

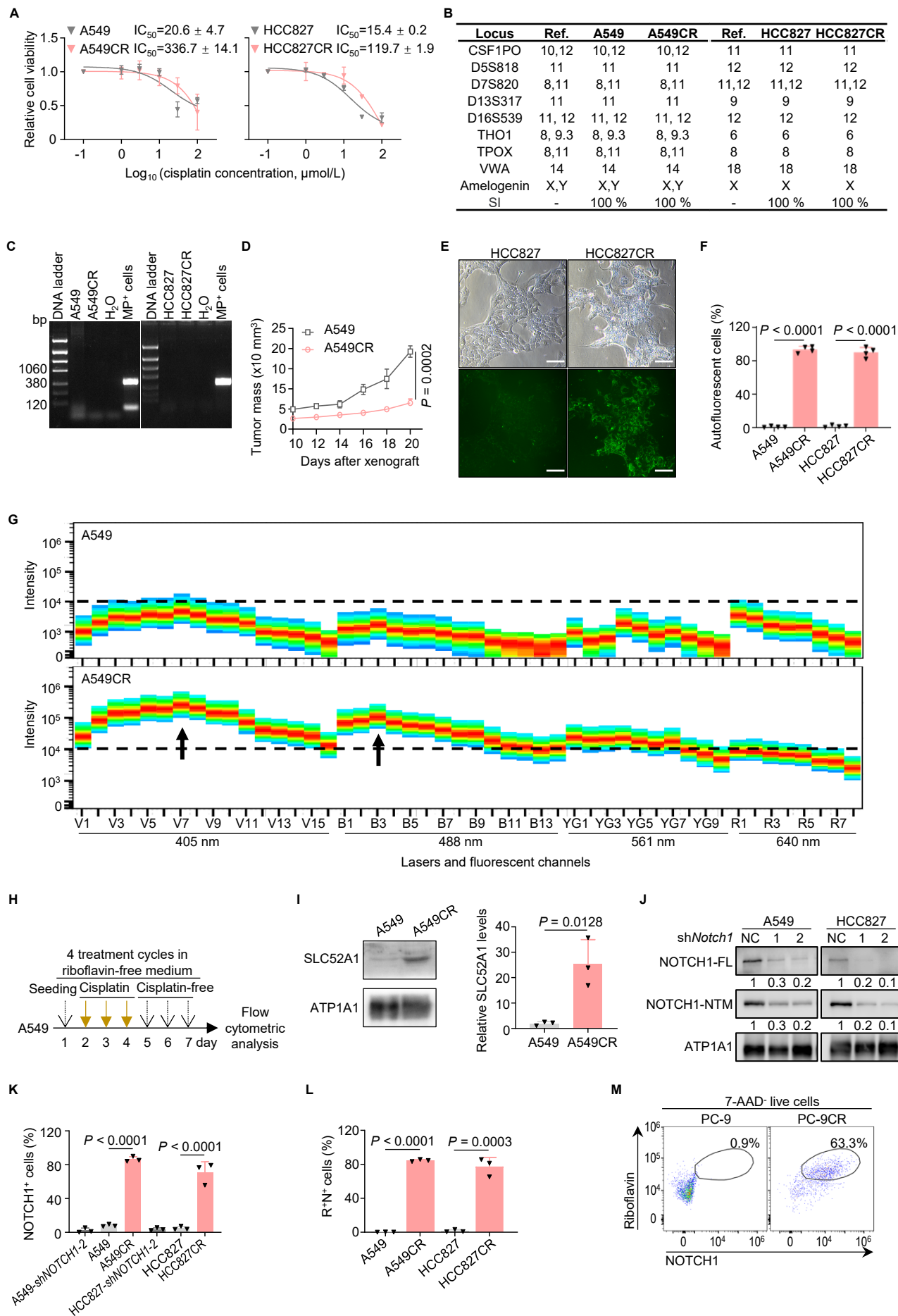


Figure S1: The riboflavin⁺NOTCH1⁺ population is identified in untreated and cisplatin-treated lung cancer cell lines. **A** Response curves of A549, A549CR, HCC827 and HCC827CR to cisplatin were determined using CCK-8 assays and used to calculate IC₅₀ (n = 2 independent experiments). **B** STR profiling of parental and surviving cells after 4 cycles of cisplatin treatment. The similarity index (SI) values in the table indicate the similarity between cisplatin-treated cells and their parental counterparts. The reference STR information was obtained from the Cellosaurus database. **C** Evaluation of mycoplasma contamination by PCR for A549CR and HCC827CR cells. MP, mycoplasma positive. **D** Growth of xenograft tumors in the NCG mice injected subcutaneously with 1 × 10⁶ A549 and A549CR cells was monitored at 2-day intervals after injection (n = 3 mice per group). **E** Representative images of autofluorescence in HCC827 and HCC827CR cells. Scale bar, 100 μm. **F** Flow cytometric quantification of autofluorescent cells (n = 4 independent experiments). See also Fig. 1D. **G** Spectrum of autofluorescence in A549 and A549CR cells was detected on a Cytex Aurora 4-laser cytometer. The arrows indicate the emission peaks of autofluorescence excited by 405 nm and 488 nm lasers, respectively. **H** Schematic diagram for assessing riboflavin fluorescence in A549CR cells, which were enriched by cisplatin treatment in a riboflavin-free medium for 28 days. **I** Western blot analysis (left panel) and quantification (right panel) of the SLC52A1 protein in A549CR cells (n = 3 independent experiments). **J** Western blot analysis of the NOTCH1 protein in A549 and HCC827 cells, in which *NOTCH1* was knocked down using 2 different shRNA. ATP1A1 was used as a loading control (n = 1 independent experiment). FL, full length; NTM, NOTCH transmembrane and intracellular domains. **K** Quantification of the NOTCH1⁺ fractions in A549/A549CR and HCC827/HCC827CR cells, as well as sh*NOTCH1*-bearing A549 and HCC827 cells. See also Fig. 1F. **L** Flow cytometric quantification of the riboflavin⁺NOTCH1⁺ (or R⁺N⁺) fractions in A549/A549CR and HCC827/HCC827CR cells (n = 3 independent experiments). See also Fig. 1G. **M** Flow cytometric analysis of the riboflavin⁺NOTCH1⁺ population in PC-9 and PC-9CR cells (n = 1 independent experiment). Error bars, mean ± SD; *P* values were calculated by Student's unpaired *t*-tests (**F**, **I**, **L**) or one way ANOVA with Tukey's tests (**K**).

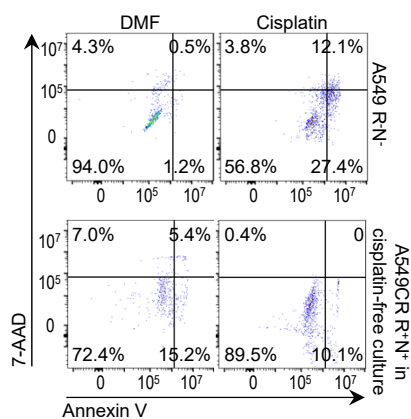
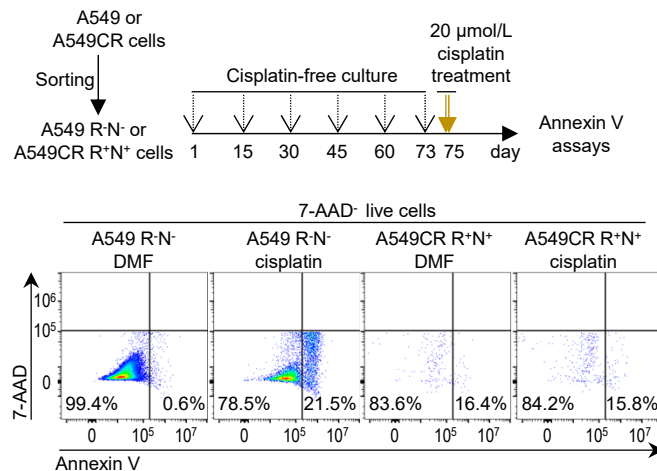
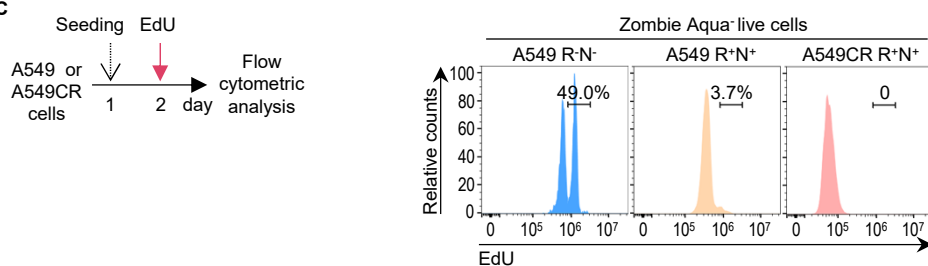
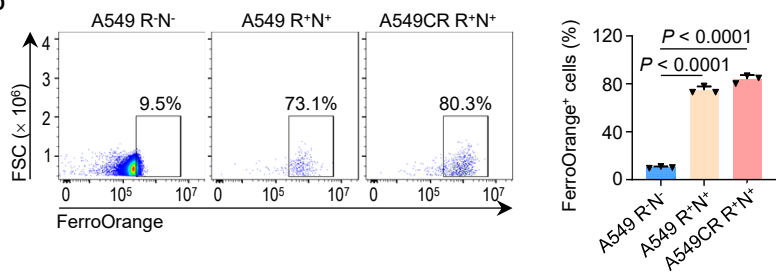
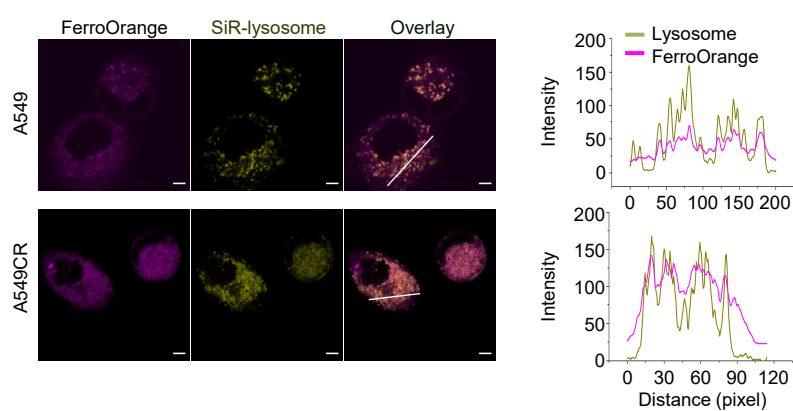
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Figure S2: Characterization of the cisplatin-naïve and cisplatin-exposed riboflavin*NOTCH1⁺ populations. **A** Representative Annexin V assay for the irreversible resistance of sorted riboflavin*NOTCH1⁺ cells after 8 days of cisplatin-free culture (n = 3 independent experiments). The experimental design is shown in Fig. 2C and the results are summarized in Fig. 2D. **B** Assessment of stable resistance of sorted riboflavin*NOTCH1⁺ A549CR cells to 2-day cisplatin treatment after 73 days of cisplatin-free culture (n = 1 independent experiment). The top panel is an experimental plot and the bottom panel is an Annexin V assay. **C** EdU proliferation assays for cisplatin-naïve and cisplatin-exposed riboflavin*NOTCH1⁺ populations gated from A549 and A549CR cells, respectively (n = 1 independent experiment). The left panel is an experimental diagram and the right panel is flow cytometric analysis. **D** Representative flow cytometric analysis (left panel) and quantification (right panel) with FerroOrange (Fe²⁺ indicator) in cisplatin-naïve and -exposed riboflavin*NOTCH1⁺ A549 cells (n = 3 independent experiments). **E** Colocalization analysis of Fe²⁺ and lysosome in A549 and A549CR cells, using a Fe²⁺ probe (FerroOrange, in rose red) and lysosome dye (SiR-lysosome, in yellow). The left panel displays representative confocal images, while the right panel presents the intensity profiles of the FerroOrange and lysosome fluorescence along a white line across cells (n = 1 independent experiment). Scale bar, 5 μ m. R⁻N⁻, riboflavin*NOTCH1⁻; R⁺N⁺, riboflavin*NOTCH1⁺; DMF, N, N-dimethylformamide (cisplatin diluent). Error bars, mean $\pm$ SD; *P* values were calculated by one way ANOVA with Tukey's tests (**D**).

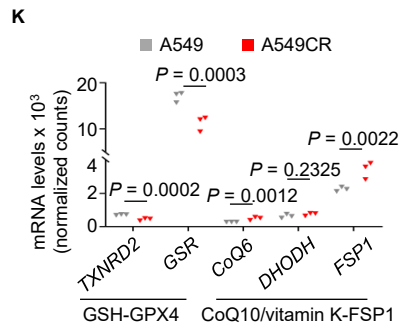
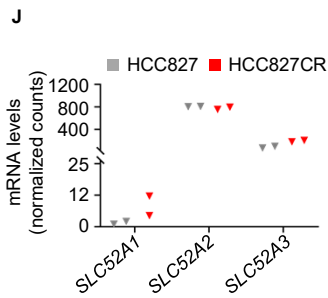
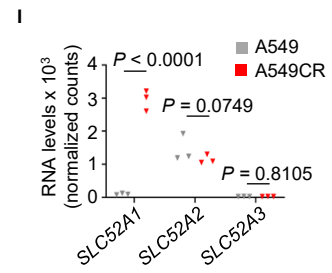
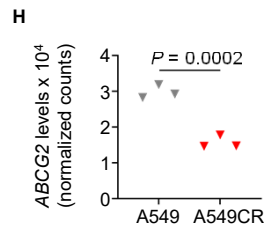
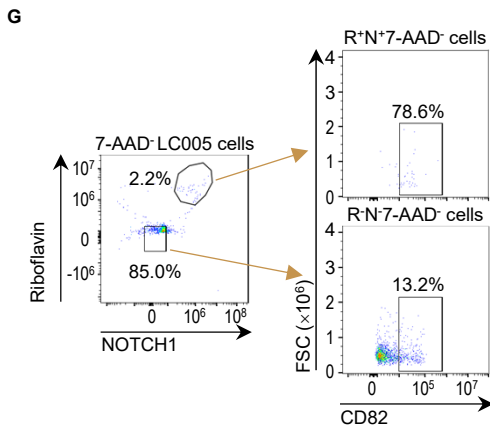
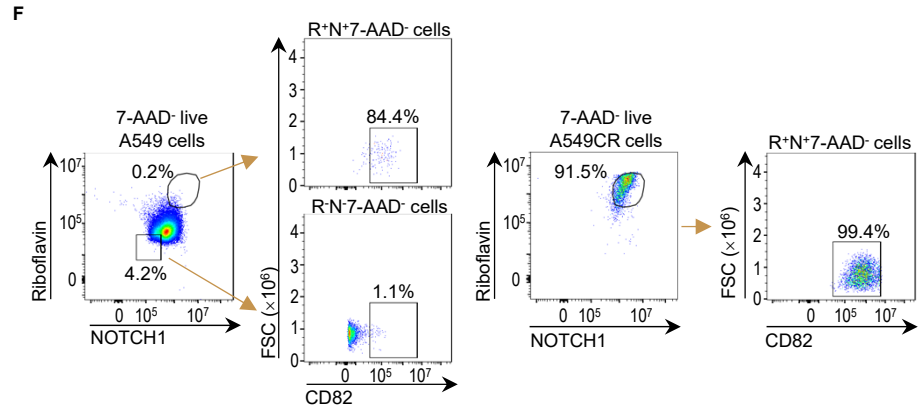
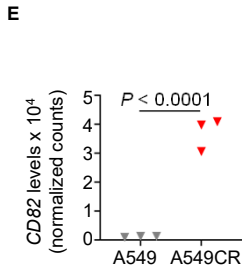
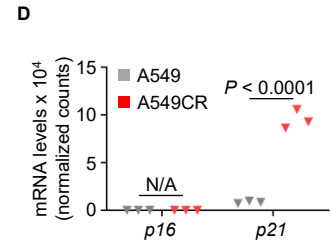
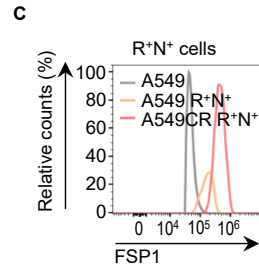
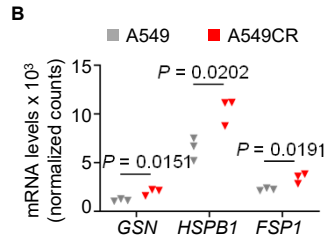
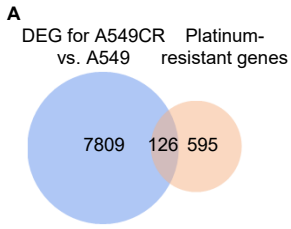


Figure S3: The molecular programs of platinum resistance, cell dormancy, and antioxidant control are associated with SRCC. **A** Venn diagram illustrating 126 platinum-resistant genes in the DEG identified by a transcriptomic comparison between A549CR and A549 cells. **B** Transcriptional comparison of the anti-apoptotic and anti-ferroptotic genes *GSN*, *HSPB1* and *FSP1* between A549 and A549CR cells, using bulk RNA-seq analysis with DEseq2 (n = 3 independent experiments). **C** Protein-flow analysis with an anti-FSP1 antibody was used to detect FSP1 expression in cisplatin-naïve and -exposed riboflavin*NOTCH1⁺ cells (n = 3 independent experiment). **D,E** Transcriptional comparison of the senescence regulatory genes *p16* and *p21* (**D**), and the dormancy marker *CD82* (**E**) between A549 and A549CR cells, using bulk RNA-seq analysis with DEseq2 (n = 3 independent experiments). **F** Representative flow cytometric analysis of CD82 expression in the riboflavin*NOTCH1⁺7-AAD⁻ and riboflavin*NOTCH1⁺7-AAD⁺ populations derived from A549 and A549CR cells. See the summarized results (n = 3 independent experiments) in Fig. 3K. **G** Representative flow cytometric analysis of CD82 expression in lung cancer specimens. See the summarized results (n = 4 patients) in Fig. 3L. **H** Transcriptional comparison of the transporter gene *ABCG2* between A549 and A549CR cells, using bulk RNA-seq analysis with DEseq2 (n = 3 independent experiments). **I** Transcriptional comparison of *SLC52A1*, *SLC52A2* and *SLC52A3* between A549CR and A549 cells, using RNA-seq analysis with DEseq2. **J** Transcriptional comparison of the riboflavin transporter genes *SLC52A1*, *SLC52A2* and *SLC52A3* between HCC827 and HCC827CR cells, using bulk RNA-seq analysis (n = 2 independent experiments). **K** Transcriptional comparison of the antioxidant genes *TXNRD2*, *GSR*, *CoQ6*, *DHODH* and *FSP1* between A549 and A549CR cells, using bulk RNA-seq analysis with DEseq2 (n = 3 independent experiments). R⁻N⁻, riboflavin*NOTCH1⁻; R⁺N⁺, riboflavin*NOTCH1⁺; the adjusted *P* values were determined using Wald tests with the Benjamini-Hochberg correction in DEseq2 (**B**, **D**, **E**, **H**, **I**, **K**).

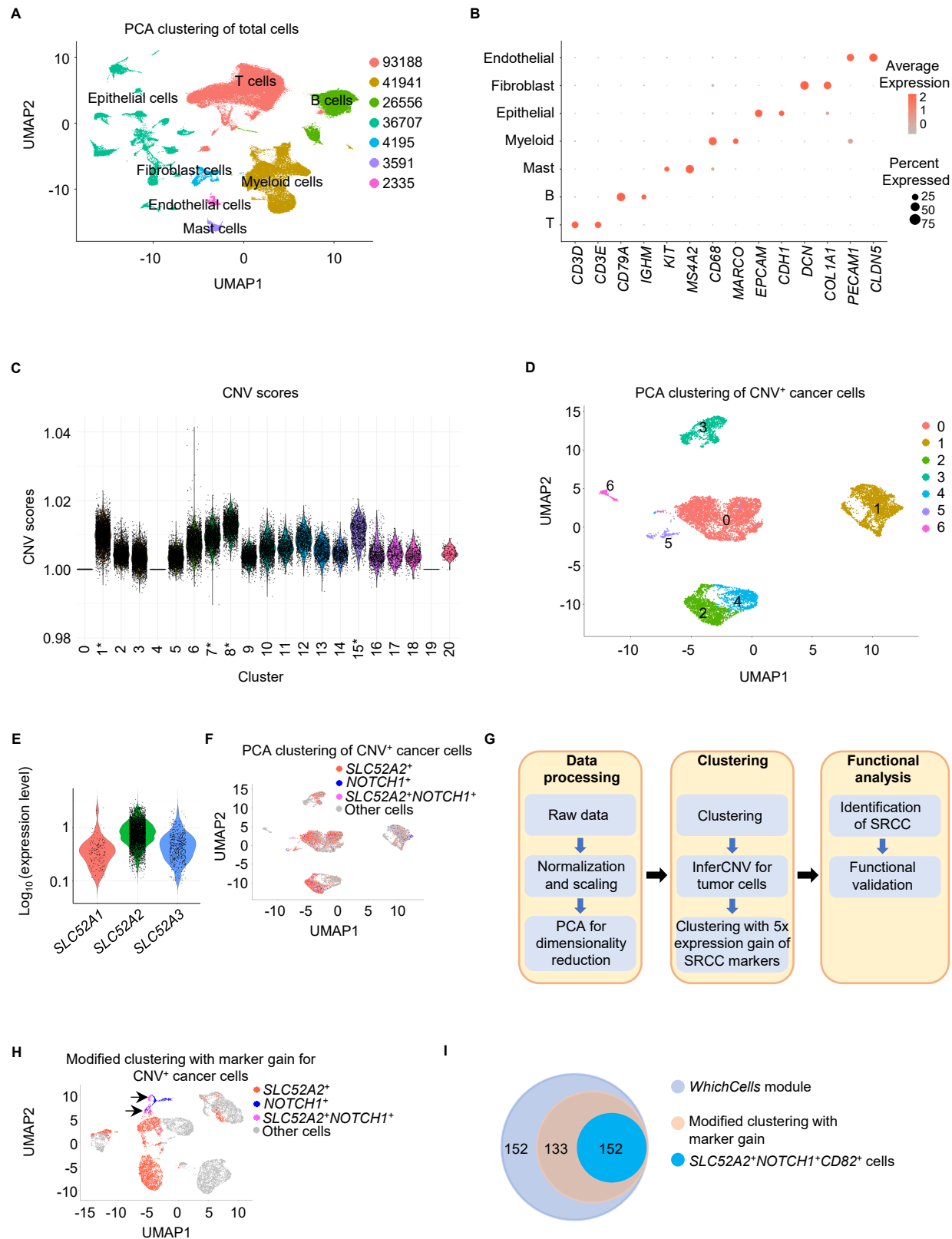


Figure S4: The *SLC52A2*⁺*NOTCH1*⁺ SRCC population is identified in scRNA-seq data derived from diagnosed NSCLC specimens. **A** PCA analysis and UMAP plot illustrated cell clusters in the dataset GSE131907 (208506 cells), derived from advanced NSCLC patients before therapy. The cell counts for individual clusters are provided on the right. **B** The expression and positive percentages of lineage marker genes were determined for the cell clusters indicated in Fig. S4A. **C** *InferCNV* analysis of the clustered epithelial, fibroblast and B cells for CNV clusters and their CNV scores (Table S6). The cells in the clusters marked with asterisks were identified as CNV⁺ tumor cells with their CNV scores > 1.009, which were used in subsequent experiments (Fig. S4D-I). **D** PCA clustering of the CNV⁺ tumor cells. **E** Violin plot showed the expression of *SLC52A1/2/3* in the CNV⁺ tumor cells selected by the *WhichCells* module. **F** Visualization of the expression of *SLC52A2* and/or *NOTCH1* on the clusters of CNV⁺ tumor cells determined by PCA clustering. **G** Schematic diagram for a modified PCA clustering technique implemented with the 5-fold pre-amplified expression of the marker genes *SLC52A2* and *NOTCH1*, which was developed to enable the clustering of rare SRCC. **H** Visualization of the expression of *SLC52A2* and/or *NOTCH1* on the cell clusters determined by modified PCA analysis with marker gain (Fig. 4A). The black arrows indicate the *SLC52A2*⁺*NOTCH1*⁺ clusters. **I** Venn diagram illustrated the *SLC52A2*⁺*NOTCH1*⁺ cells identified by the *WhichCells* module and the modified PCA clustering technique with marker gain. The *CD82*⁺ fraction was also determined in the *SLC52A2*⁺*NOTCH1*⁺ cluster identified by the modified PCA clustering technique with marker gain. See also Table S7.

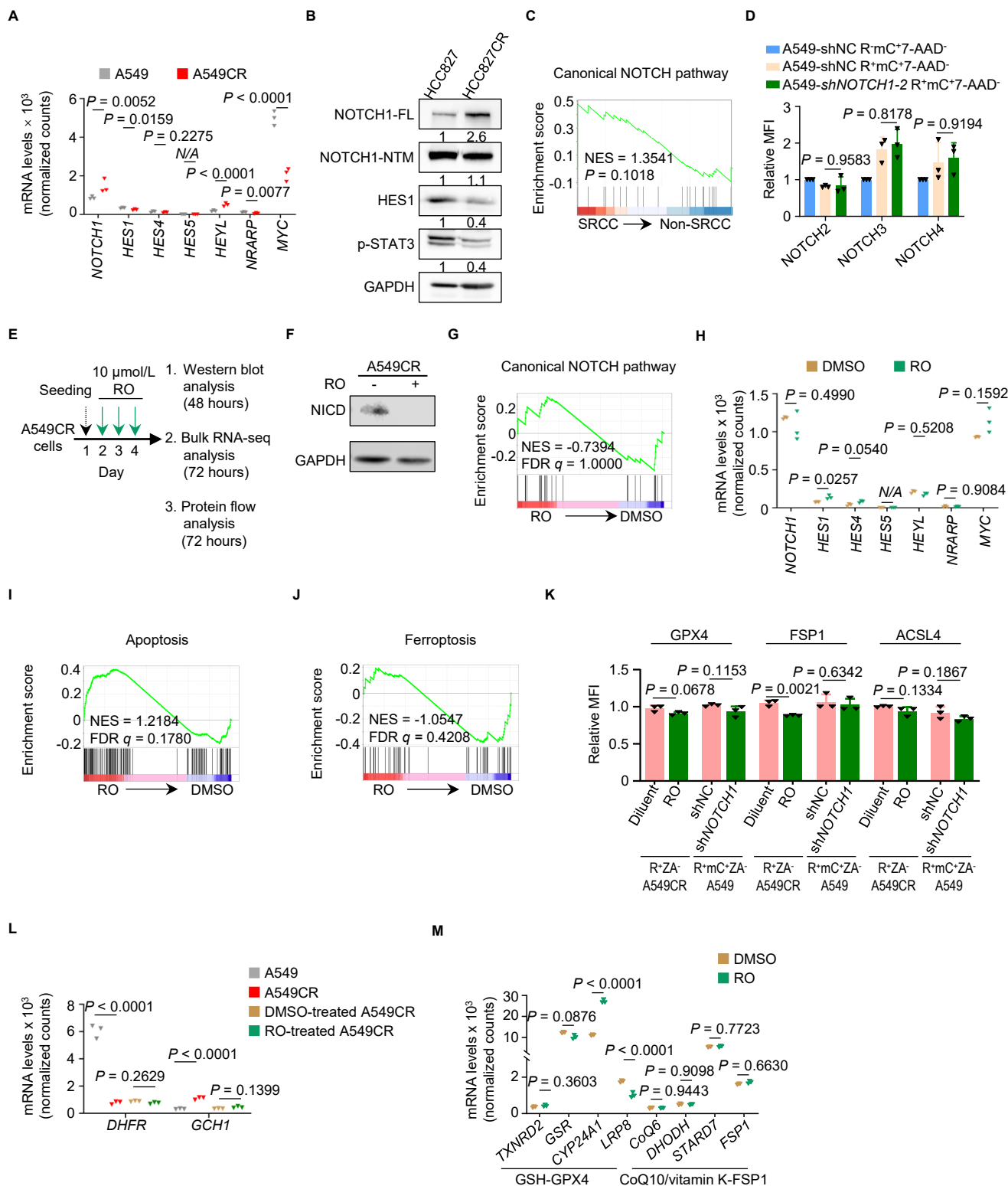


Figure S5: The non-canonical NOTCH pathway plays an important role in the regulation of SRCC. **A** Transcriptional comparison of the canonical NOTCH pathway targets between A549CR and A549 cells (n = 3 independent experiments). **B** Western blot analysis of key regulators in the canonical NOTCH pathway in the comparison of HCC827CR cells vs. HCC827 cells (n = 2 independent experiments). FL, full length, NTM, NOTCH transmembrane and intracellular region. **C** GSEA of scRNA-seq data using *GSEABase* and *fgsea* illustrated that the canonical NOTCH pathway was not enriched in SRCC compared to non-SRCC. **D** Flow cytometric analysis of NOTCH2, NOTCH3 and NOTCH4 in the surface of riboflavin⁺mCherry⁺7-AAD⁻ A549-shNC and A549-*shNOTCH1-2* cells (n = 3 independent experiments). Both A549-shNC and A549-*shNOTCH1-2* are positive for mCherry. **E** Schematic diagram for assessing the response of A549CR cells to the RO treatment for 48 hours (Western blot analysis), or 72 hours (bulk RNA-seq analysis, or protein-flow analysis). **F** Western blot analysis of the NICD protein in response to treatment with 10 $\mu\text{mol/L}$ RO for 48 hours (n = 3 independent experiments). **G** GSEA of bulk RNA-seq data illustrated that the canonical NOTCH pathway was not activated in response to treatment with 10 $\mu\text{mol/L}$ RO for 72 hours (n = 3 independent experiments). **H** Transcriptional evaluation of the canonical NOTCH pathway targets in response to RO, using bulk RNA-seq analysis with DEseq2 (n = 3 independent experiments). **I, J** GSEA assessed the correlation of the apoptotic (**I**) and ferroptotic (**J**) programs with RO treatment in A549CR cells (n = 3 independent experiments). **K** Protein-flow analysis with the monoclonal antibodies (230168A8 for GPX4 expression, 1A2B2 for FSP1 expression, and 1H5D3 for ACSL4 expression) in riboflavin⁺ZA⁻ A549CR cells treated with RO for 72 hours, or in riboflavin⁺mCherry⁺ZA⁻ A549-*shNOTCH1-2* cells (n = 3 independent experiments). Both A549-shNC and A549-*shNOTCH1-2* are positive for mCherry. **L** Transcriptional comparison of the BH4-DHFR regulatory genes *DHFR* and *GCH1* between A549CR and A549 cells, and between RO- and DMSO-treated A549CR cells, using bulk RNA-seq analysis with DEseq2 (n = 3 independent experiments). **M** Transcriptional evaluation of the GSH-GPX4 regulatory genes *TXNRD2*, *GSR*, *CYP24A1*, *LRP8*, and the CoQ10 (and vitamin K)-FSP1 regulatory genes *CoQ6*, *DHODH*, *STARD7* and *FSP1*, in A549CR cells in response to RO (n = 3 independent experiments). The adjusted *P* values were determined using Wald tests with the Benjamini-Hochberg correction in DEseq2 (see also Fig. S3B, and S3K). RO, RO4929097; R, riboflavin; mC, mCherry for *shNOTCH1-2*; ZA, Zombie Aqua fluorescence for dead cells. The data are presented as mean $\pm$ SD. *P* values were calculated by one way ANOVA with Tukey's tests (**D**), or Student's unpaired *t*-tests (**K**); the adjusted *P* values were determined using Wald tests with the Benjamini-Hochberg correction in DEseq2 (**A**, **H**, **L**, **M**).

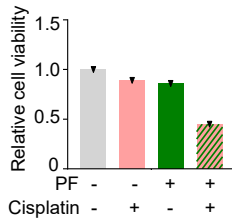
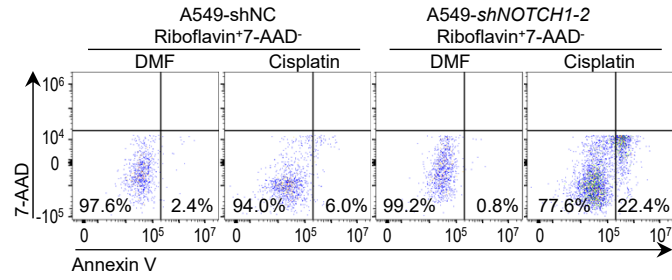
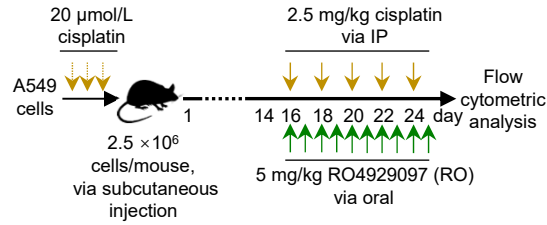
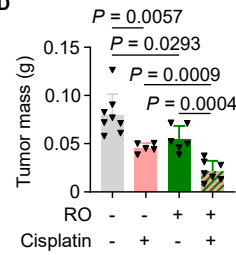
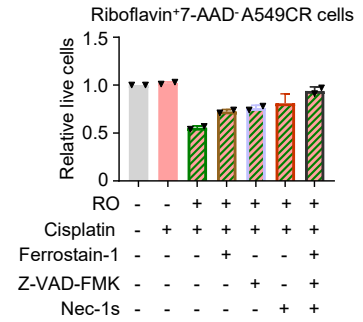
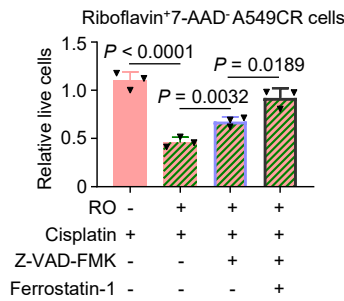
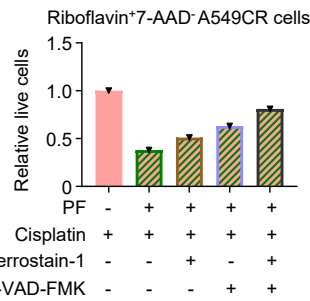
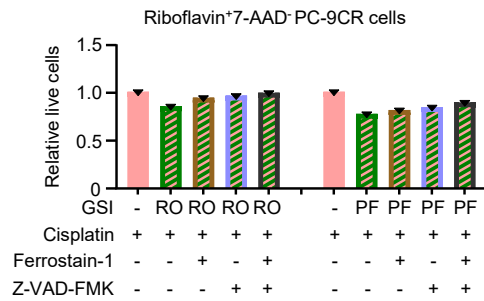
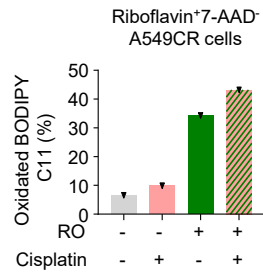
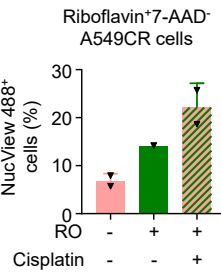
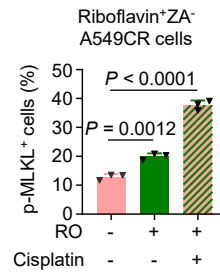
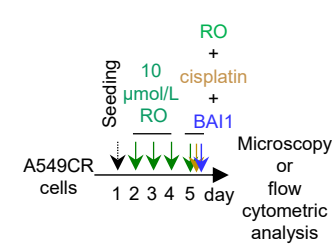
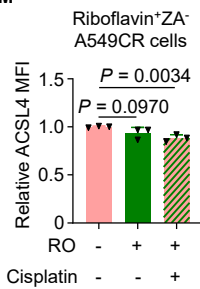
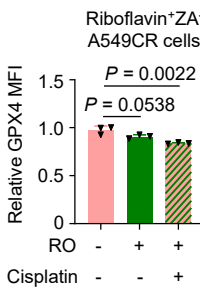
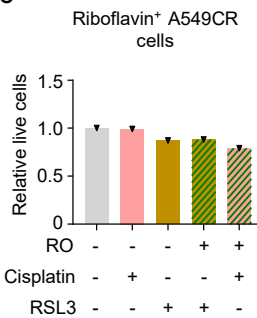
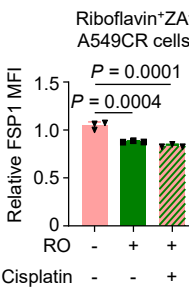
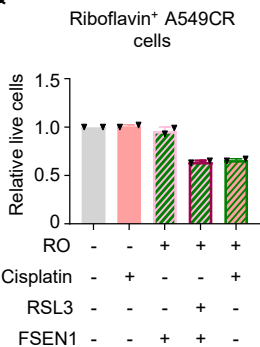
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Fig. S6: Inhibition of NOTCH1 re-sensitizes SRCC to cisplatin-mediated cytotoxicity. **A** The viability of A549CR cells in response to cisplatin, PF, or both, was assessed using CCK-8 assays ($n = 1$ independent experiment). PF is a GSI that inhibits NOTCH signaling. **B** Representative Annexin V assay for the effect of cisplatin on riboflavin⁺7-AAD⁻ A549-shNC and A549-shNOTCH1-2 cells ($n = 3$ independent experiments). Please also see summarized data in Fig. 6E. **C** Experimental design for assessing the effect of RO, cisplatin, or both on SRCC and xenograft tumors. **D** Evaluation of tumor mass in xenograft mice treated with RO alone, cisplatin alone, or both ($n = 5-8$ mice per group). **E** Annexin V assays for the effect of the ferroptosis inhibitor ferrostatin-1 (10 $\mu\text{mol/L}$), the apoptosis inhibitor Z-VAD-FMK (10 $\mu\text{mol/L}$), and the necroptosis inhibitor Nec-1s (10 $\mu\text{mol/L}$) on the death of riboflavin⁺7-AAD⁻ SRCC induced by the combinatorial treatment of RO and cisplatin ($n = 2$ independent experiments). See also representative assays in Fig. 6H. **F** Annexin V assays for the effect of the ferroptosis inhibitor ferrostatin-1 (10 $\mu\text{mol/L}$) and the apoptosis inhibitor Z-VAD-FMK (10 $\mu\text{mol/L}$) on the death of riboflavin⁺7-AAD⁻ SRCC induced by the combinatorial treatment of RO and cisplatin ($n = 3$ independent experiments). **G** An Annexin V assay for the effect of the ferroptosis inhibitor ferrostatin-1 (10 $\mu\text{mol/L}$) and the apoptosis inhibitor Z-VAD-FMK (10 $\mu\text{mol/L}$) on the death of the riboflavin⁺7-AAD⁻ A549CR cells induced by the combinatorial treatment of PF and cisplatin ($n = 1$ independent experiment). **H** Annexin V assay for the effect of the ferroptosis inhibitor ferrostatin-1 (10 $\mu\text{mol/L}$) and the apoptosis inhibitor Z-VAD-FMK (10 $\mu\text{mol/L}$) on the death of the riboflavin⁺7-AAD⁻ PC-9CR cells induced by cisplatin in combination with RO (left panel, $n = 1$ independent experiment) or PF (right panel, $n = 1$ independent experiment). **I** Flow cytometric analysis of the oxidated BODIPY 581/591 C11 fluorescence in riboflavin⁺7-AAD⁻ A549CR cells in response to RO, cisplatin, or both ($n = 1$ independent experiment). **J** Flow cytometric analysis for the cell fraction positive for activated caspase-3 (indicated by the fluorescence of NucView 488) in the riboflavin⁺7-AAD⁻ A549CR cells in response to the 24-hour treatment with RO and cisplatin ($n = 2$ independent experiments). **K** Protein-flow analysis with the monoclonal antibody EPR9514 for detecting the cell fraction positive for phosphorylated MLKL (S358) in the riboflavin⁺7-AAD⁻ A549CR compartment in response to the 24-hour treatment with RO and cisplatin ($n = 3$ independent experiments). **L** Schematic for assessing the lysosomal integrity of SRCC treated with RO and cisplatin or with RO, cisplatin, and BAI1. **M** Protein-flow analysis with the monoclonal antibody 1H5D3 for ACSL4 expression in riboflavin⁺7-AAD⁻ A549CR cells treated with RO and/or cisplatin ($n = 3$ independent experiments). **N** Protein-flow analysis with the monoclonal antibody 230168A8 for GPX4 expression in riboflavin⁺7-AAD⁻ A549CR cells treated with cisplatin and RO for 24 hours ($n = 3$ independent experiments). **O** Annexin V assay for assessing the 24-hour effect of RSL3 on riboflavin⁺ A549CR cells in the presence of RO ($n = 1$ independent experiment). **P** Protein-flow analysis with the monoclonal antibody 1A2B2 for FSP1 expression in riboflavin⁺7-AAD⁻ A549CR cells treated with cisplatin and RO for 24 hours ($n = 3$ independent experiments). **Q** Annexin V assays for assessing the 48-hour effect of RSL3 and/or FSEN1 on riboflavin⁺ A549CR cells in the presence of RO ($n = 2$ independent experiments). PF, PF-03084014; RO, RO4929097. ZA, Zombie Aqua fluorescence for dead cells. The data are presented as mean $\pm$ SD. P values were calculated by Student's unpaired t -tests (**D**) or one way ANOVA with Tukey's tests (**F**, **K**, **M**, **N**, **P**).