

SUPPLEMENTARY INFORMATION

Figure S1. Identification of CTHRC1-positive fibroblasts in EGFR-TKI resistant lung cancer, related to Figure 1

Figure S2. CTHRC1+ CAFs promote acquired resistance to EGFR-TKI in lung cancer, related to Figure 2

Figure S3. CTHRC1+ CAFs promote EGFR-TKI resistance via metabolic reprogramming in cancer cells, related to Figure 3

Table S1. Excel file containing sample clinical demographics of scRNA-seq.

Table S2. Excel file containing detail information for enrolled lung cancer patients in clinical cohort 1.

Table S3. Excel file containing detail information for enrolled lung cancer patients in clinical cohort 2.

Table S4. Excel file containing primer sequences for qRT-PCR and CUT&RUN assays. Target sequences of siRNAs and shRNAs.

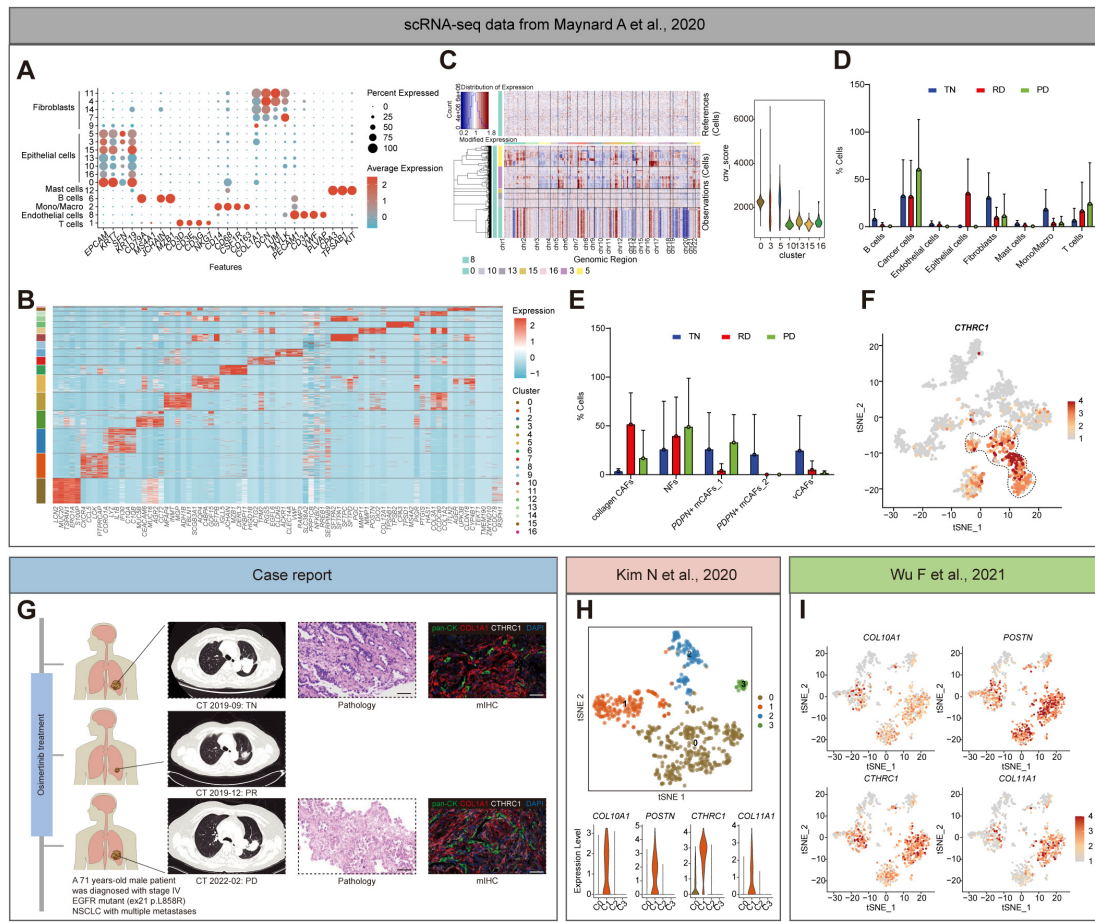


Fig. S1. Identification of *CTHRC1*-positive fibroblasts in EGFR-TKI resistant lung cancer. (A)

Dot plot showing representative marker genes across cell clusters. (B) Heatmap showing differentially expressed genes across cell clusters. Red: high expression; blue: low expression. (C) CNV patterns of epithelial sub-clusters are shown (left), with red and blue colors indicating amplifications and deletions, respectively. Violin plot showing the CNV score of each sub-cluster (right), which was calculated as quadratic sum of CNV region. (D) Bar plots showing the percentage (%) of cell types across different sample origin. (E) Bar plots showing the percentage (%) of fibroblasts sub-clusters across different sample origin. (F) t-SNE plot shows the expression of *CTHRC1* in each fibroblast subcluster. (G) The dynamic change of *CTHRC1*+ CAFs from the same patient during EGFR-TKI treatment. Chest CT images from the patient shows tumor volume changes (left). Pathological examination results at different timepoints are shown (middle). In situ

immunofluorescent staining shows the percent of CTHRC1+ CAF at different timepoints (right).

Scale bar, 50 μ m. **(H-I)** t-SNE plot and violin plots show the expression of representative top expression genes of CTHRC1+ CAFs in two published lung cancer scRNA-seq datasets.

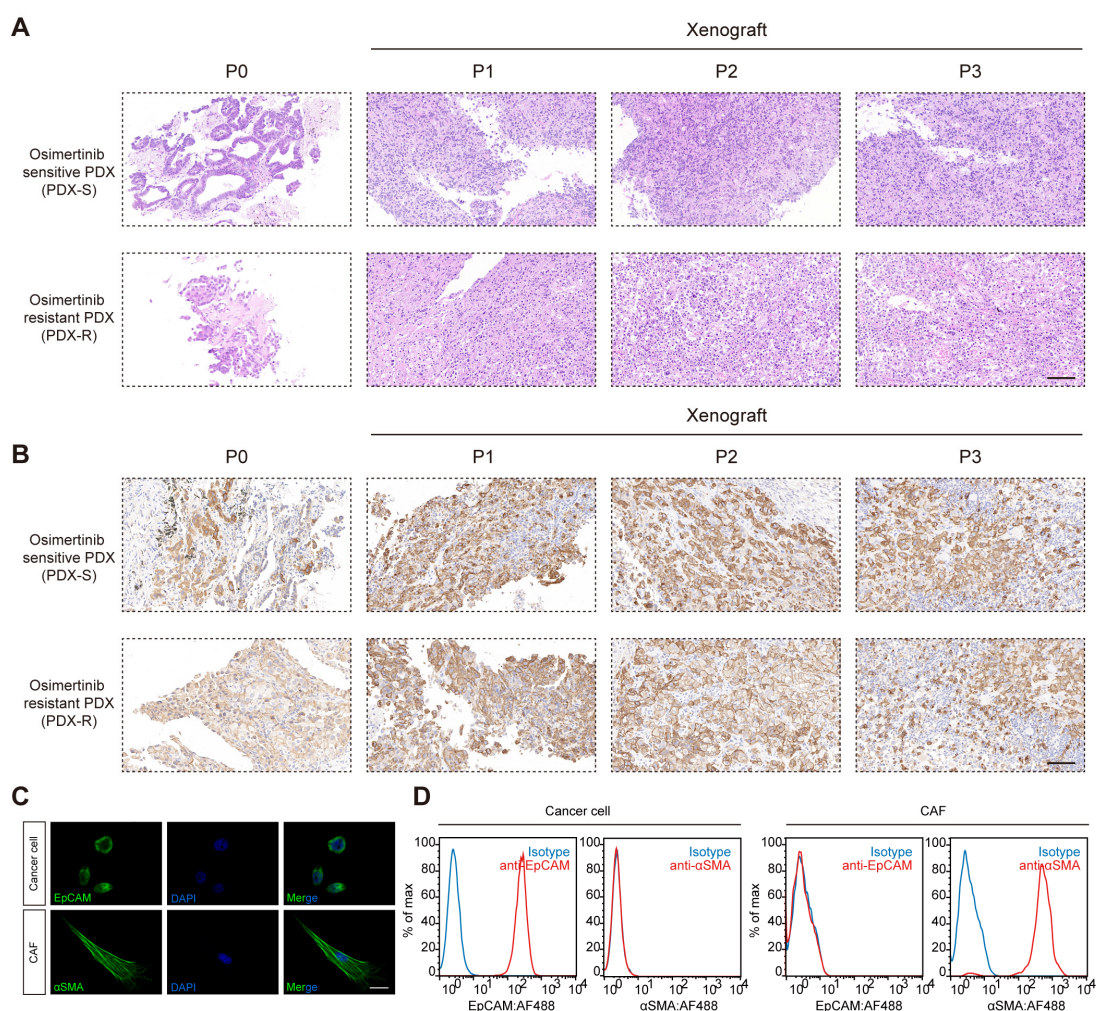


Fig. S2. CTHRC1+ CAFs promote acquired resistance to EGFR-TKI in lung cancer. (A)

Representative histology of paired patient-PDX tumors (n = 3). Scale bar, 50 μ m. **(B)** Representative

images of EGFR protein immunohistochemical staining in paired patient-PDX tumors (n = 3). Scale

bar, 50 μ m. **(C-D)** Representative immunofluorescent staining images (C) and flow cytometric

analysis (D) of CAF marker (α SMA) and epithelial cell marker (EpCAM) in primary CAFs and

cancer cells derived from PDXs (n = 3). Scale bar, 20 μ m.

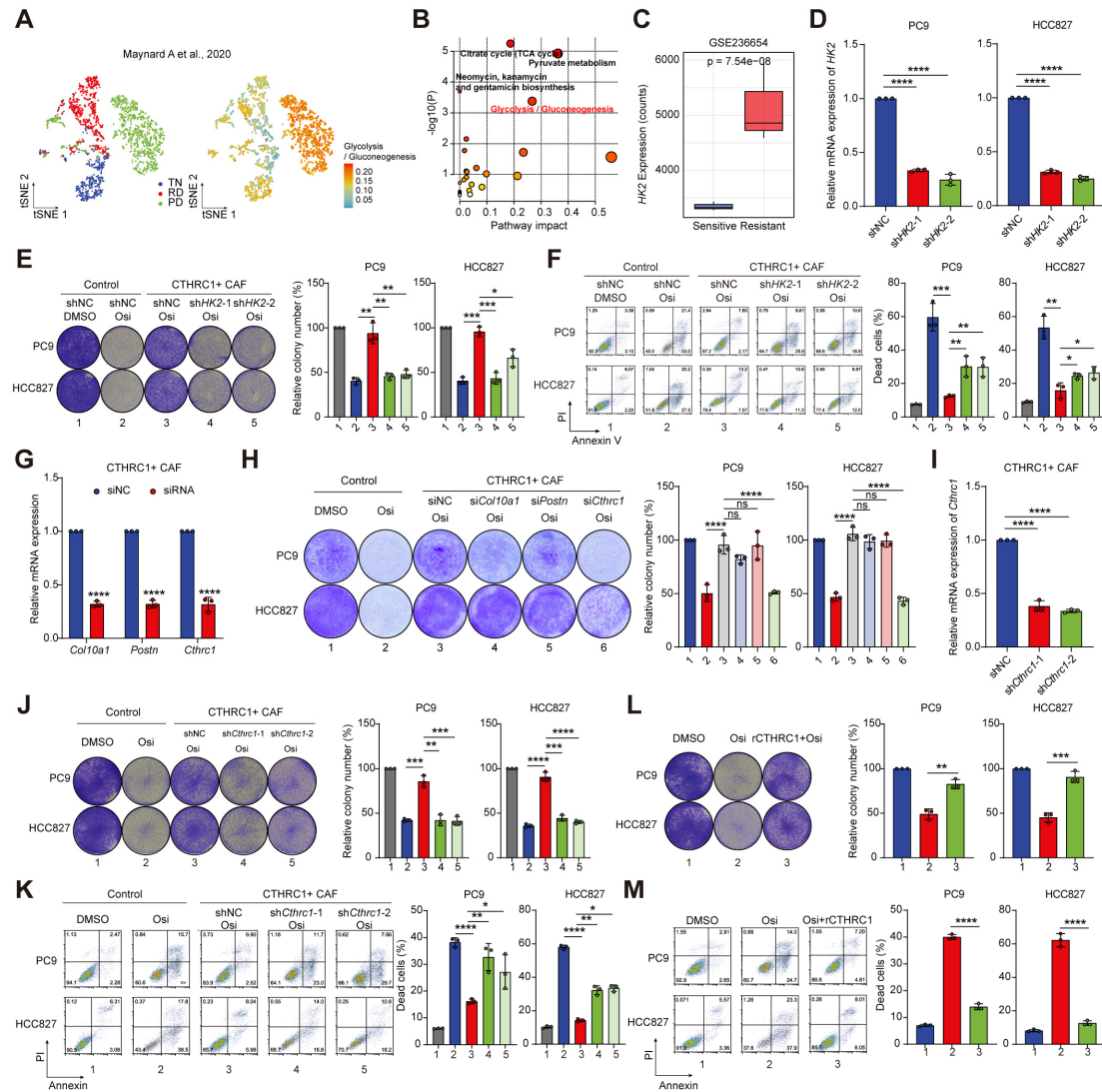


Fig. S3. CTHRC1+ CAFs promote EGFR-TKI resistance via metabolic reprogramming in cancer cells. (A) t-SNE plot of cancer cell sub-clusters colored by their sample origin (left) and the metabolic score calculated by scMetabolism (right). (B) Dot plot showing the pathway enrichment of differentially expressed metabolites in PC9 cells after being co-cultured with CTHRC1+ CAFs compared with being cultured alone. (C) *HK2* expression in osimertinib sensitive and resistant lung cancer cells from the RNA-seq data (GSE236654). (D) qPCR assays monitoring expression of *HK2* in PC9 and HCC827 cells with or without sh*HK2*. (E-F) Representative images of the colony formation (E) and the percentage of dead cells (F) in indicated lung cancer cell lines with or without sh*HK2* after being cultured with CTHRC1+ CAFs and treating

with Osi (0.01 μ M). (G) *Coll0a1*, *Postn* and *Cthrc1* knockdown efficiency verification of CTHRC1+ CAFs by qPCR. (H) Representative images of the colony formation in indicated lung cancer cell lines after being co-cultured with CTHRC1+ CAFs after knocking-down *Coll0a1*, *Postn* and *Cthrc1*. (I) qPCR assays monitoring expression of *Cthrc1* in CTHRC1+ CAFs with or without sh*Cthrc1*. (J-K) Representative images of the colony formation (J) and the percentage of dead cells (K) in indicated lung cancer cell lines after being cocultured with CTHRC1+ CAFs with or without sh*Cthrc1* and treating with Osi (0.01 μ M). (L-M) Representative images of the colony formation (L) and the percentage of dead cells (M) in indicated lung cancer cell lines treating with Osi (0.01 μ M) alone or with recombinant CTHRC1 (rCTHRC1, 500 ng/mL).