

**Figure S1.** Swimmer plot of passage number in lung tumor organoid culture (upper) and pie chart of successfully established lung tumour organoids (lower). Green, blue, and red indicates long-term (passage  $\geq$ 10), short-term, and failure models, respectively. The criteria are described in the Materials and Methods.

LTO, lung tumor organoid; ADC, adenocarcinoma; SQ, squamous cell carcinoma; SCLC, small cell lung cancer.



**Figure S2.** Bright field microscopic images besides displayed lung tumour organoids (LTOs) in Figure 1. Scale bars in high-power field indicate 100mm. DC; adenocarcinoma, SQ; squamous cell carcinoma, SCLC; small cell lung cancer, NLO; normal lung organoid.



Figure S3. Feasibility of lung tumor organoids (LTOs) for drug sensitivity test.

A. Drug sensitivity tests of LTOs and cell lines harbouring *EGFR* exon 19 deletion or *EGFR* exon 21 L858R mutation to EGFR-TKIs. LTOs were sensitive to EGFR-TKIs but the  $IC_{50}$  values were higher in LTOs than in cell lines.

B. Drug sensitivity test of LTO52 derived from KRAS G12V mutant lung adenocarcinoma and LTO43 derived from small cell lung cancer.

ETP, etoposide; CDDP, cisplatin.