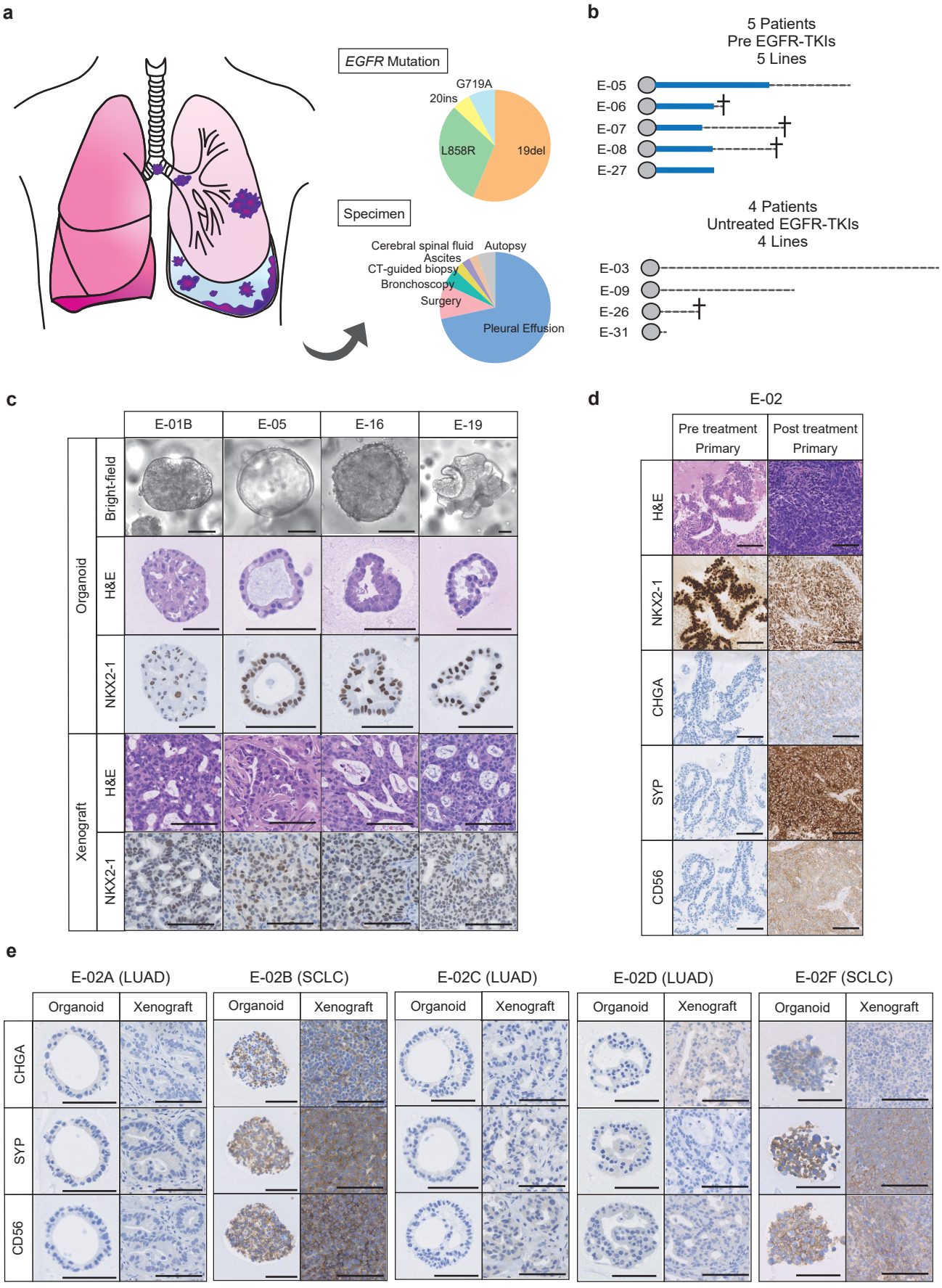
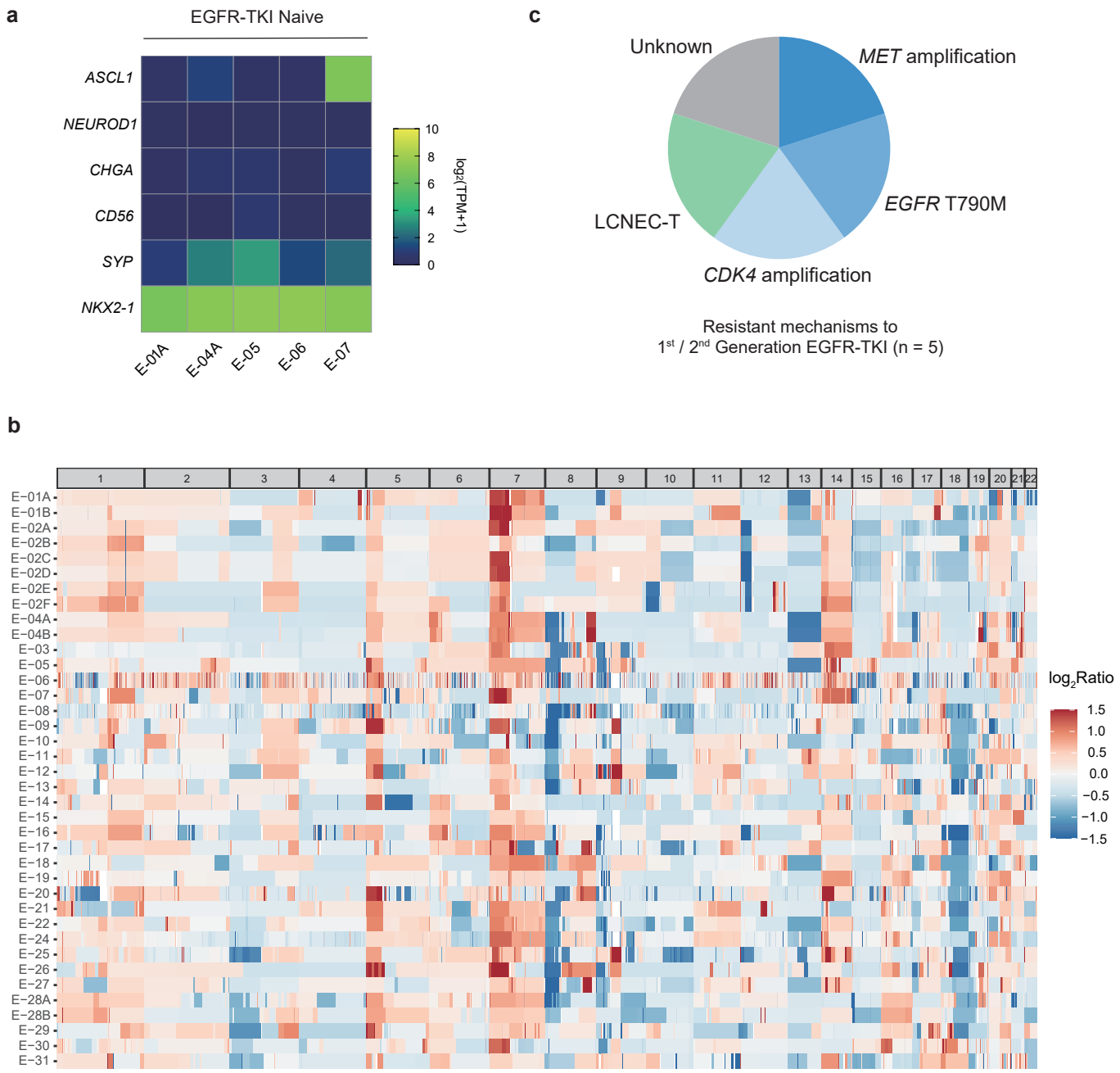
Supplementary Figure 1. Characterization of patient-derived lung cancer organoids



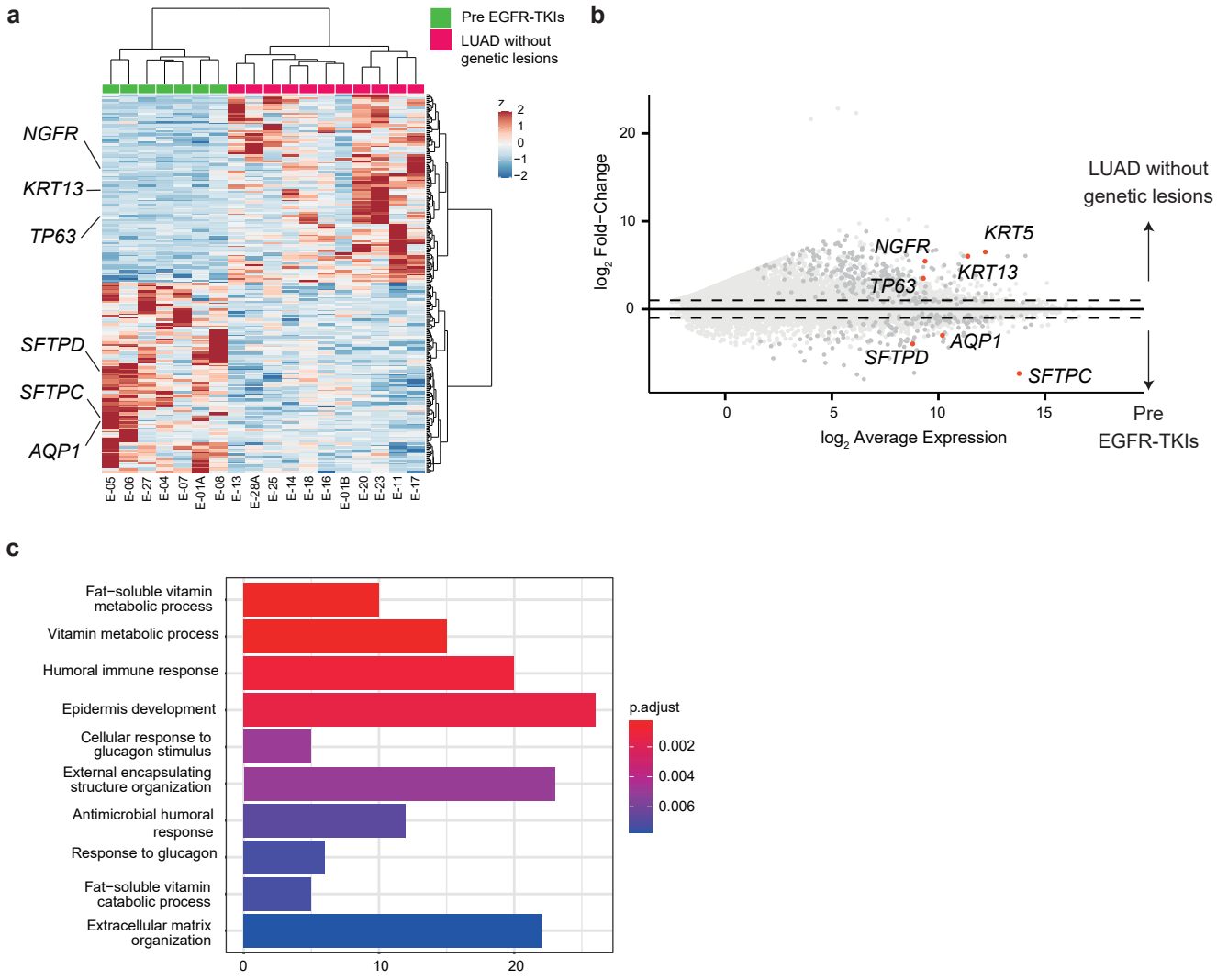
(a) Overview of ELCOL. Pie charts showing the proportion of *EGFR* mutations (top) and origin of clinical specimens (bottom). **(b)** Clinical timeline of the donors who pre-treated with EGFR-TKIs or untreated with EGFR-TKIs. Blue thick line shows the period of Osimertinib treatment, and the cross indicate patient death. **(c)** Bright-field and section staining images of the indicated organoid lines and their xenografts. Scale bar: 100 μm. **(d)** Histology of the clinical E-02 tumor sampled before and after EGFR-TKI treatment. Pre-treatment samples show LUAD histology, whereas the post-treatment tumor is SCLC. Scale bar: 100 μm. **(e)** Concordance of CHGA, SYP and CD56 staining patterns between E-02 organoids and their xenograft tumors. Scale bar: 100 μm.

Supplementary Figure 2. Molecular characterization of ELCOL



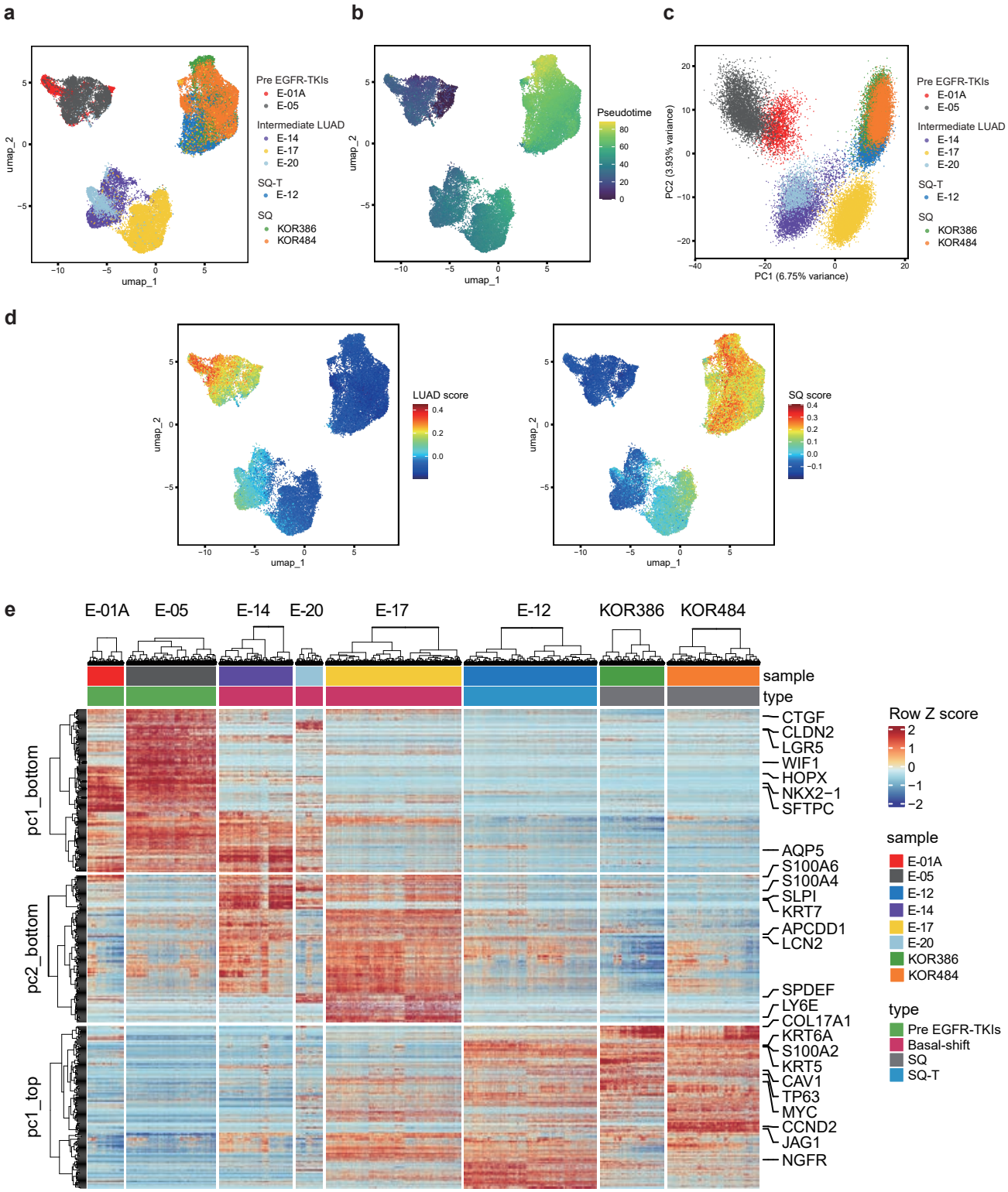
(a) Expression of neuroendocrine genes and *NKX2-1* in EGFR-TKI-naïve LUAD organoid lines. The E-07 LUAD organoid line with a high expression of *ASCL1* and *RB1* mutation was established from a tumor that later underwent SCLC transformation. Source data are provided as a Source Data file. **(b)** Copy number changes in ELCOL organoids **(c)** Cause of resistance identified in LUAD organoid lines with resistance to 1st / 2nd generation EGFR-TKIs (n = 5). Source data are provided as a Source Data file.

Supplementary Figure 3. Gene expression of EGFR-TKI resistant LUAD organoids without genetic lesions



(a) Comparative gene expression analysis between pre-EGFR-TKIs LUAD organoids (n = 7 lines) and post-EGFR-TKIs LUAD organoids without known genetic alterations (n = 11). **(b)** An MA plot of RNA-seq data between LUAD without genetic lesions lines (n = 11) and pre-EGFR-TKIs treatment lines (n = 7). **(c)** Gene-Ontology analysis result from differentially expressed genes between organoid lines without genetic lesions (n = 11) and pre-EGFR-TKIs treatment lines (n = 7).

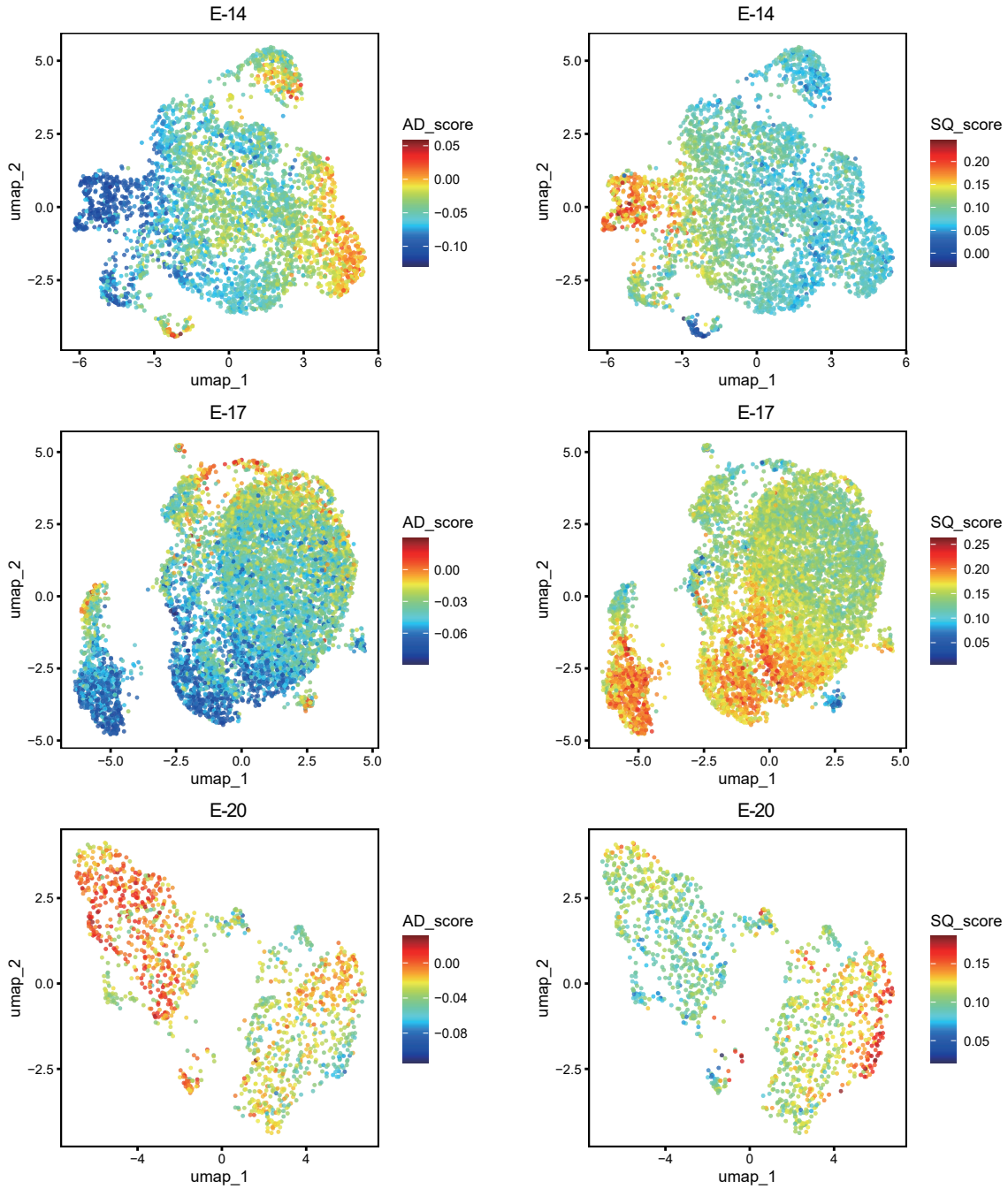
Supplementary Figure 4. scRNA-seq analysis of lung cancer organoids



(a) UMAP plotting of the integrated scRNA-seq data. The cells were colored according to the organoid line. **(b)** Pseudotime analysis of the scRNA-seq data. The cluster with the highest expression of bulk LUAD genes (log2 fold change > 2 and adjusted p-value < 0.001, LUAD vs SQ organoids in

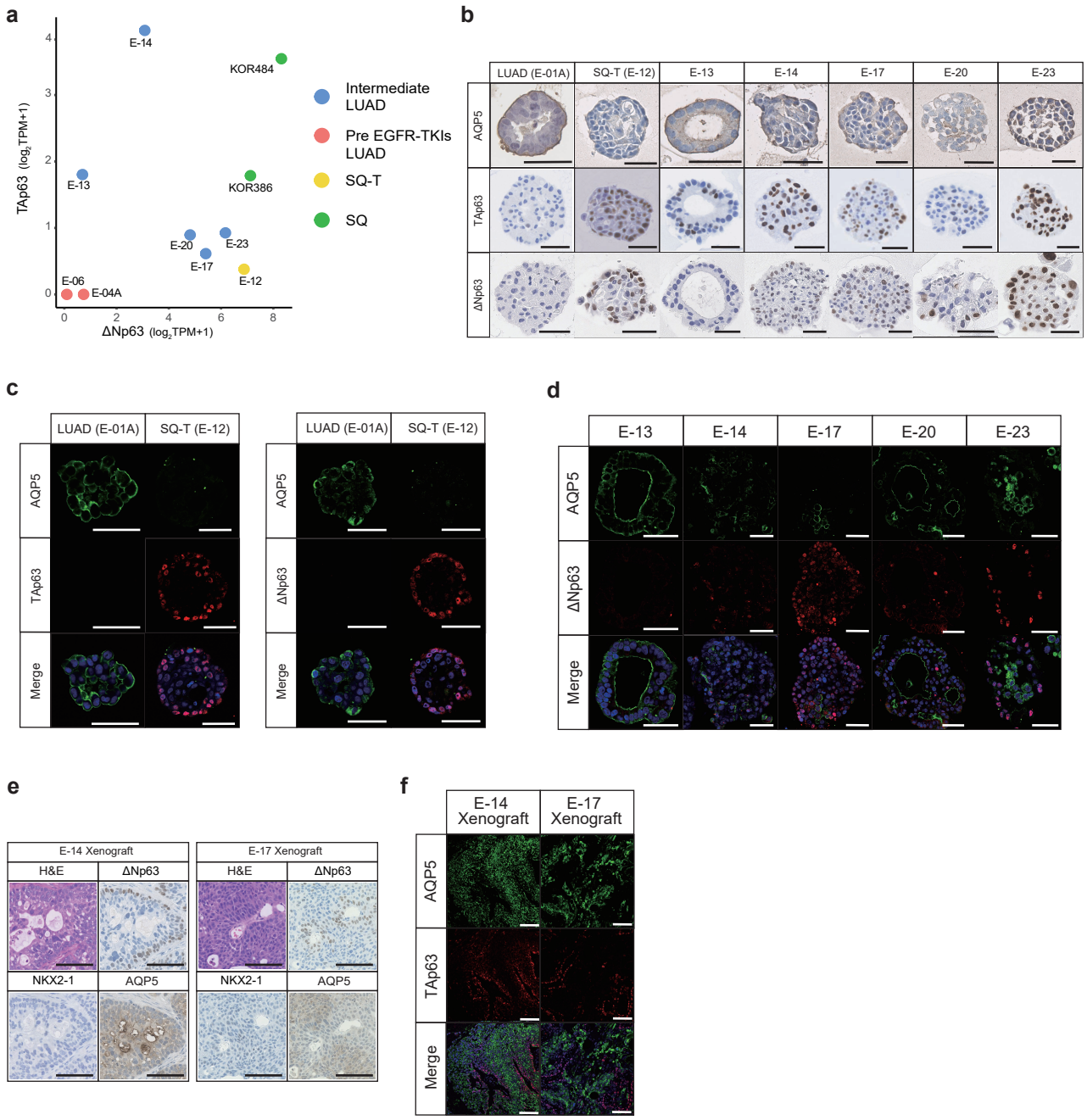
bulk RNA-seq analysis) was selected as the starting cluster. **(c)** PCA plot of the normalized scRNA-seq data before data integration. PC1 roughly separates LUAD vs SQ(-T) cells, whereas low loading in PC2 defines intermediate LUAD cells. **(d)** Enrichment of bulk LUAD (left) and SQ (right) genes (absolute fold change > 4 and adjusted p-value 0.001 in bulk RNA-seq analysis). **(e)** Gene expression profile of the cells used for scRNA-seq. Genes with a high or low loading in PC1 and a low loading in PC2 (200 genes each) were selected for visualization.

Supplementary Figure 5. Per-sample scRNA-seq analysis of intermediate LUAD organoids



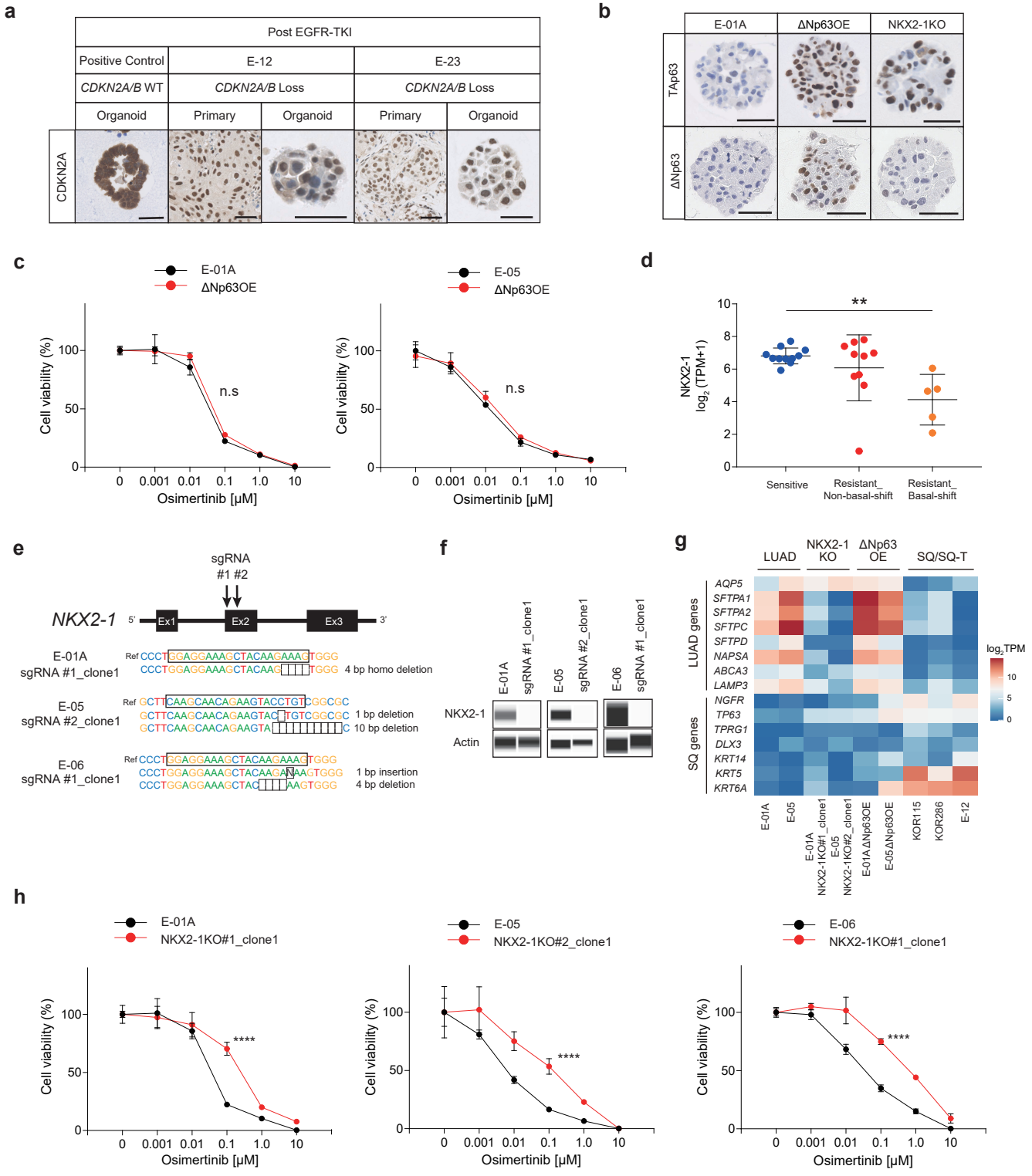
scRNA-seq analysis of intermediate (Basal-shift) organoids shown per sample. The enrichment of bulk LUAD and SQ genes are shown.

Supplementary Figure 6. Immunostaining of intermediate LUAD organoids



(a) A scatter plot showing the mRNA expression of TAp63 and ΔNp63 in intermediate LUAD, pre-EGFR-TKIs LUAD, SQ-T and SQ organoid lines. **(b)** TAp63, ΔNp63 and AQP5 immunostaining in intermediate organoid lines. Scale bar: 50 μm. **(c)** TAp63, ΔNp63 and AQP5 immunofluorescence staining in LUAD and SQ-T organoid lines. Scale bar: 50 μm. Source data are provided as a Source Data file. **(d)** Immunofluorescence ΔNp63 and AQP5 staining in E-13, E-14, E-17, E-20 and E-23 organoid lines with EGFR-TKI resistance and without known genetic lesions. Scale bar: 50 μm. **(e)** Representative H&E staining and NKX2-1, ΔNp63 and AQP5 immunostaining of E-14 and E-17 xenografts. Scale bar: 100 μm. **(f)** Immunofluorescence TAp63 and AQP5 staining of E-14 and E-17 xenografts. Scale bar: 100 μm.

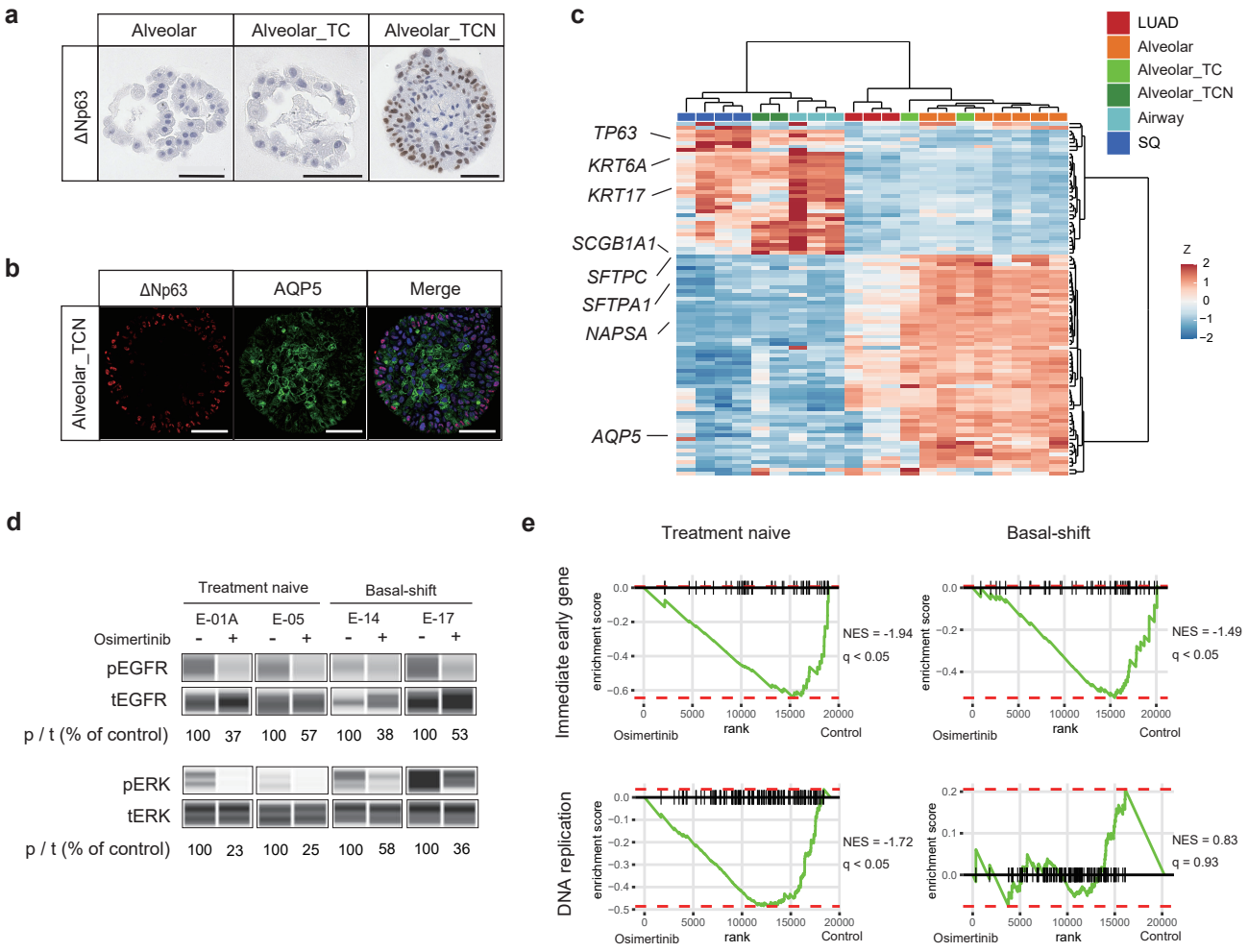
Supplementary Figure 7. Genetic modeling of basal-shift transformation in treatment-naïve LUAD organoids



(a) Immunostaining of CDKN2A in the primary tumor and organoids of the indicated lines. Scale bar: 50 μm. **(b)** TAp63 and ΔNp63 expression in E-01A organoid (treatment-naïve LUAD), E-01A with ΔNp63 overexpression (ΔNp63OE) and E-01A with NKX2-1 knockout (NKX2-1KO). Scale bar: 100 μm. **(c)** Osimertinib sensitivity of treatment-naïve LUAD organoid lines with or without ΔNp63 over-expression. Data are shown as mean ± S.D. n.s.; not significant. t-test (two-sided). Source data are

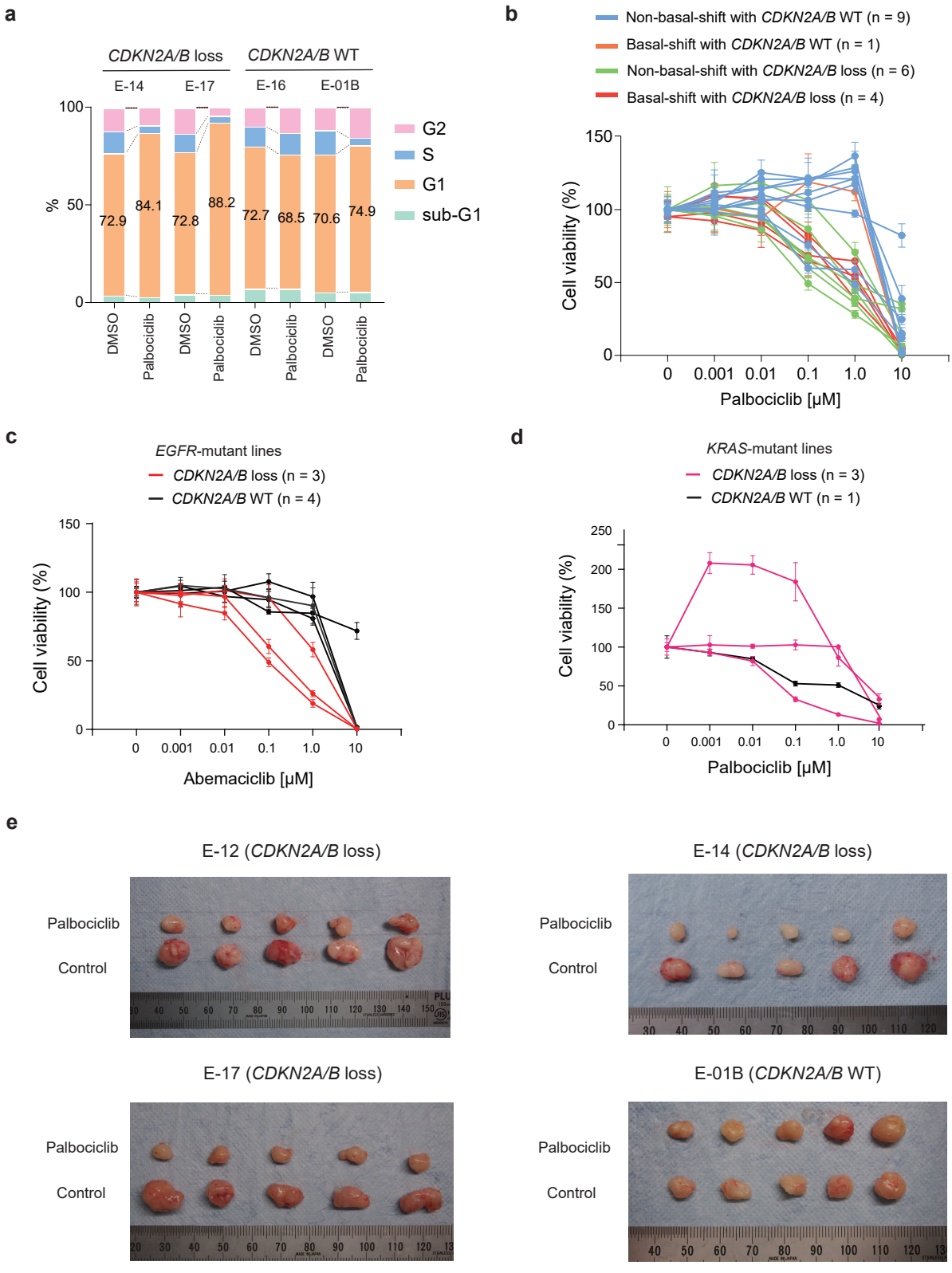
provided as a Source Data file. **(d)** The mRNA expression of *NKX2-1* in EGFR-TKI sensitive, Resistant_Non-basal-shift and Resistant_Basal-shift LUAD lines. **p= 0.0066, one-way ANOVA. Data are shown as mean ± S.D. Source data are provided as a Source Data file. **(e)** NKX2-1-KO in lung cancer organoid using CRISPR-Cas9 and its confirmation by sanger sequencing. **(f)** Confirmation of NKX2-1 knockout in E-01A, E-05 and E-06 lines with an immunoassay. Source data are provided as a Source Data file. **(g)** A heatmap showing the mRNA expression of LUAD and SQ genes in E-01A parental, NKX2-1 knockout #1_clone1 and ΔNp63-overexpressed organoids. Source data are provided as a Source Data file. **(h)** Osimertinib sensitivity of E-01A and E-01A_NKX2-1 knockout #1_clone1 lines, 0.1 μM ****p= 8.59×10^{-7} (left), E-05 and E-05_NKX2-1 knockout #2_clone1 lines, 0.1 μM ****p= 7.28×10^{-5} (middle), E-06 and E-06_NKX2-1 knockout #1_clone1 lines, 0.1 μM ****p= 3.03×10^{-6} (right). t-test (two-sided). Data are shown as mean ± S.D. Before the cell viability assay, E-05 parental, E-05 NKX2-1KO#2_clone1, E-06 parental and E-06 NKX2-1KO#1_clone1 organoids were exposed to 0.1 μM Osimertinib for 4 weeks. E-05 parental line did not tolerate Osimertinib exposure for 4 weeks. The data of E-05 parental line without Osimertinib exposure is shown. Source data are provided as a Source Data file.

Supplementary Figure 8. Genetic modeling of basal-shift transformation in alveolar organoids



(a) Representative ΔNp63 staining in normal alveolar, alveolar_TC and alveolar_TCN lines. Scale bar: 100 μm. **(b)**. Immunofluorescence staining of ΔNp63 and AQP5 in an alveolar_TCN organoid. Scale bar: 50 μm. **(c)** Comparative gene expression analysis between LUAD, SQ, alveolar and genetically engineered alveolar organoid (alveolar_TC and alveolar_TCN) lines. **(d)** Immunoblotting of the indicated proteins in E-01A, E-05, E-14 and E-17 organoids. The proteins were extracted 2 hours after Osimertinib treatment (1 μM). Source data are provided as a Source Data file. **(e)** Gene set enrichment analysis of Osimertinib-treated versus control organoids using immediate early genes and DNA replication genes. Treatment-naïve (E-01A and E-05) and basal-shift (E-14 and E-17) lines were treated with Osimertinib (1 μM) for 12 hours. NES; normalized enrichment score.

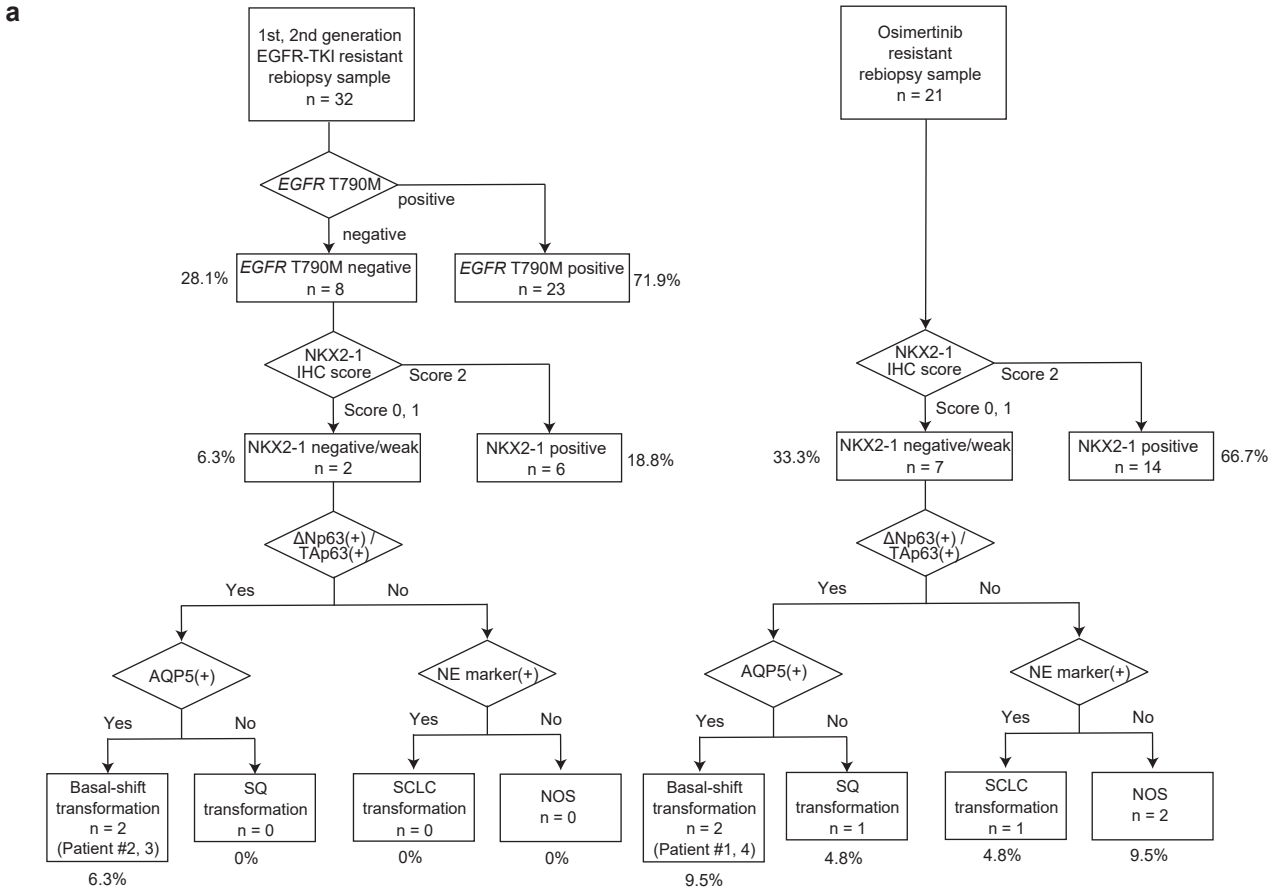
Supplementary Figure 9. CDK4/6 inhibition in LUAD organoids



(a) Flow cytometry-based cell cycle analysis for *CDKN2A/B* loss (E-14 and E-17) and *CDKN2A/B* WT (E-01B and E-16) organoid lines with or without Palbociclib treatment (1.0 μM) for 48 hours. Numbers indicate the proportion of G1-phase cells. The proportions of each cell cycle phase calculated through flow cytometry data using gating strategy and data itself are provided as a Source

Data file. **(b)** Drug sensitivity assay for Palbociclib in lung cancer organoids. The experiments were performed in quadruplicate for each line. Data are shown as mean ± S.D**.** Source data are provided as a Source Data file. **(c)** Abemaciclib sensitivity of *EGFR*-mutant lines with (n = 3) or without (n = 4) *CDKN2A/B* loss. The experiments were performed in quadruplicate for each line. Data are shown as mean ± S.D**.** Source data are provided as a Source Data file. **(d)** Palbociclib sensitivity of *KRAS*-mutant lines with (n = 3) or without (n = 1) *CDKN2A/B* loss. The experiments were performed in quadruplicate for each line. Data are shown as mean ± S.D**.** Source data are provided as a Source Data file. **(e)** Images of vehicle- or palbociclib-treated xenografts of E-12, E-14, E-17 and E-01B lines.

Supplementary Figure 10. Validation of basal-shift transformation in clinical specimens



(a) Flowcharts of immunostaining analysis of clinical samples for 1st / 2nd generation EGFR-TKI resistant rebiopsy samples (n = 32) and Osimertinib resistant rebiopsy samples (n = 21). NE marker: Neuroendocrine marker. SQ: Squamous cell carcinoma, SCLC: Small Cell Lung Cancer, NOS: Not otherwise specified. **(b)** Representative H&E staining ΔNp63, NKX2-1 and AQP5 immunostaining of clinical samples. Scale bar: 100 μm. **(c)** Immunofluorescence TAp63, NKX2-1 and AQP5 staining of clinical samples. Scale bar: 100 μm.