

Expanded View Figures

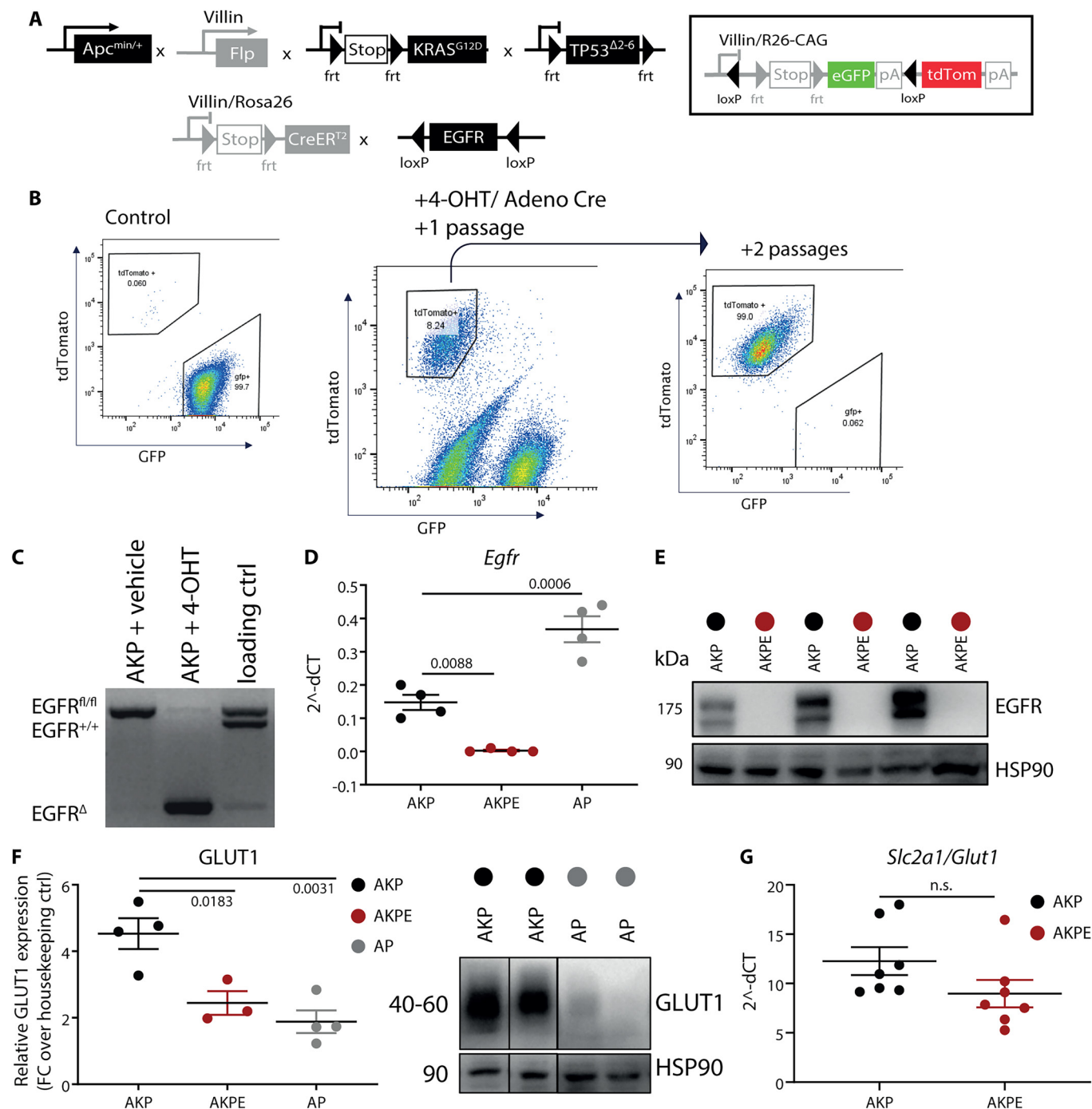
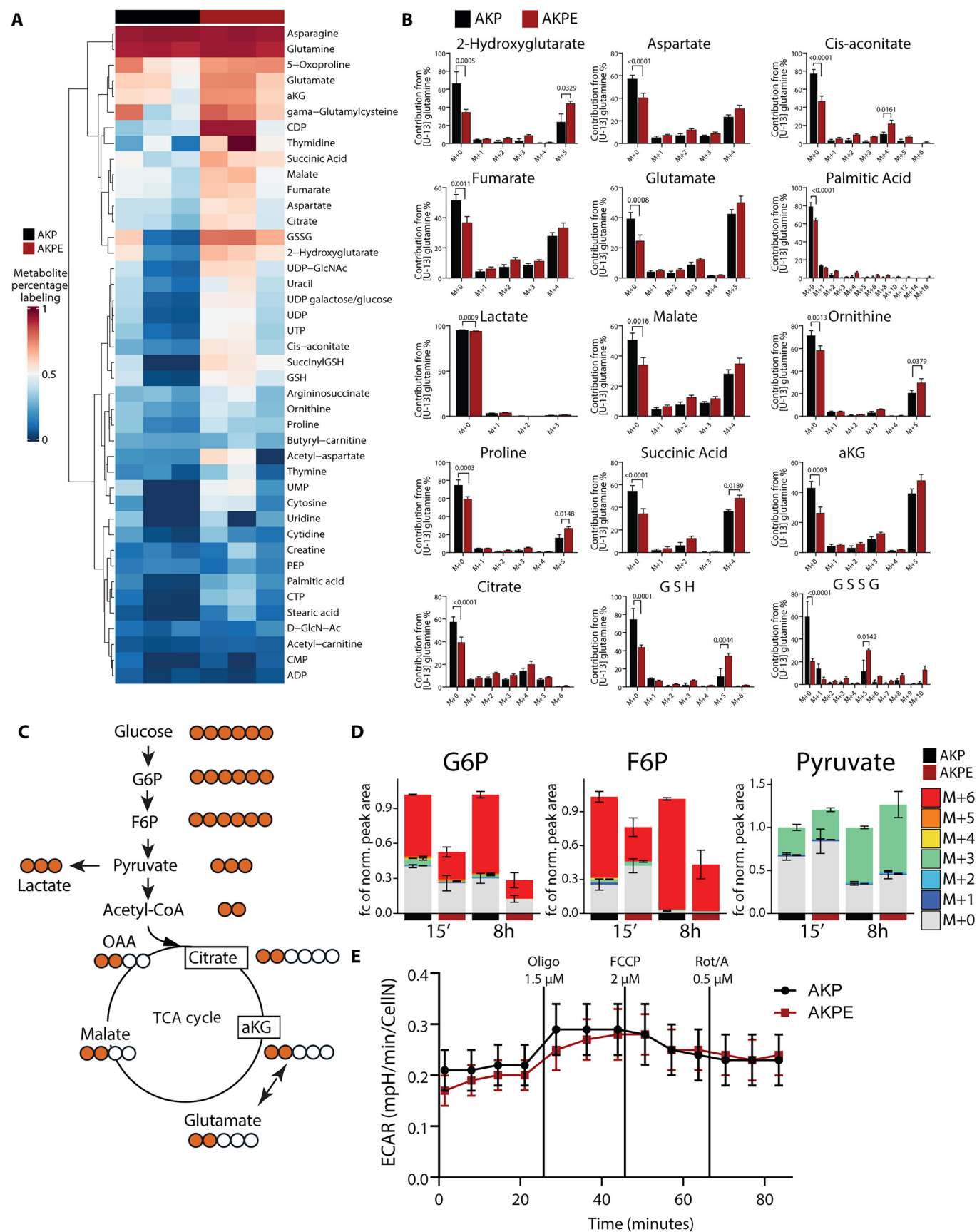


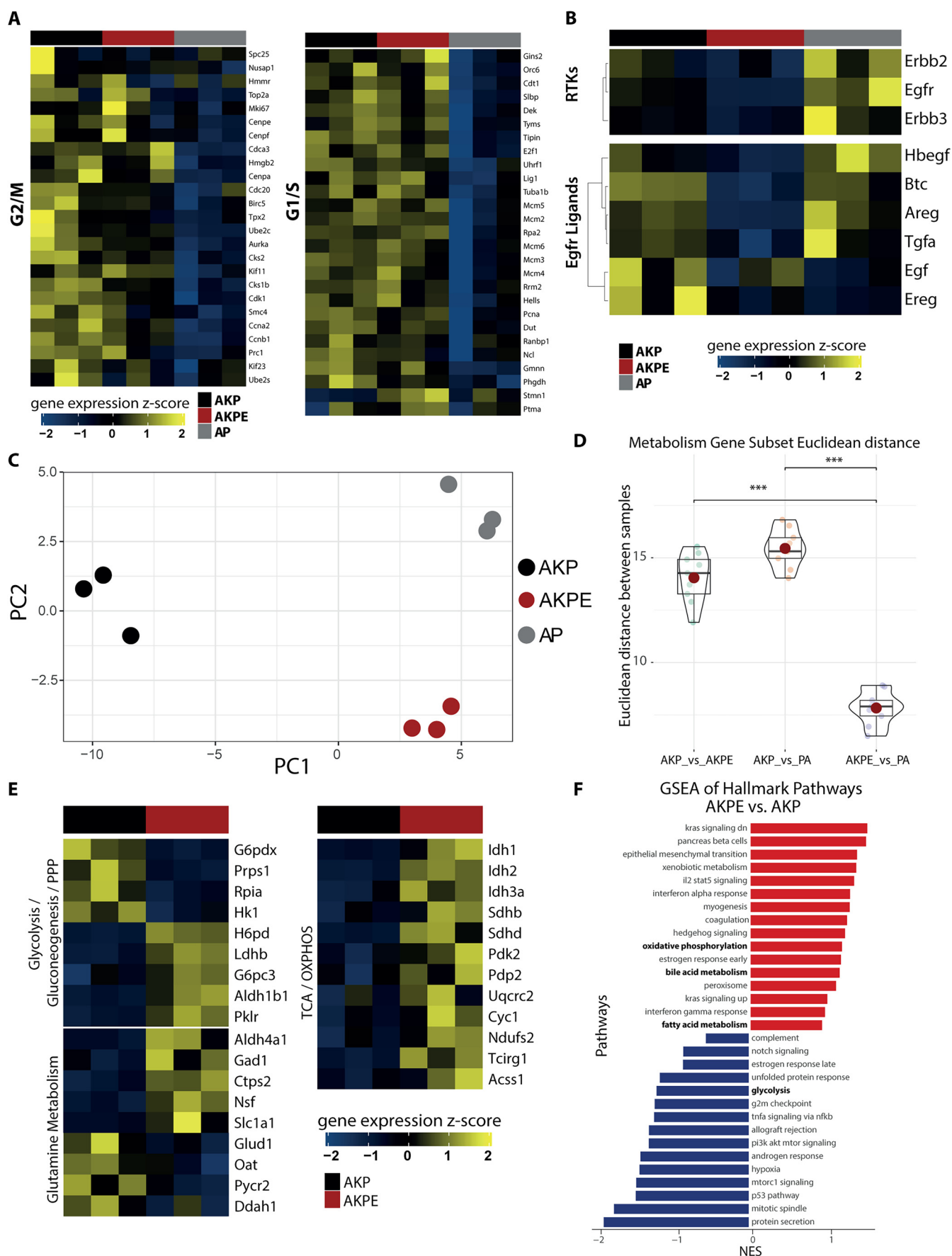
Figure EV1. Establishment and molecular characterization of EGFR-deficient organoids isolated from GEMMs.

(A) Schematic depiction of genetic crossing strategy used for generating GEMMs, from which organoids were derived. (B) Representative FACS gating strategy of AKP (green fluorescent protein (GFP)-positive), Tamoxifen (4-OHT) or Adeno-Cre induced recombination in AKP organoids after passage one (tdTomato and GFP positive) or sorted AKPE organoids (tdTomato positive), two passages after recombination (left). Depiction of genetic dual-reporter cassette (right). (C) PCR verification of genomic DNA from AKP plus vehicle, AKP plus Tamoxifen (4-OHT) or loading control (ctrl) samples for wild-type *Egfr*^{+/+}, floxed *Egfr*^{fl/+} or recombined *Egfr*^Δ alleles. (D) RT-qPCR analysis of *Egfr* mRNA in AKP, AKPE or AP organoids; *n* = 4, One-way ANOVA. (E) Absence of EGFR protein in the three independently derived EGFR-deleted AKPE organoids used in this study. (F) Quantification (left) of absolute GLUT1 protein amount between AKP, AKPE, and AP organoids assessed by western blot analysis (right), One-way ANOVA (*n* = 4, 3 or 4, respectively). (G) RT-qPCR analysis of *Slc2a1/Glut1* mRNA expression in AKP or AKPE organoids (*n* = 7). All data represent mean ± SEM. *P*-values calculated by paired, two-tailed *t*-test (between pairs of AKP and AKPE organoids) or One-way ANOVA.



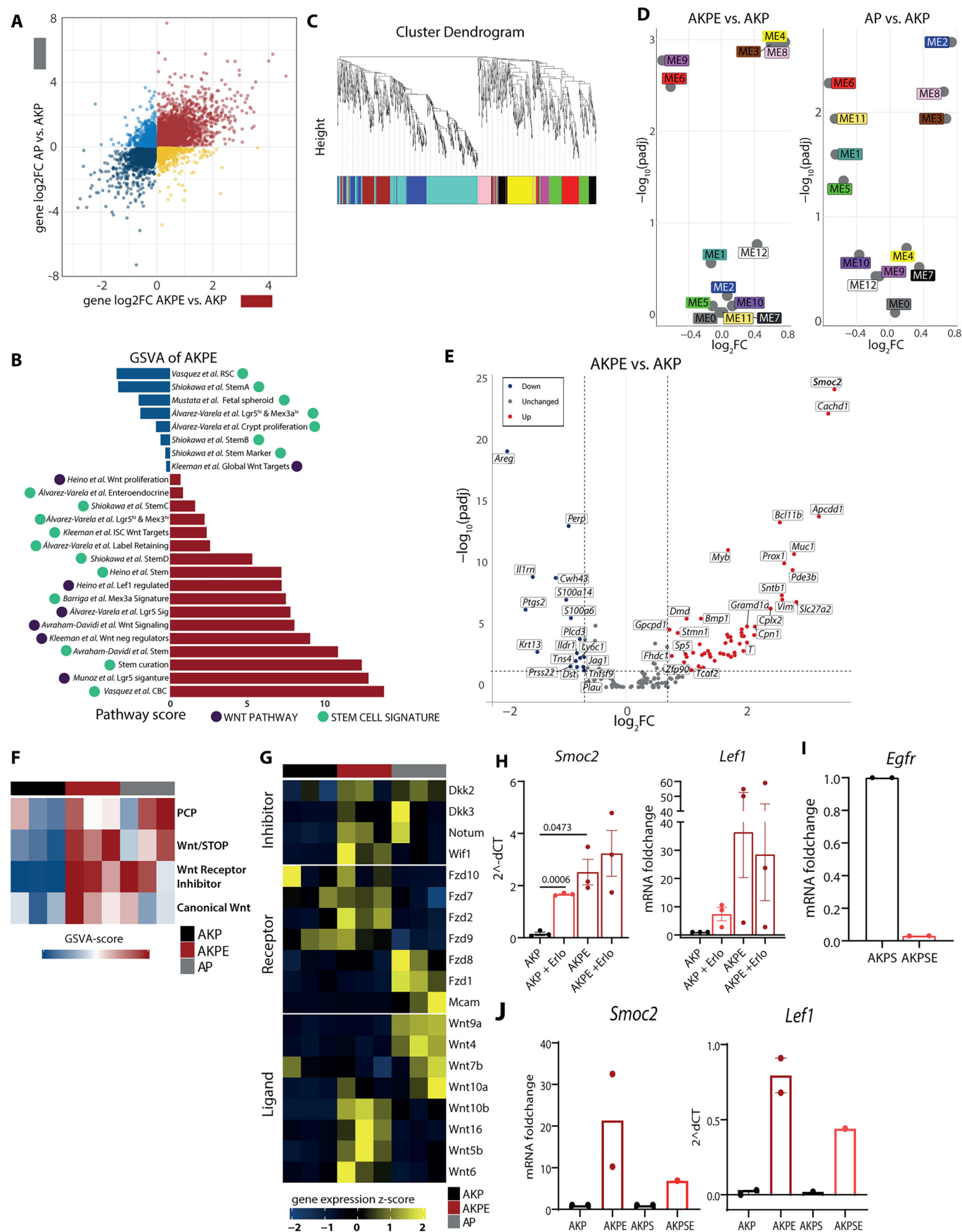
◀ **Figure EV2. Stable isotope tracing and metabolic analysis reveals differential glutamine and glucose metabolism in EGFR-deficient organoids.**

(A) Heatmap showing fractional labeling of respective metabolites in AKP or AKPE organoids. The color scale corresponds to the z-score value of relative abundance of the metabolite. (B) Fractional enrichment in ^{13}C -glutamine derived isotopologues of indicated metabolites as determined by LC-MS analysis. $\text{M} + 0$ (all carbons unlabeled) to $\text{M} + n$ isotopologues indicate number of ^{13}C atoms present in respective metabolite ($n = 3$ biological organoids with 4 technical replicates per organoid line). (C) Schematic overview of glycolytic and oxidative catabolism and fractional contribution of labeled ^{13}C carbons of indicated metabolites. (D) Glucose tracer metabolomics in AKPE ($n = 2$, biological) or AKP ($n = 2$, biological) organoids (technical replicates 6 per organoid line) showing fold-change at timepoints 15 min and 8 h of fractional labeling of glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), pyruvate. $\text{M} + 0$ (all carbons unlabeled) to $\text{M} + n$ isotopologues indicate number of ^{13}C atoms present in respective metabolite. Total abundance normalized to protein (BCA) content. (E) Representative extracellular acidification rate (ECAR) measurement of AKP and AKPE organoids ($n = 3$, biological) obtained by the Mito Stress test of the Seahorse XF analysis. Oligomycin, FCCP, Rotenone (Rot) and antimycin-A (A) were added at indicated timepoints. aKG: alpha-ketoglutarate. G6P: Glucose-6-phosphate. TCA: tricarboxylic acid. All data represent mean $\pm$ SEM. *P*-values calculated by paired, two-tailed t-test (between pairs of AKP and AKPE organoids).



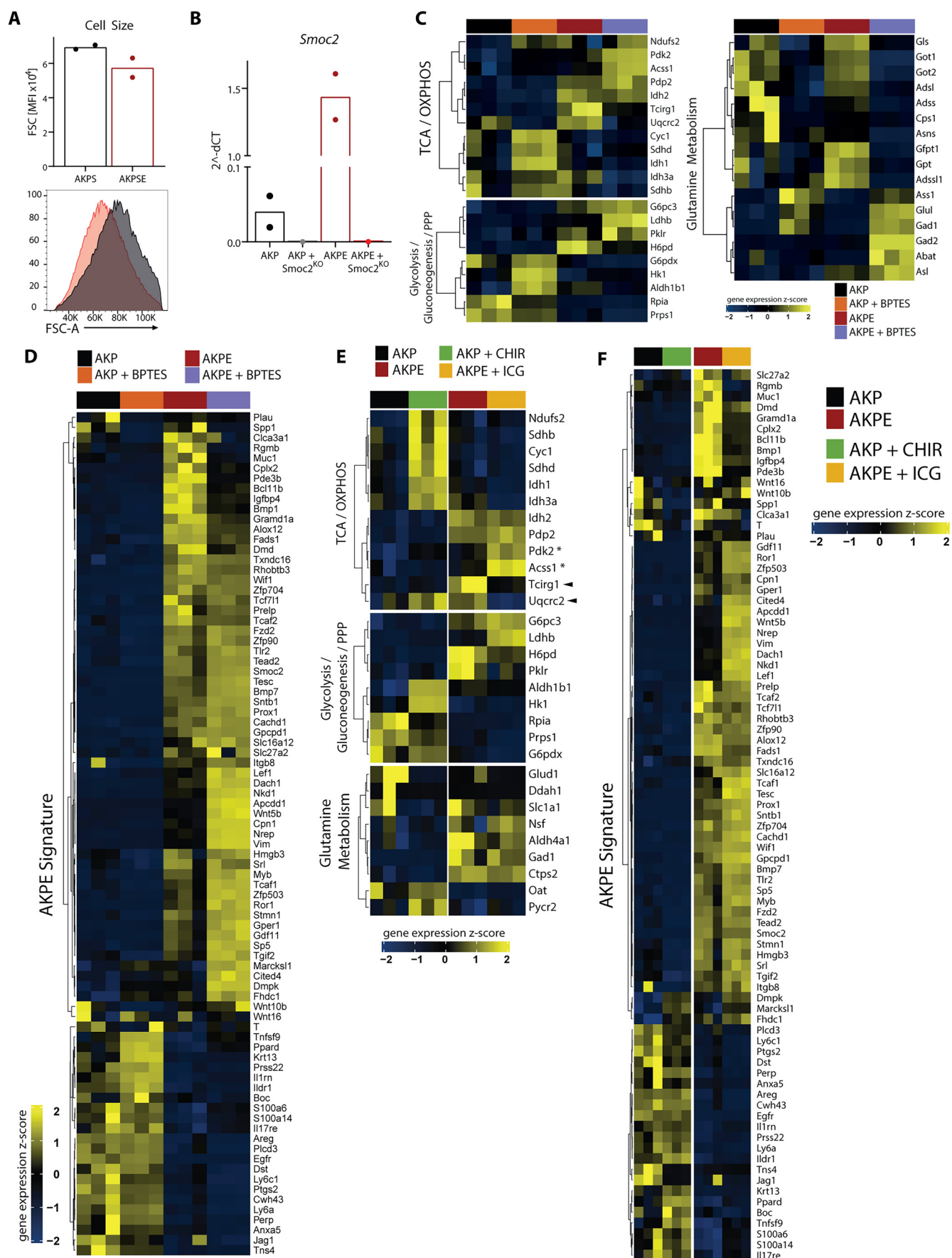
◀ **Figure EV3. Distinct transcriptional changes induced by KRAS^{G12D} expression and EGFR deletion.**

(A) Heatmap showing expression of selected cell cycle progression genes (based on (Avraham-Davidi et al, 2024)) of AKP, AKPE, and AP transcripts. (B) Heatmap showing expression of selected gene transcripts of receptor tyrosine kinases (RTKs) and EGFR ligands in AKPE, AKPE or AP organoids. (C) Principal component analysis (PCA) of metabolic gene subset of AKP, AKPE and AP organoids in steady state. Each subpopulation is depicted by indicated color. (D) Euclidean distance between samples of first and second principal component of metabolic gene subset between annotated comparisons, depicting comparisons of each sample to all other samples ($n = 9$). Horizontal lines denote the median and dots the mean. The box limits indicate 25th and 75th percentiles, whiskers extend to 1.5× of the interquartile range (IQR) from the 25th and 75th percentiles. (E) Heatmap showing expression of metabolic genes between AKP and AKPE organoids categorized according to molecular metabolic functional pathways. (F) Gene set enrichment analysis (GSEA) showing MSigDB Hallmark pathways significantly enriched in AKPE versus AKP ranked by their normalized enrichment score (NES). All data represent mean $\pm$ SEM. *P*-values calculated by One-way ANOVA.



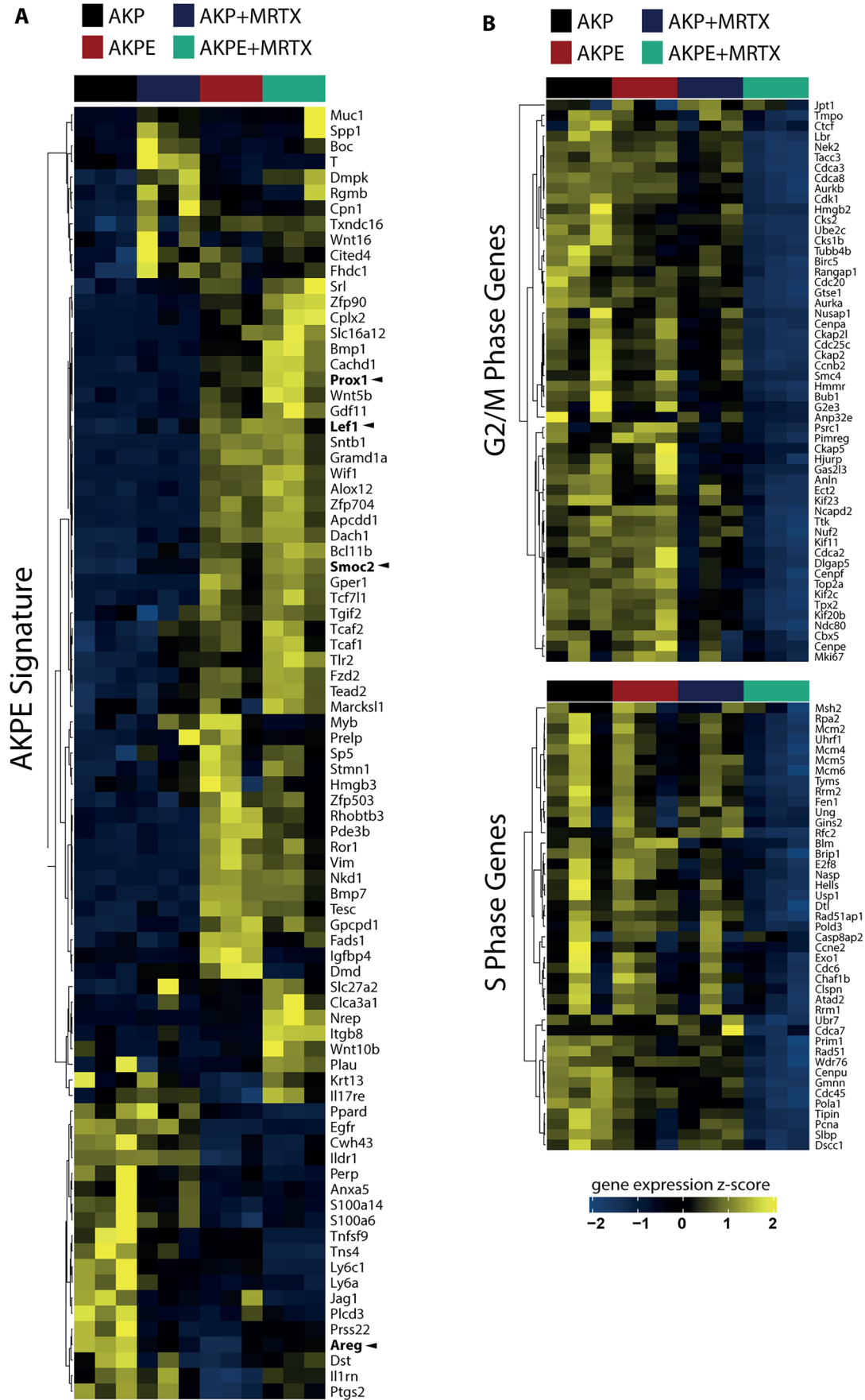
◀ **Figure EV4. Transcriptomic analysis reveals WNT pathway genes affected in EGFR-deficient colorectal organoids.**

(A) Scatter plot of fold changes from differential expression of AKPE versus AKP plotted against AP versus AKP derived fold changes. (B) Pathway enrichment of gene set variation analysis (GSVA) in AKPE organoids ranked by their pathway score. (C) Dendrogram of marker genes obtained by weighted gene correlation network analysis (WGCNA) according to modules. (D) Volcano plot of pathway-level modules derived from WGCNA analysis. (E) Volcano plot representing up or down-regulated gene expression of AKPE-associated genes according to fold-change ($n = 3$). (F) Heatmap of GSVA analysis of indicated WNT-pathways. (G) Heatmap of selected WNT-receptor and ligand interaction genes. (H) RT-qPCR analysis of *Smoc2* and *Lef1* mRNA expression in AKP, AKPE and erlotinib treated organoids ($n = 3$). (I, J) RT-qPCR analysis of *Egfr*, *Smoc2*, *Lef1* mRNA expression in AKPS or AKPSE organoids ($n = 2$). All data show mean $\pm$ SEM, or for I data mean $\pm$ SD. *P*-values calculated by paired, two-tailed t-test (between pairs of AKP and AKPE organoids).



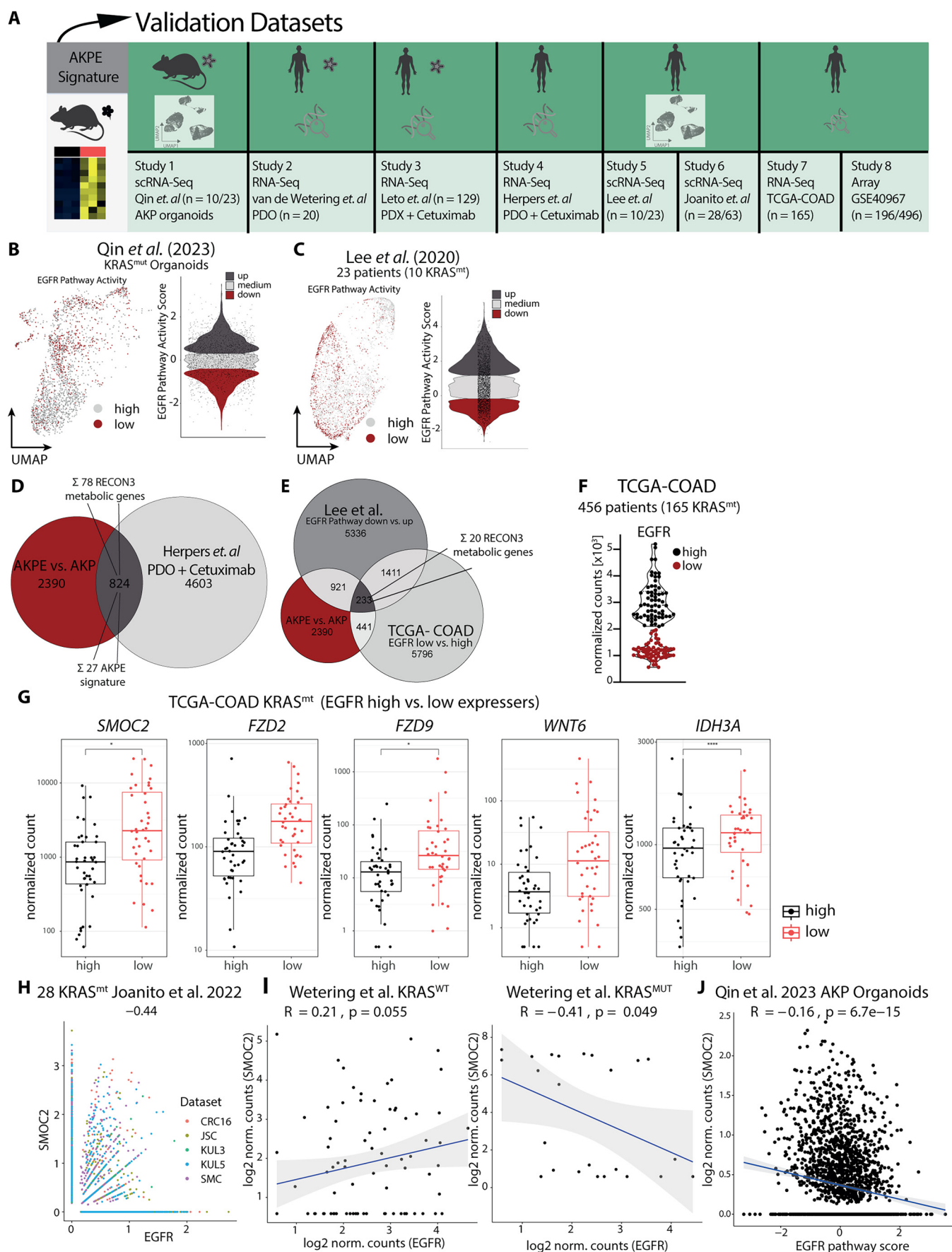
**Figure EV5. Characterisation of organoids lacking Smoc2.**

(A) Forward scatter mean fluorescent intensity (MFI) and representative histogram of AKPS and AKPSE organoids assessed by flow cytometry. Data show mean $\pm$ SEM. (B) RT-qPCR analysis of *Smoc2* mRNA in AKP, AKP + *Smoc2*^{KO} or AKPE and AKPE + *Smoc2*^{KO} organoids ($n = 2$). (C) Heatmap showing subset of metabolic genes in AKP, AKPE or AKP and AKPE treated with control vehicle or 10 μ M BPTES. (D) Heatmap showing expression of AKPE-signature genes in AKP, AKPE or AKP and AKPE treated with control vehicle or 10 μ M BPTES. (E) Heatmap showing a subset of metabolic genes in AKP, AKPE, or AKP and AKPE treated with control vehicle or 3 μ M CHIR99021 or 2 μ M ICG-001. (F) Heatmap showing expression of AKPE-signature genes in AKP, AKPE, or AKP and AKPE treated with control vehicle or 3 μ M CHIR99021 or 2 μ M ICG-001.



◀ **Figure EV6. Distinct response to KRAS inhibition highlights EGFR loss as primary driver of AKPE gene signature.**

(A) Heatmap showing expression of AKPE-signature genes in AKP, AKPE, or AKP and AKPE treated with control vehicle or MRTX1133 (100 nM). (B) Heatmap showing expression of selected cell cycle progression genes in AKP, AKPE, or AKP and AKPE treated with control vehicle or MRTX1133 (100 nM).



◀ **Figure EV7. Validation of EGFR-associated AKPE signatures across CRC datasets from mouse organoids and human tumors.**

(A) Graphical overview of accessed, publicly available mouse and human CRC datasets. *n* indicate number of KRAS^{mt} of all samples. (B) Classification of single cells based on EGFR high or low pathways activity from Qin et al, 2023 (*n* = 10 organoids). (C) Classification of single cells based on EGFR high or low pathways activity from Lee et al, 2020 (*n* = 10 patients). (D) Venn diagram of mutually expressed genes between AKPE versus AKP, EGFR high versus low expressors from Herpers et al PDOs treated with cetuximab or control vehicle. (E) Stratification of TCGA-COAD KRAS^{mt} patients into high and low EGFR expressors (*n* = 165 patients). (F) Venn diagram of mutually expressed genes between AKPE versus AKP, EGFR high vs. low expressors from TCGA-COAD and cells with down versus upregulated EGFR pathway signature from Lee et al, 2020. (G) Normalized counts of *SMOC2*, *FZD2*, *FZD9*, *WNT6* and *IDH3A* of TCGA-COAD KRAS^{mt} patients of EGFR high and low expressors (*n* = 165 patients). Horizontal lines denote the median, box limits indicate 25th and 75th percentiles and whiskers extend from the hinge to the lowest/largest value no further than 1.5x IQR from the 25th and 75th percentiles. (H) Scatter correlation plots of EGFR versus *SMOC2* expression in KRAS^{mt} single cells of the Joanito et al dataset. (I) Correlation of EGFR versus *SMOC2* expression in KRAS^{wt} or mt patients of the Wetering et al dataset. (J) Correlation of EGFR versus *SMOC2* in single cells of AKP organoids in the Qin et al dataset.