDK12</i>  | <i>CDK4</i>   |
| <i>CDK6</i>   | <i>CDKN1A</i> | <i>CDKN1B</i>  | <i>CDKN2A</i>  | <i>CDKN2B</i> | <i>CDKN2C</i> | <i>CEBPA</i>  | <i>CHEK2</i>  |
| <i>CREBBP</i> | <i>CRKL</i>   | <i>CSF1R</i>   | <i>CTNNB1</i>  | <i>DDR1</i>   | <i>DDR2</i>   | <i>DNMT3A</i> | <i>DOT1L</i>  |
| <i>EGFR</i>   | <i>EPHA3</i>  | <i>ERBB2</i>   | <i>ERBB3</i>   | <i>ERBB4</i>  | <i>ERCC2</i>  | <i>ERG</i>    | <i>ERRFI1</i> |
| <i>ESR1</i>   | <i>ETV1</i>   | <i>ETV4</i>    | <i>ETV5</i>    | <i>ETV6</i>   | <i>EWSR1</i>  | <i>EZH2</i>   | <i>FBXW7</i>  |
| <i>FGFR1</i>  | <i>FGFR2</i>  | <i>FGFR3</i>   | <i>FGFR4</i>   | <i>FLCN</i>   | <i>FLT1</i>   | <i>FLT3</i>   | <i>FLT4</i>   |
| <i>FOXL2</i>  | <i>GNA11</i>  | <i>GNAQ</i>    | <i>GNAS</i>    | <i>HDAC9</i>  | <i>HGF</i>    | <i>HRAS</i>   | <i>IDH1</i>   |
| <i>IDH2</i>   | <i>IGF1R</i>  | <i>IGF2</i>    | <i>JAK1</i>    | <i>JAK2</i>   | <i>JAK3</i>   | <i>KDR</i>    | <i>KIT</i>    |
| <i>KMT2A</i>  | <i>KRAS</i>   | <i>MAP2K1</i>  | <i>MAP2K2</i>  | <i>MAP2K4</i> | <i>MAP3K1</i> | <i>MAP3K4</i> | <i>MAPK1</i>  |
| <i>MAPK3</i>  | <i>MAPK8</i>  | <i>MCL1</i>    | <i>MDM2</i>    | <i>MDM4</i>   | <i>MED12</i>  | <i>MEN1</i>   | <i>MET</i>    |
| <i>MITF</i>   | <i>MLH1</i>   | <i>MPL</i>     | <i>MSH2</i>    | <i>MSH6</i>   | <i>MTOR</i>   | <i>MYC</i>    | <i>MYCN</i>   |
| <i>MYD88</i>  | <i>NF1</i>    | <i>NF2</i>     | <i>NFKBIA</i>  | <i>NKX2-1</i> | <i>NOTCH1</i> | <i>NOTCH2</i> | <i>NOTCH3</i> |
| <i>NOTCH4</i> | <i>NPM1</i>   | <i>NRAS</i>    | <i>NTRK1</i>   | <i>NTRK2</i>  | <i>NTRK3</i>  | <i>NUTM1</i>  | <i>PDGFB</i>  |
| <i>PDGFRA</i> | <i>PDGFRB</i> | <i>PIK3CA</i>  | <i>PIK3CB</i>  | <i>PIK3CD</i> | <i>PIK3R1</i> | <i>PIK3R2</i> | <i>POLE</i>   |
| <i>PPARG</i>  | <i>PTCH1</i>  | <i>PTEN</i>    | <i>RAB35</i>   | <i>RAD50</i>  | <i>RAF1</i>   | <i>RARA</i>   | <i>RB1</i>    |
| <i>RET</i>    | <i>RHEB</i>   | <i>RICTOR</i>  | <i>RNF43</i>   | <i>ROS1</i>   | <i>RSPO1</i>  | <i>RSPO2</i>  | <i>RUNX1</i>  |
| <i>SMAD2</i>  | <i>SMAD4</i>  | <i>SMARCA4</i> | <i>SMARCB1</i> | <i>SMO</i>    | <i>SRC</i>    | <i>STK11</i>  | <i>SYK</i>    |
| <i>TERT</i>   | <i>TET2</i>   | <i>TMPRSS2</i> | <i>TOP2A</i>   | <i>TP53</i>   | <i>TSC1</i>   | <i>TSC2</i>   | <i>VHL</i>    |
| <i>WT1</i>    | <i>XPO1</i>   | <i>ZNRF3</i>   |                |               |               |               |               |

**Table S2** Univariate analysis with clinical variables based on patient characteristics using the Cox proportional-hazard model

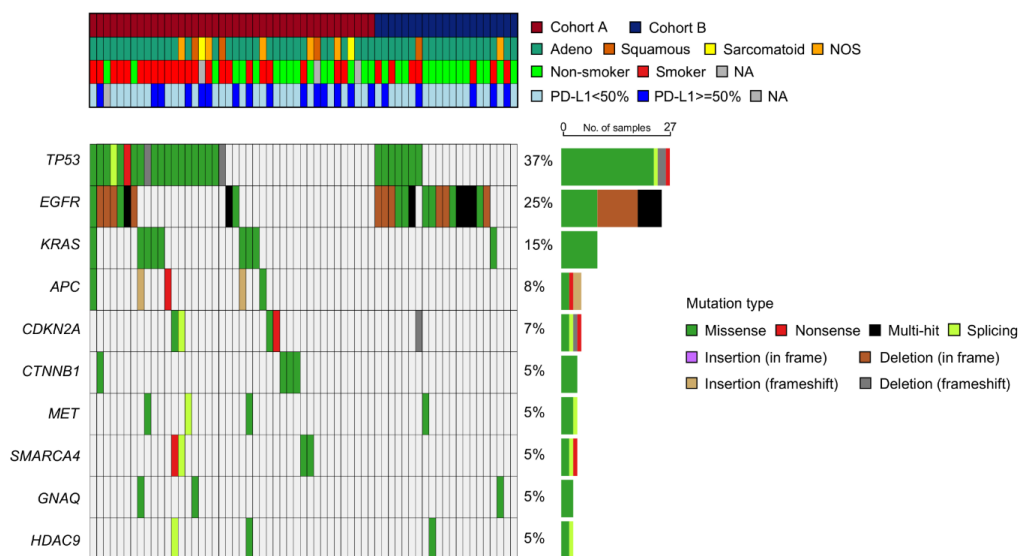
| Characteristics                                | Hazard ratio (95% CI) | P value |
|--|-----------------------|---------|
| <b>Cohort A</b>                                |                       |         |
| cfDNA concentration (high vs. low)             | 3.30 (1.00–10.94)     | 0.051   |
| Tumor mutational burden, ctDNA (high vs. low)  | 1.70 (0.49–5.88)      | 0.41    |
| Smoking (ex- or current smoker vs. non-smoker) | 2.38 (0.51–11.03)     | 0.27    |
| <b>PD-L1</b>                                   |                       |         |
| 1–50% vs. <1%                                  | 2.59 (0.50–13.37)     | 0.26    |
| >50% vs. <1%                                   | 3.10 (0.63–15.40)     | 0.17    |
| EGFR (mutant vs. wild-type)                    | 0.49 (0.10–2.38)      | 0.38    |
| ALK (mutant vs. wild-type)                     | 0.04 (0.00–2,809.74)  | 0.58    |
| <b>Cohort B</b>                                |                       |         |
| cfDNA concentration (high vs. low)             | 0.40 (0.08–2.09)      | 0.28    |
| Tumor mutational burden, ctDNA (high vs. low)  | 2.28 (0.19–27.54)     | 0.52    |
| Smoking (ex- or current smoker vs. non-smoker) | 2.50 (0.55–11.46)     | 0.24    |
| <b>PD-L1</b>                                   |                       |         |
| 1–50% vs. <1%                                  | 0.77 (0.11–5.53)      | 0.79    |
| >50% vs. <1%                                   | 1.18 (0.19–7.40)      | 0.86    |
| EGFR (mutant vs. wild-type)                    | 0.88 (0.10–7.94)      | 0.91    |
| ALK (mutant vs. wild-type)                     | 0.04 (0.00–6,874.05)  | 0.60    |



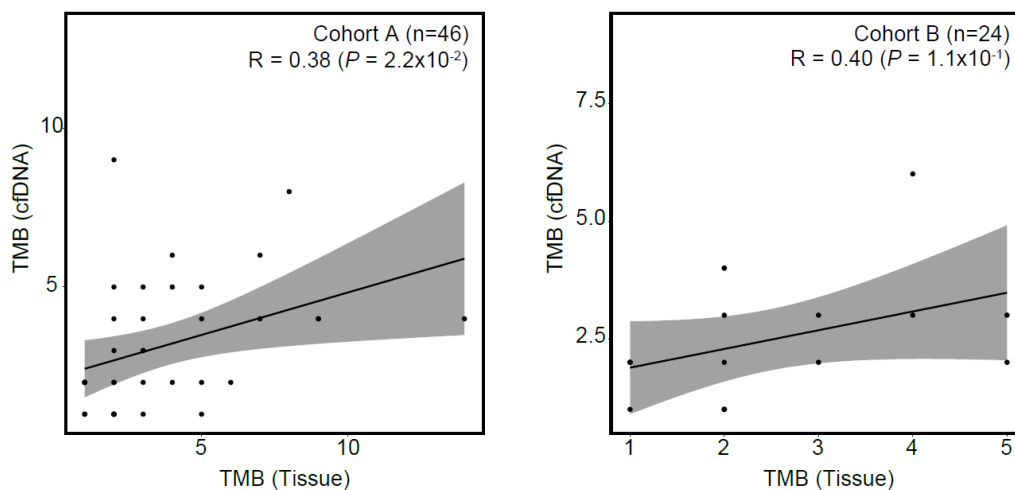
**Figure S1** The distribution of cfDNA concentration in both Cohort A and Cohort B.



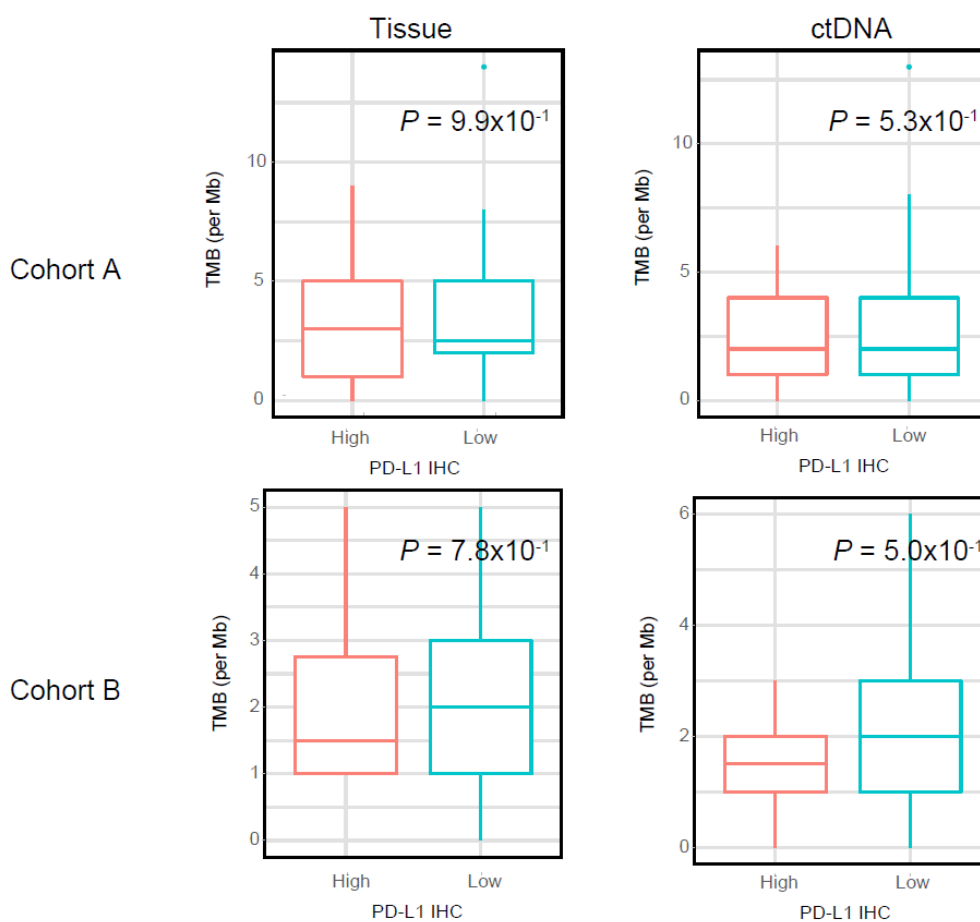
**Figure S2** cfDNA concentration between groups of sex, smoking status, and expression of PD-L1, EGFR, and ALK genotyping results.



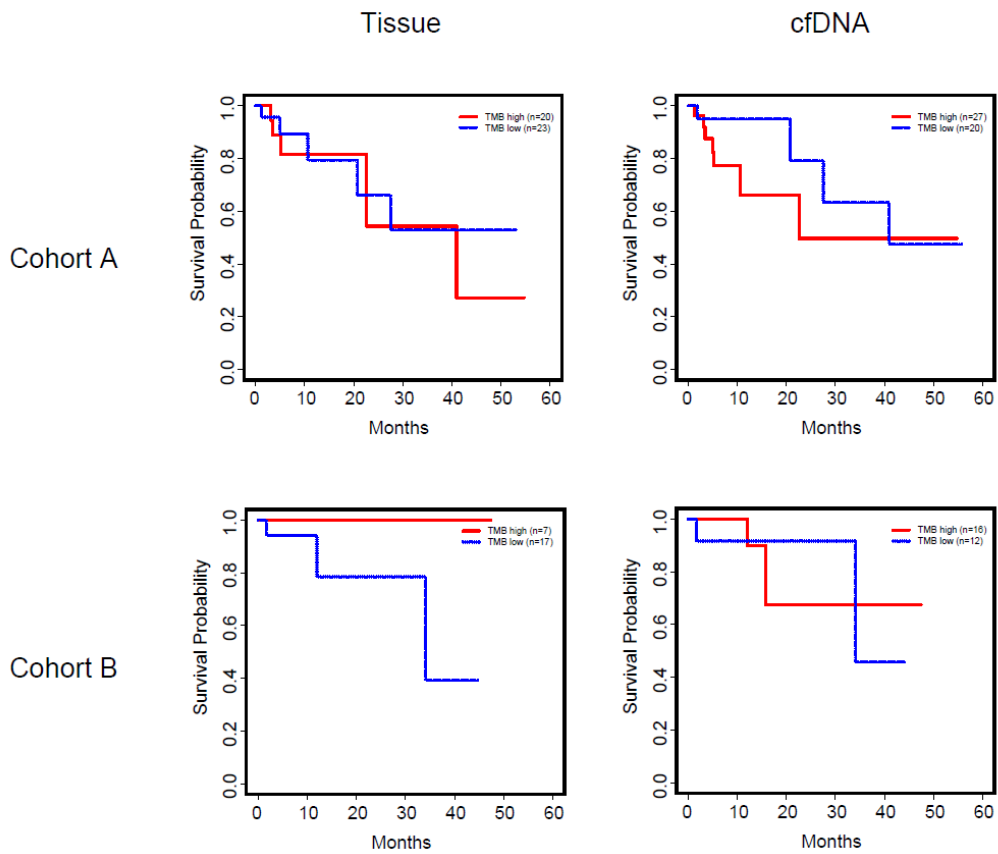
**Figure S3** Landscape of variants from tumor tissue revealed by the NGS platform with a targeted gene panel.



**Figure S4** Correlations between calculated tumor mutational burden derived from tumor tissue and ctDNA sequencing data in cohort A and cohort B (some dots in the figure contain redundant data where different samples have the same value).



**Figure S5** Comparison of TMB in PD-L1-high and -low groups using IHC results. Calculated TMB derived from tumor tissue and ctDNA sequencing data were analyzed, respectively.



**Figure S6** Kaplan-Meier analysis of OS according to the TMB groups. The TMB-high and -low groups were stratified using the median TMB values of cohort A and cohort B. Calculated TMB derived from tumor tissue and ctDNA sequencing data were analyzed, respectively.